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APORPHINOID ANTAGONISTS OF 5-HT_{2A} RECEPTORS: FURTHER EVALUATION OF RING A SUBSTITUENTS AND THE SIZE OF RING C

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Abstract

A series of ring A modified analogs of nantenine as well as structural variants in ring C were synthesized and evaluated for antagonist activity at 5-HT_{2A} and α_{1A} receptors. Halogenation improves 5-HT_{2A} antagonist potency in molecules containing a C1 methoxyl/C2 methoxyl or C1 methoxyl/C2 hydroxyl moiety. Bromination or iodination (but not chlorination) with the latter moiety also significantly increased α_{1A} antagonist potency. Homologation or contraction of ring C adversely affected antagonist activity at both receptors, implying that a six-membered ring C motif is beneficial for high antagonist potency at both receptors. Molecular docking studies suggest that the improved antagonist activity (by virtue of improved affinity) of C3 halogenated aporphines in this study, is attributable to favorable interactions with the C3 halogen and F339 and/or F340.

Keywords

5-HT_{2A}, α_{1A} , Aporphine; Nantenine; Antagonist; SAR

Aporphine alkaloids have been found to possess a diverse range of pharmacological activities including cytotoxic,(1–5) anticancer,(6) antituberculosis,(7) antiplasmodial,(4, 8) and anticholinesterase(9, 10) activities. They have been well studied as ligands for central nervous system (CNS) receptors, in particular at 5-HT_{1A},(11–16) dopamine D₁,(17) dopamine D₂₍₁₆₎ and α_1 adrenergic receptors(18, 19). Potent and selective aporphine-derived agonists or antagonists have been identified at these receptors.(11, 20, 21) (*R*)-Apomorphine (Figure 1, 1) is perhaps the most well-known aporphine alkaloid and is

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

SUPPLEMENTARY INFORMATION

Experimental details for synthesis of new compounds, NMR spectral data for new compounds and additional figures with ligand poses, are provided as supporting information.

clinically available as a treatment for Parkinson's disease (by virtue of its dopaminergic activity).(22, 23)

Given the pharmacological promiscuity across CNS targets, we consider the aporphine template to be a "privileged CNS scaffold" and one that is amenable to the identification of potent and selective receptor ligands. Our research program is particularly interested in the evaluation of aporphines as serotonin 5-HT_{2A} receptor antagonists. 5-HT_{2A} receptor antagonism has demonstrated clinical relevance in the treatment of CNS-related disorders including schizophrenia, anxiety and depression.(24–27) Recent experiments indicate that selective 5-HT_{2A} antagonism shows promise in the treatment of sleep disorders. In addition, it has become apparent that this receptor plays a significant role in psychostimulant abuse. New bioactive chemotypes will supplement our armamentarium of useful probes and pharmaceuticals relevant to neuropsychiatric diseases and stimulant abuse.

Our previous structure-activity relationship (SAR) studies have been conducted with the aporphine alkaloid nantenine (**2**) - a selective α_{1A} antagonist with modest 5-HT_{2A} antagonist properties.(28, 29) These SAR studies have largely focused on ring A of the aporphine scaffold as well as the importance of conformational flexibility. We have found that some C1 alkoxy substituents had significantly improved 5-HT_{2A} antagonist activity and selectivity vs α_{1A} receptors as compared to nantenine. Evaluation of a group of C2 alkoxy analogs indicated that small alkoxy groups (ethoxy and *n*-propyloxy) improved 5-HT_{2A} antagonist activity and α_{1A} selectivity. However, larger C2 alkoxy groups caused loss in activity at both receptors. Additionally, the C3 position of nantenine is tolerant of a bromine substituent with regards to 5-HT_{2A} antagonism. Indeed, 3-bromonantenine, **3** (the most potent aporphine-based 5-HT_{2A} antagonist identified to date) had significantly improved 5-HT_{2A} antagonist activity as compared to nantenine and had excellent selectivity vs the α_{1A} receptor. With regards to conformational flexibility, it was discovered that cleavage of ring C was detrimental to activity at both receptors in question. However, we have not investigated the effect of variation of the size of ring C to date.

In line with our efforts to further understand the structural tolerance for antagonist activity and selectivity of the aporphine scaffold at the 5-HT_{2A} receptor, we describe in this manuscript the synthesis and evaluation of a series of ring A nantenine analogs as well as various ring C aporphine congeners. Given the fact that nantenine is a selective α_{1A} antagonist, assays were also performed at the α_{1A} receptor as a first step in gauging selectivity.

METHODS AND MATERIALS

General experimental procedures

All reactions were carried out in oven-dried glassware under a nitrogen atmosphere. Dry DMSO and all other reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. A CEM Discover microwave reactor was used to carry out all direct arylation reactions. HRESIMS spectra were obtained using an Agilent 6520 QTOF instrument. ¹H NMR and ¹³C NMR spectra were recorded using Bruker DPX-500 spectrometer (operating at 500 MHz for ¹H; 125 MHz, respectively,

for ^{13}C) using CDCl_3 as solvent. Tetramethylsilane (δ 0.00 ppm) served as an internal standard in ^1H NMR and CDCl_3 (δ 77.0 ppm) in ^{13}C NMR as solvent. Reactions were monitored by TLC with Whatman Flexible TLC silica gel G/UV 254 precoated plates (0.25 mm). TLC plates were visualized by UV (254 nm) and by staining with vanillin spraying reagent (2 gm vanillin in 1 L of 10% H_2SO_4) followed by heating. Flash column chromatography was performed with silica gel 60 (EMD Chemicals, 230–400 mesh, 0.063 mm particle size). All chemicals and reagents were obtained from Sigma-Aldrich and Fischer Scientific (USA) and were used without further purification.

K_e determination in calcium assays

Compounds that showed no intrinsic activity in the functional assay and inhibited the increase in basal fluorescence elicited by EC_{80} of 5-HT by at least 50%, had their K_e (apparent affinity in a functional assay) determined. The K_e data were obtained by monitoring the ability of test compounds to inhibit serotonin-mediated stimulation of the human 5-HT $_2\text{A}$ receptor heterologously expressed in HEK293 cells. Agonist dose response curves were run in the presence or absence of a single concentration of test compound. K_e values were determined by running an 8-point half-log 5-HT concentration response curve in the presence and absence of a single concentration of antagonist. EC_{50} values were calculated for 5-HT (A) and 5-HT + test compound (A'), and these values used to calculate the test compound K_e using the formula: $K_e = [\text{L}]/(\text{DR}-1)$, where [L] equals the concentration of test compound in the assay and DR equals the dose ratio or A'/A. At least two different concentrations of test compound were used to calculate the K_e , and their concentrations were chosen such that the agonist EC_{50} exhibited at least a 4-fold shift to the right. K_e data were obtained similarly for the $\alpha_{1\text{A}}$ receptor via inhibition of norepinephrine-mediated stimulation of the human adrenergic $\alpha_{1\text{A}}$ receptor expressed in CHO K1 cells.

ICM Pro Procedures

Ligand Preparation—Nantenine and all analogs were drawn as 2D structures with a formal positive charge centered on the nitrogen, and then energy minimized through Chem3D Ultra version 9.0/MOPAC, Job Type: Minimum RMS Gradient of 0.010 kcal/mol and RMS distance of 0.1 Å, and saved as MDL MolFiles (*.mol) for purpose of docking with ICM Pro.

Homology Modeling, Docking and Visualization—Details for preparation of the 5-HT $_2\text{A}$ homology model may be found in a prior publication.(29) The following is a brief description of the docking procedure. The binding site of homology receptors was identified. The binding site was reviewed and adjusted: ICM made a box around the ligand binding site based on the information entered in the receptor setup section. The position of the box encompassed the residues expected to be involved in ligand binding. Then the receptor maps were made: Energy maps of the environment within the docking box were constructed. Resolution of the maps was set to 0.5. Interactive docking was used for all compounds. The thoroughness level was set to the maximum value of 10. Once docked all the receptor-ligand complexes were energy minimized and re-docked, and visualized in 3D using ICM Pro v 3.6. ICM calculated a score for the complexes. Poses depicted in this manuscript are for the highest ranked complexes. H-bonds and hydrophobic interactions were calculated and

plotted with Maestro. H-bonds are indicated by a solid line between the atoms involved, while hydrophobic residues are represented by green spheres and hydrophilic residues by blue spheres in Maestro.

RESULTS AND DISCUSSION

Our previous work suggested that ring A substituents play a crucial role in imparting antagonist activity of the nantenine template at the 5-HT_{2A} receptor. Optimal placement of ring A substituents is likely to be very important for obtaining high potency antagonists. With this in mind, we decided to further investigate the tolerability of halogenation at the C3 position as well as the requirement for a C2 methoxy group.

Phenol **4**, prepared as described previously,(28) was used as a key intermediate for synthesizing several ring A analogs. With compound **4** in hand, the ring A analogs **5**, **7a–c** and **9** were prepared as depicted in Scheme 1. Compound **5** was obtained by first removing the *N*-boc protecting group and then methylating the resulting secondary amine.

Halogenation (bromination, chlorination and iodination) of the C3 position of **4** was achieved with the use of the respective *N*-halosuccinimides. To obtain compounds **7a–c** we initially tried reduction of compounds **6a–c** with LAH. However, this was accompanied by substantial dehalogenation. Alternatively, compounds **7a–c** were prepared via *N*-Boc-deprotection of **6a–c** followed by reductive amination as was done for compound **5**. *O*-methylation of **6a** via standard Williamson ether synthesis afforded compound **8**. This compound was then transformed to the target analog **9** via a similar deprotection/reductive amination sequence as previously described for compounds **5** and **7a–c**.

For the synthesis of analog **14** Scheme 2 was deployed. Triflation of **4** followed by Sonogashira coupling afforded the C2 alkynes **11**. Removal of the TMS protecting group furnished compound **12**. Saturation of the alkyne bond of **12** by catalytic hydrogenation gave **13**. Subsequently, *N*-Boc deprotection and *N*-alkylation via reductive amination afforded compound **14**.

Homoaporphine **15** (Figure 2) was prepared as previously described.(30) Analogs **16–18** were synthesized by LAH reduction of the corresponding *N*-ethylcarbamate derivatives (**19**; prepared as previously described)(31)

All analogs were screened at 10μM in multi-well format for intrinsic (agonist) and antagonist activity at the human 5-HT_{2A} receptor using FLIPR-based (Molecular devices, Sunnydale, CA) functional assays that detect receptor-mediated mobilization of internal calcium with a calcium sensitive fluorescent dye (as previously described). (28) A similar set of assays was performed for the α_{1A} - adrenergic receptor. Data from these evaluations are presented in Table 1.

Ring A – C3 halogenated analogs

Replacement of the C2 methoxyl group of nantenine (**2**) with a hydroxyl group (ie compound **5**) resulted in a 3-fold increase in 5-HT_{2A} antagonism; this change caused diminished (7-fold) α_{1A} antagonist activity as compared to nantenine. The addition of a

halogen substituent to C3 of compound **5** (ie compounds **7a–c**) was associated with a doubling of 5-HT_{2A} antagonist potency, irrespective of the halogen group present. Antagonism of the α_{1A} receptor was highest for the C3 bromo and C3 iodo derivatives (**7b** and **7c** respectively), representing a quadrupling in activity as compared to **5**. However, the presence of a C3 chloro group (compound **7a**) caused a drop in α_{1A} antagonism with reference to compound **5** (1279 vs 255 nM for **7a** and **5** respectively). The C3 chloro derivative **9** showed significant improvement in 5-HT_{2A} antagonist activity as compared to nantenine. In fact, compound **9** was the most potent 5-HT_{2A} antagonist identified in this study. When compound **9** is compared to **7a**, it is apparent that methylation of the C2 phenol of **7a** results in improved 5-HT_{2A} potency (130 vs 63 nM for **7a** and **9** respectively). The analogous 3-bromonantenine (**3**) displayed similar 5-HT_{2A} activity to 3-chloronantenine (**9**). However, **9** showed greater antagonist potency at the α_{1A} receptor than 3-bromonantenine, indicating that C3 bromination is associated with greater 5-HT_{2A} selectivity than C3 chlorination. One generalization from analysis of this series is that halogenation is very well tolerated at the C3 position for 5-HT_{2A} antagonism when either a C2 hydroxyl or C2 methoxyl group is present. A C2 methoxyl substituent also seems to be slightly better for 5-HT_{2A} antagonism than a C2 hydroxyl substituent when a C3 halo group is present (ie compare **7a** and 3-chloronantenine, **9**; **7b** and 3-bromonantenine, **3**).

The effect of C3 halogenation has been studied by others with other 1,2,9,10-tetrasubstituted aporphines at dopamine D₁ and D₂ receptors.(32) C3 halogenation was associated with improved D₁ affinity and selectivity vs D₂ receptors in the case of the aporphines boldine (**20**) and predicine (**21**). However, C3 halogenation of another aporphine alkaloid glaucine (**22**), resulted in decreased affinity for D₁ and D₂ receptors.

Ivorra *et al* have studied the effect of C3 halogenation of boldine (**20**) on affinity for α_1 adrenergic receptor subtypes (ie α_{1A} , α_{1B} and α_{1D} receptors).(33) Here it was found that bromination or chlorination at C3 improved affinity and selectivity for α_{1A} receptors. Based on the structural similarity of compounds **7a–c** and **20** with regards to the ring A moiety, our work supports prior observations of improved α_{1A} affinity of C2 hydroxyl/C1 methoxyl substituted aporphines upon C3 bromination. However, in the case of chlorination, a reduction in α_{1A} antagonist activity is observed which tends to suggest that the substituent groups on ring D are also important in this instance.

Ring A – C2 analogs

As mentioned earlier, previous SAR studies indicated that small alkoxy groups at the C2 position of the nantenine parent template imparted marginally increased 5-HT_{2A} antagonist activity. However, the importance of the C2 oxygen atom was not determined. Evaluation of compound **14** suggests that the C2 oxygen atom is somewhat important for antagonism of the receptor since its removal gave reduced activity at both receptors. (The fact that moderate activity is retained in compound **14** at both receptors, tends to suggest that the C2 oxygen atom, though important is not *absolutely* required for antagonism of both receptors). Given the magnitude of this activity (though moderate), it does not seem likely that the C2 oxygen atom is involved in very strong hydrogen-bonding interactions with either receptor.

Ring C analogs

When compared to nantenine (**2**), homologation of ring C by one carbon atom to provide a homoaporphine skeleton (compound **15**) decimated antagonist activity at both receptors. A similar situation ensued upon contraction of ring C by one carbon atom to give an indeno[1,2,3-*ij*]isoquinoline scaffold (compound **16**). As compared to **16**, there was a slight improvement in α_{1A} antagonist activity when the methylenedioxy unit in ring D was replaced with a 9,10-dimethoxy moiety (ie compound **17**). The absence of substituents in ring D however (compound **18**) reduced antagonist activity at both receptors. It is possible that other combinations of substituent groups in ring D of the homoaporphine or indeno[1,2,3-*ij*]isoquinoline scaffold may engender higher antagonist activity at these receptors. However the group of compounds examined is too small to validate such a premise. It also remains to be determined the extent to which substituents in ring A affect activity at both receptors with either homoaporphine or indeno[1,2,3-*ij*]isoquinoline scaffolds. Overall, evaluation of this set of analogs reinforces our previous conclusions from ring C truncated analogs - the tetracyclic nucleus of aporphines is preferred for antagonism of 5-HT_{2A} and α_{1A} receptors.

In order to rationalize the improved affinity of the C3 halogenated compounds at the 5-HT_{2A} receptor, we the compounds into a homology model of the 5-HT_{2A} receptor. The homology model was prepared as previously described with ICM Pro using a human β_2 -adrenoceptor template.(28) Docking was performed with ICM Pro software as described previously.(29)

Figure 4 shows an overlay of binding poses for compounds **3**, **7b** and **9**. These compounds allow for some qualitative comparison of the effect of different halogen atoms at C3 (ie **3** vs **9**) as well as the effect of the presence of a methoxyl or hydroxyl group at C2 (ie **3** vs **7b**). It is evident from this figure that these compounds have very similar binding poses. In all three compounds the halogen atom resides in close proximity to F339 and F340.

Figure 5 shows a 2-dimensinal representation of the binding interactions for compounds **7a–7c**. Apart from the important Asp155 interaction with the protonated nitrogen atom in the ligand, several hydrophobic residues as well as the hydrophilic residues Asn343, Ser239, Ser242, Ser159 and Asn363 are important for the binding of these compounds. In general, all the C3 halogenated compounds (**3**, **7a–c** and **9**) had similar binding poses (see Supplementary Information for additional figure) which probably accounts for their similar affinities. Compound **5** which is devoid of a C3 halogen group, had a completely different binding orientation in the receptor as compared to the halogenated compounds; figure 6 shows the relative orientations of **5** and **7b**. Compounds **15–18** were also oriented differently as compared to the higher affinity analogs (see Supplementary Information showing overlap of **15–18** with compound **7b**).

ICM docking scores (Table 1) did not show any correlation with the affinities obtained. Of compounds **15 – 18** which all lacked any appreciable affinity, compounds **16 – 18** had the lowest ICM scores. However compound **15** had the second highest ICM score. As others have noted, docking scores are not always useful for predicting rank order of affinities/potencies; they are more useful for predicating activity vs non-activity.(34) Among all the compounds evaluated, the predicted affinity of compound **15** is an anomaly. Nevertheless,

an ICM score less than -56.6 seems to be predictive of inactivity in this series of compounds.

Overall, the docking studies suggest that the presence of a C3 halogenated group allows for a different orientation of the halogenated analogs in the receptor as compared to non-halogenated analogs. The proximity of the halogen atoms in the ligands with F339/F340 suggests that this interaction is a significant one and may in part be responsible for the higher affinity observed in the C3 halogenated compounds.

CONCLUSIONS AND FUTURE DIRECTIONS

To summarize, we have synthesized and evaluated a novel series of ring A analogs of nantenine as well as a number of ring C analogs in order to examine the effect of structural modifications in these regions of the molecule on antagonism of 5-HT_{2A} receptors and selectivity vs α_{1A} receptors. In general, halogenation at the C3 position was found to improve 5-HT_{2A} antagonist potency. The magnitude of the increase in potency is affected by the C2 oxygenated substituent; for a given halogen, slightly higher 5-HT_{2A} antagonism is seen with C2 methoxyl substitution as compared to C2 hydroxyl substitution. A C2 hydroxyl substituent also seems to be beneficial for α_{1A} antagonism and molecules with very good α_{1A} potency were identified with this structural feature. Deletion of the oxygen atom from the C2 position and replacement of the C2 methoxyl group with an alkyl substituent, diminishes but does not completely obliterate activity at the two receptors investigated. Thus, the C2 oxygen atom is important but is perhaps not a critical feature for activity at both receptors. Ring expansion or contraction of ring C gave analogs with significantly reduced antagonist activities, reinforcing earlier findings that the tetracyclic aporphine scaffold is important for activity at both receptors.

Molecular docking studies suggest that the improved antagonist activity (by virtue of increased affinity) of the C3 halogenated compounds at the 5-HT_{2A} receptor, may be partially due to interactions of the C3 halogen with F339/F340. Contacts with other hydrophobic and polar residues in the receptor pocket may also contribute to the improved affinity of the halogenated compounds. Evaluation of these compounds has provided valuable insight in our quest to obtain more potent and selective 5-HT_{2A} antagonists. In that regard investigation of the tolerance for other hydrophobic groups at the C3 site seems to be a promising direction for improving 5-HT_{2A} antagonist potency. We are continuing to work in this vein and will report on our findings in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Structures of Apomorphine (1), Nantenine (2) and 3-Bromonantenine (3)

Figure 2.
Structures of compounds **15–18**

Figure 3.
Structures of boldine (20), predicentrine (21) and glaucine (22)

Figure 4.
Compounds **3** (purple), **7b** (green) and **9** (cyan) docked in the 5-HT_{2A} receptor

Figure 5.
Interactions of compounds **7a**, **7b** and **7c** in the 5-HT_{2A} receptor

Figure 6.
Docking poses of compounds **5** (purple) and **7b** (black) in the 5-HT_{2A} receptor

Scheme 1.

Reagents and conditions: (a) *N*-halosuccinimide, DCM, rt, 68–83%; (b) (i) ZnBr_2 , DCM, rt, 1h; (ii) 37% HCHO , DCM, $\text{NaBH}(\text{OAc})_3$, rt, 1h; 58–63% over two steps (c) MeI , acetone, K_2CO_3 , reflux, 6h, 76% (via **6a**)

Scheme 2.

Reagents and conditions: (a) Trf_2O , 2,6-lutidine, DCM, 69%; (b) $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$, CuI, LiCl, Et_2NH , DMF, μwaves , 5 min., 120 °C, 76%; (c) TBAF, THF, 5 min, 69%; (d) 10% Pd/C, H_2 , EtOH, 88%; (e) (i) ZnBr_2 , DCM; (ii) 37% HCHO, DCM, $\text{NaBH}(\text{OAc})_3$, rt, 1h; 52% over two steps.

Table 1

K_e values for analogs at 5-HT_{2A} and α_{1A} receptors

Compd.	R ₁	R ₂	R ₃	n	X	K _e ± SEM ^a (nM)		ICM score (kcal/mol)
						5-HT _{2A}	α _{1A}	
5	OH	-	-	-	H	282 ± 37	255 ± 56	-59.1
7a	OH	-	-	-	Cl	130 ± 6	1279 ± 143	-58.1
7b	OH	-	-	-	Br	126 ± 30	68 ± 18	-63.3
7c	OH	-	-	-	I	133 ± 27	60 ± 9	-62.7
9	OMe	-	-	-	Cl	63 ± 16	1273 ± 120	-59.4
14	C ₂ H ₅	-	-	-	H	518 ± 121	275 ± 12	-70.3
2	OMe	-	-	-	H	830 ± 16	36 ± 7 ^b	-66.1
3	OMe	-	-	-	Br	48 ± 2	>10,000 ^c	-58.8
15	-	-OCH ₂ O-	-	2	-	>3000	>3000	-66.5
16	-	-OCH ₂ O-	-	0	-	>3000	2707 ± 341	-56.6
17	-	OMe	OMe	0	-	>3000	1264 ± 127	-52.0
18	-	H	H	0	-	>3000	>3000	-48.9

^aValues represent mean ± SEM for three independent experiments

^bData from ref. 29

^cData from ref. 28