SYNTHESIS AND EVALUATION OF THE MEDICINAL POTENTIAL OF NOVEL 4-HYDROXYCOUMARIN DERIVATIVES

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ABSTRACT

This research has focused on the synthesis and biological evaluation of a broad range of compounds characterised by the presence of the pharmacologically significant 4-hydroxycoumalin scaffold. The compounds were designed to contain additional pharmachophoric centres to enhance bioactivity and generate lead compounds with dual-action potential. The use of 4-hydroxycoumarin as the primary synthon enabled access to various series of 4-hydroxycoumarin conjugates, the reactive 3-position on the 4-hydroxycoumarin moiety being exploited for regioselective construction of the targeted compounds in several steps. Some of the reactants required in the construction of these compounds were specially synthesised and included propargyloxy benzaldehydes, benzyloxy benzaldehydes and 2,3-dihydroxysuccino-dihydride. Overall, eight different families of novel compounds were accessed, comprising conjugates of 4-hydroxycoumarin with bis-ethylidenesuccinohyrazide, trifluoroacetamide, amino, benzyloxyphenyl-iminoethyl, benzylidenehyrazinyl-thiazoyl, benzylidenehydrazonoethyl, propargyloxy-benzylidenehydrazonoethyl and phenylacryloyl moieties using protocols that required minimal work-up and purification.

The eighty novel compounds synthesised in the study were fully characterised using HMRS and advanced NMR techniques. Cytotoxicity, HIV-1 IN and PR inhibitory, and anti-trypanosomal, antimalarial and anti-*Mtb* assays were conducted on the synthesised coumarin derivatives. Several compounds exhibited activity against HIV-1 IN, the most potent being a bis-ethylidenesuccinohyrazide with an IC_{50} value of 3.5 μM. Various compounds exhibited anti-malarial activity (% pLDH viability in the range 62-77%), anti-trypanosomal activity (the most potent with an IC_{50} = 0.9 μM against *T.b. brucei*) and a measure of anti-*Mtb* activity. Apart from two chalconyl derivatives, none of the synthesised compounds exhibited significant cytotoxicity. Conflicting results were obtained from the *in silico* docking studies; in some cases supporting the observed *in vitro* assay data while, in others, exhibiting no correlation.

ACKNOWLEDGEMENTS

The fatherly support, guidance and advice offered on this PhD journey by my supervisor, Prof. Emeritus, Perry Theodore Kaye is heartily appreciated. I cherish the journey you took me on by the hand. It has been a great learning process and I consider it a divine favour to have you as the one to take me through. May the good Lord continue blessing you and enabling you to bear fruit to the praise of his name. It is worthy of all acceptation that many that have passed through your hands have greatly benefited from the experience and continue to influence others for good wherever they are. Special thanks to my co-supervisor, Prof. Rui Krause for all input to the PhD journey. May the good Lord's favour be with you in all your endeavours.

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'Blessed be the name of God forever and ever, for wisdom and might are His. And He changes the times and the seasons; He removes kings and raises up kings; He gives wisdom to the wise and knowledge to those who have understanding. He reveals deep and secret things; He knows what is in the darkness, and light dwells with Him.' Without his gracious provision for 'He gives to all life, breath, and all things'-nothing could have been done. May his name be praised.

My family in Zimbabwe, my spiritual family in different parts of the world, the Msindo, Moratau and Marungise families-thanks for all. Muwanirwe nyasha dzakasarudzika pazvose. Last but not least thanks to Medical Research Council for financial support.

LIST OF ABBREVIATIONS

AIDS - acquired immunodeficiency syndrome

ART - anti-retroviral therapy

AZT - azidothymidine

DNA - deoxyribonucleic acid

FDA - food and drug administration

HAART - highly active antiretroviral therapy

HCT - HIV counselling and testing

HIV - human immunodeficiency virus

IN - integrase

MSM - men who have sex with men

NMR - nuclear magnetic resonance

NNRTI - Non-nucleoside reverse-transcriptase inhibitors

NRTIs - nucleoside reverse transcriptase inhibitors

NtRTIs - nucleotide reverse transcriptase inhibitors

PR - protease

PEP - post exposure prophylaxis

PrEP - pre-exposure prophylaxis

PWID - people who inject drugs

RT - reverse transcriptase

STI - sexually transferred infection

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1. INTRODUCTION

1.1. BRIEF DRUG DISCOVERY BACKGROUND

Nature can be described as an age-old pharmacy that inspired drug discovery leading to a number of therapeutic agents, some of which are natural products while, for others, natural scaffolds have been lead structures.[1] Plants have been the main contributor in ancient times for treatments with records going as far back as 2600 B.C. in Mesopotamia where records show the use of oils, such as those of *Cupressus sempevirens* (cypress), *Cedras* species (cedar) and *Commiphora* species (myrrh), which are still in use for a range of sicknesses.[2] The first record of the Chinese Materia Medica, containing over 50 prescriptions was made over 2000 years ago, while records in the Indian Ayurvedic system started over 3000 years ago.[2,3] Drug discovery continues to benefit from this immense resource through the provision of new pharmacophores, chemotypes and molecular frameworks for the development of new therapeutics for a vast array of medical conditions.[4] Among the compounds that nature has bequeathed to humankind are coumarins, a class of compounds which have inspired research on their properties that could be useful to mankind. These compounds will be discussed in some length in this work and will focus on their relevance in the treatment of Human Immunodeficiency Virus infection and Acquired Immune Deficiency Syndrome HIV/AIDS, a health challenge of significant proportions in Africa. Apart from HIV/AIDS, drug discovery also offers potential solutions to other diseases Africa faces, which include Malaria[5], Tuberculosis (TB),[6] Ebola,[7] and Yellow fever.[8] The *Plasmodium falciparum* malaria parasite is responsible for the majority of malaria cases in Africa, mainly in sub-Saharan Africa (SSA) where it accounts for 90 % of the global infections and mortality (over 600 000 per year).[9] The same region carries most of the world's TB/HIV load and over half of the TB patients are also infected with HIV.[10] The emergence of multi drug resistant tuberculosis in SSA is a further health challenge.[11] Ebola has been present in Central Africa for some time but a major outbreak occurred recently in West Africa, with Sierra Leone, Guinea and Liberia being the worst affected.[12] Generally, the outbreak is taken as having started in 2013 in Meliandou, Guinea.[13] By early 2016 over 11000 deaths had been recorded.[14] Yellow fever is having a resurgence in Africa after the progress made through extensive vaccination programmes in the 1940s to 1960s[15] but, recently, there have been significant outbreaks in the Republic of the Congo, Angola and the Democratic Republic of Congo.[16]

1.2. HIV/AIDS

Acquired Immune Deficiency Syndrome was first identified in the early 1980s. A 1981 report of 5 cases on the syndrome titled "*Pneumocystis* pneumonia — Los Angeles" was the first one on the syndrome which later came to be referred to as AIDS. The report noted that the health challenge had associated with it a dysfunction of the immune system rendering victims susceptible to opportunistic infections.[17] The causative agent HIV-1 (Human Immunodeficiency Virus-1) was first described by Barre-Sinoussi *et al*. in 1983[18] and then positively linked to AIDS by Gallo *et al*. in 1984.[19] HIV-2, closely related to HIV-1, also causes AIDS but is less virulent and is largely found in West Africa unlike HIV-1 which has a global presence; HIV-2 infections may, at times not lead on to AIDS.[20,21] HIV is a member of the genus *Lentivirinae* in the *Retroviridae* virus family which has the prefix "retro" as its members reverse transcribe ribonucleic acid (RNA) to double-stranded deoxyribonucleic acid (DNA).[22,23] The Lentivirus sub-family is made up of non-primate and primate retroviruses, both of which cause immune system dysfunction in hosts.[22] **Figure 1**[24] shows the main stages in the live cycle of HIV-1. After attachment to the host cell, the viral envelop opens, releasing the capsid which then uncoats to release the contents (viral enzymes integrase (IN), protease (PR) and reverse transcriptase (RT) and the RNA genome). In the host cells, the RT converts the RNA to double-stranded DNA and the DNA is imported into the host-cell nucleus for integration by IN into host-cell DNA.[25,24] The host-cell machinery is then commandeered to make new viral proteins which, together with viral RNA, move to the plasma membrane where assembly takes place to produce non-infectious HIV. The action of PR on the immature HIV proteins converts the immature HIV into virions (mature infectious HIV) which are released in the host to reproduce.[26,27]

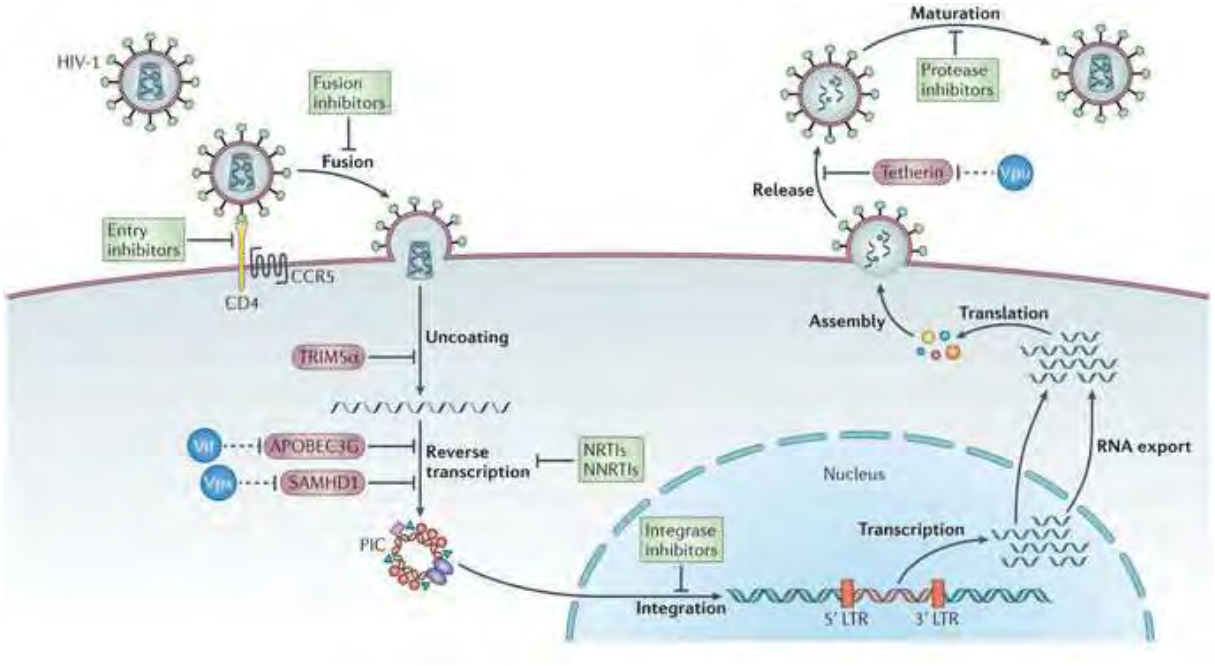


Figure 1. HIV-1 life cycle[28] (Reproduced with permission)

1.2.1. The HIV-1/AIDS pandemic

HIV-1 infection is a global challenge whose resultant mortality and morbidity levels are still significant (about a million deaths annually and over 37 million infections worldwide) even after two decades of quite positive pharmacological developments which have enabled some management of the virus; a cure, however, remains elusive.[29] New infections per year remain high, with 2 million being reported in 2014 (5 600 infections daily) and in certain groups, such as people who inject drugs (PWID), men who have sex with men (MSM) and in transgender people, HIV spread has shown an upward trend.[30] In adults infected by HIV-1, the main mode of entry (> 80%) is via mucosal surfaces and for the larger part of the remainder infection has been intravenous or percutaneous.[31] The entry via mucosal surfaces makes HIV-1 primarily a sexually transmitted infection.[20] The major mode of transmission for HIV-1 is through heterosexual sex.[32] Mother-to-child, i.e. vertical transmission, which can happen before birth, during birth and through breast feeding is another significant mode of transmission.[33]

Historically 70 million people have been infected and half of them have died.[34] The HIV pandemic has had its epicentre in sub-Saharan Africa during the last 30 years.[35] Southern

Africa with one third of the global burden is the worst affected.[36] Participation by Africans, particularly those of Southern Africa, in finding solutions for the HIV/AIDS challenges is something that should come naturally as it has direct benefits to the Southern Africans themselves as inhabitants of the most affected region. Historically the region has contributed to medical advancement with the milestone of the first human-to-human heart transplant as a case in point. On December 3 1967 the first human-to-human heart transplant was accomplished by Christiaan Barnard and his team in Cape Town at the Groote Schuur Hospital.[37]

1.2.1.1. HIV-1 Interventions

There are a number of evidence-based interventions that are described in literature and some of them will be covered below.

1. ***HIV/AIDS information campaigns*** have been run in different places to disseminate information on HIV/AIDS as the first line of defence in stemming the tide of the pandemic.[38] Armed with knowledge one is more likely to make the right decisions.
2. ***HIV counselling and testing (HCT)*** is a vital part of HIV/AIDS prevention and treatment programmes, and has been shown to be a cost effective preventative measure which also results in the reduction of risky sexual behaviours.[30]
3. ***Antiretroviral therapy (ART)*** has been reported to have averted the deaths of millions of people and it has been shown that a reduced viral load, due to use of ART, reduces sexual transmission of HIV.[30,39,40] The late 1990s saw a significant turn for the better in the use of ART with the introduction of combination ART which involves the use of several agents at the same time which resulted in the lowering of replication of the HIV-1 to levels that changed HIV/AIDS to a health challenge that could be managed with significantly reduced progression and fatalities.[33] ART went through an evolutionary process beginning with single therapy using azidothymidine (AZT), then a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) and, finally, highly active antiretroviral therapy (HAART).[41]HAART involves a combination of at least three anti-HIV agents aimed at exploiting the synergistic possibilities among a variety of agents acting on varying targets, and results in the reduction of individual drug toxicities by lowering each drug's dosage, thus lowering the chances of drug resistance developing.[42] ART regimens need to be efficacious, lowering HIV in blood to levels that are not

detectable within a few weeks after commencement of therapy, tolerable, and activating continued CD4 T-cell count recovery.[43] HAART regimens are frequently made up of two RTIs (reverse transcriptase inhibitors), an NNRTI (non-nucleoside reverse-transcriptase inhibitor), a PRI (protease inhibitor), or an INI (integrase inhibitor). The new recommended drug regimens in comparison to the older PR dependent ones, are designed to be taken less frequently, have a smaller pill burden and higher efficacy.[33] It is recommended that ART be started in all cases where CD4 T-cell counts are decreasing, even in the face of unobservable viral loads, and in cases with detectable viremia irrespective of the CD4 T-cell count.[44] In Southern Africa, a critical region with regards to HIV/AIDS prevalence, the wide-spread use of ART has greatly reduced morbidity and mortality.[45] The acquisition of HIV infection for high-risk cases can be reduced by ART as pre-exposure prophylaxis (PrEP) or post-exposure prophylaxis (PEP).[46] Oral (PrEP) has shown positive results in the prevention of sexual transmission of HIV.[47,48] Use of ART to prevent mother-to-child transmission has shown positive results with the success in Cuba being worthy of note where, in mid-2015, the country became the first to eradicate this transmission mode.[49]

4. ***Implants or inserts carrying anti-HIV-1 drugs***, such as flexible vaginal rings loaded with ARVs, e.g. Dapivirine, have been explored for long-term drug release for PrEP, while tenofovir alafenamide (TAF) implanted subdermally has also shown good release profiles[50].
5. ***Sexually transmitted infections (STI) treatment.*** STIs, especially those that cause rapture of epithelial tissues in genitals or genital inflammation, predispose those not infected to infection by HIV and those already infected to become more infectious and, hence, there is a clear benefit to treating STIs in order to reduce HIV transmission.[51,52]
6. ***Use of condoms***. The use of condoms correctly and consistently has been reported to be an effective method in fighting the spread of HIV.[38,40] A recent study conducted by Giannou *et al*. to evaluate the effectiveness of condoms in reducing transmission of HIV heterosexually revealed that the risk of transmission was significantly lower for couples who consistently used condoms than for those who were inconsistent or those who never used condoms.[53]
7. ***Vaccines***. So far, a successful vaccine has not been developed, but studies are ongoing.[54] The challenges faced include high HIV genetic polymorphism, lack of immunogenic antigens, lack of animal models, and difficulty in establishing what may be considered immunity that is protective.[41,33] The clinical trial of RV144 in Thailand, which reduced

acquisition of HIV by 31%, is the only one to have shown some promise in the wake of a number of failures by other candidates.[33]

According to Coates *et al*. progress in reducing HIV transmission has been due to behavioural change as a product of information strategies which they describe as any plan that aims at reducing sexual partners, availing of HIV counselling and testing, deferring the first sexual intercourse, reducing drug use, reducing sharing of injecting apparatus, ensuring the increase of protected sex and correct use of biomedical remedies for prevention of transmission.[55] These interventions also aim to prevent new infections. Prevention of new infections as a strategy to control HIV/AIDS is critical and includes approaches that may be described as: *biomedical* (which seek to reduce infectivity and prevent infection); *structural* (which seek to change the factors that predispose people to risk and vulnerability); and *behavioural* (those that encourage behavioural change in individuals and social groups).[56,55]

1.2.1.2. Some Challenges in the use of HAART

In spite of the successes with HAART, drug resistance is a major concern. Resistant forms of HIV-1 develop rapidly for several reasons which include: the heterogeneous nature of HIV-1; drug pressure and mutations which are fuelled by error-prone RT; and the extremely high viral replication rate which can be as high as ~ 10^{10} per day in the absence of treatment.[57,58] Some of the resistant forms are even multi-drug resistant and this curtails treatment choices and, hence, there is an urgent need for novel agents that are effective in the face of the resistant strains.[59] Novel agents may use different mechanisms which may work positively in fighting resistance.[60] For PRI resistance, increasing interactions between PR and the inhibitor by promoting comprehensive hydrogen bonding between the active-site residues and the inhibitor backbone atoms is a strategy that minimises the occurrence of resistance.[59] The ongoing development of novel compounds can lead to the widening of available drug options, more efficacious drugs and drug regimens and less harmful drug reactions.[61]

HAART cannot totally eliminate HIV-1 and there is a need for life-long drug therapy which minimises the exposure of patients to the indeterminate side-effects of long-term use of drugs which, so far, have been shown to exhibit toxicity, cause kidney, bone, heart and CNS (central nervous system) complications among other negative effects.[43] ART generally presents patients with adverse drug reactions which can be grouped into: *Haematological* (blood stained urine, bilirubinemia, and anaemia); *Digestive tract* (constipation, pancreatitis,

hepatotoxicity, dyspepsia, diarrhoea, and nausea); *CNS* (vision challenges, insomnia, tingling, pain or numbness in extremities, memory challenges, olfactory problems, and dizziness); *Musculoskeletal* (chest pain and body aches); *Metabolic* (anorexia, weight gain, lipodystrophy, and dyspnea); *Dermatological* (facial discoloration, itching, and rashes); *Psychological* (depression, hallucination, confusion, nightmares, and restlessness); and *Indiscriminate* (fever, non-regular menses, hypersensitivity, and mouth ulcers).[62] One of the reasons the virus is difficult to eradicate is its ability to go into latency, a state in which HIV-1 genomes reside in host cells without replicating, and this takes two forms, the short-lived pre-integration form and then the post-integration, long-lived form which acts as the viral reservoir.[63] ART cannot deal with HIV in the latently infected T cells and in the CNS, lymphatic tissue and the digestive tract which are anatomical reservoirs.[33] Epidemic control using ART continues to be hindered by challenges to do with treatment interruptions and lack of adherence which can lead to drug resistance due to lower than optimal drug levels.[64,65,66] Compliancy to drug protocols can be improved by simplifying how the drugs are administered, e.g. reduction of the daily pill burden, and the use of long-acting ARVs - an approach which is still under study.[67,66]

1.2.2. Antiretroviral Therapy (ART)

The comprehensive search for inhibitors of HIV began soon after its identification as the cause of AIDS, and suramin was the first successful inhibitor both *in vivo* and *in vitro* but, in 1987, zidovudine became available as the first drug approved for clinical use having first been reported in 1985 as an *in vitro* inhibitor of HIV.[68,42,69] The search for appropriate Antiretroviral agents (ARVs) for ART has targeted the stages of the HIV life cycle and progress in this regard has been reported extensively. Several classes of inhibitors have been discovered, including: fusion inhibitors and co-receptor antagonists (viral entry); RT inhibitors (reverse transcription); IN inhibitors (integration); PR inhibitors (maturation of virus).[70]

1.2.2.1. HIV-1 RT inhibitors

These inhibitors target reverse transcription, a process catalysed by viral RT, an RNA-dependent polymerase used in the retro-transcription of the single-stranded viral RNA to proviral DNA which is double-stranded; there are three classes, nucleoside RT inhibitors (NRTIs), nucleotide RT inhibitors (NtRTIs) and non-nucleoside RT inhibitors (NNRTIs).[42] The NRTIs and NtRTIs are chain terminators which block the lengthening of the DNA chain by binding competitively to the RT catalytic site, and they do this in their activated, phosphorylated form. The mechanism of NNRTIs involves interaction with an allosteric site about 15 Å from the active site which causes conformational rearrangements that affect RT action.[71,42,72] The success of RT inhibitors is constantly disrupted by mutation-associated resistance.[73] Reverse transcription, which lacks proof reading systems in contrast to DNA synthesis, is prone to errors introduced at the enzyme level and estimated to involve a mis-incorporation in every 10^4 nucleotide incorporations leading to the production of mutants some of which may be resistant to the therapeutic in use.[43,74]

1.2.2.2. HIV-1 PR inhibitors

These are mainly peptidomimetic inhibitors which mimic normal cleavable peptides but are themselves not cleavable; they bind competitively to the PR active site thus preventing normal function of PR and blocking viral maturation.[27,57] The first PR inhibitors (first generation PR inhibitors) were mainly peptidic leading to low half-lives and low bioavailability, resulting in the need for high dosing frequency; additionally, they had considerable side-effects and were prone to drug resistance. Consequently, research to address these challenges resulted in the second generation PR inhibitors.[43] Since PR processes HIV-1 encoded Gag-Pol and Gag polyproteins into active and mature proteins, its inhibition would deprive the HIV-1 life cycle of proteins needed for successful maturation. There are at least 10 PR inhibitors that have been approved so far for clinical use.[75] Despite the successes in PR therapeutics there are challenges related to side-effects, such as dyslipidemia, lipodystrophy syndrome and diabetes mellitus – apart from resistance challenges.[76,77] In pregnant women, use of PR to prevent mother to child HIV-1 transmission has been linked to low birth-weight and pre-term delivery.[78]

1.2.2.3. HIV-1 IN inhibitors

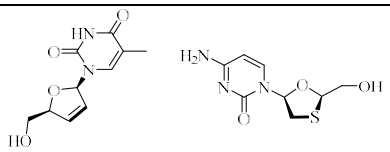
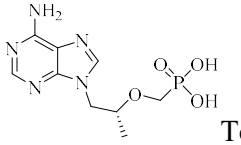
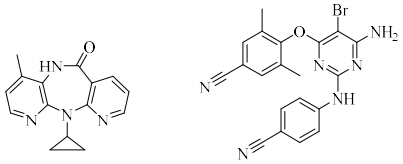
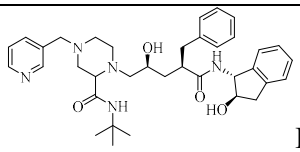
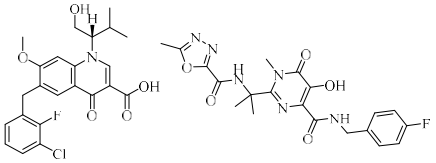
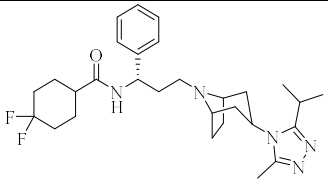
Integration, the process in which the viral DNA is inserted into the host cell genome is catalysed by HIV-1 IN and involves a number of steps, including strand transfer (ST) and 3′ endonucleolytic processing (3′-P), both of which need divalent metal ions, e.g. Mg^{2+}.[79] IN inhibitors generally fall into 2 groups, those that target (3′-P) and those that target (ST).[80,81] Based on chemical structure, IN inhibitors may be classified as diketo acid-containing aromatics, catechol-containing hydroxylated aromatics, non-catechol-containing aromatics and quinolones.[80] The first HIV-1 IN inhibitor to be licensed by the US Food and Drug Administration (FDA) for clinical use was the diketo acid analogue, Raltegravir, and this was followed by the quinolone carboxylic acid, Elvitegravir, and then Dolutegravir.[82,81] All three are ST inhibitors and have been reported to exhibit some robustness against drug resistance with the least prone being Dolutegravir.[83] However, resistance issues now preclude use of Dolutegravir as a monotherapy.[84] The inhibitors' side-effects are relatively few as the tolerability profile is reported to be very good.[71]

1.2.2.4. HIV-1 Entry inhibitors

These inhibitors are designed to block the entry of HIV-1 into the host cell. This is quite a complex process comprising virion attachment to chemokine receptors (CCR5 or CXCR4) and CD4 receptors and progressing to the fusion of the cell membranes of the host-cell and virion.[41] Dang *et al*. have reported quinolizidines exhibiting 1-5 μM anti-HIV-1 entry activity whose mechanism is suggested to involve blocking the fusion between the viral and host-cell membranes.[85] There are two entry inhibitors that are in clinical use, while co-receptor inhibitors, namely cenicriviroc (CVC) and PRO-140, and anti-attachment agents TNX-355 and BMS-663068 are reported to be in clinical trials.[86]

At least 30 agents have been developed and licensed as inhibitors of HIV-1 since the late 1980s[54] and these mainly fall into 3 groups, those that target RT, those targeting PR and those that target IN. **Table 1** shows the different classes of inhibitors, some examples in clinical use and the structures of some members of each family.

Table 1. HIV-1 Inhibitors in clinical use

Inhibitor	Examples	Structures of some members	Refs
NRTIs NtRTIs NNRTIs	AZT; Stavudine (d4T); Didanosine (ddI); Emtricitabine ((-)FTC); Alcitabine (ddC); Lamivudine (3TC); and Abacavir (ABC). Tenofovir Nevirapine; Etravirine; Rilpivirine; Efavirenz; Delavirdine.	 Stavudine Lamivudine  Tenofovir  Nevirapine Etravirine	42,87,88,27
Anti-PR: Mainly peptidomimetics	Ritonavir; Indinavir; Saqunavir; Tipranavir; Darunavir; Nelfinavir; Fosamprenavir; Lopinavir;	 Indinavir	42,57,27,71
Anti-IN: DKA analogues or isosteres	Elvitegravir; Dolutegravir; Raltegravir.	 Elvitegravir Raltegravir	42,27,79
Entry inhibitors	Maraviroc (HIV-1 co-receptor CCR5 antagonist - blocks attachment of HIV-1 to cell); Enfurvitide (prevents fusion).	 Maraviroc	27,89,90

1.3. COUMARINS

Coumarin **1** (**Figure 2**) is a naturally occurring compound first isolated in 1820 from the Tonka bean, *Coumarou*, from which the name coumarin is derived.[91] From the isolation of coumarin **1** and its first synthesis in 1868 by Perkin, considerable knowledge on the properties of coumarin and its possible uses has been generated.[92] The ongoing interest in coumarins is reflected in continuing bioorganic chemistry and biochemistry research.[93] Coumarin **1**, which has also been referred to as 2*H*-1-benzopyran-2-one, *cis*-*o*-coumarinic acid lactone, 1,2-benzopyrone or coumarinic anhydride, can be considered as the parent compound of an extensive family of naturally occurring cinnamic acid derived phenolic compounds which have come to be known as coumarins.[94,92] Coumarins can be divided into four sub-types, namely, the *simple coumarins* (e.g., **1** and **2**) which have hydroxyl, alkyl or alkoxy substituents on the benzene ring, *pyrone-substituted coumarins* (e.g., **3** and **4**), *pyranocoumarins* with a six-membered ring fused with the coumarin moiety (e.g., **5** and **6**) and *furanocoumarins* with a five membered ring fused to the coumarin scaffold (e.g., **7** and **8**).[91,95]

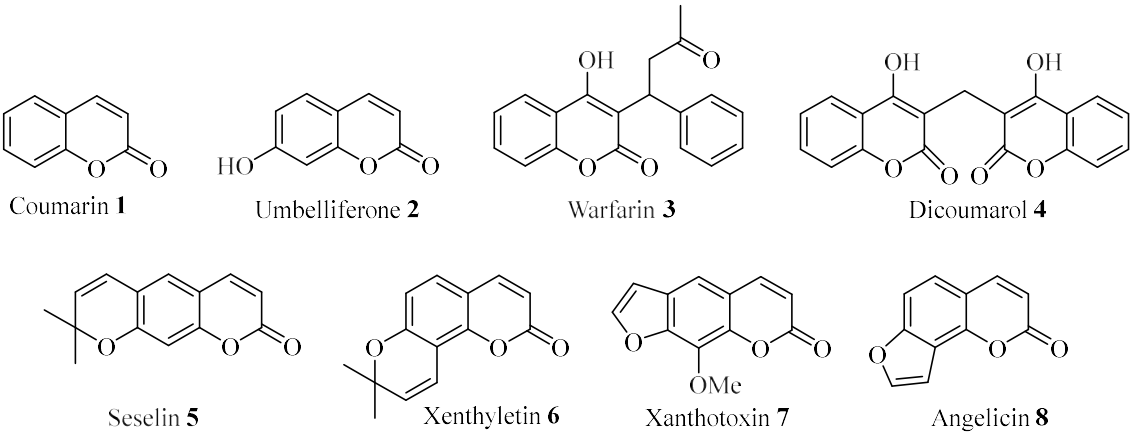


Figure 2. Coumarins from different classes[95]

Natural coumarins make up a broad class of compounds present in many plants with high levels in essential oils, such as lavender oil and cassia leaf oil.[96] Higher plants like the *Umbelliferae* and *Rutaceae* have the highest concentrations of coumarins, with the fruits having the highest level, then the roots, followed by the stems and, lastly, the leaves.[91] Microorganisms, bacteria and fungi are also sources of natural coumarins and their

derivatives.[97] One of the main drivers of interest in coumarins stems from the fact that over 1300 coumarins are present as secondary metabolites in bacteria, fungi and plants - a fact that has prompted research into the isolation of the metabolites and the study of their biological activities.[98] Novobiocin **9** (from *Streptomyces niveus* and *Streptomyces spheroides*) and Chartreusin **10** (isolated from *Streptomyces chartreusis*) are examples of bacteria-derived coumarins.[99] Aflatoxins, which are highly toxic fungal metabolites from *Aspergillus* species and which are represented by Aflatoxin B_1 **11** are fungal coumarins.[91] Apart from terrestrial organisms, marine organisms are also sources of coumarins. Coumarins are among the phenolic compounds that have been extracted from marine micro- and macro-algae.[100]

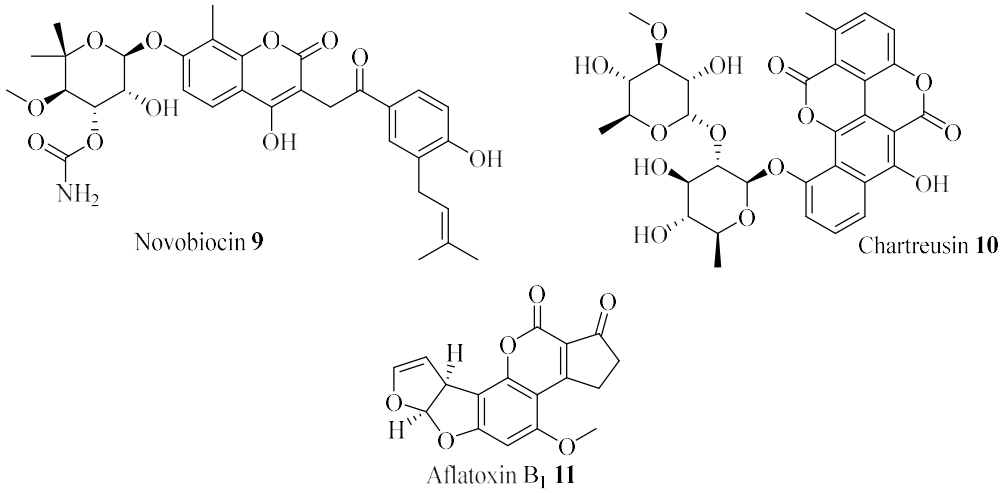


Figure 3. Bacteria-derived natural coumarins **9** and **10**[12] and the fungi-derived coumarin **11**[101]

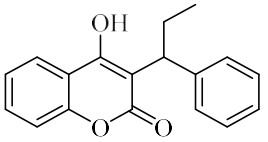
1.3.1. Bioactivities and other properties of coumarins

Coumarins have shown a wide variety of medicinal properties which include anti-fungal, anti-parasitic, anti-mutagenic, anti-HIV, anti-tumour, anti-depressive, serine protease inhibitory, anti-bacterial, anti-cancer, anti-diabetic, anti-coagulant, analgesic, anti-neurodegenerative, anti-oxidative, anti-viral, anti-inflammatory activities, and inhibitory activity against cholinesterase, lipoxygenase, monoamine oxidase and cyclooxygenase.[95,102,103] They also find application in brightening agents, supramolecular medicinal drugs, biological stains, fluorescent sensors, cosmetics, food additives, molecular photonic devices, agrochemicals and laser dyes.[95,104] The vast number of important activities and applications of coumarins have stimulated considerable research into these compounds,

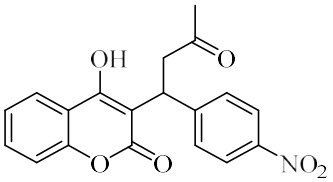
of both synthetic and natural origin. Some of the biological activities and applications will be discussed in this report.

1.3.1.1. Anticoagulant properties

In recent years, thrombotic events have become a grave threat to health and resulted in high fatality levels thus stimulating research on anti-thrombotic agents, such as anti-coagulant, thrombolytic and anti-platelet compounds.[105] Anti-coagulants are used in treatments for deep vein thrombosis, intravascular embolism, and stroke; they are also used to prevent the growth of blood clots in thrombophlebitis and the formation of clots in patients who have artificial heart valves.[104] The discovery of dicoumarol **4 (Figure 2**) as an anticoagulant in spoiled clover in the 1930s led to the development of coumarin anticoagulants, with warfarin **3** being introduced as a medicinal drug in the early 1950s after its use as a rat poison had started in the early 1940s.[106] Coumarin anti-coagulants act as vitamin K antagonists and inhibit vitamin K epoxide reductase to cause the anti-coagulant effect and are among the most widely used medications.[107] For more than 60 years coumarins, exemplified by warfarin **3** acenocoumarol **13**, and phenprocoumon **12** (**Figure 4**) have been the main anti-coagulants in clinical use.[108] All three compounds are derivatives of 4-hydroxycoumarin and are orally administered in their racemic form.[109] Minimum structural requirements for the anti-coagulant properties of these compounds are the 4-hydroxycoumarin moiety and a carbon chain at position 3 in the pyranone ring.[110]



Phenprocoumon **12**



Acenocoumarol **13**

Figure 4. The structures of the anticoagulants[111]

Coumarin derivatives have been found to be relatively safe when used in valve thrombosis cases in pregnant women who have mechanical valve prostheses, compared to the use of the anti-thrombosis agent, heparin.[112] Management and prevention of thromboembolic diseases using coumarin anti-coagulants, however, has some challenges of which drug to drug interactions is one.[113] The dose has to be individually determined as it is not fixed.[114] The

main detrimental effect of anti-coagulant therapy with coumarin agents is severe bleeding risks which are increased by drug to drug interactions.[115]

1.3.1.2. Anti-inflammatory properties

Inflammation is a health challenge that has been with mankind from the dawn of history and it is the immune system's response to infection and tissue damage. This response is complex and may involve heat sensation, oedema, redness, pain and loss of function; Celcius (30-38 B.C.) named the first four symptoms of inflammation while Galen (A.D. 130-200) named the fifth.[116] Inflammation is a defence mechanism response triggered by unfavourable stimuli in a particular body area and is driven by specialised immune and inflammatory cells with the objective of restoring normal functions and structure.[117] The response is designed to deal with disturbances to homeostatic values which may have intrinsic or external sources.[118] According to Kirsch *et al*. inflammation is a vital reaction to offensive stimuli and is indicated by a general or specifically located response involving, among other processes, an arachidonic biosynthetic cascade resulting in the formation of leukotrienes, hydroxyeicosatetraenoic acids, and prostaglandins; blocking the cascade may be a remedy to inflammation.[119] When not checked, inflammation may lead to auto-inflammatory and auto-immune malfunction, cancer or neurodegenerative illness.[120] An inflammatory process may be considered to be made up of four elements: inflammatory inducers, such as tissue damage or diseased state; sensors, such as dendritic cells and macrophages; mediators of inflammation, such as biological amines and different cytokines; and, finally, the tissues targeted. Controlling inflammation would require the removal of the inducers, blockage of the sensors, mediator inhibition or direct action on targeted tissues.[121]

The coumarin nucleus has been reported extensively as a possible candidate for the design of anti-inflammatory agents and natural coumarins, such as umbelliferone, visniadin, columbiatnetin and marmin, have been reported as potent anti-inflammatory agents, while derivatives that have been synthesised have been found to exhibit moderate to very potent anti-inflammatory activities.[122] **Figure 5** shows some examples of plant-derived coumarins that exhibit anti-inflammatory activity.

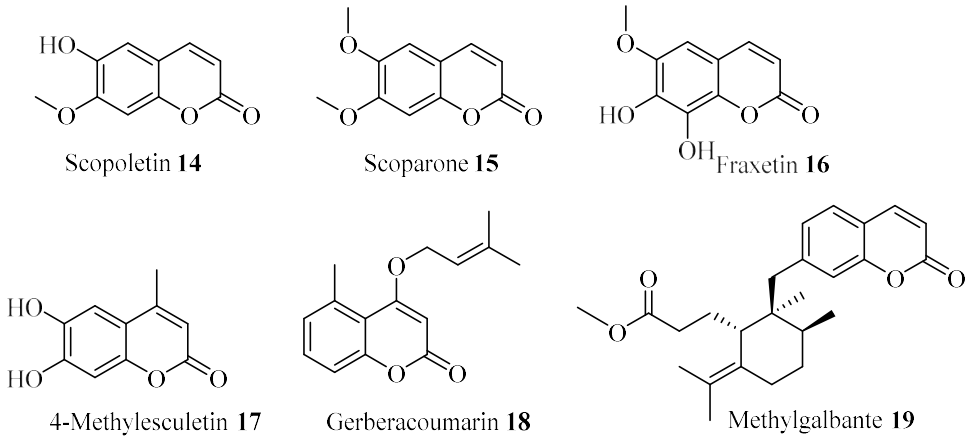


Figure 5. Plant-derived coumarins with anti-inflammatory activity[119]

4-Methylesculetin and esculetin have been found to have intestinal anti-inflammatory activity which is linked to their anti-oxidant properties.[123] A study was conducted by Wiaicenis *et al*.[124] on the plant-derived coumarins, scoparone (6,7-dimethoxycoumarin), esculin (6,7-dihydroxy-6-*o*-glucosylcoumarin), scopoletin (6-methoxy-7-hydroxy-coumarin), daphnetin (7,8-dihydroxycoumarin) and 4-methylumbeliferone (4-methyl-7-hydroxycoumarin) to determine whether potential anti-inflammatory activity was associated with anti-oxidant activity. The findings suggested that the anti-inflammatory properties of these compounds are linked to their antioxidant activities, and that foods rich in coumarins or their derivatives, such as scoparone, esculin and daphnetin, could prevent intestinal inflammation. Anti-oxidants mop up free radicals or inhibit the production of reactive oxygen species.[125] During the inflammatory process macrophages produce free radicals and such reactive oxygen species have been reported to be involved in the formation of cyclooxygenase- and lipoxygenase-mediated pro-inflammatory intermediates from arachidonic acid.[126] Coumarin itself and its derivatives function as inhibitors of lipoxygenase and cyclooxygenase pathways in the metabolism of arachidonic acid.[127] The development of coumarin-based anti-inflammatory remedies, such as cloricromine (**Figure 6**) has provided an alternative to corticosteroids which, although having excellent levels of efficacy as anti-inflammatory agents, have side effects that limit their use.[128]

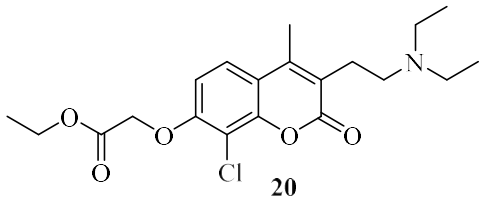


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Figure 6. Cloricromine

1.3.1.3. Antibacterial properties of coumarins

Goth (1945) established the antibacterial properties of coumarins by showing that dicoumarol was significantly active against a number of bacterial strains.[129] In the 1950s, novobiocin **9**, an aminocoumarin antibiotic, then known as streptonivicin, was first reported by Hoeksema *et al*.[130] Challenges from bacterial resistance to drugs have always made the search for novel molecules, whether synthetic, semisynthetic or natural, highly attractive. Smyth *et al*. carried out a study on the antibacterial activities of over 40 synthetic and naturally occurring coumarins in which it was established that half of the compounds which showed good activity against *Staphylococcus aureus* strains were also active against eight methicillin-resistant *Staphylococuccs aureus* (MRSA) strains of clinical origin, and that sixteen of the compounds enhanced the effect of the anti-microbial, oxacillin, on an MRSA hospital isolate through resistance-modifying activity.[131]

Yang *et al*.[132] investigated the bactericidal properties of a number of hydroxycoumarins against the phytopathogen *Ralstonia solanacearum*. In the investigation, which was narrowed to hydroxycoumarins after the screening of eighteen coumarins, they found that hydroxylation at the positions, C-6, C-7 or C-8, increased activity against the pathogen. A series of coumarin triazoles, synthesised from resorcinol and phloroglucinol by Shi and Zhou, were evaluated against MRSA, and a number of the compounds exhibited high activities with the best one being a bis-triazole with a minimum inhibitory concentration of 4 μg/mL.[133] A significant number of the compounds tested showed superior or comparable activity to the clinical drugs, Chloromycin and Enoxacin. **Figure 7** shows some of the compounds exhibiting high potency.

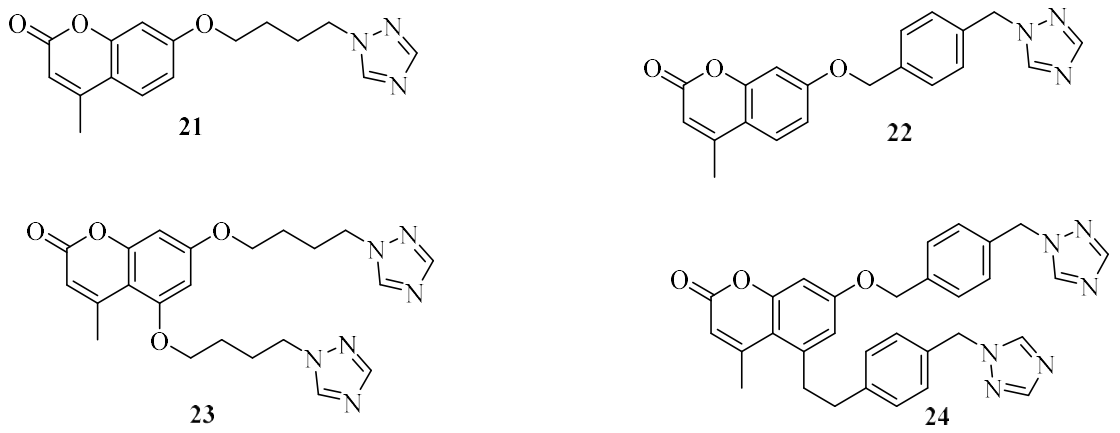


Figure 7. Coumarin triazoles with potent antibacterial activity [133]

Arshad *et al*. synthesised two series of hydrazinyl thiazolyl coumarin derivatives and evaluated them *in vitro* against several bacterial strains including *Mycobacterium tuberculosis* (*Mtb*), and three of the compounds (**Figure 8**) showed significant activity, with minimum inhibitory concentrations (MIC) in the 15-17 μM range against *Mtb*.[134]

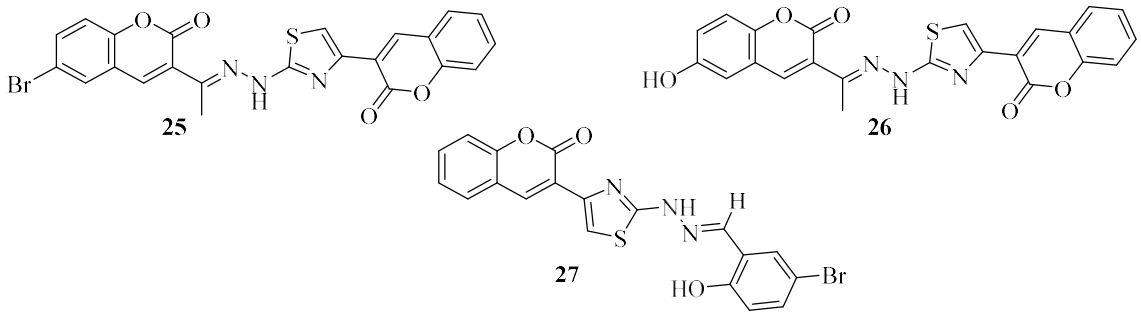


Figure 8. Hydrazinyl thiazoyl coumarins potent against *Mtb*[134]

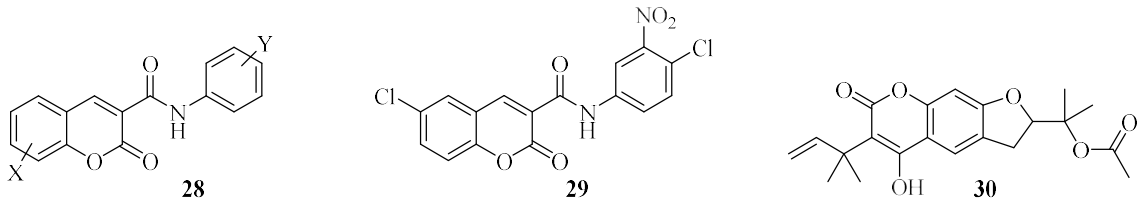
1.3.1.4. Anti-cancer properties

Cancer is a major threat to health globally and numerous studies continue to be undertaken in the search for novel therapeutic agents. There are many anti-cancer drugs on the market, but drug resistance, the high level of undesirable effects and limited efficacy are real drawbacks to their usefulness and reinforce the worldwide need for novel anti-cancer compounds.[135,136] The prevention of cell differentiation, uncontrolled generation of clonal cells and suppression of apoptosis typifies the disease.[104] A more comprehensive description is given by Hanahan and Weinburg when they describe cancer as having certain complementary abilities, which they refer to as hallmarks, which promote the development of tumours and metastatic

propagation.[137] These hallmarks include avoidance of growth controllers, sustenance of proliferative signalling, activation of abnormal angiogenesis, resistance to apoptosis, tissue invasion activation and metastasis.[138]

Plants have always been an invaluable source for the discovery of novel anti-cancer compounds and plant-derived coumarins, such as imperatorin, osthol and esculetin have been reported as anti-cancer agents.[99,136] There has been extensive exploration of coumarins as anti-cancer agents[96] and a wide range of coumarins have been shown to exert cytotoxicity on animal cancer models and diverse cancer cell lines.[110] Among the promising chemotherapeutic agents is esculetin which exhibits anti-proliferative properties on a number of human cancer cell lines, including oral cancer cell lines on which it has been found to induce apoptosis and inhibit cell growth.[139] In human leukemic cells, esculetin manifests its anti-proliferative nature by inducing apoptosis and, in human hepatocellular carcinoma cells, it has been shown to enhance taxol-induced apoptosis.[110] Esculetin has also been found to show anti-proliferative activity and to trigger cell death in HCT116 and HT-29 human colon cancer cells respectively.[140,140b] Further, in a recent study on esculetin's activity against prostate cancer cells, it was shown to induce apoptosis and to inhibit both cell migration and cell multiplication.[141] Rutamarin **30** has been shown to be active against HT-29 human colon cancer cells (IC_{50} = 5.6 μM).[142] The first set of coumarins designed for cancer treatment, which target the Hsp90 protein, was made up of a library of novobiocin analogues which exhibited some activity, shown, among other things, to be promoted by the presence of 3-amido, 6-*o*-propoxy and 8-methoxy substituents.[143]

In their anticancer activity, coumarins target various pathways, including heat shock protein inhibition, sulfatase inhibition, kinase inhibition, angiogenesis inhibition, aromatase inhibition, anti-mitotic activity and telomerase inhibition.[144,96,145] Three positions, *viz*. C-3, C-4 and C-7 out of the six available for substitution, are most important for anticancer properties.[146] Pharmacophoric modifications at C-3 have included the introduction of imine, hydrazine, heteroaryl and amide moieties; at C-7, *o*-substituted and 7-hydroxy moieties; and at C-4, 4-methylene-substituted and 4-aryl substituents.[147] *N*-arylcoumarincarboxamides **28** (**Figure 9**) were synthesised and their anticancer activity evaluated against human lung fibroblasts and breast cancer cell lines. The most potent compound, with an IC_{50} of 0.166 μM, was the 6-chlorocoumarin derivative **29**.[147-148]



X = H, EtO, Br, Cl

Y = Br, NO_2, I, NH_2, Cl

Figure 9. *N*-arylcoumarin- carboxamides anticancer candidates[147,148] and Rutamarin **30**.

Miri *et al*.[136] studied the anticancer activities of at least twenty 4-methylcoumarins against chronic breast adenocarcinoma, myelogenous leukemia and human colon adenocarcinoma cell lines. The coumarins included 7-diacetoxy-4-methylcoumarin, 7-hydroxy-4-methylcoumarin and 7,8-dihydroxy-4-methylcoumarin, and it was found that the 7,8-dihydroxy-4-methylcoumarin exhibited the highest cytotoxic effects, followed by 7-diacetoxy-4-methylcoumarin. A coumarin, 667coumate (**Figure 10**), is a Phase 1 clinical trial candidate to treat breast cancer, an oestrogen-dependent carcinoma in women.[104] Coumarin compounds have been shown to conjugate with oestrogen and also selectively modulate the oestrogen receptor, thus exhibiting potential as anti-breast cancer therapeutics.[149]

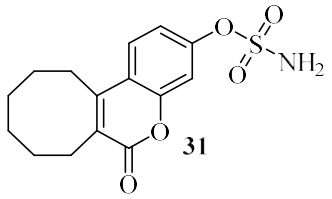


Figure 10. Coumarin 667coumate,[16]

1.3.1.5. Anti-viral properties

A number of fatal epidemic diseases such as hepatitis B and C, Middle East respiratory syndrome, HIV/AIDS, severe acute respiratory syndrome, smallpox, influenza, chikungunya and ebola are caused by viruses.[150] Globally, viral diseases have caused significant morbidity and mortality and there are also newer pandemics like the Zika virus and H1N1 (Influenza A).[150,151] Resistance to available therapeutic agents and viral mutations have presented challenges in the treatment and management of viral diseases and there is a need for novel agents with novel mechanisms of action.[150,152] It is encouraging that there are a number of

anti-viral therapeutic agents in clinical trials.[150] In the last 30 years, there have been concerted efforts in the study and development of coumarins as antiviral agents as shown by the abundance of reports, many of which reveal coumarins as a group of compounds with significant potential activity against a wide range of viruses.[99,150,153]

The pharmacophoric benzo-α-pyrone scaffold consists of a flat aromatic nucleus fused with a protein-binding lactone moiety, which also serves as a hydrogen bond acceptor; the scaffold lends itself to the design of novel compounds that display specificity and affinity for a variety of viral targets.[154,155] The attention focussed on coumarins has also been spurred by their potential as lead scaffolds for the development of non-peptidic, orally bioavailable, anti-viral drugs and, consequently, a significant number of molecules containing the coumarin moiety have been discovered, some of which are at different stages of drug advancement.[150,25] A few examples will be described briefly and a detailed discussion will then be given on anti-HIV-1 properties of coumarins.

***4-Hydroxycoumarins* (4-HCs) -** The tetramer of 4-HC, NSC 158393 **32**, exhibits anti-HIV-1 IN, PR and RT activity.[150] Analogues of warfarin, U-96988 **33** and tipranavir **34** are anti-HIV-1 PR agents.[156,157] A derivative of bis-(4-hydroxycoumarin) **35** has been found to show broad inhibitory activity against several viruses, namely, vaccinia virus, herpes simplex virus type 1 and also type 2.[158]

Pyranocoumarins - The khellactone suksdorfin **36**, is an HIV-1 RT inhibitor and (+)-calanolide A **37** is active against AZT-resistant G-9106 and pyridinone-resistant A17 HIV strain.[150]

Furanocoumarins - The coumarin derivative BPRHIV001 **38**, inhibits the Tat-dependent transcription of HIV-1 and is also a potent inhibitor of oseltamivir-resistant influenza viruses.[159,160] Rutamarin **30**, exhibits activity against human gamma-herpes virus, Epstein-Barr virus (EBV) and also Kaposi's sarcoma-associated herpes virus.[161,162] Wedelolactone **39**, which belongs to a related class of compounds, the coumestans, is an inhibitor of the hepatitis C virus (HCV) NS5B polymerase.[163]

7-Hydroxycoumarin analogues - Osthole **40**, has been found to be active against hepatitis B virus (HBV).[164] Some of its analogues have exhibited activity against HCV, the respiratory syncytial virus and the bovine viral diarrhoea virus.[150]

Conjugates – Benzouracil-coumarin-arene **41** conjugates show inhibitory activity against the Chikungunya virus (CHIKV),[165] while 7-diethylamino coumarin **42** has shown potency against (CHIKV) and Semliki forest virus.[166]

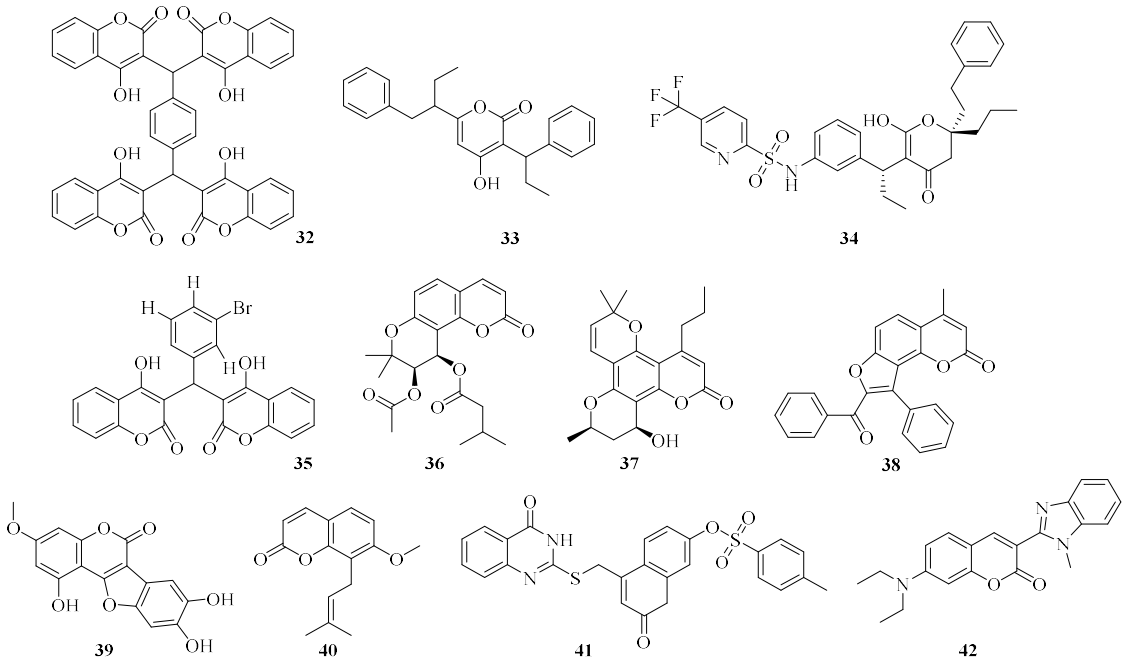


Figure 11. Coumarin antiviral agents.

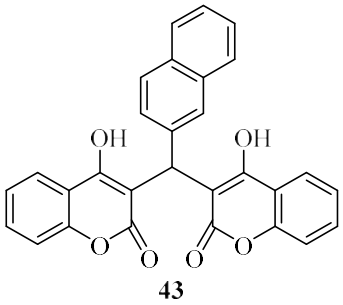
1.3.2. Anti-HIV properties of coumarins

Compounds containing the coumarin nucleus are among the large number of natural products which have been found to show anti-HIV activity.[99] Natural e.g. suksdorfin **36** and (+)-calanolide A **37**, and synthetic anti-HIV agents e.g. NSC 158393 **32** and BPRHIV001 **38**, have been studied in the quest for effective coumarin anti-HIV therapeutic agents. The abundant literature reports on the anti-viral activity of coumarins concentrate mainly on their anti-HIV potential as compared to the other anti-viral activities.[167,168] A number of reports, as will be shown later, have focussed on the discovery of coumarins and the exploration of their structure-activity inter-connections.

The HIV replicative cycle has a number of stages which can be targeted by anti-viral agents. These include adsorption of the virus on the host cell membrane, binding of the viral casing to the cell membrane, opening of the viral nucleocapsid, conversion of viral RNA to proviral DNA through reverse transcription, integration, replication of DNA, transcription, maturation of viral proteins and budding.[25] In recent studies, coumarins have been shown to be effective inhibitors of viral adsorption, and the critical HIV PR, HIV IN enzymes .[167]

1.3.2.1. HIV-1 IN inhibitors

The tetramer (NSC 158393) **32** (**Figure 11**) which inhibits 3'-processing and strand transfer has been reported to be one of the most potent inhibitors of integrase IN.[169] The tetramer's structural features are consistent with those observed in a majority of IN inhibitors with high potency. These are the presence of two aryl units connected to each other by a linker and, in a number of cases, 1,2-dihydroxy substituents on at least one of the aryl moieties.[170] It has also been reported that increasing the hydrophobicity of the linker or linker system increases activity.[150] Zhao *et al*. investigated the ways by which the structurally complex agent **32** (IC_{50} = 1.5 μM) could be simplified while still retaining potency and found that 3,3'-(2-naphthalenomethylene)bis[4,7-dihydroxycoumarin] **43** (IC_{50} = 4.2 μM) gave the best results.[171]



43

Figure 12. Simplified analogue **43** of the 4-hydroxycoumarin tetramer IN inhibitor **32**. [171]

Su *et al*. synthesised nineteen bis-coumarins **44** containing benzoyloxyphenyl linkers and thirteen of them proved to have anti-HIV-1 IN activities, the most active IC_{50} values < 3 μM (3'-processing).[172] Among the most potent 3'-processing IN inhibitors reported in literature are the polyphenolic acid conjugates **45** (IC_{50} = 1.9 μM) and **46** (IC_{50} = 1.5 μM).[173] A study of bis- and tetra-coumarins conducted by Chiang *et al*. produced analogues, some of which were more potent than the original lead, tetramer (NSC 158393) **32,** the most active being compound **47**. The three most potent had IC_{50} values of 0.49, 0.58 and 0.96 μM, respectively.[174,175]

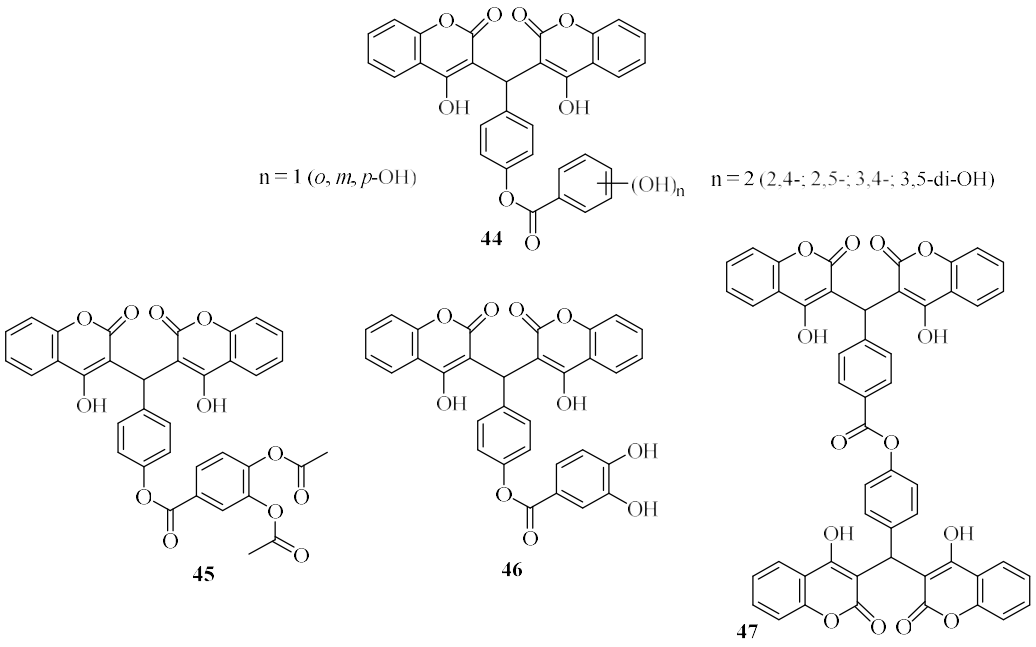


Figure 13. Modified bis-coumarins and tetra-coumarin

The study of coumarins as HIV enzyme inhibitors has also involved the use of computer-aided rational design. Veselinovic *et al*.[176] developed a quantitative structure–activity relationship (QSAR) model which they used to predict the anti-HIV IN activity of 4-phenyl hydroxycoumarins. The best results, (pIC_{50} values of *ca* 3 for both 3' processing and integration) suggested that 4-phenyl hydroxycoumarins could potentially be used as lead compounds for novel of HIV-1 IN inhibitors. Jain *et al*.[177] studied the features of a number of coumarin IN inhibitors to develop predictive 3D-QSAR models for use in the design of novel IN inhibitors. The pharmacophore models they developed could predict within a reasonable range of the literature pIC_{50} values of the evaluated compounds, thereby providing a way in which new lead compounds could be developed or identified. Patil and Sanjay using a group of 57 coumarins built a 4D-QSAR model for coumarin derivatives as potential inhibitors of HIV-1 IN which can be used in the study of 3'-processing IN inhibitors.[178]

1.3.2.2. HIV-1 Reverse transcriptrase inhibitors

Calanolides and inophyllums are coumarin natural products which exhibit novel HIV RT inhibitory activities.[99,179] A series of inophyllums have been found in the snail *Achatina fulica*.[99] **Figure 14** shows some of the members of the series. Inophyllums have also been

isolated from the Malaysian tree *Canophyllum inophyllum* Linn.[99,180] IC_{50} values of 38 nM for inophyllum B **48** and 130 nM for inophyllum P **49** for their inhibitory action against HIV-1 RT have been reported.[180]

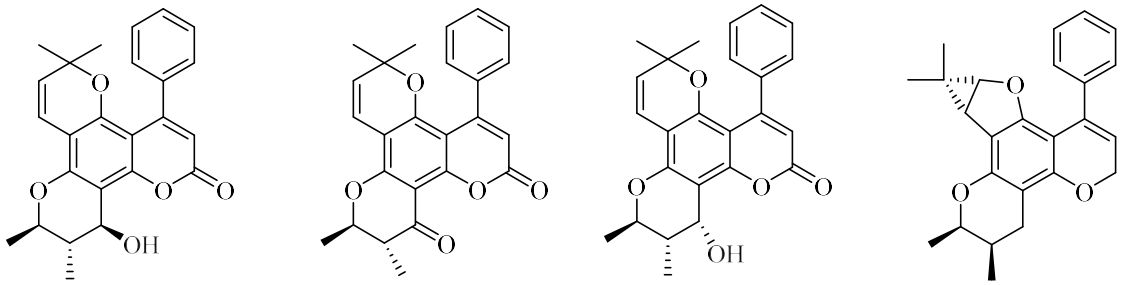


Figure 14. Inophyllums[99]

(+)-Calanolide A **37** has been reported as the first secondary metabolite to be found to have RT inhibitory activity.[181] It is an NNRTI which also exhibits synergistic anti-HIV activities with other anti-HIV agents such as protease inhibitors (PRs) and other NNRTIs.[182,183] Calanolide A belongs to the pyranocoumarin class of coumarins and its isomers, (-)-calanoid B (costatolide) **52** and (-)-dihydroxycalanolide (dihydrocostatolide) **53** are also NNRTIs and these have been found to show high potency against clinical strains.[184] Clinical trials of (+)-Calanoid A for use in HIV combination therapy have passed through phase I/II trials and have revealed minimal toxicity, although some unfavourable effects, like nausea and dizziness, have been noted.[150]

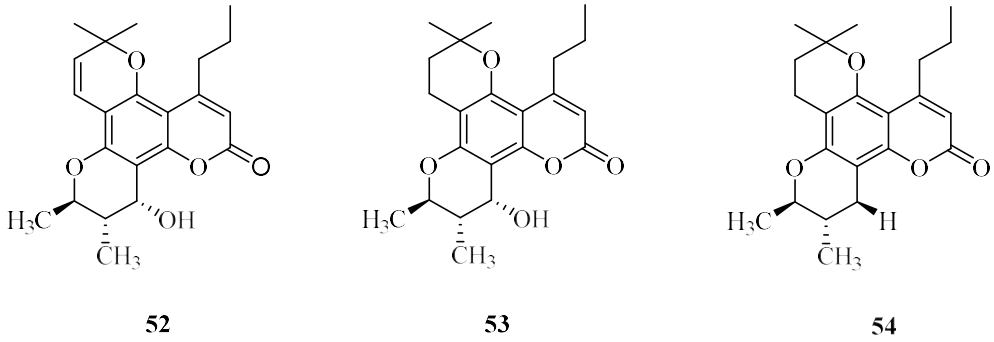
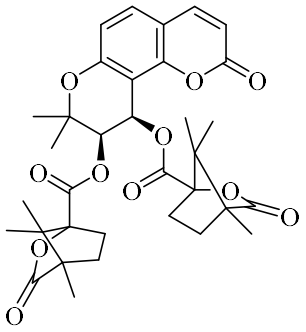


Figure 15. Calanolides[99, 69]

Khellactone coumarins are a small group of natural plant coumarins which have been found to have anti-HIV among many other bioactivities.[185] Suksdorfin **36**, a khellactone coumarin isolated from *Lomatium suksdorfii*, became a lead compound for the development of anti-

HIV khellactone derivatives.[186] 3',4'-Di-*o*-(*S*)-camphanoyl-(+)-*cis*-khellactone (DCK, **55**), a suksdorfin derivative, became a lead compound for many khellactone derivatives (KDs) after it had been found to inhibit HIV in a unique way.[187] The usual mechanism involves the blocking of the production of single-stranded DNA from RNA; in contrast, DCK prevents the single-stranded DNA intermediate from generating double-stranded viral DNA.[188] The mechanism opened up a way by which novel NNRTIs, which continue to be active in the face of multi-drug resistant HIV strains, could be explored.[189] The high potency of DCK has resulted in the synthesis and investigation of over a hundred structurally related compounds designed to keep the 3'*R*,4'*R* stereochemistry which is a necessary feature for the activity of surksdorfin.[167,190]



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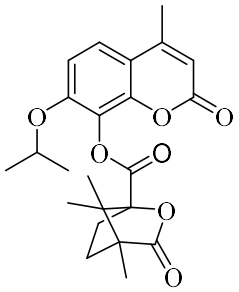

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Figure 16. Khellactone[25] and DCK analogue[191]

Tang *et al*. designed and synthesised novel DCK analogues several of which, on screening *in vitro*, showed moderate activity with the most active (**56**) showing an EC_{50} value of 0.058 μM and a therapeutic index of 1000. This compound was found to be active against a multi-RT inhibitor resistant strain which is typically unresponsive to the majority of DCK derivatives.[191]

1.3.2.3. Protease inhibitors

The clinically available protease inhibitors have typically been peptidomimetics which, although having good binding properties, have poor bioavailability and retention and are readily susceptible to the development of resistance.[192] Non-peptide agents with better biopharmaceutical characteristics have become a potential solution to these challenges.[156] 4-Hydroxycoumarins with the desirable characteristics of being orally available, small

molecules and having lower biliary excretion rates are already in therapeutic use as alternatives to peptidomimetics.[150]

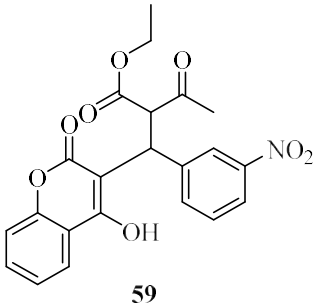
A study in the 1990s on warfarin as a non-peptidic anti-HIV-1 inhibitor showed that the oral coagulant could inhibit HIV infections, and this led to studies on 4-hydroxycoumarins as lead compounds for PR inhibitors.[193] Protein crystallography studies revealed that the binding of oral 4-hydroxycoumarin coagulants and similar compounds involved the formation of two hydrogen bonds, between the lactone and C-4 hydroxy groups of the ligand and the isoleucine and aspartic active-site protein residues. This was comparable to the binding mode of peptidic compounds, further establishing the 4-hydroxycoumarin scaffold as a lead structure for the development of more potent agents.[150] Identification *via* structure-based design, of phenprocoumon **12** as a suitable template, led to the design of a potent compound, 4-hydroxyl-2-pyrone (U-96988) **33** which became a pioneer clinical candidate for 4-hydroxy-2-pyrone anti-HIV-1 protease analogues.[194]

A mass screening technique used by Lunney and his group led to the discovery of the protease inhibitor 4-hydroxy-3-(3-phenoxypropyl)-2*H*-1-benzopyran-2-one **57**, and molecular docking studies of this compound in the protease active-site led to the design of analogues with better binding profiles; the best of these compounds was 4,7-dihydroxy-3-[4-(2-methoxyphenyl)butyl]-2*H*-1-benzopyran-2-one **58** (**Figure 16**).[195]

Figure 17. Benzopyran-2-one PR inhibitors

A study by Kirkiacharian *et al*., which involved the preparation, screening and structure-activity relationships evaluation of a variety of 4-hydroxycoumarin derivatives as HIV-1 PR inhibitors, revealed the importance for potency of groups at positions 5 and 7.[196] Stanchev *et al*. synthesised a series of 4-hydroxycoumarins and determined their interactions with HIV-1 PR. The docking investigations they conducted showed that the formation of hydrogen bonds with the PR involved the hydroxy group and the carbonyl oxygen of the lactone moiety and the oxygen of the pyran ring. Two of the compounds, compounds **59** and **60** (**Figure 18**) were

predicted to be active against PR and, in biological assays for anti-HIV-1 replication in MT-4 cells, **59** exhibited an IC_{50} value of 0.01 nM.[192]



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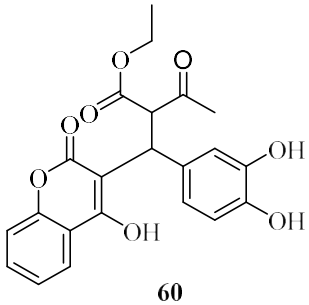


60

Figure 18. 4-Hydroxycoumarin derivatives evaluated for HIV-1 PR activity[192]

Using a 3D data base search, Wang *et al*.[197] discovered some 4-hydroxycoumarin non-peptide PR inhibitors whose *in-vitro* activities were quite encouraging (ID_{50} values of 0.32 μM and 1.7 μM for the two most potent). Compounds with higher numbers of the 4-hydroxycoumarin moiety, i,e, tetramers and dimers, generally showed better binding to PR than those with one moiety and, hence, better activity. The tetramer NSC158393 **32**, in addition to showing anti-protease activity, also showed activity against HIV-1 RT and HIV-1 IN thus indicating its potential as a lead compound for the development of multi-target anti-HIV-1 enzymes agents.[197]

1.3.2.4. Other anti-HIV-1 properties of coumarin derivatives

There are a number of reports which show that some coumarins have anti-HIV-1 activity that is dependent on targets other than RT, IN and PR. A study carried out by Sancho *et al*. showed that imperatorin **61** inhibited HIV replication without targeting reverse transcription or integration but through a mechanism that involves inhibition of Sp1, a transcription factor.[198] Uchiumi *et al*.[199] established that the activity of the HIV promoter, which is induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) could be suppressed by natural compounds which included 3-phenylcoumarin. This opened up the possibility of developing coumarin derivatives which target the promoter. Xian *et al*. reported that latent HIV-1 reservoirs could be activated using scoparone **15** or hymecromone, a discovery that may open avenues for the elimination of such reservoirs in host cells.[200] Mesuol **62** and isomesuol **63**, 4-phenylcoumarins isolated from *Marila pluricostata,* have also been reported to inhibit HIV through a mechanism that does not involve integration or transcription suppression. The

mechanism involves the long terminal repeat (LTR) transcription activity mediated by the tumour necrosis factor alpha (TNFα) and may serve as a target for the discovery of drugs that are effective against strains of HIV that have proven to be resistant to the known drugs.[201]

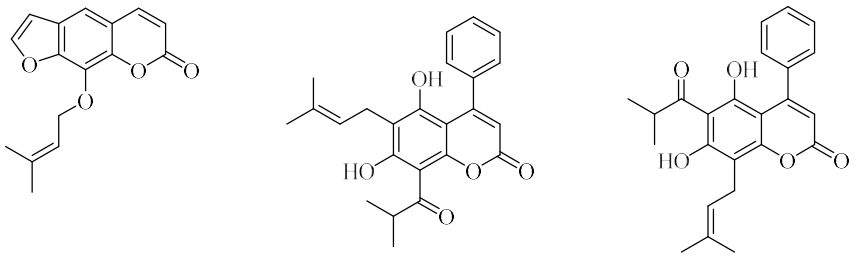


Figure 19. Imperatonin[202] and Anti-HIV 4-Phenylcoumarins[201]

A series of hybrid coumarin derivatives, including the hydrazine derivative **64**, the thiazolidinone **65** and the oxadiazoline **66** were developed and evaluated as anti-HIV agents. The activities of these compounds were found to be quite promising, with the minimum inhibition concentrations (MIC) below 0.3 mg/mL.[203]

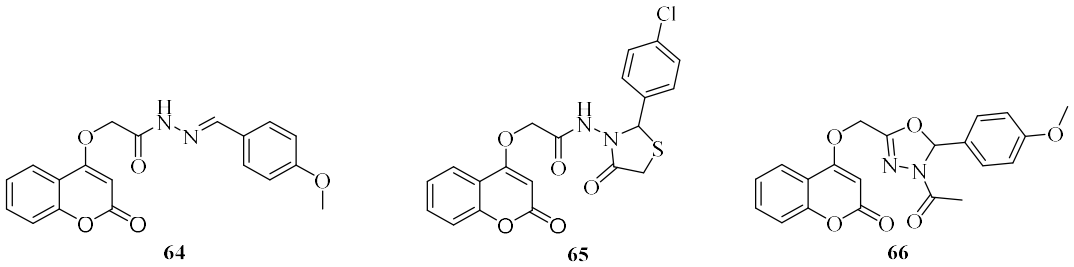


Figure 20. Hybrid coumarin anti-HIV-1 derivatives[203]

Marine-derived pyrrole alkaloids containing coumarin scaffolds, called lamellarins, have potent anti-HIV activity. Lamerallin **67**, an α-sulfate derivative, was shown to inhibit viral replication and integration in cell culture.[204] Lycopyranocoumarins and glycylcoumarins have also been reported to show anti-viral adsorption activities.[167]

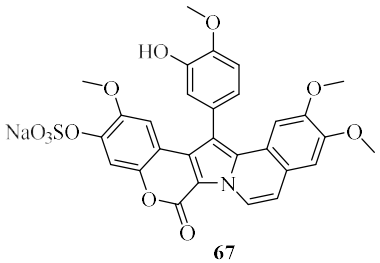
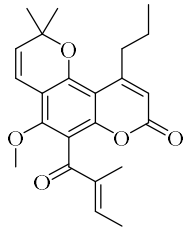


Figure 21. Lamerallin α sulfate derivative[204]

A series of 3-phenylcoumarins was assessed for activity against HIV using the Trans-activator of transcription (Tat) function and transcription as targets. Several of these compounds were found to be antagonistic to both targets[205]. Lin *et al*. demonstrated that the coumarin BPRHIV001 **38** could strongly inhibit HIV-1 Tat transactivity and hence inhibit HIV-1, thus presenting itself as a potential lead for the development of novel anti-HIV-1 compounds.[159] The evaluation of GUT-70 **68**, a tricyclic coumarin derivative extracted from *Chlophyllum brasiliense* bark, for activity against HIV-1 showed that its anti-HIV-1 replication action occurred through the inhibition of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells).[206] The same compound has been reported to target a second HIV-1 mechanism; Matsuda *et al*.[207] established that GUT-70 was also an anti-membrane fusion inhibitor. They reported that the natural product reduced the fluidity of the cell membrane, a modification which has the effect of preventing the entrance of the HIV-1 virions into potential host cells.



68

Figure 22. Coumarin derivative GUT-70[206]

It is quite evident that coumarins are well represented as medicinally active heterocyclic systems. What has been covered in this section is only a part of the extensive record of their medicinal and bioactive properties. In the following section, attention is turned to some of the photophysical properties of this interesting class of compounds.

1.3.3. Photophysical and photochemical properties of coumarin derivatives

In recent times, there has been an increase in the use of fluorescence in various scientific fields, including clinical chemistry, genetic analysis, environmental studies, cellular imaging and in tests such as enzyme-based immunoassays.[208] Coumarins, as a family, have drawn considerable attention due to their fluorescent properties, and many photophysical studies have been conducted on coumarin derivatives.[209,208] The parent compound, 2-oxo-2*H*-chromene does not exhibit significant fluorescence but strategically substituted derivatives are extremely fluorescent and have, as a consequence, found use in physics, biology,

medicine and chemistry;[210] they typically have large Stokes shifts, high extinction coefficients and quantum yields.[211] The position and nature of the substituents on the coumarin scaffold have a strong bearing on the fluorescence exhibited as do the environment and the viscosity and polarity of the medium.[208-209] Derivatives substituted with electron-donating groups at position 7 show intense fluorescence.[212] With the afore-mentioned properties, the different lipophilicities, shape and size of coumarins enables them to be used as fluorescent probes in different environments.[209] Finke *et al*. demonstrated the use of the florescent dye, Coumarin 6 **69**, commonly used to enable *in vitro* traceability in drug delivery studies; its fluorescence was used to show where the 'drug' model was and its distribution in the various parts of the system.[213] García-Beltrán *et al*. developed three coumarin-based fluorescent probes for dynamics studies in cells and membranes. The probes, exemplified by 3-acetyl-7-[(6-bromohexyl)oxy]-2*H*-chromen-2-one **70**, enabled labelling of the plasma membrane to which they adhere.[214] Novel solvatochromic coumarins have been developed for use in studying biochemical processes in cultured cells in which there are environmental polarity changes.[211]

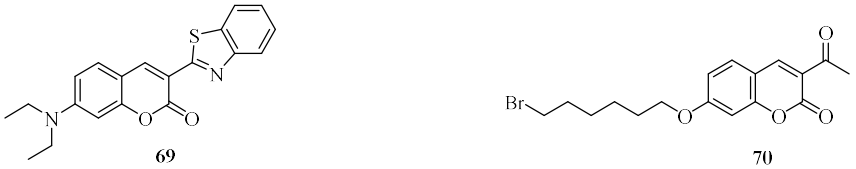


Figure 23. Structures of Coumarin 6[213] **69** ; fluorescent probe **70**[214]

Fluorescent coumarins have been synthesised and assessed as tryptophan intrinsic fluorescence resonance energy transfer (iFRET) acceptors in specific proteins. This technique enables real-time label-free fluorescence spectroscopic studies of biomolecules.[215] Wu *et al*. developed a method by which metal cations, such as Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Hg^{2+} and Pb^{2+}, could be detected using a fluorescence 'turn-on' mechanism in coumarin derivatives which was dependent on isomerisation of C=N.[216] The unbridged free ligand **71** has very low fluorescence due to decay *via* C=N isomerization whereas the complex **72**, formed on addition of $Zn(ClO_4)_2$, exhibits intense fluorescence (**Figure 24**).[216]

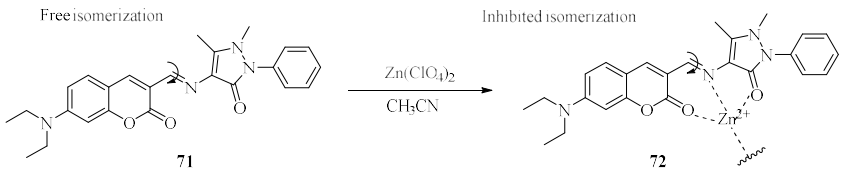


Figure 24. Metal ion detection by fluorescence

A probe that uses both fluorescence and colorimetry for CN^- detection was developed by Xu *et al*.[217] The chemodosimeter, based on a coumarinyl indole, showed excellent selectivity for CN^- in water and could be used over a wide pH range. Table **2** shows a summary of some photophysical and photochemical applications of coumarins.

Table 2. Photophysical and photochemical applications of coumarins

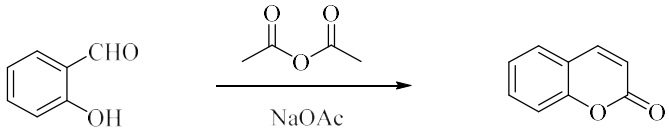
Application	Description	Reference
Increasing fluorescence of phthalocyanines	Coumarin derivatives were introduced into the outer ring of phthalocyanines to enhance the photochemical and photophysical properties.	218
CN^- detection	Michael addition conjugate based on coumarin–indanedione to ratiometrically and colorimetrically detect CN^- using fluorescence.	219
Photodynamic therapy	Photosensitizers made from coumarin derivatives functionalised with triethylene glycol.	220
Thiol imaging in cells	Ratiometric fluorescent thiol biosensor based on tetrakis(4-hydroxyphenyl)porphyrin–coumarin capable of use for thiol imaging in live cells.	221
Protein dynamics study	L-(7-hydroxycoumarin-4-yl)ethylglycine, a fluorescent amino acid was encoded in *E. coli* genetically in a technique which enables monitoring of interactions between proteins, conformational changes in proteins, trafficking and localization of proteins.	222
Dye-sensitised solar cells	Ethylenedioxythiophene was used as a low band gap chromophore in coumarin derivative dyes in dye-sensitised solar cells.	223
Organic light emitting diodes (OLEDs)	Material containing 7-phenylamino-bis(4-methylcoumarin) which emitted blue light was used in the production of an OLED that was not doped.	224
Thermally Activated Delayed Fluorescence Emitters (TADFs)	9-(10*H*-phenoxazin-10-yl)-6*H*-benzo[*c*]chromen-6-one and 3-methyl-6-(10*H*-phenoxazin-10-yl)-1*H*-isochromen-1-one were found to have requisite properties for TADF and used in the design of promising OLEDs	225
Study of micelles	Measurement of fluorescence changes on coumarin probe that are caused by the micelles can be used to determine interior polarity, microviscosity and critical micelle concentrations.	209

1.3.4. Synthesis of Coumarins

The wide spectrum of applications of coumarins has led to considerable interest in their synthesis and numerous methods have been developed. These include the Perkin, Baylis-Hillman and Wittig reactions and the Knoevenagel and Pechmann condensations.[95] These methods appear to be the more established ones and to have been studied extensively. Some of these reactions will be discussed to some detail.

1.3.4.1. Perkin reaction

The Perkin reaction was first reported by Perkin in 1868[226] and is also known as the Perkin synthesis, Perkin condensation, Perkin coumarin synthesis or Perkin cinnamic acid synthesis.[227] It involves the base-catalysed synthesis of derivatives of cinnamic acid through the combination of an aromatic aldehyde and an aliphatic acid anhydride, or other carboxylic acid derivatives, at elevated temperature.[228,227] In the Perkin reaction, the coumarin is formed through the condensation of acid anhydrides and *ortho*-hydroxybenzaldehyde, catalysed by a basic salt of the corresponding acid as illustrated in **Scheme 1**.[229]

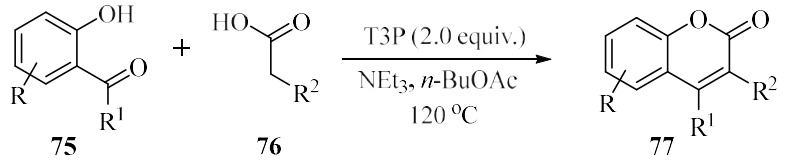


73 74

Scheme 1. Perkin synthesis of coumarin

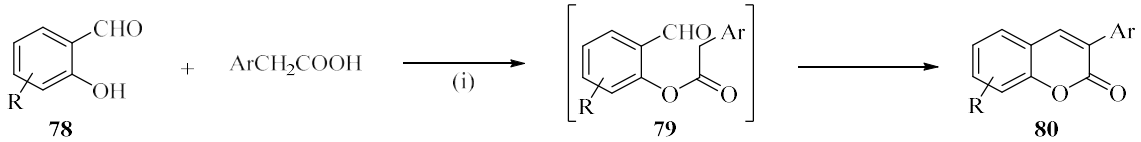
For simple and direct access to substituted coumarins, the classical Perkin method has been the method of choice but it has several draw-backs, which include: harsh reaction conditions; limited reactant range; poor yields; the need for several reaction steps at times; and complex isolation of products.[230,231] There are a number of reports on what has been done to improve the Perkin synthesis. Sodium fluoride has been used as a catalyst in the improvement of the acetic anhydride reaction with salicylaldehyde to produce coumarin.[229] To increase the yields of 3-substituted coumarins, condensing agents, such as $PhPOCl_2/Et_3N$, have been employed.[231]

Augustine and co-workers[230] demonstrated a one-pot propylphosphonic anhydride (T3P) aided synthesis of coumarin (**Scheme 2**) as an improvement to the Perkin synthesis. The method enables the synthesis of substituted coumarins from a wide range of alkanoic acids and 2-hydroxyarylcarbonyls under mild conditions which accommodate a variety of functional groups, and the yields are good.



Scheme 2. T3P mediated Perkin coumarin synthesis[230]

A single-step synthesis of 3-substituted coumarins was reported by Mashraqui *et al.*[231] who employed the Mukaiyama conditions for ester formation. The reaction (**Scheme 3**) involves formation of an aryl acetic acid-salicyladehyde ester followed by Claisen condensation to yield 3-arylcoumarins in good yields, and the method is substrate flexible.

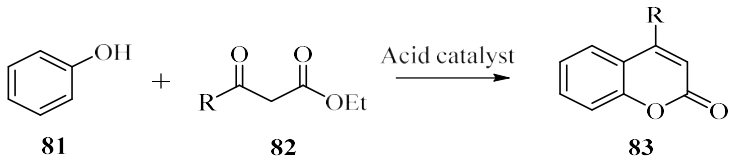


(i) 2-chloro-1-methylpyridinium iodide/Et_3N in CH_3CN, heat, N_2

Scheme 3. Mukaiyama esterification aided Perkin coumarin synthesis[231]

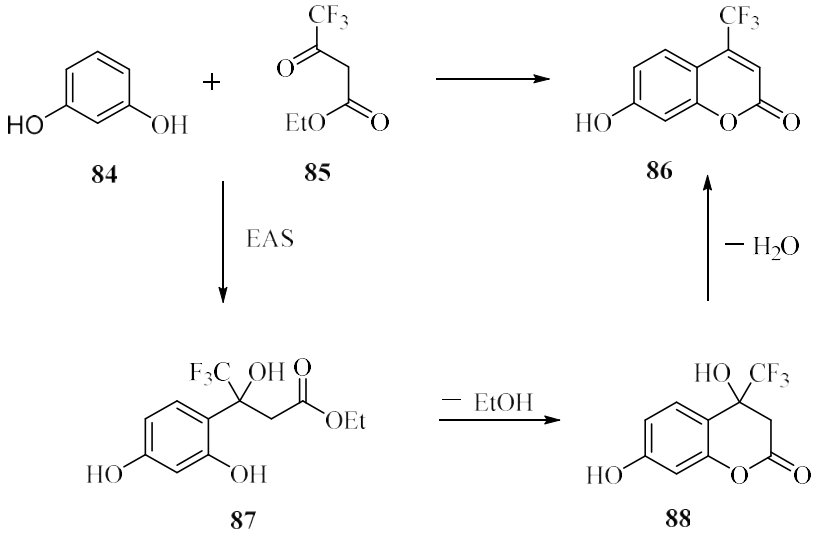
1.3.4.2. Pechmann condensation

The Pechmann condensation, Pechmann synthesis or Pechmann coumarin synthesis was first described by Hans von Pechmann in 1883, and involves the reaction of a β-ketocarboxylic acid or β-keto ester with a phenol to yield coumarins in an acidic medium (**Scheme 4**).[232,227,233] The acids that have been used in the classical method include hydrochloric acid, phosphorus pentoxide, sulfuric acid, trifluoroacetic acid and hydrofluoric acid.[232] The reaction consists of an esterification or transesterification followed by cyclisation and loss of water.[227] With relatively cheap and easily obtainable reactants, the synthesis has been the most widely used to access coumarins.[234] Yields are good and substitution can be in both the benzene and pyrone rings or in either of the two.[235]



Scheme 4. General Pechmann reaction scheme[233]

The mechanism of the reaction is yet to be established as there is still no consensus.[236] In an effort to determine the possible mechanism, Tyndall *et al.*[236] used nuclear magnetc resonance (NMR) studies to follow the reaction of resorcinol (**84**) with trifluoroacetic anhydride. The group isolated and identified the two intermediates **87** and **88** which, they suggested, are evidence that the reaction sequence involves electrophilic aromatic substitution (EAS), followed by transesterification and, finally, loss of water (**Scheme 5**). A recent study by Pornsatitworakul *et al.*[237] suggests a similar mechanism. In their study, resorcinol and ethyl acetoacetate were reacted to produce coumarin with sulfuric acid catalysis. Reaction rates were used to establish the apparent energy of activation and M06-2X density functional theory studies undertaken. The activation energy was found to be 34.7 kJ/mol in the temperature range 273-313 K and the mechanism was seen as a three-step process, with transesterification being the first, followed by intramolecular hydroxyalkylation and, finally, dehydration.

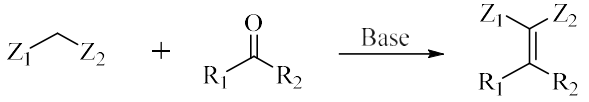


Scheme 5. Proposed mechanism of the Pechmann condensation

The Pechmann synthesis has been used widely in making coumarin derivatives under a variety of conditions, with a variety of catalysts.[232] There has been a deliberate movement away from the use of classical acids like phosphorus oxychloride, sulphuric acid, phosphoric acid and aluminium chloride, as they are irrecoverable and also hazardous, to substances such as zeolites, Filtrol and zeokard 225.[238] Bouasla *et al.*[239] optimised the Pechmann reaction synthesis of 7-hydroxy-4-methylcoumarin from phenol, resorcinol and ethyl acetoacetate. They evaluated a number of heterogenous solid acid catalysts in microwave-assisted solvent-free reactions. The catalysts studied were sulfonic acid-functionalized silica, zeolite-β and Amberlyst-15. Amberlyst-15 was found to be the best catalyst under the conditions used.Other improvements to mitigate the extreme conditions under which the original reaction was conducted have been the use of ion-exchange resins, ionic liquids and heteropoly acids.[234]

1.3.4.3. Knoevenagel condensation

The reaction, which involves nucleophilic addition of an active methylene-containing entity to a carbonyl compound to give an olefin with loss of water, was first reported in 1894 by Emil Knoevenagel.[227] Weak bases, such as amines, are used as the main catalysts although strong bases can also be used.[240,227] Lewis acids like $ZnCl_2$, CdI_2, $BiCl_3$ can also be used to catalyse the reaction.[241] The general scheme is shown below in **Scheme 6**.



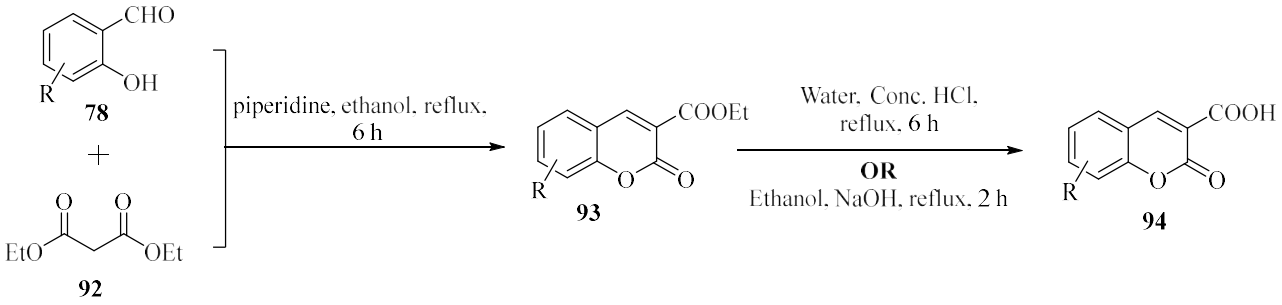
89 **90** **91**

Z_1, Z_2 = COOH, Ar, SO_2R, CN, CO_2R; R_1 = alkyl, aryl; R_2 = H, alkyl, aryl

Scheme 6. Knoevenagel condensation reaction scheme[227]

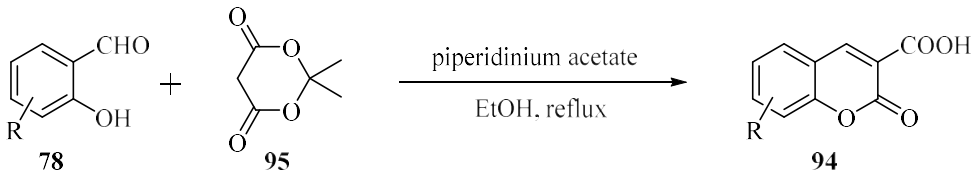
The method became vital at the beginning of the 1900s as a means to access coumarin-3-carboxylic acids, and it has been modified for use under a wide variety of conditions and with a range of substrates to access a diverse range of products.[242,227] Piperidine, a common organic catalyst in the Knoevenagel synthesis of coumarins, has been used in the modified versions of the reaction. A protocol, in which diethyl malonate was bound to a resin and reacted with *ortho*-hydroxybenzaldehydes and piperidine as a catalyst to give coumarin-3-

carboxylic acids, was designed by Watson *et al.*[243] The products were obtained in high purity under mild conditions. Creaven *et al.*[95] showed that coumarin-3-caboxylate derivatives could be accessed from diethyl malonate and *ortho*-hydroxy aryl aldehydes under reflux in ethanol with piperidine as catalyst. This protocol, as shown in **Scheme 7**, results in the conversion of the esters to carboxylic acids *via* base- or acid-catalysed hydrolysis.



Scheme 7. Synthesis of coumarin-3-carboxylic acids[95]

Traditionally, the Knoevenagel condensation has been used to prepare 3-carboxycoumarins from *ortho*-hydroxyaryl aldehydes and malonic, cyanoacetic acid or cyanoacetic ester.[244,245] A Knoevenagel condensation of *ortho*-hydroxybenzaldehydes and Meldrum's acid, conducted under reflux in ethanol in the presence of piperidinium acetate, produced coumarin-3-carboxylic acids. The method (**Scheme 8**) afforded the products in high purity and yield and can also be adjusted to handle ketones to access 4-alkylcoumarin-3-carboxylic acids by first converting the ketones to ketimines and then condensing the ketimines with Meldrum's acid.[245]



Scheme 8. Synthesis of coumarin-3-carboxylic acids

Kumar *et al*. developed an improved synthesis of coumarin-3-carboxylic acids which is cleaner and faster and also gives high yields. In this approach, the reactants, 2-hydroxybenzaldehydes and Meldrum's acid, are ground together with a little water at room temperature to give 3-carboxycoumarins in 20 minutes.[246] Recent applications of the Knoevenagel method of synthesising coumarin derivatives have employed various catalysts including: the heterogenous solid catalysts, clay KSF and alumina, HZSM-5 zeolite[95] and

natural kaolinitic clays EPZG and EPZ10;[247] and the heterogeneous and recoverable catalysts cellulose-sulfonic acid[248] and silica-sulfuric acid.[249] Ionic liquids and microwave irradiation have also been used to improve the reaction.[250,95] Recent methods reflect the desire to develop protocols that are cleaner, faster, more efficient and versatile.[95]

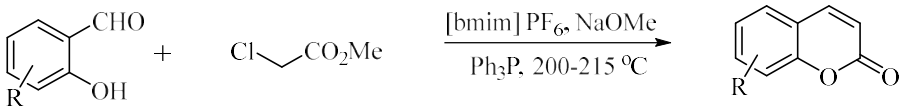
1.3.4.4. Wittig reaction

The reaction was first reported in 1953 by Wittig and Geissler and involves the reaction of a phosphonium ylide with a ketone or an aldehyde to give the respective alkene and phosphine oxide.[251,252] The reaction, an established carbonyl olefination method (**Scheme 9**), affords alkenes with stereocontrol.[252]

96 **97** **98** **99**

Scheme 9. Wittig reaction[252]

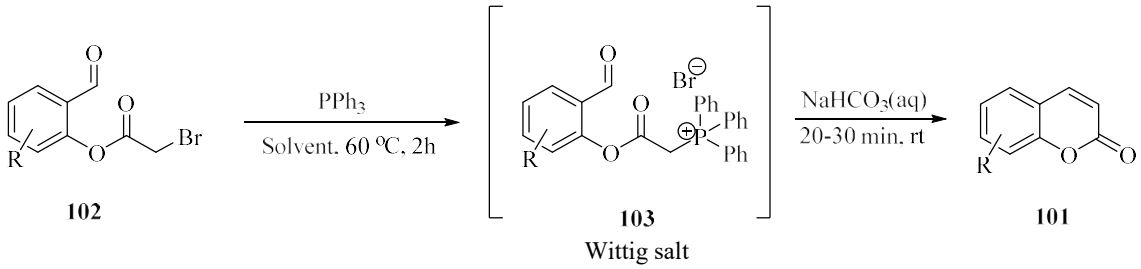
There are a number of literature reports on the use of the Wittig reaction in the synthesis of coumarins. Valizadeh and Vaghefi reported a one-pot NaOMe-mediated Wittig synthesis of coumarin derivatives from *o*-hydroxybenzaldehydes, chloroacetate esters and triphenylphosphine[253]. The reaction (**Scheme 10**) was carried out in ionic liquids at high temperature and gave good yields.



78 **100** **101**

Scheme 10. NaOMe-mediated Wittig reaction synthesis of coumarins[253]

Patre *et al*. designed a Wittig one-pot synthesis of the coumarins, balsamiferone, 6,8-diphenyl umbelliferone and gravelliferone, from 2,4-diprenyloxybenzaldehyde and involving a Wittig reaction, a double Claisen and a Cope rearrangement.[254] Belavag *et al*. reported an intramolecular Wittig reaction for the synthesis of coumarins from 2-formylphenyl 2-bromoacetate precursors (**Scheme 11**) at room temperature in a saturated solution of $NaHCO_3$. The products were isolated readily and in good yields.[255]

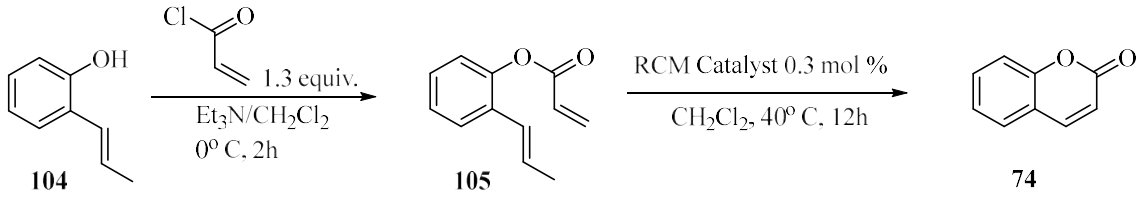


Scheme 11. Synthesis of coumarins *via* an intramolecular Wittig reaction[255]

In an intramolecular Wittig reaction synthesis of coumarins described by Jang *et al.*, furo[3,4-*c*]coumarins and similar compounds were synthesised from tributylphosphine, *α,β*-unsaturated ketones and acyl chlorides under mild conditions.[256]

1.3.4.5. Other methods for preparing coumarins

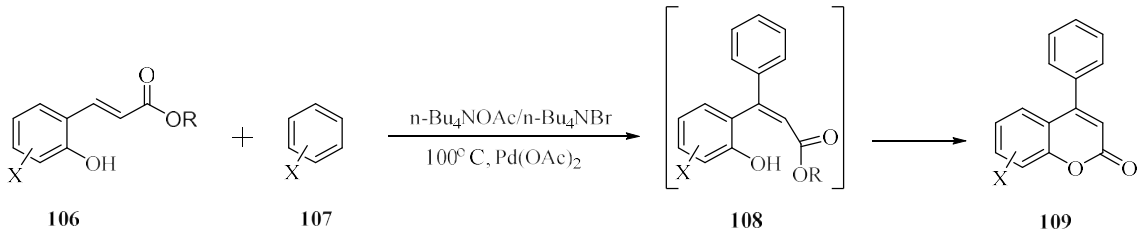
There are a number of other methods that have been developed in response to the high value of the coumarin motif, which is found in compounds that have a wide range of applications, and to the challenges inherent in the use of the more traditional syntheses. Chatterjee *et al.*[257] developed a general method to access coumarins *via* ring-closing metathesis (RCM) of olefins. They demonstrated that second-generation ruthenium catalysts could enhance the reaction of electron deficient olefins intramolecularly to access 3 and 4 substituted coumarins as well as tetrasubstituted ones. **Scheme 12** shows the general reaction.



Scheme 12. RCM synthesis of coumarin[257]

A similar method in which RCM is used to produce coumarins was developed by Nguyen Van *et al.*[258] Phenolic substrates are *o*-allylated and then subjected to an *ortho*-Claisen rearrangement, followed by base-induced isomerization to afford 2-(1-propenyl)phenol precursors; treatment with acryloyl chloride leads to the desired diene intermediate which

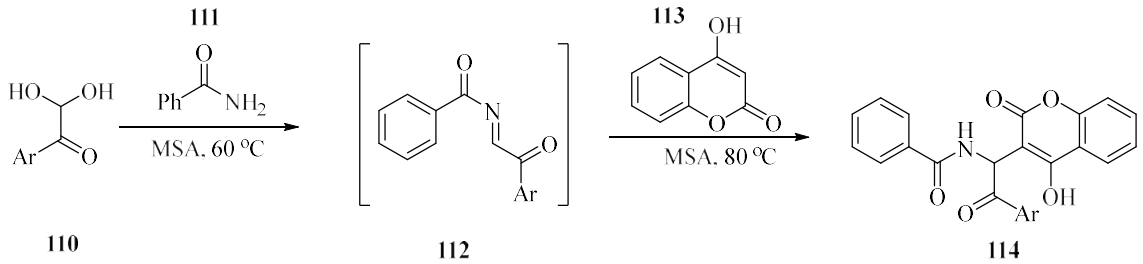
undergoes RCM with a second generation Grubbs ruthenium catalyst to yield the coumarin in good yields. Battistuzzi *et al.*[259] reported a non-classical method for 4-arylcoumarins synthesis in which aryl iodides and bromides are reacted with methyl or butyl 3-(*o*-hydroxyaryl) acrylates in a Domino Heck Reaction/Cyclization process, which is carried out at 100 °C (**Scheme 13**) in a mixture of *n*-Bu_4NOAc and *n*-Bu_4NBr and catalysed by $Pd(OAc)_2$. This approach produced 4-arylcoumarins in good to high yields.



106 107 108 109

Scheme 13. 4-Arylcoumarins synthesis[259]

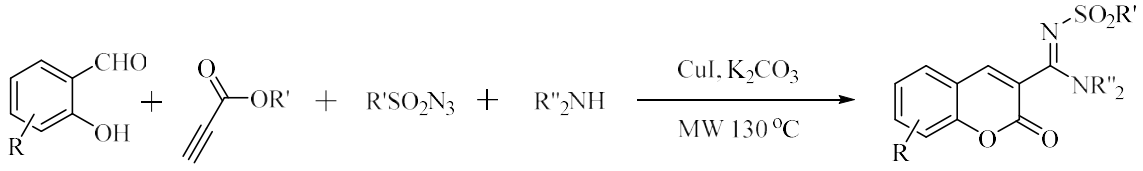
A one-pot multi-component reaction of 4-hydroxycoumarins, benzamides, and aryl glyoxals catalysed by molybdate sulphuric acid (MSA) afforded 3-aroylamido coumarins **114** (**Scheme 14**).[260,103]



110 112 114

Scheme 14. 3-Aroylamido coumarin synthesis[260]

3-*N*-Sulfonylamidine coumarins were synthesised by Murugavel and Punniyamurthy, *via* a one-pot 4-component microwave assisted reaction (**Scheme 15**).[261] Sulfonyl azides, propiolates, secondary amines, and salicylaldehydes were coupled to afford the products.



78 115 116

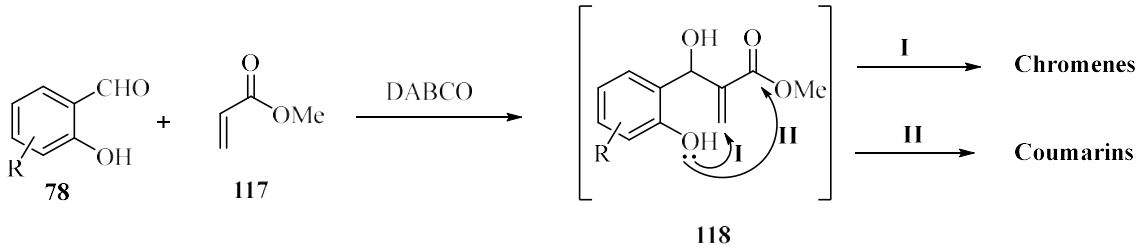
Scheme 15. 3-*N*-Sulfonylamidine coumarins synthesis[261]

The reaction enabled direct access to the products in high yields. Microorganisms such as the fungi, *Ascomycetes* and *Basidiomycetes* and bacteria, *Escherichia coli,* have been used in the

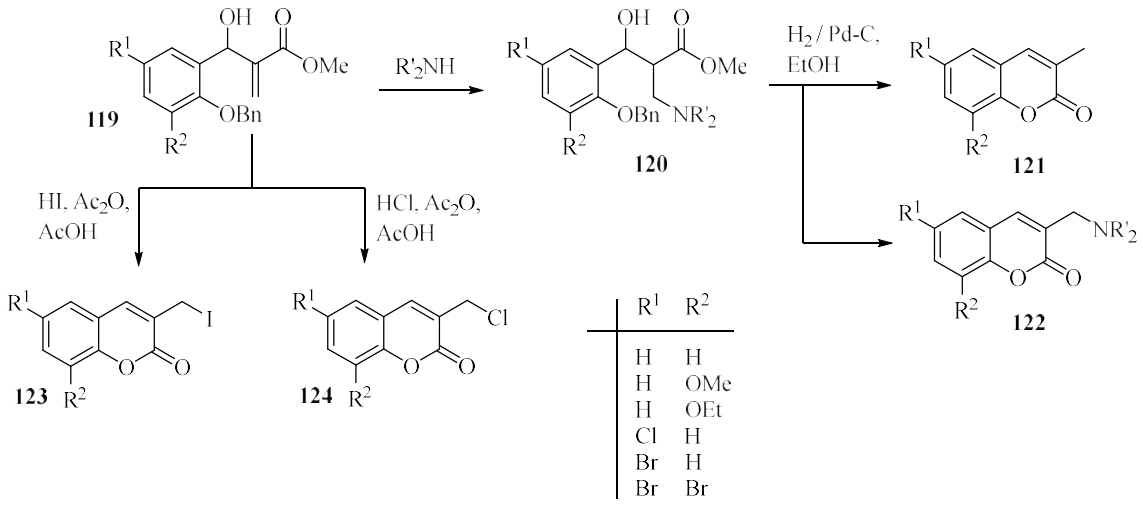
artificial biosynthesis of coumarins.[103] Lin *et al*. using such strategies employed modified *Escherichia coli* to produce scopoletin and umbelliferone from ferulate, phenylpropanoid acid precursors and 4-coumarate.[262] In a different artificial biosynthesis of coumarins, *Escherichia coli* was engineered to express the genes *4CL* and *F6'H* that encode 4-coumarate CoA:ligase and feruloyl CoA 6′ hydroxylase respectively; exposure to a culture containing caffeic, *p*-coumaric, and ferulic acids, led to the synthesis of three coumarins, namely, esculetin, scopoletin, and umbelliferone.[263] The Rhodes University group has also been responsible for developing Baylis-Hillman approaches to the preparation of coumarins, and this will be addressed in the next section.

1.3.5. Earlier research on coumarins at Rhodes University (RU)

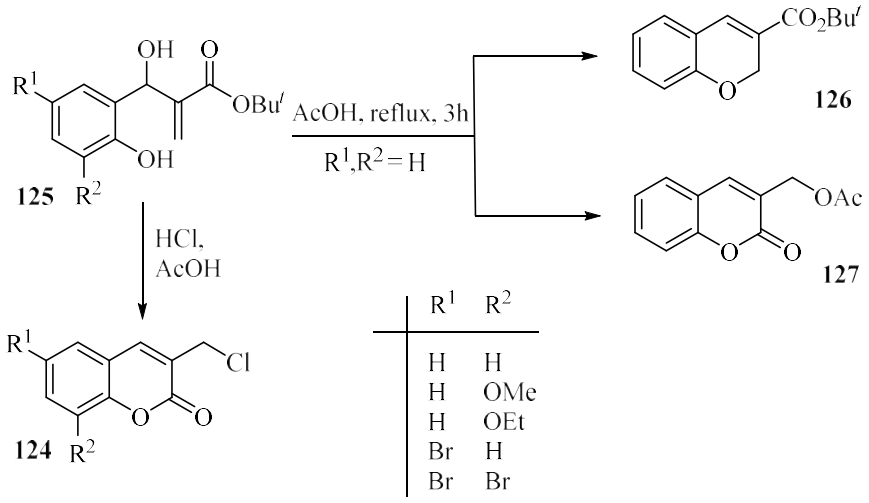
Our group at Rhodes has developed Baylis-Hillman based approaches to access various benzannulated heterocycles, including coumarins. Early studies showed that cyclisation of unprotected Baylis-Hillman ester adducts from acrylate esters and salicylaldehyde precursors resulted in a mixture of coumarin and chromene derivatives **Scheme 16**.[264,265] In subsequent studies protecting groups were employed to access 3-substituted coumarins **Scheme 17**.[266,265,] This strategy inhibited the formation of chromene derivatives. In a 2003 report, coumarins were synthesised from Baylis-Hillman adducts of salicylaldehyde without the use of protecting groups. 3-(Chloromethyl)coumarins **124** were synthesised *via* acid catalysed cyclisation of *tert*-butyl ester adducts **125**; however, one adduct, under reflux with acetic acid, afforded two compounds, a chromene **126** and coumarin **127** (**Scheme 18**).[267,268]



Scheme 16. Non-regioselective cyclisation of Baylis-Hillman adducts



Scheme 17. Protection groups strategy to 3-substituted coumarins



Scheme 18. Synthesis of 3-substituted coumarins by direct cyclisation of unprotected Baylis-Hillman adducts

The use of *tert*-butyl acrylate as opposed to methyl acrylate in the Baylis-Hillman reaction enables the isolation of the *o*-hydroxybenzaldehyde adducts that can then be cyclised to 3-substituted coumarin derivatives chemoselectively without recourse to protecting groups.[268,269,270] The Baylis-Hillman based approaches have enabled access to several classes of novel coumarin derivatives which include Ritonavir analogues,[271] phosphonated 3-(benzylaminomethyl)coumarins,[272] Michaelis–Arbuzov products,[273] and coumarin-AZT conjugates.[274,275]

1.4. COMPUTER-AIDED DRUG DISCOVERY

The rapid development of high performance computing has been viewed as a positive for the acceleration of biopharmaceutical innovation by affording, through virtual means, the reduction of time needed in testing ligands against potential targets.[276] The use of virtual platforms in drug discovery is considered desirable as it offers possibilities for the identification of potential drug molecules, evaluation of their potential activity and mechanism of operation before they are synthesised and before any *in vivo* tests are conducted.[277] The virtual space also enables creation and maintenance of much more extensive libraries of compounds in considerbly less time than combinatorial chemistry. Additionally, *in silico* studies cover a much wider space, chemical-wise, than combinatorial chemistry.[278] In the late 1800's, Emil Fischer suggested the "lock and key" model to explain binding between an enzyme and its substrate.[279] The understanding of binding, an important concept in the study of ligand-protein interactions has come a long way since then with various models being developed and improved over time to better understand and explain these molecular interactions, and computer technology has been employed extensively in this regard.[280] From its birth in the 1980s, interest in Computer-Aided Drug Design (CADD) has been growing and targets mainly the following: novel compound design; hit identification and optimisation; lead optimisation; and virtual screening.[281,280,282] CADD, as an interdisciplinary approach to drug discovery, is employed by chemists and biologists as a vital component in drug discovery and evaluation as, among other things. it permits the cutting of time and costs in drug discovery and development.[283]

Early examples of therapeutics that owe their discovery mainly to CADD include the 1990-approved anti-hypertension drug, cilazapril, the 1994-approved dorzolamide, an inhibitor of carbonic anhydrase, the anti-HIV-1 drugs , saquinavir (1995) and indinavir and ritonavir (1996) and, finally, the 1998-approved tirofiban, an antiplatelet agent.[284] CADD has played a part in the development of many drugs that have gone on to clinical trials and many that have been approved.[285,286] CADD, also referred to as *in silico* drug design, involves the employment of several strategies some of which are shown in **Table 3**. [283] In a general sense, the strategies can be narrowed into two, *viz*., i) structure-based drug discovery (SBDD). which requires knowledge of both the receptor and ligand structures and and ii) ligand-based drug discovery (LBDD) with methods that include quantitative structure–activity relationship (QSAR) studies, molecular descriptors and ligand-based pharmacophores. [284] Outcomes from

CADD are utilised in the design of compounds that will then be chemically synthesised and biologically tested, and the information obtained is used for SAR improvement and enhancement of other drug properties that encompass metabolism, distribution, absorption and elimination.[287] A generalised flow of processes in CADD is shown in **Figure 25**.

Table 3. Strategies in CADD.[283]

Strategy	Description
Structure Based Drug Design (SBDD)	Approach used when both drug target/receptor and ligand structure are known. These can be accessed from databases such as the PDB and PDBe. Alternatively, homology/theoretical modelling is employed if 3-D structures unavailable in PDB.
Ligand Based Drug Design (LBDD)	Normally employed in the absence of knowledge of drug target. Methods, such as QSAR/QSPR with 2-D/3-D descriptors are engaged to find potential ligands/drug candidates.
Fragment Based Drug Design (FBDD)	Employed in the generation of biologically active chemical fragments/scaffolds of low molecular weight which allows for their elaboration and optimisation.
Pharmacophore Based Drug Design (PBDD)	Premised on assumption that a ligand, if active, should have its requisite parental pharmacophore features at the right locations. Modelling methods include 3-D-QSAR, common feature and 3-D-pharmacophore modelling. Pharmacophore theory is applicable to receptors and ligands.

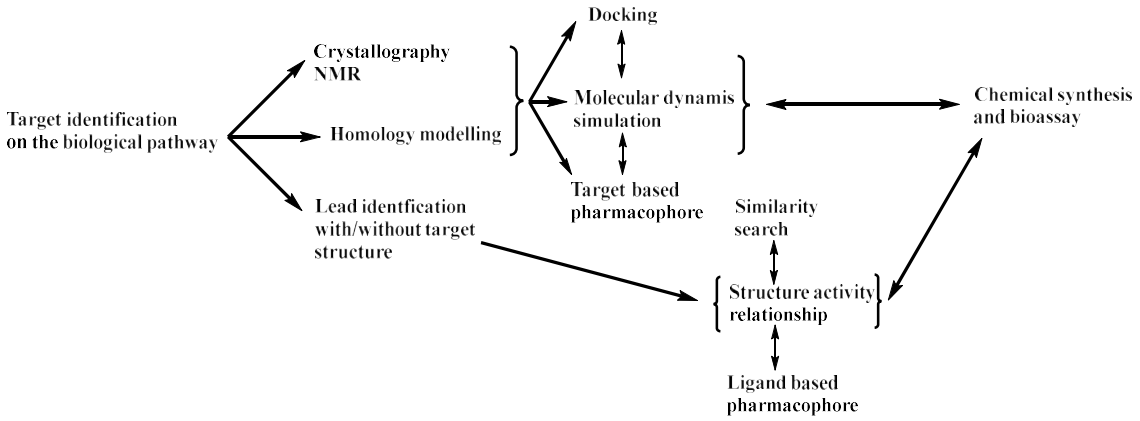


Figure 25. Flow of processes in CADD.[287] (Used with permission)

1.4.1. Docking

The docking of small molecules into receptors/macromolecules, usually proteins, and related computer technologies are integral parts of CADD.[288] It permits the binding modes and affinities of ligands in binding pockets of receptors to be evaluated. This investigation on ligand-receptor interactions is a direct beneficiary of the recent and continued developments in protein data bases and computer capacities.[289] Docking affords the identification of novel ligands, prediction of binding affinities and the modes of action of known/unknown ligands.[290] Molecular docking generates poses; orientations and conformations of the ligand in the receptor binding pocket and consists of two stages, the sampling of conformations then the association of a score to every predicted conformation.[280] Ranking of docked ligands is enabled through analysis of binding energetics calculations run by the docking algorithms.[291] The ligand-receptor complex with the lowest Gibbs free energy is considered as the most stable and its associated ligand the best ligand.[288] There is a number of available docking software packages and these are differentiated by the type of conformation sampling technique used to create the poses and the particular scoring function applied in the description of the ligand-target interaction.[287] Available docking programs include FlexX, AutoDock Vina, Glide, AutoDock, MOE-Dock, GOLD and LeDock.[290,292] Docking techniques are classified according to the level of flexibility giving 3 main groups, Rigid, Semi-flexible and Flexible docking (**Table 4**).[280]

Table 4. Docking Techniques.[280,284]

Docking technique	**Features**
Rigid docking	Ligand and receptor regarded as rigid structures and in sampling only three rotational and three translational degrees of freedom are allowed. Model is normally employed in situations where there are too many conformational degrees of freedom for sampling - as in the case with protein-protein docking. Can be used for initial selection for onward refinement *via* flexible methods
Semi-flexible docking	Only the ligand is flexible so it is the only component whose degrees of freedom with respect to confirmation are sampled besides the six translational and rotational degrees of freedom.
Flexible docking	Both protein and ligand are flexible resulting in multiple degrees of freedom. With many energy coordinates the docking demands more computer power.

Docking algorithms classically designed and applied have some drawbacks some of which can be addressed through the use of Molecular dynamics (MD), a growing technique, that among other things, is aimed at addressing the limitations which include the inability of docking algorithms to take into account water coordination effects (these are critical in the production of some ligand-target complexes), adequate flexibility which is a prerequisite for the prediction of correct poses and, finally, kinetic parameters which have been found crucial in estimating drug efficacy *in vivo* as they are more dependable than affinity.[293] Molecular dynamics (MD) reveals the time-dependent ligand-target complex shape and has shown that some ligands with high binding affinity, when subjected to MD simulations, may assume different poses and, in some worst case scenarios, even move or fall out of the binding pocket. Consequently, MD can be used as a validation method for docking outcomes and to predict the docking-derived ligand-protein complex stability.[288,291] Another strategy that has been developed to address the challenges associated with water molecules and the flexibility of receptors/proteins is multiple receptor conformations docking/ensamble docking.[294,278] In this approach, multiple conformations of the receptor are generated and, into these, ligands are docked with subsequent merging of the results leading to richer docking outcomes.[278] CADD as a technique, like any system in virtual space, has to be validated by real systems and, in a number of cases, candidates indicated as promising have failed on testing in biological systems. In the drug pipeline, only four in ten CADD identified leads may make it to the end; hence the continued development and constant updates of databases and other CADD tools, such as computer hardware and software.[289]

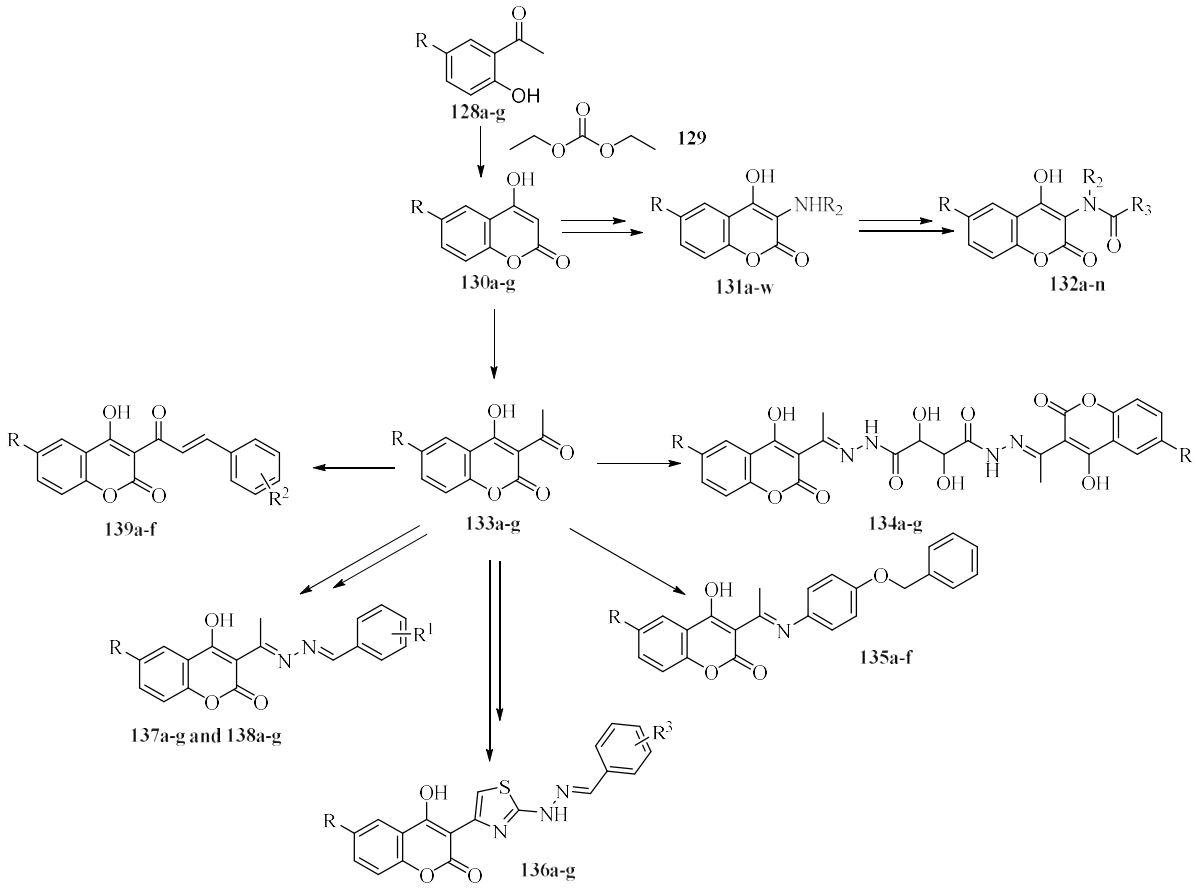
1.5. AIMS OF THE CURRENT STUDY

The study is aimed at the synthesis and evaluation of the medicinal potential of novel 4-hydroxycoumarin derivatives. Focus will be on the following objectives:

1) Synthesis of 4-hydroxycoumarins and related compounds as intermediates in the preparation of biologically active systems.
2) Evaluation of the compounds as potential inhibitors of critical HIV-1 enzymes.
3) Evaluation of the bioactivities of the compounds with respect to Malaria, Trypanosomiasis, Tuberculosis and cytotoxicity.
4) Docking studies of selected series of compounds with relevant proteins from the Protein data bank (PDB) for HIV-1 IN, HIV-1 PR, Malaria, Trypanosomiasis and Tuberculosis.

2. RESULTS AND DISCUSSION

The discussion will cover the synthesis of 4-hydroxycoumarin derivatives as potential HIV-1 IN and PR inhibitors and their bioactivity against these HIV-1 enzymes as well as their anti-malarial, anti-trypasonomal and, toxicity and anti-tuberculosis potential. *In silico* docking studies of selected potential inhibitors of HIV-1 IN and PR and other disease-specific receptors will be discussed in respective sections. Overall reaction plans to access the required 4-hydroxycoumarin derivatives are shown in **Scheme 19**.



Scheme 19. Proposed synthetic pathways to 4-hydroxycoumarin derivatives

2.1. SYNTHESIS OF 4-HYDROXYCOUMARIN DERIVATIVES

2.1.1. Synthesis of bis-4-hydroxycoumarinyl succinohydrazides

The azomethine (-NHN=CH-) proton-containing hydrazones have drawn considerable interest and have been extensively studied due to their important and diverse biological activities which include anti-cancer, anti-trypanosome, anti-oxidant, anti-arthritis, anti-mycobacteria, anti-inflammatory, anti-viral and anti-malaria.[295] The azomethine moiety present in hydrazine-hydrazone derivatives has also been found to be useful in the development of drugs based on these compounds.[296] Furazolidone **140** and nitrofurazone **141**, extensively used medicinal agents, contain the hydrazide-hydrazone motif. Another positive feature of hydrazones is that the blocking of the free NH_2 group results in compounds that are less toxic than the parent hydrazides.[297] It was attractive to explore the combination of the established biological potentials of 4-hydroxycoumarin and hydrazone moieties in single molecules.

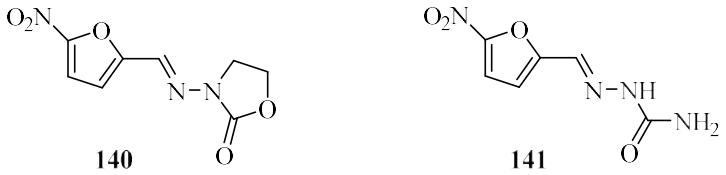
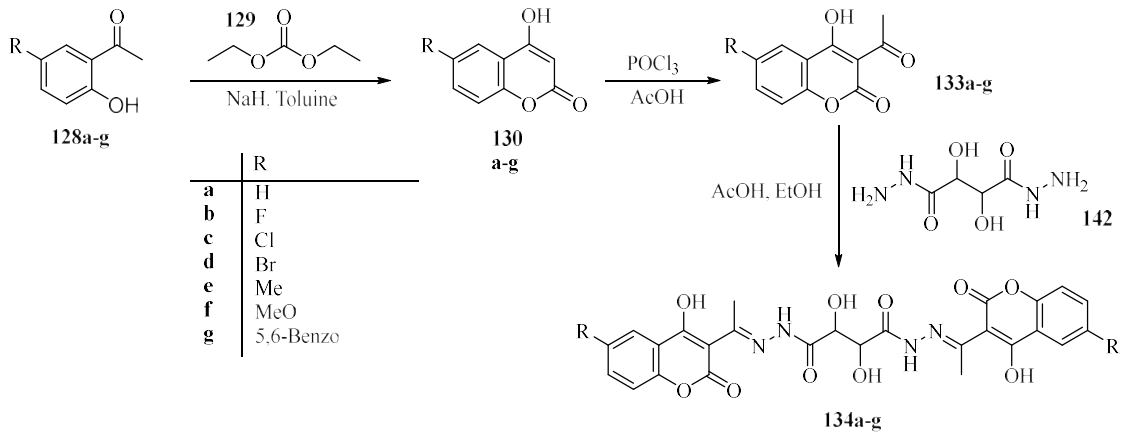


Figure 26. Medicinal agents containing the hydrazide-hydrazone motif

Several steps were required to access the novel compounds, *viz.*, i) the synthesis of 4-hydroxycoumarins **130a-g**; ii) acetylation of the coumarins at positon 3 using $POCl_3$ and acetic acid to give **133a-g**; and finally iii) the reaction of the 3-acetylated coumarins with 2,3-dihydroxysuccinohydrazide to give compounds **134a-g** (**Scheme 20**).

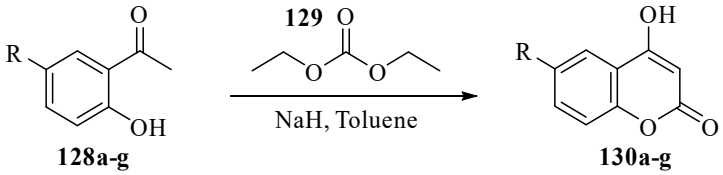


Scheme 20. Pathway to bis-4-hydroxycoumarinyl succinohydrazides.

2.1.1.1 Synthesis of 4-hydroxycoumarins

The synthesis of the 4-hydroxycoumarins **130a-g** was based on the method reported by Zhao *et al.*;[298] with some modifications, namely the use of dimethyl carbonate in some of the syntheses instead of diethyl carbonate and skipping the recrystallisation from ethanol when it was observed, from the NMR analyses, that this was not needed to isolste the products in satisfactory purity. The 2-hydroxy acetophenones **128a-g** were reacted with diethyl or dimethyl carbonate in the presence of sodium hydride (60% in mineral oil). Work up afforded the 4-hydroxycoumarins **130a-g** in yields 67-87% (**Table 5**). The ^{1}H NMR spectrum of 6-chloro-4-hydroxy-2*H*-chromen-2-one **126c** (**Figure 27**) shows the 3-H proton signal as a singlet at 4.52 ppm and the 7-H aromatic proton resonating as a doublet of doublets at 7.48 ppm and reflecting its coupling to the 8- and 5-H aromatic protons. In the ^{13}C NMR spectrum, the signal at 85.4 ppm corresponds to C-3 while the remaining eight signals correspond to the rest of the aromatic carbons and the lactone carbonyl carbon (**Figure 28**).

Table 5. Yields of 4-hydroxycoumarins **130a-g**.



Compound	R	Isolated Yields (%)
130a	H	69
130b	F	67
130c	Cl	87
130d	Br	86
130e	Me	60
130f	MeO	72
130g	5,6-Benzo	74

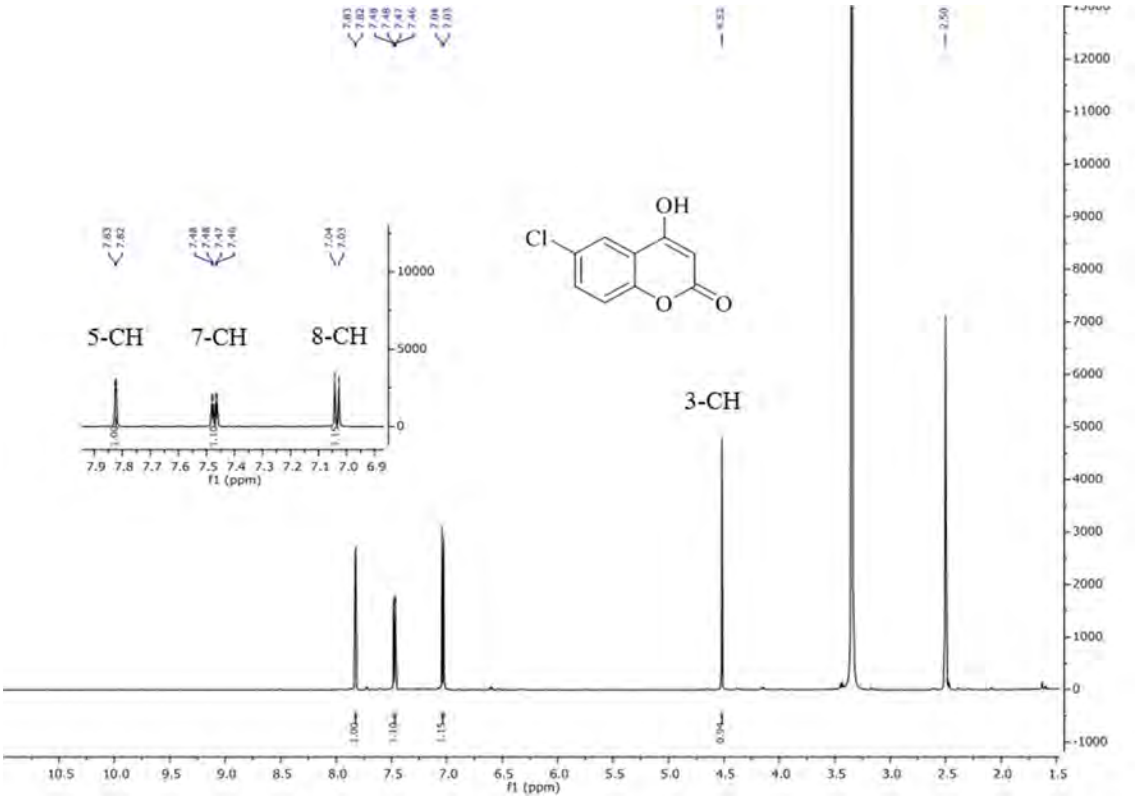


Figure 27. 600 MHz ^{1}H NMR spectrum of compound **130c** in DMSO-d_6.

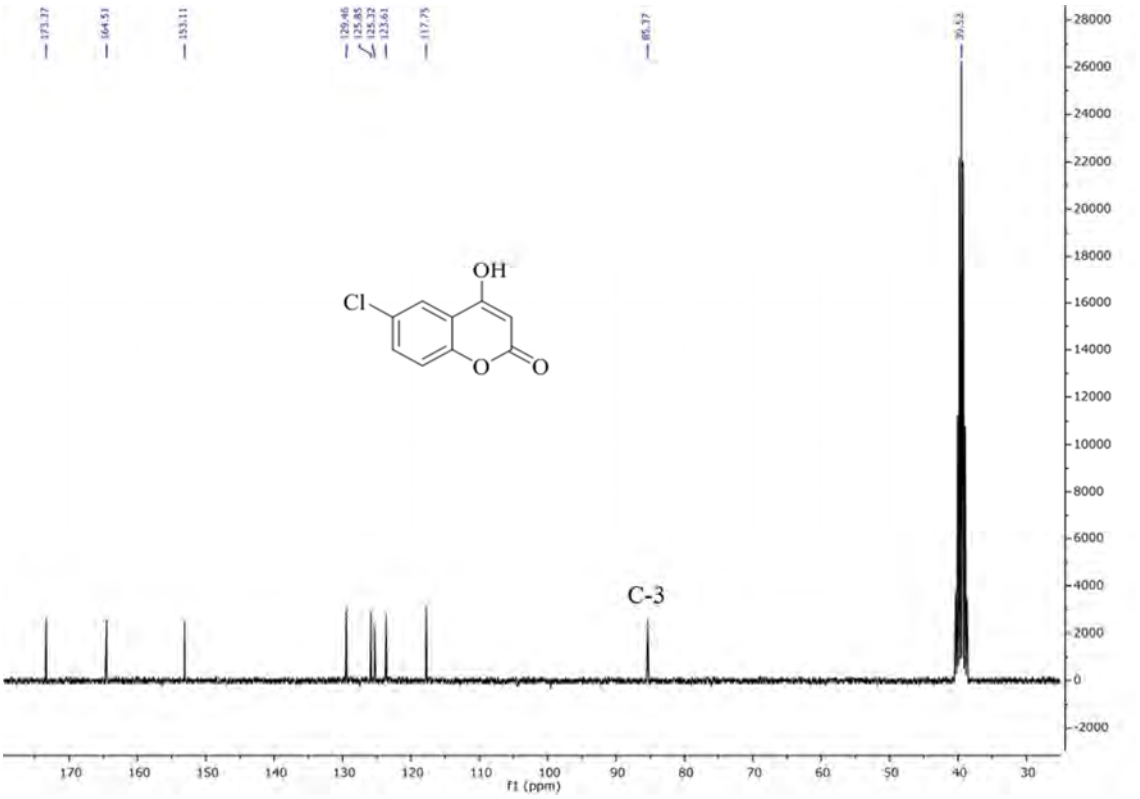
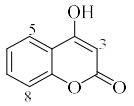


Figure 28. 150 MHz ^{13}C NMR spectrum of compound **130c** in DMSO-d_6.

The 3-H signal appears at 4.52 ppm (non-aromatic) and the corresponding C-3 signal at a high field position (85.4 ppm). This is consistent with NMR spectroscopy studies that have been conducted on 4-hydroxycoumarin.[299] The results shown in **Table 6** from the work conducted on 4-hydroxycoumarin by Traven *et al*.,[299,300] show the ^{1}H and ^{13}C NMR chemical shifts and ^{1}H signal multiplicities. The observed pattern seems to be repeated in substituted 4-hydroxy coumarins and derivatives of 4-hydroxycoumarin isolated in the current study. Tautomerism in the 4-hydroxycoumarin moiety has been reported to involve 2,4-chromandione (I), 4-hydroxy-2-chromenone (II) and 2-hydroxy-4-chromenone (III) tautomeric forms (**Figure 29**).[300] A study on derivatives of 4-hydroxycoumarin conducted by Špirtović-Halilović *et al*.,[301] reports that tautomerism between the C=O and the C-OH group is quite pronounced and that fast H-D exchange can render the hydroxyl group invisible in ^{1}H NMR spectra. The keto-enol tautomerism would help account for the generally higher field ^{13}C chemical shift for C-3 in compounds containing the 4-hydroxycoumarin moiety. In the current study, the hydroxyl proton was generally not visible in ^{1}H spectra.

Table 6. ^{1}H and ^{13}C NMR 4-hydroxycoumarin data reported by Traven *et al*.[299,300] (DMSO-d_6 as solvent)



^{1}H NMR			**^{13}C NMR**	
H	δ (ppm)	Multiplicity	C	δ (ppm)
3	5.61	s	2	161.8
			3	90.9
5	7.84	d	4	165.5
			5	123.9
6	7.66	t	6	123.1
			7	132.6
7	7.36	t	8	116.3
			9	153.4
8	7.39	d	10	116.7

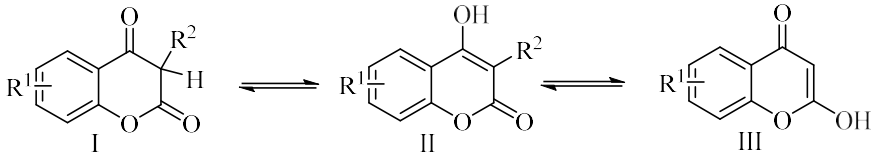


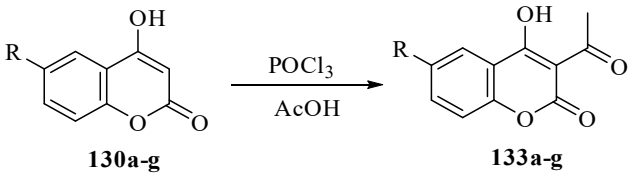
Figure 29. 4-hydroxycoumarin tautomeric structures

2.1.1.2. Synthesis of 3-acetylated 4-hydroxycoumarins

The 3-acetyl-4-hydroxycoumarins **133a-g** were synthesised using a modified form of the method described by Sukdolak *et al*.[302] It was observed that yields were better with longer reaction times (at least 1 hour) than the reported, 30 minutes and, moreover the purity of the compounds was satisfactory after washing the precipitate with methanol thereby replacing the crystallisation step. Thus, the 4-hydroxycoumarins **130a-g** were each reacted with $POCl_3$ in glacial acetic acid under reflux for at least 1 hour, after which the resulting precipitates were filtered and washed with methanol and dried to give the respective products in yields ranging from 41 % to 90 % (**Table 7**). The ^{1}H NMR spectrum of compound **133f** reveals the two expected methyl singlet signals, one for the acetyl methyl at 2.59 ppm and the other for the 6-methoxy group at 3.69 ppm; the expected aromatic proton signals appear between 6.5 and 7.5 ppm (**Figure 30**). The ^{13}C NMR spectrum of compound **133f** shows the acetyl methyl carbon signal at 30.3 ppm and the methoxy carbon signal at 56.0 ppm. The remaining 8 aromatic and

2 carbonyl carbon signals are revealed as expected (**Figure 31**). The NMR spectra of the compounds clearly reflect their purity.

Table 7. Yields of 3-acetylated 4-Hydroxycoumarins **133a-g**



130a-g **133a-g**

Compound	R	Isolated Yields (%)
133a	H	90
133b	F	53
133c	Cl	42
133d	Br	43
133e	Me	52
133f	MeO	62
133g	5,6-Benzo	41

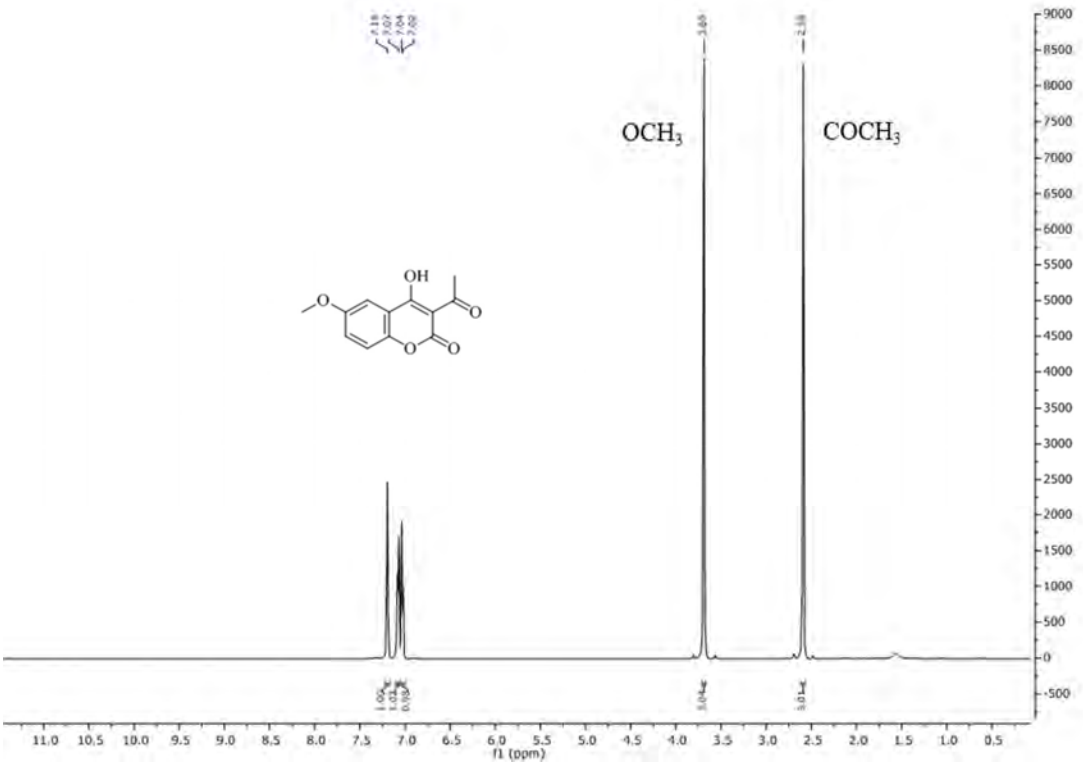


Figure 30. 600 MHz ^{1}H NMR spectrum of compound **133f** in $CDCl_3$.

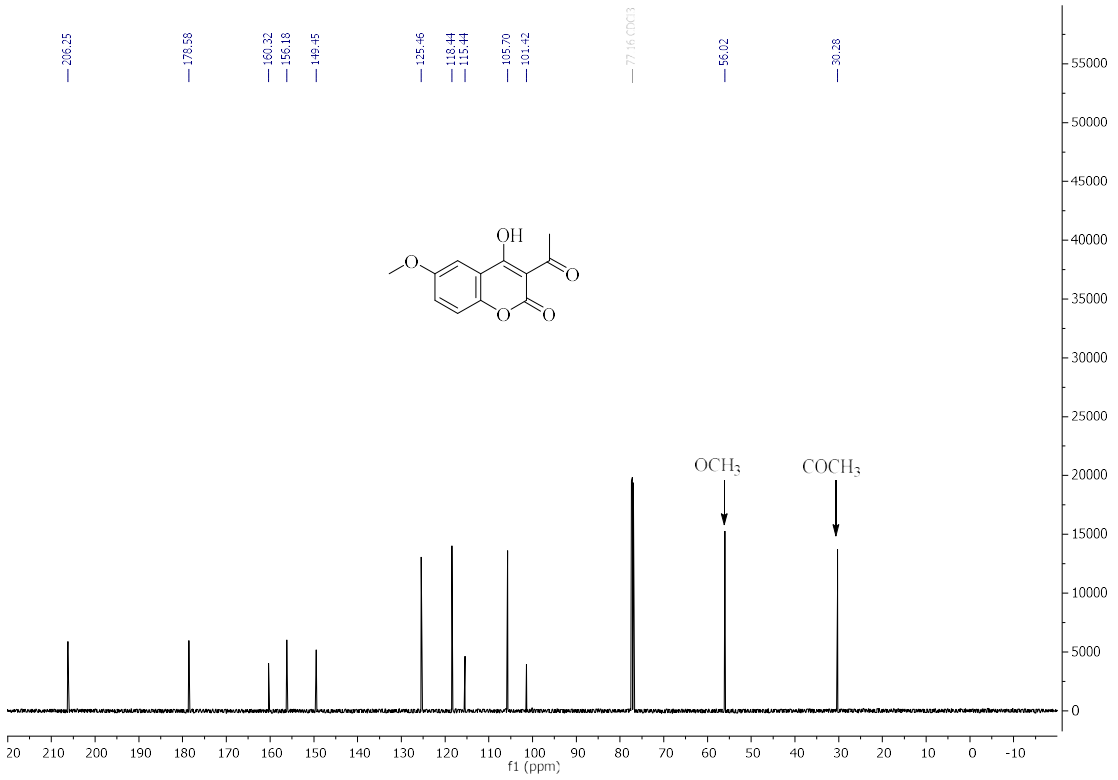
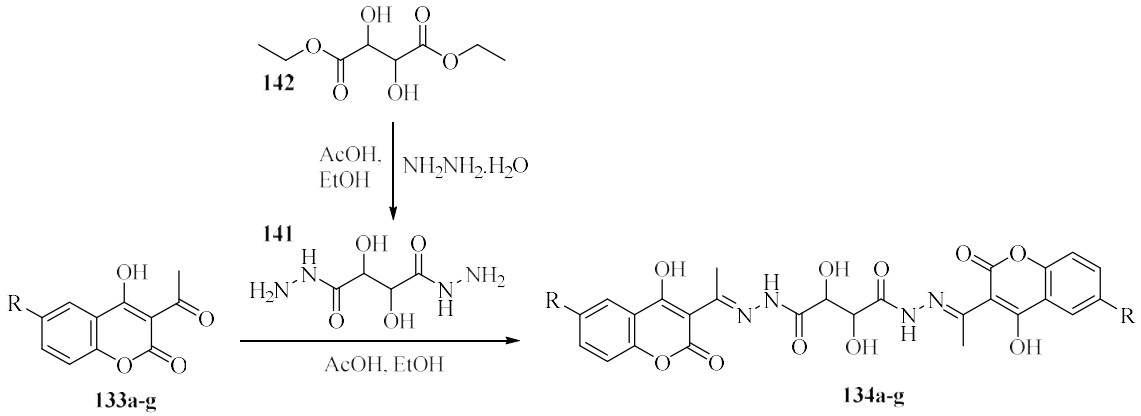


Figure 31. 150 MHz ^{13}C NMR spectrum of compound **133f** in $CDCl_3$.

2.1.1.3. Reaction of 3-acetylated 4-hydroxycoumarins with 2,3-dihydroxysuccino-dihydrazide

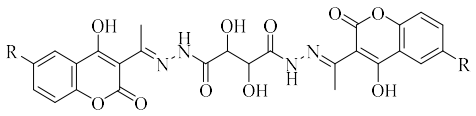
The 2,3-dihydroxysuccino-dihydrazide **141** required for the reactions was synthesised using an acid (HCl or AcOH)-catalysed acyl substitution reaction between racemic diethyl tartrate **142** and hydrazine hydrate under reflux in ethanol.[303] The resulting precipitate was recrystallised from methanol to give the desired dihydrazide as a white solid, which was then reacted with the 3-acetylated 4-hydroxycoumarins **133a-g** in ethanol under reflux for at least 2 hours (**Scheme 21**).[304] This nucleophilic substituition reaction was also acid-catalysed. The precipitate formed in each reaction was filtered, washed with methanol and dried to give the required dihydrazones **134a-g** in yields of 41% to 58% (**Table 8**). The dihydrazones' purity, as revealed by NMR analysis, was satisfactory. The representative ^1H NMR spectrum of compound **134c** reveals the expected methyl proton singlet signal at 2.66 ppm, the methine proton signal as a singlet at 4.62 ppm and the expected aromatic proton signals between 7.0 and 8.0 ppm (**Figure 32**). Further evidence for the dimerization is given by the presence of the CHOH signal at 6.40 ppm, the NH signal at 11.33 pm and finally the 4-OH signal at

15.01 ppm. The ^{13}C NMR spectrum of compound **134c** shows the methyl carbon signal at 18.3 ppm and the methine carbon signal at 73.4 ppm; the rest of the expected 11 carbon signals are also clearly evident (**Figure 33**). The presence of 13 signals for the 26 carbon atoms clearly confirms the formation of the symmetrical dimer. The compounds are novel and NMR (1D and 2D), HRMS and IR techniques were used to characterise them fully.



Scheme 21. Synthesis of bis-(4-hydroxycoumarinyl succinohydrazides)

Table 8. Yields of bis-(4-hydroxycoumarin succinohydrazides) **134a-g**



Compound	R	Isolated Yields (%)
134a	H	58
134b	F	58
134c	Cl	45
134d	Br	42
134e	Me	41
134f	MeO	43
134g	5,6-Benzo	45

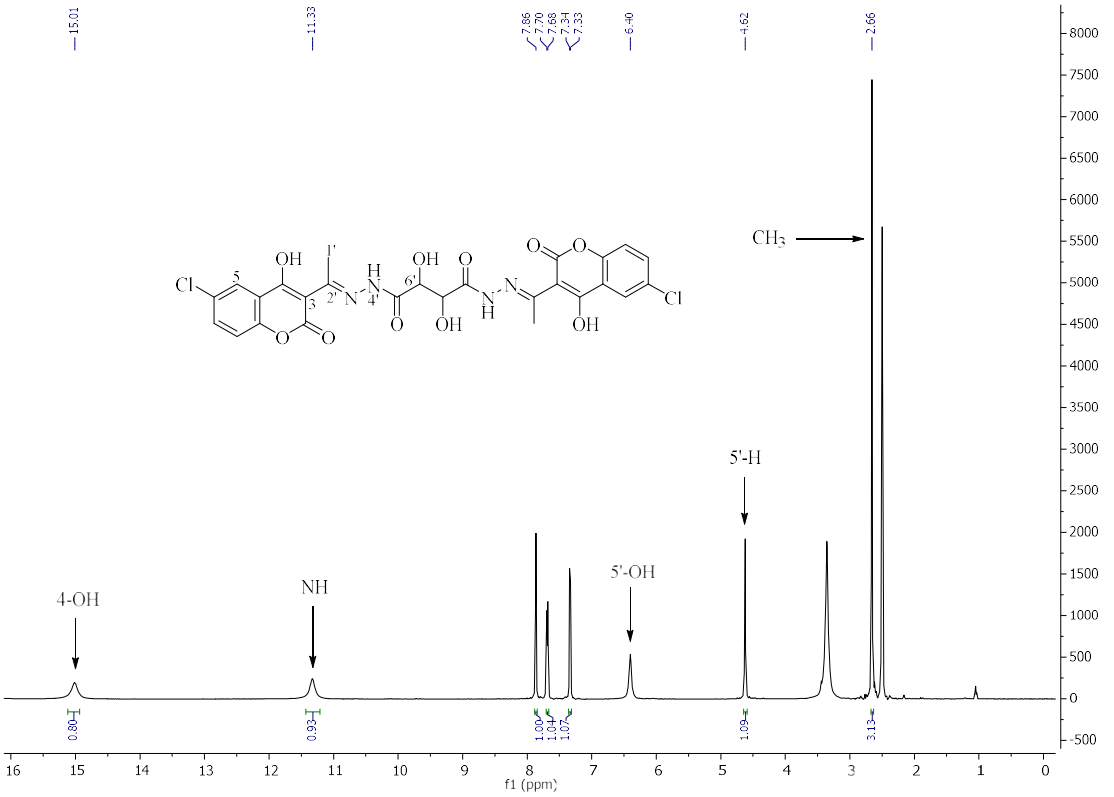


Figure 32. 600 MHz ^{1}H NMR spectrum of compound **134c** in DMSO-d_6.

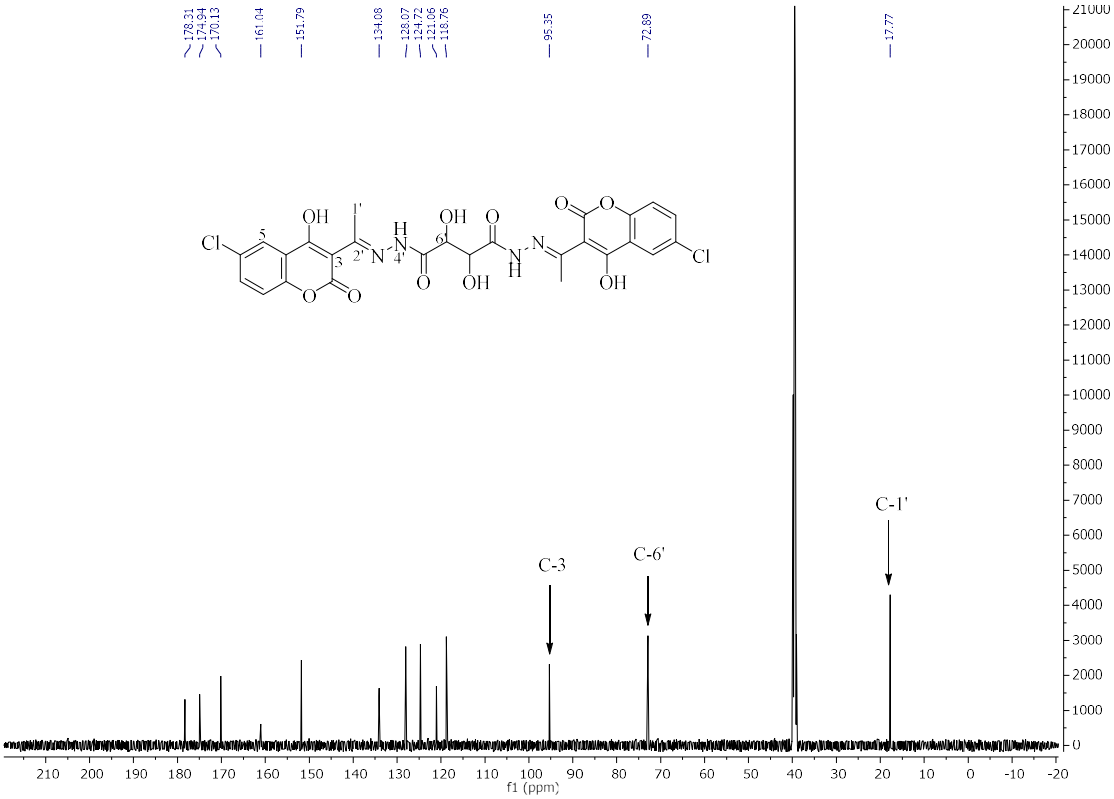


Figure 33. 150 MHz ^{13}C NMR spectrum of compound **134c** DMSO-d_6.

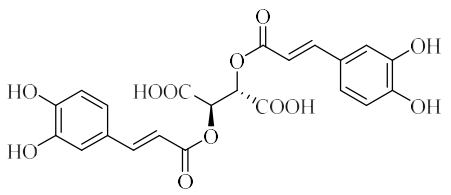
2.1.1.4. Biological studies of the bis-4-hydroxycoumarin succinohydrazides

2.1.1.4.1. *In vitro* studies

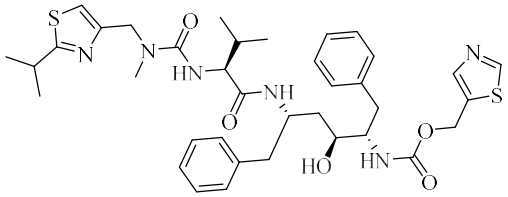
Synthetic compounds **134a-g** were evaluated for HIV-1 IN and PR inhibition and anti-plasmodium falciparum (pLDH), anti-trypanosome (*T.b. brucei*), anti-TB and cytotoxicity activity. Results of the bioassays are shown in **Table 9**.

HIV-1 IN and PR inhibition assays

The compounds were tested for inhibitory potential against the critical HIV-1 IN and PR enzymes, at a concentration of 20 μM concentrations. Chicoric acid **143** and ritonavir **144** were used as the control standards for HIV-1 IR and PR, respectively. The compounds showed some activity against HIV-1 IN but essentially none against HIV-1 PR (**Table 9**). The unsubstituted 4-hydroxcoumarin derivative showed the highest activity against HIV-1 IN (59 % inhibition at 20 μM) with an IC_{50} value of 3.5 μM. It is interesting that moving from the unsubstituted compound **134a** through the halogenated derivatives, (R = F, Cl, Br), the activity decreases until none is observable with the bromo substituted derivative **134d**. The activity seems to follow either the size of the halogen substituents in an inverse relationship, or the electronegativity of the substituents in a direct relationship. From the methyl-substituted derivative **134e** to the 5,6-benzo derivative **134g** the activity seems to increase with the electron delocalisation potential of the methoxy and 5,6-benzo substituents. However, it would appear that having no substituent at the 6-position favours inhibition. The 4-hydroxycoumarin derivatives reported in the literature as showing significant activity against HIV-1 IN have generally been unsubstituted, regardless of the number of 4-hydroxycoumarin moieties present in the molecule (**32**,[150], **43**,[171], **45** and **46**,[173], **47**[174]). Activity against HIV-1 PR was only exhibited by compound **134e** and then at a very low level of 8.3 %.



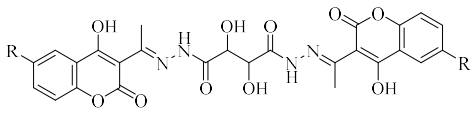
143



144

Figure 34. Chicoric acid **143** and ritonavir **144**

Table 9. Bioassay data for compounds **134a-g**, showing % inhibition of IN and PR, and % viability of pLDH, *T.b. brucei* and HeLa cells.



Compound	R	% HIV-1 IN inhibition [a]	% HIV-1 PR inhibition [b]	PLDH % parasite viability [c]	*T.b. brucei* % viability [d]	Cytotoxicity % HeLa cell viability [e]
134a	H	59.4 (3.5 μM) [f]	0.0	90.7	42.7	78.4
134b	F	35.4	0.0	92.8	32.8	84.6
134c	Cl	27.6	0.0	93.8	44.6	100.0
134d	Br	0.0	0.0	100.0	100.0	100.0
134e	Me	26.4	8.3	100.0	100.0	85.3
134f	MeO	34.6	0.0	100.0	100.0	84.6
134g	5,6-Benzo	35.7	0.0	100.0	100.0	98.1
Control		100.0 [a]	99.8 [b]	IC_{50} = 0.012 μM [c]	IC_{50} = 0.039 μM [d]	IC_{50} = 0.013 μM [e]

Controls: [a] chicoric acid; [b] ritonavir; [c] chloroquine; [d] pentamidine and [e] emetine.
[f] IC_{50} value

Anti-malarial studies

The compounds were evaluated for anti-malarial activities at a concentration of 20 μM against the parasite lactate dehydrogenase (pLDH) enzyme in cultures. An anti-malarial drug, chloroquine, was employed as a drug standard. The compounds showed no significant activity in the pLDH screen as shown by the results in **Table 9**. The highest reduction of parasite viability was about 9 % (**134a**) then 7 % (**134b**) and finally 6 % (**134c**). For the remaining compounds **134d-g**, there was no reduction in pLDH viability.

Anti-trypasonome studies

The compounds were tested at 20 μM against *Trypanosoma brucei brucei* (*T.b. brucei*) to assess anti-trypanocidal activity. Pentamidine, a drug used in the treatment for trypanosomiasis was used as a reference standard. Compounds **134a-c** showed some activity against *T.b. brucei* as shown in **Table 9** with parasite viabilities at 43, 33 and 45% respectively. The remaining compounds showed no reduction in the viability of the parasite.

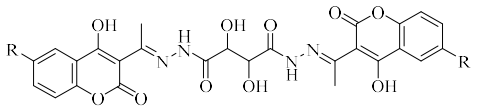
Cytotoxicity studies

The compounds **134a-g** were tested at a concentration of 20 μM against HeLa cells and none of the compounds exhibited significant cytotoxicity, *i.e.* none of them reduced the viability of the HeLa cells to less than 50 % (**Table 9**). Emetine was used as the reference standard.

Anti-tuberculosis studies

Mycobacterial (TB) assay for the 7 compounds gave the results shown in **Table 10** for the visual and calculated MIC90 7D 7H9 GLU CUS Tx assay. Rifampicin was used as the reference standard. Three of the compounds **134c**, **134d** and **134f** showed promising anti-mycobacterial potential with visual MIC_{90} values of 31.25, 15.63 and 31.25 μM and calculated MIC_{90} values of 54.25, 18.91 and 29.46 μM.

Table 10. Anti-mycobacteria assay results



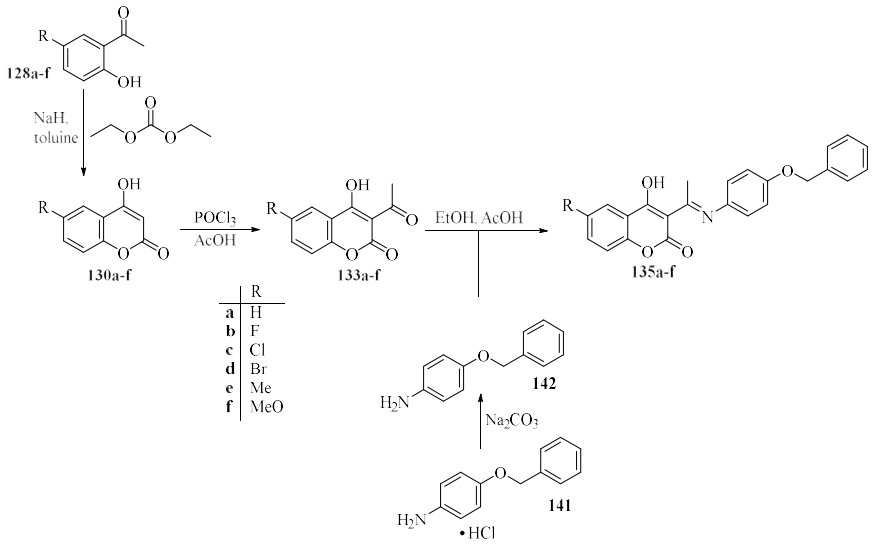
COMPOUND	R	VISUAL MIC90 7D 7H9 GLU CAS Tx (μM)	CALCULATED MIC90 7D 7H9 GLU CAS Tx (μM)
134a	H	>125	>125
134b	F	>125	>125
134c	Cl	31.25	54.25
134d	Br	15.63	18.91
134e	Me	>125	>125
134f	MeO	>125	>125
134g	5,6-Benzo	31.25	29.46
Rifampicin		0.019	0.007

2.1.2. 3-[(*N*-4-Benzyloxyphenyl)-iminoethyl]-4-hydroxycoumarins

2.1.2.1. Synthesis of 3-[(N-4-benzyloxyphenyl)iminoethyl]-4-hydroxycoumarins 135a-f

The desired compounds **135a-f** were accessed from the reactions of compounds **134a-f** with 4-benzyloxyaniline **142** (**Scheme 22**), which was specifically prepared by the neutralisation of 4-benzyloxyaniline hydrochloride **141**. The products **135a-f** are imines. Hugo Schiff in 1864 pioneered the synthesis of such primary amine-carbonyl condensation products - hence

the name "Schiff" bases for these compounds which are also known as azomethines.[323] Imines have drawn considerable interest due to their diverse physical, chemical and biological properties. Among the wide variety of biological activities that imines possess are anti-cancer, anti-inflammatory, anti-viral, anti-fungal, anti-bacterial, anti-lipoxygenase, analgesic, anti-toxic, anti-urease, anti-glycation and anti-β-secretase (useful in the treatment of Alzheimer's disease).[323,324,325] The Schiff base C=N bond has been found to be critical for the exhibited biological activities and the imines' ability to form ligands has also been exploited in designing medicinally important complexes.[324,326] A number of methods to access imines have been developed which include: i) the reaction between primary amines and aldehydes or ketones accompanied with the elimination of water (the traditional method) - normally acid-catalysed; ii) oxidative generation from alcohols and amines; iii) reaction of aromatic ketones with metal amine salts; and iv) the reaction of nitriles with phenolic ethers or phenols.[327]

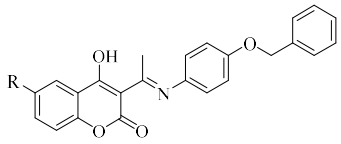


Scheme 22. Pathway to 3-[(*N*-4-benzyloxyphenyl)iminoethyl]-4-hydroxycoumarins

Each of 3-acetyl-4-hydroxycoumarins **133a-f** was reacted with compound **142** in an acid-catalysed reaction in methanol under reflux for at least an hour. The resulting precipitates were filtered, washed with methanol and dried to give the targeted products **135a-f** in yields 82% to 94% without the need for further purification as confirmed by NMR analysis. The ^{1}H NMR spectrum of **135d** reveals the expected methyl proton singlet at 2.68 ppm, the methylene protons resonating as a singlet at 5.13 ppm and the expected aromatic proton

signals between 7.0 and 8.5 ppm, integrating for the required number of 12 protons (**Figure 39**). The hydroxyl group signal appears at 15.58 ppm. The DEPT 135 NMR spectrum (**Figure 40**) shows the *O*-methylene carbon resonating at 70.5 ppm, the methyl carbon at 20.9 ppm and eight expected aromatic methane carbon signals between 115 and 137 ppm. Data from the COSY, HSQC and HMBC was used to confirm the assignments. The COSY (**Figure 41**) and HSQC (**Figure 42**) spectra are shown. The compounds in this series are all novel and besides the NMR analyses, IR and HRMS techniques were also used to characterise them fully.

Table 13. Yields of 3-[(*N*-4-benzyloxyphenyl)-iminoethyl]-4-hydroxycoumarins **135a-f**



Compound	R	Isolated Yields (%)
135a	H	93
135b	F	94
135c	Cl	91
135d	Br	86
135e	Me	95
135f	MeO	82

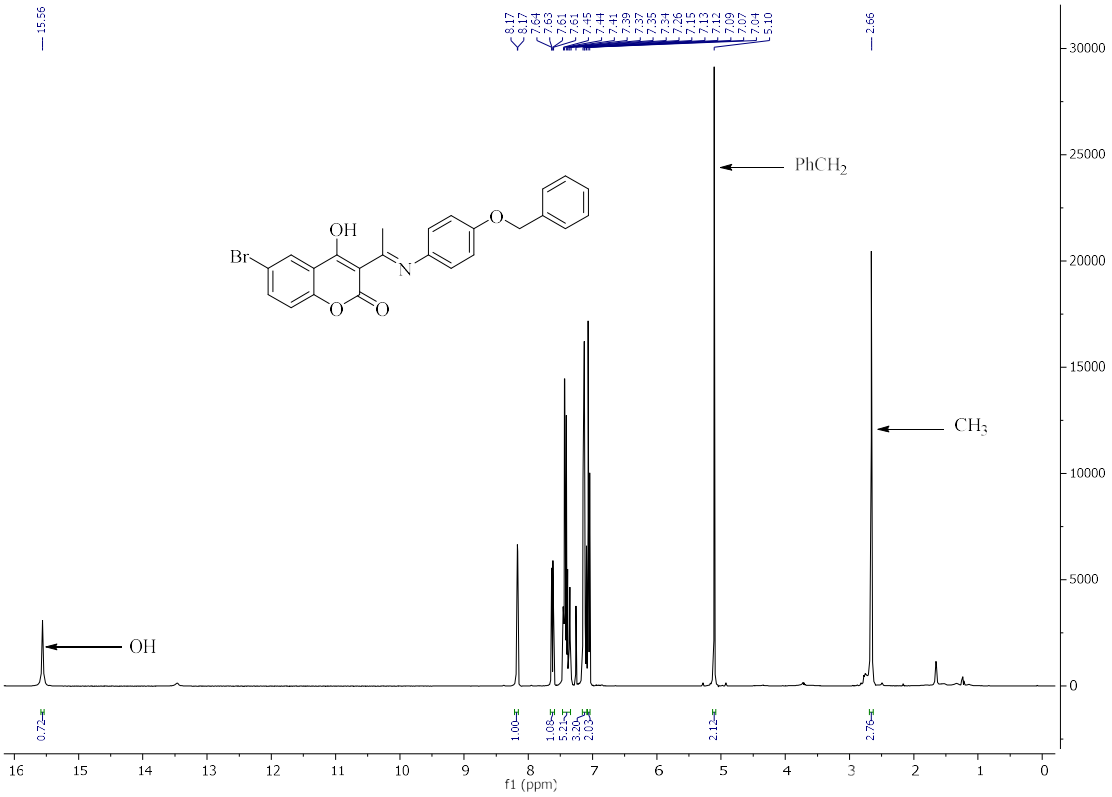


Figure 39. 400 MHz ^{1}H NMR spectrum of compound **135d** in $CDCl_3$.

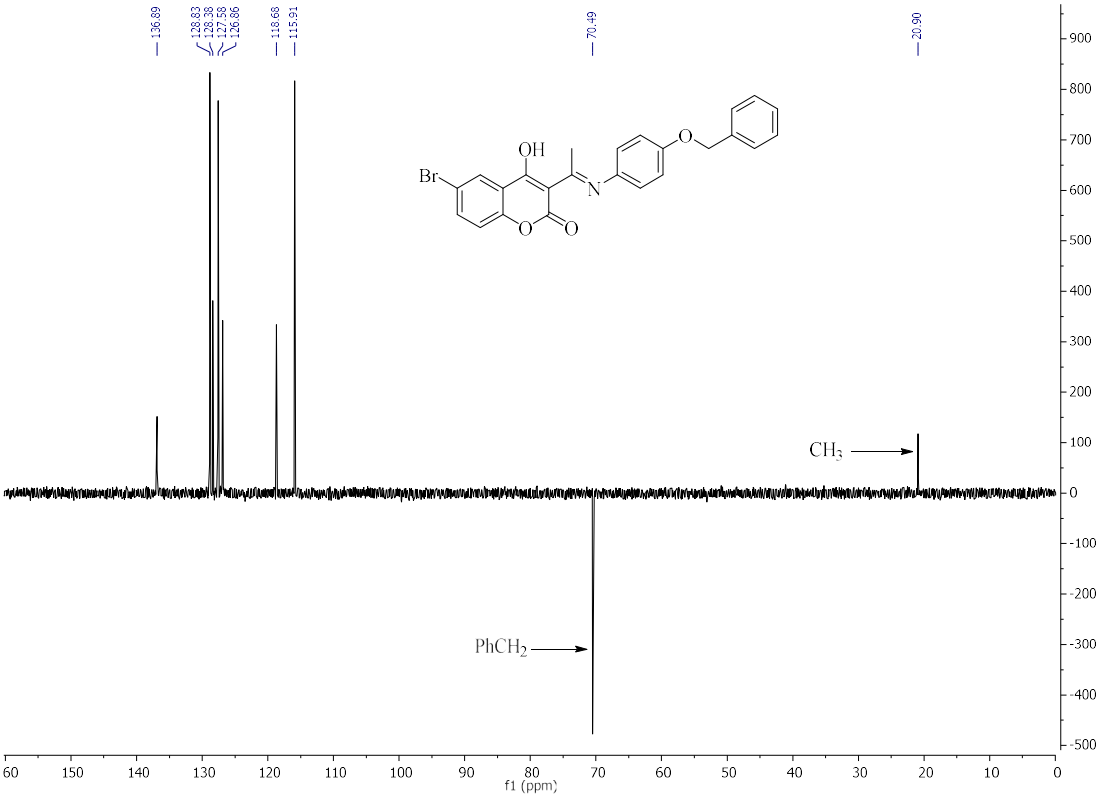


Figure 40. DEPT 135 NMR spectrum of compound **135d** in $CDCl_3$.

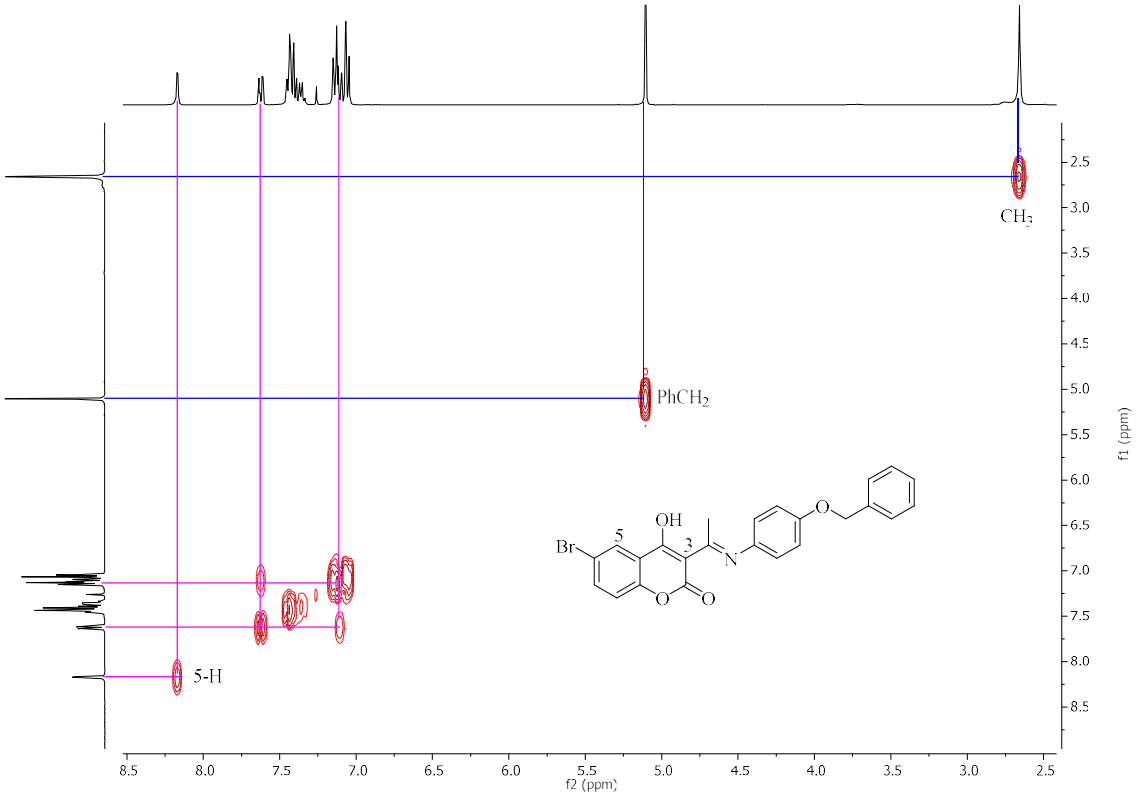


Figure 41. 400 MHz COSY spectrum of compound **135d** in $CDCl_3$.

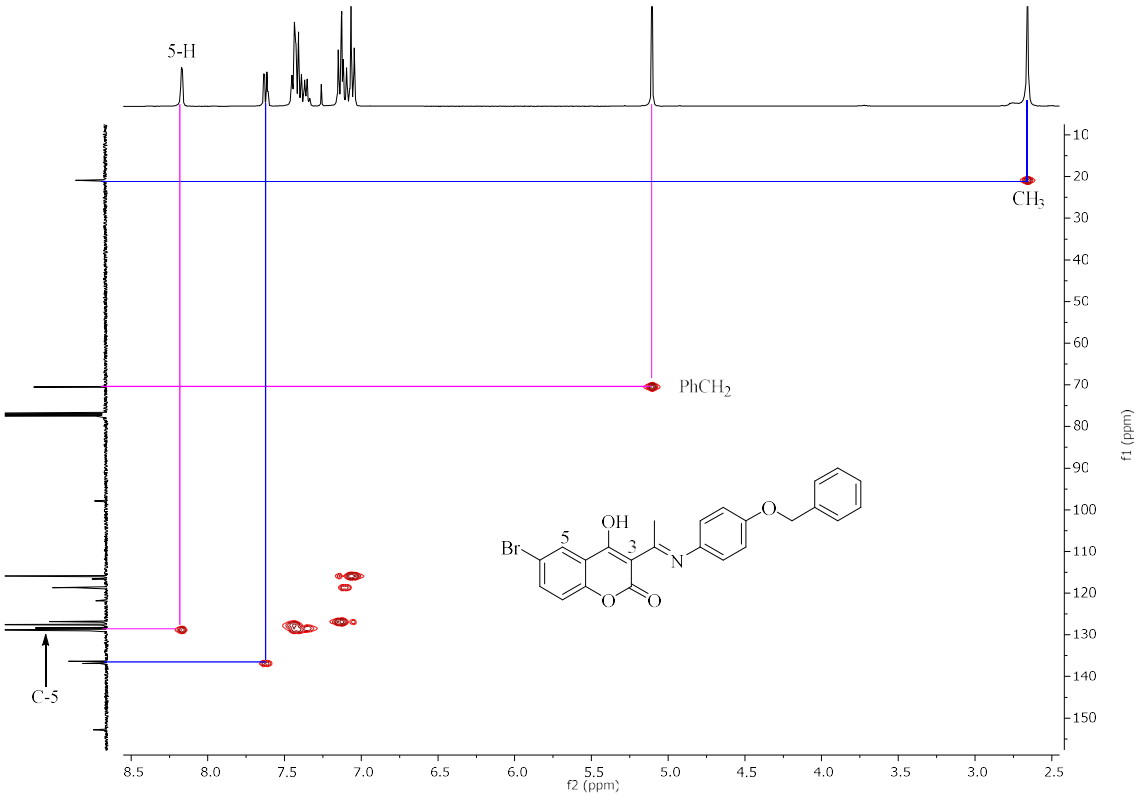


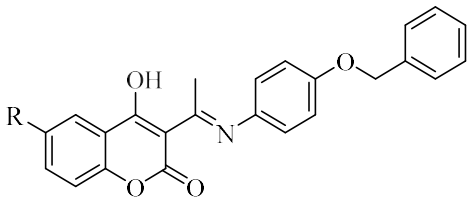
Figure 42. HSQC spectrum of compound **135d** in $CDCl_3$.

2.1.2.2 Biological studies of 3-[(*N*-4-benzyloxyphenyl)iminoethyl]-4-hydroxycoumarins

HIV-1 IN and PR, PLDH, Trypanocidal and Cytotoxicity Assays

The compounds were tested against HIV-1 IN and PR for their inhibitory potential at 20 μM concentrations, but failed to show any significant activity against these enzymes. The pLDH screen revealed that the compounds were not active against the malarial parasite. The trypanocidal screen, however, revealed that four of the six compounds were active against the *T.b. brucei* at 20 μM. Compounds **135a-d** reduced parasite viability to below 34% with the most active compound **135d** effecting a 23.0% decrease in viability (**Table 14**) and an IC_{50} value of 27.88 μM. The standard, pentamidine exhibited an IC_{50} = 0.00627 μM. The cytotoxicity test showed that the compounds were not cytotoxic under the test conditions. The potential showed by the compounds against *T.b. brucei* is quite promising, and further studies on these could result in more potent compounds.

Table 14. Bioassay data for compounds **135a-g**, showing % inhibition of IN and PR, and % viability of pLDH, *T.b. brucei* and HeLa cells.



Compound	R	HIV-1 % Integrase inhibition [a]	HIV-1% Protease inhibition [b]	PLDH % parasite viability [c]	*T.b. brucei* % parasite viability [d]	Cytotoxicity % HeLa cells viability [e]
135a	H	0.0	5.7	93.0	33.5	82.6
135b	F	11.4	0.0	100.0	37.9	77.7
135c	Cl	0.0	0.0	94.2	31.3	96.3
135d	Br	0.0	0.0	100.0	23.3	100.0
135e	Me	0.0	0.0	100.0	96.4	100.0
135f	MeO	0.0	0.0	71.1	89.1	100.0
Controls		100.0 [a]	99.83 [b]	IC_{50} = 0.010μM [c]	IC_{50} = 0.022 μM [d]	IC_{50} = 0.019 μM [e]

Controls: [a] chicoric acid; [b] ritonavir; [c] chloroquine; [d] pentamidine and [e] emetine

2.1.3. 3-[2-(Benzylidenehydrazinyl)thiazo-2-yl]-4-hydroxycoumarins

The compounds were designed to incorporate, in one molecule, a number of entities with potential biological activity, *viz*., 4-hydroxycoumarin, thiazole and hydrazinyl motifs. Novel compounds incorporating heterocyclic moieties draw continuing research interest as therapeutics since the heterocyclic motifs serve as frameworks upon which a number of additional groups can be built. Moreover, the heterocyclic scaffolds may be found in many compounds of biological and pharmacological importance.[328] The thiazole nucleus is present in a number of molecules that show biological activities, such as anti-viral (*e.g.*, RM-4848 **145)**; anti-HIV (ritonavir contains the thiazole moiety); anti-schizophrenia; anti-thrombotic; anti-barbiturate (e.g. amiphenazole **146**); anti-fungal (an antifungal agent abafungin **147** contains the thiazole nucleus); anti-tumour; anti-cancer and anti-bacterial. [329,328,330,331,332]

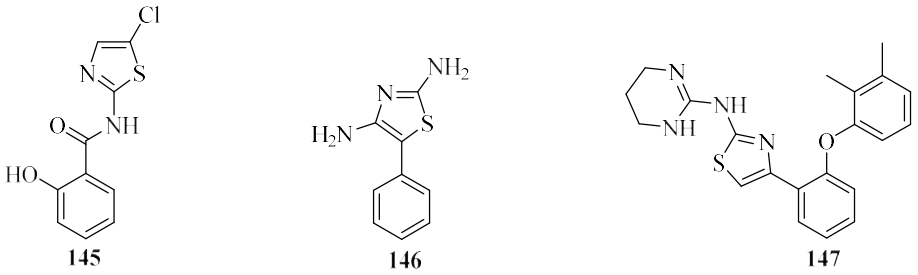
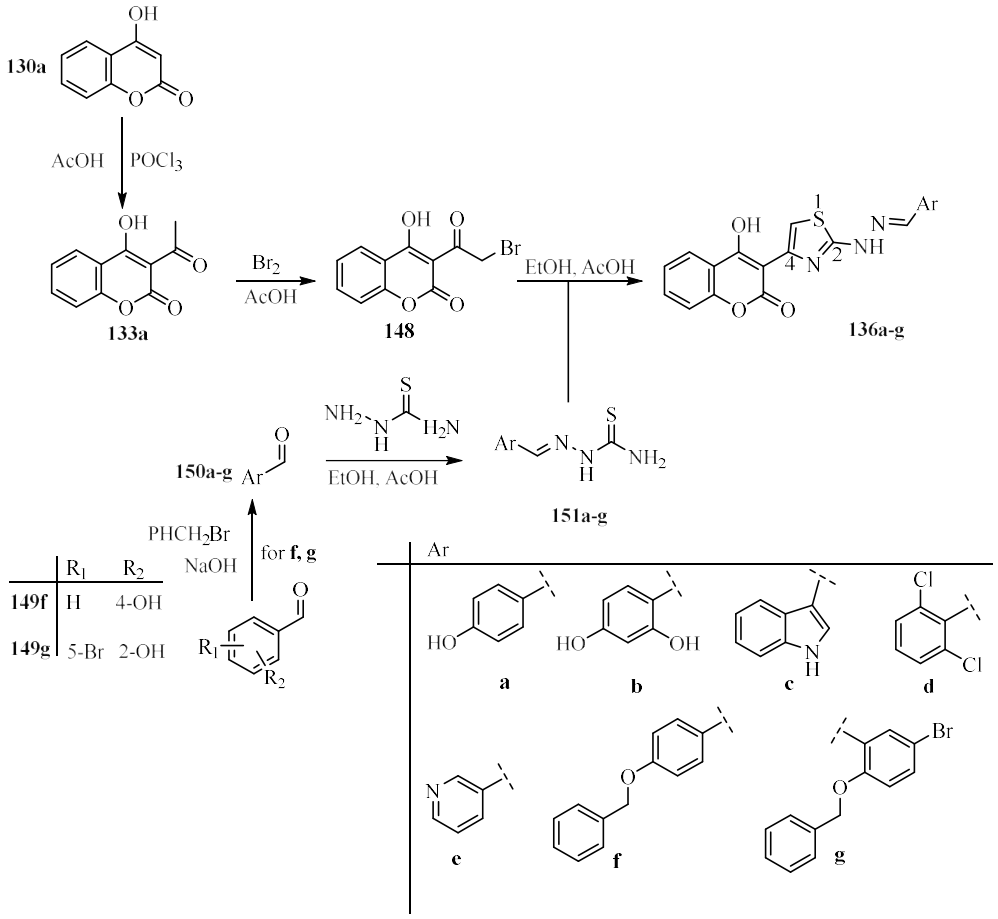


Figure 37. Medicinal agents containing the thiazole moiety[329,332]

2.1.3.1. Synthesis of 3-[2-(benzylidenehydrazinyl)thiazo-2-yl]-4-hydroxycoumarins 136a-g

The targeted molecular hybrids were synthesised by reacting 3-(2-bromoacetyl)-4-hydroxy-2*H*-chromen-2-one **148** with thiosemicarbazone derivatives **151a-g**. The steps to the desired final products are shown in **Scheme 23**.



Scheme 23. Route to 3-[2-(benzylidenehydrazinyl)thiazo-2-yl]-4-hydroxycoumarins **136a-g**

2.1.3.2. Synthesis of 3-(2-bromoacetyl)-4-hydroxycoumarin

2-Acetyl-3-hydroxycoumarin **133a** was heated with bromine at 100° C in acetic acid for an hour in the fume-hood. The precipitate which formed was filtered off and recrystallized from acetic acid to give the product **148** as flaky shiny crystals in a yield of 70 %. The ^{1}H NMR spectrum (**Figure 43**) reveals the expected methylene proton singlet at 4.82 ppm and the aromatic proton signals between 7.3 ppm and 8.0 ppm. The methyl singlet for the starting material is absent, confirming formation of the desired intermediate. The DEPT 135 NMR spectrum reveals the methylene carbon signal at 35.5 ppm and the signals for the remaining hydrogen-bearing carbons in the range 117 ppm to 136 ppm (**Figure 44**).

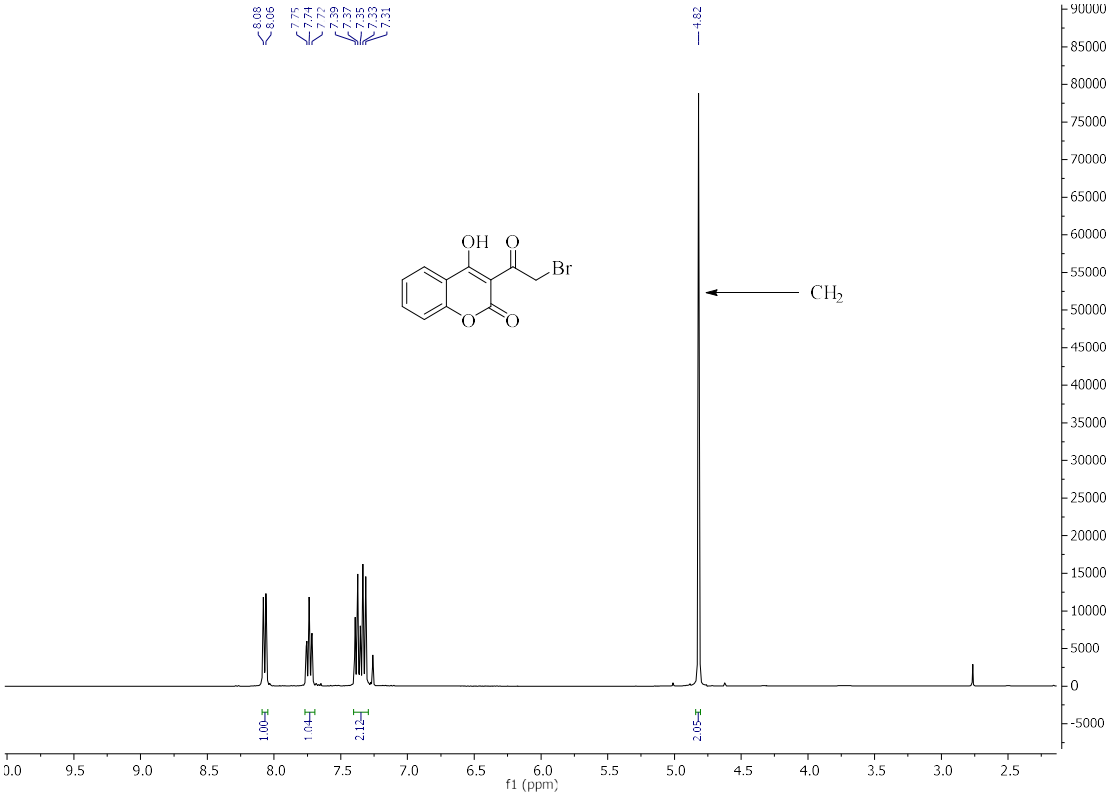


Figure 43. 400 MHz ^{1}H NMR spectrum of compound **149** in $CDCl_3$.

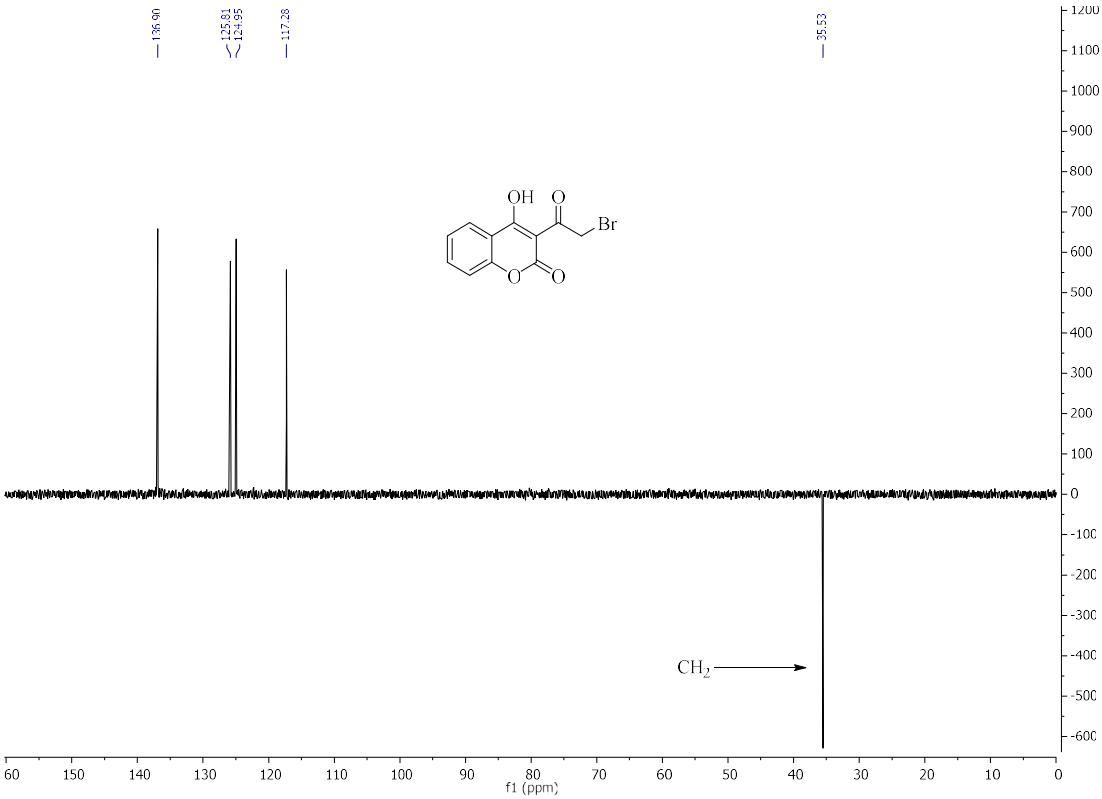


Figure 44. DEPT 135 NMR spectrum of compound **149** in $CDCl_3$.

2.1.3.3. Synthesis of thiosemicarbazone derivatives

Commercial (**150a**, **b**, **c**, **d**, **e**) and non-commercial (**150f** and **150g** synthesised as part of the study) aromatic aldehydes were each refluxed with thiosemicarbazide in ethanol for at least an hour (**Scheme 23**). The precipitate formed in each case was washed with methanol and used without further purification as the products were revealed through NMR analysis to be satisfactorily pure. The thiosemicarbazone derivatives' yields were in the range 65% to 76% (**Table 15**). The ^{1}H NMR spectrum for compounds **151e** reveals the azomethine proton signal as a singlet at 8.06 ppm, the expected four aromatic proton signals in the range 7.35-8.95 ppm and, finally, the NH signal at 11.58 ppm (**Figure 45**). The HSQC spectrum for compound **151e** shows the correlations between the aromatic protons and their respective carbons and also the azomethine proton and its respective carbon (**Figure 46**). The 5-ArH proton resonating at 7.40 ppm correlates with its corresponding carbon at 123.8 ppm, the azomethine proton (N=C$^{1'}$H) resonating at 8.06 ppm with the corresponding N=CH carbon at 139.3 ppm and the 2-pyridyl proton (N=C^{2}H), resonating at 8.91 ppm as a singlet, with the corresponding carbon at 148.8 ppm.

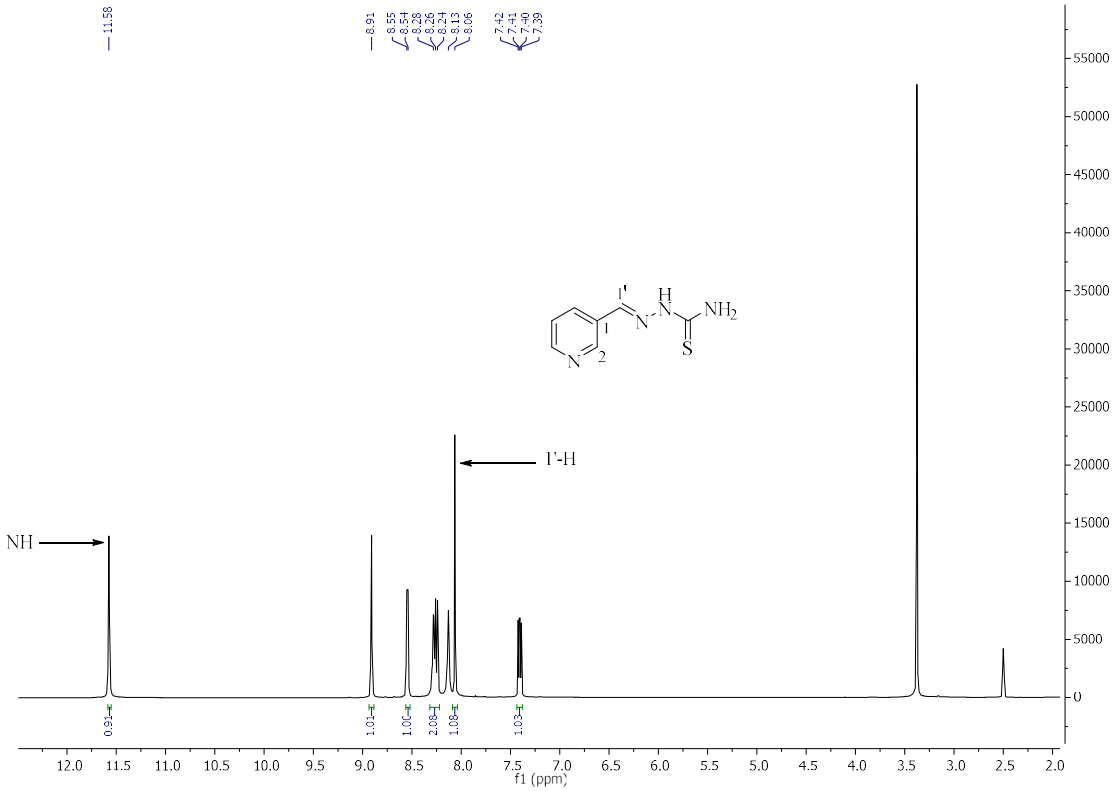


Figure 45. 400 MHz ^{1}H NMR spectrum of compound **151e** in DMSO-d_6.

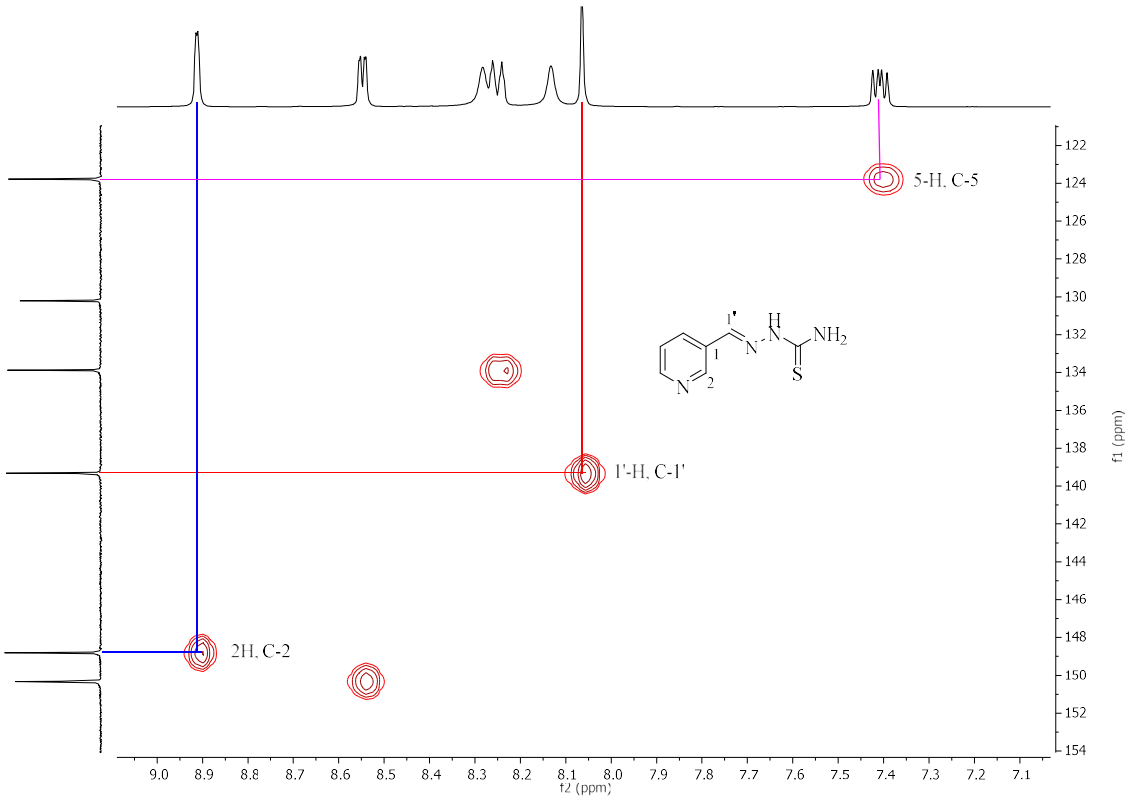
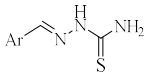
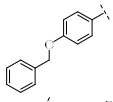
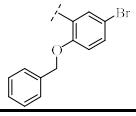


Figure 46. HSQC spectrum of compound **151e** in DMSO-d_6.

Table 15. Yields of thiosemicarbazone derivatives **151a-g**

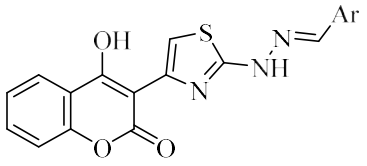


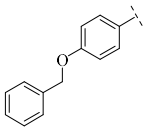
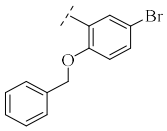
Compound	Ar	Isolated Yields (%)
151a		70
151b		68
151c		74
151d		76
151e		65
151f		69
151g		66

2.1.3.4. Synthesis of 3-[2-(Benzylidinehydrazono)thiazol-4-yl]-4-hydroxycoumarins

The targeted compounds, 2-(*N'*-arylidinehyrazinyl)-4-(4-hydroxycoumarin-3-yl)thiazoles **136a-g**, were synthesised similarly to their precursors **151a-g** as illustrated in **Scheme 23**. Thus, the 3-(2-bromoacetyl)-4-hydroxycoumarin **148** was reacted under reflux with the thiosemicarbazone derivatives. The desired products were isolated in good to very good yields (**Table 16**) without the need for further purification as NMR analysis revealed satisfactory purity. The compounds **136a-g** are all novel and were fully characterised. The ^{1}H NMR spectrum of compound **136d** shows the thiazole ring proton resonating at 7.60 ppm as a singlet, as expected, and the azomethine proton resonating at 8.31 ppm also as a singlet (**Figure 47**). The expected total of eight aromatic proton signals, are also evident and they resonate in the range 7.38 to 8.33 ppm. The NH and OH signals appear at 12.7 and 15.5 ppm, respectively. The ^{13}C NMR spectrum of compound **136d** reveals the signal of the thiazole ring carbon (the one carrying a proton) resonating at 106.4 ppm and the azomethine group carbon signal at 138.3 ppm (**Figure 48**). The spectrum also shows the 17 aromatic carbon signals as expected. The COSY (**Figure 49**), HSQC (**Figure 50**) and HMBC (**Figure 51**) spectra for compound **136d** are shown. The selected section of the COSY spectrum clearly shows that neither the azomethine proton nor the thiazole ring proton has a neighbouring proton. The triplet at 7.66 ppm corresponds to 4'-H and this proton interacts with two protons at 7.55 ppm and clearly has no correlation with the doublet at 7.94 ppm which corresponds to a coumarin moiety proton. For the HSQC spectrum only the portion showing aromatic ^{1}H and ^{13}C correlations is shown. The signal at 7.39 ppm clearly shows that the three protons belong to three different carbon atoms. The HMBC spectrum clearly reveals the expected two carbon atoms that correlate (*via* 2- and 3- bond inreractions) with the azomethine proton (at 129.1 ppm and at 133.7 ppm). The thiazole ring proton correlates with two carbon atoms at 142.8 and 166.9 ppm. The signal at 166.9 ppm is clearly corresponds to the thiazole ring carbon connected to two N atoms and the S atom.

Table 16. Yields of 3-[2-(Benzylidinehydrazono)thiazol-4-yl]-4-hydroxycoumarin **136a-g**



Compound	Ar	Isolated Yields (%)
136a		72
136b		67
136c		74
136d		73
136e		70
136f		66
136g		67

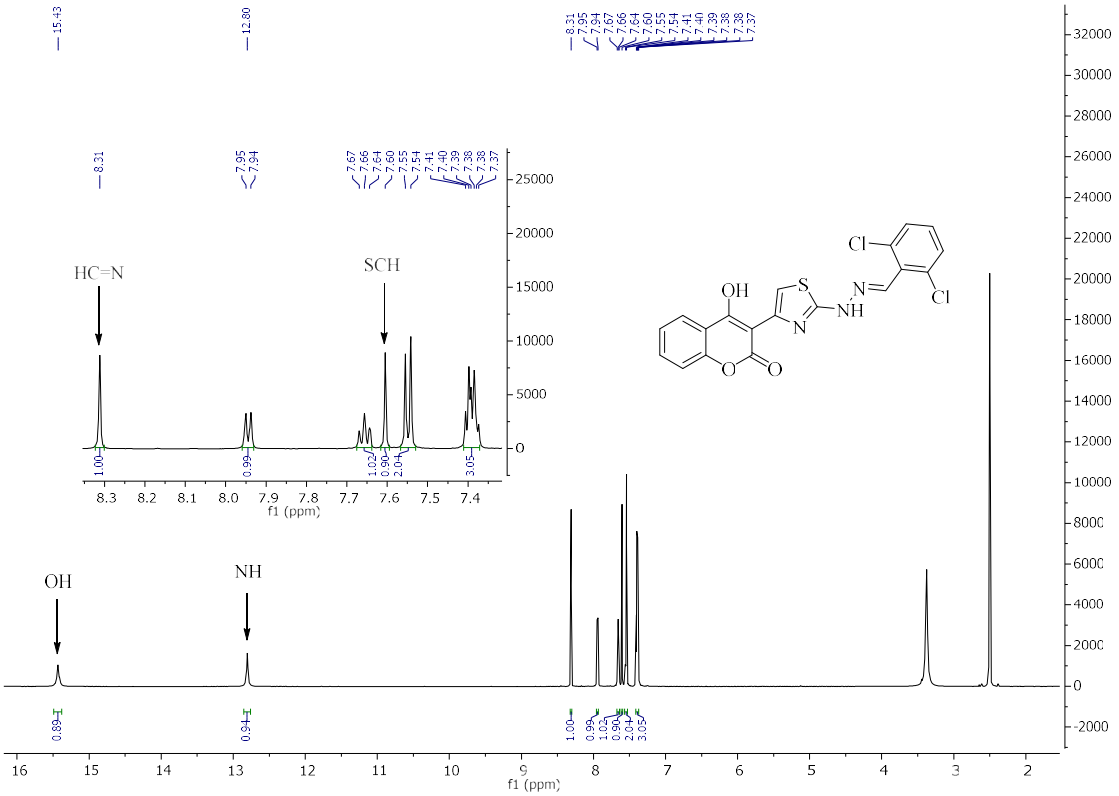


Figure 47. 600 MHz ^{1}H NMR spectrum of compound **136d** in DMSO-d_6.

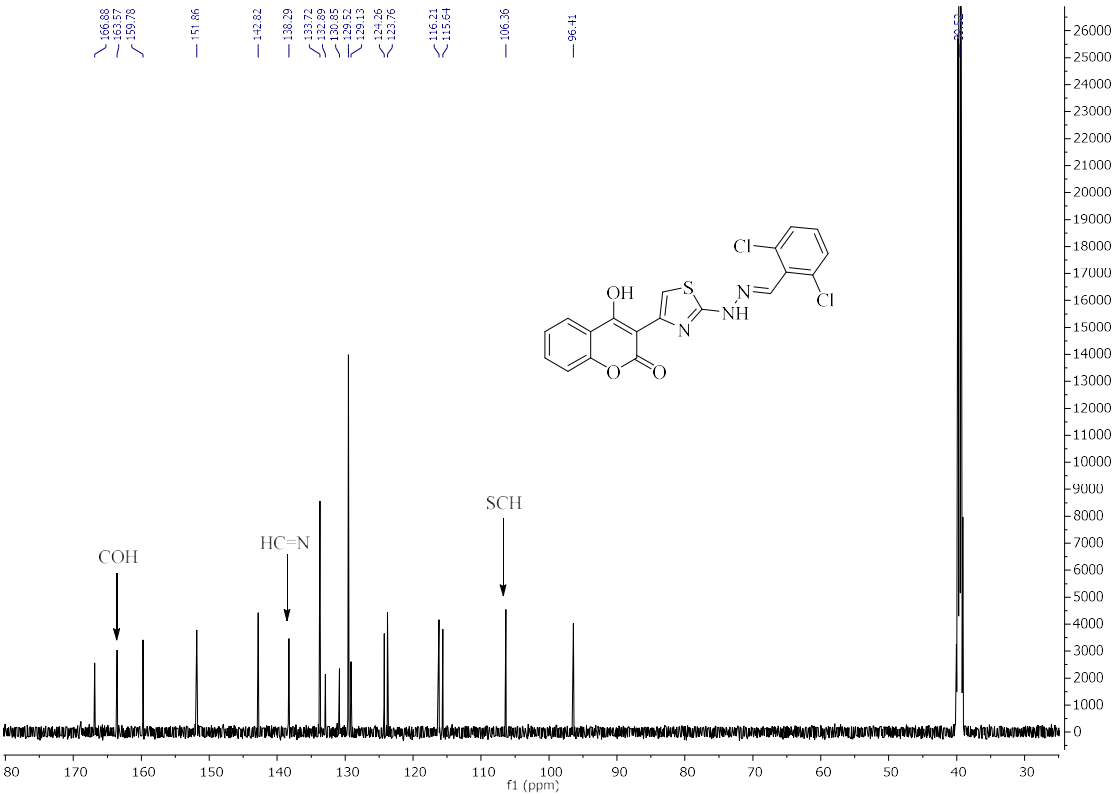


Figure 48. 150 MHz ^{13}C NMR spectrum of compound **136d** in DMSO-d_6.

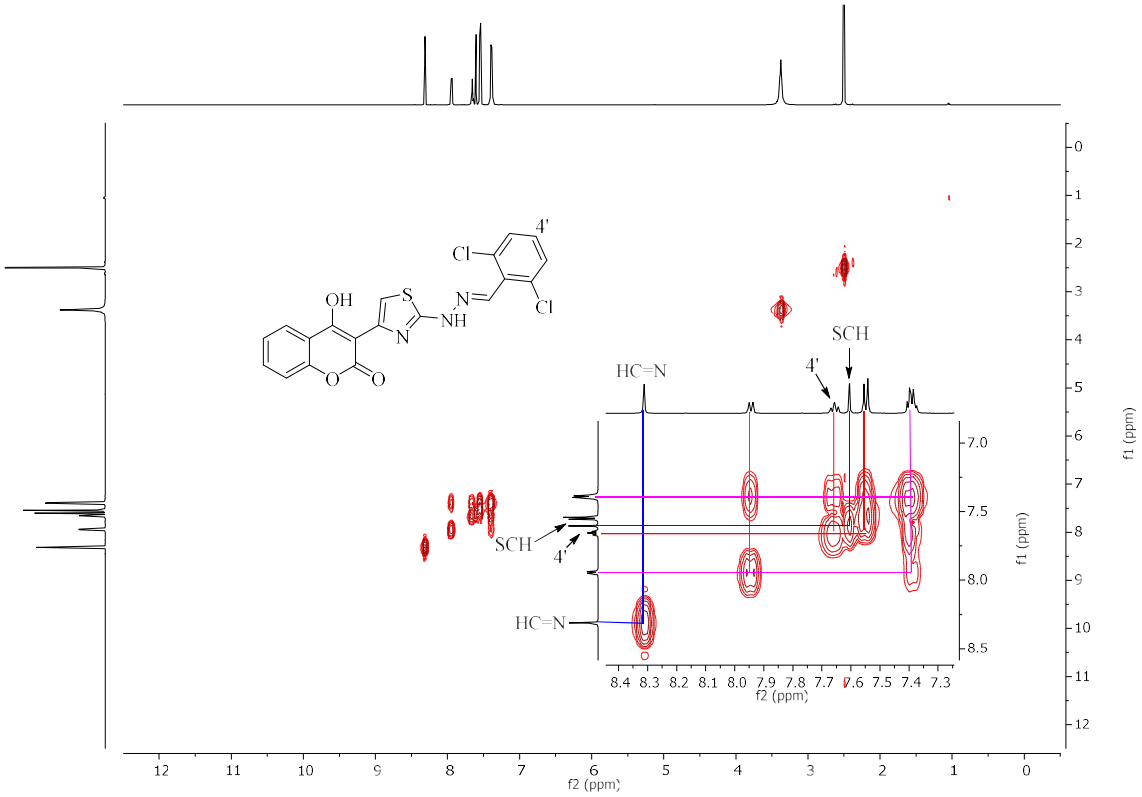


Figure 49. 600 MHz COSY NMR spectrum of compound **136d** in DMSO-d_6.

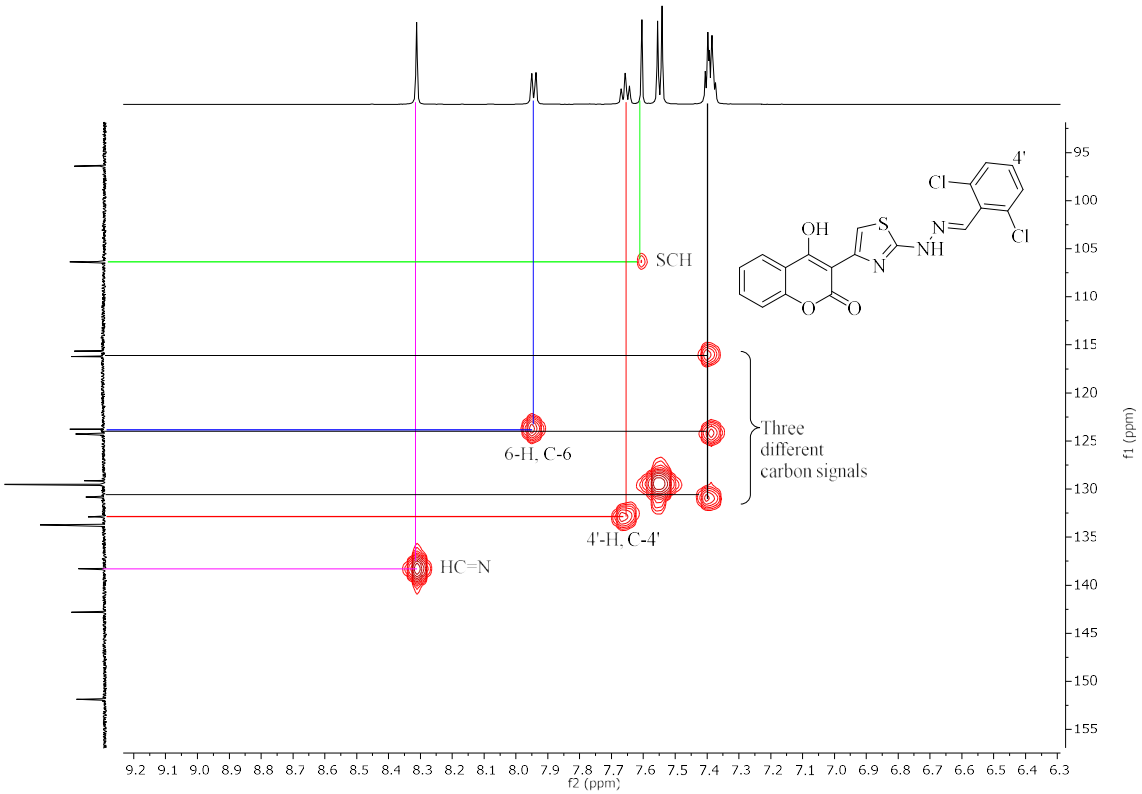


Figure 50. Partial HSQC NMR spectrum of compound **136d** in DMSO-d_6.

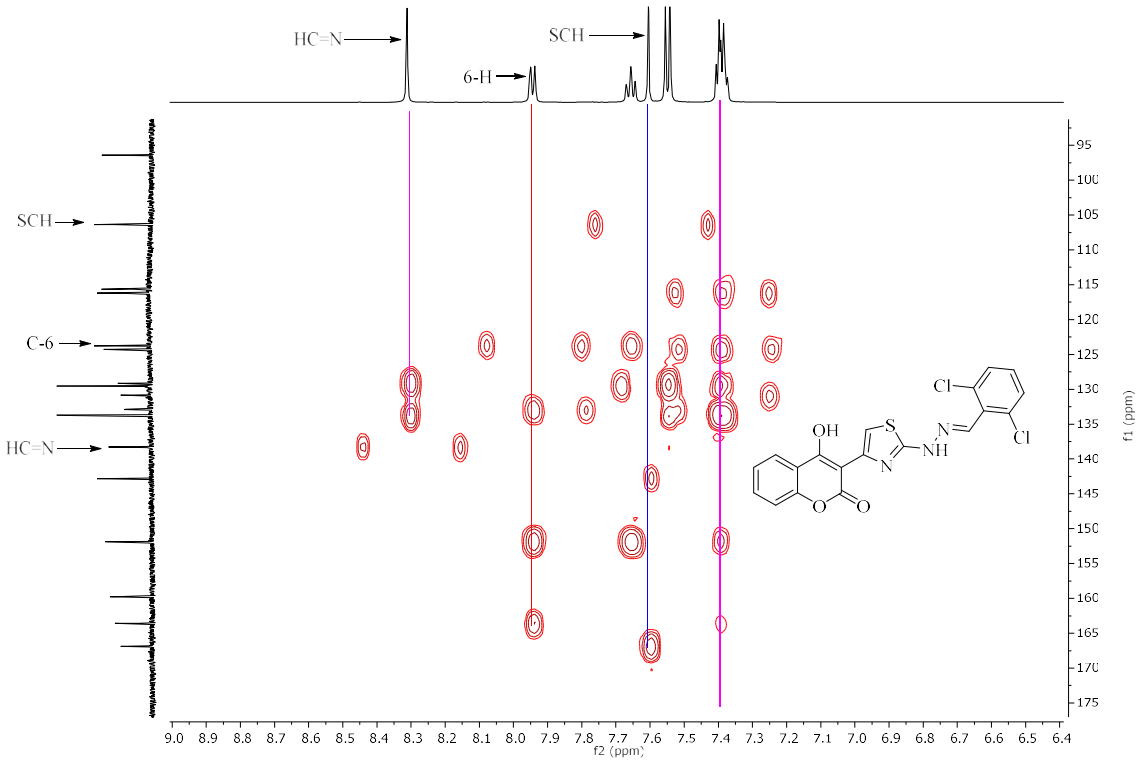


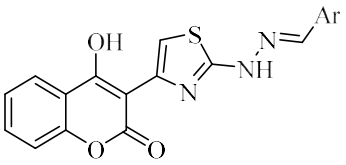
Figure 51. HMBC NMR spectrum of compound **136d** in DMSO-d_6.

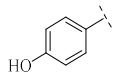
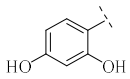
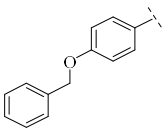
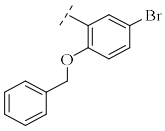
2.1.3.4. Biological studies of the 3-[2-(benzylidinehydrazono)thiazol-4-yl]-4-hydroxycoumarin derivatives 136

2.1.3.4.1. *In vitro* studies

Anti-malarial, trypanocidal, cytotoxicity and anti-mycobacterial studies were conducted on compounds **136a-g** as described in section **2.1.1.4**. Docking studies were undertaken with respect to HIV-1 IN and PR. **Table 17** shows the bioassay results for the compounds, none of which exhibited significant (< 50% viability) activity against malaria, trypanosomiasis or significant cytotoxicity against HeLa cells. In the anti-mycobacterial tests, however, compounds **136a** and **136b** showed significant anti-mycobacterial potential with MIC_{90} values of 31.25 and 15.63 μM (visual), and 15.63 and 31.25 μM (calculated), respectively. Compound **136f** exhibited weak activity with an MIC_{90} value of 73.07 μM. The rest of the compounds had values above 125 μM. It would appear the nature of the benzylidene moiety plays a significant role in the anti-mycobacterial activity of the compounds, either promoting or diminishing activity as the rest of their structures are common to all the molecules in the set.

Table 17. Bioassay data for compounds **136a-g**, showing % viability of pLDH, *T.b. brucei* and HeLa cells at 20 µM, and activity against mycobacteria



Compound	Ar	PLDH % viability [a]	*T.b. brucei* % viability [b]	HeLa cells % viability [c]	Visual MIC90 7D 7H9 GLU CAS Tx (µM)	Calculated MIC90 7D 7H9 GLU CAS Tx (µM)
136a		65.26	77.85	100.00	31.25	15.63
136b		73.18	52.03	74.92	>125	>125
136c		80.42	98.26	75.34	15.63	31.25
136d		80.03	84.25	79.54	>125	>125
136e		87.93	85.81	80.79	>125	>125
136f		88.88	88.14	85.64	>125	73.07
136g		96.87	85.70	94.51	>125	>125
Control		IC_{50} = 0.010µM [a]	IC_{50} = 0.006µM [b]	IC_{50} = 0.034µM [c]	0.019 [d]	0.007 [d]

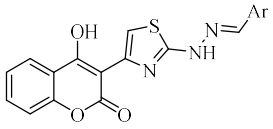
Controls. [a] chloroquine; [b] pentamidine; [c] emetine; [d] rifampicin

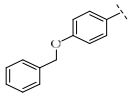
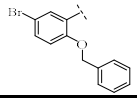
2.1.3.4.2. *In silico* docking studies

Studies were conducted on the *in silico* docking of compounds (**136**) in HIV-1 IN and PR, and *Plasmodium falciparum*, *T.b. brucei* and *Mtb* enzymes as described in section **2.1.1.4.2**. The results for the particular PDB structures used for docking are presented in **Table 18**. No *in vitro* studies were conducted for HIV-1 IN and PR. The binding affinities for 5FRN which ranged from -5.531 kcal/mol (**136d**) to -7.558 kcal/mol (**136c**) may suggest their potential as inhibitors of HIV-1 IN in view of the fact that most of the binding affinities are quite close to that of the control, L-chicoric acid (-8.403 kcal/mol). The compounds exhibited strong

binding to 1YT9 (in the range -6 to -7.4 kcal/mol) compared to the control. The ligand with the strongest binding affinity, compound **136c** (-7.341 kcal/mol) exhibited better binding than the control ligand, ritonavir (-7.309 kcal/mol) and three other compounds exhibited binding affinities that were greater than -7 kcal/mol. The binding to 1ZP8 (in the range -5.4 kcal/mol to -6.3 kcal/mol) was weaker than to 1YT9 but still compared favourably with the control, ritonavir (-7.042 kcal/mol). The strongest binding ligand was compound **136b** (-6.306 kcal/mol). It is interesting that compound **136c** was also the best ligand in the docking to 5FRN and 1YT9 and the second best with 1ZP8, suggesting potential for this compound to serve as an inhibitor of both HIV-1 IN and PR. A single compound exhibiting potential activity against two of the critical HIV-1 enzymes *in silico* is quite desirable. In general, the compounds (**136**) exhibited potential *in silico* as HIV-1 IN and PR inhibitors as suggested by the binding affinities.

Table 18. Binding affinities for the docking of ligands **136** in the active sites of various enzymes.



Ligand	Ar	HIV-1 IN 5FRN (kcal/mol)	HIV-1 PR 1YT9 (kcal/mol)	HIV-1 PR 1ZP8 (kcal/mol)	*Pf* 1T2C (kcal/mol)	*Pf* 1V0O (kcal/mol)	*T.b. brucei* 3FWN (kcal/mol)	*Mtb* 4BFW (kcal/mol)
136a		-5.981	-6.933	-5.935	-7.961	-3.219	-4.668	-7.479
136b		-5.666	-7,257	-6.306	-8.294	-4.460	-5.337	-5.899
136c		-7.558	-7,341	-6.169	-8.849	-3.750	-4.906	-5.714
136d		-5.531	-7.127	-5.666	-7.823	-3.439	-3.240	-4.774
136e		-6.845	-7,191	-5.985	-8.118	-4.075	-4.325	-6.197
136f		-6.138	-6.960	-5.183	-6.701	-3.554	-4.521	-5.841
136g		-6.085	-6,092	-5.439	-5.226	-3.296	-3.630	-5.913

Controls: As shown in **Table 12**.

The *in silico* results show the compounds to exhibit quite high binding to the *Pf* enzyme 1T2C (binding affinitities in the range -5.0 to -8.8 kcal/mol and above the -4.680 kcal/mol exhibited by the control ligand, chloroquine). This suggests potential for the compounds as pLDH inhibitors. However, the fact that the compounds in the *in vitro* assay only exhibited weak activities (% pLDH viability for the best four being **136a-d** 65.26, 73.18, 80.42 and 80.03, respectively) suggests the existence of other critical factors that could not be accounted for in the docking protocol. It is noteworthy that the best three ligands (**136a-c**) in the *in vitro* assay are among the best four ligands in the *in silico* assay but the order is different. The binding affinities for 1V0O were relatively low. The weakest binding was exhibited by compound **136a** (-3.219 kcal/mol) and the strongest, **136b** (-4.460 kcal/mol). Interestingly the best ligand for 1V0O **136b** exhibited binding affinity slightly weaker than that exhibited by chloroquine (-4.680 kcal/mol), was second-best with 1T2C and the second most active compound in the *in vitro* assay.

In the evaluation of the compounds' activity against *T.b. brucei* using 4FWN, the binding affinity ranged from -3.240 kcal/mol (compound **136d**) to -5.337 kcal/mol (compound **136b**). The three best ligands (**136a-c**) though weaker in binding to 4FWN than the control, pentamidine (-6.017 kcal/mol), exhibited binding affinities within 1.4 kcal/mol of the control. The two compounds that exhibited significant activity against *T.b. brucei in vitro*, **136a** and **b** are among the three best ligands *in silico* and interestingly compound **136b** exhibited the best activity in both evaluations which may suggest its potential as an anti-trypasomal agent. The binding affinities exhibited by the compounds **136** to 4BFW suggest their potential as *Mt*PanK inhibitors. Only one compound (**136d**) had a binding affinity that was weaker than -5.7 kcal/mol. Compound **136a** (-7.479 kcal/mol) exhibited the strongest binding and compound **136e** (-6.197 kcal/mol) exhibited the second strongest. Of note is the fact that compound **136a** exhibited the best activity against *Mtb* in both evaluations thus suggesting its potential as an anti-TB agent.

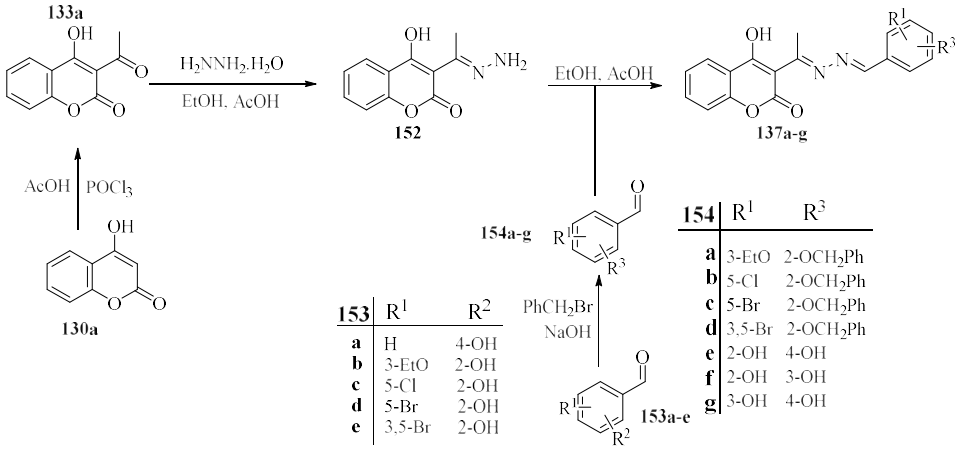
2.1.4 3-[1-(Benzylidenehydrazono)ethyl]-4-hydroxycoumarins

The hydrazono derivatives **137a-j** were designed to contain a diimine (bis-Schiff's base or bis-imine) moiety linking, on one end, to the 3-acetylated 4-hydroxycoumarin motif and, on the other end, to an aromatic aldehyde derivative. The biological potential of imines was

discussed earlier and is an important consideration in the design of the compounds. The literature reveals that diimines are an important class of compounds pharmacologically, physico-chemically and as precursors in the synthesis of important compounds such as pyrimidines, piperazines, bis-ketenes, metal complexes and polymers.[333]

2.1.4.1 Synthesis of the 3-[1-(benzylidenehydrazono)ethyl]-4-hydroxycoumarins 137

The pathway to the desired compounds outlined in **Scheme 24** involved the reaction of 3-acetyl-4-hydroxycoumarin **133a** with hydrazine hydrate; the hydrazide so formed was then reacted with selected aromatic aldehyde derivatives **154a-g** to yield the final compounds **137a-g**. Five of the aromatic aldehydes (**154a-e**) were obtained in 65-75% yields by prior *o*-benzylation of their phenolic precursors **153a-e** using benzyl bromide and 10% aqueous NaOH. The other two compounds (**153f-g**) were commercially available.



153	R^1	R^2
a	H	4-OH
b	3-EtO	2-OH
c	5-Cl	2-OH
d	5-Br	2-OH
e	3,5-Br	2-OH

154	R^1	R^3
a	3-EtO	2-OCH_2Ph
b	5-Cl	2-OCH_2Ph
c	5-Br	2-OCH_2Ph
d	3,5-Br	2-OCH_2Ph
e	2-OH	4-OH
f	2-OH	3-OH
g	3-OH	4-OH

Scheme 24. Route to 3-[1-(benzylidenehydrazono)ethyl]-4-hydroxycoumarins **137a-g**.

2.1.4.1.1 Synthesis of 3-(1-hydrazonoethyl)-4-hydroxycoumarin 152

Compound **133a**, obtained from 4-hydroxycoumarin **130a** as described earlier, was reacted with hydrazine hydrate under reflux for 1 hour using ethanol as the solvent and acetic acid as the catalyst. The precipitate that formed was filtered off and washed with methanol and dried to yield the product **152** (80%), a new compound, which was used without further purification. The ^{1}H NMR spectrum of compound **152** reveals the methyl protons resonating at 2.65 ppm, the NH_2 signal at 6.05 ppm and the expected aromatic proton signals in the

range 7.5-8.0 ppm (**Figure 52**). Appearance of the OH signal so far downfield at 15.53 ppm suggests that it is involved in hydrogen-bonding and, hence, the presence of rotamer I which would be stabilised by the 6-membered hydrogen-bonded chelate. **Figure 53** shows the ^{13}C spectrum of compound **152** with the methyl carbon signal at 15.8 ppm and the C=N signal at 166.3 ppm; the rest of the carbon signals appear as expected. Comparison of ^{13}C spectrum of compound **152** (top) with that of its precursor **133a** (bottom) (**Figure 54**) also confirms the conversion of compound **133a** to **152**. As revealed in **Figure 54** the 3-acetyl carbon signal in **133a** resonates at 206.2 ppm consistent with a ketone carbonyl, whereas in the product the C=N signal is shifted up-field to 166.3 ppm. Although most of the signals exhibit minor shifts due to the different NMR solvents used ($CDCl_3$ for **133a** and DMSO-d_6 for **152**) the 40 ppm difference between the signals assigned to the C=O and C=N groups is very significant and is clear evidence of the conversion.

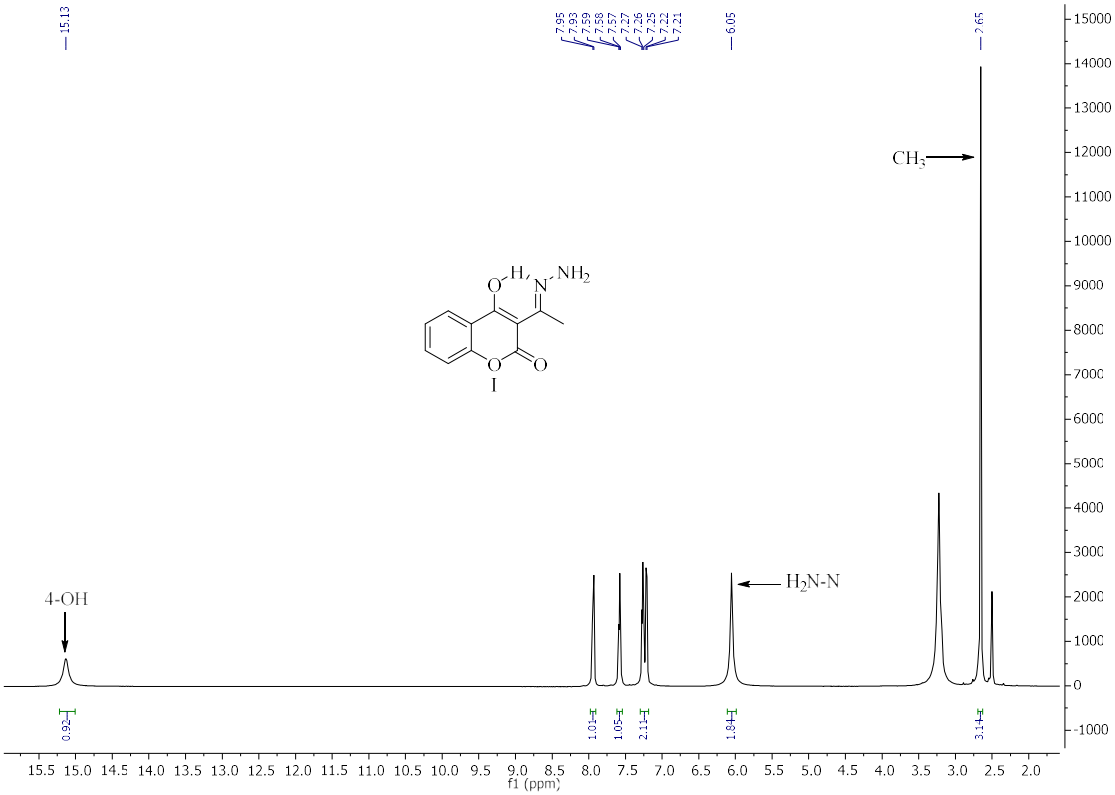


Figure 52. 600 MHz ^1H NMR spectrum of compound **152** in DMSO-d_6.

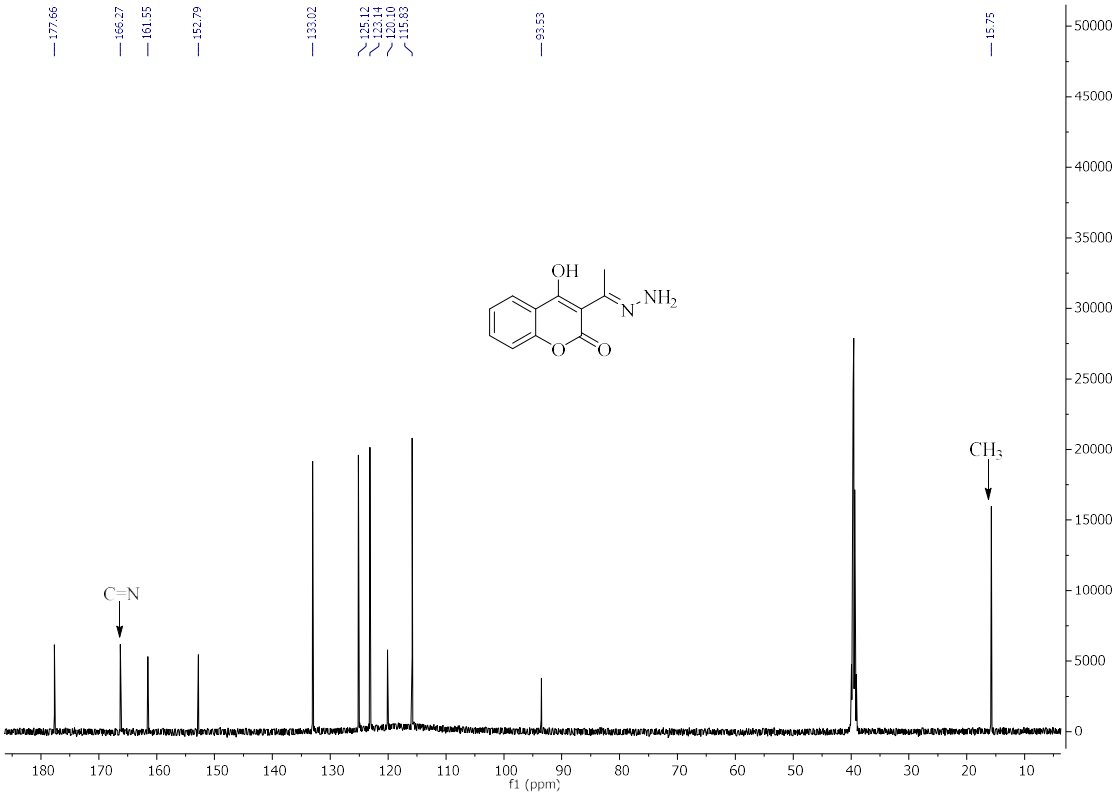


Figure 53. 150 MHz ^{13}C NMR spectrum of compound **152** in DMSO-d_6.

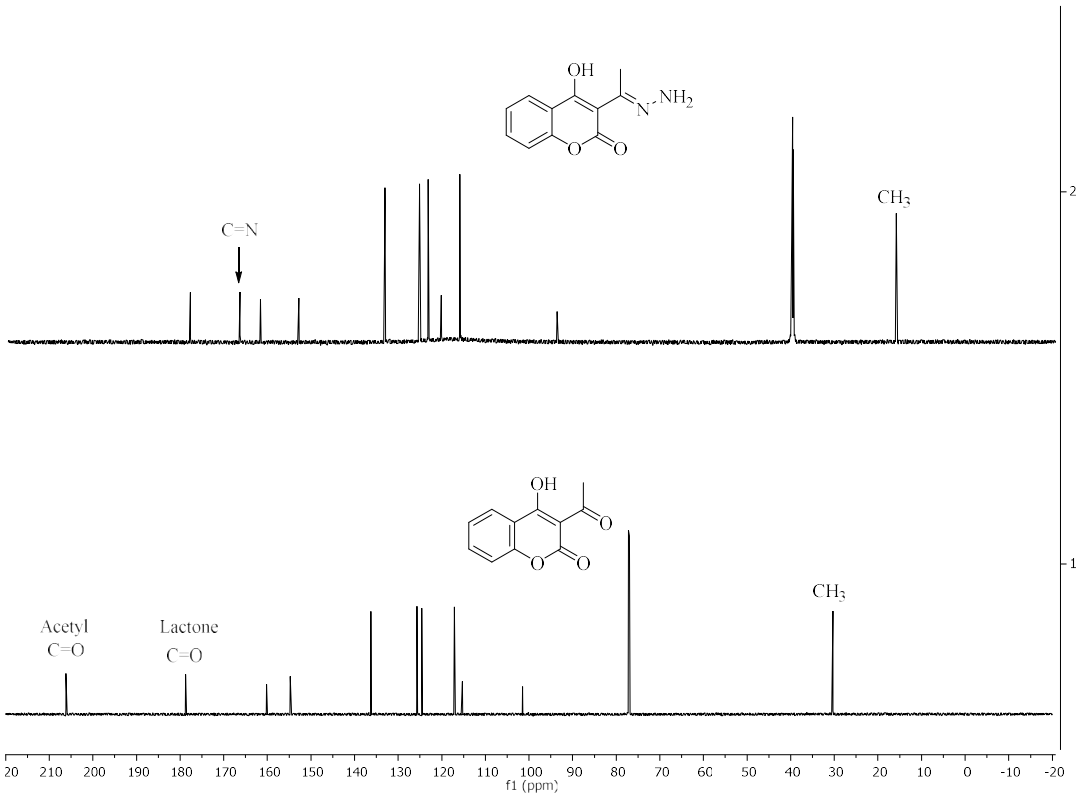
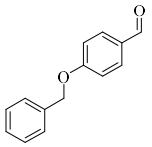
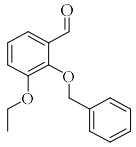
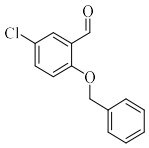
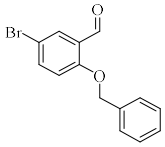
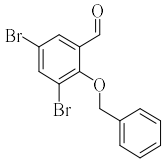


Figure 54. 150 MHz ^{13}C NMR spectra overlay for compound **152** in DMSO-d_6 (top) and **133a** in $CDCl_3$ (bottom).

2.1.4.1.2 Synthesis of the benzyloxy benzaldehydes

Each of aromatic aldehydes **153a-e** was heated under reflux with benzyl bromide in a 10% aqueous NaOH solution (**Scheme 24**) for thirty minutes. After cooling to room temperature, water was added and the solids which formed were filtered, washed with water, dried and finally crystallised from hexane. Yields for the dried, final products were in the range 55-70 %. The ^{1}H NMR spectrum of compound **154c** reveals the methylene protons resonating as a singlet at 5.18 ppm, the 6-methine proton resonating as a singlet at 7.80 ppm, and the aldehydic proton resonating at 10.48 ppm (**Figure 55**). In the corresponding DEPT 135 spectrum the methylene proton signal is observed at 71.0 ppm and the six expected proton-bearing aromatic carbon signals in the range 114-136 ppm (**Figure 56**). **Figure 57** shows the crystal structure of compound **154c**.

Table 19. Yields of benzyloxy benzaldehydes **154a-e**.

Compound	Structure	Isolated Yields (%)
154a		65
154b		55
154c		69
154d		70
154e		66

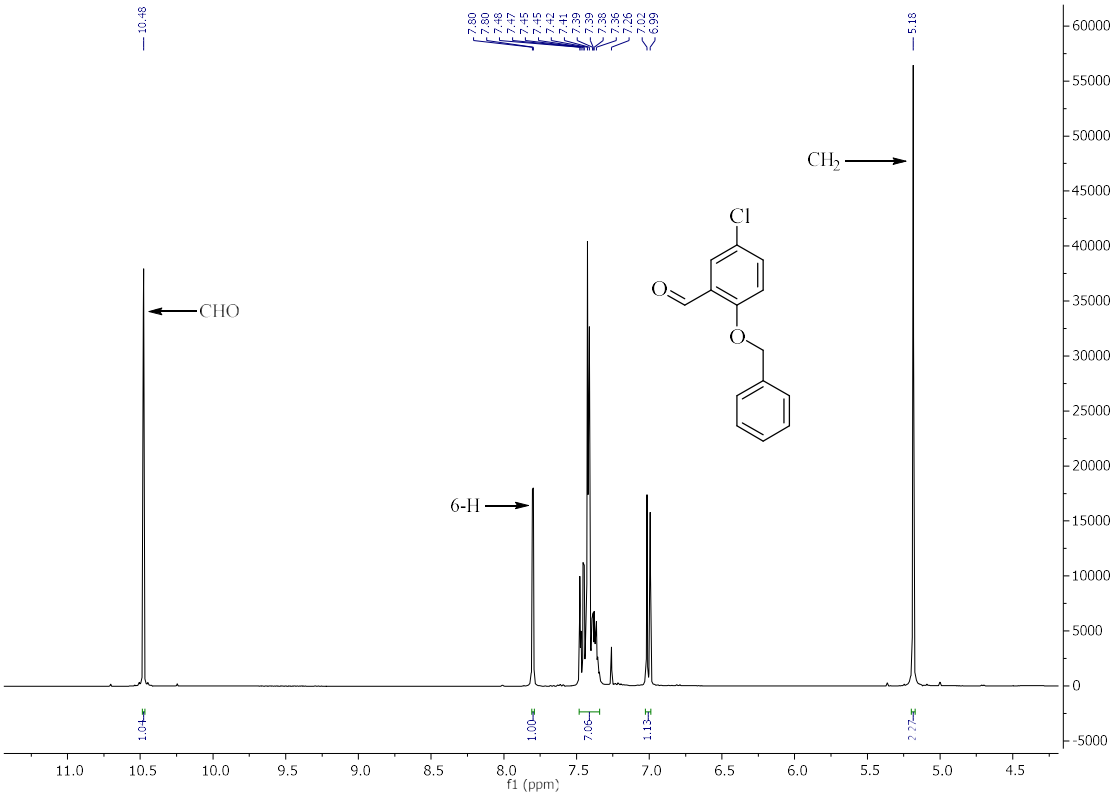


Figure 55. 400 MHz ^{1}H NMR spectrum of compound **154c** in $CDCl_3$.

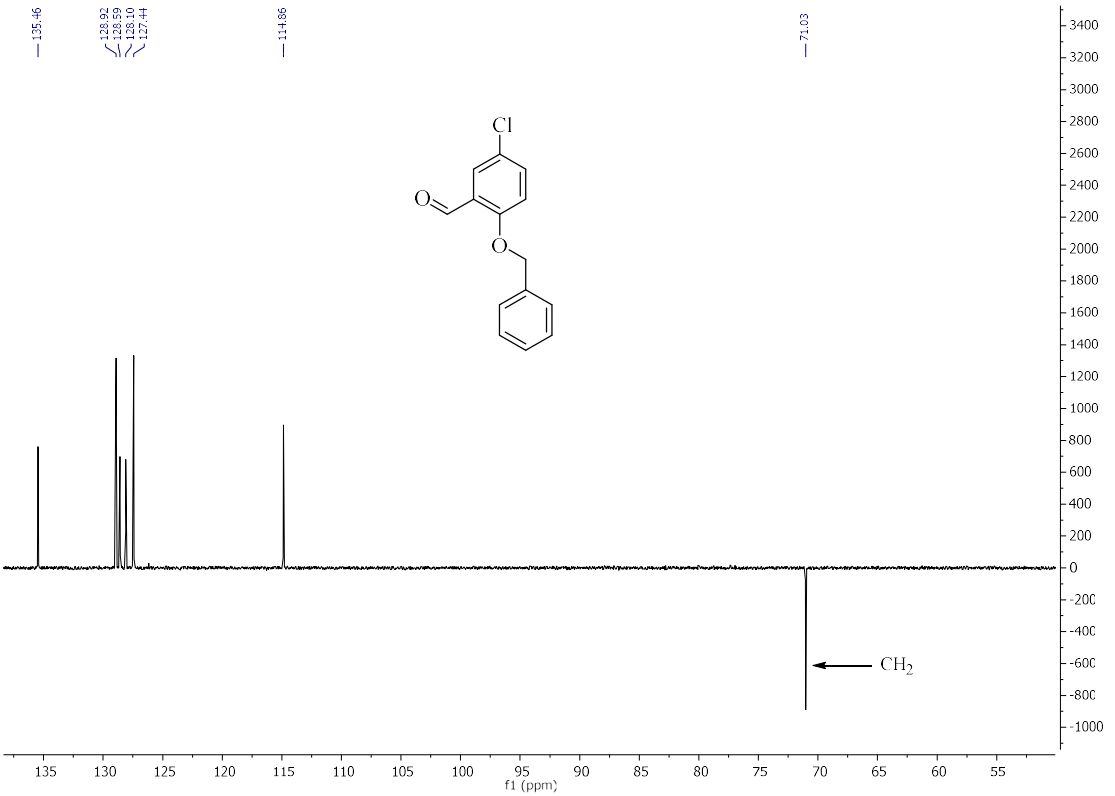


Figure 56. DEPT 135 NMR spectrum of compound **154c** in $CDCl_3$.

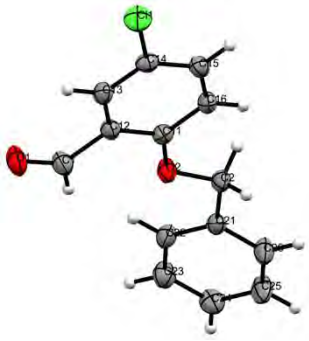


Figure 57. Crystal structure of compound **154c.**

2.1.4.2 Synthesis of the diimine compounds

The aromatic aldehydes **154b-e** synthesised as part of the study and commercially available aromatic aldehydes **154e-g** (**Figure 58**) were each refluxed with the hydrazone **152** in ethanol for at least an hour (**Scheme 24**). The precipitates formed were washed with methanol to give the final products **137a-g**, which were shown to be pure by NMR anlysis and require no further purification. The novel diimines were isolated in yields ranging from 51% to 76% (**Table 20**) and were fully characterised. The ^{1}H NMR spectrum of one of these novel compounds **137f** reveals the methyl protons signal at 2.86 ppm, the seven aromatic proton signals in the range 6.5-8.0 ppm; the signals corresponding to 5'-CH and 8-CH at 6.73 and 7.92 ppm, respectively, are highlighted in **Figure 59**. The azomethine proton resonates at 8.76 ppm and the two OH signals are clearly visible at 9.63 and 9.73 ppm. **Figure 60** shows the ^{13}C spectrum of compound **137f** in which the expected 18 carbon signals are clearly revealed. The methyl, C-3 and the azomethine carbons resonate at 17.3, 95.5 and 153.5 ppm, respectively. In the COSY NMR spectrum (**Figure 61**) the expected ^{1}H correlations are revealed for the eight protons in the compound. In the expanded portion showing the correlations for the seven aromatic protons, the respective 5'-CH and 5-CH correlations are highlighted. The partial HSQC NMR spectrum selected to show the aromatic proton signals of **137f** reveals the expected seven ^{1}H signals and the corresponding ^{13}C signals (**Figure 62**).

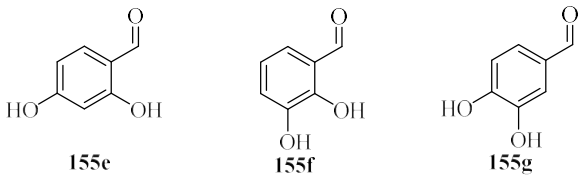
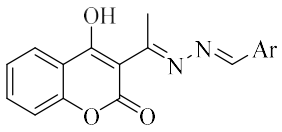
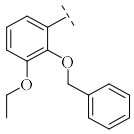
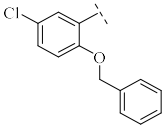
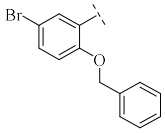
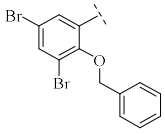
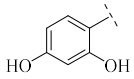
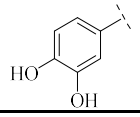


Figure 58. Commercially available aromatic aldehydes **154e-g**.

Table 20. Yields of the 3-[1-(benzylidenehydrazono)ethyl]-4-hydroxycoumarins **137a-g**.



Compound	Ar	Isolated Yields (%)
137a		51
137b		75
137c		76
137d		72
137e		67
137f		66
137g		68

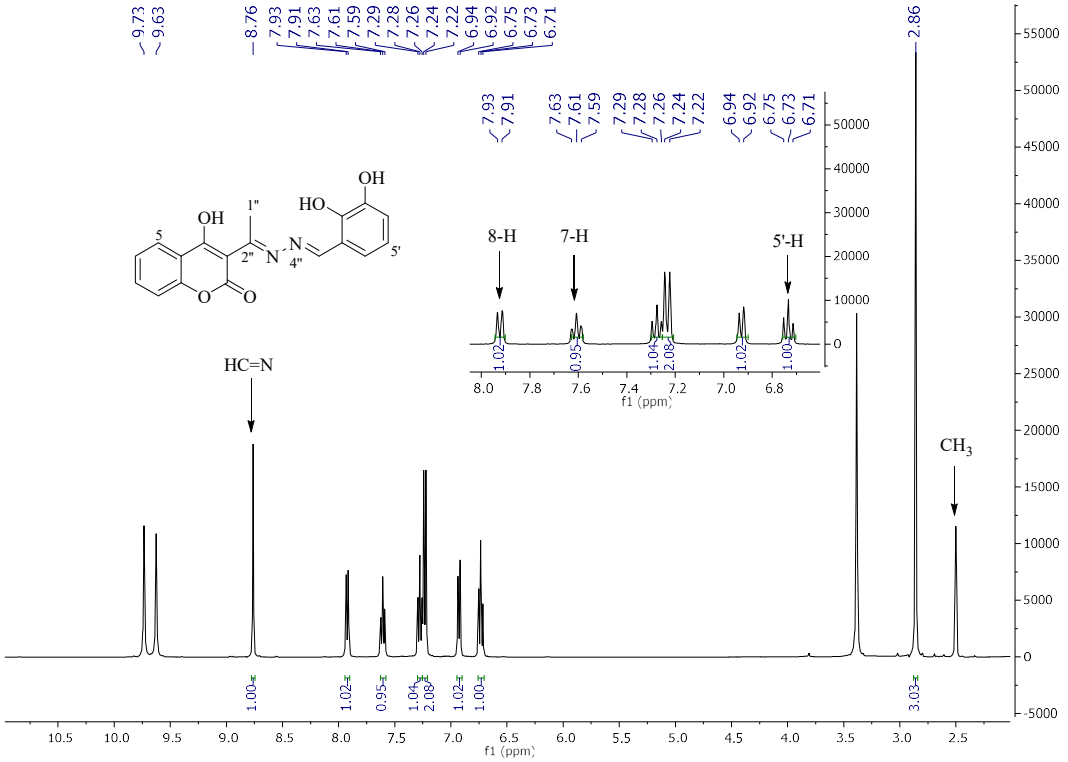


Figure 59. 400 MHz ^{1}H NMR spectrum of compound **137f** in DMSO-d_6.

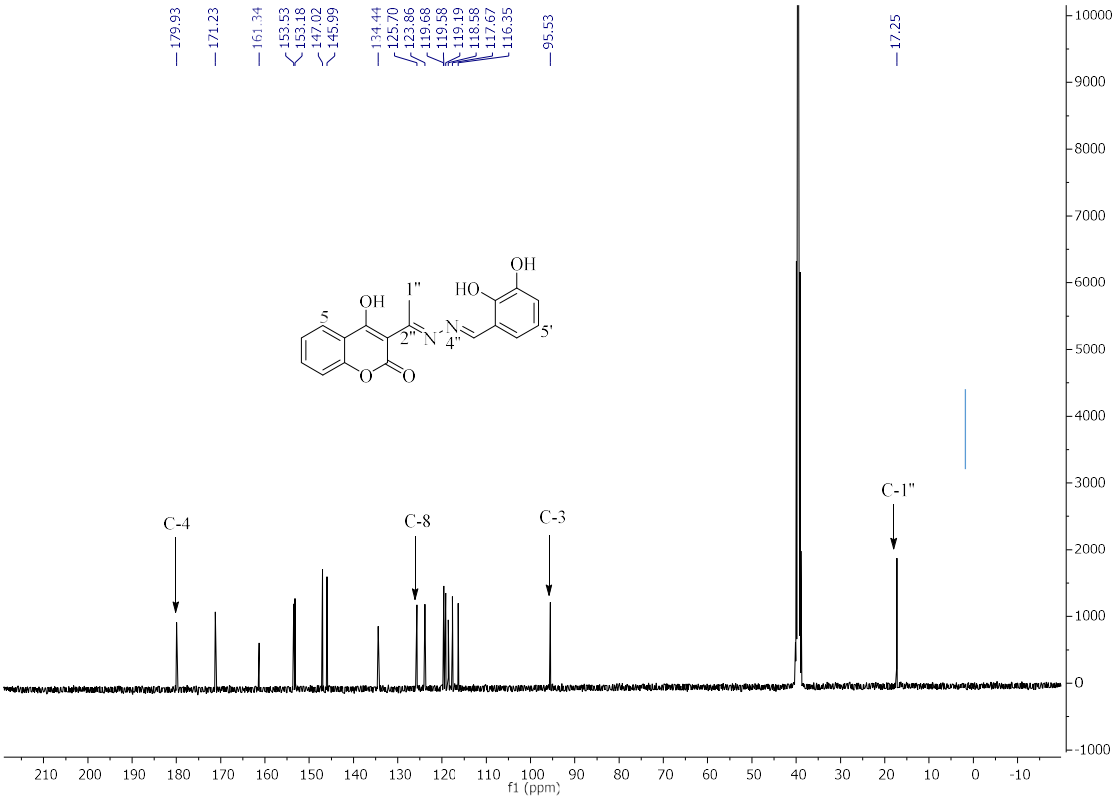


Figure 60. 100 MHz ^{13}C NMR spectrum of compound **137f** in DMSO-d_6.

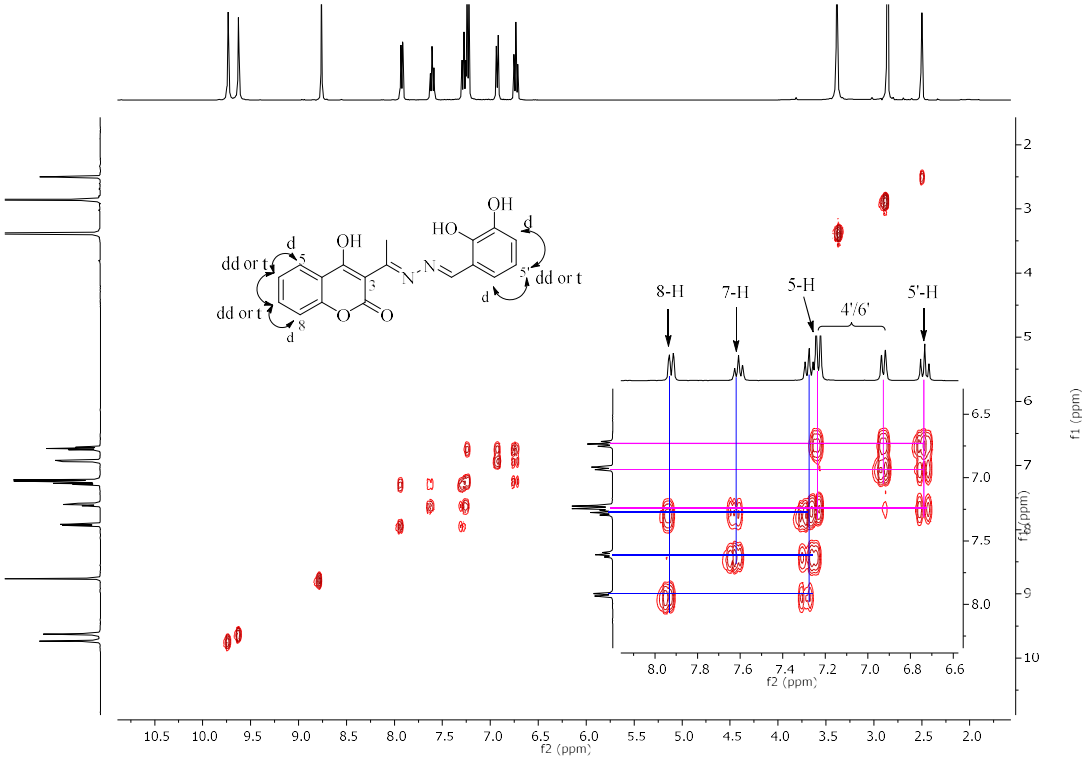


Figure 61. 400 MHz COSY NMR spectrum of compound **137f** in DMSO-d_6.

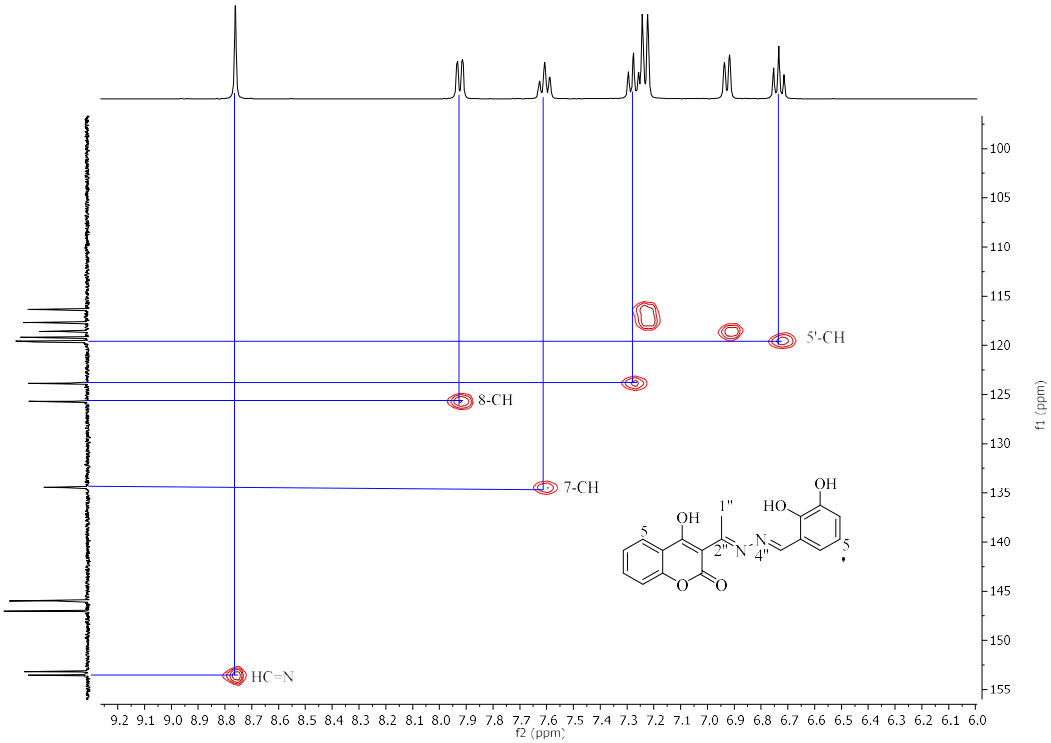
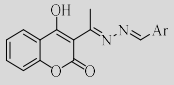
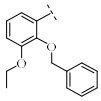
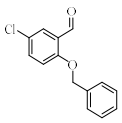
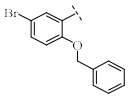
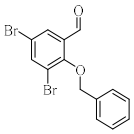
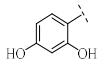
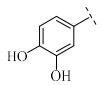


Figure 62. Partial HSQC NMR spectrum of compound **137f** in DMSO-d_6.

2.1.4.3. Biological studies of the 3-[1-(benzylidenehydrazono)ethyl]-4-hydroxycoumarins

Biological studies on the compounds **137a-g** for anti-malarial, trypanocidal, cytotoxicity and anti-mycobacterial activity were conducted as described in section **2.1.1.4**. **Table 21** shows the bioassay results for the compounds, none of which exhibited significant activity against malaria or significant cytotoxicity against HeLa cells. However, compound **137g** reduced the *T.b. brucei* viability to 1.53% and exhibited an IC_{50} value of 0.90 μM. In the anti-mycobacterial (anti-TB) tests, compound **137g** exhibited weak activity with MIC_{90} values of 62.50 μM (visual) and 62.44 μM (calculated), while the rest of the compounds exhibited no significant activity. Compared to the data obtained for compounds **136a-g** it would appear that the benzylidene moiety plays a significant role in the biological activity.

Table 21. Bioassay data for compounds **137a-g**, showing % viability of pLDH, *T.b. brucei* and HeLa and activity against mycobacterial cells at 20 μM concentrations.

	**Ar**	**PLDH % viability**[a]	***T.b. brucei* % viability** [b]	**% HeLa cells viability** [c]	**Visual MIC90 7D 7H9 GLU CAS Tx (μM)**	**Calculated MIC90 7D 7H9 GLU CAS Tx (μM)**
137a		100.00	97.40	96.98	>125	>125
137b		100.00	52.34	92.26	>125	>125
137c		100.00	80.86	89.75	125	>125
137d		100.00	100.00	83.50	-	-
137e		63.93	100.22	67.78	>125	>125
137f		81.62	58.11	59.80	>125	>125
137g		86.51	**1.53 (IC_{50} 0.90)**	65.25	62.50	62.44
Control		IC_{50} = 0.01 μM [a]	IC_{50} = 0.022 μM [b]	IC_{50} = 0.021 μM [c]	0.019 [d]	0.007 [d]

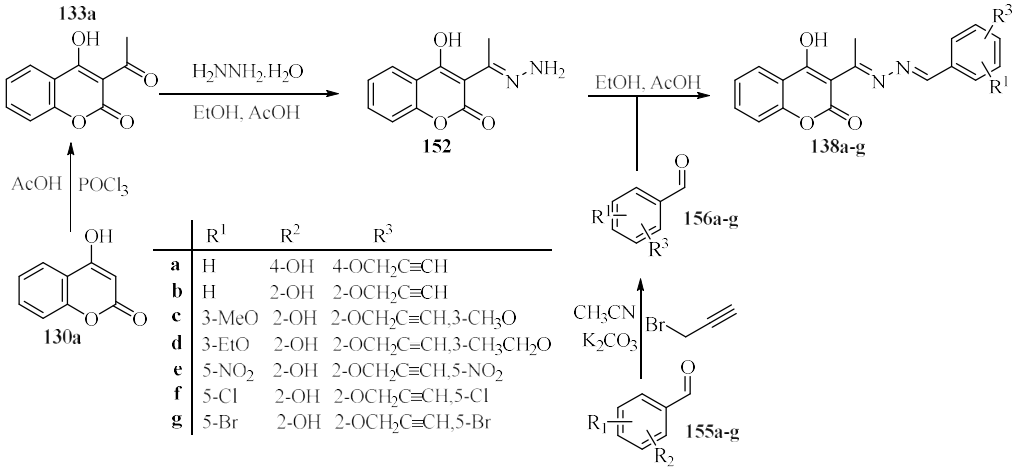
Controls. [a] Chloroquine [b] Pentamidine [c] emetine [d] Rifampicin [e] IC_{50} value

2.1.5. 3-{1-[(Prop-2-yn-1-yloxy)benzylidenehydrazono]ethyl}-4-hydroxycoumarins

The compounds are similar to the 3-[1-(benzylidenehydrazono)ethyl]-4-hydroxycoumarins **137a-g** as they have the same hyrazono moiety but instead of a benzyloxy substituent they have a propargyloxy substituent on the phenyl ring. The change of substituents was intended to investigate the effect of the different substituent on the bioactivity of the 4-hydroxycoumarin derivatives. The propargyloxy moiety also offers possibilities for click reactions thus allowing further conjugation and further biochemical exploration.

2.1.5.1 Synthesis of 3-{1-[(prop-2-yn-1-yloxy)benzylidenehydrazono]ethyl}-4-hydroxycoumarins

The pathway to the desired compounds was similar to the one described in section **2.1.4.1**. 3-Acetyl-4-hydroxycoumarin **133a** was reacted with hydrazine hydrate; the hydrazide so formed was then reacted with selected aromatic aldehyde derivatives to yield the final compounds (**Scheme 25**).



	R^1	R^2	R^3
a	H	4-OH	4-$OCH_2C{\equiv}CH$
b	H	2-OH	2-$OCH_2C{\equiv}CH$
c	3-MeO	2-OH	2-$OCH_2C{\equiv}CH$,3-CH_3O
d	3-EtO	2-OH	2-$OCH_2C{\equiv}CH$,3-CH_3CH_2O
e	5-NO_2	2-OH	2-$OCH_2C{\equiv}CH$,5-NO_2
f	5-Cl	2-OH	2-$OCH_2C{\equiv}CH$,5-Cl
g	5-Br	2-OH	2-$OCH_2C{\equiv}CH$,5-Br

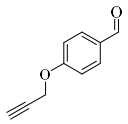
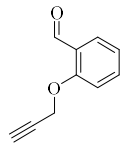
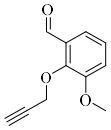
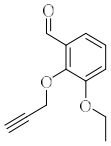
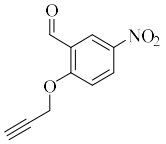
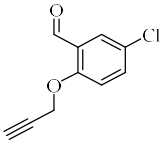
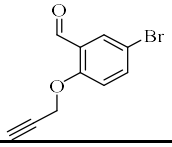
Scheme 25. Route to 3-{1-[(prop-2-yn-1-yloxy)benzylidenehydrazono]ethyl}-4-hydroxycoumarins **138a-g**

2.1.5.1.1 Synthesis of (prop-2-yn-1-yloxy)benzaldehydes

Each of salicylaldehydes **155a-g** was heated with propargyl bromide at 80 ^{o}C in CH_3CN in the presence of K_2CO_3 for 4 hours, after which the mixture was filtered and the solvent evaporated. The crude product was dissolved in DCM and washed with water. The organic

layer was dried and the residue recrystallized from hexane to obtain the 2-(prop-2-yn-1-yloxy)benzaldehydes **156a-g** in yields of 60-85% (**Table 22**). The ^{1}H NMR spectrum of compound **156g** reveals the acetylenic proton (C≡CH) signal as a triplet at 2.59 ppm (**Figure 63**), the propargyl methylene protons' signal at 4.82 ppm and the aldehydic proton signal at 10.38 ppm. It is interesting (as shown in the enlargements) to note that the methylene signal appears as a doublet and the acetylenic methine proton as a triplet. This is due to the coupling between the methylene protons and the acetylenic proton. The full complement of three aromatic proton signals is revealed in the range 7.0-8.0 ppm. The DEPT135 NMR spectrum of compound **156g** reveals a doublet (enlargement) at 77.2 ppm for the 3'-acetylenic carbon, a phenomenon which is due to the large $^{2}J_{C,H}$ coupling to C-2' (**Figure 64**). The COSY NMR spectrum confirms the interactions between the acetylenic proton and the propargyl methylene protons (**Figure 65**) and also reveals the aromatic protons correlations.

Table 22. Yields of the (prop-2-yn-1-yloxy)benzaldehydes (**156**).

Compound	Structure	Isolated Yields (%)
156a		63
156b		84
156c		66
156d		76
156e		60
156f		78
156g		85

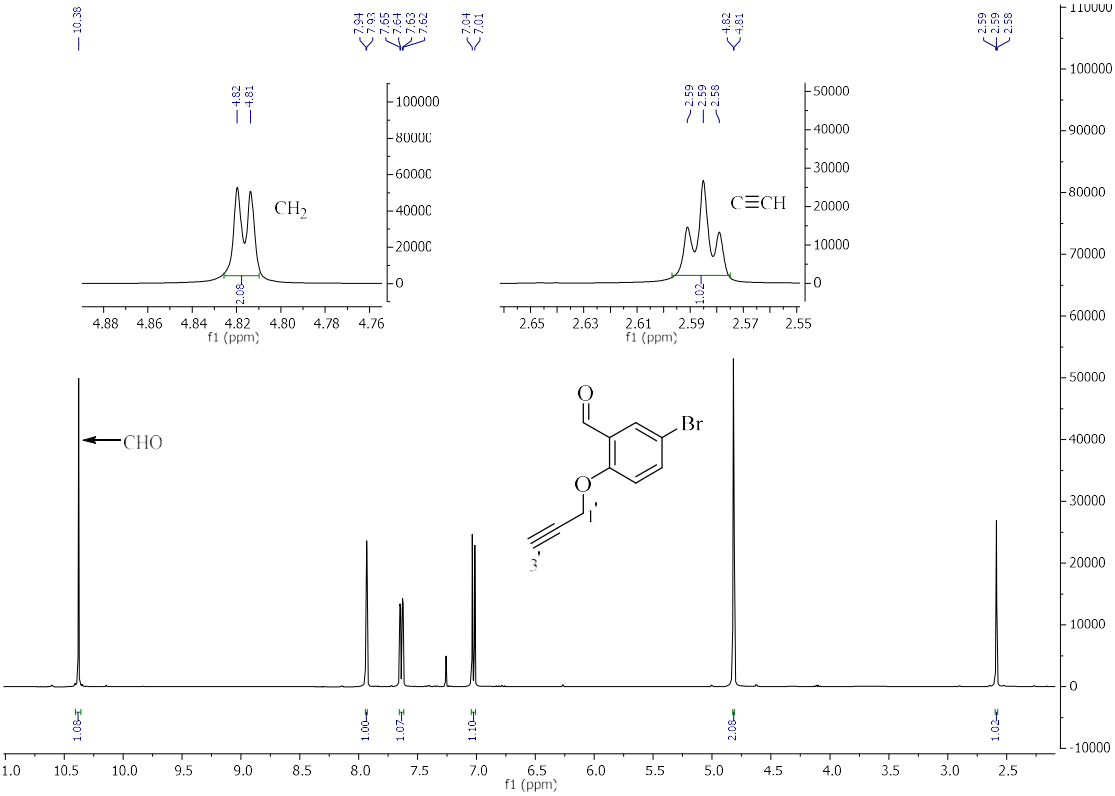


Figure 63. 400 MHz ^{1}H NMR spectrum of compound **157g** in $CDCl_3$.

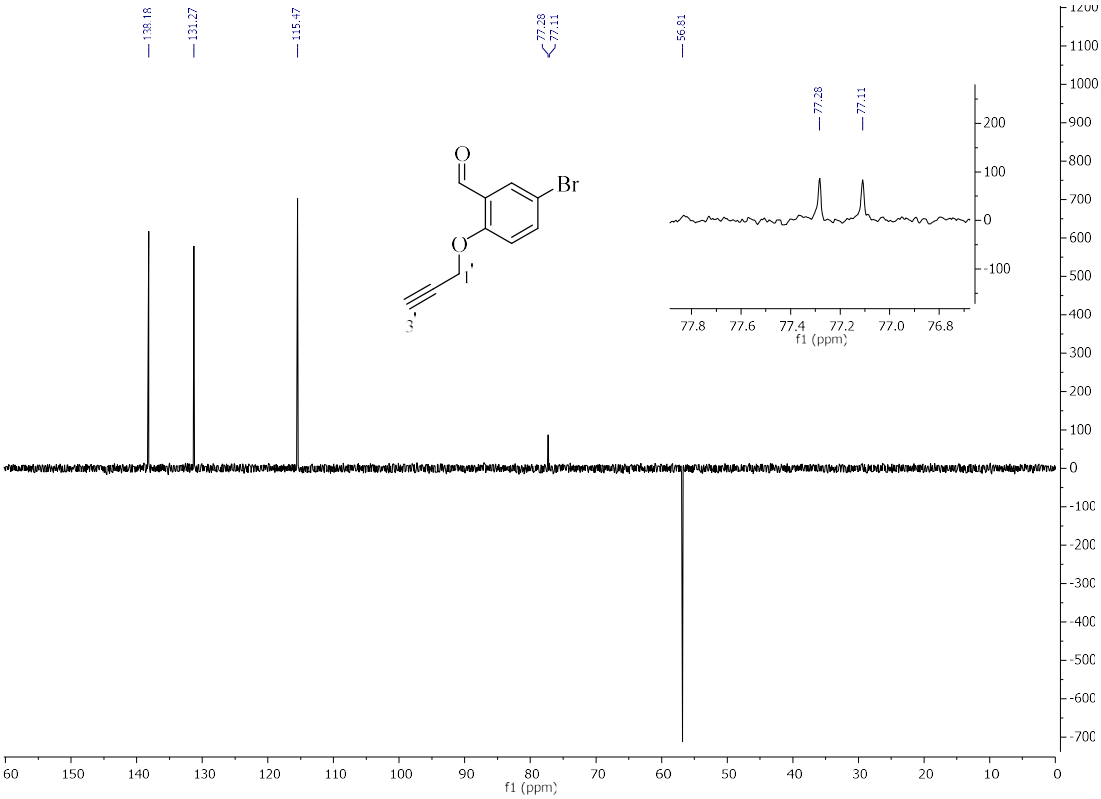


Figure 64. DEPT 135 NMR spectrum of compound **157g** in $CDCl_3$.

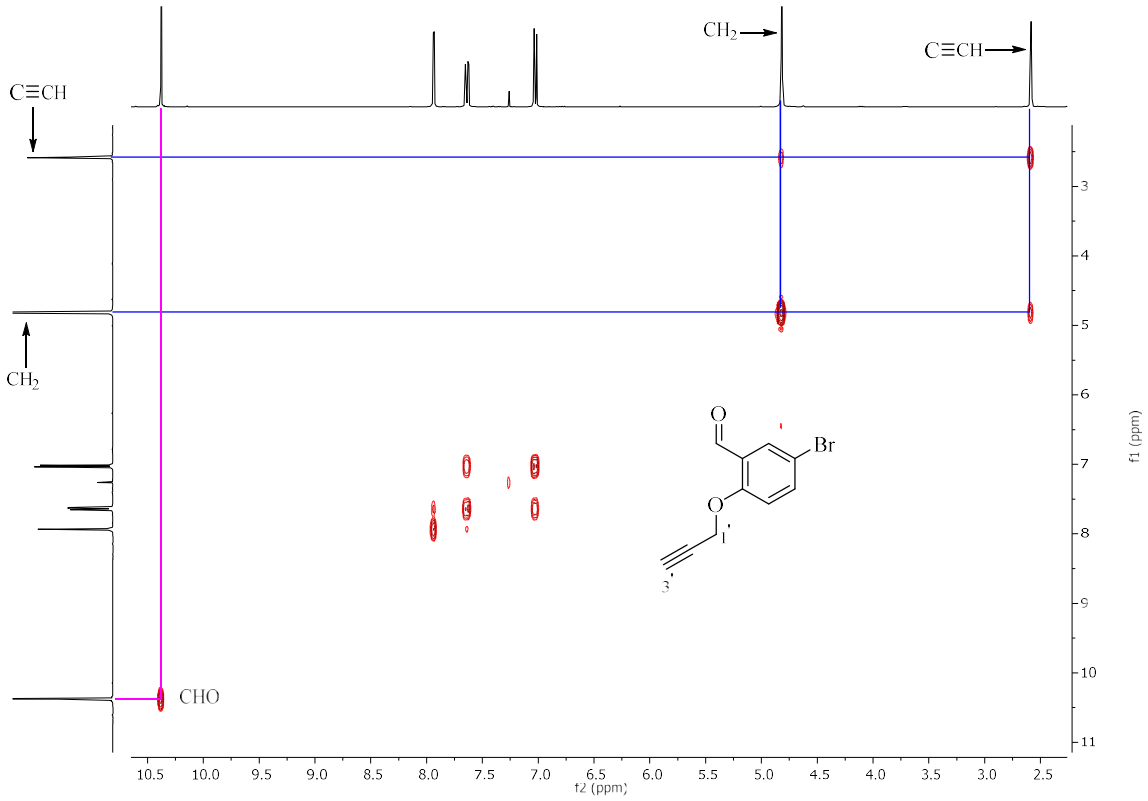


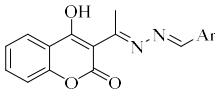
Figure 65. 400 MHz COSY NMR spectrum of compound **157g** in $CDCl_3$.

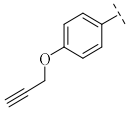
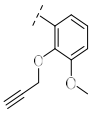
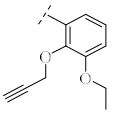
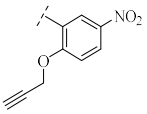
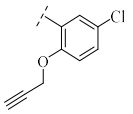
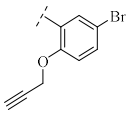
2.1.5.1.2. Reaction of 3-(1-hydrazonoethyl)-4-hydroxycoumarin 153 with the prop-2-yn-1-yloxy benzaldehydes

Each of the 2-(prop-2-yn-1-yloxy)benzaldehydes **156a-g** was refluxed with compound **152** in ethanol for at least an hour (**Scheme 25**). The precipitate formed was washed with methanol giving the final product without the need for further purification as confirmed by NMR analysis. The novel diimines were isolated in yields ranging from 65% to 80% (**Table 23**) and were fully characterised. The ^{1}H NMR spectrum of compound **138a** reveals the acetylenic proton (C≡CH) signal at 2.57 ppm as a triplet (**Figure 66**), the methyl protons resonating at 3.06 ppm as a singlet, as expected, the methylene protons at 4.76 ppm as a doublet and the azomethine proton at 8.31ppm. It is interesting (as shown in the enlargements) to note that the methylene signal appears as a doublet and the alkyl methine proton as a triplet. This is due to the weak coupling (J = 2.38 Hz) between the methylene protons and the acetylenic proton. The full complement of eight aromatic proton signals is revealed resonating in the range 7.0-8.4 ppm. The ^{13}C NMR spectrum of compound **138d** reveals the methyl carbon signal at 17.8 ppm, the methylene carbon signal at 56.1 ppm, the

terminal alkynyl carbon signal at 76.4 ppm, the carbon bearing the azomethine proton at 154.4 ppm and the expected total number of 19 carbon signals (**Figure 67**). The COSY NMR spectrum (**Figure 68**) shows some of the ^{1}H-^{1}H correlations. The interaction between the methylene protons and the alkynyl methine proton are clearly revealed. The HSQC spectrum reveals the ^{1}H-^{13}C correlations, in particular, the correlations involving the propargyl group protons (**Figure 69**).

Table 23. Yields of 3-[1-(prop-2-yn-1-yloxy)benzylidene)hydrazono)ethyl]-4-hydroxycoumarins **138a-g**.



Compound	Ar	Isolated Yields (%)
138a		65
138b		67
138c		70
138d		78
138e		67
138f		80
138g		77

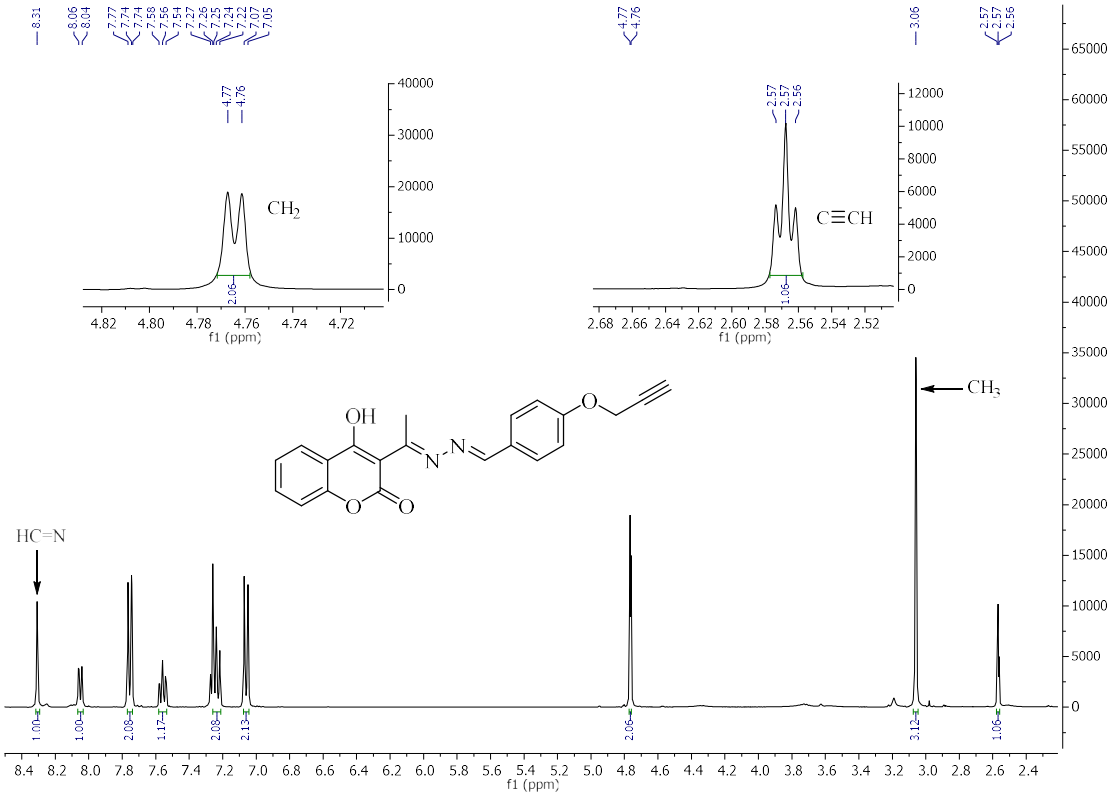


Figure 66. 400 MHz ^{1}H NMR spectrum of compound **138a** in $CDCl_3$.

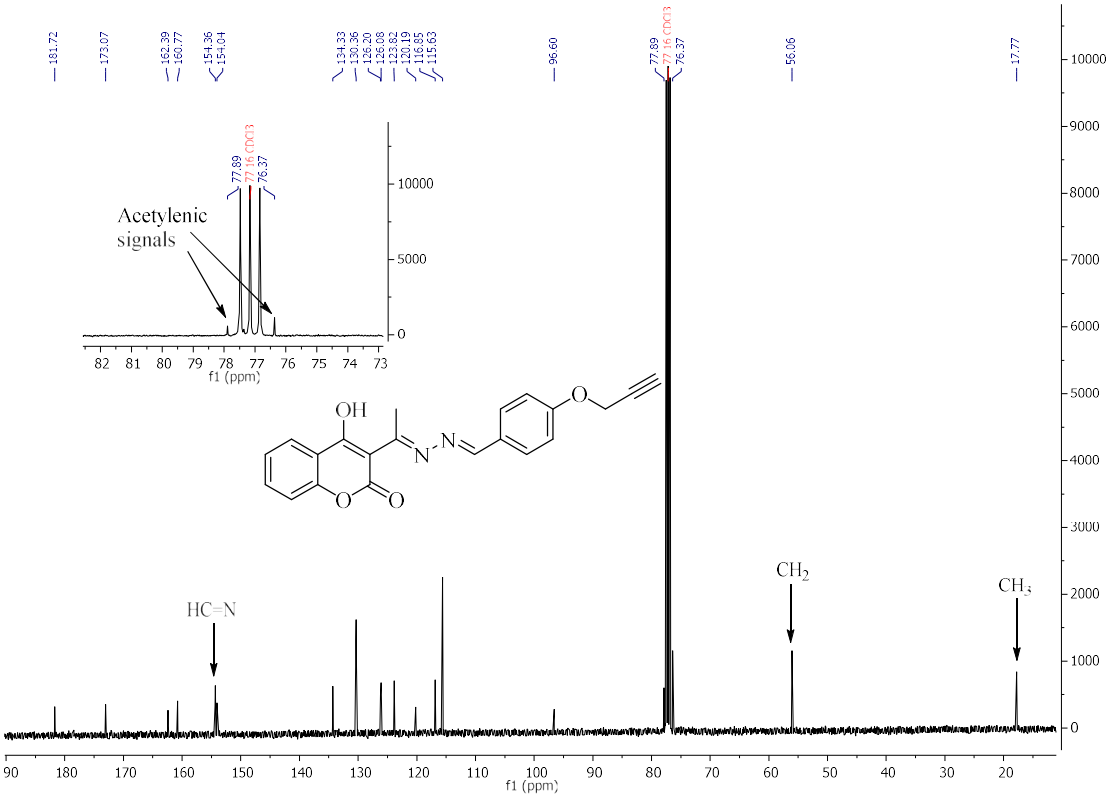


Figure 67. 100 MHz ^{13}C NMR spectrum of compound **138a** in $CDCl_3$.

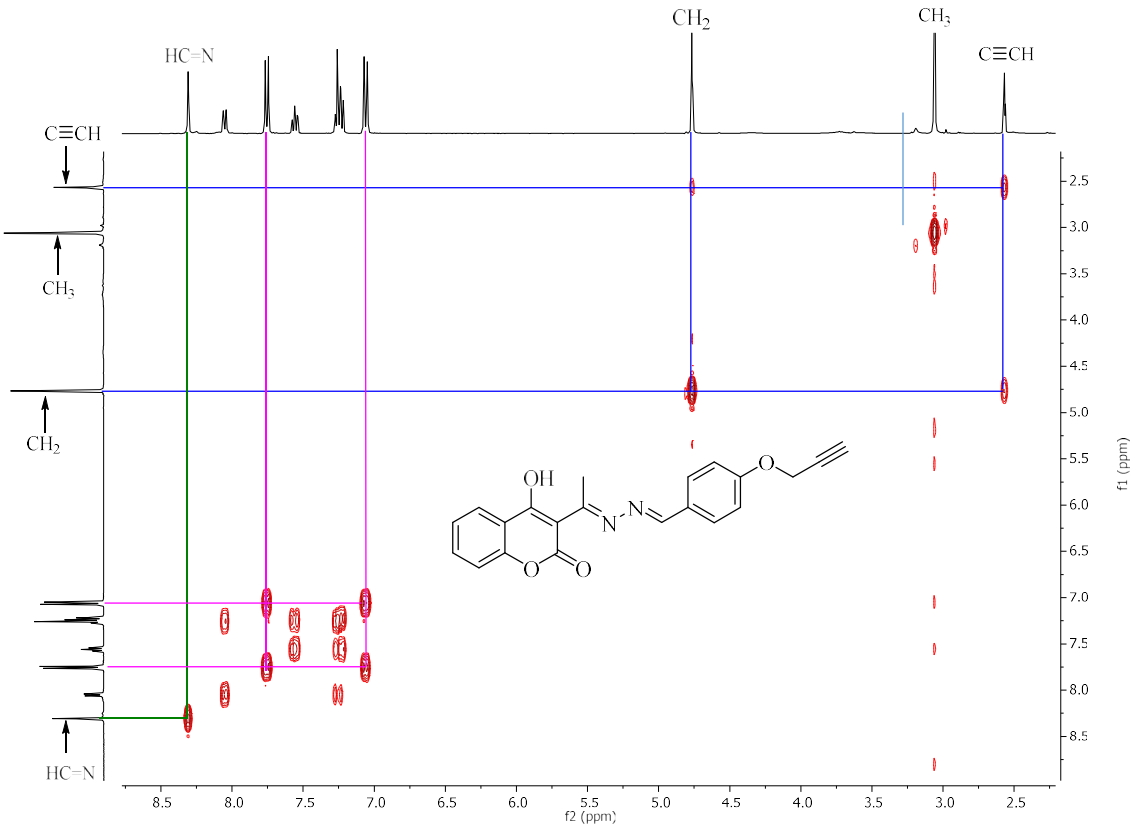


Figure 68. 400 MHz COSY NMR spectrum of compound **138a** in $CDCl_3$.

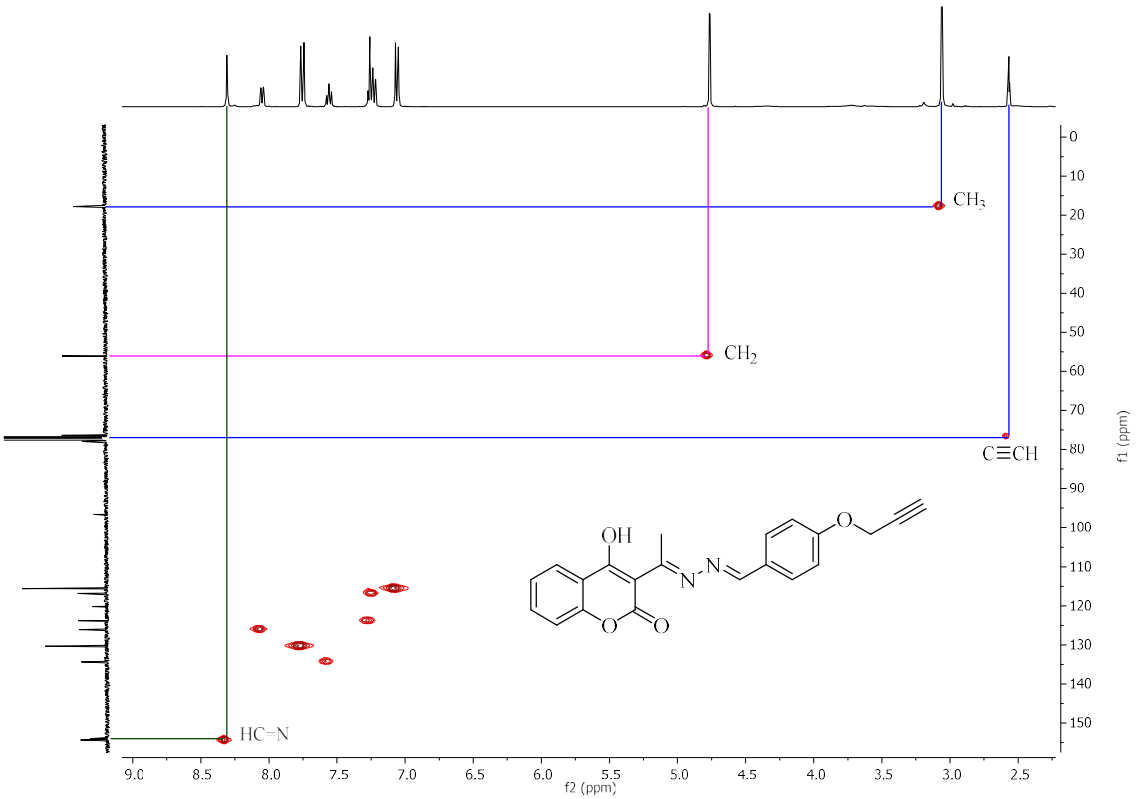
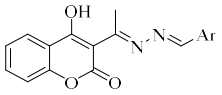


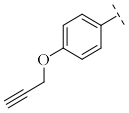
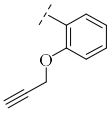
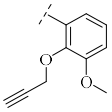
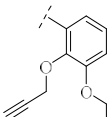
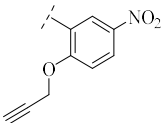
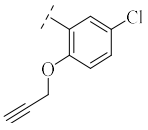
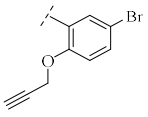
Figure 69. HSQC NMR spectrum of compound **138a** in $CDCl_3$.

2.1.5.2. Biological studies of 3-{1-[(prop-2-yn-1-yloxy)benzylidenehydrazono]ethyl}-4-hydroxycoumarins

Biological studies on the compounds **138a-g** for anti-malarial and anti-trypanosomal activity and for cytotoxicity were conducted as described in section **2.1.1.4**. **Table 14** shows the bioassay results for the compounds, none of which exhibited significant activity against malarial pLDH enzyme or *T.b. brucei*. The compounds were also not significantly cytotoxic against HeLa cells. The propargyloxy group appears to decrease the bioactivity observed for the benzyloxy compounds **137a-d** and dihydroxy analogues (compounds **137e-g**).

Table 24. Bioassay data for compounds **138a-g**, showing % activity against pLDH, *T.b. brucei* and HeLa cells 20 μM.



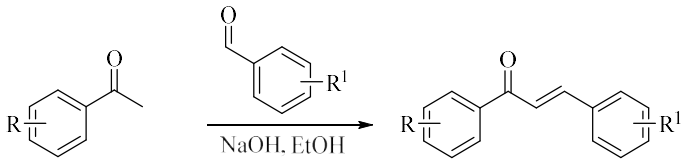
Compound	Ar	PLDH % viability [a]	*T.b. brucei* % viability [b]	Cytotoxicity % HeLa cells viability [c]
138a		100.00	96.35	78.49
138b		100.00	100.00	78.04
138c		100.00	94.57	84.71
138d		100.00	90.96	80.70
138e		100.00	64.77	72.24
138f		100.00	90.01	89.55
138g		100.00	80.49	79.38
Control		IC_{50} = 0.01μM [a]	IC_{50} = 0.022 μM [b]	IC_{50} = 0.021 μM [c]

Controls. [a] chloroquine [b] pentamidine [c] emetine

2.1.6. 3-(3-Phenylacryloyl)-4-hydroxycoumarins

These compounds were designed as chalcones containing a 4-hydroxy coumarin moiety and substituted phenyl groups. Chalcones and their analogues have been reported to exhibit a number of biological activities that include anti-viral, anti-oxidant, anti-protozoal, cytotoxic, anti-malarial, anti-tubercular, anti-fungal, antibacterial, anti-histaminic and anti-ulcer,[334,335] while their cytotoxicity has drawn attention to their potential as anti-cancer compounds.[336] The varied bioactivities of chalcones have a downside in terms of challenges with target selectivity as it has been discovered that a number of chalcones, natural and synthetic, have exhibited what Zhou *et al.* refer to as 'potential promiscuous target profiles'.[337] Such selectivity impact on the development of clinical chalcone-based agents, and ongoing studies on chalcone scaffolds have been aimed at gaining a greater understanding of their multi-target behaviour for possible guidance on motifs to enhance desirable effects while minimising the undesirable effects for specific targets.[337,338]

The synthesis of chalcones typically involves an acid- or base-catalysed Claisen-Schmit condensation of a ketone and aldehyde, and subsequent dehydration (**Scheme 26**).[335] The ease of synthesis of chalcones and the wide variety of biological activities have made them attractive research targets.[337] Natural and synthetic chalcones are receiving attention in the quest for novel and potent therapeutic agents for diseases, such as HIV, cancer, malaria and TB.[339] The loss or partial removal of the –CO-CH=CH- group (the ketoethylenic group or α,β-unsaturated ketone motif) results in the loss of observed bioactivity and, hence, this motif is considered to be the major pharmacophore.[340,341] This highly reactive electrophilic group can bond irreversibly with biological molecules leading to effects, such as carcinogenicity, induction of mutations and allergenic responses, which explain some of the negative biological effects of chalcones.[338] In a study on potential HIV-1 IN inhibitors, the chalcone **157** was found to be the most potent and exhibited an $IC_{50} = 2 \pm 1$ μM for both strand transfer and 3'-processing (**Figure 70**).[342,343] Cole *et al.*[344] reported the synthesis of chalcones **159** and **160** (**Figure 70**) which exhibited HIV inhibition of greater than 92% at 10 μM. Chalconoid **160** (**Figure 70**) is plant-derived and has been reported to show anti-HIV activity with an EC_{50} of 0.022 μg/mL.[335]



Scheme 26. Synthesis of chalcones.

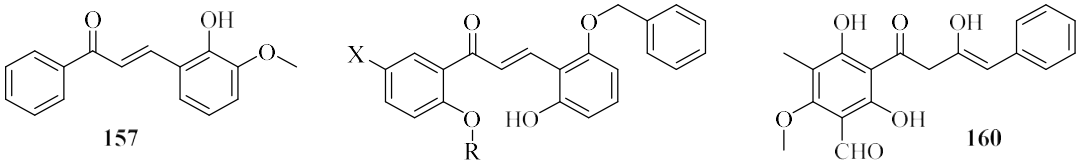
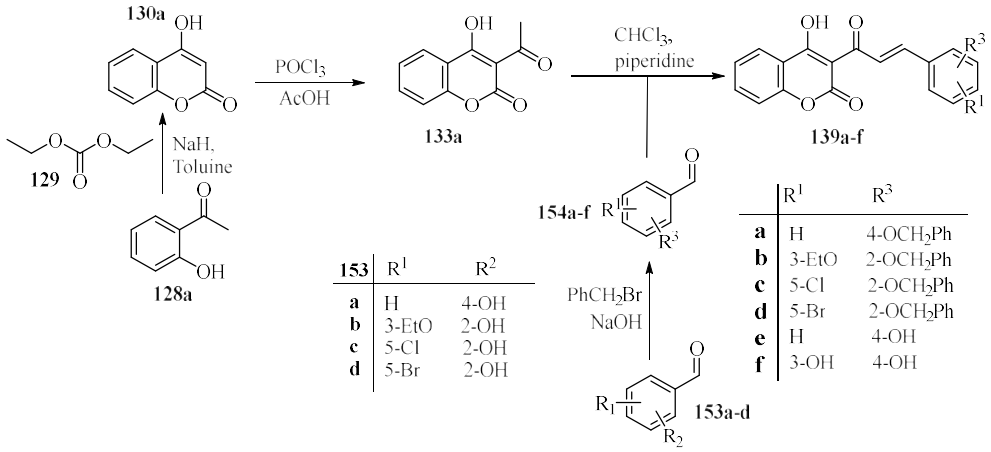


Figure 70. Anti-HIV chalcones.

2.1.6.1. Synthesis of 3-(3-phenylacryloyl)-4-hydroxycoumarins

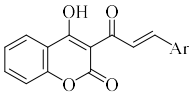
The pathway to 3-(3-phenylacryloyl)-4-hydroxycoumarins **139a-f** involved the reaction of 3-acetyl-4-hydroxycoumarin **133a**, (obtained, as described earlier, in two steps from 2-hydroxy acetophenone) with aldehydes **154a-f**. The aldehydes **154e** and **f** were available commercially and the *o*-benzylated analogues **154a-d** were prepared by the benzylation of the corresponding phenolic precursors **153a-d**. 3-Acetyl-4-hydroxycoumarin **133a** was reacted with each of the aromatic aldehydes **154a-f** in $CHCl_3$ at 80 ^{o}C with piperidine as the catalyst. The solvent was distilled off and the residue was washed with methanol to give the desired product in yields of 54-70%. The ^{1}H NMR spectrum of **139b** reveals the expected methyl proton triplet at 1.42 ppm, the ethyloxy-methylene protons resonating as a quartet at 4.04 ppm, the benzyloxy-methylene protons as a singlet at 5.04 ppm and the aromatic proton signals between 6.8 and 8.1 ppm, integrating for the required number of 12 protons (**Figure 71**). The two doublets at 8.26 and at 8.37 ppm represent the 2'-CH and 3'-CH vinylic protons; the large coupling constant of 16 Hz suggests *trans*-stereochemistry of the double bond. The ^{13}C NMR spectrum contains the expected total of 25 signals and reveals the methyl carbon signal at 15.1 ppm, the ethyloxy-methylene carbon signal at 64.6 ppm, the benzyloxy-methylene carbon signal at 76.0 ppm and the coumarin C-3 signal at 100.8 ppm. The vinylic carbons (C-2 and C-3) resonate at 123.3 and 142.6 ppm, while the coumarin and ketone carbonyl signals are evident at 160.3 and 192.5 ppm. (**Figure 72**). The DEPT 135 NMR spectrum (**Figure 73**) shows the expected two methylene carbon signals at 64.6 and 76.0 ppm respectively. The COSY spectrum (**Figure 74**) clearly reveals the correlations between the *o*-ethyl group protons. The HSQC and HMBC spectra (**Figures 75** and **76**) permitted

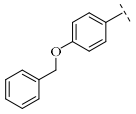
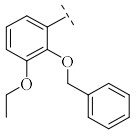
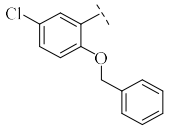
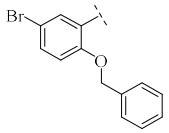
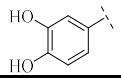
correlation of the vinylic ^{1}H and ^{13}C NMR signals. The correlations between the vinylic proton signal at 8.37 ppm (3'-CH) and the carbonyl carbon signals at 192.5 ppm (C-1') and 148.3 revealed in **Figure 76** helped confirm that the vinylic proton resonating at 8.37 ppm was the one further away from the carbonyl group. The 2'-CH signal clearly does not correlate with the signal at 148.3 ppm. Compounds **139a-f** are all novel and were fully characterised.



Scheme 27. Synthesis of 3-(3-phenylacryloyl)-4-hydroxycoumarins **139a-f**.

Table 25. Yields of 3-(3-phenylacryloyl)-4-hydroxycoumarins **139a-f**.



Compound	Ar	Isolated Yields (%)
139a		63
139b		50
139c		58
139d		63
139e		65
139f		65

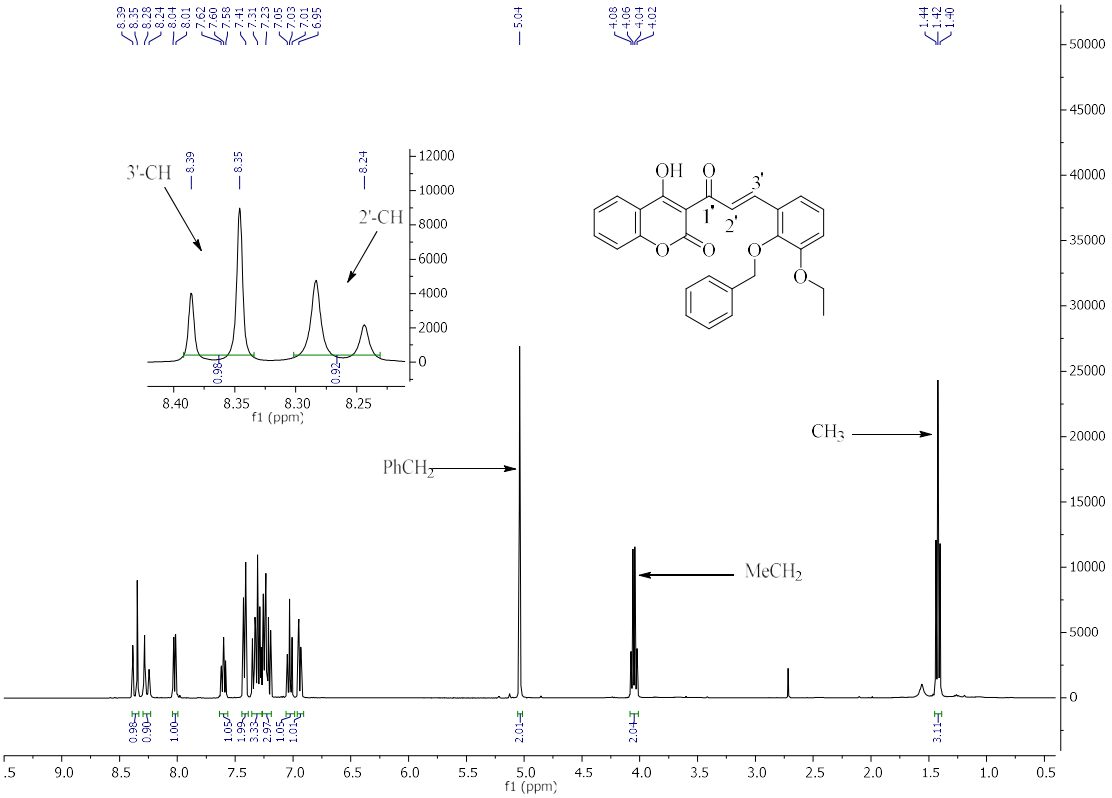


Figure 71. 400 MHz ^{1}H NMR spectrum of compound **139b** in $CDCl_3$.

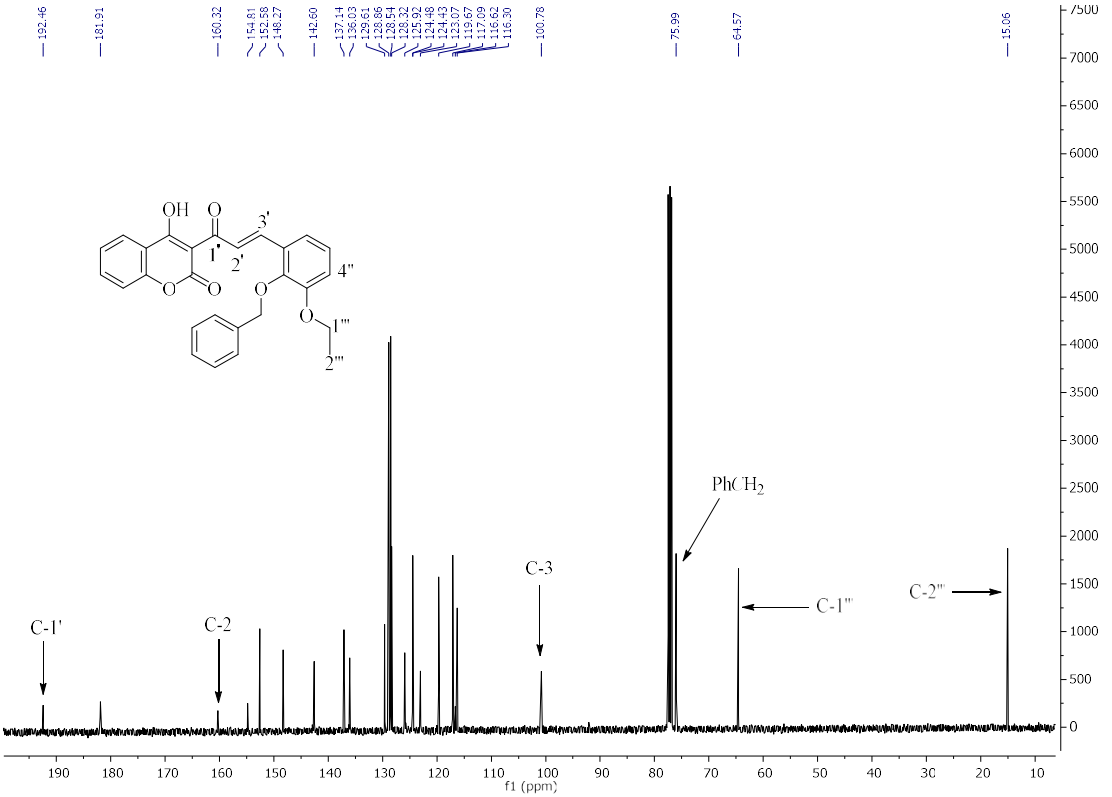


Figure 72. 100 MHz ^{13}C NMR spectrum of compound **139b** in $CDCl_3$.

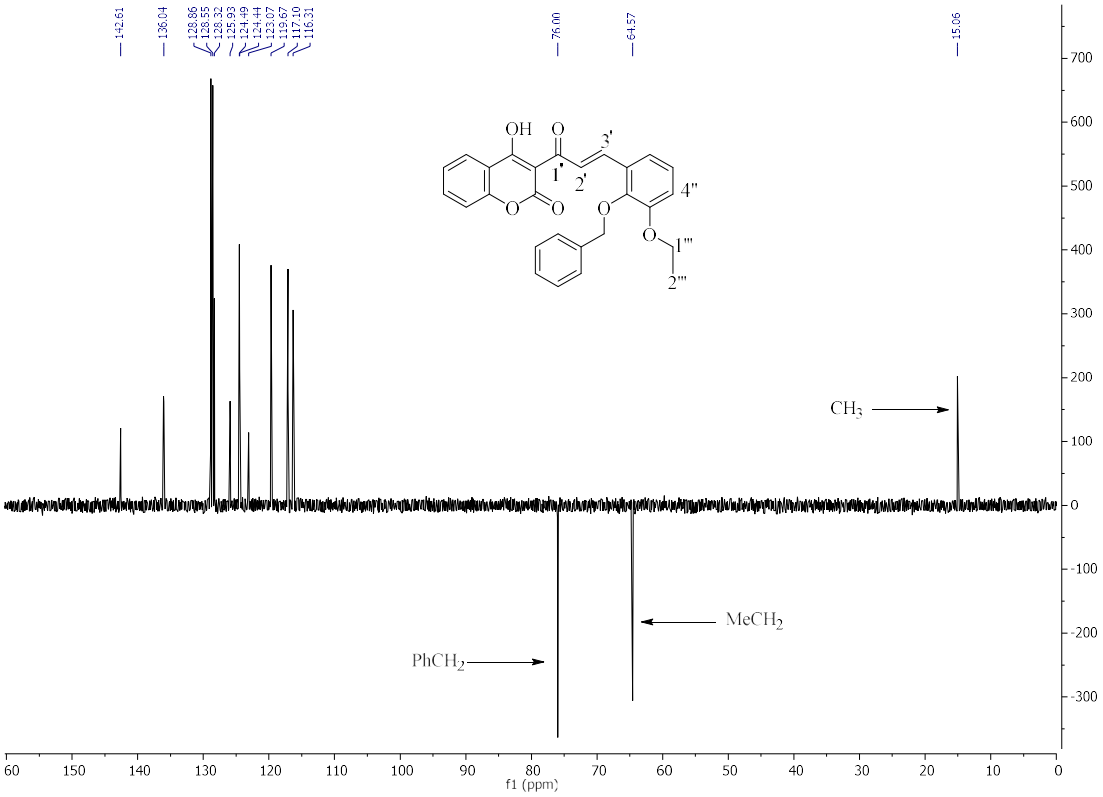


Figure 73. DEPT135 NMR spectrum of compound **139b** in $CDCl_3$.

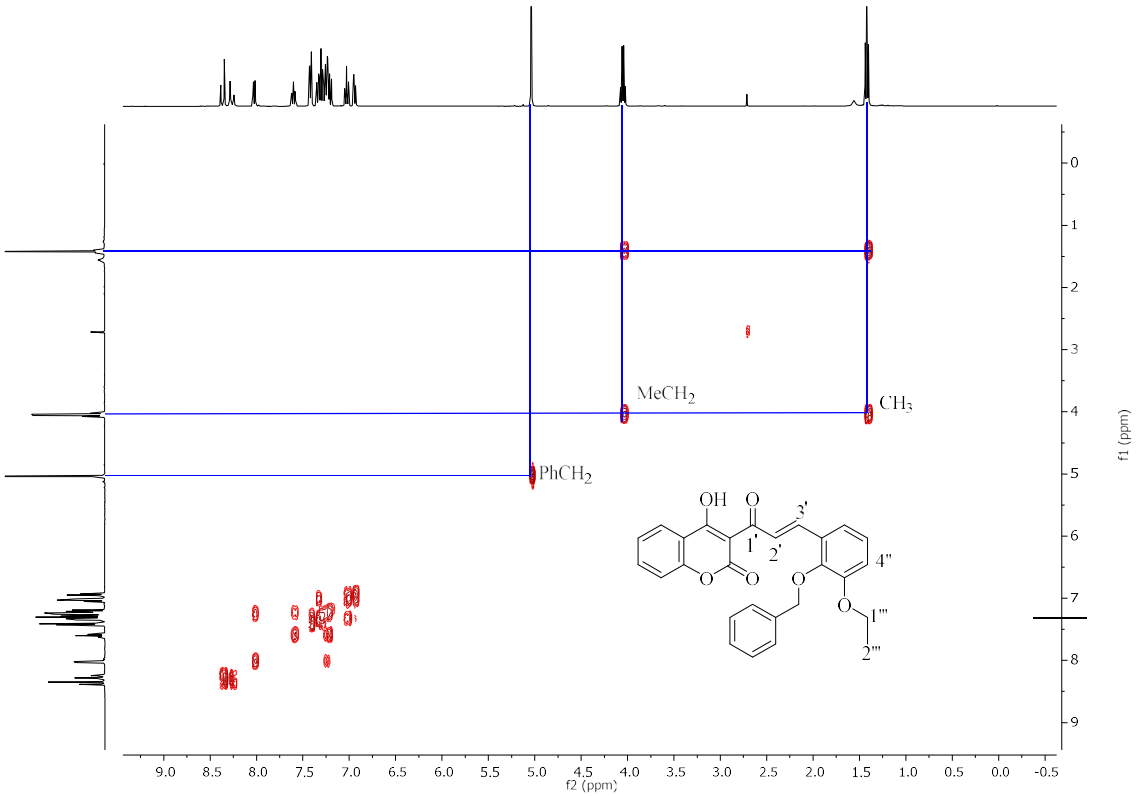


Figure 74. 400 MHz COSY NMR spectrum of compound **139b** in CDCl3.

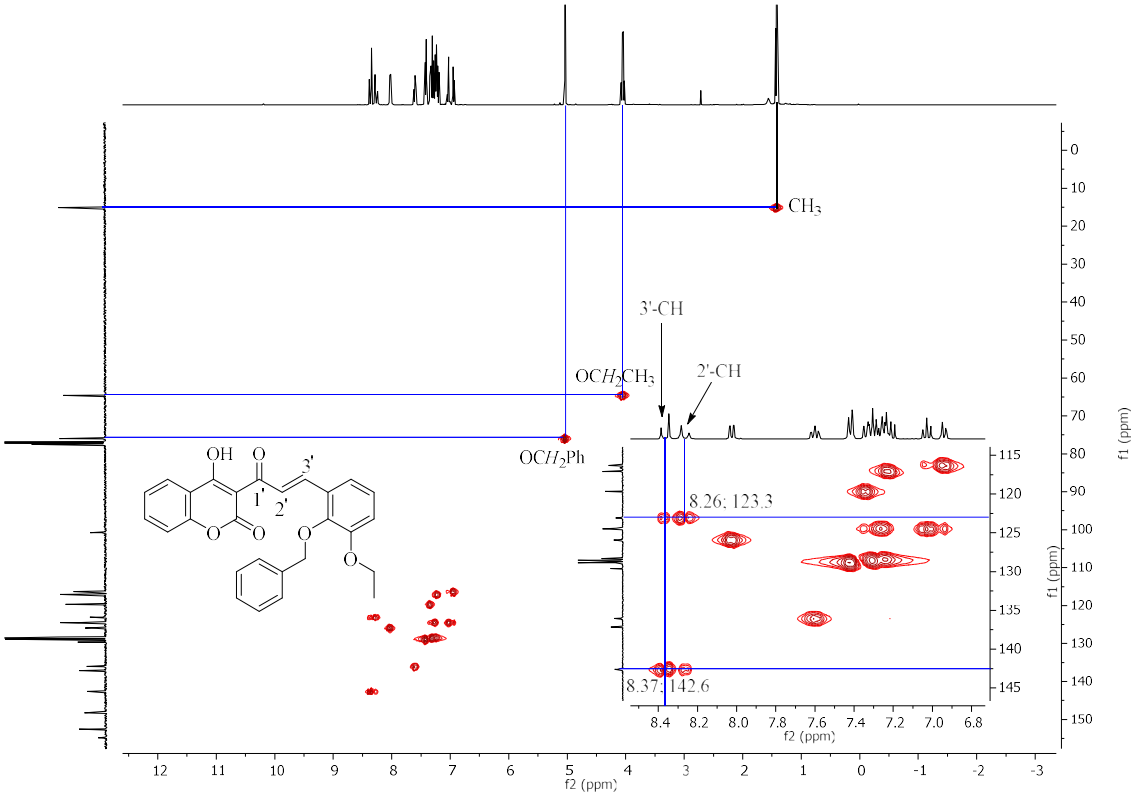


Figure 75. HSQC NMR spectrum of compound **139b** in $CDCl_3$.

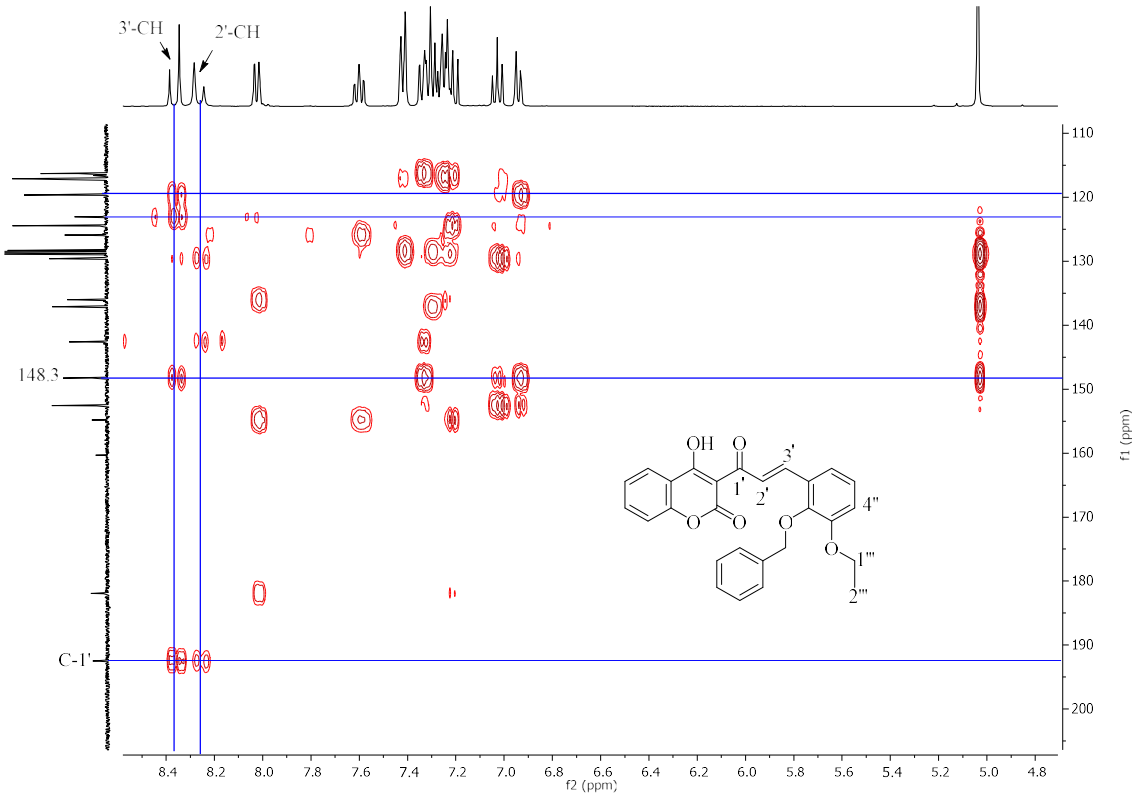


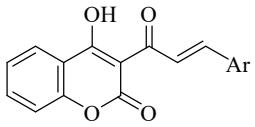
Figure 76. HMBC NMR spectrum of compound **139b** in $CDCl_3$.

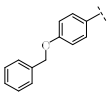
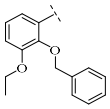
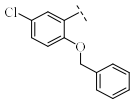
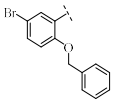
2.1.6.2. Biological studies of 3-(3-phenylacryloyl)-4-hydroxycoumarins 139a-f

2.1.6.2.1. *In vitro* studies

Biological studies on 3-(3-phenylacryloyl)-4-hydroxycoumarins **139a-f** for anti-HIV-1 IN, anti-HIV-1 PR, anti-malarial, trypanocidal and cytotoxicity were conducted as described in section **2.1.1.4**. **Table 26** shows the bioassay results for the compounds. The compounds exhibited moderate activity against HIV-1 IN with half of the compounds inhibiting HIV-1 IN by about 50%. HIV-1 PR inhibition, however, was very poor. Only two compounds **139e** (% inhibition of 26.40) and **139f** (% inhibition of 18.34) exhibited any anti-HIV-1 PR activity. These had hydroxy substituents which may suggest the importance of the OH group for anti-HIV-1 PR and IN activity for these compounds. It is apparent that none of the compounds exhibited significant anti-malarial activity. Half of the compounds exhibited activity against *T.b. brucei* with parasite viabilities below 24% at 20 μM. The most potent compound, **139f** reduced *T.b. brucei* viability to 7.86% followed by **139e** at 15.54% and **139c** at 23.17%. While the anti-*T.b. brucei* activity potential of the three compounds are encouraging, the compounds showed significant toxicity against HeLa cells at 20 μM. For the more bioactive compounds only compound **139c** had % viability for HeLa cells of above 50%. Due to toxicity concerns the compounds that showed significant bioactivity were not carried over to IC_{50} testing. A study on how the compounds can be made less toxic may permit some benefit to be drawn from these results. The cytotoxicity of the chalcone derivatives appears to be consistent with general literature reports on the anti-cancer, anti-proliferation activities and multi-target activities of chalcone derivatives.

Table 26. Bioassay data for compounds **139a-g**, showing % inhibition of IN and PR, and % viability of pLDH, *T.b. brucei* and HeLa cells at 20 µM.



Compound	Ar	HIV-1 % Integrase inhibition [a]	HIV-1% Protease inhibition [b]	PLDH % viability [c]	*T.b. brucei* % viability [d]	HeLa cells % viability [e]
139a		38.94	0.00	100.00	100.00	88.87
139b		44.69	0.00	100.00	98.48	89.55
139c		48.67	0.00	100.00	23.17	51.64
139d		48.67	0.00	100.00	82.39	84.58
139e		31.86	26.40	100.00	15.54	29.19
139f		41.59	18.34	98.95	7.86	25.51
		95.58 [a]	100.00 [b]	IC_{50} = 0.011µM [c]	IC_{50} = 0.022 µM [d]	IC_{50} = 0.021 µM [e]

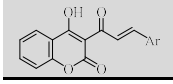
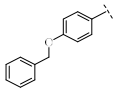
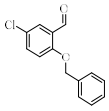
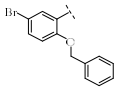
Controls. [a] Chicoric acid; [b] ritonavir; [c] chloroquine [d] pentamidine [e] emetine

2.1.6.2.2. *In silico* studies

There were no *in vitro* studies conducted for *Mtb* for compounds **139** but studies on the docking of these compounds in HIV-1 IN and PR and in *P. falciparum*, *T.b. brucei* and *Mtb* enzymes were conducted as described in section **2.1.1.4.2**. The results for the particular PDB structures used for docking are presented in **Table 27**. Generally the compounds exhibited strong binding to 5FRN with two of them (compounds **139e**: -9.296 kcal/mol; and **139f**: -9.456 kcal/mol) exhibiting stronger binding than the control, L-chicoric acid (-8.403 kcal/mol). This would suggest that the compounds could serve as good inhibitors of HIV-1 IN. The weakest binding ligand was compound **139a** (-6.426 kcal/mol) while the strongest binding was compound **139f** (-9.456 kcal/mol). The two best-binding ligands, compounds

139f (-9.456 kcal/mol) and **139e** (-9.296 kcal/mol) have OH substituents on the aryl moiety and this may again reflect the importance of the OH group in hydrogen-bonding interactions between the ligand and the receptor. Compound **139f** which has two OH groups on the aryl moiety exhibited the strongest binding and this suggests that the interactions may be enhanced by increasing the number of OH substitiuents on the aryl moiety. However these results bear little correlation with the *in vitro* results in terms of which ligands were good or bad. The docking protocol may not have covered some factors which were critical *in vitro* hence the disparity. Affinity for the HIV-1 PR enzyme 1YT9 was also high ranging from -7.175 kcal/mol (compound **139d**) to -8.768 kcal/mol (**139e**). The strongest binding ligand exhibited binding that was stronger than the control ligand ritonavir, whose binding affinity was -8.403 kcal/mol. The high binding affinities suggest their potential as inhibitors of HIV-1 PR but this was not corroborated generally by the *in vitro* studies. However, it is worth noting that compound **139e** exhibited the best activity in both evaluations. The binding affinities for the HIV-1 PR enzyme 1ZP8 were lower than for 1YT9. The strongest binding ligand was compound **139e** (-6.213 kcal/mol). The four best-binding ligands exhibited binding affinities that ranged from -5.610 kcal/mol (**139c**) to -6.213 kcal/mol (**139e**). These compare favourably with the -7.042 kcal/mol binding affinity exhibited by the control ligand, ritonavir. Interestingly compound **139e** was one of the two best-binding ligands in the evaluation with 5FRN and the best-binding ligand with both 1YT9 and 1ZP8 and exhibited some *in vitro* activity against HIV-1 PR. This suggests potential for this compound as an agent that might target both HIV-1 IN and HIV-1 PR.

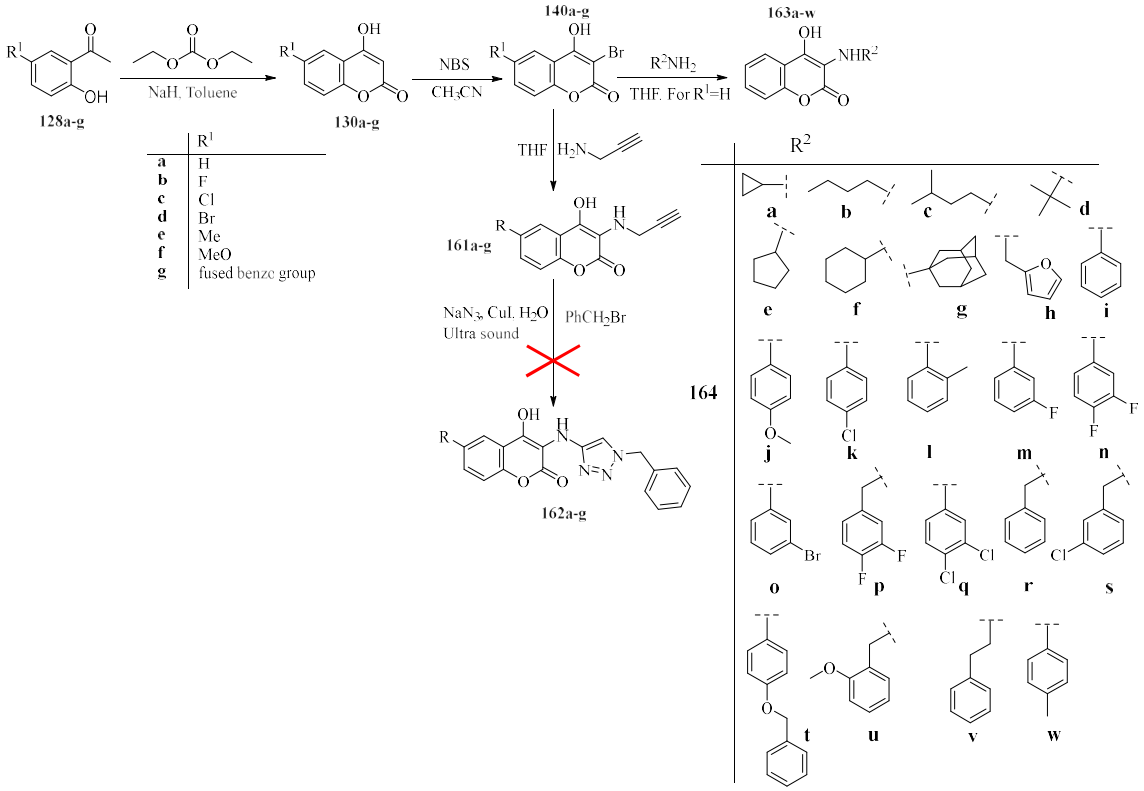
Table 27. Binding affinities for the docking of ligands **139** in the active sites of various enzymes.

	Ar	HIV-1 IN 5FRN (kcal/mol)	HIV-1 PR 1YT9 (kcal/mol)	HIV-1 PR 1ZP8 (kcal/mol)	*Pf* 1T2C (kcal/mol)	*Pf* 1V0O (kcal/mol)	*T.b. brucei* 3FWN (kcal/mol)	*Mtb* 4BFW (kcal/mol)
139a		-6.426	-7.178	-3.875	-6.761	-4.612	-4.277	-4.413
139b		-8.176	-7.849	-6.185	-6.032	-4.772	-4.153	-6.252
139c		-7.970	-7.876	-5.610	-6.823	-3.408	-3.341	-4.910
139d		-7.821	-7.175	-5.408	-6.304	-3.232	-3.109	-5.137
139e		-9.296	-8.768	-6.213	-7.265	-4.373	-4.174	-4.523
139f		-9.456	-7.353	-5.938	-6.883	-4.406	-4.948	-4.496

The compounds exhibited strong affinity for the *P. falciparum* 1T2C. The weakest binding was compound **139b** (-6.032kcal/mol) while the strongest binding was compound **139e** (-7.265 kcal/mol). All the compounds exhibited stronger binding affinities than chloroquine; however, none of the compounds exhibited significant activity *in vitro*. This may indicate the presence of essential factors critical for activity which could not be taken into account in the docking protocol. The binding affinity for *P. falciparum* enzyme 1V0O was not as high as that exhibited for 1T2C. The majority of the compounds exhibited affinities above -4 kcal/mol. The compounds did not exhibit strong binding towards the *T.b. brucei* enzyme 3FWN in comparison with pentamidine (-6.017 kcal/mol). The affinities were in the range -3 kcal/mol to -5 kcal/mol. However, the strongest binding compound **139f** (-4.948 kcal/mol) was also the most active *in vitro* where it decreased *T.b. brucei* viability to 7.86 %. The other significantly active compounds *in vitro*, **139c** and **e** decreased *T.b. brucei* viability to 23.17 % and 15.54% but failed to exhibit significant binding affinities *in silico*. Two of the compounds [**139b** (-6.252 kcal/mol) and **139d** (-5.137 kcal/mol)] exhibited strong binding for the *Mtb* enzyme 4BWN suggesting their potential for anti-*Mtb* activity.

2.1.7. 3-Amino-4-hydroxycoumarins

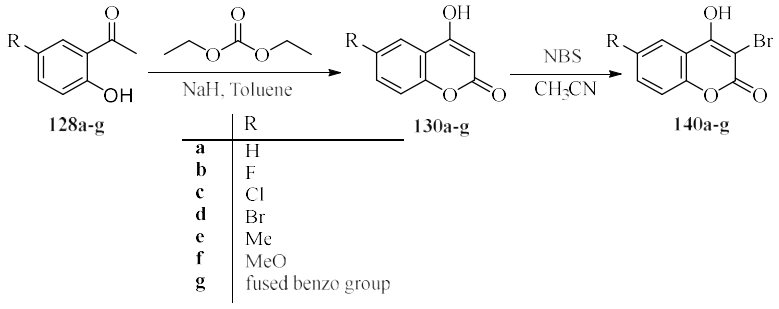
Functional groups that contain nitrogen and oxygen are prevalent in molecules of biological importance. Both nitrogen and oxygen have, among other properties, the capacity to participate in molecular interactions like hydrogen-bonding.[345] The diverse and significant biological activities of nitrogen-containing compounds have triggered considerable interest in these compounds lately.[346] Amines and *N*-heterocyclic moieties are typical constituents of many drug molecules.[347] The synthesis of 3-amino-4-hydroxycoumarins was selected as one way of introducing this all important element (N) into the 4-hydroxycoumarin derivatives to enhance their potential for biological activity. The route to the target compounds **(Scheme 28)** involved the conversion of 4-hydroxycoumarins (**130**) to 3-bromo-4-hydroxycoumarin derivatives (**140**) which were then converted to amino derivatives in two ways. Firstly, by reacting the brominated derivatives (**140**) with propargylamine to introduce the alkynyl group for use in click chemistry reactions and, secondly, through reactions with various primary amines to form secondary amines which were to be converted, in turn, to amides.



Scheme 28. Route to 3-amino-4-hydroxycoumarins.

2.1.7.1. Synthesis of 3-bromo-4-hydroxycoumarins 140a-g

Brominated aromatic compounds are important in organic synthesis. They are normally synthesised through electrophilic bromination and are used as precursors in the synthesis of compounds such as dyes, pharmaceuticals and agrochemicals.[348,349] A number of agents are used for such bromination and these include: *N*-halosuccinimides, NBS-$Pd(OAc)_2$, NBS-Al_2O_3, NBS-PTSA, *N*-halosaccharin, *N*-methylpyrrolidin-2-one hydrotribromide-H_2O_2, and NBS-DMF.[350] The 3-brominated 4-hydroxycoumarin derivatives (**140**) were synthesised as intermediates for the two series of compounds, the propargylamines (**161**) and the secondary amine derivatives (**163**). Each of 4-hydroxycoumarins **130a-g** was reacted with *N*-bromosuccinimide (NBS) in acetonitrile (CH_3CN) for at least 2 hours at room temperature (**Scheme 29**).[349] The solvent was then evaporated and the residue washed with acidified water, filtered and then washed with water and dried to give the products in yields of 21-73% (**Table 28**). The ^1H NMR spectrum of compound **140f** (**Figure 77**) reveals the methoxy protons resonating at 3.81 ppm as a singlet and the three aromatic proton signals in the range 7.2 to 7.5 ppm. The splitting of these is of interest. The 5-CH proton resonates at 7.40 ppm as a doublet instead of a singlet due to *meta*-coupling with the 7-CH proton resonating at 7.24 ppm (J = 2.95 Hz), which also couples with the 8-CH proton resonating at 7.34 ppm; hence the 7-CH signal appears as a doublet of doublets due to both *ortho* (J = 9.04 Hz) and *meta*-coupling (2.96 Hz). The ^{13}C NMR spectrum of compound **140f** reveals the methoxy carbon signal at 55.8 ppm, the C-3 signal at 89.6 ppm, the C-4 signal at 162.2 ppm and the expected total of ten carbon signals (**Figure 78**). Two-dimensional NMR spectroscopy COSY, HSQC and HMBC was employed to aid in the assignments.



	R
a	H
b	F
c	Cl
d	Br
e	Me
f	MeO
g	fused benzo group

Scheme 29. 3-Bromination of 4-hydroxycoumarins **130a-g**.

Table 28. Yields of the 3-bromination of 4-hydroxycoumarins **130a-g**.

Compound	R	Isolated Yield (%)
140a	H	73
140b	F	68
140c	Cl	42
140d	Br	43
140e	Me	68
140f	MeO	32
140g	5,6-Benzo	21

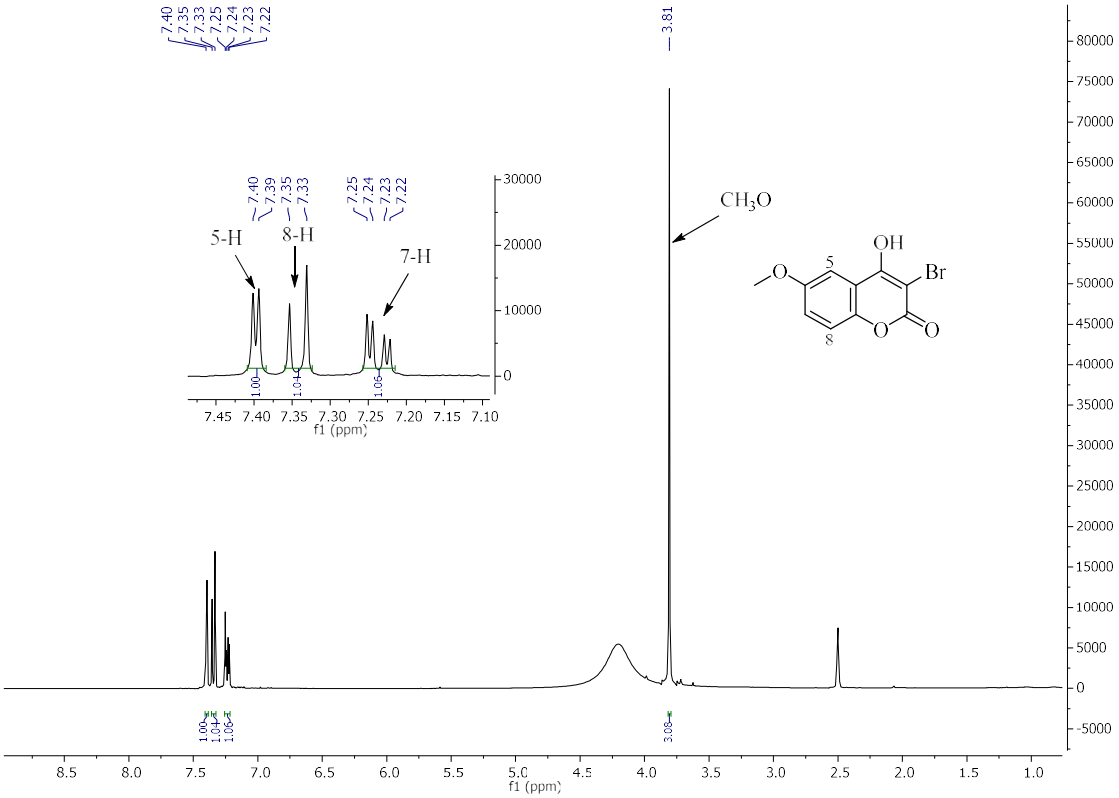


Figure 77. 400 MHz ^{1}H NMR spectrum of compound **140f** in DMSO-d_6.

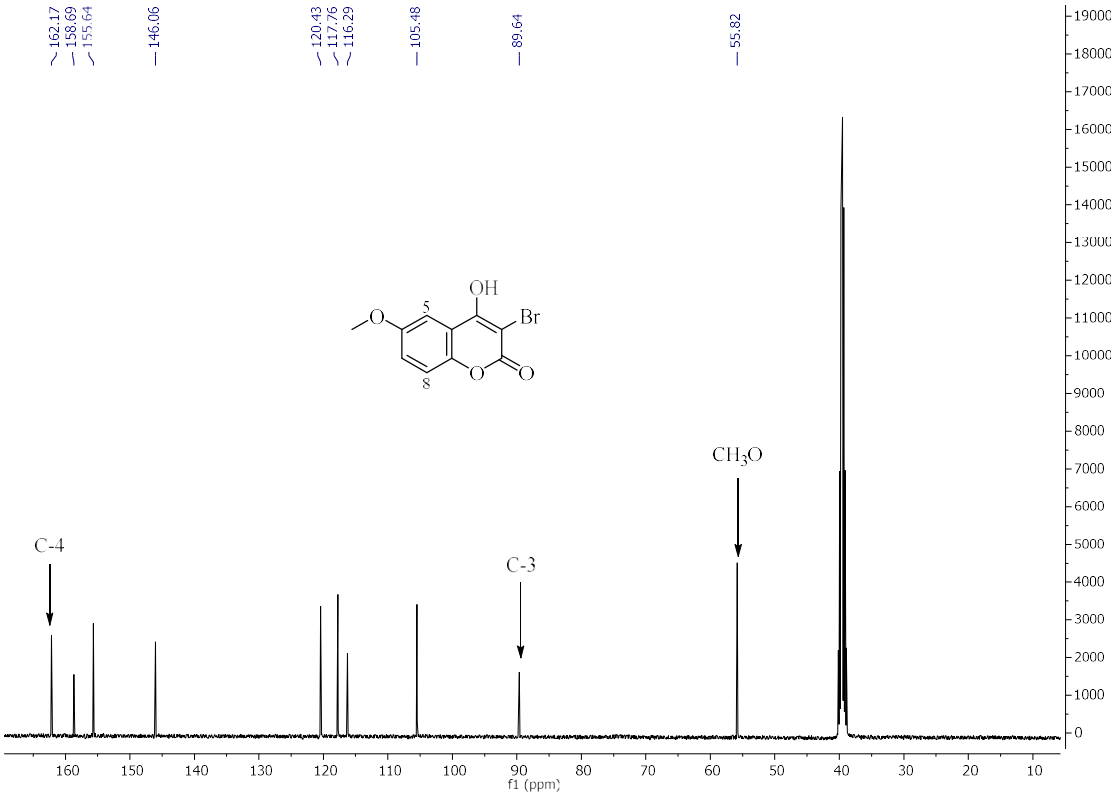
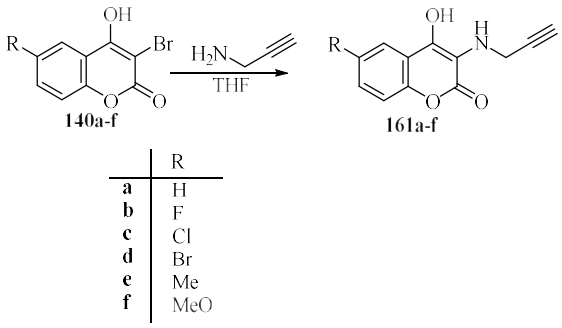


Figure 78. 100 MHz ^{13}C NMR spectrum of compound **140f** in DMSO-d_6.

2.1.7.2. Synthesis of 4-hydroxy-3-(prop-2-yn-1-ylamino)coumarins

A mixture of each of the compounds **140a-f** (synthesised as described in section **2.1.7.1**) and propargylamine in THF was stirred at room temperature for at least 2 hours (**Scheme 30**), after which, the solvent was evaporated off and the residue was washed with DCM and filtered to give the desired product in yields of 36-68% and in high purity (**Table 29**). The ^{1}H NMR spectrum of compound **161c** is shown in **Figure 78**. The methine proton signal (4'-CH) appears as a triplet at 3.59 ppm and the methylene protons' signal (2'-CH_2) as a doublet at 3.76 ppm. The splitting is due to the coupling between the methylene protons and the acetylenic proton. The 8-CH proton at 7.19 ppm resonates as a doublet due to *ortho*-coupling with the 7-CH (J = 8.68 Hz), the 7-CH (at 7.44 ppm) as a doublet of doublets due to *ortho*-coupling with 8-CH and *meta*-coupling with (J = 8.68 and 2.68 Hz respectively) 5-CH. The appearance of the 5-CH signal (at 7.74 ppm) as a doublet is due to *meta*-coupling (J = 2.68 Hz) with 7-CH proton, while the NH signal appears at 8.24 ppm. The ^{13}C NMR spectrum of compound **161c** reveals the methylene carbon signal at 28.5 ppm, the C-3 signal at 84.0 ppm and the C-4 signal at 167.9 ppm (**Figure 79**). The DEPT135 NMR spectrum of compound

161c reveals two quartenary carbon signals at 76.9 and 78.0 ppm respectively, a phenomenon due to the large $^{2}J_{C,H}$ coupling (**Figure 80**).



Scheme 30. Synthesis of 4-hydroxy-3-(prop-2-yn-1-ylamino)-coumarins **162a-f**.

Table 29. Yields of compounds **161a-f**.

Compound	R	Isolated Yield (%)
162a	H	68
162b	F	36
162c	Cl	53
162d	Br	40
162e	Me	58
162f	MeO	57

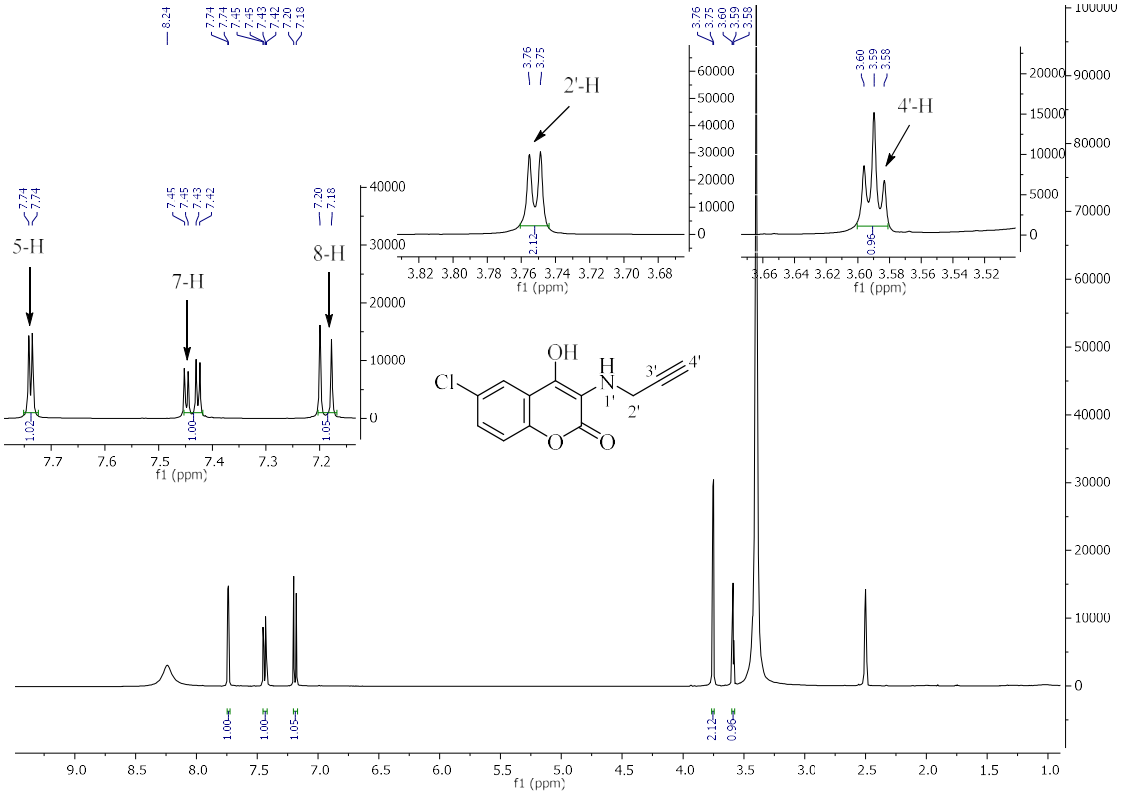


Figure 78. 400 MHz ^{1}H NMR spectrum of compound **161c** in DMSO-d_6 (inserted expansions show the respective signal multiplicities).

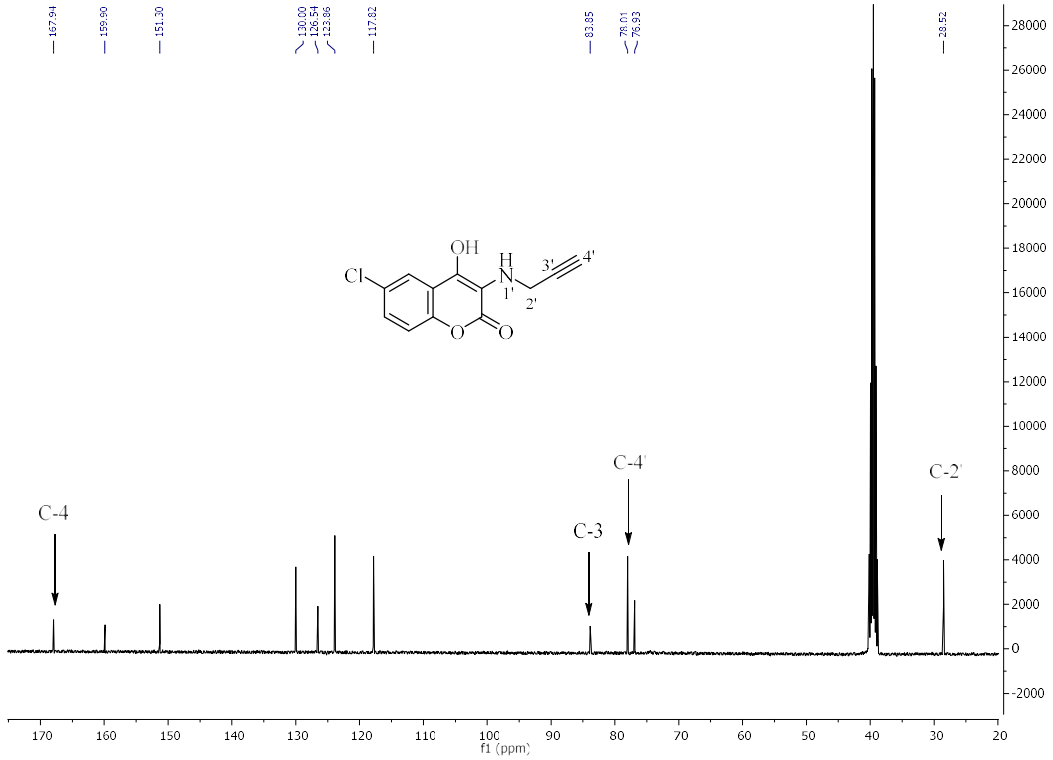


Figure 79. 100 MHz ^{13}C NMR spectrum of compound **161c** in DMSO-d_6.

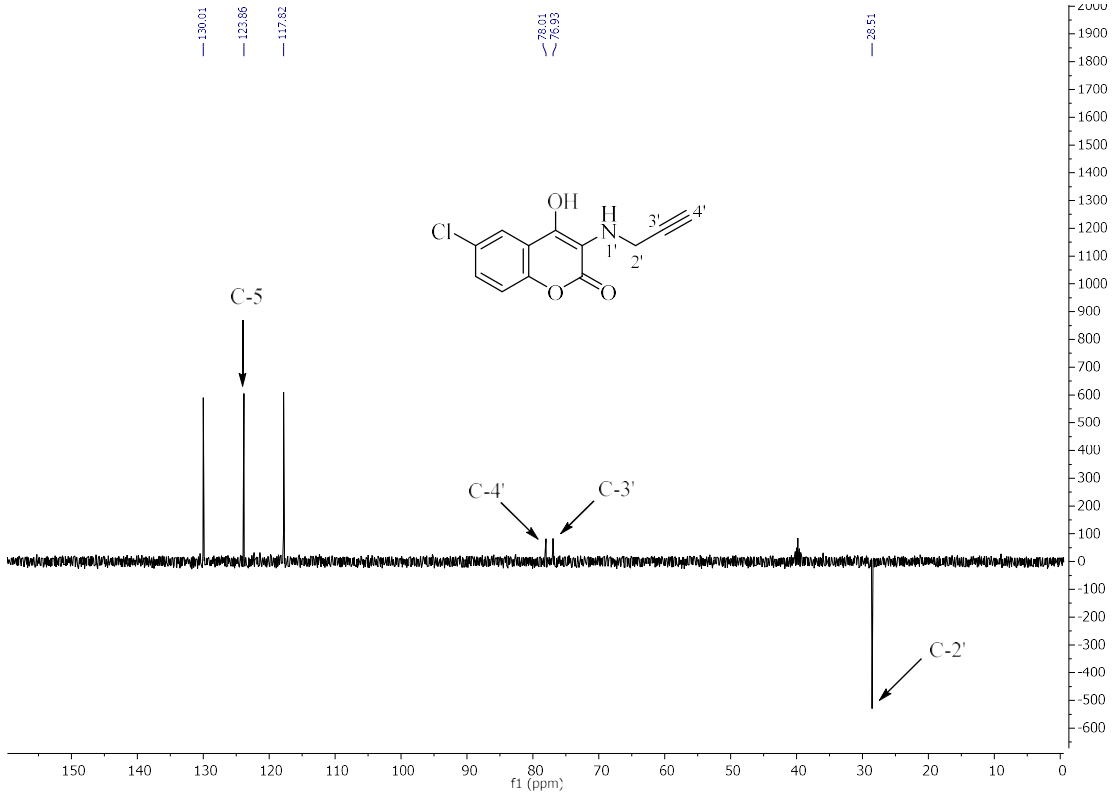
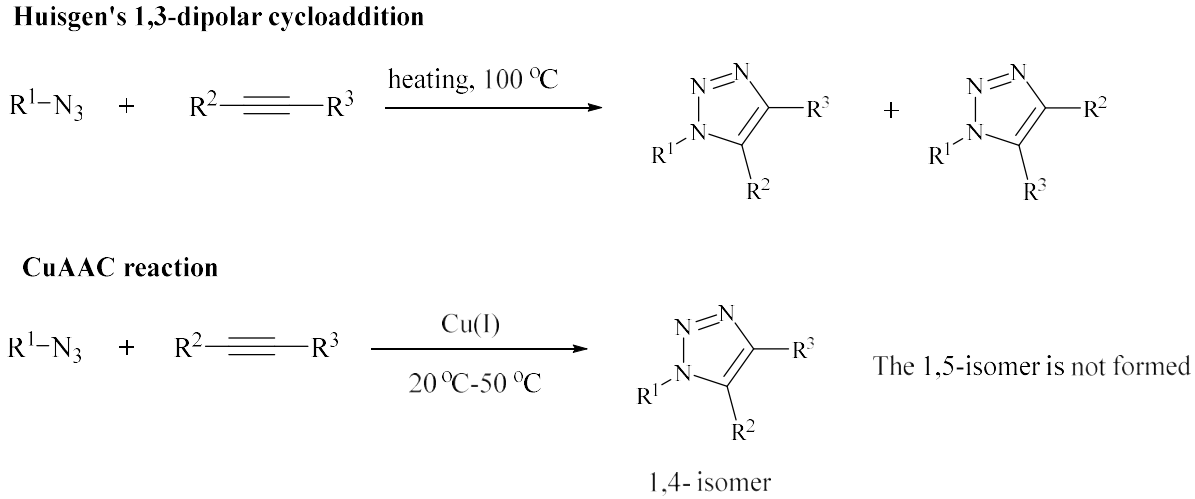


Figure 80. DEPT135 NMR spectrum of compound **161c** in DMSO-d_6.

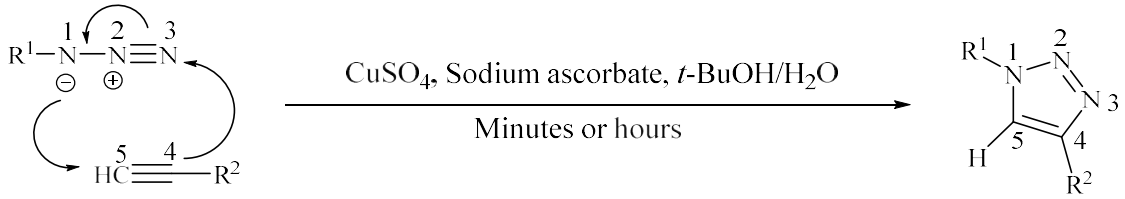
2.1.7.3. Click chemistry reactions with 4-hydroxy-3-(prop-2-yn-1-ylamino)-coumarins

"Click chemistry", as first described by Sharpless *et al.*,[351] applies to reactions that meet the criteria of being "…modular, wide in scope, give very high yields, generate only inoffensive by-products that can be removed by non-chromatographic methods, and be stereospecific (but not necessarily enantio-selective). The required process characteristics include simple reaction conditions (ideally, the process should be insensitive to oxygen and water), readily available starting materials and reagents, the use of no solvent or a solvent that is benign (such as water) or easily removed, and simple product isolation." There are a number of "click reactions" of which the Cu^{I}-catalyzed azide/alkyne cycloaddition (CuAAC) is the most widely used; other examples include pyridyl sulphide, oxime, Michael addition, Diels-Alder and thiol-ene reactions.[352] The CuAAC has greatly improved the synthesis of 1,2,3-triazoles by affording selectivity (yielding only 1,4-disubstituted triazoles), mild reaction conditions coupled with much faster reaction rates in comparison with the older Huisgen reaction which results in a mixture of 1,4 and 1,5-products.[352,353] **Scheme 31** shows the Huisgen 1,3-dipolar

cycloaddition and the CuAAC reaction, while **Scheme 32** shows the conditions for a typical CuAAC reaction.



Scheme 31. Huisgen 1,3-dipolar cycloaddition reaction and the CuAAC reaction.[352]

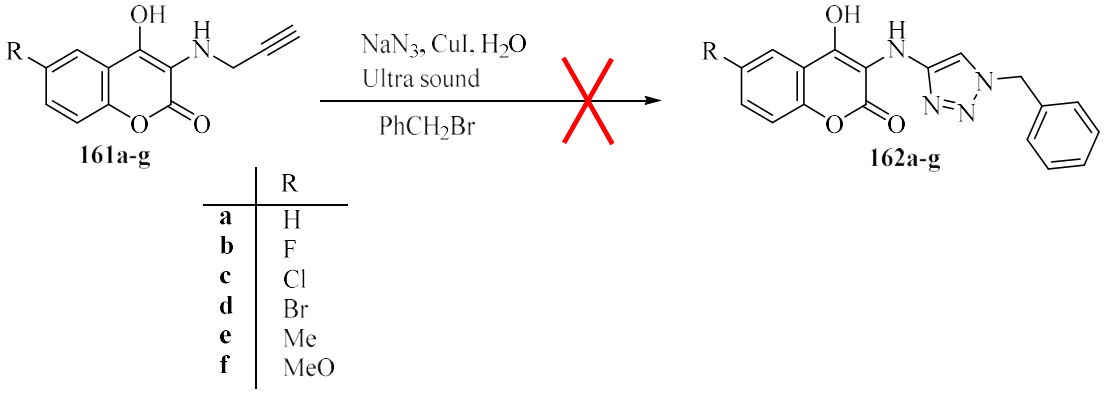


Scheme 32. Typical CuAAC reaction.[353]

In 2005 Zhang *et al.*,[354] reported an analogous reaction, *viz.*, RuAAC, through which 1,5-disubstituted triazoles can be accessed selectively. The RuAAC and CuAAC reactions have been vital tools in enhancing the versatility of the azide-alkyne click methodology which has been applied in diverse areas such as materials chemistry, drug discovery and combinatorial chemistry.[355,356] 1,2,3-Triazoles have been shown to exhibit diverse biological activities including anti-HIV, anti-tubecular, anti-cancer, anti-neuropathic, anti-bacterial, anti-fungal, anti-diabetic, β_3-adrenergic receptor agonist, and anti-histaminic.[356,357] The triazole moiety is present in a number of drugs, *e.g.* hexaconazole (anti-fungal drug), rizatriptan (anti-migrane drug), alprazolam (hyptonic, sedative and tranquilizer drug), and trazodone (anti-depressant drug).[358] The triazole nucleus having three nitrogen atoms interacts readily and non-covalently with receptors and enzymes - an important factor in their biological activities.[357]

The synthesis of the triazole derivatives **162a-g** was attempted following the protocol reported by Sreedhar and Reddy, in which ultra-sound is used to access 1,4-disubstituted 1,2,3-triazoles.[359] Each of the terminal alkynes **161a-f**, benzyl bromide, sodium azide and CuI

were mixed with water and sonicated for at least 20 minutes (**Scheme 33**) after which more water was added with the aim of precipitating the product. In each case, no precipitation was observed and the expected product failed to form.

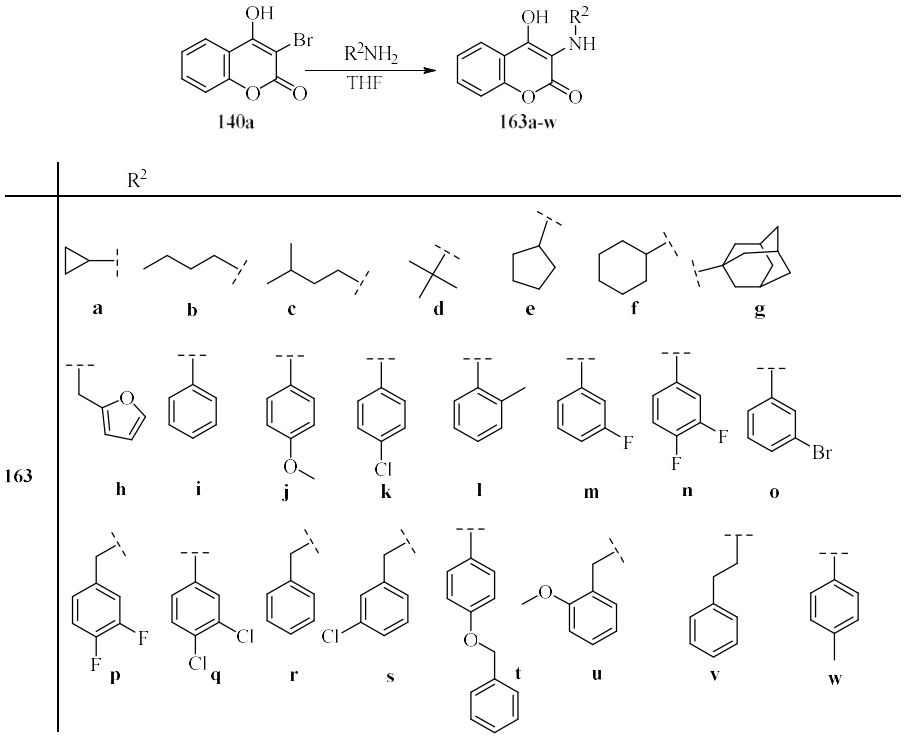


	R
a	H
b	F
c	Cl
d	Br
e	Me
f	MeO

Scheme 33. Attempted synthesis of 1,4-disubstituted 1,2,3-triazole 4-hydroxycoumarin derivatives.

2.1.7.2. Synthesis of 3-amino-4-hydroxycoumarins

A mixture of compound **140a** (synthesised as described in section **2.1.7.1**) and each of primary amines R^2NH_2 in THF was stirred at room temperature for at least 2 hours (**Scheme 34**) after which the solvent was evaporated off and, in cases where the residue was a solid, it was washed with DCM and filtered off to give the desired product. In other cases which gave liquid residues, the residue was treated with a 1:3 hexane:DCM mixture to precipitate out the product. In all cases the products were obtained in high purity and further purification was not considered necessary.

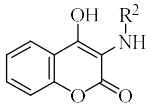


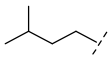
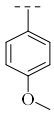
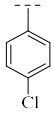
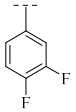
Scheme 34. Synthesis of 3-amino-4-hydroxycoumarins

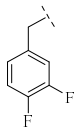
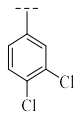
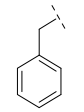
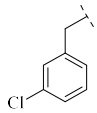
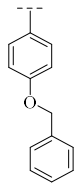
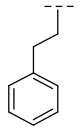
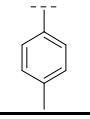
Yields of the compounds were in the range 27-87% (**Table 30**). The ^{1}H NMR spectrum of compound **163b**, is shown in **Figure 81**. The methyl proton signal appears as a triplet at 0.85 ppm due to its interaction with the 3$^{'}$-CH_2 protons, the signal for the 3$^{'}$-CH_2 protons, appears as a sextet at 1.30 ppm as it is split by the comparable coupling of three protons of the neighbouring methyl group and the two protons of the neighbouring methylene group 2$^{'}$-CH_2. The 2$^{'}$-CH_2 protons resonate as a quintet at 1.54 ppm due to comparable coupling with the neighbouring protons of the 1$^{'}$-CH_2 and 3$^{'}$-CH_2 groups. The 1$^{'}$-CH_2 methylene protons resonate as an apparent triplet. However, the relative intensities of the peaks do not match the expected 1:2:1 ratio for coupling to two equivalent neighbouring protons, and the observed "triplet" is attributed to overlap of the triplet due to coupling with the vicinal 2'-methylene proton and a doublet due to coupling with the NH proton. The expected aromatic proton signals appear in the range 7.0-8.0 ppm and integrate for the expected 4 protons also confirmed *via* COSY. The ^{13}C NMR spectrum of compound **163b** reveals the methyl carbon signal at 13.9 ppm and the three other alkyl carbon signals at 20.7, 30.6 and 40.5 ppm respectively (**Figure 82**). The C-3 signal appears at 86.8 ppm, the carbonyl carbon signal at 173.7 ppm, and the expected total of 13 carbon signals are clearly revealed in the spectrum.

The DEPT135 NMR spectrum of compound **163b** reveals the methyl carbon signal at 13.9 ppm, the three methylene carbon signals (pointing downwards) at 20.7, 30.6 and 40.5 ppm and the four expected aromatic CH carbon signals belonging to the coumarin moiety (**Figure 83**).

Table 30. Yields of 3-amino-4-hydroxycoumarins **163a-w**



Compound	R^2	Isolated Yield (%)
163a		74
163b		75
163c		66
163d		79
163e		67
163f		75
163g		77
163h		79
163i		79
163j		66
163k		71
163l		70
163m		83
163n		64
163o		60

163p		87
163q		27
163r		72
163s		74
163t		82
163u		87
163v		77
163w		87

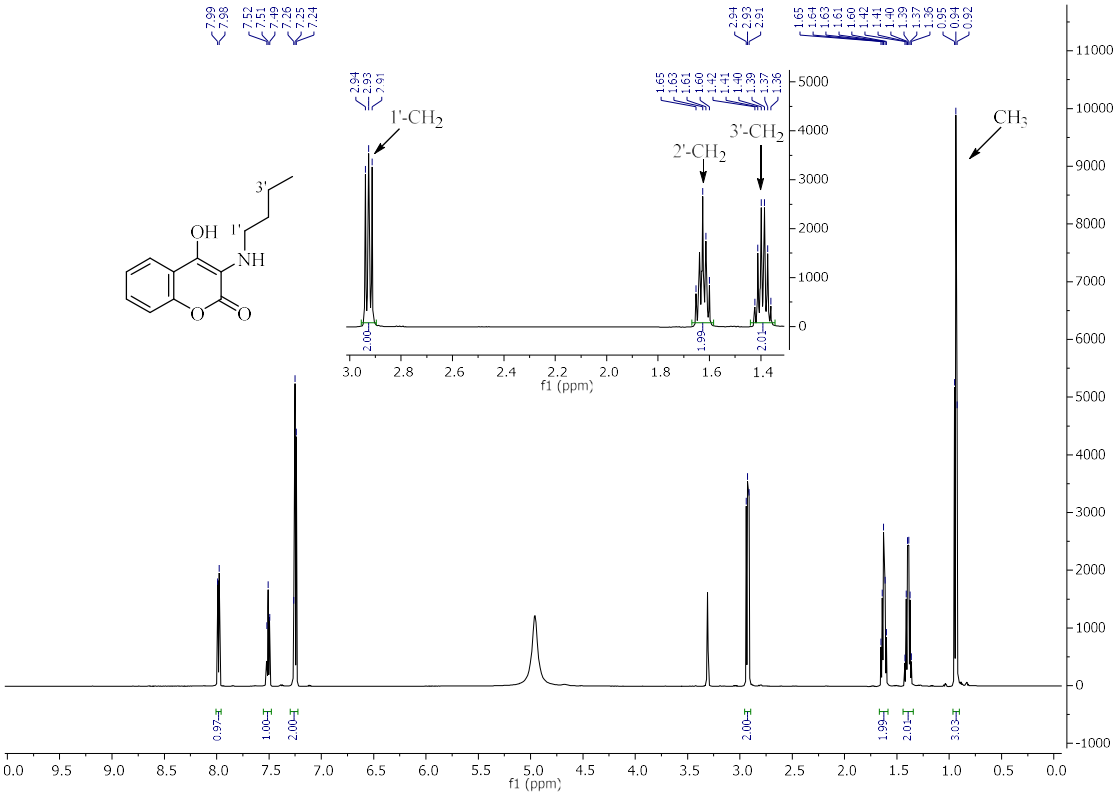


Figure 81. 600 MHz ^{1}H NMR spectrum of compound **163b** in methanol-d_4.

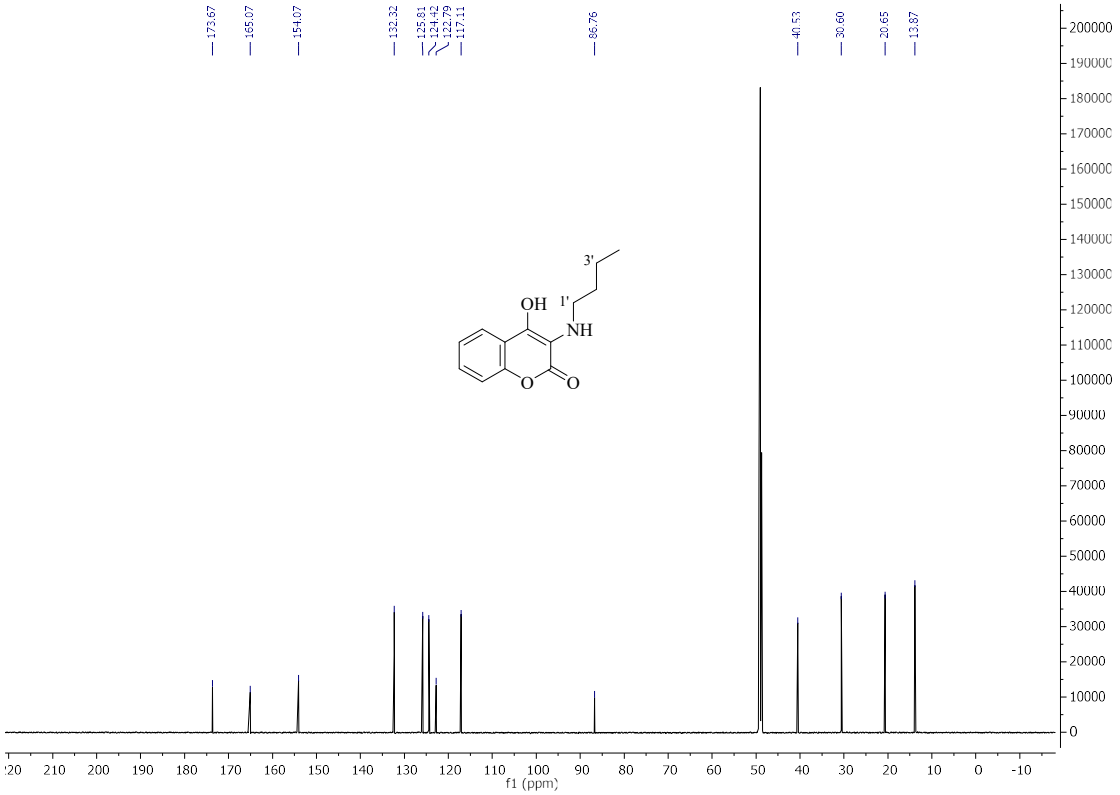


Figure 82. 150 MHz ^{13}C NMR spectrum of compound **163b** in methanol-d_4.

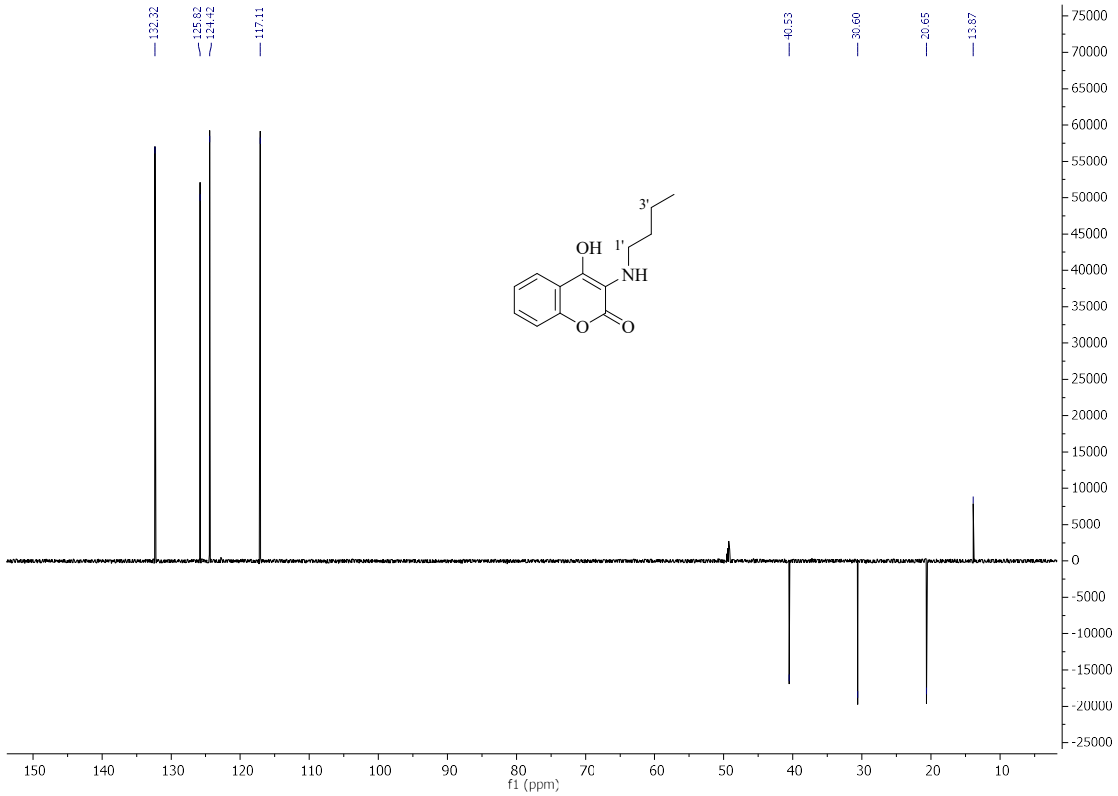
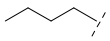
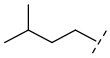
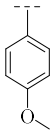
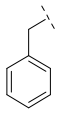


Figure 83. DEPT135 NMR spectrum of compound **163b** in methanol-d_4.

2.1.7.3. Biological studies of the 3-amino-4-hydroxycoumarins

Bioassays were conducted on samples of selected compounds, namely **163a-f, j and r** for anti-HIV-1 IN, anti-HIV-1 PR, anti-malarial and trypanocidal activity and for cytotoxicity. The assays were conducted using 20 μM solutions except for the cytotoxicity assays which were conducted at 25 μM. The results were disappointing as none of the compounds showed significant activity (**Table 31**). The highest % HIV-1 IN inhibition was effected by compound **163e** at 12.27%, while the highest % HIV-1 PR inhibition was exhibited by compound **163c** at 9.60%. All of the compounds exhibited a measure of activity against pLDH with **163f and 163j** decreasing % PLDH viability to 76.93 and 62.58%, respectively. Only two compounds, **163e** and **163f** exhibited any activity against *T. brucei*, but the effects were less than 4%. The cytotoxicity results revealed that a number of the compounds decreased HeLa cell viability but the effects are not considered significant.

Table 31. Bioassay data for compounds **163a-g**, showing % inhibition of IN and PR, and % activity against pLDH, *T.b. brucei* at 20 μM and HeLa cells at 25 μM and HeLa cells concentration.

Compound	R^2	HIV-1 % Integrase inhibition [a]	HIV-1% Protease inhibition [b]	PLDH % viability [c]	*T.b. brucei* % viability[d]	HeLa cells% viability [e]
163a		3.37	4.54	86.04	100.00	70.89
163b		0.00	0.00	91.27	100.00	94.67
163c		0.00	9.60	96.06	100.00	100.00
163d		4.36	0.00	89.09	100.00	85.47
163e		12.27	0.83	89.29	98.40	100
163f		1.08	0.09	76.93	96.16	73.07
163j		0.00	6.55	62.58	100.00	68.65
163r		0.00	0.00	91.77	100.00	100.00
Controls		100.0 [a]	99.75 [b]	IC_{50} = 0.010μM [c]	IC_{50} = 0.062 μM [d]	IC_{50} = 0.019 μM [e]

Controls: [a] chicoric acid; [b] ritonavir; [c] chloroquine; [d] pentamidine; and [e] emetine.

2.1.8. Amidation of the 3-Amino-4-hydroxycoumarins

Amides can be considered simply as ammonia derivatives in which an acyl group has replaced one of the hydrogen atoms or, more comprehensively, as derivatives of carboxylic acids in which the hydroxyl group has been substituted by an amino group. This class of compounds also includes compounds in which one nitrogen atom is bonded to 3 acyl groups.[360,361] The amide moiety occurs in a diversity of important compounds covering natural products, pharmaceuticals (25% of available drugs contain the amide bond), and many biologically active compounds, and its formation ranks among the most essential conversions in biochemistry and organic chemistry.[362,363] Examples of amide-containing pharmaceuticals are shown in **Figure 84**. A number of lubricants, detergents and polymers also contain the amide functionality.[364] Interest in amides and their derivatives is driven by

their presence in compounds known to exhibit a range of biological activities that include anti-viral, anti-fungal, anti-inflammatory, anti-microbial, anti-thrombin, anti-hypoxant, anti-cancer, anti-malarial and cytotoxic.[365,366]

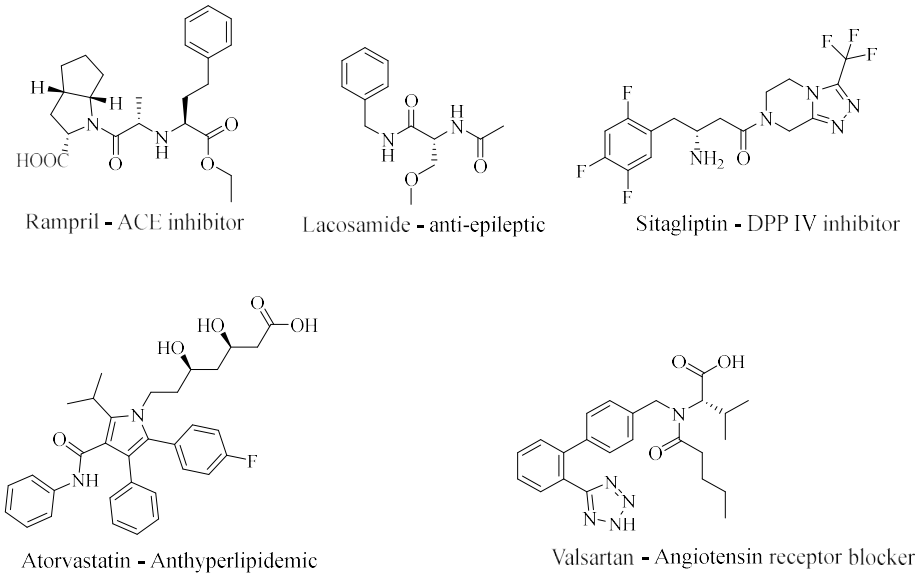


Figure 84. Some amide pharmaceuticals.[367,362]

Amides can be accessed *via* classical and non-classical approaches. Classically, amides can be synthesised through the reaction of amines with activated derivatives of carboxylic acids *e.g.* anhydrides, chlorides and esters or by coupling agent-mediated condensation of an amine and a carboxylic acid, an approach which has become common.[362,368] The classical amide-forming reactions include the Staudinger, Schmidt, Ritter, Schotten–Baumann, the multi-component Ugi reaction and the Beckmann rearrangement.[369] Classical approaches to amides have generally been found to produce large quantities of waste and are thus environmentally unfavourable, exhibiting poor atom economy.[370] These drawbacks have resulted in the research and development of new synthetic methods, including a number of non-classical approaches involving oxidative amidation of aldehydes, metal catalysed N–C bond formation, amino carbonylation of haloarenes, aminolysis of esters, transamidation of primary amides, direct reaction of acids and amines (classically or microwave-assisted or boron-catalysed).[370,369,363] Some synthetic routes to amides are shown in **Figure 85**.

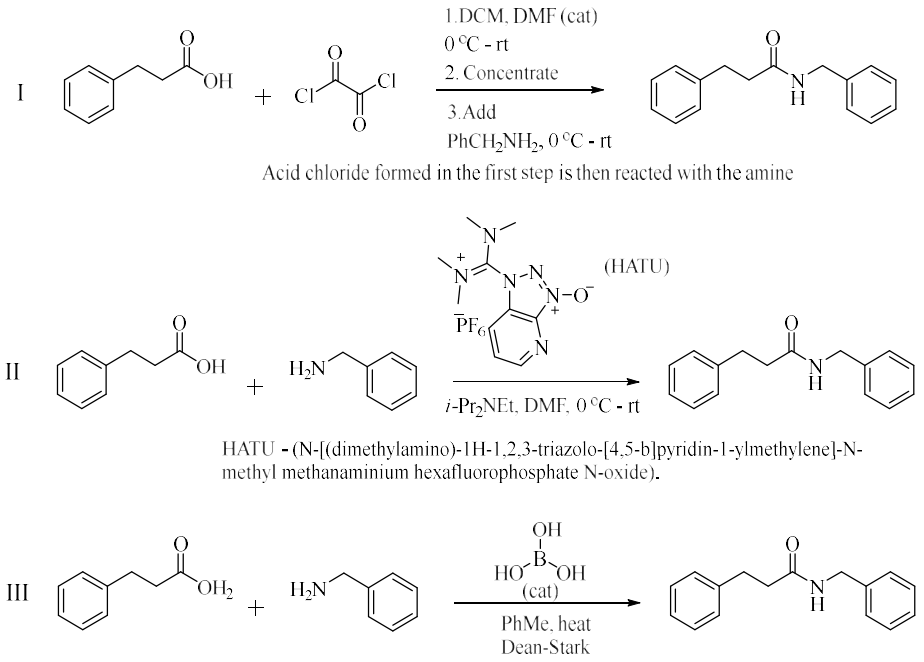


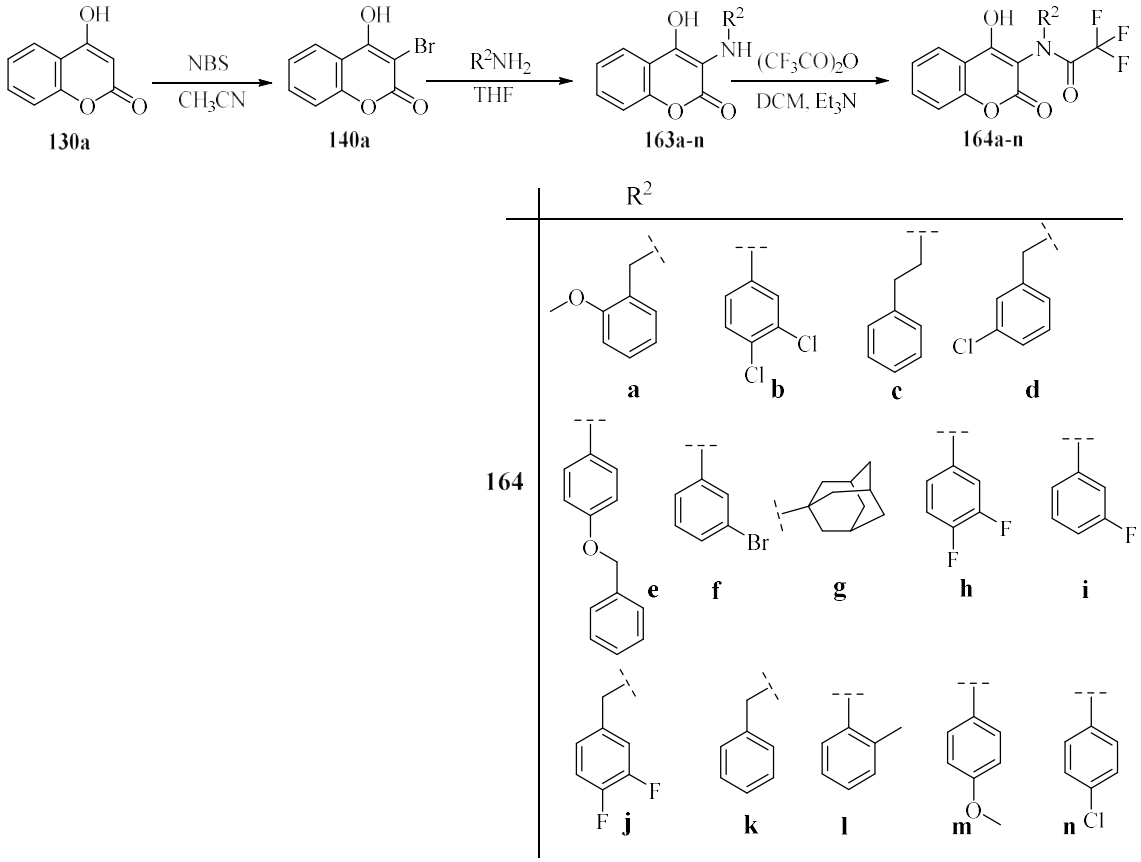
Figure 85. Some synthetic pathways to amides.[370]

I: Acid chloride pathway. II: Coupling reagent pathway. III: Boric acid catalysed pathway.

2.1.8.1. 3-(*N*-Substituted-trifluoroacetamido)-4-hydroxycoumarins

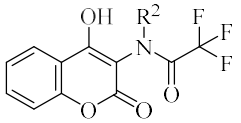
A number of attempts using different amidation approaches were conducted with unsatisfactory results before successful amidation of the secondary amines (**163**) was achieved using trifluoroacetic as the activated carboxylic acid derivative. The attempted syntheses will be discussed in **Section 2.1.8.4**. Herein, the amidation using trifluoroacetic anhydride is addressed. Mixtures of each of 14 of the 3-amino-4-hydroxycoumarins (**163**), synthesised as described earlier, trifluoroacetic anhydride and trimethylamine in DCM were stirred at 0 °C for 30 minutes; the temperature was then allowed to rise to room temperature and stirring was continued for a further 2 hours (**Scheme 35**) after which water was added and the products were extracted using DCM. The organic extracts were passed through a short silica column and the solvent was evaporated to yield the products in high purity. The amide product yields ranged from 27 to 87% (**Table 32**). The ^{1}H NMR spectrum of one of the compounds, **164k**, is shown in **Figure 86**. The methylene protons signal at *ca* 4.39 ppm is split due to restricted rotation of the N-CO bond resulting in rotamers in which the methylene protons are diastereoscopic. The nine expected aromatic proton signals appear in the range 7.0-8.0 ppm. The ^{13}C NMR spectrum of compound **164k** reveals the methylene carbon signal

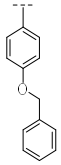
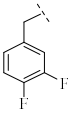
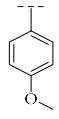
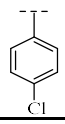
at 42.6 ppm, the C-3 signal at 88.9 ppm; the CF_3 carbon signal at 116.1 ppm and the amide C=O signal at 156.4 ppm are revealed as quartets with coupling constants, $J_{C,F}$ = 286.50 and 36.00 Hz, respectively (**Figure 87**). The phenomenon is due to ^{19}F-^{13}C coupling. The DEPT135 NMR spectrum (**Figure 88**), reveals the benzylic protons signal at 42.6 ppm and the expected 7 aromatic C-H signals.



Scheme 35. Route to 3-(*N*-substituted-trifluoroacetamido)-4-hydroxycoumarins **164a-n**

Table 32. Yields of 3-(*N*-substituted-trifluoroacetamido)-4-hydroxycoumarins **164a-n**



Compound	R^2	Isolated Yield (%)
164a		66
164b		77
164c		74
164d		65
164e		83
164f		56
164g		39
164h		83
164i		44
164j		50
164k		61
164l		61
164m		84
164n		68

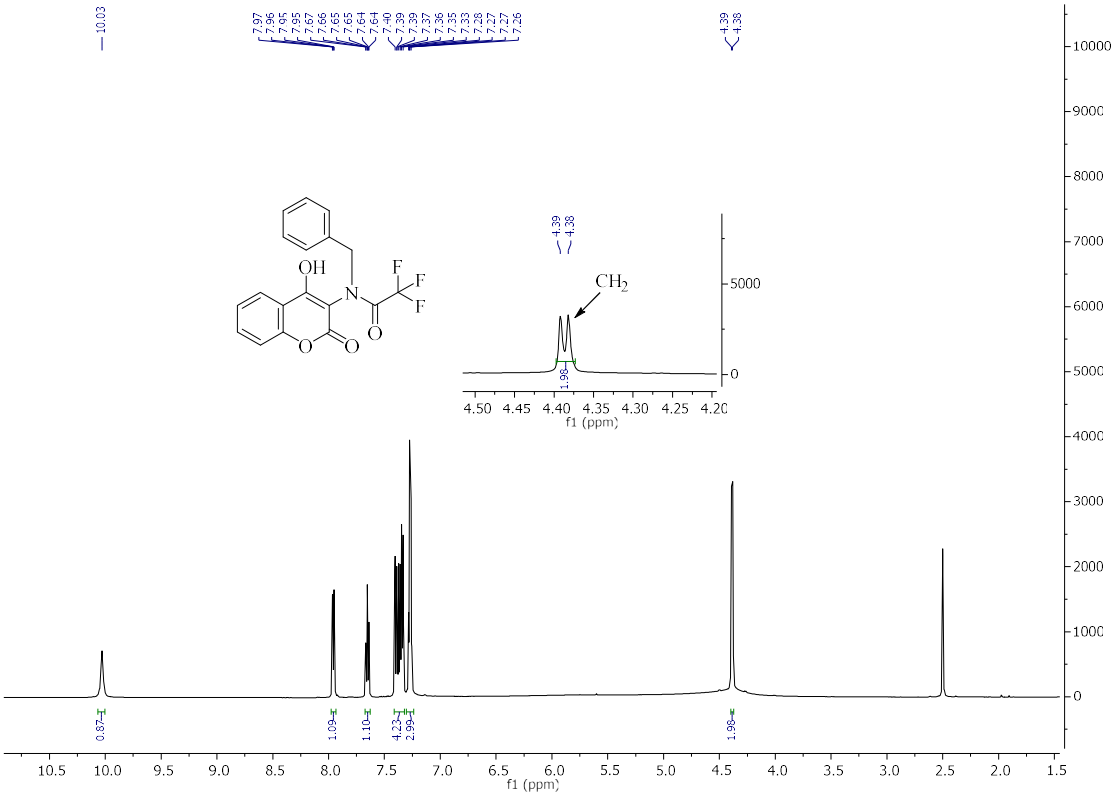


Figure 86. 600 MHz ^{1}H NMR spectrum of compound **164k** in DMSO-d_6.

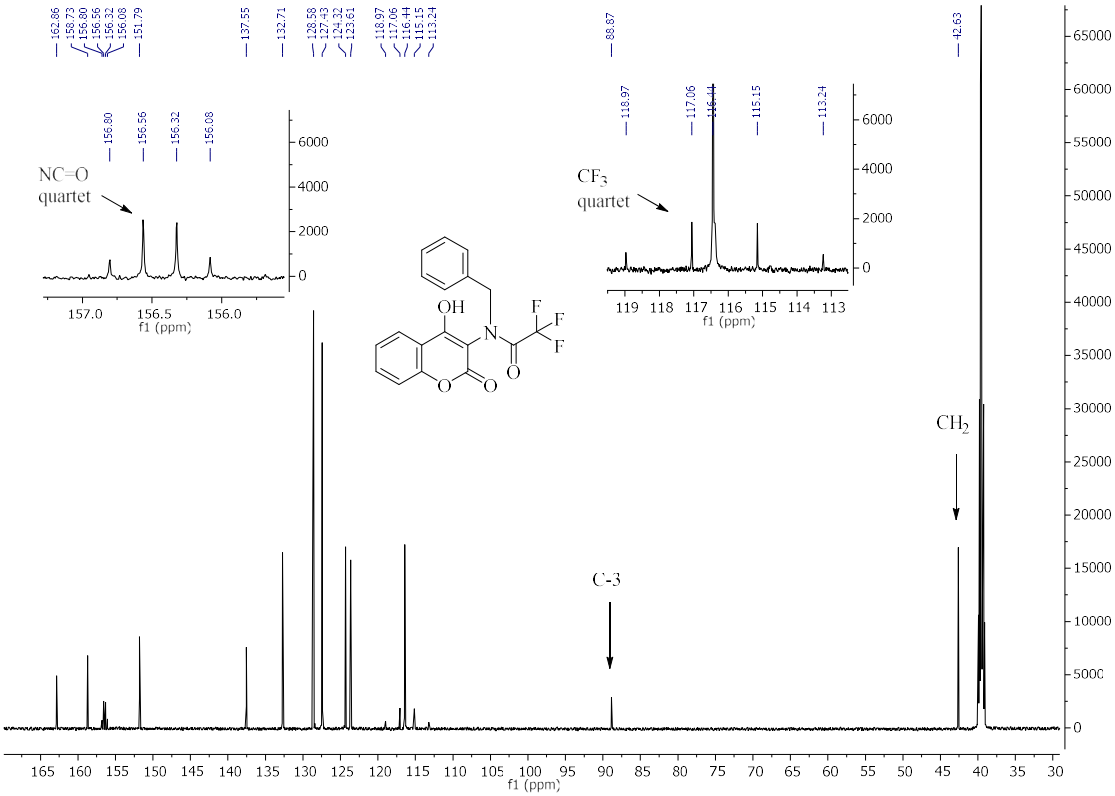


Figure 87. 150 MHz ^{1}H NMR spectrum of compound **164k** in DMSO-d_6.

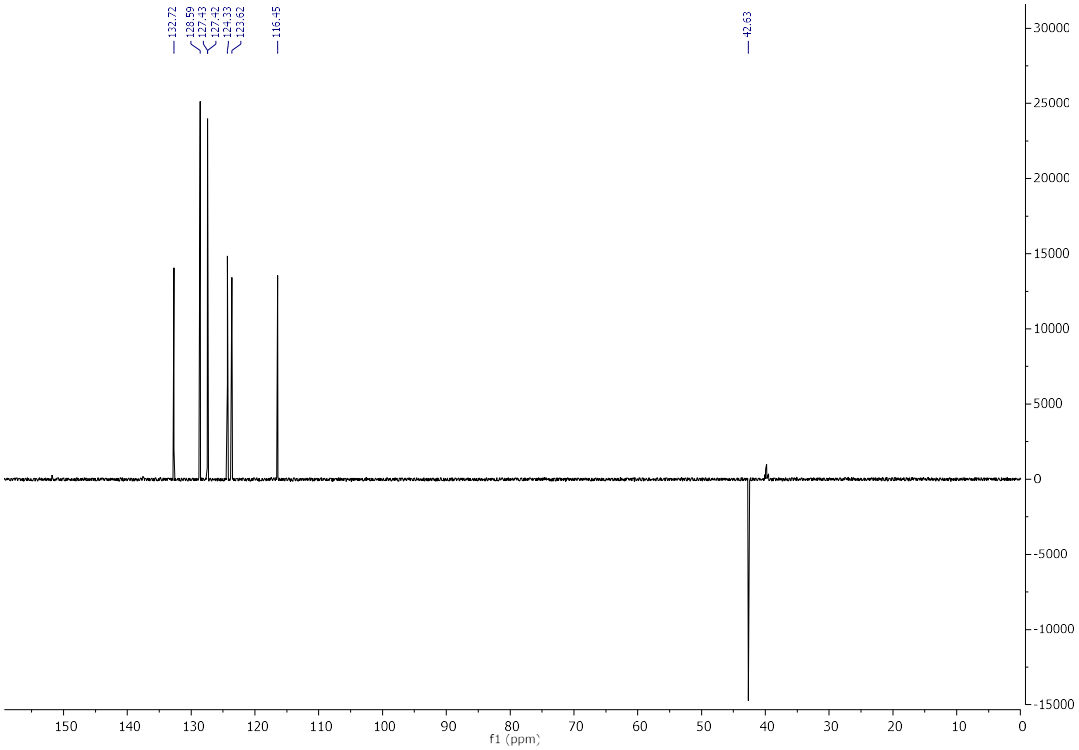


Figure 88. DEPT135 NMR spectrum of compound **164k** in DMSO-d_6.

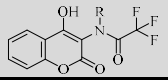
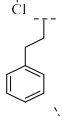
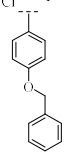
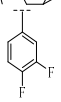
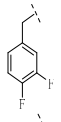
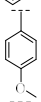
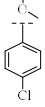
2.1.8.2. Biological studies of 3-(*N*-substituted-trifluoroacetamido)-4-hydroxycoumarins

2.1.8.2.1. *In vitro* studies

Biological studies on the compounds **164a-n** for anti-HIV-1 IN, anti-malarial, anti-trypanosomal and cytotoxicity were conducted as described in section **2.1.1.4**. **Table 33** shows the bioassay results for the compounds. Eight of the compounds showed HIV-1 IN inhibition in the range 42-49% with the remaining compounds showing less than 37% inhibition at 20 μM. The four most active compounds had % inhibition values quite close to one another [**164e** (48.47 %), **164g** (48.47%), **164h** (47.85%) and **164i** (48.19%)]. It is noteworthy that among the least active compounds were the only chlorinated members of the series, *viz* compounds **164b** and **164d** with HIV-1 IN % inhibition of 0.00 and 6.13, respectively. The anti-HIV-1 IN activities although not very high appear to show potential for improvement that could be effected through SAR studies. Compounds **164e** and **164h** were most active against pLDH resulting in pLDH % viability values of 67.56 and 75.19%, respectively. All of the compounds showed some activity against *T.b. brucei* with %

viabilities ranging from 67 to 82%, while most of the compounds showed relatively low cytotoxicity levels against HeLa cells.

Table 33. Bioassay data for compounds **164a-n**, showing % inhibition against HIV-1 IN and % viability of pLDH, *T.b. brucei* and HeLa cells at 20 μM concentration.

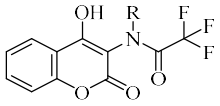
	R	% HIV-1 IN inhibition [a]	PLDH % viability [b]	*T.b. brucei* % viability [c]	HeLa cell % viability [d]
164a		45.09	100.00	70.84	100.00
164b		0.00	100.00	67.39	77.03
164c		0.00	100.00	71.91	84.60
164d		6.13	90.22	71.32	84.35
164e		48.47	67.56	70.08	88.05
164f		42.64	86.99	68.95	91.31
164g		48.47	88.62	67.73	89.40
164h		47.85	75.19	73.71	100.00
164i		48.19	100.00	71.73	75.18
164j		45.40	100.00	72.19	76.53
164k		45.83	100.00	72.18	97.87
164l		25.53	89.06	73.22	66.10
164m		36.20	87.36	72.57	78.90
164n		20.86	91.32	81.60	65.68
Controls		96.93 [a]	IC_{50} = 0.012μM [b]	IC_{50} = 0.053μM [c]	IC_{50} = 0.023 μM [d]

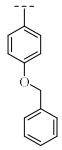
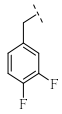
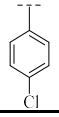
Controls: [a] chicoric acid; [b] chloroquine; [c] pentamidine; and [d] emetine.

2.1.8.2.2. *In silico* studies

Docking studies were conducted on the compounds **164** for HIV-1 IN and PR. *P. falciparum*, *T.b. brucei* and *Mtb* as described in section **2.1.1.4.2**. The results for the particular PDB structures used for docking are presented in **Table 34**. (No *in vitro* studies were conducted for HIV-1 PR and for *Mtb*). Generally, the compounds exhibited strong binding to the HIV-1 enzyme 5FRN with three of them, namely **164b** (-8.677 kcal/mol), **164c** (-9.036 kcal/mol) and **164k** (-8.843 kcal/mol) exhibiting stronger binding than the one of the control, L-chicoric acid (-8.403 kcal/mol). This may suggest that the compounds are potentially good inhibitors of HIV-1 IN; however, in the *in vitro* studies the activities exhibited were not very high, with only eight compounds (**164a**, **e**, **f**, **g**, **h**, **i**, **j** and **k**) exhibiting % HIV-1 IN inhibition of at least 42%, the rest being below that. It seems that *in vitro* and *in silico* results bear no meaningful correlation with each other. Some compounds which were inactive *in vitro* exhibited activity *in silico*, e.g., compounds **164b** and **c** exhibited no activity *in vitro* whereas *in silico* they were in the top three in terms of binding affinity with compound **164c** actually exhibiting the strongest binding affinity (-9.036 kcal/mol). Compound **164g** exhibited the highest % HIV-1 IN inhibition (48.47) *in vitro* but failed to dock in the 5FRN active site. Compound **164e** was joint most active with **164g** but *in silico* it exhibited weak binding affinity (-5.802 kcal/mol). The lack of correlation may indicate that the *in vitro*-active compounds may be binding at a completely different enzyme or site. Binding affinities for the HIV-1 PR enzyme 1YT9 were also generally high suggesting potential for the compounds **164** as HIV-1 PR inhibitors. Only one of the compounds **164g** exhibited binding affinity that was weaker than -6 kcal/mol and nearly half of the compounds exhibited binding affinities that were stronger than -7 kcal/mol. Two compounds, **164c** (-7.668 kcal/mol) and **164d** (-7.315 kcal/mol) exhibited binding affinities that were stronger than ritonavir (-7.309 kcal/mol). It is noteworthy that compound **164c** which had the strongest binding for 5FRN also had the strongest binding for 1YT9 suggesting potential for being an inhibitor of both HIV-1 IN and PR. There are seven other compounds, **164a**, **b**, **d**, **h**, **j**, **k** and **l** whose binding activities for both 5FRN and 1YT9 also exhibit the same trend. The compounds were generally less strongly binding to the HIV-1 PR enzyme 1ZP8. The strongest binding was compound **164k** (-5.726 kcal/mol) and it was followed closely by compounds **164i** (-5.621 kcal/mol) and **164j** (-5.490 kcal/mol). These binding affinities were weaker than that exhibited by ritonavir (-7.042 kcal/mol) but indicate some potential of the compounds as inhibitors of HIV-1 PR.

Table 34. Binding affinities for the docking of ligands **164** in the active sites of various enzymes.



Ligand	R	HIV-1 IN 5FRN (kcal/mol)	HIV-1 PR 1YT9 (kcal/mol)	HIV-1 PR 1ZP8 (kcal/mol)	*Pf* 1T2C (kcal/mol)	*Pf* 1V0O (kcal/mol)	*T.b. brucei* 3FWN (kcal/mol)	*Mtb* 4BFW (kcal/mol)
164a		-6,452	-7.203	-5.155	-6.427	-3.565	-3.462	-4.121
164b		-8.677	-6.547	-4.669	-5.605	-3.192	-2.938	-2.575
164c		-9.036	-7.668	-5.178	-7.896	-3.155	-3.724	-4.534
164d		-6.409	-7.315	-4.919	-6.134	-3.687	-3,253	-.3.519
164e		-5.802	-6.868	-3.261	-6.206	-	-2.641	-.4.562
164f		-6.060	-6.020	-5.309	-5.008	-2,835	-2.569	-3.512
164g		-	-5.688	-2.542	-	-2.685	-1.878	-1.338
164h		-6.371	-7.001	-3.572	-5.599	-3.226	-3.078	-4.261
164i		-5.780	-7.244	-5.621	-5.653	-3.992	-3.165	-4.198
164j		-8.311	-6.944	-5.490	-7.665	-3.198	-3.050	-.3.751
164k		-8.843	-7.167	-5.726	-7.204	-2,789	-3.224	-4.571
164l		-8.022	-6.195	-3.332	-5.482	-3.394	-3.200	-2.774
164m		-6.402	-6.239	-4.052	-6.603	-	-3.837	-3.875
164n		-5.789	-6.402	-4.434	-5.232	-3.844	-2.767	-4.124

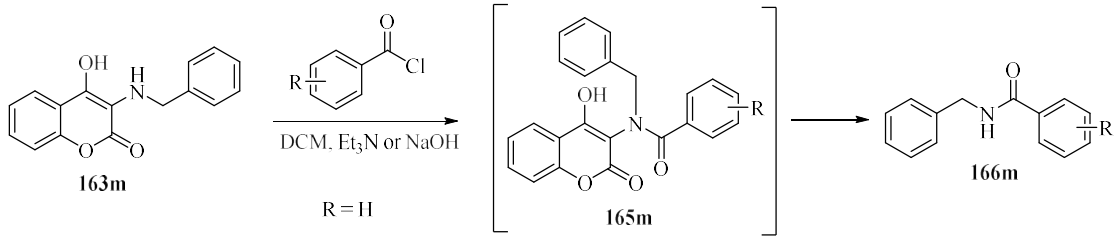
The compounds generally exhibited strong affinity for the *P. falciparum* enzyme 1T2C which may suggest their potential as anti-*P. falciparum* agents. All the thirteen compounds that successfully docked in the active site of 1T2C exhibited stronger *in silico* affinity for 1T2C than chloroquine. The *in vitro* results however, reveal weak activity against pLDH where the five best active compounds exhibited the following pLDH % viabilities, 67.56 (**164e**), 75.19 (**164h**), 86.99 (**164f**), 87.36 (**164m**) and 88.62 (**164g**). The strongest binding ligand *in silico*, compound **164c** did not show any activity against pLDH *in vitro*. In the light of the *in vitro* assay results the high binding activities exhibited suggest that factors such as solubility, kinetic and thermodynamic effects, which are not accounted for adequately in the docking protocol, may be critical. Affinity for the *P. falciparum* enzyme 1V0O was generally weak. Two compounds **164e** and **164m** failed to dock in the active site of 1V0O. The four best binding ligands were compounds **164i** (-3.992 kcal/mol), **164n** (-3.844 kcal/mol), **164d** (-3.687 kcal/mol) and **164a** (-3.565 kcal/mol). The weak binding affinities suggest that the compounds have little potential as inhibitors of 1V0O and hence *Pf*PK5. The weak binding affinities for the *T.b. brucei* enzyme 3FWN suggest that the compounds have little potential as inhibitors of 3FWN. The binding affinities exhibited by the three strongest binding compounds were -3.837 kcal/mol (**164m**), -3.724 kcal/mol (**164c**) and -3.462 kcal/mol. These were much lower than the -6.017 kcal/mol exhibited by pentamidine. Interestingly, all of the compounds (**164**) exhibited some activity against *T.b. brucei* in the *in vitro assay*, resulting in *T.b. brucei* viabilities of about 30%. The potential for the compounds as anti-*Mtb* agents appears to be quite low as generally the compounds exhibited weak binding to the *Mtb* enzyme 4BFW. The three strongest binding were **164k** (-4.571 kcal/mol), **164e** (-4.562 kcal/mol) and **164c** (-4.534 kcal/mol) and the weakest binding was compound **164g** (-1.338).

It would appear that the presence of the adamantyl moiety disposed ligands to weak binding as shown by the fact that such ligands would fail to dock as in the cases for 5FRN and 1T2C or be the weakest binding, as in the cases for 1YT9 (-5.688 kcal/mol), 1ZP8 (-2.542 kcal/mol), 1V0O (-2.685 kcal/mol), 3FWN (-1.878 kcal/mol) and 4BFW (-1.338 kcal/mol). The adamantly moiety may have reduced interactions and fitting in the respective active sites due to its steric and hydrophobicity factors.

2.1.8.3. Attempted amidations

Using benzoyl chlorides as the activated carboxylic acid derivatives

Some of the secondary amine 4-hydroxycoumarin derivatives *e.g.* compound **163m** were reacted with benzoyl chloride derivatives in the presence of trimethylamine as a base and DCM as solvent (**Scheme 36**) giving white products. The reaction was repeated using NaOH as base. In both cases, NMR analysis revealed the white products to be the simple amides (**166**) which lacked the coumarin moiety in the expected products (**165**). Representative spectra for one of the products, *N*-benzylbenzamide **166m** (R=H) are shown in **Figures 89-91**. The ^{1}H NMR spectrum for compound **166m** reveals the methylene protons signal at 4.49 ppm, signals corresponding to ten aromatic protons in the range 7.0-8.0 ppm and the NH signal at 9.08 ppm (**Figure 89**). The ^{13}C NMR spectrum (**Figure 90**) shows the methylene carbon signal at 42.6 ppm, the amide carbonyl carbon signal at 166.2 ppm and as expected total of 10 carbon signals. The DEPT135 spectrum reveals signals for six aromatic, hydrogen-bearing carbons in the range 125-132 ppm (**Figure 91**).



Scheme 36. Attempted amidation using benzoyl chlorides.

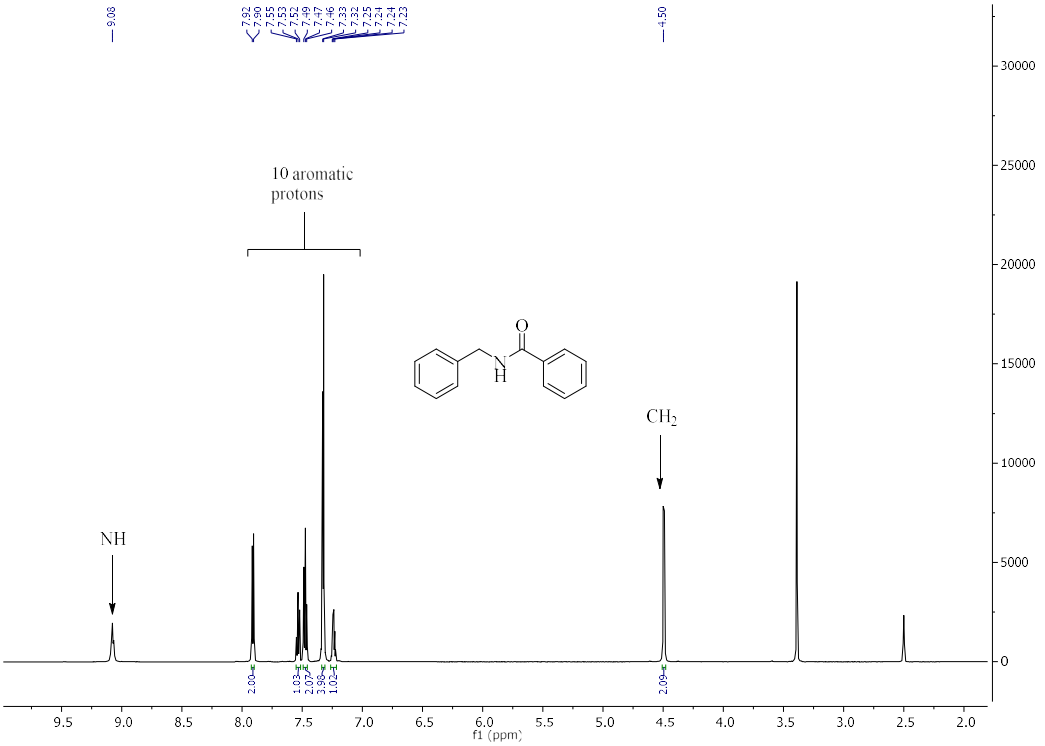


Figure 89. 600 MHz ^{1}H NMR spectrum of compound **166m** in DMSO-d_6.

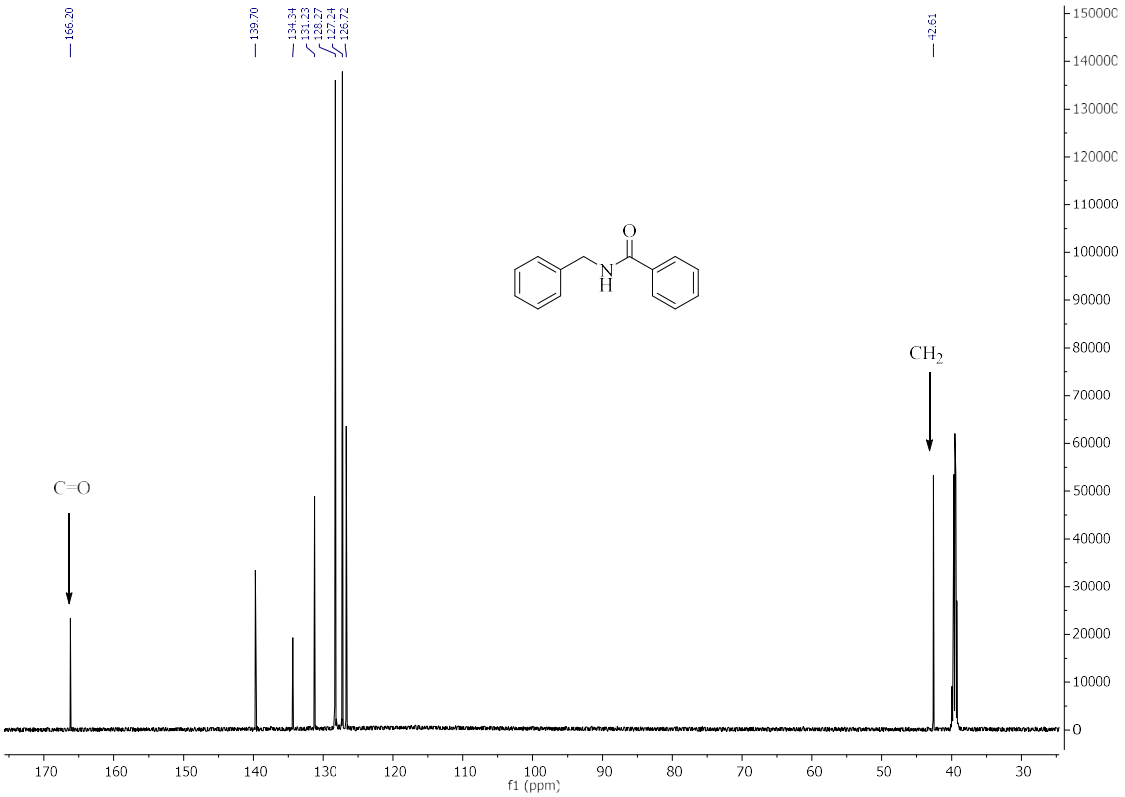


Figure 90. 150 MHz ^{1}H NMR spectrum of compound **166m** in DMSO-d_6.

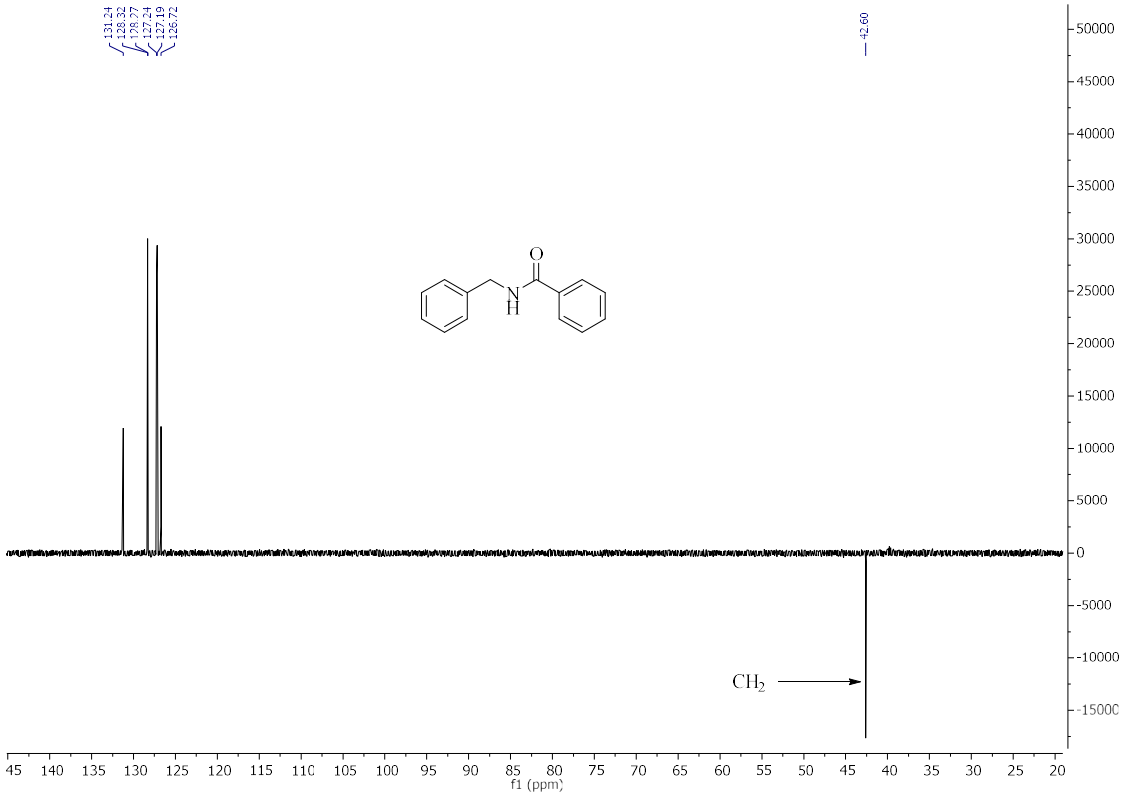
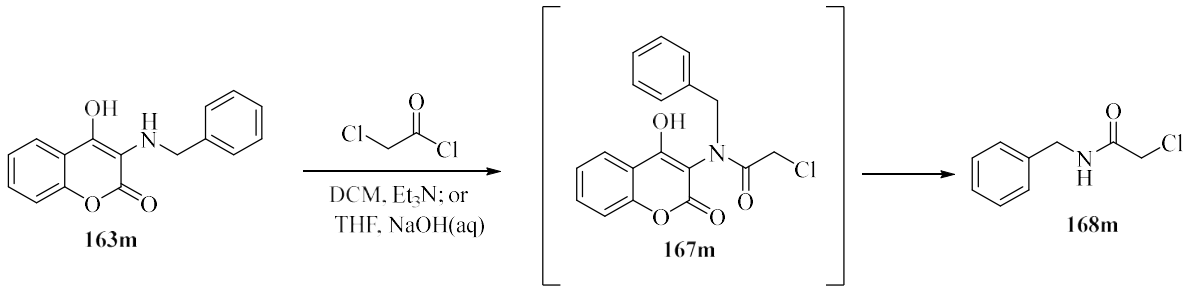


Figure 91. DEPT135 NMR spectrum of compound **166m** in DMSO-d_6.

Using chloroacetyl chloride as the activated carboxylic acid derivative

Chloroacetyl chloride was added dropwise to a mixture of the secondary, amino 4-hydroxycoumarin derivative **163m** (**Scheme 37**) and trimethylamine in DCM at 0 ºC, and the mixture was stirred for at least 2 hours with the temperature being allowed to rise to room temperature. The reaction mixture was quenched by the addition of water and the resulting mixture was extracted using DCM; the solvent was evaporated to give the product. In another attempt, aqueous sodium hydroxide was used as the base and THF as the solvent in a Schotten–Baumann approach to the amidation. Chloroacetyl chloride was added dropwise to a mixture of the amine **163m** and aqueous sodium hydroxide in THF, after which the mixture was refluxed for 3 hours. The solvent was then evaporated and the residue washed with water, filtered and dried. In both attempts the coumarin moiety was lost leaving an amide (**168m**) comprising the chloroacetyl moiety and benzylamine – *i.e.* the product that would result in an amidation reaction between a primary amine and chloroacetyl chloride (**Scheme 37**). NMR spectra are shown below for the amide product **168m** obtained from the attempted amidation reaction between 3-(benzylamino)-4-hydroxycoumarin and chloacetyl chloride

using the Schotten–Baumann approach. Several trials using different members of the compound series (**163**) (alkyl and aromatic amine derivatives) yielded similar results, *i.e.* simple secondary amides instead of the desired tertiary amides.



Scheme 37. Attempted amidation using chloroacetyl chloride.

The ^{1}H NMR spectrum for compound **168m** (**Figure 92**) reveals the two methylene proton signals at 4.12 and 4.31ppm (the latter signal being split due to the presence of amide rotamers), signals for five aromatic protons in the range 7.0-8.0 ppm and the NH signal at 8.73 ppm. The ^{13}C NMR spectrum (**Figure 93**) shows the methylene carbon signals at 42.5 and 42.7 ppm, the carbonyl carbon signals at 166.0 and the expected total of four aromatic carbon signals. The DEPT 135 spectrum reveals the two methylene carbon signals and three aromatic hydrogen-bearing carbon signals (**Figure 94**). The formation of the unexpected products **166** and **168** is attributed to rapid decomposition of the presumed, sterically hindered acylated products. It was observed that for the trifluoro acetamide derivatives (**164**) though isolable, on HRMS the molecular ion could not be found instead the respective acetamide anion was the main fragment.

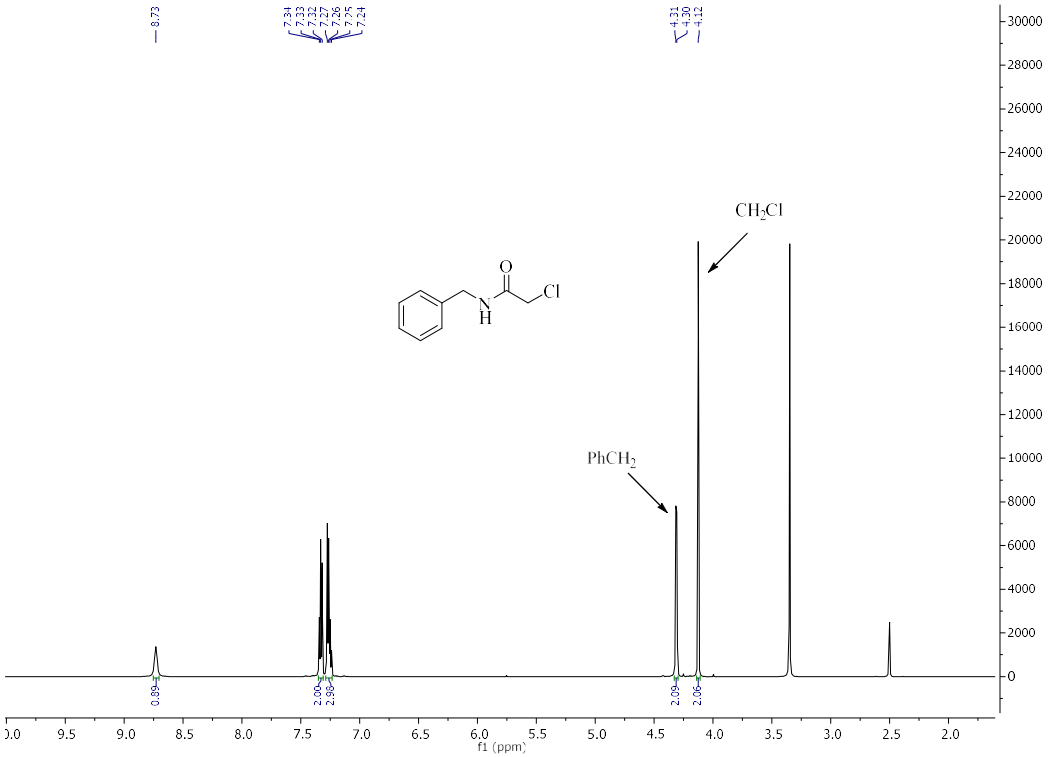


Figure 92. 600 MHz ^{1}H NMR spectrum of compound **168m** in DMSO-*d*$_6$.

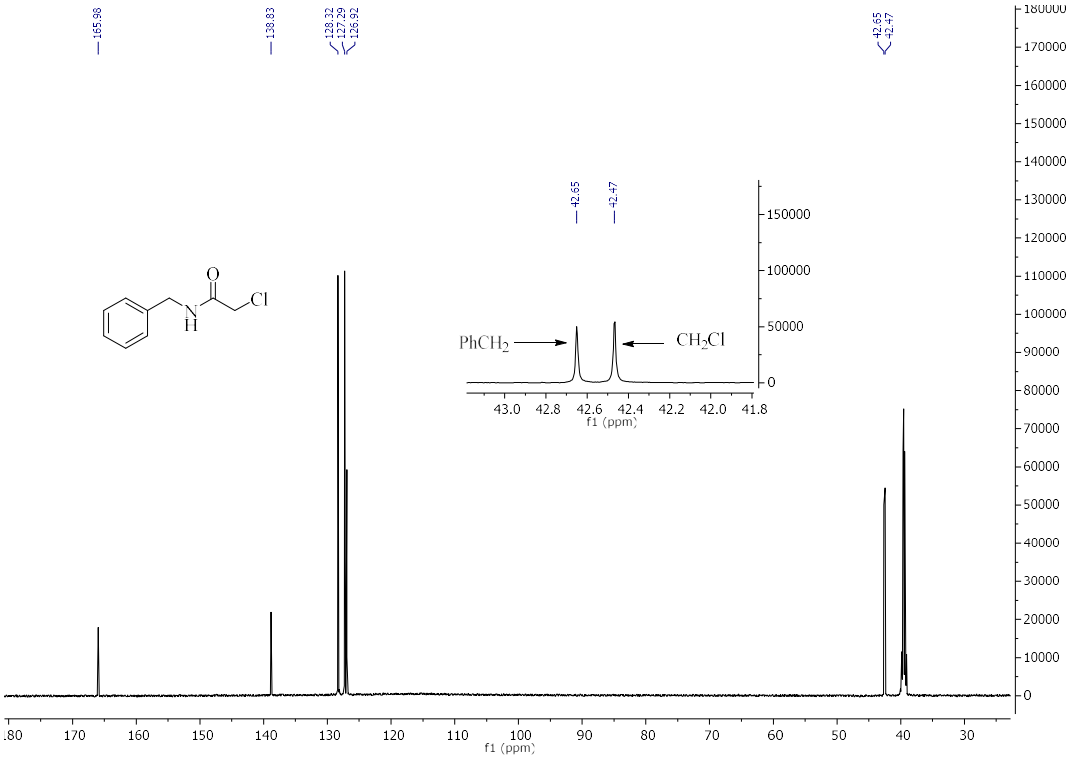


Figure 93. 150 MHz ^{13}C NMR spectrum of compound **168m** in DMSO-*d*$_6$.

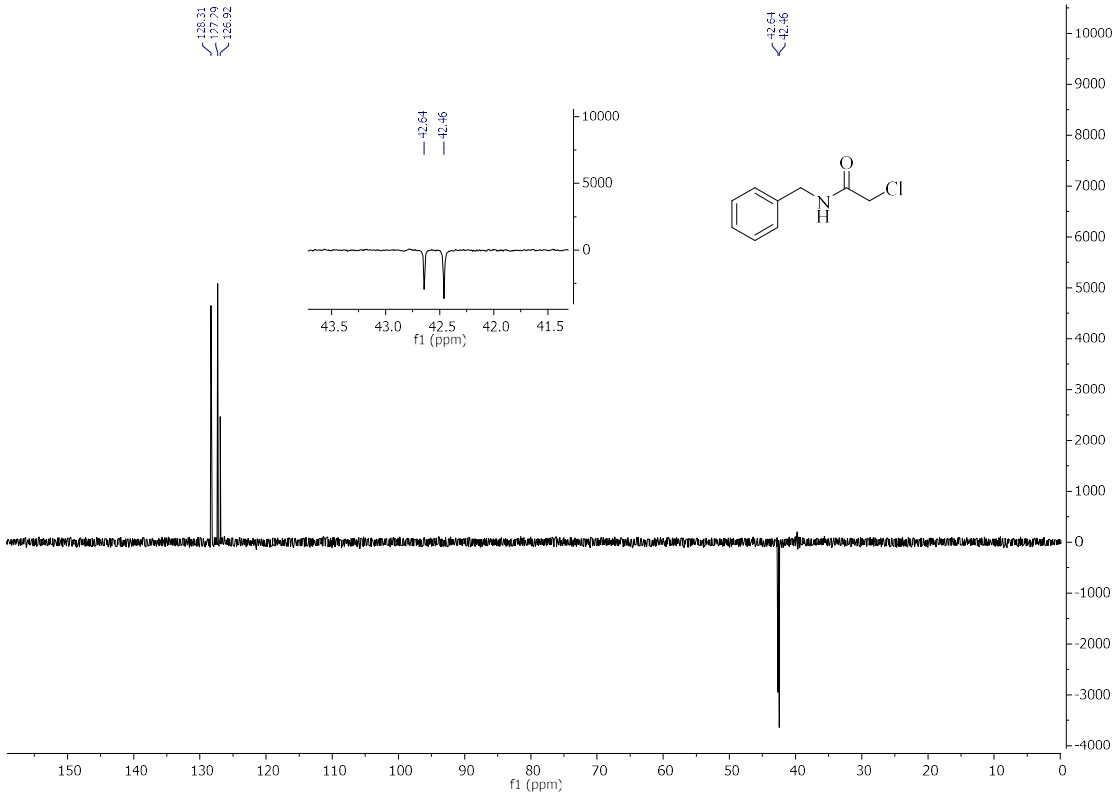


Figure 94. DEPT135 NMR spectrum of compound **168** in DMSO-d_6.

2.2. CONCLUSIONS

Following the aims of this investigation, several series of novel 4-hydroxycoumarins have been successfully synthesised and derivatised in good to very good yields. The series include succinohydrazide dimeric compounds, iminyl-, thiazolyl-, chalconyl-, amino-, and trifluoroacetamido derivatives. In fact, in excess of eighty new compounds were prepared and NMR (1 and 2D), IR and HRMS analytical techniques were employed to fully characterise them. Initial attempts to access the potentially bioactive 4-hydroxycoumarin derivatives using Baylis-Hillman methodology had proved unsuccessful and, consequently, readily available 4-hydroxycoumarin was identified as the primary synthon to access a variety of 4-hydroxycoumarin derivatives. The approaches used to access these compounds generally afforded very pure products, requiring minimal work-up and purification.

The 4-hydroxycoumarin scaffold is an established bioactive moiety and was combined with other moieties also known to possess biological activities to generate molecules which might exhibit diverse medicinal activities. The bis-4-hydroxycoumarinyl succinohydrazides **134a-g**

were successfully prepared through the reaction of 3-acetyl-4-hydroxycoumarin derivatives with 2,3-dihydroxysuccinohydrazide, the latter obtained from the reaction of diethyl 2,3-dihydroxysuccinate with hydrazine hydrate. These molecular conjugates thus contained several pharmachophoric groups, namely the two coumarin and two Schiff base (iminyl) moieties; in addition, they contained hydroxyl and carbonyl groups which are useful in hydrogen-bonding to receptor amino acid residues.

The reaction of 3-acetyl-4-hydroxycoumarin derivatives (**133**) with 4-benzyloxyaniline enabled access to the novel condensation products **135a-f**, while the 3-(1-hydrazonoethyl)-4-hydroxycoumarin **152** was employed as the amine in similar reactions to access the 3-[1-(benzylidenehydrazono)ethyl]-4-hydroxycoumarins **137a-g** and the 3-{1-[(propargyloxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarins **138a-g**. These compounds all contain the 4-hydroxycoumarin moiety, substituted benzene rings, methyl and C=N groups as important centres for biological interactions.

The series of novel molecular hybrids, the 3-[2-(benzylidenehydrazinyl)thiazo-2-yl]-4-hydroxycoumarins **136a-e**, were synthesised by reacting 3-(2-bromoacetyl)-4-hydroxy-2*H*-chromen-2-one **148**, a product of the bromination of 3-acetylated 4-hydroxycoumarin **133a**, and thiosemicarbazone derivatives **151a-g** accessed, in turn, by reacting aldehydes with thiosemicarbazide. Chalconyl derivatives were accessed *via* a three-step pathway culminating in the reaction of 3-acetylated 4-hydroxycoumarin with various aldehydes (some of which were specially synthesised) in the presence of piperidine.

Regioselective bromination of various 4-hydroxycoumarins using NBS afforded the 3-bromo derivatives **140a-g** which enabled access to 3-amino-4-hydroxycoumarins *via* nucleophilic substitution reactions between the 3-brominated coumarins and various primary amines. Subsequent acylation of some of these 3-amino-4-hydroxycoumarins using trifluoroacetic anhydride afforded the corresponding *N*-(4-hydroxcoumarin-3-yl)trifluoroacetamides **165a-n** in four steps overall. Several attempts to effect the amidation of the 3-amino-4-hydroxycoumarins were not successful and resulted in cleavage of the coumarin moiety and yielded simple secondary amides. Eventually, however, it was discovered that use of trifluoroacetic acetic anhydride as the activated acylating agent yielded the desired 4-hydroxycoumarin trifluoroacetamide derivatives.

The aldehydes **154a-e** and **156a-g** were specially synthesised for use as precursors in the syntheses of compounds, such as the 3-[1-(benzylidenehydrazono)ethyl]-4-

hydroxycoumarins (**137**) and the 3-{1-[(propargyloxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarins (**138**). The resulting aldehydes **154a-e** and **156c** were isolated as crystals which were subjected to single-crystal X-ray diffraction analysis; the resulting novel structures exhibited interesting solid state arrangements of the molecules and the data have been deposited with the Cambridge Crystallographic Data Centre (CCDC).

Bilogical evaluations were conducted on the series of synthesised coumarin derivatives and these included cytotoxicity, HIV-1 IN and PR inhibitory potential, and anti-malarial, anti-trypanosomal and anti-*Mtb* assays. A bis-4-hydroxycoumarinyl succinohydrazide, compound **134a**, exhibited the highest potency against HIV-IN (inhibition of 59.4% at 20 μM and an IC_{50} value of 3.5 μM). Several other compounds in the same series exhibited HIV-1 IN inhibition of ca. 35%. The majority of the trifluoroacetamides (**165)** exhibited activity against HIV-1 IN, with inhibition levels in the range 42-49%. However, none of the compounds tested exhibited significant anti-HIV-1 PR activity *in vitro*.

Seven of the compounds from across the series exhibited % pLDH viability in the range 62-77%, the three most potent being the amino-4-hydroxycoumarin derivative **164j** (62.6%), the thiazole moiety-containing derivative **136a** (65.3%) and the diimine **137e** (63.9%). Apart from the bis-4-hydroxycoumarinyl succinohydrazides (**134**) and amino-4-hydroxycoumarins (**164**), which exhibited no significant anti-trypanosomal activity, all the other series had at least one compound that exhibited some activity. Of note was compound **137g**, a diimine which reduced *T.b. brucei* viability to 1.5% (IC_{50} = 0.9 μM), and four of the imino-ethyl compounds (**135**), which decreased *T.b. brucei* viability to below 40%, the most potent compound in that series being **135d** which decreased *T.b. brucei* viability to below 23.3%.

The bis-4-hydroxycoumarinyl succinohydrazides (**134**), the thiazolyl-derivatives (**136**) and the diimine compounds (**137**) were evaluated for anti-*Mtb* activity. The activities exhibited by some of the compounds, though poorer than those of the drug standard, revealed the potential of the 4-hydroxycoumarin derivatives as anti-*Mtb* agents. Apart from two compounds in the chalconyl series (**139)** which reduced the viability of HeLa cells to less than 50%, none of the compounds in the study exhibited any significant cytotoxicity. It is, of course, desirable for potential medicinal compounds to exhibit insignificant cytotoxicity unless deliberately designed to do so.

In silico docking studies produced mixed results, some confirming what had been observed *in vitro* while others showed no correlation with the *in vitro* results. Generally, however, the

compounds exhibited high binding affinities towards the selected receptors and, in a number of cases, the affinities were stronger than those exhibited by the drug standards.

The initial objectives of the study have clearly been addressed. The various series of 4-hydroxycoumarin derivatives were successfully synthesised and evaluated for biological activity against a range of pathogens or their enzymes.

Future work in this area could address objectives.

i) SAR studies to improve the biological potential observed in the study.
ii) Chemical reduction of selected imino and alkenyl analogues to assess the effect of saturation on their activities.
iii) The CuAAC synthesis of triazole derivatives using the terminal alkyne group present in the diimino derivatives and an exploration of their potential bioactivity.
iv) Conducting *in silico* docking studies at an advanced level to achieve better correlation between the *in vitro* and *in silico* data.

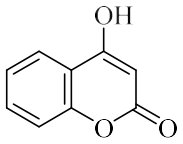
3. EXPERIMENTAL

3.1. GENERAL

Reagents were used as supplied by Sigma-Aldrich without further purification. Thin layer chromatography (TLC) was carried out on pre-coated Merck silica gel F254 plates, and spots were viewed under UV light (254 / 365 nm) or after exposure to iodine as needed. Merck silica gel 60 (particle size 0.040-0.063 mm) was used for flash chromatography. Bruker Fourier 300 MHz, AMX 400 MHz and Biospin 600 MHz spectrometers were used to record NMR spectra and referenced using residual protonated solvent signals (δ_H: 3.31 ppm for CD_3OD, 7.26 ppm for $CDCl_3$ and 2.50 ppm for DMSO-d_6; δ_C: 49.0 ppm for CD_3OD, 77.2 ppm for $CDCl_3$ and 39.5 ppm for DMSO-d_6). High resolution mass spectra were recorded on a Waters API QTOF Ultima spectrometer (University of Stellenbosch, South Africa) and on a Bruker Compact QTOF Mass spectrometer (Rhodes University, South Africa). The Perkin-Elmer FT-IR Spectrum 100 spectrometer was used to record IR spectra from neat samples. Melting points were measured using a Stuart SMP30 apparatus, and are uncorrected.

3.2 SYNTHESIS OF 4-HYDROXYCOUMARINS 130

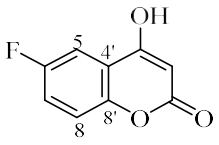
4-Hydroxycoumarin 130a



2'-hydroxyacetophenone (1.45 mL, 12 mmol) was added to NaH (1.5 g, 38 mmol, 60 % in mineral oil) in toluene (100 mL). After the evolution of hydrogen had stopped, diethyl carbonate (2 mL) in toluene (5 mL) was added slowly to the stirring mixture. The mixture was then refluxed for 3 hours, cooled to room temperature, filtered, washed with toluene and air dried. The dried residue was then mixed with water and acidified with 2N HCl until no further precipitate formed, after which the mixture was filtered and the residue washed with water and air dried in an oven yielding 4-hydroxycoumarin **130a** as a white solid (1.34 g, 69 %), m.p.204-206 ^oC (lit.[371] 206 ^oC); ν_{max}/cm^{-1} 3344 (O-H) and 1700 (C=O); δ_H (400 MHz; DMSO-d_6) 5.6 (1H, s, 3-H), 7.29-7.38 (2H, m, 5- and 6-H overlapping), 7.63 (1H, m, 7-H),

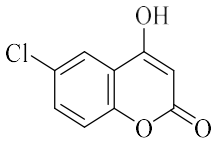
7.81 (1H, dd, J = 7.8, 1.4 Hz, 5-H); δ_C (100 MHz; DMSO-d_6) 91.1 (C-3), 115.9, 116.5, 123.3, 124.1, 132.8 and 153.6 (Ar-C), 162.1 (C=O) and 165.8 (C-OH).

6-Fluoro-4-hydroxycoumarin 130b



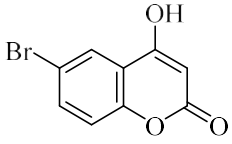
The procedure described for the synthesis of compound **130a** was followed using 5-fluoro-2-hydroxyacetophenone (1.85 g, 12 mmol). Work-up afforded 6-fluoro-4-hydroxycoumarin **130b** as a cream solid (1.45 g, 67%), m.p. 247-248 ^oC (lit.[372] 247-248 ^oC); ν_{max}/cm^{-1} 1734 (C=O);); δ_H (400 MHz; DMSO-d_6) 5.61 (1H, s, 3-H), 7.36 (1H, m, 8-H), 7.45 (2H, m, 5- and 7-H overlapping); δ_C (100 MHz; DMSO-d_6) 91.9 (C-3), 109.2 (d, J_{CF} = 25.0 Hz, C-5), 117.5 (d, J_{CF} = 8.8 Hz, C-4'), 119.0 (d, J_{CF} = 8.5 Hz, C-8), 120.7 (d, J_{CF} = 24.5 Hz, C-7), 150.2 (d, J_{CF} = 1.6, C-8') and 158.5 (d, J_{CF} = 241.2 Hz, C-6) (Ar-C) 162.9 (C=O) and 165.8 (d, J_{CF} = 1.9 Hz, C-OH).

6-Chloro-4-hydroxycoumarin 130c



The procedure described for the synthesis of compound **130a** was followed using 5-chloro-2-hydroxyacetophenone (2.05 g, 12 mmol). Work-up afforded 6-chloro-4-hydroxycoumarin **130c** as a white solid (2.06 g, 87%), m.p. 264-266 ^oC (lit.[373] 266-268 ^oC); ν_{max}/cm^{-1} 1714 (C=O); δ_H (600 MHz; DMSO-d_6) 4.52 (1H, s, 3-H), 7.04 (1H, d, J = 8.6 Hz, 8-H), 7.47 (1H, dd, J = 8.6, 2.6 Hz, 7-H), 7.82 (1H, d, J = 2.6 Hz, 5-H); δ_C (150 MHz; DMSO-d_6) 85.4 (C-3), 117.8, 123.6, 125.3, 125.9, 129.5 and 153.1 (Ar-C), 164.5 (C=O) and 173.4 (C-OH).

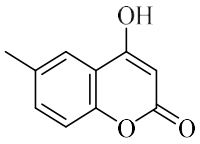
6-Bromo-4-hydroxycoumarin 130d



The procedure described for the synthesis of compound **130a** was followed using 5-bromo-2-hydroxyacetophenone (2.58 g, 12 mmol). Work-up afforded 6-bromo-4-hydroxycoumarin

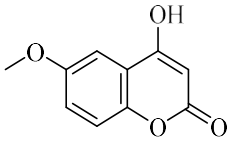
130d as a white solid (2.48 g, 86%), m.p. 245 °C (decomp) (lit.[374] 255 °C); ν_{max}/cm^{-1} 1710 (C=O); δ_H (600 MHz; DMSO-d_6) 4.52 (1H, s, 3-H), 7.04 (1H, d, J = 8.6 Hz, 8-H), 7.47 (1H, dd, J = 8.6, 2.6 Hz, 7-H), 7.82 (1H, d, J = 2.6 Hz, 5-H); δ_C (150 MHz; DMSO-d_6) 85.4 (C-3), 117.8, 123.6, 125.3, 125.9, 129.5 and 153.1 (Ar-C), 164.5 (C=O) and 173.4 (C-OH).

6-Methyl-4-hydroxycoumarin 130e



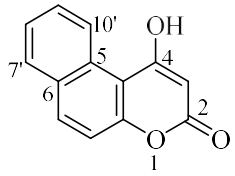
The procedure described for the synthesis of compound **130a** was followed using 5-methyl-2-hydroxyacetophenone (1.80 g, 12 mmol). Work-up afforded 4-hydroxy-6-methylcoumarin **130e** as an off white solid (1.27 g, 60 %), m.p. 260-262 °C (lit.[372] 261-262 °C); ν_{max}/cm^{-1} 1670 (C=O); δ_H (600 MHz; DMSO-d_6) 2.35 (3H, s, CH_3), 5.56 (1H, s, 3-H), 7.23 (1H, d, J = 8.4 Hz, 8-H), 7.41(1H, dd, J = 8.4, 2.0 Hz, 7-H), 7.82 (1H, d, J = 1.2 Hz, 5-H); δ_C (150 MHz; DMSO-d_6) 20.4 (CH_3) 91.0 (C-3), 115.5, 116.1, 122.8, 133.1, 133.5 and 151.7 (Ar-C), 162.1 (C=O) and 165.7 (C-OH).

6-Methoxy-4-hydroxycoumarin 130f



The procedure described for the synthesis of compound **130a** was followed using 5-methoxy-2-hydroxyphenylacetophenone (1.99 g, 12 mmol). Work-up afforded 4-hydroxy-6-methoxy-2H-chromen-2-one **130f** as an off white solid (1.67 g, 72%), m.p. 264 °C (decomp) (lit.[375] 254.4-254.7 °C; ν_{max}/cm^{-1} 1674 (C=O); δ_H (400 MHz; DMSO-d_6) 3.79 (3H, s, OCH_3), 5.59 (1H, s, 3-H), 7.19 (2H, m, 5- and 7-H overlapping), 7.28 (1H, d, J = 9.8 Hz, 8-H); δ_C (100 MHz; DMSO-d_6) 55.7 (OCH_3) 91.3 (C-3), 105.0, 116.3, 117.7, 120.4, 147.9 and 155.3(Ar-C), 162.2 (C=O) and 165.5 (C-OH).

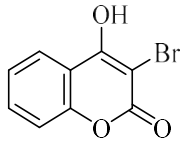
5,6-Benzo-4-hydroxycoumarin 130g



The procedure described for the synthesis of compound **130a** was followed using 1-acetyl-2-hydroxynaphthalene (2.23 g, 12 mmol). Work-up afforded 5,6-benzo-4-hydroxycoumarin **130g** as a brown solid (1.88 g, 74%), m.p. 238-240 °C (lit.[376] 238-241 °C); ν_{max}/cm^{-1} 3340 (O-H) and 1669 (C=O); δ_H (600 MHz; DMSO-d_6) 5.76 (1H, s, 3-H), 7.49 (1H, d, J = 9.0 Hz, 8-H), 7.57 (1H, t, J = 7.4 Hz, 9'-H), 7.67 (1H, t, J = 6.0 Hz, 8'-H), 8.00 (1H, d, J = 7.7 Hz, 7-H), 8.16 (1H, d, J = 9.0 Hz, 7'-H), 9.26 (1H, d, J = 8.7 Hz, 10'-H); δ_C (150 MHz; DMSO-d_6) 91.8 (C-3), 108.6, 117.2, 125.6, 126.0, 128.3, 128.9, 128.9, 130.3, 134.2 and 154.9 (Ar-C), 161.4 (C=O) and 169.4 (C-OH).

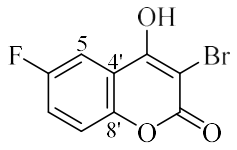
3.3. SYNTHESIS OF 3-BROMINATED 4-HYDROXYCOUMARINS 140

3-Bromo-4-hydroxycoumarin 140a



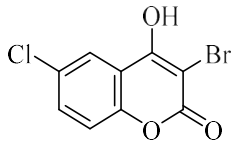
To a stirred solution of 4-hydroxycoumarin (2 g, 12.4 mmol) in CH_3CN (40 mL) was added *N*-bromosuccinamide (NBS) (12.6 mmol) and the mixture was allowed to stir for at least 2 hours at room temperature after which the solvent was evaporated and the dry residue was washed with acidified water, filtered, and the solid was then washed with water and dried to give 3-bromo-4-hydroxycoumarin **140a** as a white solid (2.17 g, 73%), m.p. 195-197 °C (lit.[377] 192-194 °C); ν_{max}/cm^{-1} 1697 (C=O); δ_H (400 MHz; DMSO-d_6) 7.33-7.43 (2H, m, 5- and 6-H overlapping), 7.66 (1H, t, J = 7.8 Hz, 7-H) and 7.95 (1H, d, J = 7.8 Hz, 8-H); δ_C (100 MHz; DMSO-d_6) 89.1 (C-3), 115.9, 116.4, 123.5, 124.4, 132.8 and 151.7 (Ar-C), 158.5 (C=O) and 163.3 (C-OH).

3-Bromo-6-fluoro-4-hydroxycoumarin 140b



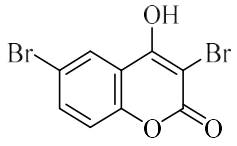
The procedure described for the synthesis of compound **140a** was followed using 6-fluoro-4-hydroxycoumarin (2.23 g, 12.4 mmol). Work-up afforded 3-bromo-6-fluoro-4-hydroxycoumarin **140b** as a white solid (2.20 g, 68%), m.p. 225-227 ^{o}C; [HRMS: *m/z* calculated for $C_9H_5BrFO_3$ (MH^+) 258.9406. Found 258.9412]; ν_{max}/cm^{-1} 3245 (O-H) and 1697 (C=O); δ_H (600 MHz; methanol-d_4) 7.17-7.44 (2H, m, 7 and 8-H overlapping) and 7.55 (1H, dd, J = 8.6, 2.9 Hz, 5-H); δ_C (150 MHz; DMSO-d_6) 90.8 (C-3), 110.0 (d, J_{FC} = 26.0 Hz, C-5), 118.2 (d, J_{FC} = 9.0 Hz, C-4'), 119.6 (d, J_{FC} = 8.5 Hz, C-8), 121.2 (d, J_{FC} = 25.0 Hz, C-7), 149.67 (d, J_{FC} = 1.9 Hz, C-8'), 160.3 (d, J_{FC} = 243.2 Hz, C-6), 160.9 (C=O) and 163.2 (d, J_{FC} = 2.6 Hz, C-OH).

3-Bromo-6-chloro-4-hydroxycoumarin 140c



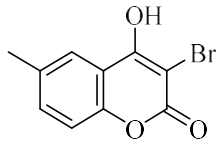
The procedure described for the synthesis of compound **140a** was followed using 6-chloro-4-hydroxycoumarin (2.44 g, 12.4 mmol). Work-up afforded 3-bromo-6-chloro-4-hydroxycoumarin **140c** as a white solid (1.45 g, 42%), m.p. 235-237 ^{o}C; [HRMS: *m/z* calculated for $C_9H_5BrClO_3$ (MH^+) 274.9111. Found 273.9122]; ν_{max}/cm^{-1} 3285 (O-H) and 1710 (C=O); δ_H (600 MHz; DMSO-d_6) 7.43 (1H, d, J = 8.8 Hz, 8-H), 7.67 (1H, d, J = 8.8 Hz, 7-H) and 7.91 (1H, s, 5-H); δ_C (150 MHz; DMSO-d_6) 90.5 (C-3), 117.9, 119.0, 123.1, 128.9, 132.8 and 150.7 (Ar-C), 158.6 (C=O) and 161.8 (C-OH).

3,6-Dibromo-4-hydroxycoumarin 140d



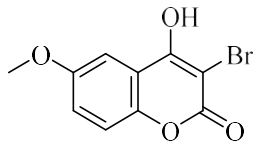
The procedure described for the synthesis of compound **140a** was followed using 6-bromo-4-hydroxycoumarin (2.99 g, 12.4 mmol). Work-up afforded 3,6-dibromo-4-hydroxycoumarin **140d** as a white solid (1.71 g, 43%), m.p. 230-232 °C (lit.[134] 70-72 °C); ν_{max}/cm^{-1} 3300 (O-H), 1691 (C=O); δ_H (600 MHz; DMSO-d_6) 7.36 (1H, d, J = 8.8 Hz, 8-H), 7.78 (1H, dd, J = 8.8, 2.3 Hz, 7-H) and 8.04 (1H, d, J = 2.2 Hz, 5-H); δ_C (150 MHz; DMSO-d_6) 89.8 (C-3), 116.1, 118.1, 118.8, 125.6, 135.0 and 150.7 (Ar-C), 158.2 (C=O) and 161.5 (C-OH).

3-Bromo-6-methyl-4-hydroxycoumarin 140e



The procedure described for the synthesis of compound **140a** was followed using 3-bromo-6-methyl-4-hydroxycoumarin (2.18 g, 12.4 mmol). Work-up afforded 3-bromo-4-hydroxy-6-methylcoumarin **140e** as a light brown crystalline solid (2.14 g, 68 %), m.p. 240-242 °C; [HRMS: *m/z* calculated for $C_{10}H_8BrO_3$ (MH^+) 254.9657. Found 254.9666]; ν_{max}/cm^{-1} 1670 (C=O); δ_H (600 MHz; DMSO-d_6) 2.37 (3H, s, CH_3), 7.27 (1H, d, J = 8.4 Hz, 8-H), 7.45 (1H, dd, J = 8.4, 1.8 Hz, 7-H) and 7.72 (1H, s, 5-H); δ_C (150 MHz; DMSO-d_6) 20.4 (CH_3) 89.1 (C-3), 115.46, 116.2, 123.0, 133.6, 133.7 and 149.8 (Ar-C), 158.6 (C=O) and 162.2 (C-OH).

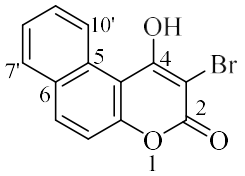
3-Bromo-6-methoxy-4-hydroxycoumarin 140f



The procedure described for the synthesis of compound **140a** was followed using 3-bromo-6-methoxy-4-hydroxycoumarin (2.18 g, 12.4 mmol). Work-up afforded 3-bromo-4-hydroxy-6-methoxycoumarin **140f** as a light brown crystalline solid (1.08 g, 32 %), m.p.188-190 °C; [HRMS: *m/z* calculated for $C_{10}H_8BrO_4$ (MH^+) 270.9606. Found 270.9615]; ν_{max}/cm^{-1} 3430 (O-H) and 1703 (C=O); δ_H (400 MHz; DMSO-d_6) 3.81 (3H, s, OCH_3), 7.24 (1H, dd, J = 9.0,

3.0 Hz, 7-H), 7.34 (1H, d, *J* = 9.0 Hz, 8-H) and 7.40 (1H, d, *J* = 2.9 Hz, 5-H); δ_C (100 MHz; DMSO-d_6) 55.8 (OCH_3) 89.6 (C-3), 105.5, 116.3, 117.8, 120.4, 146.1 and 155.6 (Ar-C), 158.7 (C=O) and 161.2 (C-OH).

5,6-Benzo-3-bromo-4-hydroxycoumarin 140g

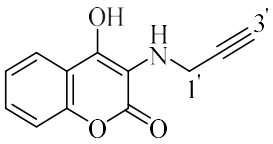


The procedure described for the synthesis of compound **140a** was followed using 5,6-benzo-4-hydroxycoumarin (2.63 g, mmol). Work-up afforded 5,6-benzo-3-bromo-4-hydroxycoumarin **140g** as a brown solid (0.77 g, 21%), m.p. 205-207 ^oC; [HRMS: *m/z* calculated for $C_{13}H_8BrO_3$ (MH^+) 290.9664. Found 290.9657]; ν_{max}/cm^{-1} 3454 (O-H) and 1660 (C=O); δ_H (400 MHz; DMSO-d_6) 7.53 (1H, d, *J* = 9.0 Hz, 8-H), 7.59 (1H, ddd, *J* = 8.0, 7.0, 1.1 Hz, 9'-H), 7.71 (1H, ddd, *J* = 8.7, 6.9, 1.5 Hz, 8'-H), 8.03 (1H, d, *J* = 7.4 Hz, 7-H), 8.20 (1H, d, *J* = 8.9 Hz, 7'-H) and 9.36 (1H, d, *J* = 8.8 Hz, 10'-H); δ_C (150 MHz; DMSO-d_6) 90.3 (2-C), 109.2, 117.0, 125.8, 126.0, 128.5, 128.7, 129.1, 130.6, 134.4, 152.9 (Ar-C), 158.2(C=O) and 165.8 (C-OH).

3.4. SYNTHESIS OF SECONDARY 4-HYDROXYCOUMARIN AMINES 162 and 163

3.4.1. 4-hydroxy-3-(propargylamino)coumarins

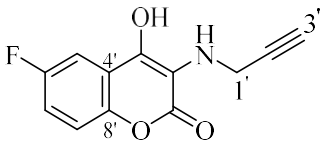
4-Hydroxy-3-(propargylamino)coumarin 162a



A mixture of 140**a** (0.72 g, 3 mmol) and propargylamine (200 µL, 3.1 mml) in THF was stirred at room temperature for at least 2 hours after which the solvent was evaporated in vacuo and the residue was washed with DCM and filtered off to give the desired product 4-hydroxy-3-(propargylamino)coumarin **162a** as a white solid (0.44 g, 68%), m.p. 196 ^oC (decomp); ν_{max}/cm^{-1} 2157 (C≡C) and 1680 (C=O); δ_H (600 MHz; methanol-d_4) 3.11 (1H, t, *J*

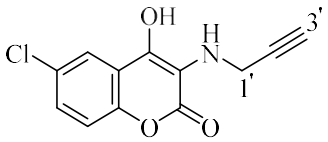
= 2.6 Hz, C≡CH), 3.84 (2H, d, *J* = 2.6 Hz, CH_2), 7.21-7.32 (2H, m, 6 and 8-H overlapping), 7.53 (1H, ddd, *J* = 8.3, 7.2, 1.7 Hz, 7-H) and 8.00 (1H, dd, *J* = 8.2, 1.7 Hz, 5-H); δ_C (150 MHz; DMSO-d_6) 30.0 (C-1'), 76.3 (C-2'), 77.5 (C-3'), 86.8 (C-3), 117.1, 122.8, 124.4, 125.8, 132.3 and 154.1 (Ar-C), 165.1 (C=O) and 173.6 (C-OH).

6-Fluoro-4-hydroxy-3-(propargylamino)coumarin 162b



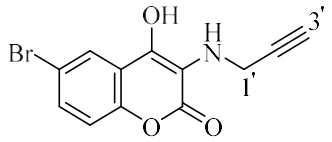
The procedure described for the synthesis of compound **162a** was followed using 3-bromo-6-fluoro-4-hydroxycoumarin (0.78 g, 3 mmol). Work-up afforded 6-fluoro-4-hydroxy-3-(propargylamino)coumarin **162b** as an off white solid (0.25 g, 36 %), m.p. 175 (decomp); ν_{max}/cm^{-1} 3287 (≡C-H) and 2131 (C≡C); δ_H (600 MHz; DMSO-d_6) 3.60 (1H, t, *J* = 2.5 Hz, C≡CH), 3.77 (2H, d, *J* = 2.6 Hz, CH_2), 7.20 (1H, dd, *J* = 8.9, 4.4 Hz, 8-H), 7.27 (1H, td, *J* = 8.5, 3.2 Hz, 7-H) and 7.47 (dd, *J* = 9.0, 3.2 Hz, 5-H); δ_C (150 MHz; DMSO-d_6) 28.6 (C-1'), 77.0 (C-2'), 78.0 (C-3'), 84.1 (C-3), 109.7 (d, J_{CF} = 23.7 Hz, C-5), 117.5 (d, J_{CF}= 14.5 Hz, C-7), 117.6 (d, J_{CF} = 2.0 Hz, C-8), 123.7 (d, J_{CF} = 6.8 Hz, C-4'), 148.9 (d, J_{CF} = 1.4 Hz, C-8'), 157.7 (d, J_{CF} = 238.5 Hz, 6-H) 160.2 (C=O) and 168.3 (d, J_{CF} = 1.9 Hz, C-OH).

6-Chloro-4-hydroxy-3-(propargylamino)coumarin 162c



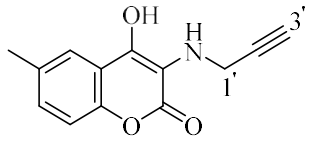
The procedure described for the synthesis of compound **162a** was followed using 3-bromo-6-chloro-4-hydroxycoumarin (0.83 g, 3 mmol). Work-up afforded 6-chloro-4-hydroxy-3-(propargylamino)coumarin **162c** as a white solid (0.4 g, 53%), m.p. 185 ^oC (decomp); ν_{max}/cm-1 3406 (N-H) and 2156 (C≡C); δ_H (400 MHz; DMSO-d_6) 3.59 (1H, t, *J* = 2.6 Hz, C≡CH), 3.75 (2H, d, *J* = 2.6 Hz, CH_2), 7.19 (1H, d, *J* = 8.7 Hz, 8-H), 7.44 (1H, dd, *J* = 8.7, 2.7 Hz, 7-H) and 7.74 (1H, d, *J* = 2.7 Hz, 5-H); δ_C (100 MHz; DMSO-d_6) 28.5 (C-1'), 76.9 (C-2'), 78.0 (C-3'), 83.5 (C-3), 117.8, 122.8, 123.9, 126.5, 130.0 and 151.3 (Ar-C), 159.9 (C=O) and 167.9 (C-OH).

6-Bromo-4-hydroxy-3-(propargylamino)coumarin 162d



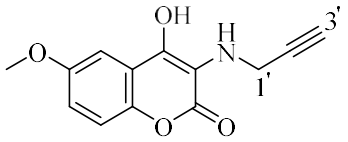
The procedure described for the synthesis of compound **162a** was followed using 3,6-dibromo-4-hydroxycoumarin (0.96 g, 3 mmol). Work-up afforded 6-bromo-4-hydroxy-3-(propargylamino)coumarin **162d** as a pale yellow solid (0.35 g, 40%), m.p. 184 ^{o}C (decomp); ν_{max}/cm^{-1} 3281 (≡C-H) and 2129 (C≡C); δ_H (600 MHz; DMSO-d_6) 3.60 (1H, m, C≡CH), 3.77 (2H, m, CH_2), 7.15 (1H, d, J = 8.6 Hz, 8-H), 7.57 (1H, dd, J = 8.6, 2.6 Hz, 7-H) and 7.88 (1H, s, 5-H); δ_C (150 MHz; DMSO-d_6) 28.6 (C-1'), 77.0 (C-2'), 78.0 (C-3'), 84.0 (C-3), 114.5, 118.3, 124.2, 126.9, 132.9 and 151.7 (Ar-C), 159.9 (C=O) and 168.0 (C-OH).

4-Hydroxy-6-methyl-3-(propargylamino)coumarin 162e



The procedure described for the synthesis of compound **162a** was followed using 6-methyl-4-hydroxycoumarin (0.77 g, 3 mmol). Work-up afforded 4-hydroxy-6-methyl-3-(propargylamino)coumarin **162e** as a yellow solid (0.4 g, 58%), m.p. 164 ^{o}C (decomp); ν_{max}/cm^{-1} 3267 (≡C-H) and 2124 (C≡C); δ_H (400 MHz; DMSO-d_6) 2.32 (3H, s, CH_3), 3.59 (1H, t, J = 2.1 Hz, C≡CH), 3.76 (2H, d, J = 1.9 Hz, CH_2), 7.02 (1H, d, J = 8.3 Hz, 8-H), 7.22 (1H, m, 7-H) and 7.61 (1H, s, 5-H); δ_C (100 MHz; DMSO-d_6), 20.5 (CH_3), 28.6 (C-1'), 77.0 (C-2'), 78.0 (C-3'), 84.0 (C-3), 115.4, 122.0, 124.5, 131.17, 139.12 and 150.8 (Ar-C), 160.4 (C=O) and 169.4 (C-OH).

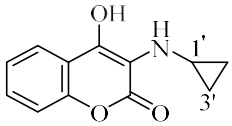
4-Hydroxy-6-methoxy-3-(propargylamino)coumarin 162f



The procedure described for the synthesis of compound **162a** was followed using 6-methoxy-4-hydroxycoumarin (0.81 g, 3 mmol). Work-up afforded 4-hydroxy-6-methoxy-3-(propargylamino)coumarin **162f** as a yellow solid (0.42 g, 57%), m.p. 160 ^{o}C (decomp); ν_{max}/cm^{-1} 3267 (≡C-H) and 2157 (C≡C); δ_H (400 MHz; DMSO-d_6) 3.59 (1H, t, J = 2.2 Hz, C≡CH), 3.76 (5H, m, overlapping CH_2 and CH_3), 7.00 (1H, dd, J = 8.8, 3.1 Hz, 7-H), 7.08 (1H, d, J = 8.8 Hz, 8-H) and 7.29 (1H, d, J = 3.0 Hz, 5-H); δ_C (100 MHz; DMSO-d_6), 28.5 (C-1') 55.4 (OCH_3), 77.0 (C-2'), 78.0 (C-3'), 84.2 (C-3), 106.7, 116.7, 122.9, 147.0 and 154.5 (Ar-C), 160.5 (C=O) and 169.1 (C-OH).

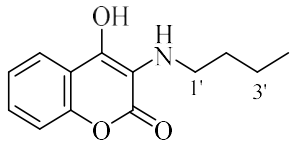
3.4.2. 3-amino-4-hydroxycoumarins

3-(Cyclopropylamino)-4-hydroxycoumarin 163a



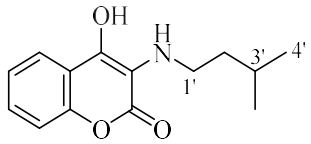
To a stirred solution of 3-bromo-4-hydroxycoumarin **140a** (0.6 g, 2.5 mmol) in THF (8 mL) was added cyclopropylamine (194 µL, 2.8 mmol) and the mixture allowed to stir for at least 2 hours at room temperature after which the solvent was evaporated in vacou and the dry residue was washed with DCM, filtered off and then dried to give 3-(cyclopropylamino)-4-hydroxycoumarin **163a** as a white solid (0.40 g, 74%), m.p. 126-128 ^{o}C; ν_{max}/cm^{-1} 3473 (N-H) and 1623 (C=O); δ_H (300 MHz; methanol-d_4) 0.51-0.82 (4H, overlapping m, 2' and 3'-CH_2), 2.51 (1H, tt, J = 7.0, 3.8 Hz, 1'-H), 7.09-7.18 (2H, m, 6- and 8-H overlapping), 7.39 (1H, t, J = 7.7 Hz, 7-H) and 7.86 (1H, d, J = 8.2 Hz, 5-H); δ_C (75 MHz; methanol-d_4) 4.2 (C-2' and C-3'), 23.8 (C-1'), 86.8 (C-3), 117.1, 122.8, 124.4, 125.8, 132.3 and 154.1 (Ar-C), 165.1 (C=O) and 173.7 (C-OH).

3-(Butylamino)-4-hydroxycoumarin 163b



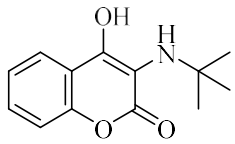
The procedure described for the synthesis of compound **163a** was followed using butylamine (277 µL, 2.8 mmol). Work-up afforded 3-(butylamino)-4-hydroxycoumarin **163b** as a white solid (0.44 g, 75%), m.p. 110-112 ^{o}C; ν_{max}/cm^{-1} 3473 (N-H) and 1657 (C=O); δ_H (600 MHz; methanol-*d*$_4$) 0.85 (3H, t, *J* = 7.4 Hz, CH_3), 1.30 (2H, sextet, *J* = 7.2 Hz, 3'-CH_2), 1.54 (2H, quintet, *J* = 7.5 Hz, 2'-CH_2), 2.84 (2H, t, *J* = 9.0 Hz, 1'-CH_2), 7.13-7.19 (2H, m, 6- and 8-H overlapping), 7.42 (1H, td, *J* = 7.6, 1.6 Hz, 7-H) and 7.89 (1H, dd, *J* = 8.3, 1.6 Hz, 5-H); δ_C (150 MHz; methanol-*d*$_4$) 13.9 (CH_3), 20,7 (C-3'), 30.6 (C-2'), 40.5 (C-1'), 86.8 (C-3), 117.1, 122.8, 124.4, 125.8, 132.3 and 154.1 (Ar-C), 165.1 (C=O) and 173.7 (C-OH).

4-Hydroxy-3-(isopentylamino)coumarin 163c



The procedure described for the synthesis of compound **163a** was followed using 3-methylbutylamine (326 µL, 2.8 mmol). Work-up afforded 4-hydroxy-3-(isopentylamino)coumarin **163c** as a white solid (0.41 g, 66%), m.p. 134-136 ^{o}C; ν_{max}/cm^{-1} 1632 (C=O); δ_H (600 MHz; DMSO-*d*$_6$) 0.85 (6H, d, *J* = 6.6 Hz, 4'-CH_3 and 5'-H_3), 1.43 (2H, m, 2'-CH_2), 1.60 (1H, septet, *J* = 7.2 Hz, 3'-CH), 2.81 (2H, t, *J* = 6.0 Hz, 1'-CH_2), 7.12-7.18 (2H, m, 6 and 8-H overlapping), 7.42 (1H, m, 7-H) and 7.82 (1H, dd, *J* = 8.2, 1.7 Hz, 5-H); δ_C (150 MHz; DMSO-*d*$_6$) 22.2 (2xCH_3), 25.0 (C-3'), 35.9 (C-2'), 37.5 (C-1'), 84.0 (C-3), 115.6, 122.3, 122.4, 124.7, 130.5 and 152.7 (Ar-C), 160.3 (C=O) and 169.5 (C-OH).

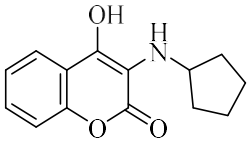
3-(*tert*-Butylamino)-4-hydroxycoumarin 163d



The procedure described for the synthesis of compound **163a** was followed using *tert*-butylamine (294 µL, 2.8 mmol). Work-up afforded 3-(*tert*-butylamino)- 4-hydroxycoumarin **163d** as a white solid (0.46 g, 79%), m.p. 230-232 ^{o}C; ν_{max}/cm^{-1} 1637 (C=O); δ_H (300 MHz; DMSO-*d*$_6$) 1.26 (9H, s, 3xCH_3), 7.12-7.19 (2H, m, 6- and 8-H overlapping), 7.43 (1H, ddd, *J*

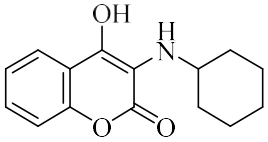
= 8.3, 7.1, 1.8 Hz, 7-H) and 7.82 (1H, dd, *J* = 8.0, 1.8 Hz, 5-H); δ_C (75 MHz; DMSO-d_6) 27.5 (3xCH_3), 51.4 (C-1'), 84.4 (C-3), 116.0, 122.7, 122.8, 125.1, 130.9 and 153.1 (Ar-C), 160.7 (C=O) and 169.8 (C-OH).

3-(Cyclopentylamino)-4-hydroxycoumarin 163e



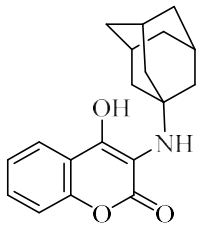
The procedure described for the synthesis of compound **163a** was followed using cyclopentylamine (325 μL, 2.8 mmol). Work-up afforded 3-(cyclopentylamino)-4-hydroxycoumarin **163e** as a white solid (0.40 g, 67%), m.p. 146-148 °C; ν_{max}/cm^{-1} 1624 (C=O); δ_H (300 MHz; DMSO-d_6) 1.44-1.98 (8H, series of m, cyclopentyl CH_2), 3.43-3.55 (1H, m, CHN), 7.10-7.20 (2H, m, 6- and 8-H overlapping), 7.42 (1H, ddd, *J* = 8.3, 7.2, 1.8 Hz, 7-H) and 7.82 (1H, dd, *J* = 8.1, 1.7 Hz, 5-H); δ_C (75 MHz; DMSO-d_6) 23.5 (NCH_2CH_2), 30.7 (2xNCHCH_2) 51.3 (CHN), 83.8 (C-3), 115.5, 122.3, 124.7, 130.3 and 152.7 (Ar-C), 160.3 (C=O) and 169.4 (C-OH).

3-(Cyclohexylamino)-4-hydroxycoumarin 163f



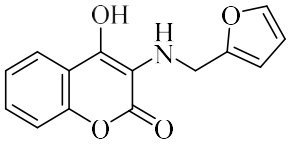
The procedure described for the synthesis of compound **163a** was followed using cyclohexylamine (320 μL, 2.8 mmol). Work-up afforded 3-(cyclohexylamino)-4-hydroxycoumarin **163f** as a white solid (0.47 g, 75%), m.p. 164-165 °C; ν_{max}/cm^{-1} 3458 (N-H) and 1625 (C=O); δ_H (600 MHz; DMSO-d_6) 1.12-1.84 (10H, series of m, cyclohexyl CH_2), 3.01-3.10 (1H, m, CHN), 7.21-7.28 (2H, m, 6- and 8-H overlapping), 7.51 (1H, ddd, *J* = 8.9, 7.5, 1.7 Hz, 7-H) and 7.98 (1H, dd, *J* = 8.3, 1.6 Hz, 5-H); δ_C (150 MHz; DMSO-d_6) 25.3 ($NCHCH_2CH_2$), 25.9 (NCH_2CH_2), 31.9 (2xNCHCH_2) 51.5 (CHN), 86.8 (C-3), 117.1, 122.8, 124.4, 125.8, 132.3 and 154.1 (Ar-C), 165.1 (C=O) and 173.7 (C-OH).

3-(Adamantanylamino)-4-hydroxycoumarin 163g



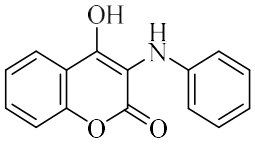
The procedure described for the synthesis of compoundn **163a** was followed using 1-amantylamine (0.42 g, 2.8 mmol). Work-up afforded 3-(adamantylamino)-4-hydroxycoumarin **163g** as a white solid (0.60 g, 77%), m.p. 224-225 ^{o}C; ν_{max}/cm^{-1} 1652 (C=O); δ_H (400 MHz; DMSO-d_6) 1.52-1.68 (6H, m, 3xCH$_2$), 1.76-1.79 (6H, m, 3xCHC*H$_2$*), 2.07 (3H, s, 3xCH), 7.10-7.19 (2H, m, 6- and 8-H overlapping), 7.42 (1H, td, *J* = 7.9, 1.7 Hz, 7-H) and 7.82 (1H, dd, *J* = 7.9, 1.4 Hz, 5-H); δ_C (100 MHz; DMSO-d_6) 28.4 (3xCH$_2$), 35.1 (3xCH*C*H$_2$), 40.1 (3xNC*C*H$_2$) 51.0 (CNH), 83.9 (C-3), 115.6, 122.3, 122.4, 124.7, 130.4 and 152.7 (Ar-C), 160.4 (C=O) and 169.4 (C-OH).

3-(Furfurylamino)-4-hydroxycoumarin 163h



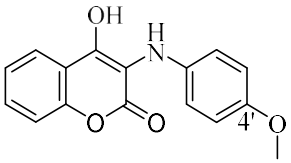
The procedure described for the synthesis of compound **163a** was followed using furfurylamine (247 μL, 2.8 mmol). Work-up afforded 3-(furfurylamino)-4-hydroxycoumarin **163h** as a pale yellow solid (0.51 g, 79%), m.p. 151-153 ^{o}C; ν_{max}/cm^{-1} 1643 (C=O); δ_H (600 MHz; DMSO-d_6) 4.11 (2H, s, CH$_2$), 6.49 (1H, dd, *J* = 3.2, 1.9 Hz, ArH), 6.53 (1H, d, *J* = 3.2 Hz, ArH), 7.12-7.16 (2H, m, ArH), 7.41 (1H, m, ArH), 7.73 (1H, dd, *J* = 1.8, 0.7, Hz, ArH) and 7.81 (1H, dd, *J* = 7.6, 1.4 Hz, ArH); δ_C (150 MHz; DMSO-d_6) 35.2 (CH$_2$), 83.7 (C-3), 110.3, 111.0, 115.5, 122.2, 122.4, 124.7, 130.3, 143.7, 147.6 and 152.7 (Ar-C), 160.3 (C=O) and 169.3 (C-OH).

4-Hydroxy-3-(phenylamino)coumarin 163i



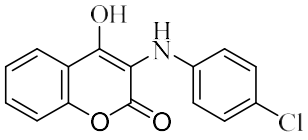
The procedure described for the synthesis of compound **163a** was followed using aniline (256 μL, 2.8 mmol). Work-up afforded 4-hydroxy-3-(phenylamino)coumarin **163i** as a white solid (0.5 g, 79%), m.p. 157-158 °C; ν_{max}/cm^{-1} 1648 (C=O); δ_H (300 MHz; DMSO-d_6) 6.95-7.06 (3H, m, overlapping ArH), 7.22-7.32 (4H, m, overlapping ArH), 7.54 (1H, ddd, *J* = 9.4, 6.7, 1.7 Hz, 7-H) and 7.91 (1H, dd, *J* = 8.3, 1.6 Hz, 5-H); δ_C (75 MHz; DMSO-d_6) 86.4 (C-3), 116.0, 119.0, 119.2, 122.6, 123.3, 124.1, 129.4, 131.5, 139.3 and 152.2 (Ar-C), 159.4 (C=O) and 166.1 (C-OH).

4-Hydroxy-3-[(4-methoxyphenyl)amino]coumarin 163j



The procedure described for the synthesis of compound **163a** was followed using *p*-anisidine (0.34 g, 2.8 mmol). Work-up afforded 4-hydroxy-3-[(4-methoxyphenyl)amino]coumarin **163j** as a white solid (0.47 g, 66%), m.p. 167-168 °C; ν_{max}/cm^{-1} 1659 (C=O); δ_H (300 MHz; DMSO-d_6) 2.20 (3H, s, CH_3) 6.91 (1H, td, *J* = 7.3, 1.5 Hz, ArH), 7.02 (1H, dd, *J* = 7.8, 1.6 Hz, ArH), 7.06-7.16 (2H, m, ArH), 7.22-7.30 (2H, m, 6 and 8-H overlapping), 7.54 (1H, ddd, *J* = 9.4, 6.7, 1.7 Hz, 7-H) and 7.91 (1H, dd, *J* = 8.2, 1.7 Hz, 5-H); δ_C (75 MHz; DMSO-d_6) 55.7 (CH_3), 85.0 (C-3), 115.1, 116.0, 121.6, 123.0, 123.7, 124.8, 126.0, 131.1, 152.7 and 158.3 (Ar-C), 160.6 (C=O) and 169.1 (C-OH).

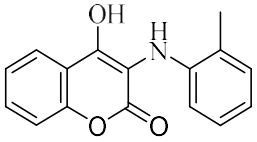
3-[(4-Chlorophenyl)amino]-4-hydroxycoumarin 163k



The procedure described for the synthesis of compound **163a** was followed using 4-chloroaniline (0.36 g, 2.8 mmol). Work-up afforded 3-[(4-chlorophenyl)amino]-4-hydroxycoumarin **163k** as a white solid (0.51g, 71%), m.p. 157-159 °C; ν_{max}/cm^{-1} 1663 (C=O); δ_H (400 MHz; DMSO-d_6) 6.76-6.82 (2H, m, ArH), 7.14-7.19 (2H, m, ArH), 7.31-7.37

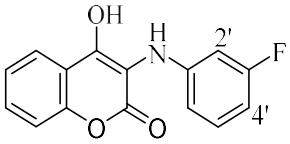
(2H, m, 6 and 8-H overlapping), 7.58-7.63 (1H, m, 7-H) and 7.93 (1H, dd, *J* = 7.9, 1.2 Hz, 5-H); δ_C (100 MHz; DMSO-d_6) 88.0 (C-3), 116.3, 117.4, 118.1, 122.8, 123.8, 124.0, 128.9, 132.3, 142.9 and 152.0 (Ar-C), 159.0 (C=O) and 164.1 (C-OH).

4-Hydroxy-3-(o-tolylamino)coumarin 163l



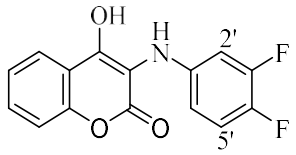
The procedure described for the synthesis of compound **163a** was followed using *o*-toluidine (306 μL, 2.8 mmol). Work-up afforded 4-hydroxy-3-(*o*-tolylamino)coumarin **163l** as a white solid (0.47 g, 70%), m.p. 140-141 °C; ν_{max}/cm^{-1} 1656 (C=O); δ_H (300 MHz; DMSO-d_6) 2.20 (3H, s, CH_3) 6.91 (1H, td, *J* = 7.3, 1.5 Hz, ArH), 7.02 (1H, dd, *J* = 7.8, 1.6 Hz, ArH), 7.06-7.16 (2H, m, ArH), 7.22-7.30 (2H, m, ArH), 7.54 (1H, ddd, *J* = 9.4, 6.7, 1.7 Hz, 7-H) and 7.91 (1H, dd, *J* = 8.2, 1.7 Hz, 5-H); δ_C (75 MHz; DMSO-d_6) 17.09 (CH_3), 86.5 (C-3), 116.0, 119.0, 119.2, 122.7, 123.3, 124.1, 126.8, 130.7, 131.5, 137.9 and 152.2 (Ar-C), 159.4 (C=O) and 166.0 (C-OH).

3-[(3-Fluorophenyl)amino]-4-hydroxycoumarin 163m



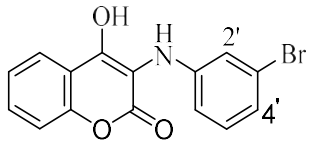
The procedure described for the synthesis of compound **163a** was followed using 3-fluoroaniline (268 μL, 2.8 mmol). Work-up afforded 3-[(3-fluorophenyl)amino]-4-hydroxycoumarin **163m** as a pale yellow solid (0.56 g, 83%), m.p. 149-150 °C; ν_{max}/cm^{-1} 1643 (C=O); δ_H (400 MHz; DMSO-d_6) 6.50-6.34 (3H, m, ArH), 7.07 (1H, q, *J* = 7.8 Hz, ArH), 7.31-7.40 (2H, m, ArH), 7.63 (1H, m, 7-H) and 7.94 (1H, dd, *J* = 7.9, 1.3 Hz, 5-H); δ_C (100 MHz; DMSO-d_6) 88.5 (C-3), 101.6 (dd, $^2J_{CF}$ = 24.3 Hz, 12.9 Hz, C-2'), 103.6 (dd, $^2J_{CF}$ = 21.3, 15.4 Hz, C-4'), 111.4 (m, C-6'), 116.4, 116.8, 123.7, 124.2, 130.5 (d, $^3J_{CF}$ = 10.2 Hz, C-5'), 132.6, 148.4 (d, $^3J_{CF}$ = 11.3 Hz, C-1') and 151.9 (Ar-C), 158.9 (C=O), 163.22 (1C, d, $^1J_{CF}$ = 240.2 Hz, C-3') and 163.4 (C-OH).

3-[(3,4-Difluorophenyl)amino]-4-hydroxycoumarin 163n



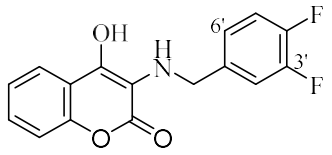
The procedure described for the synthesis of compound **163a** was followed using 3,4-difluoroaniline (278 μL, 2.8 mmol). Work-up afforded 3-[(3,4-difluorophenyl)amino]-4-hydroxycoumarin **163n** as a white solid (0.46 g, 64%), m.p. 149-151 °C; ν_{max}/cm^{-1} 1619 (C=O); δ_H (400 MHz; DMSO-d_6) 6.46 (1H, m, ArH), 6.63 (1H, ddd, J = 12.9, 7.0, 2.4 Hz, ArH), 7.10 (1H, m, ArH), 7.33-7.40 (2H, m, ArH), 7.64 (1H, m, 7-H) and 7.95 (1H, dd, J = 7.9, 1.3 Hz, 5-H); δ_C (100 MHz; DMSO-d_6) 88.5 (C-3), 103.6 (d, $^2J_{C\text{-}3'F}$ = 19.8 Hz, C-2'), 111.1 (m, C-6') , 116.4, 116.7, 117.5 (dd, $^2J_{C\text{-}4'F}$ = 17.6, $^3J_{C\text{-}3'F}$ = 1,2 Hz, C-5'), 123.7, 124.2, 132.6, 142.3 (dd, $^1J_{C\text{-}4'F}$ = 234.5 Hz, $^2J_{C\text{-}3'F}$ = 11.6 Hz, C-4'), 143.6 (m, C-1'), 149.8 (dd, $^1J_{C\text{-}3'F}$ = 242.0, $^2J_{C\text{-}4'F}$ = 11.6 Hz, C-3') and 151.8 (Ar-C), 158.8 (C=O) and 163.3 (C-OH).

3-[(3-Bromophenyl)amino]-4-hydroxycoumarin 163o



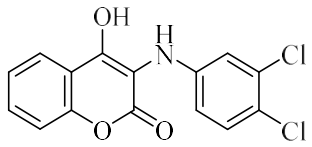
The procedure described for the synthesis of compound **163a** was followed using 3-bromoaniline (304 μL, 2.8 mmol). Work-up afforded 3-[(3-bromophenyl)amino]-4-hydroxycoumarin **163o** as a white solid (0.50 g, 60%), m.p. 143-144 °C; ν_{max}/cm^{-1} 1661 (C=O); δ_H (400 MHz; DMSO-d_6) 6.64 (1H, dd, J = 8.1, 1.3 Hz, ArH), 6.74 (1H, dd, J = 7.9, 1.0 Hz, ArH), 6.84 (1H, m, ArH), 7.00 (1H, t, J = 8.0 Hz, ArH), 7.33-7.41 (2H, m, 6 and 8-H overlapping), 7.64 (1H, ddd, J = 8.5, 7.4, 1.5 Hz, 7-H) and 7.95 (1H, dd, J = 7.9, 1.4 Hz, 5-H); δ_C (100 MHz; DMSO-d_6) 88.7 (C-3), 114.1, 116.4, 116.5, 117.3, 119.7, 122.1, 123.6, 124.3, 130.9, 132.6, 148.3 and 151.8 (Ar-C), 158.8 (C=O) and 163.1 (C-OH).

3-[(3,4-Difluorobenzyl)amino]-4-hydroxycoumarin 163p



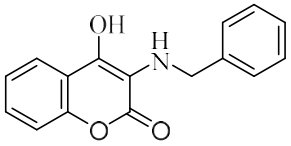
The procedure described for the synthesis of compound **163a** was followed using 3,4-difluorobenzylamine (331 μL, 2.8 mmol). Work-up afforded 3-[(3,4-difluorophenyl)amino]-4-hydroxycoumarin **163p** as a white solid (0.66 g, 87%), m.p. 142-143 ^{o}C; ν_{max}/cm^{-1} 1649 (C=O); δ_H (600 MHz; DMSO-d_6) 4.08 (2H, s, CH_2), 7.11-7.18 (2H, m, ArH), 7.34 (1H, m, ArH), 7.42 (1H, t, J = 7.6 Hz, ArH), 7.49 (1H, m, ArH), 7.59 (1H, m, ArH) and 7.80 (1H, d, J = 7.2, ArH); δ_C (150 MHz; DMSO-d_6) 41.4 (CH_2), 84.0 (C-3), 115.6, 117.7 (d, $^2J_{CF}$ = 17.2 Hz, C-5'), 118.3 (d, $^2J_{CF}$ = 17.8 Hz, C-2'), 122.2, 122.3, 124.7, 126.3 (dd, $^3J_{CF}$ = 6.7, $^4J_{CF}$ = 3.4 Hz, C-6'), 130.4, 131.7 (dd, $^3J_{CF}$ = 6.1, $^4J_{CF}$ = 3.9 Hz, C-1'), 149.1 (dd, $^1J_{C\text{-}4'F}$ = 245.6, $^2J_{C\text{-}3'F}$ =12.4 Hz, C-4'), 149.4 (dd, $^1J_{C\text{-}3'F}$ = 246.5, $^2J_{C\text{-}4'F}$ =12.1 Hz, C-3') and 152.6 (Ar-C), 160.3 (C=O), and 169.4 (C-OH).

3-[(3,4-Dichlorophenyl)amino]-4-hydroxycoumarin 163q



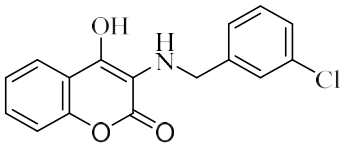
The procedure described for the synthesis of compound **163a** was followed using 3,4-dichloroaniline (0.45 g, 2.8 mmol). Work-up afforded 3-[(3,4-dichlorophenyl)amino]-4-hydroxycoumarin **163q** as a white solid (0.22 g, 27%), m.p. 136-137 ^{o}C; ν_{max}/cm^{-1} 1643 (C=O); δ_H (400 MHz; DMSO-d_6) 6.55 (1H, dd, J = 8.7, 2.6 Hz, ArH), 6.77 (1H, d, J = 2.6 Hz, ArH), 7.20 (1H, d, J = 8.7 Hz, ArH), 7.40 (2H, m, ArH), 7.67 (1H, m, ArH), and 7.96 (1H, dd, J = 7.9, 1.2 Hz, ArH); δ_C (100 MHz; DMSO-d_6) 89.0 (C-3), 114.6, 115.1, 116.2, 116.5, 116.8, 123.6, 124.4, 130.6, 131.1, 132.8, 148.4 and 151.8 (Ar-C), 158.7 (C=O) and 162.7 (C-OH).

3-(Benzylamino)-4-hydroxycoumarin 163r



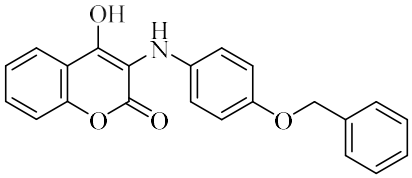
The procedure described for the synthesis of compound **163a** was followed using benzylamine (306 g, 2.8 mmol). Work-up afforded 3-(benzylamino)-4-hydroxycoumarin **163r** as a white solid (0.48 g, 72%), m.p. 145-147 ^{o}C; ν_{max}/cm^{-1} 1636 (C=O); δ_H (300 MHz; DMSO-d_6) 4.07 (2H, s, CH_2) 7.12-7.17 (2H, m, ArH), 7.34-7.51 (6H, m, ArH) and 7.81 (1H, dd, J = 7.9, 1.8 Hz, ArH); δ_C (75 MHz; DMSO-d_6) 42.4 (CH_2), 83.9 (C-3), 115.6, 122.3, 122.4, 124.8, 128.6, 128.7, 128.9, 130.4, 134.0 and 152.7 (Ar-C), 160.3 (C=O) and 169.4 (C-OH).

3-[(3-Chlorobenzyl)amino]-4-hydroxycoumarin 163s



The procedure described for the synthesis of compound **163a** was followed using 3-chlorobenzylamine (331 μL, 2.8 mmol). Work-up afforded 3-[(3-chlorobenzyl)amino]-4-hydroxycoumarin **163s** as a white solid (0.56 g, 74%), m.p. 152-153 ^{o}C; ν_{max}/cm^{-1} 1636 (C=O); δ_H (600 MHz; DMSO-d_6) 4.10 (2H, s, CH_2) 7.11-7.18 (2H, m, ArH), 7.38-7.48 (4H, m, ArH), 7.60 (1H, s, ArH) and 7.81 (1H, d, J = 7.4 Hz, ArH); δ_C (150 MHz; DMSO-d_6) 41.8 (CH_2), 84.0 (C-3), 115.6, 122.2, 122.3, 124.7, 127.7, 128.4, 128.9, 130.4, 130.5, 133.1, 136.4 and 152.7 (Ar-C), 160.3 (C=O) and 169.4 (C-OH).

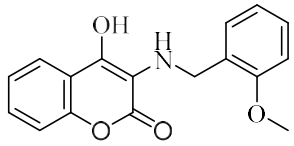
3-{[4-(Benzyloxy)phenyl]amino}-4-hydroxycoumarin 163t



The procedure described for the synthesis of compound **163a** was followed using 4-(benzyloxy)aniline (0.56 g, 2.8 mmol). Work-up afforded 3-{[4-(benzyloxy)phenyl]amino}-4-hydroxycoumarin **163t** as a white solid (0.74 g, 82%), m.p. 172-173 ^{o}C; ν_{max}/cm^{-1} 1641

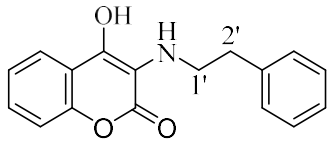
(C=O); δ_H (400 MHz; DMSO-d_6) 5.09 (2H, s, CH_2) 7.06 (2H, d, J = 7.9 Hz, ArH), 7.16-7.21 (4H, m, ArH), 7.32 (1H, t, J = 7.1 Hz, ArH), 7.37-7.51 (5H, m, ArH) and 7.83-7.90 (1H, m, ArH); δ_C (100 MHz; DMSO-d_6) 69.6 (CH_2), 84.9 (C-3), 115.7, 115.8, 121.0, 122.7, 122.8, 124.5, 127.4, 127.7, 127.9, 128.4, 130.8, 136.8, 152.5 and 156.5 (Ar-C), 159.9 (C=O) and 168.1 (C-OH).

4-Hydroxy-3-[(2-methoxybenzyl)amino]coumarin 163u



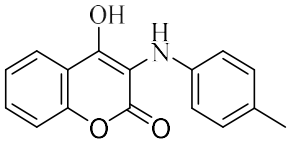
The procedure described for the synthesis of compound **163a** was followed using 2-methoxybenzylamine (366 µL, 2.8 mmol). Work-up afforded 4-hydroxy3-[(2-methoxybenzyl)amino]coumarin **163u** as a white solid (0.65 g, 87%), m.p. 164-165 ^oC; ν_{max}/cm^{-1} 1632 (C=O); δ_H (300 MHz; DMSO-d_6) 3.82 (3H, s, CH_3), 4.01 (2H, s, CH_2) 6.97 (1H, t, J = 7.4 Hz, ArH), 7.05 (1H, d, J = 8.3 Hz, ArH), 7.10-7.18 (2H, m, ArH), 7.33-7.45 (3H, m, ArH) and 7.81 (1H, dd, J = 8.0, 1.8 Hz, ArH); δ_C (75 MHz; DMSO-d_6) 38.0 (CH_2), 55.5 (CH_3), 83.8 (C-3), 110.9, 115.5, 120.3, 121.6, 122.2, 122.3, 124.7, 130.3, 130.4, 152.7 and 157.2 (Ar-C), 160.3 (C=O) and 169.3 (C-OH).

4-Hydroxy-3-(phenethylamino)coumarin 163v



The procedure described for the synthesis of compound **163a** was followed using phenethylamine (353 µL, 2.8 mmol). Work-up afforded 3-(phenethylamino)-4-hydroxycoumarin **163v** as a white solid (0.54 g, 77%), m.p. 167-168 ^oC); ν_{max}/cm-1 1665 (C=O); δ_H (600 MHz; DMSO-d_6) 2.88 (2H, t, J = 6.0 Hz, 2'-CH_2), 3.08 (2H, t, J = 7.6 Hz, 1'-CH_2), 7.16 (2H, t, J = 6.9 Hz, ArH) 7.25 (3H, m, ArH), 7.31 (2H, t, J = 7.3 Hz, ArH), 7.43 (1H, t, J = 7.6 Hz, ArH) and 7.84 (1H, d, J = 7.8 Hz, ArH); δ_C (150 MHz; DMSO-d_6) 33.2 and 40.2 (CH_2), 84.0 (C-3), 115.6, 122.3, 122.4, 124.8, 126.8, 128.68, 128.72, 130.5, 137.3 and 152.7 (Ar-C), 160.3 (C=O) and 169.5 (C-OH).

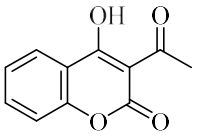
4-Hydroxy-3-(*p*-tolylamino)coumarin 163w



The procedure described for the synthesis of compoundn **163a** was followed using *p*-toluidine (308 μL, 2.8 mmol). Work-up afforded 4-hydroxy-3-(p-tolylamino)coumarin **163w** as a white solid (0.58 g, 87%), m.p. 192-194 ^{o}C; ν_{max}/cm^{-1} 1691 (C=O); δ_H (600 MHz; DMSO-d_6) 2.26 (3H, s, CH_3) 7.09 (2H, d, J = 8.1 Hz, ArH), 7.16-7.23 (4H, m, ArH), 7.49 (1H, td, J = 7.8, 1.6 Hz, 7-H) and 7.87 (1H, d, J = 7.3, Hz, 5-H); δ_C (150 MHz; DMSO-d_6) 20.5 (CH_3), 85.4 (C-3), 115.9, 120.6, 121.0, 122.9, 124.5, 130.1, 131.1, 133.2, 134.5 and 152.5 (Ar-C), 159.9 (C=O) and 167.8 (C-OH).

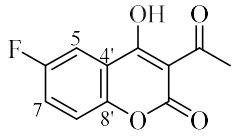
3.5. 3-ACETYL-4-HYDROXYCOUMARINS 133

3-Acetyl-4-hydroxycoumarin 133a



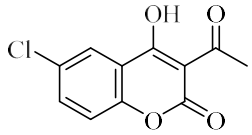
A mixture of 4-hydroxycoumarin **130a** (1.5 g, 9.3 mmol) and $POCl_3$ (2.8 mL, 30 mmol) in glacial acetic acid (8 mL) was refluxed for at least an hour after which the remaining precipitate was filtered, washed with methanol and dried to give to give 3-acetyl-4-hydroxycoumarin **133a** as colourless needle shaped crystals (1.70 g, 90%), m.p. 134-136 ^{o}C (lit.[378] 134-136 ^{o}C); ν_{max}/cm^{-1} 1711 (C=O); δ_H (600 MHz; $CDCl_3$) 2.77 (3H, s, CH_3), 7.27-7.36 (2H, m, ArH), 7.69 (1H, t, J = 7.9 Hz, ArH), 8.04 (1H, d, J = 7.6 Hz, ArH); δ_C (150 MHz; $CDCl_3$) 30.3 (CH_3), 101.5 (C-3), 115.3, 117.3, 124.6, 125.7, 136.3, 154.8 (Ar-C), 160.2 (C=O), 178.8 (C-OH) and 206.2 (CH_3*C*=O).

3-Acetyl-6-fluoro-4-hydroxycoumarin 133b



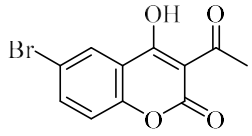
The procedure described for the synthesis of compound **133a** was followed using 6-fluoro-4-hydroxycoumarin **130b** (1.68 g, 9.3 mmol). Work-up afforded 3-acetyl-6-fluoro-4-hydroxycoumarin **133b** as a white fluffy solid (1.10 g, 53%), m.p. 168-170 °C (lit.[379] 170-171 °C); ν_{max}/cm^{-1} 1724 (C=O); δ_H (600 MHz; $CDCl_3$) 2.77 (3H, s, CH_3), 7.28 (1H, dd, *J* = 9.1, 4.2 Hz, ArH), 7.40 (1H, ddd, *J* = 9.0, 7.9, 3.1 Hz, ArH), 7.68 (1H, dd, *J* = 7.8, 3.1 Hz, ArH); δ_C (150 MHz; $CDCl_3$) 30.1 (CH_3), 101.6 (C-3), 111.0 (d, $^2J_{CF}$ = 24.9 Hz, C-5) 116.3 (d, $^3J_{CF}$ = 8.7 Hz, C-4'), 119.0 (d, $^3J_{CF}$ = 7.9 Hz, C-8), 124.0 (d, $^2J_{CF}$ = 24.7 Hz, C-7), 151.1 (d, $^4J_{CF}$ = 1.8 Hz, C-8'), 158.8 (d, $^1J_{CF}$= 246.0 Hz, C-6), 159.8 (C=O), 178.1 (C-OH) and 206.2 (CH_3C=O).

3-Acetyl-6-chloro-4-hydroxycoumarin 133c



The procedure described for the synthesis of compound **133a** was followed using 6-chloro-4-hydroxycoumarin **130c** (1.83 g, 9.3 mmol). Work-up afforded 3-acetyl-6-chloro-4-hydroxycoumarin **133c** as a white crystalline solid (0.93 g, 42%), m.p. 179-181 °C (lit.[380] 176-178 °C); ν_{max}/cm^{-1} 1719 (C=O); δ_H (600 MHz; $CDCl_3$) 2.77 (3H, s, CH_3), 7.24 (1H, d, *J* = 8.9 Hz, ArH), 7.61 (1H, dd, *J* = 8.8, 2.3 Hz, ArH), 7.99 (1H, d, *J* = 2.2 Hz, ArH); δ_C (150 MHz; $CDCl_3$) 30.1 (CH_3), 101.7 (C-3), 116.5, 118.8, 125.1, 130.3, 136.2 and 153.2 (Ar-C), 159.6 (C=O), 177.9 (C-OH) and 206.2 (CH_3C=O).

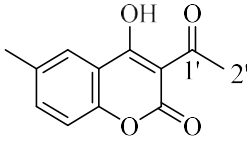
3-Acetyl-6-bromo-4-hydroxycoumarin 133d



The procedure described for the synthesis of compound **133a** was followed using 6-bromo-4-hydroxycoumarin **130d** (2.24 g, 9.3 mmol). Work-up afforded 3-acetyl-6-bromo-4-hydroxycoumarin **133d** as a white solid (1.12 g, 43%), m.p. 195-197 °C (lit.[381] 196-197 °C); ν_{max}/cm^{-1} 1714 (C=O); δ_H (600 MHz; $CDCl_3$) 2.78 (3H, s, CH_3), 7.20 (1H, d, *J* = 8.7 Hz,

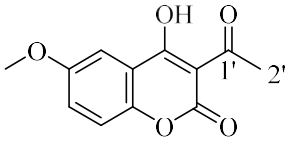
ArH), 7.77 (1H, d, *J* = 8.6 Hz, ArH), 8.16 (1H, s, ArH); δ_C (150 MHz; $CDCl_3$) 30.2 (CH_3), 101.7 (C-3), 116.9, 117.4, 119.0, 128.2, 139.0 and 153.6 (Ar-C), 159.6 (C=O), 177.8 (C-OH) and 206.3 (CH_3C=O).

3-Acetyl-4-hydroxy-6-methylcoumarin 133e



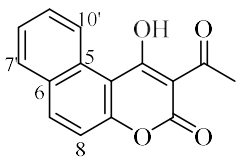
The procedure described for the synthesis of compound **133a** was followed using 4-hydroxy-6-methylcoumarin **130e** (1.64 g, 9.3 mmol). Work-up afforded 3-acetyl-4-hydroxy-6-methylcoumarin **133e** as a pale yellow solid (1.05 g, 52%), m.p. 145-147 °C (lit.[382] 145-147 °C); ν_{max}/cm^{-1} 1710 (C=O); δ_H (600 MHz; $CDCl_3$) 2.41 (3H, s, CH_3), 2.77 (3H, s, 2'-CH_3), 7.17 (1H, d, *J* = 8.5 Hz, ArH), 7.47 (1H, dd, *J* = 8.5, 1.8 Hz, ArH), 7.80 (1H, s, ArH); δ_C (150 MHz; $CDCl_3$) 20.9 (CH_3) 30.1 (2'-CH_3), 101.5 (C-3), 115.0, 116.9, 125.2, 134.4, 137.4 and 153.1 (Ar-C), 160.3 (C=O), 178.8 (C-OH) and 206.1 (1'-C=O).

3-Acetyl-4-hydroxy-6-methoxycoumarin 133f



The procedure described for the synthesis of compound **133a** was followed using 4-hydroxy-6-methoxycoumarin **130f** (1.79 g, 9.3 mmol). Work-up afforded 3-acetyl-4-hydroxy-6-methoxycoumarin **133f** as a khaki solid (1.36 g, 62%), m.p. 148-150 °C (lit.[383] 153 °C); ν_{max}/cm^{-1} 1701 (C=O); δ_H (600 MHz; $CDCl_3$) 2.59 (3H, s, 2'-CH_3), 3.69 (3H, s, OCH_3), 7.03 (1H, dd, *J* = 9.3, 2.4 Hz, ArH), 7.08 (1H, m, ArH), 7.19 (1H, s, ArH); δ_C (150 MHz; $CDCl_3$) 30.3 (2'-CH_3), 56.0 (OCH_3) 101.4 (C-3), 105.7, 115.4, 118.4, 125.5, 149.5 and 156.2 (Ar-C), 160.3 (C=O), 178.6 (C-OH) and 206.3 (1'-C=O).

3-Acetyl-5,6-benzo-4-hydroxycoumarin 133g

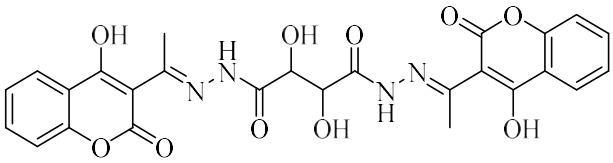


The procedure described for the synthesis of compound **133a** was followed using 5,6-benzo-4-hydroxycoumarin **130g** (1.97 g, 9.3 mmol). Work up afforded 3-acetyl-5,6-benzo-4-

hydroxycoumarin **133g** as a white solid (0.97 g, 41%), m.p. 199-201 ^{o}C; [HRMS: *m/z* calculated for $C_{15}H_{11}O_4$ (MH^+) 255.0657. Found 255.0664]; ν_{max}/cm^{-1} 1713 (C=O); δ_H (600 MHz; $CDCl_3$) 2.81 (3H, s, CH_3), 7.36 (1H, s, ArH), 7.59 (1H, s, ArH), 7.72 (1H, s, ArH), 7.88 (1H,s, ArH), 8.10 (1H, s, ArH) and 9.37 (1H, s, ArH); δ_C (150 MHz; $CDCl_3$) 30.1 (2'-CH_3), 101.2 (C-3), 108.5, 117.2, 126.3, 126.5, 129.3, 130.0, 130.6, 138.6 and 157.2 (Ar-C), 160.0 (C=O), 182.7 (C-OH) and 206.1 (CH_3*C*=O).

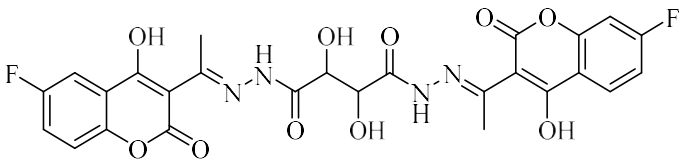
3.6. BIS-(4-HYDROXYCOUMARIN)SUCCINOHYDRAZIDES 134

2,3-Dihydroxy*-N,N'-*bis[(4-hydroxycoumarin-3-yl)ethylidene]succinohydrazide 134a



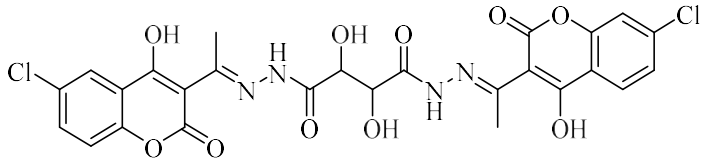
A mixture of 3-acetyl-4-hydroxycoumarin (0.10 g, 0.5 mmol) and 2,3-dihydroxysuccinohydrazide **141** (0.045 g, 0.25 mmol), was dissolved in ethanol (5 mL); and one drop of glacial acetic acid was then added after which the mixture was refluxed for at least 2 hours and then allowed to cool. The resulting precipitate was washed with methanol and dried to yield the product 2,3-dihydroxy-*N*,*N*'-bis[(4-hydroxycoumarin-3-yl)ethylidene]-succinohydrazide **134a** as a pale yellow solid (0.16 g, 58%), m.p. 240 ^{o}C (decomp); [HRMS: *m/z* calculated for $C_{26}H_{22}N_4NaO_{10}$ (M+Na) 573.1228. Found 573.1222]; ν_{max}/cm^{-1} 3454 (O-H) and 1679 (C=O); δ_H (600 MHz; DMSO-d_6) 2.66 (6H, s, 2xCH_3), 4.62 (2H, s, 2xCOC*H*OH), 7.29 (2H, d, *J* = 8.3 Hz, ArH), 7.32 (2H, t, *J* = 7.5 Hz ArH), 7.67 (2H, t, *J* = 7.7 Hz, ArH), 7.97 (2H, d, *J* = 7.7 Hz, ArH) and 11.25 (2H, s, NH); δ_C (150 MHz; DMSO-d_6) 17.7 (CH_3), 72.9 (CHOH), 95.5 (coumarin C-3, 3'), 116.4, 119.6, 123.9, 125.7, 134.5 and 153.2 (Ar-C), 161.4 (C=O), 170.1 (NC=O), 175.0 (CH_3*C*=N) and 179.7 (C-OH).

N,N'-*Bis[(6-fluoro-4-hydroxycoumarin-3-yl)ethylidene]-2,3-dihydroxysuccinohydrazide 134b*



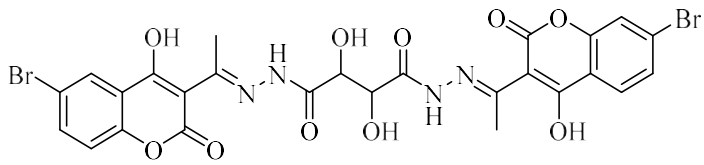
The procedure described for the synthesis of compound **134a** was followed using 3-acetyl-6-fluoro-4-hydroxycoumarin **133b** (0.11 g, 0.5 mmol). Work-up afforded *N*,*N*'-bis[(6-fluoro-4-hydroxycoumarin-3-yl)ethylidene]-2,3-dihydroxysuccinohydrazide **134b** as a pale yellow solid (0.17 g, 58 %), m.p. 242 °C (decomp); [HRMS: *m/z* calculated for $C_{26}H_{20}F_2N_4NaO_{10}$ (M+Na) 609.1040. Found 609.1024]; ν_{max}/cm^{-1} 3212 (O-H) and 1665 (C=N); δ_H (600 MHz; DMSO-d_6) 2.66 (6H, s, 2xCH_3), 4.62 (2H, s, COC*H*OH), 7.36 (2H, m, ArH), 7.53 (1H, m, ArH), 7.63 (2H, s, ArH) and 11.29 (2H, s, NH)); δ_C (150 MHz; DMSO-d_6) 17.7 (CH_3), 72.9 (*C*HOH), 95.2 (C-3), 110.7 (d, $^2J_{CF}$ = 21.6 Hz, C-5, 5'), 118.8, 120.9, 121.9 (d, $^2J_{CF}$ = 22.8 Hz, C-7, 7'), 149.5, 158.1 (d, $^1J_{CF}$ = 241.2 Hz, C-6, 6'), 161.3 (C=O), 170.1 (NC=O), 175.0 (CH_3C=N) and 178.6 (C-OH).

N,N'-*Bis[(6-chloro-4-hydroxycoumarin-3-yl)ethylidene]-2,3-dihydroxysuccinohydrazide 134c*



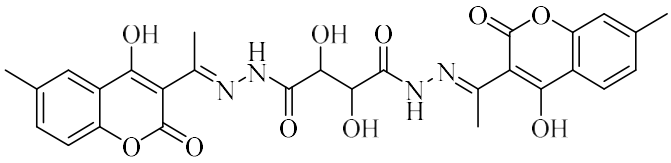
The procedure described for the synthesis of compound **134a** was followed using 3-acetyl-6-chloro-4-hydroxycoumarin **133c** (0.12 g, 0.5 mmol). Work-up afforded *N*,*N*'-bis[(6-chloro-4-hydroxycoumarin-3-yl)ethylidene]-2,3-dihydroxysuccinohydrazide **134c** as a white solid (0.14 g, 45%), m.p. 242 °C (decomp); [HRMS: *m/z* calculated for $C_{26}H_{20}Cl_2N_4NaO_{10}$ (M+Na) 641.0449. Found 641.0432]; ν_{max}/cm^{-1} 3212 (O-H) and 1666 (C=O); δ_H (600 MHz; DMSO-d_6) 2.66 (6H, s, 2xCH_3), 4.62 (2H, s, COC*H*OH), 7.33 (2H, d, *J* = 8.7 Hz, ArH), 7.69 (2H, d, *J* = 8.5 Hz ArH), 7.86 (2H, s, ArH) and 11.33 (2H, s, NH); δ_C (150 MHz; DMSO-d_6) 17.8 (CH_3), 72.9 (*C*HOH), 95.4 (C-3, 3'), 118.8, 121.1, 124.7, 128.1, 134.1 and 151.8 (Ar-C), 161.0, (C=O), 170.1 (NC=O), 174.9 (CH_3C=N) and 178.3 (C-OH).

N,N'-*Bis[(6-bromo-4-hydroxycoumarin-3-yl)ethylidene]-2,3-dihydroxysuccinohydrazide 134d*



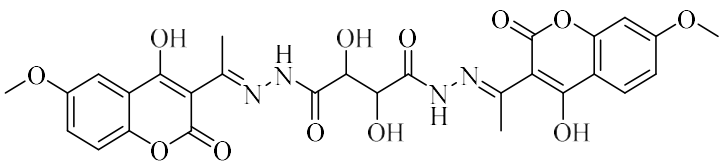
The procedure described for the synthesis of compound **134a** was followed using 3-acetyl-6-bromo-4-hydroxycoumarin **133d** (0.14 g, 0.5 mmol). Work up afforded *N*,*N*'-bis[(6-bromo-4-hydroxycoumarin-3-yl)ethylidene]-2,3-dihydroxysuccinohydrazide **134d** as a white solid (0.15 g, 42 %), m.p. 241 ºC (decomp); [HRMS: *m/z* calculated for $C_{26}H_{20}Br_2N_4NaO_{10}$ (M+Na) 728.9438. Found 728.9424]; ν_{max}/cm^{-1} 3203 (O-H) and 1666 (C=O); δ_H (600 MHz; DMSO-d_6) 2.66 (6H, s, 2xCH_3), 4.61 (2H, s, COC*H*OH), 7.29 (2H, d, *J* = 8.4 Hz, ArH), 7.82 (2H, d, *J* = 7.6 Hz ArH), 8.02 (2H, s, ArH) and 11.29 (2H, s, NH); δ_C (150 MHz; DMSO-d_6) 17.8 (CH_3), 72.9 (*C*HOH), 95.4 (C-3, 3'), 115.8, 119.1, 121.5, 127.8, 136.9 and 152.2 (Ar-C), 161.0, (C=O), 170.1 (NC=O), 174.9 (CH_3*C*=N) and 178.2 (C-OH).

2,3-Dihydroxy*-N,N'-*bis[(4-hydroxy-6-methylcoumarin-3-yl)ethylidene]succinohydrazide 134e



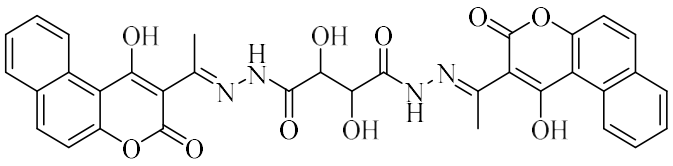
The procedure described for the synthesis of compound **134a** was followed using 3-acetyl-4-hydroxy-6-methylcoumarin **133e** (0.11 g, 0.5 mmol). Work-up afforded 2,3-dihydroxy-*N*,*N*'-bis[(4-hydroxy-6-methylcoumarin-3-yl)ethylidene]succinohydrazide **134e** as a white solid (0.12 g, 41%), m.p. 244 ºC (decomp); [HRMS: *m/z* calculated for $C_{28}H_{26}N_4NaO_{10}$ (M+Na) 601.1541. Found 601.1533]; ν_{max}/cm^{-1} 3325 (O-H) and 1659 (C=O); δ_H (400 MHz; DMSO-d_6) 2.36 (6H, s, 2xArCH_3) 2.65 (6H, s, 2xCH_3), 4.61 (2H, s, COC*H*OH), 7.18 (2H, d, *J* = 8.4 Hz, ArH), 7.47 (2H, dd, *J* = 8.4, 2.4 Hz ArH), 7.74 (2H, s, ArH) and 11.20 (2H, s, NH); δ_C (100 MHz; DMSO-d_6) 17.7 and 20.4 (CH_3), 72.9 (*C*HOH), 95.5 (C-3, 3'), 116.3, 119.3, 125.3, 133.1, 135.4 and 151.3 (Ar-C), 161.6, (C=O), 170.1 (NC=O), 175.0 (CH_3*C*=N) and 179.7 (C-OH).

2,3-Dihydroxy*-N,N'-*bis[(4-hydroxy-6-methoxycoumarin-3-yl)ethylidene]succinohydrazide 134f



The procedure described for the synthesis of 3-Acetyl-4-hydroxycoumarin **134a** was followed using 3-acetyl-4-hydroxy-6-methoxycoumarin **133f** (0.12 g, 0.5 mmol). Work-up afforded 2,3-dihydroxy-*N*,*N*'-bis[(4-hydroxy-6-methoxycoumarin-3-yl)ethylidene]succinohydrazide **134f** as a white solid (0.13 g, 43%), m.p. 246 ^{o}C (decomp) (lit.[134] 70-72 ^{o}C); [HRMS: *m/z* calculated for $C_{28}H_{26}N_4NaO_{12}$ (M+Na) 633.1439. Found 633.1440]; ν_{max}/cm^{-1} 3326 (O-H) and 1657 (C=O); δ_H (400 MHz; DMSO-d_6) 2.66 (6H, s, 2xCH_3C=N), 3.81 (6H, s, 2xOCH_3) 4.62 (2H, s, COC*H*OH), 7.19-7.28 (4H, m, ArH), 7.38 (2H, s, ArH) and 11.22 (2H, s, NH); δ_C (100 MHz; DMSO-d_6) 17.6 (CH_3), 55.6 (OCH_3), 72.9 (*C*HOH), 95.3 (C-3, 3'), 107.1, 117.8, 120.1, 122.3, 147.5 and 155.4 (Ar-C), 161.6, (C=O), 170.1 (NC=O), 174.8 (CH_3*C*=N) and 179.3 (C-OH).

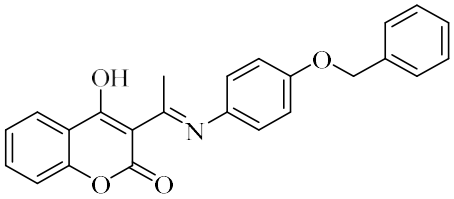
N,N'-*Bis[(5,6-benzo-4-hydroxycoumarin -3-yl)ethylidene]-2,3-dihydroxysuccinohydrazide 134g*



The procedure described for the synthesis of compound **134a** was followed using 2-acetyl-1-hydroxy-3*H*-benzo[f]chromen-3-one **133g** (0.13 g, 0.5 mmol). Work-up afforded *N*,*N*'-bis[(5,6-benzo-4-hydroxycoumarin -3-yl)ethylidene]-2,3-dihydroxysuccinohydrazide **134g** as a white solid (0.15 g, 45%), m.p. 243 ^{o}C (decomp); [HRMS: *m/z* calculated for $C_{34}H_{26}N_4$ NaO_{10} (M+Na) 673.1541. Found 673.1540]; ν_{max}/cm^{-1} 3314 (O-H) 1657 (C=O); δ_H (300 MHz; DMSO-d_6) 2.71 (6H, s, CH_3), 4.69 (2H, s, COC*H*OH), 7.41 (2H, d, *J* = 8.9 Hz, ArH), 7.56 (2H, t, *J* = 7.0 Hz, ArH), 7.69 (2H, t, *J* = 7.8 Hz, ArH), 7.99 (2H, d, *J* = 7.7 Hz, ArH), 8.20 (2H, d, *J* = 9.0 Hz, ArH), 9.75 (2H, d, *J* = 8.7 Hz, ArH) and 11.32 (2H, s, NH); δ_C (75 MHz; DMSO-d_6) 17.8 (CH_3), 72.9 (*C*HOH), 96.3 (C-3, 3'), 111.5, 117.1, 125.5, 125.6, 128.9, 129.0, 130.2, 136.1 and 154.5 (Ar-C), 161.0, (C=O), 170.2 (NC=O), 174.6 (CH_3*C*=N) and 182.9 (C-OH).

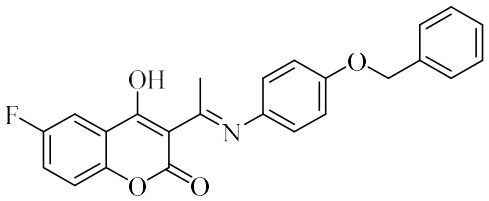
3.7. 3-[(*N*-4-BENZYLOXYPHENYL)IMINOETHYL]-4-HYDROXY COUMARINS 135

3-[1-(N-4-Benzyloxyphenyl)iminoethyl]-4-hydroxycoumarin 135a



A mixture of 3-acetyl-4-hydroxycoumarin **133a** (0.10 g, 0.5 mmol) and 4-(benzyloxy)aniline (0.1 g, 0.5 mmol) was dissolved in ethanol (5 mL)and one drop 2N HCl was added, after which the mixture was refluxed for at least 1 hour and then cooled and filtered to collect the resulting precipitate which was washed with methanol and dried to yield 3-[1-(*N*-4-benzyloxyphenyl)iminoethyl]-4-hydroxycoumarin **135a** as a white crystalline product (0.18 g, 93 %), m.p. 136-137 ^{o}C; [HRMS: *m/z* calculated for $C_{24}H_{20}NO_4$ (MH^+) 386.1392. Found 386.1395]; ν_{max}/cm^{-1} 1710 (C=O); δ_H (400 MHz; $CDCl_3$) 2.54 (3H, s, CH_3), 4.98 (2H, s, $PhCH_2$), 6.90-6.95 (2H, m, ArH), 6.99-7.04 (2H, m, ArH), 7.09-7.16 (2H, m, ArH), 7.24 (1H, m, ArH), 7.27-7.35 (4H, m, ArH), 7.44 (1H, ddd, *J* = 8.7, 7.3, 1.8 Hz, ArH) and 7.95 (1H, dd, *J* = 7.8, 1.8 Hz, ArH); δ_C (100 MHz; $CDCl_3$) 20.9 (CH_3), 70.5 ($PhCH_2$), 98.0 (C-3), 115.8, 116.8, 120.3, 123.7, 126.1, 126.9, 127.6, 128.4, 128.8, 129.2, 134.2, 136.4, 154.0 and 158.6 (Ar-C), 162.6 (C=O), 176.3 (C=N) and 181.9 (C-OH).

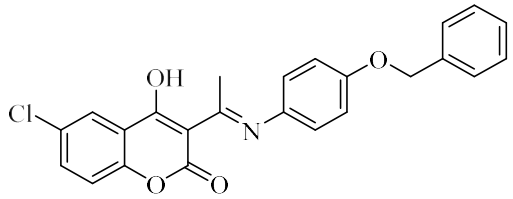
3-[1-(N-4-Benzyloxyphenyl)iminoethyl]-6-fluoro-4-hydroxycoumarin 135b



The procedure described for the synthesis of compound **135a** was followed using 3-acetyl-6-fluoro-4-hydroxycoumarin **133b** (0.10 g, 0.5 mmol) and 4-(benzyloxy) aniline (0.11 g, 0.5 mmol). Work-up afforded 3-[1-(*N*-4-benzyloxyphenyl)iminoethyl]-6-fluoro-4-hydroxy coumarin **135b** as a white crystalline product (0.19 g, 94%), m.p. 145-147 ^{o}C; [HRMS: *m/z* calculated for $C_{24}H_{19}FNO_4$ (MH^+) 404.1298. Found 404.1296]; ν_{max}/cm^{-1} 1702 (C=O); δ_H (400 MHz; $CDCl_3$) 2.53 (3H, s, NCH_3), 4.97 (2H, s, $PhCH_2$), 6.92 (2H, d, *J* = 8.9 Hz, ArH), 7.00 (2H, d, *J* = 8.8 Hz, ArH), 7.06 (1H, dd, *J* = 8.9, 4.3 Hz, ArH), 7.13 (1H, m, ArH), 7.22

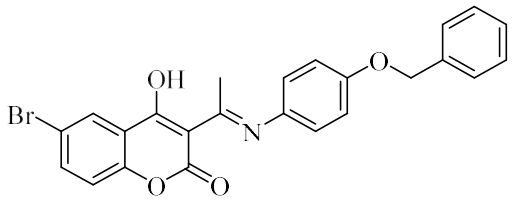
(1H, dd, J = 8.4, 5.4 Hz, ArH), 7.25-7.34 (4H, m, ArH) and 7.57 (1H, dd, J = 8.3, 2.7 Hz, ArH); δ_C (100 MHz; $CDCl_3$) 20.9 (CH_3), 70.5 ($PhCH_2$), 97.8 (C-3), 111.4 (d, $^2J_{CF}$ = 24.2 Hz, C-5), 115.9 and 118.4 (Ar-C), 121.3 (d, $^3J_{CF}$ = 7.8 Hz, C-10), 121.7 (d, $^2J_{CF}$ = 24.4 Hz, C-7), 126.9, 127.6, 128.4, 128.8, 129.1, 136.4, 150.0 and 158.7(Ar-C), 158.8 (d, $^1J_{CF}$ = 243.0 Hz, C-6), 162.3 (C=O), 176.5 (C=N) and 180.8 (C-OH).

3-[1-(*N*-4-Benzyloxyphenyl)iminoethyl]-6-chloro-4-hydroxycoumarin 135c



The procedure described for the synthesis of compound **135a** was followed using 3-acetyl-6-chloro-4-hydroxycoumarin **133c** (0.10 g, 0.5 mmol) and 4-(benzyloxy) aniline (0.12 g, 0.5 mmol). Work-up afforded 3-[1-(*N*-4-benzyloxyphenyl)iminoethyl]-6-chloro-4-hydroxy coumarin **135c** as a white crystalline product (0.19 g, 91%), m.p. 162-163 ^oC; [HRMS: *m/z* calculated for $C_{24}H_{19}ClNO_4$ (MH^+) 420.1003. Found 420.1005]; ν_{max}/cm^{-1} 1695 (C=O); δ_H (400 MHz; $CDCl_3$) 2.66 (3H, s, CH_3), 5.11 (2H, s, $PhCH_2$), 7.04-7.08 (2H, m, ArH), 7.11-7.20 (3H, m, ArH), 7.35-7.51 (6H, m, ArH) and 8.02 (1H, d, J = 2.5 Hz, ArH); δ_C (100 MHz; $CDCl_3$) 21.0 (CH_3), 70.4 ($PhCH_2$), 97.8 (C-3), 115.9, 118.4, 121.4, 125.7, 126.9, 127.6, 128.4, 128.8, 129.0, 129.3, 134.1, 136.3, 152.3 and 158.7 (Ar-C), 162.1 (C=O), 176.4 (C=N) and 180.5 (C-OH).

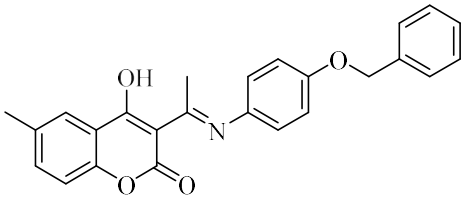
3-[1-(*N*-4-Benzyloxyphenyl)iminoethyl]-6-bromo-4-hydroxycoumarin 135d



The procedure described for the synthesis of compound **135a** was followed using 3-acetyl-6-bromo-4-hydroxycoumarin **133d** (0.10 g, 0.5 mmol) and 4-(benzyloxy) aniline (0.14 g, 0.5 mmol). Work-up afforded 3-[1-(*N*-4-benzyloxyphenyl)iminoethyl]-6-bromo-4-hydroxy coumarin **135d** as a white crystalline product (0.20 g, 86%), m.p. 161-162 ^oC; [HRMS: *m/z* calculated for $C_{24}H_{19}BrNO_4$ (MH^+) 464.0497. Found 464.0497]; ν_{max}/cm^{-1} 1701 (C=O); δ_H (400 MHz; $CDCl_3$) 2.66 (3H, s, CH_3), 5.10 (2H, s, $PhCH_2$), 7.04-7.16 (5H, m, ArH), 7.35 (1H, m, ArH), 7.38-7.46 (4H, m, ArH), 7.62 (1H, dd, J = 8.7, 2.5 Hz, ArH) and 8.17 (1H, d, J

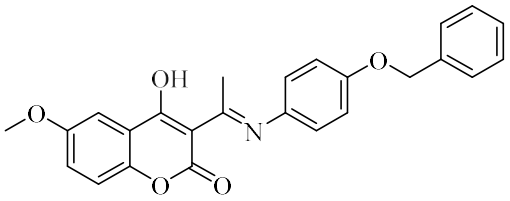
= 2.2 Hz, ArH); δ_C (100 MHz; $CDCl_3$) 20.9 (CH_3), 70.5 ($PhCH_2$), 97.9 (C-3), 115.9, 116.6, 118.7, 121.8, 126.9, 127.6, 128.4, 128.8, 129.0, 136.4, 136.9, 152.8 and 158.7 (Ar-C), 162.0 (C=O), 176.4 (C=N) and 180.4 (C-OH).

3-[1-(*N*-4-Benzyloxyphenyl)iminoethyl]-4-hydroxy-6-methylcoumarin 135e



The procedure described for the synthesis of compound **135a** was followed using 3-acetyl-6-methyl-4-hydroxycoumarin **133e** (0.10 g, 0.5 mmol) and 4-(benzyloxy) aniline (0.11 g, 0.5 mmol). Work-up afforded 3-[1-(*N*-4-benzyloxyphenyl)iminoethyl]-4-hydroxy-6-methyl coumarin **135e** as a white crystalline product (0.19 g, 95%), m.p. 142-144 ^oC; [HRMS: *m/z* calculated for $C_{25}H_{22}NO_5$ (MH^+) 400.1549. Found 400.1548]; ν_{max}/cm^{-1} 1650 (C=O); δ_H (400 MHz; $CDCl_3$) 2.40 (3H, s, CH_3), 2.66 (3H, s, CH_3C=N), 5.10 (2H, s, $PhCH_2$), 7.05 (2H, m, ArH), 7.10-7.16 (3H, m, ArH), 7.33-7.38 (2H, m, ArH), 7.38-7.46 (4H, m, ArH) and 7.85 (1H, s, ArH); δ_C (100 MHz; $CDCl_3$) 20.9 (CH_3), 20.9 (*C*H_3C=N), 70.5 ($PhCH_2$), 98.0 (C-3), 115.8, 116.5, 119.9, 125.8, 126.9, 127.6, 128.3, 128.8, 129.3, 133.3, 135.3, 136.4, 152.1 and 158.5 (Ar-C), 162.8 (C=O), 176.2 (C=N) and 182.0 (C-OH).

3-[1-(*N*-4-Benzyloxyphenyl)iminoethyl]-4-hydroxy-6-methoxycoumarin 135f



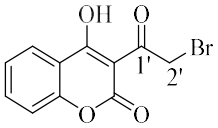
The procedure described for the synthesis of compound **135a** was followed using 3-acetyl-6-methoxy-4-hydroxycoumarin **133f** (0.10 g, 0.5 mmol) and 4-(benzyloxy) aniline (0.12 g, 0.5 mmol). Work-up afforded 3-[1-(*N*-4-benzyloxyphenyl)iminoethyl]-4-hydroxy-6-methoxy coumarin **135f** as a white crystalline product (0.17 g, 82%), m.p. 136-137 ^oC; [HRMS: *m/z* calculated for $C_{25}H_{22}NO_5$ (MH^+) 416.1498. Found 416.1500; ν_{max}/cm^{-1} 1691 (C=O); δ_H (400 MHz; $CDCl_3$) 2.66 (3H, s, CH_3), 3.85 (3H, s, OCH_3), 5.10 (2H, s, $PhCH_2$), 7.05 (2H, m, ArH), 7.11-7.17 (4H, m, ArH), 7.35 (1H, dd, *J* = 8.3, 5.5 Hz, ArH), 7.38-7.46 (4H, m, ArH) and 7.48 (1H, s, ArH); δ_C (100 MHz; $CDCl_3$) 20.9 (CH_3), 55.9 (OCH_3), 70.5 ($PhCH_2$), 97.9

(C-3), 106.8, 115.9, 118.0, 120.5, 123.0, 126.8, 127.6, 128.3, 128.8, 129.3, 136.4, 148.5, 155.9 and 158.6 (Ar-C), 162.7 (C=O), 176.3 (C=N) and 181.6 (C-OH).

3.8. 3-[2-(BENZYLIDENEHYDRAZINYL)THIAZOL-2-YL]-4-HYDROXYCOUMARINS 136

The targeted molecular hybrids were synthesised by a reacting 3-(2-bromoacetyl)-4-hydroxycoumarin **148** and thiosemicarbazone derivatives **152a-g**.

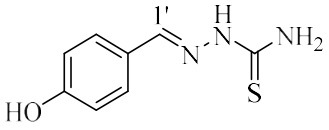
3.8.1. Synthesis of 3-(2-bromoacetyl)-4-hydroxycoumarin



3-Acetyl-4-hydroxycoumarin **133a** (2.5 g, 12.2 mmol) was heated with bromine (0.64 mL, 12.5 mmol) at 100^{o} C in acetic acid (5 mL) for 1 h in the fume-hood. The precipitate which formed was filtered off and recrystallized from acetic acid to give 3-(2-bromoacetyl)-4-hydroxycoumarin **148** as flaky shiny colourless crystals (2.65 g, 77 %), m.p. 140-142 ^{o}C (lit.[378] 144-146 ^{o}C); ν_{max}/cm^{-1} 1712 (C=O); δ_H (400 MHz; $CDCl_3$) 4.82 (2H, s, CH_2), 7.30-7.41 (2H, m, ArH), 7.74 (1H, t, *J* = 6.0 Hz, ArH), 8.07 (1H, d, *J* = 8.0 Hz, ArH); δ_C (100 MHz; $CDCl_3$) 35.5 (CH_3), 100.1 (C-3), 114.6, 117.3, 125.0, 125.8, 136.9 and 154.9 (Ar-C), 159.6 (C=O), 178.2 (C-OH) and 198.4 (1'-C=O).

3.8.2. Synthesis of thiosemicarbazone derivatives

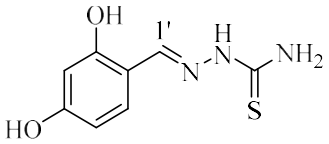
N^1-*[4-Hydroxybenzylidene]*-N^2-thiocarbamoylhydrazine *151a*



A mixture of 4-hydroxybenzaldehyde **150a** (0.24 g, 2 mmol), thiosemicarbazide (0.19, 2.1 mmol) and one or two drops 2N HCl was refluxed for at least 1.5 hours after which the solvent was dried and washed with several small volumes of methanol, filtered and air dried to afford *N*^{1}-[4-hydroxybenzylidene]-*N*^{2}-thiocarbamoylhydrazine **151a** as a pale yellow solid (0.21 g, 54%), m.p. 230-232 ^{o}C (lit.[384] 230-231 ^{o}C); ν_{max}/cm^{-1} 3464 and 3353 (N-H); δ_H (400 MHz; DMSO-d_6) 6.78 (2H, d, *J* = 8.6 Hz, ArH), 7.60 (2H, d, *J* = 8.6 Hz, ArH), 7.93 (2H, d, *J*

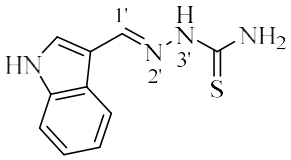
= 91.5 Hz, NH_2), 7.96 (1H, s, 1'-CH), 9.86 (1H, s, 4-OH), 11.23 (1H, s, NH); δ_C (100 MHz; DMSO-d_6) 115.6, 125.2 and 129.1 (Ar-C), 142.8 (C=N), 159.3(C-OH) and 177.5 (C=S).

N^1-*[2,4-Dihydroxybenzylidene]*-N^2-*thiocarbamoylhydrazine 151b*



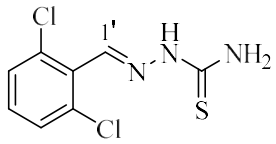
The procedure described for the synthesis of compound **151a** was followed using 2,4-dihydroxybenzaldehyde **150b** (0.28 g, 2 mmol. Work-up afforded *N*^{1}-[2,4-dihydroxybenzylidene]-*N*^{2}-thiocarbamoylhydrazine **151b** as a cream solid (0.24 g, 57 %), m.p. 238-240 ^oC (lit.[385] 237-239 ^oC); ν_{max}/cm^{-1} 3467 and 3320 (N-H); δ_H (600 MHz; DMSO-d_6) 6.26 (1H, d, *J* = 8.2 Hz, ArH), 6.29 (1H, s, ArH), 7.67 (1H, d, *J* = 6.1 Hz, ArH), 7.76 (1H, s, NH_2, H_a), 7.96 (1H, s, NH_2, H_b), 8.23 (1H, s, 1'-CH), 9.80 (2H, s, 2-OH and 4-OH),11.19 (1H, s, NH); δ_C (150 MHz; DMSO-d_6) 102.4, 107.9, 111.9 and 128.4 (Ar-C), 140.9 (C=N), 158.1 (C-2-OH), 160.5 (C-4-OH) and 177.0 (C=S).

N^1-*[(1*H*-indol-3-yl)methylidene]*-N^2-*thiocarbamoylhydrazine 151c*



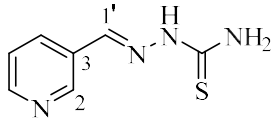
The procedure described for the synthesis of compound **151a** was followed using 1*H*-indole-3-carbaldehyde **150c** (0.29 g, 2 mmol. Work-up afforded *N*^{1}-[(1*H*-indol-3-yl)methylidene]-*N*^{2}-thiocarbamoylhydrazine **151c** as a pink solid (0.39 g, 89%), m.p. 235-237 ^oC (lit.[386] 230-232 ^oC); ν_{max}/cm^{-1} 3438 and 3303 (N-H); δ_H (400 MHz; DMSO-d_6) 7.13 (1H, t, *J* = 7.4 Hz, ArH), 7.19 (1H, t, *J* = 7.5 Hz, ArH), 7.41 (1H, s, NH_2, H_a), 7.42 (1H, d, *J* = 8.0 Hz, ArH), 7.80 (1H, d, *J* = 2.6 Hz, ArH), 8.01 (1H, s, NH_2, H_b), 11.17 (1H, s, 3'-NH) and 11.58 (1H, s, NH); δ_C (100 MHz; DMSO-d_6) 111.1, 111.8, 120.7, 122.1, 122.7, 124.0, 131.0 and 137.1 (Ar-C), 140.9 (C=N) and 176.5 (C=S).

N^1-*(2,6-Dichlorobenzylidene)*-N^2-*thiocarbamoylhydrazine 151d*



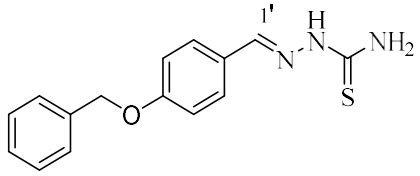
The procedure described for the synthesis of compound **151a** was followed using 2,6-dichlorobenzaldehyde **150d** (0.35 g, 2 mmol. Work-up afforded *N*^{1}-[2,6-dichlorobenzylidene]-*N*^{2}-thiocarbamoylhydrazine **151d** as a white solid (0.40 g, 81 %), m.p. 246-248 ^{o}C (lit.^{387} 245-246 ^{o}C); ν_{max}/cm^{-1} 3407 and 3260 (N-H); δ_H (400 MHz; DMSO-*d*$_6$) 7.40 (1H, dd, *J* = 8.8, 7.3 Hz, ArH), 7.45 (1H, s, NH$_2$, H$_a$), 7.53 (2H, d, *J* = 8.1 Hz, ArH), 8.31 (1H, s, 1'-CH), 8.39 (1H, s, NH$_2$, H$_b$), 11.75 (1H, s, NH); δ_C (100 MHz; DMSO-*d*$_6$) 129.3, 129.8, 131.0 and 134.0 (Ar-C), 137.7 (C=N) and 178.5 (C=S).

N^1-*(Pyridin-3-ylmethylidene)*-N^2-*thiocarbamoylhydrazine 151e*



The procedure described for the synthesis of compound **151a** was followed using 3-pyridinecarboxaldehyde **150e** (188 µL, 2 mmol). Work up afforded *N*^{1}-(pyridin-3-ylmethylidene)-*N*^{2}-thiocarbamoylhydrazine **151e** as a pale yellow solid (0.26 g, 72 %), m.p. 224 ^{o}C (decomp) (lit.^{388} 222-223 ^{o}C (decomp)); ν_{max}/cm^{-1} 3333 and 3386 (N-H), 1622 (C=N); δ_H (400 MHz; DMSO-*d*$_6$) 7.41 (1H, dd, *J* = 7.9, 4.8 Hz, ArH), 8.06 (1H, s, 1'-CH), 8.21 (2H, d, *J* = 60.1 Hz, NH$_2$), 8.25 (1H, d, *J* = 8.0 Hz, ArH), 8.55 (1H, dd, *J* = 4.8, 1.8 Hz, ArH), 8.91 (d, *J* = 2.4 Hz, 2-CH), 11.58 (1H, s, NH); δ_C (100 MHz; DMSO-*d*$_6$) 123.8, 130.2 and 133.9 (Ar-C), 139.3 (N=C-1'), 148.8, 150.3 (Ar-C) and 178.2 (C=S).

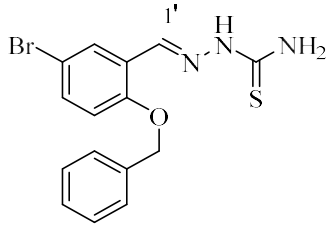
N^1-*(4-(Benzyloxy)benzylidene)*-N^2-*thiocarbamoylhydrazine 151f*



The procedure described for the synthesis of compound**151a** was followed using 4-(benzyloxy)benzaldehyde **150f** (0.44 g, 2 mmol) Work-up afforded (*N*^{1}-(4-(benzyloxy)benzylidene)-*N*^{2}-thiocarbamoylhydrazine **151f** as a white solid (0.48 g, 84%), m.p. 178-180 ^{o}C (lit.^{389} 188 ^{o}C); ν_{max}/cm^{-1} 3442 and 3303 (N-H), 1228 (C-O); δ_H (400 MHz;

DMSO-d_6) 5.15 (2H, s, $PhCH_2$), 7.04 (1H, d, J = 8.7 Hz, ArH), 7.33 (1H, t, J = 7.3 Hz, ArH), 7.39 (2H, t, J = 7.5 Hz, ArH), 7.45 (2d, J = 7.3 Hz, ArH), 7.74 (2H, d, J = 8.7 Hz, ArH), 7.91 (1H, s, NH_2, H_a), 7.99 (1H, s, 1'-CH), 8.10 (1H, s, NH_2, H_b) 11.31 (1H, s, NH); δ_C (100 MHz; DMSO-d_6) 69.3 ($PhCH_2$), 115.0, 127.0, 127.7, 127.9, 128.4, 128.9 and 136.8 (Ar-C), 142.1 (C=N), 159.7 (Ar-C) and 177.6 (C=S).

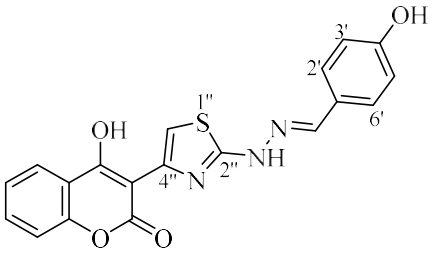
N^1-*[2-(Benzyloxy)-5-bromobenzylidene]* -N^2-*thiocarbamoylhydrazine 151g*



The procedure described for the synthesis of compound **151a** was followed using 2-(benzyloxy)-5-bromobenzaldehyde **150g** 0.58, 2 mmol). Work-up afforded N^1-[2-(benzyloxy)-5-bromobenzylidene] -N^2-thiocarbamoylhydrazine **151g** as a pale yellow solid (0.14 g, 30%), m.p. 160-161 ^oC); [HRMS: *m/z* calculated for $C_{15}H_{15}BrN_3OS$ (MH^+) 364.0119. Found 364.0115]; ν_{max}/cm^{-1} 3431 and 3297 (N-H); δ_H (600 MHz; DMSO-d_6) 5.17 (2H, s, $PhCH_2$), 7.14 (1H, d, J = 8.6 Hz, ArH), 7.30-7.37 (1H, m, ArH), 7.39-7.44 (2H, m, ArH), 7.46-7.52 (3H, m, ArH), 8.20 (2H, d, J = 17.2 Hz, NH_2), 8.36 (1H, s, ArH), 8.46 (1H, s, 1'-CH), 11.53 (1H, s, NH); δ_C (150 MHz; DMSO-d_6) 69.9 ($PhCH_2$), 113.2, 115.5, 125.0, 127.4, 127.9, 128.2, 128.5, 133.3 and 136.4 (Ar-C), 136.5 (C=N), 155.9 (Ar-C), 178.0 (C=S).

3.8.3. Synthesis of 3-[2-(Benzylidenehydrazinyl)thiazo-2-yl]-4-hydroxycoumarins

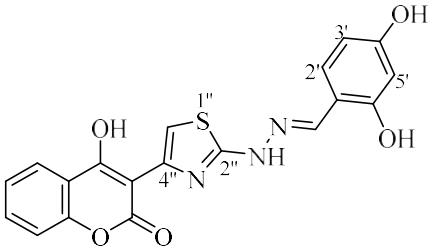
4-Hydroxy-3-{[2-(4-hydroxybenzylidene)hydrazinyl]thiazol-4-yl}coumarin 136a



A mixture of 3-(2-bromoacetyl)-4-hydroxycoumarin **148** (0.071 g, 0.25 mmol), the hydrazine derivative **151a** (0.051 g, 0.26 mmol) and one or two drops of 2N HCl was refluxed in ethanol for at least 2 hours, after which the resulting precipitate was filtered out and washed

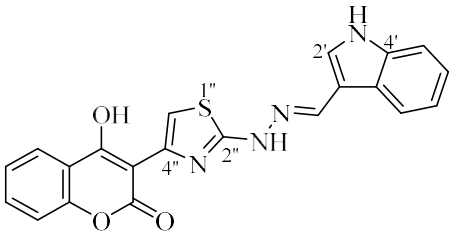
with methanol and then dried to afford 4-hydroxy-3-{[2-(4-hydroxybenzylidene)hydrazinyl]thiazol-4-yl}coumarin **136a** as a white solid (0.073 g, 77%), m.p. 250 °C (decomp); [HRMS: *m/z* calculated for $C_{19}H_{14}N_3O_4S$ (MH^+) 380.0705. Found 380.0705]; ν_{max}/cm^{-1} 1671 (C=N); δ_H (600 MHz; DMSO-d_6) 6.84 (2H, d, J = 8.6 Hz, ArH), 7.35-7.39 (2H, m, ArH), 7.51 (1H, s, NN=CH), 7.53 (2H, d, ArH), 7.64 (1H, t, J = 7.6 Hz, ArH), 7.94 (1H, d, J = 7.3 Hz, ArH), 7.99 (1H, s, 5''-CH), 9.96 (1H, 4'-OH), 12.36 (1H, s, NH) and 15.82 (1H, s, 4-OH); δ_C (150 MHz; DMSO-d_6) 96.0 (C-3), 104.6, 115.8, 116.1, 116.2, 123.9, 124.2, 124.9, 128.5, 132.8, 142.5, 144.4, 152.0 and 159.3 (Ar-C), 160.0 (C=O), 164.3 ((C-4)-OH) and 166.9 (C-2'').

3-{[2-(2,4-Dihydroxybenzylidene)hydrazinyl]thiazol-4-yl}-4-hydroxycoumarin 136b



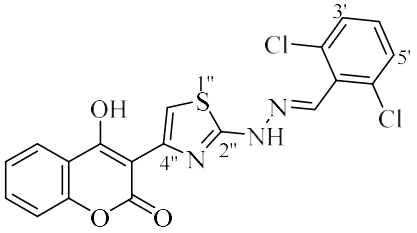
The procedure described for the synthesis of compound **136a** was followed using the hydrazine derivative **151b** (0.055 g, 0.25 mmol). Work-up afforded 3-{[2-(2,4-dihydroxybenzylidene)hydrazinyl]thiazol-4-yl}-4-hydroxycoumarin **136b** as a yellow solid (0.074 g, 75%), m.p. 270 °C (decomp); [HRMS: *m/z* calculated for $C_{19}H_{14}N_3O_5S$ (MH^+) 396.0661. Found 396.0662]; ν_{max}/cm^{-1} 1665 (C=N); δ_H (600 MHz; DMSO-d_6) 6.35 (2H, d, J = 6.4 Hz, ArH), 7.38 (2H, t, J = 8.1 Hz, ArH), 7.48 (1H, d, J = 9.2 Hz, ArH), 7.51 (1H, s, NN=CH), 7.65 (1H, t, J = 7.8 Hz, ArH), 7.95 (1H, d, J = 7.9 Hz, ArH), 8.27 (1H, s, 5''-CH), 9.90 (1H, 4'-OH), 10.10 (1H, 6'-OH), 12.35 (1H, s, NH) and 15.83 (1H, s, 4-OH); δ_C (150 MHz; DMSO-d_6) 96.0 (C-3), 102.5, 104.3, 108.1, 111.3, 116.1, 116.2, 123.9, 124.2, 128.2, 132.8, 142.5, 142.9, 152.0, 158.0 and 160.0 (Ar-C), 160.6 (C=O), 164.4 ((C-4)-OH) and 166.5 (C-2'').

4-Hydroxy-3-{[2-(1H-indol-3-yl)hydrazinyl]thiazol-4-yl}coumarin 136c



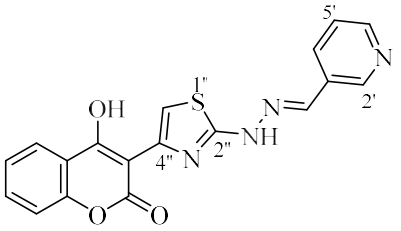
The procedure described for the synthesis of compound **136a** was followed using the hydrazine derivative **151c** (0.055 g, 0.26 mmol). Work-up afforded 4-hydroxy-3-{[2-(1*H*-indol-3-yl)hydrazinyl]thiazol-4-yl}coumarin **136c** as a white solid (0.085 g, 84%), m.p. 240 ^{o}C (decomp); [HRMS: *m/z* calculated for $C_{21}H_{15}N_4O_3S$ (MH^+) 403.0865. Found 403.0876]; ν_{max}/cm^{-1} 1696 (C=O); δ_H (600 MHz; DMSO-d_6) 7.19-7.26 (2H, m, ArH), 7.36-7.42 (2H, m, ArH), 7.48 (1H, dd, *J* = 6.2, 2.3 Hz, ArH), 7.52 (1H, s, NN=CH), 7.66 (1H, t, *J* = 7.6 Hz, ArH), 7.86 (1H, d, *J* = 2.8 Hz, ArH), 7.98 (1H, d, *J* = 8.2 Hz, ArH), 8.24 (1H, d, *J* = 6.7 Hz, ArH), 8.34 (1H, s, 5''-CH), 11.63 (1H, s, 3'-NH), 12.33 (1H, s, NH) and 16.06 (1H, s, 4-OH); δ_C (150 MHz; DMSO-d_6) 95.6 (C-3), 103.5, 111.2, 112.1, 116.2, 116.5, 120.8, 121.6, 122.8, 123.9, 124.0, 124.1, 130.8, 132.7, 137.2, 142.1, 142.2 and 152.0 (Ar-C), 160.1 (C=O), 165.1 ((C-4)-OH) and 166.7 (C-2'').

3-{[2-(2,6-Dichlorobenzylidene)hydrazinyl]thiazol-4-yl}-4-hydroxycoumarin 136d



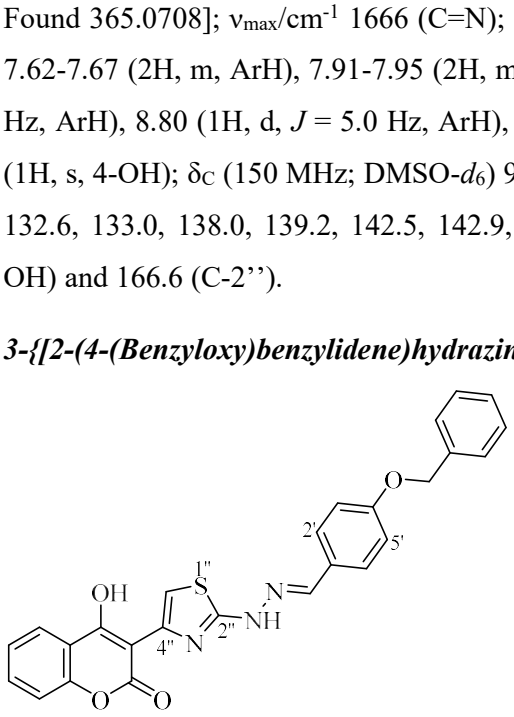
The procedure described for the synthesis of compound **136a** was followed using the hydrazine derivative **151d** (0.065 g, 0.26 mmol). Work-up afforded 3-{[2-(2,6-dichlorobenzylidene)hydrazinyl]thiazol-4-yl}-4-hydroxycoumarin **136d** as a white solid (0.08 g, 74%), m.p. 275 ^{o}C (decomp); [HRMS: *m/z* calculated for $C_{19}H_{12}Cl_2N_3O_3S$ (MH^+) 431.9976. Found 431.9963]; ν_{max}/cm^{-1} 1676 (C=N); δ_H (600 MHz; DMSO-d_6) 7.36-7.41 (3H, m, ArH), 7.55 (2H, d, *J* = 8.1 Hz, ArH), 7.60 (1H, s, NN=CH), 7.66 (1H, t, *J* = 7.7 Hz, ArH), 7.94 (1H, d, *J* = 7.5 Hz, ArH), 8.31 (1H, s, 5''-CH), 12.80 (1H, s, NH) and 15.43 (1H, s, 4-OH); δ_C (150 MHz; DMSO-d_6) 96.4 (C-3), 106.4, 115.6, 116.2, 123.8, 124.3, 129.1, 129.5, 130.9, 132.9, 133.7, 138.3, 142.8 and 151.9 (Ar-C), 159.8 (C=O), 163.6 (C-OH) and 166.9 (C-2'').

4-Hydroxy-3-{[2-(pyridin-3-ylmethylene)hydrazinyl]thiazol-4-yl}coumarin 136e



The procedure described for the synthesis of compound **136a** was followed using the hydrazine derivative **151e** (0.047 g, 0.26 mmol). Work-up afforded 4-hydroxy-3-{[2-(pyridin-3-ylmethylene)hydrazinyl]thiazol-4-yl}coumarin **136e** as a yellow solid (0.079 g, 87%), m.p. 290 ^{o}C (decomp); [HRMS: *m/z* calculated for $C_{18}H_{13}N_4O_3S$ (MH^+) 365.0716. Found 365.0708]; ν_{max}/cm^{-1} 1666 (C=N); δ_H (600 MHz; DMSO-d_6) 7.35-7.40 (2H, m, ArH), 7.62-7.67 (2H, m, ArH), 7.91-7.95 (2H, m, ArH), 8.18 (1H, s, NN=CH), 8.59 (1H, d, *J* = 8.2 Hz, ArH), 8.80 (1H, d, *J* = 5.0 Hz, ArH), 9.06 (1H, s, 5''-CH), 13.00 (1H, s, NH) and 15.34 (1H, s, 4-OH); δ_C (150 MHz; DMSO-d_6) 96.4 (C-3), 106.7, 115.7, 116.3, 123.8, 124.4, 126.5, 132.6, 133.0, 138.0, 139.2, 142.5, 142.9, 144.1 and 151.9 (Ar-C), 159.9 (C=O), 163.7 (C-OH) and 166.6 (C-2'').

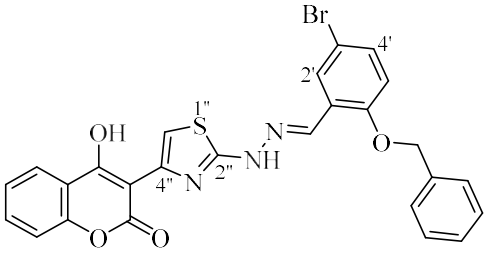
3-{[2-(4-(Benzyloxy)benzylidene)hydrazinyl]thiazol-4-yl}-4-hydroxycoumarin 136f



The procedure described for the synthesis of compound **136a** was followed using the hydrazine derivative **151f** (0.074 g, 0.26 mmol). Work-up afforded 3-{[2-(4-(benzyloxy)benzylidene)hydrazinyl]thiazol-4-yl}-4-hydroxycoumarin **136f** as a white solid (0.085 g, 72%), m.p. 285-287 ^{o}C; [HRMS: *m/z* calculated for $C_{26}H_{20}N_3O_4S$ (MH^+) 470.1175. Found 470.1181]; ν_{max}/cm^{-1} 1671 (C=N); δ_H (600 MHz; DMSO-d_6) 5.13 (2H, s, CH_2), 7.07 (2H, d, *J* = 7.9 Hz, ArH), 7.32-7.35 (1H, m, ArH), 7.36-7.42 (4H, m, ArH), 7.45 (2H, d, *J* = 7.1 Hz, ArH), 7.54 (1H, s, NN=CH), 7.60-7.67 (3H, m, ArH), 7.94 (1H, d, *J* = 7.3 Hz, ArH), 8.02 (1H, s, 5''-CH), 12.43 (1H, s, NH) and 15.77 (1H, s, 4-OH); δ_C (150 MHz; DMSO-d_6) 69.4 (CH_2), 96.1 (C-3), 104.9, 115.2, 116.0, 116.2, 123.8, 124.2, 126.6, 127.8, 128.0, 128.3,

128.5, 132.8, 136.7, 142.5, 143.8, 151.9 and 159.8 (Ar-C), 159.9 (C=O), 164.2 (C-OH) and 166.9 (C-2'').

3-{[2-(2-(Benzyloxy)-5-bromobenzylidene)hydrazinyl]thiazol-4-yl}-4-hydroxycoumarin 136g

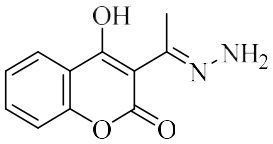


The procedure described for the synthesis of compound **136a** was followed using the hydrazine derivative **151g** (0.095 g, 0.26 mmol). Work-up afforded 3-{[2-(2-(benzyloxy)-5-bromobenzylidene)hydrazinyl]thiazol-4-yl}-4-hydroxycoumarin **136g** as a white solid (0,085 g, 62%), m.p. 255-257 ^{o}C; [HRMS: *m/z* calculated for $C_{26}H_{19}BrN_3O_4S$ (MH^+) 548.0280. Found 548.0297]; ν_{max}/cm^{-1} 1675 (C=N); δ_H (600 MHz; DMSO-d_6) 5.13 (2H, s, CH_2), 7.08 (1H, d, J = 8.8 Hz, ArH), 7.30-7.35 (2H, m, ArH), 7.38 (2H, q, J = 7.6 Hz, ArH), 7.44 (2H, t, J = 7.3 Hz, ArH), 7.50 (2H, d, J = 7.5 Hz, ArH), 7.52 (1H, s, NN=CH), 7.60 (1H, t, J = 7.6 Hz, ArH), 7.77 (1H, s, ArH), 7.88 (1H, d, J = 7.7 Hz, ArH), 8.24 (1H, s, 5''-CH), 12.54 (1H, s, NH) and 15.49 (1H, s, 4-OH); δ_C (150 MHz; DMSO-d_6) 70.1 (CH_2), 96.2 (C-3), 105.5, 112.8, 115.4, 115.7, 116.1, 123.7, 124.1, 124.4, 126.9, 127.8, 128.1, 128.6, 132.7, 133.0, 136.4, 137.0, 142.7, 151.8 and 155.4 (Ar-C), 159.8 (C=O), 163.6 (C-OH) and 166.6 (C-2'').

3.9. 3-[1-(BENZYLIDENEHYDRAZONO)ETHYL]-4-HYDROXY COUMARINS 137

The targeted molecular hybrids were synthesised by a reacting 3-(1-hydrazonoethyl)-4-hydroxycoumarin **152** and aldehydes **154a**-**g**.

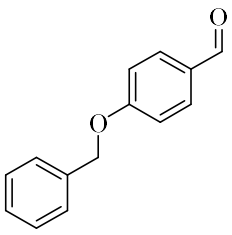
3.9.1. Synthesis of 3-(1-hydrazonoethyl)-4-hydroxycoumarin



A mixture of 3-acetyl-4-hydroxycoumarin **133a** (2g, 10 mmol) hydrazine hydrate (0.53 mL, 10.1 mmol) and 3 drops of glacial acetic acid was heated under reflux in ethanol for an hour. The precipitate that formed was filtered off and washed with methanol and dried to yield 3-(1-hydrazonoethyl)-4-hydroxycoumarin **152** as a yellow solid (g, 80%), m.p. 207-209 ^{o}C (lit.[390] 185 ^{o}C); ν_{max}/cm^{-1} 1684 (C=O); δ_H (600 MHz; DMSO-d_6) 2.65 (3H, s, CH_3), 6.05 (1H, s, NH_2), 7.11-7.31 (2H, m, ArH), 7.30-7.35 (2H, m, ArH), 7.58 (1H, t, J = 7.9 Hz, ArH) 7.44 (2H, t, J = 7.3 Hz, ArH), 7.94 (1H, d, J = 7.9 Hz, ArH) and 15.13 (1H, s, 4-OH); δ_C (150 MHz; DMSO-d_6) 15.8 (CH_3), 93.5 (C-3), 115.8, 120.1, 123.1, 125.1, 133.0 and 152.8 (ArC), 161.6 (C=O), 166.3 (C=N) and 177.7 (C-OH).

3.9.2. Benzyloxy benzaldehydes

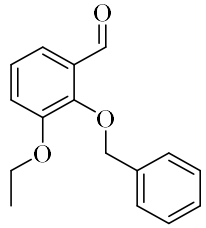
4-(Benzyloxy)benzaldehyde 154a



A mixture of 4-hydroxybenzaldehyde (1.22g, 10 mmol) and benzyl bromide (1.18 mL, 10 mmol) in 10% aqueous NaOH (10 mL) was refluxed for 30 min and then cooled to room temperature. Water was then added and the resulting solid was filtered, washed with water, dried and finally crystallised from hexane to afford 4-(benzyloxy)benzaldehyde **154a** as an

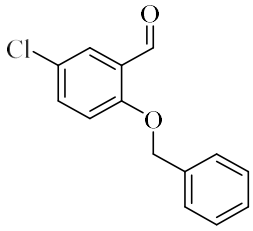
off white crystalline solid (0.8 g, 38%) m.p. 65-67 °C (lit.[391] 67-68 °C); ν_{max}/cm^{-1} 1682 (C=O); δ_H (400 MHz; $CDCl_3$) 5.15 (2H, s, CH_2), 7.08 (2H, d, *J* = 8.7 Hz, ArH), 7.34-7.47 (5H, m, ArH), 7.84 (2H, d, *J* = 8.8 Hz, ArH) and 9.89 (1H, s, HC=O); δ_C (100 MHz; $CDCl_3$) 70.4 (CH_2), 115.2, 127.6, 128.4, 128.8, 130.2, 132.1, 136.0 and 163.8 (ArC) and 190.9 (C=O).

2-(Benzyloxy)-3-ethoxybenzaldehyde 154b



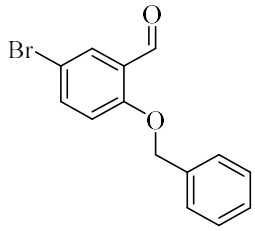
The procedure described for the synthesis of compound **154a** was followed using 3-ethoxy-2-hydroxybenzaldehyde (1.66 g, 10 mmol). Work-up afforded 2-(benzyloxy)-3-ethoxybenzaldehyde **154b** as a white fluffy solid (0.86 g, 34%), m.p. 37-39 °C (lit.[392] 39-40 °C); ν_{max}/cm^{-1} 1678 (C=O); δ_H (600 MHz; $CDCl_3$) 1.51 (3H, t, *J* = 7.0 Hz, CH_3), 4.15 (2H, q, *J* = 7.0 Hz, CH_2) 5.20 (2H, s, $PhCH_2$), 7.11 (IH, t, *J* = 7.9 Hz, ArH), 7.17 (1H, dd, *J* = 8.0, 1.4 Hz, ArH), 7.32-7.43 (6H, m, ArH overlapping) and 10.27 (1H, s, HC=O); δ_C (150 MHz; $CDCl_3$) 15.0 (CH_3), 64.9 (CH_2), 76.4 ($PhCH_2$), 119.2, 119.4, 124.3, 128.6, 128.7, 128.8, 130.5, 136.7, 151.5 and 152.5 (ArC) and 190.4 (C=O).

2-(Benzyloxy)-5-chlorobenzaldehyde 154c



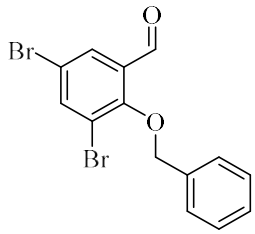
The procedure described for the synthesis of compound **154a** was followed using 5-chloro-2-hydroxybenzaldehyde (1.57 g, 10 mmol). Work-up afforded 2-(benzyloxy)-5-chlorobenzaldehyde **154c** as a white crystalline solid (2.13g, 86 %), m.p. 77-79 °C (lit.[391] 78-79 °C); ν_{max}/cm^{-1} 1676 (C=O); δ_H (400 MHz; $CDCl_3$) 5.18 (2H, s, CH_2), 7.00 (1H, d, *J* = 8.8 Hz, 3-H), 7.34-7.44 (5H, m, ArH overlapping), 7.46 (1H, dd, *J* = 8.9, 2.8 Hz, 4-H), 7.80 (1H, d, *J* = 2.8 Hz, 6-H) and 10.48 (1H, s, HC=O); δ_C (100 MHz; $CDCl_3$) 71.0 (CH_2), 114.9, 126.2, 126.8, 127.4, 128.1, 128.6, 128.9, 135.5, 135.7 and 159.5 (ArC) and 188.5 (C=O).

2-(Benzyloxy)-5-bromobenzaldehyde 154d



The procedure described for the synthesis of compound **154a** was followed using 2-(benzyloxy)-5-bromobenzaldehyde (2.01 g, 10 mmol). Work-up afforded 2-(benzyloxy)-5-bromobenzaldehyde **154d** as a white crystalline solid (1.66 g, 57%), m.p. 68-70 ^{o}C (lit.[393] 70-71 ^{o}C); ν_{max}/cm^{-1} 1674 (C=O); δ_H (400 MHz; $CDCl_3$) 5.18 (2H, s, CH_2), 6.95 (1H, d, J = 8.9 Hz, 3-H), 7.34-7.46 (5H, m, ArH overlapping), 7.60 (1H, dd, J = 8.9, 2.8 Hz, 4-H), 7.94 (1H, d, J = 2.8 Hz, 6-H) and 10.46 (1H, s, HC=O); δ_C (100 MHz; $CDCl_3$) 71.0 (CH_2), 114.0, 115.3, 126.6, 127.5, 128.6, 128.9, 131.2, 135.7, 138.3 and 160.0 (ArC) and 188.4 (C=O).

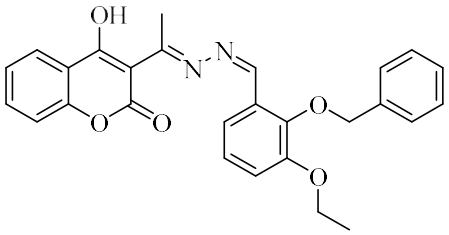
2-(Benzyloxy)-3,5-dibromobenzaldehyde 154e



The procedure described for the synthesis of compound **154a** was followed using 3,5-dibromobenzaldehyde (2.80 g, 10 mmol). Work-up afforded 2-(benzyloxy)-3,5-dibromobenzaldehyde **154e** as a white fluffy solid (1.62g, 44%), m.p. 98-100 ^{o}C lit.[394] 109.5- 110.5 ^{o}C; ν_{max}/cm^{-1} 1685 (C=O); δ_H (400 MHz; $CDCl_3$) 5.12 (2H, s, CH_2), 7.38-7.41 (5H, m, ArH overlapping), 7.86 (1H, d, J = 2.4 Hz, 6-H), 7.98 (1H, d, J = 2.6 Hz, 4-H) and 9.96 (1H, s, HC=O); δ_C (100 MHz; $CDCl_3$) 78.13 (CH_2), 118.4, 119.6, 129.0, 129.1, 129.3, 130.5, 132.5, 134.8, 141.5, 157.5 (ArC) and 187.6 (C=O).

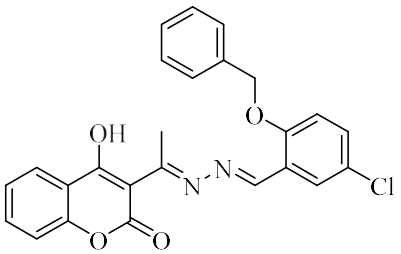
3.9.3. 3-[1-(Benzylidenehydrazono)ethyl]-4-hydroxycoumarins

3-{[2-(Benzyloxy-3-ethoxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarin 137a



A solution of 3-(1-hydrazonoethyl)-4-hydroxycoumarin **152** (0.11 g, 0.5 mmol), 2-(benzyloxy)-3-ethoxybenzaldehyde **154b** (0.13 g, 0.5 mmol) and one drop of 2N HCl in ethanol (5 mL) was refluxed for at least one hour and then cooled to room temperature. The resulting precipitate was filtered off, washed with methanol and air dried to afford 3-{[2-(benzyloxy-3-ethoxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarin **137a** as a yellow solid (0.20 g, 88%) m.p. 190-192 ^{o}C; [HRMS: *m/z* calculated for $C_{27}H_{25}N_2O_5$ (MH^+) 457.1763. Found 457.1766]; ν_{max}/cm^{-1} 1738 (C=O); δ_H (600 MHz; $CDCl_3$) 1.31 (3H, t, *J* = 7.0 Hz, C*H*$_3$CH$_2$), 2.79 (3H, s, CH$_3$C=N), 3.93 (2H, q, *J* = 6.9 Hz, CH$_3$C*H*$_2$), 4.94 (2H, s, PhCH$_2$), 6.84 (1H, d, *J* = 7.5 Hz, ArH), 6.90 (1H, t, *J* = 8.0 Hz, ArH), 7.03 (1H, d, *J* = 8.2 Hz, ArH), 7.07 (1H, t, *J* = 7.5 Hz, ArH), 7.12 (1H, t, *J* = 7.2 Hz, ArH), 7.18 (2H, t, *J* = 7.5 Hz, ArH), 7.22 (2H, d, *J* = 7.2 Hz, ArH), 7.31 (1H, d, *J* = 7.5 Hz, ArH), 7.36 (1H, t, *J* = 7.7 Hz, ArH) 7.89 (1H,d, *J* = 7.7 Hz, ArH) and 8.24 (1H s, CH=N); δ_C (150 MHz; $CDCl_3$) 15.0 and 17.7 (2xCH$_3$), 64.5 (CH$_3$*C*H$_2$), 76.0 (PhCH$_2$), 96.5 (C-3), 116.3, 116.8, 118.1, 120.2, 123.8, 124.6, 126.1, 127.3, 128.6, 128.7, 129.0, 134.3, 136.5 and 148.1 (Ar-C), 151.2 (ArC=N), 152.3 and 153.9 (Ar-C), 162.4 (C=O), 173.1 (CH$_3$*C*=N) and 181.6 (C-4).

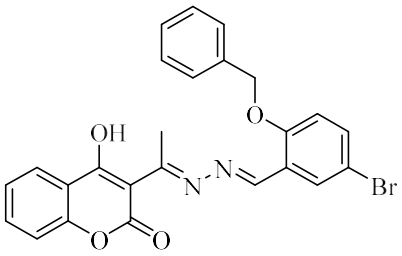
3-{[2-(Benzyloxy-5-chlorobenzylidene)hydrazono]ethyl}-4-hydroxycoumarin 137b



The procedure described for the synthesis of compound **137a** was followed using 2-(benzyloxy)-5-chlorobenzaldehyde **154c** (0.12 g, 0.5 mmol). Work-up afforded 3-{[2-(benzyloxy-5-chlorobenzylidene)hydrazono]ethyl}-4-hydroxycoumarin **137b** as a yellow

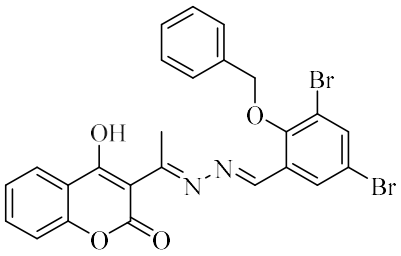
solid (0.19 g, 85%), m.p. 230-232 °C; [HRMS: *m/z* calculated for $C_{25}H_{20}ClN_2O_4$ (MH^+) 447.1112. Found 447.1111]; ν_{max}/cm^{-1} 1694 (C=O); δ_H (600 MHz; $CDCl_3$) 2.98 (3H, s, CH_3) 5.08 (2H, s, $PhCH_2$), 6.83 (1H, d, *J* = 8.9 Hz, ArH), 7.14 (1H, m, ArH), 7.17 (1H, d, *J* = 7.6 Hz, ArH), 7.25 (1H, dd, *J* = 8.9, 2.6 Hz, ArH), 7.28-7.37 (5H, m, ArH), 7.48 (1H, t, *J* = 7.7 Hz, ArH) 7.88 (1H, d, *J* = 2.6 Hz, ArH), 7.94 (1H, d, *J* = 7.8 Hz, ArH) and 8.64 (1H s, CH=N); δ_C (150 MHz; $CDCl_3$) 17.9 (CH_3), 71.1 ($PhCH_2$), 96.8 (C-3), 114.4, 116.8, 120.1, 122.9, 123.9, 126.0, 126.7, 126.8, 127.7, 128.7, 129.0, 133.0, 134.5, 135.7 (Ar-C), 149.3 (ArC=N), 154.0 and 156.7 (Ar-C), 162.3 (C=O), 173.7 (CH_3C=N) and 181.9 (C-4).

3-{[2-(Benzyloxy-5-bromobenzylidene)hydrazono]ethyl}-4-hydroxycoumarin 137c



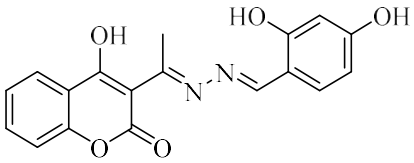
The procedure described for the synthesis of compound **137a** was followed using 2-(benzyloxy)-5-bromobenzaldehyde **154d** (0.15 g, 0.5 mmol). Work-up afforded 3-{[2-(benzyloxy-5-bromobenzylidene)hydrazono]ethyl}-4-hydroxycoumarin **137c** as a yellow solid (0.19 g, 77%), m.p. 232-234 °C; [HRMS: *m/z* calculated for $C_{25}H_{20}BrN_2O_4$ (MH^+) 491.0606. Found 491.0608]; ν_{max}/cm^{-1} 1686 (C=N); δ_H (600 MHz; $CDCl_3$) 2.98 (3H, s, CH_3) 5.09 (2H, s, $PhCH_2$), 6.79 (1H, d, *J* = 8.9 Hz, ArH), 7.13-7.19 (2H, m, ArH), 7.28-7.36 (5H, m, ArH), 7.39 (1H, dd, *J* = 8.8, 2.3 Hz, ArH), 7.48 (1H, t, *J* = 7.7 Hz, ArH) 7.95 (1H, d, *J* = 7.5 Hz, ArH), 8.02 (1H, d, *J* = 2.2 Hz, ArH) and 8.64 (1H s, CH=N); δ_C (150 MHz; $CDCl_3$) 17.9 (CH_3), 71.1 ($PhCH_2$), 96.8 (C-3), 114.0, 114.8, 116.9, 120.1, 123.5, 123.9, 126.1, 126.7, 128.7, 129.1, 129.8, 134.5, 135.7 and 135.8 (Ar-C), 149.2 (ArC=N), 154.0 and 157.2 (Ar-C), 162.2 (C=O), 173.7 (CH_3C=N) and 181.9 (C-4).

3-{[2-(Benzyloxy-3,5-dibromobenzylidene)hydrazono]ethyl}-4-hydroxycoumarin 137d



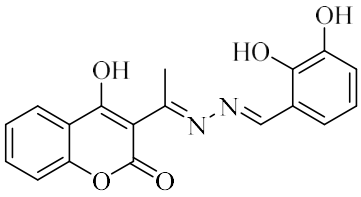
The procedure described for the synthesis of compound **137a** was followed using 2-(benzyloxy)-3,5-dibromobenzaldehyde **154e** (0.14 g, 0.5 mmol). Work-up afforded 3-{[2-(benzyloxy-3,5-dibromobenzylidene)hydrazono]ethyl}-4-hydroxycoumarin **137d** as a yellow solid (0.22 g, 77%), m.p. 219-221 ^{o}C; [HRMS: *m/z* calculated for $C_{25}H_{19}Br_2N_2O_4$ (MH^+) 568.9712. Found 568.9719]; ν_{max}/cm^{-1} 1694 (C=O); δ_H (600 MHz; $CDCl_3$) 2.84 (3H, s, CH_3) 4.95 (2H, s, $PhCH_2$), 7.10-7.17 (2H, m, ArH), 7.20 (1H, m, ArH), 7.24-7.29 (4H, m, ArH), 7.46 (1H, t, *J* = 7.1 Hz, ArH), 7.73 (1H, s ArH), 7.83 (1H, s ArH), 7.95 (1H, s, CH=N) and 7.96 (1H, s, ArH); δ_C (150 MHz; $CDCl_3$) 17.8 (CH_3), 77.5 ($PhCH_2$), 96.9 (C-3), 116.9, 118.3, 119.3, 120.0, 123.9, 126.2, 128.8, 129.1, 129.2, 129.4, 130.4, 134.6, 134.8 and 138.9 (Ar-C), 148.5 (ArC=N), 154.0 and 154.4 (Ar-C), 162.2 (C=O), 173.8 (CH_3C=N) and 181.9 (C-4).

3-{[2,4-(Dihydroxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarin 137e



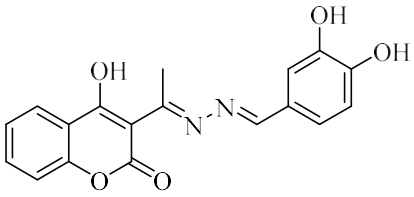
The procedure described for the synthesis of compound **137a** was followed using 2,4-dihydroxybenzaldehyde (0.07 g, 0.5 mmol). Work-up afforded 3-{[2,4-(dihydroxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarin **137e** as a yellow solid (0.15 g, 87%), m.p. 238-240 ^{o}C; [HRMS: *m/z* calculated for $C_{18}H_{15}N_2O_5$ (MH^+) 339.0981. Found 339.0981]; ν_{max}/cm^{-1} 3206 (O-H) and 1669 (C=N); δ_H (600 MHz; DMSO-d_6) 2.81 (3H, s, CH_3) 6.36 (2H, dd, *J* = 4.3, 2.3 Hz, ArH), 7.20 (1H, d, *J* = 8.2 Hz, ArH), 7.25 (1H, t, *J* = 7.5 Hz, ArH), 7.56-7.60 (2H, m, ArH), 7.90 (1H, d, *J* = 6.6 Hz, ArH), 8.59 (1H s, CH=N), 10.24 and 10.37 (2H, 2xs, 2xOH); δ_C (150 MHz; DMSO-d_6) 17.1 (CH_3), 95.2 (C-3), 102.4, 116.4, 108.7, 110.4, 116.3, 119.7, 123.7, 125.6, 129.5, 134.2 and 153.1 (Ar-C), 153.2 (ArC=N), 161.4 (Ar-C), 162.9 (C=O), 169.9 (CH_3C=N) and 179.6 (C-4).

3-{[2,3-(Dihydroxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarin 137f



The procedure described for the synthesis of compond **137a** was followed using 2,3-dihydroxybenzaldehyde (0.07 g, 0.5 mmol). Work-up afforded 3-{[2,3-(dihydroxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarin **137f** as a yellow solid (0,12 g, 71%), m.p. 260-261 ^{o}C; [HRMS: *m/z* calculated for $C_{18}H_{15}N_2O_5$ (MH^+) 339.0981. Found 339.0983]; ν_{max}/cm^{-1} 3412 (O-H) and 1701 (C=O); δ_H (400 MHz; DMSO-d_6) 2.86 (3H, s, CH_3) 6.73 (1H, t, *J* = 7.8 Hz, ArH), 6.93 (1H, d, *J* = 7.5 Hz, ArH), 7.23 (2H, d, *J* = 8.2 Hz, ArH), 7.28 (1H, t, *J* = 7.6 Hz, ArH), 7.61 (1H, t, *J* = 7.7 Hz, ArH) 7.92 (1H, d, *J* = 7.2 Hz, ArH), 8.76 (1H s, CH=N), 9.63 and 9.73 (2H, 2xs, 2xOH); δ_C (100 MHz; DMSO-d_6) 17.3 (CH_3), 95.5 (C-3), 116.4, 117.7, 118.6, 119.2, 119.6, 119.7, 123.9, 125.7, 134.4, 146.0, 147.0 and 153.2 (Ar-C), 153.5 (ArC=N), 161.3 (C=O), 171.2 (CH_3*C*=N) and 179.9 (C-4).

3-{[3,4-(Dihydroxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarin 137g



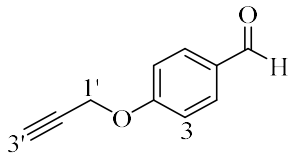
The procedure described for the synthesis of compound **137a** was followed using 3,4-dihydroxybenzaldehyde (0.07 g, 0.5 mmol). Work-up afforded 3-{[3,4-(dihydroxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarin **137g** as yellow solid (0.14 g, 83%), m.p. 285-286 ^{o}C; [HRMS: *m/z* calculated for $C_{18}H_{15}N_2O_5$ (MH^+) 339.0981. Found 339.0987]; ν_{max}/cm^{-1} 3333 (O-H) and 1684 (C=N); δ_H (400 MHz; DMSO-d_6) 2.88 (3H, s, CH_3) 6.86 (1H, d, *J* = 8.1 Hz, ArH), 7.15 (1H, dd, *J* = 8.2, 1.8 Hz, ArH), 7.25 (1H, d, *J* = 8.2 Hz, ArH), 7.29 (1H, t, *J* = 7.5 Hz, ArH), 7.33 (1H, d, *J* = 1.8 Hz, ArH), 7.62 (1H, t, *J* = 7.1 Hz, ArH) 7.93 (d, *J* = 6.7 Hz, ArH), 8.48 (1H s, CH=N), 9.50 and 9.89 (2H, 2xs, 2xOH); δ_C (100 MHz; DMSO-d_6) 17.7 (CH_3), 95.7 (C-3), 114.2, 116.2, 116.8, 120.2, 123.3, 124.2, 124.5, 126.1, 134.7, 146.3, 150.5 and 153.6 (Ar-C), 157.1 (ArC=N), 161.8 (C=O), 171.2 (CH_3*C*=N) and 180.2 (C-4).

3.10. SYNTHESIS OF 3-{[4-(PROPARGYLOXY) BENZYLIDENE]HYDRAZONO)ETHYL}-4-HYDROXYCOUMARINS 138

The targeted molecular hybrids were synthesised by a reacting 3-(1-hydrazonoethyl)-4-hydroxycoumarin **152** with aldehydes **156a-g**.

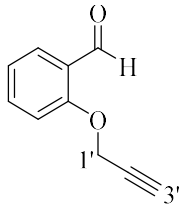
3.10.1. Synthesis of propargyloxy benzaldehydes

4-(Propargyloxy)benzaldehyde 156a



A mixture of 4-hydroxybenzaldehyde (3.0 g, 25 mmol), propargyl bromide (1.93 mL, 25.5 mmol) and K_2CO_3 (6.9 g, 50 mmol) was heated at 80 ^oC in CH_3CN for four hours. The solvent was then evaporated and the crude product was dissolved in DCM and washed with water. The organic layer was dried and the residue recrystallized from hexane to obtain 4-(propargyloxy)benzaldehyde **156a** as a cream solid (3.85g, 96%), m.p. 77-79 ^oC (lit.[395] 79-80 ^oC); ν_{max}/cm^{-1} 1676 (C=O); δ_H (400 MHz; $CDCl_3$) 2.57 (1H, t, *J* = 2.4 Hz, 3'-CH), 4.76 (2H, d, *J* = 2.4 Hz, 1'-CH_2), 7.07 (2H, d, *J* = 8.8 Hz, 3 and 5-H), 7.84 (2H, d, *J* = 8.7 Hz, 2- and 6-H) and 9.88 (1H, CHO); δ_C (100 MHz; $CDCl_3$) 56.1 (C-1'), 76.5 (C-3'), 77.7 (C-2'), 115.3, 130.7, 132.0 and 162.5 (Ar-C) and 190.8 (C=O).

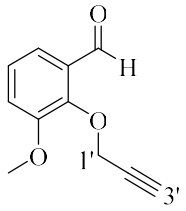
2-(Propargyloxy)benzaldehyde 156b



The procedure described for the synthesis of compound **156a** was followed using salicylaldehyde (2.61 mL, 25 mmol). Work-up afforded 2-(propargyloxy)benzaldehyde **156b** as a grey crystalline solid (3.37 g, 84%), m.p. 68-70 ^oC (lit.[396] 68-70 ^oC); ν_{max}/cm^{-1} 1678 (C=O); δ_H (400 MHz; $CDCl_3$) 2.57 (1H, t, *J* = 2.4 Hz, 3'-CH), 4.82 (2H, d, *J* = 2.4 Hz, 1'-CH_2), 7.04-7.13 (2H, m, 3- and 5-H), 7.55 (1H, m, 4-H), 7.84 (1H, dd, *J* = 7.7, 1.8 Hz, 6-H)

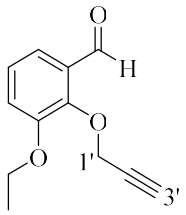
and 10.47 (1H, CHO); δ_C (100 MHz; $CDCl_3$) 56.5 (C-1'), 76.6 (C-3'), 77.8 (C-2'), 113.3, 121.8, 125.6, 128.6, 135.8 and 159.8 (Ar-C) and 189.6 (C=O).

3-Methoxy-2-(propargyloxy)benzaldehyde 156c



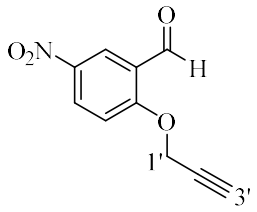
The procedure described for the synthesis of compound **156a** was followed using 2-hydroxy-3-methoxybenzaldehyde (3.80 g, 25 mmol). Work-up afforded 3-methoxy-2-(propargyloxy)benzaldehyde **156c** as a pale yellow solid (4.37 g, 92%), m.p. 48-49 °C (lit.[397] 51-52.5 °C); ν_{max}/cm^{-1} 1680 (C=O); δ_H (400 MHz; $CDCl_3$) 2.47 (1H, t, *J* = 2.4 Hz, 3'-CH), 3.90 (3H, s, CH_3), 4.87 (2H, d, *J* = 2.4 Hz, 1'-CH_2), 7.16 (2H, m, 4- and 5-H), 7.44 (1H, dd, *J* = 7.0, 2.4 Hz, 6-H) and 10.48 (1H, CHO); δ_C (100 MHz; $CDCl_3$) 56.2 (CH_3O), 61.0 (C-1'), 77.0 (C-3'), 78.4 (C-2'), 117.9, 119.0, 125.0, 131.3, 149.6 and 152.9 (Ar-C) and 190.7 (C=O).

3-Ethoxy-2-(propargyloxy)benzaldehyde 156d



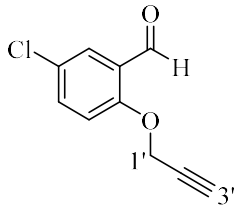
The procedure described for the synthesis of compound **156a** was followed using 2-hydroxy-3-ethoxybenzaldehyde (4.15 g, 25 mmol). Work-up afforded 3-ethoxy-2-(propargyloxy)benzaldehyde **156d** as a cream crystalline solid (4.92 g, 96%), m.p. 66-68 °C (lit.[398] 112-114 °C); ν_{max}/cm^{-1} 1679 (C=O); δ_H (400 MHz; $CDCl_3$) 1.46 (3H, t, *J* = 7.0 Hz, CH_3), 2.47 (1H, t, *J* = 2.4 Hz, 3'-CH), 4.10 (q, *J* = 7.0 Hz, CH_2), 4.90 (2H, d, *J* = 2.4 Hz, 1'-CH_2), 7.10-7.15 (2H, m, 4- and 5-H), 7.42 (1H, dd, *J* = 5.6, 3.8 Hz, 6-H) and 10.48 (1H, CHO); δ_C (100 MHz; $CDCl_3$) 14.9 (CH_3), 60.8 (C-1'), 64.7 (CH_2), 76.9 (C-3'), 78.5 (C-2'), 118.8, 118.9, 124.9, 131.2, 149.8 and 152.2 (Ar-C) and 190.7 (C=O).

5-Nitro-2-(propargyloxy)benzaldehyde 156e



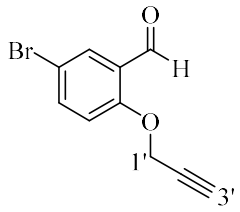
The procedure described for the synthesis of compound **156a** was followed using 2-hydroxy-5-nitrobenzaldehyde (4.18 g, 25 mmol). Work-up afforded 5-nitro-2-(propargyloxy)benzaldehyde **156e** as a light brown solid (1.22 g, 24%), m.p. 82-84 °C (lit.[397] 91.5-93°C); ν_{max}/cm^{-1} 1683 (C=O); δ_H (400 MHz; $CDCl_3$) 2.55 (1H, t, *J* = 2.2 Hz, 3'-CH), 4.86 (2H, d, *J* = 2.4 Hz, 1'-CH_2), 7.17 (2H, d, *J* = 9.2 Hz, 3-H), 8.32 (1H, dd, *J* = 9.2, 2.8 Hz, 4-H), 8.57 (d, *J* = 2.8 Hz, 6-H) and 10.32 (1H, CHO); δ_C (100 MHz; $CDCl_3$) 56.3 (C-1'), 76.3 (C-3'), 78.0 (C-2'), 113.8, 124.7, 125.3, 130.4, 142.3 and 163.4 (Ar-C) and 187.3 (C=O).

5-Chloro-2-(propargyloxy)benzaldehyde 156f



The procedure described for the synthesis of compound **156a** was followed using 2-hydroxy-5-chlorobenzaldehyde (3.9 g, 25 mmol). Work-up afforded 5-chloro-2-(propargyloxy)benzaldehyde **156f** as a khaki solid (4.63 g, 95%), m.p. 70-72 °C (lit.[399] 74-76 °C); ν_{max}/cm^{-1} 1668 (C=O); δ_H (400 MHz; $CDCl_3$) 2.58 (1H, t, *J* = 2.4 Hz, 3'-CH), 4.82 (2H, d, *J* = 2.4 Hz, 1'-CH_2), 7.08 (1H, d, *J* = 8.9 Hz, 3-H), 7.49 (1H, dd, *J* = 8.9, 2.8 Hz, 4-H), 7.79 (1H, d, *J* = 2.7 Hz, 6-H) and 10.39 (1H, CHO); δ_C (100 MHz; $CDCl_3$) 56.9 (C-1'), 77.1 (C-3'), 77.3 (C-2'), 115.1, 126.5, 127.6, 128.1,135.3 and 158.3 (Ar-C) and 188.3 (C=O).

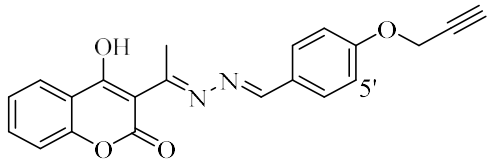
5-Bromo-2-(propargyloxy)benzaldehyde 157g



The procedure described for the synthesis of compoud **156a** was followed using 2-hydroxy-5-bromobenzaldehyde (5.03 g, 25 mmol). Work-up afforded 5-Bromo-2-(propargyloxy)benzaldehyde **156g** as a pale yellow solid (5,71 g, 96%), m.p. 90-92 ^{o}C (lit.[400] 89-91 ^{o}C); ν_{max}/cm^{-1} 1679 (C=O); δ_H (400 MHz; $CDCl_3$) 2.59 (1H, t, *J* = 2.4 Hz, 3'-CH), 4.82 (2H, d, *J* = 2.4 Hz, 1'-CH_2), 7.03 (1H, d, *J* = 8.9 Hz, 3-H), 7.64 (1H, dd, *J* = 8.9, 2.6 Hz, 4-H), 7.93 (1H, d, *J* = 2.6 Hz, 6-H) and 10.38 (1H, CHO); δ_C (100 MHz; $CDCl_3$) 56.8 (C-1'), 77.1 (C-3'), 77.3 (C-2'), 114.7, 115.5, 126.9, 131.3,138.2 and 158.7 (Ar-C) and 188.2 (C=O).

3.10.2. Synthesis of 3-{1-[(propargyloxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarins

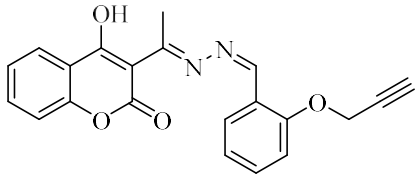
4-Hydroxy-3-{1-[(4-propargyloxybenzylidene)hydrazono]ethyl}coumarin 138a



A solution of 3-(1-hydrazonoethyl)-4-hydroxycoumarin **152** (0.22 g, 1 mmol), 4-(propargyloxy)benzaldehyde **156a** (0.17 g, 1.1 mmol) and one drop of 2N HCl in ethanol (5 mL) was refluxed for at least one hour and then cooled to room temperature. The mixture was filtered to remove the resulting precipitate which was then washed with methanol and dried to afford 4-hydroxy-3-{1-[(4-propargyloxybenzylidene)hydrazono]ethyl}coumarin **138a** as a yellow solid (0.4 g, 83%) m.p. 209-211 ^{o}C; [HRMS: *m/z* calculated for $C_{21}H_{17}N_2O_4$ (MH^+) 361.1188. Found 361.1191]; ν_{max}/cm^{-1} 1695 (C=O); δ_H (400 MHz; $CDCl_3$) 2.57 (1H, t, *J* = 2.4 Hz, C≡CH) 3.06 (3H, s, CH_3), 4.76 (2H, d, *J* = 2.4 Hz, CH_2) 7.06 (2H, d, *J* = 8.8 Hz, ArH), 7.21-7.28 (2H, m, ArH), 7.56 (1H, t, *J* = 8.5 Hz, ArH), 7.75 (2H, d, *J* = 8.8 Hz, ArH), 8.05 (1H, d, *J* = 9.0 Hz, ArH) and 8.31 (1H, s, CH=N); δ_C (100 MHz; $CDCl_3$) 17.8 (CH_3), 56.1 (CH_2), 76.4 (C≡*C*H). 77.9 (*C*≡CH), 95.6 (C-3), 115.6, 116.9, 120.2, 123.8, 126.1, 126.2,

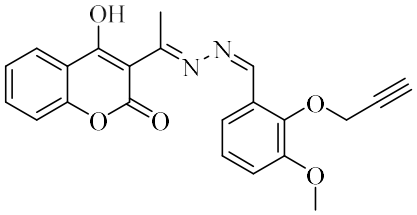
130.4, 134.3 and 154.0 (Ar-C), 154.4 (ArC=N), 160.8 (Ar-C), 162.4 (C=O), 173.1 (CH_3*C*=N) and 181.7 (C-4).

4-Hydroxy-3-{1-[(2-propargyloxybenzylidene)hydrazono]ethyl}coumarin 138b



The procedure described for the synthesis of compound **138a** was followed using 2-(propargyloxy)benzaldehyde **156b** (0.17 g, 1.1 mmol). Work-up afforded 4-hydroxy-3-{1-[(2-propargyloxy)benzylidene)hydrazono]ethyl}coumarin **138b** as a pale yellow solid (0.32 g, 89%), m.p. 188-190 ^oC; [HRMS: *m/z* calculated for $C_{21}H_{17}N_2O_4$ (MH^+) 361.1188. Found 361.1190]; ν_{max}/cm^{-1} 1695 (C=O); δ_H (300 MHz; $CDCl_3$) 2.48 (1H, t, *J* = 2.4 Hz, C≡CH) 2.96 (3H, s, CH_3), 4.70 (2H, d, *J* = 2.4 Hz, CH_2) 6,90-7.01 (2H, m, ArH), 7.21-7.28 (2H, m, ArH), 7.36 (1H, t, *J* = 7.9 Hz, ArH), 7.45 (1H, t, *J* = 7.7 Hz, ArH), 7.94 (2H, t, *J* = 7.5 Hz, ArH) and 8.70 (1H, s, CH=N); δ_C (75 MHz; $CDCl_3$) 17.8 (CH_3), 56.4 (CH_2), 76.6 (C≡*C*H). 77.9 (*C*≡CH), 96.6 (C-3), 113.0, 116.8, 120.2, 121.9, 122.1, 123.8, 126.1, 127.4, 130.4, 133.4 and 134.3 (Ar-C), 150.8 (ArC=N), 154.0, 157.1 (Ar-C), 162.3 (C=O), 173.3 (CH_3*C*=N) and 181.7 (C-4).

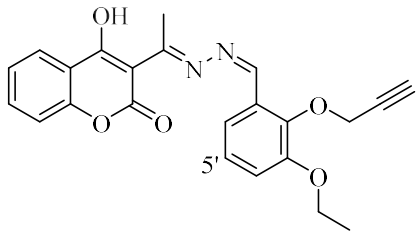
4-Hydroxy-3-{1-[(3-methoxy-2-propargyloxybenzylidene)hydrazono]ethyl}coumarin 138c



The procedure described for the synthesis of compound **138a** was followed using 3-methoxy-2-(propargyloxy)benzaldehyde **156c** (0.19 g, 1.0 mmol). Work-up afforded 4-hydroxy-3-{1-[(3-methoxy-2-propargyloxybenzylidene)hydrazono]ethyl}coumarin **138c** as a pale yellow solid (0.35 g, 90%), m.p. 192-194 ^oC; [HRMS: *m/z* calculated for $C_{22}H_{19}N_2O_5$ (MH^+) 391.1294. Found 391.1295]; ν_{max}/cm^{-1} 1713 (C=O); δ_H (300 MHz; $CDCl_3$) 2.52 (1H, t, *J* = 2.4 Hz, C≡CH) 3.08 (3H, s, CH_3), 3.91 (3H, s, CH_3O), 4.87 (2H, d, *J* = 2.4 Hz, CH_2) 7.04 (1H, d, *J* = 9.4 Hz, ArH), 7.17 (1H, t, *J* = 8.0 Hz, ArH), 7.21-7.29 (2H, m, ArH), 7.57 (1H, t, *J* = 8.5 Hz, ArH), 7.65 (1H, d, *J* = 9.1 Hz, ArH), 8.08 (1H, d, *J* = 8.9 Hz, ArH) and 8.85 (1H, s, CH=N); δ_C (75 MHz; $CDCl_3$) 17.9 (CH_3), 56.1 (CH_3O), 60.7 (CH_2), 77.0 (C≡*C*H). 78.6

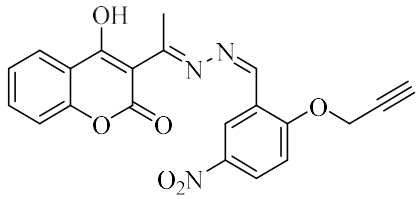
(*C*≡CH), 96.7 (C-3), 115.4, 116.8, 118.4, 120.2, 123.9, 125.2, 126.2, 127.9, 134.4 and 146.9 (Ar-C), 151.7 (ArC=N), 152.9, 154.1 (Ar-C), 162.4 (C=O), 173.5 (CH_3*C*=N) and 181.9 (C-4).

3-{1-[(3-Ethoxy-2-propargyloxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarin 138d



The procedure described for the synthesis of compound **138a** was followed using 3-ethoxy-2-(propargyloxy)benzaldehyde **156d** (0.21 g, 1.0 mmol). Work up afforded 3-{1-[(3-ethoxy-2-propargyloxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarin **138d** as a pale yellow solid (0.37 g, 91%), m.p. 202-204 ºC; [HRMS: *m/z* calculated for $C_{23}H_{21}N_2O_5$ (MH^+) 405.1450. Found 405.1457]; ν_{max}/cm^{-1} 1705 (C=O); δ_H (600 MHz; $CDCl_3$) 1.30 (3H, t, *J* = 7.0 Hz, C*H*$_3$CH$_2$) 2.34 (1H, s, C≡CH) 2.88 (3H, s, CH_3), 3.91 (2H, q, *J* = 6.9 Hz, CH_3C*H*$_2$), 4.72 (2H, d, *J* = 1.8 Hz, CH_2) 6.83 (1H, d, *J* = 8.0 Hz, ArH), 6.95 (1H, t, *J* = 8.0 Hz, ArH), 7.03-7.09 (2H, m, ArH), 7.38 (1H, t, *J* = 7.7 Hz, ArH), 7.44 (1H, d, *J* = 7.9 Hz, ArH), 7.88 (1H, d, *J* = 7.8 Hz, ArH) and 8.65 (1H, s, CH=N); δ_C (150 MHz; $CDCl_3$) 14.9 and 17.9 2xCH_3, 60.5 (CH_2), 64.5 (*C*H$_2$CH$_3$), 76.9 (C≡*C*H). 78.7 (*C*≡CH), 96.6 (C-3), 116.2, 116.8, 118.1, 120.1, 123.8, 125.1, 126.1, 127.8, 134.3, 146.8 (Ar-C), 151.7 (ArC=N), 152.1, 153.9 (Ar-C) and 162.4 (C=O), 173.4 (CH_3*C*=N) and 181.8 (C-4).

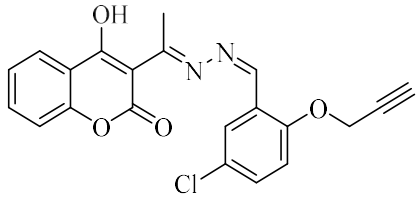
4-Hydroxy-3-{1-[(5-nitro-2-propargyloxybenzylidene)hydrazono]ethyl}coumarin 138e



The procedure described for the synthesis of compound **138a** was followed using 5-nitro-2-(propargyloxy)benzaldehyde **156e** (0.22 g, 1.1 mmol). Work-up afforded 4-hydroxy-3-{1-[(5-nitro-2-propargyloxybenzylidene)hydrazono]ethyl}coumarin **138e** as a pale yellow solid (0.35 g, 86%), m.p. 222-224 ºC; [HRMS: *m/z* calculated for $C_{21}H_{16}N_3O_6$ (MH^+) 406.1039. Found 406.1035]; ν_{max}/cm^{-1} 1688 (C=O); δ_H (400 MHz; $CDCl_3$) 2.64 (1H, s, C≡CH) 3.09 (3H, s, CH_3), 4.92 (2H, s, CH_2) 7.15-7.24 (3H, m, ArH), 7.56 (1H, t, *J* = 6.7 Hz, ArH), 8.02

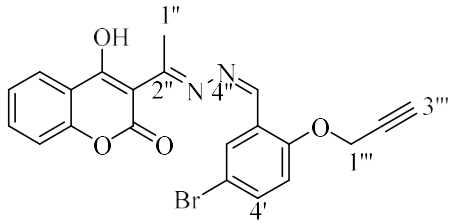
(1H, d, *J* = 7.4 Hz, ArH), 8.33 (1H, d, *J* = 8.5 Hz, ArH), 8.73 (1H, s, CH=N) and 8.86 (1H, s, ArH); δ_C (100 MHz; $CDCl_3$) 17.9 (CH_3), 57.2 (CH_2), 76.4 (C≡*C*H). 78.0 (*C*≡CH), 97.2 (C-3), 113.1, 117.0, 120.0, 122.7, 123.1, 124.0, 126.1, 128.1, 134.7 and 142.5 (Ar-C), 148.2 (ArC=N), 154.1, 160.7 (Ar-C), 162.1 (C=O), 174.4 (CH_3*C*=N) and 182.2 (C-4).

3-{1-[(5-Chloro-2-propargyloxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarin 138f



The procedure described for the synthesis of compound **138a** was followed using 5-chloro-2-(propargyloxy)benzaldehyde **156f** (0.19 g, 1.0 mmol). Work-up afforded 3-{1-[(5-chloro-2-propargyloxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarin **138f** as a pale yellow solid (0.36 g, 91%), m.p. 232-234 °C; [HRMS: *m/z* calculated for $C_{21}H_{16}ClN_2O_4$ (MH^+) 395.0799. Found 395.0793]; ν_{max}/cm^{-1} 1694 (C=O); δ_H (600 MHz; $CDCl_3$) 2.55 (1H, t, *J* = 2.4 Hz, C≡CH) 3.04 (3H, s, CH_3), 4.77 (2H, d, *J* = 2.3 Hz, CH_2) 7.00 (1H, d, *J* = 8.9 Hz, ArH), 7.18-7.24 (2H, m, ArH), 7.37 (1H, dd, *J* = 8.9, 2.6 Hz, ArH), 7.54 (1H, t, *J* = 8.5 Hz, ArH), 7.95 (1H, d, *J* = 2.6 Hz, ArH), 8.01 (1H, d, *J* = 7.8 Hz, ArH) and 8.69 (1H, s, CH=N); δ_C (150 MHz; $CDCl_3$) 17.9 (CH_3), 56.8 (CH_2), 77.0 (C≡*C*H). 77.4 (*C*≡CH), 96.9 (C-3), 114.5, 116.9, 120.1, 123.4, 123.9, 126.1, 126.8, 127.6, 132.9, 134.5, (Ar-C), 149.3 (ArC=N), 154.1 and 155.5 (Ar-C), 162.3 (C=O), 173.8 (CH_3*C*=N) and 182.0 (C-4).

3-{1-[(5-Bromo-2-propargyloxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarin 138g

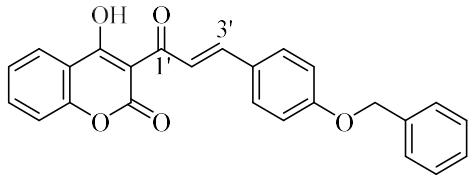


The procedure described for the synthesis of compound **138a** was followed using 5-bromo-2-(propargyloxy)benzaldehyde **156g** (0.24 g, 1.0 mmol). Work-up afforded 3-{1-[(5-bromo-2-propargyloxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarin **138g** as a pale yellow solid (0.39 g, 89%), m.p. 234-235 °C; [HRMS: *m/z* calculated for $C_{21}H_{16}BrN_2O_4$ (MH^+) 439.0293. Found 439.0298]; ν_{max}/cm^{-1} 1695 (C=O); δ_H (400 MHz; $CDCl_3$) 2.56 (1H, t, *J* = 2.2 Hz, C≡CH) 3.05 (3H, s, CH_3), 4.77 (2H, d, *J* = 2.2 Hz, CH_2) 6.96 (1H, d, *J* = 8.9 Hz, ArH), 7.17-

7.27 (2H, m, ArH), 7.50-7.57 (2H, m, ArH), 8.03 (1H, d, J = 7.1 Hz, ArH), 8.10 (1H, d, J = 2.3 Hz, ArH) and 8.68 (1H, s, CH=N); δ_C (100 MHz; $CDCl_3$) 17.9 (CH_3), 56.7 (CH_2), 77.1 (C≡*C*H). 77.4 (*C*≡CH), 96.9 (C-3), 114.8, 114.9, 116.9, 120.1, 123.8, 123.9, 126.1, 129.8, 134.5 and 135.8 (Ar-C), 149.2 (ArC=N), 154.1, 156.0 (Ar-C), 162.3 (C=O), 173.8 (CH_3*C*=N) and 182.0 (C-4).

3.11. SYNTHESIS OF 4-HYDROXY-3-[3-(PHENYL)ACRYLOYL) COUMARINS 139

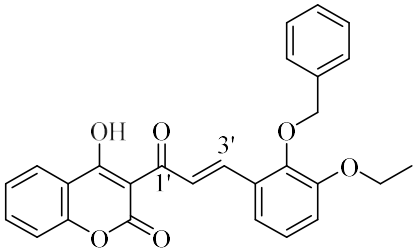
(E)-*3-{3-[4-(Benzyloxy)phenyl]acryloyl}-4-hydroxycoumarin 139a*



A solution of 3-acetyl-4-hydroxycoumarin **133a** (0.2 g, 1 mmol), 4-(benzyloxy)benzaldehyde **154a** (0.23 g, 1.1 mmol) and piperidine (66 µL) in chloroform was heated at 80 ºC for at least 2 hours, after which the solvent was evaporated in vacuo and the residue washed with methanol to afford (*E*)-3-{3-[4-(benzyloxy)phenyl]acryloyl}-4-hydroxycoumarin **139a** as a yellow solid (0.25g, 63%) m.p. 160-161 ºC; [HRMS: *m/z* calculated for $C_{25}H_{17}O_5$ (M^-) 397.1076. Found 397.1063]; ν_{max}/cm^{-1} 1707 (C=O); δ_H (400 MHz; $CDCl_3$) 5.03 (2H, s, $PhCH_2$), 6.91-6.95 (2H, m, ArH), 7.17-7.23 (2H, m, ArH), 7.26 (1H, m, ArH), 7.29-7.36 (4H, m, ArH), 7.54-7.62 (3H, m, ArH), 7.93-8.01 (2H, m, ArH and 2'-CH) and 8.24 (1H, d, J = 15.7 Hz*, 3'-CH); δ_C (100 MHz; $CDCl_3$) 70.2 ($PhCH_2$), 100.5 (C-3), 115.4, 117.0 (Ar-C), 120.0 (C-3'), 124.3, 125.8, 127.5, 127.7, 128.2, 128.7, 131.5, 135.8, 136.1 and 136.3 (Ar-C), 147.5 (C-2'), 154.6 (Ar-C), 161.7 (C=O), 178.7, 181.7 (C-4) and 191.9 (C-1').

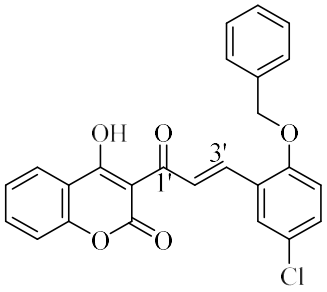
* High J_{vic} values confirm expected (*E*)-geometry

(E)-*3-{3-[2-(Benzyloxy)-3-ethoxyphenyl]acryloyl}-4-hydroxycoumarin 139b*



The procedure described for the synthesis of compound **139a** was followed using 2-(benzyloxy)-3-ethoxybenzaldehyde **155b** (0.28 g, 1.1 mmol). Work-up afforded (*E*)-3-{3-[2-(benzyloxy)-3-ethoxyphenyl]acryloyl}-4-hydroxycoumarin **139b** as a yellow solid (0.22 g, 50%), m.p. 180-182 ^{o}C; [HRMS: *m/z* calculated for $C_{27}H_{21}O_6$ (M^-) 441.1342. Found 441.1338]; ν_{max}/cm^{-1} 1702 (C=O); δ_H (400 MHz; $CDCl_3$) 1.42 (3H, t, *J* = 7.0 Hz, C*H*$_3$CH$_2$), 4.05 (2H, q, *J* = 7.0 Hz, CH$_3$C*H*$_2$), 5.04 (2H, s, PhCH$_2$), 6.94 (1H, d, *J* = 8.1 Hz, ArH), 7.03 (1H, t, *J* = 8.0 Hz, ArH), 7.18-7.26 (3H, m, ArH), 7.27-7.36 (3H, m, ArH), 7.42 (2H, d, *J* = 7.1 Hz, ArH), 7.60 (1H, t, *J* = 8.6 Hz, ArH), 8.02 (1H, dd, *J* = 7.9, 1.4 Hz, ArH), 8.26 (1H, d, *J* = 15.9 Hz, 2'-CH) and 8.37 (1H, d, *J* = 15.9 Hz, 3'-CH); δ_C (100 MHz; $CDCl_3$) 15.1 (CH$_3$*C*H$_2$), 64.6 (C-1'''), 76.0 (PhCH$_2$), 100.8 (C-3), 116.3, 116.6, 117.1 and 119.7 (Ar-C), 123.1 (C-2'), 124.4, 124.5, 125.9, 128.3, 128.5, 128.9, 129.6, 136.0 and 137.1 (Ar-C), 142.6 (C-3'), 148.3, 152.6 and 154.8 (Ar-C), 160.3 (C=O), 181.9 (C-4) and 192.5 (C-1').

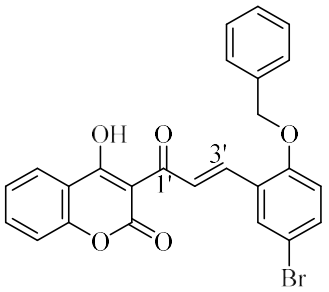
3-{3-[2-(Benzyloxy)-5-chlorophenyl]acryloyl}-4-hydroxycoumarin 139c



The procedure described for the synthesis of compound **139a** was followed using 2-(benzyloxy)-5-chlorobenzaldehyde **155c** (0.32 g, 1.1 mmol). Work-up afforded 3-(3-(2-(benzyloxy)-5-chlorophenyl)acryloyl)-4-hydroxycoumarin **139c** as a pale yellow solid (0.25 g, 58%), m.p. 185-187 ^{o}C; [HRMS: *m/z* calculated for $C_{25}H_{16}ClO_5$ (M^-) 431.0686. Found 431.0687]; ν_{max}/cm^{-1} 1712 (C=O); δ_H (400 MHz; $CDCl_3$) 5.04 (2H, s, PhCH$_2$), 6.75 (1H, d, *J* = 8.8 Hz, ArH), 6.85 (1H, d, *J* = 8.9 Hz, ArH), 7.22 (6H, m, ArH), 7.53 (1H, t, *J* = 7.2 Hz, ArH), 7.59 (1H, d, *J* = 2.3 Hz, ArH), 7.65 (1H, d, *J* = 2.6 Hz, ArH), 7.94 (1H, d, *J* = 7.3 Hz,

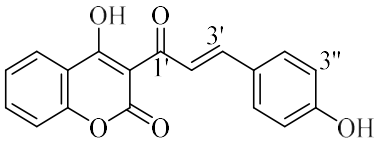
ArH) and 8.28 (2H, m, 2'-CH and 3'-CH); δ_C (100 MHz; $CDCl_3$) 71.0 ($PhCH_2$), 101.0 (C-3), 114.4, 114.9, 117.1 (Ar-C), 124.0 (C-2'), 124.5, 125.8, 125.9, 126.2, 126.5, 127.4, 128.3, 128.9, 135.5, 136.1 and 136.2 (Ar-C), 140.6 (C-3'), 154.8 and 156.6 (Ar-C), 159.5 (C=O), 181.5 (C-4) and 192.6 (C-1').

3-{3-[2-(Benzyloxy)-5-bromophenyl]acryloyl}-4-hydroxycoumarin 139d



The procedure described for the synthesis of compound **139a** was followed using 2-(benzyloxy)-5-bromobenzaldehyde **155d** (0.32 g, 1.1 mmol). Work-up afforded 3-{3-[2-(benzyloxy)-5-bromophenyl]acryloyl}-4-hydroxycoumarin **139d** as a pale yellow solid (0.30 g, 63%), m.p. 183-185 ^oC; [HRMS: *m/z* calculated for $C_{25}H_{16}BrO_5$ (M^-) 475.0181. Found 475.0180]; ν_{max}/cm^{-1} 1701 (C=O); δ_H (600 MHz; $CDCl_3$) 5.19 (2H, s, $PhCH_2$), 6.84 (1H, d, *J* = 8.8 Hz, ArH), 7.29-7.35 (3H, m, ArH), 7.38-7.45 (5H, m, ArH), 7.69 (1H, t, *J* = 7.7 Hz, ArH), 7.87 (1H, s, ArH), 8.09 (1H, d, *J* = 7.8 Hz, ArH) and 8.41 (2H, m, *J* = 3.2 Hz, 2'-CH and 3'-CH); δ_C (150 MHz; $CDCl_3$) 70.9 ($PhCH_2$), 101.0 (C-3), 113.8, 114.8, 116.4 and 117.2 (Ar-C), 124.0 (C-2'), 124.5, 125.9, 126.2, 127.3, 128.4, 128.9, 131.6, 135.1, 136.1 and 136.2 (Ar-C), 140.6 (C-3'), 154.9, 157.1 (Ar-C), 160.3 (C=O), 181.6 (C-OH) and 192.7 (C-1').

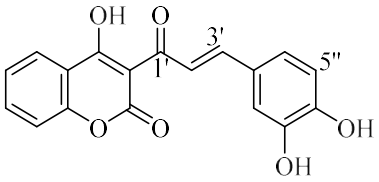
(E)-*4-Hydroxy-3-{3-[4-hydroxyphenyl]acryloyl}-coumarin 139e*



The procedure described for the synthesis of compound **139a** was followed using 4-hydroxybenzaldehyde **153a** (0.12 g, 1.0 mmol). Work-up afforded (*E*)-4-hydroxy-3-{3-[4-hydroxyphenyl]acryloyl}-coumarin **139e** as a pale yellow solid (0.20 g, 65%), m.p. 270-271 ^oC (lit.[401] 268-269 ^oC); [HRMS: *m/z* calculated for $C_{18}H_{11}O_5$ (M^-) 307.0606. Found 307.0601]; ν_{max}/cm^{-1} 1683 (C=O); δ_H (400 MHz; DMSO-d_6) 6.88 (2H, d, *J* = 8.6 Hz, ArH), 7.38 (2H, t, *J* = 7.7 Hz, ArH), 7.62 (2H, d, *J* = 8.6 Hz, ArH), 7.77 (1H, td, *J* = 8.1, 1.5 Hz,

ArH), 7.95-8.02 (2H, m, ArH and 2'-CH), 8.11 (1H, d, J = 15.6 Hz*, 3'-CH) and 10.40 (1H, s, OH); δ_C (75 MHz; DMSO-d_6) 100.1 (C-3), 116.1, 116.3 and 116.8 (Ar-C), 117.8 (C-2'), 124.6, 125.3, 125.4, 131.8 and 136.4 (Ar-C), 147.9 (C-3'), 154.1 (Ar-C), 161.6 (C=O), 181.3 (C-4) and 190.7 (C-1'). * Indicates (*E*) configuration.

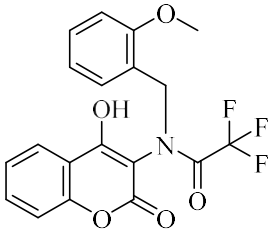
(E)-*4-Hydroxy-3-{3-[3,4-dihydroxyphenyl]acryloyl}-coumarin 139f*



The procedure described for the synthesis of 3compound **139a** was followed using 3,4-dihydroxybenzaldehyde **154g** (0.14 g, 1.0 mmol). Work up afforded (*E*)-4-hydroxy-3-{3-[3,4-dihydroxyphenyl]acryloyl}-coumarin **139f** as an orange solid (0.21 g, 65%), m.p. 270-272 ^oC; [HRMS: *m/z* calculated for $C_{18}H_{11}O_6$ (M^-) 323.0556. Found 323.0539]; ν_{max}/cm^{-1} 1668 (C=O); δ_H (300 MHz; DMSO-d_6) 6.84 (1H, d, J = 8.2 Hz, ArH), 7.10 (1H, d, J = 8.2 Hz, ArH), 7.23 (1H, s, ArH), 7.39 (2H, t, J = 7.6 Hz, ArH), 7.78 (1H, t, J = 7.8 Hz, ArH), 7.89-8.01 (2H, m, ArH and 2'-CH), 8.08 (1H, d, J = 15.6 Hz, 3'-CH), 9.50 and 9.95 (2H, 2xs, 2xOH); δ_C (75 MHz; DMSO-d_6) 100.1 (C-3), 114.5, 116.1, 116.3 and 116.8 (Ar-C), 117.6 (C-2'), 124.5, 124.6, 125.3, 125.9, 136.4 and 146.0 (Ar-C), 148.5 (C-3'), 150.7 and 154.0 (Ar-C), 159.7 (C=O), 181.4 (C-4) and 190.5 (C-1').

3.12. SYNTHESIS OF *N*-SUBSTITUTED 4-HYDROXY-3-(TRIFLUOROACETAMIDO)COUMARINS 164

4-Hydroxy-3-[N-(2-methoxybenzyl)trifluoroacetamido]coumarin 164a

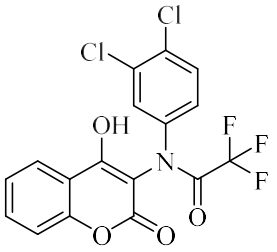


To a mixture of 4-hydroxy-3-[(2-methoxybenzyl)amino]coumarin **163u** (0.15g, 0.5 mmol) and triethylamine (84 µL, 0.6 mmol) in DCM at 0 ^oC was added trifluoroacetic anhydride (85 µL, 0.6 mmol). After stirring at 0 ^oC for 30 minutes, the temperature of the mixture was

allowed to rise to room temperature and stirring was continued for a further 2 hours, after which water was added and the product extracted using DCM. The organic layer solution was passed through a short silica column and the solvent was then evaporated to yield 4-hydroxy-3-[*N*-(2-methoxybenzyl)trifluoroacetamido]coumarin **164a** as a white solid (0.13 g, 66%), m.p. 125-127 ^{o}C; [HRMS: *m/z* calculated for (M-$C_9H_5O_3^*$)$^-$ 232.0585. Found 232.0576]; ν_{max}/cm^{-1} 1678 (C=O); δ_H (400 MHz; DMSO-d_6) 3.80 (3H, s, CH_3O), 4.35 (2H, d, J = 5.8 Hz, CH_2), 6.93 (1H, t, J = 7.4 Hz, ArH), 7.00 (1H, d, J = 8.2 Hz, ArH), 7.12 (1H, d, J = 6.7 Hz, ArH), 7.28 (1H, t, J = 8.5 Hz, ArH), 7.36-7.44 (2H, m, ArH), 7.67 (1H, t, J = 7.8 Hz, ArH), 7.97 (1H, d, J = 6.6 Hz, ArH) and 9.84 (1H, s, OH) ; δ_C (100 MHz; DMSO-d_6) 37.9 (CH_2), 55.4 (CH_3O) 89.0 (C-3), 110.8 (Ar-C), 116.1 (q, J_{CF} = 287 Hz, CF_3), 116.5, 120.3, 123.6, 124.4, 124.7, 127.7, 128.8, 132.8 and 151.8 (Ar-C), 156.4 (q, J_{CF} = 36.0 Hz, NC=O), 156.7 (Ar-C), 158.7 (C=O) and 162.6 (C-4).

* Corresponding to loss of the 4-hydroxycoumarin moiety.

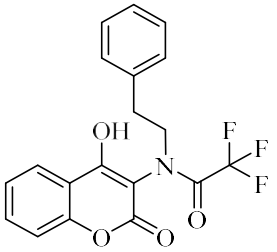
3-[N-(3,4-Dichlorophenyl)trifluoroacetamido]-4-hydroxycoumarin 164b



The procedure described for compound **164a** was followed using 3-[(3,4-dichlorophenyl)amino]-4-hydroxycoumarin **163q** (0.16 g, 0.5 mmol). Work-up afforded 3-[*N*-(3,4-dichlorophenyl)trifluoroacetamido]-4-hydroxycoumarin **164b** as a white solid (0.16 g, 77%), m.p. 100-102 ^{o}C; [HRMS: *m/z* calculated for (M-$C_9H_5O_3^*$)$^-$ 255.9544. Found 255.9537]; ν_{max}/cm^{-1} 1678 (C=O); δ_H (600 MHz; DMSO-d_6) 7.36-7.40 (2H, m, ArH), 7.63-7.68 (3H, m, ArH), 7.94-7.98 (2H, m, ArH) and 11.51 (1H, s, OH); δ_C (150 MHz; DMSO-d_6) 88.9 (C-3), 115.6 (q, J_{CF} = 287.0 Hz, CF_3), 116.2, 116.4, 121.1, 122.4, 123.5, 124.3, 127.5, 131.0, 131.3, 132.7, 136.5 and 151.7 (Ar-C), 154.7 (q, J_{CF} = 37.5 Hz, NC=O), 158.6 (C=O) and 162.6 (C-OH).

* Corresponding to loss of the 4-hydroxycoumarin moiety.

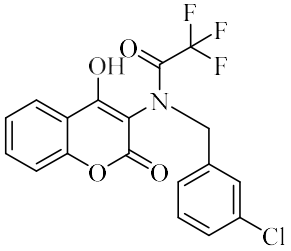
4-Hydroxy-3-[N-(phenethyl)trifluoroacetamido]coumarin 164c



The procedure described for the synthesis of compound **164a** was followed using 4-hydroxy-3-(phenethylamino)coumarin **163v** (0.14 g, 0.5 mmol). Work-up afforded 4-hydroxy-3-[*N*-(phenethyl)trifluoroacetamido]coumarin **164c** as a white solid (0.14g, 74%), m.p. 145-147 ^{o}C; [HRMS: *m/z* calculated for (M-$C_9H_5O_3^*$)$^-$ 255.9544. Found 255.9537]; ν_{max}/cm^{-1} 1694 (C=O); δ_H (600 MHz; $CDCl_3$) 2.91 (2H, t, *J* = 7.1 Hz, $PhCH_2$), 3.64 (2H, q, *J* = 6.8 Hz, NCH_2), 7.21 (2H, d, *J* = 7.4 Hz, ArH), 7.27 (1H, m, ArH), 7.34 (1H, t, *J* = 7.5 Hz, ArH), 7.38 (1H, d, *J* = 8.1 Hz, ArH), 7.64 (1H, t, *J* = 8.5 Hz, ArH) and 7.93 (1H, d, *J* = 8.4 Hz, ArH); δ_C (150 MHz; $CDCl_3$) 35.1 ($PhCH_2$), 41.2 (NCH_2), 91.0 (C-3), 114.4 (Ar-C), 115.9 (q, J_{CF} = 287.0 Hz, CF_3), 116.9, 123.6, 124.8, 127.1, 128.8, 129.0, 133.2, 137.7 and 152.3 (Ar-C), 157.4 (q, J_{CF} = 36.0 Hz, NC=O), 158.7 (C=O) and 161.1 (C-4).

* Corresponding to loss of the 4-hydroxycoumarin moiety.

3-[N-(3-Chlorobenzyl)trifluoroacetamido]-4-hydroxycoumarin 164d

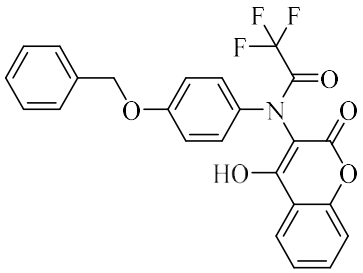


The procedure described for the synthesis of compound **164a** was followed using 3-[(3-chlorobenzyl)amino]-4-hydroxycoumarin **163s** (0.15 g, 0.5 mmol). Work-up afforded 3-[*N*-(3-chlorobenzyl)trifluoroacetamido]-4-hydroxycoumarin **164d** as a white solid (0.13 g, 65%), m.p. 140-141 ^{o}C; [HRMS: *m/z* calculated for (M-$C_9H_5O_3^*$)$^-$ 236.0090. Found 236.0076]; ν_{max}/cm^{-1} 1696 (C=O); δ_H (600 MHz; $CDCl_3$) 4.50 (2H, d, *J* = 5.8 Hz, CH_2), 7.17 (1H, m, ArH), 7.27-7.29 (3H, m, ArH), 7.33-7.37 (2H, m, ArH), 7.63 (1H, t, *J* = 8.6 Hz, ArH) 7.90 (1H, dd, *J* = 7.9, 2.0 Hz, ArH); δ_C (150 MHz; $CDCl_3$) 43.4 (CH_2), 91.0 (C-3), 114.3, 115.9 (q,

J_{CF} = 285.0 Hz, CF_3), 116.9, 123.6, 124.8, 126.2, 128.2, 128.6, 130.4, 133.3, 134.9, 138.0 and 152.2 (Ar-C), 157.5 (q, J_{CF} = 36.0 Hz, NC=O), 158.8 (C=O) and 161.1 (C-4).

* Corresponding to loss of the 4-hydroxycoumarin moiety.

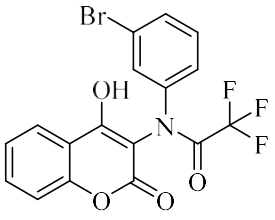
3-[N-(4-Benzyloxyphenyl)trifluoroacetamido]-4-hydroxycoumarin 164e



The procedure described for the synthesis of 2compound **164a** was followed using 3-{[4-(benzyloxy)phenyl]amino}-4-hydroxycoumarin **163t** (0.18 g, 0.5 mmol). Work-up afforded 3-[*N*-(4-benzyloxyphenyl)trifluoroacetamido]-4-hydroxycoumarin **164e** as a white solid (0.19 g, 83%), m.p. 138-140 ^oC; [HRMS: *m/z* calculated for $(M\text{-}C_9H_5O_3^*)^-$ 294.0742. Found 294.0743]; ν_{max}/cm^{-1} 1690 (C=O); δ_H (400 MHz; DMSO-d_6) 5.10 (2H, s, CH_2), 7.04 (2H, d, *J* = 9.0 Hz, ArH), 7.32 (1H, t, *J* = 7.1 Hz, ArH), 7.36-7.47 (6H, m, ArH), 7.57 (2H, d, *J* = 9.0 Hz, ArH), 7.67 (1H, t, *J* = 8.5 Hz, ArH), 7.96 (1H, d, *J* = 9.0 Hz, ArH) and 11.15 (1H, s, OH); δ_C (100 MHz; DMSO-d_6) 69.4 (CH_2), 89.0 (C-3), 115.1 (Ar-C) 116.0 (q, J_{CF} = 287.0 Hz, CF_3), 116.2, 116.5, 122.7, 123.4, 124.4, 127.8, 127.9, 128.5, 129.4, 132.8, 137.0 and 151.8 (Ar-C), 154.2 (q, J_{CF} = 36.0 Hz, NC=O), 155.9, 158.7 (C=O) and 162.6 (C-4).

* Corresponding to loss of the 4-hydroxycoumarin moiety.

3-[N-(3-Bromophenyl)trifluoroacetamido]-4-hydroxycoumarin 164f

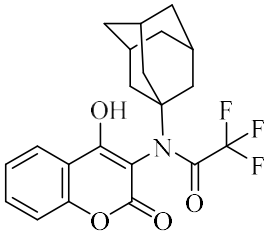


The procedure described for the synthesis of compound **164a** was followed using 3-[(3-bromophenyl)amino]-4-hydroxycoumarin **163o** (0.17 g, 0.5 mmol). Work-up afforded 3-[*N*-(3-bromophenyl)trifluoroacetamido]-4-hydroxycoumarin **164f** as a white solid (0.12 g, 56%), m.p. 102-104 ^oC; [HRMS: *m/z* calculated for $(M\text{-}C_9H_5O_3^*)^-$ 265.9428. Found 265.9435];

ν_{max}/cm^{-1} 1704 (C=O); δ_H (600 MHz; DMSO-d_6) 7.33-7.39 (3H, m, ArH), 7.42 (1H, d, J = 8.3 Hz, ArH), 7.61-7.67 (2H, m, ArH), 7.95-7.93 (2H, m, ArH) and 11.41 (1H, s, OH); δ_C (150 MHz; DMSO-d_6) 88.3 (C-3), 115.6 (q, J_{CF} = 288.0 Hz, CF_3), 116.6, 116.9, 119.9, 121.6, 123.5, 123.7, 124.1, 128.4, 131.0, 132.5, 137.9 and 151.9 (Ar-C), 154.7 (q, J_{CF} = 36.0 Hz, NC=O), 158.8 (C=O) and 163.4 (C-4).

* Corresponding to loss of the 4-hydroxycoumarin moiety.

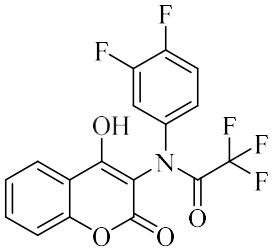
3-[N-(adamantan-1-yl)-trifluoroacetamido]-4-hydroxycoumarin 164g



The procedure described for the synthesis of compound **164a** was followed using 3-[*N*-(adamantan-1-yl)amino]-4-hydroxycoumarin **163g** (0.15 g, 0.5 mmol). Work-up afforded 3-[*N*-(adamantan-1-yl)-trifluoroacetamido]-4-hydroxycoumarin **164g** as a white solid (0.08 g, 39%), m.p. 145-147 ^oC; [HRMS: *m/z* calculated for $(M-C_9H_5O_3^*)^-$ 246.1106. Found 246.1101]; ν_{max}/cm^{-1} 1691 (C=O); δ_H (600 MHz; DMSO-d_6) 2.09 and 2.44 (12H, 2xs, adamantyl CH_2), 2.98 (3H, s, adamantyl CH), 7.84-7.90 (2H, m, 5- and 6-H overlapping), 8.14 (1H, ddd, J = 8.5, 7.4, 1.5 Hz, 7-H), 8.43 (1H, dd, J = 8.0, 1.4 Hz, 8-H) and 9.11 (1H, s, OH); δ_C (150 MHz; DMSO-d_6) 28.7 and 35.7 (adamantyl CH_2), 40.1 (adamantyl CH), 52.7 (adamantyl C-1), 89.0 (C-3), 115.6 (q, J_{CF} = 288.0 Hz, CF_3), 116.1, 116.5, 123.5, 124.4, 132.8 and 151.7 (Ar-C), 155.2 (q, J_{CF} = 36.0 Hz, NC=O), 158.6 (C=O) and 162.5 (C-4).

* Corresponding to loss of the 4-hydroxycoumarin moiety.

3-[N-(3,4-Difluorophenyl)trifluoroacetamido]-4-hydroxycoumarin 164h

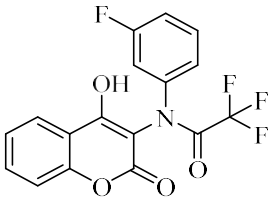


The procedure described for the synthesis of compound **164a** was followed using 3-[(3,4-difluorophenyl)amino]-4-hydroxycoumarin **163n** (0.16 g, 0.5 mmol). Work-up afforded 3-[*N*-

(3,4-difluorophenyl)trifluoroacetamido]-4-hydroxycoumarin **164h** as a white solid (0.16 g, 83%), m.p. 146-148 ^{o}C; [HRMS: *m/z* calculated for (M-$C_9H_5O_3^*$)$^-$ 224.0135. Found 224.0123]; ν_{max}/cm^{-1} 1691 (C=O); δ_H (600 MHz; DMSO-d_6) 7.36-7.40 (2H, m, ArH), 7.48 (2H, m, ArH), 7.66 (1H, t, J = 7.8 Hz, ArH), 7.76 (1H, dd, J = 12.3, 7.4 Hz, ArH), 7.95 (1H, d, J = 7.9 Hz, ArH) and 11.46 (1H, s, OH); δ_C (150 MHz; DMSO-d_6) 88.9 (C-3), 110.4 (d, J_{CF} = 21.6 Hz, C-2') 115.7 (q, J_{CF} = 288.0 Hz, CF_3), 116.2 and 116.4 (Ar-C), 117.7-118.0 (overlapping, C-5' and C-6'), 123.6, 124.3 and 132.7 (Ar-C), 133.3 (dd, J_{CF} = 8.9, 3.2 Hz, C-1'), 146.8 (dd, J_{CF} = 244.4, 12.5 Hz, C-4'), 148.9 (dd, J_{CF} = 244.7, 13.4 Hz, C-3'), 151.8 (Ar-C), 154.6 (q, J_{CF} = 37.0 Hz, NC=O), 158.7 (C=O) and 162.7 (C-4).

* Corresponding to loss of the 4-hydroxycoumarin moiety.

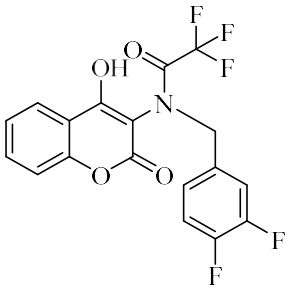
3-[N-(3-Fluorophenyl)trifluoroacetamido]-4-hydroxycoumarin 164i



The procedure described for the synthesis of compound **164a** was followed using 3-[(3-fluorophenyl)amino]-4-hydroxycoumarin **163m** (0.14 g, 0.5 mmol). Work-up afforded 3-[*N*-(3-fluorophenyl)trifluoroacetamido]-4-hydroxycoumarin **164i** as a white solid (0.08 g, 44%), m.p. 130-132 ^{o}C; [HRMS: *m/z* calculated for (M-$C_9H_5O_3^*$)$^-$ 206.0229. Found 206.0223]; ν_{max}/cm^{-1} 1690 (C=O); δ_H (600 MHz; DMSO-d_6) 7.07 (1H, m, ArH), 7.35-7.40 (2H, m, ArH), 7.45 (1H, m, ArH), 7.50 (1H, m, ArH), 7.57 (1H, dt, J = 11.1, 2.2 Hz, ArH), 7.65 (1H, ddd, J = 8.5, 7.4, 1.6 Hz, ArH), (1H, dd, J = 7.9, 1.4 Hz, ArH) and 11.44 (1H, s, OH); δ_C (150 MHz; DMSO-d_6) 88.6 (C-3), 108.0 (d, $^2J_{CF}$ = 26.3 Hz, C-2'), 112.3 (d, $^2J_{CF}$ = 20.9 Hz, C-4'), 115.6 (q, J_{CF} = 287.5 Hz, CF_3), 116.4 and 116.6 (Ar-C), 116.9 (d, $^4J_{CF}$ = 3.0 Hz, C-6'), 123.6 and 124.2 (Ar-C), 130.8 (d, $^3J_{CF}$ = 9.3 Hz, C-5'), 132.6 (Ar-C), 138.0 (d, $^3J_{CF}$ = 10.8 Hz, C-1'), 151.8 (Ar-C), 154.7 (q, J_{CF} = 36.0 Hz, NC=O), 158.7 (C=O), 161.9 (d, $^3J_{CF}$ = 241.5 Hz, C-3') and 163.1 (C-4).

* Corresponding to loss of the 4-hydroxycoumarin moiety.

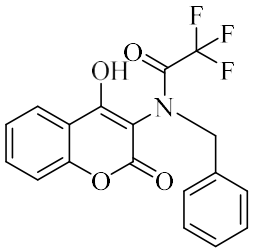
3-[N-(3,4-Difluorobenzyl)trifluoroacetamido]-4-hydroxycoumarin 164j



The procedure described for the synthesis of compound **164a** was followed using 3-[(3,4-difluorobenzyl)amino]-4-hydroxycoumarin **163p** (0.15 g, 0.5 mmol). Work-up afforded 3-[*N*-(3,4-difluorobenzyl)trifluoroacetamido]-4-hydroxycoumarin **164j** as a white solid (0.10 g, 50%), m.p. 133-135 ^{o}C; [HRMS: *m/z* calculated for (M-$C_9H_5O_3^*$)$^-$ 238.0291. Found 238.0285]; ν_{max}/cm^{-1} 1692 (C=O); δ_H (600 MHz; DMSO-d_6) 4.38 (2H, d, J = 6.0 Hz, CH_2), 7.13 (1H, m, ArH), 7.34 (1H, m, ArH), 7.37-7.44 (2H, m, ArH), 7.67 (1H, t, J = 8.5 Hz, ArH), 7.96 (1H, d, J = 9.3 Hz, ArH) and 10.01 (1H, s, OH); δ_C (150 MHz; DMSO-d_6) 41.7 (CH_2), 88.8 (C-3), 115.9 (q, J_{CF} = 286.5 Hz, CF_3), 116.2 and 116.4 (Ar-C), 116.6 (d, J_{CF} = 17.4 Hz, C-5'), 117.6 (d, J_{CF} = 17.4 Hz, C-2'), 123.5, 124.2 (m, C-6'), 124.3, 132.7 (Ar-C), 135.2 (dd, J_{CF} = 5.4, 3.9 Hz, C-1'), 148.7 (dd, J = 245.0, 12.5 Hz, C-4'), 149.3 (dd, J = 245.8, 12.8 Hz, C-3'), 151.7 (Ar-C), 156.5 (1C, q, J_{CF} = 36.0 Hz, NC=O), 158.6 (C=O) and 162.6 (C-4).

* Corresponding to loss of the 4-hydroxycoumarin moiety.

3-[N-(Benzyl)trifluoroacetamido]-4-hydroxycoumarin 164k

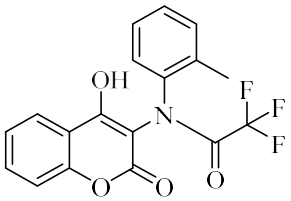


The procedure described for the synthesis of compound **164a** was followed using 3-(benzylamino)-4-hydroxycoumarin **163r** (0.13 g, 0.5 mmol). Work-up afforded 3-[*N*-(benzyl)trifluoroacetamido]-4-hydroxycoumarin **164b** as a white solid (0.11 g, 61%), m.p. 144-146 ^{o}C; [HRMS: *m/z* calculated for (M-$C_9H_5O_3^*$)$^-$ 202.0480. Found 202.0468]; ν_{max}/cm^{-1} 1691 (C=O); δ_H (600 MHz; DMSO-d_6) δ_H (600 MHz; $CDCl_3$) 4.40 (2H, d, J = 6.0 Hz, CH_2),

7.26-7.29 (3H, m, ArH), 7.33-7.41 (4H, m, ArH), 7.65 (1H, t, J = 8.6 Hz, ArH), 7.90 (1H, dd, J = 7.9, 1.5 Hz, ArH) and 10.03 (1H, s, OH); δ_C (150 MHz; $CDCl_3$) 42.6 (CH_2), 88.9 (C-3), 116.1 (q, J_{CF} = 286.5 Hz, CF_3), 116.4, 123.6, 124.3, 127.41, 127.43, 128.6, 132.7, 137.6 and 151.8 (Ar-C), 156.4 (q, J_{CF} = 36.0 Hz, NC=O), 158.7 (C=O) and 162.9 (C-4).

* Corresponding to loss of the 4-hydroxycoumarin moiety.

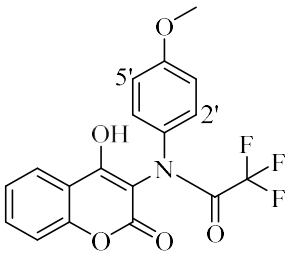
4-Hydroxy-3-[N-(o-tolyl)trifluoroacetamido]coumarin 164l



The procedure described for the synthesis of compound **164a** was followed using 4-hydroxy-3-[(o-tolyl)amino]coumarin **163l** (0.14 g, 0.5 mmol). Work-up afforded 4-hydroxy-3-[*N*-(o-tolyl)trifluoroacetamido]coumarin **164l** as a white solid (0.11 g, 61%), m.p. 137-139 ^oC; [HRMS: *m/z* calculated for $(M\text{-}C_9H_5O_3^*)^-$ 202.0480. Found 202.0470]; ν_{max}/cm^{-1} 1691 (C=O); δ_H (300 MHz; DMSO-d_6) 2.18 (3H, s, CH_3), 7.24-7.34 (4H, m, ArH), 7.35-7.43 (2H, m, ArH), 7.67 (1H, ddd, J = 8.5, 7.3, 1.6 Hz, ArH), 7.97 (1H, dd, J = 7.9, 1.5 Hz, ArH) and 10.95 (1H, s, OH); δ_C (75 MHz; DMSO-d_6) 17.3 (CH_3), 88.9 (C-3), 116.1 (q, J_{CF} = 286.5 Hz, CF_3), 116.4, 123.5, 124.3, 126.4, 126.7, 127.6, 130.7, 132.7, 133.3, 134.0 and 151.7 (Ar-C), 155.1 (q, J_{CF} = 36.3 Hz, NC=O), 158.6 (C=O) and 162.6 (C-4).

* Corresponding to loss of the 4-hydroxycoumarin moiety.

4-Hydroxy-3-[N-(4-methoxyphenyl)trifluoroacetamido]coumarin 164m

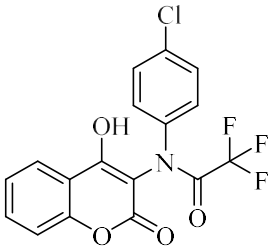


The procedure described for the synthesis of compound **164a** was followed using 4-hydroxy-3-[(4-methoxyphenyl)amino]coumarin **163j** (0.15 g, 0.5 mmol). Work-up afforded 4-hydroxy-3-[*N*-(4-methoxyphenyl)trifluoroacetamido]coumarin **164m** as a white solid (0.16 g, 84%), m.p. 106-108 ^oC; [HRMS: *m/z* calculated for $(M\text{-}C_9H_5O_3^*)^-$ 218.0429. Found

218.0424]; ν_{max}/cm^{-1} 1690 (C=O); δ_H (300 MHz; DMSO-d_6) 3.75 (3H, s, CH_3O), 6.96 (2H, d, J = 8.5 Hz, ArH), 7.34-7.44 (2H, m, ArH), 7.57 (2H, d, J = 8.5 Hz, ArH), 7.67 (1H, t, J = 7.8 Hz, ArH), 7.97 (1H, d, J = 7.9 Hz, ArH) and 11.09 (1H, s, 4-OH); δ_C (75 MHz; DMSO-d_6) 55.2 (CH_3O), 89.0 (C-3), 114.1 (Ar-C) 115.9 (q, J_{CF} = 286.5 Hz, CF_3), 116.0, 116.4, 122.7, 123.5, 124.3, 129.2, 132.7 and 151.7 (Ar-C), 154.2 (q, J_{CF} = 36.7 Hz, NC=O), 156. 9 (Ar-C), 158.6 (C=O) and 162.5 (C-4).

* Corresponding to loss of the 4-hydroxycoumarin moiety.

3-[N-(4-Chlorophenyl)trifluoroacetamido]-4-hydroxycoumarin 164n



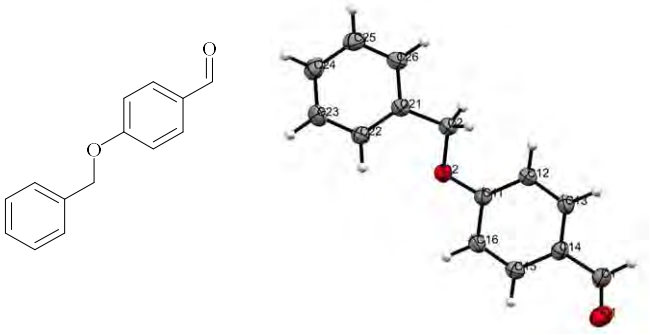
The procedure described for the synthesis of compound **164a** was followed using 3-[(4-chlorophenyl)amino]-4-hydroxycoumarin **163k** (0.14 g, 0.5 mmol). Work-up afforded 3-[*N*-(4-chlorophenyl)trifluoroacetamido]-4-hydroxycoumarin **164n** as a white solid (0.13 g, 68%), m.p. 116-118 ^oC; [HRMS: *m/z* calculated for $(M\text{-}C_9H_5O_3^*)^-$ 221.9934. Found 221.9925];ν_{max}/cm^{-1} 1695 (C=O); δ_H (300 MHz; DMSO-d_6) 7.35-7.50 (4H, m, ArH), 7.62-7.74 (3H, m, ArH), 7.96 (1H, d, J = 8.9 Hz, ArH) and 11.35 (1H, s, OH); δ_C (75 MHz; DMSO-d_6) 89.0 (C-3), 115.7 (q, J_{CF} = 288.0 Hz, CF_3), 116.0, 116.4, 122.6, 123.5, 124.3, 128.9, 129.5, 132.7, 135.3 and 151.7 (Ar-C), 154.5 (q, J_{CF} = 37.3 Hz, NC=O), 158.6 (C=O) and 162.5 (C-4).

* Corresponding to loss of the 4-hydroxycoumarin moiety.

3.13. X-RAY CRYSTAL STRUCTURE DATA

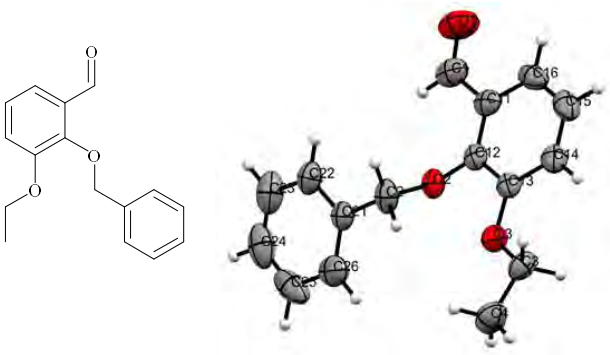
(As provided by Dr. Eric C. Hosten, Nelson Mandela University).

X-ray data for Compound 155a (CCDC number 1895105)



Formula, $C_{14}H_{12}O_2$; Formula weight, 212,25; Crystal system, orthorhombic; Space group Pna21 (No. 33); a, b, c [Angstrom] 11.5088(5) 12.9889(6) 7.2608(4); V [Ang**3] 1085.39(9); Z 4; D(calc) [g/cm**3] 1.299; Mu(MoKa) [/mm] 0.086; F(000) 448; Crystal size [mm] 0.19 x 0.25 x 0.62; Data Collection: Temperature (K) 200; Radiation [Angstrom] MoKa 0.71073; Theta min-max [Deg] 2.4, 28.3; Dataset -14: 15 ; -17: 17 ; -9: 9; Tot., Uniq. Data, R(int) 19779, 2698, 0.015; Observed data [I >2.0 sigma(I)] 2581; Refinement: Nref, Npar 2698, 145; R, wR2, S 0.0295, 0.0822, 1.05; w = ^2^(FO^2^)+(0.0474P)^2^+0.1324P] Where P=(FO^2^+2FC^2^)/3'; Max. and Av. Shift/Error 0.00, 0.00; Flack x -0.01(15); Min. and Max. Resd. Dens. [e/Ang^3] -0.16, 0.21.

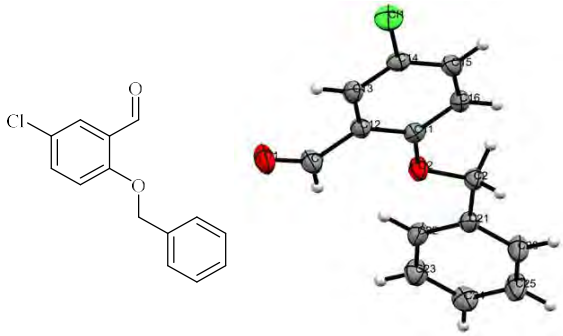
X-ray data for Compound 155b (CCDC number 1898081)



Formula, $C_{16}H_{16}O_3$; Formula weight, 256.29; Crystal system, monoclinic; Space group P21/c (No. 14); a, b, c [Angstrom] 15.3768(15) 4.5578(5) 19.4729(19); alpha, beta, gamma [deg] 90 99.697(5) 90; V [Ang**3] 1345.3(2); Z 4; D(calc) [g/cm**3] 1.265; Mu(MoKa) [/mm] 0.087; F(000) 544; Crystal size [mm] 0.06 x 0.09 x 0.63; Data Collection: Temperature (K)

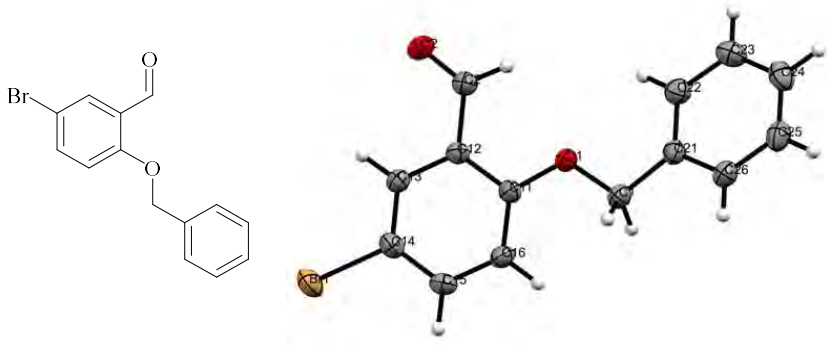
200; Radiation [Angstrom] MoKa 0.71073; Theta min-max [Deg] 2.1, 28.4; Dataset -15: 20 ; -4: 6 ; -25: 25; Tot., Uniq. Data, R(int) 12325, 3329, 0.064; Observed data [I >2.0 sigma(I)] 1762; Refinement: Nref, Npar 3329, 173; R, wR2, S 0.0625, 0.1769, 1.02; w = ^2^(FO^2^)+(0.0764P)^2^+0.2803P] Where P=(FO^2^+2FC^2^)/3'; Max. and Av. Shift/Error 0.00, 0.00; Min. and Max. Resd. Dens. [e/Ang^3] -0.22, 0.43.

X-ray data for Compound 155c (CCDC number 1898075)



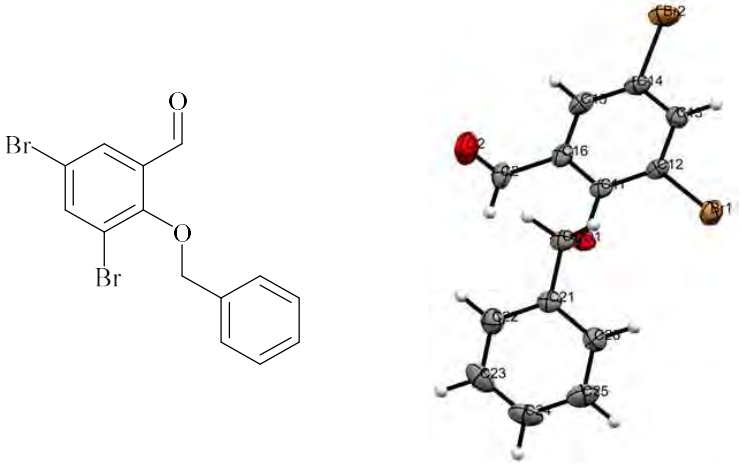
Formula, $C_{14}H_{11}ClO_2$; Formula weight, 246.68; Crystal system, monoclinic; Space group P21/n (No. 14); a, b, c [Angstrom] 4.9219(2) 16.3089(6) 14.7245(5); alpha, beta, gamma [deg] 90 99.171(2) 90; V [Ang**3] 1166.84(8); Z 4; D(calc) [g/cm**3] 1.404; Mu(MoKa) [/mm] 0.312; F(000) 512; Crystal size [mm] 0.10 x 0.13 x 0.50; Data Collection: Temperature (K) 200; Radiation [Angstrom] MoKa 0.71073; Theta min-max [Deg] 1.9, 28.3; Dataset -6: 6 ; -19: 21 ; -17: 19; Tot., Uniq. Data, R(int) 24526, 2917, 0.023; Observed data [I >2.0 sigma(I)] 2378; Refinement: Nref, Npar 2917, 154; R, wR2, S 0.0337, 0.0880, 1.05; w = ^2^(FO^2^)+(0.0398P)^2^+0.3701P] Where P=(FO^2^+2FC^2^)/3'; Max. and Av. Shift/Error 0.00, 0.00; Min. and Max. Resd. Dens. [e/Ang^3] -0.19, 0.32.

X-ray data for Compound 155d (CCDC number 1898076)



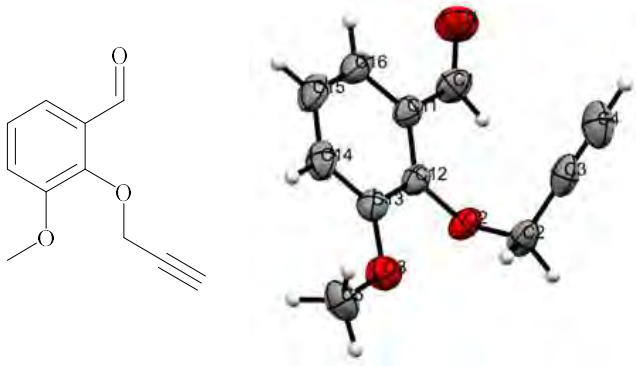
Formula, $C_{14}H_{11}BrO_2$; Formula weight, 291.14; Crystal system, monoclinic; Space group P21/n (No. 14); a, b, c [Angstrom] 7.316(4) 13.239(10) 12.300(8); alpha, beta, gamma [deg] 90 100.36(3) 90; V [Ang**3] 1171.9(13); Z 4; D(calc) [g/cm**3] 1.650; Mu(MoKa) [/mm] 3.493; F(000) 584; Crystal size [mm] 0.20 x 0.21 x 0.31; Data Collection: Temperature (K) 200; Radiation [Angstrom] MoKa 0.71073; Theta min-max [Deg] 2.3, 28.4; Dataset -9: 9 ; 0: 17 ; 0: 16; Tot., Uniq. Data, R(int) 2920, 2920, 0.000; Observed data [I >2.0 sigma(I)] 2393; Refinement: Nref, Npar 2920, 155; R, wR2, S 0.0263, 0.0618, 1.05; w = ^2^(FO^2^)+(0.0285P)^2^+0.2957P] Where P=(FO^2^+2FC^2^)/3'; Max. and Av. Shift/Error 0.00, 0.00; Min. and Max. Resd. Dens. [e/Ang^3] -0.33, 0.31.

X-ray data for Compound 155e (CCDC number 1898074)



Formula, $C_{14}H_{10}Br_2O_2$; Formula weight, 370.04; Crystal system, orthorhombic; Space group P212121 (No. 19); a, b, c [Angstrom] 4.0992(2) 17.1619(7) 18.9382(8); V [Ang**3] 1332.30(10); Z 4; D(calc) [g/cm**3] 1.845; Mu(MoKa) [/mm] 6.074; F(000) 720; Crystal size [mm] 0.04 x 0.04 x 0.60; Data Collection: Temperature (K) 200; Radiation [Angstrom] MoKa 0.71073; Theta min-max [Deg] 1.6, 28.3; Dataset -5: 5 ; -22: 22 ; -25: 25; Tot., Uniq. Data, R(int) 34685, 3341, 0.033; Observed data [I >2.0 sigma(I)] 3010; Refinement: Nref, Npar 3341, 164; R, wR2, S 0.0218, 0.0472, 1.04; w = ^2^(FO^2^)+(0.0229)^2^+0.2003P] Where P=(FO^2^+2FC^2^)/3'; Max. and Av. Shift/Error 0.00, 0.00; Flux x 0.015(11); Min. and Max. Resd. Dens. [e/Ang^3] -0.26, 0.31.

X-ray data for Compound 157c (CCDC number 1898078)



Formula, $C_{11}H_{10}O_3$; Formula weight, 190.19; Crystal system, triclinic; Space group P-1(No. 2); a, b, c [Angstrom] 7.7110(4) 7.9405(4) 9.1857(5); alpha, beta, gamma [deg] 65.896(2) 85.990(2) 70.155(2); V [Ang**3] 467.43(8); Z 2; D(calc) [g/cm**3] 1.312; Mu(MoKa) [/mm] 0.096; F(000) 200; Crystal size [mm] 0.23 x 0.38 x 0.61; Data Collection: Temperature (K) 200; Radiation [Angstrom] MoKa 0.71073; Theta min-max [Deg] 2.4, 28.4; Dataset -10: 10 ; -10: 10 ; -12: 12; Tot., Uniq. Data, R(int) 12649, 2396, 0.015; Observed data [I >2.0 sigma(I)] 1985; Refinement: Nref, Npar 2396, 128; R, wR2, S 0.0366, 0.1024, 1.04; w = ^2^(FO^2^)+(0.0443P)^2^+0.0905P] Where P=(FO^2^+2FC^2^)/3'; Max. and Av. Shift/Error 0.00, 0.00; Min. and Max. Resd. Dens. [e/Ang^3] 0.19, 0.18.

4. REFERENCES

(1) Saleta, V.-R.; Maria Joao, M.; Fernanda, B.; Eugenio, U.; Lourdes, S. *Curr. Top. Med. Chem.* **2015**, *15*, 1755.

(2) Cragg, G. M.; Newman, D. J. *Biochim. Biophys. Acta* **2013**, *1830*, 3670.

(3) Dev, S. *Environ. Health Perspect.* **1999**, *107*, 783.

(4) Veeresham, C. *J. Adv. Pharm. Technol. Res.* **2012**, *3*, 200.

(5) Berthi, W.; González, A.; Rios, A.; Blair, S.; Cogollo, Á.; Pabón, A. *Malar. J.* **2018**, *17*, 151.

(6) Eshetie, S.; Gizachew, M.; Dagnew, M.; Kumera, G.; Woldie, H.; Ambaw, F.; Tessema, B.; Moges, F. *BMC Infect. Dis.* **2017**, *17*, 219.

(7) Pigott, D. M.; Deshpande, A.; Letourneau, I.; Morozoff, C.; Reiner, R. C.; Kraemer, M. U. G.; Brent, S. E.; Bogoch, I. I.; Khan, K.; Biehl, M. H.; Burstein, R.; Earl, L.; Fullman, N.; Messina, J. P.; Mylne, A. Q. N.; Moyes, C. L.; Shearer, F. M.; Bhatt, S.; Brady, O. J.; Gething, P. W.; Weiss, D. J.; Tatem, A. J.; Caley, L.; De Groeve, T.; Vernaccini, L.; Golding, N.; Horby, P.; Kuhn, J. H.; Laney, S. J.; Ng, E.; Piot, P.; Sankoh, O.; Murray, C. J. L.; Hay, S. I. *Lancet, Lond.* **2017**, *390*, 2662.

(8) Zhao, S.; Stone, L.; Gao, D.; He, D. *PLoS Negl. Trop. Dis.* **2018**, *12*, e0006158.

(9) Maitland, K. *N. Engl. J. Med.* **2016**, *375*, 2416.

(10) Zumla, A.; Petersen, E.; Nyirenda, T.; Chakaya, J. *Int. J. Infect. Dis.* **2015**, *32*, 46.

(11) Musa, B. M.; Adamu, A. L.; Galadanci, N. A.; Zubayr, B.; Odoh, C. N.; Aliyu, M. H. *PloS one* **2017**, *12*, e0185105.

(12) Awah, P. K.; Boock, A. U.; Kum, K. A. *Pan Afr. Med. J.* **2015**, *22*, 18.

(13) Koch, T.; Oxford University Press, 2016.

(14) Jessica, R. S.; Elizabeth, E.; Jonathan, S. T.; Pierre, E. R.; Stuart, T. N. *Emerg. Infect Dis.* **2016**, *22*, 956.

(15) Garske, T.; Van Kerkhove, M. D.; Yactayo, S.; Ronveaux, O.; Lewis, R. F.; Staples, J. E.; Perea, W.; Ferguson, N. M.; for the Yellow Fever Expert, C. *PLoS Med.* **2014**, *11*, e1001638.

(16) Shearer, F. M.; Longbottom, J.; Browne, A. J.; Pigott, D. M.; Brady, O. J.; Kraemer, M. U. G.; Marinho, F.; Yactayo, S.; de Araújo, V. E. M.; da Nóbrega, A. A.; Fullman, N.; Ray, S. E.; Mosser, J. F.; Stanaway, J. D.; Lim, S. S.; Reiner, R. C., Jr.; Moyes, C. L.; Hay, S. I.; Golding, N. *Lancet Glob. Health*, *6*, e270.

(17) *MMWR Morb. Mortal. Wkly. Rep.* **1981**, *30*, 250.

(18) Barre-Sinoussi, F.; Chermann, J. C.; Rey, F.; Nugeyre, M. T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vezinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. *Science* **1983**, *220*, 868.

(19) Gallo, R. C.; Salahuddin, S. Z.; Popovic, M.; Shearer, G. M.; Kaplan, M.; Haynes, B. F.; Palker, T. J.; Redfield, R.; Oleske, J.; Safai, B.; et al. *Science* **1984**, *224*, 500.

(20) Sharp, P. M.; Hahn, B. H. *Cold Spring Harb. Perspect. Med.* **2011**, *1*, a006841.

(21) de Silva, T. I.; Cotten, M.; Rowland-Jones, S. L. *Trends Microbiol.* **2008**, *16*, 588.

(22) German Advisory Committee Blood, S. A. o. P. T. b. B. *Transfus. Med. Hemother.* **2016**, *43*, 203.

(23) Ramezani, A.; Hawley, R. G. *Curr. Protoc. Mol. Biol.* **2002**, *Chapter 16*, Unit 16.21.

(24) Sakuma, T.; Barry, Michael A.; Ikeda, Y. *Biochem. J.* **2012**, *443*, 603.

(25) Kostova, I.; Raleva, S.; Genova, P.; Argirova, R. *Bioinorg. Chem. Appl.* **2006**, *2006*.

(26) Nakayama, E.; Shioda, T. *Front. Microbiol.* **2012**, *3*.

(27) Chereshnev, V.; Bocharov, G.; Bazhan, S.; Bachmetyev, B.; Gainova, I.; Likhoshvai, V.; Argilaguet, J.; Martinez, J.; A Rump, J.; Mothe, B.; Brander, C.; Meyerhans, A. *Int. Rev. Immunol.* **2013**, *32*, 282.

(28) Barré-Sinoussi, F.; Ross, A. L.; Delfraissy, J.-F. *Nat. Rev. Microbiol.* **2013**, *11*, 877.

(29) Soliman, M.; Srikrishna, G.; Balagopal, A. *Curr. HIV/AIDS Rep.* **2017**, *14*, 101.

(30) Baxter, C.; Abdool Karim, S. *Afr. J. AIDS Res.* **2016**, *15*, 109.

(31) Cohen , M. S.; Shaw , G. M.; McMichael , A. J.; Haynes , B. F. *N. Engl. J. Med.* **2011**, *364*, 1943.

(32) Simon, V.; Ho, D. D.; Karim, Q. A. *Lancet* **2006**, *368*, 489.

(33) Maartens, G.; Celum, C.; Lewin, S. R. *Lancet* **2014**, *384*, 258.

(34) Fajardo-Ortiz, D.; Lopez-Cervantes, M.; Duran, L.; Dumontier, M.; Lara, M.; Ochoa, H.; Castano, V. M. *PloS one* **2017**, *12*, e0178293.

(35) Shanaube, K.; Bock, P. *Curr. HIV/AIDS Rep.* **2015**, *12*, 231.

(36) Williams, B. G.; Gouws, E.; Somse, P.; Mmelesi, M.; Lwamba, C.; Chikoko, T.; Fazito, E.; Turay, M.; Kiwango, E.; Chikukwa, P.; Damisoni, H.; Gboun, M. *Curr. HIV/AIDS Rep.* **2015**, *12*, 196.

(37) Brink, J. G.; Hassoulas, J. *Cardiovasc. J. Afr.* **2009**, *20*, 31.

(38) Lammers, J.; van Wijnbergen, S. J. G.; Willebrands, D. *HIV AIDS (Auckl)* **2013**, *5*, 283.

(39) Fideli, U. S.; Allen, S. A.; Musonda, R.; Trask, S.; Hahn, B. H.; Weiss, H.; Mulenga, J.; Kasolo, F.; Vermund, S. H.; Aldrovandi, G. M. *AIDS Res. Hum. Retrov.* **2001**, *17*, 901.

(40) Liu, H.; Su, Y.; Zhu, L.; Xing, J.; Wu, J.; Wang, N. *PloS one* **2014**, *9*, e111175.

(41) Desai, M.; Iyer, G.; Dikshit, R. K. *Indian J. Pharmacol.* **2012**, *44*, 288.

(42) De Clercq, E. *Int. J. Antimicrob. Agents* **2009**, *33*, 307.

(43) Ghosh, A. K.; Osswald, H. L.; Prato, G. *J. Med. Chem.* **2016**, *59*, 5172.

(44) Volberding, P. A. *Top. Antivir. Med.* **2017**, *25*, 17.

(45) Delva, W.; Abdool Karim, Q. *Curr. HIV/AIDS Rep.* **2014**, *11*, 99.

(46) Günthard, H. F.; Saag, M. S.; Benson, C. A.; et al. *JAMA* **2016**, *316*, 191.

(47) Baeten , J. M.; Donnell , D.; Ndase , P.; Mugo , N. R.; Campbell , J. D.; Wangisi , J.; Tappero , J. W.; Bukusi , E. A.; Cohen , C. R.; Katabira , E.; Ronald , A.; Tumwesigye , E.; Were , E.; Fife , K. H.; Kiarie , J.; Farquhar , C.; John-Stewart , G.; Kakia , A.; Odoyo , J.; Mucunguzi , A.; Nakku-Joloba , E.; Twesigye , R.; Ngure , K.; Apaka , C.; Tamooh , H.; Gabona , F.; Mujugira , A.; Panteleeff , D.; Thomas , K. K.; Kidoguchi , L.; Krows , M.; Revall , J.; Morrison , S.; Haugen , H.; Emmanuel-Ogier , M.; Ondrejcek , L.; Coombs , R. W.; Frenkel , L.; Hendrix , C.; Bumpus , N. N.; Bangsberg , D.; Haberer , J. E.; Stevens , W. S.; Lingappa , J. R.; Celum , C. *N. Engl. J. Med.* **2012**, *367*, 399.

(48) Kahle, E. M.; Sullivan, S.; Stephenson, R. *JMIR Public Health Surveill.* **2018**, *4*, e13.

(49) The, L. *Lancet* **2015**, *386*, 104.

(50) Nyaku, A. N.; Kelly, S. G.; Taiwo, B. O. *Curr. HIV/AIDS Rep.* **2017**, *14*, 63.

(51) Chen, L.; Jha, P.; Stirling, B.; Sgaier, S. K.; Daid, T.; Kaul, R.; Nagelkerke, N. *PloS One* **2007**, *2*, e1001.

(52) Glynn, J. R.; Biraro, S.; Weiss, H. A. *Aids* **2009**, *23*, 1595.

(53) Giannou, F. K.; Tsiara, C. G.; Nikolopoulos, G. K.; Talias, M.; Benetou, V.; Kantzanou, M.; Bonovas, S.; Hatzakis, A. *Expert Rev. Pharmacoecon. Outcomes Res.* **2016**, *16*, 489.

(54) Frydas, A.; Leptokaridou, E.; Partsanakis, E.; Kourklidou, M.; Sarantopoulos, A. *J Autoimmune Disord.* **2018**, *4*, 56.

(55) Coates, T. J.; Richter, L.; Caceres, C. *Lancet* **2008**, *372*, 669.

(56) Mayaphi, S. H.; Martin, D. J.; Olorunju, S. A. S.; Williams, B. G.; Quinn, T. C.; Stoltz, A. C. *PloS one* **2018**, *13*, e0192357.

(57) Goldfarb, N. E.; Dunn, B. M. In *Handbook of Proteolytic Enzymes*; Salvesen, G., Ed.; Academic Press, **2013**.

(58) Arts, E. J.; Hazuda, D. J. *Cold Spring Harb. Perspect. Med.* **2012**, *2*, a007161.

(59) Bai, X.; Yang, Z.; Zhu, M.; Dong, B.; Zhou, L.; Zhang, G.; Wang, J.; Wang, Y. *Eur. J. Med. Chem.* **2017**, *137*, 30.

(60) Cihlar, T.; Fordyce, M. *Curr. Opin. Virol.* **2016**, *18*, 50.

(61) Daar, E. S. *F1000Research* **2017**, *6*, 759.

(62) Bhatti, A. B.; Usman, M.; Kandi, V. *Cureus* **2016**, *8*, e515.

(63) Liu, C.; Ma, X.; Liu, B.; Chen, C.; Zhang, H. *BMC Med.* **2015**, *13*, 284.

(64) Hosseinipour, M. C.; Gupta, R. K.; Van Zyl, G.; Eron, J. J.; Nachega, J. B. *J. Infect. Dis.* **2013**, *207 Suppl. 2*, S49.

(65) Spreen, W. R.; Margolis, D. A.; Pottage, J. C. *Curr. Opin. HIV AIDS* **2013**, *8*, 565.

(66) Margolis, D. A.; Boffito, M. *Curr. Opin. HIV AIDS* **2015**, *10*, 246.

(67) Mobula, L.; Barnhart, M.; Malati, C.; Rakhmanina, N.; Minior, T.; Amzel, A.; Ferris, R.; Phelps, B. *J. AIDS Clin. Res.* **2015**, *6*, 2.

(68) Mitsuya, H.; Popovic, M.; Yarchoan, R.; Matsushita, S.; Gallo, R.; Broder, S. *Science* **1984**, *226*, 172.

(69) Gupta, S. *Trends Pharmacol. Sci.***1986**, *7*, 393.

(70) Zhan, P.; Pannecouque, C.; De Clercq, E.; Liu, X. *J. Med. Chem.* **2016**, *59*, 2849.

(71) Bernardino, J. I.; Arribas, J. R. In *Infectious Diseases (Fourth Edition)*; Powderly, W. G., Opal, S. M., Eds.; Elsevier, **2017**.

(72) de Béthune, M.-P. *Antiviral Res.***2010**, *85*, 75.

(73) Galilee, M.; Alian, A. *PLoS Pathog*. **2018**, *14*, e1006849.

(74) Das, K.; Arnold, E. *Curr. Opin. Virol.* **2013**, *3*, 111.

(75) Lv, Z.; Chu, Y.; Wang, Y. *HIV AIDS (Auckl)* **2015**, *7*, 95.

(76) Meher, B. R.; Patel, S. In *Adv. Protein. Chem. Struct. Biol.*; Karabencheva-Christova, T., Ed.; Academic Press, **2013**; Vol. 92.

(77) Siberry, G. K.; Hazra, R. In *Principles and Practice of Pediatric Infectious Diseases (Fourth Edition)*; Content Repository Only!: London, **2012**.

(78) Papp, E.; Mohammadi, H.; Loutfy, M. R.; Yudin, M. H.; Murphy, K. E.; Walmsley, S. L.; Shah, R.; MacGillivray, J.; Silverman, M.; Serghides, L. *J. Infect. Dis.* **2015**, *211*, 10.

(79) Summa, V.; Petrocchi, A.; Bonelli, F.; Crescenzi, B.; Donghi, M.; Ferrara, M.; Fiore, F.; Gardelli, C.; Gonzalez Paz, O.; Hazuda, D. J.; Jones, P.; Kinzel, O.; Laufer, R.;

Monteagudo, E.; Muraglia, E.; Nizi, E.; Orvieto, F.; Pace, P.; Pescatore, G.; Scarpelli, R.; Stillmock, K.; Witmer, M. V.; Rowley, M. *J. Med. Chem.* **2008**, *51*, 5843.

(80) Dąbrowska, M. M.; Wiercińska-Drapało, A. *HIV AIDS Rev.***2007**, *6*, 10.

(81) Métifiot, M.; Johnson, B. C.; Kiselev, E.; Marler, L.; Zhao, X. Z.; Burke, T. R.; Marchand, C.; Hughes, S. H.; Pommier, Y. *Nucleic Acids Res.* **2016**, *44*, 6896.

(82) Nishana, M.; Nilavar, N. M.; Kumari, R.; Pandey, M.; Raghavan, S. C. *Cell Death Dis.* **2017**, *8*, e2852.

(83) Han, Y.-S.; Mesplède, T.; Wainberg, M. A. *Infect. Genet. Evol.* **2016**, *46*, 286.

(84) Kuritzkes, D. R. *J. Infect. Dis.* **2018**, *218*, 673.

(85) Dang, Z.; Xie, H.; Zhu, L.; Zhang, Q.; Li, Z.; Huang, L.; Chen, C. H. *ACS Med. Chem. Lett.* **2017**, *8*, 1199.

(86) Fengyan, X.; Edward, P. A.; Liyu, L.; Yingchun, H.; Juan, Y.; Corenna, K.-W.; Qingshang, Z.; Jihan, H.; Kun, W. *Curr. Drug Metab.* **2017**, *18*, 769.

(87) El Safadi, Y.; Vivet-Boudou, V.; Marquet, R. *Appl. Microbiol. Biotechnol.*, **2007**, *75*, 723.

(88) Padberg, S. In *Drugs During Pregnancy and Lactation (Third Edition)*; Academic Press: San Diego, **2015**.

(89) Berkhout, B.; Eggink, D.; Sanders, R. W. *Curr. Opin. Virol.* **2012**, *2*, 50.

(90) Ahn, K. W.; Root, M. J. *J. Biol. Chem.* **2017**, *292*, 16498.

(91) Aoife, L. *Curr. Pharm. Des.* **2004**, *10*, 3797.

(92) Egan, D.; O'Kennedy, R.; Moran, E.; Cox, D.; Prosser, E.; Thornes, R. D. *Drug Metab. Rev.***1990**, *22*, 503.

(93) Abernethy, J. L. *J. Chem. Educ.* **1969**, *46*, 561.

(94) Singh, D.; Pathak, D. P. *Int. J. Pharm. Sci. Res.* **2016**, *7*, 482.

(95) Vekariya, R. H.; Patel, H. D. *Synth. Commun.* **2014**, *44*, 2756.

(96) Dandriyal, J.; Singla, R.; Kumar, M.; Jaitak, V. *Eur. J. Med. Chem.* **2016**, *119*, 141.

(97) Costa, T. M.; Tavares, L. B. B.; de Oliveira, D. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 6571.

(98) Revankar, H. M.; Bukhari, S. N. A.; Kumar, G. B.; Qin, H.-L. *Bioorg. Chem.* **2017**, *71*, 146.

(99) Venugopala, K. N.; Rashmi, V.; Odhav, B. *Biomed. Res. Int.* **2013**, *2013*, 14.

(100) Hamed, I.; Özogul, F.; Özogul, Y.; Regenstein, J. M. *Compr. Rev. Food Sci. Food Saf.* **2015**, *14*, 446.

(101) Luo, X.; Wang, R.; Wang, L.; Wang, Y.; Chen, Z. *Food Control* **2013**, *31*, 331.

(102) Jameel, E.; Umar, T.; Kumar, J.; Hoda, N. *Chem. Biol. Drug Des.* **2016**, *87*, 21.

(103) Stefanachi, A.; Leonetti, F.; Pisani, L.; Catto, M.; Carotti, A. *Molecules* **2018**, *23*, 250.

(104) Xin-Mei, P.; Guri, L. V. D.; Cheng- He, Z. *Curr. Pharm. Des.* **2013**, *19*, 3884.

(105) Bhatia, M. S.; Ingale, K. B.; Choudhari, P. B.; Bhatia, N. M.; Sawant, R. L. *Bioorg. Med. Chem.* **2009**, *17*, 1654.

(106) Karatzas, N. B. *Hellenic J. Cardiol.* **2014**, *55*, 89.

(107) Ufer, M. *Clin. Pharmacokinet.* **2005**, *44*, 1227.

(108) Antonio, G.-O.; Suarez-Gea, M. L.; Gonzalo, C.-R.; Ramon, L.; Eduardo, R.; Carmen, P.-H.; Ana Isabel, T.-F.; Emilio, V.-C. *Curr. Drug Discov. Technol.* **2012**, *9*, 83.

(109) Verhoef, T. I.; Redekop, W. K.; Daly, A. K.; van Schie, R. M. F.; de Boer, A.; Maitland-van der Zee, A.-H. *Br. J. Clin. Pharmacol.* **2014**, *77*, 626.

(110) Riveiro, M. E.; De Kimpe, N.; Moglioni, A.; Vazquez, R.; Monczor, F.; Shayo, C.; Davio, C. *Curr. Med. Chem.* **2010**, *17*, 1325.

(111) Daly, A. K. *Arch. Toxicol.* **2013**, *87*, 407.

(112) Pieper, P. G.; Balci, A.; Van Dijk, A. P. *Neth. Heart J.* **2008**, *16*, 406.

(113) Penning-Van Beest, F. J. A.; Koerselman, J.; Herings, R. M. C. *J. Thromb. Haemost.* **2008**, *6*, 284.

(114) Mekaj, Y. H.; Mekaj, A. Y.; Duci, S. B.; Miftari, E. I. *Ther. Clin. Risk. Manag.* **2015**, *11*, 967.

(115) Schalekamp, T.; Klungel, J. H.; Souverein, P. C.; de Boer, A. *Arch. Intern. Med.* **2008**, *168*, 180.

(116) Punchard, N. A.; Whelan, C. J.; Adcock, I. *J. Inflamm.* **2004**, *1*, 1.

(117) Gallo, J.; Raska, M.; Kriegova, E.; Goodman, S. B. *J. Orthop. Translat.* **2017**, *10*, 52.

(118) Chovatiya, R.; Medzhitov, R. *Mol. Cell* **2014**, *54*, 281.

(119) Kirsch, G.; Abdelwahab, A.; Chaimbault, P. *Molecules* **2016**, *21*, 1322.

(120) Dinarello, C. A. *Cell* **2010**, *140*, 935.

(121) Medzhitov, R. *Nature* **2008**, *454,* 428.

(122) Bansal, Y.; Sethi, P.; Bansal, G. *Med. Chem. Res.* **2013**, *22*, 3049.

(123) Witaicenis, A.; Seito, L. N.; Di Stasi, L. C. *Chem. Biol. Interact.* **2010**, *186*, 211.

(124) Witaicenis, A.; Seito, L. N.; da Silveira Chagas, A.; de Almeida, L. D.; Luchini, A. C.; Rodrigues-Orsi, P.; Cestari, S. H.; Di Stasi, L. C. *Phytomedicine* **2014**, *21*, 240.

(125) Halliwell, B.; Aeschbach, R.; Löliger, J.; Aruoma, O. I. *Food Chem. Toxicol.* **1995**, *33*, 601.

(126) Garrido, G.; González, D.; Delporte, C.; Backhouse, N.; Quintero, G.; Núñez-Sellés, A. J.; Morales, M. A. *Phytother. Res.* **2001**, *15*, 18.

(127) Melagraki, G.; Afantitis, A.; Igglessi-Markopoulou, O.; Detsi, A.; Koufaki, M.; Kontogiorgis, C.; Hadjipavlou-Litina, D. J. *Eur. J. Med. Chem.* **2009**, *44*, 3020.

(128) Maltese, A.; Maugeri, F.; Ward, K. W.; Bucolo, C. *Biomed. Chromatogr.* **2007**, *21*, 351.

(129) Goth, A. *Science* **1945**, *101*, 383.

(130) Hoeksema, H.; Johnson, J. L.; Hinman, J. W. *J. Am. Chem. Soc.* **1955**, *77*, 6710.

(131) Smyth, T.; Ramachandran, V. N.; Smyth, W. F. *Int. J. Antimicrob. Agents*, *33*, 421.

(132) Yang, L.; Ding, W.; Xu, Y.; Wu, D.; Li, S.; Chen, J.; Guo, B. *Molecules* **2016**, *21*, 468.

(133) Shi, Y.; Zhou, C. H. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 956.

(134) Arshad, A.; Osman, H.; Bagley, M. C.; Lam, C. K.; Mohamad, S.; Zahariluddin, A. S. *Eur J. Med. Chem.* **2011**, *46*, 3788.

(135) Karan, G.; Ajay, S.; Ridhima, A.; Rohit, S.; Girish, K. G.; Anil, K. S. *Anticancer Agents. Med. Chem.* **2016**, *16*, 1.

(136) Miri, R.; Nejati, M.; Saso, L.; Khakdan, F.; Parshad, B.; Mathur, D.; Parmar, V. S.; Bracke, M. E.; Prasad, A. K.; Sharma, S. K.; Firuzi, O. *Pharm. Biol.* **2016**, *54*, 105.

(137) Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57.

(138) Hanahan, D.; Weinberg, Robert A. *Cell* **2011**, *144*, 646.

(139) Cho, J. H.; Shin, J.; Cho, J.; Choi, Y. H.; Shim, J.; Chae, J. *Int. J. Oncol.* **2015**, *46*, 265.

(140) (a) Lee, S.-Y.; Lim, T.-G.; Chen, H.; Jung, S. K.; Lee, H.-J.; Lee, M.-H.; Kim, D. J.; Shin, A.; Lee, K. W.; Bode, A. M.; Surh, Y.-J.; Dong, Z. *Cancer Prev. Res. (Phila)* **2013**, *6*, 10.1158/1940 (b) Kim, A. D.; Han, X.; Piao, M. J.; Hewage, S. R.; Hyun, C. L.; Cho, S. J.; Hyun, J. W. *Environ. Toxicol. Pharmacol.* **2015**, *39*, 982.

(141) Turkekul, K.; Colpan, R. D.; Baykul, T.; Ozdemir, M. D.; Erdogan, S. *J. Cancer Prev.* **2018**, *23*, 10.

(142) Suhaimi, S. A.; Hong, S. L.; Abdul Malek, S. N. *Pharmacogn. Mag.* **2017**, *13*, S179.

(143) Donnelly, A. C.; Mays, J. R.; Burlison, J. A.; Nelson, J. T.; Vielhauer, G.; Holzbeierlein, J.; Blagg, B. S. J. *J. Org. Chem.* **2008**, *73*, 8901.

(144) Nasr, T.; Bondock, S.; Youns, M. *Eur. J. Med. Chem.* **2014**, *76*, 539.

(145) Thakur, A.; Singla, R.; Jaitak, V. *Eur. J. Med. Chem.* **2015**, *101*, 476.

(146) Manjinder, K.; Swarandeep, K.; Sonali, S.; Yogita, B.; Gulshan, B. *Anticancer Agents Med. Chem.* **2015**, *15*, 1032.

(147) Emami, S.; Dadashpour, S. *Eur. J. Med. Chem.* **2015**, *102*, 611.

(148) Reddy, N. S.; Gumireddy, K.; Mallireddigari, M. R.; Cosenza, S. C.; Venkatapuram, P.; Bell, S. C.; Reddy, E. P.; Reddy, M. V. R. *Bioorg. Med. Chem.* **2005**, *13*, 3141.

(149) Jiménez-Orozco, F. A.; Rosales, A. A. R.; Vega-López, A.; Domínguez-López, M. L.; García-Mondragón, M. J.; Maldonado-Espinoza, A.; Lemini, C.; Mendoza-Patiño, N.; Mandoki, J. J. *Eur. J. Pharmacol.* **2011**, *668*, 35.

(150) Hassan, M. Z.; Osman, H.; Ali, M. A.; Ahsan, M. J. *Eur. J. Med. Chem.* **2016**, *123*, 236.

(151) Lozano, R.; Naghavi, M.; Foreman, K.; Lim, S.; Shibuya, K.; Aboyans, V.; Abraham, J.; Adair, T.; Aggarwal, R.; Ahn, S. Y.; AlMazroa, M. A.; Alvarado, M.; Anderson, H. R.; Anderson, L. M.; Andrews, K. G.; Atkinson, C.; Baddour, L. M.; Barker-Collo, S.; Bartels, D. H.; Bell, M. L.; Benjamin, E. J.; Bennett, D.; Bhalla, K.; Bikbov, B.; Abdulhak, A. B.; Birbeck, G.; Blyth, F.; Bolliger, I.; Boufous, S.; Bucello, C.; Burch, M.; Burney, P.; Carapetis, J.; Chen, H.; Chou, D.; Chugh, S. S.; Coffeng, L. E.; Colan, S. D.; Colquhoun, S.; Colson, K. E.; Condon, J.; Connor, M. D.; Cooper, L. T.; Corriere, M.; Cortinovis, M.; de Vaccaro, K. C.; Couser, W.; Cowie, B. C.; Criqui, M. H.; Cross, M.; Dabhadkar, K. C.; Dahodwala, N.; De Leo, D.; Degenhardt, L.; Delossantos, A.; Denenberg, J.; Des Jarlais, D. C.; Dharmaratne, S. D.; Dorsey, E. R.; Driscoll, T.; Duber, H.; Ebel, B.; Erwin, P. J.; Espindola, P.; Ezzati, M.; Feigin, V.; Flaxman, A. D.; Forouzanfar, M. H.; Fowkes, F. G. R.; Franklin, R.; Fransen, M.; Freeman, M. K.; Gabriel, S. E.; Gakidou, E.; Gaspari, F.; Gillum, R. F.; Gonzalez-Medina, D.; Halasa, Y. A.; Haring, D.; Harrison, J. E.; Havmoeller, R.; Hay, R. J.; Hoen, B.; Hotez, P. J.; Hoy, D.; Jacobsen, K. H.; James, S. L.; Jasrasaria, R.; Jayaraman, S.; Johns, N.; Karthikeyan, G.; Kassebaum, N.; Keren, A.; Khoo, J.-P.; Knowlton, L. M.; Kobusingye, O.; Koranteng, A.; Krishnamurthi, R.; Lipnick, M.; Lipshultz, S. E. *Lancet* **2012**, *380*, 2095.

(152) King, D. H. *J. Am. Acad. Dermatol.* **1988**, *18*, 176.

(153) Neyts, J.; Clercq, E. D.; Singha, R.; Chang, Y. H.; Das, A. R.; Chakraborty, S. K.; Hong, S. C.; Tsay, S.-C.; Hsu, M.-H.; Hwu, J. R. *J. Med. Chem.* **2009**, *52*, 1486.

(154) Penta, S. In *Advances in Structure and Activity Relationship of Coumarin Derivatives*; Academic Press: Boston, **2016**.

(155) Torres, F. C.; Brucker, N.; Andrade, S. F.; Kawano, D. F.; Garcia, S. C.; von Poser, G. L.; Eifler-Lima, V. L. *Curr. Top. Med. Chem.* **2014**, *14*, 2600.

(156) Thaisrivongs, S.; Tomich, P. K.; Watenpaugh, K. D.; Chong, K.-T.; Howe, W. J.; Yang, C.-P.; Strohbach, J. W.; Turner, S. R.; McGrath, J. P. *J. Med. Chem.* **1994**, *37*, 3200.

(157) Cooper, D. A.; Cordery, D. V.; Zajdenverg, R.; Ruxrungtham, K.; Arasteh, K.; Bergmann, F.; Neto, J. L.; Scherer, J.; Chaves, R. L.; Robinson, P. *PloS one* **2016**, *11*, e0144917.

(158) Završnik, D.; Muratović, S.; Makuc, D.; Plavec, J.; Cetina, M.; Nagl, A.; Clercq, E. D.; Balzarini, J.; Mintas, M. *Molecules* **2011**, *16*, 6023.

(159) Lin, P.-H.; Ke, Y.-Y.; Su, C.-T.; Shiao, H.-Y.; Hsieh, H.-P.; Chao, Y.-K.; Lee, C.-N.; Kao, C.-L.; Chao, Y.-S.; Chang, S.-Y. *J. Virol.* **2011**, *85*, 9114.

(160) Shih, S.-R.; Horng, J.-T.; Poon, L.; Chen, T.-C.; Yeh, J.-Y.; Hsieh, H.-P.; Tseng, S.-N.; Chiang, C.; Li, W.-L.; Chao, Y.-S.; T-A Hsu, J. *J. Antimicrob. Chemother.* **2009**, *65*, 63.

(161) Lin, Y.; Wang, Q.; Gu, Q.; Zhang, H.; Jiang, C.; Hu, J.; Wang, Y.; Yan, Y.; Xu, J. *J. Nat. Prod.* **2017**, *80*, 53.

(162) Xu, B.; Wang, L.; Gonzalez-Molleda, L.; Wang, Y.; Xu, J.; Yuan, Y. *Antimicrob. Agents Chemother.* **2014**, *58*, 563.

(163) Cadierno, V. In *Green Synthetic Approaches for Biologically Relevant Heterocycles*; Elsevier: Boston, **2015**.

(164) Zhang, Z.-R.; Leung, W. N.; Cheung, H. Y.; Chan, C. W. *Evid. Based Complement. Alternat. Med.* **2015**, *2015*, 10.

(165) Hwu, J. R.; Kapoor, M.; Tsay, S.-C.; Lin, C.-C.; Hwang, K. C.; Horng, J.-C.; Chen, I. C.; Shieh, F.-K.; Leyssen, P.; Neyts, J. *Antiviral Res.***2015**, *118*, 103.

(166) Pohjala, L.; Utt, A.; Varjak, M.; Lulla, A.; Merits, A.; Ahola, T.; Tammela, P. *PloS one* **2011**, *6*, e28923.

(167) Yu, D.; Suzuki, M.; Xie, L.; Morris-Natschke, S. L.; Lee, K.-H. *Med. Res. Rev.* **2003**, *23*, 322.

(168) Irena, K. *Curr. HIV Res.* **2006**, *4*, 347.

(169) Mazumder, A.; Wang, S.; Neamati, N.; Nicklaus, M.; Sunder, S.; Chen, J.; Milne, G. W. A.; Rice, W. G.; Burke, T. R.; Pommier, Y. *J. Med. Chem.* **1996**, *39*, 2472.

(170) Kostova, I.; Mojzis, J. *Fut. HIV Ther.* **2007**, *1*, 315.

(171) Zhao, H.; Neamati, N.; Hong, H.; Mazumder, A.; Wang, S.; Sunder, S.; Milne, G. W. A.; Pommier, Y.; Burke, T. R. *J. Med. Chem.* **1997**, *40*, 242.

(172) Su, C.-X.; Mouscadet, J.-F.; Chiang, C.-C.; Tsai, H.-J.; Hsu, L.-Y. *Chem. Pharm. Bull.* **2006**, *54*, 682.

(173) Dayam, R.; Gundla, R.; Al-Mawsawi, L. Q.; Neamati, N. *Med. Res. Rev.* **2008**, *28*, 118.

(174) Chiang, C.-C.; Mouscadet, J.-F.; ccedil; ois; Tsai, H.-J.; Liu, C.-T.; Hsu, L.-Y. *Chem. Pharm. Bull.* **2007**, *55*, 1740.

(175) Ramkumar, K.; Serrao, E.; Odde, S.; Neamati, N. *Med. Res. Rev.* **2010**, *30*, 890.

(176) Veselinović, J.; Veselinović, A.; Toropov, A.; Toropova, A.; Damnjanović, I.; Nikolić, G. In *Acta Facultatis Medicae Naissensis*, **2014**; *31*, 95.

(177) Jain, S. V.; Sonawane, L. V.; Patil, R. R.; Bari, S. B. *Med. Chem. Res.* **2012**, *21*, 165.

(178) Patil, R. B.; Sawant, S. D. *Med. Chem. Res.* **2015**, *24*, 3062.

(179) Wang, L.; Ma, T.; Liu, G. Recent Progress in Calophyllum Coumarins as Potent Anti-HIV Agents. In *Medicinal Chemistry of Bioactive Natural Products*; Liang, X. and Fang, W., Ed; John Wiley & Sons, Inc., **2006,** 325.

(180) Patil, A. D.; Freyer, A. J.; Eggleston, D. S.; Haltiwanger, R. C.; Bean, M. F.; Taylor, P. B.; Caranfa, M. J.; Breen, A. L.; Bartus, H. R. *J. Med. Chem.* **1993**, *36*, 4131.

(181) Kashman, Y.; Gustafson, K. R.; Fuller, R. W.; Cardellina, J. H.; McMahon, J. B.; Currens, M. J.; Buckheit, R. W.; Hughes, S. H.; Cragg, G. M.; Boyd, M. R. *J. Med. Chem.* **1992**, *35*, 2735.

(182) Currens, M. J.; Gulakowski, R. J.; Mariner, J. M.; Moran, R. A.; Buckheit, R. W., Jr.; Gustafson, K. R.; McMahon, J. B.; Boyd, M. R. *J. Pharmacol. Exp. Ther.***1996**, *279*, 645.

(183) Xue, H.; Lu, X.; Zheng, P.; Liu, L.; Han, C.; Hu, J.; Liu, Z.; Ma, T.; Li, Y.; Wang, L.; Chen, Z.; Liu, G. *J. Med. Chem.* **2010**, *53*, 1397.

(184) Dighe, N. S.; Patton, S.; Dengale, S. S.; Musmade, D. S.; Shelar, M.; Tambe, V.; Hole, M. B. *Arc. Apl. Sci. Res.* **2010**, *2*, 65.

(185) Yu, D.; Suzuki, M.; Xie, L.; Morris-Natschke, S. L.; Lee, K. H. *Med. Res. Rev.* **2003**, *23*, 322.

(186) Lee, T. T.-Y.; Kashiwada, Y.; Huang, L.; Snider, J.; Cosentino, M.; Lee, K.-H. *Bioorg. Med. Chem.* **1994**, *2*, 1051.

(187) Huang, L.; Kashiwada, Y.; Cosentino, L. M.; Fan, S.; Chen, C.-H.; McPhail, A. T.; Fujioka, T.; Mihashi, K.; Lee, K.-H. *J. Med. Chem.* **1994**, *37*, 3947.

(188) Huang, L.; Yuan, X.; Yu, D.; Lee, K. H.; Ho Chen, C. *Virology* **2005**, *332*, 623.

(189) Yu, D.; Morris-Natschke, S. L.; Lee, K.-H. *Med. Res. Rev.* **2007**, *27*, 108.

(190) Liu, H.; Xu, S.; Cheng, M.; Chen, Y.; Xia, P.; Qian, K.; Xia, Y.; Yang, Z.-Y.; Chen, C.-H.; Morris-Natschke, S. L.; Lee, K.-H. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5831.

(191) Tang, J.; Qian, K.; Zhang, B.-N.; Chen, Y.; Xia, P.; Yu, D.; Xia, Y.; Yang, Z.-Y.; Chen, C.-H.; Morris-Natschke, S. L.; Lee, K.-H. *Bioorg. Med. Chem.* **2010**, *18*, 4363.

(192) Stanchev, S.; Jensen, F.; Hinkov, A.; Atanasov, V.; Genova-Kalou, P.; Argirova, R.; Manolov, I. *ISRN Pharm.* **2011**, *2011*, 137637.

(193) Bourinbaiar, A. S.; Tan, X.; Nagorny, R. *AIDS* **1993**, *7*, 129.

(194) Thaisrivongs, S.; Romero, D. L.; Tommasi, R. A.; Janakiraman, M. N.; Strohbach, J. W.; Turner, S. R.; Biles, C.; Morge, R. R.; Johnson, P. D.; Aristoff, P. A.; Tomich, P. K.; Lynn, J. C.; Horng, M.-M.; Chong, K.-T.; Hinshaw, R. R.; Howe, W. J.; Finzel, B. C.; Watenpaugh, K. D. *J. Med. Chem.* **1996**, *39*, 4630.

(195) Lunney, E. A.; Hagen, S. E.; Domagala, J. M.; Humblet, C.; Kosinski, J.; Tait, B. D.; Warmus, J. S.; Wilson, M.; Ferguson, D. *J. Med. Chem.* **1994**, *37*, 2664.

(196) Kirkiacharian, S.; Thuy, D. T.; Sicsic, S.; Bakhchinian, R.; Kurkjian, R.; Tonnaire, T. *Farmaco* **2002**, *57*, 703.

(197) Wang, S.; Milne, G. W. A.; Yan, X.; Posey, I. J.; Nicklaus, M. C.; Graham, L.; Rice, W. G. *J. Med. Chem.* **1996**, *39*, 2047.

(198) Sancho, R.; Márquez, N.; Gómez-Gonzalo, M.; Calzado, M. A.; Bettoni, G.; Coiras, M. T.; Alcamí, J.; López-Cabrera, M.; Appendino, G.; Muñoz, E. *J. Biol. Chem.* **2004**, *279*, 37349.

(199) Uchiumi, F.; Hatano, T.; Ito, H.; Yoshida, T.; Tanuma, S.-i. *Antiviral Res.***2003**, *58*, 89.

(200) Xian, L.; Hanxian, Z.; Pengfei, W.; Lu, L.; Lin, L.; Pinyi, Z.; Yuanzhe, F.; Panpan, L.; Huanzhang, Z. *Curr. HIV Res.* **2016**, *14*, 484.

(201) Márquez, N.; Sancho, R.; Bedoya, L. M.; Alcamí, J.; López-Pérez, J. L.; Feliciano, A. S.; Fiebich, B. L.; Muñoz, E. *Antiviral Res.***2005**, *66*, 137.

(202) Maneerat, W.; Prawat, U.; Saewan, N.; Laphookhieo, S. *J. Braz. Chem. Soc.* 2010, *21*, 665.

(203) Elhafez, O. M. A.; Khrisy, E. E. D. A. M. E.; Badria, F.; Fathy, A. E. D. M. *Arch. Pharm. Res.* **2003**, *26*, 686.

(204) Reddy, M. V. R.; Rao, M. R.; Rhodes, D.; Hansen, M. S. T.; Rubins, K.; Bushman, F. D.; Venkateswarlu, Y.; Faulkner, D. J. *J. Med. Chem.* **1999**, *42*, 1901.

(205) Olmedo, D.; Sancho, R.; Bedoya, L. M.; López-Pérez, J. L.; del Olmo, E.; Muñoz, E.; Alcamí, J.; Gupta, M. P.; San Feliciano, A. *Molecules* **2012**, *17*.

(206) Kudo, E.; Taura, M.; Matsuda, K.; Shimamoto, M.; Kariya, R.; Goto, H.; Hattori, S.; Kimura, S.; Okada, S. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 606.

(207) Matsuda, K.; Hattori, S.; Kariya, R.; Komizu, Y.; Kudo, E.; Goto, H.; Taura, M.; Ueoka, R.; Kimura, S.; Okada, S. *Biochem. Biophys. Res. Commun.* **2015**, *457*, 288.

(208) Ranjith, C.; Vijayan, K. K.; Praveen, V. K.; Kumar, N. S. S. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2010**, *75*, 1610.

(209) Wagner, B. *Molecules* **2009**, *14*, 210.

(210) Donovalová, J.; Cigáň, M.; Stankovičová, H.; Gašpar, J.; Danko, M.; Gáplovský, A.; Hrdlovič, P. *Molecules* **2012**, *17*, 3259.

(211) Signore, G.; Nifosì, R.; Albertazzi, L.; Storti, B.; Bizzarri, R. *J. Am. Chem. Soc.* **2010**, *132*, 1276.

(212) Raju, B. B.; Costa, S. M. B. *J. Phys. Chem. B* **1999**, *103*, 4309.

(213) Finke, J. H.; Richter, C.; Gothsch, T.; Kwade, A.; Büttgenbach, S.; Müller-Goymann, C. C. *Eur. J. Lipid Sci. Technol.* **2014**, *116*, 1234.

(214) García-Beltrán, O.; Yañez, O.; Caballero, J.; Galdámez, A.; Mena, N.; Nuñez, M. T.; Cassels, B. K. *Eur. J. Med. Chem.* **2014**, *76*, 79.

(215) Kim, J. H.; Sumranjit, J.; Kang, H. J.; Chung, S. J. *Mol. Biosyst.* **2014**, *10*, 30.

(216) Wu, J.-S.; Liu, W.-M.; Zhuang, X.-Q.; Wang, F.; Wang, P.-F.; Tao, S.-L.; Zhang, X.-H.; Wu, S.-K.; Lee, S.-T. *Org. Lett.* **2007**, *9*, 33.

(217) Xu, Y.; Dai, X.; Zhao, B.-X. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2015**, *138*, 164.

(218) Çamur, M.; Bulut, M.; Kandaz, M.; Güney, O. *Polyhedron* **2009**, *28*, 233.

(219) Zhou, X.; Lv, X.; Hao, J.; Liu, D.; Guo, W. *Dyes Pigm* **2012**, *95*, 168.

(220) Zou, Q.; Fang, Y.; Zhao, Y.; Zhao, H.; Wang, Y.; Gu, Y.; Wu, F. *J. Med. Chem.* **2013**, *56*, 5288.

(221) Cao, X.; Lin, W.; Yu, Q. *J. Org. Chem.* **2011**, *76*, 7423.

(222) Wang, J.; Xie, J.; Schultz, P. G. *J. Am. Chem. Soc.* **2006**, *128*, 8738.

(223) Seo, K. D.; Song, H. M.; Lee, M. J.; Pastore, M.; Anselmi, C.; De Angelis, F.; Nazeeruddin, M. K.; Gräetzel, M.; Kim, H. K. *Dyes Pigm.* **2011**, *90*, 304.

(224) Kim, S.; Lee, K. J.; Lee, J.; Shin, H.; Kay, K.-Y.; Park, J. *Mol. Cryst. Liq. Cryst.* **2015**, *620*, 139.

(225) Chen, J.-X.; Liu, W.; Zheng, C.-J.; Wang, K.; Liang, K.; Shi, Y.-Z.; Ou, X.-M.; Zhang, X.-H. *ACS Appl. Mater. Interfaces* **2017**, *9*, 8848.

(226) Perkin, W. H. *J. Chem. Soc.* **1868**, *21*, 53.

(227) Wang, D.; Yang, F.; Liu, Z.; Dong, Z.; Zhao, W. *Chin. J. Org. Chem.* **2014**, *34*, 204.

(228) Johnson, J. R. *Org. React.***1942**, *1*, 211.

(229) Borges, F.; Roleira, F.; Milhazes, N.; Santana, L.; Uriarte, E. *Curr. Med. Chem.* **2005**, *12*, 887.

(230) Augustine, J. K.; Bombrun, A.; Ramappa, B.; Boodappa, C. *Tetrahedron Lett.* **2012**, *53*, 4422.

(231) Mashraqui, S. H.; Vashi, D.; Mistry, H. D. *Synth. Commun.* **2004**, *34*, 3129.

(232) Abhay, S. Z.; Firoz, A. K. K.; Sureshchandra, P. Z.; Shantanu, D. S.; Jaiprakash, N. S. *Curr. Org. Chem.* **2016**, *20*, 798.

(233) Li, J. J. In *Name Reactions: A Collection of Detailed Mechanisms and Synthetic Applications*; Springer Berlin Heidelberg: Berlin, Heidelberg, **2009**.

(234) Daru, J.; Stirling, A. *J. Org. Chem.* **2011**, *76*, 8749.

(235) Joshi, R.; Chudasama, U. *J. Sci. Ind. Res. (India)* **2008**, *67*, 1092.

(236) Tyndall, S.; Wong, K. F.; VanAlstine-Parris, M. A. *J. Org. Chem.* **2015**, *80*, 8951.

(237) Pornsatitworakul, S.; Boekfa, B.; Maihom, T.; Treesukol, P.; Namuangruk, S.; Jarussophon, S.; Jarussophon, N.; Limtrakul, J. *Monatshefte für Chemie - Chemical Monthly* **2017**, *148*, 1245.

(238) Al Haj Hussien, F.; Keshe, M.; Alzobar, K.; Merza, J.; Karam, A. *Int. lett. chem. phys. astron.* **2016**, *69*, 66.

(239) Bouasla, S.; Amaro-Gahete, J.; Esquivel, D.; López, M.; Jiménez-Sanchidrián, C.; Teguiche, M.; Romero-Salguero, F. *Molecules* **2017**, *22*, 2072.

(240) Li, J. J. In *Name Reactions: A Collection of Detailed Mechanisms and Synthetic Applications Fifth Edition*; Springer International Publishing: Cham, **2014**.

(241) Ren, Z.; Cao, W.; Tong, W. *Synth. Commun.* **2002**, *32*, 3475.

(242) Trenor, S. R.; Shultz, A. R.; Love, B. J.; Long, T. E. *Chem. Rev.* **2004**, *104*, 3059.

(243) Watson, B. T.; Christiansen, G. E. *Tetrahedron Lett.* **1998**, *39*, 6087.

(244) Bigi, F.; Chesini, L.; Maggi, R.; Sartori, G. *J. Org. Chem.* **1999**, *64*, 1033.

(245) Song, A.; Wang, X.; Lam, K. S. *Tetrahedron Lett.* **2003**, *44*, 1755.

(246) Kumar, D.; Kumar, S.; Makrandi, J. K. *Green Chem. Lett. Rev.* **2015**, *8*, 21.

(247) P. Bandgar, B.; S. Uppalla, L.; S. Kurule, D. *Green Chem.* **1999**, *1*, 243.

(248) Gholap, S. S.; Deshmukh, U. P.; Tambe, M. S. *Iran. J. catal.* **2013**, *3*, 171.

(249) Hekmatshoar, R.; Rezaei, A.; Beheshtiha, S. Y. S. *Phosphorus Sulfur Silicon Relat. Elem.* **2009**, *184*, 2491.

(250) Shaabani, A.; Ghadari, R.; Rahmati, A.; Rezayan, A. H. *J. Iran. Chem. Soc.* **2009**, *6*, 710.

(251) Wittig, G.; Schöllkopf, U. *Chem. Ber.* **1954**, *87*, 1318.

(252) Maryanoff, B. E.; Reitz, A. B. *Chem. Rev.* **1989**, *89*, 863.

(253) Valizadeh, H.; Vaghefi, S. *Synth. Commun.* **2009**, *39*, 1666.

(254) Patre, R. E.; Shet, J. B.; Parameswaran, P. S.; Tilve, S. G. *Tetrahedron Lett.* **2009**, *50*, 6488.

(255) Belavagi, N. S.; Deshapande, N.; Sunagar, M. G.; Khazi, I. A. M. *RSC Advances* **2014**, *4*, 39667.

(256) Jang, Y.-J.; Syu, S.-e.; Chen, Y.-J.; Yang, M.-C.; Lin, W. *Org. Biomol. Chem.* **2012**, *10*, 843.

(257) Chatterjee, A. K.; D. Toste, F.; D. Goldberg, S.; H. Grubbs, R. *Pure Appl. Chem.* **2003**, *75*, 421.

(258) Nguyen Van, T.; Debenedetti, S.; De Kimpe, N. *Tetrahedron Lett.* **2003**, *44*, 4199.

(259) Battistuzzi, G.; Cacchi, S.; De Salve, I.; Fabrizi, G.; Parisi, L. M. *Adv. Synth. Catal.* **2005**, *347*, 308.

(260) Khodabakhshi, S.; Karami, B.; Eskandari, K. *Res. Chem. Intermediat.* **2015**, *41*, 7263.

(261) Murugavel, G.; Punniyamurthy, T. *J. Org. Chem.* **2015**, *80*, 6291.

(262) Lin, Y.; Sun, X.; Yuan, Q.; Yan, Y. *Metab. Eng.* **2013**, *18*, 69.

(263) Yang, S.-M.; Shim, G. Y.; Kim, B.-G.; Ahn, J.-H. *Microb. Cell Fact.* **2015**, *14*, 65.

(264) Kaye, P. T.; Robinson, R. S. *Synth. Commun.* **1996**, *26*, 2085.

(265) Kaye, P. T.; Musa, M. A. *Synth. Commun.* **2003**, *33*, 1755.

(266) Kaye, P. T.; Musa, M. A. *Synthesis* **2002**, *2002*, 2701.

(267) T. Kaye, P.; Musa, M.; W. Nocanda, X. *Synthesis* **2003**, *4*, 531.

(268) Kaye, P. T.; Musa, M. A.; Nocanda, X. W.; Robinson, R. S. *Org. Biomol. Chem.* **2003**, *1*, 1133.

(269) Kaye, P. T.; Musa, M.; Nocanda, X. W. *Synthesis* **2003***, 4,* 531.

(270) Olasupo, I.; Rose, N. R.; Klein, R.; Adams, L. A.; Familoni, O. B.; Kaye, P. T. *Synth. Commun.* **2014**, *44*, 251.

(271) T. Kaye, P.; Musa, M.; T. Nchinda, A.; W. Nocanda, X. *Synth. Commun.* **2004**, *34*, 2575.

(272) Rashamuse, T. J.; Klein, R.; Kaye, P. T. *Synth. Commun.* **2010**, *40*, 3683.

(273) Rashamuse, T. J.; Musa, M. A.; Klein, R.; Kaye, P. T. *Int. J. Chem. Res.* **2009**, *2*, 302.

(274) Olomola, T. O.; Klein, R.; Lobb, K. A.; Sayed, Y.; Kaye, P. T. *Tetrahedron Lett.* **2010**, *51*, 6325.

(275) Olomola, T. O.; Klein, R.; Mautsa, N.; Sayed, Y.; Kaye, P. T. *Bioorg. Med. Chem.* **2013**, *21*, 1964.

(276) Ge, H.; Wang, Y.; Li, C.; Chen, N.; Xie, Y.; Xu, M.; He, Y.; Gu, X.; Wu, R.; Gu, Q.; Zeng, L.; Xu, J. *J. Chem. Inf. Model.* **2013**, *53*, 2757.

(277) Islam, M. A.; Pillay, T. S. *J. Mol. Graph. Model.* **2015**, *56*, 20.

(278) Strecker, C.; Meyer, B. *J. Chem. Inf. Model.* **2018**, *58*, 1121.

(279) Fischer, E. *Berichte der deutschen chemischen Gesellschaft* **1894**, *27*, 2985.

(280) Salmaso, V.; Moro, S. *Front. Pharmacol.* **2018**, *9*, 923.

(281) Van Drie, J. H. *J. Comput. Aided Mol. Des.* **2007**, *21*, 591.

(282) Kitchen, D. B.; Decornez, H.; Furr, J. R.; Bajorath, J. *Nat. Rev. Drug Discov.***2004**, *3*, 935.

(283) Usha, T.; Shanmugarajan, D.; Goyal, A. K.; Kumar, C. S.; Middha, S. K. *Curr. Top. Med. Chem.* **2017**, *17*, 3296.

(284) Sliwoski, G.; Kothiwale, S.; Meiler, J.; Lowe, E. W., Jr. *Pharmacol. Rev.* **2014**, *66*, 334.

(285) Jorgensen, W. L. *Science* **2004**, *303*, 1813.

(286) Macalino, S.; Basith, S.; Clavio, N.; Chang, H.; Kang, S.; Choi, S. *Molecules* **2018**, *23*, 1963.

(287) Yu, W.; MacKerell, A. D., Jr. *Methods Mol. Biol.* **2017**, *1520*, 85.

(288) Chen, Y.-C. *Trends Pharmacol. Sci.***2015**, *36*, 78.

(289) Baig, M. H.; Ahmad, K.; Rabbani, G.; Danishuddin, M.; Choi, I. *Curr. Neuropharmacol.* **2018**, *16*, 740.

(290) Bartuzi, D.; Kaczor, A. A.; Targowska-Duda, K. M.; Matosiuk, D. *Molecules* **2017**, *22*.

(291) Ferreira, L. G.; Dos Santos, R. N.; Oliva, G.; Andricopulo, A. D. *Molecules* **2015**, *20*, 13384.

(292) Pagadala, N. S.; Syed, K.; Tuszynski, J. *Biophys. Rev.* **2017**, *9*, 91.

(293) Gioia, D.; Bertazzo, M.; Recanatini, M.; Masetti, M.; Cavalli, A. *Molecules* **2017**, *22*, 2029.

(294) Tian, S.; Sun, H.; Pan, P.; Li, D.; Zhen, X.; Li, Y.; Hou, T. *J. Chem. Inf. Model.* **2014**, *54*, 2664.

(295) Narang, R.; Narasimhan, B.; Sharma, S. *Curr. Med. Chem.* **2012**, *19*, 569.

(296) Łukasz, P.; Anna, B. *Chem. Biodivers.* **2017**, *14*, e1700075.

(297) Khan, M. S.; Siddiqui, S. P.; Tarannum, N. **2017**.

(298) Zhao, P.-L.; Wang, L.; Zhu, X.-L.; Huang, X.; Zhan, C.-G.; Wu, J.-W.; Yang, G.-F. *J. Am. Chem. Soc.* **2010**, *132*, 185.

(299) Abdou, M. M.; El-Saeed, R. A.; Bondock, S. *Arab. J. Chem.* **2019**, *12*, 88.

(300) Traven, V. F.; Negrebetsky, V. V.; Vorobjeva, L. I.; Carberry, E. A. *Can. J. Chem.* **1997**, *75*, 377.

(301) Špirtović-Halilović, S.; Salihović, M.; Trifunović, S.; Roca, S.; Veljović, E.; Osmanović, A.; Vinković, M.; Završnik, D. *J. Serb. Chem. Soc.* **2014**, *79*, 1405.

(302) S., S.; S., S.; N., M.; N., V.; J., K. L. *J. Heterocycl. Chem.* **2004**, *41*, 593.

(303) Tian, F.-F.; Jiang, F.-L.; Han, X.-L.; Xiang, C.; Ge, Y.-S.; Li, J.-H.; Zhang, Y.; Li, R.; Ding, X.-L.; Liu, Y. *J. Phys. Chem. B* **2010**, *114*, 14842.

(304) Shakdofa, M. *Indian J. Adv. Chem. Sci.* **2016**, *4*, 114.

(305) Bonthu, S.; Bindu, H. *Int. J. Eng. Technol.* **2018**, *4*, 10.

(306) Fillbrunn, A.; Dietz, C.; Pfeuffer, J.; Rahn, R.; Landrum, G. A.; Berthold, M. R. *J. Biotechnol.* **2017**, *261*, 149.

(307) Zhao, X. Z.; Smith, S. J.; Maskell, D. P.; Metifiot, M.; Pye, V. E.; Fesen, K.; Marchand, C.; Pommier, Y.; Cherepanov, P.; Hughes, S. H.; Burke, T. R., Jr. *ACS Chem. Biol.* **2016**, *11*, 1074.

(308) Tsiang, M.; Jones, G. S.; Niedziela-Majka, A.; Kan, E.; Lansdon, E. B.; Huang, W.; Hung, M.; Samuel, D.; Novikov, N.; Xu, Y.; Mitchell, M.; Guo, H.; Babaoglu, K.; Liu, X.; Geleziunas, R.; Sakowicz, R. *J. Biol. Chem.* **2012**, *287*, 21189.

(309) Yeung, C. M.; Klein, L. L.; Flentge, C. A.; Randolph, J. T.; Zhao, C.; Sun, M.; Dekhtyar, T.; Stoll, V. S.; Kempf, D. J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2275.

(310) Brik, A.; Alexandratos, J.; Lin, Y. C.; Elder, J. H.; Olson, A. J.; Wlodawer, A.; Goodsell, D. S.; Wong, C. H. *Chembiochem* **2005**, *6*, 1167.

(311) Asojo, O. A.; Afonina, E.; Gulnik, S. V.; Yu, B.; Erickson, J. W.; Randad, R.; Medjahed, D.; Silva, A. M. *Acta Crystallogr. D Biol. Crystallogr.* **2002**, *58*, 2001.

(312) Holton, S.; Merckx, A.; Burgess, D.; Doerig, C.; Noble, M.; Endicott, J. *Structure* **2003**, *11*, 1329.

(313) Cameron, A.; Read, J.; Tranter, R.; Winter, V. J.; Sessions, R. B.; Brady, R. L.; Vivas, L.; Easton, A.; Kendrick, H.; Croft, S. L.; Barros, D.; Lavandera, J. L.; Martin, J. J.; Risco, F.; Garcia-Ochoa, S.; Gamo, F. J.; Sanz, L.; Leon, L.; Ruiz, J. R.; Gabarro, R.; Mallo, A.; Gomez de las Heras, F. *J. Biol. Chem.* **2004**, *279*, 31429.

(314) Miller Iii, W. A.; Teye, J.; Achieng, A. O.; Mogire, R. M.; Akala, H.; Ong'echa, J. M.; Rathi, B.; Durvasula, R.; Kempaiah, P.; Kwofie, S. K. *Curr. Top. Med. Chem.* **2019**, *18*, 2022.

(315) Zogota, R.; Kinena, L.; Withers-Martinez, C.; Blackman, M. J.; Bobrovs, R.; Pantelejevs, T.; Kanepe-Lapsa, I.; Ozola, V.; Jaudzems, K.; Suna, E.; Jirgensons, A. *Eur. J. Med. Chem.* **2019**, *163*, 344.

(316) Rumsh, L. D.; Mikhailova, A. G.; Mikhura, I. V.; Prudchenko, I. A.; Chikin, L. D.; Mikhaleva, I. I.; Kaliberda, E. N.; Dergousova, N. I.; Mel'nikov, E. E.; Formanovskii, A. A. *Russ. J. Bioorgan. Chem.* **2008**, *34*, 660.

(317) Eubanks, A. L.; Perkins, M. M.; Sylvester, K.; Ganley, J. G.; Posfai, D.; Sanschargrin, P. C.; Hong, J.; Sliz, P.; Derbyshire, E. R. *ChemMedChem* **2018**, *13*, 2479.

(318) Waters, N. C.; Geyer, J. A. *Expert Opin. Ther. Targets* **2003**, *7*, 7.

(319) Scotti, L.; Mendonca, F. J.; da Silva, M. S.; Scotti, M. T. *Curr. Protein Pept. Sci.* **2016**, *17*, 243.

(320) Chen, Y. Y.; Ko, T. P.; Chen, W. H.; Lo, L. P.; Lin, C. H.; Wang, A. H. *J. Struct. Biol.* **2010**, *169*, 25.

(321) Bjorkelid, C.; Bergfors, T.; Raichurkar, A. K.; Mukherjee, K.; Malolanarasimhan, K.; Bandodkar, B.; Jones, T. A. *J. Biol. Chem.* **2013**, *288*, 18260.

(322) Ali, M. T.; Blicharska, N.; Shilpi, J. A.; Seidel, V. *Sci. Rep.* **2018**, *8*, 12238.

(323) Ali, E.; Naimi-Jamal, M. R.; Dekamin, M. G. *Sci. Iran.* **2013**, *20*, 592.

(324) Girija, C. *Journal of Advances in chemistry* **2014**, *10*, 2705.

(325) Hameed, A.; al-Rashida, M.; Uroos, M.; Abid Ali, S.; Khan, K. M. *Expert Opin. Ther. Pat.* **2017**, *27*, 63.

(326) Andiappan, K.; Sanmugam, A.; Deivanayagam, E.; Karuppasamy, K.; Kim, H.-S.; Vikraman, D. *Sci. Rep.* **2018**, *8*, 3054.

(327) Qin, W.; Long, S.; Panunzio, M.; Biondi, S. *Molecules* **2013**, *18*, 12264.

(328) Gupta, V.; Kant, V. *Sci. Int.* **2013**, *1*, 253.

(329) Mahesh, T. C.; Shivani, P.; Palmi, M.; Pathik, S. B. *Curr. Top. Med. Chem.* **2016**, *16*, 2841.

(330) Siddiqui, N.; Faiz Arshad, M.; Ahsan, W.; Alam, M. *Int. J. Pharm. Sci. Drug Res.* **2009**, *1*, 136.

(331) Raghav, M.; Kumar, S. P.; Kumar, V. P.; Isha, T.; Garima, M.; Kumar, D. P. *J. Heterocycl. Chem.* **2017**, *54*, 2103.

(332) Gomha, S. M.; Abdelaziz, M. R.; Kheder, N. A.; Abdel-aziz, H. M.; Alterary, S.; Mabkhot, Y. N. *Chem. Cent. J.* **2017**, *11*, 105.

(333) Thirunarayanan, G. *Bull. Chem. Soc. Ethiop.* **2014**, *28*, 73.

(334) Deng, J.; Sanchez, T.; Al-Mawsawi, L. Q.; Dayam, R.; Yunes, R. A.; Garofalo, A.; Bolger, M. B.; Neamati, N. *Bioorg. Med. Chem.* **2007**, *15*, 4985.

(335) K. Sahu, N.; S. Balbhadra, S.; Choudhary, J.; V. Kohli, D. *Curr. Med. Chem.* **2012**, *19*, 209.

(336) Perdana, F.; Eryanti, Y.; Zamri, A. *Procedia Chem.* **2015**, *16*, 129.

(337) Zhou, B.; Xing, C. *Med. Chem.* **2015**, *5*, 388.

(338) Gomes, M. N.; Muratov, E. N.; Pereira, M.; Peixoto, J. C.; Rosseto, L. P.; Cravo, P. V. L.; Andrade, C. H.; Neves, B. J. *Molecules* **2017**, *22*.

(339) Singh, P.; Anand, A.; Kumar, V. *Eur. J. Med. Chem.* **2014**, *85*, 758.

(340) Daniela Ilieva, B.; Iva Todorova, T. *Curr. Clin. Pharmacol.* **2010**, *5*, 1.

(341) Verma, S.; Srivastava, A. K.; Pandey, O. *PharmaTutor* **2018**, *6*, 22.

(342) Deng, J.; Kelley, J. A.; Barchi, J. J.; Sanchez, T.; Dayam, R.; Pommier, Y.; Neamati, N. *Bioorg. Med. Chem.* **2006**, *14*, 3785.

(343) Wadhwa, P.; Jain, P.; R A Jadhav, H.; Rudrawar, S. *Curr Drug Discov Technol.* **2018**, *15*, 2.

(344) Cole, A. L.; Hossain, S.; Cole, A. M.; Phanstiel, O. *Bioorg. Med. Chem.* **2016**, *24*, 2768.

(345) Hili, R.; Yudin, A. K. *Nat. Chem. Biol.* **2006**, *2*, 284.

(346) Kouznetsov, V.; Puerto, C.; Vargas, L.; Meléndez, C. Simple Preparation of New Potential Bioactive Nitrogen-Containing Molecules and Their Spectroscopy Analysis. In *Chromatography and Its Applications*; Dhanarasu S., Ed.; Intechopen, **2012**, 87.

(347) Blakemore, D. C.; Castro, L.; Churcher, I.; Rees, D. C.; Thomas, A. W.; Wilson, D. M.; Wood, A.Nat. Chem. **2018**, *10*, 383.

(348) Li, H.-J.; Wu, Y.-C.; Dai, J.-H.; Song, Y.; Cheng, R.; Qiao, Y. *Molecules* **2014**, *19*, 3401.

(349) Das, B.; Venkateswarlu, K.; Majhi, A.; Siddaiah, V.; Reddy, K. R. *J. Mol. Catal. A-Chem.* **2007**, *267*, 30.

(350) Baharfar, R.; Alinezhad, H.; Azimi, S.; Salehian, F. *J. Chil. Chem. Soc.* **2011**, *56*, 863.

(351) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.***2001**, *40*, 2004.

(352) Liang, L.; Astruc, D. *Coord. Chem. Rev.* **2011**, *255*, 2933.

(353) Pasini, D. *Molecules* **2013**, *18*, 9512.

(354) Zhang, L.; Chen, X.; Xue, P.; Sun, H. H. Y.; Williams, I. D.; Sharpless, K. B.; Fokin, V. V.; Jia, G. *J. Am. Chem. Soc.* **2005**, *127*, 15998.

(355) Singh, M. S.; Chowdhury, S.; Koley, S. *Tetrahedron* **2016**, *72*, 5257.

(356) Kazemi, S. S.; Keivanloo, A.; Nasr-Isfahani, H.; Amin, A. H. *J. Heterocycl. Chem.* **2018**, *55*, 1651.

(357) Zhou, C. H.; Wang, Y. *Curr. Med. Chem.* **2012**, *19*, 239.

(358) Sahu, J. K.; Ganguly, S.; Kaushik, A. *Chin. J. Nat. Med.* **2013**, *11*, 456.

(359) Sreedhar, B.; Surendra Reddy, P. *Synth. Commun.* **2007**, *37*, 805.

(360) Aftab, K.; Aslam, K.; Kousar, S.; Nadeem, M. J. *Pak. J. Pharm. Sci.* **2016**, *29*, 423.

(361) In *The Manual of Scientific Style*; Rabinowitz, H., Vogel, S., Eds.; Academic Press: San Diego, **2009**.

(362) Rajput, P.; Sharma, A. *J. Pharm. Med. Chem.* **2018**, *2*.

(363) Lanigan, R. M.; Starkov, P.; Sheppard, T. D. *J. Org. Chem.* **2013**, *78*, 4512.

(364) Greenberg, A.; Liebman, J. F. *The amide linkage: Structural significance in chemistry, biochemistry, and materials science*; John Wiley & Sons, **2000**.

(365) Koz'minykh, V. O. *Pharm. Chemi. J.* **2006**, *40*, 8.

(366) Saeedi, M.; Goli, F.; Mahdavi, M.; Dehghan, G.; Faramarzi, M. A.; Foroumadi, A.; Shafiee, A. *Iran. J. Pharm. Res.* **2014**, *13*, 881.

(367) Lanigan, R. M.; Karaluka, V.; Sabatini, M. T.; Starkov, P.; Badland, M.; Boulton, L.; Sheppard, T. D. *Chem. Commun.* **2016**, *52*, 8846.

(368) Lanigan, R. M.; Sheppard, T. D. *Eur. J. Org. Chem.* **2013**, *2013*, 7453.

(369) Drageset, A.; Bjørsvik, H.-R. *Eur. J. Org. Chem.* **2018**, *2018*, 4436.

(370) Fennie, M. W.; Roth, J. M. *J. Chem. Educ.* **2016**, *93*, 1788.

(371) Park, S.-J.; Lee, J.-C.; Lee, K.-I. *Bull. Korean Chem. Soc.* **2007**, *28*, 1203.

(372) Chen, Z.-c.; Tong, L.; Du, Z.-b.; Mao, Z.-f.; Zhang, X.-j.; Zou, Y.; Yan, M. *Org. Biomol. Chem.* **2018**, *16*, 2634.

(373) Buckle, D. R.; Cantello, B. C. C.; Smith, H.; Spicer, B. A. *J. Med. Chem.* **1975**, *18*, 391.

(374) Deželić, M.; Trkovnik, M. *J. Med. Chem.* **1964**, *7*, 284.

(375) Gao, W.-T.; Hou, W.-D.; Zheng, M.-R.; Tang, L.-J. *Synth. Commun.* **2010**, *40*, 732.

(376) Nolan, K. A.; Doncaster, J. R.; Dunstan, M. S.; Scott, K. A.; Frenkel, A. D.; Siegel, D.; Ross, D.; Barnes, J.; Levy, C.; Leys, D.; Whitehead, R. C.; Stratford, I. J.; Bryce, R. A. *J. Med. Chem.* **2009**, *52*, 7142.

(377) Eisenhauer, H. R.; Link, K. P. *J. Am. Chem. Soc.* **1954**, *76*, 1647.

(378) Sukdolak, S.; Solujić, S.; Manojlović, N.; Vuković, N.; Krstić, L. J. *J. Heterocycl. Chem.* **2004**, *41*, 593.

(379) Daněk, O. *Collect. Czech. Chem. Commun.* **1964**, *29*, 1035.

(380) Athanasellis, G.; Melagraki, G.; Chatzidakis, H.; Afantitis, A.; Detsi, A.; Igglessi-Markopoulou, O.; Markopoulos, J. *Synthesis* **2004**, *2004*, 1775.

(381) Ziegler, E.; Junek, H. *Monatshefte für Chemie und verwandte Teile anderer Wissenschaften* **1955**, *86*, 506.

(382) Karia, D.; Manvar, A.; Trangadia, V.; Shah, A. *Ind. J. Org. Chem.* **2007**, *3*, 170.

(383) Badcock, G. G.; Dean, F. M.; Robertson, A.; Whalley, W. B. *J. Chem. Soc. (Resumed)* **1950**, 903.

(384) Rajak, H.; Deshmukh, R.; Aggarwal, N.; Kashaw, S.; Kharya, M. D.; Mishra, P. *Archiv der Pharmazie* **2009**, *342*, 453.

(385) Chipeleme, A.; Gut, J.; J Rosenthal, P.; Chibale, K. *Bioorg Med Chem.* **2007**, *15*, 273.

(386) Weller, L. E.; Sell, H. M.; Gottshall, R. Y. *J. Am. Chem. Soc.* **1954**, *76*, 1959.

(387) Ovidiu, O.; Joseph Thierry, N.; Nastasa, C.; Brindusa, T.; Oniga, S.; Pîrnău, A.; Vlase, L.; Verité, P. *Farmacia* **2012**, *60*, 785.

(388) Gardner, T. S.; Smith, F. A.; Wenis, E.; Lee, J. *J. Org. Chem.* **1951**, *16*, 1121.

(389) Buu-Hoi, N. P.; Welsch, M.; Dechamps, G.; Bihan, H. L.; Binon, F.; Xuong, N. D. *J. Org. Chem.* **1953**, *18*, 121.

(390) Mustafa, A.; Hishmat, O. H.; Nawar, A. A.; Khalil, K. H. M. A. *Justus Liebigs Annalen der Chemie* **1965**, *684*, 194.

(391) Lin, C.-F.; Yang, J.-S.; Chang, C.-Y.; Kuo, S.-C.; Lee, M.-R.; Huang, L.-J. *Bioorg. Med. Chem.* **2005**, *13*, 1537.

(392) Profft, E. *Journal für Praktische Chemie* **1957**, *5*, 175.

(393) Pouysegu, L.; Chassaing, S.; Dejugnac, D.; Lamidey, A. M.; Miqueu, K.; Sotiropoulos, J. M.; Quideau, S. *Angew. Chem. Int. Ed. Engl.* **2008**, *47*, 3552.

(394) Raiford, L. C.; Tanzer, L. K. *J. Org. Chem.* **1941**, *06*, 722.

(395) Hans, R. H.; Guantai, E. M.; Lategan, C.; Smith, P. J.; Wan, B.; Franzblau, S. G.; Gut, J.; Rosenthal, P. J.; Chibale, K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 942.

(396) Hu, J.; Zhang, M.; Yu, L. B.; Ju, Y. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4342.

(397) Khoshkholgh, M. J.; Balalaie, S.; Gleiter, R.; Rominger, F. *Tetrahedron* **2008**, *64*, 10924.

(398) Muthusamy, S.; Gangadurai, C. *Tetrahedron Lett.* **2018**, *59*, 1501.

(399) Vedachalam, S.; Wong, Q.-L.; Maji, B.; Zeng, J.; Ma, J.; Liu, X.-W. *Adv. Synth. Catal.* **2011**, *353*, 219.

(400) Janietz, D.; Rudorf, W.-D. *Tetrahedron* **1989**, *45*, 1661.

(401) Wang, D.; Yang, F.; Liu, Z.; Dong, Z.; Zhao, W. *Chin. J. Org. Chem.* **2014**, *34*, 204.