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C4 phenyl aporphines with selective *h*5-HT_{2B} receptor affinity

Nirav Kapadia^{a,b} and Wayne W. Harding^{a,b,*}

^aChemistry Dept., Hunter College, CUNY, 695 Park Avenue, NY, 10065, USA

^bThe Graduate Center, City University of New York, 365 5th Ave., NY 10016, USA

Abstract

A group of aporphine alkaloids related to (±)-nantenine (**1**) and bearing a C4 phenyl and various C1 or *N*-substituents, was synthesized and evaluated for affinity to *h*5-HT receptors. In general, unlike nantenine, the analogues lack affinity for the *h*5-HT_{2A} receptor and other 5-HT receptors but bind selectively to the *h*5-HT_{2B} receptor. With regards to 5-HT_{2B} affinity, there appears to be a low tolerance for bulky C1 or *N*-substituents when the C4 phenyl moiety is present. Compound **5a** had the highest 5-HT_{2B} affinity of the compounds tested, was found to be an antagonist and is selective vs other CNS receptors.

Graphical Abstract

Keywords

5-HT_{2A}; 5-HT_{2B}; Aporphine; CNS; Nantenine

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

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For experimental details please refer to the PDSP website <http://pdsp.med.unc.edu/> and click on "Binding Assay" or "Functional Assay" on the menu bar.

Supplementary Material

Synthetic procedures for compounds and spectral characterization data.

Aporphine alkaloids display a wide range of pharmacological activities and have been shown to exhibit antimicrobial, antiviral, antimalarial and cytotoxic activities among others.^{1–6} Moreover, in the central nervous system (CNS), aporphines have been investigated as acetylcholinesterase inhibitors^{7,8} and as ligands for serotonin, adrenergic and dopamine receptors.^{9–14}

Research in our group has mainly focused on the synthesis and evaluation of aporphines as ligands for the 5-HT_{2A} receptor.^{15–17} The 5-HT_{2A} receptor plays a significant role in various neuropsychiatric disorders such as schizophrenia, anxiety depression and psychostimulant abuse.^{18–22} Selective 5-HT_{2A} antagonists are being pursued as novel therapeutics to treat sleep disorders.^{23–25}

Our prior structure-activity relationship (SAR) studies on aporphines have focused on modifications on aryl ring A, of the lead molecule (±)-nantenine (**1**, Figure 1), resulting in the identification of a number of potent 5-HT_{2A} antagonists such as compounds **2** and **3**.^{26,15,27} These prior SAR studies indicate that a variety of C1 alkoxy substituents are beneficial for 5-HT_{2A} receptor affinity and selectivity. Small alkoxy groups are tolerated at the C2 position, though the gains in affinity are somewhat moderate compared to that seen for the C1 analogues. C3 halogenated analogues were shown to possess strong affinity for the 5-HT_{2A} receptor.

In an effort to further decipher structural motifs that may be accommodated on the aporphine core for high affinity, antagonism and selectivity for the 5-HT_{2A} receptor, we decided to prepare a series of nantenine analogues bearing a phenyl ring substituent at the C4 position (exemplified by **4**). We expected that the incorporation of a C4 phenyl group would engender improved affinity of the aporphine scaffold for the 5-HT_{2A} receptor, based on structural resemblance to 9-aminomethyl- 9,10-dihydroanthracene (AMDA, **5**) – a potent 5-HT_{2A} receptor antagonist.^{28,29} Surprisingly, we have found that these novel C4 phenyl analogues lack affinity for the 5-HT_{2A} receptor but have selective affinity for the 5-HT_{2B} receptor.

Herein we describe the synthetic and biological experiments that led to this interesting serendipitous discovery.

Since C1 alkoxy analogues (eg **2** and **3**) had displayed improved affinity for the 5-HT_{2A} receptor, it was decided that a C1 alkoxy moiety would be retained in the series of C4 phenyl analogues. Prior SAR studies on nantenine indicated that *N*-substituent groups larger than methyl were not tolerated for 5-HT_{2A} receptor affinity. Nevertheless, we also wanted to determine the extent to which small *N*-alkyl substituents could be tolerated when a C4 phenyl substituent was present on the nantenine template. Synthesis of the requisite C1 alkoxy/C4 phenyl and *N*-substituted/C4 phenyl analogues was achieved via the intermediacy of a key phenolic aporphine (**15**), prepared as outlined in Scheme 1. Thus, commercially available aldehyde **6** was protected as the benzyl ether and the resulting aldehyde condensed with nitromethane to afford nitrostyrene **7**. The Michael acceptor **7** was reacted with phenylmagnesium bromide to furnish compound **8**. Reduction of the nitro group of **8** was accomplished with SnCl₂. The primary amine **9** thus formed was coupled to bromoacid **10**

to give amide **11**. Bischler-Napieralski reaction on **11** followed by reduction of the intermediate imine gave the secondary amine **12**. Amine **12** was protected as the Boc derivative, **13**. Thereafter, microwave-assisted direct arylation provided the *O*-benzyl protected aporphine **14**. Removal of the benzyl protecting group of **14** was accomplished via catalytic hydrogenolysis to produce the key precursor **15**. With **15** in hand we proceeded to prepare target C4 phenyl analogues **5a** – **5j**. The synthesis of these analogues from **15** is depicted in Scheme 2. The phenol handle of **15** was used to install a variety of substituent groups via etherification reactions. Subsequently, Bocdeprotection and *N*-methylation via reductive amination afforded target analogues **5a–h**. Phenol analog **5i** was prepared from **15** via Boc-deprotection followed by *N*-methylation. **5j** was prepared from precursor **15** via the intermediacy of *N*-acyl derivative **16**. Compound **16** in turn was synthesized from **15** by sequential *O*-methylation, Boc-deprotection and *N*-amidation with acetic acid. LAH reduction of **16** afforded **5j**. **5k** was accessed from **15** in three steps *viz* *O*-methylation, Bocdeprotection and *N*-alkylation as shown.

Analogues **5a** – **5k** were evaluated for affinity across various 5-HT receptors at the Psychoactive Drug Screening Program (PDSP). The compounds were tested initially in a primary radioligand binding assay that measures inhibition of serotonin binding, at a compound concentration of 10 μ M. Compounds with inhibition values less than 50% at a particular receptor, are deemed inactive at that receptor. Compounds that displayed more than 50% inhibition of serotonin binding in the primary assays, were evaluated for determination of K_i values in secondary assays.

The results of the primary (% inhibition) assay are compiled in Table 1. To our surprise, the compounds demonstrated a remarkable selectivity profile; they were inactive at the 5-HT_{2A} receptor and at most other 5-HT sites. Interestingly, the majority of the compounds were active at the 5-HT_{2B} receptor (save for compound **5h**). Compounds **5c** and **5f** also showed activity at the 5-HT_{1B} receptor.

Data from the follow-up secondary (K_i determination) assay are provided in Table 2. As shown, the compounds display moderate to low affinity (K_i values ranging from 96 to 1,429 nM) for the 5-HT_{2B} receptor, with compound **5a** having the highest affinity. Nantenine was found to have poor affinity for the 5-HT_{2B} receptor (K_i = 534 nM).³⁰ Thus when nantenine is compared to compound **5a** it is apparent that the C4 phenyl substituent positively impacts 5-HT_{2B} affinity and selectivity. When one compares the homologous series **5a** – **5e**, it is evident that an increase in the C1 alkoxy chain length is not good for 5-HT_{2B} affinity; increasing C1 alkoxy chain length coincided with a progressive decrease in 5-HT_{2B} receptor affinity.

The cyclopropylmethyl analogue **5f** had similar affinity to the *n*-propyl analog **5c** (K_i values of 307 and 299 nM respectively) which tends to suggest that some degree of alkyl chain branching is tolerated. The allyl analogue **5g** had diminished affinity as compared to its saturated counterpart **5c**, so saturation (at this position in the alkyl chain) is not well tolerated for 5-HT_{2B} affinity. Compound **5i** had lower affinity than most other analogues (except for compound **5k** and compound **5h** which was inactive in the primary assay). This indicates that the C1 phenolic group is not beneficial for 5-HT_{2B} receptor affinity. In

comparing *N*-alkyl analogues **5a**, **5j** and **5k**, the trend in affinities again coincides with the size of the alkyl group, i.e. the larger the *N*-alkyl substituent the lower is the 5-HT_{2B} receptor affinity. Thus, smaller *N*-alkyl groups are better accommodated for 5-HT_{2B} receptor affinity.

To further characterize the pharmacological activity of the compounds, compound **5a** (which had the highest 5-HT_{2B} receptor affinity among the tested compounds) was selected for further assays as a representative of the series. In functional activity testing, **5a** was found to be a 5-HT_{2B} receptor antagonist with an IC₅₀ of 1 μ M. No 5-HT_{2B} agonist activity was detected. A broad panel screening of **5a** at several other CNS receptor sites revealed the impressive 5-HT_{2B} receptor selectivity of the compound. No affinity (<50% inhibition in primary assay) was observed at the following receptor sites: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2C}, 5-HT₃, 5-HT_{5A}, 5-HT₇, α_{1A} , α_{1B} , α_{1D} , β_1 , β_2 , β_3 , BZP rat brain site, CB₂, D₁, D₂, D₃, D₄, D₅, DAT, DOR, GABA_A, H₁, H₂, H₃, H₄, KOR, M₂, M₃, M₄, M₅, MOR, NET, NMDA, SERT, sigma-1 and sigma-2. Compound **5a** showed affinity for six receptor sites other than the 5-HT_{2B} receptor, but the affinity was significantly lower than that for the 5-HT_{2B} receptor. The following affinities were obtained: 5-HT₆ (627 nM), α_{2A} (719 nM), α_{2B} (3,220), α_{2C} (433 nM), M1 (>10,000 nM) and PBR (2,897 nM).

The 5-HT_{2B} receptor has emerged as a novel target for the treatment of irritable bowel syndrome as well as pulmonary disorders.^{31,32} A few selective agents are known but none is yet clinically approved.³³ For example, RS-127445 is one of the most potent 5-HT_{2B} receptor antagonists known and is the standard pharmacological tool compound for investigating 5-HT_{2B} receptor functions.³³ RS-127445 shows nanomolar 5-HT_{2B} receptor affinity ($pK_i = 9.5$), with 1000 fold selectivity as compared to other 5-HT receptor sites. Compound **5a** is lower in 5-HT_{2B} receptor affinity than RS-127445, but shows comparable selectivity and is thus a promising starting point for further optimization.³³

In summary, nantenine analogues bearing a C4 phenyl substituent were synthesized and evaluated at 5-HT receptors. The addition of a phenyl ring to the C4 position of nantenine and nantenine analogues is detrimental to 5-HT_{2A} receptor affinity. A C4 phenyl moiety improves affinity and selectivity of nantenine for the 5-HT_{2B} receptor versus other 5-HT receptors. Small alkyl groups are better tolerated at the C1 and N6 positions than larger substituents, suggesting that these sites of the aporphine template associate with shallow hydrophobic pockets in the 5-HT_{2B} receptor. Compound **5a** was found to be a low potency, selective 5-HT_{2B} receptor antagonist. The unexpected discoveries described herein provides a backdrop for future SAR studies to delineate the molecular features of aporphines that are required for 5-HT_{2B} receptor selectivity. Since the prepared analogues are mixtures of diastereomers, it remains to be determined the extent to which the two chiral centers present impact 5-HT_{2B} receptor affinity and activity. Optimization of the 5-HT_{2B} receptor affinity, selectivity and antagonist activity of **5a** in future will provide opportunities for the development of novel 5-HT_{2B} receptor selective pharmacological tools and potential therapeutics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Structures of nantenine [(±)-**1**], C1 analogues (**2** and **3**), C4 phenyl analogues (**4**) and
AMDA (**5**)

Scheme 1.

Reagents and conditions: a) BnBr, K₂CO₃, acetonitrile, reflux, 4h, 94%; b) CH₃NO₂, NH₄OAc, CH₃COOH, reflux, 4h, 82%; c) phenyl magnesium bromide, THF, 0 °C-rt, 18h, 32%; d) SnCl₂, EtOH, reflux, 16h, 65%; e) CDI, THF, 0 °C-rt, 16h, 60%; f) PCl₅, DCM, 0 °C-rt, 16h; g) NaBH₄.MeOH, 0 °C-rt, 4h, 88% crude yield over 2 steps; h) (BoC)₂O, DIPEA, DMAP, DCM, rt, 16h, 85%; i) Pd(OAc)₂, Di-*tert*-butyl(methyl)phosphonium tetrafluoroborate, K₂CO₃, DMSO, 135 °C, microwaves, 200W, 88%; j) H₂, Pd/C, THF:MeOH 1:1, rt, 6h, 90%

Scheme 2.

Reagents and conditions: a) MeI (for **5a**, **5j** and **5k**) or appropriate alkyl halide (for **5b-5h**), K_2CO_3 , acetonitrile, 16h; b) $ZnBr_2$, DCM, rt, 18 h; c) HCHO (for **5i** and **5a-h**) or appropriate aldehyde (for **5j, 5k**), $Na(OAc)_3BH$, DCM, rt, 16 h; d) AcOH, CDI, THF, 0 °C-rt, 16 h; e) LAH, THF, 0 °C-rt

Table 1

Primary assay data for analogues at 5-HT receptors

Compound	Percent inhibition (%) ^a												
	5-HT _{1A}	5-HT _{1B}	5-HT _{1D}	5-HT _{1E}	5-HT _{2A}	5-HT _{2B}	5-HT _{2C}	5-HT ₃	5-HT _{5A}	5-HT ₇			
5a	z5.5	22.6	34.5	10.1	33.5	92.2	31.9	8	-12.2	36.1			
5b	-0.9	20.6	29.7	10.3	40.1	83.3	13.5	-2.7	-7.6	26			
5c	27	51.7	22.4	2.1	32.3	84	13.1	0.7	5.2	8.6			
5d	18.4	41.4	24	7.3	40.9	76.1	48	11.6	17.6	-5.1			
5e	-13.5	4.3	13.4	2.4	19.9	63.6	49.3	0.8	-6.2	-14.6			
5f	-9.8	60.2	31.9	27.4	42.9	77.1	32.4	7.7	5.2	6.9			
5g	19.2	19.4	22.6	3.3	47.8	72.1	28.8	8.3	-9.8	24.7			
5h	25.9	2.1	9.8	-0.1	15.8	24.4	-6.6	-7.4	-9.3	-22.6			
5i	24.2	-5.2	15.8	11	29.2	50.3	-2.3	4.1	1.8	31.3			
5j	3.3	23.6	27.4	5	12.8	88.4	-1.6	-4.1	-12.1	8.9			
5k	13.3	16.2	22.4	3.5	9.6	73.7	9.4	-13.3	-18.2	18.3			
1^b	na ^c	100.3	na ^c	62	89.3	92.8	103.0	44.6	92.8	96.7			

^a Hot ligands: [3H]-8-OH-DPAT (5-HT_{1A}), [3H]GR125743 (5-HT_{1B}), [3H]GR125743 (5-HT_{1D}), [3H]5HT (5-HT_{1E}), [3H]ketanserin (5-HT_{2A}), [3H]LSD (5-HT_{2B}), [3H]mesulergine (5-HT_{2C}), [3H]LY278584 (5-HT₃), [3H]LSD (5-HT_{5A}), [3H]LSD (5-HT₇);

^b Corresponding K_i data previously reported in reference 30;

^c na – data not available

Table 2

Secondary assay data for analogues

Compound	K _i (nM)	
	5-HT _{1B} ^a	5-HT _{2B} ^b
5a	nd ^c	96±12
5b	nd	209±27
5c	1,608±210	307±40
5d	nd	601±78
5e	nd	663±86
5f	2,466±320	299±39
5g	nd	416±54
5h	nd	nd
5i	nd	715±92
5j	nd	419±54
5k	nd	1,429±180
1	100±3	543±53

^a Ergotamine used as positive control (average K_i = 10 nM); hot ligand is [3H]GR125743;^b SB206553 used as positive control (average K_i = 23 nM); hot ligand is [3H]LSD;^c nd = not determined (<50% inhibition in primary assay)