Notes

Protein Tyrosine Phosphatase 1B Inhibitors: Catechols

Jin Hee Ahn, Sung Yun Cho, Jae Du Ha, Seung Kyu Kang, Sun Ho Jung, Hye-Min Kim, Sung Soo Kim, Kwang Rok Kim, Hyae Gyeong Cheon, Sung-Don Yang, and Joong-Kwon Choi*

Medicinal Science Division, Korea Research Institute of Chemical Technology, 100 Jang-dong, Yuseong-gu, Daejeon 305-600, Korea
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As most intracellular signaling takes place via cascades of phosphorylation and dephosphorylation of tyrosines, protein tyrosine phosphatases have emerged as new and promising targets. Among them, protein tyrosine phosphatase 1B (PTP-1B) negatively regulates insulin signaling by dephosphorylation of key tyrosine residues within the regulatory domain of the β-subunit of the insulin receptor, thereby attenuating receptor tyrosine kinase activity.[1] Echelby *et al*. have demonstrated that PTP-1B knock-out mice fed with high-fat diet showed enhanced insulin sensitivity without any adverse symptoms.[2]

Thus, PTP-1B inhibitor could potentially ameliorate insulin resistance and normalize plasma glucose and insulin without inducing hypoglycemia.[3] Recently, small molecule inhibitors of PTP-1B as well as peptide mimetics were reported in literatures. They included catechol **A**, *o*-quinones **B**-**D**, and carboxylic acids **E**-**G**.[4] One of the inhibitors, Ertiprotafib (**G**) went to clinical trial, but was discontinued in Phase II due to insufficient efficacy and dose-dependent side effects.

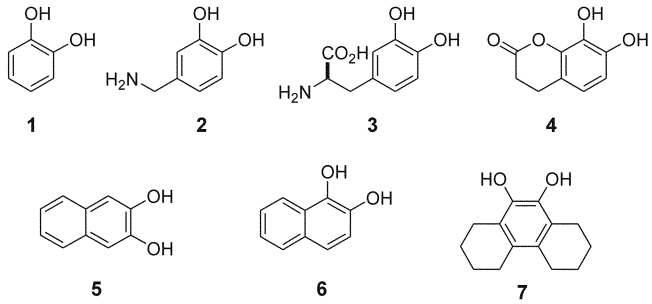


Figure 2. Catechol Derivatives.

Some catechol derivatives were discovered as hits from high-throughput screening of the library of Korea Chemical Bank.[5] As phenolic compounds with anti-oxidant activities are known to be beneficial in the treatment of diabetes and related disorders, and catechol **A** and similar compounds

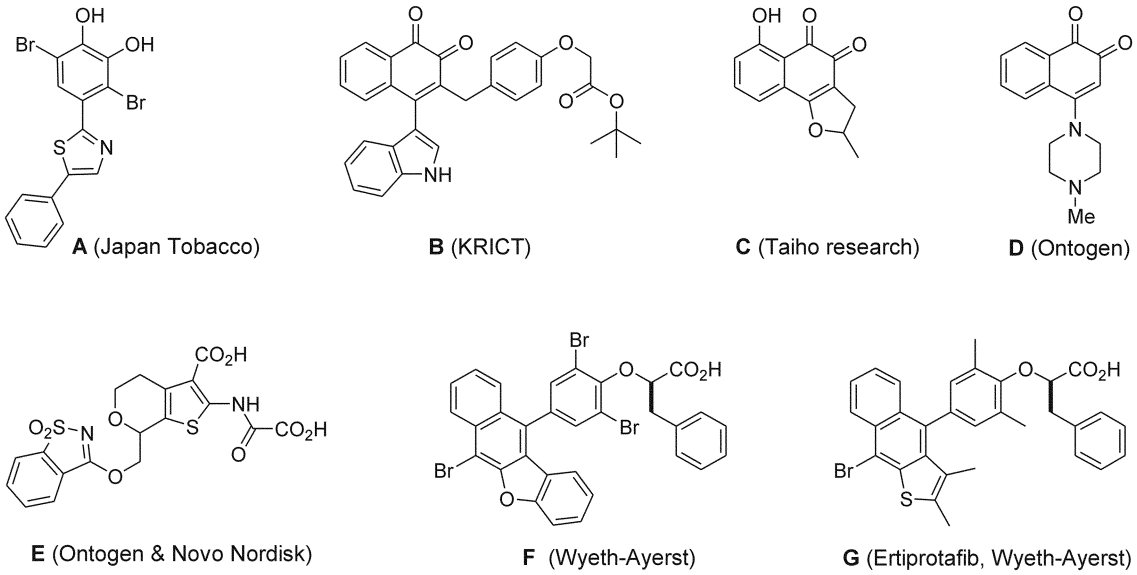
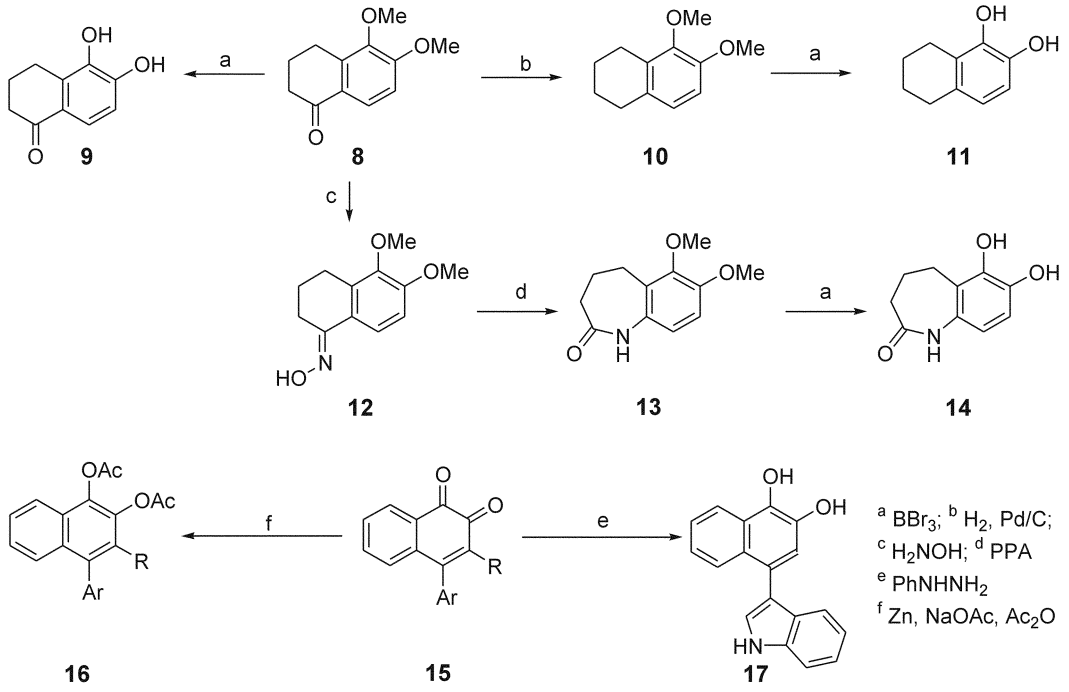


Figure 1. PTP-1B Inhibitors.

*Corresponding Author. E-mail: jkchoi@krict.re.kr



a. Ar=3-indolyl, R=H; **b**. Ar=Ph, R=CH_2Ph-4-OMe; **c**. Ar=3-indolyl, R=CH_2Ph-4-OCH_2CO_2-t-Bu

Scheme 1. Synthesis of Catechol Derivatives.

were reported to be PTP-1B inhibitors, catechol derivatives were chosen to be tested as possible inhibitors of PTP-1B.

First, commercially available catechols were tested. While **1**-**4** were not active and 2,3-naphthalenediol **5** showed moderate inhibition, 1,2-naphthalenediol **6** and octahydrophenanthrenediol **7** showed strong inhibition with IC_{50} of 1.25 and 3.65 μM, respectively. From these results, 1,2-naphthalenediol seemed well suited for further study.

Thus, some related structures were prepared from 5,6-dimethoxytetralone **8** as shown in Scheme 1.[6] Simple demethylation gave dihydroxytetralone **9**. The tetralone **8** was reduced and demethylated to **11** and also converted to benzazepinone **14** by sequential treatment with hydroxylamine, polyphosphoric aicd, and BBr_3. Ketone **9** and lactam **14** were not active and only catechol **11** showed some activity. From this observation, the modification of aromatics to saturated hydrocarbons was detrimental to the activity of parent 1,2-naphthalenediols. Then 1,2-naphthoquinone **15a** from the earlier work[5] was reduced to 1,2-dihydroxynaphthalene **17** using phenyl hydrazine.[7] As the catechol derivatives were easily oxidized back to naphthoquinone derivatives by atmospheric oxygen and metabolic instability was also expected, the acetylated derivatives **16** were prepared in one pot using zinc, sodium acetate, and acetic anhydride[8] to implement the pro-drug character with stability. As expected from modest activities of catechols **7** and **11**, diacetoxynaphthalene **16a** showed low *in vitro* inhibitory activity. Unexpectedly, diacetoxynaphthalenes **16b** and **16c** with substituents at 3-position showed remarkable *in vitro* activity, comparable to their naphthoquinone homologs.[5] Also 1,2-naphthalenediol **17** substituted with 3-indolyl group at 4 position showed the best inhibitory activity from this series.

Thus **17** was chosen for further study. As the selectivity of the inhibitors is important to minimize the undesirable side effects, the selectivity was tested against nine phosphatases and the result is shown in Table 2. The compound **17** showed better selectivity comapred to **F**. Then **17** was also evaluated

Table 1. Inhibitory Activity against PTP-1B

No	% inhibition	IC_{50}	No	% inhibition	IC_{50}
1	4.4		**9**	3.0	
2	5.6		**11**	23.9	
3	1.0		**14**	na	
4	7.8		**16a**	na	
5	49.6		**16b**	97.6	3.89
6	99.2	1.25	**16c**	101.8	1.69
7	66.5	3.65	**17**	109.4	1.61

% inhibition at 20 μM or IC_{50} (μM), na – not active

Table 2. Isozyme Selectivity (IC_{50}, μM)

	PTP-1B	Yop	VHR	PP1	CD45	LAR	cdc25A	cdc25B	cdc25C	PP2A
17	1.61	>>10	>10	>10	>>10	>>10	>10	1.30	~10	>>10
F	0.29	0.20	3.08	1.71	0.41	>10	1.50	0.17	2.76	~10

Table 3. *In vivo* Efficacy

	0	1 d	3 d	5 d
0.5% CMC	100	100	100	100
Rosiglitazone	100	87	75	57
17	100	90	99	53

in vivo for their ability to reduce plasma glucose levels in the age- and sex-matched diabetic *ob*/*ob* mice (n = 4-6) at an oral dose of 25 mg/kg/10 mL in 0.5% CMC. The plasma glucose level was reduced by 47% after 5 days of treatment.

In conclusion, a series of catechols were prepared and tested as PTP-1B inhibitors. The 4-(3-indolyl)-1,2-naphthalenediol (**17**) could reduce plasma glucose levels in the diabetic *ob/ob* mice by oral administration.

Experimental Section

5,6,7,8-Tetrahydronaphthalene-1,2-diol (11). A mixture of 5,6-dimethoxy-3,4-dihydro-2*H*-naphthalen-1-one **8** (100 mg, 0.48 mmol) and 10% Pd/C (10.3 mg) in ethanol was hydrogenated for 1 h at 60 psi. The resulting mixture was filtered through Celite and concentrated to afford 81 mg (87 %) of 5,6-dimethoxy-1,2,3,4-tetrahydronaphthalene **10**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.70-6.82 (m, 2H), 3.85 (s, 3H), 3.81 (s, 3H), 2.72-2.81 (m, 4H), 1.73-1.79 (m, 4H). To a solution of tetralin **10** (36 mg, 0.18 mmol) in methylene chloride was added BBr_3 (0.50 mL, 0.90 mmol) at -78 °C. The resulting mixture was stirred for 0.5 h at -78 °C and 2.5 h at room temperature followed by quenching with methanol at -78 °C. The concentrated residue was purified by column chromatography to afford catechol **11** (23 mg, 75%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.67-6.52 (m, 2H), 2.71-2.65 (m, 4H), 1.81-1.76 (m, 4H); EI-MS *m/z* (relative intensity) 164 (M^+, 98), 146 (26), 136 (100), 107 (19), 91 (18), 77 (23), 57 (28), 51 (23).

6,7-Dihydroxy-2,3,4,5-tetrahydro-1*H*-benzoazepin-2-one (14). A mixture of naphthalenone **8** (50 mg, 0.24 mmol), $NH_2OH{\cdot}H_2O$ (67 mg, 0.97 mmol), and NaOAc (80 mg, 0.97 mmol) in 3 mL of methanol and 1 mL of water was stirred for 6 h at 70 °C and concentrated. The residue was purified by column chromatography to afford oxime **12** (50 mg, 93 %): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.65 (d, *J* = 8.8 Hz, 1H), 6.80 (d, *J* = 8.8 Hz, 1H), 3.89 (s, 3H), 3.80 (s, 3H), 2.70-2.85 (m, 4H), 2.82 (quint, *J* = 6.2 Hz, 2H). A mixture of oxime **12** (100 mg, 0.452 mmol) and 2.0 g of PPA was stirred at 125 °C for 20 min followed by addition of ice water. The mixture was extracted with CH_2Cl_2. The organic layer was concentrated *in vacuo* and the residue was purified by column chromatography to afford lactam **13** (38 mg, 38%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.43 (brs, 1H) 6.70-6.85 (m, 2H) 3.86 (s, 3H) 3.81 (s, 3H) 2.87 (t, *J* = 7.0 Hz 2H), 2.10-2.40 (m, 4H); EI-MS *m/z* (relative intensity) 221 (M^+, 100), 206 (23), 166 (51), 151 (75). To a solution of lactam **13** (144 mg, 0.65 mmol) in methylene chloride was added BBr_3 (5.0 mmol) at -78 °C. The resulting mixture was stirred for 0.5 h at -78 °C and 2.5 h at room temperature followed by quenching with methanol at -78 °C. The concentrated residue was purified by column chromatography to afford catechol **14** (113 mg, 90%): ^{1}H NMR (200 MHz, CD_3OD) δ 6.65 (d, *J* = 8.8 Hz, 1H) 6.45 (d, *J* = 8.8 Hz, 1H) 2.80 (t, *J* = 6.2 Hz, 2H) 2.40-2.10 (m, 4H); EI-MS *m/z* (relative intensity) 193 (M^+, 29), 138 (42), 82 (32), 80 (38), 43 (100).

4-(3-Indolyl)-1,2-diacetoxynaphthalene (16a). A mixture of 4-(3-indolyl)-1,2-naphthoquinone **15a** (200 mg, 0.731 mmol), Zn (720 mg), and NaOAc (240 mg) in acetic anhydride (20 mL) was stirred for 12 h at room temperature. The resulting mixture was partitioned between ice water and methylene chloride and the organic layer was dried with $MgSO_4$, and concentrated *in vacuo*. The product was solidified by ether-hexane to afford **16a** (180 mg, 70%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.45 (brs, 1H), 8.05 (d, *J* = 8.2 Hz, 1H), 7.90 (d, *J* = 8.2 Hz, 1H), 7.57-7.11 (m, 8H), 2.52 (s, 3H), 2.36 (s, 3H); EI-MS *m/z* (relative intensity) 359 (M^+, 11), 317 (30), 275 (100), 246 (13), 43 (49).

4-Phenyl-3-(4-methoxybenzyl)-1,2-diacetoxynaphthalene (16b) and 4-(3-indolyl)-3-(4-*t*-butoxycarbonylmethoxybenzyl)-1,2-diacetoxynaphthalene (16c) were prepared like **16a** from corresponding quinones **15** in 40% and 46% yields, respectively.

16b: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.80-6.60 (m, 13H), 4.41 (s, 1H), 3.79 (s, 3H), 3.65 (s, 2H), 2.42 (s, 3H), 2.09 (s, 3H); EI-MS *m/z* (relative intensity) 440 (M^+, 7), 398 (24), 356 (74), 248 (85), 219 (100), 202 (15), 189 (18).

16c: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.32 (brs, 1H), 7.80 (d, *J* = 8.2 Hz, 1H), 7.60-6.59 (m, 12H), 4.41 (s, 1H), 4.02-3.76 (m, 2H), 2.42 (s, 3H), 2.10 (s, 3H), 1.47 (s, 9H); EI-MS *m/z* (relative intensity) 579 (M^+, 19), 537 (8), 481 (4), 439 (20), 287 (18), 57 (34), 43 (100).

4-(3-Indolyl)-1,2-dihydroxynaphthalene (17). Phenylhydrazine (79 mg, 0.731 mmol) was added to a solution of 4-(3-indolyl)-1,2-naphthoquinone **15** (200 mg, 0.731 mmol) in acetonitrile (10 mL) at room temperature and the resulting solution was stirred for 2 h followed by evaporation to afford catechol **17** (197 mg, 99%): ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.31 (s, 1H), 9.31 (s, 1H), 8.82 (s, 2H), 8.12-7.02 (m, 10H); EI-MS *m/z* (relative intensity) 275 (M^+, 100), 246 (17), 228 (29), 217 (18), 200 (9), 189 (11), 114 (33).

Biological Activity. The tests were performed against recombinant human PTP-1B using fluorescein diphosphate (FDP) as the substrate. The medium was 30 mM Tris, 75 mM NaCl, 0.67 mM EDTA in 1 mM DTT (pH 8.0) buffer with 20 μM FDP, and 0.1 μg of PTP-1B. After an hour at room temperature with inhibitor, the enzyme activity was determined by measuring the fluorescence of the product, fluorescein monophosphate (FMT) at 485 nm (excitation) and 538 nm (emission). IC_{50} (μM) values were determined from direct regression curve analysis. Isozyme selectivity was determined likewise using appropriate phosphatases.

In vivo efficacy was evaluated in the age- and sex-matched diabetic *ob/ob* mice (n = 4-6). Animals were treated *p.o.* with 0.5% CMC (vehicle control), rosiglitazone (positive control, 10 mg/kg/10 mL in 0.5% CMC) and **17** (25 mg/kg/10 mL in 0.5% CMC). Compounds were given at 10:00 am and blood

were withdrawn on days 1, 3, and 5 at 2:00 pm through peri-orbital venous sinus using heparin-treated capillary tubes. Blood glucose levels were measured using Vitalab Selectra 2 (Vital Scientific, Spankeren, Netherlands) and representative data as % inhibition compared to control are shown.

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