

# Computer-Based Conformational Analysis of Acetylcholine and Muscarine Combined with an Overview of the Receptor–Ligand Interaction

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Models are simple representations of real objects and physical phenomena. Modeling comprises building and manipulation of models with the objective of acquiring a more thorough comprehension of the represented entities. Molecular modeling involves the construction, manipulation, and representation of realistic molecular structures and the calculation of physicochemical properties. Computational modeling takes advantage of theoretical chemistry as a mathematical instrument and computational graphics as a valuable tool in handling models. Nowadays, molecular modeling systems are equipped with powerful tools for the construction, visualization, analysis, and storage of molecular models to facilitate the interpretation of the relationships between structure and biological activity.

Molecular modeling can be applied to the development of drugs using an indirect or direct approach. The former is useful when the receptor structure is not available and it aims to obtain electronic and steric parameters to study structure–biological activity relationships. The direct approach is used when the three-dimensional structure of the target molecule is known and it focuses on understanding interactions of the ligand–receptor complex. Both approaches aim to optimize the docking of the molecule with the receptor. Molecular modeling and its graphical representations make possible the exploration of three-dimensional aspects of molecular recognition, the formulation of hypotheses concerning drug development, and proposals of new ligands to be synthesized in a more advanced stage.

The goals of computer-assisted drug design are to calculate properties for individual molecules (including a complete description of the geometry of stable conformations and their energies, charges, atomic interactions, electrostatic potentials, orbitals, enthalpies of formation,  $pK_a$  values, partition coefficients, and dipole moments); calculate the properties and energies of associated molecules (to describe the interactions between molecules, for example, solvation and drug–receptor interaction); exhibit, superimpose, and compare geometric and electronic molecular models; find and exhibit quantitative and qualitative relationships between molecular representations and biological activity; manipulate and manage biological and chemical databases.

Molecular modeling applied to drug development has pharmacophore discovery as one of its objectives. A pharmacophore can be defined as the minimum collection of atoms adequately positioned in space to produce a biological response. This definition has been refined to include three-dimensional and topographic restrictions (1, 2).

The knowledge about specific stereochemistry of enzyme active sites and some receptor sites justifies the conformational study of drugs, which can interact with these sites. The

drug–receptor interaction produces a conformational change that is ultimately observed as the pharmacological response. A receptor site can only bind to a single conformation from many possible conformations. This pharmacophoric conformation has all the atoms of the drug correctly positioned in space to fit the receptor binding site. Molecules adopting a conformation capable of binding to the receptor can act as either agonists or as antagonists. The former mimic the action of receptor's normal substrate, while the latter bind to the receptor without being able to produce a biological response.

Drug development is among the topics studied in the pharmaceutical chemistry course for pharmacy undergraduates of Universidade Federal de Minas Gerais, Brazil. Computational advances in the last 20 years together with the availability of user-friendly software have promoted the development of simple computer-assisted molecular-modeling experiments for bioactive molecules. This article describes the conformational analyses of acetylcholine and muscarine using the systematic search method and the superimposition of their preferred conformations using the PCMODEL 7.0 software (3). As a supplemental exercise, we also have students match these conformations with the proposed structure of the muscarinic acetylcholine receptor.

## Experimental Procedure

Acetylcholine (ACh) is a neurotransmitter, especially important in the parasympathetic nervous system. It is used as a prototype in the development of cholinergic drugs, that is, those that mimic ACh in their mode of action. Two types of effects are produced by acetylcholine: nicotinic and muscarinic. The nicotinic effects are analogous to those caused by nicotine, that is, on ganglia and the motor end plate: stimulus and increase in tone of skeletal muscles. The muscarinic effects are similar to those produced by muscarine and pilocarpine, that is, on the postganglionic parasympathetic receptors: cardiac inhibition, peripheral vasodilation, pupil contraction, increase of salivation and glandular secretion, and contractions and peristaltic action of gastrointestinal and urinary tracts. Therefore, it is assumed that for acetylcholine there are two types of receptors: nicotinic and muscarinic (4).

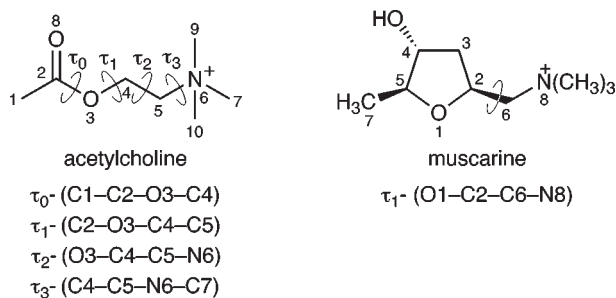
To start students use the PCMODEL tools to draw acetylcholine and optimize the geometry. The model on the screen is visualized using different view modes (renderings). Acetylcholine is a flexible molecule with four torsional angles ( $\tau_0$ ,  $\tau_1$ ,  $\tau_2$ , and  $\tau_3$ ) that can be rotated (Scheme 1). Among these angles,  $\tau_1$  and  $\tau_2$  determine the major conformational changes in the molecule. Nonetheless it is  $\tau_2$  (O3–C4–C5–N6) that determines the relative position between the two

atoms (O3 and N6) that bind to the muscarinic cholinergic receptor. As such, we have students set all  $\tau$  angles to  $180^\circ$  initially, then vary only  $\tau_2$ .

In the procedure the torsional angle  $\tau_2$  is rotated by  $10^\circ$  increments using the "dihedral driver" option in the program. In the dihedral driver one to four torsional angles can be marked and rotated by steps with determined values. For each rotation all marked atoms are kept rigid and all other degrees of freedom of the molecule are totally flexible to permit the geometry optimization. The geometries obtained are stored in a multiple structure file and the graph of potential energy surface (PES) with the data output (torsional angle and steric energy) is plotted. Based on the analysis of the PES it is possible to identify the geometries according to a minimum of energy (each geometry corresponds to a  $\tau_2$  angle). Minimum energy geometries are located in the multiple structure file produced by dihedral driver. These are used as the starting point for complete minimization and acquisition of acetylcholine conformers. Analysis of these conformers (measurements of distance and dihedral angle) permits comparison with literature data.

The same procedure is repeated for muscarine, a natural alkaloid with muscarinic cholinergic properties. The conformational analysis of muscarine is performed rotating the torsional angle  $\tau_1$  (O1–C2–C6–N8) (Scheme I). The lower-energy conformations of acetylcholine and muscarine are superimposed to demonstrate the steric similarity between these molecules.

The students perform individually the proposed calculations using a personal computer. The instructor demonstrates each stage by using a multimedia projector connected to a computer.



Scheme I. Acetylcholine with four torsional angles ( $\tau_0$ ,  $\tau_1$ ,  $\tau_2$ , and  $\tau_3$ ) that can be rotated and muscarine with one torsional angle.

**Table 1. Acetylcholine Minimum Energy Conformations**

Conformation	$\tau_2$ angle/deg	Steric Energy/(kJ mol <sup>-1</sup> )
A	180	56.0
B	-70	54.8
C	70	56.5

**Table 2. Muscarine Minimum Energy Conformations**

Conformation	$\tau_1$ angle/deg	Steric Energy/(kJ mol <sup>-1</sup> )
A	180	85.8
B	-40	89.1
C	60	74.9

## Exercises

1. Draw the structure of acetylcholine using PCMODEL drawing tools.
2. Perform the conformational analysis of acetylcholine using the systematic search method by rotating the  $\tau_2$  angle (O3–C4–C5–N6).
3. Classify the lower-energy conformations of acetylcholine. Determine the steric energy and  $\tau_2$  values.
4. Write down the Newman projections for acetylcholine lower-energy conformations.
5. Draw the structure of muscarine using PCMODEL drawing tools.
6. Perform the conformational analysis of muscarine using the systematic search method by rotating the  $\tau_1$  angle (O1–C2–C6–N8).
7. Classify the lower-energy conformations of muscarine. Determine the steric energy and  $\tau_1$  values.
8. Write down Newman projections for muscarine lower-energy conformations.
9. Superimpose the lowest-energy conformation of acetylcholine and muscarine.

## Results

Acetylcholine and muscarine are both found in three minimum-energy conformations. Common values of torsional angles and steric energy of acetylcholine and muscarine are represented in Tables 1 and 2, respectively.

## Conclusion

The proposed computational procedure represents an opportunity for the pharmaceutical chemistry students to have contact with molecular modeling software. The students draw biologically-active molecules, analyze their conformations, compare the conformations of different molecules, match the preferred conformations of the Ach with the proposed structure of the acetylcholine receptor, and compare the results with literature data.

## Supplemental Material

Handouts for students, including all technical details, and notes for the instructor are available in this issue of *JCE Online*.

## Literature Cited

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