Novel anchored nitrogen-containing heterocycles of potential biological activity from 2,3-dioxobutanethioanilide 2-arylhydrazone

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The synthesis of arylhydrazono derivatives of different nitrogen-containing heterocycles from 2,3-dioxobutanethioanilide 2-arylhydrazone was described. The structures assigned to the products were substantiated by spectroscopic studies. The antibacterial and antifungal activities for the prepared compounds were determined. Additionally, the antitumour activity together with the toxicological effect of some of the prepared compounds were studied.

Описано получение арилгидразоно-производных различных азотсодержащих гетероциклов из 2,3-диоксобутантиоанилида 2-арилгидразона. Строение, приписанное полученным соединениям, было обосновано на основе спектроскопических исследований. Определены антибактериальная и противогрибковая активности синтезированных соединений. Кроме того, изучалась противоопухолевая активность и токсическое действие некоторых полученных соединений.

Sulfur-containing compounds are particularly known for their effectiveness against bacteria and fungi. From another point of view, many dyes have proved to be of great value in medicine, especially those used for diagnostic purposes [1] or as agents for treatment of protozoal diseases.

On the basis of these findings and prompted by the fact that antitumour activity was found to be associated with numerous heterocyclic moieties such as pyrazolones [2], pyrimidinones [3—5], and diazepinone derivatives [6], it seems of interest to use the thio-analogue of 2,3-dioxobutananilide 2-arylhydrazone *II* as a versatile intermediate for synthesis of different heterocyclic moieties that might have less toxic and high potent biological activities. The antibacterial, antifungal, and antitumour activities of the prepared compounds were assessed.

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Experimental

All melting points are uncorrected. Infrared spectra were determined using KBr wafer technique on a Unicam SP 2000 spectrophotometer. ^{1}H NMR spectra ($CDCl_3$) were recorded on a Varian model T-60 spectrometer using tetramethylsilane as internal standard.

2,3-Dioxobutanethioanilide 2-arylhydrazone IIa—IIi

To a cold solution of 3-oxobutanethioanilide *IV* (0.01 mol) in ethanol (50 cm^3), sodium acetate trihydrate (0.01 mol) was added and the mixture was stirred for 15 min. The appropriate freshly prepared arenediazonium salt (0.01 mol) was added gradually with stirring. The solid product *II* that separated was filtered and recrystallized from ethanol in 40—82 % yield (Table 1). IR spectra of these products exhibited absorption bands at $\tilde{\nu}/cm^{-1}$: 3460, 3170 ν(NHC$_6$H$_5$ or NH—N=C), 1665 ν(COCH$_3$), 1570 ν(C=N), 1120 ν(C=S).

5-Arylhydrazono-4-methyl-6-phenylamino-2,5-dihydropyrimidine-2-thione Va—Vc

A mixture of *II* (0.01 mol) and thiourea (0.01 mol) in absolute methanol (30 cm^3) was heated under reflux for 8 h in the presence of 3 % solution of potassium methoxide and left to cool. The product that separated on acidification with dilute acetic acid was filtered off, washed with water and recrystallized from ethanol in 70—80 % yield. The products obtained together with their physical data are listed in Table 1. IR spectrum of *Va* exhibited absorption bands at $\tilde{\nu}/cm^{-1}$: 3500, 3200 ν(NHC$_6$H$_5$ or NHN=C), 1390, 1535 ν(C=S) [7], 1625, 1600 ν(*exo* and *endo* C=N). ^{1}H NMR spectrum of the same compound revealed signals at δ/ppm: 7.3—8.1 (9H, m, H$_{arom}$), 3.7 (3H, s, OCH$_3$), 3.25 (1H, s, =N—NH), 2.5 (1H, s, NHC$_6$H$_5$), 2.3 (3H, s, C—CH$_3$).

4-Arylazo-1-carbamoyl-3-methyl-5-phenylaminopyrazole VIa—VId

To a mixture of *II* (0.005 mol) and semicarbazide hydrochloride (0.006 mol) in ethanol (30 cm^3) sodium acetate trihydrate (0.015 mol) was added. The mixture was refluxed for 3 h and cooled. The product that precipitated was collected, washed with water and recrystallized from ethanol to give the arylazo derivatives *VIa—VId* in 80 % average yield. Physical properties of these compounds are listed in Table 1. IR spectrum of *VIa* as a representative example exhibited absorption bands at $\tilde{\nu}/cm^{-1}$: 3250—3460 ν(CONH$_2$ or NHC$_6$H$_5$), 1690 ν(amide Ist band), and 1620 ν(C=N).

Table I

Characterization of compounds *II*, *V*—*IX*

Compound	Formula M_r	w_i(calc.)/% w_i(found)/%					M. p.	Colour
		C	H	N	S	Cl	°C	
IIa							112	Orange[a]
IIb	$C_{16}H_{14}N_4O_3S$	56.13	4.12	16.37	9.36		115	Yellow
	342.36	56.34	4.07	16.23	9.58			
IIc	$C_{16}H_{14}N_3OSCl$	57.91	4.25	12.66	9.66	10.69	90	Yellow
	331.81	57.73	4.21	12.35	9.52	10.45		
IId							104	Orange[b]
IIe	$C_{16}H_{14}N_3OSCl$	57.91	4.25	12.66	9.66	10.69	133	Green
	331.81	58.16	4.40	12.48	9.38	10.78		
IIf	$C_{17}H_{17}N_3O_2S$	62.36	5.24	12.83	9.79		138	Brown
	327.39	62.30	5.36	12.64	9.98			
IIg	$C_{17}H_{17}N_3OS$	65.57	5.50	13.49	10.30		120	Yellow
	311.39	65.29	5.58	13.61	10.59			
IIh	$C_{16}H_{14}N_4O_3S$	56.13	4.12	16.37	9.36		168	Yellow
	342.36	56.29	4.26	16.55	9.72			
IIi	$C_{17}H_{15}N_3O_3S$	59.81	4.43	12.31	9.39		225	Red
	341.37	60.04	4.52	12.44	8.92			
Va	$C_{18}H_{17}N_5OS$	61.52	4.88	19.93	9.12		75	Yellow
	351.42	61.38	5.02	19.58	9.27			
Vb	$C_{17}H_{14}N_6O_2S$	55.72	3.85	22.94	8.75		108	Orange
	366.39	55.59	4.00	22.68	8.66			
Vc	$C_{17}H_{14}N_5SCl$	57.38	3.97	19.69	9.01	9.97	132	Yellow
	355.84	57.61	4.11	19.34	8.84	10.19		
VIa	$C_{17}H_{16}N_6O$	63.73	5.04	26.23			232	Orange
	320.35	63.95	4.91	25.91				
VIb	$C_{17}H_{15}N_7O_3$	55.88	4.14	26.84			140	Yellow
	365.35	56.00	4.31	26.97				
VIc	$C_{17}H_{15}N_6OCl$	57.55	4.26	23.69		9.99	148	Orange
	354.80	57.68	4.41	23.95		10.23		
VId	$C_{18}H_{16}N_6O_3$	59.33	4.43	23.07			> 300	Red
	364.36	59.44	4.49	22.88				
VIIIa							219	Yellow[c]
VIIIb	$C_{16}H_{14}N_5Cl$	61.64	4.53	22.47		11.37	72	Yellow
	311.77	61.60	4.63	22.61		11.15		
VIIIc	$C_{17}H_{17}N_5$	70.08	5.88	24.04			235	Yellow
	291.35	70.29	5.97	23.89				
IX	$C_{22}H_{19}N_5$	74.76	5.42	19.82			220	Brown
	353.41	74.81	5.52	20.03				

Ref. [7] gives *a*) m. p. = 110 °C; *b*) m. p. = 104 °C; *c*) m. p. = 219 °C.

4-Arylhydrazono-3-methyl-5-phenylamino-4H-pyrazole VIIIa—VIIIc

A mixture of *II* (0.01 mol) and hydrazine hydrate (0.01 mol) in absolute ethanol (25 cm^3) was refluxed for 6 h and cooled. Dilution of the reaction mixture with water afforded the required products, which were filtered off and recrystallized from 70 % aqueous solution of ethanol in 40—55 % yield (Table 1). IR spectrum of *VIIIa* revealed absorption bands at $\tilde{\nu}/cm^{-1}$: 3500, 3210 ν(NHC$_6$H$_5$ or =N—NH), 1610, 1590 ν(*exo* and *endo* C=N). ^{1}H NMR spectrum of *VIIIb* revealed signals at δ/ppm: 7.2—7.8 (9H, m, H_{arom}), 3.3 (1H, br, NHN=C), 2.5 (1H, br, NHC$_6$H$_5$), and 2.25 (3H, s, CH_3).

2-Methyl-4-phenylamino-3-phenylhydrazono-3H-benzo[b]-1,5-diazepin (IX)

A mixture of *II* (0.01 mol) and *o*-phenylenediamine (0.01 mol) in glacial acetic acid (20 cm^3) was refluxed for 30 min and filtered while hot. The brownish solid that separated on cooling was filtered off and recrystallized from acetic acid to give the product in 68 % yield. IR spectrum exhibited absorption bands at $\tilde{\nu}/cm^{-1}$: 3520, 3180 ν(NHC$_6$H$_5$ or =N—NH), 1630, 1590 ν(*exo* and *endo* C=N). ^{1}H NMR spectrum revealed signals at δ/ppm: 7.25—7.7 (14H, m, H_{arom}), 3.4 (1H, s, NH—N=C), 2.7 (1H, s, NHC$_6$H$_5$), and 2.55 (3H, s, CH_3).

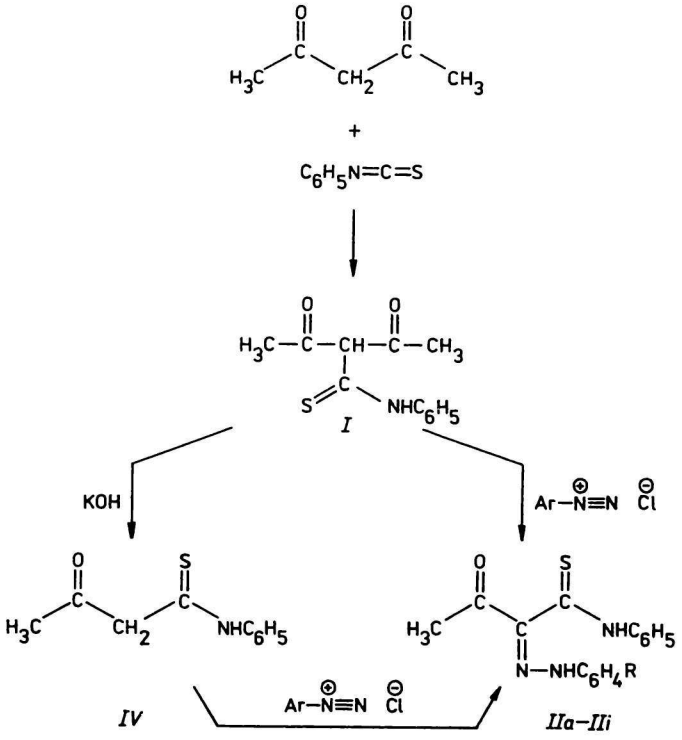
Results and discussion

Synthesis and structure

The preparation of 2,3-dioxobutanethioanilide 2-arylhydrazones (*IIa—IIi*) involves the reaction of 2,4-pentanedione with phenyl isothiocyanate (Scheme 1) followed by coupling of the product with different arenediazonium salts to give the required dyes through Japp—Klingemann reaction. Using this reaction sequence *Amer et al.* [8] reported the synthesis of some of these arylhydrazono derivatives. Recently *Dubenko et al.* [9] reported that coupling of 3-phenylthiocarbamoyl-2,4-pentanedione (*I*) with different arenediazonium salts yielded 3-arylazo-3-phenylthiocarbamoyl-2,4-pentanedione *III*.

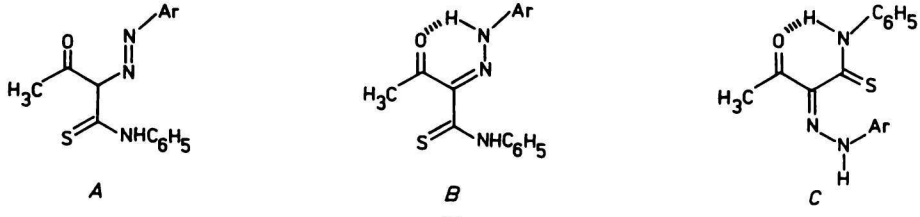
In this investigation, we report the synthesis of a series of *IIa—IIi* (Table 1), through coupling of 3-oxobutanethioanilide (*IV*) — which is readily obtainable [10] *via* treatment of *I* with diluted potassium hydroxide solution at room temperature — with different arenediazonium salt solutions. The products were found to be identical (m. p.'s and mixed m. p.'s) with those obtained by direct coupling of *I* with arenediazonium salts (Scheme 1).

The presence of an absorption band assignable to —C=N group (see Experimental) is considered as an evidence for the existence of these compounds in the hydrazo form *B* rather than the azo form *A*. The low carbonyl absorption may



Scheme 1

be due to both the conjugation and the possible intramolecular hydrogen bonding (*cf.* structures *B* and *C*). The fact that these compounds show evidence for intramolecular hydrogen bonding is in favour of the hydrazone structure.

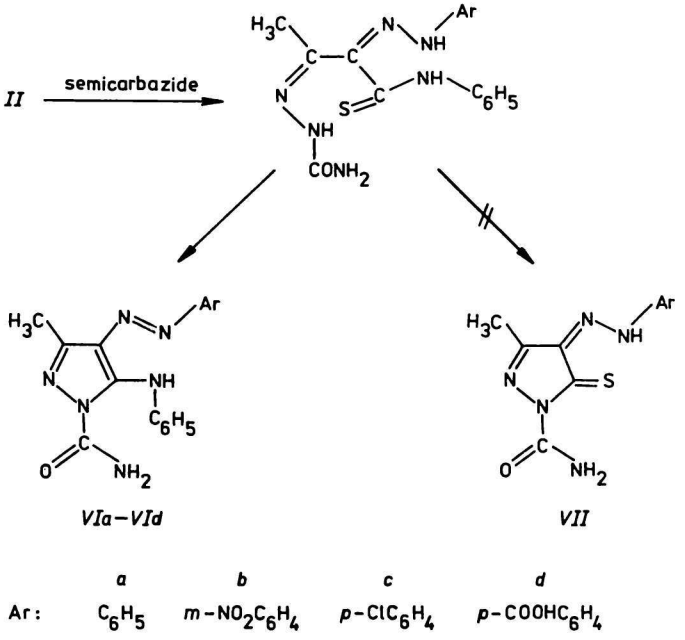


A *B* *C*

II

Treatment of *II* with thiourea in methanolic potassium methoxide solution at reflux temperature afforded 5-arylhydrazono-4-methyl-6-phenylamino-2,5--dihydropyrimidine-2-thione *Va—Vc* in good yield.

Refluxing *II* with semicarbazide hydrochloride in sodium acetate buffered solution gave 4-arylazo-1-carbamoyl-3-methyl-5-phenylaminopyrazole *VIa—VId* rather than the possible 4-arylhydrazono-1-carbamoyl-3-methyl-4*H*--pyrazole-5-thione *VII* (Scheme 2).

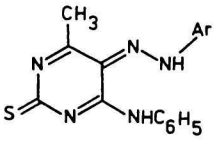


Scheme 2

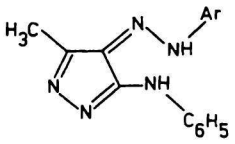
Besides correct analytical data for products *VIa—VId*, none of them exhibited any absorption bands corresponding to the C=S group.

Another pyrazole derivatives, 4-arylhydrazono-3-methyl-5-phenylamino--4*H*-pyrazole *VIIIa—VIIIc*, were obtained *via* the reaction of *II* with hydrazine hydrate in ethanol at reflux temperature.

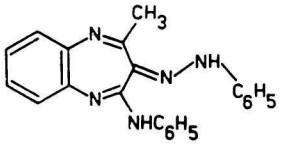
When *IIa* was allowed to react with *o*-phenylenediamine in boiling acetic acid, 2-methyl-4-phenylamino-3-phenylhydrazono-3*H*-benzo[*b*]-1,5-diazepin (*IX*) was isolated with elimination of both water and hydrogen sulfide molecules.



Va–Vc



VIIIa–VIIIc



IX

		a	*b*	
V	Ar:	m-$CH_3OC_6H_4$	m-$NO_2C_6H_4$	p-ClC_6H_4
VIII	Ar:	C_6H_5	p-ClC_6H_4	p-$CH_3C_6H_4$

Biological screening

Toxicity and antitumour activity

Some of the prepared compounds were chosen to be tested for their antitumour activity *in vivo* against Ehrlich ascites carcinoma. All of the tested compounds revealed high activity at different concentrations. The percentage of the mean survival time of the test animals (T) to that of the control animals (C) was found to be higher than 125*, **.

The acute toxicological study of these compounds in tumour-bearing mice showed that compounds *IIa*, *Vb*, and *VIIIc* have a relatively high value of maximum tolerative dose (MTD = 90, 80, and 80 mg/kg body mass, respectively), whereas the other compounds showed lower values (about 30 mg/kg body mass).

The divided dose (Chronic toxicological study) succeeded in keeping the high antitumour activity and lowering the toxicological effect of the compounds under investigation**.

Antimicrobial activities

The antimicrobial activity of the newly synthesized compounds was tested at three different concentrations against *Staphylococcus albus*, *Staphylococcus aureus*, *Escherichia coli*, and diplococcal *Neisseria catarrhalis* using the agar diffusion sensitivity test [11]. So, compound *VIIIb* showed a marked inhibitive activity against *S. albus*, compounds *Va*, *Vc*, and *VIIIb* showed high inhibitive

* % $T/C \geq 125$ denotes a significant antitumour activity.

** The details of study of antitumour activity, chronic toxicological study together with LD_{50} values for the tested compounds will be published separately.

Table 2

In vitro biological action of compounds *II*, *V*—*IX* on bacteria and fungi

Compound	w^a / ppm	Inhibition zone/cm[a]				Change in absorbance[b]
		S. albus	*S. aureus*	*E. coli*	*N. catarrhalis*	*S. cerevisiae*
IIa	1	−	−	−	−	
	2	−	−	−	−	+
	3	−	−	+	−	
IIb	1	−	−	−	−	
	2	−	−	−	−	++
	3	−	−	+	+	
IIc	1	−	−	−	−	
	2	−	−	−	−	++
	3	+	−	+	−	
Va	1	++	+++	++	++	
	2	++	++	+	+	++
	3	+	++	+	++	
Vb	1	++	++	+	++	
	2	++	++	−	++	++
	3	++	+	−	++	
Vc	1	++	+++	++	++	
	2	+	++	+	+	+++
	3	−	++	+	−	
VIa	1	++	++	+	+	
	2	++	++	+	+	++
	3	−	++	−	−	
VIb	1	++	++	++	++	
	2	+	+	+	+	++
	3	−	+	+	−	
VIIIa	1	++	++	+	++	
	2	+	+	+	++	++
	3	−	+	−	++	
VIIIb	1	+++	+++	−	+++	
	2	+++	+++	−	++	+
	3	++	++	−	++	
VIIIc	1	−	−	−	−	
	2	−	−	−	−	+++
	3	−	−	−	−	
IX	1	++	++	+	++	
	2	+	+	−	+	+++
	3	+	+	−	+	

a) For antibacterial activity mass fractions are: 1 = 500, 2 = 250, 3 = 125 ppm; inhibition zone: − <1 cm, + 1—1.5 cm, ++ 1.6—2 cm, +++ >2 cm.

b) For antifungal activity $w = 50$ ppm; change in absorbance: + <0.01, ++ 0.01—0.03, +++ >0.03.

activity against *S. aureus*. Compound *VIIIb* exhibited marked antibacterial activity against *N. catarrhalis*. Compounds *IIa—IIc* and *VIIIc* showed no activity against all types of bacteria used in this study at different concentrations. These results are tabulated in Table 2.

The antifungal activity was studied against *Saccharomyces cerevisiae* using the turbidimetric method [12]. All of the prepared compounds showed positive effect. Compounds *Vc*, *VIIIc*, and *IX* exhibited marked activity. The change in absorbance was measured spectrophotometrically at $\lambda = 540$ nm (Table 2).

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