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Synthesis and evaluation of aporphine analogues containing C1 allyl isosteres at the *h*5-HT_{2A} receptor

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Abstract

A series of C1 aporphine analogues related to compound **5** and that contain substituted allylic, alkynyl, nitrile, ester and benzyl groups was synthesized and evaluated for affinity at *h*5HT_{2A} and α_{1A} receptors in functional activity assays that measure calcium release. The presence of branched allylic substituent groups diminished affinity for the *h*5HT_{2A} receptor. Likewise, the alkynyl, nitrile and ester derivatives evaluated displayed lower 5-HT_{2A} receptor affinity as compared to **5**. Hydrophobic, steric and electronic effects impact the affinity of *p*-substituted benzyl derivatives **8i** – **8k** but in different ways. High hydrophobicity and size favor 5-HT_{2A} affinity whereas, high electronegativity disfavors 5-HT_{2A} affinity. *p*-bromobenzyl analogue **8k** was identified as a 5-HT_{2A} receptor selective ligand, with the highest 5-HT_{2A} receptor affinity of any aporphine known to date. Most of the other analogues were selective for the 5-HT_{2A} versus the α_{1A} receptor. ChemScore binding energies from docking studies correlated qualitatively with the observed trends in affinity for **8i** - **8k**, although the binding energies were not well differentiated quantitatively.

Graphical abstract

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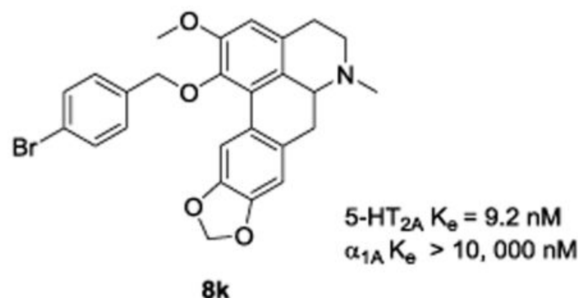

Keywords

aporphine; 5-HT_{2A}; antagonist; adrenergic; nantenine

The neurotransmitter serotonin (5-HT) plays a significant role in a variety of central nervous system (CNS) processes such as mood, anxiety, cognition, feeding behavior, aggression, thermoregulation, sleep, pain and stimulant abuse.[1] Peripherally, 5-HT is involved in the regulation of smooth muscle contraction, gastrointestinal function and cardiovascular function. These central and peripheral functions are mediated by a group of fourteen 5-HT receptors, comprising seven distinct families: 5-HT_1 - 5-HT_7. Some of these receptor families are divided further into subtypes; the 5-HT_2 family has three subtypes, namely, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}.

A handful of highly selective and potent 5-HT_{2A} antagonists have been developed as potential sleep disorder medicaments and have shown promising results in clinical trials.[2-5] Representatives from this group - eplivanserin (**1**), pruvanserin (**2**) and ritanserin (**3**) are shown in Figure 1. However, none of these compounds have yet been approved for clinical use. In light of the preceding, the identification of new, selective 5-HT_{2A} antagonists would be a valuable addition to the pipeline of therapeutics germane to the treatment of sleep disorders. Furthermore, such compounds could serve as pharmacologically interesting research tools and potential therapeutics for other CNS-related disorders where 5-HT_{2A} blockade is therapeutically beneficial.

Aporphine alkaloids have been extensively explored as ligands for dopamine D_1 and D_2 receptors.[6] That being said, aporphines are a “privileged template” and representatives of this structural class have also shown good affinity for 5-HT receptors.[7-9] This makes the scaffold an attractive one for the development of novel, selective 5-HT receptor ligands.

In contrast to structure-activity relationship (SAR) studies done on aporphines at dopamine D_1 and D_2 receptors, there are comparatively few such studies on aporphines with the 5-HT_{2A} receptor as the focus. A major thrust of our research has been to understand the molecular features of aporphines that impact affinity, activity and selectivity to 5-HT_{2A} receptors as a prerequisite for the design of novel, potent and selective 5-HT_{2A} antagonist tools and therapeutics. In that context, our previous SAR studies based on the aporphine alkaloid nantenine (**4**, Figure 2) have resulted in the identification of new aporphinoid 5-HT_{2A} receptor antagonists with significantly improved affinity and selectivity as compared

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to nantenine.[10-13] In continuing efforts in that vein, we wish to report an SAR study that has resulted in the identification of the most potent aporphine-derived 5-HT_{2A} receptor antagonist known to date.

Our prior SAR studies have been largely directed towards the C1 and C2 positions of the aporphine scaffold of nantenine.[11,13,14] These studies have shown that substituents at the C1 position of nantenine may be manipulated to improve 5-HT_{2A} affinity and selectivity. *n*-Alkyl substituents were particularly well tolerated. Among several derivatives synthesized and evaluated, the C1 allyl analogue **5** is one of the most potent that was identified (K_e = 70 nM). Compound **5** showed twelve-fold improvement in 5-HT_{2A} receptor affinity as compared to nantenine.[14] Moreover, unlike nantenine, compound **5** was devoid of affinity for the α_{1A} adrenergic receptor. We considered whether the improvement in 5-HT_{2A} receptor affinity and selectivity was due to the electron-rich nature of the allyl group or to other effects. In order to help to clarify this issue, we decided to synthesize and evaluate a series of C1 nantenine analogues related to **5**, with diverse electronic, steric and hydrophobic characteristics.

The analogues were synthesized from compound **6** as depicted in scheme 1 Compound **6** was obtained as we have detailed in previous publications.[15,10] Reaction of **6** with various alkyl halides provided phenol ether derivatives **7a** - **7k**. Thereafter, removal of the Boc protecting group with zinc bromide ensued. The secondary amine thus produced, was methylated via reductive amination with formaldehyde/$NaBH(OAc)_3$ to afford C1 analogues **8a** - **8k**.

Analogues **8a** - **8k** were then evaluated for 5-HT_{2A} receptor affinity in a functional assay. In order to gauge selectivity of the compounds, the analogues were also evaluated at the α_{1A} adrenergic receptor (since nantenine itself is selective for this receptor subtype). Briefly, the 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. 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. 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: $K_e = [L]/(DR-1)$, where [L] equals the concentration of test compound in the assay and DR equals the dose ratio or A′/A. A similar set of assays was performed for the α_{1A} - adrenergic receptor. Data from these evaluations is presented in Table 1.

Compounds **8a** - **8c** all possessed an allylic moiety directly attached to C1. In the case of **8a**, which has an (*E*)-2-butenyl moiety, a reduction in affinity for the 5-HT_{2A} receptor was observed. The methallyl analog **8b**, had improved affinity as compared to **8a** but was still 2-fold lower in affinity than **5**. The 2,2-dimethyl allyl analog **8c** had the lowest affinity of all three compounds, being 30-fold lower in 5-HT_{2A} receptor affinity than **5**. These results

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indicate that there is a preference for an unsubstituted allyl group at the C1 position. The steric bulk introduced by the additional methyl groups in **8a** - **8c** may be partially responsible for this trend. Electronic effects might not play a large role here, since if that was the case it would be expected that the electronically similar **8a** and **8b** would have similar affinities. However, it appears that the additional steric bulk is better accommodated at the internal alkene carbon rather than the terminal alkene carbon of the allyl group (i.e. compare **8b** with **8a/8c**). Analog **8d** with a 2-butynyl substituent, showed diminished 5-HT_{2A} receptor affinity when compared to **8a**. This might be due to increased electron density in the alkyne moiety of **8d**. It is also possible that the geometry of the substituent has some effect - the terminal methyl group being linear in **8d**. No affinity was seen for the C1 (*E*)-cinnamyl analogue **8e**. As was the case with **8c**, this result with **8e** is perhaps attributable to a steric intolerance for groups (methyl in **8c**; phenyl in **8e**) attached to the terminal alkene carbon of the allyl moiety. Compound **8f** lacked affinity for the 5-HT_{2A} receptor, suggesting that the double bond unit is better tolerated at an allylic position, as in **5**. Replacing the alkene moiety of **5** with a nitrile group (**8g**), also decimated affinity at the 5-HT_{2A} receptor. Given the similarity in size of the C1 substituent groups in **5** and **8g**, and based on their different electronic character, we surmise that there is an electronic factor operating in the reduced affinity of **8g**. It seems reasonable to speculate that the addition of a nitrogen atom in **8g**, leads to a reduction in hydrophobic contacts of the C1 substituent and thus a reduced 5-HT_{2A} receptor affinity. The ester analogue **8h** had low affinity for the 5-HT_{2A} receptor. When the data from **8g** and **8h** are considered together with data from compounds **8a**-**8c**, it may be reasoned that polar groups are less tolerated at the C1 position than similar sized allyl groups.

In a previous SAR study, we found that a nantenine analogue with a C1 benzyl group had moderate 5-HT_{2A} receptor affinity.[11] Since benzyl groups contain an allyl substructure, we were interested in ascertaining to what extent substituted benzyl groups could serve as surrogates for an allyl group as it pertains to 5-HT_{2A} receptor affinity. We reasoned that perhaps substituents on the phenyl ring of a benzyl moiety might be appropriately placed to allow for optimal electronic character of the phenyl ring for binding to the 5-HT_{2A} receptor. It was with this motive in mind that compounds **8i** - **8k** were synthesized and evaluated. The *p*-chlorobenzyl analog **8i** lacked any appreciable affinity for the 5-HT_{2A} receptor. This situation was made slightly better when a CF_3 group replaced the chloro group (i.e. compound **8j**). Compound **8k** with a *p*-bromo group however, showed a dramatic increase in 5-HT_{2A} receptor affinity (9.2 nM, representing a seven-fold increase as compared to **5**), superseding any known aporphine in this regard. The reason for the significant increase in 5-HT_{2A} receptor affinity of **8k** was not absolutely clear, especially when one compares the chasm in affinities between **8i** and **8k** (bearing electronically similar halogen groups). Thus, the 5-HT_{2A} receptor affinity in the **8i** - **8k** subset of analogues, does not seem to be due to the electron-withdrawing power of the *p*-substituents alone. Bromine has a higher hydrophobicity and is larger than chlorine, and we presume that the combination of these effects is favorable for 5-HT_{2A} affinity i.e. the larger and more hydrophobic the *p*-substituent, the higher is the 5-HT_{2A} affinity. This would explain the higher affinity of compound **8k** as compared to **8i**. However, this logic disintegrates when one considers compound **8j**, which has a more hydrophobic and larger CF_3 group, and would thus be

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expected to have the highest 5-HT_{2A} receptor affinity of the three. The powerful electron-withdrawing effects of the CF_3 group may be the reason for the lower than "expected" affinity of **8j**. That is the stronger the electron-withdrawing effect of the phenyl substituent, the lower is the affinity.

The analogues assayed were inactive or had only moderate affinity for the α_{1A} receptor. In the **8a** - **8c** series, compound **8a** displayed the highest (though moderate) α_{1A} receptor affinity; this suggests that branching on the allyl system is not desirable for α_{1A} affinity, probably due to steric intolerance. Analogues with allyl or benzyl substituents showed selectivity for the 5-HT_{2A} receptor; however analogues with alkynyl, nitrile or ester groups (**8d**, **8g** and **8h**) showed selectivity for the α_{1A} receptor. It seems that size, while important, is not the only important factor that controls selectivity of these analogues since the allyl containing **5** is 5-HT_{2A} selective while the similar sized nitrile containing **8g** is α_{1A} selective. It may be that in the case of **8g** and **8h**, the presence of polar functionalities capable of functioning as hydrogen bond acceptors or which disfavor hydrophobic interactions with the receptor, favors this reversal in selectivity. It is less clear what factors favor the switch to α_{1A} selectivity in **8d**, although it seems plausible that differences in electronic density play a role (compare **8c** - with an alkene functionality and **8d**). Further SAR studies will be required to obtain a clearer picture. Compounds **8i** - **8k** all lacked affinity for the α_{1A} receptor, probably a consequence of the size of the benzyl group in these analogues. When one considers the smaller allyl-containing (**8a – 8c**) and the benzyl group-containing (**8i – 8k**) analogues it appears that these functionalities are worse for α_{1A} affinity than for 5-HT_{2A} affinity. As a consequence smaller allyl moieties as well as benzyl moieties confer 5-HT_{2A} selectivity.

To determine the broader selectivity of **8k** at other CNS receptor sites, the compound was submitted for screening at the Psychoactive Drug Screening Program (PDSP). Details of the assay protocols may be found in the PDSP assay protocol book available online (https://pdspdb.unc.edu/html/tutorials/UNC-CH%20Protocol%20Book.pdf). This screening confirmed the selectivity of **8k** for the 5-HT_{2A} receptor. **8k** retained affinity for the 5-HT_{2A} receptor (185 nM) but had no affinity (less than 50% inhibition in a primary assay) at the following receptor sites: 5-HT_{1A}, 5-HT_{5A}, α_{1A} α_{1B} α_{1D}, β1, β2, β3, BZP rat brain site, D_2, D_4, D_5, DOR, $GABA_A$, H_1, H_3, H_4, KOR, M_1, M_2, M_3, M_4, MOR, NET, SERT. Poor affinity ($K_i > 900$ nM) was seen for the following sites: 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2B}, 5-HT_3, 5-HT_7, $\alpha_{2B,}$ $\alpha_{2C,}$ D_1, D_3, H_2, M_5, PBR. Moderate affinity (300 nM < K_i < 900 nM) was seen at 5-HT_{2C}, 5-HT_6, $\alpha_{2A,}$ DAT, sigma-1 and sigma-2 receptor sites. The discrepancy between the K_i values and K_e values at the 5-HT_{2A} receptor for **8k** (9.2 vs 185 nM) is likely attributable to differences in the assay protocols. The K_e assay is a functional assay and gives an indirect measurement of affinity (i.e. calculated based on blockade of calcium release by the compound). Nevertheless, both assays are in agreement with regards to the 5-HT_{2A} versus α_{1A} selectivity of **8k**.

Docking of compounds **8i, 8j** and **8k** at the 5-HT_{2A} receptor was performed in order to unravel the binding interactions among this group of substituted benzyl analogues. Analysis of the docking outcomes and the nature of the key protein-ligand interactions provides insights into the diverse affinities of the three compounds that are essentially in agreement

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with the above deductions derived from the experimental data. In the current work, the relevant ligands were docked into a homology model of the human 5-HT$_{2A}$ receptor that we constructed previously. Full details of the model construction and evaluation can be found in a prior publication[9]. Briefly, the 5-HT$_{2A}$ receptor homology model was built using a human β2-adrenergic receptor structure (PDB code: 2RH1) as a template. The 2RH1 crystal structure was solved at high resolution (2.40Å) and the sequence identity of the 5-HT$_{2A}$ receptor and the β2-adrenergic template is close to 30%. Furthermore, the two receptors have functional similarities as they are both categorized as neurotransmitter GPCRs. The sequences were aligned using the ClustalX[16] and ICM Pro sequence alignment tools[17] and the homology model was constructed with the ICM Pro model building program[17]. As secondary structure elements are highly conserved in these sequences, the alignments were manually ameliorated to achieve complete alignment of the residues known to be well conserved in the GPCR superfamily[18]. The model was subsequently relaxed by 500 steepest descent energy minimization steps via the Charmm molecular modeling package.

The docking experiments were performed using our in-house developed drug discovery platform. The algorithm invokes a simulated annealing protocol to find the best energetic fit between the ligand and the protein binding site[19, 20]. 3D atomic coordinates of the homology model serve as input to the program as well as the definition of the rectangular binding site box, which encompasses residues assumed to be important for ligand binding based on our previous docking studies of a series of nantenine analogs to the same 5-HT$_{2A}$ receptor homology model[9]. The annealing simulation evolves the ligand through a series of random moves, including rigid body translations and bond rotations. A score is calculated at each step based on the empirically derived ChemScore estimate of the binding energy[21], as well as receptor-ligand clashes. The Metropolis condition is applied to either accept or reject the modified structure. The length of the simulation is defined by the Markov chain length and the number of chains, and the temperature is slowly lowered after each chain. A single run yields a single docked structure and for each ligand, thirty docked structures were generated, clustered by ligand rmsd and ranked according to the ChemScore binding energy value. Receptor flexibility is incorporated into the docking protocol by allowing for conformational rearrangements of the amino acid side chain χ torsion angles. This represents an additional type of transition that is invoked during the simulated annealing process. Several levels of side-chain flexibility were exploited involving all residues within 4 or 5Å of the evolving ligand or by specifying a subset of side chains that typically clash with the evolving ligand structure in rigid receptor simulations. The docking outputs were found to be very similar in all these flexible side-chain scenarios.

A representation of the docked poses for the ligands **8i, 8j** and **8k** within the 5-HT$_{2A}$ receptor binding pocket is depicted in Figure 3. Overall, each ligand generates three different binding modes. They are not differentiated particularly well energetically, nevertheless, the structures depicted in the Figure correspond to the top ranked poses according to ChemScore. The estimated binding energies are -30.6, -31.6 and -34.4 kJ/mol for **8i**, **8j** and **8k**, respectively. This qualitatively correlates well with the observed affinity measurements, although quantitatively the binding energy differences are relatively small compared to the wide variation in affinity, particularly between compounds **8i** and **8k**. This

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is not particularly unexpected as no single scoring function can correctly rank order and quantitatively differentiate all protein-ligand complexes. Hence, docking scores are typically exploited to simply categorize ligands as active or inactive, rather than rank ordering. Furthermore, in this work, the tolerance of the binding site for multiple ligand binding modes and the relatively small set of compounds assessed in biological assays are other potential reasons for the lack of clear correlation between the docking results and the measured affinities measurements are needed for a larger, more structurally. It is clear that further computational simulations and affinity diverse library of compounds to gain binding energy for the latter analog is primarily due to better hydrophobic interactions with the receptor pocket involving the bromine substituent (-30.2 kJ/mol for the lipophilic term for **8k**) compared to chlorine (-27.2 kJ/mol for the lipophilic term for **8i**) as postulated above based on hydrophobicity and atomic size. For compound **8j** with the relatively large, hydrophobic CF_3 substituent, the hydrophobic contribution to the estimated binding energy is very similar to that for ligand **8k** with the bromine atom. This appears reasonable based on the close similarity of the binding poses and the distance from hydrophobic residues. The lipophilic term is however, slightly less favorable for **8j** presumably due to the relatively close proximity of the electron withdrawing CF_3 group and the protonated Lys350 side chain, making it difficult for the simulation to identify an orientation for H-bonding interactions while eliminating clashes. Overall, this translates to a binding energy for **8j** that is intermediate to that of compounds **8k** and **8i**.

Finally, compounds **5** and **8g** were docked into the 5-HT_{2A} receptor binding site in order to explore the huge difference in affinity for these systems with similar sized C1 substituents in terms of their receptor-ligand contacts. The top-ranked docking outcomes for these ligands are illustrated in Figure 4. As above for the benzyl substituent groups, the estimated binding energies are in qualitative accordance with the affinity measurements (-32.4 and -30.1 kJ/mol for compounds **5** and **8g**, respectively), however, the predicted energetic difference is too small compared to the diverse affinity data to provide definitive quantitative insight. The reasons postulated for this are similar to those explicated above for compounds **8i, 8j** and **8k**. Despite this limitation, as speculated earlier, the better binding energy for the allyl analog, **5**, derives mainly from an improved lipophilic term (-27.5 and -26.0 kJ/mol for compounds **5** and **8g**, respectively, for the hydrophobic interactions). Hence, replacement of the C1 allyl substituent with a more polar nitrile group leads to a reduction in favorable contacts in this hydrophobic region of the binding pocket.

In summary, modifications at the C1 position of **5** with various allyl isosteres has resulted in the identification of compound **8k**, the most potent *h*5-HT_{2A} receptor antagonist with an aporphine core reported thus far. Our SAR study indicates that hydrophobic, steric and electronic effects are important to the 5-HT_{2A} receptor affinity of the analogues; the relative contribution of each parameter depends on the particular C1 substituent. Substituted allyl, alkynyl, nitrile and ester functionalities are not well tolerated at C1 for high 5-HT_{2A} affinity. In the case of *p*-substituted benzyl analogues, hydrophobicity and larger steric size favors, while electron-withdrawing ability of the *p*-substituent disfavors 5-HT_{2A} receptor affinity. For compounds **8i** – **8k**, the 5-HT_{2A} receptor model does not clearly differentiate compounds with high affinity from compounds with low affinity when absolute binding

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energies are considered. Nevertheless, the binding energies for **8i – 8k** were consistent with the trend in ligand affinities observed and this was also paralleled in comparing **5** and **8g**. The extent to which the SAR trends observed above may be generalized as well as the capabilities and limitations of the 5-HT_{2A} receptor model for prospective ranking, will benefit from evaluations of larger libraries of analogues. The results obtained herein form the basis for such future extrapolations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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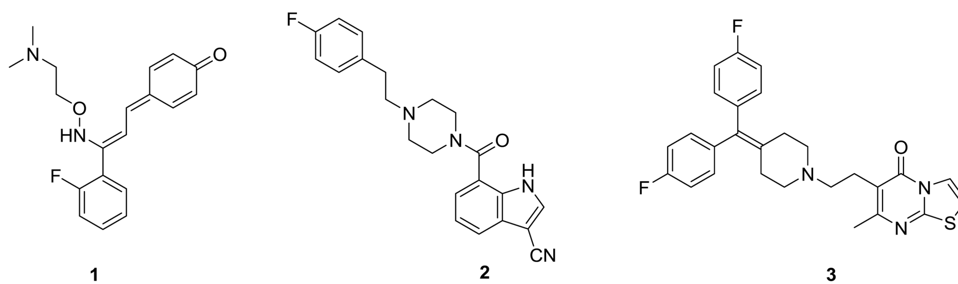

Figure 1. Structures of eplivanserin (1), pruvanserin (2) and ritanserin (3)

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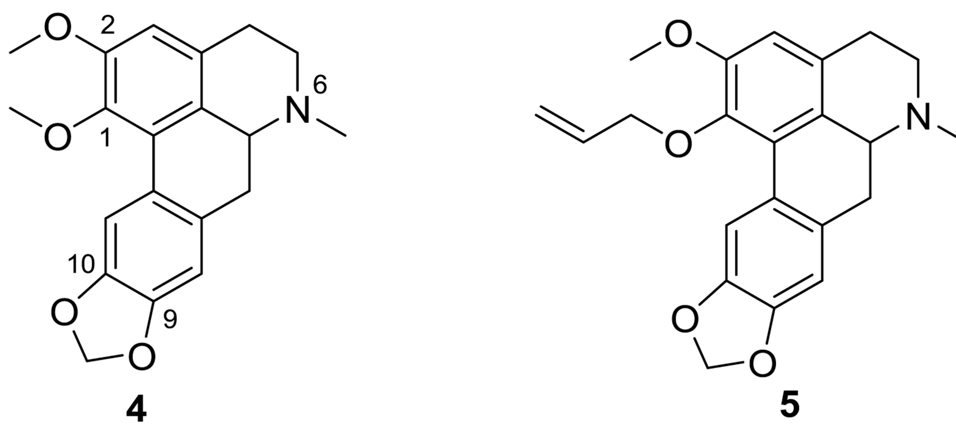

Figure 2. Structures of nantenine (4) and compound 5

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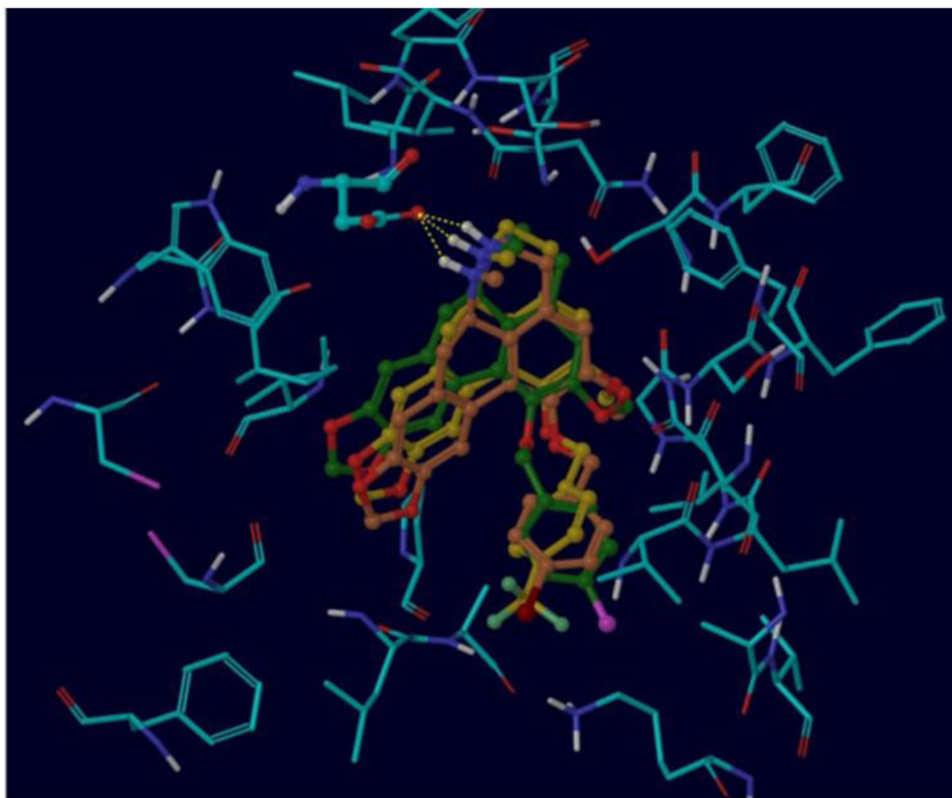

Figure 3.

Superimposed binding poses for compounds **8i** (C atoms in green), **8j** (C atoms in yellow) and **8k** (C atoms in brown). The key H-bonding interaction between the ligand quaternary N and Asp155 is depicted by the dashed line.

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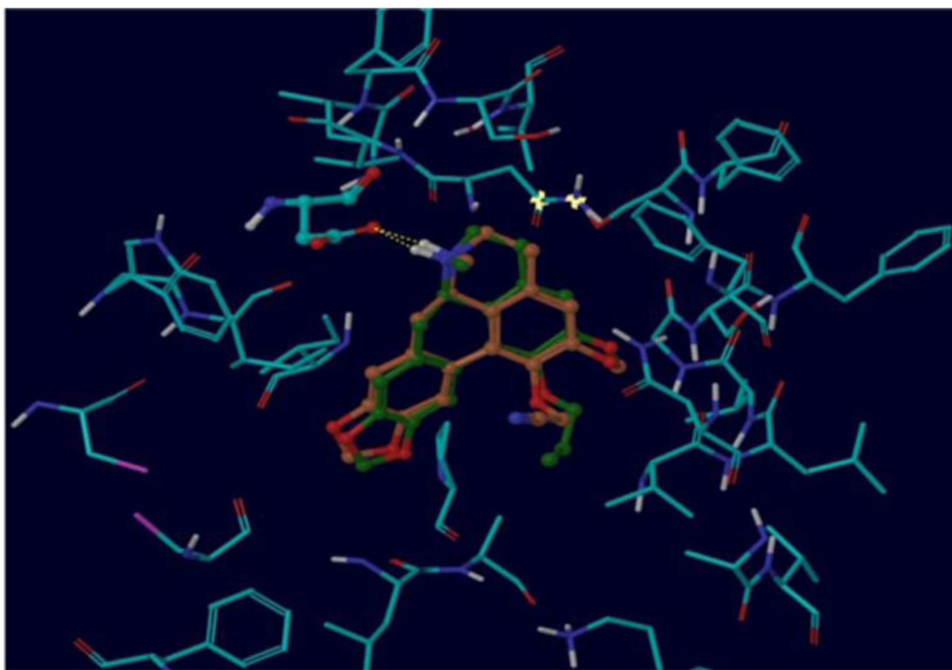

Figure 4.

Superimposed binding poses for compounds **5** (C atoms in green) and **8g** (C atoms in brown). The key H-bonding interaction between the ligand quaternary N and Asp155 is depicted by the dashed line.

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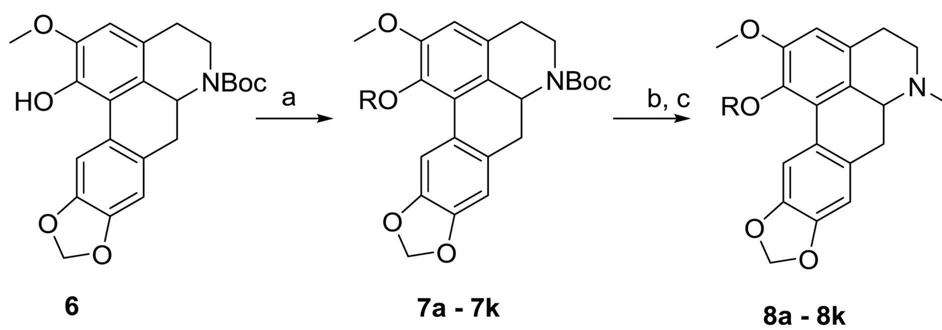

Reagents and conditions: (a) corresponding alkyl bromide, K_2CO_3, acetone; (b) $ZnBr_2$, CH_2Cl_2; (c) HCHO, $NaBH(OAc)_3$, CH_2Cl_2

Scheme 1. Synthesis of analogs 8a - 8k

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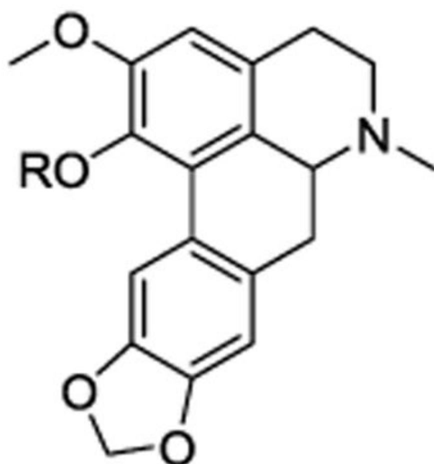
Table I

Apparent affinity (K_e) and selectivity of compounds 8a - 8k at 5-HT_{2A} and α_{1A} receptors



Compound	R	Affinity (K_e in nM)[a]		Selectivity
		5-HT_{2A}	α_{1A}	α_{1A}/5-HT_{2A}
8a	-CH_2CH=$CHCH_3$ (*E*)	723 ± 68	1,980 ± 455	2.7
8b	-$CH_2C(CH_2)CH_3$	172 ± 71	>10,000	> 58
8c	-CH_2CH=$C(CH_3)_2$	2,074 ± 230	>10,000	> 5
8d	-$CH_2C{\equiv}CCH_3$	2,690 ± 806	557 ± 111	0.2
8e	-CH_2CH=CHPh (*E*)	>10,000	>10,000	-
8f	-$CH_2CH_2CH_2CH$=CH_2	>10,000	>10,000	-
8g	-CH_2CN	>10,000	711 ± 64	< 0.07
8h	-$CH_2CH_2OCOCH_3$	1,913 ± 288	591 ± 81	0.3
8i	-$CH_2C_6H_4$-*p*-Cl	6,046 ± 3076	>10,000	> 1.7
8j	-$CH_2C_6H_4$-*p*-CF_3	2,458 ± 184	>10,000	> 4.1
8k	-$CH_2C_6H_4$-*p*-Br	9.2 ± 2.6	>10,000	>1,086
5	-CH_2CH=CH_2	70±15	>10,000	>143
4	CH_3	850 ± 5.8	36 ± 7	0.04
ketanserin		32[b,c]	nd[d]	
prazosin		nd[d]	1.1 ± 0.4	

[a] K_e (apparent affinity) values are means of at least two experiments carried out in triplicate;

[b] Experiment run once;

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

[d] not determined – compounds used as positive controls.

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