Preparation and Biological Activity of 6*H*-5,1,3-Benzothiadiazocines

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Dedicated to Professor Dr. P. Kristián, in honour of his 65th birthday

Synthesis and antibacterial activities of some 4-substituted 2-morpholino-6*H*-5,1,3-benzothiadiazocines obtained from easily available *N*-(2-chloromethylphenyl)carbimidoyl dichloride are described. Analytical data, as well as IR, mass, ^{1}H NMR, and ^{13}C NMR spectral characteristics are presented.

1,4-Benzodiazepines and 1,5-benzodiazocines can be considered cyclic analogues of 1,3-benzodiazines, *e.g.* quinazolines. Accordingly, similar properties and similar starting material for their preparation can be supposed. On the other hand, the expansion of azine ring brings about changes of spatial structure as well as of the properties. Thus, while a wide spectrum of pharmacological and phytoeffectual properties was found for quinazolines [1, 2], benzodiazepines and benzodiazocines, respectively, act mainly on CNS [3]. After psychotropic effects of diazepines have been found, the quest for novel active compounds led to analogues with various substitution patterns and different heteroatoms present in the ring [3].

In the present paper we describe a similar type of compounds 5,1,3-benzothiadiazocines, the type not described in the literature as yet, with the hope to find the novel active compounds.

We have found that the mentioned benzothiadiazocines can be effectively prepared starting from easily available *N*-(2-chloromethylphenyl)carbimidoyl dichloride *I* (see Scheme 1). By the reaction of *I* with morpholine we have obtained *N*-(2-chloromethylphenyl)-*N*′,*N*′-(3-oxapentamethylene)formamidinoyl chloride *II*. This bifunctional compound reacts with potassium thiocyanate by chlorine of the amidinoyl group affording the corresponding *N*-(2-chloromethylphenyl)-*N*′,*N*′-(3-oxapentamethylene)formamidinoyl isothiocyanate *III*. The primary amines can react with *III* by nucleophilic substitution of chlorine atom of chloromethyl group or by addition on heterocumulene grouping.

We have found in all cases, using aromatic, aliphatic, and arylaliphatic amines, respectively, only addition reaction on the NCS group. According to this reaction, series of corresponding amidinoyl thioureas *IVa*—*IVj* were prepared. Analytical and some IR spectral properties are presented in Table 1.

The heat-induced cyclization of *IVa*—*IVj* in the presence of triethylamine led to the formation of products in 35—75 % yields, these compounds being assigned the structure of 6*H*-5,1,3-benzothiadiazocines *Va*—*Vj* (see Tables 2 and 3).

Taking into account the existence of tautomerism in amidinoyl thioureas we can identify in their molecules three reactive sites (Scheme 2), namely:

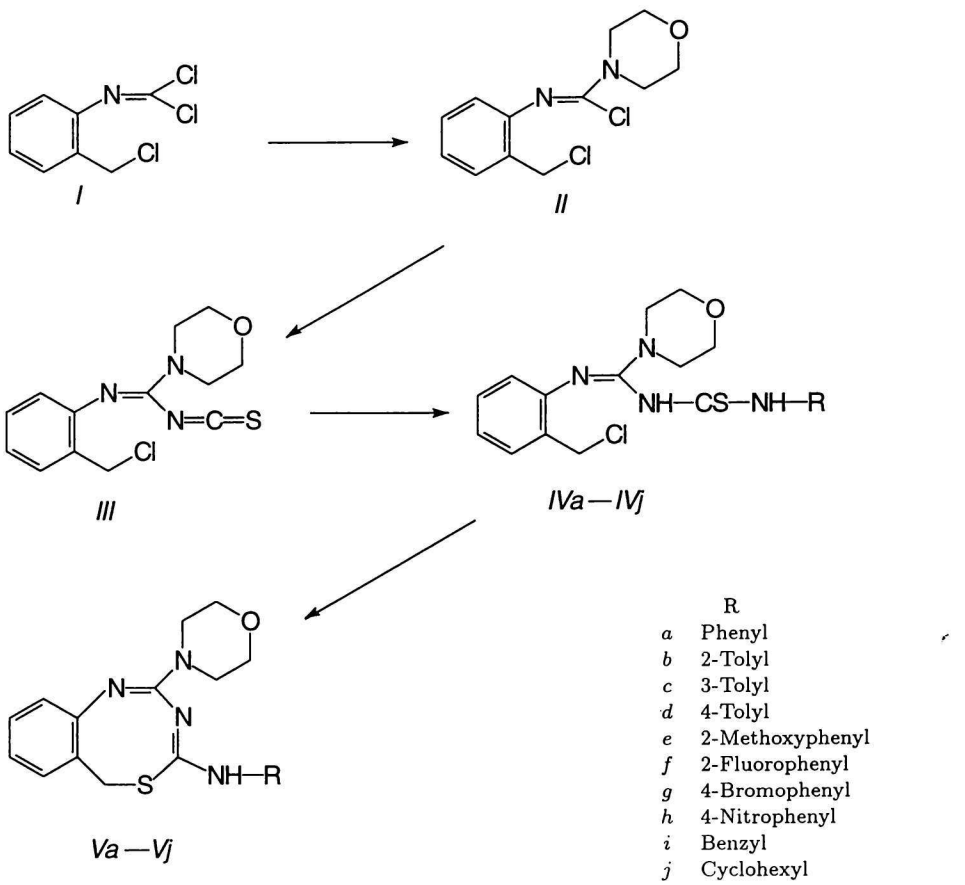
1. Urea nitrogen adjacent to the amidine. Cyclization at this atom affords 3-thiocarboxamido-4*H*-quinazolines (*A V*).

2. Terminal nitrogen of the amine. Cyclization there leads to the corresponding 6*H*-1,3,5-benzotriazocines (*B V*).

3. Sulfur atom of the thiol group of thiourea. Cyclization *via* the thio site produces 5,1,3-benzothiadiazocines *Va*—*Vj*.

On the basis of spectral (IR, ^{1}H NMR, ^{13}C NMR) properties of obtained compounds and similar reaction described in the literature we have attributed the structure of 5,1,3-benzothiadiazocines *V* to them.

The similar reactions of *o*-xylyl dibromides or 2-bromomethylbenzoyl bromide with various substituted thioureas are described in the literature [4, 5]. By heating with the base, the corresponding benzothiazepines were obtained, *e.g.* cyclization takes place *via* the sulfur atom. It is also known, that the reaction of benzyl chloride or alkyl bromides with N^1,N^2-disubstituted thioureas led to the *S*-alkyl derivatives due to higher nucleophilicity of sulfur atom than that of nitrogen. The above-mentioned facts and spectral properties of compounds obtained confirm



Scheme 1

the structure of 3-substituted 2-morpholino-6*H*-5,1,3-benzothiadiazocines.

In the IR spectra of compounds *Va*—*Vj* (Table 3) the most intensive absorption band at $\tilde{\nu} = 1630$ cm^{-1} can be observed due to the ν(C=N) vibration of thiadiazocine ring. At $\tilde{\nu} = 3100$ cm^{-1} a broad band can be observed and attributed to the vibration of the associated NH group.

In the ^{1}H NMR spectra (Table 3) the characteristic doublet of methine hydrogenes at C-6 can be observed. This splitting with $J_{A,B} = 12$ Hz is characteristic also of all chloromethyl groups in the present work and due probably to restricted rotation and rigid structure of the thiadiazocine ring. Splitting of methine hydrogen in a compound of the similar type, *e.g.* 1,4,5-benzothiadiazocine was also observed ($J_{A,B} = 9$—12 Hz) [6].

The ^{13}C NMR spectra of 2-morpholino-4-anilino-6*H*-5,1,3-benzothiadiazocine (*Va*) display the following signals at $\delta = 32.8$ belonging to C-6 of thiadiazocine ring; 45.1 and 65.9 of C-2 and C-3 of the morpholine; 119.2, 121.6, 122.1, 122.8, 123.8, 124.8, 128.6, 129.2, 129.8, 131.9, 140.0, 148.2 (benzene rings) and 151.5 and 154.8, belonging to C-2 and C-4, respectively, of the thiadiazocine ring. Some of these values are in accord with the data for C-7 of the methylene group (33.3) and C-2 of 1,3-benzo[*e*]thiazepine (140.3) published in the literature [5].

An alternative mode of cyclization of *IVa*—*IVj* would have produced 3-thiocarboxamidoquinazolines (*A V*), signals of the methylene group would have been shifted to higher δ values by about 50 and there would have been a C=S type carbon at $\delta = 186$.

Another possibility, namely the cyclization of *IVa*—*IVj* to benzotriazocines (*B V*) would have produced structure, in which the CH_2 group signal would be expected at $\delta \approx 57$ and the carbon of C=S group at $\delta = 186$. The reference data were taken over from the computer-simulated spectra.

The incessant guest for novel pharmacologically active compounds has now been extended to eight-membered heterocycles with several heteroatoms. Therefore it seemed justified to test the prepared amidinoyl thioureas *IVa*—*IVj* and final benzothiadiazocines *Va*—*Vj*.

The scope of *in vitro* antimicrobial activity of these compounds is presented in Tables 4 and 5. As seen from these tables, the derivatives with the nitro and methoxy group, respectively (*IVe, IVh* and *Ve, Vh*) were completely inactive, while others were active only

Table 1. Analytical and IR Spectral Data of Compounds *IVa*—*IVj*

Compound	Formula	w_i(calc.)/% w_i(found)/%			Yield	M.p.	IR, $\tilde{\nu}$/cm^{-1}	
	M_r	C	H	N	%	°C	ν(C=N)	ν(CH$_{aliph}$)
IVa	$C_{19}H_{21}N_4ClSO$	58.68	5.44	14.41	45	230—233	1624	2990
	388.9	58.81	5.11	14.30				2856
IVb	$C_{20}H_{23}N_4ClSO$	59.62	5.75	13.90	43	215—217	1628	2957
	402.9	59.55	5.53	13.67				2855
IVc	$C_{20}H_{23}N_4ClSO$	59.62	5.75	13.90	49	212—215	1641	2922
	402.9	59.77	5.61	13.81				2839
IVd	$C_{20}H_{23}N_4ClSO$	59.62	5.75	13.90	38	208—212	1628	2976
	402.9	59.50	5.49	13.77				2849
IVe	$C_{20}H_{23}N_4ClSO_2$	57.34	5.53	13.37	39	218—220	1624	2955
	418.9	57.11	5.41	13.08				2853
IVf	$C_{19}H_{20}N_4ClFSO$	56.08	4.95	13.77	45	225—228	1624	2916
	406.9	56.19	4.87	13.61				2856
IVg	$C_{19}H_{20}N_4BrClSO$	48.78	4.31	11.98	34	220—225	1641	2920
	467.8	48.51	4.20	11.81				2810
IVh	$C_{19}H_{20}N_5ClSO_2$	52.59	4.65	16.14	20	175—178	1647	2910
	433.9	52.63	4.50	15.98			1616	2851
IVi	$C_{20}H_{23}N_4ClSO$	59.62	5.75	13.90	45	215—218	1624	2970
		59.91	5.52	13.81				2851
IVj	$C_{19}H_{26}N_4ClSO$	57.93	6.65	14.22	35	151—153	1634	2938
	394.0	58.11	6.44	14.10				2858

Table 2. Characterization of Compounds *Va*—*Vj*

Compound	Formula	w_i(calc.)/% w_i(found)/%			Yield	M.p.
	M_r	C	H	N	%	°C
Va	$C_{19}H_{20}N_4SO$	64.75	5.72	15.90	48	180—181
	352.5	64.81	5.53	15.73		
Vb	$C_{20}H_{22}N_4SO$	65.55	6.05	15.29	33	154—155
	366.5	65.19	6.19	15.41		
Vc	$C_{20}H_{22}N_4SO$	65.55	6.05	15.29	48	192—193
	366.5	65.22	6.15	15.15		
Vd	$C_{20}H_{22}N_4SO$	65.55	6.05	15.29	44	195—196
	366.5	65.44	6.13	15.33		
Ve	$C_{20}H_{22}N_4SO_2$	62.81	5.80	14.65	68	183—184
	382.5	63.02	5.71	14.44		
Vf	$C_{19}H_{19}N_4FSO$	61.60	5.17	15.12	48	156—158
	370.5	61.48	5.33	15.01		
Vg	$C_{19}H_{19}N_4BrSO$	52.91	4.44	12.99	65	207—208
	431.3	52.88	4.31	12.63		
Vh	$C_{19}H_{19}N_5SO_3$	57.42	4.82	17.62	73	221—222
	397.5	57.13	4.77	17.55		
Vi	$C_{20}H_{22}N_4SO$	65.55	6.05	15.29	65	161—162
	366.5	65.44	6.10	15.20		
Vj	$C_{19}H_{25}N_4SO$	63.84	7.05	15.67	44	175—177
	357.5	63.59	7.11	15.43		

against gram-positive bacteria, yeast or filamentous fungi. The wide antimicrobial range has been manifested by phenyl, 4-bromophenyl, and 3-tolyl derivatives, respectively. The highest effects on the yeasts were demonstrated also by 4-bromophenyl and 3-tolyl derivatives. The selective effect on tested filamentous fungi can be observed. Also starting isothiocyanate *III* manifested interesting activities (Table 4).

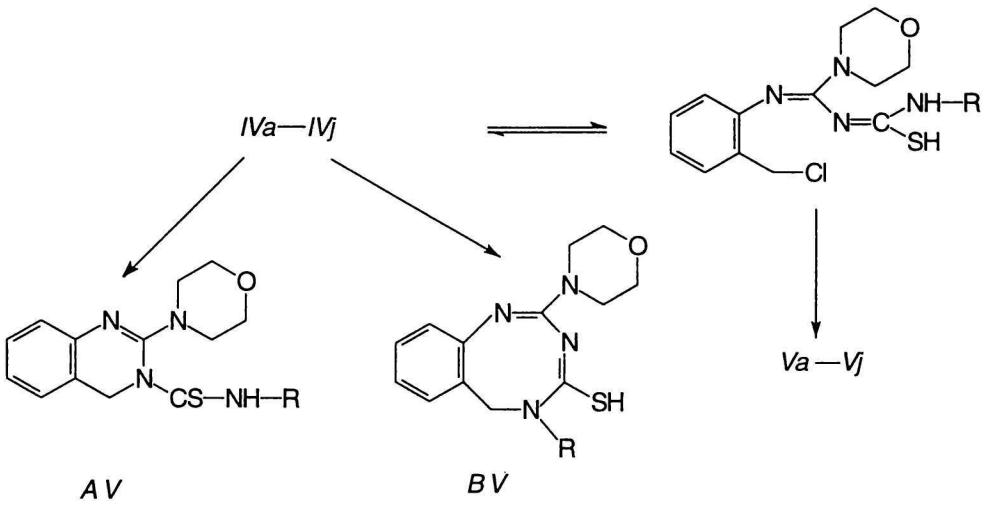
Based on the results obtained, one can add the bromothiourea derivative *IVg* and isothiocyanate *III* to the new potentially antimicrobial substances and *Va*, *Vg*, and *IVg* compounds to the potentially biologically active compounds.

EXPERIMENTAL

Infrared spectra (KBr discs) were taken with a Philips PU-9800 FTIR spectrometer. The ^{1}H NMR

Table 3. Spectral Data of Compounds *Va*—*Vj*

Compound	IR, $\tilde{\nu}/cm^{-1}$			MS	^1H NMR, δ				
	ν(C=N)	$\nu(CH_{aliph})$	ν(NH)	M^+	H_{arom}	H_{NH}	H_{CH_2}	$H_{morpholine}$	Other
Va	1638	2849—2999	3175 3121	352	6.83—7.35 (m, 9H)	6.52 (bs, 1H)	4.63 (d, 1H) 4.48 (d, 1H)	3.26—3.67 (m, 8H)	–
Vb	1628	2855—2959	3105	–	7.04—7.25 (m, 8H)	6.63 (bs, 1H)	4.65 (d, 1H) 4.50 (d, 1H)	3.55—3.75 (m, 8H)	1.90 (s, 3H, CH_3)
Vc	1628 1622	2855—2964	3204 3147	–	6.85—7.32 (m, 8H)	6.45 (bs, 1H)	4.66 (d, 1H) 4.50 (d, 1H)	3.37—3.84 (m, 8H)	2.25 (s, 3H, CH_3)
Vd	1636 1603	2853—2963	3221 3169	–	6.85—7.35 (m, 8H)	6.41 (bs, 1H)	4.62 (d, 1H) 4.47 (d, 1H)	3.29—3.81 (m, 8H)	2.25 (s, 3H, CH_3)
Ve	1624	2745—2955	3150 3106	–	6.79—8.15 (m, 8H)	6.49 (bs, 1H)	4.65 (d, 1H) 4.50 (d, 1H)	3.36—3.77 (m, 8H)	3.75 (s, 3H, OCH_3)
Vf	1624	2797—2916	3109	–	6.82—8.15 (m, 8H)	6.64 (bs, 1H)	4.65 (d, 1H) 4.50 (d, 1H)	3.39—3.83 (m, 8H)	–
Vg	1638	2849—2959	3209 3157	432 430	6.83—7.38 (m, 8H)	6.43 (bs, 1H)	4.64 (d, 1H) 4.48 (d, 1H)	3.38—3.82 (m, 8H)	–
Vh	1643 1612	2851—2972	3209 3144	–	6.82—8.18 (m, 8H)	6.54 (bs, 1H)	4.64 (d, 1H) 4.50 (d, 1H)	3.38—3.82 (m, 8H)	–
Vi	1631	2858—2976	3161	–	6.80—7.38 (m, 9H)	4.98 (bs, 1H)	4.60 (d, 1H) 4.42 (d, 1H)	3.11—3.75 (m, 8H)	4.15—4.41 (m, 2H, benzyl)
Vj	1630	2853—2976	3323	358	6.79—7.29 (m, 4H)	4.0 (bs, 1H)	4.54 (d, 1H) 4.40 (d, 1H)	3.23—3.84 (m, 8H)	1.05—1.60 (m, 11H)



Scheme 2

Table 4. Biological Activity ($IC_{50}/(\mu g\ cm^{-3})$) of Compounds *IVa—IVj*[a]

Compound	Bacteria[b]		Yeast	Filamentous fungi			
	B. subtilis	*S. aureus*	*S. cerevisiae*	*B. cinerea*	*A. solani*	*F. culmorum*	*M. gypseum*
II	> 500	135[d]	500	> 500	140	220	70
III	25*	70**	109[c]	500	120	450	145
TTC	0.3***	0.5***	–	–	–	–	–
IVa	170[d]	135	185[c]	300	> 500	> 500	> 450
IVb	195[d]	82[d]	205[c]	> 500	> 500	> 500	> 500
IVc	28**	70**	94[c]	320	380	> 500	210
IVd	24.5**	190	186[c]	490	270	> 500	> 500
IVe	250	> 500	500	> 500	> 500	> 500	> 500
IVf	175[d]	220	215[c]	> 500	350	> 500	> 500
IVg	26*	140	> 500	> 500	100	> 500	> 500
IVh	> 500	> 500	> 500	> 500	> 500	> 500	> 500
IVi	115**	160	260	> 500	> 500	> 500	310
IVj	190**	125	> 500	> 500	> 500	> 500	> 500

TTC – tetracycline, *a*) MIC or MBC of other compounds were higher than 500 $\mu g\ cm^{-3}$; *b*) All tested compounds were inactive with *E. coli* and *P. fluorescens*; *c*) MIC is 500 $\mu g\ cm^{-3}$; *d*) The highest concentration tested (500 $\mu g\ cm^{-3}$) caused bacteriostatic effect, * MIC and MBC is 100 $\mu g\ cm^{-3}$, ** MIC and MBC is 500 $\mu g\ cm^{-3}$, *** MIC is 10 $\mu g\ cm^{-3}$, MBC is 100 $\mu g\ cm^{-3}$.

spectra of deuterochloroform solution containing tetramethylsilane as an internal standard were recorded with a Tesla BS 587 (80 MHz) spectrometer, ^{13}C NMR spectra of 2-morpholino-4-anilino-6*H*-5,1,3-benzothiadiazocines were measured with a Jeol FX-100 spectrometer, mass spectra with an MS 902 S spectrometer (AEI Manchester).

Preparation of starting 2-tolylcarbimidoyl dichloride was reported in Ref. [7].

The antimicrobial activity of prepared compounds was evaluated using the G^- *Escherichia coli* CCM 5172, *Pseudomonas fluorescens* (isolated from patients) and G^+ *Bacillus subtilis* CCM 1718 and *Staphylococcus aureus* CCM 3824; the yeasts *Saccharomyces cerevisiae* 6C Valtice; the filamentous fungi *Botrytis cinerea* CCM F-16, *Alternaria solani* CCM F-167, *Fusarium culmorum* CCM F-21, *Microsporum gypseum* (isolated from patients). Compositions ($\rho/(\mu g\ cm^{-3})$) 500, 100, 10, and 1 of tested compounds were used. Chromatographically pure derivatives were dissolved in dimethyl sulfoxide; its final concentration never exceeded 1.0 vol. % in either control or treated samples.

Inhibitory concentration IC_{50} (*i.e.* such concentration of a derivative which in comparison to the control inhibits the growth of microorganisms to 50 %) and minimum inhibitory concentration (MIC) were determined by the dilution method in Difco-made nu-

Table 5. Biological Activity ($IC_{50}/(\mu g\ cm^{-3})$) of Compounds *Va*—*Vj*[a]

Compound	Bacteria[b]		Yeast	Filamentous fungi			
	B. subtilis	*S. aureus*	*S. cerevisiae*	*B. cinerea*	*A. solani*	*F. culmorum*	*M. gypseum*
Va	230[d]	60[d]	86[c]	150	> 500	> 500	> 500
Vb	260[d]	86[d]	160[c]	260	> 500	370	440
Vc	41[d]	72	> 500	170	> 500	> 500	> 500
Vd	33[d]	> 500	> 500	140	400	> 500	> 500
Ve	> 500	> 500	> 500	> 500	> 500	> 500	> 500
Vf	205[d]	> 500	> 500	200	> 500	> 500	> 500
Vg	35**	13	150[c]	110	300	> 500	> 500
Vh	> 500	> 500	> 500	> 500	> 500	> 500	> 500
Vi	120	> 500	> 500	300	> 500	> 500	> 500
Vj	> 500	> 500	> 500	148	> 500	> 500	> 500

a) MIC or MBC of other compounds were higher than 500 $\mu g\ cm^{-3}$; *b*) All tested compounds were inactive with *E. coli* and *P. fluorescens*; *c*) MIC is 500 $\mu g\ cm^{-3}$; *d*) The highest concentration tested (500 $\mu g\ cm^{-3}$) caused bacteriostatic effect, ** MIC and MBC is 500 $\mu g\ cm^{-3}$.

trient broth (bacteria), Sabourod's medium (yeasts), Sabourod's agar medium (*M. gypseum*), and malt agar medium (other fungal strains). IC_{50} and MIC values were read from the toxicity curves.

MIC experiments on subculture dishes were used to assess the minimum bactericidal concentration (MBC) values. Subcultures were prepared separately in Petri dishes containing Nutrient broth agar and incubated at 37 °C for 48 h. The MBC value was taken as the lowest concentration which showed no visible growth of bacterial colonies in the subculture dishes. The data of the biological activity are given in Tables 4 and 5.

N-(2-Chloromethylphenyl)carbimidoyl Dichloride (*I*)

Mixture of 2-tolylcarbimidoyl dichloride (10 g; 0.053 mol), sulfuryl chloride (5 cm^3), and bis(isoazobutyronitrile) (0.2 g) in absolute benzene (100 cm^3) was refluxed for 4 h. Then the solvent and excess of sulfuryl chloride were removed under reduced pressure. The residue was distilled *in vacuo*. B.p./°C (p/kPa) = 170—175 (95), yield 11.8 g, 80 %. For $C_8H_6NCl_3$ (M_r = 222.5) w_i(calc.): 43.19 % C, 2.72 % H, 6.30 % N, 47.80 % Cl; w_i(found): 43.30 % C, 2.52 % H, 6.11 % N, 47.55 % Cl. ^{1}H NMR spectrum, δ: 4.48 (d, 2H, CH_2), $J_{A,B}$ = 12 Hz, 6.84—7.95 (m, $4H_{arom}$).

N-(2-Chloromethylphenyl)-*N*′,*N*′-(3-oxapentamethylene)formamidinoyl Chloride (*II*)

The treatment of *I* with the equimolar amount of morpholine gave the title compound *II* as yellow oil, which was used directly in the following reaction without isolation, because of the decomposition during the separation and purification.

N-(2-Chloromethylphenyl)-*N*′,*N*′-(3-oxapentamethylene)formamidinoyl Isothiocyanate (*III*)

To the solution of *II* (10 g; 0.045 mol) in absolute acetone (50 cm^3) the equimolar amount of triethylamine (6.4 cm^3, 0.045 mol) was added. Within further 1 h, morpholine (4 cm^3, 0.045 mol) was added drop by drop with stirring and cooling at −10—+5 °C. The mixture was then stirred at room temperature for 2 h.

The formed precipitate (triethylammonium chloride) was removed by filtration; to the filtrate during stirring and cooling (−10—0 °C) a solution of potassium thiocyanate (4.4 g; 0.04 mol) in acetone (30 cm^3) was added. The mixture was stirred at −5 °C for 1 h, the precipitate of potassium bromide was filtered off, the filtrate was concentrated to the dryness *in vacuo*. The raw product was purified by crystallization from n-heptane. M.p. = 90—93 °C, yield 11.8 g, 89 %. For $C_{13}H_{14}N_3ClSO$ (M_r = 295,8) w_i(calc.): 52.79 % C, 4.77 % H, 14.21 % N, 10.84 % S; w_i(found): 52.55 % C, 4.51 % H, 14.37 % N, 11.01 % S. IR spectrum, $\tilde{\nu}/cm^{-1}$: 2046 ν(NCS), 1624 ν(C=N), 2850—2999 $\nu(CH_{aliph})$.

In the following reaction the isothiocyanate *III* can be used without isolation and purification.

N-(2-Chloromethylphenyl)-*N*′,*N*′-(3-oxapentamethylene)formamidinoyl Thioureas (*IVa*—*IVj*)

To the solution of *III* (0.045 mol) in absolute acetone (30 cm^3) the corresponding primary amine (0.045 mol) was added in small portions. The mixture was stirred at room temperature for 24 h. The precipitate of the formed thiourea *IV* was filtered and recrystallized from ethanol. Physical constants and IR spectral data are given in Table 1.

2,4-Disubstituted-6*H*-5,1,3-benzothiadiazocines (*Va*—*Vj*)

A mixture of corresponding thiourea (0.045 mol) and triethylamine (6.4 cm^3, 0.045 mol) in absolute benzene (70 cm^3) was stirred and refluxed for 4 h. During the reaction a precipitate was formed (triethylammonium chloride) which was removed by filtration of hot solution. The benzothiadiazocines were obtained by cooling and crystallized from methanol—chloroform (φ_r = 1 : 1). Physical and spectral data are given in Tables 2 and 3.

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