Protein Tyrosine Phosphatase 1B Inhibitors: Catechols

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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 \textit{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, \(\alpha\)-quinones B-D, and carboxylic acids E-G.\(^4\) One of the inhibitors, Ertiprotatib (G) went to clinical trial, but was discontinued in Phase II due to insufficient efficacy and dose-dependent side effects. 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.

Figure 2. Catechol Derivatives.

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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\textsubscript{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. 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 acid, and BBr\textsubscript{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\textsuperscript{5} was reduced to 1,2-dihydroxynaphthalene 17 using phenyl hydrazine.\textsuperscript{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\textsuperscript{8} to implement the pro-drug character with stability. As expected from modest activities of catechols 7 and 11, diacetoxynaphthalene 16a showed low \textit{in vitro} inhibitory activity. Unexpectedly, diacetoxynaphthalenes 16b and 16c with substituents at 3-position showed remarkable \textit{in vitro} activity, comparable to their naphthoquinone homologs.\textsuperscript{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 compared to F. Then 17 was also evaluated

Table 1. Inhibitory Activity against PTP-1B

<table>
<thead>
<tr>
<th>No</th>
<th>% inhibition</th>
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</tr>
<tr>
<td>2</td>
<td>5.6</td>
<td></td>
<td>11</td>
<td>14</td>
<td>na</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td></td>
<td>14</td>
<td>na</td>
<td>97.6</td>
</tr>
<tr>
<td>4</td>
<td>7.8</td>
<td></td>
<td>16a</td>
<td>na</td>
<td>3.89</td>
</tr>
<tr>
<td>5</td>
<td>49.6</td>
<td></td>
<td>16b</td>
<td>97.6</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>99.2</td>
<td>1.25</td>
<td>16c</td>
<td>101.8</td>
<td>1.69</td>
</tr>
<tr>
<td>7</td>
<td>66.5</td>
<td>3.65</td>
<td>17</td>
<td>109.4</td>
<td>1.61</td>
</tr>
</tbody>
</table>

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

Table 2. Isozyme Selectivity (IC\textsubscript{50}, µM)

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<tr>
<th></th>
<th>PTP-1B</th>
<th>Yop</th>
<th>VHR</th>
<th>PP1</th>
<th>CD45</th>
<th>LAR</th>
<th>cdc25A</th>
<th>cdc25B</th>
<th>cdc25C</th>
<th>PP2A</th>
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<td>17</td>
<td>1.61</td>
<td>&gt;&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;&gt;10</td>
<td>&gt;10</td>
<td>1.30</td>
<td>~10</td>
<td>&gt;&gt;10</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>0.29</td>
<td>0.20</td>
<td>3.08</td>
<td>1.71</td>
<td>0.41</td>
<td>&gt;10</td>
<td>1.50</td>
<td>0.17</td>
<td>2.76</td>
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5,6,7,8-Tetrahydronaphthalene-1,2-diol (11). A mixture of 5,6-dimethoxy-3,4-dihydro-2H-naphthalen-1-one 8 (100 mg, 0.48 mmol) and 10% Pd/C (10.3 mg) in ethanol was quenched with methanol at -78 °C. The resulting mixture was solidified by ether-hexane to afford 16a (180 mg, 70%): 1H NMR (200 MHz, CDCl3) δ 7.82 (s, 1H), 7.39 (s, 1H), 7.65 (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-Phenyl-3-(4-methoxybenzyl)-1,2-diacetoxynaphthalene (16b) and 4-(3-indolyl)-3-(4-tert-butoxycarbonylmethoxy-benzyl)-1,2-diacetoxynaphthalene (16c) were prepared like 16a from corresponding quinones 15 in 40% and 46% yields, respectively.

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%).: 1H NMR (200 MHz, DMSO-d6) 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). IC50 (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 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-naphthelenediol (17) could reduce plasma glucose levels in the diabetic ob/ob mouse by oral administration.

**Experimental Section**

5,6,7,8-Tetrahydronaphthalene-1,2-diol (11). A mixture of 5,6-dimethoxy-3,4-dihydro-2H-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: 1H NMR (200 MHz, CDCl3) 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 BBr3 (5.0 mL, 9.0 mmol) 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 lactam 13 (28, 38%): 1H NMR (200 MHz, CDCl3) δ 7.45 (brs, 1H), 7.39 (s, 1H), 7.65 (d, J = 8.2 Hz, 1H), 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).

7,6-Dihydroxy-2,3,4,5-tetrahydro-1H-benzoazepin-2-one (14). A mixture of naphthalenone 8 (50 mg, 0.24 mmol), NH2OH·H2O (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%): 1H NMR (200 MHz, CDCl3) 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 (q, 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 CH2Cl2. The organic layer was concentrated in vacuo to afford lactam 13 (38 mg, 38%): 1H NMR (200 MHz, CDCl3) 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 BBr3 (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%): 1H NMR (200 MHz, CDCl3) 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 MgSO4, and concentrated in vacuo. The product was solidified by ether-hexane to afford 16a (180 mg, 70%): 1H NMR (200 MHz, CDCl3) 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).

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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References