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PTP-1B inhibitors: Cyclopenta[*d*][1,2]-oxazine derivatives

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Abstract—A series of novel cyclopenta[*d*][1,2]-oxazine derivatives was prepared and evaluated for their inhibitory activity toward protein tyrosine phosphatase 1B (PTP-1B). Compound **6s** was found to be an inhibitor of PTP-1B with nanomolar IC_{50} value and high level of selectivity over other recombinant phosphatases.

Protein tyrosine phosphatase 1B (PTP-1B) plays a crucial role in the modulation of insulin signaling pathway through dephosphorylation of the activated insulin receptor.[1] Since two independent groups reported that PTP-1B knock-out mice showed improved insulin sensitivity and resistance to weight gain,[2] PTP-1B inhibitor could potentially ameliorate insulin resistance and normalize plasma glucose and insulin without inducing hypoglycemia.[3] Thus, PTP-1B has emerged as an attractive therapeutic target for treatment of insulin resistance related to Type 2 diabetes.[4] Recently, small molecule inhibitors of PTP-1B as well as peptide mimetics were reported in literatures.[5] In our continued effort in search of PTP-1B inhibitors, the 1,2-naphthoquinone and other heterocycle derivatives were reported as new classes of inhibitors.[6]

A class of compounds with cyclopenta[*d*][1,2]-oxazine skeleton was identified as a hit with micromolar IC_{50} through high-throughput screening (HTS).[11] Because of novelty of cyclopenta[*d*][1,2]-oxazine skeleton as a small molecule PTP-1B inhibitor, it was decided to develop potent and selective PTP-1B inhibitors through introduction and modification of substituents on cyclopenta[*d*][1,2]-oxazine skeleton.

The cyclopenta[*d*][1,2]-oxazines described in this paper were prepared according to the synthetic procedures in Scheme 1. The desired cyclopenta[*d*][1,2]-oxazine skeleton was prepared by cyclocondensation of fulvenes with substituted chlorooximes in the presence of triethylamine in ether at ambient temperature. The requisite fulvenes were prepared from the suitable amides by treatment with dimethyl sulfate and cyclopentadienyl sodium as shown.[7] For instance, fulvene **3** (R^1 = Me) was readily obtained from *N*,*N*-dimethylactamide by treatment with dimethyl sulfate followed by cyclopentadienyl sodium. The preparation of chlorooximes **2**, the other counterpart in the coupling, was straightforward from the appropriate aldehydes **1** by treatment with hydroxylamine and then with *N*-chlorosuccinimide in DMF at room temperature.[8] The aldehydes **1** were prepared by alkylation of phenol with appropriate α-bromo esters, such as α-bromoacetate, α-bromobutyrolactone or Mitsunobu condensation with (*R*)-3-phenyllactic acid methyl ester.[9] The bromo aldehyde (**1**, R^2 = Br, $R^3 = CH_2CO_2Et$) was prepared from the 4-hydroxybenzaldehyde **1** (R^2, R^3 = H) by bromination and subsequent alkylation by ethyl α-bromoacetate with KI and K_2CO_3 in DMF.

The introduction of acyl group R^4 to oxazine **4** could be accomplished in moderate yields by Friedel–Crafts acylation with suitable acyl chlorides. The esters of oxazine **4** (R^3= lactone or ester) and **9** were converted to the corresponding acids **6** and **11**, respectively, with LiOH in $CH_3OH/THF/H_2O$ (1:1:1) at ambient temperature.

Keywords: Cyclopenta[*d*][1,2]-oxazine; PTP-1B; Inhibitor; Diabetes.
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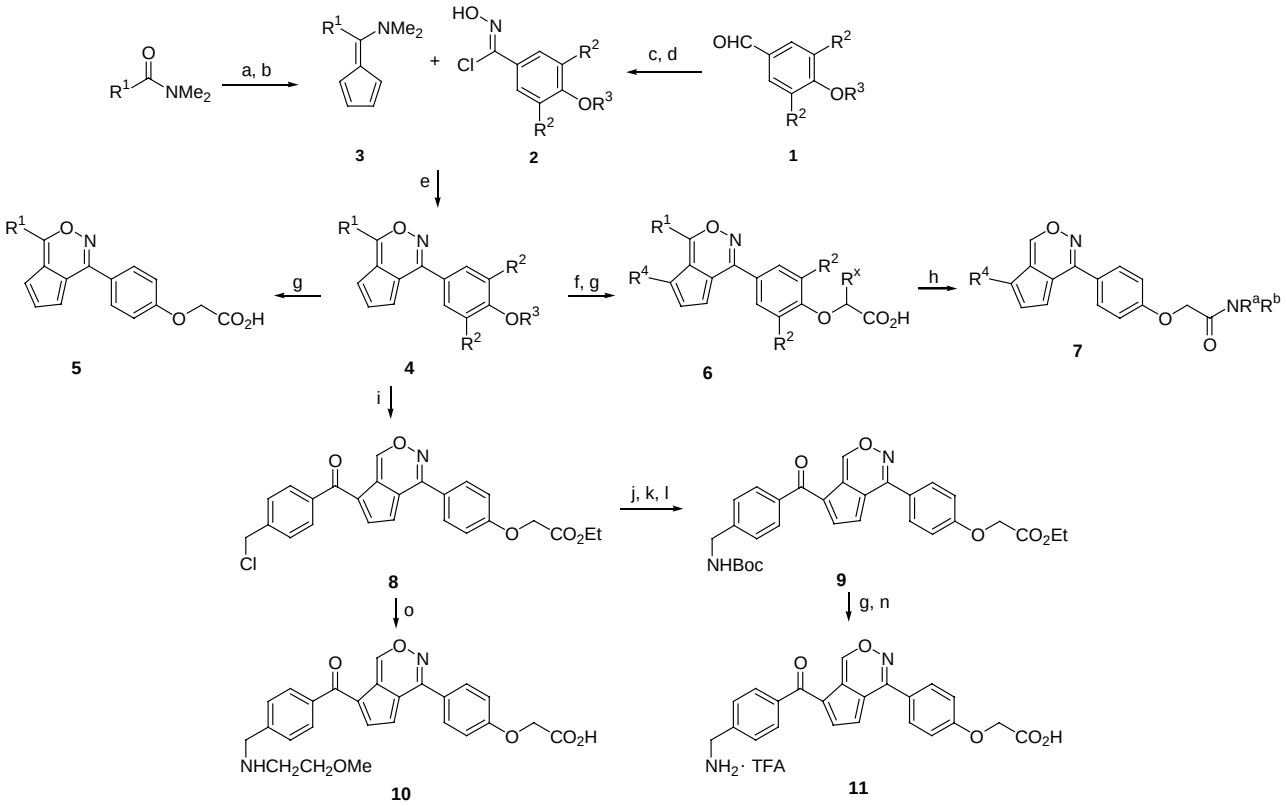


Scheme 1. Reagents and conditions: (a) Me_2SO_4; (b) cyclopentadienyl sodium/THF; (c) NH_2OH HCl, Py/EtOH; (d) NCS, HCl/DMF; (e) NEt_3/ether; (f) R^4X, $AlCl_3/CH_2Cl_2$; (g) LiOH; (h) HNR^aR^b, DCC/THF; (i) 4-chloromethylbenzoyl chloride, $AlCl_3/CH_2Cl_2$; (j) NaN_3, KI/DMF; (k) PPh_3, H_2O/MeCN; (l) $(Boc)_2O$, $NaHCO_3$; (n) TFA/CH_2Cl_2; (o) $H_2N(CH_2)_2OMe$/DMF.

The oxazine amide **7** was prepared by condensation of oxazine carboxylic acid **6** with appropriate amines in the presence of DCC in THF. *N*-Benzyl-1,4-piperazine was prepared from 1,4-piperazine through a three-step process.[10]

The introduction of basic nitrogen into R^4 acyl group was achieved by Friedel–Crafts acylation of oxazine **4** ($R^3 = CH_2CO_2Et$) with 4-chloromethylbenzoyl chloride to oxazine **8** and subsequent modifications as shown in Scheme 1. Secondary amine derivative **10** was readily obtained by reacting 1,2-oxazine **8** with appropriate amine in wet DMF at room temperature. Treatment of **8** with NaN_3 and KI in DMF afforded the azide derivative, and subsequent reduction of the azides with triphenylphosphine and water afforded oxazines containing amine functionality. Quenching of amine with Boc_2O immediately after reduction gave the protected amine derivative **9**. The amino acid **11** was prepared by hydrolysis of esters with LiOH and subsequent deprotection of Boc with 30% trifluoroacetic acid in CH_2Cl_2 at room temperature.

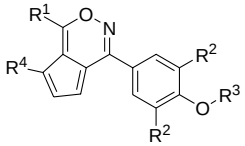
The inhibitory activity of oxazine derivatives was tested toward recombinant human PTP-1B by use of fluorescein diphosphate (FDP) as a substrate (Table 1). Enzyme activity was assayed by measuring the fluorescence of the product, fluorescein monophosphate at 485 nm (excitation) and 538 nm (emission). The medium was 30 mM Tris, 75 mM NaCl, and 0.67 mM EDTA in 1 mM DTT (pH 8.0) buffer with 20 μM FDP, 0.1 μg PTP-1B, and compound for 1 h at room temperature. The total reaction volume was 200 μL and IC_{50} (μM) values were determined from a direct regression curve analysis.[6d]

Our initial assessment[11] indicated that the oxazines (**4a**, **4b**) with no substituent at position-6 ($R^4 = H$) displayed no inhibitory activity. It appeared benzoyl moiety as R^4 (**4c**, **4d**) or acetic acid (**5a**, $R^1 = H$) was needed for displaying the inhibitory activity. Introduction of methyl or phenyl group as R^1 did not provide improved activity (**5b**, **5c**). Ester was not as active as their corresponding acid (**4e**, **5a**).

First, the effect of various acyl groups was tested while keeping the oxyacetic acid group constant. Introduction of small acyl groups or acrylate (**6a**–**c**) abolished the activity. While the introduction of moderate-sized carbonyl groups tends to make weak inhibitors (**6c**, **6d**, **6g**, and **6h**), the introduction of long-chain alkyl group (**6i**) greatly enhanced the activity as expected from earlier results.[6c] While changing R^3 from acetic acid to malonic acid improved the activity (**6e**), the introduction of hydroxyethyl group abolished the activity (**6f**).

Next, the substituent effect of benzoyl group was further explored. While most of the substitution was well tolerated, ortho-substitutions were detrimental to the activity (**6j**–**m**). Also small para- or meta-substituents, except methoxy (**6s**, **6o**), showed diminished activities (**6n**, **6p**, and **6q**). Since **6s** showed sub-micromolar inhibitory

Table 1. Inhibitory activity of 1,2-oxazine derivatives against PTP-1B



Compound	R^4	R^1	R^2	R^3	%[a]	IC_{50}[b]
4a	H	H	H	H	18.5	
4b	H	H	H	Me	3.0	
4c	4-MeOPhCO	H	H	Me	32.0	
4d	2,4-Cl_2PhCO	H	H	Me	100.6	3.40
4e	H	H	H	CH_2CO_2Et	2.1	
5a	H	H	H	CH_2CO_2H	27.4	
5b	H	Me	H	CH_2CO_2H	NA	
5c	H	Ph	H	CH_2CO_2H	1.1	
6a	$EtO_2CCH{=}CH–$	H	H	CH_2CO_2H	NA	
6b	MeCO	H	H	CH_2CO_2H	NA	
6c	2-Furoyl	H	H	CH_2CO_2H	NA	
6d	(*c*-C_5H_9)CO	H	H	CH_2CO_2H	15.7	
6e	(*c*-C_5H_9)CO	H	H	$CH(CO_2H)_2$	76.7	8.19
6f	(*c*-C_5H_9)CO	H	H	$CH(CH_2CH_2OH)CO_2H$	NA	
6g	4-MeOPhCH_2CO	H	H	CH_2CO_2H	85.5	5.63
6h	(*c*-C_5H_9)CH_2CH_2CO	H	H	CH_2CO_2H	42.4	
6i	$Me(CH_2)_{13}$CO	H	H	CH_2CO_2H	100.6	0.27
6j	2-FPhCO	H	H	CH_2CO_2H	11.9	
6k	2-MeOPhCO	H	H	CH_2CO_2H	3.8	
6l	2-HO-5-MeOPhCH_2CO	H	H	CH_2CO_2H	48.7	
6m	2,4-Cl_2PhCO	H	H	CH_2CO_2H	38.0	
6n	3-CF_3PhCO	H	H	CH_2CO_2H	68.9	7.55
6o	3-MeOPhCO	H	H	CH_2CO_2H	87.4	1.96
6p	3-NCPhCO	H	H	CH_2CO_2H	NA	
6q	4-ClPhCO	H	H	CH_2CO_2H	14.3	
6r	4-$Me(CH_2)_{13}$PhCO	H	H	CH_2CO_2H	100.6	0.14
6s	4-MeOPhCO	H	H	CH_2CO_2H	81.5	0.80
6t	4-MeOPhCO	H	H	$CH(CO_2H)_2$	3.3	
6u	4-MeOPhCO	H	H	(S)-$CH(CH_2Ph)CO_2H$	80.1	7.82
6v	4-MeOPhCO	H	Br	CH_2CO_2H	100.9	1.64
6w	4-MeOPhCO	Me	H	CH_2CO_2H	NA	
7a	4-MeOPhCO	H	H	$CH_2CONHCH_2$(4-morpholinyl)	NA	
7b	4-MeOPhCO	H	H	$CH_2CONHCH_2CH_2OH$	NA	
7c	4-MeOPhCO	H	H	CH_2CO(1-benzylpiperazine-4-yl)	68.8	5.48
10	4-$MeO(CH_2)_2NHCH_2$PhCO	H	H	CH_2CO_2H	10.2	
11	4-NH_2CH_2PhCO	H	H	CH_2CO_2H	97.2	2.16

[a] % inhibition at 10 μM, NA means no inhibition at 10 μM.
[b] IC_{50} values in μM. IC_{50} of vanadate as a reference 7.70 μM.

activity and conformed to the Lipinski's Rule of Five,[12] a further substituent effect was studied based on **6s**. While substitution of R^2 to bromine (**6v**) or changing acetic acid to phenyllactic acid[13] (**6u**) retained inhibitory activity, substitution of R^1 to methyl (**6w**) or changing acetic acid to malonic acid (**6t**) practically abolished the inhibitory activity. Though there was an improvement of activity of **6e** compared to **6d**, the same effect was not observed for **6t** compared to **6s**.

The basic nitrogen was introduced hoping to improve aqueous solubility and bioavailability by reducing the overall lipophilicity of oxazine **6s**.[14] Among the amides (**7a**–**c**), only **7c** exhibited modest inhibitory potency. Though amine **11** marginally retained the inhibitory activity, the other modification of benzoyl moiety was detrimental to the potency (**10**). The introduction of nitrogen functionality might have increased the polar surface areas with a higher chance of low bioavailability.[15]

Five compounds were further tested for selectivity against nine phosphatases using the same concentration of FDP as substrate. While **6s** and its benzyl analog **6u** showed good to reasonable selectivity, a long hydrocarbon unit of **6r** resulted in complete loss of selectivity. Also introduction of amino group for **11** resulted in significant loss of selectivity. Results are summarized in Table 2.

As a conclusion, a series of novel 1,2-oxazine derivatives was prepared as inhibitors of PTP-1B. The compound **6s**, the best compound in this class with high level of selectivity, normalized plasma glucose level in the *ob*/*ob* mice through iv administration (10 mg/kg/day), was

Table 2. Selectivity of selected inhibitors[a]

	6s	6u	6v	6r	11
PTP1B	0.81	5.70	1.20	0.15	2.5
Yop	5.71	96.8%	101%	0.55	1.19
VHR	181.2	−6.83%	53.4%	—	—
PP1	35.2	63.5%	106%	0.22	1.3
CD45	28.8	95.7%	100%	0.15	1.23
LAR	61.7	17.3%	57.7%	0.69	≫10
cdc25A	73.3	10.3%	40.5%	1.33	∼10
cdc25B	16.1	89.7%	100%	0.12	∼10
cdc25C	61.7	—	—	2.27	≫10
PP2A	≫10	−143%	41.8%	—	—

[a] IC_{50} (μM) values or % inhibition at 10 μM.

only weakly active after an oral dosing (25 mg/kg/day) presumably due to low bioavailability.

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