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Marine Cyanobacterial Fatty Acid Amides Acting on Cannabinoid Receptors

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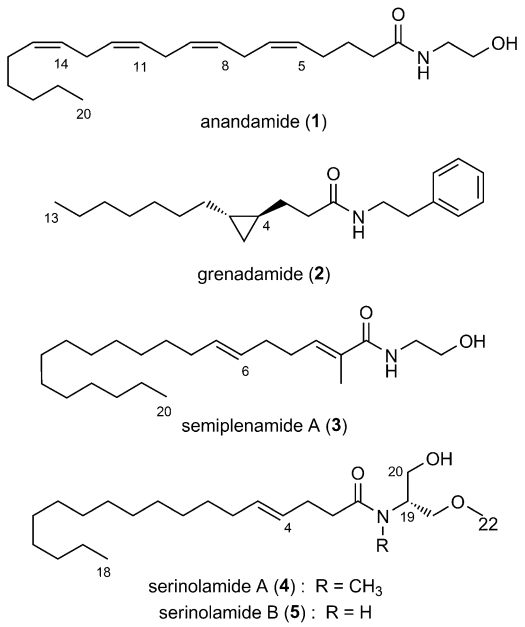
The fact that lipids are utilized by diverse organisms suggests an evolutionarily conserved role of this class of compounds.[1] Indeed, lipidomics is emerging as a crucial field of research because of the key role of lipid biomolecules in a wide array of physiological functions.[2] Research has also uncovered some vital lipid–protein interactions in which lipid molecules bind to specific protein domains to mediate physiological effects. The endocannabinoid system is a representative example; here two characterized G protein-coupled cannabinoid receptors CB_1 and CB_2 are modulated by endogenous lipids known as endocannabinoids. Importantly, this system has been implicated in various pathophysiologies, including neurodegenerative diseases, eating disorders, pain, inflammation, and cancer.[3–7] Therefore, a better understanding of this system has become of significant interest, and the cannabinoid receptors are viewed as possible targets for different diseases.[1,5] The classical concept that all agonists at a given GPCR induce a similar repertoire of downstream events is now uncertain, and the latest experimental evidence supports the existence of ligand-specific functional selectivity at the cannabinoid receptors.[3] Consequently, the identification of new structural scaffolds that can bind to the cannabinoid receptors remains an essential tool for digging further into this complex system.

Anandamide (*N*-arachidonoylethanolamine, **1**; Scheme 1) was the first endogenous ligand to be identified among the endocannabinoid family.[8] The structure of this fatty acid amide suggested that other natural and synthetic fatty acid amides might also function as cannabinoid receptor ligands. Marine cyanobacteria of the genus *Lyngbya* have a characteristic metabolic profile that is rich in fatty acid amides (in addition to peptides),[9] and they therefore represent a potential source of new model compounds that would act on the cannabinoid receptors. Support for this assumption comes from reports of metabolites isolated from *Lyngbya* samples that can interact with the cannabinoid receptors. To our knowledge, only five marine cyanobacterial fatty acid amides with binding affinities to the cannabinoid receptors have been identified; grenadamide (**2**),[10] semiplenamides A (**3**), B, and G,[11] and the recently reported metabolite serinolamide A (**4**)[12] (Scheme 1). However, none of them has been tested in functional assays before, so it remains unknown whether those metabolites act as receptor agonists or antagonists.



Scheme 1. Structures of the endocannabinoid anandamide and fatty acid amides from marine cyanobacteria with binding affinities to the cannabinoid receptors.

From a *Lyngbya* sample from the Piti Bomb Holes in Guam, we isolated and then characterized the new fatty acid amide serinolamide B (**5**; Scheme 1), a closely related analogue of serinolamide A. Based on structural features, we evaluated the ability of compound **5** to bind to both human cannabinoid receptors CB_1 and CB_2 with a functional outcome. The marine cyanobacteria *Lyngbya* spp. are also well known for the production of a large class of fatty acid amides known as malyngamides.[13] More than 30 malyngamide analogues with a broad spectrum of bioactivities are known. Although malyngamides usually contain different amine portions and sometimes vary in the length of the fatty acyl chain, they generally have a unique and characteristic structural scaffold of an N-substituted amide of a long-chain 7-methoxy fatty acid with mono-unsaturation at C4 (Scheme 2). As malyngamides are the most abundant fatty acid amides found in *Lyngbya* spp., we examined whether the malyngamide-type structural features could also bind to the cannabinoid receptors, although they usually possess more complicated amine portions and somewhat different fatty acid side chains from the known endogenous cannabinoids. For this, we tested malyngamide B (**6**; Scheme 2)[14] for its potential as a cannabimimetic compound. Interestingly, malyngamide B can bind to both CB_1 and CB_2 with moderate potencies. Here, we report the results of our studies on two marine cyanobacterial metabolites; the newly identified analogue serinolamide B (**5**) and a member of a large group of cyanobacterial fatty acid amides, malyngamide B (**6**).

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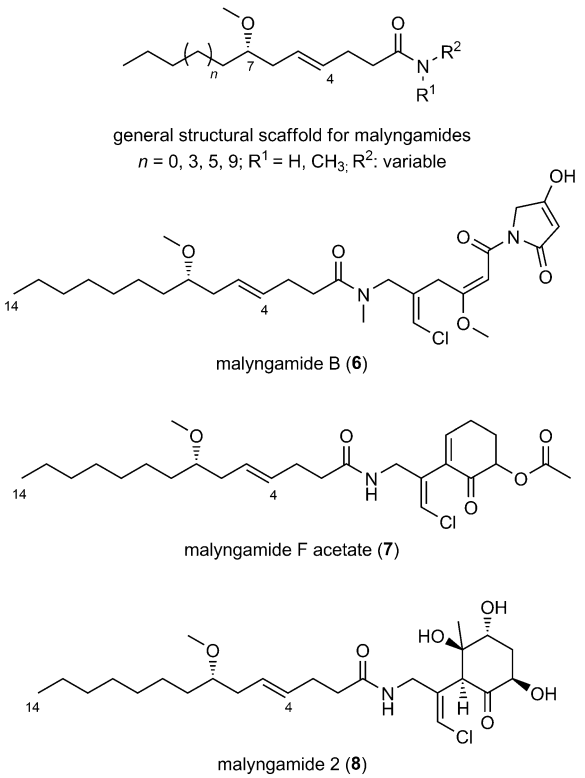

Scheme 2. General and specific structures of malyngamides.

Table 1. ^{1}H and ^{13}C NMR spectroscopy data for serinolamide B (**5**) in $CDCl_3$ at 600 MHz.

Unit	C/H #	δ_C	δ_H (J [Hz])	HMBC[a]
fatty acid	1	172.9 qC		2, 3, 19, NH
	2	36.4 CH_2	2.27 dd (6.6, 2.1)	3b, 4
	3a	28.4 CH_2	2.28 d (6.9)	2, 4, 5
	3b		2.23 m	
	4	127.8 CH	5.39 m	3a, 3b, 5, 6
	5	132.1 CH	5.49 m	3b, 4, 6, 7
	6	32.3 CH_2	1.96 ddd (7.7, 7.5, 6.8)	4, 5, 7, 8
	7	29.3 CH_2	1.32 m	6, 8
	8–15[b]	29.4 CH_2	1.25 m	6, 7
	16	31.8 CH_2	1.25 m	17, 18
	17	22.4 CH_2	1.29 m	16, 18
	18	13.9 CH_3	0.88 t (6.8)	16, 17
serinol ether	19	50.3 CH	4.07 m	20a, 21a, 21b, NH
	20a	64.2 CH_2	3.82 dd (11.2, 4.1)	19, 21a, 21b
	20b		3.66 br d (11.2)	
	21a	73.6 CH_2	3.58 dd (9.3, 4.2)	19, 21a, 20b, 22
	21b		3.53 dd (9.3, 4.2)	
	22	59.2 CH_3	3.36 s	21a, 21b
	NH		6.16 d (6.8)	

[a] Protons showing long-range correlation to indicated carbon. [b] Overlapping peaks.

A cyanobacterial sample from Guam was extracted three times with EtOAc/MeOH. Solvent partitioning of the organic extract yielded 7.2 g of a semipolar *n*BuOH fraction that was then fractionated by silica gel chromatography. Compound **5** was purified by reversed-phase HPLC from a silica column fraction that also contained pitipeptolides.[15] The molecular formula $C_{22}H_{43}NO_3$ was established by high-resolution electrospray ionization mass spectrometry (HRESIMS; *m/z* 370.3316 $[M+H]^+$). NMR profiles of this compound were characteristic of a fatty acid derivative, and the typical chemical shifts of a mono-unsaturated fatty acid chain were prominent (Table 1): ^{1}H and ^{13}C NMR spectra showed a number of overlapping methylene groups ($\delta_H \approx 1.2$ ppm and $\delta_C \approx 23$ ppm), a carbonyl ($\delta_C = 172.9$ ppm), an α-methylene group ($\delta_H = 2.27$ ppm and $\delta_C = 36.4$ ppm), a terminal methyl group ($\delta_H = 0.88$ ppm and $\delta_C = 13.9$ ppm), and characteristic olefinic methines ($\delta_H =$ 5.39 ppm, $\delta_C = 127.8$ ppm and $\delta_H = 5.49$, $\delta_C = 132.1$ ppm). Additionally, the compound appeared to be a fatty acid amide; the amide proton at $\delta_H = 6.16$ ppm showed HMBC correlation to the carbonyl carbon as well as a COSY correlation to a methine proton at 4.07 ppm ($\delta_C = 50.3$ ppm). Three oxygenated groups were also identified based on their chemical shifts; two methylene groups ($\delta_H = 3.58$, 3.53 ppm, $\delta_C = 73.6$ ppm; and $\delta_H =$ 3.66, 3.82 ppm, $\delta_C = 64.2$ ppm) and a methoxy group ($\delta_H =$ 3.36 ppm and $\delta_C = 59.2$ ppm); the latter showed HMBC correlation to the first oxygenated methylene group ($\delta_C = 73.6$ ppm; Table 1). Further analysis of COSY, TOCSY, and HMBC data allowed the amine part of this molecule to be constructed as a monomethyl serinol and located the olefinic system in the fatty acid chain between C4 and C5 (Scheme 1, Table 1). Finally, to complete the molecular formula suggested by the MS data, the number of methylene groups forming the fatty acid chain was assigned to construct an 18-carbon mono-unsaturated fatty acid.

The absolute configuration of the chiral center in the serinol moiety was determined through Jones oxidation of the primary alcohol to its corresponding carboxylic acid, followed by acid hydrolysis to liberate *O*-Me serine. Enantioselective analysis revealed an *S* configuration of the amino acid and consequently an *R* configuration in the parent compound. Additionally, the double-bond geometry was assigned as *trans* based on the chemical shifts of the adjacent methylene groups C3 and C6[12,16] and the absence of NOESY correlations between the two olefinic protons. While we were investigating the biological activity of this metabolite, the Gerwick group reported the closely related analogue **4** (Scheme 1).[12] Notably, ^{1}H and ^{13}C chemical shifts and the stereochemical assignments for **5** match those reported for **4**.

Given the structural similarity of serinolamide A (**4**) to the endocannabinoids anandamide (**1**) and 2-arachidonoyl glycerol, it was tested for binding to the human cannabinoid receptors CB_1 and CB_2; it appeared to possess more than fivefold selectively for the CB_1 receptor, with a moderate binding affinity ($K_i = 1.3$ μM).[12] As the only structural difference between the two serinolamide analogues is a secondary amide in **5** instead of the tertiary amide in **4**, we evaluated the cannabimimetic activity of serinolamide B. This compound can also bind to both CB_1 and CB_2 receptors with moderate to weak binding affinities (Figure 1 A, Table 2). However, **5** showed an opposite trend in binding affinities compared to **4**, as it exhibited a moderate affinity and higher selectivity for CB_2 ($K_i = 5.2$ μM) over

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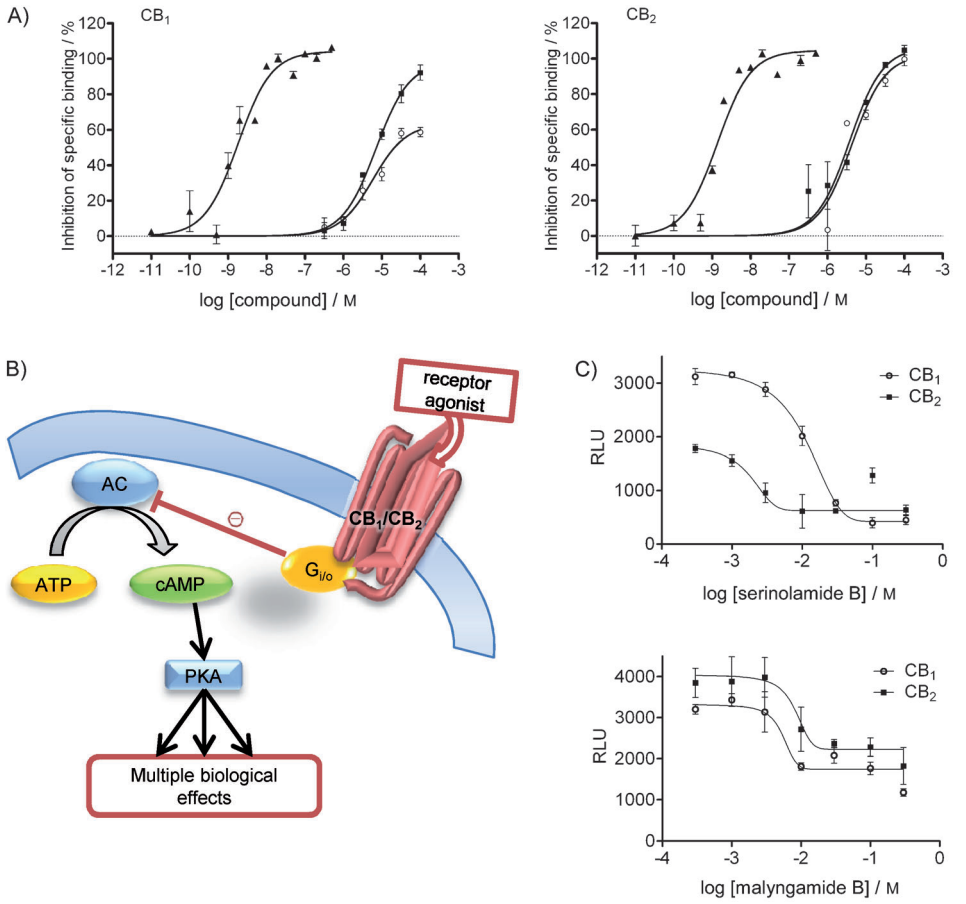

Figure 1. A) Binding of compounds **5** (○) and **6** (■) as well as HU-210 (▲) to the receptors CB_1 (left) and CB_2 (right), represented as percent inhibition of the binding of a radioactive ligand. B) Simplified representation of the cannabinoid receptors and the consequences of agonist binding. C) Effect of compounds **5** (top) and **6** (bottom) on forskolin-induced cAMP accumulation. A higher level of cAMP produces a higher luminescence reading (RLU).

Table 2. The affinities of compounds **4** and **5** for the cannabinoid receptors (K_i) and consequent functional effects on cAMP accumulation (EC_{50}).

	CB_1		CB_2	
Compound	K_i [μM]	EC_{50} [μM][a]	K_i [μM]	EC_{50} [μM][a]
5	16.4	11.8	5.2	1.8
6	3.6	5.3	2.6	8.8
HU-210[b]	0.00069	–	0.0011	–
CP 55940[c]	–	0.00024	–	0.00036

[a] Results from cAMP functional assays. EC_{50} is the agonist concentration required to cause half-maximal inhibition of forskolin-induced cAMP accumulation. [b] Positive control for binding assays. [c] Positive control for functional assays.

CB_1 (K_i = 16.4 μM). Notably, the endocannabinoid **1** shows higher selectivity for CB_1 (K_i for CB_1 = 32 nM; K_i for CB_2 = 1.9 μM),[17] thus suggesting that the presence of a secondary rather than a tertiary amide is not the main determinant for receptor selectivity.

Malyngamides have been reported with different biological activities, including cytotoxic, anti-inflammatory, and quorum-sensing actions.[18–20] Yet, to the best of our knowledge, the malyngamide structural features were not probed before for cannabinoid receptor interactions. Accordingly, we were interested in testing a representative analogue so as to determine whether this molecular architecture is capable of possessing cannabimimetic effects. We obtained malyngamide B (**6**) from our marine natural products library and tested it for CB_1 and CB_2 binding. Interestingly, **6** appeared to possess moderate binding affinities to both receptors with K_i values of 3.6 μM for CB_1 and 2.6 μM for CB_2 (Figure 1 A, Table 2). Those results are noteworthy because **6** has a more complex amine portion than the endocannabinoids and other cyanobacterial fatty acid amides that possess the same biological activity (Schemes 1 and 2).

It has not yet been determined whether the marine cyanobacterial fatty acid amides that can bind to the cannabinoid receptors act as agonists or antagonists. In order to clarify this, we tested the functional response induced by the binding of **5** and **6** to the cannabinoid receptors. Cannabinoid receptors are G protein-coupled receptors that are functionally coupled to the inhibition of adenylyl cyclase and subsequent inhibition of cAMP accumulation (Figure 1 B).[6] Compounds **5** and **6** were able to inhibit forskolin-stimulated cAMP accumulation through both CB_1 and CB_2 receptors with moderate potencies (Figure 1 C, Table 2); this proves that those metabolites act as cannabinoid receptor agonists. Intriguingly, serinolamide B (**5**) appeared to be more CB_2-receptor-selective in the binding as well as the functional assays, but malyngamide B (**6**) appeared to bind to both receptors similarly with comparable functional outcomes (Figure 1 C, Table 2).

It is known that anandamide signaling is terminated by the enzyme fatty acid amide hydrolase (FAAH), which catalyzes anandamide hydrolysis. Therefore, one emerging pharmacological approach to augment endocannabinoid activity is directed towards FAAH inhibition.[21] Thus, we tested the ability of metabolites **5** and **6** to inhibit this enzyme. However, no considerable inhibitory effects were detected for either compound at 10 and 100 μM.

It has been repeatedly shown that cannabimimetic compounds can also mediate anti-inflammatory responses.[1,7,22] From that perspective, we tested the ability of compounds **5**

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and **6** to exert anti-inflammatory effects in lipopolysaccharide (LPS)-induced murine macrophages RAW 264.7. Serinolamide B showed a weak effect with an $IC_{50} > 25$ μM; however, malyngamide B was more potent, inhibiting NO production with an IC_{50} of 6.2 μM without affecting cellular viability at up to 25 μM. Although the evidence suggests that the anti-inflammatory effects of some cannabimimetic compounds are mediated through cannabinoid receptors, particularly CB_2, it is questionable whether this is also the case with malyngamide B. Notably, Mukhopadhyay et al. showed that no detectable CB_2 receptors were apparent in RAW 264.7 cells unless they were stimulated by LPS.[23] Therefore, the effect of **6** on LPS-induced inflammation is probably not totally mediated through the cannabinoid receptors, as it was able to prevent the early stimulation by LPS. Further experiments with CB_2-receptor-selective antagonists or CB_2-receptor-deficient cells will help ascertain the presence or absence of a cannabinoid-receptor-mediated anti-inflammatory effect for **6**.

Some malyngamides can also reduce NO accumulation under similar anti-inflammatory assay conditions; malyngamide F acetate (**7**) and malyngamide 2 (**8**; Scheme 2) have IC_{50} values of 7.1[20] and 8 μM,[19] respectively. Acetate **7** was shown to possess a distinctive cytokine profile and appeared to selectively inhibit the MyD88-dependent pathway.[20] Notably, **7** and **8** share common structural features such as oxidized cyclohexyl rings, whereas compound **6** has a significantly different amine entity (Scheme 2). To our knowledge, this is the first report of such activity for an anti-inflammatory malyngamide with the pyrrolidone ring in the amine portion rather than the common six-membered cyclic ketone or lactone.

We also tested the cytotoxic effects of compounds **5** and **6** against cancer cells. Serinolamide B failed to show significant cytotoxicity against HT-29 colon adenocarcinoma and MCF7 breast cancer cell lines at up to 100 μM. It is important to point out here that serinolamide A showed some cytotoxic properties in a different cell line.[12] In contrast, malyngamide B is known as a feeding deterrent[24] and, in our hands, was cytotoxic to HT-29 cells with an IC_{50} value of 26 μM, but it remains unclear if its cannabimimetic activity contributes to this cytotoxic effect.

In this report, we have identified the new cannabimimetic marine cyanobacterial fatty acid amide serinolamide B (**5**). Serinolamide B with a secondary amide had higher CB_2 receptor selectivity and lower cytotoxicity than its analogue with a tertiary amide. In agreement, other reports showed that several structural features can increase affinity for the CB_2 receptor, including an *E* double bond at position 4, an amide proton, and additional substituents in the amine part.[1,22] Testing analogues **4** and **5** side by side under the same experimental conditions will unequivocally clarify this comparison. We also show that malyngamide B (**6**) also possesses cannabimimetic properties; this provides new insight into the biological activities of malyngamides, the most abundant marine fatty acid amide class in *Lyngbya* spp. This finding introduces a new structural lead to the cannabimimetic field from the marine environment, and should foster the cannabimimetic evaluation of further analogues. Additionally, our finding that both metabolites act as receptor agonists implies that they can mediate certain physiological effects through this pathway, and therefore opens more research avenues. Several malyngamides have been subjected to total chemical syntheses and some well-established synthetic routes are already available,[25] these can assist structural optimization efforts towards more potent analogues.

Experimental Section

General experimental procedures: The optical rotation was measured on a PerkinElmer 341 polarimeter. UV and optical activity were measured on a SpectraMax M5 (Molecular Devices), and IR data were obtained on a PerkinElmer Spectrum One FTIR Spectrometer. The ^1H and 2D NMR spectra were recorded on a Bruker Avance II 600 MHz spectrometer. All spectra were obtained in $CDCl_3$ by using residual solvent signals ($\delta_H = 7.26$ ppm, $\delta_C = 77.16$ ppm) as internal standards. HSQC and HMBC experiments were optimized for $^1J_{CH} = 145$ Hz and $^1J_{CH} = 7$ Hz, respectively. HRMS data were recorded on an Agilent LC-TOF mass spectrometer equipped with an APCI/ESI multimode ion source detector in positive-ion mode. LC-MS data were obtained by using an API 3200 triple quadrupole MS (Applied Biosystems) equipped with a Shimadzu LC system.

Extraction and isolation: The sample of the marine cyanobacterium *Lyngbya majuscula* (recollection of UOG strain VP627) was collected at Piti Bomb Holes, Guam, in February 2000. A voucher sample (voucher specimen number EC025) has been preserved at the Smithsonian Marine Station at Fort Pierce, FL. The freeze-dried organism was extracted with EtOAc/MeOH (1:1, 3×) to give a crude organic extract (35.5 g), which was partitioned between hexanes and 80% aqueous MeOH. After the methanolic phase had been dried, the residue was partitioned between *n*BuOH and H_2O. The concentrated *n*BuOH residue (7.2 g) was subjected to flash chromatography over silica gel, eluting with increasing gradients of *i*PrOH in CH_2Cl_2, and finally with MeOH. The fraction eluting with 4% *i*PrOH/CH_2Cl_2 was fractionated on a semi-preparative reversed-phase HPLC column (YMC-Pack ODS-AQ, 250×10 mm, 5 μm, 2 mL min^{-1}; UV detection at 220/254 nm) by using a linear gradient of MeOH/H_2O (75–100% aqueous MeOH over 30 min, and then 100% MeOH for 10 min) to afford ten fractions. Repurification of five fractions yielded pitiprolamide[26] and pitipeptolides.[15] Compound **5** eluted as a single peak (fraction 10) at $t_R = 28.8$ min.

Serinolamide B (**5**): colorless, amorphous solid; $[\alpha]_D^{20} = -7.9$ ($c = 0.075$, $CHCl_3$); ^1H NMR, ^{13}C NMR and HMBC data, see Table 1; IR (film): $\nu_{max} = 3290$, 3077, 2955, 2920, 2851, 1641, 1542, 1465, 1377 cm^{-1}; HRESI/APCIMS: *m/z* calcd for $C_{22}H_{44}NO_3$: 370.3316 $[M+H]^+$, found: 370.3324.

Jones oxidation and enantioselective amino acid analysis by HPLC/MS: Compound **5** (1 mg) was dissolved in acetone (1 mL), then freshly prepared Jones reagent (CrO_3 in diluted H_2SO_4, 50 μL) was added. The reaction mixture was stirred at room temperature for 1 h. The reaction was then quenched by the addition of a few drops of *i*PrOH, and the mixture was filtered through a pad of celite. The reaction mixture was dried down under nitrogen, and the residue was redissolved in water and partitioned between water and EtOAc three times. The organic layer was dried under nitrogen, and the crude product was purified by HPLC (YMC-Pack ODS-AQ, 250×10 mm, 5 μm, 2 mL min^{-1}; UV detection at 220/200 nm) using a MeOH/0.05% aq. TFA linear gradient (75–100% aqueous MeOH over 20 min, then 100% MeOH for 10 min) to give the oxidized compound (0.7 mg) at $t_R = 27.7$ min (68% yield). Then,

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HCl (6 N, 400 μL) was added to the product (100 μL), and the mixture was stirred at 110 °C overnight. The reaction mixture was dried, reconstituted in water (100 μL) and subjected to HPLC/MS enantioselective analysis. For the standards, (*S*)-*O*-Me-Ser standard (0.3 mg; Waterstone Technology, Carmel, IN, USA) was subjected to partial epimerization to obtain the (*R*)-*O*-Me Ser standard. The compound was dissolved in water (80 μL), then triethylamine (32 μL) and acetic anhydride (32 μL) were added. The reaction mixture was stirred at 60 °C for 1 h and then dried down. The residue was redissolved in HCl (6 N, 100 μL), and the solution was stirred at 110 °C overnight, then dried again. The *S*/*R* enantiomer ratio obtained from the partial epimerization reaction was 9:1. Standards as well as the test compound were subjected to HPLC/MS chiral analysis (MRM monitoring) under the following conditions: CUR 10, CAD medium, IS 5500, TEMP 600, GS1 55, GS2 55, positive-ion mode, MRM pair [120→74], t_R: (*S*)-*O*-Me-Ser: 10.6 min, (*R*)-*O*-Me-Ser: 15.4 min, oxidized moiety from compound **5** (10.6 min).

Cannabinoid CB_1/CB_2 receptor binding assays: Assays were done by Caliper Life Sciences (Hanover, MD, USA). Human recombinant CB_1 (B_{max} = 1.5 pmol per mg protein) or CB_2 (B_{max} = 8 pmol per mg protein) receptors were expressed in HEK-293 cells. [^{3}H]CP-55940 (0.5 nM) was used as the radioligand (K_d for CB_1 = 0.6 nM and for CB_2 = 4.2 nM). HU-210 (1 μM) was used as nonspecific binding determinant (K_i values of 1.1 and 3.0 nM for CB_1 and CB_2, respectively). Reactions were carried out in Tris·HCl buffer (50 mM, pH 7.4) containing EDTA (2.5 mM), $MgCl_2$ (5 mM), and BSA (0.1%) at 30 °C for 90 min. The reaction was terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters was determined by liquid scintillation spectrometry and compared to control values in order to ascertain any interactions of the test compound with the CB_1 or CB_2 binding sites.

cAMP functional assay: CHO-K1 cells expressing CB_1 or CB_2 receptors were used, and cAMP levels were determined after forskolin stimulation by using cAMP Hunter express GPCR assay kits (DiscoverX, Fremont, CA, USA) according to the manufacturer's procedures. Briefly, cells were seeded in 96-well plates (3×10^4 cells per well) and incubated at 37 °C in humidified air with 5% CO_2. After 24 h, the medium was aspirated, and cell assay buffer with cAMP antibody reagent was added to the wells. Test compound and forskolin were dissolved in DMSO. The cells were then stimulated with different concentrations of the test compound in the presence of forskolin (20 μM) for 30 min at 37 °C. Cell lysis and chemiluminescent signal detection were performed with the detection reagents according to the recommended protocol.

FAAH inhibitor enzyme assay: The FAAH inhibitor screening assay kit was purchased from Cayman Chemical (Ann Arbor, MI, USA) and used as recommended. In a black 96-well plate, FAAH enzyme (10 μL) was added to the assay buffer (170 μL), followed by the addition of the test compound or the solvent control (10 μL). The reactions were initiated by adding the substrate AMC arachidonoyl amide (10 μL, 20 μM), and the plate was incubated for 30 min at 37 °C. After incubation, the signal was detected at λ_{ex} = 350 nm and λ_{em} = 455 nm by using a microplate reader.

NO assay: RAW 264.7 mouse macrophage cells were cultured and maintained in DMEM supplemented with 10% FBS in a humidified environment with 5% CO_2. Cells were seeded in 96-well plates (2×10^4 cells per well) and, after 24 h, the cells were treated with different concentrations of the test compound or solvent control (1% EtOH), followed by LPS (0.5 μg mL^{-1}) to stimulate an inflammatory response. The production of NO was assessed by measuring the nitrite concentration in the culture medium after 24 h by using Griess reagent. Briefly, sulfanilamide (1% *w*/*v*) in phosphoric acid (5% *v*/*v*; 50 μL) was added to the cell culture supernatant (50 μL), and the culture was incubated for 5 min at room temperature in the dark, then naphthylethylenediamide·HCl (0.1% *w*/*v*, 50 μL) was added. After 5 min of incubation at room temperature in the dark, the absorbance of the reaction mixture was measured at 540 nm by using a microplate reader. Assays were run in duplicate. Nitrite quantification was determined relative to a nitrite standard curve (0–100 μM).

Cell viability assays: Cells were propagated and maintained in Dulbecco's modified Eagle medium (Invitrogen) supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, UT) at 37 °C under humidified air with 5% CO_2. Cells were seeded in 96-well plates (MCF7: 10500 cells per well; HT-29: 11000 cells per well). After 24 h, cells were treated with various concentrations of the test compound or solvent control (1% EtOH). After 48 h of incubation, cell viability was measured by using MTT (Promega) according to the manufacturer's instructions.

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Keywords: amides · anti-inflammatory agents · cannabimimetics · malyngamides · marine cyanobacteria

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