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Design, synthesis and docking studies on phenoxy-3-piperazin-1-yl-propan-2-ol derivatives as protein tyrosine phosphatase 1B inhibitors ☆

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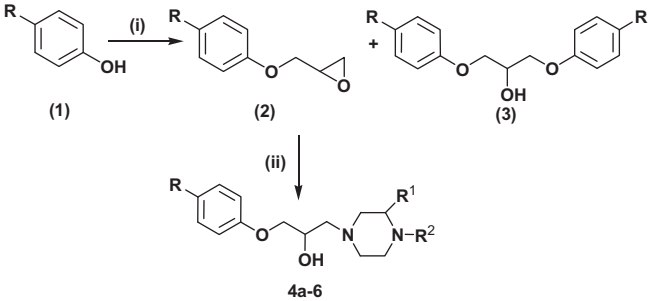
ABSTRACT

A series of substituted phenoxy-3-piperazin-1-yl-propan-2-ols has been synthesized and evaluated for PTP1B inhibitory activity in vitro and for antidiabetic activity in vivo. Two molecules viz. **4a** and **5b** showed PTP1B inhibition of 31.58% and 35.90% at 100 μM concentration. The compound **4a** also showed 40.3% normalization of plasma glucose levels at 100 mg/kg in Sugar-loaded model (SLM) and 32% activity in Streptozodocin model (STZ). The docking studies of these molecules revealed that hydrogen bond formation with Arg221 is important for activity.

Protein tyrosine phosphatases (PTPases) belong to the protein phosphatases which include protein serine/threonine phosphatases (PSTPs). The PTPases are often related to cellular dysfunction and play an important role in different diseases viz. diabetes, obesity, inflammation, cancer and neurodegeneration.[1–3] Among these, type-2 diabetes and obesity are related with the deficiency in insulin receptor signaling and is supposed to be recovered by the inhibition of a certain PTPase(s) including prolonged phosphorylation of the insulin receptor kinase.[4–7] The protein tyrosine phosphatase 1B (PTP1B) plays a key role in cellular signaling and in several human diseases, particularly diabetes and obesity.[8–10] It is an intracellular non-receptor PTPase and is expressed in a large variety of human tissues.[11] It inhibits the insulin receptor (IR) through dephosphorylation. The PTP1B knockout mice improve glucose tolerance and enhance insulin sensitivity as a result of improved insulin action in skeletal and liver muscle without affecting insulin action in adipose tissue. In addition, disorder of PTP1B gene in mice unexpectedly protects the mice against obesity when placed on a high fat diet.[4,12] Hence potent, orally active and selective PTP1B inhibitors are considered as potential pharmacological agents for the treatment of obesity and NIDDM.[13] Several series of PTP1B inhibitors including 2-(oxalylarylamino)-benzoic acids,[14] α-keto acids,[15] aminothiazoles,[16] formylchromanes,[17] isothiazolidinone derivatives,[18] 2-(4-methoxyphenyl) ethyl] acetamide derivatives,[19] have been reported in recent years. In view of the above and using the substructural analysis approach, a series of phenoxy-3-piperazin-1-yl-propan-2-ol was designed and evaluated for in vitro PTP1B inhibitory activity and in vivo antidiabetic activity in SLM and STZ models. The general route for the synthesis of phenoxy-3-piperazin-1-yl-propan-2-ol derivatives is outlined in Scheme 1. The condensation of various substituted phenols (**1**) with epichlorohydrin in the presence of bases like NaOH or NaH, in THF at 100 °C gave two products; 2-(phenoxymethyl)oxirane (**2**) and 1,3-diphenoxy-propan-2-ol (**3**), which were characterized by the PMR and mass spectroscopy. The condensation of *para* substituted 2-(phenoxymethyl) oxirane (**2**) with different substituted piperzines in ethanol at 80 °C gave the required compounds[20] (**4a**–**6**). The synthesized compounds were evaluated in vitro for their PTP1B inhibitory activity at 100 μM dose according to Goldstein



Scheme 1. Synthesis of compound **4a-6**. Reagents and conditions: (i) epichlorohydrin, NaOH or NaH, THF, 100 °C; (ii) ethanol, 80 °C, various substituted piperzines.

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method using Na-orthovanadate and peroxovanadate as a reference standard.[21] The molecules were screened for in vivo activity in SLM and STZ models in rat. The results of the in vitro inhibition assays are reported in Table 1, which indicated that the inhibitory effects of these analogues differed markedly due to the substituent variation in the phenoxyphenyl ring. Among these, the most active analogue **5b** having 4-Cl group at R and 4-nitrophenyl group at R^2 showed good in vitro inhibitory activity (% inhibition = 35.9% at 100 μM). The replacement of 4-Cl group with 4-F group (**6**) at R position resulted in slight reduction in PTP1B inhibitory activity (% inhibition = 24.0% at 100 μM). The compound **4a**, with 4-CN group at R, Me group at R^1 and 4-methylphenyl substituents at R^2 resulted in almost equal activity (% inhibition = 31.58% at 100 μM). Insertion of 4-fluorophenyl (**4c**), 3-chlorophenyl (**4d**) 2-methoxyphenyl (**4e**), phenyl (**4f**), 2-pyridine (**4g**), 5-Me-benzo1, 3 dioxole (**4h**) and benzyl group (**4i**) at R^2 position keeping 4-CN group constant at R position, resulted in total loss in the activity whereas the compound **4b**, with 4-CN group at R and 3-CF_3 group at R_2 was two time less active than the lead compound **5b.** A careful analysis revealed that the Cl group at the *para* position of the phenyl ring was favorable for the inhibitory activity. To confirm this finding, we synthesized four analogues with 4-Cl substituent at R and 4-methoxyphenyl (**5a**), 3,4-dichlorophenyl (**5c**), cinnamyl (**5d**) and 4-Me (**5e**) at R^2 position, respectively. Since these analogues showed no enhancement in the activity, it was thought that the reason behind these unexpected results may be due to the poor solubility of these compounds.

In order to add more insight into the binding mode of these compound with the enzyme of known X-ray structure, the docking studies were carried out using Genetic Optimisation for Ligand Docking (GOLD) software version 2.2 on windows based PC.[22] The reported crystal structure of PTP1B with co-crystal of sulfamic acid derivative with 2.0 Å resolution, was downloaded from Protein Data Bank (PDB) ID 2F70 and was used for the current docking studies. The docked poses were scored using GOLD score. Originally the protein was considered without ligand and water molecule for the purpose of docking studies. The Protein–ligand complexes of PTP1B (PDB-2F70) were minimised up to a gradient of 0.01 kcal/(mol Å) and hydrogens were added using the CHARMm force field, available in the software discovery studio 2.0.[23] On concluding point of docking process the resulting conformation poses of 1-(4-chlorophenoxy)-3-(4-(4-nitrophenyl)piperazin-1-yl)propan-2-ol (**5b**) and 4-[2-hydroxy-3-(3-methyl-4-*p*-tolyl-piperazin-1-yl) propoxy]benzonitrile (**4a**) in the binding sites of PTP1B were considered. Detailed binding pattern of compound **5b** exhibiting the highest inhibition value of 35.90% at 100 μM and GOLD score value 60.03, is shown in Figure 1. The one oxygen of the nitro group of **5b** showed one hydrogen bonding interaction with the $-NH_2$ of Arg221 and two with Cys215 while the other oxygen of the nitro group of **5b** showed three hydrogen bonding interactions with the $-NH_2$ of Arg221 only. The one –NH group of piperazine moiety of compound **5b** also showed hydrogen bond interaction with water molecule (HOH:590) similar to the one observed in case of –NH in the sulfamic acid.[19] The docking studies of the compound **1a** having inhibition value of 31.58% at 100 μM and GOLD score value of 52.81 showed three hydrogen bonding interactions between the cyano group attached to the *para* position of the phenyl ring and Arg221. The –OH of the compound **4a** also showed hydrogen bonding interaction with the carbonyl group of Gln262 (Fig. 2). It was also similar to the one shown by the –NH in the case of sulfamic acid.[19] These studies correlated well with the observed PTP1B inhibitory activity. In view of their good inhibitory activity, these compounds were further evaluated for their antidiabetic activities in the SLM and STZ models in rats, essentially according to the recently reported procedure from our laboratory.[24] The most

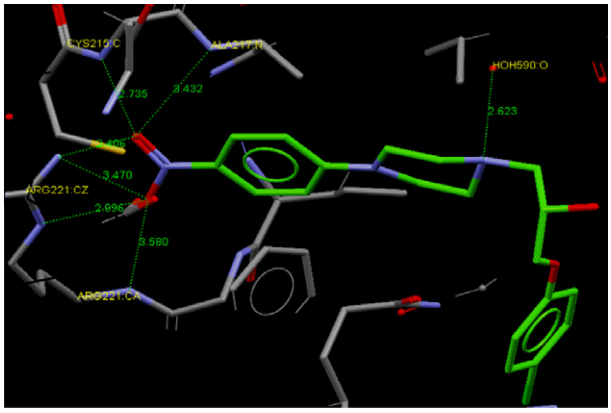

Figure 1. Interaction of the **5b** (green) with the amino acids in the active site of PTP-1B (PDB ID 2F70).

Table 1
In vitro PTP1B enzyme inhibitory and in vivo antihyperglycemic activity in SLM and STZ model for compounds **4a–6**

Compd	R	R^1	R^2	% Inhibition (100 μM)	SLM[a]	STZ[b]	
						5 h	24 h
4a	CN	Me	4-MePh	−31.58	−40.3	26.8	32
4b	CN	H	3-CF_3Ph	−18.8	+49.7	—	—
4c	CN	H	4-FPh	−3.60	+38.6	—	—
4d	CN	H	3-ClPh	−1.50	+16.5	—	—
4e	CN	H	2-OMePh	−2.6	+33.5	—	—
4f	CN	H	Ph	−4.6	+56.7	—	—
4g	CN	H	2-Pyridine	−1.80	+39.6	—	—
4h	CN	H	5-Me-benzo1,3dioxole	−0.50	+14.0	—	—
4i	CN	H	Benzyl	−5.10	−39.6	25.3	14.7
5a	Cl	H	4-OMePh	−9.21	−4.60	—	—
5b	Cl	H	4-NO_2Ph	−35.9	−24.8	18.9	13.6
5c	Cl	H	3,4-diClPh	+24.6	+3.52	—	—
5d	Cl	H	Cinnamyl	−7.63	−14.6		
5e	Cl	H	4-Me	+2.4	+2.61		
6	F	H	4-NO_2Ph	−24.0	−40.2	14.4	10.8
	Na-orthovanadate	—	—	−39.4	—	—	—
	Peroxovanadate	—	—	−56.0	—	—	—
	Metformin	—	—	—	−42.0	28.0	29.8

[a] Sucrose loaded mouse (SLM).
[b] Streptozodocin (STZ) model.

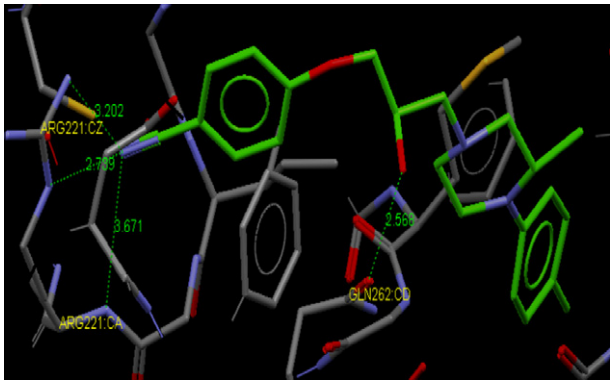

Figure 2. Interaction of the **4a** (green) with the amino acids in the active site of PTP-1B (PDB ID 2F70).

active compound **5b** showed 24.8% activity in SLM model and 18.9% (5 h) and 13.6% (24 h) in STZ model while the compound **4a** which was the second most active analogue in vitro, showed 40.3% blood glucose lowering activity in SLM model and 26.8% (5 h) and 32% (24 h) in STZ model (Figs. 3 and 4). Unlike in vitro, where the analogue **6** and **4i** which showed lesser activity viz. 24.0% and 5.10% at 100 µM, respectively than **5b** (35.9%) lowered blood glucose level very significantly 40.2% and 39.6%, respectively. These compounds were also active in STZ model where compound **6** showed lowering of blood glucose level 11% (5 h) and 4.48% (24 h) and the compound **4i** showed 25.3% (5 h) and 14.7% (24 h), respectively. Thus new lead series of phenoxy-3-piperazin-1-yl-propan-2-ol derivatives may be useful in the optimization of PTP1B inhibitory activity.

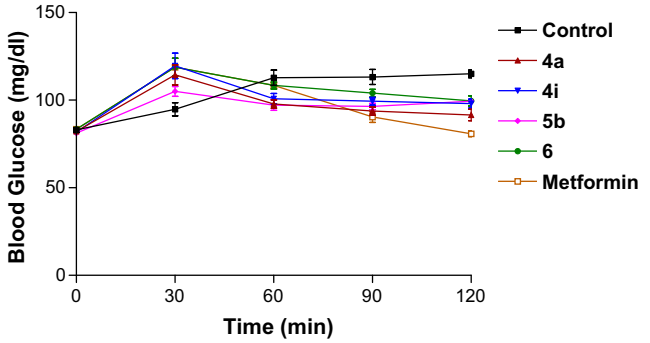

Figure 3. Effect of Compounds **4a**, **4i**, **5b** and **6** (100 mg/kg), and metformin (100 mg/kg) on the blood glucose levels of normoglycemic rats.

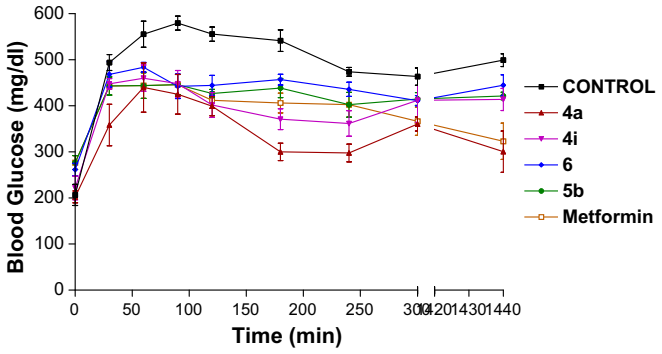

Figure 4. Blood glucose levels in STZ-induced diabetic rats before and up to 24 h after administration of vehicle, compound **4a**, **4i**, **5b, 6** and metformin.

Acknowledgements

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20. *General method for the synthesis of oxiranylmethoxy-benzonitrile* (***2***): A solution of 4-cynophenol (57, 2.38 g, 0.02 mol) in ethanol was stirred with sodium hydroxide (0.80 g, 0.02 mol) for ½ h at rt. Epichlorohydrin (1.84 g, 0.02 mol) was added and reaction mixture was stirred at room temperature for 12 h. The mixture was concentrated under reduced pressure, extracted with ethyl acetate (3 × 10 ml), dried on sodium sulphate and was concentrated. Yield: 57.1%; mp: 80 °C; ^{1}H NMR ($CDCl_3$, 200 Hz): δ 2.74–2.78 (m, 1H), 2.91–2.95 (m, 1H), 3.34–3.39 (m, 1H), 3.92–4.01 (m, 1H), 4.29–4.36 (m, 1H), 7.55–7.62 (m, 2H), 6.94–7.00 (m, 2H); FTIR (KBr): cm^{-1} 767, 1089, 1222, 1505, 2923, 2225; FAB-MS: *m/z* 176 $(M+1)^+$. *1,3-Bis-p-cyanophenoxy-propan-2-ol* (***3***): Yield: (70.5%), mp 120 °C; ^{1}H NMR ($CDCl_3$, 200 Hz): δ 2.60 (br s, 1H), 4.44 (m, 1H), 4.20–4.22 (m, 4H), 6.99 (d, *J* = 9.0 Hz, 4H), 7.60 (d, *J* = 9.0 Hz, 4H); FTIR (KBr): cm^{-1} 832, 1055, 1261, 1603, 2218, 2932, 3421; FAB-MS: *m/z* 295 $(M+1)^+$. Anal. Calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.76; N, 9.52. Found: C, 69.40; H, 4.77; N, 9.53. *4-[2-Hydroxy-3-(3-methyl-4-p-tolyl-piperazin-1-yl)-propoxy] benzonitrile* (***4a***): A solution of 4-oxiranylmethoxy-benzonitrile (**2**, 0.875 g, 0.005 mol) and 2-methyl-1-*p*-tolylpiperazine (1.05 ml, 0.005 mol) in ethanol (10.0 ml) was stired at rt for 6 h. The mixture was concentrated under reduced pressure and crystallized by methanol. Yield: 70.8%; mp: 125 °C; ^{1}H NMR ($CDCl_3$, 200 Hz): δ 1.03 (d, *J* = 6.2, 3H), 2.28 (s, 3H), 2.48-2.84 (m, 7H), 3.14–3.70 (m, 2H), 3.72 (br s, 1H), 4.07–4.17 (m, 3H), 6.85 (d, *J* = 8.0 Hz, 2H), 6.97–7.10 (m, 4H), 7.59 (d, *J* = 8.6 Hz, 2H); FTIR (KBr): cm^{-1} 670, 769, 1257, 1352, 1600, 2221, 2936, 3430; FAB-MS: *m/z* 366 $(M+1)^+$. Anal. Calcd for $C_{22}H_{27}N_3O_2$: C, 72.32; H, 7.39; N, 11.53. Found: C, 72.35; H, 7.41; N, 11.55. Compounds **4b**–**6** were synthesized similarly.
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