SYNTHESIS, ANTIVIRAL ACTIVITY, AND INTERACTION WITH DNA OF DERIVATIVES OF 2-DIALKYLAMINOMETHYL-5-(PYRIDYL-2-OXY)INDOLE

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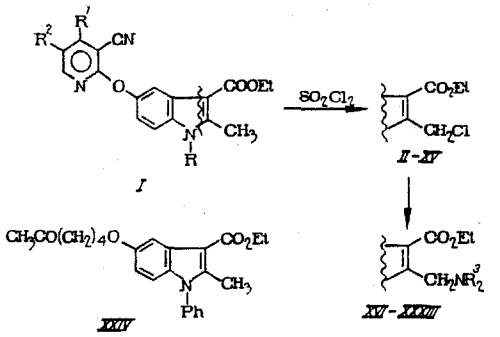
Derivatives of 5-oxyindole react with substituted 2-chloropyridines in DMFA in the presence of anhydrous K_2CO_3 to give 5-(pyridyl-2-oxy)indoles (I), some of which show antiviral activity [6].

The antiviral activity of diaryl and aryl-heteroaryl ethers can be explained, at least partially, by their ability to bind to viral proteins [9]. Therefore, it appeared interesting to introduce further substituents into these molecules, which are capable of different kinds of interactions. As such a substituent, we chose the 2-dialkylaminomethyl group. This is capable of interacting with both cationic regions (due to the presence of a free electron pair at the nitrogen atom) and, in its protonated form, with anionic regions (either electrostatically or via hydrogen bond formation). Finally, it should be noted that some indole-derived Mannich bases show a pronounced antiviral activity [2, 4, 5].

As starting materials for the preparation of the desired 2-dialkylaminomethyl-5-(pyridyl-2-oxy)indoles (XVI-XXXIII) we used the previously unknown 2-chloromethyl substituted 5-(pyridyl-2)oxyindoles (II-XV), which were prepared by chlorination of I with SO_2Cl_2 in $CHCl_3$. Compounds XVI-XXXIII were obtained in good yields upon heating of II-XV with secondary amines in benzene or with bis(dimethylamino)methane in dioxane.



R=Me (VIII, XXVI), Ph (XV, XXXIII), $C_6H_4Me_p$ (II, IV, IX, XI, XVI, XIX—XXI, XXVII, XXIX), $C_6H_4OMe_p$ (III, VI, VII, XII, XIV, XVII, XVIII, XXIII — XXV, XXX, XXXII), C_6H_4Br-p (V, X, XIII, XXII, XXVIII, XXXI), R^1=H (II, III, XVI — XVIII), NHPh (IV — VII, XIX — XXV), $NHC_6H_4Me_p$ (VIII, IX, XIII — XV, XXVI, XXVII, XXXI — XXXIII), NHC_6H_4OMe-p (X — XII, XXVIII—XXX); R^2=H (II — VI, VIII — XXIV, XXVI — XXXIII), CN (VII, XXV); R^3=Et (XVI, XVII, XX, XXII, XXIII, XXV — XXXI, XXXIII, Me (XVIII, XIX, XXIV, XXXII; R^3+R^3= $=(CH_2)_2O(CH_2)_2$(XXI).

Compound XXXIV was synthesized by reaction of 1-chloro-5-hexanone [3] with 1-phenyl-2-methyl-3-ethoxycarbonyl-5-oxyindole in acetone in the presence of anhydrous K_2CO_3.

Many antiviral compounds interact with DNA [1, 7, 8]. Therefore, we studied both the antiviral activity of the compounds prepared and their interaction with DNA.

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TABLE 1. Characterization of Compounds II-XXXIV

Compound	Mp, °C	Yield, %	Molecular formula
II	132—3	84	$C_{25}H_{20}ClN_3O_3$
III	180—1	63	$C_{25}H_{20}ClN_3O_4$
IV	188—9	64	$C_{31}H_{25}ClN_4O_3$
V	215—6	71,3	$C_{30}H_{22}ClBrN_4O_3$
VI	190—1	62,8	$C_{31}H_{25}ClN_4O_4$
VII	177—8	94,3	$C_{32}H_{24}ClN_5O_4$
VIII	232—3	35,1	$C_{26}H_{23}ClN_4O_3$
IX	185—6	42,2	$C_{32}H_{27}ClN_4O_3$
X	185—6	59,7	$C_{31}H_{24}ClBrN_4O_4$
XI	196—7	38,1	$C_{32}H_{27}ClN_4O_4$
XII	159—61	71,4	$C_{32}H_{27}ClN_4O_5$
XIII	225—6	47,8	$C_{31}H_{24}BrClN_4O_3$
XIV	187—8	71,0	$C_{32}H_{27}ClN_4O_4$
XV	173—4	40,4	$C_{31}H_{25}ClN_4O_3$
XVI	115—6	78	$C_{29}H_{30}N_4O_3$
XVI·HCl	145—7	80,9	$C_{29}H_{31}ClN_4O_3$
XVII	105—6	64,3	$C_{29}H_{30}N_4O_4$
XVII·HCl	137—9	79,4	$C_{29}H_{31}ClN_4O_4$
XVIII	174—5	68,1	$C_{27}H_{26}N_4O_4$
XIX	205—6	72,3	$C_{32}H_{31}N_5O_3$
XIX·HCl	215—7	84	$C_{33}H_{32}ClN_5O_3$
XX	200—1	73,7	$C_{35}H_{35}N_5O_3$
XX·HCl	185—7	64	$C_{35}H_{36}ClN_5O_3$
XXI	203—4	53,1	$C_{35}H_{33}N_5O_4$
XXI·HCl	173—4	79,2	$C_{35}H_{34}ClN_5O_4$
XXII	201—2	83	$C_{34}H_{33}BrN_5O_3$
XXII·HCl	225—6	91	$C_{34}H_{34}BrClN_5O_3$
XXIII	207—8	73,5	$C_{35}H_{35}N_5O_4$
XXIV	211—2	79,8	$C_{33}H_{31}N_5O_4$
XXV	140—1	68,7	$C_{36}H_{34}N_6O_4$
XXVI	182—3	74,3	$C_{30}H_{33}N_5O_3$
XXVII	191—9	75,7	$C_{36}H_{37}N_5O_3$
XXVIII	201—2	81,3	$C_{35}H_{34}BrN_5O_4$
XXIX	206—7	84	$C_{36}H_{37}N_5O_4$
XXX	183—4	81,4	$C_{36}H_{37}N_5O_5$
XXXI	200—1	79,3	$C_{35}H_{34}BrN_5O_3$
XXXII	215—6	77,4	$C_{34}H_{33}N_5O_4$
XXXIII	222—3	80,3	$C_{35}H_{35}N_5O_3$
XXXIV	92—3	51,3	$C_{24}H_{27}NO_4$

Notes. Compounds II, IV, VI, VII, and XIII-XV were recrystallized from hexane/ethyl acetate; III and V, from ethyl acetate; VIII, from $CHCl_3$; IX-XII, from hexane/acetone; XVI, XIX-XXI, XXIII, XXIV, XXVI, and XXX, from i-PrOH; the hydrochlorides of XVI and XVII, from acetone/alcohol; XVIII, XXII, XVII-XXIX, and XXXI-XXXIII, from i-PrOH/dioxane; IX-XXI, from acetone; XXII, from acetone/ether; XXV, from aqueous ethanol; and XXXIV, from hexane/benzene.

EXPERIMENTAL

Chemical Synthesis

Table 1 gives the melting points and yields of the compounds prepared. Satisfactory elemental analysis data were obtained.

1-(p-Tolyl)-2-chloromethyl-3-ethoxycarbonyl-5-(3-cyanopyridyl-2-oxy)indole (III). To a solution of 2 g (0.005 mole) of 1-(p-tolyl)-2-methyl-3-ethoxycarbonyl-5-(3-cyanopyridyl-2)-oxyindole [6] in 50 ml of $CHCl_3$ was added at −5°C 0.6 ml (0.0075 mole) of SO_2Cl_2 at such a rate that the temperature of the reaction mixture did not exceed −5°C to 0°C. After the addition, the mixture was held at room temperature for 30 min and washed with water until neutral. After drying over Na_2SO_4 and removal of the solvent, the residue was crystallized by the addition of petroleum ether, and filtered. The yield of II was 1.8 g.

Compounds III-XV were prepared similarly.

1-(p-Tolyl)-2-diethylaminomethyl-3-ethoxycarbonyl-5-(3-cyanopyridyl-2-oxy)indole hydrochloride (XVI). A mixture of 4.6 g (0.01 mole) of compound II, 30 g (0.4 mole) diethylamine, and 60 ml of dry benzene was refluxed. The course of the reaction was followed chromato-

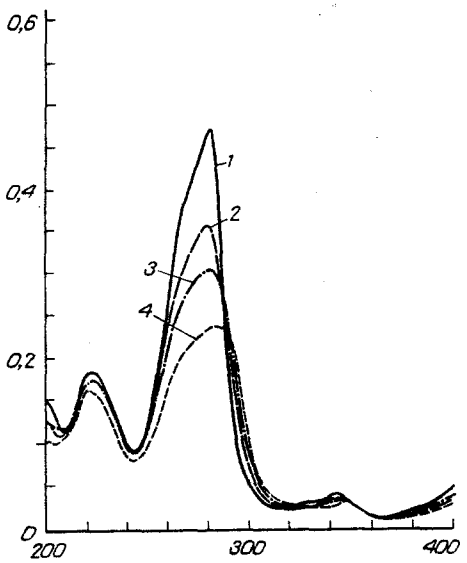

Fig. 1

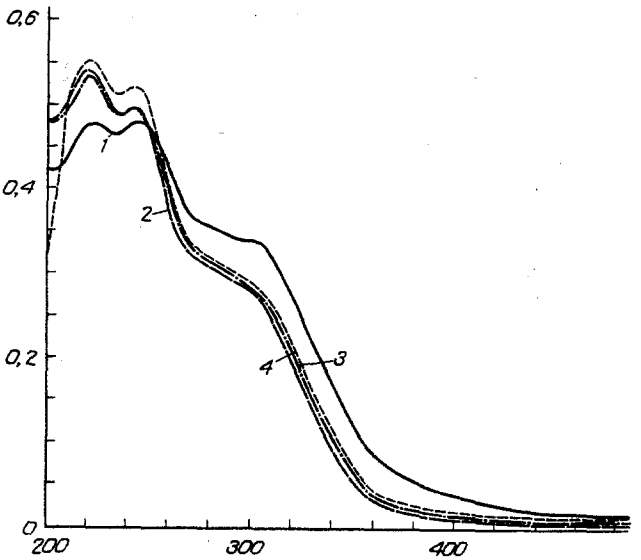

Fig. 2

Fig. 1. Absorption spectrum of acriquine in the presence of DNA. 1) Free acriquine; 2, 3, 4) acriquine in the presence of DNA at 2P/D = 5, 10, and 20, respectively. On the abscissa: wavelength, nm, on the ordinate: optical density.

Fig. 2. Absorption spectrum of XXXIV in the presence of DNA. 1) Free XXXIV; 2, 3, 4) XXXIV in the presence of DNA at 2P/D = 5, 10, and 20, respectively. On the abscissa: wavelength, nm: on the ordinate: optical density.

graphically. Upon completion of the reaction, the separated $Et_2NH \cdot HCl$ was filtered off and washed with dry benzene. The filtrate was evaporated to dryness and the residue crystallized with i-PrOH. The product XVI (3.8 g) was recovered by filtration.

The hydrochloride salt of XVI was obtained by acidification of its acetone solution with ethereal HCl. Yield: 3.3 g.

Compounds XVII-XXXIII and hydrochlorides of XVI, XVII, and XIX-XXII were prepared similarly.

1-Phenyl-2-methyl-3-ethoxycarbonyl-5-(5-oxohexyloxy)indole (XXXIV). A mixture of 2.7 g (0.009 mole) of 1-phenyl-2-methyl-3-ethoxycarbonyl-5-oxyindol, 5.2 g (0.038 mole) 1-chloro-5 hexanone, and 2.6 g (0.019 mole) of anhydrous K_2CO_3 was refluxed in 30 ml of acetone. The completion of the reaction was determined chromatographically. K_2CO_3 was removed by filtration, the acetone evaporated in vacuum, and the residue crystallized from hexane. Yield of XXXIV: 1.8 g.

Biological Activity

The antiviral activity of compounds XVI-XXXIV and of the hydrochlorides of XVI, XVII, and XIX-XXII against DNA and RNA viruses was tested. Herpes simplex virus, antigenic type I (HSV-1, strain L_2) was used as a representative of the DNA viruses, and the influenza virus EPV (H7N7) was taken as a typical RNA virus. As a test system, a primary hen embryo fibroblast cell culture (HEF), infected to a level of infection of 10-100 TCD_{50} was used. In preliminary experiments, the maximal transferable concentration (MTC) for the compounds tested was determined and found not to exceed 20 μg/ml. After adsorption of the virus to the cells the compounds under investigation were added to a final concentration of 0.25-0.5 of the corresponding MTC. The extent of repression of viral reproduction due to the compounds studied was determined by the prevention of the cytopathic activity of the virus on the cells and by the decrease of the titer of infection in comparison to the control experiments. This decrease was quantitatively expressed in terms of log TCD_{50}.

It was found that compounds XX, XXI, and XXVI-XXXI reproducibly inhibit the reproduction of HSV in a HEF cell culture. Compound XXIX showed the highest activity. When used at concentrations of 5 and 10 μg/ml, it decreased the titer of viral infection to a value of 1.75 log TCD_{50}

and 2.0 log TCD_{50}, respectively. At a concentration of 2.5 μg/ml, the value was decreased to 1.25 log TCD_{50}, which corresponds to a chemical therapeutic index (CTI) of 8. A pronounced antiviral activity was also found for compounds XXVI and XXX, which at concentrations of 5 and 10 μg/ml decreased the titer of viral infection to a value of 1.5-1.75 log TCD_{50}, corresponding to a CTI of 4-8. All other compounds were less active.

The activity of compounds XXVI-XXX was studied on a model mouse herpes virus. Mice were given these compounds per os, once daily, at doses of 30, 60, and 100 mg/kg, in the course of 5 days. None of these compounds showed any therapeutic effect.

None of the compounds tested showed any activity against the RNA virus EPV.

In another series of experiments we studied the interaction of derivatives of 5-(pyridyl-2)oxyindoles with DNA using different model systems. Thereby we have taken into account the antiviral activity of these compounds against DNA viruses (see above). Compounds XX, 1-(p-methoxyphenyl)-, and 1-(p-bromophenyl)-2-methyl-3-ethoxycarbonyl-5-(3-cyano-4-phenylaminopyridyl-2-oxy)indoles (Ia and Ib, prepared according to [5]) were used as models. As a reference, we used compound XXXIV, which has been found inactive against both HSV and FPV.

To study the interaction of Ia, Ib, XX, and XXXIV with DNA we used carp's sperm DNA (Sigma, USA). Freshly prepared mixtures of this DNA and the compounds studied were used in a buffer containing 10^{-2} M Tris-HCl, pH 6.9, $5 \cdot 10^{-4}$ M EDTA, and 10^{-3} M NaCl.

The optical densities of solutions of the compounds and of the DNA were recorded on a Perkin-Elmer spectrophotometer in the region 210-450 nm. Molar concentrations of the polynucleotide (P) and of the ligand (D) were used.

The absorption spectra of solutions of the free compounds Ia, Ib, XX, and XXXIV were first recorded. Then, the same spectra were recorded in the presence of carp's sperm DNA at varying DNA/ligand ratios (2P/D = 5, 10, and 20) [7].

As a control, the spectrum of the genuine intercalating reagent acriquine was used. In the case of this compound, there are stacking interactions between the aromatic radical and the nucleic acid bases.

Using ^{3}H-thymidine, we studied the effect of the compounds on the biosynthesis of cellular DNA. To this end, 2.5 ml aliquots of the HEF cell culture (10^6 cells/ml) or of the M-19 cell culture were placed in sterile scintillation vials. After formation of monolayers of cells the cultures were infected with a virus (A/WSN, 5-10 pfu/cell). Infected and noninfected cells were incubated with the compounds under study (10 μg/ml at 37°C, 1 h or 20 h, ^{3}H-thymidine added for 1 h). Two different cell cultures were used: hen embryo fibroblasts (HEF), and human fibroblast cells (M-19). The incorporation of ^{3}H-thymidine (20-40 μg/ml) into the cellular monolayer was taken as a measure of DNA synthesis. The cultural supernatant was decanted, the monolayer washed with cold physiological solution, and the cells fixed with a cold 5% TCA solution. In order to remove the acid-soluble radioactivity the TCA solution was then decanted and the monolayer washed with a 2:1 ethanol/ether mixture for removal of lipids. The monolayer was then dried and the acid-insoluble radioactivity counted using a Tricarb scintillation counter.

To characterize the interaction of the title compounds with DNA, their absorption spectra in the region 200-450 nm were recorded in the presence of the carp's sperm DNA at various DNA/ligand ratios.

In the case of acriquine, which is a known DNA intercalator, the addition of increasing concentrations of DNA (in molar ratios of 1:5, 1:10, and 1:20) leads to a decrease of the absorption of this compound, which is accompanied by a bathochromic shift (Fig. 1). No similar intercalating effect could be detected in the case of compounds Ia, Ib, XX, and XXXIV; however, the absorption spectra of these compounds were altered in the presence of DNA. Compound XXXIV, which has no activity against DNA and RNA viruses, does not show any changes in its absorption spectrum upon intercalation with DNA (Fig. 2).

The most significant changes in the absorption spectra were observed in the case of compound XX. The addition of increasing concentrations of DNA to a solution of XX led to an increased absorption in the short wavelength region of the spectrum and to a decrease in absorption in the 260-420 nm region. A further increase of the DNA concentration (1:20) did not lead to any further changes in the absorption spectrum. Therefore, the kinetics of complex formation between compound XX and DNA was studied. The kinetics of this complex formation was

TABLE 2. Effect of Derivatives of 5-Oxyindole on the Biosynthesis of DNA in Cell Cultures

Cell culture	Compound	Synthesis of DNA, counts/min		inhibition of DNA synthesis, %	
		1 h	20 h	1 h	20 h
HEF	Ia	3324,0	1387,0	5,58	0
	Ib	2827,3	1624,6	19,68	0
	XX	3104,6	1615,6	11,81	0
	XXXIV	2725,3	1160,6	22,59	0
	none	3520,3	1089,0	—	—
M-19	Ia	5551,6	2179,6	0	37,16
	Ib	5512,3	3048,6	0	12,1
	XX	4109,3	3378,3	0	2,6
	XXXIV	4492,0	703,3	0	79,73
	none	4119,0	3468,3	—	—

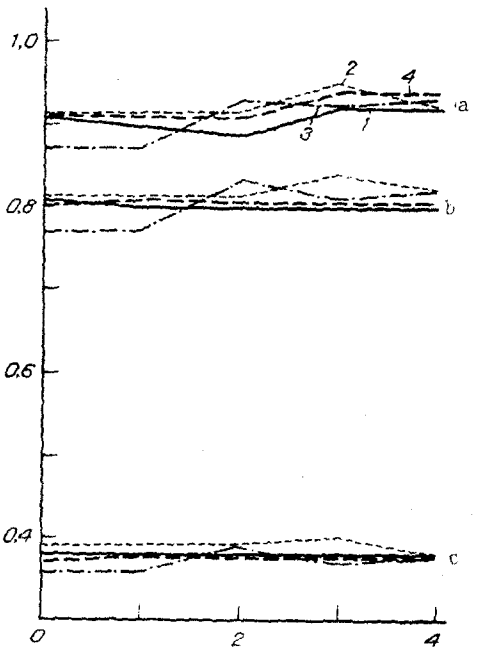

Fig. 3. Kinetics of interaction of XX with DNA. Set a: at 213 nm, set b: at 237 nm, set c: at 300 nm. 1) Free XX; 2, 3, 4) XX in the presence of DNA at 2P/D = 5, 10, and 20, respectively. On the abscissa: duration of incubation, h: on the ordinate: optical density of XX in the presence of DNA.

followed for 5 h at different wavelengths. The observed increase of the optical density at 213 and 237 nm might be an indication of complex formation between compound XX and DNA (Fig. 3).

The data presented lead to the conclusion that, depending on the chemical structure and antiviral activity, the oxyindole derivatives studied show different modes of interaction with DNA. Compounds with an antiviral activity are probably capable of complex formation with DNA (compound XX), and change their absorption spectra in the presence of DNA (compounds Ia and Ib). Compound XXXIV, which does not show any antiviral activity, does not interact with DNA. The capability of the compounds studied to interact with DNA leaves open the possibility of their selective activity against DNA viruses by selective inhibition of the viral DNA biosynthesis.

The toxicity of the title compounds was investigated by probing their effect on the biosynthesis of cellular DNA in two different cell cultures. As shown in Table 2, the compounds studied had varying inhibitory activity on the biosynthesis of the cellular DNA depending on

the cell culture and the duration of action. When a HEF culture is used the inhibition ranged from 5.5% to 22.5% after 1 h of incubation, whereas no inhibition was detectable after 20 h of incubation. With a human fibroblast cell culture (M-19), no inhibition could be detected after 1 h, but after 20 h of incubation a degree of inhibition of cellular DNA biosynthesis ranging from 2.6% to 79.7% was observed.

The biologically inactive compound XXXIV inhibits the DNA biosynthesis in HEF and M-19 cell cultures to 79% and 23%, respectively, which indicates its potential toxicity. Compounds Ia, Ib, and XXXIV, which have antiviral activity, show very low inhibition of cellular DNA biosynthesis. Most significantly, these compounds have different inhibitory effects on the DNA biosynthesis in the different cell cultures tested. Thus, both the toxicity and specificity of these compounds are dependent on the type of viral infection and the metabolic characteristics of the particular cell culture.

Compound XX, which possesses antiviral activity and is capable of complex formation with DNA, shows a weak inhibition of cellular DNA biosynthesis (3-12%). It can be assumed that its antiviral activity against DNA viruses is due to an inhibition of viral DNA synthesis.

The data presented in this paper on the antiviral activity, low toxicity, and capability of complex formation with DNA of the compounds tested show that this is a promising class of compounds for the search for biologically active substances.

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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF A NUMBER OF 1,2,3-TRIAZOLE DERIVATIVES

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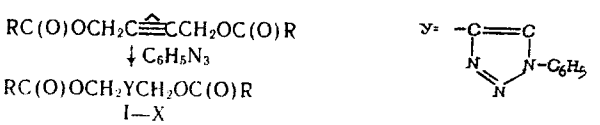
The reaction of 1,3-dipolar ring-attachment of organic azides to compounds containing an acetylene bond forms a variety of 1,2,3-triazole derivatives [2, 5].

We have synthesized a number of new triazole derivatives based on acetylene esters, i.e., derivatives of benzic acid and phenylazide (PA).

The syntheses were carried out according to the following scheme:



R=Ph (I), 2-ClC$_6$H$_4$ (II); 2,4-Cl$_2$C$_6$H$_3$ (III); 2-BrC$_6$H$_4$ (IV), 3-BrC$_6$H$_4$ (V), 2-IC$_6$H$_4$ (VI), 3-IC$_6$H$_4$ (VII), 2-NO$_2$C$_6$H$_4$ (VIII), 3-NO$_2$C$_6$H$_4$ (IX), 4-NO$_2$C$_6$H$_4$ (X).

The resulting products are stable in normal conditions, and are crystalline substances, soluble in organic solvents and insoluble in water.

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