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RESEARCH ARTICLE

Synthesis and evaluation of new thiazole-containing rhodanine-3-alkanoic acids as inhibitors of protein tyrosine phosphatases and glutathione *S*-transferases

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Abstract: Thiazole-containing derivatives of rhodanine-3-alkanoic acids with propanoic or undecanoic acid groups were synthesized and evaluated as inhibitors of some protein tyrosine phosphatases and glutathione *S*-transferases. The rhodanines bearing longer carboxylated *N*-alkyl chain were found to inhibit PTP1B, MEG1, MEG2, and VE-PTP as well as GST from equine liver and GSTA1-1 with IC_{50} values in the low micromolar range. The inhibitory effect on protein tyrosine phosphatase activity depends on substituent at position 2 of the thiazole ring. The best compound showed a competitive type of VE-PTP inhibition. In case of GST from equine liver, the inhibition was of mixed or non-competitive type with respect to glutathione or CDNB substrate, respectively. Possible binding modes of the inhibitors were discussed based on molecular docking calculations.

Keywords: rhodanine; thiazole; protein tyrosine phosphatase; glutathione *S*-transferase; enzyme inhibition; molecular docking.

Introduction

Thiazole derivatives represent a number of natural and synthetic biologically active compounds with anticancer [1], antibacterial [2], and antiviral activities [3]. Many of rhodanine-based compounds turned out to have low toxicity, exhibit antidiabetic, anti-inflammatory, anti-Alzheimer's, anticancer, antibacterial, antifungal, and antiviral activities [4]. The best known anti-hyperglycemic, hypoglycemic and hypolipidemic effects of rhodanine derivatives are based on their agonist activity against PPARs and FFAR1 receptors, as well as inhibition of ALR2, PTP1B, and α-glucosidase [5]. Anticancer activity of the compounds can be attributed to the inhibition capacity against pan-PIM kinases [6-7], protooncogene transcription factor *c*-Myc [8-9], protein disulfide isomerase (PDI) [10], histone acetyltransferases [11], topoisomerase II [11], as well as Bcl-XL and Mcl-1 families of the proteins [13]. Antibacterial effects of rhodanine derivatives can be realized *via* inhibition of bacterial penicillin-binding proteins [14] and β-lactamases [15-16].

The protein tyrosine dephosphorylation is a fundamental regulatory mechanism of many signal transduction pathways in processes of growth, proliferation, differentiation, or survival of eukaryotic cells. It was shown that elevated activity of classical non-receptor and receptor-like protein tyrosine phosphatases (PTPs) coincides with a number of pathologies [17-18]. PTP1B, being a negative regulator of insulin and leptin signaling is considered as a promising therapeutic target for treatment of type 2 diabetes and obesity [19]. Overexpression of this phosphatase was also found to contribute to tumorigenesis of cells [20-21]. Megakaryocyte protein tyrosine phosphatase MEG-2 which involved in regulating of hematopoietic signaling and blood glucose homeostasis is of interest as a therapeutic target for treatment of type 2 diabetes and myeloproliferative disorders [23-25]. PTPβ, also called as vascular endothelial-protein tyrosine phosphatase (VE-PTP), downregulates Tie2 signaling and often associated with development of variety endothelial dysfunctions. In this connection, a number of chemical compounds were developed as inhibitors of the protein tyrosine phosphatases.

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Among them, aryl and hetaryl compounds [26], including derivatives of rhodanine [27-29] and its closely related analog, thiazolidinedione [30-31], were studied as PTPs inhibitors. Compounds bearing carboxylic [32], phosphonic [33-34], sulfonic groups [35] were designed as pTyr mimetics for inhibition of PTP1B. As a drug for treatment of diabetic macular edema, diabetic retinopathy, and ocular hypertension, the inhibitor of VE-PTP AKB-9778/Razuprotafib was developed [36-38].

It was reported previously that thiazolyl-2,4-thiazolidinedione/rhodanine compounds possess anticancer activities against hepatocellular carcinoma cell lines having resistance to chemotherapeutic agents [39]. The drug resistance caused by drug-metabolizing enzymes is considered a serious problem in treatment of cancer diseases [40]. Glutathione *S*-transferases (GSTs) comprise a superfamily of multifunctional phase II detoxification enzymes catalyzing the conjugation of glutathione (GSH) with a variety of exogenous and endogenous xenobiotics. The glutathione conjugates having less cytotoxicity and greater hydrophilicity excreted from the cells *via* the mercapturic acid pathway [41]. Overexpression of GSTs was noticed in a number of tumor cases [42-44]. Chemotherapeutic alkylating agents such as busulfan, melphalan, chlorambucil, brostallicin, and immunosuppressant azathioprine described as the substrates of these enzymes. Therefore, many compounds including analogs of glutathione and its conjugates as well as derivatives of benzoquinone, benzophenone, nitrobenzoxadiazole, and curcumin were designed as inhibitors of GSTs [41-44].

Previously, a series of rhodanine-3-alkanoic acid derivatives were described as potential inhibitors of protein tyrosine phosphatase [45]. In the current study, we report synthesis of thiazole-containing rhodanine-3-alkanoic acids and their *in vitro* evaluation as inhibitors of PTPs and GSTs.

Results and discussion

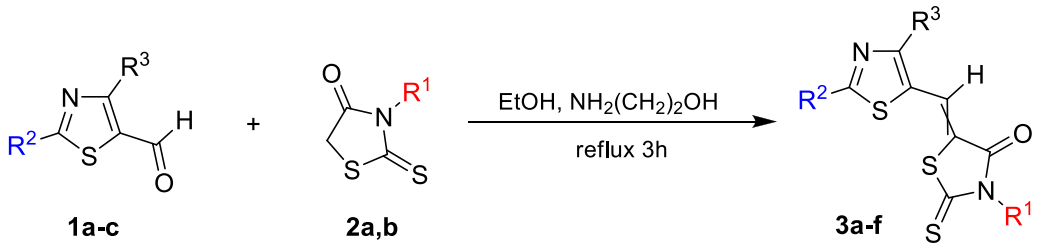
Synthesis of 5-(thiazol-5-ylmethylene)-2-thioxothiazolidin-4-one derivative

The synthesis of thiazole-containing rhodanine-3-carboxyalkyl acids **3a-f** is outlined in Scheme 1. The compounds were obtained by Knoevenagel condensation of corresponding rhodanines with aldehydes. Ethanol solution containing a proper 1,3-thiazolecarbaldehyde (compounds **1a-c**), *N*-alkyl carboxylated rhodanine (compounds **2a**, **b**), and 2-aminoethanol was heated for 3h. The obtained precipitate was filtered off and recrystallized from ethanol [46]. The compounds were obtained in moderate to good yield and were characterized by ^{1}H NMR, ^{13}C NMR and mass spectra. The data of NMR spectra showed that the newly synthesized compounds are represented by one of two Z/E isomeric forms. It should be noted that similar thiazole-contained rhodanine derivatives were described as *Z*-isomers [39].

Biological evaluation of thiazole-containing rhodanine-3-alkanoic acids as inhibitors of protein tyrosine phosphatases and glutathione S-transferases

Human recombinant protein tyrosine phosphatases PTP1B, VE-PTP, MEG1, and MEG2 were used for *in vitro* assays of the synthesized thiazole-containing rhodanine-3-carboxyalkyl acids **3a-f**. Values of the half maximal inhibitory concentrations (IC_{50}) for the compounds are presented in Table 1.

As can be seen from Table 1, rhodanine derivatives **3a-c** containing shorter alkyl carboxylated group were less effective inhibitors of PTPs than derivatives **3d-f** bearing longer *N*-alkyl chain. Compound **3a**, which possesses butyric acid fragment in *N*-3 position of rhodanine scaffold and phenyl substituent at *C*-2 position of 1,3-thiazol-5-ylmethylene moiety, was found to be a weak inhibitor of



	R^1	R^2	R^3
3a	$(CH_2)_3$-COOH	Ph	H
3b	$(CH_2)_3$-COOH	C_6H_4-p-Cl	H
3c	$(CH_2)_3$-COOH	$N(CH_3)_2$	Ph
3d	$(CH_2)_{10}$-COOH	Ph	H
3e	$(CH_2)_{10}$-COOH	C_6H_4-p-Cl	H
3f	$(CH_2)_{10}$-COOH	$N(CH_3)_2$	Ph

Scheme 1. Synthesis of thiazole-containing rhodanine-3-alkanoic acids.

Table 1. IC_{50} values of thiazole-containing rhodanine derivatives **3a-f** as inhibitors of protein tyrosine phosphatases*

Compd	IC_{50}, μM			
	PTP1B	MEG1	MEG2	VE-PTP
3a	> 25	> 25	> 25	> 25
3b	17.0 ± 4.4	>25	18.7±3.2	> 25
3c	> 25	> 25	> 25	> 25
3d	4.1 ± 1.2	23.1 ± 3.9	2.4 ± 0.4	2.4 ± 0.7
3e	0.82 ± 0.17	12.4 ± 3.5	0.54 ± 0.12	0.43 ± 0.07
3f	2.5 ± 0.6	11.0 ± 1.4	2.4 ± 0.5	3.1 ± 0.6

*IC_{50} values represent the mean of 2–3 assays ± standard deviation.

PTPs. The introduction of a chlorine substituent into the *para*-position of phenyl group of this rhodanine derivative slightly increases inhibitory effects of compound **3b** against PTP1B and MEG2. However, the replacement of 4-chlorophenyl residue by dimethylamino one, as well as introduction of phenyl group at *C*-4 position of 1,3-thiazol-5-ylmethylene moiety (compound **3c**) caused a decrease in inhibitory effects. The rhodanine derivative **3d** bearing undecanoic acid fragment at position *N*-3 showed significant increase in inhibition of PTP1B, VE-PTP, and MEG2 as compared to structure of compound **3a** with butyric acid fragment. Similarly to the effects obtained for rhodanine derivative **3b**, the introduction of chlorine atom in the *para*-position of phenyl group of 1,3-thiazol-5-ylmethylene moiety of compound **3d** led to increased inhibitory potential of rhodanine derivative **3e**. IC_{50} values of this compound were 0.82 μM, 0.54, and 0.43 μM μM for PTP1B, MEG2, and VE-PTP, respectively.

Kinetic studies were carried out to elucidate the possible mechanism of protein tyrosine phosphatases inhibition by the thiazole-containing rhodanine derivatives. According to Lineweaver-Burk plots (Figure 1), compound **3e** is a competitive-type inhibitor of protein tyrosine phosphatase VE-PTP with the calculated value of inhibition constant K_i of 0.20 μM.

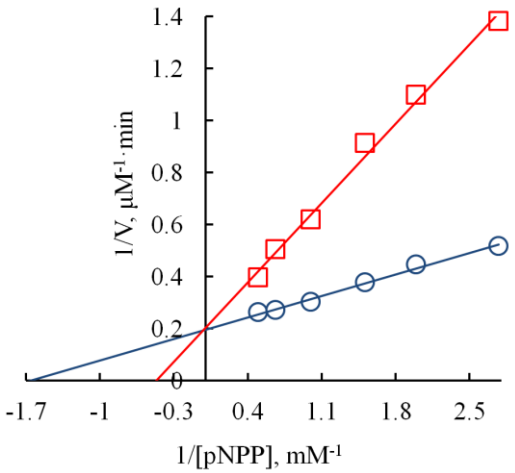


Figure 1. Lineweaver-Burk plots for inhibition of VE-PTP by compound **3e**. The inhibitor concentrations were 0 (○) and 0.5 μM (□).

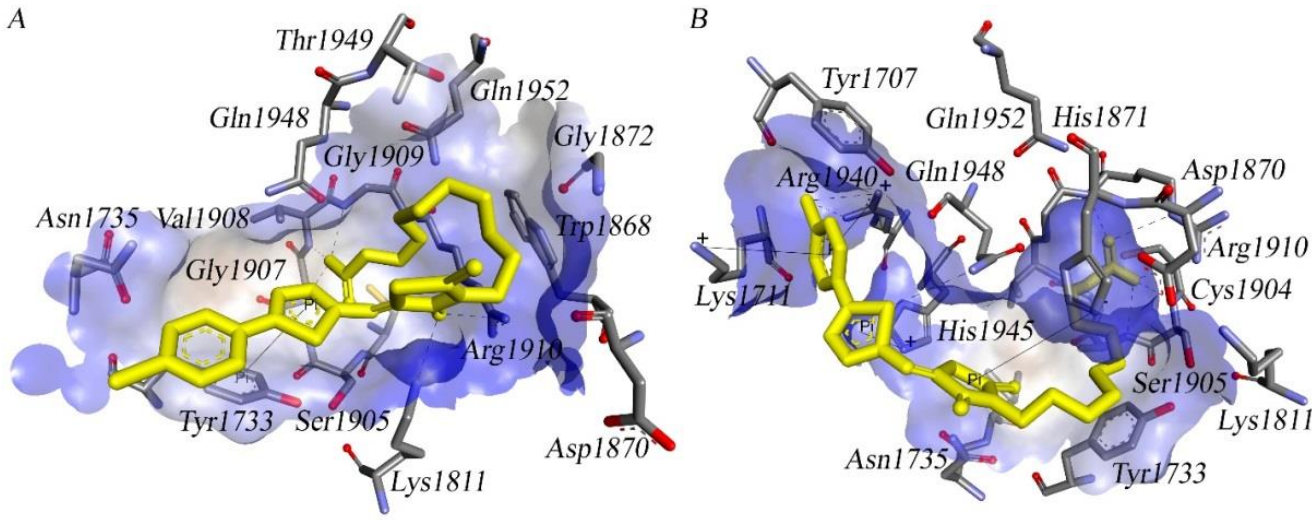


Figure 2. Possible binding modes of rhodanine derivative **3e** to human recombinant VE-PTP with open (A) and closed (B) conformations.

Molecular docking was performed to predict possible binding mode of carboxylated rhodanine derivative with PTPs. Synthetic studies described 5-ene-rhodanines mainly as *Z*-isomeres [4, 39]. In this connection, *Z*-isomer of compound **3e** was chosen to be docked into the active site region of the open and closed conformations of human VE-PTP. The estimated affinity of carboxylated rhodanine derivative **3e** to the active site of VE-PTP in open conformation was found to be -5.9 kcal/mol. The rhodanine scaffold of the inhibitor formed hydrogen bonds with amino acids residues of Lys1811 and Arg1910. The carboxylic group of the alkyl chain occupies active site near catalytic Cys1904 forming hydrogen bonds with amino acid residues Ala1906, Gly1906, Val1908, and Gly1909, while thiazole ring provided π-stacking interaction with Tyr1733 (Figure 2A).

In case of closed enzyme conformation, the estimated binding energy was -6.4 kcal/mol. The obtained model indicates that compound may be oriented into the active site of VE-PTP by carboxylic group of alkyl chain. This fragment form hydrogen bonds with amino acid residues Ser1905, Ala1906, and Arg1910. The thiazole ring of compound **3e** is involved in hydrogen bond formation with Gln1948 and π-cation interaction with His1945. The 2-chlorophenyl group forms π-cation interactions with Lys1711 and Arg1940 as well as halogen bond of chlorine atom with Arg1940 (Figure 2B).

The thiazole-containing rhodanine-3-carboxyalkyl acids were studied as inhibitors of GST from equine liver and human recombinant GSTA1-1. The obtained results (Table 2) demonstrated that compounds **3d-f** were more potent inhibitors in comparison with derivatives **3a-c** bearing butyric acid fragment at *N*-3 position of rhodanine scaffold. Better inhibition effects against GST from equine liver were observed in the case of compounds **3d-f** modified at *N*-3 position of rhodanine ring with undecanoic acid group. Further studies showed that these compounds can also inhibit the recombinant form of human GSTA1-1 with IC_{50} values in the low micromolar range.

Table 2. Inhibition activity of 5-(thiazol-5-ylmethylene)-2-thioxothiazolidin-4-one derivatives **3a-f** against GST from equine liver and human recombinant GSTA1-1*.

Compd	IC_{50}, μM	
	GST from equine liver	GSTA1-1
3a	> 25	-
3b	24.1 ± 2.7	-
3c	> 25	-
3d	5.2 ± 1.4	1.1 ± 0.2
3e	4.5 ± 0.5	0.83 ± 0.22
3f	6.2 ± 0.9	2.7 ± 0.7

*IC_{50} values are the means of 2-3 assays ± standard deviation.

Lineweaver-Burk plots (Figure 3) showed mixed-type or non-competitive inhibition of GST from equine liver by compound **3d** toward glutathione or CDNB substrate. According to the mixed-type inhibition, the calculated values of inhibition constants K_i and K_i' were 12.9±3.7 μM and 20.2±5.8 μM, respectively, while the non-competitive inhibition constant K_i was 6.8±1.8 μM.

The results of computer modeling suggest that *Z*-isomer of rhodanine derivative **3d** may occupy interdomain cavity near active site of human GSTA1-1 (Figure 4A) with the calculated docking energy of -9.3 kcal/mol. Deregulation of the interdomain contacts in structure of GSTA1-1 was shown to lead to disruption of the enzyme catalytic functions [47] which may explain the inhibition of the enzyme. The compound position (Figure 4B) is characterized by interaction of the inhibitor with hydrophobic amino acid residues Thr68, Leu72, Ile96, Ile99, Ala100, Ile106, Leu107, and Leu163. Thiazole ring at *C*-5 position of rhodanine scaffold formed π-stacking interaction with Tyr166, while phenyl fragment at *C*-2 position of the thiazole ring provided π-cation interaction with Arg13. Hydrogen bond was observed between oxygen atom of rhodanine scaffold and Ser18. Carboxylate group of the inhibitor is adjacent to the active G-site and provides

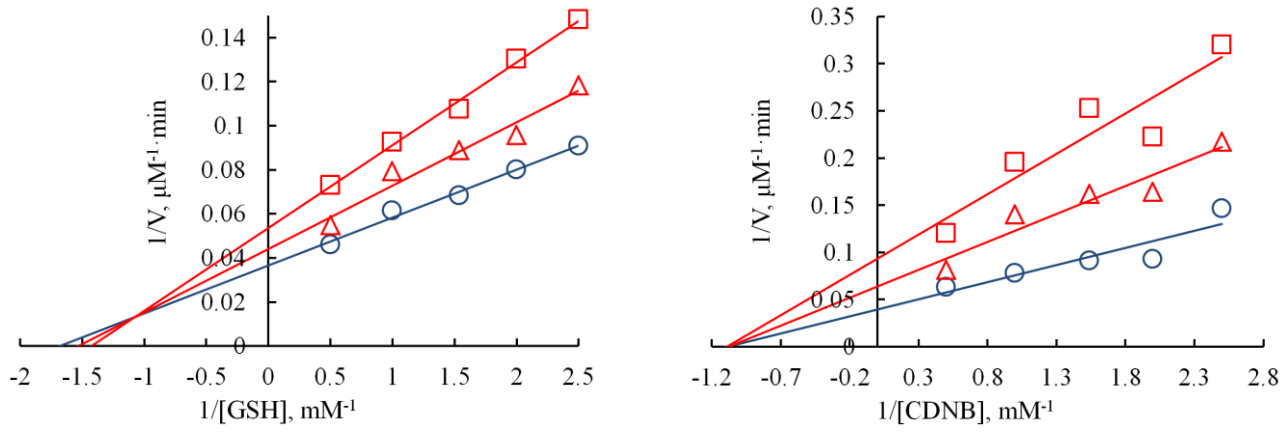


Figure 3. Lineweaver-Burk plots for inhibition of GST from equine liver by compound **3d**. The inhibitor concentrations were 0 (○), 5 μM (Δ) and 7.5 μM (□).

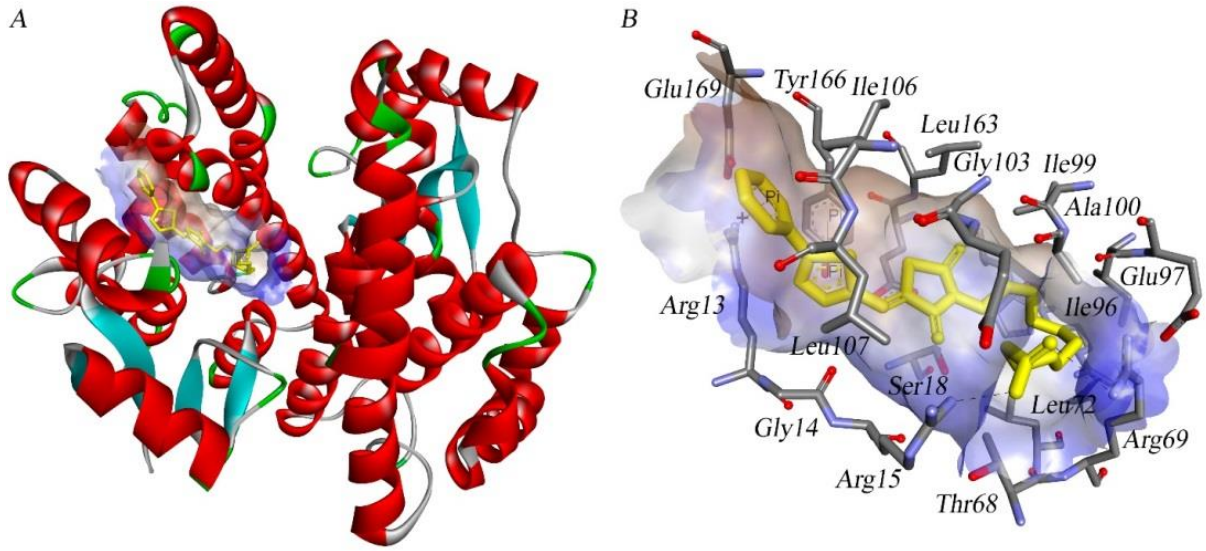


Figure 4. Possible binding mode of rhodanine derivative **3d** to homodimer structure of human GSTA1-1.

hydrogen bonds with amino acid residues Arg69 and Arg15 which is involved in interaction with GSH.

Conclusions

The study reported synthesis of new rhodanine-3-alkanoic acids with propanoic or undecanoic acid groups. The data obtained showed that compounds **3d-f** bearing undecanoic acid group at *N*-3 position of rhodanine scaffold possess good inhibitory effects against PTP1B, MEG1, MEG2, and VE-PTP as well as GSTA1-1. According to kinetic data, thiazole-containing rhodanines can be competitive-type inhibitors of VE-PTP. In case of GST from equine liver, the compounds can be considered as mixed-type inhibitors toward GSH and non-competitive toward CDNB substrate. Molecular docking results indicate that the inhibitors may occupy VE-PTP active site, while inhibition of GSTA1-1 might be explained by the location of rhodanine derivative between *C*- and *N*-terminal subunits of the enzyme.

Experimental section

Chemistry

^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on Bruker Avance DRX 500 spectrometer in DMSO-d_6 solution. IR spectra were recorded on a Vertex 70 spectrometer from KBr pellets. Melting points were measured with a Büchi melting point apparatus and are uncorrected. LC-MS spectra were obtained using HPLC apparatus, Agilent 1100 Series, equipped with diode-matrix and mass-selective detector Agilent LC/MSD SL.

*General procedure for synthesis of compounds **3a-f***

A solution of 0.002 mol of rhodanine derivative **2a** or **2b** in 5 mL of ethanol and 0.02 mL of 2-aminoethanol were added to a solution of 0.002 mol of corresponding aldehyde (**1a-c**) in 5 mL of ethanol. The mixture was refluxed for 3 h and cooled. The precipitate was filtered off and recrystallized from EtOH.

*4-{4-Oxo-5-[(2-phenyl-1,3-thiazol-5-yl)methylidene]-2-sulfanylidene-1,3-thiazolidin-3-yl}butanoic acid (**3a**).*

Yield: 0.531 g (68%); yelow crystals; mp 190-191 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.11 (br s, 1H, COO*H*), 8.48 (s, 1H, C*H*), 8.12 (s, 1H, C^4-$H_{thiazol}$), 8.03 (d, *J* 7.7 Hz, 2H, Ph), 7.49-7.59 (m, 3H, Ph), 4.06 (t, *J* 6.8 Hz, 2H, CH_2), 2.30 (t, *J* 7.2 Hz, 2H, CH_2), 1.84-1.95 (m, 2H, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ 192.5, 174.1, 172.8, 166.8, 151.2, 133.7, 132.6, 132.0, 129.9, 127.1, 123.7, 123.2, 44.4, 31.4, 22.4. LC/MS (CI) m/z 391 $(M)^+$. Anal. Calcd. for $C_{17}H_{14}N_2O_3S_3$: C, 52.29; H, 3.61; N, 7.17; S, 24.63. Found: C, 52.35; H, 3.60; N, 7.10; S, 24.64.

*4-(5-{[2-(4-Chlorophenyl)-1,3-thiazol-5-yl]methylidene}-4-oxo-2-sulfanylidene-1,3-thiazolidin-3-yl)butanoic acid (**3b**).*

Yield: 0.484 g (57%); yelow crystals; mp 201-202 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.12 (br s, 1H, COO*H*), 8.47 (s, 1H, C*H*), 8.10 (s, 1H, C^4-$H_{thiazol}$), 8.02 (d, *J* 8.5 Hz, 2H, C$_6H_4$-p-Cl), 7.56 (d, *J* 8.5 Hz, 2H, C$_6H_4$-p-Cl), 4.05 (t, *J* 6.8 Hz, 2H, CH_2), 2.31 (t, *J* 7.1 Hz, 2H, CH_2), 1.83-1.93 (m, 2H, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ 190.2, 171.9, 169.0, 164.6, 148.9, 134.4, 131.8, 129.2, 127.7, 126.5, 121.7, 120.8, 42.2, 29.2, 20.1. LC/MS (CI) m/z 426 $(M)^+$. Anal. Calcd. for $C_{17}H_{13}ClN_2O_3S_3$: C, 48.05; H, 3.08; N, 6.59; S, 22.64. Found: C, 48.14; H, 3.04; N, 6.53; S, 22.54.

*4-(5-{[2-(Dimethylamino)-4-phenyl-1,3-thiazol-5-yl]methylidene}-4-oxo-2-sulfanylidene-1,3-thiazolidin-3-yl)butanoic acid (**3c**).*

Yield: 0.702 g (81%); yelow crystals; mp 179-180 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.63 (s, 1H, C*H*), 7.50-7.62 (m, 5H, Ph), 3.98 (t, *J* 6.5 Hz, 2H, CH_2), 3.20 (s, 6H, N(CH_3)$_2$), 2.16 (t, *J* 7.2 Hz, 2H, CH_2), 1.74-1.87 (m, 2H, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ 191.2, 174.2,

172.2, 166.4, 161.8, 133.6, 129.7, 129.4, 128.7, 125.4, 116.9, 113.8, 44.0, 41.7, 32.0, 22.5. LC/MS (CI) m/z 434 $(M)^+$. Anal. Calcd. for $C_{19}H_{19}N_3O_3S_3$: C, 52.64; H, 4.42; N, 9.69; S, 22.19. Found: C, 52.68; H, 4.39; N, 9.68; S, 22.12.

*11-{4-Oxo-5-[(2-phenyl-1,3-thiazol-5-yl)methylidene]-2-sulfanylidene-1,3-thiazolidin-3-yl}undecanoic acid (**3d**).*

Yield: 0.733 g (75%); yelow crystals; mp 141-142 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.43 (s, 1H, C*H*), 8.07 (s, 1H, C^4-*H*$_{thiazol}$), 7.99 (d, *J* 8.1 Hz, 2H, Ph), 7.46-7.56 (m, 3H, Ph), 3.94 (t, *J* 7.3 Hz, 2H, C*H*$_2$), 2.16 (t, *J* 7.4 Hz, 2H, C*H*$_2$), 1.54-1.64 (m, 2H, C*H*$_2$), 1.41-1.51 (m, 2H, C*H*$_2$), 1.16-1.29 (m, 12H, (C*H*$_2$)$_6$). ^{13}C NMR (125 MHz, DMSO-d_6) δ 192.1, 174.9, 172.8, 166.6, 151.3, 133.6, 132.6, 132.0, 129.8, 127.1, 123.5, 123.4, 44.9, 34.1, 29.3, 29.3, 29.2, 29.0, 29.0, 26.7, 26.6, 25.0. LC/MS (CI) m/z 489 $(M)^+$. Anal. Calcd. for $C_{24}H_{28}N_2O_3S_3$: C, 58.99; H, 5.78; N, 5.73; S, 19.68. Found: C, 59.10; H, 5.73; N, 5.70; S, 19.69.

*11-(5-{[2-(4-Chlorophenyl)-1, 3-thiazol-5-yl]methylidene}-4-oxo-2-sulfanylidene-1,3-thiazolidin-3-yl)undecanoic acid (**3e**).*

Yield: 0.764 g (73%); yelow crystals; mp 173-174 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.76 (br s, 1H, COO*H*), 8.41 (s, 1H, C*H*), 8.06 (s, 1H, C^4-*H*$_{thiazol}$), 7.99 (d, *J* 7.7 Hz, 2H, C$_6$*H*$_4$-p-Cl), 7.54 (d, *J* 7.7 Hz, 2H, C$_6$*H*$_4$-p-Cl), 3.94-4.00 (m, 2H, C*H*$_2$), 2.16 (t, *J* 7.4 Hz, 2H, C*H*$_2$), 1.58-1.65 (m, 2H, C*H*$_2$), 1.44-1.51 (m, 2H, C*H*$_2$), 1.19-1.30 (m, 12H, (C*H*$_2$)$_6$). ^{13}C NMR (125 MHz, DMSO-d_6) δ 189.8, 172.4, 169.1, 164.4, 148.9, 134.4, 131.8, 129.3, 127.7, 126.5, 121.7, 120.9, 42.7, 31.9, 27.0, 26.9, 26.9, 26.7, 26.7, 24.4, 24.3, 22.7. LC/MS (CI) m/z 524 $(M)^+$. Anal. Calcd. for $C_{24}H_{27}ClN_2O_3S_3$: C, 55.10; H, 5.20; N, 5.35; S, 18.39. Found: C, 55.14; H, 5.15; N, 5.32; S, 18.37.

*11-(5-{[2-(Dimethylamino)-4-phenyl-1,3-thiazol-5-yl]-methylidene}-4-oxo-2-sulfanylidene-1,3-thiazolidin-3-yl)undecanoic acid (**3f**).*

Yield: 0.744 g (70%); yelow crystals; mp 114-115 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.67 (s, 1H, C*H*), 7.47-7.63 (m, 5H, Ph), 3.89-3.99 (m, 2H, C*H*$_2$), 3.21 (s, 6H, N(C*H*$_3$)$_2$), 2.15 (t, *J* 6.6 Hz, 2H, C*H*$_2$), 1.54-1.62 (m, 2H, C*H*$_2$), 1.42-1.50 (m, 2H, C*H*$_2$), 1.16-1.29 (m, 12H, (C*H*$_2$)$_6$). ^{13}C NMR (125 MHz, DMSO-d_6) δ 189.3, 172.5, 170.5, 164.5, 160.0, 131.9, 127.6, 126.9, 123.7, 123.6, 115.1, 112.0, 42.5, 32.0, 26.9, 26.9, 26.8, 26.8, 26.7, 26.6, 24.5, 24.3, 22.8. LC/MS (CI) m/z 532 $(M)^+$. Anal. Calcd. for $C_{26}H_{33}N_3O_3S_3$: C, 58.73; H, 6.26; N, 7.90; S, 18.09. Found: C, 58.79; H, 6.26; N, 7.84; S, 18.03.

Biological tests

In vitro study of thiazole-containing rhodanine-3-alkanoic acid derivatives as inhibitors of protein tyrosine phosphatases and glutathione S-transferases

Protein tyrosine phosphatases were purchased in Sigma-Aldrich. Prior to experiments, the defined volume of PTP1B, VE-PTP, MEG1 and MEG2 were diluted in a solution of 50 mM Bis-Tris buffer (pH 7.2) containing 30% glycerol, 3 mM EDTA, 2 mM DTT, 75 mM NaCl, and 0.05% Tween-20. The system for inhibition study consisted of 50 mM Bis-Tris buffer (pH 7.2), 100 mM NaCl, 3 mM EDTA, 1 mM DTT, 1 vol. % DMSO, inhibitor and enzyme. The mixture was thermostated at 30 ºC during 5 min and reaction was started by adding the substrate (pNPP) at concentration near K_m value for each of the enzymes. The activity of enzymes was measured spectrophotometrically at 410 nm. The molar extinction coefficient of 18300 $M^{-1}cm^{-1}$ was used for calculation of *p*-nitrophenol concentration.

GST from equine liver and human recombinant GSTA1-1 was purchased from Sigma-Aldrich. Before use in the experiments, 0.25 mg of GST from equine liver was diluted in 1 ml of distillated water, and 25 µL of GSTA1-1 was diluted in 1 ml of solution consisted of 50 mM Tris-HCl buffer (ph 7.5), 50 mM NaCl, 1 mM DTT, 5 mM EDTA and 50 vol. % glycerol. The rhodanine derivatives were dissolved in DMSO. *In vitro* studies were carried out in system consisting of 0.1 M sodium-phosphate buffer (pH 6.5), 0.1 mM EDTA, 2.5 vol. % DMSO, water, 20 µl of enzyme solution and inhibitor. After incubation of this mixture at 25 ºC during 5 min, the reaction was started by addition 200 µL of 10 mM reduced L-glutathione (GSH) and 20 µL of 100 mM 1-chloro-2,4-dinitrobenzene (CDNB). The enzyme activity was monitored spectrophotometrically at 340 nm. The molar extinction coefficient of 9600 $M^{-1}cm^{-1}$ was used for calculation of dinitrophenyl-*S*-glutathione concentration [48].

Molecular docking calculation

Crystal structures of open and closed conformation of VE-PTP (PDB code 2AHS and 2H02, respectively) and GSTA1-1 (PDB code 6ATO) were downloaded from PDB server (https://www.rcsb.org) [49]. Before docking calculation, the ligands, water molecules and amino acids conformers were removed from obtained PDB files. The structure of thiazole-containing rhodanine derivatives were drawn using MarvinSketch [50] and optimized with MMFF94s force field in Avogadro software [51]. Docking files were prepared using AutoDockTools (version 1.5.6) [52]. The docking calculations were carried out by Autodock Vina software [53]. The models visualizations and analysis was performed using Discovery Studio 3.5 Visualizer (Accelrys Inc., San Diego, CA, USA).

Notes

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Author contributions. **O. L. K.**: conceptualization, supervision, molecular docking simulation, writing-original draft. **V. O. S.**: synthesis of compounds, investigation, formal analysis. **Y. V. S.**: investigation of bioactivity. **V. M. B.**: investigation of bioactivity. **D. M. H.**:

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Синтез та оцінка нових тіазоловмісних роданін-3-алканових кислот як інгібіторів протеїнтирозинфосфатаз та глутатіон-*S*-трансфераз

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Резюме: Тіазоловмісні похідні роданін-3-алканової кислоти, модифіковані залишками пропанової чи ундеканової кислот, синтезовано та оцінено як інгібітори деяких протеїнтирозинфосфатаз та глутатіон-*S*-трансфераз. Сполуки отримано за реакцією Кновевенагеля взаємодією відповідних роданінів з альдегідами. Встановлено, що роданіни з довшим карбоксильованим *N*-алкільним ланцюгом інгібують активність PTP1B, MEG1, MEG2 та VE-PTP, а також GST з печінки коня та людську рекомбінантну GSTA1-1 зі значеннями IC_{50} у низькому мікромолярному діапазоні. Інгібувальний ефект на активність протеїнтирозинфосфатаз залежав від замісника в 2 положенні тіазолового кільця, тоді як природа замісників у положенні 2 та 4 мала незначний вплив на інгібувальну активність сполук щодо глутатіон-*S*-трансфераз. Найкраща сполука, 11-(5-{[2-(4-хлорфеніл)-1,3-тіазол-5-іл]метиліден}}-4-оксо-2-сульфаніліден-1,3-тіазолідин-3-іл)ундеканова кислота, продемонструвала конкурентний тип інгібування VE-PTP. У випадку GST з печінки коня сполука виявилась змішаним інгібітором при використанні GSH як субстрату та неконкурентним інгібітором у разі CDNB. Результати молекулярного докінгу вказують на те, що інгібітор може займати активний центр протеїнтирозинфосфатази VE-PTP, тоді як інгібування людської рекомбінантної GSTA1-1 може бути пояснено розташуванням похідної роданіну між *C*- та *N*-кінцевими доменами однієї з субодиниць ферменту.

Ключові слова: роданін; тіазол; протеїнтирозинфосфатаза; глутатіон-*S*-трансфераза; інгібування ферменту; молекулярний докінг.