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Note

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Tetramic Acid Analogues Produced by Coculture of *Saccharopolyspora erythraea* with *Fusarium pallidoroseum*

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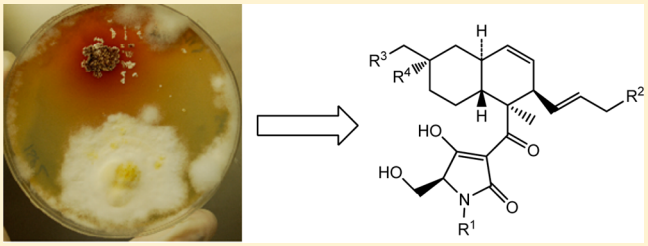
Supporting Information

ABSTRACT: Coculture of the fungus *Fusarium pallidoroseum* with the bacterium *Saccharopolyspora erythraea* was found to produce three new decalin-type tetramic acid analogues related to equisetin. The structures were determined by spectroscopic methods. The absolute configurations were established by circular dichroism spectroscopy and comparing the data with those of equisetin.

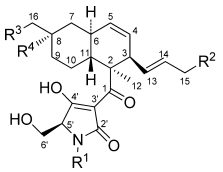


Natural products continue to play an integral role in the discovery and development of drugs used to treat various diseases. From genomic sequencing in the early 2000s of the actinomycetes *Streptomyces avermitilis*[1] and *S. coelicolor*[2] it became apparent that there were 10 to 20-plus putative (cryptic) secondary metabolite clusters in the genomes of these microbes. In the middle 2000s, from investigations of the genomes of certain *Aspergillii*, the Keller group reported that in contrast to the then current dogma, putative secondary metabolite biosynthetic clusters in the *Aspergillii* were on single chromosomes, rather than being spread among a number, as is the case in fungal primary metabolism.[3,4] Thus over the past few years, a number of investigators have been attempting to activate these cryptic biosynthetic clusters by a variety of means, including activation by exogenous epigenetic modifiers such as demethylase or histone acetylase/deacetylase activators/inhibitors.[5]

Using exogenous molecules does work, but the levels required are purely empirical in nature and costs become significant as fermentation volume increases. One very useful approach to overcoming the increasing difficulty in discovering new active structures by activation of the cryptic clusters is to mimic the competitive microbial ecosystems employed in the natural environment through mixed culture of two or more organisms known to produce bioactive compounds. The resulting interactions may lead to induced production of previously unreported secondary metabolites or an improved titer of known and at times unidentified low level analogues, as we and others have demonstrated.[6−13]

As part of our ongoing study of metabolites produced by cocultures, we analyzed the mixed culture of *Fusarium pallidoroseum* with *Saccharopolyspora erythraea*. The genus *Fusarium* includes a large group of filamentous fungi widely distributed in soil and in association with plants. It produces a broad array of active metabolites including the HDAC inhibitor apicidin,[14] the immunosuppressor cyclosporine A,[15] and the antibiotic and insecticidal beauvericin,[16] just to name a few examples. *S. erythraea* is a Gram-positive bacterium producer of the antibiotic erythromycin A.[17]

Another metabolite isolated from a number of species of *Fusarium* is equisetin,[18−23] a tetramic acid-containing analogue with antibiotic,[18,21] cytotoxic,[24] and phytotoxic[25] activities and potent inhibitor of mitochondrial ATPases[26] and HIV-1 integrase.[19,27] Equisetin is active against several genera of Gram-positive bacteria, including *Bacillus subtilis* and *Staphylococcus aureus*, and inhibits the growth of the acid-fast bacteria *Mycobacterium phlei*.[18,21] Several close analogues of equisetin have been reported, including its C-5′ epimer epi-equisetin,[21] its enantiomer phomasetin,[19] ophiosetin,[28] and trichosetin.[29]



Equisetin	R^1=CH_3, R^2=H, R^3=H, R^4=H
Ophiosetin	R^1=CH_3, R^2=H, R^3=OH, R^4=H
N-demethyl-ophiosetin (**1**)	R^1=H, R^2=H, R^3=OH, R^4=H
Pallidorosetin A (**2**)	R^1=CH_3, R^2=H, R^3=H, R^4=OH
Pallidorosetin B (**3**)	R^1=CH_3, R^2=OH, R^3=OH, R^4=H

The LCMS chromatogram of the extract from the coculture of *S. erythraea* with *F. pallidoroseum* showed the presence of four peaks that were not observed in either of the controls (Figure 1, peaks 1−4). After further analysis it was also evident that although the peak labeled as 5 was present in the chromatogram of the extract of the *Fusarium* control, its area on the coculture was 30 times larger. These peaks exhibited absorbance bands in the UV spectrum with maxima at 250 and 290 nm. This UV profile was very similar to that reported for the known *Fusarium* metabolite equisetin.[20]

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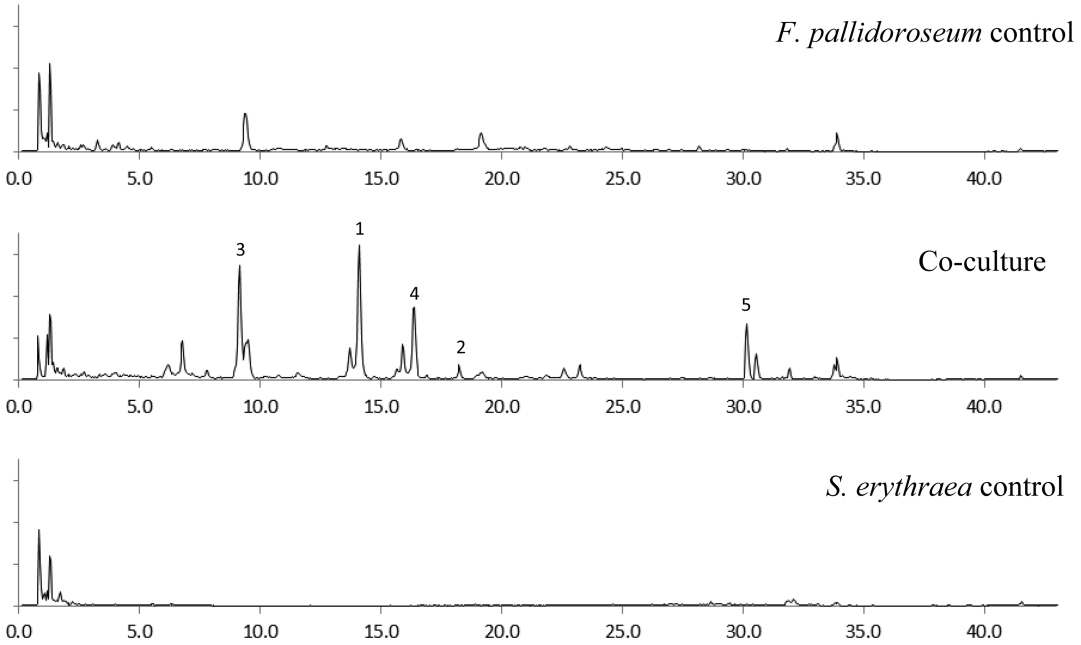

Figure 1. Chromatogram in positive ion mode (m/z 350−450 uma) of the extracts of *Fusarium pallidoroseum* (top), *Saccharopolyspora erythraea* (bottom), and coculture (middle) showing the new peaks observed only in the coculture (1−3 are new metabolites, 4 is ophiosetin, and 5 is equisetin).

Scale-up of this coculture allowed us to isolate those compounds produced only in the mixed fermentation. The analogues were identified as three new metabolites and two known compounds identified as equisetin[18] and ophiosetin[28] by comparison with reported data. Ophiosetin has been reported from the fungus *Elaphocordyceps ophioglossoides*[28] but had never been reported from the genus *Fusarium* or *Saccharopolyspora*.

The molecular weight of compound **1** was determined as 375. The UV−vis spectrum showed two maxima at 250 and 287 nm, which were consistent with the spectrum reported for equisetin.[20] The signals in the ^{1}H NMR spectrum (DMSO-d_6) of compound **1** were broad, supporting the possibility that it was an equisetin analogue since it is known that several tautomers occur with tetramic acid analogues.[30] Despite the broad signals, it was possible to assign all of the signals in the spectrum. The ^{1}H NMR spectrum showed two signals integrating for 3H each that were assigned to methyl groups [one singlet at δ_H 1.30 and one doublet at δ_H 1.48 (J = 4.8 Hz)]. The spectrum also showed signals for an additional nine protons resonating between 0.7 and 2 ppm, six protons between 3.2 and 3.7 ppm, one D_2O exchangeable proton (δ_H 4.36), and four vinylic protons. 2D NMR experiments including COSY, HSQC, and HMBC allowed us to assign all of the signals in the decalin core. The HSQC correlations between δ_C 67.0 and δ_H 3.20 and 3.23 and between δ_C 62.8 and δ_H 3.47 and 3.63 suggested that four of the signals between 3.1 and 3.8 ppm corresponded to two methylene groups carrying hydroxyl groups. The HMBC correlation between one of these methylene groups (δ_H 3.22, H-16) and δ_C 37.0 (C-7), δ_C 41.4 (C-8), and δ_C 30.4 (C-9) and between H-7a (δ_H 0.76), H-7b (δ_H 1.86), H-8 (δ_H 1.46), and H-9a (δ_H 0.99) and C-16 (δ_C 67.0) indicated that the CH_3-16 present in equisetin was oxidized to a primary alcohol. The second primary alcohol was assigned to C-6′ on the tetramic acid part of the molecule by HMBC correlations between H-6′b (δ_H 3.63) and C-4′ (δ_C 191.2) and C-5′ (δ_C 62.5). Thus analysis of the NMR data suggested that compound **1** had a structure very similar to ophiosetin. Comparison of the NMR data obtained in DMSO-d_6 of both molecules indicated that the main difference was the absence of the N-methyl group in the new analogue. The relative configuration of the decalin skeleton of compound **1** was determined by NOESY and tROESY experiments. tROESY correlations between H-6 (δ_H 1.77) and H-8 (δ_H 1.46) and CH_3-12 (δ_H 1.30) and NOESY correlation between H-3 (δ_H 3.62) and CH_3-12 (δ_H 1.30) indicated a *syn* relationship among these protons. The absence of correlation between H-6 and H-11 supported a *trans* fusion of the two six-membered rings in the decalin skeleton. NOESY correlations between H-11 (δ_H 1.54) and H-13 (δ_H 5.14) suggested a *syn* relationship but on the other side of the molecule's plane. The optical rotation ($[\alpha]^{25}_D$ −334) was the same sign as equisetin. Comparison of the CD spectra obtained for compound **1** [λ_{max} ($\Delta\varepsilon$) 232 (−3.3), 250 (−2.3), 285 (−8.4), 323 (+0.7) nm] and equisetin allowed us to establish that the absolute configuration was the same as the one reported for equisetin. Compound **1** was named *N*-demethylophiosetin.

The molecular weight of compound **2** was established as 389, which corresponds to the same molecular weight as ophiosetin. However, careful analysis of the NMR data and comparison with an authentic sample indicated that compound **2** was not ophiosetin. Its ^{1}H NMR spectrum showed three singlets (δ_H 1.08, 1.24, and 2.77) and one doublet (δ_H 1.46) integrating for three protons each. Three of the methyl groups were assigned by 2D NMR correlations to the methyl groups C-12, C-15, and N-CH_3; however the singlet at highest field (δ_H 1.08) was not observed in the ^{1}H NMR spectrum of ophiosetin. HMBC correlation between this signal (δ_H 1.08) and C-7 (δ_C 46.4), C-8 (δ_C 68.1), and C-9 (δ_C 40.1) suggested that this methyl group was on C-8. The low-field chemical shift for C-8 (δ_C 68.1) in the ^{13}C NMR spectrum together with HMBC correlations between one D_2O exchangeable proton (δ_H 3.91) and C-8 (δ_C 68.1) and C-16 (δ_C 31.8) indicated the presence of a hydroxyl group on C-8. To the best of our knowledge this is the first report of an equisetin-type analogue bearing a hydroxyl on C-8.

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This substitution had been reported in tanzawaic acid[31] and in betaenones,[32] both metabolites containing a decalin core. Comparison of the NMR data with those reported for tanzawaic acid for CH_2-7 (δ_H 1.04 and 1.59, δ_C 46.4) C-8 (δ_C 68.1), CH_2-9 (δ_H 1.30 and 1.50, δ_C 40.1), and CH_3-16 (δ_H 1.08, δ_C 31.9) confirmed the presence of the hydroxyl group at C-8. The coupling constant H-13/H-14 of 15.4 Hz indicated that the configuration of the double bond of the side chain was *E*. The relative configuration of the decalin ring was determined by tROESY experiments. Correlations between CH_3-12 (δ_H 1.24) and H-3 (δ_H 4.09) and H-6 (δ_H 2.10) and between H-6 (δ_H 2.10) and OH-8 (δ_H 3.91) indicated a *syn* relationship among all these protons, and correlations between H-11 (δ_H 1.43) and H-13 (δ_H 5.14) supported these two protons being in a *syn* relationship. The absence of correlation between H-6 and H-11 suggested that these two protons were on opposite sides of the molecule's plane, indicating a *trans* fusion of the two rings of the decalin skeleton. The optical rotation ($[\alpha]^{25}_D$ −153) and the CD spectrum [λ_{max} ($\Delta\varepsilon$) 228 (−4.0), 256 (−2.5), 292 (−11.5), 324 (+2.5) nm] were the same sign as that determined for equisetin, indicating the same absolute configuration. We proposed the name pallidorosetin A for compound **2**.

Compound **3** was more polar than the other isolated metabolites, as indicated by its shorter retention time on reversed-phase chromatography. Its molecular weight was determined to be 405, indicating that it contains one oxygen more than ophiosetin. The absence of the doublet assigned to CH_3-15 in the ^{1}H NMR spectra of all the previous analogues, together with a COSY correlation between H-15 (δ_H 3.74) and a vinylic proton on the lateral chain (H-14, δ_H 5.22) and the signal for one of the D_2O exchangeable protons (δ_H 4.38), suggested the presence of a hydroxyl group on the side chain, accounting for the extra oxygen in the molecular formula. HMBC correlations between H-15 (δ_H 3.74) and C-13 (δ_C 132.9) and C-14 (δ_C 130.5) and between H-13 (δ_H 5.30) and H-14 (δ_H 5.22) and C-15 (δ_C 62.1) confirmed the position of the new hydroxyl group on C-15. Two pairs of signals were assigned to CH-5′ (δ_H 3.19, δ_C 65.3 and δ_H 3.42, δ_C 72.8) and two to CH_2-6′ (δ_H 3.54 and 3.67; δ_C 62.2 and δ_H 3.29 and 3.36; δ_C 63.4). These data suggested that compound **3** was a mixture of epimers at C-5′.

A similar mixture of epimers at C-5′ had been described previously for equisetin,[19] phomasetin,[19] and the melophlins.[33] For equisetin and phomasetin the epimerization at C-5′ was also reported in pyridine.[21] The coupling constant $J_{H\text{-}13/H\text{-}14}$ = 15.4 Hz indicated that the configuration of the double bond of the side chain was *E*. The relative configuration of compound **3** was determined by analysis of a tROESY experiment. The lack of correlation between H-6 and H-11 together with the correlation between H-11 (δ_H 1.48) and H-13 (δ_H 5.30) and between CH_3-12 (δ_H 1.24) and H-6 (δ_H 1.67) and H-3 (δ_H 4.16) indicated *trans* fusion of the two six-membered rings on the decalin skeleton and the relative configuration of C-2 and C-3. Correlations between H-16 (δ_H 3.21) and H-11 (δ_H 1.48) with H-9a (δ_H 0.95) and H-7a (δ_H 0.71) indicated a *syn* relationship among these protons, while correlation between H-8 (δ_H 1.44) and H-7b (δ_H 1.82) and H-9b (δ_H 1.69) also indicated a *syn* relationship but on the other side of the molecule's plane. The optical rotation obtained for compound **5** ($[\alpha]_D$ −142) had the same sign as those observed for all previous analogues, and its CD spectrum was also qualitatively similar [λ_{max} ($\Delta\varepsilon$) 237 (−1.6), 250 (−1.6), 293 (−4.4), 323 (+1.0) nm], confirming that the absolute configuration for all the centers, except for C-5′, which could not be determined, was the same as the one reported for equisetin. We propose the name pallidorosetin B for compound **3**.

The spectral data for equisetin and ophiosetin were in agreement with those reported in the literature.

All the isolated analogues were tested using a Kirby-Bauer disk diffusion assay[34] against the Gram-positive bacteria *Staphylococcus erythraea* and *S. aureus*. Only equisetin was active (minimum inhibitory concentrations were <1.25 μg against *S. aureus* and 2.5 μg against *S. erythraea*). The inhibition of *S. aureus* is in agreement with the data previously reported.[18] Hydroxylation of C-16 was reported as detrimental for the antimicrobial activity based on the comparison of activity observed between equisetin and ophiosetin.[23] Compounds **1** and **3** also bear a hydroxyl group on C-16 and were both inactive against the bacteria screened. Compound **2**, which has a hydroxyl group on C-8 instead of C-16, also was inactive. All the inactive analogues are more polar than equisetin, which could suggest that the antibacterial activity has a negative correlation with the polarity of the metabolite. Analogues containing a tetramic acid moiety were proposed to have antibacterial properties by inhibiting the formation of biofilms in Gram-positive bacteria.[35] It is possible that *Fusarium* increases the production of equisetin-type analogues as a defense mechanism against *Saccharopolyspora*. Mixed cultures of *Fusarium* and other Gram-positive bacteria (*Bacillus* and *Staphylococcus*) cultured in our laboratory also increased production of equisetin (data not shown). Interestingly, the tetramic acid trichosetin[29] was isolated from a coculture of the fungus *Trichoderma harzianum* with the callus of *Catharanthus roseus* and not produced by either of the organisms alone.

The cytotoxicity of all the isolated metabolites was assessed in the NCI-60 cell line screen.[36,37] Equisetin (NSC 772378) exhibited low μM GI_{50} (Supporting Information) against all the cell lines and GI_{50} = 144 nM against the leukemia cell line CCRF-CEM. The rest of the compounds were inactive (GI_{50} > 20 μM).

EXPERIMENTAL SECTION

General Experimental Procedures. UV spectra were acquired in spectroscopy grade MeOH using an Agilent 8453 UV−vis spectrophotometer; NMR spectra were recorded on Bruker Avance III 600 MHz (Bruker Biospin) and Varian Inova 500 MHz NMR spectrometers. Measured chemical shifts were referenced to residual solvent (δ_H 2.50 and δ_C 39.5 in DMSO-d_6). LCMS analyses and HRESIMS measurements were performed with an XTerra MS C_{18} (2.1 × 50 mm, 3.5 μm, Waters) column on a Waters Acquity UPLC system coupled to a Waters LCT Premier TOF mass spectrometer, a Waters Acquity PDA detector, and a Sedex 75 evaporative light scattering detector. Matrex C_{18} adsorbent (Varian) was used for flash chromatography. Preparative-scale purification was performed with a Dynamax 60 Å C_{18} column (19 × 300 mm, Varian) on an HPLC system consisting of a Waters 600 pump, a Waters 3100 mass detector, a Waters 996 photodiode array detector, and a Shimadzu ELSD-LT2 evaporative light scattering detector.

Material. Both microorganisms were purchased from the American Type Culture Collection (ATCC). *Fusarium pallidoroseum* (ATCC 74289) was maintained on Difco potato dextrose agar at room temperature. *Saccharopolyspora erythraea* (ATCC 31772) was maintained on International Streptomyces Project Medium 2 (ISP2) agar, consisting of malt extract, 10 g/L; dextrose, 4 g/L; and yeast extract, 4 g/L, at 27 °C.

Fermentation and Isolation. *S. erythraea* and *F. pallidoroseum* were grown on agar for two weeks and one week, respectively, before

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Table 1. ^{1}H (500 MHz) and ^{13}C (150 MHz) NMR Data for Compounds 1−3 in DMSO-d_6

	1		2		3	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C,[a] type	δ_H (J in Hz)
1	199.5, C		198.1, C		197.7, C	
2	49.6, C		48.6, C		49.9, C	
3	43.9, CH	3.62[b]	42.2, CH	4.09 m	41.8, CH	4.16 br s
4	127.6, CH	5.36 ddd (10.2, 4.7, 2.1)	128.3, CH	5.34 ddd (10.0, 5.2, 2.6)	128.2, CH	5.38 br s
5	130.5, CH	5.42 d (10.2)	130.0, CH	5.27 d (10.0)	130.4, CH	5.38 br s
6	38.4, CH	1.77[b]	33.3, CH	2.10 t (10.7)	38.5, CH	1.67 m
7	37.0, CH_2	0.76 q (12.0)	46.4, CH_2	1.04 d (11.5)	37.1, CH_2	0.71 q (11.8)
		1.86 d (12.0)		1.59 d (11.5)		1.82 d (11.8)
8	41.4, CH	1.46*	68.1, C	3.91 s (OH)	41.2, CH	1.44 m
9	30.4, CH_2	0.99 q (12.0)	40.1, CH_2	1.30 m	30.5, CH_2	0.95 br d (13.8)
		1.75 m		1.50 d (12.9)		1.69 m
10	27.7, CH_2	0.89 q (12.0)	22.9, CH_2	1.10 m	27.2, CH_2	0.73[b]
		1.96 br s		1.61 br d (11.9)		1.92 br d (10.0)
11	40.6, CH	1.54 m	40.4, CH	1.43 m	40.4, CH	1.48 m
12	14.4, CH_3	1.30 s	14.7, CH_3	1.24 s	14.6, CH_3	1.24 s
13	132.6, CH	5.14 m	134.3, CH	5.14 dd (15.4,6.9)	132.9, CH	5.30 dd (15.4, 7.0)
14	125.8, CH	5.14 m	123.1, CH	5.07 dq (15.4, 6.0)	130.5, CH	5.22 dt (15.4, 5.4)
15	18.3, CH_3	1.48 d (4.8)	18.1, CH_3	1.46 d (5.6)	62.1, CH_2	3.74 br s
16	67.0, CH_2	3.20 d (11.6)	31.8, CH_3	1.08 s	67.0, CH_2	3.21 br d (4.2)
		3.23 d (11.6)				
2′	178.6, C		175.0, C		175.4, C	
3′	101.2, C		101.5, C		ND	
4′	191.2, C		188.5, C		189.2, C	
5′	62.5, CH	3.64 m	64.7, CH	3.21 br s	65.3, CH (A)	3.19[b] (A)
					72.8, CH (B)	3.42 dd (10.9, 5.7) (B)
6′	62.8, CH_2[a]	3.47 dd (11.1, 5.0)	61.8, CH_2	3.54 dd (10.5, 5.1)	62.2, CH_2 (B)	3.36[b] (a)
		3.63 dd (11.1, 2.8)		3.66 dd (10.5, 2.3)		3.29 dd (10.8, 5.7) (b)
					63.4, CH_2 (A)	3.67 dd (10.8, 3.0) (a)
						3.54 dd (10.8, 5.0) (b)
NCH_3			27.0, CH_3	2.77 s	27.6, CH_3	2.77 s
OH-16		4.36 br s				4.32 br s
OH-15						4.38 br s

[a] δ_C determined by 2D experiments, [b] Superposed with another signal; δ_H determined by analysis of 2D experiments.

inoculation into 100 mL of ISP2 seed medium in 500 mL Erlenmeyer flasks. Both organisms were incubated at room temperature for 24 h on a rotary shaker (150 rpm), after which the seeds were used to inoculate 1 L Erlenmeyer flasks containing 200 mL of ISP2 broth. The inoculum per 1 L flask was 20 mL of *S. erythraea* and 1 mL of *F. pallidoroseum* with a ratio of packed cell volume to media of <0.5 mL/15 mL and 1 mL/15 mL, respectively. Fermentation was carried out for 4 days at 27 °C on a rotary shaker (200 rpm). Single control cultures of *S. erythraea* and *F. pallidoroseum* were grown under identical conditions.

The full volume (3.5 L) of culture filtrate was concentrated to 300 mL *in vacuo* (<40 °C) and partitioned three times with equal volumes of ethyl acetate. Evaporation of the pooled organic layers yielded 1.45 g of an oily brown crude extract. The extract was applied to a 10 mL SPE cartridge packed with 1.5 g of C_{18} silica. The cartridge was eluted in a stepwise fashion with increasing percentages of MeOH in H_2O. Fractions containing the compounds of interest were pooled and lyophilized, yielding 52.4 mg of enriched extract. Further purification was performed with a stepwise gradient (H_2O/AcN w/0.1% F.A.; 0−20 min 65:35, 20−60 min 60:40; 60−80 min 55:45; 10 mL/min) on a preparative-scale reversed-phase HPLC system. Analysis and pooling of the resulting fractions afforded ophiosetin (2.53 mg), *N*-demethylophiosetin (**1**) (2.86 mg), pallidorosetin A (**2**) (1.59 mg), pallidorosetin B (**3**) (1.06 mg), and equisetin (5.07 mg).

*N-Demethylophiosetin (**1**):* $[\alpha]^{25}_D$ −334 (*c* 0.025, $CHCl_3$); CD (*c* 0.1 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 232 (−3.3), 250 (−2.3), 285 (−8.4), 323 (+0.7) nm; UV (MeOH) λ_{max} (log ε) 250 (3.73), 287 nm (3.92); ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) see Table 1; HRESIMS *m*/*z* 376.2108 $[M + H]^+$ (calc for $C_{21}H_{30}NO_5$, 376.2124).

*Pallidorosetin A (**2**):* $[\alpha]_D$ −153 (*c* 0.025, $CHCl_3$); CD (*c* 0.1 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 228 (−4.0), 256 (−2.5), 292 (−11.5), 324 (+2.5) nm; UV (MeOH) λ_{max} (log ε) 251 (3.98), 292 nm (3.99); ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) see Table 1; HRESIMS *m*/*z* 390.2262 $[M + H]^+$ (calc for $C_{22}H_{32}NO_5$, 390.2280).

*Pallidorosetin B (**3**):* $[\alpha]_D$ −142 (*c* 0.025, $CHCl_3$); CD (*c* 0.1 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 237 (−1.6), 250 (−1.6), 293 (−4.4), 323 (+1.0) nm; UV (MeOH) λ_{max} (log ε) 250 (3.76), 290 nm (3.73); ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) see Table 1; HRESIMS *m*/*z* 388.2115 $[M - H_2O + H]^+$ (calc for $C_{22}H_{30}NO_5$, 388.2124).

Equisetin: $[\alpha]_D$ −258 (*c* 0.025, $CHCl_3$); CD (*c* 0.1 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 234 (−4.5), 255 (−1.7), 292 (−8.3), 326 (+0.6) nm.

Ophiosetin: $[\alpha]_D$ −241 (c 0.025, $CHCl_3$); CD (*c* 0.1 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 234 (−4.2), 256 (−2.2), 292 (−7.7), 328 (+0.3) nm.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR, ^{13}C NMR, COSY, gHSQC, gHMBC, NOESY, TROESY, and CD spectra for compounds **1**−**3** together with dose response NCI-60 screen data for equisetin. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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