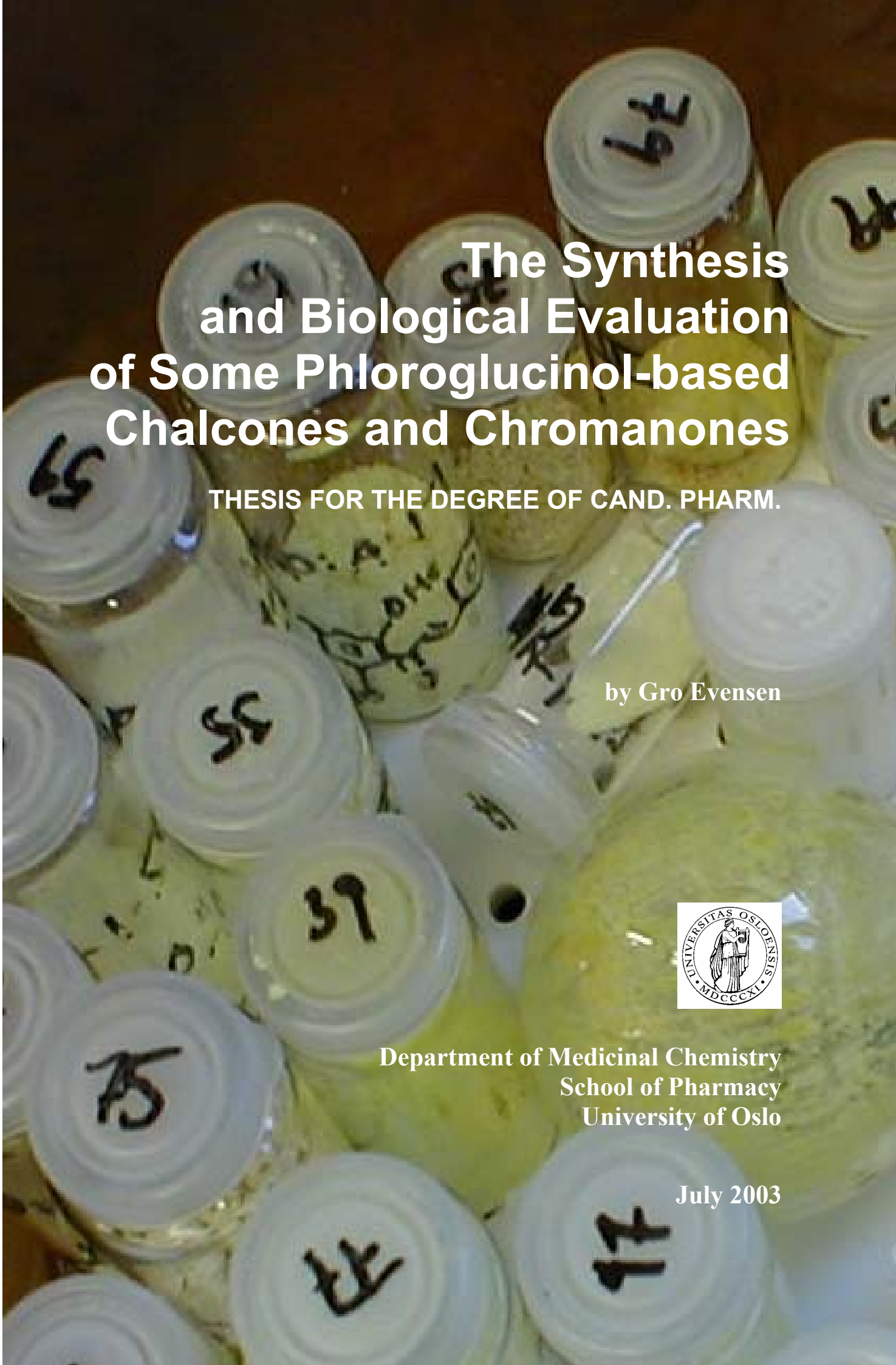

The Synthesis and Biological Evaluation of Some Phloroglucinol-based Chalcones and Chromanones

THESIS FOR THE DEGREE OF CAND. PHARM.

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Preface

This study has been carried out at the Department of Medicinal Chemistry at the School of Pharmacy, University of Oslo, under the supervision of professors Arne Jørgen Aasen, Lars Skattebøl and dr. scient. Trond Vidar Hansen.

I would like to thank everyone at the Department of Medicinal Chemistry, as well as the Department of Chemistry, for answering whatever question I might have had. I am also extremely grateful to the department for financing my participation at Organisk Kjemisk Vintermøte 2003, which gave me an opportunity to excel at poster-design.

Thank you to professor Karl Egil Malterud – if I ever go to Mongolia I shall buy you some chocolate.

Mum and dad – if you should happen to contract malaria during your expatriation, do not fear. I should know something about the treatment now.

Ragnhild – I'll be there if you ever need to spill out any frustration. Good luck with your master degree and keep eating apples, they are full of antioxidants.

Dagfinn – mine hender er fulle av blomster. Til deg.

Oslo, 27.6.2003

Soon-to-be c*and. pharm.* Gro Evensen.

Contents

Summary

A novel series of 2',4',6'-trimethoxychalcone derivatives were prepared using one-step Claisen-Schmidt condensations of aldehydes with methyl ketones. The compounds were evaluated for effects against malaria, 15-lipoxygenase and radical scavenging activity. Of the twenty-five chalcone derivatives, only one exhibited significant scavenging of DPPH radical: *E*-1-(2,4,6-trimethoxyphenyl)-3-(3,4-dihydroxyphenyl)-2-propen-1-one (**44**). Almost all chalcone compounds showed potent inhibition of 15-lipoxygenase.

Two 2',4'-dimethoxy-6'-hydroxychalcone derivatives were synthesized by the same method. In most cases, chalcones containing a 6'-hydroxyl group cyclized spontaneously to chromanone. This provided four chroman-4-one derivatives, which were alkylated to yield chromanol derivatives. Hydrogenation of the chromanols afforded the corresponding chromans.

Abbrevations

b	broad
calc.	calculated
conc.	concentrated
COSY	correlated spectroscopy
δ	chemical shift (in ppm)
d	doublet
Δ	heat
DCM	dichloromethane
DEPT	distortionless enhancement by polarization transfer
DHP	3,4-dihydro-2*H*-pyran
DMP	Dess Martin periodinane
DMSO	dimethyl sulphoxide
DPPH$^{\bullet}$	diphenylpicrylhydrazyl radical
EI	electron bombardment ionisation
EtOAc	ethyl acetate
h/hrs	hour/hours
HRMS	high resolution mass spectroscopy
IR	infrared spectroscopy
J	coupling constant (in Hz)
LDL	low density lipoprotein
lit.	literature
LO	lipoxygenase
m	multiplet
m/z	mass/charge
min	minutes
mp	melting point
MS	mass spectroscopy
n.d.	not determined
NMR	nuclear magnetic resonance
ppm	parts per million
ROS	reactive oxygen species
s	singlet
t	triplet
p-TsOH	*para*-toluenesulphonic acid
THF	tetrahydrofuran
THP	tetrahydropyran
TLC	thin layer chromatography
TMS	trimethylsilyl
ν_{max}	infrared absorption frequency (in wavenumbers: cm^{-1})

1. Introduction

1.1 Flavonoids

Flavonoids have been known for more than a hundred years, and constitute one of the largest and most diverse groups of natural phenols. The flavonoids are usually yellow-coloured compounds, and contribute to the colours of flowers and fruits. Their biological activity was discovered around 1940 and they were for a short while designated “P vitamins” because of their ability to heal capillary fragility, a property similar to that of vitamin C. This effect was never entirely proven, but since then their biological role has been studied extensively, and thousands of articles and several books have been published on the theme.[1]

Flavonoids occupy a variety of structural forms, which have the flavan skeleton structurally in common, see Figure 1.

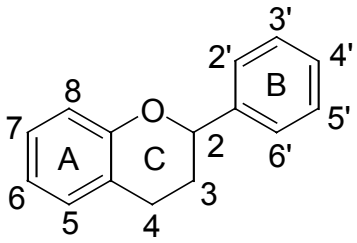

Figure 1. The structure and numbering pattern of flavan

Flavonoids show a wide variety of biological activities. In addition to their antioxidant effects,[2] they have been reported to among other things modulate enzyme activity,[3] have immunomodulating and anti-inflammatory activities,[4] antibiotic effects,[5] oestrogenic activity,[6] anticarcenogenic activity,[7] antithrombotic effects[8] and counteract vascular permeability.[9] Silybin (**1**), a flavonoid from the milk thistle *Silybum marianum*, has hepatoprotective effect and is used in combination with

penicillin in the treatment of mushroom poisoning. Drugs containing milk thistle extract are used to treat various types of liver disease.[10]

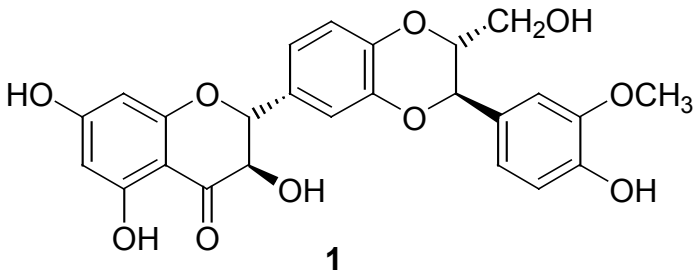

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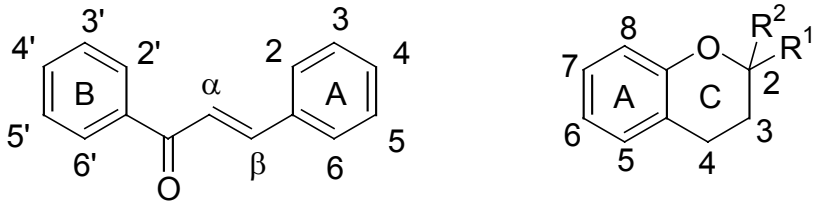
*Figure 2. Silybin (**1**) - a flavonoid in therapeutic use*

Chalcones, often entitled 'open chain flavonoids', possess a 1,3-diphenyl-2-propen-1-one skeleton. With regard to substituents the carbon atoms of the benzene rings are numbered as shown in Figure 3, which is the pattern adopted by Chemical Abstracts. It should be noted that the numbering in the chalcone framework is reversed from that of the other flavonoids. The bridge carbons are marked relative to the carbonyl function as C-α and C-β.



I **II**

*Figure 3. The basic skeleton and numbering pattern of chalcones **I** and chromans **II***

In plants, chalcones are important intermediates in the biosynthesis of flavonoids. Chalcones have shown a wide range of biological activities depending on the substitution pattern on the two aromatic rings. These include antibiotic,[11] modulation of enzyme activity,[12] modulation of P-glycoprotein mediated multidrug resistance,[13] tuberculostatic,[14] antileishmanial,[15] oestrogenic,[16] anticarcinogenic,[17] anti-inflammatory[18] and antimalarial activity.[19]

Derivatives of flavan, in which the B ring has been replaced by other ring structures or aliphatic chains are designated chromans. Chromans having a carbonyl group at C-4 are named chromanones.

1.2 Robustadial

The structural elucidation of the naturally occurring chroman derivatives robustadials A and B[20] combined with a general interest in oxygen heterocycles, triggered Skattebøl and Aukrust to attempt their synthesis. While the robustadials A and B were first isolated in 1984, their structures were not correctly established until 1988.[21] Robustadial A and B (**2**) are diastereomeric chroman derivatives from the leaves of *Eucalyptus robusta,* and they have been reported to exhibit antimalarial properties.

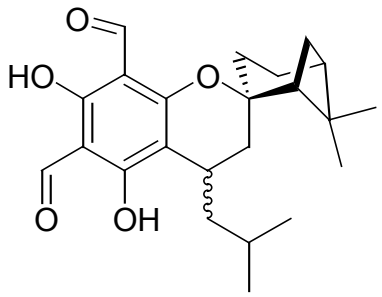


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Figure 4. The structure of robustadials A and B

Aukrust and Skattebøl described a synthesis of robustadial A in 1996.[22] According to the depicted retrosynthetic route (Figure 5), a chromanone bearing the general structure **3** would serve as an intermediate.

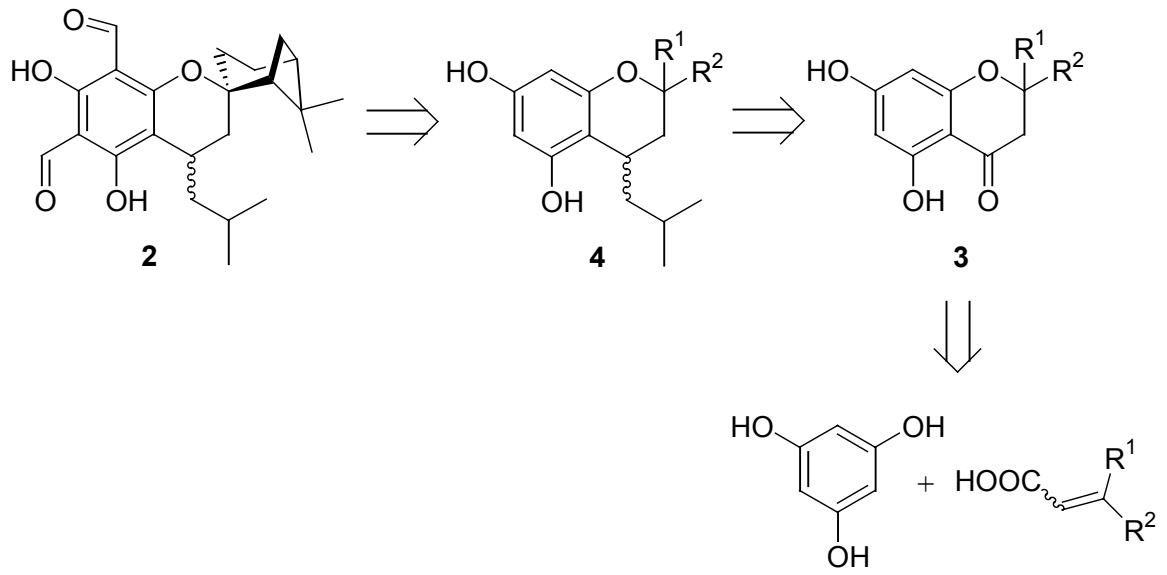

Figure 5. Retrosynthesis of robustadials

The synthetic scheme involved four steps starting from phloroglucinol and an α,β-unsaturated acid derivative via the corresponding chromanone **3**. The isobutyl group was attached with methallylzinc bromide, followed by hydrogenation, yielding the desired chroman **4**. The formylation step produced the target molecule **2**.

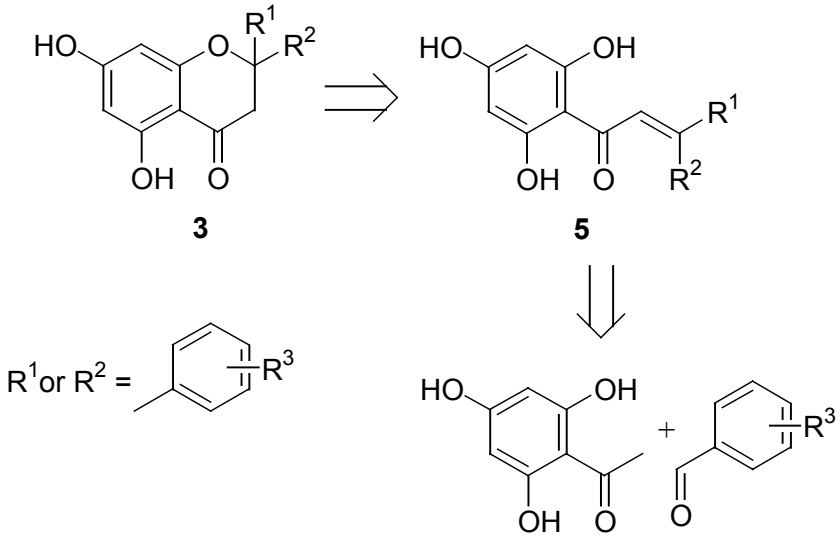

Figure 6. Alternative retrosynthesis of chromanone intermediates

Retrosynthetic studies revealed that chalcones with the general structure **5** served as valuable intermediates towards analogues of robustadial A and B. The starting material would then consist of substituted acetophenone and an aromatic aldehyde.

Thus, there are several ways towards the synthesis of robustadial intermediates bearing chalcone or chroman skeletons.

1.3 Searching for new therapies

There is urgent demand for novel and improved therapeutical compounds in many medicinal fields. The search for new drugs can be started in a rational way by identifying a biological target, i.e. an enzyme, which is essential for the pathological process. Modulators of enzyme activity can be designed based on the structural knowledge about the target enzyme. By identifying the structural requirements that might be necessary for the molecule to exert an effect on the target, one can construct a molecule containing these structural features. This lead compound then serves as a template for other potentially bioactive compounds. By testing derivatives in which the chemical structure of the lead has been modified, the optimal structural requirements for pharmacological activity might be identified and a potential drug agent unveiled. This was the case for the recent unearthing of the malaria protease inhibitory activity of certain chalcones.[23] Using a model structure of a parasitic protease, chalcones and their derivatives were identified as potential antimalarials. *In vitro* testing demonstrated antimalarial activity for several of the compounds, supporting the predicted structure-activity relationship.

Very few drugs have been developed on a rational basis despite more detailed knowledge of potential targets, such as three-dimensional structures of enzymes, metabolic pathways, and genome sequences. To date, most pharmaceuticals have been developed in an empirical way and their targets identified afterwards. This pathway involves screening of large libraries of chemicals and examination of plant extracts. Often, one identifies the constituents of herbal remedies used in traditional medicine and validates their biological activity against the maladies that the plants are used to treat. Pharmacologically active, natural substances often serve as models for

the synthesis of compounds that retain or improve the pharmacological properties. The development of the anti-inflammatory drug sodium cromoglycate (**6**) is an excellent example of this. It was derived from the structure of a naturally occurring 2-chromen-4-on, khellin, formerly used to treat stomach complaints and asthma.

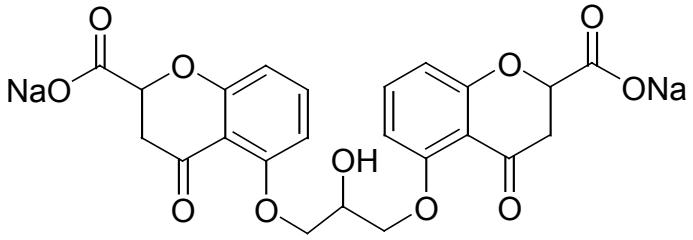

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*Figure 7. Sodium cromoglycate (**6**) - an anti-inflammatory drug derived from a naturally occurring flavonoid.*

Simple analogues of robustadial have shown antimalarial effects, albeit weak,[24] and recent reports give evidence of the antimalarial effects of some chalcone derivatives. Flavonoids have shown antioxidative and enzyme-inhibitory properties which may be beneficial in the prevention of coronary heart disease, inflammatory conditions, and cancer. With this in mind, we set about to synthesize chalcone and chroman derivatives that were subjected to biological testing. In this study, focus has been on the antimalarial activities of the synthesized compounds, their radical scavenging properties and their inhibition of the enzyme 15-lipoxygenase (15-LO).

The next sections contain brief reviews of the pathology of malaria and conditions that are associated with radical-mediated and oxidative processes: inflammation and atherosclerosis.

1.4 Malaria

The World Health Organization (WHO) estimates that globally about 500 million individuals suffer from acute cases of malaria and, at a minimum, 1 million – more probably 2 million - people die from malaria every year. The majority of deaths, at

least 70 %, are among children under the age of five living in sub-Saharan Africa. Today, 90 % of all fatalities due to malaria are found in this region.[25] In addition, Africa includes the majority of the world's poorest countries. Malaria is a curable disease if the illness is diagnosed and treated at an early stage, but in countries lacking a sustainable infrastructure, medical expertise, and basic services, very few have access to effective and affordable therapy.

The rapid increase in resistance to commonly used antimalarials, increasing global travel and global warming all suggest that the impact of malaria on the world is likely to rise. Malaria is now reappearing in some countries in which it had previously been eliminated.[26]

Malaria in humans is caused by four species of *Plasmodium* protozoa: *P. vivax, P. falciparum, P. malariae* and *P. ovale* of which the first two are the most common. The parasites are transmitted by the bite of the female *Anopheles* mosquito. Nearly all deaths caused by malaria are due to infection by *P. falciparum* and this species develops drug resistant strains at a tremendous rate.

Plasmodia undergo several morphological changes in both the human and mosquito host, see Figure 8. As she feeds on an infected person, the mosquito ingests parasites in the form of gametocytes. While in the mosquito's stomach, the parasites reproduce to generate sporozoites, which travel to the insect's salivary glands. During the next feeding, the victim is infected by the sporozoites. In the bloodstream, the parasites make their way to the liver, where they reproduce to generate thousands of merozoites, which pass back into the blood and infect erythrocytes (red blood cells). The merozoites digest host haemoglobin and plasma and generate more merozoites, each of which is capable of invading a new red blood cell. The infected erythrocytes lyse, releasing new merozoites as well as toxins and erythrocyte debris into the bloodstream. This sudden release of exogenous products induces the chills and fever so characteristic of malaria.[27]

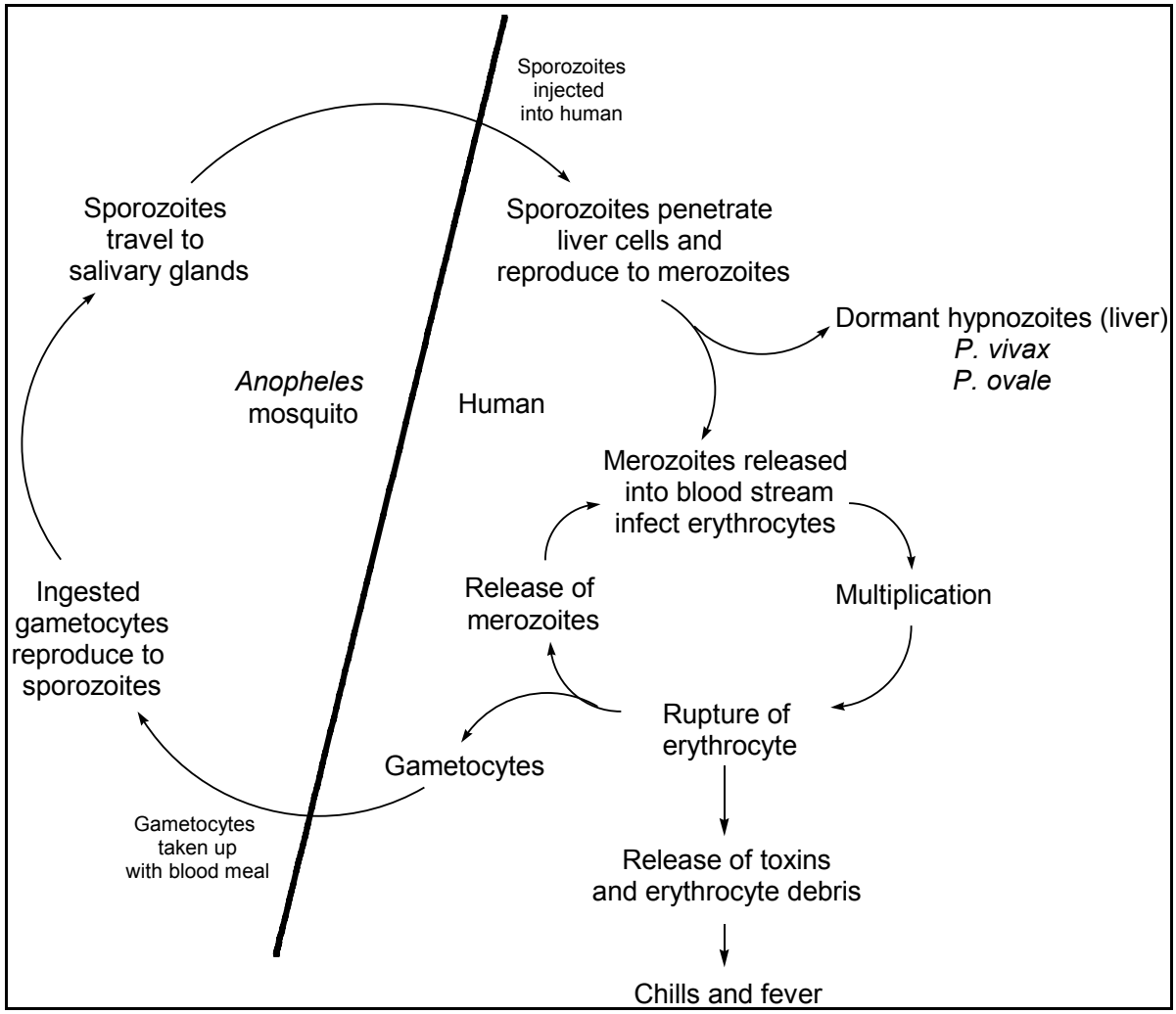

Figure 8. Life cycle of the malaria parasite. Not all plasmodia generate hypnozoites.

Malaria parasites can reproduce in the body at a phenomenal rate. Destruction of 94% of the parasites every 48 hours will only maintain status quo, and will not reduce their number or their ability to proliferate.[28] Occasionally, merozoites generate gametocytes that are ingested by another mosquito. In some forms of malaria, the sporozoites on entering the liver cells form dormant hypnozoites (also termed "secondary schizonts"), which may liberate merozoites at a later stage several months, or even years, after initial infection.

1.4.1 Antimalarial natural products

The majority of drugs used against malaria today have been developed from natural products. The oldest and still-used drug quinine (**7**) is obtained from the bark of the *Cinchona*-tree. The structure of quinine was established in 1908[29] and it has been the template for numerous other synthetic quinoline antimalarials with fewer side effects, such as chloroquine (**8**), primaquine (**9**), and mefloquine (**10**).

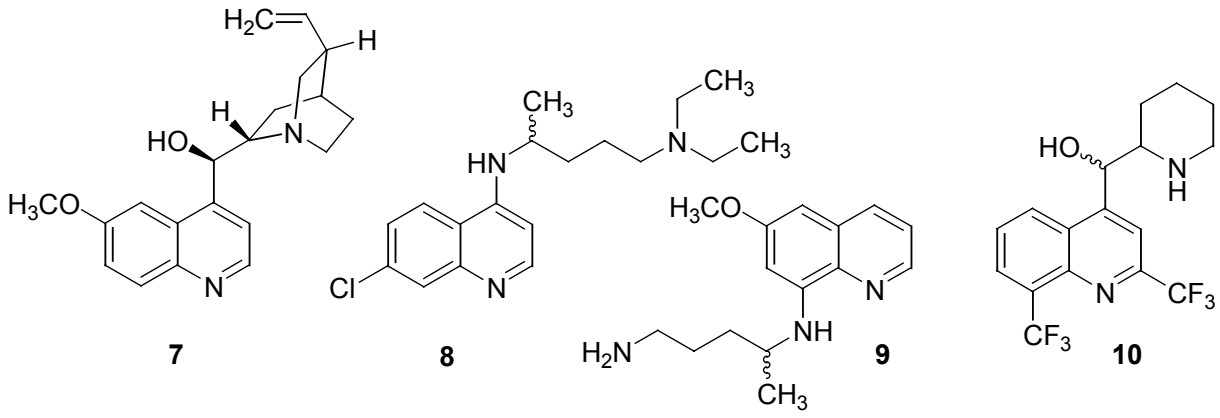

*Figure 9. Quinine (**7**) and quinoline antimalarials*

In 1972, artemisinin (**11**) was isolated from *Artemisia annua,* an ancient Chinese herb used for malaria therapy, and identified as the active compound. Today, synthetic derivatives artemether (**12a**) and sodium artesunate (**12b**) are being used in clinical treatment of the infection.

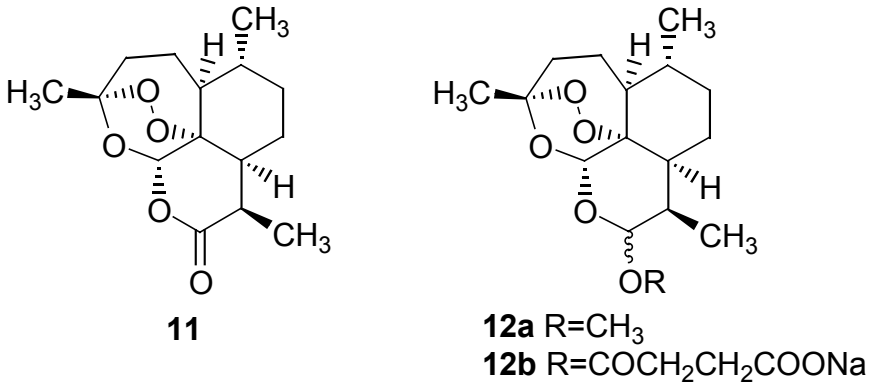

*Figure 10. Artemisinin (**11**) and synthetic derivatives **12a** and **12b***

Extracts of the leaves of *Eucalyptus robusta* are used in Chinese herbal medicine for the treatment of dysentery, malaria, and bacterial diseases. In 1984, three compounds with antimalarial activity were isolated from the extract: robustaol A (**13**) and robustadial A and B (**2**), of which the latter two had considerably greater activity than substance **13**.[20]

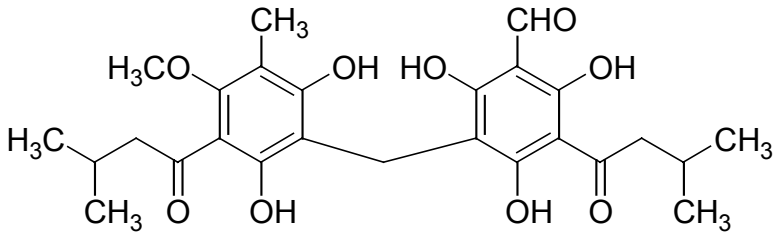

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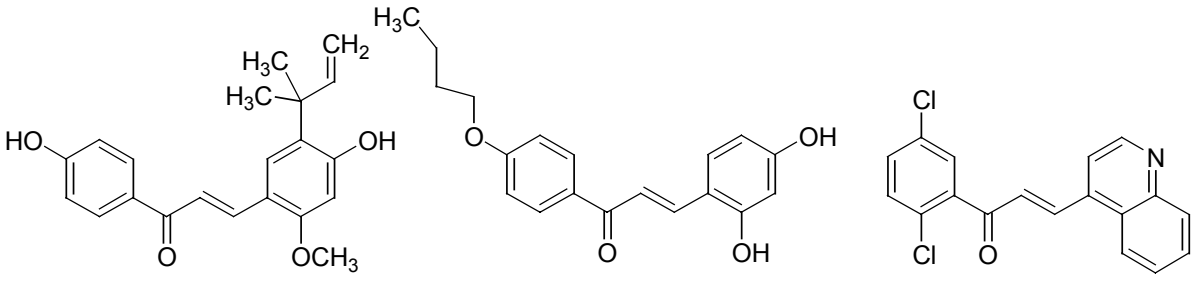
Figure 11. Robustaol A

The antimalarial activity of chalcones was first noted in 1994, when licochalcone A (**14**), a natural product isolated from Chinese liquorice roots, was reported to exhibit potent *in vitro* and *in vivo* antimalarial activity.[30] Since then, a large number of chalcones and their derivatives have been synthesized and evaluated for activity against *Plasmodia*.[19,23] An analogue of licochalcone A, 1-(4-butoxyphenyl)-3-(2,4-dimethoxyphenyl)-2-propen-1-one (**15**), exhibited potent antimalarial activity.[31] A drug design study reported 1-(2,5-dichlorophenyl)-3-(quinolin-4-yl)-2-propen-1-one (**16**) to be the most active of over 200 chalcone derivatives.[23] The structures are depicted in Figure 12.



14 **15** **16**

Figure 12. Chalcones with antimalarial activity

Biflavanone **17**, isolated from an Arabic traditional medicinal plant, has shown moderate activity against *P. falciparum*.[32]

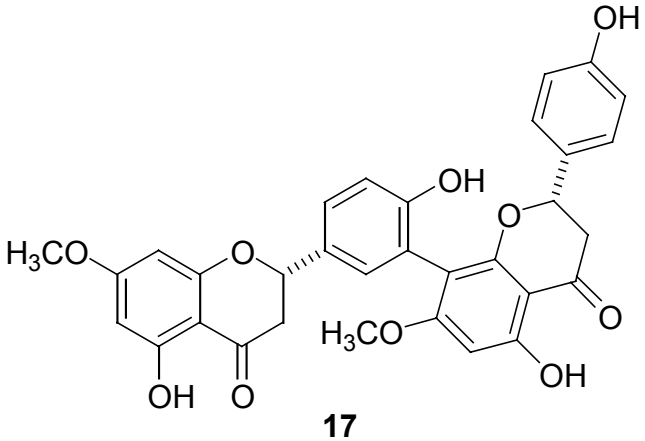


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Figure 13. (2S,2''S)-7,7''-di-O-methyltetrahydroamentoflavone

1.4.2 Antimalarials – current chemotherapy

The specific mechanisms of current drug therapies in inhibiting malaria parasite growth are not known. Their modes of action are often complex, involving several different targets. Most antimalarial drugs act primarily at the erythrocytic stage of the parasite life cycle. The so-called blood schizonticidal agents (Figure 14, I) can cure infection caused by parasites that do not produce hypnozoites.

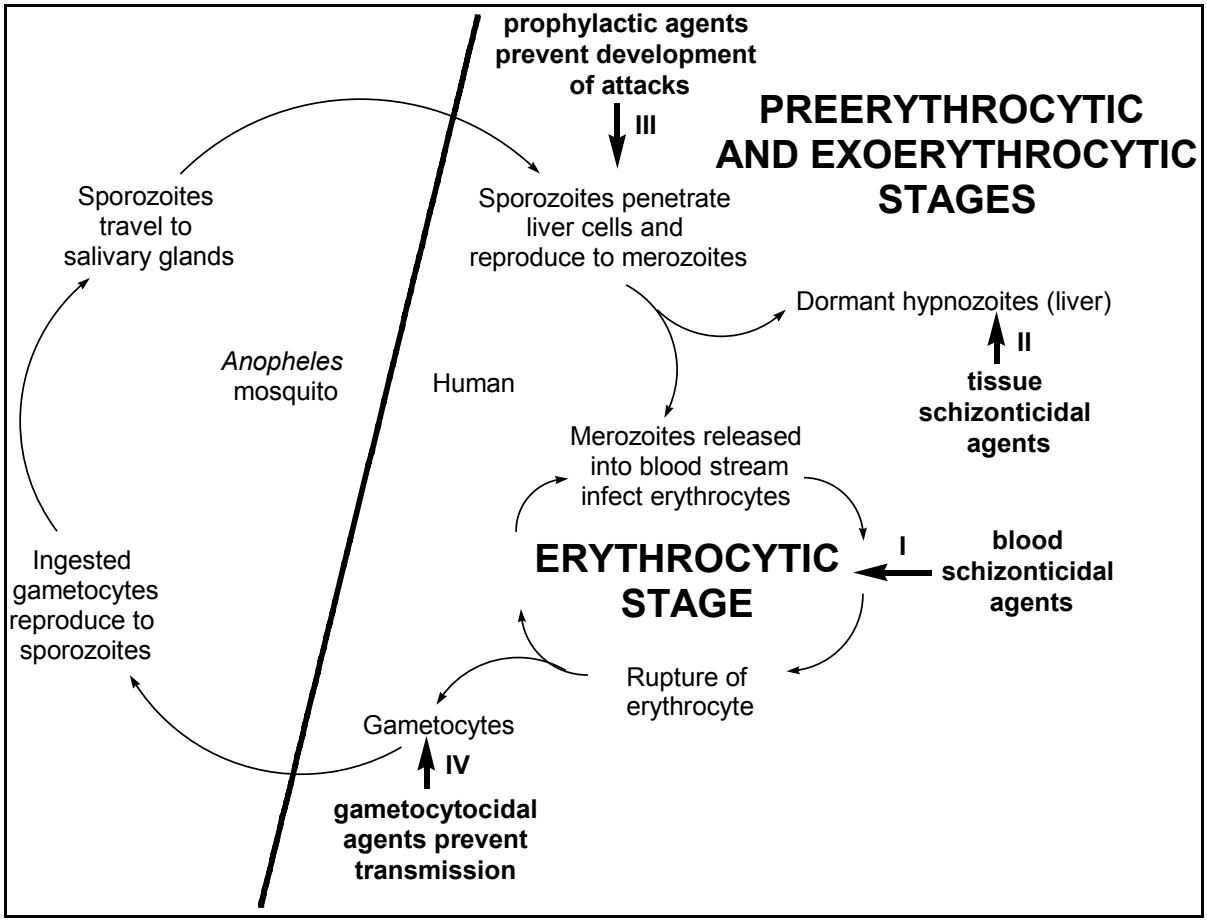


*Figure 14. The sites of action (**I-IV**) of antimalarial drugs.*

Inhibition of haem polymerisation, through different mechanisms, is believed to be the mode of action of the quinoline antimalarials **7, 8** and **10** and artemisinin (**11**). The haem remaining after the parasites digest host haemoglobin is toxic to the parasites, which convert the haem to polymeric haemozoin. Inhibition of haemozoin formation will eventually kill the parasite.

Primaquine (**9**) is active against the parasites in the liver, hence the term tissue schizonticidal agent (Figure 14, II). This drug effects cure of parasites having an exoerythrocytic stage.

Drugs used for chemoprophylaxis (Figure 14, III) act on the merozoites when they emerge from the liver and prevent the development of malarial attacks. Drugs used

for this purpose are chloroquine (**8**), mefloquine (**10**), proguanil (**18**), pyrimethamine (**19**), dapsone (**20**) and doxycyline (**21**).

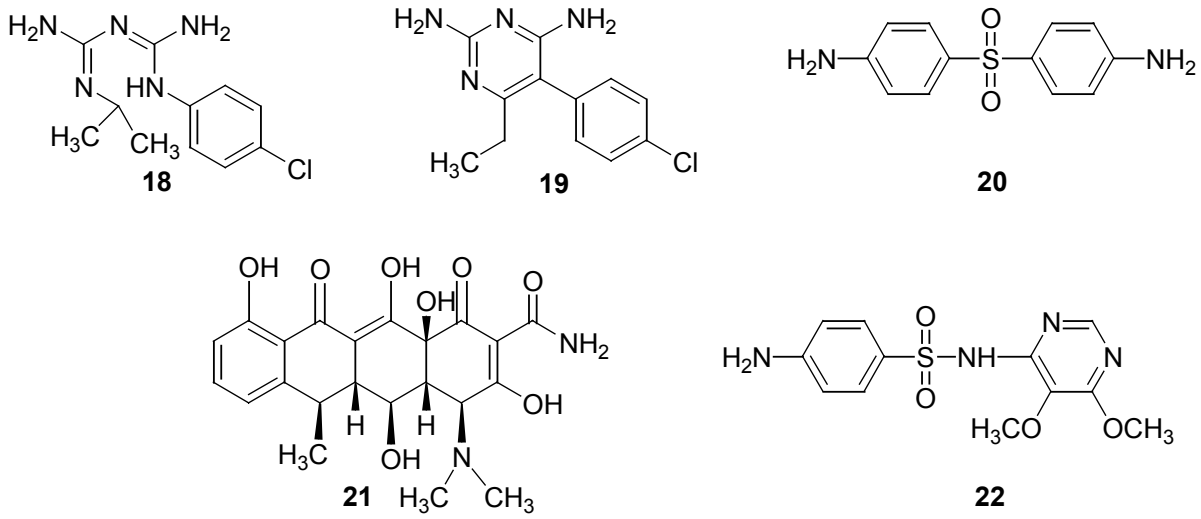


Figure 15. Structures of several widely used drugs in antimalarial chemotherapy

Drugs **18**-**21** and sulphadoxine (**22**) all inhibit, by different mechanisms, metabolic pathways and precursors essential for the growth of the plasmodia.

Primaquine (**9**), proguanil (**18**) and pyrimethamine (**19**) destroy gametocytes (Figure 14, IV), thus reducing the spread of infection, but they are rarely used for this action alone.

Treatment of malaria infection usually involves combinations of drugs with different mechanisms of action. This hinders the development of drug resistance in the parasite, and many compounds exhibit synergistic effects when combined. Atovaquone (**23**) is ineffective against malaria parasites alone, but is effective in combination with proguanil (**18**). Artemether (**12a**) is frequently administered in combination with lumefantrin (**24**).

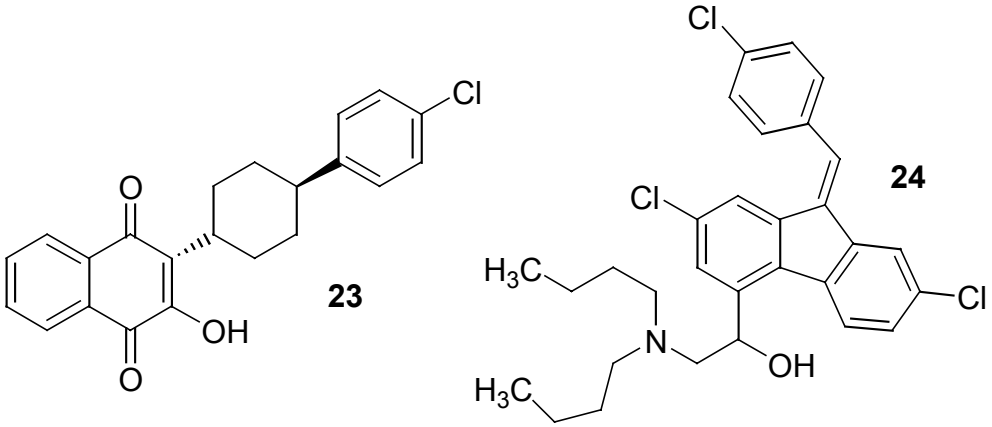


*Figure 16. Atovaquone (**23**) is only active in combination with proguanil (**18**). Lumefantrine (**24**) synergises with artemether (**12a**).*

Some compounds, of which pyronaridine (**25**) holds most promise, are under clinical development. Pyronaridine inhibits DNA topoisomerase, an enzyme involved in the replication of DNA.[33]

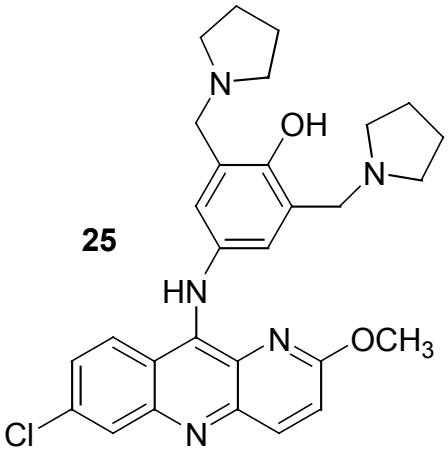


*Figure 17. Pyronaridine (**25**) is being evaluated in clinical trials.*

A number of novel potential targets have been identified, of which a few are inhibitors of phospatidylcholine biosynthesis, which is required for the growth of the parasite[34] and others are inhibitors of parasitic proteases, which it uses to digest host haemoglobin. Chalcones are thought to act through inhibition of proteases, preventing haemoglobin degradation.[23]

Advances in genomics, including the elucidation of the complete genome sequence of the *Anopheles* mosquito and the parasite *P. falciparum*, offer promising new approaches for drug and vaccine development.[35]

Mode of action of artemisinin (11)

The mechanisms of action of artemisinin (**11**) are worth special mentioning. Several theories on the mechanisms of the compound and its derivatives have been postulated in numerous articles.[36] The compound contains a unique 1,2,4-trioxane structure, in which the endoperoxide bridge appears to be necessary for antimalarial activity. Since reactive oxygen species such as hydroxyl radicals and superoxide are generated from peroxides, it has been suggested that free radicals might be involved in the antimalarial mechanism. It has been shown that artemisinin interacts with intraparasitic heme or iron, maybe causing the endoperoxide bridge to break open and form free radicals. Once formed, the artemisinin-derived free radicals attack vital targets in the parasite, most likely through alkylation of specific malaria proteins. This might explain the parasite-killing action of the drug.

1.5 Oxidation of biological molecules

1.5.1 Free radicals

Free radicals play an important part in phagocytosis, the production of some biologically essential compounds and possibly in cell signaling. At the same time, they may cause oxidative modification of biological molecules, leading to oxidative damage and eventually to various diseases.[37] Lipids, proteins and DNA are the main targets of free radicals and reactive oxygen species (ROS). Above all, the polyunsaturated fatty acids and their esters are susceptible to radical attack and oxidation. Their oxidation, referred to as lipid peroxidation, has been extensively studied and the mechanisms are now fairly well understood.

Lipid peroxidation by non-enzymatic pathways may proceed either by free radical-mediated or non-radical mechanisms. Lipid radicals ($L^{\bullet}$) are generated by both radical and non-radical initiators (Figure 18, I) during arachidonic acid metabolism, NO synthesis, cigarette smoking, UV-radiation and by reactive metabolites of some drugs. A bisallylic H atom is abstracted to give a pentadienyl radical, to which oxygen reacts rapidly to produce lipid peroxy radical ($LOO^{\bullet}$). The oxygen adds, not necessarily to the position from which H was removed in the first place, but to mesomeric adjacent radical sites owing to stabilization of the conjugated diene formed. The resulting peroxy radical reacts with bisallylic hydrogen on another polyunsaturated fatty acid, giving rise to the corresponding lipid hydroperoxide (LOOH) and generating further lipid peroxy radicals. LOOH can also break down to toxic secondary products such as unsaturated aldehydes.

Initiation $LH + I \longrightarrow IH + L^{\bullet}$

Propagation $L^{\bullet} + ROS \longrightarrow LOO^{\bullet}$

$LOO^{\bullet} + LH \longrightarrow LOOH + L^{\bullet}$

Figure 18. Lipid peroxidation. LH = lipid, I = initiator, $L^{\bullet}$ = lipid radical, $LOO^{\bullet}$ = lipid peroxy radical, LOOH =lipid hydroperoxide

ROS such as superoxide radicals are generated *in vivo* to kill bacteria and other microorganisms by phagocytosis and are essential for defence against bacterial infections, but sometimes the production of such radicals might exceed the requirement. ROS and the products of lipid peroxidation can attack many key molecules, including enzymes, membrane lipids and DNA. Oxidative stress occurs when excessive amounts of highly reactive free radicals are produced, and is involved in the pathogenesis of both cancerous and cardiovascular diseases.

Enzymatic defence mechanisms, such as superoxide dismutase and catalase, and antioxidants such as ascorbic acid (vitamin C), gluthatione and α-tocopherol (vitamin E) can trap the free radicals, thereby breaking the peroxidative cascade reaction and

preventing biological damage. The radical formed from α-tocopherol is stabilised through delocalisation over the aromatic ring structure, and forms non-radical products. The mechanism of action of α-tocopherol is illustrated in Figure 19.

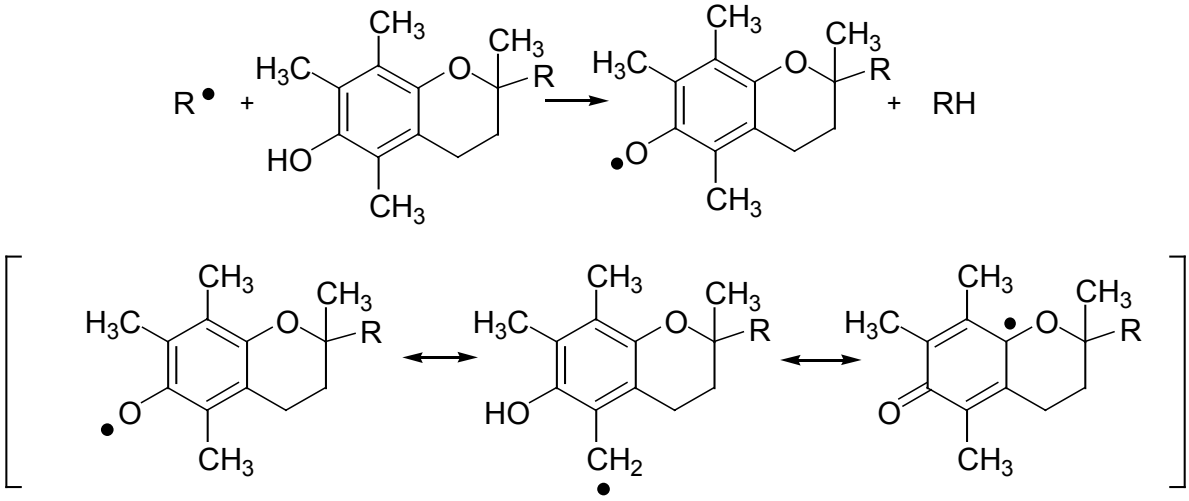


Figure 19. The radical (R•) scavenging action of α-tocopherol and some of the resonance structures of the resulting α-tocopherol radical

Free radical-mediated processes in biological systems have been extensively studied over the past years. These reactions have been implicated in several pathological conditions such as atherosclerosis, inflammatory conditions (rheumatoid arthritis), neurodegenerative disorders, asthma, psoriasis, malaria and various forms of cancer.[38]

Table 1. Conditions associated with the action of free radicals

Ischaemia and damage following reperfusion	Alcohol-induced damage
Inflammatory diseases	Ageing
Degenerative diseases	Cigarette-smoking
Damage caused by irradiation	Some forms of cancer
Hyperchromatosis	Atherosclerosis

Thus, radical scavengers of the pathogenic active radicals have potential as therapeutic agents in a wide variety of illnesses. The role and beneficial effects of antioxidants against such oxidative stress support this view.

1.5.2 15-Lipoxygenase

Lipoxygenases comprise a family of enzymes capable of mediating oxidation of polyunsaturated fatty acids to the corresponding hydroperoxide and are important oxidants *in vivo*. These enzymes, in contrast to the products of free radical-mediated oxidation, give regio-, stereo- and enantio-specific hydroperoxides.[39]

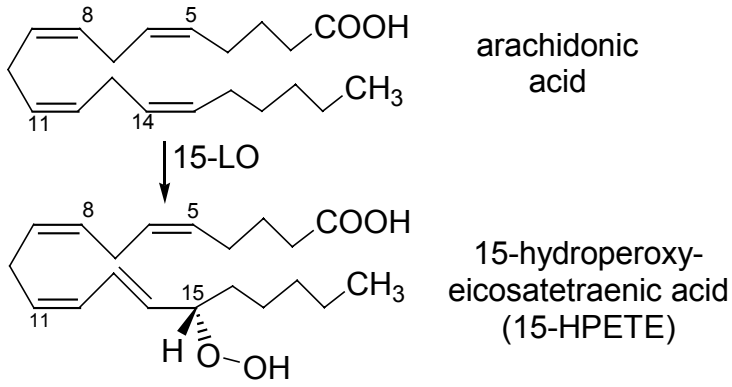


Figure 20 The action of 15-lipoxygenase on a polyunsaturated fatty acid

For example, mammalian 15-lipoxygenase (15-LO) oxidises arachidonic acid to give mainly 15(S)-5*Z*,8*Z*,11*Z*,13*E*-hydroperoxyeicosatetraenic acid (15-HPETE). Products of 15-LO are shown to be potent signal transduction modifiers and appear to influence the inflammatory responses. Production of the eicosanoid lipoxins requires the action of 15-LO, and evidence suggests that the enzyme also contributes to the oxidation of LDL and the development of atherosclerosis.

In the human body, arachidonic acid as well as eicosapentanoic acid serve as precursors of the so-called eicosanoids, which are implicated in the control of many physiological prosesses. Free arachidonic acid is metabolised by several pathways: cyclooxygenase (COX) initiates the biosynthesis of prostaglandins and thromboxanes, while the lipoxygenases (5-,12- or 15-LO) initiate the synthesis of leukotrienes, lipoxins and other compounds, see Figure 21.

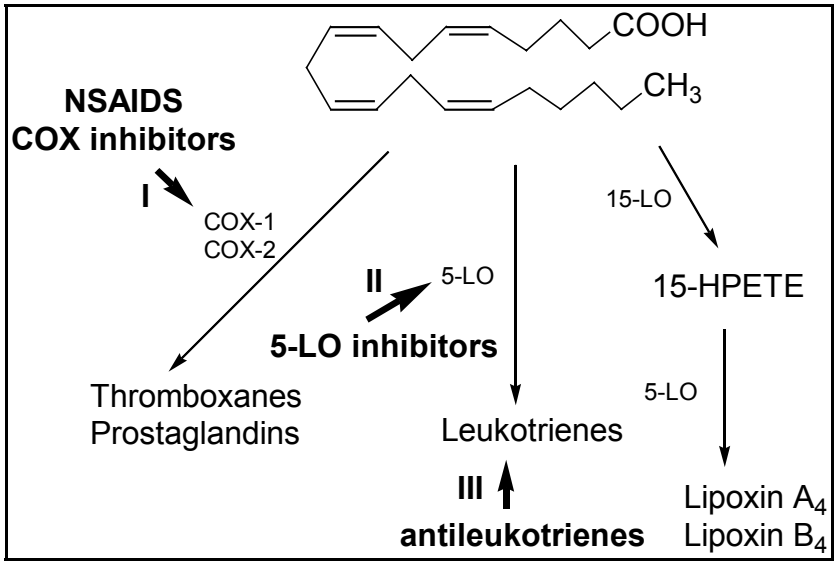


Figure 21 Arachidonic acid metabolism and generation of eicosanoids. Arachidonic acid is the precursor of many lipid mediators. The sites of action of some anti-inflammatory drugs are shown.

1.6 Inflammation

Many eicosanoids are associated with pro-inflammatory responses, and inhibiting the arachidonic acid metabolism has shown to be an effective means of reducing inflammation. Several drugs have been developed that modify the enzymatic pathways to lipid metabolites. Non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX inhibitors (Figure 21, I) block the synthesis of prostaglandins and thromboxanes, reducing inflammatory responses. Anti-leukotrienes either reduce leukotriene synthesis by inhibiting 5-lipoxygenase (Figure 21 II), or antagonize the most relevant of their receptors (Figure 21, III) and are a relatively new class of anti-asthma drugs. Lipoxins, contrary to the other eicosanoids, appear to have anti-inflammatory effects, although their role in inflammatory responses needs further investigation.[40] Antioxidants affecting the 15-LO pathway to lipid mediators might be of therapeutical use in asthma and other inflammatory reactions.

1.7 Atherosclerosis

Low-density lipoprotein (LDL) transports lipids and cholesterol through the bloodstream and is essential to life, but excessive concentrations in plasma increase the risk of coronary artery disease. The cells that line the blood vessels bind LDL and, when activated, these cells generate free radicals and other factors which oxidise LDL.

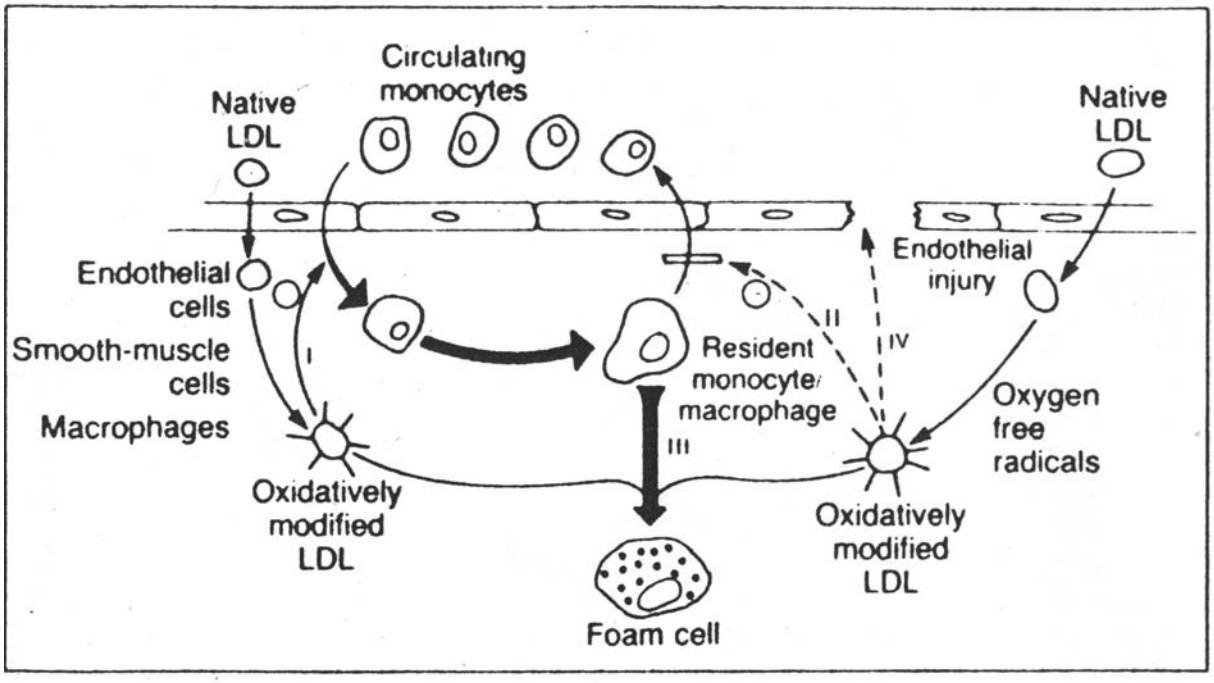


Figure 22. Mechanisms by which the oxidation of LDL may contribute to atherogenesis.[41] *Oxidized LDL releases chemotactic factors that recruit circulating monocytes (I) and prevent resident macrophages from leaving the inner lining of the blood vessels (II). Enhanced rate of uptake of oxidized LDL by macrophages leads to the generation of foam cells (III). Endothelial injury occurs due to the cytotoxic factors released by oxidized LDL (IV).*

Oxidized LDL is cytotoxic, and may harm the cells lining the blood vessels. It is taken up by macrophages, forming foam cells, which migrate beneath the inner lining of the blood vessels. This subendothelial collection of foam cells and other cells comprises the "fatty streaks" indicative of atherosclerosis. They release factors that induce deposition of fibrous tissue and stiffening of the blood vessel walls, eventually forming a dense fibrotic plaque. Oxidized LDL appears to release factors stimulating platelet aggregation and development of thrombosis. The plaque may rupture,

releasing aggregatory mediators, and form a thrombus, which may interfere with the blood supply to various organs and tissues, resulting in impaired function. A portion of the thrombus may break away, forming an embolus, which lodges downstream, causing infarction.[42] Atherosclerotic lesions develop over many decades and once symptoms occur, the disease has reached an advanced stage. Evidence suggests that 15-LO contributes to the oxidation of LDL.[43] Therefore, inhibitors of 15-LO might limit the progression of atherosclerosis.

1.8 Project description

In the present study a series of chalcone and chroman derivatives of the general structures depicted in Figure 23 were synthesized and tested for antimalarial activity, radical scavenging actions and 15-LO inhibition (antioxidants).

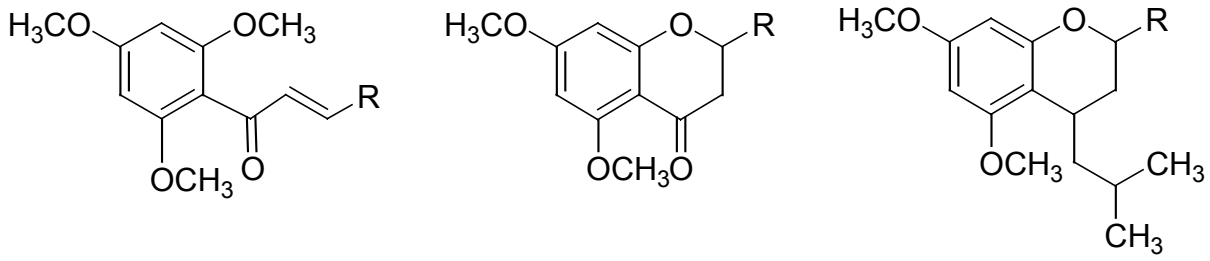


Figure 23. General structures of the chalcone and chroman derivatives

Chalcones

The choice of alkoxylated substituents on the B ring (see Figure 3) was based on information obtained from literature on structure-activity relationships, and the feasibility of the synthesis was also taken into account. Hydroxylated compounds proved more difficult to work with due to the reactivity of the phenolic groups. Substituted benzene and alternative ring systems such as pyridine, quinoline, furan, thiophene and indole as ring A were synthesized and tested in order to provide more information on the structural requirements of the A ring for biological activities.

Chroman derivatives

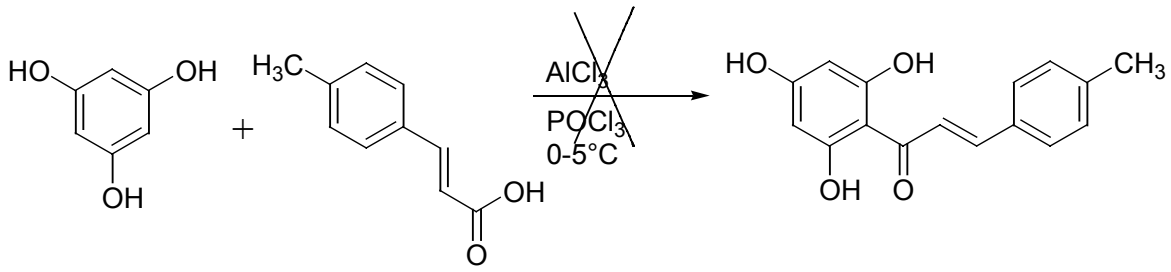
The 1,3-ketoxy structure of 5-alkoxylated chromanones may have Fe-chelating properties, producing both antioxidant and antimalarial effects. The ketone moiety was removed to provide information on whether this particular structural feature is important for activity. This might also provide some indications as to the lipophilic requirements for biological activity.

2. Results and Discussion

2.1 Syntheses

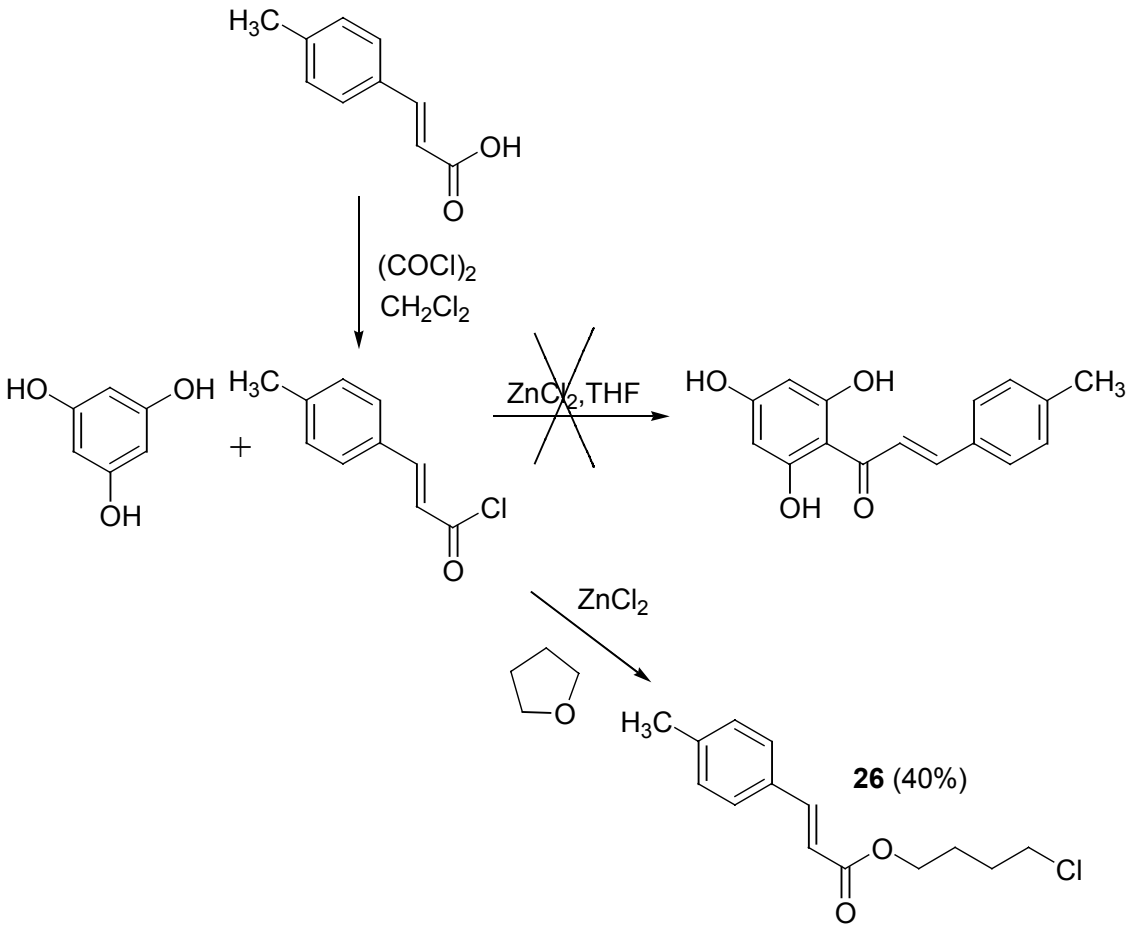
2.1.1 Friedel-Crafts reaction between phloroglucinol and cinnamic acid derivatives

Our first aim was to synthesize a chalcone with hydroxyl substituents in the 2',4'- and 6'-positions. This was attempted prepared by the Friedel-Crafts acylation reaction of phloroglucinol with α,β-unsaturated *para*-methylcinnamic acid, using an excess of Lewis acid catalyst. The reaction is outlined in Scheme 1. NMR analysis of the crude product showed that the reaction produced only minute amounts of the chalcone in a complex mixture, and consequently we chose another approach.



Scheme 1

Substituting the acid with the acid chloride and using different solvents, such as THF, DCM and ether, did not improve the outcome of the reaction. Reaction in THF solution did not provide the desired chalcone, but compound 4-chlorobutyl *E*-3-(4-tolyl)-2-propenoate (**26**) was formed (Scheme 2). This occurred most probably by acid-catalyzed opening of the tetrahydrofuran ring to 4-chloro-1-butanol, which subsequently reacted with the acid chloride.



Scheme 2

The structure of the product **26** was assigned based on the NMR, IR and MS data. ^{1}H NMR spectra of the compound showed two doublets (J 16.0 Hz) at 7.6 and 6.4 ppm, respectively, indicating the presence of an olefinic double bond with an *E* configuration.[44] The aromatic protons exhibit two doublets (J 8.0 Hz) of the same integral height in the range 7.4-7.1 ppm, characteristic for *para*-substituted benzene. The methyl substituent on the aromatic ring appears as a singlet at 2.4 ppm. The aliphatic protons α to ester and α to Cl give rise to triplets (J 6.0 Hz) at 4.2 and 3.6 ppm, respectively, whereas the methylene protons between them are not well differentiated, and appear as a multiplet at 1.9 ppm. In the HH COSY diagram, coupling interactions can be seen between the methylene multiplet at 1.9 and both triplets, as well as between the methyl substituent and the protons on the benzene ring, the protons further upfield showing the strongest coupling.

The ^{13}C NMR spectrum disclosed twelve chemically non-equivalent carbons, and the CH COSY spectrum reveals to which proton they are bonded. The signal at 21.4 ppm arises from the methyl group attached to the phenyl group, whereas the strong signals at 129.5 and 128.0 ppm constitute the methine carbons. The signals at 116.8 and 144.8 ppm represent the olefinic carbons α and β to the carbonyl group, respectively. Four C atoms with shift values 26.1, 29.1, 44.4 and 63.5 ppm form the butyl chain, of which the latter two characterize the carbons α to ester and α to Cl. The carbon at 167.0 ppm shows no connectivity to proton in the HC COSY diagram. This lack of HC connectivity and the high shift value confirm the presence of an ester group. No connectivity is observed for the carbon atoms resonating at 131.5 and 140.7 ppm. The DEPT spectra verify the carbon connectivity in structure **26**.

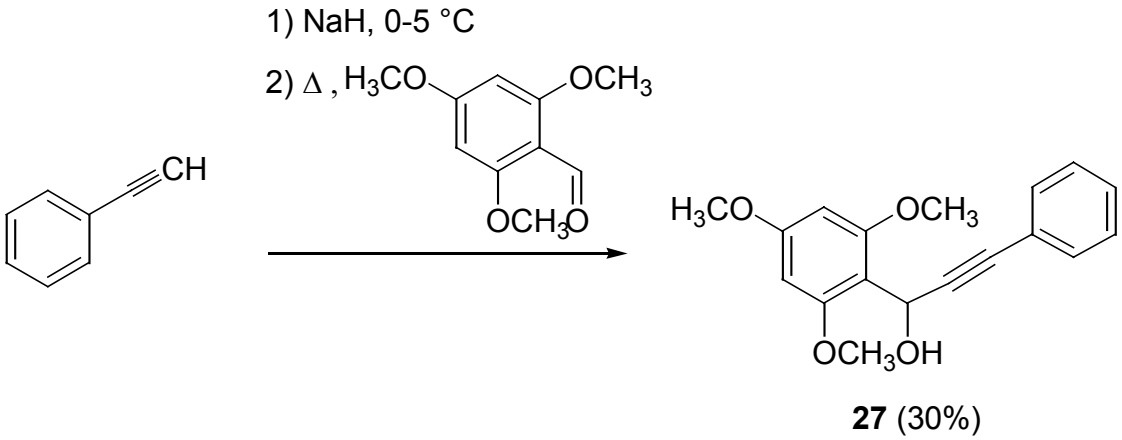
In the infrared spectrum a broad peak was observed in the region 3036-2850 cm^{-1}, and a peak at 1703 cm^{-1} is due to an α,β-unsaturated carbonyl group. EI-MS showed a signal for the molecular ion at m/z=252 and 254 in a 3:1 relation, respectively, indicating that the molecule contains a Cl atom. The peak at m/z=162 lacks this isotopic relationship and corresponds to a fragment formed by cleavage α to the ester function. The strongest mass peak was at m/z=145, arising from fragmentation of the ester. The data strongly supports the predicted structure.

Protection of the hydroxyl moieties on phloroglucinol as methyl or tetrahydropyranyl ethers produced in both cases a mixture of mono-, di- and tri-protected phloroglucinol, which were difficult to separate by flash chromatography. The same tendency was seen when benzyl bromide was used as protecting agent.

Failure to achieve adequate yields of chalcone in a one-step reaction between phloroglucinol and cinnamic acid derivatives led us to try a different pathway for the preparation of chalcones. Due to the reactivity of the phenolic groups, and difficulties experience in protecting them, we concentrated our efforts on methoxylated phloroglucinol-based compounds.

2.1.2 Nucleophilic addition to C=O with acetylide anion

Phenylacetylene reacted with NaH to generate the acetylide anion, followed by reaction with 2,4,6-trimethoxybenzaldehyde affording 1-(2,4,6-trimethoxyphenyl)-3-phenyl-2-propyn-1-ol (**27**) in very low yield (< 2%). Using the same reagents, but heating the reaction, increased the yield to 30%, see Scheme 3. Replacing NaH with MeLi at -78°C, did not improve the outcome (13%).



Scheme 3

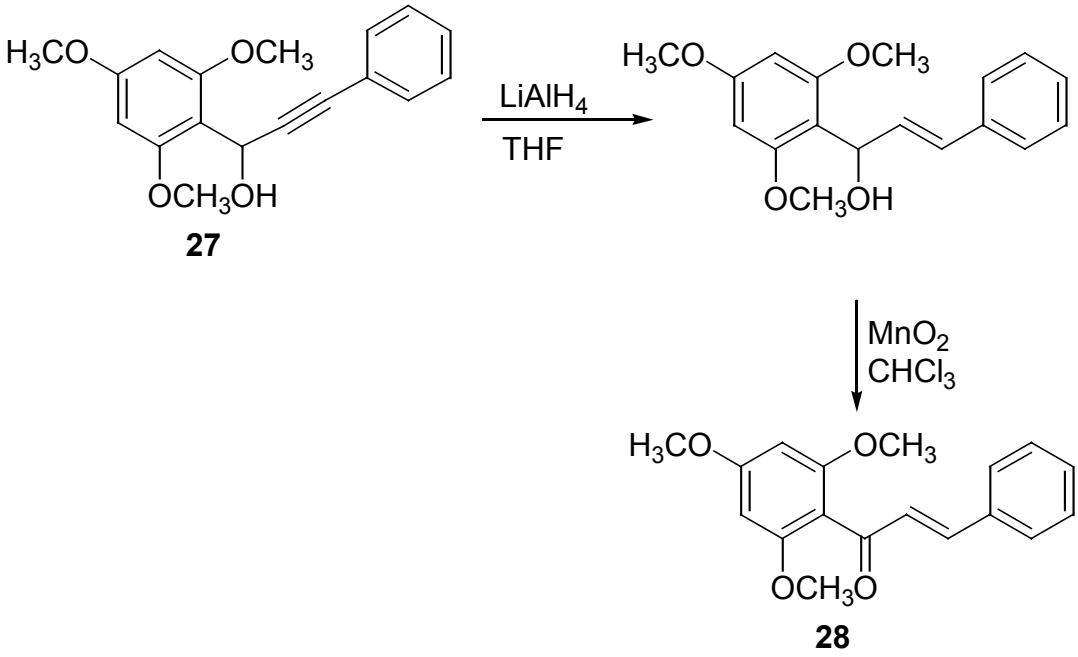
The ^{1}H NMR spectrum of the alkynol **27** showed two multiplets with integral levels in a ratio of 2:3 at aromatic shifts around 7.4 and 7.2 ppm, respectively, matching the five protons in the unsubstituted A ring. The aromatic protons in the trimethoxy substituted B ring appear as a singlet at 6.2 ppm, whereas the *para*-methoxy group gives rise to a singlet at 3.8 ppm. The protons in the *ortho*-methoxy groups cannot be differentiated from the alcoholic proton, and they appear together in a multiplet in the range 3.9-3.86 ppm. The doublet (J 11.3 Hz) at 6.0 ppm arises from the proton α to the alcohol. Coupling between the doublet at 6.0 ppm and part of the multiplet is observed in the HH COSY diagram.

The thirteen chemically non-equivalent C atoms in structure **27** appear as thirteen distinctive signals in the ^{13}C NMR spectrum. With the aid of the DEPT spectra and the HCCOSY and HCCOLOC diagrams, each peak signal can be assigned to specific carbon nuclei. There are six carbons lacking HC connectivity, of which those at

161.1, 158.3 and 110.6 ppm are from the substituted benzene B ring, and the signals at 90.5 and 82.6 ppm arise from the ethynyl carbons α and β to the alcohol, respectively. The last carbon not bonded to a proton connects the A ring to the propynol moiety and exhibits a signal at 123.4 ppm. Because of the symmetry, the A ring methine carbons give rise to three signals at 131.7, 128.0 and 127.9 ppm. The peaks at 55.9 and 55.3 ppm in relation 2:1 correspond to the *ortho*- and *para*-methoxy substituents of the B ring, in that order, and the signal at 91.3 ppm arises from the methine groups on the same ring. The alcoholic carbon has a peak signal of 56.6 ppm.

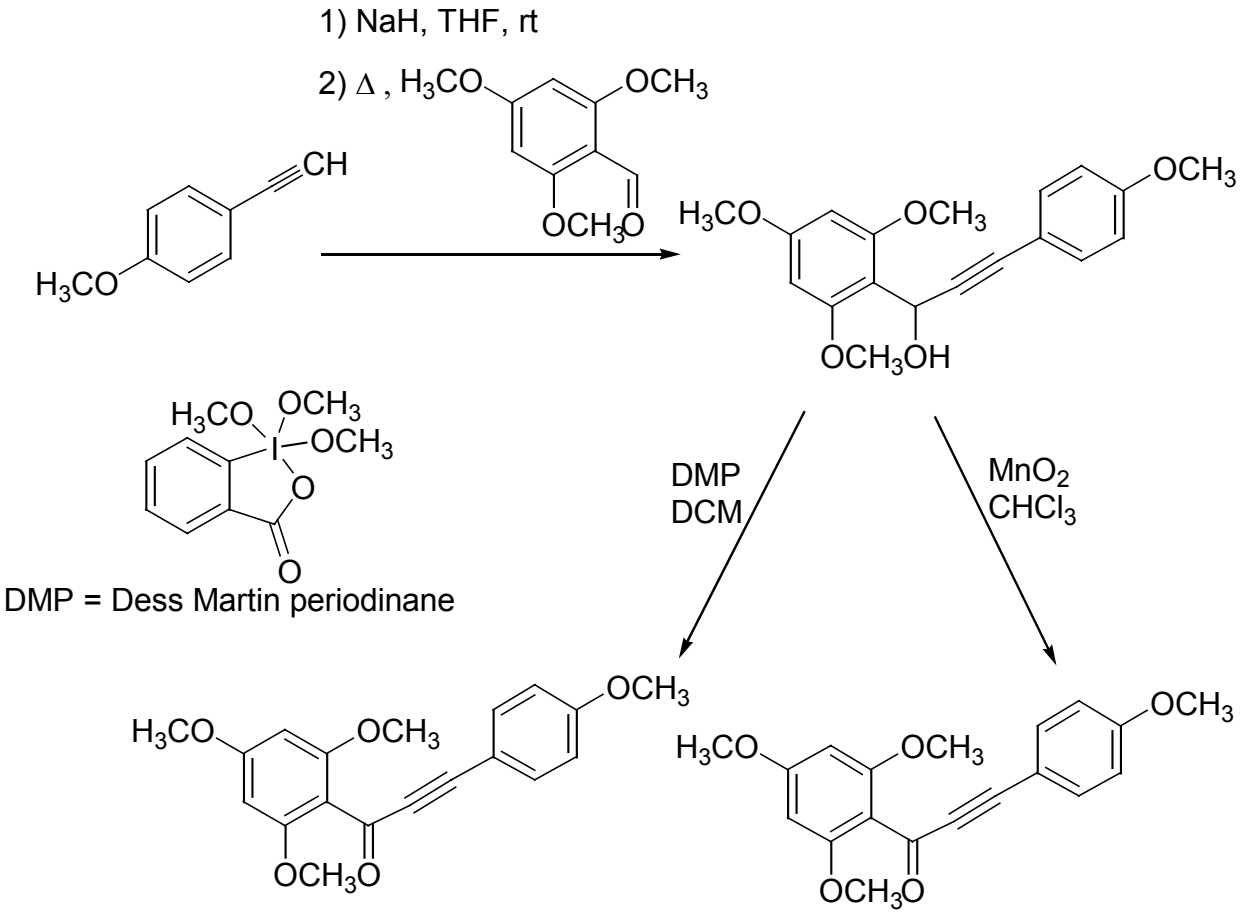
In the infrared spectrum peaks were observed at 3545 (O-H) and 2258 cm^{-1} (C≡C). In the MS spectrum, the molecular ion appeared at m/z=298, and fragmentation ions at m/z=283 and 267 are apparently derived from loss of one and two methyl groups, respectively. The strong peak at m/z=168 results most likely from fragmentation of the bond between the alcoholic carbon and the ethynyl group.

$LiAlH_4$ reduction of compound **27** under thermodynamic control in THF afforded an α,β-unsaturated alcohol.[45] Subsequent oxidation of the intermediate with MnO_2 furnished *E*-1-(2,4,6-trimethoxyphenyl)-3-phenyl-2-propen-1-one (**28**) as a complex mixture, see Scheme 4. Comparison of the ^{13}C NMR spectrum and IR data of compounds **27** and **28** provides evidence for the reduction of the ethynyl group. Compound **28** shows no IR absorbance characteristic of a triple bond and the ethynyl ^{13}C signals that were observed for compound **27** are absent. The ^{13}C spectrum shows a signal at 194.0 ppm, indicating the presence of a carbonyl carbon. The yields were not established since the products could not be isolated by column chromatography.



Scheme 4

Although compound **28** was not purified, information regarding the structure can be deduced from the NMR spectrum of the crude product. The ^{1}H NMR spectrum indicates the presence of an *E*-olefinic proton by a doublet, J 16.0 Hz, appearing at 7.0 ppm. The aromatic protons and the methoxy substituents on the B ring give rise to a pattern similar to the one described for compound **27**; a singlet at 6.15 ppm and two singlets in the region 3.7-4.0 ppm.



Scheme 5

Nucleophilic addition of 4-methoxyphenylacetylene to 2,4,6-trimethoxybenzaldehyde with subsequent oxidation was attempted (Scheme 5). Oxidation of the crude product was carried out using either Dess Martin periodinane or MnO_2 in $CHCl_3$. Both reactions produced inseparable mixtures of compounds, which were discarded.

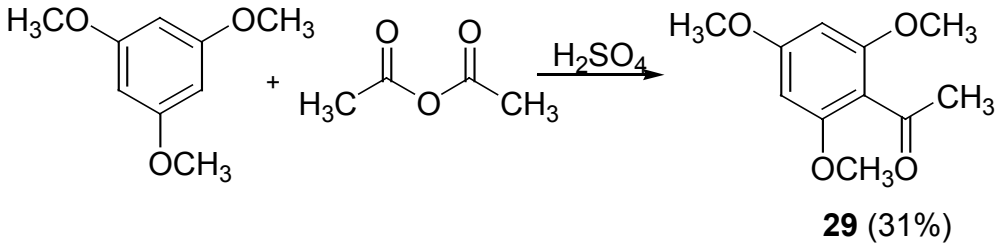
In summary, nucleophilic addition of acetylide anions to the 2,4,6-trimethoxybanzaldehyde provided products that were difficult to purify. High purity is an absolute prerequisite for compounds that are to be tested in biological assays, and consequently we chose a different strategy.

2.1.3 Synthesis of starting materials

The following section describes Claisen-Schmidt condensation reactions involving either 2,4,6-trimethoxyacetophenone (**29**) or 2,4-dimethoxy-6-hydroxyacetophenone (**30**) as a starting material.

Synthesis of 2,4,6-trimethoxyacetophenone (29)

Compound **29** was synthesised in 31% yield by a Friedel-Crafts acylation of acetic anhydride with 1,3,5-trimethoxybenzene, according to Scheme 6. The NMR spectra of compound **29** were identical to the spectra of 2,4,6-trimethoxyacetophenone obtained from a commercial source.

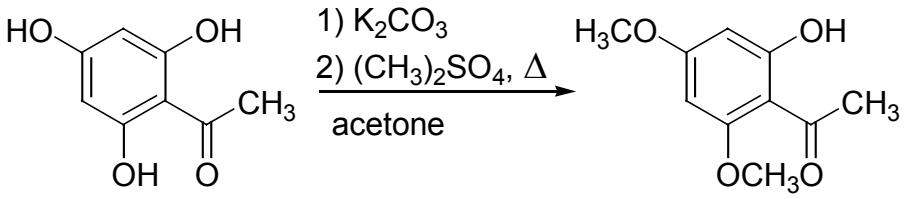


29 (31%)

Scheme 6

Synthesis of 2,4-dimethoxy-6-hydroxyacetophenone (30)

The compound was obtained from a methylation reaction of phloroacetophenone with dimethyl sulphate[46] in 68% yield as depicted in Scheme 7. The NMR data are identical to the NMR data taken of a commercially produced sample of compound **30**.

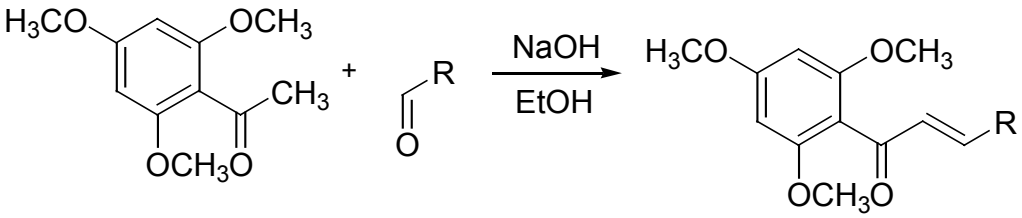


30 (68%)

Scheme 7

2.1.4 Claisen-Schmidt condensation of trisubstituted acetophenone with aromatic aldehyde

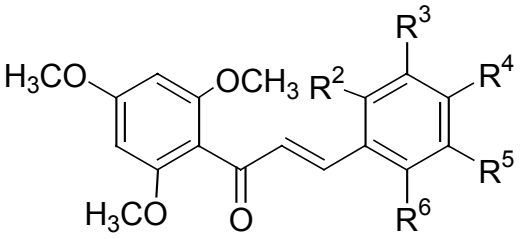
The Claisen-Schmidt condensation of aromatic aldehyde with a substituted acetophenone (**29**) in the presence of NaOH or KOH in protic solvent has frequently been used to synthesize chalcones in high yields.



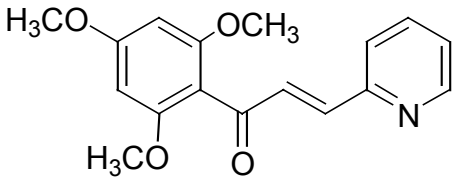
28,**31**-**54** (8-90%)

Scheme 8

A variety of chalcone derivatives (**28**,**31-54**) was synthesized in this manner (Scheme 8), see Figure 24 and Table 2. Yields range from 8% to 90%, but were not optimised. All the compounds were crystalline, their colours ranging from creamy white through yellow to orange, except for *E*-1-(2,4,6-trimethoxyphenyl)-3-(pyridin-4-yl)-2-propen-1-one (**47**) which was a yellow oil. A search of the literature revealed different melting point values for chalcone **47**: 101-103[47] and 80[48] °C. Polymorphism has been reported for many chalcones, and this may explain some of the discrepancies observed between our melting points and the values reported in the literature.[49]



28,**31**-**44**



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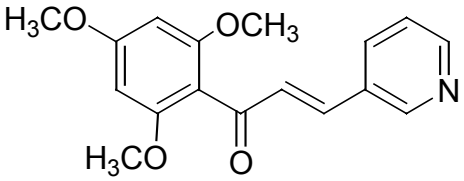

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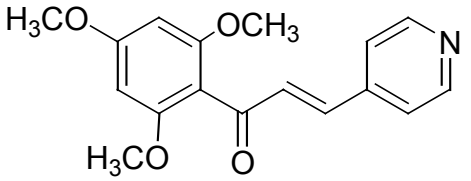

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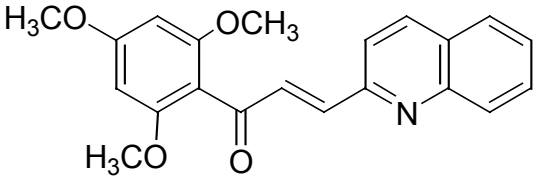

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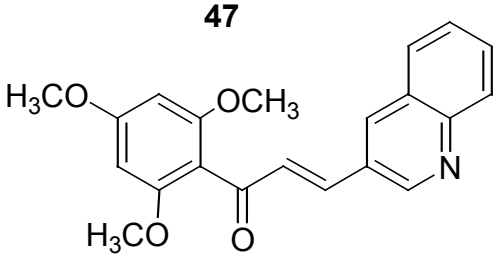

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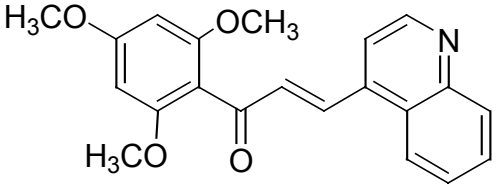

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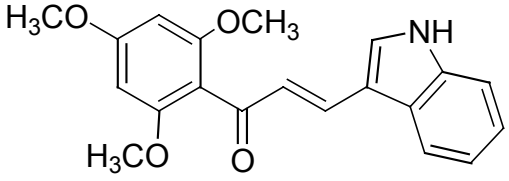

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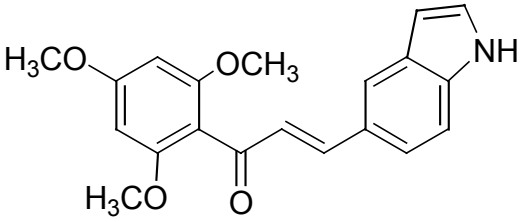

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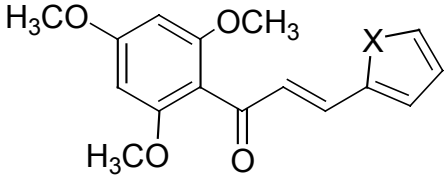


53 (X = S)
54 (X = O)

Figure 24 Structures of chalcone derivatives

Table 2 Chalcone derivatives prepared in this study

Nr	R^2	R^3	R^4	R^5	R^6	Formula	Yield (%)
28	H	H	H	H	H	$C_{18}H_{18}O_4$	76
31	NO_2	H	H	H	H	$C_{18}H_{17}NO_6$	82
32	H	NO_2	H	H	H	$C_{18}H_{17}NO_6$	87
33	H	H	NO_2	H	H	$C_{18}H_{17}NO_6$	87
34	Br	H	H	H	H	$C_{18}H_{17}BrO_4$	80
35	H	H	Br	H	H	$C_{18}H_{17}BrO_4$	63
36	H	H	CH_3	H	H	$C_{19}H_{20}O_4$	80
37	H	H	*t*-Bu	H	H	$C_{22}H_{26}O_4$	55
38	H	H	OCH_3	H	H	$C_{19}H_{20}O_5$	58
39	OCH_3	OCH_3	OCH_3	H	H	$C_{21}H_{24}O_7$	70
40	OCH_3	H	OCH_3	OCH_3	H	$C_{21}H_{24}O_7$	50
41	OCH_3	H	OCH_3	H	OCH_3	$C_{21}H_{24}O_7$	8
42	H	OCH_3	OCH_3	OCH_3	H	$C_{21}H_{24}O_7$	70
43	H	H	OH	H	H	$C_{18}H_{18}O_5$	30[a]
44	H	OH	OH	H	H	$C_{18}H_{18}O_6$	14[a]
45	-	-	-	-	-	$C_{17}H_{17}NO_4$	25
46	-	-	-	-	-	$C_{17}H_{17}NO_4$	48
47	-	-	-	-	-	$C_{17}H_{17}NO_4$	40
48	-	-	-	-	-	$C_{21}H_{19}NO_4$	75
49	-	-	-	-	-	$C_{21}H_{19}NO_4$	85
50	-	-	-	-	-	$C_{21}H_{19}NO_4$	85
51	-	-	-	-	-	$C_{20}H_{19}NO_4$	n.d.[b]
52	-	-	-	-	-	$C_{20}H_{19}NO_4$	19
53	-	-	-	-	-	$C_{16}H_{16}SO_4$	72
54	-	-	-	-	-	$C_{16}H_{16}O_5$	36

[a] Over two steps [b] Not determined

The structures of the compounds **28** and **31-54** were confirmed by NMR, MS and, occasionally, IR analysis. The ^{1}H NMR spectra for the compounds had several proton signals in common. The aromatic protons at C-3' and C-5' appear as a singlet around 6.3-6.0 ppm, whereas the three methoxyl groups of the same ring B give rise to two singlets in the integral ratio 6:3 at 4.0-3.7 ppm. The olefinic protons give rise to two doublets (J ca. 16 ppm), α-H in the range 7.3-6.7 and β-H in the range 8.1-7.1 ppm. The α proton appears further upfield compared to the β proton due to the shielding effect of the carbonyl group. The coupling constant of 16 ppm strongly indicates that the protons have a *trans* configuration, which is consistent with the observation that the more stable *trans* isomers are produced in the synthesis of chalcones.[50]

The ^{13}C NMR spectra also demonstrate some common features. The carbonyl carbon appears at the low field of 197-192 ppm. The aromatic C-3' and C-5' of the trimethoxylated B ring provide a signal at 92-90 ppm, while the methoxy carbons give rise to two peaks in the range 57-55 ppm with a ratio of 2:1.

The ^{1}H NMR signals arising from the A ring differ in chemical shifts, caused by the different shielding effects of the varying substituents and ring structures. In the case of the unsubstituted benzene ring A in compound **28**, five H atoms usually appear as three signals, due to magnetic equivalence of protons at C-2 and C-3 with C-6 and C-5, respectively. In our case, the signals of C-3, C-4 and C-5 were not differentiated, and gave rise to a multiplet together with the olefinic α proton. A similar phenomenon is observed for symmetrically substituted A rings such as the 4-monosubstituted chalcones **33**, **35-38** and **43**, the 2,4,6-trisubstituted chalcone **41**, the 3,4,5-trisubstituted compound **42**, as well as for the pyridin-4-yl **47**. The signals due to the A ring protons appear in the range 6.0-9.1 ppm, of which the highest chemical shifts are observed for the protons of heterocyclic compounds containing shielding N atom. Electron-rich substituents such as the nitro group also shift the proton signals further downfield due to their shielding effect.

The ^{13}C NMR spectral analysis of 2',4',6'-trimethoxychalcone **28** displays the expected thirteen signals corresponding to the eighteen carbon atoms. Chalcones with symmetrically substituted A rings also display only thirteen peaks in their ^{13}C NMR spectra, caused by the chalcone nucleus. In the presence of an unsymmetrical ring, all the A ring carbons display distinct signals in addition to the carbon-containing functional groups.

MS analyses were performed on all structures, except the indole derivatives **51** and **52** because of their instability. For HRMS mass determination, deviations were less than 5 ppm. In EIMS, all compounds exhibited a fragment at 195, corresponding to

cleavage of the bond between the carbonyl group and the double bond, providing a trimethoxybenzaldehyde ion, see Figure 25.

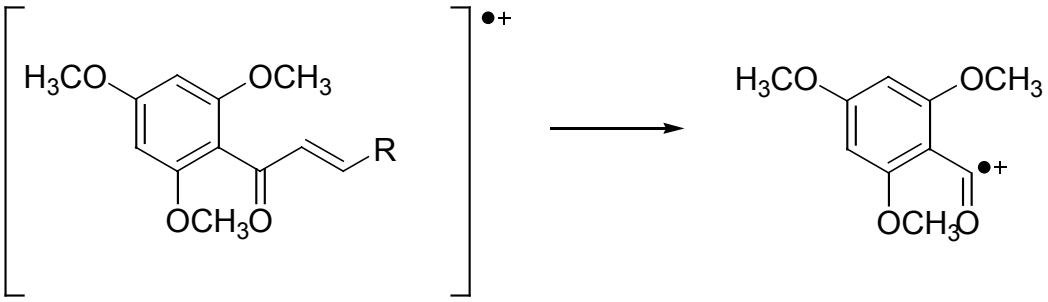


*Figure 25 The fragmentation ion (m/z=195) of chalcone derivatives **25** and **31-54***

Br-substituted compounds **34** and **35** showed characteristic Br-peaks in 1:1 relation at $[M^+]$ and $[M^+ + 2]$. The chalcones **31-33** showed characteristic IR absorption frequencies for the nitro group around 1510-1565 and 1345-1385 cm^{-1}.

Condensation of 2,4,6-trimethoxyacetophenone (**29**) with indolecarboxaldehydes provided the unstable indole A ring products **51** and **52**. *E*-1-(2,4,6-Trimethoxyphenyl)-3-(3-indolyl)-2-propen-1-one (**51**) was obtained in a very low yield and was difficult to purify by flash chromatography, and thus was not isolated and the yield has not been determined. Due to their instability, the indole derivatives **51** and **52** were not used in further reactions or subjected to any biological tests.

Some aldehyde derivatives do not undergo reaction by the Claisen-Schmidt pathway. 3-Methoxyfuraldehyde did not undergo condensation at all. This might be explained by the electron-donating properties of the methoxy substituent, which might promote cleavage of the furan ring.

Claisen-Schmidt reaction of 2,4,6-trimethoxyacetophenone (**29**) with hydroxy-substituted benzaldehydes proved difficult and protection of the hydroxyl groups was required. This observation is supported by earlier findings that the reactivity of the carbonyl carbon in *para*-hydroxybenzaldehyde is lowered in alkaline medium.[51] This

lower reactivity of the carbonyl carbon has been explained by the delocalisation of the negative charge on the phenolic oxygen (Figure 26).

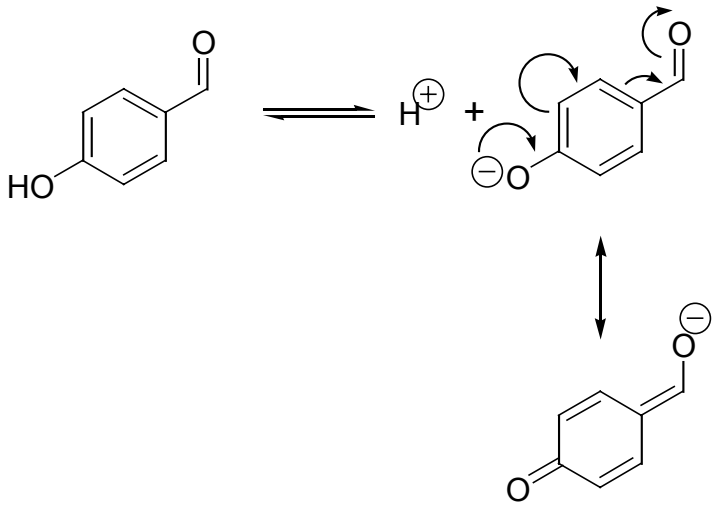
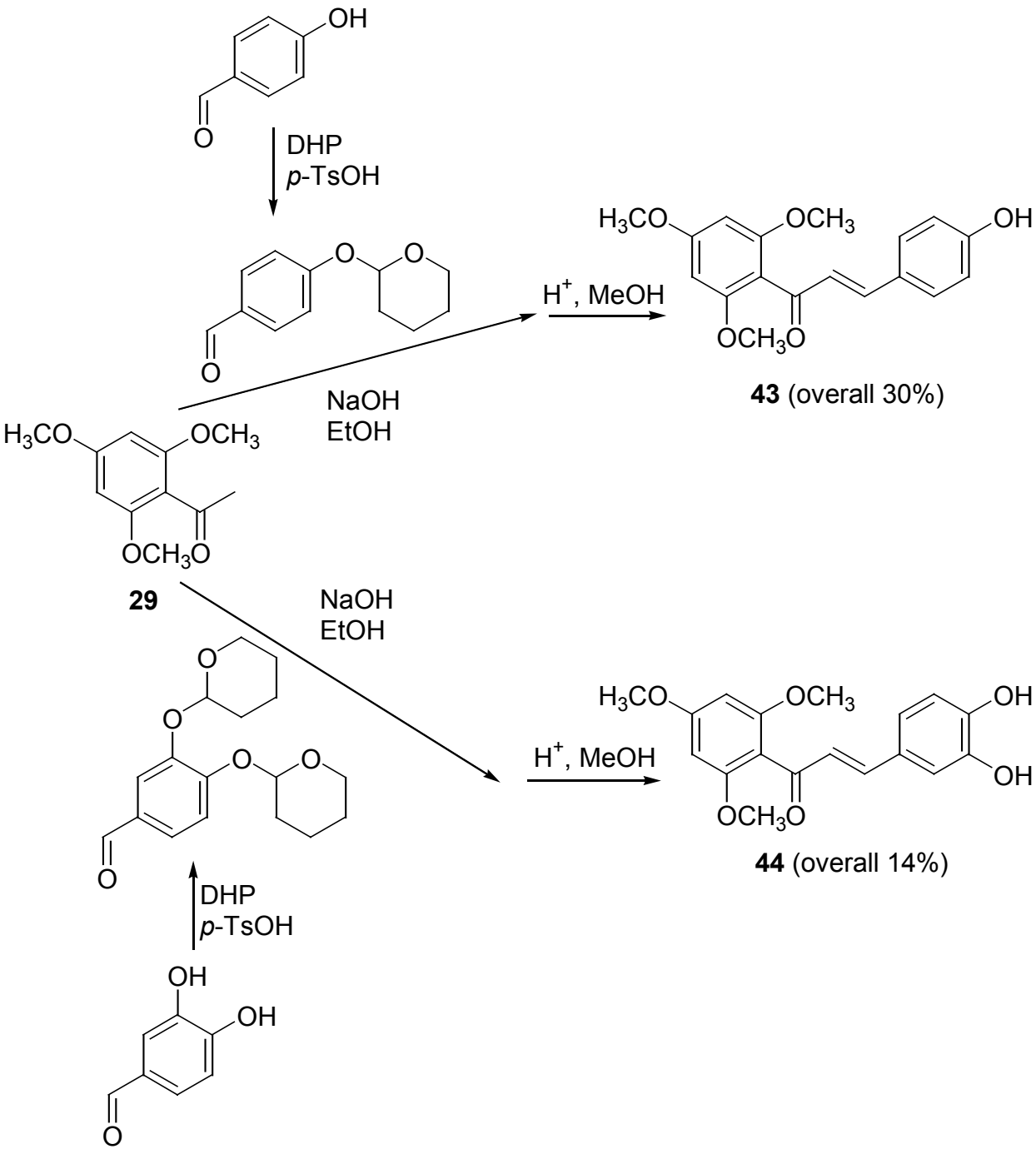


Figure 26 Delocalization of negative charge in phenolic benzaldehyde

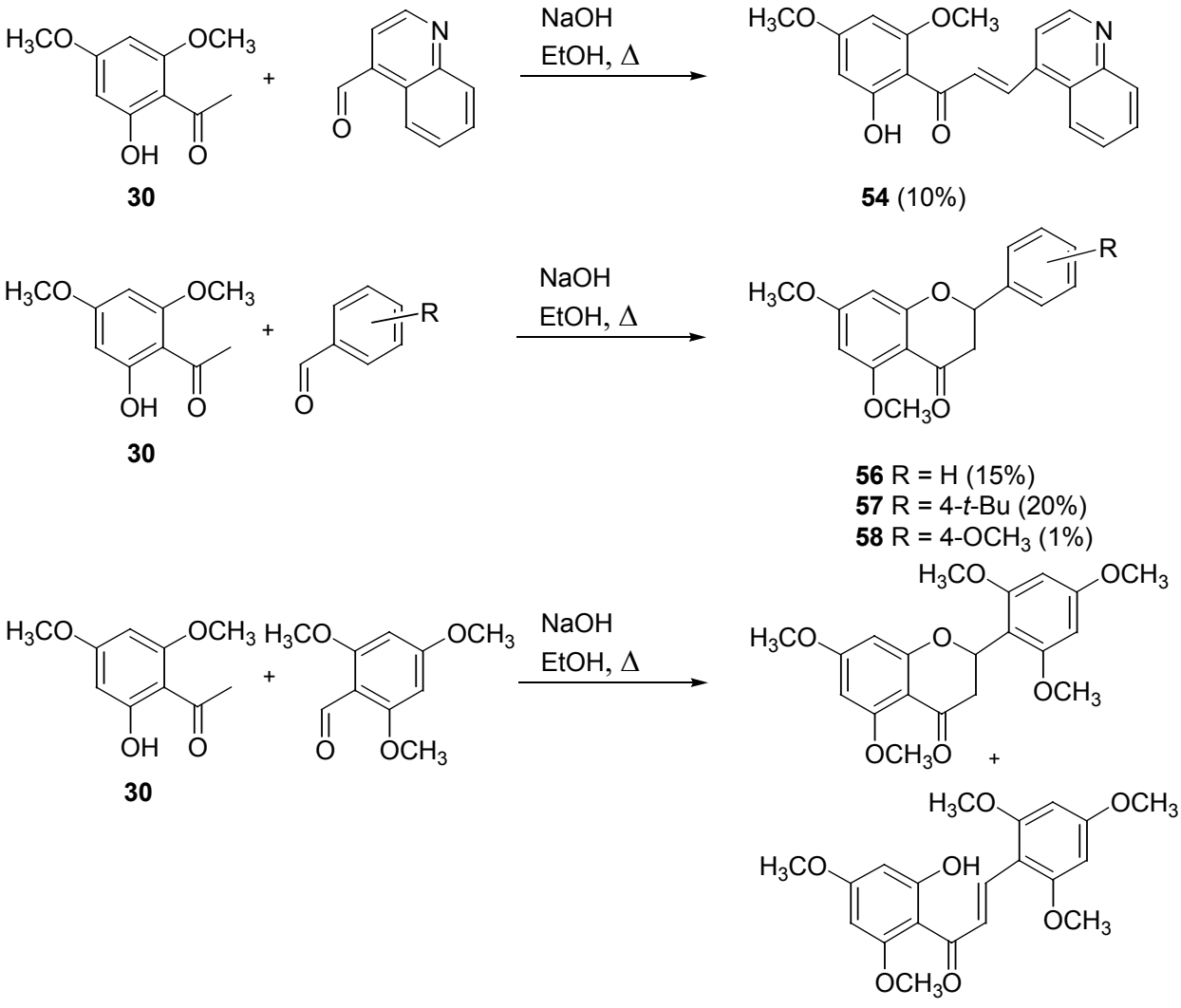
Following protection of the hydroxyl moieties on the aldehyde with THP, reaction with substituted acetophenone and subsequent removal of the protecting groups by acid hydrolysis gave the chalcones **43** and **44** in overall yields of 30 and 14%, respectively. The reaction sequence is shown in Scheme 9. Protection with other groups, such as acetyl and TMS was attempted, but with poor results.



Scheme 9

Thermally induced Claisen-Schmidt condensation of 2,4-dimethoxy-6-hydroxyacetophenone (**30**) with various substituted aldehydes afforded in only one case the pure 2',4'-dimethoxy-6'-hydroxychalcone derivative **55**, while in others the

corresponding chromanones were formed, see **56-58**, Scheme 10. Clearly, the 6'-hydroxy substituent promotes the cyclization. Furthermore, in the reaction of compound **30** with 2,4,6-trimethoxybenzaldehyde, NMR data of the product suggests that a mixture of the corresponding chromanone and the 2',4'-dimethoxy-6'-hydroxychalcone was formed. For further information on the cyclized products **56-58**, see section 2.1.5.



Scheme 10

Due to extremely poor solubility, the structure of *E*-1-(2,4-dimethoxy-6-hydroxyphenyl)-3-(quinolin-4-yl)-2-propen-1-one (**55**) has been assigned based

solely on the ^{1}H NMR and MS data. The ^{1}H NMR spectrum, in deuterated MeOH at 50 °C, indicates the presence of thirteen non-equivalent protons, which is consistent with the predicted structure. The B ring is, unlike the chalcone derivatives **28** and **31-54**, not symmetrical. Therefore, the aromatic protons in compound **55** give rise to two signals: doublets (*J* 2.3 Hz) at 6.05 and 6.00 ppm, the proton at C-3' position giving rise to the higher shift value due to the shielding 2'-hydroxyl moiety. The methoxy protons appear as singlets at 3.81 and 3.71 ppm. The five protons on the quinoline ring provide peaks in the region 7-9 ppm, but the information supplied by the spectral data is not sufficient to assign a particular proton to a specific signal. The same applies to the olefinic protons of the α,β-unsaturated ketone and the hydroxyl proton on the B ring. In the EIMS the mass at m/z=335 corresponds to the molecular ion M^{+}, while the mass at 353 most likely arises from $[M^{+}+H_2O]$. The major peak appears at m/z=181, which is analogous to the characteristic fragmentation previously observed for all the 2',4',6'-trimethoxychalcones (Figure 25).

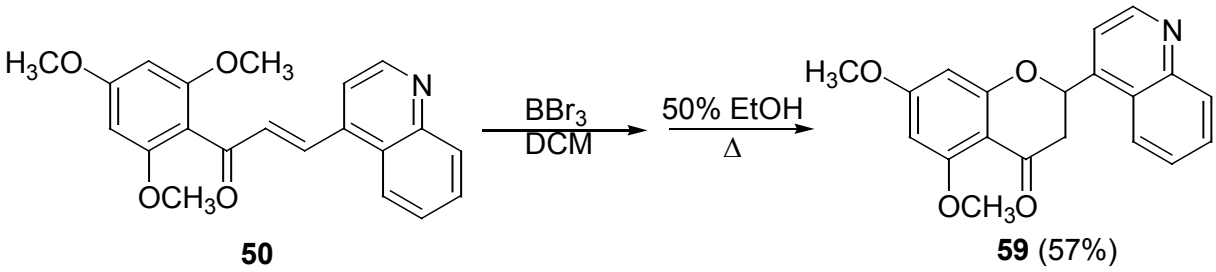
No reaction occurred between 2,4-dimethoxy-6-hydroxyacetophenone (**30**) and the pyridinyl aldehydes.

The Claisen-Schmidt reaction between a tri-substituted acetophenone and a substituted benzaldehyde proved to be the most efficient method of chalcone synthesis, providing products that were easily purified.

2.1.5 Cyclization: chalcones to chromanones

The ring closure of chalcones to chromanones requires a hydroxyl moiety in C-2' of the chalcone.[52] The majority of our chalcone derivatives were 2',4',6'-trimethoxysubstituted chalcones and therefore selective demethylation to provide a 2'-hydroxychalcone had to be performed prior to cyclization. Search of the literature provided us with several pathways for such a demethylation.

The first method involved BBr_3, which has been reported to demethylate a flavanone at the 5-position.[53] The reaction of *E*-1-(2,4,6-trimethoxyphenyl)-3-(quinolin-4-yl)-2-propen-1-one (**50**) with BBr_3 afforded the chromanone **59** in 57% yield, see Scheme 11. Cyclization was unexpected and occurred most likely during the work-up procedure, which involved trituration with boiling 50% EtOH to remove remaining BBr_3 that might have complexed with the heteroatom in the quinoline structure.



50 **59** (57%)

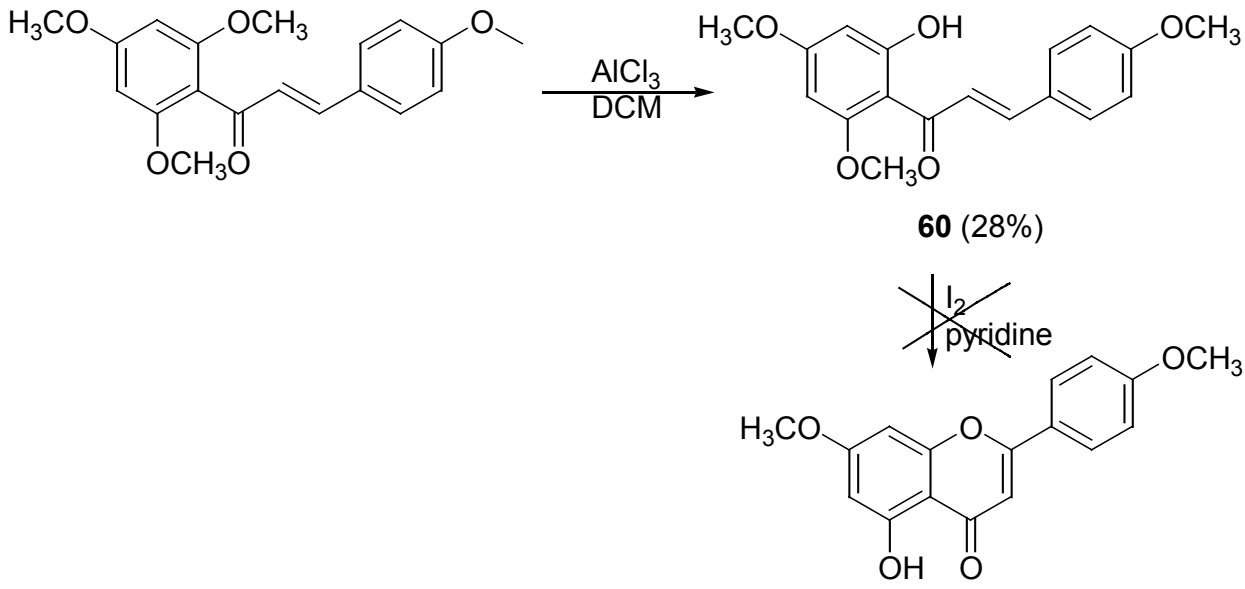
Scheme 11

NMR analysis of compound **59** supports the chromanone structure. Chromanones have some common, distinctive features in their ^{1}H NMR spectra. The proton at C-2 appears as a doublet of doublets (*J* ca. 13 Hz trans, *J* ca. 3 Hz cis) centered at about 5.3-5.0 ppm. The protons at C-3 also give rise to doublets of doublets at 4.0-2.5 ppm due to coupling with each other (*J* ca. 16 Hz) and with the proton at C-2. As was the case for the 2'-hydroxy chalcone derivatives mentioned previously, there is no symmetry in the aromatic ring, and the aromatic A ring protons exhibit two distinct signals. The proton NMR spectrum of chromanone **59** shows all these characteristics. The six protons of the quinoline structure give rise to several signals between 9.0-7.9 ppm, whereas the protons of the methoxy substituents appear together with a proton from C-3, as a multiplet at 3.8-3.7 ppm.

Due to very poor solubility of compound **59**, ^{13}C NMR and 2-D analysis could not be performed. Increasing the probe temperature to 50 °C did not improve the solubility. MS analysis revealed the molecular ion at m/z=335 and relatively strong peaks at m/z=207 and 143. Bond breakage between the quinolinyl moiety and the bicyclic

system most likely gives rise to the observed fragment at m/z=207. The melting point could not be determined as the compound decomposed upon heating.

Another method reported the use of $AlCl_3$ for demethylation of chalcone **60** in 91% yield,[54] but did not specify the use of solvent for the reaction. Our first endeavour was with 1,2-dichloroethane as solvent, affording *E*-1-(2,4-dimethoxy-6-hydroxyphenyl)-3-(4-methoxyphenyl)-2-propen-1-one (**60**) in only 3% yield. Correspondence with the author provided us with the correct solvent, and dichloromethane was indeed a better option; furnishing the chalcone **60** in 28% yield, see Scheme 12. This is a poor result compared to the yield stated in the literature, and is most likely caused by the work up process, which we did not optimise. The structure of compound **60** was ascertained by comparison of the spectral data with those published.

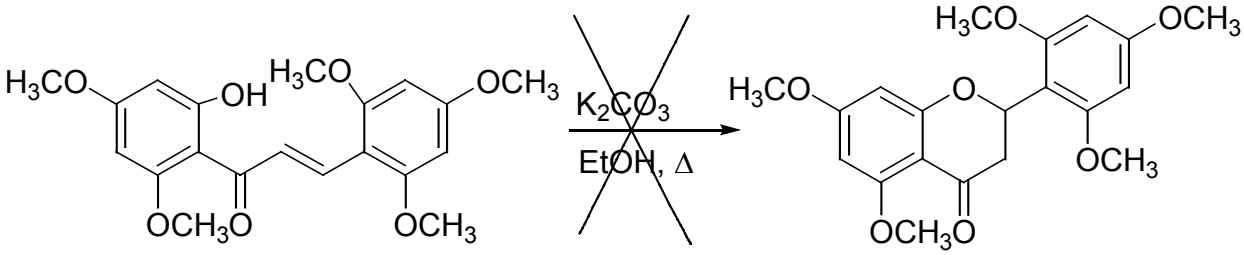


Scheme 12

Demethylation to *E*-1-(2,4-dimethoxy-6-hydroxyphenyl)-3-(4-methoxyphenyl)-2-propen-1-one (**60**) produces an unsymmetrical B ring, giving rise to a signal pattern similar to that of the B ring in 2',4'-dimethoxy-6'-hydroxychalcone **55**. A singlet at 14.41 ppm in the ^{1}H NMR spectrum represents the 6'-hydroxy substituent, the high chemical shift value indicates that it is most likely hydrogen-bonded. This is

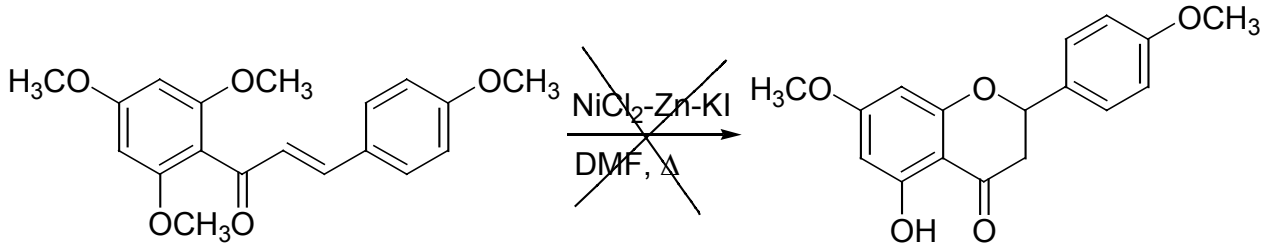
supported by the broad IR absorption band displayed at 3584 cm^{-1}, characteristic of a hydrogen-bonded hydroxyl group. Apart from the data of ring B, the NMR spectra exhibit the same features as the trimethoxy chalcones (see 2.1.4). MS data revealed the highest peak at m/z=314 to be M^+, the fragments at m/z=207 and 180 are the same as those observed for compounds **59** and **55**, respectively. Further cyclization of the chalcone **60** to a chromone with I_2/pyridine[54] failed to provide the desired product.

The chromanones are isomeric with the chalcones and are obtained from the latter by acid- or base-catalysed ring closure. Thermally induced cyclization of chalcone **55** with K_2CO_3 in EtOH did not provide the desired products (Scheme 13). Instead, the reaction afforded an inseparable mixture that was poorly soluble in common reagents such as $CHCl_3$, DMSO, acetone, hexane and MeOH, and the complexes did not move on a TLC plate.



Scheme 13

A literature search revealed that high-yield synthesis of 5-hydroxyflavanone could be carried out using a $NiCl_2$-Zn-KI reagent system without prior demethylation,[55] see Scheme 14. The experimental procedure was followed, but no reaction was observed. Lack of time prevented us from investigating this further.



Scheme 14

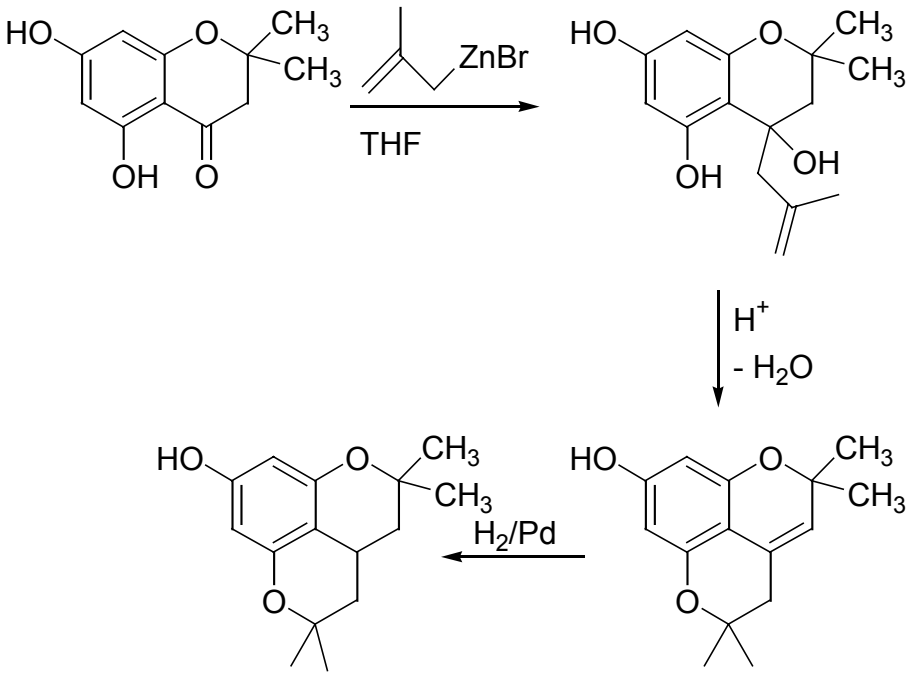
The chromanone structures of **56-58** (Scheme 10) were established by NMR and MS. The ^{1}H NMR spectra show the characteristic chromanone signals mentioned for compound **59**. The B ring of chromanone **56** is an unsubstituted phenyl, and the protons appear as a multiplet at 7.36-7.20 ppm. Para-substitution on the A rings of chromanones **57** and **58** provides symmetry, thus the aromatic protons give rise to two distinct doublets (*J* 8.6-8.7 Hz) in the range 7.4-6.9 ppm. The methoxyl group on the A ring of compound **58** is presented by a singlet in the methoxyl region, whereas the *tert*-butyl group of compound **57** produces a singlet at 1.28 ppm. This is supported by the ^{13}C NMR spectra, which show the same number of peaks as the number of magnetically non-equivalent carbon atoms. In the ^{13}C NMR peaks for chromanones, signals due to the C-2 and C-3 carbons of the C ring appear at about 80 and 45 ppm, respectively.

Two additional protons are observed in the ^{1}H NMR spectrum of chroman **58**: a singlet at 13.74 ppm and a signal within the multiplet at 3.44-3.28 ppm, both with integral ratios of 1:1. The lowfield chemical shift of around 14 ppm is most likely from a H-bonded hydroxyl group, and the signals were thought to arise from a hydrate. The compound was dried in a vacuum oven over five days, but still the signals persisted. The MS data do not show any sign of a hydrate, but revealed the molecular ion at m/z=314. The same ^{1}H NMR signals were observed for a similar chroman derivative in which the methoxyl group was replaced by a nitro group. Unfortunately, the nitro compound was hard to isolate by flash chromatography, and we did not pursue this any further.

Chromanones have been synthesised by several pathways. The best results were obtained with BBr_3, furnishing a 57% yield of the chromanone **59**. Compounds **56-58** were obtained in 1-20% yields by spontaneous cyclization, see 2.1.4, owing to the hydroxyl substituent in the 2'-position. Cyclization of chalcones to chromanones seems to occur during work-up procedures, as has been suggested for the reaction of **59**.

2.1.6 Synthesis of chroman derivatives

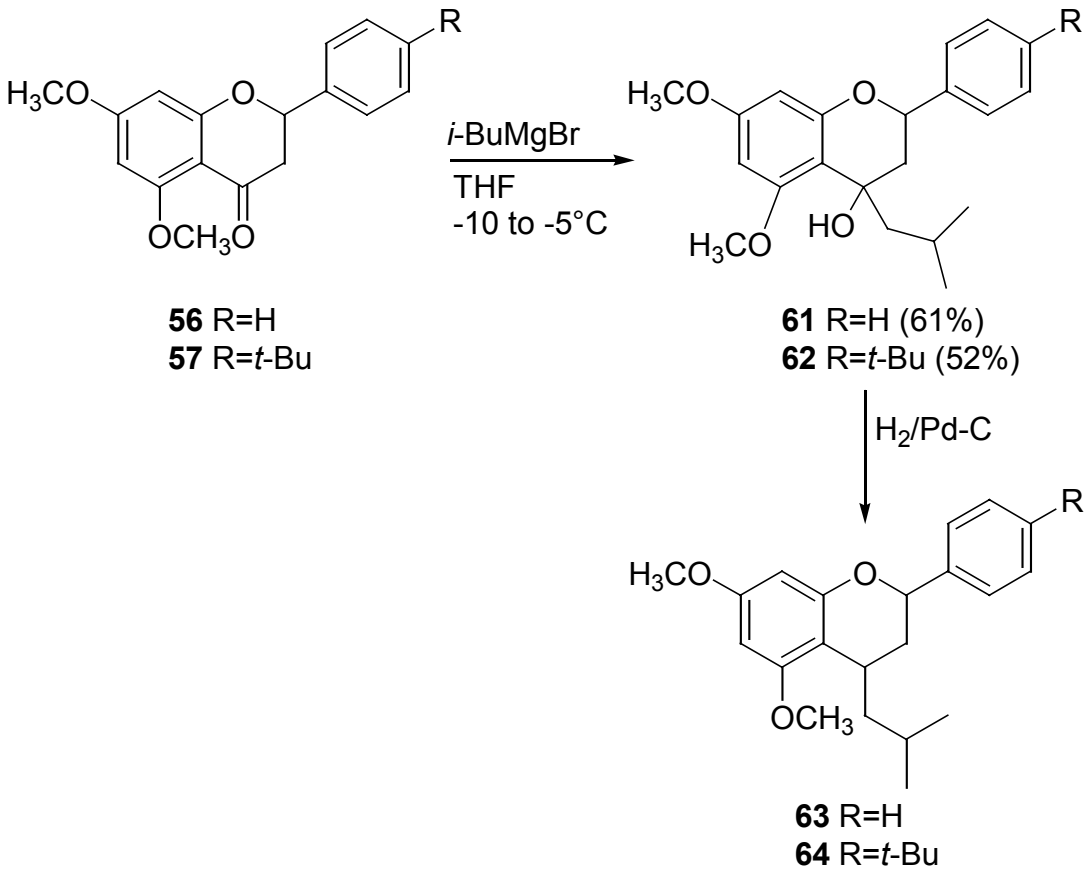
To evaluate the importance of the carbonyl group in relation to biological activity, compounds with this structural feature replaced were prepared. Alkylsubstituted chroman derivatives have been reported previously.[56] The reaction involved alkylation with methallylzinc bromide, followed by acid treatment and catalytic hydrogenation to provide good yields of polycylic chromans, see Scheme 15.



Scheme 15

With this in mind, we attempted to alkylate our chromanones **56** and **57** using a similar reaction sequence without the additional dehydrogenation step of the

intermediate. Reactions with isobutylmagnesium bromide furnished the corresponding homoallylic alcohols **61** and **62** in 61% and 52% yields, respectively (Scheme 16).



56 R=H
57 R=*t*-Bu

61 R=H (61%)
62 R=*t*-Bu (52%)

63 R=H
64 R=*t*-Bu

Scheme 16

Intermediates **61** and **62** were purified and their structures established by NMR. Both compounds contain two stereogenic centres, at C-2 and C-4. The signal pattern for the protons of the methoxyl groups in compound **62** indicate that the alcohol was obtained as a mixture of diatereomers, in a 1:5 ratio (see Figure 27). This feature was not observed for chroman **61**, suggesting that it was obtained in higher diasteromeric purity. The ^{13}C NMR spectrum of chroman **62** is complex and exhibits multiple signals for many of the carbon atoms owing to the stereogenic centre.

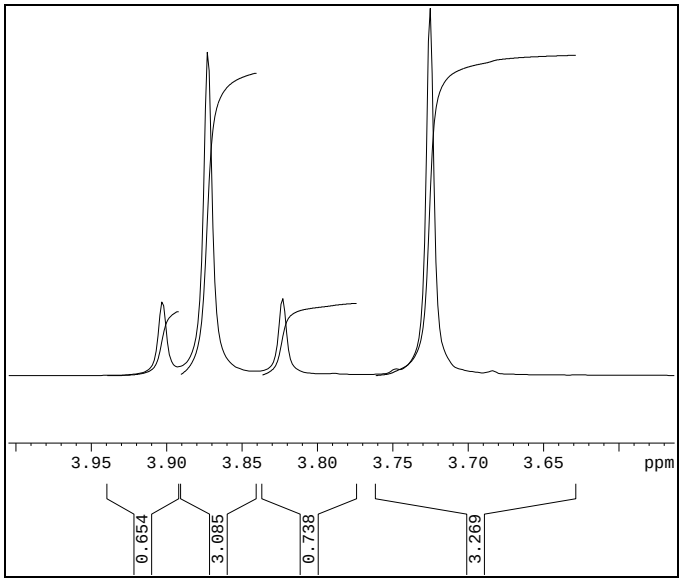


*Figure 27. Extract from the ^{1}H NMR spectrum of (±)-2-(4-tert-Butylphenyl)-4-isobutyl-5,7-dimethoxy-chroman-4-ol (**62**). The methoxy protons in the diastereomers appear at differentiable chemical shifts*

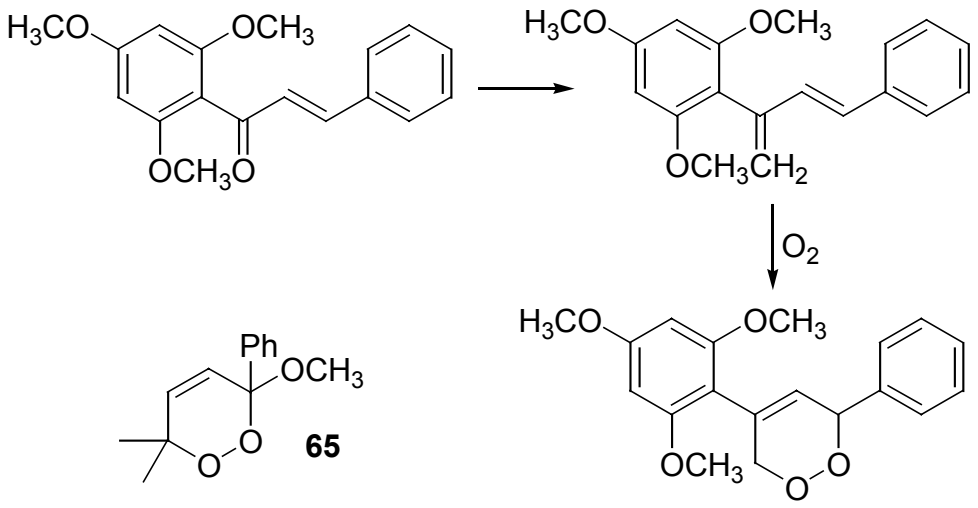
Chroman-4-ols **61** and **62** were subjected to the last step of Scheme 16, catalytic hydrogenation, to provide chromans **63** and **64**, respectively. Compound **63** exhibits a slightly different ^{1}H and ^{13}C NMR signal pattern than its corresponding chromanol **61**. In the ^{13}C NMR spectra, the C-4 signal at 71.48 ppm for the chromanol **61** has been replaced by a signal at 21.01 ppm for compound **63**, suggesting that reduction has taken place. Furthermore, the singlet at 4.14 ppm of the ^{1}H spectrum representing the hydroxyl proton of the chromanol is absent in the spectrum of compound **63**. The ^{1}H and ^{13}C NMR spectra of chroman **64** displayed a large number of signals, owing to its chirality, and they have not been interpreted. Lack of time prevented us from purifying the products, thus the yields were not established.

No reaction was observed in an attempt to alkylate 5,7-dimethoxy-2-(quinolin-4-yl)-chroman-4-one (**59**). Heating the reaction mixture of chromanone **59** and allylmagnesium bromide did not promote alkylation, suggesting that the quinoline structure somehow deactivates the organometallic compound.

In summary, four chroman derivatives were synthesized, giving stereoisomeric products of varying diastereomeric ratio.

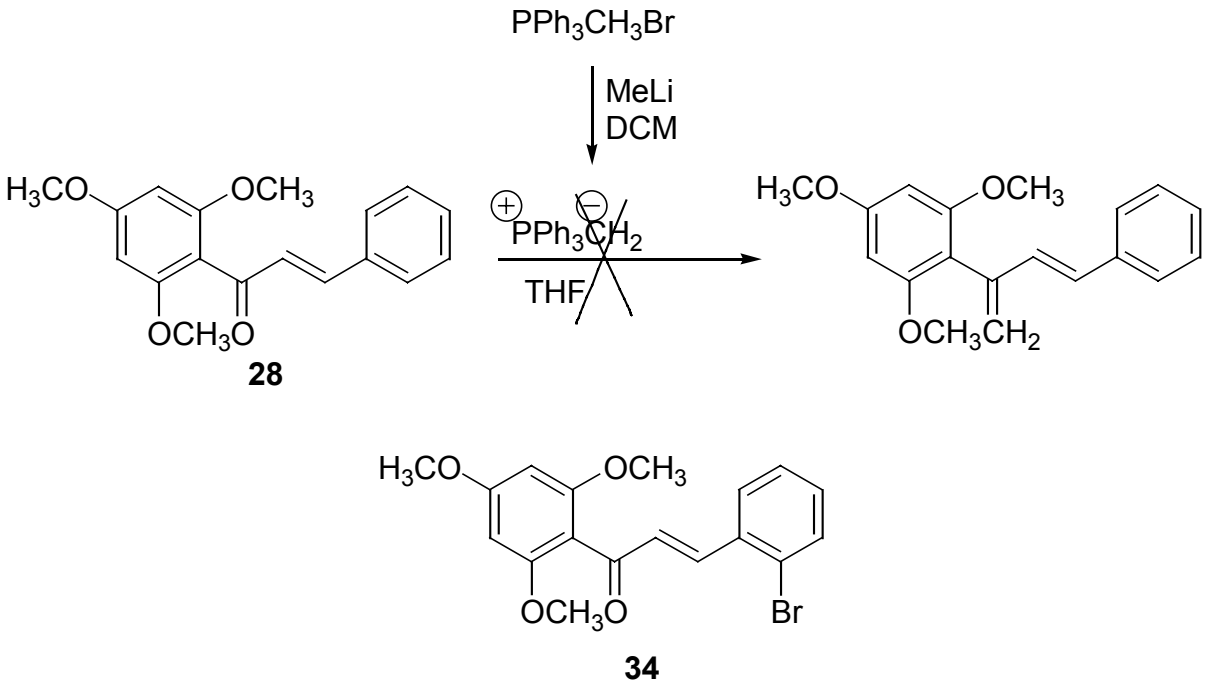
2.1.7 Carbonyl olefination

The antimalarial activity of artemisinin has been related to the presence of an endoperoxide bridge in its structure (see pg. 15). Recently, antimalarial activity has been reported for cyclic peroxy ketals (**65**).[57] Inspired by this evidence, we wanted to attempt a synthesis of a structurally similar dioxine, starting with our conjugated enones. After olefination, oxygenation of the diene function should provide a 1,2-dioxine, see Scheme 17.



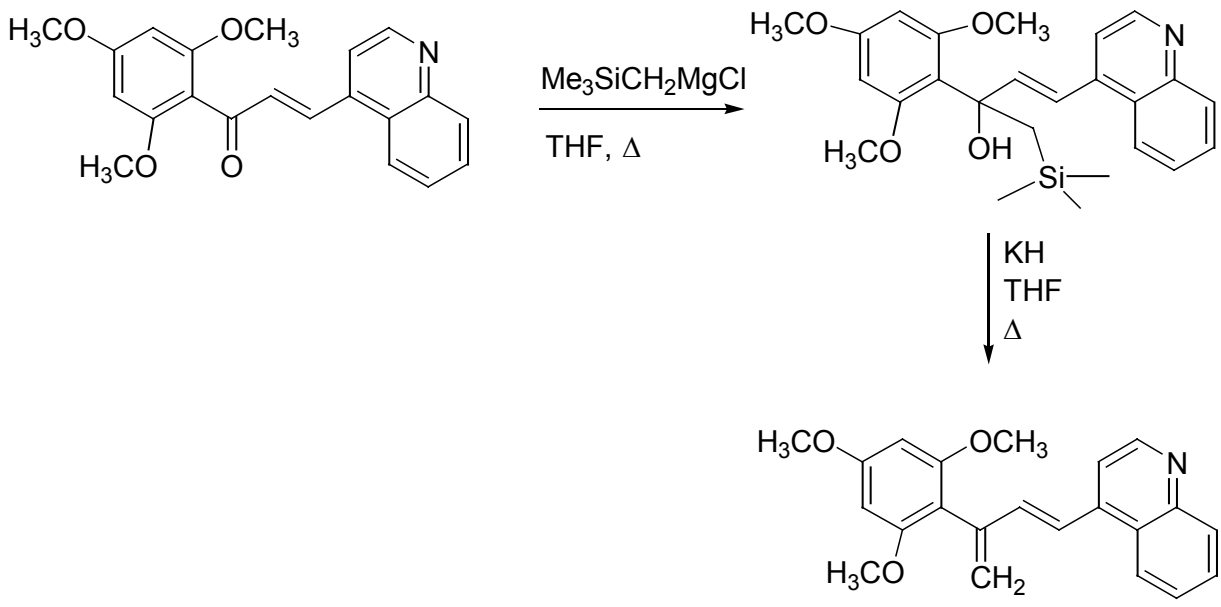
Scheme 17

Olefination of the carbonyl group proved difficult. *E*-1-(2,4,6-trimethoxyphenyl)-3-phenyl-2-propen-1-one (**28**) was reacted with methylene triphenylphosphorane in a classical Wittig reaction under various conditions, but failed to produce the olefin, probably due to steric effects. Instead, to our surprise, we isolated compound **34**, see Scheme 18. Ortho lithiation is known to occur on phenyl rings bearing an electron-rich substituent.[58]



Scheme 18

Methylenation with trimethylsilylmagnesium bromide provides an alternative to the Wittig reagents. Although the alkylsilyl Grignard reagent is sterically demanding, it is known to react with sterically hindered ketones when a Wittig reagent fails, producing a β-hydroxysilane intermediate. Forcing conditions (KH, THF, 150°C) were used to effect elimination of H_2O.[59] This provided us with the compound **66** as part of a complex mixture (Scheme 19).

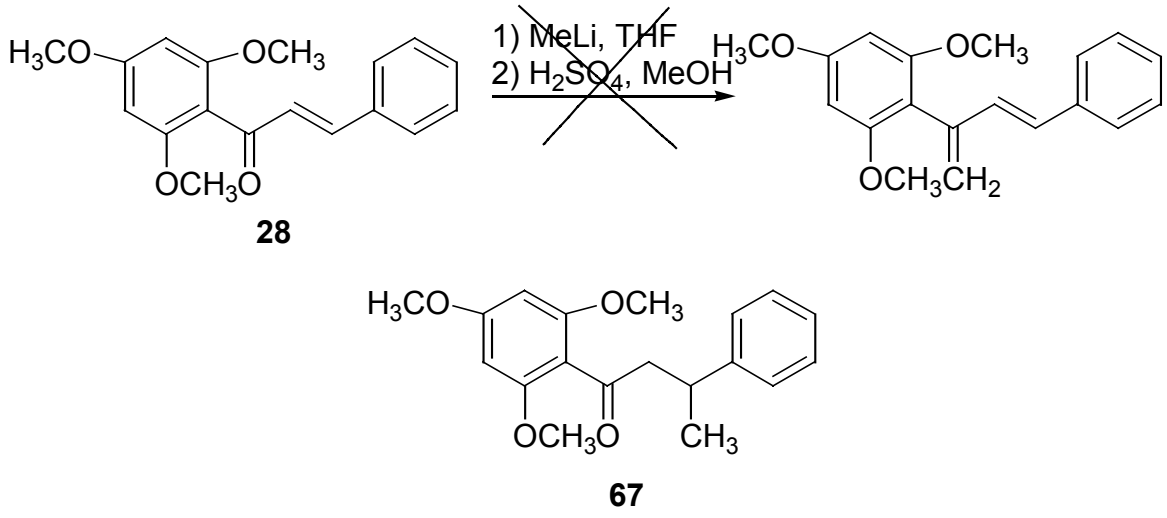


66

Scheme 19

The ^{1}H NMR spectrum of compound **66** indicates that olefination has occurred, but a great deal of unreacted intermediate remains. The compounds were difficult to isolate by flash chromatography, thus the yields were not established.

Methylenation has also been achieved by reaction of carbonyl with lithium alkyls and aryls, followed by elimination of water.[60] Reaction of *E*-1-(2,4,6-trimethoxyphenyl)-3-phenyl-2-propen-1-one (**28**) with methyl lithium, followed by treatment with acid (March, p. 920.) gave the 1,4-adduct **67** and not the anticipated 1,2-adduct, see Scheme 20.



Scheme 20

The structure of the substituted butanone **67** was established with the aid of NMR and MS. A similar NMR signal pattern to that for the chalcone derivatives in section 2.1.4 was observed for the trimethoxy substituted B ring. In addition, a multiplet at 7.26-7.13 ppm occurred for the five protons on the unsubstituted phenyl A ring. There were no olefinic protons present and a doublet (*J* 6.9 Hz) with an integral ratio of 3 appears at 1.30 ppm, representing the methyl group. This was supported by the observation of a characteristic peak at 21.88 ppm in the ^{13}C NMR spectrum. The proton on C-3 of the butanone gives rise to a multiplet at 3.54-3.38 ppm as it couples with the C-4 methyl protons and the methylene protons at C-2, whereas the latter contribute two distinct doublets of doublets (*J* 16.6 Hz geminal coupling, *J* 5.6 and 8.7 Hz vicinal coupling). In the EIMS a small signal for the molecular ion at m/z=314 is present, whereas the peaks of highest intensity are observed at m/z=297 and 195; the former corresponds to a demethylation of the compound **67** while the latter is due to a fragmentation characteristic for our chalcone derivatives, see section 2.1.4.

Our attempts to olefinate the α,β-unsaturated carbonyl group were not successful. Most likely, the ketone moiety is too sterically hindered for reaction with Wittig reagents. Peterson olefination provided a complex mixture of compounds. Reaction with alkyl lithium produced the 1,4-Michael adduct and not the desired 1,2-adduct.

2.2 Antimalarial activity

2.2.1 Structure-activity relationships of robustadial

As part of the work of the Skattebøl group, a series of robustadial chroman and chromanone analogues were synthesised and assessed for their antimalarial activity, providing information on the relationship between chemical structure and biological activity.[24]

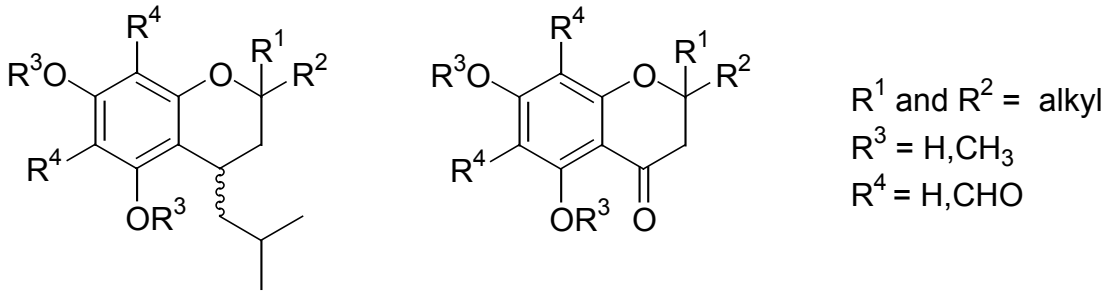


Figure 28. Structures of various chroman and chroman-4-one analogues of robustadial

Analogues without formyl groups on the phenyl ring show only slight changes in activity when compared to their aldehyde counterparts, implying that the aldehyde group is not important for activity. The chroman derivatives possess higher activity than the chromanone derivatives, indicating that the lipophilic properties of the molecule influence activity. This is supported by the observation that the hydroxylated compounds had slightly higher antimalarial activity than their methoxylated analogues. Comparison of structures with various substitution patterns at C-2 showed that the size of the alkyl groups R^1 and R^2 influence activity. Structures with spiro constellation of ring sizes n > 6 had low antimalarial activity compared to compounds with smaller substituents at C-2. Attachment of substituted phenyl groups at C-2 of the chromanones did not improve activity, neither did replacement of the phenyl group with thiophenyl or furanyl rings.

2.2.2 Chalcones

Very few studies on structure-activity relationships of antimalarial chalcones have been reported. Results from screening of more than 200 chalcone derivatives, revealed that (i) chalcones having electron-donating substitution on the B ring and electron-withdrawing substituents on the A ring, are associated with good antimalarial activity, (ii) both chloro-substituted and non-substituted quinolinyl groups as the A ring yield compounds with good activity no matter which substitution pattern was displayed on the B ring, (iii) di- and trimethoxy-substituted B ringS increase activity regardless of the substitution positions, and (iv) the α,β-unsaturated ketone moiety is essential for activity in the series.[19a,23]

2.2.3 Results

The results from the biological testing are not available.

2.3 Antioxidant properties

2.3.1 Scavenging of DPPH radical

Structure-activity relationship of flavonoids and chalcones as antioxidants

A large number of antioxidants and radical scavengers have been reported in the literature. The flavonoids have received considerable attention in this respect, as they have potent antioxidant and radical scavenging properties. Studies on structure-activity relationships of these compounds have shown that the presence of adjacent hydroxyl groups in the B ring of the flavonoid (3',4'-OH) and hydroxyl groups at C-3 and C-5 seems to promote radical scavenging activity. The double bond between C-2 and C-3 also appears to contribute to the antioxidant activity.[61] Phenolic hydroxyl

groups are likely to act as hydrogen donors in the scavenging of free radicals, and this might explain their importance for the activity, see below.

The scavenging effects of chalcone derivatives on the diphenylpicrylhydrazine radical (DPPH$^{\bullet}$) are shown in Table 3. Only *E*-1-(2,4,6-Trimethoxyphenyl)-3-(3,4-dihydroxyphenyl)-2-propen-1-one (**44**) showed significant activity. This is consistent with previous findings that chalcones with 3,4-dihydroxy substituted rings act as DPPH radical scavengers. The proposed reaction mechanism consists of hydrogen atom transfer from a phenol donor[62] to give DPPH, thereby forming a phenoxyl radical. The radical is stabilised by delocalisation over the aromatic ring structure, see Figure 29.

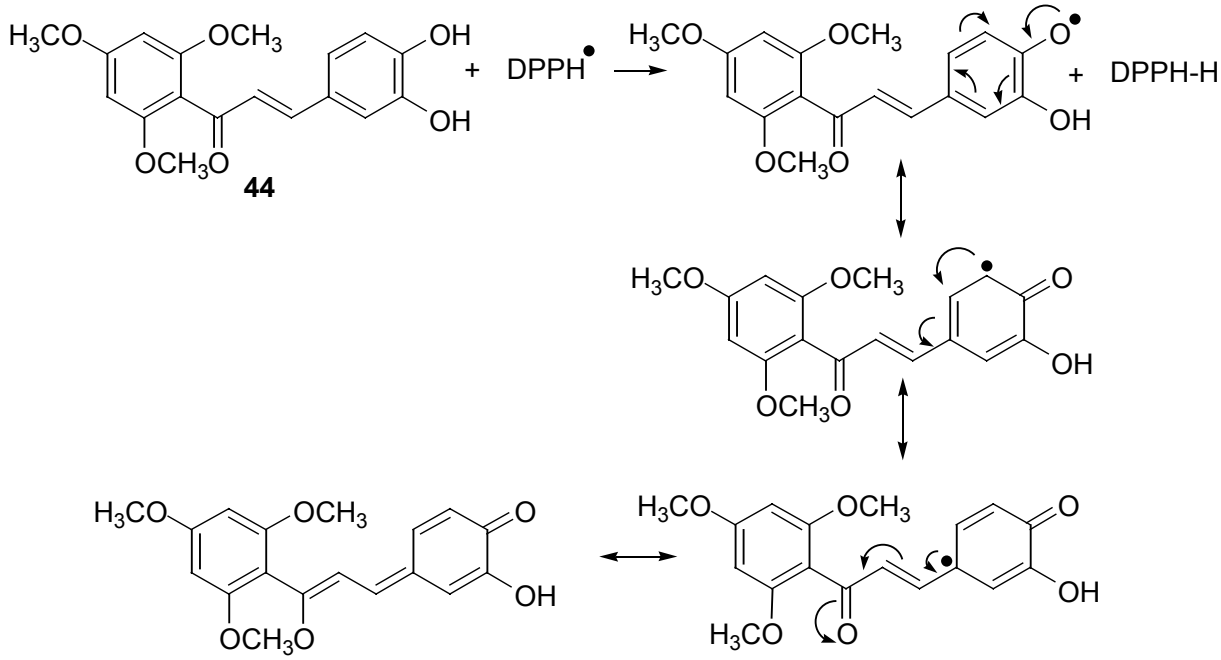


*Figure 29 Reaction of catecholic chalcone **44** with DPPH radical and resonance-stabilisation of phenoxyl radical*

The chalcone structures bear many similarities to the flavonoid structures and it is not surprising that they show related structure-activity relationships. It has been proposed that hydroxy and methoxy substituents on both the A and B rings are essential for

antioxidant and radical scavenging activities of chalcone derivatives.[63] The α,β-double bond and a 2'-hydroxyl group also appear to be important for activity.[16]

2.3.2 Inhibition of 15-LO

15-LO from soybeans was used for the biological testing. It has been reported that there is a good correlation of inhibitory activity between this enzyme and mammalian 15-LO, even though they are not identical.[64] The enzyme oxidises linoleic acid, which serves as substrate in our study, to 13(S)-9*Z*,11*E*-hydroperoxyoctadienic acid, see Figure 30.[65]

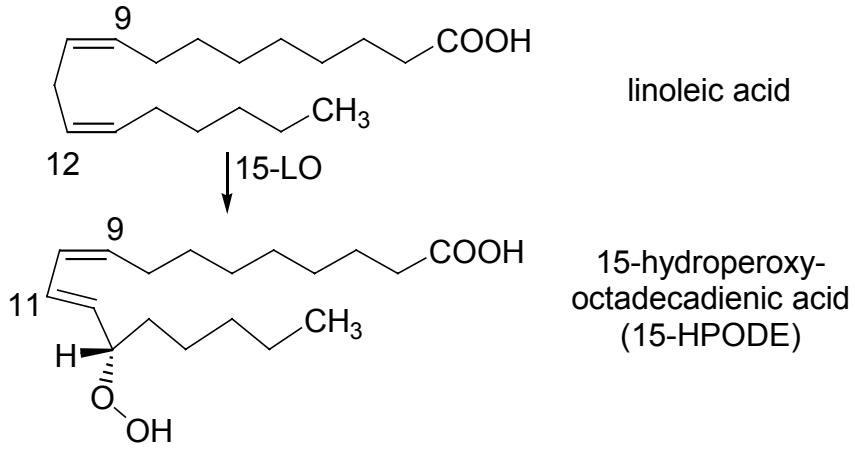
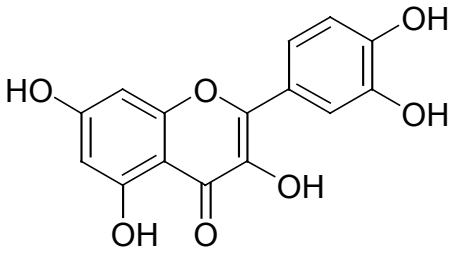


Figure 30. Peroxidation of linoleic acid by 15-LO.

The degree of formation of conjugated double bonds by the action of 15-LO on the substrate can be followed spectroscopically at 234 nm. The relative change in the absorbance of the solution provides a measurement of the lipoxygenase-modulating effect of the test substance.

All of the chalcone derivatives exhibited activity as enzyme inhibitors and IC_{50} values were determined. The inhibition was statistically significant ($p<0.05$) at concentrations of 75 μM for all substances, see Table 3. Highest activity was observed for compounds **35**, **37**, **41** and **48** with IC_{50} values of 55, 59, 43 and 56 μM, respectively. Most of the 15-LO inhibitors reported herein have IC_{50} values in the

range 40 to 140 μM. This compares favourably with 15-LO inhibiting effects of the natural product quercetin (**68**) (IC_{50} = 38 μM), which functions as the standard in this study. The data are insufficient to predict a rational structure-activity relationship for the 15-LO inhibitory action of the compounds. Earlier research has shown that the α,β-unsaturated ketone moiety is required for activity,[47] which may be mediated by conjugate addition of a biological nucleophile to the chalcone.



68

Figure 31 The structure of quercetin

There are some test values missing because of difficulties in the experimental determination. Furthermore, certain compounds (**31**,**33**,**38**,**53**) gave less reproducible results due to poor solubility in the test solution.

Table 3. Scavenging of DPPH radical and inhibition of 15-lipoxygenase

Nr	A-ring	% DPPH• scavenging after 5 min (conc. 300 μM)	Inhibition of 15-LO (conc. 75 μM)	IC_{50}[a]
28	C_6H_5	0.1 ± 0.3[b]	37 ± 5	91 ± 7
31	2-$NO_2C_6H_4$	0.9 ± 0.2	25 ± 5	142 ± 18[c]
32	3-$NO_2C_6H_4$	0.5 ± 0.2	22 ± 11	88 ± 5
33	4-$NO_2C_6H_4$	0.7 ± 0.3	34 ± 7	> 75[c]
35	4-BrC_6H_4	0.6 ± 0.1	65 ± 3	55 ± 8
37	4-*t*-BuC_6H_4	0.2 ± 0.1	62 ± 3	59 ± 5
38	4-$OCH_3C_6H_4$	0.3 ± 0.2	41 ± 3	> 75[c]
39	2,3,4-$(OCH_3)_3C_6H_2$	0.4 ± 0.2	37 ± 8	110 ± 13
40	2,4,5-$(OCH_3)_3C_6H_2$	0.4 ± 0.1	48 ± 9	78 ± 11
41	2,4,6-$(OCH_3)_3C_6H_2$	0.4 ± 0.3	73 ± 1	43 ± 9
42	3,4,5-$(OCH_3)_3C_6H_2$	0.3 ± 0.2	56 ± 3	67 ± 4
43	4-OHC_6H_4	0.6 ± 0.1[b]	42 ± 5	87 ± 7
44	3,4-OHC_6H_3	90.8 ± 0.1[b]	45 ± 4	81 ± 5
45	pyridin-2-yl	0.2 ± 0.2	42 ± 3	86 ± 4
46	pyridin-3-yl	0.1 ± 0.1	30 ± 2	107 ± 5
47	pyridin-4-yl	0.2 ± 0.2	38 ± 4	91 ± 5
48	quinolin-2-yl	0.3 ± 0.1	72 ± 4	56 ± 5
53	2-tiophenyl	0.4 ± 0.1	30 ±3	114 ± 13[c]
54	2-furanyl	5.3 ± 1.4	29 ± 6	109 ± 9
68	-	n. d.[d]	84 ± 1	38 ± 2

[a] Data are shown ± SD. [b] Concentration 150 μM. [c] Poor solubility. [d] Not determined.

2.3.3 Conclusion

Significant inhibition of the enzyme 15-LO was observed for all substances tested. No obvious correlation between enzyme inhibition and DPPH radical scavenging was observed. This lack of correlation indicates that the chalcones inhibit 15-LO by binding to the enzyme rather than by scavenging radicals arising from 15-LO oxidation of the fatty acid substrate. This is in accordance with previously reported results. Chalcones contain an electrophilic enone moiety and may act as alkylating agents. In view of the fact that the compounds have shown low activity as DPPH radical scavengers, they may be regarded as non-oxidant inhibitors of 15-LO.

3. Experimental

3.1 General

Melting points were determined with an Electrothermal IA9200 Melting Point apparatus and were recorded uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 200 or 300 MHz, and 50 or 75 MHz, respectively, with Bruker Avance DPX 200 or 300 instruments. All NMR analyses were performed at 25 ºC and as solutions in $CDCl_3$ with $CDCl_3$ as reference (^{1}H δ 7.24, ^{13}C δ 77.0), unless stated otherwise. A ChemNMR prediction program was used as an aid in analysis of NMR spectra. An interval is given for chemical shifts when two chemically non-equivalent nuclei cannot be differentiated.

IR spectra were acquired with a Perkin-Elmer 1310 Spectrometer. Mass spectra were recorded at 70eV with a Fisons VG Pro spectrometer.

All reactions were carried out under an atmosphere of nitrogen or argon. After aqueous work-up of reaction mixtures, organic solutions were routinely dried with anhydrous Na_2SO_4 or $MgSO_4$ and 'evaporation under reduced pressure' refers to removal of solvent on a rotary evaporator. Thin layer chromatography was carried out on aluminium sheets coated with 60 F_{254} silica gel (Merck). Compounds were detected by UV light and dipped in a solution of phosphomolybdic acid in ethanol, followed by heating. Column chromatography was performed as flash chromatography on silica gel (Silice 60, 40-63 μm, SDS) using gradients of EtOAc and hexane eluent, unless specified otherwise.

3.2 Assays

3.2.1 **DPPH radical scavenging**

Scavenging activity of the DPPH radical was measured as the decrease in absorbance at 517 nm of a MeOH solution (A_{517}=1.0, 2.95 mL) over a five min period after the addition of 50 µl DMSO solution of the test substance. Appropriate corrections were made for dilution and for absorbance of the reaction product (reduced DPPH). Calculation of radical scavenging activity was carried out as previously described.[66]

3.2.2 Inhibition of 15-lipoxygenase

Lipoxygenase activity was measured in borate buffer solutions (0.2M, pH 9.00) by the increase in absorbance at 234 nm from 30 to 90 s after the addition of the enzyme, using linoleic acid (134 µM) as substrate.[67] The final enzyme concentration was 167 U/mL. Test substances were added as DMSO solutions (final DMSO conc. 1.6%) and DMSO alone was added in uninhibited control experiments. Six or more parallels of controls and three or more parallels for each test substance solution were measured. The enzyme solution was kept on ice, and controls were measured at regular intervals to ensure constant enzyme activity throughout the experiment.

Calculation of the enzyme activity was carried out as previously described and IC_{50} values were determined by linear interpolation between the measuring points closest to 50% activity. Values were expressed as means ± SD while Student's *t*-test was employed for determination of statistical significance.

3.3 Syntheses

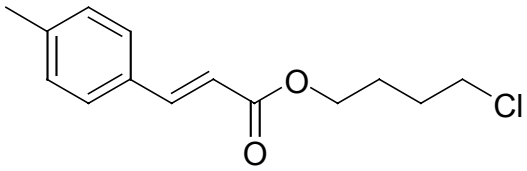
Preparation of some of the following compounds has been reported previously, references are given.

General procedure to prepare 2',4',6'-trimethoxychalcone derivatives

Aldehyde (2.0 mmol) and 2,4,6-trimethoxyacetophenone (**29**) (0.42 g, 2.0 mmol) were dissolved in EtOH (20-30 mL) containing NaOH (47 mg, 1.18 mmol). The mixture was stirred at ambient temperature until TLC showed complete conversion (8-42 h). Water (30 mL) was added, the reaction mixture extracted with ether (3 x 40 mL) and the organic phase dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude product purified by flash chromatography (SiO_2, EtOAc:hexane gradient). In certain cases, the product precipitated from the reaction mixture and could be directly collected by filtration, recrystallized and dried under vacuum.

Attempt to prepare *E*-1-(2,4,6-hydroxyphenyl)-3-(4-tolyl)-2-propen-1-one

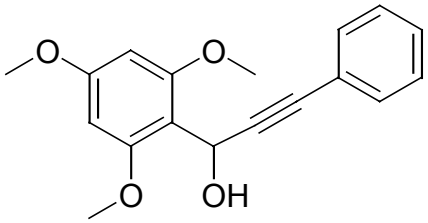
To a stirred solution of 4-methylcinnamic acid (1.63 g, 10 mmol) in dry DCM (25 mL), oxalyl chloride (1.52 g, 12 mmol) was added dropwise. The reaction mixture was stirred for 2 hrs before the solvent was evaporated under reduced pressure. The residue was dissolved in dry THF (5 mL) and added dropwise to a solution of phloroglucinol (0.32 g, 2.5 mmol) and $ZnCl_2$ (1.36 g, 10 mmol) in dry THF (20 mL). After 3 hrs, 10 % $NaHCO_3$ (50 mL) was added slowly whilst on a cooling bath. The reaction mixture was extracted with DCM (2 x 50 mL) and washed with brine, followed by 10 % $NaHCO_3$ and saturated $NaHCO_3$. The organic phase was dried (Na_2SO_4) and the solvent evaporated. The product was purified by flash chromatography to yield 0.25 g (40 %) of a white crystalline product, which was found to be **4-chlorobutyl *E*-3-(4-tolyl)-2-propenoate (26)**.



R_f 0.58 (EtOAc:hexane 1:1); IR (film, KBr) ν_{max}/cm^{-1} 1703 (C=O), 809 (C-Cl) ; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.64 (d, *J* 16.0 Hz, 1H), 7.41 (d, *J* 8.0 Hz, 2H), 7.17 (d, *J* 8.0 Hz, 2H), 6.37 (d, *J* 16.0 Hz, 1H), 4.22 (t, *J* 6.0, 2H), 3.58 (t, *J* 6.0 Hz, 2H),

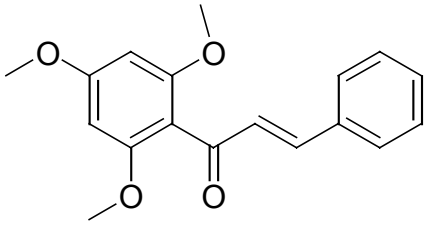
2.36 (s, 3H), 1.94-1.81 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.00, 144.80, 140.35, 131.54, 129.54, 127.99, 116.76, 63.46, 44.43, 29.13, 26.11, 21.36; EIMS *m/z* (70 eV) 252 (39), 217 (17), 162 (65), 145 (100), 117 (23), 115 (27); HRMS calcd for $C_{14}H_{17}O_2Cl^{35}$ [M^+]: 252.0917, found 252.0924.

(±)-1-(2,4,6-Trimethoxyphenyl)-3-phenyl-2-propyn-1-ol (27)



Phenylacetylene (2.19 mL, 20.0 mmol) in dry THF (25 mL) was added dropwise to a 0-5°C suspension of NaH (80 %, 0.86 g, 24.0 mmol) in dry THF (25 mL). The mixture was stirred for 30 min before 2,4,6-trimethoxybenzaldehyde (3.92 g, 20.0 mmol) in dry THF (100 mL) was added dropwise. The reaction was monitored by TLC. The mixture was heated under reflux overnight before it was quenched with saturated NH_4Cl (50 mL), extracted with diethyl ether (3 x 70 mL) and the organic phase washed with brine (50 mL). After drying (Na_2SO_4), the solvent was evaporated under reduced pressure. The products were separated by column chromatography, affording 1.80 g (30%) of the title compound **27** as cream-coloured crystals; mp n.d.; R_f 0.27 (EtOAc:hexane 40:60); IR (film, KBr) ν_{max}/cm^{-1} 3545 (O-H), 2258 (C≡C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.37 (m, 2H), 7.25-7.23 (m, 3H), 6.15 (s, 2H), 6.01 (d, *J* 11.3 Hz, 1H), 3.90-3.86 (m, 7H), 3.79 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.07, 158.27, 131.65, 128.02, 127.85, 123.35, 110.55, 91.27, 90.51, 82.63, 56.64, 55.94, 55.32; EIMS *m/z* (70 eV) 298 (24), 283 (38), 267 (43), 168 (100); HRMS calcd for $C_{18}H_{18}O_4$ [M^+]: 298.1205, found 298.1194.

***E*-1-(2,4,6-Trimethoxyphenyl)-3-phenyl-2-propen-1-one (28)**

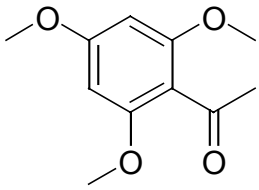


A solution of **27** (0.31 g, 1.0 mmol) in dry THF (15 mL) was added slowly dropwise to a roundbottomed flask containing $LiAlH_4$ (0.036 g, 0.94 mmol) in dry THF (20 mL). The mixture was heated under reflux overnight. The reaction was quenched with the following (in sequence): H_2O (0.1 mL), NaOH (10 % aq., 0.1 mL) and H_2O (0.3 mL). The procedure was repeated until no more precipitate formed. The mixture was filtrated and the precipitate washed with diethyl ether. The filtrate was dried (Na_2SO_4) and the solvent evaporated under reduced pressure to yield crude α,β-unsaturated alcohol intermediate, which was dissolved in $CHCl_3$ (20 mL). MnO_2 (1.04 g, 12.0 mmol) was added gradually to the mixture under magnetic stirring. After TLC indicated product, the mixture was filtered over Celite, Na_2SO_4 and SiO_2, providing a complex mixture for which NMR indicates the presence of title compound **28**.

Alternative synthesis of compound 28

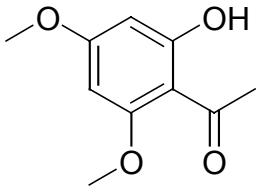
This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and benzaldehyde affording 0.57 g (76%) as yellow oily solid crystals; mp 76-78 ºC (lit.[47] 79-81 ºC); R_f 0.25 (EtOAc:hexane 40:60); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.49 (m, 2H), 7.37-7.31 (m, 4H), 6.94 (d, *J* 16.1 Hz, 1H), 6.14 (s, 2H), 3.83 (s, 3H), 3.76 (s, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 194.23, 162.40, 158.83, 144.06, 135.00, 130.12, 129.00, 128.76, 128.33, 111.76, 90.68, 55.88, 55.42; EIMS *m/z* (70 eV) 298 (23), 270 (100), 195 (90); HRMS calcd for $C_{18}H_{18}O_4$ [M^+]: 298.1205, found 298.1197.

2,4,6-Trimethoxyacetophenone (29)



Acetic anhydride (9.64 mL, 102 mmol) was added dropwise to a stirred solution of 1,3,5-trimethoxybenzene (16.82 g, 100 mmol) and concentrated H_2SO_4 (4 drops) in 1,2-dichloroethane (50 mL). The mixture was heated under reflux for 18 hrs. A colour change from pale green through dark blue to reddish brown occurred. The contents were cooled and poured into H_2O (100 mL) containing EtOH (10 mL). Concentrated HCl (8 drops) was added and the mixture heated under reflux for 45 min to decompose excess anhydride. The contents were cooled once again, and the reaction mixture extracted with diethyl ether (3 x 70 mL). The organic phase was washed with (in sequence) H_2O (2 x 70 mL), $NaHCO_3$ (10 % aq., 50 mL) and saturated $NaHCO_3$ (50 mL). The solvent was evaporated under reduced pressure and the residue purified by flash chromatography to yield 6.47 g (31%) of the title product as a creamy white powder; R_f 0.26 (EtOAc:hexane 3:7); ^1H NMR ($CDCl_3$, 300 MHz) δ 6.07 (s, 2H), 3.79 (s, 3H), 3.76 (s, 6H), 2.43 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 201.61, 162.25, 158.25, 113.60, 90.49, 55.71, 55.31, 32.41.

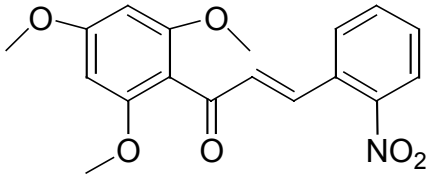
2,4-Dimethoxy-6-hydroxyacetophenone (30)[53]



To a solution of phloroacetophenone (20.5 g, 110 mmol) and K_2CO_3 (22.1g, 160 mmol) in acetone (150 mL) was added dimethyl sulphate (27.7 g, 220 mmol) slowly over 30 min with mechanical stirring. The solution was heated at reflux overnight and then poured into H_2O (500 mL), which produced a creamy white precipitate. The material was collected on a Büchner funnel, rinsed with cold H_2O (1 L) and air-dried.

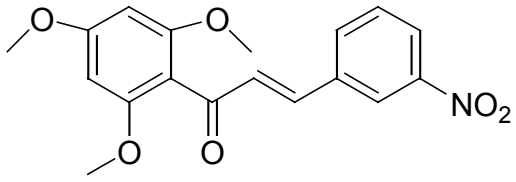
Recrystallisation from 90% MeOH (100 mL) provided 14.7 g (68%) of the desired product as creamy white needles; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 13.98 (s, 1H), 6.03 (d, *J* 2.4 Hz, 1H), 5.89 (d, *J* 2.4 Hz, 1H), 3.82 (s, 3H), 3.79 (s, 3H), 2.58 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 203.59, 167.97, 166.51, 163.32, 106.41, 93.89, 91.15, 55.92, 51.20, 33.27.

_E_-1-(2,4,6-Trimethoxyphenyl)-3-(2-nitrophenyl)-2-propen-1-one (31)



This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and 2-nitrobenzaldehyde affording 1.40 g (82%) as a yellow crystalline powder; mp 157-159 ºC; R_f 0.16 (EtOAc:hexane 40:60); IR (film, KBr) ν_{max}/cm^{-1} 1703 (C=O), 1520, 1363 (N=O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.98 (dd, *J* 8.1 Hz, 1.1 Hz, 1H), 7.70-7.60 (m, 3H), 7.49 (m, 1H), 6.79 (d, *J* 16.0 Hz, 1H), 6.13 (s, 2H), 3.83 (s, 3H), 3.77 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 194.21, 162.71, 158.91, 148.25, 139.66, 133.47, 133.41, 131.25, 130.00, 129.20, 124.79, 110.46, 90.59, 55.79, 55.38; EIMS *m/z* (70 eV) 343 (21), 195 (100); HRMS calcd for $C_{18}H_{17}NO_6$ [M^+]: 343.1055, found 343.1063.

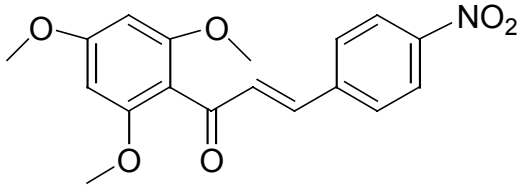
_E_-1-(2,4,6-Trimethoxyphenyl)-3-(3-nitrophenyl)-2-propen-1-one (32)



This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and 3-nitrobenzaldehyde yielding 1.50 g (87%) as a yellow crystalline powder; mp 148-151 ºC (lit.[47] 146-148 ºC); R_f 0.26 (EtOAc:hexane 40:60); IR (film, KBr) ν_{max}/cm^{-1} 1695 (C=O), 1514, 1375 (N=O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.33 (m, 1H), 8.18 (m, 1H), 7.82 (d, *J* 7.9 Hz, 1H), 7.54 (t, *J* 7.9 Hz,

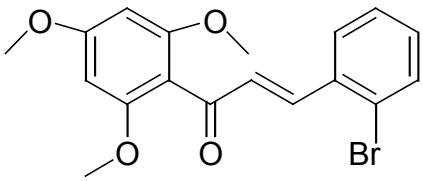
1H), 7.41 (d, *J* 16.1 Hz, 1H), 7.04 (d, *J* 16.1 Hz, 1H), 6.15 (s, 2H), 3.85 (s, 3H), 3.77 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 192.94, 162.86, 159.10, 148.60, 140.09, 136.93, 133.73, 131.40, 129.80, 124.21, 122.65, 111.36, 90.75, 55.92, 55.45; EIMS *m/z* (70 eV) 343 (17), 315 (100), 195 (39); HRMS calcd for $C_{18}H_{17}NO_6$ [M^+]: 343.1055, found 343.1055.

_E_-1-(2,4,6-Trimethoxyphenyl)-3-(4-nitrophenyl)-2-propen-1-one (33)



2,4,6-Trimethoxyacetophenone (**29**) and 4-nitrobenzaldehyde were reacted together according to the general procedure. The title compound was obtained in 1.49 g (87%) yield as a yellow crystalline powder; mp 179-180 ºC (lit.[47] 176-178 ºC); R_f 0.26 (EtOAc:hexane 40:60); IR (film, KBr) ν_{max}/cm^{-1} 1700 (C=O), 1524, 1367 (N=O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.19 (d, *J* 7.0 Hz, 2H), 7.64 (d, *J* 7.0 Hz, 2H), 7.41 (d, *J* 16.0 Hz, 1H), 7.04 (d, *J* 16.0 Hz, 1H), 6.14 (s, 2H), 3.84 (s, 3H), 3.76 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 193.11, 163.38, 159.64, 148.69, 141.90, 140.16, 132.85, 129.19, 124.45, 111.82, 91.19, 56.37, 55.90; EIMS *m/z* (70 eV) 343 (13), 315 (100), 195 (41); HRMS calcd for $C_{18}H_{17}NO_6$ [M^+]: 343.1055, found 343.1056.

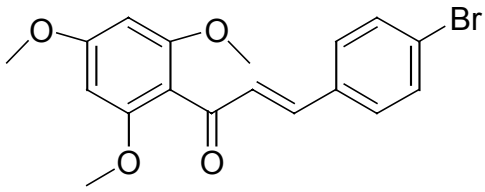
_E_-1-(2,4,6-Trimethoxyphenyl)-3-(2-bromophenyl)-2-propen-1-one (34)



This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and 2-bromobenzaldehyde yielding 0.60 g (80%) as a pale yellow crystalline powder; mp 115-117 ºC; R_f 0.26 (EtOAc:hexane 40:60); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.70 (d, *J* 16.0 Hz, 1H), 7.63 (dd, *J* 7.8, 1.8 Hz, 1H), 7.55 (dd, *J* 7.8, 1.3 Hz, 1H), 7.29 (m, 1H), 7.17 (dt, *J* 7.4, 1.8 Hz, 1H), 6.83 (d, *J*

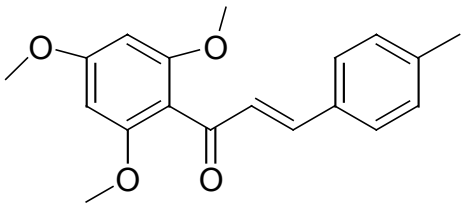
16.0 Hz, 1H), 6.14 (s, 2H), 3.83 (s, 3H), 3.76 (s, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 194.02, 162.54, 158.90, 142.61, 135.04, 133.25, 131.37, 130.96, 127.89, 127.62, 125.49, 111.31, 90.66, 55.85, 55.40; EIMS *m/z* (70 eV) 378 (19), 376 (19), 350 (98), 348 (100), 195 (99); HRMS calcd for $C_{18}H_{17}Br^{79}O_4$ [M^+]: 376.0310, found 376.0292.

***E*-1-(2,4,6-Trimethoxyphenyl)-3-(4-bromophenyl)-2-propen-1-one (35)**



This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and 4-bromobenzaldehyde furnishing 0.72 g (63%) as a creamy white crystalline powder; mp 133-135 ºC; R_f 0.25 (EtOAc:hexane 40:60); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46 (d, *J* 7.0 Hz, 2H), 7.33 (d, *J* 7.0 Hz, 2H), 7.28 (d, *J* 16.1 Hz, 1H), 6.91 (d, *J* 16.1 Hz, 1H), 6.13 (s, 2H), 3.83 (s, 3H), 3.75 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 193.70, 162.54, 158.90, 142.25, 133.97, 131.98, 129.67, 129.46, 124.28, 111.64, 90.71, 55.89, 55.42; EIMS *m/z* (70 eV) 378 (15), 376 (15), 350 (98), 348 (100), 195 (55); HRMS calcd for $C_{18}H_{17}Br^{81}O_4$ [M^+]: 378.0289, found 378.0285, $C_{18}H_{17}Br^{79}O_4$ [M^+]: 376.0310, found 376.0318.

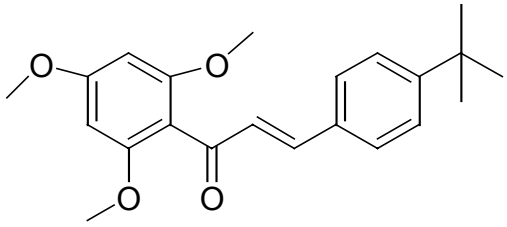
***E*-1-(2,4,6-Trimethoxyphenyl)-3-(4-tolyl)-2-propen-1-one (36)**



This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and 4-tolualdehyde affording 0.61 g (80%) as a creamy white crystalline powder; mp 122-124 ºC; R_f 0.35 (EtOAc:hexane 1:1); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.39 (d, *J* 8.1 Hz, 2H), 7.31 (d, *J* 16.0 Hz, 1H), 7.15 (d, *J* 8.1 Hz, 2H), 6.89 (d, *J* 16.0 Hz, 1H), 6.14 (s, 2H), 3.84 (s, 3H), 3.74 (s, 6H), 2.34 (s,

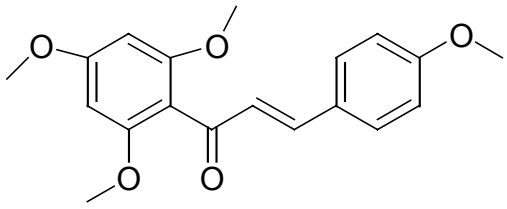
3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 194.41, 162.32, 158.78, 144.34, 140.61, 132.25, 129.52, 128.37, 128.14, 111.98, 90.70, 55.89, 55.43, 21.46; EIMS *m/z* (70 eV) 312 (22), 284 (100), 195 (33); HRMS calcd for $C_{19}H_{20}O_4$ [M^+]: 312.1361, found 312.1372.

_E_-1-(2,4,6-Trimethoxyphenyl)-3-(4-*tert*-butylphenyl)-2-propen-1-one (37)



This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and 4-*tert*-butylbenzaldehyde yielding 0.49 g (55%) as yellow needles; mp 115-118 ºC; R_f 0.38 (EtOAc:hexane 50:50); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.33 (d, *J* 8.4 Hz, 2H), 7.27-7.22 (m, 3H), 6.83 (d, *J* 16.1 Hz, 1H), 6.06 (s, 2H), 3.71 (s, 3H), 3.64 (s, 6H), 1.18 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 193.92, 162.06, 158.41, 153.35, 143.69, 131.81, 127.97, 127.83, 125.40, 111.35, 90.36, 55.42, 55.01, 34.42, 30.73; EIMS *m/z* (70 eV) 354 (22), 326 (100), 311 (58), 195 (29); HRMS calcd for $C_{22}H_{26}O_4$ [M^+]: 354.1831, found 354.1824.

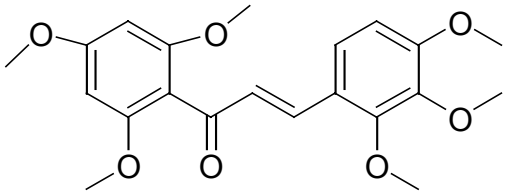
_E_-1-(2,4,6-Trimethoxyphenyl)-3-(4-methoxyphenyl)-2-propen-1-one (38)



This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and anisaldehyde yielding 0.94 g (90%) as a yellow-white crystalline product; mp 119-120 ºC (lit.[68] 119-121 ºC); R_f 0.28 (EtOAc:hexane 50:50); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.45 (d, *J* 8.8 Hz, 2H), 7.29 (d, *J* 15.9 Hz, 1H), 6.84 (d, *J* 8.8 Hz, 2H), 6.82 (d, *J* 15.9 Hz, 1H), 6.14 (s, 2H), 3.83 (s, 3H), 3.80

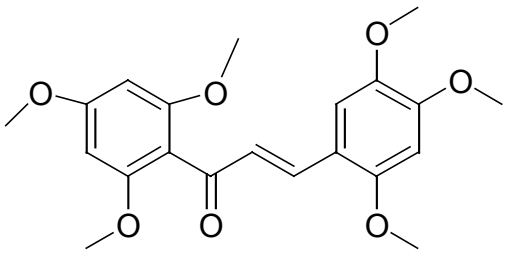
(s, 3H), 3.74 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 194.36, 162.24, 161.37, 158.72, 144.20, 130.05, 127.68, 127.00, 114.24, 111.99, 90.72, 55.90, 55.42, 55.35; EIMS *m/z* (70 eV) 328 (33), 300 (100), 195 (35); HRMS calcd for $C_{19}H_{20}O_5$ [M^+]: 328.1310, found 328.1316.

***E*-1-(2,4,6-Trimethoxyphenyl)-3-(2,3,4-trimethoxyphenyl)-2-propen-1-one (39)**



This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and 2,3,4-trimethoxybenzaldehyde yielding 0.55 g (70%) as a yellow-white crystalline powder; mp 167-168 ºC; R_f 0.27 (EtOAc:hexane 50:50); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.53 (d, *J* 16.2 Hz, 1H), 7.26 (d, *J* = 8.8 Hz, 1H), 6.86 (d, *J* 16.2 Hz, 1H), 6.65 (d, *J* = 8.8 Hz, 1H), 6.11 (s, 2H), 3.84 (s, 3H), 3.80 (s, 6H), 3.78 (s, 3H), 3.71 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 194.67, 162.14, 158.59, 155.37, 153.22, 142.25, 139.46, 128.12, 123.13, 121.96, 111.87, 107.63, 90.63, 61.34, 60.79, 55.95, 55.76, 55.32; EIMS *m/z* (70 eV) 357 (100), 195 (11); HRMS calcd for $C_{21}H_{24}O_7$ [M^+]: 388.1522, found 388.1510.

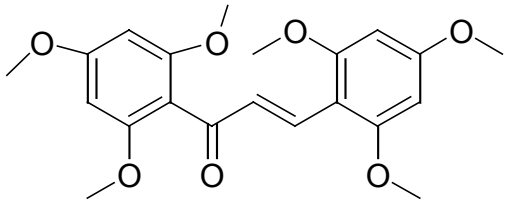
***E*-1-(2,4,6-Trimethoxyphenyl)-3-(2,4,5-trimethoxyphenyl)-2-propen-1-one (40)**



This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and 2,4,5-trimethoxybenzaldehyde yielding 0.33 g (50%) as a yellow powder; mp 107-109 ºC; R_f 0.17 (EtOAc:hexane 50:50); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.63 (d, *J* 16.1 Hz, 1H), 7.02 (s, 1H), 6.87 (d, *J* 16.1 Hz, 1H),

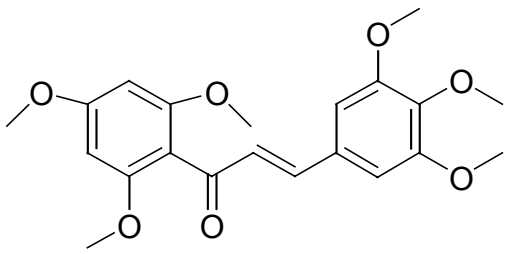
6.45 (s, 1H), 6.14 (s, 2H), 3.89 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.79 (s, 3H), 3.74 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 195.29, 162.51, 159.10, 154.52, 152.64, 143.71, 140.14, 127.60, 116.01, 111.34, 97.43, 91.24, 91.00, 56.89, 56.44, 56.34, 56.22, 55.84; EIMS *m/z* (70 eV) 388 (14), 357 (100), 195 (12); HRMS calcd for $C_{21}H_{24}O_7$ [M^+]: 388.1522, found 388.1523.

***E*-1,3-bis-(2,4,6-Trimethoxyphenyl)-2-propen-1-one (41)**



This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and 2,4,6-trimethoxybenzaldehyde yielding 0.36 g (46%) as a pale yellow crystalline powder; mp 158-160 ºC (lit.[69] 157 °C); R_f 0.10 (EtOAc:hexane 50:50); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.76 (d, *J* 16.3 Hz, 1H), 7.28 (d, *J* 16.3 Hz, 1H), 6.13 (s, 2H), 6.05 (s, 2H), 3.82 (s, 3H), 3.80 (s, 3H), 3.78 (s, 6H), 3.72 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 196.37, 162.87, 161.77, 161.35, 158.60, 136.42, 129.15, 112.72, 106.40, 90.80, 90.47, 55.88, 55.67, 55.37, 55.30; EIMS *m/z* (70 eV) 388 (10), 357 (100), 195 (13); HRMS calcd for $C_{21}H_{24}O_7$ [M^+]: 388.1522, found 388.1505.

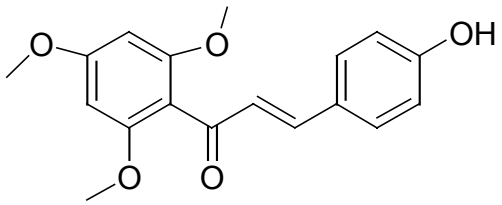
***E*-1-(2,4,6-Trimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-2-propen-1-one (42)**



This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and 3,4,5-trimethoxybenzaldehyde to yield 1.36 g (70%) as a pale yellow crystalline powder; mp 137-140 ºC; R_f 0.40 (EtOAc:hexane

3:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.22 (d, *J* 16.0 Hz, 1H), 6.84 (d, *J* 16.0 Hz, 1H), 6.72 (s, 2H), 6.15 (s, 2H), 3.84 (s, 12H), 3.75 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 194.70, 162.80, 159.11, 153.74, 144.78, 140.43, 130.85, 128.90, 111.98, 105.89, 91.09, 61.29, 56.51, 55.90, 55.83; EIMS *m/z* (70 eV) 388 (100), 360 (79), 345 (95), 195 (38); HRMS calcd for $C_{21}H_{24}O_7$ [M^+]: 388.1522, found 388.1509.

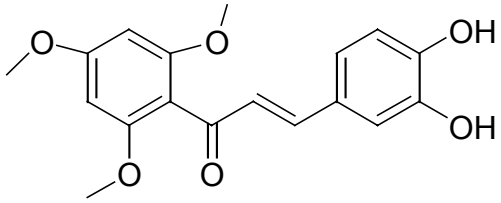
***E*-1-(2,4,6-Trimethoxyphenyl)-3-(4-hydroxyphenyl)-2-propen-1-one (43)**



4-Hydroxybenzaldehyde (2.44 g, 20 mmol) and *para*-toluenesulphonic acid (0.091 g, 0.48 mmol) were stirred in dry DCM (80 ml), and then 3,4-dihydro-2*H*-pyran (5.05 g, 60 mmol) in dry DCM (20 ml) was added dropwise. The reaction mixture was stirred at room temperature until all the components dissolved, then left for 1 hour after which H_2O (50 ml) and then aqueous $NaHCO_3$ (30 ml) was added. The mixture was washed with 10 % $NaHCO_3$ (2 x 50 ml), once with H_2O (40 ml), then dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was purified by flash chromatography yielding the crude 4-(tetrahydropyran-2-yloxy)-benzaldehyde. The title compound was prepared from 2,4,6-trimethoxyacetophenone (**29**) and 4-(tetrahydropyran-2-yloxy)-benzaldehyde according to the general procedure. 3M HCl (5 mL) in MeOH (5 mL) was added dropwise to a solution of the crude residue in MeOH (20 mL). After 2 hrs, 10 % $NaHCO_3$ solution (5 mL) was added and the mixture extracted with DCM (3 x 40 mL). The organic phase was dried (Na_2SO_4) and the solvent evaporated under reduced pressure. The residue was purified by column chromatography furnishing 0.30 g (total yield 30%) as a bright yellow powder; mp 198-200 ºC; R_f 0.17 (EtOAc:hexane 1:1); ^{1}H NMR (acetone-d_6, 300 MHz) δ 7.49 (dm, *J* 8.7 Hz, 2H), 7.20 (d, *J* 16.1 Hz, 1H), 6.87 (dm, *J* 8.7 Hz, 2H), 6.75 (d, *J* 16.1 Hz, 1H), 6.29 (s, 2H), 3.85 (s, 3H), 3.73 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 196.55, 162.62, 159.54, 158.83, 146.40, 130.46, 126.37, 125.63, 116.11, 111.10,

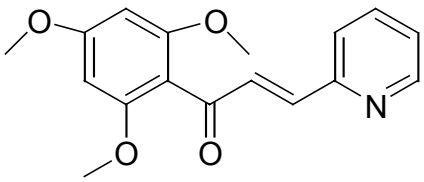
90.68, 55.81, 55.39; EIMS *m/z* (70 eV) 314 (25), 286 (100), 195 (33); HRMS calcd for $C_{18}H_{18}O_5$ [M^+]: 314.1154, found 314.1157.

***E*-1-(2,4,6-Trimethoxyphenyl)-3-(3,4-dihydroxyphenyl)-2-propen-1-one (44)**



3,4-Bis-(tetrahydropyran-2-yloxy)-benzaldehyde was obtained using six equivalents of 3,4-dihydro-2*H*-pyran according to the procedure for 4-(tetrahydropyran-2-yloxy)-benzaldehyde. The title compound was synthesised from 2,4,6-trimethoxyacetophenone (**29**) and crude 3,4-bis-(tetrahydropyran-2-yloxy)-benzaldehyde following the general procedure. Flash chromatography furnished 0.19 g (total yield 14%) of the title compound as a bright yellow crystalline powder; mp 173-177 ºC; R_f 0.73 (DCM:MeOH 60:40); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.53 (bs, 1H), 7.36 (bs, 1H), 7.25 (d, *J* 15.9 Hz, 1H), 7.04 (d, *J* 1.5 Hz, 1H), 6.80-6.73 (m, 3H), 6.08 (s, 2H), 3.78 (s, 3H), 3.66 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 196.31, 162.62, 158.77, 147.57, 146.90, 144.44, 127.14, 125.96, 122.92, 115.14, 114.46, 110.96, 90.64, 55.71, 55.34; EIMS *m/z* (70 eV) 330 (24), 302 (100), 195 (40); HRMS calcd for $C_{18}H_{18}O_6$ [M^+]: 330.1103, found 330.1095.

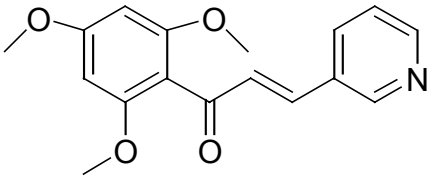
***E*-1-(2,4,6-Trimethoxyphenyl)- 3-(pyridin-2-yl)-2-propen-1-one (45)**



This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and pyridin-2-aldehyde affording 0.15 g (25%) as a bright yellow crystalline powder; mp 100-102 ºC (lit.[47] 100-102 ºC); R_f 0.10

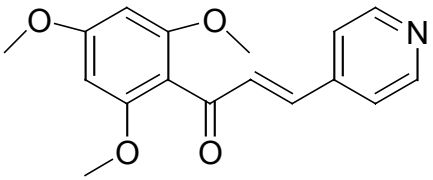
(EtOAc:hexane 40:60), 0.32 (EtOAc:hexane 1:1); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.60 (dd, *J* 4.8, 0.7 Hz, 1H), 7.68 (ddd, *J* 7.7, 7.7, 0.9 Hz, 1H), 7.47 (m, 1H), 7.39 (d, *J* 15.9 Hz, 1H), 7.29 (d, *J* 15.9 Hz, 1H), 7.20 (m, 1H), 6.11 (s, 2H), 3.82 (s, 3H), 3.74 (s, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 194.30, 162.55, 158.91, 153.83, 149.93, 142.50, 136.63, 132.33, 123.91, 123.86, 111.56, 90.65, 55.84, 55.42; EIMS *m/z* (70 eV) 299 (35), 240 (100), 195 (32); HRMS calcd for $C_{17}H_{17}NO_4$ [M^+]: 299.1157, found 299.1160.

***E*-1-(2,4,6-Trimethoxyphenyl)-3-(pyridin-3-yl)-2-propen-1-one (46)**



This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and pyridin-3-aldehyde affording 0.29 g (48%) as yellow crystalline powder; mp 119-123 ºC (lit.[47] 123-125 ºC); R_f 0.18 (EtOAc:hexane 3:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.62 (d, *J* 1.3 Hz, 1H), 8.47 (dd, *J* 4.6, 1.0 Hz, 1H), 7.77 (dt, *J* 8.0, 1.8 Hz, 1H), 7.28 (d, *J* 16.1 Hz, 1H), 7.22 (dd, *J* 8.0, 4.6 Hz, 1H), 6.94 (d, *J* 16.1 Hz, 1H), 6.09 (s, 2H), 3.76 (s, 3H), 3.68 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 192.92, 162.47, 158.70, 150.43, 149.59, 139.24, 134.08, 130.52, 130.42, 123.42, 111.02, 90.46, 55.59, 55.15; EIMS *m/z* (70 eV) 299 (25), 271 (100), 195 (48); HRMS calcd for $C_{17}H_{17}NO_4$ [M^+]: 299.1157, found 299.1149.

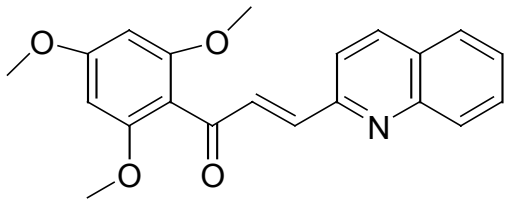
***E*-1-(2,4,6-Trimethoxyphenyl)-3-(pyridin-4-yl)-2-propen-1-one (47)**



This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and pyridin-4-aldehyde affording 0.24 g (40%) as a yellow oil; R_f 0.18 (EtOAc:hexane 3:1); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.78 (d, *J* 6.7

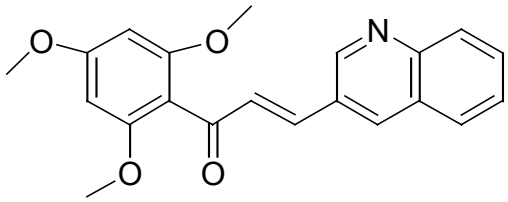
Hz, 2H), 7.56 (d, *J* 6.7 Hz, 2H), 7.50 (d, *J* 16.0 Hz, 1H), 7.28 (d, *J* 16.0 Hz, 1H), 6.15 (s, 2H), 4.02 (s, 3H), 3.95 (s, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 193.24, 163.32, 159.50, 150.61, 142.84, 140.07, 133.07, 122.42, 111.53, 91.09, 55.24, 55.81; EIMS *m/z* (70 eV) 299 (30), 271 (100), 195 (83); HRMS calcd for $C_{17}H_{17}NO_4$ [M^+]: 299.1157, found 299.1154.

***E*-1-(2,4,6-Trimethoxyphenyl)-3-(quinolin-2-yl)-2-propen-1-one (48)**



This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and 2-quinolinecarboxaldehyde yielding 0.51 g (75%) as a beige powder; mp 105-108 ºC; R_f 0.21 (EtOAc:hexane 40:60), 0.33 (EtOAc:hexane 50:50); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.14 (d, *J* 8.5 Hz, 1H), 8.04 (d, *J* 8.5 Hz, 1H), 7.79 (d, *J* 7.8 Hz, 1H), 7.72-7.67 (m, 2H), 7.56-7.50 (m, 2H), 7.33 (d, *J* 16.2 Hz, 1H), 6.14 (s, 2H), 3.84 (s, 3H), 3.76 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 194.82, 162.58, 158.89, 154.28, 148.22, 143.85, 136.52, 133.81, 129.93, 129.59, 127.89, 127.51, 127.22, 119.86, 111.17, 90.74, 55.82, 55.43; EIMS *m/z* (70 eV) 349 (56), 290 (100), 195 (30); HRMS calcd for $C_{21}H_{19}NO_4$ [M^+]: 349.1314, found 349.1323.

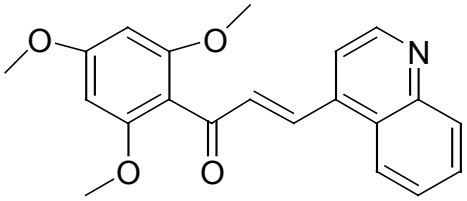
***E*-1-(2,4,6-Trimethoxyphenyl)-3-(quinolin-3-yl)-2-propen-1-one (49)**



This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and 3-quinolinecarboxaldehyde yielding 0.74 g (85%) as a pale yellow crystalline powder; mp 158-160 ºC; R_f 0.09 (EtOAc:hexane 2:3),

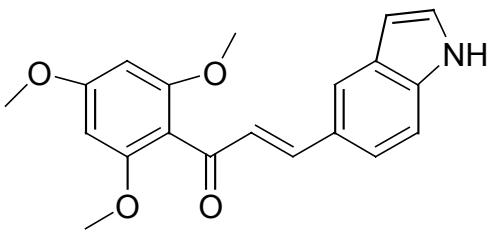
0.19 (EtOAc:hexane 1:1); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 9.06 (d, *J* 2.0 Hz, 1H), 8.21 (d, *J* 2.0 Hz, 1H), 8.07 (d, *J* 8.4 Hz, 1H), 7.81 (dd, *J* 8.4, 1.0 Hz, 1H), 7.71 (dt, *J* 8.4, 1.5 Hz, 1H), 7.57-7.49 (m, 2H), 7.16 (d, *J* 15.9 Hz, 1H), 6.15 (s, 2H), 3.84 (s, 3H), 3.76 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 193.62, 163.18, 159.51, 149.96, 148.86, 140.23, 136.01, 130.92, 130.87, 129.75, 128.72, 128.51, 128.11, 127.73, 111.95, 90.98, 56.36, 55.89; EIMS *m/z* (70 eV) 349 (19), 321 (100), 195 (27); HRMS calcd for $C_{21}H_{19}NO_4$ [M^+]: 349.1314, found 349.1308.

***E*-1-(2,4,6-Trimethoxyphenyl)-3-(quinolin-4-yl)-2-propen-1-one (50)**



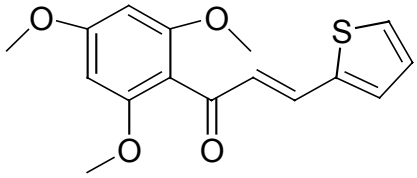
This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and 4-quinolinecarboxaldehyde yielding 0.58 g (85%) as pale yellow crystals; mp 139-143 ºC (lit.[47] 139-141 ºC); R_f 0.12 (EtOAc:hexane 3:1), 0.13 (EtOAc:hexane 50:50); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.90 (d, *J* 4.5 Hz, 1H), 8.14 (d, *J* 16.0 Hz, 1H), 8.09 (t, *J* 8.8 Hz, 2H), 7.73 (dt, *J* 6.9, 1.3 Hz, 1H), 7.63-7.53 (m, 2H), 7.13 (d, *J* 16.0 Hz, 1H), 6.20 (s, 2H), 3.80 (s, 3H), 3.79 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 192.32, 162.98, 159.33, 150.00, 148.54, 140.76, 136.67, 134.45, 130.05, 129.52, 126.98, 126.18, 123.33, 118.10, 111.42, 90.71, 55.87, 55.40; EIMS *m/z* (70 eV) 349 (21), 321 (100), 290 (18), 195 (49); HRMS calcd for $C_{21}H_{19}NO_4$ [M^+]: 349.1314, found 349.1321.

***E*-1-(2,4,6-Trimethoxyphenyl)-3-(5-indolyl)-2-propen-1-one (52)**



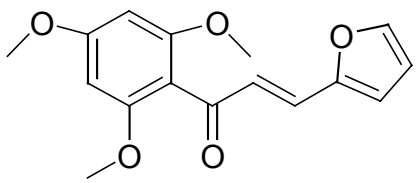
This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and indole-5-carboxaldehyde yielding 0.13 g (19%) as yellow crystals; R_f 0.20 (EtOAc:hexane 50:50); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.58 (bs, 1H), 7.74 (s, 1H), 7.48 (d, *J* 16.0 Hz, 1H), 7.67 (dd, *J* 8.6, 1.5 Hz, 1H), 7.33 (d, *J* 8.6 Hz, 1H), 7.18 (t, *J* 4.5 Hz, 1H), 6.94 (d, *J* 16.0 Hz, 1H), 6.51 (m, 1H), 6.15 (s, 2H), 3.84 (s, 3H), 3.72 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 194.98, 162.17, 158.68, 147.17, 137.15, 128.13, 126.85, 126.45, 125.34, 122.86, 121.83, 111.61, 103.28, 90.75, 55.89, 55.43. MS analysis was not performed and the melting point was not determined due to the instability of the compound.

***E*-1-(2,4,6-Trimethoxyphenyl)-3-(thiophen-2-yl)-2-propen-1-one (53)**



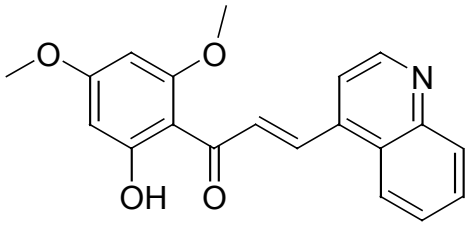
This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and thiophene-2-carboxaldehyde furnishing 0.56 g (72%) as a yellow crystalline powder; mp 89-93 ºC; R_f 0.23 (EtOAc:hexane 40:60); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.45 (d, *J* 15.7 Hz, 1H), 7.35 (d, *J* 5.1 Hz, 1H), 7.20 (d, *J* 3.7 Hz, 1H), 7.01 (dd, *J* 5.1, 3.7 Hz, 1H), 6.74 (d, *J* 15.7 Hz, 1H), 6.13 (s, 2H), 3.83 (s, 3H), 3.77 (s, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 193.25, 162.17, 158.46, 139.90, 136.15, 130.87, 128.42, 127.89, 127.71, 111.11, 90.38, 55.52, 55.10; EIMS *m/z* (70 eV) 304 (14), 276 (100), 195 (23); HRMS calcd for $C_{16}H_{16}SO_4$ [M^+]: 304.0769, found 304.0761.

***E*-1-(2,4,6-Trimethoxyphenyl)-3-(furan-2-yl)-2-propen-1-one (54)**



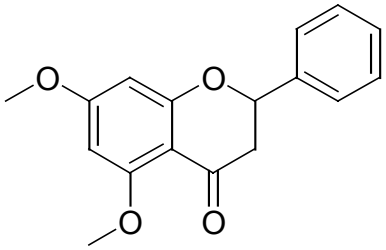
This compound was prepared according to the general procedure from 2,4,6-trimethoxyacetophenone (**29**) and 2-furaldehyde yielding 0.26 g (36%) as a bright yellow powder; mp 91-93 ºC; R_f 0.21 (EtOAc:hexane 40:60); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.45 (d, *J* 1.3 Hz, 1H), 7.10 (d, *J* 15.8 Hz, 1H), 6.81 (d, *J* 15.8 Hz, 1H), 6.57 (d, *J* 3.3 Hz, 1H), 6.43 (dd, *J* 3.3, 1.7 Hz, 1H), 6.12 (s, 2H), 3.82 (s, 3H), 3.74 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 193.66, 162.38, 158.82, 151.45, 144.73, 130.24, 126.69, 115.12, 112.40, 111.71, 90.69, 55.87, 55.41; EIMS *m/z* (70 eV) 288 (16), 260 (100), 195 (21); HRMS calcd for $C_{16}H_{16}O_5$ [M^+]: 288.0997, found 288.0997.

***E*-1-(2,4-Dimethoxy-6-hydroxyphenyl)-3-(quinolin-4-yl)-2-propen-1-one (55)**



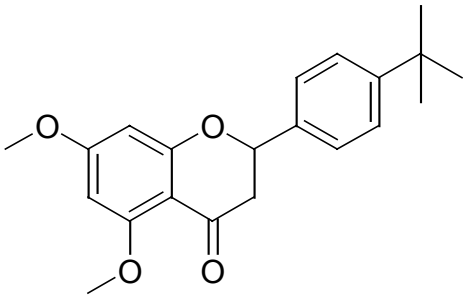
4-Quinolinecarboxaldehyde (1.57 g, 10 mmol) in EtOH (30 mL) was added slowly dropwise to a solution of 2,4-dimethoxy-6-hydroxyacetophenone (**30**) (1.96 g, 10 mmol) and NaOH (0.24 g, 5.9 mmol) in EtOH (50 mL). The mixture turned green. The reaction was monitored by TLC. After 5 h, water (50 mL) was added. The mixture was extracted with diethyl ether (3 x 50 mL), dried ($MgSO_4$) and the organic phase evaporated. The residue was purified by flash chromatography, affording 0.32 g (10%) as an orange product; mp 162-164 °C; R_f 0.12 (EtOAc:hexane 3:1); ^{1}H NMR (methanol-d_4, 300 MHz, 50ºC) δ 8.91 (d, *J* 4.6 Hz, 1H), 8.17 (d, *J* 8.6 Hz, 1H), 8.05 (d, *J* 8.6 Hz, 1H), 7.74 (m, 1H), 7.69 (d, *J* 4.6 Hz, 1H), 7.61 (m, 1H), 6.11 (m, 1H), 6.05 (d, *J* 2.3 Hz, 1H), 6.00 (d, *J* 2.3 Hz, 1H), 3.81 (s, 3H), 3.71 (s, 3H), 3.63 (m, 1H), 3.48 (m, 1H); ^{13}C NMR not available due to poor solubility; EIMS *m/z* (70 eV) 353 (11), 335 (10), 207 (17), 196 (20), 181 (100); HRMS calcd for $C_{20}H_{17}NO_4$ [M^+]: 335.1157, found 335.1153.

(±)-5,7-Dimethoxy-2-phenyl-chroman-4-one (56)



NaOH (0.24 g, 5.9 mmol) in EtOH (50 mL) was added slowly to a roundbottomed flask containing 2,4-dimethoxy-6-hydroxyacetophenone (**30**) (1.96 g, 10 mmol) and benzaldehyde (1.06 g, 10 mmol). The mixture was heated at reflux overnight with mechanical stirring. H_2O (70 mL) was added, the mixture extracted with diethyl ether (3 x 70 mL) and the organic phase dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by flash chromatography yielding 0.42 g (15%) as greyish-white crystals; mp 139-141 °C; R_f 0.21 (EtOAc:hexane 1:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.36-7.20 (m, 5H), 6.03 (d, *J* 2.3 Hz, 1H), 5.97 (d, *J* 2.3 Hz, 1H), 5.27 (dd, *J* 13.0, 3.0 Hz, 1H), 3.76 (s, 3H), 3.68 (s, 3H), 2.88 (dd, *J* 16.5, 13.0 Hz, 1H), 2.66 (dd, *J* 16.5, 3.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 188.53, 165.49, 164.50, 161.83, 138.45, 128.34, 128.19, 125.71, 105.53, 93.18, 92.69, 78.69, 55.66, 55.15, 45.15; EIMS *m/z* (70 eV) 284 (66), 207 (19), 180 (100), 152 (29), 137 (23); HRMS calcd for $C_{17}H_{16}O_4$ [M^+]: 284.1048, found 284.1061.

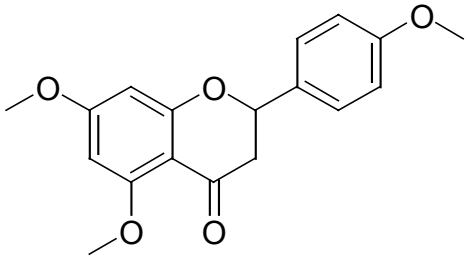
(±)-2-(4-*tert*-Butylphenyl)-5,7-dimethoxy-chroman-4-one (57)



NaOH (0.24 g, 5.9 mmol) in EtOH (40 mL) was added slowly to a roundbottomed flask containing 2,4-dimethoxy-6-hydroxyacetophenone (**30**) (1.96 g, 10 mmol) and 4-*tert*-butylbenzaldehyde (1.62 g, 10 mmol) with mechanical stirring. The mixture

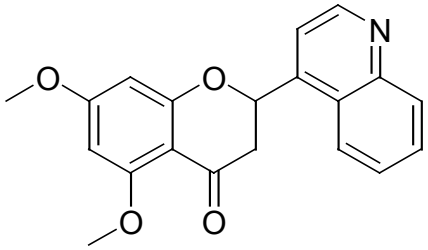
was heated at reflux for 2-3 hrs, cooled and left at room temperature overnight. H_2O (50 mL) was added, the mixture extracted with diethyl ether (3 x 70 mL) and the organic phase dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by flash chromatography affording 0.69 g (20%) as pale yellow crystals; mp 147-150 °C; R_f 0.29 (EtOAc:hexane 1:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.36 (m, 4H), 6.08 (d, *J* 2.3 Hz, 1H), 6.02 (d, *J* 2.3 Hz, 1H), 5.32 (dd, *J* 13.0, 3.0 Hz, 1H), 3.82 (s, 3H), 3.74 (s, 3H), 2.98 (dd, *J* 16.5, 13.0 Hz, 1H), 2.72 (dd, *J* 16.5, 3.0 Hz, 1H), 1.28 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 189.26, 165.84, 164.98, 162.20, 151.70, 135.59, 125.92, 125.62, 105.94, 93.48, 93.01, 79.04, 56.06, 55.48, 45.29, 34.56, 31.22; EIMS *m/z* (70 eV) 340 (66), 207 (26), 180 (100), 152 (17), 145 (13), 137 (13); HRMS calcd for $C_{21}H_{24}O_4$ [M^+]: 340.1674, found 340.1669.

(±)-5,7-Dimethoxy-2-(4-methoxyphenyl)-chroman-4-one (58)



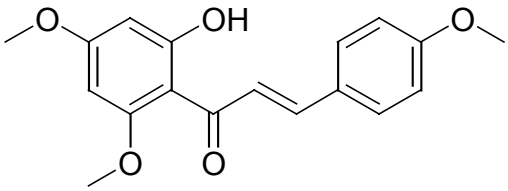
The compound was prepared from 2,4-dimethoxy-6-hydroxyacetophenone (**30**) and 4-methoxybenzaldehyde in the same way as described for compound **57**, furnishing 0.03 g (1%) as pale yellow crystals; mp 105-106 °C (lit.[70] 117-118 °C); R_f 0.33 (EtOAc:hexane 1:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 13.74 (s, 1H), 7.32 (d, *J* 8.7Hz, 2H), 6.93 (d, *J* 8.7Hz, 2H), 6.05 (d, *J* 2.3 Hz, 1H), 5.88 (d, *J* 2.3 Hz, 1H), 5.21 (m, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H), 3.44-3.28 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 204.21, 167.76, 166.44, 162.86, 158.93, 135.57, 127.11, 113.79, 105.88, 93.69, 90.96, 69.75, 55.59, 55.55, 55.23, 52.63; EIMS *m/z* (70 eV) 314 (13), 196 (26), 181 (100), 135 (41); HRMS calcd for $C_{18}H_{18}O_5$ [M^+]: 314.1154, found 314.1155.

(±)-5,7-Dimethoxy-2-(quinolin-4-yl)-chroman-4-one (59)



To a solution of 1-(2,4,6-trimethoxyphenyl)-3-(quinolin-4-yl)-2-propen-1-one (**50**) (1.75g, 5 mmol) in anhydrous DCM (30 mL) was added a 1M solution of BBr_3 (5.5 mL, 5.5 mmol) in DCM dropwise over 15 min at room temperature with magnetic stirring. A reddish-brown precipitate was formed during the addition. After stirring for 6 hrs, the reaction was quenched by addition of EtOH (30 mL) and the solvent was evaporated under reduced pressure. The red residue was triturated with boiling 50% EtOH (60 mL). After cooling to room temperature, the red solid was collected on a Büchner funnel, washed with 50% EtOH (100 mL) and air-dried. Recrystallisation from 80% EtOH (300 mL) provided 0.95 g (57 %) of the title product as a red powder; mp n.d. (decomposed upon heating); R_f 0.14 (EtOAc:hexane 3:1); ^{1}H NMR (methanol-d_4, 300 MHz) δ 9.02 (d, *J* 5.5 Hz, 1H), 8.52 (d, *J* 8.5 Hz, 1H), 8.17-7.94 (m, 4H), 6.08 (m, 2H), 5.11 (dd, *J* 8.2, 4.2 Hz, 1H), 4.03 (dd, *J* 14.9, 4.2 Hz, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 3.76-3.71 (m, 1H); ^{13}C NMR n.d. due to very poor solubility; EIMS *m/z* (70 eV) 335 (100), 207 (57), 143 (47); HRMS calcd for $C_{20}H_{17}NO_4$ [M^+]: 335.1157, found 335.1165.

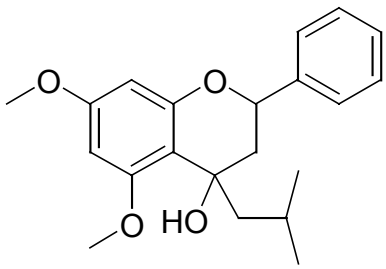
***E*-1-(2,4-Dimethoxy-6-hydroxyphenyl)-3-(4-methoxyphenyl)-2-propen-1-one (60)**



1/3 of the $AlCl_3$ (total 1.33 g, 10 mmol) was added to a solution of *E*-1-(2,4,6-trimethoxyphenyl)-3-(4-methoxyphenyl)-propenone (**38**) (1.64 g, 5 mmol) in dry DCM (30 mL). The mixture was heated on reflux for 1 h, after which another 1/3 of

the $AlCl_3$ was slowly added. The reaction was left with magnetic stirring for 30 min before the remaining $AlCl_3$ was added. When a new product was detected on TLC (hvor lenge), the reaction mixture was dissolved in DCM until complete dissolution had occurred. The mixture was extracted with H_2O. HCl was added for better separation of the phases. The organic phase was dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The residue was purified by column chromatography, yielding 0.45 g (28%) as yellow crystals; mp 117-118 °C (lit.[68] 113-114 ºC); R_f 0.28 (EtOAc:hexane 1:1); IR (film, KBr) ν_{max}/cm^{-1} 3584 (OH, H-bonded); ^1H NMR ($CDCl_3$, 300 MHz) δ 14.33 (s, 1H), 7.89 (d, *J* 15.5 Hz, 1H), 7.75 (d, *J* 15.5 Hz, 1H), 7.67 (d, *J* 8.4 Hz, 1H), 6.98 (d, *J* 8.4 Hz, 1H), 6.08 (s, 2H), 3.98 (s, 3H), 3.85 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 192.56, 168.34, 165.99, 162.43, 161.33, 142.42, 130.07, 128.29, 125.10, 114.33, 106.33, 93.79, 91.19, 55.80, 55.53, 55.36; EIMS *m/z* (70 eV) 314 (100), 207 (33), 180 (34); HRMS calcd for $C_{18}H_{18}O_5$ [M^+]: 314.1154, found 314.1147.

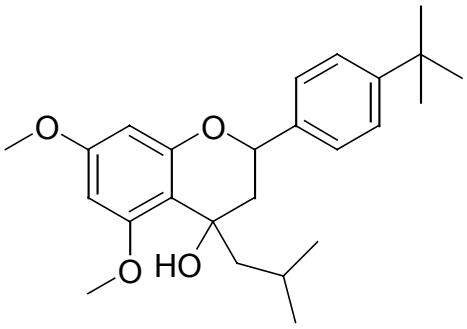
(±)-4-Isobutyl-5,7-dimethoxy-2-phenyl-chroman-4-ol (61)



(±)-5,7-Dimethoxy-2-phenyl-chroman-4-one (**56**) (0.35 g, 1.2 mmol) in dry THF (20 ML) was cooled between –10 and -5°C in a freezing mixture of crushed ice and $CaCl_2$. Isobutylmagnesium bromide (2.0M in diethyl ether, 0.75 mL, 1.5 mmol) was added slowly to the mixture under magnetic stirring. After 1 h, a saturated NH_4Cl solution (10 mL) was added slowly, while still on ice, and then H_2O (20 mL) was added before extraction with diethyl ether (2 x 20 mL). The organic phase was dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography, yielding 0.25 g (61 %) as a yellow solid; mp n.d.;

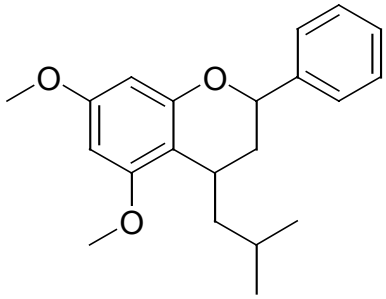
R_f 0.65 (EtOAc:hexane 3:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46-7.33 (m, 5H), 6.10 (s, 2H), 5.07 (dd, *J* 12.5, 1.9 Hz, 1H), 4.14 (s, 1H), 3.87 (s, 3H), 3.73 (s, 3H), 2.42 (dd, *J* 13.8, 1.9 Hz, 1H), 2.21 (m, 1H), 2.12-1.89 (m, 3H), 1.06 (d, *J* 6.3 Hz, 3H), 0.97 (d, *J* 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.03, 158.52, 155.12, 140.73, 128.63, 128.22, 126.24, 111.04, 94.03, 92.69, 76.30, 71.48, 55.58, 55.29, 48.99, 40.68, 25.32, 24.72, 23.64.

(±)-2-(4-*tert*-Butylphenyl)-4-isobutyl-5,7-dimethoxy-chroman-4-ol (62)



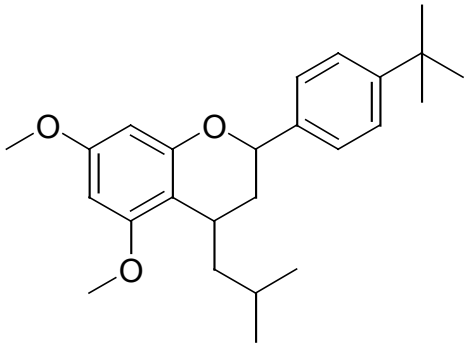
The compound was obtained from 2-(4-*tert*-butyl-phenyl)-5,7-dimethoxy-chroman-4-one (**58**) and isobutylmagnesium bromide in the same procedure as described for **61**, yielding 0.27 g (52 %) of the title compound; mp n.d.; R_f 0.68 (EtOAc:hexane 3:1); ^{1}H NMR ($CDCl_3$, 200 MHz) δ diastereomer 1: 7.47-7.37 (m, 4H), 6.11 (s, 2H), 5.06 (dd, *J* 12.3, 2.1 Hz, 1H), 4.16 (s, 1H), 3.87 (s, 3H), 3.73 (s, 3H), 2.44 (dd, *J* 2.1, 13.8 Hz, 1H), 2.23 (dd, *J* 12.8, 13.8 Hz, 1H), 2.06-1.96 (m, 3H), 1.34 (s, 9H), 1.08 (d, *J* 6.2 Hz, 3H), 0.98 (d, *J* 6.2 Hz, 3H), diastereomer 2: 3.90 (s, 0.6H), 3.82 (s, 0.6H); ^{13}C NMR not analysed due to multiple signals; EIMS *m/z* (70 eV) 380 (7), 341 (100), 323 (23), 181 (28).

(±)-4-Isobutyl-5,7-dimethoxy-2-phenyl-chroman (63)



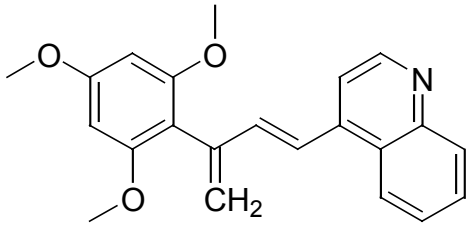
(±)-4-Isobutyl-5,7-dimethoxy-2-phenyl-chroman-4-ol (**61**) (0.28 g, 0.8 mmol) was dissolved in EtOH (10 mL) and 5 drops of conc. H_2SO_4 were added. The mixture was hydrogenated at atmospheric pressure for 32 hrs using Pd/C (0.15 g, 10%) as catalyst. The crude product was purified by dry-column flash chromatography; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.45-7.26 (m, 5H), 6.15 (d, *J* 2.4 Hz, 1H), 6.10 (d, *J* 2.4 Hz, 1H), 4.83 (dd, *J* 2.4, 10.7 Hz, 1H), 3.78 (s, 3H), 3.74 (s, 3H), 3.11 (m, 1H), 2.41 (ddd, *J* 2.4, 8.2, 14.2 Hz, 1H), 1.94-1.81 (m, 2H), 1.64 (m, 1H), 1.06 (m, 1H), 0.93 (d, *J* 6.4 Hz, 3H), 0.81 (d, *J* 6.4 Hz, 3H); ^{13}C ($CDCl_3$, 50 MHz) δ 159.23, 159.00, 157.50, 141.84, 128.46, 127.69, 125.69, 109.34, 93.93, 92.54, 77.99, 55.25, 55.19, 45.03, 38.23, 28.98, 25.53, 24.21, 21.01.

(±)-2-(4-*tert*-Butylphenyl)-4-isobutyl-5,7-dimethoxy-chroman (64)



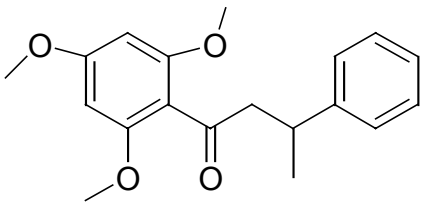
A solution of (±)-2-(4-*tert*-Butyl-phenyl)-4-isobutyl-5,7-dimethoxy-chroman-4-ol (**62**) (0.27 g, 0.7 mmol) and 5 drops of conc. H_2SO_4 in EtOH (15 mL) was hydrogenated on a Parr apparatus for 20 hrs using Pd/C (0.20g, 10%) as catalyst. The mixture was purified by dry-column flash chromatography; ^{1}H NMR and ^{13}C NMR not analyzed due to multiple signals caused by stereogenic centre.

4-[3-(2,4,6-Trimethoxyphenyl)-buta-1,3-dienyl]-quinoline (66)



To a stirred solution of trimethylsilylmethylmagnesium chloride (0.52 g, 3.6 mmol) in dry THF (5 mL) was added a solution of *E*-1-(2,4,6-trimethoxyphenyl)-3-(quinolin-4-yl)-2-propen-1-one (**50**) (1.05 g, 3 mmol) in dry THF (5 mL), slowly dropwise. The resulting mixture was refluxed with continued stirring for 1 h, allowed to cool and left at room temperature for another 3 hrs. H_2O (20 mL) was added along with $NaHCO_3$ (10 %, 10 mL), the mixture extracted with diethyl ether (3 x 30 mL) the organic phase dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The structure of the intermediate alcohol **65** was analysed by NMR and IR. Potassium hydride (1.20 g 30% dispersion in oil, 0.36 mmol) was washed with hexane (10 mL), which was removed by a pipette. To the residue was added dry THF (10 mL), and then a solution of the intermediate **65** in dry THF (10 mL) was added. The mixture was heated under reflux overnight. The mixture was poured into cold NH_4Cl (10 %, 50 mL) overlaid with diethyl ether (40 mL). The aqueous phase was extracted with diethyl ether (50 mL), the organic phases combined, washed with brine (40 mL), dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The products could not be separated by flash chromatography, thus the yield was not established.

(±)-1-(2,4,6-Trimethoxyphenyl)- 3-phenyl-butan-1-one (67)



MeLi (1.6M in diethyl ether, 2.25 mL) was added carefully to a solution of *E*-1-(2,4,6-trimethoxyphenyl)-3-phenyl-2-propen-1-one (**28**) (0.884 g, 3.0 mmol) in dry THF (20 mL). After 2 hrs, the reaction was quenched with saturated NH_4Cl (10 mL), and the mixture was extracted with diethyl ether (2 x 40 mL). The organic phase was washed with brine, dried ($MgSO_4$) and the solvent evaporated under reduced pressure. Half of the crude intermediate was dissolved in MeOH (10 mL), concentrated H_2SO_4 (1 drop) was added and TLC after 3 min showed formation of a product. A 10 % solution of $NaHCO_3$ (10 mL) was added and the mixture was extracted with DCM (2 x 20 mL). The organic phase was dried ($MgSO_4$) and the solvent evaporated giving 0.12 g (overall 25%) of the title compound **67** as reddish-brown crystals; mp n.d.; R_f 0.36 (EtOAc:hexane 30:70); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.26-7.13 (m, 5H), 6.07 (s, 2H), 3.80 (s, 3H), 3.70 (s, 6H), 3.54-3.34 (m, 1H), 3.09 (dd, *J* 16.6, 5.6 Hz, 1H), 2.97 (dd, *J* 16.6, 8.7 Hz), 1.30 (d, *J* 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 203.04, 162.14, 158.06, 146.89, 128.13, 126.83, 125.78, 113.48, 90.44, 55.59, 55.27, 52.95, 35.35, 21.88; EIMS *m/z* (70 eV) 314 (8), 297 (86), 195 (100), 168 (32); HRMS calcd for $C_{19}H_{22}O_4$ [M^+]: 314.1518, found 314.1520.

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