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New Antiinfective and Human 5-HT2 Receptor Binding Natural and Semisynthetic Compounds from the Jamaican Sponge *Smenospongia aurea*

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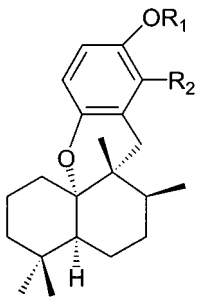
In addition to the sesquiterpene-phenol aureols (**1**), 6′-chloroaureol (**2**), and aureol acetate (**3**), eight indole alkaloids including the new *N*-3′-ethylaplysinopsin (**9**) have been isolated from the Jamaican sponge *Smenospongia aurea*. Makaluvamine O (**10**), a new member of the pyrroloiminoquinone class, was also isolated. The structures were characterized by spectroscopic methods, and two new derivatives of aureol were prepared to optimize the biological activity. Aureol *N*,*N*-dimethyl thiocarbamate (**1a**) and 6-bromoaplysinopsin (**7**) exhibit significant antimalarial and antimycobacterial activity in vitro. Compound **6** showed activity against the *Plasmodium* enzyme plasmepsin II. The 6-bromo-2′-de-*N*-methylaplysinopsin (**6**), 6-bromoaplysinopsin (**7**), and *N*-3′-ethylaplysinopsin (**9**) displaced high-affinity [^{3}H]antagonist ligands from cloned human serotonin 5-HT$_2$ receptor subtypes, whereas the other compounds tested did not. Remarkably, the 6-bromo-2′-de-*N*-methylaplysinopsin (**6**) showed a >40-fold selectivity for the 5-HT$_{2C}$ subtype over the 5-HT$_{2A}$ subtype.

As a part of our continuing investigation of Porifera for agents to treat infectious diseases,[1,2] the sponge *Smenospongia aurea* was collected at Discovery Bay (Jamaica). Analysis of the ethanol extract provided the following known sesquiterpene metabolites:[3,4] aureol (**1**), 6′-chloroaureol (**2**), and naturally occurring aureol acetate (**3**). Unlike the Belize[3] and Salvador[4] sponges, the Jamaican sample proved to be rich in indole alkaloids. The known 3-carboxylindole, *N*,*N*-dimethyltryptamine, isoplysin A (**4**),[5] 2′-de-*N*-methylaplysinopsin (**5**),[6] 6-bromo-2′-de-*N*-methylaplysinopsin (**6**),[6] 6-bromoaplysinopsin (**7**),[7] and *N*-3′-methylaplysinopsin (**8**)[8,9] as well as new *N*-3′-ethylaplysinopsin (**9**) were isolated. A halogenated pyrroloiminoquinone, related to makaluvone[10] (less a methyl group at *N*-1), joining the known highly cytotoxic makaluvamines A−F from a Fijian sponge *Zyzzya fuliginosa*,[10] makaluvamine G from *Z. fuliginosa* from Indonesia (given as *Histodermella* sp.),[11] and makaluvamines H−N from the sponge *Z. fuliginosa*,[12,13] was also obtained and named makaluvamine O (**10**).

All of the naturally occurring compounds and two new aureol derivatives (**1a** and **1b**) were assayed for their antimalarial activity in vitro as well as for their ability to inhibit the malarial enzyme plasmepsin. Plasmepsins are aspartic proteases that appear to be indispensable for parasite survival. Aspartic protease inhibitors have a profound effect on parasite multiplication in vitro, thus validating these enzymes as antimalarial drug targets.[14] Active recombinant plasmepsin-2 has previously been expressed and characterized and was therefore selected for these studies.[15] Aureol *N*,*N*-dimethyl thiocarbamate (**1a**) and 6-bromoaplysinopsin (**7**) exhibited significant activity



1. $R_1 = R_2 = H$
1a. $R_1 = (CH_3)_2NC(S)$, $R_2 = H$
1b. $R_1 = CH_3$, $R_2 = H$
2. $R_1 = H$, $R_2 = Cl$
3. $R_1 = Ac$, $R_2 = H$

at 87 and 340 ng/mL with a selectivity index of 55 and 14, respectively, against *Plasmodium falciparum*.

Compound **1a** was assayed for in vitro activity against TB, providing an MIC of <6.25 μg/mL with 100% inhibition. The new pyrroloiminoquinone **10** and aplysinopsins **4**−**9** were screened for their ability to displace the binding of radiolabeled drugs from cloned serotonin receptors, because these compounds contain an indoleamine pharmacophore like the endogenous ligand serotonin.

Results and Discussion

N-3′-Methylaplysinopsin (**8**) was reported previously as methylaplysinopsin,[8,9] but its NMR data have not been reported until now (Table 1).

The molecular formula $C_{16}H_{18}N_4O$ was established for **9** from a HRESIMS signal at *m*/*z* 283.1558 [M + H]$^+$ (calcd for $C_{16}H_{19}N_4O$, 283.1555, Δ +0.3 mmu). The IR spectrum indicated the presence of an amino (3420 cm^{-1}) and an amide carbonyl (1650 cm^{-1}) group. The ^{1}H NMR spectrum ($CDCl_3$) of **9** showed proton signals due to two *N*-methyl groups at δ 3.28 (6H, s), one imino *N*-ethyl group at δ 4.54

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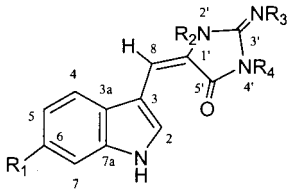
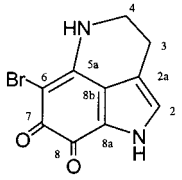


4. $R_1 = R_4 = H$, $R_2 = R_3 = CH_3$
5. $R_1 = R_2 = R_3 = H$, $R_4 = CH_3$
6. $R_1 = Br$, $R_2 = R_3 = H$, $R_4 = CH_3$
7. $R_1 = Br$, $R_2 = R_4 = CH_3$, $R_3 = H$
8. $R_1 = H$, $R_2 = R_3 = R_4 = CH_3$
9. $R_1 = H$, $R_2 = R_4 = CH_3$, $R_3 = C(1'')H_2C(2'')H_3$



10

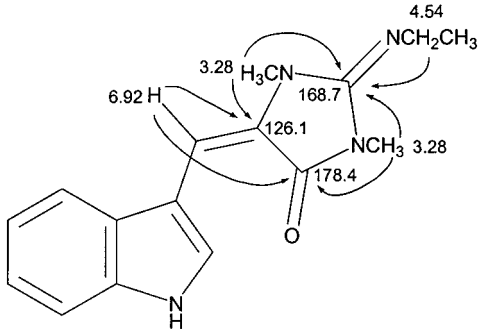
(2H, q, $J = 7.0$ Hz) and 1.48 (3H, t, $J = 7.0$ Hz), an olefin at δ 6.92 (1H, s), and a monosubstituted indole at δ 7.16 (2H, m), 7.30 (1H, br d, $J = 7.9$ Hz), 7.83 (1H, br d, $J = 8.0$ Hz), 8.61 (1H, br s), and 9.61 (1H, br s, D_2O-exchangeable proton). When the NMR solvent was changed to DMSO-d_6, the D_2O-exchangeable proton at *N*-1 appeared at δ 11.45, which was identical to those for **4**−**8**[5−9] and aplysinopsin.[16,17] The observed UV absorption maxima at 219, 276, and 409 nm supported **9** as a tryptophan derivative.[5] The assignment of the ethyl group and the two methyl groups was deduced from the HMBC experiment (Figure 1). The *E*-configuration of the double bond at C-8 was assigned on the basis of a ^1H, ^{13}C heteronuclear coupling constant (9.5 Hz)[18] between H-8 at δ 6.92 and C-5′ at δ 178.38. Thus, **9** was determined to be *N*-3′-ethyl-aplysinopsin.

Makaluvamine O (**10**) was obtained as a brown-purple solid. Like those of **6** and **7**, its LRESIMS showed an $[M + H]^+$ and an $[M + Na]^+$ ion at *m*/*z* 267 and 289 with ions of almost equal intensity at *m*/*z* 269 and 291, which indicated the presence of a single bromine atom. The molecular formula $C_{10}H_7BrN_2O_2$ was determined by the pseudo molecular ion at *m*/*z* 266.9781 $[M + H]^+$ (calcd for $C_{10}H_8$-$^{79}BrN_2O_2$, 266.9768, Δ +1.3 mmu), 268.9762 $[M + H]^+$ (calcd for $C_{10}H_8{}^{81}BrN_2O_2$, 268.9747, Δ +1.5 mmu).

Table 1. ^1H and ^{13}C NMR Data for Compounds **8** and **9**[a]

	8			**9**		
position	δ_H	mult., *J*	δ_C	δ_H	mult., *J*	δ_C
2	8.50	br s	129.87 (d)	8.61	br s	130.97 (d)
3			109.99 (s)			109.99 (s)
3a			127.65 (s)			127.80 (s)
4	7.84	br d, 7.8	119.02 (d)	7.83	br d, 8.0	118.91 (d)
5	7.22	m	123.03 (d)	7.16	m	122.97 (d)
6	7.22	m	120.98 (d)	7.16	m	120.93 (d)
7	7.37	br d, 7.2	111.94 (d)	7.30	br d, 7.9	112.07 (d)
7a			136.32 (s)			136.43 (s)
8	6.89	s	110.01 (d)	6.92	s	110.22 (d)
1′			125.56 (s)			126.13 (s)
2′-Me	3.28	s	38.12 (q)	3.28	s	38.38 (q)
3′			168.51 (s)			168.65 (s)
4′-Me	3.28	s	38.12 (q)	3.28	s	38.38 (q)
5′			177.34 (s)			178.38 (s)
1″	4.12	s	57.58 (q)	4.54	q, 7.0	66.91 (t)
2″				1.48	t, 7.0	14.83 (q)
N (1) H	9.37	br s		9.61	br s	

[a] Measured in $CDCl_3$ at 400 MHz for ^1H and 100 MHz for ^{13}C, respectively. *J* values are in Hz.



9

Figure 1. Significant HMBC correlations of **9**.

Table 2. ^1H, ^{13}C, and ^{15}N NMR Data for **10** (in DMSO-d_6)[a]

position	δ_H	mult., *J*	δ_C/δ_N
2	7.12	s	125.82 (d)
2a			118.34 (s)
3	2.74	t, 6.8	19.95 (t)
4	3.85	t, 6.8	43.09 (t)
5a			151.75 (s)
6			88.26 (s)
7			169.17 (s)
8			172.94 (s)
8a			125.37 (s)
8b			124.54 (s)
N (1) H	12.52	br s	161.51[b]
N (5) H	8.13	br s	102.35[b]

[a] Measured at 400 MHz for ^1H, 100 MHz for ^{13}C, and 50 MHz for ^{15}N NMR, respectively. [b] Chemical shift is relative to liquid ammonia. *J* values are in Hz.

The ^1H NMR spectrum (DMSO-d_6) of **10** (Table 2) showed an aromatic proton at δ 7.12 (1H, s), a spin system comprising a pair of methylene triplets at δ 2.74 and 3.85 ($J = 6.8$ Hz), and two D_2O-exchangeable protons at δ 12.52 (1H, br s) and 8.13 (1H, br s). The backbone of pyrroloiminoquinone for **10** was deduced from ^1H, ^{13}C (DEPT) together with HMQC and HMBC NMR experiments as well as initial proton and carbon assignments made by comparison with the published data for makaluvone[10] and damirone B.[10,19] Long-range $^1H-^{13}C$ correlations in the HMBC experiment were observed from the methylene protons at δ 2.74 (H-3) to δ 43.09 (C-4), 118.34 (C-2a), 124.54 (C-8b), and 125.82 (C-2) and from the protons at δ 3.85 (H-4) to δ 19.95 (C-3), 118.34 (C-2a), and 151.75 (C-5a). The aromatic singlet at δ 7.12 was attributed to H-2 of the pyrroloiminoquinone ring system, which revealed long-range correlations to the pyrrole carbons at δ 118.34 (C-2a), 124.54 (C-8b), and 125.37 (C-8a) as well as the methylene at δ 19.95 (C-3), respectively. Consistent with those of **4**−**9** in DMSO-d_6, the D_2O-exchangeable proton at δ 12.52 was bonded to *N*-1 in the pyrrole ring. The remaining D_2O-exchangeable proton (δ 8.13) and quaternary carbons (δ 172.94, 169.17, 88.26) were assigned by comparison with makaluvone.[10] The chemical shifts of the two nitrogen atoms were assigned unambiguously by a $^1H-^{15}N$ HMBC NMR experiment. The olefinic carbon C-6 was the site of bromination, judging from its upfield shift at δ 88.26. Therefore, the structure of makaluvamine O was depicted to be **10**.

Aureol (**1**) was used to generate two new semisynthetics (**1a** and **1b**) by reaction with commercially available reagents in one step (Experimental Section). All of the naturally occurring compounds and the aureol derivatives

Table 3. In Vitro Activities Against the D6 Clone of *Plasmodium falciparum* (I) and Against *Mycobacterium tuberculosis* (strain H37Rv) (II), as Well as Affinities for Human Serotonin 5-HT_2 Receptor Subtypes (III)

	I		II	III[e]	
compound	IC_{50} (μg/mL)	S.I.[a]	% inh. (at 6.25 μg/mL)	III-1[c]	III-2[d]
1	NA		31	NT	NT
1a	0.087	>55	100	NT	NT
1b	NA		53	NT	NT
2	3.4	>1.4	38	NT	NT
3	NA		34	NT	NT
4	0.97	>4.9		NA	NA
5	NA			NA	NA
6	1.1	>4.3		>100[f]	2.3
7	0.34	>14		2.0	0.33
8	NA			NA	NA
9	NA			1.7	3.5
10	0.94	>5.1		NA	NA

[a] S.I. (selectivity index) = IC_{50} (Vero cells)/IC_{50} (*P. falciparum*). Cytotoxicity was measured at 4.76 μg/mL. NA = no activity. NT = not tested. [b] MIC = minimum inhibitory concentrations. [c] III-1: equilibrium affinity constant (K_i, μM) at 5-HT_{2A} receptors. [d] III-2: equilibrium affinity constant (K_i, μM) at 5-HT_{2C} receptors. [e] In this case no activity means no displacable binding detected at 10 μM. Note that none of the samples displace [^{3}H]methylspiperone from the cloned human D4.2 receptor. The standard errors for all affinity values are <15%. [f] This affinity value is only an estimate based upon extrapolation (~10% inhibition was achieved at the highest concentration tested, 30 μM).

were tested against the D6 clone of *Plasmodium falciparum* for their in vitro antimalarial activity. Among them, aureol *N*,*N*-dimethyl thiocarbamate (**1a**) and 6-bromoaplysinopsin (**7**) exhibited activity at endpoints of 0.087 and 0.34 μg/mL with a selectivity index of 55 and 14, respectively. Other compounds such as isoplysin A (**4**), 6-bromo-2′-de-*N*-methylaplysinopsin (**6**), and makaluvamine O (**10**) showed moderately activity at 0.97, 1.1, and 0.94 μg/mL with a selectivity index of >4.9, >4.3, and >5.1, respectively (Table 3). Moreover, compound **6** inhibited the antimalarial target plasmepsin II enzyme with IC_{50} 53 μM (FRET) and 66 μM (FP). Cytotoxicity was determined by testing the compounds against Vero cells (African Green Monkey kidney cells), and none of them were cytotoxic at the tested concentration (4.76 μg/mL). **1a** and **7** were further assayed for in vivo antimalarial activity.[20] Both compounds showed no antimalarial activity.

Compounds **1** and **1a** were assayed for activity against *Mycobacterium tuberculosis* (strain H_{37}Rv) in vitro with an MIC of >6.25 and <6.25 μg/mL with 31% and 100% inhibition, respectively (Table 3). This shows that the thiocarbamate moiety can highly improve the activity against TB.

The 6-bromo-2′-de-*N*-methylaplysinopsin (**6**), 6-bromoaplysinopsin (**7**), and *N*-3′-ethylaplysinopsin (**9**) all displaced high-affinity [^{3}H]antagonist binding from cloned human serotonin 5-HT_{2C} receptors expressed in a mammalian cell line (Table 3). Compounds **7** and **9** also displaced high-affinity [^{3}H]antagonists binding from the 5-HT_{2A} receptor subtype, whereas compound **6** showed only partial displacement at the 5-HT_{2A} subtype at the highest concentration tested (Table 3). None of the other aplysinopsins (**4**, **5**, and **8**) displaced [^{3}H]antagonist binding to either serotonin 5-HT_2 receptor subtype at the highest concentration tested (Table 3). None of the aplysinopsins displaced [^{3}H]antagonist binding to cloned human dopamine D4.2 receptors (data not shown). Compound **7** had the highest overall affinity of the series of aplysinopsins, which was similar to that found for the endogenous ligand serotonin at the 5-HT_{2C} receptor subtype. The other aplysinopsins (**6** and **9**) that significantly displaced [^{3}H]-antagonist binding from serotonin receptors had 5-fold to 27-fold lower affinity than serotonin. Previously reported affinity values for serotonin are K_i = 0.32 μM at the 5-HT_{2A} receptor and K_i = 0.13 μM at the 5-HT_{2C} receptor.[21] Compound **9** displayed no significant selectivity for the two serotonin receptor subtypes, while compound **7** showed limited selectivity (~6-fold) for the 5-HT_{2C} subtype. In contrast, compound **6** demonstrated substantial selectivity (>40-fold) for the 5-HT_{2C} subtype over the 5-HT_{2A} subtype. However, due to its limited solubility, the selectivity ratio of compound **6** for the two serotonin receptor subtypes is currently only an estimate based upon extrapolation—the selectivity could be even greater.

Structure–activity comparisons of the aplysinopsins isolated from *S. aurea* reveal a role for the R1, R2, and R3 functional groups at positions 6, 2′, and 3′, respectively, in the binding to human serotonin 5-HT_2 receptors. First, the length of the alkyl chain at the R3 position appears to be important for aplysinopsins binding to serotonin receptors. For example, compounds **8** and **9** are identical except that compound **9** has an ethyl group at the R3 position and it has measurable binding activity, while compound **8**, which has no detectable binding activity, has a methyl group at the R3 position. Second, in the absence of ethylation at the R3 position, bromination at position R1 seems important for activity. Compounds **5** and **6** are identical except that compound **6** is R1-brominated, and only it has measurable binding activity. Furthermore, compound **7** has no R3-alkyl group, but it is R1-brominated and has binding activity. In addition to being important for the binding affinity of aplysinopsins, bromination at the R1 position is also important for their selective binding to the 5-HT_{2C} receptor subtype, since both compounds **6** and **7** are brominated and both selectively bind the 5-HT_{2C} receptor subtype over the 5-HT_{2A} receptor subtype. Third, methylation at the R2 position facilitates binding to the 5-HT_{2A} receptor subtype, since only compound **7**, but not its R2-desmethyl derivative (**6**), has significant affinity for the 5-HT_{2A} receptor subtype. Consequently, the R2 position appears to regulate subtype selective binding to 5-HT_{2C} over 5-HT_{2A} receptors. However, more compounds will be required to clearly define these structure–activity relationships.

Experimental Section

General Experimental Procedures. Melting points are uncorrected. 1D and 2D NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer. Chemical shifts, with δ values expressed in parts per million (ppm), are referenced to the residual solvent signals with resonances at δ_H/δ_C 2.48/39.7 (DMSO-d_6) and 7.26/77.0 ($CDCl_3$). ESI-FTMS analyses were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron HR HPLC-FT spectrometer by direct injection into an electrospray interface. IR spectra were recorded on an AATI Mattson Genesis Series FTIR spectrometer. UV spectra were scanned on a Perkin-Elmer Lambda 3B UV/vis spectrometer. Si gel (200–400 mesh) and Sephadex LH-20 (Pharmacia) for column chromatography were obtained from Natland International Corporation (www.natland.com) and Sigma Chemical Co. (USA), respectively. TLC was performed on aluminum sheets (Si gel 60 F_{254}, Merck KGaA, Germany).

Animal Material. The sponge was collected from "LTS-1", Discovery Bay, Jamaica, from a depth of 16–30 m using closed circuit underwater breathing apparatus (scuba) during July 1998. In life the sponge is a lobate cavernous mass with apical oscules, the surface of which is honeycombed. In life the sponge is yellowish brown with a yellow-olive green interior; the fibers are very dense, knotted, and dark. The sponge is *Smenospon-*

gia aurea (Hyatt), which is presently recognized within the family Thorectidae, order Dictyoceratida. A voucher specimen has been deposited in the Natural History Museum, London (BMNH 2000.7.17.20). Due to the very similar characteristics of *Smenospongia*, *Zyzzya*, and *Strongylodesma* from Jamaica, we cannot be certain that these species have not been collected together, although the voucher is clearly *Smenospongia*.

Extraction and Isolation. The sponge (1 kg) was freeze-dried for two weeks to afford semidried material (285 g). This material was extracted with EtOH (5 × 800 mL, total amount 4 L) in a blender. The combined extracts, after filtration, were concentrated in vacuo until dried to give a black residue (ca. 50 g). This material was chromatographed on Si gel (column: 4.5 × 62 cm) with a $CHCl_3$−MeOH gradient (10:1−1:1) to yield five fractions. Fraction 1 ($CHCl_3$−MeOH, 10:1, ca. 450 mg) was chromatographed on Si gel (column: 2.5 × 45 cm) with a hexane−ethyl acetate gradient (30:1−5:1) to yield **2** (5 mg) and **3** (6 mg). Fraction 2 ($CHCl_3$−MeOH, 8:1, ca. 9 g) was rechromatographed on Si gel (column: 4.5 × 70 cm) with a $CHCl_3$−MeOH gradient (10:1) to yield **1** (500 mg). 3-Carboxylindole (4 mg), **4** (30 mg), **5** (8 mg), **6** (7 mg), **7** (25 mg), **8** (6 mg), **9** (12 mg), and **10** (15 mg) were obtained from fraction 4 ($CHCl_3$−MeOH, 5:1−3:1, ca. 910 mg) and purified by repetitive Si gel column chromatography (column: 4.5 × 82 cm, eluted with $CHCl_3$−MeOH, 8:1) followed by gel permeation chromatography on Sephadex LH-20 (column: 4.9 × 76 cm) in MeOH. Fraction 5 ($CHCl_3$−MeOH, 3:1−1:1, ca. 850 mg) yielded *N*,*N*-dimethyltryptamine (30 mg), also purified by gel permeation chromatography on Sephadex LH-20 in MeOH.

***N*-3′-Methylaplysinopsin (8):** yellowish gum; ^{1}H and ^{13}C NMR ($CDCl_3$) data, see Table 1; ESI (−)-FTMS *m*/*z* 267 $[M - H]^-$ and ESI (+)-FTMS 269 $[M + H]^+$; HRESI-FTMS *m*/*z* 269.1392 $[M + H]^+$ (calcd for $C_{15}H_{17}N_4O$, 269.1399, Δ −0.7 mmu).

***N*-3′-Ethylaplysinopsin (9):** yellowish gum; IR (dry film) ν_{max} 3420 (br s), 2925, 2855, 1650, 1599, 1562, 1517, 1461, 1423, 1385, 1347, 1284, 1259, 1233, 905, 748 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 219 (4.13), 276 (3.74), and 409 (4.11) nm; ^{1}H and ^{13}C NMR ($CDCl_3$) data, see Table 1; ESI-FTMS *m*/*z* 283 $[M + H]^+$; ESI-FTMS *m*/*z* 283.1558 $[M + H]^+$ (calcd for $C_{16}H_{19}N_4O$, 283.1555, Δ +0.3 mmu).

Makaluvamine O (10): brown-purple solid; mp 282−283 °C (dec); IR (dry film) ν_{max} 3177 (br s), 1675, 1599, 1524, 1416, 1334, 1145, 975 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 244 (4.01), 337 (3.75) nm; ^{1}H and ^{13}C NMR (DMSO-d_6) data, see Table 2; ESI-FTMS *m*/*z* 267 $[M + H]^+$, 289 $[M + Na]^+$; HRESI-FTMS *m*/*z* 266.9781 $[M + H]^+$ (calcd for $C_{10}H_8{}^{79}BrN_2O_2$, 266.9768, Δ +1.3 mmu), 268.9762 $[M + H]^+$ (calcd for $C_{10}H_8{}^{81}BrN_2O_2$, 268.9747, Δ +1.5 mmu), 288.9608 $[M + Na]^+$ (calcd for $C_{10}H_7{}^{79}BrN_2O_2$-Na, 288.9588, Δ +2.0 mmu), and 290.9574 $[M + Na]^+$ (calcd for $C_{10}H_7{}^{81}BrN_2O_2Na$, 290.9567, Δ +0.7 mmu).

Preparation of Aureol *N*,*N*-Dimethylthiocarbamate (1a). A mixture of 1 molar equiv (12.1 mg) of activated zinc[22] dust and an excess of dimethylthiocarbamoyl chloride (dissolved in 25 mL of dry CH_2Cl_2) was stirred for 15 min, and then **1** (58.6 mg, dissolved in 20 mL of dry CH_2Cl_2) was added in several portions. The mixture was refluxed overnight. The product was washed with $CHCl_3$ and then filtered. The filtrate was evaporated under reduced pressure, and the residue was subjected to Si gel chromatography (230−400 mesh, 25 × 410 mm) with *n*-hexane/EtOAc (20:1−1:1) to yield pure **1a** (44.9 mg, 60%) as a colorless powder: mp 191−192 °C; $[\alpha]_D^{20}$ +35° (*c* 0.5, $CHCl_3$); IR (dry film) ν_{max} 2962, 2931, 2865, 1601, 1490, 1459, 1356, 1225, 1168, 960 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 294 (3.43) and 215 (3.68) nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.07 (3H, s, H-11), 0.78 (3H, s, H-12), 0.92 (3H, s, H-13), 2.01 (1H, d, *J* = 16.9 Hz, H-14a), 3.42 (1H, d, *J* = 16.9 Hz, H-14b), 1.11 (3H, d, *J* = 7.5 Hz, H-15), 6.70 (1H, d, *J* = 8.6 Hz, H-3′), 6.76 (1H, brd, *J* = 8.6 Hz, H-4′), 6.74 (1H, brs, H-6′), 3.29 (3H, s) and 3.44 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.69 (t, C-1), 18.74 (t, C-2), 34.21 (t, C-3), 34.31 (s, C-4), 44.69 (d, C-5), 22.61 (t, C-6), 28.26 (t, C-7), 39.74 (d, C-8), 38.39 (s, C-9), 83.34 (s, C-10), 30.33 (q, C-11), 32.34 (q, C-12), 20.59 (q, C-13), 37.60 (t, C-14), 17.73 (q, C-15), 122.11 (s, C-1′), 147.08 (s, C-2′), 122.69 (d, C-3′), 117.18 (d, C-4′), 149.82 (s, C-5), 121.27 (d, C-6′), 38.99 (q), 43.65 (q), and 188.75 (s); ESI-FTMS *m*/*z* 402 $[M + H]^+$; HRESI-FTMS *m*/*z* 402.2460 $[M + H]^+$ (calcd for $C_{24}H_{35}NO_2S$, 402.2458, Δ +0.2 mmu).

Preparation of *O*-Methylaureol (1b). To 35.7 mg of **1** dissolved in dry acetone (20 mL) were added an excess of anhydrous potassium carbonate (K_2CO_3, 5 g) and 15 mL of methyl iodide, and the mixture was refluxed for 16 h. The product was diluted with $CHCl_3$ and then filtered. The solvent was evaporated under reduced pressure, and the residue was subjected to Si gel chromatography (230−400 mesh, 25 × 390 mm) with *n*-hexane/EtOAc (10:1−5:1) to yield pure **1b** (32.8 mg, 87%) as an oil: $[\alpha]_D^{20}$ +77° (*c* 0.5, $CHCl_3$); IR (dry film) ν_{max} 2968, 2922, 2867, 1599, 1491, 1458, 1208, 955 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 297 (3.47) and 216 (3.67) nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.09 (3H, s, H-11), 0.80 (3H, s, H-12), 0.94 (3H, s, H-13), 2.02 (1H, d, *J* = 17.1 Hz, H-14a), 3.42 (1H, d, *J* = 17.1 Hz, H-14b), 1.13 (3H, d, *J* = 7.5 Hz, H-15), 6.67 (1H, br s, H-3′), 6.67 (1H, br s, H-4′), 6.56 (1H, br s, H-6′) and 3.75 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.74 (t, C-1), 18.80 (t, C-2), 34.29 (t, C-3), 34.34 (s, C-4), 44.42 (d, C-5), 22.65 (t, C-6), 28.34 (t, C-7), 39.78 (d, C-8), 38.55 (s, C-9), 82.73 (s, C-10), 30.28 (q, C-11), 32.38 (q, C-12), 20.65 (q, C-13), 37.99 (t, C-14), 17.79 (q, C-15), 122.28 (s, C-1′), 146.14 (s, C-2′), 113.88 (d, C-3′), 113.24 (d, C-4′), 153.17 (s, C-5), 117.60 (d, C-6′), and 55.96 (q); ESI-FTMS *m*/*z* 329 $[M + H]^+$; HRESI-FTMS *m*/*z* 329.2479 $[M + H]^+$ (calcd for $C_{22}H_{33}O_2$, 329.2472, Δ +0.7 mmu).

Plasmepsin-2. Proplasmepsin-2 (generously provided by Dr. Moon, Roche, Basel) was activated, and the FRET assays were carried out as described in the literature.[23] Briefly, the reactions contained enzyme at 75 ng/mL, 10 μM substrate peptide, and samples dissolved in DMSO at varying concentrations. The assays were carried out in a 50 μL final volume in 384-well black microtiter plates and read in a Tecan Ultra or SpectraFluor Plus using 360/465 excitation/emission filters. Under these conditions the assay was linear over at least 2 h. Details of the fluorescence polarization assay will be published elsewhere (Flotow et al., manuscript in preparation).

Transfection of Cells with Cloned Receptors. The pcDNA3 plasmid construct containing either the human serotonin 5-HT2A or 5-HT2C receptors was transiently transfected into African green monkey (COS-7) cells as described previously[24] using a calcium phosphate ($CaPO_4$) precipitation method. Briefly, 20 μg of purified plasmid DNA was mixed with a final volume of 1 mL of $CaPO_4$/HEPES solution, and the resulting precipitate was added dropwise to a 150 cm^2 plate containing COS-7 cells at ∼30% confluence. The following day, the media was removed by aspiration and replaced with fresh media. Cells were grown to confluence before harvesting.

Preparation of Crude Membranes for Binding Assays. Cell membranes were prepared as described previously.[24] Briefly, COS-7 cells expressing the desired receptor were dislodged by a 10 min incubation in Earle's balanced saline solution (EBSS) lacking Ca^{2+} and Mg^{2+} and supplemented with 5 mM EDTA. Dislodged cells were pelleted in EBSS by centrifugation at 900*g* for 10 min. After centrifugation, the cell pellets were lysed in lysis buffer (5 mM Tris, 5 mM $MgCl_2$, pH = 7.4). The lysate was glass−glass homogenized (10 strokes) and the membranes were isolated by centrifugation at 35000*g* for 30 min. The membrane pellets were resuspended in 50 mM Tris, pH 7.4 at 25 °C, and washed by recentrifugation. The washed membrane pellets were resuspended by light homogenization in binding buffer (50 mM Tris, pH 7.4 at 25 °C) immediately before use.

Radioligand Binding Assays of Crude Membranes. Membranes expressing cloned human serotonin 5-HT2A receptors were assayed for specific [^{3}H]methylspiperone (NET-856, 84 Ci/mmol, Dupont NEN) binding activity, while those expressing serotonin 5-HT2C receptors were assayed for specific [^{3}H]mesulergine binding activity (TRK-845, 79 Ci/mmol, Amersham) by saturation isotherm analysis as described previously.[24] Nonspecific binding was determined in the presence of 10 μM mianserin. The reaction was allowed 2 h to reach equilibrium at 25 °C prior to rapid filtration through GF/C filters pretreated with 0.3% polyethyleneimine. The wash buffer consisted of ice-cold binding buffer (pH = 7.4). Radio-

activity bound to the filters was quantified by scintillation spectroscopy. Membrane protein concentrations were determined using the bicinchonic acid (BCA) protein reagent (Pierce, IL) and a bovine serum albumin standard curve. Alpysinogen binding affinity (K_i) values were determined by equilibrium competition binding with the above-mentioned radioligands. All purified aplysinopsins were dissolved at a concentration of 10 mM in DMSO and diluted at least 1:100 v/v in the final assay solution.

Calculations and Data Analysis. All points were sampled in triplicate for each experiment. All experiments were repeated three times, and geometric mean values were reported with their associated errors. The inhibition constant (K_i) values for all purified aplysinopsins were calculated from IC_{50} values using the competitive form of the Cheng−Prusoff equation: $K_i = IC_{50}/(1 + [\text{ligand}]/K_D)$.[25] A 95% confidence interval was employed for all curve-fitting procedures using Graphpad's Prism software. The statistical measures of curve fitting were the F-test, the run test, and a correlation coefficient.

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