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Y-shaped bis-arylethenesulfonic acid esters: Potential potent and membrane permeable protein tyrosine phosphatase 1B inhibitors

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ABSTRACT

Known PTP1B inhibitors with bis-anionic moieties exhibit potent inhibitory activity, good selectivity, however, they are incapable of penetrating cellular membranes. Based upon our finding of a new pharmacophoric group in inhibition of PTP1B and the structural characteristics of the binding pocket of PTP1B, a series of bis-arylethenesulfonic acid ester derivatives were designed and synthesized. These novel molecules, particularly Y-shaped bis-arylethenesulfonic acid ester derivatives, exhibited high PTP1B inhibitory activity, moderate selectivity, and great potential in penetrating cellular membranes (compound **7p**, CLog P = 9.73, $P_{app} = 9.6 \times 10^{-6}$ cm/s; IC_{50} = 140, 1290 and 920 nM on PTP1B, TCPTP and SHP2, respectively). Docking simulations suggested that these Y-shaped inhibitors might interact with multiple secondary binding sites in addition to the catalytic site of PTP1B.

Substantial evidence indicated that protein tyrosine phosphatase 1B (PTP1B), the first isolated member of the PTP family, negatively regulate insulin signal transduction by directly or indirectly dephosphorylating the phosphotyrosine of many related signal proteins, such as insulin receptor (IR), insulin receptor kinase (IRK), and insulin receptor substrates (IRS) etc. PTP1B is a potential drug target for the treatment of type 2 diabetes and obesity.[1]

Due to promising application for type 2 diabetes, PTP1B inhibitors have become an attractive research area over the past several decades.[2] Zhang and coworkers reported, a phosphotyrosine (PTyr) mimic in 1997, and it was the first dimeric type of PTP1B inhibitor with low molecular weight but high affinity (BPPM, Fig. 1, **I**).[3] A number of PTyr mimics with bis-anionic moieties were later discovered, including bis-α,α-difluoromethylphosphonic acid (DFMP, Fig. 1, **II**)[4] and bis-benzoylformic acid (Fig. 1, **III**).[5] They exhibited more potent activity and better selectivity than their analogues with single anionic moiety. Nevertheless, these anionic inhibitors which matched well with the positively charged catalytic site of PTP1B, suffered from an apparent drawback that they are incapable of penetrating cellular membranes.[6] In order to improve molecular membrane permeability, efforts were focused on developing uncharged or nonionic PTyr mimics.

Some new scaffolds of inhibitors emerged in response to this demand, such as thioxothiazolidinone derivative (Fig. 1, **IV**)[7] and sulfonamide derivatives (Fig. 1, **V** & **VI**)[8] despite the fact that phenolic hydroxyl and diimide moieties could be ionized in basic water solution. Moreover, our previous research indicated that arylethenesulfonic acid ester derivatives (Fig. 1, **VII** & **VIII**)[9], not being hydrolyzed and ionized at physiological pH, could also exhibit comparable activity to those ionic PTP1B inhibitors. This suggested that sulfonic acid ester is an effective bioisostere of phosphoric acid and carboxylic acid moiety in the inhibition of PTP1B. These results prompted us to further develop more potent PTP1B inhibitors with improved membrane permeability.

In addition, the catalytic site of PTP1B, where PTyr residues of the IRK activation peptide are dephosphorylated, is a highly conserved site of all PTP family members, thus making it difficult to develop selective inhibitors.[10] However, complex crystals and molecular docking simulations of some inhibitors with branched linkers revealed multiple secondary binding sites (B, C, D and/or E site) around the catalytic site (A site) of PTP1B.[11] Therefore, a feasible method to design potent and selective PTP inhibitors with improved membrane permeability is to engage both the active site and peripheral binding sites thorough tethering appropriately functionalized moieties to a nonionic PTyr mimic.[12]

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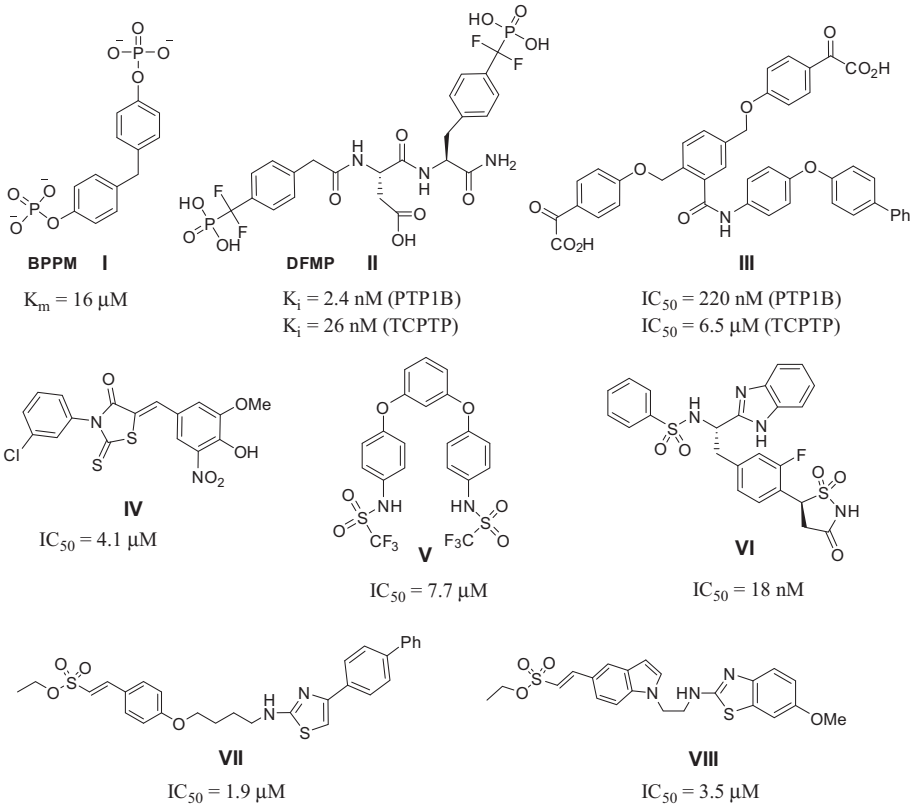
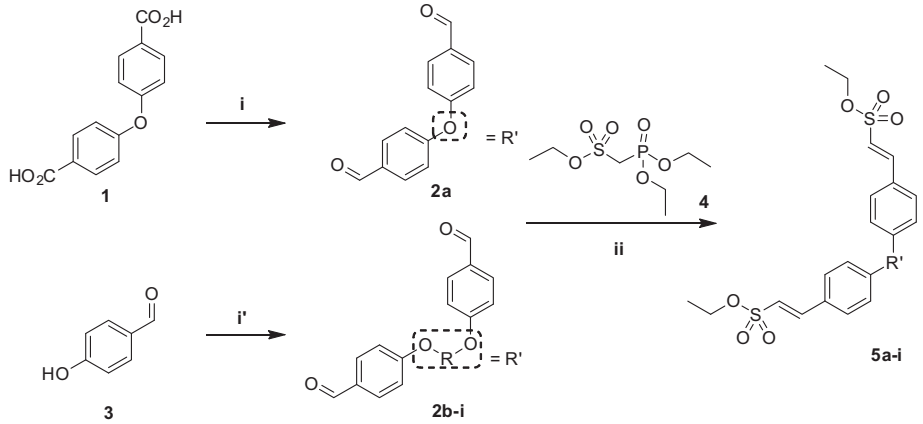


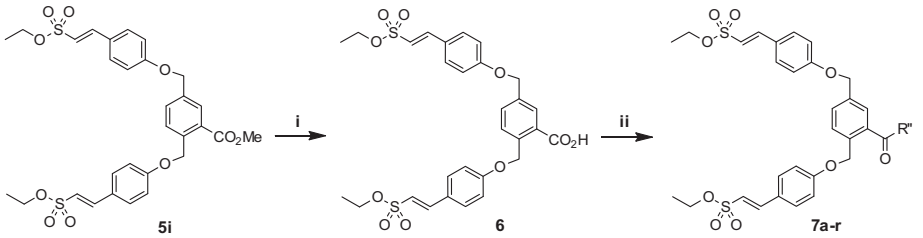
Fig. 1. Representative PTP1B inhibitors.

In this report, we continued to design, synthesize and evaluate a series of nonionic PTyr mimics with bis-arylethenesulfonic acid ester moiety. Preliminary results indicated that Y-shaped bis-arylethenesulfonic acid esters are potent, membrane permeable and moderate selective PTP1B inhibitors, which could interact with multiple peripheral binding sites (probably, C and E site) as well as the catalytic site of PTP1B.

Strategies for synthesis of bis-arylethenesulfonic acid ester derivatives (**5a**–**5i** and **7a**–**7r**) are outlined in Schemes 1 and 2. Compound **5a** was prepared from 4,4′-oxydibenzoic acid (**1**) through three step reactions. Thus, **1** was first reduced by $LiAlH_4$ to a diol intermediate, which was then oxidized by PCC to afford 4,4′-oxydibenzaldehyde (**2a**).[13] **2a** underwent a simple Wittig-Horner reaction by treating with ethyl (diethoxyphosphoryl) methanesulfonate (**4**) in the presence of NaH in THF at room temperature, affording the target compound **5a** in 94.0% yield. Other bis-arylethenesulfonic acid esters (**5b**–**5i**) were prepared from 4-hydroxybenzaldehyde (**3**) via two steps. Firstly, two molecules of 4-hydroxybenzaldehyde were coupled using dihalohydrocarbons or dimethanesulfonates as linking reagents, affording bis-benzaldehydes intermediates (**2b**–**2i**).[14] Then **2b**–**2i** underwent the same reaction as **2a** to provide bis-arylethenesulfonic acid ester derivatives (**5b**–**5i)** with high yields (84.7%–95.6%). Subsequently, bis-arylethenesulfonic acid ester derivatives (**7a**–**7r**) were prepared from the above synthesized **5i** via two steps (Scheme 2). **5i** was hydrolyzed with LiOH to give the benzoic acid intermediate



Scheme 1. The synthesis of bis-arylethenesulfonic acid ester derivatives **5a**–**5i**. Reagents and conditions: (i) a: $LiAlH_4$, THF, 60 °C, 2 h; b: PCC, DCM, rt, 3 h; (i′) dihalohydrocarbons, K_2CO_3, ACN, refluxed, 16 h; or NaOH, DMSO, 100 °C, 8 h; (ii) NaH, THF, rt, 6 h.



Scheme 2. The synthesis of bis-arylethenesulfonic acid ester derivatives **7a**–**r**. Reagents and conditions: (i) 1 N LiOH/THF, rt, 16 h; (ii) arylamines, HATU, DIPEA, DMF, rt, 6 h.

(**6**) and the latter condensed with various arylamines in the presence of HATU to form bis-arylethenesulfonic acid ester derivatives **7a**–**7r**.

The design of PTP1B inhibitors and their SARs

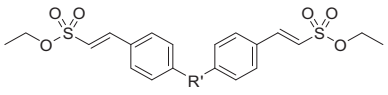
Due to the higher potency exhibited by known PTP1B inhibitors with bis-anionic moieties, several unbranched bis-arylethenesulfonic acid esters (**5a**–**5h**) were initially designed, which used different length and flexibility of alkylenedioxyl bridges (diether linkages) as linker. As shown in Table 1, and with the exception of compound **5e**, all the other tested bis-arylethenesulfonic acid ester derivatives showed moderate to potent PTP1B inhibitory activity (IC_{50} = 1–40 μM). This proved true regardless of whether the alkylenedioxyl bridges were linear alkylidenes, cyclohexylidene or aralkylidenes. Increasing the length of linear alkylidene from C0 to C2 slightly enhanced PTP1B inhibitory activity of bis-arylethenesulfonic acid esters (**5a**/**5b**/**5c**). Further increasing the length from C2 to C4 resulted in a rapid decline of molecular activity (**5c**/**5d**/**5e**). This seems to indicate that the length of alkylenedioxyl bridges exert a critical influence over the inhibitory activity of these derivatives. However, 1,4-cyclohexylidene, with similar length but different flexibility to 1,4-butylidene, endued corresponding derivative (**5f**) with moderate activity. Further, linear aralkylidenes derivatives, particularly *p*-xylene derivatives (**5h**) which have longer chain length, exhibited the most potent activity (IC_{50} = 1.2 μM). These results strongly suggested that the PTP1B inhibitory activity of chain bis-arylethenesulfonic acid esters depend largely on their spatial configurations rather than the lengths of alkylenedioxyl bridges. Excess of molecular flexibility caused by linkers will reduce bis-arylethenesulfonic acid esters' affinity.

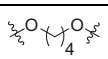
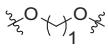
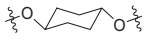
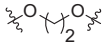
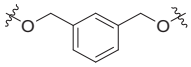
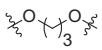
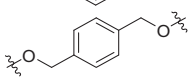
Based on compound **5h** which is a standard dimeric form, we designed a set of branched bis-arylethenesulfonic acid ester derivatives in order to discover additional binding interactions with PTP1B. As shown in Table 2, short branch-chains such as methoxycarbonyl (**5i**), carboxyl (**6**) and anilinocarbonyl groups (**7a**–**7d**) couldn't improve the inhibitory activity of bis-arylethenesulfonic acid esters. Further expansion of branch-chain with aryl groups enhanced the PTP1B inhibitory activity of compound **5h** for 3–9 times, such as compounds **7j**–**7m** and **7p**. This suggests additional interactions between these Y-shaped bis-arylethenesulfonic acid esters with multiple peripheral binding sites of PTP1B.

The selectivity and membrane permeability of PTP1B inhibitors

For those Y-shaped bis-arylethenesulfonic acid esters with IC_{50} values below 500 nM on PTP1B, we further tested their inhibitory activity on other PTPs (TCPTP and SHP2) and evaluated their membrane permeability. As shown in Table 3, most of these Y-shaped bis-arylethenesulfonic acid esters showed moderate selective inhibitory activity for PTP1B over TCPTP and SHP2. Specifically, 5–9-fold selectivity for PTP1B over TCPTP was observed in compounds **7j**, **7k**, **7l**, **7m** and **7p**, and 5–6-fold selectivity for PTP1B over SHP2 was observed in compounds **7j**, **7l**, **7m** and **7p**. Furthermore, these Y-shaped bis-arylethenesulfonic acid esters have significantly higher CLogP values than that of bis-anionic PTP1B inhibitors such as BBPM and DFMP (CLogP = 0.20 & 1.20 respectively). And as we expected, sufficient membrane permeabilities of this kind of inhibitors are demonstrated by parallel artificial membrane

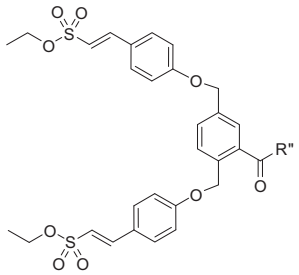
Table 1
PTP1B inhibitory activities of bis-arylethenesulfonic acid esters.[a]

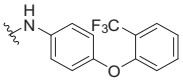
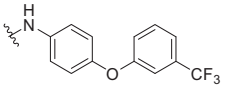
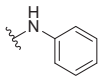
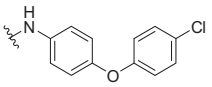
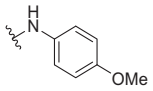
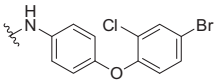
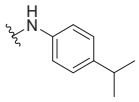
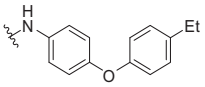
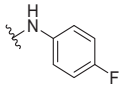
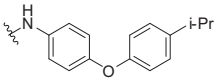
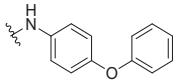
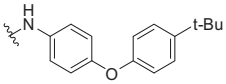
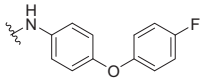
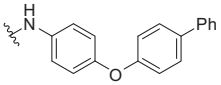
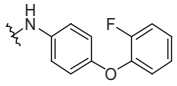
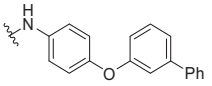
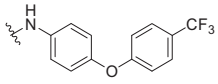
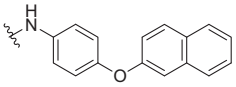


Cmpd	R′	IC_{50} (μM)	Cmpd	R′	IC_{50} (μM)
5a		11.8	**5e**		>100
5b		8.7	**5f**		29.2
5c		3.0	**5g**		18.4
5d		37.3	**5h**		1.2

[a] Values are means of triplicates, repeated two times.

Table 2
PTP1B inhibitory activities of branched bis-arylethenesulfonic acid ester derivatives.[a]



Cmpd	R″	IC_{50} (μM)	Cmpd	R″	IC_{50} (μM)
5i	OCH_3	3.4	**7i**		0.87
6	OH	1.3	**7j**		0.35
7a		1.4	**7k**		0.40
7b		2.6	**7l**		0.16
7c		1.3	**7m**		0.19
7d		1.1	**7n**		0.75
7e		0.86	**7o**		0.91
7f		1.3	**7p**		0.14
7g		0.85	**7q**		1.1
7h		0.82	**7r**		0.79

[a] Values are means of triplicates, repeated two times.

permeation assay (PAMPA).[15] Compared with DFMP which is one of the most potent PTP1B inhibitors so far, compound **7p**, despite relatively weak potency (IC_{50} = 140 nM), not only exhibit similar selectivity for PTP1B but have a great potential in penetrating cellular membranes.

Molecular docking simulation

Molecular docking of the most potent and selective compound **7p** for PTP1B was performed on GOLD 5.0.1 and presented in PyMol 1.1r1. As showed in Fig. 2, compound **7p** extended deep into the active site (A site), forming several hydrogen bonds and hydrophobic interactions with key residues including Ala215, Arg221, Gly218, Ile219, Gly220 and the backbone NHs of Ser216. The phenyl ring (A1 cycle) interacts by π-π stacking interaction with the phenyl ring of Tyr46, and the phenyl ring (A2 cycle) interacts with the phenyl ring of Phe182. The sulfonic acid ester group forms hydrogen bond with the backbone NHs of Lys36 in the second sulfonic acid ester-binding site (C site). Additionally, a hydrophobic interaction may be established between the benzyl ring (E1 and E2 ring) of the ligands and Thr263 and Ala264 in E site. These multiple interactions contribute to improvements of the inhibitory activity and selectivity of compound **7p**.

Table 3
The inhibitory activities and membrane permeability of partial Y-shaped bis-arylethenesulfonic acid esters on PTP1B, TCPTP and SHP2.

Cmpd	PTP1B IC_{50} (nM)[a]	TCPTP IC_{50} (nM)[a]	SHP2 IC_{50} (nM)[a]	CLog P[b]	P_{app} (10^{-6} cm/s)[c]
Na_3VO_4	46	14	NT	−0.92	ND
BBPM	16000[d]	NT	NT	0.20	ND
DFMP	2.4[e]	26[c]	NT	1.20	ND
5h	1200	3390	4700	5.58	ND
7j	350	1620	1700	8.73	6.9
7k	400	2970	940	8.56	9.1
7l	160	1430	1050	9.19	12.6
7m	190	1320	1100	8.87	8.5
7p	140	1290	920	9.73	9.7
Atenolol[f]	–	–	–	–	1.5
Propranolol[f]	–	–	–	–	18.7

[a] Values are means of triplicates, repeated two to three times.
[b] Predicted by ChemBioDraw Ultra 13.0.
[c] Values of parallel artificial membrane permeation assay (PAMPA).
[d] K_m value, reported by literature.3
[e] K_i values, reported by literature.4
[f] Controls in PAMPA.

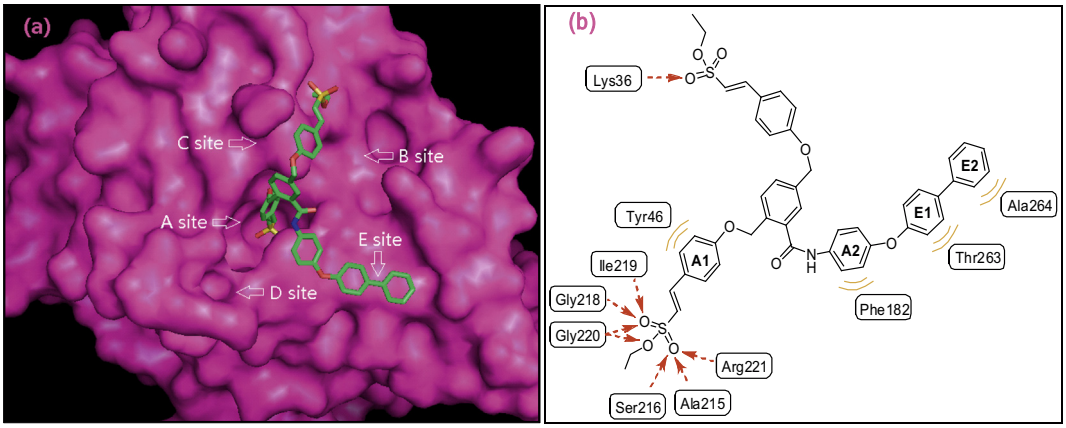


Fig. 2. Docking of compound **7p** to PTP1B (PDB code 1G1H). (a) Binding conformation of compound **7p** in PTP1B. (b) Schematic representation of the interactions between compound **7p** and PTP1B. Hydrogen bonds are depicted with red arrows, and π-π interactions or hydrophobic interactions are depicted with brown discs.

In summary, the design and synthesis of a series of bis-ethenesulfonic acid ester derivatives as potential PTP1B inhibitors, possessing high potency and excellent membrane permeability, was carried out. Preliminary research demonstrated that bis-ethenesulfonic acid esters, particularly Y-shaped bis-ethenesulfonic acid ester derivatives are potent and selective PTP1B inhibitors (compound **7p**, IC_{50} = 140, 1290 and 920 nM on PTP1B, TCPTP and SHP2, respectively). More importantly, they have a great advantage in penetrating cellular membranes. This provides useful information for further design and discovery of new PTP1B inhibitors with high potency, selectivity and good membrane permeability.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.03.060.

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