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Benzothiazole benzimidazole (*S*)-isothiazolidinone derivatives as protein tyrosine phosphatase-1B inhibitors

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Abstract—Benzothiazole benzimidazole (*S*)-isothiazolidinone ((*S*)-IZD) derivatives **5** were discovered through a peptidomimetic modification of the tripeptide (*S*)-IZD protein tyrosine phosphatase 1B (PTP1B) inhibitor **1**. These derivatives are potent, competitive, and reversible inhibitors of PTP1B with improved caco-2 permeability. An X-ray co-crystal structure of inhibitor **5**/PTP1B at 2.2 Å resolution demonstrated that the benzothiazole benzimidazole forms bi-dentate H-bonds to Asp48, and the benzothiazole interacts with the surface of the protein in a solvent exposed region towards the C-site. The design, synthesis, and SAR of this novel series of benzothiazole benzimidazole containing (*S*)-IZD inhibitors of PTP1B are presented herein.

Many cellular processes are controlled through phosphorylation and dephosphorylation of intracellular proteins by protein tyrosine phosphatases (PTPs) and protein tyrosine kinases (PTKs). Many disease states, including diabetes, cancer, and rheumatoid arthritis, are associated with the aberrant regulation of these signaling transduction pathways.[1] While the success of PTK inhibitors is well documented, the discovery of drug candidates for PTP drug targets remains elusive.

Recently, we described the discovery of potent, competitive, and reversible PTP1B peptide and non-peptide inhibitors containing the novel heterocyclic (*S*)-isothiazolidinone ((*S*)-IZD) pTyr mimetic.[2] We report herein the design and synthesis of a less polar benzothiazole benzimidazole scaffold containing the (*S*)-IZD heterocyclic pTyr mimetic which demonstrates improved caco-2 permeability.

We recently reported a peptidomimetic strategy that resulted in the replacement of the two N-terminal amides with an aryl sulfonamide to afford potent benzimidazole sulfonamide PTP1B inhibitors.[3] The ortho-substituted derivatives, such as **2**, exhibited the first reported cellular activity (pIR) for this new class of PTP1B inhibitors bearing the heterocyclic (*S*)-IZD pTyr mimetic.

Consistent with the modest cellular activity and high polar surface area (PSA = 138) was the measured low cell permeability for this benzimidazole sulfonamide series of compounds (caco-2 Pm $0.1–0.4 \times 10^{-6}$ cm/s). We surmised that further reduction in the PSA would be necessary for obtaining the requisite membrane permeability to drive cellular potency (Table 1). Analysis of the substituent contributions to the PSA of the benzimidazole sulfonamides led us to search for replacement of the highly polarizable sulfonamide functionality. Synthesis of a variety of functional groups appended to the primary amine of **6** identified the benzothiazole compound **5**, as a lead. Compound **5** contains the (*S*)-IZD as determined by X-ray crystallography studies. Consistent with our previous report,[3] compound **5** is the only active

Keywords: Protein tyrosine phosphatase 1B; Phosphotyrosine mimetics; Phosphatase inhibitors; Isothiazolidinone heterocycle; Benzothiazole benzimidazole.
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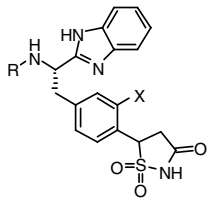
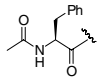
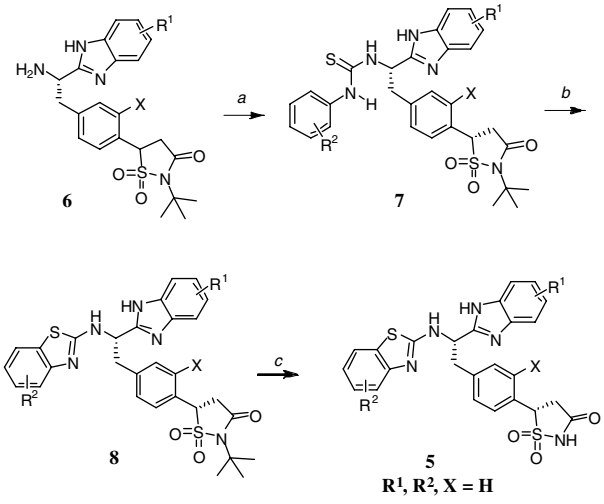
Table 1. Selected IZD inhibitors of PTP1B



Compound	R	IZD	X	PTP1B[a] IC_{50} (nM)	pIR[c] (at 80 μM)	Caco-2[b] Pm ($\times 10^{-6}$ cm/s)	PSA
1		*S*	H	35	Inactive	<0.1	150
2		*S*	Me	35	2.8 ± 0.8	0.4	138
3		*R*/*S*	H	480	Inactive	1.3	116
4		*R*	H	>10,000	Inactive	NT	116
5		*S*	H	270	Inactive	0.7	116

[a] pNPP enzyme assay.
[b] NT, not tested.
[c] Fold shift versus DMSO.

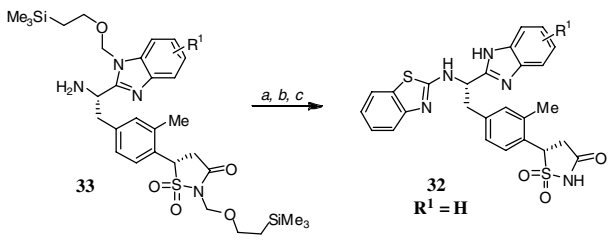
isomer. This non-peptidic small molecule is a moderately potent (PTP1B IC_{50} = 270 nM), competitive, and reversible inhibitor with significantly lower PSA (116). Most notably, the reduced PSA of this derivative correlated with an observed increase in cell penetration as measured by a caco-2 assay (Pm = 0.7×10^{-6} cm/s). Optimization of **5** by further elaboration of the benzothiazole scaffold was thus warranted (Scheme 1).



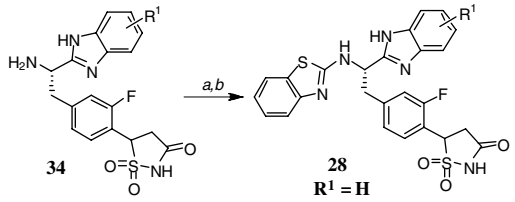
Scheme 1. Synthesis of benzothiazole benzimidazole (*S*)-IZD inhibitors. Reagents and conditions: (a) PhNCS, TEA, DCM, rt, 1 h, 57%; chiral separation of (*R*/*S*)-IZD diastereomers was performed on a chiral OD column; (b) benzyltrimethylammonium tribromide, HOAc, 15 min, rt, 88%; (c) TFA, 130 °C, μW, 1 min, 70%.

Benzothiazoles containing the (*S*)-IZD heterocyclic pTyr mimetic were synthesized starting from the previously reported benzimidazole amine **6** in a two-step process (Supporting Information). Thiourea **7** was initially formed by reacting **6** with phenylisothiocyanate. Cyclization of **7** to give benzothiazole **8** was achieved via bromination of the thiourea sulfur and subsequent electrophilic addition to the ortho position of the phenyl ring.[4] Removal of the *tert*-butyl protecting group on the (*S*)-IZD via microwave irradiation at 130 °C for 1 min afforded the desired benzothiazole benzimidazole products in high yields.

Synthesis of the *ortho*-methyl benzothiazole benzimidazole **32** proceeded in a similar manner from the literature bis-sem-protected intermediate **33** (Scheme 2).[3] The *ortho*-fluoro derivative **28** was synthesized using the known unprotected (*S*)-IZD heterocycle as shown in Scheme 3.



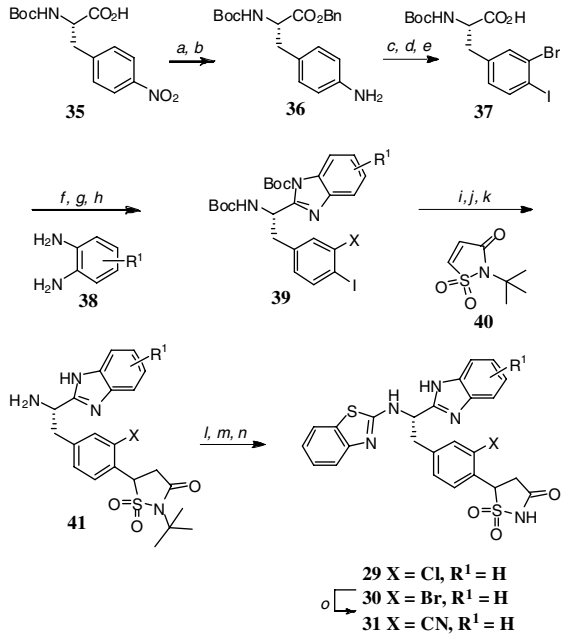
Scheme 2. Synthesis of *ortho*-methyl benzothiazole benzimidazole IZD inhibitors. Reagents and conditions: (a) PhNCS, TEA, DCM, rt, 1.5 h, 72%; (b) benzyltrimethylammonium tribromide, HOAc, 1 h, rt; (c) TFA, 130 °C, μW, 2 min, 45% (2 steps).



Scheme 3. Synthesis of *ortho*-fluoro benzothiazole benzimidazole IZD inhibitors. Reagents and conditions: (a) PhNCS, TEA, DCM, rt, 1 h, 70%; (b) benzyltrimethylammonium tribromide, HOAc, 1.5 h, rt, 62%.

Synthesis of the *ortho*-bromo derivatives **30** and *ortho*-chloro derivatives **29** required the development of a new synthetic route as shown in Scheme 4. Previously, we reported a novel chemoselective and regioselective Heck reaction that was used on the methylester of **37** to access the *ortho*-halogenated IZD intermediate **41**. In our new strategy, the Heck coupling is performed later in the synthesis with the bis-Boc-protected benzimidazole **39** to afford the requisite aminobenzimidazole after deprotection.

The synthesis of the *ortho*-chloro derivative **29** was performed in an analogous fashion, in similar yields, using NCS in the halogenation step (c). The *ortho*-cyano derivative **31** was obtained through Negishi coupling to the *ortho*-bromo derivative **30**.



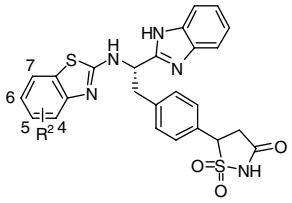
Scheme 4. Synthesis of *ortho*-bromo benzothiazole benzimidazole IZD inhibitors. Reagents and conditions: (a) BnBr, DBU, THF, 60 °C, 2 h, 80%; (b) Zn^0, NH_4Cl, MeOH, H_2O, 78%; (c) NBS, DMF, rt, 30 min, 51%; (d) $NaNO_2$, 1 N aq. HCl, KI, 44%; (e) NaOH, H_2O, CH_3CN, rt, 2 h; (f) BOPCl, *i*-Pr_2NEt, **38**, DMF, rt, 2 h, 98% (2 steps); (g) AcOH, 40 °C, 2 h; (h) Boc_2O, NaOH, H_20, THF, 35 °C, 2 h, 68% (2 steps); (i) $Pd(OAc)_2$, Bu_4NCl, **40**, Et_3N, DMF, 70 °C, 2.5 h, 34%; (j) L-Selectride, THF, −78 °C, 10 min, 78%; (k) TFA, DCM, 1:4, rt, 1 h, 58%; (l) PhNCS, TEA, DCM, rt, 1 h; (m) benzyltrimethylammonium tribromide, HOAc, 16 h, rt; (n) TFA, 130 °C, μW, 1 min, 36% (3 steps); (o) tetrakis(triphenylphosphine)Pd°, $Zn(CN)_2$, DMF, 175 °C, μW, 4 min.

A variety of substitutions at the 4-, 5-, and 6-positions of the benzothiazole were synthesized and tested in the PTP1B enzyme assay. The SAR revealed only minor substitution effects on enzyme potency (Table 2). An X-ray co-crystal structure of benzothiazole benzimidazole **5**/PTP1B solved to 2.2 Å resolution (PDB deposition number 2CNF) confirmed the presence of the bidentate H-bonding mode with Asp48 and the position of the benzimidazole in the, so-called E-site of PTP1B (Fig. 1). The benzothiazole binds toward the C-site above Tyr46. The nitrogen lone pair of the benzothiazole forms a hydrogen bond to a water molecule, but is otherwise solvent exposed on the surface of the enzyme.

Caco-2 permeability was significantly enhanced for this series of non-peptidic benzothiazole benzimidazole IZD inhibitors. The most permeable compounds were the fluoro substituted benzothiazoles (**12**, **13**, and **14**) with caco-2 Pm values of 0.9, 1.3, and 1.7×10^{-6} cm/s, respectively.

A representative set of substituted benzimidazoles is shown in Table 3. Again, only small changes in the enzyme potency were achieved. This SAR is consistent with the binding mode observed in the X-ray structure in which the benzimidazole binds in the solvent exposed E-site. Indeed, similar SAR was observed for the benzimidazole sulfonamide series **3** where the benzimidazole was shown to bind in an identical conformation[3] (Table 4).

Table 2. Benzothiazole SAR of IZD inhibitors of PTP1B



Compound	R^2	PTP1B[a] IC_{50} (μM)	Caco-2[b] Pm ($\times 10^{-6}$ cm/s)
3	H	0.48	1.3
9	6-CF_3	0.33	0.1
10	5-CF_3	1.30	NT
11	4-CF_3	0.74	0.1
12	6-F	0.49	0.9
13	5-F	0.42 (0.34)[c]	1.3
14	4-F	0.66	1.7
15	6-Cl	0.44	0.8
16	5-Cl	0.97	0.8
17	4-Cl	2.40	NT
18	6-OMe	0.35	0.5
19	4-OMe	0.55	1.0
20	6-CN	0.47	0.4
21	5-CN	1.10	NT
22	6-Br	0.50	0.2
23	5-Br	0.62	1.3
24	4-Br	1.10	NT

[a] pNPP enzyme assay.
[b] NT, not tested.
[c] The pNPP data for the separated single diastereomer.

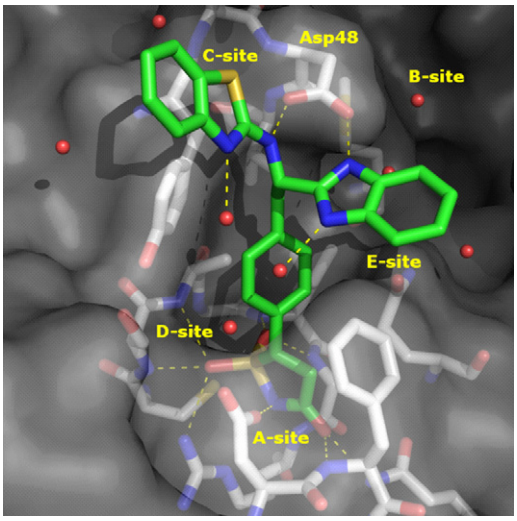
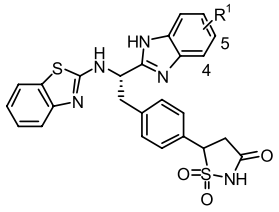


Figure 1. Co-crystal structure of **5**/PTP1B showing the (*S*)-IZD heterocycle in the active site, the bi-dentate hydrogen bonding to Asp-48, the benzimidazole in the E-site, and the benzothiazole in the C-site. PDB deposition number 2CNF.

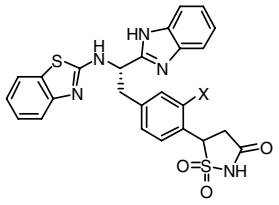
Table 3. Benzimidazole SAR of IZD inhibitors of PTP1B



Compound	R^1	PTP1B[a] IC_{50} (μM)	Caco-2 Pm ($\times 10^{-6}$ cm/s)
3	H	0.48	1.3
25	5-CF_3	0.15	0.4
26	5-Cl	0.41	0.8
27	5-CN	0.47	0.4

[a] pNPP enzyme assay.

Table 4. IZD inhibitors of PTP1B



Compound	X	PTP1B[a] IC_{50} (μM)	pIR[c] (at 80 μM)	Caco-2 Pm[b] ($\times 10^{-6}$ cm/s)	PSA
3	H	0.48	Inactive	1.3	116
28	F	0.23	Inactive	0.8	116
29	Cl	0.28	Inactive	0.7	116
30	Br	1.60	Inactive	NT	116
31	CN	0.40	Inactive	0.5	140
32	Me	0.46	Inactive	0.5	116

[a] pNPP enzyme assay.
[b] NT, not tested.
[c] Fold shift versus DMSO.

We reported earlier[3] that the benzimidazole sulfonamide series afforded cellular active PTP1B inhibitors only when they were substituted ortho to the (*S*)-IZD heterocyclic pTyr mimetic on the aryl ring. A series of ortho substituted benzothiazole derivatives were therefore synthesized and assayed in the PTP1B enzyme and pIR cellular assays. The *ortho*-fluoro derivative **28** and the *ortho*-chloro derivative **29** were the most potent of the benzothiazole benzimidazole inhibitors, but gave modest increases in enzyme potency (2-fold) as compared to the unsubstituted parent. Unfortunately, unlike the benzimidazole sulfonamide series, no improvements in the caco-2 permeability or pIR cellular activity were observed. This is believed to be due to the modest potencies of these derivatives.

In summary, optimization of the tripeptidic (*S*)-IZD protein tyrosine phosphatase 1B (PTP1B) inhibitors **1** led to the discovery of novel non-peptidic benzothiazole benzimidazoles **5** with reduced PSA. These derivatives are potent, competitive, and reversible inhibitors of PTP1B. A co-crystal structure revealed that the benzothiazole binds in the solvent exposed C-site while the benzimidazole binds in the E-site. As a result of the solvent exposed nature of the E- and C-sites, substitution of the benzothiazole and benzimidazole gave only modest improvement in potency for this new series. Caco-2 permeability was significantly enhanced for this series of non-peptidic benzothiazole benzimidazole IZD inhibitors presumably due to the lower PSA. Although no activity was observed in our cellular pIR assay these results demonstrate that non-peptidic compounds bearing the (*S*)-IZD pTyr mimetic with low PSAs can have improved caco-2 permeabilities.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.10.079.

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