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MINIREVIEW

Tautomerism in Computer-Aided Drug Design

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ABSTRACT

Tautomers are often disregarded in computer-aided molecular modeling applications. Little is known about the different tautomeric states of a molecule and they are rarely registered in chemical databases. Tautomeric forms of a molecule differ in shape, functional groups, surface, and hydrogen-bonding pattern. Calculation of physical–chemical properties and molecular descriptors differ from one tautomeric state to the other as it is demonstrated with an example of the $\log P$ calculation, similarity index, and the complementarity pattern to the targeted protein. Considering tautomery in ligand–protein interactions therefore has a significant impact on the prediction of the ligand binding using various docking techniques. This article points on hitherto unaddressed issue of tautomerism in computer-aided drug design.

Key Words: Tautomer; Chemical database; Tautomer binding site; Virtual screening.

1. INTRODUCTION

Numerous collections of chemical compounds are stored in electronic form and various techniques are used to filter out compounds having the so-called "drug-like" properties (1,2). Using automatized molecular docking programs, small molecules are

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placed in a target protein and their affinity and binding modes may be predicted (3,4). Recent advances in this domain are to consider the flexibility of ligands as well as of protein amino-acid side chains, and to include the solvation–desolvation term in energy calculation, which are described in several reviews (3–8). However, the issue of considering tautomers of small molecules both in chemical databases and in ligand–protein interactions is commonly omitted. This paper refers to what is generally overlooked when neglecting the existence of tautomers in drug-design. In the second section, examples are given concerning the compounds in databases. In the third section examples of some cases of tautomer–protein interaction are presented. The last part gives an outlook pleading to include tautomers in molecular docking.

2. TAUTOMERS IN CHEMICAL DATABASES

Tautomerism by definition concerns all molecules which can readily interconvert into isomers by transfer of a chemical group.[a] Tautomerism is very complex and is related to several phenomena: different types of migrating groups [electrofuge (e.g., H^+) or a nucleofuge], cationotropic and anionotropic properties, valence tautomerism, zwitterionic tautomerism, tautomerism related to migration of neutral groups in molecules, migration of bonds or ring-chain tautomerism (9–11). As a subtype of cationotropy, the proton migration (prototropy) is the most commonly known tautomeric phenomenon. It concerns the movement of H atom between discrete sites of the "same" molecule (in contrast to ionization or protonation where H atom leaves or comes from another molecule). Even if only the case of prototropic tautomers is considered, the problem of tautomery in chemical collections remains complex.

Compounds in chemical databases are stored in their canonical forms to which the tautomeric or ionic form of a compound can be reduced using strongly defined chemical rules. Many commercial and non-registered databases often contain pairs of tautomers registered under different names and even prices (11,12). In a recently reported work, Trepalin et al. estimated that up to 0.5% of commercially available compound collections for bioscreening contain tautomers (11). Conversely, a large amount of tautomers are missing. At the early stage of drug discovery this presence of paired structures and the omission of tautomeric structures in chemical databases has an immense impact on bioscreening. On the other hand, if a database is used for computer-aided lead finding, enriching one's database by energetically similar tautomers may significantly improve the success rates in computer-aided drug design.

Actually the problems of tautomeric structures in chemical databases can be reduced to (1) the search for tautomers and (2) the creation of missing tautomers.

1. Though in large compound registries such as Beilstein and CAS, tautomers can be found in automated fashions using high-performance computational technologies (11,13–15), tautomers in non-registered databases (if present) are difficult to be found.

[a]We refer to the commonly drawn structure of substances as "compounds" and to rare tautomeric form(s) of the compound as "tautomer(s)." Here "commonly drawn" means the form in which a molecule was saved in a database.

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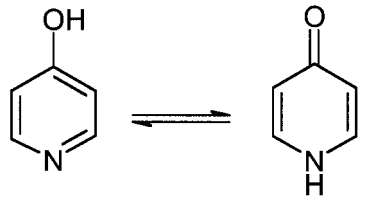
To solve this problem, Trepalin et al. (11) presented an algorithm and software allowing exact structural search for tautomers in large corporate databases. This approach is based on the generation of a canonical structure. In contrast to global chemical registry systems the management of such a wide number of tasks is available for large corporate databases using moderate computational requirements. In large collections (Beilstein, CAS), compounds can be identified using a special tautomeric identification code with links to the original patents and publications; however it only allows searching for a single compound entry. Physico-chemical properties of tautomeric entries are described inconsistently and were measured by different methods. In contrast, the proposed algorithm does not provide information concerning the preferable tautomeric form, as the tautomeric equilibrium is a function of a complex number of micro- and macro-environmental factors such as concentration, temperature, pH or type of solvent.

2. There are several programs available which are able to create tautomers, however for only one single compound at a time. Among them are listed the commercial ACD/Tautomer, Cliff, Cactvs, MDL, or Chemosoft (16–20). The widely used ACD/Tautomers proposes the tautomeric form, but not necessarily correct one (16). There are several reports about tautomeric construction and search algorithms although in most cases the algorithms are not explained. To contribute to automatic tautomerization of databases, we have designed the program AGENT (21,22) whose algorithm is based on general knowledge of legitimate chemical representations of tautomers. AGENT is able to create tautomers from large compound databases and allows the user to specify an energy threshold up to which a tautomer should be created. The relative tautomeric stabilities are calculated semiempirically based on Gibbs free energies of formation by employing the Molecular Orbital PACkage (MOPAC) (23). AGENT delivers an output file ready for molecular docking (for more applications see Sec. 4) and thus represents a valuable tool for ligand-based as well as for structure-based design.

2.1. Effect of Tautomers on Molecular Descriptors

One of the problems related to tautomeric representation is the prediction of molecular descriptors; since the outcome of every calculation crucially depends on what tautomeric form the calculation was based on. The following example shows the problem of log *P* prediction if the "wrong" tautomer is used and it is ignored, which tautomer in octanol/water partition may be favored. The molecules 4-hydroxypyridine (**1**) and 4-pyridone (**2**) (Sch. 1) have a calculated CLOGP = 0.93 for **1**, and CLOGP = −1.31 for **2** (24)! [CLOGP values varied based on method (in-house calculation), nevertheless, the difference between **1** and **2** remains huge]. However, in solution tautomer 4-pyridone (**2**) is the predominant molecule. The experimentally measured log *P* for 4-hydroxypyridine is = −1.3, which is very close to the value calculated for **2**. This clearly illustrates that obtaining the appropriate form of the tautomer improves the quality of the property prediction. Usual log *P* "calculators" use various methods of calculation based on molecular fragments and molecular properties (25,26). The fragment-based method depends on the way the fragments are produced, their number, size, and the training sets. Thus, missed or incorrectly selected tautomers for the training set lead to wrong correlations and cause the log *P* prediction to fail (3).



1 2

Scheme 1.

The second example concerns the similarity search index. Screening databases based on similarity [topological descriptors, putative pharmacophores, and molecular fields (27,28)] can be heavily influenced by tautomerism. An example is given for 4-nitrosophenol (**3**) and [1,4]benzoquinone monooxime (**4**) (Sch. 2). The Tanimoto index (27,29) for **3** and **4** is very low (0.196) and the molecules are dissimilar even if one takes into account that these are two tautomeric states of one and the same molecule. Hence, enriching databases with tautomers will improve the quality of similarity-based clustering and diversity analysis.

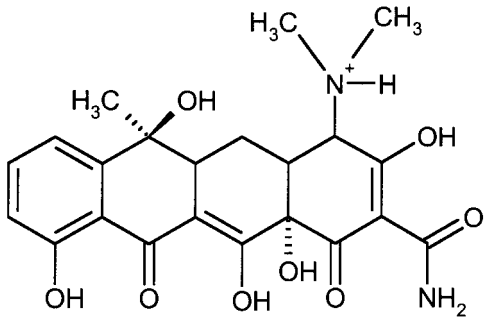
The following example depicts how tautomerism can influence chemical behavior and bioactivity of a molecule. Duarte et al. (30) reported the case of tetracyclines, a group of broad-spectrum antibiotics. Tautomerism of tetracyclines has been subject to acid/base properties and chemical behavior studies in different mediums (30). Semiempirical calculations revealed that tetracyclines exist in equilibrium of different tautomers. Indeed, considering four deprotonations, there are 64 different tautomers of tetracycline! Tetracycline drawn in the "normal" way as used to describe its structure is depicted in Sch. 3. Duarte et al. (30) concluded that the tetracyclines seem to be a sort of a chameleon molecule with high capability to modifying itself (chemical bonds as well as geometry) in response to the chemical context.

Assuming tetracycline would be the compound designated for designing an inhibitor, the choice of only one tautomeric structure for molecular docking may lead to misleading results. Furthermore, ignoring tautomers of tetracyclines can heavily bias structural search, physico-chemical data prediction and interpretation (30).

The last example concerns the intramolecular H-bond due to the tautomeric interchange (31,32). López-Rodrigríguez et al. (32) studied the prototropic equilibrium in the series of serotonin 5-HT4 receptor ligands. Two classes of benzimidazole derivatives were analyzed to gain insight into the bioactive conformation of these novel ligands. Their results from NMR and IR techniques and theoretical methods confirm the presence of important intramolecular hydrogen bonds between the benzimidazole ring and adjacent

3 4

Scheme 2.



Scheme 3.

side chain groups. These hydrogen bonds are possible also due to the tautomeric hydrogen shift on benzimidazole ring. Thus, the molecular skeleton as well as the energy required for conformational changes has the effect on adopting a bioactive conformation for ligand–receptor interaction. Such structural studies [see also in Ref. (32)] provide important insights to guide the design and synthesis of new compounds with predetermined pharmacological activities.

3. TAUTOMERS IN LIGAND BINDING INTERACTION

The tautomeric equilibrium is influenced by a number of variables including concentration, temperature, pressure, solvent type, and pH. Tautomers differ in heat and energy of formation, proton affinities, dipole moments, and ionization potentials [see examples in Refs. (33,34)]. There are numerous studies of tautomers in gas or aqueous solution, however, little is known about tautomerism of ligands in the binding site of proteins. Let us imagine a simple case of a transfer of one hydrogen atom on the same molecule-ligand. As indicated in Sec. 2, tautomery can alter the skeleton of a given molecule, which in principle can be seen as a new distinct molecule with different complementarity to the target.

Thus, several questions arise: does a molecule bind preferably in one distinct tautomer? Is the most stable tautomeric form in aqueous solution also the most stable form in the active site of a protein? What can be the binding contribution of a ligand in its excited tautomeric state in contrast to its "normal" tautomeric state, e.g., its low energy configuration? How to treat a compound, whose proton shift induces different stereoisomery?

Compared to the large amount of data available on ligand-binding interactions, little is known about the binding modes of distinct tautomers of a ligand molecule. One example can be the evidence of tautomer-bound state reported by Brandstetter et al. (35). 8-barbiturate inhibitor (RO200-1770) was bound as its enol-isomer to the active site of a matrix metalloproteinase (MMP-8) (Fig. 1). The lone pair oxygen O_2 contributes to the coordination of Zn^{2+}, while the hydrogen of O_2-hydroxyl is involved in H-bond with Glu198. Thus, it is the tautomeric enol form of the barbiturate that is favored by the protein matrix over the keto form, which dominates in solution. The H atom on N_1 is bound to the carbonyl of Ala161 and the ketone O_6 to the adjacent amides of Ala160 and Ala161.

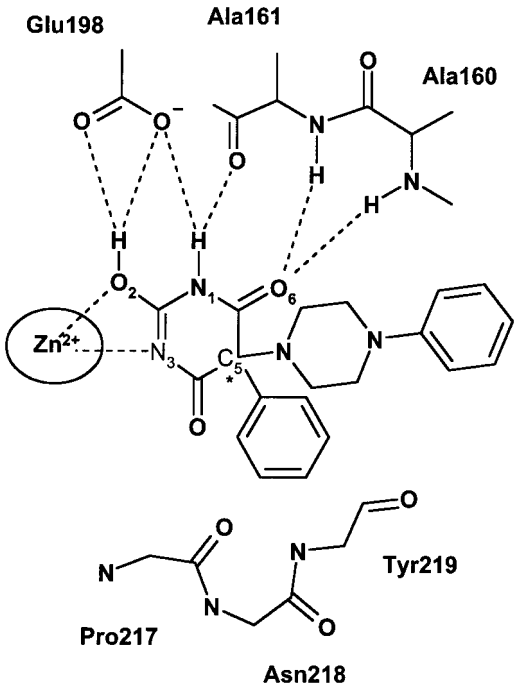


Figure 1. Schematic representation of the interaction of 2-hydroxypyrimidinedione inhibitor with the MMP-8 active site. The inhibitor binds in a tautomeric form which is unfavorable in aqueous solution. *Key:* * annotates a stereochemical center that can switch from R to S and vice-versa or become pseudocenter depending on the hydrogen shift. Dashed lines represent H-bonds.

Different hydrophobic groups of phenyl and piperidyl rings involve the hydrophobic subpockets of the binding site. In addition, 8-barbiturate inhibitor is in an enantiomeric form (chiral center C_5) which due to the hydrogen shifting may acquire another enantiomeric or prochiral form (12).

The inhibitor of Ricin toxin A-chain (RTA) is another example. Ricin toxin A-chain is a *N*-glucosidase that attacks ribosomal RNA at a highly conserved adenine residue. The inhibitor is a pterin derivative and crystallographic studies show that the pterin-based ring can bind to the active site of ricin (36). Pterins are able to form four tautomers (Fig. 2). Yan et al. (36) demonstrated that it is a tautomer of pterin, which is not in the energetically lowest form neither in the gas phase nor in aqueous solution (both calculated ab initio), that interacts best with the enzyme. Previous crystallographic studies of the RTA complexed with the inhibitor showed that pterin (**3**) (Fig. 2) is the preferred form, which was confirmed by molecular modeling net interaction energy calculation (lowest interaction energy $= -28.2$ kcal/mol). Pterin (**1**) which was calculated to be the most stable form in aqueous solution was predicted to have the net interaction energy of the complex with the enzyme $= -21.5$ kcal/mol (36).

Several other indications postulate tautomeric binding: pterin (**3**) is able to form two more hydrogen bonds than pterin (**1**), one between the hydrogen of N_1 and the backbone carbonyl oxygen of the Gly121, and another one connecting the NH group of the Val81

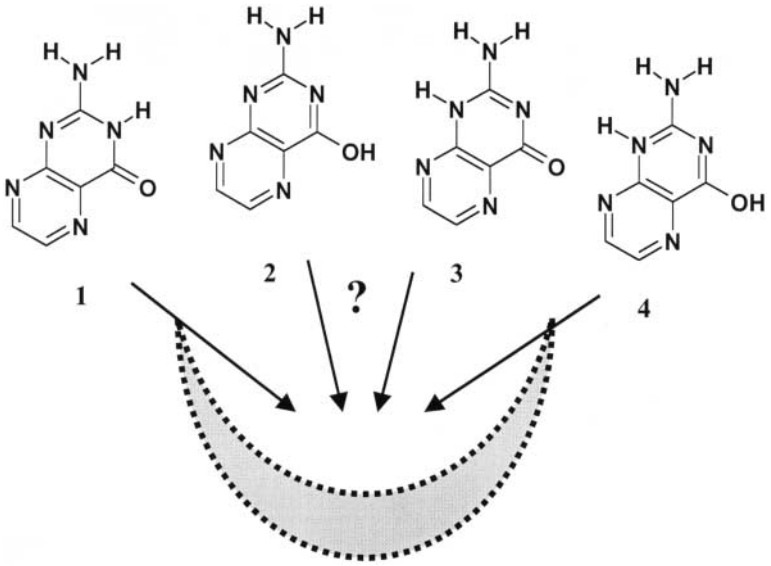


Figure 2. Four tautomers of pterin. Which of them is the preferred tautomer-bound form?

backbone with N_3 (Fig. 3). The distances between the oxygen of Gly121 and N_1 and N_{12} of pterin are close, which corresponds to strong hydrogen bonds contributing to the stability of the RTA–pterin (**3**) complex. In addition, Yan et al. postulated that the RTA first recognizes pterin (**1**) followed by the proton shift from N_3 to N_1, thus generating the pterin (**3**) complex in situ (Fig. 3).

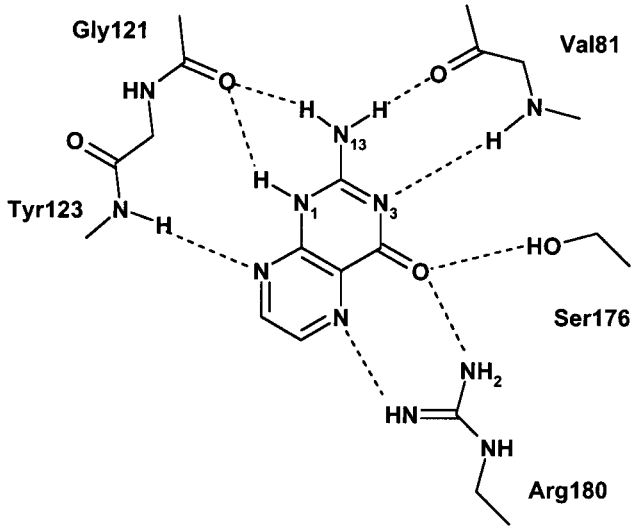


Figure 3. Interactions between RTA and pterin (**3**) tautomer. Dashed lines represent H-bonds.

The general view is that the binding environment within a protein is a very specific one. It is different from the environment of the aqueous solution or the vacuum. Apolar or polar, acidic or basic side-chains create local pHs, shift their p*K* values and subsequently influence the functional groups of the ligand. Presence of a ligand, metal cations, and water also influence the pH (and the p*K*) conditions in close proximity of amino acid side-chains and the process of catalysis (37). In such a context, ligands may be ionized or can achieve its excited tautomeric state.

Many enzymes of therapeutical relevance, such as nucleoside kinases, telomerases or DNA-polymerases, are able to accommodate purine or pyrimidine derivatives in their active sites. Hence, nucleic acid bases are molecules with well-known occurrence of tautomery. For example there is the phenomenon of tautomer base mispairing in the DNA-strand as a source of DNA replication errors (38,39). Nucleobases reveal always one tautomer, which are for stability reasons incorporated into the nucleic acid. The ability of pyrimidine and purine structures to form hydrogen bonds is linked intimately with the potential existence of tautomeric structures. Similarly, the phenomenon of tautomerism can be observed on histamines and in proteins on histidine side chains (40,41).

Tautomers have different molecular shapes and different hydrogen-bond donor and acceptor properties resulting in a significant impact on molecular recognition. As it was shown in this section, tautomers are able to bind to the active site of a protein. Nevertheless up to now, tautomers have been neglected, even omitted in automated molecular docking.

4. INCLUDING TAUTOMERS IN MOLECULAR DOCKING APPLICATIONS

Recent advances in molecular docking occurred in various fields such as ligand–protein flexibility, scoring function or automatized processing (6,8,42,43). However, little progress has been achieved in the simulation of the immediate environment within the active site. This would demand predictions of terms such as solvation-desolvation, temperature of the system, microenvironment of the active site in proximity of the ligand and protein side-chains, pH, ionization, protonation/deprotonation, and tautomerism of ligands upon binding.

In the field of drug design, virtual screening of 3D-structural chemical databases became a major discipline. Most algorithms accept chemical structures as they are imported by the user from databases without tautomers. Thus, including tautomers could be seen as enhancing the number of degrees of freedom to be considered by docking programs. It can be a fast and relatively precise method to cover for incertitudes caused by the variability of the effective p*K* in different parts of a receptor binding site.

Including tautomerism in virtual screening procedures should improve the reliability of the screening due to the following reasons: (i) it enlarges the chemical space covered by the database and (ii) it takes into account that a compound can bind in its tautomeric state, which raises the chance of detecting a hit. Hence, this approach can be considered as a large improvement of the virtual screening procedure itself. Subsequent selection of the best-ranked compounds or tautomers may also reveal new characteristics of ligand binding. If a docked compound is stabilized in its tautomeric state in a given binding

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site environment, this can lead to the recognition of a putative ligand, which would not be detected using classical screening protocols.

Few investigations have addressed this topic. ProtoPlex, a program developed by Pearlmann et al. generates all protomers of drug-sized compounds (tautomer and/or protonation state) and yields false negatives if the protomer in the screening library is not preferred by the receptor (44). Similarly, Sadowski et al. addressed the issue of tautomery together with protonated molecules using a tautomer and protonation preprocessor for virtual screening (45). In our group, we have used the generator of tautomers AGENT (in-house program) to generate a database of energetically probable tautomers, which in parallel with the database of compounds can be docked to the target of interest (22,46). Thousand nucleobase analogs enriched by tautomers have been screened on viral thymidine kinase known for its large acceptance of nucleobase derivatives. Subsequently, the top scored compounds and tautomers were compared to each other and revealed new characteristics of the ligand binding (47). It can be postulated that when the active site favors the tautomeric form over the compound, this could reveal new lead structures, which would be omitted in a classical, tautomerism-disregarded screening. Moreover, finding tautomeric hits may initiate new case studies of tautomery-dependent ligand–protein interaction.

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