The investigations with the ECHO virus were carried out on monolayer cultures of passaged human embryonic cutaneous-muscular cells; investigations with the remaining viruses were carried out on primary chicken embryo cells (CECs).

It was established that the compounds (I), (VI), and (VIII) possess antiviral properties (Table 2).

The substances indicated were mainly active in regard to the infection induced by VCBP. The hydrochloride of the amine (VIII) possesses a wide spectrum of activity, also showing inhibitory properties toward experimental infections of CECs induced by VSV, NDV, VVS, and VVEE.

LITERATURE CITED

1. V. M. Votyakov and E. I. Boreko, Methodical Problems of the Scientific Development of Antiviral Agents [in Russian], Minsk (1977), pp. 10-14.
2. I. Ya. Grava, Ya. Yu. Polis, M. Yu. Lidak, et al., Khim.-farm. Zh., No. 10, 42-45 (1980).
3. L. V. Denisova, L. I. Nikonovich, V. A. Saikovskaya, et al., Molecular Biology of Viruses: Chemotherapy and Chemoprophylaxis of Viral Infections [in Russian], Minsk (1974). pp. 111-119.
4. V. M. Plakhotnik, V. Yu. Kovtun, N. A. Leont'eva, et al., Khim.-farm. Zh., No. 9, 1060-1063 (1982).
5. K. Aigami, J. Inamoto, N. Takaishi, et al., J. Med. Chem., 18, 713-721 (1975).
6. H. A. Bruson, J. Am. Chem. Soc., 64, 2457-2461 (1942).
7. R. C. Fort, Adamantane: The Chemistry of Diamond Molecules, New York (1976), pp. 327-357.
8. P. Link, B. Rada, and D. Blaskovic, Ann. N.Y. Acad. Sci., 130, 31-43 (1965).
9. M. I. Marcialis, M. L. Schivo, P. Ucchedu, et al., Experientia, 29, 1442-1443 (1973).

SYNTHESIS AND ANTIVIRAL ACTIVITY OF 2-(1-ARYLOXY) AND 2-(1-ARYLAMINO-HYDROXYPROPYLAMINO)PYRIMIDINES AND THEIR ACYCLIC ANALOGS

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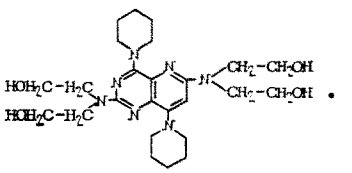
UDC 615.281.8:547.853].012.1

The search for antiviral drugs has been carried out amongst various types of chemical and biological compounds, and drugs in current use [4]. For example, antiviral activity has been found in dipyridamole, a coronary vasodilator and antithrombic drug:



Dipyridamole has been found to inhibit a wide range of viruses [11, 12], and some derivatives have been prepared and found to be possess antiviral activity [14]. In its mode of action, dipyridamole is an inhibitor of nucleoside transport in the cell, and totally suppresses the synthesis of viral RNA [13]. However, the synthesis of analogs of dipyridamole for this purpose has not been extensively pursued. For this reason, it appeared to us to be of interest to synthesize broader groups of pyrimidines and guanidines containing a propylamine moiety attached to nitrogen, which we regard as distant analogs of this drug.

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Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 21, No. 4, pp. 459-464, April, 1987. Original article submitted August 13, 1985.

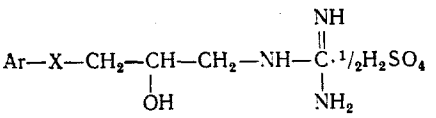
0091-150X/87/2104-0291$12.50

TABLE 1. Characteristics of Ions in the Mass Spectra of Aryl- and Hetarylpropanolamines

Compound	m/z, %							
	M	F_1	F_2	F_3	F_4	F_5	F_6	F_7
XVIII	286* (1)	171* (11)	184* (11)	72 (15)	102 (6)	268* (6)	59 (100)	155* (7)
XXII	236 (18)	121 (49)	134 (100)	72 (56)	102 (9)	218 (28)	59 (30)	105 (28)
XXV	278 (1)	163 (42)	—	72 (45)	102 (6)	260 (4)	59 (14)	105 (12)
	236 (9)**	121 (41)**	134 (100)**	—	—	218 (13)**		
XXVIII***	287 (3)	108 (10)	121 (6)	136 (96)	166 (100)	269 (1)	123 (8)	91 (6)
XXXII	286 (4)	107 (18)	120 (8)	136 (22)	166 (100)	268 (14)	123 (18)	91 (9)

*Ions containing ^{79}Br.
**Ion $(F_1-CH_2{=}CO)^+$
***In the case of (XXVIII), ions F_1, F_2, and F_5 contained O in place of NR^1.

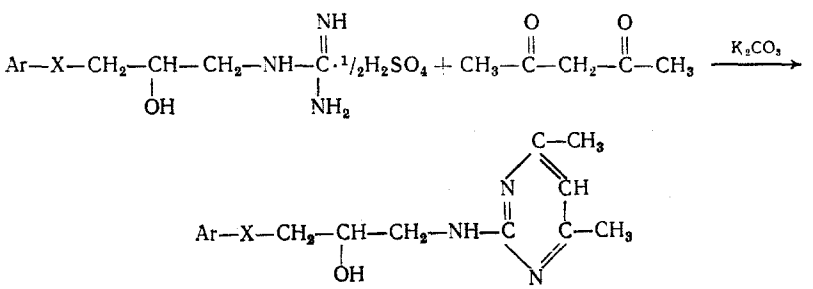
Such guanidines, which also display cardiovascular activity, were obtained by a method developed previously [5, 6].



I — XXVI

I, Ar = C_6H_5, X = O; II, Ar = $C_6H_4CH_3$-2, X = O; III, Ar = $C_6H_4CH_3$-3, X = O; IV, Ar = $C_6H_4CH_3$-4, X = O; V, Ar = C_6H_4Cl-2, X = O; VI, Ar = $C_6H_3Cl_2$-2,4, X = O; VII, Ar = C_6H_4Br-2, X = O; VIII, Ar = C_6H_4Br-4, X = O; IX, Ar = $C_6H_4CH_3O$-3, X = O; X, Ar = $C_6H_4CH_3O$-4, X = O; XI, Ar = $C_6H_4CH_2CH=CH_2$-2, X = O; XII, Ar = = $C_6H_3(CH_3)_2$-2,6, X = O; XIII, Ar = $C_6H_3CH_3$-3-$CH(CH_3)_2$-4, X = O; XIV, Ar = = $C_{10}H_7$-1, X = O; XV, Ar = C_6H_5, X = NC_2H_5; XVI, Ar = C_6H_5, X = NC_6H_5; XVII, Ar + X = 1,2,3,4-tetrahydroquinol-1-yl; XVIII, Ar = C_6H_4Br-3, X = NH; XIX, Ar = = $C_6H_3(CH_3)_2$-2,5, X = NH; XX, Ar = $C_6H_4CH_3$-2, X = NH; XXI, Ar = $C_6H_4CH_3O$-2, X = NH; XXII, Ar = $C_6H_3(CH_3)_2$-2,4, X = NH; XXIII, Ar = C_6H_5, X = NH; XXIV, Ar = $C_6H_4CH_3$-4, X = NH; XXV, Ar = $C_6H_3(CH_3)_2$-2,4, X = $NCOCH_3$; XXVI, Ar = = $C_6H_4CH_3$-4, X = $NCOCH_3$.

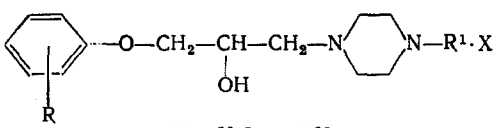
Condensation of these compounds with acetylacetone at 20°C in the presence of potassium carbonate gave the pyrimidines:



XXVII — XXXVI

XXVII, Ar = C_6H_5, X = O; XXVIII, Ar = $C_6H_4CH_3$-2, X = O; XXIX, Ar = =$C_6H_4CH_3$-3, X = O; XXX, Ar = $C_6H_4CH_3O$-4, X = O; XXXI, Ar = $C_6H_3Cl_2$-2,4, X = O; XXXII, Ar=$C_6H_4CH_3$-2, X = NH; XXXIII, Ar = C_6H_4Cl-2, X = NH; XXXIV, Ar = C_6H_4Br-2, X = NH; XXXV, Ar = $C_6H_4Cl_2$-2,6 X = NH; XXXVI, Ar = $C_6H_3CH_3$-4-Br-6, X = NH.

Propanol derivatives of piperazine, obtained as described in [7], are also of some pharmacological interest.



XXXVII — XLV

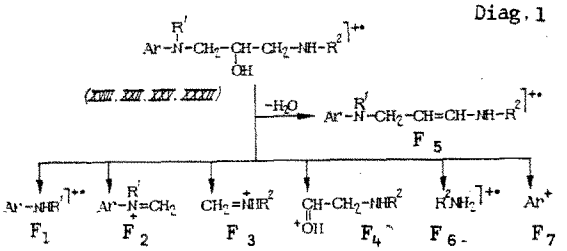
XXXVII, R = H, R^1 = C(=NH)NH_2; X = 1/2H_2SO_4; XXXVIII, R = 3-CH_3, R^1 = C(=NH)NH_2, X = 1/2H_2SO_4; XXXIX, R = 3-CH_3, R^1 = $CH_3CH(OH)CH_2OC_6H_4CH_3$-3, X = 2HCl; XL, R = H, R^1 = $CH_3CH(OH)CH_2C_6H_5$, X = 2HCl; XLI, R = 3-CH_3, R^1 = $CH_3COC_6H_4Br$-4, X = 2HCl; XLII, R = 3-CH_3, R^1 = $CH_2CONHC_6H_3(CH_3)_2$-2,4, X = 2HCl; XLIII, R = 3-CH_3, R^1 = H, X = 2HCl; XLIV, R = 2-Br, R^1 = H, X = 2HCl; XLV, R = $C_{10}H_7$-1, R^1 = H, X = 2HCl.

The structures and purities of the products were confirmed by elemental and chromatographic analyses, and in some of the compounds, by their UV and mass spectra.

The lipophilicities (R_m^0, see Experimental Chemistry) of the 1-aryloxy-and 1-arylamino-2-hydroxy-3-guanidinopropanes (I-XXVI) were almost identical in both series, ranging from 0.50 to 1.74. Within the series, the most lipophilic compounds contained bulky radicals in the aromatic ring, or halogens. The lipophilicity of the pyrimidines (XXVII-XXXVI) was much greater than that of the starting guanidines (apart from (XXX) and (XXXVI), the R_m^0 values ranging from 1.24 to 3.92 (Table 2). The lipophilicity index of the piperazines (XXXVII-XLV) varied from 0.16 to 1.67, NN'-disubstituted piperazines were more lipophilic, except for (XXXVIII) and (XL), the lipophilicites of which were 0.57 and 1.25 respectively.

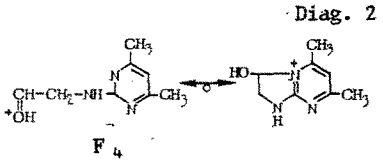
The UV spectra of the pyrimidines (XXVII-XXXVI) showed three characteristic [9] absorption bands, which were absent in the original guanidines (I-XXVI) [5, 6]. A short wavelength band of high intensity was seen at 202-204 nm for the aryloxy-compounds (XXVII-XXXI) and at 205-212 nm for the arylamino-derivatives (XXXII-XXXVI). A second band of the same intensity occurred at 226-239 and 241-251 nm, respectively. The third band, which was weak, was situated at 280-306 nm, vibrational structure being seen in some of the compounds.

Diag. 1



The mass spectra of the aryl- and hetaryl-1,3-diaminopropan-2-ols were characterized by relatively low-intensity molecular ion peaks (Diagram 1 and Table 1), which lose a molecule of water (ion F_5), or Fragment preferentially by the amine-type fragmentation of N-alkylaryl-amines (ions F_1, F_2, F_3, F_6, and F_7). To a small extent, alcohol-type fragmentation also takes place (ion F_4) which, however, becomes more likely when the cation formed can be stabilized by the presence of nitrogen atom in R^2. For example, the peak for ion F_4 becomes most intense in the spectrum of (XXXII) (Diagram 2). Fragmentation of the aryloxy-compound (XXVIII) takes place similarly.

Diag. 2



An examination of the mass spectra of pharmacologically active propanolamines and 1,3-diaminopropan-2-ols thus enables their structures to be confirmed on the one hand, and identifies characteristic fragments for the subsequent mass-fragmentographic examination of metabolites of these compounds in the body.

The presence of the same ions in representatives of the different series provides further confirmation of their similar structures.

The mode of cleavage of the piperazines under electron impact is exemplified by compound (XL) (Diagram 3). Diagram 3 gives the m/z values and relative intensities as percentages (in brackets).

TABLE 2. 2-[(1-Aryloxy) (1-Arylamino)-2-hydroxypropylamino]pyrimidines

Compound	Yield, %	mp., °C	k_j^*	R_m^0	Found, %		Empirical formula	Calculated, %		λ_{max}, nm (lgε)
					N	Cl (Br)		N	Cl (Br)	
XXVII	69	86—89	0,38	2,03	15,16	—	$C_{15}H_{19}N_3O_2$	15,38	—	202 (4,12), 239 (4,24), 280 (3,38), 302 (3,59)
XXVIII	72	100—101	0,38	2,47	14,35	—	$C_{16}H_{21}N_3O_2$	14,63	—	203 (4,33), 239 (4,23), 280 (3,43), 302 (3,51)
XXIX	74	80—81	0,39	2,41	14,23	—	$C_{16}H_{21}N_3O_2$	14,63	—	204 (4,36), 238 (4,22), 281 (3,55), 302 (3,48)
XXX	75	144—146	0,48	0,25	13,62	—	$C_{16}H_{21}N_3O_3$	13,86	—	203 (4,22), 226 (4,03), 290 (3,37)
XXXI	58	151—152	0,42	3,92	12,02	20,32	$C_{15}H_{17}Cl_2N_3O_2$	12,28	20,76	204 (4,56), 236 (4,40), 294 (3,73)
XXXII	83	65—66	0,36	2,32	19,30	—	$C_{16}H_{22}N_4O$	19,58	—	205 (4,54), 241 (4,45), 300 (3,73)
XXXIII	62	91—92	0,37	2,70	13,52	11,31	$C_{15}H_{19}ClN_4O$	13,70	11,58	206 (4,46), 243 (4,35), 297 (3,78)
XXXIV	57	96—98	0,46	2,96	15,59	22,41	$C_{15}H_{19}BrN_4O$	15,95	(22,77)	208 (4,53), 244 (4,43), 301 (3,80)
XXXV	85	171—173	0,39	1,24	16,13	20,50	$C_{15}H_{18}Cl_2N_4O$	16,42	20,82	215 (4,62), 251 (4,19), 306 (3,48)
XXXVI	60	163—164	0,48	1,23	15,01	(21,45)	$C_{16}H_{21}BrN_4O$	15,34	(21,89)	210 (4,60), 247 (4,22), 306 (3,48)

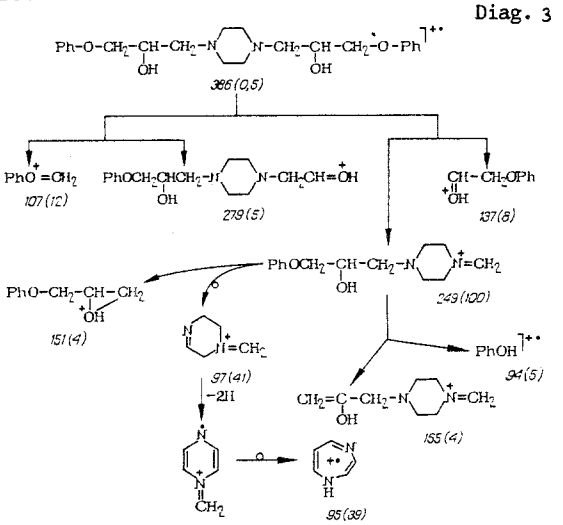
Note. Compounds (XXVII)-(XXXIV) and (XXXVI) were crystallized from aqueous ethanol (1:1), and (XXXV) from aqueous dimethylformamide (1:1).

TABLE 3. Antiviral activity of Pyrimidines (XXX) and (XXXVI) and the Piperazine Analogs (XXXVIII) and (XI)

Compound	Virus	Concentration used, μg/ml	Antiviral activity: platelet reduction test (fall in viral titer), log PFU/ml	Antiviral activity: tests with inclusion in the supporting medium: retardation in the development of cytopathic effect	Antiviral activity: tests with inclusion in the supporting medium: fall in viral titer, log PFU/ml
XXX	CAPV	100*	0,37	50	0,74
		50	0,37	50	0,41
		25	0,30	25	0,81
		12	0,43	25	0,95
		6	0,62	0	0,92
		3	0,30	0	0,77
		1,5	0,47	0	0,72
XXXVI	CAPV	12*	1,33	0	...
		6	0,67	0	...
		3	0,69	0	...
		1,5	0,08	0	...
XXXVI	VVV	12*	0,95	0	...
		6	1,60	0	...
		3	0,12	0	...
XXXVIII	VSV	200*	0,08	25	0,74
		100	0	25	0,43
XL	VVV	100*	2,11	0	...
		50	2,11	0	...
		25	0,26	0	...
		12	0,53	0	...

*MTC for CEC.

Diag. 3



Testing for antiviral activity showed that the pryimidines (XXX) and (XXXVI) and the propanol analogs of piperazine (XXXVIII) and (XI) possessed antiviral activity differing in type and level.

EXPERIMENTAL CHEMICAL

TLC of the 2-substituted pyrimidines was carried out on Silufol UV-254 plates (Czech SSR) in the solvent system dichloroethane–benzene (1:2). The spots were visualized with iodine vapor. Lipophilicites were measured by reversed-phase TLC [8]. UV spectra were recorded on a Specord UV-VIS spectrometer (East Germany) in 95% ethanol, concentration 10^{-4} M. Mass spectra were recorded on a Varian MAT 212 mass spectrometer (USA), ionizing electron energy 70 eV, with direct introduction of the sample into the ion source.

2-(1-Phenoxy-2-hydroxypropylamino)pyrimidine (XXVII). To a mixture of 6.42 g (25 mmole) of the guanidine (I) and 7.0 g (50 mmole) of potassium carbonate in 15 ml of water was added portionwise with stirring 5.0 g (50 mmole) of acetylacetone. The mixture was kept at room temperature for two days, and the solid filtered off, washed with water, and crystallized repeatedly from aqueous ethanol (1:1) to give pure (XXVII).

Compounds (XXVIII-XXXVI) were obtained similarly from other aryloxy- and arylaminopropanol derivatives of guanidine (Table 2).

Measurement of Lipophilicity by Reversed Phase TLC. To Silufol UV-254 plates impregnated with a 5% hexane solution of liquid paraffin were applied droplets each containing 2-3 μg (0.01 mmole) of the compound in solution in acetone. The temperature during chromatography was maintained at 18 ± 1°C. The mobile phase was a phosphate buffer of pH 7.4 with the addition of acetone (from 10 to 50% by volume). The spots were visualized with iodine vapor. The R_m values were calculated using the formula $R_m = \log (1/R_f - 1)$, for each concentration of the organic component, followed by extrapolation to find the value of R_m^0 corresponding to zero concentration of the organic component, which also serves as a measure of lipophilicity. The results are shown in Table 2.

EXPERIMENTAL BIOLOGICAL

Antiviral activity was measured in tissue cultures infected by herpes simplex virus type 1 (HSV), variola vaccine virus (VVV), classical avian plague virus (CAPV), Newcastle disease virus (NDV), vesicular stomatitis virus (VSV), Venezuelan horse encephalomyelitis virus (VHEV), and ECHO virus, using the primary selection test [10] followed by determination of the quantitative parameters for antiviral activity by the platelet reduction method (incorporated in the coating built up on the cells after infection) and inhibition of the development of cytopathic effects and the accumulation of infective viral progeny following introduction of the test compounds into the sustaining medium [1-3]. Measurement of the quantitative characteristics of antiviral activity was preceded by determination of the maximum tolerated concentration (MTC) for the tissue cultures.

In the case of the ECHO virus, tests were carried out in monolayer cultures of passaged musculocutaneous cells of the human embryo (PMCHE), and with the other viruses, in cultures of chick embryo primary cells (CEC).

Antiviral properties were sought in the test compounds, and activity was found in four compounds (Table 3). The greatest suppression of VVV and CAPV in CEV (seen only in the platelet reduction method and obtained in the presence of the compounds only in the MTC and similar concentrations) was obtained with the piperazine (XI) and the pyrimidine (XXXVI). A smaller reduction in the CAPV titer (although shown by all the methods and over a wide range of concentrations) was given by the pyrimidine (XXX).

LITERATURE CITED

1. V. I. Votyakov, The Molecular Biology of Viruses: Chemotherapy and Chemoprophylaxis of Viral Infections [in Russian], Minsk (1974), pp. 10-28.
2. V. I. Votyakov and E. I. Boreko, Methodological Topics in the Scientific Development of Antiviral Drugs [in Russian], Minsk (1977), pp. 10-14.
3. L. V. Denisova, L. I. Nikonovich, V. A. Saikovskaya, et al., The Molecular Biology of Viruses: Chemotherapy and Chemoprophylaxis of Viral Infections [in Russian], Minsk (1974), pp. 111-119.
4. N. P. Mishaeva, V. I. Votyakov, and T. I. Samoilova, Antiviral Drugs [in Russian], Minsk (1984), pp. 103-108.
5. L. M. M. Stankyavichene, V. K. Vizas, A. N. Kost, et al., Khim.-farm. Zh., No. 1, 76-80 (1977).
6. L. M. M. Stankyavichene, R. S. Smailene, A. P. Stankyavichyus, and V. K. Vizas, Khim.-farm Zh., No. 5, 47-53 (1978).
7. L. M. M. Stankyavichene, V. K. Vizas, G. É. Dudenas, and A. P. Stankyavichyus, Theory and Practice of Disturbances of Rhythm in Ischemic Cardiac Disease [in Russian], Kaunas (1983), pp. 146-155.
8. A. P. Stankyavichyus, L. I. Mazhilis, V. N. Garalene, et al., Khim.-farm. Zh., No. 12, 31-34 (1981).
9. C. J. Timmons and E. S. Shtern, Introduction to Electronic Absorption Spectroscopy in Organic Chemistry, 3rd edn., St. Martin's Press, New York (1971).
10. P. Link, B. Rada, and B. Blaskovic, Ann. N. Y. Acad. Sci., 130, 1, 31-43 (1965).
11. H. Oehring and J. Schmidt, Z. Ges. Hyg., 24, 447-449 (1978).
12. M. Tonew, E. Tonew, and R. Menthel, Acta Virol., 21, 146-150 (1977).
13. M. Tonew and D. Dzeguze, Chemotherapy (Basel), 23, 149-158 (1977).
14. M. Tonew, W. Laass, E. Tonew, et al., Acta Virol., 22, 287-295 (1978).