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# Synthesis of functionalized acetophenones as protein tyrosine phosphatase 1B inhibitors☆

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**Abstract**—Protein tyrosine phosphatase 1B (PTP1B) is an enzyme that plays a critical role in down-regulating insulin signaling through dephosphorylation of the insulin receptor. Studies have shown that PTP1B knock-out mice showed increased insulin sensitivity in muscle and liver as well as resistance to obesity. A series of functionalized acetophenones were synthesized and evaluated for their PTP1B inhibitory activity. Some of the screened compounds displayed good inhibitory activity.


## 1. Introduction

Protein kinases and phosphatases are a large family of enzymes that control several fundamental cellular functions via phosphorylation and de-phosphorylation reactions. In recent years, studies on protein tyrosine phosphatases (PTPs) as drug targets for controlling intracellular signaling have been rapidly increasing.[1] Among PTPs, PTP-1B has received much attention due to its pivotal role in type 2 diabetes and obesity as a negative regulator of the insulin and leptin-signaling pathway.[2] Clinical studies have demonstrated that PTP-1B is primarily responsible for dephosphorylation of the insulin receptor and thus down regulates insulin signaling.[3] Therefore, the search for potent small molecule protein tyrosine phosphatase 1B inhibitors is a major thrust area in the management of type 2 diabetes mellitus.[4]

Several synthetic and naturally occurring PTP inhibitors reported in the literature have shown efficacy in in vitro and in vivo models. The majority of them possess tyrosine mimetic structures functionalized with negatively charged moieties such as phosphonates,[5] malonates,[6] carboxylates,[7] or cinnamates.[8] Umezawa et al.[9] discovered a naturally occurring PTPase inhibitor, dephostatin, isolated from the culture filtrate of *Streptomyces* sp. MJ742-NF5. Later on, they prepared alkyl 3,4-dephostatin (**I**) as a stable, selective inhibitor of PTP1B (Fig. 1). Recently, Pei and co-workers[10] have reported several α-haloacetophenone (**II**) derivatives as potent neutral protein tyrosine phosphatase inhibitors, which covalently alkylate the conserved catalytic cysteine residue in the PTP active site. Several formyl chromone (**III**) derivatives were reported as human PTP1B possessing inhibitory activity in the micromolar range.[11] Recently, Hu and co-workers[12] have shown that the ethanolic extract of the roots of *Broussonetia papyrifera* (L.) Vent, which are composed of several flavonoids including a flavonol **IV**, showed potent inhibitory activity against PTP1B enzyme. Based on the reported PTP1B

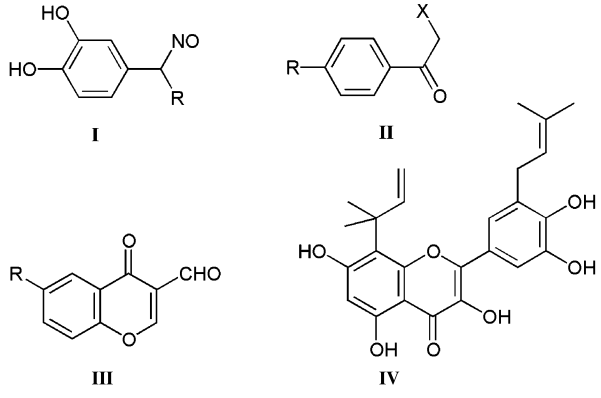


**Figure 1.** Structures of PTP1B inhibitors.

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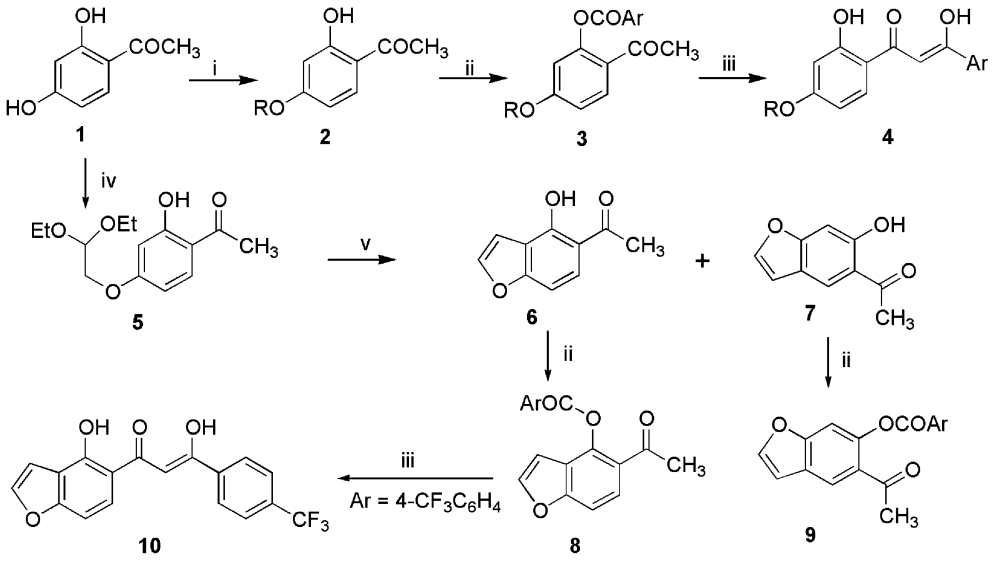


**Scheme 1.** Reagents and conditions: (i) RBr, $K_2CO_3$, acetone; (ii) ArCOCl, pyridine, rt; (iii) *t*-BuOK, pyridine, rt; (iv) $BrCH_2CH(OC_2H_5)_2$, DMF, 140 °C; (v) Amberlyst 15, toluene, 120 °C.

inhibitors, we have synthesized various small molecule acetophenones functionalized with alkoxy and aroyloxy moieties as potential PTP1B inhibitors.

The precursor used for the synthesis of various hydroxy chalcones and functionalized acetophenones was 2,4-dihydroxyacetophenone **1**, which on reaction with alkyl halide in the presence of a base afforded **2** in 60–70% yield as depicted in Scheme 1. The reaction of **2** with aroyl chloride in pyridine gave products **3a–c** in good yields. The benzoylated acetophenone **3** was stirred with potassium tertiary butoxide in pyridine to yield hydroxy chalcone derivatives **4** in 70–80% yield. The 2,4-dihydroxyacetophenone was further exploited to prepare benzofuran-based hydroxy ketone compounds, which are useful precursors for several naturally occurring furanoflavonoids that possess diverse biological activities. Compound **1** was alkylated with bromoacetaldehyde diethyl acetal in the presence of potassium carbonate to yield corresponding alkylated product **5**, which was cyclized with Amberlyst 15 by refluxing in toluene as described earlier by us.[13] Both the products **6** and **7** were benzoylated separately to the corresponding derivative **8** and **9**, respectively. Compound **8c** was further transformed to corresponding chalcones in the presence of potassium tertiary butoxide as shown in Scheme 1. All the synthesized compounds were characterized by spectroscopic data and elemental analysis.[14]

## 2. Result and discussion

Vanadate is a non-selective inhibitor of PTPs, and studies have shown that treatment with vanadate can normalize blood glucose level in diabetics.[2b,15] Taking sodium vanadate as a control, we have evaluated PTP1B inhibitory activity of functionalized acetophenones at 100 μM concentration and their results are summarized in Table 1. Some of the screened compounds demonstrated moderate to good PTP1B inhibitory activity at 100 μM concentration. Compounds **8a** and **b** displayed 43.2% and 54.1% inhibition against PTP1B enzyme.

**Table 1.** In vitro PTP1B enzyme inhibitory activity for the compounds **2a–e**, **3a–c**, **4a–c**, **6**, **7**, **8a–c**, **9a–e**, and **10**

| Entry | R | Ar | Inhibition[a](%) |
|---|---|---|---|
| **2a** | $CH_2CN$ | — | 22.4 |
| **2b** | $CH_2CH{=}CH_2$ | — | NI |
| **2c** | $CH_2CH_2C_6H_5$ | — | 38.6 |
| **2d** | $CH_2CH_2OH$ | — | NI |
| **2e** |  | — | 22.4 |
| **3a** | $CH_2CN$ | $C_6H_5$ | 12.8 |
| **3b** | $CH_2CN$ | 4-$FC_6H_4$ | NI |
| **3c** | $CH_2CH_2C_6H_5$ | 4-$FC_6H_4$ | NI |
| **4a** | $CH_2CN$ | $C_6H_5$ | NI |
| **4b** | $CH_2CN$ | 4-$FC_6H_4$ | NI |
| **4c** | $CH_2CH_2C_6H_5$ | 4-$FC_6H_4$ | NI |
| **6** | — | — | NI |
| **7** | — | — | 19.1 |
| **8a** | — | $C_6H_5$ | 43.2 |
| **8b** | — | 3,4-$Cl_2C_6H_3$ | 54.1 |
| **8c** | — | 4-$CF_3C_6H_4$ | 32.4 |
| **9a** | — | $C_6H_5$ | NI |
| **9b** | — | 4-$OCH_3C_6H_4$ | 13.5 |
| **9c** | — | 3,4-$(OMe)_2C_6H_3$ | NI |
| **9d** | — | 3-$OCH_3C_6H_4$ | 8.1 |
| **9e** | — | 3,4-$CH_2O_2C_6H_3$ | 34 |
| **10** | — | — | NI |
| $Na_3VO_4$ | (Control) | — | 56.2 |

[a] Values are means from three independent sets of experiments tested at 100 μM concentration; NI means no inhibition.

### 2.1. Protein tyrosine phosphatase inhibitory activity[16]

The effect of test compounds on protein tyrosine phosphatase was studied by pre-incubating 100 μM of the test chemicals in the reaction system for 10 min and the residual protein tyrosine phosphatase activity deter-

mined according to the method of Goldstein et al.[16] Activity of PTPase (LAR) was evaluated using *p*-nitrophenylphosphate (PNPP) as the substrate. Assay mixture was made up to 1 mL containing 10 mM PNPP in 50 mM HEPES buffer (pH 7), with 1 mM EDTA and DTT. The reaction was stopped by the addition of 500 μL of 0.1 N NaOH and absorbance was determined at 410 nm. A molar extinction coefficient of $1.78 \times 10^4\ M^{-1}\ cm^{-1}$ was used to calculate the concentration of *p*-nitrophenolate ions produced in the reaction mixture.

The structure–activity relationship of the screened 4-alkoxy-2-hydroxyacetophenones revealed that bulky nonpolar moiety at the terminus of alkyl group possesses good inhibitory activity (38.6%). None of the benzoylated acetophenones (**3a**–**c**) and their corresponding chalcones (**4a**–**c**) showed inhibition except **3a**, which showed little activity. Various benzofuran derivatives have shown to possess PTP1B inhibitory activity in the low micromolar range.[17] Thus, various hydroxy benzofuran methyl ketones and their benzoylated derivatives have been evaluated as PTP1B inhibitors. It is evident from the activity profile that 4-aroyloxy-5-acetyl-benzofuran (**8a**–**c**), showed good inhibitory activity comparable to reference compound sodium vanadate. One of these compounds, 4-(3,4-dichlorobenzoyloxy)-5-acetyl-benzofuran (**8b**), showed 54% inhibition against PTP1B at 100 μM concentration. The rest of the compounds were either inactive or possessed a low range activity.

In conclusion, we have identified a series of functionalized acetophenones as protein tyrosine phosphatase inhibitors. This is an initial report and optimization of these compounds is in progress.

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## References and notes

1. (a) Ostman, A.; Bohmer, F. D. *Trends Cell Biol.* **2001**, *11*, 258; (b) Denu, J. M.; Dixon, J. E. *Curr. Biol.* **1998**, *2*, 633; (c) Zhan, X.-L.; Wishart, M. J.; Guan, K.-L. *Chem. Rev.* **2001**, *101*, 2477; (d) Zhang, Z.-Y. *Curr. Opin. Chem. Biol.* **2001**, *5*, 416; (e) Neel, B. G.; Tonks, N. K. *Curr. Opin. Cell Biol.* **1997**, *9*, 193.
2. (a) Elchelby, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Lee Loy, A.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C.-C.; Ramanchandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P. *Science* **1999**, *283*, 1544; (b) Kennedy, B. P. *Biomed. Pharmacother.* **1999**, *53*, 466.
3. (a) Kenner, K. A.; Anyanwu, E.; Olefsky, J. M.; Kusari, J. *J. Biol. Chem.* **1996**, *271*, 19810; (b) Chen, H.; Wertheimer, S. J.; Lin, C. H.; Katz, S. L.; Amrein, K. E.; Burn, P.; Quon, M. J. *J. Biol. Chem.* **1997**, *272*, 8026; (c) Walchli, S.; Curchod, M. L.; Gobert, R. P.; Arkinstall, S.; Hooft van Huijsduijnen, R. *J. Biol. Chem.* **2000**, *275*, 9792.
4. (a) Szczepankiewicz, B. G.; Liu, G.; Hajduk, P. J.; Abad-Zapatero, C.; Pei, Z.; Xin, Z.; Lubben, T. H.; Trevillyan, J. M.; Stashko, M. A.; Ballaron, S. J.; Liang, H.; Huang, F.; Hutchins, C. W.; Fesik, S. W.; Jirousek, M. R. *J. Am. Chem. Soc.* **2003**, *125*, 4087; (b) Liu, G.; Xin, Z.; Pei, Z.; Hajduk, P. J.; Abad-Zapatero, C.; Hutchins, C. W.; Zhao, H.; Lubben, T. H.; Ballaron, S. J.; Haasch, D. L.; Kaszubska, W.; Rondinone, C. M.; Trevillyan, J. M.; Jirousek, M. R. *J. Med. Chem.* **2003**, *46*, 4232.
5. (a) Burke, T. R., Jr.; Kole, H. K.; Roller, P. P. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 129; (b) Taylor, W. P.; Zhang, Z.-Y.; Widlanski, T. S. *Bioorg. Med. Chem.* **1996**, *4*, 1515; (c) Taylor, S. D.; Kotoris, C. C.; Dinaut, A. N.; Wang, Q.; Ramachandran, C.; Huang, Z. *Bioorg. Med. Chem.* **1998**, *6*, 1457; (d) Taing, M.; Keng, Y.-F.; Shen, K.; Wu, L.; Lawrence, D. S.; Zhang, Z.-Y. *Biochemistry* **1999**, *38*, 3793; (e) Shen, K.; Keng, Y.-F.; Wu, L.; Guo, X.-L.; Lawrence, D. S.; Zhang, Z.-Y. *J. Biol. Chem.* **2001**, *276*, 47311.
6. (a) Ye, B.; Akamatsu, M.; Shoelson, S. E.; Wolf, G.; Giogetti-Peraldi, S.; Yan, X.; Roller, P. P.; Burke, T. R., Jr. *J. Med. Chem.* **1995**, *38*, 4270; (b) Burke, T. R., Jr.; Ye, B.; Akamatsu, M.; Ford, H.; Yan, X. J.; Kole, H. K.; Wolf, G.; Shoelson, S. E.; Roller, P. P. *J. Med. Chem.* **1996**, *39*, 1021; (c) Akamatsu, M.; Roller, P. P.; Chen, L.; Zhang, Z.-Y.; Ye, B.; Burke, T. R., Jr. *Bioorg. Med. Chem.* **1997**, *5*, 157; (d) Roller, P. P.; Wu, L.; Zhang, Z.-Y.; Burke, T. R., Jr. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2149.
7. (a) Sarmiento, M.; Wu, L.; Keng, Y.-Y.; Song, L.; Luo, Z.; Huang, Z.; Wu, G.-Z.; Yuan, A. K.; Zhang, Z.-Y. *J. Med. Chem.* **2000**, *43*, 146; (b) Iversen, L. F.; Andersen, H. S.; Branner, S.; Mortensen, S. B.; Peters, G. H.; Norris, K.; Olsen, O. H.; Jeppesen, C. B.; Lundt, B. F.; Ripka, W.; Møller, K. B.; Møller, N. P. H. *J. Biol. Chem.* **2000**, *275*, 10300; (c) Wrobel, J.; Li, Z.; Sredy, J.; Sawicki, D. R.; Seestaller, L.; Sullivan, D. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1535; (d) Chen, Y. T.; Onaran, M. B.; Doss, C. J.; Seto, C. T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1935; (e) Malamas, M. S.; Sredy, J.; Moxham, C.; Katz, A.; Xu, W. X.; McDevitt, R.; Adebayo, F. O.; Sawicki, D. R.; Seestaller, L.; Sullivan, D.; Taylor, J. R. *J. Med. Chem.* **2000**, *43*, 1293.
8. (a) Moran, E. J.; Sarshr, S.; Cargill, J. E.; Shahbaz, M. M.; Lio, A.; Mjalli, A. M. M.; Armstrong, R. W. *J. Am. Chem. Soc.* **1995**, *117*, 10787; (b) Cao, X.; Moran, E. J.; Siev, D.; Lio, A.; Ohashi, C.; Mjalli, A. M. M. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2953.
9. Umezawa, K.; Kawakami, M.; Watanabe, T. *Pharmacol. Ther.* **2003**, *99*, 15.
10. Arabaci, G.; Guo, X.-C.; Beebe, K. D.; Coggeshall, K. M.; Pei, D. *J. Am. Chem. Soc.* **1999**, *121*, 5085.
11. Shim, Y. S.; Kim, K. C.; Chi, D. Y.; Lee, K.-H.; Cho, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2561.
12. Chen, R. M.; Hu, L. H.; An, T. Y.; Li, J.; Shen, Q. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3387.
13. Goel, A.; Dixit, M. *Synlett* **2004**, 1990.
14. General procedure for the synthesis of compounds **2**, **3** and **4**: A mixture of 2,4-dihydroxy-acetophenone (**1**, 6 mmol, 912 mg) with alkyl halide (6 mmol) and potassium carbonate (2 equiv) was refluxed in acetone for 8–10 h. The resulting mixture was filtered to remove the potassium carbonate. The filtrate was concentrated to dryness to get the pure compound (**2**) in 60–70% yield. An acetophenone derivative (**2**, 6 mmol) and aroyl chloride (6 mmol) in dry pyridine (5 mL) was stirred at room temperature for an hour before heating at 100 °C for

10 min under anhydrous condition. The resulting solution was poured into 1 M HCl containing crushed ice, which yielded a solid benzoate (**3**) in 80–85% yield. The potassium tertiary butoxide was slowly added to magnetically stirred solution of the benzoate (**3**) for 4–5 h in dry pyridine. The resulting solution mixture was added to 10% acetic acid solution. The yellow solid thus obtained was filtered, dried, and crystallized in hexane–ethyl acetate to give yellow needle-like crystals in good yield. Compound **4a**: Yield 73%; mp: 119–120 °C; MS (FAB): *m*/*z* 296 ($M^+$+1); IR (KBr): 1605 (CO), 2218 (CN), 3422 $cm^{-1}$ (OH); $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 4.81 (s, 2H, $CH_2$), 6.53–6.59 (m, 2H, ArH), 6.74 (s, 1H, CH), 7.49–7.57 (m, 3H, ArH), 7.78 (d, 1H, $J$ = 8.9 Hz, ArH), 7.93 (d, 2H, $J$ = 8.1 Hz, ArH), 12.56 (s, 1H, OH), 15.36 (s, 1H, OH). Compound **8a**: Yield 80%; mp: 79–81 °C; MS (FAB): *m*/*z* 281 ($M^+$+1); IR (KBr): 1683 (CO), 1742 $cm^{-1}$ (CO); $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 2.60 (s, 3H, $CH_3$), 6.74 (d, 1H, $J$ = 2.0 Hz, CH), 7.48 (d, 1H, $J$ = 6.0 Hz, ArH), 7.54–7.59 (m, 2H, ArH), 7.66 (d, 1H, $J$ = 2.0 Hz, CH), 7.67–7.72 (m, 1H, ArH), 7.89 (d, 1H, $J$ = 6.0 Hz, ArH), 8.27–8.30 (m, 2H, ArH). Compound **8b**: Yield 85%; mp: 109–110 °C; MS (FAB): *m*/*z* 349 ($M^+$+1); IR (KBr): 1685 (CO), 1748 $cm^{-1}$ (CO); $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 2.60 (s, 3H, $CH_3$), 6.75 (d, 1H, $J$ = 2.0 Hz, CH), 7.51 (d, 1H, $J$ = 8.6 Hz, ArH), 7.62–7.68 (m, 2H, ArH), 7.88 (d, 1H, $J$ = 8.6 Hz, ArH), 8.10 (d, 1H, $J$ = 8.4 Hz, ArH), 8.35 (d, 1H, $J$ = 2.0 Hz, CH).

15. Sekar, N.; Li, J.; Shechter, Y. *Crit. Rev. Biochem. Mol. Biol.* **1996**, *31*, 339.
16. Goldstein, B. J.; Bittner-Kowalezyk, A.; White, M. F.; Harbeck, M. *J. Biol. Chem.* **2000**, *275*, 4283.
17. Malamas, M. S.; Sredy, J.; Moxham, C.; Katz, A.; Xu, W.; Devitt, R. M.; Adibayao, F. O.; Sawicki, D. R.; Seestaller, L.; Sullivan, D.; Taylor, X., Jr. *J. Med. Chem.* **2000**, *43*, 1293.