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Samin-derived flavonolignans, a new series of antidiabetic agents having dual inhibition against α-glucosidase and free radicals

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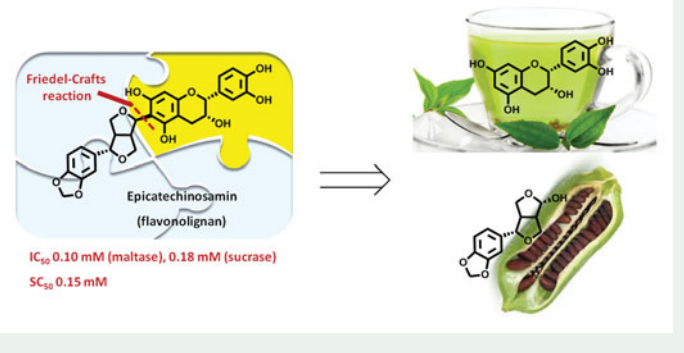
ABSTRACT

A series of novel flavonolignans were synthesized by the reaction between a lignan named samin (**1**) and a range of flavonoids. This simple and rapid approach allowed direct assembly of these two bulky motifs in good yields without the formation of byproducts. Upon evaluation of antidiabetic activity of the synthesized products, epicatechinosamin (**β-2g**) was the most active α-glucosidase inhibitor toward maltase and sucrase. The kinetic study indicated that **β-2 g** inhibited the enzymes in a mixed manner of competitive and noncompetitive inhibition.



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Flavonolignan; diabetes; glucosidase; antioxidant; Friedel-Crafts; oxocarbenium

1. Introduction

Prolonged postprandial hyperglycemia commonly detected in Diabetes mellitus (DM) patients has been recognized to trigger glucose autoxidation and free radical overproduction, eventually leading to oxidative stress and inducing β-cell dysfunction or failure (Rahimi et al. 2005). Therefore, type 2 DM therapy and treatment of complications can be successfully achieved by simultaneously attenuating hyperglycemia and suppressing glucose autoxidation. However, oral administration of combined antihyperglycemic drugs and antioxidants

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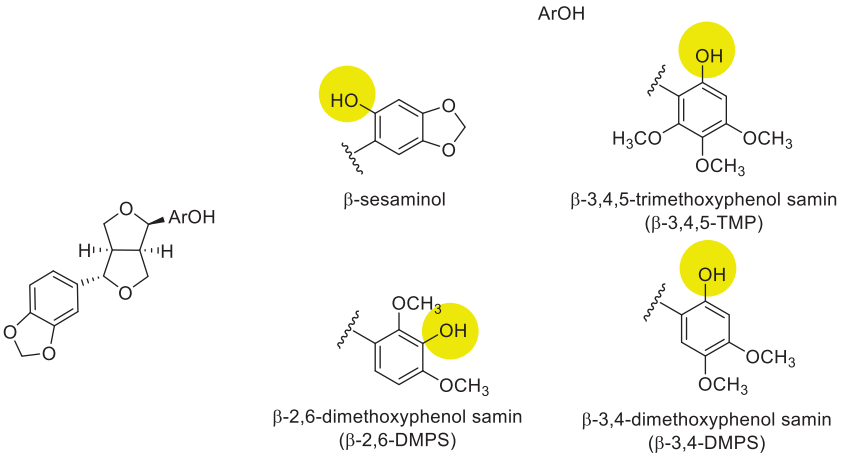


Figure 1. Structures of synthesized antidiabetic lignans.

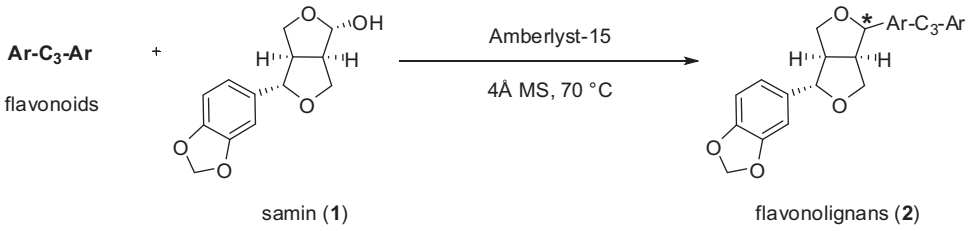
must be carefully performed due to Drug-drug interactions and unexpected adverse effects. To avoid this concern, the idea of 'One Drug Dual Function (ODDF)' has been introduced as an alternative approach (Wang et al. 2012). We adopted the ODDF approach to synthesize such compounds and assess antihyperglycemic activity through α-glucosidase inhibition as well as antioxidation through free radical scavenging. The success of this strategy resulted in the discovery of new ODDFs such as quercitylcinnamates (Rattanangkool et al. 2013) and *N*-arylmethylaminoquercitols (Worawalai et al. 2015). To our knowledge, acarbose, a widely used antihyperglycemic drug possessing α-glucosidase inhibition, has not been reported for benefit in suppressing DM-associated complications.

In our continuing work on synthesis of new ODDFs, we recently synthesized a new series of furofuran lignans (Worawalai et al. 2016) by direct coupling between a lignan named samin and a variety of phenolic compounds. Of twenty products synthesized, lignans encompassing at least one free phenolic (Ar-OH) moiety (Figure 1) showed more potent inhibition against α-glucosidases and free radicals than their parent starters. This observation suggests that inhibitory potency would largely depend on the number of free phenolics. To further explore more potent ODDFs and gain insight into the role of the phenolic group on inhibiting α-glucosidase and free radicals, a new series of furofuran lignans containing multiphenolic residues were synthesized. In the present study, we plan to apply flavonoids (Ar-C_3-Ar) as multiphenolics and samin (**1**) as a more reactive lignan starter (Scheme 1). We expect that the newly generated products, collectively named flavonolignans (**2**), would display more potent inhibition than furofuran lignans previously reported.

2. Results and discussion

2.1. Synthesis and characterization

Samin (**1**), used as starting lignan, was obtained from sesamolin, a major lignan isolated from sesame (*Sesamum indicum*) seed oil, *via* acid hydrolysis (Scheme S1, Supplementary material, Worawalai et al. 2016). With starting samin (**1**) in hand, we first evaluated the scope of flavonoids capable of undergoing Friedel-Crafts reaction. We screened six different types of flavonoids, namely dihydrochalcone, chalcone, flavanone,



Scheme 1. Synthesis of flavonolignans.

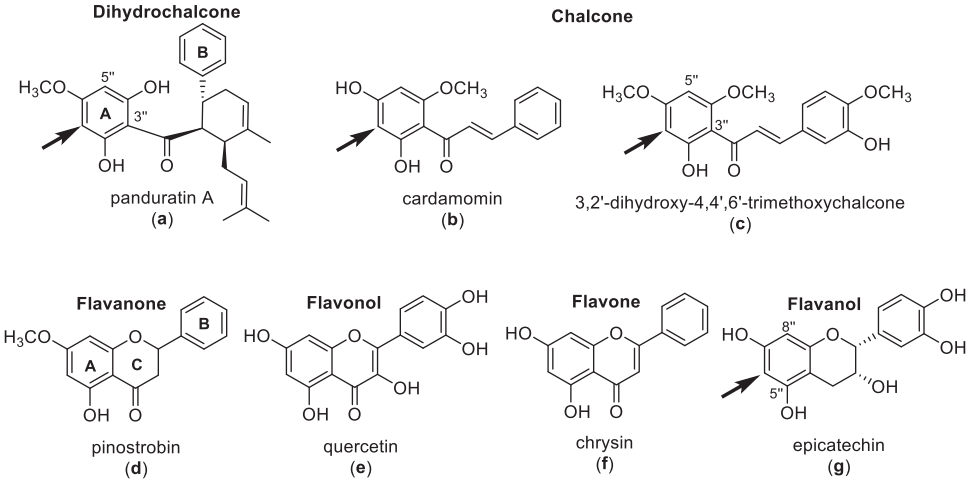


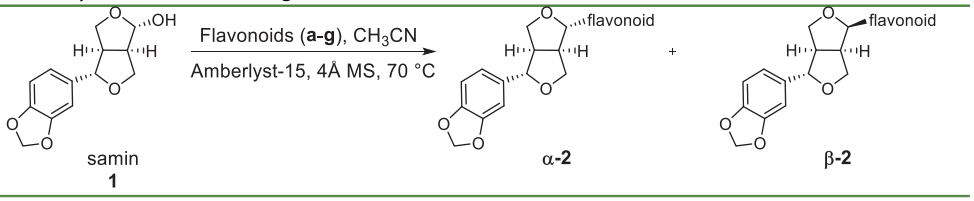
Figure 2. Flavonoids (**a**–**g**) used in synthesis of flavonolignans. The bold arrow indicates where the bonding between flavonoid and furan moiety was formed.

flavonol, flavone, and flavanol (Figure 2). It was expected that ring A of flavonoids would be substituted by samin (**1**) as oxocarbenium ion. When the process was complete, the reactions between samin (**1**) and three different flavonoids, namely dihydrochalcone (**a**), chalcones (**b** and **c**) and flavanol (**g**), furnished the expected products.

The reaction between samin (**1**) and panduratin A (**a**) in an acidic solution (Amberlyst-15, CH_3CN) at 70 °C furnished a single spot of flavonolignan, trivially named panduratinosamin (**α–2a**), in 33% yield (Table 1). The connectivity between samin (**1**) and flavonoid **a** in **α–2a** was proved by HMBC correlations shown in Figure S1a. The relative configuration of the newly generated chiral center C-2 on the furan motif was determined by analysis of multiplicity of the neighboring proton H-4 (Worawalai et al. 2016). The α-isomer revealed doublet of doublet (dd) for both H-4_{ax} and H-4_{eq} while the β-isomer showed the dd pattern only for H-4_{ax}, but a unique doublet (d) for H-4_{eq}. This observation was fully proved by NOESY analysis and also provided an effective means for rapid recognition of α- and β-isomers. We therefore adopted the above observation to predict the C-2 configuration of panduratinosamin (**α–2a**). The diastereomeric methylene protons H-4 displayed dd signals at δ_H 3.83 and 4.57 ppm, thus indicating α-configuration.

With the success on the synthesis of flavonolignan **α–2a** in hand, we subsequently examined the reactions between samin (**1**) and chalcones **b** and **c**. Under similar conditions, a pair of diastereomeric products named α-cardamominosamin (**α–2b**, 40%)

Table 1. Synthesis of flavonolignans.



	Isolated product (% Yield)	
Entry	α–2	β–2
1	**α–2a** (33%)	**β–2a** (NI)[a]
2	**α–2b** (40%)	**β–2b** (34%)
3	**α–2c** (75%)	**β–2c** (NI)
4	**α–2d** (NR)[b]	**β–2d** (NR)
5	**α–2e** (NR)	**β–2e** (NR)
6	**α–2f** (NR)	**β–2f** (NR)
7	**α–2g** (NI)	**β–2g** (52%)

[a]Not isolated. Due to trace amount, structure characterization and bioactivity evaluation could not be accomplished.
[b]No reaction.

and β-cardamominosamin (**β-2b,** 34%) were produced via the reaction between **1** and cardamomin (**b**). The occurrence of both **α–2b** and **β–2b** clearly supported the mechanistic formation of flavonolignans through a Friedel-Crafts reaction (Figure S2). Similarly, flavonolignan **α-2c**, named 3,2′-dihydroxy-4,4′,6′-trimethoxychalconosamin, was also generated a 75% yield from **1** and **c**. In addition, epicatechinosamin (**β–2g**) with 52% yield was also generated by the reaction between samin (**1**) and epicatechin (**g**). However, the reaction between samin (**1**) and flavonoids having ring C or the cyclic acyl group, namely flavanone **d**, flavonol **e** and flavone **f**, afforded no desired product, thus indicating that the acyl group deactivates aromaticity of ring A.

2.2. α-Glucosidase inhibitory activity

All of the synthesized flavonolignans were evaluated for α-glucosidase inhibition toward rat intestinal maltase and sucrase, compared with furofuran lignans having one free hydroxy group on phenolic moiety (Figure 3). Generally, all flavonolignans and furofuran lignans inhibited maltase more selectively than sucrase. The inhibitory activity of them tends to increase with the increasing of the number of phenolic hydroxy group except for **α–2c**. However, **α–2a** showed weak inhibition against only sucrase. Of flavonolignans examined, **β–2g**, having four phenolic hydroxy groups, was the most potent inhibitor against maltase and sucrase. We hypothesize that the presence of the catechol group as ring B of the flavonoid moiety might participate in chelation with enzymes, thus enhancing inhibitory activity (Kim et al. 2018). Noticeably, comparable inhibitory effects of two epimeric flavonolignans **α–2b** and **β–2b** suggested that the configuration of C-2 plays no role in bioactivity.

2.3. Antioxidant activity

All of the synthesized flavonolignans were also evaluated for radical scavenging, compared with furofuran lignans having one free hydroxy group on phenolic moiety (Figure S5). For this assay, phenolic hydroxy group could donate hydrogen radical to

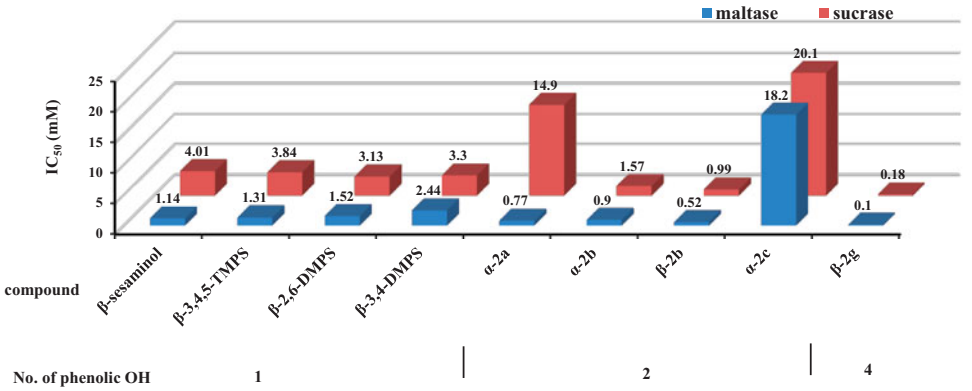


Figure 3. α-Glucosidase inhibitory effects of flavonolignans against maltase and sucrase.

terminate radical propagation, thus preventing the onset of cellular impairment. Of the lignans examined, β-sesaminol, β-2,6-DMPS, **α–2c**, and **β–2g** showed strong inhibition against free radicals in the range of 0.15-0.94 mM. Among them, **β–2g**, having four phenolic hydroxy groups, was the most potent inhibitor.

2.4. Kinetic study

In order to gain further insight into how these flavonolignans interact with rat intestinal maltase and sucrase, the inhibition mode of **β–2g**, the most active inhibitor chosen as representative, was analyzed in a kinetic study. The Lineweaver-Burk plot of **β–2g** against maltase (Figure S4a) showed a series of straight lines; all of which intersected in the second quadrant. Kinetic analysis subsequently showed that V_{max} decreased with elevated K_m in the presence of increasing concentrations of **β–2g**. This behaviour suggests that **β–2g** inhibited maltase in a mixed-type manner comprising two different pathways: competitive and noncompetitive. The observed result was elaborated by simultaneous formation of enzyme-inhibitor (EI) and enzyme-substrate-inhibitor (ESI) complexes in competitive and noncompetitive manners, respectively (Scheme S2).

We further investigated the pathway in which **β–2g** was preferentially preceded by determining dissociation constants of EI (K_i) and ESI (K'_i) complexes (Table S1). Apparently, the secondary plots (Figures S6a and S6b) demonstrate K_i and K'_i values of 0.15 and 0.40 mM, respectively, thus indicating that **β–2g** was predominantly bound to maltase (EI) rather than the formed ESI complex. The putative inhibitory mechanism is summarized in Scheme S2. The inhibitory mechanism of **β–2g** against sucrase (Figure S4b) was also examined using the above methodology. Apparently, **β–2g** inhibited sucrase *via* both competitive and noncompetitive manners (mixed-type inhibition) with K_i and K'_i values of 0.42 and 0.84 mM, respectively (Figures S7a and S7b).

3. Experimental section

3.1. Extraction and isolation of natural flavonoids (a and b) and sesamolin

Panduratin A (**a**) and cardamomin (**b**) were isolated from fingerroots (*Boesenbergia rotunda*). Sesamolin was obtained from sesame seed oil using the methodology

described elsewhere with minor modification (Reshma et al. 2010). The detail of isolation and purification of flavonoids (**a** and **b**) and sesamolin as well as their ^{1}H NMR data were supplied as supplementary material.

3.2 Chemistry

3.2.1. Synthesis of samin (1)

To a solution of sesamolin (0.3 mmol) in a mixture of acetonitrile-H_2O (9.5:0.5, 10 mL) was added Amberlyst-15 (1 mg/0.005 mmol of sesamolin). After stirring at 70 °C for 5 h, the reaction mixture was filtrated, evaporated to dryness and purified by silica gel chromatography using 1:1 EtOAc-hexane as mobile phase to afford samin (**1**, 95%) as colorless oil. NMR data of **1** were nearly identical to those reported previously (Maiti et al. 1995).

3.2.2. General procedure for synthesis of flavonolignans 2

To a solution of samin **1** (1 equiv) in acetonitrile (1.0 mL/0.1 mmol of **1**) was reacted with flavonoids (1.5–2 equiv), Amberlyst-15 (1 mg/0.005 mmol of **1**) and 4 Å molecular sieve. After stirring at 70 °C for 8 h, the reaction mixture was evaporated to dryness and purified by flash or Sephadex LH-20 chromatography to yield flavonolignans **2**. The NMR data and spectra of flavonolignans **2** were supplied as supplementary material.

3.3. α-Glucosidase inhibitory activity

α-Glucosidase inhibitory activity against rat intestinal maltase and sucrase was determined according to our previous report (Ramadhan and Phuwapraisirisan 2015). For details of the experiment, see suplementary material.

3.4. Kinetic study of α-glucosidase inhibition

For kinetic analyses of maltase by the active compound, enzyme and active compound were incubated with increasing concentrations of maltose (2–20 mM). The type of inhibition was determined by the Lineweaver-Burk plot. For calculation of K_i and K_i' values, slope and intercept from the Lineweaver-Burk plot were replotted vs. [I], which gave the secondary plot.

3.5. Antioxidant activity

Radical scavenging activity was validated using DPPH colorimetric method (Hirose et al. 2013).

4. Conclusion

In conclusion, we first synthesized a series of novel flavonolignans *via* a single-step reaction between samin (**1**) and a variety of flavonoids in the presence of acidic resin. Of the synthesized flavonolignans, **β–2g** possessed the strongest inhibition against

α-glucosidase (maltase and sucrase) and free radicals. The preliminary structure-activity relationships displayed two free hydroxyl groups on ring A and a catechol moiety on ring B in the newly introduced flavonoid unit enhanced activities. The highly potent antioxidant activity and α-glucosidase inhibition of **β–2g** should be beneficial in diabetes treatments as well as in preventing the onset of its complications.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

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