Bioorganic & Medicinal Chemistry Letters 20 (2010) 935–938

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

9-Aminomethyl-9,10-dihydroanthracene (AMDA) analogs as structural probes for steric tolerance in 5-HT_{2A} and H_1 receptor binding sites

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ARTICLE INFO

Article history:
Received 17 November 2009
Revised 14 December 2009
Accepted 15 December 2009
Available online 23 December 2009

Keywords:
5-HT_{2A} receptor
H_1 receptor
G protein-coupled receptor (GPCR)
GPCR modeling
AMDA
Phenylethylamines

ABSTRACT

Synthesis, radioligand binding and molecular modeling studies of several 9-aminomethyl-9,10-dihydroanthracene (AMDA) analogs were carried out to determine the extent of the steric tolerance associated with expansion of the tricyclic ring system and amine substitution at 5-HT_{2A} and H_1 receptors. A mixture of (7,12-dihydrotetraphene-12-yl)methanamine and (6,11-dihydrotetracene-11-yl)methanamine in a 75–25% ratio was found to have an apparent K_i of 10 nM at the 5-HT_{2A} receptor. A substantial binding affinity for (7,12-dihydrotetraphene-3-methoxy-12-yl)methanamine at the 5-HT_{2A} receptor (K_i = 21 nM) was also observed. Interestingly, this compound was found to have 100-fold selectivity for 5-HT_{2A} over the H_1 receptor (K_i = 2500 nM). *N*-Phenylalkyl-AMDA derivatives, in which the length of the alkyl chain varied from methylene to *n*-butylene, were found to have only weak affinity for both 5-HT_{2A} and H_1 receptors (K_i = 223 to 964 nM). Our results show that large rigid annulated AMDA analogs can be sterically accommodated within the proposed 5-HT_{2A} binding site.

Over the past few years, much information has been generated about 5-HT_2 receptor subtypes (5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}) and their ligands.[1,2] However, it is still not clear exactly how serotonergic ligands (agonists/partial agonists/antagonists/inverse agonists) including 5-HT itself interact with the receptors. In the absence of a crystal structure for any member of the 5-HT_2 receptor family, homology models generated from a representative GPCR such as rhodopsin or a β-adrenoceptor are used to study possible drug–receptor interactions.[3–5] Homology modeling and ligand docking, supported by experimental data, have allowed us to propose possible ligand-receptor interactions responsible for the observed affinity of the lead compound 9-aminomethyl-9,10-dihydroanthracene (AMDA) and its analogues at the human 5-HT_{2A} and H_1 receptors.[6–10] This work expands upon these earlier structure–affinity relationship studies via new AMDA analogs designed to probe the stereoelectronic accessibility of the receptor binding sites. In particular, annulated analogs of AMDA were synthesized and tested to determine the extent of bulk tolerance within the binding site of both 5-HT_{2A} and H_1 receptors. Further, to explore the potential differences between the 5-HT_{2A} and H_1 receptors in the vicinity of the ammonium ion binding site (known to be $D^{3.32}$ based on experimental and modeling data),[11,12] *N*-phenylalkyl analogues of AMDA were synthesized and tested.

The concept of using annulated analogues of established ligands as dimensional probes for ligand binding sites has been reported but not widely employed. Examples where this concept has been successfully applied include analogs of adenosine,[13] ATP,[14] cyclophosphamide,[15] isotryptamine[16] and allocolchicinoids.[17] By carrying out a systematic annulation and substitution, one can infer information about the dimensions as well as the interactions within the binding site.[18,19] AMDA and its analogues contain a rigid tricyclic scaffold. Previous SAR studies by Westkaemper and coworkers[5,7] using the 5-HT_{2A} receptor have indicated that there may be a substantial amount of steric tolerance around the rigid tricyclic scaffold of AMDA, as determined by substituent effects at the C3-position. However, the largest substituents examined to date (3-phenylpropyl-AMDA, K_i = 3.2 nM; 3-*n*-hexyl-AMDA, K_i = 7 nM; 3-*n*-pentyloxy-AMDA, K_i = 23 nM)[7] have a high degree of rotational freedom due to alkyl chain flexibility. There are currently no known 5-HT_{2A} ligands whose rigid core exceeds the dimensions of the annulated AMDA analogs reported here. For example, in the case of tricyclic antidepressants, ergot alkaloids and apomorphine the longest dimension is equivalent to three linearly fused rings. As a more stringent test of bulk tolerance, rigid and elongated structures were constructed by addition of fused rings to the tricyclic structure of AMDA. If accommodated, the AMDA pharmacophore expanded via benzfusion would also provide a new rigid scaffold for the introduction of substituents that may have access to additional binding site residues more distant from the tricyclic core than those accessible with previously-employed scaffolds.

The initial compounds for which syntheses were attempted were dihydrotetracenes (**1a** and **1b**, Table 1). However, the

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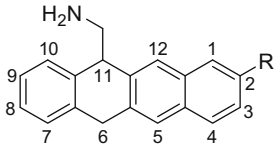
doi:10.1016/j.bmcl.2009.12.064

Table 1
Observed binding affinity data for 6,11-dihydrotetracene (**1a** and **1b**), 7,12-dihydrotetraphene (**2a** and **2b**) and 9,10-dihydroanthracene (**3a–d**) structural probes at 5-HT_{2A} and H_1 receptors



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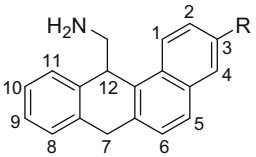

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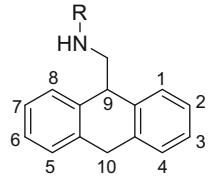


3

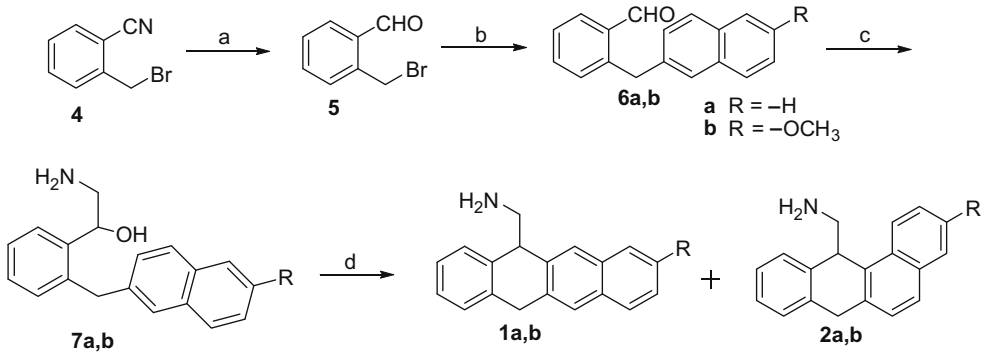
Compd	R	K_i, nM (±SD)[a]	
		5-HT_{2A}	H_1
1a + **2a**	–H	10 (±1)[b]	ND
1b	$-OCH_3$	ND	ND
2b	$-OCH_3$	21 (±1)	2640 (±528)
3a	$-CH_2C_6H_5$	721 (±223)[c]	ND
3b	$-(CH_2)_2C_6H_5$	400 (±34)	242 (±27)
3c	$-(CH_2)_3C_6H_5$	223 (±5)	725 (±128)
3d	$-(CH_2)_4C_6H_5$	365 (±17)	964 (±148)

[a] [^{3}H]Ketanserin-labeled cloned h5-HT_{2A} or [^{3}H]pyrilamine-labeled cloned hH_1 sites. Values represent the mean of computer-derived K_i estimates (using GraphPad PRISM) of multiple determinations. ND = not determined.
[b] Represents a mixture (75:25) of **2a** and **1a**. See text for details.
[c] From Runyon et al.[9]

synthetic methodology chosen for **1a** (Scheme 1) resulted in a mixture (75:25) of positional isomers of dihydrotetraphene **2a** and dihydrotetracene **1a**, respectively. In retrospect this is consistent with the greater stability of the intermediate carbocation formed as a result of electrophilic addition at the α-carbon versus the β-carbon of the naphthalene ring, giving rise to the kinetically-favored α-substituted product. The binding affinity data obtained for this mixture produced an apparent K_i value of 10 nM (5-HT_{2A}). Thus it was concluded that at least one of the isomers would be predicted to bind with $K_i \leqslant 10$ nM. The annulated methoxy-analogue **1b** was also synthesized using a similar procedure. The attempted synthesis also resulted in a mixture of two isomers (**1b** and **2b**). Due to the challenges faced in the final cyclization step and difficulty in the separation of products, the reaction resulted in a very low yield of the final products. While a mixture was obtained, it was possible to separate the methoxy-substituted isomers, although only dihydrotetraphene isomer **2b** was obtained preparatively. Dihydrotetraphene **2b** was determined to have substantial affinity for the 5-HT_{2A} receptor (K_i = 21 nM) while possessing very low affinity for the H_1 receptor (K_i = 2500 nM).

In order to explore potential differences between the 5-HT_{2A} and H_1 receptor binding sites in the vicinity of the ligand ammonium ion, various phenylalkylamine analogs of AMDA (**3a–d**) were synthesized and tested. It has previously been shown[10] that increasing either the length of the alkyl linker connecting the dihydroanthracene core to the amine or the degree of N-methylation progressively increases the affinity of AMDA analogs for H_1, but not for 5-HT_{2A}. The binding affinity of the larger *N*-benzyl-AMDA (**3a**) has also been reported[9] and shown to have a K_i value of 721 nM for the 5-HT_{2A} receptor, dramatically lower than for the parent compound AMDA (K_i = 20 nM). In order to further investigate the possibility that the ligand *N*-substituent may interact with more remote hydrophobic regions of the receptor in a manner similar to what has been proposed[7] for 3-phenylpropyl AMDA, analogs **3b–d** containing longer flexible linkers between the amine and the phenyl ring were synthesized and tested. The observed affinity data showed that each of the compounds **3b–d** had relatively low affinity (K_i = 223–964 nM) for both 5-HT_{2A} and H_1 receptors with practically no selectivity, indicating that the larger phenylalkylamine substituents are not sterically well-tolerated in either receptor.

In order to provide potential insights into how the benz-fused ligands interact with their receptors at an atomic level, automated ligand docking was carried out using an existing 5-HT_{2A} homology model, the construction and refinement of which has previously been described in detail.[6,7] This process is briefly described here. The initial step consisted of sequence alignment of the h5-HT_{2A} (P28233) sequence with several related class A GPCRs[20] using the CLUSTALX program.[21] This produced an unambiguous alignment in the transmembrane (TM) helical regions of h5-HT_{2A} sequences with that of both bovine rhodopsin and the β_2-adrenoceptor. This alignment, along with a file containing the atomic coordinates of the template receptor (PDB ID = 1U19), was used as input to the MODELLER software package[22] to generate a population of 100



Scheme 1. Reagents and conditions: (a) DIBAL-H, CH_2Cl_2; (b) $Pd(PPh_3)_4$, K_3PO_4, 2-naphthylboronic acid/6-methoxy-2-naphthylboronic acid; (c) TMSCN, ZnI, CH_2Cl_2, LAH, THF; (d) CH_3SO_3H.

different h5-HT_{2A} homology models. Each of these receptors was subsequently energy-minimized. The automated docking program GOLD version 3.01 (Cambridge Crystallographic Data Centre, Cambridge, UK)[23] was then used to dock selected ligands into the 5-HT_{2A} populations. Based on the fitness function values, steric and electronic interactions of the docked poses and reported site-directed mutagenesis data, one receptor model was selected to represent the AMDA-antagonist binding site of the h5-HT_{2A} receptor. This model was subsequently analyzed using PROCHECK and the ProTable facility within SYBYL to assess the geometric integrity of various structural elements (bond lengths, torsion angles, etc.) within the receptor. The target compounds with explicit consideration of stereoisomers were built and energy-minimized. These structures were docked into the receptor binding site using GOLD as previously described.[7] Visual inspection of the docked poses in conjunction with the ChemScore fitness function was used to select the final solution for each isomer. The GOLD program was able to place the 5-HT_{2A} ligands **2a** and **2b** in the previously-predicted AMDA-antagonist binding site (Fig. 1).

In each solution, the ligand ammonium ion interacts with the D155$^{3.32}$ residue in the binding site. Compound **2a**, with its extended aromatic structure, exhibited extensive lipophilic interactions with residues surrounding the dihydrotetraphene core (Fig. 1a and b). The region of the binding site occupied by the dihydrotetraphene core is the same as that previously proposed[6] for the dihydroanthracene core of 3-methoxy-AMDA. Like the stereoisomers of 3-methoxy-AMDA, the additional methoxy group of (*R*)-**2b** or (*S*)-**2b** may be accommodated in the binding pocket through a slight shift in the position of the polycyclic core, while the methoxy group may act as an H-bond acceptor for serine residues S159$^{3.36}$ or S77$^{1.35}$/S131$^{2.61}$, respectively (Fig. 1c and d). The observation that the methoxy-substituted compound **2b** showed substantial affinity (K_i = 21 nM) suggests that the non-linear dihydrotetraphene analog **2a** is a significant contributor to the observed affinity of the **2a**:**1a** mixture at 5-HT_{2A}. Our homology modeling and docking studies, however, indicate that the dihydrotetracenes **1a** and **1b** may also be accommodated in, and have substantial affinity for, the proposed binding site in a manner analogous to that of the dihydrotetraphenes **2a** and **2b** (ChemScores: (*R*)-**1a**, 40.75; (*S*)-**1a**, 38.23; (*R*)-**1b**, 36.49; (*S*)-**1b**, 40.65; (*R*)-**2a**, 38.28; (*S*)-**2a**, 40.03; (*R*)-**2b**, 41.15; (*S*)-**2b**, 38.05).

The target compounds were synthesized as shown in Schemes 1 and 2. In the case of the annulated analogs (Scheme 1), commercially available α-bromo-*o*-tolunitrile **4**, on treatment with DIBAL-H in anhydrous CH_2Cl_2 and work up using aqueous HBr, gave the aldehyde **5** in quantitative yields.[24] Palladium-catalyzed cross-coupling of 2-naphthylboronic acid or 6-methoxy-2-naphthylboronic acid with the halide **5** gave the coupled aldehydes **6a** and **6b**. Cyanosilylation of the aldehydes **6a** and **6b** using TMSCN gave the cyano trimethylsilyl ether as intermediates, which were

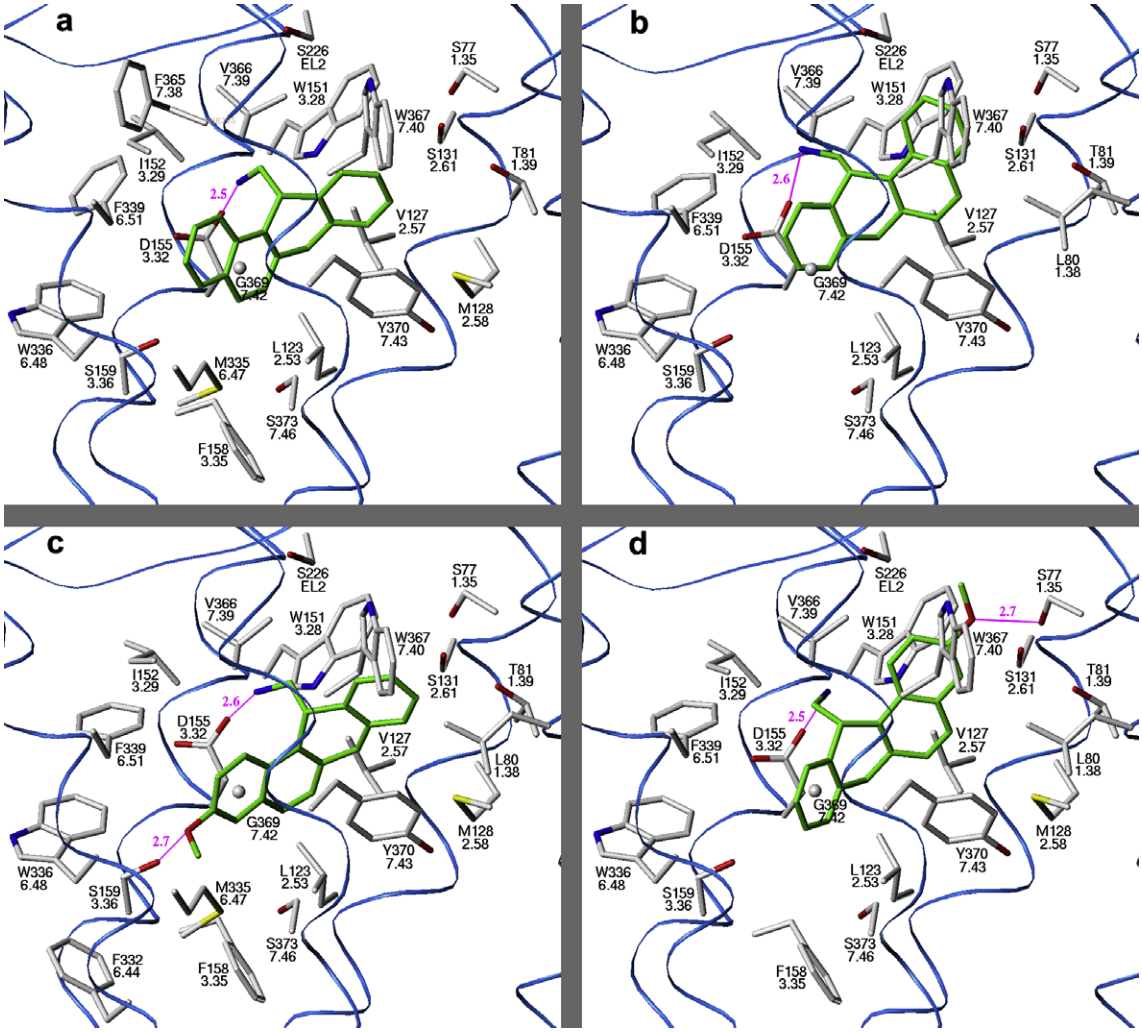
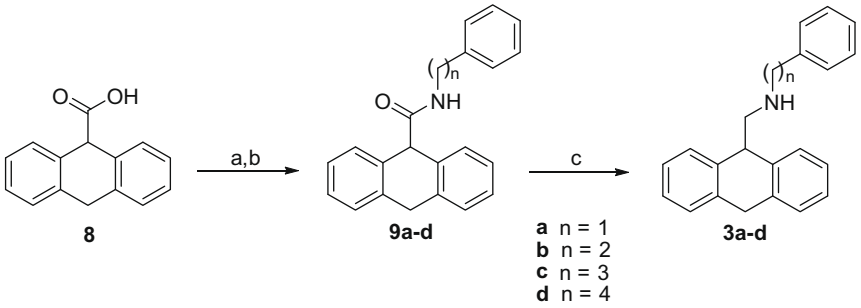


Figure 1. Proposed binding mode of isomers of annulated AMDA analogs (a) (*R*)-**2a**, (b) (*S*)-**2a**, (c) (*R*)-**2b** and (d) (*S*)-**2b** within the binding site of the 5-HT_{2A} receptor. Residues within 4 Å of the bound ligands are displayed. Hydrogen bonds are shown in magenta with donor–acceptor distances in Ångströms.



Scheme 2. Reagents: (a) $SOCl_2$, benzene; (b) phenylethylamine, phenylpropylamine or phenylbutylamine; (c) BH_3·THF.

reduced with LAH to give the respective amino alcohols **7a** and **7b**. Cyclodehydration of amino alcohols using methanesulfonic acid gave both the isomers of cyclized products **1a** and **1b**, and **2a** and **2b**, respectively. The synthesis of 9-(*N*-benzylaminomethyl)-9,10-dihydroanthracene (**3a**) was reported previously.[9] The phenylalkylamines **3b–d** were prepared by reduction (BH_3·THF) of amides obtained by the treatment of acid chloride with phenylethyl-, phenylpropyl- and phenylbutylamine, respectively (Scheme 2).

Binding assays and data analysis were performed through the NIMH Psychoactive Drug Screening Program (PDSP) using cloned human receptors. The 5-HT_{2A} competitive binding assay employs [^{3}H]ketanserin (a 5-HT_{2A} antagonist) as the radioligand, and the H_1 competitive binding assay employs [^{3}H]pyrilamine (an H_1 antagonist) as the radioligand. Binding data were analyzed using PRISM (GraphPad Software, Inc., San Diego, CA). Details of the binding assay protocol may be found at the PDSP home page, http://pdsp.med.unc.edu.

Acknowledgments

This work was supported by United States Public Health Service Grant R01-MH57969 (R.B.W.), R01-MH61887 (B.L.R.) and the NIMH Psychoactive Drug Screening Program (B.L.R.).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.064.

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