

Hydrogen Bonding: The Last Mystery in Drug Design?

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1. Introduction

Life on earth depends on water, on hydrogen bonds, and on hydrophobic interactions. DNA and proteins are held together in their defined three-dimensional structures primarily by hydrogen bonds. The double helix of DNA, RNA structures, peptide and protein secondary structures, like α -helices, β -sheets, β - and γ -loops, and the tertiary structures of proteins are formed by hydrogen bonds (enthalpic contributions) and by hydrophobic contacts (primarily entropic contributions). With a few exceptions, *e.g.*, the binding of retinol to RBP and of some ligands to the aromatic-hydrocarbon (Ah) receptor, also the formation of ligand-protein complexes depends on hydrogen bonding.

In ligand binding, three different contributions arise from hydrogen bonds:

- 1) Orientation of the ligand by a binding partner, sometimes associated by a conformational distortion of the molecules.
- 2) Recognition of substrates, inhibitors, agonists, and antagonists; differentiation between sterically similar but chemically different ligands, *e.g.*, the steroid hormones; recognition of nucleic-acid bases in DNA replication and translation.
- 3) Affinity of ligands – the most important issue in ligand design.

Of course, hydrogen bonding also affects membrane transport, as well as the distribution of drugs within the biological system; these issues are addressed in many other chapters.

Whereas hydrophobic interactions between lipophilic surfaces of a ligand and hydrophobic areas of its binding site always contribute to affinity in a positive manner (sometimes reducing water solubility to such an extent that

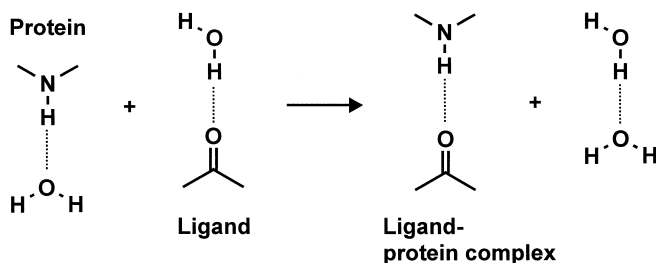


Fig. 1. Solvation and desolvation in the formation of a ligand-protein complex

unfavorable pharmacokinetic properties result), the influence of hydrogen bonding needs a more careful inspection. In ligand-protein complex formation, surrounding water molecules compete with binding. Desolvation of the free ligand and the binding site has to be taken into consideration. Binding of the ligand to its specific site will be favored if the energy of the hydrogen bonds in the complex and the entropy gain in releasing some bound water molecules are more favorable than the free-energy contribution of the hydrogen bonds between the binding partners, in their free state, and these water molecules (*Fig. 1*) [1–4].

Several examples of this chapter illustrate the role of hydrogen bonds in ligand binding and the unexpected affinity changes that occur after replacement of polar groups of ligands. Sometimes, such groups can be removed without loss in affinity, especially if a different conformation of the binding site changes the surface properties. Other examples demonstrate the influence of hydrogen-bond patterns on the binding mode of ligands, the effect of the replacement of water molecules within the binding site, and the ‘use’ of water molecules by a ligand to enhance its affinity by several orders of magnitude.

2. Orientation and Conformation of Ligands in Their Binding Site

Ligands that interact by hydrogen bonds with certain functional groups of a binding site can only bind in a certain orientation. The directionality of hydrogen bonds, demanding optimum distances and angles, is well understood and supported by statistics from the Cambridge Structural Database (*Fig. 2*) [2][5–9].

However, there are distinct differences in the strength of such hydrogen bonds. In competitive situations (*e.g.*, the sp^2 -oxygen atom vs. the sp^3 -oxygen atom in esters; nitrogen vs. oxygen atoms in methoxypyridines, oxazoles, and isoxazoles), hydrogen bonds are formed to sp^2 -oxygen atoms and to

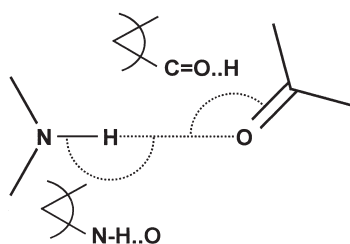


Fig. 2. *Geometry of a hydrogen bond.* The distance $N\cdots O$ is typically between 2.8 and 3.2 Å and the angle $N-H\cdots O$ is most often larger than 150° . The angle $C=O\cdots H$ has a much broader range; typical values are between 100 and 180° .

Table 1. *Lipophilicity Values of Hydrocarbons and Ethers* (octanol/water log P^* values; MedChem database)

Compound	X = -CH ₂ -	X = -O-	$\Delta O/CH_2$
Et-X-Et	3.39	0.89	-2.50
Phe-X-Et	3.72	2.51	-1.21
Phe-X-Phe	4.14	4.21	+0.07

nitrogen atoms, respectively. A theoretical study and statistical analyses of oxygen and nitrogen atoms as hydrogen-bond acceptors suggest that hydrogen bonds to sp^3 -oxygen atoms that are directly linked to an sp^2 -carbon atom (like in esters, aromatic ethers, and furans) are rather rare [10]. The poor hydrogen-bond-formation capacity of such oxygen atoms is also reflected by a significant decrease of their polarity in going from aliphatic to araliphatic and to aromatic ethers $R-O-R'$ (Table 1) [11].

The significant contribution of hydrogen bonds to ligand orientation can be demonstrated by the binding modes of dihydrofolate (DHF) and methotrexate (MTX) to dihydrofolate reductase (DHFR) (Fig. 3). A different binding mode of the DHF complex was predicted from the 3D structure of the MTX/DHFR complex [12] and later experimentally confirmed by the crystallographic 3D-structure determination of the DHF/DHFR complex [13].

In comparing the binding geometries of ligands, it makes quite a difference whether substrates, inhibitors, allosteric effector molecules, receptor agonists, or antagonists are considered. A substrate of an enzyme has to be converted into a product; thus, the enzyme will distort this ligand into the direction of the transition-state geometry. A well-known example is the binding of creatine and carbamoylsarcosin to creatinase, in conformations with nonplanar guanidino and urea systems [2]. The situation is different with enzyme inhibitors that most often bind with high affinity. Different ligand conformations may be observed in solution, in the crystal structure, and in the

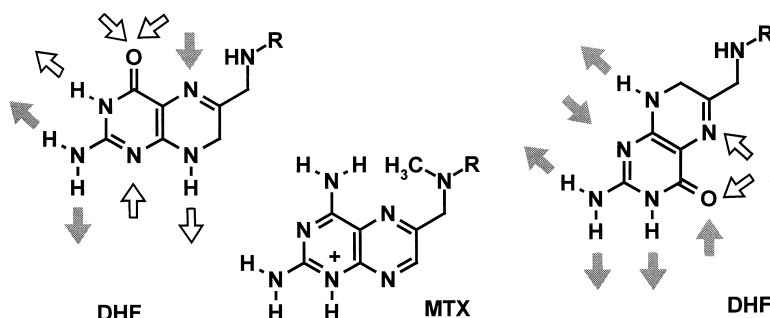


Fig. 3. The chemical structures of DHF and MTX look very similar. However, a closer inspection of the hydrogen-bond donor and acceptor patterns of both compounds shows that by a simple atom-by-atom superposition of both molecules only three donor and acceptor functions point in the same direction (left; filled arrows indicate identical hydrogen-bond directions). In the other binding mode (right), DHF has six donor and acceptor functions in the same place as in the MTX complex.

ligand-protein complex; however, if too much free energy of binding is wasted to distort the inhibitor and/or the protein into an energetically unfavorable geometry, the affinity of the ligand and, correspondingly, its activity will be significantly reduced.

3. Hydrogen Bonds and Ligand Recognition

Enzymes and receptors most often show high specificities for their substrates and agonists, respectively. Exceptions are some metabolic enzymes, *e.g.*, the cytochromes that oxidize a large number of different drugs, and some transporters. An oligopeptide-binding protein (OppA) from *Salmonella typhimurium*, binding peptides of two to five amino-acid residues without regard to their sequence, shows a remarkable lack of substrate specificity. The OppA crystal structure reveals that the ligands are completely buried within the protein. The preference for short-chain peptides is achieved by utilizing the hydrogen bonds and the electrostatic potential of the main-chain functional groups; the rest of the binding pocket is filled with water molecules to accommodate very different amino-acid side chains [14].

On the other hand, steroid-binding proteins show high specificity [15]. All steroid receptors have a conserved arginine in the binding site for the steroid ring A. Receptors for 3-keto steroids have, in addition, a conserved glutamine, whereas only the oestrogen receptor has a glutamate in this position, which allows to accommodate the 3-hydroxy group of the aromatic ring A of oestrone and its analogues (*Fig. 4*) [16][17].

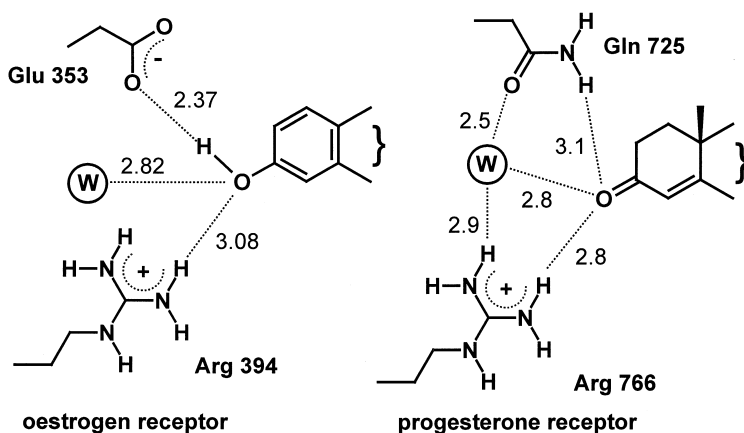


Fig. 4. Schematic representation of the hydrogen-bond network in ligand binding at the oestrogen and progesterone receptors (distances in Å)

Crystallographic investigations of steroid-binding antibodies reveal that some 4,5-didehydro-steroids bind in a regular mode, with the 18- and 19-methyl groups pointing 'upwards'; some 5β -H analogues bind in a 'bottom-up' mode, where the whole steroid-ring system flips upside-down and the methyl groups point 'downwards' [15]. Superpositions of 3-keto-17-hydroxysteroids and 3-hydroxy-17-ketosteroids, using a modified version of the computer program SEAL [18][19], indicate that these steroids may bind to the corticosteroid-binding globulin in a different mode. Whereas all 3-keto-17-hydroxysteroids, including the 3,20-diketo-21-hydroxycorticosteroids, bind in a normal mode, the 3-hydroxy-17-ketosteroids most probably bind in a 'head-to-tail' mode, with the 17-keto group mimicking the 3-keto group and the 3-hydroxy group mimicking the 17-hydroxy group [20]. In these orientations, the overall shape of the analogues has a high degree of similarity. This result clearly indicates the importance of hydrogen-bonding potentials in molecular-superposition programs.

In drug design, it is most important to realize that ligands are recognized by properties, not by their molecular structure. Analogues which exert similar interactions with the functional groups of the binding site have similar affinities, even if their chemical structures are quite different. Scytalone-dehydratase inhibitors are just one example; salicylamides as well as quinazolines are subnanomolar inhibitors of this enzyme, despite the differences in their chemical structures (*Fig. 5*) [21]. More examples are found in the literature [3][22].

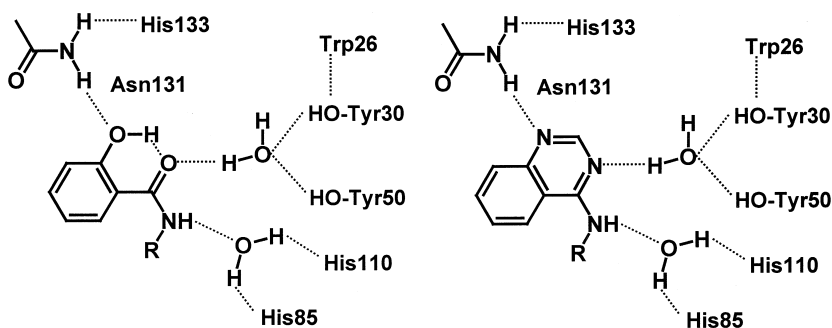


Fig. 5. Salicylamides ($R = -\text{CH}(\text{CH}_3)\text{C}_6\text{H}_4\text{-p-Br}$, $K_i = 0.14 \text{ nM}$) and quinazolines ($R = -\text{CH}_2\text{-CH}_2\text{-CH}(\text{C}_6\text{H}_5)_2$; $K_i = 0.15 \text{ nM}$) bind to scytalone dehydratase in a comparable manner. The hydrogen bonds between the interacting groups are identical, despite the differences in the chemical structures.

4. Ligand Affinities: The Replacement of Polar Groups by Nonpolar Groups

The affinities of ligands to their binding sites depend on many different factors [2][4][23] but only the influence of hydrogen bonds shall be discussed here. From the very first investigations on tyrosyl-AMP binding to tyrosyl-tRNA-synthase mutants, values of $2\text{--}6 \text{ kJ} \cdot \text{mol}^{-1}$ for neutral hydrogen bonds and up to $20 \text{ kJ} \cdot \text{mol}^{-1}$ for ionic hydrogen bonds have repeatedly been reported in the literature, corresponding to an affinity enhancement by factors of $2\text{--}15$ and up to 3000 , respectively (for a review, see [4]). However, there are many exceptions to these values and, in general, we have to understand that there is no clear relationship between the number of hydrogen bonds of a ligand to its binding site and the corresponding binding affinity [1] [2].

The allosteric effector 2,3-diphosphoglycerate forms seven ionic hydrogen bonds to human hemoglobin but, nevertheless, shows only millimolar affinity [24]; on the other hand, the sulfate ion, forming seven neutral (!) hydrogen bonds to its binding site, has an affinity value $K_i = 120 \text{ nM}$ to the sulfate-binding protein of *Salmonella typhimurium* [25].

An extreme effect of a single ionic hydrogen bond has been observed in a Gln102Arg mutant of lactate dehydrogenase. Whereas the wild-type enzyme has a selectivity for lactate, as compared to malate, of $1000:1$, it is converted into a malate dehydrogenase on introduction of Arg102. Due to the new ionic hydrogen bond between the malate γ -carboxylate group and the arginine side chain, the reaction rates are now $10\,000$ times faster for malate than for lactate [26], resulting in a selectivity change of seven orders of magnitude!

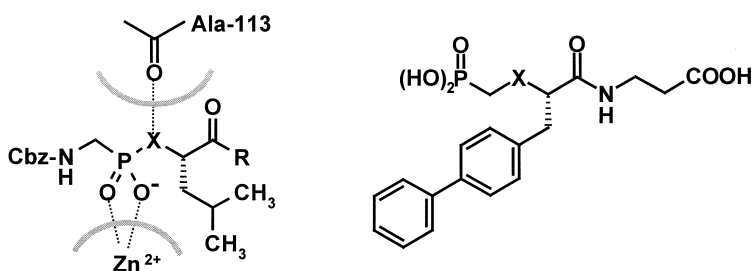


Fig. 6. The binding mode of thermolysin inhibitors ($X = -NH-$, $-O-$, $-CH_2-$; left) and the chemical structure of NEP 24.11 inhibitors (right)

The influence of replacing an $>NH \cdots O = C <$ hydrogen bond has been extensively studied in a series of thermolysin inhibitors (Fig. 6). The replacement of the $-NH-$ group of the ligands by $-O-$ reduces binding affinities by a factor of 1 000, which has been explained by the lack of the $>NH \cdots O = C <$ hydrogen bond and a mutual repulsion of the two electronegative oxygen atoms. On the other hand, it has been predicted that $-CH_2-$ analogues should have about the same affinities as the $-NH-$ analogues, which turned out to be true – one of the rare cases of a correct quantitative prediction from molecular-modelling studies [27]. The reason for this surprising effect is that there is no hydrogen bond between $-CH_2-$ and $O = C <$, but there is neither repulsion between these two groups nor a negative effect of desolvation of the $-CH_2-$ group in the ligand.

However, such structure-activity relationships cannot be transferred to other series. In some NEP 24.11 inhibitors (Fig. 6), the $-O-$ and $-NH-$ analogues show about the same affinities ($IC_{50} = 1.6$ and 10 nM), whereas the $-CH_2-$ and $-S-$ analogues are much less active ($IC_{50} = 1000$ and 800 nM) [28]; in this case it has to be assumed that the binding partner of these functional groups is a donor/acceptor function, like serine, threonine, or tyrosine hydroxy groups, or the side chains of asparagine or glutamine.

Thrombin inhibitors are investigated because of their therapeutic potential to prevent coagulation disorders. Several pairs of ligands, where either the $-NH-$ or $>C = O$ groups that form hydrogen bonds to Gly216 of the enzyme have been replaced, show affinity differences that vary in an unpredictable manner. If the $-NH-$ group of *N*-methyl-D-phenylglycyl-Pro-ArgH is replaced by $-CH_2-$ or if the NH_2 group of D-Phe-Pro-Arg-H is eliminated, affinities are only reduced by a factor of 8 (Table 2) [29][30].

If, on the other hand, the ligand carbonyl groups that interact with the Gly216 $-NH-$ group are replaced by $-CH_2-$, creating a more flexible molecule and an (additional) basic center, affinities are reduced by factors of 400–10 000 [29–31]. If in a bicyclic imide inhibitor the carbonyl group that

Table 2. Influence of the Modification of Polar Groups on Ligand Affinities

Pair of thrombin inhibitors	Thrombin inhibition	Change	Ratio	Ref.
<i>N</i> -Me-D-Phenylglycyl-Pro-Arg-H 2-Phenylbutyryl-Pro-Arg-H	$IC_{50} = 0.009 \mu\text{g/ml}$ $IC_{50} = 0.07 \mu\text{g/ml}$	-NH- to -CH ₂ -	8	[29]
D-Phe-Pro-Arg-H Phenylpropionyl-Pro-Arg-H	$IC_{50} = 49 \text{ nM}$ $IC_{50} = 390 \text{ nM}$	-NH ₂ to -H	8	[30]
3-Cyclohexylpropionyl-Pro-Arg-H 4-Cyclohexylbutyl-Pro-Arg-H	$IC_{50} = 0.15 \mu\text{M}$ $IC_{50} = 56 \mu\text{M}$	>C=O to -CH ₂ -	400	[30]
Boc-D-Phe-Pro-Arg-H 3-Phenyl-1,2-(Boc-NH)-propyl-Pro-Arg-H	$IC_{50} = 0.028 \mu\text{g/ml}$ $IC_{50} = 52 \mu\text{g/ml}$	>C=O to -CH ₂ -	2000	[29]
<i>N</i> -Acetyl,4- <i>O</i> -benzyl-Hyp-Arg-CMK <i>N</i> -Ethyl,4- <i>O</i> -benzyl-Hyp-Arg-CMK	$IC_{50} = 0.9 \text{ nM}$ $IC_{50} = 8600 \text{ nM}$	>C=O to -CH ₂ -	10000	[31]
Bicyclic ETH inhibitor, imide Bicyclic ETH inhibitor, lactame	$K_i = 0.5 \mu\text{M}$ $K_i = 2.0 \mu\text{M}$	>C=O to -CH ₂ -	4	[32]

Abbreviations: Arg-CMK = arginine chloromethylketone, Arg-H = arginine aldehyde, Boc = *tert*-butoxycarbonyl, Hyp = 4-hydroxyproline, Me = methyl, Phe = phenylalanine, Pro = proline.

interacts with Gly216 is reduced to -CH₂-, affinity decreases only by a factor of 4 [32].

Sometimes, it seems that exact shape similarity is more important for affinity than the formation of hydrogen bonds. 2,4-Difluorotoluene, sterically mimicking thymine, is a striking example. Although this analogue does not form hydrogen bonds, it codes specifically and efficiently for adenine in DNA replication, due to a perfect steric mimicry [33][34].

Some more problems in affinity predictions and in rational drug design arise from the flexibility of binding sites [2-4][22]. Neuraminidase of the influenza virus (common flu) cleaves sialic acid from cell-surface carbohydrate chains to enable the virus to enter and leave the cell in which it is reproduced. Already several years ago, the low-affinity ligand Neu5Ac2en (*Fig. 7*) was designed from a postulated transition state of the enzymatic reaction; in addition to some other important interactions, this ligand forms hydrogen bonds between its glycerol side chain and Glu276 of the enzyme. A detailed analysis of the binding site with the computer program GRID [35] showed that basic groups in the 4-position should enhance affinity by interaction with two other acidic residues, Glu119 and Glu227. Indeed, Zanamivir (4-Guandino-Neu5Ac2en; *Relenza*[®], *Glaxo Wellcome*, UK; *Fig. 7*) is a subnanomolar neuraminidase inhibitor that is systemically active after inhalation [36][37]. However, by rearrangement of the Glu276 side chain, this area becomes a hydrophobic pocket that can accommodate a lipophilic side chain, as in the *Gilead Sciences* neuraminidase inhibitor GS 4071 [38][39]; its prodrug

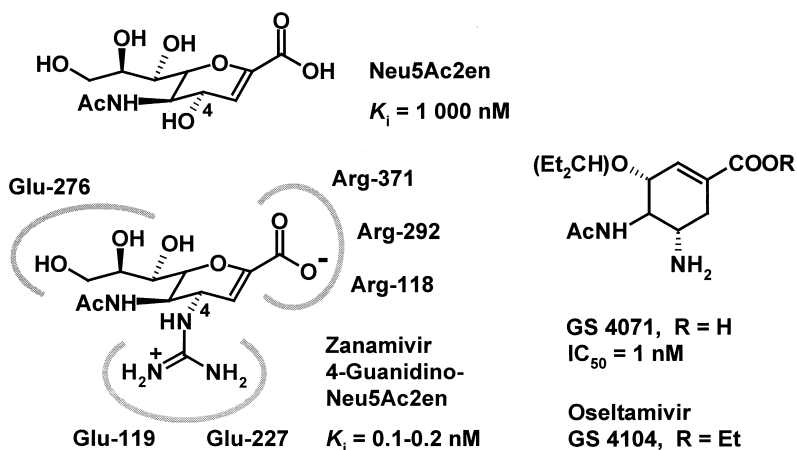


Fig. 7. The neuraminidase inhibitor Zanamivir (GG 167, *Relenza*[®]) was designed with the computer program GRID, starting from the weak transition-state inhibitor Neu5Ac2en (left). GS 4071 has a lipophilic ether group instead of the glycerol side chain of Zanamivir; the high affinity results from a rearrangement of some amino acids within the binding pocket. Its ethyl ester Oseltamivir (GS 4104, *Tamiflu*[®]) is an orally active drug.

Oseltamivir (GS 4104; *Tamiflu*[®], *Hoffmann-La Roche*, Switzerland; Fig. 7) is orally active.

5. Conserved Water Molecules in the Binding Site

Various ligand-protein 3D-structures demonstrate the importance of conserved water molecules in the ligand-binding site [3][22][40]. Most often, hydrogen-bond networks between the ligand and the protein include such water molecules (compare, *e.g.*, Figs. 4 and 5). The attempt to replace such water molecules most often fails; reduced affinities result if the energy of desolvation is larger than the energy contribution of the new hydrogen bond. Only one example for such an ‘unexpected’ lower affinity is the binding of a modified cyclosporin A, 2-(5-hydroxynorvaline)-cyclosporin A, to cyclophilin; the newly introduced $-\text{CH}_2\text{OH}$ group indeed replaces a conserved water molecule in the binding pocket, but affinity is reduced by about one order of magnitude [41].

However, there are never rules without exceptions. In quinoline analogues of scytalone-dehydratase inhibitors, one aromatic N-atom of the quinazoline ring (Fig. 5; R = $-\text{CH}_2-\text{CH}_2-\text{CH}(\text{C}_6\text{H}_5)_2$; $K_i = 0.15\ \text{nM}$) is replaced by a C–H moiety (Fig. 8). Obviously, the aromatic hydrogen atom interferes sterically with the conserved water molecule, and biological activity is reduced by

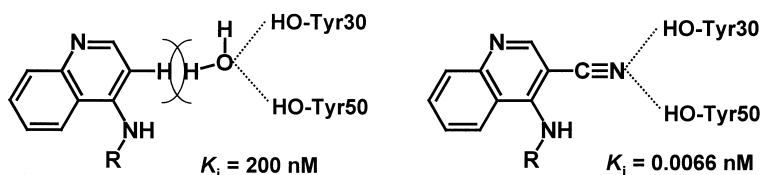


Fig. 8. Replacement of one aromatic nitrogen of the quinazoline inhibitor (Fig. 5; $R = -CH_2-CH_2-CH(C_6H_5)_2$; $K_i = 0.15$ nM) by $-CH-$ leads to an overlap of the aromatic hydrogen atom with the conserved water molecule; biological activity is reduced by about 3 orders of magnitude. On the other hand, exchange of this disturbing hydrogen atom against a cyano group leads to a replacement of the conserved water molecule; biological activity is enhanced by a factor of 30000.

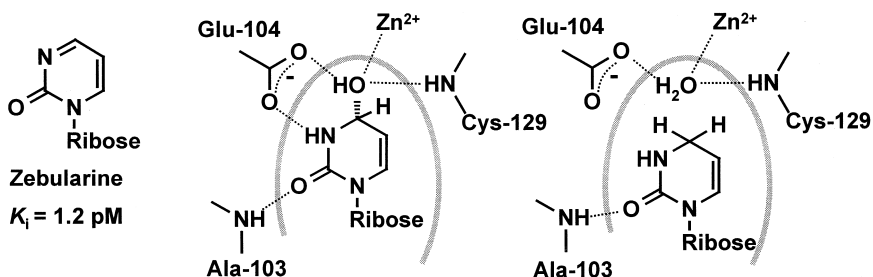


Fig. 9. The cytidine-deaminase inhibitor zebularine (left) 'uses' a conserved water molecule to mimic the transition state of the enzymatic reaction (middle). 3,4-Dihydrozebularine ($K_i = 30$ μ M) can neither add this water molecule nor replace it; affinity is reduced by more than seven orders of magnitude.

about 3 orders of magnitude. On the other hand, exchange of this hydrogen atom by a cyano group leads to a replacement of the conserved water molecule (Fig. 8); and biological activity is enhanced by a factor of 30000. Seemingly, the cyano group binds more strongly to Tyr30 and Tyr50 than the water molecule because there is still a 20-fold affinity increase, as compared to the quinazoline inhibitor (Fig. 5) [21]. An additional favorable entropic contribution may arise from the release of the bound water molecule. With respect to the 'two' hydrogen bonds of the cyano nitrogen atom, one should consider that crystallographic 3D-structures are a time-averaged reproductions of reality.

A significant biological difference between two chemically highly similar analogues is observed in cytidine-deaminase inhibitors. The natural product zebularine (Fig. 9) can add a conserved water molecule to become a highly potent transition-state inhibitor with $K_i = 1.2$ pM. Its 3,4-dihydro analogue, with only two additional H-atoms, can neither add the conserved water mole-

cule, nor replace it. Thus, the water molecule hinders binding, and affinity is reduced by more than 7 orders of magnitude to 30 μM [42][43].

6. Conclusions

Significant progress has been achieved in the derivation of scoring functions for docking and *de novo* design of ligands [44][45]. However, considering the examples presented here, it is not surprising that these affinity estimations still lack sufficient precision, especially with respect to the influence of hydrogen bonding on ligand affinities. Despite all attempts to arrive at a better understanding of the role of water and of hydrogen bonds in biological systems, we are far from a satisfactory situation.

For rational drug design, all the necessary tools are available: we can generate meaningful 3D-structures of ligands from scratch, we can convert them into multiple low-energy conformations, we can calculate their steric, electrostatic, and lipophilic properties, and we can dock them in a flexible manner into their binding sites (for reviews see, *e.g.*, [46][47]). We can even construct ligands in a combinatorial manner [47][48], but in the very end we fail because we do not have a sufficient understanding of all the individual enthalpy and entropy terms that are involved in desolvation, hydrogen-bond formation, and hydrophobic interactions. The challenge for the current decade will be to further understand and explain the mystery of hydrogen bonding. However, it might take much longer than ten years until we will arrive at reliable scoring functions. Then, the way to tailor-made ligands seems to be straightforward.

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