

Synthesis of Novel Derivatives on the Basis of 4-Hydrazinyl-6-methyl-2-(alkylthio)pyrimidines and Their Preliminary Biological Evaluation

Abstract:

By functionalization of 4-hydrazinyl-6-methyl-2-(alkylthio)pyrimidines the corresponding acetohydrazides, N-phenylhydrazine-1-carboxamides(thioamides) were obtained. The heterocyclization of the same hydrazides lead to nonfused pyrazolyl-pyrimidines and pyridazinyl-pyrimidines and a series of 3-S-substituted derivatives of fused 1,2,4-triazolo[4,3-c]pyrimidine. The preliminary in vitro tests have shown that among the synthesized compounds there are substances exhibiting growth promoting properties.

Keywords: 4-hydrazinyl-6-methyl-2-(alkylthio)pyrimidines; N-phenylhydrazine-1-carboxamides and thioamides; heterocyclization; pyrazolyl-pyrimidines; pyridazinyl-pyrimidines; 1,2,4-triazolo[4,3-c]pyrimidines; growth stimulant activity.

1. INTRODUCTION

Pyrimidine derivatives exhibit a wide spectrum of biological activity. The pyrimidine cycle is the part of the nucleic acids, vitamins, antibiotics, certain medicines, strong poisons, co-enzymes. In agriculture, pyrimidine derivatives are used mainly as herbicides, especially the compounds of a sulfonylurea series (sulfurones). Pyrimidine derivatives are also used in agriculture as fungicides, insecticides and acaricides [1]. However, over time the pests and pathogens acquire resistance to applied crop protection chemicals. This fact requires regular replenishment of their assortment with new preparations having different mechanisms of action. For this reason, the targeted synthesis of new compounds with combination of pyrimidine and the other pharmacophore heterocycles, in particular pyrazole, pyridazine or 1,2,4-triazole in a molecule, may lead to new biologically active derivative, with the respect to which the resistance has not formed yet.

The choice of the listed heterocycles is due to the fact that their derivatives themselves exhibit a wide spectrum of biological activity. Substituted pyrazoles are used as herbicides, fungicides, insecticides and acaricides [1]. With the continuing search for new physiologically active substances among the pyrazole derivatives the new compounds with herbicidal [2-13], fungicidal [14-19], insecticidal [20-25], acaricidal [26] and antiviral [27] activities were discovered. Maleic hydrazide itself is a quite effective plant growth regulator. In very small doses, the drug temporarily inhibits the growth of plants, so that is used to prevent early flowering [28-31], and in high application rates is used for weed control. Many pyridazin-3(2H)-one derivatives exhibit pronounced herbicidal [32-35], fungicidal [36] and growth stimulant [37] properties.

Over the past two decades there is an increased interest to the fused bicyclic heterosystems with different conjunction of pyrimidine and 1,2,4-triazole rings. Thus, the studies of 1,2,4-triazolo[4,3-c]pyrimidine derivatives revealed among them the novel compounds possessing anticonvulsant [38,39], anticancer [40,41], antimicrobial [40,42], neurotropic [43] anxiolytic [44] activities, antagonistic effect on adenosine receptors [45]. In agriculture, the herbicides of sulfonanilide series are mainly used [1]. The aim of this study was to develop a simple, convenient and accessible methods for the synthesis of non-condensed and condensed bi-heterocyclic systems containing two pharmacophore heterocycles and a preliminary evaluation of their biological activity.

2. EXPERIMENTAL DETAILS

2.1 General

The ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded at 30 $^{\circ}\text{C}$ on Varian Mercury-300 spectrometer with standard pulse sequences operating in the mixture of solvents DMSO- d_6 and CCl_4 (1:3) using tetramethylsilane (0.0 ppm) as internal standard. The NMR multiplicities brs, s, d, t, q,

and m stand for broad singlet, singlet, doublet, triplet, quartet and multiplet, respectively. Mass spectra were obtained on Agilent LC-MS system and X-ray analysis was done at room temperature on automated Enraf-Nonius CAD-4 diffractometer. The reaction progress and purity of the obtained substances were checked by using the TLC method on "Silufol UV-254" plates and acetone/hexane mixture (2:1) as eluent. All melting points were determined in open capillaries and are uncorrected.

2.2 Synthesis of compounds 1,2

To a solution of 2-mercapto-6-methylpyrimidin-4-ol (0.01 mol) and NaOH (0.02 mol) in 15 mL of water, at cooling with icy water 0.01 mol of DMS or (chloromethyl)benzene was added dropwise with continual stirring and the mixture was allowed to stand overnight. The precipitate was filtered off and dried.

2.2.1 6-Methyl-2-(methylthio)pyrimidin-4-ol (1). White crystals; mp. 213-215 °C; yield 83%. ¹HNMR: 2.20 (s, 3H, CCH₃); 2.47 (s, 3H, SCH₃); 5.80 (s, 1H, CH); 11.4 (brs, 1H, OH). Anal. Calcd for C₆H₈N₂OS: C 46.14, H 5.16, N 17.93, S 20.52%. Found: C 46.01, H 5.08, N 17.71; S 20.30%.

2.2.2 2-(Benzylthio)-6-methylpyrimidin-4-ol (2). Flesh-colored crystals; mp. 180-182 °C; yield 87%. ¹HNMR: 2.23 (s, 3H, CCH₃); 4.35 (s, 2H, SCH₂); 5.86 (s, 1H, CH); 7.18-7.39 (m, 5H, C₆H₅); 12.24 (brs, 1H, OH). ¹³CNMR: 23.27, 33.74, 106.86, 126.68, 127.88, 128.67, 136.85, 161.23, 163.20, 163.35. Anal. Calcd for C₁₂H₁₂N₂OS: C, 62.05; H, 5.21; N, 12.06; S, 13.80%. Found: C, 62.18; H, 5.29; N, 11.81; S, 13.58%.

2.3 Synthesis of compounds 3,4

The suspension of compound **1** or **2** in 3 mL of POCl₃ was boiled for 2 h. The excess of POCl₃ was evaporated, the residue was processed with icy water, neutralized with NH₄OH (25%) and filtered off.

2.3.1 4-Chloro-6-methyl-2-(methylthio)pyrimidine (3). Yellow crystals; mp. 57-58 °C; yield 75%. ¹HNMR: 2.25 (s, 3H, CH₃); 2.38 (s, 3H, SCH₃); 6.54 (s, 1H, CH-pyrim.). Anal. Calcd for C₆H₇ClN₂S: C 41.26, H 4.04, Cl 20.30, N 16.04, S 18.36%. Found: C 41.12, H 4.12, Cl 20.39, N 16.04, S 18.50%.

2.3.2 2-(Benzylthio)-4-chloro-6-methylpyrimidine (4). Brown crystals; mp. 47-48 °C; yield 85%. ¹HNMR: 2.24 (s, 3H, CH₃); 4.33 (s, 2H, SCH₂); 6.50 (s, 1H, CH-pyrim.); 7.20-7.40 (m, 5H, C₆H₅). Anal. Calcd for C₁₂H₁₁ClN₂S: C 57.48, H 4.42, Cl 14.14, N 11.17, S 12.79%. Found: C 57.21, H 4.33, Cl 14.01, N 11.38, S 12.51%.

2.4 Synthesis of compounds 5,6

The suspension of compound **3** or **4** in 3 mL of hydrazine hydrate (63%) was stirred at 20 °C for 6 h and allowed to stand overnight. The mixture was processed with 10 mL of icy water and filtered off.

2.4.1 4-Hydrazinyl-6-methyl-2-(methylthio)pyrimidine (5). White crystals; mp. 138-140 °C; yield 90%. ¹HNMR: 2.21 (s, 3H, CH₃); 2.41 (s, 3H, SCH₃); 3.45 (brs, 2H, NH₂); 6.18 (brs, 1H, CH-pyrim.); 7.95 (brs, 1H, NH). Anal. Calcd for C₆H₁₀N₄S: C 42.33, H 5.92, N 32.91, S 18.83%. Found: C 42.21, H 5.79, N 32.65, S 18.60%.

2.4.2 2-(Benzylthio)-4-hydrazinyl-6-methylpyrimidine (6). Lightyellow crystals; mp. 110-112 °C; yield 70%. ¹HNMR: 2.2.18 (s, 3H, CH₃); 4.30 (s, 2H, SCH₂); 4.31 (brs, 2H, NH₂); 6.27 (brs, 1H, CH-pyrim.); 7.18-7.43 (m, 5H, C₆H₅); 8.31 (brs, 1H, NH). ¹³CNMR: 23.44, 33.72, 96.86, 126.68, 128.19, 128.87, 138.87, 163.94, 164.96, 168.19. Anal. Calcd for C₁₂H₁₄N₄S: C 58.51, H 5.73, N 22.74, S 13.01%. Found: C 58.62, H 5.81, N 22.55, S 12.79%.

2.5 Synthesis of compounds 7,8

To a solution of compound **5** or **6** (0.01 mol) in 15 mL of acetic acid, 10 mL of pentane-2,4-dione was added. In 24 h the transparent solution was steamed, processed with water and filtered off.

2.5.1 4-(3,5-Dimethyl-1H-pyrazol-1-yl)-6-methyl-2-(methylthio)pyrimidine (7). White crystals; mp. 128-130 °C; yield 82%. ¹HNMR: 2.21 (s, 3H, CH₃); 2.41 (s, 3H, SCH₃); 2.50 и 2.63 (ss, 6H, 3- and 5-

CH₃-pyraz.), 6.00 (s, 1H, CH-pyraz.); 7.43 (s, 1H, CH-pyrim.); 7.18-7.40 (m, 5H, C₆H₅). Anal. Calcd for C₁₁H₁₄N₄S: C 56.38, H 6.02, N 23.91, S 13.68%. Found: C 56.22, H 6.11, N 23.66, S 13.41%.

2.5.2 2-(Benzylthio)-4-(3,5-dimethyl-1H-pyrazol-1-yl)-6-methylpyrimidine (8). Flesh-colored crystals; mp. 65-67 °C; yield 98%. ¹HNMR: 2.21 (s, 3H, CH₃); 2.50 и 2.64 (ss, 6H, 3- and 5-CH₃-pyraz.); 4.20 (s, 2H, SCH₂); 6.00 (s, 1H, CH-pyraz.); 7.43 (s, 1H, CH-pyrim.); 7.18-7.40 (m, 5H, C₆H₅). ¹³CNMR: 13.09, 14.92, 23.56, 104.42, 110.09, 126.47, 127.80, 128.31, 136.73, 141.74, 150.22, 158.64, 167.63, 169.56. Anal. Calcd for C₁₇H₁₈N₄S: C 65.78; H 5.85; N 18.05; S 10.33%. Found: C 65.55; H 5.76; N 18.29; S 10.06%.

2.6 Synthesis of compounds 9,10

To a suspension of compound **5** or **6** (0.01 mol) in 10 mL of water, the solution of HCl (35%) was added dropwise till full dissolving of hydrazide, then 0.01 mol of maleic anhydride. The mixture was allowed to stand overnight, the transparent solution was acidified with NaHCO₃ till pH 7, and the precipitate was filtered off and dried.

2.6.1 6-Hydroxy-2-(6-methyl-2-(methylthio)pyrimidin-4-yl)pyridazin-3(2H)-one (9). White crystals; mp. 194-197 °C; yield 70%. ¹HNMR: 2.25 (s, 3H, CH₃); 2.43 (s, 3H, SCH₃); 6.10 (s, 1H, CH-pyrim.); 6.23 and 6.47 (dd, J=12.2, CH=CH); 9.38 (brs, 1H, OH). Anal. Calcd for C₁₀H₁₀N₄O₂S: C 47.99, H 4.03, N 22.39, S 12.81%. Found: C 47.83, H 4.12, N 22.09, S 12.58%.

2.6.2 2-(2-(Benzylthio)-6-methylpyrimidin-4-yl)-6-hydroxypyridazin-3(2H)-one (10). White crystals; mp. 178-180 °C; yield 80%. ¹HNMR: 2.41 (s, 3H, CH₃); 4.48 (s, 2H, SCH₂); 6.26 and 6.52 (dd, J=12.2, 2H, CHCH); 6.57 (s, 1H, CH-pyrim.); 7.20-7.48 (m, 5H, C₆H₅); 10.88 (brs, 1H, OH). Anal. Calcd for C₁₆H₁₄N₄O₂S: C 58.88, H 4.32, N 17.17, S 9.82%. Found: C 58.77, H 4.21, N 17.40, S 9.62%.

2.7 Synthesis of compounds 11,12

The mixture of compound **5** or **6** and 0.01 mol of acetic anhydride in 10 ml of ethyl acetate was heated at 50-60 °C for 5 h. After ethyl acetate evaporation the residue was processed with water, then with NaHCO₃ (5%) and filtered off.

2.7.1 N'-(6-Methyl-2-(methylthio)pyrimidin-4-yl)acetohydrazide (11). Flesh-colored crystals; mp. 178-180 °C; yield 83%. ¹HNMR: 1.93 (s, 3H, COCH₃); 2.25 (s, 3H, CH₃); 2.43 (s, 3H, SCH₃); 6.04 (s, 1H, CH-pyrim.); 8.75 (s, 1H, NH); 9.63 (s, 1H, NH). ¹³CNMR: 13.13, 20.20, 23.40, 96.95, 163.70, 164.51, 168.38, 169.46. Anal. Calcd for C₈H₁₂N₄OS: C 45.27, H 5.70, N 26.39, S 15.10%. Found: C 45.16, H 5.66, N 26.18, S 15.34%.

2.7.2 N'-(2-(Benzylthio)-6-methylpyrimidin-4-yl)acetohydrazide (12). White crystals; mp. 158-160 °C; yield 58%. ¹HNMR: 1.93 (s, 3H, COCH₃); 2.27 (s, 3H, CH₃); 4.30 (s, 2H, SCH₂); 6.04 (s, 1H, CH-pyrim.); 7.14-7.40 (m, 5H, C₆H₅); 8.86 (brs, 1H, NH); 9.65 (brs, 1H, NH). Anal. Calcd for C₁₄H₁₆N₄OS: C 58.31, H 5.59, N 19.43, S 11.12%. Found: C 58.22, H 5.50, N 19.21, S 10.83%.

2.8 Synthesis of compounds 13-16

To a mixture of compound **5** or **6** in 15 mL of toluene, at 20 °C 0.01 mol of phenyl isocyanate or phenyl isothiocyanate and 2 drops of pyridine were added. The mixture was heated at 100-120 °C for 2 h. The precipitate was filtered off and dried.

2.8.1 2-(6-Methyl-2-(methylthio)pyrimidin-4-yl)-N-phenylhydrazine-1-carboxamide (13). White crystals; mp. 213-215 °C; yield 71%. ¹HNMR: 2.29 (s, 3H, CH₃); 2.42 (s, 3H, SCH₃); 6.22 (s, 1H, CH-pyrim.); 6.95-7.50 (m, 5H, C₆H₅); 8.05, 8.61 and 8.88 (brs, 3H, 3×NH). Anal. Calcd for C₁₃H₁₅N₅OS: C 53.96, H 5.23, N 24.20, S 11.08%. Found: C 53.79, H 5.12, N 24.01, S 11.29%.

2.8.2 2-(2-(Benzylthio)-6-methylpyrimidin-4-yl)-N-phenylhydrazine-1-carboxamide (14). White crystals; mp. 210-212 °C; yield 65%. ¹HNMR: 2.28 (s, 3H, CH₃); 4.32 (s, 2H, SCH₂); 6.20 (s, 1H, CH-pyrim.); 6.85-7.48 (m, 10H, 2×C₆H₅); 8.01, 8.54 and 8.82 (brs, 3H, 3×NH). ¹³CNMR: 23.35, 33.99, 97.45, 118.158, 121.24, 126.13, 127.66, 127.91, 128.57, 138.23, 139.34, 155.06, 164.48, 164.57,

168.60. Anal. Calcd for $C_{19}H_{19}N_5OS$: C 62.45, H 5.24, N 19.16, S 8.77%. Found: C 62.29, H 5.11, N 19.39, S 8.51%.

2.8.3 2-(6-Methyl-2-(methylthio)pyrimidin-4-yl)-N-phenylhydrazine-1-carbothioamide (15). Flesh-colored crystals; mp. 170-171 °C; yield 82%. ^1H NMR: 2.34 (s, 3H, CH_3); 2.42 (s, 3H, SCH_3); 6.21 (s, 1H, CH-pyrim.); 7.08-7.60 (m, 5H, C_6H_5); 9.13 9.57 and 9.59 (brs. 3H, 3 $\times$ NH). Anal. Calcd for $C_{13}H_{15}N_5S_2$: C 51.12, H 4.95, N 22.93, S 20.99%. Found: C 51.29, H 4.81, N 22.69, S 20.70%.

2.8.4 2-(2-(Benzylthio)-6-methylpyrimidin-4-yl)-N-phenylhydrazine-1-carbothioamide (16). Flesh-colored crystals; mp. 106-108 °C; yield 71%. ^1H NMR: 2.34 (s, 3H, CH_3); 4.34 (s, 2H, SCH_2); 6.21 (s, 1H, CH-pyrim.); 7.08-7.60 (m, 5H, C_6H_5); 9.13 (brs. 1H, NH); 9.59 (brs. 2H, 2 $\times$ NH). Anal. Calcd for $C_{19}H_{19}N_5S_2$: C 59.82, H 5.02, N 18.36, S 16.81%. Found: C 59.73, H 5.09, N 18.16, S 16.60%.

2.9. Synthesis of compounds 17,18

To a alcoholic solution of compound 5 or 6, at cooling 0.01 mol of KOH, then 0.015 mol of CS_2 were added and the mixture was allowed to stand overnight. After distillation of 2/3 part of alcohol, 20 ml of water was added, the residue was neutralized with HCl till pH 7, filtered off and dried.

2.9.1 7-methyl-5-(methylthio)-[1,2,4]triazolo[4,3-c]pyrimidine-3(2H)-thione (17). Flesh-colored crystals; mp. 242-244 °C; yield 83%. ^1H NMR: 2.31 (s, 3H, CH_3); 2.48 (s, 3H, SCH_3); 6.70 (s, 1H, CH-pyrim.); 14.06 (brs, 1H, NH). ^{13}C NMR: 14.49, 22.79, 101.58, 146.77, 151.44, 154.79, 160.16. Anal. Calcd for $C_7H_8N_4S_2$: C 39.60, H 3.80, N 26.39, S 30.20%. Found: C 39.49, H 3.71, N 26.18, S 30.45%.

2.9.2 5-(Benzylthio)-7-methyl-[1,2,4]triazolo[4,3-c]pyrimidine-3(2H)-thione (18). Pale yellow crystals; mp. 208-210 °C; yield 78%. ^1H NMR: 2.35 (s, 3H, CH_3); 4.34 (s, 2H, SCH_2); 6.73 (s, 1H, CH-pyrim.); 7.20-7.45 (m, 5H, C_6H_5); 14.12 (brs. 1H, NH). ^{13}C NMR: 22.66, 35.84, 101.98, 126.66, 127.80, 129.03, 135.87, 146.79, 151.36, 153.63, 160.01. Anal. Calcd for $C_{13}H_{12}N_4S_2$: C 54.14, H 4.19, N 19.43, S 22.23%. Found: C 54.30, H 4.28, N 19.20, S 22.02%.

2.10 Synthesis of compounds 19a,20a

To a aqueous solution of potassium salt of compound 17 or 18 (obtained from 0.01 mol of 17 or 18 and 0.01 mol of KOH in 10 mL of water), at cooling 0.01 mol of DMS was added dropwise. The reaction mixture was allowed to stand at 20 °C for 24 h. The precipitate was filtered off and dried.

2.10.1 7-Methyl-3,5-bis(methylthio)-[1,2,4]triazolo[4,3-c]pyrimidine (19a). White crystals; mp. 178-180 °C; yield 98%. ^1H NMR: 2.42 (s, 3H, CH_3); 2.68 (s, 3H, SCH_3); 2.76 (s, 3H, SCH_3); 7.05 (s, 1H, CH-pyrim.). ^{13}C NMR: 14.35, 17.34, 22.56, 102.68, 141.27, 149.15, 149.29, 151.04. MS: 226 (M^+). Anal. Calcd for $C_8H_{10}N_4S_2$: C 42.46, H 4.45, N 24.76, S 28.33%. Found: C 42.31, H 4.36, N 24.50, S 28.03%.

Crystal data for $C_8H_{10}N_4S_2$, monoclinic, space group P21/c, $a=8.2697(17)\text{\AA}$, $b=9.2331(18)\text{\AA}$, $c=13.396(3)\text{\AA}$, $\beta=92.19(3)^\circ$, $V=1022.1(4)\text{\AA}^3$, $Z=4$, $T=293(2)\text{K}$, $d_x=1.471\text{g cm}^{-3}$, $\mu(\text{MoK}\alpha)=0.486\text{mm}^{-1}$, 6128 data were collected up to $\theta_{\text{max}}=30^\circ$ ($R_{\text{int}}=0.0216$, $R_\sigma=0.0198$). Final R-indices for 2472 reflections with $I>2\sigma(I)$ and 134 refined parameters are: $R_1=0.0342$, $wR_2=0.0917$ ($R_1=0.0433$, $wR_2=0.0975$ for all 2973 data).

Crystallographic data for compound 19a have been deposited with the Cambridge Crystallographic Data Centre, deposition No. CCDC 991573.

2.10.2 5-(Benzylthio)-7-methyl-3-(methylthio)-[1,2,4]triazolo[4,3-c]pyrimidine (20a). White crystals; mp. 166-168 °C; yield 83%. ^1H NMR: 2.42 (s, 3H, CH_3); 2.74 (s, 3H, SCH_3); 4.55 (s, 2H, SCH_2); 7.06 (s, 1H, CH-pyrim.); 7.20-7.44 (m, 5H, C_6H_5). Anal. Calcd for $C_{14}H_{14}N_4S_2$: C 55.60, H 4.67, N 18.53, S 21.20%. Found: C 55.49, H 4.59, N 18.24, S 21.58%.

2.11 Synthesis of compounds 19,20 b-e

To a mixture of KOH (0.01 mol) in 10 mL of DMF (or acetone), at stirring 0.01 mol of compound 17 or 18 was added till the complete formation of the potassium salt, then at cooling 0.01 mol of alkyl halide. The mixture was allowed to stand overnight at 20 °C then heated at 70-80 °C for 2 h. DMF was evaporated at low pressure, the residue was processed with water, filtered off and dried.

2.11.1 7-Methyl-5-(methylthio)-3-(propylthio)-[1,2,4]triazolo[4,3-c]pyrimidine (19b). Yellow crystals; mp. 78-80 °C; yield 75%. ¹HNMR: 1.08 (t, J=7.2, 3H, CH₃-prop.); 1.78 (m, 2H, CCH₂C); 2.43 (s, 3H, CH₃); 2.67 (s, 3H, SCH₃); 3.22 (t, J=7.2, SCH₂); 7.07 (s, 1H, CH-pyrim.). Anal. Calcd for C₁₀H₁₄N₄S₂: C 47.22, H 5.55, N 22.03, S 25.21%. Found: C 47.10, H 5.44, N 22.30, S 25.59%.

2.11.2 5-(Benzylthio)-7-methyl-3-(propylthio)-[1,2,4]triazolo[4,3-c]pyrimidine (20b). Light brown crystals; mp. 58-60 °C; yield 65%. ¹HNMR: 1.09 (t, J=7.2, 3H, CH₃-prop.); 1.77 (m, 2H, CCH₂C); 2.44 (s, 3H, CH₃); 3.24 (t, J=7.2, SCH₂); 4.53 (s, 2H, SCH₂C₆H₅); 7.05 (s, 1H, CH-pyrim.); 7.20-7.45 (m, 5H, C₆H₅). Anal. Calcd for C₁₆H₁₈N₄S₂: C 58.15, H 5.49, N 16.95, S 19.40%. Found: C 58.00, H 5.31, N 16.64, S 19.13%.

2.11.3 3-(Benzylthio)-7-methyl-5-(methylthio)-[1,2,4]triazolo[4,3-c]pyrimidine (19c). White crystals; mp. 140-142 °C; yield 78%. ¹HNMR: 2.42 (s, 3H, CH₃); 2.65 (s, 3H, SCH₃); 4.47 (s, 2H, SCH₂); 7.08 (s, 1H, CH-pyrim.); 7.18-7.37 (m, 5H, C₆H₅). Найдено %: N, 18.31; S, 21.39.. Anal. Calcd for C₁₄H₁₄N₄S₂: C 55.60, H 4.67, N 18.53, S 21.20%. Found: C 55.46, H 4.59, N 18.44, S 21.05%.

2.11.4 3,5-Bis(benzylthio)-7-methyl-[1,2,4]triazolo[4,3-c]pyrimidine (20c). Yellow crystals; mp. 40-42 °C; yield 70%. ¹HNMR: 2.45 (s, 3H, CH₃); 4.54 (s, 2H, 5-SCH₂C₆H₅); 4.59 (s, 2H, 3-SCH₂C₆H₅); 7.08 (s, 1H, CH-pyrim.); 7.20-7.50 (m, 10H, 2×C₆H₅). Anal. Calcd for C₂₀H₁₈N₄S₂: C 63.46, H 4.79, N 14.80, S 16.94%. Found: C 63.29, H 4.66, N 14.59, S 16.69%.

2.11.5 7-Methyl-5-(methylthio)-3-((2-phenoxyethyl)thio)-[1,2,4]triazolo[4,3-c]pyrimidine (19d). White crystals; mp. 128-130 °C; yield 77%. ¹HNMR: 2.43 (s, 3H, CH₃); 2.64 (s, 3H, SCH₃); 3.62 (t, J=6.1, 2H, SCH₂); 4.34 (t, J=6.1, 2H, OCH₂); 7.10 (s, 1H, CH-pyrim.); 6.80-7.23 (m, 5H, C₆H₅). Anal. Calcd for C₁₅H₁₆N₄OS₂: C 54.19, H, 4.85, N 16.85, S 19.29%. Found: C 54.05, H, 4.90, N 16.66, S 19.04%.

2.11.6 5-(Benzylthio)-7-methyl-3-((2-phenoxyethyl)thio)-[1,2,4]triazolo[4,3-c]pyrimidine (20d). Flesh-colored crystals; mp. 40-42 °C; yield 70%. ¹HNMR: 2.43 (s, 3H, CH₃); 3.60 (t, J=6.1, 2H, SCH₂); 4.36 (t, J=6.1, 2H, OCH₂); 4.54 (s, 2H, 5-SCH₂C₆H₅); 7.10 (s, 1H, CH-pyrim.); 6.80-7.25 (m, 10H, 2×C₆H₅). Anal. Calcd for C₂₁H₂₀N₄OS₂: C 61.74, H 4.93, N 13.71, S 15.69%. Found: C 61.86, H 4.80, N 13.53, S 15.49%.

2.11.7 Methyl 2-((7-methyl-5-(methylthio)-[1,2,4]triazolo[4,3-c]pyrimidin-3-yl)thio)acetate (19e). White crystals; mp. 120-122 °C; yield 70%. ¹HNMR: 2.44 (s, 3H, CH₃); 2.71 (s, 3H, SCH₃); 3.70 (s, 3H, OCH₃); 4.07 (s, 2H, SCH₂); 7.11 (s, 1H, CH-pyrim.). ¹³CNMR: 14.42, 22.61, 36.30, 51.81, 102.76, 139.19, 149.15, 149.67, 151.23, 167.28. Anal. Calcd for C₁₀H₁₂N₄O₂S₂: C 42.24, H 4.25, N 19.70, S 22.55%. Found: C 42.16, H 4.17, N 19.33, S 22.28%.

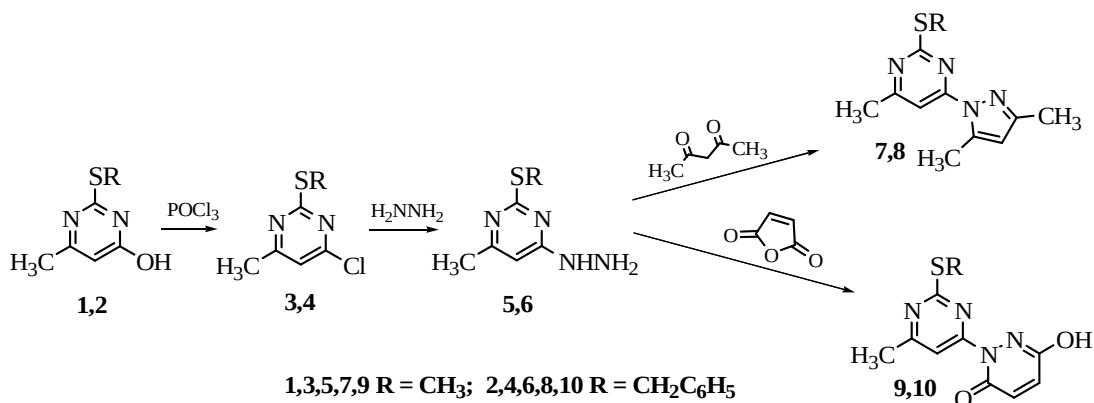
2.11.8 Methyl 2-((5-(benzylthio)-7-methyl-[1,2,4]triazolo[4,3-c]pyrimidin-3-yl)thio)acetate (20e). White crystals; mp. 64-66 °C; yield 70%. ¹HNMR: 2.47 (s, 3H, CH₃); 3.64 (s, 3H, OCH₃); 4.06 (s, 2H, SCH₂CO); 4.58 (s, 2H, SCH₂); 7.18 (s, 1H, CH-pyrim.); 7.20-7.50 (m, 5H, C₆H₅). Anal. Calcd for C₁₆H₁₆N₄O₂S₂: C 53.32, H 4.47, N 15.54, S 17.79%. Found: C 53.21, H 4.40, N 15.39, S 17.85%.

3. RESULTS AND DISCUSSION

Initial 2-mercapto-6-methylpyrimidin-4-ol has multiple nucleophilic centers, but at alkylation mainly formed the alkylthio derivatives, however, it is not excluded also O- or N-alkylation. We have found that 6-methyl-2-(methylthio)pyrimidin-4-ols (**1,2**) are obtained in good yield in the aqueous alkaline medium by the reaction of 2-mercapto-6-methylpyrimidin-4-ol with dimethyl sulfate (DMS) or benzyl chloride (Scheme 1). This was supported by the ¹H NMR spectra, where at 2.47 and 4.35 ppm the signals of S-CH₃ and S-CH₂ groups are observed, respectively.

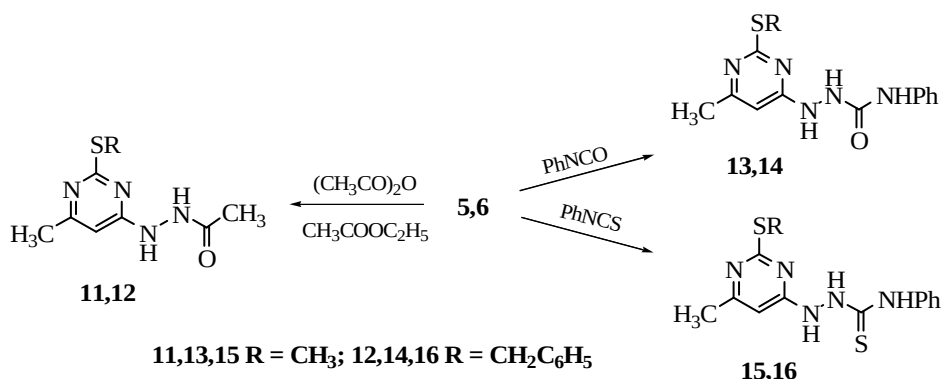
The chlorination of obtained compounds (**1,2**) with phosphorus oxychloride (POCl₃) afforded 2-(methylthio)- and 2-(benzylthio)-substituted 4-chloro-6-methyl-pyrimidines (**3,4**). These compounds under the influence of 63% hydrazine hydrate were converted into the corresponding **hydrazinyl derivatives (5,6)**.

The heterocyclization of **these compounds (5,6)** with acetylacetone and maleic anhydride led to 4-(3,5-dimethyl-1H-pyrazol-1-yl)-6-methyl-2-(alkylthio)pyrimidines (**7,8**) and 6-hydroxy-2-(6-methyl-2-(alkylthio)pyrimidine-4-yl)pyridazin-3(2H)-ones (**9,10**) (Scheme 1).



Scheme 1. Synthesis of nonfused biheterocyclic systems

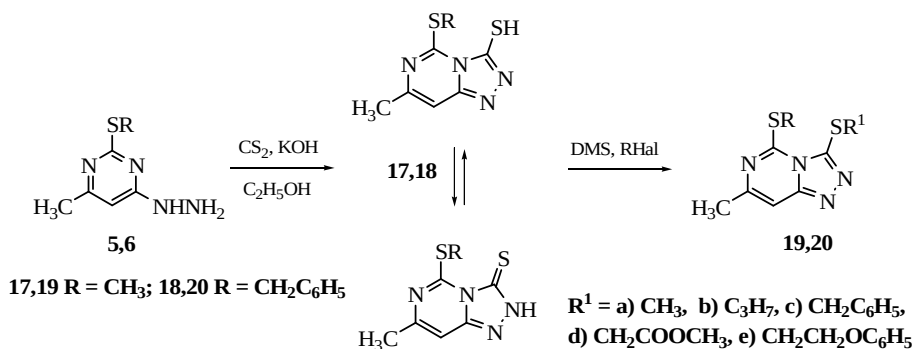
When **compounds 5,6** were heated (50-60 °C) with acetic anhydride in ethyl acetate medium for 5 hours the 2-(methylthio) and 2-(benzylthio)-substituted N'-(6-methyl-pyrimidin-4-yl)acetohydrazides (**11,12**) were formed (Scheme 2).



Scheme 2. Transformations of compounds 5,6

Via heating of **5,6** with phenylisocyanate and phenylisothiocyanate in toluene in the presence of catalytic amounts of pyridine, 2-(6-methyl-2-(alkylthio)pyrimidin-4-yl)-N-phenylhydrazine-1-carboxamides (**13,14**) and 2-(6-methyl-2-(alkylthio)pyrimidin-4-yl)-N-phenylhydrazine-1-carbothioamides (**15,16**) were synthesized, respectively (Scheme 2).

For the purpose to synthesize the fused pyrimidine derivatives the reaction of starting hydrazides **5,6** with carbon disulfide in alcoholic KOH solution were carried out. At room temperature the intramolecular cyclization was occurred and bicyclic products (**17,18**) were formed (Scheme 3).



Scheme 3. Synthesis of S-substituted 1,2,4-triazolo[4,3-c]pyrimidine derivatives

These compounds **17,18** may exist in two thiol or thione tautomeric forms. However, when the alkylation reactions of these compounds were carried out, only 3-S-substituted products (**19,20**) were obtained in good or high yields (65-98%). Their structure were confirmed with ¹H NMR spectra, where the signals of corresponding S-alkyl groups were observed.

The X-ray diffraction study of 7-methyl-3,5-bis(methylthio)-[1,2,4]triazolo[4,3-c]pyrimidine (**19a**) was also carried out, which is clearly proved that the substitution takes place at the sulfur atom (Fig. 1).

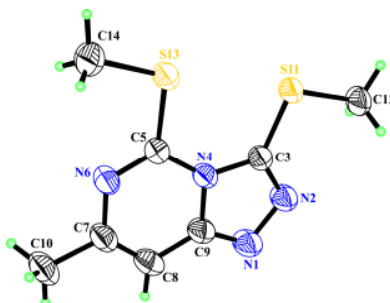


Fig. 1 The structure of 7-methyl-3,5-bis(methylthio)-[1,2,4]triazolo[4,3-c]pyrimidine (**19a**) according to X-ray analysis

3.1 BIOLOGICAL PROPERTIES OF SYNTHESIZED COMPOUNDS

The growth regulating activity of the synthesized compounds was determined in vitro using the methods adopted in phytopathology. To assess their growth-regulating properties the solutions, emulsions and suspensions of the compounds at concentrations of 25 and 50 mg/L were used. As a test object the seeds of common bean (*Phaseolus vulgaris* L) were selected. The data were compared with solutions of IAA at the same concentrations. The preliminary tests have shown that among the synthesized compounds there are substances exhibiting growth promoting properties. However, these experiments and also the screening of herbicidal and fungicidal properties of the synthesized compounds are ongoing.

4. CONCLUSION

The functionalization and heterocyclization of 4-hydrazinyl-6-methyl-2-(alkylthio)pyrimidines led to the corresponding acetohydrazides, N-phenylhydrazine-1-carboxamides(thioamides) and nonfused pyrazolyl-pyrimidines and pyridazinyl-pyrimidines and a series of 3-S-substituted derivatives of fused 1,2,4-triazolo[4,3-c]pyrimidine. The preliminary in vitro tests have shown that among the synthesized compounds there are substances exhibiting growth promoting properties. The synthesized series can be of interest in the search of novel plant growth regulators.

REFERENCES

1. Available:http://www.alanwood.net/pesticides/class_pesticides.html
2. Hong-Ju Ma, Yong-Hong Li, Qian-Fei Zhao, Tao Zhang, Ru-Liang Xie, Xiang-Dong Mei and Jun Ning. Synthesis and Herbicidal Activity of Novel N-(2,2,2)-Trifluoroethylpyrazole Derivatives. J. Agric. Food Chem. 2010;58:4356.
3. Vicentini C, Guccione S, Giurato L, Ciaccio R, Mares D, Forlani G. Pyrazole Derivatives as Photosynthetic Electron Transport Inhibitors: New Leads and Structure-Activity Relationship. J. Agric. Food Chem. 2005;53:3848.
4. Vicentini C, Mares D, Tartari A, Manfrini M, Forlani G. Synthesis of Pyrazole Derivatives and Their Evaluation as Photosynthetic Electron Transport Inhibitors. J. Agric. Food Chem. 2004;52:1898.
5. Clark R. Synthesis and QSAR of Herbicidal 3-Pyrazolyl α,α,α -Trifluorotolyl Ethers. J. Agric. Food Chem. 1996;44:3643.
6. Waldrep Th, Beck J, Lynch M, Wright F. Synthesis and herbicidal activity of 1-aryl-5-halo and 1-aryl-5-(trifluoromethyl)-1H-pyrazole-4-carboxamides. J. Agric. Food Chem. 1990;38:541.

7. Fang-Zhong Hu, Gui-Feng Zhang, Bin Liu, Xiao-Mao Zou, You-Quan Zhu, Hua-Zheng Yang. A new method for the synthesis and herbicidal activity of 3-phenoxy-6-(1H-(substituted)pyrazol-1-yl) pyridazines. *J. Heterocycl. Chem.* 2009;46:584.
8. Wei-Min Liu, You-Quan Zhu, Yi-Feng Wang, Bin Liu, Xiao-Mao Zou, Hua-Zheng Yang. Synthesis and herbicidal activity of 2-(3-(trifluoromethyl)-5-(alkoxy)-1H-pyrazol-1-yl)-4-aryloxy pyrimidine derivatives. *J. Heterocycl. Chem.* 2007;44:967.
9. Jun-Fei Li, You-Quan Zhu, Xin Wang, Hua-Zheng Yang. Synthesis and herbicidal activities of a series of di(aminopyrazolyl) ketone derivatives. *J. Heterocycl. Chem.* 2007;44:749.
10. Jun-Fei Li, You-Quan Zhu, Xin Wang, Hua-Zheng Yang. Synthesis and herbicidal activities of a series of di(aminopyrazolyl) ketone derivatives. *Pest Manag. Sci.* 2007;63:429.
11. Meazza G, Bettarini F, La Porta P, Piccardi P, Signorini E, Portoso D, Fornara L. Synthesis and herbicidal activity of novel heterocyclic protoporphyrinogen oxidase inhibitors. *Pest Manag. Sci.* 2004;60:1178.
12. Siddall Th, Ouse D, Benko Z, Garvin G, Jackson J, McQuiston J, Ricks M, Thibault Th, Turner J, VanHeertum J, Weimer M. Synthesis and herbicidal activity of phenyl-substituted benzoylpyrazoles. *Pest Manag. Sci.* 2002;58:1175.
13. Ohno R, Watanabe A, Matsukawa T, Ueda T, Sakurai H, Hori M, Hirai K. Synthesis and Herbicidal Activity of New Pyrazole-4-carboxamide Derivatives. *J. Pestic. Sci.* 2004;29:15.
14. Miao Li, Chang-Ling Liu, Ji-Chun Yang, Jin-Bo Zhang, Zhi-Nian Li, Hong Zhang and Zheng-Ming Li. Synthesis and Biological Activity of New (E)- α -(Methoxyimino)benzeneacetate Derivatives Containing a Substituted Pyrazole Ring. *J. Agric. Food Chem.* 2010;58:2664.
15. Pei-Liang Zhao, Fu Wang, Ming-Zhi Zhang, Zu-Ming Liu, Wei Huang and Guang-Fu Yang. Synthesis, Fungicidal, and Insecticidal Activities of β -Methoxyacrylate-Containing N-Acetyl Pyrazoline Derivatives. *J. Agric. Food Chem.* 2008;56:10767.
16. Song Tu, Long-He Xu, Li-Yi Ye, Xi Wang, Yong Sha and Zong-Yuan Xiao. Synthesis and Fungicidal Activities of Novel Indene-Substituted Oxime Ether Strobilurins. *J. Agric. Food Chem.* 2008;56:5247.
17. Vicentini Ch, Romagnoli C, Andreotti E, Mares, Donatella. Synthetic Pyrazole Derivatives as Growth Inhibitors of Some Phytopathogenic Fungi. *J. Agric. Food Chem.* 2007;55:10331.
18. Yan Li, Hong-Quan Zhang, Jie Liu, Xiang-Ping Yang, and Zhao-Jie Liu. Stereoselective Synthesis and Antifungal Activities of (E)- α -(Methoxyimino)benzeneacetate Derivatives Containing 1,3,5-Substituted Pyrazole Ring. *J. Agric. Food Chem.* 2006;54:3636.
19. Chen H, Li Z, Han Y. Synthesis and Fungicidal Activity against *Rhizoctonia solani* of 2-Alkyl (Alkylthio)-5-pyrazolyl-1,3,4-oxadiazoles (Thiadiazoles). *J. Agric. Food Chem.* 2000;48:5312.
20. Qiqi Zhao, Yongqiang Li, Lixia Xiong and Qingmin Wang, Design. Synthesis and Insecticidal Activity of Novel Phenylpyrazoles Containing a 2,2,2-Trichloro-1-alkoxyethyl Moiety. *J. Agric. Food Chem.* 2010;58:4992.
21. Hong Dai, Yong-Qiang Li, Ding Du, Xue Qin, Xin Zhang, Hai-Bo Yu and Jian-Xin Fang. Synthesis and Biological Activities of Novel Pyrazole Oxime Derivatives Containing a 2-Chloro-5-thiazolyl Moiety. *J. Agric. Food Chem.* 2008;56:10805.
22. Rowberg K, Even Mark, Martin E, Hopfinger A. QSAR and Molecular Shape Analyses of Three Series of 1-(Phenylcarbamoyl)-2-pyrazoline Insecticides. *J. Agric. Food Chem.* 1994;42:374.
23. Shin, E-Hyun, Young In Park, Hee Il Lee, Won Ja Lee, Young Hack Shin, Jae Chul Shim. Insecticide Susceptibilities of *Anopheles sinensis* (Diptera: Culicidae) Larvae from Paju-shi, Korea. *Entomological Research.* 2003;33:33.
24. Finkelstein B, Strock Ch. Synthesis and Insecticidal Activity of Novel Pyrazole Methanesulfonates. *Pest Sci.* 1997;50:324.
25. Hasan R, Nishimura K, Ueno T. Quantitative structure - activity relationships of insecticidal pyrazolines. *Pest. Sci.* 1994;42:291.
26. Tada I, Motoki M, Takahashi N, Miyata T, Takechi T, Uchida T, Takagi Y. Synthesis and Structure-Activity Relationships of Miticidal 4,5-Dihydropyrazole-5-thiones. *Pest. Sci.* 1996;48:165.
27. Guiping Ouyang, Xue-Jian Cai, Zhuo Chen, Bao-An Song, Pinaki S. Bhadury, Song Yang, Lin-Hong Jin, Wei Xue, De-Yu Hu and Song Zeng. Synthesis and Antiviral Activities of Pyrazole Derivatives Containing an Oxime Moiety. *J. Agric. Food Chem.* 2008;56:10160.
28. Currier H, Crafts A. Maleic hydrazide, a selective herbicide. *Science.* 1950;111:152-155.
29. Schoene D, Hoffman O. Maleic hydrazide, a unique growth regulant. *Science.* 1949;109:588-590.
30. White D. Maleic hydrazide derivatives. *Science.* 1950;111:303-305.
31. Wittwer S, Jackson H, Watson D. Control of Seedstalk Development in Celery by Maleic Hydrazide. *Am. J. Bot.* 1954;41:435-439.

32. Fischer A. Herbicidal mixture of a benzofuranyl methanesulfonate and a substituted pyridazone. Pat. US 3933460,(1976).
33. Takashi W, Sumio Y, Michio I, Yoshitake Y, Ouchi S. Substitutes 6-(2,4-dinitrophenoxy)-3(2H)-pyridazinone herbicides. (Chemical Industrial Co. Ltd Japan). Pat. Jap 78,12,979. (1971), C.A. 1971 v81, 11929c.
34. Takashi W, Sumio Y, Michio I, Yoshitake Y, Ouchi S. Pyridazone insecticides. Pat. Jap 78,17,652.(1971). C.A. 1978 v89, 158764z.
35. Yih R, Roy Y, Johnson W, Kollman O. Selective herbicidal activity of fluoroaminopyridazines. Pat US 4,083.713, (1978), C.A. 1978, v89, 101922v.
36. Takeshiba J. Pyridazine derivatives and their use as agricultural fungicides, Pat. US 4279908, 21 Jul. 1981.
37. Hoffman O, Patel I, Natu R. 1-Benzoyl-3-hydroxy-6(1H)-pyridazinones and their use as plant growth regulators. Pat. US 4,256,881, (1981), C.A. 1981, v95, 19717x.
38. Mohana K, Kumar B, Mallesha L. Synthesis and biological activity of some pyrimidine derivatives. Drug Invention Today. 2013;5;216-222.
39. Shi-Ben Wang, Guang-Chun Piao, Hong-Jian Zhang and Zhe-Shan Quan. Synthesis of 5-Alkoxythieno[2,3-e][1,2,4]triazolo[4,3-c]pyrimidine Derivatives and Evaluation of Their Anticonvulsant Activities. Molecules. 2015;20:6827-6843.
40. Fathalla O, Zeid I, Haiba M, Soliman A, Abd-Elmoez Sh. I, El-Serwy W. Synthesis, Antibacterial and Anticancer Evaluation of Some Pyrimidine Derivatives. World Journal of Chemistry. 2009;4:127-132.
41. Kandeel M, Mounir A, Refaat H, Kassab A. Synthesis of Effective Anticancer Thieno[2,3-d]pyrimidine-4-ones and Thieno[3,2-c]triazolo[4,3-c]pyrimidines. Int. J. Pharm. Pharm. Sci. 2012;4:438-448.
42. Panchamukhi S, Mulla J, Shetty N, Khazi M, Khan A, Kalashetti M, Khazi I. Benzothieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidines: synthesis, characterization, antimicrobial activity, and incorporation into solid lipid nanoparticles. Arch Pharm (Weinheim). 2011;344:358-365.
43. Paronikyan E, Akopyan Sh, Noravyan A, Dzhagatspanyan I, Paronikyan R, Nazaryan I, Akopyan A. Synthesis and neurotropic activity of condensed thieno[2,3 e][1,2,4]triazolo[4,3-c]pyrimidine derivatives. Pharm. Chem. J. 2012;46:154-156.
44. Hardy R, Jr, Baker J., Quinones N. (Substituted-phenyl)-1,2,4-triazolo[4,3-c]-pyrimidines and (substituted-phenyl)-1,2,4-triazolo[1,5-c]pyrimidines. US patent, 4,269,980, May 26 1981
45. Harris J, Neustadt J, Hao J, Stamford A. 1,2,4-Triazolo[4,3-c]pyrimidin-3-one and pyrazolo[4,3-e]-1,2,4-triazolo[4,3-c]pyrimidin-3-one compounds for use as adenosine a2a receptor antagonists. US Pat. 8222259 B2, 2012