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Evaluation of structural effects on 5-HT$_{2A}$ receptor antagonism by aporphines: identification of a new aporphine with 5-HT$_{2A}$ antagonist activity

Shashikanth Ponnala[a], **Junior Gonzales**[a,b], **Nirav Kapadia**[a,b], **Hernan A. Navarro**[c], and **Wayne W. Harding**[a,b,*]

[a]Chemistry Dept., Hunter College, CUNY, 695 Park Avenue, NY 10065, USA

[b]The Graduate Center, City University of New York, 365 5[th]Ave., NY 10016, USA

[c]Center for Organic and Medicinal Chemistry, Research Triangle Institute, Research Triangle Park, NC 27709, USA

Abstract

A set of aporphine analogs related to nantenine was evaluated for antagonist activity at 5-HT$_{2A}$ and α_{1A} adrenergic receptors.

With regards to 5-HT$_{2A}$ receptor antagonism, a C2 allyl group is detrimental to activity. The chiral center of nantenine is not important for 5-HT$_{2A}$ antagonist activity, however the *N*6 nitrogen atom is a critical feature for 5-HT$_{2A}$ antagonism.

Compound **12b** was the most potent 5-HT$_{2A}$ aporphine antagonist identified in this study and has similar potency to previously identified aporphine antagonists **2** and **3**. The ring A and *N*6 modifications examined were detrimental to α_{1A} antagonism. A slight eutomeric preference for the *R* enantiomer of nantenine was observed in relation to α_{1A} antagonism.

Keywords

Aporphine; Nantenine; 5-HT$_{2A}$; α_{1A}; Antagonist; Structure-activity relationship (SAR)

The tetracyclic aporphine template is a privileged scaffold that is endowed with several biological activities.[1–8] As central nervous system (CNS) receptor ligands, aporphines have been found to possess high affinity for a number of dopamine receptors (predominantly D_1 and D_2),[9–12] serotonin (5-HT) receptors[13–15] and α-adrenergic receptors.[6,16] Furthermore, aporphines are known with both agonist and antagonist activity at neuroreceptor sites. The

*Corresponding author. Tel.: +212-772-5359; fax: +212-772-5332; whardi@hunter.cuny.edu.

Supplementary Material
Experimental procedures on synthesis of all new compounds, procedure for biological assays and NMR spectral data on all analogs.

continued exploration of the aporphine template over the last few decades has been driven to some extent by the opportunity/promise for discovery of new ligands with high potency and selectivity for subtypes of the above-mentioned receptors. Such molecules will continue to supplement our toolbox of CNS receptor ligands that will be useful as novel biological probes, as new imaging agents and as leads for drug discovery efforts relevant to psychiatric disorders and drug abuse.

We are primarily interested in evaluating the potential of aporphines as ligands for 5-HT_{2A} receptors. 5-HT_{2A} receptors are implicated in several neuropsychiatric maladies including schizophrenia, depression, anxiety and insomnia.[17,18] 5-HT_{2A} receptors are also involved in the actions of some stimulant drugs as recent reports have revealed.[19–21]

Several potent 5-HT_{2A} receptor antagonists are known; in particular compounds with a mixed D_2/5-HT_{2A} antagonist profile (eg risperidone, clozapine) are quite prominent and are used clinically to manage schizophrenic symptoms.[22–24] However, there are no highly selective 5-HT_{2A} antagonists (>100-fold selectivity vs all other common neuroreceptor targets) clinically available. Nevertheless, such promising compounds (eg eplivanserin) have recently been or are currently being investigated in clinical trials as anti-insomnia medications. Thus, the identification of new highly selective and therapeutically useful 5-HT_{2A} receptor antagonists is still of topical interest.

Our research team has engaged structure-activity relationship (SAR) studies on the aporphine alkaloid nantenine (**1**, Figure 1) and have identified a number of new nantenine analogs with antagonist activity at the 5-HT_{2A} receptor.[25–28] We were guided by previous SAR studies on nantenine for the present study. Nantenine itself is a high affinity α_{1A} adrenergic receptor antagonist with moderate 5-HT_{2A} receptor antagonist potency (see Table 1). Compounds **2** and **3** (Figure 1) are two of the most potent 5-HT_{2A} antagonists we have obtained to date. These compounds lack affinity for the α_{1A} adrenergic receptor. Our prior investigations have mainly focused on the ring A portion of nantenine and in general have indicated a reasonable degree of tolerance for other types and patterns of substitution in the A ring in obtaining high 5-HT_{2A} potency and selectivity vs the α_{1A} receptor. However, the identity and optimal placement of substituents requires further research for maximal potency and selectivity.

To continue to expand our understanding of the structural tolerance of aporphines as 5-HT_{2A} receptor antagonists as well as selectivity vs the α_{1A} receptor, we have synthesized and evaluated a new set of aporphine analogs. These compounds were prepared in order to probe three regions in the northern portion of the aporphine template, namely: ring A, the chirality center and the nitrogen atom. As alluded to earlier, our previous studies have indicated that substituents on the ring A moiety are important in controlling the 5-HT_{2A} receptor antagonist activity and selectivity. However, we have not investigated the effect of the chiral center on 5-HT_{2A} antagonism before. This is an important task especially since the existing literature suggests that aporphines exhibit a stereochemical preference for activation of dopamine D_1 and D_2[29,30] receptors as well as the related 5-HT_{1A} receptor[31] *R* enantiomers being predominantly agonists and *S* enantiomers being predominantly antagonists in both cases. With regards to the effect of the *N*6 nitrogen atom, our prior studies suggest that a

basic nitrogen atom is required since the *N*-acetamide and *N*-methylsulfonamide derivatives were devoid of activity.[25] We sought herein to obtain experimental proof of the absolute requirement for a nitrogen atom for antagonist activity.

Along those lines we have: 1) synthesized and evaluated new ring A analogs containing features of compounds **1**, **2** or **3**; 2) evaluated nantenine enantiomers - in order to begin to probe the effect of the chirality center of aporphines on receptor antagonism ; and 3) synthesized and evaluated compounds that possess a nitrogen - oxygen isosteric replacement - to investigate the importance of the nitrogen atom on receptor antagonism. Details of these studies are described henceforth.

We were interested in examining the extent to which an allyl group would be accommodated at the C2 position since an allyloxy group seems to be beneficial for antagonism (see data for compound **2**, Table 1). The synthetic practicability of obtaining this structural feature via a Claisen rearrangement (a reaction rarely employed with aporphines) supported this impetus.[32]

To obtain the required ring A analogs Scheme 1 was engaged. Commercially available amine **4** was coupled to bromoacid **5** to give amide **6**. Bischler-Napieralski cyclization of **6** was followed immediately by reduction of the dihydroisoquinoline thus formed to the secondary amine **7**. Protection of the amine as the *N*-ethyl carbamate gave compound **8**. Microwave-assisted direct arylation[33] on **8** afforded compound **9**. The benzyl ether **9** was deprotected revealing the phenol functionality in the key intermediate **10**. Reduction of **10** with lithium aluminium hydride (LAH) gave compound **12a**. Compound **12b** was prepared from **10** via allylation to afford compound **11** and subsequent LAH reduction. Claisen rearrangement of the allyl ether **11** provided compound **13**. Reduction of **13** gave the phenol analog **14**. Analogs **15a–15c** were prepared from **13** in two steps via etherification and reduction as shown. Nantenine enantiomers (*R*)-**1** and (*S*)-**1** were prepared by resolution of racemic nantenine as described previously.[28] The isochroman analogs **16a** and **16b** were prepared via a sequence involving oxa Pictet-Spengler cyclization and direct arylation as presented in a recent report.[34]

All analogs were screened at 10 μM in multi-well format for intrinsic (agonist) and antagonist activity at the human 5-HT_{2A} receptor using Fluorescence Imaging Plate Reader (FLIPR) -based (Molecular devices, Sunnydale, CA) functional assays that detect receptor-mediated mobilization of internal calcium with a calcium sensitive fluorescent dye as reported previously.[25–27] A similar set of assays was performed for the α_{1A} - adrenergic receptor. Data from these evaluations are presented in Table 1.

As shown in Table 1, compound **12a** lacked any appreciable activity at either receptor. The placement of an allyloxysubstitutent at C2 (ie compound **12b**) resulted in a significant increase in 5-HT_{2A} antagonist activity (> 60-fold as compared to the parent phenol **12a**). Antagonism of the α_{1A} receptor also increased although the magnitude of this increase was less (4-fold increase as compared to **12b**). However, when **12b** is compared to nantenine (**1**), a significant decrease in α_{1A} antagonist activity was seen. The above data tends to suggest that the C2 methoxyl substituent of nantenine is not required for 5-HT_{2A} antagonist activity.

Compound **14** lacked antagonist activity for both receptors indicating that an allyl substituent at C2 is not well tolerated at either receptor. A comparison of **15a** with **1** reveals a slight improvement in 5-HT_{2A} antagonism but a decrease in α_{1A} antagonist activity upon replacement of the C2 methoxyl group with an allyl substituent. The antagonist activity of **15a** was higher at both receptors than compound **14** which is indicative of a greater tolerance for an alkoxy substituent than a phenol at C1. Compound **15b** had a diminished affinity at both receptors as compared to **15a**. When **15b** is compared to compound **12b**, a significant decrease in antagonist activity of **15b** at both receptors manifests. This suggests that an allyl substituent at C2 is not well accommodated at either receptor. Compound **15c** had activity and selectivity that was similar to **15b**. If a comparison of the **15b/15c** pair is made with the **2/3** pair of compounds it may be surmised that C1 allyloxy and C1 cyclopropylmethyloxy groups endow the aporphine template with very similar 5-HT_{2A} antagonist potency irrespective of the identity of the C2 substituent. That is, it appears that the allyl and cyclopropylmethyl functionalities are bioisosteric with respect to 5-HT_{2A} receptor antagonism. From our previous studies, an allyloxy or cyclopropylmethyloxy substituent (ie compounds **2** and **3** respectively) imparted high 5-HT_{2A} antagonist activity and selectivity to the nantenine template. The analysis of compounds **15a, 15b** and **15c** showed a reversal in this trend and again points to a considerable lack of tolerance for a C2 allyl group at the 5-HT_{2A} receptor.

Both compounds **16a** and **16b** (that lack the *N*6 moiety) were devoid of antagonist activity. This supports previous SAR evidence that a basic *N*6 atom is critical for affinity to both receptors. This is also in line with previous molecular docking studies which suggest that the protonated *N*6 atom is involved in a H-bonding interaction with an aspartate residue in the 5-HT_{2A} receptor binding pocket.[25]

Evaluation of (*R*)-**1** and (*S*)-**1** indicates that the chiral center of nantenine is not critical for 5-HT_{2A} antagonism although the (*S*) enantiomer is slightly more potent. Interestingly, there seems to be a reversal of this trend at the α_{1A} receptor; the (*R*)-enantiomer seems to be slightly more potent than the (*S*)-enantiomer at the α_{1A} receptor (approximately 3-fold).

In summary, this study has revealed some useful qualitative information concerning the antagonism of aporphines at the 5-HT_{2A} receptor. The data suggest that the C2 position is not tolerant of an allyl moiety. However, a C1 allyloxy substituent is well tolerated when the C2 substituent is hydrogen implying that the C2 methoxyl group of nantenine is not required for high 5-HT_{2A} antagonist potency. This modification also improves selectivity vs the α_{1A} receptor (though this selectivity is moderate as compared to that seen in **2** and **3**). Of note, the most potent 5-HT_{2A} aporphine antagonist identified in this study was compound **12b** which rivals **2** and **3** in terms of 5-HT_{2A} antagonist potency.

The chiral center of nantenine does not engender any significant preference for either enantiomer towards 5-HT_{2A} antagonism. Somewhat unsurprisingly, the *N*6 nitrogen is critical for antagonist activity of nantenine analogs at both receptors.

The evaluation of this set of compounds has further expanded our fundamental knowledge concerning the viability of the aporphine template for development as selective 5-HT_{2A}

receptor antagonists. For certain, evaluation of larger series of analogs will enable a better understanding of the extent to which the SAR information extracted up to this point may be generalized. Compound **12b** identified herein as well as compounds **2** and **3** identified earlier, are useful starting points for further SAR exploration and optimization studies. We are continuing in this vein and will report our findings in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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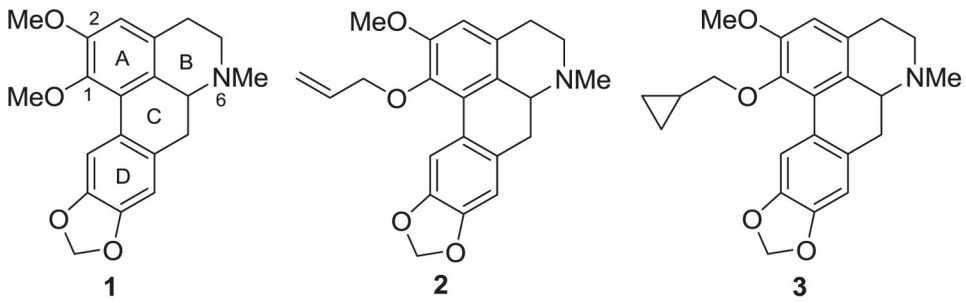
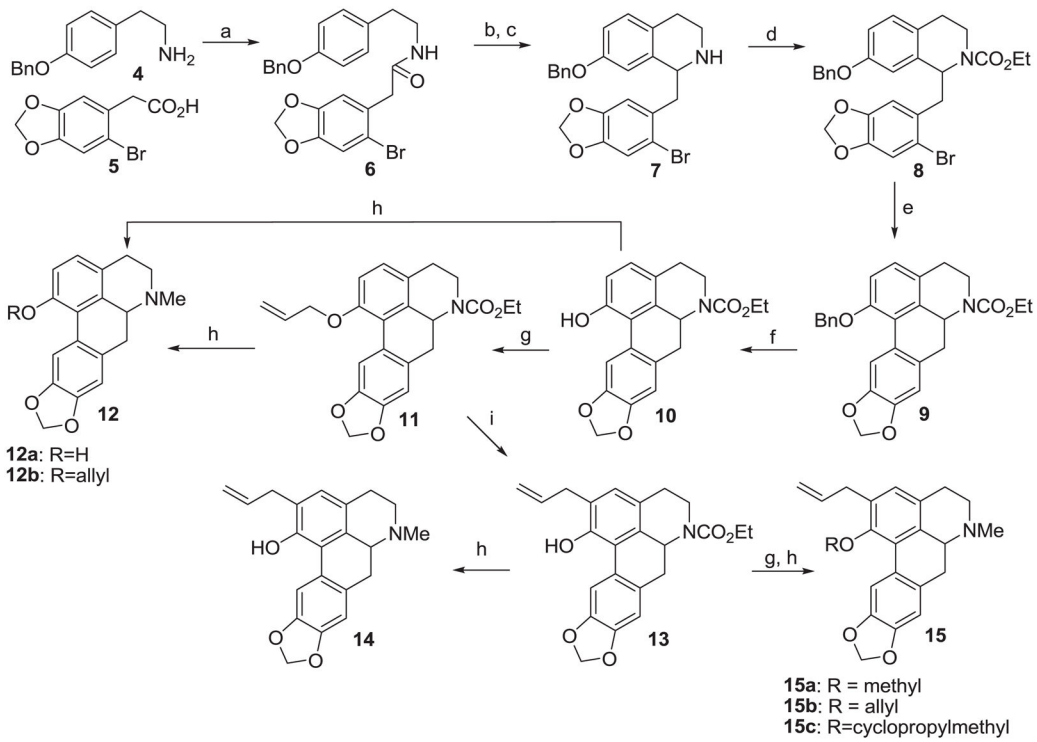


Figure 1.
Structures of nantenine (**1**), compound **2** and compound **3**



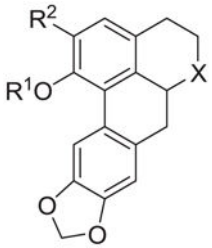
Scheme 1.

Synthesis of ring A analogs

Reagents and conditions: (a) 1,1′-carbonyldiimidazole (CDI), THF, 0 °C - rt, 5 h, 80% ; (b) trifluoromethanesulfonic acid, pyridine, DCM, 0 °C - rt, 4 h; (c) $NaBH_4$, MeOH, 0 °C, 2 h, 88% over two steps; (d) Ethyl chloroformate, K_2CO_3, DCM, rt, 3 h, 85% ; (e) $Pd(OAc)_2$, di-tert-butyl(methyl)phosphonium tetrafluoroborate, K_2CO_3, $(CH_3)_3CCOOH$, DMSO, 135 °C, microwaves, 6 min, 50% ; (f) H_2/Pd, rt, 8 h, 95% ; (g) alkyl bromide, KI, K_2CO_3, acetone, 70 °C, 6 h, 60–70% ; (h) LAH, THF, 0 °C, 10 h, 50–60% ; (i) *N*,*N*-diethylaniline, 215 °C, microwaves, 6 min, 90%

Table 1

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

	Compd.	R^1	R^2	X	$K_e \pm SEM^a$ (nM)		Selectivity 5-HT_{2A}/α_{1A}
					5-HT_{2A}	α_{1A}	
	**12a**	H	H	*N*Me	>3000	2950 ± 457	<1.01
	12b	allyl	H	*N*Me	47 ± 5	744 ± 74	0.06
	14	H	allyl	*N*Me	>3000	>3000	-
	15a	Me	allyl	*N*Me	485 ± 123	566 ± 112	0.85
	15b	allyl	allyl	*N*Me	1374 ± 405	>3000	<0.45
	15c	cyclopropylmethyl	allyl	*N*Me	963 ± 103	>3000	<0.32
	16a	Me	OMe	O	>3000	>3000	-
	16b	allyl	OMe	O	>3000	>3000	-
	(*R*)-1	Me	OMe	*N*Me	946 ± 61	70 ± 10	13.5
	(*S*)-1	Me	OMe	*N*Me	657 ± 89	196 ± 3	3.4
	±-(1)[b]	Me	OMe	*N*Me	850 ± 6	36 ± 7	23.6
	2[c]	allyl	OMe	*N*Me	70 ± 15	>10000	<0.007
	3[d]	cyclopropylmethyl	OMe	*N*Me	68 ± 8	>10000	<0.007
	prazosin	-	-	-	-	1.1 ± 0.4	-
	ketanserin[b,e]	-	-	-	32	-	-

[a] Values represent mean ± SEM for at least three independent experiments;

[b] K_i, Data from ref. 28;

[c] Data from ref. 27;

[d] Data from ref. 26;

[e] IC_{50} determined in the presence of 5-HT EC_{80}