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CT2108A and B: New Fatty Acid Synthase Inhibitors as Antifungal Agents

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A systematic screen for new natural products that displayed antifungal activity by inhibition of fungal fatty acid synthase (FAS) led to the discovery of two new fungal metabolites, designated CT2108A (**1**) and CT2108B (**2**). The metabolites were produced by *Penicillium solitum* (Westling) strain CT2108 and were classified as azaphilones. The structures of these new metabolites were determined using a variety of 1D and 2D NMR experiments, including COSY, HMQC, and HMBC. The chemical conversion of CT2108A to CT2108B was effected using WCl_6. The related metabolite, patulodin (**3**), was also isolated from the fermentation culture of this *P. solitum* isolate. Both new compounds inhibited fungal FAS, and neither was found to significantly inhibit human FAS activity.

The occurrence of fungal infections has escalated significantly in recent years. Increases in the number of patients presenting with such infections has been particularly profound in those immunocompromised by disease or therapies. Currently, only a limited number of antifungal drugs are available for treatment of fungal infections. In addition, use of the most effective antifungal agents, azole derivatives and amphotericin B, suffers from drawbacks including toxicity, fungistatic versus fungicidal activity, and the development of drug resistance. The paucity of effective agents is also due in part to the lack of suitable novel targets for drug screening.

Recent evidence suggests that the enzyme fatty acid synthase (FAS) is a good potential antifungal drug target.[1,2] FAS catalyzes the assembly of fatty acids from acetyl, malonyl, and NADPH substrate via seven enzymatic reactions. In bacteria and plants, seven genes encode the seven polypeptides that form an FAS complex.[3] In fungi, FAS is a multifunctional enzyme with the seven component activities distributed over two nonidentical polypeptides, α and β, arranged in an $\alpha_6\beta_6$ configuration.[4] Conversely, in vertebrates all seven enzymatic reactions are on a single polypeptide in a homodimer configuration and encoded by a single gene.[5,6] Despite the diversity of arrangements, all FAS complexes perform the same set of reactions, suggesting that the spatial structure of the reactive sites should be similar. Indeed, comparisons of the deduced polypeptide sequences of FAS from prokaryotic, lower eukaryotic, and higher eukaryotic sources show high degrees of relatedness at catalytic domains and substrate binding sites;[7−10] however, major structural differences, including spacing of reactive domains as well as their linear order, are apparent. Because of such structural differences, it is possible that natural products or chemically designed molecules might be identified that differentially affect human and fungal

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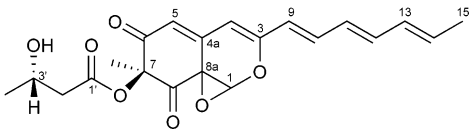
FAS and that also have a broad spectrum of activity against fungi.[11]

Previous work with analogues of the FAS inhibitor cerulenin had shown that compounds that inhibit fungal FAS and not human FAS could be identified.[11] None of these compounds proved effective as antifungal agents due to irreversible nonspecific protein binding. However, it remained likely that other agents that exploited differences between the respective human and fungal FAS could be discovered from a natural products library. Thus, a mechanism-based screen was employed to identify natural product extracts that (1) inhibited fungal FAS enzyme activity and not human FAS enzyme, (2) showed reversible inhibition of *Candida albicans* growth in liquid cultures, and (3) exhibited in vitro fungicidal activity.

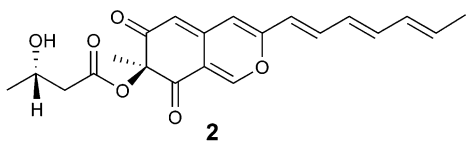
In the course of applying this mechanism-based screen using fungal and actinomycete natural product extracts, two new structurally related azaphilones, designated CT2108A (**1**) and CT2108B (**2**), were identified. The producing organism was a strain of *Penicillium solitum* (CK2108) that was obtained from a duff sample collected in Wyoming. Patulodin (**3**),[12] a related metabolite with antifungal activity, was also isolated from this strain. In this report, the fermentation, isolation, and biological activity of the new compounds are described. The physicochemical properties and details of the structure elucidation and semisynthesis will also be presented.

Results and Discussion

A total of 10 880 extracts, obtained from 1360 actinomycetes and 1360 fungi, were screened. Eighty-two extracts showed reproducible FAS inhibition and *C. albicans* growth inhibition. Of these, 30 had no activity against human FAS enzyme. This was a crucial selection criterium, since achieving a good therapeutic index requires that the inhibitor be fungal specific. The organisms yielding the 30 candidate extracts were fermented a second time to identify those cultures that reproducibly yielded active material. Following the re-fermentation, extracts were prepared and again tested for bioactivity; eight strains (six fungi and two actinomycetes) consistently produced an active agent. One of these eight strains was identified as *Penicillium solitum* Westling (CT2108) and yielded two new compounds, designated CT2108A (**1**) and CT2108B (**2**).



1



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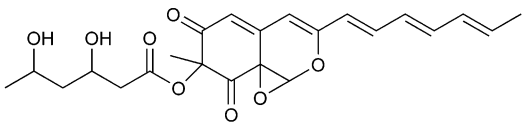

3

The 3 L fermentation of CT2108 yielded 12.4 g of crude ethyl acetate extract. Preparation of an aliquot for countercurrent partition chromatography (CPC) yielded a large amount of insoluble material. CPC fractionation of the soluble material revealed two sets of fractions with biological activity: CPC fractions 17−22 and 26−30. Pooled fraction 17−22 showed good activity against FAS; pooled fraction 26−30 yielded poor FAS activity, yet was equally effective in the susceptibility assay on a per mass basis. UV and ^{1}H NMR analysis suggested that CPC 17−22 and the insoluble material contained the same compound. The two pooled CPC fractions were further analyzed by HPLC, and the fractions were analyzed for biological activity. Fractionation of CPC 17−22 yielded a single HPLC peak (compound **1**) that exhibited FAS inhibitory and growth inhibitory activity. HPLC fractionation of CPC 26−30 yielded a single fraction (compound **2**) that retained growth inhibitory activity, lacked FAS inhibitory activity, but retained reversible growth inhibition.

Examination of the HRFABMS data indicated that the molecular formulas for **1** and **2** differed by only one oxygen atom; the molecular formula of **1** was determined to be $C_{21}H_{22}O_7$, and the molecular formula of **2** was found to contain one less oxygen atom. The UV spectra for these compounds were also similar, strengthening the premise that these metabolites were closely related. Because **2** was the most interesting biologically, and because the NMR data obtained for **2** were better than the data obtained for **1**, the structure of **2** was pursued first.

Identification of two distinct spin systems for **2** was possible based on analysis of the 1D and 2D ^{1}H NMR data (see Table 1). The first spin system, a series of three double bonds, comprised H-9 through H_3-15. A large J value was found for each of these vinylic proton signals, indicating that the three double bonds were trans. The only other spin system identified for **2** was a hydroxypropyl group (H-2′ through H_3-4′). Four singlet ^{1}H NMR signals (three downfield-shifted and one methyl group) accounted for the remaining signals in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum showed evidence of 12 vinylic/aromatic carbon atoms and three carbonyl groups. Since there were 11 unsaturations present in this molecule, two ring systems were expected. Also present in the ^{13}C NMR/DEPT spectra were signals corresponding to the hydroxypropyl group (a methyl, methylene, and methine group), an oxygenated quaternary carbon, and two methyl groups.

The one-bond ^{1}H−^{13}C assignments were made by analyzing the HMQC[13] spectrum. These assignments were straightforward and can be found in Table 1. The long-range correlations (obtained from the HMBC[14] spectrum) were used to determine the connectivity of **2**. HMBC correlations between H-2′a, H-2′b, and H-3′ to the carbon signal at δ 171.1 (C-1′) clearly indicated that this moiety is a hydroxybutanoic acid unit. The spin system comprising the three trans double bonds could be extended to four double bond units (adding C-3 and C-4) on the basis of HMBC data. The methyl group at δ 1.48 was correlated to a quaternary carbon at δ 85.7 and to the two carbonyl carbons at δ 192.6 and 193.5. This methyl group must therefore be attached to C-7, which was in turn flanked by the two carbonyl carbon atoms (C-6 and C-8). Additional HMBC correlations, together with chemical shift information, allowed placement of the two additional double bonds adjacent to the two carbonyl groups at C-6 and C-8. Several key HMBC correlations allowed all the fragments to be connected, thereby forming the requisite two rings. Correlations between H-5 and C-8a and between H-1 and C-4a allowed closure of one ring. A correlation between H-1 and C-3 was key to assigning the pyran ring system. After all

Table 1. ^{1}H and ^{13}C NMR Spectral Data for CT2108A (**1**) and CT2108B (**2**)[a]

	1			**2**		
no.	^{1}H	^{13}C	HMBC[b]	^{1}H	^{13}C	HMBC[b]
1	5.89 s	82.1	3	8.05 s	154.7	3, 4a, 8, 8a
3		154.5			156.8	
4	6.11 s	105.4	3, 5, 8a, 9	6.52 s	110.5	3, 5, 8a, 9
4a		146.7			143.7	
5	6.28 m	120.7	4, 7, 8a	5.53 m	108.0	4, 7, 8a
6		192.5[c]			192.6	
7		85.8			85.7	
7-CH_3	1.62 s	22.2	6, 7, 8	1.48 s	22.8	6, 7, 8
8		197.4[c]			193.5	
8a		55.5			115.5	
9	6.19 d (15.2)	123.88	3, 4, 10, 11	6.30 d (15.0)	121.8	3, 4, 11
10	6.95 dd (15.1, 11.3)	136.7	3, 12	7.04 dd (15.3, 11.2)	137.1	3, 9, 11, 12
11	6.31 dd (14.9, 11.4)	129.9	9, 10, 13	6.35 m	129.7	9, 13
12	6.56 dd (14.8, 10.8)	139.8	10, 13, 14	6.60 dd (15.8, 10.8)	140.4	10, 13, 14
13	6.22 ddd (16,10.9,1)	132.6	15	6.22 dd (14.9, 11.3)	132.6	11, 12, 15
14	5.94 dd (14.8, 7.0)	134.5	12, 15	5.95 dd (14.8, 7.1)	134.9	12, 15
15	1.79 dd (6.7, 0.6)	18.6	13, 14	1.79 br d (6.7)	18.6	13, 14
1′		171.1			171.1	
2′a	2.56 dd (14.3, 7.0)	43.2	1′, 3′, 4′	2.52 dd (14.3, 7.0)	44.2	1′, 3′, 4′
2′b	2.44 dd (14.4, 6.0)		1′, 3′, 4′	2.42 dd (14.4, 6.0)		1′, 3′, 4′
3′	4.11 m	64.9		4.10 m	65.1	1′
4′	1.20 d (6.2)	23.2	2′, 3′	1.20 d (6.2)	23.1	2′, 3′

[a] δ in ppm, J in Hz. Data were recorded at 500 and 125 MHz, respectively in acetone-d_6. [b]All correlations represent 2- or 3-bond couplings. $^nJ_{CH}$ was optimized for 8 Hz. [c] These carbon atoms may be interchanged.

connections were made (based on available NMR data), there remained only two open valences. The only option was to attach C-1′ to C-7 via an ester linkage. All other HMBC correlations are consistent with the proposed structure.

The stereochemistry of **2** was deduced using two methods. The absolute configuration at C-7 of the azaphilones has been clearly established by optical methods.[15,16] The sign of the Cotton effect at the longest wavelength in the CD spectrum depends on the stereochemistry at C-7. The CD curve for **2** ($\Delta\epsilon_{390}$ −1.7) provided clear evidence for the *S*-configuration at C-7. The only other asymmetric carbon atom in **2** was C-3′. Application of Horeau's method[17] established the absolute stereochemistry at C-3′ to be *S*.

The gross structure of **1** was determined on the basis of comparison to the structure determined for **2** and the NMR data obtained for **1**. The molecular formula for **1** contained one additional oxygen atom compared to **2**. In the ^{13}C NMR spectrum, two vinylic carbon signals were replaced by two downfield-shifted aliphatic signals. This suggested that a double bond had been replaced by an epoxide functionality. Based on examination of 1D NMR data alone, it appeared that it was the C-1/C-8a double bond that had been replaced by the epoxide. Data from ^{1}H−^{1}H COSY, HMQC, and HMBC experiments confirmed our assignment of the structure of CT2108A as **1**. Further corroboration for this structure was obtained when the ^{13}C NMR data for **1** were compared to the published NMR data for the related metabolite patulodin (**3**).[12] The chemical shifts for the carbon atoms in **1** differed by less than 2 ppm from the published values for **3** (excluding those carbon atoms in the divergent side chain at C-7). The assignments for C-6 and C-8, unfortunately, could not be made unequivocally. On the basis of chemical shift values, it seemed likely that the carbon atom at δ 197.4 be assigned to C-8. The carbonyl at C-6 was conjugated and therefore would probably resonate further upfield (δ 192.5).

Biosynthetic considerations suggested that **1** and **2** would likely have the same stereochemistry. Because **1** was synthetically converted to **2** (see below), and the semisynthetic **2** gave the same CD curve as the natural product, the stereochemistry at C-7 could be assigned as *S* for **1** as well. Likewise, the stereochemistry at C-3′ for **1** was assumed to be the same as was determined for **2** because the epoxide could be converted to the alkene with retention of optical properties. The stereochemistry of the epoxide was not determined.

Further support for the stereochemical relationship between **1** and **2** was obtained by chemically converting **1** to **2**. This conversion was also sought because the isolated yield of **2** from the fermentation culture was substantially less than the yield for **1**, and additional material was desired for further biological testing. A variety of methods to chemically convert epoxide **1** to alkene **2** were evaluated by analyzing the resulting crude reaction product mixtures by LC-MS. These trials included treating **1** with a variety of phosphorus reagents (Ph_3P,[18] $(EtO)_3P$,[19] Ph_3PS-Ph_3P,[20] and $[(PhO)_3PMe]I-BF_3OEt_2$[21]), zinc reagents (Zn−HOAc,[22] Zn−NaI−HOAc−NaOAc,[23] and Zn−Cu[24]), and other reducing metal/Lewis acid combinations ($TiCl_3-LiAlH_4$[25] and WCl_6−2 n-BuLi[26]). At the end, utilization of the WCl_6−2 n-BuLi reagent proved most efficacious. This reagent allowed for the preparation and isolation of gram quantities of pure **2**, whose physicochemical properties (^{1}H NMR, MS, CD, and UV) were identical to those of the natural product.

The biological activity of CT2108A and B is listed in Table 2. Compound CT2108A inhibited fungal FAS activity in vitro with an IC_{50} of 160 μg/mL. Most important, neither compound inhibited human FAS. Both compounds exhibited reversible growth inhibition of *C. albicans*. The MIC_{90} values were 6.25 and 3.124 μg/mL using a microdilution susceptibility test for **1** and **2**, respectively. The minimal fungal concentrations (MFC) were 25 and 6.25 μg/mL, respectively. In the disk diffusion assay, both compounds produced a clear zone of inhibition. Addition of long-chain fatty acids (Tween-40 and mysteric acid) to the culture medium partially reversed the growth inhibition. This fatty acid mixture had no effect on enzyme inhibition of either compound, implying that an interaction of the compounds with the fatty acids was not the likely cause of reversal. Rescue from growth inhibition by exogenous fatty acids suggests that the intracellular target of the compounds is FAS; however, a secondary target cannot be definitively

Table 2. Effect of CT2108A (**1**) and CT2108B (**2**) on FAS Activity and Growth of *C. albicans*

	CT2108A	CT2108B	cerulenin
FAS inhibition[a]			
IC_{50} (*Candida* FAS)	160	250	0.5
IC_{50} (human FAS)	>1000	>1000	0.5
growth inhibition			
MIC	6.25	3.13	5
MIC + fatty acids[b]	50	50	>1000
MFC	25	6.25	5
DDA	8 clear	10−13 clear	20−30 hazy

[a] IC_{50}, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values are expressed in μg/mL. Disk diffusion assay (DDA) zone of inhibition is expressed in mm. [b] Medium was supplemented with 1.0% Tween 40 and 0.03% mysteric acid. The addition of Tween 40 or mysteric acid to an enzyme assay has no effect on inhibition.

Table 3. Minimum Inhibitory Concentration of CT2108A (**1**) and CT2108B (**2**) against Clinical Isolates[a]

		CT2108A		CT2108B	
microorganism	no. tested	24 h	48 h	24 h	48 h
Candida albicans	4	3.9	7.8	7.8	7.8
C. tropicalis	1	NT	NT	6.25	6.25
C. lusitaniae	3	8.3	37.5	16.3	41.6
C. guillurmondii	1	<1.6	1.6	<1.6	1.6
C. krusei	3	12.5	25	25	50
C. glabrata	3	29.2	83.3	25	50
C. parapsilosis	4	15.6	62.5	14.8	50
C. dublinensis	2	9.4	18.8	12.5	12.5
S. cerevisiae	4	5.9	20.3	8.2	25
Cryptococcus neoformans[b]	2	25	50	25	50
		NG	3.12	<1.6	<1.6
Cryptococcus laurintic[b]	2	NG	6.25	<1.6	<1.6
		NG	6.25	<1.6	25

[a] The MICs, in μg/mL, are the average values for the strain group. NT = not tested, NG = no growth. [b] Two isolates of each *Cryptococccus* sp. were tested and gave very different results, and in two cases MICs values were not obtained.

ruled out at this time. A larger spectrum of activity was observed when each compound was tested against several species of *Candida*, *Cryptococcus*, and *Saccharoymces* (Table 3). The MICs ranged from less than 1.5 μg/mL to 83.3 μg/mL.

CT2108A and B belong to a class of compounds referred to as azaphilones. These fungal metabolites possess pyranoquinone ring systems and are named azaphilones because of their affinity for NH_3 (forming vinylogous γ-pyridones).[27] Patulodin was the first reported metabolite of this class that possessed an epoxide functionality. CT2108A is the only other reported azaphilone with the epoxide group. Both new compounds exhibited selective inhibition of *C.albicans* FAS and had fungicidal activity. Importantly, there was no observable inhibition of human FAS activity. This finding suggests that it may be possible to identify other more potent and selective FAS inhibitors that would impart a good therapeutic index. The finding that FAS is essential to *Candida* for establishing and maintaining an infection,[1,2] coupled with the evidence presented here, demonstrates FAS is a potentially attractive target for chemotherapeutic intervention and that natural products remain a good source of drug leads.

Experimental Section

General Experimental Procedures. All NMR data were acquired using a Bruker 500 MHz DRX spectrometer. Individual spectra were recorded in acetone-d_6 using the corresponding solvent signals (δ 2.04 for ^{1}H and δ 29.8 for ^{13}C) as references. DEPT experiments were used to determine carbon multiplicities, which are in agreement with the carbon assignments. A Fisons VG 70SEQ Tandem Hybrid MS/MS spectrometer (FAB) was used to acquire MS data. UV and IR spectra were recorded on Perkin-Elmer Lambda 6 and Perkin-Elmer 1600 FTIR spectrophotometers, respectively. A Perkin-Elmer 243B polarimeter was used to procure optical rotation data. Circular dichroism data were obtained on a Jasco J-720 spectropolarimeter.

Preparation of Yeast FAS. FAS was obtained from cell-fee extracts of freeze-dried *Saccharomyces cerevisiae* (Fleischman) or *Candida albicans strain SC5314*.[1] The extracts were prepared after first rehydrating yeast in 5 volumes of deionized water for 15 min. Cells were subsequently harvested by centrifugation at 3000*g* for 10 min at 4 °C, washed once with 5 volumes of cold 0.125 M K_2HPO_4, pH 6.6, containing 1 mM EDTA, 1 mM DTT, 0.7 μg/mL pepstatin, 0.2 μg/mL aprotinin, and 0.2 μg/mL leupeptin (yeast lysis buffer), and then resuspended in 2 volumes of yeast lysis buffer. Cells were mechanically disrupted by treatment in a bead mill for 5 min with an equal volume of 0.5 mm of acid-washed glass beads. After removal of the cell debris by centrifugation at 30000*g* for 30 min at 4 °C, ammonium sulfate was added to the supernatant to a final concentration of 50% w/v. The mixture was stirred slowly on ice for 30 min and the precipitate collected by centrifugation at 10000*g* for 10 min at 4 °C. The pellet was dissolved in 1/20 volumes of yeast lysis buffer and dialyzed (100 000 MWCO dialysis tubing) against three changes of 2 L of 125 mM K_2HPO_4, pH 6.6, 1 mM EDTA, and 1 mM DTT during a 24 h period. The extract was recovered and clarified by centrifugation at 10000*g* for 10 min and stored at 4 °C.

Preparation of Human FAS. Human breast cancer cell line ZR-75-1 was used as the source of human FAS. Cultured cells were harvested by scraping and stored at −70 °C. Lysates were prepared by suspending the frozen cells in 5 volumes of cold lysis buffer (5 mM K_2HPO_4, pH 6.6, 1 mM EDTA, and 1 mM DTT) and homogenized by 20 strokes in a Dounce-type homogenizer on ice. The lysate was clarified by centrifugation 2× for 30 min at 30000*g*, dialyzed against 125 mM K_2HPO_4 pH 6.6, 1 mM EDTA, 1 mM DTT, and 3 mM NaN_3, and stored at 4 °C.

FAS Assays. Fungal FAS assays were performed in 96-well microtiter dishes. A 105 μL portion of extract was dispensed per well of a polypropylene microplate, and 5 μL of compound solution, NP extract, cerulenin (positive control for inhibition), or solvent was added. After 20 min incubation at 24 °C, duplicate 50 μL aliquots were removed to low-protein binding polystyrene microplates. Reactions were initiated by addition of a 50 μL 2×-cocktail (final reaction conditions: 125 mM K_2HPO_4, pH 6.6, 1 mM DTT, 0.5 mM NADPH, 0.5 mM malonyl-CoA, and 40 μM acetyl-CoA). Activity was measured in a Molecular Devices UVMax spectrophotometer by a decrease in absorbance at 340 nm and the initial rate of the reactions calculated for the first 2 min of the reaction. Assays of human FAS were identical, except the final concentration of NADPH and malonyl-CoA was 100 μM. The assays were performed with the same units of enzyme activity. FAS enzyme obtained from *S. cerevisiae*, which was functionally identical to *C. albicans* enzyme, was used in the screening**.** Inhibition by a compound or natural product extract was determined by calculating the percent enzyme inhibition.

Susceptibility and Rescue Assays. Susceptibility of *C. albicans* strains to an extract or compound was determined by a microdilution spectrophotometric method (described elsewhere[28]) with the exception that YNB medium supplemented with 1% glucose and 1.5 μg/mL asparagine was used in place of RPMI-1640. The MIC endpoint was defined as the lowest drug concentration yielding 90% growth inhibition relative to the drug-free control. Rescue from growth inhibition was demonstrated using the susceptibility assay, except the medium was supplemented with 0.5% Tween 40 and 0.03% mysteric acid.

Taxonomy. *Penicillium solitum* (Westling) strain CT2108 (purchased from Panlabs Inc.) was isolated from soil in Wyoming and characterized according to Raper[29] and Samson.[30] The organism producing CT2108A and CT2108B was

identified as *P. solitum* Westling. Strain CT2108 exhibited broad columns of blue-green conidia from compactly branched penicilli. It had smooth, uncolored stripes up to 300 or 400 μm tall, which were weakly to strongly fasciculated. Structural dimensions were as follows: rami, 11−14 × 3.2 μm; metulae, 8−14 × 3 μm, nonvesiculate; phialides slender 8−10 × 2.3−2.5 μm. The conidia were strongly elliptical in early stage and became more broadly elliptical 4−4.5 × 3−2.5 μm in size with smooth walls and light pigmentation. Larger conidia, up to 6.5 × 4.0 μm, were also observed. The strain sporulated on Czapek, CYA, YES, and MEA media. An exudate was common along with a strong musty odor. There was no observable growth at 37 °C. Growth was restricted on malt agar.

Fermentation. Strain CT2108 was maintained on nutrient agar. Seed cultures were grown in 25 mL of broth (per liter: glucose 20.0 g, Pharmamedia (Traders Oil) 15.0 g, $(NH_4)_2SO_4$ 3.0 g, $ZnSO_4 \cdot 7H_2O$ 0.03 g, $CaCO_3$ 4.0 g, yeast extract 5.0 g) at 28 °C for 2 days with shaking at 250 rpm. The seed culture was used to inoculate 3 L of fermentation broth (per liter: glucose 20.0 g, sucrose 5.0 g, Pharmamedia (Traders Protein) 20.0 g, $NaNO_3$ 1.0 g, K_2HPO_4 0.5 g, KCl 0.7 g, l-histidine 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.014 g). The fermentation was incubated for 5 days at 28 °C with shaking at 250 rpm.

Isolation and Purification. The whole fermentation mixture (3 L) was vacuum filtered through cheesecloth, then Whatman #113 filter paper. The filtrate was extracted with EtOAc (3 × 2.5 L). The solid material was covered with EtOAc and left overnight. The solids were then extracted with EtOAc (3 × 0.5 L). All extracts were combined and dried under reduced pressure to yield a dark red oil (12.4 g). CPC was performed on a P.C., Inc. high-speed countercurrent chromatograph, equipped with an Ito multilayer coil column. The solvent system consisted of an equilibrated mixture of 1:3:3:3 *n*-hexane/EtOAc/MeOH/H_2O (v/v/v/v). Initially, the lower phase was used as the stationary phase, and the upper phase served as the mobile phase, which was pumped at 3 mL/min. The column rotation speed was 1040 rpm. An aliquot (400 mg) of crude extract was prepared by dissolving it in a mixture of upper and lower phases. This procedure yielded a substantial quantity of insoluble material (217 mg), which was removed (by filtration) prior to loading onto the column. The soluble material (182 mg) was loaded onto the column, and after 2 h, the upper and lower phases were switched. A Waters 991 photodiode array detector was used to monitor peak elution. Eighty 9 mL fractions were collected and assayed. Two fractions eluting from 48 to 66 min. (CPC 17−22, 48.0 mg) and from 75 to 90 min. (CPC 26−30, 10.2 mg) were found to be active. These active fractions were further separated using reversed-phase HPLC (LiChrospher, 10 μm 10 × 250 mm, C_{18}). Elution was monitored at 270 nm, and 5 mL fractions were collected. Aliquots (5 mg) of the CPC fractions were dissolved in DMSO, and a linear gradient starting at 70:30 CH_3CN/H_2O to 100% CH_3CN over 25 min was used to isolate **1** (23 mg) and **2** (3.5 mg).

A subsequent fermentation (12 L) of CT2108 yielded 30.4 g of crude EtOAc extract. A mixture of CPC solvents (200 mL, upper:lower phases, 3:1) was added. The insoluble material (11.6 g) was removed by vacuum filtration. An aliquot of the insoluble material (600 mg) was loaded onto a silica gel column (24 cm to bottom of 1 L bulb × 4.5 cm ext. diameter, wet-packed with silica gel in 30% ethyl acetate in hexane, packing height 20 cm). The column was eluted using positive pressure with 30% EtOAc in hexane (300 mL), 40% EtOAc (1500 mL), 50% EtOAc (450 mL), 60% EtOAc (500 mL), and 70% EtOAc (500 mL) and washed with 100% EtOAc. After the 40% EtOAc was eluted, six 300 mL fractions were collected. Fraction 6 contained pure **3** (11.2 mg). This sample had physical characteristics (MS, ^{1}H and ^{13}C NMR) that matched published data.[12]

CT2108A (1): yellow-orange powder; mp 210 °C (dec); $[\alpha]^{22}_D$ −303.5° (*c* 0.0004, MeOH); UV (MeOH) λ_{max} (log ϵ) 384 (4.44), 347 (4.31), 278 (4.11) nm; IR ν_{max} (KBr) 3436, 2978, 1731, 1666, 1578, 1085, 997, 885 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; FABMS *m/z* 409 $[M + Na]^+$, 387 $[M + H]^+$; HRFABMS *m/z* $[M + H]^+$ 387.1416 (calcd for $C_{21}H_{22}O_7$+H, 387.1444).

CT2108B (2): orange-brown solid; mp 130 °C; $[\alpha]^{22}_D$ −317.6° (*c* 0.0004, MeOH); UV (MeOH) λ_{max} (log ϵ) 370 (4.29), 348 (4.26), 273 (4.12) nm; CD (MeOH) $[\theta]_{390}$ −1.7, $[\theta]_{357}$ 0, $[\theta]_{340}$ +0.2, $[\theta]_{335}$ 0, $[\theta]_{315}$ −0.4, $[\theta]_{295}$ 0, $[\theta]_{283}$ +0.4, $[\theta]_{270}$ 0, $[\theta]_{260}$ −0.3, $[\theta]_{252}$ 0, $[\theta]_{225}$ + 2.2; IR ν_{max} (KBr) 3436, 2966, 2355, 1713, 1619, 1602, 1531, 1120 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; FABMS *m/z* 393 $[M + Na]^+$, 371 $[M + H]^+$; HRFABMS *m/z* $[M + H]^+$ 371.1490 (calcd for $C_{21}H_{22}O_6$+H, 371.1495).

Semisynthesis of CT2108B (2). A dry 1 L round-bottom flask was flushed with nitrogen, charged with 400 mL of dry THF, and cooled to −78 °C. Tungsten hexachloride (14.32 g, 36.1 mmol) was introduced. While the cold suspension was stirred, n-BuLi (1.6 M in hexane, 45 mL, 72 mmol) was added slowly. The resulting mixture was stirred and warmed to room temperature over 2 h. After stirring at room temperature for 20 min, the mixture was again cooled to −78 °C and the epoxide (7.20 g, 18.6 mmol) was introduced. The cooling bath was removed, and the mixture was stirred overnight. The reaction mixture was poured into an aqueous potassium sodium tartrate solution (prepared by dissolving 75 g of potassium sodium tartrate tetrahydrate in 400 mL of water). This mixture was transferred to a separatory funnel with EtOAc (500 mL) and water (100 mL), and the organic layer was collected. The aqueous layer contained a gray precipitate, which was filtered through a Buchner funnel and washed with EtOAc (500 mL). The filtrate was transferred to a separatory funnel, and the organic layer was collected. The aqueous layer was extracted again with EtOAc (500 mL), and the combined organic layers were washed with a brine solution (200 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated to give a brown viscous oil, which was purified by column chromatography on 300 g of silica gel. Elution with a solution consisting of EtOAc, hexane, and MeOH (65:34:1) yielded a fraction containing a small amount of nonpolar material followed by a fraction corresponding to starting material and finally the desired product. Mixed fractions were combined and similarly purified on 150 g of silica gel. The appropriate fractions were combined to afford starting material (2.05 g) and pure product (2.83 g, 41% yield).

Reaction of (±)-2-Phenylbutyric Acid Anhydride with CT2108B (2). Dry THF (2 mL) was added to a vial containing CT2108B (20.0 mg). (±)-2-Phenylbutyric acid anhydride (43 μL) was added, followed by pyridine (25 μL), and the reaction mixture was allowed to stand at RT. After 4 days, H_2O was added (4 drops). Two hours later, H_2O (0.5 mL) was again added. $NaHCO_3$ was added, and the mixture was extracted with EtOAc (3 × 2 mL). The EtOAc extract was washed with H_2O (2 mL), $NaHCO_3$ (2 mL), and H_2O (2 mL) again. The aqueous layers were combined, acidified (pH = 2) with 1 N HCl, and extracted with benzene (2 × 5 mL). The 2-phenylbutyric acid obtained after drying had an optical rotation ($[\alpha]^{22}_D$) of −4.1 (*c* 0.05, benzene), which corresponded to an optical yield of 18.7%.

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