Chapter 1: Flavonoids and their potential health benefits

The flavonoids are a class of plant secondary metabolites with phenolic chemical structures. They were known for their beneficial effects on health long before the active compounds were isolated (Nijveldt *et al.* 2001). Flavonoids are widely found in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine. Thus far, more than 4000 structurally unique flavonoids have been identified from plant sources (Middleton *et al*., 2000). The basic flavonoid structure is a flavan nucleus, which consists of two benzene rings (A and B) linked through a heterocyclic pyran ring C (Figure 1.1) (Harborne, 1994).

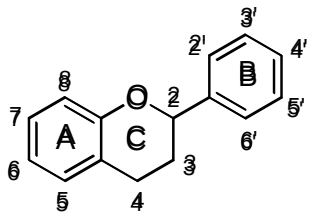


Figure 1.1 Basic flavonoid structure

Flavonoids are further divided into various classes on the basis of their molecular structures, *i.e*. chalcones, flavones, flavanones, flavonols, isoflavones, flavanonols, flavan-3-ols, anthocyanidins, biflavones, aurones, coumarins, and catechins. (Harborne, 1994; Pietta, 2000). Among the classes of flavonoids, those of particular interest in this study are chalcones, flavones, flavanones, and flavonols (Figure 1.2).

Flavonoids are important components in the human diet. Intake of flavonoids can range between 20 and 800 mg/day, depending on the amount of consumption of fruit, vegetables, red wine, tea, and beer (Beecher, 2003; Larson, 1988).

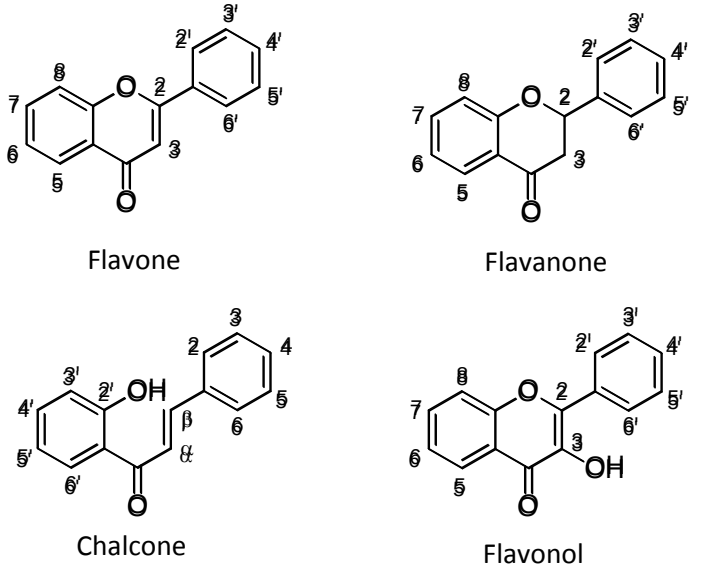


Figure 1.2 The molecular structures of flavone, flavanone, chalcone, and flavonol

Various epidemiologic studies have been conducted on flavonoid consumption and its potential health benefits. The most commonly known property of flavonoids is their capacity to act as antioxidant (Nijveldt *et al.* 2001; Pietta, 2000).

1.1 Muscarinic receptor binding effects

Natural flavonoids present in plants have been shown to ameliorate amyloid-beta peptide-induced neurotoxicity. Recently, Jin *et al.* tested several flavonoids compounds for their effects on amyloid-beta peptide-induced learning impairment in mice. They showed that fustin treatment prevented decreases in acetylcholine levels, choline acetyltransferase activity and gene expression induced by beta-amyloid peptide. In addition, fustin has been shown to attenuate decreases in muscarinic M1 receptor binding activity by modulating extracellular signal-regulated kinase ½ (ERK 1/2) and cAMP response-element binding protein (CREB) phosphorylation and brain-derived neurotrophic factor (BDNF) expression (Jin *et al.* 2009).

Our recent interest in this class of compounds has been stimulated by the potential health benefits arising from the muscarinic receptor cholinergic activity of meliternatin and 3,5,8-trimethoxy-3',4',6,7-bismethylenedioxyflavone (Chung *et al.*, 2008). The structures of these polyoxygenated flavones differ from majority of known ligands for muscarinic receptors, *i.e.* they lack a tertiary or quarternary amino group. However, the methylenedioxy groups of the flavones resemble part of the structures of darifenacin and zamifenacin (M3 antagonists) (Broadley & Kelly, 2001), which has been found to allosterically regulate muscarinic receptor activity.

1.2 Antioxidative effects

The capacity of flavonoids to act as antioxidants is due to their ability to reduce free radical formation and to scavenge free radicals. According to Halliwell and Gutteridge, the mechanisms for antioxidant action can include (1) suppressing reactive oxygen species formation either by inhibition of enzymes or chelating trace elements involved in free radical production; (2) scavenging reactive oxygen species; and (3) upregulating or protecting antioxidant defenses. (Halliwell & Gutteridge 1998). Polyhydroxylated flavonols and catechins are the most powerful flavonoids in protecting the body against reactive oxygen species (Pietta, 2000).

Free radicals and reactive oxygen species are produced in the body during normal oxygen metabolism and induced by exogenous damage (de Groot, 1994). The presence of free radicals and reactive oxygen species in the body cells and tissues can result in cellular membrane damage and eventually lead to the cell death, inflammatory response and tissue damage. The body excretes enzymes such as superoxide dismutase, catalase, glutatione peroxidase, and also nonenzymatic counterparts such as glutathione, ascorbic acid, and α-tocopherol to counteract the impact of free radicals (Nijveldt *et al.* 2001). Flavonoids may have an additive effect in preventing injury caused by free

radicals and reactive oxygen species through three different ways: (1) direct scavenging of free radicals (Korkina & Afanas'ev, 1997; Hanasaki *et al.* 1994). (2) reduction of nitric oxide (Huk *et al.*, 1998; van Acker, 1995); and (3) inhibition of xanthine oxidase (van Acker *et al.* 1995; Sanhueza *et al.* 1992).

1.3 Antiinflammatory effects

Quercetin has been shown to inhibit cyclo-oxygenase (COX) and 5-lipoxygenase and prevent the release of arachidonic acid that induces inflammatory response (Loke *et al.* 2008; Boots *et al.* 2008; Valerio *et al.* 2009). A number of other flavonols have also been shown to inhibit eicosanoid biosynthesis and neutrophil degranulation, hence diminishing the release of arachidonic acid by neutrophils and other immune cells (Damas *et al.* 1985; Liu *et al.* 2008, Rao *et al.* 2003). Chrysin has been shown to downregulate prostaglandins, a group of key pro-inflammatory (Liang *et al.* 2001).

1.4 Anticancer effects

Flavones such as apigenin, fisetin, and luteolin have been reported to be potent inhibitors of cell proliferation (Fotsis *et al.* 1997). Wogonin, baicalein, and baicalin have been shown cytotoxic to various human tumor cell lines *in vitro* and inhibit tumor growth *in vivo* (Ueng *et al.* 2001). Quercetin and apigenin have been found to inhibit melanoma growth and influence the invasive and metastatic potential in mice (Caltagirone *et al.* 2000). High intake of quercetin has been reported to reduce the risk of lung cancer (Knekt *et al.* 1997). Flavonoids have been shown to suppress reactive oxygen species that cause cellular damage, which can subsequently induce mutations (Verma & Pratap 2010). In addition, it has been reported that flavonoids can inhibit

angiogenesis. However, the mechanism behind this antiangiogenetic effect of flavonoids is unclear (Fotsis *et al.* 1997).

1.5 Antithrombogenic effects

Quercetin, kaempferol, and myricetin have been shown to be effective inhibitors of platelet aggregation in animals (Osman *et al.* 1998). Platelet aggregation contributes to both the development of atherosclerosis and acute platelet thrombus formation, which causes the narrowing of arteries. Flavonols were shown to be antithrombotic because they directly scavenge free radicals, thereby maintaining proper concentrations of endothelial prostacyclin and nitric oxide (Gryglewski *et al.* 1987). Flavone-8-acetic acid profoundly reduces platelet-dependent thrombosis and vasoconstriction after deep arterial injury *in vivo* (Mruk *et al.* 2000).

1.6 Antiviral effects

The antiviral activity of flavonoids was shown in a number of studies. Flavonoid derivatives modified from morin and quercetin were showed to have inhibitory effects on the Epstein-Barr virus early antigen (EBV-EA) activation by a short-term *in vitro* assay (Iwase *et al.* 2001). Kaul *et al.* reported the effect of some dietary flavonids on the infectivity and replication of herpes simplex virus type 1 (HSV-I), polio-virus type 1, parainfluenza virus type 3 (Pf-3), and respiratory syncytial virus (RSV). Quercetin has also been reported to exhibit both antiinfective and antireplicative abilities against those viruses (Kaul *et al.* 1985). Baicalin inhibits HIV-1 replication in peripheral blood mononuclear cells (Kitamura et al. 1998). Tan *et al.* recently reported that panduratin A and its derivatives exhibited good competitive inhibitory activities towards dengue 2 virus NS3 serine protease (Tan *et al.* 2006). Glaranine and 7-*O*-methylglabranine have also been shown to inhibit dengue viral

growth at a concentration of 25µM (Sanchez *et al.* 2000). Poncirin, rhoifolin, naringin and marmesin, isolated from *Poncirus trifoliate* have been shown to be effective mosquito repellent for up to 60 hours (Rajkumar *et al.* 2008). However, most studies of the effects on viruses were performed *in vitro* and little is known about the antiviral effect of flavonoids *in vivo* (Nijveldt *et al.* 2001).

1.7 Scope and objectives of this thesis

Flavonoids have been the subject for medical research over the past 20 years and a variety of potential beneficial effects have been identified. However, most of the flavonoid compounds isolated from nature sources are invariably so small that are unlikely to provide enough material for drug development studies. Isolation of pure flavonoid compounds from natural products is very difficult and often costly. Synthesis is an alternative route to acquiring these compounds. Elaboration of synthetic approach toward bioactive flavonoid compounds allows the preparation of structural diverse derivatives and their analogues in reasonable quantities, which may leads ultimately to the rational design of a better drug candidate. Therefore, in our effort to develop effective synthetic routes to the bioactive flavonoids, the present study was conducted with the following objectives:

1. To rationally design and synthesise bioactive flavonoids with potential muscarinic receptor binding activity

2. To develop an efficient one-pot synthesis of flavones

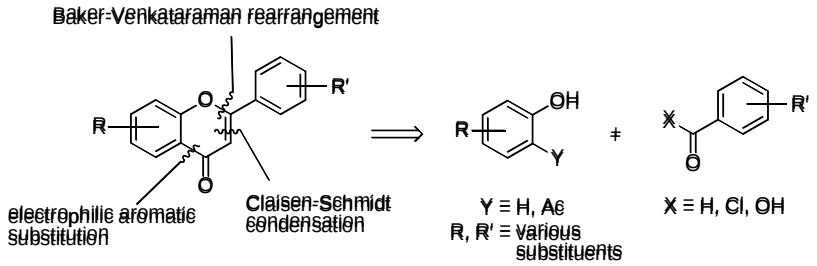
2. To synthesise panduratin A and isopanduratin A

3. To synthesise kuwanon V and dorsterone methyl esters

Chapter 2 gives an overview of the most relevant synthetic routes and methods that have been applied to the synthesis of flavonoids. The development of an efficient one-pot synthesis of flavones is elaborated in Chapter 3. The syntheses of flavonoids analogues, panduratin A and isopanduratin A are described in Chapters 4 and 5, respectively. Chapter 6 describes the synthesis of kuwanon V and dorsterone methyl esters.

Chapter 2: Synthetic routes to the flavonoids

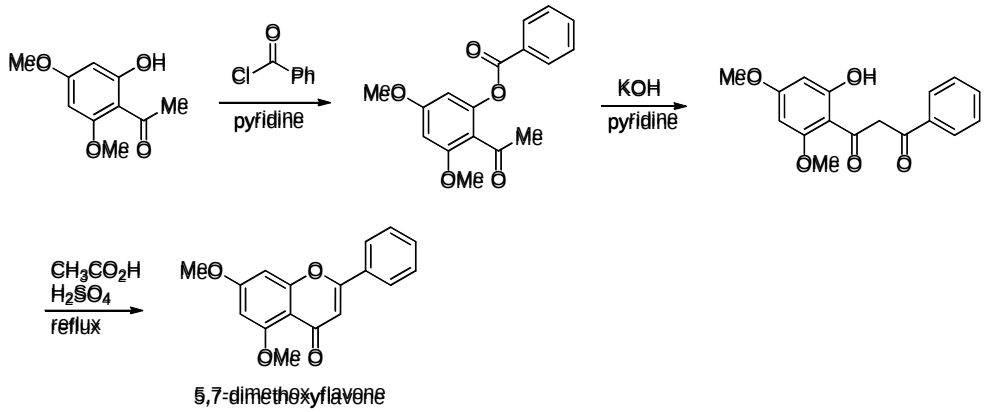
The synthesis of flavonoid compounds in particular, flavones, has been well-reported in the literature. Retrosynthetic analysis of flavone presented three primary disconnections that provided ample opportunity for diversification without a lengthy protection-deprotection strategy (Scheme 2.1).



Scheme 2.1. Retrosynthetic analysis of flavones

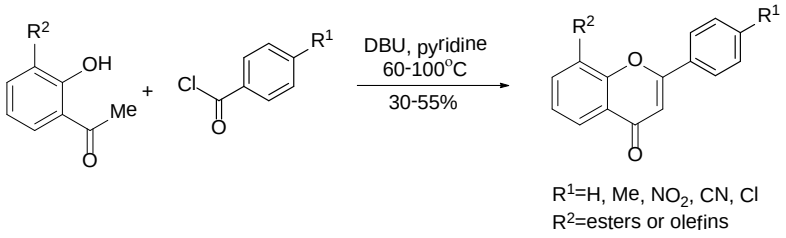
2.1 Baker-Venkataraman synthesis

The most common method to prepare flavones is though the Baker-Venkataraman synthesis (Baker, 1933; Mahal and Venkataraman, 1934). In this process, a 2′-hydroxyacetophenone is first converted into a benzoyl ester, which is then treated with a strong base such as KOH to give a 1,3-diphenylpropane-1,3-dione. Treatment of this diketone with glacial acetic acid containing a catalytic amount of sulphuric acid leads to the generation of a desired flavone. Scheme 2.2 illustrates an application of the Baker-Venkataraman method to synthesise 5,7-dimethoxyflavone**.** Despite moderate yields (50-60%) and incompatibility of sensitive substituents, the Baker-Venkataraman method is simple and convenient and can be used in large scale pharmaceutical production.



Scheme 2.2. Baker-Venkataraman synthesis of 5,7-dimethoxyflavone

Recently, the experimental conditions for the Baker-Venkataraman reaction have been improved to allow the synthesis of flavones to be carried out in one-pot. Riva and co-workers have found that by heating 2′-hydroxyacetophenone and acyl chloride in the presence of DBU in dry pyridine, the corresponding flavones could be obtained in reasonable yield (Scheme 2.3) (Riva, *et al.* 1997).

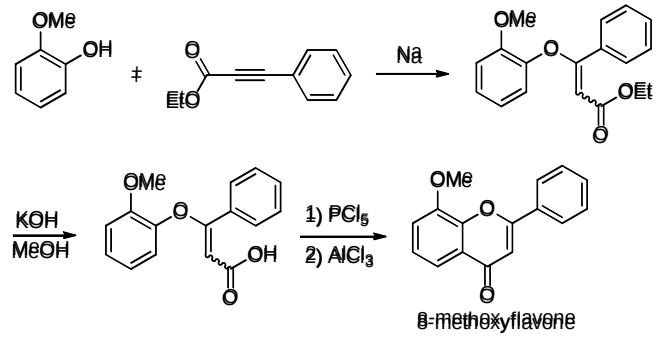


Scheme 2.3. A modified Baker-Venkataraman one-pot synthesis of flavones

2.2 Ruhemannz process

The Ruhemannz process, as outlined in Scheme 2.4, has been utilised to synthesize the A-ring-substituted flavones. In this process, conjugate addition of the sodium salt of a substituted phenol to ethyl phenylpropiolate produced the unsaturated ester as an (*E*)- & (*Z*)-mixture of regioisomers. The ester was then subjected to saponification and intramolecular Friedel-Crafts acylation to give the desired flavone.

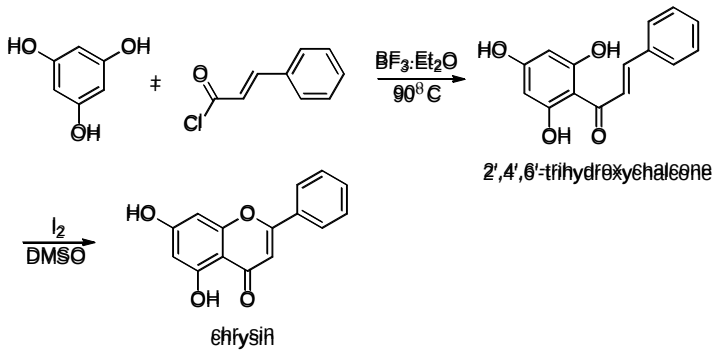
Ruhemannz utilized this procedure to prepare 8-methoxyflavone (Ruhemannz, 1900; 1913).



Scheme 2.4. Ruhemannz synthesis of 8-methoxyflavone

2.3 Friedel-Crafts reaction

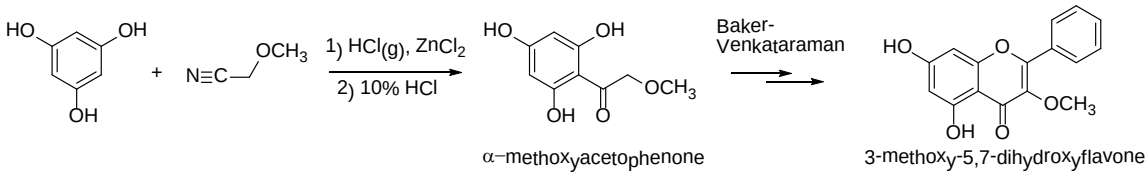
One of the more convenient route to prepare flavones is via a Friedel-Crafts reaction. The reaction first affords a chalcone following an electrophilic substitution of a phenolic compound with cinnamoyl chloride or cinnamic acid anhydride in the presence of a Lewis acid catalyst. The chalcones are then refluxed in I_2/DMSO to give the flavones in relatively good yields (60-98%) (Scheme 2.5). Conventional Lewis acids such as $AlCl_3$, BF_3, & $ZnCl_2$ and new generations of environmental friendly catalysts such as K-10 and beta zeolite have been used in this reaction (Kantam, *et al*. 2005).



Scheme 2.5. Synthesis of chrysin via a Friedel-Craft reaction

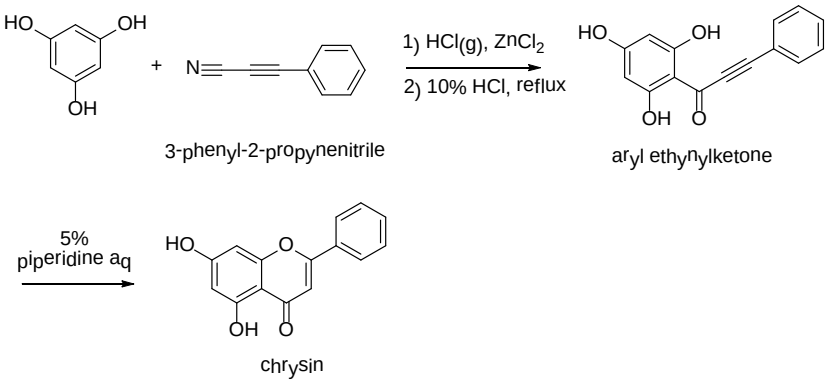
2.4 Houben-Hoesch reaction

In another approach, the electrophilic aromatic substitution of a phenolic compound with an aryl nitrile using Houben-Hoesch conditions provides the precursor to prepare 3-methoxyflavones (Hoesch, 1915; Houben, 1926). In this reaction, a α-methoxyacetophenone is prepared from phloroglucinol and methoxyacetonitrile, which is then subjected to Baker-Venkataraman reaction to give 3-methoxy-5,7-dihydroxyflavone **3** (Scheme 2.6).



Scheme 2.6. Synthesis of 3-methoxyflavone via a Houben-Hoesch reaction

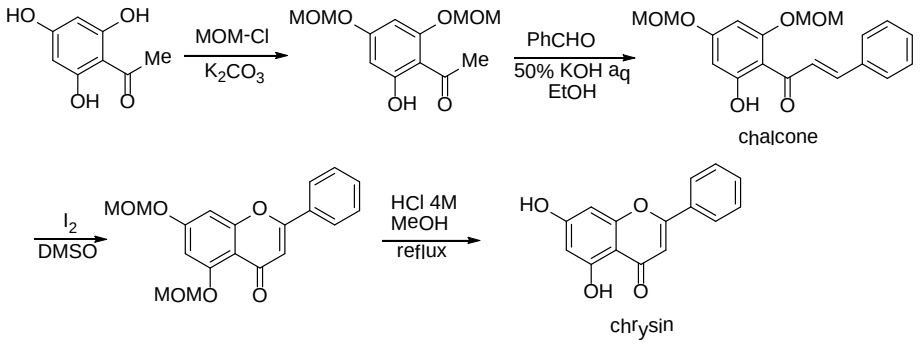
A variety of nitriles can be used in the Houben-Hoesch reaction. As outlined in Scheme 2.7, chrysin has been synthesised via Houben-Hoesch reaction involved the coupling of phloroglucinol and 3-phenyl-2-propynenitrile to afford an aryl ethynylketone, which was cyclised in aqueous piperidine to give chrysin (10%) (Tan, 2008).



Scheme 2.7. Synthesis of chrysin via a Houben-Hoesch reaction

2.5 Claisen-Schmidt condensation

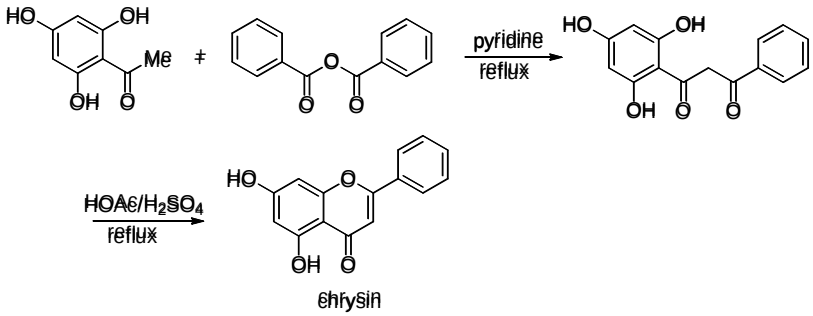
Flavone can be prepared from chalcone, an important precursor which is widely found in natural products. Chalcones can be synthesised via two well-established methods; *i.e.*, through a base-catalysed Claisen-Schmidt condensation or acid mediated aldol reaction of 2′-hydroxyacetophenone and benzaldehyde (Marais, 2005; Narender and Papi Reddy 2007). Treatment of 2′-hydroxyacetophenone and benzaldehyde in the presence of 50% potassium hydroxide in ethanol for 24 hours produced 2′-hydroxychalcone that can be subsequently cyclised to give the flavone (Scheme 2.8). Protection of 2′-hydroxyl group of acetophenone is necessary to prepare 5,7-dihydroxyflavone. However, the low yields obtained in the conversion of the chalcone to the flavone and formation of undesired side products limit the application of Claisen-Schmidt reaction in the synthesis of flavones.



Scheme 2.8. Synthesis of chrysin via a protected chalcone

2.6 Allan-Robinson synthesis

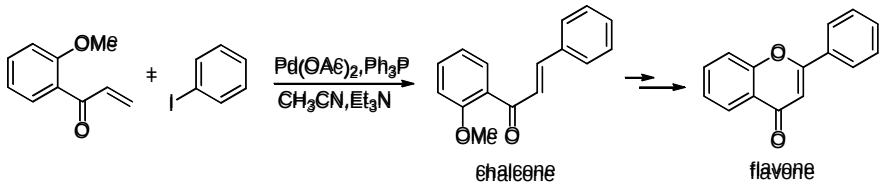
Allan-Robinson synthesis or Konstanecki reaction involves reaction of 2′-hydroxyacetophenone and benzoic acid anhydride in hot pyridine to give a 1, 3-diketone as the key intermediate, and subsequent cyclisation in acidic medium gave the flavones (Scheme 2.9). However, very few studies have been done using this method thus far (Allan and Robinson, 1924).



Scheme 2.9. Allan-Robinson Synthesis of chrysin

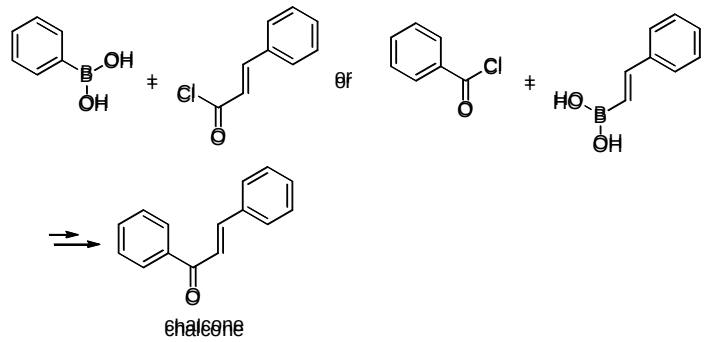
2.7 The Heck and Suzuki coupling reactions

The Heck and Suzuki palladium-catalysed cross coupling reactions have been used to synthesise chalcones. In the Heck reaction, an aryl vinyl ketone is first coupled with an aryl iodine in the presence of $Pd(OAc)_2$, PPh_3, triethylamine and acetonitrile to give the corresponding chalcone, which is then cyclised to give the flavone (60-85%) (Scheme 2.10). A drawback of this reaction is the requirement for hydroxyl group protection.



Scheme 2.10. Synthesis of flavone via a Heck coupling reaction

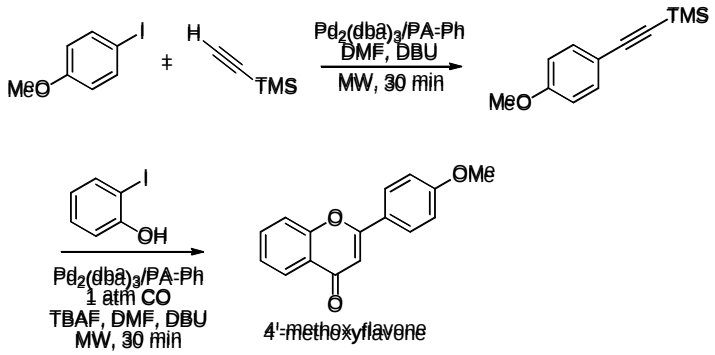
The Suzuki coupling reaction (Scheme 2.11) involves phenylboronic acid and benzoyl chloride in the presence of a base and palladium catalyst (Haddach and McCarthy 1999). The coupling reaction can be performed on cinnamoyl chloride and phenylboronic acid or benzoyl chloride with phenylvinylboronic acid (Eddarir, *et al.* 2003).



Scheme 2.11. Synthesis of chalcone via a Suzuki coupling reaction

2.8 Sonogashira-Carbonylation-Annulation reaction

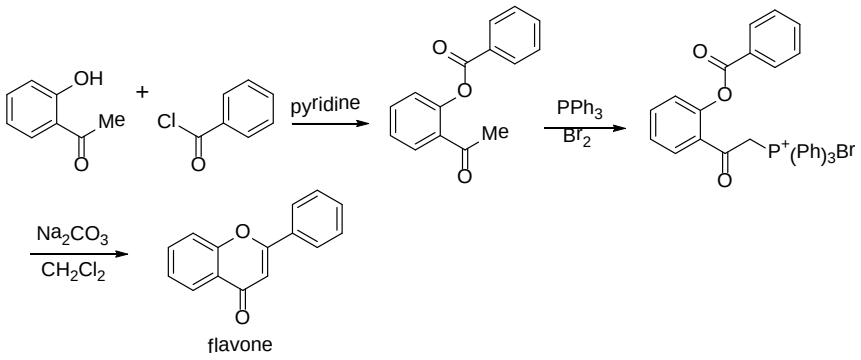
Another attractive approach to prepare flavones is via the Pd catalyzed Sonogashira-Carbonylation-Annulation reaction between 2-iodophenols and terminal alkynes as reported by Awuah and Capretta (Awuah and Capretta 2009). They first generated an aryl alkyne via a microwave-assisted Sonogashira reaction, which is then converted to the flavones through palladium catalysed carbonylation-annulation with 2-iodophenol (81-92% yields) (Scheme 2.12).



Scheme 2.12. Synthesis of 4′-methoxyflavone via a Sonogashira-Carbonylation-Annulation reaction

2.9 Intramolecular Wittig reaction

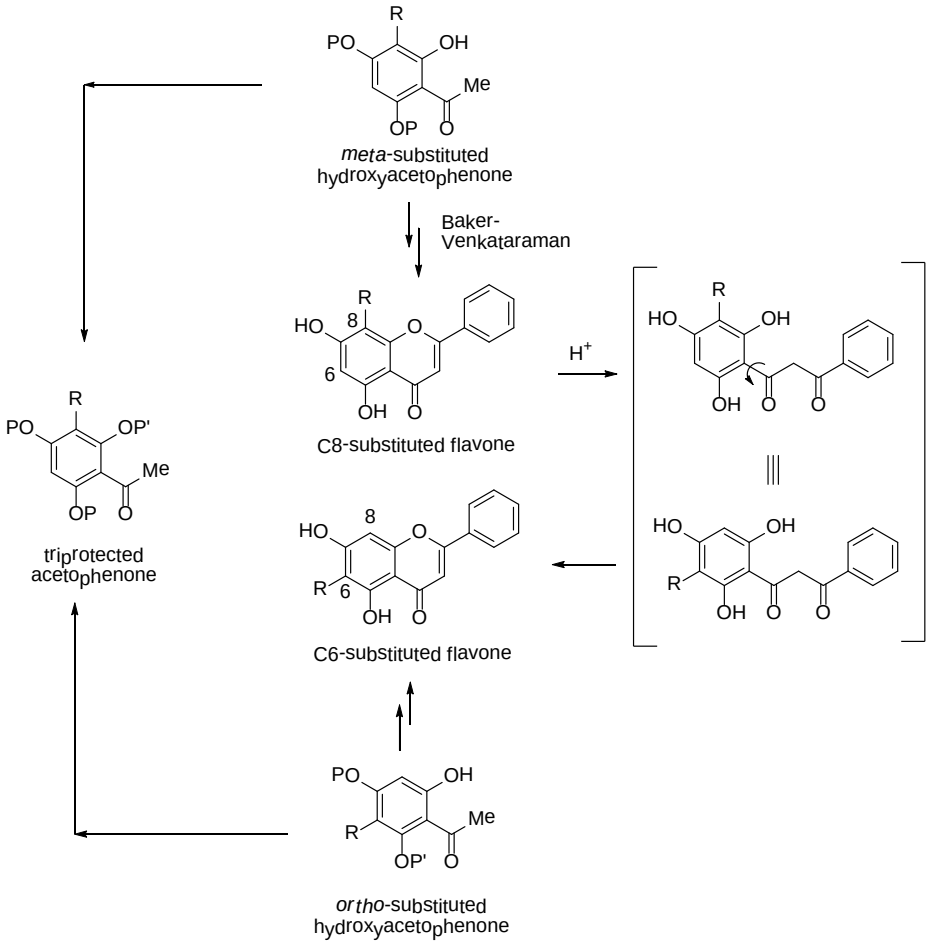
The Wittig reaction has not been extensively employed in the synthesis of flavones. The reaction utilizes 2′-hydroxyacetophenone as the starting material (Scheme 2.13). Acylation of the acetophenone with benzoyl chloride followed by reaction with bromine triphenylphosphine gives the corresponding phosphonium bromide, which then undergoes ring closure via intramolecular olefination of ester carbonyl group to afford the flavone (55-80%) (Cotelle, N. 2001).



Scheme 2.13. Synthesis of flavone via a Wittig reaction

2.10 Wessely-Moser Rearrangement of Flavones

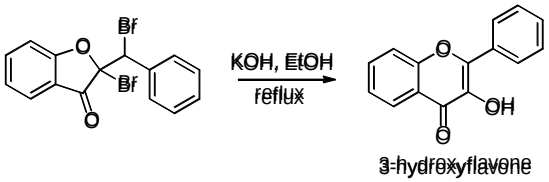
The Wessely-Moser rearrangement interconverts C-8- and C-6-substituted flavones through a diaroylmethane intermediate that can freely rotate around the C-4a/C-7 axis. The C-8- and C-6-substituted flavones are available by the Baker-Venkataraman synthesis from their corresponding protected, *meta*-substituted hydroxyacetophenone. This operation translates differences in terms of relative orientation of the free hydroxyl and the substituent (*ortho* and *para*) at the acetophenone stage into a C-6 or a C-8 flavone substitution pattern (19-31% overall yield) (Scheme 2.14). (Wessely and Moser 1930; Minassi *et al.* 2008)



Scheme 2.14. Wessely-Moser rearrangement of flavones and regiodivergent synthesis of 8- and 6-substituted flavones (P and P′ = protecting groups)

2.11 Auwers flavone synthesis

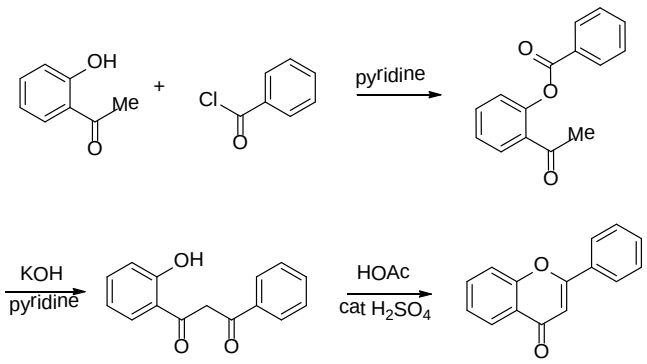
The Auwers flavones synthesis involves treatment of a dibromoaurone with alcoholic alkali to give flavonol or 3-hydroxyflavone (Scheme 2.15). The formation of flavonol is dependant upon the position of the substituent on the coumarone ring. When a methyl or methoxy group is *meta*-substituted to the coumarone ring oxygen, flavonol formation is hindered, whilst methyl, methoxy, and chlorine substituents at the *ortho* and *para* positions are conducive to flavonol formation (Auwers, 1908).



Scheme 2.15. Auwers 3-hydroxyflavone synthesis

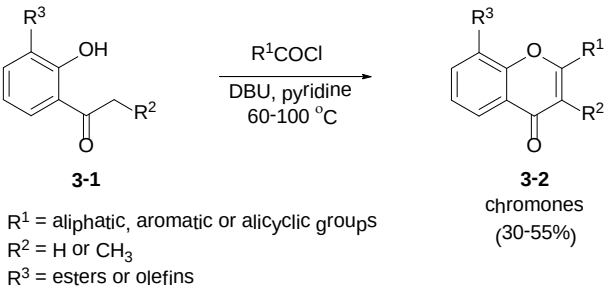
Chapter 3: An efficient one-pot synthesis of flavones

As mentioned in Chapter 2, the Baker-Venkataraman method is one of the most practical methods for the preparation of flavones. In this three-step method (Scheme 3.1), a 2′-hydroxyacetophenone is first converted to the benzoyl ester which is then treated with a base to induce the Baker-Venkataraman rearrangement to give the β-diketone. Finally, the β-diketone is cyclised by heating it in glacial acetic acid containing a catalytic amount of sulphuric acid to give the flavone (Baker, 1933; Mahal and Venkataraman, 1933).



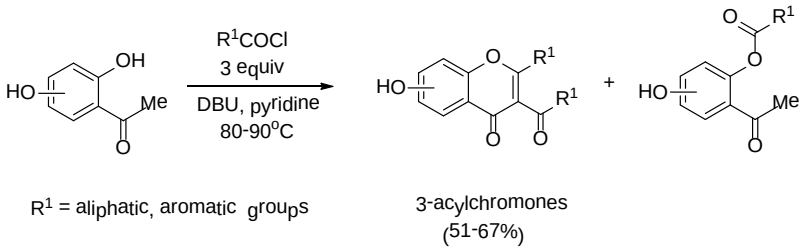
Scheme 3.1. Conventional Baker-Venkataraman synthesis of flavones

Recent reports on one-pot syntheses of flavones using modified Baker-Venkataraman reactions have caught our attention. In particular, Riva *et al.* (1997) found that heating 2′-hydroxyacetophenone **3-1** and an equivalent amount of acyl chloride in the presence of two equivalents of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dry pyridine produced the corresponding chromones **3-2** in reasonable yields (Scheme 3.2).



Scheme 3.2. Modified Baker-Venkataraman method reported by Riva *et al.* 1997

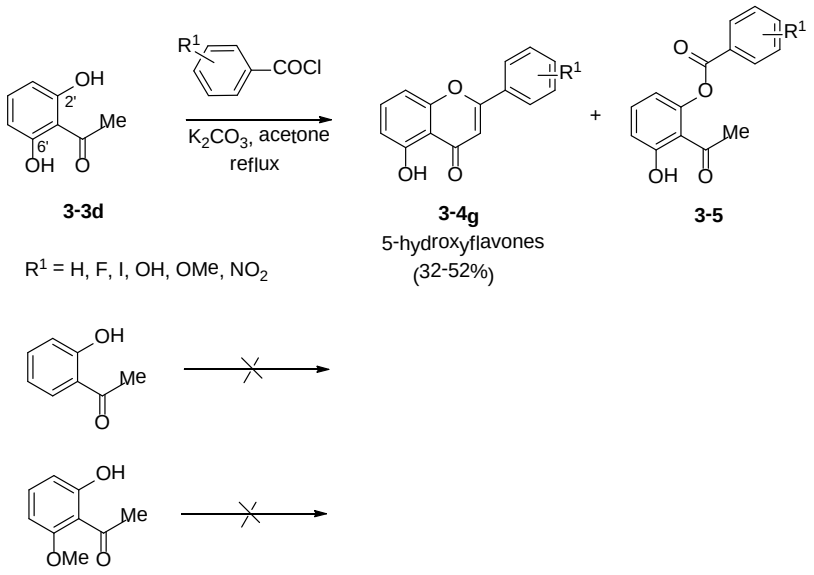
Ganguly *et al.* (2005, 2006) extended this work using three equivalents of both the acyl chloride and DBU. However, this reaction did not yield the expected chromones; instead they obtained 3-acylchromones, together with the phenolic esters in some instances (Scheme 3.3).



Scheme 3.3. Modified Baker-Venkataraman method reported by Ganguly *et al.* 2005, 2006.

In another approach, Boumendjel *et al.* (1999) heated 2′,6′-dihydroxyacetophenone **3-3d** with an equivalent of benzoyl chloride in the presence of potassium carbonate in dry acetone, to obtain 5-hydroxyflavone **3-4g**, together with a small amount of unreacted acetophenone and the corresponding phenolic ester **3-5** (Scheme 3.4). However, acetophenones with no OH group or with a masked OH group at the 6′-position, when subjected to the same experimental conditions, did not give flavones.

Intrigued by the above observations, we decided to screen a variety of parameters for the reaction of 2′-hydroxyacetophenone **3-3a** and benzoyl chloride, including the use of different solvents, bases, and temperatures (Table 3.1).

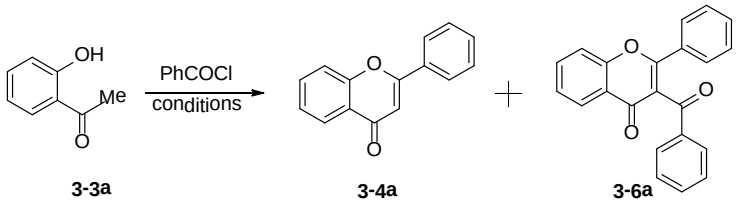


Scheme 3.4 Modified Baker-Venkataraman method reported by Boumendjel *et al.* 1999

Under the best conditions that we have tested, excess benzoyl chloride led to the formation of 3-benzoylflavone **3-6a** as the major product (Table 3.1, entries 4-6 and 11), while the use of a stoichiometric amount of benzoyl chloride gave only flavone **3-4a** (entries 3 and 10). In the absence of a base (entries 1 and 2) or when triethylamine (Et_3N) was used in conjunction with N,N'-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in dichloromethane (entry 7), only the ester 2′-benzoyloxyacetophenone was obtained. Employing the stronger bases potassium *tert*-butoxide (KO*t*Bu/THF, entry 8) or sodium hydride (NaH/THF, entry 9) led to formation of the intermediate β-diketone, 1-(2-hydroxyphenyl)-3-phenyl-1,3-propanedione. A surprising change was observed when 2′-hydroxyacetophenone was heated with excess benzoyl chloride in an open K_2CO_3/acetone system (entry 12): the yield of flavone **3-4a**

increased to 65% compared to 12% when the reaction was conducted under nitrogen (entry 11).

Table 3.1. Screening of the reaction conditions[a]



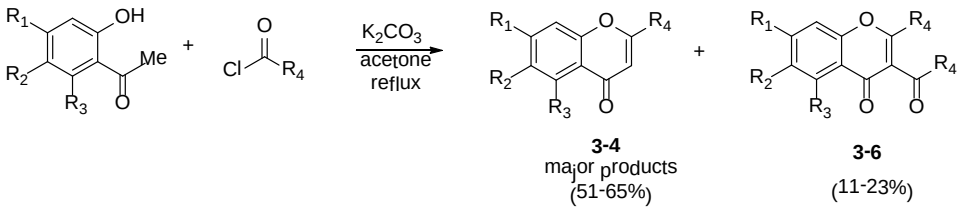
3-3a **3-4a** **3-6a**

Entry	Base (equiv)	Solvent	Temp (°C)	Benzoyl chloride (equiv)	Product; Yield[b] (%)
1	-	pyridine	110	1	[d]
2	-	pyridine	110	3	[d]
3	DBU (3)	pyridine	110	1	**3-4a** (25)
4	DBU (3)	pyridine	110	3	**3-6a** (55)
5	KOH (3)	pyridine	110	3	**3-6a** (50)
6	K_2CO_3 (10)	pyridine	110	3	**3-6a** (40)
7	Et_3N (5)[c]	CH_2Cl_2	rt	3	[d]
8	KO*t*Bu (3)	THF	-78 to rt	3	[e]
9	NaH (3)	THF	-78 to rt	3	[e]
10	K_2CO_3 (10)[f]	acetone	60	1	**3-4a** (5)
11	K_2CO_3 (10)	acetone	60	3	**3-4a** (12), **3-6a** (47)
12	K_2CO_3 (10)[f]	acetone	60	3	**3-4a** (65), **3-6a** (20)

[a] All reactions were allowed to run for 24 h using acetophenone (1 mmol) under an N_2 atmosphere (except where stated below).
[b] Isolated yield calculated from 2′-hydroxyacetophenone.
[c] In the presence of DCC (2 equiv) and DMAP (2 equiv).
[d] Only formation of the ester 2′-benzoyloxyacetophenone was observed.
[e] Only formation of the β-diketone 1-(2-hydroxyphenyl)-3-phenyl-1,3-propanedione was observed.
[f] The reaction was carried out in an open atmosphere.

Using the conditions described in entry 12, Table 3.1, we performed reactions using various combinations of 2′,4′-dihydroxy-, 2′,5′-dihydroxy-, 2′,6′-dihydroxy-, and 2′,4′,6′-trihydroxyacetophenones and acyl chlorides **3-7a**~**3-7e**, and obtained flavones **3-4a~3-4i** (51%-65%) and 3-acylflavones **3-6a~3-6h** (11%-23%) (Scheme 3.5). In contrast to the findings of Boumendjel (Bois, 1999), our results observed suggested that the presence of an additional OH group at the 6′-position of the 2′-hydroxyacetophenone was not a requirement for the formation of flavones. Table 3.2

shows the different flavones and 3-aroylflavones prepared by this method and the yields obtained.



3-3a $R^1 = R^2 = R^3 = H$
3-3b R^1= OH, $R^2 = R^3 = H$
3-3c $R^1 = R^3 = H$, R^2 = OH
3-3d $R^1 = R^2 = H$, R^3 = OH
3-3e $R^1 = R^3$ = OH, R^2= H

3-7a $R^4 = C_6H_5$-
3-7b R^4 = 4-$OCH_3C_6H_4$-
3-7c R^4 = 4-ClC_6H_4-
3-7d $R^4 = C_2H_5$-
3-7e $R^4 = OCH_3C_2H_4$-

3-4a and **3-6a** $R^1 = R^2 = R^3 = H$, $R^4 = C_6H_5$-
3-4b and **3-6b** R^1 = OH, $R^2 = R^3 = H$, $R^4 = C_6H_5$-
3-4c and **3-6c** R^1 = OH, $R^2 = R^3 = H$, R^4 = 4-$OCH_3C_6H_4$-
3-4d and **3-6d** R^1 = OH, $R^2 = R^3 = H$, R^4 = 4-ClC_6H_4-
3-4e and **3-6e** $R^1 = R^3 = H$, R^2 = OH, $R^4 = C_6H_5$-
3-4f and **3-6f** $R^1 = R^2 = H$, R^3 = OH, $R^4 = C_6H_5$-
3-4g and **3-6g** $R^1 = R^3$ = OH, $R^2 = H$, $R^4 = C_6H_5$-
3-4h and **3-6h** $R^1 = R^2 = R^3 = H$, $R^4 = C_2H_5$-
3-4i $R^1 = R^2 = R^3 = H$, $R^4 = OCH_3C_2H_4$-

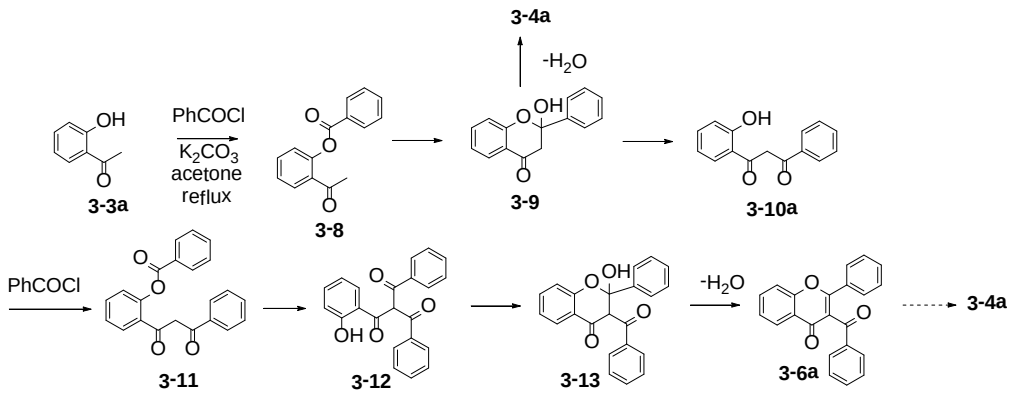
Scheme 3.5. Yields of flavones obtained via one-pot Baker-Venkataraman method

Table 3.2. Yields and melting points of the flavones **3-4** and 3-acylflavones **3-6**.[a]

Entry	Flavone (yield %)[b]	m.p. (°C) found	m.p. (°C) reported	3-Aroylflavone (yield %)[b]	m.p. (°C) found	m.p. (°C) reported
1	**3-4a** (65%)	99	99[c]	**3-6a** (20 %)	132	121-122[d]
2	**3-4b** (55%)	243	241-242[d]	**3-6b** (19 %)	265	270-271[d]
3	**3-4c** (63%)	263	260-26[e]	**3-6c** (12 %)	amorphous	-
4	**3-4d** (60%)	280	268-270[e]	**3-6d** (16 %)	amorphous	-
5	**3-4e** (51%)	232	235.5-236.5[f]	**3-6e** (23 %)	162	-
6	**3-4f** (58%)	160	158-159[g]	**3-6f** (11 %)	174	177-178[g]
7	**3-4g** (53%)	285	284-285[d]	**3-6g** (19 %)	190	193-194[d]
8	**3-4h** (50%)	oil	-	**3-6h** (5%)	oil	-
9	**3-4i** (59%)	oil	-	-		

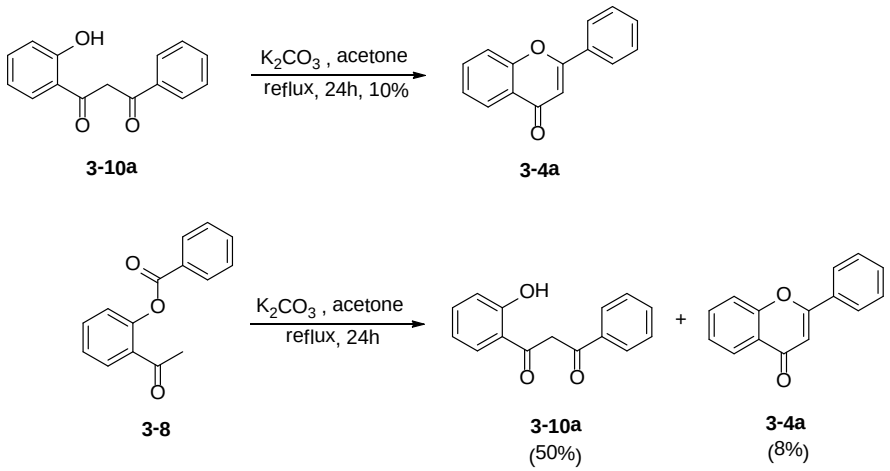
[a] All products were identified by ^{1}H and ^{13}C-NMR spectroscopy; [b] Isolated yield calculated from the corresponding acetophenone;
[c] Jain *et al.* 1982; [d]Ganguly *et al*. 2005; [e]Costantino *et al*. 1996; [f]Looker *et al.* 1962, 381; [g]Looker *et al*. 1962, 3261.

The proposed mechanism for the formation of flavone **3-4a** and 3-benzoylflavone **3-6a** is shown in Scheme 3.6. When 2′-hydroxyacetophenone **3-3a** was treated with benzoyl chloride and K_2CO_3, addition of the first equivalent of benzoyl chloride produced 2′-benzoyloxyacetophenone **3-8**. In the presence of a base, the enolate of the acetyl group is formed and attacked the carbonyl of the ester to give the hemiacetal **3-9**. This hemiacetal may either dehydrate to produce flavone **3-4a** or undergo ring opening to give the β-diketone **3-10a**, the Baker-Venkataraman rearrangement product. In an excess of benzoyl chloride, the phenolic group of β-diketone **3-10a** underwent esterification with another equivalent of benzoyl chloride to form benzoyloxydiketone **3-11**. Rearrangement of the benzoyloxydiketone **3-11** gave triketone intermediate **3-12** which finally cyclodehydrated to produce 3-benzoylflavone **3-6a** via hemiacetal **3-13**.



Scheme 3.6. Proposed mechanism for the formation of flavone and 3-benzoylflavone

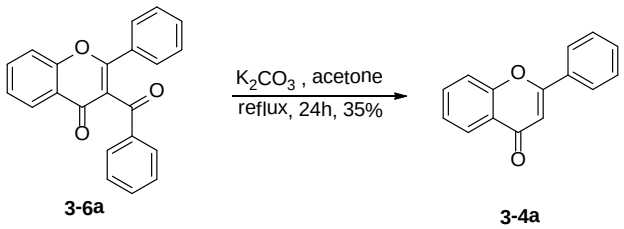
While it is known that β-diketones such as **3-10a** are readily cyclised by heating in strongly acidic medium such as (HOAc/cat.H_2SO_4) or $BF_3{\cdot}Et_2O$, cyclisation under basic conditions is uncommon, although it is known to occur in methanolic KOH (Baker, 1933). However, we observed the formation of flavones during the one-pot Baker-Venkataraman reaction in the K_2CO_3/acetone system even without the acidification of the crude product in the work-up. The presence of a flavone in the reaction mixture was further verified by GC-MS, whereby portions were sampled and the molecular weight of each compound was analysed. The results revealed that compounds **3-4a**, **3-6a**, **3-8**, **3-10a** and **3-11** all existed during the course of the reaction, but it was unclear whether the formation of flavone **3-4a** occurred via the cyclisation of 2′-benzoyloxyacetophenone **3-8** or β-diketone **3-10a** or from cleavage of the benzoyl group from 3-benzoylflavone **3-6a**.



Scheme 3.7. Control experiments of **3-10a** and **3-8** in K_2CO_3/acetone system

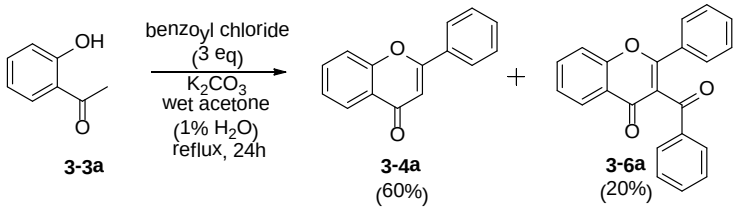
A control experiment was therefore performed by heating β-diketone **3-10a** in a K_2CO_3/acetone system in the absence of benzoyl chloride and the reaction was again monitored by GC-MS. The result revealed that β-diketone **3-10a** slowly cyclised to form flavone **3-4a** (10% yield after treatment for 24 h). When the control experiment

was performed on ester **3-8** instead, GC-MS showed the formation of β-diketone **3-10a** and flavone **3-4a** (Scheme 3.7). Thus, cyclisation of β-diketones under basic conditions was possible, but only occured slowly. Hence the formation of flavone **3-4a** in low yield in reactions using one equivalent of benzoyl chloride can be explained (Table 3.1, entries 3 and 10). However, this explanation is not sufficient to account for the higher yields of flavone that were obtained using three equivalents of benzoyl chloride in the open K_2CO_3/acetone system. Another control experiment was therefore performed by heating 3-aroylflavone **3-6a** in a K_2CO_3/acetone system to afford a 35% yield of flavone **3-4a** (Scheme 3.8). Presumably the open K_2CO_3/acetone system absorbed moisture from the air which enabled cleavage of the 3-aroyl moiety (compare entries 11 and 12 in Table 3.1). It was later confirmed that the one-pot reaction of 2′-hydroxyacetophenone and benzoyl chloride in the presence of K_2CO_3 in wet acetone (containing water 1% $^{v}/_{v}$) produced similar yield of flavone (Scheme 3.9). Furthermore, using the same conditions on the crude product, obtained from the initial reaction between acetophenone and aroyl chloride after evaporation of the acetone solvent, we were able to increase the yield of the 3-unsubstituted flavone at the expense of the 3-aroylflavone.



3-6a **3-4a**

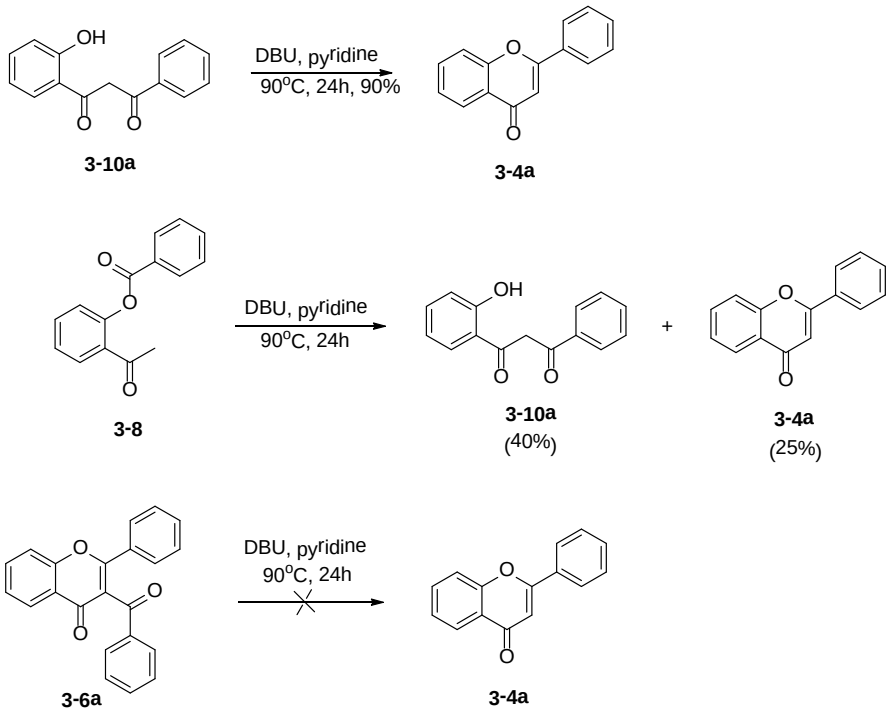
Scheme 3.8. Control experiment of **3-6a** in K_2CO_3/acetone system



3-3a **3-4a** (60%) **3-6a** (20%)

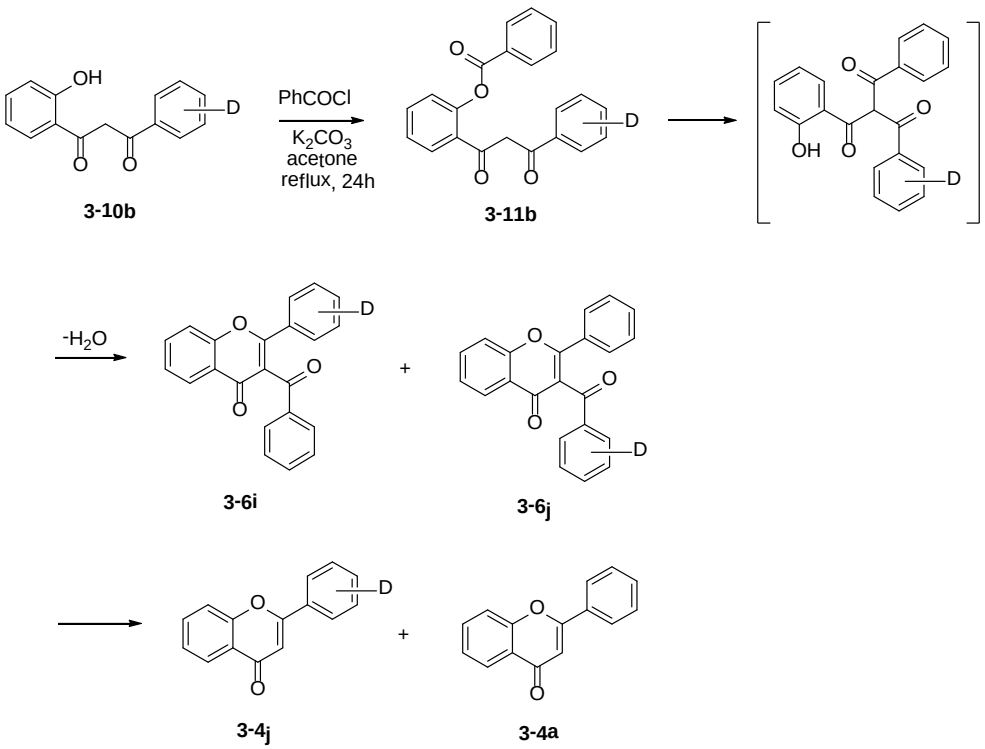
Scheme 3.9. One-pot synthesis of **3-4a** in wet acetone (1% $^{v}/_{v}$ H_2O)

A higher yield of flavone was observed when the same experiments were conducted in the presence of DBU in dry pyridine. Attempt to remove the 3-aroyl moiety of **3-6a** in DBU/pyridine was unsuccessful (Scheme 3.10).



Scheme 3.10. Control experiments of **3-6a**, **3-8**, and **3-10a** in DBU/pyridine

In another experiment, we found that heating the deuterated β-diketone **3-10b** and an equivalent amount of benzoyl chloride in K_2CO_3/acetone gave deuterated 3-benzoylflavones **3-6i** and **3-6j** which were deacylated under the one-pot conditions to give a mixture of deuterated flavones **3-4j** and flavone **3-4a** (Scheme 3.11). The ratio of flavones **3-4j**: **3-4a** was quantified as 1:1 based on the ^{1}H NMR analysis. However, a crystal structure of deuterated benzoyloxydiketone **3-11b** obtained from the reaction (Figure 3.1) indicated that acylation of β-diketone first occured on the phenolic group rather than on methylene carbon atom, which is contrary to Baker's hypothesis (Baker, 1933).



Scheme 3.11. Control experiment of deuterated **3-10b** in K_2CO_3/acetone

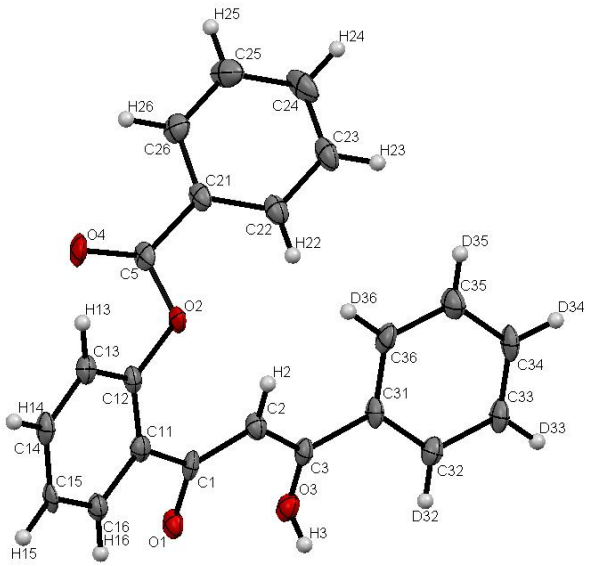
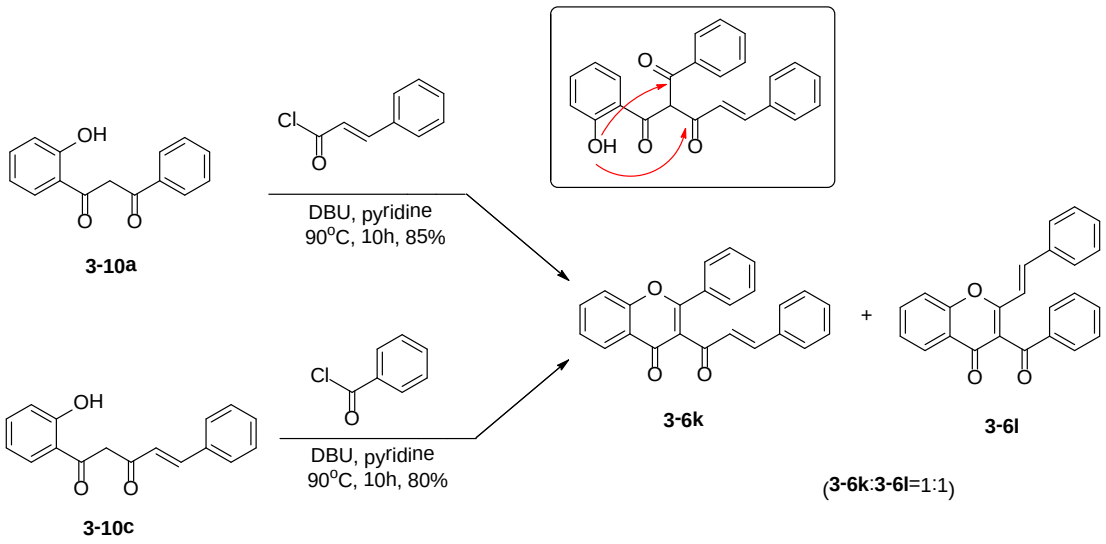


Figure 3.1. ORTEP representation of the X-ray crystal structure of 1-(2-benzoyloxyphenyl)-3-($^2H_5$)phenyl-1,3-propanedione **3-11b** with thermal ellipsoids at 50% probability. Atoms are labeled anonymously. Crystallographic data can be referred from appendix E.

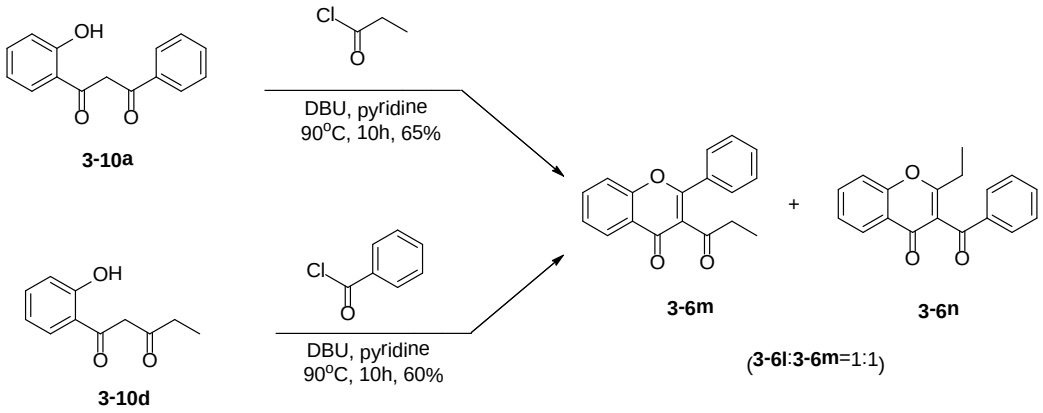
A 1:1 ratio of isomeric products **3-6k** and **3-6l** was obtained by heating either β-diketone **3-10a** with cinnamoyl chloride in pyridine or 1-(2-hydroxyphenyl)-3-styryl-1,3-propanedione **3-10c** with benzoyl chloride in pyridine (Scheme 3.12). Similarly,

products **3-6m** and **3-6n** were obtained by heating β-diketone **3-10a** with propanoyl chloride or 1-(2-hydroxyphenyl)-pentan-1,3-dione **3-10d** with benzoyl chloride in pyridine (Scheme 3.13). These findings provide evidence that the reaction does indeed proceed via a triketone intermediate which can undergo ring closure by two possible pathways (see insertion of scheme 3.12).



Scheme 3.12. Isomeric products **3-6k** and **3-6l** obtained by heating either β-diketone **3-10a** with cinnamoyl chloride or β-diketone **3-10c** with benzoyl chloride in pyridine/DBU

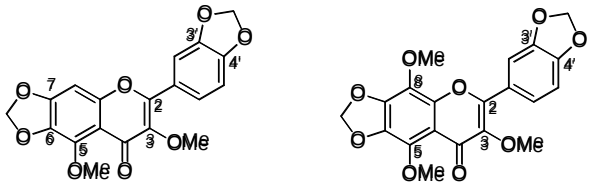
In summary, the reactions between 2′-hydroxyacetophenone and acyl chlorides under different conditions have been investigated. We found that when 2′-hydroxyacetophenone was heated with a stoichiometric amount of acyl chloride, either in a DBU/pyridine system or in an open K_2CO_3/acetone system, only the flavone was obtained, but in modest yield. However, when heated with excess of acyl chloride in a DBU/pyridine system, the 3-acylflavone was the only product, while treatment in an open K_2CO_3/acetone system afforded the flavone as the major product along with a smaller amount of 3-aroylflavone. The latter method has been successfully applied to the synthesis of flavones bearing a variety of substituents.



Scheme 3.13. Isomeric products **3-6m** and **3-6n** obtained by heating either β-diketone **3-10a** with propanoyl chloride or β-diketone **3-10d** with benzoyl chloride in pyridine/DBU

Chapter 4: Synthesis of flavonoid analogues with potential muscarinic receptor binding activity

Through a competitive [^{3}H]NMS-muscarinic receptor binding assay screening of Malaysian medicinal plant extracts, Chung and co-workers discovered meliternatin and 3,5,8-trimethoxy-3',4',6,7-bismethylenedioxyflavone from *Melicope subunifoliolata* (Figure 4.1) to be fairly potent inhibitors of muscarinic receptor, with K_i values of 2.42 and 3.19 μM, respectively (Chung *et al*., 2008). The mechanism of action of these flavonoid molecules is still unclear and their exact binding site on the protein structure has not yet been identified. In addition, structural-activity relationships for this type of compounds have not been examined. The aim of the present investigation was to explore the structural requirements for the interactions of flavones with the muscarinic M1 subtype receptor in order to ascertain potential directions for synthetic lead-optimisation studies.



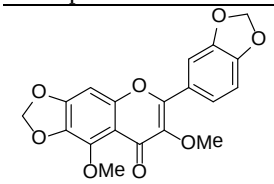
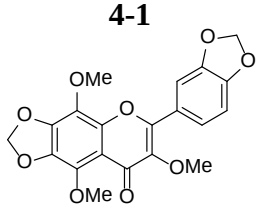
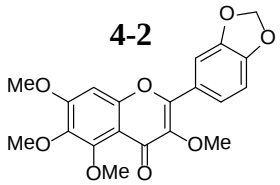
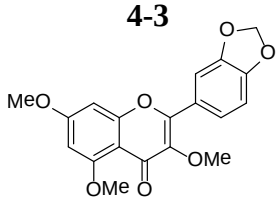
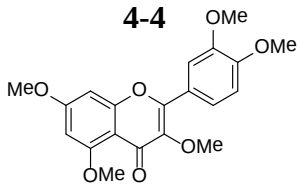
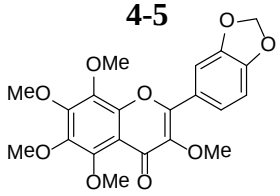
meliternatin
(*K*i = 2.42 μM)

3,5,8-trimethoxy-3',4',6,7-bismethylenedioxyflavone
(*K*i = 3.19 μM)

Figure 4.1. Meliternatin and 3,5,8-trimethoxy-3',4',6,7-bismethylene-dioxyflavone, muscarinic receptor active principle isolated from *Melicope subunifoliolata* (Chung *et al*., 2008)

Preliminary structural-activity relationship information was obtained from the six isolated flavones that bind to the muscarinic receptor (Table 4.1). Methoxy substitution caused a decrease in inhibition potency (an increase of K_i value). Subsitution of the methylenedioxy groups with methoxy groups at positions C-6/C-7 and C-3'/C-4' also led to decreases in bioactivity (Chung *et al.* 2008).

Table 4.1. IC_{50} and *K*i values for reference flavones isolated from *Melicope subunifoliolata* on muscarinic receptor binding activity

Compound	IC_{50} (M)	*K*i (μM)
 **4-1**	$2.29 \pm 0.20 \times 10^{-5}$	2.42
 **4-2**	$3.02 \pm 0.14 \times 10^{-5}$	3.19
 **4-3**	$7.41 \pm 0.07 \times 10^{-5}$	7.82
 **4-4**	$1.62 \pm 0.06 \times 10^{-4}$	17.0
 **4-5**	$4.79 \pm 0.13 \times 10^{-4}$	50.5
 **4-6**	$7.24 \pm 0.15 \times 10^{-4}$	76.5

Inhibition experiments were performed in triplicates by incubating 200 μL of rats brain membrane homogenate (36 μg protein/well) with 25 μL of [^{3}H]NMS (0.5 nM) in the presence of each compound (11 concentrations for each); the values shown for IC_{50} were the mean (n = 3) ± SEM; *K*i values were calculated using Cheng–Prusoff equation (obtaining from Chung *et al*., 2005).

Based on the preliminary SAR information, we explored our compound collection for molecules by incorporating various oxygenated (*e.g*. OH, OMe, methylenedioxy, OBz) and nitrogen containing groups. We focused on the hydroxy-, methoxy- and methylenedioxy substituted flavones and studied the influence of these

electron rich substituents on the muscarinic receptor activity. Preliminary computer modelling result (personal communication) suggested that the electron rich hydroxy and methoxy groups in the molecule could form hydrogen bonding with the amino acid residues at the muscarinic protein structure, allowing better protein-ligand interaction.

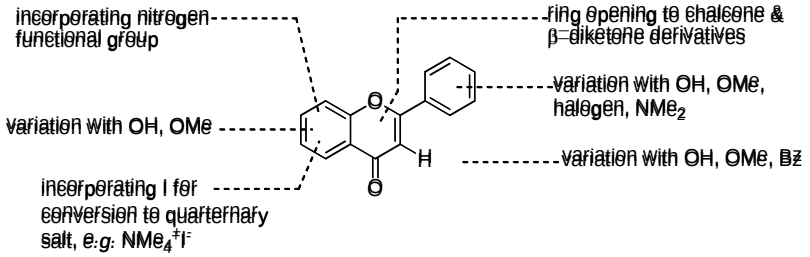
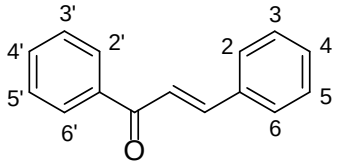


Figure 4.2. General strategy to prepare flavonoids analogues

The general strategy to further explore this template is depicted in Figure 4.2. A library of flavonoid analogues (Table 4.2-4.4 and Scheme 4.1) was prepared in the hope to gain useful information on the substitution of different functional groups and its molecular interactions with muscarinic receptor. To further build upon this knowledge, our plan was to explore the substitution of other functional groups for better bioactivity.

The substituted chalcones C1-C46 (Table 4.2) were prepared via a Claisen-Schmidt reaction except for C29, which was synthesised via a Friedel-Craft reaction. Flavones F1-F25 (Table 4.3) were constructed via a Baker-Venkaraman reaction except F17-F19, which were prepared from methylation of quercetin. Flavanones D1-D8 (Table 4.4) were prepared from the corresponding chalcones. Flavones containing nitrogen functional group N1-N4 were synthesised via a Mannich reaction (see appendix B for experimental). However, we are still waiting for the muscarinic receptor binding studies of these flavonoids compounds performed by Chung and co-workers (personal communications).

Table 4.2. Yields and melting points of chalcones synthesised



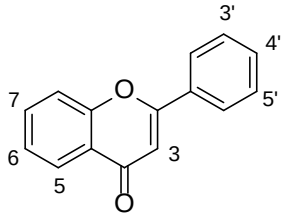
compd	C-2′	C-3′	C-4′	C-5′	C-6′	C-2	C-3	C-4	C-5	yield (%)[a]	mp (˚C) found	mp (˚C) reported
C1	H	H	H	H	H	H	H	H	H	85	55	56-57
C2	OH	H	H	H	H	H	H	H	H	78	89	81-83
C3	H	H	OMe	H	H	H	H	H	H	90	105	107-108
C4	OH	H	H	H	H	Br	H	H	H	45	104	104-105
C5	OH	H	H	H	H	H	H	Br	H	50	138	150
C6	OH	H	H	H	H	H	H	Cl	H	42	145	148-151
C7	OH	H	H	H	H	H	H	OH	H	55	160	167
C8	OH	H	H	H	H	H	H	OMe	H	55	92	92-94
C9	OH	H	H	H	H	H	H	NMe_2	H	57	170	172
C10	OH	H	H	H	H	H	H	Me	H	65	117	117-119
C11	H	Br	H	H	H	H	H	NMe_2	H	60	80	83
C12	H	Cl	H	H	H	H	H	NMe_2	H	55	85	-
C13	Cl	H	H	H	H	H	H	NMe_2	H	58	83	-
C14	H	H	OH	H	H	H	H	OH	H	49	198	197
C15	OMe	H	OMe	H	H	H	H	H	H	75	80	81
C16	OAc	H	OMe	H	H	H	H	H	H	45	68	-
C17	OAc	H	OAc	H	H	H	H	H	H	40	oil	-
C18	OH	H	OCH_2O		H	H	H	H	H	50	120	117-119
C19	OH	H	H	H	H	H	OCH_2O		H	65	138	136-138
C20	OH	H	H	H	H	H	OMe	OH	H	45	130	129

'**Table 4.2**, continued'

compd	C-2′	C-3′	C-4′	C-5′	C-6′	C-2	C-3	C-4	C-5	yield (%)[a]	mp (˚C) found	mp (˚C) reported
C21	OH	H	H	OH	H	H	H	OH	H	35	230	223-225
C22	OH	H	H	H	H	H	OH	OH	H	38	186	185-186
C23	OH	H	H	H	H	H	OMe	OMe	H	70	113	112-113
C24	OH	H	OH	H	H	H	H	OH	H	22	203	200
C25	OH	H	OH	H	H	H	H	OMe	H	25	184	188-190
C26	OMe	H	OMe	H	H	H	H	OMe	H	70	89	89
C27	OH	H	OH	H	OMe	H	H	H	H	25	205	207
C28	OMe	H	OMe	H	OMe	H	H	H	H	80	110	127-128
C29	OH	H	OH	H	OH	H	H	H	H	15[b]	177	175-176
C30	OH	H	OMe	H	OMe	H	H	H	H	65	152	152
C31	OH	H	OCH_2O		H	H	OCH_2O		H	40	186	178-180
C32	OH	H	OMe	H	OMe	Br	H	H	H	55	147	146-147
C33	OH	H	OMe	H	OMe	H	H	Br	H	50	150	150-151
C34	OH	H	OMe	H	OMe	H	H	OMe	H	62	112	113-114
C35	OH	H	OMe	H	OMe	H	H	NMe_2	H	60	122	-
C36	OH	H	OMe	H	OMe	H	H	Me	H	65	130	132-133
C37	OMe	H	OMe	H	OMe	H	H	OMe	H	75	119	113-114
C38	OH	H	H	H	H	OH	Cl	H	Cl	40	125	-
C39	OMe	Br	OMe	Br	H	H	H	OMe	H	35	89	-
C40	OH	H	H	H	H	H	OMe	OMe	OMe	62	160	160
C41	OH	H	OMe	H	OMe	H	OMe	OMe	H	50	154	149-151
C42	OH	H	OMe	H	OMe	H	OMe	OH	H	42	75	73
C43	OH	H	OMe	H	OMe	H	OCH_2O		H	60	163	163
C44	OMe	H	OMe	H	OMe	H	OMe	OMe	H	85	118	117
C45	OH	H	OMe	OMe	H	H	OMe	OMe	H	35	125	-
C46	OMe	H	OMe	OMe	H	H	OMe	OMe	H	68	150	155

a Isolated yield. All products were identified by ^{1}H and ^{13}C-NMR; *b* Prepared via a Friedel-Craft reaction.

Table 4.3. Yields and melting points of flavones synthesised



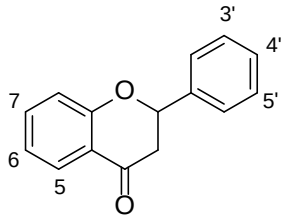
compd	C-3	C-5	C-6	C-7	C-3′	C-4′	C-5′	yield (%)[a]	mp (˚C) found	mp (˚C) reported
F1	H	H	H	H	H	H	H	68	99	99
F2	OH	H	H	H	H	H	H	54	169	170
F3	H	H	OCH_2O		H	H	H	28	208	210-212
F4	H	H	H	H	OCH_2O		H	55	202	206
F5	H	H	OCH_2O		OCH_2O		H	10	168	-
F6	H	H	OMe	OMe	H	H	H	18	188	189-190
F7	H	H	H	H	OMe	OMe	H	58	155	154-155
F8	H	OH	H	OH	H	H	H	35	285	284-285
F9	H	OH	H	OBz	H	H	H	30	154	-
F10	H	OMe	OMe	OMe	H	H	H	38	162	164-165
F11	H	H	H	H	OMe	OMe	OMe	52	175	175-176
F12	OH	H	H	H	OH	OH	H	15	300	-
F13	OH	H	H	OH	OH	OH	H	17	325	-
F14	H	H	OMe	OMe	OCH_2O		H	15	245	250
F15	H	OMe	H	OMe	OCH_2O		H	20	245	245-246
F16	H	OMe	H	OMe	OMe	OMe	H	40	190	190-194
F17	OH	OH	H	OMe	OH	OMe	H	35[b]	204	201-203
F18	OMe	OH	H	OMe	OH	OMe	H	55[b]	173	172-174
F19	OMe	OH	H	OMe	OMe	OMe	H	65[b]	160	158-159

‘**Table 4.3**, continued’

compd	**C-3**	**C-5**	**C-6**	**C-7**	**C-3′**	**C-4′**	**C-5′**	**yield (%)[a]**	**mp (˚C) found**	**mp (˚C) reported**
F20	OH	H	OMe	OMe	OMe	OMe	H	8	226	226
F21	OH	H	OMe	OMe	OCH_2O		H	5	256	258
F22	H	OMe	OMe	OMe	OCH_2O		H	14	177	178-179
F23	H	OMe	H	OMe	OMe	OMe	OMe	40	195	198-199
F24	H	OMe	OMe	OMe	OMe	OMe	H	28	176	174-176
F25	H	OMe	OMe	OMe	OMe	OMe	OMe	20	150	151-152

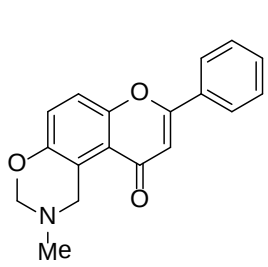
a Isolated yield. All products were identified by ^{1}H and ^{13}C-NMR; *b* Prepared from quercetin.

Table 4.4. Yields and melting points of flavanones synthesised [a]

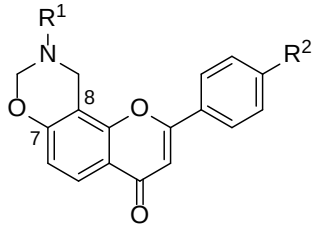


compd	C-4′	C-5′	C-5	C-6	C-7	C-8	yield (%)[b]	mp (˚C) found	mp (˚C) reported
D1	H	H	H	H	H	H	65	75	75-76
D2	OH	H	H	H	OH	H	50	209	211-212
D3	OMe	H	H	H	OME	H	35	94	93-94
D4	OMe	H	H	H	OH	H	38	150	155-163
D5	OMe	H	H	H	OMe	Br	25	74	-
D6	OMe	H	H	Br	OH	Br	39	55	-
D7	H	H	OH	H	OMe	H	20	92	89-90
D8	H	H	OMe	H	OMe	H	47	145	145-146

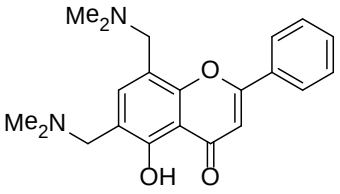
a Isolated yield. All products were identified by ^{1}H and ^{13}C-NMR spectroscopy.



N1



N2: R^1 = Bz; R^2 = H
N3: R^1 = Me; R^2 = OMe



N4

Scheme 4.1 Nitrogen-containing flavones

Chapter 5: Synthesis of (±)-panduratin A and derivatives

Panduratin A and its regioisomer isopanduratin A are cyclohexenyl chalcone derived natural products that have shown a wide spectrum of biological activity (Tuntiwachwuttikul *el al.*1984; Pandji *el al.*1993; Gu *el al.*2002; Win *el al.* 2007; Morikawa *el al.*2008). It has recently been reported that panduratin A and hydroxypanduratin A (Figure 5.1), isolated from *Boesenbergia rotunda* (L.) show good inhibitory activities towards dengue-2 virus NS3 protease with K_i values of 25 and 21 μM, respectively (Tan *et al.* 2006). Further insight into the protein-ligand interaction study revealed that these cyclohexenyl chalcones are bound to the catalytic triad of DEN2 NS2B/NS3 in the homology model (Lee *et al.* 2007). While a number of syntheses of cyclohexenyl chalcones have been described (Shibata *et al.* 2000; Cong *et al.* 2008; Jung *et al.* 2008; Corbett, *et al.* 2008), surprisingly the syntheses of panduratin A and isopanduratin A have never been reported. Thus, in this study, we attempted the synthesis of (±)-panduratin A and (±)-isopanduratin A through Diels–Alder cyclization of 2′-hydroxy-4′-methoxy-6′-ethoxymethoxychalcone and (*E*)-ocimene.

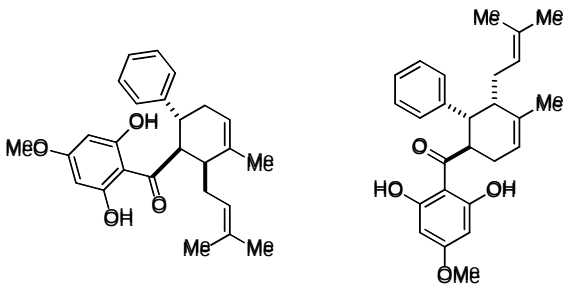
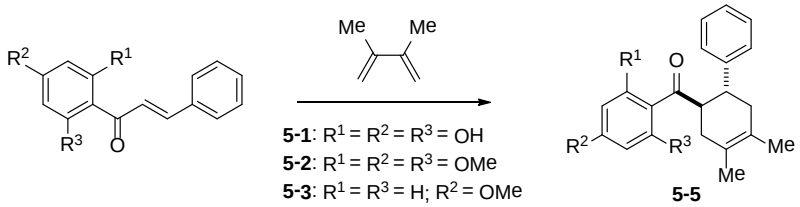


Figure 5.1. Panduratin A and isopanduratin A

Our studies began with model reactions between 2′,4′,6′-trihydroxychalcone **5-1** and 2,3-dimethyl-1,3-butadiene, carried out in various solvents as well as without solvent. Heating the two compounds for an extended period of time at 110°C resulted in

the formation of 5,7-dihydroxyflavanone (Table 5.1). The use of Lewis acid catalysts such as $BF_3{\cdot}Et_2O$, $AlCl_3$ and $ZnCl_2$, however, resulted in extensive polymerization of the diene.

Table 5.1. Screening of the reaction conditions[a]



Entry	Dienophile	Temp (˚C)	Time (h)	Catalyst (equiv)	Solvent	Product; yield (%)[b]
1	**5-1**	110	2	-	-	*c*
2	**5-1~5-3**	-78 (1h)-rt	24	$ZnCl_2$ (1)	Et_2O	*d*
3	**5-1, 5-2**	0 (1h)-rt	24	$AlCl_3$ (1)	toluene	*d*
4	**5-1, 5-2**	0 (1h)-rt	24	BF_3 (1)	Et_2O	*d*
5	**5-2**	rt	30	-	toluene	-
6	**5-2**	50	18	-	toluene	**5-5** (30)[e]
7	**5-2**	120	24	-	-	**5-5** (93)[e]

[a] Reaction conditions: dienophile (1 mmol), N_2 atmosphere.
[b] Isolated yield.
[c] Formation of 5,7-dihydroflavanone.
[d] Complex mixture ontained but no adduct was formed.
[e] Reaction performed in a pressure tube.

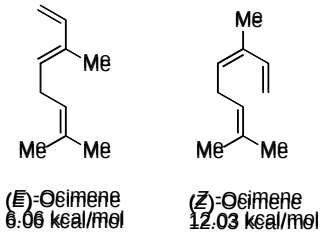
The reaction was also carried out with 2′,4′,6′-trimethoxychalcone **5-2** (as the dienophile) and 2,3-dimethyl-1,3-butadiene. An initial reaction (Table 5.1, entry 5) performed at room temperature with 1 equivalent of diene in dry toluene gave no product even after 30 h of stirring. Increasing the number of equivalents of diene also resulted in a similar observation. When the reactants were placed in a pressure tube and heated at 50 °C for 18 h, a small amount of the expected product was obtained (Table 5.1, entry 6). However, increasing the temperature to 120 °C and stirring overnight in a pressure tube resulted in the Diels–Alder adduct **5-5** being isolated in 93% yield (Table 5.1, entry 7).

Following the conditions described in Table 5.1, entry 7, compounds **5-4**, **5-6**~ **5-8** (*meta*-type cycloadducts) and their regioisomers **5-4a**, **5-6a**, **5-7a** and **5-8a** (*para*-type cycloadducts) were prepared in excellent yields and no polymerization of the diene (described as a complex mixture in Table 5.1) was observed. The products were isolated in a 3:2 *para*/*meta* ratio and the structures of the products for each reaction is given in Table 5.2.

The Diels–Alder reaction (Table 5.2, entry c) was first carried out under the same reaction conditions as those of entry 7 (Table 5.1) where excess ocimene was used and the reaction mixture was heated at 120 °C in a pressure tube. However, no Diels–Alder adduct was isolated even after the reaction was left for 24 h. The isolated product indicated polymerization of ocimene instead. The use of various Lewis acids to catalyze the reaction also resulted in polymerization of the acid-sensitive terminal conjugated double bond in ocimene.

When pure (*Z*)-ocimene was reacted with chalcone **5-2**, no Diels–Alder adduct was isolated. Instead, polymerization of the ocimene was observed. We realized that ocimene can exist in the *E*- or *Z*-configuration in nature (Scheme 5.1). However, when a mixture of (*E*)- and (*Z*)-ocimene was used in the Diels–Alder reaction with chalcone **5-2**, the adducts **5-6** and **5-6a** were isolated (Table 5.2). Presumably, only the (*E*)-ocimene underwent the Diels–Alder reaction. Examination of (*Z*)-ocimene indicated that the presence of a Z-prenyl substituent as part of the 1,3-butadiene moiety reduces its reactivity by hindering the approach of the dienophile. This limitation is not observed with (*E*)-ocimene. Molecular Mechanics calculations (MM+) revealed the (*E*)-ocimene to be more stable than the *Z* isomer by about 6 kcal/mol. This observation could provide some rationale for our findings, where the reaction between trimethoxychalcone and a

mixture of (*E*)- and (*Z*)- ocimene resulted in the formation of adducts **5-6** and **5-6a** while no product was observed when the reaction was conducted with (*Z*)-ocimene only.

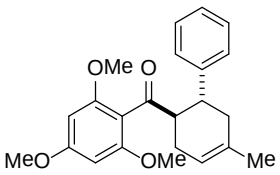
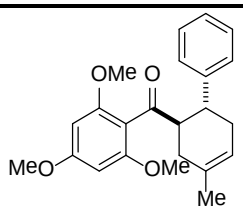
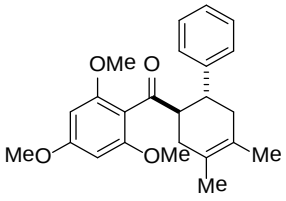
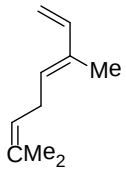
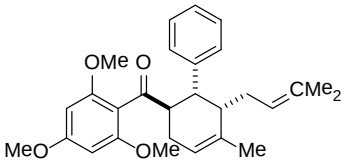
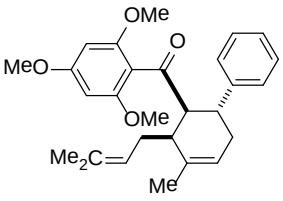
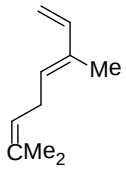
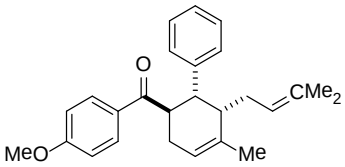
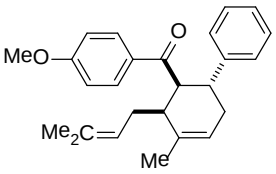
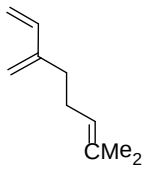
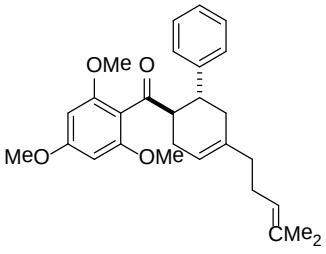
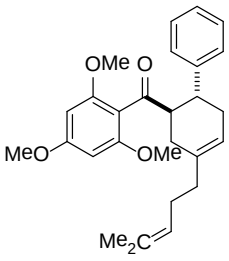


Scheme 5.1. *s-cis* Conformation of the (*E*)- and (*Z*)-ocimene

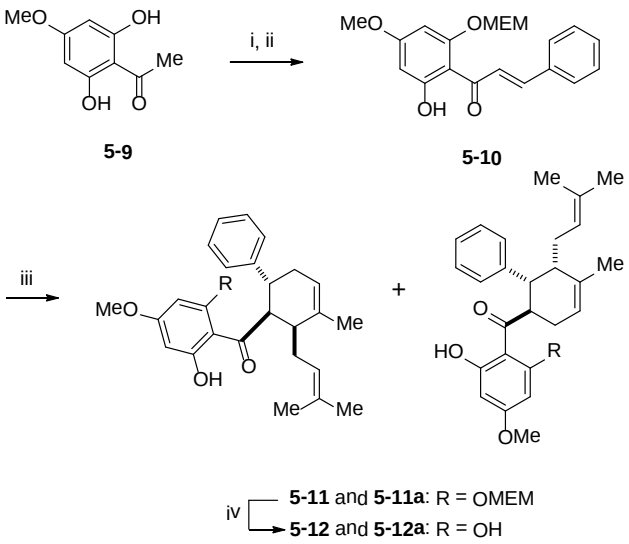
The synthesis of panduratin A and its regioisomer is illustrated in Scheme 5.2. Protection of the hydroxy group of commercially available 2′,6′-dihydroxy-4′-methoxyacetophenone **5-9** with 2-methoxyethoxymethyl chloride (MEM-Cl) followed by Claisen condensation with benzaldehyde in the presence of aqueous KOH in ethanol gave the chalcone **5-10** in 85% yield. Reaction of chalcone **5-10** with 5 equiv of (*E*)-ocimene at 150 °C led to the formation of a mixture of cycloadduct **5-11** and **5-11a** in quantitative yield. Deprotection of the MEM group gave panduratin A **5-12** and isopanduratin **5-12a** as a mixture in 89% yield.

In conclusion, the four-step synthesis of panduratin and isopanduratin A was completed in 75% overall yield. The syntheses involved a Diels–Alder reaction with a variety of dienes under moderate conditions (100–150 °C, neutral environment and no catalyst).

Table 5.2. Syntheses of panduratin A derivatives[a]

Entry	Dienophile;diene	Conditions	Product[b] (yield, %)
1	Dienophile =**5-2**; diene = 	120 ˚C, pressure tube, 24 h	 **5-4a**  **5-4** para/meta: 3:2 (86)
2	Dienophile =**5-2**; diene = 	120 ˚C, pressure tube, 18 h	 **5-5** (93)
3	Dienophile =**5-2**; diene =  (*E*)-Ocimene	150 ˚C, pressure tube, 15 h	 **5-6a**  **5-6** para/meta: 3:2 (99)
4	Dienophile =**5-3**; diene =  (*E*)-Ocimene	150 ˚C, pressure tube, 14 h	 **5-7a**  **5-7** para/meta: 3:2 (96)
5	Dienophile =**5-2**; diene =  Myrcene	120 ˚C, pressure tube, 24 h	 **5-8a**  **5-8** para/meta: 3:2 (89)

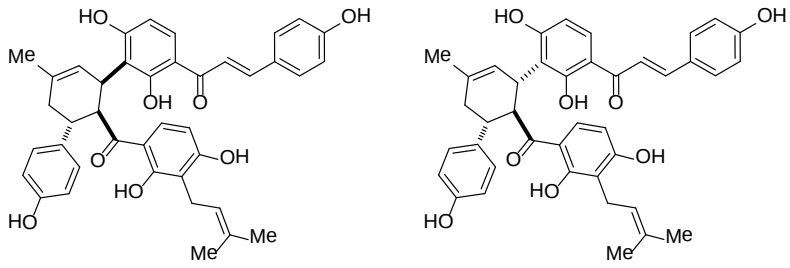
[a] All products were identified by ^{1}H, ^{13}C, DEPT, COSY, HMQC, HMBC, H2BC NMR;
[b]Isolated yields (mixture of regioisomers), calculated from chalcone.



Scheme 5.2. Reagents and conditions: (i) MEM-Cl, dry acetone, K_2CO_3, rt, 8h (ii) benzaldehyde, KOH 50 % aq, EtOH, 24h, 85 % for two steps (iii) β-*trans*-ocimene, 150˚C, pressure tube, 24h (iii) HCl 3M, MeOH, 80 ˚C, 10 min, 89 % for two steps

Chapter 6: Synthesis of (±)-kuwanon V and (±)-dorsterone methyl ethers

Kuwanon V, isolated from *Morus bombycis* and *Morus alba* L. (mulberry tree), has been reported to inhibit cAMP phosphodiesterase and hypoxia-induced factor-1 accumulation (Figure 6.1) (Dat *et al.* 2009; Hoang *et al.* 2009).

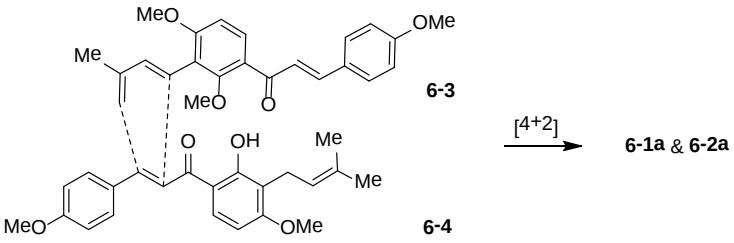


6-1 **6-2**

Figure 6.1. Naturally occurring prenylchalcones: kuwanon V **6-1** from *Morus alba* L. and *Morus bombycis*, dorsterone **6-2** from *Dorstenia barteri.*

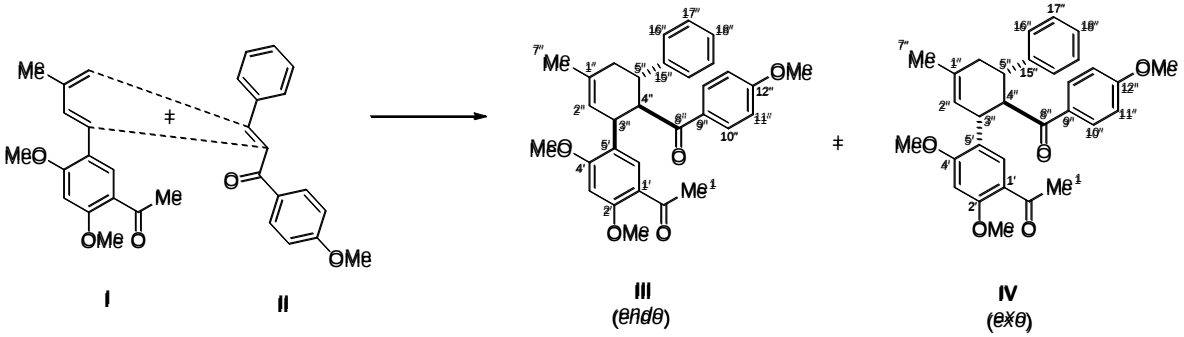
In the course of investigating the structure-activity relationship (SAR) for the Dengue-2 inhibitors (±)-panduratin A and (±)-isopanduratin A, we came across kuwanon V and dorsterone (Tsopmo *et al*. 1999), which have bigger substitutions on the cyclohexyl ring than panduratin A and isopanduratin A. However, there has been no report, thus far, on the syntheses of these cyclohexenyl chalcones. We therefore decided to attempt a practical synthesis of kuwanon V and dorsterone via a biomimetic intermolecular [4+2] cycloaddition reaction for further use of these compounds in biological activity testing.

The synthesis of kuwanon V **6-1a** and dorsterone **6-2a** from chalcones **6-3** & **6-4** was postulated via a [4 + 2] cycloaddition reaction between the diene **6-3** and dienophile **6-4**, as shown in Scheme 6.1 (Yoshio *et al.* 1992; Tsopmo *et al.* 1999).



Scheme 6.1. Postulated biosynthesis of kuwanon V (**6-1a**) and dorsterone (**6-2a**) via a Diels-Alder reaction

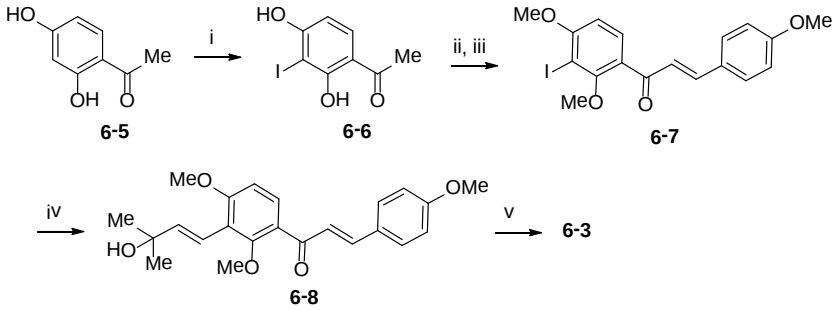
To test the feasibility of the cycloaddition reaction between these electron-rich dienophile and Lewis acid sensitive diene derived from chalcone, we carried out model study which involved a simple diene **I** and dienophile **II** as shown in Scheme 6.2. Heating compounds **I** and **II** in toluene in a pressure tube at 160˚C for 18 h afforded the *endo* and *exo* adducts *cis, trans*-**III** and *trans,trans*-**IV**, respectively, in a 1:1 ratio of 60% yield. Surprisingly, only a single regioisomer was observed as the product for the unsymmetrical diene **I**. Under the same conditions the cycloaddtion reaction between (*E*)-ocimene and dienophile **II** produced two regioisomeric adducts (Chee *et al.* 2010). The feasibility of this [4+2] cycloaddition reaction warrants the synthesis of kuwanon V and dorsterone.



Scheme 6.2. Model study of the [4+2] cycloaddition reaction using diene **I** and dienophile **II**

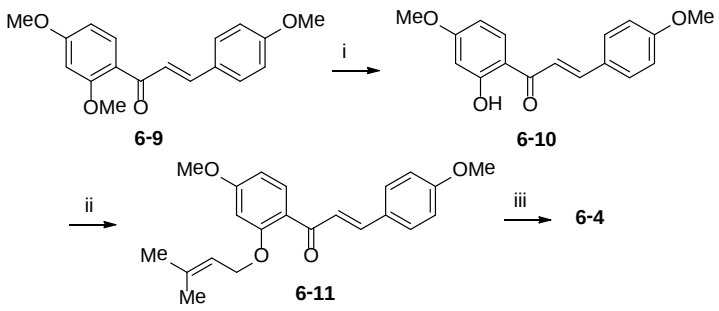
The synthesis of diene **6-3** was accomplished in five steps (Scheme 6.3) from the commercially available 2′, 4′-dihydroxyacetophenone. First, the acetophenone **6-5** was iodinated with ICl/CH_2Cl_2 to give the C-3′ iodinated acetophenone **6-6** in 40% yield.

Methylation of aryl iodide **6-6** followed by Claisen-Schmidt condensation with 4-methoxybenzaldehyde led to formation of chalcone **6-7** in 85% isolated yield. Subjecting the chalcone **6-7** to Heck coupling ($Pd(OAc)_2$, (*o*-tolyl)$_3$-P, Et_3N, DMF) with 2-methyl-3-buten-2-ol (Arkoudis *et al.* 2009) gave **6-8** in 75% yield which was easily dehydrated to diene **6-3** on treatment with acetyl chloride/pyridine (Harrington *et al.* 1987).



Scheme 6.3. Synthesis of diene **6-3**. Reagents and conditions: (i) ICl, CH_2Cl_2, rt, 24h, 40%; (ii) Me_2SO_4, K_2CO_3, acetone, rt, 8h; (iii) 4-OMebenzaldehyde, 50% aq KOH, EtOH, 24h, 85% over two steps; (iv) 2-methyl-3-buten-2-ol, $Pd(OAc)_2$, (*o*-tolyl)$_3$P, Et_3N, DMF, 90°C, 18h, 75%; (iv) AcCl, pyridine, benzene, 60°C, 8h, 95%

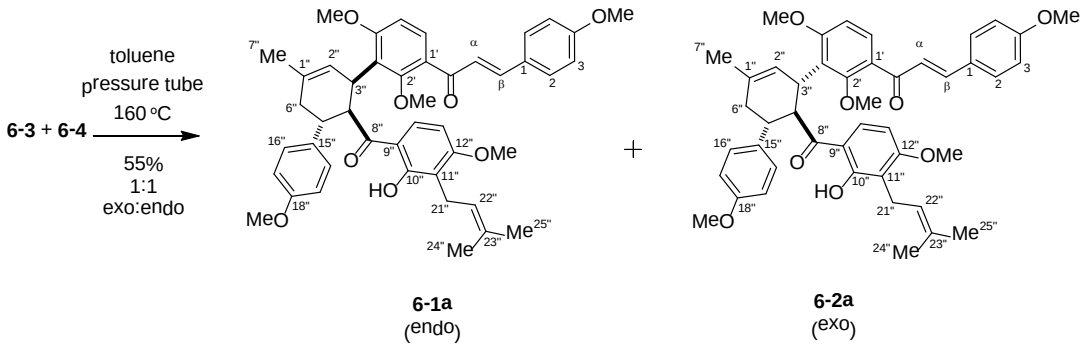
The dienophile **6-4** was synthesized from chalcone **6-9** as shown in Scheme 6.4. Selective cleavage of methoxy ether **6-9** followed by prenylation led to the formation of prenyl ether **6-11**. The prenyl ether **6-11** then was subjected to a Montmorillonite K10 promoted [1,3]-sigmatropic rearrangement (Sugamoto *et al.* 2008) to give the C-3 prenylated dienophile **6-4** in 45% yield.



Scheme 6.4. Synthesis of dienophile **6-4**. Reagents and conditions: (i) BCl_3, CH_2Cl_2, 0°C, 24h, 70%; (ii) prenyl bromide, K_2CO_3, acetone, reflux, 8h, 85%; (iii) Montmorillonite K10, CH_2Cl_2, 0°C, 2h, 45%

The chalcones **6-3** & **6-4** was then subjected to the [4 + 2] cycloaddition reaction. Heating these two compounds in toluene in a pressure tube at 160˚C for 18 h afforded the *endo* and *exo* adducts *cis, trans*-**6-1a** and *trans,trans*-**6-2a**, respectively, in a 3:2 ratio of 55% yield (Scheme 6.5). Longer period of reaction led to polymerisation due to the sensitive diene terminal. Isolation of the products **6-1a** and **6-2a** was easily performed by using silica gel chromatography.

The stereochemistry for *endo* **6-1a** and *exo* **6-2a** were assigned based on the comparison of the key coupling constant between H3′′, H4′′ and H5′′ (6.8 Hz for *cis* and 10.0 Hz for *trans*-configuration) with those reported for related natural products (Tsopmo *et al*, 1999). The configuration of **6-1a** was further confirmed by NOE experiment where irradiation at δ 4.32 (H4′′) gave 3% enhancement at H3′′ while H5′′ collapsed to doublet of doublet (J = 10.8, 5.7 Hz). In the NOESY experiment, correlations were observed between H5′′ and H6′′β, and between H3′′, H4′′, and H6′′α. It was observed from the NMR spectra that **6-2a** undergoes epimerisation in solution to yield an equilibrium mixture of **6-1a** and **6-2a**. The NMR signals of **6-2a** were resolved by measurement in DMSO-d_6 at 80 °C.



Scheme 6.5. Synthesis of pentamethyl ethers of kuwanon V (**6-1a**) and dorsterone (**6-2a**)

Further studies were then undertaken to optimize the yield of the reaction. Upon perusal of the recent literature, we were attracted to the work of Porco (Cong *et al.* 2008; 2010), in which a combination of ZnI_2/Bu_4NBH_4/CoI_2/1,10-phenanthroline catalytic system was able to promote highly efficient syntheses of cyclohexenyl chalcones. We applied this catalytic conditions in the syntheses of kuwanon V and dorsterone.

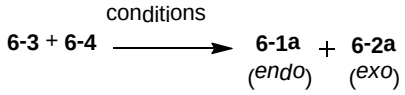
Table 6.1. Screening of the catalytic reaction conditions using Porco's catalysts[a]

Entry	ZnI_2 (mol %)	Bu_4NBH_4 (mol %)	CoI_2 (mol %)	1,10-phenanthroline (mol %)	Yield (%)[b]
1	100	100	100	100	[c]
2	100	50	100	100	5
3	100	50	-	-	5
4	50	50	-	-	4
5	50	10	-	-	55
6	60	10	-	-	60
7	60	10	10	10	62
8	70	10	-	-	62

[a]Condition: 0.1mmol of **I** & **II** in 3 mL of CH_2Cl_2 in a pressure tube (25 mL), 60°C, 18h. [b]Isolated yield. [b] Formation of dihydrochalcone.

Optimisation of the catalytic reaction conditions was carried by using diene **I** and dienophile **II**. Initial studies revealed that use of equal molar equivalent of tetrabutylammonium borohydride resulted in reduction of **II** to form dihydrochalcone without giving any cycloaduct (Table 6.1, entry 1). Addition of CoI_2 and 1,10-phenanthroline has no significant effect on the cycloaddition reaction (Table 6.1, entries 6 & 7): the yield of cycloadducts is 62% compared to 60% of the catalylic system without CoI_2 and 1,10-phenanthroline. The yield of cycloadducts was not further improved with additional molar amount of ZnI_2 catalyst. We found that combination of ZnI_2/Bu_4NBH_4/CoI_2/1,10-phenanthroline in 60/10/10/10 mol% gave the highest yield of cycloadducts (62%) and was subsequently used for cycloaddition reaction of diene **6-3** and dienophile **6-4**.

Table 6.2. Screening of the catalytic reaction conditions using various catalysts



Entry	Catalyst system[a, b]	Yield (%)[c]	*endo*:*exo*[d]
1	$ZnI_2/Bu_4NBH_4/CoI_2$/1,10-phenanthroline (60/10/10/10 mol%)	48	63:37
2	ZnI_2/Bu_4NBH_4 (60/10 mol%)	45	58:42
3	$AgOTf/Bu_4NBH_4$ (60/10 mol%)	65	60:40
4	CoI/ Bu_4NBH_4 (60/10 mol%)	-	
5	$CuOTf/Bu_4NBH_4$ (60/10 mol%)	-	
6	$Cu(OTf)_2$ (100 mol%)	-	
7	CuI (100 mol%)	-	
8	$CoCl_2/PPh_3$(50/50 mol%)	-	
9	$CoCl_2/PPh_3$/Zn (10/40/100 mol%)	-	

[a]Condition: 0.1mmol of **6-3** & **6-4** in 5 mL of CH_2Cl_2 in a pressure tube (25 mL), 60°C, 18h. [b]Formulations of mol % are based on the best result obtained from modal study. [c]Isolated yield. [d]Based on ^{1}H NMR integration.

Cycloaddition reaction of diene **6-3** and dienophile **6-4** in the combination of $ZnI_2/Bu_4NBH_4/CoI_2$/1,10-phenanthroline in 60/10/10/10 mol% gave lower yield of cycloadducts (48%, Table 6.2, entry 1) than that of obtained under thermal condition (55%). We then considered alternative modes of catalysis. On the basis of a recent report involving Diels-Alder dimerisation of piperine (Wei *et al.* 2005), we evaluated a series of Co(I) & Cu(I) catalysis for cycloaddtion. However, none of these catalysts gave desired cycloadduct in the reaction (Table 6.2, entries 4-9). We found that employing the $AgOTf/Bu_4NBH_4$ in 60/10 mol% gave a higher yield of the desired cycloadducts **6-1a** and **6-2a** in 65% yield (Table 6.2, entry 3). Nevertheless, none of these catalytic systems gave diastereomeric access to selectively produce *endo*-**6-1a** or *exo*-**6-2a**. Unfortunately, several attempts to remove the methoxy ethers of **6-1a** and **6-2a** by boron trichlorides only led to decomposition.

In conclusion, we have developed the first successful synthesis of the methyl ether derivatives of **6-1a** and **6-2a** of the mulberry kuwanon V **6-1** and dorsterone **6-2**, respectively through a [4+2] cycloaddition Diels -Alder reaction. Investigation of these compounds on the inhibitory activities against dengue 2 virus protease is underway.

Chapter 7: Conclusion

In conclusion, nearly a hundred flavonoids have been synthesised from established routes and as well as newly developed one-pot synthetic method. The one-pot method has successfully been applied toward the preparation of flavone analogues in reasonable quantities, thus allowing for the early stage of muscarinic receptor binding study.

(±)-Panduratin A and (±)-isopanduratin A, have been synthesised in four steps from commercially available 2′,6′-dihydroxy,4′-methoxyacetophenone. The key step involved a biomimetic intermolecular [4+2] cycloaddition between a chalcone and (*E*)-ocimene. Critical to the success of the Diels-Alder reaction was the use of diene (*E*)-ocimene rather than (*Z*)-ocimene.

The syntheses of kuwanon V and dorsterone have been completed in eight steps from 2′,4′-dihydroxyacetophenone and an overall yield of 16%. Neither of the thermal and catalytic ($AgOTf/Bu_4NBH_4$ in 60/10 mol%) [4+2] cycloaddition gave good enantioselectivity to kuwanon V and dorsterone.

Further studies are underway to enantioselectively synthesise panduratin A as well as kuwanon V and dorsterone.