Bioorganic & Medicinal Chemistry Letters 21 (2011) 4306–4309

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Pyrrolo[2,3-*c*]azepine derivatives: A new class of potent protein tyrosine phosphatase 1B inhibitors

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ARTICLE INFO

Article history:
Received 8 April 2011
Revised 2 May 2011
Accepted 17 May 2011
Available online 27 May 2011

Keywords:
Protein tyrosine phosphatase 1B
Inhibitor
Aldisin
Marine natural product
Synthesis

ABSTRACT

A series of pyrrolo[2,3-*c*]azepine derivatives was designed, synthesized, and evaluated as a new class of inhibitors against protein tyrosine phosphatase 1B (PTP1B) in vitro. The results demonstrated that compounds bearing a biphenyl moiety were proved to markedly influence the potency of these inhibitors. Particularly, compounds **29**, **35** and **36** showed interesting inhibition with IC_{50} value of 16.36, 14.93 and 13.92 μM, respectively.

Reversible phosphorylation on tyrosine residues represents an important means by which cells regulate signal transduction and to control a wide variety of cellular functions, such as growth, differentiation, survival, apoptosis, metabolism and gene transcription.[1,2] The tyrosine phosphorylation level in vivo is controlled by two opposing phosphatases, the protein tyrosine kinases (PTKs), which catalyse protein phosphorylation, and the protein tyrosine phosphatases (PTPs), which are responsible for dephosphorylation.[3]

The protein tyrosine phosphatese 1B (PTP1B), as the first identified one of PTPs, has recently emerged as an attractive therapeutic target for human disorders such as diabetes, obesity and cancer.[4] Following the two independent studies on PTP1B knockout mice enhancing their insulin sensitivity and resistance to weight gain,[5,6] a variety of PTP1B inhibitors (including recently reported triazole-linked glycosylated α-ketocarboxylic acid derivatives during our manuscript preparation[7]) have been disclosed among academic and industrial laboratories.[8] However, it is still a challenge to identify the highly potent, selective and more bioavailable PTP1B inhibitors for medicinal chemists.

Aldisin (6,7-dihydropyrrolo[2,3-*c*]azepine-4,8(1*H*,5*H*)-dione, **4**), a marine sponge secondary metabolite, was originally isolated from the sponges of *Hymeniacidon aldis* de Laubenfels, which was collected at Guam Island.[9] Since then, several compounds possessing a pyrrolo[2,3-*c*]azepine skeleton, have been isolated from certain species of marine sponges.[10] Moreover, biological evaluation of these natural occurring compounds[10b,c,11] and their synthetic derivatives[12] demonstrated that they exhibited several activities, such as cytotoxicity, antiplatelet aggregation, antithrombotic, inhibition on kinases and so on. However, pyrrolo[2,3-*c*]azepine containing compounds, to our knowledge, have seldom been used as the candidates of PTP1B inhibitors. Considering aldisin-based derivatives can be easily synthesized and aldisin itself showed certain inhibition against PTP1B in our preliminary bio-array, we herein report our efforts in preparing and evaluating the potency against PTP1B of aldisin analogues.

Synthetic pathways for preparation of the two key intermediates (**4** and **8**) were shown in Scheme 1 using the procedure of Papeo[13] and Tepe.[12d] The commercially available 2-trichloroacetylpyrrole **1** and 2-indolecarboxylic acid **5**, condensed with the ethyl ester of β-alanine provided the corresponding amides (**2** and **6**). Followed by hydrolysis of ester to give acids (**3** and **7**) and by intramolecular cyclization, desired compounds **4** and **8** were obtained in moderate overall yield, respectively.

The first series of target compounds **9**–**21** listed in Table 1 were prepared as outlined in Scheme 2. The reaction of **4** with benzyl bromide and 4-(bromomethyl)biphenyl in the presence of K_2CO_3 afforded **9** and **10**, and then reduction of **9** and **10** with $NaBH_4$ gave the corresponding 4-hydroxy compound **11** and **12**. Dehydrated of **11** to give the 4,5-unsaturated compound **13**. Further N-alkylation of **9** afforded **14**, which reacted with $NH_2OH{\cdot}HCl$ to give the (*E*)-oxime

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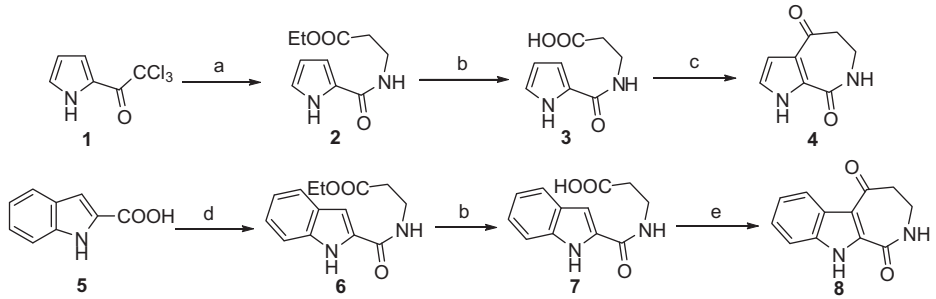
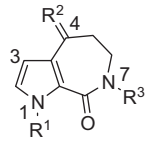
doi:10.1016/j.bmcl.2011.05.052



Scheme 1. Synthesis of compounds **4** and **8**. Reagents and conditions: (a) $NH_2CH_2CH_2COOEt{\cdot}HCl$, Et_3N, CH_3CN, rt; (b) 2 N NaOH, then HCl, rt; (c) PPA, P_2O_5; (d) $NH_2CH_2CH_2COOEt{\cdot}HCl$, EDCI·HCl, DMAP, CH_2Cl_2; (e) P_2O_5, CH_3SO_3H.

Table 1
Chemical structure of compounds **4**, **9–21**

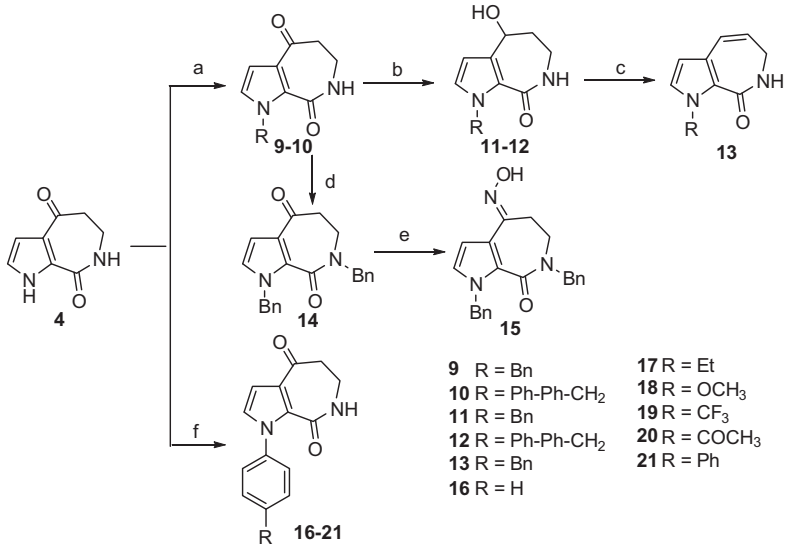


Compound	R^1	R^2	R^3
4	H	O	H
9	$PhCH_2$	O	H
10	4-$PhPhCH_2$	O	H
11	$PhCH_2$	OH	H
12	4-$PhPhCH_2$	OH	H
13	$PhCH_2$	H, $\Delta^{4,5}$	H
14	$PhCH_2$	O	$PhCH_2$
15	$PhCH_2$	NOH	$PhCH_2$
16	Ph	O	H
17	4-EtPh	O	H
18	4-OMePh	O	H
19	4-CF_3Ph	O	H
20	4-$COCH_3$Ph	O	H
21	4-PhPh	O	H

15.[12b] The copper-catalyzed C–N coupling reaction **4** with arylboronic acids produced **16–21**.[14]

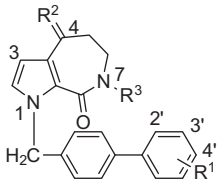
The second series of target compounds **22–36** listed in Table 2 were synthesized as showed in Scheme 3. Treatment of **4** with six different 1-bromomethyl biphenyls afforded **25–30** using conditions the same as that for the synthesis of **9** and **10**. Then, hydrolysis of **30** with 90% H_2SO_4 readily provided amide **31**. The intermediate boronate **38** was obtained from **37** reacting with borolane, followed by coupling with *para*- or *meta*-substituted aryl bromides in the presence of palladium catalyst and aqueous base in DMF afforded the biphenyl **22–24**, and **32–33**.[15] Reacting **25** with CH_3I or 2-hydrazinylpyridine gave **34** and **35**, respectively. Compound **36** was obtained by the same method as for **25**, except for the replacement **4** with **8**. All compounds synthesized were characterized by chemical and spectral methods.

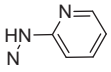
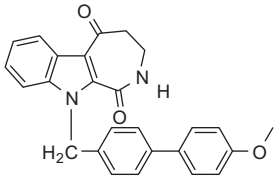
The recombinant human PTP1B protein was amplified by hGST-PTP1B-BL21 *Escherichia coli* pellets and purified by GST beads column. The dephosphorylation of *para*-nitrophenyl phosphate (*p*NPP) was catalysed to *para*-nitrophenol (*p*NP) by PTP1B. The amount of *p*-nitrophenol produced was measured at 405 nm wavelength using Microplate spectrophotometer (uQuant, Bio-tek, USA). The inhibition of 50% (IC_{50}) was evaluated using a Sigmoidal dose–response (variable slope) curve-fitting program with GraphPad Prism 4.0 software. All compounds were dissolved in 100% dimethyl sulfoxide (DMSO). The compound BPV(phen) (potassium bisperoxo (1,10-phenanthroline) oxo-vanadate (V)) (Calbiochem, Germany) was used as reference compound. And the inhibitory rate of compound **4**, and **9–36** was summarized in Figure 1.



Scheme 2. Synthesis of compounds **9–21**. Reagents and conditions: (a) $ArCH_2Br$, K_2CO_3, CH_3CN, reflux; (b) $NaBH_4$, CH_3OH, rt; (c) MsCl, Et_3N; (d) BnBr, NaH, DMF; (e) $NH_2OH{\cdot}HCl$, AcONa, CH_3OH; (f) $ArB(OH)_2$, $Cu(OAc)_2$, pyr., CH_2Cl_2.

Table 2
Chemical structure of compounds **22**–**36**



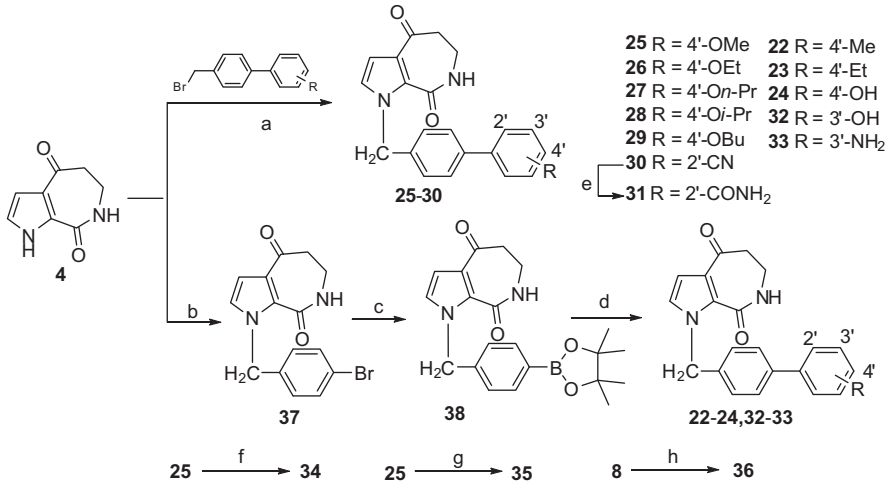
Compound	R^1	R^2	R^3
22	4′-Me	O	H
23	4′-Et	O	H
24	4′-OH	O	H
25	4′-OMe	O	H
26	4′-OEt	O	H
27	4′-O*n*-Pr	O	H
28	4′-O*i*-Pr	O	H
29	4′-O*n*-Bu	O	H
30	2′-CN	O	H
31	2′-$CONH_2$	O	H
32	3′-OH	O	H
33	3′-NH_2	O	H
34	4′-OMe	O	Me
35	4′-OMe		H
36			

As shown in Figure 1, compound **4** without any substituent at position-1, 4, and 7 exhibited the best inhibitory activity in the first series target compounds. Reduction of carbonyl group to hydroxyl group or introduction of an (*E*)-hydroxyimino group at the 4-position produced the similar potency (**9** vs **11**, **10** vs **12**, and **14** vs **15**), while the 4,5-unsaturated compound was much weaker (**9** vs **13**). These results revealed that the ability to form hydrogen bonds between the 4-position of pyrrolo[2,3-*c*]azepine ring and PTP1B enzyme seemed to be critical for the inhibitory activity. It should be noted that the rigid functionalized 1-phenyl derivatives (**16**–**21**) did not provide any better inhibitory activity against PTP1B.

In the first series, although compound **10** with biphenyl at 1-position of pyrrolo[2,3-*c*]azepine ring showed no inhibitory activity, interestingly, compound **22**, substituted with methyl at the 4′-positon of biphenyl, resulted in marked increases of the activity in vitro (**10** vs **22**). Inspired by this result, the second series of analogues was designed and synthesized (**23**–**33**). As indicated in Figure 1, compared to **24** (hydroxyl group at 4′-positon), the substituents such as a cyano (**30**), a carbamoyl (**31**), a hydroxyl (**32**) or an amino group (**33**) at 2′-, or 3′-positions, resulted in marked loss of the activity. Interestingly, replacement of O–H with O-Me on the 4′-position providing almost the same potency (**24** vs **25**) indicated that both a hydrogen-bond donor and a hydrogen-bond acceptor worked. Furthermore, the size of the alkoxy side chain moiety also had a little contribution to the potencies, for example, **27** and **29** exhibited similar results. Removal of the hydrogen-bond donor on the 7-position of pyrrolo[2,3-*c*]azepine ring and replacing it with *N*-Bn or *N*-Me caused a decrease in inhibitory activity (**9** vs **14**, and **25** vs **34**). The hydrazone and indoloazepine analogues exhibited a significant increase in inhibitory activities, probably due to the aromatic group, which can increase the van der Waals interactions between the inhibitors and the enzyme (**35**, **36** vs **25**).

To further obtain the more accurate inhibitory activities of these inhibitors, compound **29**, **35** and **36** were selected to evaluate the IC_{50} value. As shown in Table 3, low micromolar IC_{50} value were observed for these three compounds, and **36** was the most potent with IC_{50} value of 13.92 μM.

In conclusion, a series of pyrrolo[2,3-*c*]azepine derivatives were designed and synthesized to identify a new class of PTP1B inhibitors. Among these compounds, **29**, **35**, and **36** were the most active compounds, with IC_{50} value of 16.36, 14.93, and 13.92 μM against PTP1B in vitro respectively. Primary structure–activity relationships (SARs) analysis indicated that (1) the biphenyl moiety at 1-positon and aromatic groups at 4-positon contributed to the increase of inhibitory activity; (2) the indoloazepine analogue (**36**) exhibiting comparable inhibitory potency implied another scaffold for design of PTP1B inhibitor. The further optimization for inhibitors' structures and potential of this new class of pyrroloazepine and indoloazepine derivatives are currently under investigation in our laboratory and will be reported shortly.



Scheme 3. Synthesis of compounds **22**–**36**. Reagents and conditions: (a) K_2CO_3, CH_3CN, reflux; (b) 4-bromobenzyl bromide, K_2CO_3, CH_3CN, reflux; (c) $PdCl_2(dppf)\cdot CH_2Cl_2$, bis(pinacolato)diboron, AcOK, DMF; (d) $PdCl_2(dppf)\cdot CH_2Cl_2$, ArBr, 2 N Na_2CO_3, DMF; (e) 90% H_2SO_4; (f) NaH, CH_3I, DMF; (g) 2-hydrazinylpyridine, concd HCl, EtOH; (h) 4-(bromomethyl)-4′-methoxybiphenyl, K_2CO_3, CH_3CN, reflux.

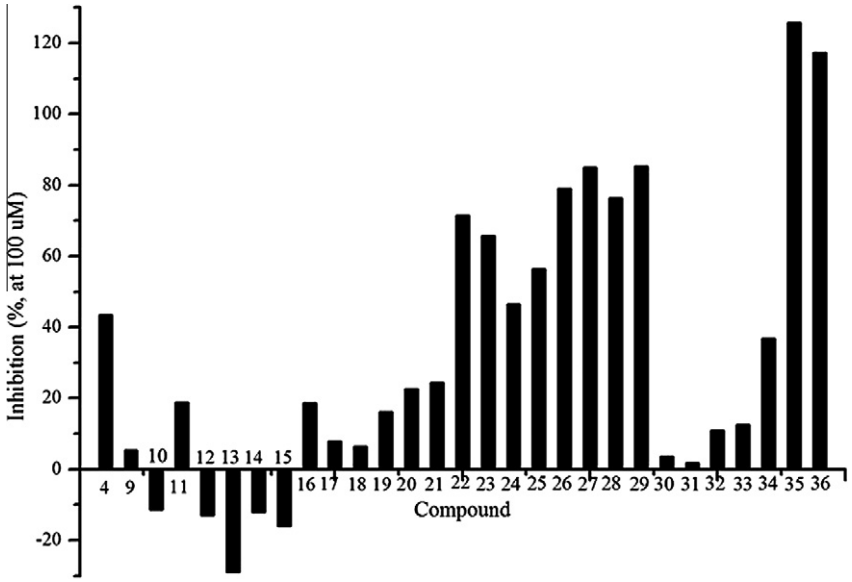


Figure 1. Inhibitory activity assays of the compound **4**, **9**–**36** at 100 μM against the PTP1B.

Table 3
Inhibitory activity against PTP1B of compounds **29**, **35**, and **36**[a]

Compound	IC_{50}[b] (μM)
29	16.36
35	14.93
36	13.92
BPV(phen)	127.90

[a] Assays were performed in 100% DMSO.
[b] The IC_{50} values represent the average of three independent experiments.

Acknowledgments

We acknowledge the National High Technology Research and Development Program of China (863 Program) (No. 2006AA 09Z446) and the National Natural Science Foundation of China (Nos. 20872182, 20802095) for their financial support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.052.

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