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PPAR Modulating Polyketides from a Chinese *Plakortis simplex* and Clues on the Origin of Their Chemodiversity

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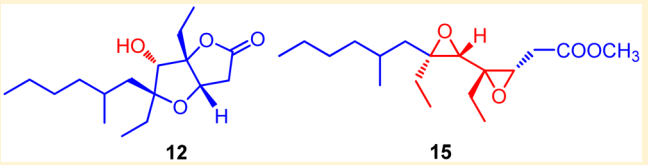
Supporting Information

ABSTRACT: Fifteen polyketides, including the first hydroxylated plakortone (**12**) and plakdiepoxide (**15**), the first polyketide to embed a vicinal diepoxide, have been isolated from the Chinese sponge *Plakortis simplex*. The structures of the new metabolites were elucidated by analysis of spectroscopic data, Mosher's derivatization, and DFT computational calculations. The reactivity of the major endoperoxide of this sponge was investigated, suggesting that furan, furanylidene, and plakilactone derivatives, well-known classes of natural products, could actually be chemical degradation products. Plakdiepoxide is a potent and selective modulator of peroxisome proliferator-activated receptor (PPAR)-γ, while the diunsaturated C_{12} fatty acid monotriajaponide (**13**) activates both PPAR-α and PPAR-γ, a dual activity of potential great importance for the treatment of metabolic disorders.



INTRODUCTION

The last couple of decades have witnessed intense research activity toward marine sponges belonging to the family Plakinidae, in particular, the genus *Plakortis*.[1] This resulted in the isolation of a variety of secondary metabolites characterized by different molecular architectures, spanning from unique alkaloids (e.g., plakohypaphorines[2] and thiaplakortones[3]) to highly rearranged steroids,[4] and in the discovery of promising bioactivities. However, the chemical and biological potential of *Plakortis* sponges is undoubtedly associated with their prolific production of 1,2-dioxane derivatives, exemplified by the antimalarial plakortin[5] and related plakortides.[6] These molecules are believed to share a propionate/butyrate-based polyketide biosynthetic origin, a hypothesis supported by the co-occurrence of analogues differing for the ketide unit (e.g., propionate in place of butyrate etc.) but, nevertheless, not yet demonstrated. Another open question about these metabolites involves the real producer: the metabolic contribution of symbiotic microorganisms, present in large percentages in the spongal tissues,[7] has been postulated but never confirmed unambiguously.

We have been working in this field for several years and first discovered the antimalarial potential of plakortin,[8] defined its mechanism of action in detail,[9] and designed the two-step total synthesis of simplified analogues.[10] Moreover, we have also discovered the potent antitrypanosoma activity of another class of *Plakortis* polyketides, named manadoperoxides after their isolation from an Indonesian *Plakortis* sample.[11]

In the frame of a Sino-Italian collaboration, we have jointly investigated a Chinese specimen of *Plakortis simplex* and recently described its antimalarial endoperoxide composition,[12] which included both 1,2-diox-4-ene and 1,2-dioxane analogues. Herein, we report the results of a detailed characterization of the nonendoperoxide polyketides of the same organism, thus completing the description of its polyketide composition. The complex mixture of nonendoperoxide polyketides has been deconvoluted into 15 pure compounds, **1**−**15**, belonging to seven different structural classes. The structures of the new plakorsin D methyl ester (**5**), plakilactone I (**7**), plakortone Q (**12**), and plakdiepoxide (**15**) have been determined on the basis of a combination of spectral and computational data. In addition, all of the isolated polyketides have been evaluated for their agonistic effect on PPAR-γ and PPAR-α, transcription factors involved in the regulation of cellular differentiation, development, and metabolism.

RESULTS AND DISCUSSION

Isolation and Structural Elucidation. A specimen of *Plakortis simplex* was collected along the coasts of the Xisha Islands, in the South China Sea, and exhaustively extracted with methanol. The obtained residue was then extracted in sequence with *n*-hexane, CH_2Cl_2 and *n*-BuOH, thus concentrating the apolar polyketides into the CH_2Cl_2 phase. This was subjected

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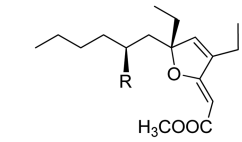

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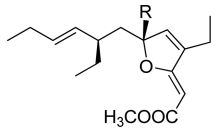

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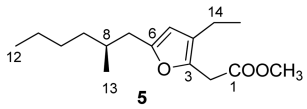

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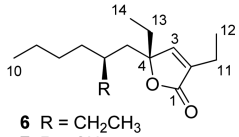
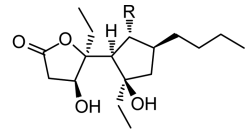


6 R = CH_2CH_3
7 R = CH_3



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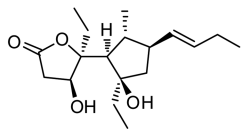
9 R = H



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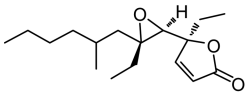

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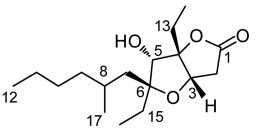

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to repeated column and HPLC chromatography to afford compounds **1**−**15** in the pure state. The known furanylidene derivatives **1**−**4**,[13,14] plakilactone A (**6**),[15] simplextones A−C (**8**−**10**),[16,17] plakortoxide A (**11**),[17] monotriajaponide A (**13**),[18] and woodylide C (**14**)[19] were identified on the basis of a comparison of their spectral data with those reported in the literature. The configuration at the two stereogenic centers C-6 and C-8 of **13** had been left unassigned.[18] By comparing the experimental CD curve with those simulated for the two enantiomers at C-6 using the TDDFT approach, we have determined the *R* configuration at C-6 of monotriajaponide A (**13**) (Supporting Information).

Compound **5** ($C_{16}H_{26}O_3$ by HR-MS) was easily assigned as the methyl ester of the known plakorsin D,[17] since ^{1}H and ^{13}C NMR spectra of the two compounds were practically identical, with the single exception of an additional methoxy group (δ_H 3.72, δ_C 52.2) present in the spectra of **5**. The signal at δ_H 3.72 exhibited a diagnostic HMBC cross-peak with the carbonyl carbon resonating at δ_C 170.4. The absolute configuration at C-8 of plakorsin D had been left unassigned;[17] however, we have defined its *S* configuration upon chemical conversion from dihydrohaterumadioxin A (see below). Since methanol has been used as solvent for extraction, we cannot exclude that compound **5** is an isolation artifact.

Similarly, the structural elucidation of the new plakilactone I (**7**), was aided by comparison with data of plakilactone A (**6**). The molecular formula of **7** ($C_{15}H_{26}O_2$) lacked only a $-CH_2-$ unit compared to that of **6**. Since ^{1}H and ^{13}C NMR signals of the γ-lactone moiety of **7**, including the two attached ethyl groups, were practically identical to parallel signals of **6**, the structural difference must be located in the long alkyl side chain. The presence of a methyl doublet signal at δ_H 0.89 (CH_3-15), coupled with H-6 in the COSY spectrum, indicated the replacement of the C-6 ethyl branching of **6** with a methyl one in **7**. Chemical conversion of dihydrohaterumadioxin A into **7** under basic conditions (see below) unambiguously indicated the 4*R*,6*S* configuration for plakilactone I (**7**).

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Plakortone Q (12) and Plakdiepoxide (15) in $CDCl_3$

	12		15	
pos	δ_H, mult (*J*, Hz)	δ_C, mult	δ_H, mult (*J*, Hz)	δ_C, mult
1		174.8, C		172.0, C
2	2.68, d (18.6)	37.9, CH_2	2.79, dd (17.2, 6.8)	33.8, CH_2
	2.77, dd (18.6, 5.8)		2.89, dd (17.2, 5.8)	
3	4.33, d (5.8)	77.2, CH	3.14, dd (6.8, 5.8)	56.7, CH
4		95.0, C		60.4, C
5	3.87, d (6.5)	81.6, CH	3.04, s	62.8, CH
5-OH	2.25, d (6.5)			
6		88.3, C		63.0, C
7	1.36[a]	42.2, CH_2	0.87[a]	41.8, CH_2
	1.48[a]		2.03, dd (14.0, 4.2)	
8	1.57[a]	28.5, CH	1.53[a]	30.0, CH
9	1.15[a]	38.3, CH_2	1.23[a]	37.9, CH_2
	1.35[a]			
10	1.29[a]	29.5, CH_2	1.78, m	29.4, CH_2
11	1.28[a]	23.0, CH_2	1.28[a]	23.1, CH_2
12	0.89, t (7.0)	14.1, CH_3	0.87, t (7.0)	10.0, CH_3
13	1.79, m	29.1, CH_2	1.60[a]	26.9, CH_2
	1.92, m		1.90, m	
14	1.04, t (7.0)	7.9, CH_3	1.01, t (7.0)	9.0, CH_3
15	1.52[a]	26.0, CH_2	1.66[a]	22.4, CH_2
	1.62[a]		1.72[a]	
16	0.91, t (7.0)	8.2, CH_3	1.03, t (7.0)	12.3, CH_3
17	0.95, d (7.0)	21.0, CH_3	0.94, d (7.0)	19.0, CH_3
1-OMe			3.70, s	51.5, CH_3

[a]Overlapped with other signals.

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HR-ESIMS experiments indicated for plakortone Q (**12**) the molecular formula $C_{17}H_{30}O_4$, compatible with three indices of hydrogen deficiency. The ^{1}H NMR spectrum of **12** (Table 1) showed four methyl signals (three triplets and one doublet), two oxymethine resonances (δ_H 4.33, d; 3.87, s), and a series of partially overlapped multiplets located between δ_H 2.77 and 1.15. These signals were unambiguously deconvoluted with the aid of the 2D NMR HSQC experiment; thus, in addition to the four methyl groups, three sp^3 methines (including the two oxymethines) and seven sp^3 methylenes were disclosed. The three remaining unprotonated carbon atoms resonated at δ_C 174.8 (an ester carbonyl), 95.0, and 88.3. This preliminary analysis indicated a bicyclic structure for plakortone Q.

The 2D NMR COSY spectrum of **12** arranged the proton multiplets into four spin systems (Figure 1), namely a

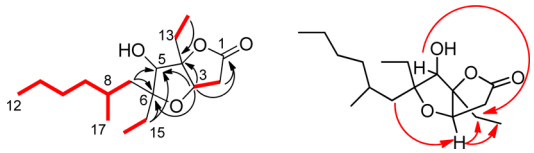


Figure 1. Key 2D NMR correlations detected for plakortone Q. (Left) COSY (bold) and HMBC (arrows). (Right) ROESY.

methylhexyl chain similar to that of the other coisolated polyketides, two ethyl groups, and a small spin system including a diastereotopic methylene and an oxygenated methine (δ_H 4.33, δ_C 77.2). This moiety was attached at the ester carbonyl on the basis of the HMBC correlations H_2-2/C-1 and H-3/C-1, while cross-peaks of both H_2-15 and H_2-7 with the unprotonated C-6 (δ_C 88.3) and with the oxymethine C-5 (δ_C 81.6) defined attachment of the two side chains and of the oxymethine at C-6. The HMBC correlations of H-3 with C-4 (δ_C 95.0), C-5 and C-6 and that of Me-14 with C-4 were only compatible with a bicyclic system of the plakortone type, thus defining the planar structure of the new plakortone Q (**12**).

The ROESY spectrum of **12** provided information to completely define the relative configuration of the four stereogenic centers around the bicyclic system (Figure 1). The correlations H-3/H_2-13; H-3/H_2-7, and H-5/H_2-13 defined the *cis* orientation of these groups. On the other hand, the free-rotating nature of the C-6/C-7 single bond prevented any extension of this relative configuration to the nonfunctionalized C-8.

The presence of a secondary alcohol functionality at C-5 of **12** suggested the possibility to upgrade this relative configuration to the absolute one through the modified Mosher's method,[20] whose application was, however, expected to be complicated by the absence of hydrogen atoms at the adjacent positions C-4 and C-6. Thus, two aliquots of plakortone Q (**12**) were dissolved in dry pyridine and allowed to react overnight with (*R*)- and (*S*)-MTPA chloride, affording in high yields the (*S*)- and (*R*)-MTPA esters **12a** and **12b** (Supporting Information), respectively. ^{1}H NMR assignment of these compounds, aided by inspection of COSY spectra, allowed an analysis of the $\Delta\delta_{(S-R)}$ values. As shown in Figure 2, the pattern observed for protons neighboring C-5 appeared consistent in indicating, following the Mosher model, the *S* configuration at C-5 of **12**.

Plakortone Q (**12**) is a new member of plakortone family, a group of compounds characterized by a tetrahydrofuro[3,2-*b*]furan-2(5*H*)-one bicyclic system whose members commonly

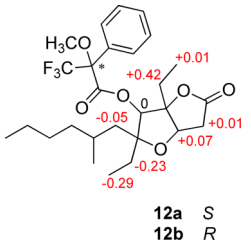
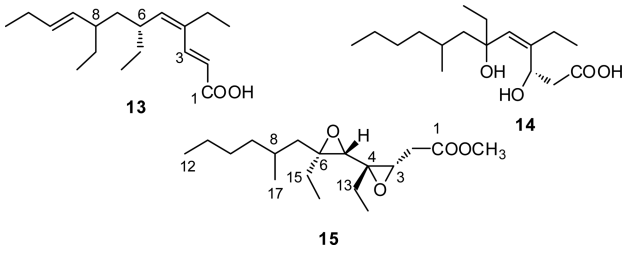


Figure 2. Application of the Mosher's method to plakortone Q. Values are expressed as $\Delta\delta_{(S-R)}$.

differ for the short alkyl appendages at C-4 and C-6 (methyl or ethyl groups) and for the long alkyl chain at C-6, with fully saturated, mono- or diunsaturated, or even phenyl-containing side chains having been reported.[21] Plakortone Q is the first member of this class to show a hydroxy group in the ring system.

Plakortones have been the object of intense synthetic efforts,[21,22] and in this context, Wong et al. have recently reported a biomimetic synthesis of plakortone B.[23] They successfully obtained plakortone B from the corresponding dioxolane derivative (plakortide E methyl ester) through reductive ring opening followed by intramolecular oxa-Michael addition and subsequent lactonization (Scheme 1).

In principle, plakortone Q (**12**) could be the product of a direct hydroxylation reaction; alternatively, it could derive from an epoxylactone epimeric to plakortoxide A through nucleophilic epoxide opening by water and subsequent oxa-Michael addition to the α,β-unsaturated γ-lactone ring (the mechanism has been reported in Scheme 1 in a concerted fashion). Of course, using hydride as nucleophile, the nonhydroxylated plakortones could be obtained. Thus, plakortones could be the result of two alternative biogenetic routes converging into the same structural scaffold.



Plakdiepoxide (**15**) was obtained as a colorless amorphous solid with the molecular formula $C_{18}H_{32}O_4$ (by HR-ESIMS), implying three degrees of unsaturation. The ^{1}H NMR spectrum of **15** ($CDCl_3$, Table 1) showed signals of a methoxy singlet at δ_H 3.70, four methyls (δ_H 0.87, 0.94, 1.01, and 1.03), a singlet at δ_H 3.04, and a series of multiplets between δ_H 3.14 and 0.87. Correlations of the 2D COSY spectrum built up four spin systems (Figure 3) that closely paralleled those above identified for plakortone Q (**12**). Indeed, a 7C-branched alkyl chain, two ethyl groups, and a $-CH_2CH-$ moiety were disclosed also for plakdiepoxide (**15**).

The HMBC cross-peaks H_3-16/C-6, H_2-7/C-6, and H_2-7/C-15 indicated the attachment of the C_7 alkyl chain and of an ethyl group at the same unprotonated carbon C-6 (δ_C 63.0). Similarly, the $-CH_2CH-$ moiety and the second ethyl group were attached at the same unprotonated carbon C-4 (δ_C 60.4) on the basis of the HMBC cross-peaks H-3/C-4, H_3-14/C-4, H_2-2/C-4. The uncoupled oxymethine at C-5 (δ_H 3.04, s; δ_C

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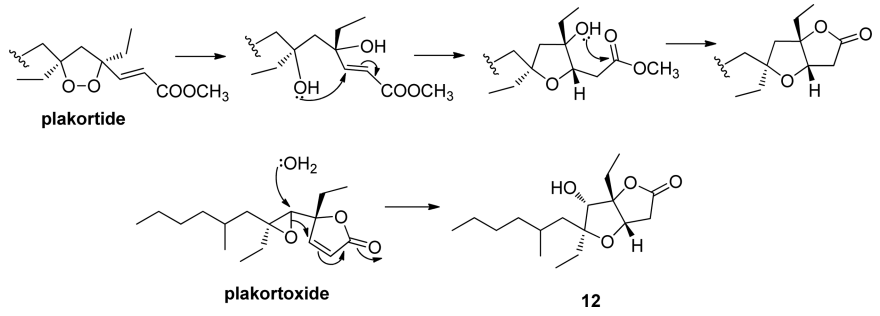
Scheme 1. (Top) Postulated[23] Biosynthetic Origin of Plakortones from Plakortides. (Bottom) Possible Derivation of Plakortone Q (12) from a Plakortoxide



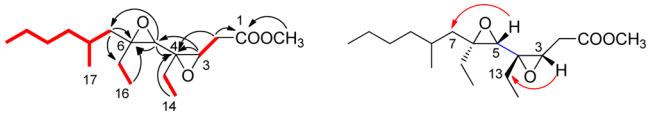


Figure 3. Key 2D NMR correlations detected for plakdiepoxide. (Left) COSY (red) and HMBC (arrows). (Right) ROESY.

62.8) should be the connection point between these two moieties, as indicated by the HMBC cross-peaks H-5/C-4, H-3/C-5, H-5/C-6, and H-5/C-7. Finally, a methyl ester group was placed at C-1, based on the HMBC correlation of both H_2-2 and the methoxy singlet with the ester carbonyl at δ_C 172.0. In order to account for the two remaining unsaturations and the two further oxygen atoms implied by the molecular formula, the four consecutive functionalized carbons from C-3 to C-6 must be involved into two oxygenated rings. ^{1}H (H-3 at δ_H 3.14; H-5 at δ_H 3.04) and ^{13}C NMR (δ_C 56.7, 60.4, 62.8 and 63.0, respectively) resonances at these positions were only compatible with the presence of two directly attached epoxide rings, thus defining the planar structure of **15**.

The relative configuration around the two three-membered rings was easily defined by the ROESY correlations H-5/H_2-7 and H-3/H_2-13 (Figure 3). However, since C-4/C-5 is a free-rotating single bond, the ROESY spectrum could not provide unambiguous information to connect each other these two relative configurations. Hence, we adopted a computational approach based on the comparison between experimental and quantum-mechanically calculated ^{13}C NMR resonances. Since the alkyl side chain was anticipated to have a negligible impact on the resonances of the oxygenated carbon atoms of the ring systems, and considering also that the relative configuration at C-8 had not been defined, we decided to use a simplified model for the computational calculations. Thus, the conformational behavior around the C-4/C-5 bond was explored in terms of the dihedral angle (θ) C-3/C-4/C-5/C-6 for the two model diastereomers **15a** and **15b** (Figure 4) through a density functional theory (DFT) calculation using the Gaussian03 software.[24]

This systematic search afforded 15 rotamers for each diastereomer, which were geometrically optimized at DFT level using a B3LYP functional and 6-31G(d) basis set. The relative energies of all conformations were calculated, and then the equilibrium room-temperature Boltzmann populations were obtained. Structure **15a** was characterized by two dominant rotamers (θ = −110.2° accounting for 51.1% of total population (tp); θ = −86.2° for 40.1% of tp), and similarly, two rotamers (θ = 109.9° for 82.2% of tp; θ = 134° for 14.0% of tp) were found for structure **15b** (Figure 4).

^{13}C NMR chemical shifts were then calculated for these conformers at the same level with the GIAO (Gauge Including Atomic Orbitals) option and the mPW1PW91/6-31G(d,p) DFT method (see the Supporting Information). Using the ab initio standard free energies as weighting factors, a Boltzmann average of ^{13}C NMR chemical shifts for any given carbon atom was independently calculated for the two diastereomers. The computed chemical shifts for **15b** appeared to match the experimental values of **15** better than those of **15a** [corrected mean absolute errors (CMAEs) were 1.71 for **15b** vs 2.97 for **15a**]. The two possible diastereomers were also compared by using the recent DP4+ probability method,[25] and also in this case, structure **15b** appeared to be the most likely (see the Supporting Information). On the basis of these computational data, the relative configuration of **15b** was suggested for plakdiepoxide. This relative configuration could not be

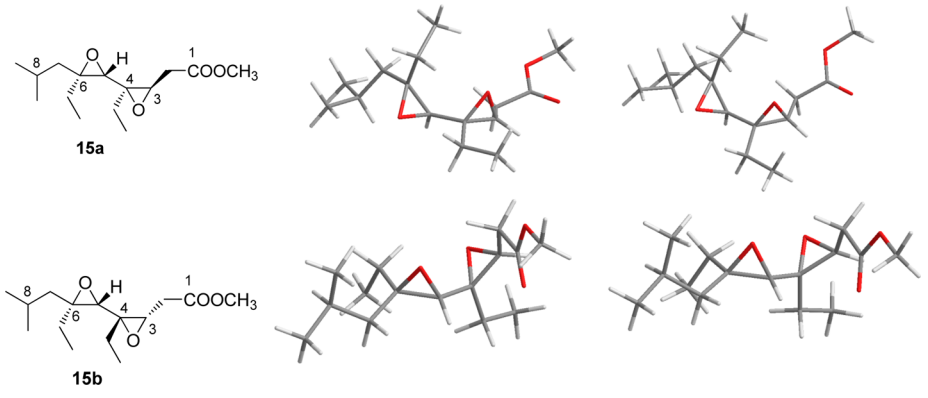


Figure 4. Two simplified diastereomers **15a** and **15b** used for the DFT calculation and the corresponding lowest energy conformers calculated.

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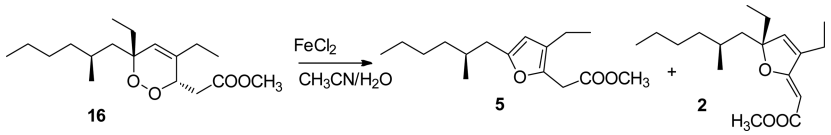
Scheme 2. Products of the Reaction of Endoperoxide 16 with $FeCl_2$



extended at C-8, and therefore, the configuration at this center has been left unassigned.

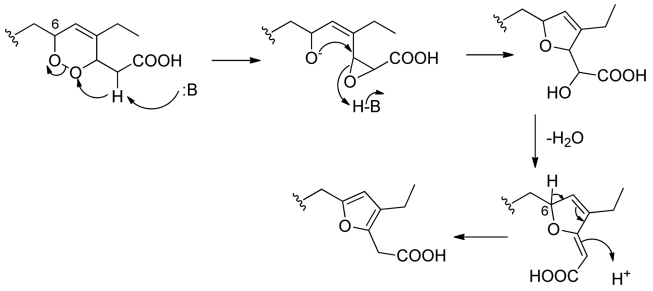
To our knowledge, plakdiepoxide (**15**) represents the first polyketide containing two vicinal epoxides on an acyclic chain. Only very few acyclic diepoxides have been isolated from natural sources, e.g., gummiferol (an acetylenic fatty acid)[26] and spatol (a terpene),[27] while vicinal diepoxides on cyclic systems are relatively more common, and a recent example is given by elysiapyrone A.[28] Interestingly, a biogenetic derivation from the corresponding bicyclic unsaturated endoperoxides has been proposed for these diepoxides,[28] and the transformation has been synthetically obtained via base (Et_3N),[28] transition-metal catalysis,[29] and photochemical rearrangement.[30] By analogy, plakdiepoxide (**15**) should derive from **16**, one of the major endoperoxides of the organic extract of *P. simplex*;[12] however, to our knowledge, this kind of transformation has never been described for monocyclic endoperoxides, much less prone to rearrangement compared to their bicyclic counterparts. For example, O'Shea and Foote have reported[31] that the same catalyst-inducing rearrangement of unsaturated bicyclic endoperoxides into diepoxides in high yields gave no diepoxide formation when the reaction was applied to 3,6-dimethyl-1,2-dioxene. Thus, although plakodiepoxide (**15**) co-occurred with the corresponding endoperoxide **16**, a direct biogenetic link between these two compounds is unlikely. Accordingly, there is no report in the literature of vicinal diepoxides similar to **15**, in spite of the dozens of unsaturated monocyclic endoperoxides reported from *Plakortis* and related sponges.

Reactivity of 1,2-Diox-4-ene Polyketides. In order to shed light on the biogenetic origin of plakdiepoxide, taking advantage of the high amounts (about 4.2%) of endoperoxide **16** in the organic extract of *P. simplex*, we investigated the reactivity of this dioxene metabolite under a variety of conditions.

Compound **16** proved to be remarkably unreactive in acidic solutions (CH_3COOH 1% in MeOH; H_2SO_4 1% in MeOH; $AlCl_3$ in $CHCl_3$) and upon thermal treatment (100 °C for 4 h). On the contrary, and not surprisingly, treatment of **16** under reducing conditions ($FeCl_2$ in CH_3CN/H_2O 4:1) caused an extensive degradation, mainly yielding two products, which were readily identified as plakorsin D methyl ester (**5**, about 72% yield) and the furanylidene derivative **2** (about 20% yield), both isolated as natural products from this specimen of *P. simplex* (Scheme 2).

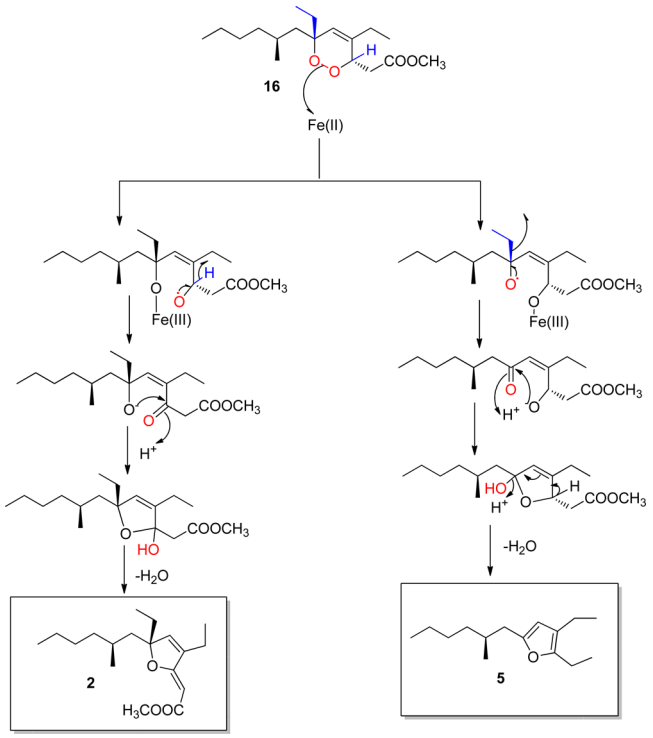
Interestingly, a biogenetic derivation from a 1,2-diox-4-ene analogue had been already postulated for both these classes of compounds, hypothesizing, however, the need for basic conditions. Andersen et al.[32] proposed that glanvillic acids, close analogues of plakorsins, could derive from a 1,2-diox-4-ene derivative lacking the methyl/ethyl branching at C-6 through a base-promoted rearrangement, as shown in Scheme 3. This hypothesis closely paralleled the Faulkner's biosynthetic proposal[33] for furanylidene derivatives, where a methyl/ethyl group at C-6 of the 1,2-diox-4-ene derivative prevents the final aromatization.

Scheme 3. Biosynthetic Origin of *Plakortis* Furan Derivatives Proposed by Andersen et al.[32]



We have now discovered that both plakorsin (or glanvillic) and furanylidene derivatives could be obtained from the corresponding 1,2-diox-4-enes upon treatment with Fe(II) salts. A plausible mechanism for this reaction, reported in Scheme 4, involves the one-electron opening of the endoperoxide ring with formation of the oxygen radical. The subsequent formation of the carbonyl group should cause expulsion of the alkyl radical at C-6 or of the H radical at C-3. This step is then followed by five-membered ring formation and dehydration, directly yielding products **2** and **5**. The exclusive formation of the *Z* diastereomer at $\Delta^{2,3}$ of **2** is likely the result of the steric hindrance of the neighboring vinylic ethyl group.

Scheme 4. Proposed Mechanism for the Formation of Compounds 2 and 5 from Endoperoxide 16



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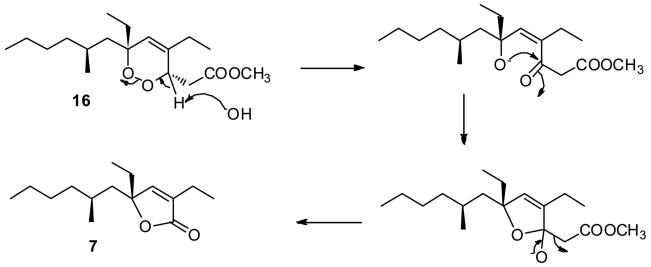
It can be anticipated that parallel reactions on a related endoperoxide bearing an ethyl group in place of the methyl at C-8 (haterumadioxin B) would yield compound **1** and the ethyl analogue of plakorsin D. Similarly, compounds **3** and **4** should derive from the 1,2-diox-4-enes possessing a double bond in the alkyl side chain, also found as metabolites of this sponge.[12]

Interestingly, a base-promoted Kornblum−DeLaMare-type[34] rearrangement of dioxenes into furanylidenes via γ-hydroxy-α,β-unsaturated ketones has been recently proposed by Norris et al.[35] but not demonstrated experimentally. In order to check the Faulkner/Andersen/Norris hypothesis, we treated endoperoxide **16** under basic conditions (NaOH 5% in $MeOH/H_2O$) and obtained, in low yields (25%), a mixture of plakilactone I (**7**) and the corresponding carboxylate, with no detectable amounts of furanylidene derivatives. A plausible mechanism for this conversion, reported in Scheme 5, could involve a retro-Claisen reaction leading to the expulsion of the $-CH_2COOCH_3$ residue.

Scheme 5. Plausible Mechanism for the Formation of Plakilactone I (7) from Endoperoxide 16 under Basic Conditions (NaOH 5% in $MeOH/H_2O$)



In summary, we have found that some polyketides commonly obtained from *Plakortis* and related sponges could be formed upon treatment of 1,2-diox-4-enes under reducing (furan and furanilydenes) or basic (plakilactones) conditions. On the contrary, the diepoxide derivative plakdiepoxide (**15**) was not obtained in detectable amounts in these conditions.

Activity on PPAR-α and PPAR-γ. The polyketides obtained from *P. simplex* (with exception of **7**, **9**, and **12**) have been evaluated for their activity on peroxisome proliferator-activated receptors (PPARs), ligand-activated transcription factors which constitute an important subfamily of nuclear receptors.[36] The three distinct PPAR subtypes (α, β, and γ) play a key role in glucose and lipid metabolism: PPAR-α is mainly deputed to fat degradation, while PPAR-γ controls glucose metabolism and insulin resistance. Existing modulators of PPAR-γ such as thiazolidinediones are highly effective for the treatment of type II diabetes, but they also possess several side effects, leading to the withdrawal from the market for some of them.[37]

The effect of *P. simplex* polyketides on PPARα and PPARγ transcriptional activity was determined by using the luciferase assay in HepG2 cells. The furanylidene acetates **1**−**3** and plakodiepoxide (**15**) proved to be selective ligands of PPAR-γ (Table 2), causing a 2-fold induction at 50 μM. The branched unsaturated fatty acid monotriajaponide (**13**) was the single compound found to be a potent agonist of both PPAR-γ and PPAR-α (50 μM = 2.13 fold induction; 25 μM = 1.85 fold induction; 12.5 μM = 1.42 fold induction), a dual activity of potential great importance for the treatment of metabolic disorders.

Table 2. Effect of *P. simplex* Polyketides on PPARγ Transcriptional Activity As Determined by Luciferase Assay in HepG2 Cells.[a,b]

compd	50 μM	25 μM	12.5 μM
1	2.1	1.6	1.4
2	2.0	1.6	1.3
3	2.4	1.7	1.2
4	1.7	1.6	1.5
5	1.8	2.0	1.8
6	1.4	1.5	1.3
8	1.3	1.6	1.4
10	1.5	1.3	1.5
11	1.6	1.3	1.4
13	3.0	2.0	2.2
14	1.6	1.5	1.5
15	2.0	1.6	1.5
rosiglitazone		3.0	2.8

[a]HepG2 cells were transfected with PPRE-luc together with pCMV-PPARγ. Twenty-four hours after the transfection, cells were treated with *Plakortis* compounds for an additional 24 h. [b]Values are fold induction compared to the control.

These three bioactive chemotypes share the presence of electrophilic sites: furanylidene derivatives and monotriajaponide are potential Michael acceptors, while plakdiepoxide includes two reactive epoxide rings. Therefore, these compounds could act as covalent ligands of PPARs; however, it should be noted that electrophilic sites are also present in the structures of the inactive/moderately active plakilactone **6** and plakortoxide A (**11**). Interestingly, plakilactone analogues embedding a strongly electrophilic α,β-unsaturated ketone in the alkyl side chain have been reported to act as PPAR-γ ligands in transactivation assays.[15]

CONCLUSION

Chemical investigation of the Chinese sponge *P. simplex* afforded 15 polyketides, including the first hydroxylated plakortone (**12**) and plakdiepoxide (**15**), a unique vicinal diepoxide. *Plakortis* polyketides are well-known antiprotozoal leads, but they can also have potential in other fields, such as PPAR modulation.[15] In the present study, plakdiepoxide has been characterized as a selective modulator of PPAR-γ, while the α,γ-diunsaturated C_{12} fatty acid monotriajaponide (**13**) has been disclosed as a potent dual activator of PPAR-α and PPAR-γ.

By investigating the reactivity of the major 1,2-diox-4-ene metabolite of this sponge (**16**), we discovered that treatment in reducing conditions afforded furan and furanylidene derivatives while treatment under basic conditions yielded plakilactones, three well-known classes of natural products, also isolated from this organism. We believe that this finding can be of general relevance and suggests that some of the nonendoperoxide polyketides isolated from *Plakortis* and related sponges are actually "degradation" products of the corresponding endoperoxides. Plakortethers,[38] first isolated in our laboratory from a Caribbean *Plakortis* sponge, and later obtained upon treatment of plakortin with Fe(II) salts,[11] represent a parallel example supporting this view. Most likely, the endoperoxide polyketides are utilized by sponges as defensive weapons, possibly against pathogen microorganisms, taking advantage of their oxidizing potential. Consequently, some of the nonendoperoxide polyketides would be simply the products of the (re)activity

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of endoperoxide precursors in the spongal cells and not genetically encoded secondary metabolites. Following Firn's "screening hypothesis",[39] this strategy adds molecular weapons to the marine invertebrate, but it also provides us with a parade of molecular architectures, whose chemodiversity is still fascinating and surprising after 30 years of investigation.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations ($CHCl_3$) were measured at 589 nm on a digital polarimeter. Low- and high-resolution ESI-MS spectra were performed on a LTQ Orbitrap mass spectrometer. IR spectra were recorded on an FT-IR 750 spectrometer. ^1H NMR spectra were measured on 700 and 500 MHz spectrometers. Chemical shifts were referenced to the residual solvent signal ($CDCl_3$: δ_H 7.26, δ_C 77.0). Homonuclear ^1H connectivities were determined by COSY experiments. Through-space ^1H connectivities were evidenced using a ROESY experiment with a mixing time of 500 ms. One-bond heteronuclear $^1H-^{13}C$ connectivities was determined by the HSQC experiment: two- and three-bond $^1H-^{13}C$ connectivities by gradient-HMBC experiments optimized for a $^{2,3}J$ of 8 Hz. Medium-pressure liquid chromatography was performed using a silica gel (230−400 mesh) column. The HPLC apparatus was equipped with a refractive index detector and SI60 (5 μ, 250 × 10 mm or 250 × 4 mm) or C18 (2.6 μ, 100 × 4.6 mm) columns.

Animal Material, Extraction, and Isolation. A specimen of *Plakortis simplex* (order Homosclerophorida, family Plakinidae) was collected around Yongxing Island and in the South China Sea in June 2007 and identified by Prof. Jin-He Li (Institute of Oceanology, Chinese Academy of Sciences, China). A voucher sample (No. B-3) was deposited in the Laboratory of Marine Drugs, Department of Pharmacy, Changzheng Hospital. The air-dried and powdered sponge (2.0 kg, dry weight) was extracted with MeOH (3 × 500 mL, 12 h each), and the crude extract was concentrated under reduced pressure at 45 °C to yield 500 g of residue. The residue was then extracted successively with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH. Part of the dichloromethane extract (15 g) was subjected to chromatography over a silica gel column (230−400 mesh) eluting with a solvent gradient of increasing polarity from *n*-hexane to methanol. Fractions eluted with *n*-hexane/EtOAc 95:5 were further fractionated by HPLC (*n*-hexane/EtOAc 97:3, flow 2.5 mL/min) to afford plakorsin D methyl ester (**5**, 4.3 mg), **6** (1.0 mg), plakortoxide A (**11**, 1.8 mg), and plakilactone I (**7**, 2.3 mg) in the pure state and a fraction whose further purification by HPLC (*n*-hexane/EtOAc 96:4, flow 0.6 mL/min) yielded plakdiepoxide (**15**, 1.4 mg). Fractions eluted with *n*-hexane/EtOAc 90:10 afforded compounds **1** (20.0 mg), **2** (50.0 mg), and **3** (120.5 mg) and a fraction further purified by HPLC (*n*-hexane/EtOAc 95:5, flow 3.5 mL/min) to obtain compounds **4** (1.0 mg) and **13** (10.0 mg) in the pure state. Fractions eluted with *n*-hexane/EtOAc 85:15 were further purified by HPLC (*n*-hexane/EtOAc 95:5, flow 3.5 mL/min) to afford plakortone Q (**12**, 3.1 mg) and **14** (4.4 mg). Fractions eluted with *n*-hexane/EtOAc 7:3 and 6:4 were combined and then purified by HPLC (*n*-hexane/EtOAc 7:3, flow 3.5 mL/min) to afford compound **10** (1.0 mg), and a fraction was further purified by RP-HPLC (MeOH/H_2O 78:22, flow 0.5 mL/min) to afford compounds **8** (0.9 mg) and **9** (1.2 mg).

Plakorsin D methyl ester (5): colorless amorphous solid; $[\alpha]_D$ +1.6 (*c* 0.6, $CHCl_3$); IR (neat) ν_{max} 1745, 1372, 1028 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ_H 5.83 (1H, bs, H-5), 3.72 (3H, s, 1-OMe), 3.55 (2H, s, H-2), 2.49 (1H, dd, *J* = 14.8, 5.6 Hz, H-7a), 2.32 (1H, overlapped, H-7b), 2.31 (2H, q, *J* = 7.7 Hz, H-13), 1.78 (1H, m, H-8), 1.23 (2H, overlapped, H-9), 1.23 (2H, overlapped, H-10), 1.16 (2H, overlapped, H-11), 1.10 (3H, t, *J* = 7.7 Hz, H-14), 0.84 (3H, d, *J* = 6.5 Hz, H-15); ^{13}C NMR ($CDCl_3$, 125 MHz) 170.4 (C-1), 154.2 (C-6), 140.3 (C-3), 124.1 (C-4), 107.6 (C-5), 52.2 (1-OMe), 35.9 (C-7), 35.5 (C-9), 32.2 (C-2), 30.1 (C-8), 28.2 (C-10), 23.0 (C-11), 20.1 (C-15), 18.1 (C-13), 14.8 (C-14), 14.5 (C-12); (+) ESI-MS *m*/*z* 267 $[M + H]^+$, 289 $[M + Na]^+$; HRMS (ESI) for $C_{16}H_{26}NaO_3$ $[M + Na]^+$ (*m*/*z* 289.1780) found *m*/*z* 289.1776.

Plakilactone I (7): colorless amorphous solid; $[\alpha]_D$ −23.0 (*c* 0.25, $CHCl_3$); IR (neat) ν_{max} 1749, 1282 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ_H 6.80 (1H, bt, *J* = 1.5, H-3), 2.31 (2H, dq, *J* = 7.5, 1.8 Hz, H-11), 1.81 (1H, m, H-13a), 1.71 (1H, overlapped, H-13b), 1.71 (1H, overlapped, H-6), 1.68 (2H, dd, *J* = 5.6, 1.5 Hz, H-5), 1.20 (2H, overlapped, H-9), 1.19 (3H, t, *J* = 7.5 Hz, H-12), 1.18−1.15 (4H, overlapped, H-7 and H-8), 0.89 (3H, d, *J* = 6.5 Hz, H-15), 0.88 (3H, t, overlapped, H-10), 0.82 (3H, t, *J* = 7.1 Hz, H-14); ^{13}C NMR ($CDCl_3$, 125 MHz) 174.1 (C-1), 150.7 (C-3), 136.3 (C-2), 90.5 (C-4), 45 (C-5), 38 (C-7), 31.7 (C-13), 29 (C-8), 28 (C-6), 23 (C-9), 19.7 (C-11), 17.1 (C-15), 14 (C-10), 12.3 (C-12), 8.8 (C-14); (+) ESI-MS *m*/*z* 239 $[M + H]^+$, 261 $[M + Na]^+$; HRMS (ESI) for $C_{15}H_{26}NaO_2$ $[M + Na]^+$ (*m*/*z* 261.1830) found *m*/*z* 261.1833.

Plakortone Q (12): colorless amorphous solid; $[\alpha]_D$ −2.4 (*c* 0.4, $CHCl_3$); IR (neat) ν_{max} 3488, 2920, 2850, 1790, 1470, 1265 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) Table 1; (+) ESI-MS *m*/*z* 299 $[M + H]^+$, 321 $[M + Na]^+$; HRMS (ESI) for $C_{17}H_{30}NaO_4$ $[M + Na]^+$ (*m*/*z* 321.2042) found *m*/*z* 321.2045.

Application of the Mosher's Method to Plakortone Q (12). Two aliquots of plakortone Q (**12**) (1.0 mg, 3.3 μmol) were treated with (*R*)-MTPA and (*S*)-MTPA chloride (30 μL) in 400 μL of dry pyridine with a catalytic amount of DMAP overnight at rt. Then the solvent was removed, and the products were purified by HPLC (*n*-hexane/EtOAc, 97:3) to obtain, respectively, the (*S*)-MTPA ester **12a** (1.2 mg) and the (*R*)-MTPA ester **12b** (1.3 mg).

*(S)-MTPA ester **12a**:* colorless amorphous solid; ^1H NMR ($CDCl_3$, 500 MHz) selected values δ_H 4.96 (1H, s, H-5), 4.41 (1H, d, *J* = 5.8 Hz, H-3), 2.75 (1H, dd, *J* = 18.6, 5.8 Hz, H-2a), 2.65 (1H, d, *J* = 18.6, Hz, H-2b), 2.22 (1H, m, H-13a), 1.88 (1H, m, H-13b), 1.46 (1H, overlapped, H-7a) 1.43 (2H, overlapped, H_2-15), 1.42 (1H, overlapped, H-7b), 1.27 (1H, m, H-8), 0.99 (3H, t, *J* = 7.0 Hz, H_3-14), 0.87 (3H, d, *J* = 7.0 Hz, H_3-17), 0.48 (3H, t, *J* = 7.0 Hz, H_3-16); (+) ESI-MS *m*/*z* 515 $[M + H]^+$, 537 $[M + Na]^+$.

*(R)-MTPA ester **12b**:* colorless amorphous solid; ^1H NMR ($CDCl_3$, 500 MHz): selected values δ_H 4.96 (1H, s, H-5), 4.34 (1H, d, *J* = 5.8 Hz, H-3), 2.74 (1H, dd, *J* = 18.6, 5.8 Hz, H-2a), 2.64 (1H, d, *J* = 18.6, Hz, H-2b), 1.88 (1H, m, H-13a), 1.80 (1H, m, H-13b), 1.66 (2H, overlapped, H_2-15), 1.51 (1H, overlapped, H-7a), 1.47 (1H, overlapped, H-7b), 1.28 (1H, m, H-8), 0.98 (3H, t, *J* = 7.0 Hz, H_3-14), 0.87 (3H, d, *J* = 7.0 Hz, H_3-17), 0.77 (3H, t, *J* = 7.0 Hz, H_3-16); (+) ESI-MS *m*/*z* 515 $[M + H]^+$, 537 $[M + Na]^+$.

Plakdiepoxide (15): colorless amorphous solid; $[\alpha]_D$ + 17.4 (*c* 0.06, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) Table 1; (+) ESI-MS *m*/*z* 313 $[M + H]^+$, 335 $[M + Na]^+$; HRMS (ESI) for $C_{18}H_{32}NaO_4$ $[M + Na]^+$ (*m*/*z* 335.2198) found *m*/*z* 335.2200.

Computational Calculations. DFT calculations were performed on a Intel(R) Core(TM) i5-4440 processor at 3.0 GHz using the Gaussian03 package (Multiprocessor). A Systematic Conformational Search for the models **15a** and **15b** around the C2−C3/C4−C6 bond was carried out at the B3LYP level using the 6-31G(d) basis set (range: + 180 to −180; step: 24°; number of conformers = 15) in the gas phase. All of the conformers obtained were subsequently optimized at the B3LYP level using the 6-31G(d,p) basis set. GIAO ^{13}C calculations were performed using the mPW1PW91 functional and 6-31G(d,p) basis set using the geometry previously optimized at the mPW1PW91/6-31G(d) level as input.

Reaction of Endoperoxide 16 with $FeCl_2$. Compound **16** (19.5 mg, 62 μmol) was dissolved in CH_3CN/H_2O 4:1 (3 mL), and then $FeCl_2 \cdot 4H_2O$ (62 mg, 0.31 mmol) was added. The reaction mixture was left under stirring at room temperature for 2 h, excluding light from the reaction. Then the obtained mixture was partitioned between water and EtOAc, and the organic phase, dried over Na_2SO_4, was purified by HPLC (SI60, *n*-hexane/EtOAc 9:1) to afford compounds **2** (4.0 mg, 13.5 μmol, 20%) and **5** (11.5 mg, 44.5 μmol, 72%) in the pure state.

Reaction of Endoperoxide 16 with NaOH. Compound **16** (5.0 mg, 16 μmol) was dissolved in 2 mL of a 5% NaOH solution in MeOH/H_2O 5:1. The reaction mixture was allowed to reflux under

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stirring at 110 °C for 2 h and quenched with HCl 0.5 N. Then the mixture was partitioned between water and EtOAc, and the organic phase, dried over Na_2SO_4, was concentrated in vacuo and purified by HPLC (SI60, *n*-hexane/EtOAc 9:1) to afford compounds 7 (1.0 mg, 4.0 μmol, 25%).

Assay for PPARα and PPARγ Agonistic Activity. The activation of PPAR-α and PPAR-γ was determined by a reporter gene assay as described previously.[40] In brief, human hepatoma (HepG2) cells were cultured in DMEM supplemented with 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin. For the assay, cells were transfected with either pSG5-PPARα and PPRE X3-tk-luc or pCMV-rPPARγ and pPPREaP2-tk-luc plasmid DNAs (25 μg/1.5 mL cell suspension) by electroporation at 160 V for single 70 ms pulse using a Square electroporator T820 (BTX, San Diego, CA). The transfected cells were plated in the wells of 96-well tissue culture plates (5×10^4 cells/well) and incubated for 24 h for confluency. The cells were then treated with various concentrations of the test compounds (12.5, 25.0, 50.0 μM), drug controls (ciprofibrate or rosiglitazone, 10 μM), or solvent control (DMSO, 0.5%). After incubation for 24 h with the samples, the luciferase activity was measured using a Luciferase assay system (Promega, Madison, WI). Light output was detected on a SpectraMax plate reader. The fold induction of luciferase activity in the sample treated cells was calculated in comparison to the vehicle treated cells (control).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b00695.

1D and 2D NMR spectra for the new metabolites; characterization data of known compounds; computational calculation data (PDF)

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Notes

The authors declare no competing financial interest.

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