SYNTHESIS AND BIOLOGICAL PROPERTIES OF 4-PHENYL-AMINO- AND 4-DIMETHYLAMINO-3-CYANOPYRIDINE-2-THIONES AND THE THIENO[2,3-b]PYRIDINES OBTAINED FROM THEM

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The synthesis of new sulfur-containing derivatives of 4-aminopyridines has been undertaken in continuation of our investigations on the synthesis and study of biologically active compounds of this type of heterocycle.

Initially 4-phenylamino-3-cyanopyridine-2-thione (I) was synthesized by a method developed previously for obtaining 4-amino substituted pyridine-2-thiones [2]. Cyanothioacetamide was taken as starting material and was reacted with the diethyl acetal of dimethylacetamide to give α-cyano-β-dimethylaminocrotonic acid thioamide (III) in good yield. The transamination of enamine (III) with aniline in acetic acid medium enabled the previously undescribed examine (IV) to be synthesized. The reaction of compound (IV) with an excess of the acetal of dimethylformamide (DMF) led to the pyridine intermediate (V) which was treated with aqueous alkali without isolation. Ring opening was accompanied by subsequent recyclization with the formation of the desired pyridinethione (I).

Another approach to the synthesis of pyridine-2-thiones is by the reaction of 2-chloro-3-cyanopyridines (VIa, b) with thiourea. The reaction was carried out in boiling toluene. After treatment of the intermediate isothiouronium salt with aqueous alkali the 4-phenylamino- and 4-dimethylaminopyridinethiones (I) and (VII) were obtained in yields of 63 and 72%, respectively.

Of the two existing syntheses of 4-amino substituted 3-cyanopyridine-2-thiones the direct replacement of a chlorine atom by a thione grouping is preferable since it uses thiourea, a sulfur-containing synthetic equivalent, which is more available than cyanothioacetamide.

Numerous examples are known in the literature of the use of 3-cyanopyridine-2-thiones in syntheses of derivatives of 3-aminothieno[2,3-b]pyridine [1], however 4-amino substituted 3-cyanopyridine-2-thiones have not as yet been used for this. Compounds (I) and (II) were alkylated with chloroacetic acid derivatives (ethyl ester, carbamide, nitrile) and with phenacyl and 4-nitrophenacyl bromides with the aim of synthesizing 4-phenylamino- and 4-dimethylaminothieno[2,3-b]pyridines. As a rule 3-cyanopyridine-2-thiones are used in alkylation reactions as the potassium salts in a medium of aqueous DMF. The S-alkylation reaction is most frequently accompanied by a spontaneous Torp–Ziegler cyclization with the formation of 3-aminothieno[2,3-b]pyridine derivatives [1]. All the alkylation reactions of the potassium salts of 3-cyanopyridine-2-thiones (I, VII) were carried out under standard conditions (aqueous DMF at 5-7°C) in the present work with the aim of studying the effect of the nature of the amino substituent at the 4 position of the pyridine ring. It was established that in the case of the 4-phenylaminopyridine-2-thione (I) the intermediate S-alkyl derivative (like VIII) is isolated under these conditions only in the case of R^3 = COOEt (VIIIa). In the remaining examples the alkylation is accompanied by a Torp–Ziegler cyclization with the formation of condensed thiophenes. The alkylation of 4-dimethylaminopyridine-2-thione (VII) under similar conditions gave the Torp–Ziegler cyclization product only in the case of R^3 = CN and COPh (IXh, i), i.e., the process of thiophene cyclization is slower when using (VII) compared to (I) which enables the pyridine intermediate (VIIIc, d) to be isolated. Such a difference in behavior of the intermediate S-alkyl derivatives is explained by the significantly larger electron-donating effect of the dimethylamino group compared with the aniline fragment in position 4 of the pyridine ring. It is understandable that such a positive mesomeric effect leads to an increase in the electron density on the carbon of the cyano group and correspondingly to a reduction of its ability

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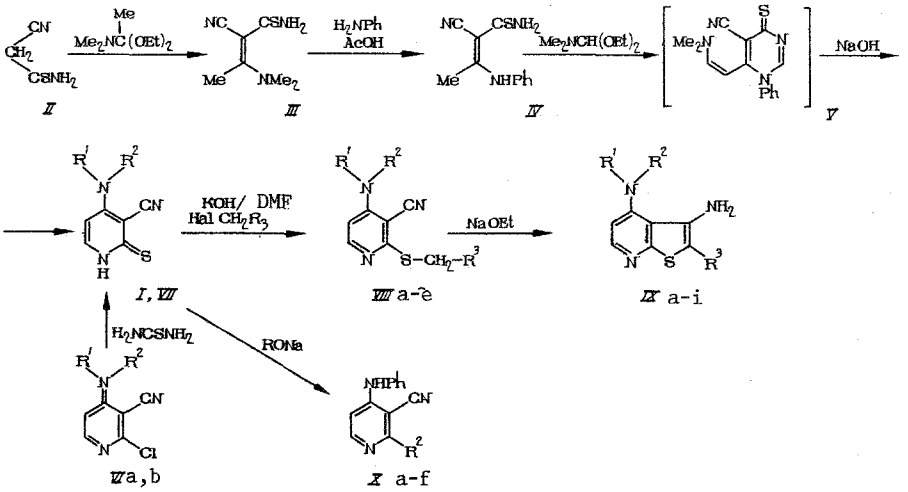
to condense at the anionic CH-fragment which is characteristic of the Torp–Ziegler reaction. The use of an equimolar quantity of alkali to obtain the potassium salts of the pyridinethiones (I, VII) enables the process to be stopped at the stage of forming the S-alkylated compounds (VIII) even when the Torp–Ziegler cyclization usually proceeds at the highest rate. Even the most readily cyclizable S-cyanomethyl derivative (VIIIb) was successfully isolated in this way. Several other 2-methylthio-3-cyano-pyridines (VIIIa-d) were isolated under the usual conditions and these compounds were converted by Torp–Ziegler with alcoholic sodium ethylate into the corresponding 4-amino derivatives of thieno[2,4-b]pyridine (IX).



R^1=H(I, VIa VIIIa, b, IXa-e), CH_3(VIb VII, VIIIc-e IXf-i); R^2=Ph(I, VIa VIIIa, b IXa-e), CH_3(VIb, VII, VIIIc-e, IXf-i); R^3=COOEt(VIIIa, c, IXa, f), CN(VIIIb, IXb, h), $CONH_2$(VIIId IXc, g), COC_6H_4NO-p(VIIIe IXd, i).

The methyl- and benzylthio derivatives (Xa, b) were synthesized under the conditions described above for S-alkylation with the aim of obtaining new derivatives of 3-cyano-4-phenylaminopyridine. In addition the 2-phenoxy and 2-methoxy substituted pyridines (Xc, d) were synthesized by the interaction of 2-chloro-3-cyanopyridine (VIa) with sodium phenolate or methylate. The physicochemical properties of the compounds synthesized are given in Table 1.

EXPERIMENTAL (CHEMICAL)

The mass spectra of the compounds synthesized were obtained on a MAT 112 spectrometer, ionizing voltage 50 eV, ionization chamber temperature 140°C. The ^{1}H NMR spectra were obtained on an XL 200 spectrometer, internal standard was TMS. Melting points were determined on a Boetius hot stage.

The values of elemental analyses found corresponded with calculated values.

α-Cyano-β-dimethylaminocrotonamide (III). The diethyl acetal of DMF (1.93 g: 12 mmoles) was added to a suspension of cyanothioacetamide (1.0 g: 10 mmoles) in acetonitrile (5 ml) at 20°C. The mixture was stirred for 1 h, cooled, and compound (III) (1.3 g) was filtered off.

α-Cyano-β-phenylaminocrotonamide (IV). Aniline (1.08 g: 11.5 mmoles) was added to a mixture of compound (III) (1.52 g: 9 mmoles) and glacial acetic acid (3 ml) and heated at 80°C for 1 h. The reaction mixture was cooled, and examine (IV) (1.55 g) filtered off.

3-Cyano-4-phenylamino-1,2-dihydropyridine-2-thione (I). Method A. The acetal of DMF (3.09 g: 21 mmoles) was added to a solution of compound (IV) (1.52 g: 7 mmoles) in toluene (10 ml). The reaction mixture was boiled for 2 h, evaporated in vacuum, 10% NaOH (10 ml) added to the residue, and the mixture heated at 70°C for 10 min. After cooling the solution was acidified to pH 7 and pyridinethione (I) (1.2 g) filtered off.

Method B. Thiourea (9.9 g: 13 mmoles) was added to a solution of the chloropyridine (VIa) (2.3 g: 10 mmoles) in toluene (30 ml). The reaction mixture was boiled for 4 h, cooled to 60°C, ethanol (50 ml) added, and boiled for 30 min. The precipitated solid was filtered off and washed with ethanol. Purification was carried out by reprecipitation through the Na salt or by crystallization from aqueous DMF. M^+ 227. ^{1}H NMR (DMF_d): 6.29 (1H, d, 5-CH), 7.29-7.44 (5H, m, Ph), 7.49 (1H, d, 6-CH), 9.25 (1H, s, PhNH), 12.61 (1H, br.s, 1-NH).

TABLE 1. Physicochemical Properties of the Compounds Synthesized

Compound	Yield, %	mp, °C (solvent)	Empirical formula	IR spectrum, λ_{max}, cm^{-1}		
				CO	CN	NH, NH_2
I	76*	279—82 (aq. DMF)	$C_{12}H_9N_3S$	—	2210	3130 br., 3350
III	76	148—51 (acetonitrile)	$C_7H_{11}N_2S$	—	2180	3110, 3240, 3330
IV	79	171—2 (acetonitrile)	$C_{11}H_{11}N_3S$	—	2180	3180, 3280, 3340
VII	60	238—42 (aq. DMF)	$C_8H_9N_3S$	—	2180	3310
VIII a	86	107—8 (aq. alcohol)	$C_{16}H_{15}N_3SO_2$	1745	2210	3310
VIII b	69	175—7 (propan-2-ol)	$C_{14}H_{10}N_4S$	—	2210	3320
VIII c	77	93—4, (hexane–benzene)	$C_{12}H_{15}N_3SO_2$	1745	2190	—
VIII d	85	213—6 (aq. DMF)	$C_{10}H_{12}N_4SO$	1670	2195	3150, 3380
VIII e	56	157—60 (aq. DMF)	$C_{16}H_{14}N_4SO$	1690	2195	—
IX a	89	174—5 (acetontrile)	$C_{16}H_{15}N_3SO_2$	1670	—	3170, 3280, 3350
IX b	98	256—9 (acetonitrile–DMF)	$C_{11}H_{10}N_4S$	—	2180	3150, 3340, 3440
IX c	87	230—2 (aq. DMF)	$C_{14}H_{12}N_4SO$	1670	—	3100 br., 3260, 3340
IX d	63	236—8 (aq. DMF)	$C_{20}H_{15}N_3SO$	1560—60		3180, 3270, 3300 br.
IX e	85	281—3 (DMF)	$C_{20}H_{14}N_4SO_3$	1575 br.		3320, 3280, 3420
IX f	86	86—8 (aq. alcohol)	$C_{12}H_{11}N_3SO_2$	1670	—	3340, 3450
IX g	85	204—6 (aq. DMF)	$C_{10}H_{12}N_4SO$	1670		3120, 3280 br.
IX h	95	227—9 (acetonitrile)	$C_{10}H_{10}N_4S$	—	2180	3190, 3320, 3430
IX i	96	174—5 (acetonitrile)	$C_{16}H_{15}N_3SO$	1560—80	—	3220, 3340
X a	84	161—3 (toluene)	$C_{13}H_{15}N_3S$	—	2210	3280
X b	83	131—3 (tolüene)	$C_{13}H_{11}N_3S$	—	2210	3280
X c	76	157—8 (methanol)	$C_{13}H_{13}N_3O$	—	2220	3280
X d	92	155—7 (acetonitrile)	$C_{13}H_{11}N_3O$	—	2220	3280

*By method A (method B, 69% yield).

3-Cyano-4-dimethylamino-1,2-dihydropyridine-2-thione (VII). Obtained in an analogous manner to compound (I) (method B).

General Method of Synthesis of 2-alkylthio-3-cyano-pyridines (VIIIa-e, Xa, b) and 4-Phenylamino- and 4-Dimethylamino-3-amino-2R-thieno[2,3-b]pyridines (IXa-i). A solution of KOH (12 mmoles) in water (2 ml) was added to a suspension of the initial pyridinethione (10 mmoles) in DMF (5 ml), the mixture stirred until complete solution, and cooled to 5°C. The alkylating agent (10 mmoles) (if solid in DMF solution) was added, the solution kept for 15 min, diluted with water, and the solid filtered off.

The intermediate functionally substituted 3-cyano-2-methylthiopyridines (VIIIa-e) were cyclized by boiling in alcoholic NaOEt solution.

3-Cyano-4-phenylamino-2-phenoxypyridine (Xc). A mixture of the chloropyridine (VIa) (2.3 g: 10 mmoles), K_2CO_3 (2.76 g: 20 mmoles), phenol (1.13 g: 12 mmoles), and DMF (40 ml) was boiled with stirring for 4 h, the reaction mixture filtered, and the filtrate evaporated in vacuum. The residue was crystallized from acetonitrile.

3-Cyano-2-methoxy-4-phenylaminopyridine (Xd). The chloropyridine (VIa) (2.3 g: 10 mmoles) was added to a solution of MeONa prepared from Na (1.15 g: 50 mmoles) and methanol (30 ml). The reaction mixture was heated in an autoclave at 150°C for 10 h, evaporated in vacuum, the residue washed with water, and crystallized from methanol.

EXPERIMENTAL (BIOLOGICAL)

Investigation of the antiviral activity of the compounds synthesized [3] showed that only one of them (IXg) displayed weak virus-inhibiting action toward Herpes simplex virus in a culture of chick embryo fibroblasts and reduced the infection titer of the virus to 1.0 log TCD when used at a concentration of 10 μg/ml.

In view of the fact that 4-aminopyridine derivatives possess antihypertensive and analgesic activity [5], compounds (IXa-c) were studied for acute toxicity and effect on arterial blood pressure (AP) following administration intravenously and intragastrically and for analgesic activity.

Methods of Investigation. 1) The acute toxicity of compounds was determined in random-bred white male mice of weight 18-20 g on intraperitoneal administration. The LD_{50} was calculated according to the method in [9].

2) The effect of compounds on AP was studied in spontaneously hypertensive (SH) male rats of weight 250-280 g anesthetized with urethane (1 g/kg, intraperitoneally) by directly recording AP in the left common carotid artery of animals with

TABLE 2. Effect of 3-Cyano-4-phenylaminopyridine Derivatives on the Threshold of Pain Sensitivity (TPS) in Mice in Various Nociceptor Stimulation Models**

Compound	Change of TPS, % control		% spasm suppresion
	hot plate	tail flick	
I	+22*	+10	−18*
Xa	+13*	+7	−15*
Xb	0	0	0
Xc	+21*	+24*	−35*
Analgin	+25*	+20*	−50*

*Significantly different from control $p < 0.05$.
**Intragastric dose 50 mg/kg.

a Trantec (USA) pressure sensor and a Gemini 7070 (Ugo Basile, Italy) automatic recorder. Compounds were administered through a catheter in the jugular vein at doses of 0.1, 0.5, 1.0, and 2.0 mg/kg.

3) The effect of compounds on AP was studied following intragastric administration to unanesthetized SH male rats. Recording of AP was carried out by an indirect method in the caudal artery of animals using a set of equipment from IITC (USA) [6]. Compounds were introduced into the stomach with a probe at doses of 25 and 50 mg/kg. The AP was measured before administration of the compound (initial level) and at 1, 2, and 3 h after.

4) The analgesic activity of compounds was studied in the hot plate [10] and tail flick [8] models and in a chemical pain stimulation model (spasms caused by intraperitoneal administration of 1% acetic acid [7]) in mice.

Compounds were administered intragastrically at a dose of 50 mg/kg 1 h before application of the pain stimulus. Analgin (at the same dose) was used as reference preparation.

Statistical processing of the experimental results was carried out by determining values of the arithmetic mean and the standard error of the mean. Values were compared using the Student t test.

Results. It was shown on determining the acute toxicity of compounds that their LD_{50} values (intragastrically) exceeded 1000 mg/kg and these derivatives therefore are weakly toxic according to Sidorov's classification [4].

By studying the effect of the 3-cyano-4-phenylaminopyridine derivatives on AP in anesthetized SH rats it was established that on intravenous injection the compounds caused a transient (15-20 min) reduction in AP, the size and duration of the effect depended on the dose.

Compound (Xc) was the most active. Its hypotensive effect was observed beginning at 0.5 mg/kg (reduction in AP 15 mm Hg) and increasing with increasing dose. The ΔAP was 20 and 25 mm Hg respectively for doses of 1.0 and 2.0 mg/kg intravenously. Compound (I) was less active, compound (Xa) caused a transient fall in AP at the highest dose used (2 mg/kg) and compound (Xb) showed no effect over the dose range examined.

When studying the effect of compounds on AP in unanesthetized SH rats by intragastric administration it was established that compounds (I) and (Xc) at a dose of 50 mg/kg caused a fall in AP (by 15-20 mm Hg relative to the initial level). The antihypertensive effect of the compounds reached a maximum 1 h after intragastric administration and continued for 2 h. Compounds (Xb) and (Xa) did not show a reliable effect on the AP of SH rats in this series of experiments.

The results of investigating the analgesic activity of compounds (I) and (Xa-c) are given in Table 2.

As is seen from the data, compounds (I) and (Xa) show an analgesic effect in the hot plate and spasm models. Compound (I) caused a large inhibition of the nociceptor reaction and compound (Xa) was somewhat less active. Compound (Xc) was the most active among the 3-cyano-4-phenylaminopyridine derivatives represented. It caused an analgesic effect in all three models of pain stimulation in mice on intragastric administration at 50 mg/kg and was comparable in activity to the reference preparation (analgin). Compound (Xb) was inactive in these experiments.

On analysis of the chemical structure–biological effects it may be noted that in spite of the small number of compounds the presence of a methylthio group in the 2 position of pyridine leads to the disappearance of antihypertensive and analgesic activity in compound (Xb) compared to (I). The introduction of a phenoxy group into the 2 position of pyridine causes the appearance in compound (Xc) of weakly expressed antihypertensive properties and analgesic activity.

Among the derivatives of 3-cyano-4-phenylaminopyridine represented the compound (Xc) is the most interesting from a pharmacological point of view since it possesses the elements of antihypertensive activity and analgesic action comparable with but not exceeding the analgesic effect of analgin.

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