A Bastadin with Potent and Selective δ-Opioid Receptor Binding Affinity from the Australian Sponge *Ianthella flabelliformis*

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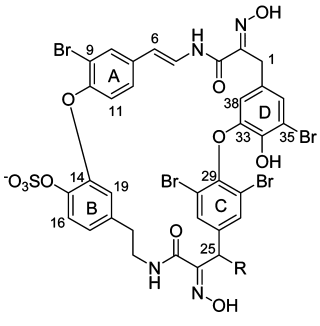
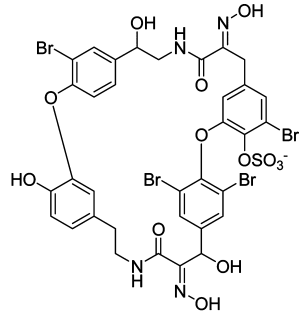
Three new bastadins, bastadin 25 (**1**), 15-*O*-sulfonatobastadin 11 (**2**), and bastadin 26 (**3**), were isolated from a MeOH extract of the Australian marine sponge *Ianthella flabelliformis.* Their structures were determined by interpretation of 1D and 2D NMR spectra and mass spectrometry. Bastadin 26 (**3**) showed potent affinity for the guinea pig δ-opioid receptors with a K_i value of 100 nM. The other two bastadins had a 100-fold lower affinity. The three compounds were also tested for their affinity to guinea pig μ- and κ-opioid receptors and shown to have either no affinity or only very weak affinity toward both of these opioid receptors.

Sponges from the genus *Ianthella* consistently produce macrocyclic alkaloids known as the bastadins.[1−15] These alkaloids comprise two tyramino tyrosines units that have been oxidatively coupled to yield macrocyclic ethers. To date, over 20 compounds from this class have been isolated. The compounds possess a range of biological activities including cytotoxic,[1−4,9,14,15] antimicrobial,[1,6] anti-inflammatory,[3] inhibition of tumor angiogenesis,[16,17] inhibition of the enzymes topoisomerase-II,[7] dihydrofolate reductase,[7] and inosine-1-phosphate dehydrogenase, and modulation of Ca^{2+} release from the sarcoplasmic reticulum by binding to the RyR1-FKBP12 Ca^{2+} channel.[10,13,18] Our interest in the MeOH extract of a sample of the marine sponge *Ianthella flabelliformis,* collected from Darwin Harbor, was sparked by its ability to potently inhibit the binding of the opioid peptide [^{3}H]DPDPE to δ-opioid receptors in membranes from guinea pig forebrain at 5 μg/mL. The same MeOH extract did not inhibit the binding of [^{3}H]DAMGO to μ-opioid receptors in membranes from guinea pig forebrain or [^{3}H]U69593 to κ-opioid receptors in membranes from guinea pig cerebellum. The high selectivity and potency for δ-opioid receptors of this extract warranted further investigation, and we report herein the bioassay-guided purification, structure determination, and biological activities of three active constituents, bastadin 25 (**1**), 15-*O*-sulfonatobastadin 11 (**2**), and bastadin 26 (**3**), isolated from *I. flabelliformis*.



1 R = OH
2 R = H



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A small sample of the freeze-dried, ground sponge *I. flabelliformis* was extracted sequentially with CH_2Cl_2, MeOH, and H_2O, and each extract tested for δ-, μ-, and κ-opioid binding affinity. The CH_2Cl_2 extract was inactive, while the MeOH extract was 100-fold more active than the H_2O extract on the δ-opioid assay. Analytical gradient C_{18} Si gel HPLC separation of the MeOH and H_2O extracts and bioassay of collected fractions indicated that the active component(s) eluted in approximately 60% MeOH/40% H_2O in both extracts. A larger amount of the sponge was extracted sequentially with CH_2Cl_2, MeOH, and H_2O, and the latter two extracts were combined. The combined extract was desalted by filtering through C_{18} with H_2O followed by MeOH. The MeOH fraction was purified by C_{18} Si gel HPLC employing a gradient from 55% 0.1 M NH_4OAc(aq)/45% MeOH to 30% 0.1 M aqueous NH_4OAc/70% MeOH. Three active fractions were obtained: an early potently active fraction that was still impure and two later weakly active fractions that were pure bastadin 25 (**1**) and 15-*O*-sulfonatobastadin 11 (**2**). The early eluting active fraction was further purified by C_{18} Si gel HPLC with a gradient from 50% MeOH/50% 0.1 M NH_4OAc(aq) to 60% MeOH/40% 0.1 M NH_4OAc(aq), yielding pure bastadin 26 (**3**).

Bastadin 25 (**1**) was obtained as a white, optically active, amorphous solid. The negative ESIMS of **1** displayed a $[M - H^+]^-$ isotopic cluster centered at *m*/*z* 1032 that could be assigned to a tetrabromo compound. A fragment ion 80 Da smaller than the molecular ion but retaining the same isotopic cluster pattern indicated that the molecule contained a sulfate ester. An accurate mass measurement of the $[M - H^+]^-$ cluster of **1** could not be obtained since the compound decomposed before a measuremnt could be obtained. A molecular formula of $C_{34}H_{26}Br_4N_4O_{12}S$ was assigned to **1** on the basis of low-resolution MS and detailed NMR analysis. The ^{1}H NMR spectrum (Table 1) of bastadin 25 (**1**) contained signals for six aliphatic protons, an oxygenated benzylic proton, 10 olefinic or aromatic protons, and two amide protons (an additional signal integrating for two aromatic protons was observed only when the ^{1}H NMR spectrum was acquired at 50 °C). Interpretation of the ^{1}H NMR spectrum and correlations observed in the gCOSY and gHMQC spectra suggested that **1** possessed two 1,2,4-trisubstituted phenyls, an unsymmetrical 1,2,3,5-tetrasubstituted phenyl, a symmetrical 1,2,3,5-tetrasubstituted phenyl, an ethyleneamido, an ethylamido, and isolated benzylic methylene and methine groups. One of the 1,2,4-trisubstituted phenyls was attached to the ethyleneamido group because a benzylic coupling was observed between δ_H 6.33 (H-6) and δ_H 7.41 (H-8), while the other was attached to an ethylamido group on the basis of a benzylic coupling observed between δ_H 2.73 (20-CH_2) and δ_H 6.70 (H-19). The unsymmetrical 1,2,3,5-tetrasubstituted phenyl group was attached to an isolated methylene on the basis of benzylic couplings observed between δ_H 3.70 (H_2-1) and δ_H 6.20 (H-38) and 7.06 (H-

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Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Spectroscopic Data for **1**− **3**[a]

	bastadin 25 (**1**)		15-*O*-sulfonatobastadin 11 (**2**)		bastadin 26 (**3**)	
position	δ_C[b]	δ_H (*J* in Hz)	δ_C[b]	δ_H (*J* in Hz)	δ_C[b]	δ_H (*J* in Hz)
1	27.4	3.70, s	27.3	3.63, s	27.4	3.48, d (12.6) 3.56, d (12.6)
2	151.6		151.4		150.6	
3	161.3		161.1		161.8	
4		10.12, d (7.2)		10.12, d (7.2)		7.67, t (5.4)
5	123.5	7.29, dd (7.2, 14.4)	123.5	7.25, dd (7.2, 14.4)	42.5	3.09, m 3.34, m
6	110.9	6.33, d (14.4)	110.9	6.41, d (14.4)	70.0	4.64, dd (4.2, 7.2)
6-OH						5.47, br s
7	133.2		133.5		139.5	
8	130.9	7.41, d (1.8)	130.0	7.53, d (1.8)	129.6	7.59, d (1.8)
9	112.7		113.0		111.2	
10	151.5		152.0		152.9	
11	120.1	6.81, d (8.4)	120.3	6.80, d (8.4)	117.2	6.75, d (8.4)
12	123.7	7.52, dd (1.8, 8.4)	124.1	7.41, dd (1.8, 8.4)	126.8	7.12, dd (1.8, 8.4)
14	146.2		146.0		142.6	
15	142.5		142.4		146.8	
16	122.9	7.55, d (8.4)	121.2	7.52, d (8.4)	116.5	6.85, d (8.4)
17	126.2	6.93, dd (1.8, 8.4)	124.5	6.89, dd (1.8, 8.4)	125.5	6.82, dd (1.8, 8.4)
18	134.3		134.4		130.2	
19	118.2	6.70, d (1.8)	118.6	6.62, d (1.8)	119.9	6.72, d (1.8)
20	33.4	2.73, m	33.2	2.74, m	34.0	2.55, m 2.80, m
21	39.4	3.38, m 3.43, m	38.3	3.39, m	n.o.[c]	3.23, m 3.42, m
22		7.95, t (5.4)		7.77, t (5.4)		8.20, t (5.4)
23	162.8		162.8		163.1	
24	153.3		150.0		153.8	
25	64.9	5.86, s	28.8	3.50, s	64.0	5.91, s
26	142.4		137.6		142.2	
27/31	131.3	7.75, br s[d]	133.3	7.45, s	130.5	7.55, br s
28/30	116.8		116.8		n.o.[c]	
29	146.8		145.7		n.o.[c]	
33	144.6		144.8		150.5	
34	142.1		141.9		138.6	
35	109.8		110.0		118.6	
36	127.0	7.06, d (1.8)	126.7	7.06, d (1.8)	126.1	7.05, d (1.8)
37	127.4		127.6		133.3	
38	111.4	6.20, d (1.8)	112.3	6.10, d (1.8)	113.7	6.29, d (1.8)

[a] Spectra recorded in d_6-DMSO at 30 °C. [b] ^{13}C chemical shifts obtained from gHSQC and gHMBC spectra. [c] n.o. = not observed. [d] Observed only at 50 °C.

36). These data suggested that **1** was a bastadin alkaloid and that the broad aromatic signal H-27/30 (δ_H 7.75) observed only at 50 °C in combination with an oxygenated benzylic methine (H-25, δ_H 5.86, δ_C 64.9) was consistent with hydroxylation of one of the methylenes next to an oxime, as this results in restricted rotation about the phenyl group.[15] Correlations observed in a gHMBC spectrum confirmed the placement of the four side chains attached to the phenyl groups. Correlations were observed from the methylene proton (H_2-1) to an oxime carbon (C-2) at δ_C 151.6, an amide carbon (C-3) at δ_C 161.3, and the aromatic carbons C-36, C-37, and C-38. HMBC correlations from H-36 to carbons at δ_C 27.4 (C-1), 109.8 (C-35), 111.4 (C-38), and 142.1 (C-34) and from H-38 to carbons at δ_C 27.4 (C-1), 127.1 (C-36) 142.2 (C-34), and 144.6 (C-33) were consistent with a 1-methylene-3-bromo-4,5-dioxy-substituted phenyl group being present in the molecule. Furthermore the ^{13}C chemical shift for the methylene C-1 was indicative of *E* geometry for the adjacent oxime.[1−15] A weak correlation was observed from the olefinic proton (H-5) to the amide carbon C-3, indicating that the ethyleneamido nitrogen formed an amide bond with the amide carbonyl C-3. HMBC correlations from the aromatic protons H-8 and H-12 to an oxygenated quaternary carbon at δ_C 151.5 (C-10) and from H-8 and H-11 to an upfield quaternary carbon at δ_C 112.7 (C-9) indicated that the phenyl attached to the ethyleneamido group was substituted at C-9 by a bromine and C-10 by an oxygen. The ethylamido group was attached to a phenyl that was substituted by two oxygens on the basis of $^3J_{CH}$ correlations observed between H-19 and H-17 and an oxygenated quaternary carbon at δ_C 142.5 (C-15), while H-19 and H-16 correlated to a second oxygenated quaternary carbon at δ_C 146.2 (C-14). The oxygenated methine, H-25, correlated to an oxime carbon at δ_C 153.3 (C-24), an amide carbonyl carbon at δ_C 162.8 (C-23), and aromatic carbons at δ_C 131.3 (C-27/31) and 142.2 (C-26). These aromatic carbon chemical shifts were very similar to those observed in bastadin 24, a molecule that also has an oxymethine substitutent at C-25, suggesting that **1** contained a 2,6-dibromophenoxy group attached to the oxymethine.[15] The remaining two aromatic carbon signals C-28/30 and C-29 showed no HMBC correlations but had almost identical chemical shifts to those observed in bastadin 24. HMBC correlations from H-21a, H-21b, H-22, and H-25 to an amide carbonyl carbon at δ_C 162.8 (C-23) provided evidence that a second amide bond was present between N-24 and C-23. Comparison of the ^{13}C NMR data for carbons in rings C and D with those for bastadins 4, 8, 9, 11, and 24 showed good agreement, suggesting that an ether linkage between C-29−C-33 was present in **1**. On biogenetic grounds the most probable linkage for the second ether was between C-10 and C-14 since the oxygen *para* to the side chain should be present in both tyramines and oxidative coupling normally occurs through coupling via a phenoxide radical and a radical generated at the position *ortho* to the phenol of a second aromatic group. Comparison of the ^{1}H and ^{13}C NMR data for ring B in **1** with those of bastadins 9 and 11, both of which contain a C-10−C-14 ether linkage, however showed significantly differences.[3] In particular the proton and carbon resonances for position 16 were downfield shifted 0.7 and 0.6 ppm, respectively, in **1** compared to those in bastadins 9 and 11. However, since bastadin 25 contains a sulfate ester and since it has been reported that the carbons and protons *ortho* to the site of sulfonation undergo a significant downfield shift, the site of sulfonation was proposed to be O-15, and therefore the ether linkage was between C-10 and C-14 in **1**.[19]

Compound **2**, isolated as a white, amorphous solid, displayed a molecular ion cluster at *m*/*z* 1017 $[M - H^+]^-$ and at *m*/*z* 937 $[M - SO_3H]^-$ in the (−)-LRESIMS spectra. Similar to **1** a HRMS measurement could not be obtained for **2** because it decomposed prior to HRMS analysis, and so a molecular formula of $C_{34}H_{26}Br_4N_4O_{11}S$ was assigned to **2** on the basis of MS and detailed NMR data analysis. The ^{1}H NMR spectrum of **2** (Table 1) was very similar to that of **1** except that the oxymethine in **1** was replaced with a methylene at δ_H 3.50 and a sharp, two-proton singlet was observed at δ_H 7.45 in **2**. This suggested that **2** was the 25-deoxy derivative of **1** and the 15 sulfate of bastadin 11, and this was confirmed from analysis of correlations observed in gCOSY, gHMQC, and gHMBC spectra for **2**. The report that the *ipso* and *ortho* carbons are shifted upfield and downfield, respectively, upon sulfonation suggests that the ^{13}C chemical shifts reported for C-15 and C-14 in bastadin 11 should be reversed (i.e., C-14 δ_C 143.0.and C-15 δ_C 146.7).[3,19]

Bastadin 26 (**3**) was isolated in low yield as a colorless, optically active gum. Negative HRESIMS analysis of the $[M - H^+]^-$ ion at *m*/*z* 1051.8037 allowed a molecular formula of $C_{34}H_{28}Br_4N_4O_{13}S$ to be assigned to **3**. A fragment ion centered at *m*/*z* 971 due to loss of SO_3 pointed to the presence of an *O*-sulfate ester. The ^{1}H NMR spectrum of **3** (Table 1) possessed many similarities to that of **1**, the major differences being the replacement of the olefinic protons associated with the ethyleneamido group in **1** by a methylene, an oxygenated methine, and a saturated amide proton in **3**. Correlatons observed in a gCOSY spectrum indicated that C-6 was hydroxylated since H-6 (δ_H 4.64) correlated to an aromatic proton at δ_H 7.59 (H-8), methylene protons at δ_H 3.09 and 3.34, and an exchangeable proton at δ_H 5.47 (6-OH). The methylene protons H-5a and H-5b also correlated to an amide proton at δ_H 7.67 (H-4). Small sample

size precluded the acquisition of a ^{13}C NMR spectrum, but correlations observed in HMQC and HMBC spectra allowed the chemical shifts of all but four of the carbons to be assigned. The same substitution pattern for the four aromatic groups as that found in **1** was deduced from analysis of correlations observed in gCOSY, gHMQC, and gHMBC spectra. The chemical shifts of signals associated with ring B were almost identical with those of bastadin 11, inferring the absence of an *O*-sulfonate at C-15.[3] The chemical shifts of the carbon signals in ring D in **3** were also different from those in bastadin 25 (**1**) since C-33 and C-35 were 4.9 and 8.8 ppm downfield shifted, respectively, and C-34 was 3.5 ppm upfield shifted. These data affirmed that bastadin 26 was the 34-*O*-sulfonato-6,25-dihydroxy derivative of bastadin 9. The small amount of bastadin 26 that was isolated did not allow stereochemical investigations to be undertaken, and the relative and absolute configurations of the two stereogenic centers, C-6 and C-25, remain to be assigned.

Bastadin 25 (**1**), 15-*O*-sulfonatobastadin 11 (**2**), and bastadin 26 (**3**) were tested for their ability to displace [^{3}H]DPDPE from δ-opioid receptors in membranes obtained from guinea pig forebrain. Bastadin 26 (**3**) was the most active compound, displaying an IC_{50} value of 206 nM and a K_i of 100 nM. The reference compound naloxone displayed a K_i of 30 nM. Bastadin 25 (**1**) and 15-*O*-sulfonatobastadin (**2**) inhibited the binding of [^{3}H]DPDPE only at 100 μM (86% and 87%, respectively). The three compounds were also tested for their ability to displace [^{3}H]DAMGO binding to μ-opioid receptors in membranes from guinea pig forebrain, and **1** and **2** inhibited binding by 39% and 57%, respectively, at 100 μM, while **3** was inactive at this dose. The reference compound naloxone had a K_i of 3.9 nM. The three compounds were also tested for their ability to displace [^{3}H]U69593 binding to κ-opioid receptors in membranes from guinea pig cerebellum, and all compounds were inactive at concentrations up to 100 μM. The reference compound naloxone had a K_i of 2.6 nM. Bastadin 26 (**3**) is therefore an interesting lead compound because it is a potent δ-opioid binder and is >1000 times more selective for the δ-opioid receptor than the μ- and κ-opioid receptors. Because **3** was isolated only in very small amount, there was not sufficient quantity of compound to determine if it is an agonist or antagonist of the δ-opioid receptor.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter (10 cm cell). UV spectra were recorded on a CAMSPEC M501 UV/vis spectrophotometer, and IR spectra were recorded on a Bruker Tensor 27 spectrometer. NMR spectra were recorded on a Varian Inova 600 MHz NMR spectrometer. Samples were dissolved in d_6-DMSO, and chemical shifts were calculated relative to the DMSO solvent peak (δ_H 2.49 and δ_C 39.5). 2D NMR spectra were recorded at 30 °C using standard Varian pulse sequences gCOSY, gHMQC, and gHMBC. LRESIMS were recorded on a Fisons VG platform II mass spectrometer in negative mode. HRESIMS were recorded on a Mariner Biospectrometry TOF workstation using negative electrospray ionization, mobile phase 1:1 MeOH/H_2O. Alltech Davisil 30−40 μm 60 Å C_{18} bonded Si gel was used for desalting. HPLC separations were achieved using a Rainin microsorb C_{18} bonded Si gel analytical column (3 μm, 4.6 mm ×50 mm) and a Rainin microsorb C_{18} bonded Si gel semipreparative column (3 μm, 10 mm ×50 mm). All solvents used were Omnisolv HPLC grade.

Animal Material. The sponge *Ianthella flabelliformis* was collected by scuba (−19 m) in November 1993, from Darwin Harbor. A voucher specimen, G303371, is deposited at the Queensland Museum.

Extraction and Isolation. The freeze-dried and ground sponge (200 mg) was exhaustively extracted with CH_2Cl_2 (8 mL), MeOH (8 mL), and H_2O (8 mL). The three extracts were dried and resuspended in DMSO (800 μL), and aliquots were serially diluted and tested for δ-opioid binding affinity. The MeOH and H_2O extracts showed bioactivity (92% and 40%, respectively, at 5 μg/mL). The MeOH and H_2O extracts were individually analyzed by analytical C_{18} HPLC using a linear gradient from H_2O to MeOH at a flow rate of 1 mL/min over 8 min, collecting 40 × 12 s fractions into a 96-well microtiter plate. The fractions were tested for δ-opioid binding affinity, and a band of fractions eluting around 60% MeOH showed bioactivity. A second sample of ground sponge (1.0 g) was extracted sequentially with CH_2Cl_2 (50 mL), MeOH (50 mL), and H_2O (50 mL), and the MeOH and H_2O extracts were combined and evaporated to dryness, yielding a dark brown gum (285 mg). The combined extract was redissolved in 10% MeOH/90% H_2O and filtered through C_{18} Si gel (200 mg), eluting with H_2O (10 mL) followed by MeOH (20 mL). Both fractions were tested for δ-opioid binding affinity, and only the MeOH fraction retained activity. The MeOH fraction (48 mg) was purified by semipreparative C_{18} Si gel HPLC eluting with a gradient from 55% NH_4OAc(aq)/45% MeOH to 30% NH_4OAc(aq)/70% MeOH over 7 min. Six fractions were collected, and fractions 3, 4, and 5 were bioactive. Fraction 4 was pure bastadin 25 (**1**) (10.6 mg, 1.06%), and fraction 5 was pure 15-*O*-sulfonatobastadin 11 (**2**) (5.4 mg, 0.54%). Fraction 3 (3.2 mg) was still impure and was further separated by C_{18} HPLC with a gradient from 50% NH_4OAc(aq)/50% MeOH to 40% NH_4OAc(aq)/60% MeOH over 6 min, yielding pure bastadin 26 (**3**) (0.7 mg, 0.07%).

Bastadin 25 (1): white, amorphous solid; $[\alpha]^{17}_D$ +8.5 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 229 (5.21), 252 (5.37), 313 (4.81), 335 (4.54) nm; IR (KBr) ν_{max} 3395 br, 2924, 1702, 1660, 1632, 1606, 1512, 1467, 1280, 1248, 1185, 1124, 1033 cm^{-1}; ^1H NMR (600 MHz, d_6-DMSO) and ^{13}C NMR (150 MHz, d_6-DMSO) see Table 1; (−)-LRESIMS 1029, 1031, 1033, 1035, 1037, 949, 951, 953, 955, 957.

15-*O*-Sulfonatobastadin 11 (2): white, amorphous solid; UV (MeOH) λ_{max} (log ε) 227 (4.69), 278 (4.24), 335 (3.5) nm; IR (KBr) ν_{max} 3369 br, 2945, 1700, 1665, 1492, 1452, 1426, 1248, 1123, 1003 cm^{-1}; ^1H NMR (600 MHz, d_6-DMSO) and ^{13}C NMR (150 MHz, d_6-DMSO) see Table 1; (−)-LRESIMS 1013, 1015, 1017, 1019, 1021, 933, 935, 937, 939, 941.

Bastadin 26 (3): colorless gum; $[\alpha]^{25}_D$ +59 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 221 (5.29), 272 (4.59) nm; ^1H NMR (600 MHz, d_6-DMSO) and ^{13}C NMR (150 MHz, d_6-DMSO) see Table 1; (−)-LRESIMS 1047, 1049, 1051, 1053, 1055, 967, 969, 971, 973, 975; (−)-HRESIMS *m/z* 1051.8037 $[M - H^+]^-$ (calcd for $C_{34}H_{27}{}^{79}Br_2{}^{81}Br_2N_4O_{13}S$, 1051.8076).

Preparation of Membranes for δ-, μ-, and κ-Opioid Receptor Binding Assays. Guinea pig brains were obtained from the Institute of Medical and Veterinary Science, South Australia, and were IMVS colored, adult, and mixed sex. Whole brains were frozen at −80 °C. Frozen guinea pig brains were rehydrated in 50 mM Tris·HCl (pH 7.4 at 4 °C), then dissected. For the δ-opioid and μ-opioid assays forebrains were pooled. For the κ-opioid assay cerebellums were pooled. Tissue was homogenized in a 7-fold volume of 50 mM Tris·HCl (pH 7.4 at 4 °C) and centrifuged at 20000*g* for 20 min at 4 °C. Pellets were resuspended in buffer, incubated at 37 °C for 45 min to remove endogenous ligands, then washed twice in buffer. Pellets were pooled and resuspended at 200 mg/mL wet weight of tissue and stored at −70 °C until used. An aliquot of membrane was used for bicinchoninic protein estimation using bovine serum albumen in Dulbecco's phosphate-buffered saline as a standard.

Receptor Binding Assay Methods for δ-, μ-, and κ-Opioid Receptors. Incubations were performed in 210 mL final volume containing (μ-opioid assay) 1 nM [^{3}H]DAMGO (60 Ci/mmol) and 10 μM naloxone for determination of nonspecific binding and reactions started by the addition of 315 μg of membrane protein; (δ-opioid assay) 1 nM [^{3}H]DPDPE (40.7 Ci/mmol) and 10 μM naloxone for determination of nonspecific binding and reaction started by the addition of 315 μg of membrane protein; (κ-opioid assay) 1 nM [^{3}H]U69593 (64.0 Ci/mmol) and 10 μM naloxone for determination of nonspecific binding and reactions started by the addition of 190 μg of membrane protein. Incubation was for 90 min at rt. Reactions were stopped by rapid filtration onto glass fiber B filter mats for μ- and δ-opioid assays and glass fiber B filter mats presoaked with poyethylenimine (0.01%) for the κ-opioid assay. Filter mats were dried and then counted in a Betaplate liquid scintillation counter. Potency of compounds was estimated by screening or from concentration−response curves. For screening, compounds were solubilized in 100% DMSO and tested at 100 μM, with a final DMSO concentration of 2%. For concentration−response curves, experiments were performed with 11 treatment levels and a final DMSO concentration of 2%. All determinations were completed in duplicate. The radioligands [^{3}H]DAMGO and [^{3}H]U69593 were obtained from Amersham, and [^{3}H]DPDPE was from DuPont NEN.

Guinea pig brains were purchased from the Institute of Medical and Veterinary Science, South Australia.

Data Analysis. Counts per minute were averaged, and % activity was calculated as 100 − % control.

$$\%\ \text{control} = \frac{\text{total binding(compound)} - \text{nonspecific binding(compound)}}{\text{total binding(control)} - \text{nonspecific binding(control)}} \times 100$$

The potency (IC_{50}, K_i) was determined by nonlinear least-squares analysis, and model testing by analysis of variance (GraphPad Inplot 4.02).

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Supporting Information Available: 1D and 2D NMR spectra for bastadin 25, 15-*O*-sulfonatobastadin 11, and bastadin 26. A δ-opioid receptor concentration−response curve for bastadin 26. This material is available free of charge via the Internet at http://pubs.acs.org.

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