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Acylsulfonamide-Containing PTP1B Inhibitors Designed to Mimic an Enzyme-Bound Water of Hydration

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Abstract—Previously, it had been reported that 6-(phosphonodifluoromethyl)-2-naphthoic acid binds to the protein-tyrosine phosphatase PTP1B with its 2-carboxyl group interacting only indirectly through a bridging water molecule. Reported herein is a family of new analogues that utilize acylsulfonamido functionality both to mimic this water of hydration and to provide an additional new site for elaboration not found in the parent carboxyl-containing analogue. Target acylsulfonamides were prepared in two steps from commercially available primary sulfonamides, which were selected based on in silico screening for their potential ability to interact with one of three binding surfaces proximal to the PTP1B catalytic site. In general, modest potency enhancements were observed. Arylacylsulfonamides represent a structure-based extension of inhibitor design that may have broader utility in the development of PTP1B inhibitors.

As the first protein-tyrosine phosphatase (PTP) to have its X-ray crystal structure reported,[1] PTP1B has served both as a general model for the design of PTP inhibitors[2] and as a novel target for the treatment of obesity and diabetes.[3] Starting with our initial report of the high PTP1B-binding affinity of a phosphonodifluoromethyl phenylalanyl (F_2Pmp, **1**) containing hexapeptide,[4] and our subsequent finding that structurally simpler naphthyls such as **2** bearing phosphonodifluoromethyl-based phospho-mimicking functionality can also exhibit reasonable PTP1B affinity,[5] we have undertaken a number of related studies with naphthyl ring systems. Our approach has been characterized by its use of X-ray crystallographic analysis of lead inhibitors bound to the PTP1B catalytic site as starting points for further inhibitor optimization (Fig. 1).[5,6]

In one of these studies dipeptide mimetic **3** was designed with the intent of its Glu γ-carboxyl group interacting with the positively charged Arg47 side chain, which is located proximal to the PTP1B phosphoryl-binding catalytic pocket.[7] During this work, the protected analogue 6-((bis-(*tert*-butyl))phosphono-difluoromethyl)-2-naphthoic acid (**4**) was prepared as a synthetic intermediate, and the binding affinity of its free phosphonic acid form (**5**) was also examined. Surprisingly, although the 2-carboxyl group of **5** lacked extension sufficient to reach the Arg47 residue, it exhibited an 8-fold higher binding affinity ($K_i = 22\,\mu M$) than the corresponding analogue (**2**), which lacks a 2-carboxyl group ($K_i = 179\,\mu M$).[7] As shown by X-ray crystallographic analysis of **5** complexed to PTP1B,[8] enhanced binding is achieved by interaction of its 2-carboxyl group with the protein backbone indirectly through a bridging H_2O molecule (Fig. 2).

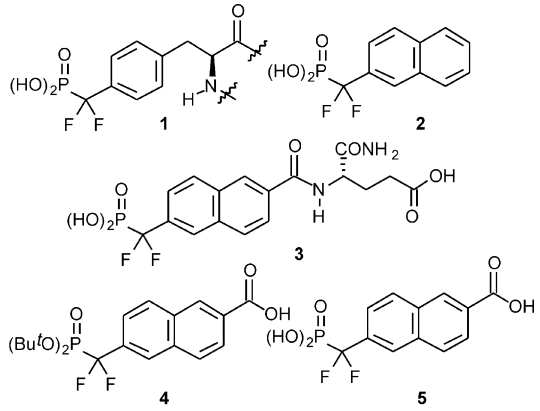

Figure 1. Structures of F_2Pmp and naphthyl-containing analogues.

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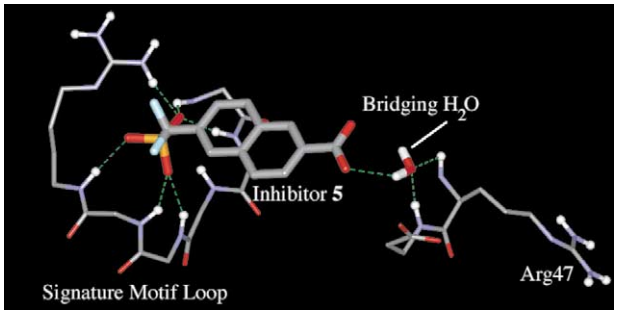
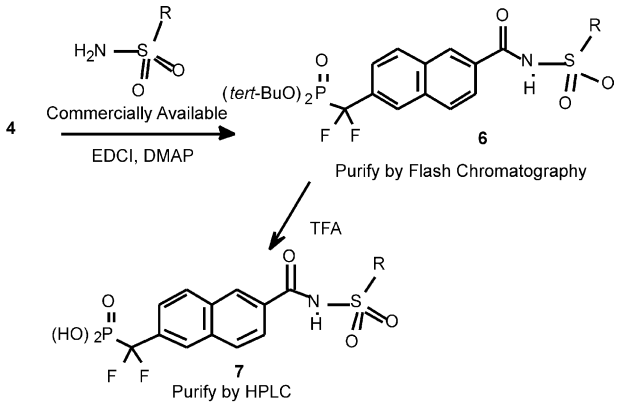


Figure 2. X-ray crystal structure of **5** bound to PTP1B showing the bridging H_2O molecule. The crystal structure was originally reported in ref 8, where compound **5** was designated as 'INH' and has PDB code 1BZJ.



Scheme 1. Synthetic route to acylsulfonamides.

Since inclusion of H_2O-mimicking functionality can potentially be useful in improving ligand binding,[5,9,10] it was of interest to examine whether the 2-carboxyl group could be modified to include and replace the bridging H_2O molecule. Using molecular modeling, we observed that a sulfonamido moiety could potentially achieve the desired effect. One advantage of such acylsulfonamides is that they could afford a new position 'R' for introduction of additional functionality not present in the carboxyl-containing **5**. In order to select potential R groups, all commercially available primary sulfonamides were virtually screened according to their predicted ability to interact with at least one of three lipophilic binding sites on the enzyme surface proximal to the pTyr binding cavity (Fig. 3). The program Catalyst 4.0 (Accelrys Inc.) was used to search the Sigma-Aldrich catalogue (1999 version) for commercially available sulphonamides. Selection of sulfonamides was based on a 3D-pharmacophore query that was established around three lipophilic centers using the 'best-Flexible option.' Location constraints with radii of 1.5 Å were constructed for the sulphonamide nitrogen and two oxygen atoms of **7a** bound to the catalytic site. Hydrophobic functions with 3 Å location constraints were set up around centers **Lip a** (corresponding to Ile 219 and VAl 49), **Lip b** (corresponding to Tyr46 and Arg47) and **Lip c** (corresponding to Phe182) (Fig. 3). Protein and ligand (**5** without a carboxy group) and heavy atoms (obtained from the crystal structure PDF-code 1BZJ[8] within 12 Å radius of the ligand were represented as excluded volumes with radii of 1 Å for **a**, 1.5 Å for **b** and 1.25 Å for **c**. A total of 49 hits were retrieved.

Using commercially available primary sulfonamides that were identified in this fashion, synthesis of final naphthoyl-sulfonamides was achieved by coupling to the common precursor **4**. This yielded *tert*-butyl-protected intermediates **6**, which were purified by silica gel flash chromatography, then deprotected by treatment with TFA and purified by HPLC to afford final products **7a**–**7i**. (Scheme 1)[11]

Figure 4 illustrates the potential binding pattern of one of the selected sulfonamide derivatives, **7e**, to PTP1B. The phenyl group is expected to make hydrophobic interactions with side chain of Arg47, which is a component of LIP b.

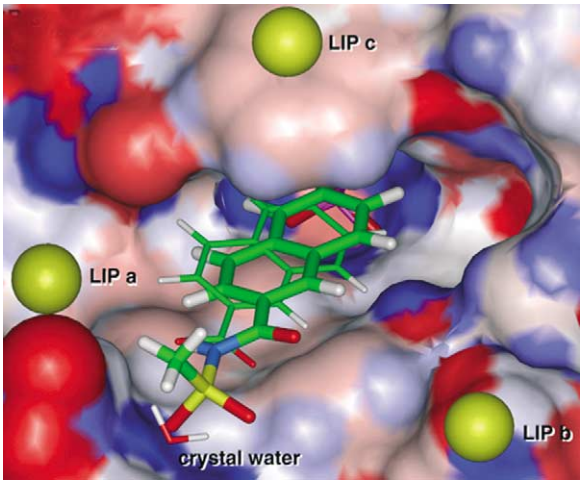


Figure 3. Overlap of X-ray crystal structure of **5** (thin bonds) with molecular dynamics-fitted methylsulfonamide **7a** (fat bonds) showing positions of three surface regions (Lip a, b and c) used to screen for compatible 'R' groups for target inhibitors **7**.

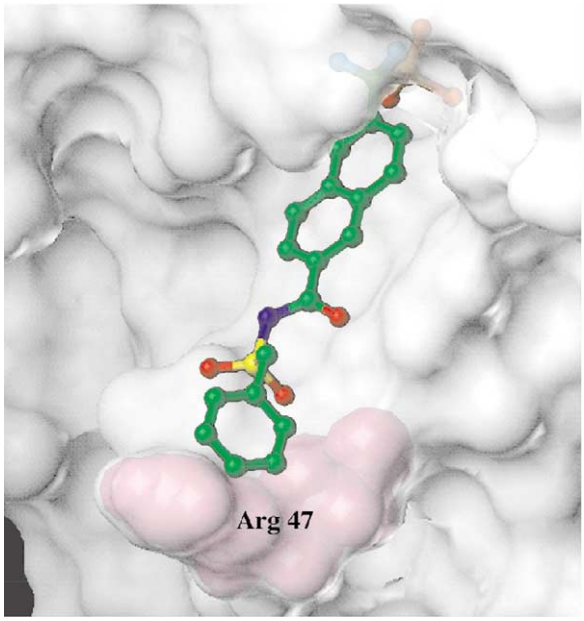
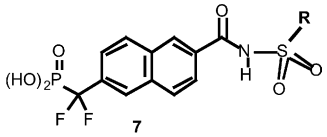


Figure 4. Simulated binding of benzyl sulfonamide **7e** to PTP1B.[12]

Table 1. Inhibition constants against PTP1B for acylsulfonamides **7a**–**7i** as determined in ref 12



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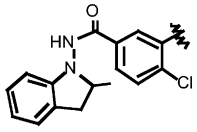
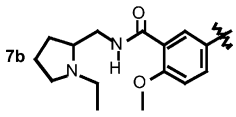
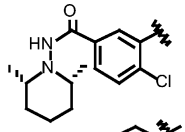
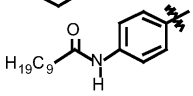
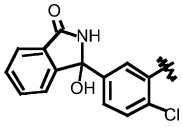
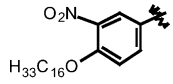
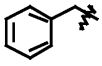
No.	R	IC_{50} (μM)	No.	R	IC_{50} (μM)
7a		41±2 (K_i = 17 μM)	**7f**		20±2
7b		46±2	**7g**		19±1
7c		30±2	**7h**		18±2
7d		25±1	**7i**		0.35
7e		24±2			

Inhibitory potencies of naphthoylsulonamides were determined against recombinant human PTP1B as previously desribed[13] with results being given as IC_{50} values in Table 1. The simplest acylsulfonamide (**7a**, R = Me) was shown to have binding affinity (K_i = 17 μM) equivalent to the parent **5**. Of the eight remaining sulfonamides selected through the in silico screening process, seven exhibited binding affinities greater than the parent methyl sulfonamide-containing **7a**. (Only analogue **7b** showed reduced affinity.) An average 1.8-fold potency enhancement relative to the parent methyl sulfonamide **7a** was observed for most analogues, and compound **7i** exhibited an even greater, 100-fold potency enhancement. The basis for the exceptional affinity of **7i** is not yet understood.

Acylsulfonamides represent a structure-based extension of inhibitor design that may have broader utility in the development of PTP1B inhibitors. The ability of sulfonamido functionality to mimic caboxyl·H_2O motifs may be useful in other systems.

Acknowledgements

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11. Compounds were homogeneous to HPLC and provided NMR and FAB-MS consistent with their assigned structures.
12. Molecular modeling was performed based on the X-ray coordinate 1BZJ using Macromodel 8.0 (Schrödinger, L.L.C.) and Sybyl 6.8 (Tripops, Inc.). Conformational analyses and minimizations were performed on MacroModel using MMFF94 (Merck Molecular Force Field) force field with a continuum solvation model.
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