First synthetic report and antioxidant potential of natural product (±)-5,7-dihydroxy-8-methyl-3-(2′,4′-dihydroxybenzyl)chroman-4-one from Chinese medicine Gan Luo Xin pill

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ABSTRACT

The first synthetic route of naturally occurring (±)-5,7-dihydroxy-8-methyl-3-(2′,4′-dihydroxybenzyl)chroman-4-one (1) from Gan Luo Xin pill was successfully accomplished. The synthetic route has been developed retro-synthetically in 9 simple steps with a high yield of ~80%. The synthetic protocol was developed using readily available starting material phloroglucinol. The key intermediate 2,4,6-trihydroxy-3-methyl acetophenone (4) was synthesized via Vilsmeier–Haack reaction, followed by reduction using sodium cyanoborohydride and acylation reaction. LC-MS, IR, 1H NMR, 13C NMR of 1 have been analyzed to confirm the structure of (±)-5,7-dihydroxy-8-methyl-3-(2′,4′-dihydroxybenzyl)chroman-4-one (1) and found in agreement with the natural molecule. The target compound showed 97% and 87% antioxidant activity in DPPH and ABTS assay at 1 mg/ml concentration, respectively. The compound (1) also showed ferric ion reducing activity with the absorbance of 0.18 at 700 nm. The present study could be useful in developing synthetic routes of other potential naturally occurring homoisoflavonoid.

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1. Introduction

Natural products are untapped sources of enormous valuable substances. Researchers periodically explore them, just like digging the mines for valuable minerals and getting recognition (Ahmad et al. 2016, 2017). To date, innumerable natural products are...
isolated and evaluated for bioprospecting potential (Atanasov et al. 2021). Still, the luster of natural products never declined with time. Secondary metabolites produced by plants and fungi exhibit various pharmaceutical properties (Hussein and El-Anssary 2018). They remain an integral part of traditional medicine in many countries. The Gan Luo Xin pill is a traditional Chinese medical formula composed of 20 types of herbs (such as Panax ginseng, Astragalus membranaceus, Polygonatum sibiricum, and Crataegus pinnatifida, etc.) that has been shown effective for the treatment of hepatitis B (Li et al. 2014). It exhibits immunomodulatory and anti-inflammatory activities in therapeutics and has been proven curative in a clinical trial. In 2014, Li et al. reported the isolation and structural determination of two new homoisoflavonoids, (±)-5,7-dihydroxy-8-methyl-3-(2',4'-dihydroxybenzyl)chroman-4-one (1) and (±)-5,7-dihydroxy-6,8-dimethyl-3-(2',4'-dihydroxybenzyl)chroman-4-one (2), along with two known homoisoflavonoids, 5,7-dihydroxy-6-methyl-3-(2',4'-dihydroxybenzyl)chroman-4-one (3) and disporopsin (4). The structures of compounds 1-4 reported from the Gan Luo Xin pill by Li et al. (2014) have been displayed in Figure 1. Dimethyl analogs of homoisoflavonoid (2) reported from Genus Polygonatum have anti-inflammatory activity (Zhao et al. 2019) and insulin-stimulated glucose uptake (Zhou et al. 2015) activity. Compound 3 is also reported from Liriope platyphylla roots with anti-platelet activity (Tsai et al. 2013). Disporopsin (4) possesses cytotoxicity against MCF7 and A549 cell lines (Nguyen et al. 2006). Natural product isolation is reasonably necessary but suffers from limitations such as tedious isolation, environmental stress, low yield, seasonal and species variation. The synthesis of natural products can be helpful to overcome these limitations so that they can be available throughout the year. Our research group has previously developed the synthetic route for other natural products such as rugosaflavonoid A and podocarflavone A and evaluated them for biological activities (Puranik and Srivastava 2017; Puranik et al. 2021). Therefore, we focused on developing the synthetic route via retrosynthesis followed by characterization of recently isolated (±)-5,7-dihydroxy-8-methyl-3-(2',4'-dihydroxybenzyl)chroman-4-one (1) by the easy, economical and convenient method and evaluated it for antioxidant activity by DPPH, ABTS and ferric ion reducing assays. All the synthesized intermediates and target molecule (1) were spectroscopically evaluated by FT-IR, 1H NMR, 13C NMR, followed by LCMS analysis.

Figure 1. Structures of compounds isolated from the Gan Luo Xin pill.
2. Results and discussion

The literature survey on Structure-Activity Relationship (SAR) studies of homoisoflavonoid demonstrated that the methyl group at C-6/C-8 positions is responsible for biological activity (Nguyen et al. 2006). Therefore, retrosynthetic analysis of (±)-5,7-dihydroxy-8-methyl-3-(2',4'-dihydroxybenzyl) chroman-4-one (1) was planned by two different routes Figure 2: (Route 1, Route 2). It has been observed that substituted chalcone (5) and 1-(2,4,6-trihydroxy-3-methylphenyl)ethanone (4) are essential intermediates to complete its synthesis.
2.1. Retrosynthetic analysis

Route 1

(±)-5,7-Dihydroxy-8-methyl-3-(2’,4’-Dihydroxybenzyl)chroman-4-one (1) can be synthesized by hydrogenation of its unsaturated analog 3-(2,4-dihydroxybenzyl)-5,7-dihydroxy-8-methyl-4H-chromen-4-one (7) using Pd/C. The intermediate 7 can be prepared by cyclization of analog 3-(2,4-dihydroxyphenyl)-1-(2,4,6-trihydroxy-3-methylphenyl)propan-1-one (6). The chalcone 3-(2,4-dihydroxyphenyl)-1-(2,4,6-trihydroxy-3-methylphenyl)prop-2-en-1-one (5) can be used for hydrogenation using Pd/C to prepare 6. With the involvement of aldol condensation between 1-(2,4,6-trihydroxy-3-methylphenyl)ethanone (4) and 2,4-dihydroxy benzaldehyde, synthesis of chalcone 5 can be accomplished. The intermediate 4 is the basic requirement, which can be obtained via direct methylation of 1-(2,4,6-trihydroxyphenyl)ethanone (3). The substituted ethanone 3 can be synthesized (Route 1) via acylation of phloroglucinol. Therefore, readily and economically available phloroglucinol has been considered as a starting material for synthesizing homoisoflavonoid 1.

Route 2

Route 2 involves the synthesis of (±)-5,7-dihydroxy-8-methyl-3-(2’,4’-dihydroxybenzyl)chroman-4-one (1) by demethylation of 3-(2,4-dihydroxybenzyl)-5,7-dimethoxy-8-methylchroman-4-one (9). The compound 3-(2,4-dihydroxybenzyldiene)5,7-dimethoxy-8-methylchroman-4-one (8) could be used to prepare 9 by reducing the double bond via hydrogenation reaction using Pd/C. The intermediate 8 can be obtained by refluxing 2,3-dihydro-5,7-dimethoxy-8-methylchromen-4-one (7) with 2,4 dihydroxy benzaldehyde in piperidine. The intermediate 7 can be generated by hydrogenation of 5,7-dimethoxy-8-methyl-4H-chromen-4-one (6) using 10% Pd/C. The intermediate 6 can be synthesized by protecting the hydroxyl groups via methylation of 5,7-dihydroxy-8-methyl-4H-chromen-4-one (5). The intermediate 5 can be synthesized by cyclization of key intermediate 1-(2,4,6-trihydroxy-3-methylphenyl)ethanone (4). The intermediate 4 can be obtained via the acylation reaction of 2-methylphloroglucinol (3). The intermediate 3 can be obtained via the conversion of the aldehyde group of 2,4,6-trihydroxybenzaldehyde (2) to the methyl group. The intermediate 2 can be synthesized.
by introducing the formyl group in phloroglucinol at the desired position. Therefore, in Route 2, for the synthesis of 1, phloroglucinol has been chosen as the starting material.

In Route 1, acylation of phloroglucinol in the presence of AlCl₃/acetyl chloride in DCM: nitrobenzene (1:1), yielded 80% of 1-(2,4,6-trihydroxyphenyl)ethanone (3) (Zhou et al. 2017). Attempt to synthesize the target molecule via Route 1 was unsuccessful because the methylation reaction on 1-(2,4,6-trihydroxyphenyl)ethanone (3) using methyl iodide (Hawranik et al. 2009) as a methylating agent, K₂CO₃ as a base, and acetone as solvent at 0°C for 9 h led to the formation of multiple side products due to methylation of oxygen atoms. Therefore, instead of direct methylation of 3, Route 2 was considered to synthesize homoisoflavonoid 1. Following the planned Route 2, phloroglucinol was formylated using the Vilsmeier-Haack reaction. (Li et al. 2016) resulted in 2,4,6-trihydroxybenzaldehyde (2) with a 90% yield. The reaction of intermediate 2 with sodium cyanoborohydride in methanol produced 2-methylphloroglucinol (3) with a 65% yield (Elliger 1985). Based on constructive evidence, the synthesis of 1-(2,4,6-trihydroxy-3-methylphenyl)ethanone (4) was accomplished by the acylation of 3 using acetic anhydride/BF₃.etherate at 120°C (Li et al. 2016). The reaction of 4 with BF₃.etherate/mesyl chloride/DMF at 120°C yielded 70% of 5,7-dihydroxy-8-methyl-4H-chromen-4-one (5) (Zheng et al. 2013). O-methylation of 5 was carried out using dimethyl sulphate/K₂CO₃/acetone at reflux condition (Liu et al. 2015) for 3 hours to obtain intermediate 5,7-dimethoxy-8-methyl-4H-chromen-4-one (6, Yield 70%). Hydrogenation reaction was conducted using 10% Pd/C under H₂ atmosphere in ethyl acetate at room temperature (Damodar et al. 2018) to reduce double bond in 6 to achieve intermediate 2,3-dihydro-5,7-dimethoxy-8-methylchromen-4-one (7, Yield 80%). The reaction of 7 with 2,4-dihydroxy benzaldehyde in piperidine, at refluxed condition, provided intermediate 3-(2,4-dihydroxybenzylidene)5,7-dimethoxy-8-methyl-chroman-4-one (8) (Shaikh et al. 2012) with 60% yield. Reduction of 8 using 10% Pd/C under H₂ atmosphere in ethyl acetate at room temperature produced intermediate 3-(2,4-dihydroxybenzyl)-5,7-dimethoxy-8-methylchroman-4-one (9, Yield 75%) (Shaikh et al. 2013), which was finally demethylated using BBr₃ in DCM to afford the targeted natural product (±)-5,7-dihydroxy-8-methyl-3-(2’,4’-dihydroxybenzyl)chroman-4-one (1) with 80% yield (Lee et al. 2014). Scheme 1 represents the final synthetic route of (±)-5,7-dihydroxy-8-methyl-3-(2’,4’-dihydroxybenzyl)chroman-4-one.

The comparative structural characterization of both isolated (Li et al. 2014) and synthesized homoisoflavonoid (1) has been represented in Table 1 (Supplementary file 1: ST1), which has been found in good agreement with each other. Therefore, the synthesis of (±)-5,7-dihydroxy-8-methyl-3-(2’,4’-dihydroxybenzyl)chroman-4-one was successfully accomplished in 9 steps.

**Antioxidant activity**

The pure isomer (3R)-5,7-dihydroxy-8-methyl-3-(2’,4’-dihydroxybenzyl)-chroman-4-one of compound (1) reported from *Polygonatum odoratum* possess antioxidant potential (Zhou et al. 2015); hence the antioxidant activity of the compound (1) was also evaluated by DPPH, ABTS and Ferric ion reducing assays. The target homoisoflavonoid (1) showed DPPH radical scavenging activity of 97% at 1 mg/ml concentration. It also
showed ABTS radical scavenging activity of 87% at the same concentration. Ferric ion reducing ability can be observed by the increase in absorbance at 700 nm in the ferric ion reducing assay. In this regard, the synthesized homoisoflavonoid (1) showed increased absorbance at 700 nm with an increasing concentration from 0.06-1 mg/ml. The results are compared with BHA, BHT, and ascorbic acid. The % inhibition graphs of DPPH and ABTS radicals have been given in supplementary file 1 (Figure S5).

3. Experimental section

All the intermediates 2-9 are synthesized and characterized by 1H NMR. Their methodologies are given in supplementary file 1 (Supplementary file 1). It also contains the LCMS, 1H NMR, and 13C NMR spectra of compound 1 and graphs of DPPH, ABTS, and ferric ion reducing assays.

4. Antioxidant activity of homoisoflavonoid (1)

The compound was tested for free radical scavenging assays using DPPH, ABTS as per the reported protocol (Srivastava et al. 2012). The ferric ion reducing assay was performed to identify its reducing ability at 700 nm. The detail protocols are mentioned in Supplementary file 1.

5. Conclusion

Natural products represent a wide variety of molecules with enormous bioprospecting potential. The development of synthetic routes can enhance their availability throughout the year to reduce environmental stress by cutting the plants repeatedly. The developed synthetic route of (±)-5,7-dihydroxy-8-methyl-3-(2',4'-dihydroxybenzyl) chroman-4-one (1) in 9 steps with economically available reagents will work as an insight for the chemist to do biologically active natural product synthesis. The synthesized natural product (1) showed antioxidant activity, enhancing its application as a ROS scavenger.

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Disclosure statement

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