ANTIVIRAL ACTIVITY OF 1-AMINO-1,2,3,4-TETRAHYDROCARBAZOLES

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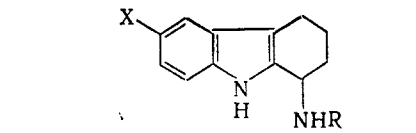
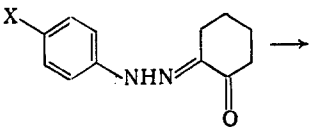
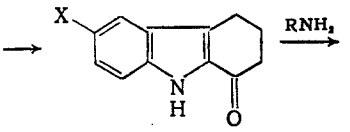
We have shown in the preceding paper [2] that 1-aminotetrahydrocarbazoles (I-XI) suppress the growth of tuberculosiss myobacteria in in vitro experiments. During more extensive biological studies of compounds I-XI we have found that compounds VIII-XI have an inhibiting action on the reproduction of the herpes virus. Therefore we have also synthesized the 1-aminotetrahydrocarbazoles (XII-XX) and studied their antiviral activity.



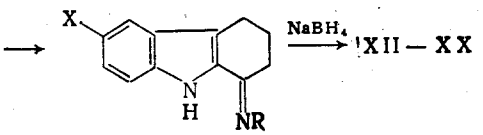
I—XX



XXI



XXII



XIIi—XXi

R = $CH_2CH_2NEt_2$ (I), $(CH_2)_3OH$ (II), $CHEtCH_2OH$ (III), $CH_2CH(OH)CH_2Ph$ (IV), CHMePh (V), $CHMeCH_2Ph$ (VI), cyclohexyl (VII), Et (VIII), CH_2CH_2OH (IX), CH_2Ph (X, XIX), $CH_2CH_2C_6H_3(OMe)_2$-3,4 (XI), $CH_2CH(OMe)_2$ (XII), $(CH_2)_3NMe_2$ (XIII), CH_2CH_2OPh (XIV, XX), $CH_2CH_2OC_6H_4F$-4 (XV), CH_2CH_2Ph (XVI—XVIII); X=Me (I—XVI), H (XVII), F (XVIII), Cl (XIX, XX).

The syntheses were carried out according to a general scheme: reaction of ketocarbazoles (XXII) with primary amines yielded 1-iminotetrahydrocarbazoles (XIIi-XXi),* which were reduced to amino compounds XII-XX. Some of the iminotetrahydrocarbazoles were isolated in the form of their hydrochlorides (Table 1). Reductions of the hydrochlorides and the free basis proceed under the same conditions: in ethanol at room temperature with an equimolar amount of $NaBH_4$.

*In the text of this article and in Table 1, the iminotetrahydrocarbazoles are designated by the same numbers as the corresponding aminotetrahydrocarbazoles but with the index "i".

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TABLE 1. 1-Imino- and 1-Amino-1,2,3,4-tetrahydrocarbazoles XIIi-XXi and XII-XX

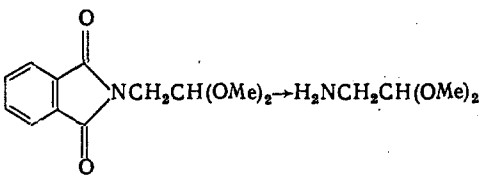
Compound	Yield, %	mp, °C	Empirical formula
XIIi	84	90—3	$C_{17}H_{22}N_2O_2$
XIIi	88	71—6	$C_{18}H_{28}N_2$
XIVi	86	114—7	$C_{21}H_{22}N_2O$
XVi	88	130—2	$C_{21}H_{21}FN_2O$
XVIi	80	120—3	$C_{21}H_{23}N_2$
XVIIi·HCl	93	205—8	$C_{20}H_{20}N_2 \cdot HCl$
XVIIIi·HCl	96	210—4	$C_{20}H_{19}FN_2 \cdot HCl$
XIXi	99	127—9	$C_{19}H_{17}ClN_2$
XXi	90	136—8	$C_{20}H_{19}ClN_2O$
XII·HCl	50	150*	$C_{17}H_{14}N_2O_2 \cdot HCl$
XIII·HCl	77	146—8	$C_{18}H_{27}N_2 \cdot HCl$
XIV·HCl	93	165*	$C_{21}H_{24}N_2O \cdot HCl$
XV	96	116—7	$C_{21}H_{23}FN_2O$
XV·HCl	85	150*	$C_{21}H_{23}FN_2O \cdot HCl$
XVI·HCl	81	175*	$C_{21}H_{24}N_2 \cdot HCl$
XVII·HCl	92	170*	$C_{20}H_{23}N_2 \cdot HCl$
XVIII·HCl	88	178—9	$C_{20}H_{21}FN_2 \cdot HCl$
XIX	98	112—3	$C_{19}H_{19}ClN_2$
XIX·HCl	83	180*	$C_{19}H_{19}ClN_2 \cdot HCl$
XX	98	74—6	$C_{20}H_{21}ClN_2O$
XX·HCl	82	160*	$C_{20}H_{21}ClN_2O \cdot HCl$

*Compound melts with decomposition.

Starting ketones XXII were prepared by the Fisher indole synthesis [3] from the corresponding monoarylhydrazones of cyclohexanedione (XXI). We have found that fluorinated hydrazone XXI (X = F, $C_{12}H_{13}FN_2O$) melts at 184-185°C, which is considerably higher than the value 147-148°C reported in [4]. However, the melting points of ketone XXII (X = F), prepared by us and in [4], correspond.

Most of the starting amines (RNH_2) are commercially available, but 2-phenoxyethylamine was prepared by hydrazinolysis of the N-substituted phthalimide [5] and 2-(p-fluorophenoxy)-ethylamine by reduction of p-fluorophenoxyacetamide [6].

The synthesis of 2,2-dimethoxyethylamine (XXIV), described in [1], demands the use of an autoclave and special techniques. We have found a more convenient laboratory method for the preparation of amine XXIV: potassium phthalimide is alkylated with bromoacetaldehyde dimethyl acetal and the phthaloyl derivative (XXIII) obtained is split with ethanolamine. The use of ethanolamine makes it possible to convert imide XXIII to aminoacetal XXIV in high yield.



XXIII XXIV

Biological investigations of aminotetrahydrocarbazoles I-XX are reported for the hydrochlorides. Physical properties of compounds I·HCl-XI·HCl are mentioned in [2], the properties of compounds XII·HCl-XX·HCl and those of some free bases are given in Table 1.

EXPERIMENTAL (CHEMICAL)

Mass spectra were recorded on a MAT-112 spectrometer (FRG). Found and calculated values of elemental analyses correspond.

Dimethyl Acetal of Phthalimidoacetaldehyde (XXIII). A mixture of 72.2 g (0.39 mole) of potassium phthalimide, 50.7 g (0.3 mole, 35 ml) of bromoacetaldehyde dimethyl acetal, and 200 ml of DMF was heated at 130-135°C for 20 h. The reaction mixture was filtered, the filtrate was evaporated under vacuum, and to the residue was added 50 ml of hot water. The precipitate formed was filtered off, washed on the filter with 50 ml of a MeOH—water mixture (1:1), and dried. The dried product was extracted with 350 ml of hot benzene, the benzene extract was evaporated, the pasty product was transferred to a filter, pressed out, and washed with 20 ml of i-PrOH. The yield of phthalimide derivative XXIII is 39.6 g (56%), mp 104-108°C. Crystallization from MeOH raised the mp to 108-109°C; $C_{12}H_{13}NO_4$.

TABLE 2. Antiherpes Activity of 1-Amino-1,2,3,4-tetrahydrocarbazoles (herpes simplex type I virus, strain L_2)

Compound	MBC,, μg/ml	Dose, μg/ml	Lowering of the infectious titer, log TCD_{50}
VIII·HCl	20,0	10,0	1,0
		5,0	1,0
		2,5	1,0
IX·HCl	10,0	5,0	0,75
		2,5	0,5
X·HCl	10,0	5,0	1,0
		2,5	0,75
XI·HCl	10,0	5,0	1,0
		2,5	0,75
XV·HCl	20,0	10,0	1,0
		5,0	1,0
		2,5	0,5
XVI·HCl	20,0	10,0	1,75
		5,0	0,75
		2,5	0,5
XX·HCl	20,0	10,0	1,75
		5,0	1,0

Dimethoxyethylamine (XXIV). A mixture of 70.5 g (0.3 mole) of phthalimide XXIII and 90 ml of monoethanolamine was heated at 170-180°C in a flask equipped with a dephlegmator. Amine XXIV was distilled with the rate at which it was formed. The fraction with bp 135-145°C (lit. [1] bp 137-139°C) was collected. Yield 28.7 g (91%) of XXIV).

1-(2,2-Dimethoxyethylimino)-6-methyl-1,2,3,4-tetrahydrocarbazole (XIIi). A mixture of 19.9 g (0.1 mole) of ketone XXII (X = Me), 15.8 g (0.15 mole, 16.5 ml) of amine XXIV, and 0.4 g of p-toluenesulfonic acid in 30 ml of toluene was refluxed in a flask equipped with a Dean–Stark separator until the separation of water stopped (5 h). The toluene was evaporated under vacuum, the residue was treated with hexane, the crystals were filtered off, and crystallized from hexane (with addition of activated carbon). Yield 24.2 g (84%) of XIIi, mp 90-93°C. Mass spectrum (relative intensity): 286 (45), 255 (9), 223 (16), 211 (100), 198 (7), 182 (14).

In the same way imino compounds XIIIi-XXi (see Table 1) were prepared from ketones XXII (X = Me, H, F, Cl). Compounds XIIIi and XIXi were crystallized from hexane, XIVi-XVIi and XVII·HCl from ethanol, and XVIII·HCl and XX from MeOH.

Hydrochloride of 1-(2,2-Dimethoxyethylamino)-6-methyl-1,2,3,4-tetrahydrocarbazole (XII. HCl). To a solution of 11.45 g (0.04 mole) of imine XIIi in 100 ml of ethanol was added 1.51 g (0.04 mole) of $NaBH_4$. The mixture was stirred at 20°C for 1 h, then the excess of reductant was decomposed with 50 ml of water, the ethanol was evaporated under vacuum, and the product was extracted from the residue with 50 ml of benzene. The benzene extract was washed with water, dried, the benzene was evaporated, and the residue was dissolved in 40 ml of MeOH and treated with 40 ml of an ethereal HCl solution. The precipitated product was crystallized from a MeOH–ether (1:1) mixture. Yield 6.6 g (50%), mp 150-153°C (dec.). Mass spectrum (relative intensity): 288 (22), 228 (11), 184 (100), 183 (89), 182 (72), 169 (11), 168 (22), 167 (33).

Compounds XIII·HCl-XX·HCl were prepared in the same way. In three cases the crystalline free bases of the 1-aminotetrahydrocarbazoles could be prepared (see Table 2). Compound XV was crystallized from ethanol, XIII·HCl from an ethanol–ether mixture, XIV·HCl-XIX·HCl, XIX, and XX from MeOH, and XX·HCl from a MeOH–ether mixture.

EXPERIMENTAL (BIOLOGICAL)

The antiviral activity of compounds I·HCl-XX·HCl was studied in relation to representatives of DNA viruses (herpes simplex virus type I of the antigenic strain L_2) and RNA viruses (virus group A) in cell cultures and in animal experiments.

To study the antiherpes activity of the compounds in vitro, use was made of a pretrypsinized cell culture of fibroblasts of chicken embryos (FCE). A two-day-old monolayer of a cell culture was injected with dilutions of the virus containing from 10 to 1000 TCD_{50} in 0.4

ml. The maximum bearable and lower concentrations were added within 1 h after infecting the cell monolayer with the virus. The results were judged after 48 h by the ability of the compounds under investigation to prevent the cytostatic activity of the virus on the cells and to lower the infectious titer of it in comparison with a control.

In preliminary experiments the cytotoxic activity of the compounds on intact cells was studied in order to determine the maximum bearable concentration (MBC). It was found that all compounds I·HCl-XX·HCl are characterized by a good tolerance: the MBC for FCE cell cultures is 20-10 μg/ml.

In the series of compounds I·HCl-XX·HCl that we have studied, seven compounds (see Table 2) have inhibiting activity on the reproduction of the herpes simplex virus and lower the infectious titer of the virus.

The chemotherapeutic activity of four compounds was studied. By means of the model of influenzal pneumonia in mice, induced by intranasal infection of the animals with the A/Aichi/68(H3N2) virus, the activity of compounds VIII·HCl and XVI·HCl was studied. The compounds were administered perorally at doses of 100 and 50 mg/kg (0.2 and 0.1 of LD_{50}) once daily for 5 days. The activity was judged by the lowering of the mortality (in %) of the treated animals in comparison with the control. Compound XVI·HCl decreases the mortality of the mice by 40% ($p < 0.05$) at a dose of 100 mg/kg. When the dose was lowered to 50 mg/kg, activity was not observed. Compound VIII·HCl did not show therapeutic activity.

With the model of generalized herpes in mice, induced by intranasal infection of the animals with herpes simplex type I virus (strain L_2), the activity of compounds VIII·HCl, X·HCl, XI·HCl, and XVI·HCl was studied. The compounds were administered perorally once daily for 6 days at doses from 200 to 12.5 mg/kg. Of the compounds studied only XVI·HCl had therapeutic activity: when 50 mg/kg was given, a lowering of the mortality by 30% ($p < 0.05$) was observed in comparison with the control.

Of the twenty 1-aminotetrahydrocarbazoles investigated, seven compounds are able to inhibit the reproduction of the herpes simplex virus in a cell culture—the chemotherapeutic index reaches 8. Compound XVI showed therapeutic activity on in vivo models with respect to both the herpes virus and the influenza virus.

The virus-inhibiting activity of 1-aminotetrahydrocarbazoles clearly depends on the molecular structure. For example, compound XVI (X = Me, R = CH_2CH_2Ph) is active, but its close analogs isomer V (X = Me, R = CHMePh) and homolog XVII (X = H, R = CH_2CH_2Ph) are inactive. Because the virus-inhibiting activity of 1-aminotetrahydrocarbazoles depends on the nature of the substituents at the heterocycle, we consider it advisable to continue the search for structural factors that increase the antiviral effect.

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