SYNTHESIS AND ANTIVIRAL ACTIVITY OF NEW BISACRIDINYLHYDRAZIDES OF ARYLOXYACETIC ACIDS

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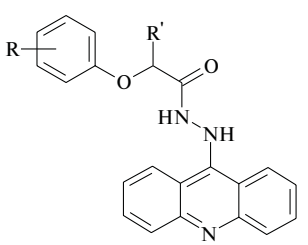
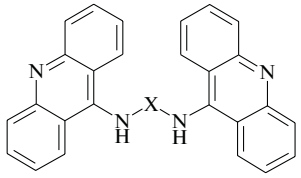
The class of aminoacridine derivatives contains compounds possessing antitumor [1], antimicrobial [2], and antiviral [3] properties. As for acridinylhydrazine derivatives, it was only established that some aryloxy acid acridinylhydrazides (I) exhibited fungicidal and schistosomiacidal activity [4 – 8]. Previously [9], we reported that biodegradable bisacridines (II, where X is a fragment containing amide or ester bonds) may possess significant antiviral properties [9].



I



II

In this context, we have synthesized and characterized a series of bisacridinylhydrazides of dicarboxylic acids XXV – XXXII. The target compounds were synthesized according to the scheme. In the first step, 4,4′-dihydroxydiphenyl (III), 2,2-bis(4-hydroxyphenyl)propane (IV), and 1,3- and 1,4-dihydroxybenzene (V, VI) of *meta*- and *para*-hydroxybenzoic acid methylates (VII, VIII) were alkylated by methylbromoacetate in boiling acetone in the presence of potassium carbonate. The resulting diesters IX – XIV, as well as methyl esters of isophthalic and terephthalic acids (XV, XVI), were subjected to hydrazinolysis to obtain hydrazides XVII – XXIV. These hydrazides were acridinylated by boiling with methoxyacridine in methanol for 2 – 5 days. The target bisacridinylhydrazides XXV – XXXII were obtained in a chromatographically pure state with a yield of 75 – 85% directly from the reaction mixtures.

Purity of the target and intermediate compounds was checked by TLC on Silufol UV-254 plates eluted in various solvent systems. The compositions were confirmed by elemental analyses; the proposed structures, by the data of IR spectroscopy and mass spectrometry. The IR spectra exhibited absorption bands characteristic of the intermolecularly associated N–H bonds (3300 – 3250 cm^{-1}), aromatic and aliphatic C–H bonds (3010 – 2930 cm^{-1}), ester and hydrazide C=O groups (1710 – 1700 and 1650 – 1620 cm^{-1}, respectively), and double aromatic bonds (1500 – 1400 cm^{-1}). The mass spectra of all synthesized compounds displayed the peaks due to molecular ions with intensities decreasing in the following order: diester > dihydrazide > bisacridinylhydrazide. The yields and physicochemical characteristics of the synthesized compounds are summarized in Table 1.

The antiviral activity of the synthesized compound was studied with respect to the influenza strain A2/Honkong H_1N_1 (viral diagnosticum) in reference to amyxin [10]; the tests were performed *in ovo* by conventional methods [11]. The results of these investigations are summarized in Table 2.

Some of the synthesized compounds (XXV – XXVII) showed significant antiviral activity, as characterized by high values (above 66%) of the protection index (PI). Some other products were much less active (PI ≤ 50%), while two compounds (XXX and XXXI) exhibited no antiviral effect at all while being rather toxic (leading to the loss of one and three test embryos in a group of six, respectively, in the absence of any loss in the control group). Tested under analogous conditions, amyxin was characterized by PI = 66.67%. Thus compounds XXVI and XXVII exceeded amyxin in the antiviral activity, while compound xXV was comparable with the reference drug in this respect. All tested substances led to a decrease in the viral titer, except for compound XXXI where the decrease was not as reliable relative to control ($p > 0.05$).

The obtained results confirm our previous data concerning the antiviral activity of bisacridines [9]. Moreover, now

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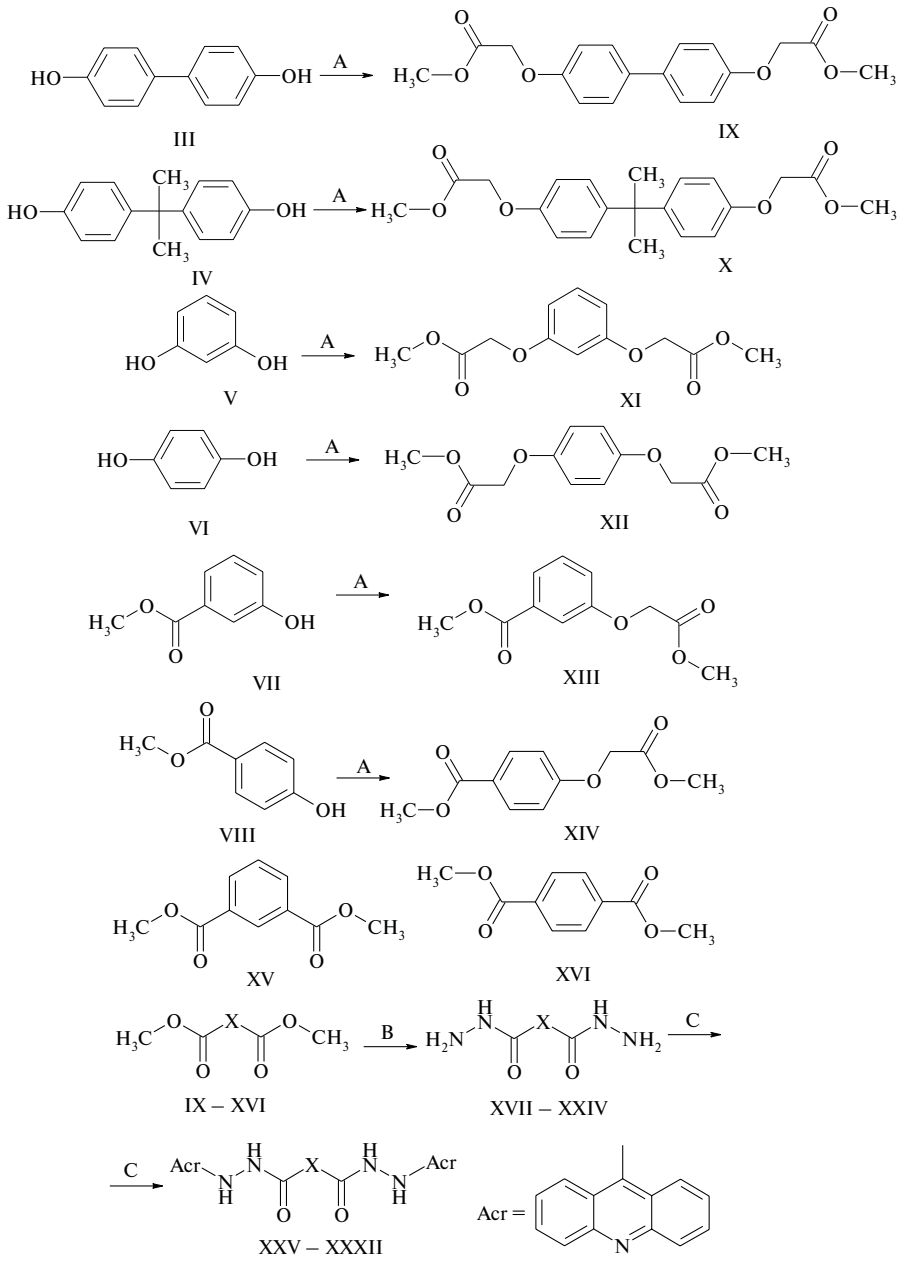
Scheme



A: $BrCH_2COOCH_3$, K_2CO_3, $(CH_3)_2CO$, 55 °C, 8 h
B: $N_2H_4 \cdot H_2O$, CH_3OH, 65 °C, 8 – 10 h
C: $AcrOCH_3$, CH_3OH, 65 °C, 50 – 120 h

we can establish certain relationships between the antiviral properties and structure of these compounds. In particular, the derivatives of isophthalic and terephthalic acids (XXXI, XXXII) exhibit virtually no activity (PI = 0 and 16.67%, respectively). Introduction of the oxymethylene fragment between carbonyl group and aromatic ring led to a decrease in the viral titer and even to an increase in the PI value. The second oxymethylene fragment introduced between the aromatic ring and another carbonyl increases the PI values of compounds XXVIII and XXVII up to 16.67 and 83.33%, respectively (the average viral titer being 3.17 and 1.59, respectively).

Thus, an increase in the distance between acridine chromophores and in the flexibility of a linker fragment enhances the antiviral activity of compounds, this rule being valid in the series of both *meta* and *para* derivatives. The antiviral activity significantly increases upon going from *para* derivatives with a single aryl fragment to their analogs containing two benzene rings. The antiviral properties of the latter compounds are comparable with those of amyxin, while the resorcinol derivative XXVII exceeds the reference drug both in the PI value and in the viral titer characteristic.

Thus the synthesized bisacridines are of interest for the further study as potential antiviral agents.

EXPERIMENTAL
CHEMICAL PART

The mass spectra were recorded with an MX-1321 instrument using a direct sample introduction scheme. The ion source was operated at an electron impact energy of 70 eV and a temperature of 220°C. The IR spectra were measured in chloroform, carbon tetrachloride, or Vaseline oil in a Specord 75 spectrophotometer. The UV spectra were recorded with Specord M-40 and Perkin-Elmer Lambda 9 spectrophotometers. TLC was performed on Silufol UV-254 and UV-366 plates. The syntheses were conducted with commercial reagents of not less than 97% purity relative to the main component.

1,4-Bis(carbomethoxy)methoxybenzene (XII). A mixture of 2.2 g (0.02 mole) hydroquinone, 6.9 g (0.05 mole) potassium carbonate, and 3.97 ml (0.042 mole) of methylbromoacetate was boiled for 10 h with intensive stirring (preferably, under inert atmosphere), cooled to room temperature, and filtered. The filtrate was evaporated under vacuum to dryness and the dry residue was recrystallized from aqueous methanol to obtain 3.32 g (65%) of compound XII.

Analogous procedures with phenols III – V, VII, and VIII were used to obtain bis-esters IX – XI, XIII, and XIV, respectively (Table 1).

2-[3-(Hydrazinocarbonylmethoxy)phenoxy]acetylhydrazine (XIX). To a solution of 3.3 g (0.013 mole) of bis-ester

TABLE 1. Yields and Physicichemical Properties of the Synthesized Compounds

Compound	Yield, %	M.p.,°C	Solvent	Molecular weight.	Empirical formula	$[M]^+$	TLC mobility	
							R_f	Eluent**
IX	53	188 – 189	Dioxane	330.34	$C_{18}H_{18}O_6$	330	0.46	Chl–EA, 10 : 1
X	97	††	…	372.42	$C_{21}H_{24}O_6$	372	0.58	Chl–Ace, 5 : 1
XI	66	62 – 63	MeOH–H_2O	254.24	$C_{12}H_{14}O_6$	254	0.63	Chl–Ace, 7 : 1
XII	65	98 – 99	MeOH–H_2O	254.24	$C_{12}H_{14}O_6$	254	0.55	Chl–Ace, 7 : 1
XIII	98	††	…	224.22	$C_{11}H_{12}O_5$	224	0.54	Chl–Ace, 7 : 1
XIV	71	95 – 96	MeOH–H_2O	224.22	$C_{11}H_{12}O_5$	224	0.63	Chl–Ace, 7 : 1
XVII	47	> 326 p†	MeOH	330.35	$C_{16}H_{18}N_4O_4$	330	0.08	Chl–Ace, 3 : 1
XVIII	74	191 – 192	MeOH	372.43	$C_{19}H_{24}N_4O_4$	372	0.20	Chl–MeOH, 5 : 1
XIX	95	246 – 247	MeOH	254.25	$C_{10}H_{14}N_4O_4$	254	0.07	BuOH–EtOH–H_2O, 2 : 2 : 1
XX	93	262 – 263	MeOH	254.25	$C_{10}H_{14}N_4O_4$	254	0.04	MeOH–DMF–AcOH, 10 : 1 : 3
XXI	41	158 – 159	MeOH	224.22	$C_9H_{12}N_4O_3$	224	0.38	Chl–MeOH, 5 : 1
XXII	83	202 – 203	MeOH	224.22	$C_9H_{12}N_4O_3$	224	0.33	Chl–MeOH, 5 : 1
XXIII	70	238 – 239	MeOH	194.19	$C_8H_{10}N_4O_2$	194	0.46	MeOH–TEA–DMF, 20 : 1 : 2
XXIV	94	> 360 p	MeOH	194.19	$C_8H_{10}N_4O_2$	194	0.00	MeOH–TEA–DMF, 20 : 1 : 2
XXV	69	> 272 p	MeOH	684.76	$C_{42}H_{32}N_6O_4$	684	0.72	MeOH–TEA–DMF, 20 : 1 : 2
XXVI	44	> 188 p	MeOH	726.84	$C_{45}H_{38}N_6O_4$	726	0.11	Bnz–TEA–MeOH, 10 : 1 : 1
XXVII	90	> 256 p	MeOH	608.66	$C_{36}H_{28}N_6O_4$	608	0.06	Bnz–TEA–MeOH, 10 : 1 : 1
XXVIII	76	> 298 p	MeOH	608.66	$C_{36}H_{28}N_6O_4$	608	0.13	Bnz–TEA–MeOH, 10 : 1 : 1
XXIX	22	> 220 p	MeOH	578.64	$C_{35}H_{26}N_6O_3$	578	0.20	Bnz–TEA–MeOH, 10 : 1 : 1
XXX	41	224 – 225	MeOH	578.64	$C_{35}H_{26}N_6O_3$	578	0.56	Chl–MeOH, 5 : 1
XXXI	78	> 362 p	MeOH	548.61	$C_{34}H_{24}N_6O_2$	548	0.51	MeOH–TEA, 20 : 1
XXXII	84	> 374 p	MeOH	548.61	$C_{34}H_{24}N_6O_2$	548	0.00*	MeOH–TEA, 20 : 1

Notes: † (p), decomposition on melting; †† noncrystallizable oily product; * this substance was not eluted in any of the systems employed, while all initial reagents and potential intermediate products were eluted to a certain extent; ** solvents: Ace = acetone, BuOH = butanol, Bnz = benzene, DMF = dimethylformamide, MeOH-methanol, TEA = triethylamine, AcOH = acetic acid, Chl = chloroform, EtOH = ethanol, EA = ethyl acetate.

XI in 20 ml of methanol was added 1.5 ml (0.03 mole) of 100% hydrazine hydrate and the mixture was boiled with stirring for 8 h and cooled. The precipitate was separated by filtration, washed on the filter with water (3 × 10 ml) and methanol (3 × 10 ml), and dried under vacuum (10 – 15 Torr) at room temperature for 8 h to obtain 3.142 g (95%) of compound XIX.

Analogous procedures with bis-esters IX, X, and XII – XVI were used to obtain compounds XVII, XVIII, and XX – XXIV, respectively (Table 1).

N-[3-(N′-Acridin-9-yl-hydrazinocarbonylmethoxy]-phenoxy)acetyl-(N′-acridin-9-yl)hydrazine (XXVII). To a suspension of 0.254 g (0.001 mole) of bishydrazide XIX in 10 ml of methanol was added 0.627 g (0.003 mole) of methoxyacridine and the mixture was boiled with stirring for 50 h. The course of the reaction was monitored by TLC. After terminating the process and cooling the mixture, the precipitate was separated by filtration, washed on the filter with methanol (3 × 10 ml), and dried under vacuum (10 – 15 Torr) at room temperature for 8 h to obtain 0.554 g (90%) of compound XXVII.

Analogous procedures with bishydrazides XVII, XVIII, and XX – XXIV were used to obtain compounds XXV, XXVI, and XXVIII – XXXII, respectively (Table 1).

EXPERIMENTAL BIOLOGICAL PART

The antiviral activity of the synthesized compounds was studied by simultaneously introducing a sample and the test viral species into allantochorion cavity of a 10-day chicken

TABLE 2. Antiviral Activity of Compounds XXV – XXXII

Compound	Number of embryos			PC	PI	GMT
	total	infected	%			
XXV	6	2	33.33	3	66.67	1 : 2
XXVI	6	1	16.67	6	83.33	1 : 2.24
XXVII	6	1	16.67	6	83.33	1 : 1.59
XXVIII	6	5	83.33	1.2	16.67	1 : 3.17
XXIX	6	3	50	2	50	1 : 2.52
XXX	5	5	100	1	0	1 : 8
XXXI	3	3	100	1	0	1 : 10.1
XXXII	6	5	83.33	1.2	16.67	1 : 16
Amyxin	6	2	33.33	3	66.67	1 : 2
Control	6	6	100	–	–	1 : 128

embryo. The synthesized compounds were dissolved in DMSO to a concentration of 1.5 mg/ml and introduced in a dose of 0.1 ml per embryo. The viral species were diluted 1 : 100 with DMSO and introduced into control and test embryos in an amount of 0.1 ml. Each test and the control experiment were performed with sic embryos. The inoculated embryos were incubated in a thermostat for 48 h.

The antiviral activity was evaluated in terms of the protection index (PI) calculated by the formula $PI = ((PC - 1)/PC) \times 100\%$, where PC is the protection coefficient determined as the ratio of the fraction of virus-infected embryos in the control to that in the test group. For the same PI value, a higher activity is assigned to a substance characterized by a lower geometric mean titer (GMT) of the virus.

The GMT value was determined as the geometric mean of titers for each embryos in a group of six (the virus-free embryos were assigned a unity titer). The titration was performed with respect to the developed hemagglutination reaction with human erythrocytes of the zeroth group. The viral titer was determined as a maximum dilution of the allantois fluid for which erythrocyte agglutination was still observed in the form of a film with scalloped lacy edge. The results of titration were statistically processed relative to the control in terms of the Wilcoxon criterion for independent data sets [12].

REFERENCES

1. B. Granzen, D. E. Graves, B. D. Baguley, et al., *Oncol. Res.*, **4**, 489 – 496 (1992).
2. P. Chavalitshewinkoon, P. Wilairat, S. Gamage, et al., *Antimicrob. Agents Chemother.*, **37**, 403 – 406 (1993).
3. S. A. Lyakhov, Y. I. Suveyzdis, L. K. Berezina, et al., *Die Pharmazie*, **49**, 926 – 927 (1994).
4. US Patent No. 4711889 (1987); *Chem. Abstr.*, **108**, 167319x (1987).
5. P. B. Pathan and S. C. Bahel, *Bokin Bobai*, **8**, 247 – 250 (1980); *Chem. Abstr.*, **94**, 15532 (1981).
6. R. S. Sharma and S. C. Bahel, *Bokin Bobai*, **10**, 111 – 114 (1982); *Chem. Abstr.*, **97**, 6125 (1982).
7. V. K. Mishra and S. C. Bahel, *Bokin Bobai*, **11**, 103 – 105 (1983); *Chem. Abstr.*, **99**, 5493 (1983).
8. A. Srivastava, R. B. Pathak, and S. C. Bahel, *J. Indian. Chem. Soc.*, **62**, 486 – 487 (1985).
9. S. A. Lyakhov, *Abstracts of Papers. The 6th Int. Symp. on Molecular Aspects of Chemotherapy*, Gdansk (1997), p. 137.
10. S. A. Andronati, L. A. Litvinova, and N. Ya. Golovenko, *Zh. Akad. Med. Nauk Ukr.*, **5**(1), 53 – 65 (1999).
11. L. A. Litvinova, S. A. Andronati, G. V. Lempart, et al., *Khim.-Farm. Zh.*, **17**(2), 19 – 20 (1983).
12. A. M. Merkov and L. E. Polyakov, *Statistics iun Sanitary* [in Russian], Meditsina, Leningrad (1974), pp. 96 – 98.