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Formylchromone Derivatives as a Novel Class of Protein Tyrosine Phosphatase 1B Inhibitors

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Abstract—Formylchromone inhibits a human protein tyrosine phosphatase PTP1B with a IC_{50} value of 73 μM. The chemical reactivity of formylchromone was adjusted by substitution at various positions of the formylchromone skeleton. In an initial assessment of the structure–activity relationship, the most potent inhibitor showed an IC_{50} of 4.3 μM against PTP1B and strong or medium selectivity against other human PTPases, LAR and TC-PTP. This compound, however, was not selective against microbial PTPases, YPTP1 and YOP. The potency and selectivity of the formylchromone derivatives expecting further improvements provides a novel pharmacophore for the design of drugs for the treatmenrt of type 2 diabetes and obesity.

Protein tyrosine phosphatases (PTPases), together with protein tyrosine kinases, regulate the phosphorylation level of tyrosine residue of cellular proteins.[1] Many of those proteins are known to be involved in a diverse signal transduction pathways and the dynamic control of the tyrosine phosphorylation is found to be critical in many cellular processes.[1b,2] As anticipated from the importance of tyrosine phosphorylation, PTPases are implicated in a diverse human diseases including diabetes, obesity, autoimmune diseases, infectious diseases, inflammation, cancer, osteoporosis and neurodegeneration.[2b,3] Among those, type 2 diabetes is believed to be associated with the defect in insulin receptor signaling which begins with the receptor autophosphorylation and the receptor tyrosine kinase activation.[4] Insulin signaling is negatively regulated by dephosphorylation of the receptor by PTPases and, therefore, the defect in insulin sensitivity is possibly recovered by the inhibition of the relevant PTPases.[5] The most likely candidates include PTP1B, LAR, PTPα and SHP-2.[6] Among those, PTP1B has been most intensively studied as a target for the development of inhibitors aiming at the treatment of type 2 diabetes and obesity.

A variety of inhibitors for PTP1B and/or other PTPases has been designed and reported in the literature. Many of those incorporated phosphate-mimicking functionalities such as $–CH_2PO_4^{2-}$, $–CF_2PO_4^{2-}$, $–COCO_2^-$, or CO_2^- to list a few.[7] The pharmacophores without charge were also precedented.[8]

In the course of the search for PTP1B inhibitors, we found that 3-formylchromone 1 (4-oxo-4H-1-benzopyran-3-carboxaldehyde) inhibits PTP1B with a potency of $IC_{50} = 73$ μM. Formylchromone is a neutral molecule without charge and the examination of the structure reveals that it is well suited to derivatization. For initial assessment of the structure–activity relationship, the chemical reactivity of formylchromone derivatives was adjusted by substitution at various positions of the formylchromone skeleton (Fig. 1). Some of the compounds (**1**, **2**, **4**–**10**) were purchased and other derivatives (**3**, **11**–**14**) were synthesized starting from appropriate aryl alcohols using Vilsmeier–Haack reaction as a key step for the construction of the formylchromone skeleton (Scheme 1). Compound **14** was prepared by introducing 4-phenylphenyl moiety with Suzuki coupling reaction followed by Vilsmeier–Haack reaction (Scheme 2).

The inhibitory activities of the formylchromone derivatives were determined with minor modifications of the procedure described in the literature using p-nitrophenyl phosphate (pNPP) as a substrate at 37 °C.[9] IC_{50} values against PTP1B are summarized in Table 1. In compounds **13** and **14**, phenyl substituent(s) at C-6 of

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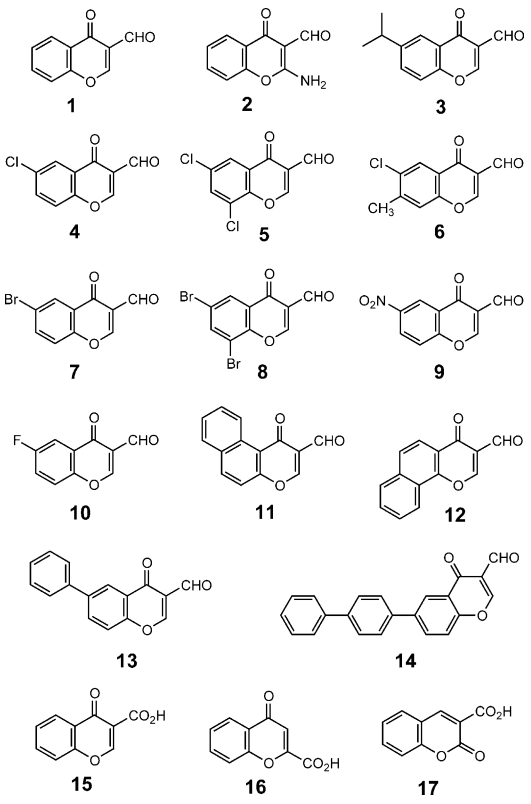
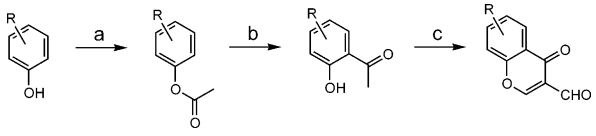


Figure 1. Structures of formylchromone derivatives used in this study.



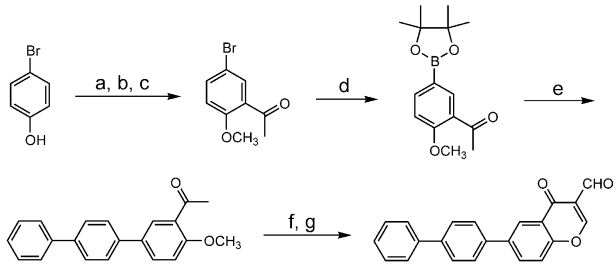
Scheme 1. Reagents and conditions: (a) $(CH_3O)_2O$, pyridine, CH_2Cl_2, rt, 70%; (b) $AlCl_3$, 1,4-dichlorobenzene, 100 °C, 20–30%; (c) $POCl_3$, DMF, 50 °C, 50%.

formylchromone increased the potency of the inhibitor. Compound **14** with an extended biphenyl moiety was demonstrated to be the most potent inhibitor with an IC_{50} value of 4.3 μM. The 3-fold higher inhibitory activity compared to compound **13** is probably due to extended interactions of the extra phenyl ring with the surface near the active site of the enzyme. Aldehyde functionality in this series of compounds is critical in their binding to PTP1B because neither chromone-3-carboxylic acid (**15**) nor chromone-2-carboxylic acid (**16**) possesses significant inhibitory activity against PTP1B. Coumarin-3-carboxylic acid (**17**) with structural similarity was not an effective inhibitor either.

Table 1. Inhibition of PTP1B by formylchromone derivatives[a]

Compd	IC_{50} (μM)	Compd	IC_{50} (μM)
1	73	**10**	55
2	>1000	**11**	14
3	22	**12**	25
4	28	**13**	14
5	25	**14**	4.3
6	18	**15**	>1000
7	20	**16**	>1000
8	20	**17**	>1000
9	91		

[a]IC_{50} values were usually derived from single experiments using a range of inhibitor concentration. For inhibition assay, inhibitor (5 μL in DMSO) was added to a mixture containing enzyme (5 μL), 5× reaction buffer (10 μL, 0.5 M Hepes, 25 mM EDTA, pH 7.0) and water (25 μL) and it was incubated at 37 °C for 10 min. The reaction was initiated by addition of pNPP (5 μL, 0.1 M) and, after 5 min at 37 °C, the reaction was quenched by addition of NaOH solution (950 μL, 0.5 M). The progress of the reaction was determined for the formation of *p*-nitrophenolate by measuring the absorbance at 405 nm. PTP1B was diluted before use to an appropriate concentration (typically 40 μg/mL) by enzyme dilution buffer (25 mM Hepes, 5 mM EDTA, 1 mM DTT, 1 mg/mL BSA, pH 7.3).



Scheme 2. Reagents and conditions: (a) $(CH_3O)_2O$, pyridine, CH_2Cl_2, rt, 99%; (b) $AlCl_3$, 180 °C, 75%; (c) CH_3I, K_2CO_3, acetone, 45 °C, 98%; (d) Bis(pinacolato)diboron, $PdCl_2(dppf)_2$, DMSO, 80 °C, 87%; (e) 4-phenylphenol trifluoromethanesolfonate, $PdCl_2(dppf)_2$, K_3PO_4, DMF, 80 °C, 78%; (f) BBr_3, CH_2Cl_2, rt, 68%; (g) $POCl_3$, DMF, 50 °C, 76%.

The selectivity of compounds **11**, **13** and **14** was evaluated against PTPases (Table 2). PTP1B, LAR-D1 and TC-PTP are human PTPases, and YOP and YPTP1 are from *Yersinia enterocolitica* and *Saccharomyces cerevisiae* respectively. The inhibitors showed good selectivity against human enzymes. They were >100-fold selective against LAR-D1 and >10-fold selective against TC-PTP. Little selectivity was observed against microbial PTPases, YPTP1 and YOP. The amino acid sequence similarity between the catalytic domain of PTP1B and those of other PTPases are 41% for LAR-D1, 72% for TC-PTP, 20% for Yop and 29% for YPTP1.[10] The homology in amino acid sequence between PTPases

Table 2. Inhibition of PTPases by selected compounds[a]

Compd	IC_{50} (μM)				
	PTP1B	LAR	TC-PTP	YPTP1	YOP
11	14±5	>1000	780±340	56±2	23±2
13	14±3	>1000	240±81	24±1	15±2
14	4.3±0.1	>1000	51±4	5.4±0.3	3.7±0.4

[a]IC_{50} values were usually derived from single experiments using a range of inhibitor concentration. LAR, TC-PTP and YOP are purchased from New England Biolabs, Inc. (Beverly, USA) and YPTP1 was prepared as described (Kwon, M.; Oh, M.; Han, J.; Cho, H. *J. Biochem. Mol. Biol.* **1996**, *29*, 386). The enzymes were diluted and assayed in the buffers used for PTP1B assay. The quantity of enzymes used for 50 μL reaction was 1.25 units (manufacturer's definition) for LAR, TC-PTP and YOP and 30 ng for YPTP1.

does not correlate with the selectivity data. Three-dimensional structure at or near the active site is the more likely determinant of the selectivity. Nevertheless, LAR is well differentiated from PTP1B in its reaction with the inhibitors and this observation suggests the possibility that further excavation of the formylchromone derivatives might lead to a highly potent and selective PTP1B inhibitor despite the high homology between PTPases and the effort is in progress.

In summary, we have shown that formylchromone and its derivatives are capable of inhibiting PTP1B. Initial derivatizations of the formylchromone identified compound **14** as a potent inhibitor against PTP1B with strong or medium selectivity against human PTPases, LAR and TC-PTP. The selectivity together with the ongoing improvement of the potency suggests that formylchromone could be a novel lead for the design of a potent and selective PTP1B inhibitor.

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References and Notes

1. (a) Tonks, N. K.; Neel, B. G. *Curr. Opin. Cell Biol.* **2001**, *13*, 182. (b) Östman, A.; Böhmer, F. D. *Trends in Cell Biol.* **2001**, *11*, 258. (c) Denu, J. M.; Dixon, J. E. *Curr. Biol.* **1998**, *2*, 633.
2. (a) Zhan, X.-L.; Wishart, M. J.; Guan, K.-L. *Chem. Rev.* **2001**, *101*, 2477. (b) Zhang, Z.-Y. *Curr. Opin. Chem. Biol.* **2001**, *5*, 416. (c) Neel, B. G.; Tonks, N. K. *Curr. Opin. Cell Biol.* **1997**, *9*, 193.
3. (a) Van Huijsduijnen, R. H.; Bombrun, A.; Swinnen, D. *Drug Discov. Today* **2002**, *7*, 1013. (b) Li, L.; Dixon, J. E. *Semin. Immunol.* **2000**, *12*, 75.
4. (a) Zick, Y. *Trends Cell Biol.* **2001**, *11*, 437. (b) Bailey, C. *J. Biochem. Pharm.* **1999**, *58*, 1511. (c) Taylor, S. I. *Cell* **1999**, *97*, 9. (d) Kido, Y.; Burks, D. J.; Withers, D.; Bruning, J. C.; Kahn, C. R.; White, M. F. *J. Clinical Invest.* **2000**, *105*, 199. (e) Cheng, A.; Dubé, N.; Gu, F.; Tremblay, M. L. *Eur. J. Biochem.* **2002**, *269*, 1050.
5. (a) Goldfine, A. B.; Simonson, D. C.; Folli, F.; Patti, M. E.; Kahn, C. R. *Mol. Cell. Biochem.* **1995**, *153*, 217. (b) Huyer, G.; Liu, S.; Kelly, J.; Moffat, J.; Payette, P.; Kennedy, B.; Tsaprailis, G.; Gresser, M. J.; Ramachandran, C. *J. Biol. Chem.* **1997**, *272*, 843. (c) Goldstein, B. J. *J. Clin. Endocrinol. Metab.* **2002**, *87*, 2474.
6. (a) Zinker, B. A.; Rondinone, C. M.; Trevillyan, J. M.; Gum, R. J.; Clampit, J. E.; Waring, J. F.; Xie, N.; Wilcox, D.; Jacobson, P.; Frost, L.; Kroeger, P. E.; Reilly, R. M.; Koterski, S.; Opgenorth, T. J.; Ulrich, R. G.; Crosby, S.; Butler, M.; Murray, S. F.; McKay, R. A.; Bhanot, S.; Monia, B. P.; Jirousek, M. R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11357. (b) Kennedy, B. P.; Ramachandran, C. *Biochem. Pharm.* **2000**, *60*, 877. (c) Elchebly, M.; Cheng, A.; Tremblay, M. L. *J. Mol. Med.* **2000**, *78*, 473. (d) Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A. L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C. C.; Ramachandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P. *Science* **1999**, *283*, 1544.
7. (a) Zhang, Z.-Y. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 209 and references cited therein. (b) Burke, T. R., Jr.; Zhang, Z.-Y. *Biopolymers* **1998**, *47*, 225 and references cited therein. (c) Ahn, J. H.; Cho, S. Y.; Ha, J. D.; Chu, S. Y.; Jung, S. H.; Jung, Y. S.; Baek, J. Y.; Choi, I. K.; Shin, E. Y.; Kang, S. K.; Kim, S. S.; Cheon, H. G.; Yang, S.-D.; Choi, J.-K. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1941 and references cited therein. (d) Burke, Jr., T. R.; Gao, Y.; Yao, Z.-J. In *Biomedical Chemistry*, Torrence, P. F., Ed.; John Wiley & Sons, Inc., England, 2000, pp 189–210.
8. (a) Liljebris, C.; Martinsson, J.; Tedenborg, L.; Williams, M.; Barker, E.; Duffy, J. E. S.; Nygren, A.; James, S. *Bioorg. Med. Chem.* **2002**, *12*, 3197. (b) Wipf, P.; Aslan, D. C.; Southwick, E. C.; Lazo, J. S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 313. (c) Malamas, M. S.; Sredy, J.; Gunawan, I.; Mihan, B.; Sawicki, D. R.; Seestaller, L.; Sullivan, D.; Flam, B. R. *J. Med. Chem.* **2000**, *43*, 995. (d) Watanabe, T.; Suzuki, T.; Umezawa, Y.; Takeuchi, T.; Otsuka, M.; Umezawa, K. *Tetrahedron* **2000**, *56*, 741. (e) Ham, S. W.; Park, J.; Lee, S.-J.; Yoo, J. S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 185.
9. Park, D.; Shim, Y. S.; Kim, K. C.; Park, J.; Yang, D.; Cho, H. *J. Korean Chem. Soc.* **2002**, *46*, 296.
10. (a) James, P.; Hall, B. D.; Whelen, S.; Craig, E. A. *Gene* **1992**, *122*, 101. (b) Stuckey, J. A.; Schubert, H. L.; Fauman, E. B.; Zhang, Z. Y.; Dixon, J. E.; Saper, M. A. *Nature* **1994**, *370*, 571. (c) van Huijsduijnen, R. H. *Gene* **1998**, *225*, 1.