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SYNTHESIS AND ANTIVIRAL ACTIVITY OF INDOLE AND BENZOFURAN SULFIDES

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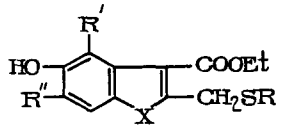
New arbidol analogs of the indole and benzofuran series were synthesized. The phenyl in them was replaced with substituted phenyl or separated from the sulfide bridge by a methylene group. The antivirus activity of the synthesized compounds was studied using enzyme-linked immunoassay and a murine influenza-induced pneumonia model. Both 5-hydroxyindole and 5-hydroxybenzofuran derivatives were found to have antiviral activity.

Arbidol (hydrochloride, monohydrate of 1-methyl-2-phenylthiomethyl-3-ethoxycarbonyl-4-dimethylaminomethyl-5-hydroxy-6-bromindole) is a highly effective drug with an antiviral, interferon-inducing, and immunostimulating effect [1].

We were interested in synthesizing analogs of arbidol in the indole and benzofuran series in which the phenyl is replaced with substituted phenyl or is separated from the sulfide bridge by a methylene group, and then studying their antiviral activity.

The starting compounds used were the previously described 2-bromomethyl-3-ethoxycarbonyl-5-acetoxybenzofuran (I) [2] and 1-methyl-2-bromomethyl-3-ethoxycarbonyl-5-acetoxy-6-bromoindole (II) [3]. By condensing them with *p*-methyl-, *p*-ethyl-, *p*-ethoxy-, *p*-chloro-, *o*-hydroxymethyl-, and *o*-nitrothiophenol, and also with benzylthiol, we obtained derivatives of the benzofuran and indole series. The latter, without separation, were deacetylated by the action of hydrochloric acid or potassium hydroxide up to the corresponding 5-hydroxy derivatives. This technique was used to prepare 2-*p*-methyl- (IIIa), 2-*p*-ethyl- (IIIb), 2-*p*-ethoxy (IIIc), 2-*p*-chloro- (IIId), 2-*o*-hydroxymethyl- (IIIe), and 2-*o*-nitrophenylthiomethyl-3-ethoxycarbonyl-5-hydroxybenzofuran (IIIf), and also the analogous 2-derivatives of 1-methyl-3-ethoxycarbonyl-5-hydroxy-6-bromoindole (IIIa – e). For the 2-*o*-nitrophenylthiomethyl indole derivative, the use of an equimolar amount of potassium hydroxide for deacetylation of the intermediate 5-acetoxy derivative failed to produce the desired result, and 1-methyl-2-*o*-nitrophenylthiomethyl-3-ethoxycarbonyl-5-acetoxy-6-bromoindole (IVf) was separated with a yield of 39%. When a two-fold excess of KOH was used for deacetylation of the intermediate IVf, the corresponding 5-hydroxyindole (IVg) was obtained with a yield of 27%. Condensation of compound II with benzylthiol and subsequent basic deacetylation yielded the corresponding 5-hydroxy-2-benzylthiomethyl derivative of indole IVh.

By aminomethylation of the compounds IIIa – e and IVa – d, g, h with bis-dimethylaminomethane, we obtained the corresponding 4-dimethylaminomethyl derivatives and the hydrochlorides (V – XV).



R = *p*-$CH_3C_6H_4$ (IIIa, IVa, V, X),
p-$C_2H_5C_6H_4$ (IIIb, IVb, VI, XI),
p-$C_2H_5OC_6H_4$ (IIIc, IVc, VII, XII),
p-ClC_6H_4 (IIId, IVd, VIII, XIII),
o-$HOCH_2C_6H_4$ (IIIr, IVe, IX),
o-$NO_2C_6H_4$ (IIIf, IVg, XIV), $CH_2C_6H_5$ (IVh, XV);
R' = H (IIIa – e; IVa – h), $CH_2N(CH_3)_2$ (V – XV);
R'' = H (IIIa – e, V – IX), Br (IVa – h, X – XV);
X = O (IIIa – f, V – IX), NCH_3 (IVa – h, X – XV).

The aminomethyl group in one of the sulfur-containing derivatives of indole, namely, in the arbidol base, was replaced with an acetoxymethyl group by heating the arbidol with Ac_2O. We isolated 1-methyl-2-phenylthiomethyl-3-ethoxycarbonyl-4-acetoxymethyl-5-acetoxy-6-bromoindole (XVI) with a yield of 80.6%.

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CHEMICAL EXPERIMENTAL PART

The elemental analysis results and the molecular weights correspond to the calculated values ones. The synthesized compounds are characterized in Table 1.

General method for preparing 2-R-thiomethyl-3-ethoxycarbonyl-5-hydroxybenzofurans (IIIa – f). To a solution of 0.02 mol of KOH in 95 ml of methanol at room temperature, we added 0.02 mole of a mercaptan, stirred it for 10 min, added 0.02 mole of compound I, and stirred the mixture at room temperature for three hours. We added 20 ml of concentrated HCl to the reaction mixture and after boiling for 0.5 h poured the suspension into water. We separated the precipitate, washed it with water, dried it, and purified it in a column with silica gel with subsequent recrystallization.

General method for preparing 1-methyl-2-R-thiomethyl-3-ethoxycarbonyl-5-hydroxy-6-bromoindoles (IVa – h). To a solution of 0.06 mole of KOH (for compound IVg – 0.08 mole of KOH) in 120 ml of methanol at room temperature, we added 0.02 mole of a mercaptan, stirred it for 10 min, added 0.02 mole of compound II in a single dose, stirred the mixture for three hours at room temperature, and acidified it to a pH of 7.0 with 5% AcOH. We separated the precipitate, washed it with water, dried it, and purified it in a column with silica gel or by recrystallization.

General method of preparing Mannich bases (V – XV). We boiled a solution (0.03 mole) of compound IIIa in 150 ml of dioxane for three hours with 0.08 mol of bis-dimethylaminomethane. We distilled off the solvent and excess amine, and purified the residue in a column.

We neutralized a suspension of the base in ether with an ether solution of hydrogen chloride. We purified compound V by recrystallization.

Compounds VI – XV were prepared similarly to compound V.

1-Methyl-2-phenylthiomethyl-3-ethoxycarbonyl-4-acetoxymethyl-5-acetoxy-6-bromoindole (XVI). We boiled a solution of 4.77 g (0.01 mole) of the arbidol base in 10 ml of acetic anhydride for six hours. We diluted the reaction mixture with water. We separated the precipitate, washed it with

TABLE 1. Characteristics of Synthesized Sulfur-Containing Derivatives of Benzofuran and Indole

Compound	Molecular formula	Molecular weight	Yield, %	M. p., °C (solvent for crystallization)
IIIa	$C_{19}H_{18}O_4S$	342	96	135 – 137 (iso-PrOH)
IIIb	$C_{20}H_{20}O_4S$	356	58.4	124 – 126 (iso-PrOH– tef)
IIIc	$C_{20}H_{20}O_5S$	372	70	123 – 124 (iso-PrOH– tef)
IIId	$C_{18}H_{15}CLO_4S$	362	73.9	128 – 130 (iso-PrOH– tef)
IIIe	$C_{19}H_{18}O_5S$	358	10	107 – 109 (iso-PrOH– tef)
IIIf	$C_{18}H_{15}NO_6S$	373	2.1	192 – 194 (eth)
IVa	$C_{20}H_{20}BrNO_3S$	433	74	195 – 197 (a)
IVb	$C_{21}H_{22}BrNO_3S$	447	54	157 – 160 (alc)
IVc	$C_{21}H_{22}BrNO_4S$	463	57.6	189 – 192 (dioxane)
IVd	$C_{19}H_{17}BrClNO_3S$	453	75.6	182 – 185 (benz)
IVe	$C_{20}H_{20}BrNO_4S$	449	8.4	155 – 158 (with decomp.) (iso-PrOH— alc)
IVf	$C_{21}H_{19}BrN_2O_6S$	506	39	115 (a)
IVg	$C_{19}H_{17}BrN_2O_5S$	464	27	> 250 (DMF)
IVh	$C_20H_{20}BrNO_4S$	433	49	164 – 11167 (a)
V	$C_{22}H_{25}NO_4S \cdot HCl$		25	185 – 188 (ac—MeOH—eth)
VI	$C_{23}H_{27}NO_4S \cdot HCl$		85.5	184 – 185 (ac–MeOH–eth)
VII	$C_{23}H_{27}NO_5S \cdot HCl$		92.3	169 – 170 (ac–MeOH–eth)
VIII	$C_{21}H_{22}ClNO_4S \cdot HCl \cdot H_2O$		83.3	183 – 5 (ac–MeOH–eth)
IX	$C_{22}H_{25}NO_5S \cdot HCl$		55	187 – 190 (iso-PrOH–eth)
X	$C_{23}H_{27}BrN_2O_3S$	490	71	121 – 123 (iso-PrOH)
	$C_{23}H_{27}BrN_2O_3S \cdot HCl \cdot H_2O$		64	148 – 150 (ac–MeOH)
XI	$C_{24}H_{29}BrN_2O_3S \cdot HCl \cdot H_2O$		30.5	100 – 102 (ac–MeOH–eth)
XII	$C_{24}H_{29}BrN_2O_4S$	520		127 – 129 (a)
	$C_{24}H_{29}BrN_2O_4S \cdot HCl \cdot H_2O$		40.4	162 – 164 (ac–MeOH)
XIII	$C_{22}H_{24}BrClN_2O_3S$		61.6	144 – 146 (chlf)
	$C_{22}H_{24}BrClN_2O_3S \cdot HCl$		31.1	182 – 184 (with decomp.) (iso-PrOH–MeOH–eth)
XIV	$C_{22}H_{24}BrN_3O_5S$	521	45	158 – 160 (ac)
XV	$C_{22}H_{24}BrN_3O_5S$		44.1	103 – 105 (iso-PrOH)
	$C_{25}H_{31}BrN_2O_3S$		68	144 – 146 (ac–MeOH–eth)
	$C_{25}H_{31}BrN_2O_3S \cdot HCl$		24.7	175 – 177 (ac–MeOH–eth)

water, and recrystallized it from methanol. We obtained 4.3 g (80.6%) of compound XVI, m.p. 148 – 149°C.

BIOLOGICAL EXPERIMENTAL PART

Study of the antiviral activity by enzyme-linked immunosorbent (ELIS). We studied the antiviral activity of the arbidol analogs by using the modified ELIS method described previously [4]. We used the influenza virus A/Japan (H_2N_2) together with MDSC cells that we cultivated in 96-well plates from the Costar company in medium 199 with the addition of 10% fetal calf serum and 10 mm of glutamine. We used all the compounds being tested in a final concentration of 10 μg /ml. After incubation of the preparation (30 min at 37°C), we added 10 μliter of the virus into the wells (excluding the cell control); the virus dilutions were prepared in medium 199 with trypsin. The cells were incubated for 15 – 18 h at 37°C. The monoclonal antibodies to the viral proteins (NP, M) of the influenza virus A were kindly provided by Dr. Kendal (the WHO Center for Infections Disease Control, Atlanta, USA). The maximum value of OD_{490} of the control was subtracted from the rest of the OD values. For each virus dilution, we found the mean value of OD_{490}, and determined the average percent of reduction of OD_{490} by the compound being tested (8 wells). We studied the cytotoxic effect of the chemical substances using an automatic analyzer.

In the comparative studies for determining the activity of the compounds being investigated, we selected the conditions of the experiment so that the inhibition of the virus reproduction by arbidol would be about 60%. The activity in the ELIS was expressed in percent of OD_{490} suppression.

Study of the antiviral activity using a model of murine influenza-induced pneumonia. We studied the antiviral activity of the compounds with respect to the influenza A virus/Bethesda/63 (H_2N_2) using a model of murine influenza-induced pneumonia caused by internasal innoculation with 10 LD_{50} of the virus, which caused the death of 80 – 90% of the animals in the control group. The substances being studied were administered internally in doses of 120 – 60 – 30 mg/kg daily for five days. Each dose was administered to a group of 20 animals. The observation period was 14 days. We determined the activity of the compounds from the drop in lethality in percent relative to the control group.

RESULTS AND DISCUSSION

The compounds IIIc, VI, VII, and VIII in concentrations of 5 – 10 μg /ml exhibit a cytotoxic effect and were not selected for further study.

Among the compounds studied in the ELIS system, the compounds IIIe, IVa, IVb, IVd – g, IX, and XIV do not suppress viral reproduction. The compounds IIIb, IVc, VI, and XIII exhibit low activity (the suppression of OD_{490} was 12 – 30%). Among the derivatives of 5-hydroxybenzofuran, a high (in comparison with the 60% of arbidol) antiviral activity was exhibited by compounds IIIa (48%) and V (54%), and among the derivatives of 5-hydroxyindole by compounds X (54%), XI (48%), XII (55%), XV, and XVI (54%). In the model of influenza-induced pneumonia, compound V in a dose of 60 mg /kg lowers lethality by 35%, and compounds X, XI, XII, XIII, XV, and XVI lower lethality by 30, 20, 15, 20, 30, and 20% in comparison with the 60% for arbidol.

Consequently, our restuls, like those obtained earlier [3], show that a number of derivatives of 5-hydroxyindole and 5-hydroxybenzofuran exhibit a comparatively high antiviral activity in vitro and in vivo, which points to good prospects for continuing searches in these directions.

REFERENCES

1. USSR Inventors Certificate No. 1685933, *Otkrytiya*, No. 39. (1991).
2. F. A. Trofimov, N. G. Tsyshkova, and A. N. Grinev, *Khim. Geterotsikl. Soed.*, No. 3, 308 – 311 (1973).
3. S. A. Zotova, T. M. Korneeva, V. I. Shvedov, et al., *Khim-Farm. Zh.*, **26**(1), 181 – 186 (1992).
4. N. I. Fadeeva, I. A. Leneva, E. K. Panisheva, et al., *Khim.-Pharm. Zh.*, **26**(9 – 10), 17 – 20 (1992).