Eight new biflavonoids with lavandulyl units from the roots of *Sophora flavescens* and their inhibitory effect on PTP1B

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A B S T R A C T

In the course of studying the components from the roots of *Sophora flavescens*, eight new unusual biflavonoids consisting of a flavanone fused with a dihydrochalcone skeleton were isolated. These new chemical structures were elucidated by means of UV, IR, HRESIMS, NMR and ECD spectroscopic data and a comparison of experimental ECD spectra with calculated ECD spectra. Some compounds were subjected to an antidiabetic bioassay on human recombinant PTP1B inhibition, and showed strong inhibitory activity.

1. Introduction

Biflavonoids generally consist of two identical or nonidentical flavonoid moieties, and can be considered a subclass of plant flavonoids [1]. Pharmacological investigations on biflavonoids have indicated their anti-inflammatory, anti-cancer, anti-diabetic, anti-microbial, anti-viral, anticlotting, anti-bacterial and anti-oxidant activities[2–4]. Moreover, the bioactivity of a biflavonoid is stronger than that of its composition monomers under many circumstances[5–7]. According to their conjugation patterns, biflavonoids are further divided into three linkage types: C—C type[8–14], C—O—C type[6,15–18], and a combined C—C/C—O—C type[19–20]. Literature research has revealed that two flavonoid units are most commonly dimerized through a C—C bond. Within the C—C linkage type, the majority of the biflavonoids are formed through an interflavonoid linkage between the C-8/6 and C-3′, C-2′, C-3, C-6 or C-8.

Sophora flavescens Aiton (Leguminosae) is a shrub widely distributed in Asia[21] from which a large number of flavonoids[22–25] have been found. Our preliminary screening assay of the ethyl acetate fraction of the ethanol extract of *S. flavescens* displayed remarkable inhibitory activity against PTP1B with an IC_{50} value of $1.37\,\mu g \cdot ml^{-1}$. Further study of the chemical components of the ethyl acetate fraction contributed to the isolation of eight new, uncommon C—C type biflavonoids formed through a rare $C_5\text{-}C_\beta$ or $C_3\text{-}C_\beta$ bond. Their structures were elucidated by extensive spectroscopic methods, and the inhibition effects on PTP1B were evaluated in *vitro*.

2. Experimental

2.1. General procedures

The optical rotations and UV and ECD spectra were measured on JASCO P-2000, JASCO V-650, and JASCO J-815 spectrometers (JASCO, Easton, MD, USA), respectively. IR spectra were recorded on a Nicolet 5700 spectrometer using an FT-IR microscope transmission method (Thermo Scientific, Waltham, MA, USA). NMR spectra were obtained on a Bruker AVIII-500 spectrometer (Bruker-Biospin, Billerica, MA, USA). HRESIMS were performed on an Agilent 6520 HPLC-Q-TOF (Agilent Technologies, Waldbronn, Germany). The reversed-phase preparative HPLC was performed utilizing a Shimadzu LC-10AT instrument equipped with a YMC-Pack ODS-A column (250 mm × 20 mm, 5 μm; YMC Corp., Kyoto, Japan). Silica gel (100–200 mesh, Qingdao Marine Chemical Inc. Qingdao, People's Republic of China), RP-C_{18} (50 μm, YMC, Kyoto, Japan) and Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden) were employed for column chromatography. The HPLC experiments were performed using an Agilent 1260 system with an Apollo C_{18} column (250 × 4.6 mm, 5 μm, Grace Davison). TLC was carried out with glass precoated silica gel GF254 plates. Spots were visualized under UV light or by spraying with 10% H_2SO_4 in absolute EtOH followed by heating.

2.2. Plant material

The roots of *S. flavescens* were collected in Weichan City (Hebei

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Province, China) in July 2010. The plant material was identified by Prof. L. Ma. A voucher specimen (ID-5-2438) was deposited at the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, China.

2.3. Extraction and isolation

The dried powdered roots of *S. flavescens* (39.0 kg) were extracted with 95% EtOH (2 × 2 h) and 70% EtOH (1 × 2 h) under reflux. The filtrate was evaporated in *vacuo* to give a crude extract (8.4 kg), which was suspended in H_2O and extracted successively with petroleum ether, ethyl acetate, and *n*-butanol. The EtOAc fraction (1.5 kg) was chromatographically separated on a silica gel column eluted with PE, PE/ EtOAc (1:1), EtOAc, EtOAc/acetone (1:1), acetone, acetone/methanol (1:1) and methanol to give fractions A-G. Fraction C (127 g) was further subjected to a silica gel column eluted with a gradient of CH_2Cl_2-MeOH (40: 1–2: 1), yielding fractions C1-C15. Fraction C10 (16.3 g) was further purified on an RP-C_{18} column eluted with H_2O/MeOH (from 80:20 to 0:100) to give 16 fractions (C10.1–C10.16) based on TLC and HPLC analysis. Fraction C10.14 (125 mg) was separated by preparative HPLC (MeOH/H_2O, 80:20, v/v, detection wavelength UV 280 nm) to provide **1** (57.1 mg) and **3** (9.1 mg). Fraction C10.13 (72 mg) was separated by preparative HPLC (MeOH/H_2O, 78: 22, v/v, detection wavelength UV 280 nm) to obtain **2** (5.5 mg) and **5** (12.1 mg). Fraction C10.9 (50 mg) was separated by preparative HPLC (MeOH/H_2O, 75: 25, v/v, HOAc 0.05%, detection wavelength UV 280 nm) to give **4** (5.0 mg). Fraction C6 (4.27 g) was chromatographically separated by means of an RP-C_{18} column using MeOH/H_2O (40–100%, with 10% stepwise increase of MeOH) to yield 14 fractions. Fraction C6.9 (1.17 g) was purified by column chromatography on Sephadex LH-20 eluted with MeOH/H_2O (75: 25, v/v) to give fractions C6.9.1-C6.9.9. Subsequently, fraction C6.9.7 (253.9 mg) was chromatographed on Sephadex LH-20 eluted with MeOH/H_2O (60: 40, v/v) to afford **6** (5.2 mg), **7** (7.8 mg) and **8** (29.2 mg).

2.3.1. Sophobiflavonoid A (1)

Orange, amorphous powder; [α]20 D − 61.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 290 (4.37), 226 sh (4.52) nm; ECD (*c* 1.0 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 337 (+6.22), 294 (−15.44), 241 (+6.60), 217 (−32.68) nm; IR ν_{max} 3269, 1603, 1503, 1450 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 899.3946 [M + Na]$^+$ (calcd for $C_{52}H_{60}O_{12}Na$, 899.3977).

2.3.2. Sophobiflavonoid B (2)

Yellowish, amorphous powder; [α]20 D + 38.7 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 290 (4.41), 226 sh (4.55) nm; ECD (*c* 1.0 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 337 (−4.81), 295 (+15.52), 240 (−3.88), 214 (+46.41) nm; IR ν_{max} 3294, 1602, 1503, 1449 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 899.3933 [M + Na]$^+$ (calcd for $C_{52}H_{60}O_{12}Na$, 899.3977).

2.3.3. Sophobiflavonoid C (3)

Orange, amorphous powder; [α]20 D − 19.9 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 290 (4.23), 226 sh (4.39) nm; ECD (*c* 1.0 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 337 (−1.92), 293 (+6.26), 243 (+2.53), 226 (−2.54), 204 (−8.98) nm; IR ν_{max} 3264, 1602, 1504, 1450 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 899.3963 [M + Na]$^+$ (calcd for $C_{52}H_{60}O_{12}Na$, 899.3977).

2.3.4. Sophobiflavonoid D (4)

Orange, amorphous powder; [α]20 D − 25.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 290 (4.82), 225 sh (4.96) nm; ECD (*c* 1.0 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 335 (+2.21), 293 (−6.25), 245 (−1.65), 226 (+4.03), 207 (+11.61) nm; IR ν_{max} 3396, 3310, 1597, 1503, 1449 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 917.4105 [M + Na]$^+$ (calcd for $C_{52}H_{62}O_{13}Na$, 917.4083).

2.3.5. Sophobiflavonoid E (5)

Yellowish, amorphous powder; [α]20 D − 20.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 291 (4.22), 226 sh (4.42) nm; ECD (*c* 1.0 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 337 (+2.29), 302 (−4.75), 278 (+1.21), 245 (−1.65), 240 (+9.17), 215 (−19.23) nm ; IR ν_{max} 3351, 1605, 1505, 1449 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 915.3902 [M + Na]$^+$ (calcd for $C_{52}H_{60}O_{13}Na$, 915.3926).

2.3.6. Sophobiflavonoid F (6)

Yellowish, amorphous powder; [α]20 D − 83.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 294 (4.34), 225 sh (4.46) nm; ECD (*c* 1.0 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 294 (−11.78), 249 (+4.62), 233 (−2.87), 213 (+36.28) nm ; IR ν_{max} 3387, 1613, 1509, 1451 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 3 and 2; HRESIMS *m/z* 899.3955 [M + Na]$^+$ (calcd for $C_{52}H_{60}O_{12}Na$, 899.3977).

2.3.7. Sophobiflavonoid G (7)

Yellowish, amorphous powder; [α]20 D + 129.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 297 (4.46), 225 sh (4.57) nm; ECD (*c* 1.0 mg/ mL, MeOH) λ_{max} ($\Delta\varepsilon$) 310 (+24.33), 286 (−13.38), 217 (+28.71) nm ; IR ν_{max} 3409, 1613, 1508, 1452 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 3 and 2; HRESIMS *m/z* 899.3958 [M + Na]$^+$ (calcd for $C_{52}H_{60}O_{12}Na$, 899.3977).

2.3.8. Sophobiflavonoid H (8)

Yellowish, amorphous powder; [α]20 D − 152.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 298 (4.43), 226 sh (4.53) nm; ECD (*c* 1.0 mg/ mL, MeOH) λ_{max} ($\Delta\varepsilon$) 311 (−40.25), 285 (+23.27), 216 (−20.46) nm; IR ν_{max} 3409, 1605, 1508, 1452 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 3 and 2; HRESIMS *m/z* 899.3969 [M + Na]$^+$ (calcd for $C_{52}H_{60}O_{12}Na$, 899.3977).

2.4. PTP1B inhibition assay

The PTP1B inhibition assay used the same method as described in the reference[26].

3. Results and discussion

3.1. Phytochemical investigation

Sophobiflavonoid A (**1**) was obtained as an orange amorphous powder, giving a typical flavanone skeleton absorption with maxima at 226 (sh) and 290 nm in the UV spectrum. The molecular formula of **1** was established as $C_{52}H_{60}O_{12}$ on the basis of the HRESIMS peak at *m/z* 899.3946 [M + Na]$^+$ (calcd 899.3977). The IR spectrum showed absorption bands attributable to hydroxyl groups (3269 cm^{-1}), conjugated carbonyls (1603 cm^{-1}) and aromatic rings (1503 and 1450 cm^{-1}).

The ^{1}H NMR spectrum of **1** (Table 1) showed an ABX spin system with aromatic protons at δ_H 6.80 (1H, d, *J* = 8.5 Hz, H-6‴), 6.19 (1H, d, *J* = 2.5 Hz, H-3‴) and 6.14 (1H, dd, *J* = 2.5, 8.5 Hz, H-5‴), four singlet aromatic protons at δ_H 7.15 (1H, s, H-6′), 6.27 (1H, s, H-3′), 6.02 (1H, s, H-6) and 5.89 (1H, s, H-6″) for 1,2,4,5-tetrasubstituted or pentasubstituted benzene rings, and diagnostic flavanone signals at δ_H 5.46 (1H, dd, *J* = 3.0, 12.5 Hz, H-2), 2.76 (1H, dd, *J* = 12.5, 16.5 Hz, H-3a) and 2.59 (1H, dd, *J* = 3.0, 16.5 Hz, H-3b). In addition, a methine signal at δ_H 5.01 (1H, t, *J* = 7.0 Hz, H-β), two methylene protons at δ_H 3.69 (1H, overlap, H-α) and 3.57 (1H, dd, *J* = 7.0, 16.5 Hz, H-α), two methoxy groups at δ_H 3.73 (3H, s, OMe-5) and 3.71 (3H, s, OMe-5″) and two sets of lavandulyl group[24,27] signals at δ_H [1.36 (3H, s), 1.45 (3H, s), 1.49 (3H, s), 1.87 (2H, m), 2.37 (1H, m), 2.54 (2H, m), 4.38 (1H, d, *J* = 2.0 Hz), 4.44 (1H, d, *J* = 2.0 Hz), 4.85 (1H, overlap)] and [1.46 (3H, s), 1.55 (3H, s), 1.60 (3H, s), 1.97 (2H, m), 2.42 (1H, m), 2.49 (2H, m), 4.45 (1H, dd, *J* = 1.5, 2.5 Hz), 4.50 (1H, dd, *J* = 1.5, 2.5 Hz), 4.94 (1H, t, *J* = 7.0 Hz)] were observed. The ^{13}C NMR (Table 2) and HSQC

Table 1

^{1}H NMR data for compounds **1–5** (500 MHz in CD$_3$OD, δ in ppm, J in Hz).

position	1	2	3	4	5
2	5.46 dd (3.0, 12.5)	5.36 dd (3.0, 13.0)	5.33 dd (4.0, 12.0)	5.43 dd (4.5, 11.5)	5.26 d (11.5)
3	2.59 dd (3.0, 16.5)	2.57 dd (3.0, 17.0)	2.44 overlap	2.50 overlap	4.38 d (11.5)
	2.76 dd (12.5, 16.5)	2.66 dd (13.0, 17.0)	2.50 overlap	2.59 overlap	
6	6.02 s	6.03 s	6.04 s	6.03 s	6.03 s
3′	6.27 s	6.26 s	6.24 s	6.24 s	6.30 s
6′	7.15 s	7.23 s	7.29 s	7.27 s	7.18 s
1a	2.54 m	2.47 m	2.52 m	2.55 m	2.37 m
2a	2.37 m	2.25 m	2.31 m	2.28 m	2.34 m
3a	1.87 m	1.86 m	2.03 m	1.07 m, 1.25 m	1.87 m
4a	4.85 overlap	4.91 t (7.0)	4.92 m	1.07 m, 1.25 m	4.84 overlap
6a	1.36 s	1.44 s	1.47 s	0.94 s	1.38 s
7a	1.45 s	1.48 s	1.51 s	0.90 s	1.45 s
9a	4.38 d (2.0)	4.20 br s	4.22 br s	4.47 m	4.35 d (2.0)
	4.44 d (2.0)	4.39 br s	4.39 d (2.0)	4.53 br s	4.42 overlap
10a	1.49 s	1.31 s	1.34 s	1.55 s	1.46 s
5-OCH$_3$	3.73 s	3.73 s	3.74 s	3.74 s	3.75 s
α	3.69 overlap	3.80 dd (7.0, 17.0)	3.86 m	3.82 dd (9.0, 16.5)	3.75 m
	3.57 dd (7.0, 16.5)	3.52 dd (7.0, 17.0)	3.46 m	3.45 dd (6.5, 16.5)	3.55 dd (7.0, 17.0)
β	5.01 t (7.0)	5.03 t (7.0)	5.07 dd (6.5, 9.0)	5.05 dd (6.5, 9.0)	5.04 t (7.0)
6″	5.89 s	5.88 s	5.83 s	5.85 s	5.88 s
3‴	6.19 d (2.5)	6.19 d (2.5)	6.23 d (2.5)	6.23 d (2.5)	6.18 d (2.0)
5‴	6.14 dd (2.5, 8.5)	6.15 dd (2.5, 8.5)	6.14 dd (2.5, 8.5)	6.13 dd (2.5, 8.5)	6.13 dd (2.0, 8.5)
6‴	6.80 d (8.5)	6.83 d (8.5)	6.78 d (8.5)	6.75 d (8.5)	6.83 d (8.5)
1a′	2.49 m	2.51 m	2.45 m	2.43 m	2.49 m
2a′	2.42 m	2.43 m	2.39 m	2.39 m	2.41 m
3a′	1.97 m	1.96 m	1.93 m	1.93 m	1.95 m
4a′	4.94 t (7.0)	4.94 t (7.0)	4.93 m	4.92 t (7.0)	4.94 t (7.0)
6a′	1.46 s	1.47 s	1.45 s	1.45 s	1.47 s
7a′	1.55 s	1.55 s	1.54 s	1.54 s	1.55 s
9a′	4.45 dd (1.5, 2.5)	4.44 br s	4.40 dd (1.5, 2.5)	4.39 d (2.0)	4.43 overlap
	4.50 dd (1.5, 2.5)	4.50 br s	4.47 dd (1.5, 2.5)	4.46 m	4.49 dd (1.5, 2.5)
10a′	1.60 s	1.61 s	1.58 s	1.57 s	1.60 s
5″–OCH$_3$	3.71 s	3.72 s	3.71 s	3.73 s	3.72 s

spectra revealed 52 carbon resonances, including 15 flavanone skeleton carbons, two carbons assignable to methoxy groups and 20 carbons belonging to two lavandulyl groups. Apart from the aforementioned carbons, the remaining 15 carbons at δ_C [205.4, 166.1, 164.0, 162.5, 157.2, 156.1, 129.7, 123.8, 108.5, 107.7, 106.1, 103.6, 91.0, 49.8, 33.5] indicated the existence of a dihydrochalcone moiety, which suggested compound **1** was a biflavonoid composed of one flavanone and one dihydrochalcone monomer. The linkage through a C$_5$~C$_\beta$ bond of these two units was unambiguously established by the HMBC correlations (Fig. 2) from H-β (δ_H 5.01) to C-4′ (δ_C 156.2) and C-6′ (δ_C 127.1) and from H-α (δ_H 3.69, 3.57) to C-5′ (δ_C 124.2). The HMBC cross peaks of MeO-5 (δ_H 3.73) with C-5 (δ_C 161.8) and MeO-5″ (δ_H 3.71) with C-5″ (δ_C 162.5) confirmed that the two methoxy groups were located at C-5 and C-5″, respectively. One of the two lavandulyl groups showing HMBC correlations from H-1a (δ_H 2.54) to C-7 (δ_C 164.7) and C-9 (δ_C 164.9), and from H-2a (δ_H 2.37) to C-8 (δ_C 109.5), was attached to C-8. The other was located at C-8″ with similar HMBC correlations of H-1a′ (δ_H 2.49) to C-7″ (δ_C 164.0) and C-9″ (δ_C 166.1), and of H-2a′ (δ_H 2.42) to C-8″ (δ_C 108.5). Ten oxygenated aromatic carbons observed in the ^{13}C NMR spectrum evidenced the presence of oxygenation at C-5, C-9 and C-5″, as well as seven hydroxyl groups located on benzene rings. Based on the above comprehensive spectroscopic analysis, the planar structure of **1** was determined as shown in Fig. 1, in which the two monomers were identified as 7,2′,4′-trihydroxy-8-lavandulyl-5-methoxy flavanone and 7″, 9″, 2‴, 4‴-tetrahydroxy-8″ -lavandulyl- 5″-methoxydihydrochalcone.

Sophobiflavonoid B (**2**) and sophobiflavonoid C (**3**) were obtained as yellowish and orange amorphous powders, respectively, and their UV and IR spectra for characteristic functional groups were similar to those of **1**. Meanwhile, their deduced molecular formula C$_{52}$H$_{60}$O$_{12}$ by HRESIMS was consistent with **1**. Careful analysis of their NMR data (Tables 1 and 2) also revealed the biflavonoid subunits of a flavanone and a dihydrochalcone, two methoxy and two lavandulyl groups, and

ten oxygenated aromatic carbons with the same substituent patterns as in **1**. Their linkage and positions were identical to **1** as evidenced by HMBC correlations and coupling patterns. The above information suggested compound **2** and **3** had the same planar structure as **1**.

Sophobiflavonoid D (**4**) was obtained as an orange amorphous powder, giving a typical flavanone UV spectrum. According to the HRESIMS ion peak at m/z 917.4105 [M+Na]$^+$ (calcd 917.4083), its molecular formula was established as C$_{52}$H$_{62}$O$_{13}$. The IR spectrum also showed absorptions for hydroxyl (3396 cm^{-1}, 3310 cm^{-1}), conjugated carbonyl (1597 cm^{-1}) and aromatic moieties (1503 and 1449 cm^{-1}). Compared to the NMR data (Tables 1 and 2) of **1**, compound **4** revealed a unique difference in that one of the two lavandulyl groups was replaced by a 5-hydroxy-2-isopropenyl-5-methylhexyl moiety[23], whose NMR signals were at δ_H [0.90 (3H, s), 0.94 (3H, s), 1.07 (2H, m), 1.25 (2H, m), 1.55 (3H, s), 2.28 (1H, m), 2.55 (2H, m), 4.47 (1H, m), 4.53 (1H, br s)] and δ_C [149.6, 111.7, 71.6, 48.3, 42.6, 29.2, 28.8, 28.7, 27.8, 19.0]. This was further confirmed by the molecular formula of C$_{52}$H$_{62}$O$_{13}$, which had one more oxygen atom and two more hydrogen atoms than that of **1**. The position of the 5-hydroxy-2-isopropenyl-5-methylhexyl moiety was assigned to C-8 (δ_C 109.4) based on HMBC correlations from H-1a (δ_H 2.55) to C-7 (δ_C 164.7) and C-9 (δ_C 164.9) and from H-2a (δ_H 2.28) to C-8 (δ_C 109.4). Along with the 2D NMR spectra, the planar structure of **4** was elucidated as a 7,2′,4′-trihydroxy-8-(5-hydroxy-2-isopropenyl-5-methylhexyl)-5-methoxyflavanone and 7″,9″,2‴,4‴-tetrahydroxy-8″-lavandulyl-5″-methoxydihydrochalcone biflavonoid linked by a C$_5$~C$_\beta$ bond.

The absolute configurations at C-2 and C-β for compounds **1–4** were determined by comparison of the experimental ECD spectra and calculated ECD data. Compounds **1** and **2** exhibited completely opposite Cotton effects (CEs) in their ECD spectra (Fig. 4) suggesting that they may have contrary absolute configurations. The ECD spectra of compounds **3** and **4** also displayed opposite CEs (Fig. 4). In consideration that the flexible lavandulyl groups can produce diverse conformations

Table 2
^{13}C NMR data for compounds **1–8** (125 MHz in CD$_3$OD, δ in ppm).

position	1	2	3	4	5	6	7	8
2	75.5	75.7	75.6	75.4	79.1	77.7	71.9	71.9
3	45.4	46.2	46.0	45.7	73.6	54.7	52.6	52.6
4	193.9	194.0	194.0	194.0	193.9	207.0	205.5	205.7
5	161.8	161.8	161.8	161.8	161.6	161.9	162.4	162.4
6	93.2	93.2	93.2	93.3	93.2	90.9	91.4	91.3
7	164.7	164.7	164.5	164.7	165.0	164.4	164.7	164.4
8	109.5	109.9	109.8	109.4	109.5	108.4	108.9	108.8
9	164.9	165.0	164.9	164.9	164.2	165.9	166.4	166.4
10	105.7	105.6	105.6	105.7	103.6	107.5	107.1	107.2
1′	118.0	118.6	118.6	118.0	115.6	117.6	119.0	119.0
2′	154.3	153.8	153.8	154.0	156.2	157.4	158.0	158.0
3′	103.6	103.5	103.6	103.6	103.8	103.3	103.8	103.8
4′	156.2	156.2	156.0	156.3	156.1	159.2	159.3	159.3
5′	124.2	124.1	123.4	123.3	124.1	107.4	107.7	107.7
6′	127.1	126.8	126.8	126.6	128.7	130.6	130.2	130.3
1a	28.2	28.0	28.2	28.7	28.0	28.0	27.9	28.0
2a	48.1	48.9	48.8	48.3	48.1	47.7	47.7	47.9
3a	32.4	32.9	32.9	27.8	32.5	32.4	32.5	32.5
4a	124.8	124.9	124.9	42.6	124.9	125.1	124.9	125.0
5a	131.9	131.9	132.1	71.6	131.9	131.7	131.8	131.9
6a	17.9	18.1	18.1	28.8	18.0	17.9	18.0	17.9
7a	25.9	25.9	25.9	29.2	25.9	26.0	26.0	26.0
8a	149.5	149.2	149.3	149.6	149.6	149.4	149.6	149.7
9a	111.3	111.2	111.2	111.7	111.2	111.5	111.4	111.3
10a	19.2	18.6	18.8	19.0	19.1	19.0	18.9	19.0
5-OCH$_3$	55.9	55.9	55.9	55.9	56.0	55.9	56.2	56.2
α	49.8	49.0	48.9	49.0	49.7	50.6	49.4	49.3
β	33.5	33.2	33.8	34.0	33.3	37.1	36.2	35.9
4″	205.4	205.4	205.8	205.9	205.4	204.6	205.6	205.4
5″	162.5	162.6	162.4	162.3	162.6	162.4	162.2	162.2
6″	91.0	91.0	91.0	91.0	90.9	91.0	90.9	90.9
7″	164.0	164.0	164.0	164.0	164.0	164.1	164.3	164.2
8″	108.5	108.6	108.5	108.5	108.6	108.6	108.6	108.6
9″	166.1	166.2	165.8	165.7	166.1	165.9	165.9	166.0
10″	106.1	106.1	106.5	106.6	106.1	106.0	106.3	106.3
1‴	123.8	123.9	124.2	124.2	124.0	119.4	117.9	118.0
2‴	156.1	156.2	156.1	156.1	156.7	157.5	156.5	156.5
3‴	103.6	103.6	103.5	103.6	103.6	104.2	103.8	103.8
4‴	157.2	157.2	157.2	157.2	157.1	157.3	158.1	158.0
5‴	107.7	107.8	107.7	107.8	107.8	109.7	108.5	108.6
6‴	129.7	129.7	129.6	129.7	129.8	129.5	130.5	130.6
1a′	28.1	28.2	28.0	28.0	28.1	28.0	28.0	27.9
2a′	47.9	47.9	47.8	47.8	47.9	47.9	48.0	47.8
3a′	32.3	32.3	32.3	32.3	32.3	32.5	32.4	32.4
4a′	125.1	125.1	125.0	125.0	125.1	125.0	125.0	125.1
5a′	131.7	131.7	131.7	131.7	131.7	131.8	131.8	131.8
6a′	17.9	17.9	17.9	17.9	17.9	17.9	18.0	18.0
7a′	26.0	26.0	25.9	26.0	26.0	26.0	26.0	26.0
8a′	149.7	149.8	149.6	149.7	149.7	149.7	149.8	149.5
9a′	111.2	111.2	111.2	111.2	111.7	111.2	111.2	111.4
10a′	19.0	19.0	19.0	19.0	19.0	19.0	19.0	18.9
5″–OCH$_3$	55.9	56.0	55.9	56.0	56.0	55.8	55.5	55.6

and had an insignificant influence on the chiral centers C-2 and C-β, the simplified planar structure (Fig. 3) of **1a** was used for the ECD calculation. A total of four pairs of enantiomers (2S, βR; 2R, βS; 2S, βS and 2R, βR) of **1a** were employed for systematic conformational analysis by means of molecular mechanics force field (MMFF94) calculations. Subsequently, the optimized conformations were obtained using the time–dependent density functional theory (TDDFT) method at the B3LYP/6–31 + G (d, p) level, and the overall calculated ECD spectra were generated through Boltzmann-population weighting. The good match of the theoretically calculated ECD curves with experimental ECD curves permitted the assignment of the absolute configurations of **1–4** as shown in Fig. 4, and the four compounds were named sopho-biflavonoid A–D according to their identified order.

It is worth noting that the absolute configuration at C-2 of flava-nones is routinely established by CD spectra[28]. The characteristic CEs at 270–290 nm ($\Delta\varepsilon$: **–**) and 320–330 nm ($\Delta\varepsilon$: +) based on the $\pi \to \pi^*$ and $n \to \pi^*$ transitions, respectively, of flavanone indicate the 2S

configuration. In contrast, flavanones with a 2R configuration will exhibit a positive CE in the $\pi \to \pi^*$ absorption band and a negative CE in the $n \to \pi^*$ absorption band. Given that the dihydrochalcone unit may influence the CEs of C-2, we employed calculated ECD spectra to determine the absolute configurations of compounds **1–4**. Consequently, calculated CEs attributable to the $\pi \to \pi^*$ and $n \to \pi^*$ transitions of fla-vanone were in accordance with the empirical rule, suggesting the rule is still applicable for determining the absolute configuration at C-2 of obtained biflavonoids composed of one flavanone and one dihy-drochalcone monomer. Interestingly, the CEs in the range of less than 250 nm might also show certain regularity for the absolute configuration of C-β. The ECD spectra (Fig. 4) exhibit a negative absorption at approximately 205–215 nm and a positive absorption at approximately 240 nm when the configuration of C-β is R, whereas the positive absorption at approximately 205–215 nm and negative absorption at approximately 240 nm correspond to C-β S configuration. This requires substantiation by further study on the same type of biflavonoids.

Sophobiflavonoid E (**5**) was obtained as a yellowish amorphous powder, showing UV and IR spectra comparable to those of **1**. The 1D and 2D NMR data showed great similarity to **1** in shifts, coupling patterns and substituent positions except for both the proton and carbon signals at C-2 and C-3. Compared with the NMR data of **1**, compound **5** exhibited two doublets at δ_H 5.26 (1H, d, $J = 11.5$ Hz, H-2) and 4.38 (1H, d, $J = 11.5$ Hz, H-3) in the ^{1}H NMR (Table 1) with a 3.6 ppm downfield shift for C-2 (δ_C 79.1) and a large deshielded resonance for C-3 (δ_C 73.6) in the ^{13}C NMR (Table 2), implying the flavanone unit in **1** existed as a flavanonol in **5**. The molecular formula of **5** established as C$_{52}$H$_{60}$O$_{13}$ by the HRESIMS ion peak at m/z 915.3902 [M + Na]$^+$ (calcd 915.3926) also proved an additional hydroxyl group on it. The large coupling constant between H-2 and H-3 indicated their *trans* orientation, and the absolute configuration was assigned to be 2R, 3R [28] owing to the positive absorption at 337 nm ($\Delta\varepsilon$: + 2.29) in the CD spectrum (Fig. S38, Supporting Information). The absolute configuration for C-β was determined as R by comparison of the CD peaks (Figs. S6 and S38, Supporting Information) with **1** and the (2R, 3R, βR) configuration was further confirmed by comparing experimental and calculated ECD spectra of **5** (Fig. 4).

Sophobiflavonoid F (**6**) was obtained as a yellowish amorphous powder, also giving a typical flavanone skeleton absorption with maxima at 225 (sh) and 294 nm in the UV spectrum. The molecular formula of **6** was established as C$_{52}$H$_{60}$O$_{12}$ by the HRESIMS at m/z 899.3955 [M + Na]$^+$ (calcd 899.3977). The IR spectrum showed absorption consistent with hydroxyl groups (3387 cm^{-1}), conjugated carbonyls (1613 cm^{-1}) and aromatic rings (1509 and 1451 cm^{-1}).

The ^{1}H NMR (Table 3) spectrum of **6** showed two sets of ABX spin system aromatic protons at δ_H 6.95 (1H, d, $J = 8.5$ Hz, H-6′), 6.08 (1H, d, $J = 2.5$ Hz, H-3′) and 6.04 (1H, dd, $J = 2.5, 8.5$ Hz, H-5′), and 6.86 (1H, d, $J = 8.5$ Hz, H-6‴), 6.22 (1H, d, $J = 2.5$ Hz, H-3‴) and 6.29 (1H, dd, $J = 2.5, 8.5$ Hz, H-5‴), two singlet aromatic protons at δ_H 5.65 (1H, s, H-6) and 5.84 (1H, s, H-6″) for two pentasubstituted benzene rings, an oxymethine proton at δ_H 5.24 (1H, d, $J = 9.5$ Hz, H-2), two methine signals at δ_H 4.87 (1H, dd, $J = 9.5, 9.5$ Hz, H-3) and 4.00 (1H, m, H-β), and two methylene proton signals at δ_H 3.39 (1H, dd, $J = 5.5, 18.5$ Hz, H-α) and 3.29 (1H, dd, $J = 5.0, 18.5$ Hz, H-α). In addition, two methoxy groups at δ_H 3.55 (3H, s, OMe-5) and 3.65 (3H, s, OMe-5″), and two sets of lavandulyl group [24,27] signals at δ_H [1.45 (3H, s), 1.54 (3H, s), 1.56 (3H, s), 1.92 (2H, m), 2.41 (1H, m), 2.53 (2H, m), 4.33 (1H, d, $J = 2.0$ Hz), 4.42 (1H, d, $J = 2.0$ Hz), 4.93 (1H, m)] and [1.48 (3H, s), 1.56 (3H, s), 1.61 (3H, s), 1.97 (2H, m), 2.34 (1H, m), 2.46 (2H, m), 4.44 (1H, dd, $J = 1.5, 2.5$ Hz), 4.49 (1H, dd, $J = 1.5, 2.5$ Hz), 4.96 (1H, m)] were also observed. Analysis of the ^{13}C NMR (Table 2), HSQC and HMBC spectra revealed 52 carbon resonances, comprising two carbonyls, 24 carbons of benzene rings, 20 carbons of two lavandulyl groups, two methoxys, three methines (one oxygenated) and one methylene. A detailed NMR spectrum comparison with sophobiflavonoid A (**1**) implied that compound **6** possessed the same flavanone–fused

Table 3
^{1}H NMR data for compounds **6–8** (500 MHz in CD$_3$OD, δ in ppm, J in Hz).

Position	6	7	8	Position	6	7	8
2	5.24 d (9.5)	5.75 d (11.0)	5.73 d (11.0)	α	3.39 dd (5.5, 18.5)	3.60 dd (5.5, 14.5)	3.62 dd (6.0, 15.0)
					3.29 dd (5.0, 18.5)	2.99 dd (9.0, 14.5)	3.01 dd (8.5, 15.0)
3	4.87 dd (9.5, 9.5)	4.73 dd (4.0, 11.0)	4.75 dd (4.5, 11.0)	β	4.00 m	3.85 m	3.92 m
6	5.65 s	5.91 s	5.91 s	6″	5.84 s	5.79 s	5.80 s
3′	6.08 d (2.5)	6.21 d (2.5)	6.21 d (2.5)	3‴	6.22 d (2.5)	6.15 d (2.5)	6.16 d (2.5)
5′	6.04 dd (2.5, 8.5)	6.10 dd (2.5, 8.5)	6.10 dd (2.5, 8.5)	5‴	6.29 dd (2.5, 8.5)	6.13 dd (2.5, 8.5)	6.13 dd (2.5, 8.5)
6′	6.95 d (8.5)	6.88 d (8.5)	6.88 d (8.5)	6‴	6.86 d (8.5)	6.50 d (8.5)	6.56 d (8.5)
1a	2.53 m	2.51 m	2.49 m	1a′	2.46 m	2.55 m	2.52 m
2a	2.41 m	2.42 m	2.46 m	2a′	2.34 m	2.47 m	2.40 m
3a	1.92 m	1.95 m	1.93 m	3a′	1.97 m	2.00 m	1.99 m
4a	4.93 t (7.0)	4.94 t (7.0)	4.95 t (7.0)	4a′	4.96 m	4.98 t (7.0)	4.98 t (7.0)
6a	1.45 s	1.46 s	1.45 s	6a′	1.48 s	1.50 s	1.50 s
7a	1.54 s	1.55 s	1.55 s	7a′	1.56 s	1.57 s	1.57 s
9a	4.33 d (2.0)	4.37 d (2.0)	4.46 dd (1.5, 2.5)	9a′	4.44 dd (1.5, 2.5)	4.46 dd (1.5, 2.5)	4.40 d (2.0)
		4.42 d (2.0)	4.45 d (2.0)	4.51 dd (1.5, 2.5)		4.49 dd (1.5, 2.5)	4.54 dd (1.5, 2.5)	4.47 overlap
10a	1.56 s	1.58 s	1.62 s	10a′	1.61 s	1.64 s	1.56 s
5-OCH$_3$	3.55 s	3.70 s	3.72 s	5″-OCH$_3$	3.65 s	3.41 s	3.46 s

dihydrochalcone skeleton with a different mode of connecting. The linkage by a C$_3$-C$_\beta$ bond of the two units was demonstrated by the HMBC correlations (Fig. 2) from H-2 (δ_H 5.24) to C-β (δ_C 37.1), from H-3 (δ_H 4.87) to C-α (δ_C 50.6) and H-α (δ_H 3.39, 3.29) to C-3 (δ_C 54.7). The locations of the two methoxy and the two lavandulyl groups were also assigned by HMBC correlations as displayed in Fig. 2.

Sophobiflavonoid G (**7**) and sophobiflavonoid H (**8**) were both obtained as yellowish amorphous powders and exhibited the same molecular formula (C$_{52}$H$_{60}$O$_{12}$), UV and IR characteristics as **6**. A careful comparison of the 1D NMR data (Tables 2 and 3) and 2D NMR spectra among the three compounds suggested that they had the same planar structure.

The absolute configurations at C-2, C-3 and C-β for compounds **6–8**

were established via a combination of ECD spectra, coupling constant and conformation analysis. The large coupling constants (9.5 Hz or 11.0 Hz, Table 3) between H-2 and H-3 indicated their *trans* orientation, and compounds **6** and **7** were assigned to have (2S, 3R) configuration due to the negative CEs at 270–290 nm in the ECD spectra (Figs. S46 and S54, Supporting Information), while compound **8** exhibited a positive absorption at 285 nm ($\Delta\varepsilon$: +23.27) and negative absorption at 311 nm ($\Delta\varepsilon$:–40.25) (Fig. S62, Supporting Information), which was in agreement with the (2R, 3S) configuration[27]. Therefore, there were four possible stereoisomers for **6–8**, of which (2S, 3R, βS) or (2S, 3R, βR) configurations were attributed to **6** or **7**, and either the (2R, 3S, βS) or (2R, 3S, βR) configuration was for **8**. The Karplus equation can describe quantitatively the correlation of the experimental vicinal
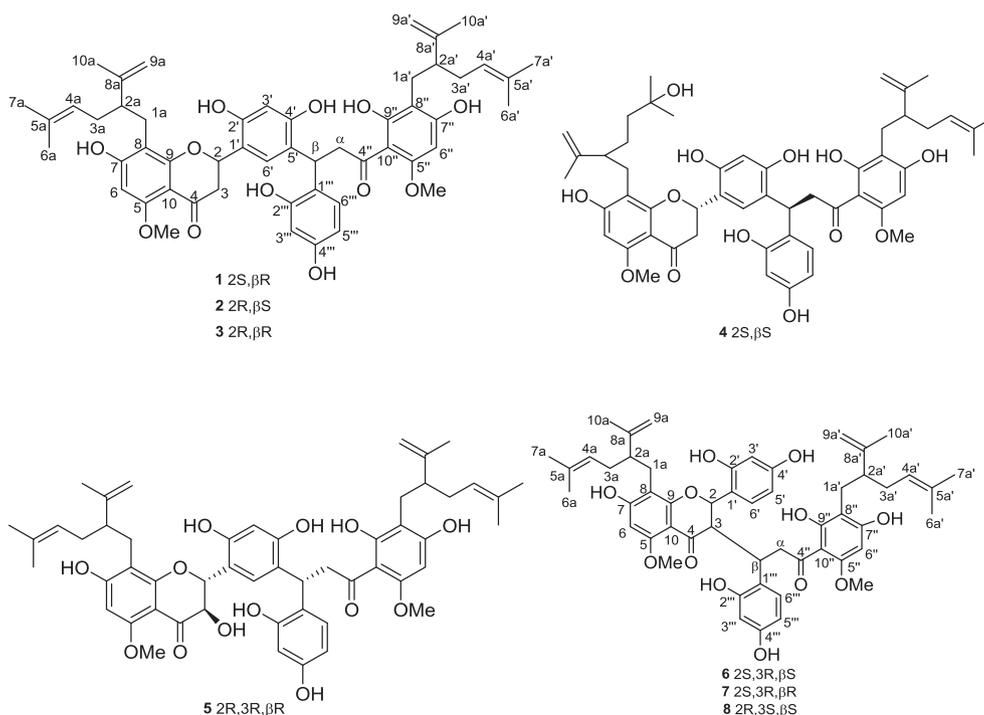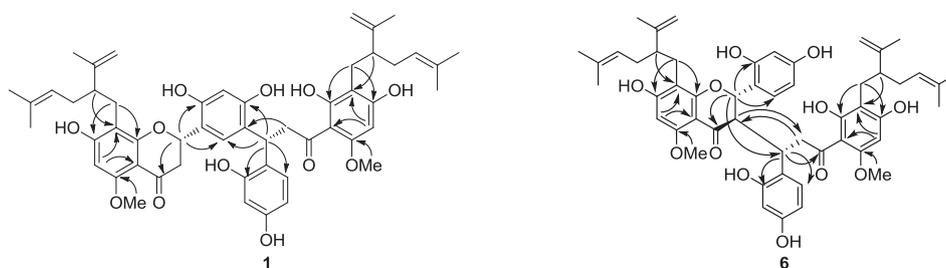

Fig. 1. Chemical structures of **1–8**.
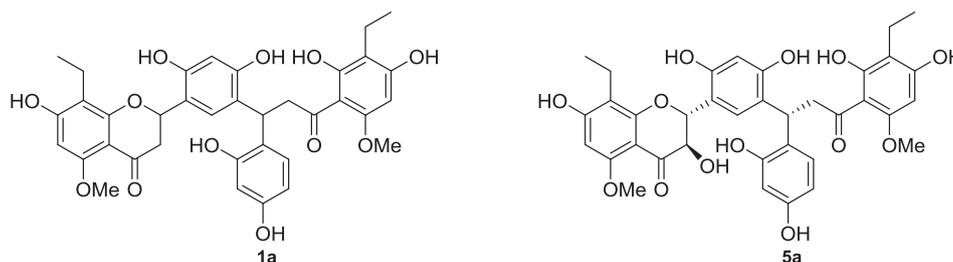
Fig. 2. Key HMBC correlations of compounds **1** and **6**.

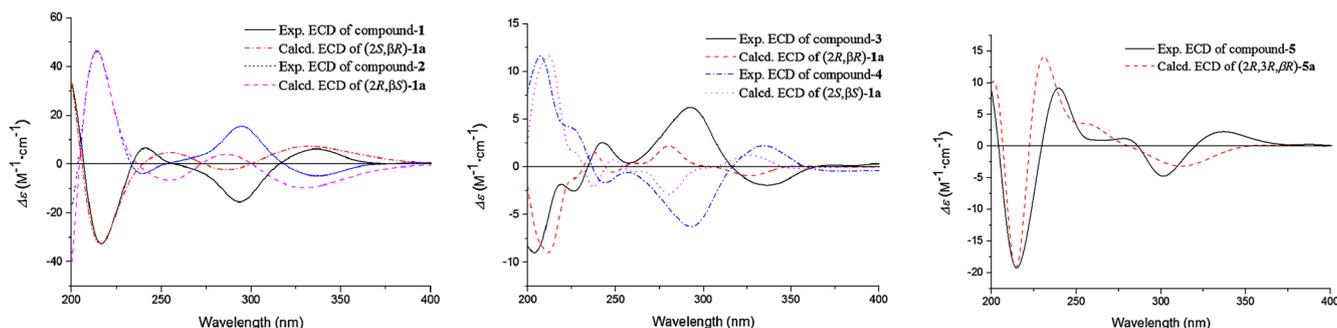**Fig. 3.** The structures of **1a** and **5a**.

Fig. 4. Experimental ECD and calculated ECD spectra of **1–5** in MeOH.

coupling constant to the H—C—C′-H′ dihedral angle and has been used very successfully to derive molecular structures[29]. To the best of our knowledge, the ^3J coupling constant between the two coupled protons is at a maximum when the dihedral angle is near to 0° or 180°, but at a minimum when the dihedral angle is near to 90° [30]. Accordingly, a molecular geometry optimization for the four stereoisomers with specific absolute configurations was performed in ChemBio3D (version 14.0) through the MM2 calculation method and the conformer with the minimum energy for every configuration was considered to be the optimal and used for dihedral angle measurements. The dihedral angles of H_3-C_3-C_β-H_β of the four stereoisomers and their optimal conformers with minimum energy are presented in Fig. 5. The 2S, 3R, βS-configuration whose dihedral angle of H_3-C_3-C_β-H_β was 175.3° was attributed to **6** ($^3J_{H3-H\beta}$ = 9.5 Hz), and the 2S, 3R, βR-configuration displaying an acute angle of 59.1° between the H_3-C_3-C_β plane and the H_β-C_β-C_3 plane was assigned to **7** ($^3J_{H3-H\beta}$ = 4.0 Hz). Similarly, compound **8** ($^3J_{H3-H\beta}$ = 4.5 Hz) was deduced to be of the 2R, 3S, βS-configuration with a dihedral angle of 94.8° for H_3-C_3-C_β-H_β, while the 2R, 3S, βR-configuration was assumed to be matched with a large coupling constant between H_3 and H_β on account of its dihedral angle of 157.1° for H_3-C_3-C_β-H_β.

3.2. Inhibitory effect on PTP1B

Due to deficient quantities of compounds **2**, **4** and **6**, only compounds **1**, **3**, **5**, **7** and **8** were evaluated for their inhibitory activity against PTP1B in *vitro* at a concentration of 10 μM, and they exhibited plummy inhibitory ratios of 95.7%, 96.0%, 96.6%, 94.1% and 93.0%,

respectively. Moreover, large quantities of compounds **1** and **3** remained, and IC₅₀ values were determined to be 0.33 and 0.35 μM, respectively. In a previous report [31], the two monomers dimerized in compounds **1** and **3** showed IC₅₀ values of more than 16 μM in their PTP1B inhibition tests, which implies that dimerization might significantly increase the inhibitory effect of lavandulyl flavonoids from *S. flavescens* roots on PTP1B.

4. Conclusions

Eight new unusual biflavonoids with lavandulyl groups were isolated from the EtOAc extracts of *Sophora flavescens*. Their flavanone and dihydrochalcone monomers were connected by a C_5-C_β or C_3-C_β bond. Comparison of the experimental ECD and calculated ECD spectra of compounds **1–5** verified that the empirical rule for determining C-2 absolute configuration of the flavanone unit was not affected by the linked dihydrochalcone unit. Moreover, the Cotton effects at the range of less than 250 nm in the ECD spectra of the biflavonoids formed by a C_5-C_β bond seemed to show certain regularity for the absolute configuration of C-β, and this requires further verification. In the in *vitro* PTP1B inhibition experiment, both the EtOAc fraction and the tested compounds showed strong inhibitory activity, suggesting the antidiabetic bioactivity of *Sophora flavescens* deserves future investigation.

Conflicts of interest

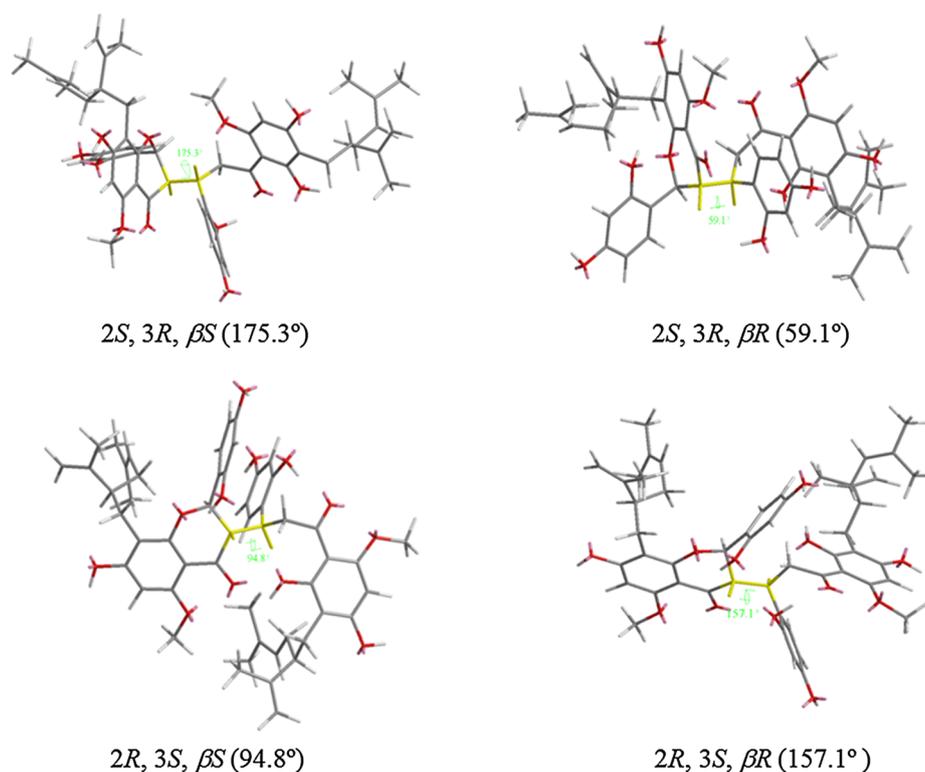The authors declare no competing financial interest.

2*S*, 3*R*, β*S* (175.3°)

2*S*, 3*R*, β*R* (59.1°)

2*R*, 3*S*, β*S* (94.8°)

2*R*, 3*S*, β*R* (157.1°)

Fig. 5. The optimal conformers with minimum energy of four stereoisomers and their dihedral angles of H_3-C_3-C_β-H_β.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.01.058.

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