Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2657–2660

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Synthesis and Characterization of a Potent and Selective Protein Tyrosine Phosphatase Inhibitor, 2-[(4-Methylthiopyridin-2-yl)methylsulfinyl]benzimidazole

Takuya Hamaguchi,[a,*] Akiko Takahashi,[a] Terumi Kagamizono,[a] Akira Manaka,[a] Masakazu Sato[a] and Hiroyuki Osada[b]

[a]*Medical Research Laboratories, Taisho Pharmaceutical Co., Ltd, Yoshino-cho 1-403, Omiya-shi, Saitama 330-8530, Japan*
[b]*Antibiotics Laboratory, The Institute of Physical and Chemical Research (RIKEN), Hirosawa 2-1, Wako-shi, Saitama 351-01, Japan*

Received 24 July 2000; accepted 16 September 2000

**Abstract**—The synthesis and biological activity of a series of 2-[(4-methylthiopyridin-2-yl)methylsulfinyl]benzimidazoles are described. These compounds have potent inhibitory effects against the protein tyrosine phosphatase activity of CD45. Enzymatic analysis with several phosphatases revealed that compound **5a** had high specificity for CD45 compared with serine/threonine phosphatases (PP1, PP2A), tyrosine phosphatases (LAR, PTP1B and PTP-S2) and dual phosphatase (VHR). 

## Introduction

Tyrosine phosphorylation of receptors is a feature of cell activation in a variety of biological systems. Tyrosine phosphorylation level is determined by the balances of activities of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPases). While PTKs have been extensively studied, the characterization of the equally important PTPases has recently been undertaken. For example, CD45 is a typical receptor-type PTPase, which consists of a putative ligand-binding extracellular domain, a transmembrane segment, and an intracellular catalytic region. The intracellular catalytic regions of receptor-type PTPases are highly homologous both to each other and to the non-receptor-type PTPases. A large number of studies have shown that CD45 dephosphorylates the phosphotyrosine of Src-family kinases, permitting these kinases to become activated in T and B cells.[1–5] CD45 is a possible target for drugs in the treatment of several immune diseases.

PTPase-specific inhibitors could potentially serve as useful tools in elucidating the physiological significance of protein tyrosine dephosphorylation in cellular signal transduction pathways. In contrast to tyrosine kinases, elucidation in detail of the roles of PTPase has been hampered by the absence of PTPase-specific agents. Sodium orthovanadate[6] and phenylarsine oxide (PAO)[7] are known to be PTPase inhibitors. However, both compounds have no specificity for PTPases. Therefore, more selective PTPase inhibitors are needed.

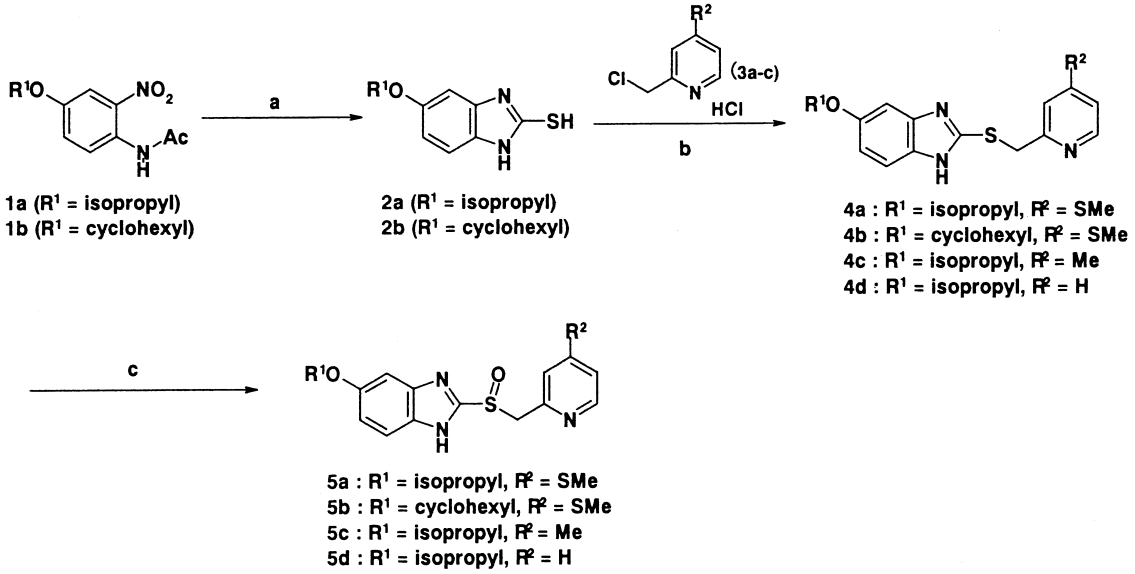
To discover new immunosuppressive reagents, we have been screening new PTPase inhibitors from among both synthetic compounds and microbial metabolites. Previously, we found potent and specific PTPase inhibitors, such as RK-682[8] and the stevastelins.[9] Using them, we evaluated the roles of PTPases in cellular signal transduction. In subsequent screening for PTPase inhibitors, we found benzimidazole derivatives, which exhibited 95% inhibition of human CD45 PTPase activity at 5 μg/mL concentration. A variety of benzimidazole compounds are known, but their PTPase inhibitory effects have not been reported. In addition, these compounds had potent and specific inhibitory effects on the PTPase activity of CD45. In this study, 2-[(4-methylthiopyridin-2-yl)methylsulfinyl]benzimidazoles were synthesized and evaluated for their ability to inhibit the several PTPase activities. Specific inhibitors against CD45 are useful for the treatment of several immune diseases.

## Chemistry

For structure–activity relationship studies, we prepared benzimidazole derivatives. The syntheses of the

*Corresponding author. Tel.: +81-48-663-1111; fax: +81-48-652-7254; e-mail: s14795@ccm.taisho.co.jp

0960-894X/00/$ - see front matter 
PII: S0960-894X(00)00539-4




**Scheme 1.** (a) (i) concd HCl/AcOH, reflux; (ii) $H_2$, 10% Pd/C/EtOH, rt; (iii) $EtOCS_2K/H_2O$, reflux; (b) 2 N NaOH aq/EtOH, 50–90 °C; (c) *m*-chloroperbenzoic acid/$CHCl_3$, −50 °C.

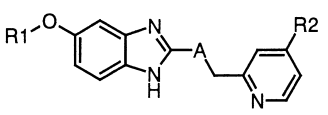
compounds (**4a**, **5a**–**d**) are outlined in Scheme 1. 5-Substituted 2-mercapto-1*H*-benzimidazoles (**2a**, **b**) were synthesized from corresponding acetanilide derivatives (**1a**, **b**) in the previously described manner.[10] 4-Substituted 2-(chloromethyl)pyridines (**3a**–**c**) were purchased or derived from corresponding picoline-1-oxides via Pummerer-like rearrangement and chlorination with thionyl chloride in chloroform. Coupling reactions of **3a**–**c** with **2a** or **2b** were performed in aqueous basic conditions to obtain the sulfenyl derivatives (**4a**–**d**), and continuous selective oxidation of the benzimidazolyl sulfur atom using *m*-chloroperbenzoic acid were subjected to obtain sulfoxides **5a**–**d**.

## Biological Studies

The effects of compounds on CD45 were examined using *p*-nitrophenyl phosphate as a substrate at 37 °C and pH 6.5.[11] $IC_{50}$ values are summarized in Table 1. Compound **5a** had a potent inhibitory effect on CD45 with an $IC_{50}$ value of 0.28 μM. On the other hand, **4a** had no inhibitory effect on CD45 at a concentration of 1000 μM. The substituents at the 5-position and 2-sulfinyl moiety of benzimidazole were required for this inhibitory effect. With a change from the 4-methylthiopyridyl moiety to 4-methylpyridyl **5c** or pyridyl moiety **5d**, inhibitory effects were decreased in a graded fashion. The 4-methylthiopyridyl moiety was required for inhibitory activity.

Protein phosphatases are categorized into three groups:[12] the protein serine/threonine phosphatases (PPases), PTPases and dual-specificity phosphatases (DSPases). These protein phosphatases play important roles in regulating a variety of fundamental cellular processes. Since **5a** was most potent against CD45, we investigated the inhibitory specificity of **5a** against PPases (PP1 and PP2A), PTPases (CD45, LAR, PTP1B and PTP-S2) and DSPase (VHR).[13] As shown in Table 2, **5a** had no inhibitory effect on either PP1 or PP2A activities at a concentration of 1000 μM. On the other hand, it exhibited inhibitory effects on CD45, LAR, PTP1B and PTP-S2 with $IC_{50}$ values of 0.28, 18.2, 55.4 and 55.2 μM, respectively. Sodium orthovanadate, a non-specific PTPase inhibitor, also inhibited only PTPases activities. These findings indicated that **5a** was an inhibitor of PTPases and had potent inhibitory effects on receptor-type PTPases (CD45 and LAR) compared with non-receptor-type PTPase (PTP1B and PTP-S2). Furthermore, **5a** also exhibited a slight inhibition of VHR, with an $IC_{50}$ of 50.2 μM. Since vanadate inhibited CD45 and LAR with $IC_{50}$ values of 34.2 and 30.8 μM, respectively, **5a** had some degree of enzyme specificity for CD45.

**Table 1.** Inhibitory effects on PTPase activity of CD45

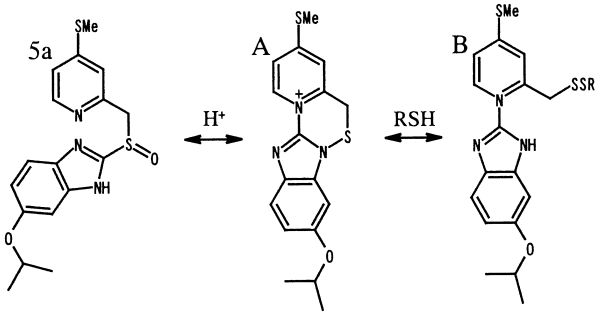



| Compound | R1 | R2 | A | $IC_{50}$ (μM) |
|---|---|---|---|---|
| **4a** | *i*-Propyl | $SCH_3$ | S | >1000 |
| **5a** | *i*-Propyl | $SCH_3$ | S=O | 0.28 |
| **5b** | Cyclohexyl | $SCH_3$ | S=O | 80.5 |
| **5c** | *i*-Propyl | $CH_3$ | S=O | 380 |
| **5d** | *i*-Propyl | H | S=O | 120 |

**Table 2.** Specificity of inhibition by **5a** of several phosphatases

| | Activity $IC_{50}$ (μM) | | | | | | |
|---|---|---|---|---|---|---|---|
| | PPases | | PTPases | | | | DSPase |
| Compound | PP1 | PP2A | CD45 | LAR | PTP1B | PTP-S2 | VHR |
| **5a** | >1000 | >1000 | 0.28 | 18.2 | 55.4 | 55.2 | 50.2 |
| Vanadate | >1000 | >1000 | 34.2 | 30.8 | 30.2 | 38.1 | 105.8 |

The 2-[(2-pyridylmethyl)sulfinyl]benzimidazoles, such as omeprazole, have attracted considerable attention as potential therapeutics for the treatment of peptic ulcer.[14] Some evidence suggests that omeprazole oxidizes SH groups of the ($H^+-K^+$)-ATPase resulting in enzyme inhibition after omeprazole is transformed into the active form by acid.[15,16] This mechanism of action is supported by chemical evidence in that 2-[(2-pyridylmethyl)sulfinyl]benzimidazole is transformed to intermediate cyclic sulfenamides followed by coupling with adequate thiol to form disulfide[17] (Scheme 2).




**Scheme 2.** Possible mechanism for acid-transformation of **5a**. **5a** is transformed into the intermediate cyclic sulfenamide (A), followed by coupling with adequate thiols (RSH) to form the ultimate disulfides (B).

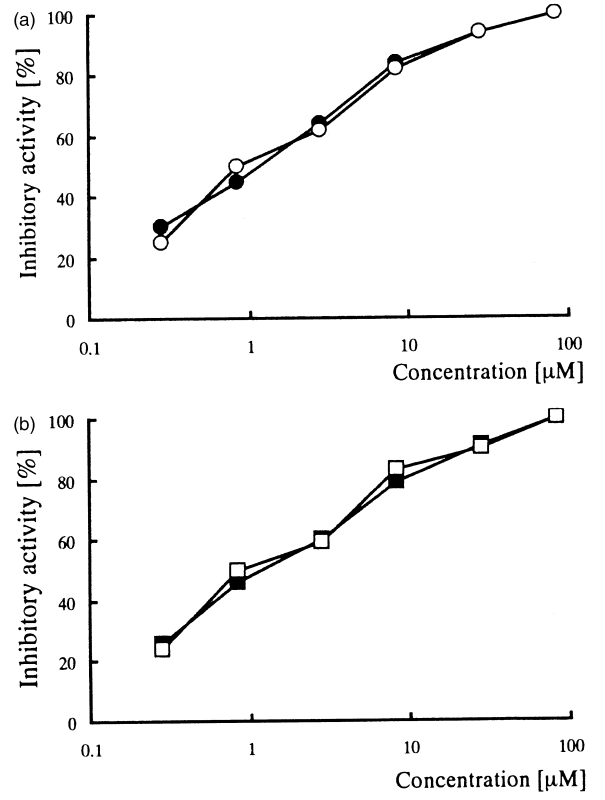



**Figure 1.** Effect of GSH on inhibition by **5a** of PTPase activity of CD45: (a) various concentrations of **5a** were measured with (closed circles) or without (open circles) 10 mM glutathione (GSH) in PTPase assay buffer; (b) **5a** was preincubated in medium (0.1M HCl) at 37 °C for 30 min. Inhibitory effects of aliquots of the medium were measured with (closed squares) or without (open squares) 10 mM GSH in PTPase assay buffer. Results are expressed as a percentage inhibition of control activity.

Without exception, all PTPases contain an active site signature motif, (I/V)HCXAGXGR(S/T)G, which enfolds the catalytic cysteinyl residue.[18] To investigate whether **5a** must be transformed to the active form for showing an inhibitory effect on PTPase, we evaluated the influence of glutathion (GSH) on the inhibitory effect of **5a** against CD45. GSH is an SH-containing compound, which disrupts the binding of omeprazole to ($H^+-K^+$)-ATPase.[19,20] Compound **5a** preincubated in acidic conditions (in 0.1 M HCl) was added to PTPase assay medium with or without 10 mM GSH at 37 °C for 30 min.[11] As shown in Fig. 1a, **5a** inhibited the PTPase activity of CD45 without affecting GSH. In addition, the inhibitory effects of **5a** were the same with or without treatment in acidic conditions (Fig. 1b). We investigated the inhibitory effect of omeprazole on PTPase activity, but found it had no effect up to 1 mM (data not shown). The difference in structures between omeprazole and **5a** is the absence of a 4-methylthiopyridyl group in the former. These findings indicated that the mechanism of inhibition by **5a** did not involve formation of a disulfide bond between the intermediate cyclic sulfenamide and the catalytic cysteinyl residue of PTPase.

CD45 is a transmembrane PTPase expressed on most hematopoietic cells. The exact function of CD45 in lymphocyte activation is currently under intense investigation in many laboratories. Accumulating evidence has shown that CD45 is an essential component of receptor signaling pathways, since it dephosphorylates the negative regulatory tyrosine residues of Src-family kinases. In T cell activation, the PTPase activity of CD45 activates Lck by dephosphorylation of a C-terminal tyrosine residue.[5] In immature B cells, another Src family PTK, Fyn, appears to be a selective substrate for CD45, compared with Lck and Syk.[21] Some studies have reported that CD45 is necessary for activation of FcεRI-associated Lyn,[22] the dephosphorylation of receptor subunits,[23] and the initiation of calcium influx in mast cell activation.[24] Since **5a** suppressed histamine release from rat peritoneal mast cells and mouse passive-sensitized anaphylaxis reaction induced by monoclonal anti-DNP IgE and DNP-BSA (Hamaguchi et al., unpublished data), CD45 may be involved in these processes. These results indicate that PTPase inhibitors are useful in the treatment of allergies.

## References and Notes

1. Eiseman, E.; Bolen, J. B. *Nature* **1992**, *355*, 78.
2. Chan, A. C.; Desai, D. M.; Weiss, A. *Annu. Rev. Immunol.* **1994**, *12*, 555.
3. Cahir McFarland, E. D.; Hurley, T. R.; Pingel, J. T.; Sefton, B. M.; Shaw, A.; Thomas, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1402.
4. Koretzky, G. K. *FASEB J.* **1993**, *7*, 420.
5. Trowbridge, I. S.; Thomas, M. L. *Annu. Rev. Immunol.* **1994**, *12*, 85.
6. 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.

7. Garcia-Morales, P.; Minami, Y.; Luong, E.; Klausner, R. D.; Samelson, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9255.
8. Hamaguchi, T.; Sudo, T.; Osada, H. *FEBS Lett.* **1995**, *372*, 54.
9. Hamaguchi, T.; Masuda, A.; Morino, T.; Osada, H. *Chem. Biol.* **1997**, *4*, 279.
10. Kühler, T. C.; Swanson, M.; Shcherbuchin, V.; Larsson, H.; Mellgård, B.; Sjöström, J.-E. *J. Med. Chem.* **1998**, *41*, 1777.
11. Phosphatase assay. Assays of phosphatase activity were performed in a final volume of 105 μL, comprising 50 μL of enzyme solution, 50 μL of substrate solution, and 5 μL of inhibitor solution. Dephosphorylation of 1 mM *p*-nitrophenylphosphate (*p*Npp) by CD45 (Calbiochem-Novabiochem Co.), LAR (Calbiochem-Novabiochem Co.) and PTP1B (Upstate Biotechnology Inc., Lake Placid, NY) was measured in an assay buffer containing 25 mM MOPS, pH 6.5, 5 mM EDTA, and 1 mM dithiothreitol. Dephosphorylation of 1 mM *p*Npp by PP1 (Upstate Biotechnology Inc.) and PP2A (Upstate Biotechnology Inc.) was measured in an assay buffer containing 20 mM MOPS, pH 7.5, 60 mM 2-mercaptoethanol, 0.1 M NaCl, 1 mg/mL serum albumin, and 50% (v/v) glycerol. Bacterially-expressed glutathione-*S*-transferase (GST)-VHR fusion protein was prepared as described in ref 15. Dephosphorylation of 1 mM *p*Npp by GST-VHR was measured in an assay buffer containing 25 mM MOPS, pH 6.5, 5 mM EDTA, 1 mM dithiothreitol. In tests of enzyme inhibition, the reaction mixture contained various concentrations of compound in the range of 30 nM–1000 μM. The mixture containing CD45 (5 μg/mL), LAR (5 μg/mL), PTP1B (1 μg/mL), PP1 (1 μg/mL), PP2A (1 μg/mL), PTP-S2 (1 μg/mL) or VHR (1 μg/mL) was incubated with compound and 1 mM *p*Npp for 30 min at 37 °C. Then 20 μL of 1 N NaOH was added to measure absorbance at 405 nm. The test compound concentration producing 50% inhibition of phosphatase activity ($IC_{50}$ value) was determined by use of a nonlinear regression curve-fitting program. For investigation of the influence of glutathione (GSH) on the inhibitory effect of **5a**, compound was preincubated in the medium (0.1 M HCl) at 37 °C for 30 min. Inhibitory effects of aliquots of the medium were measured in PTPase assay buffer containing 10 mM GSH. Each value represents the averages of three independent experiments.

12. Fauman, E. B.; Saper, M. A. *Trends Biochem. Sci.* **1996**, *21*, 413.
13. Ishibashi, T.; Bottaro, D. P.; Chan, A.; Miki, T.; Aaronson, S. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 12170.
14. Fellenius, E.; Berglindh, T.; Sachs, G.; Olbe, L.; Elander, B.; Sjostrand, S. E.; Wallmark, B. *Nature* **1981**, *290*, 159.
15. Wallmark, B.; Brändström, A.; Larsson, H. *Biochem. Biophys. Acta* **1984**, *778*, 549.
16. Im, W. B.; Sih, J. C.; Blakeman, D. P.; McGrath, J. P. *J. Biol. Chem.* **1985**, *260*, 4591.
17. Sturm, E.; Krüger, U.; Senn-Bilfinger, J.; Figala, V.; Klemm, K.; Kohl, B.; Rainer, G.; Schaefer, H.; Blake, T. J.; Darkin, D. W.; Ife, R. J.; Leach, C. A.; Mitchell, R. C.; Pepper, E. S.; Salter, C. J.; Viney, N. J.; Huttner, G.; Zsolnai, L. *J. Org. Chem.* **1987**, *52*, 4573.
18. Walton, K. M.; Dixon, J. E. *Annu. Rev. Biochem.* **1993**, *62*, 101.
19. Krüger, U.; Senn-Bilfinger, J.; Sturm, E.; Figala, V.; Klemm, K.; Kohl, B.; Rainer, G.; Schaefer, H.; Blake, T. J.; Darkin, D. W.; Ife, R. J.; Leach, C. A.; Mitchell, R. C.; Pepper, E. S.; Salter, C. J.; Viney, N. J. *J. Org. Chem.* **1990**, *55*, 4163.
20. Nagaya, H.; Satoh, H.; Kubo, K.; Maki, Y. *J. Pharm. Exp. Ther.* **1989**, *248*, 799.
21. Charbonneau, H.; Tonks, N. K. *Annu. Rev. Cell Biol.* **1992**, *8*, 463.
22. Murakami, K.; Sato, S.; Nagasawa, S.; Yamashita, T. *Int. Immunol.* **2000**, *12*, 169.
23. Adamczewski, M.; Numerof, R. P.; Koretzky, G. A.; Kinet, J.-P. *J. Immunol.* **1995**, *154*, 3047.
24. Adamczewski, M.; Paolini, R.; Kinet, J.-P. *J. Biol. Chem.* **1992**, *267*, 18126.