Hasubanan Alkaloids with δ-Opioid Binding Affinity from the Aerial Parts of *Stephania japonica*

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Two new (**1** and **2**) and six known hasubanan alkaloids (**3**−**8**) and one morphinane alkaloid (**9**) were isolated from the leaves of the North Queensland rainforest vine *Stephania japonica*. The structures of **1** and **2** were determined by interpretation of their 1D and 2D NMR spectra. The hasubanan alkaloids showed affinity for the human δ-opioid receptor with IC_{50} values ranging from 0.7 to 46 μM. The compounds were also tested for their affinity to μ- and κ-opioid receptors and shown to be inactive against κ-opioid receptors, but were of similar potency against the μ-opioid receptor.

Chronic pain is a condition that causes suffering for a large number of people living with cancer, arthritis, back pain, and other conditions.[1] A recent "Gallup" survey in the United States found that 40% of the adult population experienced pain on a daily basis,[2] and in Australia ∼20% of the adult population cope with chronic pain, with 10% suffering lower back pain.[3] The occurrence of major side effects including dependence, respiratory depression, and muscle rigidity associated with drugs that modulate the μ-opioid receptor has led to the search for analgesics active against other pain targets. Agonists of the δ-opioid receptor have been shown in animal models to provide antinociception without these undesirable side effects.[4] We have screened a large natural product extract library comprising those from plants, marine organisms, insects, and soil microbes against a number of pain targets, including thyrotropin-releasing hormone receptor 2,[5,6] sensory neuron-specific G protein coupled receptor,[7] and the three opioid receptors (δ, κ, μ),[8−12] and this has yielded a number of active natural products. Screening against the human δ-opioid receptor, in particular, has been very productive, with more than 10 indolizidine alkaloids being reported to date.[8−12] In continuation of our interest in finding natural products with affinity for the δ-opioid receptor, an extract from the aerial parts of the Australian rainforest vine *Stephania japonica* (Thunb.) Miers (Menispermaceae) was targeted for chemical evaluation, as it displayed human δ-opioid receptor affinity. In this paper, we describe the bioassay-guided isolation, structure determination, and human δ-opioid receptor affinity of two new hasubanan alkaloids, *N*-methylstephisoferulin (**1**) and 6-cinnamoylhernandine (**2**), together with the known hasubanan alkaloids stephalonine E (**3**), aknadinine (**4**), longanine (**5**), aknadilactam (**6**), *N*-methylstephuline (**7**), and prostephabyssine (**8**) and the known morphinane alkaloid sinococuline (**9**), which we have identified as the bioactive components from *S. japonica*.

The ground aerial parts of *S. japonica* were exhaustively extracted with CH_2Cl_2 and MeOH and the combined extracts partitioned between CH_2Cl_2 and 2 M H_2SO_4. The aqueous layer was basified with 27% NH_4OH and partitioned with CH_2Cl_2. The second CH_2Cl_2 fraction, which showed affinity for the δ-opioid receptor, was chromatographed on aminopropyl-bonded Si gel with a stepped gradient from CH_2Cl_2 to MeOH. Aknadinine (**4**) eluted early followed by stephalonine E (**3**), longanine (**5**), *N*-methylstephisoferulin (**1**), and sinococuline (**9**), respectively. A mixture of several alkaloids eluted just after **3**, and this was further purified by HPLC on aminopropyl-bonded Si gel eluting with 1% MeOH/99% CH_2Cl_2, yielding three fractions. The first fraction was a mixture of two compounds. The second fraction was pure 6-cinnamoylhernandine (**2**), and the third fraction was pure prostephabyssine (**8**). The first fraction was partitioned between 2 M H_2SO_4 and $CHCl_3$. The $CHCl_3$ layer contained pure aknadilactam (**6**). The aqueous layer was basified with NH_4OH and partitioned with $CHCl_3$. The $CHCl_3$ layer contained pure *N*-methylstephuline (**7**). The structures of the known compounds **3**,[13] **4**,[14,15] **5**,[16] **6**,[15,17] **7**,[18] **8**,[19] and **9**[20] were assigned

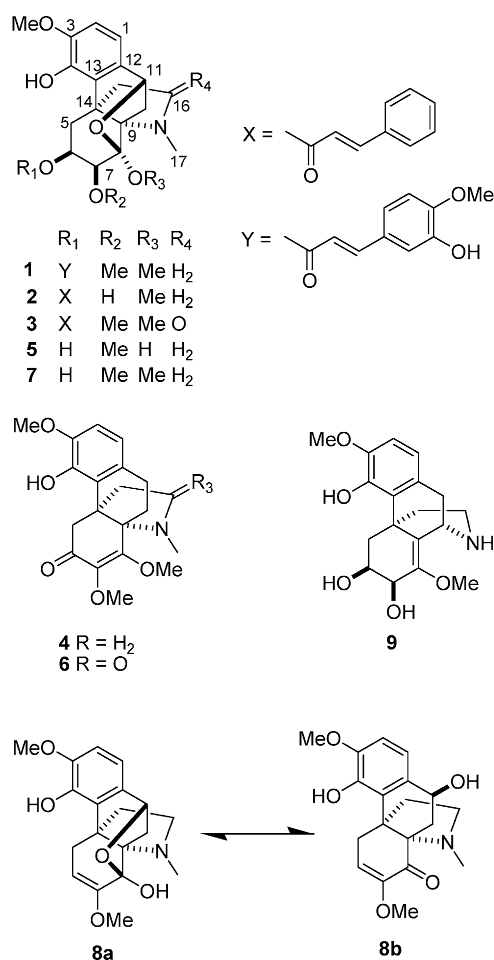

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Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data for Compounds **1** and **2**[a]

	1		**2**	
position	δ_C[b]	δ_H (J in Hz)	δ_C[b]	δ_H (J in Hz)
1	116.0	6.67 d (8.6)	115.3	6.66 d (8.4)
2	106.6	6.44 d (8.6)	106.8	6.40 d (8.4)
3	146.8		146.8	
OCH_3-3	55.2	3.35 s	55.5	3.32 s
4	143.2		143.2	
OH-4		5.70 s		5.70 s
5	31.8	2.12 dd (2.4, 15.0)	31.2	2.20 dd (2.4, 15.6)
		3.14 dd (3.6, 15.0)		3.20 dd (3.6, 15.6)
6	67.9	5.39 ddd (2.4, 3.6, 3.6)	73.1	5.14 ddd (2.4, 3.6, 3.6)
7	81.5	3.78 d (3.6)	72.5	4.20 d (3.6)
OCH_3-7	57.2	3.41 s		
8	102.9		102.6	
OCH_3-8	51.4	3.57 s	51.8	3.60 s
9	76.3		75.8	
10	28.8	1.52 d (10.8)	29.7	1.57 d (10.8)
		2.68 dd (6.6, 10.8)		2.75 dd (6.6, 10.8)
11	77.2	4.96 d (6.6)	76.6	4.95 d (6.0)
12	133.8		133.6	
13	127.7		127.9	
14	48.8		42.4	
15	34.5	1.86 ddd (7.8, 10.8, 13.2)	35.3	1.84 m
		2.50 ddd (3.6, 10.8, 12.6)		2.51 m
16	54.3	2.46 ddd (3.6, 9.0, 12.6)	53.8	2.50 m
		3.39 m		3.40 m
NCH_3-17	38.5	2.57 s	38.1	3.07 s
1′	167.0		165.9	
2′	116.9	5.33 d (16.2)	117.3	5.53 d (15.6)
3′	142.4	7.06 d (16.2)	142.9	7.12 d (15.6)
4′	128.2		134.0	
5′	113.0	6.91 d (1.8)	127.9	7.29 m
6′	145.2		128.7	7.29 m
6′-OH		5.90 brs		
7′	147.9		130.7	7.29 m
7′-OCH_3	55.9	3.94 s		
8′	110.2	6.81 d (8.4)	128.7	7.29 m
9′	121.4	6.86 dd (1.8, 8.4)	127.9	7.29 m

[a] Spectra recorded in d_6-DMSO at 30 °C. [b] ^{13}C NMR chemical shifts obtained from gHSQC and gHMBC spectra.

from analysis of their 2D NMR spectroscopic data and by comparison with literature data.

N-Methylstephisoferulin (**1**) was obtained as an optically active yellow gum. Accurate mass measurement of the $[M + H]^+$ ion at *m/z* 554.2400 in the (+) HRESIMS allowed a molecular formula of $C_{30}H_{35}NO_9$ to be assigned to **1**. An IR absorption band at 1686 cm^{-1} suggested the presence of an α,β-unsaturated ester carbonyl, and this was supported by a UV absorbance at 324 nm.

The ^{1}H NMR spectrum of *N*-methylstephisoferulin (**1**) (Table 1) in $CDCl_3$ exhibited signals for two *ortho*-coupled aromatic doublets (J = 8.6 Hz) (δ_H 6.44, 6.67), three aromatic protons assigned to a 1,3,4-trisubstituted aromatic ring (δ_H 6.81, d, J = 8.4 Hz; 6.86 dd J = 1.8, 8.4 Hz; 6.91, d, J = 1.8 Hz), two doublets (J = 16.2 Hz) assigned to a *trans*-disubstituted double bond (δ_H 5.33, 7.06), three oxygenated methine protons (δ_H 5.39, 4.96, 3.78), four methoxy singlets (δ_H 3.35, 3.41, 3.57, 3.94), an *N*-methyl (δ_H 2.57), two phenolic protons (δ_H 5.70, 5.90), and eight aliphatic multiplets. Correlations observed in the gHSQC spectrum allowed the eight aliphatic multiplets to be assigned to four methylene groups, of which one was substituted by a nitrogen (δ_H/δ_C 2.46, 3.39/54.3). Three aliphatic proton spin systems, CH_2CH_2N, CH_2CHO, and $CH_2CHORCHOR$, were deduced from correlations observed in a gCOSY spectrum. A 4-methoxy-3-hydroxycinnamate unit was assigned from HMBC correlations from δ_H 7.06 to a carbonyl carbon at δ_C 167.0 and the aromatic protonated carbons at δ_C 113.0 (C-5′) and 121.4 (C-9′), while the aromatic protons at δ_H 6.86 and 6.91 and the methoxy protons at δ_H 3.94 showed $^{3}J_{CH}$ correlations to an oxygenated aromatic carbon at δ_C 147.9. The other aromatic proton at δ_H 6.81 showed a $^{3}J_{CH}$ correlation to a second oxygenated aromatic carbon at δ_C 145.2. HMBC correlations from the oxygenated methine proton at δ_H 4.96 (H-11) to aromatic carbons at δ_C 116.0, 127.7, and 133.8 indicated that this proton is benzylic and *ortho* to H-1. The attached phenyl group was deduced to have a 1-methoxy-2-hydroxy-3,4-disubstitution pattern since correlations were observed from δ_H 6.67 (H-1) to the carbon at δ_C 127.7, an oxygenated aromatic carbon at δ_C 146.8 (which also correlated to a methoxy proton at δ_H 3.35), and the oxygenated methine carbon at δ_C 77.2 (C-11), while δ_H 6.44 (H-2) correlated to the aromatic quaternary carbon at δ_C 133.8 and a second oxygenated aromatic carbon at δ_C 143.2. A *N*-methylpyrrolidine ring was deduced from correlations between the aminomethylene proton H-16b to two aliphatic quaternary carbons at δ_C 76.3 (C-9) and δ_C 48.8 (C-14) and from the *N*-methyl protons at δ_H 2.57 to the methylene carbon at δ_C 54.3 (C-16) and the quaternary carbon C-9. The 2,3-dioxygenated propyl moiety was attached to the pyrrolidine at C-14, as the proton at δ_H 3.14 (H-5b) correlated to C-9 and C-14. A methoxy group was attached to C-7 since a correlation was observed between δ_H 3.41 and δ_C 81.5 (C-7). A tetrahydronaphthalene was also attached to the pyrrolidine at C-9/C-14, as the benzylic proton H-11 correlated to C-9, the methylene protons H-10a and H-10b correlated to C-9 and C-14, and the methylene protons H-5a and H-5b correlated to the aromatic quaternary carbon C-13. The H-10a, H-11, and the methoxy protons at δ_H 3.57 all correlated to a carbon at δ_C 102.9, suggesting it is a ketal carbon and that a tetrahydrofuran ring could be defined by an oxygen bridge between C-8 and C-11. The downfield chemical shift of H-6 suggested that the cinnamate ester is attached to C-6. The molecular formula dictated that **1** possesses another ring, and a bond between C-8 and C-9 provided the final linkage to define the planar structure. The fused tetracyclic nature of the molecule meant that only one relative configuration at C-8, C-9, C-11, and C-14 could be defined. The relative configurations of the two remaining stereogenic centers in the molecule, C-6 and C-7, were deduced from analysis of proton coupling constants and ROESY correlations. Small couplings between H-5a and H-5b and H-6 indicated that H-6 is equatorial. A ROESY correlation between H-7 and H-5a suggested that they are diaxial and that therefore the OMe-7 group is equatorial. The significant upfield chemical shift of OMe-3 (δ_H 3.35) and H-2 (δ_H 6.44) was a result of shielding from the phenyl of the axial cinnamate ester group attached at C-6. Compound **1** is therefore the *N*-methyl derivative of stephisoferulin.[18] The absolute configuration of **1** was determined by comparison of CD spectra obtained for all of the isolated hasubanan alkaloids. In particular, the absolute configuration of aknadinine (**4**) has been determined by X-ray crystallography of its 4-brosylate ester.[14] Therefore, comparison of its CD spectrum provided evidence to assign the absolute configuration of other hasubanan alkaloids within the series. All of the compounds showed negative Cotton effects at 260−290 nm. The CD spectra for the six known hasubanan alkaloids (**4**−**8**) were all very similar to each other, and to compound **1**, and this suggested that they all possess the same absolute configuration about the tetracyclic core. The CD spectrum of **1** showed a negative Cotton effect at 285 nm, consistent with an absolute configuration of 6*S*, 7*S*, 8*R*, 9*S*, 11*S*, and 14*S*.

6-Cinnamoylhernandine (**2**) was isolated as a colorless, optically active gum. Accurate mass measurement of the pseudomolecular ion at *m/z* 494.2172 in the (+) HRESIMS allowed a molecular formula of $C_{28}H_{31}NO_7$ to be assigned to **2**. The ^{1}H NMR spectrum of **2** (Table 1) was very similar to that of **1**. The only major differences were the replacement of the signals for a 4-methoxy-3-hydroxycinnamate ester with those for a cinnamate ester, one less methoxy group signal, the downfield chemical shift of H-7

Table 2. Opioid Binding Affinity for Compounds **1**−**9**

compound	δ-opioid receptor[a] IC_{50} μM	μ-opioid receptor[b] % activity at 10 μM	κ-opioid receptor[c] % activity at 10 μM
6-cinnamoyl-*N*-methylstephasunoline (**1**)	11.3	56	0
N-methylstephisoferulin (**2**)	2.5	54	0
6-cinnamoylhernandine (**3**)	5.1	74	0
aknadinine (**4**)	46.0	68	0
longanine (**5**)	0.7	68	0
aknadilactam (**6**)	17.0	49	0
N-methylstephuline (**7**)	2.1	68	0
prostephabyssine (**8**)	5.5	67	0
sinococuline (**9**)	37.0	54	0

[a] Positive control DPDPE, IC_{50} = 1.2 nM. [b] Positive control DAMGO, IC_{50} = 2.0 nM. [c] Positive control U69593, IC_{50} = 8.0 nM.

from δ_H 3.78 to 4.20, and the upfield shift of H-6 from δ_H 5.39 to 5.14. Analysis of correlations observed in the gHSQC spectrum (Table 1) indicated that the chemical shift of the protonated carbon C-6 (δ_C 73.1) was 5.2 ppm downfield and C-7 (δ_C 72.5) 9.0 ppm upfield relative to **1**. This suggested that **2** differs from **1** by the replacement of the OMe-7 group with a OH-7 group and the 3-hydroxy-4-methoxycinnamate by a cinnamate. Analysis of correlations observed in the gCOSY and gHMBC spectra of **2** confirmed the planar structure. The upfield chemical shifts of OMe-3 and H-2 were almost identical to the same protons in **1** and indicated that the 6-cinnamate is axial, while a ROESY correlation between H-7 and H-5a indicated that OH-7 is equatorial. Compound **2** is therefore the 6-cinnamate ester of hernandine.[21] A negative Cotton effect at 280 nM in the CD spectrum indicated that the absolute configuration of the six stereogenic centers in 6-cinnamoylhernandine (**2**) are 6*S*, 7*S*, 8*R*, 9*S*, 11*S*, and 14*S*.

Hasubanan alkaloids appear to be taxonomic markers for plants from the family Menispermaceae and more particularly plants from the genus *Stephania*, since nearly all of the over 50 compounds of this class isolated to date have been isolated from *Stephania* species.[22]

Screening the alkaloids for δ-, μ-, and κ-opioid binding affinity (Table 2) highlighted an interesting structure−activity relationship. This is the first report on the opioid binding affinity for hasubanan alkaloids. Longanine (**5**) was the most potent binder to the δ-opioid receptor, with an IC_{50} value of 700 nM. Methylation of C-8 reduced δ binding by a factor of 3, while additional esterification at C-6 or removing the C-6 alcohol did not reduce δ binding affinity any further. Converting the pyrrolidine to a pyrrolidone halved the δ binding affinity. Converting the alcohol at C-6 to a ketone and removing the ether linkage between C-8 and C-11 significantly reduced binding affinity by over an order of magnitude. The orientation of the phenyl group relative to the octahydroindole must therefore significantly affect binding affinity since removing the ether linkage leads to a rotation of the phenyl group. The hasubanan alkaloids (**1**−**8**) exhibit comparable binding affinity for both the μ- and δ-opioid receptors while showing no affinity for the κ-opioid receptor. The hasubanan alkaloids are only weakly active when compared to morphinan opioid ligands, and this is probably related to the absolute configuration of the phenyl ring relative to the nitrogen, being opposite of that observed for potently active morphinan ligands.[23] The morphinan sinococuline (**9**) was only weakly active, but this was not surprising since it has an absolute configuration opposite that of synthetic morphinan opioid ligands such as MCL 101.[23] It has been observed that (+) isomers of a number of morphinan opioid ligands consistently show over 100-fold weaker binding affinity compared to the corresponding (−) isomers.[24] This observation also is in agreement with the significantly weaker opioid activity observed for (+)-morphine, which has been recorded to be 10 000 times weaker than (−)-morphine.[25]

Experimental Section

General Experimental Procedures. All solvents used were Omnisolv HPLC grade. 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. CD spectra were recorded on a JASCO J-715 spectropolarimeter. NMR spectra were recorded on a Varian Inova 600 MHz NMR spectrometer. Samples were dissolved in $CDCl_3$, and chemical shifts were calculated relative to the $CDCl_3$ solvent peak (δ_H 7.26 and δ_C 77.0). 2D NMR spectra were recorded at 30 °C using standard Varian pulse sequences (gCOSY, gHMQC, gHSQC, gHMBC, ROESY). HRESIMS were recorded on a Bruker Daltonics Apex III 4.7e Fourier-transform mass spectrometer. Alltech Davisil 30−40 μm 60 Å aminopropyl-bonded Si gel was used for MPLC separations. HPLC separations were achieved using a YMC aminopropyl-bonded Si semipreparative column (5 μm, 10 mm × 150 mm). Trizma base, bovine serum albumin (BSA), unlabeled naloxone, unlabeled DAMGO ([D-Ala^2, N-Me-Phe^4, Gly^5-ol-] enkephalin), (D-Pen 2,5)-enkephalin (DPDPE), $MgCl_2$, EDTA, and polyethylenimine (PEI) were from Sigma Chemical Company; [^{3}H]-naloxone (60 Ci/mmol) and [^{3}H]-U69593 (40 Ci/mmol) were obtained from NEN Life Science Products, Inc. Unlabeled U69593 ((5α,7α,8β)-(+)-*N*-methyl-*N*-(7-[1-pyrrolidiny]-1-oxaspiro[4.5]dec-8-yl)benzeneacetamide) was from ICN. Whatman glass fiber B filter mats (GF/B), sample bags, Betaplate scintillant, and microplates (isoplates) were from Wallac Oy (Turku, Finland). Wheatgerm agglutinin-coated PVT-SPA beads and [^{125}I]-Deltorphin II (∼2000 Ci/mmol) were from Amersham. HEK cell membranes expressing recombinant human δ-opioid receptors were provided in batches from AstraZeneca R and D Montreal. Chinese hamster ovary (CHO) cell membranes expressing recombinant human μ-opioid receptor were obtained in batches from NEN Life Science Products, Inc., and human κ-opioid receptor containing membranes from human embryonic kidney (HEK) 293 cells were provided by AstraZeneca R and D Mölndal.

Plant Material. Aerial parts of *S. japonica* were collected by one of the authors (P.I.F.) in November 1997, in State Forest 144 on Mount Windsor Tableland, far North Queensland. A voucher specimen, AQ604793, is deposited at the Queensland Herbarium.

Extraction and Isolation. The ground aerial parts (30 g) of *S. japonica* were exhaustively extracted with CH_2Cl_2 (3 × 200 mL) and MeOH (3 × 200 mL) and the combined extracts (5.2 g) partitioned between CH_2Cl_2 (300 mL) and 2 M H_2SO_4 (300 mL). The aqueous layer was basified with 27% NH_4OH (100 mL) and partitioned with CH_2Cl_2 (3 × 300 mL). The second CH_2Cl_2 fraction (435 mg), which showed affinity for the δ-opioid receptor, was chromatographed on aminopropyl-bonded Si gel (20 mm ×200 mm), with a stepped gradient from CH_2Cl_2 to MeOH, and 21 fractions (100 mL) were collected. Pure aknadinine (**4**) (13.7 mg, 0.046% dry weight) eluted in fractions 2 and 3. 16-Oxo-6-cinnamoyl-*N*-methylstephasunoline (**3**) (0.7 mg, 0.002% dry weight) eluted in fraction 9, longanine (**5**) (5.2 mg, 0.017% dry weight) eluted in fraction 11, *N*-methylstephisoferulin (**1**) (6.4 mg, 0.021% dry weight) eluted in fraction 14, and sinococuline (**9**) (17.7 mg, 0.059% dry weight) eluted in fractions 18 and 19. A mixture of several alkaloids eluted in fraction 10, and this was further purified by HPLC on aminopropyl-bonded Si gel, eluting with 0.5% MeOH/99.5% CH_2Cl_2, yielding three fractions. The first fraction was a mixture of two compounds. The second fraction was pure 6-cinnamoylhernandine (**2**) (1.6 mg, 0.005% dry weight), and the third fraction was pure prostephabyssine (**8**) (1.7 mg, 0.006% dry weight). The first fraction was partitioned between 2 M H_2SO_4 (10 mL) and $CHCl_3$ (2 × 10 mL). The $CHCl_3$ layer was pure aknadilactam (**6**) (1.2 mg, 0.0004% dry weight). The aqueous layer was basified with 27% aqueous NH_4OH (20 mL) and partitioned with $CHCl_3$ (3 × 20 mL). The $CHCl_3$ layer was pure *N*-methylstephuline (**7**) (1.3 mg, 0.004% dry weight).

***N*-Methylstephisoferulin (1):** colorless gum; $[\alpha]^{25}_D$ −3.1 (*c* 0.26, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 213 (4.24), 286 (3.94), 324 (3.89) nm; CD (MeOH) λ_{ext} ([θ]) 210 (+20 500), 226 (+1475), 237 (+2984), 285 (−4695) nm; IR (KBr) ν_{max} 3392 br, 2941, 1686, 1636, 1612, 1510, 1488, 1440, 1375, 1272, 1177, 1158, 987, 666 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$), see Table 1; (+)-HRESIMS *m/z* 554.2400 [M + H$^+$]$^+$ (calcd for $C_{30}H_{36}NO_9$, 554.2384).

6-Cinnamoylhernandine (2): colorless gum; $[\alpha]^{25}_D$ +60.7 (*c* 0.07, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (3.80), 253 (3.32), 311 (3.42) nm; CD (MeOH) λ_{ext} ([θ]) 200 (+33 856), 226 (+4859), 236 (+10 056),

280 (−12 434) nm; IR (KBr) ν_{max} 3368 br, 2920, 2850, 1693, 1639, 1612, 1488, 1441, 1377, 1275, 1176, 1045, 755 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$), see Table 1; (+)-HRESIMS *m/z* 494.2172 $[M + H^+]^+$ (calcd for $C_{28}H_{32}NO_7$, 494.2173).

Stephalonine E (3): colorless gum; $[\alpha]^{25}_D$ +101 (*c* 0.02, $CHCl_3$) [lit.[13] $[\alpha]_D$ +49.6 (*c* 0.115, $CHCl_3$)]; CD (MeOH) λ_{ext} ([θ]) 221 (−1250), 237 (+4000), 282 (−12 000) nm

Aknadinine (4): yellow gum; $[\alpha]^{25}_D$ −218 (*c* 0.83 $CHCl_3$) [lit.[15] $[\alpha]_D$ −290 (*c* 0.1, $CHCl_3$), lit.[14] $[\alpha]_D$ −123 (*c* 3.46, MeOH)]; CD (MeOH) λ_{ext} ([θ]) 222 (+6041), 267 (−16 074), 281 (−24 402), 320 (−12 000) nm.

Longanine (5): colorless gum; $[\alpha]^{25}_D$ +206 (*c* 0.023, $CHCl_3$); CD (MeOH) λ_{ext} ([θ]) 211 (+18 560), 235 (+4561), 280 (−4410) nm.

Aknadilactam (6): colorless gum; $[\alpha]^{25}_D$ −130 (*c* 0.06, $CHCl_3$) [lit.[15] $[\alpha]_D$ −152 (*c* 0.58, $CHCl_3$)]; CD (MeOH) λ_{ext} ([θ]) 210 (+45 240), 234sh (+236), 264 (−19 220) nm.

***N*-Methylstephuline (7):** colorless gum; $[\alpha]^{25}_D$ +129.8 (*c* 0.05, $CHCl_3$) [lit.[18] $[\alpha]_D$ +92 (*c* 0.54, $CHCl_3$)]; CD (MeOH) λ_{ext} ([θ]) 212 (+20 441), 225 (+2327), 235 (+3804), 283 (−3977) nm.

Prostephabyssine (8): colorless gum; $[\alpha]^{25}_D$ −69.7 (*c* 0.1, $CHCl_3$) [lit.[19] $[\alpha]_D$ −105 (*c* 1.98, MeOH)]; CD (MeOH) λ_{ext} ([θ]) 212 (+3237), 231 (+68), 262 (−4505) nm.

δ-Opioid Receptor Binding Assay. Assays were performed in 50 mM Tris containing 3 mM $MgCl_2$, 1 mg/mL BSA, pH 7.4, with HEK cell membranes expressing recombinant human δ-opioid receptors (2 μg/well), [^{125}I]-deltorphin II (56 pM), and SPA beads (700 μg/well) in a total volume of 200 μL. Controls included 10 μM naloxone (for nonspecific binding) and 1 nM DPDPE (for reference). Compounds were tested at a final concentration of 2% DMSO. Microplates were shaken for 1 h at ambient temperature (ca. 23 °C), then left to reach steady state for 4 h. Microplates were counted for 1 min/well.

μ- and κ-Opioid Receptor Binding Filtration Assays. The filtration method for the μ-opioid receptor was performed using buffer containing Tris (50 mM), $MgCl_2$ (10 mM), and EDTA (1 mM, pH 7.4). The assays were performed with [^{3}H]-naloxone (0.66 nM) and CHO cell membranes (0.017 mg/mL) in a final assay volume of 200 μL. The microtiter plates were incubated for 90 min, including 60 min of shaking, at ambient room temperature (ca. 23 °C). The filtration assay for κ-opioid receptor binding contained the same binding buffer as the μ-assay, [^{3}H]-U69593 (1 nM) and HEK293 cell membranes (0.125 mg/mL) in a final volume of 200 μL. Both assays were stopped using a Tomtec Cell Harvester to filter onto presoaked PEI-treated GF/B filter mats. The mats were subsequently dried in an oven at 50 °C and then bagged into sample bags containing Betaplate scintillant. The mats were counted in a 1205 Betaplate liquid scintillation counter (Wallac Oy, Turku, Finland).

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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