CHEMICAL STUDIES OF SELECTED CHROMONE DERIVATIVES

THESIS

Submitted in fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

of

RHODES UNIVERSITY

By

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M.Sc (Yaounde)

December 2001

ABSTRACT

This investigation has been geared towards several aspects of chromone chemistry. Selected 2-(*N,N*-dimethylamino)chromones have been synthesized *via* 2-hydroxyacetophenone boron difluoride complex intermediates, and potentiometric analysis of these compounds in ethanol-water has been used to determine the influence of substituents on their basicity. The pK_a values have been found to lie within a narrow range (1.92 – 2.52), and the observed substituent effects have been rationalized with the aid of semi-empirical and *ab initio* molecular orbital calculations.

An efficient route has been developed for the synthesis of the naturally-occurring chromone, "granulosin" [7,8-(methylenedioxy)-2-propylchromone], and several C-2 side chain analogues in good yields, by condensing 2'-hydroxy-3',4'-(methylenedioxy)acetophenone with a range of ethyl carboxylate esters. These compounds show significant cytotoxic activity against the brine shrimp, *Artemia salina*, and two of them, the 2-ethyl and 2-benzyl derivatives also show 100% activity as pesticides on Beet army worms (BAW). Another naturally-occurring chromone derivative, 5-hydroxy-2-isopropyl-7-methoxychromone, and four C-2 side chain analogues have been prepared in moderate yields. These compounds also show significant cytotoxic activity against the brine shrimp, *Artemia salina*, and it is apparent that the presence of the hydroxyl group at C-5 is critical for such activity. The electron-impact mass spectra of both series of chromone derivatives have been investigated, permitting the elucidation of characteristic fragmentation patterns.

In work directed towards the synthesis of potential HIV-1 protease inhibitors, five novel chromone-containing analogues of the clinically useful drug, ritonavir, have been synthesized. The design strategy has involved the coupling of substituted chromone-2-carboxylic acids with a specially prepared, hydroxyethylene dipeptide isostere to afford ritonavir analogues containing chromone termini. An interactive docking procedure has been used to explore the docking of ritonavir and the novel chromone-containing analogues into the active site of the enzyme, and has indicated the capacity of the ritonavir analogues to form hydrogen-bonds with the HIV-1 enzyme receptor.

Various substituted chromone-3-carbaldehydes, which have been synthesized from the corresponding *o*-hydroxyacetophenones using Vilsmeier-Haack methodology, have been examined as substrates for Morita-Baylis-Hillman reactions, using 3-hydroxyquinuclidine as the catalyst and arcylonitrile and methyl acrylate as the activated alkenes. Optimization of the reaction conditions has permitted efficient conversion of the chromone-3-carbaldehydes to the Morita-Baylis-Hillman products and, in some cases, dimeric products, within 24 h. Heating of the Morita-Baylis-Hillman products, arising from reactions with methyl acrylate, at 80 °C for 3 h in the presence of DABCO as catalyst, has been shown to effect transformation to the corresponding dimers in good yield.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisor, Professor Perry T. Kaye, for his guidance, constant encouragement, enthusiasm and invaluable support throughout the entire project. Prof, it has been a great pleasure and honour for me to have been one of your many students; you have been a great source of inspiration.

Thanks also go to Prof. M.T. Davies-Coleman and Mr. Christopher A. Gray of the Marine Natural Products research group for help with the brine shrimp bioassay and the anti-pesticide tests.

I would like to thank Dr. P. Boshoff of the Cape Technikon Mass Spectrometry Unit for collecting high-resolution mass spectrometry data, and to Prof. C.W. McCleland of the University of Port Elizabeth for assisting with molecular orbital calculations.

Thank you to my research group.

I also would like to thank the NRF and Rhodes University for generous financial support.

Finally, a very special thank you to my family.

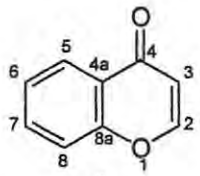
CONTENTS

LIST OF SELECTED ABBREVIATIONS

CDI	1,1'-carbonyldiimidazole
COSY	^{1}H - ^{1}H shift-correlated spectroscopy
DABCO	1,4-diazabicyclo[2.2.2]octane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	distortionless enhancement by polarization transfer
DME	1,2-dimethoxyethane (glyme)
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EDC	*N*-ethyl-*N*'-(dimethylaminopropyl)carbodiimide hydrochloride
HMBC	heteronuclear multibond coherence ($^{1}H - ^{13}C$ shift)
HMQC	heteronuclear quantum coherence ($^{1}H - ^{13}C$ shift)
HOBt	1-hydroxybenzotriazole hydrate
3-HQ	3-hydroxyquinuclidine
HREIMS	high-resolution electron-impact mass spectroscopy
IR	infrared
MO	molecular orbital
MS	mass spectroscopy
NMR	nuclear magnetic resonance
RNA	ribonucleic acid
THF	tetrahydrofuran
TLC	thin layer chromatography

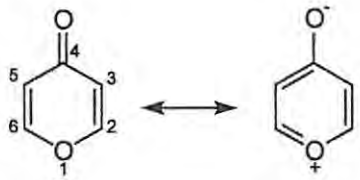
1. INTRODUCTION

Chromones constitute an important class of oxygen-containing heterocyclic compounds, many of which are widely distributed in plants, and possess useful medicinal properties.[1] The name "chromone"was first used by Bloch and Kostanecki[1] to describe coloured, naturally occurring compounds known to contain the benzopyran-4-one structure **1**. Chromones are benzannulated derivatives of γ-pyrone **2a**, and their systematic nomenclature is based on the pyran analogues **3** and **4**.[2,3] In this thesis, the chromone structure will be denoted simply as chromone rather than the systematic name, 4*H*-1-benzopyran-4-one. Chromone **1** is isomeric with coumarin **5**.



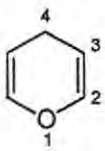
1

chromone
(4*H*-1-benzopyran-4-one)
(benzo-4*H*-pyran-4-one)
(4-oxo-4*H*-chromene)



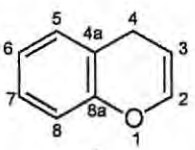
2a **2b**

γ-pyrone
(4*H*-pyran-4-one)



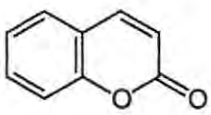
3

4*H*-pyran



4

4*H*-chromene
(4*H*-1-benzopyran)
(γ-chromene)



5

coumarin
(2*H*-1-benzopyran-2-one)

1.1 Properties of the chromone nucleus

Many properties of the heterocyclic ring of the chromone nucleus follow the pattern established by γ-pyrone, and can generally be explained in terms of the aliphatic dienone structure **2a** rather than the aromatic pyrylium betaine structure **2b**.[4] However, certain

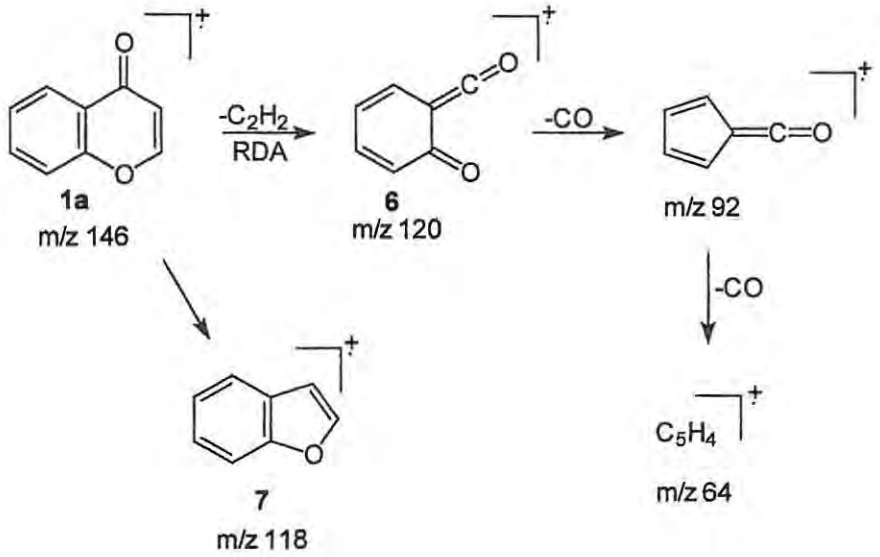
properties of the γ-pyrones and chromones, such as lack of normal ketonic behaviour and their tendency to form salts with acids, has been rationalized in terms of the pyrylium betaine structure **2b**.[5] These properties reflect π-electron delocalization in the pyrylium betaine structure, and it was Arndt in 1924,[5] who first suggested that the ether oxygen in the γ-pyrone could interact electronically with the carbonyl group, thus modifying the properties of the latter. This was, in fact, the first explanation of the theory of mesomerism, affording γ-pyrone an important place in the history of organic chemistry.[5]

Studies of the molecular orbital delocalization energy (DE), dipole moments and ^{1}H- and ^{13}C NMR data have shown that pyran-4-one and chromone possess some degree of aromaticity.[2] Nevertheless, certain spectroscopic properties of chromones may be rationalized in terms of the aliphatic dienone structure **2a**, rather than the aromatic pyrylium betaine structure **2b**. The IR carbonyl stretching frequency for chromone (ν_{max} 1660 cm^{-1}) is a little higher than that of γ-pyrone (ν_{max} 1650 cm^{-1}), but is much lower than that of coumarin **5** (ν_{max} 1710 cm^{-1}). Hence, IR spectroscopy can be used to distinguish chromones from the isomeric coumarins. The UV spectra of chromones are characterized by two strong peaks at *ca.* 225 and 290 nm; chromone itself exhibits electronic absorption bands at λ_{max} 245 nm (10000) and 297 nm (6460).[3]

In the ^{1}H NMR spectrum of chromone **1** in $CDCl_3$, the 2-H and 3-H nuclei resonates at δ 7.9 and 6.3 ppm respectively. These values are very close to those found for γ-pyrone **2** (δ 8.0 and 6.5 ppm respectively),[3] suggesting that the ring current in the heterocyclic ring is not significantly affected by benzannulation. In the ^{13}C NMR spectra of chromones, carbonyl carbon (C-4) signal is always at lowest field and is relatively unaffected by substitution in the system. Of the remaining nuclei, C-2 resonates at lower field and C-3 at higher field than all of the other carbon atoms. However, substitution at C-2 or C-3 of the chromone nucleus has a marked influence on the chemical shifts of these carbon atoms. For instance, both methyl and phenyl groups induce a downfield shift of the carbon to which they are attached, but upfield shifts of the adjacent carbon atoms.[2]

When subjected to electron-impact mass spectrometry, chromone **1** fragments *via* two main pathways, involving either loss of carbon monoxide or ring cleavage by a retro-Diels-Alder

(RDA) reaction as outlined in **Scheme 1**.[6] The base peak (*m/z* 146) is due to the molecular ion **1a**. Loss of acetylene by the RDA pathway gives the radical cation **6** (*m/z* 120), which decomposes further by subsequent losses of carbon monoxide. Substituents may, however, divert the fragmentation pattern.[6]



Scheme 1

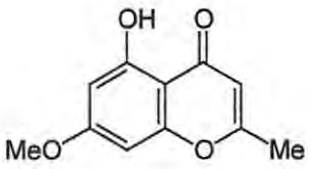
1.2 Review of chromone chemistry

The chemistry of chromones has been extensively reviewed[1,3] and, in this introduction, particular attention will be given to the more recent literature on synthetic and naturally-occurring chromone derivatives, which exhibit interesting pharmacological properties.

1.2.1 Occurrence of biologically active chromone derivatives

Naturally-occurring chromones, like the 2-phenylchromone derivatives (flavones), often contain hydroxyl or methoxy groups at C-5 and/or C-7 and a methyl group at C-2. One of the simplest chromones illustrating this substitution pattern is eugenin **8,** long known as a constituent of the wild clove *Eugenia caryophyllata L. Thunb.*[3] The continuing interest in the chemistry of chromones is largely due to their pharmacological importance. One of the most

useful naturally-occurring chromone derivatives is khellin **9**,[7,8,9] a natural furochromone isolated from the seeds of *Amni visnaga*. Extracts of this plant have been used for the treatment of bronchial asthma,[7,8,9] but unpleasant side effects, such as nausea and vomiting, have limited its clinical use. Khellin **9** also exhibits lipid-altering, antiatherosclerotic activity[10] and may thus provide a valuable therapy for reducing the risk of cardiovascular disease. The bronchial spasm of asthma is caused by antigen-induced release of histamine from mast cells, and, bronchodilators have been used to provide symptomatic relief of this condition. However, prophylactic treatment is now possible with disodium cromoglycate, which is marketed as "Intal" or "cromolyn sodium" **10** and which blocks the release of histamine and other substances which mediate hypersensitivity reactions.[11] This drug, which was developed from the lead given by khellin **9**, has to be administered by inhalation as a powder, and research has been focussed on developing analogues that may be administered orally.



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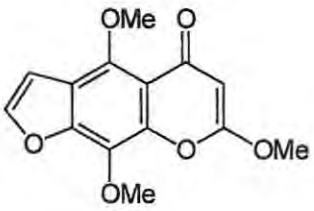
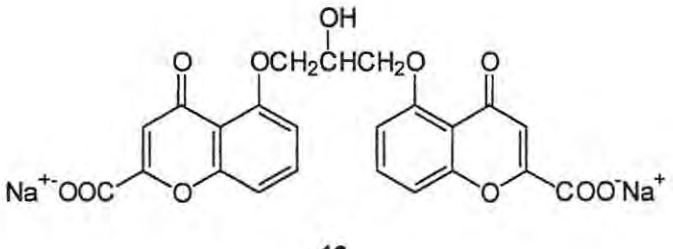


9

Two new 5-methylchromones, 2-acetonyl-7-hydroxy-8-(3-hydroxyacetonyl)-5-methyl-chromone **11** and 2-acetonyl-8-(2-furoylmethyl)-7-hydroxy-5-methylchromone **12,** have recently been isolated from dried latex from the cut leaves of *Aloe ferox* Mill (commonly known as the Cape aloe). [12] Traditional medicine recommends the application of freshly cut aloe leaves to wounds, boils and ulcers, and the use of aloe leaves and roots to treat parasitic infestations in animals and people. In the old Cape, warmed aloe leaves were used to soothe aching teeth, and legend has it that aloes were used to embalm Christ's body. Today, the aloe plant is widely used in the cosmetic industry because of its medicinal properties,[13] and bitter aloe is used as a bittering agent in alcoholic beverages.[14] *Aloe vera* has recently attracted attention as a health food although its chemical composition is far from being completely known. It is, however, rich in chromone derivatives,[14-18] and several of these constituents have shown anti-tyrosinase activity.[16-18] Robustaflavone **13,** a naturally occurring

biflavonoid, has been reported as a potent, non-nucleoside inhibitor of hepatitis B virus (HBV) replication.[19,20]



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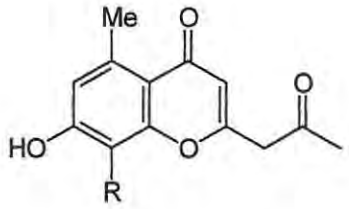
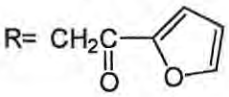
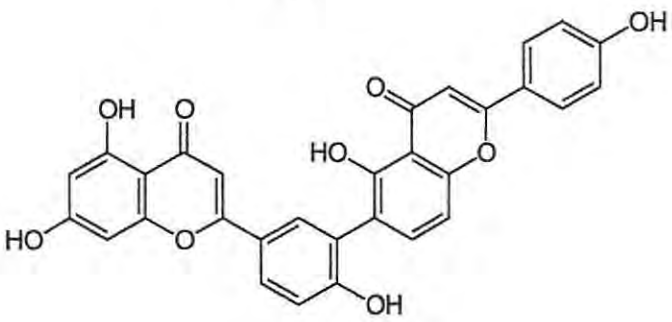


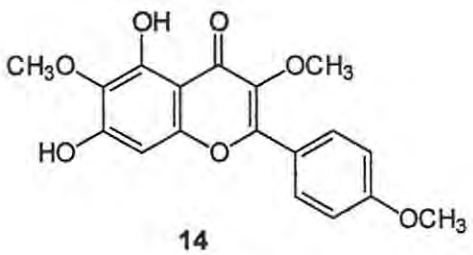
11 R= CH_2COCH_2OH

12 R= 



13

In the phytochemical studies of Spanish medicinal plants, Martinez *et al.*[21] recently isolated two anti-inflammatory flavonoids, 5,7-dihydroxy-3,6,4′-trimethoxyflavone (Santin) **14** and 5,7-dihydroxy-3,4′-dimethoxyflavone (Ermanin) **15** from the aerial parts of *Tanacetum microphyllum*.[21] An extract of this plant has been shown to exhibit significant antiedema activity on carrageenan-induced paw edema in rats and mice.[21] A new xanthone, 6,7-dihydroxy-1,3-dimethoxyxanthone **16**, was obtained from the leaves and stem of *Hypericum geminiflorum* (Guttiferae) in Taiwan and was shown to possess anti-inflammatory activity.[22]



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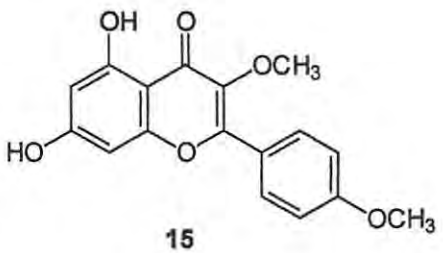
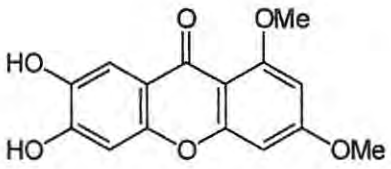


15

Natural 3-methylchromones are rare and hormothamnione **17**, isolated in small quantities from the blue-green marine algae, *cryptophyte chrysophaeum taylori* by Gerwick *et al.*,[23] is a potent cytotoxin towards several human leukemia cell lines *in vitro.* Although the mechanism for its cytotoxic activity has not been completely established, it appears to operate *via* selective inhibition of RNA synthesis.[24] Hormothamnione was the first naturally-occurring styrylchromone to be isolated, and its biological potential has stimulated interest in synthetic analogues.[24,25]



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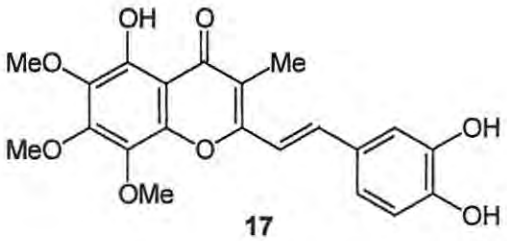

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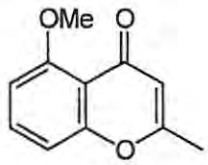

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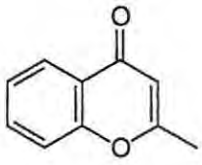

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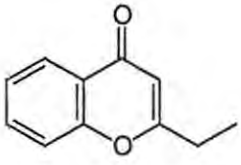
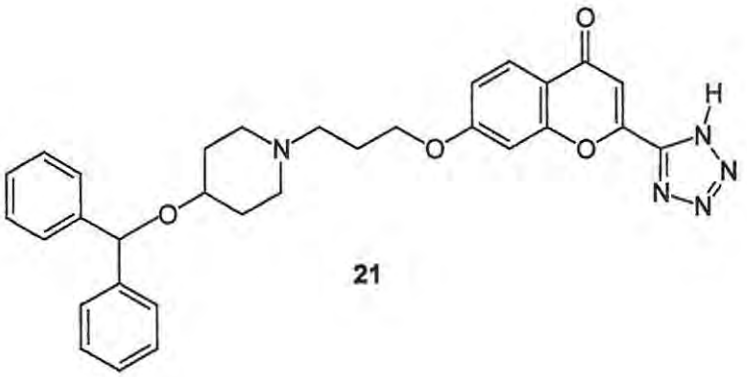


20

Simple chromone derivatives such as 5-methoxy-2-methylchromone **18**, 2-methylchromone **19** and 2-ethylchromone **20**, have recently been shown to possess gastroprotective properties.[26,27] Histamine induces various complex biological processes by interacting with specific receptors in the membranes of cell surfaces. The action of histamine on H_1-receptors stimulates many smooth muscles to contract, including those in the bronchi. Histamine also increases the permeability of the capillary walls so that more of the constituents of the plasma can escape into tissue spaces, leading to an increase in the flow of lymph (and its protein content). The chromone derivative **21**, in which the chromone moiety is connected to a (diphenylmethoxy)piperidine *via* an alkyloxy spacer, has recently been found to possess antiallergic, antiasthmatic and antihistaminic activity.[28] The chromone derivative, 3-acetyl-5-7-dihydroxy-2-methylchromone **22**, depresses the release of mediators (histamine, SRS-A, *etc.*) more strongly than 5,7-dihydroxy-2-methylchromone **23**,[29] the marked difference being attributed to the presence of the carbonyl group at C-3[30] – a structural feature which appears

to enhance the antiallergic activity of a number of chromone derivatives.[30] The presence of an acidic group at C-2 or C-3 of the chromone nucleus[31,32] is also characteristic of many chromones which exhibit antiallergic properties. The majority of these contain a carboxyl group, but its replacement by a 5-tetrazolyl ring, as in compound **24**, has been shown to result in systems with increased pharmacological activity.[31,32] 2-Aminochromones, such as compound **25**, have recently been reported to display antiplatelet properties, and find use in the treatment of unstable angina and other thrombolytic disorders.[33] The presence of both a diethylamino group at C-2 and an electron-donating group, such as ethoxy at C-7, appears to be an important feature for physiological activity.[33]



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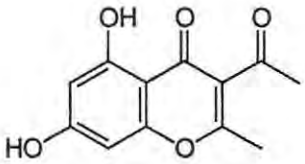

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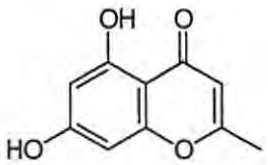

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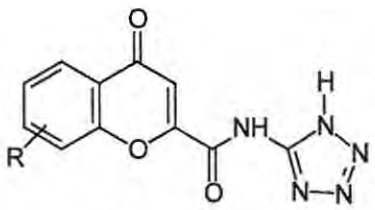

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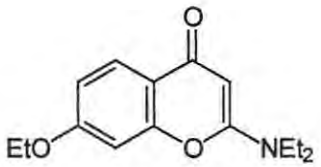
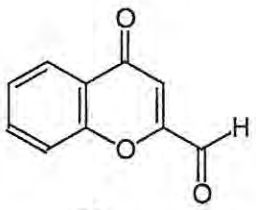


25

Miller *et al.*[34] reported that simple, non-hydroxylated chromones inhibit $p56^{lck}$ tyrosine kinase. Specific tyrosine kinases have been implicated in proliferative diseases, such as cancer and atherosclerosis, and selective tyrosine kinase inhibitors may be useful in the treatment of these diseases. More particularly, selective inhibitors of $p56^{lck}$ could be effective in the treatment of T-cell leukaemias, lymphomas and autoimmune diseases such as rheumatoid arthritis, in which activated T-cells play an important role in the pathogenesis of the disease. Miller *et al.*,[34] also pointed out that the active non-hydroxylated chromones were as potent as the polyhydroxylated flavones in inhibiting $p56^{lck}$. It is interesting to note that all

the chromones (typified by the compounds **26** and **27**), which act as $p56^{lck}$ inhibitors have an aldehyde group at the C-2 or C-3 position, the C-3 aldehydes being the more active.



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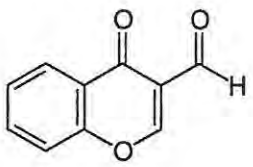
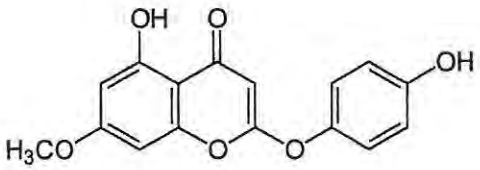


27

Natural products often exhibit useful medicinal properties and local populations may rely on these plants as a source of treatment for various maladies. This has prompted the phytochemical investigation of many medicinal plants. Recently, Huang *et al.*[35] isolated capillarsin derivative **28**, a 2-phenoxychromone, from the leaves of *Epimedium sagittatum* (Berberidaceae). This plant, called "yinyanghuo" in China, has been used for the treatment impotence, atrophy, neurasthenia, amnesia, and climacteric hypertension.[35] Visnagin **29**, a furochromone like khellin **9**, was isolated from *Pimpinella monica* (Umbelliferae) and was found to be an active feeding deterrent.[36] The production of feeding deterrents is one of the defence mechanisms that makes plants unpalatable to insect predators. It was suggested that the presence of an unsubstituted furan ring and an alkoxy substituent at C-5 of the furochromone is essential for feeding deterrent activity; both the heterocyclic (furan and pyrone) rings are also considered essential.[36]



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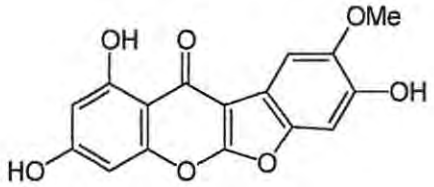
29

5,7,4'-Trihydroxy-5'-methoxycoumaronochromone (desmoxyphyllin A) **30** and 7,4'-di-hydroxy-5'-methoxycoumaronochromone (desmoxyphyllin B) **31** have been isolated from the leaves of *Desmodium oxyphyllum*.[37] Although, the activity of these compounds has not been ascertained, their importance is due to the fact that they were the first reported

coumaronochromones to be isolated from the genus *Desmodium*. The 2-hydroxymethyl-chromone derivatives, **32** and **33** have been isolated from the aerial parts of *Cnidium monnieri* (L) Cusson, a plant called `Jashoshi' in Japan, which has been used mainly for treating the swelling of women`s genitals, male impotence and ringworm.[38]



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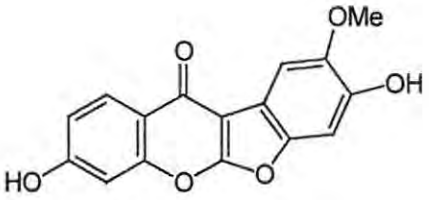
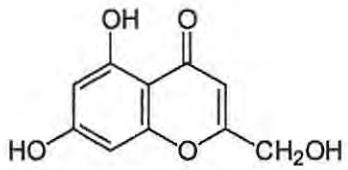


31

8-(2,3-Dihydroxy-3-methylbutyl)-5-hydroxy-7-methoxy-2-methyl-chromone **34** has been isolated from the wood of *Harrisonia perforata* (Blanko) Merr, a plant widely distributed in southeast Asia; the root of this plant is used in a folk medicine in China for the prevention and treatment of malaria and boils.[39] 5-Hydroxy-2-isopropyl-7-methoxychromone **35** has been isolated from the aerial parts of *Baeckea frutescens* L., an aromatic, low-growing shrub used in traditional Chinese medicine for treating rheumatism and snake-bite.[40]



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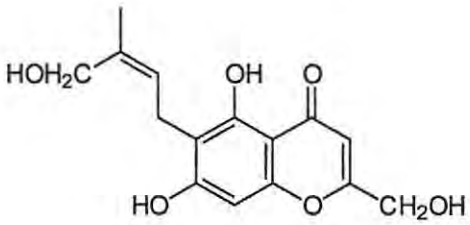

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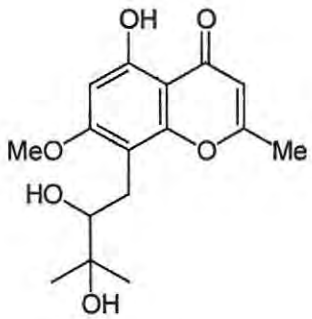

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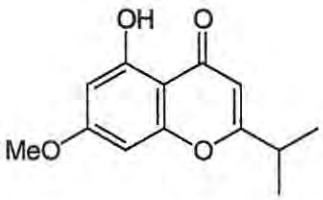
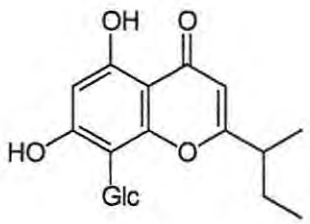


35

5,7-Dihydroxy-2-(1-methylpropyl)chromone-8-β-D-glucoside **36** is a chromone glycoside present in the aerial parts of *Hypericum japonicum* Thunb., a plant used in Chinese medicine for the treatment of infectious hepatitis; this compound has shown good coagulant activity *in vitro*.[41] 5-Hydroxy-7-methoxy-2,3-dimethylchromone **37** has recently been isolated, for the first time, from cultures of spore-derived mycobionts of the lichen, *Graphis scripta*.[42] One characteristic of lichens, is their production of diverse secondary metabolites, some of which exhibit a wide range of potentially useful biological activities, including fungicidal activity.[42]



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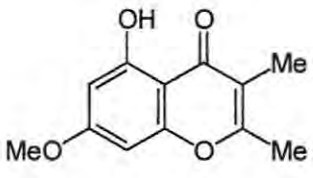
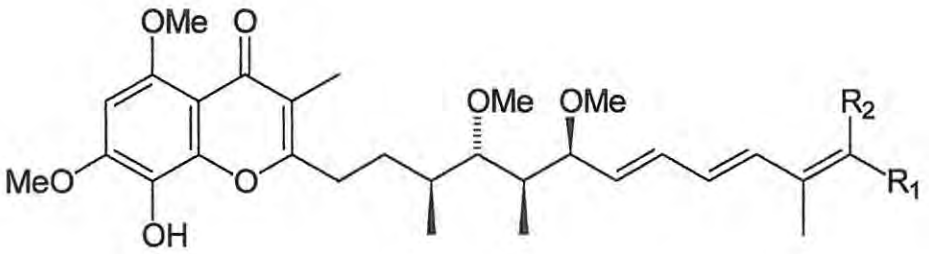


37

Stigmatellin **38**, a naturally occurring chromone isolated by Höfle[43] from a culture of the gliding bacterium *Stigmatella auriantaca*, as diastereomers **38a** and **38b**, has proved to be a potent inhibitor of the photosynthetic system. It is, consequently, a useful tool for studying relevant electron-transfer phenomena.[43,44]

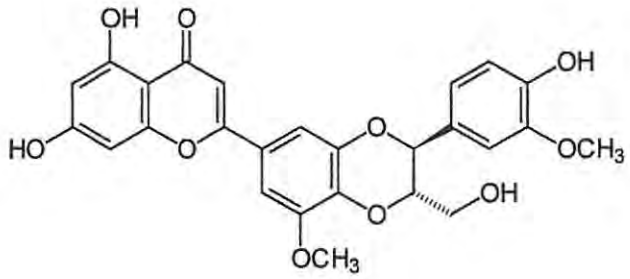


38a (R_1= Me; R_2= H)

38b (R_1= H; R_2= Me)

Major efforts continue to be directed towards the discovery and development of new antibiotics to combat serious infections caused by resistant bacteria. 5'-Methoxyhydnocarpin-D (5'-MHC-D) **39**, a chromone derivative isolated from the plant *H. wightiana*, has

recently been shown to be a potent inhibitor of a *Staphylococcus aureus* multidrug-resistant efflux pump.[45,46] The compound disables the resistance-conferring pump, permitting antibiotics to accumulate in the bacteria cells and thus kill them. It is said that this discovery could lead to the development of drugs to treat antibiotic-resistant bacteria including "superbugs", which exhibit resistance to multiple antibiotics.[45] A new chromone, granulosin [7,8-(methylenedioxy)-2-propylchromone] **40,** which contains a methylenedioxy group has recently been isolated from an ethanol extract of the bark of *Galipea granulosa* Kallunki in Costa Rica.[47] This plant has been traditionally employed in the treatment of parasitic infections, particularly cutaneous leishmaniasis, by the Indians of Bolivia, and an extract displayed activity in the brine shrimp lethality test.[47]



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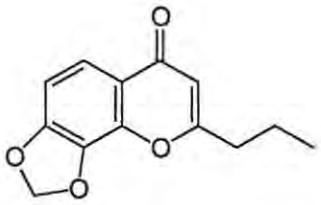
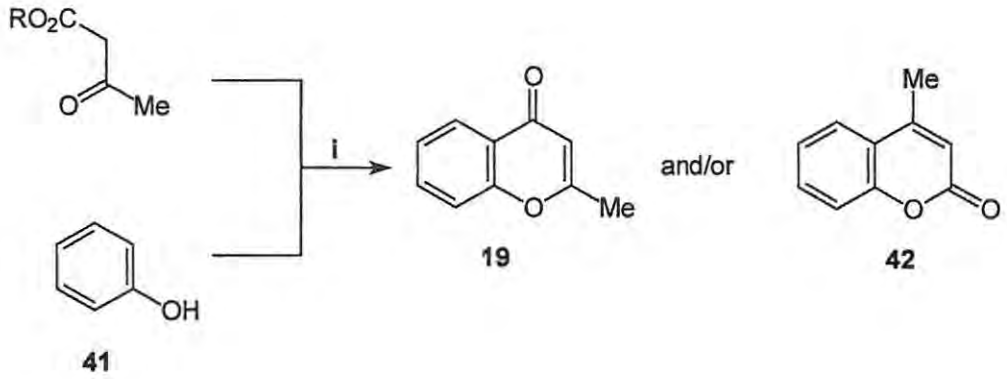


40

1.2.2 Synthesis of chromones

The synthesis of chromones and their 2-phenyl substituted derivatives (flavones) has been widely explored and extensively reviewed.[1-3] Here, a brief review of the classical synthetic methodology will be followed by a survey of more recent methods.

Two widely-used methods of chromone synthesis are the Simonis and the Kostanecki-Robinson reactions.[3] In both approaches, the general strategy involves building a side chain on to a phenolic substrate before cyclization to the desired chromone.[1] In the Simonis reaction,[3] a phenol **41** is condensed with a β-ketoester to give either a chromone **19** or a coumarin **42** (**Scheme 2**). The reaction which leads to a coumarin is called the Pechmann condensation, for which sulfuric acid is necessary as a condensing agent. Chromone formation is favoured when the phenol contains a deactivating group, such as chlorine, or when the β-ketoester is α-substituted, and when phosphorus pentoxide is used as the condensing agent.

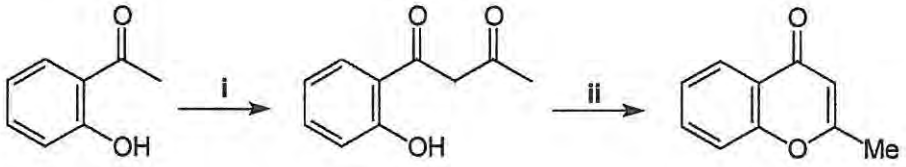


Scheme 2

Reagents: i) Condensing agent (H_2SO_4 or P_2O_5).

In the Kostanecki-Robinson method, use is made of a Claisen-type condensation. In this case, an *o*-hydroxyacetophenone **43** is condensed with an acylating agent in the presence of a strong base to form a β-diketone intermediate **44**, which is subsequently cyclized in acidic medium to the desired chromone **19** (**Scheme 3**).[2,48] The Baker-Venkataraman rearrangement of 2-acyloxyacetophenones **45** (**Scheme 4**)[2,3,49] involves intramolecular migration of an acyl

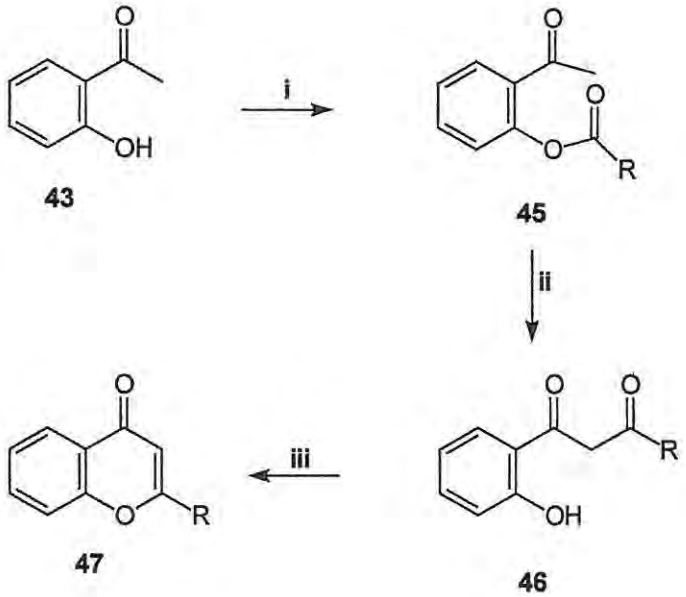
group from oxygen to the carbon atom α to the ketone carbonyl group, and provides an alternative route to the 1,3-diketone intermediate **46** encountered in the Kostanecki-Robinson approach. The migrating acyl group may be aliphatic or aromatic and, hence, this approach is useful for the preparation of flavones **47** (R=Ar)[50,51] as well as chromones **47** (R=alkyl).[50] Catalysts (other than potassium hydroxide, pyridine or potassium carbonate) recommended for the Baker-Venkataraman rearrangement include sodium, sodamide, sodium alkoxides, sodium hydride, and sodium hydroxide.[52]



43 **44** **19**

Scheme 3

Reagents: i) strong base, CH_3CO_2Et; ii) H^+.



43 **45** **46** **47**

Scheme 4

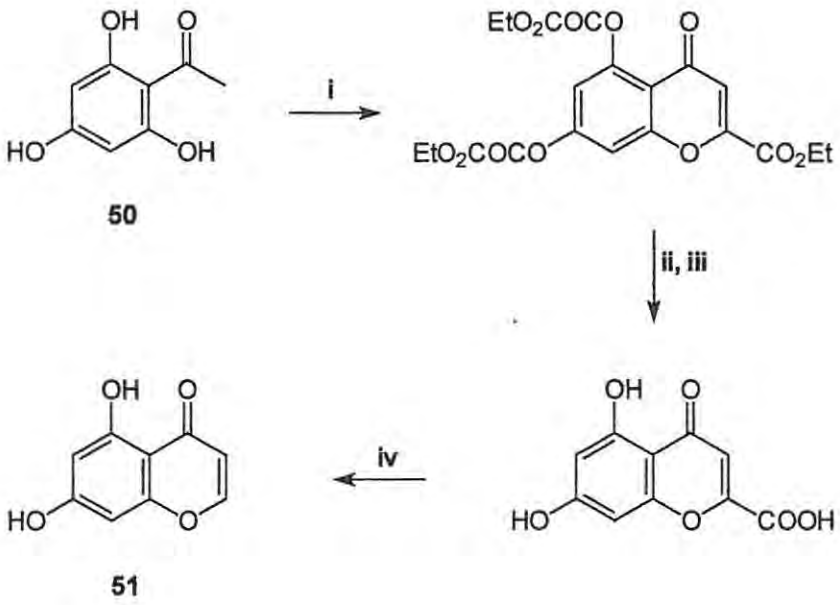
Reagents: i) RCOCl, pyridine ; ii) KOH, pyridine or K_2CO_3 ; iii) H^+.

3-Methylchromones **49**, which are used as fungicides, cardiovascular agents and in the treatment of allergic conditions or hyperacidity, have been synthesized by treating 2-hydroxypropiophenone **48** with DMF, BF_3-etherate and methanesulphonyl chloride **(Scheme 5)**.[53] 5,7-Dihydroxychromone **51**,[54] a flavonoid decomposition product found in some plant extracts, is also a germination and growth inhibitor and has been prepared by condensing 2',4',6'-trihydroxyacetophenone monohydrate **50** with ethyl oxalyl chloride, followed by hydrolysis, acidification and decarboxylation (**Scheme 6**).

48 **49**

Scheme 5

Reagents: i) DMF, BF_3-OEt_2, $MeSO_2Cl$.



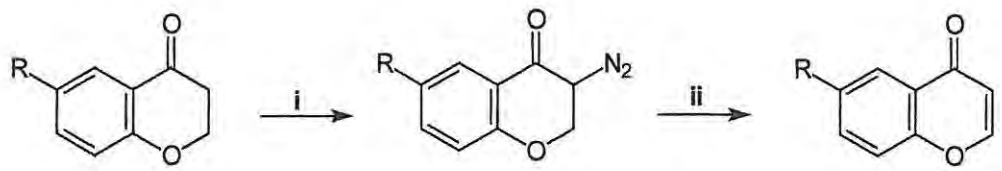
50

51

Scheme 6

Reagents: i) EtOOCCOCl, pyridine; ii) OH^-; iii) H^+; iv) heat.

The facile elimination of the diazo group from α-diazocarbonyl compounds has made such compounds very useful in organic synthesis. Thus, substituted chromones **54** have been synthesized in high yield from 3-diazochromanones **53** by Lewis acid-catalyzed elimination of the diazo group from 3-diazochromanones **53** (**Scheme 7**).[55]

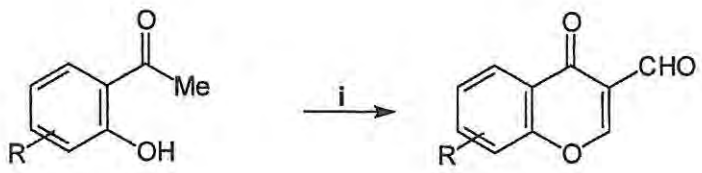


52 **53** **54**

Scheme 7

Reagents: i) (a) NaH, HCO_2Et, Et_2O (b) Et_3N, TsN_3 ; ii) $BF_3.Et_2O$, CH_2Cl_2.

Chromone-3-carbaldehydes **55**, which exhibit anti-anaphylactic properties have been synthesized in excellent yield from *o*-hydroxyacetophenones **43** by the Vilsmeier-Haack reaction (**Scheme 8**).[56,57] This one-pot synthesis has been reported as the most convenient method of synthesizing chromone-3-carbaldehyde derivatives **55** in high yield.



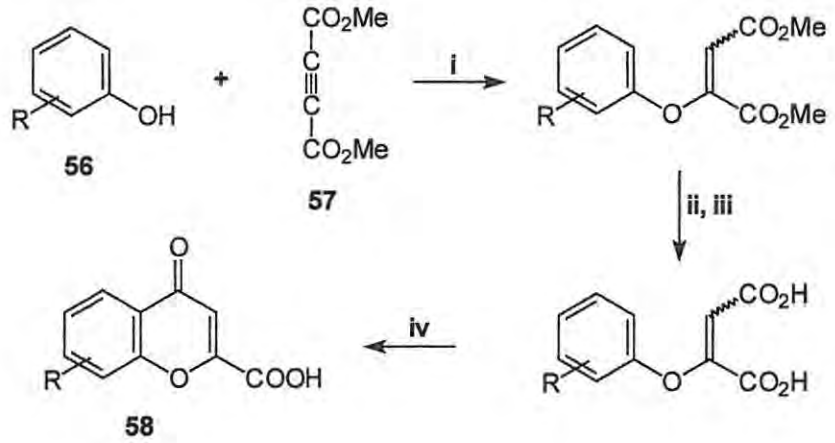
43 **55**

Scheme 8

Reagents: i) (a) DMF, $POCl_3$ (b) H_2O.

The addition of substituted phenols **56** to dimethyl acetylenedicarboxylate **57** has recently been reported as a versatile, high-yielding reaction in a three-step synthesis of chromone-2-carboxylic acids **58**. Substituted phenols, deprotonated by triethylamine, add to dimethyl

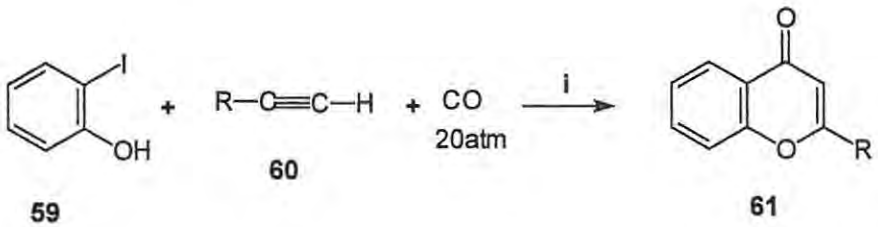
acetylenedicarboxylate **57** under mild conditions, the reaction tolerating a range of functional groups on the phenol. Moreover, the addition reaction is not stereospecific in the sense that both aryloxy fumarates and maleates are produced and can be cyclized to the desired chromones without contamination by isomeric coumarins (**Scheme 9**).[58] This reaction thus constitutes a useful general synthesis of chromones. Recently, Zatón *et al.*[59] have reported that the heterocyclic ring of chromone-2-carboxylic acids binds to human serum albumin (HAS); – a process involved in the distribution of a drug through the circulation system.



Scheme 9

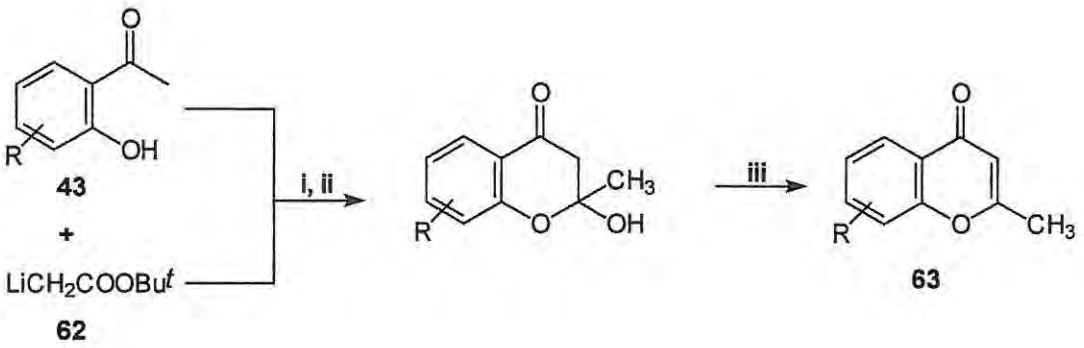
Reagents: i) Et_3N, Et_2O ; ii) OH^-, H_2O ; iii) H^+, H_2O ; iv) AcCl, H_2SO_4.

Developments in organometallic chemistry have extended the range of methods available to the synthetic chemist. In particular, palladium is known to activate aryl halides towards functionalisation, and the palladium-catalyzed carbonylative coupling of *o*-iodophenol **59** with terminal acetylenes **60** in the presence of secondary amine has been reported to be a convenient and efficient method for the synthesis of 2-substituted chromones **61**(**Scheme 10**).[60]



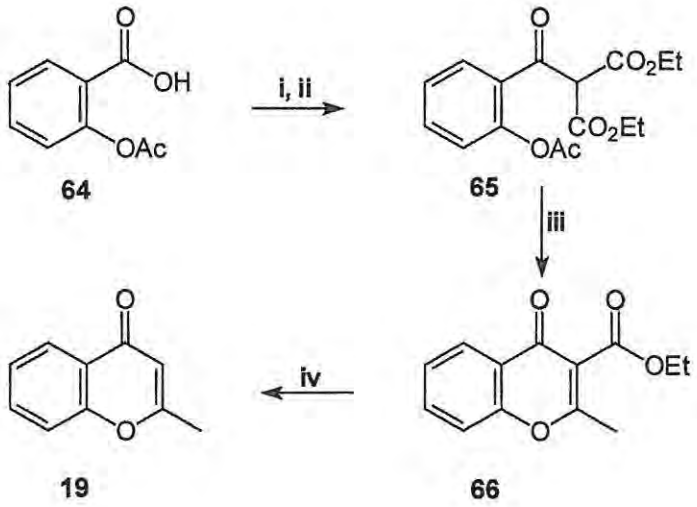
Scheme 10
Reagents: i) Pd-catalyst, Et_2NH, 120°C, 2h.

2-Methylchromones **63** (including khellin **9**) have been synthesized in high yield by the reaction of *t*-butyl lithioacetate **62** with various polysubstituted *o*-hydroxyacetophenones **43** and subsequent acid-catalyzed dehydration of the resulting hemiacetals (**Scheme 11**).[61] 2-Methylchromone **19** has also been synthesized in high yield (*ca.* 85%) by hydrolysis and decarboxylation of compound **66**, which was obtained by an unusual cyclization of the precursor **65** in the presence of *p*-toluenesulfonic acid (**Scheme 12**).[62] Compound **65** is also an important intermediate in the synthesis of 4-hydroxycoumarin, a potent anticoagulant.[62]



Scheme 11

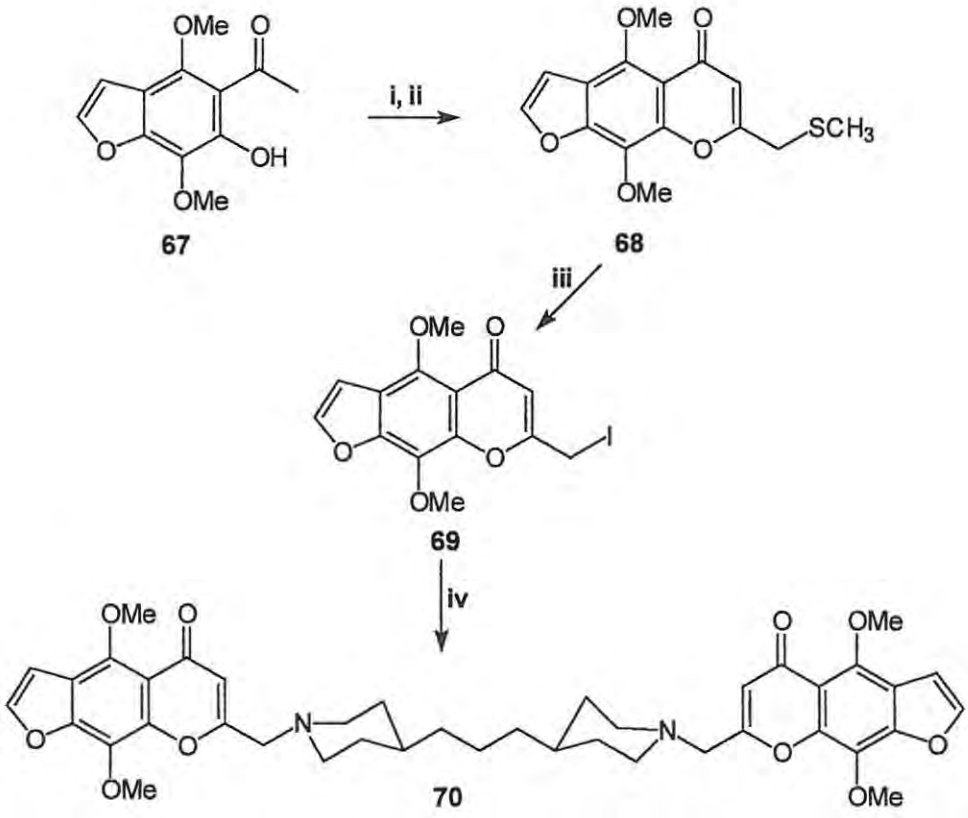
Reagents: i) Toluene, 100°C; ii) satd. aq. NaCl; iii) 20% HCl-MeOH, <10min.



Scheme 12

Reagents: i) $SOCl_2$, urea, toluene; ii) $CH_2(CO_2Et)_2$, Mg, EtOH; iii) *p*-TsOH
iv) 6**M**-HCl.

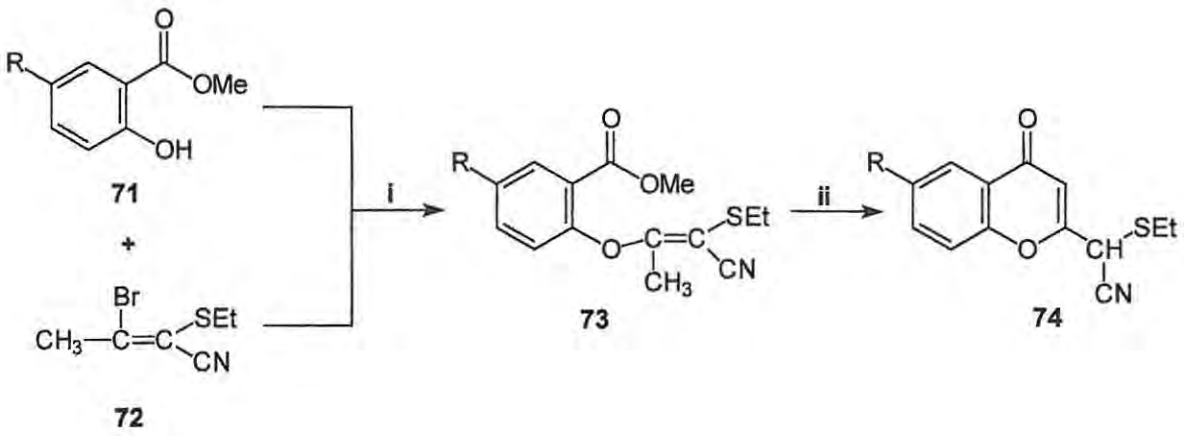
The furochromone moiety, present in the pharmacologically active khellin **9,** has attracted the interest of several groups of synthetic organic chemists. Recently, the bisaminofurochromone **70,** a bivalent inhibitor of AcylCoA: cholesterol O-acyltransferase (ACAT), one of the major regulators of cholesterol metabolism, has been synthesized by Gammil *et al.* in good yield as outlined in **Scheme 13.**[63] It has been suggested that ACAT activity increases when cells are exposed to cholesterol-rich lipoprotein. It has also been recognized that ACAT plays an important role in the intestinal absorption of cholesterol and that ACAT activity is greatest in the jejunum, where the majority of cholesterol absorption occurs. Since the intracellular accumulation of esterified cholesterol is one of the characteristic features of the atherosclerotic plaque, there is continuous interest in the hypothesis that regulation of ACAT activity is likely to be of great importance in atherosclerosis treatment.[63]



Scheme 13

Reagents: i) NaH, THF, $CH_3SCH_2CO_2Et$; ii) H_3O^+; iii) CH_3I, CH_2Cl_2,
iv) $C_{13}H_{26}N_2$, K_2CO_3, CH_3CN.

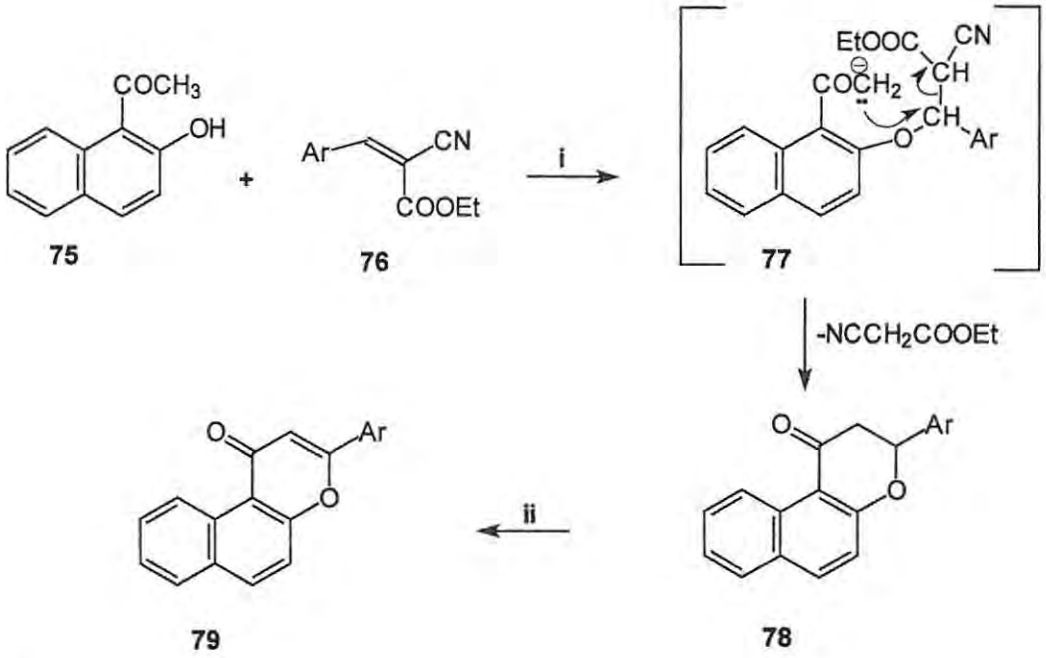
The substituted 2-methylchromones **74**, a rare class of chromone derivatives, have been prepared in overall yield (*ca.* 75%) by condensing the sodium salt of methyl salicylate **71** with the bromocrotononitrile **72** to give the intermediate vinyl ethers **73**, which are then cyclized by treatment with sodium hydride (**Scheme 14**).[64] The vinylogous cyanogen bromide **72** reacts readily with the ortho carboxylated phenols **71** to give the 2-functionalised chromone derivatives **74**, which are not easily obtained by other methods.



Scheme 14

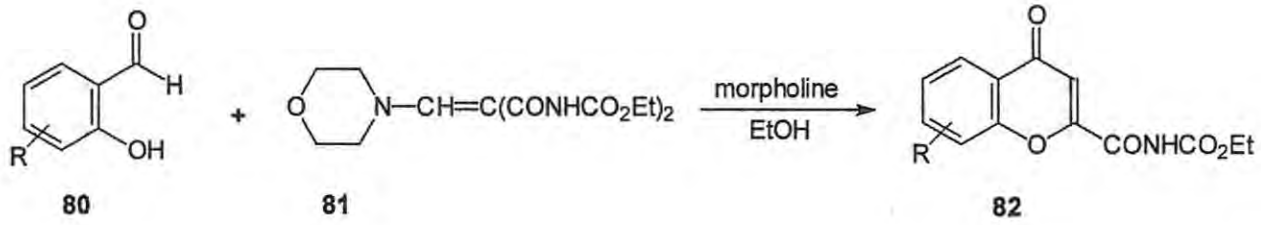
Reagents: i) NaH, dry DME; ii) NaH, dry DME, reflux.

Many synthetic flavones are known to exhibit pharmacological activity. 5,6- and 7,8-Benzoflavones have been shown to act as inhibitors of tumor induction by carcinogenic polycyclic aromatic hydrocarbons (PAHs). In contrast to most other types of tumor-inhibiting compounds, many of which exhibit toxicity, mutagenicity, and other undersirable properties, the flavone derivatives tend to show minimal side effects.[65] Recently, potentially antitumorigenic, polycyclic chromone derivatives **79** have been synthesized in excellent yield by the condensation of 2-hydroxy-1-acetonaphthone **75** with cinnamonitriles **76**, followed by cyclization and dehydrogenation as outlined in **Scheme 15**.[65]



Scheme 15

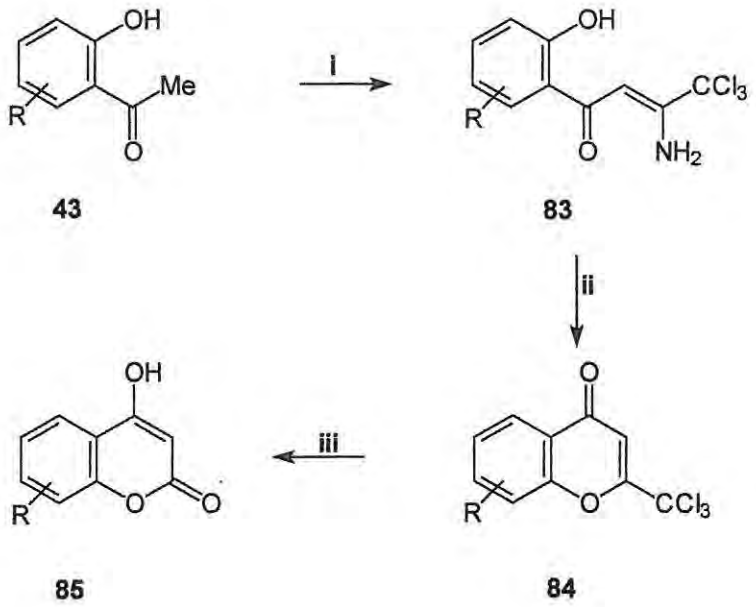
Reagents: i) Piperidine, EtOH, reflux, 6h; ii) DDQ, benzene.



Scheme 16

Ellis *et al.*[31] have shown that chromone derivatives containing a carboxamide group at C-2 possess antiallergic properties. Substituted chromone-2-carboxamides **82** are rapidly produced in high yield (>90%) by reacting salicylaldehydes **80** with the enamine **81** in ethanol (**Scheme 16**),[66] the reaction involving base-catalyzed condensation. 2-Trichloro-methylchromone derivatives **84** have been synthesized in excellent yield (*ca.* 95%) by condensing 2-hydroxyacetophenones **43** with trichloroacetonitrile in the presence of *N*-methylanilinomagnesium bromide to afford the intermediates **83**, which are converted into

the 2-trichloromethylchromones **84** upon treatment with concentrated HCl as shown in **Scheme 17**.[67] This synthetic route also provides access to 4-hydroxycoumarins **85** *via* base-catalyzed hydrolysis of the 2-trichloromethylchromone derivatives **84**.

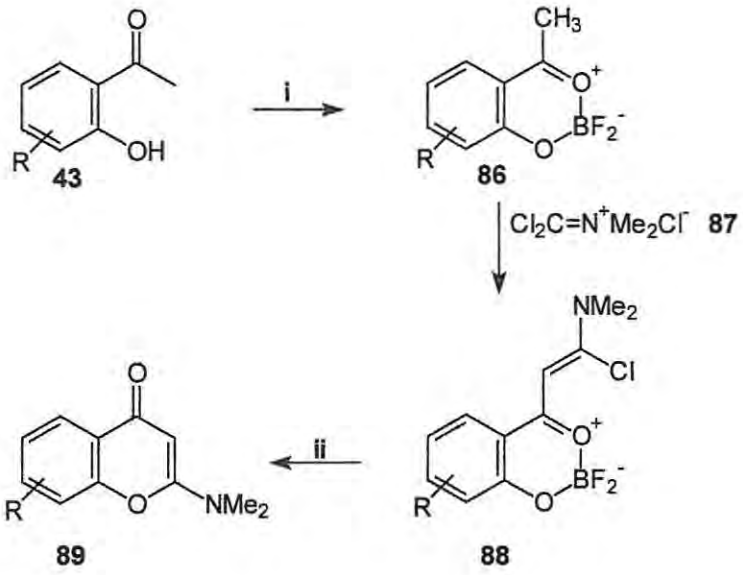


Scheme 17

Reagents: i) PhNMeMgBr, CCl_3CN, 20°C, 3h; ii) HCl, 1day
iii) KOH, MeOH, 0.5h, reflux.

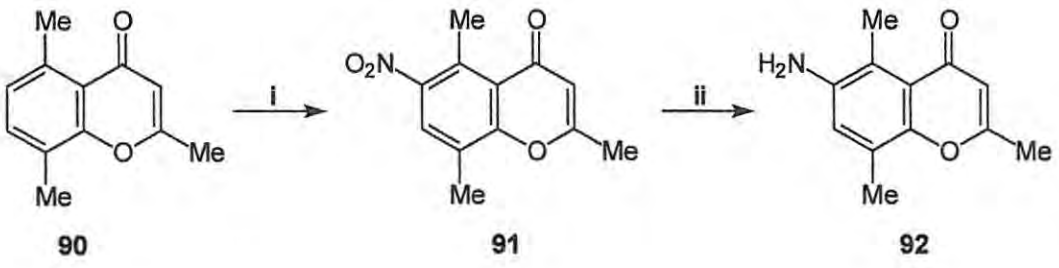
Recently, Morris and co-workers[68] reported a convenient procedure for the synthesis of substituted 2-aminochromones **89**, which exhibit antiplatelet activity, from substituted *o*-hydroxyacetophenones **43**. Thus, the substituted *o*-hydroxyacetophenones **43**, when treated with boron trifluoride etherate, afford the *o*-hydroxyacetophenone-BF_2 complexes **86**. Reaction of these complexes with the phosgeniminium salt **87** gives the β-chlorovinylogous amide complexes **88**, which undergo methanolysis to afford the 2-aminochromones **89** (**Scheme 18**). The initial protection of the phenolic hydroxyl group with boron trifluoride etherate is necessary in order to direct reaction of the phosgeniminium salt **87** to the methyl carbon of ketone group.[68]

Chromone derivatives **91** and **92**, which are useful intermediates in the synthesis of fungicides and herbicides, have been prepared in excellent yield from 2,5,8-trimethyl-chromone **90** as shown in **Scheme 19**.[69] Thus, nitration of the compound **90** occurs selectively at C-6, and reduction of the nitro group with Fe/HCl affords the amino derivative **92**.



Scheme 18

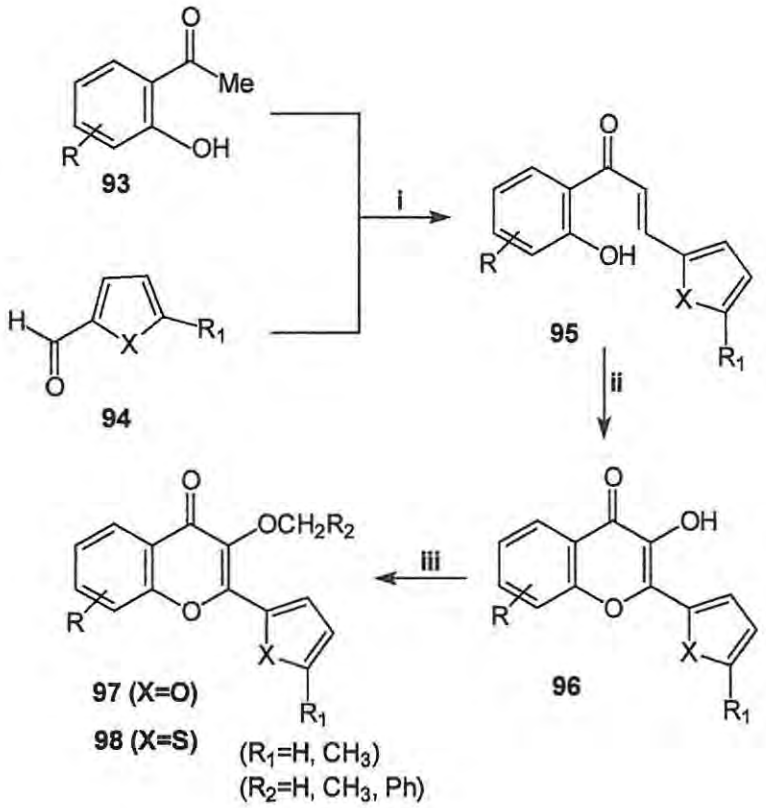
Reagents: i) $BF_3.OEt_2$, Et_2O; ii) MeOH, 50°C.



Scheme 19

Reagents: i) HNO_3, H_2SO_4; ii) Fe, HCl.

Chromone derivatives bearing a furan (**97**) or thiophene (**98**) ring at the C-2 position have been synthesized by condensing substituted 2-hydroxyacetophenones **93** with 2,5-disubstituted furans or thiophenes **94** (X=O, S) as illustrated in **Scheme 20**.[70,71] The synthesis involves a crossed-aldol condensation, followed by epoxidation using alkaline hydrogen peroxide and, finally, cyclization of the α,β-unsaturated carbonyl derivative **95**. These products (**97** and **98**) have been used to investigate the photochemistry of 3-alkoxychromones bearing furyl or thiophenyl groups, since furans themselves are known to undergo phototransformations.[70,71]

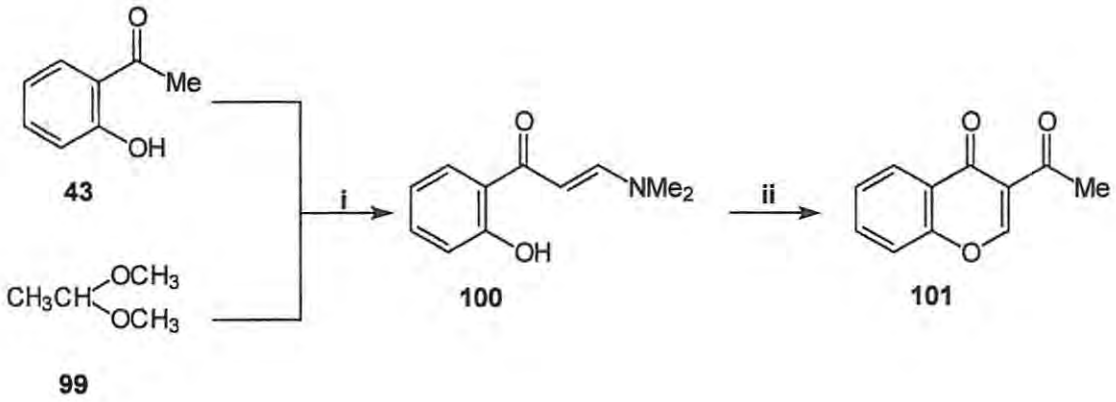


Scheme 20

Reagents: i) NaOH, EtOH; ii) H_2O_2, KOH; iii) K_2CO_3, Me_2CO, DMF, R_2CH_2Cl.

Chromone derivatives bearing a carbonyl group at C-3 appear to exhibit significant antiallergic activity.[29,30] Moreover, Dean *et al.*[72] have shown that a carbonyl group at the C-3 position strongly activates the chromone ring by its electron-withdrawing effect, permitting

selective C-2 alkylation using diazoalkanes. Recently, 3-acetyl-4*H*-1-benzopyran-4-one **101** has been synthesized by condensing 2-hydroxyacetophenone **43** with the dimethyl acetal **99** to give the intermediate 3-(dimethylamino)-1-(2-hydroxyphenyl)propen-1-one **100** in 85% yield, which, on refluxing with acetic anhydride in acetonitrile, affords the chromone **101** in *ca.* 62% yield (**Scheme 21**).[73]



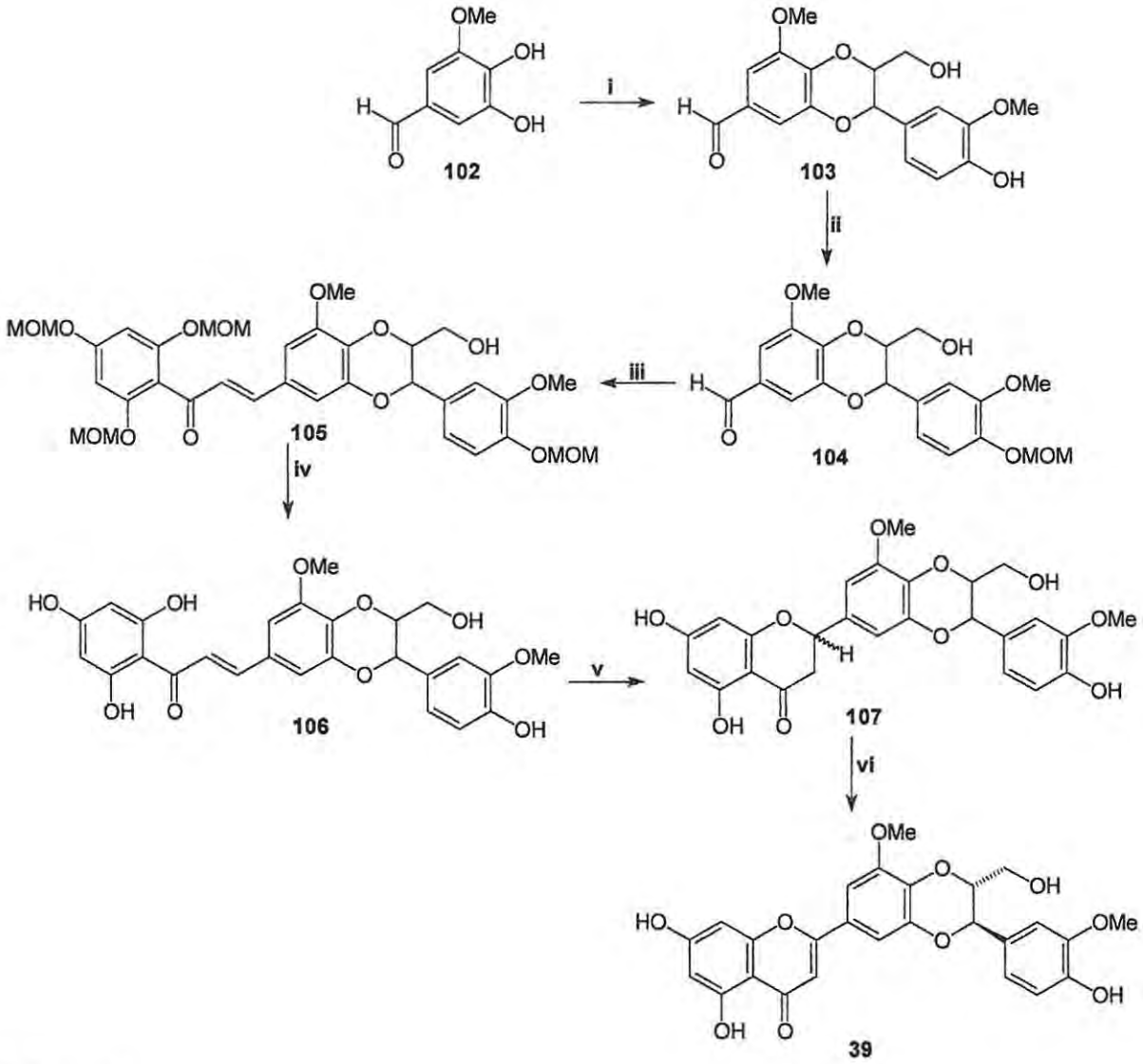
Scheme 21

Reagents: i) DMF; ii) Ac_2O, MeCN, reflux.

The chromone derivative, 5-methoxyhydnocarpin-D (5'-MHC-D) **39**, has been found to kill bacteria by first disabling their defence mechanisms; this is regarded as one of the two major discoveries in drug research in the year 2000,[44,45] the other being the discovery of the first family of compounds capable of inhibiting human immunodeficiency virus (HIV) integrase by Merck scientists. The compound, 5'-MHC-D **39**, was synthesized by coupling coniferyl alcohol with 3,4-dihydroxy-5-methoxybenzaldehyde **102** to afford compound **103**; subsequent crossed-aldol condensation, cyclization and dehydrogenation gave the chromone derivative **39** as outlined in **Scheme 22**.[44]

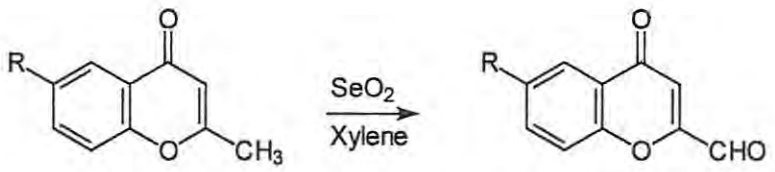
Selenium dioxide has been recognized as an effective reagent for the incorporation of an oxygen functionality at allylic positions[74] and, recently, chromone-2-carbaldehydes **109** have been synthesized in moderate yield (*ca.* 60%) by SeO_2 oxidation of 2-methylchromones **108** (**Scheme 23**).[75] Previous methods for obtaining such products have involved base-catalyzed

condensation of 2-methylchromone and *p*-nitroso-*N,N*-dimethylaniline followed by acid hydrolysis to afford the chromone-2-carbaldehyde in low yield,[76] or the oxidation of 2,2-dibromomethyl-3-methylchromone using aqueous ethanolic silver nitrate to afford chromone-2-carbaldehyde derivatives in *ca.* 65% yield.[77]



Scheme 22

Reagents: i) Coniferyl alcohol, Ag_2CO_3, C_6H_6-acetone(5:1), 60°C, 7h; ii) MOMCl, THF, NaH, rt, 7h; iii) KOH, EtOH, 2,4,6-tris(methoxymethoxy)aceto-phenone, 48h; iv) conc. HCl, MeOH, rt, 12h; v) NaOAc, MeOH, reflux 3h; vi) DDQ, dry dioxane, reflux, 36h



108 **109**

Scheme 23

1.2.3 Reactivity of chromone derivatives

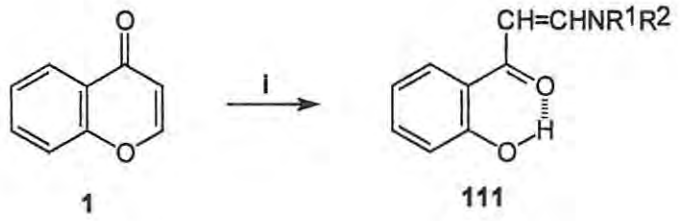
Chromones may react with nucleophiles, electrophiles or other reagents to give various derivatives. In this introduction, particular attention will be focussed on reactions of chromones with nitrogen nucleophiles to afford ring-opened and addition products.

1.2.3.1 Ring-opening reactions

Chromones readily undergo nucleophilic cleavage at the C-2 position of the heterocyclic ring,[78] and a variety of nitrogen nucleophiles have been used in the ring opening of chromones at this position. The pyran-4-one ring in chromone **1** is cleaved by oxygen nucleophiles and by primary and secondary amines to yield enamines.[79] Thus, Kostka *et al.*[80] showed that chromone **1** reacts with primary and secondary amines **110** to produce β-aminovinyl ketones **111** having either *E*- or *Z*-double-bond configurations (**Scheme 24**). Zagorevskii *et al.*[80,81] used ^{1}H NMR spectroscopy to study the detailed structure of the β-aminovinylketone products **111** and proposed a reaction mechanism to account for their formation, based on deuterium labelling data.

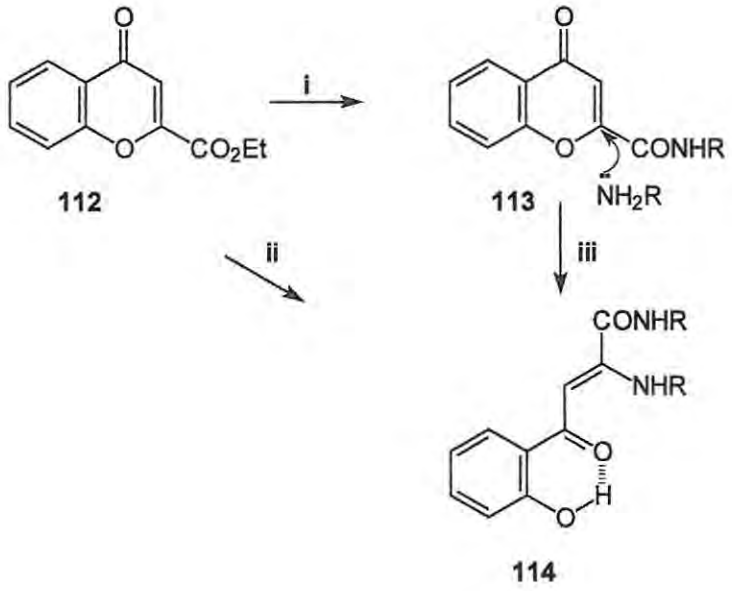
The chromone-2-carboxylate ester **112** reacts with amines or ammonia to give different products depending on the nature and quantity of the amine, and the reaction conditions used (**Scheme 25**). Secondary and tertiary carboxamides **113** may be produced under very mild, anhydrous conditions, while use of an excess of amine or prolonged reaction affords coloured acrylamides **114**.[81-85] The latter reaction is presumed to proceed *via* the carboxamide with a second mole of amine cleaving the ring, since treatment of the preformed carboxamide with benzylamine afforded the corresponding acrylamide.[86] The susceptibility of chromone

derivatives to ring-opening *via* nitrogen nucleophilic attack at C-2 prompted Kaye and co-workers to explore the amine-mediated formation of (*E*)-2-(*N,N*-dimethylamino)-3-(2-hydroxybenzoyl)acrylamides **117** from the 4-oxo-4*H*-chromone-2-carboxamides **116** (**Scheme 26**).[87,88] They also showed that the dimethylamine-mediated ring-opening of chromone-2-carboxamides **116** follows a third-order kinetics and proposed the mechanistic sequence detailed in **Scheme 26**.[87]



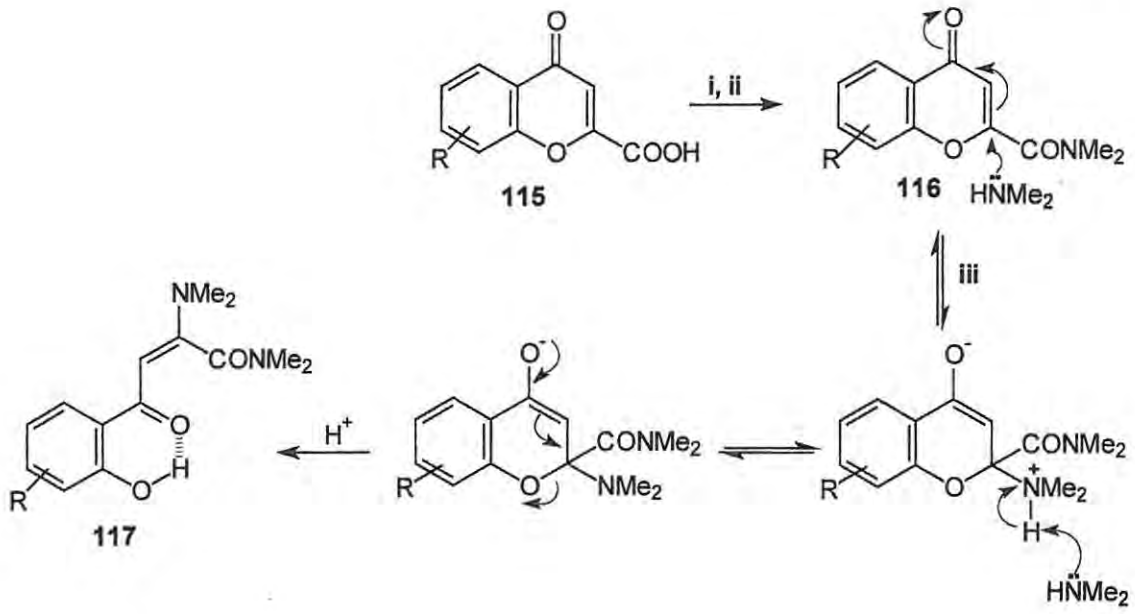
Scheme 24

Reagents: i) R^1R^2NH (**110**), 80-85°C, 2h.



Scheme 25

Reagents: i) RNH_2 (1 molar eq.)
ii) RNH_2 (2 molar eq.)
iii) RNH_2 (1 molar eq.)

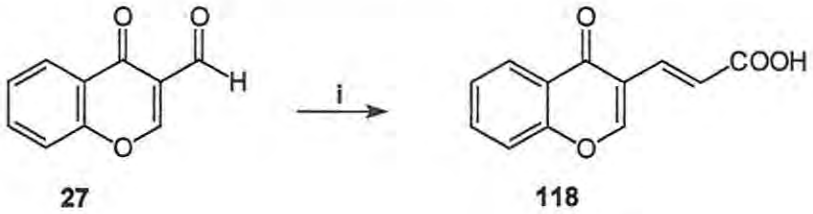


Scheme 26

Reagents: i) $SOCl_2$, DMF-$ClCH_2CH_2Cl$; ii) Me_2NH_2Cl, pyridine
iii) Me_2NH, EtOH

1.2.3.2 Nucleophilic addition to chromone carbaldehydes

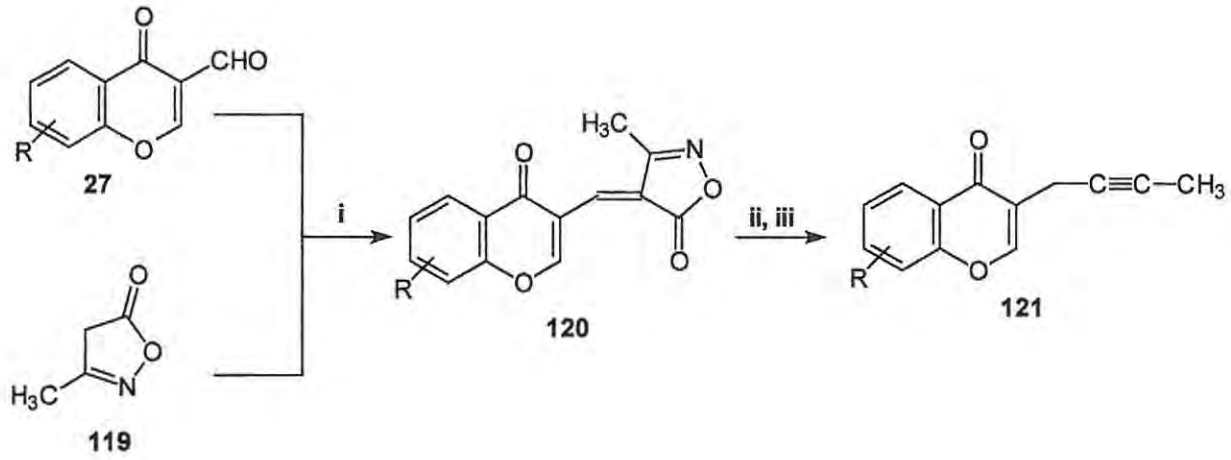
Despite the susceptibility of chromone derivatives to ring-opening of the pyran-4-one moiety by nucleophiles, chromone-3-carbaldehyde **27** generally undergoes nucleophilic addition at the formyl carbon to afford condensation products. Chromone-3-carbaldehyde **27** has been shown to react with a monofunctional nucleophile, such as malonic acid in the presence of pyridine to afford the acrylic acid derivative **118** in *ca.* 74% yield (**Scheme 27**).[89] However, synthesis of benzopyran compounds having unsaturation in the 3-alkyl side chain is generally difficult.[90] Sabitha and co-workers, however, have recently reported the synthesis of the acetylenes **121** under mild conditions by condensing chromone-3-carbaldehydes **27** with 3-methyl-5-isoxazolone **119** in ethanol at room temperature, and selectively reducing the resulting isoxazolone intermediate **120** with sodium borohydride in methanol to give the desired acetylene derivatives **121** in high yield (> 80%) (**Scheme 28**).[90]



27 **118**

Scheme 27

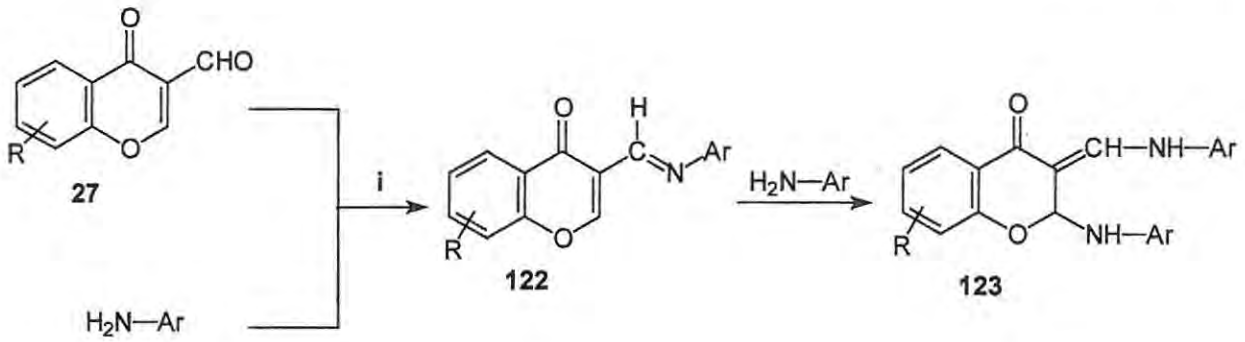
Reagents: i) $CH_2(CO_2H)_2$, pyridine.



Scheme 28

Reagents: i) Ethanol; ii) $NaBH_4$, methanol; iii) aq. $NaNO_2$, $FeSO_4$, AcOH.

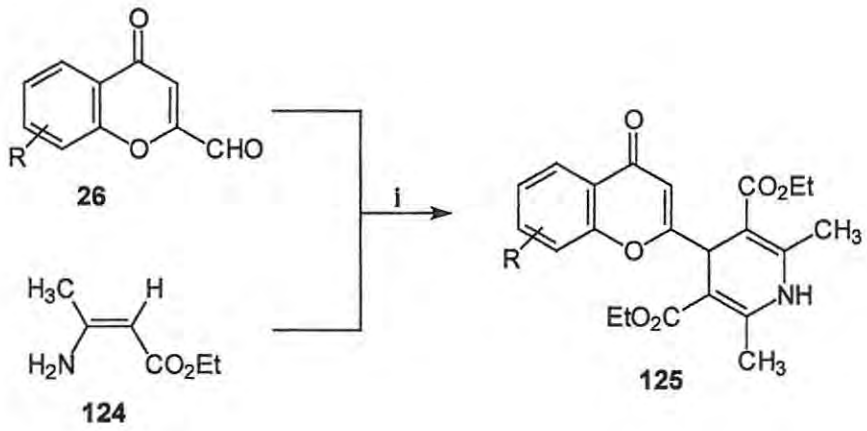
Chromone-3-carbaldehyde **27** also reacts readily with primary aromatic amines[91,92] and secondary amines such as piperidine.[93] Chromone-3-carbaldehydes **27** have been shown to condense with aromatic amines to give, firstly, the 3-aryliminomethylchromones **122** and then following the addition of a second molecule of amine to the imine intermediates, the 2-arylamino-3-arylaminomethylenechroman-4-ones **123** (**Scheme 29**).[94] The 3-arylimino-methyl group apparently stabilizes the chromone ring towards the usual ring-cleavage by aromatic amines and also facilitates the addition of other external nucleophiles which would not otherwise react with the chromone ring.[94]



Scheme 29

Reagents: i) Dry benzene, reflux 30min.

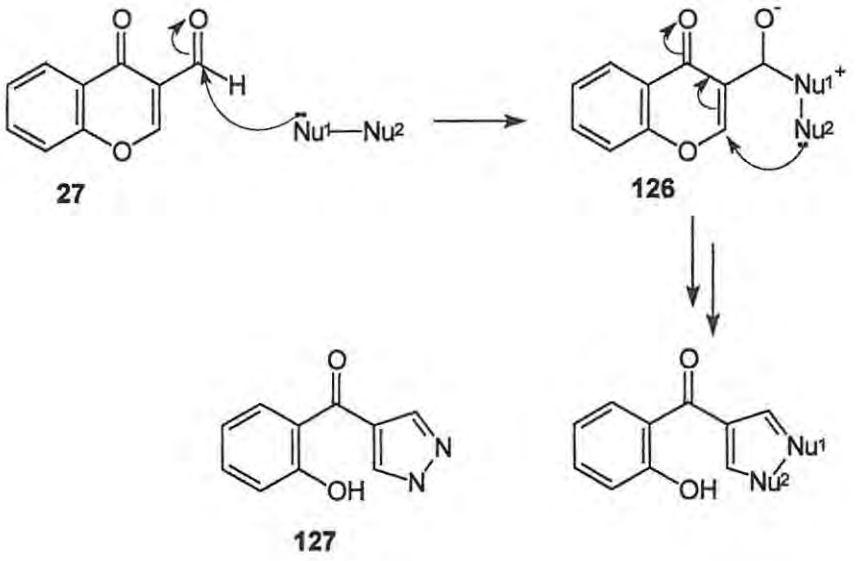
Chromone derivatives having heterocyclic substituents at C-2 and C-3 have been shown to exhibit coronary spasmolytic and bronchodilatory activities, and some are used in the treatment of asthma.[31] Jagadeesh *et al.*[75] has successfully carried out the condensation of chromone-2-carbaldehydes **26** with two equivalents of ethyl aminocrotonate **124** *via* a modified Hantzsch synthesis to give the chromone derivatives **125** in good yield (**Scheme 30**).[75]



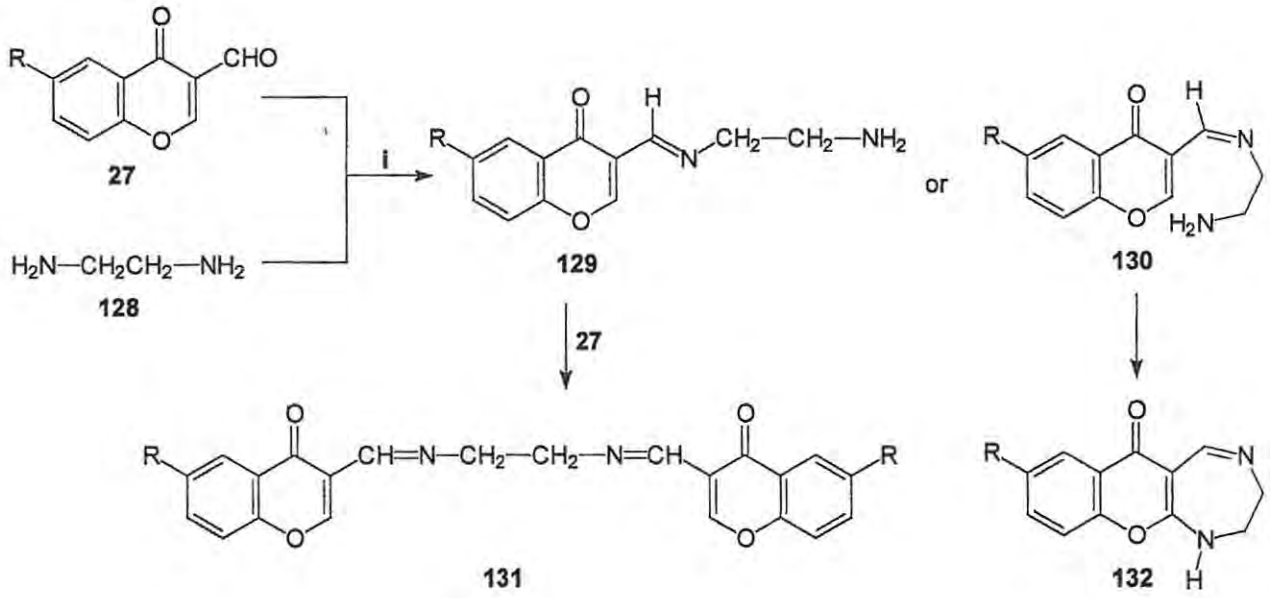
Scheme 30

Reagents: i) AcOH, rt, 96h.

Chromone-3-carbaldehyde **27** may react with a bifunctional nucleophile (Nu^1-Nu^2) to form a new heterocycle. Thus, nucleophilic attack at the formyl carbon gives, initially, an intermediate **126** which, following intramolecular attack by Nu^2 at the reactive C-2 position, results in the ring-forming/ ring-opening sequence illustrated in **Scheme 31**.[95] This approach has been used to prepare products such as 4-(2-hydroxybenzoyl)pyrazole **127**.[96]



Scheme 31



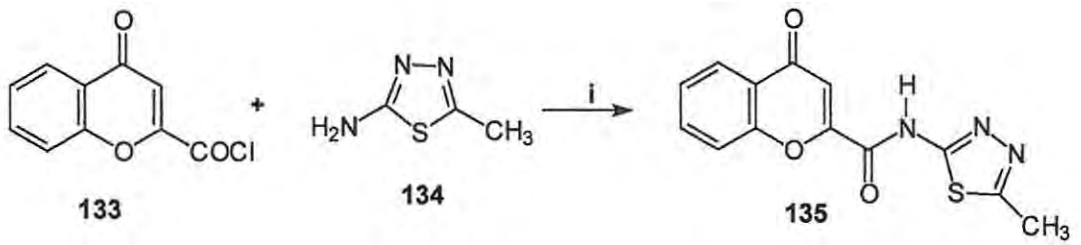
Scheme 32

Reagents: i) Dry benzene, reflux, 2h.

Condensation of chromone-3-carbaldehydes **27** with the binucleophile, 1,2-ethanediamine **128** in dry benzene affords the bis-aldimines **131** in excellent yield (**Scheme 32**).[97] The first step in the reaction involves the formation of aldimines, which may exist in two isomeric forms **129** and **130**. The *E*-isomer **129** cannot undergo intramolecular cyclization because the terminal amino group is too far away to react with either of the two electrophilic centres *viz.*, C-2 and the carbonyl carbon of the chromone moiety; it may, however, condense with a second molecule of the chromone **27** to give the corresponding bis-imine **131** (**Scheme 32**). The *Z*-isomer, on the other hand, may cyclize to afford a fused diazepine **132** as illustrated in **Scheme 32**.[97]

1.2.3.3 Other reactions of chromone derivatives

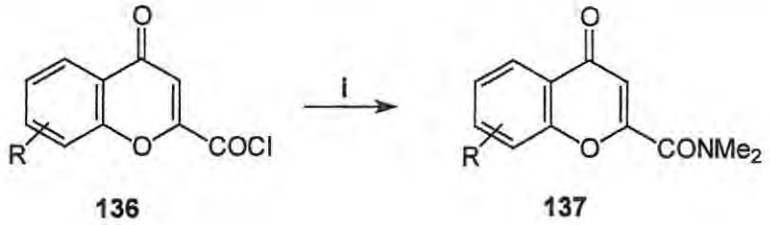
Chromones undergo many different types of reaction, some of which provide access to derivatives with interesting medicinal properties. Ellis *et al.*[31,32] have shown that the antiallergic chromones are generally those which contain a carboxamide group at C-2, and there has been a continuing interest in the synthesis of analogues of such chromone derivatives. Recently, reaction of 2-amino-5-methyl-1,3,4-thiadiazole **134** with chromone-2-carbonyl chloride **133** was shown to give the carboxamide **135** (**Scheme 33**),[98] while Davidson *et al.*[99] achieved the synthesis of *N,N*-dimethylchromone-2-carboxamides **137** in excellent yield by reacting the corresponding acid chlorides **136** with dimethylammonium chloride in dry pyridine (**Scheme 34**).[99]



133 134 135

Scheme 33

Reagents: i) Dry benzene, reflux, 3h.

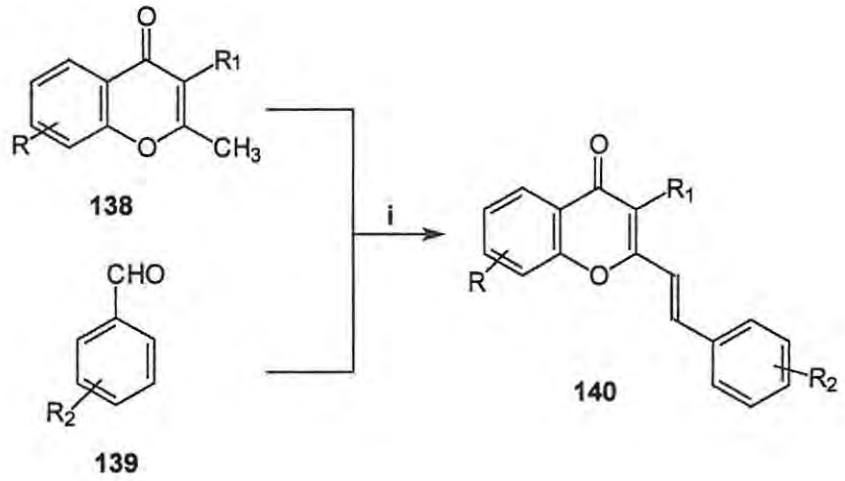


136 **137**

Scheme 34

Reagents: i) Me_2NH_2Cl, dry pyridine, 0°C.

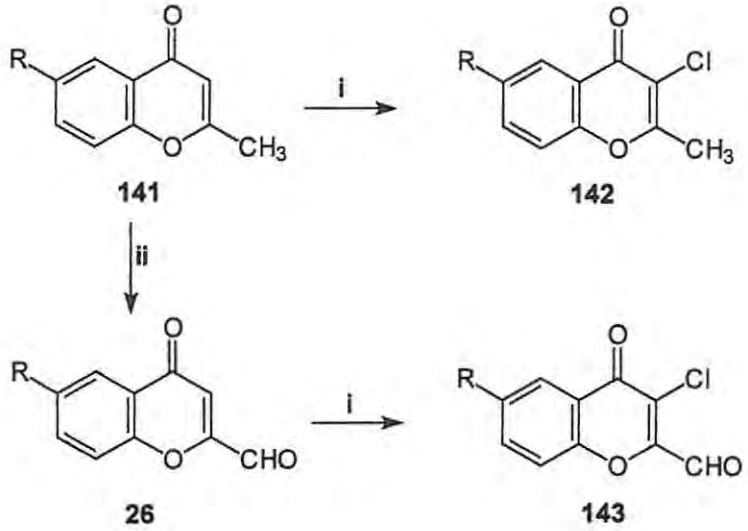
In an approach used for the synthesis of the pharmacologically active hormothamniones **17**,[24,25,100] base-induced condensation of a 2-methylchromones **138** with substituted benzaldehydes **139** afforded the 2-styrylchromones **140** (**Scheme 35**).[101]



Scheme 35

Reagents: i) NaOEt, EtOH, rt, 24h.

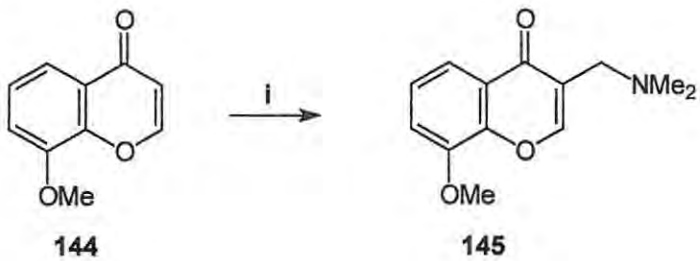
The 3-chlorochromones **142** and **143** have been prepared in excellent yield by selective C-3 monochlorination of 2-methylchromones **141** and chromone-2-carbaldehydes **26** using aqueous sodium hypochlorite and acetic acid (**Scheme 36**);[102] these products are starting materials for the synthesis of chromone derivatives having heterocyclic substituents at C-2, as discussed in Section 1.2.3.2. p.30.



Scheme 36

Reagents: i) NaOCl, AcOH; ii) SeO_2, xylene.

Chromones typically resist electrophilic attack, since electrophilic reagents are often strongly acidic and, hence, likely to protonate the pyran-4-one ring, thus inhibiting further attack by the electrophile.[2] However, aminomethylation of 8-methoxychromone **144** may be achieved under less acidic Mannich reaction conditions to afford 3-aminomethylchromone **145** (**Scheme 37**).[103] This reaction is, however, inhibited by a methyl substituent at C-2.[103] 3-Hydroxychromone **146** may be brominated, nitrated and aminated at C-2, a position which is usually electron-deficient but is presumably activated by the hydroxyl group to give the corresponding derivatives **147** as shown in **Scheme 38**.[103]



Scheme 37

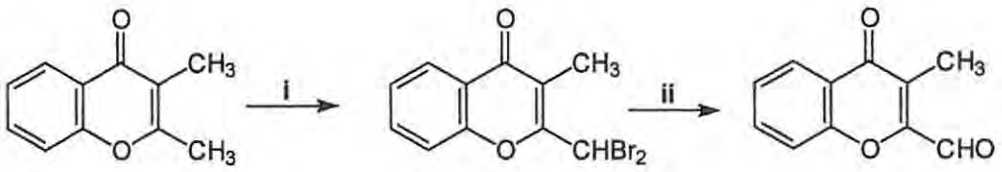
Reagents: i) HCHO, Me_2NH, AcOH.

Electrophilic reagents may react with the side chains of certain chomone derivatives to give interesting products. Thus, 2,2-dibromomethyl-3-methylchromone **149**, a key intermediate in the synthesis of 3-methylchromone-2-carbaldehyde **150**, has been synthesized in high yield (*ca.* 79%) by treating 2,3-dimethylchromone **148** with slightly more than a two-fold excess of bromine in boiling benzene (**Scheme 39**),[77] dibromination of the C-2 side chain occurring in preference to addition or substitution on the chromone nucleus.

146 **147**

R= Br, NO_2 or NHR

Scheme 38

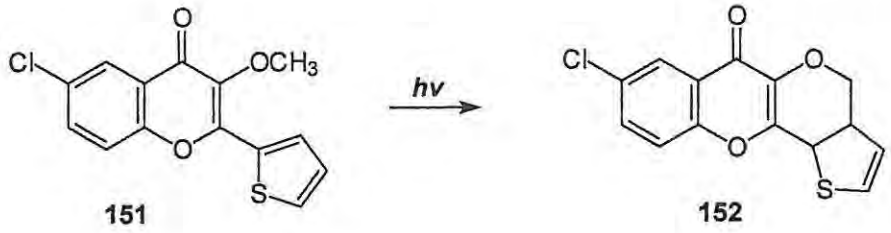


148 **149** **150**

Scheme 39

Reagents: i) Br_2, dry benzene; ii) $AgNO_3$.

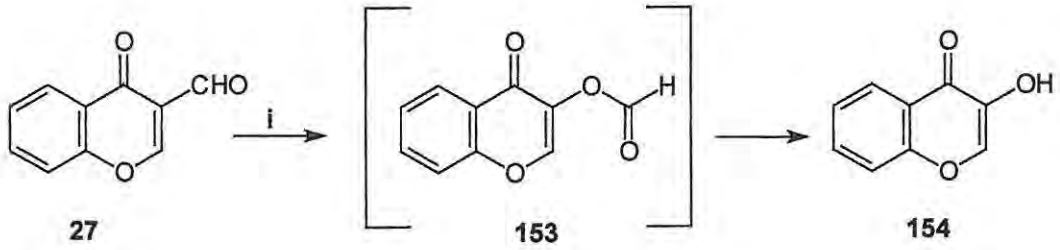
Photoirradiation of a methanolic solution of the 2-thienyl derivative **151** with pyrex-filtered UV light has been reported to afford the cyclized product **152** (**Scheme 40**).[71]



151 **152**

Scheme 40

Baeyer-Villiger oxidation of chromone-3-carbaldehyde **27** using *m*-chloroperbenzoic acid (MCPBA) in boiling dichloromethane has been reported to afford 3-hydroxychromone **154** in good yield, as compared to other methods (**Scheme 41**).[104] 3-Hydroxychromone **154** has also been prepared by simply oxidizing 2,3-unsubstituted chromones with *m*-chloroperbenzoic acid in dry benzene.[104]



27 **153** **154**

Scheme 41

Reagents: i) MCPBA, CH_2Cl_2.

1.2.4 HIV Protease inhibitors

Since the human immunodeficiency virus (HIV) epidemic outbreak some 15 years ago, an estimated 47 million or more people world-wide have been infected with the virus, and more than 2.2 million deaths were attributed to the disease in 1998. HIV/AIDS is now considered to be the fourth leading cause of death, with its impact likely to increase if the problem does not receive urgent attention.[105] The alarming rate of spread of the acquired immuno-deficiency syndrome (AIDS) has prompted widespread efforts to understand and find ways of combating this disease.[106] The identification of the molecular events, which occur during the replication stage of the virus, has permitted the selection of several chemotherapeutic strategies.[106] The genome of the HIV-1 virus encodes a proteinase (HIV-1 protease) which cleaves the *gag-pol* proteins; inhibition of this cleavage results in the production of immature virions which are non-infectious, thus, inhibiting multiplication of the virus. The chemical inhibition of this deadly viral enzyme has been recognized as a powerful strategy for the development of an effective therapy for the treatment of AIDS and other related diseases.[107] Although, the role of viral replication at particular stages of the disease progression is still being debated, there is a general consensus that inhibition of HIV replication in infected patients will result in a reduction of mortality.[108] Hence, HIV-1 protease has been recognized as an attractive target for antiviral therapy, and the discovery of potent inhibitors has been the focus of many research programmes.[109]

1.2.4.1 HIV-1 protease inhibitor design

Computer-aided drug design strategies have been applied in the development of a variety of highly potent inhibitors,[110] and an increasing number of crystal structures of HIV-1 protease inhibitors are available in the Brookhaven Protein Data Bank (PDB).[111,112] Figure 1 shows the X-ray structure (coded as **1HXW**) of HIV-1 protease complexed with ritonavir **156**,[113] a drug in clinical use. HIV-1 protease is an aspartic acid protease, which cleaves specific amide bonds (peptides) in a similar manner to renin and endothiopepsin. It is a dimer made up of two 99-amino acid monomers, each contributing an aspartic acid residue at the catalytic site.[109] The enzyme functions as a "molecular pair of scissors", hydrolyzing the viral *gag-pol* precursor proteins to produce structural proteins needed by the human immunodeficiency

virus.[109] The ideal HIV-1 protease inhibitor:- should be potent against various HIV clinical isolates; be specific for HIV-1 protease (compared with other mammalian aspartic acid proteases), so as to minimize possible adverse effects; have good oral bioavailability and duration in humans; and safe to use and well tolerated.[109,110,114]

There are currently four HIV protease inhibitors approved for marketing as drugs in the USA in the clinical treatment of AIDS. These are:- Inverase® (Saquinavir mesylate; Hoffman-La Roche) **155**; Norvir® (Ritonavir; Abbott laboratories) **156**; Crixivan® (Indinavir sulfate; Merck) **157**; Viracept® (Nelfinavir mesylate; Agouron Pharmaceuticals) **158**. Others, like Amprenavir **159**, are in late-stage clinical trials.[109,115]

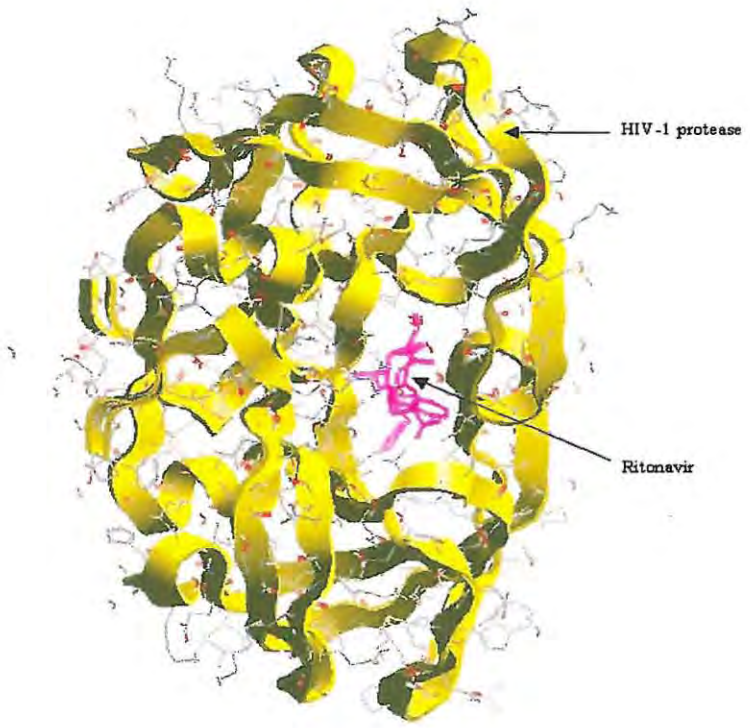
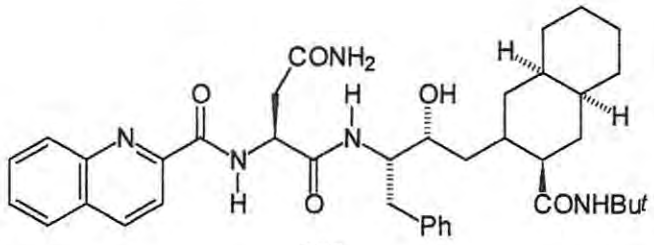


Figure 1. X-ray crystallographic structure (coded as **1HXW**) of HIV-1 protease complexed with the drug, ritonavir **156** (see ref. 113).



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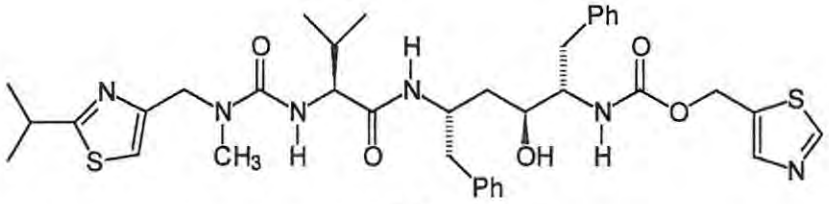

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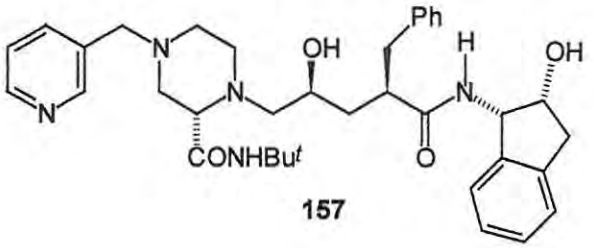

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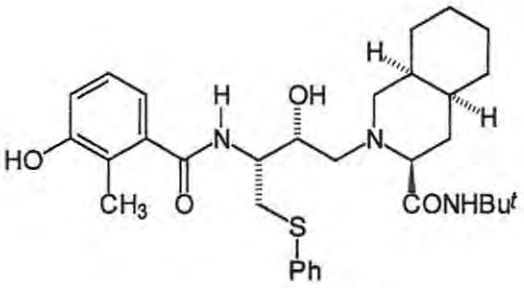

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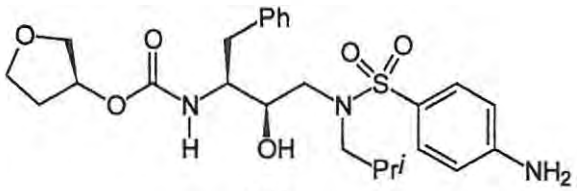
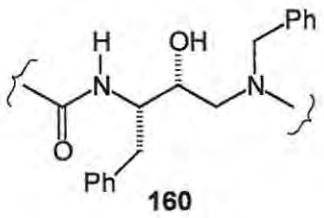


159

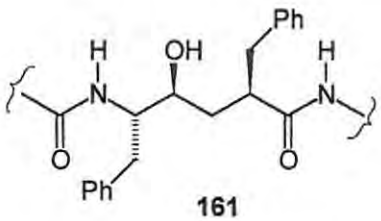
Many of the highly potent HIV-1 and HIV-2 protease inhibitors, which incorporate the non-hydrolyzable hydroxyethylamine **160** and hydroxyethylene dipeptide **161** isosteres, are active at nanomolar concentrations.[108] These inhibitors have a defined configuration at the hydroxyl-bearing asymmetric carbon, and these configurations are considered to be important

for inhibitory potency. HIV protease inhibitors, which incorporates the hydroxyethylene isostere **161**, have been shown to possess an *S*-configuration at this centre,[116,117] while inhibitors incorporating the hydroxyethylamine isostere **160** show a marked preference for an *R*-configuration.[116,118] Thus, the absolute configuration is very important for biological activity. The hydroxyl group in the isosteres has been shown to participate in hydrogen-bonding interactions with the catalytic aspartic acid residues of the HIV-protease, and the orientation of the hydroxyl group plays an important role with respect to the degree of binding.



160

Hydroxyethylamine isostere

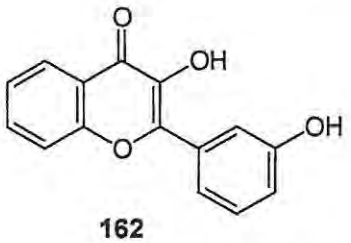


161

Hydroxyethylene dipeptide isostere

1.2.4.2 Chromones and their phenyl-substituted derivatives as HIV-1 protease inhibitors

It has been found[108,119,120] that chromones such as **162 – 164**, which bear hydroxylated phenyl substituents at C-2 (flavones) and the 3-substituted derivatives **165**, can inhibit HIV-1 protease. Such compounds are known as non-peptidic HIV-1 protease inhibitors, in which the hydroxyl substituents interact with the enzyme *via* hydrogen-bonding.[120,121] Isolicoflavonol **164** and glycyrrhisoflavone **165**, have been isolated from licorice and show antiviral properties, including anti-HIV activity, by inhibiting the cytopathic activity of the HIV virus.[122,123] 5,2',4'-Trihydroxy-6,7,5'-trimethoxyflavone **163**, a chromone derivative isolated from *Artemisia capillaris* Thunb., exhibits significant activity against HIV replication in H9 lymphocytic cells.[124,125] The plant, *A. capillaris*, is a famous traditional Chinese medicine used mainly as a choleretic, anti-inflammatory and diuretic agent in the treatment of epidemic hepatitis; in addition, the plant has found use in the traditional treatment of viral-induced liver inflammation and jaundice.[124,125]



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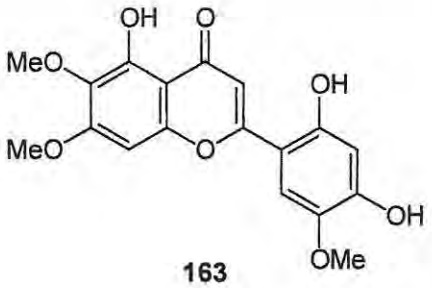

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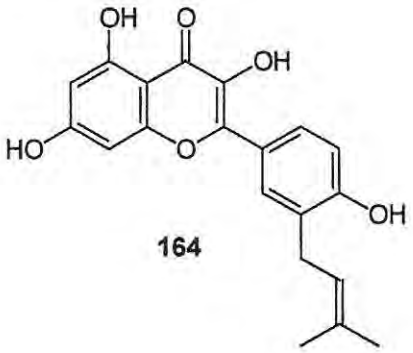
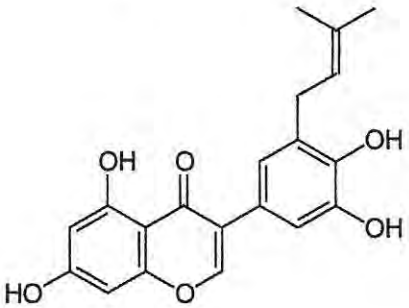


164



165

1.3 Previous work done in the group and aims of the present investigation

As a result of their varied biological activities, chromone derivatives have been the subject of considerable pharmaceutical and chemical interest, and the investigation of their chemistry, which forms the basis of this project, is part of an ongoing progamme within our research group.

Over the past years, work in this area has been focussed on synthetic and physical organic studies of chromone derivatives. Infrared studies of substituted chromone-2-carboxylate esters revealed doubling of the IR carbonyl bands, which was shown to be solvent-, substituent- and temperature-dependent, and which was rationalized in terms of rotameric equilibria between *syn-s-trans* and *anti-s-trans* forms.[126] An efficient synthesis of substituted chromone-2-carboxamides has been developed,[99] and dynamic NMR studies of these compounds revealed a temperature-dependent splitting of the *N*-alkyl ^{1}H- and ^{13}C NMR signals, which was attributed to internal rotation of the amide group.[127]

The susceptibility of chromone derivatives to ring-opening *via* nucleophilic attack at C-2 has been illustrated by the amine-mediated ring-opening of substituted chromone-2-carbox-

amides,[87] and a kinetic-mechanistic study demonstrated the influence of substituents on the ring-opening process.[88] Mass spectrometric analysis of the ring-opened, polyfunctional acrylamide derivatives has permitted elucidation of their major fragmentation patterns,[128] while dynamic NMR analysis of rotational isomerism in these systems permitted the calculation of internal rotational barriers.[129]

Dynamic ^{1}H NMR spectroscopic technique was used to explore the influence of substituents on the internal rotation of the amino group in 2-(*N,N*-dialkylamino)chromones;[130] nitrogen lone-pair delocalization was presumed to inhibit rotation about the N-C(O) bond – a property which has some influence on the basicity of these compounds, as will be discussed later. Research has also addressed the influence of various substituents on the electron density at C-2 and, hence, on the acidity in a series of 2-carboxychromones[131] and, an investigation of substituent effects on the basicity of 2-*(N,N*-dimethylamino)chromones was seen as a possible extension of these studies.

Applications of the Morita-Baylis-Hillman reaction have also enjoyed considerable attention in our group. Morita-Baylis-Hillman products have provided convenient access to 2-substituted indolizines,[132] quinoline derivatives,[133] chromenes,[134] thiochromenes[135] and coumarins.[136] Chromone-3-carbaldehydes were identified as interesting substrates for the continuation of these studies.

Consequently, the aims of this research have included the following.

1. The synthesis and characterization of a series of substituted 2-(*N,N*-dimethylamino)-chromones.
2. Potentiometric analysis of the substituted 2-(*N,N*-dimethylamino)chromones to explore the effect of substituents on their basicity.
3. Synthesis, characterization, and bioassay analysis of the natural product, granulosin [7,8-(methylenedioxy)-2-propylchromone], and its C-2 side-chain analogues.
4. Synthesis, characterization, and bioassay analysis of the natural product, 5-hydroxy-7-methoxy-2-isopropylchromone, and its C-2 side-chain analogues.
5. An investigation of the mass fragmentation patterns exhibited by the "granulosin" and 5-hydroxy-7-methoxy-2-alkylchromone series.

6. Synthesis and characterization of novel chromone-containing analogues of the HIV-1 protease inhibitor, ritonavir.
7. Computer-modelling studies of the chromone-containing analogues of the HIV-1 protease inhibitor, ritonavir, using an interactive docking programme to explore their binding to the enzyme active site.
8. Synthesis of substituted chromone-3-carbaldehydes and chromone-2-carbaldehydes, and an investigation of their reactions with acrylonitrile and methyl acrylate under Morita-Baylis-Hillman condition.

2. DISCUSSION

2.1 Synthesis and pK_a studies of 2-(*N,N*-dimethylamino)chromone derivatives

*2.1.1 Synthesis of 2-(*N,N*-dimethylamino)chromones*

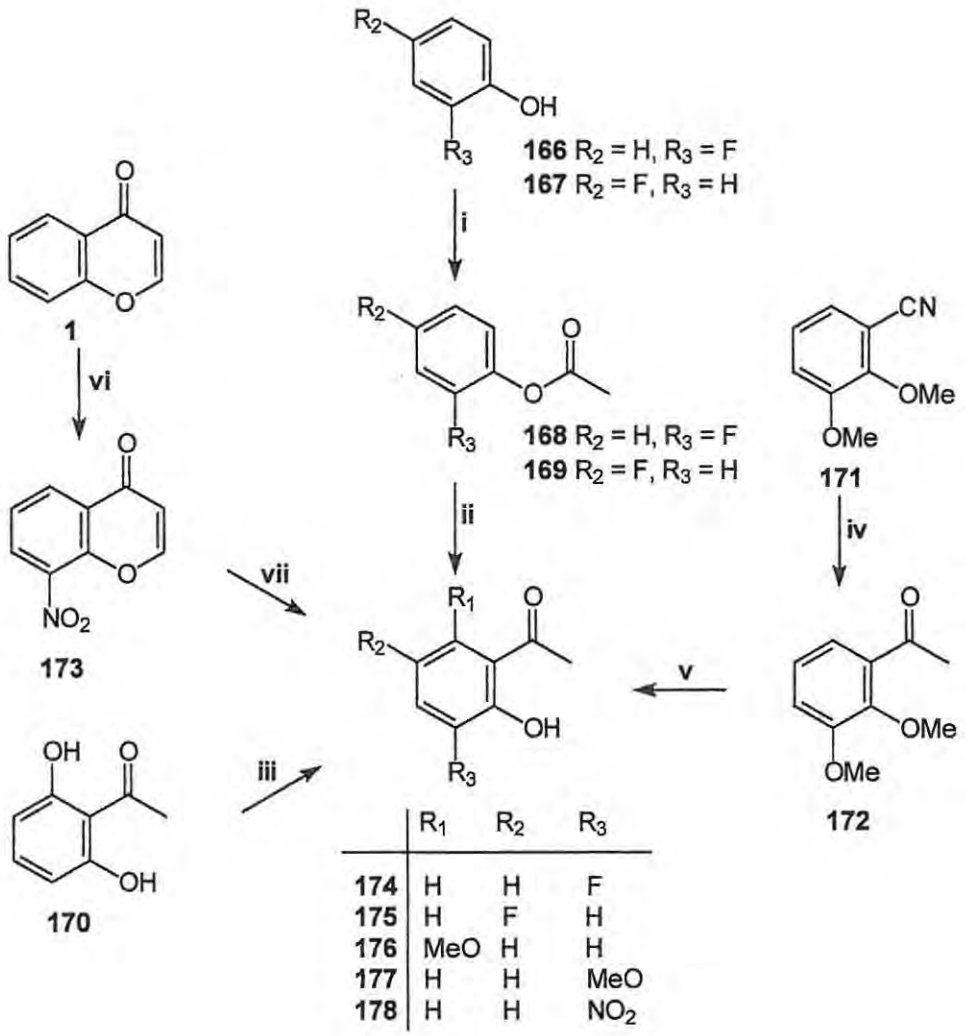
As indicated in the introduction, a number of 2-aminochromones have been shown to exhibit pharmacological activity[33] and, in this study, a range of 2-(*N,N*-dimethylamino)chromones were prepared in order to examine the effects of substituents on their basicity. The compounds were targeted to complete a study initiated in these laboratories by Sabbagh.[144] The synthetic methodology used involved the formation of phosgeniminium salt intermediates from *o*-hydroxyacetophenone precursors.

The substituted *o*-hydroxyacetophenones **174** and **175** (**Scheme 42**) were prepared *via* Fries rearrangement[2] of the corresponding substituted phenyl acetates **168** and **169** which, in turn, were obtained by acetylation of the appropriate phenols **166** and **167**, as described by Bryan *et al.*[137] The acetates were heated in an oil bath at high temperature (*ca.* 190°C) which favours rearrangement to the *o*-hydroxyacetophenones rather than the *p*-analogues.[138] The Fries rearrangement of 4-fluorophenyl acetate **169** afforded 5'-fluoro-2'-hydroxyacetophenone **175** in 70% yield, while 2-fluorophenyl acetate **168** give 3'-fluoro-2'-hydroxyacetophenone **174** in low yield (*ca.* 12%).

2'-Hydroxy-6'-methoxyacetophenone **176** was prepared in excellent yield (*ca.* 80%) by methylation of 2',6'-dihydroxyacetophenone **170**, using one equivalent of methyl iodide and one equivalent of K_2CO_3 in acetone, as described by Lau *et al.*[139] 2'-Hydroxy-3'-methoxyacetophenone **177** was obtained in moderate yield (*ca.* 52%) *via* selective demethylation of 2',3'-dimethoxyacetophenone **172** at high temperature in the presence of anhydrous $AlCl_3$, following the method described by Baker *et al.*;[50] the precursor **172** was prepared, in turn, *via* a Grignard reaction of 2,3-dimethoxybenzonitrile **171** with methyl magnesium bromide.[140]

2'-Hydroxy-3'-nitroacetophenone **178**, which was required as a precursor for the synthesis of 2-(*N,N*-dimethyamino)-8-nitrochromone **184**, is not commercially available and,

consequently, was synthesized in good yield (*ca.* 63%) as indicated in **Scheme 42.** This method involves nitration of chromone **1** using P.Da Re`s procedure,[141] followed by alkaline hydrolysis of the resulting 8-nitrochromone **173** to afford the required 2'-hydroxy-3'-nitroacetophenone **178.**[141]



Scheme 42

Reagents: i) aq. NaOH, Ac_2O, O°C; ii) $AlCl_3$, heat; iii) MeI, K_2CO_3, acetone; iv) MeMgBr, dry Et_2O; v) $AlCl_3$, dry Et_2O, reflux; vi) conc.H_2SO_4, fuming HNO_3; vii) 5% KOH, reflux.

The ^{1}H NMR spectrum of compound **178** (Figure 2) reveals the presence of an acetyl methyl singlet at δ 2.74 ppm and a low-field singlet at δ 12.85 ppm corresponding to the strongly

deshielded, hydrogen-bonded phenolic proton. Confirmation of the structure of the product, 2'-hydroxy-3'-nitroacetophenone **178**, is provided by the DEPT-135 and ^{13}C NMR spectra (Figures 3 and 4, respectively). The DEPT-135 spectrum shows 4 carbon signals corresponding to one methyl and three methine carbons, while the ^{13}C NMR spectrum shows 8 carbon signals with the acetyl methyl group resonating at 26.7 ppm and the carbonyl carbon at 203 ppm.[¶]

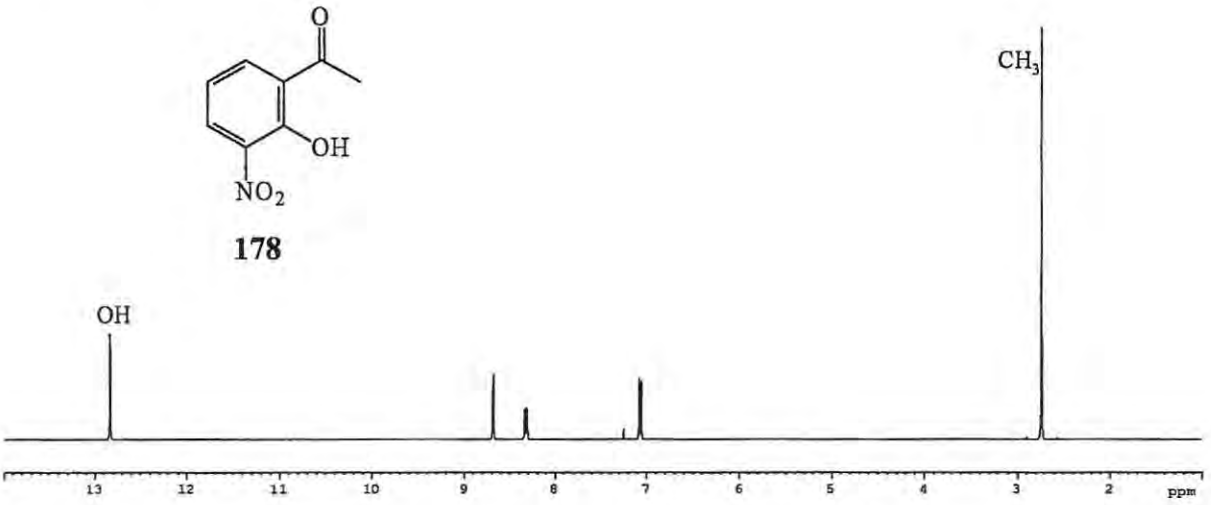


Figure 2. 400 MHz ^{1}H NMR spectrum of compound **178** in $CDCl_3$.

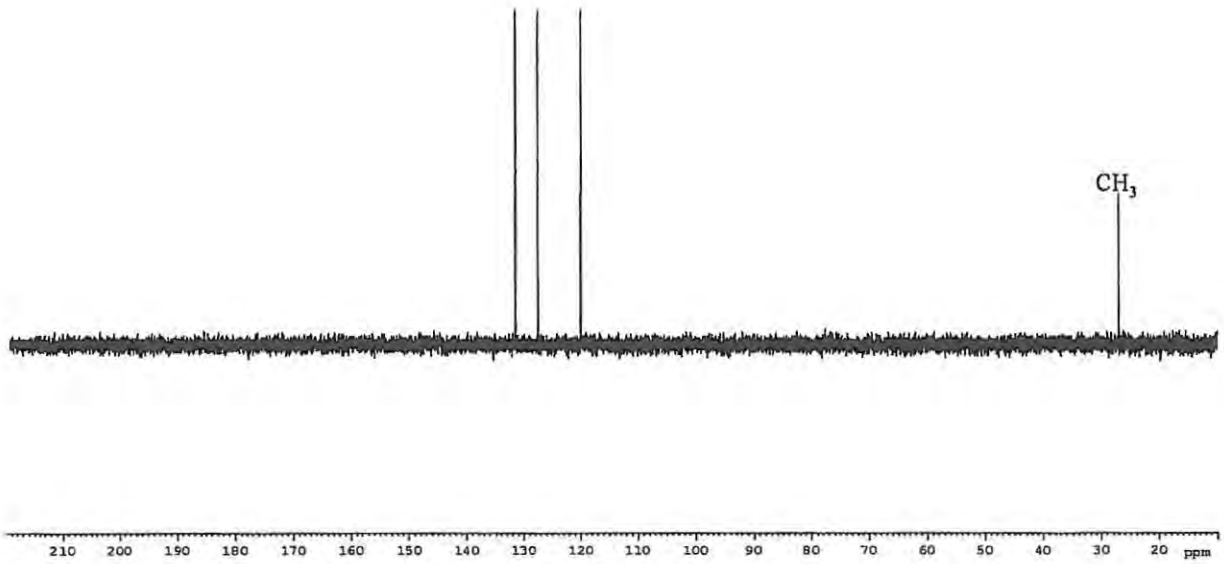


Figure 3. DEPT-135 NMR spectrum of compound **178** in $CDCl_3$.

[¶] Compound **178** has been reported previously and was prepared following a literature procedure.[141] Careful examination of the spectroscopic data, however, has revealed certain anomalies, which would also apply to the derivatives **183**, **189** and **195**. These will be addressed in future studies.

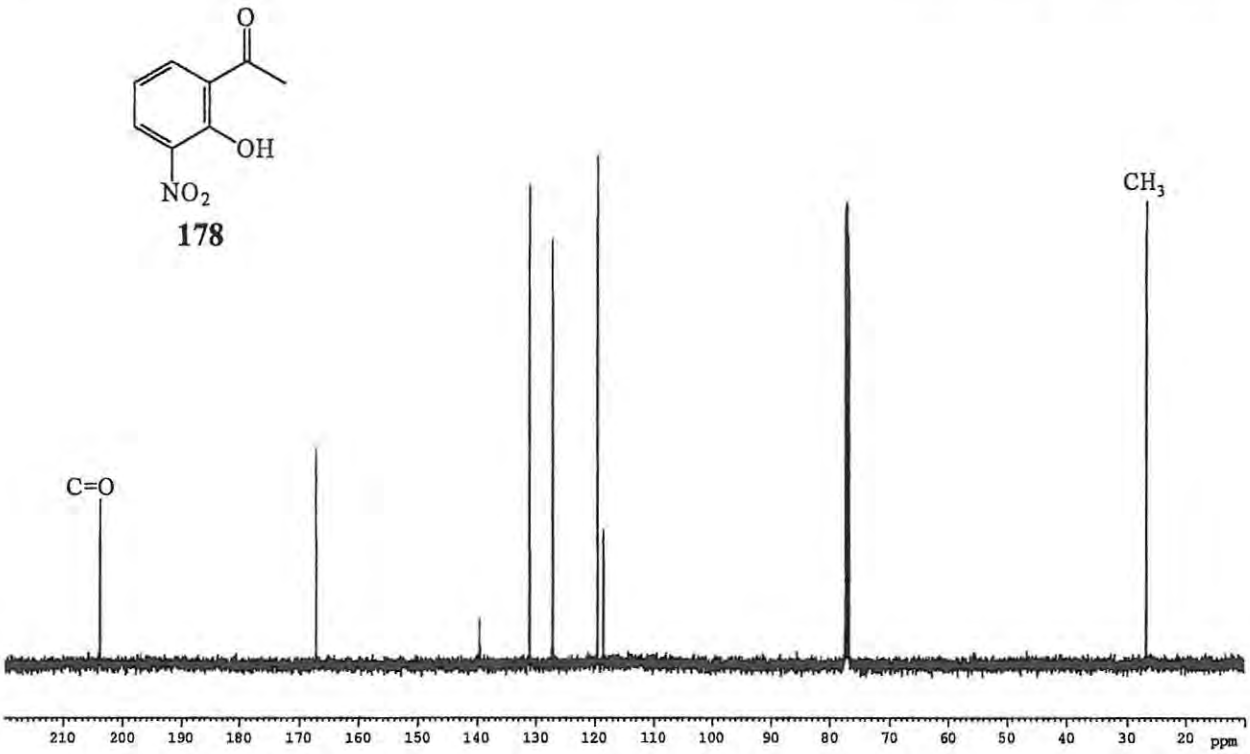
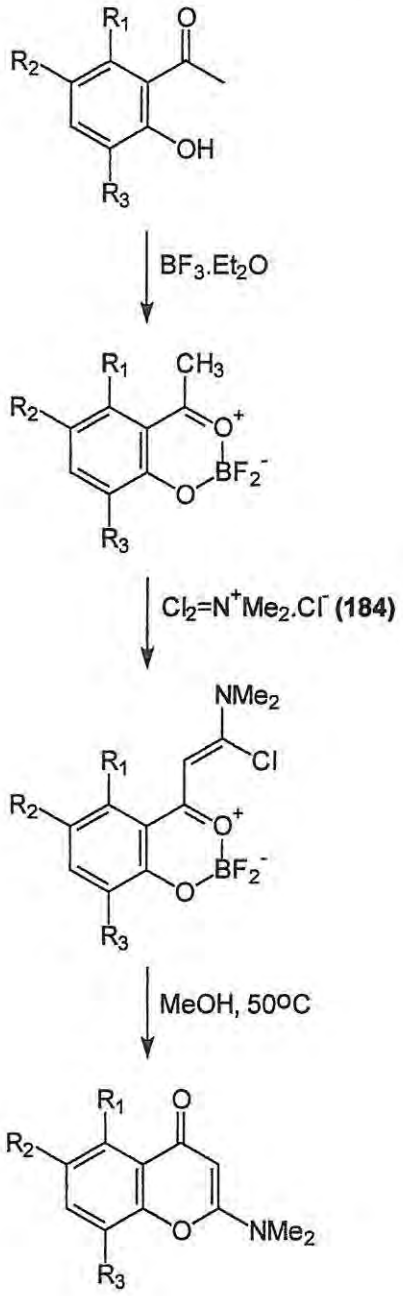


Figure 4. 100 MHz ^{13}C NMR spectrum of compound **178** in $CDCl_3$.

The 2-(*N,N*-dimethylamino)chromones **191 – 195**, most of which have not been reported previously, were prepared following the method reported by Morris *et al.*,[68] which is summarized in **Scheme 43**. Treatment of the *o*-hydroxyacetophenones **174 – 178** with boron trifluoride-etherate in dry diethyl ether gave the corresponding 2'-hydroxyacetophenone-boron difluoride complexes **179 – 183**. The boron difluoride complexes **179 – 183** were then heated with phosgeniminium chloride **184** at 80 °C to afford the corresponding 3-chloro-3-(*N,N*-dimethylamino)-1-(2-hydroxyphenyl)propenone-boron difluoride complexes **185 – 189**. The crude boron difluoride complexes were identified by ^1H NMR analysis and used without further purification. Subsequent methanolysis and cyclization at *ca.* 50 °C finally afforded the required 2-(*N,N*-dimethylamino)chromones **191 – 195** in yields ranging from 60 to 87% (Table 1); the products were recrystallized to obtain the analytically pure material necessary for the pK_a studies. The protection of the phenolic group with boron trifluoride-etherate is necessary in order to direct the reaction of the phosgeniminium salt to the methyl group instead of the hydroxyl group, as established by Morris *et al.* in their preliminary experiments.[68]



	R_1	R_2	R_3
174	H	H	F
175	H	F	H
176	MeO	H	H
177	H	H	MeO
178	H	H	NO_2

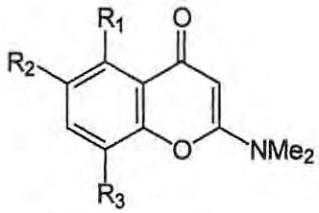
	R_1	R_2	R_3
179	H	H	F
180	H	F	H
181	MeO	H	H
182	H	H	MeO
183	H	H	NO_2

	R_1	R_2	R_3
185	H	H	F
186	H	F	H
187	MeO	H	H
188	H	H	MeO
189	H	H	NO_2

	R_1	R_2	R_3
191	H	H	F
192	H	F	H
193	MeO	H	H
194	H	H	MeO
195	H	H	NO_2

Scheme 43

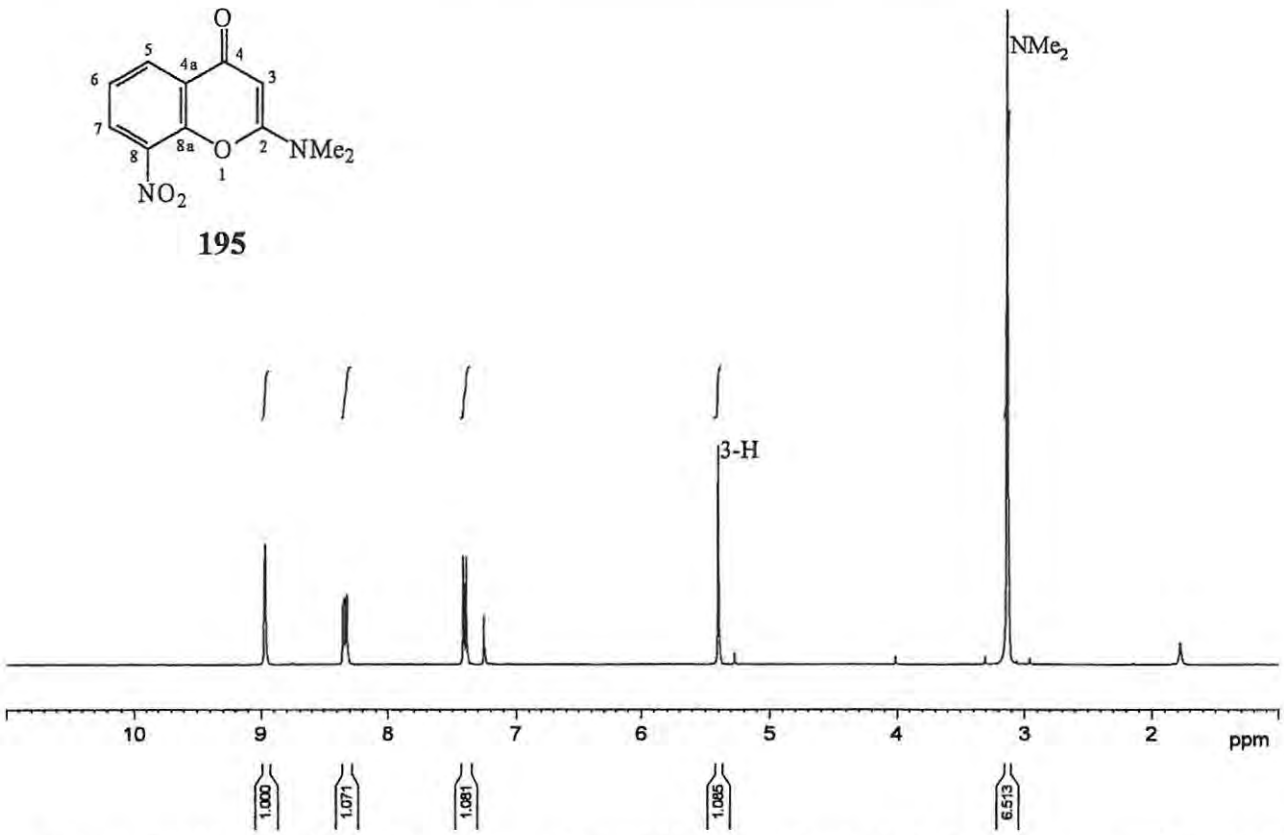
Table 1. Comparative yields (%) of the 2-(*N,N*-dimethylamino)chromones **191 – 195,** prepared as shown in **Scheme 43.**



Compd.	R_1	R_2	R_3	Yield (%)*
191	H	H	F	87
192	H	F	H	60
193	MeO	H	H	63
194	H	H	MeO	72
195	H	H	NO_2	83

* Isolated product

The 2-(*N,N*-dimethylamino)chromones **191 – 195** were fully characterized by elemental (HREIMS) and spectroscopic (IR, ^{1}H- and ^{13}C NMR) analysis, and their purity is illustrated by the ^{1}H and ^{13}C NMR spectra of the 8-nitro derivative **195** (Figures 5 and 6). The ^{1}H NMR spectrum clearly shows the dimethylamino singlet at δ 3.15 ppm, and the characteristic 3-methine proton singlet at δ 5.46 ppm, while the ^{13}C NMR spectrum reveals the presence of 10 carbon signals, with the *N,N*-dimethyl nuclei resonating as a singlet at δ 37.8 ppm and the characteristic 3-methine carbon signal at δ 86.3 ppm. Signal assignments were facilitated by the use of DEPT-135, COSY, HMQC and HMBC spectra, and it is evident that the substituent changes do not appear to influence the ^{1}H and ^{13}C chemical shifts significantly.



195

Figure 5. 400 MHz ^{1}H NMR spectrum of 2-(*N,N*-dimethylamino)-8-nitrochromone **195** in $CDCl_3$.

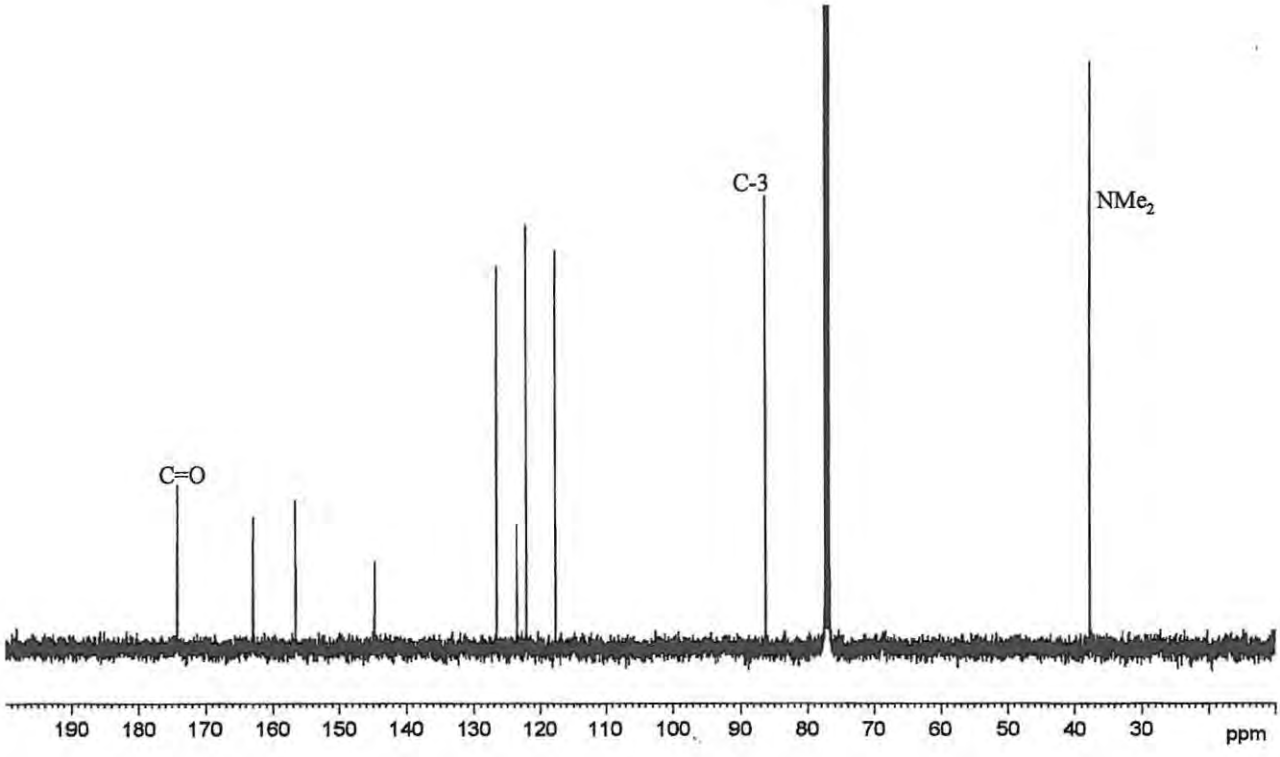


Figure 6. 100 MHz ^{13}C NMR spectrum of 2-(*N,N*-dimethylamino)-8-nitrochromone **195** in $CDCl_3$.

2.1.2 pK_a studies of 2-(N,N-dimethylamino)chromones

The pK_a studies were carried out in order to demonstrate the influence of remote substituents on the basicity of the 2-(*N,N*-dimethylamino)chromones **190 – 195**. The pK_a values were determined in ethanol-water (1:1) by potentiometric analysis, as described by Albert and Serjeant.[142] Solutions of the 2-(*N,N*-dimethylamino)chromones were titrated with hydrochloric acid (0.1063 $mol.dm^{-3}$) in 0.20 mL increments, the pH being measured after each addition and the temperature being held constant at 25 °C.

The pK_a values were calculated using the ionization constant K_a:

$$K_a = \frac{[H^+][B]}{[BH^+]}$$

thus $$pK_a = pH + \log [BH^+] - \log [B]$$

where $[BH^+]$ represents the concentration of the protonated species (*i.e.* the conjugate acid) and [B] the concentration of the free base.

The concentration on which the calculations are based is reached, in each case, at the mid-point of the titration.[142] Tables 2 and 3 summarize the analytical data for 5-methoxy-2-(*N,N*-dimethylamino)chromone **193**; BH^+ is the number of moles of hydrogen ions in the volume of acid added, while B represents the difference between the number of moles of the 2-(*N,*N-dimethylamino)chromone and BH^+. From Table 2, the observed pH values lie outside the range pH 5-10, therefore, it is necessary to make corrections for the hydrogen ion activity.[142] These corrections were effected following Albert and Serjeant's method,[142] and are summarized in Table 3, in which $[H^+]$ (column ii) expresses the hydrogen ion concentration given by the negative antilogarithm of the measured pH. This value is then multiplied by the total volume (in litres) to give the number of moles of H^+ (column iii). The pK_a values (column vi) reflect the sum of the measured pH and column v. The pK_a values in column vi cannot be averaged directly and a mean result is obtained as follows. The antilogarithms of the values in column vi are given in column vii; the average of these values is calculated, with the exclusion of the first and the last entries, (as recommended by Albert and Serjeant[142]) and converted back to pK_a. The variance about the mean value finally obtained for the pK_a (2.52) falls within the limits of ± 0.06, considered acceptable by Albert and

Serjeant.[142] All titrations were carried out in triplicate to ensure reproducibility and the results are tabulated in the experimental section. The pK_a values calculated for the series of 2-(*N,N*-dimethylamino)chromones **190 – 195** are summarized in Table 4.

Table 2. Concentration of the protonated and non-protonated 5-methoxy-2-(*N,N*-dimethylamino)chromone **193** as a function of pH.

Conc. HCl	Vol. Titrant (mL)	Mass (g)	Molar mass (g/mol)	No. of moles	pH	BH^{+} [a]	B[b]
0.1063	0.00	0.0438	219	0.0002	5.00	0.00	0.000200
0.1063	0.20	0.0438	219	0.0002	3.60	2.13E-05	0.000179
0.1063	0.40	0.0438	219	0.0002	3.24	4.25E-05	0.000157
0.1063	0.60	0.0438	219	0.0002	3.05	6.38E-05	0.000136
0.1063	0.80	0.0438	219	0.0002	2.89	8.50E-05	0.000115
0.1063	1.00	0.0438	219	0.0002	2.76	0.000106	9.37E-05
0.1063	1.20	0.0438	219	0.0002	2.66	0.000128	7.24E-05
0.1063	1.40	0.0438	219	0.0002	2.58	0.000149	5.12E-05
0.1063	1.60	0.0438	219	0.0002	2.49	0.000170	2.99E-05
0.1063	1.80	0.0438	219	0.0002	2.42	0.000191	8.66E-06
0.1063	2.00	0.0438	219	0.0002	2.34	0.000213	-1.26E-05[c]

[a] Moles of conjugate acid.
[b] Moles of residual free 2-(*N,N*-dimethylamino)chromone.
[c] The negative concentration reflects the excess concentration of titrant.

Table 3. Calculation of pK_a values for 5-methoxy-2-(*N,N*-dimethylamino)chromone **193**, corrected for hydrogen-ion activity.

(i) Total vol. (mL)	(ii) $[H^+]$	(iii) H^+	(iv) $\frac{BH^+ - H^+}{B + H^+}$	(v) log(col.iv)	(vi) pK_a	(vii) 10^{-pKa}
19.00	0.000010	1.9000E-06	-0.000949			
19.20	0.000251	4.8228E-06	0.089545	-1.047957	2.552043	0.002805
19.40	0.000575	1.1164E-05	0.185933	-0.730643	2.509357	0.003095
19.60	0.000891	1.7469E-05	0.301333	-0.520953	2.529047	0.002958
19.80	0.001288	2.5507E-05	0.423819	-0.372820	2.517180	0.003040
20.00	0.001738	3.4756E-05	0.556953	-0.254181	2.505819	0.003120
20.20	0.002188	4.4193E-05	0.714784	-0.145825	2.514175	0.003061
20.40	0.002630	5.3657E-05	0.907715	-0.042051	2.537949	0.002898
20.60	0.003236	6.6660E-05	1.070816	0.029715	2.519715	0.003022
20.80	0.003802	7.9079E-05	1.279478	0.107033	2.527033	0.002971
21.00	0.004571	9.5989E-05	1.398412	0.145635	2.485635	0.003269

$pK_a = 2.52 \pm 0.06$

The pK_a value for chromone itself, obtained by this method, was 1.77; this value is, however, lower than the value (2.00) obtained by the spectrophotometric analysis method reported by Tolmachev *et al.*[143] Protonation of chromone is favoured at the carbonyl oxygen, delocalization of the ether oxygen lone pair stabilizing the conjugate acid (Figure 7a). The increased basicity of the 2-(*N,N*-dimethylamino)chromones (pK_a 1.92-2.52) relative to chromone may be attributed to the additional delocalization of the nitrogen lone pair (Figure 7b), provided protonation occurs at the chromone carbonyl oxygen rather than at the amine nitrogen. Preferential protonation of the carbonyl oxygen is supported by the results of a ^{13}C

NMR study conducted in our laboratories by Sabbagh.[144] Moreover, AM1 semi-empirical molecular orbital calculations by C.W. McCleland[144] showed that, in these systems, protonation of the oxygen atom is favoured over nitrogen by *ca.* 27 kcal mol^{-1}.

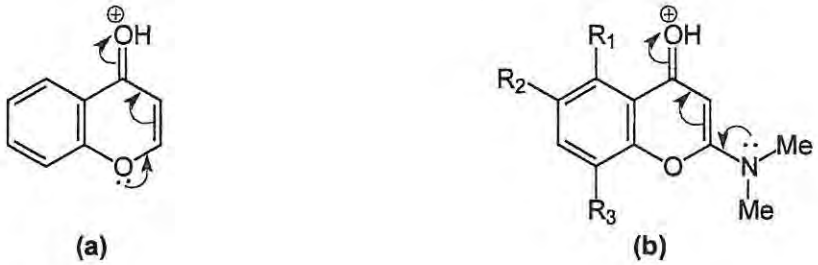
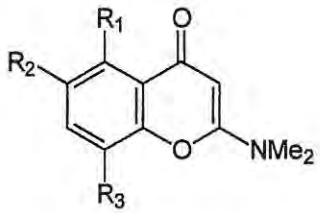


Figure 7. Lone-pair delocalization stabilizing the conjugate acids of:- (a) chromone; and (b) the 2-(*N,N*-dimethylamino)chromones **190 – 195.**

Table 4. pK_a data for the 2-(*N,N*-dimethylamino)chromones **190 – 195**



Compd.	R_1	R_2	R_3	pK_a [b]
190	H	H	H	2.47
191	H	H	F	1.98
192	H	F	H	2.20
193	MeO	H	H	2.52
194	H	H	MeO	2.50
195	H	H	NO_2	1.92

[b] At 25°C in H_2O-EtOH (1:1); estimated error ± 0.06.

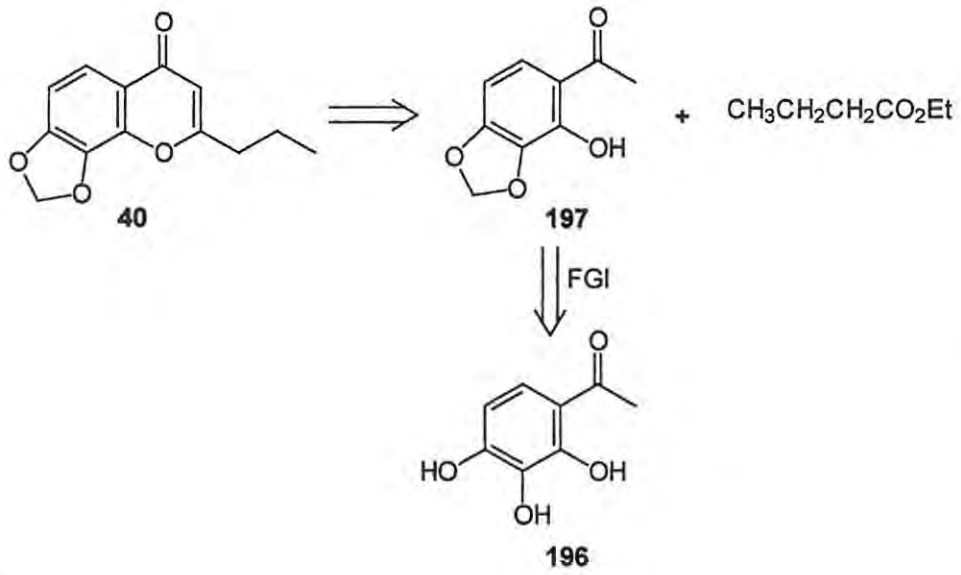
From the results detailed in Table 4, it is apparent that the measured pK_a values lie within a relatively narrow range (1.92 – 2.52), thus, indicating that the remote substituents at positions C-5, C-6 and C-8 have relatively little effect on the basicity of the 2-(*N,N*-dimethyl-amino)chromones studied. Their influence is, nevertheless, discernible. The trend observed for the pK_a values of the 8-substituted derivatives decreases in the order:- pK_a: **194** (R_3=OMe) > **190** (R_3=H) > **191** (R_3=F) > **195** (R_3=NO_2). This reflects the general expectation that basicity should be increased by electron-releasing substituents and decreased by electron-withdrawing substituents, although the pK_a value for the 8-fluoro analogue **191** is lower than might have been expected. In the case of the 8-nitro analogue **195**, the low pK_a value (1.92) clearly reflects the electron-withdrawing character of the 8-nitro substituent, while the 8-methoxy derivative **194** shows an increase in basicity (pK_a 2.50), reflecting the electron-releasing character of the methoxy group.

The 5-methoxy analogue **193** exhibits similar basicity (pK_a 2.52) to the 8-methoxy derivative **194**, but appears to have significantly more negative stabilization energy (-6.80 kcal mol^{-1}) [as calculated by C.W. McCleland][144]; this is attributed to two factors, *viz*: (i) relative destabilization of the free base **193**, and (ii) intramolecular H-bonding stabilization of the conjugate acid. Hydrogen-bonding effects may also explain the low pK_a value (1.98) measured for the 8-fluoro analogue **191**, in that the location of the fluorine and pyran oxygen atoms may permit chelation of a water molecule. Such intra-molecular hydrogen-bonding would not only inhibit lone-pair delocalization by both atoms into the chromone nucleus, but also expose the electron-withdrawing inductive effect of the fluorine substituent. The obtained pK_a values were supported by the optimized AMI semi-empirical calculations done by C.W. McCleland.[144]

2.2 Granulosin and C-2 side chain analogues

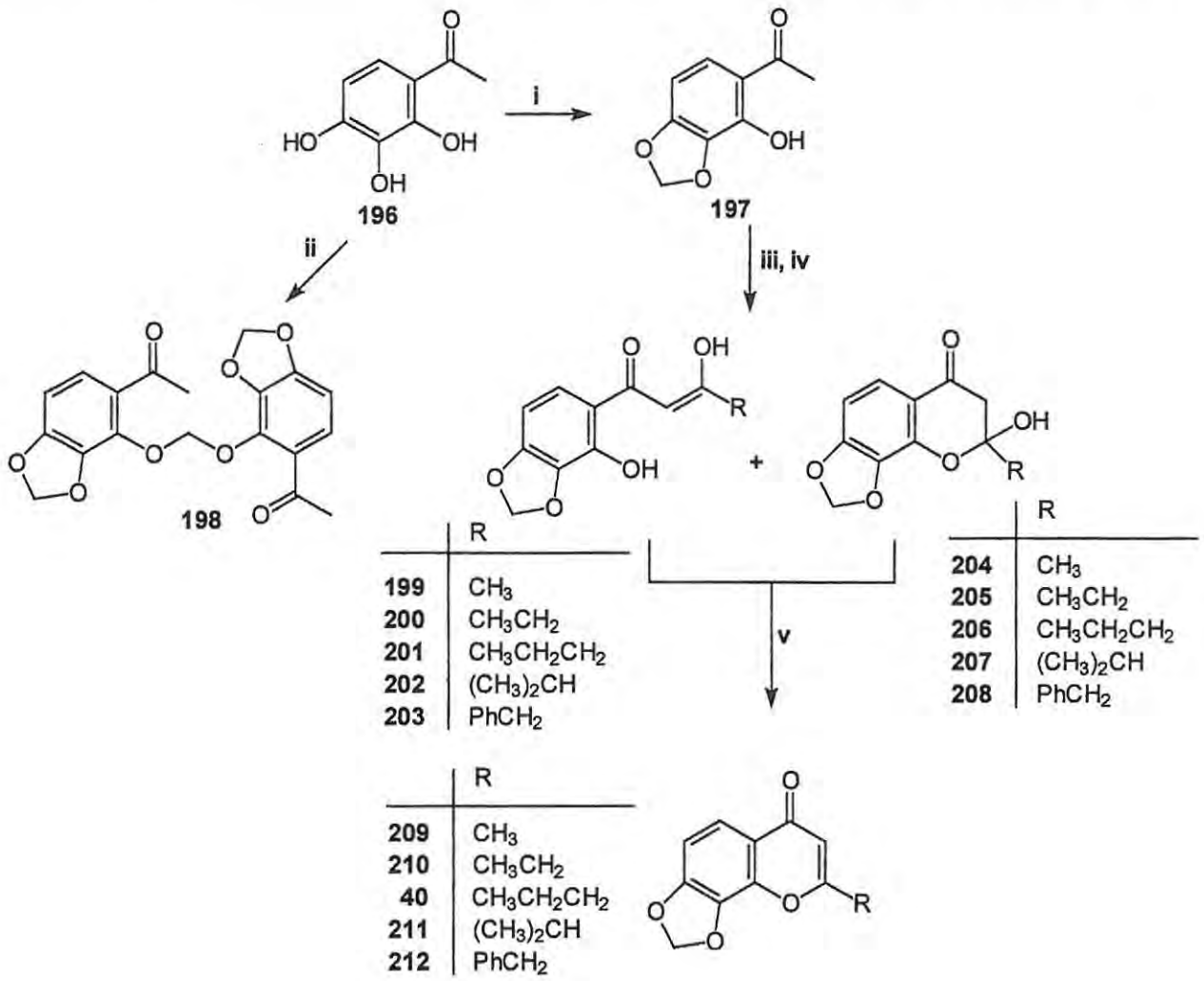
2.2.1 Synthesis of granulosin and C-2 side-chain analogues

Lõpez *et al.*[47] in their investigation of Costa Rican medicinal plants, have recently reported the isolation of a chromone derivative, granulosin **40**, from the bark of *Galipea granulosa*, a plant used by the Indians of Bolivia for the treatment of parasitic infections, particularly cutaneous leishmaniasis. The activity shown by an extract of this plant in the brine shrimp test and our interest in chromones prompted us to synthesize granulosin **40** and four structural analogues **209 – 212**. From the retrosynthetic analysis (**Scheme 44**), it was envisaged that the 2',3',4'-trihydroxyacetophenone **196** could be used as the key starting material.



Scheme 44

The first step of the synthesis involved formation of the dioxolane derivative **197** *via* regioselective acetalisation of 2',3',4'-trihydroxyacetophenone **196** (**Scheme 45**) using bromochloromethane in the presence of cesium carbonate.[145] The strong intramolecular hydrogen-bonding between the 2'-hydroxyl and acetyl carbonyl groups in the 2'-hydroxyacetophenone **196** was expected to inhibit reaction of the 2'-hydroxyl group and favour selective acetalisation of the 3'- and 4'- hydroxyl groups.



Scheme 45

Reagents: i) $BrCH_2Cl$ (1 eq.), Cs_2CO_3(1 eq.), DMF; ii) $BrCH_2Cl$ (1.5 eq.), Cs_2CO_3(1.5 eq.), DMF; iii) NaOEt, EtOH; iv) RCO_2Et; v) AcOH, H_2SO_4.

In an initial attempt to prepare the precursor **197**, using 1.5 equivalents of bromochloromethane for each equivalent of 2',3',4'-trihydroxyacetophenone **196**, the expected acetal **197** together with the unexpected product **198** was obtained in a 12 : 88 mole ratio. The unexpected, major product **198** clearly arose from reaction of all three phenolic hydroxyl groups. The ^{1}H NMR spectrum of the "dimeric" system **198** (Figure 8) reveals a four proton singlet at δ 5.95 ppm, corresponding to the two dioxolane methylene protons, while the other methylenedioxy protons resonate as a two proton singlet at δ 6.09 ppm; the singlet at δ 2.38 ppm corresponds to the two acetyl methyl groups. The ^{13}C NMR spectrum of the compound

198 (Figure 9) shows the presence of 10 distinct signals. A close inspection of the spectrum reveals the presence of an acetyl methyl carbon signal at δ 30.8 ppm, the methylenedioxy and dioxolane methylene signals at δ 94.3 and 102.0 ppm respectively (the difference in intensity consistent with the number of carbon nuclei present), and a carbonyl carbon signal at δ 197.3 ppm. Assignment of the structure was supported by COSY, HMQC and HMBC correlations. Further confirmation is provided by the IR spectrum of the "dimeric" system **198**, in which the carbonyl absorption band at 1665 cm^{-1} and the absence of a hydroxyl band are significant. High-resolution mass spectrometric analyses indicated the molecular formula to be, as expected, $C_{19}H_{16}O_8$.

When the proportion of the bromochloromethane was limited to one equivalent, the reaction proceeded smoothly to afford the required 2'-hydroxy-3',4'-(methylenedioxy)acetophenone **197** in 80% yield. The ^1H NMR spectrum of the acetal **197** (Figure 10) reveals a singlet at δ 12.27 ppm corresponding to an aromatic hydroxyl group. The down-field shift is indicative of hydrogen-bonding between the 2'-hydroxyl group and the carbonyl oxygen, hence, supporting the selective acetalisation of the 3'- and 4'- hydroxyl groups in 2',3',4'-trihydroxyacetophenone **196**. The ^1H NMR spectrum also reveals a singlet at δ 6.07 ppm corresponding to the methylenedioxy protons, while the acetyl methyl protons resonate as a singlet at δ 2.56 ppm. The ^{13}C NMR spectrum of compound **197** (Figure 11) shows 9 carbon signals with the methylene carbon of the methylenedioxy group resonating at δ 103.0 ppm. The COSY, HMQC and HMBC data were used to assigned all the signals.

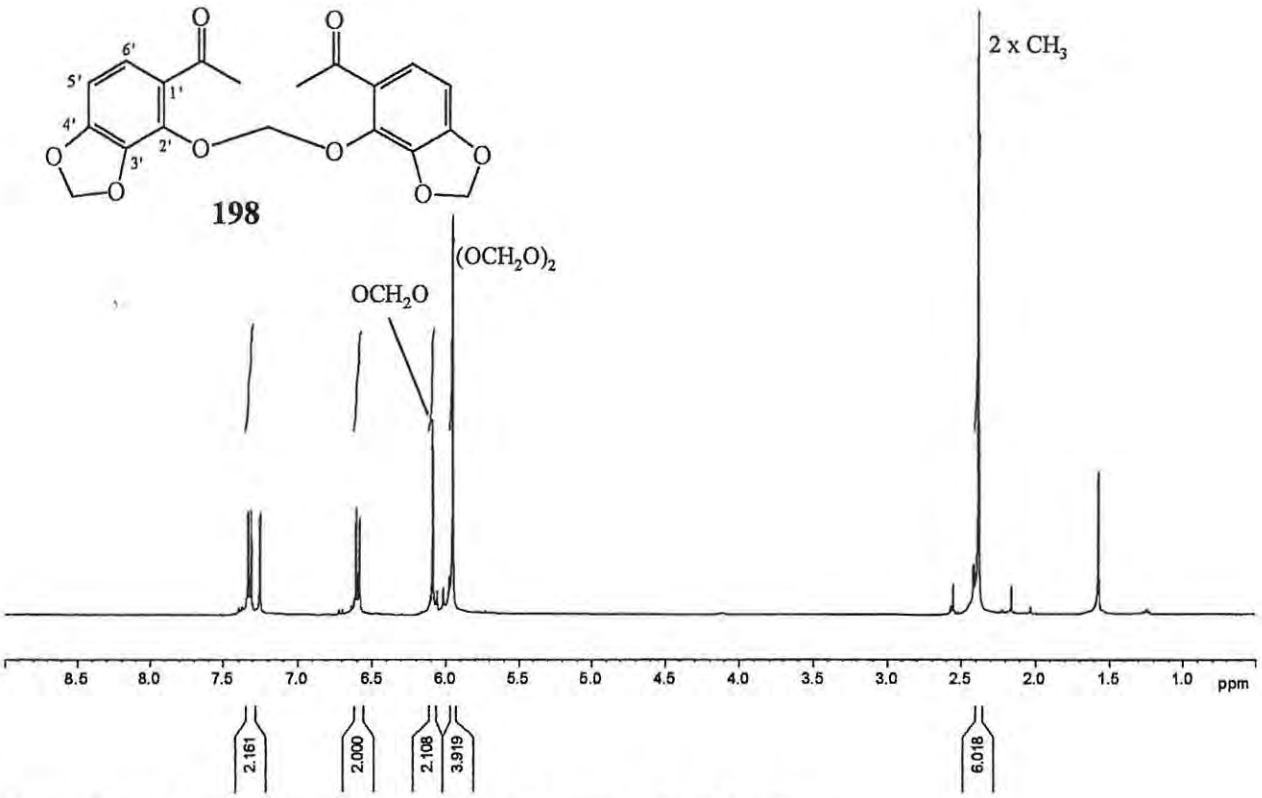


Figure 8. 400 MHz ^{1}H NMR spectrum of dimer **198** in $CDCl_3$.

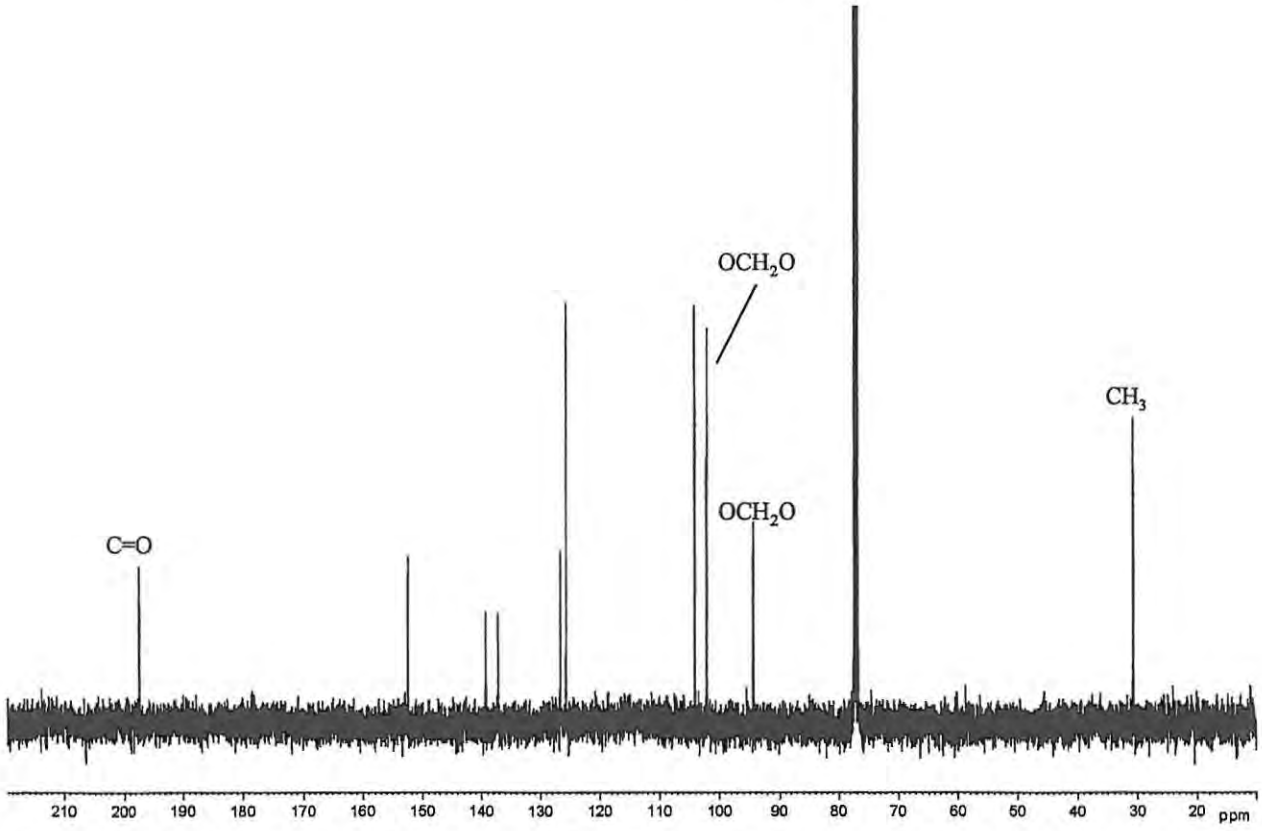


Figure 9. 100 MHz ^{13}C NMR spectrum of dimer **198** in $CDCl_3$.

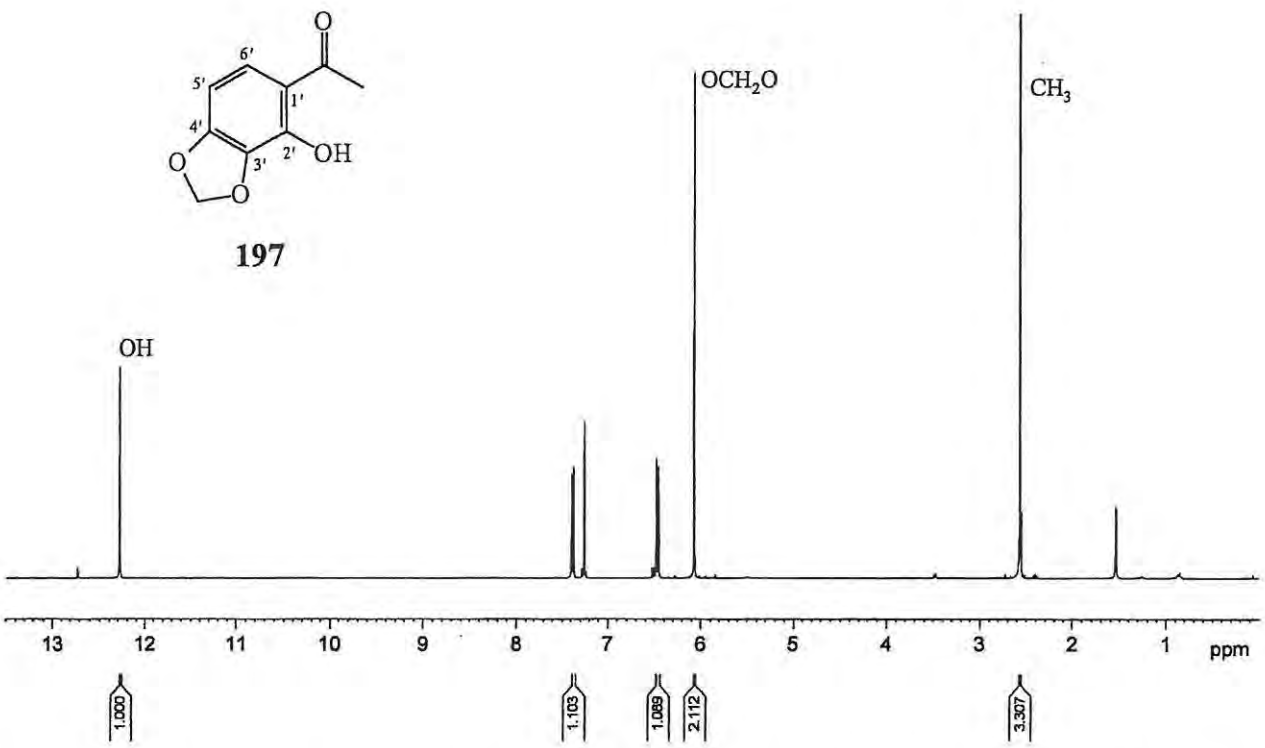


Figure 10. 400 MHz ^{1}H NMR spectrum of 2'-hydroxy-3',4'-(methylenedioxy)acetophenone **197** in $CDCl_3$.

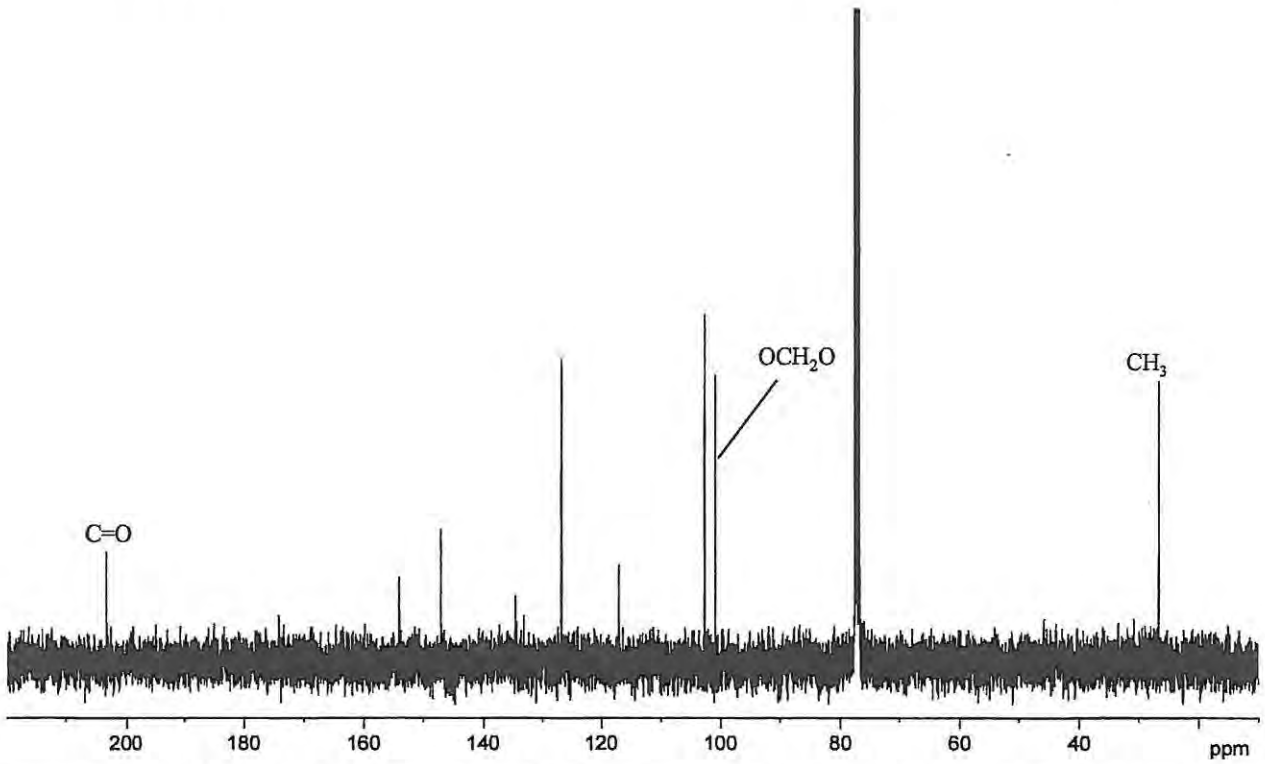


Figure 11. 100 MHz ^{13}C NMR spectrum of 2'-hydroxy-3',4'-(methylenedioxy)acetophenone **197** in $CDCl_3$.

Treatment of 2'-hydroxy-3',4'-(methylenedioxy)acetophenone **197** with two equivalents of sodium ethoxide in ethanol afforded the enolate which, on reaction with a series of ethyl carboxylate esters [R = CH_3, CH_3CH_2, $CH_3CH_2CH_2$, $(CH_3)_2CH$, $PhCH_2$] gave mixtures, indicated by ^1H NMR spectroscopy to contain the corresponding acylated products (existing, in each case, as an enol tautomer) formulated as structures **199 – 203** and their cyclised derivatives **204 – 208**. These reaction mixtures were then treated with a mixture of acetic and sulfuric acids to afford the corresponding chromone derivatives **40** and **209 – 212** in yields ranging from 58 to 85%. All of the products were purified by flash chromatography, and the structures were assigned on the basis of elemental (HREIMS) and spectroscopic (IR, ^1H and ^{13}C NMR) analysis. The ^1H NMR spectrum of granulosin **40** (Figure 12) reveals a triplet at δ 0.96 ppm corresponding to a methyl group, a multiplet and a triplet at δ 1.68 and 2.61 ppm , respectively, corresponding to the two methylene groups in the alkyl chain. The methylenedioxy nuclei (OCH_2O) resonate as a singlet at δ 6.27 ppm, and the singlet at δ 6.12 ppm corresponds to the characteristic 3-methine proton of the chromone nucleus. The ^{13}C NMR spectrum in $CDCl_3$ (Figure 13) clearly reveals, as expected, the presence of 13 distinct carbon signals, while in DMSO-d_6 (Figure 14), only 12 distinct carbon resonances, are evident due to the coincidence of the two signals at δ 119.3 ppm. Inspection of the ^{13}C NMR spectrum in DMSO-d_6 (Figure 14) reveals the presence of one methyl signal at δ 13.3 ppm and two methylene signals at δ 19.7 and 35.1 ppm corresponding to the propyl side- chain. The characteristic methylene carbon of the methylenedioxy group is observed to resonate at δ 103.6 ppm, and the chromone carbonyl carbon at δ 175.9 ppm. The signal at *ca.* δ 169 ppm is characteristic of the C-2 carbon of most chromone nuclei. The DEPT-135 spectrum (Figure 15) confirms assignment of the methylene carbons of the propyl chain and the methylenedioxy group. The aromatic signals were differentiated using the COSY spectrum and the ^1H and ^{13}C NMR chemical signal assignments were facilitated using the HMQC and HMBC data. The ^1H and ^{13}C NMR data obtained for the 2-propyl derivative **40** were shown to correspond closely to those reported[47] for granulosin in DMSO-d_6 (the ^1H chemical shifts lie within 0.03–0.19 ppm and the ^{13}C shift within 0.1-1.1 ppm of the reported values).

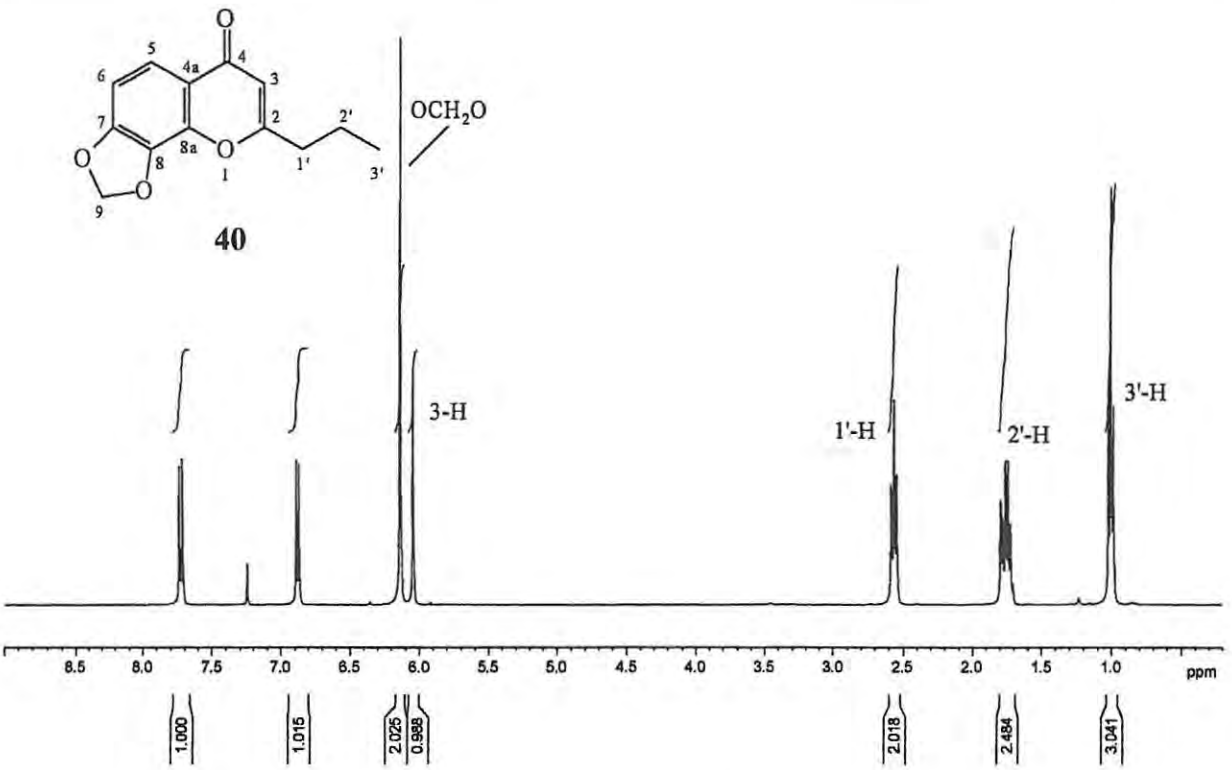


Figure 12. 400 MHz ^{1}H NMR spectrum of granulosin **40** in $CDCl_3$.

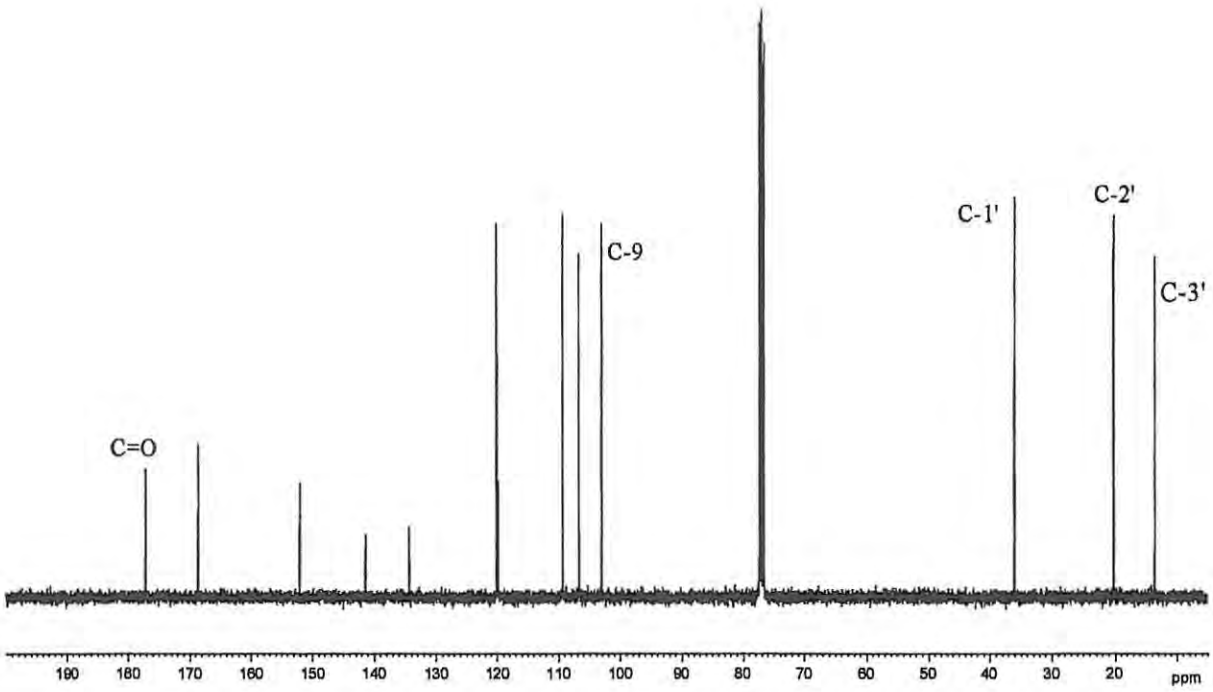


Figure 13. 100 MHz ^{13}C NMR spectrum of granulosin **40** in $CDCl_3$.

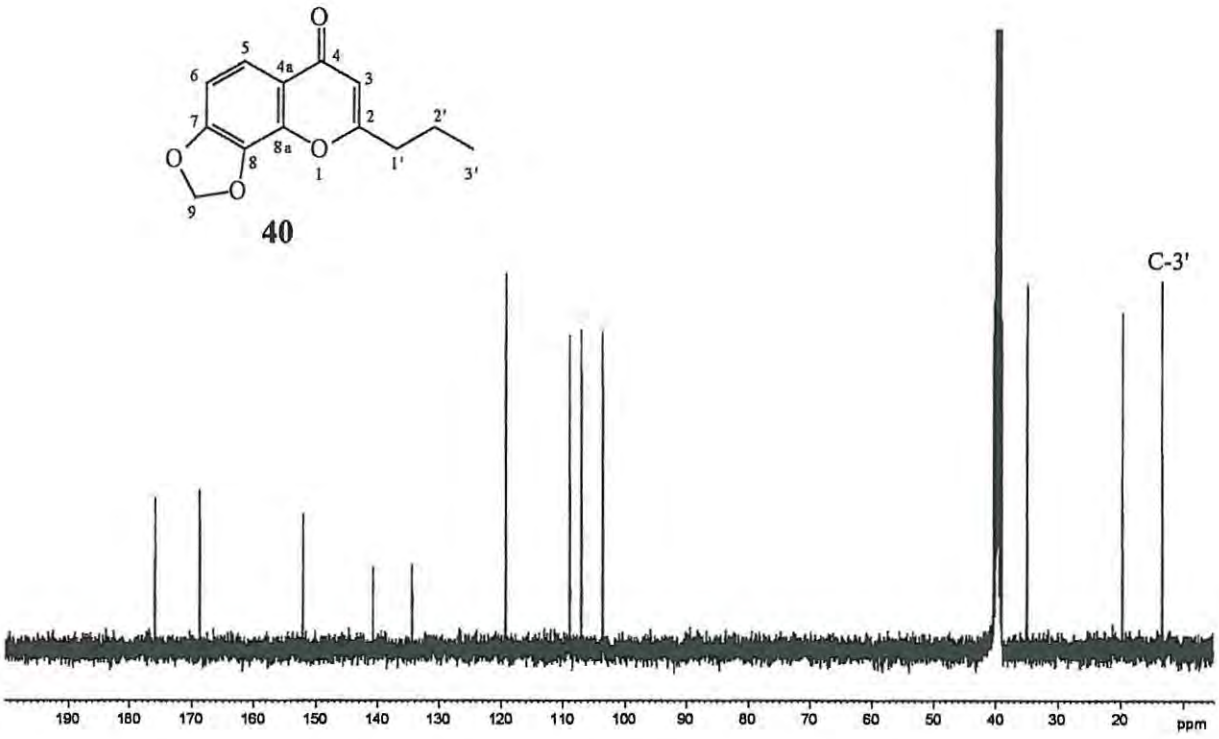


Figure 14. 100 MHz ^{13}C NMR spectrum of granulosin **40** in DMSO-d_6.

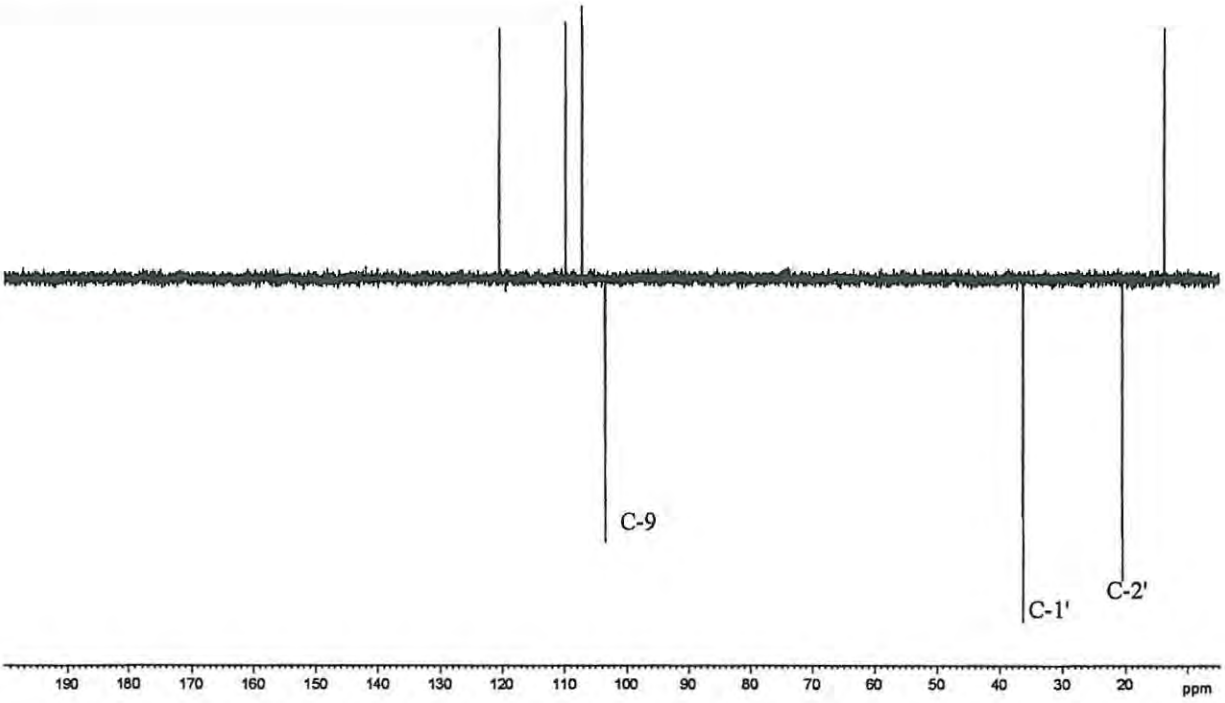
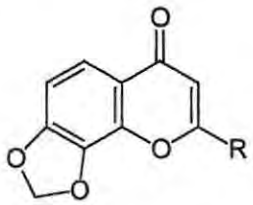


Figure 15. DEPT-135 NMR spectrum of granulosin **40** in $CDCl_3$.

2.2.2 Assessment of the biological activity of granulosin 40 *and C-2 side-chain analogues*

In the light of the reported biological activity of granulosin **40**,[47] it was decided to evaluate the biological activity of compound **40** and selected structural analogues. *Artemia salina* larvicidal bioassays were performed as described by Solis *et al.*[146] Estimates of median lethal concentrations were obtained by probit analysis[147] of *A. salina* mortality data from 12 solutions across the concentration ranges:- 25.00 – 0.586 μg/mL for granulosin **40**; and 400.0 – 12.50 μg/mL for compounds **209 – 212.**

Table 5. Summary of *Artemia salina* biological assay data[a]:



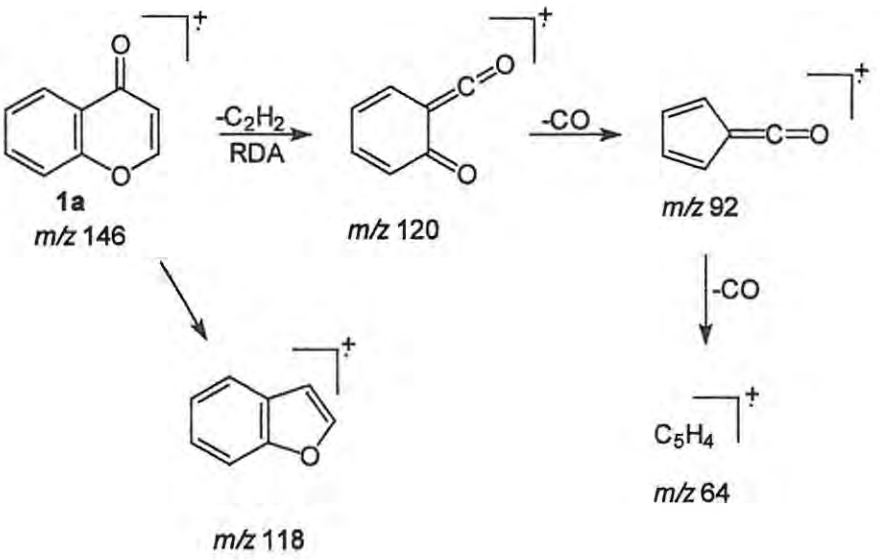
Compd.	R	LC_{50}[b] (μm/mL)	95% Confidence interval (μm/mL)		BAW[c]
			Lower limit	Upper Limit	
209	CH_3	131.8	112.6	156.3	-
210	CH_3CH_2	22.3	19.8	25.0	100/100
40	$CH_3CH_2CH_2$	4.3	3.5	5.2	-
211	$(CH_3)_2CH$	21.3	18.2	24.7	-
212	$PhCH_2$	108.6	93.4	126.7	100/100

[a]Analyses conducted by C.A. Gray.[b]
[b]The estimated median lethal concentration values (LC_{50}) represent the concentration of compound required to halve the amount of substrate cleaved during the allotted reaction period, as obtained by probit analysis.[147]
[c]BAW: Beet army worm biological assay data (insect pests on crops).

All five of the chromone derivatives **40** and **209 – 212** showed significant cytotoxic effects against the brine shrimp *Artemia salina*. LC_{50} values estimated by probit analysis[147] (Table 5) indicate an interesting range in activity with granulosin **40** being highly toxic (LC_{50} : 4 ppm), the analogues **210** and **211** displaying less toxicity (LC_{50} : 22 and 21 ppm respectively) and **209** and **212** being significantly less active (LC_{50} : 132 and 109 ppm respectively). Anti-parasitic activity assays were carried out on compounds **40** and **209 – 212** and the results also summarized in Table 5. From the Beet army worm (BAW) data obtained, only compounds **210** and **212** showed 100% BAW activity, while the other compounds **40**, **209** and **211** showed no activity. Thus, granulosin **40** despite being highly toxic to the brine shrimp *Artemia salina,* showed absolutely no activity against the beet army worm.

2.2.3 Mass spectrometric analysis of granulosin 40 *and C-2 side-chain analogues*

As indicated in the introduction (Section 1.1), when chromone **1** is subjected to electron-impact mass spectrometry, it fragments *via* two main pathways involving, initially, loss of carbon monoxide or ring-cleavage by a retro-Diels-Alder (RDA) reaction (**Scheme 1**). An electron-impact (EI) mass spectrometric study of granulosin **40** and the four structural analogues **209 – 212** was undertaken to explore the effect of the various substituents on the fragmentation patterns. Examination of the high-resolution electron-impact (EI) and low-resolution mass spectrometric data (Table 6), as well as the B/E link-scan data, has permitted elucidation of the significant peaks in the mass spectra of these compounds.



Scheme 1

The EI mass spectrum of the parent system, granulosin **40**, is illustrated in Figure 16, and the proposed fragmentation pathways are outlined in **Scheme 46**. In the mass spectrum of the parent compound **40**, the molecular ion **I** at *m/z* 232, which corresponds to the base peak, fragments *via* four major pathways (**A, B, C,** and **D**).

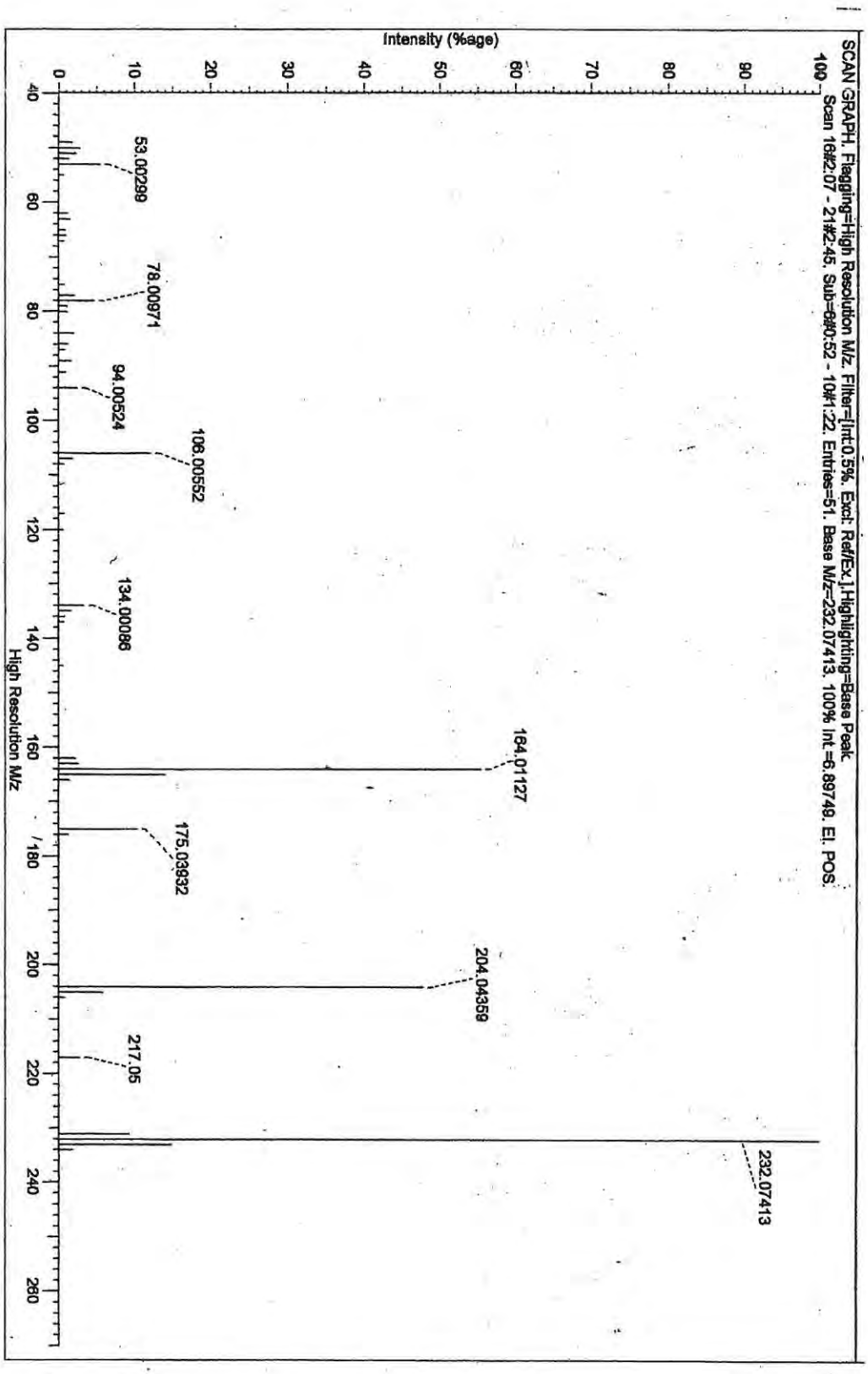
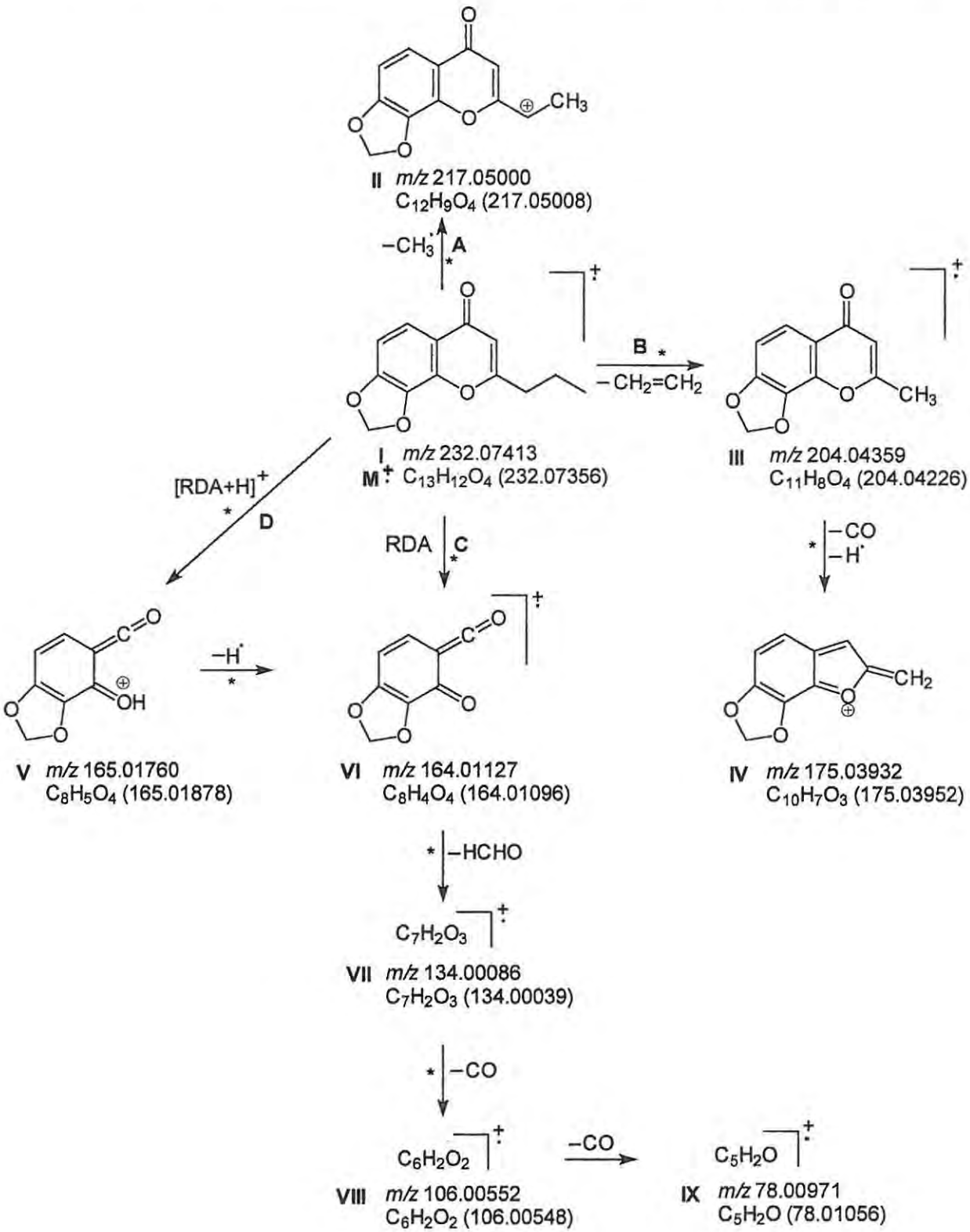


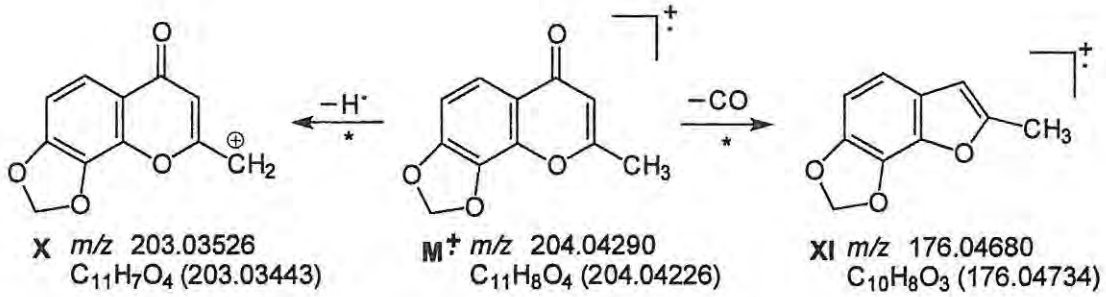
Figure 16. High-resolution EI mass spectrum of granulosin **40**



Scheme 46. EI mass fragmentation pathways for granulosin **40**. Accurate masses (*m/z*) are followed, in parentheses, by calculated formula masses; an asterisk indicates a pathway supported by the B/E link-scan data.

In path [**A**], the loss of a methyl radical from the molecular ion **I**, followed by rearrangement, gives the well-stabilized cation **II** (*m/z* 217, M^+-15). In path [**B**], loss of ethylene by a McLafferty-type rearrangement of the molecular ion **I** gives the radical-cation **III** (*m/z* 204, M^+-28), which subsequently loses CO and a hydrogen atom to give fragment **IV** (*m/z* 175). In path [**C**], loss of pent-1-yne by a retro-Diels-Alder (RDA) pathway, which is characteristic of the chromone nucleus, affords fragment **VI** (*m/z* 164, M^+-68), which then loses HCHO to give fragment **VII** $C_7H_2O_3^{+\cdot}$ (*m/z* 134). Sequential decarbonylation of fragment **VII** then affords fragment **VIII** $C_6H_2O_2^{+\cdot}$ (*m/z* 106) and fragment **IX** $C_5H_2O^{+\cdot}$ (*m/z* 78). Formation of fragment **V** (*m/z* 165) from the molecular ion **I** is attributed to an RDA-type process accompanied by proton transfer, *i.e.* $[RDA+H]^+$, which is also characteristic of the 2- and 3-alkyl chromones[6] {Path [**D**]}; subsequent deprotonation also gives the ketene radical-cation **VI**.

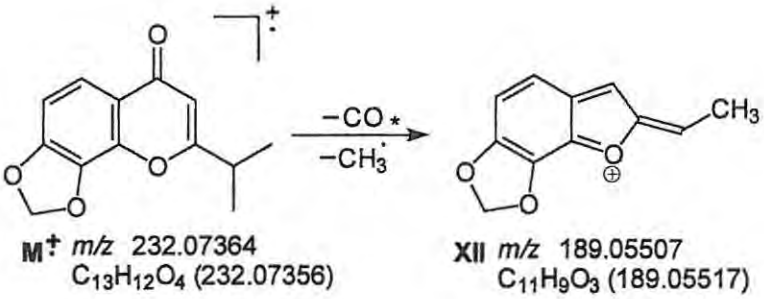
In the mass spectra of the analogues **209** and **210**, additional fragments were observed and these are illustrated in **Schemes 47 – 49**. Thus, fragment **X** arises from loss of a hydrogen atom from the molecular ion, while decarbonylation of the molecular ion gives the ring-contracted species **XI** (**Scheme 47**). The fragmentation patterns exhibited by the analogue **211** are very similar to that of granulosin **40**, a notable difference being the appearance of a peak at *m/z* 189, attributed to loss of CO and a methyl radical from the molecular ion, as illustrated in **Scheme 48**.



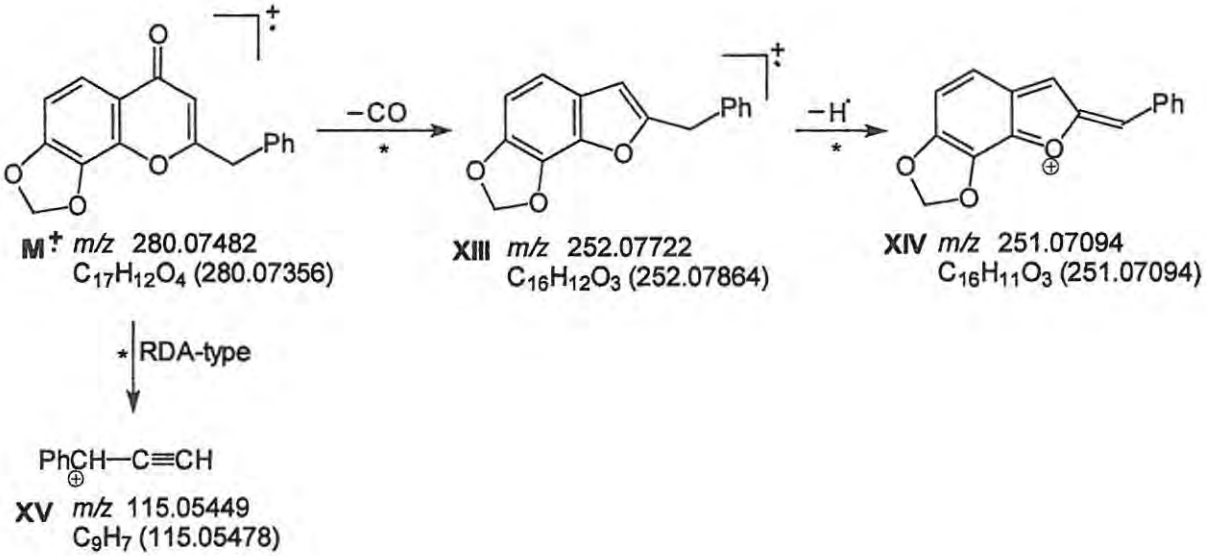
Scheme 47. Additional fragmentations of the molecular ion from compound **209**

Fragmentation of the 2-benzyl analogue **212** exhibits the additional fragments illustrated in **Scheme 49**. Fragment **XIII** (*m/z* 252) arises from decarbonylation of the molecular ion, while subsequent loss of a hydrogen atom gives the fragment **XIV** (*m/z* 251). Formation of

the fragment **XV** (*m/z* 115) is attributed to heterolytic fission of the molecular ion *via* an RDA-type process; in this case, however, effective stabilization of the benzylic carbocation presumably accounts for its formation as a cationic fragment. The fragmentation patterns of the structural analogues **209 – 212** are detailed in Table 6 and are supported by the B/E link-scan data.

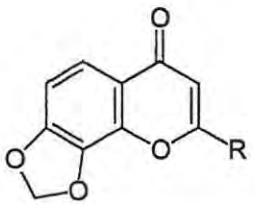


Scheme 48. Additional fragmentation of the molecular ion from compound **211**



Scheme 49. Additional fragmentations in the mass spectrum of compound **212**

Table 6. Selected peaks (*m/z*, followed, in parentheses, by % relative abundance) in the EI mass spectra of compounds **40** and **209 – 212**, classified according to ion-types **I – XV** (**Schemes 46 – 49**).



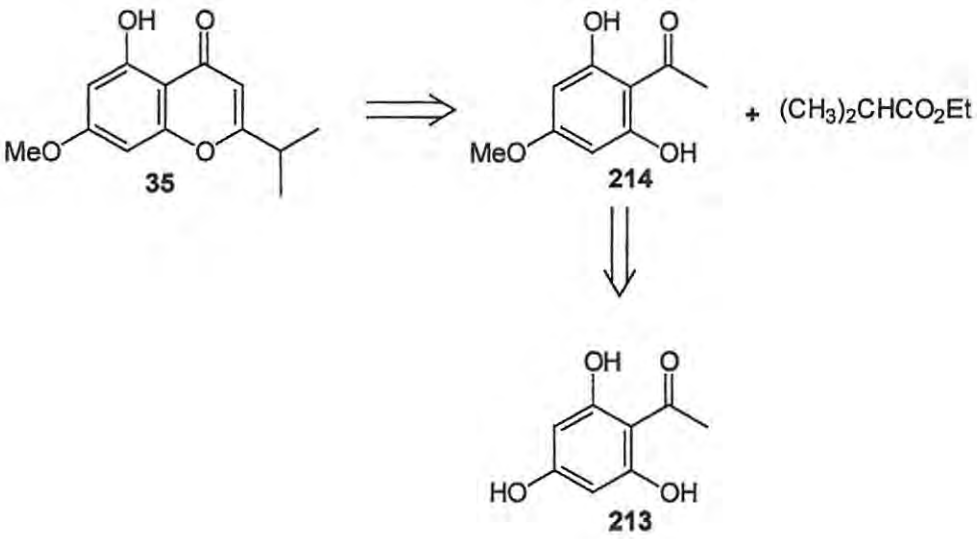
		Ion-types							
Compd	R	I	II	III	IV	V	VI	VII	VII
209	CH_3	204 (100)			175 (2)		164 (57)	134 (4)	106 (12)
210	CH_3CH_2	218 (100)			175 (4)		164 (46)	134 (3)	106 (11)
40	$CH_3CH_2CH_2$	232 (100)	217 (3)	204 (48)	175 (10)	165 (14)	164 (55)	134 (3)	106 (12)
211	$(CH_3)_2CH$	232 (100)	217 (3)			165 (8)	164 (26)	134 (2)	106 (2)
212	$PHCH_2$	280 (100)				165 (24)	164 (31)		106 (7)

		Ion-types						
Compd	R	IX	X	XI	XII	XIII	XIV	XV
209	CH_3	78 (5)	203 (29)	176 (6)				
210	CH_3CH_2	78 (4)	217 (21)	190 (2)				
40	$CH_3CH_2CH_2$	78 (5)						
211	$(CH_3)_2CH$				189 (9)			
212	$PHCH_2$					252 (1)	252 (2)	115 (6)

2.3 5-Hydroxy-2-isopropyl-7-methoxychromone 35 and C-2 side-chain analogues

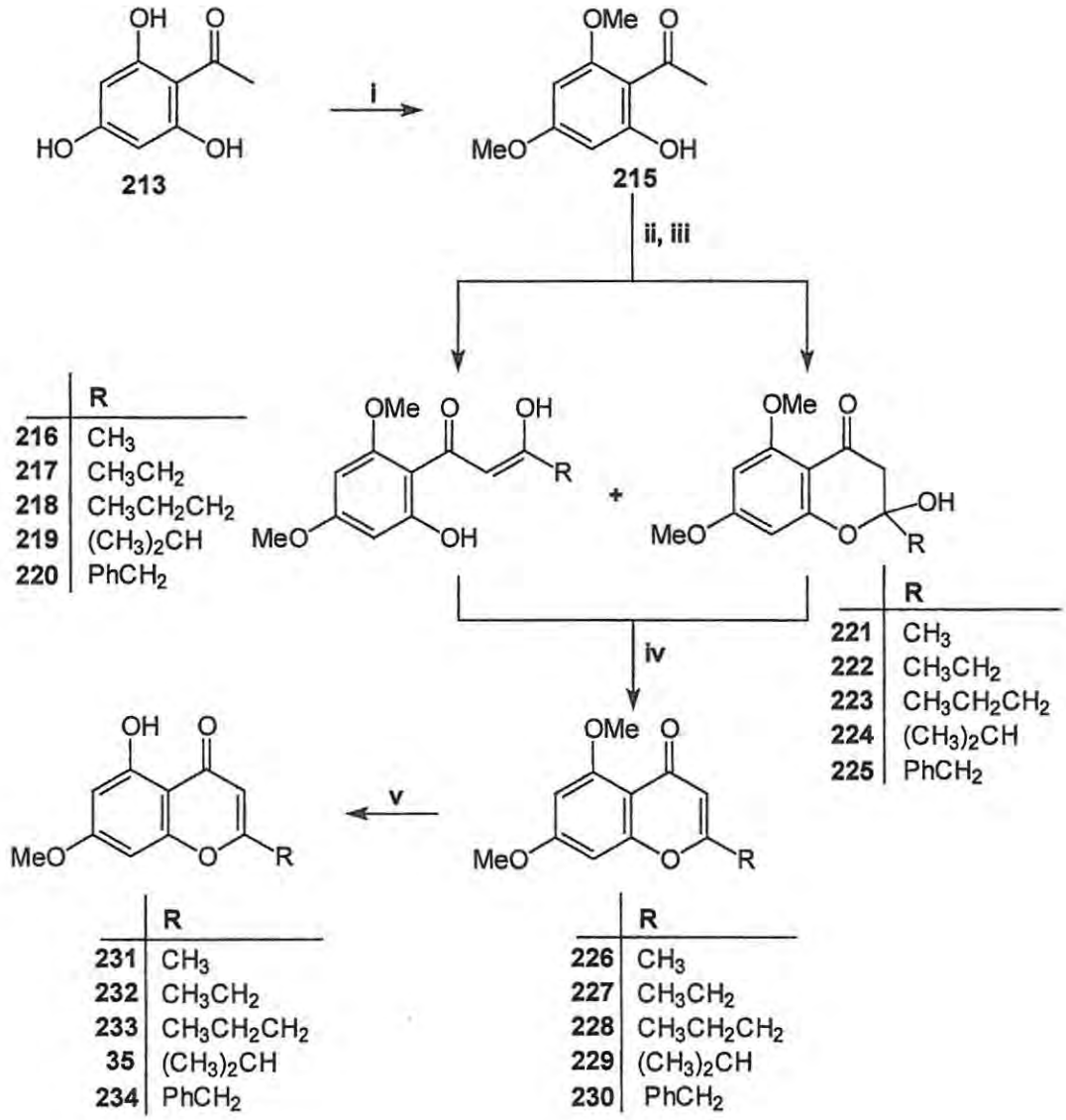
2.3.1 Synthesis of 5-hydroxy-2-isopropyl-7-methoxychromone 35 and C-2 side-chain analogues

Recently, Tsui *et al.*,[40] in their investigation of Hong-Kong medicinal plants, isolated a new chromone derivative, 5-hydroxy-2-isopropyl-7-methoxychromone **35**, from the aerial parts of *Baeckea frutescens* L., a plant used in traditional Chinese medicine for treating rheumatism and snake-bite. In previous work, Nohara and co-workers[89] have shown that the presence of hydroxyl groups on the benzene ring of the chromone system are important for biological activity. As part of the present study, we have developed an efficient synthesis and investigated the biological activity of 5-hydroxy-2-isopropyl-7-methoxychromone **35** and four structural analogues **231 – 234**. From the retrosynthetic analysis **Scheme 50**, it was apparent that the synthesis should involve 2',6'-dihydroxy-4'-methoxyacetophenone **214** as a crucial synthon.



Scheme 50

In an initial attempt to prepare compound **214**, using one equivalent of dimethylsulfoxide, one equivalent of the commercially available 2',4',6'-trihydroxyacetophenone **213** and one equivalent of K_2CO_3 as described by Haung *et al.*,[148] a mixture of products, comprising 2',6'-dihydroxy-4'-methoxyacetophenone **214**, 2'-hydroxy-4',6'-dimethoxyacetophenone **215** and the starting material, 2',4',6'-trihydroxyacetophenone **213**, was obtained. ^{1}H NMR analysis of this mixture indicated that the desired product **214** had been formed in less than 40% yield. Because of the low yield, this approach was abandoned and the strategy outlined in **Scheme 51** was adopted.



Scheme 51

Reagents: i) Me_2SO_4 (2 eq.), K_2CO_3 (2 eq.), acetone; ii) NaOEt, EtOH; iii) RCO_2Et; iv) AcOH, H_2SO_4; v) Ac_2O, HI, 115 °C, 30 min.

The first step of the synthesis involved selective methylation of 2',4',6'-trihydroxyacetophenone **213** with two equivalents of dimethylsulfoxide in the presence of two equivalents of anhydrous K_2CO_3 in acetone under reflux for 5 h. The required 2'-hydroxy-4',6'-dimethoxyacetophenone **215**[149] was obtained in 90% yield.

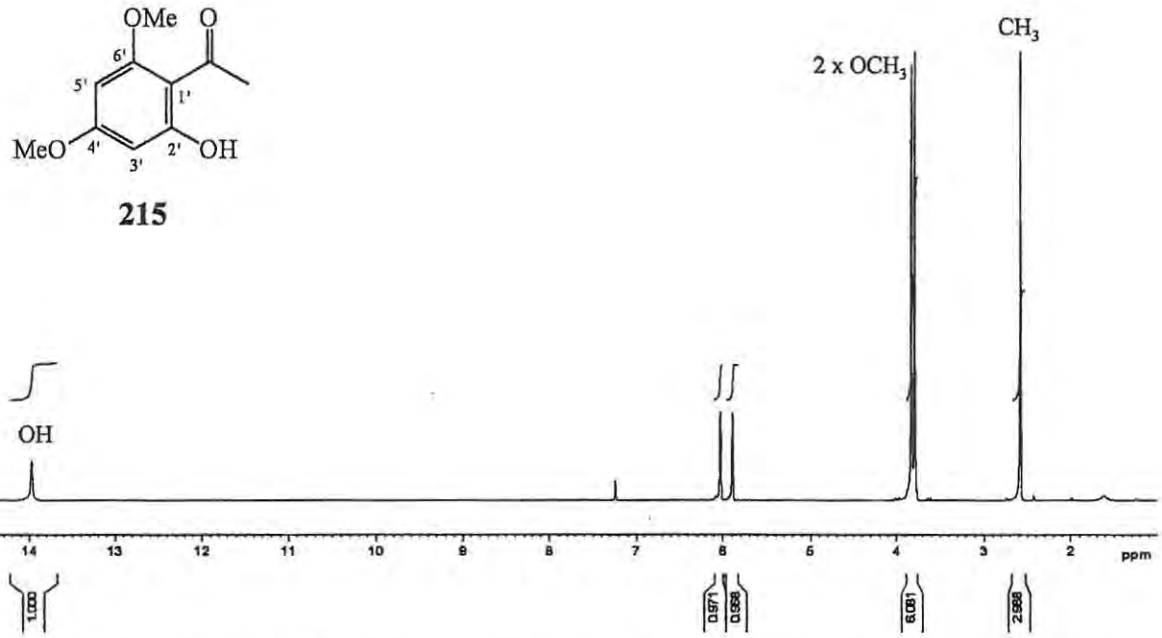


Figure 17. 400 MHz ^{1}H NMR spectrum of 2'-hydroxy-4',6'-dimethoxyacetophenone **215** in $CDCl_3$.

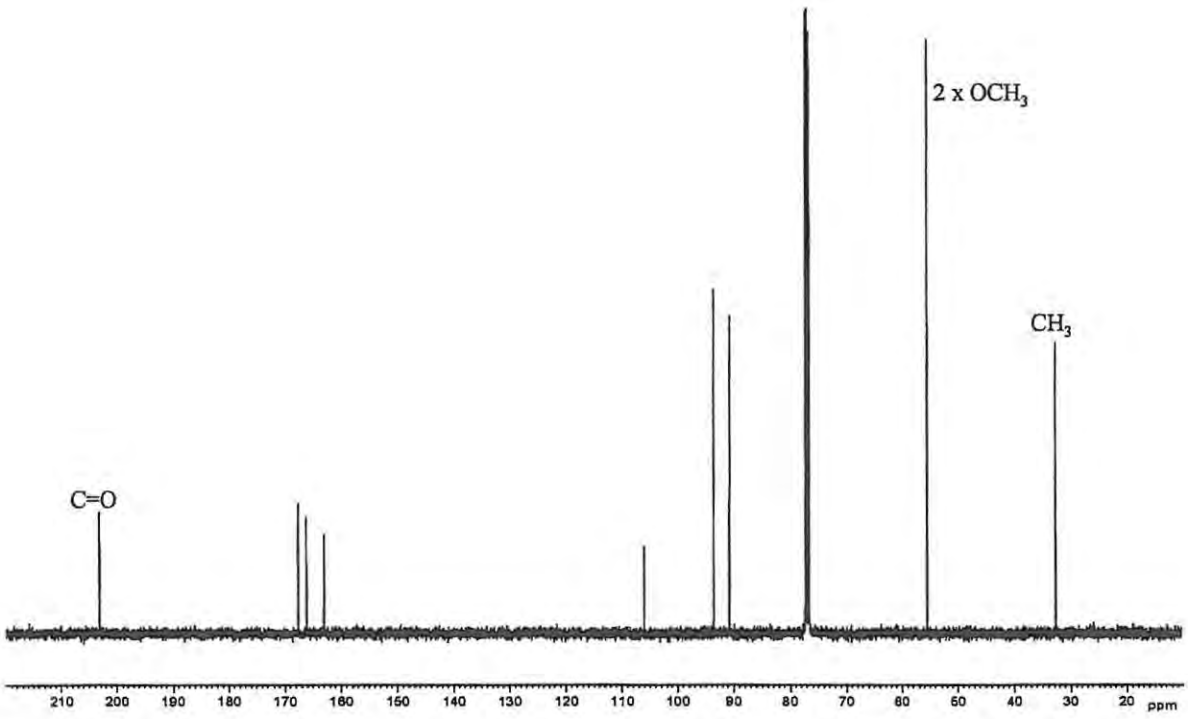


Figure 18. 100 MHz ^{13}C NMR spectrum of 2'-hydroxy-4',6'-dimethoxyacetophenone **215** in $CDCl_3$.

The ^{1}H NMR spectrum of 2'-hydroxy-4',6'-dimethoxyacetophenone **215** (Figure 17) reveals a singlet at δ 13.92 ppm corresponding to the phenolic hydroxyl group, the low-field shift being attributed to strong intramolecular hydrogen-bonding between the acetyl carbonyl and the 2'-hydroxyl group (see also, Section 2.2.1, p. 60). The ^{1}H NMR spectrum also reveals a singlet at δ 2.57 ppm corresponding to the acetyl methyl protons, two singlets at δ 3.78 and 3.82 ppm corresponding to the two methoxy groups and two doublets at δ 5.88 and 6.01 ppm, with a *meta*-coupling constant of 2.2 Hz, corresponding to the two aromatic protons, 5'-H and 3'-H, respectively. The ^{13}C NMR spectrum of 2'-hydroxy-4',6'-dimethoxyacetophenone **215** (Figure 18) shows only 9 distinct carbon signals, instead of the expected 10; the signal at δ 55.4 ppm is very intense, suggesting coincidence of the two methoxy carbon signals – a conclusion supported by the COSY, HMQC and HMBC data.

Treatment of 2'-hydroxy-4',6'-dimethoxyacetophenone **215** with two equivalents of sodium ethoxide in ethanol afforded the enolate which, on reaction with a series of ethyl carboxylate esters [R = CH_3, CH_3CH_2, $CH_3CH_2CH_2$, $(CH_3)_2CH$, $PhCH_2$] gave mixtures, indicated by ^{1}H NMR spectroscopy, to contain the corresponding C-acylated products (existing, in each case, as an enol tautomer, formulated as structures **216 – 220**) and their cyclized derivatives **221 – 225**. Treatment of these mixtures with a mixture of acetic and sulfuric acids afforded the chromone derivatives **226 – 230** in yields ranging from 40 to 70%. The dimethoxychromone derivatives **226 – 230** were then selectively demethylated at the C-5 position by treating with boiling acetic anhydride and hydriodic acid[150] to afford the desired monomethoxychromone derivatives **35** and **231 – 234** in yields ranging from 55 to 80%. The product structures were confirmed by elemental (HREIMS) and spectroscopic (IR, ^{1}H and ^{13}C NMR) analysis.

The ^{1}H NMR spectrum of the 2-isopropyl-5,7-dimethoxychromone **229** (Figure 19) reveals a doublet at δ 1.26 ppm, with a vicinal coupling constant of 6.9 Hz, corresponding to the two isopropyl methyl groups, a multiplet at δ 2.74 ppm corresponding to the methine proton signal and two singlets at δ 3.86 and 3.91 ppm corresponding to the two methoxy groups. The characteristic 3-H signal of the chromone nucleus appears at δ 6.00 ppm, while the two doublets at δ 6.32 and 6.42 ppm, with a *meta*-coupling constant of 2.2 Hz, correspond to the two aromatic protons, 6-H and 8-H, respectively.

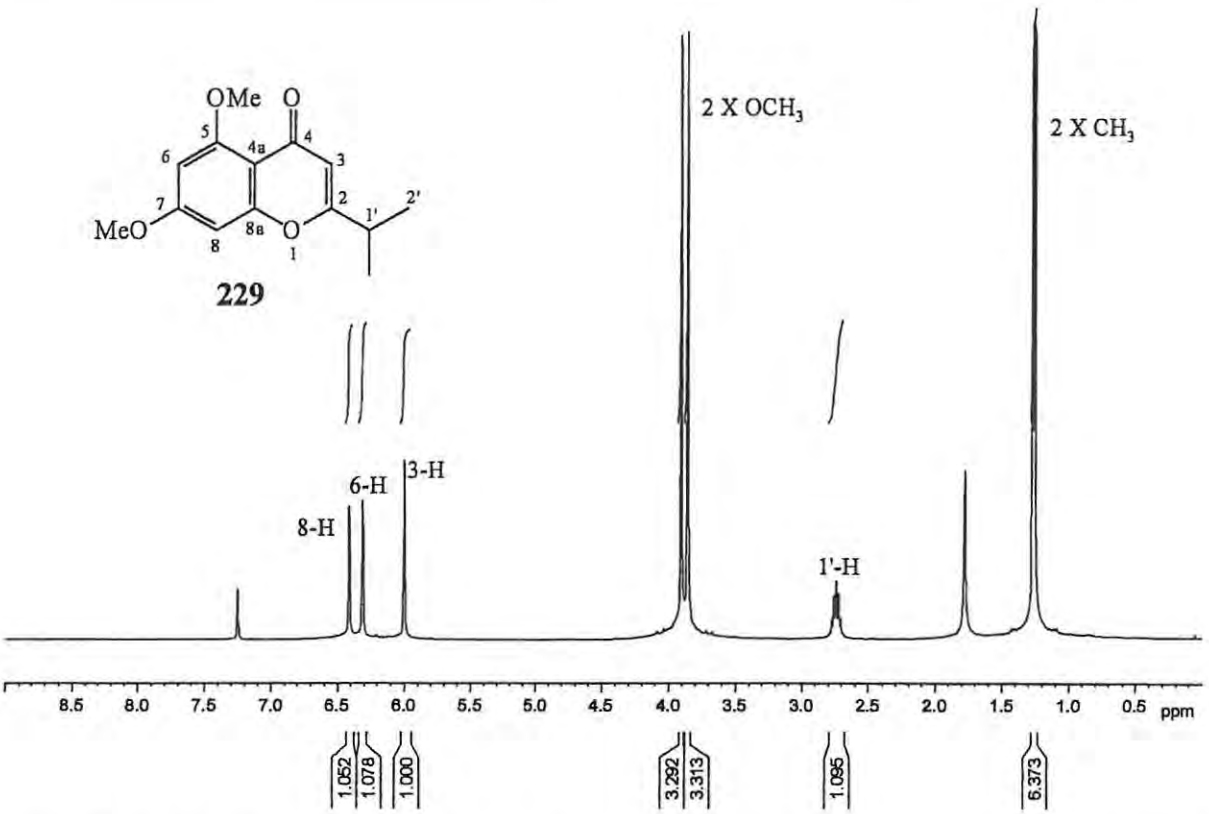


Figure 19. 400 MHz ^{1}H NMR spectrum of 2-isopropyl-5,7-dimethoxychromone **229** in $CDCl_3$.

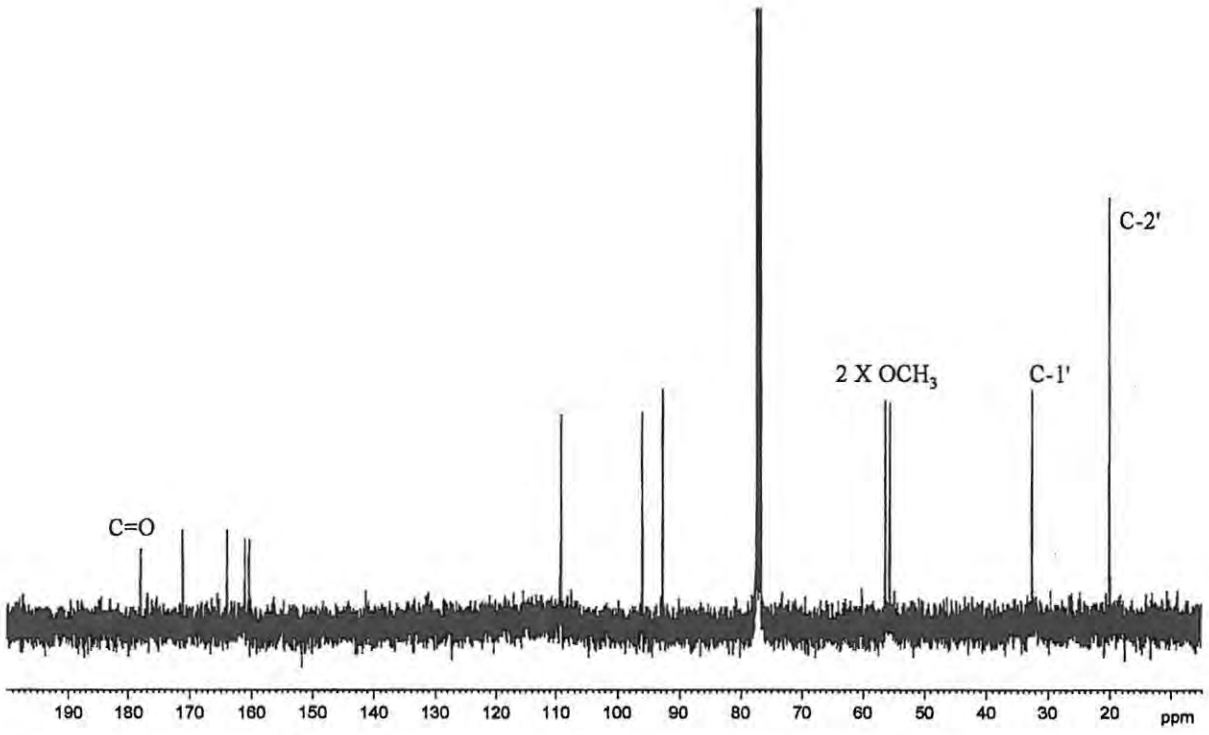


Figure 20. 100 MHz ^{13}C NMR spectrum of 2-isopropyl-5,7-dimethoxychromone **229** in $CDCl_3$.

The ^{13}C NMR spectrum (Figure 20) shows only 12 distinct carbon signals, instead of the expected 13 (with two signals overlapping at 108.92 and 108.93 ppm). The methyl and methine carbons resonating at δ 19.9 and 32.5 ppm, respectively, while the two methoxy carbons resonate at δ 55.6 and 56.3 ppm. The aromatic carbon signals were differentiated using the COSY spectrum, and all of the carbon signals were assigned using HMQC and HMBC correlations.

The ^{1}H NMR spectrum of 5-hydroxy-2-isopropyl-7-methoxychromone **35** (Figure 21) reveals a doublet at δ 1.29 ppm, with a vicinal coupling constant of 6.9 Hz due to the isopropyl methyl groups, a methine proton multiplet at δ 2.82 ppm, and a singlet at δ 3.85 ppm corresponding to the 7-methoxy group. The characteristic 3-H signal of the chromone nucleus appears at δ 6.03 ppm, while the two doublets at δ 6.32 and 6.36 ppm exhibit a *meta*-coupling constant of 2.2 Hz and correspond to the two aromatic protons, 6-H and 8-H, respectively. The low-field singlet at δ 12.70 ppm is attributed to the intramolecularly hydrogen-bonded phenolic group at C-5. The ^{13}C NMR spectrum (Figure 22) shows the expected 12 signals with the methyl and methine carbons resonating at δ 20.1 and 33.2 ppm, respectively, the methoxy group at δ 55.7 ppm and the carbonyl carbon at δ 182.8 ppm. The aromatic carbon signals were differentiated using the COSY spectrum and all of the carbon signals were assigned using HMQC and HMBC correlations. The IR spectrum shows a broad band at *ca.* 3000 cm^{-1} (absent in the IR spectrum of the dimethoxy derivatives **226 – 230**), attributed to the presence of the hydrogen-bonded phenolic group. The high-resolution mass spectrum reveals a peak at *m/z* 234 corresponding to the molecular ion. The spectroscopic data obtained for the 5-hydroxy-2-isopropyl-7-methoxychromone **35** were shown to correspond to those reported for the natural product.[40]

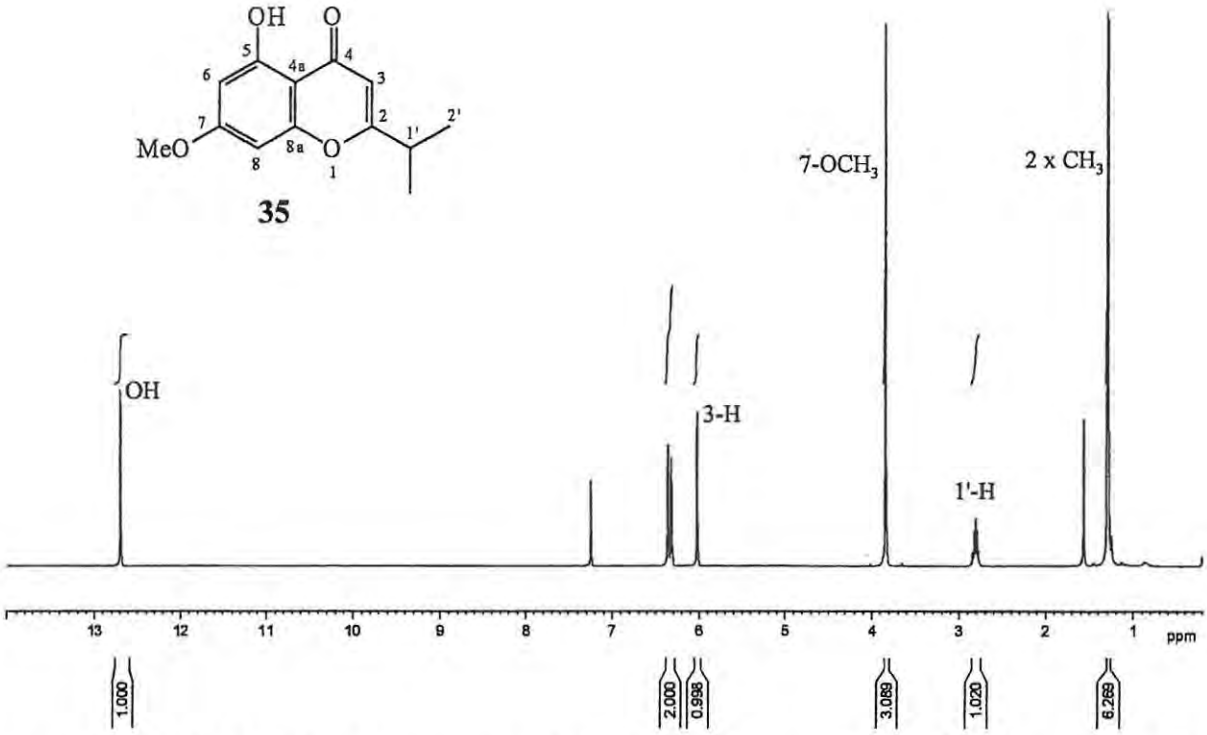


Figure 21. 400 MHz ^{1}H NMR spectrum of 5-hydroxy-2-isopropyl-7-methoxychromone **35** in $CDCl_3$.

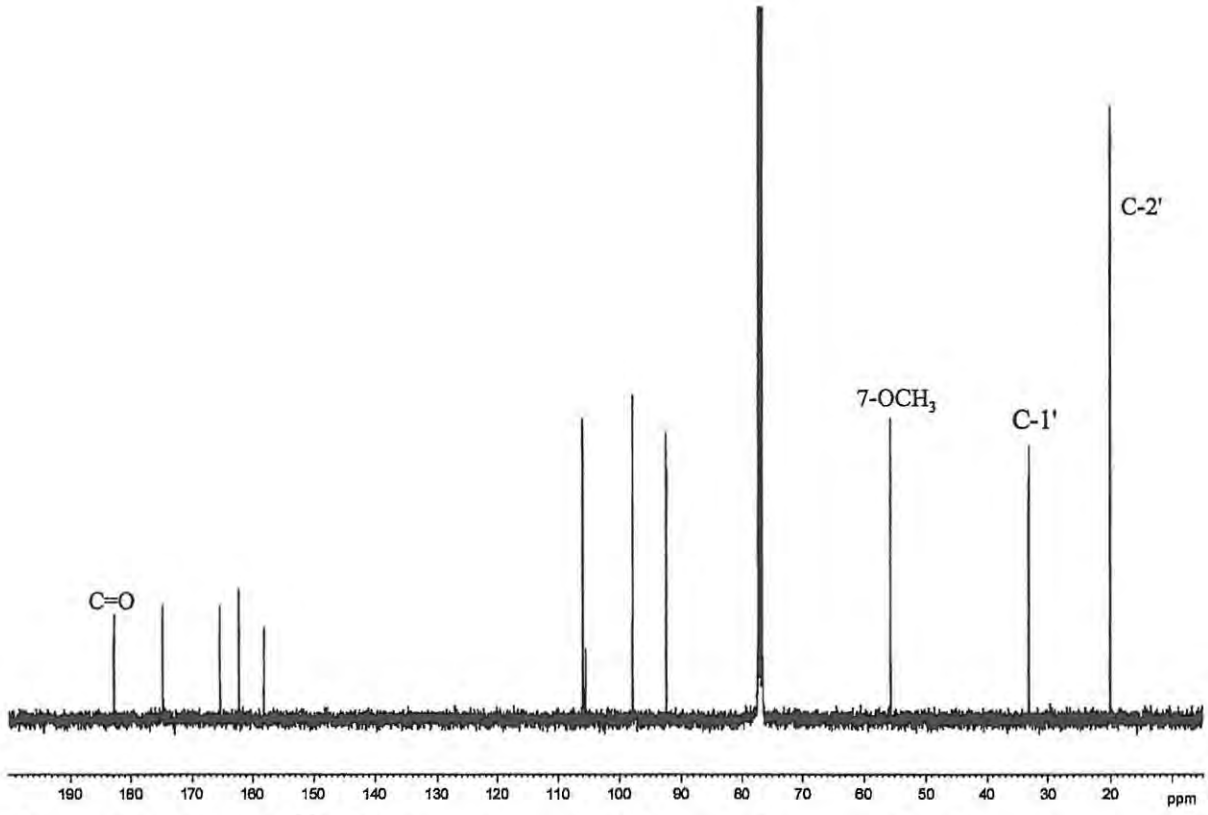
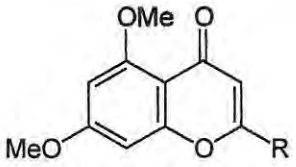


Figure 22. 100 MHz ^{13}C NMR spectrum of 5-hydroxy-2-isopropyl-7-methoxychromone **35** in $CDCl_3$.

2.3.2 Assessment of the biological activity of 5-hydroxy-2-isopropyl-7-methoxychromone **35**, *its C-2 side-chain analogues and their dimethoxy derivatives*

In order to evaluate their biological activity, *Artemia salina* larvicidal bioassays were performed using the method of Solis *et al.*,[146] as discussed in Section 2.2.2. Estimates of median lethal concentrations were obtained by probit analysis[147] (and use of the trimmed Spearman-Karber method[151]) of the *A. salina* mortality data from 12 solutions across concentration ranges of 200.0 – 10.00 μg/mL for compounds **35** and **231 – 233**, 700.0 – 300.0 μg/mL for compound **234** and 400.0 – 50.00 μg/mL for compounds **226 – 230**.

Table 7. Summary of *Artemia salina* biological assay data for compounds **226 – 230**[a]. Estimates of median lethal concentrations obtained by probit analysis[147] and the trimmed Spearman-Karber method.[151]

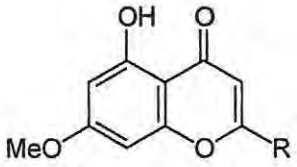


Compd.	R	LC_{50} (μm/mL)	95% Confidence interval (μm/mL)	
			Lower limit	Upper Limit
226	CH_3	315.1	223.9	443.3
227	CH_3CH_2	200.1	182.3	219.7
228	$CH_3CH_2CH_2$	106.0	95.0	118.3
229	$(CH_3)_2CH$	94.2	82.5	107.5
230	$PhCH_2$	247.3	217.1	281.8

[a]Analyses conducted by C.A. Gray

Table 7 shows the LC_{50} values of the dimethoxychromone derivatives **226 – 230**, and it is apparent that these compounds are, in general, considerably less active than the monomethoxy compounds **35** and **231 – 234** (Table 8). These data indicate that the hydroxyl group at C-5 is important for biological activity. Four of the chromone derivatives **35** and **231 – 233** showed significant cytotoxic effects against the brine shrimp *Artemia salina*. The 2-benzyl derivative **234**, however, appears to be even less active than the dimethoxy derivatives (Table 7). The LC_{50} values (Table 8) indicate an interesting range in activity with 5-hydroxy-2-isopropyl-7-methoxychromone **35** being highly toxic (LC_{50} : 23 ppm), whilst the analogues **232** and **233** display less toxicity (LC_{50} : 39 and 32 ppm respectively) and compounds **231** and **234** are significantly less active (LC_{50} : 112 and 517 ppm respectively).

Table 8. Summary of *Artemia salina* biological assay data for compounds **35** and **231 – 234**[a]. Estimates of median lethal concentrations obtained by probit analysis[147] and the trimmed Spearman-Karber method.[151]



Compd.	R	LC_{50} (μm/mL)	95% Confidence interval (μm/mL)	
			Lower limit	Upper Limit
231	CH_3	112.2	94.8	132.9
232	CH_3CH_2	39.0	35.7	42.6
233	$CH_3CH_2CH_2$	31.5	27.4	36.4
35	$(CH_3)_2CH$	23.8	21.2	26.8
234	$PhCH_2$	516.6	445.4	599.2

[a]Analyses conducted by C.A. Gray

2.3.3 Mass spectrometric analysis of 2-isopropyl-5,7-dimethoxychromone **229** *and C-2 side-chain analogues*

A study of the electron impact (EI) mass fragmentation patterns of 2-isopropyl-5,7-dimethoxychromone **229** and the C-2 side-chain analogues **226 – 230** was undertaken, using a combination of low-resolution, high-resolution and B/E link-scan data, and selected fragmentation data for these compounds are summarized in Table 9. The EI mass spectrum of the parent system, 2-isopropyl-5,7-dimethoxychromone **229** is illustrated in Figure 23, while the proposed fragmentation patterns are outlined in **Scheme 52**.

In the mass spectrum of the parent compound **229**, the base peak at *m/z* 248 corresponds to the molecular ion **I**, which fragment *via* two major pathways (**A** and **B**). In path [**A**], loss of a hydrogen atom from the molecular ion **I** gives fragment **II** (*m/z* 247) which, on loss of carbon monoxide, affords cation **III** (*m/z* 219). In path [**B**], loss of HCHO from the molecular ion **I** gives rise to the radical cation **IV** (*m/z* 218), in a fragmentation which is common in aromatic methoxy derivatives.[152] Fragment **IV** then undergoes three different fragmentation pathways (**C**, **D** and **E**). In path [**C**], loss of a hydrogen atom affords cation **V** (*m/z* 217), which then loses HCHO in a fragmentation associated with the second aromatic methyl ether group to give cation **VI** (*m/z* 187). In path [**E**], loss of 2-methyl-1-butyne by the retro-Diels-Alder (RDA) pathway, characteristic of the chromone nucleus, gives the ketene **VIII** (*m/z* 150), which then undergoes decarbonylation to give the ring-contracted fragment **IX** (*m/z* 122). Formation of the protonated ketene **VII** (*m/z* 151) from fragment **IV** is to attributed an $[RDA+H]^+$ process (path [**D**]), which is also characteristic of most chromone system; deprotonation then leads to the ketene radical cation **VIII** (*m/z* 150).

The ion-types designated **I – IX** (**Scheme 52**), were generally observed in the mass spectra of the analogues **226 – 230** (as indicated in Table 9), while the 2-benzyl analogue **230** exhibits the specific, additional fragments illustrated in **Scheme 53**. The fragment **X** (*m/z* 116) is attributed to fission of the molecular ion *via* the $[RDA+H]^+$-type process. Loss of a hydrogen atom from fragment **X** then affords the resonance-stabilized benzylic-propargylic carbocation **XI** (*m/z* 115), which was also observed in the fragmentation of 2-benzyl-7,8-(methylenedioxy)chromone **212** (see p. 70). The additional fragment **XII** (*m/z* 91) results

from the heterolytic fission of the molecular ion to give the resonance-stabilized tropylium cation **XII** typical of alkylbenzenes.

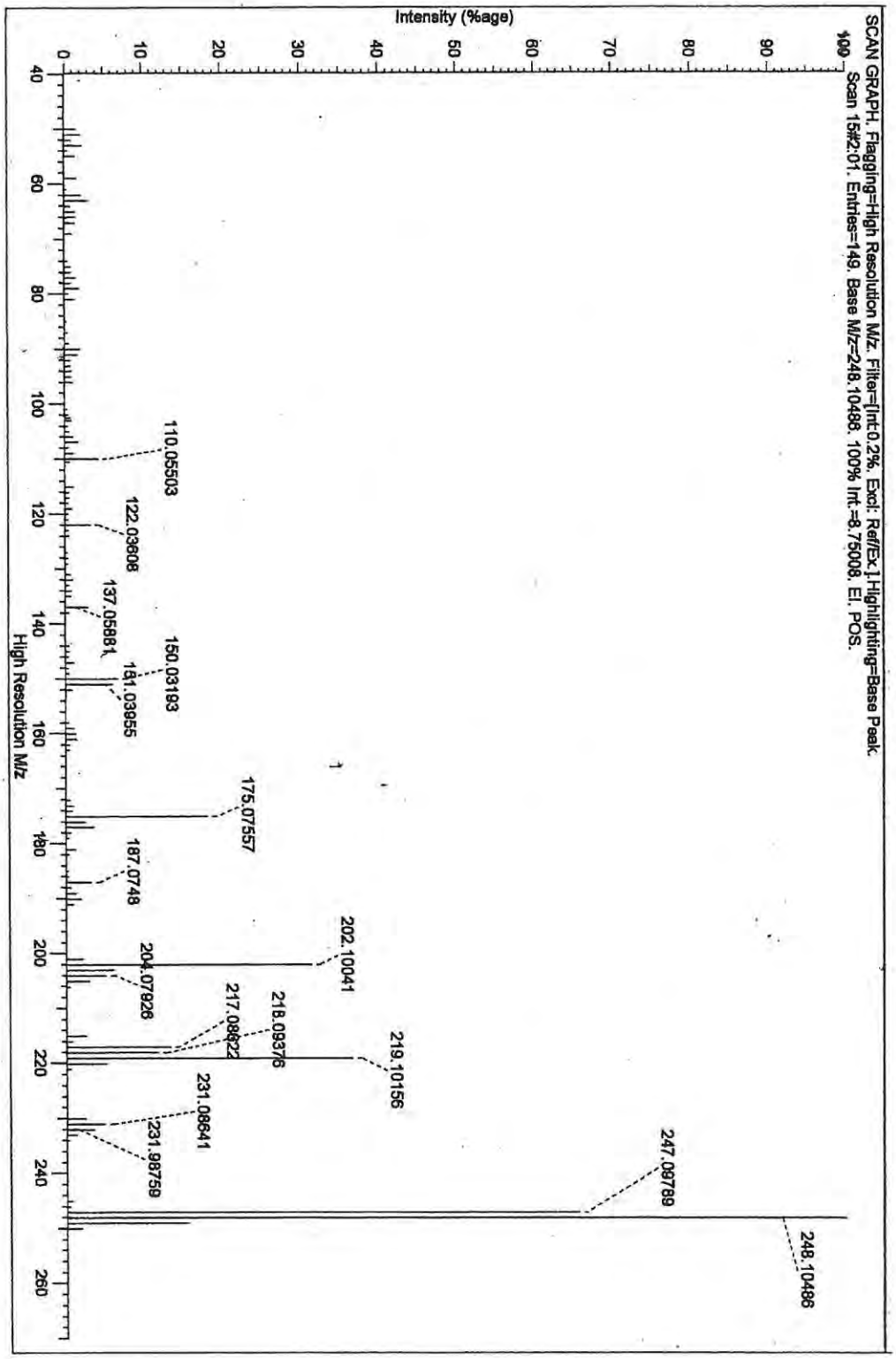
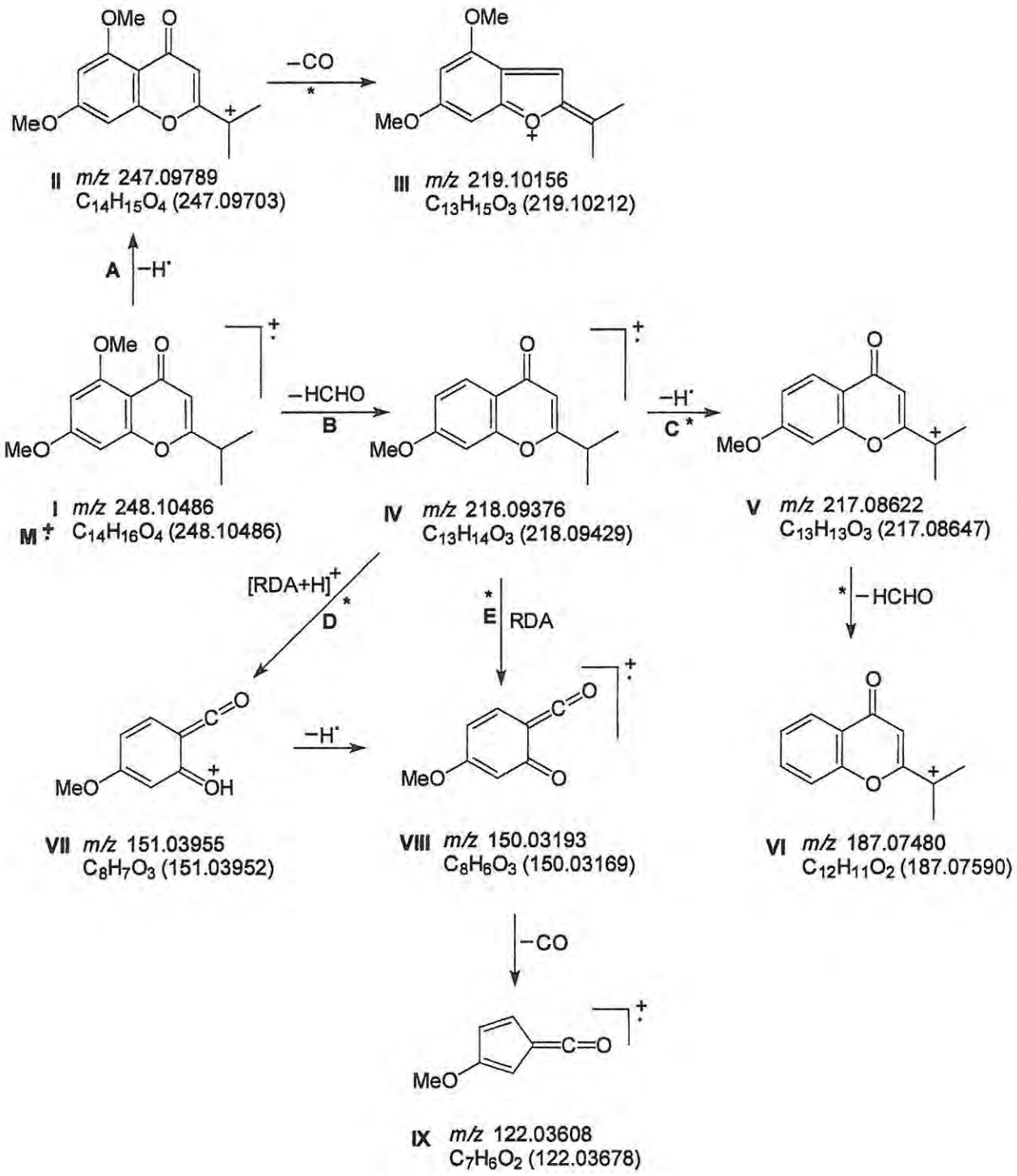
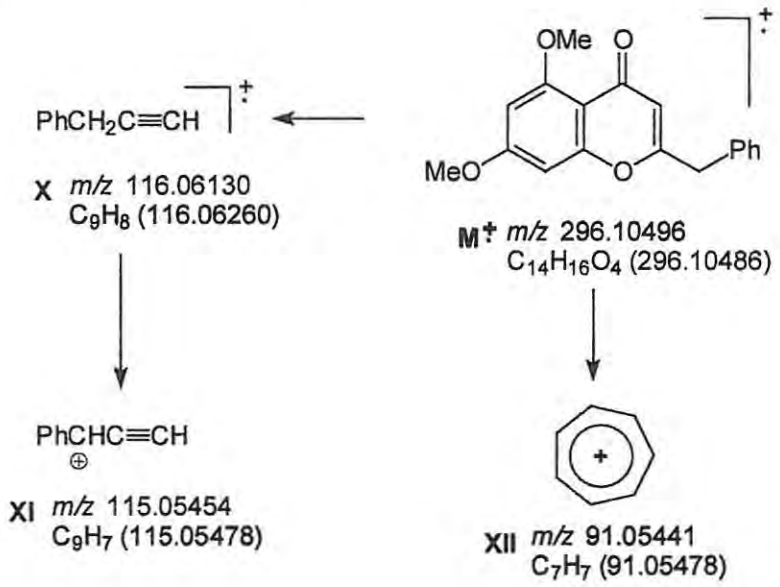


Figure 23. High-resolution EI mass spectrum of compound **229**.

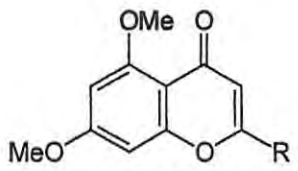


Scheme 52. EI mass-fragmentation patterns for compound **229**. Accurate masses (*m/z*) are followed, in parentheses, by calculated formula masses; an asterisk indicates a pathway supported by the B/E link-scan data.



Scheme 53. Additional fragmentations in the mass spectrum of compound **230**

Table 9. Selected peaks (*m/z*; followed, in parentheses, by % relative abundance) in the EI mass spectra of compounds **226 – 230**, classified according to ion-types **I – XII** (**Schemes 52** and **53**).



		Ion-types					
Compd	R	I	II	III	IV	V	VI
226	CH_3	220 (100)	219 (50)	191 (33)	190 (13)	189 (10)	159 (3)
227	CH_3CH_2	234 (100)	233 (60)	216 (2)	204 (13)	203 (12)	173 (3)
228	$CH_3CH_2CH_2$	248 (100)	247 (67)	219 (38)	218 (4)	217 (13)	
229	$(CH_3)_2CH$	248 (100)	247 (66)	219 (37)	218 (12)	217 (13)	187 (3)
230	$PhCH_2$	296 (100)	295 (49)	267 (28)			

		Ion-types					
Compd	R	VII	VIII	IX	X	XI	XII
226	CH_3		150 (10)	122 (6)			
227	CH_3CH_2	151 (4)	150 (9)	122 (6)			
228	$CH_3CH_2CH_2$	151 (5)	150 (13)	122 (4)			
229	$(CH_3)_2CH$	151 (6)	150 (7)	122 (3)			
230	$PhCH_2$	151 (18)	150 (4)	122 (3)	116 (3)	115 (8)	91 (6)

2.3.4 Mass spectrometric analysis of 5-hydroxy-2-isopropyl-7-methoxy-chromone **35** *and C-2 side-chain analogues*

The EI mass spectrum of the parent system, 5-hydroxy-2-isopropyl-7-methoxychromone **35**, is illustrated in Figure 24, while the proposed fragmentation pathways are outlined in **Scheme 54**.

The base peak at *m/z* 234 corresponds to the molecular ion **I**, from which five major fragmentations pathways (**A, B, C, D** and **E**) are apparent. In path [**A**], loss of a hydrogen atom from the molecular ion **I** gives the tertiary carbocation **II** (*m/z* 233), which loses carbon monoxide to give the ring-contracted cation **IV** (*m/z* 205). In path [**B**], loss of a methyl radical from the molecular ion gives the resonance-stabilized carbocation fragment **III** (*m/z* 219), which subsequently undergoes decarbonylation to give the ring-contracted fragment **VI** (*m/z* 191). In path [**C**], the elimination of HCHO, characteristic of aromatic methyl ethers,[152] affords the radical-cation **V** (*m/z* 204), subsequent decarbonylation of the radical cation **V** affords fragment **X** (*m/z* 176). In path [**D**], the molecular ion **I** undergoes the expected retro-Diels-Alder reaction (RDA), characteristic of the chromone nucleus, with the loss of 2-methyl-1-butyne to give fragment **VIII** (*m/z* 166). Fragment **VIII** then loses carbon monoxide to give the ketene **IX** (*m/z* 138), which further loses carbon monoxide to afford the radical cation **XI** ((*m/z* 110). The formation of fragment **VII** (*m/z* 167) {path [**E**]} from the molecular ion is attributed to the $[RDA+H]^+$ process, which is also common in chromone derivatives; subsequent deprotonation provides an alternative route to fragment **VIII**.

While, most of the ion-types, designated **I** – **XI**, were consistently observed in the mass spectra of the analogues **231** – **234**, additional fragments observed for the 2-benzyl derivative **234** (**Scheme 55**) arise from the presence of the phenyl group. Thus, formation of fragment **XII** (*m/z* 115) is attributed to heterolytic fission of the molecular ion *via* an RDA-type process; as in the previous case involving 2-benzylchromones (see pp. 70 and 84), effective stabilization of the benzylic-propargylic carbocation presumably accounts for its formation. The tropylium cation **XIII** (*m/z* 91) and the phenyl cation **XIV** (*m/z* 77) are typical of alkyl benzenes. The link-scan data supports these fragmentations.

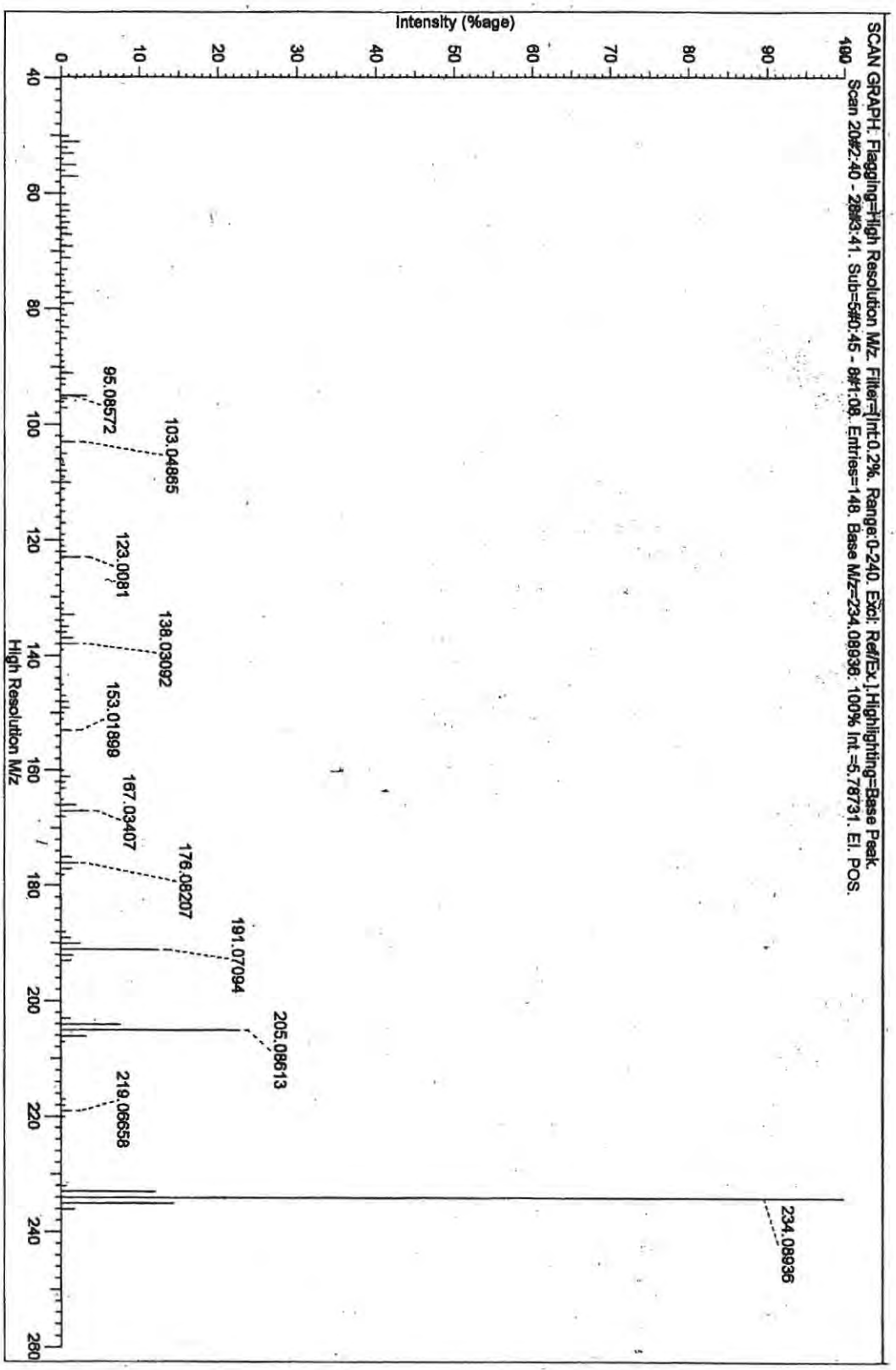
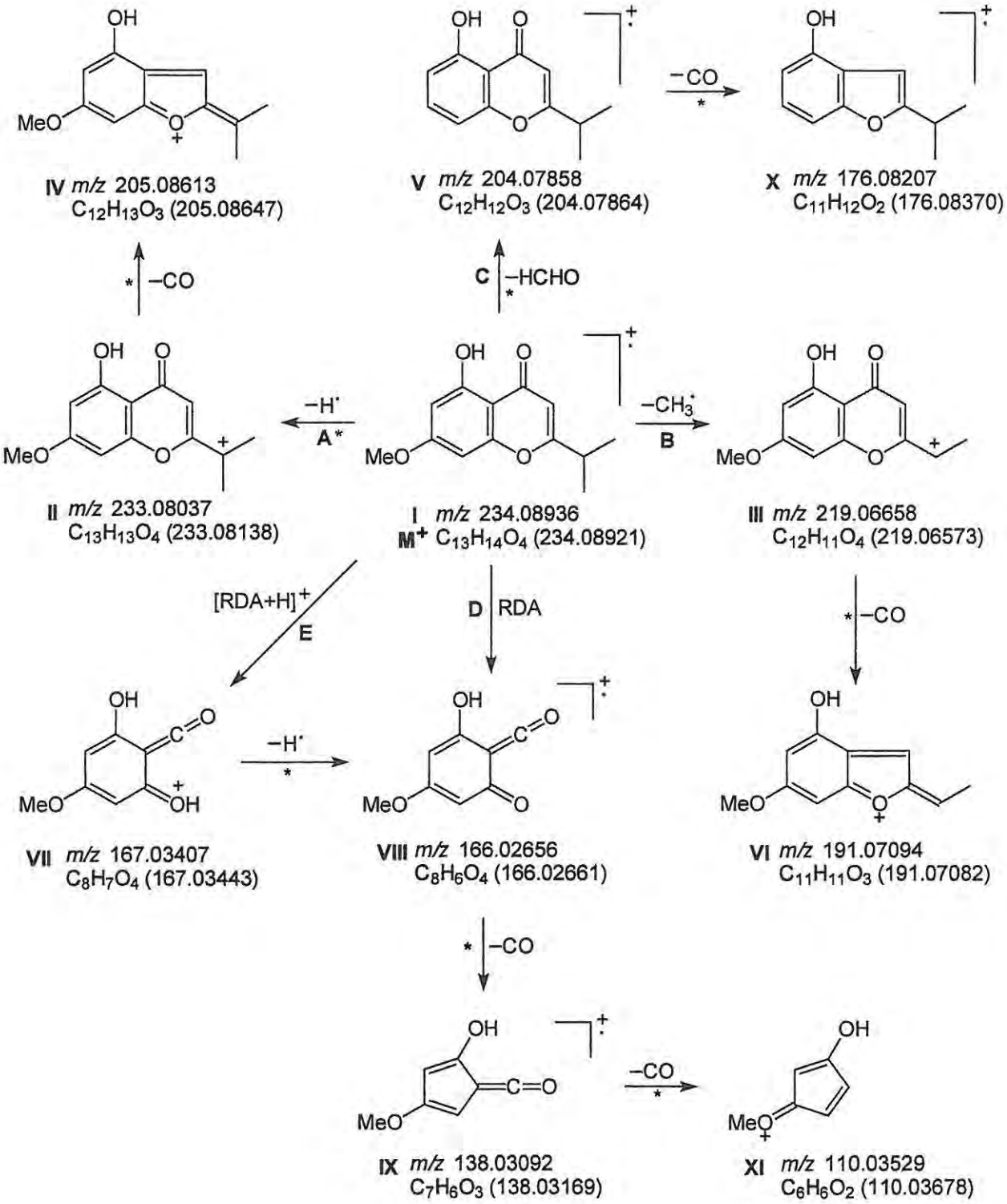
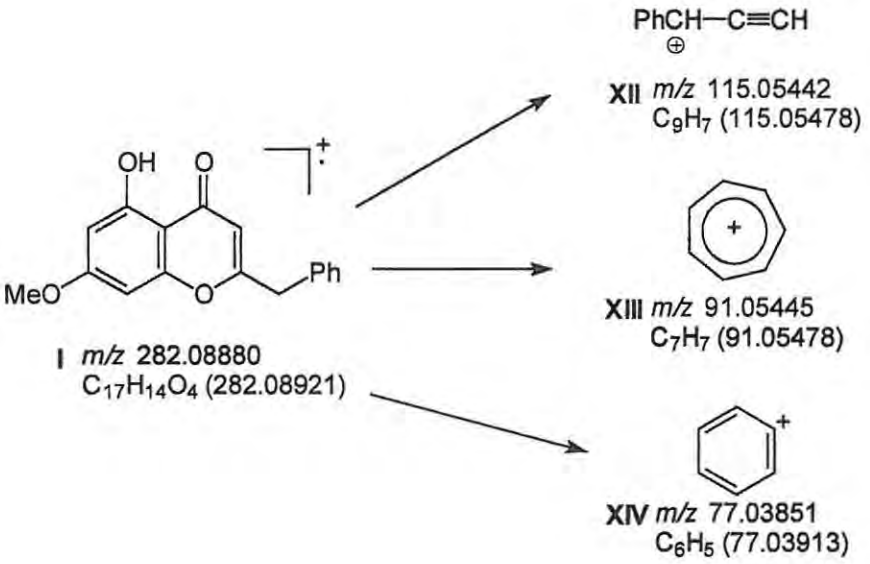


Figure 24. High-resolution EI mass spectrum of compound **35**.

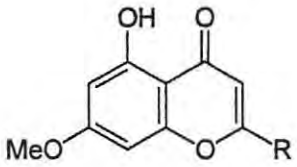


Scheme 54. EI mass-fragmentation patterns for compound **35**. Accurate masses (*m/z*) are followed, in parentheses, by calculated formula masses; an asterisk indicates a pathway supported by the B/E link-scan data.



Scheme 55. Further fragmentations of ion-type **I** from compound **234**

Table 10. Selected peaks (*m/z* followed in parentheses, by % relative abundance) in the EI mass spectra of compounds **35** and **231 – 234**, classified according to ion-types **I – XIV** (**Schemes 54** and **55**).



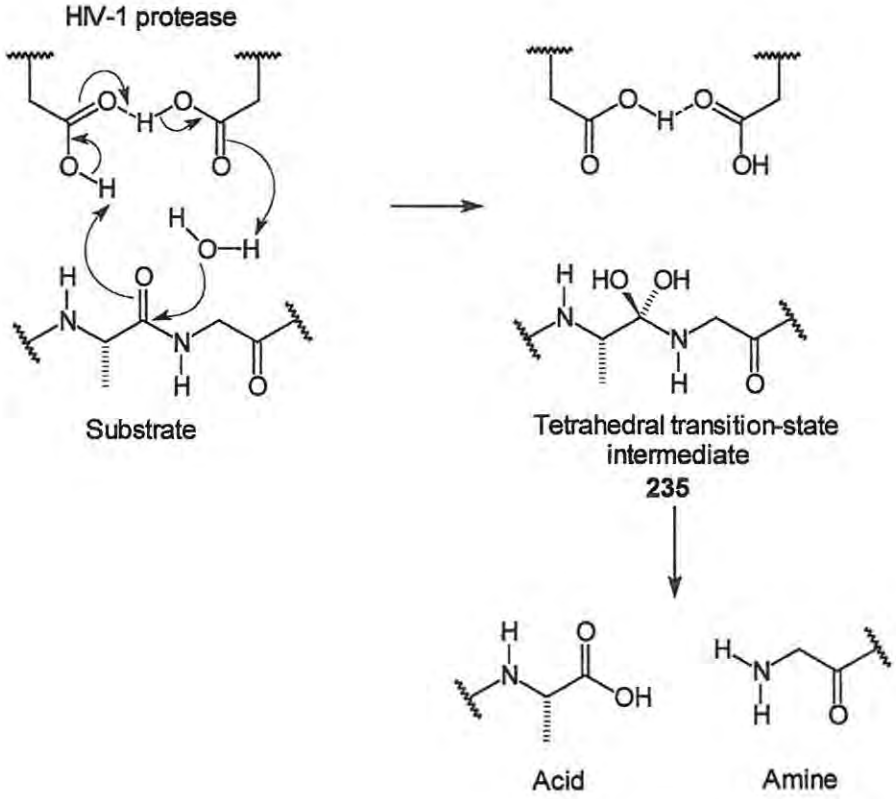
		Ion-types						
Compd	R	I	II	III	IV	V	VI	VII
231	CH_3	206 (100)	205 (15)	191 (1)	177 (48)	176 (16)	163 (9)	167[a]
232	CH_3CH_2	220 (100)	219 (15)	205 (1)	191 (37)	190 (12)	177 (9)	
233	$CH_3CH_2CH_2$	234 (100)	233 (10)	219 (1)	205 (23)	204 (6)	191 (2)	167 (3)
35	$(CH_3)_2CH$	234 (100)	233 (12)	219 (1)	205 (23)	204 (8)	191 (12)	167 (4)
234	$PhCH_2$	282 (100)	281 (8)		253 (13)	252 (4)		167 (5)

		Ion-types						
Compd	R	VIII	IX	X	XI	XII	XIII	XIV
231	CH_3	166[a]	138 (4)	148 (10)	110 (2)			
232	CH_3CH_2	166 (1)	138 (3)	162 (5)	110 (2)			
233	$CH_3CH_2CH_2$	166 (4)	138 (3)	176 (2)	110 (1)			
35	$(CH_3)_2CH$	166 (2)	138 (2)	176 (2)	110 (1)			
234	$PhCH_2$	166 (2)	138 (1)	224 (2)	110 (1)	115 (7)	91 (6)	77 (2)

[a]% relative abundance under 1

2.4 Synthesis of potential HIV-1 protease inhibitors

The rapid spread of the acquired immune deficiency syndrome (AIDS) and the identification of the human immunodeficiency virus (HIV) as the main cause of the epidemic have prompted efforts to understand the viral replication process and, hence, to establish suitable targets for antiviral therapy. HIV is known to be a member of the *Lentiviridae*, a subfamily retroviruses.[106,109] Like other retroviruses it contains 3 major genes, *viz.*, *gag*, *pol* and *env*,[109,153] with the *gag* and *pol* genes expressed as polyproteins. These proteins are catalytically cleaved by aspartic acid residues in the HIV-1 protease enzyme to produce structural protein needed by the virus for replication.[154-157] Thus, HIV-1 protease acts as a "molecular pair of scissors" that cleaves the *gag* and *pol* precursor proteins to produce viral structural proteins.[109] **Scheme 56** illustrates the proposed mechanism by which HIV-1 protease hydrolyzes and cleaves the protein substrate.[109]



Scheme 56

Catalytic hydration of the protein substrate by a water molecule affords the transition-state intermediate **235,** which undergoes fission to afford fragments with carboxylic acid and amine termini. These fragments are used by the virus for replication, and the interruption of this catatylical process results in the production of immature virions which are non-infectious.[158] Consequently, the HIV-1 protease enzyme has emerged as an important target for AIDS chemotherapy, and a number of HIV protease inhibitors are currently being used for the treatment of AIDS.[159] Recent clinical studies have shown that many HIV protease inhibitors reduce the viral load and increase the number of $CD4^+$ lymphocytes (T-cells) in patients infected with HIV. The severe decrease in the number of $CD4^+$ lymphocytes in such patients often results in opportunistic infections and diseases.[160] Many potent and selective HIV-1 protease inhibitors have been designed as transition-state **235** mimetics, which incorporate a non-hydrolyzable hydroxyethylamine **160** or hydroxyethylene **161** dipeptide isostere at the P_1-P_1' catalytic site.[114,117,161] As indicated in Section 1.2.4.2 (pp. 40-41), some chromone derivatives have been shown to inhibit HIV-1 protease activity, and belong to the class of non-peptidic HIV protease inhibitors. All five of the clinically useful HIV-1 protease inhibitors (saquinavir **155,** ritonavir **156,** indinavir **157,** nelfinavir **158** and amprenavir **159**) and other synthetic inhibitors, currently on clinical trials, incorporate either hydroxy-ethylamine or hydroxyethylene dipeptide isosteres.

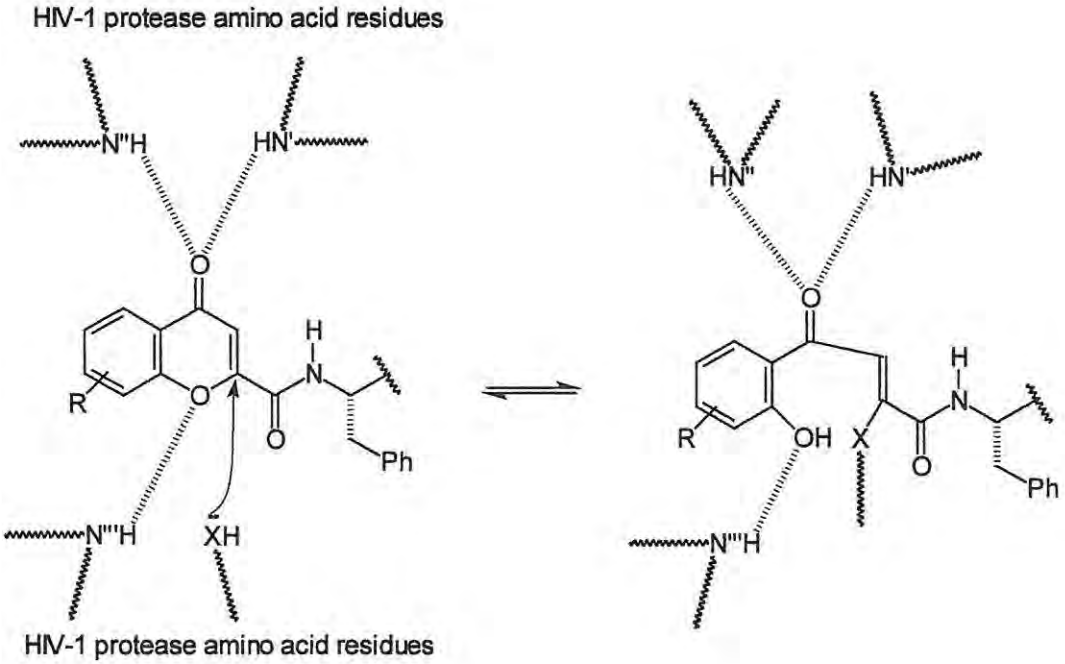
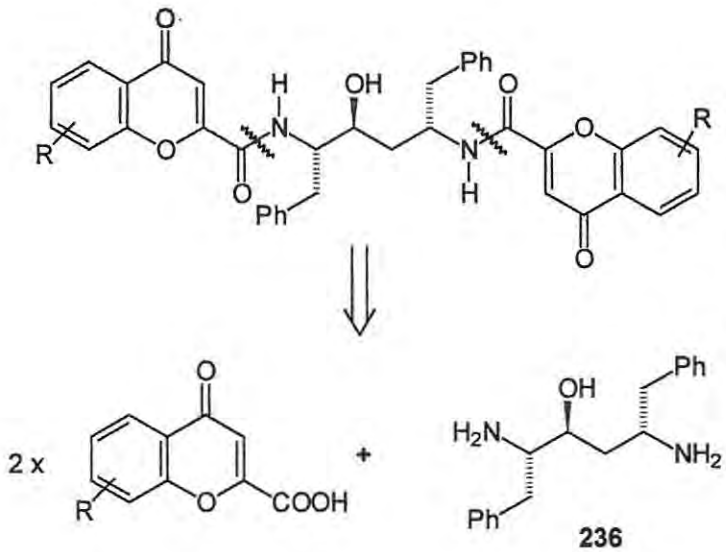


Figure 25. Hypothetical hydrogen-bonding interactions and ring-opening of the chromone ring in the HIV protease receptor cavity (X = O, S, NH).

However, despite the host of publications to date, no attention appears to have been given to the synthesis of systems containing both chromone and dipeptide isostere moieties. We hypothesized that attachment of chromone nuclei on to the hydroxyethylene dipeptide isostere **161** might:- i) improve aqueous solubility and oral bioavailability; ii) permit hydrogen-bonding between the chromone carbonyl and ether oxygens, on one hand, and structural water molecules or amino acid residues in the enzyme receptor cavity, on the other; and iii) permit nucleophilic ring-cleavage of the chromone pyran ring by suitably located amino acid residues, resulting in covalent links between the enzyme and the inhibitor (Figure 25).

Of the five HIV-1 protease inhibitors in clinical use, ritonavir **156**, is considered the single most potent but, recently, multidrug therapy has been reported to be very effective in combatting the disease.[115,162] In the present study, the synthesis of ritonavir analogues, containing chromone termini was investigated, and an interactive computer programme was used to model the docking of these analogues into the active site of the enzyme. The synthetic strategy which we adopted was to first synthesize the non-hydrolyzable hydroxyethylene dipeptide isostere **236**, which could then be coupled to chromone carboxylic acids to afford the required ritonavir analogues as illustrated in the retrosynthetic analysis in **Scheme 57.**

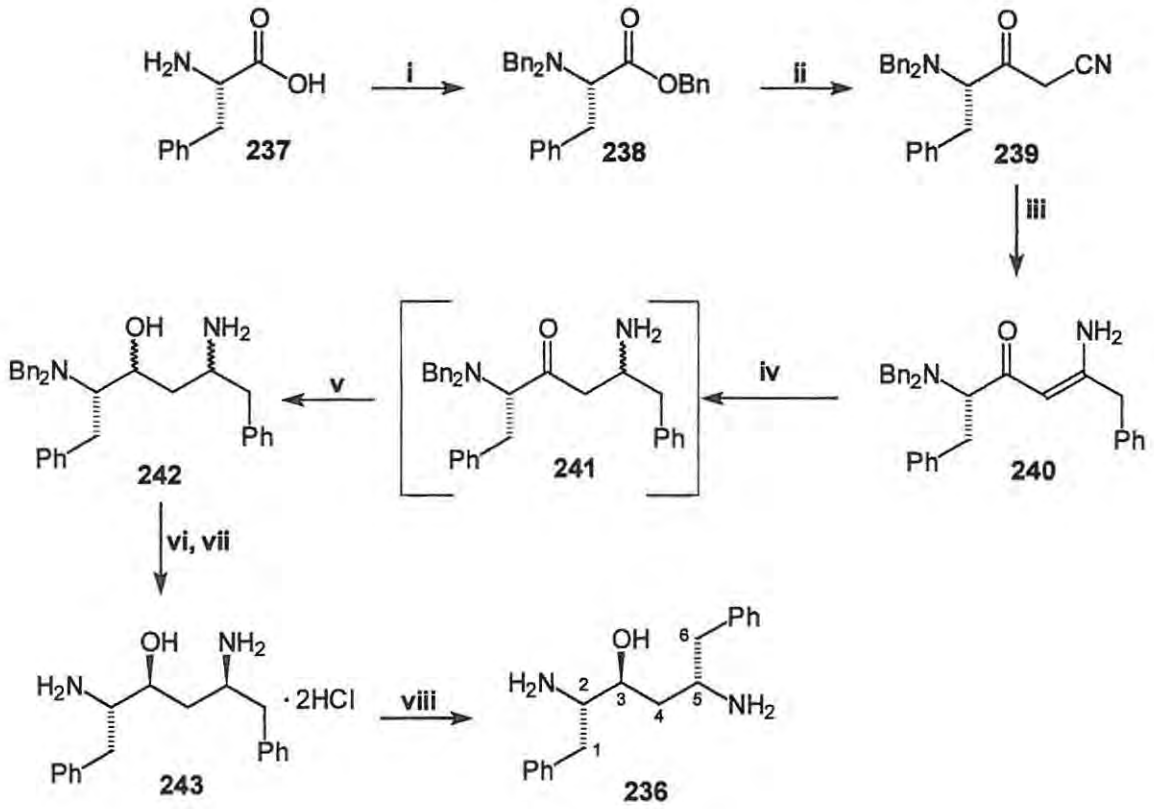


236

Scheme 57

2.4.1 Synthesis of the hydroxyethylene dipeptide isostere

The hydroxyethylene dipeptide isostere **236** was synthesized following the approach reported by Stuk *et al.*,[163] as outlined in **Scheme 58**. The synthetic sequence, which begins with commercially available L-phenylalanine **237**, involves seven steps and requires diastereo-selective reduction of the enaminone intermediate **240**. In order to ensure that this reduction affords the required alcohol, prior dibenzylation of the adjacent amino group was necessary to inhibit possible chelation-controlled hydride addition to the amino ketone intermediate **241**.



Scheme 58[163]

Reagents: i) BnCl, K_2CO_3, H_2O; ii) CH_3CN, $NaNH_2$, dry THF; iii) BnMgCl, dry THF; iv) $NaBH_4$, MsOH; v) $NaBH_3$(OTFA), vi) Pd/C, HCO_2NH_4; vii) HCl(aq), *i*PrOH; viii) $NaHCO_3$(aq).

L-phenylalanine **237** was treated with benzyl chloride under basic conditions to give the tribenzylated derivative **238**. The cyanomethyl ketone **239** was obtained *via* acyl substitution by reacting the acetonitrile-derived anion with the ester **238** at –40 °C (the temperature and mode of addition being important in minimizing racemisation). The Grignard reagent, benzyl magnesium chloride, reacted efficiently with the nitrile **239** at 20 °C, to afford the enaminone **240** in 92% yield. The next crucial step was the generation of the C-3 and C-5 chiral centers. This involved treatment of the enaminone **240** with excess sodium borohydride in the presence of methanesulphonic acid, and stereoselective reduction of the ketone intermediate **241** using $NaBH_3(OTFA)$, generated *in situ* from trifluoroacetic acid and sodium borohydride, to afford a mixture of diastereomeric amino alcohols **242**. The ratio of the diastereomers was not determined, but the ^1H and ^{13}C NMR spectroscopic data were identical with those reported by Stuk *et al.*[163] Debenzylation of the diastereomers **242** was achieved in *ca.* 68% yield using a 5% palladium-on-carbon catalyst in the presence of ammonium formate, while purification was achieved by precipitating the product from isopropyl alcohol and concentrated HCl to afford the dihydrochloride salt **243**, which was readily converted to the free diamine **236** by treatment with saturated aqueous sodium hydrogen carbonate.

One- and two-dimensional NMR spectroscopy was used to confirm the formation of compounds **236** – **243**, the ^1H and ^{13}C NMR data being consistent with those reported by Stuk *et al.*,[163] (only the m.p. of compound **239** was higher than the reported value). The ^1H NMR spectrum of the free diamine **236** (Figure 26) reveals the 3-H signal at δ 3.72 ppm, the 5-H proton signal at δ 3.11 ppm and the signals corresponding to the diastereotopic 4-methylene protons as multiplets at δ 1.56 ppm and 1.70 ppm. The ^{13}C NMR spectrum (Figure 27) reveals characteristic signals for the 3-methine carbon at δ 74.5 ppm, the 2-methine carbon at δ 57.5 ppm and the 5-methine carbon at δ 54.0 ppm. The three methylene carbons, C-1, C-4, and C-6, resonate at δ 39.1, 41.3 and 47.3 ppm, respectively, their assignment being supported by the DEPT-135 spectrum (Figure 28). The COSY, HMQC and HMBC data, confirm attachment of the three pairs of diastereotopic methylene protons to the corresponding carbon nuclei, C-1, C-4 and C-6.

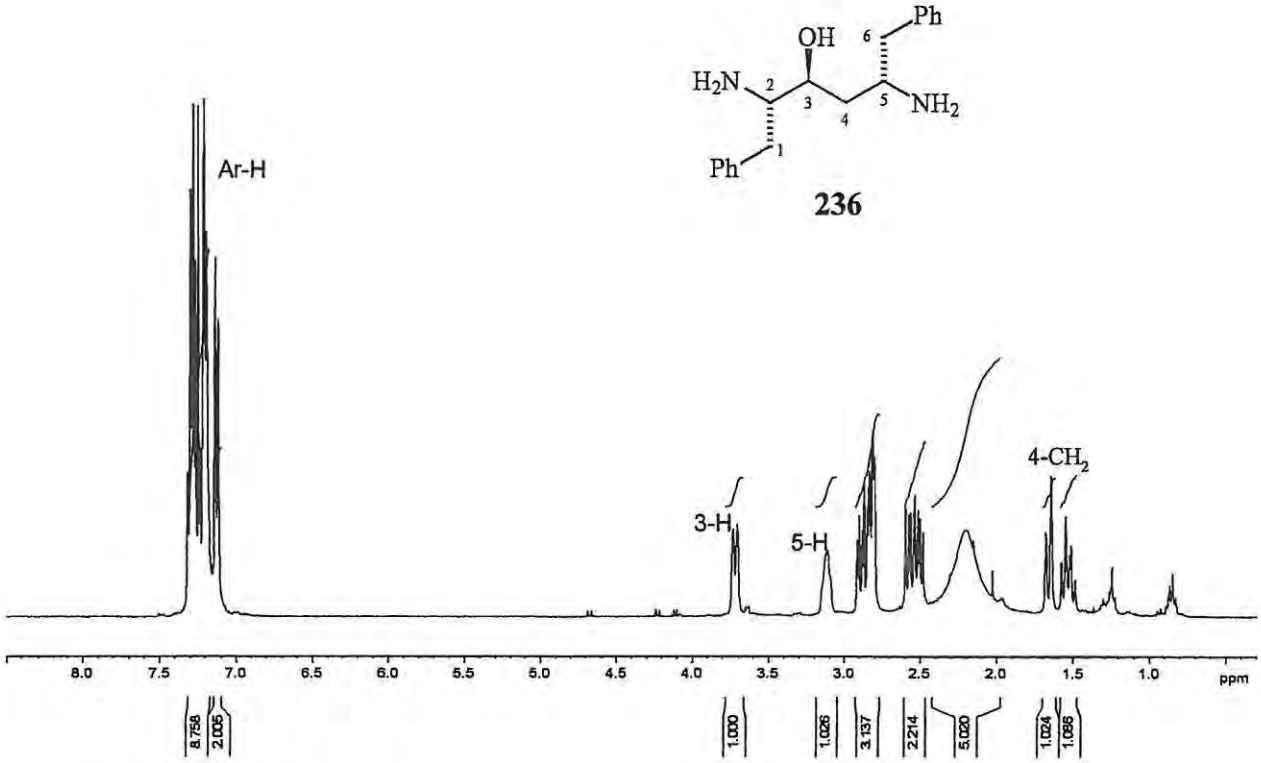


Figure 26. 400 MHz ^{1}H NMR spectrum of hydroxyethylene dipeptide isostere **236** in $CDCl_3$.

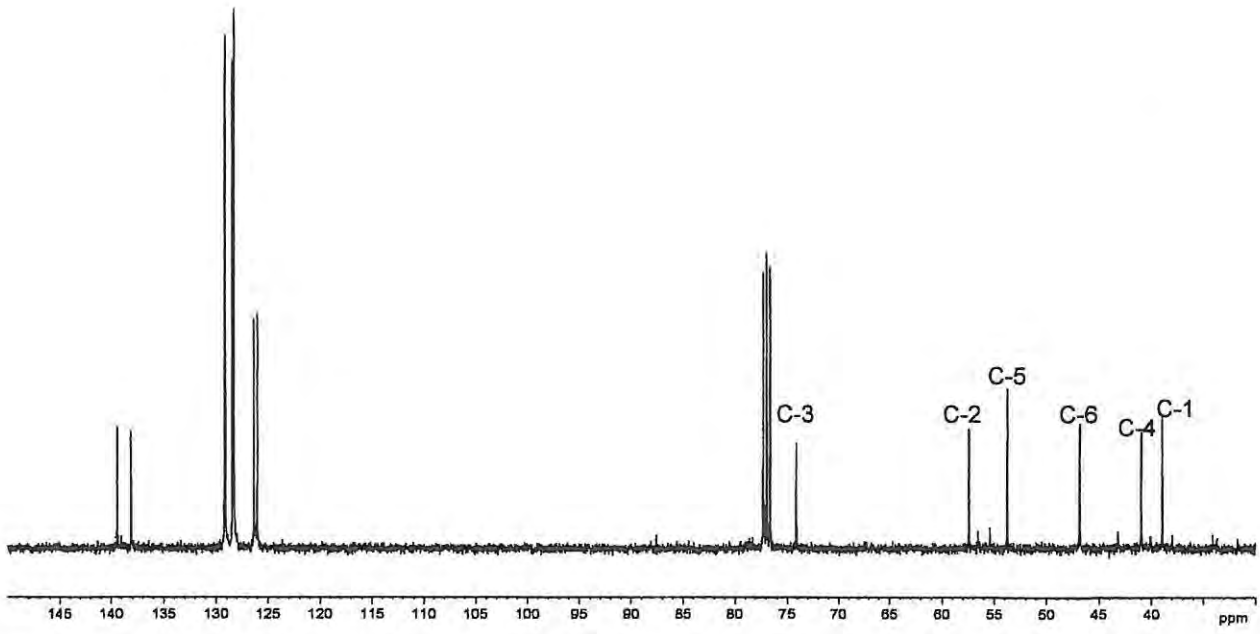
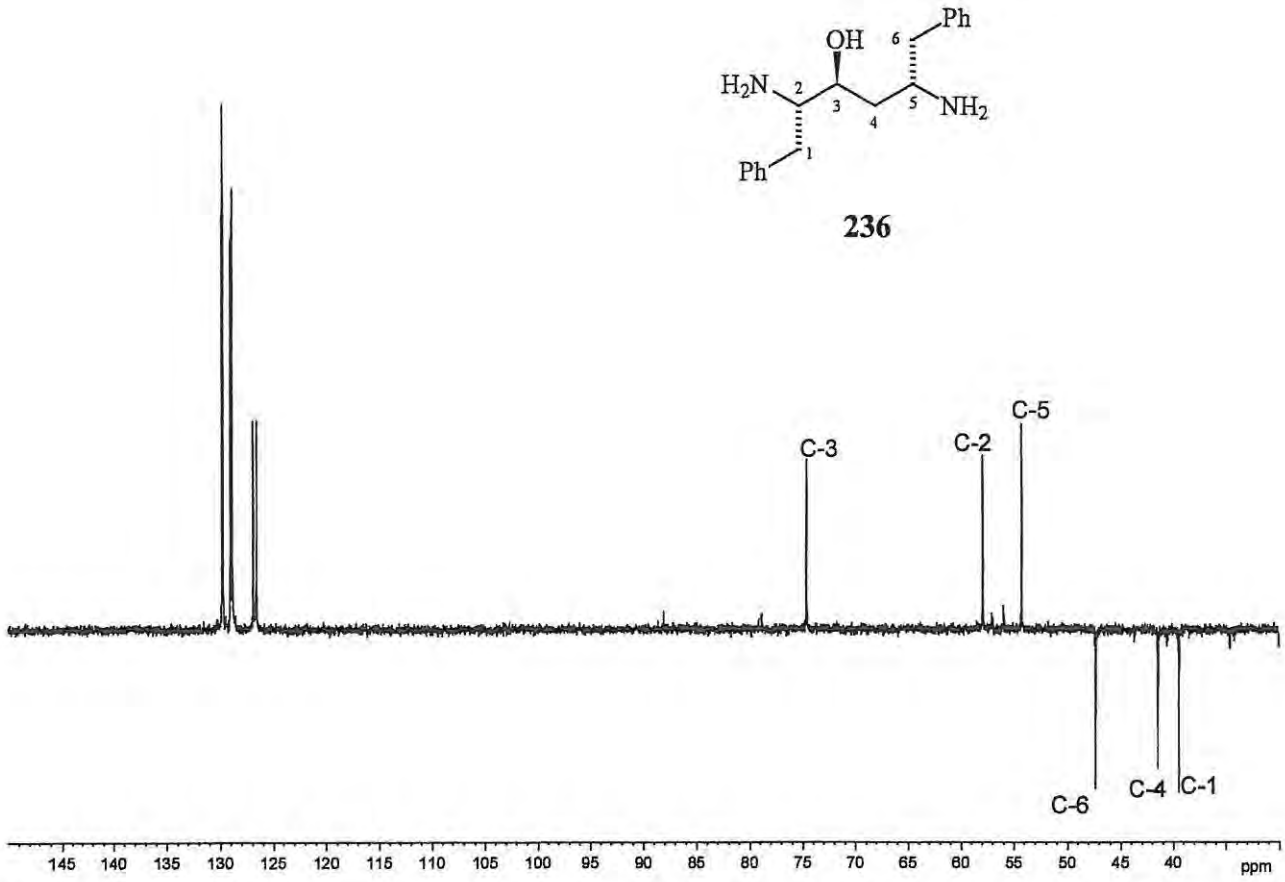


Figure 27. 100 MHz ^{13}C NMR spectrum of hydroxyethylene dipeptide isostere **236** in $CDCl_3$.

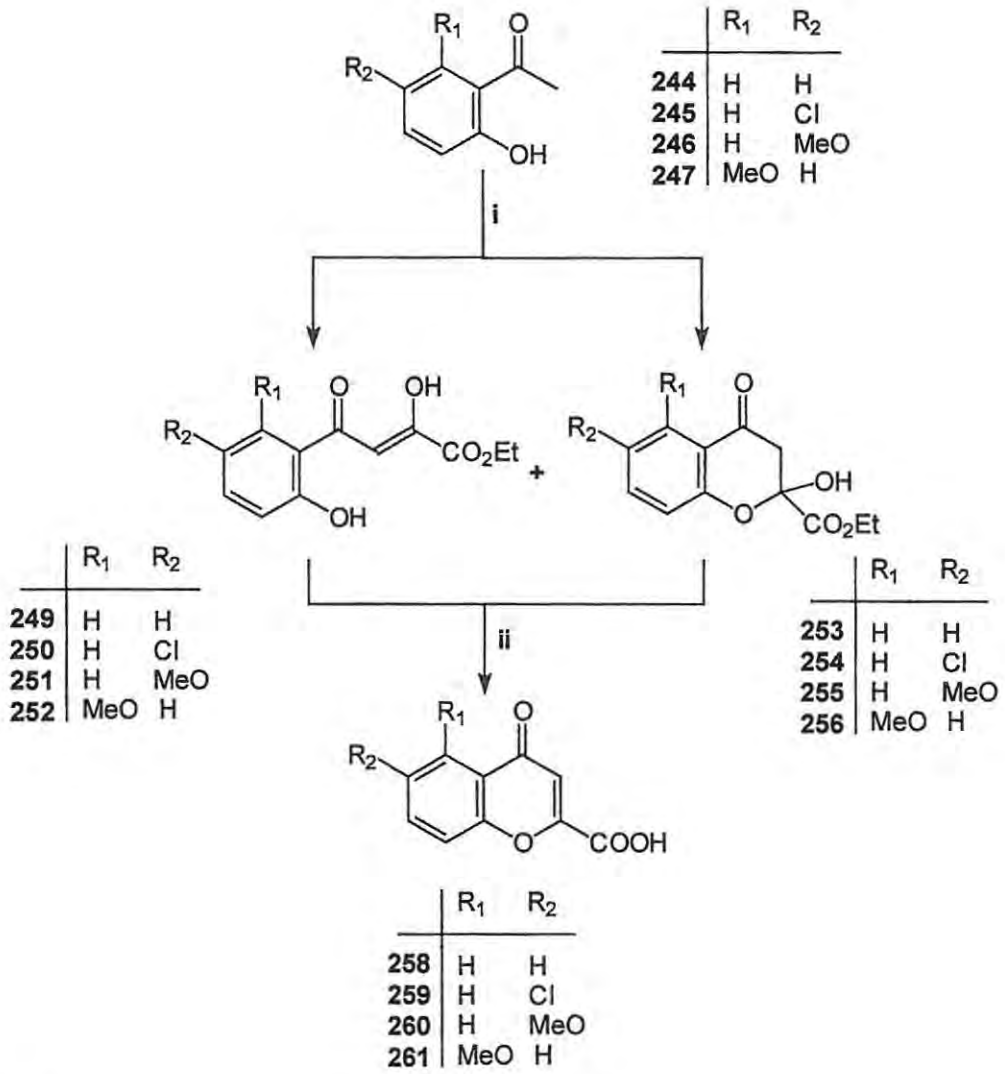


236

Figure 28. DEPT-135 NMR spectrum of hydroxyethylene dipeptide isostere **236** in $CDCl_3$.

2.4.2 Synthesis of chromone-2-carboxylic acid derivatives

The chromone-2-carboxylic acid derivatives **258** – **262**, required for coupling with the diamine **236**, were prepared as outlined in **Schemes 59** and **60**. Claisen acylation of the substituted *o*-hydroxyacetophenones **244** – **248** with diethyl oxalate in the presence of sodium ethoxide (as described by Fitton and Smalley[164] and by Ellis *et al.*[165,166]), followed by one-pot acid-catalyzed cyclization, dehydration and hydrolysis, afforded the corresponding chromone-2-carboxylic acids **258** – **261** directly. The 7-hydroxychromone-2-carboxylic acid **262** was obtained by acid-catalyzed hydrolysis of the ester **257**, isolated following the procedure reported by Ellis *et al.*[166]



	R_1	R_2
244	H	H
245	H	Cl
246	H	MeO
247	MeO	H

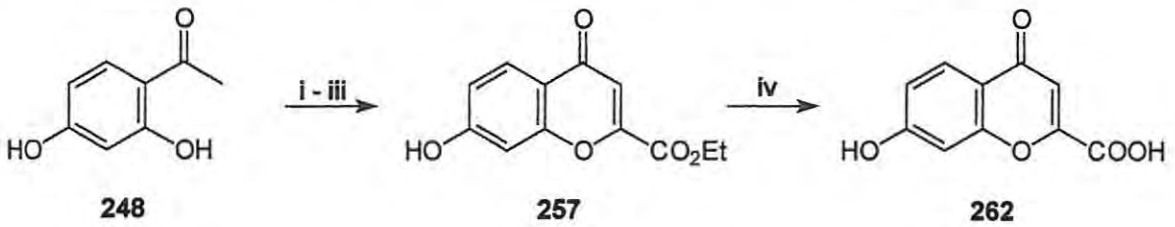
	R_1	R_2
249	H	H
250	H	Cl
251	H	MeO
252	MeO	H

	R_1	R_2
253	H	H
254	H	Cl
255	H	MeO
256	MeO	H

	R_1	R_2
258	H	H
259	H	Cl
260	H	MeO
261	MeO	H

Scheme 59

Reagents: i) NaOEt, EtOH, $(CO_2Et)_2$; ii) HCl-AcOH (1:1), heat.



248 **257** **262**

Scheme 60

Reagents: i) NaOEt, EtOH, $(CO_2Et)_2$; ii) conc. HCl; iii) charcoal, H_2O;
iv) HCl-AcOH (1:1), heat.

The reaction intermediates, *viz*, the diketones (existing as enol tautomers) **249** – **252** and the substituted 2-hydroxychromanones **253** – **256** were readily distinquished by ^{1}H NMR spectroscopy, and the crude mixtures were used in the next step without further purification.

The ^{1}H and ^{13}C NMR spectra of 6-methoxychromone-2-carboxylic acid **260** are illustrated in Figures 29 and 30. The ^{1}H NMR spectrum reveals a singlet at δ 6.88 ppm, which is characteristic of the 3-H nucleus of the chromone ring, and a singlet at δ 3.87 ppm corresponding to the methoxy protons. The carboxylic acid proton was not observed, presumably, due to rapid exchange with water present in the DMSO-d_6 used as the solvent; the IR spectrum, however, revealed a broad band in the region 3300 – 2200 cm^{-1} corresponding to the carboxylic hydroxyl group. The ^{13}C NMR spectrum clearly shows 11 carbon signals, with the methoxy carbon resonating at δ 55.7 ppm. The use of COSY, HMQC and HMBC experiments facilitated the interpretation of both the ^{1}H and ^{13}C NMR spectra.

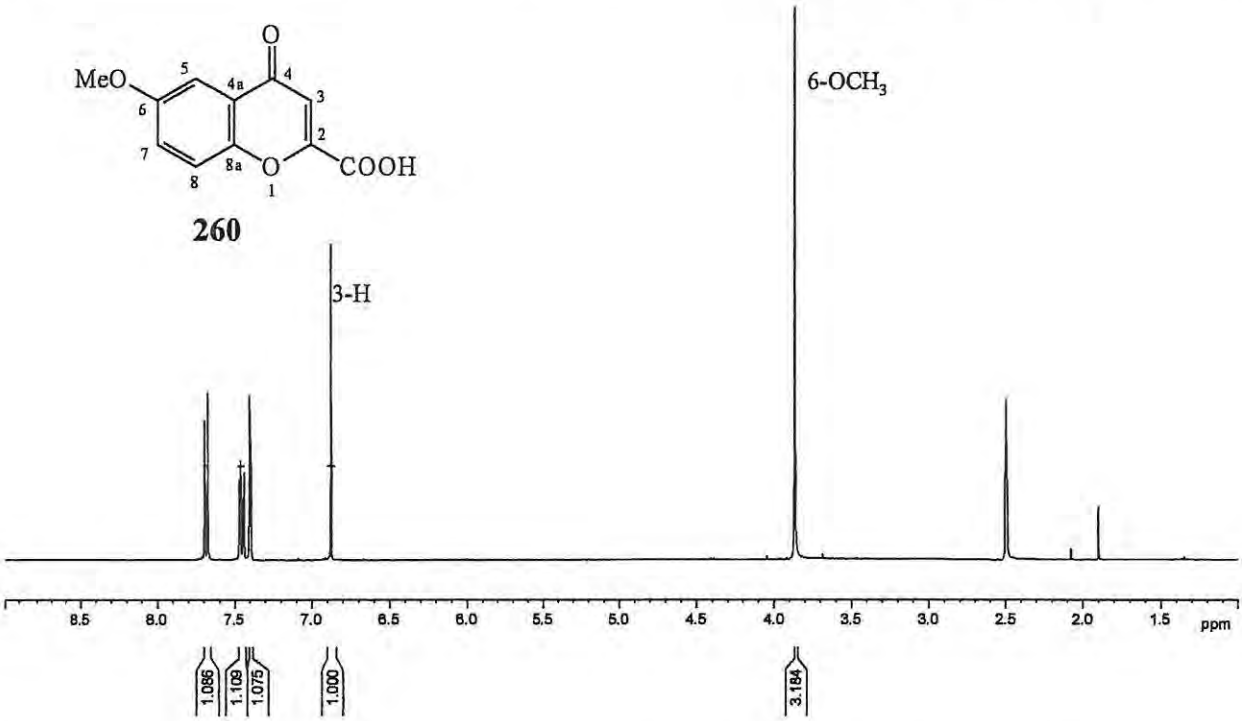


Figure 29. 400 MHz ^{1}H NMR spectrum of 6-methoxychromone-2-carboxylic acid **260** in DMSO-d_6.

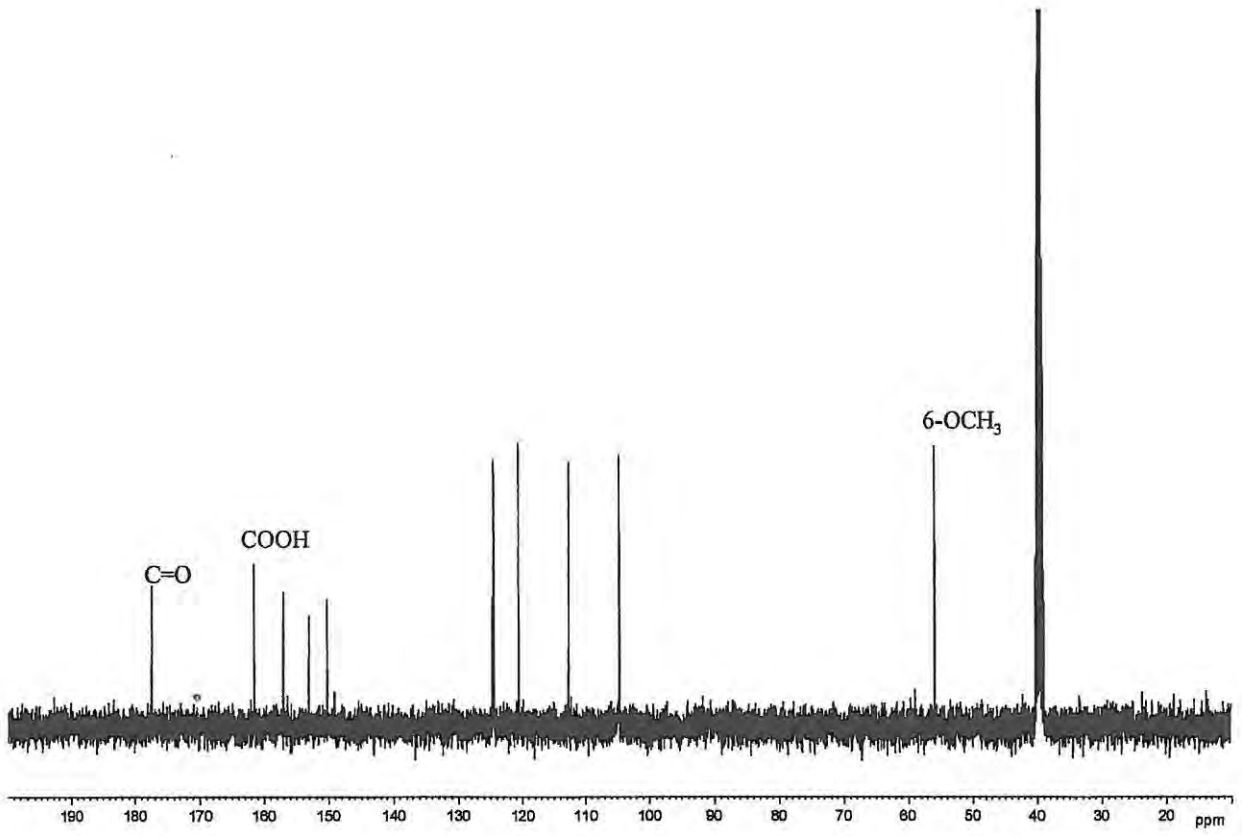
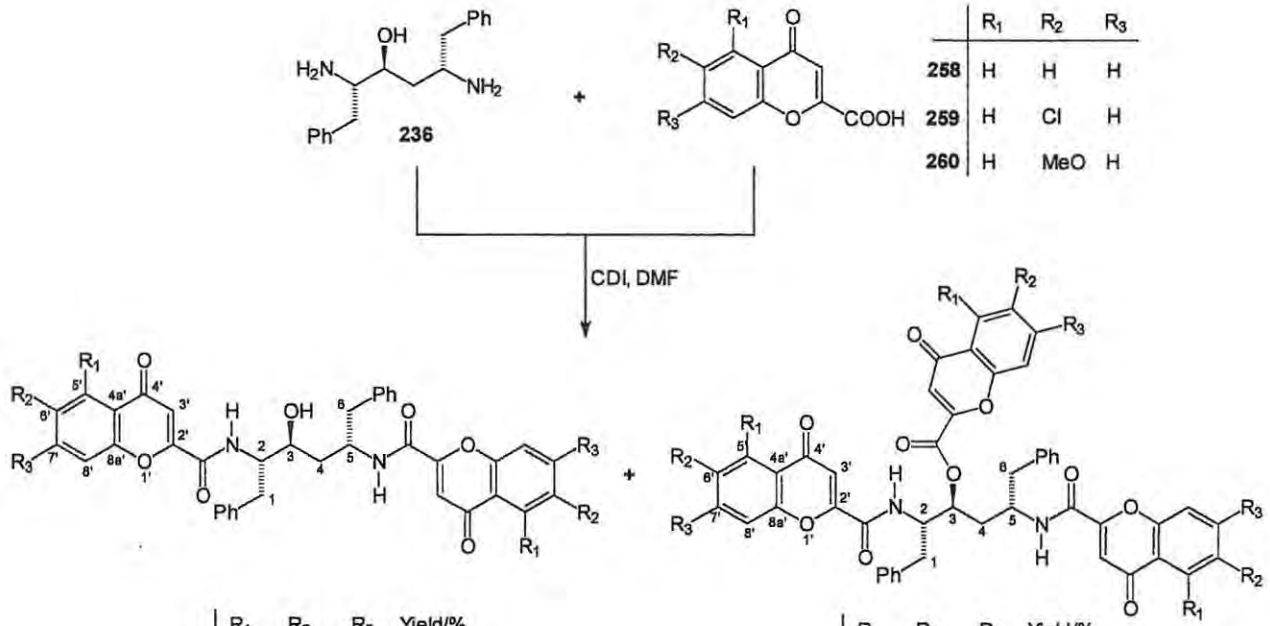


Figure 30. 100 MHz ^{13}C NMR spectrum of 6-methoxychromone-2-carboxylic acid **260** in DMSO-d_6.

2.4.3 Synthesis of chromone-containing analogues of the HIV-1 protease inhibitor, ritonavir

Initial attempts to synthesize chromone-containing analogues of ritonavir, using two equivalents of *N,N'*-carbonyldiimidazole (CDI) as the coupling agent, in the presence of dry DMF as outlined in **Scheme 61**, afforded both the *bis*- and *tris*-chromone derivatives, with the yields of the *tris*-chromone derivatives **268 – 270** being higher than those of the desired *bis*-chromones **263 – 265**. The formation of the *tris*-chromone compounds **268 – 270** was clearly due to the ability of CDI to promote the coupling of amines with acids as well as acids with alcohols.[167,168] The *tris*-chromone derivatives **268 – 270** were characterized using ^{1}H and ^{13}C NMR, IR and high-resolution mass spectrometric analysis. Thus, the ^{1}H NMR spectrum of compound **270** reveals three distinct singlets in the region δ 3.80 - 4.00 ppm corresponding to the three methoxy groups, and singlets at δ 7.00, 7.04 and 7.09 ppm corresponding to the characteristic 3'-H nuclei of the three chromone rings.



	R_1	R_2	R_3
258	H	H	H
259	H	Cl	H
260	H	MeO	H

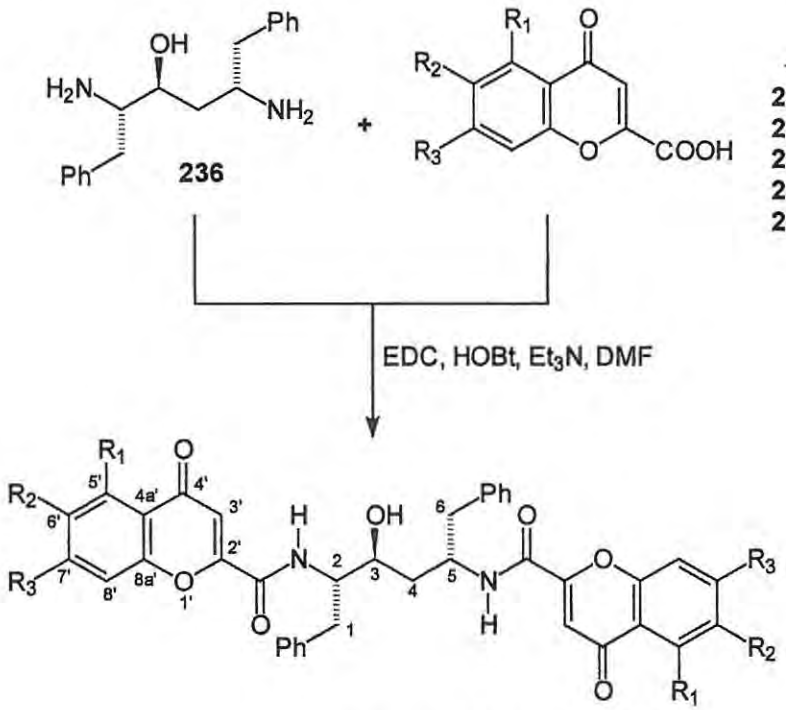
	R_1	R_2	R_3	Yield/%
263	H	H	H	31
264	H	Cl	H	27
265	H	MeO	H	35

	R_1	R_2	R_3	Yield/%
268	H	H	H	54
269	H	Cl	H	49
270	H	MeO	H	60

Scheme 61

The ^{13}C NMR spectrum clearly reveals the presence of three methoxy carbons resonating at δ 55.8, 55.9 and 56.0 ppm, while the high-resolution FAB spectrum exhibits a peak at *m/z* 891

corresponding to the molecular ion ($M+H^+$) (see experimental section for spectroscopic data). Because of our desire to improve the yield of the desired *bis*-chromone products, in which the crucial 3-hydroxyl group is available for binding with the enzyme, a search for suitable peptide coupling agents was undertaken. Use of *N*-ethyl-*N'*-(dimethylaminopropyl)-carbodiimide hydrochloride (EDC) in the presence of 1-hydroxybenzotriazole hydrate (HOBt) has been shown to effect such coupling,[117] and the diamine **236**, in dry DMF, was treated with two equivalents of each of the chromone-2-carboxylic acids **258 - 262** in the presence of two equivalents of EDC, two equivalents of HOBt and basified with drops of triethylamine. Work-up and purification of the reaction mixture by flash chromatography on silica gel afforded, in each case, the desired chromone-containing ritonavir analogues **263 – 267** as the sole products in yields ranging from 52 to 82% (**Scheme 62**).



	R_1	R_2	R_3
258	H	H	H
259	H	Cl	H
260	H	OMe	H
261	OMe	H	H
262	H	H	OH

	R_1	R_2	R_3	Yields/%
263	H	H	H	60
264	H	Cl	H	52
265	H	OMe	H	56
266	OMe	H	H	82
267	H	H	OH	78

Scheme 62

The ritonavir analogues **263** – **267** were fully characterized by elemental (HREIMS), one- and two-dimensional NMR, and IR spectroscopic analysis. The ^{1}H NMR spectrum of the parent compound **263** (Figure 31) reveals a multiplet at δ 1.83 ppm, corresponding to the diastereotopic 4-methylene protons, and a complex of multiplets at δ 2.86-3.00 ppm corresponding to the benzylic 1- and 6-methylene protons. The broad singlet at δ 3.89 ppm is characteristic of the 3-methine proton, while the 2- and 5-methine protons resonate as multiplets at *ca.* δ 4.40 ppm. The ^{1}H NMR spectrum also reveals two singlets at δ 6.96 and 7.05 ppm which are typical of the 3'-H nuclei, and a broad singlet at δ 4.24 ppm corresponding to the 3-hydroxyl group. The ^{13}C NMR spectrum (Figure 32) shows three methine carbons resonating at 69.4 ppm (C-3), 55.6 ppm (C-2) and 49.9 ppm (C-5), while the methylene carbons at C-1, C-4 and C-6 resonate at 38.2, 39.4 and 40.8 ppm respectively. The methylene and methine signal assignments were established from the DEPT-135 spectrum (Figure 33), while the COSY spectrum (Figure 34) shows the coupling between the 4-methylene protons and the 5-methine and 3-methine protons, between the 1-methylene protons and the 2-methine proton and between the 6-methylene protons and the 5-methine proton. The aromatic signals were assigned with the aid of the COSY, and the HMQC and HMBC spectra (Figures 35 and 36), while the high-resolution mass spectral data for the peak at *m/z* 628.22076 corresponds to the molecular formula of the parent system **263**, $C_{38}H_{32}N_2O_7$ (628.22095).

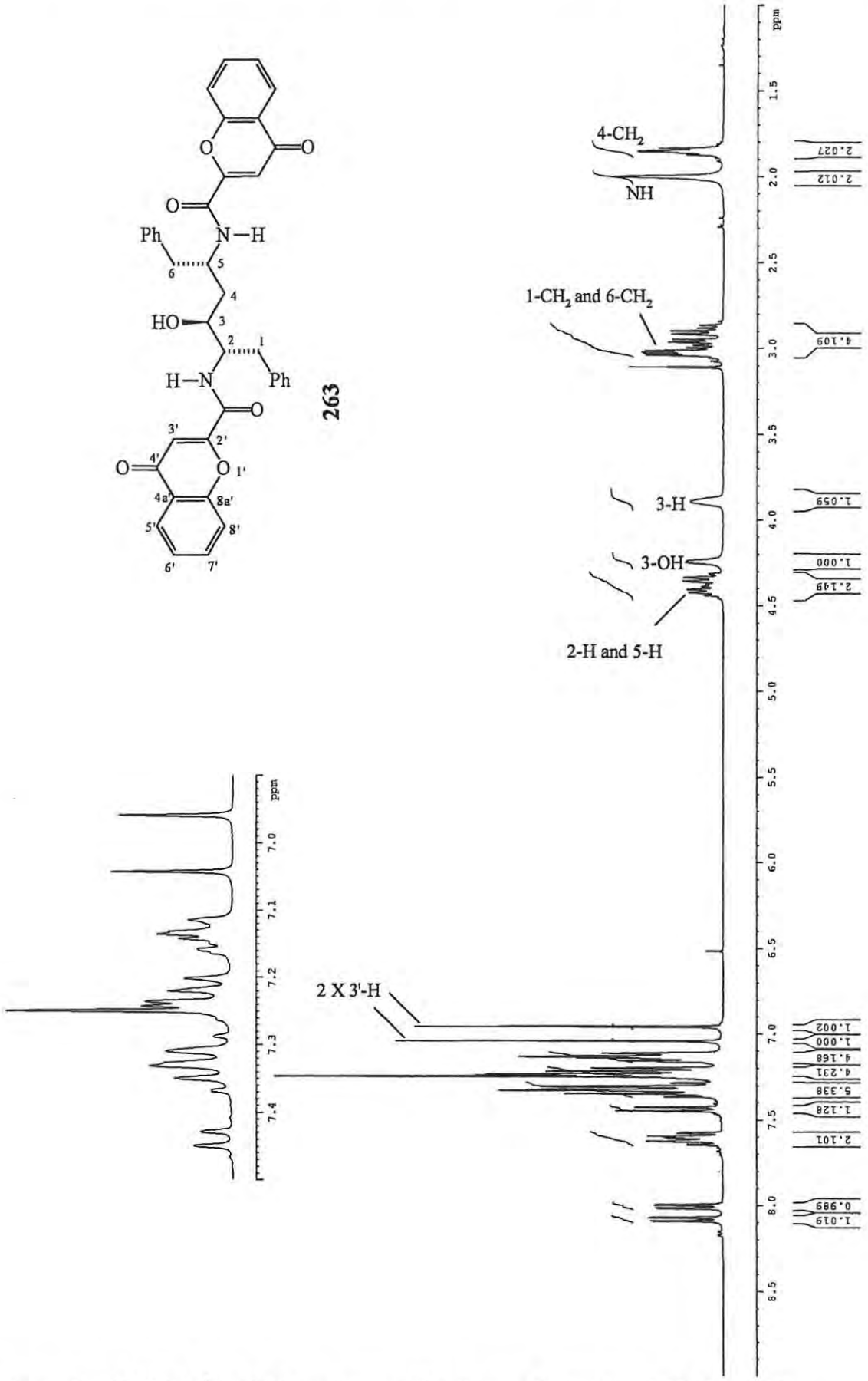


Figure 31. 400 MHz ^{1}H NMR spectrum of the " parent" compound **263** in $CDCl_3$.

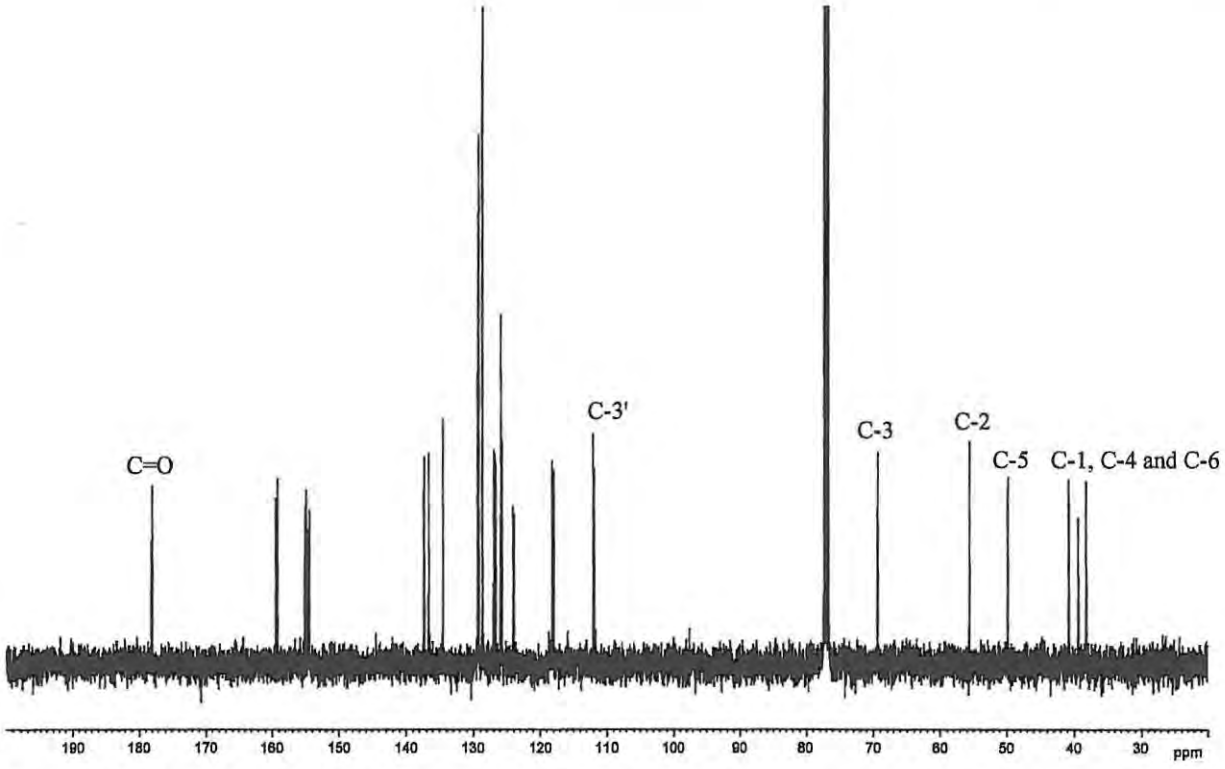


Figure 32. 100MHz ^{13}C NMR spectrum of the "parent" compound **263** in $CDCl_3$.

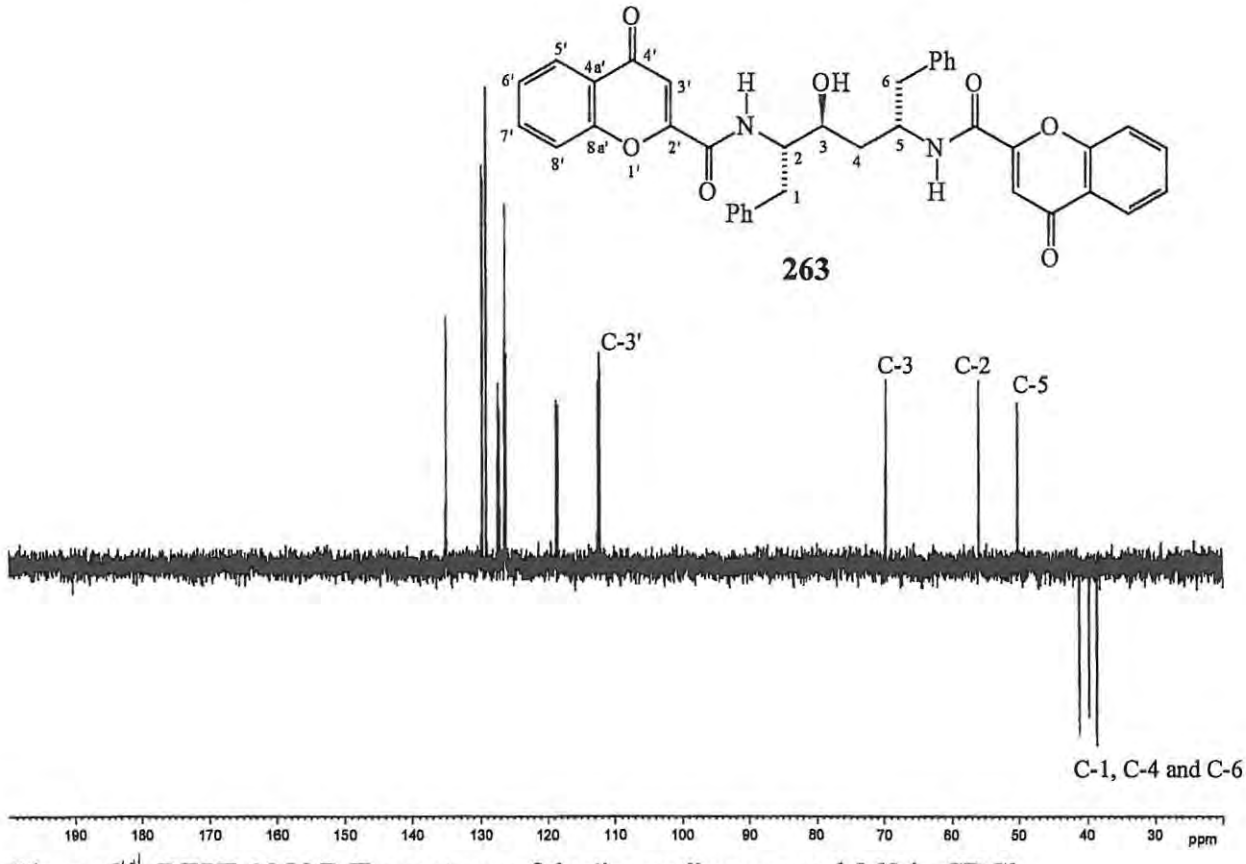


Figure 33. DEPT-135 NMR spectrum of the "parent" compound **263** in $CDCl_3$.

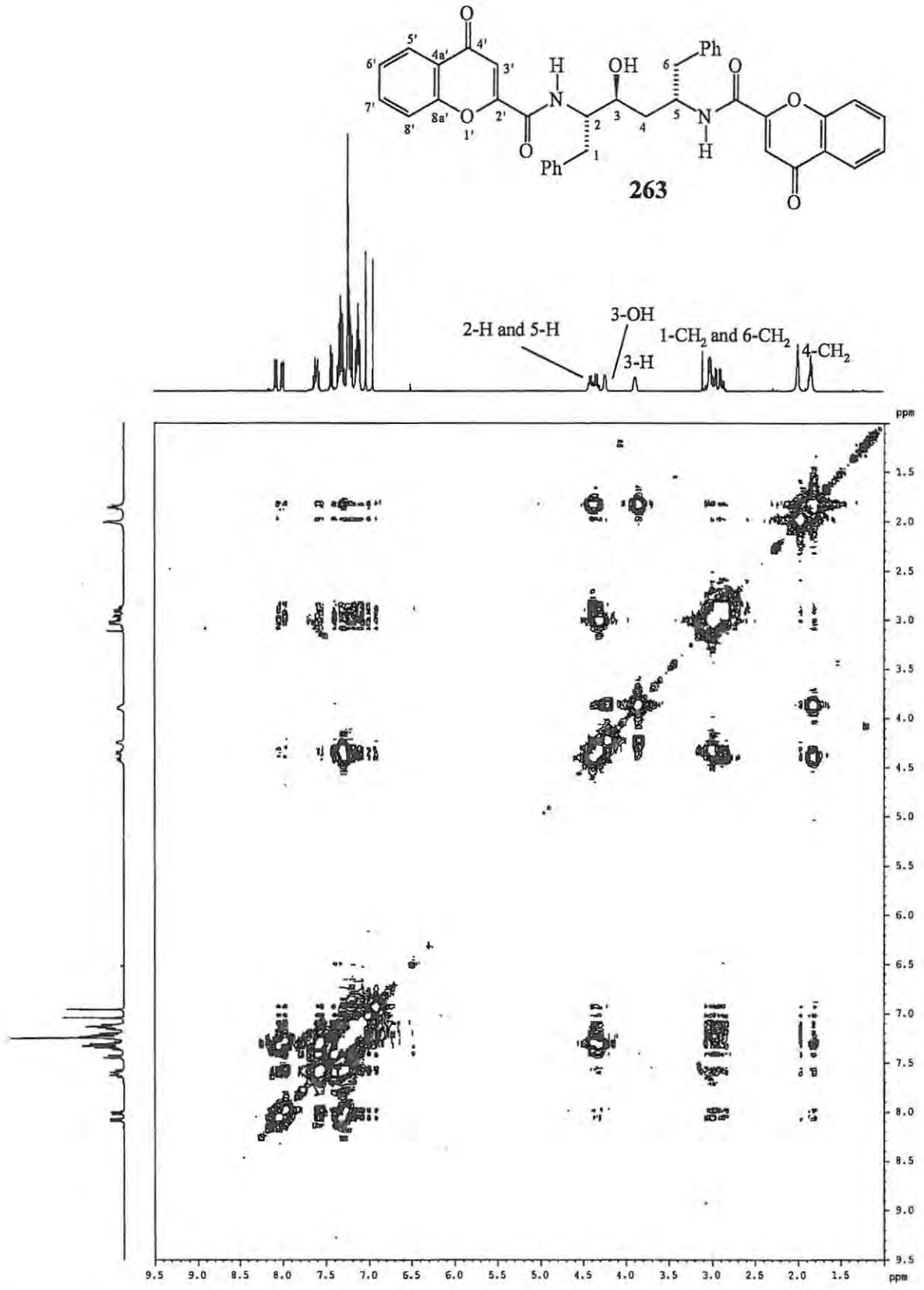


Figure 34. 400 MHz COSY NMR spectrum of the "parent" compound **263** in $CDCl_3$.

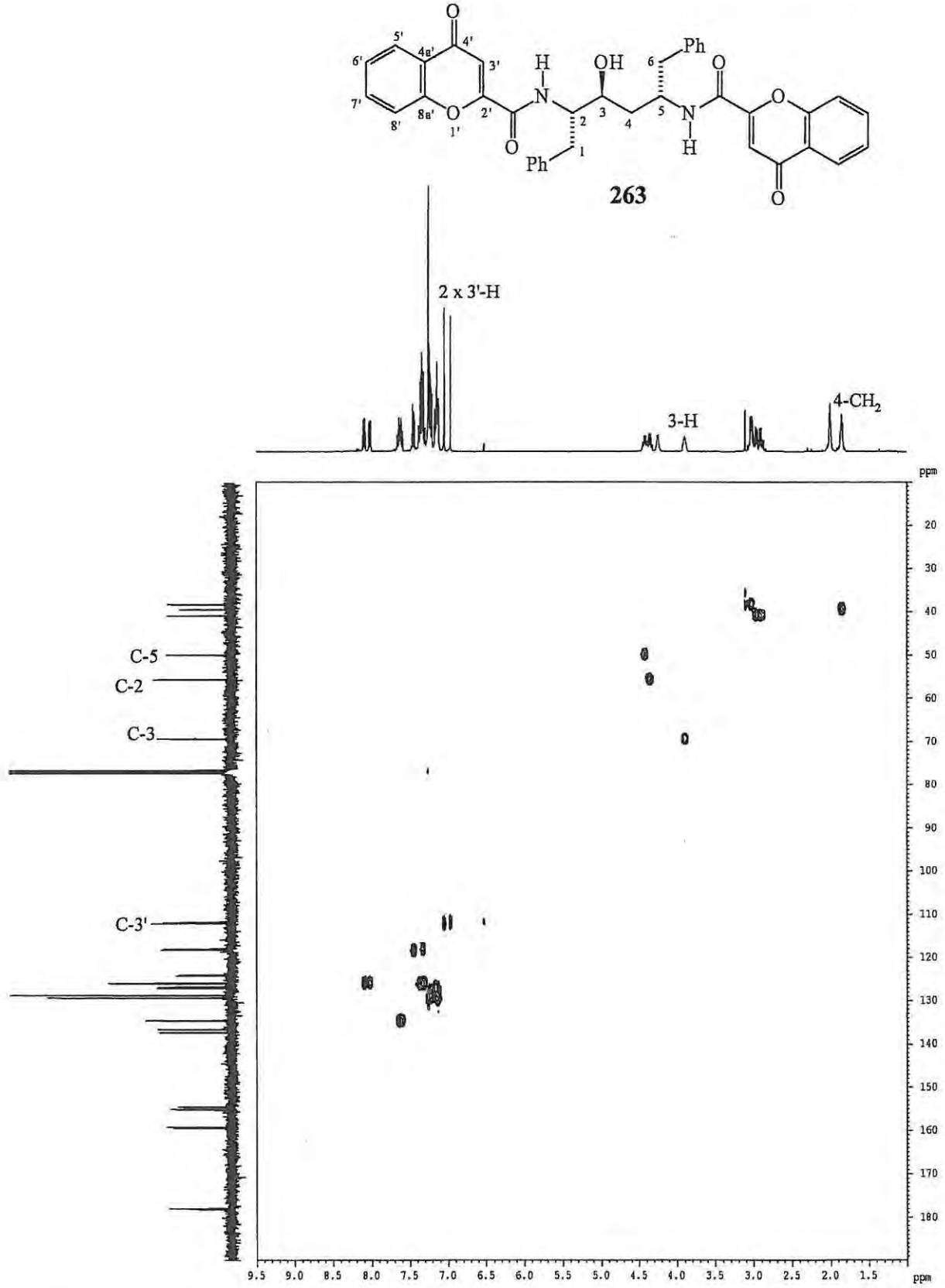


Figure 35. 400MHz HMQC NMR spectrum of the "parent" compound **263** in $CDCl_3$.

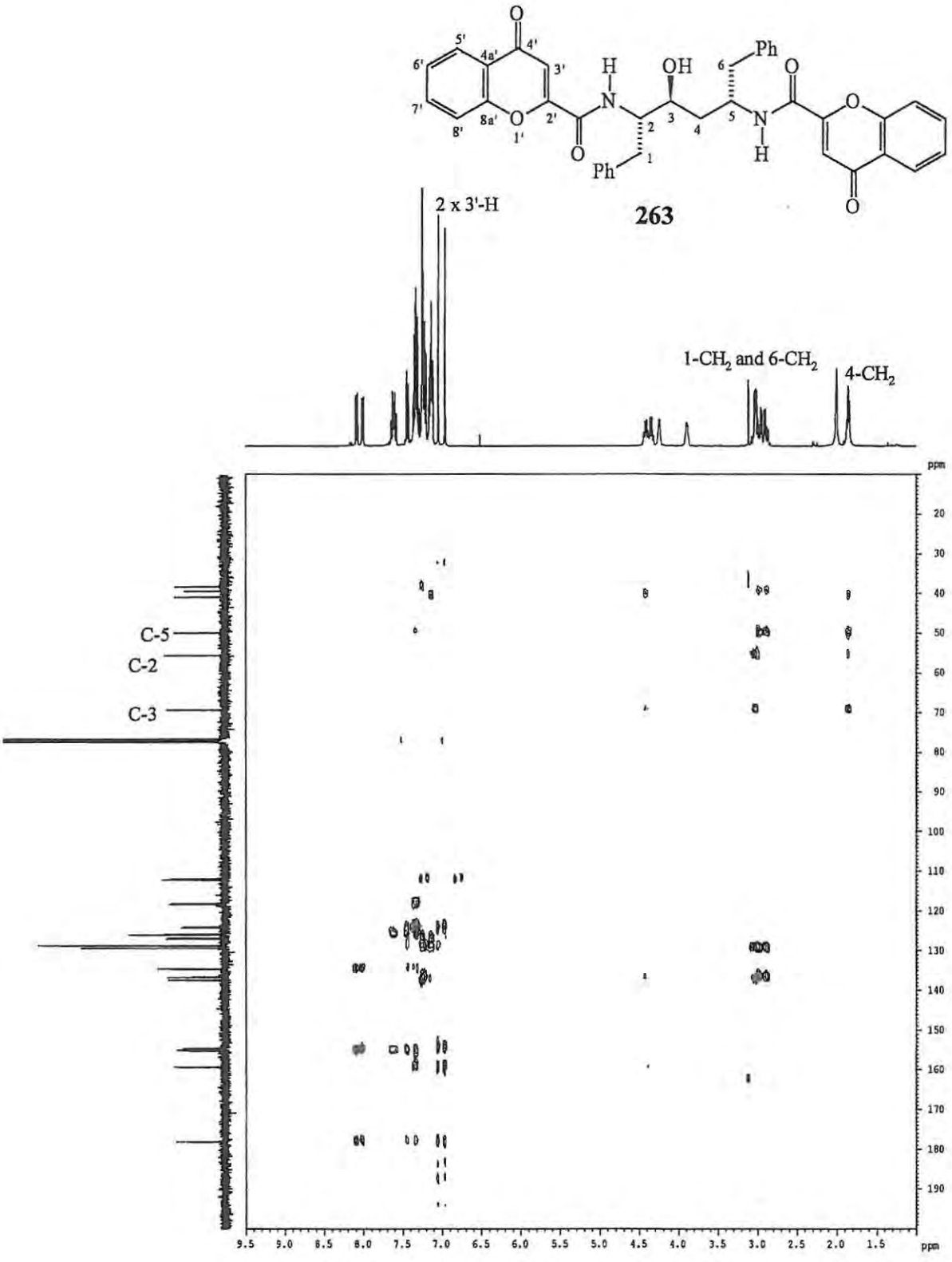
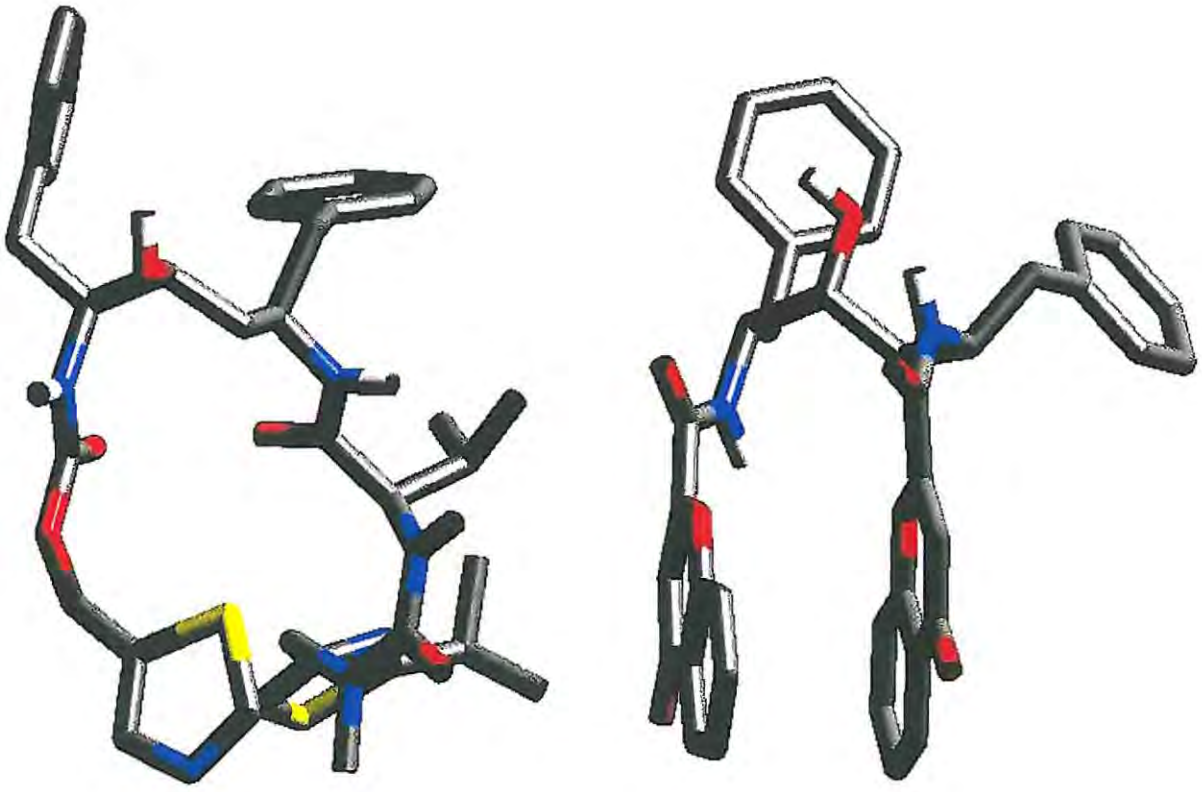


Figure 36. 400MHz HMBC NMR spectrum of the "parent" compound **263** in $CDCl_3$.

2.4.4 Computer modelling studies of the ritonavir analogues

In recent years, the developments in molecular modelling and structure-based drug design have led to the production of several highly potent HIV-1 protease inhibitors. Using a reported X-ray crystal structure[113] of ritonavir **156** and an interactive docking procedure, we have investigated the docking of the chromone-containing ritonavir analogues **263 – 267** into the active site of the enzyme. Before attempting the docking experiments, preliminary computer alignment of the five clinically used drugs **155 –159** and our ritonavir analogues **263 - 267** was carried out. The conformational energies of the ten compounds **155 – 159** and **263 – 267** were minimized by molecular dynamics simulations to obtain their minimum energy conformations.



(a) (b)

Figure 37. Energy-minimized structures of (a) ritonavir **156** and (b) the chromone-containing analogue **263**.

Figures 37a and 37b show the energy-minimized structures of ritonavir **156** and the parent chromone-containing analogue **263**, respectively. The energy-minimized structures, were aligned by first matching the corresponding atoms (*i.e.* the characteristic 3-hydroxy groups) manually. A preliminary overlay using this approach revealed the alignment of our compounds **263 – 267** and the five clinically used drugs (Figure 38) and the corresponding net receptor-binding surface (Figure 39).

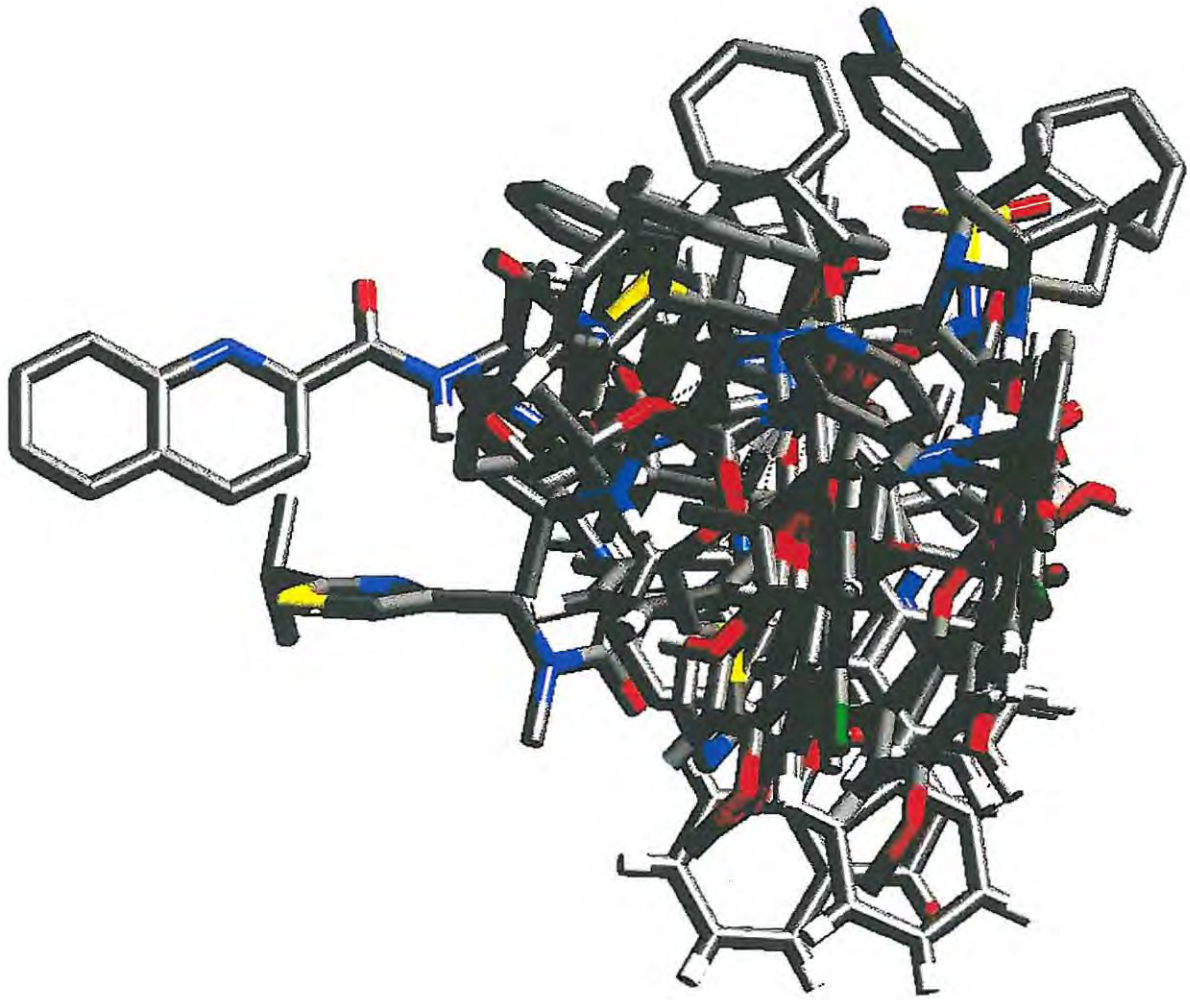


Figure 38. Alignment of the energy-minimized structures of compounds **155 – 159** and **263 – 267**.

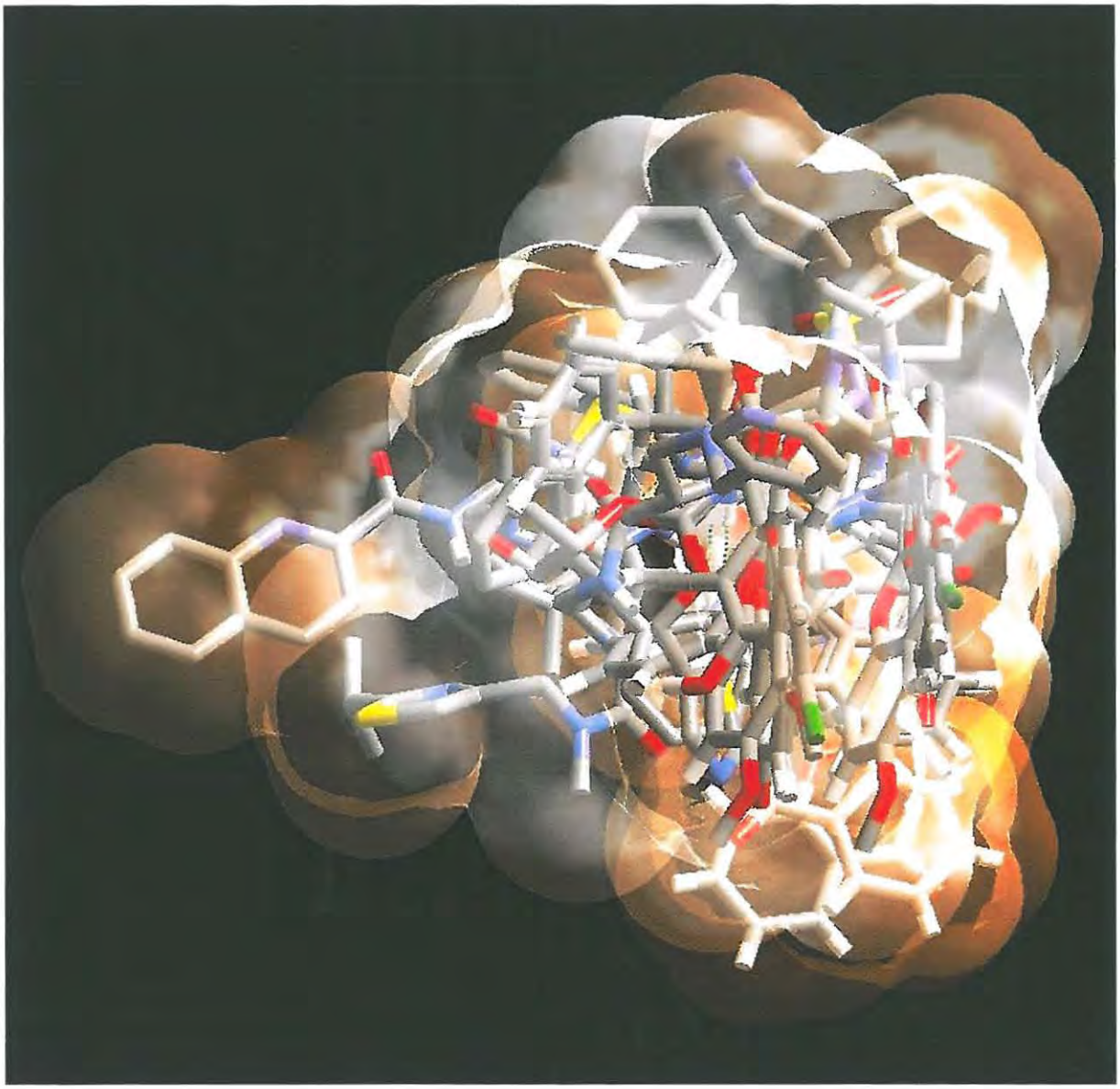


Figure 39. Receptor-binding surface containing the aligned, energy-minimized structures of compounds **155 – 159** and the chromone-containing analogues **263 - 267**.

However, it was apparent that the energy-minimized conformations bore little resemblance to the binding conformation adopted by ritonavir **156** in the enzyme active site. Consequently, it was decided to use the Cerius^2 LIGAND FIT module to determine the most favourable *bound* conformations of each of the compounds **263 – 267** and investigate their interactions with the enzyme active site.

A number of high-resolution X-ray structures of complexes of linear inhibitors with the HIV-1 protease dimer have been reported.[113,121,169,170,171] Two common features observed in these structures are illustrated in Figure 40.[110,169] The first is the presence of a structural water molecule linking the bound inhibitors to the flexible glycine-rich "flaps" of the enzyme. The structural water molecule accepts two hydrogen-bonds from the backbone amide hydrogens of the isoleucine residues, Ile 50A-NH and Ile 50B-NH, while donating two hydrogen-bonds to the carbonyl oxygens of the transition state mimetic of the inhibitor molecule. The second and probably most important, feature is the hydrogen-bonding between the hydroxyl group of the inhibitor molecule and the catalytic aspartic acid residues (Asp 25A-COO^- and Asp 25B-COO^-) of the enzyme. Residues to the left of the HIV-1 protease inhibitor cleavage site (hydroxyl group) are referred to as the P_1, P_2, P_3, *etc* positions, and those to the right as P_1', P_2', P_3', *etc*, while their respective locations in the enzyme active site are designated the S_1, S_2, S_3, *etc* and S_1', S_2', S_3', *etc* pockets.

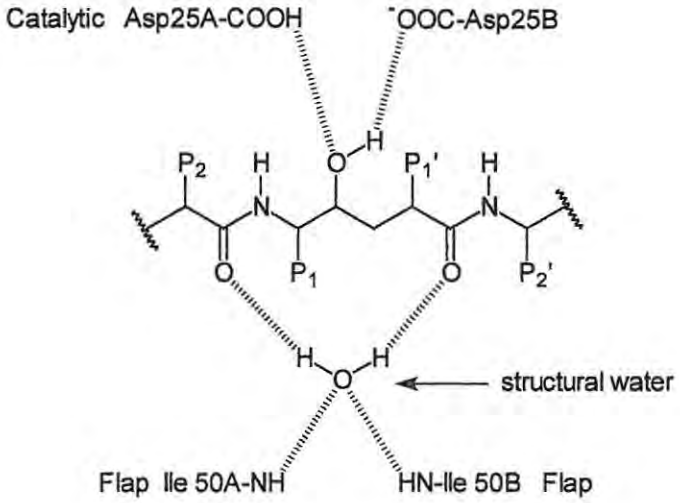


Figure 40. Schematic representation of hydrogen-bonding interactions found for a typical peptidomimetic inhibitor binding at the HIV-1 protease active site *via* bridging structural water.[110,169]

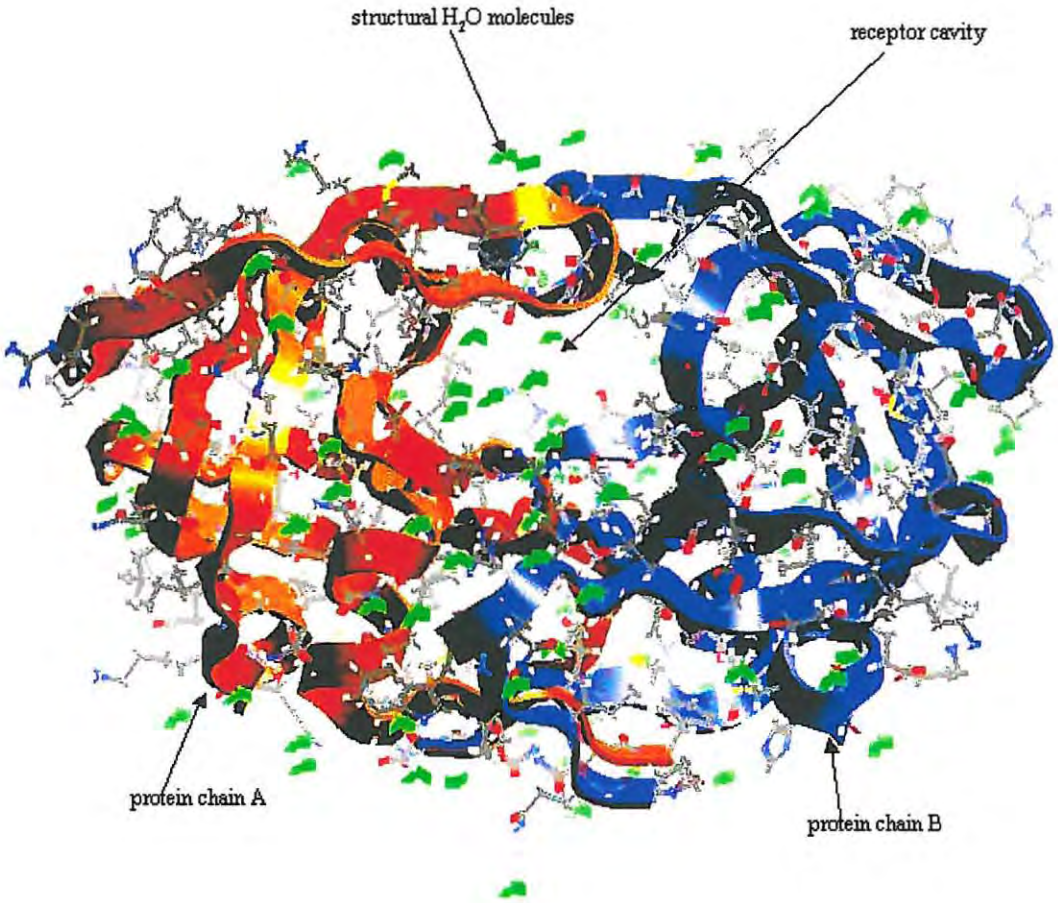


Figure 41. Active site of the dimeric HIV-1 protease enzyme, as determined by Kempf *et al.*[113]

In order to study the nature of the interactions between the HIV-1 protease enzyme and our compounds, the X-ray structure of the HIV-1 protease containing ritonavir, as determined by Kempf *et al.*,[113] was used as the starting point. Removal of the ligand, ritonavir, reveals the receptor cavity of the enzyme, (Figure 41). Three-dimensional computer models of compounds **263 – 267** were built and their energies minimized using a molecular mechanics routine. Using the interactive docking module "LIGAND FIT", the ligands **263 – 267** were each docked into the enzyme active site.

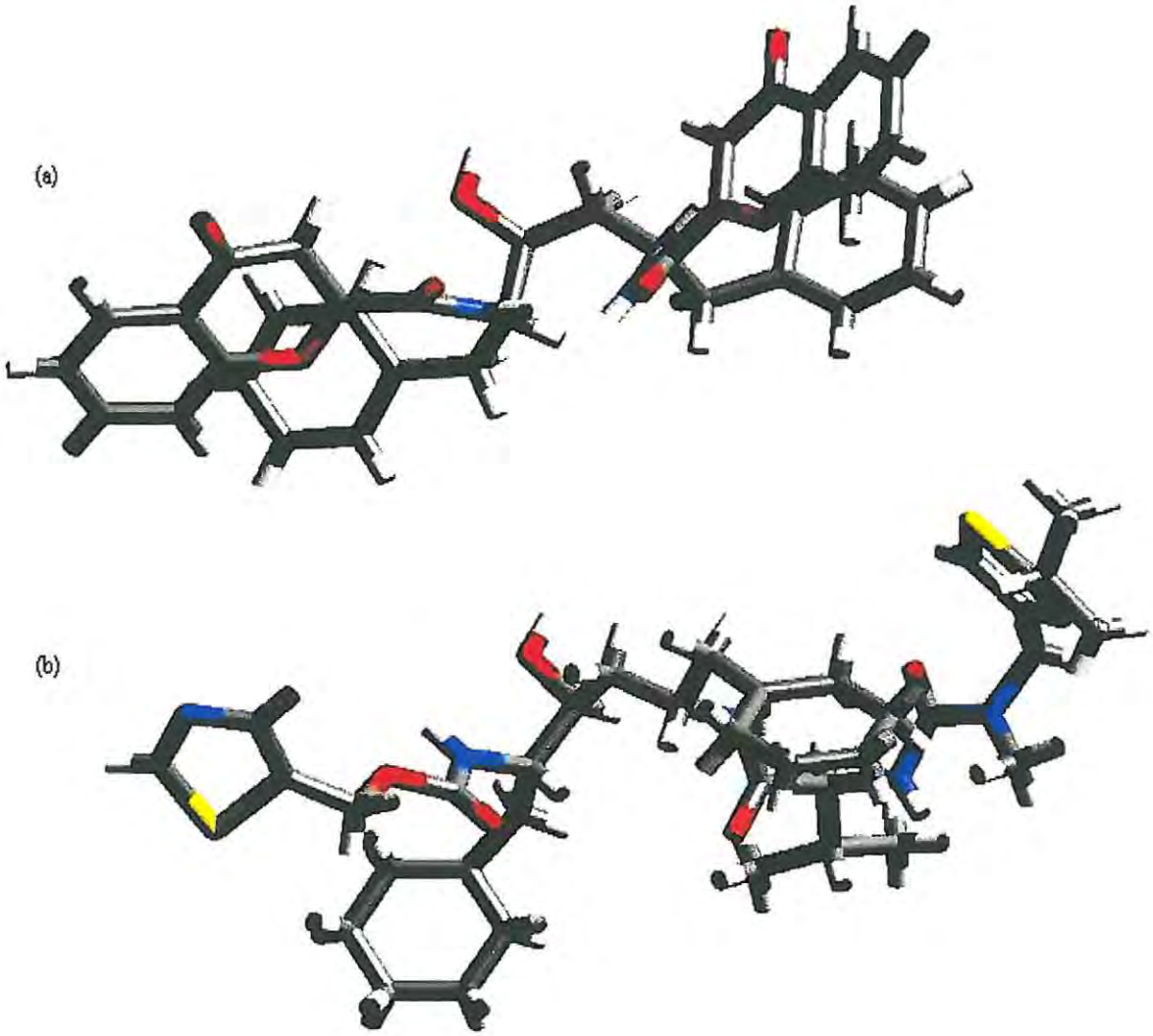


Figure 42. The most favourable bound conformations of:- a) compound **263**; and b) ritonavir **156**.

The most favourable bound conformations of the ligands in the enzyme active site were determined, by scoring different conformations of each compound to obtain their van der Waals protein-ligand interaction energy. Figure 42 shows the most favourable bound conformer of the parent system **263** and that of ritonavir **156** in the active site of the HIV-1 protease enzyme. The former has a van der Waals protein-ligand interaction energy of –60.8 Kcal mol^{-1} compared to –98.2 Kcal mol^{-1} for the latter. Although, the value for the parent system **263** is somewhat higher than that for ritonavir **156**, it is nevertheless a negative value,

indicating the relative stability of the enzyme-ligand **263** complex. Figures 43a and 43b illustrate the potential hydrogen-bonding interactions between ritonavir **156** and the receptor in the absence and presence of bridging structural water, respectively. Figures 44a illustrates the potential hydrogen-bonding interactions between the 3-hydroxyl group of the most favoured conformer of compound **263** with the side-chain catalytic aspartic acid residues, while Figure 44b illustrates the potential interactions between the 2-carbamoyl and the chromone ether oxygens, a bridging water molecule and the backbone (Ile 50A and Ile 50B) amide hydrogens. The potential interactions in the absence of structural water are detailed schematically in Figure 45a, in which it is apparent that the distances between the 3-hydroxyl group and the catalytic aspartic acid residues (Asp 25A-COO^- and Asp 25B-COO^-) of the enzyme are 4.358 Å and 3.259 Å, compared to 2.327 Å and 2.178 Å for the corresponding distances for ritonavir **156** (Figure 43a). However, the 2-carbamoyl oxygen is only 3.155 Å distant from the Ile 50A amide hydrogen compared to a distance of 3.875 Å for ritonavir **156**.

The potential hydrogen-bonding interactions between the ligand **263** and the enzyme receptor in the presence of structural water molecules are illustrated in Figure 45b. Thus, the 2-carbamoyl and chromone (P_2-P_3) ether oxygens may accept two hydrogen bonds from a structural water molecule, while the Ile 50A and Ile 50B residues each donate an amide hydrogen to the structural water molecule, the respective inter-atomic distances of 2.804 Å and 2.183 Å indicating the possibility of strong hydrogen-bonding interactions. It is also apparent that a structural water molecule could accept a hydrogen-bond from the Asp 29A amide hydrogen (2.855Å) and donate a hydrogen-bond to the chromone (P_2'-P_3') carbonyl oxygen (3.953 Å). Close examination of the docked structure reveals that the two chromone moieties fill the S_2-S_3 and S_2'-S_3' pockets of the enzyme receptor, while the two phenyl groups fill the S_1 and S_1' pockets. The phenyl group in the S_1 socket is involved in hydrophobic interactions with the Val 82A and Ile 84A residues of the enzyme at distances of 3.091 Å and 2.865 Å, respectively, while the phenyl group in the S_1' socket interacts with the Arg 8B residue at 2.107 Å. Other possible hydrophobic interactions between the bound ligand **263** and the receptor include that of the benzene ring of the chromone P_2-P_3 moiety with the Asp 29B residue (3.218 Å) and the corresponding ring of the chromone P_2'-P_3' moiety with the Pro 81B residue (2.410 Å). Figure 46 shows the most favoured bound conformer of ligand **263** in the active site of the enzyme.

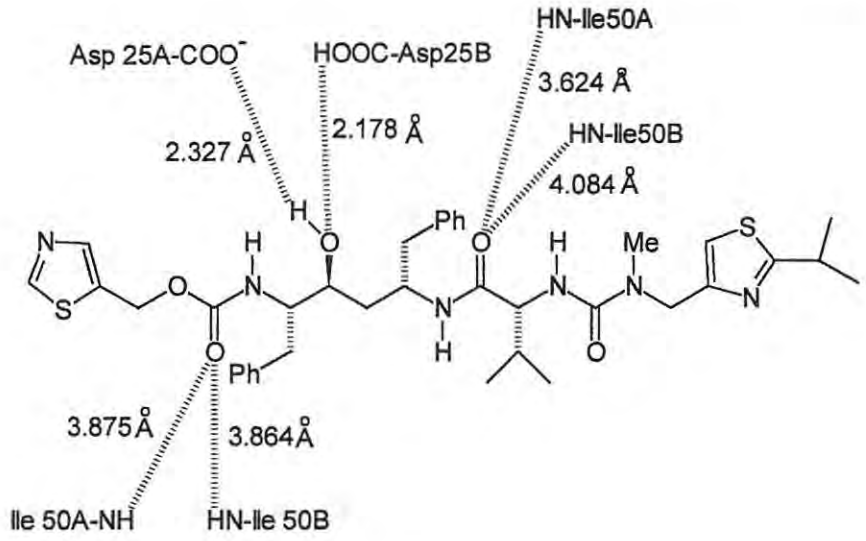


Figure 43a. Schematic representation of potential hydrogen-bonding interactions (with distances in Å) between ritonavir **156** and the HIV-1 protease enzyme in the absence of bridging structural water molecules.

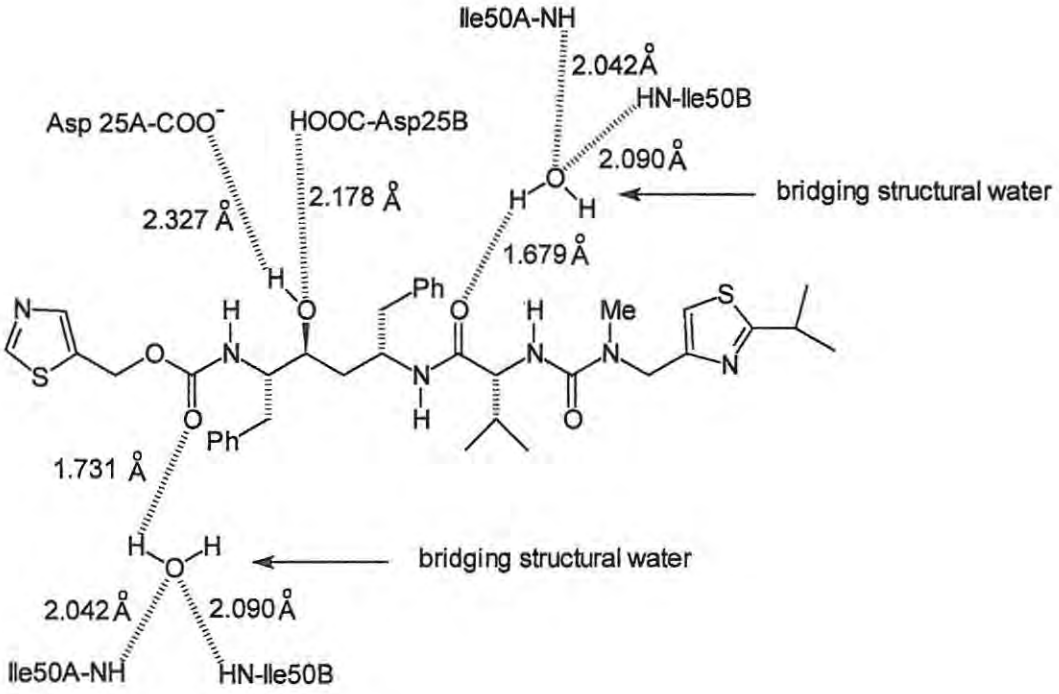


Figure 43b. Schematic representation of potential hydrogen-bonding interactions (with distances in Å) between ritonavir **156** and the HIV-1 protease enzyme in the presence of bridging structural water molecules.

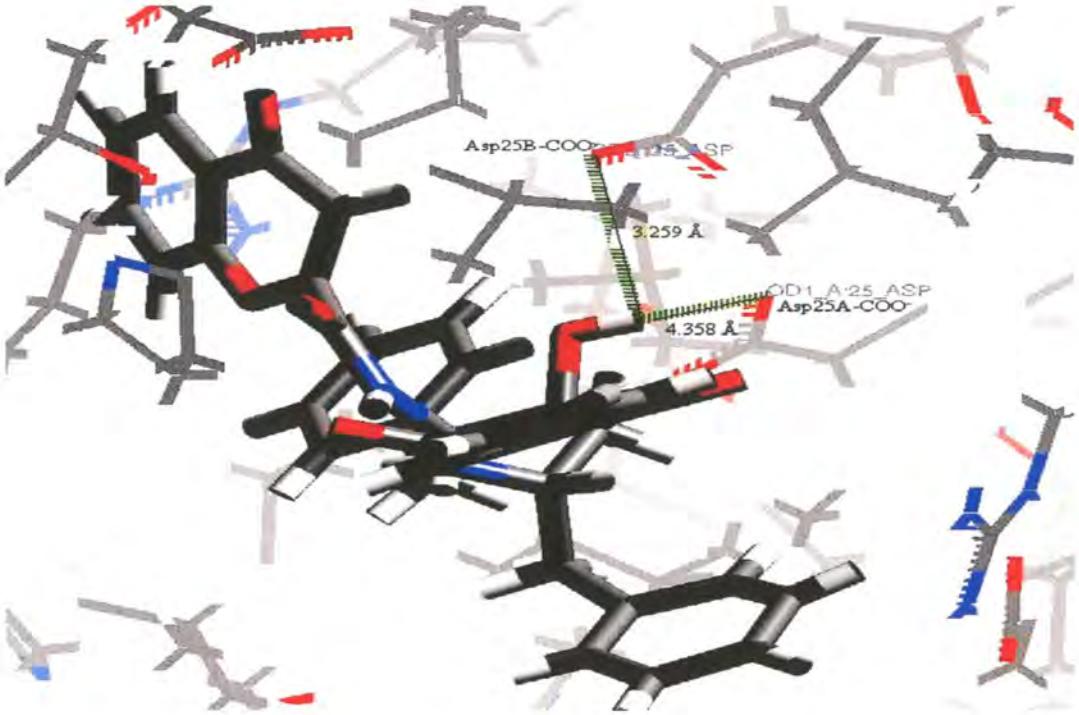


Figure 44a. Favoured bound conformer of compound **263** showing the potential hydrogen-bonding interactions between the 3-hydroxyl group and the HIV-1 protease aspartic acid residues.

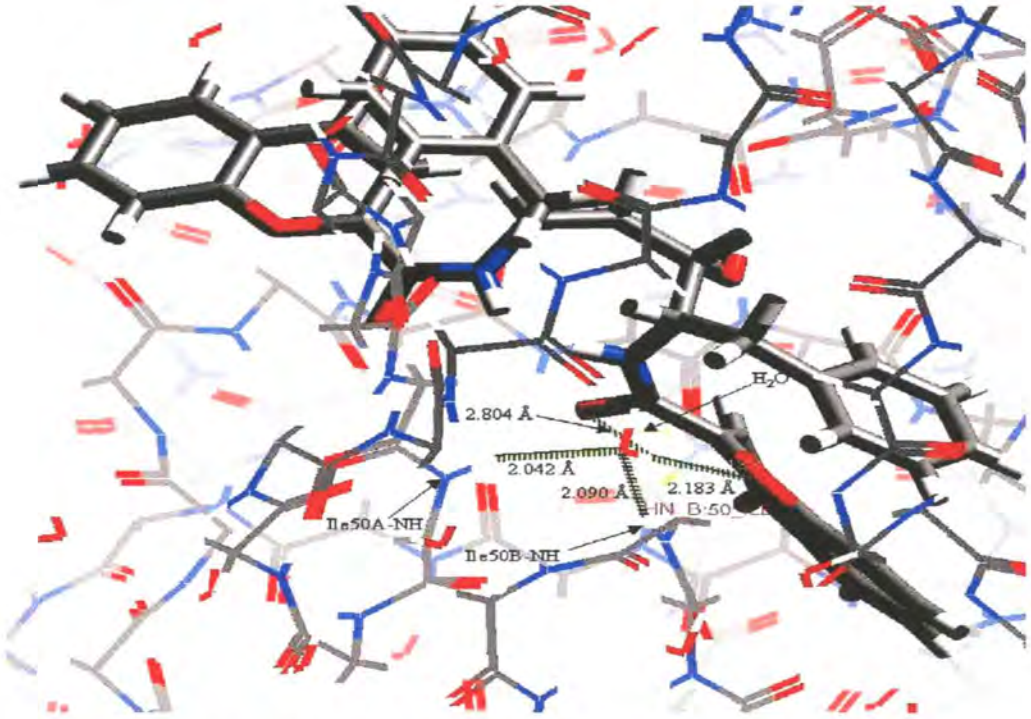


Figure 44b. Favoured bound conformer of compound **263** showing the potential hydrogen-bonding interactions between the 2-carbamoyl group and chromone ether oxygen, a bridging water molecule and the backbone (Ile 50A and Ile 50B) amide hydrogens.

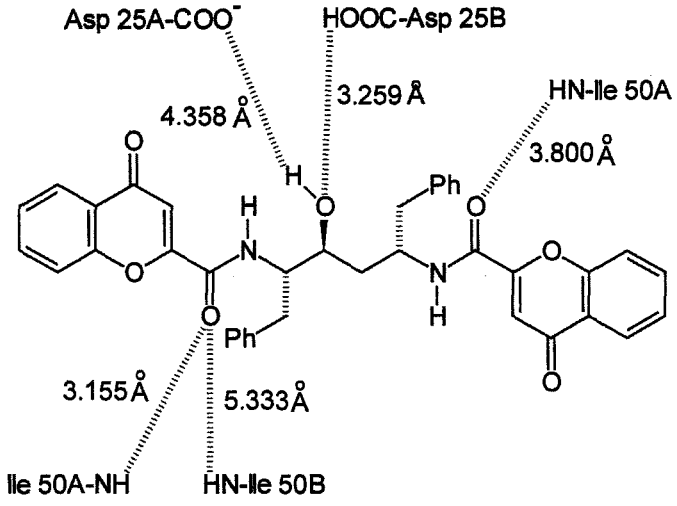


Figure 45a. Schematic representation of potential hydrogen-bonding interactions (with distances in Å) between ligand **263** and the HIV-1 protease enzyme in the absence of bridging structural water molecules.

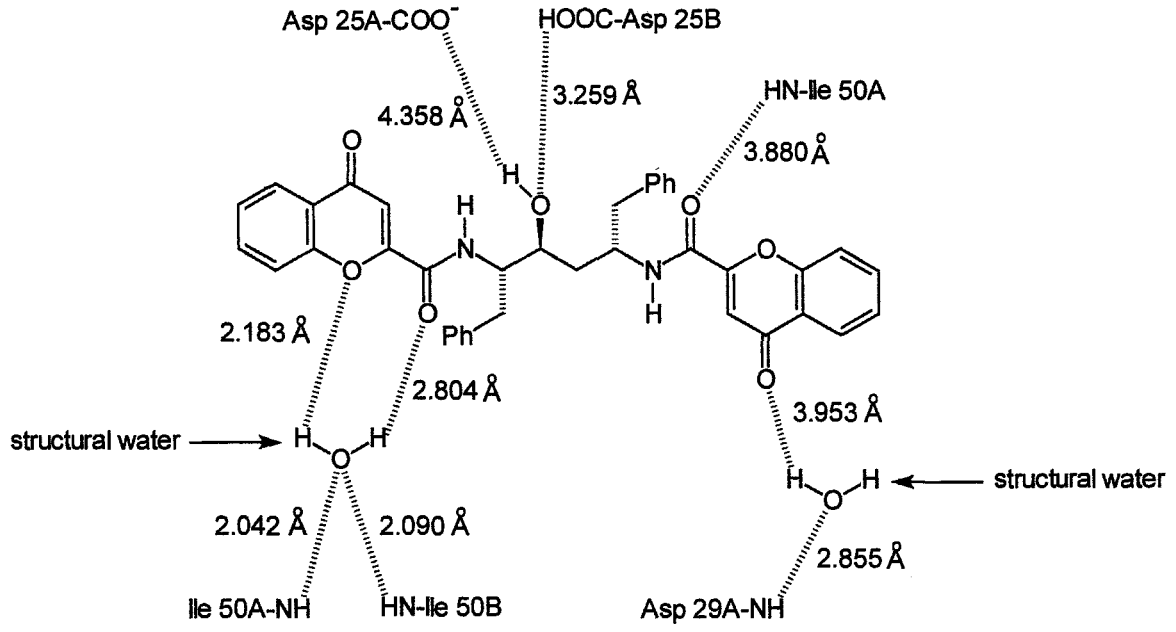


Figure 45b. Schematic represention of potential hydrogen-bonding interactions (with distances in Å) between ligand **263** and the HIV-1 protease enzyme in the presence of bridging structural water molecules.

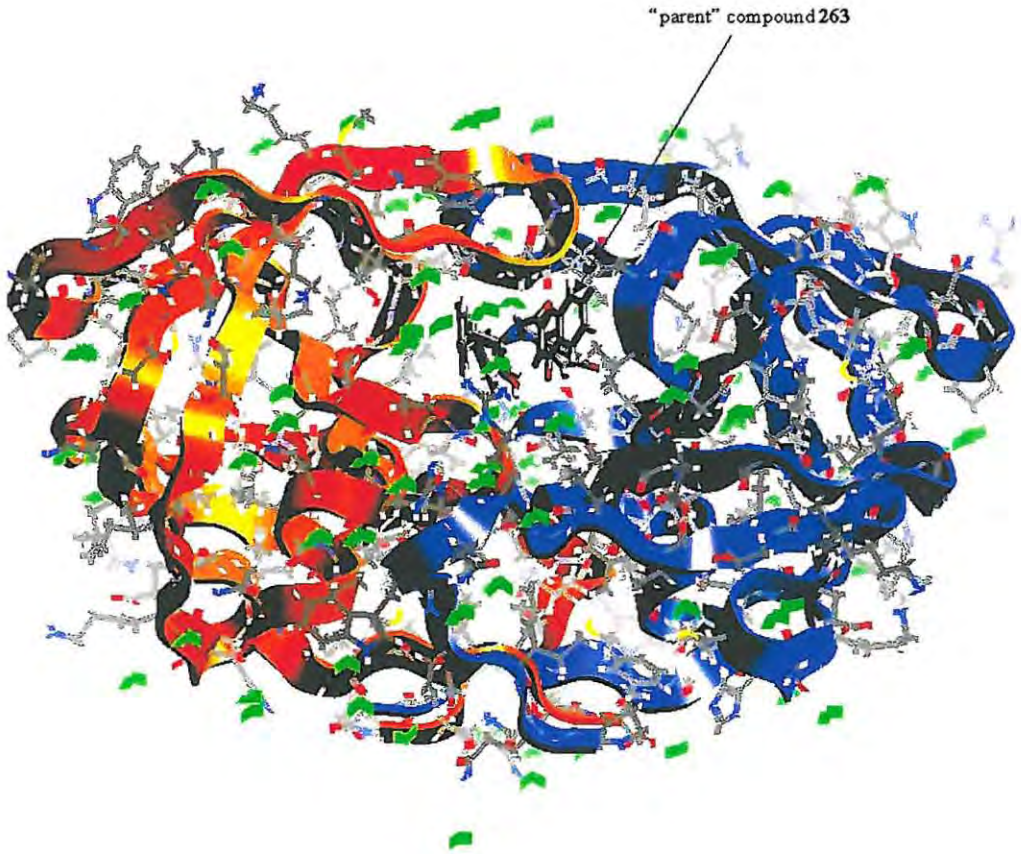


Figure 46. Illustration of the most favoured bound conformer of ligand **263** in the active site of the HIV-1 protease enzyme.

Docking experiments were also undertaken for the remaining synthetic ligands **264 – 267** and the resulting pertinent data are summarized in Table 11. In the case of ligands **265** and **267**, the 6-methoxy and the 7-hydroxyl substituents on the P_2-P_3 chromone moiety exhibit potential hydrogen-bonding interactions with the Asp 29B amide hydrogen and the Asp 29B carboxylate group with inter-atomic distances of 2.190 Å and 2.070 Å, respectively. The 6-chloro and 6-methoxy substituents on the P_2-P_3 chromone moiety (ligands **264** and **265**) were also observed to lie close enough to the Val 32B (3.199 Å) and Ile 47B (2.414 Å) residues to participate in hydrogen-bonding interactions.

Figure 47 illustrates the complementary receptor-binding surface for the aligned ligand **263** and ritonavir **156**. Alignment of the favoured bound conformers of all the ligands **263 – 267**

and ritonavir **156** was performed, and the complementary receptor-binding surface was generated in order access the structural similarities and steric demands of the various ligands (Figure 48). From Figures 47 and 48, it is seems that the ritonavir analogues **263 – 267**, have the capacity to bind in the receptor cavity.

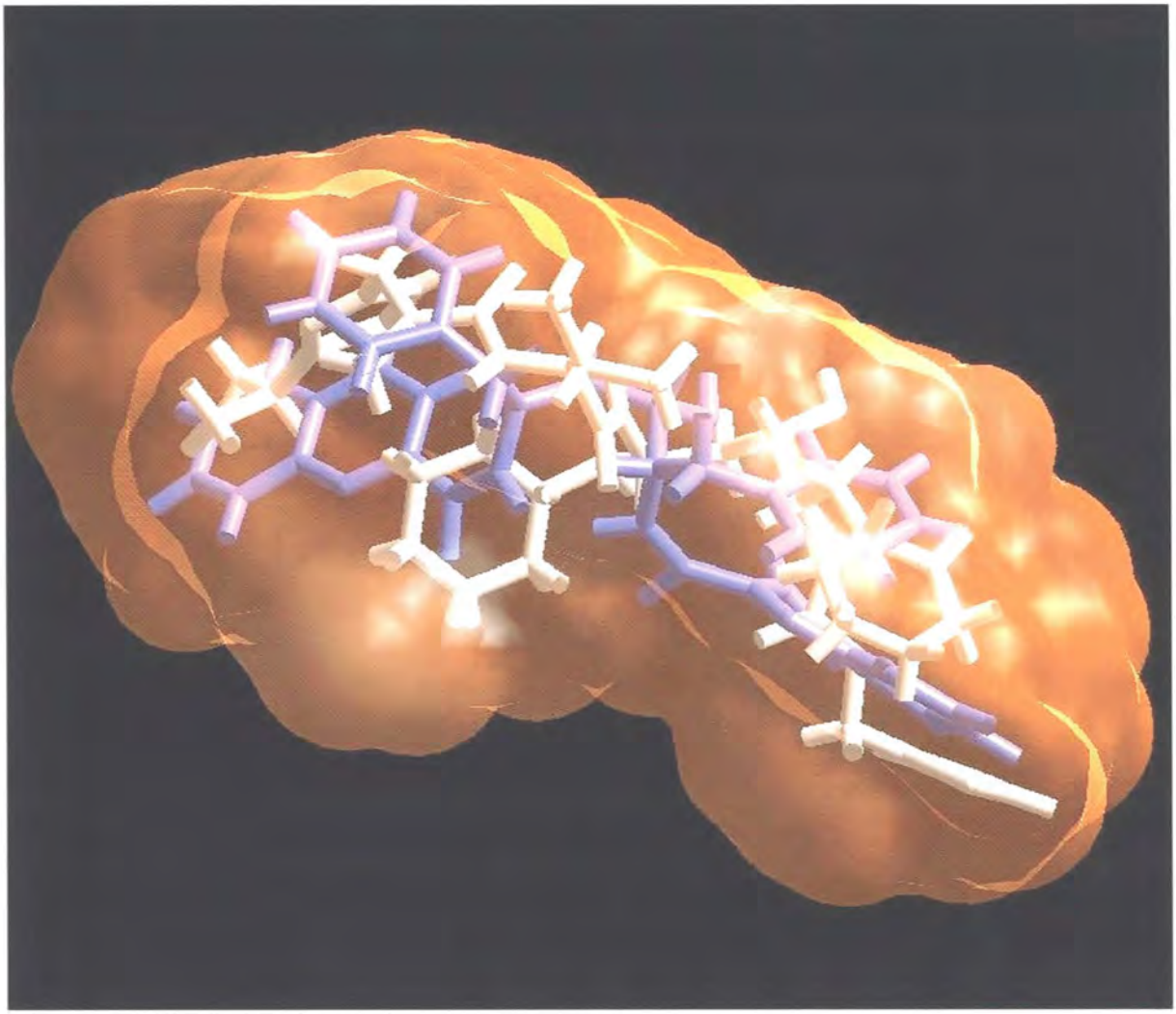


Figure 47. Complementary receptor-binding surface for the aligned ligands **263** (blue) and ritonavir **156** (white).

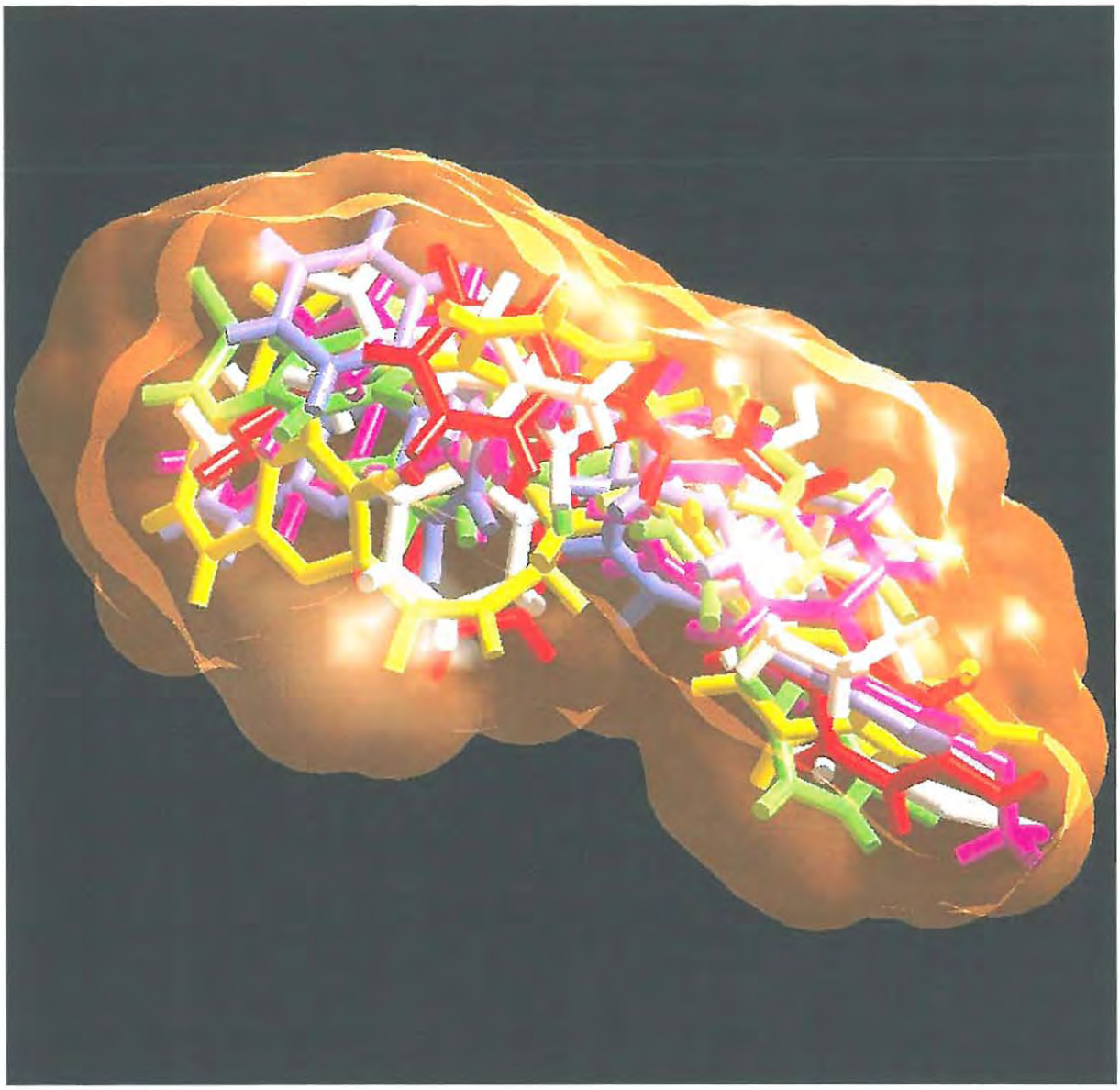


Figure 48. Complementary receptor-binding surface for the aligned ligands **263** (blue), **264** (light green), **265** (magenta), **266** (yellow), **267** (red) and ritonavir **156** (white).

Table 11. Data for the docking of ritonavir **156** and ligands **263 – 267** with the HIV-1 protease receptor.

Compd.	vdW energy[a] (Kcal mol^{-1})	Hydrogen-bonding interaction distances in Å				
		Asp25A-COO^{-}[b]	Asp25B-COO^{-}[b]	Ile50A-NH[c]		Ile50B-NH[c]
156	-98.19	2.327	2.178	1.731	-	1.679
263	-60.79	4.358	3.259	3.155	2.183[d]	2.804
264	-46.10	5.649	3.769	2.316	2.162[d]	2.471
265	-68.13	3.856	3.372	2.592	2.326[d]	2.700
266	-56.51	6.362	3.751	3.592	3.818[d]	3.436
267	-66.81	3.481	3.659	3.727	4.291[d]	2.042

[a] van der Waals' energy of the protein-ligand interactions for the most stable arrangement.
[b]interactions with the 3-hydroxyl group.
[c]interaction with the chromone-2-carbamoyl oxygen
[d]Interactions with P_2-P_3 chromone ether oxygen.

The possibility of nucleophilic ring-cleavage of the chromone-pyran ring by a suitably located nucleophilic residue of the HIV-1 enzyme was also investigated for the parent system **263**. It was observed that two structural water molecules located at an inter-atomic distances of 3.298 Å and 5.682 Å from the electrophilic centres, C-2, of the P_2-P_3 and P_2'-P_3' chromone moieties respectively, as detailed in Figure 49, could possibly attack the chromone rings. Such attack could result in ring-opening and the formation of covalent links between the inhibitor and the structural water molecules. The presence of other structural water molecules capable of forming hydrogen-bonding interactions with the chromone-pyran carbonyl oxygens (2.664 and 4.338 Å) could facilitate electron-delocalization and thus enhance electrophilicity at the C-2 position of the chromone rings making them more susceptible to nucleophilic ring-opening. Other nucleophilic centres located on the protein backbone with the potential for attacking one or other of the chromone C-2 centres are:- Asp 29B-NH, Ala 28B-NH, Val 82B-NH and Gly 49B-NH with inter-atomic distances of 5.055 Å 5.723 , 5.419 and 6.054 Å, respectively.

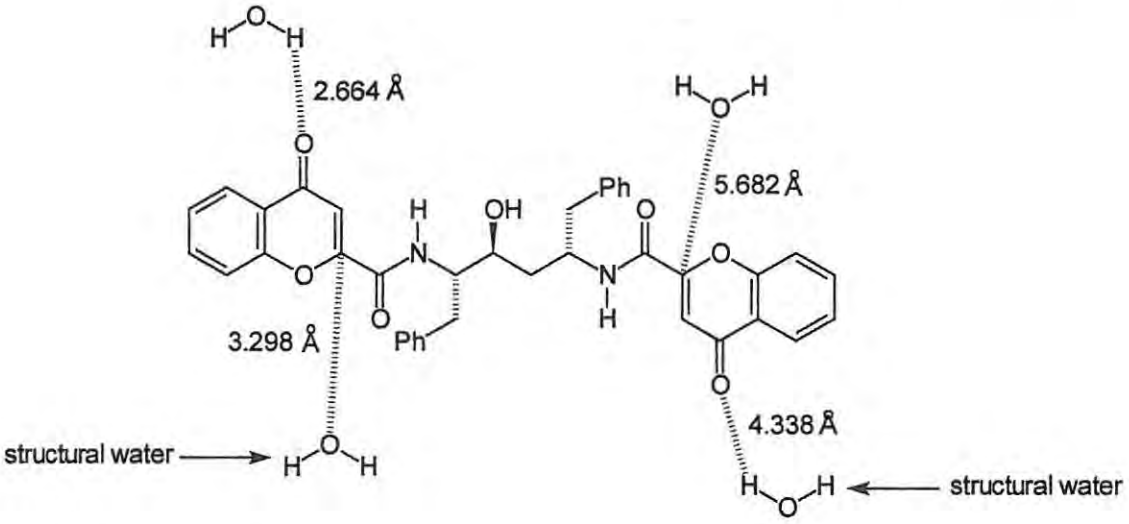
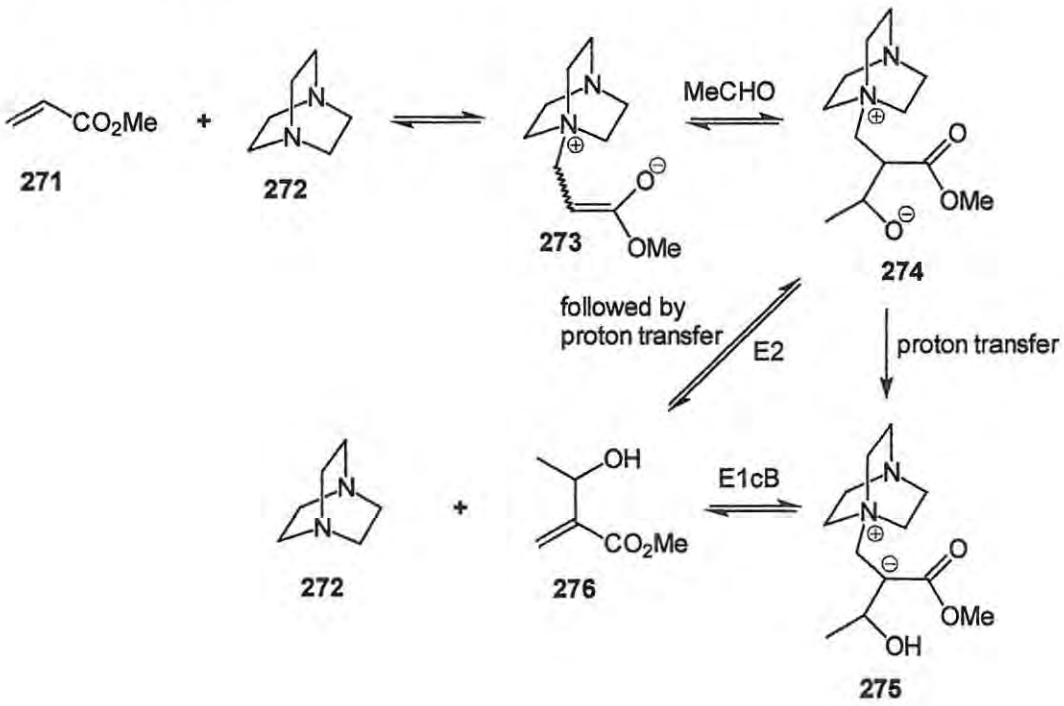


Figure 49. Schematic representation of potential interactions (distances in Å) between the ligand **263** and suitably located structural water molecules.

From the results obtained in the docking experiments, it is apparent that the favoured bound conformation adopted by each of the ligands in the enzyme active site is very different from the conformation corresponding to the respective global-minima obtained in the preliminary studies (Figures 37a and 37b). It is also expected, on the basis of the computer modelling data, that all of the synthetic ligands **263 – 267** should be capable of binding to the enzyme active site. The next phase in our programme will involve biological testing of these compounds.

2.5 Morita-Baylis-Hillman reactions of chromone-3-carbaldehydes

The Morita-Baylis-Hillman reaction involves the coupling of activated alkenes with carbon electrophiles, particularly aldehydes, in the presence of a nucleophilic catalyst.[172-175] The reaction results in the formation of a new carbon-carbon bond, a process which is fundamental to synthetic organic chemistry. These reactions typically require mild reaction conditions, and provide useful synthetic intermediates. A common drawback in Morita-Baylis-Hillman reactions is that 1,4-diazabicyclo[2,2,2]octane (DABCO)-catalyzed reactions are often exceedingly slow. Several approaches have been proposed to address this problem; these include the use of other catalysts and different reaction conditions.[174] The generally accepted mechanism[175] for the DABCO-catalyzed reaction of acetaldehyde with methyl acrylate involves nucleophilic addition of the catalyst (*i.e.* DABCO) **272** to the activated alkene **271** to afford the Baylis-Hillman zwitterion **273**, which then reacts with the electrophile to give the zwitterion **274** (illustrated in **Scheme 63**).[175] *Anti* E2 elimination of the catalyst, followed by protonation, then leads to the Morita-Baylis-Hillman product **276** and release of the catalyst. Alternatively, an internal proton transfer in the intermediate **274** may occur to give the resonance-stabilized zwitterion **275**, which proceeds to the product **276** by an E1cB elimination.[175]

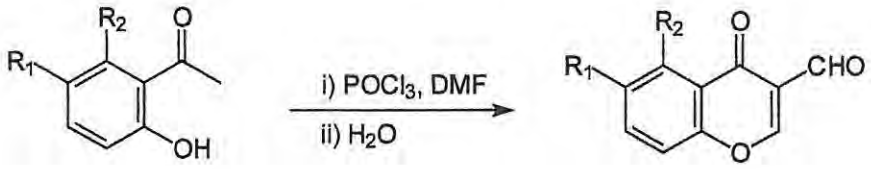


Scheme 63

Although the Morita-Baylis-Hillman reaction is enjoying attention by many research groups, no attention appears to have been given to its application to chromone-3-carbaldehydes.[95] Such reactions have been undertaken in our laboratories in the hope that novel and interesting transformations might occur.

2.5.1 Synthesis of chromone-3-carbaldehydes

The substituted chromone-3-carbaldehydes, which were required to explore substituent effects on the subsequent Morita-Baylis-Hillman reactions, were synthesized *via* Vilsmeier-Haack reaction of appropriately substituted *o*-hydroxyacetophenones and DMF, as reported by Nohara *et al.*[56] The commercially available *o*-hydroxyacetophenones **43** and **277 – 281** were treated with phosphorus oxychloride ($POCl_3$) in dry DMF at –20 °C (in a dry ice-acetone bath) to give the desired chromone-3-carbaldehydes **27** and **282 – 286** in yields ranging from 52 to 72% (**Scheme 64**).



	R_1	R_2
43	H	H
277	Br	H
278	Cl	H
279	F	H
280	OMe	H
281	H	OMe

	R_1	R_2
27	H	H
282	Br	H
283	Cl	H
284	F	H
285	OMe	H
286	H	OMe

Scheme 64

Recrystallization from acetone afforded the analytically pure products, which were fully characterized by elemental (HREIMS) and spectroscopic (IR, ^1H and ^{13}C NMR) analysis. The ^1H NMR spectrum (Figure 50) of compound **283** reveals a singlet at δ 8.52 ppm corresponding to the characteristic 2-methine proton, while the formyl proton resonates as a singlet at δ 10.33 ppm. The ^{13}C NMR spectrum (Figure 51) shows the expected 10 carbon

signals, with two signals overlapping at δ 120.2 and 120.3 ppm. The 2-methine carbon resonates at δ 160.6 ppm and the two carbonyl carbon signals appear at δ 174.8 and 188.0 ppm.

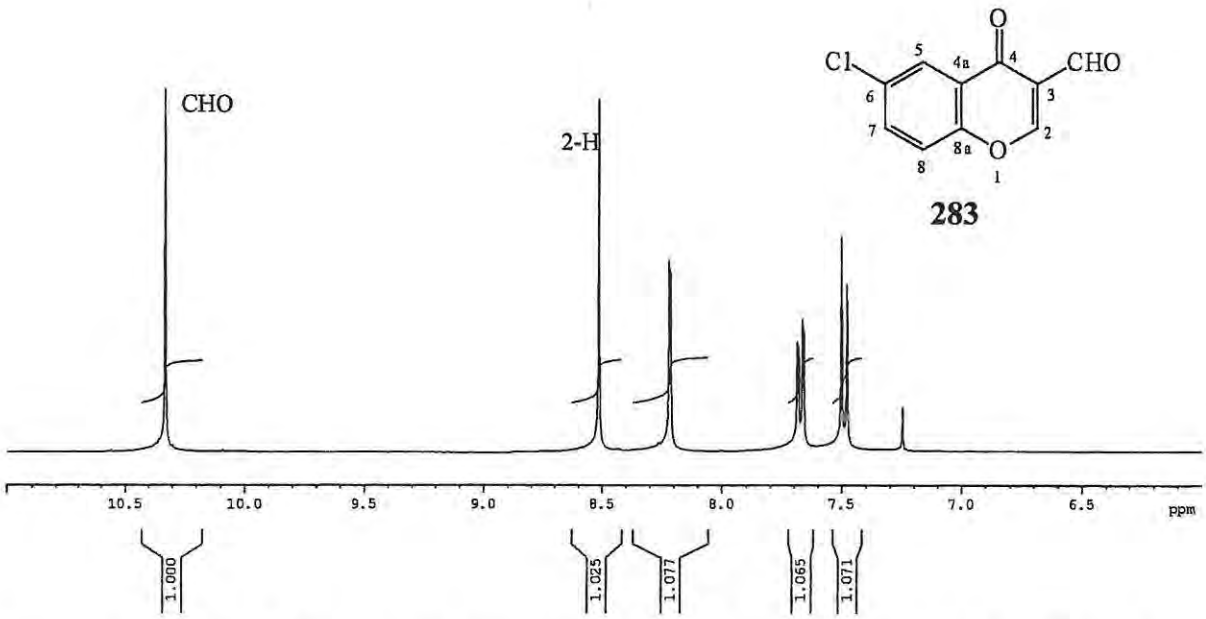


Figure 50. 400 MHz ^{1}H NMR spectrum of 6-chlorochromone-3-carbaldehyde **283** in $CDCl_3$.

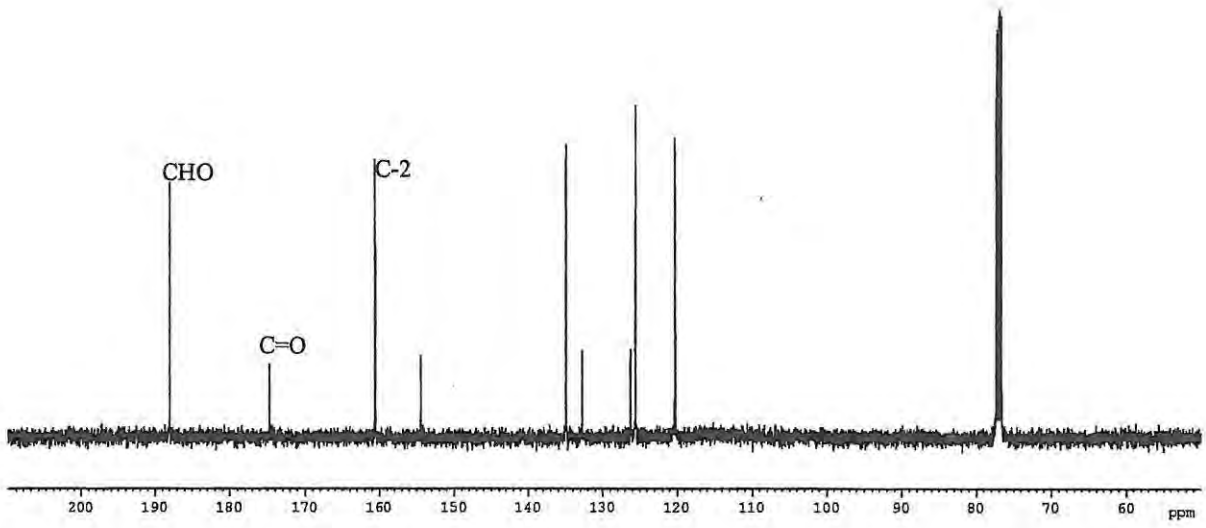
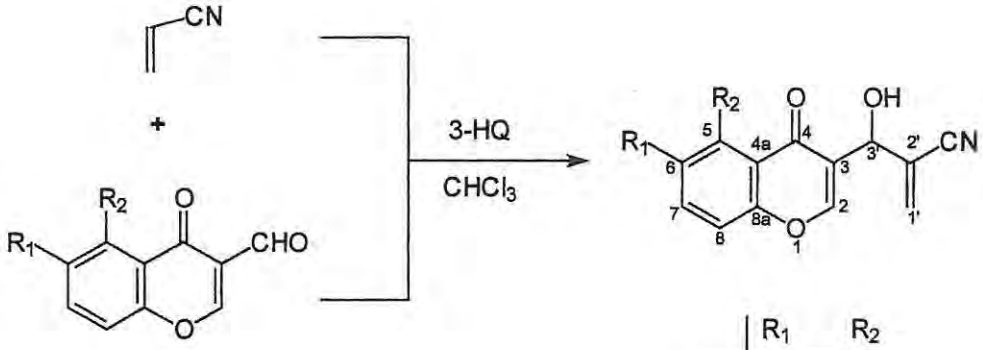


Figure 51. 100 MHz ^{13}C NMR spectrum of 6-chlorochromone-3-carbaldehyde **283** in $CDCl_3$.

2.5.2 Reactions of chromone-3-carbaldehydes with acrylonitrile

Earlier work carried out in our laboratories by Sabbagh[176] on the Morita-Baylis-Hillman reaction between chromone-3-carbaldehydes and the activated alkenes, methyl acrylate and acrylonitrile in the presence of DABCO afforded, in very low yields, the expected Morita-Baylis-Hillman products (8 to 17%), together with unexpected chromone dimers (2 to 15%). Not only were the yields very low, but reaction times of several weeks and extensive purification procedures were also required. In the present study, we extended the preliminary optimization studies undertaken by Sabbagh[176] (on an NMR tube scale) to obtain, principally, the Morita-Baylis-Hillman products. Attention was then given to the possibility of obtaining the chromone dimers in good yield.



	R_1	R_2
27	H	H
282	Br	H
283	Cl	H
284	F	H
285	OMe	H
286	H	OMe

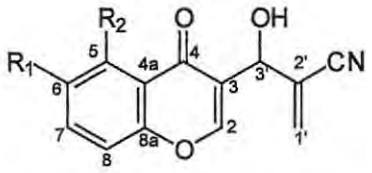
	R_1	R_2
287	H	H
288	Br	H
289	Cl	H
290	F	H
291	OMe	H
292	H	OMe

Scheme 65

After exploring various protocols, a general procedure was developed which provided efficient access to the required Morita-Baylis-Hillman products on a preparative scale. Thus, a mixture of one equivalent of each of the chromone-3-carbaldehydes **27** and **282 – 286**, 1.5 equivalents of acrylonitrile and 5 equivalents of the catalyst, 3-hydroxyquinuclidine (3-HQ) in a minimum volume of chloroform was stirred at room temperature for 24 h (**Scheme 65**).

The crude reaction mixtures were purified by flash chromatography to afford the corresponding Morita-Baylis-Hillman products **287 – 292** in isolated yields ranging from 53 to 67% (Table 12). The products were fully characterized by elemental (HREIMS) analysis and one- and two-dimensional NMR spectroscopy. The ^{1}H NMR spectrum (Figure 52) of the Morita-Baylis-Hillman product **289** reveals a doublet at δ 4.16 ppm corresponding to the 3'-hydroxyl group, a doublet at δ 5.34 ppm corresponding to 3'-H nucleus and two distinct singlets at δ 6.13 and 6.32 ppm characteristic of the 1'-methylene protons. The ^{13}C NMR spectrum (Figure 53) clearly shows the expected 13 signals. The methine carbon C-3' resonates at δ 68.7 ppm and the carbonyl carbon at δ 176.3 ppm, while the DEPT-135 spectrum (Figure 54) confirms assignment of the sole methylene signal (at δ 131.6 ppm) to C-1'. The signals were assigned with the aid of the COSY, HMQC and HMBC data.

Table 12. Comparative yields of Morita-Baylis-Hillman products **287 – 292.**



R_1	R_2	Morita-Baylis-Hillman product	
		Compd.	Yield[a] /%
H	H	**287**	64
Br	H	**288**	67
Cl	H	**289**	59
F	H	**290**	53
OMe	H	**291**	54
H	OMe	**292**	60

[a]Isolated yields

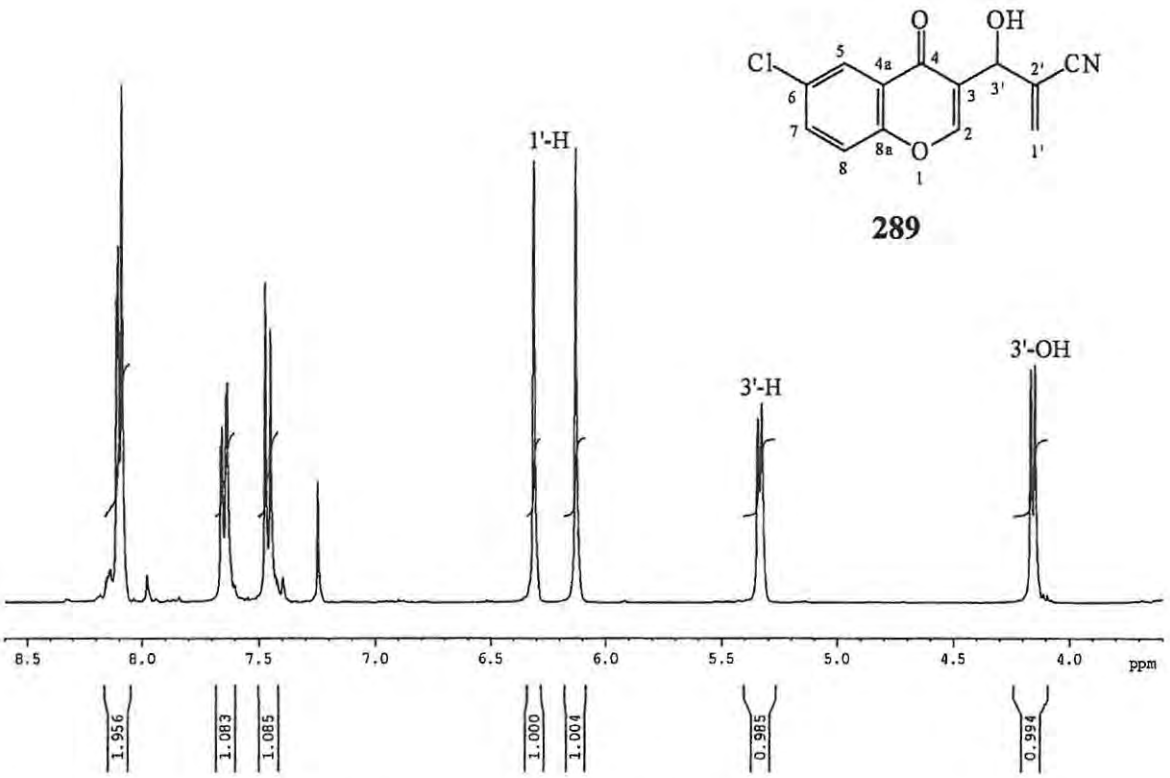


Figure 52. 400 MHz ^{1}H NMR spectrum of Morita-Baylis-Hillman product **289** in $CDCl_3$.

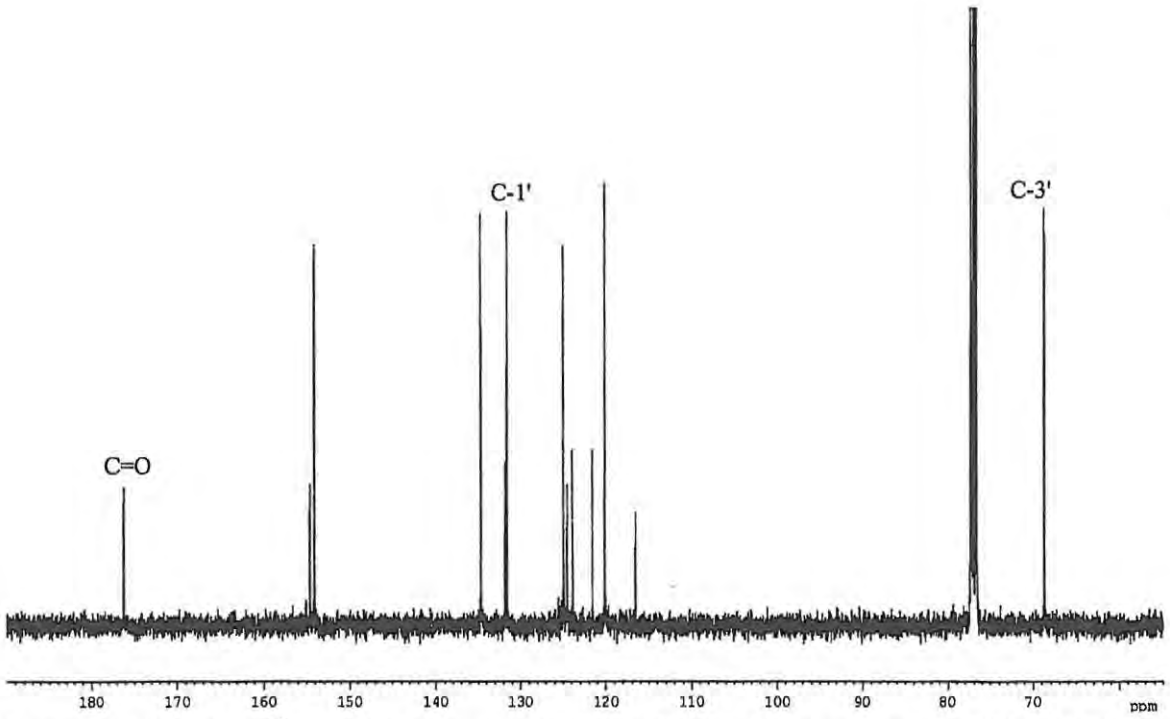


Figure 53. 100 MHz ^{13}C NMR spectrum of Morita-Baylis-Hillman product **289** in $CDCl_3$.

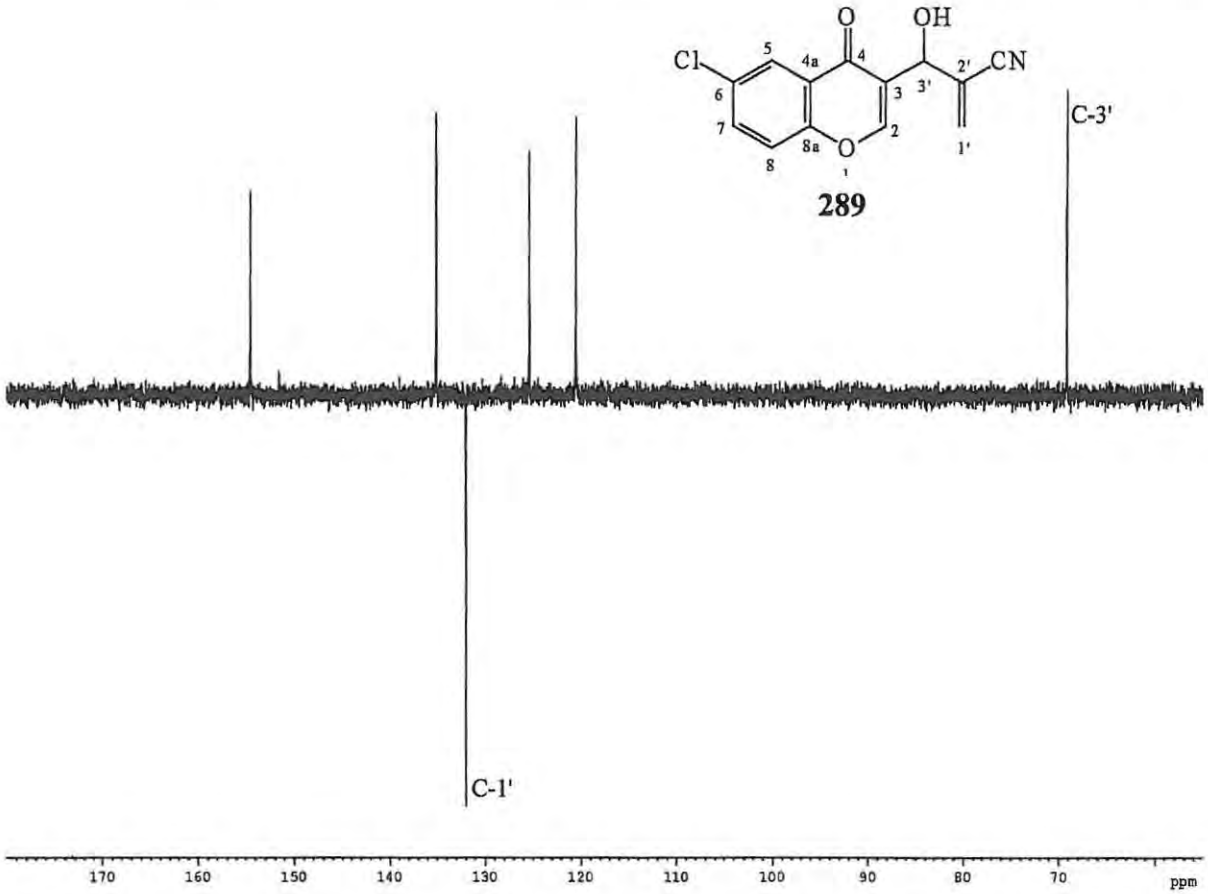
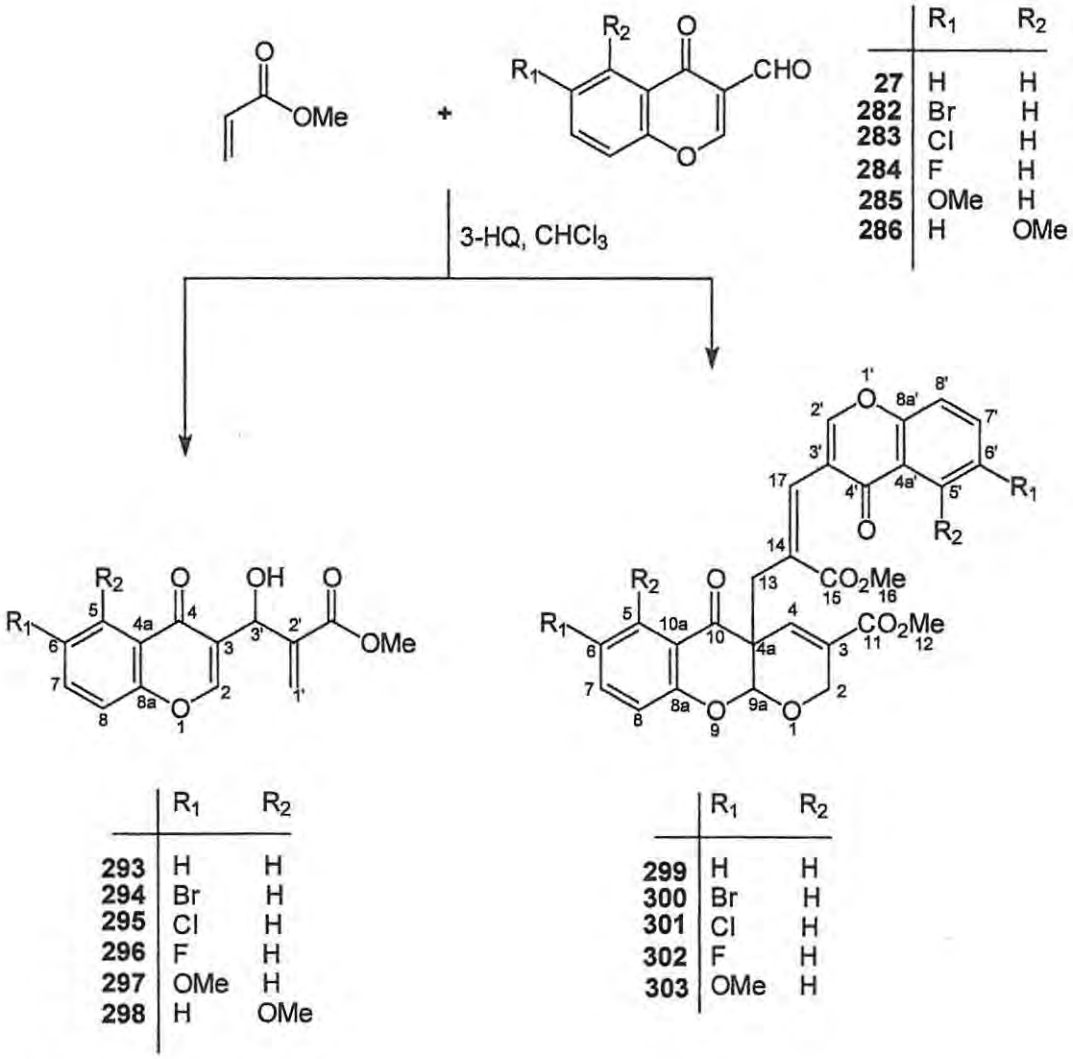


Figure 54. DEPT-135 NMR spectrum of Morita-Baylis-Hillman product **289** in $CDCl_3$.

2.5.3 Reactions of chromone-3-carbaldehydes with methyl acrylate

Encouraged by the ready accessibility of the Morita-Baylis-Hillman products **287 – 292** using the catalyst, 3-hydroxyquinuclidine (3-HQ), we extended the study to the reaction of chromone-3-carbaldehydes with methyl acrylate. As before, mixture of one equivalent of the chromone-3-carbaldehydes **27** and **282 – 286**, 1.5 equivalents of the activated alkene, in this case, methyl acrylate and 5 equivalents of the catalyst, 3-hydroxyquinuclidine (3-HQ) in a minimum volume of chloroform was stirred at room temperature for 24 h. Efficient purification of the crude reaction mixtures by flash chromatography afforded the corresponding Morita-Baylis-Hillman products **293 – 298** (**Scheme 66**) in yields ranging



	R_1	R_2
27	H	H
282	Br	H
283	Cl	H
284	F	H
285	OMe	H
286	H	OMe

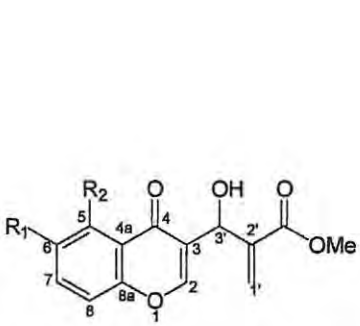
	R_1	R_2
293	H	H
294	Br	H
295	Cl	H
296	F	H
297	OMe	H
298	H	OMe

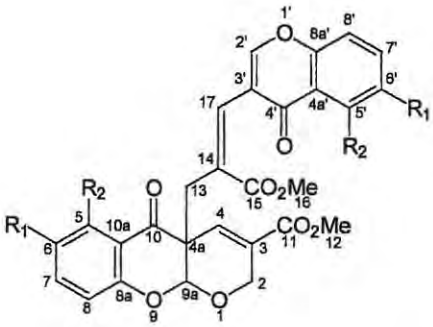
	R_1	R_2
299	H	H
300	Br	H
301	Cl	H
302	F	H
303	OMe	H

Scheme 66

from 50 to 79% (Table 13), together with the generally minor products identified as the "chromone dimers" **299** – **303** in yields ranging from 30 to 50%. The use of 3-hydroxyquinuclidine (3-HQ) as the catalyst, suggested by Sabbagh's preliminary optimization studies,[176] resulted in far better yields than those obtained using DABCO. In the case of the 5-methoxy system, however, the Morita-Baylis-Hillman product **298** was the only product to be isolated. A mechanism which accounted for the formation of the chromone dimers has been proposed by Sabbagh (**Scheme 69**, p. 138).

Table 13. Comparative yields of the Morita-Baylis-Hillman products and the corresponding dimers.





R_1	R_2	Morita-Baylis-Hillman product		Chromone dimer	
		Compd.	Yield[a] /%	Compd.	Yield[a] /%
H	H	**293**	66	**299**	34
Br	H	**294**	63	**300**	37
Cl	H	**295**	50	**301**	50
F	H	**296**	70	**302**	30
OMe	H	**297**	60	**303**	40
H	OMe	**298**	79	-	-

[a]Isolated yield.

The products were characterized using elemental (HREIMS) and spectroscopic (IR and NMR) analysis. The ^{1}H NMR spectrum (Figure 55) of the Morita-Baylis-Hillman product **298** reveals two distinct singlets at δ 3.71 and 3.95 ppm corresponding to the ester methyl and 5-methoxy protons, respectively, a poorly resolved doublets at δ 4.78 and 5.51 ppm corresponding to the 3'-hydroxyl proton and the 3'-H nucleus, respectively, and two distinct

singlets at δ 6.19 and 6.43 ppm characteristic of the 1'-methylene protons. The ^{13}C NMR spectrum (Figure 56) shows 15 carbon signals with the ester methyl and 5-methoxy carbons resonating at δ 51.8 and 56.4 ppm, respectively and the C-3' nucleus at δ 68.0 ppm. The DEPT-135 spectrum (Figure 57) confirms the presence of the C-1' methylene carbon resonating at δ 127.0 ppm. The signal assigments were facilitated with the aid of the COSY, HMQC and HMBC data.

The ^{1}H NMR spectrum (Figure 58) of the "parent" chromone dimer **299** reveals a pair of doublets at δ 3.13 and 3.37 ppm corresponding to the diastereotopic 13-methylene protons, two singlets at δ 3.61 and 3.66 ppm corresponding to the two methoxy groups, a pair of double doublets at *ca.* δ 4.50 ppm corresponding to the 2-methylene protons and a singlet at δ 5.05 ppm corresponding to the 9a proton. The added multiplicity of the signals at δ 4.50 ppm is due to coupling of the diastereotopic 13-methylene protons and the 4-H nucleus. The ^{13}C NMR spectrum (Figure 59) reveals 28 carbon signals, with the two methylene carbons (C-13 and C-2) resonating at δ 28.4 and 65.8 ppm, respectively. Assignment of these two methylene carbon signals, and the 12 quarternary carbon signals was confirmed using the DEPT-135 data (Figure 60).

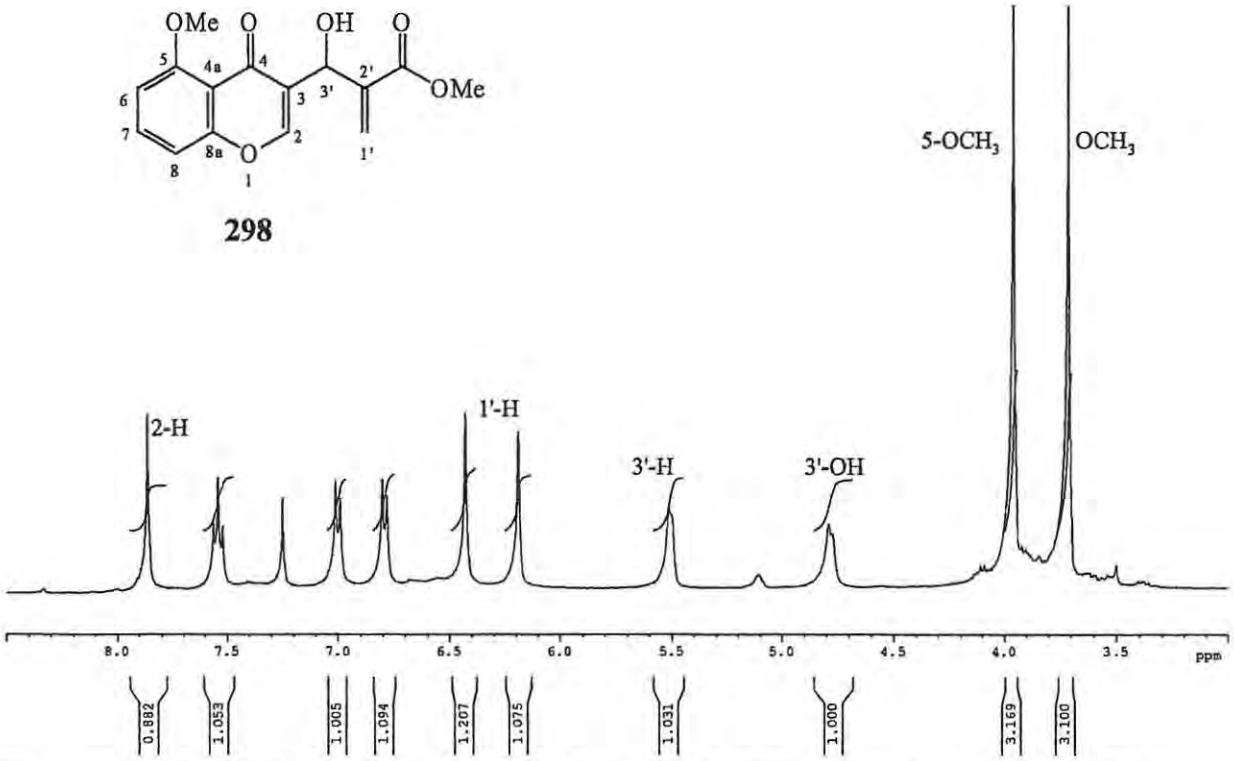


Figure 55. 400 MHz ^{1}H NMR spectrum of Morita-Baylis-Hillman product **298** in $CDCl_3$

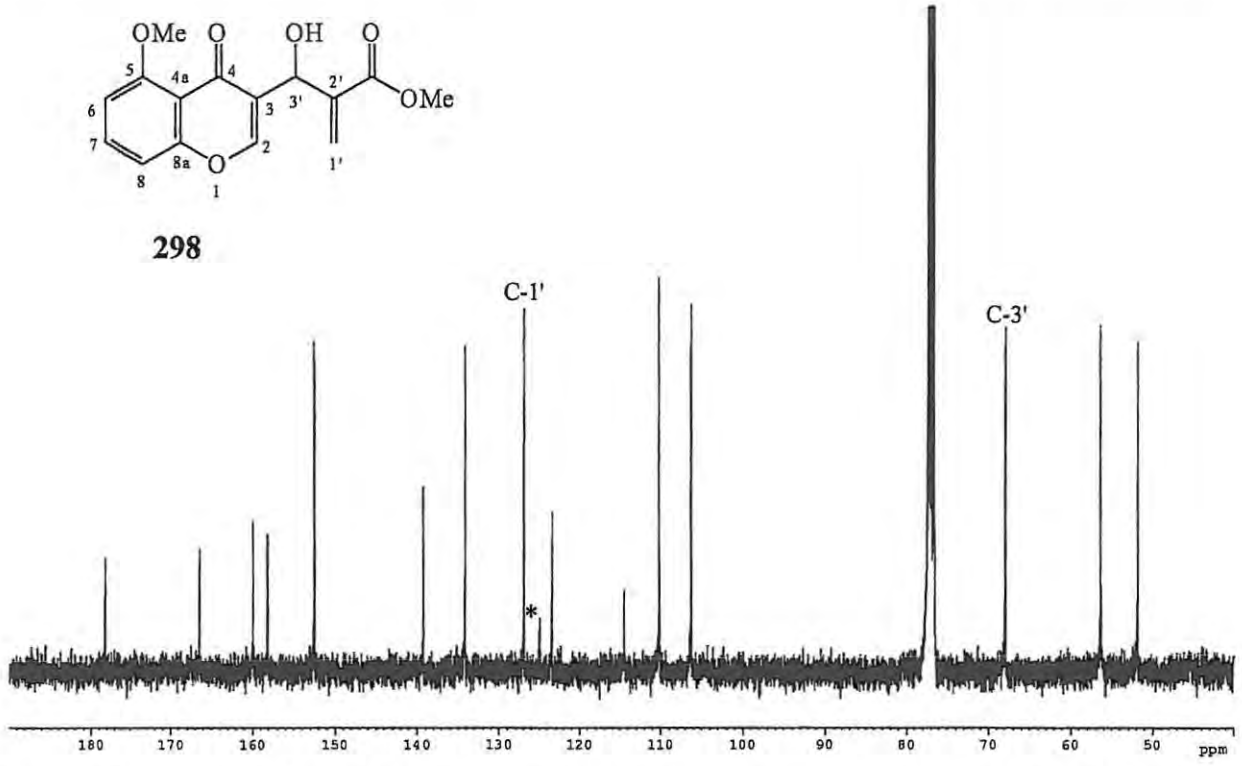


Figure 56. 100 MHz ^{13}C NMR spectrum of Morita-Baylis-Hillman product **298** in $CDCl_3$, with an asterisk indicating signal due to impurity.

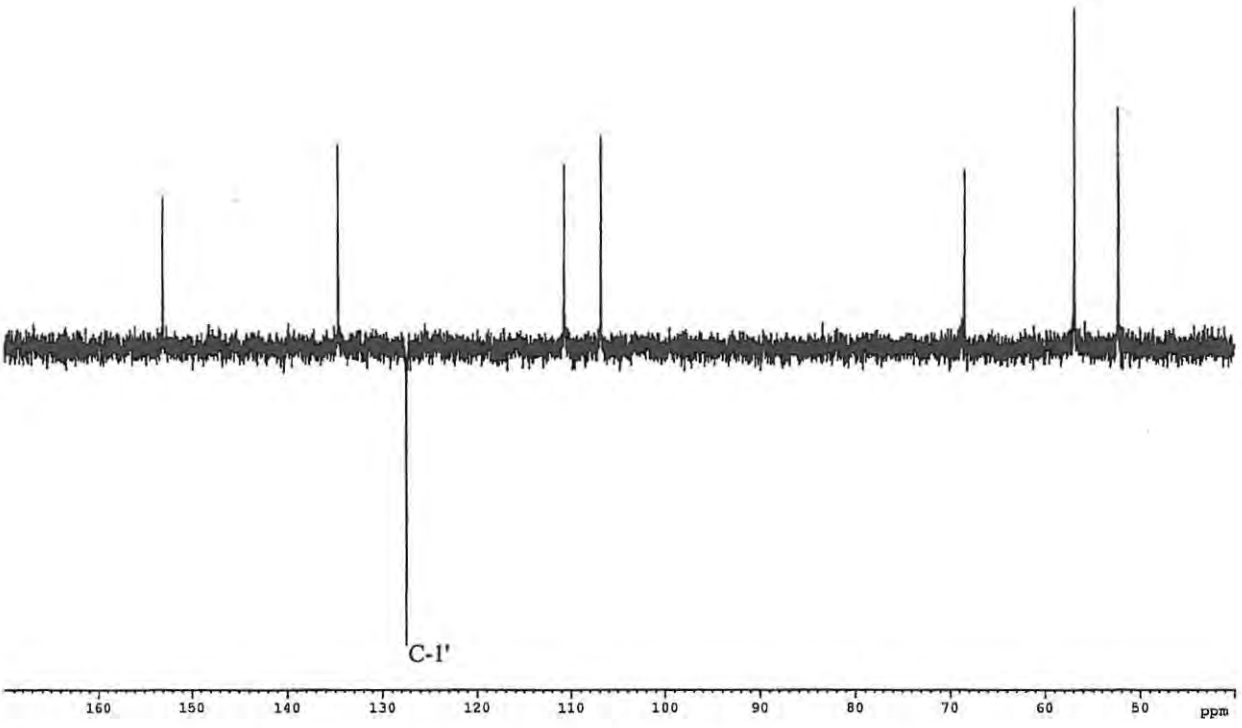


Figure 57. DEPT-135 NMR spectrum of Morita-Baylis-Hillman product **298** in $CDCl_3$.

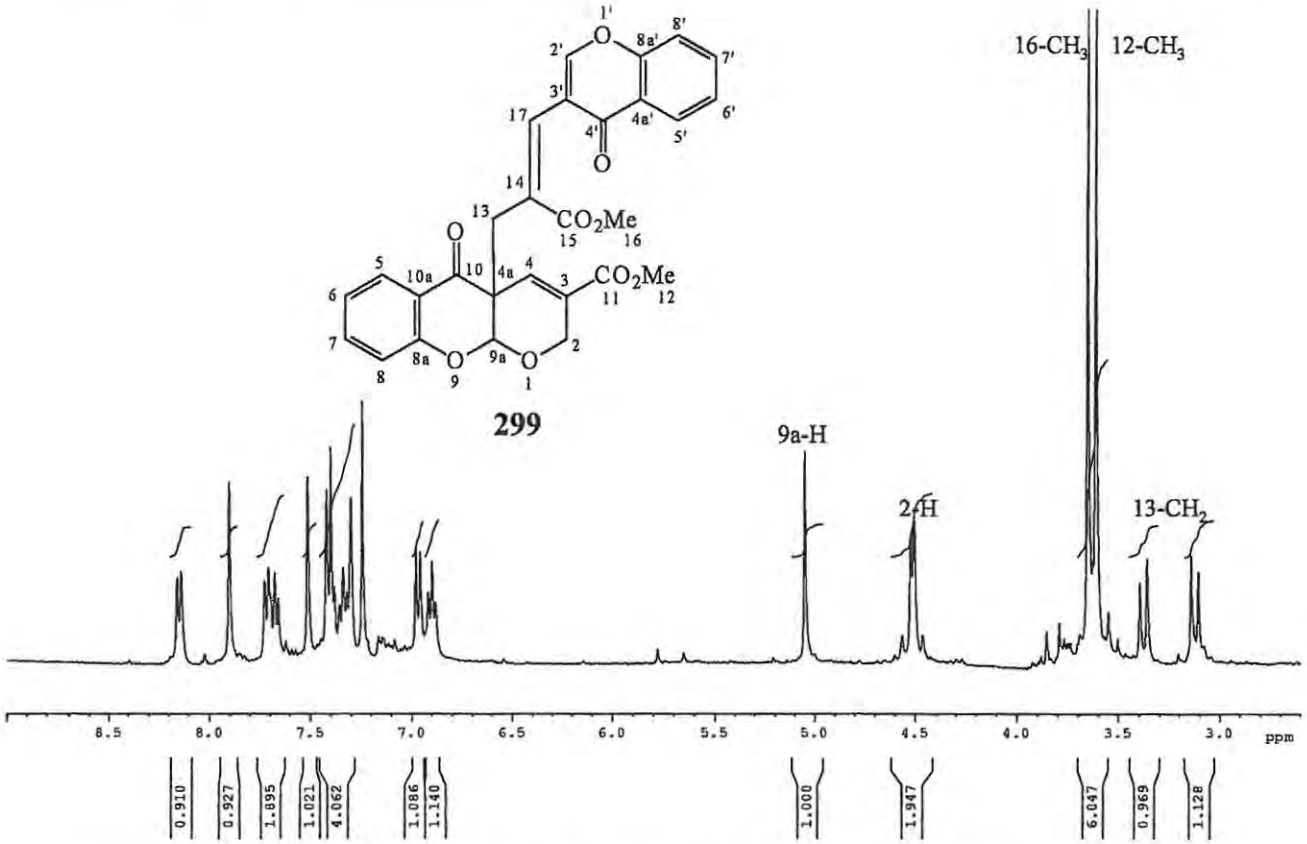


Figure 58. 400 MHz ^{1}H NMR spectrum of chromone dimer **299** in $CDCl_3$.

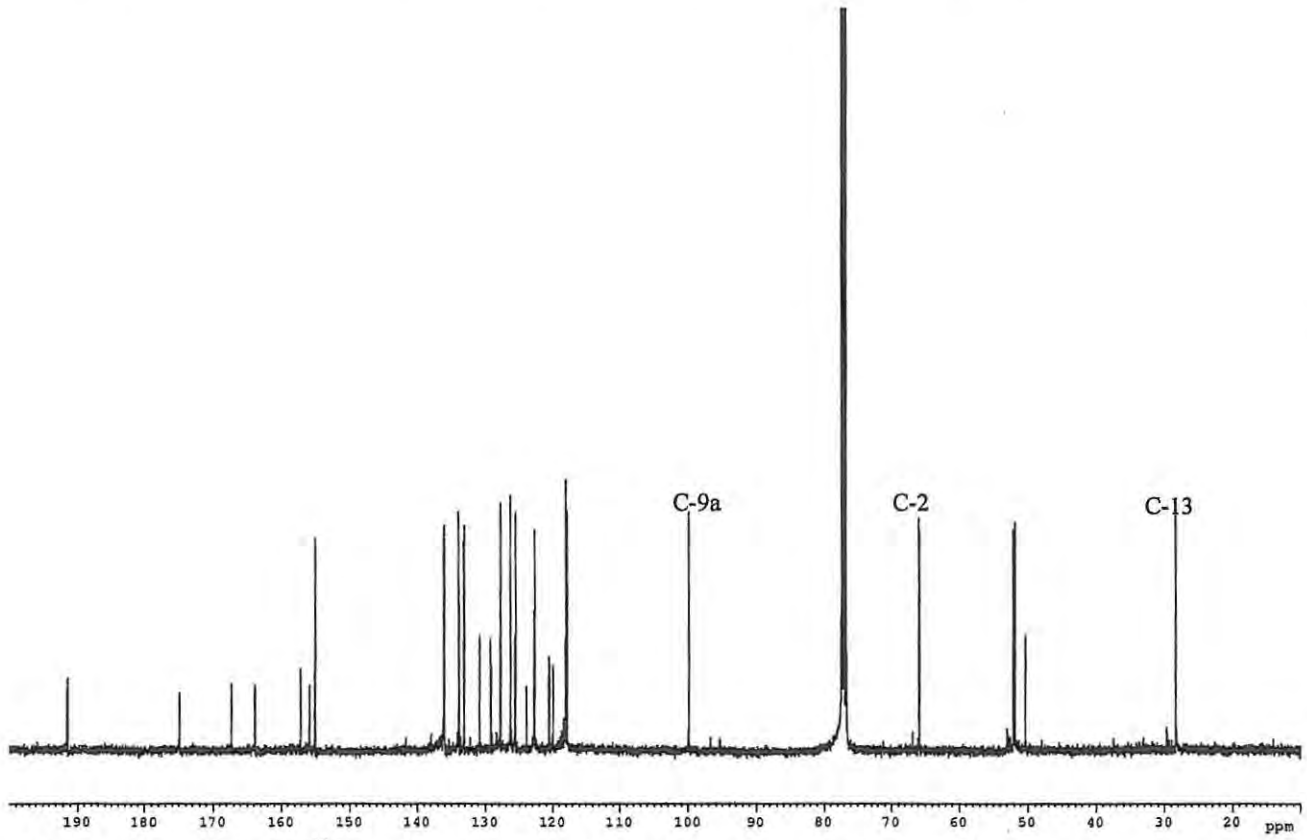


Figure 59. 100 MHz ^{13}C NMR spectrum of chromone dimer **299** in $CDCl_3$.

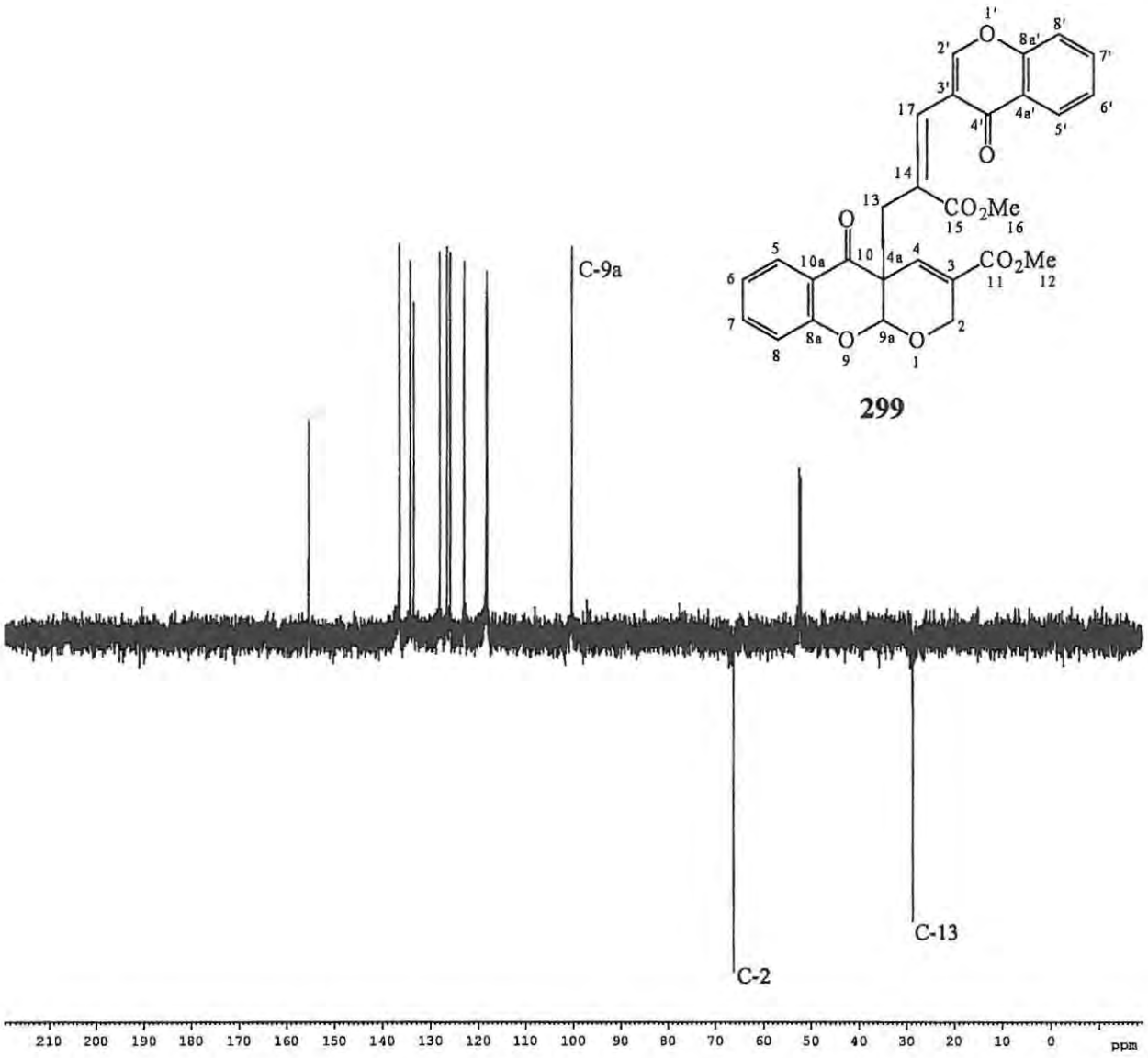


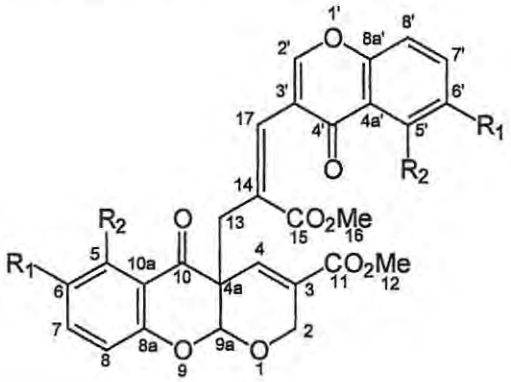
Figure 60. DEPT-135 NMR spectrum of chromone dimer **299** in $CDCl_3$.

2.5.4 "Dimer" studies

As discussed in Section 2.5.2 and 2.5.3, an earlier investigation[176] of the reaction between the chromone-3-carbaldehydes and the two activated alkenes, acrylonitrile and methyl acrylate, in the presence of DABCO afforded, in very low yields, the expected Morita-Baylis-Hillman products together with unprecedented bischromone adducts (in the case of acrylonitrile) or chromone dimers (in the case of methyl acrylate). Preliminary optimization studies undertaken by Sabbagh[176] shown that the bischromone adducts could be obtained from a

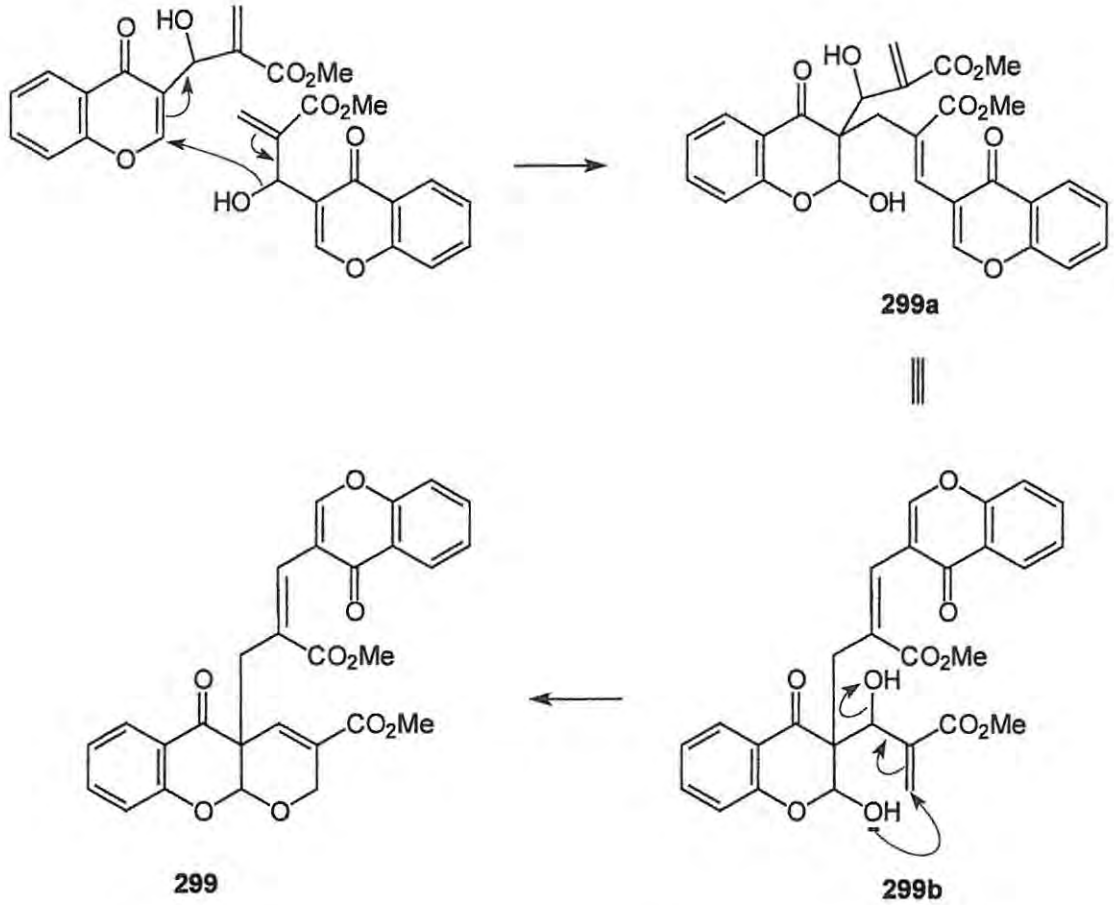
reaction between the Morita-Baylis-Hillman products and the corresponding chromone-3-carbaldehydes in the presence of the catalyst, DABCO. However, several attempts to reproduce these earlier results by reacting one equivalent of the Morita-Baylis-Hillman product (**287 – 292**), one equivalent of the corresponding chromone-3-carbaldehyde (**27** and **283 – 286**) and two equivalent of DABCO in a minimum volume of chloroform proved unsuccessful. However, when a mixture of one equivalent of the Morita-Baylis-Hillman product (**293 – 298**) and 3.0 equivalents of DABCO were heated in an oil bath at 80 °C for 3 h, the reaction being monitored by TLC, ^{1}H NMR analysis of the crude reaction mixture, in each case, showed 100% conversion to the chromone dimer (**299 – 304**). Efficient purification of the crude reaction mixtures, using the Chromatotron, afforded, principally, the desired chromone dimers **299 – 304** in yields ranging from 49 to 68% (Table 14). The mechanism proposed by Sabbagh[176] to account for the formation of the chromone dimer **299** and its substituted analogues is outlined in **Scheme 67**, p. 138.

Table 14. Comparative yields of the chromone dimers **299 – 304**.



R_1	R_2	Chromone dimer	
		Compd.	Yield[a] /%
H	H	**299**	62
Br	H	**300**	59
Cl	H	**301**	68
F	H	**302**	49
OMe	H	**303**	51
H	OMe	**304**	55

[a]isolated yields



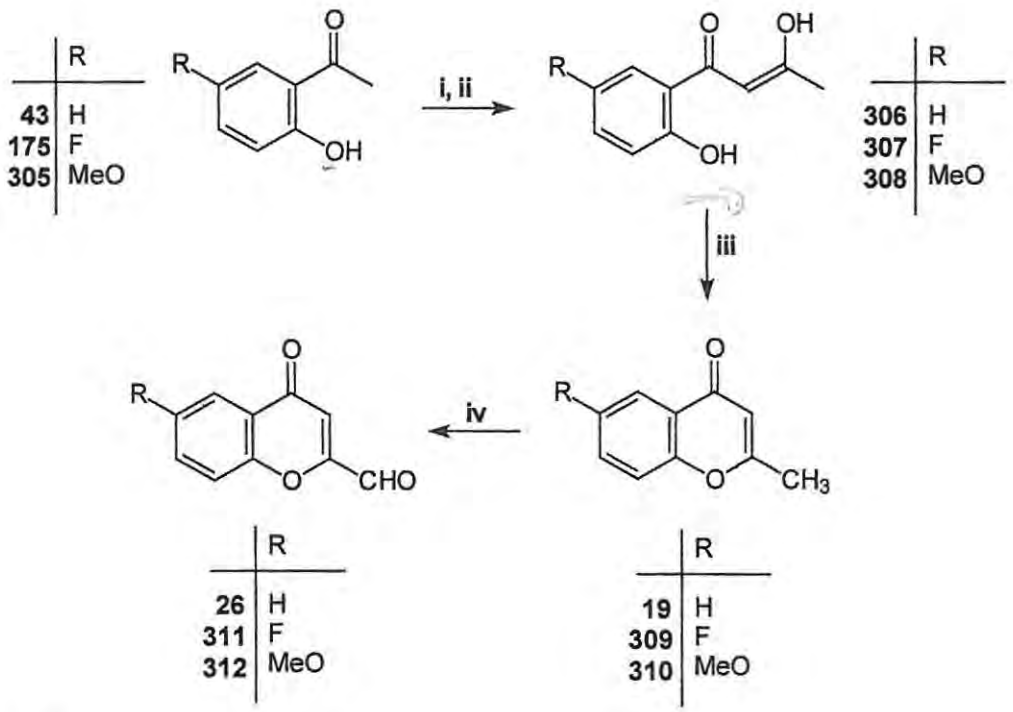
Scheme 67. Proposed mechanism for the formation of the chromone dimers.[176]

It is thus apparent that the use of 3-hydroxyquinuclidine (3-HQ) as a catalyst provides convenient access to chromone-3-carbaldehyde – derived Morita-Baylis-Hillman products on a preparative scale, and that the dimeric products **299 – 304** can be readily obtained from their respective precursors **293 – 298** using DABCO as the catalyst. Potential of these chromone dimers for elaboration to HIV-1 protease inhibitors will be explored further.

2.5.5 Synthesis of chromone-2-carbaldehydes

As discussed in the introduction, chromone-2-carbaldehydes are important intermediates, which are known to undergo a number of reactions involving the 2-formyl functionality to give a wide range of interesting products. Morita-Baylis-Hillman reactions of chromone-2-carbaldehydes had not hitherto been reported and, in view of our current interest in these reactions, we synthesized a range of chromone-2-carbaldehydes with the intention of exploring their use in Morita-Baylis-Hillman reactions.

The chromone-2-carbaldehydes **26**, **311** and **312** were synthesized *via* SeO_2 oxidation of the corresponding substituted 2-methylchromones **19**, **309** and **310**, as described by Jagadeesh *et al.*[75,102] The 2-methylchromones **19**, **309** and **310** were obtained following a method reported by Wittig *et al.*,[48] involving base-catalyzed acylation of the substituted *o*-hydroxyacetophenones **43**, **175** and **305** with ethyl acetate. The resulting intermediates **306** – **308** were then treated (without prior purification) with mixtures of acetic and sulfuric acids, to effect acid-catalyzed cyclization to the required compounds **19**, **309** and **310** (**Scheme 68**).



Scheme 68

Reagents: i) NaOEt, EtOH; ii) dry CH_3CO_2Et; iii) AcOH, conc. H_2SO_4; iv) SeO_2, xylene, heat.

Recrystallization of the crude 2-methylchromones **19**, **309** and **310** from hexane afforded the products in analytical quality in yields ranging from 71 to 88%. The ^{1}H NMR spectrum of compound **19** (Figure 61) reveals a singlet at δ 2.36 ppm corresponding to the methyl protons; the characteristic 3-H signal of the chromone nucleus appears at δ 6.15 ppm and the aromatic region of the spectrum indicates the presence of four aromatic protons. The ^{13}C NMR spectrum (Figure 62) shows the expected 10 carbon signals, with the methyl carbon resonating at δ 20.5 ppm.

The pure chromone-2-carbaldehydes **26**, **311** and **312** were obtained by flash chromatography on silica gel in yields ranging from 35 to 40%. The ^{1}H NMR spectrum of compound **26** (Figure 63) reveals a singlet at δ 6.90 ppm, corresponding to the characteristic 3-H nucleus of the chromone ring, and a downfield singlet at δ 9.79 ppm, corresponding to the 2-formyl proton. The ^{13}C NMR spectrum (Figure 64) shows the expected 10 carbon signals, with the 2-formyl carbon resonating at δ 185.4 ppm. Unfortunately, time constraints did not permit investigation of the Morita-Baylis-Hillman reactions of these chromone-2-carbaldehydes.

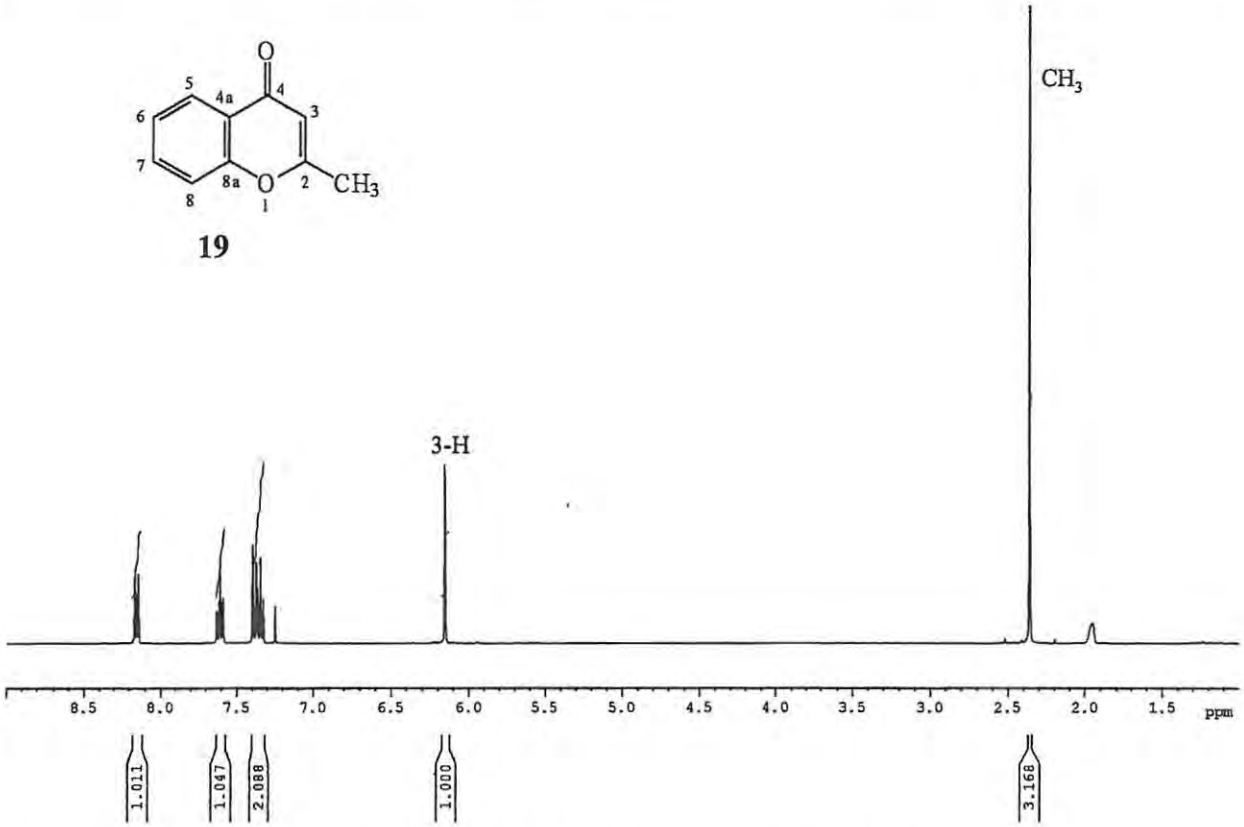


Figure 61. 400 MHz ^{1}H NMR spectrum of 2-methylchromone **19** in $CDCl_3$.

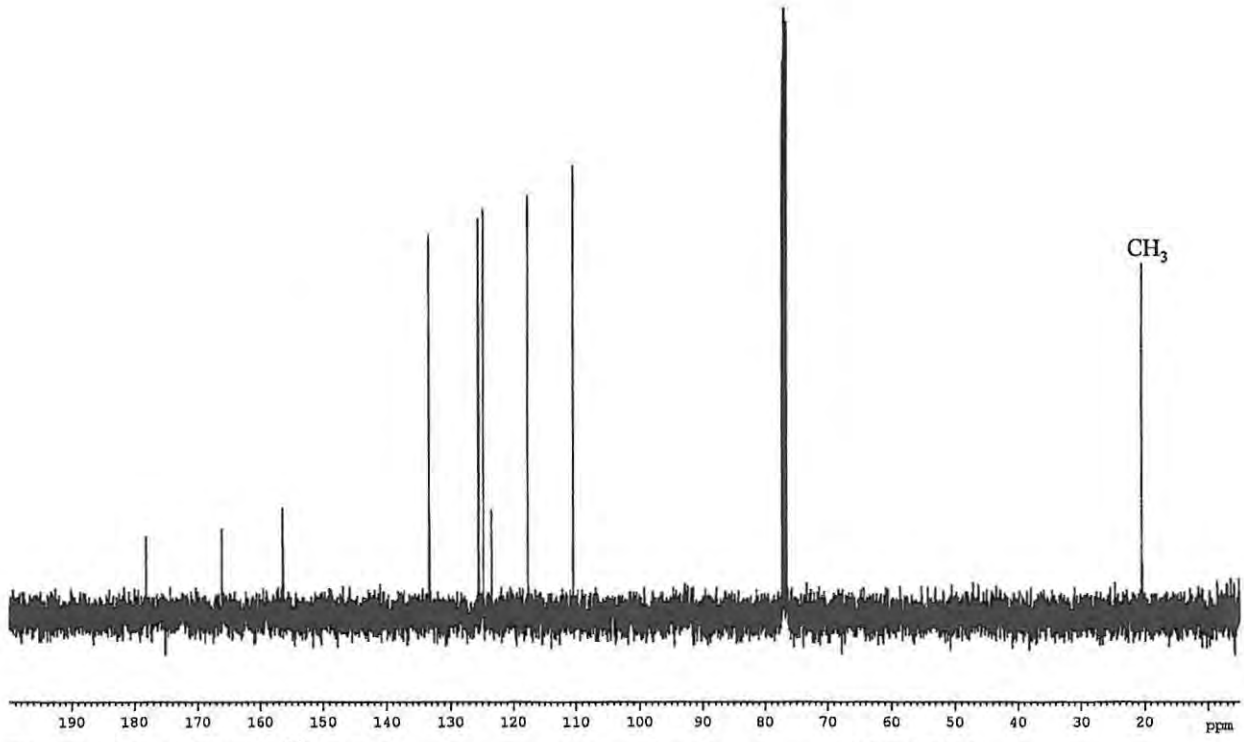


Figure 62. 100 MHz ^{13}C NMR spectrum of 2-methylchromone **19** in $CDCl_3$.

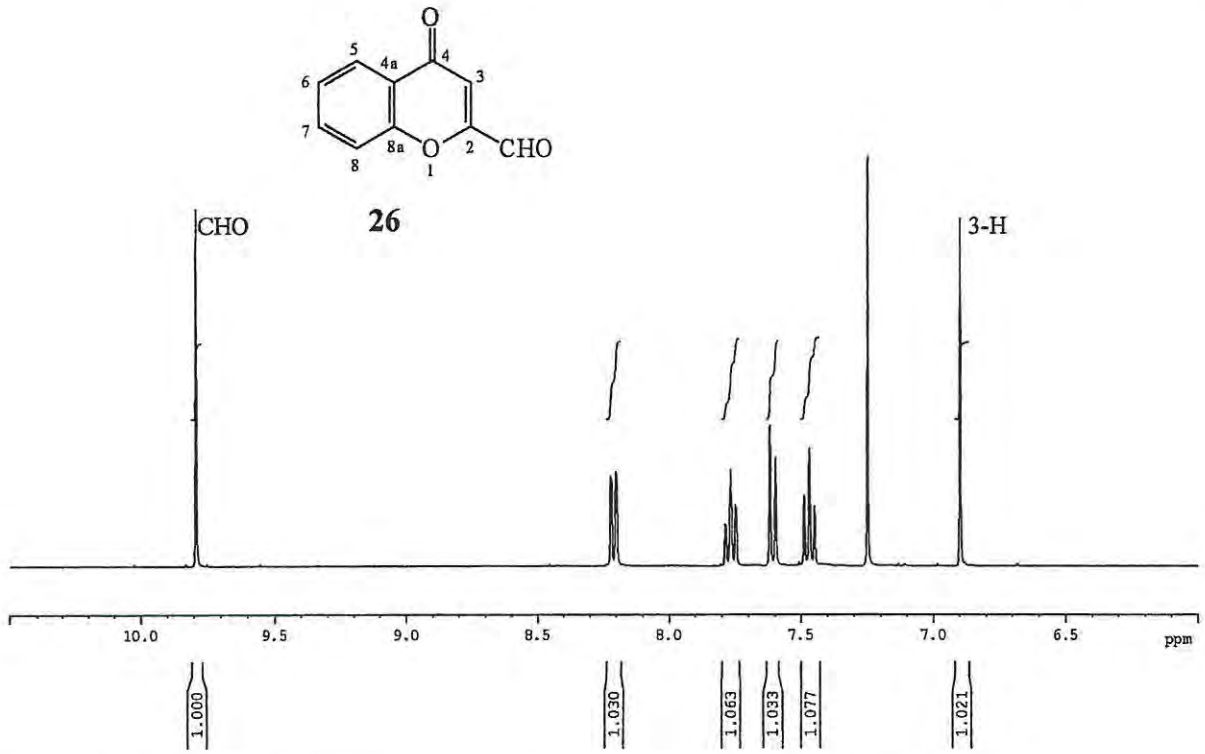


Figure 63. 400 MHz ^{1}H NMR spectrum of chromone-2-carbaldehyde **26** in $CDCl_3$.

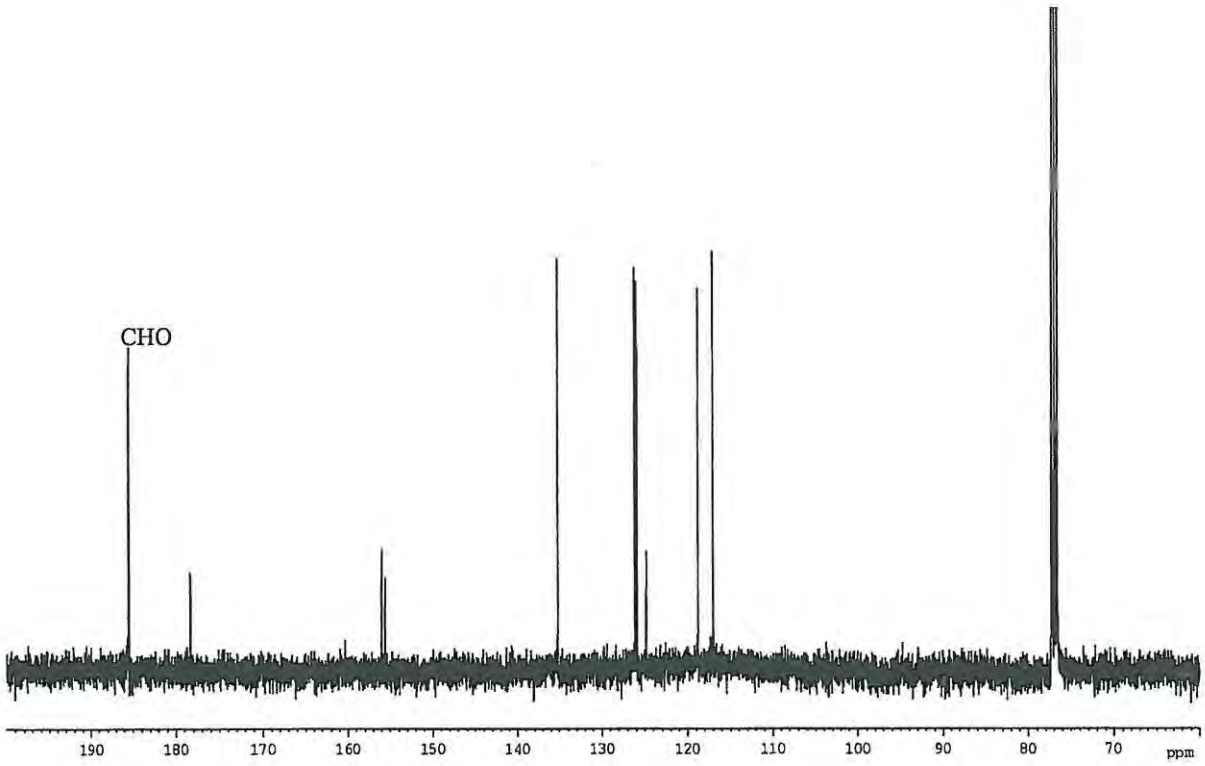


Figure 64. 100 MHz ^{13}C NMR spectrum of chromone-2-carbaldehyde **26** in $CDCl_3$.

2.6 Conclusions

Many chromone derivatives are known to exhibit medicinal properties, and the present study has focussed, largely, on the synthesis of chromones with pharmacological potential.

A series of substituted 2-(*N,N*-dimethylamino)chromones has been successfully synthesized *via* the corresponding boron difluoride complex intermediates for use as substrates in a pK_a study. The pK_a values, which were determined by potentiometric analysis and which lie within a relatively narrow range (1.92 – 2.52), provide insight into the effect of the various substituents on the basicity of the chromone carbonyl oxygen and confirm the general expectation that basicity should be increased by electron-releasing substituents and decreased by electron-withdrawing substituents. These results, which have been shown to be consistent with molecular orbital calculations at the semi empirical and *ab initio* levels, have already been published.[144]

The synthesis of the naturally-occurring chromone derivative, "granulosin" [7,8-(methylenedioxy)-2-propylchromone], and a number of its C-2 side-chain analogues has been successfully achieved by base-catalyzed condensation of 2'-hydroxy-3',4'-(methylenedioxy)-acetophenone with a range of ethyl carboxylate esters. The 2-propyl derivative (granulosin) and its 2-ethyl and 2-isopropyl analogues have been shown to exhibit significant cytotoxic activity against the brine shrimp, *Artemia salina*, while the 2-ethyl and 2-benzyl derivatives were found to possess 100% anti-pesticidic activity on Beet army worms (BAW). This synthetic methodology was extended to the synthesis of another, naturally-occurring and pharmacologically active chromone, 5-hydroxy-2-isopropyl-7-methoxychromone, and to several of its C-2 side chain analogues. This involved the preparation of the dimethoxy precursors, followed by selective C-5 demethylation. The 2-ethyl, 2-propyl and 2-isopropyl derivatives showed significant cytotoxic activity, and it was observed that demethylation to expose the 5-hydroxyl group is crucial for such activity. The electron-impact mass fragmentation patterns of both sets of chromone derivatives have been investigated using low-resolution, high-resolution and B/E link-scan MS data, permitting the identification of certain common fragmentation pathways.

A series of novel chromone-containing analogues of the HIV-1 protease inhibitor, ritonavir, have been synthesized by treating a specially prepared, hydroxyethylene dipeptide isostere[163] with a series of chromone-2-carboxylic acid derivatives using the coupling agent, *N*-ethyl-*N'*-(dimethylaminopropyl)carbodiimide hydrochloride (EDC), in the presence 1-hydroxybenzotriazole hydrate (HOBt). Use of the coupling agent, 1,1'-carbonyldiimidazole (CDI), gave, in addition to the desired *bis*-chromone derivatives, unexpected novel *tris*-chromone derivatives. An interactive docking procedure was used to explore the docking of the established HIV-1 protease inhibitor, ritonavir, and the novel chromone-containing analogues into the active site of the enzyme. The modelling results clearly demonstrate the ability of the novel, chromone-containing ritonavir analogues to form a number of significant hydrogen-bonding interactions with the HIV-1 enzyme binding site.

In a continuation of earlier work,[176] a series of substituted chromone-3-carbaldehydes were successfully synthesized using Vilsmeier-Haack methodology. The application of these compounds in Morita-Baylis-Hillman reactions has been investigated using 3-hydroxyquinuclidine (3-HQ) as catalyst and acrylonitrile and methyl acrylate as the activated alkenes, the intention being to optimize the reaction condition to establish a preparative scale synthesis of the Morita-Baylis-Hillman products and their "dimeric" derivatives. With acrylonitrile, the expected Morita-Baylis-Hillman products were finally obtained in yields ranging from 53 to 67%, and with methyl acrylate, the expected Morita-Baylis-Hillman products were obtained in yields ranging from 50 to 79%. It was also shown that, when the Morita-Baylis-Hillman products resulting from reaction with methyl acrylate were heated at 80 °C for 3 h, the corresponding chromone dimers were produced in yields ranging from 49 to 68%.

While all of the objectives of this investigation have been addressed, opportunities for further development have become apparent, and future research is expected to focus on the following areas.

i) Biological evaluation of the HIV-1 protease inhibitory and antiviral activities of the novel chromone-containing ritonavir analogues.

ii) Investigation of the reactions of substituted chromone-2-carbaldehydes under Morita-Baylis-Hillman conditions.

iii) Optimization of the reaction conditions for the synthesis of the *bis*-chromone-acrylonitrile products.

iv) Functional elaboration and investigation of the pharmacological potential of the chromone "dimers".

3. EXPERIMENTAL

3.1 General

All melting points were determined using a Kofler hot-stage apparatus, and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX400 or AVANCE 400 MHz spectrometers at 303, and were calibrated using the solvent signals. The coupling constants, where specified, are given in hertz (Hz). Where convenient, the atom numbering used in quoting NMR data follows the systematic nomenclature. IR spectra were recorded on a Perkin Elmer Spectrum 2000 FT-IR spectrometer. Low-resolution mass spectra were obtained on a Finnegan-Mat GCQ mass spectrometer, and high-resolution mass spectra were recorded on a VG70-SEQ double-focusing magnetic sector mass spectrometer (Cape Technikon Mass Spectrometry Unit).

Flash chromatography was carried out using Merck silica gel 60 [particle size 0.040 – 0.063 mm (230 – 400 mesh)] and preparative layer chromatography was achieved using Merck silica gel 60 PF_{254}. Chromatotron plates, when necessary, were prepared using silica gel 60 PF_{254} containing $CaSO_4$. Routine thin layer chromatography (TLC) was carried out on pre-coated Merck silica gel F_{254} plates, visualization being achieved by exposure to iodine or inspection under UV light (254 nm).

All dry solvents were prepared using the procedures prescribed by Perrin and Armarego.[177] Diethyl ether and THF were pre-dried over CaH_2 and then distilled from Na wire in the presence of benzophenone under nitrogen. Ethanol and methanol were dried by reaction with Mg turnings and iodine and then distilled from the resulting magnesium alkoxide under nitrogen. Chloroform and 1,2-dichloroethane were distilled from $CaCl_2$ under nitrogen, while *N,N*-dimethylformamide (DMF) was distilled from 3 Å molecular sieves under reduced pressure.

Molecular modelling was performed on a Silicon Graphics O^2 work station using the MSI CERIUS^2 version 4.5 modelling platform at 300K using the Drieding force field. The Ligand Fit module supplied by Accelrys Inc. was used to explore receptor docking interactions.

3.2 Synthesis and pK_a analysis of 2-(*N,N*-dimethylamino)chromones

3.2.1 Synthesis of substituted 2-hydroxyacetophenones

2-Fluorophenyl acetate **168**[137,138,178]

Ac_2O (15 ml, 16.0 g, 0.16 mol) was added dropwise to a stirred solution of 2-fluorophenol **166** (9.0 ml, 11.0 g, 0.10 mol) and NaOH (6.4 g, 0.16 mol) in H_2O (*ca.* 100 ml) maintained at *ca.* 0 °C in an ice-salt bath. After stirring for 2 h at 0 °C, the resulting mixture was extracted with EtOAc (3 x 50 ml); the combined extracts were washed sequentially with 5% aq. $NaHCO_3$ (2 x 50 ml) and saturated aq. NaCl (50 ml), and then dried over anhydrous $MgSO_4$. The solvent was evaporated *in vacuo* to afford a yellow oil, which was distilled to give 2-fluorophenyl acetate **168** as a colourless oil(12.5 g, 91%), b.p. 66-68 °C/10 mmHg (lit.,[178] 76.5-77 °C/11 mmHg); ν_{max} (liquid film)/cm^{-1} 1760 (CO); δ_H (400 MHz; $CDCl_3$) 2.32 (3H, s, CH_3) and 7.11-7.20 (4H, m, Ar-H).

4-Fluorophenyl acetate **169**[137,179]

The experimental procedure employed for the synthesis of 2-fluorophenyl acetate **168** was followed, using Ac_2O (7.0 ml, 7.4 g, 72 mmol), 4-fluorophenol **167** (5.0 g, 45 mmol) and NaOH (3.0 g, 72 mmol) in H_2O (*ca.* 46 ml). Work-up afforded an oil, which was distilled to give 4-fluorophenyl acetate **169** as a colourless oil (4.9 g, 71%), b.p. 55-58 °C/1.0 mmHg (lit.,[179] 85-87 °C/16 mmHg); ν_{max} (liquid film)/cm^{-1} 1755 (CO); δ_H (400 MHz; $CDCl_3$) 2.25 (3H, s, CH_3) and 7.04-7.48 (4H, m, Ar-H).

2',3'-Dimethoxyacetophenone **172**[140]

To a stirred solution of 2,3-dimethoxybenzonitrile **171** (5.0 g, 31 mmol) in dry Et_2O (25 ml) was added methylmagnesium bromide (3.0**M** solution in diethyl ether; 10.3 ml, 31 mmol), and the mixture was stirred at room temperature for 12 h under N_2. The resulting mixture was boiled under reflux for 1 h and then quenched with 10% acetic acid. The ethereal layer was washed with saturated aq. Na_2CO_3 solution (50 ml), dried over anhydrous $MgSO_4$ and the solvent evaporated *in vacuo* to afford 2',3'-dimethoxyacetophenone **172** as a brick-red oil (4.8 g, 80%), (Found: M^+, 180.07973. $C_{10}H_{12}O_3$ requires *M*, 180.07864); ν_{max} (liquid

film)/cm^{-1} 1640 (CO); δ_H (400 MHz; $CDCl_3$) 2.78 (3H, s, CH_3), 4.04 and 4.05 (6H, 2 x s, 2 x OCH_3) and 7.20 - 7.45 (3H, m, Ar-H); *m/z* 180 (M^+, 62%) and 151 (100).

8-Nitrochromone **173**[141]

Chromone **1** (5.0 g, 34 mmol) was dissolved in conc. H_2SO_4 (30 ml), and the resulting reddish-orange solution cooled in ice-water. A solution of fuming HNO_3 (1.4 ml) in conc. H_2SO_4 (5.0 ml) was added dropwise to the stirred solution over a period of 10 min. The resulting mixture was poured into ice-water (50 ml) and allowed to warm to room temperature. The precipitated solid was collected by filtration and washed well with water to give the crude product (4.2 g). Repeated recrystallization from benzene afforded 8-nitrochromone **173** as a yellow crystalline solid (2.44 g, 38%), m.p. 172-174 °C (from benzene) (lit.,[141] 173-175 °C), (Found: M^+, 191.02243. $C_9H_5NO_4$ requires *M*, 191.02186); ν_{max} (KBr)/cm^{-1} 1640 (CO); δ_H (400 MHz; $CDCl_3$) 6.42 (1H, d, *J*= 6.1 Hz, 3-H), 7.62 (1H, d, *J*= 9.2 Hz, 7-H), 7.91 (1H, d, *J*= 6.1 Hz, 2-H), 8.50 (1H, dd, *J*= 2.8 and 9.4 Hz, 6-H) and 9.05 (1H, d, *J*= 2.8 Hz, 5-H); *m/z* 191 (M^+, 100%).

3'-Fluoro-2'-hydroxyacetophenone **174**[137,178]

A stirred mixture of 2-fluorophenyl acetate **168** (4.6 g, 30 mmol) and anhydrous $AlCl_3$ (16 g, 0.12 mol) under N_2 was heated in an oil bath at *ca.* 190 °C for 3 h. The reaction mixture was cooled, the complex was decomposed with 2**M**-HCl (80 ml) and the resulting mixture steam distilled until the distillate was no longer milky. The distillate was extracted with diethyl ether (3 x 50 ml), and the ethereal solution was then extracted with 0.5**M**-KOH (3 x 50 ml). The aqueous extract was washed with diethyl ether (3 x 50 ml), acidified with 2.5**M**-H_2SO_4 and then re-extracted into ether (3 x 50 ml). The combined organic extracts were dried over anhydrous $MgSO_4$, and the solvent was evaporated *in vacuo* to afford a yellow oil, which crystallized to give 3'-fluoro-2'-hydroxyacetophenone **174** as a colourless crystalline solid (0.50 g, 12%), m.p. 71-73 °C (from hexane) (lit.,[178] 72-73 °C), (Found: M^+, 154.04429. $C_8H_7FO_2$ requires *M*, 154.04301); ν_{max} (KBr)/cm^{-1} 3000-2800 (br, OH) and 1646 (CO); δ_H (400 MHz; $CDCl_3$) 2.64 (3H, s, CH_3), 6.85, 726 and 7.52 (3H, 3 x m, Ar-H) and 12.26 (1H, s, OH); *m/z* 154 (M^+, 44%) and 139 (100).

5'-Fluoro-2'-hydroxyacetophenone **175**[137,178,180]

The experimental procedure employed for the synthesis of 3'-fluoro-2'-hydroxyacetophenone **174** was followed, using 4-fluorophenyl acetate **169** (4.5 g, 30 mmol) and anhydrous $AlCl_3$ (16 g, 0.12 mol). Work-up afforded a yellow oil, which crystallized to give 5'-fluoro-2'-hydroxyacetophenone **175** as a colourless crystalline solid (3.0 g, 70%), m.p. 46-48 °C (from hexane) (lit.,[180] 56 °C), (Found: M^+, 154.04389. $C_8H_7FO_2$ requires *M*, 154.04301); ν_{max} (KBr)/cm^{-1} 3200-3000 (br, OH) and 1648 (CO); δ_H (400 MHz; $CDCl_3$) 2.60 (3H, s, CH_3), 6.95, 7.22 and 7.40 (3H, 3 x m, Ar-H) and 11.90 (1H, s, OH); *m/z* 154 (M^+, 49%) and 139 (100).

2'-Hydroxy-6'-methoxyacetophenone **176**[138]

A mixture of 2',6'-dihydroxyacetophenone **170** (4.6 g, 30 mmol), K_2CO_3 (4.2 g, 30 mmol) and methyl iodide 4.3 g (2.0 ml, 30 mmol) in acetone (75 ml) was boiled under reflux for 22 h. The resulting mixture was filtered and the filtrate concentrated *in vacuo* to give a residue which crystallized to afford 2'-hydroxy-6'-methoxyacetophenone **176** as a yellow crystalline solid (4.0 g, 80%), m.p. 56-57 °C (from hexane) (lit.,[139] 57-58 °C), (Found: M^+, 166.06266. $C_9H_{10}O_3$ requires *M*, 166.06299); ν_{max} (KBr)/cm^{-1} 3500-2800 (br, OH) and 1620 (CO); δ_H (400 MHz; $CDCl_3$) 2.65 (3H, s, CH_3), 3.87 (3H, s, OCH_3), 6.36 (1H, d, *J*= 8.3 Hz, 3-H), 6.54 (1H, d, *J*= 8.4 Hz, 5-H), 7.32 (1H, t, *J*= 8.4 Hz, 4-H) and 13.20 (1H, s, OH); *m/z* 166 (M^+, 45%) and 151 (100).

2'-Hydroxy-3'-methoxyacetophenone **177**[50]

To a stirred suspension of anhydrous $AlCl_3$ (27 g, 0.20 mol) in dry Et_2O (90 ml) under N_2 was added 2',3'-dimethoxyacetophenone **172** (4.0 g, 22 mmol), and the resulting mixture was boiled under reflux for 12 h. The cooled reaction mixture was treated with ice-cold 2**M**-HCl (100 ml), and the resulting mixture steam distilled until the distillate was no longer milky. The distillate was extracted with Et_2O (3 x 50 ml), and the combined ethereal extracts were dried over anhydrous $MgSO_4$. The solvent was evaporated *in vacuo* to afford an oil, which crystallized to give 2'-hydroxy-3'-methoxyacetophenone **177** as a pale yellow solid (1.8 g, 52%), m.p. 50-52 °C (from hexane) (lit.,[50] 54 °C), (Found: M^+, 166.06330. $C_9H_{10}O_3$ requires *M*, 166.06299); ν_{max} (KBr)/cm^{-1} 3100-2800 (br, OH) and 1640 (CO); δ_H (400 MHz; $CDCl_3$)

2.65 (3H, s, CH_3), 3.92 (3H, s, OCH_3), 6.83 (1H, t, *J*= 7.8 Hz, 5-H), 7.04 (1H, d, *J*= 7.2 Hz, 4-H), 7.32 (1H, d, *J*= 7.5 Hz, 6-H) and 12.55 (1H, s, OH); *m/z* 166 (M^+, 62%) and 151 (100).

2'-Hydroxy-3'-nitroacetophenone **178**[141,181]

A mixture of 8-nitrochromone **173** (2.0 g, 10 mmol) and 10% aq. KOH (20 ml) was boiled under reflux for 1 h. The resulting green-yellow solution was cooled to room temperature and then acidified with 2.5**M**-H_2SO_4. The resulting mixture was then extracted with EtOAc (3 x 75 ml), the combined extracts were dried over anhydrous $MgSO_4$ and the solvent was evaporated *in vacuo* to afford 2'-hydroxy-3'-nitroacetophenone **178** as a yellow solid (1.2 g, 63%), m.p. 96-98 °C (from hexane) (lit.,[141] 103-104 °C), (Found: M^+, 181.03666. $C_8H_7NO_4$ requires *M*, 181.03751); ν_{max} (KBr)/cm^{-1} 3500-3200 (br, OH), 1648 (CO) and 1523 (Ar-NO_2); δ_H (400 MHz; $CDCl_3$) 2.74 (3H, s, CH_3), 7.07 (1H, d, *J*= 9.3 Hz, 4-H), 8.70 (1H, d, *J*= 2.7 Hz, 6-H), 8.34 (1H, dd, *J*= 2.8 and 9,2 Hz, 5-H) and 12. 85 (1H, s, OH); *m/z* 181 (M^+, 65%) and 166 (100).

3.2.2 Synthesis of 2-(N,N-dimethylamino)chromones via *phosgeniminium salt intermediates*

3'-Fluoro-2'-hydroxyacetophenone boron difluoride complex **179**[68]

$BF_3.OEt_2$ (0.83 ml, 6.5 mmol) was added to a solution of 3'-fluoro-2'-hydroxyacetophenone **174** (1.0 g, 6.5 mmol) in dry Et_2O (6.5 ml) under dry N_2, and the mixture was stirred at room temperature for 6 h. The resulting mixture was filtered and the solid material washed well with Et_2O to give *3'-fluoro-2'-hydroxyacetophenone boron difluoride complex* **179** as a greenish-yellow solid (0.70 g, 54%), [(Found: M^+, 202.04193. $C_8H_6{}^{11}BF_3O_2$ requires *M*, 202.04129); ν_{max} (KBr)/cm^{-1} 1627 (CO); δ_H (400 MHz; $CDCl_3$) 2.70 (3H, s, CH_3), 6.91, 7.48 and 7.70 (3H, 3 x m, Ar-H); *m/z* 202 (M^+, 36%) and 187 (100)], which was used without further purification.

5'-Fluoro-2'-hydroxyacetophenone boron difluoride complex **180**

The experimental procedure employed for the synthesis of 3'-fluoro-2'-hydroxyacetophenone boron difluoride complex **179** was followed, using 5'-fluoro-2'-hydroxyacetophenone **175** (1.0 g, 6.5 mmol), dry Et_2O (6.5 ml) and $BF_3.OEt_2$ (0.83 ml, 6.5 mmol). Work-up afforded *5'-fluoro-2'-hydroxyacetophenone boron difluoride complex* **180** as an orange solid (0.80 g,

61%), [(Found: M^+, 202.04142. $C_8H_6{}^{11}BF_3O_2$ requires *M*, 202.04129); ν_{max} (KBr)/cm^{-1} 1622 (CO); δ_H (400 MHz; $CDCl_3$) 2.88 (3H, s, CH_3), 7.16, 7.44 and 7.63 (3H, 3 x m, Ar-H); *m/z* 202 (M^+, 41%) and 187 (100)], which was used without further purification.

2'-Hydroxy-6'-methoxyacetophenone boron difluoride complex **181**

The experimental procedure employed for the synthesis of 3'-fluoro-2'-hydroxyacetophenone boron difluoride complex **179** was followed, using 2'-hydroxy-6'-methoxyacetophenone **176** (1.0 g, 6.0 mmol), dry Et_2O (6.0 ml) and $BF_3.OEt_2$ (0.80 ml, 6.0 mmol). Work-up afforded *2'-hydroxy-6'-methoxyacetophenone boron difluoride complex* **181** as a yellow solid (0.97 g, 76%), [(Found: M^+, 214.06144. $C_9H_9{}^{11}BF_2O_3$ requires *M*, 214.06128); ν_{max} (KBr)/cm^{-1} 1617 (CO); δ_H (400 MHz; $CDCl_3$) 2.89 (3H, s, CH_3), 3.98 (3H, s, OCH_3), 6.40 (1H, d, *J*= 8.3 Hz, 3-H), 6.70 (1H, d, *J*= 8.5 Hz, 5-H) and 7.65 (1H, t, *J*= 8.4 Hz, 4-H); *m/z* 214 (M^+, 48%)], which was used without further purification.

2'-Hydroxy-3'-methoxyacetophenone boron difluoride complex **182**

The experimental procedure employed for the synthesis of 3'-fluoro-2'-hydroxyacetophenone boron difluoride complex **179** was followed, using 2'-hydroxy-3'-methoxyacetophenone **177** (1.0 g, 6.0 mmol), dry Et_2O (6.0 ml) and $BF_3.OEt_2$ (0.80 ml, 6.0 mmol). Work-up afforded *2'-hydroxy-3'-methoxyacetophenone boron difluoride complex* **182** as a yellow solid (0.98 g, 77%), [(Found: M^+, 214.06175. $C_9H_9{}^{11}BF_2O_3$ requires *M*, 214.06128); ν_{max} (KBr)/cm^{-1} 1616 (CO); δ_H (400 MHz; $CDCl_3$) 2.87 (3H, s, CH_3), 3.95 (3H, s, OCH_3), 6.96 (1H, t, *J*= 8.2 Hz, 5-H), 7.23 (1H, d, *J*= 8.3 Hz, 4-H) and 7.35 (1H, d, *J*= 8.4 Hz, 6-H); *m/z* 214 (M^+, 63%)], which was used without further purification.

2'-Hydroxy-3'-nitroacetophenone boron difluoride complex **183**

The experimental procedure employed for the synthesis of 3'-fluoro-2'-hydroxyacetophenone boron difluoride complex **179** was followed, using 2'-hydroxy-3'-nitroacetophenone **178** (0.50 g, 2.8 mmol), dry Et_2O (6.0 ml) and $BF_3.OEt_2$ (0.82 ml, 5.6 mmol). Work-up afforded *2'-hydroxy-3'-nitroacetophenone boron difluoride complex* **183** as a brownish-yellow solid (0.24 g, 38%), [ν_{max} (KBr)/cm^{-1} 1648 (CO) and 1523 (Ar-NO_2); δ_H (400 MHz; $CDCl_3$) 2.75 (3H, s, CH_3), 7.30, 8.62 and 8.84 (3H, 3 x m, Ar-H)], which was used without further purification.

*3-Chloro-3-(*N,N*-dimethylamino)-1-(3-fluoro-2-hydroxyphenyl)propenone, boron difluoride complex* **185**[68]

A suspension of 3'-fluoro-2'-hydroxyacetophenone boron difluoride complex **179** (0.40 g, 2.0 mmol) and phosgeniminium chloride **184** (0.35 g, 2.0 mmol) in dry 1,2-dichloroethane (7.5 ml) was heated at 80 °C under N_2 for 2 h. The mixture was cooled at 0 °C, and the resulting solid was filtered off and washed with cold 1,2-dichloroethane (5.0 ml) and Et_2O (5.0 ml) to afford the crude *3-chloro-3-(*N,N*-dimethylamino)-1-(3-fluoro-2-hydroxyphenyl)propenone, boron difluoride complex* **185** as a yellow solid (0.55 g, 95%), [(Found: M^+, 291.04456. $C_{11}H_{10}{}^{11}BClF_3NO_2$ requires *M*, 291.04452); ν_{max} (KBr)/cm^{-1} 1624 (CO); δ_H (400 MHz; DMSO-d_6) 3.54 (6H, s, NMe_2), 6.32 (1H, s, 2-H), 6.95, 7.52 and 7.87 (3H, 3 x m, Ar-H); *m/z* 291 (M^+, 19%) and 256 (100)], which was used without further purification.

*3-Chloro-3-(*N,N*-dimethylamino)-1-(5-fluoro-2-hydroxyphenyl)propenone, boron difluoride complex* **186**

The experimental procedure employed for the synthesis of 3-chloro-3-(*N,N*-dimethylamino)-1-(3-fluoro-2-hydroxyphenyl)propenone, boron difluoride complex **185** was followed, using 5'-fluoro-2'-hydroxyacetophenone boron difluoride complex **180** (0.50 g, 2.4 mmol), phosgeniminium chloride **184** (0.41 g, 2.4 mmol) and dry 1,2-dichloroethane (9.5 ml). Work-up afforded the crude *3-chloro-3-(*N,N*-dimethylamino)-1-(5-fluoro-2-hydroxyphenyl)-propenone, boron difluoride complex* **186** as a yellow solid (0.63 g, 90%), [(Found: M^+, 291.04398. $C_{11}H_{10}{}^{11}BClF_3NO_2$ requires *M*, 291.04452); ν_{max} (KBr)/cm^{-1} 1566 (CO); δ_H (400 MHz; DMSO-d_6) 3.01 (6H, s, NMe_2), 6.47 (1H, s, 2-H), 7.01, 7.38 and 7.57 (3H, 3 x m, Ar-H); *m/z* 291 (M^+, 12%) and 256 (100)], which was used without further purification.

*3-Chloro-3-(*N,N*-dimethylamino)-1-(2-hydroxy-6-methoxyphenyl)propenone, boron difluoride complex* **187**

The experimental procedure employed for the synthesis of 3-chloro-3-(*N,N*-dimethylamino)-1-(3-fluoro-2-hydroxyphenyl)propenone, boron difluoride complex **185** was followed, using 2'-hydroxy-6'-methoxyacetophenone boron difluoride complex **181** (0.50 g, 2.3 mmol), phosgeniminium chloride **184** (0.40 g, 2.3 mmol) and dry 1,2-dichloroethane (7.5 ml). Work-up afforded the crude *3-chloro-3-(*N,N*-dimethylamino)-1-(2-hydroxy-6-methoxyphenyl)-propenone, boron difluoride complex* **187** as a yellow solid (0.69 g, 98%), [(Found: M^+, 303.06419. $C_{12}H_{13}{}^{11}BClF_2NO_3$ requires *M*, 303.06451); ν_{max} (KBr)/cm^{-1} 1604 (CO); δ_H (400 MHz; $CDCl_3$) 3.45 (6H, s, NMe_2), 3.90 (3H, s, OCH_3), 6.39 (1H, d, *J*= 8.4Hz, 3'-H), 6.67

(1H, d, *J*= 8.5 Hz, 5'-H), 6.76 (1H, s, 2-H) and 7.37 (1H, t, *J*= 8.4 Hz, 4'-H); *m/z* 303 (M^+, 16%) and 268 (100)], which was used without further purification.

*3-Chloro-3-(*N,N-*dimethylamino)-1-(2-hydroxy-3-methoxyphenyl)propenone, boron difluoride complex* **188**

The experimental procedure employed for the synthesis of 3-chloro-3-(*N,N*-dimethylamino)-1-(3-fluoro-2-hydroxyphenyl)propenone, boron difluoride complex **185** was followed, using 2'-hydroxy-3'-methoxyacetophenone boron difluoride complex **182** (0.60 g, 2.8 mmol), phosgeniminium chloride **184** (0.50 g, 2.8 mmol) and dry 1,2-dichloroethane (10 ml). Work-up afforded the crude *3-chloro-3-(*N,N-*dimethylamino)-1-(2-hydroxy-3-methoxyphenyl)-propenone, boron difluoride complex* **188** as a yellow solid (0.82 g, 97%), [(Found: M^+, 303.06499. $C_{12}H_{13}{}^{11}BClF_2NO_3$ requires *M*, 303.06451); ν_{max} (KBr)/cm^{-1} 1609 (CO); δ_H (400 MHz; DMSO-d_6) 3.51 (6H, s, NMe_2), 3.78 (3H, s, OCH_3), 6.22 (1H, s, 2-H), 6.86 (1H, t, *J*= 8.2 Hz, 5'-H), 7.16 (1H, d, *J*= 7.6 Hz, 4'-H) and 7.55 (1H, d, *J*= 8.2 Hz, 6'-H); *m/z* 303 (M^+, 15%) and 268 (100)], which was used without further purification.

*3-Chloro-3-(*N,N-*dimethylamino)-1-(2-hydroxy-3-nitrophenyl)propenone, boron diflluoride complex* **189**

The experimental procedure employed for the synthesis of 3-chloro-3-(*N,N*-dimethylamino)-1-(3-fluoro-2-hydroxyphenyl)propenone, boron difluoride complex **185** was followed, using 2'-hydroxy-3'-nitroacetophenone boron difluoride complex **183** (0.30 g, 1.3 mmol), phosgeniminium chloride **184** (0.50 g, 2.6 mmol) and dry 1,2-dichloroethane (5.0 ml). Work-up afforded the crude *3-chloro-3-(*N,N-*dimethylamino)-1-(2-hydroxy-3-nitrophenyl)-propenone, boron difluoride complex* **189** as a yellow solid (0.35 g, 85%), [(Found: M^+, 318.03957. $C_{11}H_{10}{}^{11}BClF_2N_2O_4$ requires *M*, 319.03902); ν_{max} (KBr)/cm^{-1} 1650 (CO); δ_H (400 MHz; DMSO-d_6) 3.02 (6H, s, NMe_2), 6.50 (1H, s, 2-H) and 7.18-8.60 (3H, 3 x m, Ar-H); *m/z* 318 (M^+, 13%) and 283 (100)], which was used without further purification.

*2-(*N,N-*Dimethylamino)-8-fluorochromone* **191**[68]

A solution of 3-chloro-3-(*N,N*-dimethylamino)-1-(3-fluoro-2-hydroxyphenyl)propenone, boron difluoride complex **185** (0.34 g, 1.0 mmol) in MeOH (20 ml) was stirred at 50 °C for 30 min. The solvent was evaporated *in vacuo*, and the residue dissolved in saturated aqueous $NaHCO_3$. The resulting mixture was extracted with CH_2Cl_2 (3 x 75 ml), and the combined

extracts were washed with brine (1 x 75 ml) and dried over anhydrous $MgSO_4$. The solvent was evaporated *in vacuo* to give *2-(*N,N-*dimethylamino)-8-fluorochromone* **191** as a white solid (0.18 g, 87%), m.p. 162-163 °C (from EtOAc), (Found: M^+, 207.06972. $C_{11}H_{10}FNO_2$ requires *M*, 207.06956); ν_{max} (KBr)/cm^{-1} 1622 (CO); δ_H (400 MHz; $CDCl_3$) 3.11 (6H, s, NMe_2), 5.40 (1H, s, 3-H), 7.15-7.30 (2H, m, 6-H and 7-H) and 7.86 (1H, d, *J*= 7.8 Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 37.5 (NMe_2), 86.3 (C-3), 118.0 and 124.1 (C-6 and C-7), 120.5 (C-5), 126.0 (C-4a), 149.1 (C-8), 151.7 (C-8a), 162.5 (C-2) and 175.5 (C=O); *m/z* 207 (M^+, 100%).

*2-(*N,N-*Dimethylamino)-6-fluorochromone* **192**

The experimental procedure employed for the synthesis of 2-(*N,N*-dimethylamino)-8-fluorochromone **191** was followed, using 3-chloro-3-(*N,N*-dimethylamino)-1-(5-fluoro-2-hydroxyphenyl)propenone, boron difluoride complex **186** (0.50 g, 1.7 mmol) and MeOH (34 ml). Work-up afforded *2-(*N,N-*dimethylamino)-6-fluorochromone* **192** as a white solid (0.21 g, 60%), m.p. 176-178 °C [from hexane-EtOAc (1:1)], (Found: M^+, 207.07103. $C_{11}H_{10}FNO_2$ requires *M*, 207.06956); ν_{max} (KBr)/cm^{-1} 1622 (CO); δ_H (400 MHz; $CDCl_3$) 3.12 (6H, s, NMe_2), 5.41 (1H, s, 3-H), 7.18-7.28 and 7.78 (3H, 3 x m, Ar-H); δ_C (100 MHz; $CDCl_3$) 37.5 (NMe_2), 85.9 (C-3), 111.0 (C-5), 117.9 and 119.5 (C-7 and C-8), 124.6 (C-4a), 149.7 (C-6), 158.1 (C-8a), 163.2 (C-2) and 175.5 (C=O); *m/z* 207 (M^+, 100%).

*2-(*N,N-*Dimethylamino)-5-methoxychromone* **193**

The experimental procedure employed for the synthesis of 2-(*N,N*-dimethylamino)-8-fluorochromone **191** was followed, using 3-chloro-3-(*N,N*-dimethylamino)-1-(2-hydroxy-6-methoxyphenyl)propenone, boron difluoride complex **187** (0.50 g, 1.6 mmol) and MeOH (32 ml). Work-up afforded *2-(*N,N-*dimethylamino)-5-methoxychromone* **193** as a white solid (0.22 g, 63%), m.p. 166-168 °C (from EtOAc), (Found: M^+, 219.08927. $C_{12}H_{13}NO_3$ requires *M*, 219.08954); ν_{max} (KBr)/cm^{-1} 1620 (CO); δ_H (400 MHz; $CDCl_3$) 3.01 (6H, s, NMe_2), 3.92 (3H, s, OCH_3), 5.28 (1H, s, 3-H), 6.73 (1H, d, *J*= 8.3 Hz, 8-H), 6.86 (1H, d, *J*= 8.3 Hz, 6-H) and 7.37 (1H, t, *J*= 8.3 Hz, 7-H); δ_C (100 MHz; $CDCl_3$) 37.2 (NMe_2), 56.4 (OCH_3), 87.4 (C-3), 106.7 (C-8), 108.9 (C-6), 113.0 (C-4a), 131.8 (C-7), 155.9 (C-5), 159.5 (C-8a), 161.6 (C-2) and 177.2 (C=O); *m/z* 219 (M^+, 100%).

*2-(*N,N-*Dimethylamino)-8-methoxychromone* **194**

The experimental procedure employed for the synthesis of 2-(*N,N*-dimethylamino)-8-fluorochromone **191** was followed, using 3-chloro-3-(*N,N*-dimethylamino)-1-(2-hydroxy-3-methoxyphenyl)propenone, boron difluoride complex **188** (0.60 g, 2.0 mmol) and MeOH (40 ml). Work-up afforded 2-(*N,N*-dimethylamino)-8-methoxychromone **194** as a white solid (0.32 g, 72%), m.p. 147-148 °C (from EtOAc) (lit.,[178] 147-148 °C), (Found: M^+, 219.09041. $C_{12}H_{13}NO_3$ requires *M*, 219.08954); ν_{max} (KBr)/cm^{-1} 1625 (CO); δ_H (400 MHz; $CDCl_3$) 3.10 (6H, s, NMe_2), 3.93 (3H, s, OCH_3), 5.40 (1H, s, 3-H), 7.05 (1H, d, *J*= 6.8 Hz, 7-H), 7.20 (1H, t, *J*= 8.0 Hz, 6-H) and 7.71 (1H, d, *J*= 8.0 Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 37.3 (NMe_2), 56.3 (OCH_3), 86.1 (C-3), 113.5 (C-7), 116.7 (C-5), 123.9 (C-6), 124.1 (C-4a), 143.8 (C-8), 147.8 (C-8a), 162.8 (C-2) and 176.6 (C=O); *m/z* 219 (M^+, 100%).

*2-(*N,N-*Dimethylamino)-8-nitrochromone* **195**

The experimental procedure employed for the synthesis of 2-(*N,N*-dimethylamino)-8-fluorochromone **191** was followed, using 3-chloro-3-(*N,N*-dimethylamino)-1-(2-hydroxy-3-nitrophenyl)propenone, boron difluoride complex **189** (0.30 g, 0.94 mmol) and MeOH (20 ml). Work-up afforded *2-(*N,N-*dimethylamino)-8-nitrochromone* **195** as a yellow solid (0.183 g, 83%); m.p. 220-222 °C (from EtOAc), (Found: M^+, 234.06462. $C_{11}H_{10}N_2O_4$ requires 234.06406); ν_{max} (KBr)/cm^{-1} 1639 (CO) and 1547 (Ar-NO_2); δ_H (400 MHz; $CDCl_3$) 3.15 (6H, s, NMe_2), 5.46 (1H, s, 3-H), 7.43 (1H, d, *J*= 9.1 Hz, 7-H), 8.37 (1H, dd, *J*= 2.8 and 9.1 Hz, 6-H) and 9.00 (1H, d, *J*= 2.8 Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 37.8 (NMe_2), 86.3 (C-3), 117.7 (C-7), 122.2 (C-5), 123.5 (C-4a), 126.6 (C-6), 144.7 (C-8), 156.6 (C-8a), 162.9 (C-2) and 174.2 (C=O); *m/z* 234 (M^+, 100%).

3.2.3 Procedure for the determination of pK_a values for the 2-(N,N-dimethylamino)chromones 190 – 195[142]

Aqueous-ethanolic solutions of chromone **1** and the 2-(*N,N*-dimethylamino)chromones **190 – 195** were prepared by dissolution of the appropriate quantity in distilled absolute EtOH (10 ml) and dilution of the resulting solution with H_2O (boiled prior to use; 9 ml) to afford, at half-neutralisation point, aliquots (20 ml) having a concentration of 0.01 mol.dm^{-3}. The stirred 2-(*N,N*-dimethylamino)chromone solutions were titrated against hydrochloric acid (0.1063 mol.dm^{-3}) at 25 ± 0.1 °C (stirring was stopped when taking pH readings). The titrant was added in 0.20 ml portions and was standardized against freshly recrystallized sodium tetraborate decahydrate. The pH was measured after each addition using Mettler Toledo MP225 pH meters fitted with a Beckman Type 39849 epoxy body calomel electrode. The pH meter was calibrated at pH 4.008 (using a potassium hydrogen phthalate buffer) and at pH 1.679 (using a potassium tetroxalate buffer). All titrations were done in a water bath to maintain a constant temperature and were replicated to ensure reproducibility. The pK_a values were determined following the method described by Albert and Serjeant.[142] Tables 15 – 26 summarize the data used to calculate the pK_a value and calculated errors for each compound; a detailed example of the method is described in Section 2.1.2 (pp. 51-54).

Table 15: Concentration of the protonated and non-protonated chromone **1** as a function of pH.

Conc. HCl	Vol. Titrant (mL)	Mass (g)	Molar mass (g/mol)	No. of moles	pH	BH^{+} [a]	B [b]
0.1063	0.00	0.0292	146	0.0002	4.65	0.00	0.000200
0.1063	0.20	0.0292	146	0.0002	3.16	2.13E-05	0.000179
0.1063	0.40	0.0292	146	0.0002	2.85	4.25E-05	0.000157
0.1063	0.60	0.0292	146	0.0002	2.67	6.38E-05	0.000136
0.1063	0.80	0.0292	146	0.0002	2.55	8.50E-05	0.000115
0.1063	1.00	0.0292	146	0.0002	2.45	0.000106	9.37E-05
0.1063	1.20	0.0292	146	0.0002	2.37	0.000128	7.24E-05
0.1063	1.40	0.0292	146	0.0002	2.30	0.000149	5.12E-05
0.1063	1.60	0.0292	146	0.0002	2.24	0.000170	2.99E-05
0.1063	1.80	0.0292	146	0.0002	2.18	0.000191	8.66E-06
0.1063	2.00	0.0292	146	0.0002	2.15	0.000213	-1.26E-05[c]

[a] Moles of conjugate acid; [b] Moles of residual free chromone; [c] The negative concentration reflects the excess concentration of titrant.

Table 16: Calculation of pK_a values for chromone **1**, corrected for hydrogen-ion activity.

(i) Total vol. (mL)	(ii) $[H^+]$	(iii) H^+	(iv) $\frac{BH^+ - H^+}{B + H^+}$	(v) log(col.iv)	(vi) pK_a	(vii) 10^{-pKa}
19.00	2.24E-05	4.2500E-07	-0.002122			
19.20	0.000692	1.3300E-05	0.041541	-1.381522	1.778478	0.016654
19.40	0.001413	2.7400E-05	0.081764	-1.087438	1.762562	0.016159
19.60	0.002138	4.1900E-05	0.122813	-0.910756	1.759244	0.016214
19.80	0.002818	5.5800E-05	0.171207	-0.766478	1.783522	0.015303
20.00	0.003548	7.1000E-05	0.214604	-0.668362	1.781638	0.015299
20.20	0.004266	8.6200E-05	0.260962	-0.583423	1.786577	0.016346
20.40	0.005012	1.0200E-04	0.303592	-0.517709	1.782291	0.016509
20.60	0.005754	1.1900E-04	0.347159	-0.459472	1.780528	0.020200
20.80	0.006607	1.3700E-04	0.369073	-0.432888	1.747112	0.019901
21.00	0.007079	1.4900E-04	0.469847	-0.328044	1.821956	0.015068

$$pK_a = 1.77 \pm 0.06$$

Table 17: Concentration of the protonated and non-protonated 2-(*N,N*-dimethylamino)-8-fluorochromone **191** as a function of pH.

Conc. HCl	Vol. Titrant (mL)	Mass (g)	Molar mass (g/mol)	No. of moles	pH	BH^{+} [a]	B [b]
0.1063	0.00	0.0413	207	0.0002	4.55	0.00	0.000200
0.1063	0.20	0.0413	207	0.0002	3.24	2.13E-05	0.000178
0.1063	0.40	0.0413	207	0.0002	2.93	4.25E-05	0.000157
0.1063	0.60	0.0413	207	0.0002	2.75	6.38E-05	0.000136
0.1063	0.80	0.0413	207	0.0002	2.62	8.50E-05	0.000114
0.1063	1.00	0.0413	207	0.0002	2.52	0.000106	9.32E-05
0.1063	1.20	0.0413	207	0.0002	2.43	0.000128	7.20E-05
0.1063	1.40	0.0413	207	0.0002	2.36	0.000149	5.07E-05
0.1063	1.60	0.0413	207	0.0002	2.29	0.000170	2.94E-05
0.1063	1.80	0.0413	207	0.0002	2.24	0.000191	8.18E-06
0.1063	2.00	0.0413	207	0.0002	2.20	0.000213	-1.31E-05 [c]

[a] Moles of conjugate acid; [b] Moles of residual free aminochromone; [c] The negative concentration reflects the excess concentration of titrant.

Table 18: Calculation of pK_a values for 2-(*N,N*-dimethylamino)-8-fluorochromone **191**, corrected for hydrogen-ion activity.

(i) Total vol. (mL)	(ii) $[H^+]$	(iii) H^+	(iv) $\frac{BH^+ - H^+}{B + H^+}$	(v) log(col.iv)	(vi) pK_a	(vii) 10^{-pKa}
19.00	2.82E-05	5.3500E-07	-0.002677			
19.20	0.000575	1.1000E-05	0.053942	-1.268071	1.971929	0.010668
19.40	0.001175	2.2800E-05	0.109722	-0.959705	1.970295	0.010708
19.60	0.001778	3.4900E-05	0.169562	-0.770672	1.979328	0.010488
19.80	0.002399	4.7500E-05	0.231785	-0.634915	1.985085	0.010349
20.00	0.003020	6.0400E-05	0.298803	-0.524614	1.995386	0.010107
20.20	0.003715	7.5100E-05	0.357193	-0.447097	1.982903	0.010402
20.40	0.004365	8.9000E-05	0.427710	-0.368851	1.991149	0.010206
20.60	0.005129	1.0600E-04	0.476958	-0.321520	1.968480	0.010753
20.80	0.005754	1.2000E-04	0.560330	-0.251556	1.988444	0.010270
21.00	0.006310	1.3300E-04	0.670745	-0.173443	2.026557	0.009407

$pK_a = 1.98 \pm 0.06$

Table 19: Concentration of the protonated and non-protonated 2-(*N,N*-dimethylamino)-6-fluorochromone **192** as a function of pH

Conc. HCl	Vol. Titrant (mL)	Mass (g)	Molar mass (g/mol)	No. of moles	pH	BH^+ [a]	B [b]
0.1063	0.00	0.0412	207	1.99E-04	5.75	0.00	0.000199
0.1063	0.20	0.0412	207	1.99E-04	3.35	2.13E-05	0.000178
0.1063	0.40	0.0412	207	1.99E-04	3.05	4.25E-05	0.000157
0.1063	0.60	0.0412	207	1.99E-04	2.86	6.38E-05	0.000135
0.1063	0.80	0.0412	207	1.99E-04	2.72	8.50E-05	0.000114
0.1063	1.00	0.0412	207	1.99E-04	2.61	0.000106	9.27E-05
0.1063	1.20	0.0412	207	1.99E-04	2.51	0.000128	7.15E-05
0.1063	1.40	0.0412	207	1.99E-04	2.44	0.000149	5.02E-05
0.1063	1.60	0.0412	207	1.99E-04	2.36	0.000170	2.90E-05
0.1063	1.80	0.0412	207	1.99E-04	2.30	0.000191	7.69E-06
0.1063	2.00	0.0412	207	1.99E-04	2.25	0.000213	-1.36E-05[c]

[a] Moles of conjugate acid; [b] Moles of residual free aminochromone; [c] The negative concentration reflects the excess concentration of titrant.

Table 20: Calculation of pK_a values for 2-(*N,N*-dimethylamino)-6-fluorochromone **192**, corrected for hydrogen-ion activity.

(i) Total vol. (mL)	(ii) $[H^+]$	(iii) H^+	(iv) $\frac{BH^+ - H^+}{B + H^+}$	(v) log(col.iv)	(vi) pK_a	(vii) 10^{-pKa}
19.00	1.78E-06	3.3800E-08	-0.000170			
19.20	0.000447	8.5800E-06	0.068064	-1.167085	2.182915	0.006563
19.40	0.000891	1.7300E-05	0.145162	-0.838147	2.211853	0.006140
19.60	0.001380	2.7100E-05	0.226262	-0.645388	2.214612	0.006101
19.80	0.001905	3.7700E-05	0.311833	-0.506078	2.213922	0.006111
20.00	0.002455	4.9100E-05	0.403346	-0.394322	2.215678	0.006086
20.20	0.003090	6.2400E-05	0.486461	-0.312952	2.197048	0.006353
20.40	0.003631	7.4100E-05	0.601473	-0.220784	2.219216	0.006036
20.60	0.004365	8.9900E-05	0.674297	-0.171149	2.188851	0.006474
20.80	0.005012	1.0400E-04	0.778028	-0.109005	2.190995	0.006442
21.00	0.005623	1.1800E-04	0.904165	-0.043752	2.206248	0.006219

$pK_a = 2.20 \pm 0.06$

Table 21: Concentration of the protonated and non-protonated 2-(*N,N*-dimethylamino)-5-methoxychromone **193** as a function of pH.

Conc. HCl	Vol. Titrant (mL)	Mass (g)	Molar mass (g/mol)	No. of moles	pH	BH^{+} [a]	B [b]
0.1063	0.00	0.0438	219	0.0002	5.00	0.00	0.000200
0.1063	0.20	0.0438	219	0.0002	3.60	2.13E-05	0.000179
0.1063	0.40	0.0438	219	0.0002	3.24	4.25E-05	0.000157
0.1063	0.60	0.0438	219	0.0002	3.05	6.38E-05	0.000136
0.1063	0.80	0.0438	219	0.0002	2.89	8.50E-05	0.000115
0.1063	1.00	0.0438	219	0.0002	2.76	0.000106	9.37E-05
0.1063	1.20	0.0438	219	0.0002	2.66	0.000128	7.24E-05
0.1063	1.40	0.0438	219	0.0002	2.58	0.000149	5.12E-05
0.1063	1.60	0.0438	219	0.0002	2.49	0.000170	2.99E-05
0.1063	1.80	0.0438	219	0.0002	2.42	0.000191	8.66E-06
0.1063	2.00	0.0438	219	0.0002	2.34	0.000213	-1.26E-05[c]

[a] Moles of conjugate acid; [b] Moles of residual free aminochromone; [c] The negative concentration reflects the excess concentration of titrant.

Table 22: Calculation of pK_a values for 2-(*N,N*-dimethylamino)-5-methoxychromone **193**, corrected for hydrogen-ion activity.

(i) Total vol. (mL)	(ii) $[H^+]$	(iii) H^+	(iv) $\frac{BH^+ - H^+}{B + H^+}$	(v) log(col.iv)	(vi) pK_a	(vii) 10^{-pKa}
19.00	0.000010	1.9000E-06	-0.000949			
19.20	0.000251	4.8228E-06	0.089545	-1.047957	2.552043	0.002805
19.40	0.000575	1.1164E-05	0.185933	-0.730643	2.509357	0.003095
19.60	0.000891	1.7469E-05	0.301333	-0.520953	2.529047	0.002958
19.80	0.001288	2.5507E-05	0.423819	-0.372820	2.517180	0.003040
20.00	0.001738	3.4756E-05	0.556953	-0.254181	2.505819	0.003120
20.20	0.002188	4.4193E-05	0.714784	-0.145825	2.514175	0.003061
20.40	0.002630	5.3657E-05	0.907715	-0.042051	2.537949	0.002898
20.60	0.003236	6.6660E-05	1.070816	0.029715	2.519715	0.003022
20.80	0.003802	7.9079E-05	1.279478	0.107033	2.527033	0.002971
21.00	0.004571	9.5989E-05	1.398412	0.145635	2.485635	0.003269

$$pK_a = 2.52 \pm 0.06$$

Table 23: Concentration of the protonated and non-protonated 2-(*N,N*-dimethylamino)-8-methoxychromone **194** as a function of pH.

Conc. HCl	Vol. Titrant (mL)	Mass (g)	Molar mass (g/mol)	No. of moles	pH	BH^+ [a]	B [b]
0.1063	0.00	0.0437	219	0.0002	5.40	0.00	0.000200
0.1063	0.20	0.0437	219	0.0002	3.60	2.13E-05	0.000178
0.1063	0.40	0.0437	219	0.0002	3.23	4.25E-05	0.000157
0.1063	0.60	0.0437	219	0.0002	3.03	6.38E-05	0.000136
0.1063	0.80	0.0437	219	0.0002	2.88	8.50E-05	0.000115
0.1063	1.00	0.0437	219	0.0002	2.76	0.000106	9.32E-05
0.1063	1.20	0.0437	219	0.0002	2.65	0.000128	7.20E-05
0.1063	1.40	0.0437	219	0.0002	2.57	0.000149	5.07E-05
0.1063	1.60	0.0437	219	0.0002	2.48	0.000170	2.95E-05
0.1063	1.80	0.0437	219	0.0002	2.41	0.000191	8.20E-06
0.1063	2.00	0.0437	219	0.0002	2.36	0.000213	-1.31E-05[c]

[a] Moles of conjugate acid; [b] Moles of residual free aminochromone; [c] The negative concentration reflects the excess concentration of titrant.

Table 24: Calculation of pK_a values for 2-(*N,N*-dimethylamino)-8-methoxychromone **194**, corrected for hydrogen-ion activity.

(i) Total vol. (mL)	(ii) $[H^+]$	(iii) H^+	(iv) $\frac{BH^+ - H^+}{B + H^+}$	(v) log(col.iv)	(vi) pK_a	(vii) 10^{-pKa}
19.00	3.98E-06	7.5600E-08	-0.000379			
19.20	0.000251	4.8200E-06	0.089769	-1.046880	2.553124	0.002798
19.40	0.000589	1.1400E-05	0.184607	-0.733753	2.496247	0.003190
19.60	0.000933	1.8300E-05	0.295272	-0.529777	2.500223	0.003161
19.80	0.001318	2.6100E-05	0.419178	-0.377601	2.502399	0.003145
20.00	0.001738	3.4800E-05	0.558940	-0.252635	2.507365	0.003109
20.20	0.002239	4.5200E-05	0.702508	-0.153349	2.496651	0.003187
20.40	0.002692	5.4900E-05	0.889066	-0.051066	2.518934	0.003027
20.60	0.003311	6.8200E-05	1.042903	0.018244	2.498244	0.003175
20.80	0.003890	8.0900E-05	1.238922	0.093044	2.503044	0.003140
21.00	0.004365	9.1700E-05	1.538342	0.187053	2.547053	0.002838

$$pK_a = 2.50 \pm 0.06$$

Table 25: Concentration of the protonated and non-protonated 2-(*N,N*-dimethylamino)-8-nitrochromone **195** as a function of pH.

Conc. HCl	Vol. Titrant (mL)	Mass (g)	Molar mass (g/mol)	No. of moles	pH	BH^+ [a]	B [b]
0.1063	0.00	0.0468	234	0.0002	5.00	0.00	0.000200
0.1063	0.20	0.0468	234	0.0002	3.22	2.13E-05	0.000179
0.1063	0.40	0.0468	234	0.0002	2.90	4.25E-05	0.000157
0.1063	0.60	0.0468	234	0.0002	2.72	6.38E-05	0.000136
0.1063	0.80	0.0468	234	0.0002	2.59	8.50E-05	0.000115
0.1063	1.00	0.0468	234	0.0002	2.50	0.000106	9.37E-05
0.1063	1.20	0.0468	234	0.0002	2.41	0.000128	7.24E-05
0.1063	1.40	0.0468	234	0.0002	2.34	0.000149	5.12E-05
0.1063	1.60	0.0468	234	0.0002	2.28	0.000170	2.99E-05
0.1063	1.80	0.0468	234	0.0002	2.22	0.000191	8.66E-06
0.1063	2.00	0.0468	234	0.0002	2.18	0.000213	-1.26E-05[c]

[a] Moles of conjugate acid; [b] Moles of residual free aminochromone; [c] The negative concentration reflects the excess concentration of titrant.

Table 26: Calculation of pK_a values for 2-(*N,N*-dimethylamino)-8-nitrochromone **195**, corrected for hydrogen-ion activity.

(i) Total vol. (mL)	(ii) $[H^+]$	(iii) H^+	(iv) $\frac{BH^+ - H^+}{B + H^+}$	(v) log(col.iv)	(vi) pK_a	(vii) 10^{-pKa}
19.00	0.000010	1.9000E-07	-0.000949			
19.20	0.000603	1.1600E-05	0.050922	-1.293098	1.926902	0.011833
19.40	0.001259	2.4400E-05	0.099486	-1.002237	1.897763	0.012654
19.60	0.001905	3.7300E-05	0.152293	-0.817321	1.902679	0.012512
19.80	0.002570	5.0900E-05	0.205881	-0.686384	1.903616	0.012485
20.00	0.003162	6.3200E-05	0.274327	-0.561731	1.938269	0.011527
20.20	0.003890	7.8600E-05	0.324265	-0.489099	1.920901	0.011998
20.40	0.004571	9.3200E-05	0.384792	-0.414774	1.925226	0.011879
20.60	0.005248	1.0800E-04	0.448957	-0.347795	1.932205	0.011689
20.80	0.006026	1.2500E-04	0.492622	-0.307486	1.912514	0.012232
21.00	0.006607	1.3900E-04	0.585469	-0.232496	1.947504	0.011285

$$pK_a = 1.92 \pm 0.06$$

Computational details

Computations were conducted for the author by Professor C.W. McCleland (University of Port Elizabeth) using HyperChem (release 4.5)[183] and MOPAC (version 6.0)[184] packages on Pentium 90 MHz/16 MB RAM and 133 MHz/32 MB RAM personal computers. Structures, drawn in HyperChem and partially refined using its MM+ molecular mechanics option, were exported to MOPAC. Geometries were then optimized without constraint through implementation of the AM1 Hamiltonian, using the eigenvector-following routine (keyword EF). Use of the keyword PRECISE ensured that all geometry optimizations achieved energy gradient norms of at least 0.01 kcal.mol^{-1} Å^{-1}. Single-point HF/3-21G *ab initio* calculations on selected structures were carried out in HyperChem to an SCF convergence limit of 1 x 10^{-1} kcal.mol^{-1}. Convergences were accelerated through application of the DIIS procedure.

3.3 Synthesis of "granulosin" [7,8-(methylenedioxy)-2-propylchromone and C-2 side-chain analogues

2'-Hydroxy-3',4'-(methylenedioxy)acetophenone **197**

To a stirred suspension of 2',3',4'-trihydroxyacetophenone **196** (0.50 g, 3.0 mmol) and Cs_2CO_3 (0.97 g, 3.0 mmol) in dry DMF (7.5 mL) was added $BrCH_2Cl$ (0.20 mL, 3.0 mmol), and the resulting mixture was boiled under reflux. After 3 h, the mixture was allowed to cool to room temperature and then filtered through a pad of Celite 545, washing with EtOAc. The filtrate and the washings were concentrated *in vacuo* almost to dryness, and the residue diluted with H_2O (20 mL) and extracted with EtOAc (3 x 50 mL). The combined extracts were washed with water (25 mL) and then with brine (25 mL), and dried over anhydrous $MgSO_4$. Evaporation of the solvent *in vacuo* gave a dark-tan solid, which was recrystallized from petroleum ether (b.p. 80-100 °C) to afford *2'-hydroxy-3',4'-(methylenedioxy)-acetophenone* **197** as a pale yellow solid (0.42 g, 80%), m.p. 96-98 °C (Found: M^+, 180.04259. $C_9H_8O_4$ requires *M*, 180.04226); ν_{max} (KBr)/cm^{-1} 1663 (CO); δ_H (400 MHz; $CDCl_3$) 2.56 (3H, s, CH_3), 6.07 (2H, s, CH_2), 6.46 (1H, d, *J*=8.4 Hz, 5'-H), 7.37 (1H, d, *J*=8.5 Hz, 6'-H) and 12.27 (1H, s, OH); δ_C (100 MHz; $CDCl_3$) 27.0 (CH_3), 101.2 (C-5'), 103.0 (CH_2), 117.4 (C-1'), 127.0 (C-6'), 134.9 (C-3'), 147.4 (C-2'), 154.4 (C-4') and 203.8 (C=O); *m/z* 180 (M^+, 54%) and 165 (100).

Bis[6-acetyl-2,3-(methylenedioxy)phenoxy]methane **198**

The experimental procedure employed for the synthesis of 2'-hydroxy-3',4'-(methylene-dioxy)acetophenone **197** was followed, using of 2',3',4'-trihydroxyacetophenone **196** (1.0 g, 6.0 mmol), Cs_2CO_3 (2.93 g, 9.0 mmol), dry DMF (15 mL) and $BrCH_2Cl$ (0.60 mL, 9.0 mmol). After heating for 2 h, the reaction mixture was worked up to afford a yellow-brown solid. Flash chromatography on silica gel [elution with hexane-EtOAc (1:1)] gave, as a white crystalline solid, *bis[6-acetyl-2,3-(methylenedioxy)phenoxy]methane* **198** (1.96 g, 88%), m.p. 133-135 °C (Found: M^+, 372.08495. $C_{19}H_{16}O_8$ requires *M*, 372.08452); ν_{max} (KBr)/cm^{-1} 1665 (CO); δ_H (400 MHz; $CDCl_3$) 2.38 (6H, s, CH_3), 5.95 (4H, s, 2xCH_2), 6.09 (2H, s, CH_2), 6.60 (2H, d, *J*=8.3 Hz, 4-H) and 7.32 (2H, d, *J*=8.3 Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 30.8 (CH_3), 94.3 and 102.0 (2 x CH_2), 104.0 (C-4), 125.5 (C-5), 126.5 (C-6), 137.2 (C-2), 139.2 (C-1), 152.4 (C-3) and 197.3 (C=O); *m/z* 372 (M^+, 2%) and 193 (100).

7,8-(Methylenedioxy)-2-propylchromone (Granulosin) **40**

A mixture of 2'-hydroxy-3',4'-(methylenedioxy)acetophenone **197** (0.50 g, 2.8 mmol) and $CH_3CH_2CH_2CO_2Et$ (1.6 mL, 12 mmol) was added dropwise to a stirred dispersion of NaOEt [generated *in situ* by adding Na metal (0.27g, 12 mmol) to dry EtOH (2.0 mL)]. The resulting dark-green mixture was boiled gently under reflux for 8 h, during which time, a thick yellow slurry was formed. After cooling, the reaction mixture was poured into Et_2O (15 mL) and, after standing for 2 h, the sodium salt was filtered off, washed with Et_2O and dissolved in ice-cold water (15mL). The resulting solution was acidified with acetic acid, and then extracted with Et_2O (3 x 25 mL); the combined ethereal extracts were dried over anhydrous $MgSO_4$ and evaporation *in vacuo* afforded a brick-red residue indicated, by ^1H NMR spectroscopy, to contain a mixture of 1-[2-hydroxy-3,4-(methylenedioxy)phenyl]-1,3-hexanedione (as an enol tautomer, formulated as **201**) and 2-hydroxy-7,8-(methylenedioxy)-2-propylchromanone **206**, which was used without further purification. The crude mixture, together with glacial acetic acid (4.0 mL) and conc. H_2SO_4 (0.1 mL), was boiled under reflux for 4 h. The hot solution was poured into ice-cold water (20 mL), the mixture was basified with 10% aq. $NaHCO_3$ (20 mL) and the resulting dark-purple precipitate filtered and washed with cold water. Flash chromatography of the precipitate [on silica gel; elution with hexane-EtOAc (1:1)] afforded a colourless solid, which was recrystallized from [petroleum ether (b.p. 80-100°C)-methanol (1:1)] to afford 7,8-(methylenedioxy)-2-propylchromone **40** as colourless crystals (0.48 g, 76%), m.p. 101-103 °C (lit.,[47] 102-103 °C) (Found: M^+, 232.07413. $C_{13}H_{12}O_4$ requires *M*, 232.07356); ν_{max} (KBr)/cm^{-1} 1658 (CO); δ_H (400 MHz; DMSO-d_6) 0.96 (3H, t, *J*=7.4 Hz, CH_3), 1.68 (2H, m, CH_3CH_2), 2.61 (2H, t, *J*=7.4 Hz, $CH_3CH_2CH_2$), 6.12 (1H, s, 3-H), 6.27 (2H, s, OCH_2O), 7.09 (1H, d, *J*=8.4 Hz, 6-H) and 7.56 (1H, d, *J*=8.4 Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 13.3 (CH_3), 19.7 (CH_3CH_2), 35.1 ($CH_3CH_2CH_2$), 103.6 (OCH_2O), 107.1 (C-6), 109.0 (C-3), 119.3 (C-4a and C-5; 119.3 and 120.1 in $CDCl_3$), 134.4 (C-8), 140.8 (C-8a), 152.1 (C-7), 168.8 (C-2) and 175.9 (C=O); *m/z* 232 (M^+, 100%).

2-Methyl-7,8-(methylenedioxy)chromone **209**

The experimental procedure employed for the synthesis of 7,8-(methylenedioxy)-2-propyl-chromone (granulosin) **40** was followed, using the crude mixture of 1-[2-hydroxy-3,4-(methylenedioxy)phenyl]-1,3-butanedione **199** and 2-hydroxy-2-methyl-7,8-(methylene-dioxy)chromanone **204** {initially prepared using 2'-hydroxy-3',4'-(methylenedioxy)aceto-phenone **197** (0.50 g, 2.8 mmol), dry CH_3CO_2Et (1.20 mL, 12 mmol) and NaOEt [generated

in situ by adding Na metal (0.27g, 12 mmol) to dry EtOH (3.0 mL)]}, glacial acetic acid (4.0 mL) and conc. H_2SO_4 (0.1 mL). Work-up afforded *2-methyl-7,8-(methylenedioxy)chromone* **209** as a white crystalline solid (0.32 g, 58%), m.p. 161-163 °C [from petroleum ether (b.p. 80-100 °C)] (Found: M^+, 204.04226. $C_{11}H_8O_4$ requires *M*, 204.04226); ν_{max} (KBr)/cm^{-1} 1657 (CO); δ_H (400 MHz; $CDCl_3$) 2.36 (3H, s, CH_3), 6.06 (1H, s, 3-H), 6.15 (2H, s, OCH_2O), 6.90 (1H, d, *J*=8.4 Hz, 6-H) and 7.74 (1H, d, *J*=8.4 Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 20.3 (CH_3), 103.1 (OCH_2O), 106.9 (C-6), 110.1 (C-3), 119.7 (C-4a), 120.2 (C-5), 134.3 (C-8), 141.4 (C-8a), 152.1 (C-7), 165.3 (C-2) and 177.1 (C=O); *m/z* 204 (M^+, 100%).

2-Ethyl-7,8-(methylenedioxy)chromone **210**

The experimental procedure employed for the synthesis of 7,8-(methylenedioxy)-2-propyl-chromone (granulosin) **40** was followed, using the crude mixture of 1-[2-hydroxy-3,4-(methylenedioxy)phenyl]-1,3-pentanedione **200** and 2-ethyl-2-hydroxy-7,8-(methylene-dioxy)chromanone **205** {initially prepared using 2'-hydroxy-3',4'-(methylenedioxy)aceto-phenone **197** (0.50 g, 2.8 mmol), $CH_3CH_2CO_2Et$ (1.40 mL, 12 mmol) and NaOEt [generated *in situ* by adding Na metal (0.27g, 12 mmol) to dry EtOH (3.0 mL)]}, glacial acetic acid (4.0 mL) and conc. H_2SO_4 (0.1 mL). Work-up afforded *2-ethyl-7,8-(methylenedioxy)chromone* **210** as a white crystalline solid (0.49 g, 82%), m.p. 106-107 °C [from petroleum ether (b.p. 80-100 °C)] (Found: M^+, 218.05884. $C_{12}H_{10}O_4$ requires *M*, 218.05791); ν_{max} (KBr)/cm^{-1} 1653 (CO); δ_H (400 MHz; $CDCl_3$) 1.29 (3H, t, *J*=7.5 Hz, CH_3), 2.63 (2H, q, *J*=7.4 Hz, CH_3CH_2), 6.06 (1H, s, 3-H), 6.15 (2H, s, OCH_2O), 6.89 (1H, d, *J*=8.5 Hz, 6-H) and 7.73 (1H, d, *J*=8.5 Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 10.9 (CH_3), 27.2 (CH_3CH_2), 103.1 (OCH_2O), 106.8 (C-6), 108.4 (C-3), 119.8 (C-4a), 120.1 (C-5), 134.4 (C-8), 141.4 (C-8a), 152.1 (C-7), 169.9 (C-2) and 177.3 (C=O); *m/z* 218 (M^+, 100%).

2-Isopropyl-7,8-(methylenedioxy)chromone **211**

The experimental procedure employed for the synthesis of 7,8-(methylenedioxy)-2-propyl-chromone (granulosin) **40** was followed, using the crude mixture of 1-[2-hydroxy-3,4-(methylenedioxy)phenyl]-4-methyl-1,3-pentanedione **202** and 2-hydroxy-2-isopropyl-7,8-(methylenedioxy)chromanone **207** {initially prepared using 2'-hydroxy-3',4'-(methylene-dioxy)acetophenone **197** (0.50 g, 2.8 mmol), $(CH_3)_2CHCO_2Et$ (1.60 mL, 12 mmol) and NaOEt [generated *in situ* by adding Na metal (0.27g, 12 mmol) to dry EtOH (3.0 mL)]},

glacial acetic acid (4.0 mL) and conc. H_2SO_4 (0.1 mL). Work-up afforded *2-isopropyl-7,8-(methylenedioxy)chromone* **211** as a brown crystalline solid (0.40 g, 63%), m.p. 119-121 °C [from petroleum ether (b.p. 80-100 °C)] (Found: M^+, 232.07364. $C_{13}H_{12}O_4$ requires *M*, 232.07356); ν_{max} (KBr)/cm^{-1} 1634 (CO); δ_H (400 MHz; $CDCl_3$) 1.31 (6H, d, *J*=6.7 Hz, 2 x CH_3), 2.85 (1H, q, *J*=6.7 Hz, CH), 6.09 (1H, s, 3-H), 6.16 (2H, s, OCH_2O), 6.90 (1H, d, *J*=8.3 Hz, 6-H) and 7.74 (1H, d, *J*=8.3 Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 20.1 (CH_3), 33.1 [$(CH_3)_2CH$], 103.1 (OCH_2O), 106.8 (C-6), 107.0 (C-3), 119.8 (C-4a), 120.1 (C-5), 134.4 (C-8), 141.4 (C-8a), 152.1 (C-7), 173.4 (C-2) and 177.6 (C=O); *m/z* 232 (M^+, 100%).

2-Benzyl-7,8-(methylenedioxy)chromone **212**

The experimental procedure employed for the synthesis of 7,8-(methylenedioxy)-2-propylchromone (granulosin) **40** was followed, using the crude mixture of 1-[2-hydroxy-3,4-(methylenedioxy)phenyl]-4-phenyl-1,3-butanedione **203** and 2-benzyl-2-hydroxy-7,8-(methylenedioxy)chromanone **208** {initially prepared using 2'-hydroxy-3',4'-(methylenedioxy)acetophenone **197** (0.50 g, 2.8 mmol), $PhCH_2CO_2Et$ (1.90 mL, 12 mmol) and NaOEt [generated *in situ* by adding Na metal (0.27g, 12 mmol) to dry EtOH (3.0 mL)]}, glacial acetic acid (4.0 mL) and conc. H_2SO_4 (0.1 mL). Work-up afforded *2-benzyl-7,8-(methylenedioxy)chromone* **212** as a white crystalline solid (0.66 g, 85%), m.p. 172-174 °C [from petroleum ether (b.p. 80-100 °C)] (Found: M^+, 280.07415. $C_{17}H_{12}O_4$ *M*, 280.07356); ν_{max} (KBr)/cm^{-1} 1657 (CO); δ_H (400 MHz; $CDCl_3$) 3.90 (2H, s, CH_2Ph), 6.00 (1H, s, 3-H), 6.14 (2H, s, OCH_2O), 6.88 (1H, d, *J*=8.5 Hz, 6-H), 7.25-7.34 (5H, m, Ar-H) and 7.72 (1H, d, *J*=8.4 Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 40.4 (CH_2Ph), 103.1 (OCH_2O), 106.9 (C-6), 110.2 (C-3), 119.7 (C-4a), 120.2 (C-5), 127.5, 128.9, 129.3 and 134.5 (Ar-C), 134.6 (C-8), 141.4 (C-8a), 152.1 (C-7), 167.3 (C-2) and 177.2 (C=O); *m/z* 280 (M^+, 100%).

3.4 Synthesis of 5-hydroxy-2-isopropyl-7-methoxychromone and C-2 side-chain analogues

2'-Hydroxy-4',6'-dimethoxyacetophenone **215**[149]

A stirred solution of 2',4',6'-trihydroxyacetophenone **213** (5.0 g, 30 mmol), Me_2SO_4 (5.3 mL, 56 mmol) and anhydrous K_2CO_3 (8.2 g, 56 mmol) in acetone (90 mL) was boiled under reflux for 5 h. The reaction mixture was filtered and evaporated *in vacuo* to give a solid. Recrystallization from petroleum-ether (b.p. 80-100 °C) – hexane (1:9) afforded 2'-hydroxy-4',6'-dimethoxyacetophenone **215** as a pale yellow solid (5.2 g, 90%), m.p. 75-77 °C (lit.,[182] 77-79 °C) (Found: M^+, 196.07405. $C_{10}H_{12}O_4$ requires *M*, 196.07356); ν_{max} (KBr)/cm^{-1} 3100 (br, OH) and 1620 (CO); δ_H (400 MHz; $CDCl_3$) 2.57 (3H, s, CH_3), 3.78 and 3.82 (6H, 2 x s, 2 x OCH_3), 5.88 (1H, d, *J*=2.2 Hz, 5'-H), 6.01 (1H, d, *J*=2.2 Hz, 2'-H) and 13.92 (1H, s, OH); δ_C (100 MHz; $CDCl_3$) 32.7 (CH_3), 55.43 and 55.44 (2 x OCH_3), 90.6 (C-5'), 93.5 (C-3'), 106.0 (C-1'), 162.9 (C-2'), 166.1 (C-6'), 167.5 (C-4'), and 203.1 (C=O); *m/z* 196 (M^+, 4%) and 181 (100).

3.4.1 Synthesis of the C-2 substituted 5,7-dimethoxychromone derivatives

2-Isopropyl-5,7-dimethoxychromone **229**

A mixture of 2'-hydroxy-4',6'-dimethoxyacetophenone **215** (1.0 g, 5.1 mmol) and ethyl iso-butyrate (3.0 mL, 22 mmol) was added dropwise to a stirred dispersion of NaOEt [generated *in situ* by adding Na metal (0.51g, 22 mmol) to dry EtOH (4.0 mL)]. The resulting mixture was boiled gently under reflux for 8 h, during which time, a thick yellow slurry was formed. After cooling, the reaction mixture was poured into Et_2O (30 mL) and, after standing for 2 h, the sodium salt was filtered off, washed with Et_2O and dissolved in ice-cold water (15mL). The resulting solution was acidified with acetic acid, and then extracted with Et_2O (3 x 30 mL); the combined ethereal extracts were dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to afford a brick-red residue indicated, by ^1H NMR spectroscopy, to contain a mixture of 1-(2-hydroxy-4,6-dimethoxyphenyl)-4-methyl-1,3-pentanedione (as an enol tautomer, formulated as **219**) and 2-hydroxy-2-isopropyl-5,7-dimethoxychromanone **224**, which was

used without further purification. The crude mixture, together with glacial acetic acid (5.0 mL) and conc. H_2SO_4 (0.1 mL), was boiled under reflux for 4 h. The hot solution was poured into ice-cold water (20 mL), and the resulting mixture was basified with 10% aq. $NaHCO_3$ (20 mL) [monitored with pH indicator paper] and extracted with Et_2O (3 x 50 mL). The combined ethereal extracts were dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to give a tan-solid. Flash chromatography on silica gel (elution with EtOAc) afforded *2-isopropyl-5,7-dimethoxychromone* **229** as a white solid (0.50 g, 40%), m.p. 54-56 °C (Found: M^+, 248.10486. $C_{14}H_{16}O_4$ requires *M*, 248.10486); ν_{max} (KBr)/cm^{-1} 1656 (CO); δ_H (400 MHz; $CDCl_3$) 1.26 (6H, d, *J*=6.9 Hz, 2 x CH_3), 2.74 (1H, q, *J*=6.8 Hz, CH), 3.86 and 3.91 (6H, 2 x s, 2 x OCH_3), 6.00 (1H, s, 3-H), 6.32 (1H, d, *J*=2.2 Hz, 6-H) and 6.42 (1H, d, *J*=2.2 Hz, 8-H); δ_C (100 MHz; $CDCl_3$) 19.9 (2 x CH_3), 32.5 (CH), 55.6 and 56.3 (2 x OCH_3), 92.6 (C-8), 95.9 (C-6), 108.9 (C-3), 109.0 (C-4a), 160.2 (C-8a), 160.9 (C-5), 163.8 (C-7), 171.0 (C-2) and 177.9 (C=O); *m/z* 248 (M^+, 100%).

5,7-Dimethoxy-2-methylchromone **226**

The experimental procedure employed for the synthesis of 2-isopropyl-5,7-dimethoxy-chromone **229** was followed, using the crude mixture of 1-[2-hydroxy-4,6-dimethoxyphenyl]-1,3-butanedione **216** and 2-hydroxy-5,7-dimethoxy-2-methylchromanone **221** {initially prepared using 2'-hydroxy-4',6'-dimethoxyacetophenone **215** (1.0 g, 5.1 mmol), dry CH_3CO_2Et (4.3 mL, 44 mmol) and NaOEt [generated *in situ* by adding Na metal (0.51g, 22 mmol) to dry EtOH (5.0 mL)]}, glacial acetic acid (5.0 mL) and conc. H_2SO_4 (0.1 mL). Work-up afforded a crude residue, which was chromatographed [flash chromatography on silica gel; elution with $CHCl_3$ – MeOH (9:1)] to afford 5,7-dimethoxy-2-methylchromone **226** as a yellow crystalline solid (0.78 g, 70%), m.p. 121-123 °C [from hexane – EtOAc (7:3)] (lit.,[150] 124-125 °C) (Found: M^+, 220.07366. $C_{12}H_{12}O_4$ requires *M*, 220.07356); ν_{max} (KBr)/cm^{-1} 1657 (CO); δ_H (400 MHz; $CDCl_3$) 2.24 (3H, s, CH_3), 3.84 and 3.90 (6H, 2 x s, 2 x OCH_3), 5.98 (1H, s, 3-H), 6.31 (1H, d, *J*=2.2 Hz, 6-H) and 6.39 (1H, d, *J*=2.2 Hz, 8-H); δ_C (100 MHz; $CDCl_3$) 19.7 (CH_3), 55.6 and 56.3 (2 x OCH_3), 92.6 (C-8), 95.9 (C-6), 108.9 (C-4a), 111.9 (C-3), 160.1 (C-8a), 160.9 (C-5), 162.9 (C-7), 163.7 (C-2) and 177.4 (C=O); *m/z* 220 (M^+, 100%).

2-Ethyl-5,7-dimethoxychromone **227**

The experimental procedure employed for the synthesis of 2-isopropyl-5,7-dimethoxychromone **229** was followed, using the crude mixture of 1-[2-hydroxy-4,6-dimethoxyphenyl]-1,3-pentanedione **217** and 2-ethyl-2-hydroxy-5,7-dimethoxychromanone **222** {initially prepared using 2'-hydroxy-4',6'-dimethoxyacetophenone **215** (1.0 g, 5.1 mmol), $CH_3CH_2CO_2Et$ (2.54 mL, 22 mmol) and NaOEt [generated *in situ* by adding Na metal (0.51g, 22 mmol) to dry EtOH (5.0 mL)]}, glacial acetic acid (5.0 mL) and conc. H_2SO_4 (0.1 mL). Work-up afforded a crude residue, which was chromatographed (flash chromatography on silica gel; elution with $CHCl_3$) to afford 2-ethyl-5,7-dimethoxychromone **227** as a white crystalline solid (0.77 g, 65%), m.p. 134-136 °C (lit.,[186] 133-134 °C) (Found: M^+, 234.08939. $C_{13}H_{14}O_4$ requires *M*, 234.08921); ν_{max} (KBr)/cm^{-1} 1661 (CO); δ_H (400 MHz; $CDCl_3$) 1.28 (3H, t, *J*=7.4 Hz, CH_3), 2.59 (2H, q, *J*=7.5 Hz, CH_3CH_2), 3.86 and 3.92 (6H, 2 x s, 2 x OCH_3), 6.01 (1H, s, 3-H), 6.32 (1H, d, *J*=2.0 Hz, 6-H) and 6.41 (1H, d, *J*=2.0 Hz, 8-H); δ_C (100 MHz; $CDCl_3$) 10.7 (CH_3), 26.7 (CH_3CH_2), 55.6 and 56.3 (2 x OCH_3), 92.6 (C-8), 95.9 (C-6), 109.1 (C-4a), 110.3 (C-3), 160.1 (C-8a), 160.9 (C-5), 163.7 (C-7), 167.5 (C-2) and 177.6 (C=O); *m/z* 234 (M^+, 100%).

5,7-Dimethoxy-2-propylchromone **228**

The experimental procedure employed for the synthesis of 2-isopropyl-5,7-dimethoxychromone **229** was followed, using the crude mixture of 1-[2-hydroxy-4,6-dimethoxyphenyl]-1,3-hexanedione **218** and 2-hydroxy-5,7-dimethoxy-2-propylchromanone **223** {initially prepared using 2'-hydroxy-4',6'-dimethoxyacetophenone **215** (1.0 g, 5.1 mmol), $CH_3CH_2CH_2CO_2Et$ (3.0 mL, 22 mmol) and NaOEt [generated *in situ* by adding Na metal (0.51g, 22 mmol) to dry EtOH (5.0 mL)]}, glacial acetic acid (5.0 mL) and conc. H_2SO_4 (0.1 mL). Work-up afforded a crude residue, which was chromatographed [flash chromatography on silica gel; elution with $CHCl_3$] to afford 5,7-dimethoxy-2-propylchromone **228** as white prisms (0.84 g, 67%), m.p. 129-131 °C (lit.,[187] 131-132 °C) (Found: M^+, 248.10515. $C_{14}H_{16}O_4$ requires *M*, 248.10456); ν_{max} (KBr)/cm^{-1} 1660 (CO); δ_H (400 MHz; $CDCl_3$) 0.98 (3H, t, *J*=7.4 Hz, CH_3), 1.70 (2H, m, CH_3CH_2), 2.46 (2H, t, *J*=7.5 Hz, $CH_3CH_2CH_2$), 3.84 and 3.90 (6H, 2 x s, 2 x OCH_3), 5.98 (1H, s, 3-H), 6.30 (1H, d, *J*=2.2 Hz, 6-H) and 6.39 (1H, d, *J*=2.2 Hz, 8-H); δ_C (100 MHz; $CDCl_3$) 13.5 (CH_3), 19.9 (CH_3CH_2), 35.4 ($CH_3CH_2CH_2$),

55.6 and 56.3 (2 x OCH_3), 92.6 (C-8), 95.8 (C-6), 109.0 (C-4a), 111.2 (C-3), 160.1 (C-8a), 160.8 (C-5), 163.7 (C-7), 166.2 (C-2) and 177.5 (C=O); *m/z* 248 (M^+, 100%).

2-Benzyl-5,7-dimethoxychromone **230**

The experimental procedure employed for the synthesis of 2-isopropyl-5,7-dimethoxychromone **229** was followed, using the crude mixture of 1-[2-hydroxy-4,6-dimethoxyphenyl]-4-phenyl-1,3-butanedione **220** and 2-benzyl-2-hydroxy-5,7-dimethoxychromanone **225** {initially prepared using 2'-hydroxy-4',6'-dimethoxyacetophenone **215** (1.0 g, 5.1 mmol), $PhCH_2CO_2Et$ (3.5 mL, 22 mmol) and NaOEt [generated *in situ* by adding Na metal (0.51g, 22 mmol) to dry EtOH (5.0 mL)]}, glacial acetic acid (5.0 mL) and conc. H_2SO_4 (0.1 mL). Work-up afforded a crude residue, which was chromatographed [flash chromatography on silica gel; elution with EtOAc] to afford *2-benzyl-5,7-dimethoxychromone* **230** as a brown crystalline solid (0.90 g, 60%), m.p. 170-171 °C (Found: M^+, 296.10496. $C_{18}H_{16}O_4$ requires *M*, 296.10486); ν_{max} (KBr)/cm^{-1} 1664 (CO); δ_H (400 MHz; $CDCl_3$) 3.80 (2H, s, CH_2Ph), 3.83 and 3.89 (6H, 2 x s, 2 x OCH_3), 5.96 (1H, s, 3-H), 6.30 (1H, d, *J*=2.0 Hz, 6-H), 6.37 (1H, d, *J*=2.1 Hz, 8-H) and 7.25-7.34 (5H, m, Ar-H); δ_C (100 MHz; $CDCl_3$) 39.9 (CH_2Ph), 55.6 and 56.3 (2 x OCH_3), 92.7 (C-8), 96.0 (C-6), 109.0 (C-4a), 112.1 (C-3), 127.3, 128.8, 129.1 and 135.0 (Ar-C), 160.1 (C-8a), 160.9 (C-5), 163.8 (C-7), 164.8 (C-2) and 177.4 (C=O); *m/z* 296 (M^+, 100%).

3.4.2 Synthesis of the C-2 substituted 5-hydroxy-7-methoxychromone derivatives

5-Hydroxy-2-isopropyl-7-methoxychromone **35**

A solution of 2-isopropyl-5,7-dimethoxychromone **229** (50 mg, 0.20 mmol), acetic anhydride (1.01 mL, 10.7 mmol) and hydriodic acid (d. 1.7; 1.52 mL, 20.2 mmol) was heated in an oil bath at 115 °C for 30 min. The reaction mixture was cooled, and then diluted with aqueous sodium bisulphite. The resulting solution was neutralized with sodium bicarbonate, and the precipitated solid filtered off and washed with water. Flash chromatography on silica gel [elution with hexane–EtOAc (5:1)] afforded 5-hydroxy-2-isopropyl-7-methoxychromone **35** as a pale yellow solid (26 mg, 55%), m.p. 44-46 °C (lit.,[40] 40-43 °C) (Found: M^+, 234.08936.

$C_{13}H_{14}O_4$ requires *M*, 234.08921); ν_{max} (KBr)/cm^{-1} 2966 (br, OH) and 1675 (CO); δ_H (400 MHz; $CDCl_3$) 1.29 (6H, d, *J*=6.9 Hz, 2 x CH_3), 2.82 (1H, m, CH), 3.85 (3H, s, OCH_3), 6.03 (1H, s, 3-H), 6.32 (1H, d, *J*=2.2 Hz, 6-H), 6.36 (1H, d, *J*=2.2 Hz, 8-H) and 12.70 (1H, s, OH); δ_C (100 MHz; $CDCl_3$) 20.1 (2 x CH_3), 33.2 (CH), 55.7 (OCH_3), 92.4 (C-8), 97.9 (C-6), 105.5 (C-4a), 105.9 (C-3), 158.1 (C-8a), 162.2 (C-5), 165.4 (C-7), 174.9 (C-2) and 182.8 (C=O); *m/z* 234 (M^+, 100%).

5-Hydroxy-7-methoxy-2-methylchromone **231**

The experimental procedure employed for the synthesis of 5-hydroxy-2-isopropyl-7-methoxychromone **35** was followed, using 5,7-dimethoxy-2-methylchromone **226** (200 mg, 0.909 mmol), acetic anhydride (4.57 mL, 48.5 mmol) and hydriodic acid (6.86 mL, 91.0 mmol). Work-up afforded a crude yellow solid, which was flash chromatographed on silica gel (elution with EtOAc) to afford 5-hydroxy-7-methoxy-2-methylchromone **231** as a pale yellow solid (108 mg, 58%), m.p. 114-115 °C [from hexane–EtOAc (85:15)] (lit.,[150] 118-119 °C) (Found: M^+, 206.05842. $C_{11}H_{10}O_4$ requires *M*, 206.05791); ν_{max} (KBr)/cm^{-1} 2980 (br, OH) and 1672 (CO); δ_H (400 MHz; $CDCl_3$) 2.33 (3H, s, CH_3), 3.83 (3H, s, OCH_3), 6.01 (1H, s, 3-H), 6.32 (1H, d, *J*=2.2 Hz, 6-H), 6.34 (1H, d, *J*=2.1 Hz, 8-H) and 12.68 (1H, s, OH); δ_C (100 MHz; $CDCl_3$) 20.4 (CH_3), 55.7 (OCH_3), 92.4 (C-8), 97.9 (C-6), 105.2 (C-4a), 108.7 (C-3), 158.1 (C-8a), 162.2 (C-5), 165.3 (C-7), 166.8 (C-2) and 182.4 (C=O); *m/z* 206 (M^+, 100%).

2-Ethyl-5-hydroxy-7-methoxychromone **232**

The experimental procedure employed for the synthesis of 5-hydroxy-2-isopropyl-7-methoxychromone **35** was followed, using 2-ethyl-5,7-dimethoxychromone **227** (200 mg, 0.855 mmol), acetic anhydride (4.30 mL, 45.3 mmol) and hydriodic acid (6.43 mL, 85.5 mmol). Work-up afforded a crude yellow solid, which was flash chromatographed on silica gel [elution with hexane–EtOAc (8:2)] to afford *2-ethyl-5-hydroxy-7-methoxychromone* **232** as a brown solid (150 mg, 80%), m.p. 101-103 °C, [from hexane-EtOAc (8:2)] (Found: M^+, 220.07272. $C_{12}H_{12}O_4$ requires *M*, 220.07356); ν_{max} (KBr)/cm^{-1} 2975 (br, OH) and 1660 (CO); δ_H (400 MHz; $CDCl_3$) 1.27 (3H, t, *J*=7.2 Hz, CH_3), 2.61 (2H, q, *J*=7.4 Hz, CH_3CH_2), 3.87 (3H, s, OCH_3), 6.02 (1H, s, 3-H), 6.32 (1H, d, *J*=2.2 Hz, 6-H), 6.35 (1H, d, *J*=2.1 Hz, 8-H) and 12.67 (1H, s, OH); δ_C (100 MHz; $CDCl_3$) 10.9 (CH_3), 27.4 (CH_3CH_2), 55.7 (OCH_3), 92.4

(C-8), 97.9 (C-6), 105.4 (C-4a), 107.2 (C-3), 158.1 (C-8a), 162.2 (C-5), 165.4 (C-7), 171.5 (C-2) and 182.7 (C=O); *m/z* 220 (M^+, 100%).

5-Hydroxy-7-methoxy-2-propylchromone **233**

The experimental procedure employed for the synthesis of 5-hydroxy-2-isopropyl-7-methoxychromone **35** was followed, using 5,7-dimethoxy-2-propylchromone **228** (200 mg, 0.806 mmol), acetic anhydride (4.03 mL, 42.7 mmol) and hydriodic acid (6.06 mL, 80.6 mmol). Work-up afforded a crude yellow solid, which was flash chromatographed on silica gel [elution with hexane–EtOAc (6:4)] to afford *5-hydroxy-7-methoxy-2-propylchromone* **233** as a pale yellow crystalline solid (125 mg, 66%), m.p. 89-91 °C (Found: M^+, 234.08937. $C_{13}H_{14}O_4$ requires *M*, 234.08921); ν_{max} (KBr)/cm^{-1} 2967 (br, OH) and 1654 (CO); δ_H (400 MHz; $CDCl_3$) 1.01 (3H, t, *J*=7.4 Hz, CH_3), 1.75 (2H, m, CH_3CH_2), 2.55 (2H, t, *J*=7.5 Hz, $CH_3CH_2CH_2$), 3.84 (3H, s, OCH_3), 6.02 (1H, s, 3-H), 6.32 (1H, d, *J*=2.1 Hz, 6-H), 6.35 (1H, d, *J*=2.1 Hz, 8-H) and 12.69 (1H, s, OH); δ_C (100 MHz; $CDCl_3$) 13.5 (CH_3), 20.2 (CH_3CH_2), 36.1 ($CH_3CH_3CH_2$), 55.7 (OCH_3), 92.5 (C-8), 97.9 (C-6), 105.5 (C-4a), 108.1 (C-3), 158.2 (C-8a), 162.2 (C-5), 165.4 (C-7), 170.3 (C-2) and 182.6 (C=O); *m/z* 234 (M^+, 100%).

2-Benzyl-5-hydroxy-7-methoxychromone **234**

The experimental procedure employed for the synthesis of 5-hydroxy-2-isopropyl-7-methoxychromone **35** was followed, using 2-benzyl-5,7-dimethoxychromone **230** (200 mg, 0.676 mmol), acetic anhydride (3.38 mL, 35.8 mmol) and hydriodic acid (5.08 mL, 67.6 mmol). Work-up afforded a crude yellow solid, which was flash chromatographed on silica gel [elution with hexane–EtOAc (8:2)] to afford *2-benzyl-5-hydroxy-7-methoxychromone* **234** as a white crystalline solid (115 mg, 61%), m.p. 152-153 °C, (from ethanol) (Found: M^+, 282.08880. $C_{17}H_{14}O_4$ requires *M*, 282.08921); ν_{max} (KBr)/cm^{-1} 3000 (br, OH) and 1677 (CO); δ_H (400 MHz; $CDCl_3$) 3.83 (3H, s, OCH_3), 3.87 (2H, s, CH_2Ph), 5.96 (1H, s, 3-H), 6.32 (1H, d, *J*=2.1 Hz, 6-H), 6.34 (1H, d, *J*=2.1 Hz, 8-H), 7.26-7.37 (5H, m, Ar-H) and 12.64 (1H, s, OH); δ_C (100 MHz; $CDCl_3$) 40.5 (CH_2Ph), 55.7 (OCH_3), 92.5 (C-8), 98.0 (C-6), 105.3 (C-4a), 108.9 (C-3), 127.5, 128.9, 129.2 and 134.5 (Ar-C), 158.1 (C-8a), 162.1 (C-5), 165.4 (C-7), 168.8 (C-2) and 182.5 (C=O); *m/z* 282 (M^+, 100%).

3.4.3 Assessment of biological activity of compounds 35, 40, 209 – 212 and 226 – 234

Artemia salina larvicidal bioassays were performed for the author by Mr C.A. Gray, following the method described by Solis *et al.*[146] Estimates of median lethal concentrations were obtained by probit analysis[147] and the trimmed Spearman-Karber method[151] of *A. salina* mortality data from 12 solutions across concentration ranges of:- 25.00 - 0.586 μg/mL for compound **40**; 400.0 – 12.50 μg/mL for compounds **209 – 212**; 200.0 – 10.00 μg/mL for compounds **35** and **231 – 233**; 700.0 – 300.0 μg/mL for compound **234** and 400.0 – 50.00 μg/mL for compounds **226 - 230**.

3.5 Synthesis of potential HIV-1 protease inhibitors

3.5.1 Synthesis of the hydroxyethylene dipeptide isostere

(L)-N,N-*Dibenzylphenylalanine benzyl ester* **238**[163]

To a homogeneous solution of L-phenylalanine **237** (10 g, 60 mmol), K_2CO_3 (27 g, 0.20 mol) and water (40 mL) was added benzyl chloride (22.7 mL, 194 mmol). The solution was boiled under reflux for 18 h (prolonged reflux is necessary to destroy excess BnCl). The reaction mixture was cooled and hexane (30 mL) and water (20 mL) were added. The organic phase was separated and washed with water–methanol (2:1; 2 x 20 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to afford (L)-*N,N*-dibenzylphenylalanine benzyl ester **238** as a pale yellow oil (24.38 g, 94%), (Found: M^+-Bn, 344.16540. $C_{30}H_{29}NO_2$ requires *M*-Bn, 344.16505); ν_{max} (nujol mull/cm^{-1}) 1732 (CO); δ_H (400 MHz; $CDCl_3$) 3.21 and 3.33 (2H, 2 x dd, *J*=8.1 and 13.9 Hz, CH_2), 3.63 (2H, d, *J*=14.0 Hz, CH_2), 3.80 (1H, t, *J*=7.7 Hz, CH), 4.00 (2H, d, *J*=14.0 Hz, CH_2), 5.19 and 5.29 (2H, 2 x d, *J*=12.3 Hz, CH_2), 7.05-7.45 (20H, m, Ar-H); δ_C (100 MHz; $CDCl_3$) 35.7 (CH_2), 54.4 (2 x CH_2), 62.4 (CH), 66.0 (CH_2), 126.2, 126.9, 128.1, 128.2, 128.3, 128.4, 128.5, 128.7, 129.3, 136.0, 138.0 and 139.2 (Ar-C) and 172.1 (C=O).

(4S)-4-(Dibenzylamino)-3-oxo-5-phenylpentanenitrile **239**[163]

A solution of the crude benzyl ester **238** (10 g, 23 mmol) in dry THF (30 mL) was cooled to –45 °C under N_2. A separate flask was charged with sodium amide (95%; 2.2 g, 55 mmol) under N_2 followed by THF (25 mL). The slurry was cooled below –45 °C and CH_3CN (3.1 mL, 59 mmol) was added over a period of 15 min; the resulting solution was then added to the ester solution over 15 min. After stirring the mixture at –45 °C for 2 h, the reaction was quenched with 25% aqueous citric acid (60 mL). The organic layer was separated, washed with 20% brine (60 mL), filtered, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a yellow viscous oil. Crystallization from ethanol (denatured with toluene) afforded *(4S)*-4-(dibenzylamino)-3-oxo-5-phenylpentanenitrile **239** as white crystals (5.4 g, 64%), m.p. 132-134 °C (lit.,[163] 84-85 °C) (Found: M^+, 368.18897. $C_{25}H_{24}N_2O$ requires *M*, 368.18886); ν_{max} (nujol mull/cm^{-1}) 2265 (CN) and 1739 (CO); δ_H (400 MHz; $CDCl_3$) 2.98 (1H, dd, *J*=3.4 and 13.4 Hz, CH), 3.00 (1H, d, *J*=19.5 Hz, CH), 3.22 (1H, dd, *J*=9.6 and 13.5 Hz, CH), 3.53 (1H, dd, *J*=3.5 and 9.5 Hz, CH), 3.57 (2H, d, *J*=13.5 Hz, CH_2), 3.80 (2H, d,

J=13.4 Hz, CH_2), 3.85 (1H, d, *J*=19.5 Hz, CH), and 7.14-7.38 (15H, m, Ar-H); δ_C (100 MHz; $CDCl_3$) 28.5 (CH_2), 30.0 (CH_2), 54.8 (2 x CH_2), 68.6 (CH), 113.8, 126.5, 127.8, 128.6, 128.8, 129.0, 129.5, and 138.1 (Ar-C), 138.4 (CN) and 196.8 (C=O); *m/z* 368 (M^+, 1%) and 91 (100).

(5S)-2-Amino-5-(dibenzylamino)-4-oxo-1,6-diphenylhex-2-ene **240**[163]

To a stirred solution of the nitrile **239** (5.0 g, 14 mmol) in dry THF (15 mL) at 10 °C was added, dropwise, a solution of benzylmagnesium chloride in dry THF (2.0**M**; 20 mL, 0.14 mol), maintaining the temperature of the mixture below 5 °C. The solution was allowed to warm to 25 °C and stirring was continued for 24 h. The mixture was then cooled to 5 °C and the reaction quenched by the slow addition of 10% aqueous citric acid (70 mL). The organic layer was separated and washed with saturated brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a yellow viscous oil. The crude oil was crystallized from ethanol (denatured with toluene) to afford *(5S)*-2-amino-5-(dibenzylamino)-4-oxo-1,6-diphenylhex-2-ene **240** as white crystals (5.71 g, 92%), m.p. 101-102 °C (lit.,[163] 101-102 °C) (Found: M^+-Bn, 369.19646. $C_{32}H_{32}N_2O$ requires *M*-Bn, 369.19669); ν_{max} (nujol mull/cm^{-1}) 3433 and 3324 (NH_2) and 1597 (CO); δ_H (400 MHz; $CDCl_3$) 2.97 and 3.15 (2H, 2 x dd, *J*=6.3 and 13.7 Hz, CH_2), 3.49 (3H, m, CH_2 and CH), 3.65 (2H, d, *J*=14.0 Hz, CH_2), 3.78 (2H, d, *J*=14.0 Hz, CH_2), 4.88 (1H, br s, NH), 5.08 (1H, s, CH), 7.10-7.42 (20H, m, Ar-H) and 9.79 (1H, br s, NH); δ_C (100 MHz; $CDCl_3$) 32.5 (CH_2), 42.4 (CH_2), 54.4 (2 x CH_2), 66.7 (CH), 97.0, 125.6, 126.7, 127.3, 128.0, 128.1, 128.7, 128.9, 129.3, 129.5, 135.8, 140.1, 140.2 and 162.8 (Ar-C and CH=C) and 198.1 (C=O).

(2S,3S,5S)-5-Amino-2-(dibenzylamino)-3-hydroxy-1,6-diphenylhexane **242**[163]

A suspension of sodium borohydride (1.0 g, 27 mmol) in dry THF (56 mL) was cooled to -5 °C. Methanesulfonic acid (4.3 mL, 66 mmol) was added at a rate such that the temperature remained below 5 °C. The reaction mixture was cooled to 0 °C, and a solution of the enaminone **240** (5.0 g, 11 mmol) in dry THF (10 mL) and isopropyl alcohol (6.0 mL) was added, and the resulting mixture was stirred for 14 h at 5 °C. In a separate flask, a dispersion of sodium borohydride (1.65 g, 43.4 mmol) in dry THF (22 mL) was cooled to 0 °C, and trifluoroacetic acid (4.2 mL, 54 mmol) was added slowly. The resulting solution was stirred for 45 min at 10 °C, and then added slowly to the enaminone reaction mixture, maintaining

the temperature of the mixture below 15 °C. The resulting mixture was stirred for 4 h and cooled to 10 °C before quenching the reaction with 3**M** NaOH (35 mL). *tert*-Butyl methyl ether (40 mL) was then, added and the organic layer was separated and washed sequentially with 2% aq. NaOH (40 mL), 20% aq. NH_4Cl (40 mL) and 6% aq. NaCl (2 x 40 mL). The organic solution was dried over anhydrous Na_2SO_4 and then concentrated *in vacuo* to afford, as a yellow oil, a mixture of diastereomers containing the required hydroxy compound **242** (5.5 g, *ca.*100%) (Found: M^+-Bn, 373.22440. $C_{32}H_{36}N_2O$ requires *M*-Bn, 373.22799); ν_{max} (nujol mull/cm^{-1}) 3400-3000 (br, OH and NH_2); δ_H (400 MHz; $CDCl_3$) 1.25 (1H, m, CH), 1.61 (1H, m, CH), 2.47 (1H, dd, *J*=14.0 Hz, CH), 2.66 (2H, m, CH_2), 3.00 (3H, m, CH_2 and CH), 3.46 (2H, d, *J*=13.5 Hz, CH_2), 3.65 (1H, m, CH), 4.16 (2H, d, *J*=13.6 Hz, CH_2) and 7.07-7.35 (20H, m, Ar-H) ; δ_C (100 MHz; $CDCl_3$) 30.3 (CH_2), 40.5 (CH_2), 46.7 (CH_2), 53.3 (CH), 55.1 (2 x CH_2), 63.7 (CH), 72.1 (CH), 125.7, 126.3, 126.8, 128.2, 128.3, 128.4, 128.9, 129.0, 129.4, 138.6, 140.3 and 141.0 (Ar-C).

(2S,3S,5S)-2,5-Diamino-3-hydroxy-1,6-diphenylhexane dihydrochloride **243**[163]

The diastereomeric mixture containing the hydroxy compound **242** (5.0 g, 11 mmol), methanol (80 mL), aqueous ammonium formate (3.4 g in 5.8 mL of water) and 5% palladium-on-carbon catalyst (50-60% water by weight; 1.0 g) was boiled under reflux for 6 h. The cooled suspension was filtered through a bed of diatomaceous earth (Celite 545), and the cake was washed with methanol (2 x 50 mL). The filtrate and washings were concentrated *in vacuo* to afford an oily residue, which was dissolved in EtOAc (50 mL) and washed sequentially with 4% aq. NaOH (50 mL), 20% aq. NaCl (50 mL) and water (30 mL). The organic solution was dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give a yellow oily residue. Isopropyl alcohol (52 mL) and conc. HCl (4.3 mL) were added to the residue, and the resulting suspension was boiled under reflux for 1 h, cooled to 25 °C and then stirred for 18 h. The solid was filtered off and washed with EtOAc to afford *(2S,3S,5S)*-2,5-diamino-3-hydroxy-1,6-diphenylhexane dihydrochloride **243** as a white crystalline solid (2.00 g, 68%), m.p. >300 °C (lit.,[163] >300 °C) (Found: M^+-Bn, 193.13469. $C_{18}H_{24}N_2O$ requires *M*-Bn, 193.13409); ν_{max} (nujol mull/cm^{-1}) 3190-2360 (br, OH and NH); δ_H (400 MHz; DMSO-d_6)[†] 1.62 and 1.80 (2H, 2 x m, 4-H), 2.90 (4H, m, 1-H and 6-H), 3.20 (1H, m, 5-H), 3.46 (1H, m, 2-H), 3.71 (1H, m, 3-H), 7.18-7.34 (10H, m, Ar-H) and 8.10 (4H, br s, 2 x

[†] The hydroxyl proton was not observed presumably, due to rapid exchange with water present in the DMSO-d_6 used as the solvent.

NH_2); δ_C (100 MHz; DMSO-d_6) 34.9 (C-1), 35.2 (C-4), 37.8 (C-6), 50.5 (C-5), 56.4 (C-2), 66.1 (C-3), 126.6, 126.7, 128.5, 129.3, 129.4, 136.0 and 136.5 (Ar-C).

(2S,3S,5S)-2,5-Diamino-3-hydroxy-1,6-diphenylhexane **236**

A solution of the dihydrochloride salt **243** (0.50 g, 1.4 mmol) in water (2.0 mL) was basified with saturated aq. $NaHCO_3$ (monitored by pH indicator paper). The resulting solution was extracted with EtOAc (3 x 50 mL). The combined extracts were dried over anhydrous Na_2SO_4, and the solvent was evaporated *in vacuo* to afford *(2S,3S,5S)*-2,5-diamino-3-hydroxy-1,6-diphenylhexane **236** as a yellow oil (0.30 g, 77%) (Found: M^+-Bn, 193.13404. $C_{18}H_{24}N_2O$ requires *M*-Bn, 193.13409); ν_{max} (hexachlorobutadiene/cm^{-1}) 3467-3090 (br, OH and NH_2); δ_H (400 MHz; $CDCl_3$) 1.56 and 1.70 (2H, m, 4-H), 2.20 (5H, br s, 3-OH and 2 x NH_2), 2.54 and 2.85 (5H, m, 2-H and 1-H and 6-H), 3.11 (1H, m, 5-H), 3.72 (1H, m, 3-H), and 7.15-7.35 (10H, m, Ar-H); δ_C (100 MHz; $CDCl_3$) 39.1 (C-1), 41.3 (C-4), 47.3 (C-6), 54.0 (C-5), 57.5 (C-2), 74.5 (C-3), 126.1, 126.5, 128.4, 128.6, 129.3, 129.4, 138.3, and 139.7 (Ar-C).

3.5.2 Synthesis of chromone-2-carboxylic acid derivatives

Chromone-2-carboxylic acid **258**[165,166]

A mixture of diethyl oxalate (15 mL, 0.11 mol) and *o*-hydroxyacetophenone **244** (12 mL, 0.10 mol) was added dropwise under N_2 to a stirred ethanolic solution of NaOEt [generated *in situ* by adding Na metal (6.9 g, 0.3 mol) to dry EtOH (200 mL)]. The resulting yellow mixture was boiled gently under reflux for 45 min, during which time a thick yellow slurry was formed. After cooling, the yellow reaction mixture was poured into Et_2O (300 mL). After standing for 1 h, the yellow sodium salt was filtered off, washed with Et_2O and acidified with 2M-HCl (200 mL), and the resulting semi-solid mixture was extracted with Et_2O (3 x 100 mL). The combined ethereal extracts were dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo* to afford a yellow oily residue [indicated, by ^1H NMR spectroscopy, to contain a mixture of ethyl 1-(2-hydroxyphenyl)-1,3-dioxobutanoate (as an enol tautomer, formulated as **249**) and ethyl 2-hydroxychromanone-2-carboxylate **253**] which was used without further purification. The crude mixture, together with glacial acetic acid (45

mL) and conc. HCl (45 mL), was boiled under reflux for 1 h. After cooling, the precipitated solid was filtered off, washed with acetic acid and recrystallized from acetic acid to afford chromone-2-carboxylic acid **258** as a colourless solid (16 g, 85%), m.p. 249-251 °C (decom.p.) (lit.,[165] 265 °C); ν_{max} (KBr)/cm^{-1} 3300-2000 (br, OH) and 1740 and 1630 (2 x CO); δ_H (400 MHz; DMSO-d_6)‡ 6.91 (1H, s, 3-H), 7.54 (1H, d, *J*=7.6 Hz, 6-H), 7.72 (1H, d, *J*=8.3 Hz, 8-H), 7.87 (1H, m, 7-H) and 8.05 (1H, m, 5-H); δ_C (100 MHz; DMSO-d_6) 113.3 (C-3), 118.7 (C-8), 123.6 (C-4a), 124.8 (C-5), 125.9 (C-6), 135.0 (C-7), 153.2 (C-2), 155.3 (C-8a), 161.3 (COOH) and 177.4 (C=O).

6-Chlorochromone-2-carboxylic acid **259**[165,166]

The experimental procedure employed for the synthesis of chromone-2-carboxylic acid **258** was followed, using the crude mixture of ethyl 1-(5-chloro-2-hydroxyphenyl)-1,3-dioxo-butanoate **250** and ethyl 6-chloro-2-hydroxychromanone-2-carboxylate **254** {initially prepared using 5'-chloro-2'-hydroxyacetophenone **245** (4.5 g, 26 mmol), diethyl oxalate (20 mL, 0.15 mol) and NaOEt [generated *in situ* by adding Na metal (2.4 g, 0.11 mol) to dry EtOH (44 mL)]}, glacial acetic acid (22 mL) and conc. HCl (11 mL). Work-up afforded 6-chlorochromone-2-carboxylic acid **259** as a white solid (5.1 g, 88%); m.p. >260 °C (decom.p.) (from ethanol) (lit.,[188] 267-269 °C) (Found: M^+, 223.98806. $C_{10}H_5ClO_4$ requires *M*, 223.98764); ν_{max} (KBr)/cm^{-1} 3200-2100 (br, OH) and 1745 and 1657 (2 x CO); δ_H (400 MHz; DMSO-d_6)‡ 6.93 (1H, s, 3-H), 7.79 (1H, d, *J*=8.9 Hz, 8-H), 7.90 (1H, dd, *J*=2.6 and 9.0 Hz, 7-H), and 7.96 (1H, d, *J*=2.5 Hz, 5-H); *m/z* 223.9 (M^+, 100%).

6-Methoxychromone-2-carboxylic acid **260**[165,166]

The experimental procedure employed for the synthesis of chromone-2-carboxylic acid **258** was followed, using the crude mixture of ethyl 1-(2-hydroxy-5-methoxyphenyl)-1,3-dioxobutanoate **251** and ethyl 2-hydroxy-5-methoxychromanone-2-carboxylate **255** {initially prepared using 2'-hydroxy-5'-methoxyacetophenone **246** (4.0 g, 24 mmol), diethyl oxalate (5.0 mL, 36 mol) and NaOEt [generated *in situ* by adding Na metal (1.7 g, 72 mol) to dry EtOH (50 mL)]}, glacial acetic acid (60 mL) and conc. HCl (30 mL). Work-up afforded 6-methoxychromone-2-carboxylic acid **260** as a pale green solid (4.5 g, 85%), m.p. >260 °C (decom.p.) (lit.,[189] 268 °C) (Found: M^+, 220.03674. $C_{11}H_8O_5$ requires *M*, 220.03717); ν_{max}

‡ The carboxylic acid proton was not observed, presumably, due to rapid exchange with water present in the DMSO-d_6 used as the solvent.

(KBr)/cm^{-1} 3200-2200 (br, OH) and 1730 and 1640 (2 x CO); δ_H (400 MHz; DMSO-d_6)‡ 3.87 (3H, s, OCH_3), 6.88 (1H, s, 3-H), 7.40 (1H, d, J=3.1 Hz, 8-H), 7.45 (1H, m, 7-H), and 7.68 (1H, d, J=9.1 Hz, 5-H); δ_C (100 MHz; DMSO-d_6) 55.7 (OCH_3), 104.5 (C-3), 112.4 (C-8), 120.4 (C-4a), 124.2 (C-5), 124.4(C-7), 150.0 (C-6), 152.9 (C-2), 156.9 (C-8a), 161.3 (COOH) and 177.1 (C=O); *m/z* 220 (M^+, 100%).

5-Methoxychromone-2-carboxylic acid **261**[165,166]

The experimental procedure employed for the synthesis of chromone-2-carboxylic acid **258** was followed, using the crude mixture of ethyl 1-(2-hydroxy-6-methoxyphenyl)-1,3-dioxobutanoate **252** and ethyl 2-hydroxy-5-methoxychromanone-2-carboxylate **256** {initially prepared using 2'-hydroxy-6'-methoxyacetophenone **247** (2.0 g, 12 mmol), diethyl oxalate (3.4 mL, 25 mol) and NaOEt [generated *in situ* by adding Na metal (1.0 g, 43 mol) to dry EtOH (25 mL)]}, glacial acetic acid (30 mL) and conc. HCl (15 mL). Work-up afforded 5-methoxychromone-2-carboxylic acid **261** as a cream solid (4.8 g, 87%); m.p. 250-252 °C (decom.p.) (lit.,[190] 252-253 °C) (Found: M^+, 220.03717. $C_{11}H_8O_5$ requires *M*, 220.03854); ν_{max} (KBr)/cm^{-1} 3300-2200 (br, OH) and 1750 and 1650 (2 x CO); δ_H (400 MHz; DMSO-d_6)‡ 3.86 (3H, s, OCH_3), 6.70 (1H, s, 3-H), 7.02 (1H, d, J=8.2 Hz, 6-H), 7.16 (1H, d, J=8.3 Hz, 8-H), and 7.72 (1H, t, J=8.4 Hz, 7-H); *m/z* 220 (M^+, 100%).

7-Hydroxychromone-2-carboxylic acid **262**[166]

A mixture of 2',4'-dihydroxyacetophenone **248** (2.0 g, 13 mmol) and diethyl oxalate (3.5 mL, 26 mmol) was added to a stirred ethanolic solution of NaOEt [generated *in situ* by adding Na metal (1.2 g, 52 mmol) to dry EtOH (30 mL)]. The resulting mixture was boiled under reflux for 5 h, and then conc. HCl (7.2 mL) was added and heating was continued for 1 h. Charcoal (0.40 g), conc. HCl (6 mL) and H_2O (12 mL) were added, and the heating was continued for a further 4 h. The resulting hot solution was filtered and evaporated *in vacuo* to give a brown residue, which was washed well with hot water (60 °C), and recrystallized from aqueous EtOH [EtOH–H_2O (2:1)] to afford a brown solid, indicated by ^{1}H NMR analysis to be ethyl 7-hydroxychromone-2-carboxylate **257**. A mixture of ethyl 7-hydroxychromanone-2-carboxylate **257**, glacial acetic acid (20 mL) and conc. HCl (20 mL) was boiled under reflux for 12 h. After cooling, H_2O (40 mL) was added and the resulting precipitate was filtered off and washed well with cold H_2O to afford 7-hydroxychromone-2-carboxylic acid **262** as a

maroon solid (1.9 g, 72%); m.p. >280 °C (decom.p.) (lit.,[166] >300 °C) (Found: M^+, 206.02152. $C_{10}H_6O_5$ *M*, 206.02079); ν_{max} (KBr)/cm^{-1} 3400-2000 (br, OH) and 1760 and 1640 (2 x CO); δ_H (400 MHz; DMSO-d_6)‡ 6.79 (1H, s, 3-H), 6.89 (1H, d, *J*=2.1 Hz, 8-H), 6.96 (1H, dd, *J*=2.2and 8.8 Hz, 6-H), and 7.89 (1H, d, *J*=8.7 Hz, 5-H); *m/z* 206 (M^+, 100%).

3.5.3 Coupling of the isostere 236 *with chromone-2-carboxylic acid derivatives using EDC and HOBt*

*(2*S, *3*S, *5*S*)-2,5-Bis(benzopyran-4-one-2-carbamoyl)-3-hydroxy-1,6-diphenylhexane* **263**

To a solution of the diamine **236** (90 mg, 0.32 mmol) in dry DMF (5.0 mL) under N_2 were added 1-hydroxybenzotriazole hydrate (HOBt) (171 mg, 1.26 mmol), *N*-ethyl-*N'*-(dimethyl-aminopropyl)carbodiimide hydrochloride (EDC) (241 mg, 1.26 mmol) and chromone-2-carboxylic acid **258** (120 mg, 0.63 mmol). Triethylamine was then added to adjust the pH of the solution to 8.5. The resulting mixture was stirred at room temperature for 24 h, and then poured into H_2O (6 mL) and extracted with EtOAc (3 x 25 mL). The combined extracts were washed sequentially with 10% citric acid, saturated aq. $NaHCO_3$ (10 mL) and saturated brine (10 mL), and then dried over anhydrous Na_2SO_4. The solvent was evaporated *in vacuo* give a yellow residue, which was chromatographed [flash chromatography on silica gel; elution with hexane–EtOAc (1:9)] to afford *(2*S, *3*S, *5*S*)-2,5-bis(benzopyran-4-one-2-carbamoyl)-3-hydroxy-1,6-diphenylhexane* **263** as a white solid (115 mg, 60%), m.p. 126-128 °C (Found: M^+, 628.22076. $C_{38}H_{32}N_2O_7$ requires *M*, 628.22095); ν_{max} (KBr)/cm^{-1} 3500-3200 (br, OH and NH), 1655 and 1649 (2 x CO); δ_H (400 MHz; $CDCl_3$) 1.83 (2H, m, 4-H), 2.01 (2H, d, *J*=6.7 Hz, 2 x NH), 2.86-3.00 (4H, m, 1-H and 6-H), 3.89 (1H, br s, 3-H), 4.24 (1H, br s, 3-OH), 4.41 (2H, m, 2-H and 5-H), 6.96 and 7.05 (2H, 2 x s, 2 x 3'-H), 7.10-7.65 (16H, m, Ar-H) 8.00 and 8.10 (2H, 2 x d , *J*=8.0, 8.1 Hz, 2 x 5'-H); δ_C (100 MHz; $CDCl_3$) 38.2 (C-1), 39.4 (C-4), 40.8 (C-6), 49.9 (C-5), 55.6 (C-2), 69.4 (C-3), 111.8, 112.0, 118.0, 118.2, 123.9, 124.1, 125.7, 125.8, 126.0, 126.7, 127.0, 128.7, 129.2, 129.3, 134.5, 134.6, 136.6, 137.4, 154.5, 154.7, 155.0 and 155.2 (C-2', C-3' and Ar-C), 159.2 and 159.4 (2 x NCO), 178.0 and 178.2 (2 x C=O); *m/z* 628 (M^+, 2%) and 91 (100).

(2S, 3S, 5S)-2,5-Bis(6-chlorobenzopyran-4-one-2-carbamoyl)-3-hydroxy-1,6-diphenylhexane **264**

The experimental procedure employed for the synthesis of (2*S*, 3*S*, 5*S*)-2,5-*bis*(benzopyran-4-one-2-carbamoyl)-3-hydroxy-1,6-diphenylhexane **263** was followed, using the diamine **236** (80 mg, 0.28 mmol), 6-chlorochromone-2-carboxylic acid **259** (126 mg, 0.56 mmol), 1-hydroxybenzotriazole hydrate (HOBt) (150 mg, 1.12 mmol), *N*-ethyl-*N'*-(dimethylaminopropyl)carbodiimide hydrochloride (EDC) (251 mg, 1.12 mmol) and dry DMF (10 mL). Work-up afforded a yellow oily residue. Flash chromatography on silica gel [elution with hexane–EtOAc (2:8)] gave *(2S, 3S, 5S)-2,5-bis(6-chlorobenzopyran-4-one-2-carbamoyl)-3-hydroxy-1,6-diphenylhexane* **264** as a cream solid (93 mg, 52%), m.p. 226-228 °C (Found: M^+, 696.14184. $C_{38}H_{30}{}^{35}Cl_2N_2O_7$ requires *M*, 696.14301); ν_{max} (KBr)/cm^{-1} 3500-3200 (br, OH and NH), 1654 and 1648 (2 x CO); δ_H (400 MHz; $CDCl_3$) 1.86 (2H, m, 4-H), 2.28 (3H, br s, 3-OH and 2 x NH), 2.98 (4H, m, 1-H and 6-H), 3.90 (1H, br s, 3-H), 4.38 (2H, m, 2-H and 5-H), 6.93 and 7.02 (2H, 2 x s, 2 x 3'-H), 7.10-7.60 (14H, m, Ar-H), 7.90 and 8.00 (2H, 2 x d, *J*=2.5, 2.5 Hz, 2 x 5'-H); δ_C (100 MHz; $CDCl_3$) 38.2 (C-1), 39.3 (C-4), 40.8 (C-6), 50.0 (C-5), 55.6 (C-2), 69.4 (C-3), 111.7, 112.0, 119.7, 120.0, 124.7, 125.0, 125.2, 125.3, 126.8, 127.0, 128.7, 129.2, 129.3, 132.1, 132.2, 134.7, 134.8, 136.5, 137.2, 153.3, 153.4, 154.7 and 154.9 (C-2', C-3' and Ar-C), 158.8 and 159.0 (2 x NCO), 176.8 and 176.9 (2 x C=O); *m/z* 696 (M^+, 1%) and 91 (100).

(2S, 3S, 5S)-3-Hydroxy-2,5-bis(6-methoxybenzopyran-4-one-2-carbamoyl)-1,6-diphenylhexane **265**

The experimental procedure employed for the synthesis of (2*S*, 3*S*, 5*S*)-2,5-*bis*(benzopyran-4-one-2-carbamoyl)-3-hydroxy-1,6-diphenylhexane **263** was followed, using the diamine **236** (80 mg, 0.28 mmol), 6-methoxychromone-2-carboxylic acid **260** (125 mg, 0.56 mmol), 1-hydroxybenzotriazole hydrate (HOBt) (150 mg, 1.12 mmol), *N*-ethyl-*N'*-(dimethylaminopropyl)carbodiimide hydrochloride (EDC) (251 mg, 1.12 mmol) and dry DMF (10 mL). Work-up afforded a yellow oily residue. Flash chromatography on silica gel [elution with hexane–EtOAc (2:8)] to gave *(2S, 3S, 5S)-3-hydroxy-2,5-bis(6-methoxybenzopyran-4-one-2-carbamoyl)-1,6-diphenylhexane* **265** as a pale yellow solid (98 mg, 56%), m.p. 119-121 °C (Found: M^+, 688.24222. $C_{40}H_{36}N_2O_9$ requires *M*, 688.24208); ν_{max} (KBr)/cm^{-1} 3470-3000 (br, OH and NH), 1654 and 1638 (2 x CO); δ_H (400 MHz; $CDCl_3$) 1.85 (2H, m, 4-H), 2.30 (2H, br s, 2 x NH), 2.86-3.06 (4H, m, 1-H and 6-H), 3.77 and 3.80 (6H, 2 x s, 2 x OCH_3), 3.91

(1H, br s, 3-H), 4.40 (2H, m, 2-H and 5-H), 4.47 (1H, br s, 3-OH), 6.90 and 7.02 (2H, 2 x s, 2 x 3'-H) and 7.10-7.40 (16H, m, Ar-H); δ_C (100 MHz; $CDCl_3$) 38.2 (C-1), 39.1 (C-4), 40.7 (C-6), 50.0 (C-5), 55.6, 55.7 and 55.8 (C-2 and 2 x OCH_3), 69.4 (C-3), 104.7, 104.8, 110.7, 111.0, 119.4, 119.6, 124.4, 124.5, 124.8, 126.7, 126.8, 128.6, 129.2, 129.3, 136.7, 137.4, 149.7, 149.9, 137.2, 154.3, 154.5, 157.3 and 157.4 (C-2', C-3' and Ar-C), 159.1 and 159.4 (2 x NCO), 177.8 and 178.0 (2 x C=O); *m/z* 688 (M^+, 1%) and 91 (100).

(2S, 3S, 5S)-3-Hydroxy-2,5-bis(5-methoxybenzopyran-4-one-2-carbamoyl)-1,6-diphenylhexane **266**

The experimental procedure employed for the synthesis of (2*S*, 3*S*, 5*S*)-2,5-*bis*(benzopyran-4-one-2-carbamoyl)-3-hydroxy-1,6-diphenylhexane **263** was followed, using the diamine **236** (150 mg, 0.53 mmol), 5-methoxychromone-2-carboxylic acid **261** (233 mg, 1.06 mmol), 1-hydroxybenzotriazole hydrate (HOBt) (286 mg, 2.12 mmol), *N*-ethyl-*N'*-(dimethylaminopropyl)carbodiimide hydrochloride (EDC) (406 mg, 2.12 mmol) and dry DMF (10 mL). Work-up afforded a brown residue. Flash chromatography on silica gel [elution with $CHCl_3$–MeOH (9:1)] gave *(2S, 3S, 5S)-3-hydroxy-2,5-bis(5-methoxybenzopyran-4-one-2-carbamoyl)-1,6-diphenylhexane* **266** as a brown powder (300 mg, 82%), m.p. 143-145 °C (Found: M^+, 688.24070. $C_{40}H_{36}N_2O_9$ requires *M*, 688.24208); ν_{max} (KBr)/cm^{-1} 3300-2500 (br, OH and NH), 1650 and 1640 (2 x CO); δ_H (400 MHz; $CDCl_3$) 1.72-1.93 (5H, br s, 4-H, 3-OH and 2 x NH), 2.86-3.00 (4H, m, 1-H and 6-H), 3.86 and 3.90 (6H, 2 x s, 2 x OCH_3), 3.92 (1H,br s, 3-H), 4.36 (2H, m, 2-H and 5-H) and 6.66-7.50 (18H, m, 2 x 3'-H and Ar-H); δ_C (100 MHz; $CDCl_3$) 38.2 (C-1), 39.2 (C-4), 41.0 (C-6), 50.0 (C-5), 55.5 (C-2), 56.3 and 56.4 (2 x OCH_3), 69.5 (C-3), 106.9, 107.0, 109.8, 110.0, 113.4, 113.6, 114.6, 114.7, 126.7, 126.9, 128.6, 128.7, 129.3, 129.4, 134.5, 136.8, 137.4, 152.7, 152.8, 157.1, 157.2, 159.3 and 159.4 (C-2', C-3' and Ar-C), 159.7 and 159.8 (2 x NCO), 177.7 and 177.8 (2 x C=O); *m/z* 688 (M^+, 2%) and 377 (100).

(2S, 3S, 5S)-3-Hydroxy-2,5-bis(7-hydroxybenzopyran-4-one-2-carbamoyl)-1,6-diphenylhexane **267**

The experimental procedure employed for the synthesis of (2*S*, 3*S*, 5*S*)-2,5-*bis*(benzopyran-4-one-2-carbamoyl)-3-hydroxy-1,6-diphenylhexane **263** was followed, using the diamine **236** (160 mg, 0.56 mmol), 7-hydroxychromone-2-carboxylic acid **262** (230 mg, 1.12 mmol), 1-hydroxybenzotriazole hydrate (HOBt) (303 mg, 2.24 mmol), *N*-ethyl-*N'*-(dimethylamino-

propyl)carbodiimide hydrochloride (EDC) (430 mg, 2.24 mmol) and dry DMF (10 mL). Work-up afforded a yellow oily residue. Flash chromatography on silica gel (elution with EtOAc) gave *(2S, 3S, 5S)-3-hydroxy-2,5-bis(7-hydroxybenzopyran-4-one-2-carbamoyl)-1,6-diphenylhexane* **267** as a yellow solid (290 mg, 78%), m.p. 196-198 °C (Found: M^++H, 661.21755. $C_{38}H_{32}N_2O_9$ requires *M*+H, 661.21861); ν_{max} (KBr)/cm^{-1} 3500-3000 (br, OH and NH), 1660 and 1645 (2 x CO); δ_H (400 MHz; DMSO-d_6) 1.76 (2H, m, 4-H), 2.84 (2H, d, *J*=6.6 Hz, 1-H), 2.96 (2H, d, *J*=6.7 Hz, 6-H), 3.32 (2H, br s, 2 x NH), 3.74 (1H, br s, 3-H), 4.40 (2H, m, 2-H and 5-H), 5.05 (1H, br s, 3-OH), 6.57 and 6.64 (2H, 2 x s, 2 x 3'-H), 6.93-7.27 (12H, m, Ar-H), 7.87 (2H, dd, *J*=4.1 and 8.5 Hz, 2 x 8'-H), 8.39 and 8.73 (2H, 2 x d, *J*=9.0 Hz, 2 x 5'-H) and 10.90 (2H, br s, Ar-OH); δ_C (100 MHz; DMSO-d_6) 36.5 (C-1), 38.1 (C-4), 40.1 (C-6), 48.3 (C-5), 54.3 (C-2), 68.1 (C-3), 102.6, 102.7, 110.2, 110.4, 115.6, 116.3, 125.7, 125.8, 126.5, 127.9, 128.9, 129.0, 138.5, 138.8, 154.9, 155.0, 156.8, and 158.3 (C-2', C-3' and Ar-C), 159.0 and 163.1 (2 x NCO), 176.0 and 176.2 (2 x C=O); *m/z* 661 ($M+H^+$, 56%) and 91 (100).

3.5.4 Coupling of the isostere 236 *with chromone-2-carboxylic acid derivatives using CDI*

The tris-chromone derivative **268** *and the bis-chromone derivative* **263**

To a solution of the diamine **236** (30 mg, 0.11 mmol) in dry DMF (1.0 mL) was added to a stirred solution of chromone-2-carboxylic acid **258** (40 mg, 0.21 mmol), 1,1'-carbonyldiimidazole (CDI) (34 mg, 0.21 mmol) in dry DMF (1.0 mL) under N_2. The reaction mixture was stirred at room temperature for 48 h, and the reaction was then quenched with H_2O (3.0 mL). The mixture was extracted with EtOAc (3 x 10 mL), and the combined extracts were sequentially washed with 10% citric acid (5.0 mL), saturated aq. $NaHCO_3$ (5.0 mL) and brine (5.0 mL). The extracts were dried over anhydrous Na_2SO_4, and evaporation of the solvent *in vacuo* afforded a yellow oily residue, which was chromatographed (flash chromatography on silica gel; elution with EtOAc) to afford the tris-*chromone derivative* **268** as a pale yellow oil (45 mg, 54%) [ν_{max} (KBr)/cm^{-1} 3300 (NH), 1740, 1653 and 1650 (3 x CO); δ_H (400 MHz; $CDCl_3$) 2.00 (2H, m, CH_2), 2.88-3.02 (4H, m, 2 x CH_2Ph), 4.58 (1H, br s, 5-H), 4.81 (2H, m, 2-H and 3-H), 6.92 (1H, d, N-H), and 7.05-8.30 (26H, m, Ar-H, 3'-H and

N-H); FABMS *m/z* 800 (M^+, 100%)] and the bis-*chromone derivative* **263** as a white solid (20 mg, 31%).

The tris-chromone derivative **269** *and the bis-chromone derivative* **264**

The experimental procedure employed for the synthesis of the tris-chromone derivative **268** and *bis*-chromone derivative **263** was followed, using the diamine **236** (320 mg, 1.13 mmol), 6-chloro-chromone-2-carboxylic acid **259** (507 mg, 2.26 mmol), CDI (366 mg, 2.26 mmol) and dry DMF (10 mL). Work-up afford a yellow oily residue. Flash chromatography on silica gel [elution with hexane-EtOAc (1:3)] gave the tris-*chromone derivative* **269** as a white solid (500 mg, 49%), m.p. 180-183 °C [ν_{max} (KBr)/cm^{-1} 3270 (NH), 1736, 1655 and 1650 (3 x CO); δ_H (400 MHz; $CDCl_3$) 2.05 (2H, m, CH_2), 2.96 (4H, m, 2 x CH_2Ph), 4.61 (1H, br s, 5-H), 4.93 and 5.32 (2H, m, 2-H and 3-H), 6.91 (1H, d, N-H), and 7.00-8.10 (23H, m, Ar-H, 3'-H and N-H); δ_C (100 MHz; $CDCl_3$) 35.9, 38.3 and 41.1 (3 x CH_2), 48.3 (C-5), 52.2 (C-2), 76.0 (C-3), 112.0, 112.3, 114.9, 119.6, 119.9, 120.3, 124.9, 125.0, 125.3, 127.1, 127.2, 128.8, 128.9, 129.0, 129.4, 132.1, 132.3, 132.5, 134.7, 134.9, 135.3, 135.7, 136.1, 151.4, 153.2, 153.4, 153.9, 154.3, 154.4, 158.5, 159.2 and 159.5 (Ar-C and C-3'), 176.6, 176.7 and 177.0 (3 x C=O); FABMS *m/z* 902 (M^+, 100%)] and the bis-*chromone derivative* **264** as a cream white solid (210 mg, 27%).

The Tris-chromone derivative **270** *and the bis-chromone derivative* **265**

The experimental procedure employed for the synthesis of the tris-chromone derivative **268** and the bis-chromone **263** was followed, using the diamine **236** (250 mg, 0.879 mmol), 6-methoxy-chromone-2-carboxylic acid **260** (390 mg, 1.76 mmol), CDI (290 mg, 1.76 mmol) and dry DMF (10 mL). Work-up afford a yellow oily residue. Flash chromatography on silica gel [elution with hexane-EtOAc (1:3)] gave the tris-*chromone derivative* ***270*** as a cream white solid (470 mg, 60%), m.p. 235-237 °C [ν_{max} (KBr)/cm^{-1} 3300 (NH), 1740, 1653 and 1650 (3 x CO); δ_H (400 MHz; $CDCl_3$) 2.02 (2H, m, CH_2), 2.93 (4H, m, 2 x CH_2Ph), 3.86, 3.89, and 3.91 (9H, 3 x s, 3 x OCH_3), 4.59 (1H, br s, 5-H), 4.90 and 5.31 (2H, m, 2-H and 3-H) and 6.70-7.60 (24H, m, Ar-H, 3'-H and 2 x N-H); FABMS *m/z* 891 ($M+H^+$, 100%)] and the bis-chromone derivative **265** as a pale yellow solid (210 mg, 35%).

3.6 Synthesis of chromone-3-carbaldehydes

Chromone-3-carbaldehyde **27**[29]

$POCl_3$ (9.4 mL, 0.10 mol) was added dropwise, during a period of 30 min, to a stirred solution of *o*-hydroxyacetophenone **43** (3.0 mL, 25 mmol) in dry DMF (25 mL) under N_2, while maintaining the temperature at –20 °C using a dry ice-acetone bath. The resulting mixture was stirred overnight at room temperature and then poured into ice-water (50 mL). The resulting precipitate was filtered off, and washed successively with water and EtOH. Recrystallization from acetone afforded chromone-3-carbaldehyde **27** as a colourless crystalline solid (2.6 g, 60%), m.p. 152-154 °C (lit.,[29] 152-153 °C); ν_{max} (KBr)/cm^{-1} 1649 and 1690 (2 x CO); δ_H (400 MHz; $CDCl_3$) 7.47 (1H, t, *J*=7.5 Hz, 6-H), 7.51 (1H, d, *J*=8.6 Hz, 8-H), 7.73 (1H, t, *J*=7.2 Hz, 7-H), 8.27 (1H, d, *J*=7.7 Hz, 5-H), 8.52 (1H, s, 2-H) and 10.35 (1H, s, CHO); δ_C (100 MHz; $CDCl_3$) 118.6 (C-8), 120.4 (C-3), 125.4 (C-4a), 126.2 (C-5), 126.6 (C-6), 134.8 (C-7), 156.2 (C-8a), 160.5 (C-2), 175.9 (C=O) and 188.5 (CHO); *m/z* 174 (M^+, 6%) and 146 (100).

6-Bromochromone-3-carbaldehyde **282**[29]

The experimental procedure employed for the synthesis of chromone-3-carbaldehyde **27** was followed, using $POCl_3$ (8.4 mL, 90 mmol), 5'-bromo-2'-hydroxyacetophenone **277** (5.0 g, 23 mmol) and dry DMF (25 mL). Work-up afforded 6-bromochromone-3-carbaldehyde **282** as a yellow crystalline solid (4.2 g, 72%), m.p. 193-194 °C (lit.,[191] 186-188 °C); ν_{max} (KBr)/cm^{-1} 1655 and 1699 (2 x CO); δ_H (400 MHz; $CDCl_3$) 7.42 (1H, d, *J*=8.8 Hz, 8-H), 7.82 (1H, dd, *J*=2.2 and 8.8 Hz, 7-H), 8.38 (1H, d, *J*=2.1 Hz, 5-H), 8.51 (1H, s, 2-H) and 10.33 (1H, s, CHO); δ_C (100 MHz; $CDCl_3$) 120.3 (C-3), 120.4 (C-6), 120.5 (C-8), 126.6 (C-4a), 128.8 (C-5), 137.8 (C-7), 155.0 (C-8a), 160.6 (C-2), 174.6 (C=O) and 188.0 (CHO); *m/z* 252 (M^+, 4%) and 226 (100).

6-Chlorochromone-3-carbaldehyde **283**[29]

The experimental procedure employed for the synthesis of chromone-3-carbaldehyde **27** was followed, using $POCl_3$ (18.7 mL, 200 mmol), 5'-chloro-2'-hydroxyacetophenone **278** (8.53 g, 50.0 mmol) and dry DMF (50 mL). Work-up afforded 6-chlorochromone-3-carbaldehyde **283** as a yellow crystalline solid (7.0 g, 67%), m.p. 166-168 °C (lit.,[29] 166-168 °C); ν_{max}

(KBr)/cm^{-1} 1655 and 1695 (2 x CO); δ_H (400 MHz; $CDCl_3$) 7.49 (1H, d, *J*=8.9 Hz, 8-H), 7.68 (1H, dd, *J*=2.6 and 8.9 Hz, 7-H), 8.22 (1H, d, *J*=2.5 Hz, 5-H), 8.52 (1H, s, 2-H) and 10.33 (1H, s, CHO); δ_C (100 MHz; $CDCl_3$) 120.2 (C-3), 120.3 (C-8), 125.6 (C-5), 126.3 (C-4a), 132.8 (C-6), 135.0 (C-7), 154.5 (C-8a), 160.6 (C-2), 174.8 (C=O) and 188.0 (CHO); *m/z* 208 (M^+, 4%) and 180 (100).

6-Fluorochromone-3-carbaldehyde **284**[29]

The experimental procedure employed for the synthesis of chromone-3-carbaldehyde **27** was followed, using $POCl_3$ (8.40 mL, 90.0 mmol), 5'-fluoro-2'-hydroxyacetophenone **279** (5.0 g, 32 mmol) and dry DMF (25 mL). Work-up afforded 6-fluorochromone-3-carbaldehyde **284** as a yellow crystalline solid (3.6 g, 58%), m.p. 157-159 °C (lit.,[176] 158 °C); ν_{max} (KBr)/cm^{-1} 1657 and 1701 (2 x CO); δ_H (400 MHz; $CDCl_3$) 7.44 (1H, m, 7-H), 7.56 (1H, m, 8-H), 7.91 (1H, dd, *J*=2.2 and 7.7 Hz, 5-H), 8.52 (1H, s, 2-H) and 10.34 (1H, s, CHO); δ_C (100 MHz; $CDCl_3$) 111.3 (J_{CF} =23 Hz, C-5), 119.7 (C-4a), 120.8 (J_{CF} =8.2 Hz, C-8), 123.0 (J_{CF} =25 Hz, C-7), 126.7 and 126.8 (C-3 and C-6), 152.4 (C-8a), 160.6 (C-2), 175.2 (C=O) and 188.1 (CHO); *m/z* 192 (M^+, 4%) and 164 (100).

6-Methoxychromone-3-carbaldehyde **285**[29]

The experimental procedure employed for the synthesis of chromone-3-carbaldehyde **27** was followed, using $POCl_3$ (13.5 mL, 144 mmol), 5'-methoxy-2'-hydroxyacetophenone **280** (6.0 g, 36 mmol) and dry DMF (25 mL). Work-up afforded 6-methoxychromone-3-carbaldehyde **285** as a yellow crystalline solid (3.88 g, 62%), m.p. 163-165 °C (lit.,[29] 164-166 °C); ν_{max} (KBr)/cm^{-1} 1650 and 1690 (2 x CO); δ_H (400 MHz; $CDCl_3$) 3.90 (3H, s, OCH_3), 7.30 (1H, dd, *J*=2.9 and 9.1 Hz, 7-H), 7.45 (1H, d, *J*=9.1 Hz, 8-H), 7.62 (1H, d, *J*=3.0 Hz, 5-H), 8.51 (1H, s, 2-H) and 10.37 (1H, s, CHO); δ_C (100 MHz; $CDCl_3$) 56.0 (OCH_3), 105.4 (C-5), 119.5 (C-3), 120.0 (C-8), 124.4 (C-7), 126.1 (C-4a), 151.0 (C-6), 157.9 (C-8a), 160.2 (C-2), 175.8 (C=O) and 188.7 (CHO); *m/z* 204 (M^+, 1%) and 176 (100).

5-Methoxychromone-3-carbaldehyde **286**[29]

The experimental procedure employed for the synthesis of chromone-3-carbaldehyde **27** was followed, using $POCl_3$ (11.2 mL, 120 mmol), 6'-methoxy-2'-hydroxyacetophenone **281** (5.0 g, 30 mmol) and dry DMF (25 mL). Work-up afforded 5-methoxychromone-3-carbaldehyde

286 as a yellow crystalline solid (3.2 g, 52%), m.p. 113-115 °C (lit.,[29] 115-116 °C); ν_{max} (KBr)/cm^{-1} 1650 and 1697 (2 x CO); δ_H (400 MHz; $CDCl_3$) 3.99 (3H, s, OCH_3), 6.87 (1H, d, *J*=8.3 Hz, 6-H), 7.05 (1H, dd, *J*=0.7 and 8.3 Hz, 8-H), 7.60 (1H, t, *J*=8.4 Hz, 7-H), 8.36 (1H, s, 2-H) and 10.32 (1H, s, CHO); δ_C (100 MHz; $CDCl_3$) 56.5 (OCH_3), 107.9 (C-6), 110.4 (C-8), 115.5 (C-4a), 121.2 (C-3), 134.9 (C-7), 158.0 (C-8a), 158.7 (C-2), 160.3 (C-5), 175.8 (C=O) and 189.1 (CHO); *m/z* 204 (M^+, 2%) and 176 (100).

3.7 Morita-Baylis-Hillman reactions of chromone-3-carbaldehydes

3.7.1 Reactions of chromone-3-carbaldehydes with acrylonitrile

*3-(3-Hydroxy-2-cyanopropen-3-yl)-4*H*-1-benzopyran-4-one* **287**

Acrylonitrile (0.56 mL, 8.6 mmol) and 3-hydroxyquinuclidine (3.63 g, 28.8 mmol) were added to a stirred solution of chromone-3-carbaldehyde **27** (1.0 g, 5.7 mmol) dissolved in a minimum volume of $CHCl_3$ (8.0 mL). The resulting intense red mixture was stirred at room temperature for 25 h. Flash chromatography on silica gel [elution with hexane-EtOAc (2:3)] afforded 3-(3-hydroxy-2-cyanopropen-3-yl)-4*H*-1-benzopyran-4-one **287** as a yellow crystalline solid (0.83 g, 64%), m.p. 69-71 °C (lit.,[176] 68-70 °C); (Found: M^+, 227.0570. $C_{13}H_9NO_3$ requires *M*, 227.0582); ν_{max} (thin film)/cm^{-1} 3430 (br, OH), 2225 (CN) and 1630 (CO); δ_H (400 MHz; $CDCl_3$) 4.41 (1H,br s, 3'-OH), 5.30 (1H, s, 3'-H), 6.13 and 6.32 (2H, 2 x s, 1'-H), 7.45 (1H, m, 6-H), 7.50 (1H, d, *J*=8.3 Hz, 8-H), 7.71 (1H, t, *J*=7.0 Hz, 7-H), 8.08 (1H, s, 2-H) and 8.18 (1H, dd, *J*=1.4 and 8.0 Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 69.2 (C-3'), 116.8 and 124.1 (C-2' or CN), 118.4 (C-8), 121.2 (C-3), 123.7 (C-4a), 125.6 (C-6), 125.8 (C-5), 131.3 (C-1'), 134.5 (C-7), 153.9 (C-2), 156,3 (C-8a) and 177.7 (C=O); *m/z* 227 (M^+, 46%) and 210 (100).

*6-Bromo-3-(3-hydroxy-2-cyanopropen-3-yl)-4*H*-1-benzopyran-4-one* **288**

The experimental procedure employed for the synthesis of 3-(3-hydroxy-2-cyanopropen-3-yl)-4*H*-1-benzopyran-4-one **287** was followed, using 6-bromochromone-3-carbaldehyde **282** (0.5 g, 2.0 mmol), acrylonitrile (0.20 mL, 3.0 mmol), 3-hydroxyquinuclidine (1.27 g, 10.0 mmol) and $CHCl_3$ (6.0 mL). Work-up afforded 6-bromo-3-(3-hydroxy-2-cyanopropen-3-yl)-4*H*-1-benzopyran-4-one **288** as an orange-yellow solid (0.40 g, 67%), m.p. 123-125 °C

(lit.,[176] 122-125 °C); (Found: M^+, 304.9724. $C_{13}H_8{}^{79}BrNO_3$ requires *M*, 304.9688); ν_{max} (thin film)/cm^{-1} 3420 (br, OH), 2225 (CN) and 1648 (CO); δ_H (400 MHz; $CDCl_3$) 4.10 (1H,br s, 3'-OH), 5.33 (1H, s, 3'-H), 6.14 and 6.32 (2H, 2 x s, 1'-H), 7.40 (1H, d, *J*=8.8 Hz, 8-H), 7.80 (1H, dd, *J*=2.3 and 8.8 Hz, 7-H), 8.09 (1H, s, 2-H) and 8.28 (1H, d, *J*=2.3 Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 68.8 (C-3'), 116.6 (CN), 119.3 (C-6), 120.3 (C-8), 121.6 (C-2'), 123.9 (C-3), 124.9 (C-4a), 128.2 (C-5), 131.6 (C-1'), 137.5 (C-7), 154.1 (C-2), 155.1 (C-8a) and 176.1 (C=O); *m/z* 305 (M^+, 44%) and 253 (100).

6-Chloro-3-(3-hydroxy-2-cyanopropen-3-yl)-4H-1-benzopyran-4-one **289**

The experimental procedure employed for the synthesis of 3-(3-hydroxy-2-cyanopropen-3-yl)-4*H*-1-benzopyran-4-one **287** was followed, using 6-chlorochromone-3-carbaldehyde **283** (0.50 g, 2.4 mmol), acrylonitrile (0.24 mL, 3.6 mmol), 3-hydroxyquinuclidine (1.53 g, 12.0 mmol) and $CHCl_3$ (7.0 mL). Work-up afforded 6-chloro-3-(3-hydroxy-2-cyanopropen-3-yl)-4*H*-1-benzopyran-4-one **289** as a yellow crystalline solid (0.37 g, 59%), m.p. 133-134 °C (lit.,[176] 131-133 °C); (Found: M^+, 261.0196. $C_{13}H_8{}^{35}ClNO_3$ requires *M*, 261.0193); ν_{max} (thin film)/cm^{-1} 3440 (br, OH), 2300 (CN) and 1653 (CO); δ_H (400 MHz; $CDCl_3$) 4.16 (1H, d, *J*=6.8 Hz, 3'-OH), 5.34 (1H, d, *J*=6.5 Hz, 3'-H), 6.13 and 6.32 (2H, 2 x s, 1'-H), 7.47 (1H, d, *J*=8.9 Hz, 8-H), 7.66 (1H, dd, *J*=2.5 and 8.9 Hz, 7-H), 8.09 (1H, s, 2-H) and 8.11 (1H, d, *J*=2.4 Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 68.7 (C-3'), 116.6 and 123.9 (C-2' or CN), 120.1 (C-8), 121.7 (C-3), 124.5 (C-4a), 125.0 (C-5), 131.6 (C-1'), 131.8 (C-6), 134.7 (C-7), 154.1 (C-2), 154.6 (C-8a) and 176.3 (C=O); *m/z* 261 (M^+, 52%) and 209 (100).

6-Fluoro-3-(3-hydroxy-2-cyanopropen-3-yl)-4H-1-benzopyran-4-one **290**

The experimental procedure employed for the synthesis of 3-(3-hydroxy-2-cyanopropen-3-yl)-4*H*-1-benzopyran-4-one **287** was followed, using 6-fluorochromone-3-carbaldehyde **284** (0.50 g, 2.6 mmol), acrylonitrile (0.26 mL, 3.9 mmol), 3-hydroxyquinuclidine (1.66 g, 13.0 mmol) and $CHCl_3$ (6.0 mL). Work-up afforded 6-fluoro-3-(3-hydroxy-2-cyanopropen-3-yl)-4*H*-1-benzopyran-4-one **290** as a yellow solid (0.34 g, 53%), m.p. 60-62 °C (lit.,[176] 58-60 °C); (Found: M^+, 245.0490. $C_{13}H_8FNO_3$ requires *M*, 245.0488); ν_{max} (thin film)/cm^{-1} 3200 (br, OH), 2235 (CN) and 1640 (CO); δ_H (400 MHz; $CDCl_3$) 4.16 (1H, d, *J*=6.0 Hz, 3'-OH), 5.34 (1H, d, *J*=4.0 Hz, 3'-H), 6.13 and 6.32 (2H, 2 x s, 1'-H), 7.46 (1H, m, 7-H), 7.52 (1H, m, 8-H), 7.80 (1H, dd, *J*=2.9 and 8.0 Hz, 5-H) and 8.10 (1H, s, 2-H); δ_C (100 MHz; $CDCl_3$) 68.8

(C-3'), 110.5 (J_{CF} =23 Hz, C-5), 116.6 and 124.0 (C-2' or CN), 120.6 (J_{CF} =8.1 Hz, C-8), 120.9 (C-3), 122.8 (J_{CF} = 25.4 Hz, C-7), 124.8 (J_{CF} =7.5 Hz, C-4a), 131.4 (C-1'), 152.6 (C-8a), 154.1 (C-2), 159.7 (J_{CF} =246.8 Hz, C-6) and 176.7 (C=O); *m/z* 245 (M^+, 57%) and 193 (100).

*3-(3-Hydroxy-2-cyanopropen-3-yl)-6-methoxy-4*H*-1-benzopyran-4-one* **291**

The experimental procedure employed for the synthesis of 3-(3-hydroxy-2-cyanopropen-3-yl)-4*H*-1-benzopyran-4-one **287** was followed, using 6-methoxychromone-3-carbaldehyde **285** (0.50 g, 2.5 mmol), acrylonitrile (0.24 mL, 3.7 mmol), 3-hydroxyquinuclidine (1.56 g, 12.3 mmol) and $CHCl_3$ (6.0 mL). Work-up afforded 3-(3-hydroxy-2-cyanopropen-3-yl)-6-methoxy-4*H*-1-benzopyran-4-one **291** as a yellow solid (0.34 g, 54%), m.p. 113-115 °C (lit.,[176] 114-117 °C); (Found: M^+, 257.0686. $C_{14}H_{11}NO_4$ requires *M*, 257.0688); ν_{max} (thin film)/cm^{-1} 3400 (br, OH), 2225 (CN) and 1650 (CO); δ_H (400 MHz; $CDCl_3$) 3.89 (3H, s, OCH_3), 4.50 (1H, s, *J*=4.0 Hz, 3'-OH), 5.33 (1H, d, *J*=3.8 Hz, 3'-H), 6.10 and 6.30 (2H, 2 x s, 1'-H), 7.27 (1H, dd, *J*=2.9 and 9.0 Hz, 7-H), 7.41 (1H, d, *J*=9.0 Hz, 8-H), 7.49 (1H, d, *J*=3.0 Hz, 5-H) and 8.08 (1H, s, 2-H); δ_C (100 MHz; $CDCl_3$) 55.9 (OCH_3), 68.8 (C-3'), 104.4 (C-5), 116.8 (C-3), 119.8 (C-8), 120.7 (C-4a), 124.2 and 124.3 (C-2' or CN), 124.6 (C-7), 131.2 (C-1'), 151.2 (C-8a), 153.7 (C-2), 157.3 (C-6) and 177.3 (C=O); *m/z* 257 (M^+, 75%) and 205 (100).

*3-(3-Hydroxy-2-cyanopropen-3-yl)-5-methoxy-4*H*-1-benzopyran-4-one* **292**

The experimental procedure employed for the synthesis of 3-(3-hydroxy-2-cyanopropen-3-yl)-4*H*-1-benzopyran-4-one **287** was followed, using 5-methoxychromone-3-carbaldehyde **286** (0.50 g, 2.5 mmol), acrylonitrile (0.24 mL, 3.7 mmol), 3-hydroxyquinuclidine (1.56 g, 12.3 mmol) and $CHCl_3$ (6.0 mL). Work-up afforded 3-(3-hydroxy-2-cyanopropen-3-yl)-5-methoxy-4*H*-1-benzopyran-4-one **292** as a yellow solid (0.38 g, 60%), m.p. 99-102 °C (lit.,[176] 97-100 °C) (Found: $M+H^+$, 258.0766. $C_{14}H_{11}NO_4$ requires *M+H*, 258.0766); ν_{max} (thin film)/cm^{-1} 3400 (br, OH), 2355 (CN) and 1650 (CO); δ_H (400 MHz; $CDCl_3$) 3.99 (3H, s, OCH_3), 4.60 (1H, s, *J*=7.2 Hz, 3'-OH), 5.18 (1H, d, *J*=5.6 Hz, 3'-H), 6.10 and 6.31 (2H, 2 x s, 1'-H), 6.84 (1H, d, *J*=8.3 Hz, 6-H), 7.15 (1H, d, *J*=8.4 Hz, 8-H), 7.60 (1H, t, *J*=8.4 Hz, 7-H) and 7.92 (1H, s, 2-H); δ_C (100 MHz; $CDCl_3$) 55.6 (OCH_3), 69.6 (C-3'), 106.8 (C-6), 110.3

(C-8), 114.4 (C-4a), 117.0 (CN), 121.9 (C-3), 124.1 (C-2'), 131.2 (C-1'), 134.7 (C-7), 152.1 (C-2), 158.3 (C-8a), 160.0 (C-5) and 177.7 (C=O); *m/z* 257 (M^+, 19%) and 239 (100).

3.7.2 Reactions of chromone-3-carbaldehydes with methyl acrylate

*3-(3-Hydroxy-2-methoxycarbonylpropen-3-yl)-4*H*-1-benzopyran-4-one* **293** *and the corresponding dimer* **299**

Methyl acrylate (0.40 mL, 4.3 mmol) and 3-hydroxyquinuclidine (1.83 g, 14.4 mmol) were added to a stirred solution of chromone-3-carbaldehyde **27** (0.50 g, 2.9 mmol) dissolved in a minimum volume of $CHCl_3$ (6.0 mL). The resulting intense red mixture was stirred at room temperature for 24 h. Evaporation of the solvent gave, *in vacuo* an intense red oily residue. Flash chromatography on silica gel {elution with a mixture of hexane-EtOAc-petroleum ether [b.p. 80-100°C]-toluene (1:1:1:1)} afforded two fractions:-

i) 3-(3-hydroxy-2-methoxycarbonylpropen-3-yl)-4*H*-1-benzo-pyran-4-one **293** as a yellow solid (0.49 g, 66%), m.p. 108-110 °C (lit.,[176] 109-112 °C); (Found: M^+, 260.0690. $C_{14}H_{12}O_5$ requires *M*, 260.0685); ν_{max} (thin film)/cm^{-1} 3420 (br, OH), 1720 and 1640 (2 x CO); δ_H (400 MHz; $CDCl_3$) 3.74 (3H, s, OCH_3), 4.58 (1H,br s, 3'-OH), 5.59 (1H, s, 3'-H), 6.14 and 6.42 (2H, 2 x s, 1'-H), 7.40 (1H, m, 6-H), 7.45 (1H, d, *J*=8.3 Hz, 8-H), 7.68 (1H, m, 7-H), 8.02 (1H, s, 2-H) and 8.18 (1H, dd, *J*=1.4 and 8.0 Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 52.0 (CO.*OCH*$_3$), 67.6 (C-3'), 118.2 (C-8), 123.0 (C-3), 124.0 (C-4a), 125.4 (C-6), 125.6 (C-5), 126.8 (C-1'), 134.0 (C-7), 139.5 (C-2'), 154.3 (C-2), 156,3 (C-8a), 166.5 (CO.O) and 177.9 (C=O); *m/z* 260 (M^+, 7%) and 200 (100); and

ii) The corresponding dimer **299** as a pale yellow solid (0..25 g, 34%), m.p. 192-195 °C (lit.,[176] 193-194 °C) (Found: M^+, 502.1250. $C_{28}H_{22}O_9$ requires *M*, 502.1257); ν_{max} (thin film)/cm^{-1} 1710, 1705, 1650 and 1631 (4 x CO); δ_H (400 MHz; $CDCl_3$) 3.12 and 3.38 (2H, dd, 13H), 3.61 (3H, s, 12-H), 3.65 (3H, s, 16-H), 4.50 (2H, dd, *J*=2.0 and 17.0 Hz, 2-H_a and 2-H_b), 5.05 (1H, s, 9a-H), 6.90 (1H, t, *J*=7.8 Hz, 6-H), 6.97(1H, d, *J*=8.4 Hz,5-H), 7.30 (1H, s, 4-H), 7.35(1H, t, *J*=8.4 Hz, 7-H), 7.40 (2H, m, 7'-H and 8'-H), 7.50 (1H, s, 17-H), 7.69 (1H, t, *J*=7.2 Hz, 6'-H), 7.72 (1H, dd, 8-H), 7.90 (1H, s, 2'-H) and 8.16 (1H, d, *J*= 7.2 Hz, 5'-H); δ_C (100 MHz; $CDCl_3$) 28.4 (C-13), 50.3 (C-4a), 51.8 (C-12), 52.1 (C-16), 65.8 (C-2), 99.9 (C-9a), 117.7 (C-8), 117.9 (C-8'), 119.9 (C-10a), 120.5 (C-4a'), 122.7 (C-6), 123.9 (C-

3'), 125.5 (C-6'), 126.2 (C-5), 127.7 (C-5'), 129.1 (C-3), 130.8 (C-14), 133.1 (C-17), 133.9 (C-7'), 136.0 (C-7), 136.1 (C-4), 154.9 (C-2'), 155.8 (C-8a'), 157.1 (C-8a), 163.8 (C-11), 167.3 (C-15), 174.9 (C-4') and 191.4 (C-10); *m/z* 502 (M^+, 24%) and 243 (100).

6-Bromo-3-(3-hydroxy-2-methoxycarbonylpropen-3-yl)-4H-1-benzopyran-4-one **294** *and the corresponding dimer* **300**

The experimental procedure employed for the synthesis of 3-(3-hydroxy-2-methoxycarbonyl-propen-3-yl)-4*H*-1-benzopyran-4-one **293** and the corresponding dimer **299** was followed, using 6-bromochromone-3-carbaldehyde **282** (0.50 g, 2.0 mmol), methyl acrylate (0.27 mL, 3.0 mmol), 3-hydroxyquinuclidine (1.26 g, 9.92 mmol) and $CHCl_3$ (3.0 mL). Work-up and flash chromatography afforded two fractions:-

i) 6-bromo-3-(3-hydroxy-2-methoxycarbonylpropen-3-yl)-4*H*-1-benzopyran-4-one **294** as a yellow solid (0.42 g, 63%), m.p. 114-116 °C (lit.,[176] 114-116 °C); (Found: M^+, 337.9790. $C_{14}H_{11}{}^{79}BrO_5$ requires *M*, 337.9789); ν_{max} (thin film)/cm^{-1} 3440 (br, OH), 1716 and 1642 (2 x CO); δ_H (400 MHz; $CDCl_3$) 3.75 (3H, s, OCH_3), 4.38 (1H, d, *J*=7.8 Hz, 3'-OH), 5.59 (1H, d, *J*= 7.8 Hz, 3'-H), 6.11 and 6.42 (2H, 2 x s, 1'-H), 7.37 (1H, d, *J*=8.9 Hz, 8-H), 7.75 (1H, dd, *J*=2.5 and 9.0 Hz, 7-H), 8.02 (1H, s, 2-H) and 8.28 (1H, d, *J*=2.5 Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 52.0 (CO.*OCH*$_3$), 67.5 (C-3'), 118.8 (C-8), 120.2 (C-6), 123.3 (C-3), 125.2 (C-4a), 127.0 (C-1'), 128.3 (C-5), 137.0 (C-7), 139.2 (C-2'), 154.5 (C-2), 155.0 (C-8a), 166.5 (CO.O) and 176.5 (C=O); *m/z* 338 (M^+, 17%) and 280 (100); and

i) The corresponding dimer **300** as a yellow solid (0.23 g, 37%), m.p. 222-224 °C (lit.,[176] 223-225 °C) (Found: M^+, 657.9471. $C_{28}H_{20}{}^{79}Br_2O_9$ requires *M*, 657.9474); ν_{max} (thin film)/cm^{-1} 1715, 1705, 1650 and 1646 (4 x CO); δ_H (400 MHz; $CDCl_3$) 3.11 and 3.33 (2H, dd, 13H), 3.65 (3H, s, 12-H), 3.69 (3H, s, 16-H), 4.51 (2H, dd, *J*=2.0 and 17.0 Hz, 2-H_a and 2-H_b), 5.02 (1H, s, 9a-H), 6.88 (1H, d, *J*=8.8 Hz, 8-H), 7.27(1H, m, 4-H), 7.35 (1H, d, *J*=8.9 Hz, 7'-H), 7.41 (1H, dd, *J*=2.5 and 9.0 Hz, 7-H), 7.48 (1H, s, 17-H), 7.78 (1H, dd, *J*=2.5 and 9.0 Hz, 8'-H), 7.81 (1H, d, *J*=2.5 Hz, 5-H), 7.88 (1H, d, *J*=1.0 Hz, 2'-H) and 8.26 (1H, d, *J*= 2.5 Hz, 5'-H); δ_C (100 MHz; $CDCl_3$) 28.4 (C-13), 50.3 (C-4a), 52.0 (C-12), 52.2 (C-16), 66.0 (C-2), 100.0 (C-9a), 115.7 (C-8), 119.2 (C-4a'), 119.8 (C-8'), 120.1 (C-10a), 120.6 (C-6), 121.3 (C-6'), 125.0 (C-3'), 128.8 (C-5'), 129.3 (C-3), 130.1 (C-5), 131.2 (C-14), 132.8 (C-17), 135.4 (C-7'), 137.0 (C-7), 138.8 (C-4), 154.4 (C-2'), 155.0 (C-8a'), 156.0 (C-8a), 163.8 (C-11), 167.1 (C-15), 173.5 (C-4') and 190.2 (C-10); *m/z* 658 (M^+,10%) and 323 (100).

6-Chloro-3-(3-hydroxy-2-methoxycarbonylpropen-3-yl)-4H-1-benzopyran-4-one **295** *and the corresponding dimer* **301**

The experimental procedure employed for the synthesis of 3-(3-hydroxy-2-methoxycarbonyl-propen-3-yl)-4*H*-1-benzopyran-4-one **293** and the corresponding dimer **299** was followed, using 6-chlorochromone-3-carbaldehyde **283** (0.50 g, 2.4 mmol), methyl acrylate (0.33 mL, 3.6 mmol), 3-hydroxyquinuclidine (1.53 g, 12.0 mmol) and $CHCl_3$ (3.0 mL). Work-up and flash chromatography afforded two fractions:-

i) 6-chloro-3-(3-hydroxy-2-methoxycarbonylpropen-3-yl)-4*H*-1-benzopyran-4-one **295** as a cream white solid (0.35 g, 50%), m.p. 110-112 °C (lit.,[176] 108-110 °C); (Found: **M**+H^+, 295.0373. $C_{14}H_{12}{}^{35}ClO_5$ requires *M*+*H*, 295.0373); ν_{max} (thin film)/cm^{-1} 3423 (br, OH), 1718 and 1640 (2 x CO); δ_H (400 MHz; $CDCl_3$) 3.75 (3H, s, OCH_3), 4.48 (1H, br s, 3'-OH), 5.60 (1H, s, 3'-H), 6.12 and 6.44 (2H, 2 x s, 1'-H), 7.42 (1H, d, *J*=9.0 Hz, 8-H), 7.61 (1H, dd, *J*=2.5 and 9.0 Hz, 7-H), 8.04 (1H, s, 2-H) and 8.13 (1H, d, *J*=2.5 Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 52.0 (CO.*OCH*$_3$), 67.5 (C-3'), 120.0 (C-8), 123.2 (C-3), 124.8 (C-6), 125.1 (C-4a), 127.0 (C-1'), 131.4 (C-5), 134.2 (C-7), 139.2 (C-2'), 154.5 (C-2), 154.6 (C-8a), 166.5 (CO.O) and 176.6 (C=O); *m/z* 294 (M^+, 3%) and 234 (100); and

ii) The corresponding dimer **301** as a yellow solid (0.25 g, 50%), m.p. 210-213 °C (lit.,[176] 210-212 °C) (Found: **M**+H^+, 572.0636. $C_{28}H_{20}{}^{35}Cl_2O_9$ requires *M*+*H*, 572.0641); ν_{max} (thin film)/cm^{-1} 1715, 1710, 1650 and 1646 (4 x CO); δ_H (400 MHz; $CDCl_3$) 3.10 and 3.35 (2H, dd, 13H), 3.67 (3H, s, 12-H), 3.69 (3H, s, 16-H), 4.52 (2H, dd, 2-H_a and 2-H_b), 5.04 (1H, s, 9a-H), 6.93 (1H, d, *J*=8.9 Hz, 8-H), 7.27(1H, m, 4-H), 7.29 (1H, m, 7-H), 7.42 (1H, d, *J*= 9.0 Hz, 8'-H), 7.48 (1H, s, 17-H), 7.61 (1H, m, 7'-H), 7.65 (1H, d, *J*=2.4 Hz, 5-H), 8.00 (1H, s, 2'-H) and 8.10 (1H, d, *J*= 2.5 Hz, 5'-H); δ_C (100 MHz; $CDCl_3$) 28.4 (C-13), 50.3 (C-4a), 51.9 (C-12), 52.2 (C-16), 66.0 (C-2), 100.0 (C-9a), 119.5 (C-8), 119.8 (C-8'), 120.5 (C-4a'), 120.8 (C-3'), 125.5 (C-5'), 127.0 (C-5), 128.5 (C-10a), 129.4 (C-3), 131.2 (C-14), 131.7 (C-6'), 132.3 (C-17), 134.3 (C-7'), 135.4 (C-7), 136.0 (C-4), 154.0 (C-8a'), 155.1 (C-2'), 155.6 (C-8a), 163.8 (C-6), 167.1 (C-11), 173.6 (C-15), 175.7 (C-4') and 190.3 (C-10); *m/z* 571 (M^+, 38%) and 277 (100).

6-Fluoro-3-(3-hydroxy-2-methoxycarbonylpropen-3-yl)-4H-1-benzopyran-4-one **296** *and the corresponding dimer* **302**

The experimental procedure employed for the synthesis of 3-(3-hydroxy-2-methoxycarbonyl-propen-3-yl)-4*H*-1-benzopyran-4-one **293** and the corresponding dimer **299** was followed, using 6-fluorochromone-3-carbaldehyde **284** (0.50 g, 2.6 mmol), methyl acrylate (0.35 mL, 3.9 mmol), 3-hydroxyquinuclidine (1.66 g, 13.0 mmol) and $CHCl_3$ (3.0 mL). Work-up and flask chromatography afforded two fractions:-

i) 6-fluoro-3-(3-hydroxy-2-methoxycarbonylpropen-3-yl)-4*H*-1-benzopyran-4-one **296** as a yellow crystalline solid (0.50 g, 70%), m.p. 138-141 °C (lit.,[176] 140-142 °C); (Found: M^+, 278.0596. $C_{14}H_{11}FO_5$ requires *M*, 278.0591); ν_{max} (thin film)/cm^{-1} 3420 (br, OH), 1710 and 1637 (2 x CO); δ_H (400 MHz; $CDCl_3$) 3.75 (3H, s, OCH_3), 4.43 (1H, d, *J*=7.8 Hz, 3'-OH), 5.60 (1H, d, *J*=8.0 Hz, 3'-H), 6.12 and 6.46 (2H, 2 x s, 1'-H), 7.40 (1H, m, 7-H), 7.48 (1H, dd, *J*=4.2 and 9.1 Hz, 8-H), 7.81 (1H, dd, *J*=3.0 and 8.3 Hz, 5-H) and 8.05 (1H, s, 2-H); δ_C (100 MHz; $CDCl_3$) 52.0 (CO.*OCH*$_3$), 67.6 (C-3'), 110.5 (C-5), 120.4 (C-8), 122.3 (C-7), 122.5 (C-3), 125.8 (C-4a), 126.9 (C-1'), 139.2 (C-2'), 152.5 (C-8a), 154.6 (C-2), 159.6 (C-6), 166.5 (CO.O) and 177.1 (C=O); *m/z* 278 (M^+, 34%) and 193 (100); and

ii) The corresponding dimer **302** as a yellow solid (0.20 g, 30%), m.p. 200-202 °C (lit.,[176] 198-200 °C) (Found: M^+, 538.1074. $C_{28}H_{20}F_2O_9$ requires *M*, 538.1075); ν_{max} (thin film)/cm^{-1} 1725, 1713, 1650 and 1646 (4 x CO); δ_H (400 MHz; $CDCl_3$) 3.10 and 3.35 (2H, dd, 13H), 3.64 (3H, s, 12-H), 3.70 (3H, s, 16-H), 4.51 (2H, dd, 2-H_a and 2-H_b), 5.01 (1H, s, 9a-H), 6.97 (1H, dd, *J*=4.0 and 9.0 Hz, 5-H), 7.05 (1H, m, 7-H), 7.25 (1H, s, 4-H), 7.33 (1H, d, *J*= 3.0 and 7.8 Hz, 8-H), 7.38-7.48 (2H, m, 5'-H and 7'-H), 7.50 (1H, s, 17-H), 7.78 (1H, dd, *J*=2.8 and 8.3 Hz, 8'-H) and 7.90 (1H, s, 2'-H); δ_C (100 MHz; $CDCl_3$) 28.4 (C-13), 50.2 (C-4a), 51.9 (C-12), 52.2 (C-16), 65.9 (C-2), 100.1 (C-9a), 111.0 (C-8'), 112.7 (C-8), 119.5 (C-5), 119.8 (C-3'), 120.2 (C-5'), 120.6 (C-10a), 122.3 (C-7'), 123.6 (C-7), 124.9 (C-4a'), 129.4 (C-3), 131.1 (C-14), 132.8 (C-17), 135.4 (C-4), 151.9 (C-8a'), 153.3 and 157.8 (C-8a and C-6'), 155.1 (C-2'), 159.7 (C-6), 163.8 (C-11), 167.1 (C-15), 174.0 (C-4') and 190.6 (C-10); *m/z* 538 (M^+, 21%) and 261 (100).

3-(3-Hydroxy-2-methoxycarbonylpropen-3-yl)-6-methoxy-4H-1-benzopyran-4-one **297** *and the corresponding dimer* **303**

The experimental procedure employed for the synthesis of 3-(3-hydroxy-2-methoxycarbonyl-propen-3-yl)-4*H*-1-benzopyran-4-one **293** and the corresponding dimer **299** was followed, using 6-methoxychromone-3-carbaldehyde **285** (0.50 g, 2.5 mmol), methyl acrylate (0.33 mL, 3.7 mmol), 3-hydroxyquinuclidine (1.56 g, 12.3 mmol) and $CHCl_3$ (3.0 mL). Work-up and flash chromatography afforded two fractions:-

i) 3-(3-hydroxy-2-methoxycarbonylpropen-3-yl)-6-methoxy-4*H*-1-benzopyran-4-one **297** as a pale yellow oil (0.42 g, 60%) (Found: M^+, 290.0780. $C_{15}H_{14}O_6$ requires *M*, 290.0790); ν_{max} (thin film)/cm^{-1} 3425 (br, OH), 1720 and 1640 (2 x CO); δ_H (400 MHz; $CDCl_3$) 3.70 (3H, s, CO.*OCH*$_3$), 3.81 (3H, s, 6-OCH_3), 4.68 (1H, d, *J*=4.5 Hz, 3'-OH), 5.60 (1H, d, *J*=2.5 Hz, 3'-H), 6.10 and 6.39 (2H, 2 x s, 1'-H), 7.18 (1H, dd, *J*=3.0 and 9.1 Hz, 7-H), 7.30 (1H, d, *J*=9.1 Hz, 8-H), 7.44 (1H, d, *J*=3.0 Hz, 5-H) and 7.98 (1H, s, 2-H); δ_C (100 MHz; $CDCl_3$) 51.7 (CO.*OCH*$_3$), 55.6 (6-OCH_3), 67.1 (C-3'), 104.3 (C-5), 119.4 (C-8), 122.2 (C-3), 123.9 (C-7), 124.3 (C-4a), 126.3 (C-1'), 139.7 (C-2'), 150.9 (C-8a), 154.0 (C-2), 156.8 (C-6), 166.3 (CO.O) and 177.4 (C=O); *m/z* 290 (M^+, 26%) and 151 (100); and

ii) the corresponding dimer **303** as a pale yellow viscous oil (0.26 g, 40%) (Found: M^+, 562.1467. $C_{30}H_{26}O_{11}$ requires *M*, 562.1475); ν_{max} (thin film)/cm^{-1} 1722, 1715, 1650 and 1646 (4 x CO); δ_H (400 MHz; $CDCl_3$) 3.11 and 3.35 (2H, dd, 13H), 3.60 (3H, s, 12-H), 3.70 (3-H, s, 6-OCH_3), 3.78 (3H, s, 16-H), 3.89 (3H, s, 6'-OCH_3), 4.39-4.53 (2H, dd, 2-H_a and 2-H_b), 5.45 (1H, s, 9a-H), 6.70 (1H, s, 4-H), 6.73 (1H, d, *J*=9.0 Hz, 8-H), 7.00 (1H, m, 7-H), 7.10 (1H, d, *J*= 3.0 Hz, 5-H), 7.26 (1H, dd, *J*=3.0 and 9.0 Hz, 7'-H), 7.38 (1H, d, *J*=9.1Hz, 8'-H), 7.50 (1H, d, *J*=3.2 Hz, 5'-H), 7.55 (1H, s, 17-H) and 7.91 (1H, s, 2'-H); δ_C (100 MHz; $CDCl_3$) 31.2 (C-13), 51.0 (C-4a), 51.7 (C-12), 52.2 (C-16), 55.7 (6-OCH_3), 55.9 (6'-OCH_3), 62.9 (C-2), 99.2 (C-9a), 105.3 (C-5'), 107.6 (C-5), 119.0 (C-10a), 119.2 (C-8), 119.4 (C-8'), 119.6 (C-3'), 123.9 (C-7'), 124.5 (C-4a'), 125.6 (C-7), 129.9 (C-3), 130.6 (C-14), 133.3 (C-17), 135.0 (C-4), 150.7 (C-8a'), 151.8 (C-8a), 154.3 (C-2'), 154.7 (C-6), 157.1 (C-6'), 163.9 (C-11), 167.8 (C-15), 175.1 (C-4') and 192.4 (C-10); *m/z* 562 (M^+, 37%) and 289 (100).

3-(3-Hydroxy-2-methoxycarbonylpropen-3-yl)-5-methoxy-4H-1-benzopyran-4-one **298**

The experimental procedure employed for the synthesis of 3-(3-hydroxy-2-methoxycarbonyl-propen-3-yl)-4*H*-1-benzopyran-4-one **293** and the corresponding dimer **299** was followed,

using 5-methoxychromone-3-carbaldehyde **285** (0.50 g, 2.5 mmol), methyl acrylate (0.33 mL, 3.7 mmol), 3-hydroxyquinuclidine (1.56 g, 12.3 mmol) and $CHCl_3$ (3.0 mL). Work-up and flash chromatography afforded 3-(3-hydroxy-2-methoxycarbonylpropen-3-yl)-5-methoxy-4*H*-1-benzopyran-4-one **298** as a yellow viscous oil (0.56 g, 79%) (Found: $M+H^+$, 291.0869. $C_{15}H_{14}O_6$ requires *M*+H, 291.0869); ν_{max} (thin film)/cm^{-1} 3420 (br, OH), 1722 and 1653 (2 x CO); δ_H (400 MHz; $CDCl_3$) 3.71 (3H, s, CO.*OCH*$_3$), 3.95 (3H, s, 5-OCH_3), 4.78 (1H, d, *J*=7.1 Hz, 3'-OH), 5.51 (1H, br s, 3'-H), 6.19 and 6.43 (2H, 2 x s, 1'-H), 6.80 (1H, d, *J*=8.2 Hz, 6-H), 7.00 (1H, d, *J*=8.4 Hz, 8-H), 7.54 (1H, t, *J*=8.3 Hz, 7-H) and 7.87 (1H, s, 2-H); δ_C (100 MHz; $CDCl_3$) 51.8 (CO.*OCH*$_3$), 56.4 (5-OCH_3), 68.0 (C-3'), 106.3 (C-6), 110.2 (C-8), 114.6 (C-4a), 123.5 (C-3), 127.0 (C-1'), 134.1 (C-7), 139.2 (C-2'), 152.5 (C-2), 158.2 (C-8a), 160.0 (C-5), 166.5 (CO.O) and 178.2 (C=O); *m/z* 290 (M^+, 41%) and 230 (100).

3.7.3 Formation of the chromone dimers

The chromone dimer **299**

A mixture of 3-(3-hydroxy-2-methoxycarbonylpropen-3-yl)-4*H*-1-benzopyran-4-one **293** (50.0 mg, 0.192 mmol) and DABCO (50.0 mg, 0.446 mmol) was heated at 80 °C in an oil bath for 3 h (the reaction being monitored by TLC). Chromatography of the reaction mixture on chromatotron [elution with hexane–EtOAc–toluene (1:1:1)] afforded the chromone dimer **299** (30 mg, 62%).

The chromone dimer **300**

The experimental procedure employed for the synthesis of the chromone dimer **299** was followed, using 6-bromo-3-(3-hydroxy-2-methoxycarbonylpropen-3-yl)-4*H*-1-benzopyran-4-one **294** (50.0 mg, 0.148 mmol) and DABCO (50.0 mg, 0.446 mmol). Work-up and chromatography afforded the chromone dimer **300** (28 mg, 59%).

The chromone dimer **301**

The experimental procedure employed for the synthesis of the chromone dimer **299** was followed, using 6-chloro-3-(3-hydroxy-2-methoxycarbonylpropen-3-yl)-4*H*-1-benzopyran-4-

one **295** (50.0 mg, 0.169 mmol) and DABCO (50.0 mg, 0.446 mmol). Work-up and chromatography afforded the chromone dimer **301** (22 mg, 68%).

The chromone dimer **302**

The experimental procedure employed for the synthesis of the chromone dimer **299** was followed, using 6-fluoro-3-(3-hydroxy-2-methoxycarbonylpropen-3-yl)-4*H*-1-benzopyran-4-one **296** (50.0 mg, 0.180 mmol) and DABCO (50.0 mg, 0.446 mmol). Work-up and chromatography afforded the chromone dimer **302** (24 mg, 49%).

The chromone dimer **303**

The experimental procedure employed for the synthesis of the chromone dimer **299** was followed, using 3-(3-hydroxy-2-methoxycarbonylpropen-3-yl)-6-methoxy-4*H*-1-benzopyran-4-one **297** (50.0 mg, 0.172 mmol) and DABCO (50.0 mg, 0.446 mmol). Work-up and chromatography afforded the chromone dimer **303** (25 mg, 51%).

The chromone dimer **304**

The experimental procedure employed for the synthesis of the chromone dimer **299** was followed, using 3-(3-hydroxy-2-methoxycarbonylpropen-3-yl)-5-methoxy-4*H*-1-benzopyran-4-one **298** (50.0 mg, 0.172 mmol) and DABCO (50.0 mg, 0.446 mmol). Work-up and chromatography afforded the *chromone dimer* **304** as a yellow viscous oil (27 mg, 55%), (Found: M^+, 562.1461. $C_{30}H_{26}O_{11}$ requires *M*, 562.1469); ν_{max} (thin film)/cm^{-1} 1725, 1719, 1647 and 1644 (4 x CO); δ_H (400 MHz; $CDCl_3$) 3.09 and 3.37 (2H, dd, 13H), 3.62 (3H, s, 12-H), 3.76 (3-H, s, 5-OCH_3), 3.79 (3H, s, 16-H), 4.00 (3H, s, 5'-OCH_3), 4.42-4.60 (2H, m, 2-H_a and 2-H_b), 5.47 (1H, s, 9a-H), 6.75 (1H, s, 4-H), 6.83 (1H, d, *J*=8.0 Hz, 6-H), 7.03 (1H, d, *J*=8.3 Hz, 8-H), 7.07 (1H, dd, 7-H), 7.09 (1H, d, *J*= 8.5 Hz, 6'-H), 7.15 (1H, d, *J*=9.0 Hz, 8'-H), 7.20 (1H, dd, 7'-H), 7.53 (1H, s, 17-H) and 7.93 (1H, s, 2'-H); δ_C (100 MHz; $CDCl_3$) 31.0 (C-13), 51.3 (C-4a), 51.9 (C-12), 52.1 (C-16), 56.4 (5-OCH_3), 56.6 (5'-OCH_3), 63.1 (C-2), 100.0 (C-9a), 106.1 (C-6'), 107.0 (C-6), 120.1 (C-10a), 120.2 (C-8), 120.4 (C-8'), 120.5 (C-3'), 124.2 (C-7'), 124.6 (C-4a'), 125.0 (C-7), 130.0 (C-3), 130.1 (C-14), 133.8 (C-17), 135.2 (C-4), 151.3 (C-8a'), 152.0 and 157.4 (C-8a and C-5'), 154.9 (C-2'), 155.5 (C-5), 163.7 (C-11), 168.0 (C-15), 175.4 (C-4') and 192.8 (C-10); *m/z* 562 (M^+, 32%) and 289 (100).

3.7.4 Attempted formation of the bischromone-acrylonitrile adduct

Method 1

3-(3-Hydroxy-2-cyanopropen-3-yl)-4*H*-1-benzopyran-4-one **287** (260 mg, 1.15 mmol), chromone-3-carbaldehyde **27** (200 mg, 1.15 mmol) and 3-hydroxyquinuclidine (292 mg, 2.30 mmol) were dissolved in a minimum volume of $CHCl_3$ (3.0 mL). The mixture was stirred at room temperature for 7 days, after which ^1H NMR analysis indicated that the starting materials were eventually unchanged.

Method 2

3-(3-Hydroxy-2-cyanopropen-3-yl)-4*H*-1-benzopyran-4-one **287** (260 mg, 1.15 mmol), chromone-3-carbaldehyde **27** (200 mg, 1.15 mmol) and DABCO (258 mg, 2.30 mmol) were dissolved in a minimum volume of $CHCl_3$ (2.0 mL). The mixture was stirred at room temperature for 7 days, after which ^1H NMR analysis indicated that the starting materials were eventually unchanged.

Method 3

3-(3-Hydroxy-2-cyanopropen-3-yl)-4*H*-1-benzopyran-4-one **287** (0.26 g, 0.68 mmol), chromone-3-carbaldehyde **27** (0.10 g, 0.68 mmol) and DABCO (130 mg, 1.15 mmol) were dissolved in a mixture of dioxane–H_2O (2:1) (3.0 mL). The mixture was stirred at room temperature for 2 days, after which ^1H NMR analysis indicated that the starting materials were eventually unchanged.

3.8 Synthesis of chromone-2-carbaldehydes

1-[2-Hydroxyphenyl]-1,3-butanedione **306**[48,50]

A mixture of *o*-hydroxyacetophenone **43** (10 mL, 83 mmol) and dry EtOAc (35 mL, 0.36 mol) was added dropwise to a stirred suspension of NaOEt [generated *in situ* by adding Na metal (8.0 g, 0.35 mol) to dry EtOH (40 mL)]. The resulting yellow mixture was boiled

gently under reflux for 8 h, during which time a thick yellow slurry was formed. After cooling, the reaction mixture was poured into Et_2O (200 mL) and, after standing for 1 h, the yellow sodium salt was filtered off, washed with Et_2O and dissolved in ice-cold water (100mL). The resulting solution was acidified with acetic acid, and the resulting precipitate filtered off and recrystallized from petroleum-ether (b.p. 60-80 °C) to afforded 1-[2-hydroxyphenyl]-1,3-butanedione **306** as a colourless solid (9.1 g, 63%), which was used immediately without further purification.

1-[2-Hydroxy-5-fluorophenyl]-1,3-butanedione **307**[48,50]

The experimental procedure employed for the synthesis of 1-[2-hydroxyphenyl]-1,3-butanedione **306** was followed, using 5'-fluoro-2'-hydroxyacetophenone **175** (2.0 g, 13 mmol), dry EtOAc (8.0 mL, 82 mmol) and NaOEt [generated *in situ* by adding Na metal (1.84 g, 79.8 mmol) to dry EtOH (9.0 mL)]. Worked-up afforded 1-[2-hydroxy-5-fluorophenyl]-1,3-butanedione **307** as a yellow solid (1.5 g, 60%), which was used immediately without further purification.

1-[2-Hydroxy-5-methoxyphenyl]-1,3-butanedione **308**[48,50]

The experimental procedure employed for the synthesis of 1-[2-hydroxyphenyl]-1,3-butanedione **306** was followed, using 5'-methoxy-2'-hydroxyacetophenone **305** (10 g, 60 mmol), dry EtOAc (26 mL, 0.26 mol) and NaOEt [generated *in situ* by adding Na metal (5.83 g, 252 mmol) to dry EtOH (29 mL)]. Worked-up afforded 1-[2-hydroxy-5-methoxyphenyl]-1,3-butanedione **308** as a yellow solid (6.3 g, 51%), which was used immediately without further purification.

2-Methylchromone **19**[48,50]

A mixture of 1-[2-hydroxyphenyl]-1,3-butanedione **306** (5.0 g, 28 mmol), glacial acetic acid (25 mL) and conc. H_2SO_4 (1.0 mL) was boiled under reflux for 4 h (until the solution becomes brick red). The hot solution was poured into ice-cold water (100 mL), the resulting mixture basified with 10% aq. $NaHCO_3$, and the precipitated solid filtered off and washed with ice-cold water. Recrystallization from hexane afforded 2-methylchromone **19** as a yellow solid (3.6 g, 80%), m.p. 70-71 °C (lit.,[49] 69-70 °C) (Found: M^+, 160.05326. $C_{10}H_8O_2$ requires *M*, 160.05243); δ_H (400 MHz; $CDCl_3$) 2.36 (3H, s, CH_3), 6.15 (1H, s, 3-H), 7.33-7.40 (2H, m, 6-H and 8-H), 7.62 (1H, m, 7-H) and 8.16 (1H, dd, *J*=1.6 and 8.0 Hz, 5-H); δ_C

(100 MHz; $CDCl_3$) 20.5 (CH_3), 110.5 (C-3), 117.7 (C-8), 123.5 (C-4a), 124.9 (C-6), 125.6 (C-5), 133.4 (C-7), 156.5 (C-8a), 166.2 (C-2) and 178.2 (C=O); *m/z* 160 (M^+, 100%).

6-Fluoro-2-methylchromone **309**[48,50]

The experimental procedure employed for the synthesis of 2-methylchromone **19** was followed, using 1-[2-hydroxy-5-fluorophenyl]-1,3-butanedione **307** (3.5 g, 18 mmol), glacial acetic acid (20 mL) and conc. H_2SO_4 (0.8 mL). Work-up afforded 6-fluoro-2-methylchromone **309** as a yellow solid (2.25 g, 71%), m.p. 101-102 °C (lit.,[192] 101-102 °C) (Found: M^+, 178.04377. $C_{10}H_7FO_2$ requires *M*, 178.04301); δ_H (400 MHz; $CDCl_3$) 2.37 (3H, s, CH_3), 6.14 (1H, s, 3-H), 7.31-7.42 (2H, m, 7-H and 8-H) and 7.78 (1H, dd, *J*=3.1 and 8.2 Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 20.5 (CH_3), 109.9 (C-3), 110.6 (C-5), 119.8 (C-7), 119.9 (C-6), 121.4 (C-8), 121.6 (C-4a), 160.6 (C-8a), 166.4 (C-2) and 177.3 (C=O); *m/z* 178 (M^+, 100%).

6-Methoxy-2-methylchromone **310**[48,50]

The experimental procedure employed for the synthesis of 2-methylchromone **19** was followed using 1-[2-hydroxy-5-methoxyphenyl]-1,3-butanedione **308** (5.0 g, 24 mmol), glacial acetic acid (25 mL) and conc. H_2SO_4 (1.0 mL). Work-up afforded 6-methoxy-2-methylchromone **310** as a yellow solid (4.0 g, 88%), m.p. 105-106 °C (lit.,[193] 107-108 °C) (Found: M^+, 190.06296. $C_{11}H_{10}O_3$ requires *M*, 190.06299); δ_H (400 MHz; $CDCl_3$) 2.35 (3H, s, CH_3), 3.86 (3H, s, OCH_3), 6.14 (1H, s, 3-H), 7.20 (1H, m, 8-H), 7.72 (1H, d, *J*=9.1Hz, 7-H) and 7.53 (1H, d, *J*=3.0Hz, 5-H); δ_C (100 MHz; $CDCl_3$) 20.5 (CH_3), 55.9 (OCH_3), 104.9 (C-5), 109.8 (C-3), 119.1 (C-7), 123.4 (C-8), 124.1 (C-4a), 151.3 (C-6), 156.8 (C-8a), 165.9 (C-2) and 178.0 (C=O); *m/z* 190 (M^+, 100%).

Chromone-2-carbaldehyde **26**[75,102]

A stirred solution of 2-methylchromone **19** (0.50 g, 3.0 mmol) and SeO_2 powder (1.7 g, 15 mmol) in xylene (10 mL) was boiled under reflux on an oil bath at 160-165 °C for 12 h. The reaction mixture was filtered while hot to remove the black selenium, and the filtrate concentrated *in vacuo*. Flash chromatography of the residue on silica gel (elution with chloroform) afforded chromone-2-carbaldehyde **26** as a yellow solid (0.20 g, 40%), m.p. 160-

162 °C (lit.,[76] 162-163 °C) (Found: M^+, 174.03178. $C_{10}H_6O_3$ requires *M*, 174.03169); ν_{max} (KBr)/cm^{-1} 1653 and 1701 (2 x CO); δ_H (400 MHz; $CDCl_3$) 6.90 (1H, s, 3-H), 7.47 (1H, t, *J*=7.4 Hz, 6-H), 7.60 (1H, d, *J*=8.4 Hz, 8-H), 7.78 (1H, m, 7-H), 8.20 (1H, d, *J*=8.0 Hz, 5-H) and 9.79 (1H, s, CHO); δ_C (100 MHz; $CDCl_3$) 117.0 (C-3), 118.8 (C-8), 124.9 (C-4a), 125.9 (C-6), 126.2 (C-5), 135.2 (C-7), 155.6 (C-8a), 156.0 (C-2), 178.3 (C=O) and 185.5 (CHO); *m/z* 174 (M^+, 100%).

6-Fluorochromone-2-carbaldehyde **311**

The experimental procedure employed for the synthesis of chromone-2-carbaldehyde **26** was followed, using 6-fluoro-2-methylchromone **309** (0.50 g, 2.8 mmol), SeO_2 powder (1.7 g, 15 mmol) and xylene (10 mL). Work-up afforded *6-fluorochromone-2-carbaldehyde* **311** as a yellow solid (0.21 g, 38%), m.p. 156-158 °C (Found: M^+, 192.02299. $C_{10}H_5FO_3$ requires *M*, 192.02227); ν_{max} (KBr)/cm^{-1} 1655 and 1704 (2 x CO); δ_H (400 MHz; $CDCl_3$) 6.89 (1H, s, 3-H), 7.50 (1H, m, 7-H), 7.63 (1H, m, 8-H), 7.84 (1H, m, 5-H), and 9.79 (1H, s, CHO); δ_C (100 MHz; $CDCl_3$) 110.8 (C-3), 111.0 (C-5), 115.8 (C-7), 121.0 (C-6), 121.1 (C-8), 123.7 (C-4a), 155.0 (C-8a), 156.1 (C-2), 177.5 (C=O) and 185.1 (CHO); *m/z* 192 (M^+, 100%).

6-Methoxychromone-2-carbaldehyde **312**

The experimental procedure employed for the synthesis of chromone-2-carbaldehyde **26** was followed using, 6-methoxy-2-methylchromone **310** (0.50 g, 2.8 mmol), SeO_2 powder (0.5 g, 4.0 mmol) and xylene (10 mL). Work-up afforded *6-methoxychromone-2-carbaldehyde* **312** as a yellow solid (0.20 g, 35%), m.p. 174-176 °C (Found: M^+, 204.04304. $C_{11}H_8O_4$ requires *M*, 204.04226); ν_{max} (KBr)/cm^{-1} 1652 and 1699 (2 x CO); δ_H (400 MHz; $CDCl_3$) 3.91 (3H, s, OCH_3), 6.88 (1H, s, 3-H), 7.36 (1H, m, 8-H), 7.53-7.56 (2H, m, 5-H and 7-H), and 9.78 (1H, s, CHO); δ_C (100 MHz; $CDCl_3$) 56.0 (OCH_3), 104.9 (C-5), 115.8 (C-3), 120.2 (C-7), 124.5 (C-8), 125.7 (C-4a), 152.0 (C-6), 156.0 (C-8a), 157.8 (C-2), 178.1 (C=O) and 185.5 (CHO); *m/z* 204 (M^+, 100%).

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