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Tautomerism in drug discovery

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Abstract The influence of tautomerism on the precise structure of drugs and thus of their potential to interact with biological systems is discussed from thermodynamic and kinetic aspects. The types of tautomerism encountered in the structure of drugs in current use are surveyed together with the effect of pH, solvent polarity, and temperature.

Keywords Tautomerism · Heterocycles · Energetics · Biological processes · Drugs

Introduction

A tautomeric equilibrium is one between two or more isomeric structures of a single compound that are interconverted by movement of an atom (usually hydrogen) or group of atoms from one site to another within the molecular structure. By contrast, two or more isomers each possess the same atomic composition but in general do not interconvert easily. It must be emphasized however, that there is no clear dividing line between tautomerism and isomerism; tautomers are simply isomers that interconvert with a relatively low activation energy below ca 20 kcals/mol.

Keto-enol is probably the best known example of tautomerism. In this case interconversion of the tautomers may be acid- or base-catalyzed. The relative stability of tautomers may be judged from bond energy differences (Fig. 1) which show that, in this instance of acetaldehyde/vinyl alcohol, the keto form is the more stable isomer by 18 k cal mol^{-1}, which corresponds to an equilibrium constant of 2.6×10^{13} at 20 °C.

Tautomeric equilibria are profoundly dependent on the dielectric constant of the medium and the ability of solvents to hydrogen bond with each tautomer with more polar solvent favoring the more polar tautomer. The equilibrium composition is also influenced by temperature, with higher temperatures increasing the proportion of the least stable tautomer (the enol form) since $\Delta G^{o} = -RTlnK$. Since temperature differences are relatively small in most biological systems, temperature effects on tautomerism are unlikely to be relevant to this article or important in drug design. The influence of medium polarity, however, may be highly significant since a biological system may involve an aqueous medium (blood or plasma) or an essentially non-protic medium such as a cell membrane or possibly an enzymatic reaction center.

Molecular structure and solvent polarity clearly define the equilibrium position of a tautomeric system but the rate of interconversion is controlled by the free energy of activation between the interconverting species. If the latter are not energetically equal (i.e. $K \neq 1$) the free energy of activation from high (less stable) to low energy (more stable) species is inevitably lower than the energy for the reverse process. Thus it is important to take both kinetic and thermodynamic factors into consideration (vide infra) when assessing the influence of tautomeric structures on biological activity.

The vast majority of pharmaceutical molecules (drugs) contain heteroaromatic systems with 4-, 5- or 6-membered rings and many of these molecules are capable of existing

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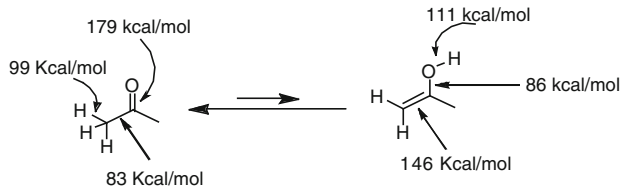


Fig. 1 Bond energy differences in acetaldehyde-vinyl alcohol, an example of keto-enol tautomerism

as two or more tautomeric structures that usually involve migration of a proton (prototropy) from one site to another within a given molecule.

The importance of prototropy is illustrated dramatically by the change in base pairing brought about by the formation of the imino tautomer of adenine. Within DNA, adenine normally pairs with thymine but the imino form of adenine pairs with cytosine (Fig. 2), thus promoting a misreading of the code: such misreadings are believed to lead to mutations and hence genetic variations that may be the basis of evolution.

Clearly therefore, it is important to recognize the potential for tautomerization in heteroaromatic systems and to assess the role of individual tautomers in biological activity.

Types of tautomerism in heteroaromatic systems

The vast majority of tautomeric equilibria in heteroaromatic molecules are prototropic, i.e. involve proton migration between (1) carbon and C, N, or O centers; (2) nitrogen and N or O centers, and (3) oxygen and O centers. Details of molecular structures falling into these three general categories have been documented extensively elsewhere [1–4] but several examples are shown in Fig. 3. In general, only those tautomeric systems in which a C–H bond is cleaved and a new C–H bond is formed are capable of isolation as separate tautomers. For example, the pairs of oxo-tautomers of many 2-hydroxy-furans, -pyrroles or -thiophens [3, 5, 6] (Fig. 3a) are separable and interconvert sufficiently slowly in solution to enable measurement of their individual physical properties.

If a labile proton attached to carbon migrates to N or O, although it is usually difficult to isolate pure individual tautomers, mixtures enriched in one tautomer can often be obtained [7–9]. However, if the migration is from N to O or O to O, proton exchange is so fast that one almost invariably obtains an equilibrium mixture, the position of which is dependent on the specific conditions (solvent/temperature). This does not mean, however, that one cannot observe individual tautomers by spectroscopic techniques–including multi-nuclear magnetic resonance, ir, uv, ms, etc.

Beyond prototropy, there are tautomeric systems involving cationic or anionic group transfers which, in some instances, manifest themselves as equilibrium mixtures of tautomers again dependent on solvent and temperature. An example of the former is the tautomeric equilibration of N-(α-aminoalkyl)-tetrazoles (Fig. 4), which is an intramolecular migration *via* ion pairs with a $\Delta G°(298\ K) \approx 0.5$ kcal mol^{-1} and a $\Delta G^{\neq}$ values of 16.3 (low to high energy) and 15.8 kcal mol^{-1} in the reverse direction [10, 11].

Tautomerism of this type, however, is not found in drugs currently widely used and will not be discussed further in this article.

Energetics

Introduction

Thermodynamic and kinetic factors are important to the understanding of tautomeric equilibria and their impact on biology.

In general if the rate at which tautomers interconvert is much slower than the biological process, the most active tautomer would be consumed but the equilibrium would not shift quickly enough to consume both tautomers. Since interaction with the biological system must be second order (at least) the concentration of the active species would influence activity and in a system,

$$T_1 \overset{K_1}{\rightleftharpoons} T_2$$

With equilibrium constant, $K_1 >> 1$ and T_1 as the active species, the rate of reaction with the biological system, S, would be given by Eq. 1 where T_T is the total tautomer concentration.

$$\text{Rate} = k_2[S][T_1] = k_2[S][T_T]/(1 + K_1) \quad (1)$$

On the other hand, if the rate of tautomeric interconversion is much faster than the biological process they

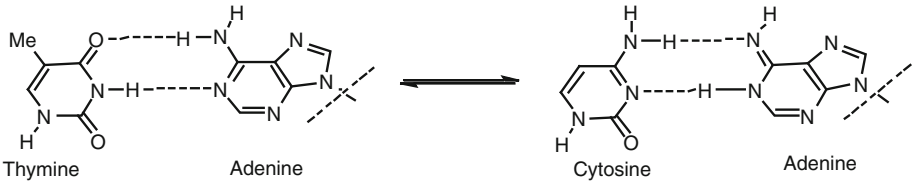
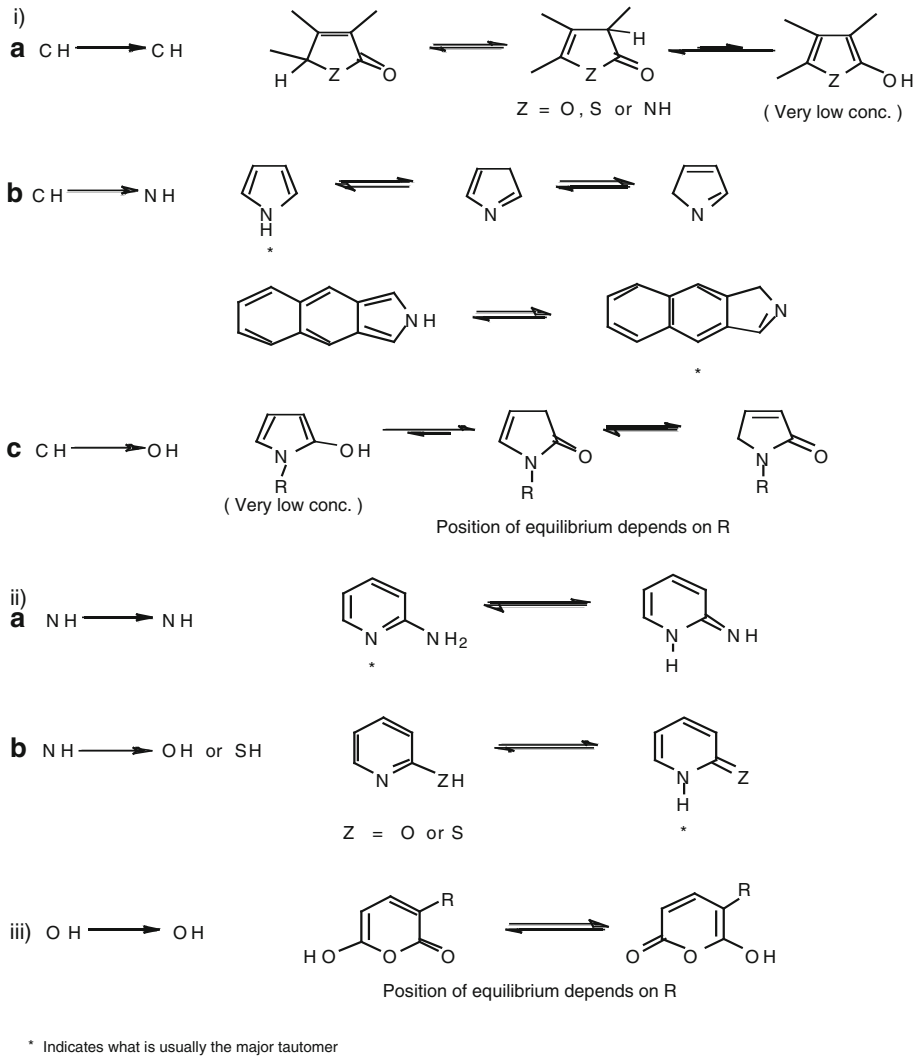


Fig. 2 Base pairing in nucleic acids: Differences for adenine between its normal amino and rare imino forms



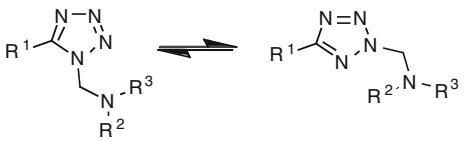


Fig. 4 Tautomeric equilibrium of N-(α-aminoalkyl)-tetrazoles

influence, the rate of interaction with the biological system again depends on the concentration of the active species but the equilibrium would shift so that all the tautomeric mixture would be consumed. Equation 1 again applies but if $K_1 << 1$, rate = $k_2[S][T_T]$ and if $K_1 >> 1$, rate = $k_2K_{-1}[T_T]$.

Energetics of biological functions

Various biological functions relative to the time scale of the life span of human kind ($t_{½}$ ca. 10^9s) are shown in Table 1 [12]. With these figures in mind, we consider the energetics of tautomer inter-conversion in terms of both thermodynamics and kinetics.

Energetics of Tautomeric Interchange

As mentioned above (Fig. 3), tautomers that interchange by CH bond cleavage/CH bond formation can often be isolated as distinct, stable species and hence the energy barrier between the tautomers must exceed 30 kcal mol^{-1} equivalent to a $t_{½}$ value $\geq 10^4$s (ca 3 h).[1] With CH to NH, OH, or SH tautomerism, however, the energy barriers are much lower and in the best-known example of keto-enol tautomerism (Scheme 1), $\Delta G^{\neq} \approx 20$ kcal mol^{-1} giving $t_{½} \approx 0.1$ s. A study of the equilibrium position for such systems is, however, available by multinuclear NMR in

[1] The indistinct line between slowly inter-converting tautomers and structural isomers is generally considered to fall somewhere in between CH/CH tautomerism ($\Delta G^{\neq} \approx 25$–$35$ kcal mol^{-1}) and *cis*/*trans* isomers ($\Delta G^{\neq} \geq 60$ kcal mol^{-1} since the average energy for cleavage of a π bond is 63 kcal mol^{-1}).

Table 1 Time scale of biological functions

Function	$\Delta G^{\neq}$ at 37 °C (kcal mol^{-1})	$t_{½}$ (s)
Macromolecular synthesis (e.g. DNA)	ca 27	$10^2 \rightarrow 10^6$
Cell division	ca 23	10^3
Muscle contraction (e.g. heartbeat)	ca 18	1
Antibody complexes, lifetime	15 → 21	$10^{-2} \rightarrow 10^2$
Enzyme-substrate complexes	10 → 13	$10^{-4} \rightarrow 10^{-6}$
Reciprocal turnovers, enzymes	11 → 16	$10^{-5} \rightarrow 10^{-2}$
Photosynthesis; α-helix base pairing	10	10^{-6}
H-bonding	4	10^{-10}

solvents with a wide range of polarity. The rates of interconversion of tautomers can also be studied by variable temperature (VT) NMR. As the temperature is increased the NMR signals from individual tautomers coalesce and at T_c, the temperature of coalescence, for a two-site exchange involving equal proportions of each tautomer, the rate of exchange at T_c (k_{Tc}) is given by Eq. 2 [13] where Δv is the maximum chemical shift difference (in Hz) of signals from each tautomer under "no exchange" conditions.

$$k_{Tc} = \pi \Delta \upsilon / \sqrt{2} \qquad (2)$$

This calculation can also be extended to tautomeric systems with unequal populations of tautomers at equilibrium [14] and thus the rate constants (and hence the $\Delta G^{\neq}$ values) at T_c in both directions may be determined. If a number of signals in any one molecular system are observed to coalesce, one may derive a series of k_{Tc} and T_c values from which an Arrhenius plot of ln k_{Tc} vs. $1/T_c$ may be generated. This applies to both ^{1}H and ^{13}C signals, or indeed, any nucleus that is observed to coalesce, and results in evaluation of E_A (and hence $\Delta H^{\neq}$ and $\Delta S^{\neq}$) over a wide range of T_c values thus obviating the inherent error of imprecise probe temperature measurements when using line shape analysis over a narrow temperature range. Knowledge of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ can provide valuable information on the mechanism (intra- or intermolecular) of tautomerism. With NH to OH or OH to OH tautomerism, the rates are very much faster and therefore rarely amenable to NMR coalescence experiments.

A survey of the top 200 drugs (of year 2010) identified some 33 containing five- or six-membered heterocyclic rings. All examples of potential tautomerism within this group involved shifts of hydrogen from N to N, N to O, O to O. These processes are all expected to be extremely fast and hence on the biological time scale, the rate of tautomerism would not impact biological processes such as DNA synthesis, cell division, muscle contraction, and probably antibody complexes. On the other hand, an understanding of the relative stability of potential tautomers may well influence enzyme activity, primary processes such as photosynthesis, and base pairing. We next discuss the various types of tautomerism found in current drug molecules.

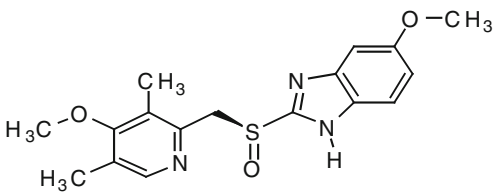
Drugs containing tautomeric systems with 5-membered rings

NH to N tautomerism

Nexium (1), a drug commonly used to inhibit gastric acid problems [15, 16] and the related compound Protonix (2) [17] both incorporate the benzimidazole unit, but there are no reported studies of their N to N prototropic tautomerism. The mode of action, however, depends on the basicity of the pyridine ring to control the proton pump of the gastrointestinal system and hence the tautomer composition is probably irrelevant (Fig. 5).

The tetrazole ring appears in a number of drugs affording N1 to N2 tautomerism, including Diovan (3) [18], Benicar (4) [19], Avapro (5) [20], Atacand (6) [21] and Hyzaar (7) [22]. ^{15}N CPMAS experiments showed that at room temperature (295 K), the four tetrazole nitrogen atoms gave a very broad signal compared to the imidazole signals, but became sharp at 253 K. These findings are

Fig. 5 Structure of Nexium (1) and Protonix (2)



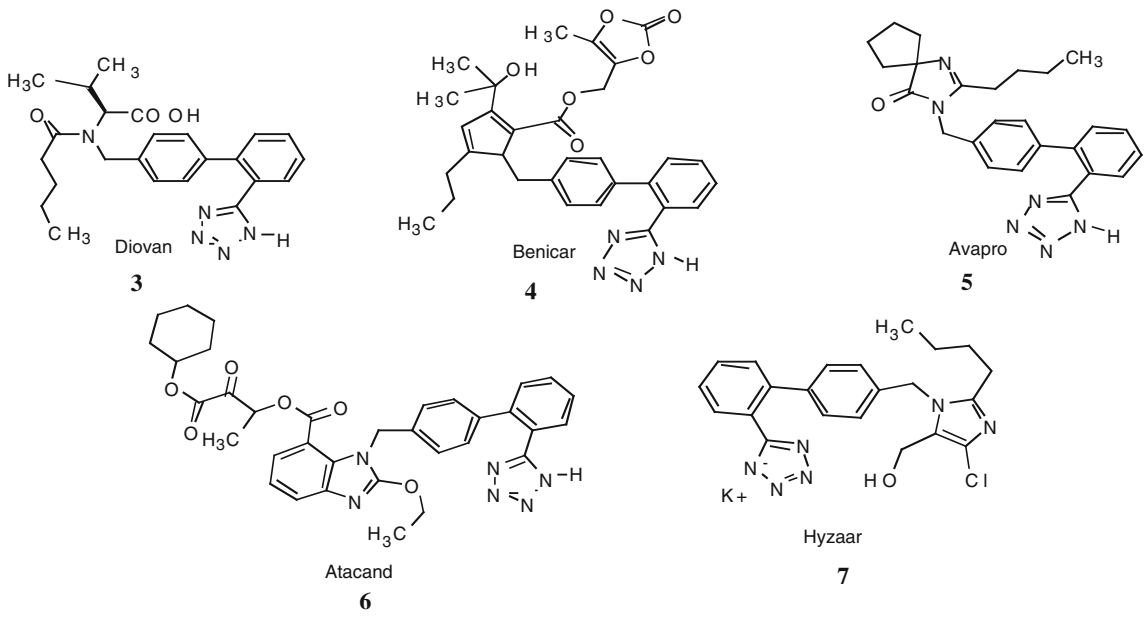


Fig. 6 Structure of drugs 3–7

consistent with fast prototropic exchange as proposed by Harris et al. [23].

These tetrazoles are generally used as cardiovascular or hypertension drugs and the acidity of the tetrazole ring system ($pK_a \sim 4.8$) [24] means they exist largely as anions in biological systems which effectively eliminates the relevance of tautomerism. Lonsartan potassium salt or Hyzaar (7), the parent drug was approved by FDA 1995 under the commercial name COZAAR. Early studies showed that losartan degrades by dimerisation [25] and the drugs act on very specific targets such as cell receptors of Angiotensin II [26] (Fig. 6).

Imidazolidine-2-imine derivatives include Catapres-TTS (**8**) and Alphagan P (**9**) [27] and NMR studies plus computational studies on the former [28] suggest the imino tautomer is more stable than the amino tautomer by ca. 7 kcal mol^{-1} ($K = 8 \times 10^4$ at 37 °C). Lack of experimental information on the relative stabilities or rates of interconversion of the tautomers however, precludes speculation on their importance to biological activity. Suffice it to note that either or both could be active. On the other hand, 2-aminothiazole derivatives exemplified by Mirapex (**10**) [29] exist, as expected, in the aminothiazole form (Fig. 7).

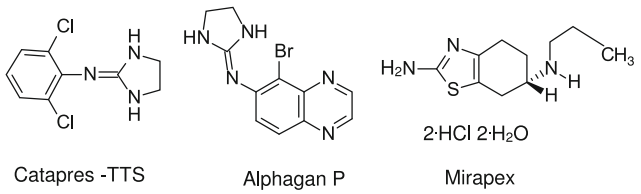


Fig. 7 Structure of drugs 8–10

NH to O tautomerism

The examples of drugs containing this type of tautomerism all involve proton migration from N to amide carbonyl oxygen as found in thiazolidine-2,4-dione derivatives Avandia (**11**) [30], Actoplus Met (**12**) [31], or oxazolidine-2-one derivatives Skelaxin (**13**) [32], and Zomig (**14**) [33]. All of these compounds exist predominantly in the NH form, although the possible significance of the alternative tautomer is recognized for Avandia. Since proton migration would be fast, one or both tautomers could again be available for biological activity (Fig. 8).

6-Membered ring drugs containing tautomeric systems

NH to N tautomerism

Drugs with potential NH to N tautomerism include those used for the treatment of HIV **15** [34, 35], **16** [36], epilepsy (**17**) [37], and skin (**18**) [38, 39], lung and pancreatic cancers (**19**) [40–42]. They all involve amine (NH_2 or NH) to imine tautomerism a change that decreases the aromaticity of each heteroaromatic system. Thus the predominant tautomers in these cases contain the amino group (Fig. 9).

NH to O tautomerism

Seven topical drugs **(20–26)** that could exhibit potential NH to OH tautomerism are all examples of cyclic amide-enamide interconversion and in most cases exist as the amide tautomer which provide centers for the formation of

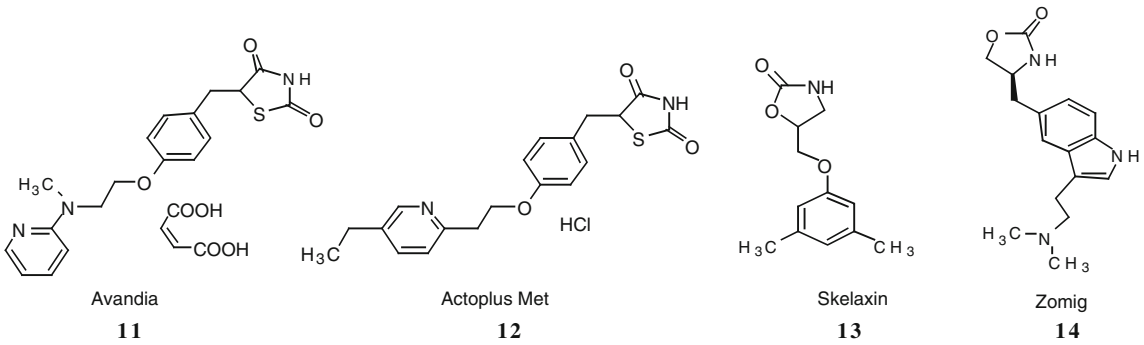


Fig. 8 Structure of drugs 11–14

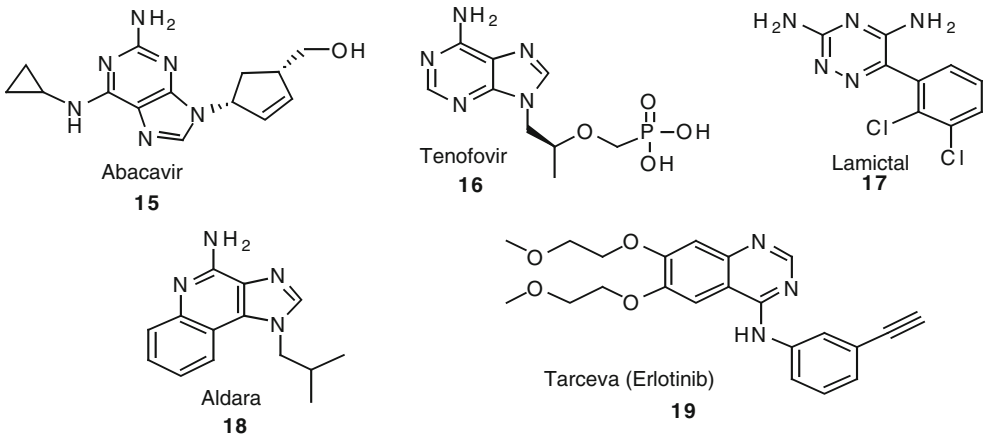


Fig. 9 Structure of drugs 15–19

intermolecular hydrogen bonds. These drugs are used as sedatives (**20** [43], **22** [44, 45]) and for the treatment of myeloma (**20** [43], **21** [46, 47]), HIV (**22** [44, 45], **24** [48]), schizophrenia (**23**) [49], and Benign prostatic hyperplasia (BPH) (**25** [50, 51], **26** [52]). No studies of tautomerism within these molecular systems have been reported (Fig. 10).

NH to N and NH to O tautomerism within a single molecule

Examples (**27–33**) (Fig. 11) of drugs with potentially both NH to N and NH to O tautomerism, all contain amino-pyridone units: (**27** [53, 54], **28** [55, 56], **29** [57–59], **31** [60–62], **32** [63–65]); these are all used as antiviral agents including the treatment of HIV. Shift of a proton from NH_2 to a heterocyclic N greatly reduces the aromatic character of the pyrimidone ring whilst generating the energetically unfavorable imino tautomer. The potential for NH to O tautomerism is again exhibited in a cyclic amide group with its usual polarity and H-bonding capacity. Xeloda (**33**) [66]–an anti-cancer agent–contains a carbamic ester group which would compete with NH to N tautomerism.

AM1-RHF type semiempirical calculations of Viagra (**30**) [67] revealed that the pyrimidone tautomers (**30A** and **30B**) are more stable than the pyrimidinol tautomer (**30C**) (Fig. 12) [68].

The influence of pH

The pKa values of most of the drugs mentioned are shown in Table 2 but it should be noted that not all of the values refer to the potentially tautomeric proton. The pKa of the tautomeric proton is, however, an important factor since, if it falls below normal physiological pH (7.2) the molecule will exist as the anion and the resultant delocalized anion would render tautomerism of the system redundant. This is the case for compounds **1–7**. The first two (Nexium and Protonix) each contain a benzimidazole unit that would be ionized at pH 7.2. Since both drugs are used to treat gastric problems, however, they would experience pH values of 1–2 under which conditions they would be protonated. However, in principle, Nexium could exist as two tautomers by virtue of the unsymmetrical structure imposed

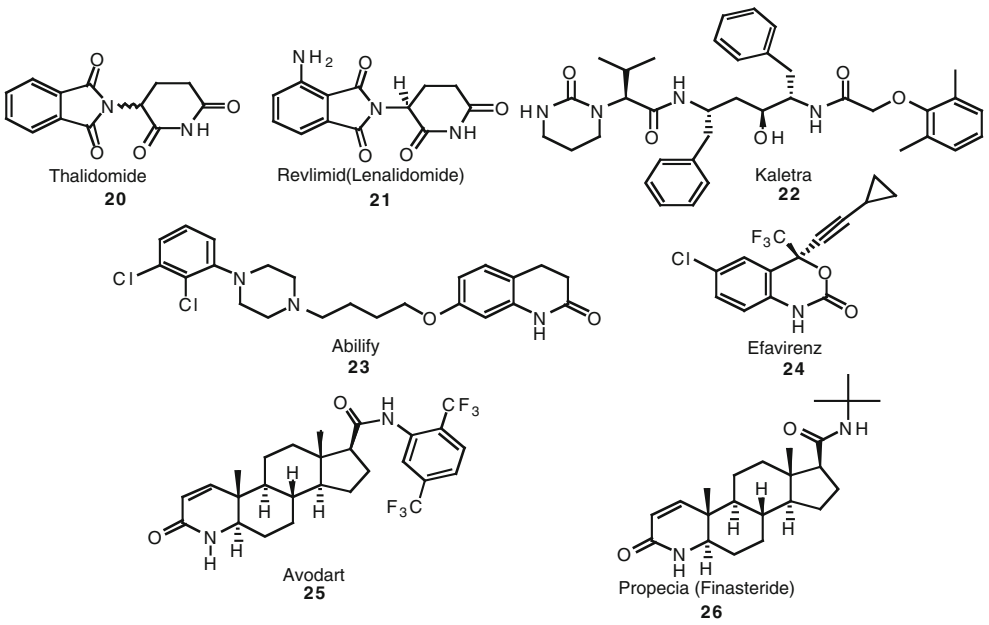


Fig. 10 Structure of drugs 20–26

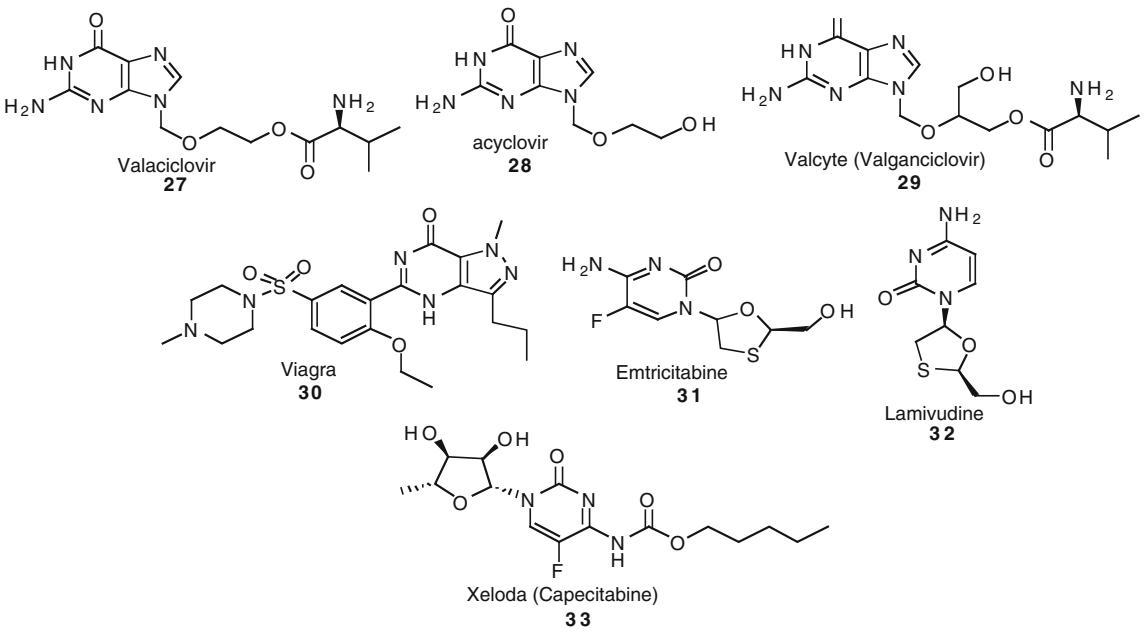


Fig. 11 Structure of drugs 27–33

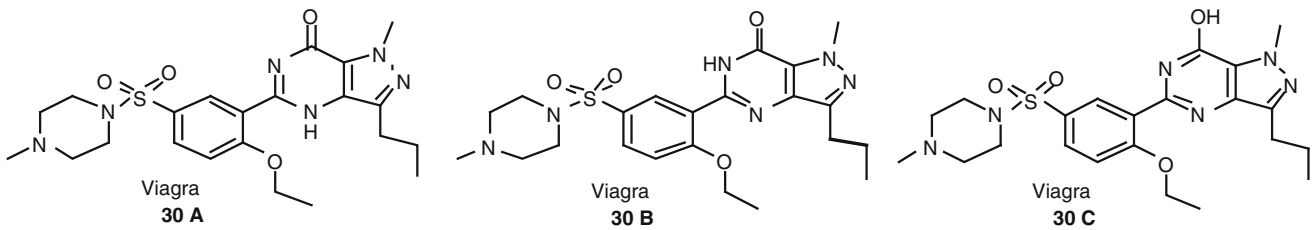


Fig. 12 Possible tautomers of Viagra

Table 2 Drug's pKa

Entry	Drug	pKa	Entry	Drug	pKa
1	Nexium	4.00 (A) and 8.80 (L)	18	Aldara	7.30 (A)
2	Protonix	≈4.00 (A)	19	Tarceva (Erlotiib)	5.40 (A)
3	Diovan	NA	20	Thalidomide	3.75 (?)
4	Benicar	4.30 (L)	21	Revlimid	NA
5	Avapro	4.20 (L)	22	Kaletra	NA
6	Atacand	NA	23	Abilify	7.60 (A)
7	Hyzaar	NA	24	Efavirenz	10.20 (?)
8	Catapress TTS	9.50 (A)	25	Avodart	NA
9	Alphagan P	7.40 (A)	26	Propecia	9.41 (?)
10	Mirapex	9.60 (A)	27	Valaciclovir HCl (Valtrex)	1.90, 7.47, and 9.43
11	Avandia	6.10 (A) and 6.80 (L)	28	Acyclovir (Zovirax)	2.27 and 9.25
12	Actoplus MET	12.40 (L)	29	Valganciclovir HCl (Valcyte)	7.60 (A)
13	Skelaxin	NA	30	Viagra	NA
14	Zomig	9.64 (A)	31	Emtricitabin (Emtriva)	2.65 (A)
15	Abacavir	5.01 (A)	32	Lamivudine	4.30 (A)
16	Tenofovir DF	3.75 (L)	33	Capecitabine (Xeloda)	5.41 (?)
17	Lamictal	5.70 (A)			

NA data are not available

In Table 2 we have recorded reported values for the pK_as of the drugs. In some cases it is clear whether these values refer to proton loss (L) or proton addition (A). However, in other cases, it is not clear what the reported pK_a values refer to, and in some cases these reported values appear to be very uncertain and unreliable (?). We have not commented in detail on these cases

by the methoxy-substituted benzimidazole unit whereas the tautomeric structures in Protonix are equivalent. Compounds **3–7**, used as cardiovascular or hypertension drugs, all contain a tetrazole unit with a pKa value of ca. 4.8. Thus, at normal physiological pH they would be present as anions and tautomerism would be irrelevant.

The pKa values of Catapress **(8)** and Alphagan P **(9)** at 9.5 and 7.4 respectively obviously refer to proton addition but tautomerism between the more stable neutral imino form and the neutral exocyclic amino structure is likely to be fast on most biological time scales so either structure could be active.

Compounds **11–14** all involve amide tautomerism with the NH form predominating but fast prototropy could render either amide or the imidol structure available for biological activity. The same applies to compounds **20-26**. Compounds **15–19** all involve NH (or NH_2) to ring N tautomerism but since the imino form substantially reduces aromaticity in the hetero ring, the amino form greatly predominates. Fast prototropy could, however, again make either structure available for therapeutic activity. The pKa values (apart from Tenofovir DF, **16**, containing the $-P(O)(OH)_2$ group at pKa 3.75) all refer to proton addition to the ring N.

Compounds **27–33** all contain both NH to N and NH to O potential tautomerism within aminopyrimidone units. At physiological pH, all of these molecules probably remain unprotonated and the pyrimidinone structures would predominate in fast prototropic equilibria.

Conclusions

Tautomerism in drug molecules almost invariably involves prototropy but in all cases the influence of a single tautomer on therapeutic activity depends on the time scale of the tautomeric equilibrium relative to that of the biological process in question. Thus in judging the influence of tautomerism on biological activity, it is essential to consider both thermodynamic and kinetic factors. Fast interconversion of tautomers relative to a specific biological process means that both tautomers may be consumed. Conversely, slow interconversion relative to the biological process may result in one tautomer being the only active species.

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