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# Exploring the Relationship Between Binding Modes of 9-(Aminomethyl)-9,10-dihydroanthracene and Cyproheptadine Analogues at the 5-$HT_{2A}$ Serotonin Receptor

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**Abstract**—Comparison of the serotonin 5-$HT_{2A}$ receptor affinities of a parallel series of structural analogues of the novel ligand 9-aminomethyl-9,10-dihydroanthracene (AMDA) and a structurally similar prototypical tricyclic amine cyproheptadine suggests that the two agents bind to the receptor in different fashions. Examination of ligand–receptor model complexes supports the experimental data and suggests a potential origin for the differences in binding modes. 

We have recently described 9-(aminomethyl)-9,10-dihydroanthracene (AMDA) as the parent member of a potentially new class of 5-$HT_{2A}$ high affinity serotonergic antagonists.[1] With the exception of its two aromatic rings and basic nitrogen, it 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. Although AMDA was initially conceived and evaluated to test a theoretical receptor model without explicit consideration of other known 5-HT ligands,[2] it became apparent that the 9-(aminomethyl)-9,10-dihydroanthracene nucleus bears a general similarity to at least two classes of known, nonselective serotonin receptor ligands: tricyclic antidepressants and phenothiazine antipsychotic agents. Both classes of agents are tricyclic amines consisting of two aromatic groups flanking a nonaromatic central ring that bears an alkylamino substituent as in AMDA. If AMDA were to share a common mode of binding with either class, given the multiple neurochemical actions of classical tricyclic amines, 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.[3] Establishment of parallel SARs between two series of compounds is one experimental approach to indirectly estimate the similarity in modes of receptor occupation. Cyproheptadine 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[4] as well as inhibiting norepinephrine and dopamine uptake.[5] The effects of central ring modifications that alter the orientation of the two aromatic rings of cyproheptadine on serotonin receptor affinity have been described.[6] Since we have observed that the 5-$HT_{2A}$ affinity of AMDA analogues is sensitive to the aromatic ring geometry,[7] we chose to evaluate the possibility that cyproheptadine and AMDA bind in similar fashions to the 5-$HT_{2A}$ receptor by evaluating a parallel series of modifications in the AMDA series.

## Results and Discussion

Structural[8,9] and molecular modeling studies[10] indicate that cyproheptadine and its derivatives are relatively rigid. The only flexibility that occurs is inversion of the chair-form piperidine ring. The plane defined by the

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piperidine atoms and that defined by the aromatic atoms of the tricyclic nucleus are nearly perpendicular (Fig. 1). Conformational analysis of AMDA indicates that a minimum energy structure exists that places the 9-methylamino group in a pseudoaxial position consistent with structural studies of 9-alkyl-9,10-dihydroanthracenes (Fig. 1).[11] Despite differences in the central ring structures, there is significant geometric similarity between cyproheptadine and AMDA; both show a symmetrical fold of the aromatic rings with comparable folding angles (124° and 147°, respectively). The obvious difference in 3-D structures is the length of the aminoalkyl side chains, cyproheptadine being about 2 Å longer than AMDA.

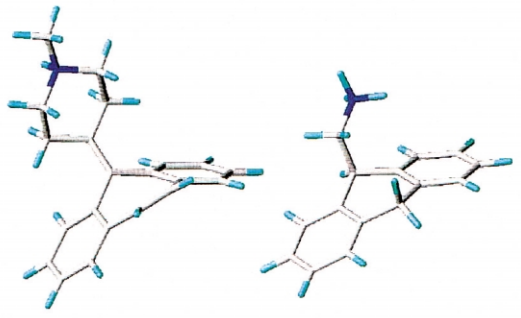
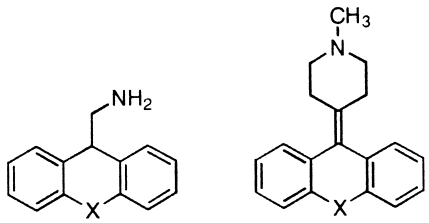


**Figure 1.** Comparison of the 3-D structures of cyproheptadine (left) and AMDA (right).

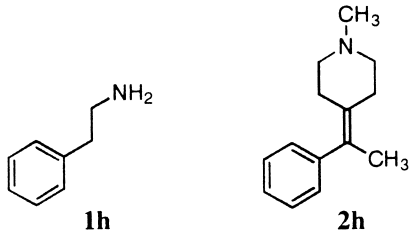
Several cyproheptadine analogues with altered cycloheptadene ring structures (**2a**–**2h**) have been synthesized and their 5-$HT_{2A}$ affinities have been reported.[6] We evaluated an analogous series of modification in the AMDA series (**1a**–**1h**) to explore the possibility that two tricyclic 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, there is a substantial range in affinities within each series (1000- and 500-fold, respectively). There is low correlation ($r^2 = 0.5$) between p$K_i$ values within the **1a**–**1h** and **2a**–**2h** series. These observations do not strongly support the hypothesis that the aromatic moieties of cyproheptadine and AMDA interact with the 5-$HT_{2A}$ receptors in similar fashions.

Computational simulations of the binding of cyproheptadine and AMDA to a 5-$HT_{2A}$ model were carried out in an attempt to identify potential similarities or differences in the modes of binding of the two ligands. Given the relative featurelessness of both structures, the only likely ligand–receptor interaction is that between the ammonium ions and Asp-155 of the third transmembrane helix (TM3). Initial studies indicated that manual docking followed by minimization and dynamics simulations (both isothermal and simulated annealing) produced results that were highly dependent on the starting configurations of the complexes. Since there are numerous potential starting configurations for complexes with either cyproheptadine or AMDA, these procedures are susceptible to an unacceptable level of operator bias.

**Table 1.** $K_i$ values for compounds **1a**–**1h** and **2a**–**2h** at ketanserin labeled 5-$HT_{2A}$ sites




**1a - 1g** **2a - 2g**




**1h** **2h**

| X | Compd | $K_i$ (nM)[a] | Compd | $K_i$ (nM)[b] |
|---|---|---|---|---|
| $-CH_2-$ | **1a** | 20 | **2a** | 0.7[a] |
| $-CH=CH-$ | **1b** | 4125 | **2b** | 1.6 |
| $-S-$ | **1c** | 65 | **2c** | 2.5 |
| $-O-$ | **1d** | 170 | **2d** | 4.0 |
| $-CH_2CH_2-$ | **1e** | 112 | **2e** | 9.0 |
| H, H | **1f** | 5700 | **2f** | 13.0 |
| — | **1g** | 20,833 | **2g** | 199 |
| | **1h** | 16,820 | — | — |
| | — | — | **2h** | 355 |

[a][$^3$H]Ketanserin labeled cloned 5-$HT_{2A}$ sites. Values represent the mean of computer-derived $K_i$ estimates (using LIGAND) of quadruplicate determinations. Standard errors typically range between 15 and 25% of the $K_i$ value.
[b][$^3$H]Ketanserin labeled 5-$HT_{2A}$ sites from rat forebrain. Data from ref 6.

Automated docking methods were similarly unsuitable because of the necessity to arbitrarily assign (lacking obvious potential functional interactions in our case) multiple points of interaction between ligand and receptor. We elected to take an approach primarily based on steric accommodation (favorable and unfavorable van der Waals interactions) to evaluate the hypothesis that cyproheptadine and AMDA **can** bind in a similar fashion. A complex of cyproheptadine and AMDA, created by flexible superimposition of AMDA with the crystal structure of cyproheptadine (RMSD = 0.49), was manually docked into the receptor model guided by a Connolly channel surface[12] representing volume accessible to a 2.0 Å probe (Fig. 2). Under these conditions there is only one plausible orientation that can sterically accommodate both ligands while maintaining an interacting distance between the ligand ammonium ions and the TM3 Asp-155. The minimized ligand–receptor complexes were used as the starting point for a systematic conformational search ensuring that the initial binding modes were similar for both ligands.

Temporary bonds were created between an ammonium ion hydrogen and the nearest Asp-155 carboxylate oxygen and systematic rotation about all resulting rotatable

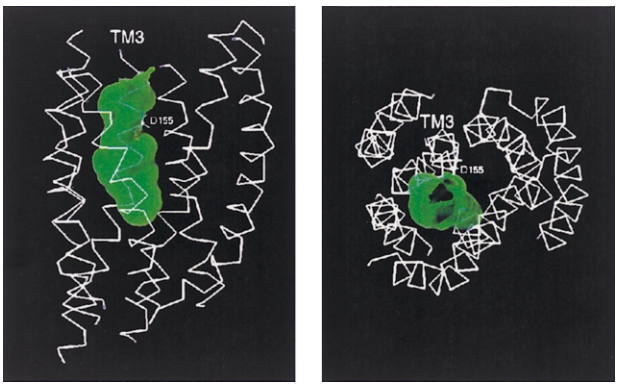
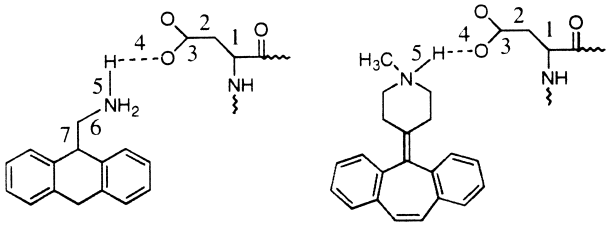



**Figure 2.** Connolly channel plot depicting the sterically accessible binding pocket of the 7 helix 5-$HT_{2A}$ receptor model. Left: side view, extracellular end up. Right: top view (right) from the extracellular end. Helices are arranged counter clockwise in numerical order.

'bonds' (seven for AMDA and five for cyproheptadine) was carried out in 30° increments. Rotatable bonds included are numbered below.



Conformations resulting in superimposition of van der Waals radii outside of specified tolerances were automatically filtered from the resulting data sets. The conformational searches produced 33 structures for cyproheptadine and 4060 for AMDA clearly indicating that AMDA is more easily accommodated within the binding cavity of the receptor model. Decreasing the torsion increment to 10° increased the number of cyproheptadine conformers to 86. While different, all of the cyproheptadine conformers remain between the TM4, 5, and 6 anchored to Asp-155 with the tricyclic nucleus nearly perpendicular to the helix axes pointed toward the intracellular side of the receptor (Fig. 3). On the other hand, AMDA can occupy many orientations, with the aggregate of conformers more completely filling the sterically accessible cavity (Fig. 3). Examination of individual members of the conformational analysis data set reveals that this is the result of smaller size and greater conformational flexibility of AMDA relative to cyproheptadine. The modeling results are consistent with the experimental data which predict that AMDA may 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 a lower affinity than cyproheptadine (20 vs 1.6 nM) may be due to differences in solvation, a greater entropic penalty for binding the more conformationally flexible AMDA, or inability of any single conformer of AMDA to optimally fill the cavity.

It may be possible to overcome an entropic disadvantage by generating a rigid analogue of AMDA. In addition, examination of Fig. 3 does suggests that

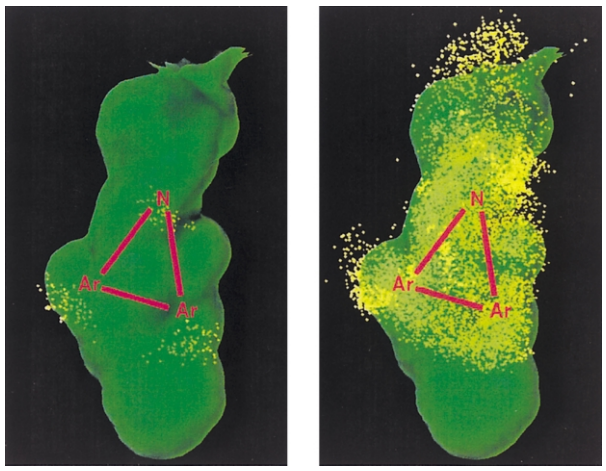



**Figure 3.** Plot of points (yellow) corresponding to N, C3, and C8 atoms for 86 sterically allowed conformers of cyproheptadine (left) and N, C3, and C7 for 4060 conformers generated from AMDA superimposed on a Connolly channel plot (green). The starting configurations of the ligands is shown schematically in red.

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. Structural modifications of AMDA with these factors in mind are currently under investigation.

## Experimental

### Affinity determinations

Binding assays and data analysis were performed as previously described using [$^3$H]ketanserin as the radioligand and stably transfected NIH3T3 cells expressing the 5-$HT_{2A}$ receptor (GF-62 cells).[13]

### Molecular modeling

Molecular modeling investigations were conducted using the SYBYL molecular modeling package (version 6.5, 1999, Tripos Associates, Inc., St. Louis, MO). Molecular mechanics minimizations were performed using the Tripos force field with Gasteiger–Huckel charges (distance dependant dielectric constant $\varepsilon = 4$, non-bonded cutoff = 8 Å) without constraints and were terminated at an energy gradient of 0.05 kcal/mol Å. The 5-$HT_{2A}$ receptor model was constructed from the backbone atom coordinates of a unique 5-$HT_{1A}$ receptor model arrived at in a de novo fashion using hydrogen bonding constraints in a distance geometry approach.[14,15] The SCWRL program was used to generate initial side-chain geometries.[16] The results were analyzed for geometrically clashing pairs of residues using the ProTable facility in SYBYL. Geometric clashes were relieved as previously described.[13] The model was minimized first with backbone atom constraints and then without constraints. Conformational analysis was carried out using the SYBYL SEARCH facility with the following van der Waals scaling factors: general = 0.6, 1–4 interaction = 0.87, H-bond = 0.65.

## Ligands

2,2-Diphenylethylamine (**1f**) and phenylethylamine (**1h**) were obtained commercially and tested as the hydrochloride salts. 9-Aminomethyl-9,10-dihydroanthracene (**1a**),[1] 5-aminomethyl-10,11-dihydro-5(*H*)-dibenzo[*a,d*]-cycloheptadiene (**1e**),[2] and 9-aminomethylfluorene (**1g**)[17] were obtained as described. 4-(9,10-Dihydroanthracene-9-ylidene)-1-methylpiperidine fumarate (**2a**) was prepared by the condensation of the lithium anion of 9,10-dihydroanthracene with 1-methyl-4-piperidone to provide 4-hydroxy-4-[9-(9,10-dihydroanthracenyl)]-1-methylpiperidine (54%) followed by elimination via the halide.[18] The elimination led to the formation of two regioisomers, the 9-anthracenyl alkene and the 3,4-piperidinyl alkene. The fumarate salt was prepared of the mixture and fractional crystallization from 2-propanol provided the desired product in very low yield. 9-Aminomethyl-9(*H*)-thioxanthene oxalate (**1c**) was prepared by reduction (borane/THF) of 9(*H*)-thioxanthene-9-carboxamide[19] which was obtained from the carboxylic acid.[20] 9-Aminomethyl-9(*H*)-xanthene[21] (**1d**) was obtained by reduction (borane/THF) of 9(*H*)-xanthene-9-carboxylamide.[22] Although 5-aminomethyl-(5*H*)dibenzo[*a,d*]-cycloheptene hydrochloride[23,24] (**1b**) obtained by reduction of the nitrile had the reported melting point (mp 276–277 °C) and satisfactory elemental analysis, repeated preparations of **1b**, using a variety of reductive conditions, consistently produced samples containing spurious $^1$H NMR signals (t, δ 3.49; d, δ 4.15) amounting to approximately 10% of the material. GC/MS (CI) analysis also revealed an impurity (approximately 10% of total) having an apparent M + 1 signal of 233.28, approximately 12 amu larger that the desired amine. Given the low affinity of the substance, the presence of the unidentified impurity is not likely to have affected interpretation of the binding data.

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