Design, Synthesis and Docking Study of 5-(Substituted Benzylidene)Thiazolidine-2,4-dione Derivatives as Inhibitors of Protein Tyrosine Phosphatase 1B

Zengtao Wang, Zhiguo Liu, Woojung Lee, Su-Nam Kim, Goo Yoon, Seung Hoon Cheon

PII: S0960-894X(14)00608-8
DOI: http://dx.doi.org/10.1016/j.bmcl.2014.05.099
Reference: BMCL 21713

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 16 April 2014
Revised Date: 26 May 2014
Accepted Date: 29 May 2014

Please cite this article as: Wang, Z., Liu, Z., Lee, W., Kim, S-N., Yoon, G., Cheon, S.H., Design, Synthesis and Docking Study of 5-(Substituted Benzylidene)Thiazolidine-2,4-dione Derivatives as Inhibitors of Protein Tyrosine Phosphatase 1B, *Bioorganic & Medicinal Chemistry Letters* (2014), doi: http://dx.doi.org/10.1016/j.bmcl.2014.05.099

Design, Synthesis and Docking Study of 5-(Substituted Benzylidene)Thiazolidine-2,4-dione Derivatives as Inhibitors of Protein Tyrosine Phosphatase 1B

Zengtao Wang [a], Zhiguo Liu[b], Woojung Lee [c], Su-Nam Kim [c], Goo Yoon[d], and Seung Hoon Cheon [a, *]

[a] College of Pharmacy and Research Institute of Drug Development, Chonnam National University; Gwangju 500-757, Korea

[b] School of Pharmacy, Wenzhou Medical College, 1210 University Town, Wenzhou, Zhejiang 325035, China, and Wenzhou Undersun Biotechnology Co., Ltd., Wenzhou, Zhejiang, China

[c] KIST Gangneung Institute, Gangneung 210-340, Korea

[d] College of Pharmacy and Natural Medicine Research Institute, Mokpo National University, Jeonnam 534-729, Korea.

Corresponding author shcheon@chonnam.ac.kr, Tel: +82625302929, Fax: +82625302911

Abstract

A series of novel 5-(substituted benzylidene)thiazolidine-2,4-dione derivatives was designed, and synthesized based on our previous studies. Also their activities were evaluated as competitive inhibitors of protein tyrosine phosphatase 1B (PTP1B). Compounds **6d-6g**, **7b**, **7c**, **7e**, **7j**, **7k**, **7m**, **14b** and **14e-14f** showed potent inhibitory effects against PTP1B, and compound **7e**, the most potent among the series, had an IC_{50} of 4.6 μM. Also a Surflex-Dock docking model of **7e** was studied. Compound **7e** showed a negative binding energy of −7.35 kcal/mol and a high affinity to PTP1B residues (Gly220, Ala217, Arg221, Asp181, Ser216, Cys215, Phe182, Gln262 and Ile219) in the active sites, indicating that it may stabilize the open form and generate tighter binding to the catalytic sites of PTP1B.

Key words: thiazolidinediones, PTP1B, synthesis, docking study

The term diabetes mellitus (DM) is used to simply describe a heterogeneous group of metabolic disorders characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism.[1] It is often accompanied by characteristic long-term complications such as retinopathy, nephropathy and neuropathy.[2] DM is more prevalent than ever, particularly in developing countries. More than 285 million people suffer from DM and this number is expected to rise to 439 million cases by 2030.[3] The most common form of DM is type 2, accounting for 90 to 95 percent of all diabetes cases, which is characterized by combining insulin resistance and lacking compensatory

∗ Corresponding author. Tel.: +82-62-530-2929; fax: +82-62-530-2911; e-mail: shcheon@chonnam.ac.kr

response to sufficiently create insulin, resulting from a failure of pancreatic islet β-cells.[4,5] In addition**,** the prevalence of type 2 diabetes mellitus (T2DM) is escalating, mainly due to the rapidly increasing incidence of overweight and obesity. More than 80 percent of people with T2DM show overweight and it has become a recognized risk factor for T2DM.[6-8] More importantly, T2DM is a leading cause of early death, heart disease, stroke, kidney disease and blindness.[9]

These warning events present grave threats to our health and quality of life and have incited a search for pharmacological agents capable of inhibiting the negative regulators of the insulin signaling pathways and/or potentiating the action of insulin. Mounting data from cellular, biochemical, mouse and human genetic and chemical inhibitor studies have strongly implicated protein tyrosine phosphatase 1B (PTP1B) as a key negative regulator of both, insulin and leptin signaling. PTP1B knockout mice exhibited improved insulin sensitivity with reduced plasma glucose and insulin levels.[10,11] Furthermore, these mice have lower adiposity and are resistant to weight gain. Thus, these studies provided a strong insight into developing inhibitors of PTP1B to treat diabetes and obesity.

We have been searching for a lead compound from natural products as a potential preclinical candidate to treat T2DM for the past few years and reported that licochalcone A and licochalcone E showed good PTP1B inhibitory activities.[12] In addition, the results of structure–activity relationship study of retrochalcones related to licochalcone E have been reported recently.[13] With our continuing interests in retrochalcones, the introduction of thiazolidinedione ring instead of a benzoyl group in retrochalcones, gave compounds with potent inhibitory activities (Figure 1). For example, compound **6b** showed IC_{50} value of 11.3 μM. Although some PTP1B inhibitors containing thiazolidine group have been reported,[14-16] most of them incorporate a large aromatic substituent or charged phosphor tyrosine (pTyr) mimetic group such as phosphonates, carboxylic acids and sulfamic acids which have proven a lack of cell membrane permeability and oral bioavailability because of the strong negative charge carried by the pTyr mimetics as well as their high molecular weight.[17] The nature of the highly charged active site and the relatively shallow of the surrounding protein surface of PTP1B provide a great challenge to medicinal chemists for the discovery of cell permeable and orally bio-available PTP1B inhibitors. Hence, there is an urgent need to develop small molecule PTP1B inhibitors devoid of any charged moieties and with good inhibitory activities that can combat T2DM.

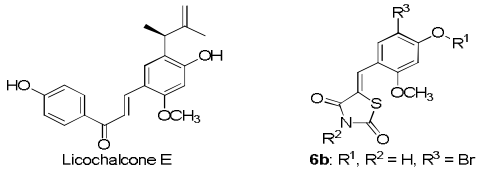
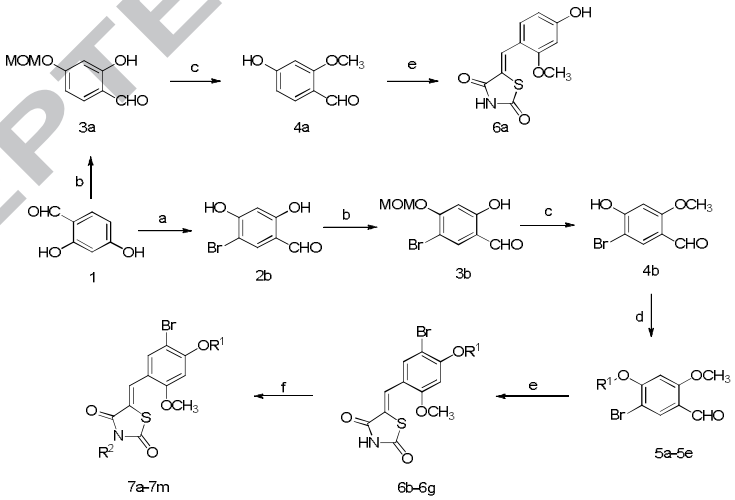


Figure 1. Structures of licochalcone E and **6b**

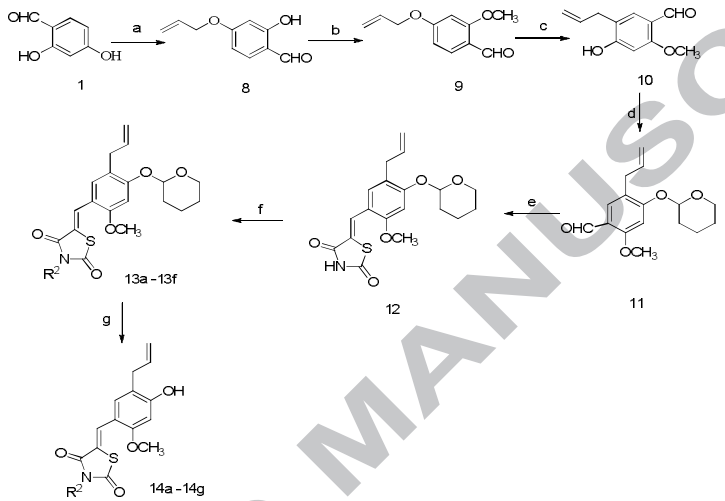
Herein we report the results of synthesis, PTP1B inhibitory activities, structure-activity relationship (SAR) and docking study of 5-(substituted benzylidene)thiazolidine-2,4-dione derivatives as a part of our efforts to discover novel non-charged small molecule PTP1B**.**

The synthesis of compounds **6a-6g** and **7a-7m** from 2,4-dihydroxybenzaldehyde **1** was carried out following the procedure depicted in Scheme 1. The bromination of **1** followed by selective MOM protection of 4-hydroxyl group provided compound **3b**, then methylated with MeI/K_2CO_3, deprotected with concentrated hydrochloric acid in methanol to form the aldehyde **4b**, which in turn reacted with correspond halides to afford intermediates **5a-5e**. The selective MOM protection of 4-phenol in compound **1** and then the methylation of 2-phenol with methyl iodide followed by acid hydrolysis of MOM yielded the compound **4a**. Compounds **6a-6g** were synthesized using Knoevenagel condensation with 2,4-thiazolidinedione and suitable benzaldehydes (**4a**, **5a-5e**) in refluxing toluene in the presence of piperidinium acetate.[18] The proper substitution at the nitrogen atom of the thiazolidinedione ring of **6b-6g** gave compounds **7a-7m**.



Scheme 1. Reagents and Conditions: (a) Br_2, acetic acid, rt, 84%; (b) MOMCl, K_2CO_3, acetone, rt, 86%; (c) CH_3I, K_2CO_3, acetone, rt, 16 h; 6N-HCl, rt, 95%; (d) R^1X, K_2CO_3, acetone, rt, 61-91%; (e) thiazolidine-2,4-dione, piperidinium acetate, toluene, reflux, 59-86%; (f) R^2X, K_2CO_3, acetone, reflux, 60-91%.

The synthesis of compounds **13a-13f** and **14a-14g** was carried out following the procedure depicted in Scheme 2. *O*-Allylphenol **10** was readily prepared in a three-step sequence from allyl bromide and 2,4-dihydroxybenzaldehyde via allylation of phenol **1** followed by methylation to **9** and subsequent Claisen rearrangement. Compound **12** was obtained via Knoevenagel condensation of the commercially available 2,4-thiazolidinedione and the aldehyde **11**. Treatment of **12** with suitable alkyl halides in the presence of potassium carbonate afforded compounds **13a-13f**. The removal of the THP under acidic conditions finally gave the compounds **14a-14g**.



Scheme 2. Reagent and Conditions: (a) Allyl bromide, K_2CO_3, acetone, rt, 79%; (b) CH_3I, NaI, K_2CO_3, acetone, rt, 90%; (c) 200°C, 24 hours, 39%; (d) 3,4-Dihydro-2*H*-pyran, PPTS, CH_2Cl_2, rt, 75%; (e) Thiazolidine-2,4-dione, piperidinium acetate, toluene, reflux, 78%; (f) R^2X, K_2CO_3, acetone, reflux, 62-89%; (g) 6N-HCl, rt, 37-65%.

The introduction of 5-arylidene moiety provided only Z isomers, as already demonstrated by X-ray diffraction studies.[19,20] The ^{1}H NMR spectra showed only one signal attributable to the resonance of the 5-methylidene proton in the range 7.81-8.24 ppm. In their ^{13}C NMR spectra, the 5-methylidene carbon and C5 of the thiazolidinedione ring resonated in the ranges 131.94-139.04 and 116.12-122.39 ppm, respectively. Spectroscopic data (^{1}H, ^{13}C NMR and IR) confirmed the structures assigned to compounds **6**-**7** and **12**-**14**.

Table1：Structures and inhibition of PTP1B of thiazolidinedione derivatives **6a**-**14g**[a]

Comp.	R^1	R^2	R^3	IC_{50} (μM)[a]	Comp.	R^1	R^2	R^3	IC_{50} (μM)[b]
6a	H	H	H	29.8	7l	Bu	Bu	Br	>30
6b	H	H	Br	11.3	7m	Bu	Bn	Br	6.6
6c	Me	H	Br	14.2	12	THP	H	Allyl	12.2
6d	Allyl	H	Br	8.1	13a	THP	Me	Allyl	>30

6e	Prenyl	H	Br	5.3	13b	THP	Isopropyl	Allyl	>30
6f	Bu	H	Br	4.9	13c	THP	Allyl	Allyl	>30
6g	Bn	H	Br	7.6	13d	THP	Prenyl	Allyl	22.7
7a	Me	Me	Br	22.3	13e	THP	Bu	Allyl	20.6
7b	Bn	Me	Br	8.1	13f	THP	Bn	Allyl	>30
7c	Prenyl	Me	Br	8.2	14a	H	Me	Allyl	>30
7d	Prenyl	Allyl	Br	17.0	14b	H	Isopropyl	Allyl	6.4
7e	Prenyl	Isopropyl	Br	4.6	14c	H	Allyl	Allyl	>30
7f	Prenyl	Bu	Br	12.3	14d	H	Prenyl	Allyl	12.0
7g	Prenyl	Prenyl	Br	10.0	14e	H	Bu	Allyl	9.6
7h	Prenyl	Bn	Br	10.3	14f	H	Bn	Allyl	7.2
7i	Bu	Me	Br	12.1	14g	H	H	Allyl	29.3
7j	Bu	Allyl	Br	7.9	Ursolic acid[c]				4.0
7k	Bu	Isopropyl	Br	9.7					

[a] General formula of the target compounds in figure 1.
[b] Results are expressed as IC_{50} values (μM).
[c] Positive control.

The synthesized 5-(substituted benzylidene)thiazolidine-2,4-dione derivatives (**6a-6g**, **7a-7m**, **12**, **13a-13g** and **14a-14g**) were evaluated as inhibitors against PTP1B using *p*-nitrophenyl phosphate (*p*NPP) as a substrate and the results are summarized in Table 1.[21] The known PTP1B inhibitor, ursolic acid (IC_{50} = 4.0 μM), was used as positive control. Compounds **6d-6g**, **7b**, **7c**, **7e**, **7j**, **7k**, **7m**, **14b** and **14e-14f** were proven to be highly active PTP1B inhibitors with IC_{50} values from 4.6 to 9.7 μM, while compounds **6b**, **6c**, **7d**, **7g-7i**, **12** and **14d** showed moderate activity with IC_{50} values from 10.0 to 17.0 μM. When R^3 was bromine, compounds with allyl, prenyl, n-butyl or benzyl as R^1 and hydrogen as R^2 showed excellent activity. But the most active compound had prenyl as R^1, isopropyl as R^2, and bromine as R^3. In general, compounds have lower activity with allyl rather than bromine as R^3. Though, compounds displayed excellent activity with hydrogen as R^1, either isopropyl, n-butyl or benzyl as R^2 and allyl as R^3. These results indicate that steric factors play important role in binding as shown in molecular modeling study (*vide infra*).

The crystallographic 3D structural information of the biomolecular targets offer tremendous opportunities for establishing novel drug design strategies to accelerate the drug discovery process. Available X-ray crystallographic data on Protein Data Bank facilitated the performance of virtual structure based drug discovery projects aiming at PTP1B as a molecular target for type 2 diabetes. In this regard, docking simulation plays a key role in the structural molecular biology and computer-assisted drug design. Binding models for receptors and ligands via a lowest energy pathway may be best represented by docking simulations. One of the most effective docking techniques is Surflex-Dock.[22] The literature review shows that Surflex-Dock has offered several fruitful advantages in the field of drug design.[23] The binding model of compound **7e** and PTP1B (PDB ID: 2QBS) is

depicted in Figure 2. The thiazolidinedione group occupies the large hydrophobic pocket directed toward Gly220, Ala217, Asp181 and Phe182. Specifically, the carbonyl group at the thiazolidinedione ring interacts with Gly220 of the PTP1B domain via the formation of a strong hydrogen bond (hydrogen bond distance is 2.88 Å). This would help to stabilize the open form and generate tighter binding to the catalytic sites of PTP1B. Furthermore, the interactions were also stabilized by the hydrophobic residues of the inner cavity, such as Ala217, Arg221, Asp181, Ser216, Cys215, Phe182, Gln262 and Ile219 as the active pocket consisted of 10 amino acid residues in Figure 2.

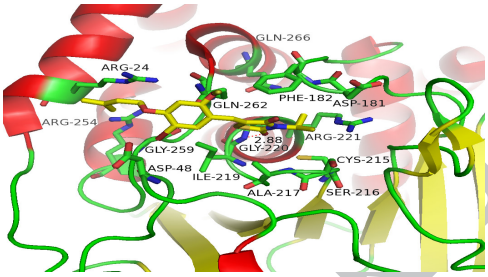


Figure 2. Binding mode of **7e** with active sites of PTP1B (2QBS). The right green coloring of residues indicates neighboring subunits that form the binding pocket for **7e**. The side chain atoms are colored as follows: carbon green, nitrogen blue, oxygen red and hydrogen gray. The **7e** atoms are colored as follows: nitrogen blue, oxygen red, carbon yellow and sulfur orange.

In the present work, we have designed and synthesized a series of 5-(substituted benzylidene)thiazolidine-2,4-dione derivatives as potent PTP1B inhibitors. Compounds **6d-6g**, **7b**, **7c**, **7e**, **7j**, **7k**, **7m**, **14b** and **14e-14f** showed potent inhibitory activities with IC_{50} values ranging from 4.6 to 9.7 μM. In particular, compound **7e** as the most potent of the present series had an IC_{50} of 4.6 μM with bromine at C-5 and *O*-prenyl group at C-4 position of the benzene ring and isopropyl group at the nitrogen of the thiazolidinedione ring. Compound **7e** showed negative binding energy of −7.35 kcal/mol and a high affinity to PTP1B residues (Gly220, Ala217, Arg221, Asp181, Ser216, Cys215, Phe182, Gln262 and Ile219) in the active sites, indicating that it may stabilize the open form and generate tighter binding to the catalytic sites of PTP1B.

Acknowledgments

This research was supported by Basic Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (grant No. 2012R1A1A2006613). We thank the

Korea Basic Science Institute (KBSI), Gwangju branch, for performing the NMR and HRMS experiments.

References

1. Baquer, N.; Gupta, D. ; Raju, J. Indian J. Clin. Biochem. 1998, 13, 63.
2. Jensen, T. ; Deckert, T. Horm. Metab. Res. Suppl Series 1992, 26, 68.
3. Shaw, J. E.; Sicree, R. A.; Zimmet, P. Z. Diabet. Res. Clin. Pract. 2010, 87, 4.
4. Diagnosis and classification of diabetes mellitus, Diabet. Care 2008, 31, Suppl 1, S55.
5. Kahn, S. E. J. Clin. Endocrin. Metab. 2001, 86, 4047.
6. The Weight-Control Information Network. Nutrition Reviews 1995, 53, 232.
7. Klein, B. E.; Klein, R.; Lee, K. E. Diabet. Care 2002, 25, 1790.
8. Mokdad, A. H.; Ford, E. S.; Bowman, B. A.; Dietz, W. H.; Vinicor, F.; Bales, V. S.; Marks, J. S. J. Am. Med. Assoc. 2003, 289, 76.
9. Stein, C. J.; Colditz, G. A. J. Clin. Endocrin. Metab. 2004, 89, 2522.
10. 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. Bio l. 2000, 20, 5479.
11. Vakili, S.; Ebrahimi, S. S.; Sadeghi, A.; Gorgani-Firuzjaee, S.; Beigy, M.; Pasalar, P.; Meshkani, R. Mol. Med. Rep. 2013, 7, 211.
12. Yoon, G.; Lee, W.; Kim, S. N.; Cheon, S. H. Bioorg. Med. Chem. Lett. 2009, 19, 5155.
13. Liu, Z.; Lee, W.; Kim, S. N.; Yoon, G.; Cheon, S. H. Bioorg. Med. Chem. Lett. 2011, 21, 3755.
14. Maccari, R.; Paoli, P.; Ottana, R.; Jacomelli, M.; Ciurleo, R.; Manao, G.; Steindl, T.; Langer, T.; Vigorita, M. G.; Camici, G. Bioorg. Med. Chem. 2007, 15, 5137.
15. Maccari, R.; Ottana, R.; Ciurleo, R.; Paoli, P.; Manao, G.; Camici, G.; Laggner, C.; Langer, T. ChemMedC hem 2009, 4, 957.
16. Bhattarai, B. R.; Kafle, B.; Hwang, J. S.; Ham, S.W.; Lee, K.H.; Park, H.; Han, I. O.; Cho, H. Bioorg. Me d. Chem. Lett. 2010, 20, 6758.
17. Zhang, S.; Zhang, Z.-Y. Drug Discovery Today 2007, 12, 373.
18. Lee, J.; Park, W.-H.; Cho, M.-K.; Yun, H.; Chung, B.-H.; Pak, Y.; Hahn, H.-G.; Cheon, S. Arch. Pharm. R es. 2005, 28, 142.
19. Ottana, R.; Maccari, R.; Barreca, M. L.; Bruno, G.; Rotondo, A.; Rossi, A.; Chiricosta, G.; Di Paola, R.; S autebin, L.; Cuzzocrea, S.; Vigorita, M. G. Bioorg. Med. Chem. 2005, 13, 4243.
20. Bruno, G.; Costantino, L.; Curinga, C.; Maccari, R.; Monforte, F.; Nicolo, F.; Ottana, R.; Vigorita, M. G. Bioorg. Med. Chem. 2002, 10, 1077.
21. PTP1B assay: Recombinant human PTP1B was purchased from BIOMOL International LP. For the inhibition assay, a sample (3 μL in DMSO) was added to a reaction mixture containing enzyme (2 μL), reaction buffer [10 μL, 50 mM citrate (pH 6.0), 0.1 M NaCl, 1 mM EDTA and 1 mM dithiothreitol], water (35 μL) and 50 μL of 4 mM *p*-nitrophenyl phosphate (*p*NPP). The reaction mixture (100 μL) was incubated at 37°C for 30 min and then quenched by addition of 10 μL of 10 N NaOH. The hydrolysis of *p*NPP was determined by measuring the absorbance at 405 nm.
22. Kellenberger, E.; Rodrigo, J.; Muller, P.; Rognan, D. Proteins 2004, 57, 225.
23. Jain, A. N. J. Comput. Aided Mol. Des. 2007, 21, 281.

Graphical Abstract

Design, Synthesis and Docking Study of 5-(Substituted Benzylidene)Thiazolidine-2,4-dione Derivatives as Inhibitors of Protein Tyrosine Phosphatase 1B

Leave this area blank for abstract info.

Zengtao Wang [a], Zhiguo Liu [b], Woojung Lee [c], Su-Nam Kim [c] Goo Yoon [3], and Seung Hoon Cheon [a,*]