CHAPTER IV
SYNTHESES OF FLAVONOLS AND ISOFLAVONES FROM FUNCTIONALISED CHALCONES
Chapter IV has been divided into three parts:

Part I; Synthesis of Seabuckthorn flavonols,
Part II; Synthesis of Soya isoflavones and
Part III; Green deprotection of methoxymethyl (MOM) groups.

Chalcones are biosynthesised through the interaction of acetate and phenyl alanine pathways (details are given in chapter I). Chalcones are the important intermediates in the biosyntheses of large number of phenolics i.e. flavonoids. These are endowed with several features which make them amenable for a variety of transformations. Taking inspiration from nature, appropriately substituted chalcones were converted into flavonols and isoflavones. Chemically, the chalcones can be synthesised by Claisen condensation of acetophenones and benzaldehydes under strong alkaline or acidic conditions. In the present chapter, part I describes the syntheses of flavonols using chalcones by modified AFO reaction and part II describes the syntheses of isoflavones from chalcones by oxidative reaction using thallium nitrate (figure 17). Additionally, this chapter also describes (part III), a green / solvent-free method for the deprotection of methoxymethyl (MOM) groups.

![Figure 17: Syntheses of flavonols and isoflavones using chalcones.](image-url)
PART I
SYNTHESIS OF FLAVONE-3-OLS USING CHALCONES;
APPLICATION TO SYNTHESIS OF SEABUCKTHORN (SBT)
FLAVONOLS

1. INTRODUCTION

1.1. Introduction to Seabuckthorn

Seabuckthorn is an herb / weed which is occurs in several countries including India, China, Germany, Canada and Russia. So far, seven species of Seabuckthorn have been reported of which the most abundant species is *Hippophae rhamnoides* (SBT). The leaves, flowers and fruits of the plants are useful for different types of medicines, nutraceuticals, beverages, cosmeceuticals etc. Seabuckthorn leaves and flowers are used for treating arthritis, gastrointestinal ulcers and skin diseases. A tea containing Seabuckthorn leaves is used as a source of vitamins, antioxidants, fatty acids and minerals\(^1\). Also, it is used for preventing and controlling diseases related to blood vessels and enhancing immunity. Seabuckthorn berries are used for preventing infections and possess anti-aging properties. The seed or berry oil is used for treating asthma and heart disorders. Also, SBT products are used as supplemental source for vitamins C, A, E, β-carotene, minerals, amino acids and fatty acids\(^2\).

Many of the remarkable biological activities of Seabuckthorn (*Hippophae sp.*, SBT) have been attributed to the presence of flavonoids\(^3\). These insights made us look into the flavonoids present in the Seabuckthorn plant. A product called, “Seabuckthorn flavone” (*SBT flavone*), is already been commercialized. However, the composition of this product has not been clearly defined. This attracted our attention to analyse the composition of commercial samples of SBT flavone.
1.2. Analysis of SBT flavones

Commercial samples of SBT flavone were obtained from Ladakh (India), China and Russia. They were subjected to careful analysis using TLC, HPLC and MASS spectra. The analysis on commercial SBT flavone revealed that they mainly contained a mixture of isorhamnetin (4), quercetin (13a) and kaempferol (13b) with traces of myrecetin in highly variable proportions and yields (15-30%) with more than 50% intractable materials. No evidence for presence of glycosides or tannins were found in SBT flavone samples. SBT flavone is reported to exhibit various bioactivities such as radio-protective\(^3\), kinase inhibitory and apoptotic activities related to cancer\(^4\). They show anti-oxygenic and metal chelating activities and have also been implicated in the conditions related to degenerative diseases (aging), wound healing and cardio-vascular problems. Ambiguously reported bioactivities and composition of SBT flavone prompted us to synthesize individual flavone-3-ols, so that pure compounds are subjected to studies on structure-activity relationship (SAR).

1.3. Synthesis of flavonols (3-hydroxyflavones)

Of the SBT flavones, isorhamnetin (4) seems to the most important for it shows a multitude of bioactivities. Recent reports suggest that the polyhydroxyflavonoids are metabolized in the biological systems into more bioavailable partially protected forms (e.g. methyl ethers) which may be the actual active entity (details are given in chapter I). For example, plasma of rats fed with quercetin (13a) were devoid of it, but contained only its 3′-O-methyl ether namely, isorhamnetin (4). Thus, preparation of partially protected compounds assumes special importance in metabolic studies. So we chose isorhamnetin (4) as a model candidate for the present synthesis.

Isorhamnetin (4) has been synthesized earlier by Allan-Robinson or by selective methylation of quercetin (13a). Allan-Robinson method involves, the condensation of \(\omega\)-O-
benzoyloxy acetophenone, sodium salt and anhydride of vanilic acid at 200°C and followed by hydrogenation (for debenzylations) to give isorhamnetin (4) in reasonable yield (scheme 1).

Though the method is apparently straightforward, it employs very harsh experimental conditions and requires ingenious selective protection and deprotection of the free hydroxyl groups with benzyl and/or benzoyl groups. Moreover, eight to eleven steps involving hazardous reagents has to be performed for the preparation of starting materials.

Alternately, isorhamnetin (4) has also been synthesised from quercetin (13a) using selective protection and deprotection of hydroxyl groups. The method involves, selective benzylolation of quercetin (13a), followed by the methylation and deprotection of benzyl groups, and provided isorhamnetin (4) in very poor yield-5% (scheme 2). This method has many disadvantages viz. i) the method gave a mixture of intermediates (tri and tetra-benzylated quercetin) which are difficult to separate, ii) advance starting material i.e. quercetin is involved and iii) the reaction yield was very low. These drawbacks made us to develop a practical method for the synthesis of isorhamnetin (4). An alternate methods viz. Algar-Flynn-Oyamada
Scheme 2. Partial methylation of quercetin.

(AFO) reaction offers flavone-3-ols directly using chalcones with hydrogen peroxide in alkaline medium. The present study describes a modified AFO reaction for flavonols syntheses.

2. PRESENT STUDY

The retro-synthesis of isorhamnetin is shown in scheme 3. The flavonol (I), scaffold can be synthesised via the Algar-Flynn-Oyamada (AFO) reaction from chalcone (II). The chalcones can be synthesised by the Aldol condensation using an acetophenone (III) and an aldehyde (IV). For the synthesis of isorhamnetin (4), phloroacetophenone forms an A ring, while the vanillin moiety forms a B ring.

Scheme 3. Retro-synthetic analysis of isorhamnetin by AFO method.
AFO reaction also requires benzyl or alkyl protection for attaining hydroxylated flavonols in reasonable yields. Earlier, we had found that methoxymethyl (MOM) protections is very useful for the preparation of flavone and biflavones (described in previous chapters, II & III). Therefore, it was of interest to explore the possible use of MOM protections in the AFO reaction. For the synthesis of isorhamnetin (4), the required 4,6-diMOM-phloroacetophenone (1) was prepared as described earlier in chapter II. 4-MOM vanillin (2) was prepared by using MOM-Cl/K$_2$CO$_3$ and vanillin at room temperature. Claisen condensation of 1 and 2 under alkaline conditions (at 0°C) was used for the synthesis of 3. The chalcone (3) was characterized by UV and IR spectra. In our hands, the conventional AFO reaction with 4,4′,6′-triMOM protected chalcone (3) resulted in the formation of mixture of compounds with low and variable yields (scheme 4). One of the reasons could be, when 6′-position substituted with 2′-O-hydroxyl free in chalcone (3); aurone formation is favoured rather than flavonol formation.

![Scheme 4: Reaction optimization.](image)

The reaction was then tried with complete protection of hydroxyls by MOM groups as in chalcone (6). 6 was prepared by the condensation of 2,4,6-triMOM phloracetophenone (5) with 4-MOM vanillin (2) in the presence of base (KOH). The chalcone (6) was obtained as a pale yellow oily product. It did not respond to Shinoda test (Mg-HCl); also, no distinct color change was observed on addition of alcoholic FeCl$_3$ suggesting that MOM protections were intact (i.e. no hydroxyl group is free). The chalcone (6) showed UV (MeOH): λ 324 nm; IR, carbonyl group absorb at IR (KBr): ν 1677 and MOM groups absorb at 2900-2920 cm$^{-1}$. The chalcone
(6) was subsequently converted to epoxide (7) using alkaline H₂O₂ in good yield. The colourless epoxide (7) showed a hypsochromic shift in the UV absorption from UV (MeOH): λ 324 to 285 nm due to the absence of double bond (and formation of epoxide).

Further, carbonyl absorption shifted in the IR spectrum from IR (KBr): ν 1677 cm⁻¹ to 1700, and a new absorption peak appeared at 883 cm⁻¹, suggesting that the double bond was transformed into epoxide. Treatment of epoxide (7) with methanolic-HCl resulted into regio-specific opening of the epoxide ring with concurrent loss of MOM protections to give dihydroisorhamnetin (8) in 55–60% yield (scheme 5). The dihydroisorhamnetin (8) showed, UV (MeOH): λ 285 nm and IR (KBr): ν 1644 (carbonyl) cm⁻¹. It responded to Shinoda’s (Mg-HCl, rose color) and Pew’s (Zn-HCl) color reactions (pink color)¹⁰. Dihydroisorhamnetin (8) is a natural product and occurs in Dillenia indica; this plant reported to have wound healing, anti-diabetic and anti-diarrheal properties¹¹. Conversions of 8 to isorhamnetin (4) was achieved by the oxidation of 8 with potassium meta-bisulfite solutions for 5h at 100°C (scheme 5). After the completion of reaction (monitored by TLC), the reaction mixture was poured into crushed ice when isorhamnetin (4) precipitated as a pale yellow solid. The solid was collected by
centrifugation (55% yield). Isorhamnetin (4) was purified by column chromatography (SiO₂); it eluted by a mixture of solvents MeOH (0.2%) and CHCl₃ (99.8%). The product showed expected UV spectra at UV (MeOH): λ 254, 370 nm, molecular mass at (M+H)+: 317, MS/MS: 168 and 153 fragments, melting point 300-303°C (lit; 307°C) and NMR spectra. Physical and spectral data are identical with the those reported for isorhamnetin¹². The details are given in the experimental section.

2.1. Synthesis of Seabuckthorn flavonols and analogs

To show the compatibility of the reaction, two other Seabuckthorn flavones viz. quercetin (13a) and kaempferol (13a) were also synthesized. For the synthesis quercetin (13a), pentaMOM-chalcone (10) was used. 10 was prepared by the condensation of 5 with 3,4-diMOM-benzaldehyde (9a) under alkaline conditions. As expected, the chalcone (10) showed expected

Scheme 6: Synthesis of Seabuckthorn flavonols and analogs by modified AFO reaction.
spectral properties; UV (MeOH): $\lambda$ 330 nm; IR (KBr): $\nu$ 1676 (carbonyl) and MOM absorb at 2989-2930 cm$^{-1}$. The chalcone (10) was converted to epoxide (11) using alkaline hydrogen peroxide (at room temperature). The epoxide (11) absorb UV (MeOH): $\lambda$ 285 nm and IR (KBr): $\nu$ 1699, 883 cm$^{-1}$. The epoxide (11) was converted to taxifolin (12a) by treatment with HCl. It may be noted that taxifolin (12a) is a natural product which occurs in Pinus roxburghii$^{13}$ and acts as an agonist of the adiponectin receptor-$2^{14}$. Oxidation of taxifolin (12a) to quercetin (13a) was carried out using potassium meta-bisulphite in 60% yield (scheme 6). 13a was characterized by different spectral analyses and compared with those reported in the literature$^{15}$; UV (MeOH): $\lambda$ 255, 371 nm: Mp; 311-315°C, lit. Mp; 315°C$^{15}$. The identity was further established by direct comparison with an authentic sample of quercetin.

Similarly, kaempferol (13b) and galangin (13c) were also synthesized by the same sequence by the reaction of 5 along with 9a and 9b respectively. Further, two other partial methyl ethers, tamarixetin (13d) and kaempferide (13e) were also synthesized by the same sequence of reactions using 5 with MOM-isovanillin (9d) and p-anisaldehyde (9e) respectively (scheme 6).

In summary, a short and practical syntheses of Seabuckthorn flavonols viz. isorhamnetin (4), quercetin (13a), kaempferol (13b) and analogs viz. galangin, tamarixetin and kaempferide have been achieved in good to moderate yield. Identity of the compounds were established by the direct comparison of the properties with authentic samples and literature data$^{16,17,18}$. The details are given in the experimental section. The synthesised compounds were evaluated for antioxidant studies.

### 3. ANTIOXIDANT ACTIVITY

Anti-oxygenic activities of flavonoids of Seabuckthorn$^{19}$ have been associated with bioactivities such as radioprotection, cancer prevention, anti-aging and prevention of
cardiovascular diseases\textsuperscript{20}. Therefore, it was of interest to carry out a comparative study of anti-oxygenic activities of SBT extracts, *SBT flavone* and other common flavonoids (Table 1, a-z). For this the free radical scavenging activities were determined by quenching DPPH (2,2-diphenyl-1-picrylhydrazyl). EC\textsubscript{50} were calculated as µg required for inhibition of 50\% of activity. Trolox (g, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a reference antioxidant.

**Table 1.**

Antioxidant activities of Seabuckthorn extracts, isolated products and synthesised compounds.

<table>
<thead>
<tr>
<th>Products</th>
<th>EC\textsubscript{50} value (µg)</th>
<th>Products</th>
<th>EC\textsubscript{50} value (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Gallic acid</td>
<td>3.8</td>
<td>n) Gossypin</td>
<td>25.5</td>
</tr>
<tr>
<td>b) Ellagic acid</td>
<td>4.8</td>
<td>o) Isorhamnetin</td>
<td>26.2</td>
</tr>
<tr>
<td>c) Gossypetin</td>
<td>5.5</td>
<td>p) Taxifolin</td>
<td>97</td>
</tr>
<tr>
<td>d) Tannic acid</td>
<td>8.8</td>
<td>q) SBT 70% acetone extract</td>
<td>&gt;100</td>
</tr>
<tr>
<td>e) Quercetagenetin</td>
<td>10.5</td>
<td>r) Kaempferide</td>
<td>&gt;100</td>
</tr>
<tr>
<td>f) Patuletin</td>
<td>10.6</td>
<td>s) SBT seed oil</td>
<td>&gt;100</td>
</tr>
<tr>
<td>g) Trolox</td>
<td>12.5</td>
<td>t) SBT pulp oil</td>
<td>&gt;100</td>
</tr>
<tr>
<td>h) PC</td>
<td>12.8</td>
<td>u) Tamarixetin</td>
<td>&gt;100</td>
</tr>
<tr>
<td>i) Quercetin</td>
<td>14.4</td>
<td>v) Dihydroisorhamnetin</td>
<td>&gt;100</td>
</tr>
<tr>
<td>j) Kaempferol</td>
<td>14.6</td>
<td>w) Dihydrotamarixetin</td>
<td>&gt;100</td>
</tr>
<tr>
<td>k) Rutin</td>
<td>15.5</td>
<td>x) Dihydrokaempferol</td>
<td>&gt;100</td>
</tr>
<tr>
<td>l) Hibifolin</td>
<td>16</td>
<td>y) Dihydrokaempferide</td>
<td>&gt;100</td>
</tr>
<tr>
<td>m) SBT flavone</td>
<td>18.5</td>
<td>z) Dihydrogalangin</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>
Amongst the SBT extracts, 70% aqueous acetone extract of SBT leaves (q) was found to be the most active; the seed and pulp oils showed lower activities. A sample enriched in phenolic concentrate (PC, h) was isolated from 70% aqueous acetone extract was found to have high anti-oxygenic activity (EC$_{50}$, 12.8µg). Analysis of PC (h) showed the presence of quercetin (13a, i), isorhamnetin (4, p) and gallic acid (a). Amongst isolated pure compounds, gallic acid (a) showed the maximum activity followed by ellagic acid (b). Both these may be artefact of hydrolysable tannins present in SBT leaves. The bio-activities reported for amla (Phyllanthus emblica)$^{21}$ and pomegranate (Punica granatum)$^{22}$ could be attributed to the presence of gallic acid and ellagic acid in the form of tannins. Gallic acid is reported to have radio-protection properties. Potent anti-oxidant property of gallic acid could be one of the reasons for the radio-protection activity. Quercetin (13a, i) and kaempferol (13b, j) showed better activity compared to the major flavonoid, isorhamnetin (4, p) of ‘SBT flavone’. Isorhamnetin (4, p; quercetin-3’-methyl ether), tamarixetin (13d, u; quercetin-3’-methyl ether), kaempferide (13e, r; kaempferol-3’-methyl ether) are less active than the parent compounds viz. quercetin (13a, i) and kaempferol (13b, j). Gossypetin (c; 8-hydroxyquercetin) and quercetagetin (e; 6-hydroxyquercetin) showed high activities. Patuletin (f; 6-methyl quercetagetin) also showed similar trend in activity. Blocking of hydroxyl by glycosylation decreases the bioactivities considerably as can be seen for rutin (k; quercetin-3-rhamnoglucoside) and gossypin (o; gossypetin-8-glucoside). 8-Glucronide of gossypetin (l; hibifolin) showed good activity. Dihydroflavonols (v–z) did not show significant activity except dihydroquercetin (12a, n; taxifolin). Thus, it may be inferred that radical scavenging activities are dependent on number and position of hydroxyl groups. Also, presence of dihydroxy (catechol) group acts as metal chelating systems.
4. CONCLUSION

A synthetic route to Seabuckthorn flavone-3-ols and analogs of biological importance has been achieved using readily accessible starting materials and mild reaction conditions. Amongst the compounds tested, gallic and ellagic acids were found to have higher anti-oxygenic activities compared to flavone-3-ols. Therefore, due importance should be given to the presence of gallic and ellagic acids for bioactivity studies of SBT extracts, in addition to flavonoids. Since many flavone-3-ols show better activities when compared to flavones from SBT, it should be feasible to develop improved bioactive formulations compared to SBT products.
PART II

CONVENIENT SYNTHESSES OF SOYA ISOFLAVONES
AND THEIR ANTI-BIOFILM ACTIVITIES

1. INTRODUCTION

1.1. Introduction to phytoestrogens

Phytoestrogens have attracted much attention in recent years due to their influence on estrogenic profiles of postmenopausal women\textsuperscript{21}. Several studies has been conducted to develop novel phytoestrogens from natural sources. The main classes of phytoestrogens are isoflavonoids (isoflavones, coumestans), lignans, stilbenoids, which mostly occur in the Leguminosae family. Isoflavones show superior phytoestrogenic properties over other groups. For the identification of phytoestrogenic components, bio-guided fractionation concepts are routinely employed. However, these approaches are time consuming, show lack of specificity and reproducibility. Thus, identification of individual phytoestrogens present logistic problems. Therefore, convenient and practical syntheses of Soya isoflavones are warranted.

1.2. Importance of Soya isoflavones

Soya products are highly beneficial to human health. Consumption of Soya products in the form of food or dietary supplements lower the rates of postmenopausal cancer and osteoporosis in women\textsuperscript{24,25,26}. Important examples of Soya isoflavones are: daidzein, genistein, formononetin, glycine and their glycosides\textsuperscript{27}. A new bioactivity of Soya extracts, namely anti-biofilm activity on food pathogens\textsuperscript{28} has been reported very recently. However no attempt was made to identify the active constituents. A recent study from CDRI (Central Drug Research Institute, Lucknow, India) has shown that an isoflavone \textit{viz.} caviunin [caviuin-7-O-(\(\beta\)-D-apiofuranosyl-(1→6)-\(\beta\)-D-glucopyranoside] from \textit{Dalbergia sissoo} has bone stimulation properties and is presently under clinical trials\textsuperscript{29,30}. It is important to note that for any drug (or food supplement) development, characterization of individual compounds are warranted. In
order to explore that phenomena, we have chosen to explore anti-biofilm activity of individual Soya isoflavones. For this study, substantial quantities of individual compounds are required. These compounds are not easily available in sufficient quantities from the natural sources. In the present study, a practical method for the synthesis of Soya isoflavones and their anti-biofilm activities are described.

1.3. Synthetic methods for isoflavones

Several methods have been reported for the synthesis isoflavones (scheme 7). Most of them involve one carbon introduction to 2-hydroxy deoxybenzoin, oxidative coupling of chalcone, chalcone epoxide rearrangement to isoflavone, 3-arylation of chromone using Suzuki coupling and more recently Wacker-Cook synthesis for isoflavones. Though the described methods are good, they are not ideal for the synthesis of Soya isoflavones.

These methods required extensive protection and deprotection of hydroxyls as benzyl, benzoyl or methoxyl protection to obtain desired isoflavones in reasonable quantities. Amongst methods available, oxidative rearrangement of chalcone to isoflavone by thallium nitrate
(TTN) seems to be a method of choice\textsuperscript{32}. In earlier studies (Chapter II, III, and IV part I) we have successfully used methoxymethyl (MOM) protection for the flavonols, flavones and biflavones syntheses. In this part, compatibility of MOM protection in the oxidative rearrangement using TTN has been explored.

2. PRESENT WORK

2.1. Synthesis of Soya isoflavones

For the TTN oxidative rearrangement, it is necessary to protect the hydroxyls by benzyl or benzoyl or methyl groups. To explore, the compatibility of MOM group in the TTN reaction, initial experiments have been tried between MOM-protected chalcone (4,2′,4′,6′-tetra-MOM chalcone, 14) and TTN reagent (scheme 8) under different reaction conditions. The reaction did not yield expected isoflavone, genistein (15). Only, deprotected chalcone and flavanone were recovered.

![Scheme 8](image_url)

Scheme 8. Reaction optimization for isoflavones.

It was felt that chalcone (18) with free 2′-hydroxyl group could enhance the rearrangement reaction of TTN. For the preparation of genistein (15), then chalcone (18) was used as a starting material. The chalcone (18) was prepared by the condensation of 4,6-diMOM phoroacetophenone (16) and 4-MOM benzaldehyde (17) under alkaline condition (at room temperature for 12 hrs). Chalcone (18) was characterized and treated with TTN (in MeOH) at room temperature (12 hrs). The reaction mixture was acidified with conc. HCl and refluxed for 2 hrs (scheme 9). As expected during the cyclodehydration reaction, MOM groups got
deprotected. This is a notable advantage over other methods, since additional step for deprotection is not required. Solvent was removed under reduced pressure and the reaction mixture was poured into ice. The white product was separated by centrifugation and was purified through SiO\(_2\) column chromatography, eluted with the mixture of solvents in the ratio of 1:1 petroleum ether and ethyl acetate.

**Scheme 9.** Preparation of genistein.

The product was characterized by UV, IR, MASS and \(^1\)H NMR spectral data. The product absorbed UV at (MeOH): \(\lambda\) 262, 329\(\text{sh}\) nm, IR (KBr): \(\nu\) 1655 cm\(^{-1}\) and has melting point of; 295-298°C (reported Mp 301°C for genistein)\(^{22}\). The structure was further, confirmed by \(^1\)H NMR spectroscopy. It showed the characteristic signal at \(\delta\) 8.3 (s) which characteristic of proton at 2-position of genistein (15). Additionally, mass spectra shows expected molecular ion peak at (M+H)\(^+\); 271 and mass spectral fragment MS/MS at m/e 243, 153, indicating typical loss of carbonyl (fragment mass 28) which is characteristic of isoflavones. A-ring loss of m/e 153 further, confirms the assigned structure of genistein (15, scheme 10). These data are found to be identical with those reported for genistein (15)\(^{22}\).
One pot synthesis of Soya isoflavones: During the course of this work, it was observed that the isoflavones can be synthesised in one pot reaction without the need of isolation of chalcone intermediate. Thus, 4,6-diMOM pholoracetopheone (16) and 4-MOM benzaldehyde (20) were taken in alkaline methanolic solution and stirred at room temperature for 12 h. The reaction was monitored by TLC for the appearance of yellow coloured (chalcone) band and disappearance of starting materials.

Scheme 11. One pot syntheses of soya isoflavones.
To the chalcone (without isolation) solution, TTN was added and stirring continued for additional 12 hrs. It was acidified with HCl to pH-2 and then, it was refluxed for 2 h (scheme 11). The methanol was removed under reduced pressure. The reaction mixture was poured into ice. The product, genistein (15) was collected by centrifugation as a white precipitate. In the modified one-pot reaction, the yield had improved from 60% to 78%.

Following the above protocol other isoflavones were also synthesised. Diadzein (21a) was prepared by the condensation of 4-MOM resacetophenone (19a) and 4-MOM benzaldehyde (20a) under alkaline condition and followed by the TTN rearrangement and cyclodehydration (indicated in scheme 10). Formononetin (21b) was prepared by the reaction of 4-MOM resacetophenone (19b) and 4-methoxybenzaldehyde (20b) through similar conditions and reactions. Biochanin-A (21c) was prepared by the similar sequence and conditions by using starting materials, 4,6-diMOM phoroacetophenone (19c) and 4-methoxybenzaldehyde (20c). Reaction procedures and characterization of Soya isoflavones are given in the experimental section.

In summary, an efficient and practical method was developed for the preparation of Soya isoflavones viz genistein (15), diadzein (21a), formononetin (21b) and biochanin-A (21c) in good yield (75-85%). It has been established that MOM groups are compatible with TTN oxidation reactions. Also this method does not require an additional step for the deprotection of MOM groups. The synthesised compounds were subjected for anti-biofilm activity.

3. ANTI-BIOFILM ACTIVITY

The need for multipronged strategies apart from use of antibiotics in tackling the menace of Multi-drug resistance (MDR) is gaining importance. One of the possible ways to stop MDR growth is through controlling the cell to cell communication amongst bacteria. Microorganisms are highly interactive and utilize a range of cell to cell communication mechanisms, a process known as quorum sensing (QS), to promote collective behaviour within a population. In
bacteria QS is exploited to facilitate gene expression at a threshold cell density. Gram-positive and Gram-negative bacteria use QS communication circuits to regulate a diverse array of physiological activities, such as secretion of virulence factors, biofilm formation, motility, sporulation, bioluminescence and the exchange of DNA, all of which facilitate bacterial pathogenesis. QS molecules have an important role in cell morphology variation which is considered to have major pathogenic role in yeast like fungi, Candida albicans.

Recent study from Chatterji et al., indicated that two novel peptides, control and protect the biofilm formation in tuberculosis bacteria (Mycobacterium tuberculosis). It was inferred that inhibition of biofilm formation is important for control MDR resistant bacterial growth which are implicated in many diseases. With this intention, the synthesized Soya isoflavones were subjected for anti-biofilm activity against Chromobacterium violaceum, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans.

**Figure 18.** Anti-biofilm activity of compounds by Soya isoflavones on 1. C. violaceum, 2. P. aeruginosa, 3. S. aureus and 4. C. albicans.
The isoflavones (figure 18), genistein (15), diadzein (21a), formononetin (21b) and biochanin-A (21c), did not show anti-biofilm activity on \( P. \) aeruginosa. Biocahnin-A (21c), inhibited biofilm formation of \( S. \) aureus (54%), however genistein (15), formononetin (21b) and diadzein (21a) did not affect the biofilm formation of \( S. \) aureus. This observation supports with those reports of Rekha et al., 28 where isoflavones extract showed a marginal activity against Methicillin resistant \( S. \) aureus (figure 18). Further, we have also examined the anti-biofilm activity of these isoflavones on \( C. \) violaceum. It was observed that diadzein (21a) could inhibit 84% and formononetin (21b) could inhibit 61.6% biofilm formation of \( C. \) violaceum. Inhibition of biofilm formation might have contributed the anti QS properties of these compounds. Hence anti QS studies of these compounds are very well support the anti-biofilm studies. We have also studied the effect of these compounds on germ tube formation of \( C. \) albicans. This study indicated that the formation of germ tubes was affected by formononetin (21b) and biochanin-A (21c).

4. CONCLUSION

MOM-protected chalcones were efficiently converted into hydroxylated (genistein; 15 and diadzein; 21a) or partial methyl ethers (formononetin; 21b and biochanin-A; 21c) of isoflavones, using thallium nitrate oxidation reaction. The synthesis has been modified into a one pot reaction. The individual synthesized compounds were subjected to anti-biofilm activity. It was found that amongst the compounds studied, diadzein (21a) inhibits \( C. \) violaceum and \( S. \) aureus bio-films. Additionally, it also control gem-tube formation of \( C. \) albicans. From this study, it was inferred that diadzein (21a) was found to be an active component in Soya extract for the inhibition of bio-film formation on food pathogens.
PART III
A RAPID, SOLVENT-FREE DEPROTECTION OF METHOXYMETHYL (MOM) ETHERS BY PTSA

1. INTRODUCTION

Protection of functional groups plays important role in the syntheses of organic compounds. Attempts have been made to develop green methods for deprotection reactions using solvent free, atom efficient syntheses and employing recyclable catalysts/reagents\(^{44,45}\). Protection and deprotection of hydroxyl functionalities are important processes in the synthesis of numerous of organic molecules. Amongst many protecting groups for hydroxyl protections, methoxymethyl (MOM) groups offer some advantages over others\(^8\). MOM groups have been utilized in the total syntheses of several complex natural products such as hennoxazole-A\(^{46}\), laulimalide\(^{47}\), verongamine and purealidin-N\(^{46}\) and anti-cancer compound, taxol\(^{48}\). MOM protection/deprotections of the hydroxyl groups has been the one of the important steps in these syntheses. In the programme on discovery of bioactive compounds, we have explored the bioactivities of lesser known flavonoids such as partial methyl ethers. This led us to develop “bioinspired diversity oriented syntheses” of oxygen heterocyclic compounds such as flavonoids\(^{12,49,50}\), isoflavonoids and biflavonoids, using mild reactions conditions, inexpensive reagents and MOM protection. We have made extensive use of MOM protections/depotetion in the syntheses of partially protected or methylated/polyhydroxy flavones\(^{12}\), flavonols\(^{49}\), biflavones\(^{50}\) and isoflavones. During the course of this work, need for the development of an eco-friendly green method for the deprotection of MOM ethers was felt. A large number of reagents have been reported for deprotection of MOM such as acid catalysts (HCl, HF, CF\(_3\)COOH and AcOH/H\(_2\)SO\(_4\))\(^8\); Lewis acids, (BF\(_3\)-etherate, AlCl\(_3\), FeCl\(_3\), InI\(_3\), ZrCl\(_4\) and NbCl\(_5\))\(^{51}\); triflates \{Sc(OTf), Zn(OTf), Bi(OTf), TESOTf and TIPSOTf\}\(^{52}\); bromo catalysts,
(catechol bromide, BBr₃, Me₂BBr₃, Me₃SiBr and MgBr)⁵³; and miscellaneous catalysts—such as Well-Dawson catalyst ⁵⁴, NaHSO₄·SiO₂ ⁵⁵ and clay catalysed reaction ⁵⁵ (scheme 1).

![Scheme 1](image)

**Scheme 1.** Reported methods for the deprotection of methoxymethyl (MOM) group.

Though the methods are good in general, they use solvents, require tedious workup procedures and often use of hazardous reagents (i.e. BBr₃). An eco-friendly and solvent-free bromination of 1,3-diketones, under solid phase conditions has been reported ⁵⁶. We report here solvent-free deprotection of MOM ethers at ambient temperature using inexpensive readily available reagent viz. p-toluene sulphonic acid (pTSA).

**2. PRESENT STUDY**

We observed that mere intimate trituration of MOM ethers with pTSA in a mortar followed by aqueous work up produced the deprotected compounds in good to excellent yields with good purity. Deprotection of MOM was selective in the presence of other groups such as methoxy, benzyl, ester, amide, allyl and lactone, except acetate. This method provides a platform for the synthesis of differently substituted compounds.
Solvents play a significant role in the course of synthetic organic transformations. Compared to solvent phase reactions, solid phase reactions offer intimate contact of the substrates with the reagents\(^5\). We have explored the possibility of use of \(\text{pTSA}\) under solid-phase conditions for the deprotection of MOM ethers. \(\text{pPTS}^5\) and \(\text{pTSA}^4\) have been used earlier for the deprotection of MOM; but the reactions were carried out in solutions and often required refluxing temperatures and the reactions were worked up in the conventional manner. We found that MOM deprotection can be carried out under solvent-free conditions in solid phase (scheme 2). Vanillin MOM (1) was chosen as a model compound for this solvent-free deprotection. In the initial experiments, 1 (1 mmol) and \(\text{pTSA}\) (0.5 mmol) were triturated in a mortar for 5 min. After monitored by TLC, it was observed that on leaving the mixture for additional 30 min at room temperature, led to the completion of reaction. Being highly soluble, \(\text{pTSA}\) and other deprotection components such as \(\text{CH}_3\text{OH, HCHO}\), could be readily removed by water (4\(^\circ\)C) leaving the reaction products as precipitates. Product analysis (TLC) showed that reaction was only partially complete (only 70\% yield). The yield could be improved by increasing the amounts of \(\text{pTSA}\). Best result (yield 95\%) was obtained when the molar ratio of substrate to \(\text{pTSA}\) (from 0.5 to 1.5). The generality of the reaction could be established by using different substrates (tables 1 and 2). The mono-MOM substrates (entries 1-4) were cleaved efficiently with excellent yield (92-96\%). An alcoholic MOM (5) was also cleaved in quantitative yield under similar conditions. MOM derivative of naturally occurring, plumbagin (6) and 5-hydroxyquinoline (7) also underwent smooth deprotection. Substrates with di-MOM
protections (entries 8 and 9) were also deprotected efficiently with good yields (95 and 98\%) respectively. Deprotection of methyl tri-MOM gallate (10) required relatively higher reaction

<table>
<thead>
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<th>Entry</th>
<th>Substrates</th>
<th>Products</th>
<th>Yield (%)</th>
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<tr>
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<tr>
<td>5</td>
<td><img src="image" alt="MeO-MeO-OMOM" /></td>
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<td><img src="image" alt="Cl-O" /></td>
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<td>97</td>
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<tr>
<td>7</td>
<td><img src="image" alt="N-OMOM" /></td>
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<td>8</td>
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<td>98</td>
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<tr>
<td>9</td>
<td><img src="image" alt="OMOM-OMOM" /></td>
<td><img src="image" alt="OH-OH" /></td>
<td>95</td>
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time (15 min). MOM could be selectively deprotected in the presence of other protecting groups such as methoxy (entries 1, 3, 4, 5), ester (10), lactone (11), amide (13), benzyl (14) and allyl (15). It was found that MOM group was selectively removed in presence of aforementioned groups (table 2). MOM deprotection was not selective for acetyl group as seen in the case of entry 12. Acetyl group also underwent deprotection along with MOM.

Table 2. MOM selectivity in presence of other functional groups.

<table>
<thead>
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<th>Entry</th>
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<th>Products</th>
<th>Yield (%)</th>
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<td><img src="image11" alt="Substrate 15" /></td>
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<td>88</td>
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3. CONCLUSION

Methoxymethyl (MOM) groups are extensively used in synthetic organic chemistry. We have shown that it can be successfully cleaved with $p$TSA by trituration at room temperature under solvent free conditions (scheme 3). Deprotection was achieved in short reaction time (30 min) with high yield ranging from 85-98%. Organic solvent-free conditions, a feature of the green chemistry concept, were successfully used not only for these reactions but also for the isolation of the products. The reaction yielded products with high purity directly. This mild reaction conditions will be useful for the multi-stage syntheses of labile complex products for the drug discovery programmes.
4. EXPERIMENTAL

PART I

4.1. Synthesis of flavonols

4.1.1. General procedure; preparation of chalcones (6 & 10);

The chalcones (7) were prepared by the condensation of 2,4,6-tri-MOM phloracetophenones (1, 1 mmol) with corresponding MOM protected aldehydes (2, 1 mmol) in DMF (5 ml) using powdered KOH (1 mmol) under anhydrous conditions at 0-4°C for 4h. After monitored by TLC, water (20 ml) was added and extracted with dichloromethane (30 ml x 2). Dichloromethane was dried over anhydrous sodium sulphate and solvent was removed by distillation. The products were purified by column (SiO\textsubscript{2}) chromatography. Chalcones were obtained as light yellow colored solid with 78-88% yield. It absorb, UV at (MeOH): \( \lambda \) 305-327 nm and IR at (KBr): \( \nu \) 1647-1676 (CO) cm\(^{-1}\).

4.1.2. General procedure; preparation of epoxides (7 & 11);

A mixture of NaOH (4.8 ml, 5%) and H\textsubscript{2}O\textsubscript{2} (5.0 ml, 30%) were added to a stirred solution of chalcones (6 & 10, 1 mmol) in methanol (20 ml) at room temperature (stirred for 4h). Water (50 ml) was added and extracted with ethyl acetate (30 ml x 2), dried with anhydrous sodium sulphate and solvent removed under reduced pressure. The products were purified by column (SiO\textsubscript{2}) chromatography. The epoxides were obtained in 70-90% yield. It showing characteristic IR spectrum for epoxide at (KBr): \( \nu \) 882-884 and adjacent carbonyl group absorb at 1696-1700 cm\(^{-1}\). It absorb UV at (MeOH): \( \lambda \) 282-285 nm.

4.1.3. General procedure; preparation of dihydroflavonols (8 & 12);

Epoxides (7 & 11, 0.9 mmol) were taken in methanol (45 ml) and concentrated HCl (5 ml) were refluxed for 20 (min) and poured into ice-water. The product was collected by centrifugation. The colourless dihydroflavonols (8 & 12) were obtained in 52-60% yield. No
distinctive change was observed in the UV (MeOH): ranges from $\lambda$ 283-292 nm but IR spectra for carbonyl is reduced to IR (KBr): $\nu$ 1634-1644 cm$^{-1}$ due to loss of epoxide ring.

4.1.4. General procedure; preparation of Flavonols (4 & 13):
Dihydroflavonols (8 & 12, 0.3 mmol) in ethanol (2.5 ml) was added to potassium metabisulphite, (5.0 ml, 20%) and heated at 100°C (5-8 h). The reaction mixture is poured into crushed ice. The centrifuged product was purified by column (SiO$_2$) chromatography. The purity of the products were established by melting point, HPLC, $^1$H NMR and MASS spectral data. Characterization of the synthesized compounds are given below.

4.1.5. Characterization of flavonols
Isorhamnetin (4)
Isorhamnetin obtained as pale yellow solid. UV (MeOH): $\lambda$ 254, 370 nm. IR (KBr): $\nu$ 1675 cm$^{-1}$. $^1$H NMR (MeOD-$d_4$); $\delta$ 3.84, s, 3H; 6.28, d, ($J$ = 2Hz), 1H; 6.43, d ($J$ = 2Hz), 1H; 6.99 d ($J$ = 8.5Hz), 1H; 7.73, d ($J$ = 8.5Hz), 1H; 7.80, d ($J$ = 2Hz), 1H.

Dihydroisorhamnetin (8)
2,3-Dihydroisorhamnetin was obtained as dull white solid. UV (MeOH): $\lambda$ 288 nm. IR (KBr): $\nu$ 1644 cm$^{-1}$. $^1$H NMR (400 MHz, DMSO-$d_6$); $\delta$ 12.91, s, 1H; 10.8, s, 1H; 9.0, s, 1H; 7.11, d ($J$ = 1.6 Hz), 1H; 6.92, dd, 1H; 6.80, d ($J$ = 8 Hz), 1H; 6.92, d ($J$ = 2 Hz), 2H; 5.73, d ($J$ = 6.4 Hz), 1H; 5.06, d ($J$ = 11.2 Hz), 1H; 4.6, dd, 1H; 3.79, s, 3H.

Quercetin (13a)
Quercetin was obtained as yellow solid. UV (MeOH): $\lambda$ 255, 371 nm. IR (KBr): $\nu$ 1676 cm$^{-1}$. $^1$H NMR (MeOD-$d_4$); $\delta$ 6.26, d, ($J$ = 2Hz), 1H; 6.42, d ($J$ = 2Hz), 1H; 6.95, d ($J$ = 2Hz), 1H; 7.65, dd ($J$ = 2Hz), 1H; 7.75, d ($J$ = 2Hz), 1H.
**Kaempferol (13b)**

Kaempferol was obtained as yellow solid. UV (MeOH): \(\lambda\) 266, 366 nm. IR (KBr): \(\nu\) 1670 cm\(^{-1}\). \(^1\)H NMR (MeOD-\(d_4\)); \(\delta\) 6.27, d (\(J = 2.5\)Hz), 1H, 6.42, d (\(J = 2.5\)Hz), 1H, 6.94, d (\(J = 8.5\)Hz), 2H, 8.10, d, (\(J = 8.5\)Hz), 2H.

**Galangin (13c)**

Galangin was obtained as pale yellow solid. UV (MeOH): \(\lambda\) 266, 310, 358 nm. IR (KBr): \(\nu\) 1670 cm\(^{-1}\). \(^1\)H NMR (MeOD-\(d_4\)); \(\delta\) 12.08 (s, 1H), 8.26 (d, 2H), 7.54 (m, 3H), 6.56 (s, 1H), 6.30 (s, 1H).

**Tamarixetin (13d)**

Tamarixetin was obtained as light yellow solid. UV (MeOH): \(\lambda\) 256, 372 nm. IR (KBr): \(\nu\) 1670 cm\(^{-1}\). \(^1\)H NMR (MeOD-\(d_4\)); \(\delta\) 3.81, s, 3H; 6.14, d (\(J = 2\)Hz), 1H; 6.48, d (\(J = 2\)Hz), 1H; 7.03, d (\(J = 8.5\)Hz), 1H; 7.63, d (\(J = 8.5\)Hz), 1H; 7.79, d (\(J = 2\)Hz), 1H.

**Kaempferide (13e)**

Kaempferide was obtained as light yellow solid. UV (MeOH): \(\lambda\) 265, 365 nm. IR (KBr): \(\nu\) 1670 cm\(^{-1}\). \(^1\)H NMR (MeOD-\(d_4\)); \(\delta\) 3.90, s, 3H; 6.28, d (\(J = 2\)Hz), 1H; 6.44, d (\(J = 2\)Hz), 1H; 7.05, d (\(J = 9\)Hz), 2H; 8.17, d (\(J = 9\)Hz), 2H.

**PART II**

5.1. One pot syntheses of isoflavones

2′-hydroxyacetophenones (1 mmol) and substituted aldehydes (1 mmol) were taken in dry methanol (20 ml). To that powdered KOH (10 mmol) was added and stirred overnight at room temperature. After observing chalcones formation by TLC (toluene; methanol; formic acid- 5; 5; 2). Thallium nitrate (2 mmol) was added and stirred for 12 hr at room temperature, to the same reaction mixture, concentrated HCl (4 ml) was added and refluxed for 2-3 h. Reaction mass poured into ice and product was separated using centrifugation. Product purified through
column chromatography using silica (SiO$_2$) and products were obtained in solvents; petroleum ether/ethyl acetate mixtures. Compounds were characterized by UV, IR, NMR, MASS, melting point and compared with its known standards.

5.1.1. Characterization of isoflavones

**Genistein (15)**

Genistein was obtained as white powder. Yield; 78%. Mp; 295-298°C. UV (MeOH): λ 262, 329sh nm. IR (KBr): ν 1655 cm$^{-1}$. $^1$H NMR (400 MHz, DMSO-d6); δ 6.2, d (J = 2 Hz), 1H; 6.3, d (J = 2 Hz), 1H; 6.8, dd (J = 2 Hz), 2H; 7.3, dd (J = 2 Hz), 2H; 8.3, s, 1H; 9.5, s, 1H; 12.9, s, 1H.

**Diadzein (21a)**

Diadzein was obtained as dull white powder. Yield; 75%. Mp; 267-270°C. UV (MeOH): λ 249, 303sh nm. IR (KBr): ν 1657 cm$^{-1}$. $^1$H NMR (400 MHz, DMSO-d6); δ 6.1, d (J = 2 Hz), 1H; 6.3, d (J = 2 Hz), 1H; 6.9, dd (J = 2 Hz), 2H; 7.4, dd (J = 2 Hz), 2H; 8.23, s, 1H; 10.8, s, 1H; 12.8, s, 1H.

**Formononetin (21b)**

Formononetin was obtained as pale yellow powder. Yield; 80%. Mp; 254-257°C. UV (MeOH): λ 248, 259sh, 311 nm. IR (KBr): ν 1660 cm$^{-1}$. $^1$H NMR (400 MHz, DMSO-d6); δ 3.9, s, 3H; 6.8, d (J = 2 Hz), 1H; 6.9, dd (J = 2 Hz), 2H; 7.3, dd (J = 2 Hz), 2H; 7.8, d, (J = 2 Hz), 1H; 8.3, s, 1H; 10.9, s, 1H.

**Biochanin-A (21c)**

Biochanin-A was obtained at dull white powder. Yield; 85%. Mp; 210-212°C. UV (MeOH): λ 260, 39sh nm. IR (KBr): ν 1658 cm$^{-1}$. $^1$H NMR (400 MHz, DMSO-d6); δ 3.9, s, 3H; 6.7, dd (J = 2 Hz), 2H; 6.8, d (J = 2.4 Hz), 1H; 7.3, dd (J = 2 Hz), 2H; 8.2, s, 1H; 9.5, s, 1H; 10.7, s, 1H.
5.2. Anti-quorum sensing activity

A standard well-diffusion assay was used to detect the anti-QS activity of compounds by using a bio-monitor strain of *C. violaceum* ATCC12472, by the method described previously. Briefly *C. violaceum* was inoculated and the compounds to be tested were added to the wells and the plates were incubated overnight at 37°C and examined for violacein production. Anti-QS activity was detected by a colourless, opaque halo zone with viable bacterial cells around the well.

5.2.1. Bacterial strains, media and growth conditions

Anti-QS activity was determined by using *Chromobacterium violaceum* ATCC 12472, purchased from the American Type Culture Collection (ATCC). Biofilm assay were done in *Staphylococcus aureus* (MTCC 96) and *P. aeruginosa* PAO1 (*P. aeruginosa* ATCC 15692). For germ tube studies *Candida albicans* (MTCC 183) was used. Bacteria were routinely grown aerobically in Luria-Bertani (LB) broth and *Candida albicans* was grown in YEPD broth.

**PART III**

6. Preparation of MOM derivatives; selected procedure for 1

To the mixture (vanillin; 6.5 mmol and K₂CO₃; 8 mmol, in acetone), methoxymethyl chloride (8.1 mmol) was added and stirred for 1-2 hr. After completion of the reaction, (monitored by TLC; solvent system; hexane-7; ethyl acetate-3) reaction mass filtered and the product (>95% yield) was obtained on removal of the solvent. The MOM-vanillin was characterized by IR (film): ν 3076, 2929, 2845, 2071 cm⁻¹, ¹H NMR (400 MHz, DMSO-d₆); δ 3.3, s, 3H; 5.2, s, 2H and ¹³C NMR (400 MHz, DMSO-d₆); δ 55, 92 spectroscopy.

7. MOM deprotection by pTSA

MOM ether (5 mmol) and pTSA.H₂O (7.7 mmol) were triturated well in a mortar for 5 min (in the case of entry 10, trituration time was about 15 min), reaction mixture was left at room
temperature for another 30 min. After completion of the reaction (monitored by TLC), cold water (4°C) was added. The products were separated by centrifugation. The yields of the products ranged from 85-98%. The purities and the identities of the products were established by direct comparison with known compounds (TLC, Mp and IR). Details are given below.

8. Spectral data of selected compounds

Characterization of compound 4

IR (flim): ν 3076, 2929, 2845, 2071, 1992, 1739, 1681, 1591, 1510, 1462, 995 cm\(^{-1}\). \(^1\)H NMR: 400MHz (CDCl\(_3\)): δ 3.36, s, 3H; 3.88, s, 3H; 5.09-5.13, m, 2H; 5.21, s, 2H; 5.92-5.99, m, 1H; 6.71-6.75, m, 2H; 7.07-7.09, d, \((J = 8 \text{ Hz})\), 2H. \(^{13}\)C NMR 100MHz (CDCl\(_3\)): δ 39.8, 55.7, 56, 95.6, 112.3, 115.6, 116.7, 120.6, 134.5, 137.5, 144.7, 149.7.

Characterization of compound 10

IR (flim): ν 3058, 2928, 2860, 2428, 1676, 1388, 1255, 1093, 1049 cm\(^{-1}\). \(^1\)H NMR: 400MHz (CDCl\(_3\)): δ 3.35, s, 3H; 3.58, s, 6H; 3.87, s, 3H; 5.19, s, 4H; 5.23, s, 2H; 7.4, s, 2H. \(^{13}\)C NMR 100MHz (CDCl\(_3\)): δ 30.7, 35.7, 52.2, 55.8, 56.5, 59.7, 94.6, 97.7, 110.4, 124.9, 140.1, 150.4, 162.3, 165.5.

Characterization of compound 11

IR (flim): ν 3076, 2995, 2920, 2364, 1728, 1647, 1560, 1384, 918 cm\(^{-1}\). \(^1\)H NMR: 400MHz (CDCl\(_3\)): δ 3.35, s, 3H; 5.48, s, 2H; 5.90, s, 1H; 7.38-7.42, m, 2H; 7.65-7.70, m, 1H; 7.8, dd, 1H. \(^{13}\)C NMR 100MHz (CDCl\(_3\)): δ 56.9, 92.2, 95, 115, 116.4, 122.8, 124.2, 132.8, 152.8, 161.3, 163.

Characterization of compound 12

IR (flim): ν 2956, 2900, 2850, 2345, 1768, 1676, 1612, 1363, 1201, 1157 cm\(^{-1}\). \(^1\)H NMR: 400MHz (CDCl\(_3\)): δ 2.34, s, 3H; 2.51, s, 3H; 3.47, s, 3H; 5.20, s, 2H; 6.76, d (\(J = 2.4 \text{ Hz})\), 1H; 6.93, dd (\(J = 2.4 \text{ Hz})\), 1H; 7.80, d (\(J = 8.8 \text{ Hz})\), 1H. \(^{13}\)C NMR 100MHz (CDCl\(_3\)): δ 21.14, 21.16, 29, 56.3, 94.2, 111.4, 113.3, 123.9, 132.3, 132.34, 151.1, 161.3, 164.4, 195.8.
Characterization of compound 13

IR (flim): ν 3398, 3062, 2985, 2898, 2339, 1678, 1653, 1541, 1514, 1153 cm\(^{-1}\). \(^1\)H NMR: 400MHz (CDCl\(_3\)): δ 3.38, s, 3H; 5.24, s, 2H; 7.04, d (\(J = 8.8 \text{ Hz}\)), 2H; 7.83, d (\(J = 8.8 \text{ Hz}\)), 2H. \(^1^3\)C NMR 100MHz (CDCl\(_3\)): δ 55.6, 93.6, 115.3, 127.4, 129.2, 159, 167.4

Characterization of compound 14

IR (flim): ν 3062, 2985, 2845, 2362, 1654, 1597, 1255, 1136, 1020 cm\(^{-1}\). \(^1\)H NMR: 400MHz (CDCl\(_3\)): δ 2.44, s, 3H; 3.38, s, 3H; 4.69, s, 2H; 5.18, s, 2H; 6.47, dd (\(J = 2.2 \text{ Hz}\)), 1H; 7.32-7.38, m, 2H; 7.40-7.46, m, 3H; 7.61, d (\(J = 8.4 \text{ Hz}\)), 1H. \(^1^3\)C NMR 100MHz (CDCl\(_3\)): δ 31.7, 34.4, 54.4, 55.8, 69.8, 100.2, 108, 119.2, 127.7, 127.9, 128.2, 128.4, 128.6, 129.2, 132, 136.4, 137.9, 160.2, 163, 195.8.

Characterization of compound 15

IR (flim): ν 3082, 2908, 2792, 2362, 1664, 1600, 1496, 1421, 1259, 1155, 1001, 837 cm\(^{-1}\). \(^1\)H NMR: 400MHz (CDCl\(_3\)): δ 2.50, s, 3H; 3.39, s, 3H; 4.67, d (\(J = 5.2 \text{ Hz}\)), 2H; 5.26, s, 2H; 5.32, d (\(J = 1.2 \text{ Hz}\)), 1H; 5.37, d (\(J = 1.6 \text{ Hz}\)), 1H; 6.0-6.14, m, 1H; 6.67, d (\(J = 2 \text{ Hz}\)), 1H; 6.69, d (\(J = 2.4 \text{ Hz}\)), 1H; 7.64, d (\(J = 8.8 \text{ Hz}\)), 1H. \(^1^3\)C NMR 100MHz (CDCl\(_3\)): δ 31.6, 55.6, 69.1, 93.7, 101.3, 107.7, 117.9, 121.4, 131.5, 133, 159.4, 161.4, 196.5.

5. REFERENCES


