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SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2996–2999

Synthesis and biological evaluation of rhodanine derivatives as PRL-3 inhibitors

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Received 7 January 2006; revised 16 February 2006; accepted 22 February 2006
Available online 10 March 2006

Abstract—A series of rhodanine derivatives was synthesized and evaluated for their ability to inhibit PRL-3. Benzylidene rhodanine derivative showed good biological activity, while compound **5e** was the most active in this series exhibiting IC_{50} value of 0.9 μM in vitro and showed a reduced invasion in cell-based assay.

The protein tyrosine phosphatases (PTPs) constitute a family of closely related key regulatory enzymes that dephosphorylate phosphotyrosine residues in their protein substrates. Malfunctions in PTP activity are linked to various diseases, ranging from cancer to neurological disorders and diabetes. Consequently, PTPs have emerged as promising targets for therapeutic intervention in recent years.[1]

Among the PTPs, the phosphatase of regenerating liver (PRL) family tyrosine phosphatases (PRL-1, PRL-2, and PRL-3) are closely related intracellular enzymes that possess the PTP active-site signature sequence CX_5R.[2,3] All are proteins of about 20 kDa with at least 75% amino acid sequence homology.

PRL-3 gene codes a 22 kDa nonclassical protein tyrosine phosphatase with a C-terminal prenylation motif. It is recently identified as a metastasis-related enzyme. It is located at the cytoplasmic membrane when prenylated and in the nucleus when nonprenylated. Overexpression of PRL-3 has been found to transform human embryonic kidney cell HEK293 and increase HEK293 cell growth.[4] Saha et al. reported that PRL-3 mRNA expression was consistently increased in the liver metastasis of colorectal cancers, suggesting that PRL-3 was associated with colorectal cancer metastasis.[5] Furthermore, Zeng et al. showed that PRL proteins promote cell motility, invasion activity and metastasis, which were directly dependent on their catalytic activity.[6] These suggest that PRL-3 is not only a putative prognostic marker but also a therapeutic target for metastastic tumors.

Thus far, only pentamidine[7] was reported as a PRL family inhibitor with anti-cancer potential via inactivation of PRL-1, -2, and -3. This prompted us to attempt systematic discovery of potent PRL-3 inhibitors. In the course of the search for PRL-3 inhibitors through high throughput screening (HTS) using chemical library of Korea Chemical Bank, rhodanine skeleton was discovered as a hit. We now report the synthesis and their

Keywords: Rhodanine; Cancer; Metastasis; PRL-3; Inhibitor.
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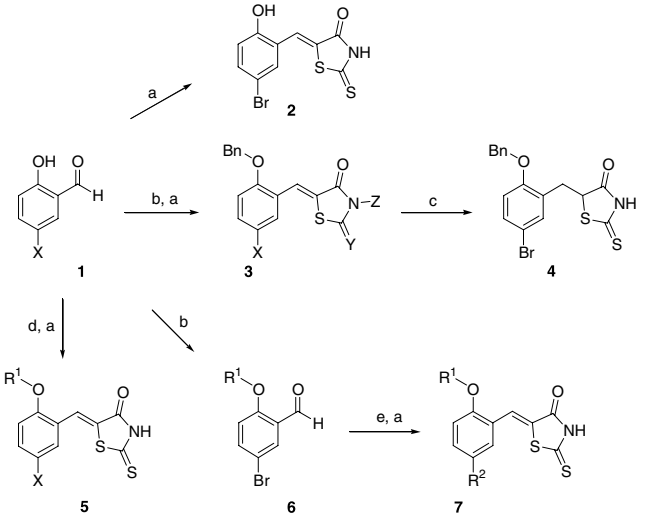
doi:10.1016/j.bmcl.2006.02.060

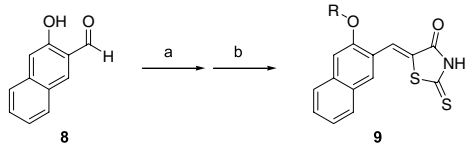
structure–activity relationship (SAR) study of rhodanine derivatives as PRL-3 inhibitors.

A series of rhodanine derivatives was synthesized according to the Schemes 1 and 2. 5-Bromosalicylaldehyde (**1a**, X = Br) was reacted with rhodanine in the presence of ammonium acetate to produce the corresponding rhodanine derivative (**2**). Also salicylaldehydes (**1**, X = H or Br) was benzylated and then coupled with rhodanine, *N*-methylrhodanine, or thiazolidinedione to afford **3a–d**. Reduction of **3a** by lithium borohydride in pyridine gave compound **4**. Next, 5-bromosalicylaldehyde (**1a**, X = Br) was treated with methanesulfonyl chloride in pyridine or the appropriate benzyl halide equivalents to provide the corresponding aldehydes, which was converted to **5a–e** by treatment with rhodanine. Also, **1** was coupled with benzyl bromide to produce **6**, which adopted diverse aryl groups at 5-position by Suzuki type coupling, followed by attaching rhodanine moiety to afford the desired compounds **7a–d**. Rhodanine derivatives with naphthalene skeleton were prepared as outlined in Scheme 2. 3-Hydroxynaphthalene-2-carbaldehyde (**8**) was benzylated by several benzyl bromides, and condensed with rhodanine to give the corresponding naphthalydene rhodanine derivatives (**9a–d**).

All compounds prepared were evaluated for their in vitro inhibitory activity against recombinant human PRL-3.



Scheme 1. Reagents and conditions: (a) rhodanine, *N*-methylrhodanine or thiazolidinedione, NH_4OAc, AcOH, benzene, reflux; (b) benzyl bromide, K_2CO_3, KI, acetone, reflux; (c) $LiBH_4$, pyridine, THF, and reflux; (d) methanesulfonyl chloride, pyridine, rt or R^1Br, K_2CO_3, KI, acetone, reflux; (e) $R^2B(OH)_2$, $Pd(dppf)_2Cl_2$, Na_2CO_3, 100 °C.



Scheme 2. Reagents and conditions: (a) RX, K_2CO_3, KI, acetone, reflux; (b) rhodanine, NH_4OAc, AcOH, benzene, reflux.

PRL-3 protein was overexpressed as His-tag fusion protein in *Escherichia coli* and purified. Assays were performed using 6,8-difluoro-4-methylumbelliferyl phosphate (DIFMUP) as a substrate at 25 °C for 5 min in 20 mM Tris–HCl (pH 8.0), 5 mM DTT, in the presence or absence of the inhibitor. After the addition of purified PRL-3 (0.3 μM) and DIFMUP (5 μM), the reaction mixture was incubated for 5 min. The reaction was stopped by the addition of sodium orthovanadate (20 mM). The phosphatase activities were measured by the absorbance changes caused by hydrolysis of the substrate at 460 nm. IC_{50} values were an average of triplicate experiments as determined from direct regression curve analysis. Pentamidine was used as a reference.

The result for the rhodanine derivatives is shown in Table 1. While 5-bromosalicylaldehyde (**1b**, X = Br) was not active, introduction of a rhodanine group provided an active PRL-3 inhibitor with an IC_{50} of 9.5 μM (**2**). Benzyl substitution of OH at 2-position exhibited enhanced potency (3.0 μM, **3a**), and was almost 20-fold more potent than reference pentamidine. Either elimination of Br at 5-position (**3b**) or introduction of thiazolidinedione instead of rhodanine abolished the activity (**3c**). N-Methylation was also detrimental to the in vitro activity (**3d**). Compound **4** produced by reduction of double bond of compound **3a** showed similar activity (4.0 μM).

These data suggest that substituents at 2- and 5-position of benzene ring influence the in vitro inhibitory activity. Introduction of sulfonyl group or pyridinylmethyl group at R^2 position showed no inhibitory activities (**5a** and **5b**). Introduction of 2-chlorobenzyl group as R^1 demonstrated comparable potency with **3a** (**5c**, 2.4 μM). Introduction of 2-bromobenzyl substituent (**5e**) exhibited enhanced potency, and is the first of our compounds to break the micromolar barrier with an IC_{50} value of 0.9 μM. Various substitution at R_2 position resulted in IC_{50} values in the range of 1.2–3.7 μM. Compound **7d** was another submicromolar inhibitor toward PRL-3 with an IC_{50} value of 0.9 μM.

Naphthalene based rhodanine derivatives were another possibility to show PRL-3 inhibition comparable to that of the benzene as shown in Table 2. The effect of R lipophilicity showed similar trend as benzylidene series. Among them, compound **9d** showed an IC_{50} of 1.7 μM.

Recently, we reported the crystal structure of PRL-1 protein for the first time.[8] Our crystal structure revealed a well-ordered active-site structure with catalytically important residues in active conformations, providing the insight into the mode of interaction between rhodanine compounds and PRL proteins. NH-Group in rhodanine ring may be deprotonated, mimicing negatively charged substrates. This moiety would be directed toward the active-site P-loop that are surrounded by positively charged guanidine group from Arg110 and several amide groups from main-chain atoms. As stated previously, N-methylation in rhodanine resulted in no inhibitory activity, supporting this notion. Further, the

Table 1. Inhibitory activity of compounds derived from salicylaldehydes against PRL-3

Compound	R^1	X or R^2	Y	Z	IC_{50}[a] (μM)
1a	—	Br	—	—	na[b]
2[9]	H	Br	S	H	9.5
3a[10]	Benzyl	Br	S	H	3.0
3b[10]	Benzyl	H	S	H	na
3c[10]	Benzyl	Br	O	H	na
3d	Benzyl	Br	S	CH_3	na
4	—	—	—	—	4.0
5a	CH_3SO_2	Br	—	—	na
5b	Pyridin-3-yl-methyl	Br	—	—	na
5c	2-Chlorobenzyl	Br	—	—	2.4
5d	4-Bromobenzyl	H	—	—	1.6
5e	2-Bromobenzyl	Br	—	—	0.9
7a[10]	Benzyl	Phenyl	—	—	1.7
7b	Benzyl	4-*N*,*N*-Dimethylaminophenyl	—	—	3.7
7c	Benzyl	Benzofuran-3-yl	—	—	1.2
7d	Benzyl	Benzo[*b*]thiophen-3-yl	—	—	0.9
Pentamidine					53.6

[a] IC_{50} values were determined from direct regression curve analysis.
[b] Not active up to 50 μM.

Table 2. Inhibitory activity of naphthalydene rhodanine derivatives against PRL-3

Compound	R	IC_{50}[a] (μM)
9a	Benzyl	2.0
9b	3,5-Dimethoxybenzyl	3.1
9c	4-Phenylbenzyl	1.9
9d	2-Chloro-6-fluorobenzyl	1.7
Pentamidine		53.6

[a] IC_{50} values were determined from direct regression curve analysis.

surroundings from the active-site pocket of PRL reveals highly hydrophobic character with large entrance comparing to other PTPs. The inhibitory activities of rhodanine compounds against PRL-3 are enhanced by the introduction of hydrophobic substituents, which well agrees with the structural information.

The compounds **5e** and **9d** were evaluated for their ability to reduce the invasiveness of tumor as shown in Figure 1. B16F10 cells (1×10^5) in routine medium were seeded on the upper chamber of 24-well BD Biocoat Matrigel invasion chambers with 8 μm polycarbonate filters (BD Bioscience, San Jose, CA). The bottom chamber contained conditioned medium from HT1080 cells as a chemoattractant. Cells were incubated in the presence and absence of the compounds for 22 h at 37 °C in a humidified incubator with 5% CO_2. Nonmigratory cells on the upper surface of the filter were removed by wiping with a cotton swab. Migrated cells to the underside of the membrane were stained with 0.5% crystal violet after fixation with methanol and observed under the microscope.

Compound **5e**, with an IC_{50} value of 0.9 μM, showed the reduced invasion comparable to 30 μg/ml of fumagillin.[11] Naphthalene based compound **9d** also exhibited similar result to **5e,** both showing higher potency compared to pentamidine.

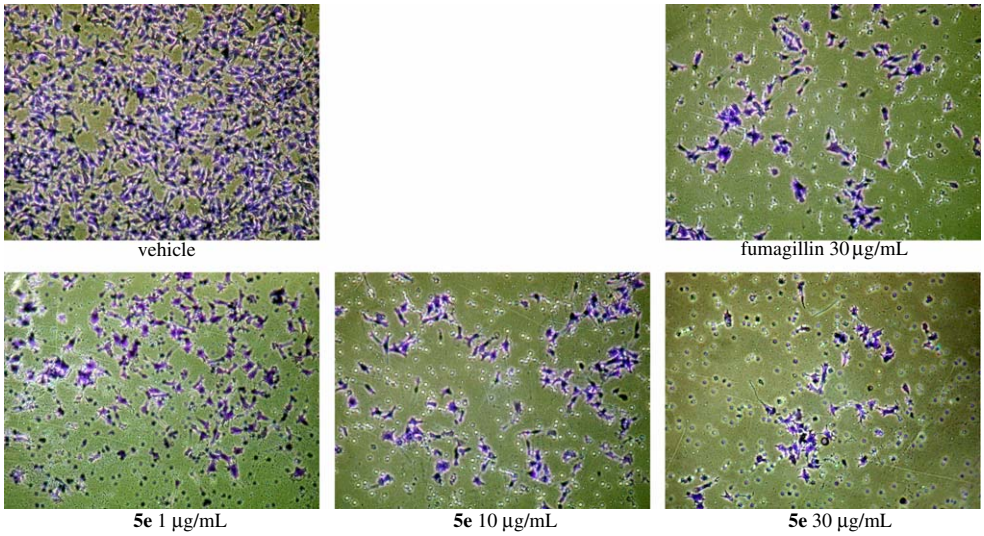


Figure 1. Effect of compound **5e** on invasion of B16F10 cells.

In conclusion, we have discovered a new series of rhodanine derivatives as inhibitors of PRL-3. Benzylidene rhodanine derivatives showed good biological activity, compound **5e** was the most active in this series, exhibited an IC_{50} value of 0.9 μM and showed a reduction of invasion in cell. Further studies aimed at improving efficacy using structure-based design are in progress and will be reported in due course.

Acknowledgments

We thank the cooperation of Korea Chemical Bank to use chemical library for HTS (http://www.chembank.or.kr). This study was supported by Ministry of Commerce, Industry and Energy (MOCIE).

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