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Ligand binding cooperativity: Bioisosteric replacement of C=O with SO_2 among thrombin inhibitors

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ABSTRACT

Ligand–protein binding is a complex process that involves the formation of number of non-covalent interactions, e.g. H-bonds and hydrophobic interactions, between the ligand and the protein host. Upon binding, ligand functional groups can act synergistically (positive cooperativity) to improve the overall ligand binding affinity beyond what would be expected from their individual contributions. In this study, using thrombin as a protein model system, we evaluated the effect of the bioisosteric replacement of a carbonyl functionality with a sulphonyl functionality on positive cooperativity between their H-bonds with thrombin and hydrophobic binding in the adjacent S3 pocket. The positive cooperativity observed was greatly reduced when replacing the carbonyl group with a sulphonyl group. Evaluating how bioisosteric replacements affect cooperativity is important for making better informed ligand optimization SAR decisions.

One of the challenges facing medicinal chemists in drug discovery is to optimize the binding affinity of the ligands for their protein target.[1–4] Unfortunately, accurately predicting a ligand's binding affinity remains a significant problem. This is mainly because some of the fundamental non-covalent factors underlying the binding process are still not fully understood and quantified. These factors include enthalpy–entropy compensation, the detailed role of water, and the intricacies of the hydrophobic effect and cooperativity.[5–12]

Scoring functions used to predict ligand binding affinity typically make the inaccurate assumption that the contribution of ligand functional groups can be treated in additive manner.[13–16] They usually don't take into consideration that ligand functional groups can have a mutual effect on each other i.e. synergistic or antagonistic. Cooperativity among ligand functional groups is observed when the differential binding energy obtained from non-covalent interactions when acting together is more favorable (positive cooperativity) or less favorable (negative cooperativity) than the sum of the differential binding energies obtained when these groups act individually. The potential error in ligand binding affinity predictions for not including cooperativity between ligand functional groups can be 1–3 orders of magnitude.[17–20] This suggests that scoring function predictions of ligand binding affinities could be improved if cooperativity were properly included. Kuhn and co-workers have started to address this significant deficiency by incorporating some elements of cooperativity into their scoring function.[21]

Bioisosteric replacement is a useful tactic for medicinal chemists when seeking to improve potency, enhancing selectivity, alter physical properties, reduce or redirect metabolism, eliminate or modify toxicophores, or to acquire new intellectual property without making major changes in the chemical structure of a molecule.[1,2] Bioisosterism also finds utility in structure based drug design. There are now a large number of available crystal structures of ligand–protein complexes, as well as for the protein hosts themselves. The availability of this structural information allows the medicinal chemist to design new molecules while attempting to determine what changes may, or may not, be tolerated in a particular location on the ligand. As a result, the decision to use a functional group bioisostere, and if so which bioisostere, can be guided by the ligand's structural information available.

Hydrogen bonds are considered one of the mostly important non-covalent interactions between the ligand and protein when forming the complex.[22,23] In this study, the bioisosteric replacement of a H-bond accepting carbonyl group (C=O) with a H-bond accepting sulfonyl group (SO_2) was performed and the effect on functional group cooperativity with an adjacent hydrophobic interaction was determined.

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Thrombin is considered a good model protein for fundamental structure-based ligand design studies. This is mainly because thrombin has a relatively rigid and well-defined binding site with numerous deposited X-ray crystal structures containing a variety of bound inhibitors. The active site of the thrombin consists of three pockets:[24] (1) The S1 pocket which is a deep hydrophobic pocket with the carboxylate of Asp189 and two backbone carbonyls at the bottom of the pocket. This Asp189 carboxylate anion is responsible for strong ionic and bifurcated H-bonding interactions with positively charged ligand amidine containing P1 residues; (2) The S2 hydrophobic pocket which mainly consists of Tyr60A and Trp60D side chains of the 60-insertion loop as well as the isobutyl group of Leu99; (3) The S3-pocket which is a well-defined hydrophobic pocket, consisting mainly of the side chains of Trp215, Ile174, and Leu99.

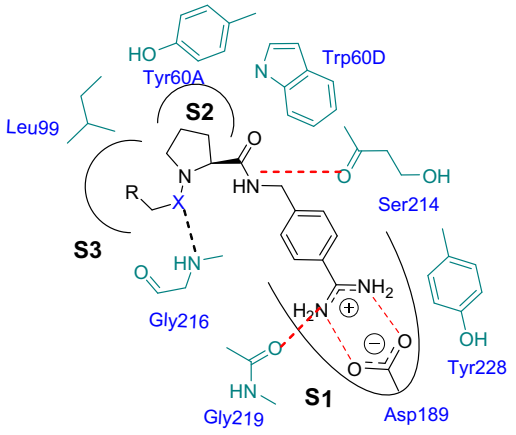
In the current study the effect of a bioisosteric replacement of the ligand carbonyl moiety with a sulfonyl moiety, both of which can accept an H-bond from thrombin Gly216, on cooperativity with the adjacent hydrophobic side chains binding in the S3 pocket was evaluated. In our previous study, this carbonyl group was found to be engaged in positive cooperativity with the hydrophobic side chain which accounted for −3.9 kJ/mol enhanced binding affinity.[18] The current study address's two important questions for predicting ligand binding affinities: (1) Is cooperativity the same when substituting one H-bond acceptor with another? (2) How does the H-bond acceptor change affect the magnitude of the hydrophobic binding in an adjacent pocket? To answer these questions, three series of thrombin inhibitors were designed, synthesized, and biologically tested. The structural features and the expected non-covalent interactions with thrombin for these inhibitors are shown in Figure 1.

As shown in Figure 1, thrombin inhibitors of series I have X = carbonyl group, which acts as a H-bond acceptor with the Gly216-NH-residue. In series II the carbonyl group of series I is replaced with a sulphonyl group to act as a H-bond acceptor bioisostere, and which is also expected to form a H-bond with the Gly216-NH-residue. In series III, the H-bond accepting moiety is removed altogether by replacing it with a methylene group. The common structural features between all of these inhibitors are: (1) A proline moiety selected to bind under Tyr60A and Trp60D in the S2 pocket, as does the natural substrate.[25] (2) The R hydrophobic side chain that binds in the thrombin S3 pocket and ranges from a methyl group to a benzyl group. (3) A *p*-benzamidine moiety to bind in the thrombin S1 pocket. This moiety forms strong ionic interactions with the protein residues through a bifurcated salt bridge with the Asp 189 carboxylate at the bottom of the S1 pocket. The amidine also forms H-bonds with the Gly219 carbonyl residue as well as with a nearby crystallographic water molecule.

The synthetic route for series II is outlined in Scheme 1. The synthesis starts with an amide coupling in DMF between commercially available *N-Boc*-L-proline and 4-cyano benzyl amine using EDC/HOAt in presence of DIEA to provide **1a**. The *Boc* protecting group was then removed by stirring in 3 N methanolic HCl to provide intermediate **1b** as hydrochloride salt. The general synthesis continues with a coupling between the commercially available sulphonyl chlorides and **1b** using triethylamine (TEA) as a base in DCM. The resulting sulfonamides **1c**–**5c** were stirred with hydroxylamine hydrochloride and DIEA in anhydrous methanol for 16 h to form the corresponding hydroxyamidines. The hydroxyamidines were carried on directly by stirred with acetic anhydride in acetic acid for 30–45 min. 10% Pd/C was then added and the mixture was hydrogenated. The resulting products were purified by reverse phase HPLC to give the final compounds **1**–**5** with a purity of >95%. We published the synthetic details for series I and III previously.[18]

All the ligands reported herein were tested for thrombin inhibition using a standard kinetic photometric assay at pH 7.4 using Pefachrome-tPA as a chromogenic substrate.[26] The IC_{50} values for the tested ligands were determined from the dose–response curves. These values were then utilized to obtain the inhibition constants 'K_i's' using the Cheng–Prusoff equation,[27] and the inhibition constants were used to calculate the binding free energies 'ΔG's' of the tested ligands using the equation $\Delta G = RT \ln(K_i)$. In this study, the hydrophobic contact surface areas between the designed inhibitors and thrombin active site were calculated using SYBYL-X following our previously published protocol.[18,19] The molecular modeling of the series II and III ligands was carried out starting with the thrombin bound crystal structures of **7** (PDB: 2ZI2) and **10** (PDB: 2ZHQ).[19] The resulting modeled complexes were then energy minimized to convergence using the Tripos force field. More details regarding the molecular modeling procedure are given in the Supporting Information. Once the ligands were docked into the thrombin active site, the hydrophobic contact surface areas were calculated. The experimentally determined K_i values, the corresponding Gibbs free energy $\Delta G°$ values, and the calculated hydrophobic contact surface areas for the series I, II and III inhibitors are provided in Table 1.

Bioisosteric replacement of the carbonyl group with a sulphonyl group: What is the effect on binding affinity, adjacent hydrophobic binding, and cooperativity? The binding affinity (K_i) of the key inhibitors **2**, **5**, **7**, **10**, **12** and **15** are compared first. These inhibitors form matched sets wherein the side chain R is either ethyl or



Series I: X = -C=O

Series II: X = -SO_2-

Series III: X = -CH_2-

R = -CH_3, -CH_2CH_3, -$CH(CH_3)_2$, -$CH_2CH(CH_3)_2$, -benzyl

Figure 1. The structural features, expected non-covalent interactions, and the binding mode of the *p*-benzamidine containing inhibitors inside the thrombin active site. The X group is carbonyl or sulphonyl or methylene.