Journal of Exploratory Research in Pharmacology

Vol. 2 (2017) e1700025

DOI: 10.14218/JERP.2016.00025

Bio-inspired syntheses of partially methylated flavonoids – untapped source of bioactivities

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Received: September 30 2016; Revised: October 18 2016; Accepted: October 26 2016

Abbreviations: MOM, Methoxymethyl; MMP, Matrix metalloproteinases; NFkB, nuclear factor kappa-light-chain-enhancer of activated B cells; AFO, Algar Flynn Oyamada; CAN, Cerium ammonium nitrate.

Abstract

Alkylation of hydroxyl groups of flavonoids is known to increase their bioavailability and metabolic stability, and also to impart *new* bioactivities. Though partially-alkylated flavonoids and related compounds occupy substantial chemical space, there is scant information on their biological activities. They are comparatively less accessible, therefore development of their general syntheses are desirable. Chalcones play a key role in the biosynthesis of an array of flavonoids. Taking cues from nature, bio-inspired, ecofriendly syntheses of polyhydroxy- and partially-alkylated flavonoids have been developed. Compared to conventional *protection* groups, methoxymethylation was found to be more useful for the protection of hydroxyl groups. A library of flavones, flavonols, isoflavones and biflavones has been prepared. A brief account of our ongoing effort towards syntheses of small molecules is summarized here. Biological screening of the synthesized compounds has led to recognition of several hitherto unreported inhibitors of biomarkers for matrix metalloproteinases, nuclear factor-kappa B, carbonic anhydrase etc.

Keywords: Flavonoids; Partial methyl ethers; Methoxymethylation; Biomarkers; Matrix metalloproteinases; Nuclear factor kappa B, NFkB; Prodrugs.

Introduction

A growing number of pathogenic microbes are acquiring resistance against the existing drug regimes. This has necessitated the search for new drug molecules. The natural products provide great chemodiversity. Prominent phytochemicals, such as quercetin, kaempferol, luteolin genistein etc, have been subjected to detailed biological investigations. But, of late, it has been realized that many of these molecules may not be the actual active principles but represent *prodrugs* and express their activities after metabolic transformation or activations.[1] The actual active principles may be the metabolites, rather than the parent compounds. Therefore, easy access to the metabolites is very desirable for drug discovery programs.

It has already been established that most of dietary polyphenols that are ingested by humans undergo rapid transformation into more soluble products by conjugation, such as glucuronidation or sulfation. For example, common flavonoids such as quercetin, kaempferol, diosmetin and resveratrol are metabolized before reaching the target organ.[2] Methoxylated flavones may undergo demethylation by the tumour-specific enzymes to hydroxy flavones.[3] On the other hand, demethylation may be a process for bioactivation of naturally occurring prodrugs. Some reports have suggested that the polyhydroxy flavonoids are metabolized in the biological systems into more bioavailable partially-protected forms (e.g., methyl ethers), which may be the actual bioactive entity. The study of biological properties of analogues of the parent compounds, such as partial-methyl ethers and other intermediates, could be a good strategy for drug discovery.

Though apparently simple, the procurement of the partial-methyl ethers may not be a straight-forward process. The natural occurrence of such compounds is sporadic, presenting logistical problems for collection and isolation. Access to these compounds by conventional syntheses is possible but involves extensive protection and deprotection of functional groups and adds additional steps to the syntheses, making them lengthy, time consuming and ecologically unsound.[4] The availability of regio-specific syntheses of methyl ethers thus becomes very crucial for drug discovery studies.

In nature, most flavonoids occur as polyhydroxy compounds. These have been subjected to many investigations, including studies in human nutrition and metabolism. Yet, not much attention has been paid to the bioactivity of small molecules containing hydroxyl as well as methoxyl groups (partial-methyl ethers), although they occupy substantial chemical space. The lack of ready availability of such compounds could be the main cause for this apparent neglect. This has led us to develop bio-inspired, divergent, green syntheses, through which an array of compounds with different molecular architectures have been prepared.

Chalcones are the central molecule in the biosynthesis of flavonoids.[5] These are suitably functionalized molecules and are amenable for a variety of chemical transformations. They are readily accessible synthetically by Claisen condensation from the commercially available compounds with varied substituents. Taking inspiration from nature, we have utilized chalcones for many of the syntheses that will be described below.

Protection of hydroxyl groups is one of the most common steps in the syntheses of polyphenolics. Many methods for protection of hydroxyl groups are known. Methylation or benzylations or benzoylation are typically used for the protection of phenolic hydroxyls. They can be deprotected by several methods, such as hydrogenolysis or dealkylation. Thus, protection and deprotection increase steps in the syntheses.

Methoxymethylation (MOM) has certain advantages for protection of hydroxyls.[6] MOM derivatives can be synthesized easily. Being hemi-acetals, they are stable to bases but can be deprotected under mild acidic conditions. We have explored the compatibility of MOM under different experimental conditions. For protection, MOM has been applied under different reaction conditions, such as in the syntheses of flavonols, dihydroflavones, flavones, isoflavones and biflavones. A brief of account of syntheses is given below. The synthesized compounds have been subjected to screening for several bioactivities, leading to discovery of previously unreported activities. The bioactivities of these compounds will be discussed elsewhere.

1. Syntheses of flavonols

Amongst several methods, the traditional Allan-Robinson synthesis is often the method of choice for the synthesis of flavonols. It gives good yields and is of general applicability, but it employs very harsh experimental conditions and requires ingenious selective protection and deprotection of the free hydroxyls with methyl or benzyl or benzoyl groups.[4] An alternate method using the Algar Flynn–Oyamada (AFO) reaction yields flavonols directly, but the yields of the reaction are variable and it generates undesirable side products.[7,8] AFO reactions are not suitable for the preparation of partial-methyl ethers. To examine the compatibility of MOM protection under AFO conditions, synthesis of isorhamnetin was undertaken as a model experiment (Fig. 1). While isorhamnetin was synthesized earlier by the Allan-Robinson method, it was more recently obtained as a mixture by non-specific methylation of quercetin.[4] In particular, syntheses of partial-methyl ethers of quercetin were carried out in multiple steps through sequential protection and deprotection of phenolic hydroxyls through benzylation and methylation.[9] The described methods have limitations, however, since they require quercetin as starting material; further, the method lacks generality.

Our attempts to use the conventional AFO reaction for the synthesis of isorhamnetin with protected chalcones resulted in the formation of a mixture of compounds with low and variable yields.[10] In the present method, the synthesis of isorhamnetin was successfully carried out using

2′, 4′, 6′, 3-tetraMOM-4-methoxychalcone as the starting compound. MOM-protected chalcone was obtained by the reaction of 2, 4, 6-triMOM phloractophenone with 4-MOM vanillin to give the chalcone, which was easily converted into epoxide using alkaline H_2O_2. Treatment of the epoxide with methanolic HCl resulted in regio-specific opening of the epoxide ring, with concurrent removal of the MOM protections to give dihydroisorhamnetin in 55–60% yield (Fig. 1). Conversion of the dihydroflavonol to isorhamnetin was achieved by treating with potassium metabisulfite solutions. After completion of the reaction (monitored by thin layer chromatography, TLC), the reaction mixture was poured into crushed ice, upon which isorhamnetin was obtained as a pale yellow precipitate (55% yield).

Using this route, two other partial-methyl ethers–namely tamarexetin and kaempferide (kaempferol -4′ - methyl ether)–were also synthesised. Polyhydroxyflavones such as galangin, quercetin and kaempferol were synthesised as well. Incidentally, quercetin and kaempferol are the main constituents of a commercially important nutraceutical called "seabuckthorn flavone". The compounds synthesised thusly were evaluated for radical quenching properties and structure-activity relationship between different compounds were studied.[11]

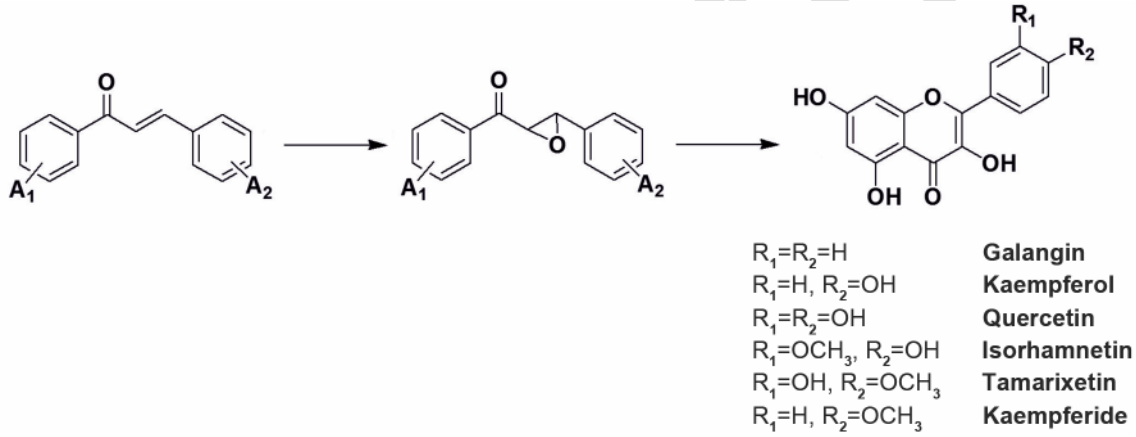

Fig. 1. Synthesis of flavonols.

2. Syntheses of flavones

Though several methods of syntheses of flavones have been reported, Baker-Venkataraman (BV) transformation is the most commonly used.[12] It is based on the acylation of aroyloxy esters of 2-hydroxyacetophenones through intramolecular Claisen condensation to the corresponding 1, 3-diketones, which upon acid catalysation of cyclodehydration yields flavones (Fig. 2). Though BV transformation is simple and works well for hydroxyl-protected (such as benzyl/ benzoyl/ methyl) compounds, they are not ideal for the preparation of polyhydroxy or partial-methyl ethers.[7] We have found that BV transformations are quite compatible with MOM-protected compounds.

Using appropriately protected starting materials, partially-methylated flavones like chrysoeriol, diosmetin, acacetin and tricin were prepared. Some of the compounds show remarkable bioactivities, such as modulation of matrix metalloproteinase (MMP), inhibition of nuclear factor-kappa B (NFkB)-regulated gene expression in the cancer cell lines HT1080 and MDA-MB-231, and inhibition of carbonic anhydrase.[13]

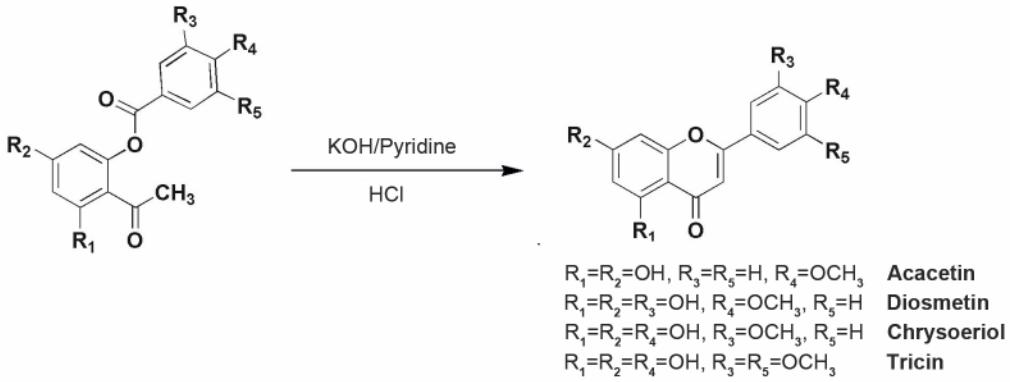

Fig. 2. Synthesis of flavones.

3. *Synthesis of biflavones*

Amongst the biflavones, (I-3, II-3)-biflavones are comparatively rare. Due to their limited occurrence, not much is known about their bioactivities. In a novel approach, 1, 3-diketones were dimerized to tetraketones using cerium ammonium nitrate oxidation (Fig. 3). The 1, 3-diketones were obtained by BV reactions, as described above. The tetraketones upon double-cyclodehydration yielded (I-3, II-3)-biflavones with good to excellent yield (78-88%). In practice, it was found that the dimerization of 1, 3-diketone and cyclodehydration takes place sequentially in the same flask without the need for isolation of the tetraketones.

Using this so-called "one pot" reaction, eleven (I-3, II-3)-biflavones, with different hydroxyl/methoxyl groups, were synthesized and screened for MMP inhibitory activities (e.g. MMP-2 and MMP-9). Among the compounds tested, biacacetin was found to be the best inhibitor [14]. *In silico* docking studies suggested that (I-3, II-3)-biacacetin inhibits the gelatinases through *non-zinc* binding interactions. The *non-zinc* binding nature of (I-3, II-3)-biacacetin is an important factor for designing selective molecules of therapeutic importance through inhibition of MMP-2 and MMP-9.

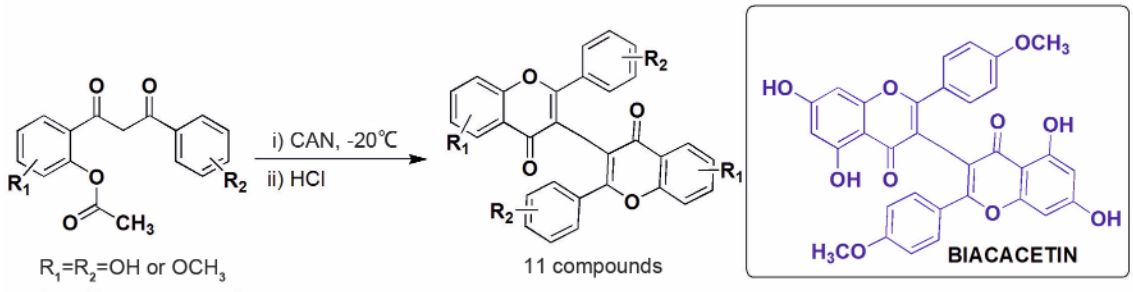

Fig. 3. "One-pot" synthesis of (I-3,II-3)-biflavones.

4. *Synthesis of isoflavones*

Isoflavones are important nutraceuticals, and are well known constituents of phytoestrogenic foods. The biological activities of soybeans, for example, have been attributed to isoflavones. The major isoflavones identified in soybean to date are genistein, biochanin-A, formononetin and daidzein. Oxidative cyclisation of chalcone by thallium (III) nitrate to

isoflavones is one of the widely used methods for their syntheses; however, this procedure does not work well with chalcones with unprotected hydroxyls.[15] We have shown that these reactions proceed smoothly with the MOM-protected chalcones.

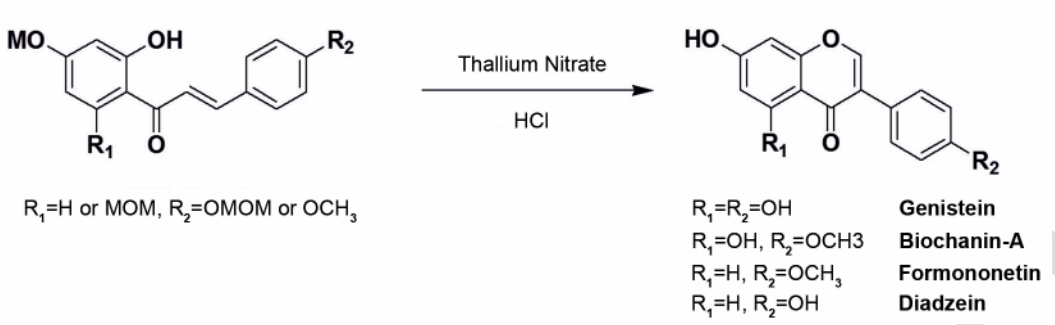

Fig. 4. Synthesis of soya isoflavones.

Using this method, MOM-protected chalcones were converted into hydroxylated/partial-methylated isoflavones using thallium nitrate (Fig. 4). Soya isoflavones such as genistein, biochanin A, formononetin and daidzein were also efficiently synthesized. Recently, the crude extracts of soya have been reported to have anti-biofilm activity, but the active principles remain to be identified and studies are underway to identify them.[16] Attempts have been made, in particular, to relate the activity with the active principles. To this end, individual pure compounds were subjected to screening for anti-quorum sensing activity. Of all the compounds tested, diadzein and formononetin were found to be effective against *Chromobacterium violaceum, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans.*

Conclusions

Due to lack of convenient synthesis, the lesser known flavones, such as partial-methyl ethers, have not received adequate attention for their bioactivities. We have developed convenient bio-inspired syntheses and generated libraries of such flavonols, flavones, isoflavones and biflavones (Fig. 5). As a result of screening of compounds, readily accessible novel inhibitors of biomarkers like MMP-2, MMP-9, NFkB and carbonic anhydrase were discovered.

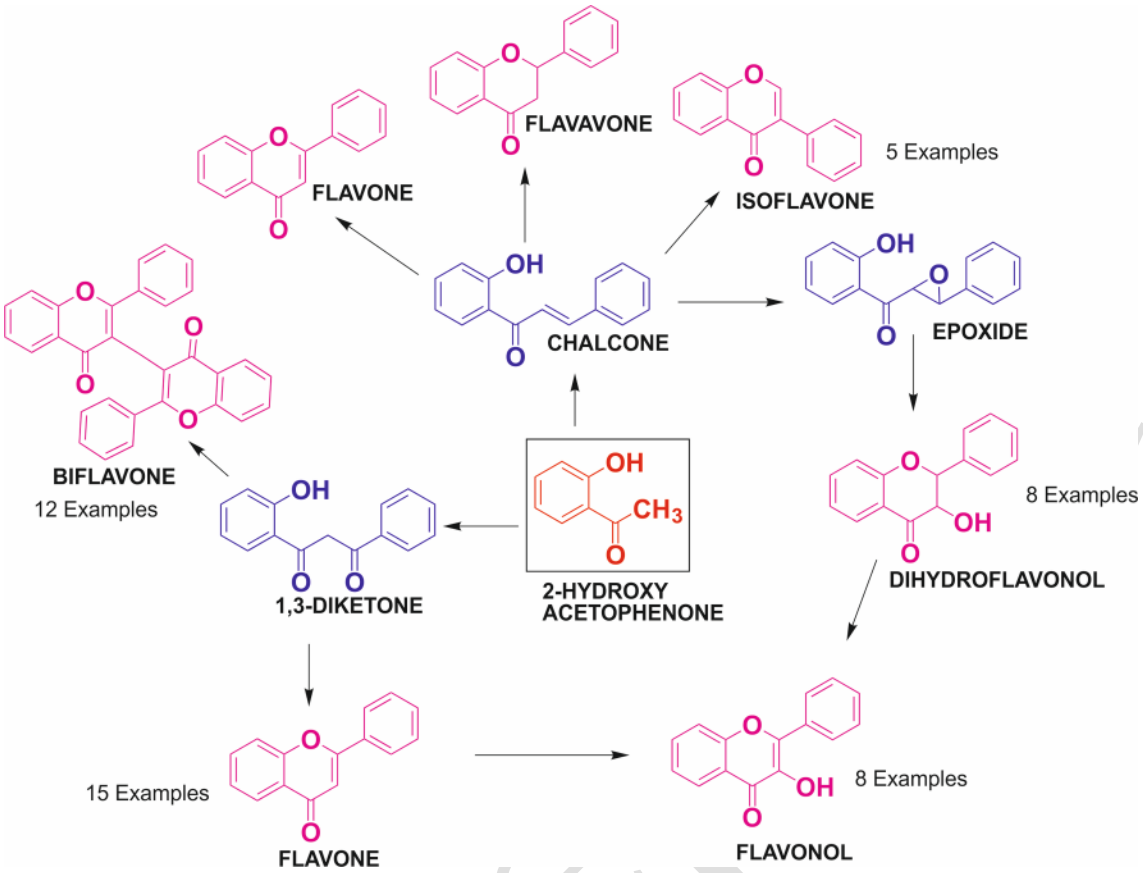

Fig. 5. Bio-inspired, diversity-oriented syntheses of oxygen heterocyclics.

Acknowledgements

Contributions of my colleagues who were associated with the work are acknowledged through the references mentioned below. I thank Dr. Bipin G. Nair, Dean, Amrita School of Biotechnology, for his keen interest in this work. I also acknowledge the help of Sri N. Pandurangan and Sri Chinchubose in the preparation of this manuscript. I gratefully acknowledge the financial support and facilities provided by the Amrita School of Biotechnology, Amrita University, Kerala, India.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Experimental design and drafting the manuscript (AB).

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