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SYNTHESIS AND BIOLOGICAL ACTIVITY OF CONDENSED PYRROLO[3,2-d]PYRIMIDINES

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Pyrrolo[3,2-d]pyrimidines are currently attracting considerable attention in view of the high biological activity of derivatives of this heterocyclic system [7].

The aim of the present investigation was to develop synthetic methods for pyrrolo[3,2-d]-pyrimidines annelated with saturated or benzene rings, and to examine their antitumor and antiviral activity.

We have previously reported [5] the synthesis of exocyclic enaminonitriles, which are capable of undergoing the Thorpe–Ziegler cyclization to give 5-cyano-6-aminopyrrolizines, which were subsequently used to synthesize pyrimido[4,5-f]- and [5,4-e]pyrrolizines, which are novel heterocyclic systems [4, 6]. Some of the latter, containing the pyrrolo[3,2-d]pyrimidine bicyclic system, showed high antitumor activity [6].

The mode of synthesis chosen for the preparation of the starting N-functionalized enamines was based on an examination of the N-alkylation of secondary enaminonitriles [8, 9]. Attempts to cyanomethylate or ethoxycarbonylmethylate β-cyano-β-ethoxycarbonylmethylenepyrrolidine (I) [2] with chloroacetonitrile or ethyl bromoacetate by heating the reactants in DMF in the presence of potassium carbonate were unsuccessful, since side reactions occurred and it was not possible to isolate the required products. For this reason, the starting material chosen was enamine with substituents which were of small bulk, and which were incapable of intramolecular hydrogen bonding in the enamine β-position, namely β,β-dicyanomethylenepyrrolidine (IIa) [2], thus presenting a more favorable situation for N-alkylation. It was found that, under the conditions described above, (IIa) was smoothly alkylated by ethyl bromoacetate to give N-ethoxycarbonyl-β,β-dicyanomethylenepyrrolidine (IIIa), isolated in 57% yield, or, more conveniently from the preparative point of view, EtONa was employed, in which case the Thorpe-Ziegler pyrrole cyclization occurred to give 5-ethoxycarbonyl-6-amino-7-cyano-1,2-dihydro-3H-pyrrolizine (IVa) in an overall yield of 75% on the enamine (IIa).

Similarly, without isolation of the intermediate enamines (IIIb, c), from β,β-dicyanomethylenepiperazine (IIb) and -hexahydroazepine (IIc) there were obtained the indolizine (IVb) and 6,7,8,9-tetrahydro-5H-pyrrolo[1,2-a]azepine (IVc).

The structure of the bicyclic products (IVa-c), in which the amino-group is flanked by cyano- and ethoxycarbonyl groups, offered the possibility of synthesizing two types of pyrrolopyrimidine systems therefrom, namely, pyrrolo[3,4-d]pyrimidines when the cyano-group is in-

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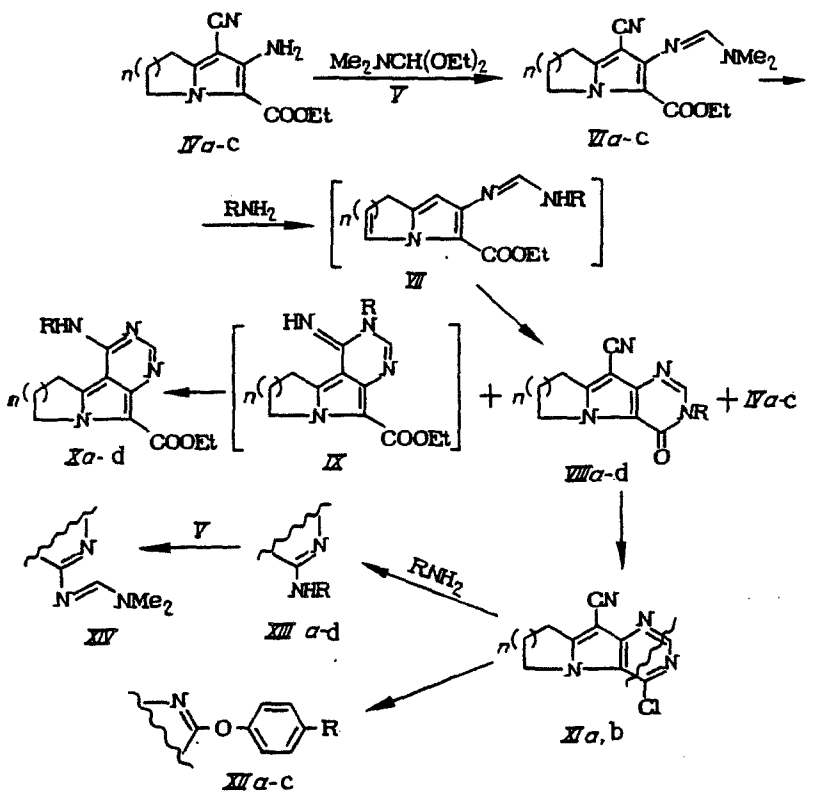
volved in the pyrimidine cyclization, and pyrrolo[3,2-d]pyrimidines when the ethoxycarbonyl group is involved.

The one-carbon component used for the closure of the pyrimidine ring was dimethylformamide diethyl acetal (V), which was reacted with the bicyclic compounds (IVa-c) to give the amidines (VIa-c). The use of such amidines for the synthesis of pyrimidines has been well covered in a review [3], and usually involves treatment with an excess of an amine. It is assumed that the intermediate amidines (VII) may be stabilized in one of three ways, namely closure of the pyrimidine ring with the involvement of the secondary amidine NH group and the cyano- or ethoxycarbonyl group [reaction products (VIII) and (X)], and cleavage of the amidine fragment to give the starting bicyclic compounds (IVa-c). The amines used were ammonia and benzylamine, the reaction with amonia being carried out at 110°C in an autoclave, and with benzylamine, in toluene solution at the boil. In several cases it was possible to isolate the final products preparatively, and in the other cases their proportions were established by ^{1}H NMR spectroscopy.

In the separation of the products and their identification when benzylamine was used, it was found that the 3-benzyl-4-iminopyrrolo[3,4-d]pyrimidines (IX) underwent the Dimroth rearrangement during the reaction (owing to the presence of excess benzylamine), to give the 4-benzylamino-compounds (Xb, c). The structures of the latter follow conclusively



R=H (VIIIa, b, Xb, XIIIa, b), CH_2Ph (VIIIc, d, Xb-d, XIIIc), Cl (XIIa), Me (XIIb), OMe (XIIc), $CH(CH_3)CH_2Ph$ (XIIId), n=1 (IVa, VIa, Xa-b), 2 (IVb, VIb, VIIIa, VIIIc, Xc, XIa, XIIIa), 3 (IVc, VIc, VIIIb, VIIId, Xd, XIb, XIIa-c, XIIIb-d)

from their ^{1}H NMR spectra. For example, in the case of 4-benzylamino-5,6-tetramethylene-7-ethoxycarbonylpyrrolo[3,4-d]pyrimidine (Xc), the following proton signals were seen: 1.43 (CH_3), 1.96-2.03 (β-CH_2 and γ-CH_2), 3.13 (δ-CH_2), 4.46 (OCH_2), 4.56 (α-CH_2), 4.81 (N–CH_2),

5.41 (NH), 7.30-7.37 (Ph), 8.50 (2-CH) ppm. The signals for the NH and benzylamine CH_2 protons (a triplet at 5.41 ppm for NH–CH_2 and a doublet at 4.81 ppm for HN–CH_2) confirm the amino-structure of the products (X), i.e., both the pyrimidine cyclization and subsequent Dimroth rearrangement occurred.

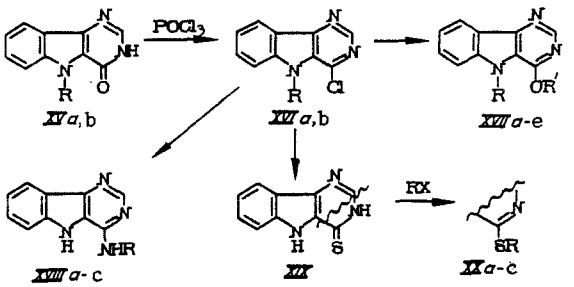
The structures of the 5,6-polymethylene-pyrrolo[3,2-d]pyrimidines (VIIIa-d) follow from their elemental analyses, IR spectra (especially the presence of CN absorption at 2200-2220 cm^{-1}), and NMR spectra (see Experimental). The yields of the pyrrolopyrimidines (VIII) and (X), and of the starting bicyclic compuonds (IV), are given in Table 1.

Examination of the data in Table 1 leads to certain conclusions as to the dependence of the course of the reactions of the amidines (VIa-c) with amines on the size of the annelated saturated ring, and the amine used. With ammonia, the formation of the starting compounds (IV) occurred to a greater extent than when benzylamine was used. To all appearances, this is due to the greater ease of addition of ammonia at the C=N bond of the amidines, followed by elimination of formamidine.

Increasing the size of the annelated saturated ring in the amidines (VIa-c) changes the direction of the reaction towards the formation of the products (VIII). The reason for this is apparent from molecular models of the intermediates (IX). It will readily be seen that in indolizines, and particularly pyrroloazepines, there is greater steric hindrance to the formation of the amines (IX), due to the close approach of the methylene and imino protons.

In the case of pyrrolizines, in which hindrance is also at a minimum, the reaction is directed predominantly towards the formation of the compounds (X), suggesting that the cyano-group in the 4-position of the pyrrole ring (the β-position) is more reactive with respect to pyrimidine cyclization than is the ethoxycarbonyl group in the 2-position (the α-position of the pyrrole ring).

The oxo-compounds obtained (VIIIa, b) were converted by treatment with phosphoryl chloride into the chlorinated tetra- and pentamethylenepyrrolo[3,2-d]pyrimidines (XIa, b). Reaction of the latter with phenols afforded the phenoxy-, and with amines, the amino-substituted tricyclic compounds (XIIa-c) and (XIIIa-d) respectively. Reaction of the amine (XIIIa) with dimethylformamide acetal gave the amidine (XIV).



R=H(XVa, XVIa, XVIIa-d), Me(XVb, XVIb, XVIIe, XXa), Ph (XVIIIa), CH_2Ph (XVIIIb, XXb), $CH(CH_3)CH_2Ph$ (XVIIIc), CH_2COOEt (XXc); R^1=Et (XVIIa, d), C_6H_4Cl-p (XVIIb), C_6H_4Me-p (XVIIc), C_6H_4OMe-p (XVIId)

In order to establish the relationship of biological activity to which ring (saturated or aromatic) was annelated to the pyrrolopyrimidine moiety, some pyrimido[5,4-b]indoles were prepared. The starting materials for the preparation of these compounds were the 4-chloropyrimido[5,4-b]indoles (XVIa, b), obtained from the corresponding oxo-compounds (XVa, b) [1]. The chlorine atom in (XVIa, b) was quite labile, and was readily replaced by the ethoxy-group on heating with EtONa (XVIIa, e). In the case of the N-unsubstituted compound (XVIa), it was found that the chlorine could also be replaced by phenoxy groups (XVIIb, d) or amino groups (XVIIIa-c). Reaction of the chloropyrimidoindole (XVIa) with thiourea proceeded smoothly to give the mercapto-derivative (XIX). Since some alkylmercapto-derivatives of pyrrolo[3,2-d]pyrimidines have shown antitumor activity, (XIX) was alkylated to give the methyl-, benzyl-, and ethoxycarbonylmethylmercaptopyrimidoindoles (XXa-c).

TABLE 1. Yields of Products of Reaction of (VI) with Amines

Starting material		Amine	Yields, %		
number	n		IV	VIII	X
VIa*	1	NH_3	56	0	44
VIb	2	NH_3	23	67	0
VIc	3	NH_3	0	100	0
VIa	1	H_2NCH_2Ph	—**	—**	79
VIb	2	H_2NCH_2Ph	0	40	42
VIc*	3	H_2NCH_2Ph	0	95	5

*Determined by ^1H NMR spectroscopy.
**Overall yield of (IV) and (VIII), 5%.

EXPERIMENTAL (CHEMISTRY)

^1H NMR spectra were recorded on a Varian XL-200 spectrometer (Switzerland), internal standard TMS. Mass spectra were obtained on a Varian MAT-112 spectrometer (West Germany). Melting points were determined on a Boetius hot plate (East Germany). The elemental analyses were in agreement with the calculated values.

1-Ethoxycarbonylmethyl-2-(cyanomethylene)pyrrolidine (IIIa). To a solution of 5.32 g (40 mmole) of the enamine (IIa) in 45 ml of dry DMF was added 5.52 g (40 mmole) of K_2CO_3 and 6.68 g (40 mmole) of ethyl bromoacetate. The mixture was stirred at 100°C for 2 h, then filtered, diluted with water (200 ml), and cooled. The solid which separated was filtered off.

1,2-Dihydro-3H-5-ethoxycarbonyl-6-amino-7-cyanopyrrolizine (IVa). Method A. The enaminonitrile (IIIa) was obtained similarly, the solution after filtration being treated with sodium ethoxide solution (1 g of sodium in ethanol) and the mixture heated at 80°c for 30 min, filtered, poured into 200 ml of water, and the solid which separated was filtered off and washed with alcohol to give (IVa).

Method B. To a solution of 4.38 g (20 mmole) of (IIIa) in 50 ml of alcohol was added a solution of sodium ethoxide in alcohol (0.2 g of sodium in 5 ml of ethanol), the mixture boiled for 30 min, and the solid which separated was filtered off. Yield 85%.

5,6,7,8-Tetrahydro-2-amino-3-ethoxycarbonyl-1-cyanoindolizine (IVb). Obtained as for (IVa), by alkylating (IIb) with ethyl bromoacetate (method A).

5H-6,7,8,9-Tetrahydro-1-cyano-2-amino-3-ethoxycarbonylpyrrolo[1,2-a]azepine (IVc). Obtained as for (IVa), by alkylating (IIc) with ethyl bromoacetate (method A).

1,2-Dihydro-3H-5-ethoxycarbonyl-6-(N,N-dimethylaminomethylene)amino-7-cyanopyrrolizine (VIa). To a solution of 6.57 g (30 mmole) of (IVa) in 50 ml of DMF was added 6.6 g (45 mmole) of the acetal (V). The mixture was heated at 100°C for 1.5 h, then evaporated under reduced pressure and the residue recrystallized to give (VIa).

5,6,7,8-Tetrahydro-2-(N,N-dimethylaminomethylene)amino-3-ethoxycarbonyl-1-cyanoindolizine (VIb). Obtained as for (IVa), from (IVb) and (V).

5H-6,7,8,9-Tetrahydro-1-cyano-2-(N,N-dimethylaminomethylene)amino-3-ethoxycarbonylpyrrolo[1,2-a]azepine (VIc). Obtained as for (IVa), from (IVc) and (V).

4-Amino-5,6-trimethylene-7-ethoxycarbonylpyrrolo[3,4-d]pyrimidine (Xa). A mixture of 5.48 g (20 mmole) of (VIa) and 60 ml of 7% alcoholic ammonia was heated in an autoclave for 6 h at 110°C (bath temp.). The mixture was then evaporated under reduced pressure to give 4.6 g of a mixture of (Xa) and (IVa) ($M_1^{+\cdot}$ 246, $M_2^{+\cdot}$ 219). The product ratio (Xa)/((IVa) was found by ^1H NMR spectroscopy.

3,4-Dihydro-5,6-tetramethylene-7-cyanopyrrolo[3,2-d]pyrimidin-4-one (VIIIa). Compound (Xa) was obtained as above, except that the reaction mixture after evaporation was treated with 10% NaOH, and the (Ivc) filtered off. The mother liquors were acidified with acetic acid to pH 7.0, and the tricyclic compound (VIIIa) filtered off.

3,4-Dihydro-5,6-pentamethylene-7-cyanopyrrolo[3,2-d]pyrimidin-4-one (VIIIb). Obtained as for (Xa).

4-Benzylamino-5,6-trimethylene-7-ethoxycarbonylpyrrolo[3,4-d]pyrimidine (Xb). A mixture of 5.48 g (20 mmole) of (VIa), 5.35 g (50 mmole) of benzylamine, and 0.01 g of toluene-p-sulfonic acid in 60 ml of dry toluene was boiled with stirring for 3 h, then evaporated under reduced pressure, excess amine removed by washing with ether, and the residue chromatographed on a column of silica gel (40/100), 25/400 mm, eluent ethyl acetate. Fraction I (R_f ~ 0.9), mass 0.1 g, mixture ($M_1^{+\cdot}$ 200, $M_2^{+\cdot}$ 219); fraction II (R_f ~ 0.1), $M^{+\cdot}$ 336 (Xb). The crude product can also be purified by crystallization from aqueous DMF.

^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.40 (3H, t, CH_2), 2.59 (2H, quint., β-CH_2), 3.15 (2H, t, γ-CH_2), 4.37-4.54 (4H, m, α-CH_2 and O–CH_2), 4.79 (1H, d, N–CH_2), 5.54 (1H, t, NH), 7.28-7.34 (5H, m, Ph), 8.48 (1H, s, 2-CH).

4-Benzylamino-5,6-tetramethylene-7-ethoxycarbonylpyrrolo[3,4-d]pyrimidine (Xc) and 3,4-dihydro-3-benzyl-7-cyano-5,6-tetramethylenepyrrolo[3,2-d]pyrimidin-4-one (VIIIc). Obtained as for (Xb), (VIIIc) and (Xc) were separated by chromatography: fraction I (R_f ~ 0.85), (VIIIc) and (Xc) were separated by chromatography: fraction I (R_f ~ 0.85), (VIIIc) (yield 40%). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.92-2.07 (4H, m, β-CH_2 and γ-CH_2), 3.05 (2H, t, δ-CH_2), 4.50 (2H, t, α-CH_2), 5.17 (2H, s, NCH_2), 7.28-7.35 (5H, m, Ph), 8.01 (1H, s, 2-CH). Fraction II (R_f ~ 0.1), (Xc) (yield 42%). ^{1}H NMR spectrum ($CDCl_3$): 1.43 (3H, t, CH_3), 1.96-2.03 (4H, m, β-CH_2 and γ-CH_2), 3.13 (2H, t, δ-CH_2), 4.46 (2H, q, O–CH_2), 4.56 (2H, t, α-CH_2), 4.81 (1H, d, N–CH_2), 5.41 (1H, t, NH), 7.30-7.37 (5H, m, Ph), 8.50 (1H, s, 2-CH).

3,4-Dihydro-3-benzyl-5,6-pentamethylene-7-cyanopyrrolo[3,2-d]pyrimidin-4-one (VIIId). Obtained as for (Xb), product ratio (VIIId/Xd 95:5 (found by ^{1}H NMR). Compound (VIIId) can also be purified by crystallization from acetonitrile.

4-Chloro-5,6-tetra(penta)methylene-7-cyanopyrrolo[3,2-d]pyrimidines (XIa, b). A mixture of 50 mmole of (VIIIa) or (VIIIb), 60 ml of $POCl_3$, and 2.75 g (20 mmole) of $Et_3N \cdot HCl$ was boiled until all the solid had dissolved (~1 h), then excess $POCl_3$ was removed under reduced pressure, and the residue poured on to ice, basified with K_2CO_3 to pH 9.0, extracted with benzene, dried over Na_2SO_4, and evaporated to give (XIa, b).

4-Amino-5,6-tetra(penta)methylene-7-cyanopyrrolo[3,2-d]pyrimidines (XIIIa-d). A solution of (Xa) or (Xb) (10 mmole) in 20 ml of alcohol was heated in an autoclave with the appropriate amine (in the case of ammonia, a 16-fold excess of ammonia was used, and heating continued for 6 h at 140°C, while with benzylamine and α-methyl-β-phenylethylamine a twofold excess was used and the mixture heated for 14 h at 180°C), then the reaction mixture was evaporated under reduced pressure, the residue triturated thoroughly with water, and the solid filtered off and washed with alcohol.

4-(N,N-Dimethylaminomethylene)amino-5,6-pentamethylene-7-cyanopyrrolo[3,2-d]pyrimidine (XIV). To a solution of 1 g (4.4 mmole) of (XIIIb) in 30 ml of DMF was added 1.3 g (8.8 mmole) of the acetal (V), and the mixture heated for 2.5 h at 100°C. It was then evaporated under reduced pressure, and the residue washed with alcohol to give (XIV).

4-Phenoxy-5,6-pentamethylene-7-cyanopyrrolo[3,2-d]pyrimidines (XIIa-c). A mixture of 1.23 g (5 mmole) of (XIb), 1.38 g (10 mmole) of anhydrous K_2CO_3, and 5.5 mmole of p-chlorophenol, p-cresol, or hydroquinone monomethyl ether in 15 ml of DMF was heated for 1 h at 100°C, the solid removed by filtration, evaporated under reduced pressure, and the residue crystallized.

5-Methylpyrimido[5,4-b]indol-4-one (XVb). A mixture of 2.2 g (10 mmole) of 1-methyl-2-ethoxycarbonyl-3-aminoindole [8] and 10 ml of formamide was heated for 2 h at 220°C under nitrogen, cooled, and the resulting solid filtered off and washed with ether to give 1 g of (XVb).

4-Chloro-5-methylpyrimido[5,4-b]indole (XVIb). To a suspension of 1 g (5 mmole) of (XVb) in 20 ml of dry dioxane was added 10 ml of $POCl_3$. The mixture was boiled for 8 h, cooled, evaporated to 2/3 of its volume under reduced pressure, poured on to ice, and the solid which separated was filtered off, washed with water, and dried to give 0.62 g of (XVIb).

4-Ethoxypyrimido[5,4-b]indoles (XVIIa, e). A solution of 8 mmole of 4-chloro-5-methylpyrimido[5,4-b]indole (XVIa) [1] or 4-chloro-5-methylpyrimido[5,4-b]indole (XVIb) in 20 ml of absolute alcohol and 7.8 ml of a solution of sodium ethoxide obtained from 11.5 g of sodium and 190 ml of absolute alcohol was boiled for 1.5 or 3 h respectively. The mixture was cooled, the solvent removed under reduced pressure, and the residue triturated with alcohol and recrystallized from 25% alcohol.

TABLE 2. Properties of Products Obtained

Compound	Yield %	Mp, °C (solvent)	Empirical formula
IIIa	57	91–3 (alcohol)	$C_{11}H_{13}N_3O_2$
IVa	75	239–41 (DMF)	$C_{11}H_{13}N_3O_2$
IVb	53	173–5 (MeCN)	$C_{12}H_{15}N_3O_2$
IVc	65	161–3 (alcohol)	$C_{13}H_{17}N_3O_2$
VIa	81	159–61 (alcohol)	$C_{14}H_{16}N_4O_2$
VIb	76	117–9 (heptane-benzene, 3:1)	$C_{15}H_{20}N_4O_2$
VIc	84	115–7 (heptane)	$C_{16}H_{22}N_4O_2$
Xb	79	223–5 (Aq. DMF)	$C_{19}H_{20}N_4O_2$
Xc	42	200–2 (MeCN)	$C_{20}H_{22}N_4O_2$
VIIIa	67	>300 (DMF)	$C_{11}H_{10}N_4O$
VIIIb	100	284–6 (DMF-alcohol, 1:1)	$C_{12}H_{12}N_4O$
VIIIc	40	168–9 (alcohol)	$C_{18}H_{16}N_4O$
VIIId	95	210–3 (MeCN)	$C_{19}H_{18}N_4O$
XIa	79	189–91 (benzene–alcohol, 1:1)	$C_{11}H_9N_4Cl$
XIb	88	135–8 (heptane–ethyl acetate, 1:1)	$C_{12}H_{11}N_4Cl$
XIIa	82	246–7 (DMF)	$C_{18}H_{15}N_3OCl$
XIIb	82	227–9 (DMF-alcohol, 1:1)	$C_{19}H_{18}N_4O$
XIIc	73	217–8 (ditto)	$C_{19}H_{18}N_4O_2$
XIIIa	61	>300 (» »)	$C_{11}H_{11}N_5$
XIIIb	93	283–5 (» »)	$C_{12}H_{13}N_5$
XIIIc	74	222–4 (» »)	$C_{19}H_{19}N_5$
XIIId	90	176–8 (» »)	$C_{21}H_{23}N_5$
XIV	95	202–3 (i-PrOH)	$C_{15}H_{16}N_6$
XVb	52	260–2 (25% alcohol)	$C_{11}H_9N_3O$
XVIb	57	145–6 (25% alcohol)	$C_{11}H_8ClN_3$
XVIIa	41	235–7 (90% alcohol)	$C_{12}H_{11}N_3O$
XVIIb	34	228–9 (80% alcohol)	$C_{18}H_{10}ClN_3O$
XVIIc	73	196–7 (50% alcohol)	$C_{17}H_{13}N_3O_2$
XVIId	43	204–5 (80% alcohol)	$C_{17}H_{13}N_3O_2$
XVIIe	33	120–1 (25% alcohol)	$C_{11}H_{13}N_3O$
XVIIIa	62	>300 (Aq. DMF)	$C_{16}H_{12}N_4$
XVIIIb	37	237–8 (benzene-alcohol, 9:1)	$C_{17}H_{14}N_4 \cdot AcOH$
XVIIIc	75	179–80 (Aq. DMF)	$C_{19}H_{17}N_4$
XIX	62	>300 (50% dioxane)	$C_{10}H_7N_3S$
XXa	80	256–7 (alcohol)	$C_{11}H_9N_3S$
XXb	83	223–4 (alcohol)	$C_{17}H_{13}N_3S$
XXc	51	178–9 (alcohol)	$C_{21}H_{17}N_3O_2S$

Phenoxy-5H-pyrimodo[5,4-b]indoles (XVIIb-d). A mixture of 1.12 g of anhydrous potassium carbonate, 20 ml of DMF, and 4.4 mmole of p-chlorophenol, hydroquinone monomethyl ether, or p-cresol was stirred for 5 min at ambient temperature. The mixture was then treated with 0.67 g (4 mmole) of 4-chloropyrimido[5,4-b]indole (XVIIa), and boiled for 7 h. On cooling, inorganic material was filtered off, the filtrate evaporated under reduced pressure, and the residue triturated with alcohol.

4-Amino-5H-pyrimido[5,4-b]indoles (XVIIIa-c). A mixture of 0.67 g (4 mmole) of 4-chloropyrimido[5,4-b]indole (XVIa) and 5 ml of the appropriate amine was heated at 120-130°C for 6 h, then the solid was filtered off, and washed with water and alcohol to give (XVIIIa, c). The acetate of (XVIIIb) was obtained by treating the reaction mixture with 60% acetic acid to pH 7.0.

4-Mercapto-5H-pyrimido[5,4-b]indole (XIX). To a solution of 0.65 g (4 mmole) of 4-chloropyrimido[5,4-b]indole (XVIa) in absolute alcohol was added 0.3 g (4 mmole) of thiourea, the mixture boiled for 0.5 h, cooled, and the solid which separated filtered off, dissolved in 10 ml of 1 N NaOH, and 5 ml of acetic acid added to pH 6.0. The solid which separated was filtered off, washed with water, and dried to give 0.5 g of (XIX).

Mercaptopyrimido[5,4-b]indoles (XXa-c). To a suspension of 5 mmole of 4-mercaptopyrimodo-[5,4-b]indole in 20 ml of absolute alcohol was added a solution of 0.2 g (5 mmole) of NaOH in 20 ml of absolute alcohol, the mixture stirred for 15 min at ambient temperautre, heated to the boil, and boiled for a further 15 min. The mixture was then cooled to 20°C, and 5 mmole of MeI, benzyl chloride, or ethyl chloroacetate added. The mixture was then boiled for 0.5 h, cooled, the solvent removed under reduced pressure, and the residue washed with water and dried.

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EXPERIMENTAL (BIOLOGY)

The antiviral activity of the compounds was examined with respect to type 1 herpes simplex virus (strain L_2) and influenza virus (strains A/FPV (H7N7) and A/Bethesda/63 (H2N2)).

The viral inhibitory activity of the compounds was assessed in primarily trypsinized cell cultures of chick embryo fibroblasts (CEF) infected with 10-100 50% tissue cytopathic doses (TCD_{50}) of virus. The maximum tolerated concentrations (MTC) of the test compounds were determined on monolayers of intact CEF cultures, and subsequently examined in concentrations of 1/4 and 1/8 of the MTC.

The therapeutic activity of the compounds was examined in model generalized herpes and influenzal pneumonia in mice induced by intranasal infection of the animals with the herpes simplex or influenza viruses. The activity of the compounds was assessed by the prevention of cytopathic effects of the virus on the cells, and reduction in the infective titer of the virus in in vitro tests, or by the mortality of the animals in in vivo tests, as compared with the control groups.

It was found that the MTC values for CEF cell cultures for (VIIIb, d), (XIa, b), (XIIa-c), (XIIIc), and (XIV), which are pyrrolopyrimidines, ranged from 20 to 40 μg/ml. The pyrimido-[5,4-b]indoles (XVIIb-d) showed greater cytotoxicity in CEF cell cultures (MTC 10 μg/ml).

Of the compounds tested ((VIIIb, d), (XIa, b), (XIIa-c), (XIIIc), (XIv), and (XVIIb-d)), (XIa) suppressed the reproduction of herpes simplex virus in CEF cell culture, reducing the infective titer by 1 lg TCD_{50} in a concentration of 10 μg/ml. It had no therapeutic activity in model generalized herpes infection in mice induced by intranasal infection with this virus.

No activity against influenza virus was found, either in vitro or in vivo, with any of the test compounds.

Of the twelve condensed pyrrolo[3,2-d]pyrimidines tested, therefore, antiviral activity against DNA-genomic herpes simplex virus has been found in (XIa), although activity against the RNA-genomic influenza virus has not been observed.

Antitumor activity was examined in 156 mongrel white rats (initial body weights 120-130 g) with transplanted Jensen's sarcoma and 302 NDF_1 hybrid mice (initial body weights 18-20 g) with carcinoma 755 and leukemia P388. The test compounds were given intraperitoneally in 10% polyvinylpyrrolidione solution (in a concentration of 1%) daily for 5-7 days, commencing three days (for Jensen's sarcoma and carcinoma 755) or 24 h (for leukemia P388) following tumor transplantation. Activity was assessed by the percentage retardation of the growth of the solid tumors in the treated animals as compared with the controls (48 h after cessation of treatment), or by the change in mean lifespan in the leukemic mice.

The 4-chloro-7-cyanopyrrolo[3,2-d]pyrimidines (XIa, b) were found to be more toxic than the corresponding 4-amino- and 4-oxo-compounds. For example, the tolerated therapeutic doses of (XIa, b) are 25 mg/kg in mice, whereas the 4-amino- (XIIIa, c, d) and 4-oxo-compounds (VIIIb, d) are well tolerated by the animals in doses of around 100 mg/kg. None of the compounds in this group were found to be active in mice with carcinoma 755 or leukemia P388, but the 4-amino- and 4-oxo-compounds slightly inhibited (by 30-40%) the growth of Jensen's sarcoma, while greater activity (approximately 50%) was shown by (VIIIb) and (XIIIa), which do not carry substituents at N(3) or the 4-amino-group of the pyrimidine ring respectively.

The 4-substituted pyrimido[5,4-b]indoles (XVIIIb, c) and (XXa-c) showed no antitumor activity.

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