



# Synthesis and structure–affinity relationships of novel small molecule natural product derivatives capable of discriminating between serotonin 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub> receptor subtypes

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## ARTICLE INFO

### Article history:

Received 17 February 2010

Revised 3 May 2010

Accepted 4 May 2010

Available online 15 May 2010

### Keywords:

5-HT<sub>2C</sub> receptor selective ligand

5-HT<sub>2C</sub>

Serotonin receptor

5-Hydroxytryptamine

Natural product analogue

Aplysinopsin

## ABSTRACT

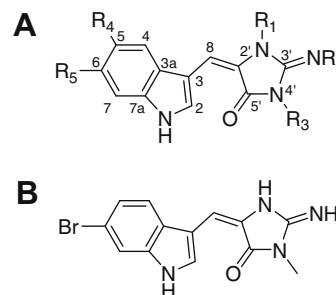
Efforts to develop ligands that distinguish between clinically relevant 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> serotonin receptor subtypes have been challenging, because their sequences have high homology. Previous studies reported that a novel aplysinopsin belonging to a chemical class of natural products isolated from a marine sponge was selective for the 5-HT<sub>2C</sub> over the 5-HT<sub>2A</sub> receptor subtype. Our goal was to explore the 5-HT<sub>2A/2C</sub> receptor structure–affinity relationships of derivatives based on the aplysinopsin natural product pharmacophore. Twenty aplysinopsin derivatives were synthesized, purified and tested for their affinities for cloned human serotonin 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, and 5-HT<sub>2C</sub> receptor subtypes. Four compounds in this series had >30-fold selectivity for 5-HT<sub>2A</sub> or 5-HT<sub>2C</sub> receptors. The compound (*E*)-5-((5,6-dichloro-1*H*-indol-3-yl)methylene)-2-imino-1,3-dimethylimidazolidin-4-one (UNT-TWU-22, **16**) had approximately 2100-fold selectivity for the serotonin 5-HT<sub>2C</sub> receptor subtype: an affinity for 5-HT<sub>2C</sub> equal to 46 nM and no detectable affinity for the 5-HT<sub>1A</sub> or 5-HT<sub>2A</sub> receptor subtypes. The two most important factors controlling 5-HT<sub>2A</sub> or 5-HT<sub>2C</sub> receptor subtype selectivity were the combined R<sub>1</sub>,R<sub>3</sub>-alkylation of the imidazolidinone ring and the type and number of halogens on the indole ring of the aplysinopsin pharmacophore.

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## 1. Introduction

Aplysinopsins (Fig. 1A) are a chemical class of natural product indole alkaloids, whose name derives from the marine sponge *Thorecta aplysinopsis* from which it was originally isolated.<sup>1</sup> Aplysinopsins have also been purified from other sponges, including *Verongia spengelii*,<sup>2</sup> *Dercitus*,<sup>3</sup> *Hyrtios erecta*,<sup>4</sup> *Smenospongia aurea*,<sup>5</sup> and other *Thorectandra*, *Smenospongia* and *Verongula* species as well.<sup>6,7</sup> Tissues from Cnidarians such as the corals *Tubastraea faulkneri*<sup>8</sup> and *Leptopsammia pruvoti*,<sup>9</sup> and from the sea anemone *Radiantius kuekenthali*<sup>10</sup> also contain aplysinopsins; however, in the latter case their presence was specifically attributed to an uniden-

tified species of dinoflagellate symbiotic with the anemone<sup>10</sup> raising the possibility that aplysinopsins are not endogenous to



**Figure 1.** (A) Aplysinopsin pharmacophore and numbering. The original aplysinopsin reported had R<sub>1</sub> = R<sub>3</sub> = CH<sub>3</sub>, R<sub>2</sub> = R<sub>4</sub> = R<sub>5</sub> = H.<sup>2</sup> (B) The structure of 6-bromo-2'-de-*N*-methylaplysinopsin or 6-bromo-R<sub>3</sub>-methylaplysinopsin reported by Hu et al.<sup>5</sup> (also known as Natural Product #6 in that report).

**Abbreviations:** 5-HT, 5-hydroxytryptamine (serotonin); 5-HT<sub>1A</sub>, serotonin receptor subtype 1A; 5-HT<sub>2A</sub>, serotonin receptor subtype 2A; 5-HT<sub>2C</sub>, serotonin receptor subtype 2C.

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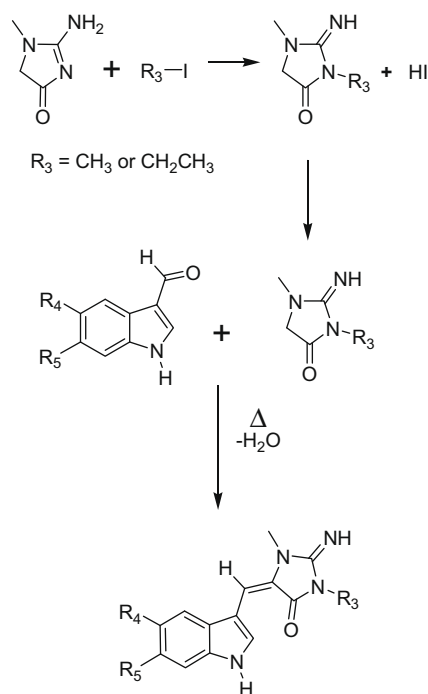
Porifera and Cnidaria, rather that they might be from a microbial source.

Although anti-tumor<sup>2</sup> and anti-filarial<sup>11</sup> activities have been ascribed to aplysinopsins, our interest relates to a previous discovery in our lab that a few aplysinopsins bind to human serotonin 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors with moderate to low affinities. One of these structures identified as 6-bromo-2'-de-N-methylaplysinopsin (Fig. 1B), exhibited considerably higher affinity (>40-fold) for the 5-HT<sub>2C</sub> receptor than the 5-HT<sub>2A</sub> receptor.<sup>5</sup> This is of interest, because finding structurally simple small molecule pharmacophores that can distinguish between the closely related and clinically important 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptor subtypes has remained a challenge.<sup>12</sup> Here, we report on the synthesis of a library of novel aplysinopsin derivatives and their structure–affinity relationships for cloned human serotonin 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors as well as another closely related and clinically important serotonin receptor subtype, the 5-HT<sub>1A</sub> receptor. The preference for 5-HT<sub>2A</sub> or 5-HT<sub>2C</sub> receptor subtypes can be controlled by changing the type and extent of halogenation on the aplysinopsin indole ring. Some of these modifications resulted in higher affinity compounds that preferentially bound either the 5-HT<sub>2A</sub> or 5-HT<sub>2C</sub> receptors. To avoid the use of 'de-methyl' designations, and for added clarity, the aplysinopsin pharmacophore will be described here without the assumption that R<sub>1</sub> and R<sub>3</sub> are methylated as in the original aplysinopsin structure, rather alkyl substitutions at R<sub>1</sub> and R<sub>3</sub> will be explicitly defined in the simplified naming throughout.

## 2. Results

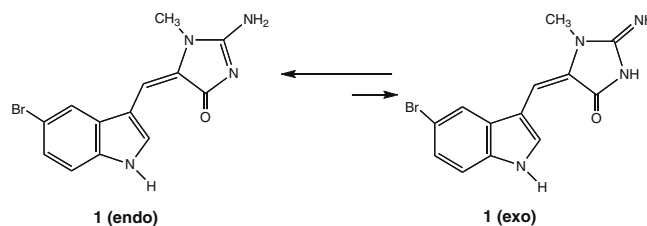
### 2.1. Synthesis of a library of aplysinopsin derivatives

A library of aplysinopsin analogues were synthesized from indole aldehydes and differentially alkylated 2-imino-imidazolidin-4-ones utilizing a scheme similar to that described by Djura and Faulkner<sup>3</sup> (Fig. 2). The indole aldehydes (indole-3-carboxaldehyde-



**Figure 2.** Synthesis of 2-imino-1,3-dimethylimidazolidin-4-one and 3-ethyl-2-imino-1-methylimidazolidin-4-one moieties and subsequent synthesis of aplysinopsin analogues.

des) were either commercially available or were synthesized using a procedure described by Jiang et al.<sup>13</sup> The 2-imino-1,3-dimethylimidazolidin-4-one and 3-ethyl-2-imino-1-methylimidazolidin-4-one were synthesized by methylation or ethylation of creatinine (2-amino-1-methyl-2-imidazolin-4-one) utilizing a procedure by Kenyon and Rowley<sup>14</sup> (Fig. 2). Single crystal structures were determined for **1** and **2** and in both cases the double bond linking the indole and imidazolidine rings (C8–C1') were in the *E*-configuration.<sup>15</sup> Compounds **1** and **3–8** can exist in two tautomeric forms: one where the imine double bond is endocyclic and the other where the imine double bond is exocyclic as shown.



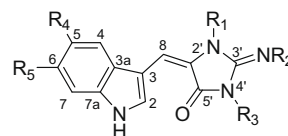
The single crystal structure of **1** shows that the imidazolidone ring exists in the endocyclic tautomeric form in the solid state. The <sup>1</sup>H NMR of **1** shows an absorption at 7.73 ppm integrating for two hydrogen atoms. This absorption must be due to the NH<sub>2</sub> group, which demonstrates that **1** exists in DMSO-*d*<sub>6</sub> solution in the endocyclic form.

### 2.2. Structure–affinity relationships for aplysinopsin derivatives

A series of 20 synthesized compounds possessing an aplysinopsin pharmacophore were examined at a high concentration (1–3 μM) for the ability to displace specifically bound radioligands from 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, and 5-HT<sub>2C</sub> receptors (Table 1). This initial screen revealed that compounds lacking R<sub>3</sub>-alkylation of the imidazolidine (**1**, **3–8**) did not strongly interact with any of the three

**Table 1**

Chemical structures and single point data for displacement of specifically bound radioligands from cloned serotonin 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, and 5-HT<sub>2C</sub> receptors



Compd No.	R3	R4	R5	% Displacement		
				1A	2A	2C
1	H	Br	H	6 ± 2	0 ± 5	0 ± 5
2	CH <sub>3</sub>	F	H	4 ± 3	10 ± 1	0 ± 6
3	H	Cl	H	9 ± 3	9 ± 1	0 ± 6
4	H	Cl	Cl	4 ± 2	0 ± 4	10 ± 5
5	H	F	H	0 ± 3	0 ± 1	19 ± 6
6	H	H	F	0 ± 4	6 ± 4	1 ± 8
7	H	H	Cl	1 ± 2	0 ± 3	0 ± 6
8	H	H	H	0 ± 6	0 ± 2	6 ± 10
9	CH <sub>2</sub> CH <sub>3</sub>	Br	H	0 ± 0	9 ± 3	18 ± 10
10	CH <sub>3</sub>	Cl	H	9 ± 2	22 ± 4	43 ± 11
11	CH <sub>3</sub>	Br	H	18 ± 2	37 ± 1	64 ± 5
12	CH <sub>2</sub> CH <sub>3</sub>	I	H	15 ± 1	35 ± 2	46 ± 6
13	CH <sub>3</sub>	H	F	2 ± 3	79 ± 3	0 ± 5
14	CH <sub>3</sub>	H	Cl	3 ± 3	0 ± 5	49 ± 5
15	CH <sub>3</sub>	H	Br	1 ± 3	8 ± 1	46 ± 8
16	CH <sub>3</sub>	Cl	Cl	0 ± 4	12 ± 2	79 ± 4
17	CH <sub>3</sub>	H	H	0 ± 1	47 ± 3	0 ± 7
18	CH <sub>2</sub> CH <sub>3</sub>	H	H	0 ± 3	50 ± 2	0 ± 5
19	CH <sub>2</sub> CH <sub>3</sub>	H	F	0 ± 1	86 ± 1	0 ± 5
20	CH <sub>2</sub> CH <sub>3</sub>	H	Br	0 ± 1	0 ± 8	49 ± 10

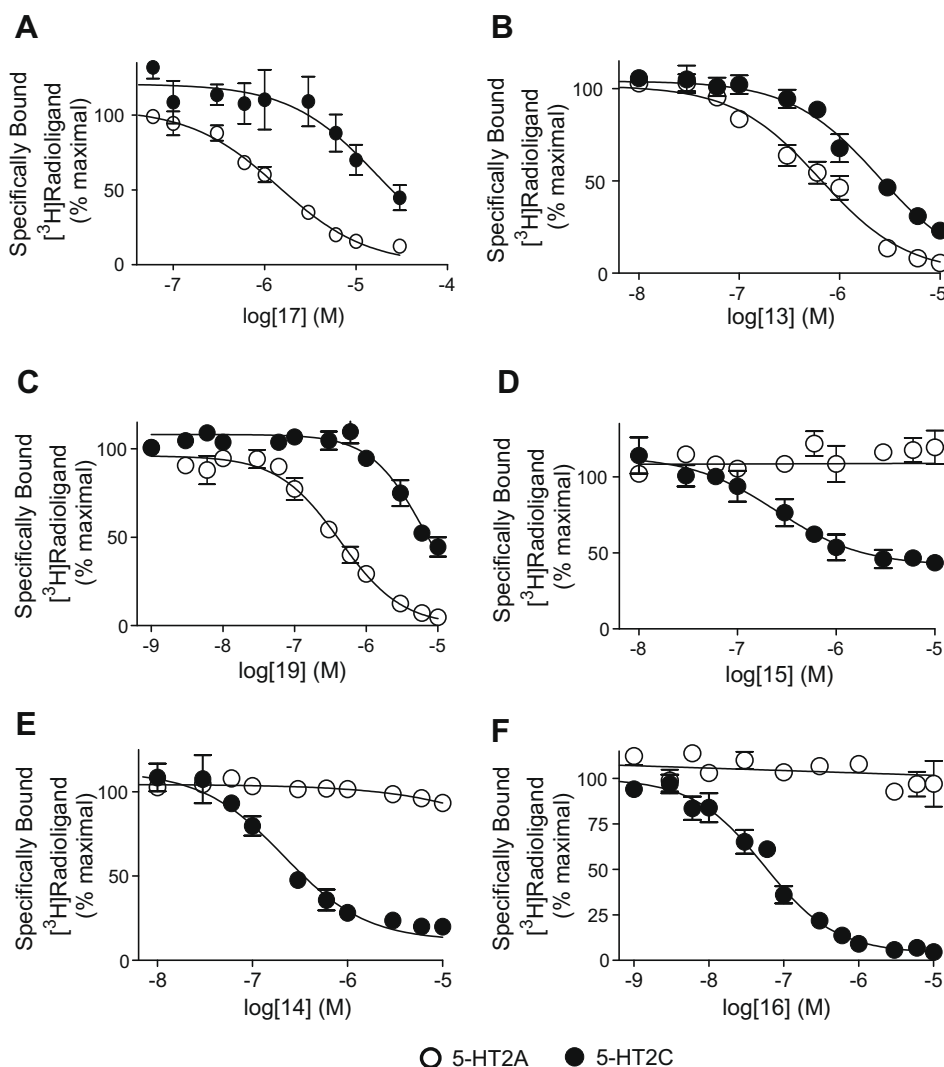
All compounds were screened at 3 μM, except **15**, which due to poor solubility had to be screened at 1 μM. For all the structures shown in the table R<sub>1</sub> = CH<sub>3</sub> and R<sub>2</sub> = H.

subtypes of serotonin receptor. With the exceptions of the 5-fluoro (**2**) and 5-bromo derivatives (**9**), all R1,R3-dimethyl and R1,R3-methylethyl derivatives (**10–20**) displaced at least 40% of specifically bound radioligand from one or more of the three receptor systems (Table 1). Of these 11 compounds, the monohalogenated 5-chloro (**10**), 5-bromo (**11**), and 5-iodo (**12**) derivatives were least able to distinguish between the three 5-HT receptor subtypes. In contrast, monohalogenation at the 6-position controlled preference for either 5-HT2A or 5-HT2C receptors over the other two receptor subtypes. For example, the 6-fluoro compound (**13**) preferentially interacted with the 5-HT2A receptor, while the 6-chloro (**14**) and 6-bromo (**15**) analogues preferred interactions with the 5-HT2C receptor (Fig. 3B, D, and E). The higher selectivity ratio of **14** relative to **15** (>602-fold vs >45-fold) is due to its higher affinity for the 5-HT2C receptor (Fig. 3D and E, Table 2). Even though the 5-monochloro derivative **10** does not display a subtype preference (Table 1), the addition of a 5-chloro substituent to **14** to make the 5,6-dichloro derivative (UNT-TWU-22, **16**) improves 5-HT2C selectivity further (>2170-fold for **16** compared to >602-fold for **14**, Fig. 3E and F, Table 2). This enhanced selectivity for **16** is due to its increased affinity for the 5-HT2C receptor.

Apysinsin derivatives that either lack indole ring halogenation (**17**, **18**) or have a 6-monofluoro substituent (**13**, **19**) preferred interactions with 5-HT2A over 5-HT1A and 5-HT2C receptors

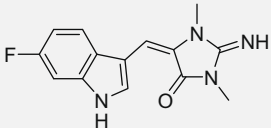
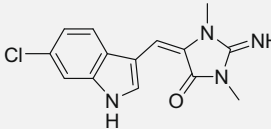
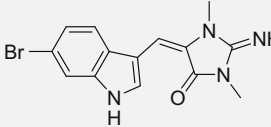
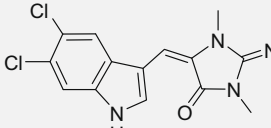
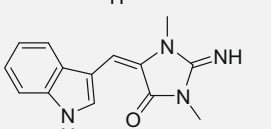
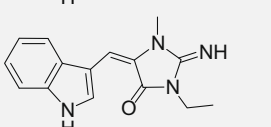
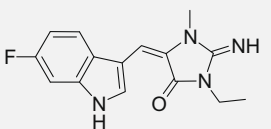
(Fig. 3 and Table 2). A methyl (**17**) to ethyl (**18**) substitution at R3 of the unhalogenated derivatives **17** and **18** or of the 6-fluoro derivatives **13** and **19** does not significantly affect the displacement profile for any of the three receptor subtypes (Table 1) or the affinity profile for 5-HT2A and 5-HT2C receptors (Table 2, Fig. 3). A similar trend for ethylation versus methylation of R3 is observed for the 6-monobromo derivatives **20** and **15** (Table 1). However, in the case of 5-monobromo derivatives the displacement profiles suggest that methylation of R3 (**11**) is preferred over ethylation (**9**) by all the serotonin subtypes (Table 1).

Interest in the apysinsin pharmacophore as a molecular scaffold with the potential to differentiate between the 5-HT2C and 5-HT2A receptor subtypes evolved from our previous work with apysinsins isolated from natural sources. One of those isolated apysinsins (Natural Product #6, 6-bromo-2'-de-N-methylapysinsin) had reasonably good selectivity (>40-fold) for the 5-HT2C over the 5-HT2A receptor albeit its affinity for the 5-HT2C receptor was quite low (2.3  $\mu$ M).<sup>5</sup> Although we did not attempt to synthesize 6-bromo-2'-de-N-methylapysinsin due to its low affinity, we did synthesize the much less selective but higher affinity Natural Product #7 (6-bromoapysinsin)<sup>5</sup> whose reported structure is identical to our **15**. This allowed us to compare affinity values when **15** was derived from natural or synthetic sources. While **15** from both sources preferentially interacts with 5-HT2C



**Figure 3.** Competition binding for selected compounds for cloned serotonin 5-HT2A (open circles) and 5-HT2C (closed circles) receptors ( $n = 3$ ).

**Table 2**  
Compound affinities for cloned human serotonin 5-HT2A and 5-HT2C receptor subtypes ( $n = 3$ )

Name	Structure	Binding affinity ( $K_i$ nM)		$K_i$ selectivity ratio <sup>a</sup> 5-HT2A/5-HT2C
		5-HT2A $\pm$ SEM	5-HT2C $\pm$ SEM	
13		235 $\pm$ 42	2114 $\pm$ 203	0.11
14		ND	166 $\pm$ 55	>602
15		ND	2202 $\pm$ 674	>45
16		ND	46 $\pm$ 8.6	>2170
17		598 $\pm$ 53	14,451 $\pm$ 5893	0.041
18		655 $\pm$ 94	8323 $\pm$ 2911	0.079
19		173 $\pm$ 42	5230 $\pm$ 858	0.033

ND = not detected at highest concentration tested.

<sup>a</sup> For those affinity values that could not be detected at the highest concentration tested (see Fig. 3), an affinity of >100,000 nM is assumed for the purpose of the selectivity calculations

over 5-HT2A receptors, the affinity values were quite different: 2.2  $\mu$ M versus 0.33  $\mu$ M at 5-HT2C and not detectable versus 2.0  $\mu$ M at 5-HT2A when derived from synthetic versus natural sources, respectively. Although the isolated natural products were screened in a COS-7 cell line transient expression system and synthetic compounds were screened in an HEK293 cell line stable expression system, the other assay conditions were the same. One possibility for the discrepancy is that the isolated natural product samples, which were gummy in consistency, might have contained impurities with radioligand displacing activity.

### 3. Discussion

A key discovery is the versatility of the aplysinopsin pharmacophore in the context of the three 5-HT receptor subtypes investigated: 5-HT1A, 5-HT2A, 5-HT2C. Small molecule aplysinopsins can be created that preferentially interact with either 5-HT2C or 5-HT2A receptor subtypes. Based upon the elaborations examined in the series presented here, much higher levels of 5-HT2C selectivity (>2100-fold) could be achieved than 5-HT2A selectivity (>30-fold). As long as the 1- and 3-positions of the imidazolidinone ring are alkylated, selectivity for one or the other of these subtypes can

be controlled by the type of halogenation at position 6 of the indole ring. Interactions with 5-HT2A over 5-HT2C receptors are favored when the indole ring contains a 6-fluoro substituent or is unhalogenated, while interactions with 5-HT2C over 5-HT2A receptors are favored for 6-chloro or 6-bromo derivatives.

Because the R1,R3-dimethyl-6-chloro-aplysinopsin (**14**) had the highest degree of 5-HT2C selectivity due to its higher affinity for this receptor subtype and lack of affinity for the 5-HT1A and 5-HT2A receptor subtypes, it was chosen as the best structure for investigating the effect of 5,6-dihalogenation of the indole. Addition of a 5-chloro to make the 5,6-dichloro-aplysinopsin (**16**) increased the affinity for 5-HT2C more than threefold beyond that of the 6-chloro congener (**14**) without increasing the affinities for the 5-HT1A or 5-HT2A receptor subtypes; the net effect was an increase in 5-HT2C selectivity from >602-fold for (**14**) to >2170-fold for (**16**). The critical role of alkylation at the R3-position of the imidazolidinone ring was also confirmed for the 5,6-dichloro derivative since removal of the R3-alkyl as in **4** results in a ligand with little or no apparent affinity for any of the three receptor subtypes.

The loss of affinity at all three serotonin receptor subtypes, when R3 lacks alkylation is likely due to the reduced probability of protonating the nitrogen attached to the 3' carbon. This nitrogen

is assumed to interact with the conserved aspartic acid approximately two helical turns from the extracellular side of the third transmembrane-spanning domain (Asp(116) for 5-HT<sub>1A</sub>, Asp(155) for 5-HT<sub>2A</sub>, and Asp(134) for 5-HT<sub>2C</sub>; biogenic amine GPCR universal indexing D3.32). This is a reasonable assumption, because a protonatable amine is required for high affinity interactions in other G protein-coupled biogenic amine receptors,<sup>16</sup> and replacing the nitrogen attached to the 3' carbon with an oxygen or sulfur results in no detectable affinity for any of the three 5-HT receptors tested (data not shown).

Although we cannot discount the effects of electronic factors, we speculate that the size of the halogen at position 6 most likely influences ligand selectivity via appropriately oriented hydrophobic bulk. If lipophilicity or ring electronegativity alone were the critical factors then 5-halogenated derivatives might be expected to have selectivity patterns similar to their 6-halogenated congeners, which was not the case. Unhalogenated (**17–18**) and 6-fluorinated (**13**, **19**) derivatives had a preference for the 5-HT<sub>2A</sub> receptor; however, when chlorine or bromine occupies position 6 (**14–16** and **20**) interactions with the 5-HT<sub>2C</sub> receptor were preferred. This further suggests that subtype preference may depend upon the size of the substituent at the 6-position, because fluorine and hydrogen are similar in size, while chlorine and bromine are considerable larger.

Indole and indoline ring halogenation, while important for serotonin receptor affinity, has only occasionally led to compounds with high selectivity between the 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptor subtypes. In a study of racemic 2-(indol-1-yl)-1-methylethylamines mixtures, the 5-methyl derivative had a 13-fold preference for 5-HT<sub>2C</sub> over 5-HT<sub>2A</sub> receptors. Replacement of the 5-methyl group with a halogen (i.e., F, Cl, or Br) increased ligand affinity for both 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> (Table 3).<sup>17</sup> Since the improvement in affinity was somewhat more pronounced for the 5-HT<sub>2C</sub> receptor this led to an increased preference for 5-HT<sub>2C</sub> (threefold

improvement for the 5-bromo) (Table 3).<sup>17</sup> The 6-fluoro derivative had an approximately twofold greater preference for 5-HT<sub>2C</sub> than did the 5-fluoro derivative (Table 3).<sup>17</sup> While comparison is difficult, the most 5-HT<sub>2C</sub> selective enantiomerically pure 5,6-difluorinated derivative, the *S*-enantiomer, only had a twofold improvement over the racemic 6-fluoro derivative. This improvement was mostly due to an increased affinity for 5-HT<sub>2C</sub> (Table 3).<sup>17</sup> In similar structures containing an indoline (i.e., 1-(1-indolinyl)-2-propylamines) instead of an indole, halogenation at the 6- or 5,6-positions results in no improved preference for 5-HT<sub>2C</sub> over 5-HT<sub>2A</sub> receptor as compared to the 6-thiomethyl, which has the highest preference in the series.<sup>18</sup>

In the case of 1-(3-pyridylcarbamoyl)indolines, the unhalogenated parent compound has low affinity for 5-HT<sub>2C</sub> (1.3 μM) and no detectable affinity (>10 μM) for 5-HT<sub>2A</sub> at the highest concentration tested (~8 μM) (Table 3).<sup>19</sup> With the exception of a 6,7-dichloro derivative, all 1-(3-pyridylcarbamoyl)indolines with mono- or dihalogenated substitutions of the indoline ring (regardless of whether the indoline ring also contains a methyl substitution) exhibited moderately increased selectivity for 5-HT<sub>2C</sub> as a function of increased affinity at both 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors (Table 3).<sup>19</sup> Specifically, a 5-chloro or 6-chloro substitution resulted in a respective 32-fold and 16-fold increased selectivity for 5-HT<sub>2C</sub> over 5-HT<sub>2A</sub> receptors. Further improvements in selectivity were observed for 5,6-dichloro or 5-iodo, 6-chloro substitutions over the 6-chloro. Due to the undesirable interaction of 'short' indoline structures with CYP4501A2,<sup>20</sup> the extended 1-[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]indolines, were synthesized and tested.<sup>21</sup> In comparison to the unhalogenated parent compound, substitution of any halogen at position 5 (i.e., F, Cl, Br, I) increased the selectivity for the 5-HT<sub>2C</sub> receptor by as much as 16-fold (Table 3).<sup>21</sup> The 6-chloro derivative was considerably less 5-HT<sub>2C</sub> selective than the 5-chloro derivative due primarily to an increase in the affinity of the 6-chloro derivative for the

**Table 3**Selected structures and affinity values from the literature highlighting the role of indole and indoline ring halogenated on compound selectivity for 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors

Reference	Structure	R group	Affinity ( <i>K<sub>i</sub></i> , nM)		2A/2C <i>K<sub>i</sub></i> ratio
			5-HT <sub>2A</sub>	5-HT <sub>2C</sub>	
17		5-Me ( <i>R,S</i> )	794	63	13
		5-F ( <i>R,S</i> )	159	6.3	25
		5-Cl ( <i>R,S</i> )	200	7.9	25
		5-Br ( <i>R,S</i> )	159	4.0	40
		6-F ( <i>R,S</i> )	251	4.0	63
		5-F, 6-F ( <i>S</i> )	100	1.0	100
		19		Unsubstituted	>10,000
5-Cl	2512			79	32
6-Cl	794			50	16
5,6-Cl	251			7.9	32
4,5-Cl	631			25	25
4-Br, 5-Me	126			7.9	16
4-I, 5-Me	2512			25	100
5-I, 6-Cl	100			3.2	32
5-Cl, 6-Me	398			10	40
5-Me, 6-Cl	159			6.3	25
5-Me, 6-Br	63			4.0	16
5-Me, 6-I	63			3.2	20
6,7-Cl	5012			794	6
21		Unsubstituted	>10,000	100	>100
		5-F	>10,000	40	>251
		5-Cl	>10,000	6.3	>1585
		5-Br	3981	3.2	1260
		5-I	1585	3.2	502
		6-Cl	316	4.00	80
		5,6-Cl	251	2.0	126
		5-F, 6-Cl	501	6.3	79

5-HT<sub>2A</sub> receptor (Table 3).<sup>21</sup> No significant improvements were observed for the 5,6-dichloro or the 5-fluoro, 6-chloro derivatives over the 5-monochlorinated derivative (Table 3).<sup>21</sup>

Here, we report on a low molecular weight aplysinopsin pharmacophore that serves as a versatile chemical scaffold for synthesizing compounds capable of differentiating between cloned human 5-HT<sub>2C</sub> and 5-HT<sub>2A</sub> receptor subtypes. To our knowledge, the aplysinopsin derivatives are the only indole-containing structural class of compounds without a substituent bound to the indole nitrogen that display a significant degree of preference between 5-HT<sub>2C</sub> and 5-HT<sub>2A</sub> receptor subtypes. An additional finding is that the aplysinopsin pharmacophore can be uniquely tuned to select for 5-HT<sub>2A</sub> over 5-HT<sub>2C</sub> (**13**, **19**) or for 5-HT<sub>2C</sub> over 5-HT<sub>2A</sub> (**14–16**, **20**) through a halogen selectivity filter at the 6-position (or 5,6-position) of the indole ring. Selectivity for the 5-HT<sub>2C</sub> receptor subtype was more readily achievable in that compounds could be made that displayed essentially no detectable affinity for 5-HT<sub>2A</sub> or 5-HT<sub>1A</sub> subtypes. Future elaborations on this natural product pharmacophore are likely to produce selective compounds with higher affinities.

## 4. Experimental section

### 4.1. Synthetic procedures: general methods

All chemicals used in this research were reagent grade and were used without purification unless specified. Melting points were determined in a OptiMelt Automated Melting Point System by Stanford Research Systems or a Mel-Temp melting point apparatus and are uncorrected. Column chromatography was carried out using Silica 200–300 mesh from Natland International Corporation. Infrared spectra were determined on nujol mulls samples between sodium chloride plates using a Nicolet Magna 560 FTIR over the frequency range of 4000–600 cm<sup>-1</sup>. <sup>1</sup>H NMR spectra were obtained in DMSO-*d*<sub>6</sub> on a Varian Mercury 300 MHz spectrometer. Chemical shifts ( $\delta$ ) are given in parts per million relative to TMS and coupling constants (*J*) are given in hertz (Hz). The high resolution mass spectra were run at the University of Minnesota using either a Finnigan MAT 95 or Bruker BioTOF mass spectrometer. Compound purities were estimated to be >90% by NMR spectroscopy. When present, peaks representing the solvent DMF are noted.

### 4.2. Synthesis of Indole-3-carboxaldehydes

The procedures utilized for the synthesis of indole-3-carboxaldehydes and their halogenated derivatives were those reported previously by Jiang et al.,<sup>13</sup> Johnson et al.,<sup>15</sup> Kalir and Szara,<sup>22</sup> Noland and Reich,<sup>23</sup> James and Snyder,<sup>24</sup> and Somei et al.<sup>25</sup> Briefly, phosphorus(V) oxychloride (4.25 g, 0.028 mol) was added dropwise to DMF (6 mL) cooled in an ice bath. The mixture was kept at 0 °C for approximately 30 min. A solution of the indole (3 g, 0.015 mol) in DMF (22 mL) was added dropwise, keeping the reaction mixture below 10 °C. After 3 h at 20 °C the solution was poured into an ice-water mixture (180 g), neutralized with 1 N NaOH, and left overnight at room temperature. The crude product was collected by filtration and recrystallized from ethanol/water to give the corresponding indole-3-carboxaldehyde. In all cases the descriptive and analytical values (e.g., melting points and NMR spectra) were identical to those reported previously. Additional data not cited in the initial reports were gathered for some of the indole-3-carboxaldehydes and are reported here: 5-fluoroindole-3-carboxaldehyde, Anal. Calcd for C<sub>9</sub>H<sub>6</sub>NFO: C, 66.26; H, 3.71; N, 8.59; F, 11.64. Found: C, 66.37; H, 3.83; N, 8.60; F, 11.89, and 5-iodoindole-3-carboxaldehyde, Anal. Calcd. For C<sub>9</sub>H<sub>6</sub>NIO: C, 39.88; H, 2.23; N, 5.17, I, 46.82. Found: C, 40.04; H, 2.36; N, 5.19;

I, 46.61. Compounds **8** and **17** have been reported previously.<sup>3</sup> Our <sup>1</sup>H NMR spectra were identical to the reported spectra.

### 4.3. Synthesis of 2-amino-1,3-dimethylimidazolidin-4-one

The procedure used for this synthesis was reported by Kenyon and Rowley.<sup>14</sup>

### 4.4. Synthesis of 2-amino-3-ethyl-1-methylimidazolidin-4-one

The procedure used for this synthesis was reported by Kenyon and Rowley.<sup>14</sup>

### 4.5. Reaction of indole aldehydes with creatinine or alkylated creatinine derivatives

The procedure for this reaction was originally reported by Djura and Faulkner.<sup>3</sup> Indole-3-carboxaldehyde and creatinine (or methylated or ethylated creatinine derivatives) were placed in a 100 mL three-neck round-bottomed flask fitted with a nitrogen gas inlet, and a probe from a J-KEM 210 temperature controller for monitoring the temperature. The flask was then heated cautiously with a Bunsen burner and the temperature was kept at approximately 153 °C for 10 min. Considerable frothing of the mixture took place during the course of the reaction. The reaction mixture was allowed to cool to room temperature and then extracted with methanol (2 × 50 mL). The remaining solid was recrystallized from DMF–water to give the desired product.

### 4.6. 2-Amino-5-(5-bromo-1H-indol-3-ylmethylene)-1-methyl-1,5-dihydro-imidazol-4-one (**1**)

This compound was synthesized and structurally characterized as reported by us recently.<sup>15</sup>

### 4.7. 5-(5-Fluoro-1H-indol-3-ylmethylene)-2-imino-1,3-dimethyl-imidazolidin-4-one (**2**)

This compound was synthesized and structurally characterized as reported by us recently.<sup>15</sup>

### 4.8. (E)-2-Amino-5-[(5-chloro-1H-indol-3-yl)methylene]-1-methyl-1H-imidazol-4(5H)-one (**3**)

5-Chloroindole-3-carboxaldehyde (5.00 g, 0.028 mol) and creatinine (3.43 g, 0.030 mol) were reacted using the procedure described above to give **3**. Recrystallization from DMF–water gave an orange solid (2.68 g, 54% yield), mp 208 °C.

IR (cm<sup>-1</sup>): 3321, 3190, 3159, 1696, 1661 1627 cm<sup>-1</sup>.

<sup>1</sup>H NMR:  $\delta$ : 11.63 (s, 1H), 9.13 (d, *J* = 2.7 Hz, 1H), 8.05 (d, *J* = 2.1 Hz, 1H), 7.96 (s, DMF), 7.73 (s, 2H), 7.44 (d, *J* = 8.7 Hz, 1H), 7.26 (dd, *J* = 8.7 Hz and 1.8 Hz, 1H), 6.55 (s, 1H), 3.30 (s, 3H), 2.86 (s, DMF), 2.70 (s, DMF).

HRMS *m/z* calcd for C<sub>13</sub>H<sub>12</sub>N<sub>4</sub>ClO = 275.0694 (M<sup>+</sup>+H); found 275.0691.

### 4.9. (E)-2-Amino-5-[(5,6-dichloro-1H-indol-3-yl)methylene]-1-methyl-1H-imidazol-4(5H)-one (**4**)

5,6-Dichloroindole-3-carboxaldehyde (1.00 g, 0.0046 mol) and creatinine (0.556 g, 0.0049 mol) were reacted using the procedure described above to afford **4** a red solid compound (0.61 g, 61% yield), mp 190 °C.

IR (cm<sup>-1</sup>): 3457, 3173, 2719, 1675, 1639, 1608, 1531 cm<sup>-1</sup>.

<sup>1</sup>H NMR:  $\delta$ : 11.68 (s, 1H), 9.15 (s, 1H), 8.28 (s, 1H), 7.67 (s, DMF), 6.54 (s, 1H), 3.29 (s, 3H).

HRMS  $m/z$  calcd for  $C_{13}H_{11}N_4C_{12}O$ , 309.0304 ( $M^+ + H$ ); found 309.0303.

**4.10. (E)-2-Amino-5-[(5-fluoro-1H-indol-3-yl)methylene]-1-methyl-1H-imidazol-4(5H)-one (5)**

5-Fluoroindole-3-carboxaldehyde (1.00 g, 0.0074 mol) and creatinine (0.759 g, 0.0071 mol) were reacted using the above procedure to give compound **5** (0.75 g, 75% yield), mp 291–292 °C.

IR ( $cm^{-1}$ ): 3375, 3266, 3151, 2729, 1650, 1621, 1564  $cm^{-1}$ .

$^1H$  NMR:  $\delta$ : 9.15 (d,  $J = 2.7$  Hz), 7.96 (s, DMF), 7.77 (dd,  $J = 10.2$  and 2.4 Hz, 1H), 7.44 (dd,  $J = 9.0$  and 4.7 Hz, 1H), 7.01 (dd,  $J = 9.0$  and 2.4 Hz, 1H), 3.29 (s, 3H), 2.89 (s, DMF), 2.74 (s, DMF).

HRMS  $m/z$  calcd for  $C_{13}H_{12}N_4FO$ , 259.099 ( $M^+ + H$ ); found 259.0992.

**4.11. (E)-2-Amino-5-[(6-fluoro-1H-indol-3-yl)methylene]-1-methyl-1H-imidazol-4(5H)-one (6)**

6-Fluoroindole-3-carboxaldehyde (1.0 g, 0.0074 mol) and creatinine (0.84 g, 0.0074 mol) were reacted according to the above procedure to give **6** (0.61, 61% yield), mp 258–260 °C.

IR ( $cm^{-1}$ ): 3231, 2725, 1667, 1589  $cm^{-1}$ .

$^1H$  NMR:  $\delta$ : 11.49 (d,  $J = 1.8$  Hz), 9.06 (d,  $J = 2.7$  Hz, 1H), 7.95 (s, DMF), 7.91 (dd,  $J = 7.8$  and 6.3 Hz, 1H), 7.71 (s, br, 2H), 7.21 (dd,  $J = 9.8$  and 2.3 Hz), 6.97 (qd,  $J = 3.0$ , 2.4, and 0.9 Hz, 1H), 6.51 (s, 1H), 3.28 (s, 3H), 2.89 (s, DMF), 2.73 (s, DMF).

HRMS  $m/z$  calcd for  $C_{13}H_{12}N_4FO$ , 259.099 ( $M^+ + H$ ); found 259.0992.

**4.12. (E)-2-Amino-5-[(6-chloro-1H-indol-3-yl)methylene]-1-methyl-1H-imidazol-4(5H)-one (7)**

6-Chloroindole-3-carboxaldehyde (1.00 g, 0.0060 mol) and creatinine (0.685 g, 0.0060 mol) were reacted using the above procedure to give **7** (0.44 g, 44% yield), mp 300–302 °C.

IR ( $cm^{-1}$ ): 3448, 3184, 3148, 3080, 1660, 1622  $cm^{-1}$ .

$^1H$  NMR:  $\delta$ : 11.57 (s, 1H), 9.10 (d,  $J = 1.8$  Hz, 1H), 7.93 (d,  $J = 8.4$  Hz, 1H), 7.75 (s, 2H), 7.48 (d,  $J = 2.1$  Hz, 1H), 7.12 (dd,  $J = 8.4$  Hz and 1.8 Hz), 3.28 (s, 3H), 2.89 (s, DMF), 2.73 (s, DMF).

HRMS  $m/z$  calcd for  $C_{13}H_{12}N_4FO$ , 289.085 ( $M^+ + H$ ); found 289.0851.

**4.13. (E)-5-[(1H-Indol-3-yl)methylene]-2-amino-1-methyl-1H-imidazol-4(5H)-one (8)**

The synthesis and structural characterization of this compound has been reported previously.<sup>3</sup> Indole-3-carboxaldehyde (2.00 g, 0.014 mol) and creatinine (1.40 g, 0.012 mol) were reacted using the above procedure to give **8** (1.15 g, 58% yield), mp 287–290 °C.

IR ( $cm^{-1}$ ): 3360, 3184, 2714, 1675, 1650, 1613  $cm^{-1}$ .

$^1H$  NMR:  $\delta$ : 11.47 (s, 1H), 9.09 (d,  $J = 2.7$ , 1H), 7.89 (d,  $J = 7.2$  Hz, 1H), 7.71 (s, 2H), 7.58 (d,  $J = 7.2$ , 1H), 7.13 (m), 6.55 (s, 1H), 3.29 (s, 2H). The NMR is identical to that reported by Djura and Faulkner, 1980.

HRMS  $m/z$  calcd 241.1087 ( $M^+ + H$ ); found 241.1084.

**4.14. (Z)-5-[(5-Bromo-1H-indol-3-yl)methylene]-3-ethyl-2-imino-1-methylimidazolidin-4-one (9)**

5-Bromoindole-3-carboxaldehyde (0.37 g, 0.0017 mol) and 2-amino-3-ethyl-1-methylimidazolidin-4-one (0.233 g, 0.0017 mol) were reacted using the above procedure to give **9** (0.26 g, 70% yield), mp 305–307 °C.

IR ( $cm^{-1}$ ): 3318, 3194, 3148, 1716, 1666, 1612  $cm^{-1}$ .

$^1H$  NMR:  $\delta$ : 12.19 (d,  $J = 8.7$ , 1H), 9.40 (s, 1H), 8.99 (d,  $J = 2.7$ , 1H), 8.39 (d,  $J = 1.5$  Hz, 1H), 7.48 (d,  $J = 8.7$ , 1H), 7.36 (dd,  $J = 8.9$  and 2.0 Hz, 1H), 7.34 (s, 1H), 3.79 (m,  $J = 7.5$  Hz, 2H), 1.19 (t,  $J = 7.2$ , 3H).

HRMS  $m/z$  calcd for  $C_{15}H_{16}N_4BrO$ , 347.502 ( $M^+ + H$ ); found 347.508.

**4.15. (E)-5-[(5-Chloro-1H-indol-3-yl)methylene]-2-imino-1,3-dimethylimidazolidin-4-one (10)**

5-Chloroindole-3-carboxaldehyde (3.00 g, 0.017 mol) and 2-amino-1,3-dimethylimidazolidin-4-one (2.31 g, 0.018 mol) were reacted according to the above procedure to give **10** 1.79 g, 60% yield, mp 245–246 °C.

IR ( $cm^{-1}$ ): 3354, 3113, 2361, 1719, 1691, 1654, 1631  $cm^{-1}$ .

$^1H$  NMR:  $\delta$ : 11.64 (s, 1H), 8.75 (d,  $J = 1.2$  Hz, 1H), 8.039 (d,  $J = 1.2$  Hz, 1H), 7.44 (d,  $J = 8.7$  Hz, 1H), 7.15 (dd,  $J = 8.7$  and 2.1 Hz, 1H), 3.27 (s, 3H), 3.066 (s, 3H).

HRMS  $m/z$  calcd for  $C_{14}H_{14}N_4ClO$ , 289.0851 ( $M^+ + H$ ); found 289.0841.

**4.16. (E)-5-[(5-Bromo-1H-indol-3-yl)methylene]-2-imino-1,3-dimethylimidazolidin-4-one (11)**

The synthesis and structural characterization of this compound has been reported previously.<sup>26</sup> 5-Bromoindole-3-carboxaldehyde (3.78 g, 0.017 mol) and 2-amino-1,3-dimethylimidazolidin-4-one (2.15 g, 0.017 mol) were reacted according to the above procedure to give **11** as a dark red solid (1.33 g, 35% yield), mp at 280–281 °C.

IR ( $cm^{-1}$ ): 3353, 3251, 3121, 2722, 1722, 1653, 1630  $cm^{-1}$ .

$^1H$  NMR:  $\delta$ : 11.62 (s, 1H), 8.74 (s, 1H), 8.18 (d,  $J = 2.1$  Hz, 1H), 7.39 (d,  $J = 8.7$  Hz, 1H), 7.26 (dd,  $J = 8.4$  and 1.8 Hz, 1H), 6.44 (s, 1H), 3.27 (s, 3H, N), 3.08 (s, 3H).

HRMS  $m/z$  calcd for  $C_{14}H_{14}N_4BrO$ , 333.0345 ( $M^+ + H$ ); found 333.0346.

**4.17. (Z)-3-Ethyl-2-imino-5-[(5-iodo-1H-indol-3-yl)methylene]-1-methylimidazolidin-4-one (12)**

5-Iodoindole-3-carboxaldehyde (0.50 g, 0.0018 mol) and 2-amino-3-ethyl-1-methylimidazolidin-4-one (0.48 g, 0.0034 mol) were reacted using the procedure described previously to give **12** (0.39 g, 78% yield), mp 290–292 °C.

IR ( $cm^{-1}$ ): 3329, 3189, 2719, 1716, 1666, 1612  $cm^{-1}$ .

$^1H$  NMR:  $\delta$ : 12.15 (s, 1H), 8.95 (d,  $J = 2.7$  Hz, 1H), 8.53 (s, 1H), 7.51 (dd,  $J = 8.9$  and 2.0 Hz, 1H), 7.36 (d,  $J = 8.7$  Hz, 1H), 7.29 (s, 1H), 3.79 (q,  $J = 6.9$  Hz, 2H), 1.19 (t,  $J = 6.9$  Hz, 3H).

HRMS  $m/z$  calcd for  $C_{15}H_{16}N_4IO$ , 395.0363 ( $M^+ + H$ ); found 395.0385.

**4.18. (E)-5-[(6-Fluoro-1H-indol-3-yl)methylene]-2-imino-1,3-dimethylimidazolidin-4-one (13)**

6-Fluoroindole-3-carboxaldehyde (0.50 g, 0.0037 mol) and 2-amino-1,3-dimethylimidazolidin-4-one (0.467 g, 0.0037 mol) were reacted according to the above procedure to give **13** (0.22 g, 44% yield), mp 260–264 °C.

IR ( $cm^{-1}$ ): 3359, 3124, 3072, 1721, 1650, 1633 1593  $cm^{-1}$ .

$^1H$  NMR:  $\delta$ : 11.51 (s, 1H), 8.68 (d,  $J = 2.1$ , 1H), 7.89 (dd,  $J = 9.0$  and 5.7 Hz, 1H), 7.21 (dd,  $J = 9.0$  and 2.4 Hz, 1H), 6.96 (td,  $J = 9.0$ , 2.3 and 1.8 Hz, 1H), 6.42 (s, 1H), 3.25 (s, 3H), 3.06 (s, 3H).

HRMS  $m/z$  calcd for  $C_{14}H_{14}N_4FO$ , 273.1146 ( $M^+ + H$ ); found 273.1143.

**4.19. (E)-5-[(6-Chloro-1H-indol-3-yl)methylene]-2-imino-1,3-dimethylimidazolidin-4-one (14)**

6-Chloroindole-3-carboxaldehyde (1.00 g, 0.0060 mol) and 2-amino-1,3-dimethylimidazolidin-4-one (0.77 g, 0.0061 mol) were reacted according to the above procedure to give **14** (0.74 g, 74% yield), mp 278–280 °C.

IR (cm<sup>-1</sup>): 3340, 3128, 3063, 1723, 1646, 1632 cm<sup>-1</sup>.

<sup>1</sup>H NMR: δ: 11.57 (s, 1H), 8.70 (s, 1H), 7.91 (d, *J* = 8.7 Hz), 7.47 (d, *J* = 1.8), 7.17 (dd, *J* = 8.4 and 1.5 Hz, 1H), 6.75 (s, 1H), 6.40 (s, 1H), 3.34 (s, 3H), 3.25 (s, 3H).

HRMS *m/z* calcd for C<sub>13</sub>H<sub>12</sub>N<sub>4</sub>ClO, 289.085 (M<sup>+</sup>+H); found 289.0851.

**4.20. 5-(6-Bromo-1H-indol-3-yl)methylene)-2-imino-1,3-dimethyl-imidazolidin-4-one (15)**

6-Bromoindole-3-carboxaldehyde (1.00 g, 0.0045 mol) and 2-imino-1,3-dimethyl-imidazolidin-4-one (0.567 g, 0.0045 mol) were reacted using the procedure described above to give **15** as orange needles (0.550 g, 56%), mp 281–282 °C.

IR (cm<sup>-1</sup>): 3333, 3122 (w), 3065 (w), 1724, 1646, 1632 cm<sup>-1</sup>.

<sup>1</sup>H NMR: δ: 11.58 (s, 1H), 8.69 (s, 1H), 7.87 (d, *J* = 9 Hz, 1H), 7.61 (d, *J* = 1.5 Hz), 7.23 (dd, *J* = 9 and 1.5 Hz, 1H), 6.75 and 6.54 (two s, br, 0.6H and 0.4H), 6.39 (s, 1H), 3.25 (s, 3H), 3.06 (s, 3H).

HRMS *m/z* calcd for C<sub>14</sub>H<sub>14</sub>N<sub>4</sub>BrO, 333.0345 (M<sup>+</sup>+H); found 333.0346.

Ultraviolet-visible spectrum: λ<sub>max</sub> 381 nm ε = 4.50.

**4.21. (E)-5-[(5,6-Dichloro-1H-indol-3-yl)methylene]-2-imino-1,3-dimethylimidazolidin-4-one (16)**

5,6-Dichloroindole-3-carboxaldehyde (0.300 g, 0.0014 mol) and 2-amino-1,3-dimethylimidazolidin-4-one (0.1907 g, 0.0015 mol) were reacted using the above procedure to give **16** (0.15 g, 50% yield), mp 214 °C.

IR (cm<sup>-1</sup>): 3341, 2719, 1723, 1698, 1655, 1632 cm<sup>-1</sup>.

<sup>1</sup>H NMR: δ: 11.7 (s, 1H), 8.76 (d, *J* = 1.5 Hz, 1H), 8.28 (s, 1H), 7.95 (s, DMF), 7.68 (s, 1H), 6.41 (s, 1H), 3.26 (s, 3H), 3.05 (s, 3H), 2.89 (s, DMF), 2.73 (s, DMF).

HRMS *m/z* calcd for C<sub>14</sub>H<sub>13</sub>N<sub>4</sub>Cl<sub>2</sub>O, 323.0461 (M<sup>+</sup>+H); found 323.0462.

**4.22. (E)-5-((1H-Indol-3-yl)methylene)-2-imino-1,3-dimethylimidazolidin-4-one (17)**

The synthesis and structural characterization of this compound has been reported previously.<sup>3</sup> Indole-3-carboxaldehyde (0.500 g, 0.0034 mol) and 2-amino-1,3-dimethylimidazolidin-4-one (0.437 g, 0.0034 mol) were reacted using the above procedure to give **17** (0.25 g, 50% yield), mp 245–247 °C.

IR (cm<sup>-1</sup>): 3417, 3303, 2729, 2662, 1712, 1634 cm<sup>-1</sup>.

<sup>1</sup>H NMR: δ: 11.48 (s, 1H), 8.70 (d, *J* = 2.7 Hz, 1H), 7.88 (d, *J* = 7.2 Hz, 1H), 7.42 (dd, *J* = 7.3 and 1.4 Hz, 1H), 7.13 (m), 6.44 (s, 1H), 3.23 (s, 3H), 3.07 (s). NMR is identical to that reported by Djura and Faulkner, 1980.

HRMS *m/z* calcd 255.1246 (M<sup>+</sup>+H); found 255.124.

**4.23. (Z)-5-((1H-Indol-3-yl)methylene)-3-ethyl-2-imino-1-methylimidazolidin-4-one (18)**

Indole-3-carboxaldehyde (0.500 g, 0.0031 mol) and 2-amino-3-ethyl-1-methylimidazolidin-4-one (0.97 g, 0.0069 mol) were reacted using the above procedure to give **18** (0.100 g, 20% yield), mp 320–324 °C.

IR (cm<sup>-1</sup>): 3450, 3238, 3189, 2725, 2662, 1729, 1663, 1662, 1546 cm<sup>-1</sup>.

<sup>1</sup>H NMR: δ: 12.08 (d, *J* = 2.4 Hz, 1H), 9.35 (s, 1H), 9.00 (d, *J* = 3 Hz, 1H), 8.08 (dd, *J* = 6.6 and 2.5, 1H), 7.52 (dd, *J* = 6.3 and 2.4 Hz, 1H), 7.35 (s, 1H), 7.25 (m), 3.8 (q, *J* = 7.2 Hz, 2H), 1.20 (t, *J* = 7.2, 3H).

HRMS *m/z* calculated for C<sub>15</sub>H<sub>17</sub>N<sub>4</sub>O, 269.1397 (M<sup>+</sup>+H); found 269.1406.

**4.24. (E)-3-Ethyl-5-[(6-fluoro-1H-indol-3-yl)methylene]-2-imino-1-methylimidazolidin-4-one (19)**

6-Fluoroindole-3-carboxaldehyde (0.500 g, 0.0037 mol) and 2-amino-3-ethyl-1-methylimidazolidin-4-one (0.43 g, 0.0031 mol) were reacted according to the above procedure to give compound **19** (0.27, 54% yield), mp 330–331 °C.

IR (cm<sup>-1</sup>): 3230, 3193, 3070, 2713, 1731, 1664, 1624 cm<sup>-1</sup>.

<sup>1</sup>H NMR: δ: 12.08 (s, 1H), 9.36 (s, 1H), 8.97 (d, *J* = 2.7 Hz, 1H), 8.11 (dd, *J* = 9.0 and 5.4 Hz, 1H), 7.33 (dd, *J* = 9.0 and 3.2 Hz, 2H), 7.12 (td, *J* = 3.0, 2.4, and 2.1 Hz), 3.80 (q, *J* = 7.2 Hz, 2H), 3.51 (s, 3H), 1.19 (t, *J* = 6.0 Hz, 3H).

HRMS *m/z* calcd for C<sub>15</sub>H<sub>16</sub>N<sub>4</sub>FO, 287.1303 (M<sup>+</sup>+H); found 287.1312.

**4.25. (E)-5-[(6-Bromo-1H-indol-3-yl)methylene]3-ethyl-2-imino-1-methylimidazolidin-4-one (20)**

6-Bromoindole-3-carboxaldehyde (0.500 g, 0.0022 mol) and 2-amino-3-ethyl-1-methylimidazolidin-4-one (0.314 g, 0.0022 mol) were reacted as previously described to give compound **20** (0.12 g, 24% yield), mp 310–315 °C.

IR (cm<sup>-1</sup>): 3205, 3091, 2725, 1737, 1682, 1633 cm<sup>-1</sup>.

<sup>1</sup>H NMR: δ: 12.11 (s, 1H), 9.37 (s, 1H), 8.97 (d, *J* = 2.7, 1H), 8.07 (d, *J* = 8.7 Hz, 1H), 7.72 (d, *J* = 0.9, 1H), 7.37 (dd, *J* = 8.4 and 1.5 Hz, 1H), 7.31 (s, 1H), 3.79 (m, *J* = 7.2 Hz, 2H), 1.19 (t, *J* = 7.05, 3H).

HRMS *m/z* calcd for C<sub>15</sub>H<sub>16</sub>N<sub>4</sub>BrO, 347.0502 (M<sup>+</sup>+H); found 347.0505.

**4.26. Preparation of crude membranes for binding assays**

Cells derived from human embryonic kidney cells (HEK293) were employed as hosts for the expression of cloned human serotonin 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors. As previously described,<sup>27</sup> HEK293 cells transfected by calcium phosphate precipitation were put under selective drug pressure to select for the stable expression of membrane bound receptor. Briefly, 20 μg of purified plasmid DNA containing genes for the receptor of interest and for selection by drug resistance was mixed to a final volume of 1 mL calcium phosphate/HEPES buffered saline solution and then added dropwise to a 150 cm<sup>2</sup> plate containing adherent HEK293 cells at 5–10% confluence in sterile growth medium (DMEM supplemented with 100 μM sodium pyruvate and 10% BCS). The transfection medium was replaced on the following morning (approximately 12 h post-transfection) with fresh growth medium containing 2 mg/mL of the selection agent geneticin (G418). Cells were grown under constant G418 selection (medium changed every 3–4 days) with colony selection by sterile cylinder isolation after several pinhead-sized colonies were visually apparent and confirmed by microscopy (approximately 2 weeks). The expression level of the serotonin receptor subtype in individual clones was determined by radioligand saturation isotherm binding assays that utilized the standard rapid filtration techniques previously described by our lab.<sup>27</sup>

Cell membranes were prepared for radioligand binding assays through a standard laboratory procedure.<sup>27</sup> Briefly, cells were detached by a 15 min incubation in 10 mL of Dulbecco's phosphate buffer saline (D-PBS) without Ca<sup>2+</sup> and Mg<sup>2+</sup> and supplemented



with 5 mM ethylenediaminetetraacetic acid (EDTA). Detached cells were pipetted into a 50 mL conical centrifugation tube and the remainder of the tube volume filled with Earle's balanced salts solution (EBSS). This cell suspension was pelleted in a Sorvall Legend RT centrifuge at 800 rpm (700g) for 10 min. The supernatant was decanted, the cell pellet was resuspended in 10 mL of lysing buffer (5 mM MgCl<sub>2</sub>, 5 mM Tris, pH 7.4 at 4 °C), homogenized using eight full strokes of a Dounce glass homogenizer and then poured into a high speed centrifugation tube. This homogenate was balanced for centrifugation through the addition of approximately 25 mL of cold binding buffer (50 mM Tris, pH 7.4 at 4 °C) before 30 min of centrifugation at 13,000 rpm (28,000g). After decanting the supernatant, the resulting crude membrane pellet was resuspended in a small amount of cold binding buffer (approximately 6 mL), rebalanced and recentrifuged a final 30 min. The resulting membrane pellet was resuspended in an appropriate volume of cold binding buffer, homogenized by four strokes of the Dounce homogenizer, and kept on ice for same day use.

#### 4.27. Radioligand binding assays

Membranes expressing cloned human 5-HT1A, 5-HT2A, or 5-HT2C receptors were assayed by saturation isotherm analysis of [<sup>3</sup>H]MPPF (4-(2'-methoxy)-phenyl-1-[2'-(N-2"-pyridinyl)-p-fluorobenzamido]ethyl-piperazine) (79.8 Ci/mmol Perkin-Elmer, NET-1109), [<sup>3</sup>H]methylpiperone (N-methyl-8-[4-(4-fluorophenyl)-4-oxobutyl]-1-phenyl-1,3,8-triazaspiro-[4.5]decan-4-one) (84 Ci/mmol, Perkin-Elmer, NET-856) or [<sup>3</sup>H]mesulergine (N'-(1-methyl-6-methylergolin-8α-yl)-N,N-dimethylsulfamide) (80 Ci/mmol, GE Healthcare, TRK845) specific binding activities, respectively. The values for 5-HT1A, 5-HT2A, and 5-HT2C radioligand affinities (K<sub>D</sub> ± SD (pM)) were 267 ± 36, 246 ± 42, and 1803 ± 726 with corresponding receptor density (pmol/mg membrane protein) values 3.1 ± 1.7, 4.2 ± 2.3, and 14 ± 7, respectively. Nonspecific binding activity was defined by 5 μM NAN-190 (1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine) for 5-HT1A or 5 μM mianserin (1,2,3,4,10,14b-hexahydro-2-methyl-dibenzo[c,f]pyrazino[1,2-a]azepine) for 5-HT2A and 5-HT2C receptors. A target concentration of approximately 500 pM radioligand was utilized for the competition binding experiments and actual concentrations of radioligand were determined by scintillation counting. Mirtazapine (1,2,3,4,10,14b-hexahydro-2-methylpyrazino[2,1-a]pyrido[2,3-c][2]benzazepine) was used during the single point binding assessments as a high affinity 5-HT2A/2C reference compound (data not shown). As described previously,<sup>27,28</sup> all radioligand assays were allowed at least 90 min to reach equilibrium at 25 °C prior to rapid filtration through GF/C filters pre-soaked for 10 min in 0.5% polyethyleneimine. Radioligand-receptor complexes were rapidly filtered and washed with 3 × 3 mL of ice-cold binding buffer (50 mM Tris pH 7.4 at 2 °C). The radioactivity of each sample was determined using a liquid scintillation counter (Perkin-Elmer Tri-Carb 2800TR). Membrane protein concentrations were determined with a bicinchoninic acid assay using a bovine serum albumin (BSA) standard curve. The aplysinopsin analogues were prepared at an initial concentration of 3 mM in DMSO with the exception of **15** which was prepared at a concentration of 1 mM in DMSO. These solutions were then diluted to 1:1000 v/v for the final assay solution. For competition assays covering a range of concentrations, stock solutions of test compounds were dissolved at the highest concentrations possible in either DMSO or 95:5 v/v of DMSO:0.01 N HCl, then diluted in binding buffer while not exceeding a 1:300 v/v ratio of non-aqueous solvent.

#### 4.28. Calculations and data analyses

Each data point was sampled in triplicate and each experiment was repeated three to four times. Data are reported as the geomet-

ric mean of three to four experiments with standard deviations (tables) or standard errors (graphs). The inhibition constants (K<sub>i</sub>) were calculated from IC<sub>50</sub> values using the Cheng-Prusoff equation:  $K_i = IC_{50}/(1 + [ligand]/K_D)$ . In cases where the displacement was >20% but less than 100% at the highest concentration of inhibitor, IC<sub>50</sub> values were calculated by setting the bottom of the curve fit to zero. A 95% confidence interval was employed for all curve-fitting procedures using Graphpad's Prism software version 4.0.

#### Acknowledgments

Special thanks to Dr. Pilar Rodriguez-Loaiza for helpful insights and suggestions regarding the synthesis strategy. The authors wish to thank Greta D'Ambrosia for assistance in early phases of this work. This study was supported by the National Institutes of Health [Grants R01-MH063162 and R25-GM55380 (J.E.J.)], the National Science Foundation DBI-0649889 (J.A.S) competitive institutional funds G67710 (J.A.S.), The Robert A. Welch Foundation M-200 (J.E.J.), and The Texas Woman's University Research Enhancement Program (J.E.J.).

#### Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.05.017.

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