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REVIEW ARTICLE

Conformational Preferences of Drug like Molecules: Implication in Drug Discovery

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Abstract

The therapeutic action of drug and drug like molecules is governed by their three-dimensional structure (conformation). The macromolecular target recognizes a specific type of conformation that is responsible for its action. At the room temperature or physiological condition, a conformationally flexible molecule is not a single conformation rather it is an ensemble of several conformations. The choice of conformation is determined by the presence of intermolecular and intramolecular sets of interactions; therefore, the preference for a particular conformation may change as per requirement of surrounding environment. Analysis of conformational preferences is very important, because several descriptors used in molecular modeling vary conformation to conformation. Reliability and predictions of 3D-QSAR and ligand based pharmacophore methods depend on alignment of several conformations. Similarly, docking and *de novo* design method deal with searching of best binding mode and conformation of a molecule.

Keywords

Conformation, Geometrical Isomerism, QSAR, Docking, Pharmacophore

Introduction

The conformations of a drug molecule are defined as the various arrangements of its atoms in the space, which can be interconverted by rotation around single bonds (Leach, 2007). A drug molecule can adopt more than one conformation, and one of the chemically acceptable conformations may be biologically active. The conformational characteristics of drugs/leads and biomolecules (e.g. amino acids) are of supreme importance for the understanding of their reactivity, affinity for the specific macromolecular targets to evaluate drug action. The low activity of the molecule may be attributed to the lower population of the active conformation in solution (Silverman, 2010). The low potency (or even adverse reaction in some cases) of a drug can be attributed to the wrong conformations of a drug being bound to a given target. The energies of these conformations determine their relative population in the equilibrium mixture in solution or biological conditions. Therefore, a high-energy bioactive conformation has poor affinity because of the relative poor population of conformation (Silverman, 2010).

Conformational preferences and its importance in drug discovery

Any flexible drug molecule can have several conformations, and these alternative conformations usually differ from each other across rotatable single bonds due to change in the torsion angles. It is commonly encountered in the conformationally flexible molecules like single bond rotamers, conformers related by ring inversion, conformers generated by inversion about sp^3 nitrogen. The number of possible conformations is dramatically increased if any molecule shows geometrical isomerism due to the presence of partial or restricted rotation across the double bond. For example, nitroethenediamine moiety containing drug molecule like ranitidine shows partial restriction about carbon-carbon double bond because the estimated barrier across this bond is found to be about 24 kcal/mol (Dhaked and Bharatam, 2013a). Therefore, nitroethenediamine based drug molecules show E/Z isomerism across carbon-carbon double bond (Fig. 1). This barrier in ranitidine across carbon-carbon bond is very low compared to barrier in carbon-carbon double bond of ethene (64 kcal/mol) and very high from carbon-carbon single bond of ethane (4-5 kcal/mol). Kemp and Pitzer, 1936 indicated that rotation across carbon-carbon single bond is not free but associated with both steric repulsions and electronic interactions among the atoms or group present on the adjacent carbons, and conformations, which minimize these repulsions and maximize the attractive interactions, are found to be preferred. Adjustment of these interactions

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can result in variations in internuclear distances and local electronic structure. Most of these conformations are energetically unfavorable and are difficult to observe experimentally.

The conformations of drug molecules are influenced by the presence of noncovalent interactions like H-bonding, cation-π, π-π, etc.(Li *et al,* 2013; Nagy, 2012; Urban *et al,* 1997). These interactions change the conformational choice of the drug molecule depending upon the surrounding environment. Intramolecular hydrogen bonds control conformations in the gas phase conditions, intermolecular hydrogen bonds control conformational polymorphism in solid state conditions, the hydrogen bonds between solute and solvent control conformational preferences, and the hydrogen bonds between drugs and macromolecules control the bioactive conformations of the drug molecules. Hydrogen bonded conformations are often preferred in the gas phase; however, the same may not be preferred in the other conditions due to weakening of this interaction. For instance, bicalutamide (BC), an antineoplastic drug shows conformational polymorphism due to conformational diversity among all reported crystal structures (Vega *et al,* 2007). All these vary in terms of H-bonding and π-π interactions. In the polymorphic form II, π-π interaction is found to be dominant (folded conformation) between two phenyl rings in the solid state whereas in the polymorphic form I, intramolecular H-bond is observed between the sulfonyl and hydroxyl group (extended conformation) (Fig. 1). Another example is dopamine, which exists in a nearly equal mixture of the extended (anti) and folded (gauche) conformations in the aqueous phase at neutral pH (Fig. 1) (Urban *et al,* 1992). Whereas, folded conformation is favored in the gas phase (acidic condition) due to the presence of favorable interaction between the positively charged ammonium group and the aromatic ring (cation-π interactions) (Nagy *et al,* 1999). In sulfonylurea class of drugs, two conformations (**SU-1** and **SU-2**) are found to be important (Fig. 1) (Kasetti *et al,* 2010). In crystal structures, **SU-2** type conformation is found frequently in several drug molecules while **SU-1** type is preferred in only a few drugs. In the gas phase, **SU-1** is found to be more stable than **SU-2** by about 4 kcal/mol due to the presence of six membered intramolecular hydrogen bond. However, in aqueous medium **SU-2,** is preferred over **SU-1** by about 1.8 kcal/mol, due to the presence of two sulfone oxygen atoms to make interaction with polar solvents. In comparison, important conformations of pyridine sulfonyl urea (PU) class of drug molecules do not follow same trends, where **PU-2** is 3.40 kcal/mol less stable over **PU-1** (Fig. 1). Thus **PU-1** remains stable in polar and nonpolar solvents (Dhaked and Bharatam, 2013b).

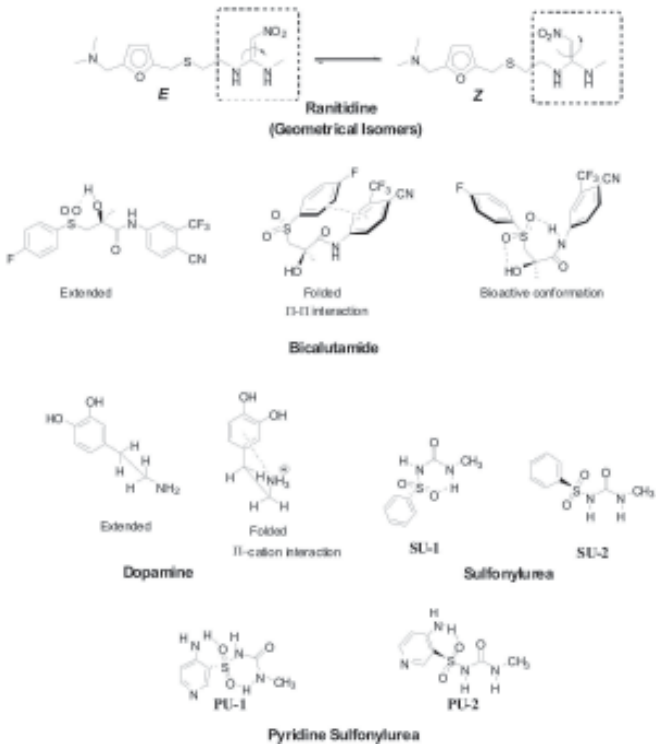

Fig. 1. Geometrical isomers of ranitidine, and extended and folded conformations of bicalutamide, dopamine, sulfonylurea and pyridine sulfonylurea.

The challenge for a medicinal chemist is to understand the structural and energetic differences between these conformations to successfully design new molecules that adopt the desired shape with little or no energetic penalty. The details of the bioactive conformations are determined experimentally by using X-ray and NMR methods. In addition, binding of drug molecules can be investigated using a number of computational methods like docking and pharmacophore search, etc. It has been observed that a good ligand must bind in a low energy conformation in order to get the favorable free energy of binding. However, in most of the cases, the binding mode of drugs does not correspond to the global or local minimum conformations. These conformational changes in the most stable conformation arise as a result of intermolecular interactions between drugs and the macromolecular targets. A study showed that about 60% ligands bind to targets in 5 kcal/mol less stable conformation (Perola and Charifson, 2004).

The comparison of the bioactive and solution conformations helps in identification of various thermodynamics and kinetics factors, which determine the binding of a drug molecule to the macromolecular target. Further, this information can be used to design conformationally restricted molecules, for example, rigid

analogues of bicalutamide have been designed (Fig. 2) (Lanter *et al*, 2006). Suppose a drug molecule acts on its three targets in three different conformations to produce two beneficial effects (antihypertensive and diuretic effects) and one major side effect, the specific agonists and/or antagonists can be developed by locking individual active conformations for corresponding targets and at the same time avoiding any unnecessary effects or side effect (Harrold, 1996).

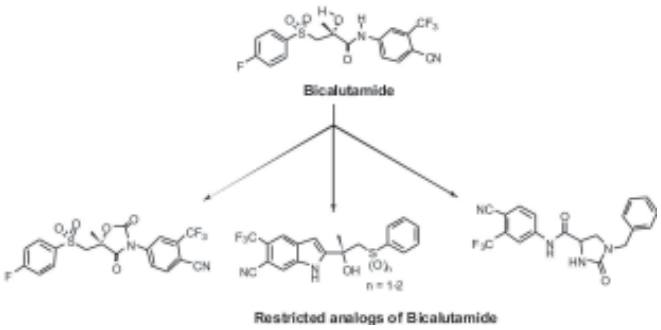

Fig. 2. Various rigid analogs of bicalutamide.

For example, chlorpromazine an antipsychotic drug binds to the dopamine receptor (Fig. 3). In the bioactive conformation of this drug, the tertiary amine group of the side chain is located on the side of a aromatic ring containing chlorine atom (Harrold, 1996). Chlorpromazine adopts a variety of inactive conformations due to conformational flexibility. Introduction of double bond in the side chain allows the tertiary amine group toward the aromatic ring containing chlorine atom and avoids generation of inactive conformation. Therefore, this restricted molecule is found to be more potent. Another example is acetylcholine neurotransmitter (Fig. 3), which shows several conformations due to the presence of four single bonds between the acetyl group and the quaternary nitrogen (Silverman, 2010). It binds to the muscarinic and nicotinic receptors in *trans* and in *gauche* conformations, respectively. In order to provide selectivity to the muscarinic receptor, its active form is restricted in trans conformation using methylene linker. Using similar approach, highly potent dopaminergetic agonists have been designed from dopamine (Fig. 3) (Anand, 1983). Dopamine has α and β conformations across C_6H_5-CH_2 bond. Restriction in b form gives a potent molecule while á form gives an inactive molecule.

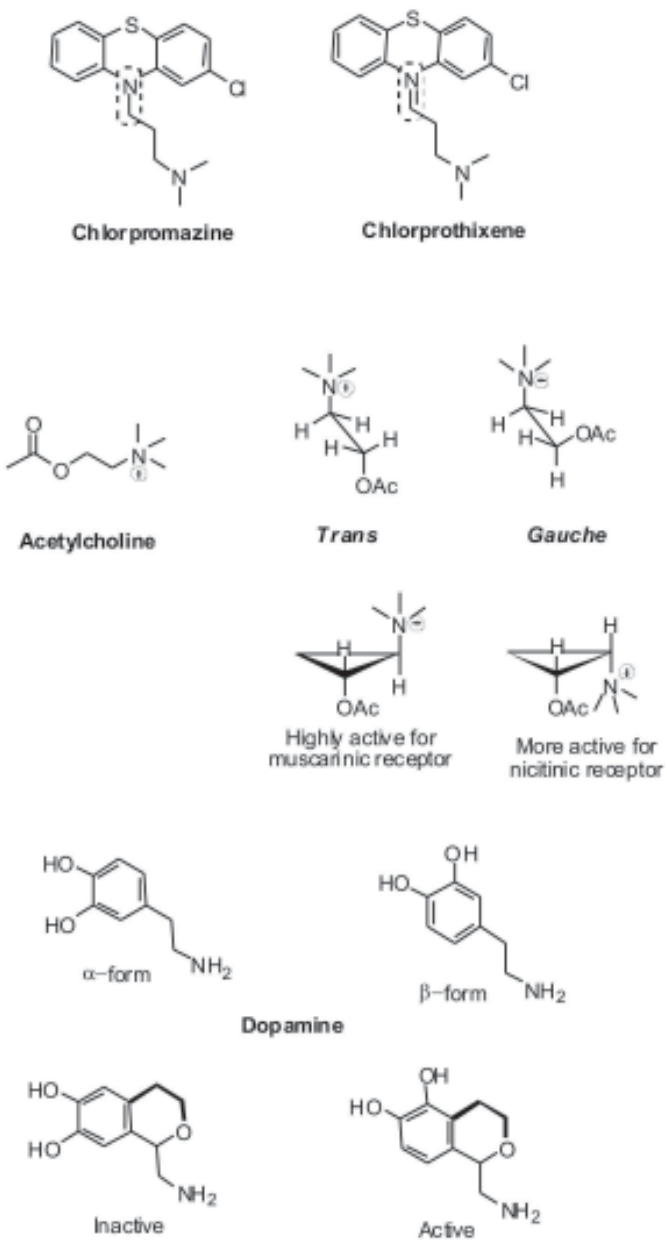

Fig. 3. Conformationally restricted analogs of chlorpromazine, acetylcholine and dopamine.

Importance of conformational preferences in computer aided drug design (CADD)

CADD is associated with several techniques of molecular modelling: docking, QSAR, pharmacophore, *de novo* design, structure based screening. All these methods directly or indirectly deal with identification of appropriate conformation of ligands, in order to predict molecular interactions or new molecules.

The docking is a process that predicts the molecular conformation, orientation and binding affinity of the

ligands in the active site of protein (Kitchen *et al,* 2004; Sousa *et al,* 2013). ENREF_14 Conformational searching is very important during docking because it is not known which conformation of a ligand binds favourably with protein. Molecular flexibility of ligands is a challenging process; even a simple molecule can have many degrees of freedom. Therefore, sampling of these degrees of freedom is critical task and need to be done with sufficient accuracy. The best identified conformation must have tighter binding in protein (better score). There are several algorithms available to treat ligand flexibility for the docking, which are roughly categorized in three types: systematic search/ stochastic algorithms/simulation methods, etc.(Brooijmans and Kuntz, 2003; Huang and Zou, 2010; Kitchen *et al,* 2004). Finally, energetically reasonable conformations are judged on the basis of different scoring functions.

***Systematic search*:** These algorithms explore all the degrees of freedom of the ligand to generate all possible ligand-binding conformations. There are several methods of systematic search: exhaustive search, conformational ensemble, etc. In exhaustive search methods, several conformations are produced systematically by rotating all possible rotatable bonds. One of the major problems of this method is that it generates huge number of conformations (which are physical states but not chemical states), which need to be handled rapidly. In conformational ensemble method, pre-generated library of ligand conformations is docked in the protein.

***Stochastic algorithms*:** In these methods, ligand binding orientations and conformations are sampled by making random changes to the ligand at each step in both the conformational space and the translational/rotational space of the ligand, respectively. A new conformation with these changes is accepted or rejected on the basis of some criteria. There are several methods, which are based on stochastic algorithms: genetic algorithms, Monte Carlo, Tabu search, etc. For example in genetic algorithms (Fig. 4), initial set of solutions are generated randomly and evaluated by fitness score. In the selection process, the most fit solutions are copied to the next generation and rest places are filled with parent chromosomes. Then genetic operation is carried out using crossover and mutation. Fitness score evaluates this new population. These cycles are repeated until best solutions are obtained.

***Simulation methods*:** This is one of the popular methods. Problem of this method is that it is unable to cross high-energy barrier in given simulation time and may trap the ligand in local energy minima. This problem can be sorted out by carrying out simulation at different positions of the ligand (Kitchen *et al,* 2004).

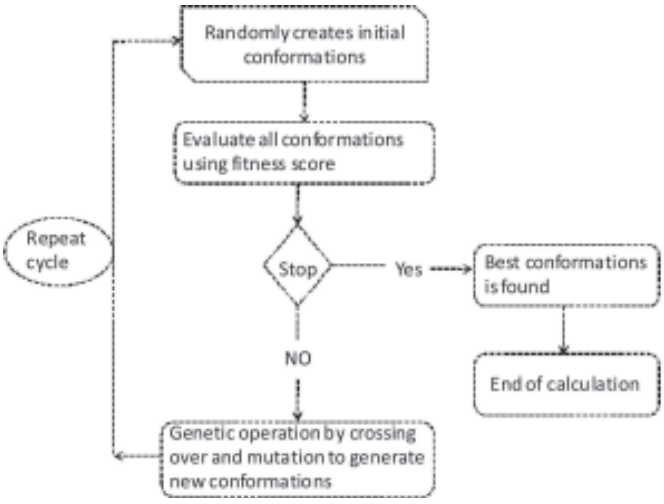

Fig. 4. Flow chart of genetic algorithm to find best conformations of docked molecule.

QSAR is a statistical method, which correlates change in chemical structure with respect to change in biological activity (Kubinyi, 1997). This method is very important in medicinal chemistry for the optimization of lead molecules and the prediction of biological activity of untested or non-synthesized compounds (Kubinyi, 1993). The results of 3D-QSAR methods depend on the identification of the lowest energy conformation and it is hypothesized that biological activity of the drug or ligand is modulated by this conformation. If the ligand molecule is rigid then it does not have conformational flexibility and does not pose problem for further QSAR analysis. For the flexible ligands, it is very difficult to have starting structure. The information regarding conformational preferences of such molecules can be obtained from Cambridge Structural Database (CSD) (Allen, 2002). More information regarding conformation can be obtained from Protein Data Bank (PDB) (Berman *et al,* 2000) if the ligand is co-crystallized with protein. The reliability of 3D-QSAR methods depends on the determination of receptor bound conformation of the ligand. These bioactive conformations are experimentally determined using X-ray crystallography and NMR spectroscopy. If the bioactive conformation details are not available for any molecule, then energy minimum conformation of the most potent molecule can be used as a template for alignment. In addition, docking based alignment can also be used for 3D-QSAR (Xu *et al,* 2004). The most crucial step in 3D-QSAR is the alignment of ligands which means the way ligands are superimposed. The better alignment of the molecules can be obtained only if proper conformational analysis has been carried out. If the alignment of molecules is carried out using improper conformation, then receptor-surface model generated from this data will be incorrect and finally it will make wrong predictions.

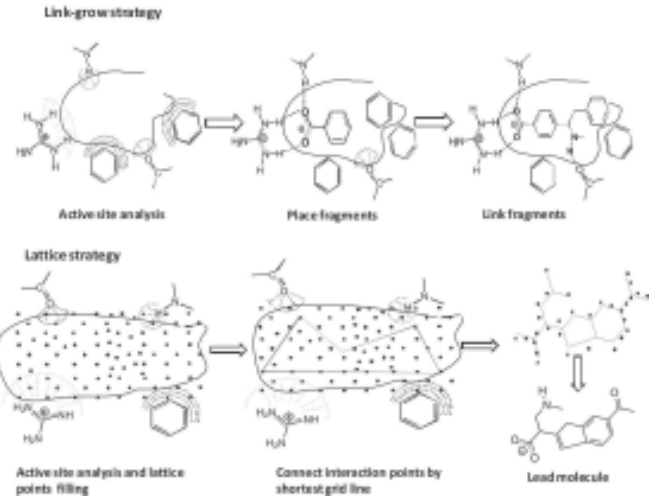

Fig. 5. Schematic representation of link-grow and lattice strategies of *de novo* design method.

De novo design is a method, which generates virtual lead compounds in the active site of macromolecules from scratch using several approaches like link-grow, lattice, fragment placing and growing (Mauser and Guba, 2008; Schneider and Fechner, 2005). In all these approaches, main aim is the identification of best conformation and orientation of designed molecule. The success of these approaches depends on how the conformational sampling is carried out. There are several *de novo* programs that have been developed in the last few decades for the generation of leads (LUDI, BUILDER, PRO_LIGAND, SYNOPSIS, BREED) (Schneider and Fechner, 2005). For example, in link-grow strategy, analysis of binding pocket of model protein is carried out (Fig. 5) and the probable interaction sites are called "hot-spots" (Schneider and Fechner, 2005). These sites may be hydrogen bond donor, hydrogen bond acceptor, hydrophobic region, etc. Several fragments are placed in the active site and best fragments on the basis of score are kept in the binding pocket. Fragments, which are making interaction with "hot-spots" are connected using suitable linker to a complete ligand. This way, best binding conformation of the ligand is generated in the active site. This link-grow strategy is implemented in LUDI programme for the development of lead molecules (Bohm, 1992). This method has been used for the design of several ligands.

In lattice strategy, binding pocket is filled with several lattice points (Fig. 5) (Schneider and Fechner, 2005). Then, interaction sites are analysed and are connected using grid line following shortest path. On the basis of interaction points and grid line, a molecular fragment is assigned, from which a molecule is generated.

Pharmacophore refers to the 3D arrangement of features that are responsible for a specific biological activity of the drug/lead molecules. There are two types of pharmacophore generation methods: direct and indirect (Dror *et al*, 2004). Direct methods use information on both ligand and protein while indirect methods use only ligands information. The alignment of the conformations of the ligands is the most critical step in pharmacophore generation (Yang, 2010). This alignment is carried out to determine the best pharmacophoric features. Conformational flexibility of ligands complicates the process of pharmacophoric map preparation. One ligand can have several conformations across single bond; this number increases rapidly with increase of number of rotatable bonds. Any of the conformation of ligand can bind in the binding site of protein. Therefore, all of the conformations of each ligand need to be considered during pharmacophore map preparation. There are several softwares available for pharmacophore map generation such as HipHop, HypoGen, MOE, GASP, etc (Yang, 2010). The most of the pharmacophore softwares use conformational search initial step or on the fly during search. Therefore, these methods employ conformation generation tools like ROTATE, CatConf, ConfGen, MacroModel, etc (Schwab, 2010). Main problem with these methods is that these generate large number of conformations. To overcome this problem, energy minimization process is used to eliminate higher energy conformations and only low energy conformations are considered (Dror *et al*, 2004). In addition, sometimes clustering analysis is used to eliminate redundant conformations. Application of pharmacophore search depends on not only the quality and accuracy but also conformational diversity of ligands, which are stored in 3D database. In addition, identification of novel lead molecules also depends on the presence of multiple conformation libraries against which these pharmacophores are searched.

Conclusions

Conformationally flexible molecules have several degrees of freedom, which leads to several conformations of a molecule. Of these, a specific conformation of a drug molecule is responsible for its drug action. In order to predict binding mode of the drug, analysis of conformations is very important. There are several tools available to search/generate conformations of a molecule, which vary with methods. Several methods of lead optimization like 3D-QSAR, docking, pharmacophore, etc. depend on quality of conformational analysis. In addition, conformations play critical role in crystal packing, reactivity of a molecule, intermolecular interactions, interpretation of experimental data, drug action, etc. Therefore, conformational analysis of a drug like molecule is very important in the drug discovery.

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