4-(*N*,*N*-Dialkylthiocarbamoylthio)-5-nitropyrimidines as new potential nitric oxide donors

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On heating at pH 6.86, 4-(*N*,*N*-dialkylthiocarbamoylthio)-5-nitropyrimidines are transformed into dithiolopyrimidines, which are either oxidized to bis(4-dialkylthiocarbamoylpyrimidin-5-yl) disulfides or converted into 4,6-diamino-5-nitropyrimidine derivatives with carbon disulfide elimination. The direction of the reaction is determined by the nature of a substituent in position 2 of pyrimidine and the bulk of the thiocarbamate substituent. Mechanistic schemes for these processes were proposed.

Key words: dialkyldithiocarbamates, 5-nitropyrimidine, 1,3-dithiolo[4,5-*d*]pyrimidine, substitution, nitrite anion.

Events that induced fundamental changes in the concepts on functioning of the very diverse biological systems have occurred during recent 15 years. It has been found that such a low-molecular-weight compound as nitrogen(II) oxide (NO) is one of the versatile and necessary regulators of functions of cell metabolism. This compound, being continuously produced in the mammalian organisms, exerts the key effect on various physiological and pathophysiological processes. The number of reviews and original publications devoted to the problem of NO grows like an avalanche.[1—14]

It has recently been found[12] that different nitro compounds exhibit distinct properties of nitric oxide donors. This suggests that promising biologically active compounds can be discovered among this type of compounds. Many active compounds, including drugs, are known in the series of the nitro derivatives. Among them, compounds with the properties of NO donors, such as nitroglycerol, nitrosorbide, molsidomin, and others,[12] play a worthy role. *O*-, *N*-, and *S*-Nitro derivatives generate nitric oxide.[1—3,8,12—14]

Considerably less attention has been given yet to the *C*-nitro derivatives, although the study of *C*-nitroheterocycles as NO donors began to develop rather actively predominantly among the five-membered heterocyclic nitro compounds.[12,15—18] The study of six-membered heterocycles in this direction is of great interest, which is also caused by the known data on many biologically active compounds in these series and relative preparative availability of this type of compounds.

The study of *vic*-nitro(thiocarbamoylthio)pyrimidine derivatives are among the problems being of undoubted interest in both chemical and biological respects. Note that a large group of compounds possessing noticeable biological activity: fungicidal, insecticidal, herbicidal, anticholesterolemic, hypotensive, antimicrobial, and antihelmintic have been found in the series of various dialkyldithiocarbamates.[19—22] However, the mechanisms of realization of these types of activity were insufficiently studied so far. Due to this, the search for biologically active substances in the series of poorly studied dithiocarbamates of nitroheterocycles is topical.

It has recently been found[23] that the thermal treatment of this type of compounds induces processes associated with removal of the nitro group. The purpose of the present work is to study denitration of 5-nitro-4-thiocarbamoylthiopyrimidines under hydrolytic conditions, *i.e.*, to reveal their ability to act as nitric oxide donors.

Using 4,6-dichloro-5-nitropyrimidines (**1a,b**) as the starting compounds, the known 4-alkoxy (aryloxy, amino) derivatives **2a—j** and then dithiocarbamates **3a—o** were synthesized (Scheme 1).

On refluxing in a mixture of EtOH with a K—Na phosphate buffer (pH 6.86), the majority of dithiocarbamates **3a—o** underwent degradation to form nitrite anions.

The determination of the nitrite anion by the Griess reaction is used to identify nitrogen(II) oxide, which serves as a measure for the NO-donor capability of tested compounds. The photocolorimetric method for nitrite anion determination is based on the formation of a colored azo dye due to dinitration followed by azocoupling[24] (Scheme 2).

The reaction is very specific and allows NO_2^- to be determined in a concentration of 3 μg L^{-1}. A 2% solution of sulfanilic acid in 1 *M* HCl was used as the diazo com-

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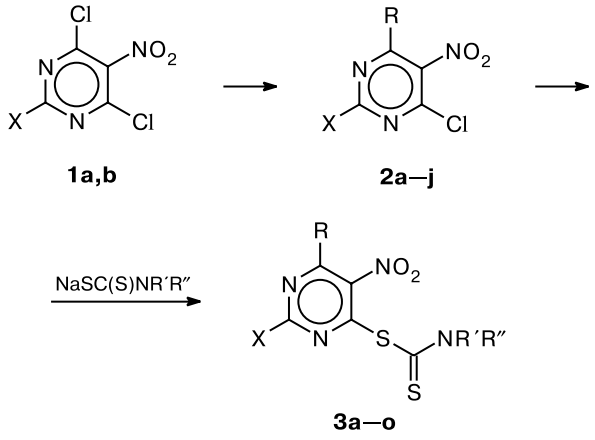
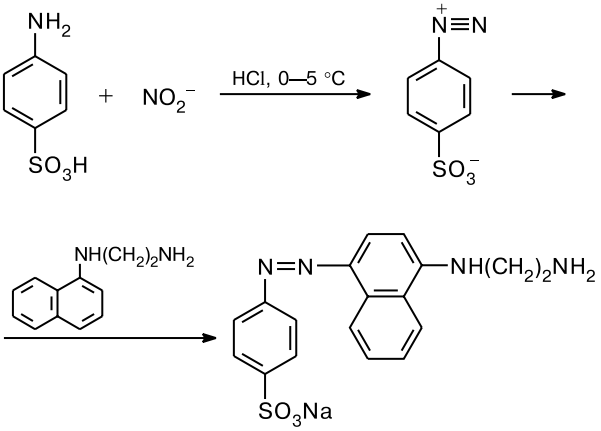
Scheme 1



1: X =H (**a**), Me (**b**)
2: X = H, R = OMe (**a**), OPh (**b**), NHMe (**c**), $NHPr^i$ (**d**), NMe_2 (**e**), NEt_2 (**f**); X = Me, R = OMe (**g**), OEt (**h**), NHMe (**i**), NMe_2 (**j**)
3: X = H, R = OMe, R′R″ = $(CH_2)_4$ (**a**),
R = OMe, R′R″ = $(CH_2)_5$ (**b**), R = OMe, R′R″ = $(CH_2)_6$ (**c**),
R = OPh, R′R″ = $(CH_2)_4$ (**d**), R = NHMe, R′R″ = $(CH_2)_4$ (**e**),
R = $NHPr^i$, R′R″ = $(CH_2)_4$ (**f**), R = NMe_2, R′R″ = $(CH_2)_4$ (**g**),
R = NEt_2, R′R″ = $(CH_2)_4$ (**h**); X = Me, R = OMe, R′R″ = $(CH_2)_4$ (**i**),
R = OEt, R′R″ = Me_2 (**j**), R = NHMe, R′R″ = MeEt (**k**),
R = NMe_2, R′R″ = Et_2 (**l**), R = NMe_2, R′R″ = $(CH_2)_4$ (**m**),
R = NMe_2, R′R″ = $(CH_2)_5$ (**n**), R = NMe_2, R′R″ = $(CH_2)_6$ (**o**)

Scheme 2



ponent, and α-naphthylethylenediamine hydrochloride (0.3% aqueous solution) was the azo component.

The selection of conditions for nitrite anion determination according to Griess revealed that the most reproducible results were obtained when solutions of the compounds under study were refluxed in a mixture of EtOH and a phosphate buffer solution with pH 6.86. The pH value remained unchanged during the reaction, which was confirmed by potentiometry. The data obtained are given in Table 1.

Table 1. Amount (N) of the nitrite anion (% of the theoretical value) evolved by (5-nitropyrimidin-4-yl) dialkyldithiocarbamates **3a—o**

Compound	X	R	R′R″	N (%)
3a	H	OMe	$(CH_2)_4$	8.3
3b	H	OMe	$(CH_2)_5$	11.2
3c	H	OMe	$(CH_2)_6$	16.0
3d	H	OPh	$(CH_2)_4$	11.4
3e	H	NHMe	$(CH_2)_4$	2.2
3f	H	$NHPr^i$	$(CH_2)_4$	2.1
3g	H	NMe_2	$(CH_2)_4$	2.4
3h	H	NEt_2	$(CH_2)_4$	3.3
3i	Me	OMe	$(CH_2)_4$	1.7
3j	Me	OEt	Me_2	2.2
3k	Me	NHMe	MeEt	0.7
3l	Me	NMe_2	Et_2	3.0
3m	Me	NMe_2	$(CH_2)_4$	1.9
3n	Me	NMe_2	$(CH_2)_5$	1.8
3o	Me	NMe_2	$(CH_2)_6$	2.7

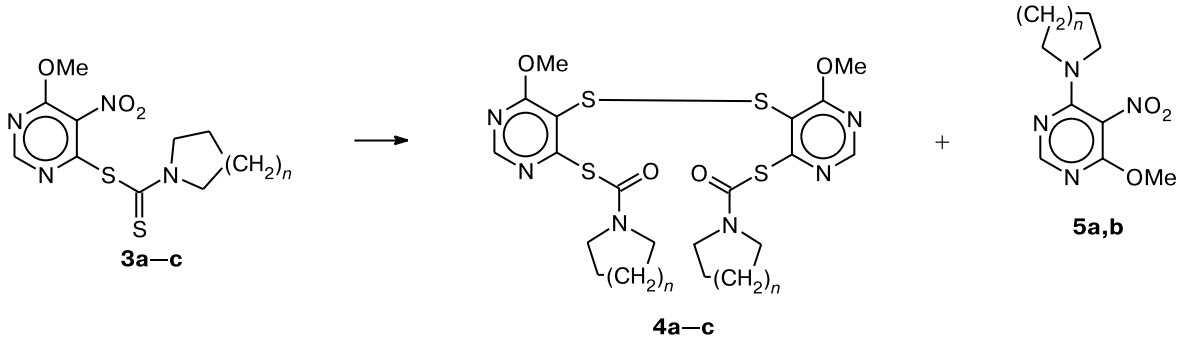
The greatest amount of the detected nitrite anion is observed (see Table 1) for compounds with the phenoxy and methoxy groups at position 6 (**3a—d**), whereas with stronger electron donors (amino groups) instead, this amount decreases.

The process of nitrite anion liberation was studied at the temperature much higher than the physiological temperature. Nevertheless, the compounds under study can be efficient NO sources in the organism, because the process rates and yields of the final products increase multiply under enzymatic catalysis conditions. The single fact of determination of significant amounts of nitrite anions during the degradation of six-membered nitroheterocycles suggests substantially that these compounds can be generators of nitric oxide in living organisms.

Using selected compounds **3** as an example, we showed that their cleavage occurs in two main routes: to form disulfides **4** and derivatives of 4-dialkylamino-5-nitro-6-R-pyrimidines (**5**). Evidently, only the first of these directions is accompanied by the elimination of the nitro group and, correspondingly, formation of the nitrite anion. Therefore, nitric oxide liberation in the living organism should be accompanied, most likely, by the formation of disulfides **4**.

The reaction mixtures formed from compounds **3a—c** with different sizes of the azacycloalkyl substituents upon refluxing in a mixture of a phosphate buffer (pH 6.86) with ethanol were studied by ^{1}H NMR spectroscopy. In the case of the pyrrolidine derivative (**3a**), a mixture of disulfide **4a** and 4-pyrrolidinopyrimidine (**5a**) in a ratio of 3 : 2 (and several nonidentified minor admixtures) is formed. For the piperidine derivative (**3b**), the **4b** : **5b** ratio is 9 : 1 (also with minor admixtures), whereas for the hexahydroazepine-substituted derivative (**3c**) the process

Scheme 3



n = 1 (**a**), 2 (**b**), 3 (**c**)

proceeds toward the formation of disulfide **4c** only with an insignificant amount of admixtures (Scheme 3).

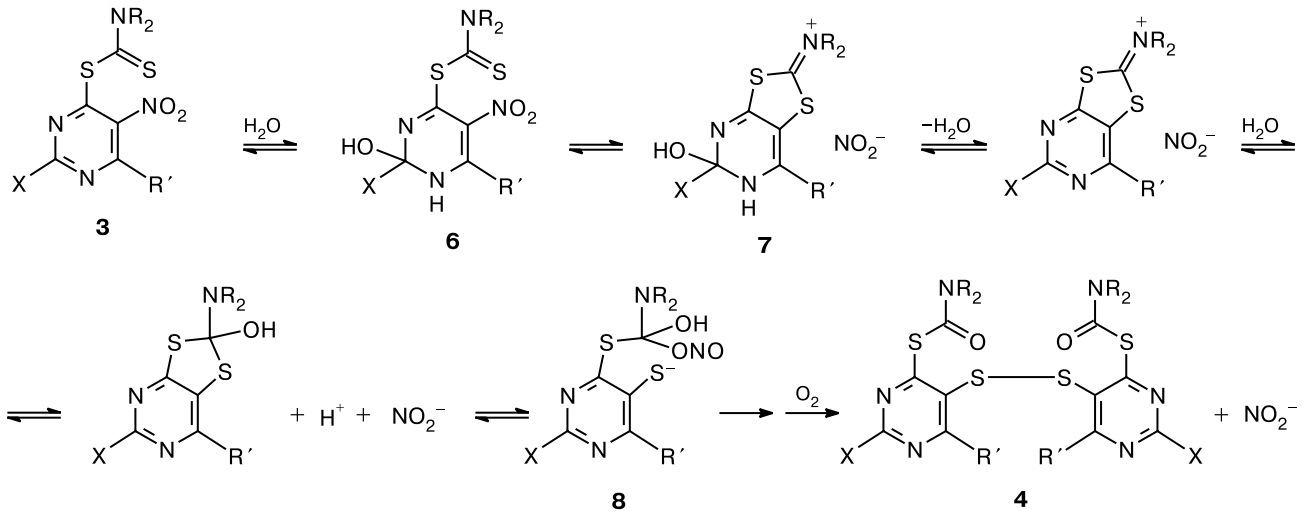
We also found that the yield of nitrite anions decreases on going to compounds containing the methyl substituent instead of a proton in position 2 of the pyrimidine cycle (*cf.* **3a** and **3i**). In addition, it was experimentally found that the nitrite anion is released during hydrolysis only under anaerobic conditions. When the process is conducted in an argon flow, the amount of liberated nitric oxide is beyond the determination limit. Compounds **3a,b** gave aminopyrimidines **5a,b** only, and no disulfides **4a,b** were detected by chromatography. A probable mechanism of the processes is presented in Scheme 4. As in other publications,[25,26] we proposed the reversible transformation of amino(hydroxy)dithiolopyrimidine into the *O*-nitroso compound.

The formation of *O*-nitroso compounds can result (probably, after the oxidation step) in the elimination of NO^+ (see Ref. 25). In turn, this process can cause nitrosation of the thiolate anionic fragment to form *S*-nitrosothiols, which are known[12–14] to be efficient nitric oxide donors.

Probably, the first step of formation of disulfides **4** (see Scheme 4) is the addition of elements of H_2O at positions 1 and 2 of the pyrimidine ring. Such an addition is observed for many reactions involving the pyrimidine ring (see, *e.g.*, Refs 27 and 28). In this case, the function of this addition is ring dearomatization, further favoring the attack of the dithiocarbamate group at position 5 of the ring with nitrite ion rejection. The formed dihydropyrimidine **6** is further transformed into cyclic immonium cation **7** and nitrite anion. These processes are reversible and, finally, the equilibrium shifts due to the irreversible oxidation of intermediate **8** to disulfide **4**. The latter process requires oxygen in the system, which is, as already mentioned, a necessary condition for nitrite ion formation in this reaction.

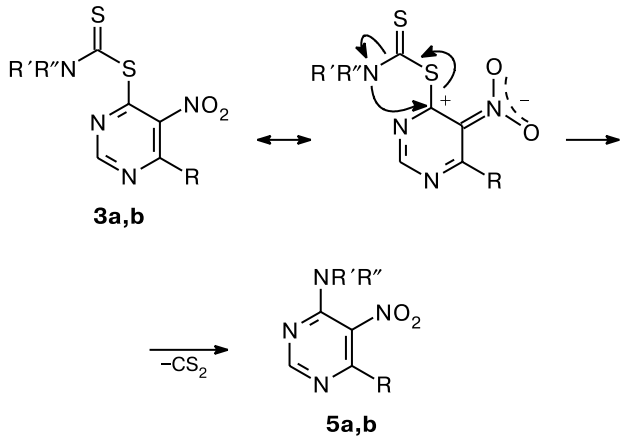
The second process, which occurs under chosen conditions, is the formation of 4-aminopyrimidine derivatives **5a,b** with liberation of carbon disulfide. The latter

Scheme 4

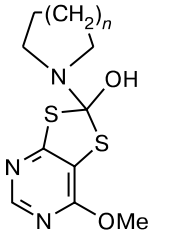


was detected in the reaction mixture by gas liquid chromatography.[23] We have earlier[23] considered this reaction (Scheme 5) based on the nucleophilic attack of the pyrimidine carbon at position 4 by a lone electron pair of the nitrogen atom in the dithiocarbamate moiety.

Scheme 5



Other non-evident facts characterizing this process can also be explained in terms of the schemes presented. For instance, in the series of compounds **9a—c**, hexahydroazepine derivative **9c** is sterically more loaded, resulting in its destabilization (to a certain extent, compared to pyrrolidine and piperidine derivatives **9a,b**) and easier oxidation. As a result, the yield of disulfide and, correspondingly, the yield of nitrite anion upon hydrolytic cleavage of hexahydroazepine derivative **3c** are higher than from compounds **3a** and **3b**.



9

n = 1 (**a**), 2 (**b**), 3 (**c**)

The yield of nitrite ion upon hydrolysis of derivatives **3** containing substituents in position 2 of the pyrimidine ring (**3i—o**) decreases, probably, because pyrimidine ring dearomatization upon the addition of a water molecule is hindered (see Ref. 23).

The processes under discussion produce some amount of nitrite ion, which is detected by the color reaction.[29]

To confirm Scheme 4, we specially synthesized dithiole **9a** by the reduction of disulfide **4a** with $NaBH_4$ (Scheme 6). The structure of dithiole **9a** was proved by mass spectrometry and ^1H and ^{13}C NMR spectroscopy. The ^1H NMR spectrum for a solution in pyridine contains no signal from the proton of the OH group, which is related, most likely, to its fast exchange. The ^1H NMR spectrum in DMSO-d_6 exhibits all expected signals but the signals for the proton of the OH group appears in an unusually low field (δ 13.90), which is probably caused by a strong intermolecular hydrogen bond between the S→O and HO groups. The structure of **9a** was additionally confirmed by the ^{13}C NMR spectrum containing a signal at δ 110 attributed to the sp^3-hybridized C(2) atom.

The reverse process of conversion of dithiole **9a** to the corresponding disulfide occurs easily but only in the presence of sodium nitrite, and a catalytic amount of the nitrite anion is sufficient for the formation of the latter. For instance, 30 min after the beginning of hydrolysis of **3a**, dithiole **9a** (0.1 mol-equiv. with respect to **3a**) was added to the reaction mixture and converted to disulfide **4** (TLC) within a short time (not more than 10 min).

An attempt to oxidize dithiocarbamate **3a** with atmospheric oxygen in the absence of a buffer solution was unsuccessful: only the starting compound and an insignificant amount of admixtures were found in the reaction mixture for 3 days.

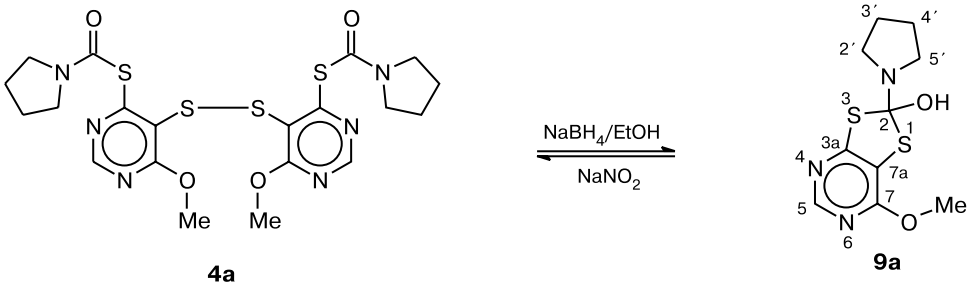
It is known[27] that amide acetals (and thioacetals) in solutions are in equilibrium with the corresponding immonium cations and alkoxy anions, which explains their high reactivity toward nucleophilic reagents (Scheme 7).

It seems probable that dithiole **9** has similar properties, and the scheme of participation of the nitrite anion in the transformation **9** → **4** can appear as follows (Scheme 8).

In this case, the nitrite anion is not consumed and its equimolar amount is not required for the process **9** → **4** to occur.

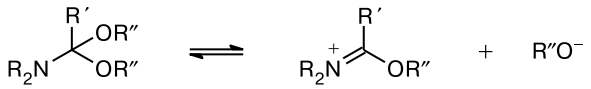
Thus, it is found that nitro derivatives of pyrimidyl dithiocarbamates release the nitrite anion under hydro-

Scheme 6

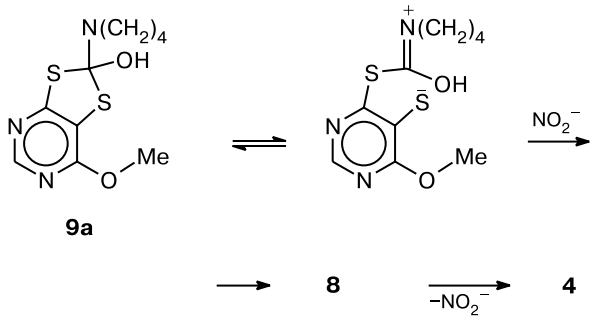


4a **9a**

Scheme 7



Scheme 8



lytic conditions, which indicates, possibly, that these compounds can act as nitrogen(II) oxide precursors in the living organism.

Experimental

NMR spectra were recorded on an Oxford Unity Plus spectrometer (Varian, 400 MHz) using Me_4Si as the internal standard. Mass spectra were obtained on a Finnigan SSQ-700 spectrometer with direct injection of substances into an ion source. IR spectra were recorded on a Perkin—Elmer 2000 FT-IR instrument. Elemental analyses were carried out on a Carlo Erba 5500 analyzer. Melting points were determined on an Electrothermal 9100 instrument (Great Britain). The purity of compounds and the course of reactions were monitored by chromatography on Silicagel 60 F_{254} plates (Merck).

The physicochemical characteristics and elemental analysis data of the compounds synthesized are given in Table 2.

The starting compounds **2a,b**,[28] **2c**,[30] **2d**,[31] **2e**,[32] **2f**,[33] **2g,i,j** (see Ref. 34), and **2h** (see Ref. 35) were synthesized according to known procedures. Sodium dialkyldithiocarbamates (Sigma—Aldrich, USA) were used as received.

4-*N*,*N*-Dialkylthiocarbamoylthio-6-R-5-nitropyrimidines 3a—o. A solution of the corresponding sodium dialkyldithiocarbamate (10.7 mmol) was added to a solution of 4-R-6-chloro-5-nitropyrimidine **2** (10.5 mmol) in EtOH (50 mL), and the mixture was kept for 2—10 h at room temperature. The course of the reaction was monitored by TLC (hexane—acetone (2 : 1) or chloroform—methanol (9 : 1)). The reaction mixture was poured

Table 2. Physicochemical characteristics and elemental analysis data for the synthesized compounds

Compound	Yield (%)	M.p. /°C	Found / Calculated (%)			Molecular formula	Mass spectrum, m/z (I_{rel} (%))
			C	H	N		
3a	74	136—138	40.08 39.99	4.11 4.03	18.67 18.65	$C_{10}H_{12}N_4O_3S_2$	300 $[M]^+$ (7)
3b	63	117—119	41.92 42.02	4.62 4.49	17.81 17.82	$C_{11}H_{14}N_4O_3S_2$	314 $[M]^+$ (8)
3c	71	127—129	43.94 43.89	4.99 4.91	17.23 17.06	$C_{12}H_{16}N_4O_3S_2$	328 $[M]^+$ (8)
3d	84	168—170	49.87 49.71	3.94 3.89	15.55 15.46	$C_{15}H_{14}N_4O_3S_2$	362 $[M]^+$ (6)
3e	32	218—220	40.03 40.12	4.50 4.38	23.92 23.99	$C_{10}H_{13}N_5O_2S_2$	299 $[M]^+$ (29)
3f	85	148—149	44.18 44.02	5.25 5.23	21.47 21.39	$C_{12}H_{17}N_5O_2S_2$	327 $[M]^+$ (32)
3g	45	154—156	42.31 42.16	4.81 4.82	22.42 22.35	$C_{11}H_{15}N_5O_2S_3$	313 $[M]^+$ (4)
3h	67	99—101	45.75 45.73	5.61 5.60	20.51 20.54	$C_{13}H_{19}N_5O_2S_2$	341 $[M]^+$ (25)
3i	81	151—153	42.09 42.02	4.55 4.49	17.87 17.82	$C_{11}H_{14}N_4O_3S_2$	314 $[M]^+$ (8)
3j	76	135—137	39.83 39.72	4.69 4.67	18.66 18.53	$C_{10}H_{14}N_4O_3S_2$	302 $[M]^+$ (19)
3k	78	132—133	39.89 39.85	4.97 5.02	23.18 23.24	$C_{10}H_{15}N_5O_2S_2$	301 $[M]^+$ (17)
3l	34	116—117	43.83 43.75	5.92 5.81	21.09 21.26	$C_{12}H_{19}N_5O_2S_2$	329 $[M]^+$ (12)

(*to be continued*)

Table 2 (*continued*)

Compound	Yield (%)	M.p. /°C	Found / Calculated (%)			Molecular formula	Mass spectrum, m/z (I_{rel} (%))
			C	H	N		
3m	51	139—141	44.02 44.05	5.23 5.26	21.39 21.40	$C_{12}H_{17}N_5O_2S_2$	327 $[M]^+$ (27)
3n	83	109—111	45.70 45.73	5.69 5.61	20.51 20.51	$C_{13}H_{19}N_5O_2S_2$	341 $[M]^+$ (36)
3o	71	103—105	47.21 47.30	6.03 5.95	19.84 19.70	$C_{14}H_{21}N_5O_2S_2$	355 $[M]^+$ (18)
4a	40	201—203	44.61 44.43	4.41 4.47	15.63 15.54	$C_{20}H_{24}N_6O_4S_4$	540 $[M]^+$ (2)
4b	13	148—150	46.32 46.46	4.87 4.96	17.91 17.78	$C_{22}H_{28}N_6O_4S_4$	568 $[M]^+$ (4)
4c	25	174—176	48.39 48.30	5.41 5.40	14.07 14.08	$C_{24}H_{32}N_6O_4S_4$	596 $[M]^+$ (7)
5a	50	121—123	48.16 48.21	5.34 5.39	25.06 24.99	$C_9H_{12}N_4O_3$	224 $[M]^+$ (29)
5b	12	132—134	50.32 50.41	6.03 5.92	23.64 23.52	$C_{10}H_{14}N_4O_3$	238 $[M]^+$ (37)
9a	89	187—189	44.26 44.27	4.83 4.79	15.48 15.53	$C_{10}H_{13}N_3O_2S_2$	271 $[M]^+$ (88)

into water, and pyrimidine **3** was filtered off as a bright yellow (**3e—h,k—o**) or white (**3a—d,i,j**) precipitate. Compounds **3a—o** were recrystallized from EtOH.

Bis(4-*N*,*N*-dialkylcarbamoylthio-6-methoxypyrimidin-5-yl) disulfides 4a—c. A solution of 4-*N*,*N*-dialkylthiocarbamoylthio-6-methoxy-5-nitropyrimidine **3a—c** (5.39 mmol) in a mixture of ethanol (40 mL) and a 0.025 *M* phosphate buffer solution, pH 6.86, (10 mL) was refluxed for 12 h. After the mixture of solvents was evaporated, an oily residue was treated with water (20 mL) and triturated. A white crystalline product was filtered off and recrystallized from isopropyl alcohol.

Bis(4-pyrrolidinocarbonylthio-6-methoxypyrimidin-5-yl) disulfide (4a). Mass spectrum, m/z (I_{rel} (%)): 540 $[M]^+$ (2), 411 $[M - SCON(CH_2)_4]^+$ (7), 271 $[1/2\ M + H]^+$ (32), 238 $[1/2\ M - S]^+$ (52), 98 $[O{=}C{=}N^+(CH_2)_4]^+$ (100), 70 $[N^+(CH_2)_4]^+$ (100). IR (KBr), ν/cm^{-1}: 1694 (C=O); 1550, 1395, 1352, 1016.

Bis(4-piperidinocarbonylthio-6-methoxypyrimidin-5-yl) disulfide (4b). Mass spectrum, m/z (I_{rel} (%)): 568 $[M]^+$ (4), 456 $[M - CON(CH_2)_5]^+$ (4), 424 $[M - SCON(CH_2)_5]^+$ (12), 285 $[1/2\ M + H]^+$ (24), 252 $[1/2\ M - S]^+$ (40), 112 $[O{=}C{=}N^+(CH_2)_5]^+$ (100), 84 $[N^+(CH_2)_5]^+$ (65). IR (KBr), ν/cm^{-1}: 1687 (C=O); 1543, 1400, 1207, 1021.

Bis(4-hexahydroazepinocarbonylthio-6-methoxypyrimidin-5-yl) disulfide (4c). Mass spectrum, m/z (I_{rel} (%)): 596 $[M]^+$ (7), 470 $[M - CON(CH_2)_6]^+$ (9), 438 $[M - SCON(CH_2)_6]^+$ (7), 299 $[1/2\ M + H]^+$ (37), 266 $[1/2\ M - S]^+$ (43), 126 $[O{=}C{=}N^+(CH_2)_6]^+$ (100), 98 $[N^+(CH_2)_6]^+$ (20). IR (KBr), ν/cm^{-1}: 1673 (C=O); 1532, 1402, 1162, 1035.

6-Methoxy-5-nitro-4-pyrrolidinopyrimidine (5a). A solution of compound **3a** (1.7 g, 5.39 mmol) in a mixture of ethanol (40 mL) and a buffer solution, pH 6.86, (10 mL) was refluxed for 12 h under argon and concentrated. The oily residue was treated with acetone (40 mL) and filtered. The mother liquor was concentrated and the residue was crystallized from ethanol.

6-Methoxy-5-nitro-4-piperidinopyrimidine (5b) was synthesized similarly to pyrimidine **5a**.

2-Hydroxy-7-methoxy-2-pyrrolidino-1,3-dithiolo[4,5-*d*]pyrimidine (9a). Sodium borohydride (0.18 g, 4.73 mmol) was added to a solution of disulfide **4a** (0.7 g, 1.29 mmol) in ethanol (30 mL), and the resulting suspension was kept for 2.5 h at room temperature with vigorous stirring. The reaction mixture was diluted with water (30 mL), acidified with 10% HCl to pH 4, and left for 16 h at 6—8 °C. A white precipitate of dithiolopyrimidine **9a** (0.61 g) was filtered off and recrystallized from ethanol. Mass spectrum, m/z (I_{rel} (%)): 271 $[M]^+$ (19), 200 $[M - HN(CH_2)_4]^+$ (22), 172 $[M - COHN(CH_2)_4]^+$ (86), 108 $[M - SSCON(CH_2)_4]^+$ (100). IR (KBr), ν/cm^{-1}: 3600—3200, 3112 (HO assoc.); 1612, 1573, 1500. ^1H NMR (DMSO-d_6), δ: 1.89 (br.s, 4 H, CH_2CH_2); 3.19 (br.s, 4 H, $N(CH_2)_2$); 3.93 (s, 3 H, OMe); 8.29 (s, 1 H, H of pyrimidine); 13,90 (br.s, 1 H, OH). ^{13}C NMR (DMSO-d_6), δ: 24.0 and 24.9 (C(3′), (C(4′)); 45.8, 46.9 (C(2′), (C(5′)); 55.4 (OMe); 110.0 (C(2)); 149.9 (C(5)); 160.2 (C(7)); 168.0 (C(7a)); 183.3 (C(3a)).

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