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Original article

Synthesis and biological evaluation of substituted (thieno[2,3-d]pyrimidin-4-ylthio)carboxylic acids as inhibitors of human protein kinase CK2

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ABSTRACT

A novel series of substituted (thieno[2,3-d]pyrimidin-4-ylthio)carboxylic acids has been synthesized and tested in vitro towards human protein kinase CK2. It was revealed that the most active compounds inhibiting CK2 are 3-{[5-(4-methylphenyl)thieno[2,3-d]pyrimidin-4-yl]thio}propanoic acid and 3-{[5-(4-ethoxyphenyl)thieno[2,3-d]pyrimidin-4-yl]thio}propanoic acid (IC_{50} values are 0.1 μM and 0.125 μM, respectively). Structure–activity relationships of 28 tested thienopyrimidine derivatives have been studied and binding mode of this chemical class has been predicted. Evaluation of the inhibitors on seven protein kinases revealed considerable selectivity towards CK2.

1. Introduction

Protein kinase CK2 has been intensively studied for more than 50 years, however, its biological role and regulation of its activity is still remains unclear [1]. Possessing constitutive catalytic activity with the ability to phosphorylate more than 300 physiological substrates (their number is growing continuously), this kinase stands out noticeably against a background of the kinase family [2]. Naturally, these features make CK2 appear at extremely diverse points of cell signaling pathways and be involved in processes leading to the development of various disorders, especially cancer. Increased CK2 expression and/or activity both have been shown in malignant cells and cells of intensively proliferating tissues [3]. This is related to the fact that CK2 can function as a general antiapoptotic agent [4], and its activity is quite important for determining whether cell will survive or die through apoptosis. Moreover, some viruses use this enzyme to phosphorylate their own proteins [5–8]; also this kinase is involved in development of inflammation processes [9]. Therefore, nowadays CK2 is considered as druggable protein kinase target and could be used for the development of antitumor, antiviral and anti-inflammatory drugs [10].

Effective approach to study the function of protein kinases is the use of small molecule inhibitors. Taking into account that CK2 is not activated in response to external stimuli and exists in cell only in active form the mentioned approach is especially useful for CK2 targeting [11]. There are many compounds inhibiting CK2 that have been discovered [12]; nevertheless, none of them is in clinical use. So far, the only one orally administered highly selective and potent CK2 inhibitor CX-4945 has entered phase I clinical trials due to its broad spectrum antiproliferative activity in multiple cancer cell lines including inflammatory breast cancer [13]. Thus, development of new potent and selective CK2 inhibitors is a task of great importance which provides a powerful tool to extend our knowledge about CK2 function as well as to regulate its activity both in case of health and disease.

The goal of our research was to identify potent and selective inhibitors of CK2 among thieno[2,3-d]pyrimidines. Compounds from this chemical class are known to inhibit activity of ErbB, PDGF, VEGFR-2, FLT3 and Tie2 kinases [14–16], but there are no data available on their activity towards CK2. Data regarding synthesis and study of (thieno[2,3-d]pyrimidin-4-ylthio)carboxylic acids are rare in literature [17]. Taking into account that many potent CK2 inhibitors published at the moment contain carboxyl group in their

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structure [18–21], we focused our studies on (thieno[2,3-d]pyrimidin-4-ylthio)carboxylic acids.

2. Experimental section

2.1. Chemistry

General synthetic procedure for compounds **5a**–**u**, **6a**–**g** was performed according to Scheme 1.

Nuclear magnetic resonance spectra were recorded on a Varian Mercury VRX-400 spectrometer using DMSO-d_6 as solvent and tetramethylsilane as internal standard. Chemical shift values (δ) are quoted in ppm, and coupling constants (J) in Hz.

2.1.1. Substituted 2-aminothiophenes ***1***

A mixture 108 mL (1 mol) of ethyl cyanoacetate, 32 g (1 mol) of elemental sulfur and 1 mol of corresponding ketone in 200 ml of diethyl ether was stirred at room temperature. To this mixture 80 ml of diethylamine was added for 12 h. The solid product was collected by filtration and washed with aqueous alcohol (1:1), yield 50–80%.

2.1.2. Substituted thieno[2,3-d]pyrimidin-4(3H)-ones ***2***

1 mol formamide was added to 1 mol 2-aminothiophene and the mixture was heated at 160–170 °C for 24 h. The mixture was diluted with isopropyl alcohol. The solid product was collected by filtration and washed with isopropyl alcohol and water and dried, yield 80%.

2.1.3. Substituted 4-chlorothieno[2,3-d]pyrimidines ***4***

A mixture of 0.33 mol thieno[2,3-d]pyrimidin-4(3H)-one, 333 mL $POCl_3$ and 0.33 mol PCl_5 was boiled until a homogeneous solution was formed. $POCl_3$ was distilled off in vacuum and equal volume of chloroform was slowly added into the warm mixture and pH was adjusted to 7 with 10% aqueous NaOH. The organic phase was separated and dried over Na_2SO_4, filtered, and then solvent was evaporated in vacuum. The residue was crystallized from isopropyl alcohol or hexane. The yield of this product was 80%.

2.1.4. Substituted (thieno[2,3-d]pyrimidin-4-ylthio)carboxylic acids ***5a–u***

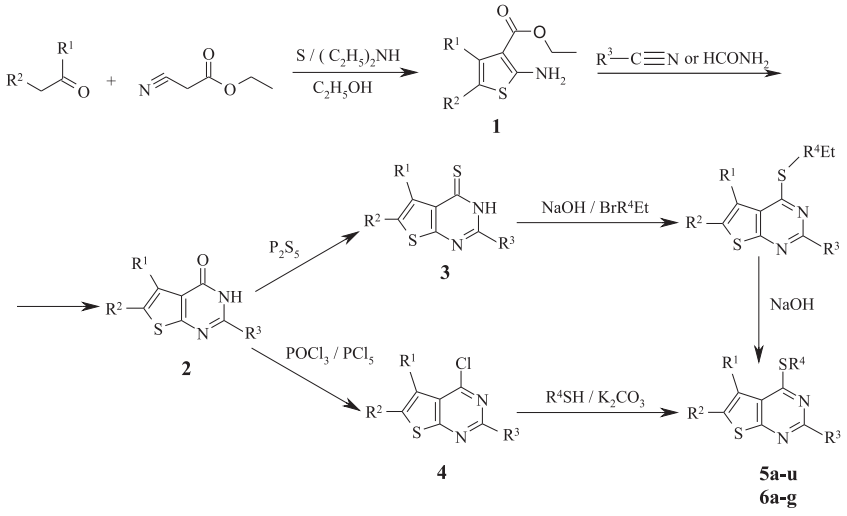
Substituted thieno[2,3-d]pyrimidine-4(3H)-thiones **3** were synthesized as described in Reference [22]. Compounds **5a**–**u** shown in Table 1 were synthesized on the base of **3**. To 0.01 mol of thieno [2,3-d]pyrimidine-4(3H)-thione in 5 mL ethanol and 0.01 mol 2 M of aqueous sodium hydroxide the 0.0105 mol of bromine substituted carboxylic acid ethyl ester was added. The mixture was refluxed for 5 min; in some reactions with ethyl 3-bromopropropionate the concentration of this reagent was slightly elevated up to 0.012 mol and the mixture was heated under reflux for 30 min. Water and dichloromethane were added to the mixture and the resulted phases were separated. The aqueous phase was extracted 2 times with CH_2Cl_2, the combined organic dried over Na_2SO_4, filtered, and concentrated in vacuum to afford the ester. The ester was hydrolyzed to solid acid.

*2.1.4.1. 2-(5,6,7,8-Tetrahydro[1]benzothieno[2,3-d]pyrimidin-4-ylthio) propanoic acid (**5a**).* Yield 80%; m.p. 162–163 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 1.58 (3H, d, CH_3 J = 7.3 Hz), 1.83 (4H, m, CH_2, $-(CH_2)_4-$), 2.82–2.98 (4H, m, CH_2, $-(CH_2)_4-$), 4.73 (1H, q, J = 7.3 Hz, J = 7.3 Hz), 8.69 (1H, s, 2-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 17.50, 21.98, 25.20, 26.16, 41.00 95.41, 126.83, 127.23, 136.57, 150.92, 161.08, 164.58, 172.57. Anal. Calc. for $C_{13}H_{14}N_2O_2S_2$: C, 53.04; H, 4.79; S, 21.78. Found: C, 53.02; H, 4.76; S, 21.77.

*2.1.4.2. 2-[(7-Methyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4-yl)thio]propanoic acid (**5b**).* Yield 81%; m.p. 134–135 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 1.06 (3H, d, CH_3, J = 6.5 Hz), 1.46 (1H, m), 1.57 (3H, d, CH_3, J = 7.3 Hz), 1.92 (2H, m), 2.42 (1H, m), 2.91 (2H, m), 3.14 (1H, m), 4.73 (1H, q, J = 7.3 Hz, J = 7.3 Hz), 8.69 (1H, s, 2-H), 12.92 (bs, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 17.58, 20.95, 25.84, 28.25, 30.08, 33.12, 40.90, 95.50, 126.45, 127.10, 135.94, 150.76, 161.09, 164.75, 172.49. Anal. Calc. for $C_{14}H_{16}N_2O_2S_2$: C, 54.52; H, 5.23; S, 20.79. Found: C, 54.50; H, 5.21; S, 20.77.

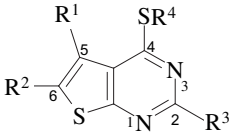
*2.1.4.3. 2-[(5,6-Dimethylthieno[2,3-d]pyrimidin-4-yl)thio]propanoic acid (**5c**).* Yield 83%; m.p. 208–209 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 1.58 (3H, d, CH_3, J = 7.3 Hz), 2.47 (3H, s, CH_3), 2.5 (3H, s, CH_3), 4.73 (1H, q, J = 7.3 Hz, J = 7.3 Hz), 8.69 (1H, s, 2-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 13.49, 14.27, 17.45, 41.00, 95.47, 124.73, 128.14, 133.40, 150.62, 161.27, 163.93, 172.55. Anal. Calc. for $C_{11}H_{12}N_2O_2S_2$: C, 49.23; H, 4.51; S, 23.90. Found: C, 49.21; H, 4.50; S, 23.88.

*2.1.4.4. [(5-Phenylthieno[2,3-d]pyrimidin-4-yl)thio]acetic acid (**5d**).* Yield 80%; m.p. 174–175 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 3.95 (2H, s), 7.48 (5H, m), 7.80 (1H, s, C_6H), 8.84 (1H, s, 2-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 31.76, 95.41, 125.15, 125.67, 127.81, 128.38,



Scheme 1. The synthesis pathway for compounds **5a**–**u**, **6a**–**g**.

Table 1
Structure and inhibitory activity towards CK2 of the (thieno[2,3-d]pyrimidin-4-ylthio)carboxylic acids.



Compound	R^1	R^2	R^3	R^4	IC_{50} (μM)
5a	$-CH_2-CH_2-CH_2-CH_2-$		H	$CH(CH_3)COOH$	17
5b	$-CH_2-CH_2-CH(CH_3)-CH_2-$		H	$CH(CH_3)COOH$	20
5c	CH_3	CH_3	H	$CH(CH_3)COOH$	>33
5d	C_6H_5	H	H	CH_2COOH	30
5e	$-CH_2-CH_2-CH(CH_3)-CH_2-$		H	CH_2COOH	1.78
5f	$-CH_2-CH_2-CH_2-CH_2-$		H	$CH(C_2H_5)COOH$	>33
5g	CH_3	CH_3	H	CH_2COOH	17.75
5h	C_6H_5	H	H	$CH(CH_3)COOH$	23
5i	4-ClC_6H_4	H	H	CH_2COOH	32
5j	4-ClC_6H_4	H	H	$CH(C_2H_5)COOH$	>33
5k	H	C_6H_5	H	CH_2COOH	1.25–1.75
5l	4-$CH_3C_6H_4$	H	H	$CH(CH_3)COOH$	31
5m	4-$CH_3C_6H_4$	H	H	$CH(C_2H_5)COOH$	>33
5n	4-BrC_6H_4	H	H	CH_2COOH	14
5o	-–$CH_2-CH_2-CH_2-CH_2-$		H	$CH_2C_6H_4COOH-p$	>33
5p	4-ClC_6H_4	H	H	CH_2-CH_2COOH	0.175
5q	CH_3	CH_3	H	$CH_2C_6H_4COOH-m$	17.75
5r	$-CH_2-CH_2-CH_2-CH_2-$		CH_3	CH_2-CH_2COOH	15.8
5s	$-CH_2-CH_2-CH_2-CH_2-$		H	$CH_2-(CH_2)_2COOH$	>33
5t	CH_3	CH_3	H	CH_2-CH_2COOH	11.3
5u	C_6H_5	CH_3	H	CH_2COOH	>33
6a	4-$CH_3C_6H_4$	H	H	CH_2-CH_2COOH	0.1
6b	3,4-$(CH_3)_2C_6H_3$	H	H	CH_2-CH_2COOH	0.175
6c	H	CH_3	H	CH_2COOH	4
6d	4-$C_2H_5OC_6H_4$	H	H	CH_2-CH_2COOH	0.125
6e	4-FC_6H_4	H	H	CH_2-CH_2COOH	1.2
6f	H	CH_3	H	CH_2-CH_2COOH	7
6g	H	C_6H_5	CH_3	CH_2-CH_2COOH	3.5

129.93, 134.82, 135.04, 151.49, 163.29, 165.80, 169.33. Anal. Calc. for $C_{14}H_{10}N_2O_2S_2$: C, 55.61; H, 3.33; S, 21.21. Found: C, 55.60; H, 3.31; S, 21.20.

*2.1.4.5. [(7-Methyl-5,6,7,8,-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4-yl)thio]acetic acid (**5e**).* Yield 85%; m.p. 209–208 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 1.06 (3H, d, CH_3, J = 6.5 Hz), 1.48 (1H, m), 1.94 (2H, m), 2.43 (1H, m), 2.93 (2H, m), 3.18 (1H, m), 4.12 (2H, s), 8.68 (1H, s). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 20.97, 25.76, 28.24, 30.06, 31.52, 33.07, 95.44, 126.47, 127.23, 136.03, 150.90, 161.21, 164.52, 169.35. Anal. Calc. for $C_{13}H_{14}N_2O_2S_2$: C, 53.04; H, 4.79; S, 21.78. Found: C, 53.03; H, 4.76; S, 21.75.

*2.1.4.6. 2-(5,6,7,8-Tetrahydro[1]benzothieno[2,3-d]pyrimidin-4-ylthio) butanoic acid (**5f**).* Yield 82%; m.p. 110–111 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 1.02 (3H, t, CH_3), 1.84 (4H, m, CH_2, $-(CH_2)_4-$), 1.89–2.04 (2H, m, CH_2), 2.84–3.01 (4H, m), 4.74 (1H, t), 8.69 (1H, s, 2-H), 12.93 (bs, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 11.48, 22.00, 24.84, 25.22, 26.22, 47.25, 95.44, 126.85, 127.32, 136.51, 150.84, 161.19, 164.59, 171.93. Anal. Calc. for $C_{14}H_{16}N_2O_2S_2$: C, 54.52; H, 5.23; S, 20.79. Found: C, 54.50; H, 5.22; S, 20.78.

*2.1.4.7. [(5,6-Dimethylthieno[2,3-d]pyrimidin-4-yl)thio]acetic acid (**5g**).* Yield 84%; m.p. 209–210 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 2.47 (3H, s, CH_3), 2.53 (3H, s, CH_3), 4.12 (2H, s, CH_2), 8.68 (1H, s, 2-H), 12.79 (bs, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 13.46, 14.20, 31.60, 95.44, 124.73, 128.29, 133.42, 150.70, 161.37, 163.71, 169.37. Anal. Calc. for $C_{10}H_{10}N_2O_2S_2$: C, 47.23; H, 3.96; S, 25.21. Found: C, 47.21; H, 3.95; S, 25.20.

*2.1.4.8. 2-[(5-Phenylthieno[2,3-d]pyrimidin-4-yl)thio]propanoic acid (**5h**).* Yield 80%; m.p. 111–112 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 1.40 (3H, d, CH_3, J = 7.3 Hz), 4.61 (1H, q, J = 7.3 Hz, J = 7.3 Hz), 7.42–7.49 (5H, m, C_6H_5), 7.78 (1H, s, 6-H), 8.84 (1H, s, 2-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 17.24, 41.18, 95.44, 125.16, 127.76, 128.36, 129.86, 134.81, 151.38, 163.19, 166.02, 172.54. Anal. Calc. for $C_{15}H_{12}N_2O_2S_2$: C, 56.94; H, 3.82; S, 20.27. Found: C, 56.93; H, 3.80; S, 20.25.

*2.1.4.9. {[5-(4-Chlorophenyl)thieno[2,3-d]pyrimidin-4-yl]thio}acetic acid (**5i**).* Yield 85%; m.p. 203–204 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 3.89 (2H, s, CH_2), 7.45 (2H, d, J = 8.5 Hz, 4-ClC_6H_4), 7.48 (2H, d, J = 8.5 Hz, 4-ClC_6H_4), 7.66 (1H, s, 6-H), 8.76 (1H, s 2-H), 12.51 (bs, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 31.78, 95.42, 125.70, 127.85, 131.70, 133.48, 151.57, 163.16, 165.79, 169.29. Anal. Calc. for $C_{14}H_9ClN_2O_2S_2$: C, 49.92; H, 2.69; S, 19.04. Found: C, 49.90; H, 2.67; S, 19.00.

*2.1.4.10. 2-{[5-(4-Chlorophenyl)thieno[2,3-d]pyrimidin-4-yl]thio} butanoic acid (**5j**).* Yield 82%; m.p. 144–145 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 0.94 (3H, t, CH_3), 174–1.90 (2H, m), 4.59 (1H, t), 7.44 (2H, d, J = 8.5 Hz, 4-ClC_6H_4), 7.49 (2H, d, J = 8.5 Hz, 4-ClC_6H_4), 7.70 (1H, s, 6-H), 8.77 (1H, s, 2-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 11.56, 24.62, 47.56, 95.47, 125.32, 125.75, 127.72, 131.57, 133.62, 151.39, 163.13, 166.07, 171.78. Anal. Calc. for $C_{16}H_{13}ClN_2O_2S_2$: C, 52.67; H, 3.59; S, 17.58. Found: C, 52.66; H, 3.57; S, 17.56.

*2.1.4.11. [(6-Phenylthieno[2,3-d]pyrimidin-4-yl)thio]acetic acid (**5k**).* Yield 86%; m.p. 171–172 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 4.15 (2H, s,

CH_2), 7.40–7.50 (3H, m), 7.79–7.84 (3H, m), 8.75 (1H, s, 2-H), 12.80 (bs, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 31.38, 95.42, 114.05, 126.36, 128.59, 129.10, 132.13, 143.41, 151.99, 161.96, 164.38, 169.28. Anal. Calc. for $C_{14}H_{10}N_2O_2S_2$: C, 55.61; H, 3.33; S, 21.21. Found: C, 55.60; H, 3.31; S, 21.20.

*2.1.4.12. 2{[5-(4-Methylphenyl)thieno[2,3-d]pyrimidin-4-yl]thio} propanoic acid (**5l**).* Yield 81%; m.p. 159–160 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 1.43 (3H, d, J = 7.3 Hz, CH_3), 2.42 (3H, s, CH_3), 4.60 (1H, q, J = 7.3 Hz, J = 7.3 Hz), 7.26 (2H, d, J = 8.1 Hz, 4-$CH_3C_6H_4$), 7.3 (2H, d, J = 8.1 Hz, 4-$CH_3C_6H_4$), 7.58 (1H, s, 6-H), 8.75 (1H, s 2-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 17.24, 20.98, 41.10, 95.44, 124.95, 125.46, 128.36, 129.71, 131.89, 135.06, 137.64, 151.33, 163.14, 166.05, 172.57. Anal. Calc. for $C_{16}H_{14}N_2O_2S_2$: C, 58.16; H, 4.27; S, 19.41. Found: C, 58.15; H, 4.26; H, 19.40.

*2.1.4.13. 2-{[5-(4-Methylphehyl)thieno[2,3-d]pyrimidin-4-yl]thio} butanoic acid (**5m**).* Yield 84%; m.p. 133–134 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 0.94 (3H, t, CH_3), 1.71–1.89 (2H, m), 2.42 (3 H, s CH_3), 4.59 (1H, t), 7.25 (2H, d, J = 7.8, 4-$CH_3C_6H_4$), 7.3 (2H, d, J = 7.8, 4-$CH_3C_6H_4$), 7.58 (1H, s, 6-H), 8.75 (1H, s, 2-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 11.59, 20.95, 24.64, 47.42, 95.40, 125.08, 125.50, 128.33, 129.76, 131.89, 135.07, 137.68, 151.39, 163.21, 166.02, 72.00. Anal. Calc. for $C_{17}H_{16}N_2O_2S_2$: C, 59.28; H, 4.68; S, 18.62. Found: C, 59.27; H, 4.66; S, 18.60.

*2.1.4.14. {[5-(4-Bromophenyl)thieno[2,3-d]pyrimidin-4-yl]thio}acetic acid (**5n**).* Yield 80%; m.p. 171–172 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 3.98 (2H, s, CH_2), 7.44 (2H, d, J = 8.2, 4-BrC_6H_4), 7,69 (2H,d, J = 8.2, 4-BrC_6H_4),7.84 (1H, s, 6-H), 8.84 (1H, s, 2-H), 12,67 (bs, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 31.77, 95.43, 122.08, 125.67, 130.76, 131.98, 133.64, 134.04, 151.56, 163.15, 165.83, 169.26. Anal. Calc. for $C_{14}H_9BrN_2O_2S_2$: C, 44.10; H, 2.38; S. 16.82. Found: C, 44.07; H, 2.36; S, 16.81.

*2.1.4.15. 4-(5,6,7,8-Tetrahydro[1]benzothieno[2,3-d]pyrimidin-4-ylthio) methyl]benzoic acid (**5o**).* Yield 83%; m.p. 257–258 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 1.80 (4H, m, –$(CH_2)_4$–), 2.82–2.98 (4H, m, –$(CH_2)_4$–), 4.67 (2H, s, CH_2), 7.58 (2H, d, J = 8.2), 7.88 (2H, d, J = 8.2), 8.76 (1H, s, 2-H), 12.92 (bs, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 21.96, 25.22, 26.17, 32.22, 95.46, 126.87, 127.35, 128.96, 129.28, 129.55, 136.35, 142.28, 150.90, 161.36, 164.55, 166.71. Anal. Calc. for $C_{18}H_{16}N_2O_2S_2$: C, 60.65; H, 4.52; C, 17.99. Found: C, 60.64; H, 4.50; S, 17.97.

*2.1.4.16. 3-{[5-(4-Chlorophenyl)thieno[2,3-d]pyrimidin-4-yl]thio} propanoic acid (**5p**).* Yield 85%; m.p. 168–169 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 2.57 (2H, t, CH_2), 3.32 (2H, t, CH_2), 7.39 (2H, d, J = 7.8, 4-ClC_6H_4), 7.44 (2H, d, J = 7.8, 4-ClC_6H_4), 7.62 (1H, s, 6-H), 8.78 (1H, s, 2-H), 12.14 (bs, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 24.43, 33.54, 95.49, 125.45, 127.67, 131.56, 133.37, 133.77, 151.60, 163.85, 165.94, 172.38. Anal. Calc. for $C_{15}H_{11}ClN_2O_2S_2$: C, 51.53; H; 3.16; S, 18.28. Found: C, 51.50; H, 3.15; S, 18.27.

*2.1.4.17. 3-{[(5,6-Dimethylthieno[2,3-d]pyrimidin-4-yl)thio]methyl} benzoic acid (**5q**).* Yield 80%; m.p. 116–117 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 2.47 (3H, s, CH_3), 2.52 (3H, s, CH_3), 4.64 (2H, s, CH_2), 7.39 (1H, t), 7.63 (1H, d), 7.82 (1H, d), 8.04 (1H, s), 8.65 (1H, s, 2-H), 12.60 (bs, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 19.51, 14.37, 95.37, 124.90, 128.04, 128.56, 129.85, 130.95, 133.45, 137.77, 150.90, 161.82, 163.85, 166.90. Anal. Calc. for $C_{16}H_{14}N_2O_2S_2$: C, 58.16; H, 4.27; S, 19.41. Found: C, 58.15; H, 4.26; S, 19.40.

*2.1.4.18. 3-[(2-Methyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4-yl)thio]propanoic acid (**5r**).* Yield 83%; m.p. 177–178 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 1.79 (4H, m, –$(CH_2)_4$–), 2.50 (2H, t), 2.58 (3H, s), 2.77–2.93 (4H, m, –$(CH_2)_4$–), 3.39 (2H, t). Anal. Calc. for $C_{14}H_{16}N_2O_2S_2$: C, 54.52; H, 5.23; S, 20.79. Found: C, 54.51; H, 5.22; 20.76.

*2.1.4.19. 4-(5,6,7,8-Tetrahydro[1]benzothieno[2,3-d]pyrimidin-4-ylthio) butanoic acid (**5s**).* Yield 81%; m.p. 153–154 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 1.88 (4H, m, –$(CH_2)_4$–), 1.95 (2H, t, CH_2), 2.36 (2H, t, CH_2), 2.84–3.05 (4H, m, –$(CH_2)_4$–), 3.32 (2H, t, CH_2), 8.58 (1H, s, 2-H), 11.94 (bs, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 22.01, 22.08, 24.17, 25.26, 26.27, 28.05, 32.51, 95.46, 127.07, 127.70, 136.07, 151.01, 162.26, 164.40, 173.46. Anal. Calc. for $C_{14}H_{16}N_2O_2S_2$: C, 54.52; H, 5.23; S, 20.79. Found: C, 54.51; H, 5.23; S, 20.76.

*2.1.4.20. 3-[(5,6-Dimethylthieno[2,3-d]pyrimidin-4-yl)thio]propanoic acid (**5t**).* Yield 87%; m.p. 189–190 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 2.47 (3H, s, CH_3), 2.53 (3H, s, CH_3), 2.69 (2H, t, CH_2), 3.46 (2H, CH_2), 8.61 (1H, s, 2-H), 12.19 (bs, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 13.52, 14.37, 24.17, 33.55, 95.44, 124.92, 128.53, 133.18, 150.88, 162.21, 163.77, 172.54. Anal. Calc. for $C_{11}H_{12}N_2O_2S_2$: C, 49.53; H, 4.51; S, 23.9. Found: C, 49.52; H, 4.50; S, 23.7.

*2.1.4.21. [(6-Methyl-5-phenylthieno[2,3-d]pyrimidin-4-yl)thio]acetic acid (**5u**).* Yield 82%; m.p. 174–175 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 2.35 (3H, s, CH_3), 3.78 (2H, s, CH_2), 7.32 (2H, m, C_6H_5), 7.47 (3H, m, C_6H_5), 8.66 (1H, s, 2-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 14.09, 31.71, 95.40, 127.02, 128.10, 128.45, 130.58, 130.91, 133.60, 136.39, 150.98, 161.75, 163.43, 169.44. Anal. Calc. for $C_{15}H_{12}N_2O_2S_2$: C, 56.94; H, 3.82; S, 20.27. Found: C, 56.93; H, 3.80; S, 20.26.

*2.1.5. Substituted (thieno[2,3-d]pyrimidin-4-ylthio)carboxylic acids **6a–g***

Compounds **6a–g** shown in Table 1 were synthesized on the base of substituted 4-chlorothieno[2,3-d]pyrimidines **4**. To a suspension of 0.01 mol of 3-mercaptopropanoic acid or mercaptoacetic acid and 0.024 mol of potassium carbonate in 10 mL DMF the 4-chlorothieno [2,3-d]pyrimidine was added. The mixture was heated on a boiling water bath for 10 min. The resulting mixture was evaporated in vacuum. The residue was diluted with water and acetic acid slowly to pH = 7. The solid acid was filtrated and crystallized from isopropyl alcohol.

*2.1.5.1. 3-{[5-(4-Methylphenyl)thieno[2,3-d]pyrymidin-4-yl]thio} propanoic acid (**6a**).* Yield 85%; m.p. 161–162 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 2.42 (3H, s, CH_3), 2.56 (2H, t, CH_2), 3.29 (2H, t, CH_2), 7.21 (2H, d, J = 7.8, 4-$CH_3C_6H_4$) 7.26 (2H, d, J = 7.8, 4-$CH_3C_6H_4$), 7.50 (1H, s, 6-H), 8.76 (1H, s, 2-H), 12.12 (bs, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 20.95, 24.42, 33.54, 95.39, 124.80, 125.79, 125.29, 129.76, 132.00, 135.19, 137.53, 151.62, 163.99, 165.85, 172.56. Anal. Calc. for $C_{16}H_{14}N_2O_2S_2$: C, 58.16; H, 4.27; S, 19.41. Found: C, 58.15; H, 4.26; S, 19.40.

*2.1.5.2. 3-{[5-(3,4-Dimethylphenyl)thieno[2,3-d]pyrimidin-4-yl]thio} propanoic acid (**6b**).* Yield 84%; m.p. 133–134 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 2.30 (3H, s, CH_3), 2.32 (3H, s, CH_3), 2.57 (2H, t, CH_2), 3.29 (2H, t, CH_2), 7.07–7.17 (3H, m, 3,4-$(CH_3)_2C_6H_3$), 7.46 (1H, s, 6-H), 8.75 (1H, s, 2-H), 12.10 (bs, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 19.27, 19.37, 24.42, 33.53, 95.39, 124.60, 125.78, 127.27, 128.78, 130.93, 130.93, 132.29, 135.45, 136.21, 150.78, 151.59, 164.00, 165.86, 172.58. Anal. Calc. for $C_{17}H_{16}N_2O_2S_2$: C, 59.28; H, 4.68; S, 18.62. Found: C, 59.27; H, 4.67; S, 18.60.

*2.1.5.3. [(6-Methylthieno[2,3-d]pyrimidin-4-yl)thio]acetic acid (**6c**).* Yield 80%; m.p. 171–172 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 2.64 (3H, s, CH_3), 4.09 (2H, s, CH_2), 7.11 (1H, s, 5-H), 8.67 (1H, s, 2-H), 12.69 (bs, OH).

^{13}C NMR (100 MHz, DMSO-d_6): δ: 16.13, 31.22, 95.38, 116.18, 127.86, 141.87, 151.44, 160.44, 164.75, 169.34, 177.40. Anal. Calc. for $C_9H_8N_2O_2S_2$: C, 44.98; 3.36; S, 26.69. Found: C, 44.97; H, 3.34; S, 26.68.

*2.1.5.4. 3-{[5-(4-Ethoxyphenyl)thieno[2,3-d]pyrimidin-4-yl]thio} propanoic acid (**6d**).* Yield 86%; m.p. 138–139 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 1.42 (3H, t, CH_3), 2.56 (2H, t, CH_2), 3.29 (2H, t, CH_2), 4.08 (2H, q, CH_2), 6.91 (2H, d, J = 8.5, 4-$CH_3CH_2OC_6H_4$), 7.26 (2H, d, J = 8.5, 4-$CH_3CH_2OC_6H_4$), 7.84 (1H, s, 6-H), 8.75 (1H, s, 2-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 14.69, 24.37, 33.53, 63.00, 95.41, 113.52, 124.50, 125.95, 126.82, 131.09, 134.99, 151.57, 158.57, 164.02, 165.77, 172.52. Anal. Calc. for $C_{17}H_{16}N_2O_2S_2$: C, 56.65; H, 4.47; S, 17.79. Found: C, 56.64; H, 4.45; S, 17.78.

*2.1.5.5. 3-{[5-(4-Fluorophenyl)thieno[2,3-d]pyrimidin-4-yl]thio} propanoic acid (**6e**).* Yield 83%; m.p. 166–167 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.57 (2H, t, CH_2), 3.30 (2H, t, CH_2), 7.16–7.21 (2H, m, 4-FC_6H_4), 7.39–7.42 (2H, m, 4-FC_6H_4), 7.59 (1H, s, 6-H), 8.77 (1H, s, 2-H), 12.13 (bs, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 24.41, 33.49, 95.39, 114.52, 114.73, 125.34, 125.77, 131.20, 132.05, 133.97, 151.71, 160.99, 163.43, 163.91, 165.75, 172.53. Anal. Calc. for $C_{15}H_{11}FN_2O_2S_2$: C, 53.88; H, 3.32; S, 19.18. Found: C, 53.86; H, 3.31; S, 19.16.

*2.1.5.6. 3-[(6-Methylthieno[2,3-d]pyrimidin-4-yl)thio]propanoic acid (**6f**).* Yield 80%; m.p. 168–169 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 2.62 (3H, s, CH_3), 2.70 (2H, t, CH_2), 3.48 (2H, t, CH_2), 7.04 (1H, s, 5-H), 8.68 (1H, s, 2-H), 12.13 (OH, bs). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 16.10, 23.91, 33.77, 95.37, 116.27, 128.14, 141.62, 151.58, 161.14, 164.68, 172.58. Anal. Calc. for $C_{10}H_{10}N_2O_2S_2$: C, 47.23; H, 3.96; S, 25.21. Found: C, 47.22; H, 3.94; S, 25.20.

*2.1.5.7. 3-[(2-Methyl-6-phenylthieno[2,3-d]pyrimidin-4-yl)thio] propanoic acid (**6g**).* Yield 82%; m.p. 215–216 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ: 2,68 (3H, s, CH_3), 2.72 (2H, t, CH_2), 3.50 (2H, t CH_2), 7.36–7.46 (3H, m, C_6H_5), 7.59 (1H, s, 5-H), 7.74–7.76 (2H, m, C_6H_5), 12.22 (bs, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 23.87, 25.62, 33.72, 95.46, 113.80, 126.10, 126.33, 128.90, 128.98, 132.37, 141.69, 161.36, 162.19, 165.02, 172.55. Anal. Calc. for $C_{16}H_{14}N_2O_2S_2$: C, 56.94; H, 3.82; S, 20.27. Found: C, 56.92; H, 3.79; S, 20.19.

2.2. Biology

Selected compounds were tested using in vitro kinase assay. Each test was performed twice in a total reaction volume of 30 μL, containing 1 μg of peptide substrate RRRDDDSDDD (Custom synthesized by Genscript); 10 units of recombinant human CK2 (New England Biolabs); 50 μM ATP and γ-labeled ^{32}P ATP, diluted to specific activity 100 μCi/μM; CK2 buffer (20 mM Tris–HCl, pH 7.5; 50 mM KCl; 10 mM $MgCl_2$) and inhibitor in varying concentrations. Incubation time was 20 min at 30 °C. The reaction was stopped by adding an equal volume of 10% o-phosphoric acid and the reaction mixture was loaded onto 20-mm discs of P-31 phosphocellulose paper (Whatman). Disks were washed three times with 1% o-phosphoric acid solution, air-dried at room temperature, and counted by Cherenkov method using a beta-counter (LKB). As negative control an equal volume of DMSO was added to the reaction mixture. Quercetin, a known CK2 inhibitor [23], was used as inhibition positive control. The final concentration of quercetin was 0.55 μM (which inhibited CK2 activity by 50%). Percent inhibition was calculated from substrate-incorporated radioactivity in the reaction relative to the radioactivity incorporated in control reactions, i.e. in the absence of inhibitor. Serial dilutions of inhibitor stock solution were used in reactions to determine their IC_{50} concentrations.

Protein kinases ASK1, JNK3, Aurora A, Rock 1, FGFR1, Met and Tie2 were assayed according to the supplier's suggestions (Millipore).

2.3. Computational methodology

For molecular docking of substituted (thieno[2,3-d]pyrimidin-4-ylthio)carboxylic acids DOCK 4.0 package was applied [24–27]. Molecular docking was performed as described previously [21].

3. Results and discussion

3.1. Biological effect and structure–activity relationships

To study CK2 inhibitory activity of substituted (thieno[2,3-d] pyrimidin-4-ylthio)carboxylic acids, 28 compounds of this class were synthesized and tested in vitro. The results showed that 21 compounds of the studied group inhibited the enzyme activity more than by 50% in the concentration ranging from 0.1 μM to 33 μM (IC_{50} values are shown in Table 1). Compounds displaying highest activity are **5e**, **5k**, **5p**, **6a**, **6b**, **6d**, **6e** and **6g**.

Kinetic studies of the most active compound **6a** (IC_{50} is 0.1 μM) showed that activity of (thieno[2,3-d]pyrimidin-4-ylthio)carboxylic acids is a result of their competition with ATP molecule for the binding site (Fig. 1). Inhibition constant for **6a** is 40 nM.

We studied structure–activity relationship of thieno[2,3-d] pyrimidine heterocycle considering substituents R^1, R^2 and R^4. It turned out that substituent R^4 affects compound's inhibitory activity the most. We observed that the heterocycles with R^4 = propionic acid are more potent inhibitors than that with R^4 = acetic acid. To compare this effect one can refer to compound pairs such as **5t**, **5g**; **5p**, **5i** (corresponding IC_{50} values are 11.3 μM, 17.75 μM; 0.175 μM, 32 μM). At the same time, R^4 = acetic acid is more active than R^4 = 2-propionic acid (see **5e**, **5b**; **5g**, **5c**, corresponding IC_{50} values are 1.78 μM, 20 μM; 17.75 μM, >33 μM), while R^4 = 2-propionic acid is more active than R^4 = 2-butyric acid and n-butyric acid (IC_{50} values of compounds **5a**, **5f**, **5s** are 17 μM, 33 μM and 33 μM, respectively). Substituents $CH_2C_6H_4COOH$-*m* and CH_2COOH equally affect the inhibitory properties of studied heterocycles.

From the obtained results we concluded that the substituent R^4 of the heterocycle could be ranked by its effect as the following series: CH_2CH_2COOH > CH_2COOH > $CH(CH_3)COOH$ > $CH_2CH_2CH_2$-$COOH$ = $CH(C_2H_5)COOH$. This points out that R^4 = propionic acid causes the highest inhibitory activity of the studied compounds, and

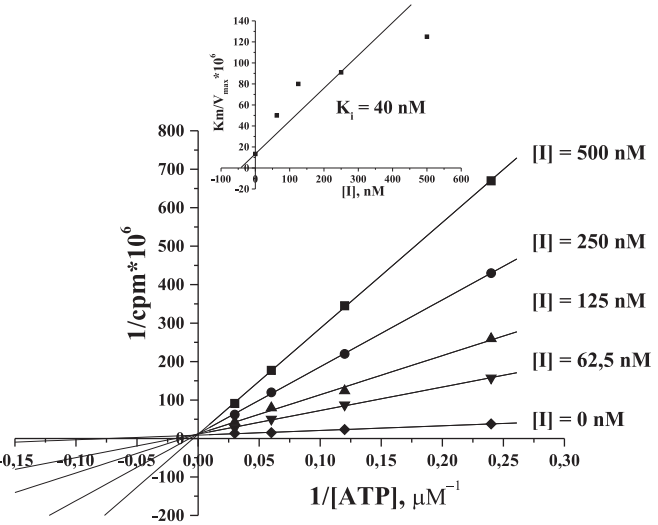


Fig. 1. Lineweaver–Burk plots of CK2 inhibition by compound **6a**. K_i value is 40 nM. Concentration of inhibitors varied from 0 nM to 500 nM.

Table 2
Specificity profile of CK2 inhibitors **6a** and **6d**[a].

Kinase	Kinase residual activity at presence of **6a**, %	Kinase residual activity at presence of **6d**, %
CK2	0.72	0.86
Jnk3	104	94
Rock1	126	115
Tie2	70	76
Ask1	92	100
Aurora A	23	48
Met	114	115
FGFR1	92	99

[a] Residual activity, determined in the presence of 10 μM of inhibitor is expressed as a percentage of the control without inhibitor. Final concentration of ATP in the experiment was 100 μM.

it should be noticed that elongation and branching of the investigated substituents decreases their ability to inhibit CK2.

Substituents R^1 and R^2 of (thieno[2,3-d]pyrimidin-4-ylthio)acetic acid also significantly affect the inhibitory activity. Compounds containing R^1 = para-substituted phenyl (**5p**, **6a**, **6b**, **6d**, **6e**) display the lowest IC_{50} values. It can be deduced that activity of these compounds is arranged as 4-FC_6H_4 < 3,4-$CH_3C_6H_3$ = 4-ClC_6H_4 < 4-$C_2H_5OC_6H_4$ < 4-$CH_3C_6H_4$ (IC_{50} = 1.2 μM, 0.175 μM, 0.175 μM, 0.125 μM, 0.1 μM, respectively).

3.2. Selectivity

Initial in vitro tests of discovered thienopyrimidine inhibitors **6a** and **6d** on four serine/threonine (ASK1, JNK3, Aurora A and Rock 1) and three tyrosine protein kinases (FGFR1, Met and Tie2) revealed remarkable specificity towards CK2 (Table 2). It can be noticed that the activity of Aurora A kinase is inhibited considerably by compound **6d**; nevertheless, we suppose that these thienopyrimidine derivatives are promising for extended selectivity evaluation.

3.3. Molecular modeling

In order to suppose the intermolecular interactions causing inhibitory activity of thienopyrimidines we inspected molecular docking complexes of the most active compounds displaying IC_{50} less than 1 μM (Fig. 2). It has been shown that these compounds have quite similar positions in the CK2 ATP-binding site, presumably because their R^1 and R^4 substituents of the thieno[2,3-d] pyrimidine heterocycle are structurally alike. Key contacts that contribute significantly to the ligand binding are Van der Waals interactions of thieno[2,3-d]pyrimidine heterocycle and aromatic substituent R^1 with a number of hydrophobic residues (Leu45, Val53, Val66, Val116 and Ile174).

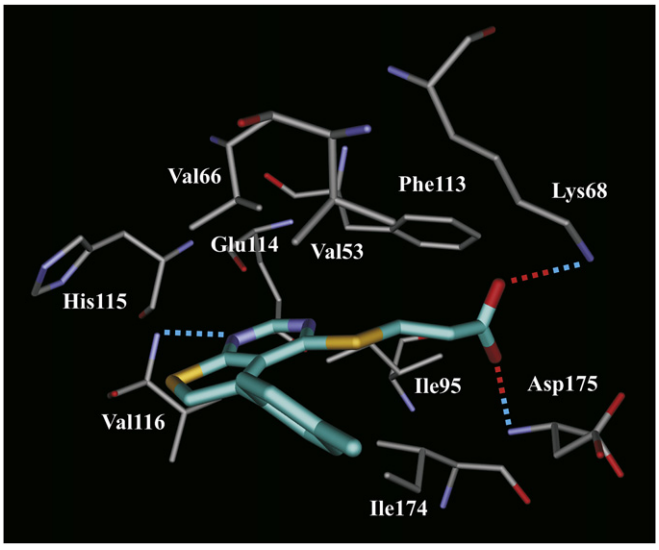


Fig. 2. **6a** bound to the active site of the CK2 catalytic subunit. The complex has been obtained with molecular docking. Intermolecular hydrogen bonds are shown as dotted lines.

In addition, these compounds form three intermolecular hydrogen bonds in CK2 ATP-binding site. One of the bonds forms between nitrogen in 1-position of the thieno[2,3-d]pyrimidine heterocycle and Val116 of the CK2 hinge region. Two other bonds are formed by the substituent R^4 (propionic acid) with side chain of Lys68 and main chain of Asp175 that are located in the hydrophobic region 1 of the active site.

Comparing SAR and modeling data it can be assumed that R^4 = propionic acid provides an optimal length between carboxyl group and heterocycle of thieno[2,3-d]pyrimidine allowing inhibitor **6a** bind effectively in the CK2 active site due to formation of hydrogen bonds in two areas (hinge region and hydrophobic region 1) of catalytic site simultaneously. Replacing this substituent with a shorter R^4 like acetic acid, branched 2-propionic and 2-butyric acids as well as with a longer 4-butyric acid resulted in decreasing of the compounds activity.

4. Conclusions

Substituted (thieno[2,3-d]pyrimidin-4-ylthio)carboxylic acids represent novel and selective class of CK2 inhibitors. The most active compounds obtained are **6a**, **6d** having IC_{50} values of 0.1 μM and 0.125 μM, respectively. These inhibitors display remarkable selectivity towards CK2, and provide an opportunity to examine the therapeutic validity against CK2 and other kinases. The importance of carboxyl group in the structure of CK2 inhibitors and role of sterical effects in their binding interactions has been shown providing the basis for further structural optimization of this chemical class.

Author contribution

Andriy G. Golub: Molecular modeling, preparation of manuscript.
Volodymyr G. Bdzhola: Molecular modeling, SAR studies, preparation of manuscript.
Nadiia V. Briukhovetska: Organic synthesis, preparation of manuscript.
Anatoliy O. Balanda: Organic synthesis.
Olexander P. Kukharenko: In vitro tests.
Igor M. Kotey: Organic synthesis.
Olga V. Ostrynska: In vitro tests.
Sergiy M. Yarmoluk: Supervision, research planning, preparation of manuscript.

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