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The Janus head of small Molecules on Drug Targets

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ABSTRACT

'Drug promiscuity' refers to a drug that can act on more than one molecular target, by binding specifically to separate proteins exhibiting similar or different pharmacological effects. Three examples of such compounds from my own past work are presented here. The focus is on the interesting phenomena of compound promiscuity and not on the vast field of DHODH biology, which is out of the scope of this short review.

Introduction

The lock and key metaphor of Emil Fischer was the first time to suggest a molecular recognition between molecules in biological systems [1]. David Koshland refined this by introducing the induced fit model, which states that a ligand causes the active site to align to this substrate to form the final shape of the complex [2]. The advance of molecular modeling with ever powerful computers and the easy access to X- ray crystallography was important to create a more predictable method for drug discovery. Medicinal chemistry, X-ray Structure of ligand bound to a protein target and computer chemistry is an integral part of medicinal chemistry today. The final proof of an active compound by an X-ray of the complex of small molecules with a protein target is the visualization of a molecule in the center of the activity and gives the rational for the blocking of the natural activity. This method has shown successful interpretations of the binding mechanism in the field of enzyme inhibitors, protein-protein interactions, protein ligand interactions. Its exiting to see a visual representation of the binding complex created by a computer graphics program in real almost real life (Figure 1).

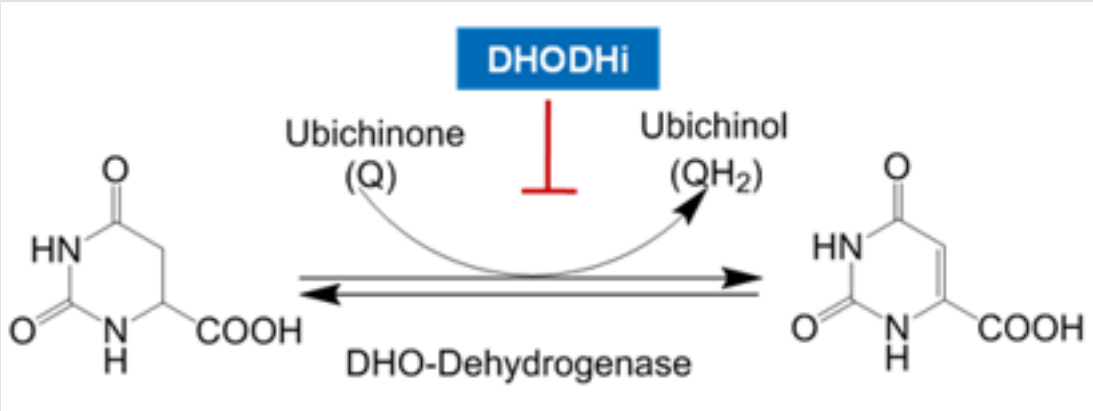

Figure 1.

I have been interested in Coenzyme Q dependent enzymes since my post doc years with K. Folkers, a pioneer in CoQ research at the UT Austin, Tx. When I joined the 4SC company in Germany, I suggested to use the computer screening platform of the company to screen for novel inhibitors of the enzyme dihydroorotate dehydrogenase (DHODH) [3-5]. Since the structure of DHODH bound to an inhibitor was published, we considered it a as an excellent target for docking studies, to find new inhibitors [6]. Such compounds had great potential as anti-inflammatory and anticancer drug candidates [7]. In the body, DHODH catalyzes the synthesis of pyrimidines, which are necessary for cell growth. An inhibition of DHODH inhibits the growth of (pathologically) fast proliferating cells, whereas cells which grow at normal speed may obtain their required pyrimidine bases from the normal metabolic cycle. The most important types of cells for the immune response, the lymphocytes, use exclusively the synthesis of pyrimidines for their growth and react par-ticularly sensitively to DHODH inhibition. Substances that inhibit the growth of lymphocytes are important medicaments for the treatment of autoimmune diseases. We found novel DHODH Inhibitors and performed extensive X-ray crystallographic studies of the compounds bound to the Enzyme. We found well-defined structures at the active site of enzyme with characteristic molecular interactions. The hydrophobic biphenyl residue binds into the hydrophobic binding site of coenzyme Q and blocked the access to the active site NADH cofactor [8].

A carboxylic group was necessary for binding to tyrosine via a water bridge. However, we found that the molecule can flip, depending on the substitution pattern on the hydrophobic pharmacophore and the carboxyl group then binds to a arginine at the active site the structure of the enzyme [8,9]. Depending on the activity of the compound we could differentiate binding poses oriented to Arg 138 or the opposite direction directed towards Tyrosine 147, we even identified a molecule which adopted two binding poses in the crystal structure determination. We suggested that the compound enzyme complex would form a mixture of crystals and the double structure would be detected as such an overlap (Figure 2). This was a great surprise since few examples of multiple binding poses of compounds in crystal structure are reported. It is common in docking studies that multiple poses are obtained, but most of these are considered artifacts. We could detect different poses in one enzyme and link the pose with the enzyme activity. DHODH inhibitors display a wide range of pharmacological effects, which are mostly linked to the mechanism of the enzyme which results in Uridine depletion. Uridine is a mayor building block for RNA and DNA [7]. First and most Uridine depletion inhibits cell proliferation, due to limitation of nuclear building blocks [9]. The proliferation inhibition assay of stimulated T-cells is therefore also often used secondary assay for DHODH inhibitors. New data have shown more complex mechanisms derived from uridine depletion, such effects as inhibition of Il-17 [8].

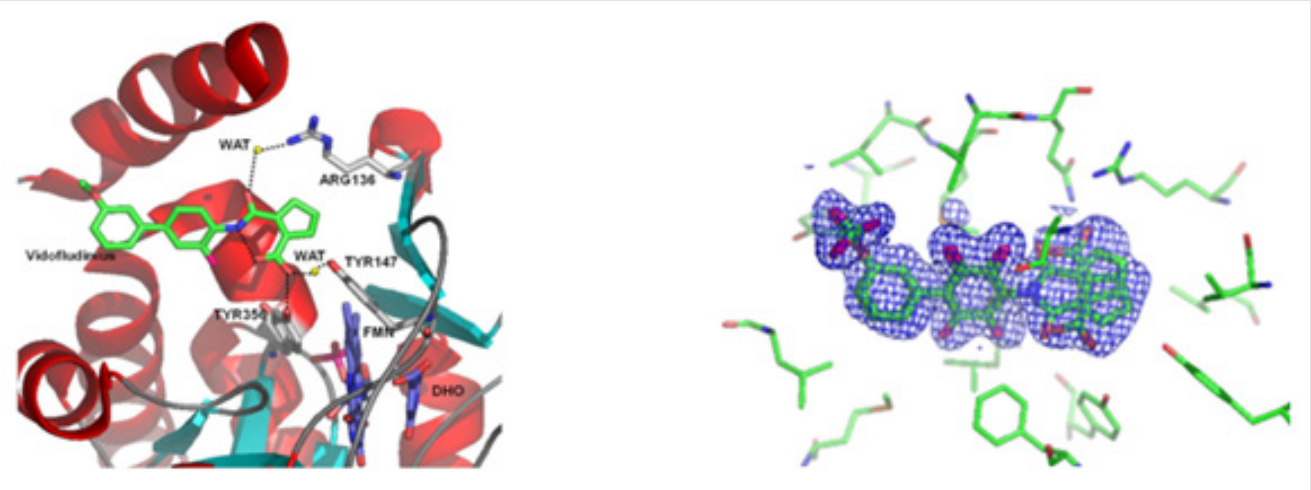

Figure 2.

Most of the biochemical activities can be reversed in vitro by uridine substitution which is also a confirmation of the DHODH as a target. However, some of these tests could not be reversed by uridine which left on some of the biochemical assay data of DHODH inhibitors in doubt. Cell reporter gen assays not good suited for testing compound like DHODH inhibitors which inhibit cell growth and metabolism as the assay is dependent on these factors. Therefore, biologist doubted early indications of DHODH inhibitors on several targets which are based on nuclear events. It was a great surprise when a group from China discovered in the case of a DHODH Inhibitor that the compound is an activator of the Nuclear farnesoid X receptor (FXR). This is confirmed by an

Xray structure and more biological assays [10]. Nuclear receptors (NRs) are ligand-inducible transcription factors that transmit physiological signals of a wide variety of ligands, such as classical steroid hormones, retinoic acid, thyroid hormone, and vitamin D. Nuclear farnesoid X receptor (FXR) plays pivotal roles in regulating inflammatory processes and metabolism and, thus, has become an important target to treat liver steatosis [10]. The binding pose is different in both X-ray structures which is surprising as the molecule is not that flexible as the structure has not many rotatable bonds, it more likely that the protein has the property to adjust to the small molecule.

The binding site clearly shows a large area of hydrophobic residues covering the biphenyl residue, and the carbonyl of the amide is hydrogen bounded to tyrosine residue. The free carboxyl group is hydrogen bounded to His and Ser the ligand binding domain. Vidofludimus binds in a unique way to the cofactor by inducing a conformation change (Figures 3 & 4). A new second generation of potent DHODH inhibitors did not follow this simple model as shown before. One class of such compounds was discovered by chance when screening commercial compounds which did not fit the previous pharmacophore model as we previously described. There was no free carboxylic acid present, and the hydrophobic groups are connected as a string of aromatic and heteroaromatic residues. The compounds were confirmed as DHODH inhibitors by X-ray crystallography (Immunic Ag [11]. The compound were potent inhibitors of the release of IL-17 from activated T-cell. This strongly hinted to the ROR (gamma)t nuclear receptor. The case of a DHODH Inhibitor it was discovered that it binds to the nuclear receptor ROR (gamma)t [11]. This was confirmed by X-ray crystallography and by the enzyme assay and T cell proliferation assay. Significant is also the strong inhibition of Il-17 release after stimulation of T-cells [12]. Further there were three patents published on many analogs of this series as inhibitors of IL-17 and IFN-gamma. Patents EP2668182B1, US 2012/0196862 A1, US 2021/00661796 A1 [13].

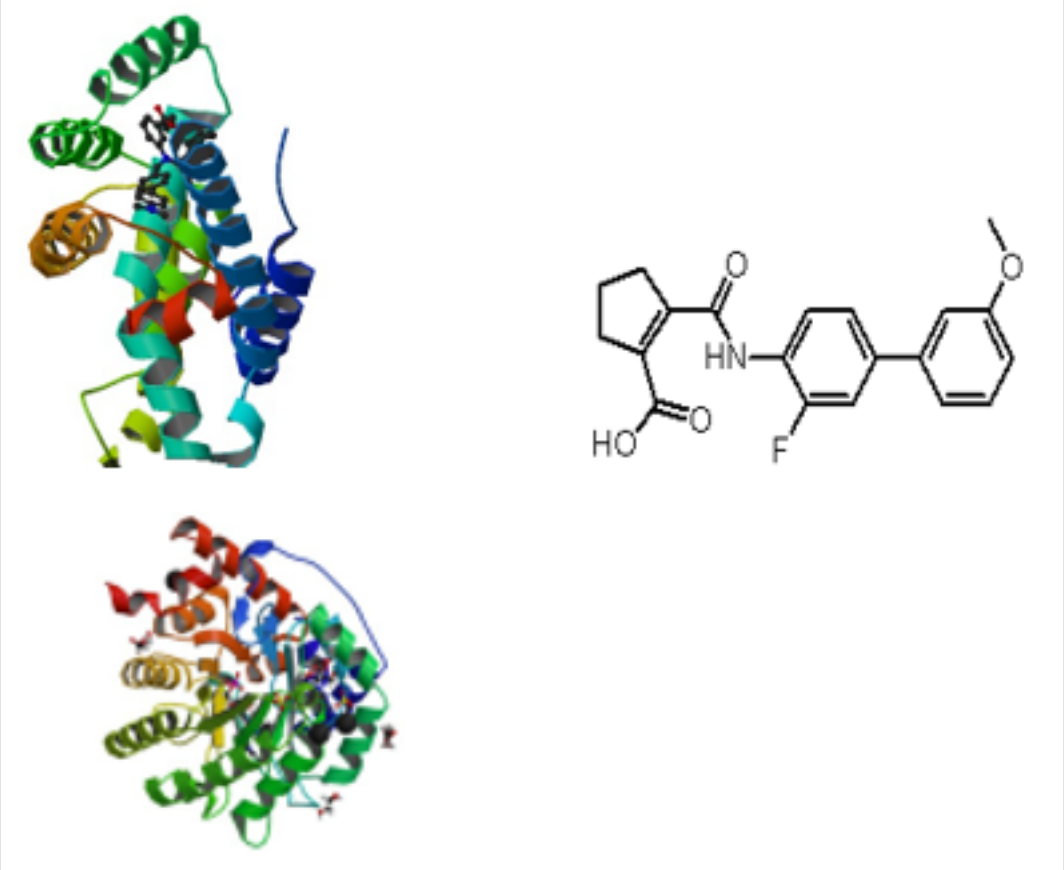

Figure 3: Vidofludimus inhibits DHODH and activates farnesoid X nuclear receptor.

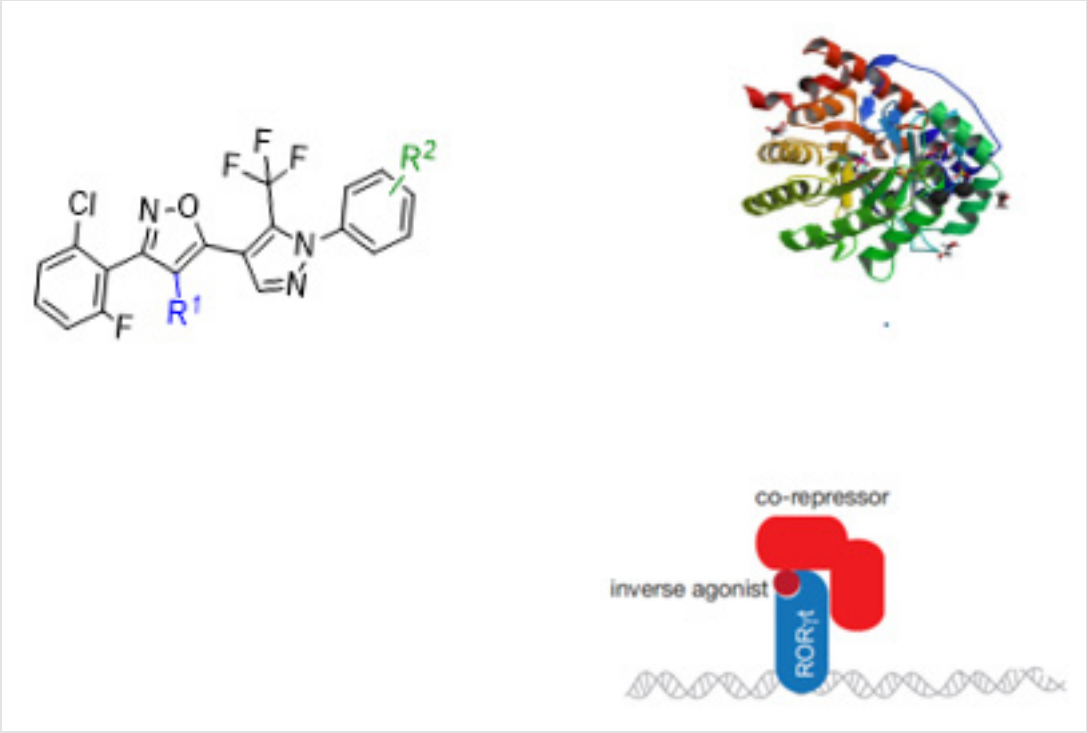

Figure 4: Pyrazoloisooxazoles as DHODH Inhibitors and ROR gamma t Inhibitors.

The first patent derives from a compound which was a chance discovery as a DHODH Inhibitor. The second Patent, focused on the replacement of the carboxymethylated on the isoxazole ring, finally the third patent deals with the modification of the phenyl ring on the right-hand side, to obtain better water solubility [12]. The compounds also inhibit IFN gamma and Il17 and only later the compounds were declared inverse agonists of the ROR gamma t nuclear receptor by the Immunic company [13]. Immunic Ag reported that IMU-935 a compound derived from the patents [11] is an orally available RORγt reverse agonist with unique properties: synergistic mechanisms of RORγt/DHODH lead to very potent inhibition of the Th17/IL-17 axis, approximately 20% basal RORγt activity at full inhibition, and no effects on thymocyte maturation in vitro. After completion of the IND-enabling studies, IMU-935 will enter phase 1 double-blind, placebo-controlled, single, and multiple ascending dose trials in healthy volunteers later this year [14,15].

Conclusion

Small flexible molecules can bind in different poses to proteins and assert this way different effects. The effect can be noticed as the strength on the activity of a target, or the compound can bin to a completely different target in another pose. GPCR receptor heterodimerization has important consequences on the pharmacology of the of these receptors. Janus head type small molecules could be a promising new target in GPCR drug discovery research [16].

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