PTP1B Inhibition and Antihyperglycemic Activity in the ob/ob Mouse Model of Novel 11-Arylbenzo[*b*]naphtho[2,3-*d*]furans and 11-Arylbenzo[*b*]naphtho[2,3-*d*]thiophenes

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Introduction. Non-insulin-dependent diabetes mellitus (type II) represents 80−90% of the human population with diabetes, and worldwide estimates approach 215 million sufferers by 2010.[1] In the patients with diabetes, maintaining tight glycemic control appears to be essential to prevent diabetic complications.[2] However, this is difficult since the current therapies for type II diabetes have inherent problems including compliance, ineffectiveness, and hypoglycemic episodes with insulin and the sulfonylureas. Recently, troglitazone (marketed as Rezulin) has been introduced as an agent that enhances insulin action and lowers plasma glucose.[3] However, troglitazone is not effective in all type II patients; therefore, there still remains a great need for more effective, orally administered agents,[4] particularly ones that normalize both glucose and insulin levels.

Excess hepatic glucose production and hepatic, skeletal muscle, and adipose tissue insulin resistance are prime factors which contribute to type II diabetes.[5] Insulin resistance is associated with a deficit in protein-tyrosine phosphorylation in the insulin signal transduction cascade. This deficit in tyrosine phosphorylation in insulin-responsive tissues, muscle, liver, and adipose, causes a reduction in the metabolic actions of insulin and hyperglycemia. The decrease in protein-tyrosine phosphorylation in cells does not appear to be due to an inherent problem in the insulin receptor (IR) tyrosine kinase but instead is caused by an elevation in protein-tyrosine phosphatase activity.[6] These enzymes (possibly both transmembrane and intracellular PTPases) dephosphorylate the active form (triphosphorylated in the regulatory domain) of the IR and attenuate its tyrosine kinase activity. One PTPase, in particular, the intracellular, nonreceptor PTPase PTP1B, appears to play a major role in the dephosphorylation of the IR on the basis of many biochemical and cellular studies[7] and according to a pivotal study with PTP1B knockout mice.[8] Furthermore, vanadium-containing inhibitors of PTPases have been shown to increase IR tyrosine phosphorylation, mimic cellular and in vivo actions of insulin, and lower plasma glucose in diabetic animal models.[9] These studies all suggest that a selective, orally active PTP1B inhibitor could function as an agent that normalizes plasma glucose without inducing hypoglycemia; therefore, such an agent could be a major advance in the treatment of type II diabetes. We disclose here some of our efforts leading to potent and selective PTP1B inhibitors that are orally active as antidiabetic agents.

Chemistry. According to Scheme 1, 2-benzylbenzothiophene[10] (**9**) and 2-benzylbenzofuran[10] (**10**) were acylated with *p*-anisoyl chloride under standard Friedel−Crafts conditions to provide 3-acyl derivatives **11** and **12**, respectively (method a). Compound **11** was demethylated with pyridinium hydrochloride and dibrominated to provide the benzbromarone analogue **2** (methods b and c). Treatment of **11** or **12** with boron tribromide etherate resulted in demethylation followed by concomitant intramolecular cyclization and aromatization to produce the 11-*p*-phenoxybenzo[*b*]naphtho[2,3-*d*]thiophene (**13**) and 11-*p*-phenoxybenzo[*b*]naphtho[2,3-*d*]-furan (**14**) derivatives, respectively (method d). Bromination of **13** and **14** with 3 equiv of molecular bromine resulted not only in dibromination ortho to the phenol moiety but also in bromination at the 6 position to afford tribromo derivatives **3** and **15** (method e).

Alkylation of phenol **3** with methyl bromoacetate followed by methyl ester hydrolysis with aqueous potassium hydroxide produced acetic acid congener **4** (method f). Phenol **3** was also alkylated with diethyl trifluoromethanesulfonoxymethylphosphonate,[11] and this (bis)-ethyl ester derivative was further converted to the phosphonic acid compound **5** using iodotrimethylsilane (method g). Treatment of either phenol **3** or **15** with (*S*)-2-hydroxy-3-phenylpropionic acid, methyl ester under Mitsunobu conditions followed by methyl ester hydrolysis with aqueous potassium hydroxide gave (*R*)-2-benzyloxyacetic acid derivatives **6** and **7**, respectively (method h). Likewise, treatment of phenol **3** with (*S*)-2-hydroxy-1,3-dioxo-2-isoindolinebutyric acid, methyl ester under Mitsunobu conditions followed by methyl ester removal using iodotrimethylsilane gave (*R*)-2-alkylated oxyacetic acid analogue **8** (method i).

Results and Discussion. The inhibitory activity of our compounds against recombinant PTP1B[12] was assessed using, as substrate, the phosphotyrosyl dodecapeptide TRDI(P)YETD(P)Y(P)YRK, corresponding to the 1142−1153 insulin receptor kinase regulatory domain, phosphorylated on the 1146, 1150, and 1151 tyrosine residues. Enzyme reaction progression was monitored via the release of inorganic phosphate as detected by the malachite green−ammonium molybdate method.[13]

Early in our lead screening program, we discovered that the potent uricosuric agent benzbromarone[14] (**1**) was a weak rat PTP1B inhibitor ($IC_{50} = 26\ \mu M$). Since this compound was a highly efficacious oral agent with excellent absorption and pharmacokinetic parameters,[15] we thought it a good starting point for a PTP1B inhibitor analogue program. Standard SAR transformations on **1** led only to modest improvements in potency.

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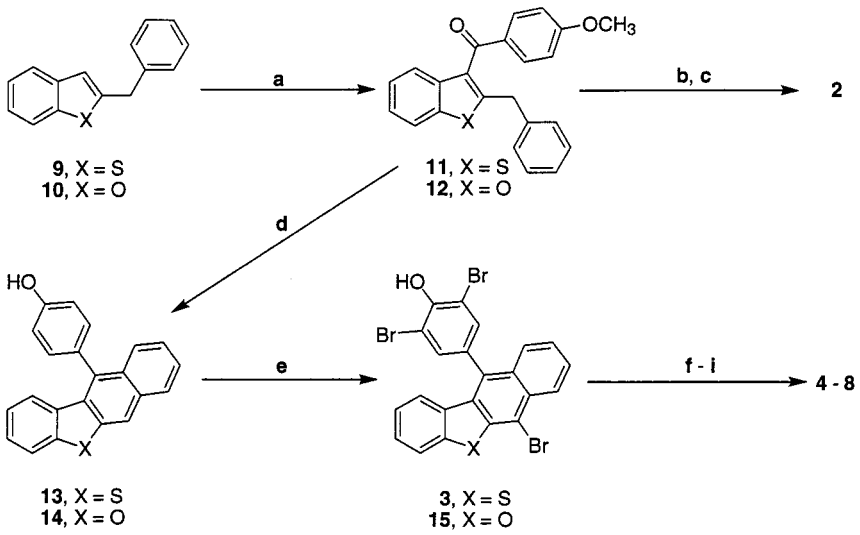
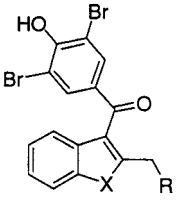
Scheme 1[a]



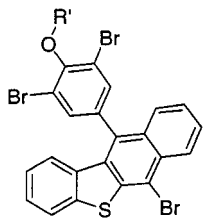
[a] Reagents: (a) *p*-anisoyl chloride, $SnCl_4$, CS_2, rt, **9** to **11** (85%), **10** to **12** (84%); (b) pyridinium hydrochloride, 228 °C (90%); (c) Br_2, HOAc, H_2O, rt (95%); (d) BBr_3, CH_2Cl_2, −78 °C to rt, **11** to **13** (56%), **12** to **14** (42%); (e) Br_2, HOAc, rt, **13** to **3** (KOAc added, 95%), **14** to **15** (83%); (f) **3** to **4**, $BrCH_2CO_2Et$, K_2CO_3, DMF, rt (98%)/KOH, H_2O, THF, MeOH (91%); (g) **3** to **5**, $TfOCH_2PO_3Et_2$, NaH, THF, 0 °C to rt (88%)/TMSI, CH_2Cl_2, 0 °C to rt (44%); (h) (*S*)-HOCH(CH_2Ph)CO_2Et, diethyl azodicarboxylate, PPh_3, benzene, reflux (**3** to **6**, 90%; **3** to **7**, 82%)/KOH, H_2O, THF, MeOH (for **6**, 67%; for **7**, 98%); (i) **3** to **8**, (*S*)-α-hydroxy-1,3-dioxo-2-isoindolinebutyric acid, methyl ester, diethyl azodicarboxylate, PPh_3, benzene, reflux (81%)/TMSI, CH_2Cl_2, −10 °C to rt (52%).

For example, extension of the 2-ethyl moiety of **1** to a benzyl group to provide benzothiophene **2** (IC_{50} = 4 μM, rPTP1B) improved inhibition values to the low micromolar range. We decided to build rigid analogues of **1** and **2** in an effort to study the effect of the conformation of the dibromophenolic residue with respect to the benzothiophene nucleus. This led to the tribrominated (benzo[*b*]naphtho[2,3-*d*]thiophene-11-yl)phenol **3** (IC_{50} = 0.23 μM, rPTP1B), the first of our analogues to break the micromolar barrier.



1, X = O, R = CH_3

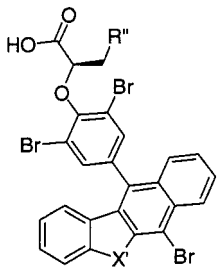
2, X = S, R = Ph



3, R' = H

4, R' = CH_2CO_2H

5, R' = $CH_2PO_3H_2$



6, X' = S, R" = Ph

7, X' = O, R" = Ph

8, X' = S, R" = CH_2–N

Table 1

compd	hPTP1B IC_{50} (nM)[a]	dose[b] (mg/kg/day)	4-day ob/ob mouse[g] (% decrease) glucose[c]	insulin[c]
3	384	100	*d*	*d*
4	386	100	23	61
5	145	90	*d*	*d*
6	61	75	50	85
		50	35	71
		25	26	72
		10	26	*d*
7	83	25	42	86
		10	29	69
		5	*d*	56
		1 (ip)	38	*e*
8	11	100	*d*	*d*
ciglitazone[f]		100	43	39
troglitazone[f]		200	34	*d*

[a] Substrate is the triphosphorylated peptide TRDI(P)YETD-(P)Y(P)YRK. The reported IC_{50} values are from direct regression curve analyses that are significant ($p < 0.05$). [b] All doses are po unless otherwise indicated. [c] All values from drug-treated mice, other than *d*, are significant vs vehicle-treated mice: $p < 0.05$. [d] Less than 15% decrease at dose tested. [e] Not determined. [f] Reference standard. [g] Plasma glucose and insulin values of 200−350 mg/dL and 300−775 μIU/mL, respectively, in untreated mice.

However, the highly lipophilic and poorly water-soluble **3** did not demonstrate efficacy at high doses in an oral study done in our primary in vivo model, the ob/ob mouse (vide infra). An acetic acid moiety was introduced onto the phenol of **3,** to afford the oxyacetic acid **4**, in an effort to improve water solubility and, hence, intestinal absorption. Compound **4** retained PTP1B potency (IC_{50} = 0.15 μM, rPTP1B) and was active orally in the ob/ob mouse model. Around this time we received the human clone of the PTP1B protein, and compounds **3** and **4** demonstrated comparable potency against this human enzyme (IC_{50}'s of 384 and 386 nM, respectively, Table 1).

We surmised that compound **4** was binding to the active site of PTP1B and, therefore, hypothesized that the acetic acid residue of **4** might bind to the side chain

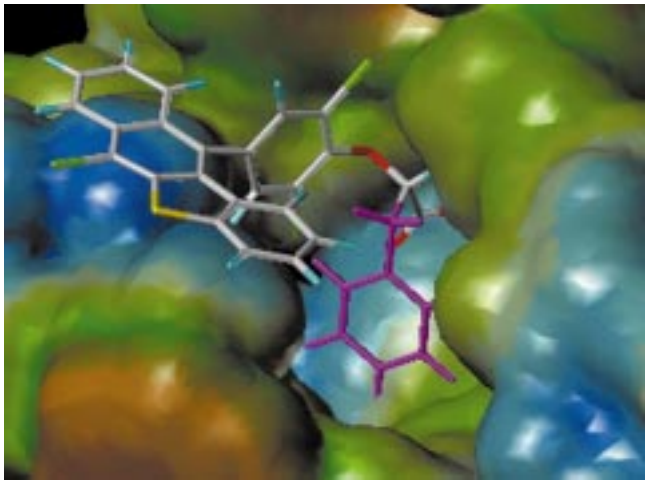


Figure 1. Compound **6** docked into the active site of PTP1B with the α-benzyl group of **6** shown in magenta.

of active site Arg 221 via a charge−charge interaction and to the main chain amides of the phosphate binding loop via hydrogen bond interactions similar to the tyrosine phosphate residue of a peptide substrate undergoing phosphate hydrolysis.[16] We were encouraged further with this hypothesis when we found that phosphonate analogue **5** showed a slight potency increase over **4** (IC_{50} of 145 versus 386 nM, Table 1). The X-ray crystal structure of PTP1B[17] was used as a starting point for our modeling efforts, and compound **4** was docked into the active site and minimized with the acetic acid moiety pointing toward the catalytic sequence residues V_{213}HCSAGIGR$_{221}$SG of PTP1B. This docking experiment revealed the existence of a large pocket of which the inhibitor **4** was not taking advantage. We believed that a lipophilic residue emanating from the acetic acid α-carbon could fill this pocket and enhance the binding interactions of the inhibitor with the enzyme. In fact, placement of a (*R*)-benzyl moiety in the α position resulted in compound **6** (Figure 1) and a 6-fold potency increase to an IC_{50} of 61 nM. The furan derivative of **6**, compound **7**, was also synthesized and showed similar potency to **6** (IC_{50} of 83 nM, Table 1).

Further optimization at the α-carbon of **6**, via replacement of the (*R*)-benzyl group by a (*R*)-2-phthalimidoethyl side chain, gave compound **8**. With an IC_{50} of 11 nM (Table 1), this compound was 6 times more potent than the benzyl derivative **6**. Furthermore a kinetic analysis, using the monophosophotyrosine-containing insulin receptor peptide TRDIYETD(P)YYRK, as substrate, revealed that **8** bound competitively with a potent K_i of 3 nM.[18]

The PTPase domains of receptor and nonreceptor PTPases are highly conserved with ∼35% mean sequence identity between known phosphatases.[19] Therefore it is critical that inhibitors of PTPases used for therapeutic purposes show requisite selectivity, especially in vivo. To address this issue at the in vitro level, compounds **4** and **7** were evaluated as inhibitors against a battery of PTPases presented in Table 2 using the triphosphorylated IR peptide as substrate. Both compounds showed only modest selectivity (3−6-fold, respectively) over LAR, a PTPase also implicated as a negative regulator of the insulin receptor.[6] On the other hand, compounds **4** and **7** inhibited PTP1B 40- and 160-fold more potently than PTPα, which has also been implicated as a negative regulator of insulin signaling. Compound **7**, however, was generally more than 25 times selective for PTP1B over other PTPases. The exception was hPTPα, where compound **7** was 7-fold selective.

Table 2

PTPase[a]	IC_{50} (μM)	
	4	**7**
hPTP1B	0.39 ± 0.1	0.08 ± 0.01
LAR	1.5 ± 0.2	0.42 ± 0.08
PTP1α (LRP)	17 ± 3	13 ± 3
CD45	9.1 ± 3.2	6.3 ± 2.4
PTP1C (SH-PTP1)	5.0 ± 3.3	9.0 ± 5
hPTPβ	0.60 ± 0.3	0.5 ± 0.2
PTP-PEST	2.3 ± 0.7	2.2 ± 0.9
HePTP360	2.7 ± 0.6	2.1 ± 0.6
VHR	7.9 ± 1.6	11 ± 3

[a] All PTPases are human. Substrate for all PTPases is the triphosphorylated peptide TRDI(P)YETR(P)Y(P)YRK at 50 μM concentration. Three independent measurements were performed for IC_{50} determinations. Similar results were obtained in multiple measurements. The reported values are the average of all experiments, and the errors are standard deviations.

The compounds were also evaluated as antidiabetic agents in the ob/ob mouse model.[20] Insulin resistance in this model has been associated with a reduction in insulin-induced protein-tyrosine phosphorylation in tissues such as liver, and markedly elevated PTPase activities in these tissues have been observed leading to the conclusion that PTPases may cause or contribute to the reduced phosphorylation of the IR.[21] Increased abundance of PTP1B in the livers of ob/ob mice has also been noted.[22]

As stated previously, compound **4** showed oral glucose- and insulin-lowering activity at a dose of 100 mg/kg/day in the ob/ob mouse model. This result served as a proof-of-concept that justified further testing of inhibitors in this model. In fact, much more robust oral activity was seen with (*R*)-benzyl congeners **6** and **7**. Both of these compounds showed a dose−response relationship in reductions of both glucose and insulin. The minimum effective dose (MED) for plasma glucose lowering for **7** was between 5 and 10 mg/kg/day, although the MED for insulin lowering with this compound was not reached in this dose−response study. Compound **7** was more potent when administered ip, showing a strong glucose-lowering response at 1 mg/kg/day. The potent inhibitor **8** was not active at the highest dose tested (100 mg/kg). Although compound **8** has a calculated log *P* value similar to that of **7**, the precise nature for the reduced potency in vivo (i.e. solubility, absorption, metabolism, etc.) was not determined. Compound **7** was further found to exhibit high mean plasma concentrations (up to 120 μg/mL at the 4-h time point) after a single 10 mg/kg oral dose in ob/ob mice over a 24-h period, and these levels were not significantly different when compared to ip administration. This demonstrated that compound **7** was well-absorbed and did not undergo extensive first-pass metabolism.

Summary. We have reported on a series of novel, potent, and selective PTPase inhibitors that function as oral antidiabetic agents. Compounds **6** and **7** are particularly notable in this regard. They were both low nanomolar inhibitors and lowered glucose in the diabetic

ob/ob mouse at doses at or below 10 mg/kg/day, po. Compound **7** generally showed selectivities against other PTPases of greater than 25-fold. These compounds and other analogues are currently being further evaluated.

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Supporting Information Available: Details of the synthesis and analysis of compounds **2**−**8** and procedures for the biological evaluations of these compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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