after its injection differs little from the control experiment using an isotonic solution of NaCl whereas heparin, even in very small amounts, retards this process.

Thus, although mannan, like heparin, activates lipoprotein lipase in both *in vivo* and *in vitro* tests, mannan differs from heparin in having no anticoagulant properties, possibly because mannan contains no negatively charged groups such as the thio group.

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THE NITROGEN COUPLING OF AROMATIC AMINES WITH 2-ARYLIDENHYDRAZINO-4-PHENYLSELENAZOLES AND 2-ARYLIDENHYDRAZINO-4-PHENYLTHIAZOLES AND THEIR ANTIMICROBIAL ACTIVITY

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UDC 615.281.015.2:[547.553+547.551.525.211.1

Continuing the work described in [1, 2], we have carried out the nitrogen coupling of aromatic amines and sulfanylamides with some previously prepared 2-arylidenhydrazino-4-phenylselenazoles [1] to give compounds I-X (Table 1). The bacteriostatic and mycostatic activity of these compounds was studied, as well as the activities of some of the compounds prepared earlier [1, 2]. To determine the effect of the selenium atom on the biological activity, a number of thio analogs — XI-XIV (Table 2) and XV-XX (Table 3) — were synthesized.

The antimicrobial activity of the compounds was studied by the method of double serial dilution in liquid nutrient medium; three microorganisms were studied — *staphylococcus*, *Escherichia coli*, and *microsporum*. Bacteriostatic activity was studied using Khottinger broth (pH 7.2-7.4), and mycostatic activity in Sabouraud's medium (pH 6.0-6.8). For bacteria the microbial loading was $2.5 \cdot 10^5$ cells per ml of medium, and for the fungus, $5 \cdot 10^5$ reproductive bodies per ml of medium. The maximum concentration of test substance employed was 200 μg/ml. Activities of the compounds were obtained from the minimum bacteriostatic and mycostatic concentrations, expressed in μg per ml of medium. The results are given in Table 4.

In agreement with earlier work, all the tested 2-arylidenhydrazino-4-phenylselenazoles [1] were found to be active against Gram-positive bacteria. This activity is higher in compounds containing o-hydroxy and 3,4-dimethoxy substituted benzylidene groups, and is not affected by the p-nitro group in the 4 position of the phenyl radical.

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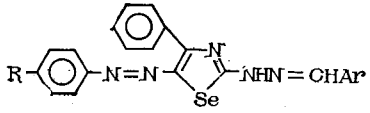
Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 13, No. 11, pp. 54-57, November, 1979. Original article submitted May 4, 1979.

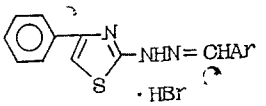
0091-150X/79/1311-1151$07.50

TABLE 1. 2-Arylidenhydrazino-4-phenyl-5-p-R-phenylazoselenazoles (I–X)



Compound	Ar	R	Yield, %	Melting point, °C	Found, % N	Empirical formula	Calculated, % N
I	2-OH-C_6H_4	SO_3H	92	187—9	13,38	$C_{22}H_{17}N_5O_4SSe$	13,30
II	C_6H_5	$COOC_2H_5$	86	222—4	14,02	$C_{25}H_{21}N_5O_2SSe$	13,95
III	2-OH-C_6H_4	$COOC_2H_5$	93	242—4	14,95	$C_{25}H_{21}N_5O_3SSe$	14,89
IV	4-$CH_3OC_6H_4$	$COOC_2H_5$	75	220—2	13,22	$C_{26}H_{23}N_5O_3SSe$	13,15
V	2-OH-C_6H_4	SO_2NH_2	92	262—4	16,08	$C_{22}H_{18}N_6O_3SSe$	15,99
VI	3-CH_3O-4-OH-C_6H_3	SO_2NH_2	94	233—5	15,21	$C_{23}H_{20}N_6O_4SSe$	15,13
VII	3,4-$(CH_3O)_2C_6H_3$	$SO_2NHCOCH_3$	78	220—2	13,82	$C_{26}H_{24}N_6O_5SSe$	13,74
VIII	4-$CH_3OC_6H_4$	$SO_2NHC(NH)NH_2$	86	235—7	19,32	$C_{24}H_{22}N_8O_3SSe$	19,28
IX	3-CH_3O-4-OH-C_6H_3	$SO_2NHC(NH)NH_2$	91	194—6	18,83	$C_{24}H_{22}N_8O_4SSe$	18,74
X	3,4-$(CH_3O)_2C_6H_3$	SO_2NH-(3,4-dimethylpyrimidyl-2)	81	173—5	16,63	$C_{30}H_{28}N_8O_4SSe$	16,58

TABLE 2. 2-Arylidenhydrazino-4-phenylthiazole Hydrobromides



Compound	Ar	Yield, %	Melting point, °C	Found, % N	Empirical formula	Calculated, % N
XI	C_6H_5	96	192—4	11,71	$C_{16}H_{13}N_3S \cdot HBr$	11,66
XII	$2\text{-}OH\text{-}C_6H_4$	89	188—90	11,23	$C_{16}H_{13}N_3OS \cdot HBr$	11,16
XIII	$4\text{-}CH_3OC_6H_4$	85	148—50	10,84	$C_{17}H_{15}N_3OS \cdot HBr$	10,76
XIV	$3\text{-}CH_3O\text{-}4\text{-}OH\text{-}C_6H_3$	82	138—40	10,41	$C_{17}H_{15}N_3O_2S \cdot HBr$	10,34

The product obtained from coupling with diazotized sulfanilic acid (I) possesses moderate activity against pathogenic fungi. The coupling of 2-arylidenhydrazino-4-phenylselenazoles and diazotized benzocaine gave compounds II-IV, which displayed negligible bacteriostatic activity.

Tests with 2-arylidenhydrazino-4-phenyl-5-p-R-sulfamoylphenylazoselenazoles confirmed that the majority of these compounds were more active against pathogenic fungi than against bacteria. The products obtained from 2-benzylidenhydrazino-4-phenylselenazole and from 2-p-methoxybenzylidenhydrazino-4-phenylselenazole with diazotized sulfanilylurea and with N^1-acetylsulfanilamide possess considerable activity against Gram-positive microorganisms. Coupling products from 2-arylidenhydrazino-4-phenylselenazoles with sulfanilamides (V and VI), N^1-acetylsulfanilamide (VII), and sulfanilylurea display the same activity as the starting compound.

The thio analogs (XI-XX) displayed no antifungal activity; compounds XVI and XX displayed antibacterial activity. The antimicrobial activity of compound XX was considerably lower than that of its selenium analog 2-benzylidenhydrazino-4-phenyl-5-[p-(5-ethyl-1,3,4-thiadiazolyl-2) sulfamoylphenylazo]selenazole. From this it can be concluded that the antimycotic activity is associated with the selenium atom, whereas the antibacterial activity is dependent not only on the sulfanilamide part of the molecule with its phenolic hydroxyl group, but also on the hydrazone group.

The results of this investigation show that the azo derivatives of the 2-arylidenehydrazino-4-phenylselenazoles, the 2-arylidenhydrazino-4-phenylselenazoles, like the parent compounds, display some biological activity. It was also shown that the selenium atom plays a role in the manifestation of antimicrobial properties.

EXPERIMENTAL METHOD

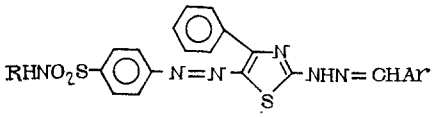
2-(4-Methoxybenzylidenhydrazino)-4-phenyl-5-p-guanidinosulfonylphenylazoselenazole (VIII). Sulfaguanidine (2.32 g, 0.01 mole) was dissolved in hydrochloric acid (10 ml, 1:3) with mixing, the solution cooled to 0°C, and a solution of sodium nitrite (0.69 g, 0.01 mole) in water (4 ml) added dropwise. This was then slowly added to a solution of 2-(4-methoxybenzylidenhydrazino)-4-phenylselenazole (4.37 g, 0.01 mole) in ethanol (10 ml) to give a thick dark orange paste. Recrystallization from ethanol gave 5.05 g (86%) of compound VIII as orange plates with mp 235-237°C. Found, %: N 19.32; Se 13.45. $C_{24}H_{22}N_8O_3SSe$. Calculated, %: N 19.28; Se 13.57. Compounds I-VII and IX-X were prepared in the same way.

2-Benzylidenhydrazino-4-phenylthiazole Hydrobromide (XI). To benzaldehyde thiosemicarbazone (3.58 g, 0.02 mole) was added α-bromoacetophenone (3.97 g, 0.02 mole) and ethanol (40 ml). On mixing, a cream colored paste separated, and this was recrystallized from propanol to give 5.42 g (96%) of compound XI as fine, pale yellow needles with mp 192-194°C. Found; %: N 11.71. $C_{16}H_{13}N_3S \cdot HBr$. Calculated, %: N 11.66.

Compounds XII-XIV were prepared in the same way.

2-Benzylidenhydrazino-4-phenyl-5-p-acetylsulfamoylphenylazothiazole (XV). To a solution of N^1-acetylsulfanilamide (2.54 g, 0.01 mole) in 10 ml hydrochloric acid (1:3) at 0°C was added dropwise a solution of sodium nitrite (0.69 g, 0.01 mole) in water (4 ml). This was added to a solution of 2-benzylidenhydrazino-4-phenylthiazole (3.64 g, 0.01 mole) in ethanol (40 ml) to give a thick orange paste. Recrystallization from propanol gave 5.63 g (96%) of

TABLE 3. 2-Arylidenhydrazino-4-phenyl-5-p-R-sulfamoylphenylazothiazoles



Compound	Ar	R	Yield, %	Melting point, °C	Found, % N	Empirical formula	Calculated, % N
XV	C_6H_5	$COCH_3$	96	267—9	16,69	$C_{24}H_{20}N_6O_3S_2$	16,62
XVI	2-OH-C_6H_4	$COCH_3$	92	260—2	16,20	$C_{24}H_{20}N_6O_4S_2$	16,14
XVII	4-$CH_3OC_6H_4$	$COCH_3$	87	208—10	15,79	$C_{25}H_{20}N_6O_4S_2$	15,72
XVIII	3-CH_3O-4-OH-C_6H_3	$COCH_3$	91	269—71	15,33	$C_{25}H_{22}N_6O_5S_2$	15,26
XIX	C_6H_5	$CONH_2$	86	180—2	19,44	$C_{23}H_{19}N_7O_3S_2$	19,39
XX	C_6H_5	5-ethyl-thiadiazol-yl-2	82	247—9	19,40	$C_{26}H_{22}N_8O_2S_3$	19,49

TABLE 4. Antimicrobial Activity of Products of Nitrogen Coupling of Aromatic Amines with 2-Arylidenhydrazino-4-phenylselenazoles and 2-Arylidenhydrazino-4-phenylthiazoles

Compound	Minimum concentrations, suppressing growth of microorganisms, μg/ml		
	Staphylococcus aureus 209-p	Escherichia coli 675	Microsporum lanosum
I	>200	>200	25
II	>200	>200	>200
III	>200	>200	50
IV	200	>200	100
V	25	>200	50
VI	>200	>200	200
VII	100	>200	200
VIII	200	>200	200
IX	100	>200	200
X	200	>200	50
XI	>200	>200	>200
XII	>200	>200	>200
XIII	>200	>200	>200
XIV	>200	>200	>200
XV	>200	>200	200
XVI	50	>200	>200
XVII	>200	>200	>200
XVIII	>200	>200	>200
XIX	>200	>200	>200
XX	12,5	>200	>200

compound XV as orange needles with mp 269-271°C. Found, %: N 16.69. $C_{24}H_{20}N_6O_3S_2$. Calculated, %: N 16.62.

Compounds XVI-XX were prepared in the same way.

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