Molecular Modeling Studies of Some Choline Acetyltransferase Inhibitors

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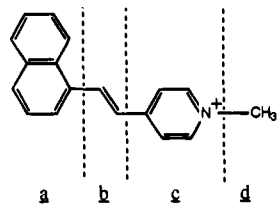
Choline acetyltransferase (ChAT) inhibitors related to *trans*-1-methyl-4-(1-naphthylvinyl)-pyridinium (NVP^+) have been assumed to depend upon a nearly or completely planar conformation for their enzyme–inhibitor interaction. In an effort to investigate the geometries and preferred conformations for these compounds, geometry optimizations using the semiempirical molecular orbital method AM1 and a modified version of the molecular mechanics method MM2(92) have been carried out. The results indicate that the active inhibitors are either planar or nearly coplanar, lying in relatively flat potential wells in the vicinity of the corresponding planar structures. When nonplanarity is favored, one ring is twisted out of the plane by approximately 30°. Where steric features significantly deter assumption of a nearly planar conformation, the analogs are inactive. The inactivity of analogs bearing tricyclic aryl groups appears to result from bulk-related hindrance to ChAT receptor binding rather than lack of coplanarity.

Compounds related to *trans*-1-methyl-4-(1-naphthylvinyl)pyridinium (**I**) have been reported to show a wide range in potency as inhibitors of choline acetyltransferase (EC 2.3.1.6).[1–6] Speculations as to possible interactions of these inhibitors with the enzyme at the molecular level included (1) charge-transfer involvement of the π-donor aryl structure **a** and the π-acceptor moiety **c** and (2) hydrophobic interactions.[2,3,7] An essentially coplanar conformation of the *trans* **a–b–c** region was believed to be important. Optimum features



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governing inhibitory potency include **a** as a bicyclic aryl group or as a phenyl moiety with π-electron donor enhancing substituents, **b** as a site of unsaturation with a double or triple bond, and **c** as a pyridinium or quinolinium system.[3] Substituent **d** is the least structurally specific.[3,6] Preference for planarity or near coplanarity of the conjugated *trans* **a–b–c** system was supported by the weak to inactive inhibitory properties of the *cis*-isomer and of analogs carrying a saturated bond in **b**, or a methyl substituent on atom C_4 of the exocyclic double bond (for numbering, see Table 1). The weak activity reported for the *cis*-isomer[8] probably resulted from traces of the corresponding *trans*-compound.

The object of this study was to determine the degree to which a planar conformation for these inhibitors is critical for their interaction with ChAT. There exists spectral evidence to support some deviation from the planar configuration of both the *trans*-naphthylvinylpyridines[9] and the structurally similar *trans*-styrylnaphthalene.[10] A relatively shallow energy profile has been reported for *trans*-stilbene with a minimum lying 0.7 kcal/mol lower than the planar conformation.[11] Furthermore, an analog of **I** in the crystalline state was found to show a 12° deviation from planarity of the aromatic–conjugated system.[12] In an attempt to correlate activity with the preferred conformations of the compounds in Figure 1, conformational searches using the semiempirical and molecular mechanics methodologies were utilized.

Methods and Results

To evaluate the geometries of this group of ChAT inhibitors, semiempirical molecular orbital calculations were carried out on Silicon Graphics 4D/310GTX workstations using the AM1 formalism,[13] as implemented by the Quantum Chemistry Program Exchange options (MOPAC 5.0[14]) within the molecular modeling program SYBYL 6.0.[15] The keywords "CHARGE", "PREC", and "GNORM=0.5" were included in all calculations. To search for conformational minima of these inhibitors, potential energy curves as a function of rotation around the $C_1–C_2–C_3=C_4$ (α) and $C_3=C_4–C_5–C_6$ (β) torsion angles were generated (for labeling see Table 1; note that C_3 in **2** is a nitrogen, while in **10** nitrogen replaces the C_4 atom). The torsional profiles were calculated by constraining the relevant torsion angles for rotation from 0° to 180° in 30° increments. While evaluating the rotational energy profiles for the dihedral angle $C_1–C_2–C_3=C_4$, the torsions about the $C_4–C_5$ bonds were not explicitly varied. Likewise, in investigating the $C_3=C_4–C_5–C_6$ torsional potentials, the calculations were carried out by constraining the $C_1–C_2–C_3=C_4$ torsion angles to their corresponding minimum values.

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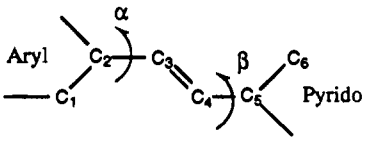
Table 1. Geometries for the ChAT Inhibitors



compd	AM1-α	AM1-β	kcal/mol[a]	MM2-α	MM2-β	kcal/mol[b]	I_{50}[c]
1	28.0	6.5	0.456	36.8	7.5	1.0447	0.4[3]
2	0.2	2.1	0.001	87.2	3.5	3.4544	1.5[4]
3	30.0	16.8	0.663	36.2	25.2	1.3660	0.4[3]
4	−11.0	−15.5	0.049	10.0	27.1	0.3172	5.0[2]
5	−5.6	−28.7	0.358	1.0	37.1	1.2338	3.5[2]
6	12.3	21.0	0.136	10.5	30.7	0.4830	19.0[3]
7	−0.3	−0.3	0.000	15.2	13.2	0.0167	13.5[2,3,5]
8	−1.1	−0.4	0.000	1.8	−1.5	0.0036	2.3[3]
9	23.1	5.1	0.134	32.3	11.6	0.4451	28.0[3]
10	25.4	78.4	1.159	37.5	86.4	3.0905	≫100[4]
11	21.5	5.4	0.215	39.9	7.4	1.1206	≫100[5]
12	40.3	37.6	3.187	46.1	145.8	5.6765	≫100[3]
13	30.2	6.8	0.675	146.2	2.3	5.3499	≫100[2,5]

[a] Energy difference from the planar structure utilizing the AM1 methodology. [b] Energy difference from the planar structure in kcal/mol utilizing the MM2(94) methodology. [c] I_{50} are reported as 10^{-6} M. Superscript numbers correspond to citations.

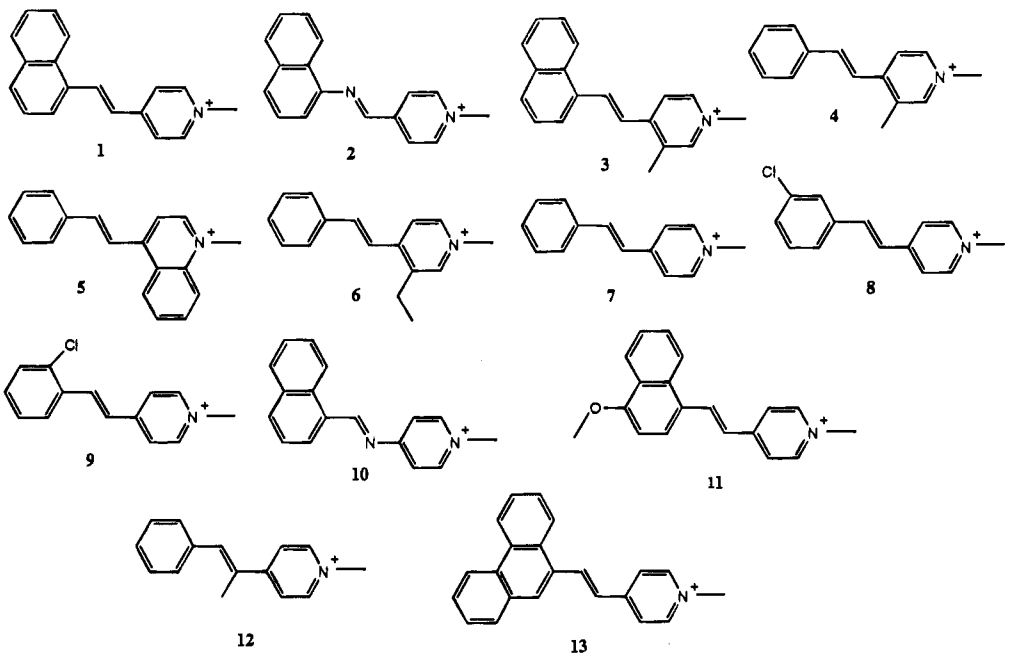


Figure 1. The nine (**1**–**9**) ChAT inhibitors and the four inactive analogs (**10**–**13**) investigated in this study.

No constraints were employed in subsequent calculations, where the low-energy geometries, computed as discussed above, were optimized with complete geometry refinement and the same Hamiltonian. In order to ensure that a minimum was located, vibrational frequency calculations utilizing the "FORCE" option were also undertaken. After a true minimum was determined, the appropriate torsions, α and β, were measured. Those values are listed in Table 1.

It has been noted that rotational barriers in conjugated systems, such as benzylideneaniline, stilbene, and azobenzene, are not well treated by MNDO.[16] In contrast, a recent investigation indicated that AM1 is far superior to MNDO in computing barriers to rotation about conjugated single bonds.[17] Furthermore, in a comparative examination of relative heats of formation calculated as a function of internal rotation in biphenyl, it was found that among the three semiempirical methods (AM1, MNDO, and PM3), only AM1 predicts a nonplanar ground-state geometry,[18] in accord with experimental studies.[19–21] Consequently, in order to elucidate conformational preferences of the compounds in Figure 1, the semiempirical method AM1 was employed. The results presented in Table 1 indicate that the low-energy conformations are either planar or nearly coplanar, with one ring twisted out of the plane by approximately 30°. It should be emphasized that the energy differences between the nonplanar geometries and the planar ones are rather low, usually favoring the nonplanar conformers.

In the two isomers in which the exocyclic double bond is replaced by an imine moiety, as in aryl-N=CH-pyrido (**2**) and aryl-CH=N-pyrido (**10**), the former has a favorable planar conformation, contrary to its inactive counterpart (Figures 2 and 3). It is uncertain whether the marked difference in enzyme inhibitory activity of isomers **2** and **10** results from conformational preferences alone or whether electronic effects also play a role. The findings for **10**, however, are comparable to the electron-diffraction studies of gaseous *N*-benzylidene-

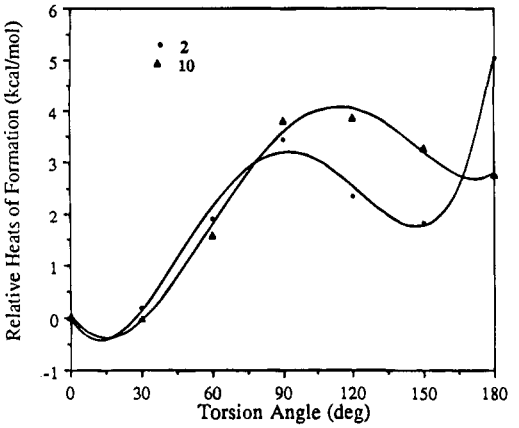


Figure 2. Relative heats of formation as a function of rotation around the bond attached to the naphthalene moiety in **2** and **10**.

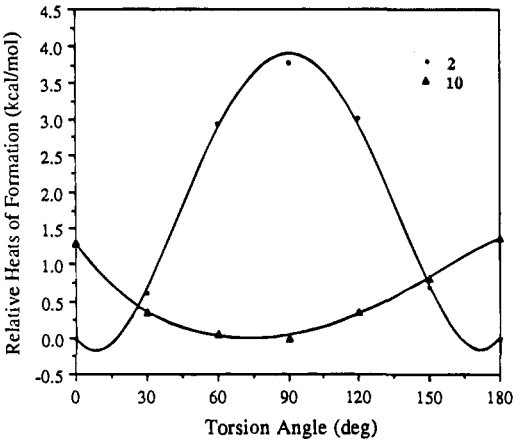


Figure 3. Relative heats of formation as a function of rotation around the bond attached to the pyridinium species in **2** and **10**.

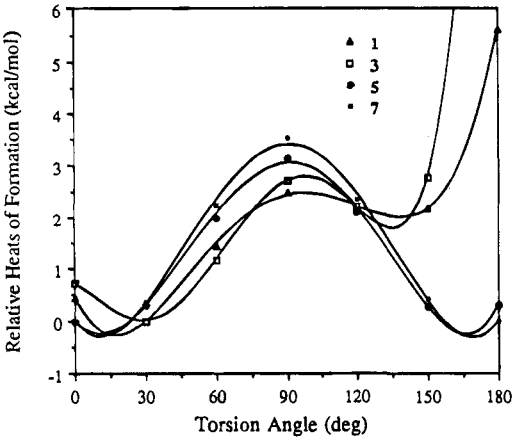


Figure 4. AM1 calculated potential for rotation about the C_{sp^2}—C_{sp^2} single bond attached to the aryl group in **1**, **3**, **5**, and **7**.

aniline (NBA), which indicated that in the minimum-energy conformation one of the phenyl groups is coplanar with the C=N bond while the other phenyl moiety bonded to the nitrogen atom is rotated approximately 52° out of this plane.[22]

Profiles of relative heats of formation as a function of the α and β torsions are illustrated in Figures 4 and 5 for compounds **1**, **3**, **5**, and **7**. Not surprisingly, the unsubstituted monocyclic rings in **7** are in equivalent relative positions at the 0° and 180° conformers as

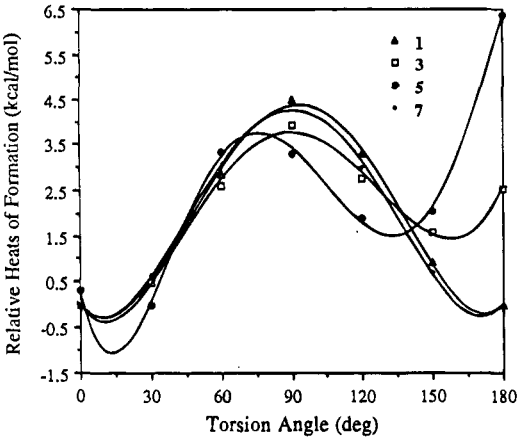


Figure 5. AM1 calculated potential for rotation about the C_{sp^2}—C_{sp^2} single bond attached to the pyridinium moiety in **1**, **3**, **5**, and **7**.

illustrated by their symmetric curves. With 1 and **5**, the curves are symmetric where the exocyclic bond is linked to a monocyclic ring; for the bicyclic ring linkages the asymmetry of the curves shows minima corresponding to conformers that deviate slightly from the relevant planar configurations. In **3**, both rings appear to have a preferred orientation relative to the other such that near-coplanarity would be favored with a *transoid* relationship between the 3-methyl substituent on the pyridine ring and the *peri*-naphthyl position.

A molecular mechanics investigation was subsequently carried out using a modified version of the MM2(92) force field for comparison with the semiempirical methodology.[23] Of course, to calculate the preferred geometries of the ChAT inhibitors using the MM2(92) force field, the charged nitrogen atom in the pyridine ring first needed to be parametrized. Therefore, high level *ab initio* calculations were performed on pyridinium and related compounds to obtain accurate rotational barriers and structures. The 6-31G** basis set was used at the Hartree–Fock level of theory for these calculations. The results were utilized to construct an MM2 force field for the aromatic positively charged nitrogen-containing species. The resulting molecular mechanics geometries and energetics compare well with those obtained through the *ab initio* calculations. These results are reported elsewhere.[23]

The dihedral driver option was used to obtain a complete energy profile about the two rotatable bonds, defined as α and β. The bonds were rotated from 0° to 180° in 15° increments. The lowest energy conformation from this driver was determined by examining the output files. The lowest energy conformation was extracted from the output, and in order to find the global minimum, a molecular mechanics calculation was subsequently carried out with no constraints. The results are shown in Table 1. In most cases, excellent agreement between the methods was observed. In cases in which a nitrogen was substituted in the **b** region on the ChAT inhibitors, disagreement between the AM1 and the MM2(92) calculations was noted. This difference could be due to the treatment of the nonaromatic nitrogen in MM2(92). As mentioned previously, electron-diffraction results for NBA, a structure analogous to compound **2**, have shown that the α torsion, as defined in Table 1, is twisted by 52°.[22] Calculations on NBA using the MM2(92) force field resulted in a global

minimum with an α torsion of approximately 88°. A twisted structure with an α torsion of approximately 48° was found to be 0.70 kcal/mol higher in energy than the global minimum. Thus, because MM2(92) does not agree with the experimental results for NBA, the minimum energy conformation of compound **2** is probably not being calculated correctly by MM2(92).

In conclusion, it would appear that an essential geometry for ChAT inhibitory activity among these compounds is one in which the **a**–**b**–**c** components of the molecule can assume a planar or nearly coplanar conformation with minimal energy expenditure. Where the aryl group *a* is tricyclic, the lack of inhibitory properties appears to result from a bulk-related deterrent to interacting with the enzyme receptor rather than from lack of coplanarity. With compound **11**, instability[5] as well as electronic or bulk characteristics may be unfavorable for activity. It is worth mentioning, however, that these calculations were carried out on molecules *in vacuo*, with two implicit assumptions. First, we assumed that the calculated conformations are representative of conformers in solution. The second assumption was that the conformation of the inhibitor bound to the receptor belongs to a set of low-energy conformations.

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