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Isolation and Synthesis of a New Aromatic Compound, Brefelamide, from *Dictyostelium* Cellular Slime Molds and Its Inhibitory Effect on the Proliferation of Astrocytoma Cells

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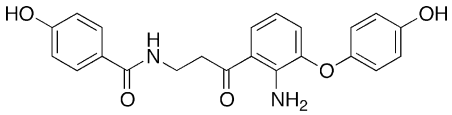

Brefelamide

We have explored the diversity of secondary metabolites produced by cellular slime molds to examine the possible use of such cellular slime molds as a resource for novel drug development. A new aromatic amide, brefelamide (**1**), was isolated from methanol extracts of the fruiting bodies of *Dictyostelium brefeldianum* and *D. giganteum*. The structure of **1** was determined by spectral means including EIMS and ^{1}H and ^{13}C NMR. The total synthesis of **1** was carried out to confirm the structure and obtain sufficient samples for performing biological evaluation. Interestingly, compound **1** inhibited the cellular proliferation of 1321N1 human astrocytoma cells.

Introduction

The cellular slime mold *Dictyostelium discoideum* is thought to be an excellent model organism for the study of cell and developmental biology because of its simple pattern of development.[1] Vegetative cells of *D. discoideum* grow as single amoebae by eating bacteria; however, when starved, they start a developmental program of morphogenesis and gather to form a slug-shaped multicellular aggregate. This aggregate then differentiates into two distinct cell types, prespore and prestalk cells, which are precursors of spores and stalk cells, respectively. At the end of its development, the aggregate forms a fruiting body consisting of spores and a multicellular stalk.

Several small molecules including differentiation-inducing factors (DIFs),[2] discadenine,[3] and cAMP[4] have been reported as development-regulating substances of cellular slime molds. DIF-1 was also found to inhibit proliferation and induce differentiation in mammalian leukemia cell lines.[5] Furthermore, an intriguing experimental result suggesting that DIF-1 may induce differentiation of vascular smooth muscle cells in vitro could lead to novel strategies for the treatment of vascular diseases.[6] A chlorine-substituted aromatic compound, AB0022A, was also isolated as an antibacterial substance.[7] However, except for these compounds, there have been no reports on secondary metabolites of cellular slime molds. We have focused on the utility of cellular slime molds as a resource for novel drug development and have studied the diversity of cellular slime mold secondary metabolites as well as their physiological and pharmacological activities. We have isolated α-pyronoids and aromatics with unique structures;[8,9] these compounds

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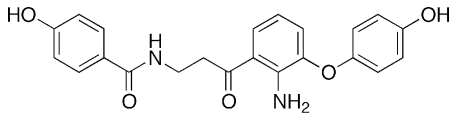
TABLE 1. ^{13}C and ^{1}H NMR Spectral Data of Brefelamide (1)[a]

	^{13}C	^{1}H
1	126.4[b]	
2,6	130.2	7.67 (2H, d, J = 8.9 Hz)
3,5	116.1	6.80 (2H, d, J = 8.9 Hz)
4	162.1	
1′	170.2	
3′	37.1	3.73 (2H, t, J = 6.8 Hz)
4′	39.9	3.30 (2H, t, J = 6.8 Hz)
5′	202.5	
1″	121.8	
2″	144.1	
3″	147.4	
4″	121.8	6.77 (1H, dd, J = 7.8, 1.3 Hz)
5″	115.4	6.52 (1H, dd, J = 8.2, 7.8 Hz)
6″	126.5[b]	7.57 (1H, dd, J = 8.2, 1.3 Hz)
1′ ″	150.6	
2′ ″,6′ ″	121.0	6.84 (2H, d, J = 9.1 Hz)
3′ ″,5′ ″	117.2	6.76 (2H, d, J = 9.1 Hz)
4′ ″	154.9	

[a] 600 MHz for ^{1}H and 150 MHz for ^{13}C in CD_3OD. [b] These signals were indistinguishable.

have demonstrated physiological activities such as control of cellular slime mold development.[10,11] This paper reports the structure elucidation and synthesis of a new aromatic compound, brefelamide (**1**). The inhibitory effect of **1** on the proliferation of 1321N1 human astrocytoma cells is also described.



Brefelamide (**1**)

Results and Discussion

Isolation and Structure Elucidation. Fruiting bodies (wet weight 335 g) of the cellular slime mold *D. brefeldianum* were cultured on plates and extracted three times with methanol at room temperature to yield an extract (12 g), which was partitioned with ethyl acetate and water. The ethyl acetate solubles (2.4 g) were separated by repeated column chromatography over SiO_2 and ODS to yield **1** (2.1 mg) as a yellow oil. Compound **1** (0.8 mg) was obtained from the fruiting bodies (wet weight 411 g) of *D. giganteum* in a similar manner.

HREIMS (*m/z* 392.1396) and ^{1}H and ^{13}C NMR spectra indicated that the molecular formula of **1** was $C_{22}H_{20}N_2O_5$ (Table 1). The ^{13}C NMR spectrum showed 16 sp^2 carbon signals (>C=O × 2, =C−O (or N) × 5, >C= × 2, and −CH= × 7) and two sp^3 methylene carbon signals, indicating the presence of symmetrical benzene rings. The ^{1}H NMR spectrum indicated the presence of a 1,2,3-trisubstituted benzene ring (δ 7.57 (1H, dd, J = 8.2, 1.3 Hz), 6.77 (1H, dd, J = 7.8, 1.3 Hz), and 6.52 (1H, dd, J = 8.2, 7.8 Hz)) (Figure 1B), two 1,4-disubstituted benzene rings (δ 7.67 and 6.80 (each 2H, d, J = 8.9 Hz) (Figure 1A), and δ 6.84 and 6.76 (each 2H, d, J = 9.1 Hz)) (Figure 1C), and an ethylene group (δ 3.73 and 3.30 (each 2H, t, J = 6.8 Hz)). HMBC correlations for H-3 and H-3′ to C-1′ and H-3′, H-4′, and H-6" to C-5′ suggested that benzene rings A and B were linked by an amide-containing unit C1′−C5′ (Figure 1D). An amino group attached to a benzene ring moves the chemical shifts of ortho and para protons further to the upper field than a hydroxy or alkoxy group. Thus, H-5" (δ 6.52) and H-4" (δ 6.77) indicated that an amino group and a hydroxy or alkoxy group were bonded at C-2″ and C-3″, respectively. Fragment ion peaks at *m/z* 228 and 121 in EIMS determined the place of benzene ring C, yielding the structure of **1** (Figure 1E).

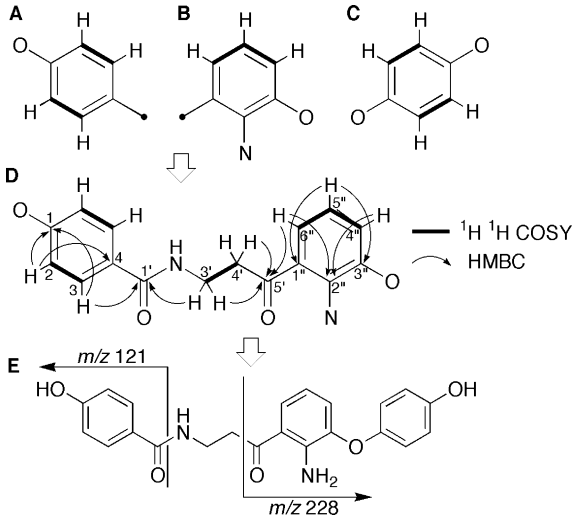


FIGURE 1. Structure elucidation of brefelamide (**1**).

Synthesis. The total synthesis of **1** was carried out to confirm the structure and obtain a sufficient quantity of sample for performing several biological evaluations (Scheme 1). An S_NAr reaction of commercially available 2-fluoronitrobenzene (**2**) and 4-(benzyloxy)phenol (**3**) with cesium carbonate produced diaryl ether **4**. Reduction of **4** to **5** was performed using an $NaBH_4$−$SnCl_2$ system[12] in good yield. 7-Aryloxytryptamine **8** was synthesized by modified Abramovitch−Shapiro tryptamine synthesis.[13,14] The reaction of diazotized **5** with 2-piperidone-3-carboxylic acid[13] formed hydrazone **6**, which was cyclized by warming with formic acid to yield tetrahydro-β-carboline **7**. Following debenzylation of **7**, alkaline hydrolysis and acidic decarboxylation provided tryptamine **8**. Compound **10** was produced by selective acylation with 4-(methoxymethoxy)benzoic acid[15] and protection of the phenolic hydroxyl group of **8**. Oxidative cleavage of the indole ring[16] in **10** by $NaIO_4$ and subsequent acidic hydrolysis allowed us to complete the synthesis of brefelamide (**1**). All of the spectral data on synthetic **1** were identical to

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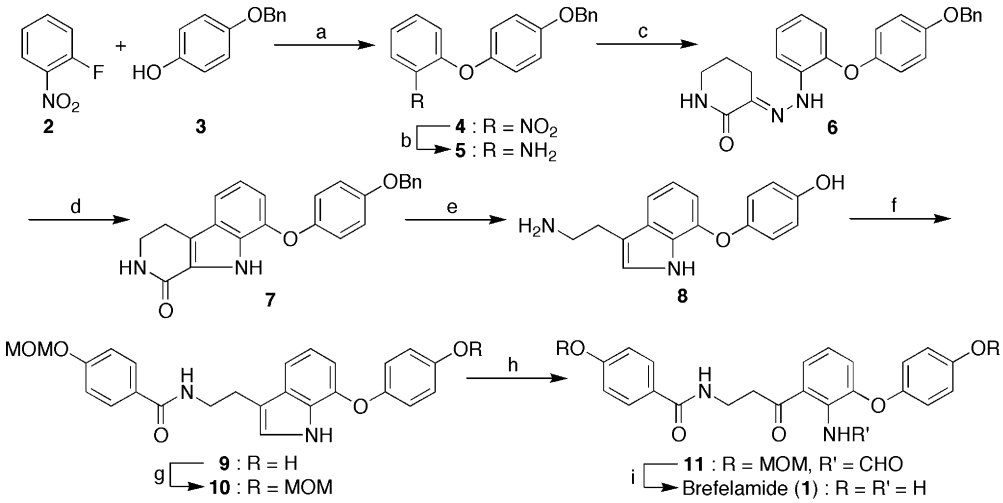
SCHEME 1. Synthesis of Brefelamide (1)[a]



[a] Reagents and conditions: (a) Cs_2CO_3, DMSO, 80 °C (77%); (b) $SnCl_2 \cdot 2H_2O$, $NaBH_4$, EtOH−EtOAc (3:1), 60 °C (87%); (c) (1) $NaNO_2$, 4.5 M HCl−DMF (2:1), 0 °C; (2) 2-piperidone-3-carboxylic acid, AcONa, 0 °C (68%); (d) HCOOH, DMF, 50 °C (56%); (e) (1) H_2, $Pd(OH)_2$/C, EtOAc−EtOH (1:1), rt; (2) 1 M KOH−EtOH (17:2), reflux; (3) concd H_2SO_4, reflux (53%); (f) 4-(methoxymethoxy)benzoic acid, WSC, DMF, 0 °C (65%); (g) MOMCl, *N*,*N*-diisopropylethylamine, CH_2Cl_2, 0 °C (52%); (h) $NaIO_4$, H_2O−MeCN (1:1), 60 °C (75%); (i) TFA, CH_2Cl_2, 0 °C (67%).

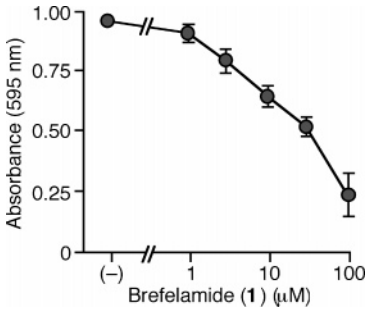


FIGURE 2. Effects of brefelamide (**1**) on the proliferation of 1321N1 human astrocytoma cells. 1321N1 Human astrocytoma cells were incubated with various concentrations of **1** for 2 days in DMEM containing 5% FCS. MTT assays were performed to determine the number of live cells. Each point represents the mean ± SE from three determinations.

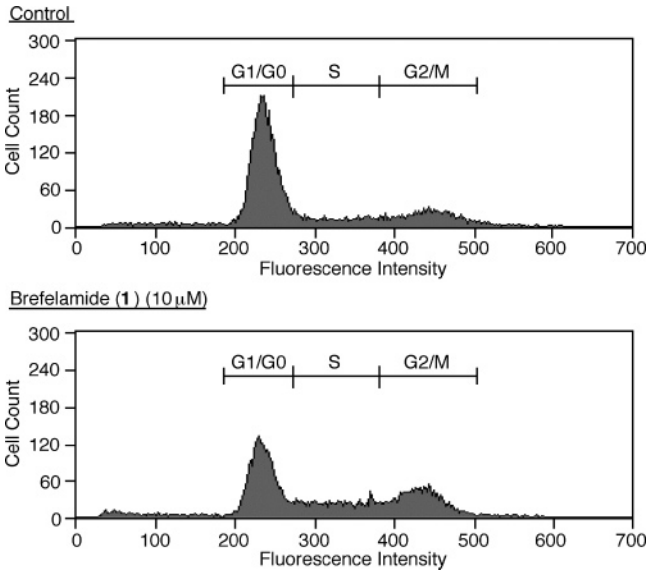


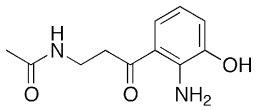
FIGURE 3. Effects of brefelamide (**1**) on the cell-cycle of 1321N1 human astrocytoma cells. 1321N1 Human astrocytoma cells were incubated with 10 μM of **1** for 2 days in DMEM containing 5% FCS. Cells were washed with PBS and fixed in cold 70% ethanol for 1−2 h at 4 °C. Fixed cells were incubated with RNase A for 20 min at 37 °C and stained with propidium iodide for 10 min at room temperature. Cell-cycle profiles were examined by flow cytometry.

those of the natural compound, confirming the proposed structure of **1** in Figure 1.

Biological Evaluation. Brefelamide (**1**) inhibited the cellular proliferation of 1321N1 human astrocytoma cells in a concentration-dependent manner with an EC_{50} value of approximately 10 μM (Figure 2). Flow cytometric analysis demonstrated that treatment with **1** resulted in cell-cycle inhibition through the accumulation of cells in G2/M phase (Figure 3). These results suggest that **1** causes G2/M phase arrest and inhibits proliferation, similar to asiatic acid[17] and isoliquiritigenin.[18]

Conclusions

We presume that brefelamide (**1**) is biosynthesized from tryptophan (Scheme 2). 3-Hydroxykynurenine, a known tryptophan metabolite,[19] is decarboxylated to afford 3-hydroxykynurenamine. This compound may be linked with *p*-hydroxybenzoic acid and hydroquinone to give **1**. Compounds similar to **1** have been only rarely described, as with erebusinone (**12**), a simple acetamide



Erebusinone (**12**)

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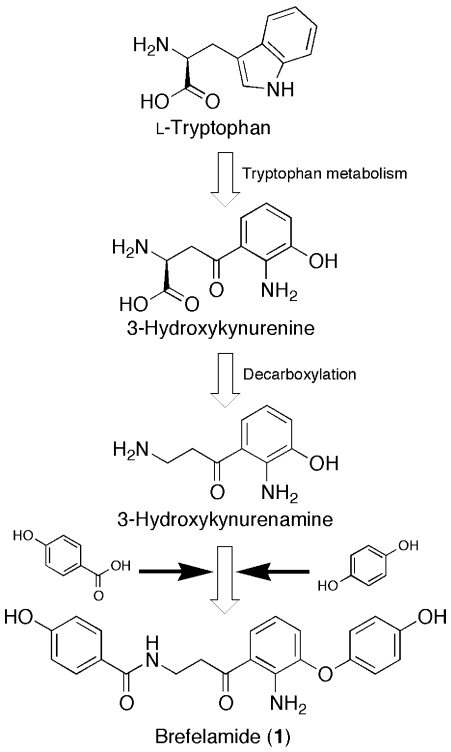
SCHEME 2. Plausible Biosynthetic Pathway for Brefelamide (1)



of 3-hydroxykynurenamine.[20] The isolation of novel classes of compounds such as DIF-1,[2] dictyopyrones,[8] and **1** shows that cellular slime molds are promising sources in natural product chemistry.

In addition, brefelamide (**1**) showed inhibitory effects on the proliferation of 1321N1 human astrocytoma cells caused by G2/M phase arrest. This result indicates that **1** may be utilized as a novel lead compound for anticancer agents.

Experimental Section

Isolation of Brefelamide (1). The cultured fruiting bodies (wet weight 335 g) of *D. brefeldianum* were extracted three times with MeOH at room temperature to give an extract (12.0 g), which was then partitioned with EtOAc and H_2O to yield EtOAc solubles (2.4 g). The EtOAc solubles were chromatographed over SiO_2, and the column was eluted with *n*-hexanes−EtOAc solutions with increasing polarity. *n*-Hexanes−EtOAc (1:3) eluent (130 mg) was further chromatographed over SiO_2 using $CHCl_3$−MeOH (49:1) to give crude brefelamide. The crude sample was purified by ODS column chromatography (H_2O−MeOH (1:1)) to give brefelamide (**1**) (2.1 mg). In a similar manner, the cultured fruiting bodies of *D. giganteum* (wet weight 411 g) yielded **1** (0.8 mg). Data for **1**: yellow oil; ^1H NMR and ^{13}C NMR data are shown in Table 1; EIMS *m/z* 392 $[M]^+$ (48%), 374 (3%), 254 (90%), 228 (28%), 121 (100%); HREIMS *m/z* 392.1396 (392.1372 calcd for $C_{22}H_{20}N_2O_5$).

2-(4-Benzyloxyphenoxy)nitrobenzene (4). To a solution of 2-fluoronitrobenzene (**2**) (8.91 g, 63.2 mmol) and the 4-benzyloxyphenol (**3**) (10.0 g, 50.0 mmol) in DMSO (100 mL) was added cesium carbonate (24.5 g, 75.1 mmol) over 1 h. After being stirred at 80 °C for 12 h, the reaction mixture was cooled to room temperature, poured into H_2O (250 mL), and extracted with EtOAc three times. The combined organic layers were washed with H_2O and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over SiO_2 and eluted by *n*-hexane−EtOAc (39:1) to give **4** (12.4 g, 38.6 mmol, 77%). Data for **4**: yellowish amorphous solid; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.91 (1H, dd, J = 8.1, 1.8 Hz), 7.44 (1H, td, J = 8.4, 1.8 Hz), 7.43 (2H, d, J = 7.3 Hz), 7.39 (2H, t, J = 7.3 Hz), 7.33 (1H, t, J = 7.3 Hz), 7.12 (1H, ddd, J = 8.4, 8.1, 1.1 Hz), 7.01 (2H, d, J = 9.2 Hz), 6.98 (2H, d, J = 9.2 Hz), 6.92 (1H, dd, J = 8.4, 1.1 Hz), 5.05 (2H, s); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 155.9, 151.8, 148.9, 140.7, 136.7, 134.0, 128.6 (2C), 128.1, 127.5 (2C), 125.7, 122.3, 121.1 (2C), 119.1, 116.2 (2C), 70.5; EIMS *m/z* 321 $[M]^+$, 91 (base); HREIMS *m/z* 321.1009 (321.1000 calcd for $C_{19}H_{15}NO_4$).

2-(4-Benzyloxyphenoxy)aniline (5). A solution of **4** (12.4 g, 38.6 mmol) and tin(II) chloride dihydrate (40.7 g, 180 mmol) in 100 mL of EtOH−EtOAc (3:1) was heated at 60 °C for 1 h. Sodium borohydride (730.1 mg, 19.3 mmol) in EtOH (10 mL) was added to this solution over a period of 1 h with stirring at 60 °C. After being stirred for further 1 h, the reaction mixture was cooled to room temperature and poured into H_2O (20 mL). The solvent was evaporated off, and the aqueous solution was alkalinized with 1 M NaOH and then extracted with EtOAc three times. The organic layers were combined, washed with H_2O and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over SiO_2 eluted by *n*-hexanes−EtOAc (24:1) to give **5** (9.81 g, 33.7 mmol, 87%). Data for **5**: brownish amorphous solid; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.42 (2H, d, J = 7.3 Hz), 7.38 (2H, t, J = 7.3 Hz), 7.32 (1H, t, J = 7.3 Hz), 6.93 (1H, ddd, J = 7.9, 7.3, 1.5 Hz), 6.92 (4H, s), 6.79 (1H, dd, J = 7.9, 1.5 Hz), 6.78 (1H, dd, J = 8.0, 1.5 Hz), 6.67 (1H, ddd, J = 8.0, 7.3, 1.5 Hz), 5.02 (2H, s), 3.81 (2H, br.s); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 154.6, 151.0, 144.4, 138.1, 137.0, 128.6 (2C), 127.9, 127.5 (2C), 124.1, 118.9 (2C), 118.8, 118.6, 116.2, 115.9 (2C), 70.5; EIMS *m/z* 291 $[M]^+$, 200, 91 (base); HREIMS *m/z* 291.1258 (291.1258 calcd for $C_{19}H_{17}NO_2$).

3-((2-(4-Benzyloxyphenoxy)phenyl)hydrazono)piperidin-2-one (6). 3-Carbethoxy-2-piperidone (6.07 g, 35.5 mmol) was dissolved in 1 M KOH (40 mL) and stirred at room temperature for 10 h. Then, 6 M HCl (4.0 mL) was added dropwise to this solution at 0 °C. The solution was added dropwise to a solution of diazotized **5**, which was prepared according to the next procedure. A solution of sodium nitrite (2.49 g, 36.1 mmol) in H_2O (9 mL) was added to a solution of **5** (9.53 g, 32.7 mmol) in 4.5 M HCl (67 mL) and DMF (33 mL) during 10 min at 0 °C and stirred for an additional 15 min. After combining the above solutions, sodium acetate (13.4 g, 164 mmol) in H_2O (32 mL) was added (pH ~4.5). The reaction mixture was stirred at 0 °C for 5 h and extracted with EtOAc three times. The organic layers were combined, washed with H_2O and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over SiO_2 eluted by *n*-hexanes−EtOAc (7:3) to give **6** (8.96 g, 22.3 mmol, 68%). Data for **6**: yellowish brown amorphous solid; ^1H NMR ($CDCl_3$, 600 MHz) δ 13.12 (1H, s), 7.60 (1H, d, J = 7.9 Hz), 7.40 (2H, d, J = 7.3 Hz), 7.36 (2H, t, J = 7.3 Hz), 7.30 (1H, t, J = 7.3 Hz), 7.04 (1H, td, J = 7.9, 2.4 Hz), 6.94 (2H, d, J = 9.2 Hz), 6.88 (2H, d, J = 9.2 Hz), 6.79 (1H, d, J = 2.6 Hz), 6.76−6.80 (2H, m), 4.98 (2H, s), 3.17 (2H, td, J = 6.0, 2.6 Hz), 2.64 (2H, t, J = 6.0 Hz), 1.85 (2H, quint, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 164.1, 154.6, 150.8, 143.5, 137.0, 135.6, 128.5 (2C), 127.8, 127.4, 127.4 (2C), 124.0, 120.4, 119.3 (2C), 118.2, 115.7 (2C), 113.0, 70.4, 41.6, 31.0, 22.5; EIMS *m/z* 401 $[M]^+$ (base), 200, 91; HREIMS *m/z* 401.1743 (401.1738 calcd for $C_{24}H_{23}N_3O_3$).

8-(4-Benzyloxyphenoxy)-1,2,3,4-tetrahydro-β-carbolin-1-one (7). To a solution of **5** (3.32 g, 8.27 mmol) in DMF (8.0 mL) was added formic acid (32 mL) dropwise. After being stirred for 15 h at 50 °C, the reaction mixture was cooled to room temperature and evaporated. The residue was chromatographed over SiO_2 eluted by *n*-hexane−$CHCl_3$ (1:4) to give **7** (1.78 g, 4.64 mmol, 56%). Data for **7**: brownish-orange amorphous solid; ^1H NMR ($CDCl_3$, 600 MHz) δ 10.07 (1H, s), 7.37 (2H, d, J = 7.3 Hz), 7.34 (2H, t, J = 7.3 Hz), 7.29 (1H, t,

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J = 7.3 Hz), 7.28 (1H, dd, J = 7.9, 0.7 Hz), 7.00 (1H, t, J = 7.9 Hz), 6.97 (1H, t, J = 2.5 Hz), 6.95 (2H, d, J = 9.2 Hz), 6.84 (2H, d, J = 9.2 Hz), 6.74 (1H, dd, J = 7.9, 0.7 Hz), 4.91 (2H, s), 3.60 (2H, td, J = 7.1, 2.5 Hz), 2.96 (2H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 163.1, 154.7, 150.5, 144.2, 136.9, 129.4, 128.4 (2C), 127.8, 127.6, 127.4 (2C), 126.7, 120.5, 120.0, 119.5 (2C), 115.7 (2C), 115.0, 112.1, 70.3, 41.8, 20.8; EIMS *m/z* 384 $[M]^+$ (base), 293, 91; HREIMS *m/z* 384.1463 (384.1473 calcd for $C_{24}H_{20}N_2O_3$).

3-(2-Aminoethyl)-7-(4-hydroxyphenoxy)indole (8). A solution of **7** (2.99 g, 7.78 mmol) in EtOAc (28 mL) was added to a suspension of palladium hydroxide (20 wt % Pd on carbon) (598 mg) in ethanol (28 mL) and stirred under H_2 atmosphere for 4 h. The reaction mixture was filtered through a Celite pad, and the filter cake was washed with MeOH and EtOAc. The combined filtrates were concentrated. The residue was dissolved in 1 M KOH (34 mL) and EtOH (4 mL). After being refluxed for 5 h, the mixture was evaporated, and the residue was dissolved in H_2O (40 mL). To this solution was added concentrated H_2SO_4 (6.0 mL) dropwise at 0 °C. After being refluxed for 5 h, the mixture was alkalinized with 5M KOH under vigorous stirring, and extracted with EtOAc and *n*-BuOH two times, respectively. The EtOAc layers were washed with H_2O and brine, dried over sodium sulfate, and evaporated. The *n*-BuOH layers were also evaporated. Each residues were combined and chromatographed over SiO_2 eluted by EtOAc-MeOH (4:1) and MeOH to give **8** (1.10 g, 4.10 mmol, 53%). Data for **8**: a brownish powder; ^{1}H NMR (CD_3OD, 600 MHz) δ 7.27 (1H, dd, J = 7.9, 0.7 Hz), 7.07 (1H, s), 6.90 (1H, t, J = 7.9 Hz), 6.88 (2H, d, J = 9.0 Hz), 6.76 (2H, d, J = 9.0 Hz), 6.49 (1H, dd, J = 7.9, 0.7 Hz), 2.96−2.98 (2H, m), 2.91−2.93 (2H, m); ^{13}C NMR (CD_3OD, 150 MHz) δ 154.6, 151.1, 145.6, 131.4, 129.7, 124.0, 121.0 (2C), 120.1, 117.0 (2C), 114.2, 113.9, 109.6, 43.0, 29.2; EIMS *m/z* 268 $[M]^+$, 238 (base); HREIMS *m/z* 268.1195 (268.1211 calcd for $C_{16}H_{16}N_2O_2$).

***N*-(2-(7-(4-Hydroxyphenoxy)indol-3-yl)ethyl)-4-(methoxymethoxy)benzamide (9).** To a solution of **8** (570 mg, 2.13 mmol) in DMF (10 mL) were added 4-(methoxymethoxy)-benzoic acid[15] (465 mg, 2.55 mmol) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (WSC) (1.22 g, 6.38 mmol) at 0 °C. The mixture was gradually warmed to room temperature. After being stirred for 2 h, it was poured into 0.3 M HCl and extracted with EtOAc three times. The organic layers were combined, washed with H_2O and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over SiO_2 eluted by *n*-hexanes−EtOAc (1:1) to give **9** (599 mg, 1.39 mmol, 65%). Data for **9**: offwhite amorphous solid; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.17 (1H, s), 7.62 (2H, d, J = 9.0 Hz), 7.36 (1H, d, J = 7.9 Hz), 7.05 (1H, s), 7.01 (2H, d, J = 9.0 Hz), 7.00 (1H, t, J = 7.9 Hz), 6.96 (2H, d, J = 9.0 Hz), 6.83 (2H, d, J = 9.0 Hz), 6.67 (1H, d, J = 7.9 Hz), 6.14 (1H, t, J = 6.0 Hz), 5.51 (1H, s), 5.20 (2H, s), 3.79 (2H, td, J = 6.6, 6.0 Hz), 3.47 (3H, s), 3.09 (2H, t, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 167.0, 159.7, 152.1, 150.0, 143.9, 129.8, 128.6 (2C), 128.1, 128.0, 122.2, 120.2 (2C), 120.1, 116.4 (2C), 115.9 (2C), 113.7, 113.6, 109.4, 94.2, 56.2, 40.2, 25.5; EIMS *m/z* 432 $[M]^+$, 251 (base); HREIMS *m/z* 432.1678 (432.1684 calcd for $C_{25}H_{24}N_2O_5$).

4-(Methoxymethoxy)-*N*-(2-(7-(4-(methoxymethoxy)phenoxy)indol-3-yl)ethyl)benzamide (10). To a solution of **9** (699 mg, 1.62 mmol) in CH_2Cl_2 (8.0 mL) were added *N*,*N*-diisopropylethylamine (700 μL, 4.02 mmol) and chloromethyl methyl ether (200 μL, 2.63 mmol) at 0 °C. The mixture was gradually warmed to room temperature. After being stirred for 12 h, it was poured into 0.2 M HCl and extracted with EtOAc three times. The organic layers were combined, washed with H_2O and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over SiO_2 eluted by *n*-hexanes−EtOAc (2:1 and 1:1) to give **10** (399 mg, 0.838 mmol, 52%) and recovered **9** (256 mg, 0.592 mmol, 37%). Data for **10**: offwhite amorphous solid; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.40 (1H, s), 7.63 (2H, d, J = 8.6 Hz), 7.35 (1H, d, J = 7.9 Hz), 6.96−7.00 (8H, m), 6.66 (1H, d, J = 7.9 Hz), 6.27 (1H, t, J = 5.9 Hz), 5.17 (2H, s), 5.13 (2H, s), 3.76 (2H, td, J = 6.6, 5.9 Hz), 3.48 (3H, s), 3.45 (3H, s), 3.06 (2H, t, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 166.9, 159.6, 153.3, 151.2, 143.5, 129.8, 128.5 (2C), 128.0, 128.0, 122.3, 119.9, 119.7 (2C), 117.6 (2C), 115.7 (2C), 113.7, 113.6, 109.5, 95.0, 94.1, 56.1, 55.9, 40.2, 25.4; EIMS *m/z* 476 $[M]^+$, 295 (base); HREIMS *m/z* 476.1947 (476.1946 calcd for $C_{27}H_{28}N_2O_6$).

***N*-(3-(2-Formylamino-3-(4-(methoxymethoxy)phenoxy)phenyl)-3-oxopropyl)-4-methoxymethoxybenzamide (11).** Sodium periodate (402 mg, 1.88 mmol) was added to a solution of **10** (222 mg, 0.467 mmol) in acetonitrile (5 mL) and H_2O (5 mL). After being stirred for 36 h at 60 °C, the reaction mixture was extracted with EtOAc three times. The organic layers were combined, washed with H_2O and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed over SiO_2 eluted by *n*-hexanes−EtOAc (1:1 and 2:3) to give **11** (178 mg, 0.349 mmol, 75%). Data for **11**: yellow oil; ^{1}H NMR (acetone-d_6, 600 MHz) δ 9.60 (1H, br.s), 8.34 (1H, br.s), 7.88 (2H, d, J = 9.0 Hz), 7.74 (1H, br.s), 7.40 (1H, br.s), 7.24 (1H, t, J = 7.9 Hz), 7.06 (2H, d, J = 9.2 Hz), 7.05 (2H, d, J = 9.0 Hz), 6.99 (1H, br.s), 6.98 (2H, d, J = 9.2 Hz), 5.23 (2H, s), 5.16 (2H, s), 3.75 (2H, td, J = 6.2, 5.5 Hz), 3.43 (3H, s), 3.41 (3H, s), 3.32 (2H, br.s); ^{13}C NMR (acetone-d_6, 150 MHz) δ 201.2, 166.8, 161.0, 160.5, 154.9, 151.6, 151.3, 137.4, 129.6 (2C), 129.6, 129.1, 126.8, 122.3, 121.0 (2C), 120.4, 118.6 (2C), 116.3 (2C), 95.5, 94.8, 56.1, 56.0, 41.1, 35.9; EIMS *m/z* 508 $[M]^+$, 165, 45 (base); HREIMS *m/z* 508.1858 (508.1844 calcd for $C_{27}H_{28}N_2O_8$).

***N*-(3-(2-Amino-3-(4-hydroxyphenoxy)phenyl)-3-oxopropyl)-4-hydroxybenzamide (Brefelamide (1)).** To a solution of **11** (12.7 mg, 0.025 mmol) in CH_2Cl_2 (900 μL) was added trifluoroacetic acid (200 μL) dropwise at 0 °C. After being stirred for 3 h, the mixture was evaporated. The residue was chromatographed over aluminum oxide eluted by $CHCl_3$−MeOH (9:1) to give brefelamide (**1**) (7.0 mg, 0.018 mmol, 67%). All spectral data of the synthetic product were identical with those of the natural product.

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Supporting Information Available: General experimental methods and NMR spectra for new compounds including natural and synthetic brefelamide (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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