What impact does tautomerism have on drug properties and development?

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Abstract

This mini-review reports on effects that tautomerism has on predicted and clinically observed small-molecule properties important for drug design and drug behaviour in the patient. We present examples of both pharmacokinetics and pharmacodynamics effects. The importance of tautomerism on drugs' formulation and possible interconversion of stereoisomers is also discussed.

1. Introduction

Tautomers are structural isomers that interconvert from one form to another. The most common, and best-known, type of tautomerism occurs by proton migration. Other forms that involve equilibrium between ring and chain form with and without proton migration are called ring-chain and valence tautomers. In textbooks and literature, the term "readily" is often used to describe the process. However, this is not correct in all cases. In the case of solid forms of drug molecules, the transformation from one form to another is very slow due to the difficult migration of protons.

To speed up and make less expensive the typically very expensive and time-consuming drug development process, numerous computational tools have been developed. Preclinical studies involve estimating absorption, distribution, metabolism, and excretion (ADME) properties and optimizing new chemical entities. Issues with these pharmacokinetic properties, as well as toxicity, have been part of the reasons for failure of drug candidates. All these properties affect the pharmacodynamic action of a drug molecule. A good candidate should have balanced ADME and good potency. This mini-review discusses the impact of tautomerism on pharmacokinetics, pharmacodynamics properties, formulation, and racemization. We emphasize here that tautomerism is not a novel phenomenon of drugs in the pill or the body.

There is, however, in our view still a limited understanding of the effects and importance of, and limited capabilities of predictive tools to take into account, tautomerism in drug design.

2. Pharmacokinetics

2.1 Absorption and Bioavailability:

The effect of tautomerism on bioavailability has not been explored much, based on the small number of pertinent research articles. Molecules capable of tautomerism are expected to behave differently when exposed to solvents or gastrointestinal fluids. They may show different tautomeric preferences based on pH, temperature, and microfluids of the intestine. These different prototropic species are expected to behave differently regarding their dissolution, disintegration, and solubility.[1] Tautomerization is also affected by drug carriers such as micelles, liposomes, cucurbit, and beta-cyclodextrin, which can have a notable effect on improving their solubility, stability, and bioavailability.

2.2 Metabolism:

Tautomerism in the field of metabolism has likewise received little attention, though most drugs are metabolized in the body. The most commonly represented tautomer is considered for analyzing the metabolism of drug molecules. Different tautomers can be metabolized by enzymes or converted non-enzymatically at different rates. Warfarin, an anti-coagulant agent, exists in two enantiomeric forms and has at least 40 tautomers ranging from open chain to ring tautomers. Studies on warfarin and analogue compounds have suggested that tautomeric S-warfarin forms are metabolized in ring form. The pharmacodynamics effect will differ if minor or major tautomers are metabolized rapidly.[2]

After one or two rounds of initial oxidation, drugs may go for tautomerization due to the addition of a hydroxyl group or epoxide in the structure. For example, cyclophosphamide is converted to 4-OH-cyclophosphamide via hydroxylation, then this ring tautomer is converted to a chain tautomer (Figure 1).[3] Another interesting example is daclatasvir, which is a selective inhibitor of the hepatitis C virus. After δ-oxidation at the pyrrolidine moiety, daclatasvir is prone to tautomeric equilibrium with a chain tautomer (aminoaldehyde) via opening of the pyrrolidine ring. This is converted to a stable metabolite by attacking the aldehyde group with imidazole nitrogen.[4]

Mechanism-based inhibition (MBI) is a special type of inhibition where a metabolite formed from a drug is quite reactive and can bind to a metabolizing enzyme covalently or non-

covalently, leading to temporary or permanent loss of metabolizing enzyme activity, hence affecting the metabolism of other drugs (drug-drug interaction).

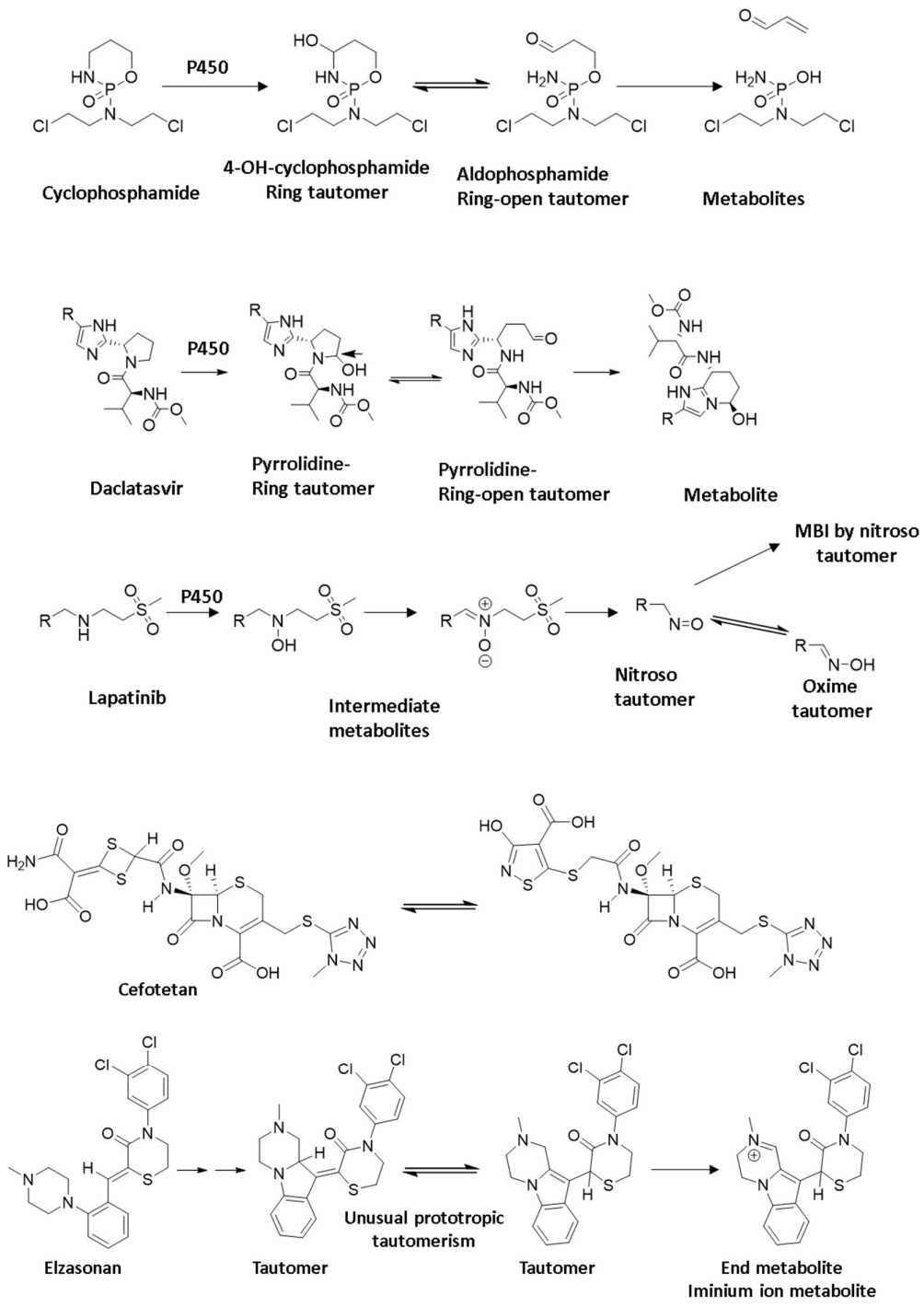

Figure 1. Examples of drugs molecules involving tautomerism during ADME processes.

These end metabolites can have the potential for tautomerization, and only one of the tautomers leads to MBI (lapatinib nitroso tautomer, (Figure 1)).[5]

2.3 Distribution

Human serum albumin (HSA) is one of the abundant transporter proteins present in the blood. HSA has multiple binding sites due to its ability to adapt to accommodate drug molecules. There is a general lack of studies as to which types of drugs or specific tautomers binds in particular HSA sites. However, there are many examples where one enantiomer is reported to bind tightly and the other weakly or with a different binding strength. Drugs usually form reversible complexes with HSA by electrostatic and hydrophobic forces, and strong binding may significantly affect the pharmacokinetic properties.

Warfarin primarily exists as the open-chain 4-hydroxycoumarin tautomer in aqueous environments. According to spectroscopic studies, the ionized form of the open-chain tautomer binds with HSA to distribute the drug to the body.[2] The binding study of 3,4,7,8-Tetrahydroxyflavone with HSA indicated that this molecule exists in both keto and enol tautomers; however, in HSA bound complex, both tautomers are deprotonated by releasing proton from hydroxyl group.[6] One of the tautomers has a significant interaction in one of the sites, and another tautomer has a weak interaction in the other site. Overall, the interaction of drugs with human HSA is a complex process and plays a significant role in drug transport, distribution, drug-drug interactions, clearance, and toxicity.

2.4 Excretion:

Most drugs lose their pharmacological activities mainly through metabolic transformation, which increases their polarity. This results in metabolites with high water solubility that are readily renally excreted. Other excretion routes are feces, sweat, and bile. We found no direct information on the excretion of tautomeric metabolites or preference of one tautomer over other in excretion.

The pharmacokinetics study of the tautomer of cefotetan revealed that it is converted to a tautomer with antibacterial activity similar to the parent drug, predominantly in the urine of rabbits and monkeys. However, this tautomer was not observed in the plasma of studied animals including humans (Figure 1).[7] Tautomerism may play a role in converting metabolites to final or intermediate metabolites that facilitate their easy excretion. For example, in the case of the drug elzasonan, tautomerism was reported via the formation of the indole iminium ion metabolite through ring closure and rearrangement via proton migration

between two carbons, and this metabolite was not detected in urine but excreted through feces.[8]

3. Pharmacodynamics

3.1 Drug-Target Interactions and Drug Action:

Tautomerism can affect the binding of a drug to its target and the subsequent drug action, including the drug's efficacy and potency. Erythromycin is a frequently prescribed antibiotic that exists in three tautomeric forms (one ketone and two cyclic hemiketals) (Figure 2). It has been proven that the ketonic form is active and recognized by ribosomes, inhibiting protein synthesis. The inactive tautomer can be present in up to 20% in the gastrointestinal tract. Therefore, erythromycin has to be given in a correspondingly larger dose.[9] The keto-enol tautomers of curcumin-based molecules were potent at both BACE-1 and GSK-3β targets, while diketo tautomer-based molecules were less active or inactive.[10]

In some cases, tautomers exist in equilibrium with cationic, anionic, and zwitterionic forms, and it is difficult to determine which form may be responsible for drug action. For example, edaravone is present in 50% anionic form and the rest as a mixture of three neutral tautomeric forms (Figure 2). Avobenzone is the only FDA-approved ultraviolet filter molecule that absorbs harmful UVA radiation. The protective effect and stability of this molecule is tautomer-dependent. The keto-enol tautomer protects from UVA, while diketo tautomer is susceptible to photodegradation.[11]

3.2 Blood-Brain Barrier Permeability:

Designing a molecule for targets of CNS diseases presents significant challenges as the blood-brain barrier (BBB) restricts the migration of molecules to the brain more strongly than to other body compartments. If the designed molecule is amenable to tautomerism, it may have multiple forms under physiological conditions. It should be present in sufficient amounts or exclusively to allow entry of the predominant tautomer. Edaravone is known to exist in anionic and tautomeric forms; however, a slight modification to the phenyl ring led to exclusively a keto tautomer which had good BBB permeability, while the replacement of the methyl group by trifluoromethyl led to only the enol form with poor BBB permeability.[12]

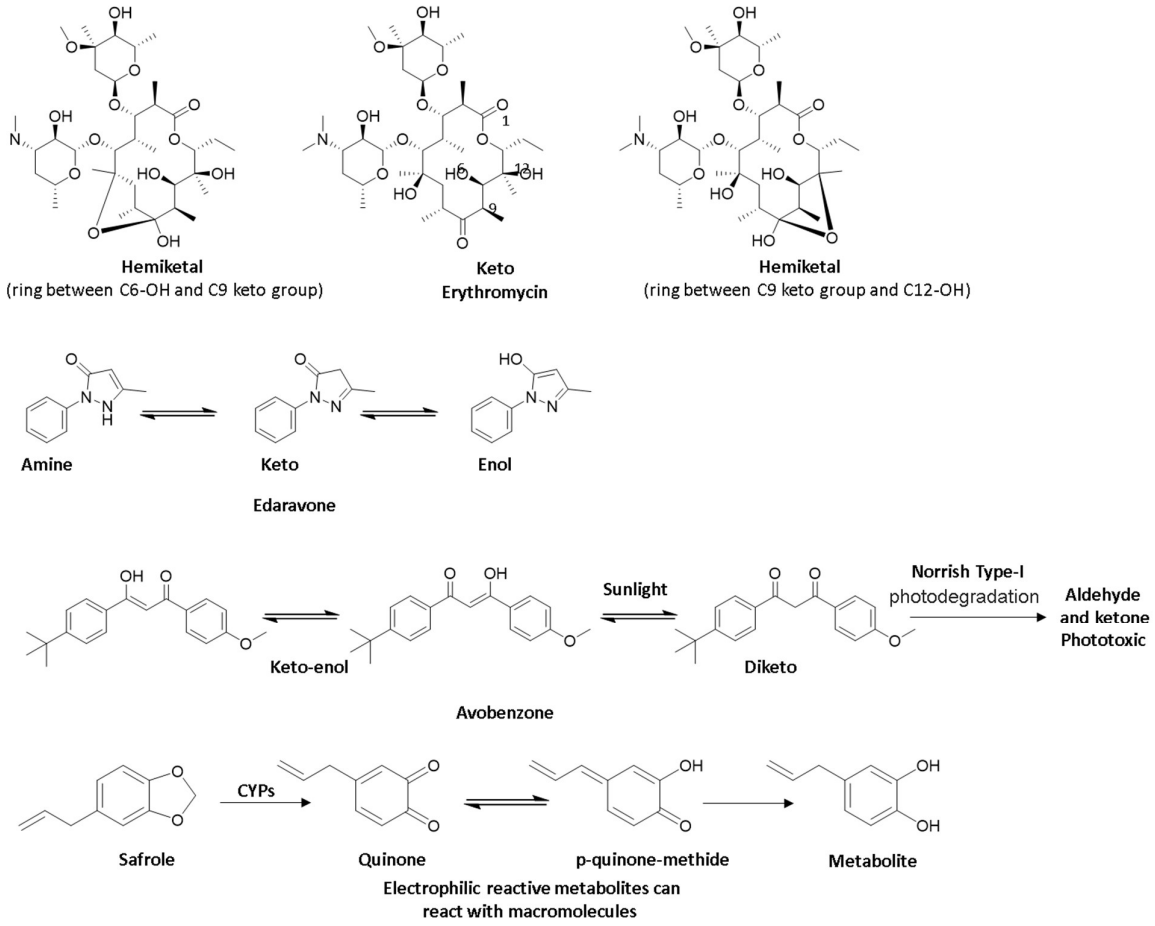

Figure 2. Examples of drugs and drug like molecules showing tautomerism-dependent drug action and toxicity.

3.3 Toxicity:

The formation of benzene-oxide is widespread in drugs containing a phenyl ring. The bicyclic benzene-oxide metabolite can equilibrate with the corresponding seven-membered oxepine by valence tautomerism. Oxepine can progress to ring opening to yield a reactive alpha, beta-unsaturated aldehyde, and trans-trans-muconaldehyde, which are known to have myelotoxicity.[13] The hepatotoxicity of methylenedioxyphenyl containing (Figure 2) molecules (safrole, and myristicin) is associated with quinone and its methide tautomer, which are generated during phase I metabolism. CYP1A2 activates safrole into the o-quinone and its methide tautomer, which leads to the inactivation of the enzyme by tightly binding with the enzyme's active site. [14] Photodegradation products of the diketo tautomer of avobenzone have phototoxic effects and cause photoirradiation, restricting its application in sunscreen products.[11]

4. Preformulation:

Tautomerism affects the solid-state properties of drug molecules in different solid states such as polymorphs, amorphous, co-crystalline, and solvates. In the solid state, proton transfer may be slow, so it may be possible to design a specific solid state with a particular tautomer. There are cases where one polymorphic form has one tautomer while the other has a different tautomer or mixture.[15] Using mechano-chemical treatment, a thermodynamically less stale tautomeric form can be transformed into a more stable tautomer. The processed solid state can be studied using FTIR, powder XRD, HPLC, UV, and optical microscopy. In addition to these, broadband dielectric spectroscopy is a very sophisticated method used to detect and monitor tautomers of drugs.[1] Different dosage forms may also affect the ratio of tautomeric forms, e.g., the thickness of the nanometric layer. The diketo form of curcumin is favored in low concentrations in liposomes. In higher concentrations, the keto-enol form is dominant in liposome-based nanoformulation.[16]

Guest systems like cyclodextrin and beta-cucurbits for pharmaceuticals have shown huge potential for improving their stability, solubility, compatibility with other ingredients, and dissolution rate. If a drug is tautomeric, these carriers selectively can entrap on tautomer over others with improved physiochemical properties. Moreover, the degree of derivatization of the carrier system can show selectivity for one tautomer over another.[17]

5. Epimerization and stereochemistry:

Rosiglitazone's antidiabetic action is related to the (S)-enantiomer. A carbonyl group is present in the proximity of the chiral centre; therefore, keto-enol tautomerism can convert the (S)-enantiomer to the (R)-enantiomer or vice versa. This rate of interconversion is very high after S-oxidation of rosiglitazone. One of the notorious examples is thalidomide, which shows therapeutic activity by the S-enantiomer, while the R-enantiomer shows teratogenicity.[3] These enantiomers undergo racemization at physiological conditions very easily via keto-enol tautomerism.

6. Expert opinion

Tautomerism has been known for over 100 years. It has significant effects on drug discovery and development. The pharmacophoric features of molecules can be changed by tautomerism, affecting the pharmacokinetics and pharmacodynamics of drugs.

Studies of thermodynamic properties (stability, solubility) are crucial in understanding and explaining drug pharmacokinetic properties and behavior during preclinical studies using in vitro and in vivo models. Tautomers are characterized using traditional spectroscopy and crystallography (X-ray) methods. Broad band spectroscopy has been instrumental in studying the behavior of tautomers in solid crystal, amorphous, and glass phases of drugs. Most drugs are represented commonly using structure determined by the X-ray method, which may be incorrect as there are many cases where the tautomer of the drug in the solid is different from the tautomer in the aqueous phase or other solvents. This tautomer seen in aqueous solution may furthermore be different under different physiological conditions.

Our recent analysis of DrugBank and other databases has indicated that around 70% of drug molecules are prone to tautomerization.[18] Currently, there are no computational tools or databases that provide information about ratios of different tautomers in aqueous solutions or other environments important for drug development. Selecting a suitable tautomer is crucial for understanding a drug molecule's binding behavior including by docking, pharmacophore mapping, 3D-QSAR, and molecular dynamics simulation.

Very fast tautomerization does not allow the separation of tautomers for clinical testing. On the other hand, half-life of tautomerization in the range of months has an impact on the formulation perspective, i.e., shelf-life of drugs. Epimerization may lead to minor tautomers as impurities if not detected during early studies. Lack of information on tautomeric preferences may affect the optimization of leads, development of a drug, its stability, drug product performance, formulation development, dose, bioavailability, manufacturing, shelf-life, and possible adverse effects and toxicities.

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Competing interests

The authors declare that they have no competing interests.

Funding Sources

The authors received funding from Department of Pharmacoinformatics, NIPER (DDK); and the NCI, NIH, Intramural Research Program (MCN).

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