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# 1,2,3,4-Tetrahydroisoquinolinyl sulfamic acids as phosphatase PTP1B inhibitors

Sean R. Klopfenstein,* Artem G. Evdokimov, Anny-Odile Colson,† Neil T. Fairweather, Jeffrey J. Neuman, Matthew B. Maier, Jeffrey L. Gray, Gina S. Gerwe, George E. Stake, Brian W. Howard, Julie A. Farmer, Matthew E. Pokross, Thomas R. Downs, Bhavani Kasibhatla and Kevin G. Peters

*Procter & Gamble Pharmaceuticals, Health Care Research Center, 8700 Mason-Montgomery Rd., Mason, OH 45040, USA*

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**Abstract**—High-throughput screening of the P&GP corporate repository against several protein tyrosine phosphatases identified the sulfamic acid moiety as potential phosphotyrosine mimetic. Incorporation of the sulfamic acid onto a 1,2,3,4-tetrahydroisoquinoline scaffold provided a promising starting point for PTP1B inhibitor design.


The PTPase PTP1B has been shown to be involved in the insulin signaling cascade as a negative regulator of insulin signaling.[1] There is a growing body of evidence to suggest that inhibition of PTP1B can enhance insulin signaling primarily through prolonged activation of the insulin receptor. Recent studies have shown that PTP1B knock-out mice exhibit increased insulin sensitivity.[2,3] In addition, treatment of animal models of type-2 diabetes with antisense oligonucleotides has been shown to normalize blood glucose levels.[4] These results have spurred the research community to develop small molecule inhibitors of PTP1B for the treatment of type-2 diabetes and several groups have reported compounds to this end.[5–7]

High-throughput screening of the P&GP corporate repository against multiple phosphatase enzymes identified a number of sulfamic acid derivatives, typified by **1**, which were potent inhibitors of several soluble phosphatases, but showed only weak inhibition of PTP1B. Based on these structures we hypothesized that a sulfamic acid moiety linked directly to an aryl group is likely to be a better mimic of the PTP1B substrate—phosphotyrosine. The assumption of the aryl sulfamic acid moiety as a potential phosphotyrosine mimetic allowed us to launch PTP1B inhibitor discovery efforts.[8] As a starting point toward this goal, simple aryl sulfamic acids were prepared which demonstrated varying levels of potency against several PTPases (Fig. 1). One of the initial

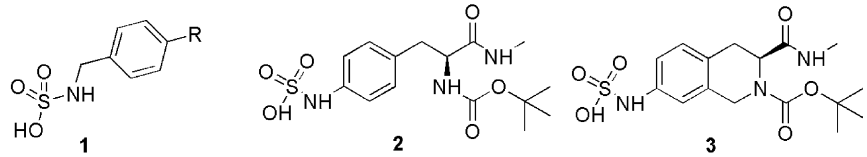


**Figure 1.** Starting points for PTP1B inhibitor design.

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* Corresponding author. Tel.: +1 513 622 3692; fax: +1 513 622 1195; e-mail: klopfenstein.sr@pg.com
† Present address: Computational Chemistry Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, 12 Center Dr, Bethesda, MD 20892, USA.

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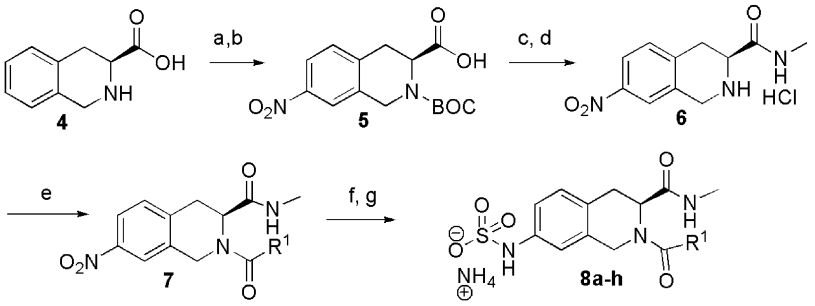
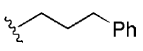
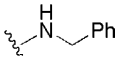
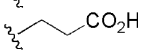
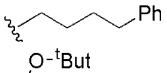
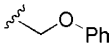
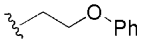
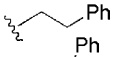
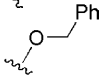
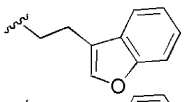
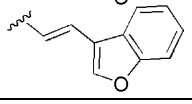



**Scheme 1.** Reagents and conditions: (a) $KNO_3$, $H_2SO_4$, 5 °C to rt; (b) $Boc_2O$, $Et_3N$, $H_2O$/MeOH; (c) EDCI, HOBt, DiPEA, DMF, $MeNH_2$; (d) 4 M HCl/1,4-dioxane; (e) $R^1CO_2H$, EDCI, HOBt, DMF, DiPEA, 0 °C or $R^1COCl$, DiPEA, DCM or $R^1NCO$, $CH_2Cl_2$, DiPEA; (f) $H_2$, Pd/C, MeOH; (g) Pyr–$SO_3$, Pyr then $NH_4OH$.

scaffolds which showed promise for further PTP1B inhibitor design was based on the 7-substituted-1,2,3,4-tetrahydroisoquinoline (TIQ) structure.[9] The initial TIQ derivative **3** provided comparable potency to the PTP1B substrate-like phenylalanine analog **2**. Also, we observed a modest 4-fold selectivity of the TIQ analog for PTP1B versus T cell protein tyrosine phosphatase (TC-PTP).[10] In addition to the in vitro screening data we were excited about the prospect of developing a constrained ring system which could provide two points of diversity for rapid SAR development. Herein, we report a new class of PTP1B inhibitors based on a sulfamic acid pTy mimetic and the 1,2,3,4-tetrahydroisoquinoline system.[11,12]

Synthesis of the described TIQ sulfamic acids was accomplished in seven steps from readily available starting materials (Scheme 1).

**Table 1.** PTP1B inhibition data for compounds **8a–m**

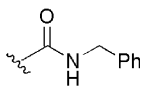
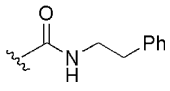
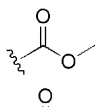
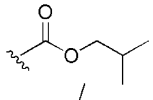
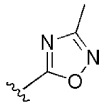
| Compound | $R^1$ | PTP1B $IC_{50}$ (μM) |
|---|---|---|
| **8a** | L-Phe(Boc) | >500 |
| **8b** |  | 285.7 |
| **8c** |  | 271.2 |
| **8d** |  | 164 |
| **8e** |  | 153.5 |
| **8f** |  | 148 |
| **8g** (*R*) |  | 134.8 |
| **8h** |  | 95.3 |
| **8i** |  | 83.6 |
| **8j** |  | 64.4 |
| **8k** |  | 37 |
| **8l** |  | 36.7 |
| **8m** |  | 24.9 |

Nitration of (*R*) or (*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**4**) with $KNO_3/H_2SO_4$ provided the 7-nitro derivative in high yield which was converted directly to the Boc derivative **5**. Bulk synthesis of the 3-methylcarboxamide derivative **6** was done using a mixed anhydride approach[13] followed by Boc deprotection with 4 M HCl/1,4-dioxane. This sequence has been routinely carried out on a multi-gram scale. Diversity at the 2-position was introduced using routine chemistry. Introduction of the sulfamic acid functionality was carried out in two steps. The 7-nitro group was reduced either by catalytic hydrogenation with 10% palladium on carbon or with $SnCl_2$ (e.g., **8k** and **m**). The resulting primary anilines were treated with pyridine–$SO_3$ complex in pyridine to give the desired products. This process generally provided the final products in 25–50% yield following RP-HPLC purification.[14]

Additional diversity was introduced at the 3-position of the TIQ ring system (Table 2) from intermediate **5** using

**Table 2.** PTP1B inhibition data for compounds **9a–f**


**9a-f**

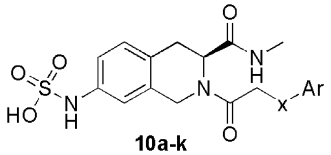
| Compound | $R^2$ | PTP1B $IC_{50}$ (μM) |
|---|---|---|
| **9a** |  | >500 |
| **9b** |  | 120.2 |
| **9c** |  | 51.4 |
| **9d** |  | 19.0 |
| **9e** |  | 16.7 |
| **9f** |  | 8.23 |

standard conditions. Following introduction of the $R^2$ substituent the synthesis was completed in a similar manner as that outlined in Scheme 1.

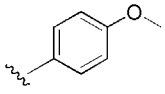
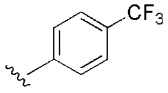
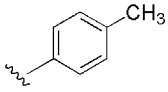
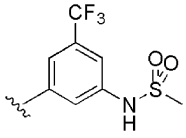
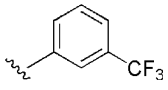
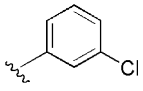
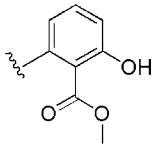
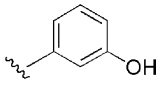
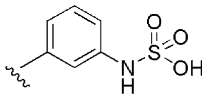
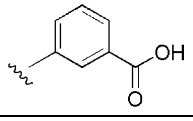
The initial TIQ sulfamic acid analog, **3**, which showed moderate PTP1B inhibition was the basis for further exploration around a core 1,2,3,4-tetrahydroisoquinoline structure. Compound **3** was shown to be a time-independent, competitive, reversible inhibitor of PTP1B with a $K_i = 24\ \mu M$.[15] Inversion of the stereochemistry to provide (*R*)-**3** (**8g**) resulted in a 3-fold loss in potency. The X-ray structures of both **3** and **8g** were obtained by soaking methods and showed opposing orientations of the side chains within the active site of the enzyme.[16] In the case of **3**, the *tert*-butyl carbamate side chain was oriented in the general direction of the previously reported second aryl phosphate binding site (P + 1 pocket), while **8g** adopted a flipped conformation and oriented the Boc side chain in the general direction of Lys41 (P − 1 pocket).[17] With this information, we decided to target the P + 1 pocket for potency enhancements by diversifying the (*S*)-TIQ scaffold. As a starting point, we chose to investigate the 2-position of the TIQ ring keeping the 3-position fixed as the methylamide similar to **3** and replacing the Boc group with simple substituents.

Incorporation of an amino acid residue, as in **8a**, resulted in a complete loss of potency. This is in contrast to

**Table 3.** SAR survey of P + 1 aryl substitution for TIQ sulfamic acids




**10a-k**

| Compound | X | Ar | PTP1B $IC_{50}$ (μM) |
|---|---|---|---|
| **10a** | $CH_2$ |  | 242 |
| **10b** | $CH_2$ |  | 224.8 |
| **10c** | $CH_2$ |  | 198 |
| **10d** | $CH_2$ |  | 183.7 |
| **10e** | $CH_2$ |  | 146 |
| **10f** | $CH_2$ |  | 101.1 |
| **10g** | O |  | 93.4 |
| **10h** | $CH_2$ |  | 82.1 |
| **10i** | $CH_2$ |  | 4.8 |
| **10j** | $CH_2$ |  | 2.5 |

what has been observed in other less rigid peptide-based inhibitors.[18–20] Initial experiments summarized in Table 1 were designed to gain insight into the ideal linker length between the TIQ scaffold and the aryl group which was to interact with residues in the P + 1 pocket. The data seem to indicate a preference for a three-atom linker between the TIQ system and the aryl ring (i.e., **8j**–**m**) and that a urea linkage (**8d**) is not well tolerated.

Table 2 provides a representative example of the SAR developed for the 3-position of the TIQ ring system. Introduction of a benzyl amide side chain in this position proved to be uniformly poor (e.g., **9b**) for all analogs regardless of the $R^1$ substituent. A phenethyl amide side chain (**9c**) returned compounds of roughly equal potency to that of **3**. Incorporation of an ester functionality in the 3-position provided a potency enhancement over **3** and this seemed to be somewhat independent of the size of the ester (**9d** and **e**). Reduction of the methyl ester **9d** to the primary alcohol **9a** resulted in a complete loss of activity, the reasons for which are unclear. Incorporation of an oxadiazole **9f** provided a 5-fold gain in potency and one of the most potent compounds from this survey.

The second phase of the investigation centered on identifying $R^1$ side chains which would be able to capture the positive interactions within the P + 1 pocket which have been previously reported.[21] These interactions center on key ionic interactions with Arg24 and Arg54 of the protein. We were fortunate to have X-ray structural data for most of the three-atom-linked aryl systems. Most notably the X-ray structure of **8k** showed the benzyl carbamate side chain to be positioned in the P + 1 pocket and to be ideally situated to interact with these residues if functionalized with the appropriate groups. Due to ease of synthesis and comparable potency to **8k**, entry **8j** was chosen as the starting point for optimization of interactions in the P + 1 pocket. We investigated substitution patterns on the aryl ring to identify elements capable of picking up the key interactions and to further probe the electronic and steric requirements of the pocket (Table 3).

Electron-withdrawing or releasing groups in the 3-position (**10e**, **f**, and **h**) did not provide gains in potency relative to **8j**. Similarly, substitution in the 4-position (**10a**–**c**) was detrimental to potency.

Incorporation of a second sulfamic acid residue in the 3-position of the side chain (**10i**) did result in a 13-fold increase in potency. X-ray crystal data of **10i** bound in the active site of PTP-1B (Fig. 3) show the second aryl sulfamic acid bound tightly in the P + 1 pocket. The second sulfamic acid moiety picks up hydrogen bonding interactions with Gln262, Arg24, and Arg254 of the protein. The charged sulfamic acid pulls Arg24 into the P + 1 and out of its native conformation to form a salt bridge. A similar result was obtained for the 3-carboxylate analog **10j** which provided a 16-fold increase in potency. Once again, X-ray crystallography showed the aryl carboxylate bound tightly in the P + 1 pocket with similar interactions to the bis-sulfamic acid **10i**. Analog **10i** was screened against a panel of 14 PTPases (Table 4). High selectivity was observed against several other therapeutically useful phosphatases

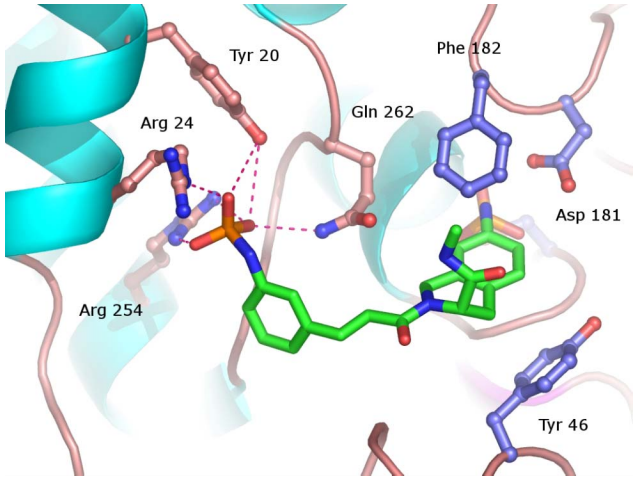



**Figure 3.** Crystal structure of **10i** bound to PTP1B. Carbon atoms of the compound are shown in green, those of the key residues in the P0 and P1 sites are colored dark blue and light brown, respectively.

**Table 4.** Selectivity of **10i** for a panel of protein phosphatases

| Phosphatases | $IC_{50}$ (μM) |
|---|---|
| PTP1B | 4.8 |
| HCPTPA | 3.1 |
| HPTPβ | 20.7 |
| SHP-1 | 203 |
| SHP-2 | >500 |
| HPTPμ | >500 |
| HPTPη | 150 |
| LAR | >500 |
| CD45 | >500 |
| PTPγ | >500 |
| STP-1 | 8.1 |
| VHR | 4.0 |
| TC-PTP38 | 9.9 |
| HPTPε | >500 |

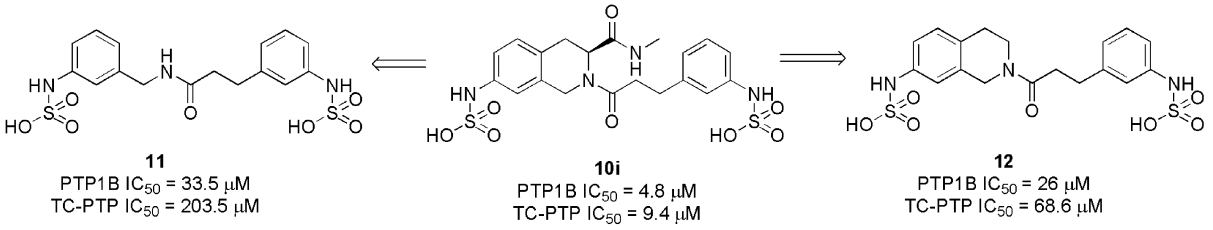



**Figure 2.** Simplification of **10i** and the effect on potency and selectivity.

(i.e., CD45, PTPγ, SHP2, etc.). Unfortunately, selectivity over the closely homologous TC-PTP was poor (2-fold).

Complete removal of the 3-position substituent from **10i** provided **12** which showed a 5-fold loss in potency (Fig. 2). Additional simplification of **10i** by removal of the constraining TIQ ring gave the benzyl amine derivative **11** which also suffered from a loss in potency (6-fold). Interestingly, the X-ray structure of **11** showed a reversal in the binding mode of the compound. In the case of **11**, the sulfamic acid residue of the propionic acid side chain was shown to bind in the P0 pocket, while the benzyl amine unit was extended into the P + 1 pocket.

High-throughput screening of the P&GP corporate repository identified the sulfamic acid functionality as a potential pTyr mimetic. Incorporation of a sulfamic acid moiety at the 7-position of the 1,2,3,4-tetrahydroisoquinoline scaffold provided compounds which showed promising initial PTP1B inhibitory properties. X-ray crystallography provided a guide to inhibitor design by determining that side chains extending from the 2-position of the natural enantiomer pointed toward the second aryl phosphate binding region (P + 1 pocket). An initial survey of side chains extending from the 2-position of the TIQ scaffold indicated that a three-atom linker between the TIQ ring system and the distal aryl ring was preferred. A second investigation provided an order of magnitude increase in potency by incorporating an acidic functionality in the 3-position of the aryl ring.

Simplification of the TIQ ring system by removal of the 3-carboxamide functionality reduced potency as did removal of the constraining saturated 6-membered ring. X-ray crystallography of these improved compounds revealed the nature of the ligand–protein interactions and should provide a stepping stone for further potency enhancements in this series of compounds.

## Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.12.051.

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14. All compounds were fully characterized by $^{1}H$ and $^{13}C$ NMR, HPLC, MS, and combustion analysis.
15. Phosphatase activity and kinetics: recombinant phosphatases (nM) were diluted in assay buffer (150 mM NaCl, 0–0.1% BSA, 5 mM DTT, and 50 mM Tris–HCl) (pH 7) or 10 mM Na/Tris acetate (pH 6) and incubated with 10 μM DiFMUP (Molecular Probes). After 15 min, the fluorescence increase was measured on a Victor V plate reader (Wallac). Inhibitors were pre-incubated with enzyme for 10 min before substrate addition. Kinetic measurements were measured using 3–5 concentrations of inhibitor around the $IC_{50}$ over a range of substrate concentrations (3–500 μM). Data were used to determine $K_m/K_i$/competitive inhibition using GraphPad Prism software.
16. Crystal structures: preparation of recombinant PTP1B catalytic domain (1–322) was essentially as described earlier (see Ref. 21). Crystallization of apo-PTP1B for soaking experiments, co-crystallization of PTP1B bound to various inhibitors, X-ray data collection, and structure solution are described in the Supplementary material.
17. All structures referred to in this article were deposited with the RCSB (www.rcsb.org/pdb) under PDB-IDs of 2F6T (**3**), 2F6Y (**8g**), 2F6V (**8k**), 2F6Z (**10i**), 2F71 (**10j**), 2F70 (**11**), and 2F6W (**13**-Supplementary material).
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