Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 1039–1042

The development of potent non-peptidic PTP-1B inhibitors

Claude Dufresne,[a,*] Patrick Roy,[a] Zhaoyin Wang,[a] Ernest Asante-Appiah,[b] Wanda Cromlish,[b] Yves Boie,[b] Farnaz Forghani,[b] Sylvie Desmarais,[b] Qingping Wang,[b] Kathryn Skorey,[b] Deena Waddleton,[b] Chidambaram Ramachandran,[b] Brian P. Kennedy,[b] Lijing Xu,[c] Robert Gordon,[c] Chi Chung Chan[c] and Yves Leblanc[a,*]

[a]*Department of Medicinal Chemistry, Merck Frosst Centre for Therapeutic Research, PO Box 1005, Pointe-Claire, Dorval, Canada H9R 4P8*
[b]*Department of Biochemistry and Molecular Biology, Merck Frosst Centre for Therapeutic Research, PO Box 1005, Pointe-Claire, Dorval, Canada H9R 4P8*
[c]*Department of Pharmacology, Merck Frosst Centre for Therapeutic Research, PO Box 1005, Pointe-Claire, Dorval, Canada H9R 4P8*

Received 16 May 2003; accepted 14 November 2003

Abstract—The SAR from our peptide libraries was exploited to design a series of potent deoxybenzoin PTP-1B inhibitors. The introduction of an *ortho* bromo substituent next to the difluoromethylphosphonate warhead gave up to 20-fold increase in potency compared to the desbromo analogues. In addition, these compounds were orally bioavailable and active in the animal models of non-insulin dependent diabetes mellitus (NIDDM).

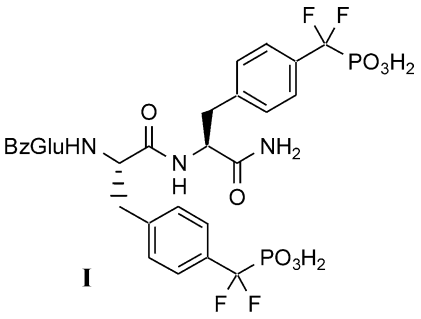
When insulin binds to the insulin receptor (IR), the intrinsic tyrosine kinase activity of the β-subunit becomes activated. This results in autophosphorylation of critical tyrosine residues in the regulatory domain which fully activates the tyrosine kinase of the receptor. The insulin receptor (IR) is then capable of phosphorylating its various substrates to propagate the insulin signal.[1] For the past 10 years since the identification of the protein tyrosine phosphatases (PTPs), it has been speculated that a specific PTP is involved in the dephosphorylation and inactivation of the insulin receptor, thus attenuating insulin signaling.[2] It has also been hypothesized that disequilibrium between the insulin receptor and the PTP could be a contributing factor to the insulin resistance observed in type 2 or non-insulin dependent diabetes mellitus (NIDDM).[3] Inhibition of the PTP specific for the insulin receptor would be beneficial in the treatment of this disease as it would result in maintenance of an activated insulin receptor and hence prolong insulin signaling.

PTP-1B is an intracellular PTP that has been implicated in the negative regulation of insulin signaling. Recently, we and others have shown that mice lacking PTP-1B have enhanced insulin sensitivity.[4,5] These mice require only half the amount of insulin to maintain normal blood glucose in the fed state. They also show increased sensitivity in glucose and insulin tolerance tests. In addition, animal models treated with the specific PTP-1B anti-sense oligonucleotide ISIS 113715 show normalization of both blood glucose and insulin levels.[6] Thus, inhibitors of PTP-1B may enhance insulin sensitivity, making them ideal for the treatment of type 2 diabetes.

Heretofore, a few research groups have shown interest in developing PTP-1B inhibitors.[7–16] PTPs have a high affinity for phosphotyrosine. As for PTP-1B inhibitors, there was an interest to develop compounds containing non-hydrolyzable phosphate mimetics. Burke et al. has shown that difluorophosphonomethyl-phenylalanine or (F_2PMP) is a mimetic of phosphotyrosine with similar binding affinity.[17] Our research group has embarked on the synthesis of peptide libraries leading to the discovery of peptide **I**.

* Corresponding author. Tel.: +1-514-428-3037; fax: +1-514-428-4900; e-mail: claude_1_dufresne@merck.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.11.048



I

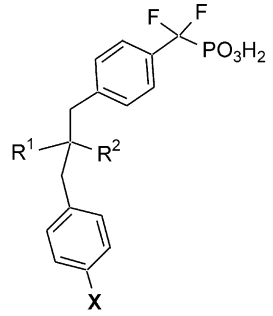
The X-ray crystal structure of peptide **I** in complex with PTP-1B shows that the C-terminal (F_2PMP) is located in the active site while the second phosphonic group interacts with R 47.[20] Also, the SAR results from these peptide libraries has revealed that monophosphonate-containing peptide inhibitors can be equipotent to bisphosphonate inhibitors.[21a]

Surprisingly, even though these peptidic inhibitors are highly charged molecules they also show cellular activity. For example the tripeptide **I** has an IC_{50} of 200 nM in an Sf9 cell-based assay.[21b]

With cell permeable peptide inhibitors in hand, we then decided to investigate the in vivo efficacy of peptide **I** in animal models. Despite its short half-life (<30 min), **I** was found to reduce the AUC in ob/ob mice by 40% in the oral glucose tolerance test[22] following a single IP dose (10 mg/kg, 30 min pre-treatment). This result was consistent with the observation made in the knockout (KO) mice and established the proof of concept for the hypothesis that PTP-1B inhibitors will prolong insulin signaling. At this point our efforts became focused on the design of low molecular weight, non-peptidic inhibitors based on the peptide SAR and structural information from the enzyme–peptide **I** complex.[20] Malonate esters (**1**) was chosen to be the first template to replace the peptide backbone. Alkylation with a benzyl bromide such as (**2**) and (**3**) was achieved as demonstrated in Scheme 1, to provide (**4**).

This approach has produced potent, selective (except for TC-PTP)[19] and reversible PTP-1B inhibitors as exemplified in Table 1 with entry **1**–**8**. The most potent bisphosphonate inhibitors are entry **2** and entry **3** based on their potency in the FDP assay and the Sf9 cell-based assay.[21b] Of the monophosphonates, entry **6** was identified to be the most potent inhibitor with an IC_{50} of 40 nM in FDP assay[18–21] and an IC_{50} of 1.9 μM in Sf9 cell-based assay thereby supporting our previous observation in the peptide series that monophosphonate inhibitors can be equipotent to bis-phosphonate inhibitors. Unfortunately, the malonate esters were predicted to be unstable to blood esterases presumably due to hydrolysis of the ester group resulting in the poor pharmacokinetics in rats when dosed PO or IV. Thus, we have turned our attention to deoxybenzoin as a possible template to introduce the phosphonate warhead (Fig. 1). Deoxybenzoin (**5**) (Scheme 2) was deprotonated with sodium hydride or potassium *tert*-butoxide and alkylated with benzyl bromide (**6**) and (**7**) giving the tetrapode (**8**). This

Table 1. In vitro potency data[18–21] for **1**–**8**



Entry	R^1	R^2	X	(IC_{50} μM)		
				FDP assay		
				PTP-1B	CD-45	Sf9 (PTP-1B)
1	CO_2Bn	CO_2Me	$CF_2PO_3H_2$	0.19	>50.0	1.00
2	CO_2Bn	CO_2Bn	$CF_2PO_3H_2$	0.06	19.0	0.54
3	CO_2iPr	CO_2iPr	$CF_2PO_3H_2$	0.11	>50.0	1.65
4	CO_2Me	CO_2H	$CF_2PO_3H_2$	0.39	>50.0	3.80
5	CH_2OBn	CH_2OBn	$CF_2PO_3H_2$	0.16	>50.0	1.30
6	CO_2Bn	CO_2Bn	CF_2CO_2H	0.04	>50.0	1.90
7	CO_2Bn	CO_2Bn	SO_2NHAc	0.09	25.0	1.70
8	CO_2Bn	CO_2Bn	SO_2Me	0.94	>50.0	7.20

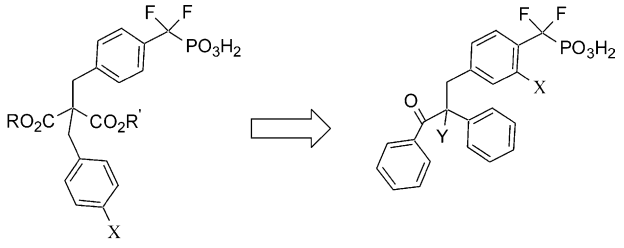
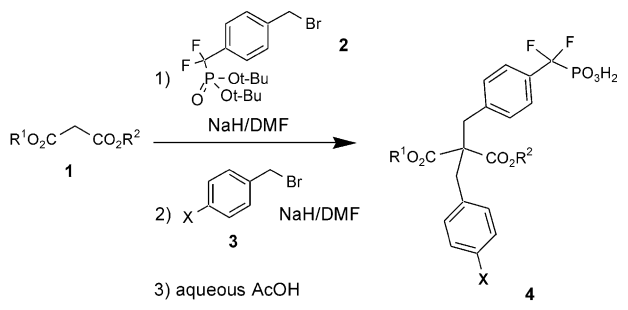
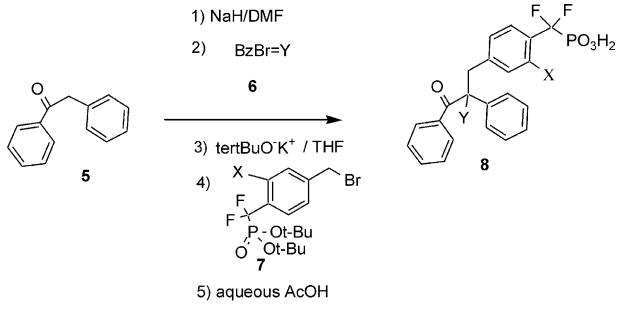


Figure 1.



Scheme 1.

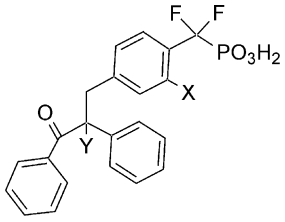


Scheme 2.

series has provided potent inhibitors as shown in Table 2, such as entry **9**. Entry **9** has an IC_{50} of 60 nM in the FDP assay and 0.58 nM in the Sf9 cell based assay. As found for peptide **I**, a statistically significant glucose lowering effect was attained in animals. A 43% AUC reduction at 10 mg/kg I.P. dosing in *ob*/*ob* mice and a 63% AUC reduction at 20 mg/kg I.P. dosing in *fa*/*fa* rat models (Table 3)[22,23] was observed.

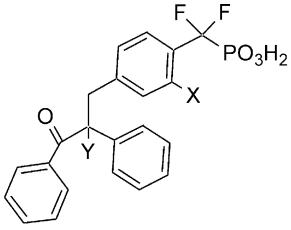
The next step was to develop orally bioavailable inhibitors. We decided to further optimize the deoxybenzoin series. The monophosphonic analogue of entry **9**, entry **13** was found to have good bioavailability in rats. This compound, however, was found to be poorly active in the enzyme assay with an IC_{50} of 2 μM (Table 2). We had observed that the introduction of a halogen *ortho* to the difluoromethylphosphonate group had the ability to increase inhibitor potency. For example entry **15** is more potent than the desbromo analogue entry **13** by a factor of 20. The tripode **15** containing the *ortho* bromo warhead was prepared using Scheme 2 with deoxybenzoin (**5**) and benzyl intermediate (**12**) (Scheme 3)

Table 2. In vitro potency data[18–21] for **9**–**16**

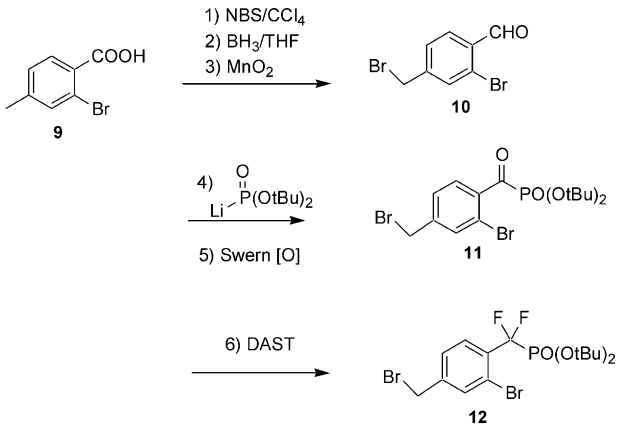


Entry	X	Y	(IC_{50} μM)		
			FDP assay		
			PTP-1B	CD-45	Sf9 (PTP-1B)
9	H	*p*-$CF_2PO_3H_2$benzyl	0.06	>50.0	0.58
10	H	*p*-$CF_2PO_3H_2$benzyl	0.19	>50.0	1.50
11	H	*p*-CO_2Hbenzyl	0.80	>50.0	6.63
12	H	Cinnamyl	0.19	5.50	2.00
13	H	H	2.00	>50.0	16.0
14	Cl	H	0.36	>50.0	2.40
15	Br	H	0.12	>50.0	1.22
16	Ph	H	16.0	>50.0	NA

Table 3. In vivo efficacy data[22,23] for **9** and **15**



Entry	X Y	X Y
	9: H 4-$CF_2PO_3H_2PhCH_2$	**15**: Br H
DIO Mice	43% reduction of AUC at 10 mg/kg IP	50% reduction of AUC at 10 mg/kg PO
Zucker rats	63% reduction of AUC at 20 mg/kg IP	50% reduction of AUC at 30 mg/kg PO



Scheme 3.

which in turn was prepared from 2-bromo-4-methyl benzoic acid (**9**). The benzoic acid (**9**) was transformed in 3 steps to benzaldehyde (**10**). Reaction of **10** with the lithium salt of diethyl phosphite followed by a Swern oxidation gave ketophosphonate (**11**). The last step required the reaction of **11** with DAST to give diethyl[2-bromo-4-(bromomethyl) phenyl] (difluoro) methylphosphonate (**12**).

The *ortho* bromo analogue entry **15** was found to be orally bioavailable ($F = 13\%$) in rats with a C_{max} of 35 μM. In the oral glucose tolerance test (OGTT) in *fa*/*fa* Zucker rats, a reduction of AUC by 50% was observed after a single PO dose of 30 mg/kg. A similar effect was observed in diet induced obesity mice (DIO mice) where the AUC decreased by 50% at a dose of 10 mg/kg (Table 3).

In summary, we have reported the design and the development of a series of deoxybenzoin PTP-1B inhibitors. The introduction of a bromine atom *ortho* to the warhead has allowed the preparation of potent analogues such as entry **15**. This inhibitor contains a single phosphonate group and showed an increase of potency by a factor of 20 compared to the desbromo analogue. This PTP-1B inhibitor **15** is both bioavailable and active in the animal models of NIDDM. The present work has served as a platform to elaborate very potent PTP-1B inhibitors using structure-based design.[24]

References and notes

1. Evans, J. L.; Jallal, B. *Exp. Opin. Invest. Drugs* **1999**, *8*, 139.
2. (a) Vague, P.; Raccah, D. *Horm. Res.* **1992**, *38*, 28. (b) Taylor, S. I.; Accili, D.; Imai, Y. *Diabetes* **1994**, *43*, 735. (c) Reaven, G. M. *Diabetes/Metab. Rev.* **1993**, *9* (Supp. 1), 5 S.
3. Kahn, C. R. *Diabetes* **1994**, *43*, 1066.
4. 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.
5. Klaman, L. D.; Boss, O.; Peroni, O. D.; Kim, J. K.; Martino, J. L.; Zabolotny, J. M.; Moghal, N.; Lubkin,

M.; Kim, Y. B.; Sharpe, A. H.; Stricker-Krongrad, A.; Shulman, G. I.; Neel, B. G.; Kahn, B. B. *Mol. Cell. Biol.* **2000**, *20*, 5479.
6. 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. *PNAS* **2002**, *99*, 11357.
7. Taing, M.; Keng, Y.-F.; Shen, K.; Wu, L.; Lawrence, D. S.; Zhang, Z.-Y. *Biochemistry* **1999**, *38*, 2793.
8. Andersen, H. S.; Iversen, L. F.; Jeppesen, C. B.; Branner, S.; Norris, K.; Rasmussen, H. B.; Moller, K. B.; Møller, N. P. *J. Biol. Chem.* **2000**, *275*, 7101.
9. Bleasdale, J. E.; Ogg, D.; Palazuk, B. J.; Jacob, C. S.; Swanson, M. L.; Wang, X. Y.; Thompson, D. P.; Conradi, R. A.; Mathews, W. R.; Laborde, A. L.; Stuchly, C. W.; Heijbel, A.; Bergdahl, K.; Bannow, C. A.; Smith, C. W.; Svensson, C.; Liljebris, C.; Schostarez, H. J.; May, P. D.; Stevens, F. C.; Larsen, S. D. *Biochemistry* **2001**, *40*, 5642.
10. Kotoris, C. C.; Chen, M. J.; Taylor, S. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3275.
11. Larsen, S. D.; Barf, T.; Liljebris, C.; May, P. D.; Ogg, D.; O'Sullivan, T. J.; Palazuk, B. J.; Schostarez, H. J.; Stevens, F. C.; Bleasdale, J. E. *J. Med. Chem.* **2002**, *45*, 598.
12. Taylor, S. D.; Kotoris, C. C.; Dinaut, A. N.; Wang, Q.; Ramachandran, C.; Huang, Z. *Bioorg. Med. Chem. Lett.* **1998**, *6*, 1457.
13. Wang, Q.; Huang, Z.; Ramachandran, C.; Dinaut, A. N.; Taylor, S. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 345.
14. Wrobel, J.; Sredy, J.; Moxham, C.; Dietrich, A.; Li, Z.; Sawicki, D. R.; Seestaller, L.; Wu, L.; Katz, A.; Sullivan, D.; Tio, C.; Zhang, Z.-Y. *J. Med. Chem.* **1999**, *42*, 3199.
15. Xian, M.; Wang, K.; Chen, X.; Hou, Y.; McGill, A.; Chen, X.; Zhou, B.; Zhang, Z.-Y.; Cheng, J.-P.; Wang, P. G. *Biochem. Biophys. Res. Commun.* **2000**, *268*, 310.
16. Wrobel, J.; Li, Z.; Sredy, J.; Sawicki, D. R.; Seestaller, L.; Sullivan, D. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1535.
17. Burke, T. R. J.; Kole, H. K.; Roller, P. P. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 129.
18. Desmarais, S.; Wang, Q.; Ly, H.; Scheigetz, J.; Govindarajan, A.; Ramachandran, C.; Zamboni, R.; Huang, Z. *J. Biomolecular Screening* **1999**, *4*, 327.
19. Asante-Appiah, E.; Ball, K.; Bateman, K.; Skorey, K.; Friesen, R.; Desponts, C.; Payette, P.; Bayly, C.; Zamboni, R.; Scapin, G.; Ramachandran, C.; Kennedy, B. P. *J. Biol. Chem.* **2001**, *276*, 26036.
20. (a) Desmarais, S.; Friesen, R. W.; Zamboni, R.; Ramachandran, C. *Biochem. J.* **1999**, *337*, 219. (b) Skorey, K. I.; Kennedy, B. P.; Friesen, R. W.; Ramachandran, C. *Anal. Biochem.* **2001**, *291*, 269. (c) Asante-Appiah, E.; Patel, S.; Dufresne, C.; Roy, P.; Wang, Q.; Patel, V.; Friesen, R. W.; Ramachandran, C.; Becker, J. W.; Leblanc, Y.; Kennedy, B. P.; Scapin, G. *Biochem.* **2002**, *41*, 9043.
21. (a) Unpublished results. (b) Cromish, W. A.; Payette, P.; Kennedy, B. *Biochem. Pharmacology* **1999**, *58*, 1539.
22. Coleman, D. L.; Hummel, K. P. *Diabetologia* **1967**, *3*, 238.
23. Peterson, R. G.; Shaw, W. N.; Neel, M.; Little, L. A.; Eichberg, J. *Inst. Lab. Animal Res. News* **1990**, *32*, 16.
24. Lau, C. K.; Bayly, C. I.; Gauthier, J. Y.; Li, C. S.; Therien, M.; Asante-Appiah, E.; Cromlish, W.; Boie, Y.; Forghani, F.; Desmarais, S.; Wang, Q.; Skorey, K.; Waddleton, D.; Payette, P.; Ramachandran, C.; Kennedy, B. P.; Scapin, G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, following paper in this issue. doi:10.1016/j.bmcl.2003.11.076.