

The Influence of Conformational Isomerism on Drug Action and Design

Marc W. Harrold¹

School of Pharmacy, Duquesne University, Pittsburgh PA 15282

COURSE INFORMATION

The following text is a summary of a two-hour lecture sequence presented in the course, Advanced Medicinal Chemistry I. This course is required for all MS and PhD students in Duquesne University's Medicinal Chemistry Graduate Program. It is also available as an elective for undergraduate students in their last semester of didactic work. The course emphasizes the basic chemical principles which underlie drug design. The lectures on conformational isomerism are constructed to emphasize several principles: (i) the identification and significance of conformational isomers; (ii) the differences between preferred and active conformations; and (iii) the advantages and disadvantages of conformational restriction. While the material included in this paper is presented in the traditional lecture style, computer based assignments using the molecular modeling software program ALCHEMY² have been used to reinforce the concepts. Reports detailing the use of ALCHEMY to teach stereochemical principles have previously appeared in *the Journal*(1,2).

INTRODUCTION

Conformational isomers (or conformers) are nonsuperimposable orientations of a molecule which result from the free rotation of atoms about single bonds. In order for a molecule to possess conformational isomers, it must possess at least one single bond that is not part of a ring system. The reason for this restriction is that it is impossible to freely rotate a single bond within a ring system without breaking the ring in the process. Additionally, neither of the atoms which are joined by this single bond can contain three identical substituents (*e.g.*, three hydrogen atoms, three methyl groups, etc.) or else rotation about the bond will be irrelevant. Since almost every drug molecule meets these criteria, conformational isomers can exist for almost every drug. Both the number of rotatable single bonds and their position determine whether a compound is classified as conformationally flexible or conformationally rigid. A rotatable bond located in the middle of a molecule allows much more flexibility than one located at either end.

The neurotransmitter acetylcholine, shown in Figure 1, has often been used to demonstrate the concept of conformational isomerism(3,4). The ethane bridge (*i.e.*, atoms 4 and 5) between the ester oxygen and the quaternary nitrogen is a freely rotatable system and gives rise to a variety of

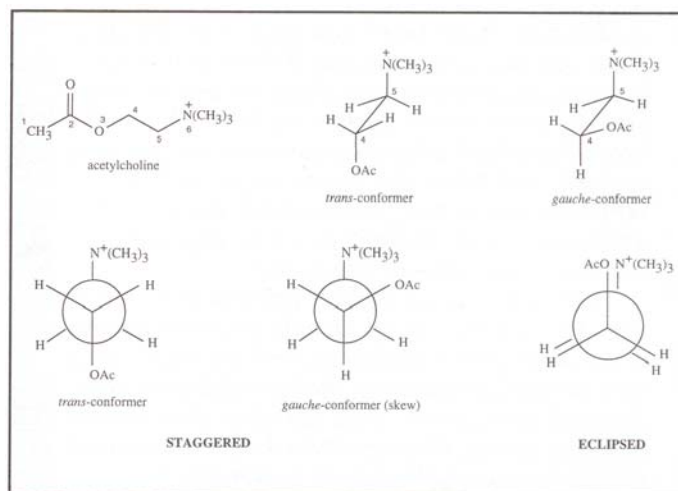


Fig. 1. Conformations of acetylcholine.

different conformations. When the acetyl group and the quaternary nitrogen are situated 180 degrees apart, the molecule is said to be in the *trans* or anti conformation. Both a sawhorse representation and a Newman projection of the *trans* conformation are illustrated in Figure 1. Rotation of the *trans* Newman projection 60 degrees in the counter-clockwise direction gives rise to the *gauche* or skew conformation. Continued rotation by another 30 degrees in the same direction provides the fully eclipsed conformation. These rotations alter the spatial arrangement of the atoms; however, unlike configurational isomers, no bonds need to be broken and reformed in order to convert one conformer to another. Therefore, conformational isomers are not distinct molecules, but rather different orientations of the same molecule.

Obviously, there are many more conformations possible for acetylcholine than the three shown in Figure 1. In fact, rotation about the ethane bond will potentially yield thousands of different conformations—albeit many will be almost identical—and that is just considering the rotation of one single bond! Rotation of the single bond between atoms 2 and 3 or the single bond between atoms 3 and 4 will also alter the conformation. In contrast, note that rotation of the single bond connecting atoms 1 (methyl group) and 2 (carbonyl carbon) is meaningless due to the fact that the methyl group contains three identical substituents. Rotation here

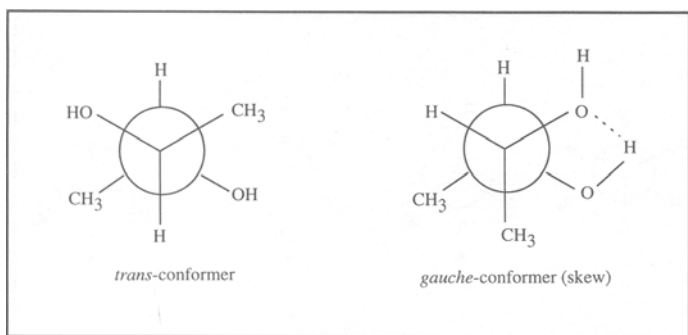


Fig. 2. Conformations of 2,3-dihydroxybutane. The gauche conformer is energetically favored due to the intramolecular hydrogen bonding.

will not change either the shape or orientation of the molecule. The same holds true for the single bond connecting atoms 5 and 6.

PREFERRED CONFORMATION

From the above discussion, it should be easy to see that rotation of carbon-carbon single bonds within a drug molecule will theoretically produce an infinite number of conformers. These conformers however are not all of equal importance. In 1936, Kemp and Pitzer(5) observed that rotation about carbon-carbon single bonds is not really "free", but is subject to an energy barrier. This energy barrier is due to both steric repulsions and electronic interactions among the atoms or groups on adjacent carbon atoms. Thus, those conformations which minimize any repulsive interactions and maximize all attractive interactions are more energetically favorable than other conformations and are said to be preferred.

From a strictly steric point of view, *gauche* or skew conformations are less energetically favorable than *trans* conformations. Therefore as a general rule for acyclic compounds, conformations in which the larger groups are staggered and separated from one another by as great a distance as possible are more energetically favorable than those in which a significant number of skew interactions occur. Exceptions to this general rule occur when forces of electronic attraction more than compensate for any steric repulsion(4). An example of this is shown in Figure 2. The skew form of 2,3-dihydroxybutane is more energetically favorable than the *trans* form because of the intramolecular hydrogen bonding which is present in the skew conformation. As an additional example, consider acetylcholine. Looking only at steric factors one would most likely predict that the *trans* conformer would be preferred. A variety of spectrographic studies have however shown that it is actually the *gauche* conformer which is preferred. The intramolecular attractive force between the quaternary nitrogen and the ester carbonyl overcomes steric barriers and stabilizes the *gauche* conformer(6). The above examples illustrate the importance of examining both steric and electronic factors before making any predictions on preferred conformations.

The discussion so far has centered on acyclic systems; however, the conformation of ring systems is equally as important. Cyclohexane and other six-membered, nonaromatic, heterocyclic ring systems (e.g., pyran, piperidine) are present in a large number of natural and synthetic molecules. The following section focuses on the factors which influence the conformation of the cyclohexane ring. Note that the generalizations which are contained in the

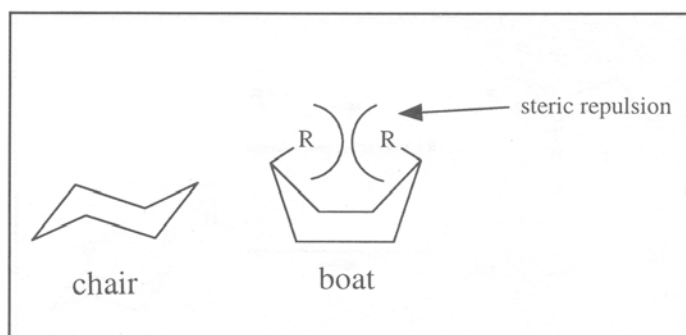


Fig. 3. Chair and boat conformations of cyclohexane.

discussion below are also applicable to six-membered heterocyclic systems.

Cyclohexane can exist in several conformations; however only two, the boat form and the chair form (Figure 3), maintain the proper tetrahedral bond angles. Of these two conformations, the chair form is of much lower energy than the boat form. The reason for this is two-fold. First, all of the bonds are staggered in the chair form, while two sets of eclipsed interactions are present in the boat form. Second, a van der Waals repulsive interaction exists between the two atoms which are directed towards each other in the boat conformation. This interaction is not present in the chair form. It may be helpful to build models of the boat and chair forms in order to visualize these differences. Thus under most circumstances, cyclohexane will adopt a chair conformation. Furthermore, those molecules which have multiple cyclohexane rings (e.g., steroids) will prefer to adopt a conformation that allows the largest number of chair forms(4).

When defining the conformation of a molecule which contains a cyclohexane ring, it is insufficient to simply state that the ring will exist in a chair conformation. The reason for this is that at room temperature, cyclohexane is capable of undergoing chair-chair inversion or "flipping" (Figure 4). In this process, all hydrogens which were originally axial (e.g., H₁) become equatorial, and all hydrogens which were originally equatorial (e.g., H₂) become axial. Thus, there are two possible chair conformations. While this fact is of only theoretical interest when dealing with cyclohexane, it becomes extremely important when considering substituted cyclohexane rings. In this case, inversion will not simply interchange hydrogen atoms but will instead switch axial substituents to equatorial and vice-versa. As an example, consider Figure 4. Chair-chair inversion of *cis*-1,3-dimethylcyclohexane produces two conformations, one in

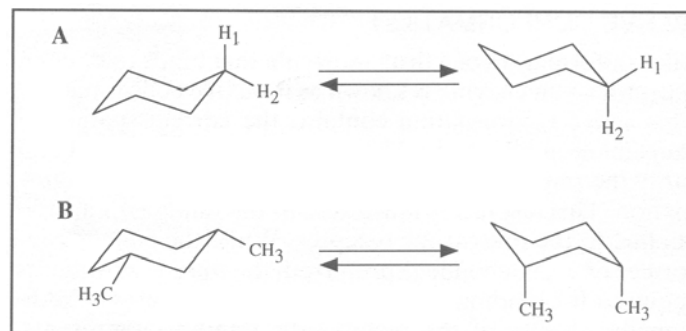


Fig. 4. Chair-chair inversion of cyclohexane, A, and *cis*-1,3-dimethylcyclohexane, B.

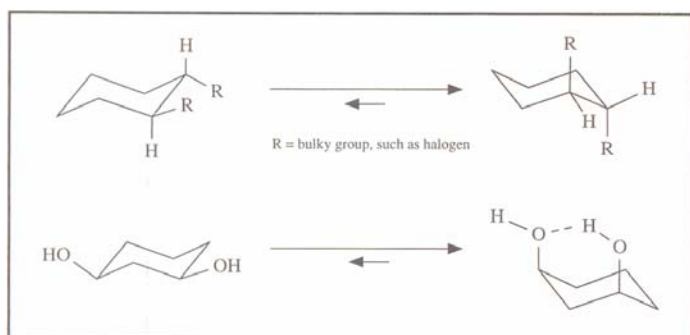


Fig. 5. Examples of cyclohexane rings where diaxial substituents are favored over diequatorial substituents.

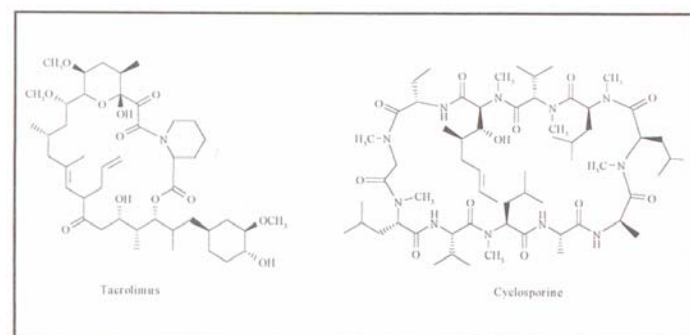


Fig. 6. Immunosuppressive agents tacrolimus and cyclosporine.

which both methyl groups are axial and one in which both methyl groups are equatorial.

Rational drug design of molecules which contain substituted cyclohexane rings is therefore dependent, at least in part, on the ability to predict which of the two chair conformations is the most energetically favorable. Fortunately a few generalizations can be made concerning the conformation of substituted cyclohexanes. Due to the presence of 1,3-diaxial interactions, equatorial substituents are more energetically favored than axial substituents. Thus, the chair conformation with the most equatorial substituents is favored. Furthermore, if one of the substituents is extremely bulky (*i.e.*, *t*-butyl group), the conformation which allows this group to be equatorial is preferred. There are also special circumstances which favor axial substituents over equatorial substituents. Occasionally, bulky groups on adjacent carbon atoms will repel one another and will prefer to adopt a diaxial conformation rather than a diequatorial conformation. Additionally, chair conformations with axial substituents are favored if attractive forces between the substituents are present. An example of this is shown in Figure 5 (4).

ACTIVE CONFORMATION

The conformation of a drug molecule that binds to either a receptor or an enzyme is known as its active conformation. This active conformation contains the correct spatial arrangement of all essential binding groups but is not necessarily the same as the most energetically preferred conformation. This last fact is illustrated in the binding of acetylcholine to the muscarinic receptor. While the *gauche* conformer of acetylcholine is preferred, the *trans* conformer is required for binding to the muscarinic receptor(4). Additionally, studies of the macrocyclic immunosuppressants, tacrolimus and cyclosporine (Figure 6), have shown that the active conformation of a compound may be radically different

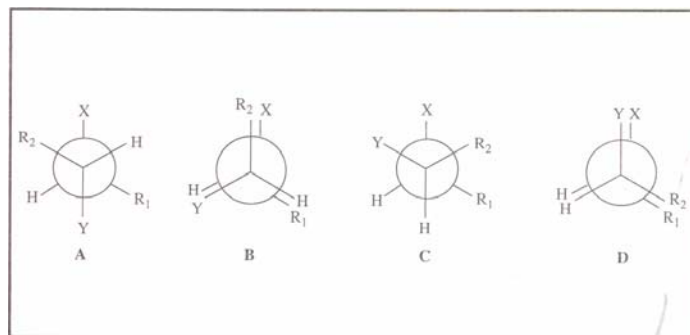


Fig. 7. Conformations of a hypothetical drug molecule.

from the preferred conformation(7). Tacrolimus and cyclosporine bind to peptidyl-prolyl-*cis,trans*-isomerases and interfere with T cell signaling. Binding of tacrolimus to its binding protein requires a substantial conformational change. Relative to its preferred solution conformation, tacrolimus must undergo *cis-trans* isomerization of the amide bond, concomitant repositioning of the homoproline ring, and movement of the pyranose ring in order to bind to its receptor. For cyclosporine, the differences between the preferred and active conformations are equally as striking. The unbound structure of cyclosporine is essentially turned inside out to achieve the active, bound conformation. Four intramolecular hydrogen bonds are lost, a peptide bond isomerizes from *cis* to *trans*, and two sets of four side chains switch sides of the ring. These examples show that flexible drug molecules are capable of undergoing substantial geometrical distortion in order to achieve a suitable binding conformation.

The energy required to change a drug molecule from its preferred solution conformation to its required active conformation can be obtained from the energy released when the drug molecule binds to its receptor or target enzyme(8). In some cases the energy barriers to rotation are too prohibitive, and the drug molecule is inactive (*i.e.*, will not bind). In many instances however the energy of binding is sufficient to overcome the barrier to the formation of an unstable conformer. These ideas are consistent with both the induced fit theory and the zipper mechanism of drug-receptor binding. In the induced fit theory, Koshland(9) states that binding of a molecule to its receptor is a dynamic process which results in a mutual plastic molding of both the molecule and the receptor. In the zipper mechanism, Burgen *et al.* (10) propose that the binding of flexible molecules to receptors occurs sequentially. In each discrete and successive step, small energy barriers are overcome as the molecule and receptor adjust their conformations to allow binding.

In summary, the use of preferred conformations in making SAR determinations is very difficult since in many instances knowledge of the conformational changes which occur during a drug-receptor interaction are unknown. Knowledge of the preferred solution conformation is still valuable and can be used in some instances to explain activity profiles. It can also be used as a good starting point for the design of new analogs; however, other conformations should not be excluded until they have been shown to be inactive or less active.

BENEFITS OF CONFORMATIONAL RESTRICTION

Restriction of conformational flexibility is a commonly used technique to "lock" a molecule into a desired conformation. Before proceeding with a discussion of how molecules can

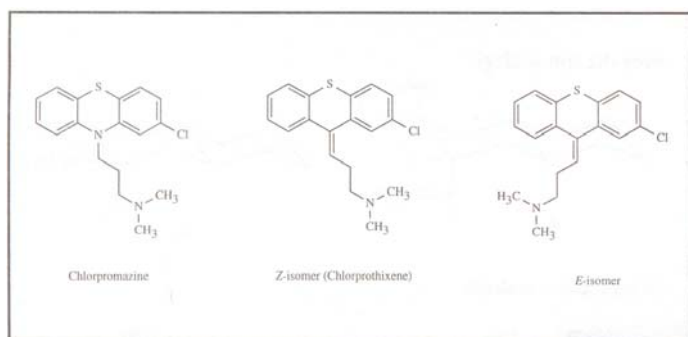


Fig. 8. Antipsychotic agents chlorpromazine and chlorprothixene.

be conformationally restricted, it is important to address the question of why it is beneficial.

Consider the following hypothetical example. Assume a drug contains two functional groups, X and Y, which are located on adjacent carbon atoms and which are essential for biological activity. Further assume that the drug is capable of binding to three different receptor types or subtypes. Given this scenario, it would be quite beneficial to determine if the drug is binding to the three receptors in similar or distinct conformations. Conformational restriction could help to provide this information. Additionally, there are many conformations of X and Y relative to one another. Of these, consider the four (A-D) shown in Figure 7. As an idealized case, suppose that previous research has determined that the drug acts on its three receptors in three different conformations to produce two beneficial effects (antihypertensive and hypoglycemic effects) and one very prevalent side effect (dry mouth). Also suppose the following is true: when the molecule is in conformation A, it binds to receptor 1 and produces an antihypertensive effect; when the molecule is in conformation B, it binds to receptor 2 and produces a hypoglycemic effect; and when the molecule is in conformation C, it binds to receptor 3 and produces the side effect. Since the active conformations are known, it should be possible to use conformational restriction to develop specific agonists and/or antagonists for these receptors. By locking the molecule in either conformation A or B, it should be possible to elicit only the desired beneficial effect without the side effect or any other unnecessary effects. This knowledge could then be used to make affinity labels specific for only one of these receptors. Carrying this idealistic case one step further, suppose the drug has a high affinity for hepatic inactivating enzymes when in conformer D. Rigid analogs of conformers A or B would then be expected to have an increased duration of action over the parent compound in addition to the above stated advantages. Even though this is an idealized case, it is not that far-fetched. Small, conformationally flexible neurotransmitters such as acetylcholine, norepinephrine and histamine are known to bind in different conformations to different receptor subtypes to produce different effects. The use of conformational restriction to produce selective acetylcholine analogs is discussed below.

In summary, conformational restriction can be used to make specific agonists or antagonists, to eliminate toxic or unnecessary effects, and to increase the duration of action. In addition, it can be used to improve the understanding of specific drug-receptor interactions. In cases where a drug binds to only one receptor, there are still some advantages to conformationally restricted analogs. They can be used to

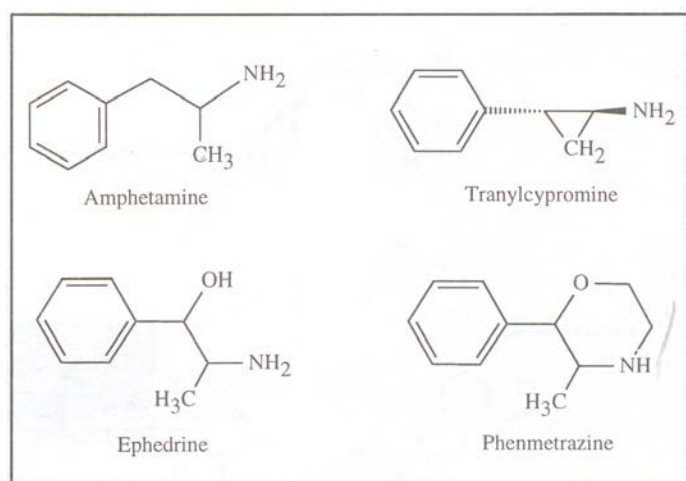


Fig. 9. Examples of ring closed analogs

help map the receptor site, and they may provide more potent compounds. It is important to note that even though a flexible drug can assume an unfavorable conformation, this requires energy. A conformationally rigid analog with all the necessary groups in the proper orientation would not require this energy and should have a higher affinity for the receptor.

STRATEGIES TO ACHIEVE CONFORMATIONAL RESTRICTION

Conformational restriction of acyclic groups can be accomplished either by introducing double bonds or by incorporating the acyclic groups into a more rigid framework. The use of double bonds to achieve conformational restriction has successfully been demonstrated with phenothiazine antipsychotics. Chlorpromazine, shown in Figure 8, produces antipsychotic effects by blocking dopamine receptors. The active conformation of chlorpromazine is that in which the side chain nitrogen is located on the same side as the chlorine containing aromatic ring(11). However, the conformational flexibility of the side chain allows the molecule to adopt a variety of inactive conformations. Introduction of a double bond gives rise to the closely related thioxanthene class of antipsychotics. The double bond results in the formation of *Z* and *E* stereoisomers and conformationally restricts rotation of the side chain. The *Z*-isomer (chlorprothixene), in which the side chain lies on the same side as the chlorine containing aromatic ring, is less likely than chlorpromazine to assume an inactive conformation and thus possesses a greater potency. In contrast, the *E*-isomer, in which the side chain is improperly restricted, is considerably less potent.

While double bonds can provide limited conformational restriction, the introduction of a rigid framework can often impart much more restriction on a molecule. The framework is usually a ring system of some type, and the process of converting an acyclic system to a cyclical one is sometimes referred to as ring closure. In addition to conformationally restricting the molecule, ring closure may lead to changes in the activity profile of a compound. This is illustrated by the two pairs of compounds shown in Figure 9. Amphetamine is a potent CNS stimulant while tranylcypromine is a potent monoamine oxidase (MAO) inhibitor. In fact, tranylcypromine is 5,000 times more potent than amphetamine in inhibiting MAO(12).

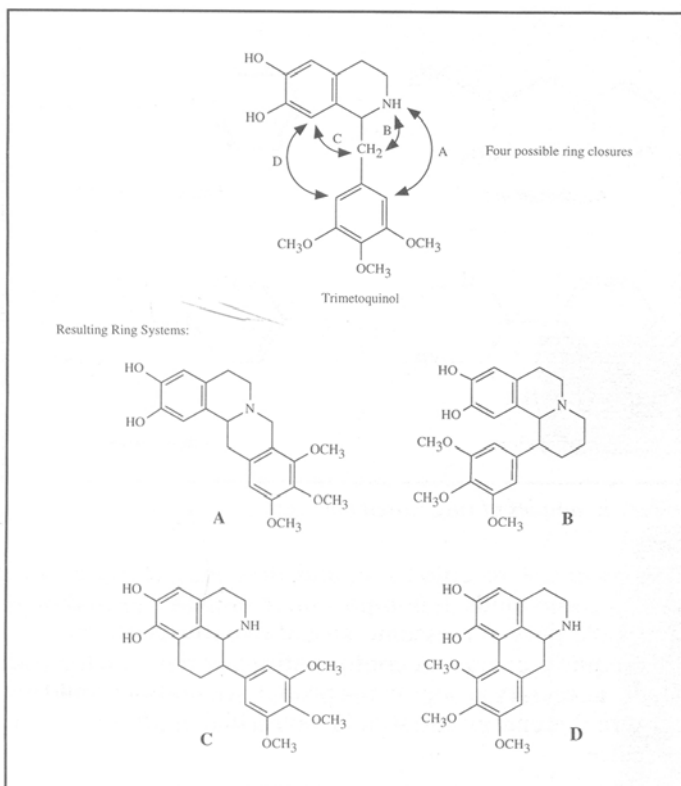


Fig. 10. Possible ring closed analogs of trimetoquinol.

Phenmetrazine is a cyclic analog of ephedrine. While ephedrine, a conformationally flexible molecule, is an indirect acting adrenergic agonist with CNS stimulating activity, phenmetrazine, a conformationally restricted molecule, retains the anorexic activity of ephedrine without pronounced CNS stimulation(13).

Two important considerations which need to be explored in designing ring closed analogs are the direction of closure and the extent of conformational restriction imposed by the closure. The direction in which the ring is closed may give rise to *cis* and *trans* isomers, and the extent of restriction may exclude potential active conformations. As an example, consider the tetrahydroisoquinoline trimetoquinol (Figure 10). This compound is a β -adrenergic agonist and a platelet antiaggregatory agent(14). It also has sufficient flexibility to allow the trimethoxy ring to adopt a variety of conformations relative to the tetrahydroisoquinoline ring. Conformational restriction of this compound via ring closure can occur in four different ways as shown in Figure 10. Two of these, B and D, place the trimethoxy ring system under the catechol ring, while the other two, A and C, place it away from the catechol ring. Additionally, note that analogs A and D are extremely rigid compounds, while analogs B and C allow the trimethoxy ring to assume a conformation perpendicular to the isoquinoline ring while

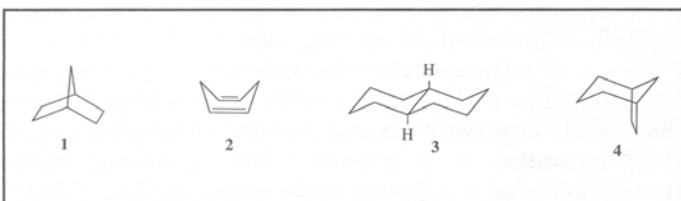


Fig. 11. Ring structures which can either lock a cyclohexane ring into a boat conformation, 1 and 2, or prevent chair-chair inversion, 3 and 4.

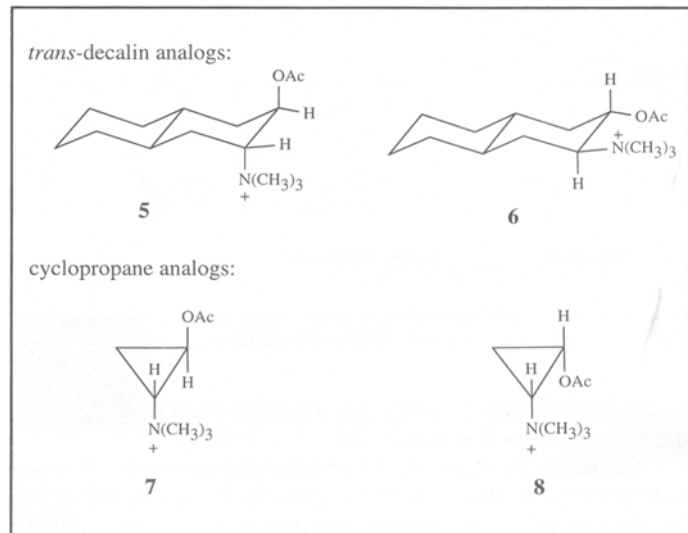


Fig. 12. Conformationally restricted analogs of acetylcholine. Compounds 5 and 7 approximate the *trans* conformer, while compounds 6 and 8 approximate the *gauche* conformer.

analog A and D do not.

Cyclohexane rings can also be constrained to lock the molecule into the unfavorable boat conformation or to prevent ring inversion (Figure 11). The introduction of a methylene group, 1, as in norborane, or a pair of double bonds, 2, will lock the cyclohexane ring into a boat conformation. Chair-chair inversion can be prevented either by expanding the cyclohexane ring to a trans-decalin, 3, system or by adding an ethylene bridge, 4.

EXAMPLES OF CONFORMATIONAL RESTRICTION

A tremendous amount of effort has been spent in the elucidation of the active conformations of acetylcholine. Acetylcholine (see Figure 1) is a flexible molecule that acts at two receptor subtypes, the muscarinic receptor and the nicotinic receptor. It is also hydrolytically inactivated by the enzyme acetylcholinesterase. Studies involving conformationally restricted analogs of acetylcholine have suggested that the *trans* conformer is required by both the muscarinic receptor and acetylcholinesterase, while the *gauche* conformer is required by the nicotinic receptor. The *trans*-decalin analogs, 5 and 6, shown in Figure 12 lock the *trans* and *gauche* conformers of acetylcholine (compare with Figure 1). Unfortunately, neither of these analogs show any appreciable activity due to the addition of so many atoms. In this instance, the *trans*-decalin systems causes substantial changes in the physical and chemical characteristics of the compound. This in turn drastically reduced any nicotinic or muscarinic activity(4).

A more successful attempt at designing rigid acetylcholine analogs was reported by Chiou and coworkers(15). These investigators added a single methylene group and prepared the cyclopropyl analogs shown in Figure 12. The (+)-*trans* isomer 7 was equiactive with acetylcholine at the muscarinic receptor, but was two orders of magnitude less active at the nicotinic receptor. It was also hydrolyzed by acetylcholinesterase at a rate equal to that of acetylcholine. The *cis* isomer 8 was considerably less active at both receptor subtypes; however, it was more active at the nicotinic receptor. The relatively poor results obtained with the *cis* isomer may in part be due to the geometry of the molecule.

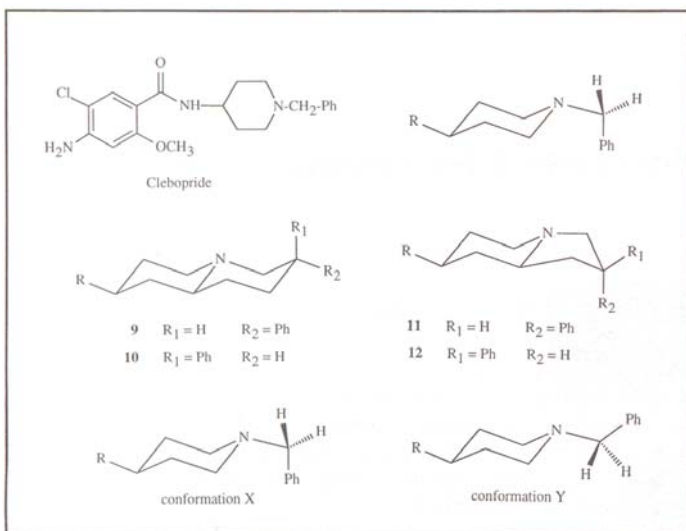


Fig. 13. Conformational restriction of the N-benzyl group of clebopride.

The *gauche* conformer of acetylcholine (see Figure 1) has a dihedral angle of 60° between the quaternary nitrogen and the acetyl group. The same dihedral angle is equal to 0° in the *cis* cyclopropyl analog.

These examples illustrate the primary drawback to conformational restriction. In order to produce a conformationally rigid analog, it is necessary to add new atoms or groups to the parent molecule. Any physical and/or chemical changes imparted by these new atoms or groups must be considered when evaluating the biological data from the rigid analogs. As is probably obvious by now, the greatest success with conformational restriction is achieved when the differences between the parent molecule and the rigid analogs are minimized.

A final example of conformational restriction is provided by the work of King and coworkers(16). These investigators were studying the pharmacological actions of benzamides and sought to determine binding requirements for central and peripheral dopamine receptors. The parent compound in their study was clebopride, a gastric prokinetic agent as well as a potent central dopamine receptor antagonist. In order to determine the conformation of the N-benzyl group with respect to the piperidine ring at both central and peripheral dopamine receptors, King *et al.* designed a series of quinolizidines **9** and **10** and indolizidines **11** and **12**, (see Figure 13). In comparing these analogs, note that the phenyl group effectively occupies a graded position in space going from **10** to **12** to **9** to **11**. The activity profiles of these four compounds helped to establish that conformation X is required at the gastric prokinetic receptor, while conformation Y is required at the central dopamine receptor.

SUMMARY STATEMENT

Conformational isomerism, present in almost all commercially available pharmaceuticals as well as compounds under clinical and pre-clinical evaluation, plays a key role in the activity of drug molecules. An understanding of the concepts discussed above—preferred conformation, active conformation, advantages and disadvantages of conformational restriction, and strategies to develop conformationally restricted analogs—is crucial to the chemical design of new compounds. Additional examples and further discussion of these concepts can be found in the references included within.

Am. J. Pharm. Educ., **60**, 192-197(1996); received 1/24/96, accepted 2/26/96.

References

- (1) Harrold, M.W., "The use of molecular modeling programs in medicinal chemistry instruction," *Am. J. Pharm. Educ.*, **56**, 158-163(1992).
- (2) Harrold, M.W., "Computer-based exercises to supplement the teaching of stereochemical aspects of drug action," *ibid.*, **59**, 20-26(1995).
- (3) Silverman, R.B., *The Organic Chemistry of Drug Design and Drug Action*, Academic Press, San Diego CA (1992) pp. 83-86.
- (4) Mathison, I.W., Solomons, W.E. and Tidwell, R.R., "Structural features and pharmacologic activity," in *Principles of Medicinal Chemistry*, 4th ed., (eds. Foye, W.O., Lemke, T.L., and Williams, D.A.), Lea & Febiger, Philadelphia PA (1995) pp. 25-49.
- (5) Kemp, J.D. and Pitzer, K.S., "Hindered rotation of the methyl groups in ethane," *J. Chem. Phys.*, **4**, 749(1936).
- (6) Lattin, D.L., "Cholinergic agonists, acetylcholinesterase inhibitors, and cholinergic antagonists," in *Principles of Medicinal Chemistry*, 4th ed., (eds. Foye, W.O., Lemke, T.L., and Williams, D.A.), Lea & Febiger, Philadelphia PA (1995) pp. 321-344.
- (7) Jorgensen, W.L., "Rusting of the lock and key model for protein-ligand binding," *Science*, **254**, 954-955(1991).
- (8) Nogrady, T., *Medicinal Chemistry, A Biochemical Approach*, 2nd ed., Oxford, New York NY (1988) pp. 31-35.
- (9) Koshland, D.E., "Application of a theory of enzyme specificity to protein synthesis," *Proc. Natl. Acad. Sci.*, **44**, 98-104(1958).
- (10) Burgen, A.S.V., Roberts, G.C.K., Feeney, J., "Binding of flexible ligands to macromolecules," *Nature*, **253**, 753-755(1975).
- (11) Horn, A.S. and Snyder, S.H., "Chlorpromazine and dopamine: conformational similarities that correlate with the antischizophrenic activity of phenothiazine drugs," *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 2325-2328(1971).
- (12) Craig, P.N., "Guidelines for drug and analog design," in Burger's *Medicinal Chemistry*; Part 1, *The Basis of Medicinal Chemistry*, 4th ed., (edit. Wolff, M.E.), Wiley, New York NY (1980) pp. 331-348.
- (13) Korolkovas, A.J., *Essentials of Medicinal Chemistry*, 2nd ed., Wiley, New York NY (1988) pp. 74-76.
- (14) Harrold, M.W., Gerhardt, M.A., Romstedt, K., Feller, D.R. and Miller, D.D., "Synthesis and platelet antiaggregatory activity of trimetoquinol analogs as endoperoxide/thromboxane A₂ antagonists," *Drug Design and Delivery*, **1**, 193-207(1987).
- (15) Chiou, C.Y., Long, J.P., Cannon, J.G. and Armstrong, P.D., "The cholinergic effects and rates of hydrolysis of conformationally rigid analogs of acetylcholine," *J. Pharmacol. Exp. Ther.*, **166**, 243-248 (1969).
- (16) King, F.D., Hadley, MS and McClelland, C.M., "Substituted benzamides with conformationally restricted side chains. 2. Indolizidine derivatives as central dopamine receptor antagonists," *J. Med. Chem.*, **31**, 1708-1712 (1988).