PRIMARY SELECTION OF VIRAL INHIBITORS IN TISSUE CULTURE

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Negative results are presented in the present paper on the selection of viral inhibitors among various types of organic compounds.

Viruses of group A2, parainfluenza type 3, ECHO6, arbovirus, fixed rabies virus, adenovirus type 3, herpes virus L2, and pox virus were used as test viruses as well as f_2 and T_2 coliphage. Screening was carried out in an initially trypsinized tissue culture of chicken embryo fibroblasts (arbovirus, pox virus, herpes virus), in passaged human musculocutaneous tissue (adenovirus, parainfluenza virus, ECHO6), in surviving fragments of chicken embryo chorioallantoic membrane (influenza virus), in *E. coli* bacterial cells (f_2 and T_2 phage), and in white mice (fixed rabies virus). Methods of selecting inhibitors and assessing their antiviral action have been described previously [1-4]. Results of testing the antiviral activity of compounds are presented in Table 1.

EXPERIMENTAL CHEMICAL PART

The isatin derivatives (I-XIII) were obtained by previously described methods [5-8].

Methods are given below for obtaining derivatives of benzimidazole (XIV-XXI) and theophylline (XXII-XXV).

5,6-Dimethyl-2-β-hydroxypropylthiobenzimidazole Hydrochloride (XVI). To a solution of 5,6-dimethyl-2-acetonylthiobenzimidazole (2.34 g; 0.01 mole) [9] in ethanol (40 ml) was added a solution of potassium hydroxide (0.5 g) in water (5 ml) and then technical sodium borohydride (0.4 g). The reaction mixture was left for 24 h at 18-20°C after which it was poured into water, acidified with acetic acid, and the precipitate filtered off. The hydrochloride was obtained in the usual way.

2-β-Hydroxyphenylethylthiobenzimidazole Hydrochloride (XVII) was obtained in a similar manner to compound (XVI) from 2-phenacylthiobenzimidazole [9].

2-Phenacylthiobenzimidazole Oxime (XIX). To a solution of 2-phenacylthiobenzimidazole (2.68 g; 0.01 mole) [9] in ethanol (50 ml) was added $NaHCO_3$ (0.84 g; 0.01 mole) and hydroxylamine hydrochloride (0.7 g; 0.01 mole). The mixture was boiled for 3 h, cooled, poured into water, and the precipitate filtered off. The oxime of 5,6-dimethyl-2-p-bromophenacylthiobenzimidazole (XIV) was obtained by a similar method using the appropriate amount of hydroxylamine hydrochloride.

2-Phenacylthiobenzimidazole Guanylhydrazone Monohydrate (XVIII). To a solution of 2-phenacylthiobenzimidazole (2.68 g; 0.01 mole) [9] in ethanol (50 ml) was added aminoguanidine hydrochloride (1.43 g; 0.013 mole). The reaction mixture was boiled for 2 h, poured into water, the solution neutralized with ammonia, and the precipitate filtered off.

1-(Prop-2-ynyl)-2-phenacylthiobenzimidazole (XV). 2-Phenacylthiobenzimidazole (5.96 g; 0.02 mole) [9] was dissolved in a solution of sodium methoxide prepared from metallic sodium (0.46 g) and methanol (50 ml), propargyl bromide (2.4 g; 0.02 mole) was added, the mixture

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TABLE 1. Antiviral Activity of Compounds (I-XLV)

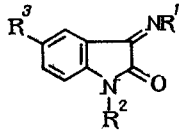
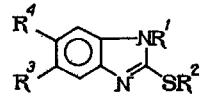
Compound	R^1	R^2	R^3	R^4	Maximal transferable concn., μg/ml	Test virus: influenza	parainfluenza	arbovirus	ECHO	rabies	f_2 coliphage	adenovirus	herpes	pox virus	T_2 coliphage
Isatins 															
I	OH	$CH_2CONHOH$	—	—	50	—	+	+	—	—	—	—	—	—	—
II	$NHCSNH_2$	$CH_2COOC_2H_5$	CH_3	—	25	—	—	—	—	—	—	—	—	—	—
III	$NHCSNH_2$	$CH_2CONHOH$	Br	—	800	—	—	+	—	—	—	—	—	—	—
IV	$NHCSNH_2$	$CH_2COOC_2H_5$	Br	—	400	—	—	+	—	—	—	—	—	—	—
V	$NHCSNH_2$	$COOC_2H_5$	CH_3	—	400	—	—	—	—	—	—	—	—	—	—
VI	$NHCSNH_2$	$CH_2CH=CH_2$	—	—	25	—	—	—	—	—	—	—	—	—	—
VII	$NHCSNH_2$	$CH_2CH=CH_2$	NO_2	—	800	—	—	—	—	—	—	—	—	—	—
VIII	$NHCSNH_2$	$CH_2CH=CH_2$	CH_3	—	400	—	—	—	—	—	—	—	—	—	—
IX	$NHCSNH_2$	$CH_2C\equiv CH$	—	—	400	—	—	—	—	—	—	—	—	—	—
X	$NHCSHN_2$	$CH_2CH\ CH_2$	—	—	400	—	—	—	—	—	—	—	—	—	—
XI	$NHCSNH_2$	$CH_2C\equiv CH$	Br	—	800	—	—	—	—	—	—	—	—	—	—
XII	$NHCSNH_2$	$CH_2C\equiv CH$	NO_2	—	800	—	—	—	—	—	—	—	—	—	—
XIII	$NHCSNH_2$	$CH_2C\equiv CH$	CH_3	—	800	—	—	—	—	—	—	—	—	—	—
Benzimidazoles 															
XIV	H	$CH_2C=N(OH)C_6H_4Br\text{-}4$	CH_3	CH_3	800	—	—	—	+	—	—	—	—	—	—
XV	$CH_2C\equiv CH$	$CH_2COC_6H_5$	—	—	800	—	—	—	—	—	—	+	—	—	—
XVI	H	$CH_2CH(OH)CH_3$	CH_3	CH_3	50	—	—	—	—	—	—	+	—	—	—
XVII	H	$CH_2CH(OH)C_6H_5$	—	—	100	+	—	—	—	—	—	+	+	—	—
XVIII	H	$CH_2C=N(NH)C=(NH)NH_2C_6H_5$	—	—	200	—	—	—	—	—	—	—	—	—	—
XIX	H	$CH_2C=N(OH)C_6H_5$	—	—	800	—	—	—	—	—	—	—	—	—	—

TABLE 1 (Continued)

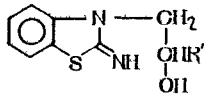
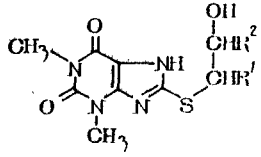
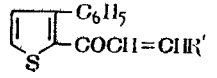
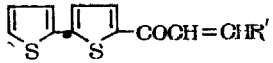
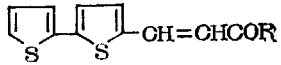
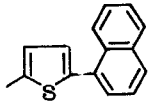
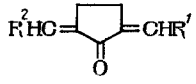
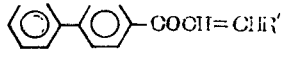
Compound	R^1	R^2	R^3	R^4	Maximal transferable concn., μg/ml	Test virus: influenza	parainfluenza	arbovirus	ECHO	rabies	f_2 coliphage	adenovirus	herpes	pox virus	T_2 coliphage
		Benzothiazolines 													
XX	H	—	—	—	50	—	—	—	—	—	—	-|-	—	—	—
XXI	C_6H_5	—	—	—	100	—	—	—	—	—	—	|-|	—	+|-	—
		Theophyllines 													
XXII	C_6H_5	C_6H_5	—	—	100	—	—	—	—	—	—	—	—	—	—
XXIII	H	C_6H_5	—	—	100	—	-|-	—	—	—	—	—	—	—	—
XXIV	H	4-$NO_2C_6H_4$	—	—	800	—	—	—	—	—	—	—	—	-|+	—
XXV	H	4-Br C_6H_4	—	—	800	—	—	—	—	—	—	—	—	—	—
		Thiophenes 													
XXVI	4-$NO_2C_6H_4$	—	—	—	800	—	-|-	—	—	—	—	—	-|-	—	—
XXVII	2-$NO_2C_6H_4$	—	—	—	200	—	—	—	—	—	—	—	—	—	—
XXVIII	3-$NO_2C_6H_4$	—	—	—	800	—	—	—	—	—	—	—	—	—	—
XXIX	4-$N(CH_3)_2C_6H_4$	—	—	—	10	—	—	—	—	—	—	—	—	—	—
XXX	Thien-2-yl	—	—	—	50	—	—	—	—	—	—	—	—	—	—
XXXI	5-Nitrothiene-2-yl	—	—	—	800	—	—	—	—	—	—	—	—	—	—
XXXII	C_6H_5	—	—		200	-|-	—	—	—	—	—	—	—	—	—

TABLE 1 (Continued)

Compound	R¹	R²	R³	R⁴	Maximal transferable concn., μg/ml	influenza	parainfluenza	arbovirus	ECHO	rabies	f_2 coliphage	adenovirus	herpes	pox virus	T_2 coliphage
	2,2'-Dithienyls	 XXXIII													
		 XXXIV-XXXV													
XXXIII	5-Nitrothiene-2-yl		—	—	400	—	—	—	—	—	—	—	—	—	—
XXXIV			—	—	25	—	—	—	—	—	—	—	—	—	—
XXXV			—	—	25	—	—	—	—	—	—	—	—	—	—
	Cyclopentanones														
XXXVI	C_6H_5	C_6H_5	—	—	200	—	—	—	—	—	—	—	—	—	—
XXXVII	β-Naphthyl	C_6H_5	—	—	200	—	—	—	—	—	—	—	—	—	—
XXXVIII	α-Furyl	C_6H_5	—	—	25	—	—	—	—	—	—	—	—	—	—
XXXIX	β- Naphthyl	α- Furyl	—	—	5	—	—	—	—	—	—	—	—	—	—
XL	α- Furyl	α- Furyl	—	—	50	—	+	—	—	—	—	—	—	—	—
XLI	α- Thienyl	α- Furyl	—	—	800	—	—	—	—	—	—	—	—	—	—
XLII	α-Naphthyl	α- Thienyl	—	—	200	—	—	—	—	—	—	—	—	—	—
XLIII	β-Naphthyl	α- Thienyl	—	—	200	—	+	—	—	—	—	—	—	—	—
	Propenones														
XLIV	$4\text{-}NO_2C_6H_4$		--	—	100	—	—	—	—	—	—	—	—	—	—
XLV	C_6H_5		—	—	800	—	—	—	—	—	—	—	—	—	—

Note. — , compound inactive; + and ++, chemotherapeutic index of compounds equal to 1 and 2 respectively.

boiled for 5 h, poured into water, and extracted with chloroform. The chloroform extract was washed with water, dried over $CaCl_2$ and the solvent distilled off in vacuum.

3-β-Hydroxyethyl-2-imino-4,5,6,7-tetrahydrobenzothiazoline Hydrobromide (XX). To a solution of 2-amino-4,5,6,7-tetrahydrobenzothiazole (7.7 g; 0.05 mole) in ethanol (40 ml) was added ethylene iodohydrin (8.6 g; 0.05 mole), the mixture was boiled for 18 h, the solvent evaporated, the residue washed with acetone, suspended in water, neutralized with ammonia, and the precipitate filtered off. The hydrobromide was obtained by the usual method.

3-β-Hydroxy-β-phenylethyl-2-iminobenzothiazoline Hydrobromide (XXI). To a solution of 3-phenacyl-2-iminobenzothiazoline (2.68 g; 0.01 mole) [10] in ethanol (30 ml) was added a solution of potassium hydroxide (1.2 g) in water (10 ml) and technical sodium borohydride (0.6 g). The reaction mixture was left for 24 h at 18-20°C, poured into water, and extracted with chloroform. The hydrobromide was obtained by the usual method.

8-β-Hydroxy-β-phenylethylthiotheophylline Monohydrate (XXIII). To a mixture of 8-phenacylthiotheophylline (3.3 g; 0.01 mole) [11] in ethanol (60 ml) was added potassium hydroxide (0.5 g) in water (15 ml) and sodium borohydride (0.7 g). The mixture was left for 24 h at 18-20°C, poured into water, neutralized with acetic acid solution, and the precipitate filtered off.

8-β-Hydroxy-β-p-bromophenylethylthio- (XXV), 8-β-hydroxy-β-p-nitrophenylethylthio- (XXIV), and 8-β-hydroxy-β-phenyl-α-phenylethylthiotheophylline (XXII) were obtained similarly from 8-β-p-bromophenacylthiotheophylline, 8-β-p-nitrophenacylthiotheophylline, and 8-α-phenylphenacylthiotheophylline [9] respectively.

The synthesis of the thiophene derivatives (XXVI-XLIII) has been reported previously [12-15].

1-(Diphenyl-4-yl)-3-phenylprop-2-en-1-one (XLV). To an alcoholic solution of 4-acetyldiphenyl (0.01 mole) and benzaldehyde (0.009 mole) was added dropwise with stirring a 20% aqueous solution of sodium hydroxide (0.3-0.6 g). The reaction mixture was left overnight. The precipitate which separated was filtered off, washed with water, and recrystallized from ethanol. Yield was 2.4 g of mp 160°C. Found, %: C 88.6; H 5.6. $C_{21}H_{16}O$. Calculated, %: C 88.7; H 5.7.

1-(Diphenyl-4-yl)-3-(4-nitrophenyl)prop-2-en-1-one (XLIV) was obtained in a similar manner to (XLV). Yield was 2.3 g of mp 210°C. Found, %: C 76.5; H 4.5. $C_{21}H_{15}NO_3$. Calculated, %: C 76.6; H 4.6.

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