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New bisthioalkyl substituted thieno[3,2-d]pyrimidines: Synthesis and antitumor activity

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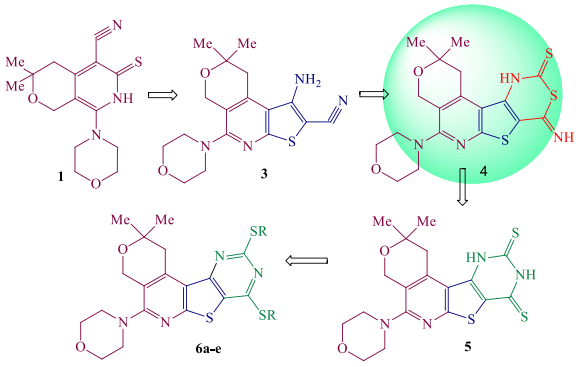
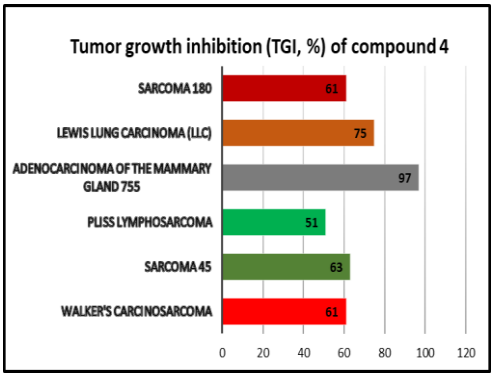
Abstract

Effective methods for synthesizing 1-amino-8,8-dimethyl-5-morpholin-4-yl-8,9-dihydro-6*H*-pyrano[4,3-*d*]-thieno[2,3-*b*]pyridine-2-carbonitrile (**3**) have been developed. A new heterocyclic system, pyrano[4'',3'':4',5']-pyrido[3',2':4,5]thieno[3,2-*d*][1,3]thiazine (**4**), has been synthesized. Based on the latter, 2,2-dimethyl-5-morpholin-4-yl-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidine-8,10(9*H*,11*H*)-di-thione (**5**) has been synthesized via the opening of the thiazine ring, rearrangement, and recyclization. New bis-S-alkyl derivatives of tetracyclic thieno[3,2-d]pyrimidine (**6a-e**) were synthesized. The antitumor activity of the synthesized compounds was studied in models of various experimental tumors in mice and rats. All compounds demonstrated antitumor activity, with the tetracyclic thieno[3,2-d][1,3]thiazine-10-thione (**4**) being particularly potent compared to 5-fluorouracil.

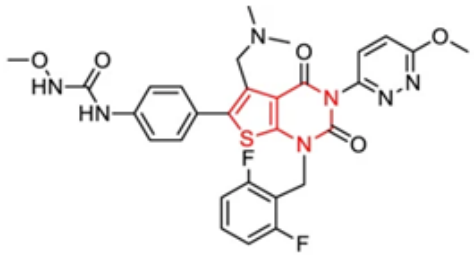




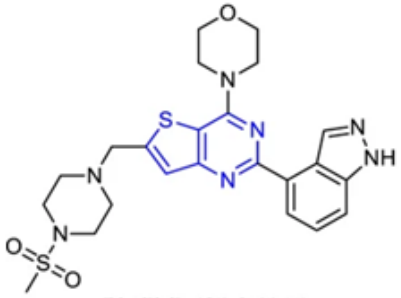
Keywords: Pyrano[3,4-*c*]pyridine; thieno[3,2-*d*][1,3]thiazine-10-thione; thieno[3,2-d]pyrimidine; *S*-bis-alkylation; rearrangement; antitumor activity.

Introduction

The most important fundamental task of synthetic organic chemistry is the development of methods for the synthesis of multifunctional carbo- and heterocyclic compounds with useful properties. Pyrimidine derivatives have a special place in them, which are universal structural units for the synthesis of complex heterocyclic systems. Pyrimidine and pyrimidine-derived structures are important in medicinal chemistry due to their ability to bind to biological targets and influence their activity. Thiophene-based compounds have been investigated for their pharmaceutical properties. Some thiophene derivatives exhibit biological activities, such as antimicrobial,[1] anticancer,[2] and anti-inflammatory[3] effects. Additionally, the combination of different ring systems allows for the exploration of new chemical space and the modulation of the properties and activities of the resulting compounds. Thienopyrimidines are a class of heterocyclic compounds that consist of a fused ring system containing a thiophene ring and a pyrimidine ring. Thienopyrimidines have gained significant interest in various fields, including medicinal chemistry, pharmaceutical research, and material science, due to their diverse biological activities and potential applications. Thienopyrimidines have been extensively studied for their potential therapeutic applications. They exhibit a broad range of biological activities, including anticancer,[4, 5] antimicrobial,[6] anti-inflammatory,[7] antiviral,[8] and kinase inhibitory properties.[9, 10] Figure 1 represents some thienopyrimidine-containing drugs with varying profiles of biological activity. Relugolix (TAK385), is a thienopyrimidine derivative that has completed phase III clinical trials and is being studied for its capacity to treat endometriosis and prostate carcinoma by acting as a gonadotropinreleasing hormone receptor (GnRHR) antagonist.[11, 12] Moreover, pictilisib (GDC-0941) is a thieno[3,2-d]pyrimidine derivative which inhibits phosphatidylinositol 3-kinase (PI3K) and is in clinical trials and was clinically investigated for the treatment of advanced solid tumors.[13]



Relugolix (TAK-385)



Pictilisib (GDC-0941)

Given their importance in medicinal chemistry, there is significant interest in developing new synthetic methods for the efficient production of thienopyrimidine derivatives.

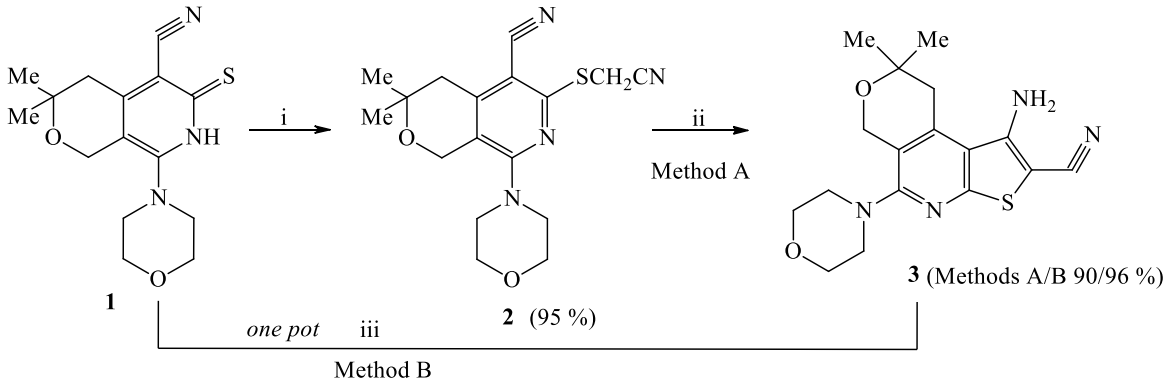
The current study is a continuation of our research on the synthesis and evaluation of antitumor activity of fused tetracyclic systems containing thieno[3,2-*d*]pyrimidine ring. Herein, we synthesized new heterocyclic system – pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*][1,3]thiazine-10-thione. The reaction mechanism for synthesis 2,2-dimethyl-5-morpholin-4-yl-1,4-dihydro-2*H*-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidine-8,10(9*H*,11*H*)-dithione from corresponding 1-amino-thieno[2,3-*b*]pyridine-2-carbonitrile was approved. Optimal method for synthesis new bis(thioalkyl) derivatives of tetracyclic pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidine was developed. The antitumor properties of the synthesized compounds was studied.

Results and Discussion

An effective methods for obtaining 1-amino-8,8-dimethyl-5-morpholin-4-yl-8,9-dihydro-6*H*-pyrano[4,3-*d*]thieno[2,3-*b*]pyridine-2-carbonitrile (**3**) based on conversion of 3,3-dimethyl-8-morpholin-4-yl-6-thioxo-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile (**1**) have been developed.[14] Synthesis of compound **3** was previously reported in our published article.[15] However, in this work, we optimized the synthetic methods for compound **3**, increasing the yields and confirming their structure.

Method for synthesis 6-[(cyanomethyl)thio]-3,3-dimethyl-8-morpholin-4-yl-3,4-dihydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile **(2)** was developed (Scheme 1). The reaction between 3,3-dimethyl-8-morpholin-4-yl-6-thioxo-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile (**1**) and chloroacetonitrile was carried out in the presence of sodium acetate in ethanol, under stirring conditions and at room temperature. As a result, compound **2** was synthesized and confirmed by physicochemical methods. In the IR-spectrum of the obtained compound **2** are absorption bands characteristic of nitrile groups attached with alkyl and aromatic ring at 2254 cm^{-1} and 2206 cm^{-1}, respectively.

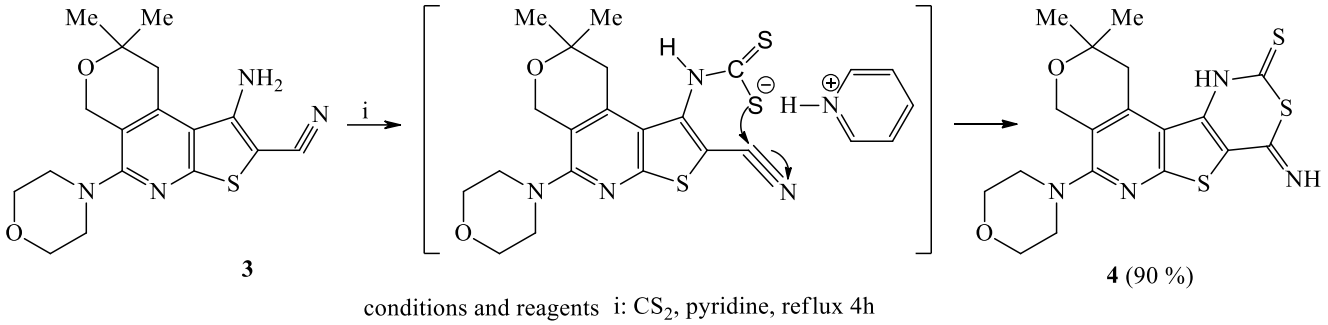
The synthesis of compound **3** carried out by two methods. Thus, by refluxing 6-[(cyanomethyl)thio]-3,3-dimethyl-8-morpholin-4-yl-3,4-dihydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile **(2)** in the present of sodium acetate in ethanol obtaining compound **3** (Method A, Scheme 1). The last was obtained via *one pot* reaction. The reaction was carried out by reacting 3,3-dimethyl-8-morpholin-4-yl-6-thioxo-3,4,6,7-tetrahydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile (**1**) with chloroacetonitrile in the presence of sodium acetate in dry ethanol under refluxing conditions (Method B, Scheme 1). As a result of the *one-pot* reaction, the closure of the thiophenic ring proceeds simultaneously with the alkylation, leading to the formation of 1-amino-8,8-dimethyl-5-morpholin-4-yl-8,9-dihydro-6*H*-pyrano[4,3-*d*]thieno[2,3-*b*]pyridine-2-carbonitrile (**3**).



Scheme 1. Synthesis of bicyclic pyrano[3,4-*c*]pyridine (**2**) and tricyclic pyrano[4,3-*d*]thieno[2,3-*b*]pyridine (**3**).

In the IR-spectrum of the obtained tricyclic thieno[2,3-*b*]pyridine **3** there are absorption bands characteristic of amino and nitrile groups at 3482, 3349, 3249 cm^{-1} and 2191 cm^{-1}, respectively. In the ^{1}H NMR spectra of compound **3** the typical signal of the amino functional group protons (NH_2) are observed at 6.00 ppm. In addition, in the ^{13}C NMR spectrum, the characteristic signal of the CN group substituted in the second position of thiophene ring was recorded at 115.3 ppm, which also confirms the structure of the synthesized compound.

1-Amino-8,8-dimethyl-5-morpholin-4-yl-8,9-dihydro-6*H*-pyrano[4,3-*d*]thieno[2,3-*b*]pyridine-2-carbonitrile (**3**) was used as a key intermediate for the synthesis of functional substituted new heterocyclic system pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*][1,3]thiazine **4** (Scheme 2). The last was obtained by the interaction of compound **3** with carbon disulfide in the presence of pyridine.

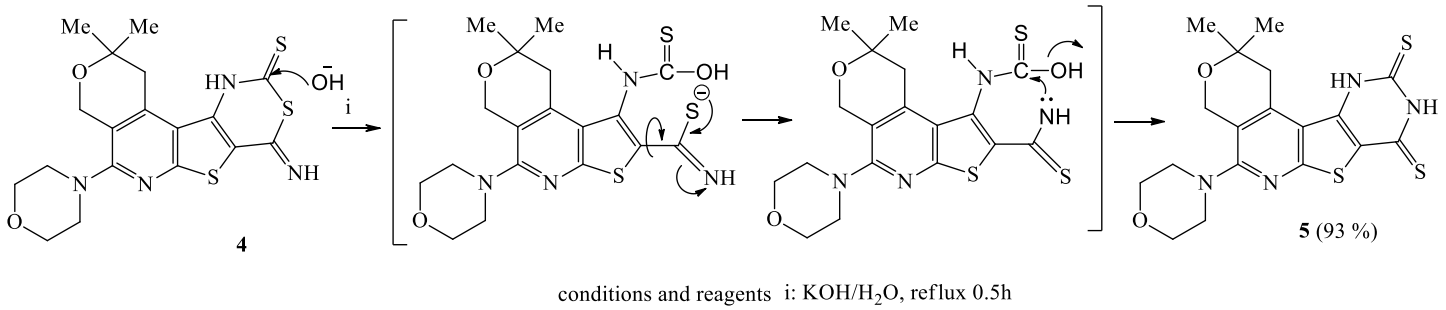


Scheme 2. Synthesis and mechanism of pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*][1,3]thiazine-10-thione **(4).**

The reaction mechanism proceeds via electrophilic substitution, wherein a carbon atom in carbon disulfide acts as an electrophile, attacking the nitrogen atom in the amine, which acts as the nucleophile.

The structure and purity of 8-imino-2,2-dimethyl-5-morpholin-4-yl-1,4,8,11-tetrahydro-2*H*,10*H*-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*][1,3]thiazine-10-thione (**4**) was confirmed by physicochemical methods. In the IR-spectrum of the obtained compounds **4** are absorption bands characteristic of secondary amino in cycle (NH), imino (C=NH) and carbon monosulfide (C=S) groups at 3417, 3108 cm^{-1} and 1262 cm^{-1}, respectively, whereas absorption bands characteristic of amino and nitrile groups are absent. In the ^{1}H NMR spectra of compound **4** the typical signal of the NH functional group protons are observed at 11.50 and 13.61 ppm. In addition, in the ^{13}C NMR spectrum, the characteristic signal of the S-C=NH and C=S groups was recorded at 171.7 and 179.2 ppm, respectively.

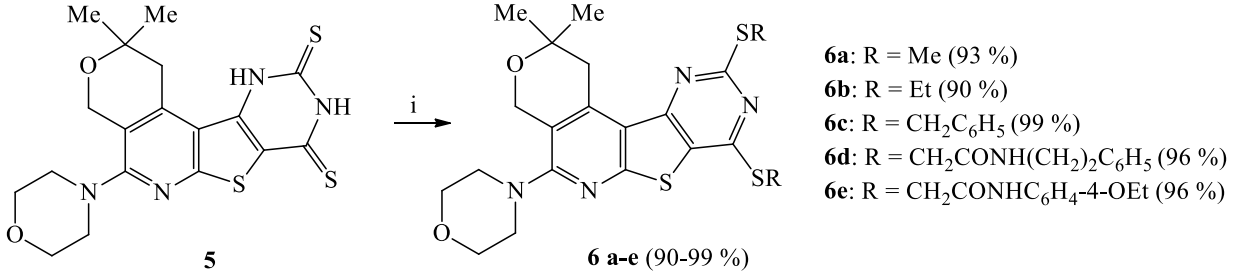
To continue our investigation, 2,2-dimethyl-5-morpholin-4-yl-1,4-dihydro-2*H*-pyrano[4'',3'':4',5']pyrido-[3',2':4,5]thieno[3,2-*d*]pyrimidine-8,10(9*H*,11*H*)-dithione (**5**) has been synthesized based on pyrano-[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*][1,3]thiazine-10-thione **4** (Scheme 3). The reaction was carried out in the presence of potassium hydroxide in a water, under stirring and heating conditions. The mechanism for obtaining compound 4 is assumed to occur in the following steps: In the presence of a base, the OH group attacks the carbon atom of the C=S bond in the thiazine ring, leading to the cleavage of the S-C=S bond and the opening of the thiazine ring. This is followed by rearrangement via rotation of the C-C bond at the 2-position of the thiophene ring, and subsequently, recyclization occurs through the attack of the NH group on the carbon atom of the C=S-OH group and in the removal of the OH group to yield the target compound.



Scheme 3. Synthesis and mechanism of pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidine-8,10-dithione (**5**).

In the IR-spectrum of the obtained dithio-thieno[3,2-*d*]pyrimidine **5** there are absorption bands characteristic of NH and C=S groups at 3430, 3130 cm^{-1} and 1298, 1283 cm^{-1}, respectively, which confirms the structure of the synthesized compound.

Synthesized pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidine-8,10-dithione (**5**) was used as key intermediate for synthesizing new bis-thioalkyl derivatives of thieno[3,2-*d*]pyrimidine **6a-e** (Scheme 4).



Scheme 4. Synthesis of bis-thioalkyl derivatives of thieno[3,2-*d*]pyrimidine (**6a-e**).

The structure and purity of compounds **6a–e** were confirmed by physicochemical methods. In the ^{1}H NMR spectra, observed the signal as a singlet (for compounds **6a,c-e**) and as a quartet (for **6b**) in the range of 2.62-4.64 ppm, which are characteristic of SCH_3 and SCH_2 group protons. In addition, signals characteristic of the thioalkyl group at in the range of 11.6–35.4 ppm were also recorded in the ^{13}C NMR spectrum of compounds **6a-e**. The results of NMR ^{1}H and ^{13}C studies confirm the structure and purity of synthesized new bis-thioalkyl derivatives of thieno[3,2-*d*]pyrimidine (**6a–e**).

The antitumor activity of the synthesized compounds was investigated using established methods.[16, 17] Initially, in acute experiments on white outbred mice weighing 19-21 g, the absolutely lethal (LD_{100}) and maximum tolerated (MTD) doses of the substances were determined. Upon assessing the acute toxicity of the tested compounds, it was observed that they exhibited low toxicity, with LD_{100} ranging from 2000-2500 mg/kg and MTD ranging from 1200-1750 mg/kg. Chemotherapy experiments revealed that compunds **5** and **6a-e** exhibited weak antitumor activity, inhibiting the growth of sarcoma 180 by 19.0-35.2% ($p \geq 0.05$), while compound **4** exhibited significant activity at 61% (Table 1).

Table 1. Antitumor activity of compounds **4**, **5** and **6a-e**

Compounds	Toxicity *mg/kg*		Antitumor activity on Sarcoma 180		
	LD_{100}	MTD	Dose (*mg/kg*)	TGI (%)	P
4	2500	1750	250	61.0	>0.05
5	>2500	–	250	19.0	>0.05
6a	2000	1200	150	21.2	>0.05
6b	>2500	–	250	19.0	>0.05
6c	2000	1200	150	21.2	>0.05
6d	2500	1750	200	28.0	>0.05
6e	2500	1500	150	35.2	=0.05
5- fluorouracil	212	102	25	45.0	>0.05

Due to the highest activity of compound **4** compared to the others, it was chosen as the most effective and has also been studied in relation to a number of other experimental tumor models of mice and rats: Walker's carcinosarcoma, Sarcoma 45, Pliss lymphosarcoma, adenocarcinoma of the mammary gland 755, and Lewis lung carcinoma (LLC) (Table 2).

Chemotherapy experiments revealed that compound **4** at doses 100-250 mg/kg causes significant growth inhibition (51-63%) Walker's carcinosarcoma, Pliss lymphosarcoma and Sarcoma 45. It should be noted that in experiments, compound **4** inhibits LLC by 75% at a dose of 250 mg/kg and exhibits high activity against breast adenocarcinoma 755, showing a 97% inhibition (Table 2).

Table 2. Antitumor activity of compound **4**

Experimental tumor models	Compound 4			5- fluorouracil		
	Dose (*mg/kg*)	Tumor growth inhibition (TGI, %)	P	Dose (*mg/kg*)	Tumor growth inhibition (TGI, %)	P
Walker's carcinosarcoma	100	61	>0.05	10	32	=0.05
Pliss lymphosarcoma	100	51	>0.05	10	41	=0.05
Sarcoma 45	100	63	>0.05	10	32	=0.05
adenocarcinoma of the mammary gland 755	250	97	>0.05	25	98	>0.05
Lewis lung carcinoma (LLC)	250	75	>0.05	25	60	>0.05

Conclusions

Effective synthesis methods were developed for 1-amino-8,8-dimethyl-5-morpholin-4-yl-8,9-dihydro-6*H*-pyrano[4,3-*d*]-thieno[2,3-*b*]pyridine-2-carbonitrile, leading to the synthesis of a new heterocyclic system incorporating pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*][1,3]thiazine-10-thione. Additionally, 2,2-dimethyl-5-morpholin-4-yl-1,4-dihydro-2*H*-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidine-8,10(9*H*,11*H*)-dithione was synthesized through cleavage, rearrangement, and recycling of thieno[3,2-

d][1,3]thiazine-10-thione. An effective method was also developed for synthesizing new bis-S-alkyl derivatives of the tetracyclic thieno[3,2-*d*]pyrimidine. The antitumor activity of all synthesized compounds was studied, confirming their efficacy in the Sarcoma 180 model. Notably, tetracyclic thieno[3,2-d][1,3]thiazine-10-thione exhibited higher activity in various experimental tumor models in mice and rats, suggesting its potential as a more effective candidate for future antitumor drug development

Experimental Section

General. All chemicals and solvents were of commercially high purity grade purchased from Sigma-Aldrich (Saint Louis, MO, USA). Melting points (m.p.) were determined on a Boetius microtable. They are expressed in degrees centigrade (°C). ^{1}H-NMR and ^{13}C NMR spectra were recorded in DMSO-d_6, $CDCl_3$, DMSO-d_6/CCl_4, 1/3, *v*/*v* solutions (300 MHz for ^{1}H and 75.462 MHz for ^{13}C) on a Varian mercury spectrometer (Varian Inc., Palo Alto, CA, USA). Chemical shifts are reported as δ (parts per million) relative to TMS (tetramethylsilane) as the internal standard. IR spectra were recorded on Nicolet Avatar 330-FTIR spectrophotometer (Thermo Nicolet, Foster, CA, USA) and the reported wave numbers are given in cm^{-1}. Mass spectra were recorded on XEVO G3 QTof spectrometers (Waters Corporation Company, Milford, Massachusetts). Elemental analyses were performed on a Euro EA 3000 Elemental Analyzer (*Euro*Vector, Pavia, Italy).

Synthesis of 6-[(cyanomethyl)thio]-3,3-dimethyl-8-morpholin-4-yl-3,4-dihydro-1*H*-pyrano[3,4-*c*]pyridine-5-carbonitrile (2). A mixture of compound **1** (1.53 g, 5 mmol), sodium acetate (1.64 g, 20 mmol) and ethanol (20 mL) was stirred 5 min, after which chloroacetonitrile (0.38 g, 5 mmol) was added and stirring continued for an additional 2 h at room temperature. The formed precipitate was filtered off, washed with water and recrystallized from ethanol to give the product as a pure white solid (1.64 g, 95%). mp: 189–190 °C. IR (*v*/cm^{-1}): 2254 (6-CN), 2206 (5-CN).^{1}H NMR (300 MHz, DMSO-d_6/CCl_4, 1/3): δ_H 1.31 (s, 6H, $C(CH_3)_2$), 2.72 (s, 2H, 4-CH_2), 3.41 (t, J = 4.6 Hz, 4H, $N(CH_2)_2$), 3.75 (t, J = 4.6 Hz, 4H, $O(CH_2)_2$), 4.14 (s, 2H, SCH_2), 4.51 (s, 2H, 1-CH_2). ^{13}C NMR (75.465 MHz, DMSO-d_6/CCl_4, 1/3) δ_C 15.0 (SCH_2), 26.3 ($2CH_3$), 39.7 (4-CH_2), 48.8 ($N(CH_2)_2$), 58.7 (1-CH_2), 65.9 ($O(CH_2)_2$), 69.3 (C^3), 97.9 (C^5), 113.9 (C), 116.2 (CN), 116.6 (CN), 148.0 (C), 155.0 (C), 157.9 (C). Anal. Calcd for $C_{17}H_{20}N_4O_2S$ (344.43): C, 59.28; H, 5.85; N, 16.27; S, 9.31. Found: C, 59.35; H, 5.81; N, 16.38; S, 9.43.

Synthesis methods of 1-amino-8,8-dimethyl-5-morpholin-4-yl-8,9-dihydro-6*H*-pyrano[4,3-*d*]thieno[2,3-*b*]pyridine-2-carbonitrile (3).

Method A. A mixture of compound **2** (1.03 g, 3 mmol), sodium acetate (0.25 g, 3 mmol) and ethanol (7 mL) was refluxed for 0.5 h. The obtained crystals were filtered off, washed with water, dried, and recrystallized from dioxane to give the product as a pure white solid (0.93 g, 90%). m.p. 321–322 °C.

Method B. A mixture of compound **1** (1.53 g, 5 mmol), sodium acetate (1.64 g, 20 mmol) and dry ethanol (20 mL) was refluxed for 3 h. The obtained crystals were filtered off, washed with water, dried, and recrystallized from dioxane to give the product as a pure white solid (1.66 g, 96%). m.p. 321–322 °C.

IR (*v*/cm^{-1}): 3482, 3349, 3249 (NH_2), 2191 (CN).^{1}H NMR (300 MHz, DMSO-d_6/CCl_4, 1/3): δ_H 1.31 (s, 6H, $C(CH_3)_2$), 3.13 (br. t, J = 4.5 Hz, 4H, $N(CH_2)_2$), 3.16 (s, 2H, 9-CH_2), 3.75 (t, J = 4.5 Hz, 4H, $O(CH_2)_2$), 4.61 (s, 2H, 6-CH_2), 6.00 (br.s, 1H, NH_2). ^{13}C NMR (75.465 MHz, DMSO-d_6/CCl_4, 1/3) δ_C 26.4 ($2CH_3$), 36.2 (9-CH_2), 49.8 ($N(CH_2)_2$), 58.9 (6-CH_2), 65.9 ($O(CH_2)_2$), 69.0 (C^8), 72.9 (C), 115.3 (CN), 117.8 (C), 117.9 (C), 141.5 (C), 151.3 (C), 157.9 (C), 158.6 (C). Anal. Calcd for $C_{17}H_{20}N_4O_2S$ (344.43): C, 59.28; H, 5.85; N, 16.27; S, 9.31. Found: C, 59.24; H, 5.87; N, 16.32; S, 9.23.

Synthesis of 8-imino-2,2-dimethyl-5-morpholin-4-yl-1,4,8,11-tetrahydro-2*H*,10*H*-pyrano[4'',3'':4',5']pyrido-[3',2':4,5]thieno[3,2-*d*][1,3]thiazine-10-thione (4). A mixture of compound **3** (1.72 g, 5 mmol), carbon disulfide (15 mL) and pyridine (20 mL) was refluxed for 4 h. The obtained crystals were filtered off, washed with water and ethanol, dried, and recrystallized from DMSO to give the product as a pure yellow solid (2.10 g, 90%). m.p. m.p. 336-337 °C. IR (ν/cm^{-1}): 3417 (NH), 3108 (NH$_{\mathbf{imino}}$), 1561 (C=N), 1262 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6/CCl_4, 1/3): δ_H 1.34 (s, 6H, C(CH$_3$)$_2$), 3.28 (br. t, J = 4.4 Hz, 4H, N(CH$_2$)$_2$), 3.34 (s, 2H, 1-CH$_2$), 3.76 (t, J = 4.4 Hz, 4H, O(CH$_2$)$_2$), 4.62 (s, 2H, 4-CH$_2$), 11.50 (br.s, 1H, NH), 13.61 (s, 1H, NH). ^{13}C NMR (75.465 MHz, DMSO-d_6/CCl_4, 1/3) δ_C 26.5 (2CH$_3$), 35.9 (1-CH$_2$), 49.4 (N(CH$_2$)$_2$), 59.1 (4-CH$_2$), 65.9 (O(CH$_2$)$_2$), 69.2 (C^2), 115.7 (C), 118.3 (C), 124.7 (C), 134.5 (C), 142.8 (C), 158.6 (C), 160.9 (C), 171.6 (S-C=NH), 179.2 (C=S). Anal. Calcd for $C_{18}H_{20}N_4O_2S_3$ (420.57): C, 51.40; H, 4.79; N, 13.32; S, 22.87. Found: C, 51.35; H, 4.74; N, 13.40; S, 22.73.

Synthesis of 2,2-dimethyl-5-morpholin-4-yl-1,4-dihydro-2*H*-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidine-8,10(9*H*,11*H*)-dithione (5). To a solution of KOH (0.34 g, 6.0 mmol) in water (15 mL) the compound **4** (0.84 g, 2.0 mmol) was added and refluxed for 0.5 h. After cooling, the reaction mixture neutralized by 15 % hydrochloric acid. The obtained crystals were filtered off, washed with water and ethanol, recrystallized from nitromethane to give the product as a pure yellow solid (0.78 g, 93%). m.p. >360 °C. IR (ν/cm^{-1}): 3430 (NH), 3130 (NH), 1298 (C=S), 1283 (C=S). TOF MS ES+ [MH]$^+$ *m*/*z* 421.0836 (calcd for $C_{18}H_{20}N_4O_2S_3$, 421.0827). Anal. Calcd for $C_{18}H_{20}N_4O_2S_3$ (420.57): C, 51.40; H, 4.79; N, 13.32; S, 22.87. Found: C, 51.48; H 4.83; N, 13.38; S, 22.69.

General procedure for the synthesis of compounds (6a-e). To a solution of KOH (0.12 g, 2.1 mmol) in 80% aqueous ethanol (15 mL) the compound **5** (0.42 g, 1.0 mmol) was added. After complete dissolution, the appropriate alkyl halide (2.1 mmol) was added, and the reaction mixture was stirred for 2 h at room temperature. The obtained crystals were filtered off, washed with water, dried, and recrystallized from EtOH.

2,2-Dimethyl-8,10-bis(methylthio)-5-morpholin-4-yl-1,4-dihydro-2*H*-pyrano[4'',3'':4',5']pyrido[3',2':4,5]-thieno[3,2-*d*]pyrimidine (6a). Reaction of compound **5** (0.42 g, 1.0 mmol) and iodomethane (0.30 g, 2.1 mmol) according to general procedure afforded 0.42 g (93%) of product **6a** isolated as a white solid: m.p. 267–268 °C. ^{1}H NMR (300 MHz, DMSO-d_6/CCl_4, 1/3): δ_H 1.36 (s, 6H, C(CH$_3$)$_2$), 2.62 (s, 3H, SCH$_3$), 2.75 (s, 3H, SCH$_3$), 3.27 (br. t, J = 4.6 Hz, 4H, N(CH$_2$)$_2$), 3.47 (s, 2H, 1-CH$_2$), 3.79 (t, J = 4.6 Hz, 4H, O(CH$_2$)$_2$), 4.67 (s, 2H, 4-CH$_2$). ^{13}C NMR (75.465 MHz, DMSO-d_6/CCl_4, 1/3) δ_C 11.6 (SCH$_3$), 13.6 (SCH$_3$), 26.6 (2CH$_3$), 37.2 (1-CH$_2$), 49.7 (N(CH$_2$)$_2$), 59.1 (4-CH$_2$), 65.9 (O(CH$_2$)$_2$), 69.0 (C^2), 118.3 (C), 118.7 (C), 120.8 (C), 143.6 (C), 154.4 (C), 159.3 (C), 159.4 (C), 161.9 (C), 166.7 (C). Anal. Calcd for $C_{20}H_{24}N_4O_2S_3$ (448.62): C, 53.54; H, 5.39; N, 12.49; S, 21.44. Found: C, 53.48; H 5.53; N, 12.38; S, 21.59.

8,10-Bis(ethylthio)-2,2-dimethyl-5-morpholin-4-yl-1,4-dihydro-2*H*-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno-[3,2-*d*]pyrimidine (6b). Reaction of compound **5** (0.42 g, 1.0 mmol) and iodoethane (0.33 g, 2.1 mmol) according to general procedure afforded 0.43 g (90%) of product **6b** isolated as a white solid: m.p. 238–239 °C. ^{1}H NMR (300 MHz, DMSO-d_6/CCl_4, 1/3): δ_H 1.35 (s, 6H, C(CH$_3$)$_2$), 1.47 (t, J = 7.3 Hz, 3H, SCH$_2$C*H*$_3$), 1.48 (t, J = 7.3 Hz, 3H, SCH$_2$C*H*$_3$), 3.19 (q, J = 7.3 Hz, 2H, SC*H*$_2$CH$_3$), 3.27 (br. t, J = 4.5 Hz, 4H, N(CH$_2$)$_2$), 3.39 (q, J = 7.3 Hz, 2H, SC*H*$_2$CH$_3$), 3.47 (s, 2H, 1-CH$_2$), 3.79 (t, J = 4.5 Hz, 4H, O(CH$_2$)$_2$), 4.67 (s, 2H, 4-CH$_2$). ^{13}C NMR (75.465 MHz, DMSO-d_6/CCl_4, 1/3) δ_C 14.2 (CH$_3$), 14.4 (CH$_3$), 23.3 (SCH$_2$), 24.7 (SCH$_2$), 26.6 (2CH$_3$), 37.1 (1-CH$_2$), 49.7 (N(CH$_2$)$_2$), 59.1 (4-CH$_2$), 65.9 (O(CH$_2$)$_2$), 69.0 (C^2), 118.3 (C), 118.6 (C), 121.0 (C), 143.4 (C), 154.5 (C), 159.3 (C), 159.4 (C), 161.7 (C), 166.1 (C). Anal. Calcd for $C_{22}H_{28}N_4O_2S_3$ (476.68): C, 55.43; H, 5.92; N, 11.75; S, 20.18. Found: C, 55.49; H 5.86; N, 11.78; S, 20.09.

8,10-Bis(benzylthio)-2,2-dimethyl-5-morpholin-4-yl-1,4-dihydro-2*H*-pyrano[4'',3'':4',5']pyrido[3',2':4,5]-thieno[3,2-*d*]pyrimidine (6c). Reaction of compound **5** (0.42 g, 1.0 mmol) and (chloromethyl)benzene (0.27 g, 2.1 mmol) according to general procedure afforded 0.60 g (99%) of product **6c** isolated as a white solid: m.p.

207–208 °C. ^{1}H NMR (300 MHz, DMSO-d_6/CCl_4, 1/3): δ_H 1.25 (s, 6H, $C(CH_3)_2$), 3.25 (br. t, J = 4.5 Hz, 4H, $N(CH_2)_2$), 3.36 (s, 2H, 1-CH_2), 3.77 (t, J = 4.5 Hz, 4H, $O(CH_2)_2$), 4.51 (s, 2H, 4-CH_2), 4.61 (s, 2H, SCH_2), 4.64 (s, 2H, SCH_2), 7.17-7.32 (m, 6H, $6CH_{Ar}$), 7.38-7.45 (m, 4H, $4CH_{Ar}$). ^{13}C NMR (75.465 MHz, DMSO-d_6/CCl_4, 1/3) δ_C 26.5 ($2CH_3$), 33.0 (SCH_2), 34.5 (SCH_2), 37.1 (1-CH_2), 49.7 ($N(CH_2)_2$), 59.1 (4-CH_2), 65.9 ($O(CH_2)_2$), 69.0 (C^2), 118.2 (C), 118.7 (C), 120.9 (C), 126.4 (CH_{Ar}), 126.8 (CH_{Ar}), 127.8 ($2CH_{Ar}$), 127.9 ($2CH_{Ar}$), 128.0 ($2CH_{Ar}$), 128.7 ($2CH_{Ar}$), 136.1 (C), 137.1 (C), 143.5 (C), 154.7 (C), 159.4 (C), 159.5 (C), 161.4 (C), 165.9 (C). Anal. Calcd for $C_{32}H_{32}N_4O_2S_3$ (600.80): C, 63.97; H, 5.37; N, 9.33; S, 16.01. Found: C, 63.88; H 5.48; N, 9.48; S, 16.19.

2,2'-[(2,2-Dimethyl-5-morpholin-4-yl-1,4-dihydro-2*H*-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidine-8,10-diyl)bis(thio)]bis[*N*-(4-ethoxyphenyl)acetamide] (6d). Reaction of compound **5** (0.42 g, 1.0 mmol) and 2-chloro-*N*-(4-ethoxyphenyl)acetamide (0.45 g, 2.1 mmol) according to general procedure afforded 0.74 g (96%) of product **6d** isolated as a white solid: m.p. 260–261 °C. IR (ν/cm^{-1}): 3304 (2NH), 1666 (2C=O). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.21 (s, 6H, $C(CH_3)_2$), 1.28 (t, J = 7.0 Hz, 3H, $OCH_2C\mathit{H}_3$), 1.30 (t, J = 7.0 Hz, 3H, $OCH_2C\mathit{H}_3$), 3.26 (br. t, J = 4.5 Hz, 4H, $N(CH_2)_2$), 3.39 (s, 2H, 1-CH_2), 3.74 (br. t, J = 4.5 Hz, 4H, $O(CH_2)_2$), 3.93 (q, J = 7.0 Hz, 2H, $OC\mathit{H}_2CH_3$), 3.96 (q, J = 7.0 Hz, 2H, $OC\mathit{H}_2CH_3$), 4.15 (s, 2H, SCH_2), 4.35 (s, 2H, SCH_2), 4.64 (s, 2H, 4-CH_2), 6.78-6.89 (m, 4H, $4CH_{Ar}$), 7.40-7.51 (m, 4H, $4CH_{Ar}$), 9.88 (s, 1H, NH), 10.21 (s, 1H, NH). ^{13}C NMR (75.465 MHz, DMSO-d_6) δ_C 14.5 (CH_3), 14.6 (CH_3), 26.7 ($2CH_3$), 34.1 (SCH_2), 35.3 (SCH_2), 37.2 (1-CH_2), 49.7 ($N(CH_2)_2$), 59.1 (4-CH_2), 62.9 ($O(CH_2)_2$), 63.0 (OCH_2), 66.0 (OCH_2), 69.6 (C^2), 114.3 ($2CH_{Ar}$), 114.4 ($2CH_{Ar}$), 117.7 (C), 119.3 (C), 120.4 (C), 120.7 ($2CH_{Ar}$), 120.8 ($2CH_{Ar}$), 131.7 (C), 131.9 (C), 144.1 (C), 154.5 (C), 154.6 (C), 154.9 (C), 159.5 (C), 159.8 (C), 161.3 (C), 164.7 (C), 165.3 (C=O), 165.9 (C=O). Anal. Calcd for $C_{38}H_{42}N_6O_6S_3$ (775.0): C, 58.89; H, 5.46; N, 10.84; S, 12.41. Found: C, 58.99; H 5.58; N, 10.68; S, 12.59.

2,2'-[(2,2-Dimethyl-5-morpholin-4-yl-1,4-dihydro-2*H*-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidine-8,10-diyl)bis(thio)]bis[*N*-(2-phenylethyl)acetamide] (6e). Reaction of compound **5** (0.42 g, 1.0 mmol) and 2-chloro-*N*-(2-phenylethyl)acetamide (0.42 g, 2.1 mmol) according to general procedure afforded 0.71 g (95%) of product **6e** isolated as a white solid: m.p. 260–261 °C. IR (ν/cm^{-1}): 3294 (2NH), 1650 (2C=O). ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.46 (s, 6H, $C(CH_3)_2$), 2.72 (t, J = 6.7 Hz, 2H, $NHCH_2C\mathit{H}_2$), 2.84 (t, J = 6.7 Hz, 2H, $NHCH_2C\mathit{H}_2$), 3.35 (br. t, J = 4.6 Hz, 4H, $N(CH_2)_2$), 3.43 (s, 2H, 1-CH_2), 3.49-3.56 (m, 2H, $NHC\mathit{H}_2CH_2$), 3.53-3.61 (m, 2H, $NHC\mathit{H}_2CH_2$), 3.67 (s, 2H, SCH_2), 3.89 (s, 2H, SCH_2), 3.90 (br. t, J = 4.5 Hz, 4H, $O(CH_2)_2$), 4.78 (s, 2H, 4-CH_2), 6.78-7.12 (m, 11H, NH and $10CH_{Ar}$), 7.38 (br. t, J = 5.8 Hz, 1H, NH). ^{13}C NMR (75.465 MHz, DMSO-d_6/CCl_4, 1/3) δ_C 27.0 ($2CH_3$), 32.8 ($NHCH_2\mathit{C}H_2$), 34.9 ($NHCH_2\mathit{C}H_2$), 35.3 (SCH_2), 35.4 (SCH_2), 37.7 (1-CH_2), 40.9 ($NH\mathit{C}H_2CH_2$), 41.2 ($NH\mathit{C}H_2CH_2$), 50.3 ($N(CH_2)_2$), 60.2 (4-CH_2), 67.0 ($O(CH_2)_2$), 70.4 (C^2), 118.6 (C), 119.3 (C), 122.6 (C), 126.3 (CH_{Ar}), 126.4 (CH_{Ar}), 128.3 ($2CH_{Ar}$), 128.4 ($2CH_{Ar}$), 128.5 ($2CH_{Ar}$), 128.7 ($2CH_{Ar}$), 138.3 (2C), 138.7 (C), 144.9 (C), 155.8 (C), 160.5 (C), 161.6 (C), 164.3 (C), 168.2 (C=O), 168.8 (C=O). Anal. Calcd for $C_{38}H_{42}N_6O_4S_3$ (742.98): C, 61.43; H, 5.70; N, 11.31; S, 12.95. Found: C, 61.54; H 5.63; N, 11.49; S, 13.08.

Biological evaluation

General. Compounds were studied for their antitumor activity, as well as for their LD100 (absolutely lethal dose) and MTD (maximum tolerated dose), using 150 outbred white mice and 50 C57BL/6 inbred strain mice weighing 19-21 g each, along with 75 white rats weighing 90-110 g, encompassing both sexes. All groups of animals were maintained at 25 ± 2 °C in the same room, on a common food ration. All the biological experiments were carried out in full compliance with the European Convention for the Protection of Vertebrate. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of "(ETS No 123, Strasbourg, 03/18/1986): Strasbourg (France). European Treaty Series – No 123, March 18, 1986. 11 P". University and Experiments were approved by the Animal Ethics Committee of the Scientific Technological Center of Organic and Pharmaceutical Chemistry of the National Academy of Sciences of the Republic of Armenia. P.№7 from 26 May 2023.

Evaluation of the antitumor activity of the synthesized compounds. In acute experiments on outbred white mice weighing 19-21 g, the absolutely lethal (LD100) and maximum tolerated (MTD) doses of substances were determined. The compounds were administered intraperitoneally to the mice once at various doses (2500, 1500, 1000 mg/kg). The animals were observed for 15 days, systematically recording mortality, changes in weight, and various toxic manifestations.

In chemotherapeutic experiments, the compounds were tested in the form of a suspension in a 0.5% carboxymethylcellulose solution and administered to animals intraperitoneally. The treatment of animals with Walker's carcinosarcoma, Sarcoma 45, and Sarcoma 180 began on the 4th-5th day after tumor vaccinations. For adenocarcinoma of the mammary gland 755 and LLC (Lewis lung carcinoma), treatment began on the 3rd day of tumor growth. Treatment for Pliss lymphosarcoma started 24 hours after tumor vaccinations. The compounds were administered daily to mice for 6 and to rats for 8 days at doses of 1/15-1/20 of their LD100. Control animals received an equivalent volume of solvent at the same time of experiment. Antitumor activity was assessed 48 hours after the last injection by calculating the percentage of tumor growth inhibition (TGI %) relative to the control. Statistical analysis of the results was performed using the Fisher test method.[18, 19]

Supplementary Material

^{1}H and ^{13}C NMR spectra of products can be found in the supplementary material file.

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