



Synthesis, structure–affinity relationships, and modeling of AMDA analogs at 5-HT_{2A} and H₁ receptors: Structural factors contributing to selectivity

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ABSTRACT

Histamine H₁ and serotonin 5-HT_{2A} receptors present in the CNS have been implicated in various neuropsychiatric disorders. 9-Aminomethyl-9,10-dihydroanthracene (AMDA), a conformationally constrained diarylalkyl amine derivative, has affinity for both of these receptors. A structure–affinity relationship (SAFIR) study was carried out studying the effects of N-methylation, varying the linker chain length and constraint of the aromatic rings on the binding affinities of the compounds with the 5-HT_{2A} and H₁ receptors. Homology modeling of the 5-HT_{2A} and H₁ receptors suggests that AMDA and its analogs, the parent of which is a 5-HT_{2A} antagonist, can bind in a fashion analogous to that of classical H₁ antagonists whose ring systems are oriented toward the fifth and sixth transmembrane helices. The modeled orientation of the ligands are consistent with the reported site-directed mutagenesis data for 5-HT_{2A} and H₁ receptors and provide a potential explanation for the selectivity of ligands acting at both receptors.

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1. Introduction

Sleep disorders and circadian rhythm abnormalities are prevalent, with 70 million Americans reporting disturbed sleep each year. Epidemiological studies indicate that between 30% and 48% of the global population find difficulty in initiating and maintaining sleep.¹ Serotonin (5-hydroxytryptamine, 5-HT), a major neurotransmitter found in the CNS and periphery, has been reported to be involved in the control of sleep and waking states.² 5-HT₂ receptors belong to the superfamily of G protein-coupled receptors (GPCRs) and consist of three subtypes: 2A, 2B, and 2C. 5-HT_{2A} receptors are involved mainly in non-rapid eye movement sleep regulation and respiratory control,³ and selective antagonism of the 5-HT_{2A} receptor has emerged as a promising new mechanism for the treatment of sleep disorders.⁴

Histamine released by tissue mast cells, basophils, and histaminergic neurons is also known to impact the ability to fall asleep and stay asleep.⁵ It has been suggested that ligands acting at H₁ receptors in the CNS may be useful in the treatment of neuropsychiatric and sleep disorders.⁶ Histamine exerts its effect by interacting with four different receptors H₁–H₄, which are GPCRs.^{7,8} Early research and development of H₁ ligands has focused largely

on antagonists that are used for their antiallergic effects in the periphery. However, first-generation H₁ receptor antagonists (diphenhydramine, mepyramine, chlorpheniramine) also exhibit high H₁ receptor occupancy in the CNS due in part to their lipophilicity, leading to sedative effects.^{9–11} This led to the development of second-generation antagonists (acrivastine, loratadine, terfenadine, cetirizine, etc.) that exhibited high selectivity and less sedative potential due to the decrease in their ability to penetrate the blood brain barrier.^{12,13} Some of these antagonists (doxepin, ketotifen, epinastine, olopatadine), which have a tri- or tetracyclic fused ring system, also showed affinity at 5-HT_{2A}, adrenaline α_1 , dopamine D₂ and muscarinic M₁ receptors, indicative of their low selectivity for the H₁ receptor.¹⁴ Among the various chemical classes of H₁ antagonists, ligands that are zwitterionic (olopatadine, acrivastine, fexofenadine) showed the highest degree of selectivity for H₁, suggesting that the carboxylate group of the ligand may interact with a residue (K191)¹⁵ in the H₁ receptor that is not present in the other GPCRs. Introduction of a carboxylate moiety thus provides one potential means of designing peripherally-acting H₁-selective antagonists.

In this study, we set out to determine if H₁ versus 5-HT_{2A} selectivity could be achieved through the systematic modification of the uncharacteristically nonpolar¹⁶ structure of 9-(aminomethyl)-9,10-dihydroanthracene (AMDA, **1a**). We have previously reported AMDA as the parent member of a potentially new class of

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high-affinity 5-HT_{2A} antagonists.^{17,18} This work builds on our earlier studies¹⁹ by creating a ‘matrix’ of AMDA-like compounds in which the aromatic ring system, degree of N-methylation and length of the aliphatic linker are systematically varied. A series of 9-(aminoalkyl)-9,10-dihydroanthracenes (DHAs) and an analogous series of diphenylalkylamines (DPAs) were synthesized and their binding affinities at H₁ and 5-HT_{2A} determined. Our results show that AMDA and related compounds exhibit varying degrees of affinity for the H₁ receptor. Homology models using the human β_2 -adrenoceptor (β_2 -AR)²⁰ as a template provide receptor-based explanations for the observed structure–affinity relationships (SAFIR) among the AMDA analogs. To our knowledge this is also the first report of a comparison of 5-HT_{2A} and H₁ homology models generated from the β_2 -adrenergic structure.

2. Results and discussion

2.1. Chemistry

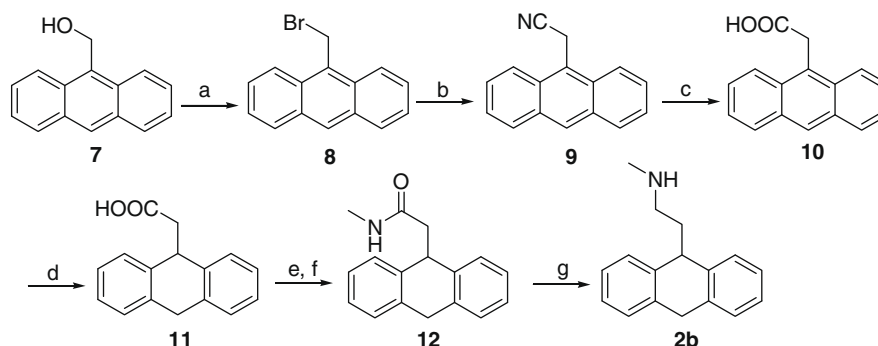
9-(Aminomethyl)-9,10-dihydroanthracene (**1a**), 9-(2-aminoethyl)-9,10-dihydroanthracene (**2a**), and 9-(2-aminopropyl)-9,10-dihydroanthracene (**3a**) were prepared as previously described.^{19,21} N-Alkylated analogs **1b**, **1c**, **3b**, and **3c** have been reported by us.¹⁹ N-Methylated analogs of **2a** were synthesized (Scheme 1) using 9-hydroxymethylanthracene **7** as the starting material. Halogenation²² followed by cyanation²³ of the alcohol gave 2-(anthracen-9-yl)acetonitrile **9** that was further hydrolyzed to the corresponding acid **10**. Anthracene ring reduction using sodium metal in *n*-pentanol gave 2-(9,10-dihydroanthracen-9-yl)acetic acid (**11**). Sequential conversion of the acid **11** to its amide **12** via acid chloride, followed by reduction, afforded 2-(9,10-dihydroanthracen-9-yl)-N-methylethanamine (**2b**). 2-(9,10-Dihydroanthracen-9-yl)-N,N-dimethylethanamine (**2c**) was obtained by reductive amination²⁴ of 2-(9,10-dihydroanthracen-9-yl)acetaldehyde (**15**), which was obtained by the oxidation of 2-(9,10-dihydroanthracen-9-yl)ethanol (**14**) (Scheme 2). N-Methyl-2,2-diphenylethanamine²⁵ (**4b**) was obtained by the N-methylation of the respective amine as reported.²⁶ N,N-Dimethyl-3,3-diphenylpropan-1-amine²⁷ (**5c**) was obtained by the same method adopted for the synthesis of **2c** from commercially available 3,3-diphenylpropanal. 4,4-Diphenylbutan-1-amine²⁸ (**6a**) was obtained by the BH₃·THF reduction of commercially available 4,4-diphenylbutanenitrile. Compounds **4a**, **4c**, **5a**, **5b**, **6b**, and **6c** were commercially obtained.

2.2. Structure–affinity relationships

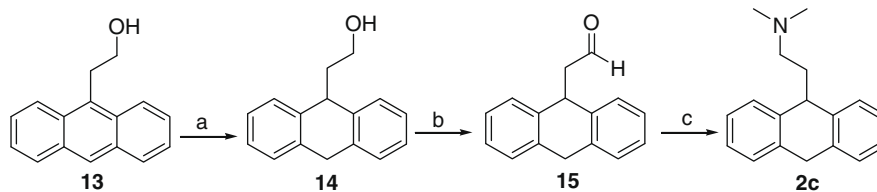
Several reviews describing structure–activity relationship studies of diphenhydramine-like antihistamines have been reported.^{29–31} The features that have been systematically varied include the

nature of the two required aromatic groups, the terminal basic amine, and the aliphatic linker between these two features.^{32,33} In this work, we examined similar structural variations using AMDA as the parent structure. These compounds closely resemble well-established H₁ antagonists: replacement of the ether oxygen of diphenhydramine by a methylene unit results in compound **6c** and replacement of the pyridin-2-yl group of the H₁ antagonist pheniramine with a phenyl group results in **5c**.

Compound affinities for the 5-HT_{2A} and H₁ receptors are given in Table 1 and displayed graphically in Figure 1. The most significant differences in affinity were observed between the 9-(aminoalkyl)-9,10-dihydroanthracene (DHA) compounds (Fig. 1a) and their corresponding diphenylalkylamine (DPA) congeners (Fig. 1b). Ring-opening of the tricyclic system uniformly produced decreases in affinity for both 5-HT_{2A} and H₁ receptors. No DPA compound exhibited high affinity (K_i < 100 nM) at 5-HT_{2A}, and only three showed high affinity for H₁ receptors (**5b**, K_i = 64 nM; **5c**, K_i = 75 nM; **6c**, K_i = 70 nM). In contrast, many of the DHA analogs were found to have high affinity at both receptors, demonstrating that dihydroanthracene is a privileged³⁴ structure. For compounds interacting with the H₁ receptor, progressively increasing either the number of methylene units in the linker or the number of N-methyl groups consistently retained or enhanced affinity in both the DHA and the DPA series. H₁ receptor affinity was insensitive to chain length for the unsubstituted amine analogs of AMDA (**1a**, K_i = 197 nM; **2a**, K_i = 137 nM; **3a**, K_i = 175 nM). In contrast, H₁ affinity increased with increasing chain length for both N-methylated (**1b**, K_i = 189 nM; **2b**, K_i = 48 nM; **3b**, K_i = 3 nM) and N,N-dimethylated analogs (**1c**, K_i = 25 nM; **2c**, K_i = 6 nM; **3c**, K_i = 0.5 nM). For the DHA analogs, N,N-dimethylation and a three-methylene linker was optimal for H₁ affinity (**3c**, K_i = 0.5 nM). The trends in affinity at 5-HT_{2A} are less uniform. Like the H₁ receptor, the affinity of the DPA compounds for 5-HT_{2A} generally increased with increasing linker length or degree of N-methylation though, as noted above, no DPA compound was found to have substantial (K_i < 700 nM) affinity for the 5-HT_{2A} receptor. As observed previously for DHA¹⁹ and compounds with a one-methylene linker, 5-HT_{2A} affinity decreased as N-methylation degree increased (**1a**, K_i = 20 nM; **1b**, K_i = 52 nM; **1c**, K_i = 540 nM). This trend was not observed for the other linker lengths. Additionally, for DHA compounds with no N-methylation, linker lengths of one (**1a**, K_i = 20 nM) and three (**3a**, K_i = 32 nM) methylene units demonstrated significantly higher affinity than the two-methylene linker (**2a**, K_i = 480 nM) compound. Affinities were more uniform for compounds with one N-methyl group (**1b**, K_i = 52 nM; **2b**, K_i = 92 nM; **3b**, K_i = 13 nM), and for those with two N-methyl groups, affinity increased with linker length by 25-fold (**1c**, K_i = 540 nM; **2c**, K_i = 84 nM; **3c**, K_i = 22 nM). In general, 5-HT_{2A} receptor affinity is less sensitive to N-methylation and chain length variation than H₁ receptor affinity.



Scheme 1. Reagents and conditions: (a) PBr₃, toluene 0 °C, 1 h; (b) KCN, DMSO, 70 °C, 1 h; (c) KOH, ethylene glycol/H₂O (1:1), reflux, 12 h; (d) Na, 1-pentanol, reflux, 30 min; (e) SOCl₂, dry benzene, reflux, 2 h; (f) methylamine–THF, 25 °C, 6 h; (g) BH₃·THF, THF reflux, 6 h.



Scheme 2. Reagents and conditions: (a) Na₂K silica gel, THF, 1 h; (b) Dess–Martin reagent, CH₂Cl₂, 1 h; (c) Me₂NH·HCl, Ti(O-*i*-Pr)₄, NEt₃, abs EtOH, 25 °C, 9 h, NaBH₄, 25 °C, 10 h.

Table 1

Observed binding affinities for 9-(aminoalkyl)-9,10-dihydroanthracene (DHA) and diphenylalkylamine (DPA) analogs at 5-HT_{2A} and H₁ receptors

Compd	X	n	R ¹	R ²	K _i (nM)	
					5-HT _{2A}	H ₁
1a	–CH ₂ –	1	–H	–H	20 ^a	197
1b	–CH ₂ –	1	–H	–CH ₃	52 ^b	189
1c	–CH ₂ –	1	–CH ₃	–CH ₃	540 ^b	25
2a	–CH ₂ –	2	–H	–H	480 ^b	137
2b	–CH ₂ –	2	–H	–CH ₃	92	48
2c	–CH ₂ –	2	–CH ₃	–CH ₃	84	6
3a	–CH ₂ –	3	–H	–H	32 ^b	175
3b	–CH ₂ –	3	–H	–CH ₃	13	3
3c	–CH ₂ –	3	–CH ₃	–CH ₃	22	0.5
4a	–H, –H	1	–H	–H	4610 ^c	>10,000
4b	–H, –H	1	–H	–CH ₃	>10,000	>10,000
4c	–H, –H	1	–CH ₃	–CH ₃	7356	5172
5a	–H, –H	2	–H	–H	>10,000	2758
5b	–H, –H	2	–H	–CH ₃	1498	64
5c	–H, –H	2	–CH ₃	–CH ₃	1636	75
6a	–H, –H	3	–H	–H	2589	1670
6b	–H, –H	3	–H	–CH ₃	754	386
6c	–H, –H	3	–CH ₃	–CH ₃	1151	70

^a From Westkaemper, et al. 2001.²¹

^b From Runyon, et al. 2001.¹⁹

^c From Runyon, et al. 2002.¹⁷

2.3. Modeling receptor–ligand interactions

2.3.1. H₁ and 5-HT_{2A} receptor models

When considering the selectivity of a particular ligand for one receptor versus another, it is useful to analyze the differences in amino acids that comprise the binding sites of the two receptors. The alignment of the human H₁ and 5-HT_{2A} receptor sequences with that of human β₂-AR is presented in Figure 2. The H₁ receptor is phylogenetically related to the 5-HT₂ receptor subtypes, with higher sequence homology found primarily in the transmembrane-spanning regions.³⁵ Based on the primary sequences, binding site analysis of both 5-HT_{2A} and H₁ receptors indicates that the major variations in amino acid constitution occur at positions 3.33, x12.52, x12.54, 5.39, 5.42, 6.55, and 7.35 (Fig. 2). Differences in steric and electrostatic sidechain properties at these and other positions are likely responsible for directing the ligand selectivity of the two receptors. The H₁ receptor amino acid residue K191^{5,39}, not commonly found at this position among aminergic GPCRs, could be potentially exploited for the design of highly selective H₁ ligands. The three-dimensional arrangement of these residues is shown in Figure 3. The residues that are conserved

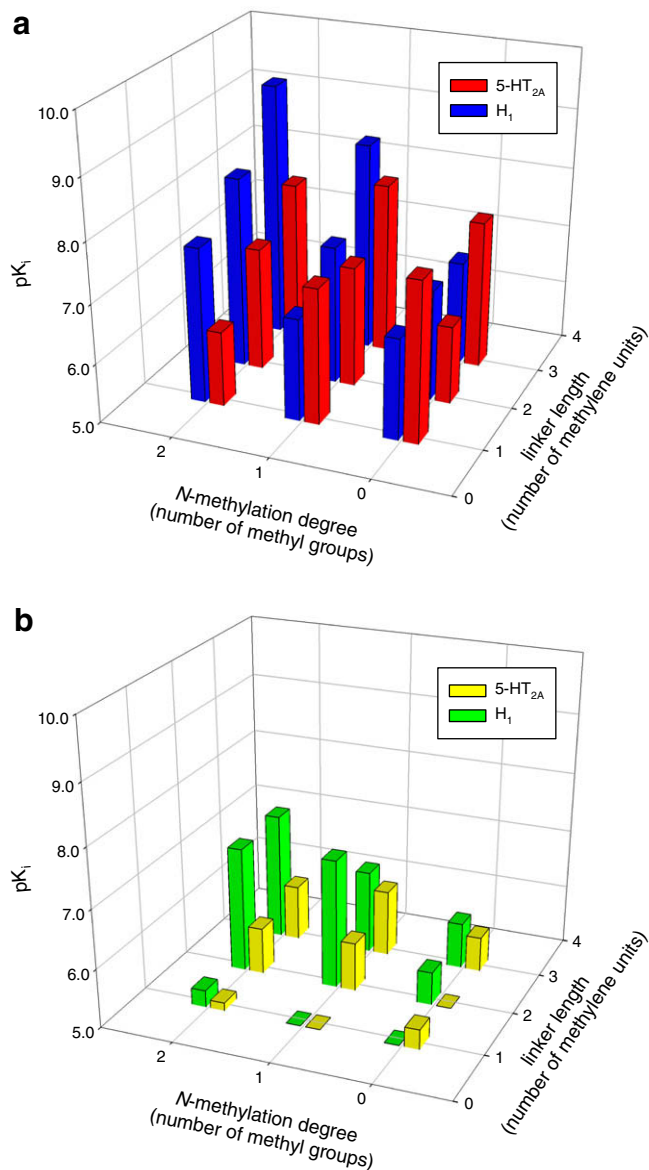


Figure 1. 3D bar graph showing the affinities at 5-HT_{2A} and H₁ for the (a) 9-(aminoalkyl)-9,10-dihydroanthracene (DHA) and (b) diphenylalkylamine (DPA) analogs listed in Table 1.

between H₁ and 5-HT_{2A} are those that tend to be highly conserved among all aminergic GPCRs. These residues, primarily located in the inner region of the binding cavity, include W^{3.28}, the amine counter-ion D^{3.32}, S^{3.36}, and T^{3.37}, and the ‘aromatic cluster’^{36–39} residues W^{6.48} and F^{6.52}, and Y^{7.43} (an H-bonding partner for D^{3.32}).

Models of human H₁ and 5-HT_{2A} receptors were generated with the MODELLER⁴⁰ program using a high-resolution crystal structure of

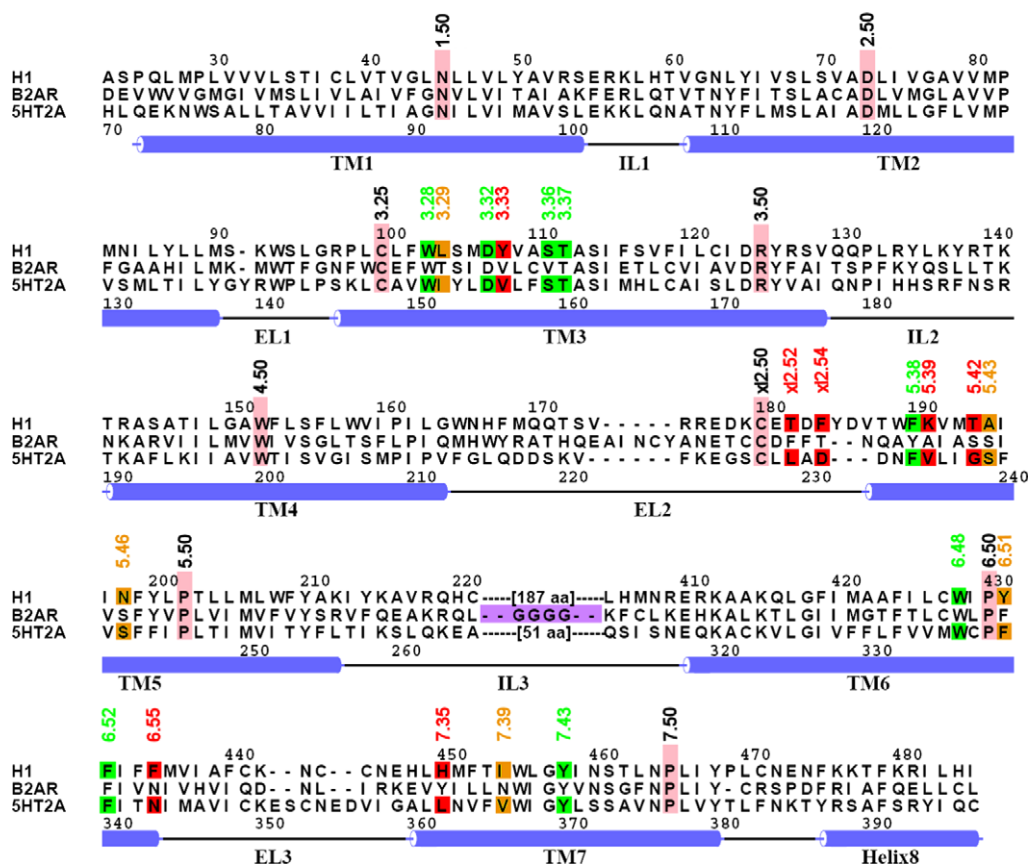


Figure 2. Alignment of the human β_2 -adrenergic, H_1 and 5-HT $_2A$ receptor sequences. Sequence positions highlighted in pink indicate highly conserved amino acids among the Class A GPCR family that serve as reference positions in the Ballesteros–Weinstein⁵² numbering system, as well as the cysteine residues of the disulfide bridge tethering EL2 to TM3. The traditional numbering shown corresponds to the hH $_1$ (top) and h5-HT $_2A$ (bottom) sequences. β_2 -Adrenergic residues highlighted in purple indicate positions in the third intracellular loop (IL3) that were mutated to glycine in the hH $_1$ and h5-HT $_2A$ sequences; these were retained in subsequent hH $_1$ and h5-HT $_2A$ models. Other residues highlighted in the hH $_1$ and h5-HT $_2A$ sequences represent the residues of the binding site and correspond to those positions that are within 5.0 Å of the carazolol ligand bound in the β_2 AR homolog. The color indicates the degree of similarity between the hH $_1$ and h5-HT $_2A$ residue at a particular position as defined by the Gonnet PAM250 similarity matrix (green = identical; yellow = strong or weak conservation; red = no conservation). The figure was created using ALSCRIPT.⁵³

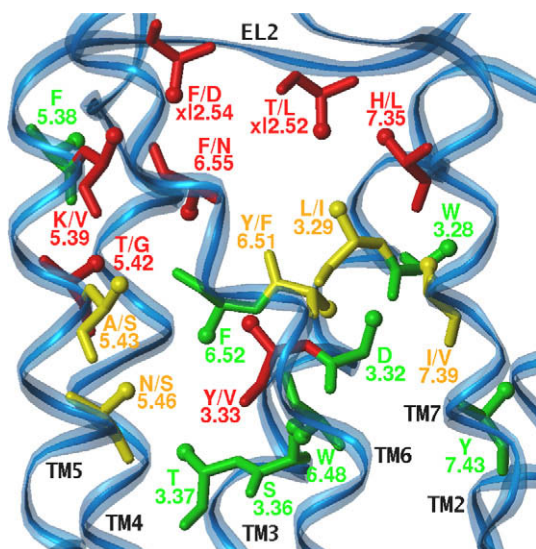


Figure 3. Illustration of the differences in binding site residue composition of the hH $_1$ and h5-HT $_2A$ receptors within the context of the β_2 AR-T4L crystal structure. Residues are truncated at the C β carbon atom (ball-and-stick representation) and are colored based on residue similarity as described in Figure 2. For those positions whose residue identity differs, the H $_1$ residue is listed first, followed by the 5-HT $_2A$ residue.

the human β_2 -AR²⁰ as the template (see Section 4). Inspection of putative receptor binding sites and ligand binding modes in our homology models (Figs. 4 and 5) indicate two common interactions that occur for each of the compounds listed in Table 1 at both 5-HT $_2A$ and H_1 receptors: 1) the highly conserved aspartic acid residue at position⁴¹ 3.32 (H $_1$, D107^{3,32}; 5-HT $_2A$, D155^{3,32}) is able to interact with the protonated amine of the docked ligand; and 2) hydrophobic residues present in transmembrane helix 6 (TM6) comprising the aromatic cluster⁴² are able to interact with the aromatic rings of the ligands. In other binding site locations variability of amino acid residues at equivalent positions in the hH $_1$ and h5-HT $_2A$ receptors influence the way the aromatic rings are oriented in the receptor, which in turn provides an explanation for the observed differences in affinity for AMDA and its analogs with varying chain lengths. The presence of hydrophobic residues surrounding the conserved D^{3,32} in H $_1$ and in 5-HT $_2A$, together with the aromatic cluster region in H $_1$ and in 5-HT $_2A$, provide sites of favorable interaction with the ligands for each receptor.

2.3.2. Binding mode analysis

Compounds listed in Table 1 were docked into the receptor binding sites of the H $_1$ and 5-HT $_2A$ receptor models using the GOLD automated docking routine.⁴³ The GOLD scoring function (steric and electrostatic interactions) was used to select the favored ligand conformation. In addition, we carried out HINT⁴⁴ (Hydro-

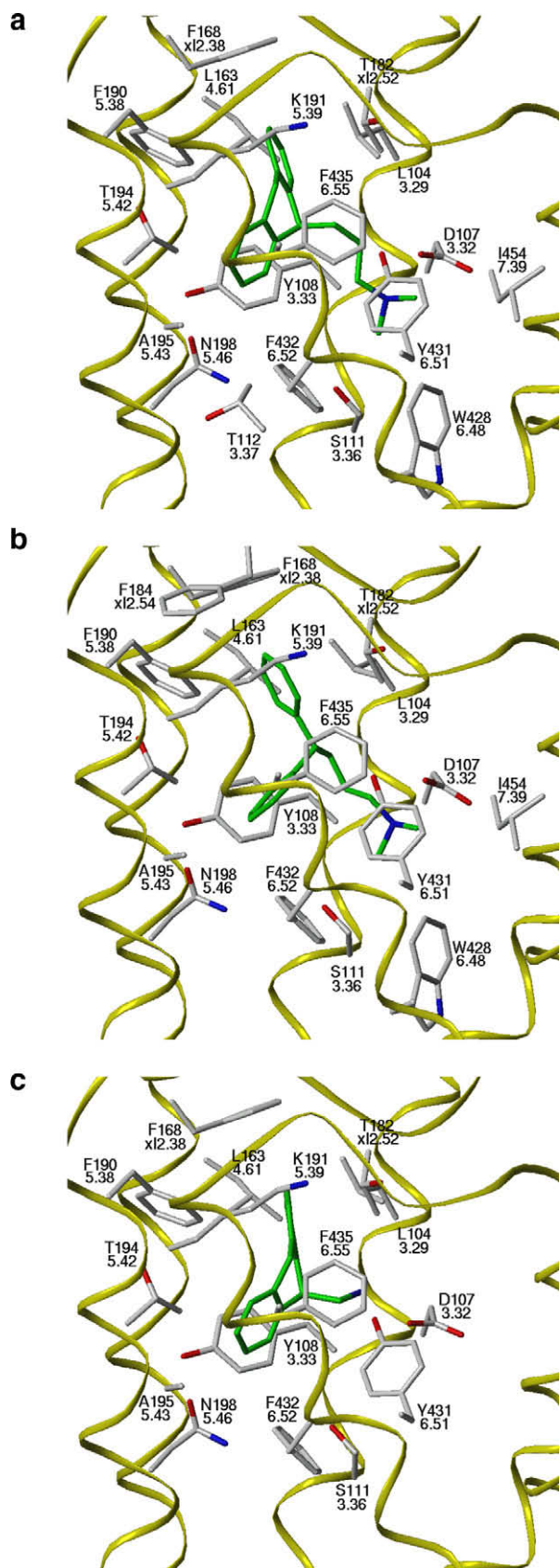


Figure 4. Docked hH1-ligand complexes. (a) 3c. (b) 6c. (c) 1a. Residues within 5 Å of the bound ligand are displayed.

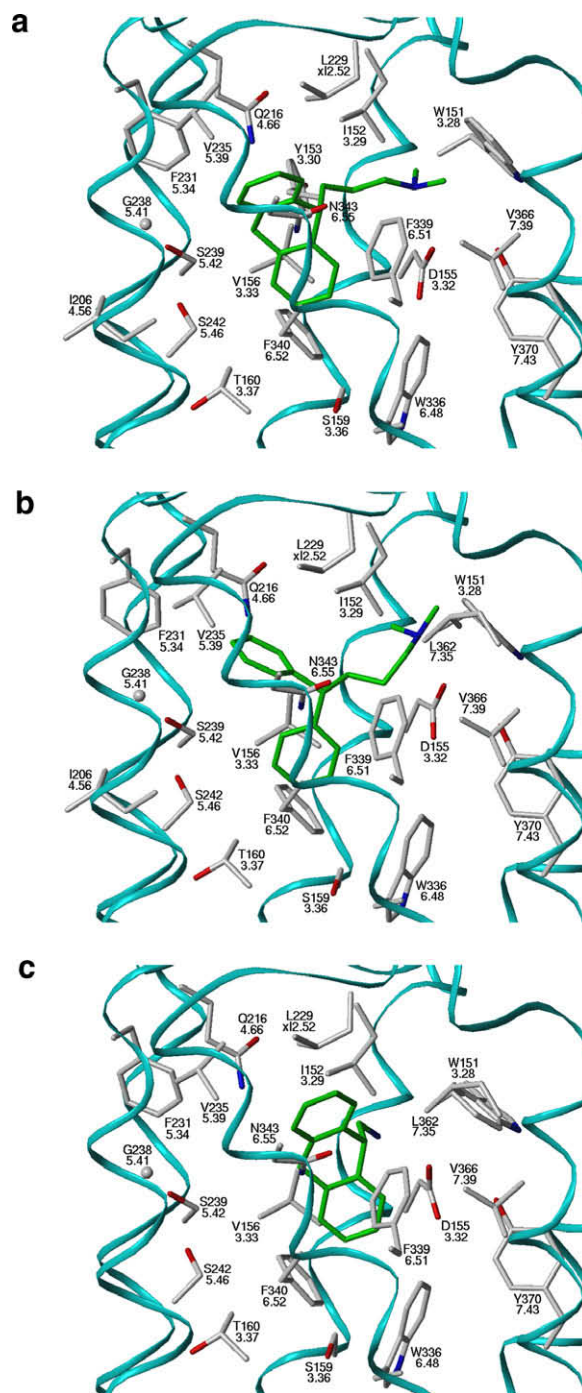


Figure 5. Docked h5-HT_{2A}-ligand complexes. (a) 3c. (b) 6c. (c) 1a. Residues within 5 Å of the bound ligand are displayed.

pathic INTERACTION) analysis to characterize the nature of the binding site interactions. HINT is a free-energy-based method that considers atom–atom interactions in a bimolecular complex using a parameter set derived from octanol/water partition coefficients.⁴⁵ Modeling observations indicate that the compounds prefer to be oriented within the binding pockets of the two receptor models in similar, but distinct binding modes (see Figs. 4 and 5). The well-known aspartate D^{3.32} residue was found to interact

with the basic amine in both receptors for each docked ligand, and the ligand aromatic rings were consistently oriented in the binding site surrounded by TM4, TM5, and TM6. In general, for the short-chain linker ligands there is a relatively small amount of ligand surface area that may interact with the surrounding hydrophobic environment in the binding pocket, accounting for the observed decreased affinity of these compounds at both 5-HT_{2A} and H₁ (Figs. 4c and 5c). However, for AMDA (1a), the observed high affinity could be due to the presence of an alternate binding mode.⁴⁶

The tricyclic DHA ring system in **3c** showed strong hydrophobic interactions (Y108^{3.33}, L163^{4.61}, F168^{x12.38}, F190^{5.38}, and F435^{6.55}), with the phenyl rings oriented toward TM5 and the aliphatic linker located deep in the H₁ binding pocket. In contrast, in the 5-HT_{2A} model, **3c** is oriented such that the phenyl rings are facing TM6 (F339^{6.51}, F340^{6.52}, and N343^{6.55}) and the aliphatic linker is positioned closer to extracellular loop 2 (EL2). For both H₁ and 5-HT_{2A}, the compound with the longest linker and highest degree of methylation (**3c**) was found to have the highest (H₁) or one of the highest (5-HT_{2A}) affinities among the compounds tested. The methylene linkers in the H₁ receptor are able to interact with residues L104^{3.29} and Y431^{6.51}, which explains the comparatively higher affinity of **3c** for H₁ than for 5-HT_{2A}. Further, the observed differences in ligand orientation within the receptor binding site can account for the preference for N-methylation at H₁, with the methyl groups more closely surrounded by hydrophobic residues as compared to 5-HT_{2A} (Figs. 4a and 5a).

For the unbridged DPA analogs, the increased conformational flexibility (as compared to DHA) and intramolecular steric repulsion produce a twisted ring orientation. This results in more unfavorable receptor–ligand interactions (steric clashes and polar–nonpolar interactions) involving the ring system and the surrounding residues (Figs. 4b and 5b).

HINT hydrophathic interaction analysis provided additional support for the proposed orientation of the ligands in the binding pocket. Figure 6 shows the HINT maps generated for compound **3c** (which has the highest affinity for both receptors) in the binding sites of the H₁ and 5-HT_{2A} receptors. In each case the tricyclic ring system fits into the hydrophobic ‘aromatic cluster’ while the amine group faces D107^{3.32} and is stabilized by both ionic and hydrogen bonding interactions. However, both the aliphatic linker region and the *N*-methyl groups engage in more extensive hydrophobic interactions in H₁ than in 5-HT_{2A}.

In addition, the receptor–ligand complexes described here are in general agreement with other studies that have implicated residues that contribute to an antagonist binding site in H₁ and 5-HT_{2A}. Besides the several key residues that have been reported by site-directed mutagenesis studies in H₁ receptor models^{15,47} (W158^{4.56}, Y200^{5.48}, F424^{6.44}, W428^{6.48}, F432^{6.52}, and F435^{6.55}) and 5-HT_{2A} receptor models⁴⁸ (F243^{5.47}, W336^{6.48}, F339^{6.51}, F340^{6.52}), we found that Y108^{3.33} and I454^{7.39} were oriented to favorably interact with ligands in the binding pocket of the H₁ receptor, and the cognate residues V156^{3.33} and V366^{7.39} for the 5-HT_{2A} receptor. The differences in the stereoelectronic character of these residues likely contribute to the differences in the way the ligand binds to these receptors, and consequently the observed differences in binding affinity.

3. Conclusions

Within the matrix of compounds synthesized and tested, the *N,N*-dimethylated chain-lengthened propylene analog of AMDA shows the highest affinity at both 5-HT_{2A} and H₁ receptors (**3c**: 5-HT_{2A}, *K*_i = 22 nM; H₁, *K*_i = 0.5 nM) and the highest selectiv-

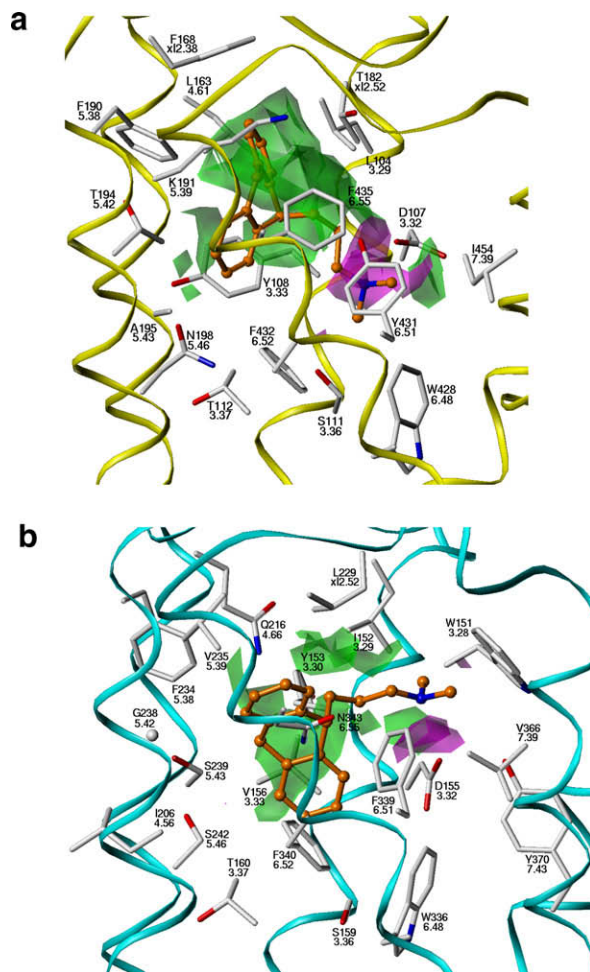


Figure 6. HINT interaction maps for compound **3c** in (a) H₁ and (b) 5-HT_{2A} binding sites. Regions of favorable hydrophobic (green) and polar (magenta) intermolecular interactions are shown as contours.

ity for the H₁ receptor (44-fold). In addition, removing the conformational restriction of the dihydroanthracene tricyclic system by ring-opening to provide a diphenyl system is detrimental to the ligand affinity for both 5-HT_{2A} and H₁ receptors. Structure–affinity relationships among these compounds show that *N*-alkylation either decreases or has little effect on 5-HT_{2A} affinity, while the propylene linker is the optimum chain length between the tricyclic system and amine for receptor affinity. Modeling studies suggest that diaryl alkylamine analogs exhibit a common binding mode within the 5-HT_{2A} and H₁ receptors. Hydropathic analysis of the modeled complexes supports the proposed role of the TM6 aromatic cluster in directing binding. The proposed differences in the binding pocket of H₁ (Y108^{3.33} and I454^{7.39}) and 5-HT_{2A} (V156^{3.33} and V366^{7.39}) may determine the way ligands bind, which in turn may determine the selectivity of the ligands for each of the two receptors. These preliminary modeling results provide a qualitative understanding of how AMDA analogs might interact with the 5-HT_{2A} and H₁ receptors. This study also provides potential insight into the mechanisms by which differences in the structures of the receptors and ligands determines receptor selectivity. We continue to synthesize and test compounds and refine our models toward the development of a more quantitative and predictive structure-based QSAR model.

4. Experimental

4.1. Chemistry

Nuclear magnetic resonance (^1H NMR and ^{13}C NMR) spectra were recorded using a Varian Gemini 300 spectrometer in CDCl_3 using tetramethylsilane as an internal standard unless otherwise specified. Melting points were determined using an OptiMelt melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., and determined values are within 0.4% of theory. All reactions were maintained under a nitrogen atmosphere. Anhydrous solvents were purchased and stored under nitrogen over molecular sieves. Medium-pressure column chromatography was carried out using silica gel 60 Å, 0.040–0.063 mm, (200–400 mesh), Sorbent Technologies.

4.1.1. 9-Bromomethylanthracene (**8**)²²

Phosphorus tribromide (0.8 ml, 8.4 mmol) was added to a suspension of 9-hydroxymethylanthracene **7** (1.5 g, 7.2 mmol) in toluene (40 ml) at 0°C via syringe. The mixture was stirred at 0°C for 1 h and then warmed to rt, during which the reaction became homogeneous. Saturated Na_2CO_3 solution (15 mL) was added slowly and the reaction mixture was stirred until it cooled to rt. The phases were separated, and the organic phase was washed with H_2O (10 mL), brine (10 mL), and dried over MgSO_4 . The yellow filtrate was concentrated to minimum volume, and then stored at 0°C for crystallization. The yellow needle-like solid was collected and dried in vacuum (1.24 g). The mother liquid was concentrated and purified using medium pressure column chromatography (0.6 g). The two parts were combined to give the product **8** (total 1.84 g, 94%) as yellow solid. Mp $143\text{--}146^\circ\text{C}$. ^1H NMR (300 MHz, CDCl_3): δ 5.3 (s, 2H, $\text{CH}_2\text{--Br}$), 7.2–8.3 (m, 9H, Ar-H). ^{13}C NMR (75 MHz, CDCl_3): δ 32.20, 125.50, 125.70, 125.80, 127.70, 128.10, 129.40, 131.60.

4.1.2. 2-(Anthracen-9-yl)acetonitrile (**9**)

A solution of 9-bromomethylanthracene **8** (1.5 g, 5.53 mmol) in DMSO (15 mL) was added over 10 min to a stirred suspension of KCN (0.54 g, 8.29 mmol) in DMSO (30 mL) at 70°C under N_2 . The mixture was stirred for an additional 40 min, cooled to rt, and diluted with H_2O . The aqueous layer was saturated with NaCl and then extracted with ether (3×25 mL). The combined extracts were washed with H_2O , dried (MgSO_4), filtered, and concentrated to yield solid product **9** (0.96 g, 80%). Mp $154\text{--}156^\circ\text{C}$. ^1H NMR (300 MHz, CDCl_3): δ 4.6 (s, 2H, $\text{CH}_2\text{--CN}$), 7.2–8.5 (m, 9H, Ar-H). ^{13}C NMR (75 MHz, CDCl_3): δ 20.50, 125.20, 125.31, 124.60, 126.10, 128.20, 130.10, 131.60.

4.1.3. 2-(Anthracen-9-yl)acetic acid (**10**)

KOH (0.97 g, 17.48 mmol) in 10 ml of H_2O was added to a suspension of 2-(anthracen-9-yl)acetonitrile **9** (0.95 g, 4.37 mmol) in ethylene glycol (50 ml). The mixture was heated at reflux for 24 h until homogenous. The hot solution was filtered, and the filtrate was acidified with dilute HCl to obtain the precipitated product **10** (1.0 g, 100%). Mp $228\text{--}230^\circ\text{C}$. ^1H NMR (300 MHz, CDCl_3): δ 4.2 (s, 2H, CH_2), 7.5–8.5 (m, 9H, Ar-H). ^{13}C NMR (75 MHz, CDCl_3): δ 34.20, 124.60, 125.30, 125.60, 126.10, 128.20, 130.50, 131.90, 173.40.

4.1.4. 2-(9,10-Dihydroanthracen-9-yl)acetic acid (**11**)

Sodium metal (10 equiv) was added slowly to a refluxing solution of 2-(anthracen-9-yl)acetic acid **10** (0.9 g, 3.8 mmol) in 1-pentanol (20 ml). The reaction mixture was stirred for 10 min until all of the sodium dissolved, then was cooled and H_2O (10 mL) was added. The solution was made acidic with 5% HCl. The reaction

mixture was concentrated under vacuum and the oily solution obtained was triturated with chloroform, dried (MgSO_4), and concentrated to yield pure product **4** (0.76 g, 84%). ^1H NMR (300 MHz, CDCl_3): δ 2.8 (d, $J = 7.8$ Hz, 2H, CH_2), 4.4 (t, $J = 7.8$ Hz, 1H, CH), 3.9 (d, $J = 18.1$ Hz, 2H, Ar- CH_2 -Ar), 4.1 (d, $J = 18.1$ Hz, 2H, Ar- CH_2 -Ar), 7.1–7.3 (m, 8H, Ar-H). ^{13}C NMR (75 MHz, CDCl_3): δ 40.1, 41.2, 45.1, 126.8, 128.7, 138.4, 139.6, 177.4.

4.1.5. 2-(9,10-Dihydroanthracen-9-yl)-N-methylacetamide (**12**)

Thionyl chloride (1.73 g, 14.6 mmol) was added under N_2 to a stirred solution of compound **11** (0.7 g, 2.9 mmol) in anhydrous benzene (5 mL). The solution was heated at reflux (2 h), allowed to cool and the excess benzene and thionyl chloride were removed under reduced pressure to provide an oil. The oil obtained was dissolved in anhydrous THF (10 mL) and cooled in an ice bath (0°C). A methylamine/THF (2 M, 5.8 mmol) solution was added dropwise into the stirred solution, and the mixture was stirred at rt (2 h). The solvent was removed under reduced pressure to give a white solid. Water (20 mL) was added, and the suspension was extracted with EtOAc (3×25 mL). The combined extracts were washed with water, brine, and dried (MgSO_4). Removal of solvent under reduced pressure gave the crude amide as a viscous oil. The resulting amide was purified using medium pressure chromatography (CH_2Cl_2 /acetone, 9:1), yield (75–80%). ^1H NMR (300 MHz, CDCl_3): δ 2.45 (s, 3H, CH_3), δ 2.67 (d, $J = 7.8$ Hz, 2H, CH_2), 3.93 (d, $J = 18.3$ Hz, 2H, Ar- CH_2 -Ar), 4.05 (d, $J = 18.3$ Hz, 2H, Ar- CH_2 -Ar), 4.56 (t, $J = 7.5$ Hz, 1H, CH), 7.16–7.37 (m, 8H, Ar-H). ^{13}C NMR (75 MHz, CDCl_3): δ 34.79, 41.0, 41.6, 42.60, 126.11, 127.47, 135.91, 138.24, 176.58.

4.1.6. 2-(9,10-Dihydroanthracen-9-yl)-N-methylethanamine (**2b**)

A borane-THF complex (1.0 M in THF, 0.537 g, 6.25 mmol) was added in a dropwise manner to a stirred solution of **12** (0.35 g, 1.25 mmol) in anhydrous THF (5 mL) under N_2 at 0°C . The mixture was slowly warmed to rt and heated at reflux (6 h). The reaction mixture was allowed to cool to rt, and HCl (6.0 M, 3 mL) was added with caution. The mixture was heated at reflux (1 h) and allowed to cool to rt, and the solvent was removed under reduced pressure. Water was added and the residue was extracted with ether (25 mL). The aqueous portion was made basic with 10% NaOH and extracted with CH_2Cl_2 (3×25 mL). The organic layer was washed with water and brine, dried (MgSO_4), and concentrated under reduced pressure to give **2b**, which was then purified by medium pressure column chromatography. CH_2Cl_2 /MeOH (9:1) yield (80–85%). Mp $202\text{--}205^\circ\text{C}$ (oxalate). ^1H NMR (300 MHz, CDCl_3): δ 1.81 (q, $J = 7.5$ Hz, 2H, CH_2), 2.38 (s, 3H, CH_3), 2.56 (t, $J = 7.5$ Hz, 2H, $\text{CH}_2\text{--NH}$), 3.89 (d, $J = 18.3$ Hz, 1H, Ar- CH_2 -Ar), 4.09 (d, $J = 18.3$ Hz, 1H, Ar- CH_2 -Ar), 4.03 (t, $J = 7.2$ Hz, 1H, Ar-CH-Ar), 7.1–7.3 (m, 8H, Ar-H). ^{13}C NMR (75 MHz, CDCl_3): δ 35.30, 36.72, 37.40, 45.41, 50.20, 126.41, 128.10, 136.40, 140.61. Anal. ($\text{C}_{17}\text{H}_{19}\text{N}\cdot\text{C}_2\text{H}_2\text{O}_4\cdot 0.25\text{H}_2\text{O}$): C, H, N.

4.1.7. 2-(9,10-Dihydroanthracen-9-yl)ethanol (**14**)

Na_2K silica gel (2 g) was added to a well-stirred solution of 2-(anthracen-9-yl)ethanol **13** (0.75 g, 4.4 mmol) in anhydrous THF and stirred continuously under nitrogen. The reaction mixture was refluxed for 15 min then allowed to cool to rt and quenched with H_2O (50 mL). The solid precipitate was filtered and washed with EtOAc (5×25 mL). The filtrate was then collected followed by extraction. The EtOAc portion was dried (MgSO_4) and concentrated under reduced pressure to provide viscous yellow oil. The resulting yellow oil was purified using medium pressure column chromatography (CH_2Cl_2 /MeOH, 9:1) to provide **14** (0.65 g, 86%) as a yellow oil. ^1H NMR (300 MHz, CDCl_3): δ 1.86 (m, 2H, CH_2), δ 3.61 (t, 2H, $\text{CH}_2\text{--OH}$), 3.90 (d, $J = 18.3$ Hz, 2H, Ar- CH_2 -Ar), 3.95 (d, $J = 18.3$ Hz, 1H, Ar- CH_2 -Ar), 4.0 (d, $J = 18.3$ Hz, 1H, Ar- CH_2 -Ar),

4.02 (t, $J = 7.5$ Hz, 1H, CH), 7.19–7.47 (m, 8H, Ar-H). ^{13}C NMR (75 MHz, CDCl_3): δ 31.29, 31.39, 52.1, 62.30, 126.11, 128.47, 137.91, 138.24.

4.1.8. 2-(9,10-Dihydroanthracen-9-yl)acetaldehyde (15)

A solution of (9,10-dihydroanthracene-9-yl)ethanol **14** (0.60 g, 1.5 mmol) dissolved in anhydrous CH_2Cl_2 (20 mL) was added to a stirred mixture of Dess–Martin oxidant (0.954 g, 2.25 mmol) in CH_2Cl_2 . The reaction mixture was stirred for 1 h, then diluted with ether (75 mL) and poured into 1.3 M NaOH (75 mL). The ether layer was separated and extracted with 1.3 M NaOH (3×15 mL) and was washed with H_2O (2×20 mL), brine (20 mL), dried (MgSO_4) and concentrated under reduced pressure to yield an oil. The resulting oil was purified using medium pressure column chromatography (petroleum ether/EtOAc 9:1) to provide **15** (0.445 g, 75%) as an oil. ^1H NMR (300 MHz, CDCl_3): δ 2.69 (d, 2H, $\text{CH}_2\text{-CHO}$), 3.87 (d, $J = 18.3$ Hz, 1H, Ar- $\text{CH}_2\text{-Ar}$), 4.06 (d, $J = 18.3$ Hz, 1H, Ar- $\text{CH}_2\text{-Ar}$), 4.55 (t, $J = 6.9$ Hz, 1H, CH), 7.19–7.34 (m, 8H, Ar-H) 9.71 (s, 1H, CHO). ^{13}C NMR (75 MHz, CDCl_3): δ 34.85, 40.77, 50.0, 126.19, 127.54, 137.21, 138.50.

4.1.9. 2-(9,10-Dihydroanthracen-9-yl)-*N,N*-dimethylethanamine oxalate (2c)

2-(9,10-Dihydroanthracen-9-yl)acetaldehyde **15** (0.4 g, 1.79 mmol), dimethylamine hydrochloride (0.292 g, 3.59 mmol) and titanium isopropoxide (1.02 g, 3.59 mmol) were added to a solution of triethylamine (0.363 g, 3.59 mmol) in absolute ethanol. The reaction mixture was stirred at rt for 12 h. NaBH_3 (0.1 g, 2.68 mmol) was then added and the mixture was stirred for 12 h. The reaction was quenched by pouring the mixture into aqueous ammonia (30 mL, 2 N). The resulting precipitate was filtered and washed with CH_2Cl_2 (5×20 mL). The filtrate was collected and extracted with CH_2Cl_2 . The CH_2Cl_2 portion was dried (MgSO_4) and concentrated under reduced pressure to provide viscous yellow oil. The resulting yellow oil was purified using medium pressure column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 9:1) to provide **2c** (0.248 g, 55%). The yellow oil was dissolved in anhydrous acetone (10 mL) and oxalic acid (0.043 g, 0.47 mmol) was added until no further precipitate formed. The oxalate salt was recrystallized from methanol/ether to provide **2c** oxalate as pale yellow crystals. Yield (55%). Mp 183–185 °C (oxalate). ^1H NMR (300 MHz, CDCl_3): δ 1.83 (q, $J = 7.5$ Hz, 2H, CH_2), 2.26 (s, 6H, $(\text{CH}_3)_2$), 2.28–2.30 (m, 2H, $\text{CH}_2\text{-N}$), 3.94 (d, $J = 18.6$ Hz, 1H, Ar- $\text{CH}_2\text{-Ar}$), 4.14 (d, $J = 18.6$ Hz, 1H, Ar- $\text{CH}_2\text{-Ar}$), 4.1 (t, $J = 7.2$ Hz, 1H, CH), 7.1–7.3 (m, 8H, Ar-H). ^{13}C NMR (75 MHz, CDCl_3): δ 32.30, 40.41, 45.32, 52.20, 56.21, 126.80, 128.71, 138.42, 140.10. Anal. ($\text{C}_{18}\text{H}_{21}\text{N}\cdot\text{C}_2\text{H}_2\text{O}_4\cdot 0.5\text{H}_2\text{O}$): C, H, N.

4.1.10. *N*-Methyl-2,2-diphenylethanamine (4b)

Yield (50%). Mp 149–151 °C (oxalate) ^1H NMR (300 MHz, CDCl_3): δ 2.47 (s, 3H, CH_3), 3.02 (d, $J = 8.1$ Hz, 2H, CH_2), 4.3 (t, $J = 6$ Hz, 1H, Ar-CH-Ar), 7.12–7.26 (m, 10H, Ar-H). ^{13}C NMR (75 MHz, CDCl_3): δ 37.20, 44.80, 60.20, 126.60, 128.50, 129.60, 143.0. Anal. ($\text{C}_{15}\text{H}_{17}\text{N}\cdot\text{C}_2\text{H}_2\text{O}_4$): C, H, N.

4.1.11. *N,N*-Dimethyl-3,3-diphenylpropan-1-amine (5c)

Yield (20%). Mp 150–153 °C (oxalate) ^1H NMR (300 MHz, CDCl_3): δ 2.52–2.56 (m, 2H, CH_2), 2.85 (s, 6H, $(\text{CH}_3)_2$), 3.0–3.02 (m, 2H, $\text{CH}_2\text{-N}$), 4.2 (t, $J = 7.4$ Hz, 1H, Ar-CH-Ar), 7.2–7.4 (m, 10H, Ar-H). ^{13}C NMR (75 MHz, CDCl_3): δ 32.20, 45.80, 50.60, 58.33, 126.30, 128.20, 129.10, 143.15. Anal. ($\text{C}_{17}\text{H}_{21}\text{N}\cdot\text{C}_2\text{H}_2\text{O}_4\cdot 0.5\text{H}_2\text{O}$): C, H, N.

4.1.12. 4,4-Diphenylbutan-1-amine (6a)

Yield (60%). Mp 198–200 °C (HCl) ^1H NMR (300 MHz, CDCl_3): δ 1.42–1.48 (m, 2H, CH_2), 2.03–2.1 (m, 2H, CH_2), 2.73 (t, $J = 6.2$ Hz, 2H, $\text{CH}_2\text{-NH}_2$), 4.1 (t, $J = 7.2$ Hz, 1H, Ar-CH-Ar), 7.1–7.3 (m, 10H,

Ar-H). ^{13}C NMR (75 MHz, CDCl_3): δ 32.30, 38.40, 42.30, 52.20, 126.80, 128.70, 129.40, 143.10. Anal. ($\text{C}_{16}\text{H}_{19}\text{N}\cdot 0.5\text{HCl}$): C, H, N.

4.2. Molecular modeling

Molecular modeling investigations were conducted using the SYBYL 7.1 molecular modeling package (Tripos LP, St. Louis, MO) on MIPS R14K- and R16K-based IRIX 6.5 Silicon Graphics Fuel and Tezro workstations. Molecular mechanics-based energy minimizations were performed using the Tripos Force Field with Gasteiger–Hückel charges, a distance-dependent dielectric constant ($\epsilon = 4$) and a non-bonded interaction cutoff of 8 Å and were terminated at an energy gradient of 0.05 kcal/(mol Å). The hH₁ (P35367) and h5-HT_{2A} (P28233) receptor sequences were retrieved from the Expasy Proteomics Server (<http://www.expasy.org/>) and aligned with a profile of several related class A GPCRs (human, dopamine D₃ (P35462), muscarinic cholinergic M₁ (P11229), vasopressin V_{1a} (P37288), adrenergic β_2 (P07550), δ -opioid (P41143), 5-HT_{2A} (P28223), dopamine D₂ (P14416), bovine rhodopsin (P02699) using the CLUSTALX program.⁴⁹ Within CLUSTALX, the slow-accurate alignment algorithm was used, the BLOSUM matrix series was employed and the gap opening penalty was increased from 10.0 to 15.0 to help maintain the continuity of the transmembrane helical segments. The alignment was carried out in two separate steps as reported by Bissantz, et al.⁵⁰ Manual adjustment of the CLUSTALX alignment was required to properly align the disulfide-forming cysteine residues in the EL2 loop. The result was an unambiguous alignment in the transmembrane (TM) helical regions of both the hH₁ and h5-HT_{2A} sequences with that of the β_2 -adrenoceptor. This alignment, along with a file containing the atomic coordinates of the adrenergic β_2 receptor (PDB ID = 2RH1), was used as input to the MODELLER⁴⁰ software package to generate a population of 100 different hH₁ or h5-HT_{2A} homology models. Each of these receptors was subsequently energy-minimized.

The automated docking program GOLD⁴³ version 3.01 (Cambridge Crystallographic Data Centre, Cambridge, UK) was then used to dock the classical antihistaminic diphenhydramine and the high-affinity ligands **3c** and AMDA (**1a**) from the synthesized dihydroanthracene matrix into each of the 100 receptor models using the ChemScore fitness function. 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 ligand binding site of the hH₁ and h5-HT_{2A} receptors. These models were subsequently analyzed using PROCHECK⁵¹ and the ProTable facility within SYBYL to assess the geometric integrity of various structural elements (bond lengths, torsion angles, etc.) of each receptor model. After checks for stereochemical integrity, the receptor models were used for the docking of all the target compounds. Ligand molecules were created within SYBYL and energy-minimized using the same parameters as were used for the receptor models. Basic amines were protonated to form ammonium ions. GOLD was used to dock each resulting ligand structure (using the parameter set defined by the 'standard default settings' option) into the final receptor model. Each receptor–ligand complex was then energy-minimized with its best-ranked docking pose.

The HINT scoring function was utilized (version 3.12) to explore and visualize hydrophobic interactions by analyzing the ligand–receptor complexes generated by the automated docking program GOLD. The interaction scores were calculated for the highest-ranked ligand conformation. The receptors (5-HT_{2A} and H₁) and ligands were partitioned as distinct molecules. The 'all hydrogen atoms' option was employed in the H-bonding model, and hydrogen atoms at unsaturated positions and alpha to heteroatoms were considered potential H-bond donors. The inferred solvent model, which considers the partition of each residue based on its hydro-

gen count, was selected. The 'Chain HBond Correction' option was set to '+20 -NH- SASA'. Finally, hydrophobic and polar interaction HINT maps were generated separately at +20% of the maximum HINT value.

4.3. Affinity determinations

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 [³H]ketanserin (a 5-HT_{2A} antagonist) as the radioligand, and the H₁ competitive binding assay employs [³H]chlorpheniramine (an H₁ 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>.

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