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Design and synthesis of potent, non-peptidic inhibitors of HPTPβ

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Abstract—The sulfamic acid phosphotyrosine mimetic was coupled with a previously known malonate template to obtain highly selective and potent inhibitors of HPTPβ. Potentially hydrolyzable malonate ester functionalities were replaced with 1,2,4-oxadiazoles without a significant effect on HPTPβ potency.

Protein tyrosine phosphorylation, regulated by the opposing actions of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPases), plays a key role in signal transduction. Disruption of the balance of the actions of PTKs and PTPases has been implicated in a variety of disease processes and hence the identification of small molecules capable of inhibiting the specific kinases[1] or phosphatases[2] has emerged as a new therapeutic intervention for a variety of diseases such as cancer, atherosclerosis, and diabetes. Most of the phosphatase inhibition programs have been focused on developing selective inhibitors for PTP1B, a cytoplasmic PTP that negatively regulates insulin receptor action, as new therapies for type-2 diabetes and obesity.[3]

Despite the growing interest of PTP1B as a therapeutic target, little attention has been given to the other phosphatases such as PTPα, HPTPβ, CD45, LAR, and SHP-1, which may also be attractive biological targets.[4] We became interested in developing non-peptidic, selective inhibitors for HPTPβ, a receptor-type phosphatase which is expressed primarily in endothelial cells. HPTPβ is associated with and negatively regulates the activation of Tie2, a receptor PTK for the angiopoietin family of polypeptide growth factors, suggesting an important role of HPTPβ in vascular biology.[5] Tie2 activation has been implicated in maintenance of the adult vasculature and in the development of collateral blood vessels that restore blood flow to ischemic tissue.[6] Thus, we hypothesize that inhibition of HPTPβ should enhance Tie2 activation and thereby preserve vascular function and enhanced blood flow to ischemic tissue. Consistent with this hypothesis, we have recently shown that a non-specific phosphatase inhibitor bis(maltolato)-oxovanadium IV that inhibits HPTPβ augments collateral blood flow in a rat model of vascular insufficiency.[7] These data provided the basis for initiating a program to develop selective, small molecule HPTPβ inhibitors for the treatment of occlusive cardiovascular disease.

Selectivity for the desired PTPase is a very important goal in a phosphatase drug discovery program because individual PTPases have specific and non-overlapping functions in a variety of important biological processes. Most phosphatase programs are hampered by the difficulty of finding selective inhibitors for the specific PTPases. Therefore, we reasoned that a set of highly selective HPTPβ inhibitors would not only be useful in providing therapies for vascular diseases, but also would serve as an important platform for other phosphatase inhibitor discovery programs.[8]

Improving upon a HTS lead identified by screening of the P&GP corporate repository, sulfamic acid functionality was identified as a potential phosphotyrosine

Keywords: Protein tyrosine phosphatases; HPTPβ; Sulfamic acid; Malonates; Oxadiazoles.
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mimetic.[9] In order to obtain novel inhibitors of HPTPβ, the sulfamic acid phosphotyrosine mimetic was coupled to known non-peptidic PTP backbones. A variety of non-peptidic scaffolds including malonates and deoxybenzoin, benzotriazoles, and 1,2-oxazines combined with various phosphotyrosine mimetics have been reported as PTPase inhibitors.[3c,10,11] One of these backbones, dimethyl malonate ester, was chosen as a template, and sulfamic acid **3** was selected as an initial target (Fig. 1). Alkylation of the commercially available dimethyl 2-(4-nitrobenzyl) malonate with benzyl bromide followed by the reduction and sulfamation of the nitro group furnished **3** (Scheme 1).[12] Compound **3** proved to be a competitive, reversible inhibitor of HPTPβ with a K_i of 0.20 μM.[13] It also showed good selectivity versus a representative panel of PTPases including PTP1B, TC-PTP, and HPTP A (Fig. 1).

The X-ray crystal structure of **3** bound to HPTPβ reveals that the sulfamic acid functionality binds in the P0 pocket making it a good phosphotyrosine mimetic (Fig. 2a).[14,15] The two ester groups of the malonate, which face opposite directions, make hydrogen-bonding interactions with Asn1735. The benzyl group attached to the 2-position of the malonate did not seem to make any significant interaction with HPTPβ.

Optimization of the newly found HPTPβ inhibitor began with substituting the phenyl ring of the benzyl group in hopes of achieving additional interactions with the enzyme. Synthesis was carried out as outlined in Scheme 1 using appropriately substituted benzyl bromides. No significant potency gain was achieved by substituting the phenyl ring at 3- or 4-positions by sulfonamides (**4a** and **4b**), esters (**4c** and **4d**), acids (**4e**) or ethers (**4f** and **4g**) (Table 1). However, the doubly charged symmetric bis-sulfamic acid **4h** showed an IC_{50} of 60 nM for HPTPβ making it the most potent inhibitor of the series.

Additional diversity at the 2-position of the malonate ester was introduced either by alkylation of 4-nitrobenzyl bromide with diethyl 2-(*tert*-butoxycarbonyl)malonate or by alkylation of dimethyl 2-(4-nitrobenzyl) malonate

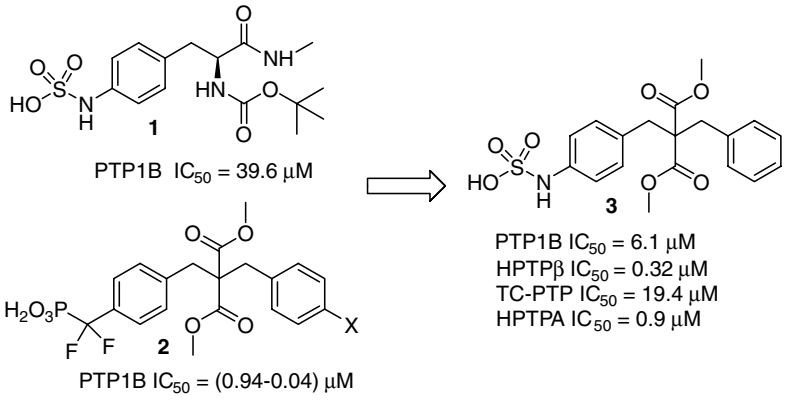
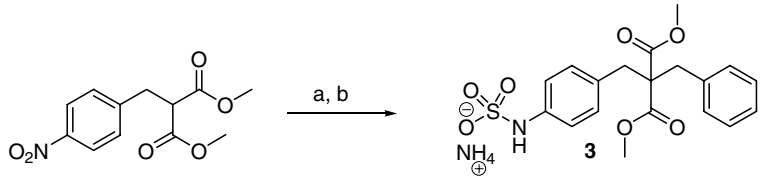


Figure 1. Design of non-peptidic inhibitors of HPTPβ.



Scheme 1. Reagents and conditions: (a) NaH, benzyl bromide, THF, 0 °C to rt. (b) i—H_2, Pd/C, MeOH; ii—Pyr–SO_3, Pyr then NH_4OH.

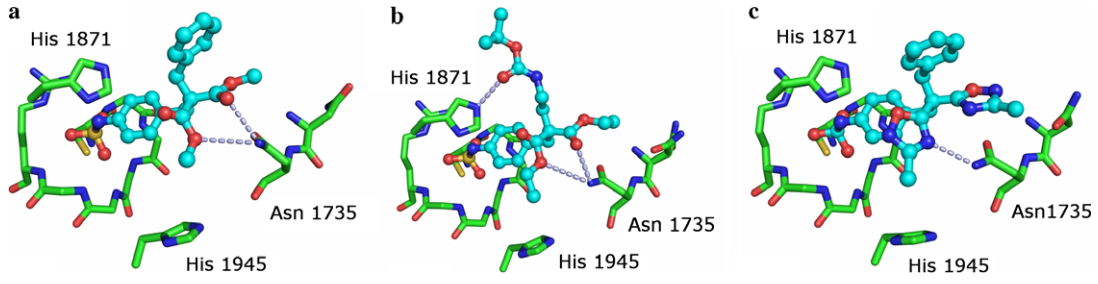
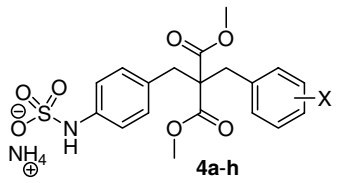


Figure 2. Crystal structures of **3** (a), **5m** (b), and **12** (c) bound to HPTPβ. Carbon atoms of the compounds are shown in blue, those of the key residues are colored green.

Table 1. HPTPβ inhibition data for compounds **4a–i**



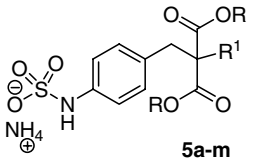
4a-h

Compound	X	HPTPβ IC_{50} (μM)
4a	4-SO_2NH_2	0.43
4b	3-SO_2NH_2	0.30
4c	4-CO_2Me	0.41
4d	3-CO_2Me	0.29
4e	4- CO_2H	0.14
4f	4-OMe	0.39
4g	3-OMe	0.33
4h	4-$NHSO_3H$	0.06
4i	3-$NHSO_3H$	0.10

with the appropriate alkyl halide followed by simple functional group transformations. Substitution of the 2-position of the malonate ester by hydrogen (**5a**) or protected amines (**5b** and **5c**) lowered the potency (Table 2). An aromatic group attached to the 2-position via a 3- or 4-atom linker (**5f** and **5g**) did not have a meaningful effect on the HPTPβ potency. Molecular modeling studies suggested that a hydrogen-bonding accepting group attached to the 2-position via a 2-atom linker would grab some additional interactions. Thus, compound **5m** with 2-Boc-amino ethane group at the 2-position of malonate was synthesized. Compound **5m** showed an IC_{50} of 90 nM for HPTPβ. The X-ray crystal structure of **5m** shows that the two ester groups of the malonate interact with Asn1735 in a similar fashion as **3** (Fig. 2b). In addition, the carbonyl oxygen atom on the Boc group makes an extra hydrogen bond with His1871. This partially explains why **5m** is more potent than other compounds of the series. Compounds **5c**, **5d**, and **5f** were screened in a panel of 10 PTPases (Table 3). All these compounds show excellent selectivity against other PTPases such as PTP 1B, HPTPA, CD-45, etc.

With highly potent and selective malonate-based HPTPβ inhibitors in hand, we decided to replace the potentially hydrolyzable ester functionalities with the ester isostere 1,2,4-oxadiazole. Ester groups were converted to two isomeric oxadiazole rings to obtain compounds **8** and **12** as outlined in Scheme 2. The bis-amidoxime obtained by the reaction of bis-nitrile **6** with hydroxyl amine in refluxing methanol was acylated with acetic anhydride and cyclized to obtain the bis-oxadiazole **7**. The nitro group was then reduced with $SnCl_2$ and the resulting aniline was combined with pyridine–SO_3 complex in pyridine to obtain the sulfamic acid **8**. Bis-oxadiazole **10**, obtained by the reaction of **9** with acetamide oxime and K_2CO_3 in refluxing toluene,[16] was alkylated with 4-nitrobenzyl bromide to obtain **11**. The bis-oxadiazole **11** was converted to the required sulfamic acid **12** in the usual manner. Both compounds were equipotent to their diester counterpart **3**. The binding pose of **12** in PTPβ is very similar to that of malonate-derived compound **3** (Fig. 2c). Only one oxadiazole

Table 2. HPTPβ inhibition data for compounds **5a-m**



5a-m

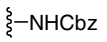
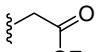
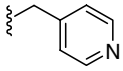
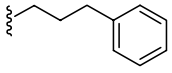
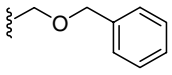
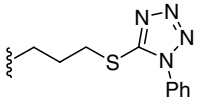
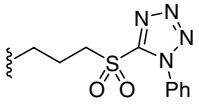
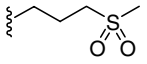
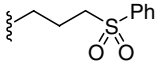
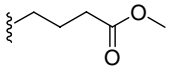
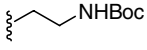
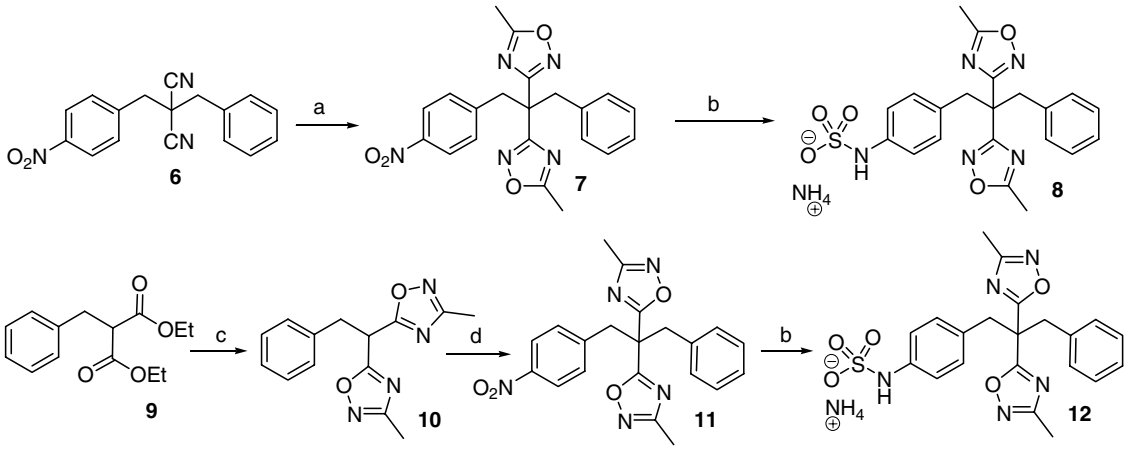
Compound	R^1	R	HPTPβ IC_{50} (μM)
5a	–H	Me	1.0
5b		Et	0.45
5c		Et	0.40
5d		Me	0.23
5e		Me	0.57
5f		Me	0.15
5g		Me	0.27
5h		Me	0.20
5i		Me	0.08
5j		Me	0.42
5k		Me	0.15
5l		Me	0.38
5m		Et	0.09

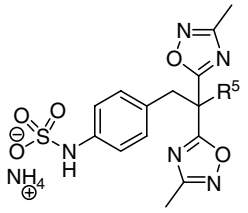
Table 3. Comparison of phosphatase inhibition potency [IC_{50} (μM)] of **5c**, **5d**, and **5f**

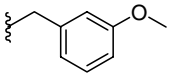
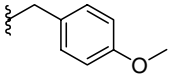
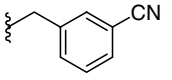
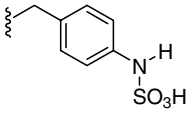
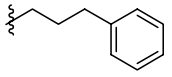
Phosphatase	Compound		
	5c	**5d**	**5f**
HPTPβ	0.40	0.23	0.15
PTP 1B	12.8	16.2	2.3
HCPTPA	82.2	4.8	7.7
HPTPε	>500	>500	>500
LAR-1	227	288	409
LAR-2	472	>500	>500
PTPγ	196	200	93
TC-PTP38	78	109	15.3
HPTPμ	>500	>500	>500
CD45	280	126	29



Scheme 2. Reagents and conditions: (a) i—NH_2OH, MeOH, reflux; ii—$(CH_3CO)_2O$, rt; iii—CH_3COOH, reflux; (b) i—$SnCl_2{\cdot}2H_2O$, EtOH, reflux; ii—Pyr–SO_3, Pyr then NH_4OH; (c) acetamide oxime, K_2CO_3, toluene, reflux; (d) NaH, 4-nitrobenzyl bromide, THF, 0 °C to rt.

Table 4. HPTPβ inhibition data for compounds **13a–e**



Compound	R^5	HPTPβ IC_{50} (μM)
13a		0.23
13b		0.34
13c		0.21
13d		0.08
13e		0.07

ring makes the pivotal hydrogen-bonding interaction to Asn1735 (Table 4).

Compound **12** was selected to introduce further diversity at the 2-position due to its slightly higher potency compared to **8** and its ease of synthesis. Compounds **13a–e** were synthesized in a similar fashion to **12** according to Scheme 2 using the appropriate alkylating agent. It is important to note that both malonate and bis-oxadiazole series showed roughly the same SAR trends around substitutions at the 2-position (Table 4). Compound **13e** with a 3-phenylpropyl group in the 2-position showed the highest HPTPβ potency of the series.

In conclusion, a structure-based approach was taken to prepare a series of novel malonate ester derived aryl sulfamic acids as inhibitors of HPTPβ. Most of the compounds turned out to be highly potent inhibitors of HPTPβ with good selectivity over several other phosphatases. Diester groups of the malonate were replaced by 1,2,4-oxadiazole, a common isostere of the ester, that retained the HPTPβ potency.

Acknowledgment

Authors thank Dr. Jennifer Tinsley for helpful discussions.

References and notes

1. (a) Toledo, L. M.; Lydon, N. B.; Elbaum, D. *Curr. Med. Chem.* **1999**, *6*, 775; (b) Rewcastle, G. W.; Denny, W. A.; Showalter, H. D. H. *Curr. Org. Chem.* **2000**, *4*, 679.
2. (a) McCluskey, A.; Bowyer, M. C.; Collins, E.; Sim, A. T. R.; Sakoff, J. A.; Baldwin, M. L. . *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1687; (b) Zhang, Z.-Y. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 209.
3. (a) Bialy, L.; Waldmann, H. *Angew. Chem., Int. Ed.* **2005**, *44*, 3814; (b) Liu, G. *Curr. Med. Chem.* **2003**, *10*, 1407; (c) Blaskovich, M. A.; Kim, H.-O. *Expert Opin.Ther. Patents* **2002**, *12*, 871.
4. (a) van Huijsduijnen, R. H.; Bombrun, A.; Swinnen, D. *Drug Discovery Today* **2002**, *7*, 1013; (b) Cheng, A.; Dubé, N.; Gu, F.; Tremblay, M. L. *Eur. J. Biochem.* **2002**, *269*, 1050.
5. (a) Fachinger, G.; Deutsch, U.; Risau, W. *Oncogene* **1999**, *18*, 5948; (b) Gale, N. W.; Yancopoulos, G. D. *Genes Dev.* **1999**, *13*, 1055; (c) Wright, M. B.; Seifert, R. A.; Bowen-Pope, D. F. *Arterioscler. Thromb. Vasc. Biol.* **2000**, *20*, 1189.
6. (a) Brindle, N. P. J.; Saharinen, P.; Alitalo, K. *Circ. Res.* **2006**, *98*, 1014; (b) Chae, J. K.; Kim, I.; Lim, S. T.; Chung, M. J.; Kim, W. H.; Kim, H. G.; Ko, J. K.; Koh, G. Y. *Arterioscler. Thromb. Vasc. Biol.* **2000**, *20*, 2573; (c) Shyu, K.; Chang, H.; Isner, J. M. *Life Sci.* **2003**, *73*, 563.
7. Carr, A. N.; Davis, M. G.; Eby-Wilkens, E.; Howard, B. W.; Towne, B. A.; Dufresne, T. E.; Peters, K. G. *Am. J. Physiol. Heart Circ. Physiol.* **2004**, *287*, 268.

8. Lund, I. K.; Andersen, H. S.; Iversen, L. F.; Olsen, O. H.; Møller, K. B.; Pedersen, A. K.; Ge, Y.; Holsworth, D. D.; Newman, M. J.; Axe, F. U.; Møller, N. P. H. *J. Biol. Chem.* **2004**, *279*, 24226.
9. Klopfenstein, S. R.; Evdokimov, A. G.; Colson, A.; Fairweather, N. T.; Neuman, J. J.; Maier, M. B.; Gray, J. L.; Gerwe, G. S.; Stake, G. E.; Howard, B. W.; Farmer, J. A.; Pokross, M. E.; Downs, T. R.; Kasibhatla, B.; Peters, K. G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1574.
10. Cho, S. Y.; Baek, J. Y.; Han, S. S.; Kang, S. K.; Ha, J. D.; Ahn, J. H.; Lee, J. D.; Kim, K. R.; Cheon, H. G.; Rhee, S. D.; Yang, S. D.; Yon, G. H.; Pak, C. S.; Choi, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 499.
11. Lau, C. K.; Bayly, C. I.; Gauthier, J. Y.; Li, C. S.; Therien, M.; Asante-Appiah, E.; Cromlish, W.; Boie, Y.; Forghani, F.; Desmarais, S.; Wang, Q.; Skorey, K.; Waddleton, D.; Payette, P.; Ramachandran, C.; Kennedy, B. P.; Scapin, G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1043.
12. All compounds were fully characterized by ^{1}H and ^{13}C NMR, HPLC, MS, and combustion analysis.
13. See Ref. 9 for experimental procedures for the phosphatase assays.
14. Preparation of the crystal structures will be discussed elsewhere, Manuscript in preparation.
15. All structures referred to in this article were deposited with the RCSB (www.rcsb.org/pdb) under PDB-IDs of 2H04, 2H03, and 2H02. Data were collected at SER-CAT 22-ID beamline and at the IMCA-CAT 17-ID at the Advanced Photon Source, Argonne National Laboratory. USE of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Science, under Contract No. W-31-109-Eng-38.
16. Amarasinghe, K. K. D.; Maier, M. B.; Srivastava, A.; Gray, J. L. *Tetrahedron Lett.* **2006**, *47*, 3629.