Application of Ligand SAR, Receptor Modeling and Receptor Mutagenesis to the Discovery and Development of a New class of 5-HT_{2A} Ligands

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Abstract: The present review describes our approach to the development of a structurally unique class of 5-HT_{2A} ligands. On the basis of an abbreviated graphics model of a 5-HT_{2A} serotonin receptor, it was hypothesized that introduction of an additional aromatic ring might enhance the affinity of phenylethylamine (an agent that lacks significant affinity for the 5-HT_{2A} receptors). Continued work with such structures, and the continual refinement of graphics receptor models, ultimately led to the identification of AMDA (**27**, 5-HT_{2A} K_i = 20 nM). AMDA is a 5-HT_{2A} antagonist that, unlike certain other tricyclic 5-HT_{2A} antagonists, binds with very low affinity at dopamine D_2 receptors, the serotonin transporter, and the norepinephrine transporter. Comparative structure-affinity studies indicate that AMDA binds in a manner distinct from the tricyclic antagonists Graphics models were employed to identify possible modes of binding. This investigation illustrates the impact of a combination of classical medicinal chemistry, receptor modeling, and molecular biology on novel drug design.

INTRODUCTION

Early Antagonists

5-HT_2 serotonin receptors were first identified in 1979. This classification was based, at least in part, on the finding that [^{3}H]spiperone labels a population of nondopaminergic brain receptors different than that labeled by [^{3}H]5-HT. Early thinking was that 5-HT_2 receptors might represent 5-HT antagonist binding sites whereas 5-HT_1 receptors represent agonist binding sites. Various 5-HT antagonists were examined at both populations of sites and were generally shown to display higher affinity for 5-HT_2 sites than 5-HT_1 sites. It is now recognized that multiple populations of 5-HT_1 receptors exist (i.e., 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}) [1], and that both agonists and antagonists bind at 5-HT_2 receptors. In any event, many of the 5-HT antagonists developed prior to 1980 did bind with high (low nanomolar) affinity at 5-HT_2 receptors; these included spiperone (**1**), pizotifen (**2**), cyproheptadine (**3**), mianserin (**4**), and cinanserin (**5**). Certain antipsychotics (e.g. chlorpromazine, **6**), tricyclic andtidepressants (e.g. amitriptyline, **7**), and lysergic acid derivatives also displayed high affinity for 5-HT_2 receptors (see reference 2 for a review of these early studies) (Scheme **1**). The high affinity of psychotherapeutic agents spurred interest in the further exploration of 5-HT_2 receptors.

Shortly after the discovery of 5-HT_2 receptors, ketanserin (**8**) and pirenperone (**9**) were introduced as the first 5-HT_2-selective antagonists (Scheme **2**). That is, these agents displayed selectivity for 5-HT_2 *versus* 5-HT_1 receptors. Ketanserin (**8**), for example, displayed much lower affinity for dopaminergic receptors than spiperone (**1**), and unlike spiperone, ketanserin was subsequently found to lack affinity for 5-HT_{1A} receptors. Although there is a general failure to recognize that ketanserin, pirenperone (and various other agents such as **1**-**7**) can bind with high affinity at histaminergic, adrenergic, dopaminergic and/or cholinergic receptors [3, 4], ketanserin was a major advance in 5-HT_2 research and is still considered a prototypical 5-HT_2 antagonist.

Since the early 1980s, numerous 5-HT_2 antagonists have been prepared. Several reviews have appeared on 5-HT_2 antagonists [5-7] and attempts have been made to classify these agents and to formulate 5-HT_2 pharmacophore models. Much of the early work with 5-HT_2 receptors must be cautiously evaluated because 5-HT_2 receptors are now realized to represent a family of receptors with 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} subpopulations [1]. Depending on the pharmacological assay, binding assay, assay conditions, or radioligands that were used, it is fairly apparent that many of the initial investigations were targeting 5-HT_{2A} receptors. Nevertheless, nearly all leads for drug development in this area came from these early studies. Today, there is evidence that 5-HT_2 receptors might be involved in schizophrenia, depression, anxiety, appetite control and cardiovascular function [8]. Roles of the specific 5-HT_2 receptor subpopulations are still not certain.

Classification

Classification of 5-HT_2 ligands has been difficult because they appear to belong to so many different chemical classes. The first attempts to classify these agents were based on

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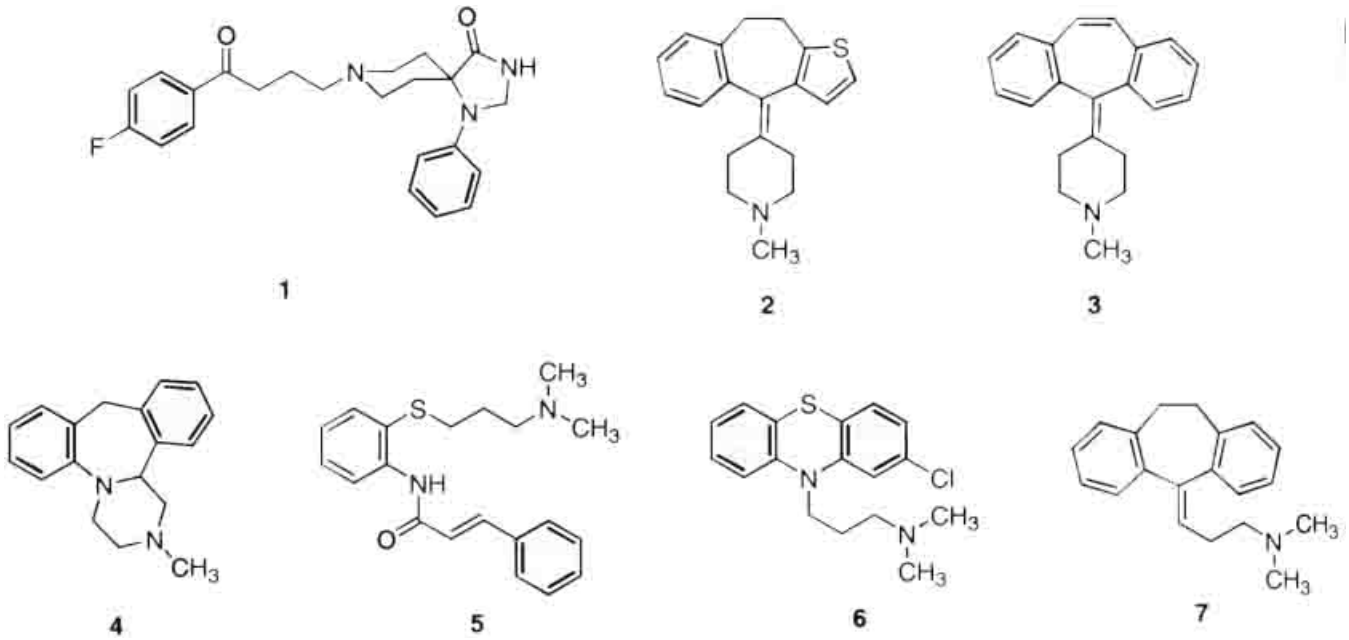


Scheme 1.

chemical structure [5, 9-11]. 5-HT_2 ligands were divided into the *indolealkylamines*, *phenylalkylamines*, *arylpiperazines*, *alkylpiperidines/alkylpiperazines*, *polycyclic/tricyclic* agents, and *other* agents. The *indolealkylamines* include serotonin and various other tryptamines and ergolines, whereas the *phenylalkylamines* include agents such as the 5-HT_2 agonist DOB (**10**) and the antagonist DOPP (**11**) (Scheme **2**). Included in the *arylpiperazine* class are agents such as quipazine (**12**) and tiospirone (**13**). Arylpiperazines (long-chain arylpiperazines or LCAPs) are usually thought of as 5-HT_{1A} ligands; however, many of these agents bind at 5-HT_2, dopamine and/or adrenergic receptors as well as at 5-HT_{1A} receptors.

The *alkylpiperidines/alkylpiperazines* are probably the largest category of 5-HT_2 ligands. They include spiperone (**1**), ketanserin (**8**) and ketanserin-related compounds, and piperazine derivatives such as irindalone (**14**) (Scheme **2**). The *polycyclic* or *tricyclic* category contains compounds such as pizotifen (**2**), cyproheptadine (**3**), mianserin (**4**), chlorpromazine (**6**), and amitriptyline (**7**). The *other* category is a catch-all group that contains compounds that could not be classified in one of the other categories; included are cinanserin (**5**) and ICI 169,369 (**15**).

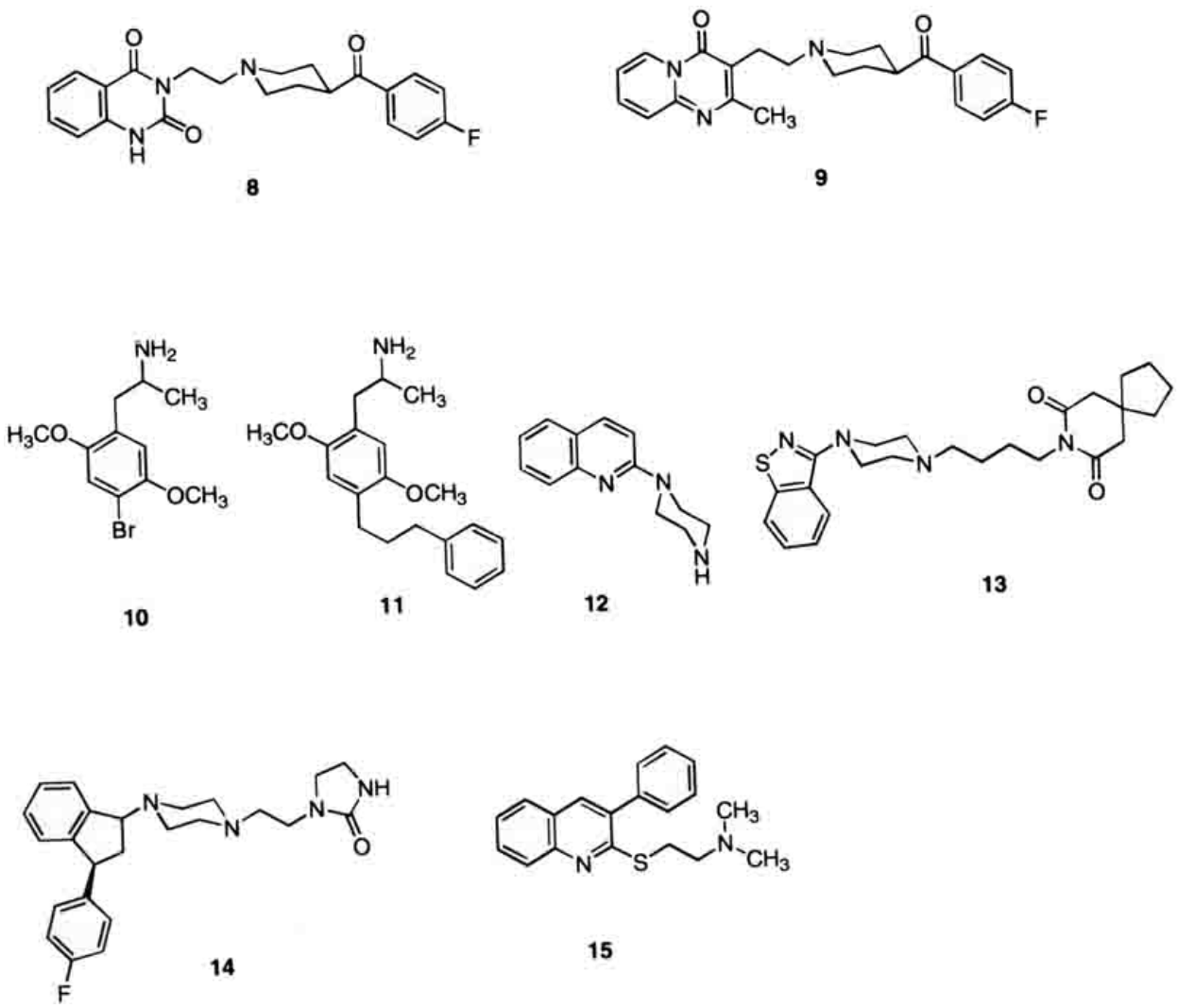
Most newer agents generally fall into one of the above classes and represent variations on a theme. The 2-aryltryptamine **16**, for example, is a new antagonist member of the *indolealkylamine* class, **17** is a novel *phenylalkylamine* antagonist, and fananserin (**18**) is a newer *arylpiperazine* antagonist (Scheme **3**). The atypical antipsychotic agents clozapine (**19**) and olanzepine (**20**) can be considered as members of the *polycyclic/tricyclic* category. It is the *other* category, however, that seems to be growing at the fastest pace.

Newer agents in this category include sarpogrelate analogs and metabolites, such as sarpogrelate metabolite **21**, and SR 46349B (**22**). For additional examples, the reader is referred to references 5, 7, and 9. The above classification scheme is far from ideal and it is apparent that certain compounds can fall into more than one category. But, the scheme does assist in discussions of the various structures that bind at 5-HT_2 receptors.

Pharmacophore Models

As of now, no comprehensive pharmacophore model has been published that accounts for the binding of all the above structure types. This is probably not unexpected given the number of structure types involved. Andersen *et al.* [12] and Mokrosz *et al.* [13] independently published pharmacophore models to describe the binding of various arylpiperazines, tricyclic, and related agents at 5-HT_2 receptors (Figure **1**). Both models require two aryl substituents, separated by distance *a*, located distance *b* and *c* from an amine moiety. A distance range was provided in the latter study and specific distances in the former; the specific distances fell within the ranges of the latter study. Distances suggested by Anderson *et al.* [12] for *a*, *b*, and *c* are 5.1, 7.5 and 8.1 Å, respectively.

Holtje and Jendretzki [14] described a model that accommodates both agonists and antagonists, and several investigators have described possible modes of receptor binding for small groups of selected agents (e.g. [10]). But, it has been argued that no single model describes the binding of all classes of compounds and that even closely related agents can bind differently [12, 15, 16]. Roth *et al.* [17], by examining multiple 5-HT_{2A} receptor mutants, have shown that the binding of spiperone and ketanserin can be differently influenced by different mutations. It is likely that multiple modes of binding are possible. Rowley *et al.* [18], for example, has attempted to divide 5-HT_2 antagonists into those with a triangular arrangement and those with a more



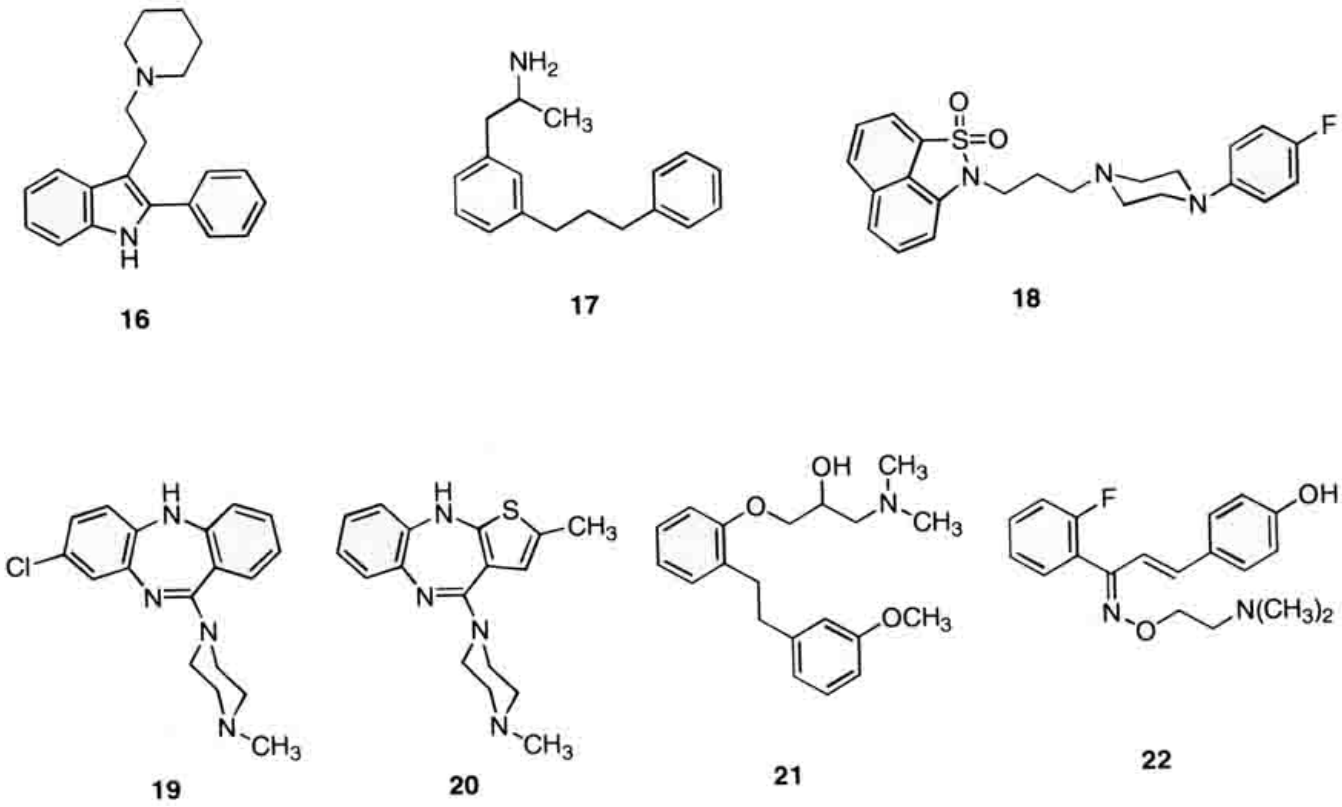
Scheme 2.

linear arrangement. There is general agreement that there are at least two different modes of antagonist binding at 5-HT_{2A} receptors.

SEROTONIN RECEPTORS AND RECEPTOR MODELS

5-HT_{2A} Receptor Structure and Ligand Design

The design of serotonergic agents with improved properties (greater selectivity, agonist vs antagonist properties) is typically accomplished by structural modification of existing lead compounds. The obvious limitation of this approach is the high probability of producing agents that may retain the disadvantageous properties of the lead compound by virtue of the inevitable structural similarities between the new agent and the parent. A de novo approach in which the structure of the receptor, not of a ligand, provides the design inspiration is possible when receptor structures are known. Receptor-structure based ligand design can then proceed with a knowledge of the nature of the ligand binding site using chemical intuition assisted by molecular graphics visualization. Proposed ligands can be evaluated computationally using simple molecular mechanics or dynamics based calculations of binding energy, the use of one of numerous empirical scoring methods with ligand-receptor complexes generated interactively or with automated docking algorithms. Ligand design carried out in this way (based on receptor structure rather than the structure of known ligands) has a high probability of leading to novel structural types, perhaps with unique and desirable characteristics. Unfortunately, experimental structures of the membrane bound neurotransmitter GPCRs have not been determined. In fact, very few structures of integral membrane bound proteins have been determined due to the difficulty in obtaining crystalline or other ordered array material necessary for diffraction methods. However, the known amino acid sequences for most neurotransmitter receptors provide a wealth of indirect structural data. Biochemical methods including site-directed mutagenesis [17, 19], substituted cysteine accessibility methods [20] and a number of spectrocopic experiments (fluorescent and spin labeling) [21, 22] provide hints as to the location of the ligand binding sites and overall architecture of the seven transmembrane (7TM) α-helical aggregate (linked by extracellular and intracellular loops of unknown secondary structure) and conformational changes that may take place on receptor activation.



Scheme 3.

Receptor Models

Graphics models constructed from a known 5-HT receptor sequence were described as early as 1991 [23, 24]. Later models relied on the experimental structure [25] of bacteriorhodopsin. Bacteriorhodopsin, also is a membrane bound receptor with seven transmembrane helices, was the only experimental structure of a protein configured in this way in existence at the time which could be used as a template to construct various GPCR models [26-29]. The difficulties in this approach are numerous. First, bacteriorhodopsin is not G-protein coupled but is a retinal containing, light-dependent proton pump. In light of these biochemical differences, the structural similarity between bacteriorhodopsin and the GPCRs has been questioned. Second, there is no usable sequence homology between bacteriorhodopsin and the neurotransmitter GPCRs. Thus, establishing a potential alignment is problematic as is establishing probable helix termini [27]. Obviously, different alignments can produce significantly different receptor models. For example, the most certain receptor feature is that D155, the ammonium ion binding residue [30, 31] must be accessible to the central aqueous pore. However, even with this constraint, D155 could be near the top, center or bottom of the aggregate depending on which alignment is chosen. Establishing the structure of the extra-

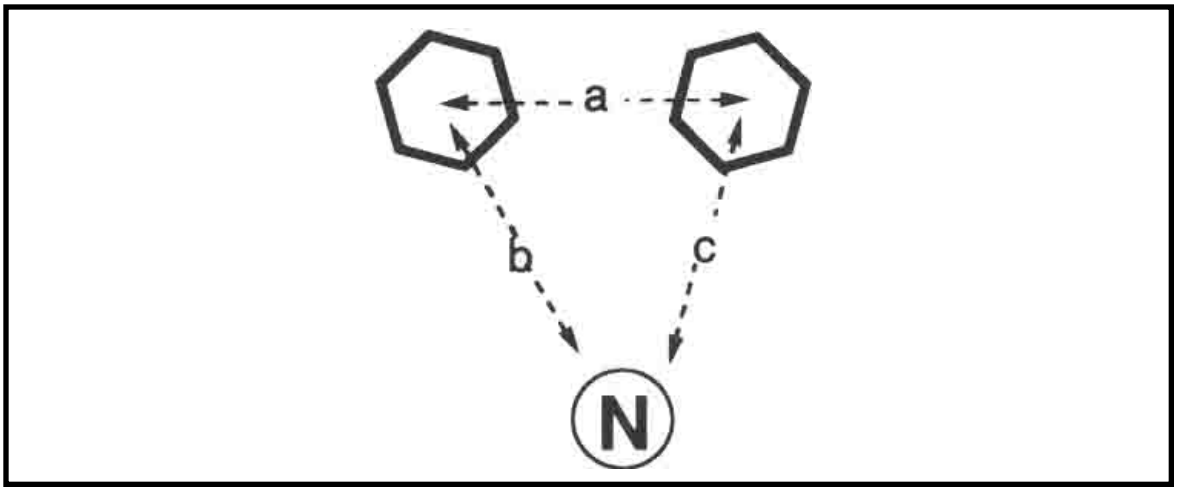


Fig. (1). A general pharmacophore model proposed by Andersen *et al* [12] and Mokrosz *et al* [13]. to account for the binding of 5-HT$_2$ antagonists.

and intracellular loops by traditional homology modeling methods is of limited utility due to the lack of sequence homologs amongst proteins of known structure. Several models have included loops of undefined secondary structure. The initial helix spanning loop structures that are constructed are typically relaxed using molecular dynamics within the distance constraints of the helix termini [32-34]. Models generated in this way almost certainly do not reflect the actual loop structure. In spite of these limitations, many investigations have reported the generation and use of bacteriorhodopsin models, and useful insights have been gained [26, 29].

Eventually several low resolution structures of rhodopsin, which is retinal-dependant visual pigment that is a GPCR, were reported [35-37]. Unfortunately, rhodopsin has very little sequence homology with the neurotransmitter GPCRs so an alignment cannot be established using typical methods. However, careful analysis of all known GPCR sequences (and all biochemical information) revealed that in each putative helical segment there is at least one uniformly or highly conserved residue throughout the hundreds sequences examined [38, 39]. These observations establish highly probable alignments between the rhodopsins and the other GPCRs. Examination of mutagenesis, conservation, and biochemical data for the rhodopsins with the low resolution projection structure of rhodopsin allowed a tentative assignment of α-carbon atoms within the experimental electron density map [40]. This model, as well as the low resolution density maps, have been widely used to generate models of neurotransmitter receptors usually devoid of extracellular and intracellular loops [16, 31, 41-43].

There have been several reports of serotonin receptor models derived in a de novo fashion without explicit consideration of the experimental structures of either bacteriorhodopsin or rhodopsin [34, 44]. In one, a particularly notable approach, helix positions and orientations were fitted using distance constraints derived from potential hydrogen bonded residue pairs unique to each specific receptor sequence [45, 46]. Models of representative sub-types of most GPCRs were constructed. The interpretive value of this approach is impressive; the models are consistent with the largest body of experimental structural data [46]. Remarkably, the 5-HT_{1A} receptor model and one derived from the α-coordinates based on a low resolution structure of rhodopsin are qualitatively very similar (backbone atom rms deviation = 3.6 Å) despite the very different types of information used to generate them.[a] Comparative evaluations of many of the serotonin receptor models up to about 1998 have been reported [19, 47]. Not unexpectedly, no single model accurately predicted all of the experimental mutagenesis data.

Recently, an x-ray crystal structure of nearly the entire molecule (including most of the intra- and extracellular loops) of the G-protein coupled visual pigment bovine rhodopsin was reported [48]. Models of the neurotransmitter GPCRs can now be based on an even better, though not perfect, experimental template than was previously available. It is likely that most new modeling studies will be conducted using serotonin models based, in some way, on the rhodopsin crystal structure.

Rhodopsin is the best structurally and biochemically characterized GPCR amongst the many known. This has been possible because pure rhodopsin can be obtained from readily available natural sources (bovine retina) in milligram quantities by selective solubilization of rod outer segment disk membranes obtained by sucrose gradient centrifugation [49]. The ease with which large amounts of pure protein can be obtained is unique to the anatomical location of the light sensitive protein on retinal rods. Of course, crystallization of integral membrane proteins is notoriously difficult. All of the early low resolution structural data obtained for rhodopsin had been gathered by cryoelectron microscopy using two-dimensional preparations consisting of a membrane layer in which relatively ordered protein aggregates. The quest for both 2D or 3D crystals of rhodopsin suitable for structural studies has been a long one, spanning at least 20 years [49] of empirically exploring crystallization conditions (e.g. temperature, protein concentration, pH, type and concentration of buffers, additives, detergents).

The production of 3D crystals of rhodopsin is a monumental accomplishment and, unfortunately, may prove to be a rare achievement. In theory, much of the empirical experience gained in the crystallization of rhodopsin could be applied to the neurotransmitter GPCRs. If sufficiently pure, milligram quantities of neurotransmitter receptors could be obtained from efficient expression systems, it would be possible to explore the hundreds to thousands of conditions usually necessary (even for soluble proteins) to produce crystals. There is no guarantee that such efforts will be successful, but attempts must and will be made. Even if such efforts bear fruit in the foreseeable future with a representative of the neurotransmitter GPCRs, it would still be necessary to use comparative molecular modeling methodologies to apply the new information to the serotonin receptors that are of interest to us. If the structure of a representative of the serotonin receptor family were to be solved, molecular modeling investigations still would be necessary to explore possible modes of interaction with the spectrum of ligands of interest, many of which are new compounds that we are generating (it is very unlikely that structures of many ligand-receptor complexes will be solved in the near future).

Unfortunately, for a variety of practical reasons, there are no experimental structures for neurotransmitter GPCRs available at this time with the single exception of the metabotropic glutamate receptor ligand binding domain [50]. The mGluRs are structurally and functionally distinct from the typical neurotransmitter GPCR's in that the ligand binding site is located extracellularly as a dimeric structure that can be expressed, isolated and crystallized separately from the transmembrane helix aggregate in aqueous media. Thus, the ligand binding domain can be treated as a soluble protein and as such is much more tractable than the neurotransmitter receptors for which the ligand binding site is within the transmembrane helices themselves (i.e., rhodopsin, serotonin, adrenergic, muscarinic, purininergic, opiate, and dopaminergic receptors).

[a]Unpublished observations.

Construction of Rhodopsin Crystal Structure-Based 5-HT_{2A} Receptor Models

The following is a description of our model building based on the crystal structure of rhodopsin.[a] Indirect testing of rhodopsin-based GPCR models using ligand SAR, site-directed mutagenesis and other molecular genetics-based methods will be necessary to evaluate and verify such models. The major points necessary to understand the strengths and weaknesses of the basic assumption that rhodopsin and the 5-HT_{2A} receptor are structurally homologous, i.e., that rhodopsin is a good 3D model for neurotransmitter GPCRs, are discussed below.

Transmembrane Helices

Unambiguous alignment of the rhodopsin and 5-HT_{2A} receptor sequences is possible by matching the highly conserved residues previously identified [39, 40]. Mutation of the helical segments of the rhodopsin sequence to that of the 5-HT_{2A} receptor helices is easily accomplished computationally. Since the identities of specific residues at any point are different, there is no reason to assume amino acid side chain geometries are comparable. Initial amino acid side-chain geometries for the 5-HT_{2A} receptor model were established from backbone-dependent libraries of rotomer preference. The helix backbone geometry of rhodopsin is transferred without change in this procedure. There are several deviations in normal helix geometry in the 5-HT_{2A} receptor model generated in this way. As would be expected, the most significant deviations in normal ideal helix geometry occurred near proline- and glycine-containing segments. Significant helix bends occurred in TM2 L126/V127 (G89/G90), TM6 P338(P267), and TM7 P377(P303) in the rhodopsin structure (nomenclature: helix number, 5-HT_{2A} sequence range, rhodopsin sequence range). Since the two proline residue sites are conserved in both sequences, the irregular geometry of the rhodopsin helices probably also occurs in the 5-HT_{2A} receptor and was retained. The TM2 L126/V127 (G89/G90) sequence marks the beginning of a significant helix bend placing the C-terminal, extracellular third of TM2 inward toward TM7 and TM1. Sequence identity of the glycine-containing segment of bovine rhodopsin TM2 (GGFT) is conserved amongst the opsins but is not conserved in the amine neurotransmitter receptors including the 5-HT_{2A} receptor (LVMP). However, a proline residue is highly conserved at the same approximate position of the rhodopsin helix bend in nearly all amine GPCRs. Therefore, it is not unreasonable that the amine receptors could have a rhodopsin-like bend in TM2 as well. The other non-homologous occurrences of proline and glycine motifs either do not perturb helix geometry or are located very near the helix termini. In summary, the major geometric perturbations observed for rhodopsin are probably mirrored in the 5-HT_{2A} receptor structure; these perturbations were retained in the 5-HT_{2A} receptor model. In addition to sites with major geometric perturbations, there are two regions in the rhodopsin structure with local geometric perturbations leading to irregular helicity that do not alter the helix axis. These regions are near A242(H211) in TM5 and Y370(K296) in TM7, the site of Schiff base formation with retinal. The most extensive perturbation is in TM7. Specifically, W336-L371(P391–S398) have either absent or altered backbone hydrogen bonding patterns. Setting phi, psi, and omega angles in the serotonin receptor model to ideal helix values and fitting the backbone atoms of the native and altered TM7 resulted only in very minor changes in helix position (backbone rmsd = 1.58 Å) and no significant changes in the disposition of key residue sidechains (e.g., Y370, V366) or alteration of the shape and dimensions of the helix aggregate cavity. There is no reason to expect that the amine neurotransmitter receptors share this irregularity with rhodopsin. However, these irregularities were retained in the current 5-HT_{2A} receptor model in the interest of fidelity to the best experimental structural template currently available.

The rhodopsin structure reveals five interhelical hydrogen bonding networks [48]. Where residue identity or functional homology is retained in the 5-HT_{2A} receptor, the geometries of interhelical hydrogen bonding networks identified for rhodopsin were also present in the 5-HT_{2A} receptor model. This observation suggests that rhodopsin is a suitable template for the 5-HT_{2A} receptor and that the method used for generation of side chain conformation produces a reasonably realistic geometry. It is remarkable that the rhodopsin crystal structure-based 5-HT_{2A} model described here is qualitatively very similar to an earlier model derived from a 5-HT_{1A} model arrived at in a de novo fashion without direct reference to experimental electron densities (backbone atom rmds = 3.2 Å) [45, 46].

Extracellular Loops

One of the most striking features of the rhodopsin structure is the complexity and compactness of a helix bundle "cap" or "plug" formed from the extracellular interhelix loops and N-terminal segment [51, 52]. Together, the N-terminal sequence, and the three loops E1 (TM2 to TM3), E2 (TM4 to TM5) and E3 (TM6 to TM7) form a layered, interlocking structure consisting partly of β-sheet loops. The bottom-most of these is an E2 β-sheet which traverses the entire helix aggregate extending from its origin at TM4 and its terminus at TM5 toward TM1 and TM7. The E2 loop is tethered to TM3 via a disulfide bond between two highly conserved cysteines. The E2 loop of rhodopsin comes within van der Waals distance of the covalently bound retinal chromophore forming the bottom of the plug (Figure **2**) and comprises a significant portion of the retinal binding site. Thus, the relevant questions become, is there an analogous structure that forms part of the ligand binding site of neurotransmitter G-protein coupled receptors? Do the extracellular loops of the 5-HT_{2A} receptor also contribute to the ligand binding site? This issue is important because modeling of loop structure is difficult due to large differences between sequence length of rhodopsin and 5-HT_{2A} receptor loops. It has been suggested that such a complete enclosure of the ligand binding site is not likely for receptors that, unlike rhodopsin, must reversibly associate with ligand [51, 52]. This issue was evaluated in

[a] Unpublished obervations.

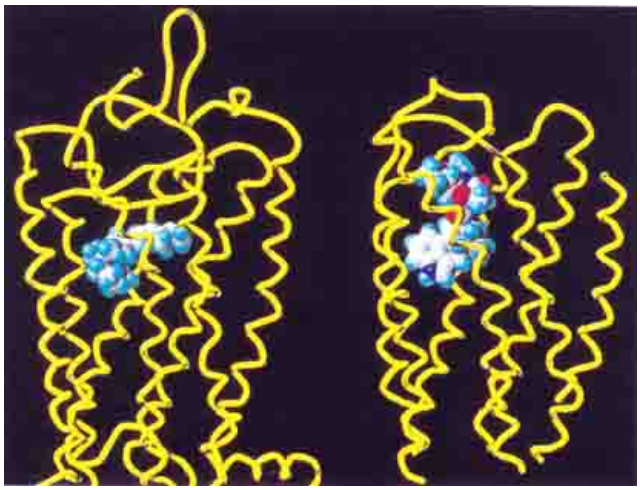


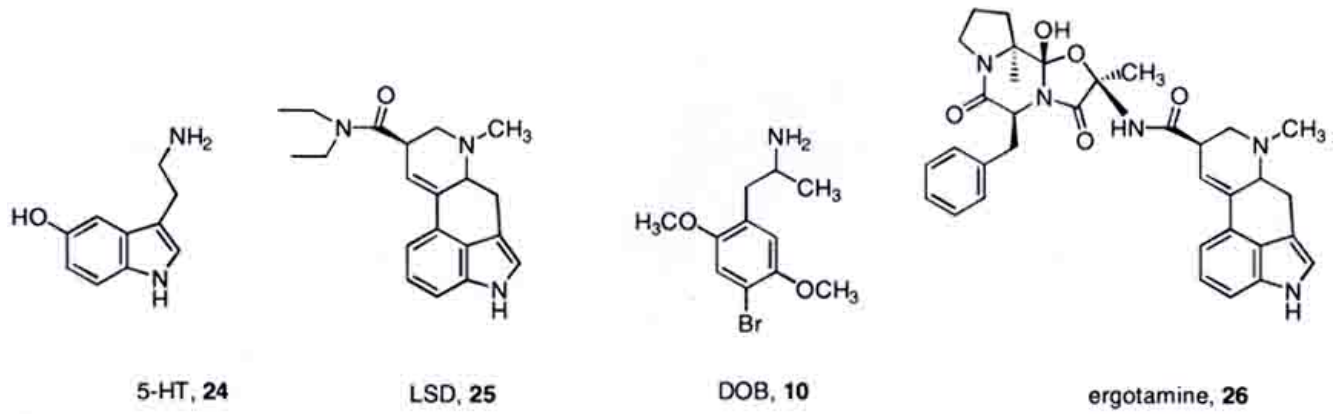
Fig. (2). Backbone atom trace of the transmembrane helices and extracellular loop domains of the experimental structure of rhodopsin with retinal bound (left) and of the modeled 5-HT_{2A} receptor with ergotamine bound (right).

the context of the 5-HT_{2A} receptor sequence. The E2 loop of the 5-HT_{2A} receptor is eight residues shorter than the corresponding E2 loop of rhodopsin. In fact, the E2 loop of the 5-HT_{2A} receptor is one of the shortest in the GPCR family [39]. This requires that the 5-HT_{2A} disulfide forming sequence CLLA span the path between the TM5 terminus (D231) and near the top of TM3 (C148), a distance (14.6 Å) which is barely achievable even with a fully extended peptide chain, a configuration that cannot form a cap-like structure. Thus, it sterically improbable for the E2 loop of the 5-HT_{2A} receptor to interact with ligand tethered to D155 of TM3. E1 and E3 are similar in length in both the 5-HT_{2A} and rhodopsin receptors; the E1 and E3 loops of both probably have similar structures with both extending beyond the helix termini. Thus the TM4-TM3-TM5 domain of E2 probably represents the nearest steric barrier (farthest down) that a ligand could encounter which is far enough from the TM3 D155 that typical ligands probably do not interact directly with it. While comparison of the lengths and sequences of the rhodopsin and 5-HT_{2A} receptors suggests that the loop domain does not extend into the ligand binding site significantly for typical ligands, the ergopeptines, bearing bulky substituents at the ergoline 8-position are probably the exception as has been previously proposed [42] (Figure **2**). The fact that ergopeptines bind at all provides evidence that the ligand binding cavity of unsubstituted ergolines, indolealkylamine, and phenylethylamines is probably not lined by the extracellular loop domain independent of the model building considerations above [42]. For these reasons, further attention was focused on a transmembrane helix - only representation of the serotonin receptor. Figure **2** shows a comparison of models of the 5-HT_{2A} and rhodopsin extracellular loops excluding the N-terminal sequence of the serotonin receptor. It should be noted that mutation of several E2 residues can affect ligand affinity for muscarinic and adrenergic receptors [53]. In addition, E2-TM5 5-$HT_{1D/1B}$ chimers show altered antagonist (ketanserin) affinity without affecting the agonist affinity [53]. On the basis of these observations, it has been suggested that E2 may, in fact, form part of the ligand binding domain even for small molecule aminergic GPCRs [53] – a conclusion contrary to our own. However, as with all mutations, these results could be due to indirect effects on the conformation or properties of a distal binding site.

Ligand-Receptor Docking

The rhodopsin side chains are tightly packed around bound retinal [48]. Mutation of the rhodopsin structure to the 5-HT_{2A} receptor structure does not radically alter the binding cavity shape or dimensions even after generating side chain geometry entirely independent of the side chain geometries present in rhodopsin. Thus, any docking method, whether interactive or automated, will probably be unduly biased by the nature of the retinal binding cavity transferred to the 5-HT_{2A} receptor model. For these reasons, potential modes of binding of 5-HT (**24**), LSD (**25**), DOB (**10**) and ergotamine (**26**) (Scheme **4**) with the model receptor were evaluated in a systematic, de novo fashion using available mutagenesis data of potential hydrogen bond forming groups.

5-HT_{2A} serine residues that have been mutated and shown to affect agonist binding are TM3 S159 [54], TM5 S239 [43, 55], and TM5 S242 (A242 in rat) [56]. These residues have been variously suggested to participate in hydrogen bond formation with the ammonium ion (S159 [54]), the 5-HT hydroxyl group (S239 [55]) and the indole NH (S239 [43]) on the basis of differential effects of residue mutation on the affinity of ligands presenting or lacking the targeted ligand functional group. Ligand-receptor complexes were constructed assuming all possible combinations of hydrogen bond participants. Each complex was subjected to molecular dynamics equilibration followed by molecular mechanics minimization. Each specific potential combination of roles was evaluated with respect to the geometry constraints each imposes along with the necessity for the ligand ammonium ion to interact with the TM3 D155 carboxylate. In this way, a minimalistic estimate of the geometric feasibility of a particular mode of binding can



Scheme 4.

be made, i.e., is a given interaction mode consistent with experimental hydrogen bond angles and distances or is it excluded by the steric constraints imposed by other receptor features?

Dynamics simulations of 5-HT constrained to interact with the appropriate receptor residues were performed to estimate the geometric probability of each of the candidate binding modes. The two most reasonable 5-HT binding modes by these criteria are those in which the 5-HT ammonium ion interacts either with the D155 carboxylate alone or with both the carboxylate and the S159 OH group with the 5-OH accepting a hydrogen bond from S239. Molecular dynamics of both produced similar trajectories with the aminoalkyl chain of 5-HT alternating between an extended and folded conformation. Averaged, minimized samples representing each bound ligand conformation were used as the starting points for docking LSD (superimposition of aromatic centroids, indole nitrogen atoms, and ammonium ions of each) which were then subjected to dynamics calculations followed by minimization of sampled average structures. The two most likely orientations of DOB in the binding site were determined by superimposition with LSD. Superimpositions of the ammoium ion nitrogens, aromatic centroids the indole nitrogen with either the 2- or 5-methoxy groups of DOB were used as starting points that were then used for dynamics simulations. Representative complexes were obtained as described for 5-HT. The dynamics simulations with the standard ligands 5-HT, LSD, and DOB followed by minimization progressively imprinted a sterically reasonable serotonin receptor ligand binding domain on the original tightly packed "retinal site" ghost. Figure **3** shows 5-HT and DOB docked into the resulting sites. Lacking key functional groups, or any unambiguous superimposition rule, potential orientations for AMDA derivatives were examined by interactive docking of AMDA within the steric confines of the binding site visualized as a Connolly channel plot.

The development of serotonin receptor models has progressed chronologically as follows: abbreviated models [23, 44], bacteriorhodopsin-based models [26-29]/de novo models [32-34], low resolution rhodopsin-based models [41-43], and finally 2.8-Å resolution rhodopsin crystal structure-based models [52, 53]. While the detailed features of each type of model are different, some general features persist. Analysis of early 5-HT_{2A} receptor models led us to consider two general areas of steric accessibility (Figure **4**): Site 1 (TM3 flanked by TM4, TM5, and TM6) and Site 2 (TM3 flanked by TM1, TM2, TM6, and TM7). Consideration of ligand SAR and receptor mutagenesis data prompted us to provisionally consider Site 1 the "agonist site" and Site 2

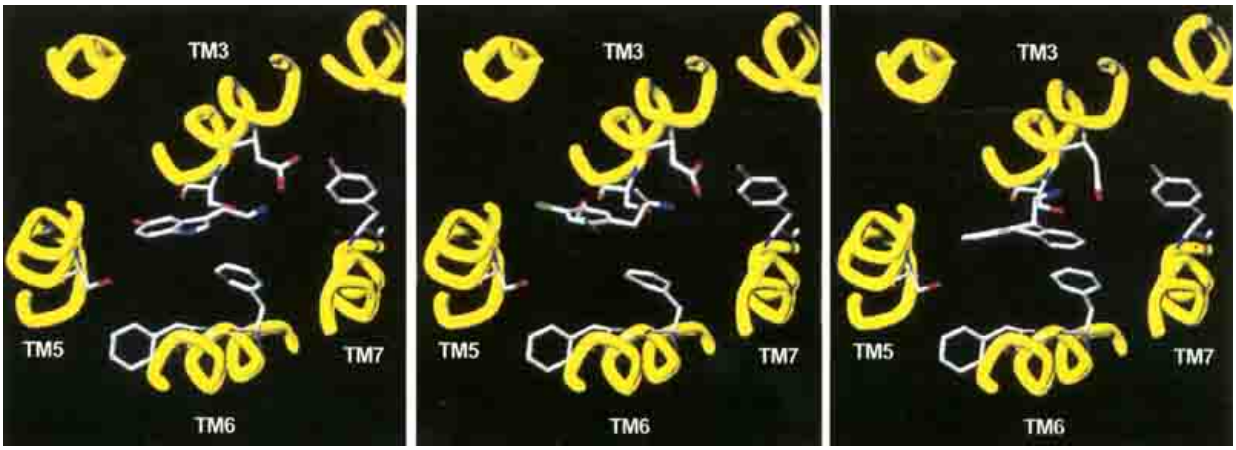


Fig. (3). 5-HT (left), DOB (center), and AMDA (right) bound to the 5-HT2A receptor model. A 14-Å slab at the position of the TM3 D155 residue is displayed. TM3 D155, S159; TM5 S239; TM6 F339, F340; and TM7 Y370 side chains only are displayed. TM1 is not shown.

the "antagonist site" [16, 27, 57]. Similar suggestions have also been made for the 5-HT_{1A} receptor [58]. The 5-HT_{2A} receptor model generated from the rhodopsin crystal structure shows two similar, overlapping areas of accessibility that are more symmetrically distributed around TM3, Site 1 defined by TM4, TM5, and TM6 with Site 2 defined by TM2, TM6, and TM7 (Figure **4**).

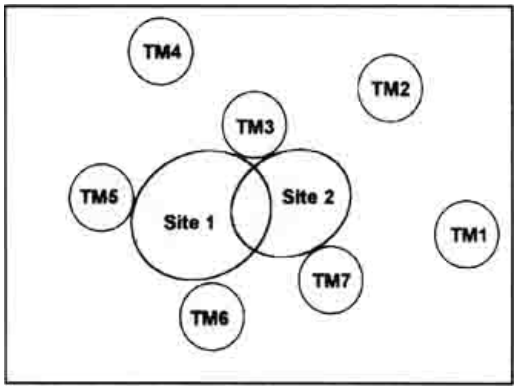


Fig. (4). Schematic representation of sterically accessible binding sites within the 5-HT_{2A} receptor provisionally considered the agonist site (Site 1) and the antagonist site (Site 2) [16, 27, 57].

DEVELOPMENT OF AMDA AND RELATED LIGANDS

Use of Receptor Models in Ligand Discovery

A number of compounds were evaluated in an iterative model-testing, model-building paradigm; some of these are shown in Scheme **5**.

Typically, simple unsubstituted phenylethylamines show very low affinity for 5-HT_2 receptors (e.g. phenylethylamine, **58**, 5-HT_{2A} K_i > 10,000 nM) [24]. Some time ago, examination of abbreviated receptor models suggested that the affinity of structures containing a phenylethylamine skeleton could be enhanced by introducing a second aromatic moiety, perhaps by participating in additional aromatic-aromatic interactions between ligand and receptor [24]. Although the original model was intentionally incomplete, calculated binding energies seemed to at least divide known test compounds into those that do not bind with measurable affinity and those that do bind. Using this model, amphetamine (i.e., α-methyl **58**), which does not bind with measurable affinity (K_i > 40,000 nM), also proved to have a poor calculated binding energy. Appropriately substituted amphetamine derivatives that have reasonably high affinities (e.g. DOB, **10**, (K_i = 24 nM) also have reasonable large calculated binding energies. Comparison of models of the receptor-amphetamine/amphetamine derivative complexes suggested that one reason for the lower affinity of the unsubstituted parent was an inability to interact favorably and simultaneously with two neighboring aromatic residues whereas the steric and electronic properties of the high affinity substituted compounds enforced a favorable bidentate interaction between the ligand and both aromatic receptor residues. We reasoned that introduction of a second aromatic ring into the phenylethylamine skeleton might increase affinity by allowing simultaneous interaction with both aromatic receptor features. Computational examination of several potential ligands led to the synthesis and evaluation of 5-aminomethyl-10,11-dihydro-5H-dibenzo[a,d]cycloheptadiene (AMDH, **31**, K_i = 112 nM) which binds with nearly 200-fold higher affinity than the parent **58** (Figure **5**) [24]. As was noted at the time, this single favorable outcome does not prove the validity of the receptor model. However it is unlikely, given the structures of the available ligands (e.g. LSD, amphetamine, DOM, DOB), that **31**, an analog of an inactive compound, would have been proposed for synthesis and evaluation in the absence of a receptor model.

Having established a reasonably high-affinity lead compound, the importance of the second aromatic ring and of the geometric relationship between the two aromatic rings was investigated [59, 60]. These explorations lead to the discovery of AMDA (**27**), a tricyclic compound with 800-fold higher affinity than the phenylethylamine skeleton, that has proven to be a 5-HT_{2A} antagonist [59].

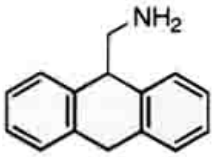
Nature of the Tricyclic Ring System

Removing one aromatic ring from AMDA (**27**; K_i = 20 nM) drastically reduces affinity as indicated by the tetrahydronaphthalene **72** (K_i > 10,000 nM). This suggests that the enhanced affinity of AMDA (**27**) over phenylethylamine (**58**; K_i = 16,800 nM) is not due solely to the presence of the central ring. The simple presence of two aromatic rings is also not sufficient for optimal affinity as demonstrated by compounds **32, 33, 73, 74**. The 2,2-diphenylethylamine **32** (K_i = 4,610 nM), while enhanced in affinity compared to phenylethylamine (**58**), has 240-fold lower affinity than AMDA (**27**). Similarly, 2-(2-benzylphenyl)ethylamine (**73**; K_i = 1,810 nM) has a 9-fold greater affinity than phenylethylamine (**58**) but, has a 90-fold lower affinity than AMDA (**27**). Thus, it appears that the high affinity of AMDA (**27**) could be attributable to its tricyclic configuration. However, incorporation of two aromatic rings fused to a central cyclopentane ring produces a compound (**33**; K_i = 4,490 nM) that has very low 5-HT_{2A} affinity. The fully aromatic derivative of AMDA, anthracene **74** (K_i = 2,300 nM), has 100-fold lower affinity than the dihydro derivative AMDA (**27**). Consideration of the binding data in Figure **5** invites the simple conclusion that compounds with a nearly coplanar (**33**, **74**) or orthogonal (**33**) orientation of the two necessary aromatic rings have low affinity while compounds with a substantial symmetrical aromatic fold can bind to the receptor with high affinity.

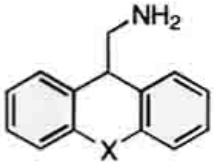
Unsubstituted dihydroanthracene adopts a symmetrical folded structure with 9- and 10-position hydrogens in either a pseudoaxial or pseudoequatorial configuration ([61] and ref therein) (Figure **6**).

The energetically preferred conformation of AMDA places the aminomethyl substituent in the axial position which is capable of adopting a *trans*, gauche (exo) or a gauche, gauche (endo) conformation of nearly equal energies (Figure **6**). While it is not known which, or if both, rotomers

AMDA and Derivatives

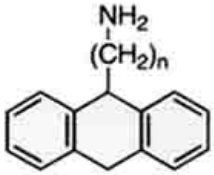


AMDA, 27



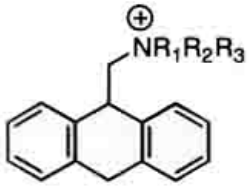
27-33

Compds	X
27	-CH_2-
28	-CH=CH-
29	-S-
30	-O-
31	-CH_2CH_2-
32	H, H
33	—



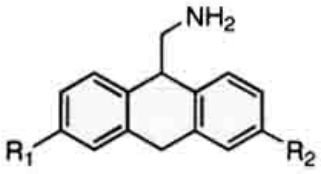
27, 34-36

Compds	n
34	0
27	1
35	2
36	3



27, 37-40

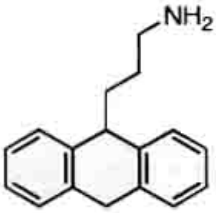
Compds	R_1	R_2	R_3
27	H	H	H
37	CH_3	H	H
38	CH_3	CH_3	H
39	CH_3	CH_3	CH_3
40	CH_2Ph	H	H



27, 41-47

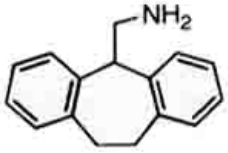
Compd	R_1	R_2
27	-H	-H
41	-Br	-H
42	-$(CH_2)_3Ph$	-H
43	-C_6H_{13}	-H
44	-OCH_3	-H
45	-$O(CH_2)_4CH_3$	-H
46	-OH	-H
47	-C_6H_{13}	-OCH_3

APDA and Derivatives

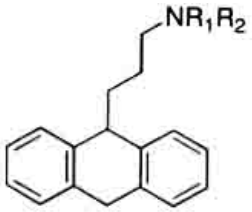


APDA, 36

AMDH and Derivatives



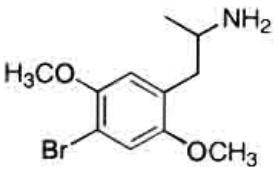
AMDH, 31



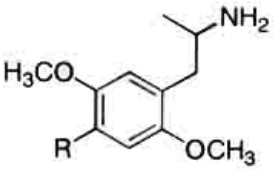
36, 48, 49

Compd	R_1	R_2
36	-H	-H
48	$-CH_3$	-H
49	$-CH_3$	$-CH_3$

DOB and Derivatives



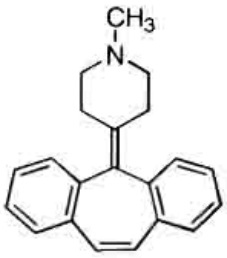
DOB, **10**



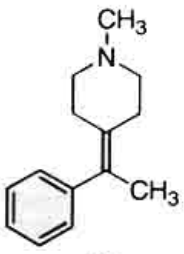
10, 11, 54-57

Compd	R
54	-H
10	-Br
11	$-(CH_2)_3Ph$
55	$-C_6H_{13}$
56	$-OCH_3$
57	-OH

Cyproheptadine and Derivatives

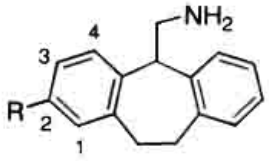


cyproheptadine, **3**



68

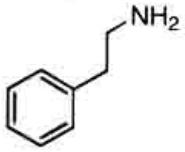
(Scheme 5) contd....



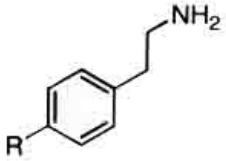
31, 50-53

Compd	R
31	-H
50	-Br
51	$-(CH_2)_3Ph$
52	$-C_6H_{13}$
53	$-OCH_3$

Phenethylamine and Derivatives



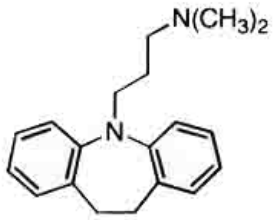
phenylethylamine, **58**



58-61

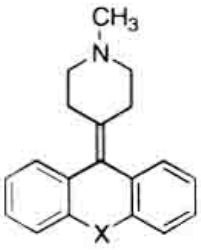
Compd	R
58	-H
59	-Br
60	$-(CH_2)_3Ph$
61	$-C_6H_{13}$

Imipramine and Derivatives



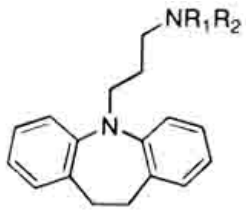
imipramine, **71**

(Scheme 5) contd....



3, 64-67

Compds	X
62	-CH_2-
3	-CH=CH-
63	-S-
64	-O-
65	-CH_2CH_2-
66	H, H
67	—



69, 70

Compd	R_1	R_2
69	-H	-H
70	-CH_3	-H
71	-CH_3	-CH_3

Scheme 5.

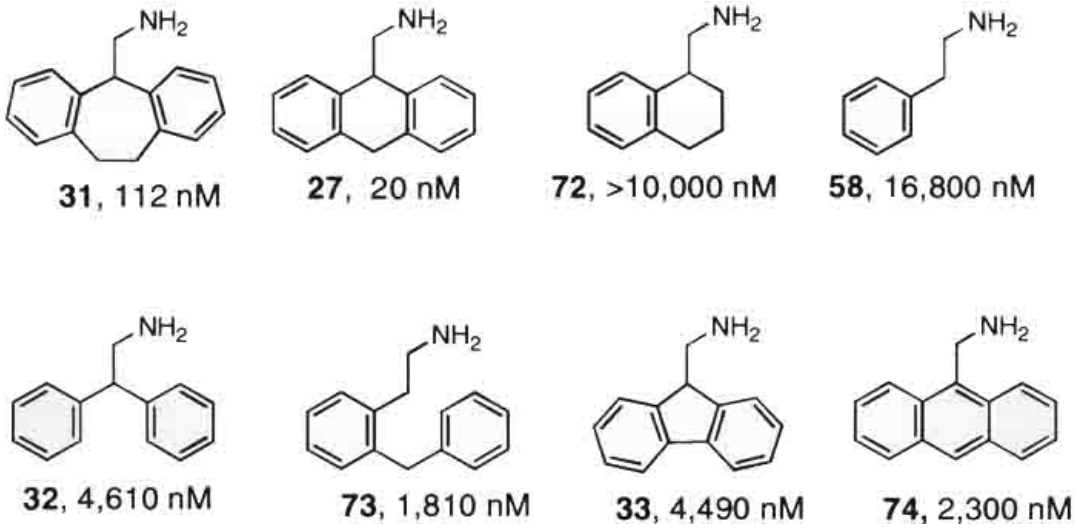


Fig. (5). Structures and receptor affinities of compounds **27, 59, 31-33, 72-74** at [^{3}H]ketanserin-labeled cloned 5-HT_{2A} sites.

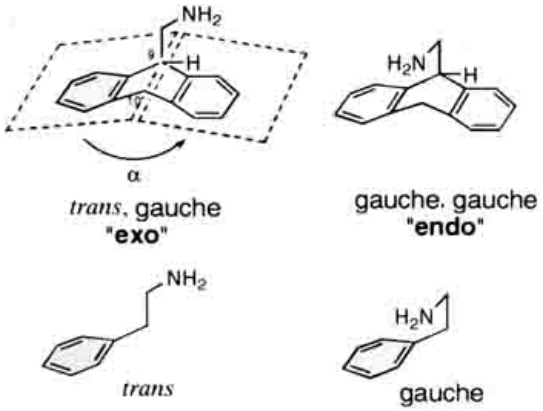


Fig. (6). Rotational conformers of AMDA and phenylethylamine.

of AMDA contribute to 5-HT_{2A} binding, the CNS activity of arylethylamines is usually attributed to the *trans* form [62]. Thus, AMDA has a significant symmetrical aromatic fold with relatively free rotation about the aminomethyl-dihydroanthracene bond producing both exo and endo minima. Compounds with less than optimal affinity at 5-HT_{2A} receptors have either a nearly planar (**33**, **74)**, folded but twisted (**31**), or orthogonal arrangement (**33**) of two aromatic rings. The relationship between biological activity and the nature of a folded tricyclic aromatic ring system has also been noted for tricyclic antipsychotics (phenothiazine and thioxanthene derivatives) and antidepressants (dibenzazepine and cyclopheptadiene derivatives) [63]. Phenothiazines and thioxanthenes that have a symmetrical fold of nearly 133-139^o (α) and fused ring torsion angle τ_1 ~ 0^o, tend to be antipsychotics (presumably D_2 antagonists) while dibenzazepine and dibenzocycloheptadienes that have a

folded aromatic configuration (α = 125° with a distinct twist (τ_1 ~ 10°) tend to be antidepressants (presumably by inhibition of neurotransmitter uptake) [63]. Thus, the geometric characteristics of AMDA and AMDA analogs are reminiscent of classical tricyclic agents. The major difference between AMDA and AMDA analogs and classical tricyclic agents is simply that the former contain a phenylethylamine skeleton while the later have a phenylbutylamine skeleton.

VARIATION OF THE AROMATIC FOLD ANGLE (unrestricted aminomethyl rotation).

Since AMDA has a significantly folded aromatic tricyclic system with free rotation about the aminomethyl bond, we synthesized and evaluated several AMDA derivatives with varying fold angles α containing either a rotatable or conformatinally restricted alkylamine (Figure **7**). In this series, the fold angle α falls between a maximum of 174° and a minimum of 111°.

The binding data in Figures **5** and **7** suggest that there may be some optimum aromatic fold angle near the value for AMDA in the 137 - 155° range. For compounds with a nearly symmetrical fold, there is a parabolic relationship between receptor affinity and the fold angle α. Since the AMDA parent is the highest affinity member in the series, it is not yet known whether AMDA has the optimal aromatic ring fold. There is no quantitative relationship (linear or parabolic) between affinity and any of the other geometric parameters evaluated [61]. It is rather remarkable that affinity appears to be sensitive to relatively small changes in aromatic fold angle.

Conformationally Restricted AMDA Analogs

Of course, the conformational disposition of all substances is not static and AMDA most certainly exists as a rapidly interconverting population of species. We have synthesized and evaluated a number of conformationally restricted AMDA variants in an attempt to delineate the AMDA pharmacophore (Figure **7**).

As with any such study, results are complicated by the necessity of introducing additional steric bulk to accomplish a decrease in rotational degrees of freedom, as well as changing other features that may be important (i.e. aromatic ring fold angle).

The [2.2.2]bicyclo derivatives **80** and **81** (K_i > 10,000

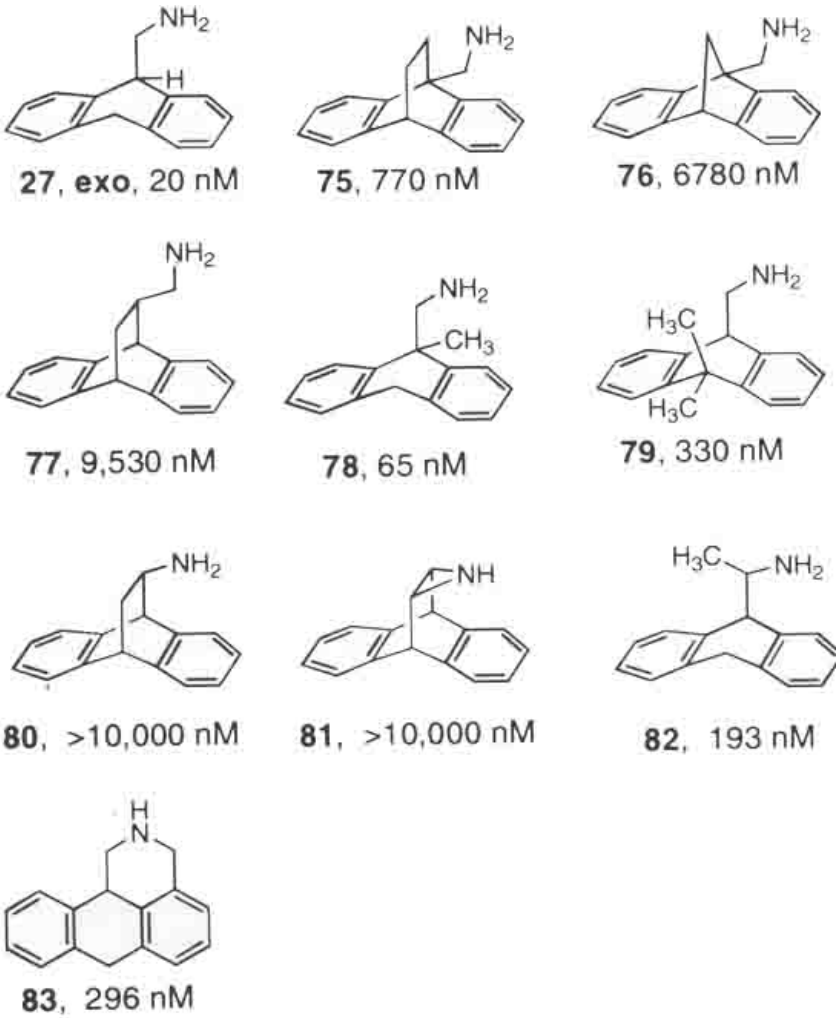


Fig. (7). Structures and receptor affinities of compounds **27** and **75-83** at [^{3}H]ketanserin-labeled cloned 5-HT$_{2A}$ sites.

nM) are both reasonable approximations of the exo and endo aminomethyl-axial AMDA conformers respectively (Figure **7**), but have no measurable 5-HT_{2A} affinity. Since compound **82** (K_i = 193 nM) does have measurable affinity, and binds with only 10-fold lower affinity than AMDA, the α-carbon bridge should be sterically tolerated. It appears that, while reasonable placement of the nitrogen can be achieved, the aromatic fold angle may be too acute (α ~ 120°) to be compatible with good receptor affinity. The aromatic fold angle of compound **83** is closer to the presumed optimum and it has reasonably good receptor affinity (K_i = 296 nM). The fused piperidine ring of 2,3,7,11b tetrahydrodibenzo [*d,e,h*]isoquinoline (**83**) has two possible orientations that place the nitrogen atom either above or below the aromatic plane. Based on the geometric considerations discussed above, we suspect that one of the aminomethyl axial conformers may be responsible for the reasonable affinity of the compound although all conformers are accessible and will likely bind to the receptor. It should be noted that none of the conformers of **83** closely resembled the endo form of AMDA. Compound **83** (K_i = 296 nM) has the highest affinity of any conformationally constrained analog in the AMDA class. This observation is particularly interesting given that 9-methyl AMDA (**78**, K_i = 65 nM, i.e., an equatorial methyl is tolerated) has modest affinity as does N-methyl AMDA (K_i = 52 nM) [64] suggesting that methylation is tolerated but decreases affinity. Compound **83** can exist in one of two conformational minima with the aminoalkyl chain either pseudo equitorial or pseudo axial. While both conformers should be energetically accessible, the former is energetically more stable by about 3 kcal/mol and is most likely the form bound to the receptor. Since compound **83** more closely resembles the exo form of **27** than the endo form, we speculate that the exo conformer of AMDA may be the bound form.

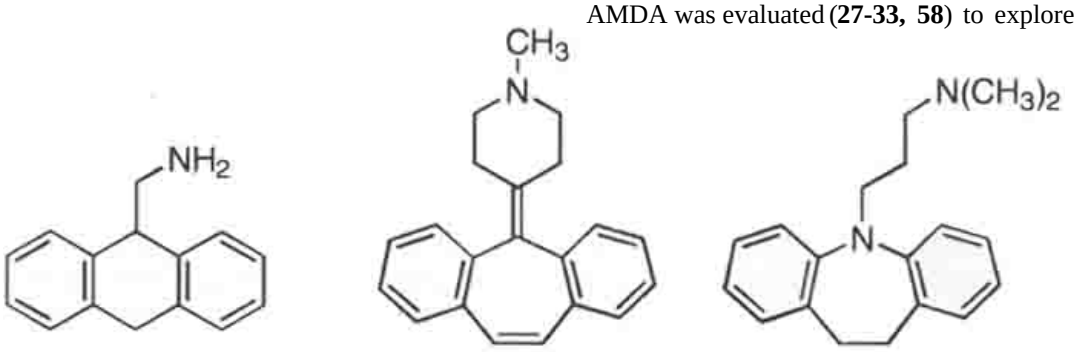
AMDA is a high affinity, 5-HT_2-selective antagonist that possesses a geometry inconsistent with previously reported 5-HT_2 antagonist pharmacophore models (see Figure **1** and related discussion). It is expected that structural variations that retain a phenylethylamine skeleton in a configuration similar to that of exo AMDA, within a tricyclic system containing two symmetrically folded aromatic rings (fold angle, α = 137° - 155°) should have high 5-HT_{2A} affinity. Based on the pharmacological properties of AMDA, compounds in this class are expected to function as 5-HT_2-selective antagonists [59].

Binding Modes of AMDA and Classical Tricyclic Amines: Have we Reinvented the Wheel?

With the exception of its two aromatic rings and basic nitrogen atom, AMDA is remarkably devoid of the pharmacophore features usually associated with high affinity receptor ligands such as the heteroatom hydrogen bonding features of the endogenous ligand serotonin. AMDA (**27**, Figure **5**), contains a phenylethylamine skeleton within a tricyclic ring system and does not fit either of the two pharmacophore models [12, 13].

The 9-(aminomethyl)-9,10-dihydroanthracene nucleus bears a general similarity to at least two classes of known, non-selective serotonin receptor ligands: tricyclic antidepressants and phenothiazine antipsychotic agents. Both classes of agents are tricyclic amines consisting of two aromatic groups flanking a non-aromatic central ring that bears an alkylamino substituent as does AMDA. Given the multiple neurochemical actions of classical tricyclic amines, if AMDA were to share a common mode of binding with either class, enthusiasm for further development based on the AMDA skeleton would be significantly diminished. An often unstated central tenet of classical drug design is that compounds with similar structural skeletons occupy similar sites when bound to receptors. However, there are numerous examples of similar compounds binding quite differently to a common receptor as well as ligands with multiple binding modes at a single receptor [65]. Establishment of parallel structure-activity relationships between two series of compounds is one experimental approach to indirectly estimate the similarity in modes of receptor occupation. The possible binding mode commonality between AMDA and classical tricyclic amines was evaluated by comparing AMDA derivatives with parallel series of cyproheptadine and imipramine derivatives (Scheme **6**).

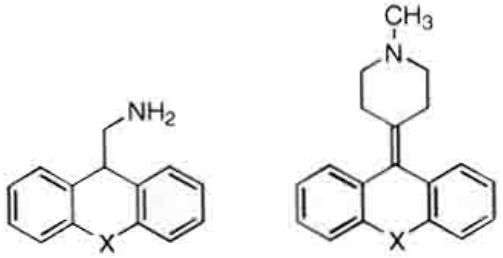
Cyproheptadine (**3**) (Scheme **6**) is approved for use as an antihistaminergic but has been used in the treatment of migraine, schizophrenia, Parkinson's disease, and as an appetite stimulant. It is structurally similar to tricyclic antidepressants and has a broad spectrum of affinities for serotonergic, adrenergic, muscarinic, dopaminergic and histaminergic receptors [66] as well as inhibiting norepinephrine and dopamine uptake [67]. Several cyproheptadine analogs with altered cycloheptadiene ring structures (**3, 62-68**) have been synthesized and their 5-HT_{2A} affinities have been reported [68]. A parallel series based on AMDA was evaluated (**27-33, 58**) to explore the possibility

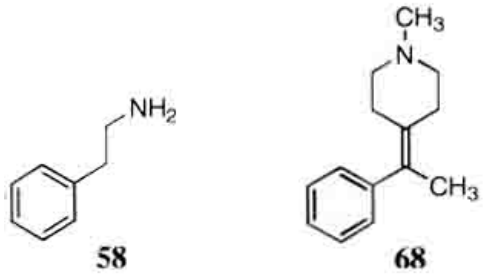


Scheme 6.

that two tricyclic amines may have similar binding sites at the receptor (Table **1**). While all of the AMDA derivatives have consistently lower affinities for 5-HT_{2A} receptors than the cyproheptadine derivatives, the range in affinities is about the same (1000-fold and 800-fold) within each series. There is low correlation (r^2 = 0.5) between pKi values within the AMDA and cyproheptadine series and no qualitative parallelism between receptor affinities of the two comparable series. These observations lends support to the hypothesis that the aromatic moieties of cyproheptadine and AMDA interact with the 5-HT_{2A} receptors in distinctly different fashions. Computational ligand-receptor docking experiments were performed to evaluate the feasibility of the hypothesis that cyproheptadine and AMDA bind to the receptor in different modes. Docking simulation consisting of systematic rotation of the receptor aspartate side chain torsion angles, the receptor carboxylate-ligand ammonium ion bond, and all rotatable bonds of the ligand indicated that while AMDA could bind in the region occupied by cyproheptadine, additional binding modes were also feasible, principally because of the greater flexibility of the AMDA aminoalkyl chain. The modeling results are consistent with the experimental data which predict that AMDA might bind in a fashion different from cyproheptadine and that AMDA may, in fact, have multiple modes of binding to the receptor. The fact that AMDA has 10-fold lower affinity than cyproheptadine may be due to a greater entropic penalty for binding the conformationally flexible AMDA or inability of any single conformer of AMDA to optimally fill the cavity. We speculated that it might be possible to overcome the entropic disadvantage by generating a rigid analog of AMDA. In addition, examination of ligand-receptor complex models suggested that aromatic substitution of AMDA may allow the derivative to more completely fill the unoccupied portion of the cavity and, perhaps, minimize occupation of multiple binding sites. Application of both of these design principles has led to AMDA derivatives with higher affinity than the parent.

Table 1. Ki Values for Compounds 27–24, 58 and 63–69 at Ketanserin Labeled 5-HT_{2A} Sites





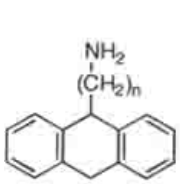
X	Compds	K_i, nM[a]	Compds	K_i, nM[b]
-CH_2-	**27**	20	**62**	0.7[a]
-CH=CH-	**28**	4,125	**3**	1.6
-S-	**29**	65	**63**	2.5
-O-	**30**	170	**64**	4.0
-CH_2CH_2-	**31**	112	**65**	9.0
H, H	**32**	5,700	**66**	13
—	**33**	20,833	**67**	199
-	**58**	16,820	-	-
-	-	-	**68**	355

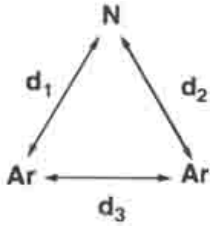
[a][^{3}H]Ketanserin labeled cloned 5-HT_{2A} sites. [b][^{3}H]Ketanserin labeled 5-HT_{2A} sites from rat forebrain.

Cyproheptadine is a useful model to study potential similarities and differences between classical tricyclic amines and AMDA derivatives, principally because it is a relatively rigid structure. However, most clinically useful classical tricyclic amines are conformationally flexible. Since chain length and *N*-alkylation are two major structural features that clearly distinguish AMDA from conformationally flexible classical tricyclic amines, we investigated the influence of chain length and *N*-alkylation in the AMDA series compared to imipramine (**69**) (Scheme **6**), a prototypical, nonselective, conformationally flexible tricyclic amine. To this end, we synthesized, examined geometric properties of, and evaluated chain lengthened and *N*-alkylated analogs of AMDA and demethylated derivatives of imipramine for comparison.

The data suggest (Table **2**) that there may be two optimal chain lengths for high 5-HT_{2A} receptor affinity in the

Table 2. The Effect of Chain Length on 5-HT_{2A} Receptor Affinity and Molecular Geometry





Compd	n	d1 (C)a	d_2 (C)[a]	d_3 (C)[a]	K_i, nM[b]
34	0	3.7	3.7	4.9	12,000
27	1	3.8	5.2	4.9	20
35	2	5.2	6.2	4.9	480
36	3	6.0	7.6	4.9	32
imipramine	-	6.5	7.2	4.8	160
cyproheptadine	-	6.1	6.1	4.9	1.6

[a]Distances measured for chain extended conformers.
[b][^{3}H]Ketanserin labeled cloned 5-HT_{2A} sites.

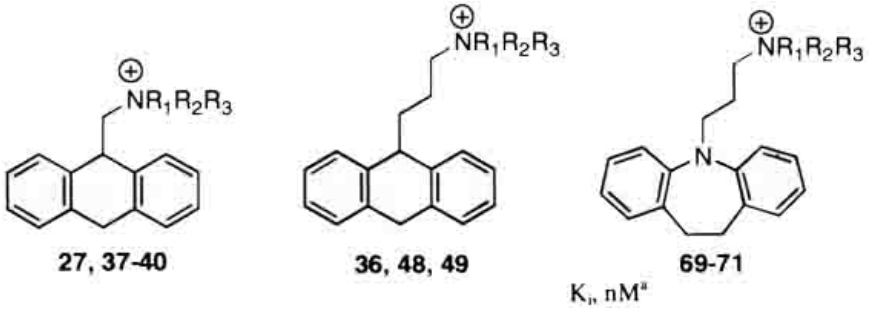
AMDA series. These are represented by an embedded phenylethyl fragment (n = 1) (AMDA, **27**) and a phenylbutyl chain length (n = 3) as found in compound **36** and the classical tricyclic antidepressants.

Several pharmacophore models for 5-HT_{2A} receptors have been proposed based on the structure–activity relationships of known antagonists (see Figure **1**). Typically, the essential geometric characteristics are described by the distances between two aromatic rings (d_3 = 4.6-7.3 Å) and the distances between each aromatic ring and the basic amine nitrogen (d_1 = 5.2-8.4 Å, d_2 = 5.7-8.5 Å). The corresponding dimensions for the minimum energy conformation of AMDA (**27**) are similar to existing agents with respect to the aromatic rings (d_3 = 4.9 Å) but deviate substantially in that AMDA is not symmetrical with respect to the two amine-ring distances (d_1 = 3.8 Å, d_2 = 5.2 Å), d_1 being much shorter than is considered optimal. The distance parameters for compound **36** are quite similar to those of classical antidepressants including cyproheptadine (**3**) and imipramine (**72**). Thus, unlike AMDA (**27**), compound **36** is consistent with existing pharmacophores (Figure **1**). Crystal structures of flexible tricyclic amines like imipramine usually show an extended aminoalkyl chain [69], but folded conformers that more closely resemble AMDA are energetically accessible. Molecular dynamics simulations of imipramine suggest that folded conformers with decreased phenyl-to-N distances (range 4.0 to 7.5 Å) do occur [70]. Thus, classical tricyclic amines can attain an AMDA-like configuration. The series, where n = 0-3, includes the "phenylethylamine" skeleton of AMDA and the "phenylbutylamine" skeleton of imipramine. Affinity is highest for the phenylethylamine and "phenylbutylamine" skeletons. Interpreted in terms of receptor binding modes, there are two limiting possibilities. AMDA (**27**) and **36** might bind in a similar fashion with **36** attaining an AMDA-like conformation. Alternatively, the aromatic moieties of AMDA and **36** might occupy different regions of the receptor allowed or enforced by the extension of the **36** side chain, i.e., **36** binds in a manner similar to imipramine. Since different classes of 5-HT_{2A} ligands show different effects with respect to *N*-alkylation [71], we explored a parallel series of *N*-alkylated derivatives of AMDA (**27**, **37**–**40**), the chain lengthened form of AMDA (**36, 48, 49**), and imipramine (**69**–**71**) to test this hypothesis. *N*-Methylation of AMDA (Table **3**) progressively decreases affinity. In contrast, mono- and dimethylation of the imipramine-like compound **36** and of the imipramine analog **69** enhances affinity.

While the number of different alkylation patterns is limited in this series, the results suggest that, at least with respect to nitrogen substitution, the short and long chain series bind differently. One possible explanation for the difference between AMDA and **36** with respect to *N*-alkylation is that, due to the proximity of the tricyclic ring system and the steric bulk of the methyl groups, dimethyl AMDA preferentially adopts a conformation with a buried ammonium ion NH. While **36** can adopt a folded conformation that places the nitrogen within a region of space similar to that for AMDA, dimethylation similarly buries the ammonium ion NH. The steric clash of a folded dimethyl **49** can be relieved by adopting a more chain extended and necessarily less AMDA-like conformation.

Classical tricyclic amines such as imipramine bind with high affinity to several neurotransmitter receptors and transporters (e.g., 5-HT_{2A} D_2, SERT, and NET) [72, 73]. Ligand SAR and ligand-receptor docking studies suggest that the imipramine-related and AMDA-related compounds

Table 3. The Effect of N-Alkylation on 5-HT_{2A} Receptor Affinity



K_i, nM[a]

			K_i, nM[a]					
R_1	**R_2**	**R_3**	**Compd**	**K_i, nM[a]**	**Compd**	**K_i, nM[a]**	**Compd**	**K_i, nM[a]**
H	H	H	27	20	36	32	69	392
CH_3	H	H	**37**	52	**48**	13	**70**	160
CH_3	CH_3	H	**38**	540	**49**	22	**71**	140
CH_3	CH_3	CH_3	**39**	4000		-		-
CH_2Ph	H	H	**40**	721		-		-

[a][^{3}H]Ketanserin labeled, cloned 5-HT_{2A} sites.

Table 4. Receptor and Transporter Selectivities of AMDA (27) and Classical Tricyclic Agents

		Ki, nM			
Compd	**5-HT$_{2A}$[a]**	**5-HT$_{2C}$[b]**	**D$_2$[c]**	**SERT[d]**	**NET[e]**
AMDA (**27**)	20	43	>10,000	>10,000	>10,000
imipramine (**71**)	94	160	726	5	16
cyproheptadine (**3**)	3	11	112	4100	290

[a][^{3}H]ketanserin
[b][^{3}H]mesulergine
[c][^{3}H]spiperone
[d][^{3}H]paroxetine
[e][^{3}H]nisoxetine radioligands.

interact with the 5-HT$_{2A}$ receptor differently. This suggests that AMDA may behave differently with respect to other neurotransmitter receptors and binding sites. Table **5** shows the results of a preliminary selectivity study.

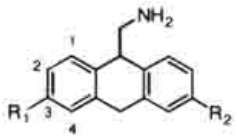
Given the high degree of receptor sequence homology, it is not surprising that the AMDA does not differentiate between 5-HT$_{2A}$ and 5-HT$_{2C}$ sites. It is, however, quite remarkable that AMDA shows at least 500-fold selectivity for 5-HT$_{2A}$ receptors vs D$_2$ receptors, and vs serotonin (SERT) and norepinephrine (NET) transporters. The results suggest that AMDA and imipramine most likely bind to the 5-HT$_{2A}$ receptor in different fashions, though the chain lengthened aminoalkyl dihydroanthracene **36** may be more imipramine-like in binding mode. While *N*-methylation decreases the 5-HT$_{2A}$ receptor affinity of AMDA, *N*-methylation increases the affinity in the imipramine and chain-lengthened AMDA series. The fact that AMDA has a high degree of selectivity and imipramine does not suggests that AMDA and imipramine bind to the D$_2$ receptors, SERT, and NET differently as well. Thus, AMDA behaves quite differently from classical tricyclic amines and may be a suitable template for the construction of structurally novel, selective 5-HT$_2$ receptor antagonists.

LIGAND-RECEPTOR COMPLEX MODELS

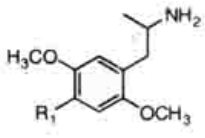
Effects of Aromatic Substitution

In part because model building studies suggested that there may be a region of bulk tolerance near the 3-position of AMDA and partly because the 3-position of AMDA corresponds to the 4-position of phenylethylamine, parallel series of AMDA, AMDH, phenylethylamine and DOX compounds were evaluated (Table **5**). The 5-HT$_{2A}$ receptor can accommodate a wide range substituents associated with the 3-position of AMDA (**27**, **41 – 47;** Table **5**).

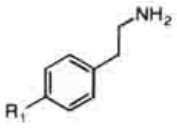
Table 5. The Effects of Aromatic Substitution on 5-HT$_{2A}$ Affinities



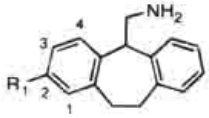
27, 41-47



10, 11, 54-57



58-61



31, 50-53

R_1	R_2	Compd	K_i, nM[a]	Compd	K_i, nM[a]	Compd	K_i, nM[a]	Compd	K_i, nM[a]
-H	-H	**27**	20	**54**	5,200	**58**	16,800	**31**	110
-Br	-H	**41**	1.3	**10**	41	**59**	1770	**50**	37
-(CH$_2$)$_3$Ph	-H	**42**	3.2	**11**	10	**60**	60	**51**	81
-C$_6$H$_{13}$	-H	**43**	7.0	**55**	2.5	**61**	78	**52**	630
-OCH$_3$	-H	**44**	7.5	**56**	1,200	-	-	**53**	800
-O(CH$_2$)$_4$CH$_3$	-H	**45**	23	-	-	-	-	-	-
-OH	-H	**46**	107	**57**	>50,000	-	-	-	-
-C$_6$H$_{13}$	-OCH3	**47**	43	-	-	-	-	-	-

[a][^{3}H]Ketanserin labeled cloned 5-HT$_{2A}$ sites.

Affinities varied only about 80-fold (**41**, K_i = 1.3 nM; **46**, K_i = 107 nM) within the series. With the exception of the 3-hydroxy compound (**46**, K_i = 107 nM), monosubstitution of AMDA (**27**, K_i = 20 nM) either does not change (**45**, K_i = 23 nM) or increases affinity to a maximum of 15-fold (**41**, K_i = 1.3 nM) regardless of steric bulk or electronic character of the substituent. The effects of 4-position substitution on the affinities of 1-(2,5-dimethoxyphenyl)-2-aminopropanes (DOX; **10**, **11**, **54**-**57**) are qualitatively similar in that each of these substituents, except 4-hydroxy (**57**, K_i > 50,000 nM), enhances affinity. However, in the DOX series, the range of affinity enhancement is much greater (**55**, K_i = 2.5 nM; **54**, K_i = 5,200 nM) than for the AMDA series with a maximum range of about 2,000-fold, excluding the 4-hydroxy compound (**57**) that shows no measurable affinity. Consistent with these observations, the lipophilic character of the 4-position substituent of DOX has been shown to modulate affinity over a broad range [73, 74]. These results suggest that the AMDA and DOX series may interact differently with the 5-HT_{2A} receptor. This is perhaps not surprising given the fact that DOB is an agonist [74] whereas AMDA is an antagonist [59]. At very least, even if the two series bind in a comparable fashion, they must interact preferentially with functionally and conformationally distinct forms of the receptor. An alternative possibility is that the binding sites of agonists and antagonists only share a common ammonium ion binding site with the remaining bulk of each type of agent occupying completely different domains within the receptor. The [*a*,*d*] dibenz fused cycloheptane **31** (AMDH) has an altered aromatic ring geometry compared with AMDA in that AMDH has a pronounced twist in addition to a fold between the two aromatic rings. Given that **31** has reasonably high affinity for the 5-HT_{2A} receptor and would probably have superior *in vivo* stability, we evaluated a series of aromatic ring 2-substituted derivatives (**31**, **50** – **53**) to examine the generality of the relationships between substituent structure and ligand-receptor binding modes as well as to provide a preliminary estimate of the suitability of AMDH for further optimization. The data in Table **5** indicate that the affinities are much more sensitive to the nature of the substituent within the DOB-like series (**10, 11, 54**-**57**; 21,000-fold) than either the AMDA series (**27**, **41 – 47**; 15-fold) or the AMDH series (**31**, **50** – **53**; 7-fold). There is no quantitative correlation between the pKi values for the AMDH series (**31**, **50** – **53)** and those in the DOB-like series (r^2 = 0.0004) and little correlation between the AMDA series and the DOB-like series (r^2 = 0.32) suggesting that the tricyclic compounds and the phenylethylamines interact with the receptor in different fashions. The affinity data for the AMDA and AMDH series are not quantitatively parallel by virtue of the parent unsubstituted members of each series, however the limited sensitivity to substitution by a range of substituents with greatly different steric and electronic characteristics is a property shared by both the AMDA and AMDH series.

Analysis of early 5-HT_{2A} receptor models led us to consider two general areas of steric accessibility (Figure **4**): Site 1 (TM3 flanked by TM4, TM5, and TM6) and Site 2 (TM3 flanked by TM1, TM2, TM6, and TM7). Consideration of ligand SAR and receptor mutagenesis data prompted us to provisionally consider Site 1 the "agonist site" and Site 2 the "antagonist site." Similar suggestions have also been made for the 5-HT_{1A} receptor [58]. The 5-HT_{2A} receptor model generated from the rhodopsin crystal structure shows two similar, overlapping areas of accessibility that are more symmetrically distributed around TM3, Site 1 defined by TM4, TM5, and TM6 with Site 2 defined by TM2, TM6, and TM7 (Figure **4**). Viewed from the perspective of the ligand in the most general terms, it can usually be observed that the structures of antagonists differ from the endogenous neurotransmitters and other agonists in that they either lack key functional groups, present molecular features in areas of space not occupied/utilized by any portion of the agonist (i.e., an "accessory site"), or both [75]. For example, while 5-methoxytryptamine is a serotonin agonist, tryptamine is a partial agonist (see ref. 10 for a review). It has recently been shown that 2-phenyltryptamines are high affinity 5-HT_{2A} antagonists [76]. Similarly, LSD is an agonist or partial agonist whereas 2-bromo LSD is an antagonist [77]. In the DOX series, compounds with small substituents at the 4-position are agonists and those with bulky substituents, such as phenylpropyl (e.g. **11**) are antagonists [75, 76]. In the latter case, the 2,5-dimethoxy groups of 2,5-dimethoxy-4-(3-phenylpropyl)phenylethlyamines, functional groups characteristically required for agonists, are no longer required and, in fact, the desmethoxy parent has comparable affinity to the 2,5-dimethoxy substituted derivative [75,76]. It has been hypothesized that phenylalkylamines with small 4-substituents (e.g. **10**, **54**, **56**) bind differently from those with bulky substituents (e.g. **11**, **55**) at this position. This would seem reasonable. Models of complexes of 5-HT_{2A} receptor and DOB support the notion that there may be limited bulk tolerance at the 4-position for some modes of binding. Bound within Site 1, large substituents at the 4-position project into TM5 (see Figure **3** for representative complexes). Whereas 4-methyl and 4-ethyl substituents appear to be tolerated in the DOB-like series; successively adding methylene units to the model of 1-(2,5-dimethoxy-4-ethylphenyl)-2-aminopropane bound to the receptor actually causes a displacement of the of the aromatic ring (2.3 Å) from the initial site on minimization. The bound ligand **55** is also rapidly displaced from its initial site during dynamics simulations (100 ps, 300º K, range constraint NH-OD155, 1.3-2.6Å, helix backbone constrained) whereas DOB (**10**) is not. It seems unlikely that substitutions that increase affinity substantially (**11**, **55**) perturb binding in this fashion. Another possible binding mode would place large 4-position substituents in Site 2 (Figure **8**). The 5-HT_{2A} receptor model with the phenylethylamine **61** bound in Site 2 does not show displacement of the aromatic ring and the ligand remains in the binding site on dynamics simulation.

AMDA lacks both agonist like functional groups (e.g., the 5-OH group of 5-HT or the 2,5-dimethoxy substituents of DOB) and presents an added feature, the "second" aromatic ring. When AMDA is fit interactively into the receptor model Connolly channel, energy minimized, and subjected to dynamics simulation, the complex formed places one aromatic ring near TM5 in Site 1 with the other pointed toward TM7 in Site 2 (Figure **3**). Because of the greater width of the tricyclic aromatic moiety compared with

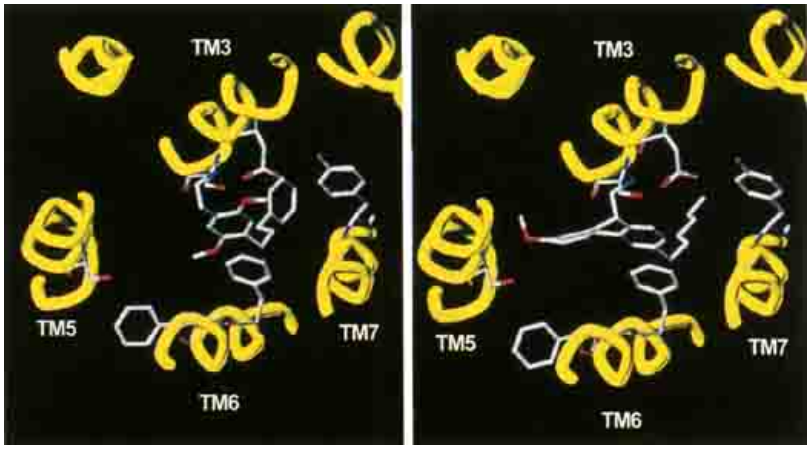


Fig. (8). DOPP (**11**, left), and the AMDA derivative **47** (right) bound to the 5-HT_{2A} receptor model. A 14-Å slab at the position of the TM3 D155 residue is displayed. TM3 D155, S159; TM5 S239; TM6 F339, F340; and TM7 Y370 side chains only are displayed. TM1 is not shown.

the single ring of phenylethylamines, the aromatic ring of AMDA that is within Site 1 is even nearer TM5 than is the phenyl ring of DOB. While 3-position substituents of AMDA could occupy either Site 1 or Site 2, the most sterically reasonable binding site is between TM3 and TM7 (Figure **8**). 3-Hexyl AMDA (**61**) constructed by successively adding methyl groups to the 3-position in Site 1 shows a pronounced displacement (1.6 Å) similar to that observed for 4-hexylDOX (**55**). In fact, Site 1 appears unable to tolerate even a 3-methyl AMDA substituent. Dynamics trajectories starting with 3-hexyl AMDA (**43**) result in expulsion of the AMDA derivative from Site 1. This is not the case for dynamics simulation of 3-hexyl AMDA bound to Site 2. Due to the steric interaction between the 3-position of AMDA and TM5 of Site1, all of the AMDA derivatives, regardless of steric bulk, place 3-position substituents in the Site 2 direction. These potential binding modes may explain the lack of parallelism between substituted AMDA and phenylalkylamine derivatives as well as the antagonist properties of AMDA and the bimodal nature of the phenylalkyamine functional properties. Thus, we hypothesize that AMDA and DOX analogs with bulky 4-substituents bind as suggested in Figure **3** with substituents positioned in Site 2 (the antagonist site) while DOB places the 4-bromo substituent in Site 1 (the agonist site).

In addition to aromatic substituent effect data, the effects of N-alkylation appear to somewhat support the notion that DOB and AMDA interact with the receptors differently. In the case of both 5-methoxytryptamine and 4-bromo-2,5-dimethoxy-2-phenylpropylamine, successive methylation decreased affinity but N-benzyl DOB and N-benzyl-5-methoxytryptamine have slightly higher affinities (2- to 6-fold) than their parents [71]. In the AMDA series, successive methylation also decreased affinity but, unlike the agonists series, N-benzylation decreased affinity (36-fold) [64].

The proposed binding pocket for 3-hexyl AMDA in Site 2 is lined with several polar residues including S159, S162 (TM3); S373, S372 (TM7) and hydrophobic residues including F158 (TM3) F339, W336 (TM6) that are positioned within 5Å of the substituent. The distribution of polar and hydrophobic residues is such that an amphiphilic cavity is created between the relatively polar faces of TM3 and TM7 and the lipophilic face of TM6. It is possible that the amphiphilic nature of the site is the characteristic that allows both relatively polar (e.g. **44**, **46**), non-polar (e.g. **42**, **43**), and mixed (e.g. **45**) groups to bind with reasonably high affinity almost without discrimination.

The bis-substituted compound **47** (Table **5**) was evaluated in an attempt to bridge and interact simultaneously with both Sites 1 and 2. The 6-methoxy group was expected to interact with a hydrogen bond donating residue of Site 1, perhaps S239 of TM5, with the hexyl group anchored in Site 2. While mono substitution with either a hexyl (K_i = 7.0 nM, **43**) or a methoxy group (K_i = 7.5 nM, **44**) enhances affinity relative to AMDA (K_i = 20 nM, **27**) to a small extent, the bis-substituted compound (K_i = 43 nM, **47**) has a lower affinity than both the unsubstituted compound (**27**) and the mono substituted derivatives (**43**, **44**). At very least, the bifunctional nature of **47** does not greatly enhance affinity. This is understandable, retrospectively, given the hypothesized orientation of AMDA within the ligand-receptor complexes where tolerance to substituents is limited in Site 1 (Figure **8**). Dynamics simulation, followed by geometry optimization suggest that **47** is not able to optimally fit the "AMDA" site and is too far (5 Å) from S239 for a productive hydrogen bond.

Information from mutagenesis experiments further suggests that AMDA and phenylalkylamines or DOX analogs with small 4-substituents (e.g. DOB, DOI) bind differently, at least with respect to F340 (3). In the current model, the aromatic ring of F340 can either be in the central cavity or at the interface between TM6 and TM5. Any effect the F340 mutation might have on ligand affinity could either be due to changes in direct ligand-receptor van der Waals interaction or by indirectly affecting the shape of the helix aggregate. The mutation F340L has been shown to decrease affinity of agonists and generally have no effect on classical antagonists [19]. AMDA (**27**) and the bromo analog **41** both bind to the mutant receptor approximately as well (3-fold decrease and no change in affinity, respectively) as to wild type receptor (Table **6**). The same mutation has little effect on ketanserin affinity but essentially abolishes DOI binding (an approximately 14,000-fold decrease) [19]. This is entirely consistent with AMDA and AMDA derivatives

binding in a completely different fashion from DOI, at least with respect to the F340 position in the receptor structure.

Table 6. The Effects of the 5-HT$_{2A}$ Receptor F340L Mutation on Ligand Affinity

	K_i (nM)[a]	
Compound	**Wild type**	**F340L**
27	20	57
41	1.3	1.8
DOI	0.9	13,700
ketanserin	0.4	0.23

[a][^{3}H]Ketanserin labeled cloned 5-HT$_{2A}$ sites.

RECEPTOR SELECTIVITY

As shown in Table **7**, AMDA and two of its high affinity analogs are quite selective for 5-HT$_2$ receptors. 5-HT$_{2A}$ affinity is between 900- and 7000-fold higher than D2 receptor affinity. There is little selectivity for 5-HT$_{2A}$ vs 5-HT$_{2C}$ receptors (2- to 9-fold).

Selectivity for the 5-HT$_{2A}$ receptor over the serotonin and norepinephrine transporters is pronounced for **27** and **41** (between 500- to 3,000-fold) and less pronounced for **43** (60- and 120-fold). Since selectivity against the D_2 receptor is not strongly influenced by the nature of the 3-substituent, the observed selectivity is probably attributable to the AMDA nucleus itself. Examination of an alignment of the 5-HT$_{2A}$ and D_2 receptor sequences allows the identification of one variant position in TM2, four in TM3, and four in TM7 that face the central cavity of the 5-HT$_{2A}$ receptor model. One of the TM7 differences is V366 which is equivalent to, in position, T413 of the D_2 receptor and is within 4 Å of the Site 2 aromatic ring of AMDA in the model (Figure **9**). Presence of an alanine or threonine instead of the asparagine residue at this position in adrenergic receptors has been shown to be responsible for subtype selectivity within the serotonin receptor family particularly with respect to the ability to bind β-adrenergic antagonists such as propranolol [41, 78]. It is possible that placement of a polar threonine near the AMDA aromatic ring may be unfavorable enough to account for the lower affinity of

Table 7. Receptor and Transporter Selectivity for Compounds 27, 41, and 43

	K_I, nM (±SEM)				
Compd	**5-HT$_{2A}$[a]**	**5-HT$_{2C}$[b]**	**D$_2$[c]**	**SERT[d]**	**NET[e]**
27[f]	20	43	>10,000	>10,000	>10,000
41	1.3	3.3	>10,000	1,200	4,490
43	7.0	62	6,280	490	845

Radioligands: [a][^{3}H]ketanserin, [b][^{3}H]mesulergine, [c][^{3}H]spiperone, [d][^{3}H]paroxetine, [e][^{3}H]nisoxetine.

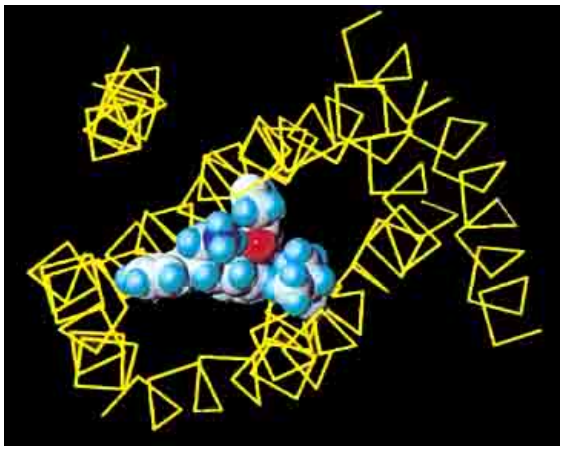


Fig. (9). AMDA (27) shown as a space-filled model bound to the 5-HT$_{2A}$ receptor. The TM3 D155 and TM7 V366 residues are also rendered as space-filling models.

AMDA and AMDA derivatives with the D_2 receptor. This hypothesis could be tested by evaluation of the V366T mutant of the 5-HT_{2A} receptor.

CONCLUSION

Numerous classes of 5-HT_2 ligands exist (see Introduction). However, examples of most of these agents were either known prior to the discovery of 5-HT_2 receptors, or were serendipitous discoveries. The present review describes an attempt to develop a 5-HT_2 ligand de novo (i.e., based on the structure of the 5-HT_{2A} receptor as opposed to exploiting an already developed lead). An early graphics model suggested that the affinity of an inactive compound (i.e., phenylethylamine, K_i = 16,800 nM) might be enhanced by the introduction of a flanking aromatic ring. The first compound synthesized in the series (AMDH, **31,** K_i = 112 nM) provided encouraging results. As time went on, the quality of the graphics models improved and additional compounds were designed and prepared. AMDA (**27**, K_i = 20 nM), a direct consequence of these investigations, proved to be a structurally unique 5-HT_2 antagonist. Some similarity exists between AMDA and both phenylalkylamine and tricyclic/polycyclic classes of 5-HT_2 ligands. However, comparative structure-affinity studies indicated that AMDA likely binds in a different manner than these seemingly related agents. In fact, AMDA does not meet the structural requirements of any of the currently existing pharmacophore models. Subsequent modeling studies addressed the issue of how AMDA might bind at 5-HT_{2A} receptors, and how this binding mode differs from that of the phenylalkylamines, tricyclic/polycyclic serotonergic agents, and indeed, 5-HT itself. Previous investigations have provided evidence that phenylalkylamines can be agonists or antagonists depending on the nature of the 4-position substituent [73, 74]. It has been speculated that the difference in functional behavior is a reflection of the possibility that agonist and antagonist phenylalkylamines bind in a different fashion with the 5-HT_{2A} receptor. Comparison of the effects of a parallel series of aromatic substituents based on the tricyclic 5-HT_{2A} antagonist AMDA suggests that the AMDA-series may bind in a fashion similar to that of antagonist phenylalkylamines with bulky aromatic substituents. Differential effects of the F340L mutation observed for the AMDA series and phenylethylamine agonists supports this hypothesis. Simulations with ligands docked into a 5-HT_{2A} model are consistent with the hypothesis that agonists bind in a fashion such that the aromatic rings are oriented toward the fifth transmembrane helix (Site 1), a region of limited bulk tolerance, whereas antagonists place the substituted aromatic ring near the seventh transmembrane helix (Site 2) in a region of greater bulk tolerance. AMDA and ring substituted analogs show a remarkable degree of selectivity for the 5-HT_2 receptors particularly in light of their relatively simple structures. The lack of selectivity for 5-HT_{2A} vs 5-HT_{2C} receptors is not unexpected. It may be possible to use comparative molecular modeling methods to ultimately elicit subtype selectivity. Our work has demonstrated that it is possible to use receptor models productively in ligand design, complementing classical SAR approaches. At very least, receptor models provide a three-dimensional context for organizing a large body of structure-relevant data and aid the formulation of testable hypotheses. The recent availability of a crystal structure for at least one GPCR (rhodopsin) will bring aminergic receptor modeling into a new era which will most likely see even more productive use of receptor models in novel ligand design and the identification of targets for site-directed mutagenesis.

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