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Microbial metabolism of cannflavin A and B isolated from *Cannabis sativa*

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Abstract

Microbial metabolism of cannflavin A (**1**) and B (**2**), two biologically active flavonoids isolated from *Cannabis sativa* L., produced five metabolites (**3–7**). Incubation of **1** and **2** with *Mucor ramannianus* (ATCC 9628) and *Beauveria bassiana* (ATCC 13144), respectively, yielded 6''*S*,7''-dihydroxycannflavin A (**3**), 6''*S*,7''-dihydroxycannflavin A 7-sulfate (**4**) and 6''*S*,7''-dihydroxycannflavin A 4'-*O*- α -L-rhamnopyranoside (**5**), and cannflavin B 7-*O*- β -D-4'''-*O*-methylglucopyranoside (**6**) and cannflavin B 7-sulfate (**7**), respectively. All compounds were evaluated for antimicrobial and antiprotozoal activity.

Keywords

Microbial metabolism; Cannflavin A; Cannflavin B; Cannabaceae; *Cannabis sativa*; *Mucor ramannianus*; *Beauveria bassiana*; Antifungal activity; Antibacterial activity; Antimalarial activity; Antileishmanial activity

1. Introduction

Cannabis sativa L. (Family: Cannabaceae), a plant originating in Central Asia, is cultivated worldwide as a source of fiber, energy, food and medicinal or narcotic preparations (Flores-Sanchez and Verpoorte, 2008). Recently, a number of new cannabis constituents were identified, increasing the total from 489 in 2005 (ElSohly and Slade, 2005) to 537 in 2009 (Ahmed et al., 2008a,b; Appendino et al., 2008; Radwan et al., 2009, 2008a,b), while the number of cannabinoids increased from 70 to 109. The cannabinoids are the most studied cannabis constituents, in particular ⁹-tetrahydrocannabinol (⁹-THC), the main psychoactive component (Galal et al., 2009); however, research has shown that some of the

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other cannabinoids also exhibit pharmacological activities, e.g., the nonpsychotropic cannabinoid cannabidiol (CBD) displays antihyperalgesic, antipsychotic, anticonvulsant, neuroprotective and antiemetic properties (Galal et al., 2009). Other classes of compounds reported for cannabis include terpenes, sugars, hydrocarbons, steroids, flavonoids, nitrogenous compounds, noncannabinoid phenols and amino acids (ElSohly and Slade, 2005).

More than 4000 flavonoids have been identified and numerous beneficial health effects have been reported, e.g., antiinflammatory (Gomes et al., 2008), antiviral (Naithani et al., 2008), anticancer (Mojziso and Mojzis, 2008), cardioprotective (Mojziso and Mojzis, 2008), antioxidant (Ibrahim et al., 2008), antiprotozoal (Fotie, 2008) and antimicrobial (Heinonen, 2007) activities.

Twenty-six flavonoids have been isolated from cannabis (ElSohly and Slade, 2005; Flores-Sanchez and Verpoorte, 2008; Radwan et al., 2008a), representing seven chemical structures (vitexin, isovitexin, apigenin, luteolin, kaempferol, orientin and quercetin) with different glycosylation, prenylation, geranylation and methylation patterns. Cannflavin A and B, two methylated isoprenoid flavones, represent the first aglycone flavonoids uniquely isolated from cannabis. The antileishmanial activity for cannflavin A and B was reported as strong (IC₅₀ 10.3 μ M) (Radwan et al., 2008a) and moderate (IC₅₀ 13.6 μ M) (Radwan et al., 2008b), respectively.

Microorganisms are used as predictive models for mammalian drug metabolism to establish the metabolic fate of biologically active compounds, while providing sufficient amounts of metabolite for structure elucidation and pharmacological evaluation. These models are also employed to obtain more active or less toxic substances and selective derivatives (Venisetty and Ciddi, 2003).

We herein report the microbial metabolism of **1** and **2** via a panel of 41 microorganisms, as well as the isolation, structure elucidation and activity (antimicrobial and antiprotozoal) of the resultant metabolites.

2. Results and discussion

Initial screening of **1** and **2** was carried out using a standard two-stage procedure (Ibrahim et al., 2008), with three and five of the 41 microorganisms showing the ability to transform **1** (three polar metabolites) and **2** (two polar metabolites), respectively. TLC analysis indicated that *Mucor ramannianus* (ATCC 9628) and *Beauveria bassiana* (ATCC 13144) had the highest transformational efficiencies for **1** and **2**, respectively, and was thus chosen for scaleup fermentation. Preparative scale fermentation of **1** with *M. ramannianus* yielded three new metabolites [6''S,7''-dihydroxycannflavin A (**3**), 6''S,7''-dihydroxycannflavin A 7-sulfate (**4**) and 6''S,7''-dihydroxycannflavin A 4'-O- α -L-rhamnopyranoside (**5**)], while preparative scale fermentation of **2** with *B. bassiana* yielded two new metabolites [cannflavin B 7-O- β -D-4'''-O-methylglucopyranoside (**6**) and cannflavin B 7-sulfate (**7**)].

Metabolite **3** was isolated as a light yellow powder with molecular formula C₂₆H₃₀O₈ based on HRESIMS and ¹³C NMR spectroscopic data (12° of unsaturation). The UV λ_{max} at 275

and 350 nm indicated a flavonoid structure (Avula et al., 2009). The ^{13}C and DEPT-135 NMR spectroscopic data displayed an sp^3 oxymethine (δ_{C} 72.3) and an sp^3 geminal dimethyl oxygenated quaternary carbon (δ_{C} 77.7), in addition to eight oxygenated carbon resonances similar to those found in **1** (Choi et al., 2004). A comparison of the ^1H (Table 1) and ^{13}C (Table 2) NMR data of **1** and **3** indicated almost identical resonances for both compounds, except for the replacement of the C-6''/C-7'' olefinic resonances in **1** [δ_{H} 5.04 (t , $J = 7.0$, H-6''); δ_{C} 125.2 (C-6''), 131.6 (C-7'')] by oxymethine [δ_{H} 3.80 (m); δ_{C} 77.7 (C-6'')] and oxygenated quaternary carbon [δ_{C} 72.3 (C-7'')] resonances in **3**. A series of HMBC (H-6''/C-4'', C-8'', C-9''; C-6''/H₂-4'', H₃-8'', H₃-9''; C-7''/H₃-8'', H₃-9''; H₂-5''/C-9'') and COSY (H-6''/H₂-5''/H₂-4'') correlations confirmed the assignment of hydroxy groups at C-6'' and C-7'', establishing **3** as 6'',7''-dihydroxycannflavin A.

Metabolite **4**, a yellow amorphous powder, was shown to have a molecular formula $\text{C}_{26}\text{H}_{30}\text{O}_{11}\text{S}$ (HRESIMS). The IR spectroscopy displayed characteristic sulfate bands at ν_{max} 1050 (C–O–S) and 1250 (S=O) cm^{-1} (Ibrahim, 2005). The ^1H (Table 1) and ^{13}C (Table 2) NMR chemical shifts of **4** and **1** were similar, with significant differences being the downfield shift of H-8, C-6, C-8 and C-10 (0.55, 2.5, 4.0 and 0.6 ppm, respectively) and the upfield shift of C-7 (1.9 ppm), supporting the sulfation of the C-7 hydroxy (Barron and Ibrahim, 1987). The C-6 geranyl group in **4** was 6'',7''-dihydroxylated as in **3** based on HMBC (H-6''/C-4'', C-8'', C-9''; C-6''/H₃-8''; C-7''/H₃-8'', H₃-9'') and COSY (H-6''/H₂-5''/H₂-4'') correlations and the absence of C-6''/C-7'' olefinic NMR resonances, establishing **4** as 6'',7''-dihydroxycannflavin A 7-sulfate.

Metabolite **5** (HRESIMS: $\text{C}_{32}\text{H}_{40}\text{O}_{12}$), isolated as a light yellow powder, displayed similar ^1H (Table 1) and ^{13}C (Table 2) NMR resonances to **3** and **4**, with additional resonances indicating a pyranose moiety [^1H NMR: δ_{H} 5.50 (1H, *br s*, H-1'''), 1.04 (3H, *d*, $J = 6.0$, H₃-6'''); ^{13}C NMR: δ_{C} 98.4 (C-1'''), 16.1 (C-6''')]. The pyranoside location was determined at C-4' via HMBC correlation of the anomeric proton and C-4' (δ_{C} 149.0), while its identity was determined through GC–MS analysis. The acetylated thiazolidine derivative of the hydrolysis product of **5** and of authentic L-rhamnose displayed the same GC–MS retention time (15.64 min) (Shukla et al., 2009). The chemical shift of the anomeric carbon [δ_{C} 98.4 (C-1''')] suggested an α -O-glycosidic linkage (Herath et al., 2008; Ibrahim et al., 2008), establishing **5** as 6'',7''-dihydroxycannflavin A 4'-O- α -L-rhamnopyranoside.

The absolute configuration at C-6'' was determined through application of the modified Mosher's method ($\delta = \delta_S - \delta_R$) for **5** (Ahmed et al., 2008a; Murata et al., 2008; Nakamura et al., 2009; Zhang et al., 2009). The ^1H NMR resonances for the H₃-8'' and H₃-9'' protons of the *S*-MTPA ester (**5a**) were observed downfield compared to those of the *R*-MTPA ester (**5b**) [$\delta(\text{H}_3\text{-8}'') = +0.02$ and $\delta(\text{H}_3\text{-9}'') = +0.03$], indicating *S* absolute configuration at C-6''. The absolute configurations at C-6'' for **3** and **4** were subsequently determined as *S* by comparison of their NMR data with that of **5** (Tables 1 and 2).

Metabolite **6**, a yellow amorphous solid with molecular formula C₂₈H₃₂O₁₁ (HRESIMS), displayed similar ^1H (Table 1) and ^{13}C (Table 2) NMR resonances to **2** (Choi et al., 2004), with additional resonances indicating a 4'''-*O*-methylglucopyranoside unit [δ_{H} 5.04 (1H, *d*, $J = 7.6$, H-1'''), 3.54 (3H, *s*, 4'''-OCH₃)]. NMR spectroscopic data (^{13}C , DEPT-135 and HMQC) confirmed the presence of the methylated glycoside moiety via an anomeric (δ_{C} 100.1), two hydroxymethine (δ_{C} 73.6, 76.4), one methoxymethine (δ_{C} 79.0), one oxygenated methine (δ_{C} 76.4), one hydroxymethylene (δ_{C} 60.2) and one *O*-methyl (δ_{C} 59.5) carbon resonance, in addition to the resonances for the cannflavin B structure. The anomeric proton coupling constant ($J = 7.6$ Hz) indicated β -*O*-glycosidic linkage, while HMBC correlation (H-1'''/C-7) revealed the location at C-7 (Herath et al., 2008, 2006; Ibrahim et al., 2008). Consequently, **6** was identified as cannflavin B 7-*O*- β -D-4'''-*O*-methylglucopyranoside. *B. bassiana* has previously been shown to produce 4-*O*-methyl-D-glucopyranoside-phenol adducts (Kikuchi et al., 2004; Zhan and Gunatilaka, 2005, 2008).

Metabolite **7**, a yellow solid, was shown to have a molecular formula C₂₁H₂₀O₉S (HRESIMS), while IR spectroscopy indicated characteristic sulfate bands at ν_{max} 1050 (C–O–S) and 1250 (S=O) cm⁻¹ (Ibrahim, 2005). The ^1H (Table 1) and ^{13}C (Table 2) NMR chemical shifts of **7** and **2** were similar, with significant differences in the downfield shift of H-8, C-6, C-8 and C-10 (0.57, 2.2, 3.8 and 0.4 ppm, respectively) and the upfield shift of C-7 (2.9 ppm), supporting the sulfation of the C-7 hydroxy (Barron and Ibrahim, 1987). HMBC correlations (H-8/C-6, C-7) confirmed the sulfation position and established **7** as cannflavin B 7-sulfate.

The isolated metabolites were devoid of significant antifungal, antibacterial, antileishmanial or antimalarial activity (Babu et al., 2006; Bharate et al., 2007; Radwan et al., 2008b).

3. Concluding remarks

In conclusion, in the present study, three metabolites of cannflavin A (**1**), produced by *M. ramannianus*, and two metabolites of cannflavin B (**2**), produced by *B. bassiana*, were isolated, with *B. bassiana* producing higher transformation yields than *M. ramannianus*. The enzymatic reactions included hydroxylation, *O*-glycosylation and sulfate conjugation, as well as combinations of these transformations. *O*-Glycosylation occurred at the C-4' and C-7 hydroxy groups of **1** and **2**, respectively, while sulfate conjugation occurred exclusively at the C-7 hydroxy. This study demonstrates the use of microbial transformation techniques to prepare new derivatives of flavonoids.

4. Experimental

4.1. General experimental procedures

NMR spectra were recorded in DMSO- d_6 on Bruker Avance DPX-500 (^1H : 500 MHz; ^{13}C : 125 MHz) and Varian AS400 (^1H : 400 MHz; ^{13}C : 100 MHz) spectrometers. IR spectra were recorded on a Bruker Tensor 27 spectrophotometer. UV spectra were obtained on a Varian Cary 50 Bio UV–visible spectrophotometer. Optical rotations were measured at ambient temperature using a Rudolph Research Analytical Autopol IV automatic polarimeter. HRESIMS was obtained using a Bruker BioApex FTMS. GC–MS analysis was carried out on a HP 6890 series GC, equipped with a split/splitless capillary injector, a HP 6890 Series injector autosampler, and a DB-5 ms column (30 m $\times$ 0.25 mm $\times$ 0.25 μm , Agilent). The GC was interfaced to a HP 5973 quadrupole mass selective detector through a transfer line set at 280 $^\circ\text{C}$. The injector temperature was 250 $^\circ\text{C}$, and 1 μL injections were performed in the split (1:10) mode. Column flow was set at a constant pressure of 20 psi, giving an initial flow of 2.2 mL/min, using helium as carrier gas. The oven temperature was raised from 150 (hold 1 min) to 250 $^\circ\text{C}$ (hold 30 min) at a rate of 10 $^\circ\text{C}/\text{min}$. The filament was operated at 70 eV, with an emission current of 35 μA . The multiplier voltage was automatically set to 2247 V. The ion source and quadrupole temperatures were 230 and 150 $^\circ\text{C}$, respectively. The acquisition range was m/z 30–800 at 1.95 scans per second, starting 3.5 min after injection. TLC was carried out on aluminum-backed plates pre-coated with silica gel F₂₅₄ (20 $\times$ 20 cm, 200 μm , 60 $\text{\AA}$, Merck) and on glass-backed plates pre-coated with silica gel F₂₅₄ (10 $\times$ 20 cm, 200 μm , 60 $\text{\AA}$, Analtech). Visualization was accomplished by spraying with *p*-anisaldehyde [0.5 mL in glacial acetic acid (50 mL) and sulfuric acid (97%, 1 mL)] spray reagent followed by heating. Flash silica gel (40–63 μm , 60 $\text{\AA}$, Silicycle) and Sephadex LH-20 (25–100 μm , lipophilic, Sigma–Aldrich) were used for column chromatography. High-performance flash chromatography (HPFC) was performed using a Horizon Biotage system (Biotage, Inc., Charlottesville, VA) with normal phase silica gel cartridges (KP-SIL, 10 g, 40–63 μm , 60 $\text{\AA}$, Biotage).

4.2. Substrates

Cannflavin A (**1**) and B (**2**) were isolated from *C. sativa* L. (Radwan et al., 2008a, 2008b) grown at the University of Mississippi. Whole buds of mature female plants were harvested, air-dried and stored in barrels at low temperature ($-24\text{ }^\circ\text{C}$).

4.3. Organisms

Microorganisms (41) housed at the National Center for Natural Products Research, University of Mississippi, were used in preliminary screening experiments to identify organisms capable of metabolizing **1** and **2**.

4.4. Analytical scale fermentation

Medium- α , consisting of dextrose (20 g), NaCl (5 g), K_2HPO_4 (5 g), bacto-peptone (5 g) (Difco Labs, Detroit, MI) and yeast extract (5 g) (Difco Labs) in distilled H_2O (1000 mL), was used in the twostage fermentation experiments. Initial screening was performed in Erlenmeyer flasks (125 mL) containing medium- α (25 mL). The media were autoclaved (20

min, 121 °C, 15 psi) before inoculation with each of the 41 microorganisms. The substrates were separately dissolved in dimethylformamide (DMF) (5 mg/500 µL) and added to 24 h old stage II cultures. Incubation (room temperature, 14 days, 100 rpm) was achieved on a rotary shaker (New Brunswick Model G10-21), while samples were monitored at 7 day intervals. Samples (5 mL) were extracted (5 mL, EtOAc/*iso*-PrOH, 8:2) and centrifuged (5 min, 3000 rpm), and the organic layers analyzed by TLC (CHCl₃/MeOH solvent systems). Culture and substrate controls were simultaneously maintained with the above fermentation experiments (Herath et al., 2006, 2008; Ibrahim et al., 2008).

4.5. Preparative scale fermentation

Preparative scale fermentations of **1** and **2** with *M. ramannianus* (ATCC 9628) and *B. bassiana* (ATCC 13144), respectively, were performed (*vide supra*) in six flasks (2 L each) containing medium-α (500 mL each). Each flask was inoculated with the relevant microorganism and the substrate (**1**: 5 × 100 mg; **2**: 1 × 100 mg), dissolved in DMF (10 mL), added. Incubation (room temperature, 14 days, 100 rpm) was followed by extraction (3 × 500 mL, EtOAc/*iso*-PrOH 8:2), evaporation of the solvent and dehydration over anhydrous Na₂SO₄ to provide the final extract (860 and 220 mg, respectively).

4.6. Isolation and purification of the metabolites

Sephadex LH-20 chromatography (MeOH) of the resulting extracts from **1** (860 mg) and **2** (220 mg) yielded **3** (5.2 mg) and **4** (6.5 mg), and **6** (31.0 mg) and **7** (7.0 mg), respectively, while **5** (10.0 mg) was subsequently purified through preparative thin layer chromatography (EtOAc/MeOH/H₂O, 30:5:4).

4.6.1. 6''S,7''-Dihydroxycannflavin A (**3**)—Light yellow powder (5.2 mg, 1.04%

yield); $[\alpha]_D^{25} -55$ (*c* 0.2, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ): 275 (4.1), 350 (4.02), +NaOMe 270, 410, +NaOAc 270, 405, +AlCl₃ 280 nm; IR (neat) $\nu_{\max}$: 3406, 1657, 1360, 1210, 1073 cm⁻¹; for ¹H and ¹³C NMR spectroscopic data, see Tables 1 and 2, respectively; HRESIMS *m/z* 469.1929 [M-H]⁻ (calcd for C₂₆H₂₉O₈, 469.1862).

4.6.2. 6''S,7''-Dihydroxycannflavin A 7-sulfate (**4**)—Yellow amorphous solid (6.5

mg, 1.3% yield); $[\alpha]_D^{25} -60$ (*c* 0.2, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ): 275 (4.1), 340 (4.02), +NaOMe 270, 410, +NaOAc 270, 405, +AlCl₃ 280 nm; IR (neat) $\nu_{\max}$: 3400, 1625, 1600, 1250 (S=O), 1050 (C–O–S) cm⁻¹; for ¹H and ¹³C NMR spectroscopic data, see Tables 1 and 2, respectively; HRESIMS *m/z* 551.1450 [M+H]⁺ (calcd for C₂₆H₃₁O₁₁S, 551.1587).

4.6.3. 6''S,7''-Dihydroxycannflavin A 4'-O-α-L-rhamnopyranoside (**5**)—Light

yellow powder (10.0 mg, 2.0% yield); $[\alpha]_D^{25} -122$ (*c* 0.5, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ): 275 (4.01), 365 (4.04), +NaOMe 275, 430, +NaOAc 290, 335, +AlCl₃ 280, 360 nm; IR (neat) $\nu_{\max}$: 3398, 1677, 1290, 1190, 1069 cm⁻¹; for ¹H and ¹³C NMR spectroscopic data, see Tables 1 and 2, respectively; HRESIMS *m/z* 639.2425 [M+Na]⁺ (calcd for C₃₂H₄₀NaO₁₂, 639.2417).

4.6.4. Cannflavin B 7-O- β -D-4'''-O-methylglucopyranoside (6)—Yellow amorphous solid (31.0 mg, 31.0% yield); $[\alpha]_{\text{D}}^{25} -30$ (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ): 275 (4.01), 340 (4.03), +NaOMe 264, 395, 405, +NaOAc 270, 345, 405, +AlCl₃ 280, 355 nm; IR (neat) ν_{max} : 3398, 1677, 1290, 1190, 1069 cm⁻¹; for ¹H and ¹³C NMR spectroscopic data, see Tables 1 and 2, respectively; HRESIMS *m/z* 545.2133 [M+H]⁺ (calcd for C₂₈H₃₃O₁₁, 545.2023).

4.6.5. Cannflavin B 7-sulfate (7)—Yellow solid (7.0 mg, 7.0% yield); UV (MeOH) λ_{max} (log ϵ): 275 (4.01), 340 (4.02), +NaOMe 264, 395, 410, +NaOAc 270, 404, 445, +AlCl₃ 280, 355 nm; IR (neat) ν_{max} : 3400, 1625, 1600, 1250 (S=O), 1050 (C–O–S) cm⁻¹; for ¹H and ¹³C NMR spectroscopic data, see Tables 1 and 2, respectively; HRESIMS *m/z* 471.0811 [M+Na]⁺ (calcd for C₂₁H₂₀NaO₉S, 471.0726).

4.7. Acid hydrolysis and identification of the sugar units

Compounds **5** and **6** (2 mg) were refluxed separately with HCl (0.5 N, 3 mL, 2 h), followed by addition of water (10 mL) and extraction with CHCl₃ (3 × 5 mL). The aqueous layers were dried to give the hexoses, which were identified through NMR spectroscopy, GC–MS analysis of their acetylated thiazolidine derivatives and through comparison of their specific rotation with authentic samples.

4.8. GC–MS analysis of the sugar units

The sugars obtained through acid hydrolysis of **5** and **6** were separately dissolved in pyridine (1 mL), added to L-cysteine methyl ester hydrochloride (0.1 M) in pyridine (2 mL), and the mixture heated (60 °C, 1 h). An equal volume of acetic anhydride (ca. 3 mL) was added and the heating continued (1 h). The acetylated thiazolidine sugar derivatives were subjected to GC–MS analysis and their retention times compared with acetylated thiazolidine derivatives of authentic sugars (Sigma–Aldrich): D-glucose, 21.32 min; L-glucose, 22.68 min; D-galactose, 22.12 min; L-rhamnose, 15.64 min; L-xylose, 16.90 min; L-arabinose, 16.11 min (Shukla et al., 2009).

4.9. Modified Mosher's method

The Mosher esters were directly prepared in two NMR tubes by reacting **5** (2 mg) in pyridine-*d*₅ (100 μ L) with (*R*)-(–)- (30 μ L) and (*S*)-(+)-MTPA-Cl (30 μ L), respectively, at room temperature (2 h) to afford the *S*- (**5a**) and *R*-MRPA (**5b**) esters, respectively. ¹H NMR data were measured directly from the reaction mixtures (Ahmed et al., 2008a).

4.10. Biological activities

The isolated metabolites were evaluated for *in vitro* antifungal [*Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258) and *Aspergillus fumigat* (ATCC 90906)], antibacterial [*Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853), *Mycobacterium intracellulare* (ATCC 23068) and *Staphylococcus aureus* (ATCC 29213)], antileishmanial (culture of *Leishmania donovani*) and antimalarial [*Plasmodium falciparum* (D6 and W2 clones)] activity (Babu et al., 2006; Bharate et al., 2007; Radwan et al., 2008b).

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Table 1

¹H NMR spectroscopic data (δ_{H}) for **1–7** in DMSO-*d*₆ (*J* in Hz).

No.	1a	3a	4b	5a	2a	6b	7a
3	6.69 (s)	6.53 (s)	6.58 (s)	6.63 (s)	6.68 (s)	6.91 (s)	6.90 (s)
8	6.63 (s)	6.83 (s)	7.18 (s)	6.64 (s)	6.63 (s)	6.93 (s)	7.20 (s)
2'	7.61 (<i>d</i> , 1.9)	7.52 (<i>br s</i>)	7.25 (<i>d</i> , 2.0)	7.23 (<i>d</i> , 2.0)	7.60 (<i>d</i> , 1.9)	7.54 (<i>d</i> , 2.0)	7.60 (<i>d</i> , 2.0)
5'	7.02 (<i>d</i> , 8.3)	6.91 (<i>d</i> , 8.8)	6.46 (<i>d</i> , 8.4)	6.60 (<i>d</i> , 8.4)	7.00 (<i>d</i> , 8.3)	7.00 (<i>d</i> , 8.0)	9.93 (<i>d</i> , 8.2)
6'	7.58 (<i>dd</i> , 1.9, 8.2)	7.50 (<i>br s</i>)	7.46 (<i>dd</i> , 2.0, 8.4)	7.47 (<i>dd</i> , 2.0, 8.4)	7.58 (<i>dd</i> , 1.9, 8.2)	7.56 (<i>dd</i> , 2.0, 8.0)	7.54 (<i>dd</i> , 2.0, 8.2)
3'-OCH ₃	3.99 (s)	3.86 (s)	3.79 (s)	3.86 (s)	3.99 (s)	3.86 (s)	3.89 (s)
1''	3.36 (<i>d</i> , 7.2)	3.21 (<i>d</i> , 7.2)	3.36 (<i>d</i> , 7.1)	3.20 (<i>d</i> , 7.1)	3.36 (<i>d</i> , 7.1)	3.50 (<i>d</i> , 7.1)	3.26 (<i>d</i> , 7.2)
2''	5.28 (<i>t</i> , 7.2)	5.20 (<i>t</i> , 6.5)	5.17 (<i>t</i> , 7.2)	5.17 (<i>t</i> , 7.2)	5.29 (<i>t</i> , 7.2)	5.20 (<i>t</i> , 7.0)	5.17 (<i>t</i> , 7.2)
4''	1.93 (<i>m</i>)	1.93 (<i>m</i>), 2.48 (<i>m</i>)	1.87 (<i>m</i>), 2.20 (<i>m</i>)	2.48 (<i>m</i>)	1.80 (s)	1.80 (s)	1.74 (s)
5''	2.03 (<i>m</i>)	1.60 (<i>m</i>)	1.60 (<i>m</i>)	1.60 (<i>m</i>)	1.65 (s)	1.60 (s)	1.66 (s)
6''	5.04 (<i>t</i> , 7.0)	3.80 (<i>m</i>)	3.92 (<i>m</i>)	3.94 (<i>m</i>)	–	–	–
8''	1.54 (s)	0.94 (s)	0.84 (s)	0.95 (s)	–	–	–
9''	1.78 (s)	0.99 (s)	0.85 (s)	0.99 (s)	–	–	–
10''	1.65 (s)	1.71 (s)	1.74 (s)	1.74 (s)	–	–	–
1'''	–	–	–	5.50 (<i>br s</i>)	–	5.04 (<i>d</i> , 7.6)	–
2'''	–	–	–	3.86 (<i>br s</i>)	–	3.19 (<i>dd</i> , 8.0, 8.0)	–
3'''	–	–	–	3.86 (<i>br s</i>)	–	3.04 (<i>dd</i> , 8.8, 8.8)	–
4'''	–	–	–	3.55 (<i>br s</i>)	–	3.49 (<i>dd</i> , 8.8, 8.8)	–
5'''	–	–	–	3.92 (<i>m</i>)	–	3.37 (<i>dd</i> , 8.8, 8.8)	–
6'''	–	–	–	1.04 (<i>d</i> , 6.0)	–	3.68 (<i>d</i> , 8.8)	–
4'''-CH ₃	–	–	–	–	–	3.54 (s)	–

^a 400 MHz.^b 500 MHz.^c Acetone-*d*₆.

Table 2

¹³C NMR spectroscopic data (δ_C) for **1–7** in DMSO-*d*₆.

No.	a ¹	a ³	a ⁴	a ⁵	2a,c	a ⁶	7a
2	164.7	164.0	168.8	162.9	164.7	164.0	164.2
3	104.4	103.6	104.4	103.6	104.4	103.3	103.0
4	183.1	182.3	179.8	179.7	183.1	182.0	182.2
5	162.4	158.4	158.2	157.6	162.5	157.6	157.7
6	112.4	111.8	114.8	112.3	112.3	112.7	114.5
7	160.2	162.0	158.2	160.7	160.1	160.6	157.2
8	94.1	94.0	98.2	94.6	94.2	93.5	98.0
9	156.6	155.8	154.3	156.2	156.5	155.0	154.2
10	105.2	103.6	105.7	105.0	105.1	105.0	105.5
1'	123.7	125.0	123.0	125.2	123.7	121.3	120.6
2'	110.5	110.0	108.3	110.2	110.4	110.4	109.9
3'	148.8	148.7	148.8	150.1	148.8	148.0	148.2
4'	151.3	151.2	151.0	149.0	151.3	151.0	151.0
5'	116.3	116.5	118.1	116.6	116.3	115.8	116.0
6'	121.2	122.1	123.5	123.1	121.2	120.4	122.2
3'-OCH ₃	56.6	56.5	55.6	56.2	56.7	56.0	56.0
1''	22.0	25.1	22.0	24.6	22.0	21.2	21.5
2''	123.2	120.8	122.0	119.1	123.2	121.9	122.2
3''	131.6	135.5	135.0	133.3	131.6	130.6	130.8
4''	40.5	37.3	37.3	36.6	25.8	25.4	25.5
5''	27.4	30.0	29.9	29.5	17.9	17.6	17.7
6''	125.2	77.7	77.6	77.7	-	-	-
7''	131.6	72.3	72.1	71.9	-	-	-
8''	16.3	25.1	25.0	24.6	-	-	-
9''	25.8	27.0	26.7	25.9	-	-	-
10''	17.7	16.8	16.6	16.2	-	-	-
1'''	-	-	-	98.4	-	100.1	-
2'''	-	-	-	69.3	-	73.6	-

^c Acetone-*d*₆.