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Synthesis and antimicrobial activity of new amino derivatives of pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidine

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ABSTRACT

Annulated thienopyrimidine derivatives attracted big interest of the scientific community due to their broad spectrum of biological activities among which are the inhibition of phosphodiesterase, antiproliferative and antimicrobial activities. As a continuation of our studies on the synthesis and biological activity of fused thieno[3,2-*d*]pyrimidine derivatives, the goal of this paper is the synthesis and study of the properties of compounds containing different heterocycles such as fused thieno[2,3-*b*]pyridine and tetrazolo[1,5-*c*] pyrimidine in the same molecule. Thus, starting from the ethyl 1-amino-5-isopropyl-8,8-dimethyl-8,9-dihydro-6*H*-pyrano[4,3-*d*]thieno[2,3-*b*]pyridine-2-carboxylate **1,** efficient methods for obtaining new 8-amino-5-isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2*H*-pyrano[4'',3'':4',5']pyrido[3',2':4,5] thieno[3,2-*d*]pyrimidines **6** and thieno[2,3-*e*]tetrazolo[1,5-*c*]pyrimidine **8** are described. The spectroscopic results showed that compound **8** in the solid state is exclusively in the tetrazolo tautomeric form, while in solution an azide-tetrazole equilibrium is present **8A/T**. The possible antimicrobial activity of newly synthesized compounds against some gram-positive and gram-negative bacilli strains has been evaluated. The biological tests evidenced that some of them showed promising antimicrobial activity. Two compounds showed similar activity to the one of the used reference drug. The study of structure-activity relationships revealed that the activity of a compound depends mostly on the nature of substituent R^1R^2. According to the predicted docking studies our compounds could be DnaG inhibitors.

Key words: amino derivatives, antimicrobial activity, azide/tetrazole equilibrium, nucleophilic substitution, thieno[3,2-*d*]pyrimidine.

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INTRODUCTION

During human history man was fighting against a variety of microorganisms that cause infections and diseases, some of them like swine appeared in the form of a pandemic. The discovery of antibiotics led to disappearance of many diseases, such as plague. Unfortunately, new infections and diseases appeared affecting large population, causing significant morbidity and mortality. The most recent example is acute immunodeficiency syndrom (AIDS).

Despite the fact that antimicrobial agents play positive role during the 20th century in the treatment of infectious diseases, the problem of microbial resistance became global, bacteria became resistant to cheap and effective drugs, contributing mostly to human diseases.

From another hand the economic crisis, high cost of industrialized medicines, inefficient public access to medical and pharmaceutical care enhanced the rate of infectious disease, becoming a big problem with increasing importance in hospitalized patients, in immuno suppressed patients with AIDS or undergoing cancer therapy and organ transplants.

A potential approach to overcome the resistance problem is to design innovative agents with different mode of action so that no cross-resistance with the present therapeuticals can occur.

One of the targets of antibacterials is inhibition of the synthesis of bacterial DNA. DNA primases are involved in this synthesis as template-dependent RNA polymerases that synthesize oligoribonucleotide primers that RNA polymerase can be extended by DNA polymerase.

Special interest towards annulated thienopyrimidine derivatives is due to their broad spectrum of biological activity (Litvinov 2004, Shnute et al. 2008, Gineinah et al. 2013). In particular, thieno[3,2-*d*]pyrimidine derivatives were found to inhibit phosphodiesterase (Chakraborti et al. 2003, Reichelt et al. 2011) and possess antiproliferative (Temburnikar et al. 2014), neurotropic (Oganisyan et al. 2003) and antimicrobial (Bakhite et al. 2002, 2004, Rateb 2007, Agarwal et al. 2007, Entsar et al. 2011) activities.

In continuation of our studies on the synthesis and biological activity of amino derivatives of fused thieno[3,2-*d*]pyrimidines (Sirakanyan et al. 2014, 2016a), in this article we report the synthesis and antimicrobial activity of new 8-amino-5-isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2*H*-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*] pyrimidines.

Furthermore, considering our previous experience in the field of azide-tetrazole equilibrium in the series of thieno[2,3-*e*]tetrazolo[1,5-*c*] pyrimidines (Sirakanyan et al. 2016b), herein we report the synthesis of new 8-azidothieno[3,2-*d*] pyrimidine/thieno[2,3-*e*]tetrazolo[1,5-*c*] pyrimidine **8A/T**.

MATERIALS AND METHODS

^{1}H NMR spectra were recorded in DMSO/CCl_4 (1/3) solution on a Varian Mercury 300VX 300 MHz spectrometer. Chemical shifts were reported as δ (parts per million) relative to TMS as internal standard. IR spectra were recorded on Nicolet Avatar 330-FT-IR spectrophotometer and the reported wave numbers were given in cm^{-1}. All melting points were determined in an open capillary and were uncorrected. Elemental analyses were performed on an Elemental Analyzer Euro EA 3000.

Compound **1** (Sirakanyan et al. 2014) was already described.

EXPERIMENTAL

Procedure for the synthesis of compound 2

A mixture of compound **1** (34.85 g, 100 mmol), benzoyl isothiocyanate (14.8 mL, 110 mmol) in dry benzene (250 mL) was refluxed for 5 h. After

cooling, the solvent was removed and diethyl ether (50 mL) was added. The separated crystals were filtered off, washed with diethyl ether, dried, and recrystallized from a mixture of ethanol–chloroform (1:2).

*Ethyl 1-{[(benzoylamino)carbonothioyl] amino}-5-isopropyl-8,8-dimethyl-8,9-dihydro-6H-pyrano[4,3-d]thieno[2,3-b]pyridine-2-carboxylate (**2**).* Colorless solid; yield 88%; mp 119–200 °C; IR ν/cm^{-1}: 3391 (NH), 1723, 1672 (C=O). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.24 (s, 6H, $C(CH_3)_2$), 1.33 (d, J = 6.7 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.34 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.04 (sp, J = 6.7 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.08 (br d, J = 16.8 Hz, 1H, CH_2), 3.20 (br d, J = 16.8 Hz, 1H, CH_2), 4.33 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 4.84 (br s, 2H, OCH_2), 7.47–7.54 (m, 2H, Ph), 7.58–7.65 (m, 1H, Ph), 8.11–8.16 (m, 2H, Ph), 11.65 (s, 1H, NH), 12.35 (s, 1H, NH). Anal. calcd. for $C_{26}H_{29}N_3O_4S_2$: C 61.03; H 5.71; N 8.21 %. Found: C 61.34; H 5.95; N 7.94 %.

Procedure for the synthesis of compound 3

To a solution of potassium hydroxide (1.12 g, 20 mmol) in ethanol (100 mL, 80 %) compound **2** (5.12 g, 10 mmol) was added and the mixture refluxed for 2 h. After cooling, water (50 mL) was added and the reaction mixture was neutralized with hydrochloric acid. The resulting crystals were filtered off, washed with water, dried, and recrystallized from DMF.

*5-Isopropyl-2,2-dimethyl-10-thioxo-1,4,10,11-tetrahydro-2H-pyrano[4'',3'':4',5'] pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8(9H)-one (**3**).* Colorless solid; yield 80%; mp 327–329 °C; IR ν/cm^{-1}: 3418, 3152, 3091 (NH), 1677 (C=O), 1259 (C=S). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.23 (d, J = 6.7 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.28 (s, 6H, $C(CH_3)_2$), 3.10 (sp, J = 6.7 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.42 (s, 2H, CH_2), 4.88 (s, 2H, OCH_2), 11.45 (br s, 1H, NH), 12.97 (br s, 1H, NH). Anal. calcd. for $C_{17}H_{19}N_3O_2S_2$: C 56.48; H 5.30; N 11.62 %. Found: C 56.82; H 5.48; N 11.41 %.

Procedure for the synthesis of compound 4

To a stirred solution of compound **3** (3.61g, 10 mmol) in DMF (50 mL) a solution of potassium hydroxide (0.62 g, 11 mmol) in water (10 mL) was added. The reaction mixture was stirred and heated until dissolution of compound **3**, then it was cooled to 0–10 °C and a solution of methyl iodide (0.68 mL, 11 mmol) in ethanol (30 mL) was dropwise added maintaining the reaction temperature at 0–10 °C. Then the mixture was stirred for 6 h at room temperature. After cooling water (50 mL) was added and the reaction mixture was neutralized with hydrochloric acid. The resulting crystals were filtered off, washed with water and then with ethanol, dried, and recrystallized from DMF.

*5-Isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5'] pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8(9H)-one (**4**).* Colorless solid; yield 78%; mp 338–340 °C; IR ν/cm^{-1}: 3198 (NH), 1647 (C=O). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.30 (s, 6H, $C(CH_3)_2$), 1.31 (d, J = 6.6 Hz, 6H, $CH(C\underline{H}_3)_2$), 2.62 (s, 3H, SCH_3), 3.04 (sp, J = 6.6 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.48 (s, 2H, CH_2), 4.86 (s, 2H, OCH_2), 12.98 (br, 1H, NH). Anal. calcd. for $C_{18}H_{21}N_3O_2S_2$: C 57.57; H 5.64; N 11.19 %. Found: C 57.89; H 5.84; N 10.93 %.

Procedure for the synthesis of compound 5

A mixture of compound **4** (3.76 g, 10 mmol) and phosphorus oxychloride (50 mL) was refluxed for 4 h. The excess of phosphorus oxychloride was distilled off to give a dry residue. Ice water and then ammonia solution were added. The separated crystals were filtered off, washed with water, dried, and recrystallized from ethanol.

8-Chloro-5-isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5']

*pyrido[3',2':4,5]thieno[3,2-d]pyrimidine (**5**)*. Colorless solid; yield 73%; mp 211–213 °C. ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.33 (d, *J* = 6.6 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.34 (s, 6H, $C(CH_3)_2$), 2.66 (s, 3H, SCH_3), 3.10 (sp, *J* = 6.6 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.50 (s, 2H, CH_2), 4.89 (s, 2H, OCH_2). Anal. calcd. for $C_{18}H_{20}ClN_3OS_2$: C 54.88; H 5.12; N 10.67 %. Found: C 55.19; H 5.31; N 10.91%.

General procedure for the synthesis of compounds 6a–v

A mixture of compound **5** (1.97 g, 5 mmol) and of corresponding amine (11 mmol) in absolute ethanol (50 mL) was refluxed for 5 h. The ethanol was distilled off to dryness, water (25 mL) was added to the residue. The separated crystals were filtered off, washed with water, dried, and recrystallized from ethanol.

*5-Isopropyl-2,2-dimethyl-10-(methylthio)-8-pyrrolidin-1-yl-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidine (**6a**)*. Colorless solid; yield 89%; mp 257–259 °C. ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.31 (d, *J* = 6.2 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.33 (s, 6H, $C(CH_3)_2$), 2.06–2.11 (m, 4H, $2CH_2$, C_4H_8N), 2.54 (s, 3H, SCH_3), 3.05 (sp, *J* = 6.6 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.56 (s, 2H, CH_2), 3.88–3.95 (m, 4H, $N(CH_2)_2$), 4.86 (s, 2H, OCH_2). Anal. calcd. for $C_{22}H_{28}N_4OS_2$: C 61.65; H 6.58; N 13.07 %. Found: C 61.93; H 6.79; N 12.85 %.

*5-Isopropyl-2,2-dimethyl-10-(methylthio)-8-piperidin-1-yl-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidine (**6b**)*. Colorless solid; yield 82%; mp 199–201 °C. ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.30 (d, *J* = 6.6 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.32 (s, 6H, $C(CH_3)_2$), 1.68–1.82 (m, 6H, $(CH_2)_3$, $C_5H_{10}N$), 2.55 (s, 3H, SCH_3), 3.04 (sp, *J* = 6.6 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.55 (s, 2H, CH_2), 3.89–3.98 (m, 4H, $N(CH_2)_2$), 4.85 (s, 2H, OCH_2). Anal. calcd. for $C_{23}H_{30}N_4OS_2$: C 62.41; H 6.83; N 12.66 %. Found: C 62.74; H 7.04; N 12.92 %.

*5-Isopropyl-2,2-dimethyl-10-(methylthio)-8-morpholin-4-yl-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidine (**6c**)*. Colorless solid; yield 86%; mp 251–253 °C. ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.31 (d, *J* = 6.6 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.33 (s, 6H, $C(CH_3)_2$), 2.56 (s, 3H, SCH_3), 3.06 (sp, *J* = 6.6 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.56 (s, 2H, CH_2), 3.76–3.83 (m, 4H, $N(CH_2)_2$), 3.90–3.96 (m, 4H, $O(CH_2)_2$), 4.87 (s, 2H, OCH_2). Anal. calcd. for $C_{22}H_{28}N_4O_2S_2$: C 59.43; H 6.35; N 12.60 %. Found: C 59.72; H 6.54; N 12.83 %.

*5-Isopropyl-2,2-dimethyl-8-(4-methylpiperazin-1-yl)-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidine (**6d**)*. Colorless solid; yield 77%; mp 224–225 °C. ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.30 (d, *J* = 6.6 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.32 (s, 6H, $C(CH_3)_2$), 2.31 (s, 3H, NCH_3), 2.48–2.57 (m, 4H, $CH_3N(C\underline{H}_2)_2$), 2.56 (s, 3H, SCH_3), 3.05 (sp, *J* = 6.6 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.55 (s, 2H, CH_2), 3.92–4.01 (m, 4H, $N(CH_2)_2$), 4.87 (s, 2H, OCH_2). Anal. calcd. for $C_{23}H_{31}N_5OS_2$: C 60.36; H 6.83; N 15.30 %. Found: C 60.72; H 7.05; N 15.07 %.

*8-(4-Ethylpiperazin-1-yl)-5-isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidine (**6e**)*. Colorless solid; yield 75%; mp 173–175 °C. ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.11 (t, *J* = 7.2 Hz, 3H, $NCH_2C\underline{H}_3$), 1.30 (d, *J* = 6.6 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.32 (s, 6H, $C(CH_3)_2$), 2.45 (q, *J* = 7.2 Hz, 2H, $NC\underline{H}_2CH_3$), 2.55 (s, 3H, SCH_3), 2.53–2.61 (m, 4H, $C_2H_5N(C\underline{H}_2)_2$), 3.06 (sp, *J* = 6.6 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.55 (s, 2H, CH_2), 3.92–4.03 (m, 4H, $N(CH_2)_2$), 4.86 (s, 2H, OCH_2). Anal. calcd. for $C_{24}H_{33}N_5OS_2$: C 61.11; H 7.05; N 14.85 %. Found: C 61.41; H 7.23; N 15.10 %.

*Ethyl 4-[5-isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5'] pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-yl] piperazine-1-carboxylate (**6f**)*. Colorless solid; yield 84%; mp 222–224 °C; IR ν/cm^{-1}: 1658 (C=O). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.29 (t, J = 7.2 Hz, 3H, $OCH_2C\underline{H}_3$), 1.31 (d, J = 6.6 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.32 (s, 6H, $C(CH_3)_2$), 2.55 (s, 3H, SCH_3), 3.06 (sp, J = 6.6 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.55 (s, 2H, CH_2), 3.57–3.65 (m, 4H, $NCO(C\underline{H}_2)_2$), 3.92–4.00 (m, 4H, $N(CH_2)_2$), 4.12 (q, J = 7.2 Hz, 2H, $OC\underline{H}_2CH_3$), 4.87 (s, 2H, OCH_2). Anal. calcd. for $C_{25}H_{33}N_5O_3S_2$: C 58.23; H 6.45; N 13.58 %. Found: C 58.51; H 6.64; N 13.82 %.

*2-{4-[5-Isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5'] pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-yl] piperazin-1-yl}ethanol (**6g**)*. Colorless solid; yield 81%; mp 241–243 °C. ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.31 (d, J = 6.6 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.32 (s, 6H, $C(CH_3)_2$), 2.45–2.52 (m, 2H, $OHCH_2$), 2.55 (s, 3H, SCH_3), 2.60–2.68 (m, 4H, $CH_2N(C\underline{H}_2)_2$), 3.05 (sp, J = 6.6 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.51–3.61 (m, 4H, $OHC\underline{H}_2C\underline{H}_2$), 3.82–3.96 (br, 1H, $O\underline{H}CH_2CH_2$), 3.92–4.00 (m, 4H, $N(CH_2)_2$), 4.87 (s, 2H, OCH_2). Anal. calcd. for $C_{24}H_{33}N_5O_2S_2$: C 59.11; H 6.82; N 14.36 %. Found: C 59.46; H 7.04; N 14.62 %.

*2-{[5-Isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5'] pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-yl] amino}ethanol (**6h**)*. Colorless solid; yield 87%; mp 242–244 °C; IR ν/cm^{-1}: 3234 (NH). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.32 (s, 6H, $C(CH_3)_2$), 1.33 (d, J = 6.6 Hz, 6H, $CH(C\underline{H}_3)_2$), 2.55 (s, 3H, SCH_3), 3.06 (sp, J = 6.6 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.55 (s, 2H, CH_2), 3.59–3.70 (m, 4H, $HNC\underline{H}_2C\underline{H}_2OH$), 4.42 (br, 1H, OH), 4.86 (s, 2H, OCH_2), 7.39 (br, 1H, NH). Anal. calcd. for $C_{20}H_{26}N_4O_2S_2$: C 57.39; H 6.26; N 13.39 %. Found: C 57.7; H 6.45; N 13.63 %.

*5-Isopropyl-N-(2-methoxyethyl)-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5] thieno[3,2-d]pyrimidin-8-amine (**6i**)*. Colorless solid; yield 79%; mp 159–161 °C; IR ν/cm^{-1}: 3317 (NH). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.31 (c, 6H, $C(CH_3)_2$), 1.33 (d, J = 6.6 Hz, 6H, $CH(C\underline{H}_3)_2$), 2.54 (s, 3H, SCH_3), 3.05 (sp, J = 6.6 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.36 (s, 3H, OCH_3), 3.55 (s, 2H, CH_2), 3.56–3.63 (t, J = 5.6 Hz, 2H, $HNCH_2C\underline{H}_2$), 3.66–3.76 (м, 2H, $HNC\underline{H}_2CH_2$), 4.86 (s, 2H, OCH_2), 7.53 (t, J = 5.6 Hz, 1H, NH). Anal. calcd. for $C_{21}H_{28}N_4O_2S_2$: C 58.30; H 6.52; N 12.95 %. Found: C 58.63; H 6.72; N 13.21 %.

*N-(2,2-Dimethoxyethyl)-5-isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5] thieno[3,2-d]pyrimidin-8-amine (**6j**)*. Colorless solid; yield 85%; mp 170–171 °C; IR ν/cm^{-1}: 3237 (NH). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.31 (s, 6H, $C(CH_3)_2$), 1.32 (d, J = 6.6 Hz, 6H, $CH(C\underline{H}_3)_2$), 2.55 (s, 3H, SCH_3), 3.05 (sp, J = 6.6 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.37 (s, 6H, $CH(OC\underline{H}_3)_2$), 3.54 (s, 2H, CH_2), 3.58–3.64 (m, 2H, $HNC\underline{H}_2$), 4.64 (t, J = 5.7 Hz, 1H, $HNCH_2C\underline{H}$), 4.86 (s, 2H, OCH_2), 7.57 (t, J = 5.7 Hz, 1H, NH). Anal. calcd. for $C_{22}H_{30}N_4O_3S_2$: C 57.12; H 6.54; N 12.11 %. Found: C 57.46; H 6.72; N 12.33 %.

*1-{[5-Isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5'] pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-yl] amino}propan-2-ol (**6k**)*. Colorless solid; yield 76%; mp 234–236 °C; IR ν/cm^{-1}: 3324 (NH). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.16 (d, J = 6.3 Hz, 3H, $CHC\underline{H}_3$), 1.32 (d, J = 6.6 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.34 (s, 6H, $C(CH_3)_2$), 2.54 (s, 3H, SCH_3), 3.05 (sp, J = 6.6 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.35 (ddd, J = 13.4, 7.4, 5.0 Hz, 1H, $NHC\underline{H}_2$), 3.54 (s, 2H, CH_2), 3.62 (ddd, J = 13.4, 6.4, 4.4 Hz, 1H, $NHC\underline{H}_2$), 3.88–3.99 (m, 1H, $C\underline{H}CH_3$), 4.50 (br, 1H, OH), 4.87 (s, 2H, OCH_2), 7.29 (t, J = 5.7 Hz, 1H, NH). Anal. calcd. for $C_{21}H_{28}N_4O_2S_2$: C 58.30; H 6.52; N 12.95 %. Found: C 58.58; H 6.75; N 13.19 %.

*N'-[5-Isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-yl]-N,N-dimethylethane-1,2-diamine (**6l**)*. Colorless solid; yield 82%; mp 183–185 °C; IR ν/cm^{-1}: 3330 (NH). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.32 (d, J = 6.6 Hz, 6H, CH(C$\underline{H}_3$)$_2$), 1.33 (s, 6H, C(CH_3)$_2$), 2.31 (s, 6H, N(CH_3)$_2$), 2.55 (s, 3H, SCH_3), 2.60 (t, J = 6.7 Hz, 2H, C$\underline{H}_2$N(CH$_3$)$_2$), 3.05 (sp, J = 6.6 Hz, 1H, C$\underline{H}$(CH$_3$)$_2$), 3.54 (s, 2H, CH_2), 3.66 (td, J = 6.5, 5.9 Hz, 2H, NHC$\underline{H}_2$), 4.86 (s, 2H, OCH_2), 7.26 (t, J = 5.5 Hz, 1H, NH). Anal. calcd. for $C_{22}H_{31}N_5OS_2$: C 59.29; H 7.01; N 15.72 %. Found: C 59.66; H 7.18; N 15.98 %.

*5-Isopropyl-2,2-dimethyl-10-(methylthio)-N-(2-morpholin-4-ylethyl)-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-amine (**6m**)*. Colorless solid; yield 73%; mp 170–172 °C; IR ν/cm^{-1}: 3357 (NH). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.31 (s, 6H, C(CH_3)$_2$), 1.32 (d, J = 6.6 Hz, 6H, CH(C$\underline{H}_3$)$_2$), 2.55 (s, 3H, SCH_3), 2.58–2.68 (m, 4H, N(CH_2)$_2$), 2.64 (t, J = 6.7 Hz, 2H, C$\underline{H}_2$N(CH$_2$)$_2$), 3.05 (sp, J = 6.6 Hz, 1H, C$\underline{H}$(CH$_3$)$_2$), 3.54 (s, 2H, CH_2), 3.56–3.62 (m, 4H, O(CH_2)$_2$), 3.66 (td, 2H, J = 6.5, 5.9 Hz, NHC$\underline{H}_2$), 4.85 (s, 2H, OCH_2), 7.28 (t, J = 5.6 Hz, 1H, NH). Anal. calcd. for $C_{24}H_{33}N_5O_2S_2$: C 59.11; H 6.82; N 14.36 %. Found: C 59.4; H 7.00; N 14.59 %.

*N'-[5-Isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-yl]-N,N-dimethylpropane-1,3-diamine (**6n**)*. Colorless solid; yield 78%; mp 169–171 °C; IR ν/cm^{-1}: 3256 (NH). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.32 (d, J = 6.7 Hz, 6H, CH(C$\underline{H}_3$)$_2$), 1.33 (s, 6H, C(CH_3)$_2$), 1.80–1.90 (m, 2H, NHCH$_2$C$\underline{H}_2$), 2.37 (s, 6H, N(CH_3)$_2$), 2.54 (s, 3H, SCH_3), 2.55 (br, 2H, C$\underline{H}_2$N(CH$_3$)$_2$), 3.05 (sp, J = 6.7 Hz, 1H, C$\underline{H}$(CH$_3$)$_2$), 3.55 (s, 2H, CH_2), 3.63 (td, 2H, J = 6.8, 5.5 Hz, NHC$\underline{H}_2$), 4.86 (s, 2H, OCH_2), 7.81 (t, J = 5.5 Hz, 1H, NH). Anal. calcd. for $C_{23}H_{33}N_5OS_2$: C 60.10; H 7.24; N 15.24 %. Found: C 60.43; H 7.45; N 15.49 %.

*3-{[5-Isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-yl]amino}propan-1-ol (**6o**)*. Colorless solid; yield 86%; mp 257–259 °C; IR ν/cm^{-1}: 3244 (NH). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.32 (s, 6H, C(CH_3)$_2$), 1.33 (d, J = 6.6 Hz, 6H, CH(C$\underline{H}_3$)$_2$), 1.77–1.87 (m, 2H, NHCH$_2$C$\underline{H}_2$), 2.55 (s, 3H, SCH_3), 3.06 (sp, J = 6.6 Hz, 1H, C$\underline{H}$(CH$_3$)$_2$), 3.50–3.60 (m, 2H, OHC$\underline{H}_2$), 3.55 (s, 2H, CH_2), 3.60–3.68 (m, 2H, NHC$\underline{H}_2$), 4.16 (br, 1H, OH), 4.86 (s, 2H, OCH_2), 7.44 (br t, J = 5.5 Hz, 1H, NH). Anal. calcd. for $C_{21}H_{28}N_4O_2S_2$: C 58.30; H 6.52; N 12.95 %. Found: C 58.65; H 6.72; N 13.17 %.

*N-(2-Furylmethyl)-5-isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-amine (**6p**)*. Colorless solid; yield 81%; mp 261–263 °C; IR ν/cm^{-1}: 3267 (NH). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.32 (s, 6H, C(CH_3)$_2$), 1.33 (d, J = 6.6 Hz, 6H, CH(C$\underline{H}_3$)$_2$), 2.55 (s, 3H, SCH_3), 3.05 (sp, J = 6.6 Hz, 1H, C$\underline{H}$(CH$_3$)$_2$), 3.55 (s, 2H, CH_2), 4.74 (d, J = 5.6 Hz, 2H, NHC$\underline{H}_2$), 4.86 (s, 2H, OCH_2), 6.22–6.32 (m, 2H, 3,4-CH_{fur}.), 7.38 (dd, J = 1.7, 0.8 Hz, 1H, 5-CH_{fur}), 8.06 (br t, J = 5.7 Hz, 1H, NH). Anal. calcd. for $C_{23}H_{26}N_4O_2S_2$: C 60.77; H 5.76; N 12.32 %. Found: C 61.05; H 5.98; N 12.56 %.

*N-Benzyl-5-isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-amine (**6q**)*. Colorless solid; yield 88%; mp 185–187 °C; IR ν/cm^{-1}: 3332 (NH). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.32 (s, 6H, C(CH_3)$_2$), 1.33 (d, J = 6.6 Hz, 6H, CH(C$\underline{H}_3$)$_2$), 2.51 (s, 3H, SCH_3), 3.06 (sp, J = 6.7 Hz, 1H, C$\underline{H}$(CH$_3$)$_2$), 3.55 (s, 2H, CH_2), 4.78 (d, J = 5.9 Hz, 2H, NHC$\underline{H}_2$), 4.87 (s, 2H, OCH_2), 7.16–7.41 (m, 5H, Ph), 8.12 (t, J = 5.7 Hz, 1H, NH). Anal. calcd. for $C_{25}H_{28}N_4OS_2$: C 64.62; H 6.07; N 12.06 %. Found: C 64.93; H 6.26; N 12.29 %.

*5-Isopropyl-2,2-dimethyl-10-(methylthio)-N-(pyridin-2-ylmethyl)-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-amine (**6r**)*. Colorless solid; yield 74%; mp 263–264 ºC; IR ν/cm^{-1}: 3225 (NH). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.32 (s, 6H, $C(CH_3)_2$), 1.32 (d, J = 6.6 Hz, 6H, $CH(C\underline{H}_3)_2$), 2.46 (s, 3H, SCH_3), 3.06 (sp, J = 6.6 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.54 (s, 2H, CH_2), 4.84 (d, J = 5.8 Hz, 2H, $NHC\underline{H}_2$), 4.85 (s, 2H, OCH_2), 7.17 (ddd, J = 7.5, 4.7, 1.0 Hz, 1H, 5-CH-C_5H_4N), 7.35 (ddd, J = 7.6, 1.1, 0.9 Hz, 1H, 3-CH-C_5H_4N), 7.65 (ddd, J = 7.9, 7.4, 1.8 Hz, 1H, 4-CH-C_5H_4N), 8.10 (t, J = 5.8 Hz, 1H, NH), 8.49 (ddd, J = 4.8, 1.8, 0.9 Hz, 1H, 6-CH-C_5H_4N). Anal. calcd. for $C_{24}H_{27}N_5OS_2$: C 61.91; H 5.84; N 15.04 %. Found: C 62.26; H 6.05; N 15.30 %.

*5-Isopropyl-2,2-dimethyl-10-(methylthio)-N-(pyridin-3-ylmethyl)-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-amine (**6s**)*. Colorless solid; yield 71%; mp 275–276 ºC; IR ν/cm^{-1}: 3229 (NH). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.32 (s, 6H, $C(CH_3)_2$), 1.33 (d, J = 6.6 Hz, 6H, $CH(C\underline{H}_3)_2$), 2.52 (s, 3H, SCH_3), 3.06 (sp, J = 6.6 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.55 (s, 2H, CH_2), 4.76 (d, J = 5.9 Hz, 2H, $NHC\underline{H}_2$), 4.86 (s, 2H, OCH_2), 7.23 (dd, J = 7.9, 4.9 Hz, 1H, 5-CH-C_5H_4N), 7.76 (dt, J = 7.8, 2.0 Hz, 1H, 6-CH-C_5H_4N), 8.16 (t, J = 5.9 Hz, 1H, NH), 8.40 (dd, J = 4.8, 2.0 Hz, 1H, 4-CH-C_5H_4N), 8.60 (t, J = 2.0 Hz, 1H, 2-CH-C_5H_4N). Anal. calcd. for $C_{24}H_{27}N_5OS_2$: C 61.91; H 5.84; N 15.04 %. Found: C 62.20; H 6.04; N 15.27 %.

*5-Isopropyl-2,2-dimethyl-10-(methylthio)-N-(2-phenylethyl)-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-amine (**6t**)*. Colorless solid; yield 79%; mp 189–191 ºC; IR ν/cm^{-1}: 3278 (NH). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.32 (s, 6H, $C(CH_3)_2$), 1.33 (д, J = 6.6 Hz, 6H, $CH(C\underline{H}_3)_2$), 2.57 (s, 3H, SCH_3), 2.99 (t, J = 7.2 Hz, 2H, $C\underline{H}_2Ph$), 3.06 (sp, J = 6.7 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.55 (s, 2H, CH_2), 3.67–3.84 (m, 2H, $NHC\underline{H}_2$), 4.87 (s, 2H, OCH_2), 7.10–7.28 (m, 5H, Ph), 7.59 (t, J = 5.7 Hz, 1H, NH). Anal. calcd. for $C_{26}H_{30}N_4OS_2$: C 65.24; H 6.32; N 11.70 %. Found: C 65.53; H 6.51; N 11.94 %.

*N,N-Diethyl-5-isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-amine (**6u**)*. Colorless solid; yield 87%; mp 223–225 ºC. ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.26–1.39 (m, 12H, $N(CH_2C\underline{H}_3)_2$, $CH(C\underline{H}_3)_2$), 1.32 (s, 6H, $C(CH_3)_2$), 2.54 (s, 3H, SCH_3), 3.06 (sp, J = 6.7 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.56 (s, 2H, CH_2), 3.82 (q, J = 7.0 Hz, 4H, $N(C\underline{H}_2CH_3)_2$), 4.86 (s, 2H, OCH_2). Anal. calcd. for $C_{22}H_{30}N_4OS_2$: C 61.36; H 7.02; N 13.01 %. Found: C 61.72; H 7.19; N 13.25 %.

*2-{Ethyl[5-isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-8-yl]amino}ethanol (**6v**)*. Colorless solid; yield 83%; mp 175–177 ºC. ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.26–1.39 (m, 9H, $CH_2C\underline{H}_3$, $CH(C\underline{H}_3)_2$), 1.32 (s, 6H, $C(CH_3)_2$), 2.54 (s, 3H, SCH_3), 3.06 (sp, J = 6.7 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.56 (s, 2H, CH_2), 3.69–3.86 (m, 4H, $C\underline{H}_2C\underline{H}_2OH$), 3.91 (q, J = 7.0 Hz, 2H, $C\underline{H}_2CH_3$), 4.55 (br, 1H, OH), 4.86 (s, 2H, OCH_2). Anal. calcd. for $C_{22}H_{30}N_4O_2S_2$: C 59.16; H 6.77; N 12.54 %. Found: C 59.45; H 6.95; N 12.79 %.

Procedure for the synthesis of compound 7

A mixture of compound **5** (5 mmol) and hydrazine hydrate (2.5 g, 50 mmol) in absolute ethanol (50 mL) was refluxed for 10 h. The reaction mixture was cooled, water (100 mL) was added, and the separated crystals were filtered off, washed with water, dried, and recrystallized from a mixture of ethanol-dichloromethane (1:2).

*8-Hydrazino-5-isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-d]pyrimidine (**7**)*.

Colorless solid; yield 87%; mp 305–306 °C; IR ν/cm^{-1}: 3354, 3317 (NH, NH_2). ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.31 (d, J = 6.7 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.32 (s, 6H, $C(CH_3)_2$), 2.54 (s, 3H, SCH_3), 3.04 (sp, J = 6.7 Hz, 1H, $C\underline{H}(CH_3)_2$), 3.56 (s, 2H, CH_2), 4.58 (br, 2H, NH_2), 4.86 (s, 2H, OCH_2), 8.78 (br s, 1H, NH). Anal. calcd. for $C_{18}H_{23}N_5OS_2$: C 55.50; H 5.95; N 17.98 %. Found: C 55.78; H 6.17; N 18.22 %.

Procedure for the synthesis of compound 8

To an ice-cold solution of compound **7** (1 mmol) in glacial acetic acid (30 mL), a solution of sodium nitrite (0.14 g, 2 mmol, dissolved in the least amount of water) was added dropwise under stirring in an ice-bath at 5 °C. The reaction mixture was maintained at room temperature for 12 h, and then water (25 ml) was added. The resulting crystals were filtered off, washed with water, dried, and recrystallized from a mixture of ethanol/dichloromethane (1:3).

*8-Azido-5-isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2H-pyrano[4'',3'':4',5'] pyrido[3',2':4,5]thieno[3,2-d]pyrimidine/11-Isopropyl-8,8-dimethyl-5-(methylthio)-7,10-dihydro-8H-pyrano[4'',3'':4',5']pyrido[3',2':4,5] thieno[2,3-e]tetrazolo[1,5-c]pyrimidine (**8A/T**)*. Light yellow solid; yield 75%; mp 185–187 °C. ^{1}H NMR (300 MHz, DMSO/CCl_4, 1/3) δ 1.25–1.41 (m, 12H, $CH(C\underline{H}_3)_2$, $C(CH_3)_2$), 2.64 (s, 2.25H, SCH_3), 2.95 (s, 0.75H, SCH_3), 3.00–3.20 (m, 1H, $C\underline{H}(CH_3)_2$), 3.51 (s, 1.5H, CH_2), 3.62 (s, 0.5H, CH_2), 4.89 (s, 1.5H, OCH_2), 4.94 (s, 0.5H, CH_2). Anal. calcd. for $C_{18}H_{20}N_6OS_2$: C 53.98; H 5.03; N 20.98 %. Found: C 54.33; H 5.23; N 21.19 %.

RESULTS AND DISCUSSION

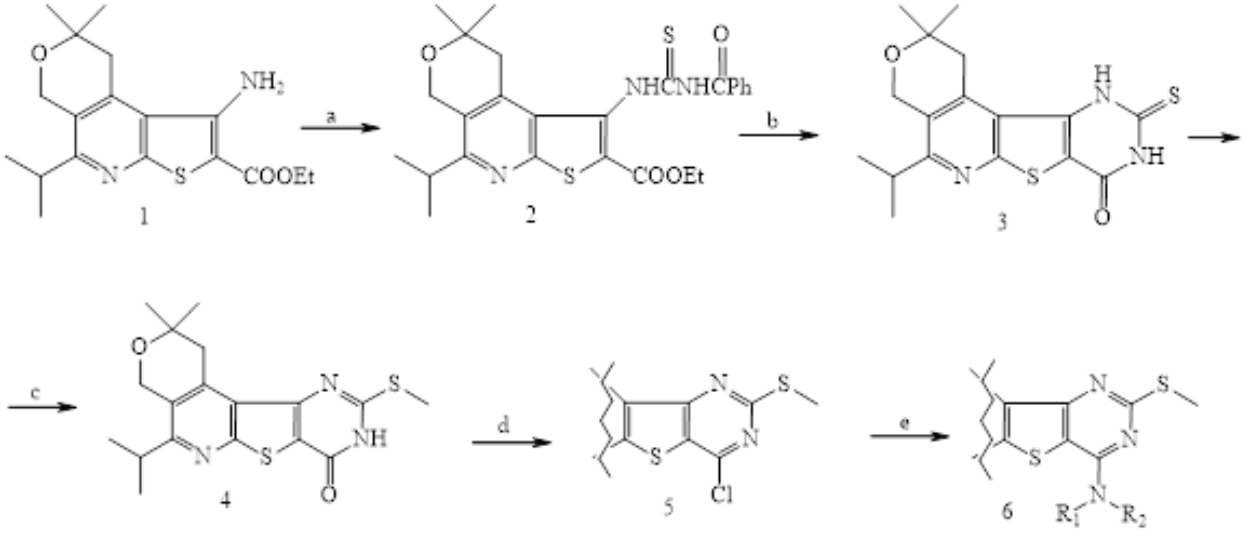
CHEMISTRY

As a starting compound ethyl 1-{[(benzoylamino) carbonothioyl]amino}-5-isopropyl-8,8-dimethyl-8,9-dihydro-6*H*-pyrano[4,3-*d*]thieno[2,3-*b*] pyridine-2-carboxylate **1** (Sirakanyan et al. 2014) was used. Compound **1** reacts with benzoyl isothiocyanate in benzene, giving the relevant thioureido derivative **2**, which in turn underwent intramolecular cyclization under the action of potassium hydroxide in ethanol. The subsequent neutralization with hydrochloric acid furnished the expected 5-isopropyl-2,2-dimethyl-10-thioxo-1,4,10,11-tetrahydro-2*H*-pyrano[4'',3'':4',5'] pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidin-8(9*H*)-one **3** (Figure 1). The IR spectrum of compound **3** showed absorption bands characteristic for the C=O (1677 cm^{-1}), C=S (1259 cm^{-1}) and NH (3418, 3152, 3091 cm^{-1}) groups. In the ^{1}H NMR spectrum the presence of two NH proton signals at 11.45 ppm and 12.97 ppm (slightly broadened) was observed.

Further compound **3** was carefully methylated with methyl iodide (ca. 1 equivalent) at 0–10 °C in the presence of a base (see Experimental) giving only the S-alkylated compound **4** (Figure 1). The IR spectrum of the S-alkylated compound **4** showed the bands of the carbonyl and NH groups of the cyclic amide fragment at ν 1647 cm^{-1} and 3198 cm^{-1}, respectively, thus excluding the presence of N- or O-alkylated derivatives. Moreover in the ^{1}H NMR spectrum of this compound the presence of the proton of NH group at 12.98 ppm and of the proton of the SCH_3 group at 2.62 ppm was observed.

In turn, compound **4** by reaction with phosphorus oxychloride led to the corresponding chloro derivative **5**, containing an 'activated' chlorine atom that could easily be displaced by nucleophiles. Thus, 8-chlorothieno[3,2-*d*] pyrimidine **5** was reacted with various amines to give a series of 8-amino-5-isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2*H*-pyrano[4'',3'':4',5'] pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidines **6a**–**v** in high yields (Figure 1).

The reaction of 8-chlorothieno[3,2-*d*] pyrimidine **5** with an excess of hydrazine hydrate in ethanol at reflux afforded the 8-hydrazino-



(a) PhCONCS, C6H6, reflux 5 h; (b) i. KOH/EtOH, reflux 2 h, ii. HCl; (c) DMF/KOH, CH3I, 0-10 oC, 2 h; (d) POCl3; reflux 4 h; (e) HNR1R2, EtOH, reflux 5 h

6a: R1-R2 = -(CH2)4-; **b:** R1-R2 = -(CH2)5-; **c:** R1-R2 = -(CH2)2O(CH2)2-; **d:** R1-R2 = -(CH2)2NMe(CH2)2-; **e:** R1-R2 = -(CH2)2NEt(CH2)2-; **f:** R1-R2 = -(CH2)2NCO2Et(CH2)2-; **g:** R1-R2 = -(CH2)2N(CH2)2OH(CH2)2-; **h:** R1 = H, R2 = CH2CH2OH; **i:** R1 = H, R2 = CH2CH2OMe; **j:** R1 = H, R2 = CH2CH(OMe)2; **k:** R1 = H, R2 = CH2CH(Me)OH; **l:** R1 = H, R2 = CH2CH2N(Me)2; **m:** R1 = H, R2 = 2-morpholinoethyl; **n:** R1 = H, R2 = (CH2)3N(Me)2; **o:** R1 = H, R2 = (CH2)3OH; **p:** R1 = H, R2 = 2-furylmethyl; **q:** R1 = H, R2 = Bn; **r:** R1 = H, R2 = 2-pyridylmethyl; **s:** R1 = H, R2 = 3- pyridylmethyl; **t:** R1 = H, R2 = CH2CH2Ph; **u:** R1 = R2 = Et; **v:** R1 = Et, R2 = CH2CH2OH.

Figure 1 - Synthesis of 8-amino-5-isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2*H*-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidines **6**.

5-isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2*H*-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidine **7** (Figure 2). The two broad singlets at 8.78 ppm and 4.58 ppm in the ^{1}H NMR spectrum supported the presence of NH and NH_2 groups, respectively, in the obtained compound **7**. Moreover the IR spectrum showed bands in the region at ν 3354, 3317 cm^{-1} (typical for the NH and NH_2 groups) thus confirming the formation of 8-hydrazinothieno[3,2-*d*]pyrimidine **7**.

Next, the 8-hydrazino derivative **7** was treated with sodium nitrite in acetic acid at 0−5 °C, giving the targeted 8-azidothieno[3,2-*d*]pyrimidine/thieno[2,3-*e*]tetrazolo[1,5-*c*]pyrimidine **8A/T** in excellent yield (87 %) (Figure 2).

In a previous paper from us (Sirakanyan et al. 2016b) we have described the synthesis of thieno[2,3-*e*]tetrazolo[1,5-*c*]pyrimidines, the structure of which was determined by the X-ray crystallography, showing that thieno[2,3-*e*]tetrazolo[1,5-*c*]pyrimidines in the crystalline state are present exclusively in the tetrazolo tautomeric form. However in solution thieno[2,3-*e*]tetrazolo-[1,5-*c*]pyrimidines exist as mixtures of azide and tetrazol isomers (Sirakanyan et al. 2016b).

The same azide/tetrazole equilibrium was observed in the new synthesized compound **8** (Figure 2). In fact, its ^{1}H NMR spectrum in DMSO/CCl_4 (1:3) showed the expected double set of signals: the **8A**/**T** isomer ratio was 3:1. Moreover all of the chemical shifts of the tetrazolo form, as a rule, appeared at lower field then those due to the azido form (Sirakanyan et al. 2016b).

In contrast, the IR spectrum of compound **8** did not show the characteristic azide bands in the region at ν 2100−2200 cm^{-1}.

Based on spectroscopic results we can conclude that as the thieno[2,3-*e*]tetrazolo[1,5-*c*]pyrimidines (Sirakanyan et al. 2016b) compound

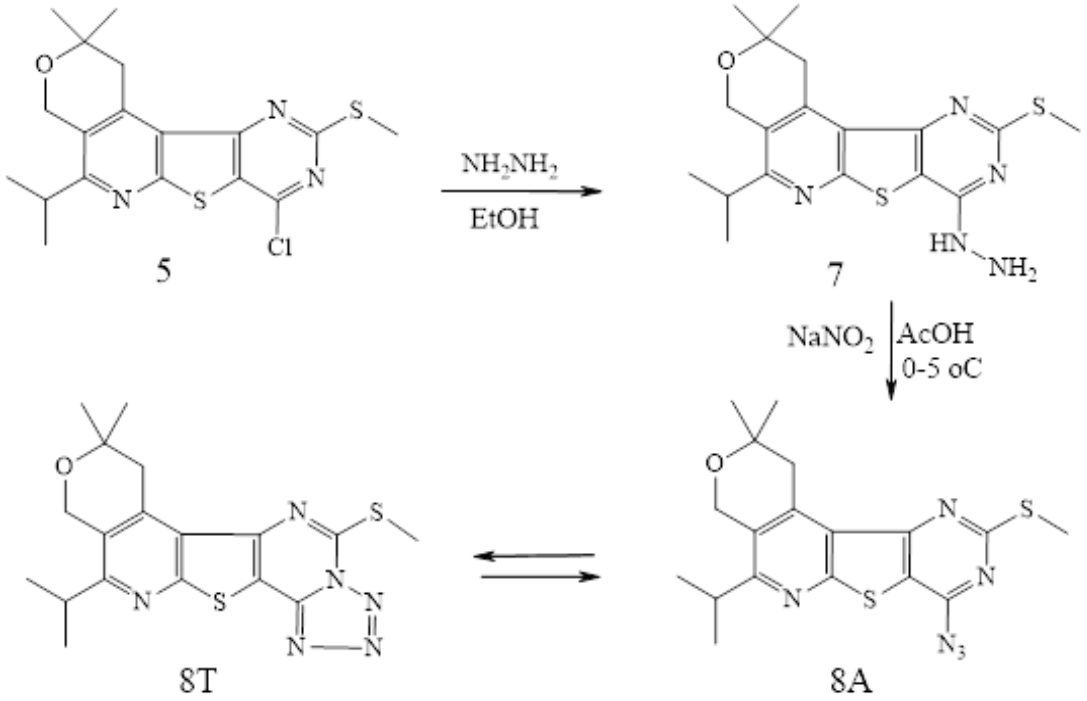


Figure 2 - Synthesis of 8-azidothieno[3,2-*d*]pyrimidine/thieno[2,3-*e*]tetrazolo[1,5-*c*]pyrimidine **8**.

8 in the solid state is presents exclusively in the tetrazolo tautomeric form, while in solution it exists as a mixture of two isomeric forms.

EVALUATION OF ANTIMICROBIAL ACTIVITY OF 8-AMINO DERIVATIVES OF THIENO[3,2-*D*] PYRIMIDINE 6a–v

The synthesized 8-amino-5-isopropyl-2,2-dimethyl-10-(methylthio)-1,4-dihydro-2*H*-pyrano[4'',3'':4',5']pyrido[3',2':4,5]thieno[3,2-*d*] pyrimidines **6a–v** were tested for their possible antimicrobial activity using the agar diffusion method in the bacterial load of 20 million microbial cells per 1 mL of medium (Mironov 2012). In experiments Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacilli (*Shigella Dysenteriae Flexneri 6858, Esherichia Coli 0-55*) strains were used. The compounds were dissolved in DMSO (which has no inhibitory activity) at concentration 1:20.

The antimicrobial activity of compounds **6** was determined by the diameter of zones (mm) of microbial growth inhibition on the place of application of substances after 24 h growing test cultures in a thermostat at 37 °C. As a positive control known drug Furazolidone (Mashkovski 2010) (its zone inhibition is 24–25 mm) under similar conditions was used.

The obtained data are presented in the Table I. The experiments indicated that all tested compounds **6a–v** showed more or less comparable activity with reference drug against all bacteria species with inhibition zone 20–24 mm. Eight compounds (**6a, 6b, 6e, 6k, 6l, 6m, 6r** and **6q**) exhibited good activity against different bacterial species, the activity of the rest was moderate. A very promising antimicrobial activity was shown by compounds **6k** and **6l** (Table I). Compound **6k** is the most active against *S. aureus*, *Sh. Dysenteriae Flexneri 6858 and E. coli* showing inhibition zone almost equal to reference drug, while compound **6l** was active only against *S. aureus and E. coli*. Compounds **6b, 6m** and **6r** also showed good activity against *S. aureus* with 23 mm inhibition zones. The same good activity possessed compound **6r** against *Sh.*

TABLE I
Antimicrobial activity of 8-amino derivatives of thieno[3,2-d]pyrimidine 6a–v.

Compound	The diameter of growth inhibition zones (mm)			
	Staphylococcus aureus		*Sh. Dysenteriae Flexneri 6858*	*E. Coli 0-55*
	209p	**1**		
6a	20	20	22	23
6b	21	21	24	23
6c	22	21	21	20
6d	20	20	20	21
6e	22	21	23	22
6f	21	21	22	23
6g	20	20	21	21
6h	22	21	22	20
6i	21	20	20	21
6j	20	20	21	22
6k	24	24	23	24
6l	24	23	22	23
6m	23	23	22	22
6n	22	21	20	21
6o	21	20	20	22
6p	22	23	21	22
6q	21	21	22	23
6r	23	23	23	22
6s	21	20	21	22
6t	22	21	21	22
6u	21	20	22	22
6v	21	20	20	21
Furazolidone	25	24	24	24

Dysenteriae Flexneri 6858, while compounds **6a**, **6b**, **6f** and **6q** exhibited activity against Gram negative bacteria *E. coli* (inhibition zone 23).

The study of structure-activity relationships revealed that the activity of compounds depends mostly on the nature of substituent R^1R^2. Thus the presence of $CH_2CHN(Me)OH$ group (**6k**) seems to have a beneficial effect on antibacterial activity against three bacteria species *S. aureus*, *Sh. Dysenteriae Flexneri 6858 and E. coli*. Replacement of this group by $CH_2CH_2N(Me)_2$ (**6l**) did not change the activity of compound against *S. aureus,* but slightly decreases the activity against *E. coli.* Introduction of 2-morpholinoethyl (**6m**) as well as 2-pyridylmethyl (**6r**) groups as R^1R^2 substituent resulted in slightly decreased activities compared to compound **6k** against *S. aureus.* The replacement of 2-pyridylmethyl group with 3-pyridylmethyl (**6s**) led to less active compound against all bacteria tested. On the other hand the presence of pyrrolidine, piperidine, $(CH_2)_2NCO_2Et(CH_2)_2$, $CH_2CH_2N(Me)_2$ and Bn groups as R^1R^2-substituents seem to play postive role in the activity against bacteria *E. coli*, while R^1R^2-substituent's such as piperidine, ethylpyperazine, $CH_2CHN(Me)OH$ and benzyl

are beneficial for activity against *Sh. Dysenteriae Flexneri 6858.*

DOCKING STUDIES

Based on the literature data (Agarwal et al. 2007) where compounds, analogous to ours, were mentioned as primase inhibitors we made docking studies to see whether our compounds can inhibit this enzyme.

Escherichia coli primase (DnaG) is a flexible molecule that is composed of three sub-domains the NH_2-terminal, the central and the COOH-terminal sub-domain. The central sub-domain forms five-stranded b sheet sandwiched by six α helices. Part of this region belongs to the topoisomerase-primase (toprim) fold family, as had been predicted by Koonin and co-workers (Aravind et al. 1998).

The NH_2-terminal and the toprim (topoisomerase-primase) sub-domains form a narrow cleft on the dipped side of the protein. This cleft consist of 14 surface-exposed residues some of which are important for primase activity, as shown by mutagenesis experiment, implying that this region is critical for DnaG function and probably is the catalytic center (Fig. 3a, b) (Ziegelin et al. 1995).

Since primase is a metal-dependent enzyme, mutation of the Glu-265 led to loss of activity (Ziegelin et al. 1995), suggesting that this region of the cleft serves as the active site for RNA chain elongation in DnaG.

The compounds were docked to *E. coli* primase (DnaG) (PDB code 1DDE) and estimated binding energies are given in the Table II. The most active compound **6k** binds to DnaG in a way that includes one favorable H-bonding interaction between the H atom of hydroxyl group and the carboxy O of the side chain of Glu265 (distance 2.02 Å). The fused rings form hydrophobic interactions with

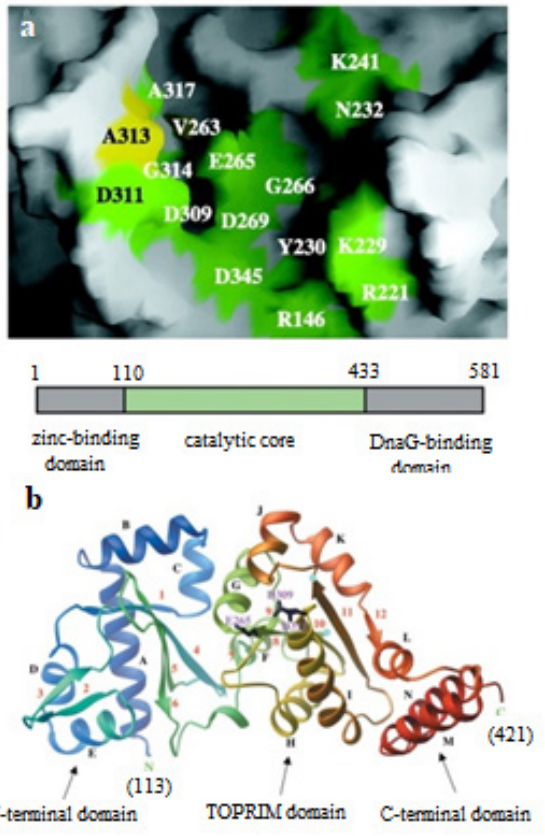


Figure 3 - (**a**) Proposed active-site of DnaG and the residues include, (**b**) Structure of DnaG.

Gly266, Asn232, Lue285, Tyr267, Ala283, Asp347, Met268, Tyr230, Lys229 and Asp269 (Fig. $4a_2$).

The second most active compound **6l** showed several hydrophobic interactions between the fused rings and the amino acids Asp269, Tyr230, Asp347, Asp309, Glu265 and Ala313 (Fig $4b_2$). Three hydrogen bonds were formed, the first hydrogen bond between the hydrogen atom of NH in the side chain of compound with the oxygen atom of hydroxyl in the side chain of Asp347 (distance 2.46 Å). Another hydrogen bond was formed between the sulfur of the side chain and the hydrogen of the side chain NH group of Tyr267 (distance 3.17Å). Furthermore a hydrogen bond formation was observed between oxygen of cyclohexane-type ring and NH of the side chain of Thr287 (distance

TABLE II
***Escherichia coli* primase (DnaG): 1DDE binding affinities.**

Comp/d	Est. binding energy(kcal/mol)	Binding affinity score	I-H	Residues
6a	-7.57	-23.12	–	–
6b	-7.29	-30.86	1	Glu265
6c	-8.45	-34.78	1	Asp269
6d	-6.55	-21.03	–	-
6e	-9.43	-37.89	2	Glu265, Tyr267
6f	-7.13	-29.77	–	–
6g	-6.41	-21.56	–	–
6h	-9.00	-36.18	1	Tyr267
6i	-6.78	-22.74	1	Asp269
6j	-6.49	-23.04	1	Asp345
6k	-12.11	-41.24	1	Glu265
6l	-11.97	-40.88	3	Tyr267, Thr287, Asp345
6m	-11.20	-39.43	2	Glu265, Asp269
6n	-8.44	-31.56	1	Asp269
6o	-6.77	-22.14	1	Asp269
6p	-10.41	-36.78	1	Ser284
6q	-7.05	-28.39	1	Tyr267
6r	-10.74	-38.22	2	Glu265, Tyr267
6s	-6.13	-19.67	–	–
6t	-9.11	-35.17	2	Tyr267, Ser284
6u	-6.98	-22.85	1	Ty267
6v	-6.72	-22.78	1	Glu265

3.21 Å). Despite this compound forms three hydrogen bonds and binding energy and affinity score are similar to those of compound **6k**, this compound lacks hydrogen bond with Glu265. It is shown that the residue Glu265 plays a major role for DnaG function (Ziegelin et al.1995); that's why compound **6k** by forming an hydrogen bond with this residue showed good antibacterial activity. Despite some other compounds (**6b, 6e, 6r** and **6v**) formed the same hydrogen bond with Glu265, their binding energies and affinity scores are higher.

CONCLUSIONS

An efficient procedure for the synthesis of new 8-amino derivatives of thieno[3,2-d]pyrimidine **6a–v** has been described starting from the ethyl 1-amino-5-isopropyl-8,8-dimethyl-8,9-dihydro-6*H*-pyrano[4,3-*d*]thieno[2,3-*b*]pyridine-2-carboxylate **1**.

Starting from the relevant 8-hydrazino derivative of thieno[3,2-*d*]pyrimidine **7**, 8-azidothieno[3,2-*d*]pyrimidine/thieno[2,3-*e*] tetrazolo[1,5-*c*]pyrimidine **8** was synthesized in excellent yield. The studies revealed that the insertion of the SCH_3 group in the pyrimidine ring in compound **8** shifted the azide-tetrazole equilibrium to the azido side.

Biological tests of the synthesized compounds evidenced that all the tested compounds exhibit similar or less antimicrobial activity than the used reference drug.

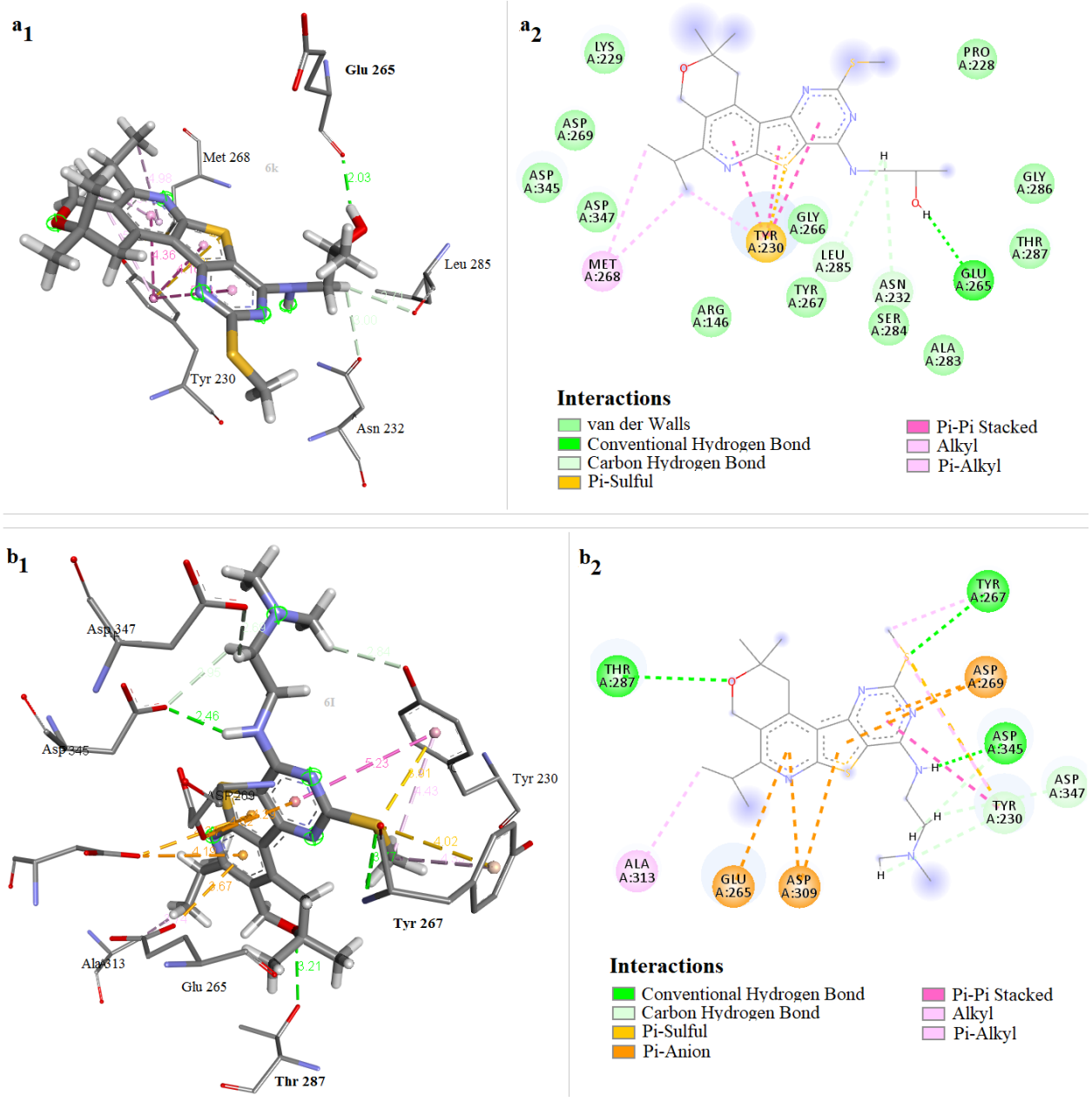


Figure 4 - (a_1, a_2) Docked conformation of the two most active compounds **6k** and **6l** (b_1, b_2) in *Escherichia coli* primase).

According to predicted docking studies our compounds could be DnaG inhibitors.

REFERENCES

AGARWAL A ET AL. 2007. Small molecule inhibitors of *E. coli* primase, a novel bacterial target. Bioorg Med Chem Lett 17: 2807-2810.

ARAVIND L, LEIPE DD AND KOONIN EV. 1998. Toprima conserved catalytic domain in type IA and II topoisomerases, DnaG-type primases, OLD family nucleases and RecR proteins. Nucleic Acids Res 26: 4205-4213.

BAKHITE EA, ABDEL-RAHMAN AE AND AL-TAIFI EA. 2004. Synthesis and antimicrobal activity of some new pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine derivatives. Phosphorus Sulfur Silicon Relat Elem 179: 513-520.

BAKHITE EA, ABDEL-RAHMAN AE, MOHAMED OS AND THABET EA. 2002. Synthesis and reactions of new thienopyridines, pyridothienopyrimidines and pyridothienotriazines. Bull Korean Chem Soc 23: 1709-1714.

CHAKRABORTI AK, GOPALAKRISHNAN B, SOBHI ME AND MALDE AK. 2003. 3D-QSAR Studies on thieno[3,2-*d*]pyrimidines as phosphodiesterase IV inhibitors. Bioorg Med Chem Lett 13: 1403-1408.

ENTSAR MA, NERMIN AM, SADIA AH AND ABEER MA. 2011. Synthesis, reactions and antimicrobial activity of some new thienopyridine and thienopyrimidine derivatives. World J Chem 6: 25-31.

GINEINAH MM, NASR MNA, BADR SMI AND EL-HUSSEINY WM. 2013. Synthesis and antitumor activity of new pyrido[2,3-*d*]pyrimidine derivatives. Med Chem Res 22: 3943-3952.

LITVINOV VP. 2004. Thienopyrimidines: synthesis, properties, and biological activity. Russ Chem Bull 53: 487-516.

MASHKOVSKI MD. 2010. Medicines, 16th ed., Moscow: Novaya Volna, 851 p.

MIRONOV AN. 2012. Manual on Preclinical Drug Trials, Moscow: Medicine.

OGANISYAN AKH, NORAVYAN AS, DZHAGATSPANYAN IA AND MELIKYAN GG. 2003. Derivatives of condensed thienopyrimidines: synthesis and neurotropic activity of new pyrano[4′,3′:4,5]thieno[3,2-e]triazolo[3,4-*b*] pyrimidine derivatives. Pharm Chem J 37: 13-14.

RATEB NM. 2007. Convenient synthesis and antimicrobial evaluation of multicyclic thienopyridines. Phosphorus Sulfur Silicon Relat Elem 182: 2393-2407.

REICHELT C, LUDWIG A, SCHULZE A, DAGHISH M, LEISTNER S, KRODEL A AND HEINICKE J. 2011. Substituted pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidines and pyrido[3',2':4,5]furo[3,2-*d*]pyrimidines used as inhibitors of the PDE-4 and/or the release of TNF-alpha. US Patent 8,058,285 B2.

SHNUTE ME ET AL. 2008. Synthesis of 4-oxo-4,7-dihydrofuro[2,3-*b*]pyridine-5-carboxamides with broad-spectrum human herpesvirus polymerase inhibition. Bioorg Med Chem Lett 18: 3856-3859.

SIRAKANYAN SN, AKOPYAN EK, PARONIKYAN RG, AKOPYAN AG AND OVAKIMYAN AA. 2016a. Synthesis and anticonvulsant activity of 7(8)-amino derivatives of condensed thieno[3,2-*d*]pyrimidines. Pharm Chem J 50: 296-300.

SIRAKANYAN SN, OVAKIMYAN AA, NORAVYAN AS, MINASYAN NS, DZHAGATSPANYAN IA, NAZARYAN IM AND AKOPYAN AG. 2014. Synthesis and neurotropic activity of 8-amino derivatives of condensed thieno[3,2-*d*]- and furo[3,2-*d*]pyrimidines. Pharm Chem J 47: 655-659.

SIRAKANYAN SN, SPINELLI D, GERONIKAKI A, KARTSEV VG, PANOSYAN HA, AYVAZYAN AG, TAMAZYAN RA, FRENNA V AND HOVAKIMYAN AA. 2016b. The azide/tetrazole equilibrium: an investigation in the series of furo- and thieno[2,3-*e*]tetrazolo[3,2-*d*] pyrimidine derivatives. Tetrahedron 72: 1919-1927.

TEMBURNIKAR KW, ZIMMERMANN SC, KIM NT, ROSS CHR, GELBMANN CH, SALOMON CHE, WILSON GM, BALZARINI J AND SELEY-RADTKE KL. 2014. Antiproliferative activities of halogenated thieno[3,2-*d*] pyrimidines. Bioorg Med Chem 22: 2113-2122.

ZIEGELIN G, LINDEROTH NA, CALENDAR R AND LANKA E. 1995. Domain structure of phage P4 alpha protein deduced by mutational analysis. J Bacteriol 177: 4333-4341.