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Scientific paper

Synthesis, Characterization and Antimicrobial Activities of Some New Heterocyclic Schiff Bases Derived from Thiocarbohydrazide

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Abstract

The reaction of pyrazolobenzothienopyrimidine-3-carbaldehyde **1** with thiocarbohydrazide afforded the Schiff's base **3**. The latter compound reacted with some electrophilic reagents to give 1,2,4-triazoles **4–6** and 1,2,4-triazines **7–9**. Treatment of compound **3** with 2-cyano-3,3-bis(methylthio)acrylonitrile gave the corresponding 5-amino-4-cyano-3-methylthiopyrazole derivative **11**. The reaction of pyrazole **11** with carbon disulfide afforded dithioxopyrazolopyrimidine **12**. Acylation of compound **11** by using acetic anhydride yielded acetamide **13**. On the other hand, the cyclocondensation of pyrazole **11** with acetic anhydride in pyridine yielded pyrazolopyrimidine derivative **14**. The reactivity of compound **11** towards formamide and phenylisothiocyanate to give the pyrazolopyrimidines **15** and **16** was studied. The newly synthesized compounds were screened for their antimicrobial activity.

Keywords: Schiff bases; triazines, triazoles; carbothiohydrazide; antimicrobial activity.

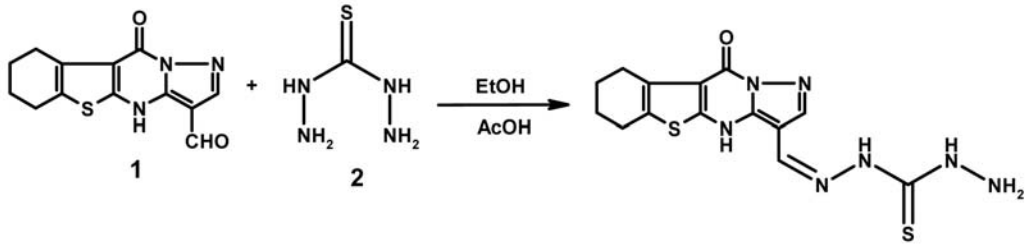
1. Introduction

Schiff bases[1,2] of heterocyclic compounds are interesting class of organic compounds possessing a wide spectrum of biological activities, such as antibacterial, antitubercular, antiinflammatory, anthelmintic, antiviral and antioxidant activities. Study of thiocarbohydrazide and its derivatives is of great interest due to the wide use of these compounds and their valuable reactions. Thiocarbohydrazides are an important class of compounds which possess applications in many fields. The chemistry of thiocarbohydrazides has gained increased interest in both synthetic organic chemistry and biological fields and has considerable value in many useful applications, such as the assessment process of the three-dimensional ultra structure examination techniques of interphase nuclei and tissues, besides their therapeutic importance.[3] Thiocarbohydrazide derivatives have attracted much attention in recent years due to their applications in the synthesis of transition metal complexes.[4–6] Thiocarbohydrazide[7–9] is the closest structural analogue of thiosemicarbazide, derivatives of which are recommended as effective antitubercular and antiviral preparations. Thiocarbohydrazides of the aromatic series exhibit high antiviral and antimicrobial activity.[10–12] In the view of these observations, we planned to synthesis some novel Schiff bases and to evaluate for antimicrobial activity.

2. Results and Discussion

2. 1. Chemistry

Novel Schiff bases were obtained from condensation of thiocarbohydrazide with substituted aldehyde giving the monothiocarbohydrazone. Thus, condensation reaction of 10-oxo-4,6,7,8,9,10-hexahydroprazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidine-3-carbaldehyde (**1**)[13] with thiocarbohydrazide **2**[14] in boiling absolute ethanol containing a catalytic amount of acetic acid gave the corresponding *N*′-[(10-oxo-4,6,7,8,9,10-hexahydropyrazolo



Scheme 1. Synthesis of thiocarbohydrazone **3**.

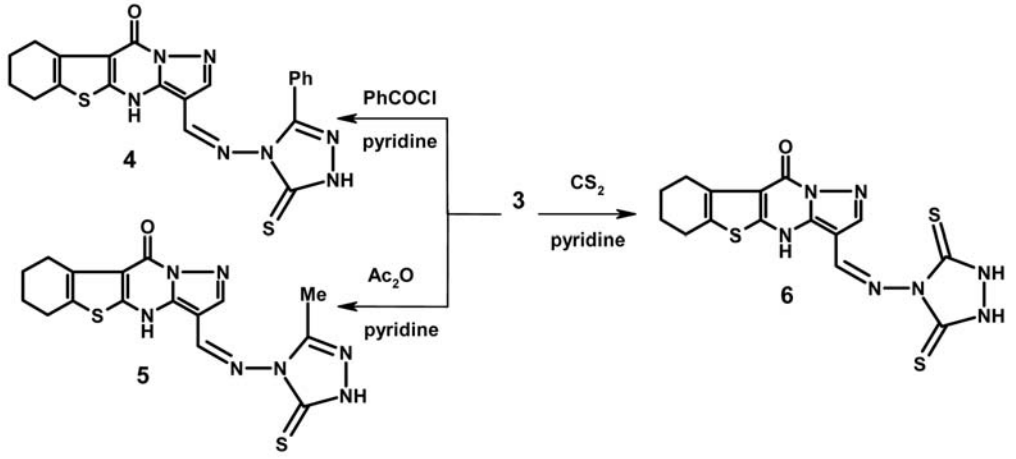
[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)methylene] thiocarbohydrazide (**3**) (Scheme 1).

The structure of the isolated product was confirmed on the basis of its elemental analysis and spectral data. Thus, the IR spectrum of compound **3** showed absorption bands at 1194, 1684, and 3198–3422 cm^{-1} corresponding to C=S, C=O, NH and NH_2 functions, respectively. Its ^1H NMR spectrum showed four D_2O-exchangeable signals at δ 3.32, 9.50, 11.36 and 12.05 ppm assigned to NH_2 and three NH protons, respectively.

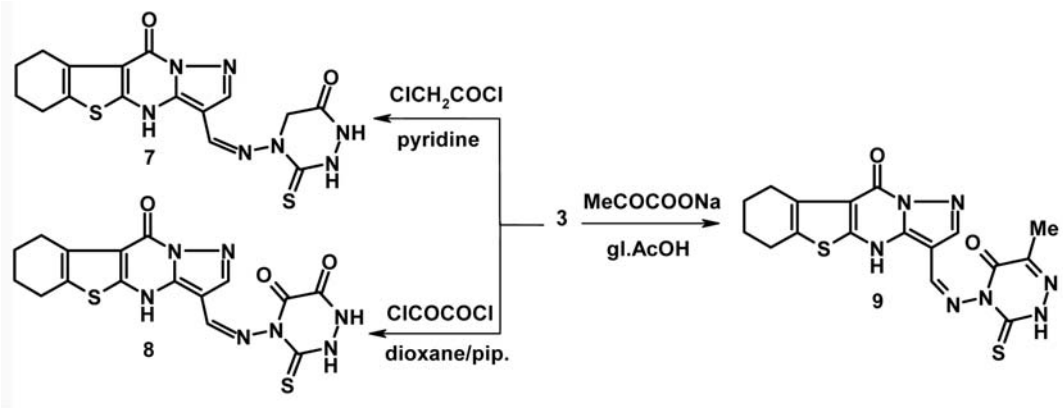
Further, it is of interest to note that the thiocarbohydrazide **3** is a convenient intermediate for the synthesis of a novel isolated heterocyclic system. Thus, heating thiocarbohydrazone **3** under reflux with benzoyl chloride in pyridine gave 3-{[(3-phenyl-5-thioxo-1,5-dihydro-4-*H*-1,2,4-triazol-4-yl)imino]methyl}-6,7,8,9-tetrahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-10(4*H*)-one (**4**), while boiling with acetic anhydride afforded 3-{[(3-methyl-5-thioxo-1,5-dihydro-4-*H*-1,2,4-triazol-4-yl)imino]methyl}-6,7,8,9-tetrahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-10(4*H*)-one (**5**). Also, reaction of **3** with carbon disulfide in boiling pyridine afforded 3-{[(3,5-dithioxo-1,2,4-triazolidin-4-yl)imino] methyl}-6,7,8,9-tetrahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-10(4*H*)-one (**6**) (Scheme 2). The structures of compounds **4**–**6** were confirmed by their elemental analysis and spectral data.

Moreover, compound **3** was used for construction of some new Schiff bases bearing 1,2,4-triazine moiety through the reaction with 1,2-bifunctional electrophiles. Thus, heterocyclization of **3** with chloroacetyl chloride, oxalyl chloride and/or sodium pyruvate led to the formation of 3-{[(6-oxo-3-thioxo-1,2,4-triazinan-4-yl)imino]methyl}-6,7,8,9-tetrahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-10(4*H*)-one (**7**), 3-{[(5,6-dioxo-3-thioxo-1,2,4-triazinan-4-yl)imino]methyl}-6,7,8,9-tetrahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-10(4*H*)-one (**8**) and 3-{[(6-methyl-5-oxo-3-thioxo-2,5-dihydro-1,2,4-triazin-4(3*H*)-yl)imino]methyl}-6,7,8,9-tetrahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-10(4*H*)-one (**9**), respectively (Scheme 3).

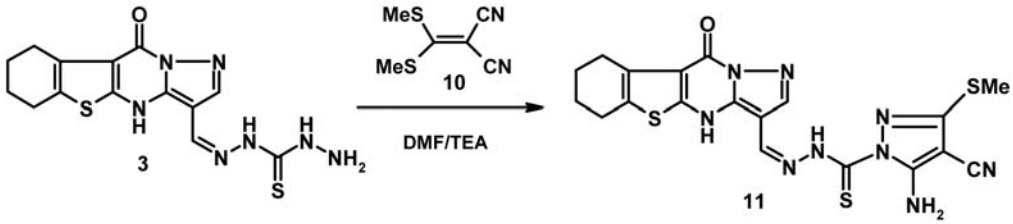
The structures of compounds **7**–**9** were characterized from their spectroscopic as well as elemental analytical data. Thus, the IR spectrum of compound **7** revealed absorption bands at 1171, 1674 and 3200–3415 cm^{-1} corresponding to C=S, C=O and NH functions, respectively. Its ^1H NMR spectrum showed D_2O-exchangeable signals at δ 7.16, 7.37 and 7.50 ppm assigned to three NH protons. The mass spectrum of compound **8** revealed the molecular ion peak at *m/z* 415 corresponding to the molecular formula $C_{16}H_{13}N_7O_3S_2$, which agrees well with the molecular weight (415.44). The mass spectrum of compound **9** revealed the molecular ion peak at *m/z* 413 corresponding to the molecular formula $C_{17}H_{15}N_7O_2S_2$, which agrees well with



Scheme 2. Formation of 1,2,4-triazole derivatives **4**, **5** and **6**.



Scheme 3. Formation of 1,2,4-triazine derivatives **7**, **8** and **9**.



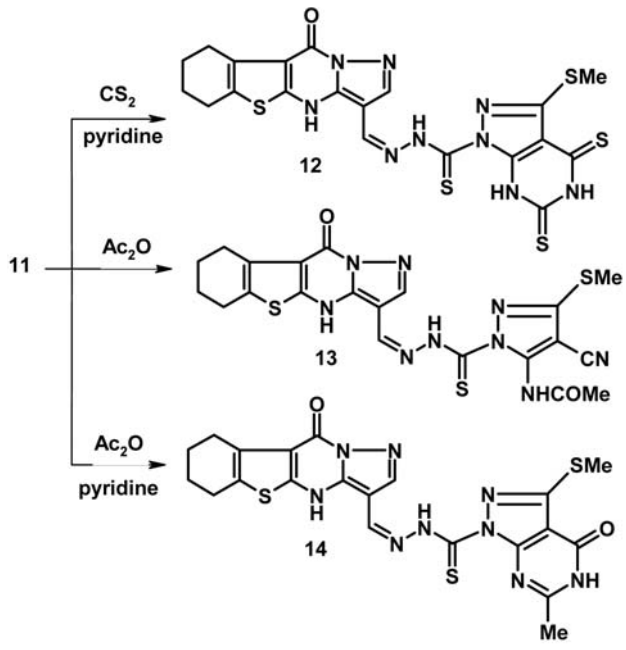
Scheme 4. Synthesis of aminocyanopyrazole **11**.

the molecular weight (413.47) and supports the identity of the structure and the base peak at *m/z* 273 (100%).

Thiocarbohydrazide **3** was treated with 2-cyano-3,3-bis(methylthio)acrylonitrile **10** in dimethylformamide containing a catalytic amount of triethylamine to give 5-amino-4-cyano-3-(methylthio)-*N*-[(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)methylene]-1*H*-pyrazole-1-carbothiohydrazide (**11**) (Scheme 4).

The IR spectrum of compound **11** showed absorption bands at 1182, 1654, 2204 and 3200–3446 cm^{-1} corresponding to C=S, C=O, C≡N, NH and NH_2 functions, respectively. Its ^1H NMR spectrum revealed signals at δ 2.41 and 9.86 due to SCH_3 and CH=N protons, respectively. It showed also three D_2O-exchangeable signals at δ 3.34, 8.30 and 8.42 ppm assigned to NH_2 and two NH protons, respectively. Also, the structure of **11** was confirmed by its mass spectrum which exhibited the molecular ion peak at *m/z* 483 with a base peak at *m/z* 80 (100%).

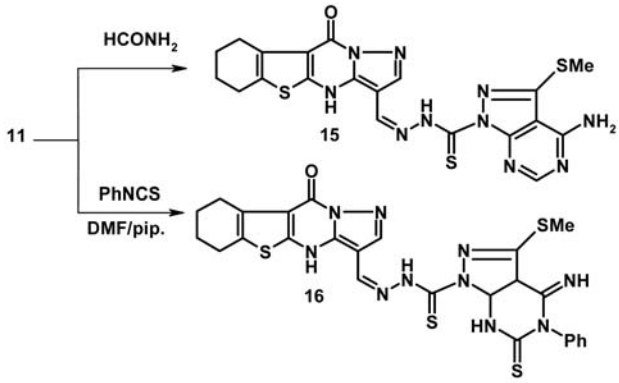
Heating **11** with an excess of carbon disulfide in pyridine afforded 3-(methylthio)-*N*-[(10-oxo-4,6,7,8, 9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)methylene]-4,6-dithioxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-1-carbothiohydrazide (**12**). Acylation of carbothiohydrazide **11** by using acetic anhydride yielded the amide, *N*-[4-cyano-3-(methylthio)-1-{[2-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)methylene]hydrazino} carbonothioyl]-1*H*-pyrazol-5-yl]acetamide (**13**). Cyclocondensation of carbothiohydrazide **11** with acetic anhydride in pyridine gave 6-methyl-3-(methylthio)-*N*-[(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)methylene]-4-oxo-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-1-carbothiohydrazide (**14**) (Scheme 5).



Scheme 5. Reaction of **11** with carbon disulfide and/or acetic anhydride.

The structures of compounds **12–14** were determined using spectroscopic as well as elemental analytical data. Thus, no evidence for the presence of an amino (NH_2) group was seen in both the IR and ^{1}H NMR spectra. The IR spectrum of compound **13** showed absorption bands at 1254, 1701, 1684, 2205 and 3447 cm^{-1} corresponding to C=S, C=O, C≡N and NH functions, respectively. Its ^{1}H NMR spectrum revealed signals at δ 2.20 and 2.42 due to $COCH_3$ and SCH_3 protons, respectively. It also showed three D_2O-exchangeable signals at δ 7.82, 8.10 and 8.13 ppm assigned to three NH protons.

Interaction of carbothiohydrazide **11** with formamide and phenyl isothiocyanate yielded 4-amino-3-(methylthio)-*N*-[(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)methylene]-1*H*-pyrazolo[3,4-*d*]pyrimidine-1-carbothiohydrazide (**15**) and 4-imino-3-(methylthio)-5-phenyl-*N*-[(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)methylene]-6-thioxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-1-carbothiohydrazide (**16**), respectively (Scheme 6).



Scheme 6. Formation of pyrazolo[3,4-*d*]pyrimidine derivatives **15** and **16**.

The ^{1}H NMR spectrum of compound **15** showed a characteristic singlet at δ 7.62 assigned to pyrimidine-H-6, in addition to three D_2O-exchangeable signals at δ 3.38, 7.52 and 7.99 ppm attributed to NH_2 and two NH protons, respectively. Also, the structure of **15** was confirmed by its mass spectrum which exhibited the molecular ion peak at *m/z* 510 (M^+, 2%) with a base peak at *m/z* 336 (100%). On the other hand, the ^{1}H NMR spectrum of compound **16** showed four D_2O-exchangeable signals at δ 8.06, 8.20, 8.33 and 8.38 ppm assigned to four NH protons, in addition to an aromatic multiplet in the region δ 6.96–7.60. The structure of **16** was also supported by its mass spectrum which showed the molecular ion peak at *m/z* 618 (7.5%) corresponding to the molecular formula $C_{26}H_{22}N_{10}OS_4$, which agrees well with the molecular weight (618.77) and supports the identity of the structure and the base peak at *m/z* 336 (100%).

2. 2. Biological Activities

The standardized disc agar diffusion method[15] was followed to determine the activity of the synthesized compounds against the sensitive organisms *Staphylococcus*

Table 1. The antimicrobial activity of the synthesized compounds

	Mean* of zone diameter , nearest whole mm.									
	Gram-positive bacteria				Gram-negative bacteria				Fungi	
Comp. No.	*S. aureus*		*B. subtilis*		*S. typhimurium*		*E. coli*		*C. albicans*	
	1 mg/mL	0.5 mg/mL	1 mg/mL	0.5 mg/mL	1 mg/mL	0.5 mg/mL	1 mg/mL	0.5 mg/mL	1 mg/mL	0.5 mg/mL
3	–	–	20	15	–	–	–	–	14	10
4	–	–	–	–	–	–	–	–	24	19
5	–	–	–	–	–	–	–	–	18	11
6	–	–	–	–	–	–	–	–	23	17
7	–	–	–	–	–	–	–	–	23	19
8	21	15	21	16	21	18	17	10	20	14
9	–	–	21	15	13	10	-	-	27	24
11	–	–	8	7	–	–	16	13	–	–
12	–	–	18	15	–	–	–	–	–	–
13	16	10	9	7	–	–	–	–	19	15
14	–	–	–	–	–	–	–	–	16	12
15	16	13	18	16	18	14	–	–	28	24
16	17	13	8	7	–	–	–	–	23	17
Control #	35	26	35	25	36	28	38	27	35	28

* = Calculated from 3 values. – = No effect. 7–10 mm: Low activity = Mean of zone diameter ≤ 1/3 of mean zone diameter of control. 11–20: Intermediate activity = Mean of zone diameter ≤ 2/3 of mean zone diameter of control. 21–28: High activity = Mean of zone diameter > 2/3 of mean zone diameter of control. #: Chloramphenicol in the case of Gram-positive bacteria, Cephalothin in the case of Gram-negative bacteria and cycloheximide in the case of fungi.

aureus and *Bacillus subtilis* as Gram-positive bacteria, *Salmonella typhimurium* and *Escherichia coli* as Gram-negative bacteria and *Candida albicans* as fungus strain. The compounds were dissolved in DMSO which has no inhibition activity to obtain concentration of 100 μg mL^{-1}. The test was performed on medium potato dextrose agars (PDA) which contains infusion of 200 g potatoes, 6 g dextrose and 15 g agar.[16] Uniform size filter paper disks (3 disks per compound) were impregnated by equal volume (10 μL) from the specific concentration of dissolved tested compounds and carefully placed on inoculated agar surface. After incubation for 36 h at 27 °C in the case of bacteria and for 48 h at 24 °C in the case of fungi, inhibition of the organisms was measured and used to calculate mean of inhibition zones.

The results depicted in Table 1 show various activities against all species of microorganisms which suggest that the variations in the structures affect the growth of the microorganisms. Thus, we can conclude from these results that some of the prepared compounds showed a low to high antimicrobial activity towards Gram-positive bacteria, Gram-negative bacteria and the fungal strain (Table 1).

3. Experimental

3. 1. General

Melting points are uncorrected and were recorded in open capillary tubes on a Stuart SMP3 melting point apparatus. Infrared spectra were recorded on FT-IR Jasco 4100 spectrophotometer using a KBr wafer technique. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ on Gemini spectrometer (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) and the chemical shift ar given as δ downfield from TMS as an internal standard, measured at the Main Laboratories of the War Chemical. Elemental microanalyses were recorded on a Perkin Elmer series II CHNS analyzer 2400. Mass spectra were obtained using gas chromatography GCMS qp-2010 and on a Shimadzu instrument mass spectrometer (70 eV) at the Cairo University Microanalytical Center. 10-Oxo-4,6,7,8,9,10-hexahydroprazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidine-3-carbaldehyde (**1**) has been prepared according to the reported method.[13]

3. 1. 1. Synthesis of N'-[(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)methylene] thiocarbohydrazide (3)

A mixture of compound **1** (0.273 g, 1 mmol) and thiocarbohydrazide **2** (0.106 g, 1 mmol) in absolute ethanol (10 mL) containing a few drops of acetic acid was heated under reflux for 1 h. After cooling, the precipitated solid was filtered off, air dried and recrystallized from ethanol to give **3** as yellow crystals. Yield 81%, mp 200–202 °C. IR (KBr) ν 3422–3198 (NH_2, NH), 3069 ($CH_{arom.}$), 2933 ($CH_{aliph.}$), 1684 (C=O), 1611 (C=N), 1194 (C=S) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.80–1.82 (m, 4H, $2CH_2$), 2.72 (t, 2H, CH_2), 2.94 (t, 2H, CH_2), 3.32 (brs, 2H, D_2O-exchangeable, NH_2), 8.12 (s, 1H, CH=N), 8.26 (s, 1H, pyrazole-H2), 9.50 (s, 1H, D_2O-exchangeable, NH), 11.36 (s, 1H, D_2O-exchangeable, NH), 12.05 (s, 1H, D_2O-exchangeable, NH). MS: *m/z* 361 (M^+, 0.4%). Anal. Calcd for $C_{14}H_{15}N_7OS_2$: C, 46.52; H, 4.18; N, 27.13; S, 17.74. Found: C, 46.51; H, 4.16; N, 27.15; S, 17.75.

3. 1. 2. Synthesis of 3-{[(3-phenyl-5-thioxo-1,5-dihydro-4*H*-1,2,4-triazol-4-yl)imino] methyl}-6,7,8,9-tetrahydropyrazolo [1,5-*a*][1]benzothieno[2,3-*d*] pyrimidin-10(4*H*)-one (4)

A mixture of compound **3** (0.361 g, 1 mmol) and benzoyl chloride (0.12 mL, 1 mmol) was added dropwise with continuous stirring to dry pyridine (10 mL). The reaction mixture was heated under reflux for 4 h. After cooling, the reaction mixture was poured onto ice/HCl. The solid obtained was filtered off, washed several times with water, and recrystallized from acetic acid to give **4** as pale violet crystals. Yield 81%, mp 229–230 °C. IR (KBr) ν 3444, 3125 (2NH), 3020 ($CH_{arom.}$), 2937 ($CH_{aliph.}$), 1682 (C=O), 1627 (C=N), 1217 (C=S) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.79–1.82 (m, 4H, $2CH_2$), 2.66 (t, 2H, CH_2), 2.90 (t, 2H, CH_2), 6.09 (s, 1H, D_2O-exchangeable, NH), 7.48–8.41 (m, 6H, ArH's and pyrazole-H2), 8.08 (s, 1H, D_2O-exchangeable, NH), 8.83 (s, 1H, CH=N). MS: *m/z* 448 (M^++1, 4.2%). Anal. Calcd for $C_{21}H_{17}N_7OS_2$: C, 56.36; H, 3.83; N, 21.91; S, 14.33. Found: C, 56.35; H, 3.82; N, 21.92; S, 14.32.

3. 1. 3. Synthesis of 3-{[(3-methyl-5-thioxo-1,5-dihydro-4*H*-1,2,4-triazol-4-yl)imino] methyl}-6,7,8,9-tetrahydropyrazolo [1,5-*a*][1]benzothieno[2,3-*d*] pyrimidin-10(4*H*)-one (5)

A mixture of compound **3** (0.361 g, 1 mmol) and acetic anhydride (10 mL) in dry pyridine (5 mL) was heated under reflux for 2 h. After cooling, the reaction mixture was poured onto ice. The solid obtained was filtered off, washed several times with water, and recrystallized from DMF to give **5** as pale brown crystals. Yield 63%, mp >300 °C. IR (KBr) ν 3421, 3200 (2NH), 3069 ($CH_{arom.}$), 2925 ($CH_{aliph.}$), 1653 (C=O), 1609 (C=N), 1183 (C=S) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.76–1.91 (m, 4H, $2CH_2$), 2.03 (s, 3H, CH_3), 2.73 (t, 2H, CH_2), 2.88 (t, 2H, CH_2), 8.30 (s, 1H, pyrazole-H2), 8.53 (s, 1H, D_2O-exchangeable, NH), 8.80 (s, 1H, CH=N), 9.11 (s, 1H, D_2O-exchangeable, NH). MS: *m/z* 385 (M^+, 0.2%). Anal. Calcd for $C_{16}H_{15}N_7OS_2$: C, 49.85; H, 3.92; N, 25.44; S, 16.64. Found: C, 49.86; H, 3.91; N, 25.43; S, 16.65.

3. 1. 4. Synthesis of 3-{[(3,5-dithioxo-1,2,4-triazolidin-4-yl)-imino]methyl}-6,7,8,9-tetrahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-10(4*H*)-one (6)

A mixture of compound **3** (0.361 g, 1 mmol) and carbon disulfide (0.06 mL, 1 mmol) in dry pyridine (10 mL) was heated under reflux for 8 h or until evolution of H_2S ceased. After cooling, the reaction mixture was poured onto ice/HCl. The solid obtained was filtered off, washed several times with water, and recrystallized from DMF/ethanol to give **6** as pale brown crystals. Yield 58%, mp >300 °C. IR (KBr) ν 3440–3280 (3NH), 3050 ($CH_{arom.}$), 2929 ($CH_{aliph.}$), 1660 (C=O), 1606 (C=N), 1181 (2C=S) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.76–1.80 (m, 4H, $2CH_2$), 2.72 (t, 2H, CH_2), 2.88 (t, 2H, CH_2), 6.96 (s, 1H, D_2O-exchangeable, NH), 7.13 (s, 1H, D_2O-exchangeable, NH), 8.38 (s, 1H, D_2O-exchangeable, NH), 8.90 (s, 1H, pyrazole-H2), 9.89 (s, 1H, CH=N). MS: *m/z* 403 (M^+, 0.5%). Anal. Calcd for $C_{15}H_{13}N_7OS_3$: C, 44.65; H, 3.25; N, 24.30; S, 23.84. Found: C, 44.66; H, 3.24; N, 24.31; S, 23.83.

3. 1. 5. Synthesis of 3-{[(6-oxo-3-thioxo-1,2,4-triazinan-4-yl)imino]methyl}-6,7,8,9-tetrahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-10(4*H*)-one (7)

A mixture of compound **3** (0.361 g, 1 mmol) and chloroacetyl chloride (0.113 mL, 1 mmol) in dry pyridine (10 mL) was heated under reflux for 6 h. After cooling, the reaction mixture was poured onto ice/HCl. The solid obtained was filtered off and recrystallized from dioxane to give **7** as pale brown crystals. Yield 63%, mp >300 °C. (KBr) ν 3415–3200 (3NH), 3055 ($CH_{arom.}$), 2932 ($CH_{aliph.}$), 1674 (2C=O), 1619 (C=N), 1171 (C=S) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.77–1.90 (m, 4H, $2CH_2$), 2.65 (t, 2H, CH_2), 2.88 (t, 2H, CH_2), 4.37 (s, 2H, CH_2), 7.16 (s, 1H, D_2O-exchangeable, NH), 7.37 (s, 1H, D_2O-exchangeable, NH), 7.50 (s, 1H, D_2O-exchangeable, NH), 8.51 (s, 1H, pyrazole-H2), 8.88 (s, 1H, CH=N). MS: *m/z* 401 (M^+, 0.1%). Anal. Calcd for $C_{16}H_{15}N_7O_2S_2$: C, 47.87; H, 3.77; N, 24.42; S, 15.97. Found: C, 47.86; H, 3.78; N, 24.43; S, 15.96.

3. 1. 6. Synthesis of 3-{[(5,6-dioxo-3-thioxo-1,2,4-triazinan-4-yl)imino]methyl}-6,7,8,9-tetrahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-10(4*H*)-one (8)

A mixture of compound **3** (0.361 g, 1 mmol) and oxalyl chloride (0.129 mL, 1 mmol) was added dropwise in dry dioxane (20 mL) containing catalytic amount of piperidine was heated under reflux for 6 h. After cooling, the reaction mixture was poured onto ice. The solid obtained was filtered off, washed several times with water, and recrystallized from DMF/H_2O to give **8** as brown crystals. Yield 61%, mp >300 °C. IR (KBr) ν 3420–3200 (3NH), 3050 ($CH_{arom.}$), 2931 ($CH_{aliph.}$), 1640 (3C=O), 1616 (C=N), 1185 (C=S) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.79–1.90 (m, 4H, $2CH_2$), 2.65 (t, 2H, CH_2), 2.93 (t, 2H, CH_2), 6.09 (s, 1H, D_2O-exchangeable, NH), 7.86 (s, 1H, pyrazole-H2), 8.73 (s, 1H, D_2O-exchangeable, NH), 9.93 (s, 1H, CH=N), 11.21 (s, 1H, D_2O-exchangeable, NH). MS: *m/z* 415 (M^+, 0.3%). Anal. Calcd for $C_{16}H_{13}N_7O_3S_2$: C, 46.26; H, 3.15; N, 23.60; S, 15.44. Found: C, 46.25; H, 3.14; N, 23.61; S, 15.45.

3. 1. 7. Synthesis of 3-{[(6-methyl-5-oxo-3-thioxo-2,5-dihydro-1,2,4-triazin-4(3*H*)-yl)imino]methyl}-6,7,8,9-tetrahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-10(4*H*)-one (9)

A mixture of compound **3** (0.361 g, 1 mmol) and sodium pyruvate (0.11 g, 1 mmol) in glacial acetic acid (10 mL) was heated under reflux for 4 h. After cooling, the reaction mixture was poured onto crushed ice. The solid obtained was filtered off, washed several times with water, and recrystallized from acetic acid to give **9** as brown crystals. Yield 71%, mp >300 °C. IR (KBr) ν 3453, 3243 (2NH), 3055 ($CH_{arom.}$), 2934 ($CH_{aliph.}$), 1689, 1642 (2C=O), 1607 (C=N), 1199 (C=S) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.78–1.90 (m, 4H, $2CH_2$), 2.16 (s, 3H, CH_3), 2.67 (t, 2H, CH_2), 2.88 (t, 2H, CH_2), 8.37 (s, 1H, D_2O-exchangeable, NH), 8.56 (s, 1H, pyrazole-H2), 9.80 (s, 1H, CH=N), 13.71 (s, 1H, D_2O-exchangeable, NH). MS: *m/z* 413 (M^+, 1.2%). Anal. Calcd for $C_{17}H_{15}N_7O_2S_2$: C, 49.38; H, 3.66; N, 23.71; S, 15.51. Found: C, 49.37; H, 3.65; N, 23.72; S, 15.52.

3. 1. 8. Synthesis of 5-amino-4-cyano-3-(methylthio)-*N*-[(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)methylene]-1*H*-pyrazole-1-carbothiohydrazide (11)

A mixture of compound **3** (0.361 g, 1 mmol) and 2-cyano-3,3-bis(methylthio)acrylonitrile **10** (0.17 g, 1 mmol) in DMF (10 mL) containing two drops of triethylamine was heated under reflux for 4 h. After cooling, the reaction mixture was poured onto ice. The solid obtained after cooling was filtered, washed with ethanol, and recrystallized from DMF/ethanol to give **11** as violet crystals. Yield 75%, mp 145–147 °C. IR (KBr) ν 3446–3200 (NH_2, 2NH), 3053 ($CH_{arom.}$), 2931 ($CH_{aliph.}$), 2204 (C≡N), 1654 (C=O), 1630 (C=N), 1182 (C=S) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.79-1.82 (m, 4H, $2CH_2$), 2.41 (s, 3H, SCH_3), 2.73 (t, 2H, CH_2), 2.92 (t, 2H, CH_2), 3.34 (brs, 2H, D_2O-exchangeable, NH_2), 7.95 (s, 1H, pyrazole-H2), 8.30 (s, 1H, D_2O-exchangeable, NH), 8.42 (s, 1H, D_2O-exchangeable, NH), 9.86 (s, 1H, CH=N). MS: *m/z* 483 (M^+, 0.1%). Anal. Calcd for $C_{19}H_{17}N_9OS_3$: C, 47.19; H, 3.54; N, 26.07; S, 19.89. Found: C, 47.18; H, 3.55; N, 26.08; S, 19.88.

3. 1. 9. Synthesis of 3-(methylthio)-*N*-[(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)methylene]-4,6-dithioxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-1-carbothiohydrazide (12)

A mixture of compound **11** (0.483 g, 1 mmol) and carbon disulfide (0.06 mL, 1 mmol) in dry pyridine (10 mL) was heated under reflux for 8 h or until evolution of H_2S ceased. After cooling, the reaction mixture was poured onto ice/HCl. The solid obtained was filtered off, washed several times with water, and recrystallized from DMF/ethanol to give **12** as brown crystals. Yield 62%, mp >300 °C. IR (KBr) ν 3421 (3NH), 3032 ($CH_{arom.}$), 2926 ($CH_{aliph.}$), 1653 (C=O), 1636 (C=N), 1184 (3C=S) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.79–1.82 (m, 4H, $2CH_2$), 2.41 (s, 3H, SCH_3), 2.69 (t, 2H, CH_2), 2.93 (t, 2H, CH_2), 7.69 (s, 1H, pyrazole-H2), 8.05 (s, 2H, D_2O-exchangeable, 2NH), 8.15 (s, 1H, D_2O-exchangeable, NH), 8.74 (s, 1H, CH=N). MS: *m/z* 558 (M^+-1, 8.5%). Anal. Calcd for $C_{20}H_{17}N_9OS_5$: C, 42.92; H, 3.06; N, 22.52; S, 28.64. Found: C, 42.91; H, 3.05; N, 22.53; S, 28.65.

3. 1. 10. Synthesis of *N*-[4-cyano-3-(methylthio)-1-{[2-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)methylene]hydrazino} carbonothioyl]-1*H*-pyrazol-5-yl] acetamide (13)

A mixture of compound **11** (0.483 g, 1 mmol) and acetic anhydride (10 mL) was heated under reflux for 1 h. After cooling, the reaction mixture was poured onto crushed ice. The solid obtained was filtered off, washed several times with water, and recrystallized from DMF to give **13** as pale brown crystals. Yield 74%, mp 198–200 °C. IR (KBr) ν 3447 (3NH), 3061 ($CH_{arom.}$), 2934 ($CH_{aliph.}$), 2205 (C≡N), 1701 ($C=O_{acetamido}$), 1684 (C=O), 1636 (C=N), 1254 (C=S) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.79–1.83 (m, 4H, $2CH_2$), 2.20 (s, 3H, $COCH_3$), 2.42 (s, 3H, SCH_3), 2.81 (t, 2H, CH_2), 2.91 (t, 2H, CH_2), 7.82 (s, 1H, D_2O-exchangeable, NH), 7.92 (s, 1H, pyrazole-H2), 8.10 (s, 1H, D_2O-exchangeable, NH), 8.13 (s, 1H, D_2O-exchangeable, NH), 8.42 (s, 1H, CH=N). Anal. Calcd for $C_{21}H_{19}N_9O_2S_3$: C, 47.99; H, 3.64; N, 23.98; S, 18.30. Found: C, 47.98; H, 3.65; N, 23.97; S, 18.31.

3. 1. 11. Synthesis of 6-methyl-3-(methylthio)-*N*-[(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)methylene]-4-oxo-4,5-dihydro-1*H*-pyrazolo[3,4-d]pyrimidine-1-carbothiohydrazide (14)

A mixture of compound **11** (0.483 g, 1 mmol) and acetic anhydride (10 mL) in dry pyridine (10 mL) was heated under reflux for 6 h. After cooling, the reaction mixture was poured onto crushed ice. The solid obtained was filtered off, washed several times with water, and recrystallized from dioxane to give **14** as brown crystals. Yield 65%, mp >300 °C. IR (KBr) ν 3421–3200 (3NH), 3048 ($CH_{arom.}$), 2933 ($CH_{aliph.}$), 1685, 1654 (2C=O), 1617 (C=N), 1182 (C=S) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.23 (s, 3H, CH_3), 1.78–1.80 (m, 4H, $2CH_2$), 2.40 (s, 3H, SCH_3), 2.67 (t, 2H, CH_2), 2.90 (t, 2H, CH_2), 7.50 (s, 1H, D_2O-exchangeable, NH), 8.01 (s, 1H, pyrazole-H2), 8.42 (s, 1H, CH=N), 9.80 (s, 2H, D_2O-exchangeable, 2NH). MS: *m/z* 525 (M^+, 12.4%). Anal. Calcd for $C_{21}H_{19}N_9O_2S_3$: C, 47.99; H, 3.64; N, 23.98; S, 18.30. Found: C, 47.99; H, 3.64; N, 23.98; S, 18.30.

3. 1. 12. Synthesis of 4-amino-3-(methylthio)-*N*-[(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)methylene]-1*H*-pyrazolo[3,4-*d*]pyrimidine-1-carbothiohydrazide (15)

A mixture of compound **11** (0.483 g, 1 mmol) and formamide (10 mL) was heated under reflux for 6 h. After cooling, the reaction mixture was poured onto crushed ice. The solid obtained was filtered off, washed several times with water, and recrystallized from ethanol to give **15** as pale brown crystals. Yield 81%, mp 194–196 °C. IR (KBr) ν 3449–3411 (NH_2, 2NH), 3058 ($CH_{arom.}$), 2931 ($CH_{aliph.}$), 1685 (C=O), 1631 (C=N), 1148 (C=S) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.77–1.82 (m, 4H, $2CH_2$), 2.39 (s, 3H, SCH_3), 2.65 (t, 2H, CH_2), 2.89 (t, 2H, CH_2), 3.38 (brs, 2H, D_2O-exchangeable, NH_2), 7.41 (s, 1H, D_2O-exchangeable, NH), 7.63 (s, 1H, pyrimidine-H6), 7.95 (s, 1H, pyrazole-H2), 7.99 (s, 1H, D_2O-exchangeable, NH), 8.13 (s, 1H, CH=N). MS: *m/z* 510 (M^+, 2%). Anal. Calcd for $C_{20}H_{18}N_{10}OS_3$: C, 47.04; H, 3.55; N, 27.43; S, 18.84. Found: C, 47.05; H, 3.54; N, 27.42; S, 18.85.

3. 1. 13. Synthesis of 4-imino-3-(methylthio)-5-phenyl-*N*-[(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)methylene]-6-thioxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-1-carbothiohydrazide (16)

A mixture of compound **11** (0.483 g, 1 mmol) and phenyl isothiocynate (0.135 mL, 1 mmol) in DMF (10 mL) containing a few drops of piperidine was heated under reflux for 4 h. After cooling, the reaction mixture was poured onto crushed ice. The solid obtained was filtered off, washed several times with water, and recrystallized from DMF/H_2O to give **16** as brown crystals. Yield 66%, mp 235–237 °C. IR (KBr) ν 3446 (4NH), 3043 ($CH_{arom.}$), 2927 ($CH_{aliph.}$), 1647 (C=O), 1558 (C=N), 1181 (C=S) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.79–1.82 (m, 4H, $2CH_2$), 2.41 (s, 3H, SCH_3), 2.73 (t, 2H, CH_2), 2.92 (t, 2H, CH_2),

6.96–7.60 (m, 5H, ArH), 7.95 (s, 1H, pyrazole-H2), 8.06 (s, 1H, D_2O-exchangeable, NH), 8.20 (s, 1H, D_2O-exchangeable, NH), 8.33 (s, 1H, D_2O-exchangeable, NH), 8.38 (s, 1H, D_2O-exchangeable, NH), 9.90 (s, 1H, CH=N). MS: *m/z* 618 (M^+, 7.5%). Anal. Calcd for $C_{26}H_{22}N_{10}OS_4$: C, 50.47; H, 3.58; N, 22.64; S, 20.73. Found: C, 50.46; H, 3.59; N, 22.63; S, 20.74.

4. Conclusions

In conclusion *N'*-[(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl) methylene] thiocarbohydrazide (**3**) has proved to be a versatile precursor for the synthesis of some new Schiff bases containing the pyrazolobenzothieno-pyrimidine ring. The synthesized compounds were screened for their antimicrobial activity.

5. References

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Povzetek

Pri reakciji med pirazolobenzotienopirimidin-3-karbaldehidom **1** in tiokarbohidrazidom nastane Schiffova baza **3**, ki lahko dalje reagira z različnimi elektrofilnimi reagenti pri čemer nastanejo 1,2,4-triazoli **4**–**6** ali 1,2,4-triazini **7**–**9**. Obdelava spojine **3** z 2-ciano-3,3-bis(metiltio)akrilonitrilom daje ustrezen 5-amino-4-ciano-3-metiltiopirazolski derivat **11**. Reakcija pirazola **11** z ogljikovim disulfidom daje ditioksopirazolopirimidin **12**. Pri aciliranju spojine **11** z acetanhidridom nastane acetamid **13**. Po drugi strani pa ciklokondenzacija pirazola **11** z acetanhidridom v piridinu daje pirazolopirimidinske derivate **14**. Raziskali smo tudi reakcijo spojine **11** s formamidom in fenilizotiocianatom ter ugotovili, da nastaneta pirazolopirimidina **15** in **16**. Določili smo tudi antimikrobno aktivnost novih spojin.