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Bioorganic & Medicinal Chemistry Letters 17 (2007) 6579–6583
Bioorganic & Medicinal Chemistry Letters

2-Aryl-3,3,3-trifluoro-2-hydroxypropionic acids: A new class of protein tyrosine phosphatase 1B inhibitors

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Received 4 September 2007; revised 19 September 2007; accepted 20 September 2007
Available online 25 September 2007

Abstract—A new series of non-peptidic, mono-acid protein tyrosine phosphatase 1B (PTP1B) inhibitors has been identified by structure-based design. Compounds with 2-(indol-3-yl)- and 2-phenyl-3,3,3-trifluoro-2-hydroxypropionic acid core units targeted at the enzyme's primary site and a hydrophobic chlorophenylthiazole extension in its 2° site exhibit 3–60 μM IC_{50}s for PTP1B inhibition in an S*f*9 cell-based assay.
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The binding of insulin to the insulin receptor (IR), a member of the receptor tyrosine kinase family, enables ATP binding and autophosphorylation of tyrosine residues in its intracellular domain. This autophosphorylation promotes the receptor's kinase activity and facilitates interaction with its intracellular substrates.[1] Protein tyrosine phosphatase 1B (PTP1B) has been shown to dephosphorylate the insulin receptor and downregulate insulin signaling.[2–5] Consequently inhibitors for PTP1B are of therapeutic interest because of their potential for suppressing dephosphorylation of the IR, thereby maintaining the receptor in an activated state and potentiating insulin signaling. Such compounds may have therapeutic utility for treating type 2 diabetes, which is characterized by insulin resistance.[6] Several series of PTP1B inhibitors have been disclosed. The majority of these act competitively and contain one or other of a variety of phosphotyrosyl (pTyr) mimetic subunits: mainly phosphonic and carboxylic acids, including α-keto, malonic, cinnamic, salicylic, and oxalylaminobenzoic acids.[6] Here, we present the design and synthesis of a new inhibitor class possessing a 2-aryl-3,3,3-trifluoro-2-hydroxypropionic acid core unit targeted at the PTP1B active site.

One of the challenges in developing PTP1B inhibitors is that minimal pTyr mimetics exhibit only modest affinity for the enzyme compared to its phosphorylated protein substrates. Indeed the enzyme makes extensive contact with its substrate proteins over the surface beyond the immediate enclosed pTyr-binding catalytic pocket. This is clearly seen in the PTP1B X-ray co-crystal structure with a peptide fragment, RDI(pY)ETD(pY)(pY)RK, taken from the IR (Fig. 1).[7] The enzyme's catalytic site is formed by a 'P-loop' motif, ^{213}VHCSAGIGRSG^{223}, which is conserved across all PTPs as a characteristic (I/V)HCXAGXXR(S/T)G signature sequence.[8] During dephosphorylation the substrate pTyr residue is engaged by the PTP1B P-loop through a salt bridge to R221 and by additional hydrogen bonds to donors in the main chain. This engagement induces the closure of a flexible

Keywords: Protein tyrosine phosphatase 1B; PTP1B; Inhibitor; Diabetes; Obesity; Structure-based design; Insulin resistance; Indole; Thiazole; Phospho-tyrosine; Insulin sensitivity.
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doi:10.1016/j.bmcl.2007.09.069

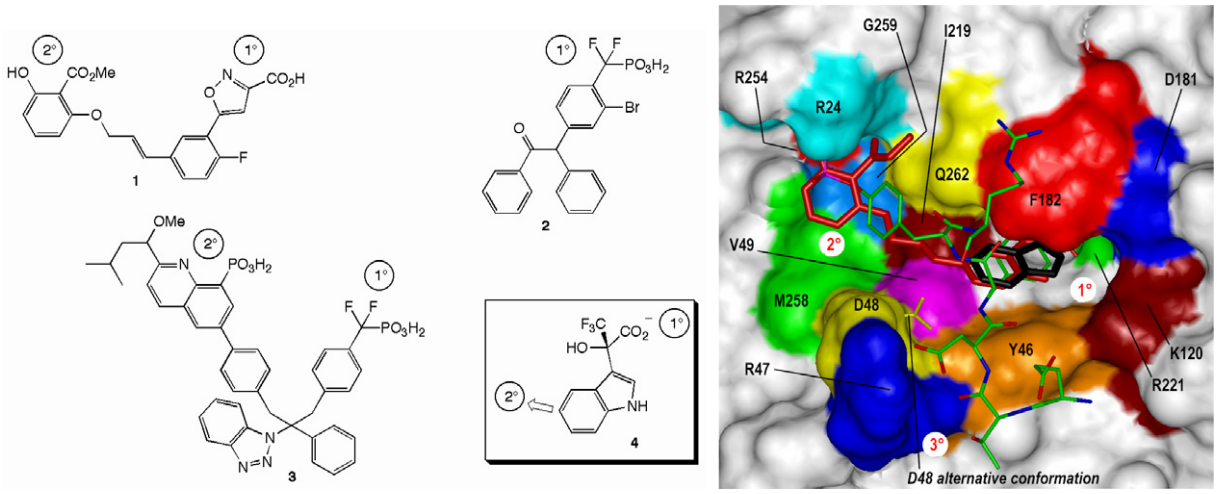


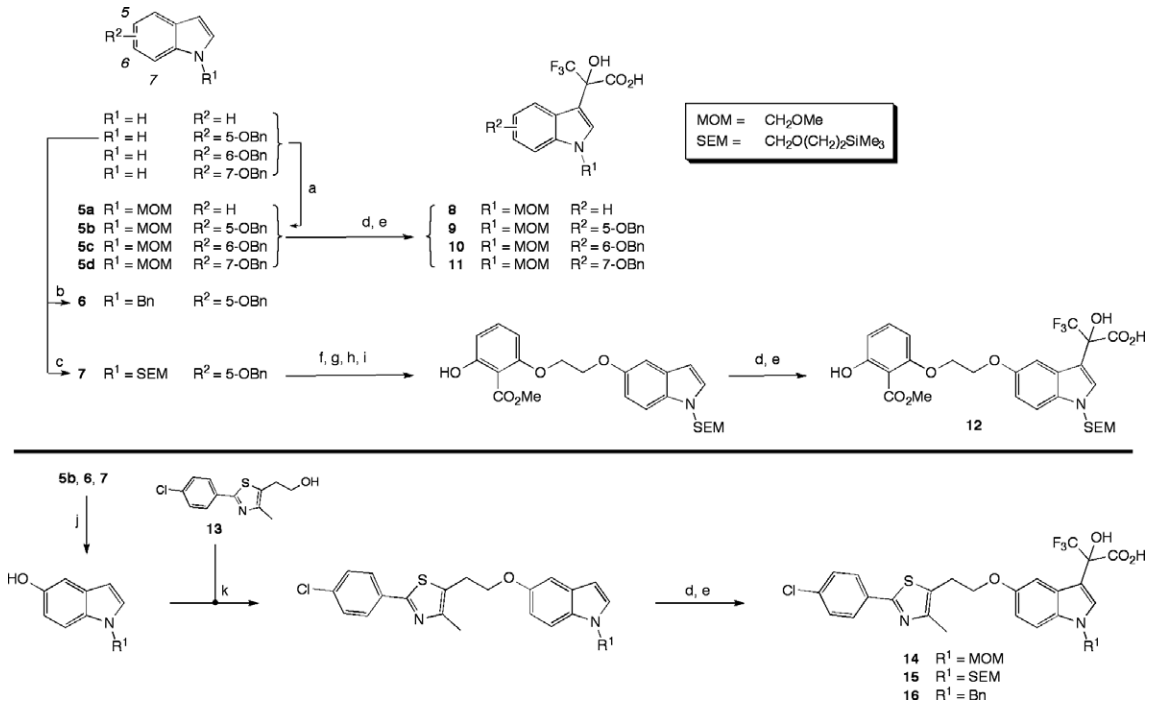
Figure 1. PTP1B inhibitors and ligand binding modes. The PTP1B co-crystal structure (PDB: 1Q1M) with Abbott inhibitor **1** (red stick) is shown. The IR fragment RDI(pY)ETD(pY)(pY)RK (thin stick) is superimposed from its co-crystal structure with PTP1B (PDB: 1G1F) and hydrogen bonds D48 in the alternative conformation marked (yellow stick). Indole **4** (black stick) is shown docked in the 1° site.

loop—the 'WPD loop'—over the top of the pTyr residue, gripping its benzene ring in a clamp between hydrophobic residues (F182 and Y46). From the primary pTyr binding site (Fig. 1, 1° site) the peptide ligand branches in two directions. On the C-terminal side a second pTyr side chain is engaged by R24 and R254 in a depression in the PTP1B surface that forms a secondary binding site (2° site). On the N-terminal side the ligand runs over Y46 and engages PTP1B with a salt bridge to R47 (3° site). The main chain of the peptide ligand is additionally engaged in hydrogen bonds from the D48 side chain (Fig. 1, yellow stick) where the ligand passes the mouth of the 1° site.

Zhang and coworkers first proposed exploitation of the 2° site as a paradigm for achieving enhanced ligand binding affinity with bisphosphonate structures that bridge to the 1° site.[9] Compounds that span these two sites have since been developed by other groups, notably by Abbott[10] (e.g., **1**) and by Merck Frosst [11] (e.g., **2** and **3**), where ligand binding modes are supported by X-ray co-crystal structures. Exploitation of the 2° site also usefully confers selectivity for inhibition of PTP1B over other PTPs, as this site and the channel communicating to the 1° site appear only to be conserved in one other phosphatase (TCPTP).[12]

The potential pharmacokinetic problems associated with peptidic, phosphonate, and multiply ionized compounds led us to consider development of a new PTP1B inhibitor series, preferably with a single acid functionality. Inspection of X-ray co-crystal structures suggested that the 3D space in the 1° site may be sub-optimally used by many pTyr mimetics. In particular we were struck by the potential of a CF_3 group in an (*S*)-configured 2-aryl-3,3,3-trifluoro-2-hydroxypropionate to occupy a frequently under-exploited cavity beneath F182. The hydroxy and acid functions in such a molecule could maintain interactions with the enzyme's P-loop. As a starting point we selected 3-indolyl as the aryl group of the core structure (**4**, Fig. 1, black stick) because computational docking studies suggested that its benzo ring should superimpose onto the fluorobenzene ring of Abbott's phenylisoxazole **1** (Fig. 1, red stick), thus allowing us to predict that extensions from the indole 5-position could be directed into the 2° site. Compound **1**, as the first published example of a PTP1B inhibitor with activity in a cell-based assay,[10a] was a logical template choice for developing an extension into the 2° site in a new cell-permeable inhibitor series.

We commenced by preparing a range of simple substituted indoles (**8**–**11**), as summarized in Scheme 1. Compounds were prepared in racemic form and, as cell-based activity was a key objective, directly assessed in a PTP1B-specific assay in S*f*9 cells.[13] Activity data are summarized in Table 1. No inhibition was observed with concentrations up to 400 μM for indole **8**. This result is understandable given the flexibility of the enzyme and the small enclosed volume of the 1° site in its WPD loop-closed state. Activity was observed, however, with the introduction of a benzyloxy side chain at the indole 6- and 7-positions, as in **10** and **11** (IC_{50} 382 and 176 μM, respectively). Clearly the activity observed for these compounds may be sensitive to differences in cell permeability, but this result was consistent with the potential for extended surface contact over the edge of Y46 and/or F182 in these compounds. The 5-benzyloxy substituted isomer (**9**), with the side chain targeted at the gateway leading to the 2° site, did not exhibit observable activity at 400 μM. Our binding model for core indole **4** suggested that an extended side chain at the 5-position should allow more effective exploitation of the enzyme's 2° site however. We therefore prepared indole **12** with a salicylate-containing side chain similar to that in the Abbott compound (**1**). The activity of this racemic compound (IC_{50} 123 μM) was comparable to that of **1** (IC_{50} 110 μM) in our S*f*9 cell assay. For reference purposes the activity of phosphonic acid **2** was also determined (IC_{50} 1.2 μM) and found to be very similar to the value previously reported by Merck Frosst using the same assay.[11a] We next investigated whether the side chain in indole **12**



Scheme 1. Reagents and conditions: (a) MOMCl, NaH, DMF, rt (74%-quant.); (b) BnBr, NaH, DMF, rt (71%); (c) SEMCl, NaH, DMF, rt (98%); (d) $F_3CC(O)CO_2Et$, PhMe, Δ (59%-quant.) (e) KOH (aq), EtOH, Δ or 1 M TBAF, THF, 40 °C (48–69%); (f) H_2, Pd-C, EtOH, rt (quant.); (g) ICH_2CO_2Et, K_2CO_3, DMF, rt (82%); (h) $LiAlH_4$, THF, 0 °C (78%); (i) methyl 2,6-dihydroxybenzoate, diisopropyl azodicarboxylate, PPh_3, THF, rt (70%); (j) H_2, Pd-C, EtOH/EtOAc, rt (67%-quant.); (k) **13** (Ref. 16), diisopropyl azodicarboxylate, PPh_3, THF, rt (25–39%).

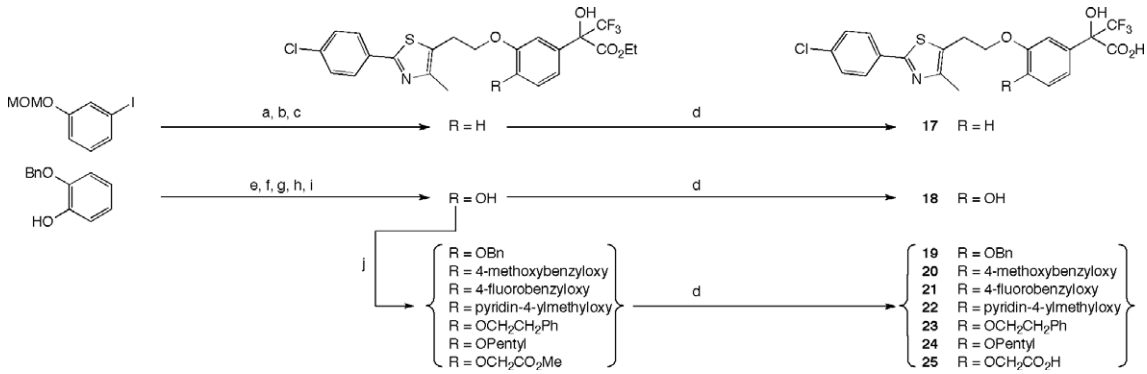
Table 1. PTP1B inhibitory activity for compounds determined in the *Sf*9 cell-based assay[13]

Compound	IC_{50}/μM
1	110
2	1.2
8	NI[a]
9	NI[a]
10	382
11	176
12	123
14	59
15	32
16	27
17	37
18	105
19	3.2
20	12
21	23
22	36
23	10
24	12
25	500

[a] No inhibition was observed for compounds **8** and **9** with concentrations up to 400 μM.

could be altered to make more effective use of hydrophobic contact over the surface of M258 (Fig. 1, green surface). The extended aromatic quinoline nucleus fulfills this role in the case of compound **3** for example. We wished to redesign the side chain without introduction of an additional acid functionality, however, and chose to test the chlorophenylthiazole unit in compounds **14**–**16**. Our modeling studies suggested that the thiazole nitrogen and methyl group should replace the salicylate ester of **12**, with the chlorophenyl group extending more fully over the surface of M258. Pleasingly the activity for these compounds was found to lie in the 27–59 μM range in our cell-based assay.

A need to avoid acidic N-deprotection conditions for the rather sensitive indole core motivated the replacement of the N(1)-MOM group in indoles **8**–**11** by SEM in **12** and **15**. In practice, cleavage of the SEM group was also found to require harsh conditions (1 M TBAF/THF at reflux) to which the indole was rather unstable. A desire to find a more robust core structure as well as to simplify the target series led us to prepare the phenyl analogue (**17**) of indoles **14**–**16**, Scheme 2. The activity of **17** (IC_{50} 37 μM) was found to be comparable to the indoles. Our earlier finding that the 6- and 7-benzyloxy groups in compounds **10** and **11** promoted activity of the parent indole (**8**) suggested that an additional benzyloxy group attached to the core of **17** might enhance activity. We envisaged that such a group would extend the surface contact between the compound and the protein through interaction with Y46 or F182. For synthetic convenience we chose to introduce the additional substituent at the position ortho to the long side chain targeted at the 2° site. We therefore prepared the trisubstituted phenyl series **18**–**25** for comparison, Scheme 2. Phenol **18** (IC_{50} 105 μM) was rather less active than parent **17**. However, the introduction of a hydrophobic group (compounds **19**–**24**) maintained or improved the activity of the parent. The benzyloxy substituted analogue (**19**) was most active in this series (IC_{50} 3.2 μM) and found to possess comparable activity (IC_{50} 3.0 μM) in a cell-free PTP1B



Scheme 2. Reagents and conditions: (a) *n*-BuLi THF, −78 °C then $F_3CC(O)CO_2Et$ (50%); (b) 10% CF_3CO_2H/CH_2Cl_2 (quant.); (c) **13** (Ref. 16), diisopropyl azodicarboxylate, PBu_3, THF, rt (25%); (d) KOH, EtOH aq, 60 °C (34%-quant.); (e) Ac_2O, Et_3N, CH_2Cl_2 (quant.); (f) H_2, Pd-C, EtOAc, rt (quant.); (g) **13** (Ref. 16), diisopropyl azodicarboxylate, PPh_3, THF, rt (79%); (h) KOH, EtOH, PhMe, 0 °C (quant.); (i) $F_3CC(O)CO_2Et$, Et_3N, PhMe, rt (30%); (j) RBr, K_2CO_3, DMF, rt [yields for **nn**-ester precursors: **19** (64%), **20** (75%), **21** (quant.), **22** (84%), **23** (40%), **24** (88%), **25** (89%)].

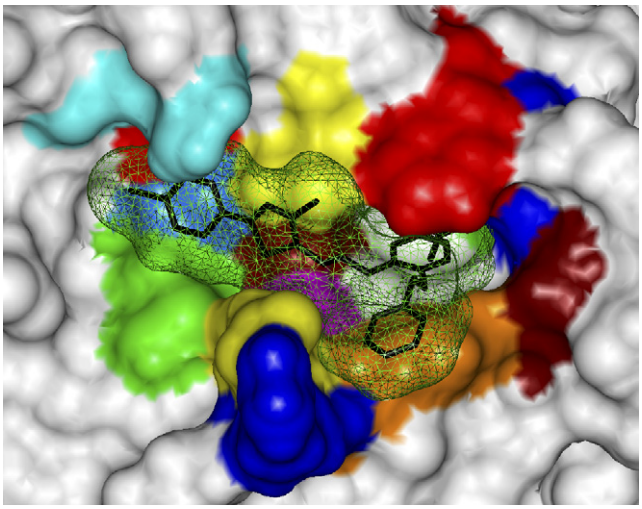


Figure 2. Docked model of (*S*)-**19** (black stick/mesh surface) in the PTP1B catalytic site; PTP1B solvent accessible surface is shown (solid; color coding as Fig. 1).

assay with *para*-nitrophenyl phosphate at pH 7.0.[14] Computational docking[15] of compound **19** (Fig. 2) supported the proposed binding model in which the chlorophenylthiazole unit occupies the enzyme's 2° site and the benzyloxy group folds over Tyr46. Interestingly, the introduction of a polar oxyacetic acid side chain (**25**) was substantially detrimental to activity. This is consistent with the requirement for a hydrophobic extension from the core structure envisaged in our binding model, but it is also possible that the additional acid functionality compromises cell permeability.

In summary, conformational mobility, a small enclosed catalytic pocket, and extensive substrate contacts outwith the active site present challenges for the design of PTP1B inhibitors. Here, we have used a structure-based approach to design novel 2-aryl-3,3,3-trifluoro-2-hydroxypropionic acid ligands for the PTP1B 1° site. These compounds exhibit PTP1B inhibitory activity in a cell-based assay and provide a starting point for a new series of non-peptidic, mono-acid PTP1B inhibitors.

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