Bioorganic & Medicinal Chemistry 17 (2009) 7816–7822

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

1,3,4-Oxadiazole-2(3*H*)-thione and its analogues: A new class of non-competitive nucleotide pyrophosphatases/phosphodiesterases 1 inhibitors

Khalid M. Khan [a,*], Naheed Fatima [a], Maimona Rasheed [a], Saima Jalil [a], Nida Ambreen [a], Shahnaz Perveen [b], M. Iqbal Choudhary [a]

[a] *H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan*
[b] *PCSIR Laboratories Complex, Karachi, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan*

ARTICLE INFO

Article history:
Received 13 July 2009
Revised 5 September 2009
Accepted 10 September 2009
Available online 19 September 2009

Keywords:
Oxadiazole
Thiadiazole
Phosphodiesterase 1
Snake venom
Nucleotide pyrophosphatase/phosphodiesterase-1 (NPP1)

ABSTRACT

A series of 1,3,4-oxadiazole-2 (3*H*)-thiones and 1,3,4-thiadiazole-2 (3*H*)-thiones were synthesized and evaluated for their inhibitory activities against the two nucleotide pyrophosphatase phosphodiesterase 1 enzymes. Dixon, as well as Lineweaver–Burk plots, and their secondary replots have indicated that the inhibition was of pure non-competitive type, against both snake venom and pure human recombinant enzymes as the V_{max} values decreases without affecting the K_m values. 5-[4-(*t*-Butyldimethylsilyloxy)-phenyl]-1,3,4-thiadiazole-2 (3*H*)-thione (**17**) and [4-(*t*-butyldimethylsilyloxy)-phenyl]-1,3,4-oxadiazole-2 (3*H*)-thione (**1**) were found to be the most active compounds with IC_{50} values 66.47 and 368 μM, respectively. The K_i values were 100 μM and 360 μM against the snake venom and human recombinant NPP1 enzyme, respectively. Most active compounds were found to be non-toxic in neutrophil viability assay.

1. Introduction

Nucleotide pyrophosphatases/phosphodiesterases (EC 3.1.4.1, NPP1) catalyze the release of nucleoside-5′-monophosphates by the hydrolysis of a variety of nucleotides or nucleotide derivatives.[1] They exist both as membrane proteins with an extra-cellular active site or as soluble proteins in body fluids. They are widely distributed in mammalian intestinal mucosa, liver cells, serum, and snake venom, as well as in various plants.[1–3] NPP1 or PC-1 (plasma cell membrane glycoprotein) is a key regulator of calcification of bone and other tissues. Over-expression of NPP1 has been associated with chondrocalcirosis,[4] while under-expression causes severe periarticular calcification in mice[5,6] and the syndrome of idiopathic infantile arterial calcification in humans.

Only a few studies have been conducted on the inhibition of nucleotide pyrophosphatases/phosphodiesterases by natural or synthetic compounds.[7–16] During this study, we randomly evaluated 1,3,4-oxadiazole-2(3*H*)-thione and 1,3,4-thiadiazole-2(3*H*)-thione derivatives for the inhibition of the nucleotide pyrophosphatases/phosphodiesterases.

A variety of biological activities are conducted for 1,3,4-oxadiazole-2(3*H*)-thione and their sulfur analogs 1,3,4-thiadiazole-2 (3*H*)-thione. This includes bactericidal[17] fungicidal,[18] and herbicidal activities.[18] Some of them are also known to be strong inhibitors of monoamine oxidase,[19] cyclooxygenase,[20] lipoxygenase,[20] and succinate dehydrogenase[19] enzymes and also active as carbonic anhydrase inhibitors.[21]

In this manuscript, we report the detail snake venom and human NPP1 (PC-1) inhibitory activities, inhibition kinetics, and cytotoxicity, of twenty-six 1,3,4-oxadiazole-2(3*H*)-thione and 1,3,4-thiadiazole-2(3*H*)-thione derivatives.

A total of 26 1,3,4-oxadiazole-2(3*H*)-thione and 1,3,4-thiadiazole-2(3*H*)-thiones were synthesized.[22] To best of our knowledge, it is the first report of the inhibitory potential of this class of compounds against NPP1. The results of preliminary screening, IC_{50} values, kinetic parameters, and cytotoxicity clearly indicate that these compounds have significant potential for inhibition of NPP1.

2. Experimental

All reagents were obtained from Aldrich and Sigma Chemicals and used without further purification. The redistilled and de-ionized water was used in all experiments.

2.1. Enzyme inhibitory assays

2.1.1. Snake venom phosphodiesterase

Activity against snake venom phosphodiesterase 1 (Sigma P 4631) (EC 3.1.4.1) was assayed by using the reported method[23,24] with the following modifications. Tris–HCl buffer 33 mM pH 8.8,

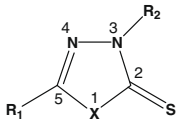
* Corresponding author. Tel.: +92 21 4824910; fax: +92 21 4819018.
E-mail address: hassaan2@super.net.pk (K.M. Khan).

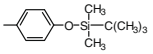
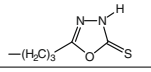
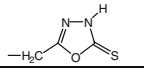
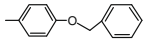
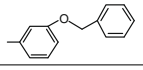
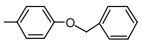
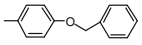
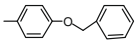
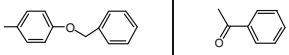
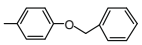
0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.09.011

30 mM Mg-acetate with 0.000742 U/well final concentrations using microtiter plate assay and 0.33 mM bis-(*p*-nitro phenyl) phosphate (Sigma N-3002) as a substrate. Cysteine and EDTA[25–27] (E. Merck) were used as positive controls, having IC_{50} = 748 μM ± 0.15 and 274 μM ± 0.07, respectively. After 30 min of incubation, the enzyme activity was monitored at 37 °C on a microtitre plate spectrophotometer (Molecular Devices, USA) by following the release of *p*-nitrophenol from *p*-nitrophenyl phosphate at 410 nm. All the reactions were performed in triplicate, and the initial rates were measured as the rates of changes in the OD/min (optical density/min) and used in subsequent calculations.

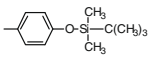
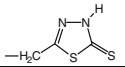
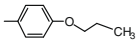
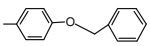
2.1.2. Human recombinant NPP1 (PC-1)

Water soluble human recombinant NPP1 (catalytic extracellular domain) was obtained as gift from Professor J. W. Goding, Monash University, Melbourne, Australia. NPP1 was assayed by the hydrolysis of *p*-nitrophenyl phenyl phosphonate (Sigma N-2881) as described by Kelly et al.[28] The assay was performed in a buffer consisting of 20 mM Tris–HCl, pH 8.0, 0.5 M NaCl and 1 mM $CaCl_2$. Enzyme activity was monitored spectrophotometrically at 37 °C on a microtitre plate spectrophotometer (Molecular Devices) at 405 nm, by following the release of *p*-nitrophenol and assays were conducted in triplicate.



Compd.	Compounds	X	R_1	R_2
1	5-[4-*t*-Butyldimethylsilyloxy phenyl]-1,3,4-oxadiazole-2(3*H*)-thione	O		H
2	*Bis*(1,3,4-oxadiazol-2-propyl)-5-thione	O		H
3	*Bis*(1,3,4-oxadiazol-2-methyl)-5-thione	O		H
4	5-[4-Benzyloxyphenyl]-1,3,4-oxadiazole-2(3*H*)-thione	O		H
5	5-[4-Hydroxyphenyl]-1,3,4-oxadiazole-2(3*H*)-thione	O		H
6	5-[4-Pyridyl]-1,3,4-oxadiazole-2(3*H*)-thione	O		H
7	5-[3-Benzyloxyphenyl]-1,3,4-oxadiazole-2(3*H*)-thione	O		H
8	5-[3-Hydroxyphenyl]-1,3,4-oxadiazole-2(3*H*)-thione	O		H
9	5-[4-Benzyloxyphenyl]-3-octyl-1,3,4-oxadiazole-2(3*H*)-thione	O		$-(CH_2)_7-CH_3$
10	5-[4-Benzyloxyphenyl]-3-benzyl-1,3,4-oxadiazole-2(3*H*)-thione	O		$-CH_2$-Ph
11	5-[4-Benzyloxyphenyl]-3-pentyl-1,3,4-oxadiazole-2(3*H*)-thione	O		$-(CH_2)_4-CH_3$
12	5-Cyclohexyl-1,3,4-oxadiazole-2(3*H*)-thione	O		H
13	5-Diphenylmethyl-1,3,4-oxadiazole-2(3*H*)-thione	O		H
14	5-[4-Benzyloxyphenyl]-3-benzoyl-1,3,4-oxadiazole-2(3*H*)-thione	O		
15	5-[4-Benzyloxyphenyl]-3-(2-hydroxyethyl)-1,3,4-oxadiazole-2(3*H*)-thione	O		$-CH_2-CH_2OH$

Scheme 1.

16	5-[1-Naphthyl]-1,3,4-oxadiazole-2(3*H*)-thione	O		H
17	5-[4-*t*-Butyldimethylsilyloxy phenyl]-1,3,4-thiadiazole-2(3*H*)-thione	S		H
18	5-[4-Hydroxyphenyl]-1,3,4-thiadiazole-2(3*H*)-thione	S		H
19	5-[4-Pyridyl]-1,3,4-thiadiazole-2(3*H*)-thione	S		H
20	Bis(1,3,4-thiadiazole-2-methyl)-5-thione	S		H
21	5-Phenyl-1,3,4-thiadiazole-2(3*H*)-thione	S		H
22	5-[4-Hydroxyphenyl]-3-benzyl-1,3,4-thiadiazole-2(3*H*)-thione	S		-CH_2-Ph
23	5-[4-Propoxyphenyl]-3-propyl-1,3,4-thiadiazole-2(3*H*)-thione	S		-CH_2-CH_3
24	5-Diphenylmethyl-1,3,4-thiadiazole-2(3*H*)-thione	S		H
25	5-[4-Hydroxyphenyl]-1,3,4-thiadiazole-2(3*H*)-thione	S		-$(CH_2)_2$-CH_3
26	5-[4-Benzyloxyphenyl]-3-benzyl-1,3,4-thiaadiazole-2(3*H*)-thione	S		-CH_2-Ph

Scheme 1 (*continued*)

2.1.3. Enzyme kinetic studies

Kinetic parameters were calculated using EZ-Fit Enzyme Kinetics Program (Perrella Scientific Inc., Amherst, USA). The assay conditions for the measurements of the residual activities of all inhibitors were identical, except that fixed concentrations of inhibiting compounds were used in the assay medium. Dissociation constants (K_i values) were determined by the interpretation of Dixon plots. Lineweaver–Burk plots and their secondary plots using initial velocities were drawn over substrate concentrations between 0.166 and 0.666 mM for snake venom and 1.0 and 2.5 mM for human recommended PC-1.

2.1.4. Determination of type of inhibition

Two different methods were applied to monitor the effect of the inhibitor on both K_m and V_{max} values. This was done firstly by plotting the reciprocal of the rate of the reactions against the reciprocal of the substrate concentration as Lineweaver–Burk plot[29] and secondly by the Dixon plot in which the reciprocal of the rate of the reactions was plotted against the inhibitor concentrations.[30] The secondary re-plot of the Lineweaver–Burk was also constructed as $1/V_{max}$ or slope against different concentrations of the respective inhibitor. The secondary replot of Dixon plot was constructed as the slope of each line of substrate concentration in original Dixon plot against the reciprocals of the substrate concentrations.

The types of inhibition were determined by the graphical analysis of Dixon plots, Lineweaver-Burk plots and their secondary plots. K_i Values (the constant of dissociation of the enzyme-inhibitor complex into free enzyme and inhibitor) were determined by the interpretation of Dixon plot, Lineweaver–Burk plot, and its secondary replots by using initial velocities.

2.1.5. Statistical analysis

Assays were conducted in triplicate at each concentration of the inhibitors. Graphs were plotted using GRAFIT program (Leather Barrow, R.J. GraFit; 4.09 ed; Erithacus Software Ltd.; Tains, U.K.). Values of the correlation coefficient, slope, intercept and their standard errors were obtained by the linear regression analysis using the same software. The correlation coefficient for all the lines of all graphs was >0.99 at each point in the constructed graphs, which represents the means of three experiments.

2.2. Cell viability assay

2.2.1. Isolation of human neutrophils

Heparinized fresh venous blood was drawn from healthy volunteers in a local blood bank and neutrophils were isolated by the method of Siddiqui et al. [31] Briefly whole blood was mixed with Ficoll paque and left for the sedimentation of unwanted red blood cells. After 30 min, buffy coat was layered on the Ficoll (3 mL) in the centrifuge tube and centrifuged for 30 min at 1500 rpm. After discarding the supernatant, unwanted RBCs was further lysed by mixing it with hypotonic ammonium chloride solution (0.83%), centrifuged and the neutrophils with MHS and resuspended at 1×10^7 cells/mL.

2.2.2. Assay protocol

We used the modified method of Berridge et al.[32] Briefly, the isolated human neutrophils (1×10^7 cells/mL) were incubated with the various conc of 200–12.5 μg/mL test compounds for 30 min. WST-1 (0.25 mM) was added and incubated in shaking water bath at 37 °C. After 3 h incubation, absorbance at 450 nm was measured with spectraMAX 340 (Molecular Devices, USA)

micro plate reader. The OD is the mean of the five experimental replicates. % Cell viability was calculated by using the following formula:

$$\%\ \text{Viability of cells} = \{(\text{OD test compound} \times 100/\text{OD control}) - 100\} - 100.$$

3. Results and discussion

A series of 26 1,3,4-oxadiazole-2(3*H*)-thiones and their sulfur analogs 1,3,4-thiadiazole-2(3*H*)-thione were synthesized according to the Scheme 1. The structures of all the compounds were deduced on the basis of spectroscopic techniques, such as UV, IR, NMR, and mass spectrometry.

All 1,3,4-oxadiazole-2(3*H*)-thione, and their sulfur analogs 1,3,4-thiadiazole-2(3*H*)-thione (Scheme 1), were evaluated for in vitro snake venom NPP1 inhibitory activity, whereas cysteine and EDTA were used as positive controls or standards. 5-[4-(*t*-Butyldimethylsilyloxy)-phenyl]-1,3,4-thiadiazole-2(3*H*)-thione (**17**) was found to be the most active compound in the series (IC_{50} = 66.47 ± 3.12 μM) against the snake venom phosphodiesterase, more potent than the standards. The other compounds **1**–**5** and **18**–**20** were also found to be active with IC_{50} values in the range of 383–960 μM. Compounds **6**–**9** exhibited a weak inhibitory activity whereas no inhibitory potential was displayed by remaining compounds. Results of snake venom NPP1 inhibitory assay are presented in Table 1.

Compounds which showed good to moderate inhibitory activity against the snake venom NPP1 were selected to study against human NPP1. 5-[4-(*t*-Butyldimethylsilyloxy)-phenyl]-1,3,4-oxadiazole-2(3*H*)-thione (**1**) demonstrated an excellent activity against the human NPP1 enzyme (IC_{50} = 368 ± 2.2 μM). Compounds **2**, **4**, **7**, **18**, and **19** were also found to be active with the IC_{50} values between 467–998 μM. However, other compounds showed either only weak or no activity (Table 2). Only those compounds which showed good inhibitory potential against NPP1 were subjected to kinetic analysis. Graphical representations compound **1**, as an example of non-competitive inhibition against the snake venom

Table 1
In vitro snake venom phosphodiesterase 1 activity of oxadiazole thione derivatives **1**–**16**

Compound	IC_{50}[a] (μM) ± SEM[b]	K_i^*[a] (μM) ± SEM[b]
1	383 ± 0.09	375 ± 1.2
2	429 ± 0.12	450 ± 0.8
3	850 ± 0.2	500 ± 2.5
4	900 ± 0.6	1400 ± 3.1
5	960 ± 0.32	1150 ± 3.4
6	>1000	—
7	>1000	—
8	1000 ± 0.52	1050 ± 2.8
9	>1000	—
10	Inactive	—
11	Inactive	—
12	Inactive	—
13	Inactive	—
14	Inactive	—
15	Inactive	—
16	Inactive	—
17	66.47 ± 3.12	100 ± 0.5
18	390 ± 0.24	350 ± 1.0
19	660 ± 0.7	525 ± 0.8
20	839 ± 0.31	1050 ± 0.38
21	>1000	—
22	Inactive	—
23	Inactive	—
24	>1000	—
25	Inactive	—
26	Inactive	—

K_i^* = is the mean of five values calculated from Lineweaver–Burk plot, its secondary replots and Dixon plot.
— = Not tested due to weak inhibitory activity
[a] IC_{50} is the 50% inhibitory concentration.
[b] SEM = Standard mean error of 3–5 experiments.

Table 2
In vitro human recombinant phosphodiesterase 1 activity of oxadiazole thiones derivatives **1**–**9**, and **17**–**19**

Compound	IC_{50}[a] (μM) ± SEM[b]	K_i^*[a] (μM) ± SEM[b]
1	368 ± 2.2	360 ± 0.14
2	467 ± 1.1	150 ± 0.025
3	>1000	—
4	628 ± 3.7	850 ± 0.02
5	Inactive	—
6	Inactive	—
7	494 ± 3.4	360 ± 0.3
8	Inactive	—
9	Inactive	—
17	>1000	—
18	900 ± 3.5	850 ± 0.8
19	998 ± 1.2	—

K_i^*[a] = is the mean of five values calculated from Lineweaver–Burk plot, its secondary replots and Dixon plot.
— = Not tested due to weak enzyme inhibitory activity.
[a] IC_{50} is the 50% inhibitory concentration.
[b] SEM = Standard mean error of 3–5 experiments.

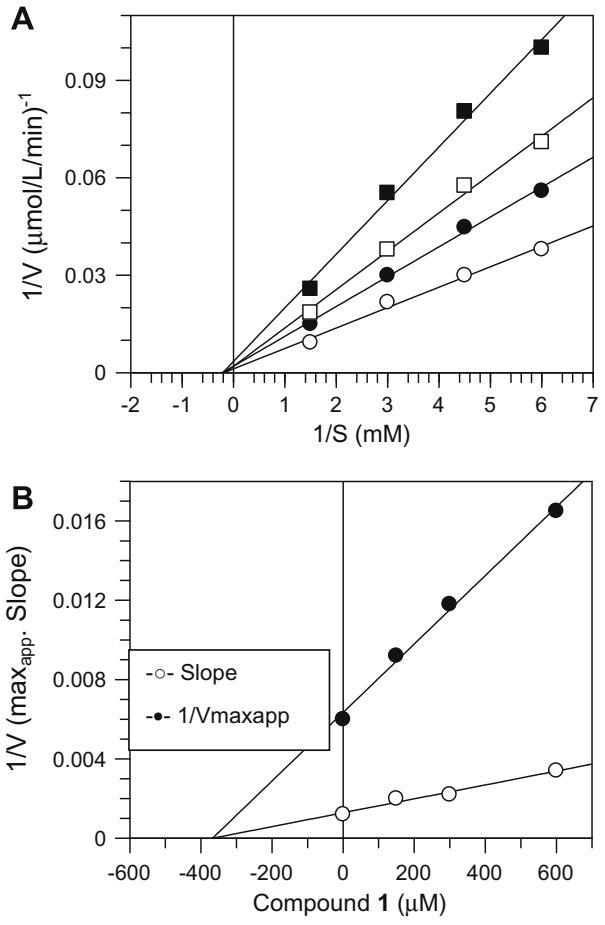


Figure 1. Steady state inhibition of snake venom NPP1 by compound **1**. (A) Lineweaver–Burk plot in the absence (○) and presence of 150 μM; (●) 300 μM (□) and 600 μM(■) of compound **1**, (B) respective secondary replots of the Lineweaver–Burk plot, that is, $1/V_{maxapp}$ and slope versus various concentrations of compound **1**. Each point in the graph represents the mean of three experiments.

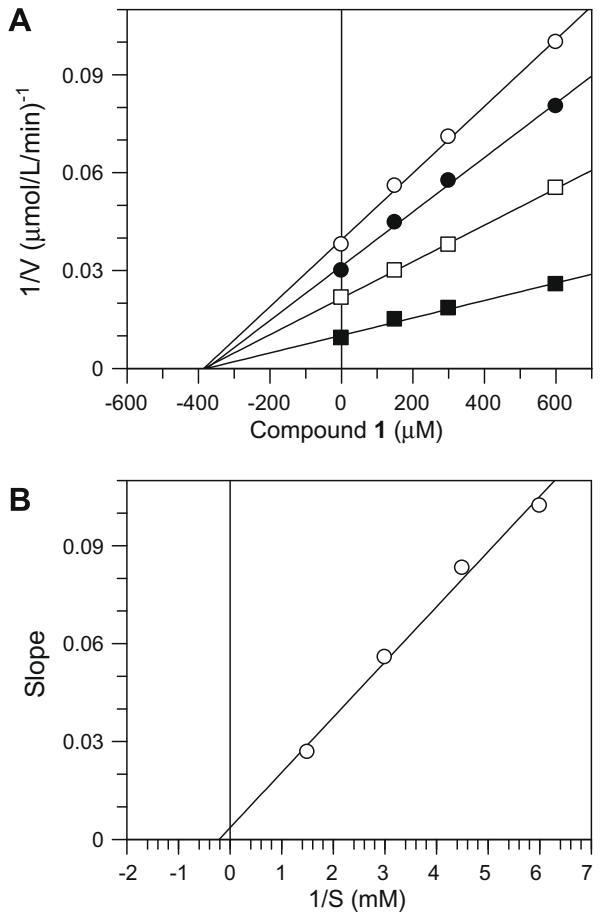


Figure 2. Steady state inhibition of snake venom phosphodiesterase by compound **1**. (A) Dixon plot at four fixed bis-(*p*-nitrophenyl) phosphate concentrations: (○) 1.5 mM; (●)3.0 mM; (□) 4.5 mM and (■) 6.0 mM, (B) secondary replots of the Dixon plot: slope versus reciprocal of substrate concentrations. Each point in the graph represents the mean of three experiments.

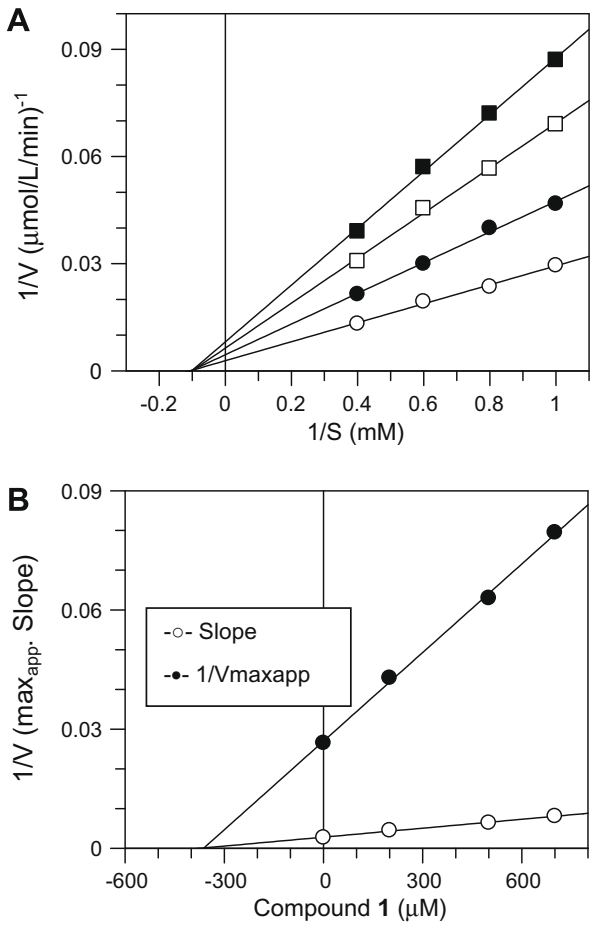


Figure 3. Steady state inhibition of human PC-1 by compound **1**. (A) Lineweaver–Burk plot in the absence (○) and presence of 200 µM (●), 500 µM (□), and 700 µM; (■) of compound **1**, (B) respective secondary replots of the Lineweaver–Burk plot, that is, $1/V_{maxapp}$ and slope versus various concentrations of compound **1**. Each point in the graph represents the mean of three experiments.

and pure human recombinant NPPI enzyme, are shown in Figures 1–5.

3.1. Structure–activity relationship (SAR) studies against snake venom nucleotide pyrophosphatases/phosphodiesterases (NPP1)

Twenty-six 1,3,4-oxadiazole-2(3*H*)-thione and 1,3,4-thiadiazole-2(3*H*)-5-thione analogs were screened for their snake venom NPP1 inhibitory potential. Snake venom is a complex mixture of enzymes and protein. Many of these proteins are harmless to humans, but some are toxins. The composition of snake venom differs from species to species. It was found that the presence of an oxygen or sulfur in five-membered heterocyclic ring, as well as the substitution of various groups at C-5 play an important role in the activity against the snake venom NPP1. It was discovered that the presence of hydrogen at C-3 of the heterocyclic ring contributes in the inhibitory activity, in addition to the above-mentioned factor. Similarly the presence of a sulfur atom in the ring also influences the inhibitory potential. 5-[4-(*t*-Butyldimethylsilyloxy)-phenyl]-1,3,4-thiadiazole-2(3*H*)-thione (**17**), which have a free hydrogen at C-3 and sulfur in the ring found to be the most active member of the series (IC_{50} = 66.47 ± 3.12 µM) against the snake venom phosphodiesterase, better than the two standards used in the study. The low activity of the structurally related 5-[4-(*t*-butyldimethylsilyloxy)-phenyl]-1,3,4-oxadiazole-2(3*H*)-thione (**1**) (IC_{50} = 383 ± 0.09 µM) may be due to replacement of sulfur by oxygen in the heterocyclic ring. In other words, hydrogen at C-3 and sulfur in the ring and *t*-butyldimethylsilyloxyphenyl at C-5 of ring are mainly responsible for the activity. Comparison of activities of closely related compounds **5** (IC_{50} = 960 ± 0.32 µM), **8** (IC_{50} = 1000), and **18** (IC_{50} = 390 ± 0.24 µM), clearly indicated the importance of sulfur in the ring, as well as hydrogen at C-3. The complete loss of inhibitory potential in compounds **22** and **25** may be due to aforementioned. The weak or lack of inhibitory activity in compounds **4**, **9**–**11**, **14**, **15**, **23,** and **26** may be due to absence of sulfur and unsubstituted position 3, or both. The difference in the activities of compounds **2**, **3**, and **20** with IC_{50} values 429, 850, and 317 µM, respectively, also indicated that sulfur in ring is responsible of activity, along with a few other structural features. It inference was supported by difference in the activities of compounds **6** and **19**. Compounds **12**, **13**, **16**, **21**, and **24** were found to be completely inactive due to reasons mentioned above.

3.2. Structure–activity relationship (SAR) studies against human nucleotide pyrophosphatases/phosphodiesterases (NPP1)

Phosphodiesterases are used to interfere with the prey's cardiac system, mainly to lower the blood pressure. Based on the preliminary activities results against the snake venom nucleotide pyrophosphatases/phosphodiesterases (NPP1), we selected compounds **1**–**9**

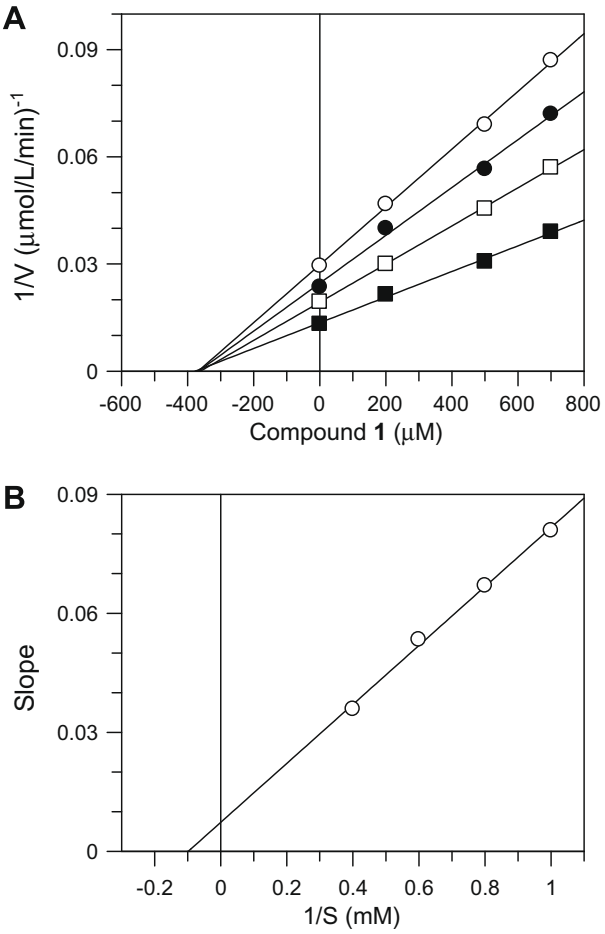


Figure 4. Steady state inhibition of human PC-1 by compound **1**. (A) Dixon plot at four fixed *p*-nitrophenyl phosphonate concentrations: (○) 0.4 mM; (●) 0.6 mM; (□) 0.8 mM and (■) 1.0 mM, (B) secondary replots of the Dixon plot: slope versus reciprocal of substrate concentrations. Each point in the graph represents the mean of three experiments.

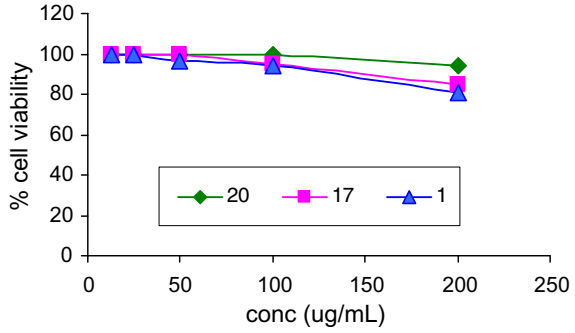


Figure 5. Graph shows % cell viability of freshly isolated human neutrophils (1×10^7 cells/mL) in the presence of various concentrations (200–12.5 µg/mL) of compound **1**, **17**, and **20**.

and **17**–**19** for screening against the human NPP1. Compounds (inhibitors) are potential candidates for the therapy of snake bites. Results of inhibitory assay are presented in Table 2. The two factors emerge out of the experimental results, that is, suitable substitution at C-5, and hydrogen at C-3 are responsible for human NPP1 inhibition. 5-[4-(*t*-Butyldimethylsilyloxy)-phenyl]-1,3,4-oxadiazole-2(3*H*)-thione (**1**) (IC_{50} = 368 ± 2.2 µM) was found to be most active compound against the human NPP1, clearly indicating that oxygen in ring is also participating in the activity. The strong activities of compounds **2**, **4**, and **7** against human NPP1 as compared to their activities against the snake venom NPP1, may be due to presence of oxygen in ring. The low activities of compounds **18**, and **19** may be due to absence of oxygen in the ring.

3.3. Evaluation of cytotoxicity

The cytotoxicities of the active compound were evaluated on human neutrophils by measuring reduction of WST-1 salt. WST-1 tetrazolium salt readily reduced to Formazan dye in the presence of cellular enzymes of metabolically active neutrophils. Five concentrations ranges 200–12.5 µg/mL of compounds **1**, **17**, and **20** were used to determine the viability of the cells. These compounds were found to be largely non-toxic as neutrophils showed over 90% viability up to 200 µg/mL concentration.

3.4. Kinetic studies

On the basis of IC_{50} values, compounds **1**–**5**, **8**, **17**–**20**, which showed inhibitory potential against the snake venom NPP1 and human NPP1 (compound **1**, **2**, **4**, **7**, **18** and **19**), were selected for kinetic studies.[31,32] The K_i values and mode of inhibition are presented in Tables 1 and 2. The K_i values (inhibitory constants) were determined directly from the Dixon plots. These values were also confirmed by plotting the $1/V_{maxapp}$ against different concentrations of respective inhibitor. $1/V_{maxapp}$ values were calculated at each intersection point of lines of every inhibitory concentration on the *Y*-axis of the Lineweaver–Burk plot. Secondly the slope of each line of inhibitory concentration on the Lineweaver–Burk plot was plotted against inhibitor concentrations. All the compounds were found to be pure non-competitive type of inhibitors as the V_{max} values decreased without affecting the K_m values. Graphical representation of 5-[4-*t*-butyldimethylsilyloxy phenyl]-1,3,4-oxadiazole-2(3*H*)-thione (**1**), as an example of non-competitive inhibition against snake venom and pure human recombinant phosphodiesterase 1 enzyme are presented in Figures 1–5. Compound **1** binds to the enzyme at a site other than the enzymes active site. This affects the rate of the reaction catalyzed by the enzyme because the presence of the inhibitor causes a change in the structure and shape of the enzyme. This change in shape means the enzyme is no longer able to bind with the structure correctly.

Acknowledgements

We are grateful to Professor J. W. Goding, Monash University, Melbourne, Australia, for a generous gift of water-soluble human recombinant NPP1 (catalytic extra cellular domain) and also for reviewing this manuscript. We also acknowledge the help of the Fatmid Foundation for the blood samples.

References and notes

1. Razzell, W. E. *Methods Enzymol.* **1963**, *6*, 236.
2. Bollen, M.; Gijsbers, R.; Ceulemans, H.; Stalmans, W.; Stefan, C. *Crit. Rev. Biochem. Mol. Biol.* **2000**, *35*, 393.
3. Goding, J. W.; Grobben, B.; Slegers, H. *Biochim. Biophys. Acta* **2003**, *1638*, 1.
4. Johnson, K.; Hashimoto, S.; Lotz, M.; Pritzker, K.; Goding, J.; Terkeltaub, R. *Arthritis Rheum.* **2001**, *44*, 1071.
5. Okawa, A.; Nakamura, I.; Goto, S.; Moriya, H.; Nakamura, Y.; Ikegawa, S. *Nat. Genet.* **1998**, *19*, 271.
6. Sali, A.; Favaloro, J. M.; Terkeltaub, R.; Goding, J. W. In *Proceedings of the Second International Workshop on Ecto-ATPases and Related Ectonucleotidases;* Diepenbeek, Belgium; Shaker Publishing BV: Maastricht, The Netherlands, 1999.
7. Ahmed, V. U.; Abbasi, M. A.; Hussain, H.; Akhtar, M.; Farooq, U.; Fatima, N.; Choudhary, M. I. *Phytochemistry* **2003**, *63*, 217.
8. Iqbal, K.; Anis, I.; Muhktar, N.; Malik, A.; Fatima, N.; Choudhary, M. I. *Heterocycles* **2003**, *60*, 151.

9. Ahmed, V. U.; Abbasi, M. A.; Zubair, M.; Fatima, N.; Farooq, U.; Choudhary, M. I. *Helv. Chim. Acta* **2004**, *87*, 67.
10. Choudhary, M. I.; Fatima, N.; Abbasi, A.; Jalil, S.; Ahmad, V. U.; Rahman, A. U. *Bioorg. Med. Chem.* **2004**, *12*, 5793.
11. Tahir, M. M.; Brezina, D.; Fatima, N.; Choudhary, M. I.; Rahman, A. U. *J. Inorg. Biochem.* **2004**, *2*, 589.
12. Abbasi, M. A.; Ahmed, V. U.; Zubair, M.; Fatima, N.; Farooq, U.; Hussain, S.; Lodhi, M. A.; Choudhary, M. I. *Planta Med.* **2004**, *70*, 1189.
13. Rahman, A. U.; Naz, H.; Fadimatou; Makhmoor, T.; Yasin, A.; Fatima, N.; Ngounou, F. N.; Kimbu, S. F.; Sondengam, B. L.; Choudhary, M. I. *J. Nat. Prod.* **2005**, *68*, 189.
14. Ahmed, V. U.; Zubair, M.; Abbasi, M. A.; Kousar, F.; Fatima, N.; Choudhary, M. I. *Z. Naturforsch.* **2005**, *60b*, 1101.
15. Choudhary, M. I.; Fatima, N.; Khan, K. M.; Jalil, S.; Iqbal, S.; Rahman, A. U. *Bioorg. Med. Chem.* **2006**, *14*, 8066.
16. Mostafa, M.; Nahar, N.; Mosihuzzaman, M.; Sokeng, S.; Fatima, N.; Rahman, A. U.; Choudhary, M. I. *Nat. Prod. Res.* **2006**, *20*, 686.
17. Abdel-ghani, E. *Indian J. Heterocycl. Chem.* **1997**, *7*, 123.
18. Edwards, L. H.; U.S. 4, 259,104; *Chem. Abst*. **1981**, *95*, 25078.
19. Soni, N.; Barthwal, J. P.; Saxena, A. K.; Bhargava, K. P. *J. Heterocycl. Chem.* **1982**, *19*, 29.
20. Boschelli, D. H.; Connor, D. T.; Bornemeier, D. A.; Dyer, R. D.; Kennedy, J. A.; Kuipers, J. P.; Okonkwo, G. C.; Schrier, D. J.; Wright, C. D. *J. Med. Chem.* **1993**, *36*, 1802.
21. (a) Almajan, G. L.; Barbuceanu, S. F. A.; Scozzafava, A.; Supuran, C. T. *J. Enz. Inhib. Med. Chem.* **2008**, *23*, 101; (b) Mohammed, K.; Hamid, A.; Atef, A.; Hafez, A.; Nawal, A.; El-Koussi; Nadia, M.; Mahfouz; Innocenti, A.; Supuran, C. T. *Bioor. Med. Chem.* **2007**, *15*, 6975; (c) Supuran, C. T.; Scozzafava, A.; Saramet, I.; Banciu, M. D. *J. Enz. Inhib.* **1998**, *13*, 177.
22. Amtul, Z.; Rasheed, M.; Choudhary, M. I.; Rosanna, S.; Khan, K. M.; Atta-ur-Rahman *Biochem. Biophys. Res. Commun.* **2004**, *319*, 1053.
23. Francoeur, A. M.; Assalian, A. *Biochemica* **1996**, *3*, 19.
24. Mamillapalli, R.; Haimovitz, R.; Ohad, M.; Shinitzky, M. *FEBS Lett.* **1998**, *436*, 256.
25. Razzell, W. E.; Khorana, H. G. *J. Biol. Chem.* **1959**, *234*, 2105.
26. Nakabayashi, T.; Matsuoka, Y.; Ikezawa, H.; Kimura, Y. *Int. J. Biochem.* **1994**, *26*, 171.
27. López-Gómez, J.; Costas, M. J.; Ribeiro, J. M.; Fernández, A.; Romero, A.; Ávalos, M.; Cameselle, J. C. *FEBS Lett.* **1998**, *421*, 77.
28. Kelly, S. J.; Dardinger, D. E.; Butler, L. G. *Biochemistry* **1975**, *14*, 4983.
29. Segal, I. *Enzyme Kinetics*, 2nd ed.; John Wiley and Sons: New York, USA, 1993. pp 125–143.
30. Dixon, M. *Biochem. J.* **1953**, *55*, 170.
31. Siddiqui, R. A.; English, D.; Cui, Y.; Martin, M. I.; Wentlands, J.; Akard, L. *J. Leukocytes Biol.* **1995**, *58*, 189.
32. Berridge, M. V.; Tan, A. S.; McCOY, K. D.; Wang, R. *Biochemica* **1996**, *4*, 14.