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The evaluation of biological activities of *N-, S*-substituted polyhalogenated butadiene compounds

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ABSTRACT

A series of N-(diphenylmethyl)piperazine, thiomorpholine and alkylthio substituted nitro-1,3-butadiene compounds have been synthesized and tested for their antimicrobial, antifungal activities and for evaluation of growth regulative action. Compounds **2b, 2c, 2d** in 0.5 % concentration showed moderate activity against *Myc.luteum* and *C.tenuis.* Biological activity prediction was carried out by using the computer program *PASS C&T* which indicated the necessity of further investigation of synthesized compounds in this order.

Keywords: N,S-substituted nitrodiene, Piperazine derivatives, thiomorpholine, antifungal activity, antimicrobial activity

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INTRODUCTION

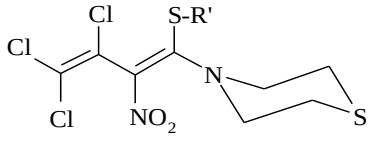
The synthesis of new biologically active molecules is important for the development of future drugs. Monoaryl- and diarylpiperazines are a significant class of organic compounds for clinical chemistry [1]. Piperazines have been reported in gene transfer reactions [2] and quaternary piperazinium salts have shown spasmolytic, anthelmintic and germicidal activity. Polycationic ligands having piperidine and piperazine rings have been reported to exhibit a substantial degree of selective RNA binding [3]. Replacement of the piperazinyl nitrogen with carbon, oxygen or sulfur, corresponding to the piperidino, morpholino, or thiomorpholino group, respectively, enhances the activity against Gram-positive bacteria, but reduces the activity against Gram-negative bacteria [4]. Some piperazine derivatives possess high biological activity for multidrug resistance in cancer and malaria [5].

Halogenated nitro-1,3-butadienes are useful precursors for the synthesis of polyfunctional derivatives of heterocyclic compounds exhibiting antibacterial, antiarrhythmic, antihypoxic, antiviral, antelmintic activity, anti-HIV-1 and antitumor activity [6].

MATERIAL AND METHODS

General procedure of Synthesis of *N,S*-substituted polyhalonitrodienes 1a-f, 2a-f (Ref 10) :

Equimolar amounts of S-substituted polyhalonitrodienes and thiomorpholine or *n*-(diphenylmethyl)-piperazine were mixed in 20 mL dichloromethane at RT (scheme 1) [7]. The mixture was stirred for 24 hr. Chloroform (30 mL) was added to the reaction mixture. The organic layer washed with water (4 × 30 mL) and dried with anhyd. Na_2SO_4. After the solvent was evaporated the residue was purified by column chromatography over silica gel. The structure of the products was determined by microanalysis, and spectroscopic data such as IR, UV, ^{1}H NMR, ^{13}C NMR and MS [7].



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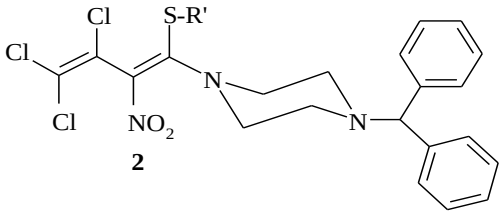

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1, 2	R'
a	CH_3-CH_2-
b	CH_3-$(CH_2)_3$-
c	CH_3-$(CH_2)_7$-
d	CH_3-$(CH_2)_9$-
e	CH_3-$(CH_2)_{11}$-
f	CH_3-$(CH_2)_{15}$-

Scheme I

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Experimental

Biological screening of antimicrobial activity of synthesized compounds **1a-f** and **2a-f** was carried out according to reported procedure [8]. Tested microorganisms included the following: bacteria *Escherichia coli, Staphylococcus aureus, Mycobacterium luteum* and fungi *Candida tenuis, Aspergillus niger*. The antibacterial activity was compared with Vankomicine, Oxacilinum, and Nistatine controls. Prediction of the biological activity spectrum was estimated on the basis of its structure [9-10].

The influence of the synthesized compounds on plantlets of *Avena* and *Lepidium sativum* with 0.01, 0.001 and 0.0001% solutions for the determination of physiology activity was carried out using the Krasilnikov method [11].

Prediction of the biological spectrum of synthesized compounds **1a-f** and **2a-f** was estimated by the program *PASS C&T.*

RESULTS AND DİSCUSİON

Activity evaluation of compounds 1a-f, 2a-f

Table I — Antimicrobial activity of compounds 1a-f and 2a-f

Compound	Concentration (%)	Diameter of inhibition of growth of microorganisms, mm				
		E.coli	*St.aureus*	*Myc.luteum*	*C.tenuis*	*A.niger*
1b	0,5	0	0	0	6,0	0
	0,1	0	0	0	0	0
2b	0,5	0	0	13,6	11,0	0
	0,1	0	0	9,3	0	0
1e	0,5	0	0	0	0	0
	0,1	0	0	0	0	0
2e	0,5	0	0	0	0	0
	0,1	0	0	0	0	0
1d	0,5	0	0	0	11,0	0
	0,1	0	0	0	0	0
2d	0,5	0	0	14,6	10,3	0
	0,1	0	0	0	0	0
1c	0,5	0	0	0	0	0
	0,1	0	0	0	0	0
2c	0,5	0	0	8,3	9,3	0
	0,1	0	0	0	7,3	0
1f	0,5	0	0	0	0	0
	0,1	0	0	0	0	0
2f	0,5	0	0	0	0	0
	0,1	0	0	0	0	0
1a	0,5	0	0	0	0	0
	0,1	0	0	0	0	0
2a	0,5	0	0	0	0	0
	0,1	0	0	0	0	0
Vankomicine	0,1	16	18	58	0	0
Nistatine	0,1	0	11	15	24	25
Oxacilinum	0,1	0	21	0	0	0

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Biological screening of antimicrobial activity compounds **1a-f** and **2a-f** was estimated using inhibition zones of microorganism test-cultures. Antimicrobial activity of compounds was evaluated by diffusion in peptone on a solid nutrient medium consisting of meat-extract agar for bacteria, and wort agar for fungi. The microbial loading is 10^9 cells /ml. The incubation period for bacteria was 24 hr at 35°C and 48-72 hr at 28-30°C for fungi.

The microorganisms that were tested included the following: bacteria *Escherichia coli, Staphylococcus aureus, and Mycobacterium luteum* and fungi *Candida tenuis, and Aspergillus niger*. The antibacterial activity was compared to Vankomicine, Oxacilinum, and Nistatine controls.

The antimicrobial activity of the synthesized compounds were listed in Table I. Compounds **2b**, **2c** and **2d** at 0.5% molar concentration exhibited moderate activity against *Myc.luteum* and *C.tenuis.* Compounds **1b**, **1d**, and **2c** showed low activity against the *C.tenuis* strain. The compounds **1a-f** and **2a-f** referred in Table 1 have not shown better activity on comparison with controls.

Table 2 — Minimal inhibition concentrations of compounds 1b-e and 2b-e

1b	**Number of tube**	**1**	**2**	**3**	**4**	**5**	**Control**
	Concentration, mkg/ml	100	50	25	12,5	6,25	0
	Microorganism						
	E.coli	+	+	+	+	+	+
	St.aureus	+	+	+	+	+	+
	Myc.luteum	+	+	+	+	+	+
	C.tenuis	+	+	+	+	+	+
	A.niger	+	+	+	+	+	+
2b	**Number of tube**	**1**	**2**	**3**	**4**	**5**	**Control**
	Concentration, mkg/ml	100	50	25	12,5	6,25	0
	Microorganism						
	E.coli	+	+	+	+	+	+
	St.aureus	+	+	+	+	+	+
	Myc.luteum	±	+	+	+	+	+
	C.tenuis	+	+	+	+	+	+
	A.niger	+	+	+	+	+	+
1e	**Number of tube**	**1**	**2**	**3**	**4**	**5**	**Control**
	Concentration, mkg/ml	100	50	25	12,5	6,25	0
	Microorganism						
	E.coli	+	+	+	+	+	+
	St.aureus	+	+	+	+	+	+
	Myc.luteum	+	+	+	+	+	+
	C.tenuis	+	+	+	+	+	+
	A.niger	+	+	+	+	+	+
	Number of tube	**1**	**2**	**3**	**4**	**5**	**Control**
	Concentration, mkg/ml	100	50	25	12,5	6,25	0
	Microorganism						
	E.coli	+	+	+	+	+	+

2e	St.aureus	+	+	+	+	+	+
	Myc.luteum	±	+	+	+	+	+
	C.tenuis	+	+	+	+	+	+
	A.niger	+	+	+	+	+	+
1d	**Number of tube**	**1**	**2**	**3**	**4**	**5**	**Control**
	Concentration, mkg/ml	100	50	25	12,5	6,25	0
	Microorganism						
	E.coli	+	+	+	+	+	+
	St.aureus	+	+	+	+	+	+
	Myc.luteum	+	+	+	+	+	+
	C.tenuis	+	+	+	+	+	+
	A.niger	+	+	+	+	+	+
2d	**Number of tube**	**1**	**2**	**3**	**4**	**5**	**Control**
	Concentration, mkg/ml	100	50	25	12,5	6,25	0
	Microorganism						
	E.coli	+	+	+	+	+	+
	St.aureus	+	+	+	+	+	+
	Myc.luteum	±	+	+	+	+	+
	C.tenuis	+	+	+	+	+	+
	A.niger	+	+	+	+	+	+
1c	**Number of tube**	**1**	**2**	**3**	**4**	**5**	**Control**
	Concentration, mkg/ml	100	50	25	12,5	6,25	0
	Microorganism						
	E.coli	+	+	+	+	+	+
	St.aureus	+	+	+	+	+	+
	Myc.luteum	+	+	+	+	+	+
	C.tenuis	+	+	+	+	+	+
	A.niger	+	+	+	+	+	+
2c	**Number of tube**	**1**	**2**	**3**	**4**	**5**	**Control**
	Concentration, mkg/ml	100	50	25	12,5	6,25	0
	Microorganism						
	E.coli	+	+	+	+	+	+
	St.aureus	+	+	+	+	+	+
	Myc.luteum	±	+	+	+	+	+
	C.tenuis	+	+	+	+	+	+
	A.niger	+	+	+	+	+	+

Note : + growth of microorganisms;
± inhibition of growth of microorganisms
- absent of growth of microorganisms

The results of minimal bacteriostatic and minimal bactericidic concentrations (MBSC and MBCC) and minimal fungicidic and minimal fungistatic concentrations (MFCC and MFSC) of the synthetic compounds using serial dilution are listed in Table II. The certain volume of solution of compounds **1b-e** and **2b-e** was brought in nutrient medium (meat-extract agar for bacteria, wort for fungi). The inoculum of bacteria and fungi was inoculated in nutrient medium. The duration of incubation of bacteria is 37°C for bacteria, and 30°C for fungi during 24-72 hr. The results are an estimation of the presence or the absence of growth of microorganisms.

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Prediction of the biological activity spectrum

Table 3 — Prediction of the biological activity spectrum of compounds 1a-f and 2a-f

Compound	Antiviral	Antibacterial	Toxic	Antifungal	Antiinflammatory	Antitussive	Antipruritic	Cardiotonic / Cardioprotectant	Neuroprotector	Antihypertensive	Antiallergic
2a	0,355	0,101	0,796	0,352	0,645	0,746	0,746	0,889	0,893	0,889	0,915
2b	0,445	0,101	0,743	0,130	0,627	0,747	0,747	0,885	0,757	0,885	0,916
2c	0,477	0,110	0,738	0,130	0,627	0,734	0,734	0,884	0,745	0,884	0,909
2d	0,477	0,103	0,738	0,130	0,627	0,734	0,734	0,884	0,745	0,884	0,909
2e	0,477	0,123	0,738	0,130	0,627	0,734	0,734	0,884	0,745	0,884	0,909
2f	0,477	0,109	0,738	0,130	0,627	0,734	0,734	0,884	0,745	0,884	0,909
1a	0,129	0,123	0,773	0,344	0,728	-	0,728	0,137 / 0,939	0,728	0,174	0,214
1b	0,478	0,123	0,694	-	0,700	0,336	0,700	0,138 / 0,925	0,700	0,172	0,230
1c	0,478	0,121	0,694	-	0,700	0,336	0,700	0,138 / 0,925	0,700	0,172	0,230
1d	0,478	0,110	0,694	-	0,700	0,336	0,700	0,138 / 0,925	0,700	0,172	0,230
1e	0,478	0,130	0,694	-	0,700	0,336	0,700	0,138 / 0,925	0,700	0,172	0,230
1f	0,478	0,123	0,694	-	0,700	0,336	0,700	0,138 / 0,925	0,700	0,172	0,230

Prediction of the biological activity spectrum was estimated on the basis of its structural formula with the computer program *PASS C&T* having Pa > 0.1 (Prediction of Activity Spectra for Substances) [9-10]. The results of prediction are presented as a list of activities with appropriate Pa estimating the probability for the compound to be active and inactive, respectively, for each type of activity from the biological activity spectrum. The value for Pa varies from 0.000 to 1.000. If Pa > 0.7, then the compound is likely to reveal activity in experiments, but the likehood of being the analogue of a known pharmaceutical agent for this compound is also high. If 0.5 < Pa < 0.7, then the compound is likely to reveal this activity in experiments, but with lower probability, and the compound is not so similar to known pharmaceutical agents. If Pa < 0.5, then the compound is unlikely to reveal activity in experiments, but if the presence of this activity is confirmed in the experiment the compound might be a New Chemical Entity. Prediction of the biological spectrum of synthesized compounds **1a-f** and **2a-f** was estimated by program *PASS C&T* when Pa > 0.1 in Table III.

The data obtained for the predictions indicated a low antibacterial activity and medium antifungal activity for **1a** and **2a** only; however, all compounds will show, in practice, cardio, antiallergic, antihypertensive, neuroprotector activity as predicted by the PASS program.

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Evaluation of growth regulative action

Table 4 — Growth regulative action of compounds 1a-f and 2a-f

Compound	Concentration, %	*Avena*			*Lepidium sativum*	
		Length, Mm		Germinability %	Length of root, mm	Germinability, %
		Root	Caulis			
1b	0,01	-	-	-	-	-
	0,001	-	-	-	-	-
	0,0001	35	52	89	7	85
2b	0,01	-	-	-	-	-
	0,001	33	43	57	5	55
	0,0001	69	113	138	12	133
1e	0,01	-	-	-	-	-
	0,001	-	-	-	-	-
	0,0001	28	47	78	-	-
2e	0,01	54	86	75	9	93
	0,001	71	115	130	15	150
	0,0001	60	61	93	9	93
1d	0,01	-	-	-	-	-
	0,001	64	83	110	10	111
	0,0001	48	55	73	9	100
2d	0,01	52	74	98	4	44
	0,001	45	47	63	8	88
	0,0001	58	103	137	12	133
1c	0,01	-	-	-	-	-
	0,001	-	-	-	-	-
	0,0001	45	55	100	8	88
2c	0,01	42	55	92	-	-
	0,001	-	-	-	-	-
	0,0001	-	-	-	-	-
1f	0,01	-	-	-	-	-
	0,001	-	-	-	-	-
	0,0001	51	45	89	8	87
2f	0,01	-	-	-	-	-
	0,001	15	20	45	7	77
	0,0001	33	45	69	8	93
1a	0,01	67	61	100	5	55
	0,001	64	68	105	10	111
	0,0001	65	73	115	15	150
2a	0,01	68	67	73	4	44
	0,001	58	91	37	5	55
	0,0001	48	63	68	5	55

- growth is absent

Note: Germinability > 100 % - stimulative concentration, < 100 % - inhibition concentration.

The influence of the synthesized compounds on plantlets of *Avena* and *Lepidium sativum* with 0.01, 0.001 and 0.0001% solutions for the determination of physiology activity was carried out using the Krasilnikov method [11]. To 0.01%, 0.001% and 0.0001% solutions of the corresponding compounds was added plantlets of *Avena* and *Lepidium sativum.* Plantlets were exposed during 24 hr and then were removed from solution and placed into Petri dishes. Germination of plantlets was carried out by irrigation of the solution: K_2HPO_4 – 0.5 g, $MgSO_4$ – 0.5 g, $FeSO_4$ – 0.1 g, agar-agar – 0.8 g during 14 days. After irrigation was performed on the solutions, measurements of length of roots and caulis, and calculation of germinability was carried out in Table IV.

The results indicated that growth regulators are among the synthesized compounds (**1a**, **1d**, **2d**, **2e –** growth stimulators in 0.001% and 0.0001% concentrations).

CONCLUSİON

A series of N-, S-substituted butadiene compounds were synthesized and tested for their antimicrobial, and antifungal activities and evaluation of growth regulative action. Compounds **2b**, **2c** and **2d** in 0.5% concentration showed moderate activity against *Myc.luteum* and *C.tenuis.* Compounds **1b**, **1d**, **2c** showed low activity against strain *C.tenuis.* The synthesized compounds were found to be substances with antimicrobial activity against *Myc.luteum* and *C.tenuis* among them. Obtained data of prediction showed a low antibacterial activity and medium antifungal activity for **1a** and **2a**.

The obtained results indicate that growth regulators are among synthesized compounds (**1a**, **1d**, **2d**, **2e –** growth stimulators in 0.001% and 0.0001% concentrations). Growth stimulators in 0.001% and 0.0001% concentrations were also found. Previous computer screening indicates the necessity of following evaluations of compounds of this order.

The synthesized compounds have thiomorpholine ring for compounds **1a-f** and piperazine ring for compounds **2a-f**. Both the thiomorpholine and piperazine rings adopt a chair conformation in solid state. The lowest energy conformation for these compounds is most likely the chair conformation in solid state. It agrees well with corresponding conformations in a similar compounds by using X-ray diffraction method [12-14].

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