LITERATURE CITED

1. R. Adams, C. Weisel, and H. Mosher, J. Am. Chem. Soc., 68, 885 (1946).
2. V. R. Jaertner, J. Org. Chem., 33, 523 (1968).
3. T. P. Johnson, L. B. Holum, and J. A. Montgomery, J. Am. Chem. Soc., 80, 6265 (1958).
4. J. H. Werner, Pharmacol. Ther., 6, 235 (1979).

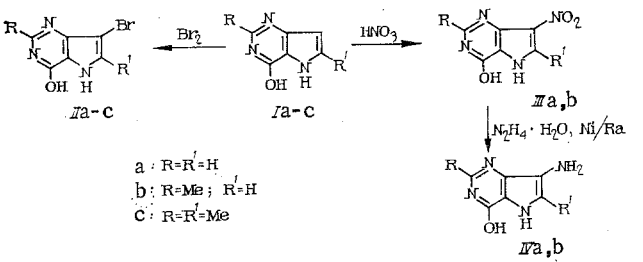
SYNTHESIS AND BIOLOGICAL ACTIVITY OF 4,7-SUBSTITUTED PYRROLO[3,2-d]PYRIMIDINES

O. S. Sizova, G. A. Modnikova, R. G. Glushkov, N. P. Solov'eva, N. A. Ryabokon', V. A. Chernov, O. V. Okinshevich, and G. N. Pershin

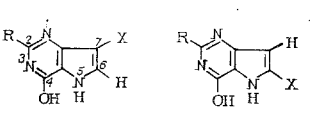
UDC 615.28+615.277.3[:547.853].012

Continuing the search for antitumor and antibacterial pyrrolo[3,2-d]pyrimidines [4, 7], we have now synthesized some new 4- and 7-substituted pyrrolo[3,2-d]pyrimidines and studied their biological activity.

Bromination of the pyrrolo[3,2-d]pyrimidines (Ia-c) with bromine in acetic acid afforded the 7-bromo derivatives (IIa-c), and nitration of (Ia) and (Ib) with nitric acid gave the 7-nitro derivatives (IIIa, b).



The structures of (IIa, b) and (IIIa, b) were proved by their PMR spectra (Table 1). The choice between the two possible positions for the substituent X in these compounds (structures A and B) was made by comparing the experimental value of the chemical shift of the proton in the five-membered ring with the values calculated for the expected structures.



X=Br, NO_2

The value of the chemical shift was calculated by an additive method: $C_7H(C_6H) = \delta H_o + Z_{ortho}$, where δH_o is the chemical shift of the C_6H or C_7H proton in the unsubstituted pyrrolopyrimidine, and Z_{ortho} is the change in δH_o following introduction of the substituent X into the five-membered ring. The increments Z_{ortho} were obtained from the benzene series: For the bromine atom, Z_{ortho} = 0.18 ppm, and for the nitro group, it is 0.95 ppm [2].

The calculated values for the chemical shifts δH for the five-membered ring in (IIa, b) and (IIIa, b) are also shown in Table 1.

It will be seen from Table 1 that the chemical shifts of the five-membered ring proton in these pyrrolo[3,2-d]pyrimidines are in good agreement with the δH values calculated for structure A. It should be noted that the differences in the experimental and calculated values for the chemical shifts δH, which are 0.14 ppm for (IIb) and 0.15 ppm for (IIIa), are

S. Ordzhonikidze All-Union Scientific-Research Institute of Pharmaceutical Chemistry, Moscow. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 18, No. 8, pp. 958-962, August, 1984. Original article submitted December 12, 1983.

0091-150X/84/1808-0567$08.50

TABLE 1. PMR Spectra of (Ia, b), (IIa, b), and (IIIa, b)

Compound	Experimental values of chemical shifts, ppm			Calculated values of chemical shifts, ppm	
	δC_2H or δC_2-CH_3	δC_7H	δC_6H	for structure A	for structure B
Ia	7,77	6,35	7,34	—	—
Ib	2,39	6,35	7,46	—	—
IIa	7,84		7,53	7,52	6,52
IIb	2,33		7,50	7,64	6,53
IIIa	8,05		8,44	8,29	7,30
IIIb	2,48		8,47	8,41	7,30

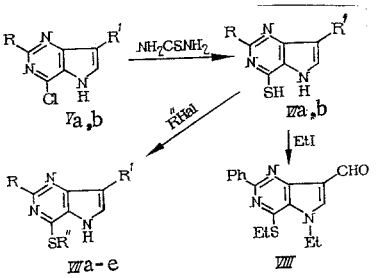
Note. The PMR spectra of (Ia), (IIa), and (IIIa) were obtained in DMSO-d_6, and of (Ib), (IIb), and (IIIb), in DMF-d_7.

evidently due to the fact that the increments used were for the benzene series. These differences, however, do not prevent an unambiguous choice being made between structures A and B.

The nitro compounds (IIIa, b) were reduced with hydrazine hydrate in the presence of Raney nickel to the 7-aminopyrrolopyrimidines (IVa, b).

Reaction of the 4-chloro-pyrrolo[3,2-d]pyrimidines (Va, b) with thiourea followed by treatment of the resulting thiouronium salt with sodium hydroxide solution gave the 4-mercaptopyrrolopyrimidines (VIa, b) and from these the thioethers (VIIa-e) were obtained.

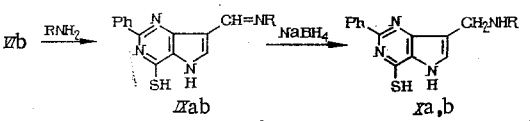
It should be emphasized that the alkylation of (VIc) with EtBr selectively affords the ethylthio derivative (VIIc), whereas the use of EtI in this reaction results in attack at two centers (SH and NH) with the formation of (VIII).



Va, VIa: R=R¹=H; Vb, VIb: R=Ph, R¹-CHO; VIIa: R=R′=H, R″=Et; VIIb: R=R¹H, R″=CH_2CH_2OH, VIIc: R=Ph, R¹=CHO, R″=Et; VIId: R=Ph, R¹=CHO; R″=CH_2Ph; VIIe: R=R¹=H, R″=CH_2Ph; VIIa, c: Hal=Br;, VII6 d, e: Hal=Cl.

Reaction of the aldehyde (VIb) with amines gave the Schiff's bases (IXa, b), which were then reduced with $NaBH_4$ to the 4-mercapto-7-aminomethylpyrrolopyrimidines (Xa, b).

The IR spectra of the Schiff's bases (IXa, b) showed absorption characteristic of C=N at 1645 cm^{-1}, which was absent from the spectra of the reduced compounds (Xa, b).



IXa, Xa: R = Bu; IXb, Xb: R = cyclohexen-1-ylethyl

The yields, melting points, and elemental analyses of the compounds obtained are given in Table 2.

The antimicrobial activity of the compounds prepared was studied in *in vitro* experiments by the methods described in [3] against nine species of gram-positive and gram-negative bacteria, three species of acid-resistant mycobacteria, and five species of pathogenic fungi.

The test compounds were virtually inactive against gram-negative bacteria and pathogenic fungi, the minimum inhibitory concentrations being greater than 500 μg/ml.

TABLE 2. Pyrrolo[3,2-d]pyrimidines

Compound	Yield, %	mp, °C	Found, %					Empirical formula	Calculated, %				
			C	H	Br	N	S		C	H	Br	N	S
Ia	70,0	300 (decomp.)	33,53	1,68	36,96	19,79	—	$C_6H_4BrN_3O$	33,67	1,88	37,33	19,63	—
IIb	58,5	292—3	36,73	2,77	35,09	. . .	—	$C_7H_6BrN_3O$	36,86	2,65	35,04	18,43	—
IIc	64,5	283—5	39,59	3,57	33,10	17,35	—	$C_8H_8BrN_3O$	39,69	3,33	33,01	17,36	—
IIIa	79,0	300	40,33	1,75	—	31,41	—	$C_6H_4N_4O_3$	40,0	2,24	—	31,11	—
IIIb	69,2	300	43,32	2,95	—	28,80	—	$C_7H_6N_4O_3$	43,31	3,12	—	28,86	—
IVa	44,3	300	47,50	4,20	—	37,28	—	$C_6H_6N_4O$	48,00	4,03	—	37,32	—
IVb	40,0	249 (decomp.)	50,80	4,85	—	34,01	—	$C_7H_8N_4O$	51,21	4,91	—	34,13	—
VIa	76,0	320	47,90	3,70	—	27,85	21,73	$C_6H_5N_3S$	47,67	3,33	—	27,79	21,21
VIb	85,3	265—8	61,21	3,58	—	16,71	12,20	$C_{13}H_9N_3OS$	61,16	3,54	—	16,46	12,55
VIIa	55,0	164—5	53,78	5,05	—	23,35	17,92	$C_8H_9N_3S$	53,61	5,06	—	23,44	17,89
VIIb	51,0	208—10	49,09	4,70	—	21,59	16,43	$C_8H_9N_3OS$	49,21	4,65	—	21,52	16,42
VIIc	59,0	185—7	63,37	4,81	—	14,71	11,29	$C_{15}H_{13}N_3OS$	63,58	4,62	—	14,83	11,31
VIId	59,0	190—2	69,73	4,09	—	11,88	9,32	$C_{20}H_{15}N_3OS$	69,54	4,37	—	12,16	9,28
VIIe	65,0	178—81	64,89	4,63	—	17,60	—	$C_{13}H_{11}N_3S$	64,60	4,56	—	17,37	—
VIII	55,0	142—4	65,90	5,45	—	13,30	10,33	$C_{17}H_{17}N_3OS$	65,57	5,50	—	13,49	10,29
IXa	58,0	214—6	65,55	5,80	—	18,01	10,61	$C_{17}H_{18}N_4S$	65,77	5,84	—	18,05	10,32
IXb	91,5	69—71	69.47	6,42	—	15,51	8,64	$C_{21}H_{22}N_4S$	69,58	6,12	—	15,46	8,84
Xa	60,0	175—6	65,07	6,51	—	17,96	10,29	$C_{17}H_{20}N_4S$	65,35	6,45	—	17,93	10,26
Xb	75,0	169—71	69,21	6,50	—	15,18	8,96	$C_{21}H_{24}N_4S$	69,20	66,4	—	15,37	8,80

Note. Compounds (IIa, b) and (IIIb) were crystallized from DMF, (IIc), (IVb), (VIa, b), (VIIa-d), (VIII), and (Xa) from ethanol, (IVa) from isoamyl alcohol, (IXa) from benzene, (Xb) and (VIIe) from aqueous ethanol, and (IIIa) was purified via its sodium salt.

The greatest activity against gram-positive bacteria was shown by (VIId), which inhibited the growth of *Staphylococcus aureus, Streptococcus hemolyticus, Corynebacterium diphtheriae,* and anthracoid spores in concentrations of 2-3.9 μg/ml.

Compound (IXb) was moderately active against gram-positive bacteria.

Compounds (IXa) and (Xa) displayed tuberculostatic activity.

4-Oxo-2-phenyl-7-(β-cyclohexenyl)ethylaminomethyl-3,4-dihydropyrrolo[3,4-d]'pyrimidine has been found to inhibit strongly the enzyme dihydrofolate reductase [4]. Replacement of the hydroxy group by mercapto in the pyrrolopyrimidine (Xb) resulted in a decrease in the antireductase activity ($I_{50} = 1.10^{-5}$ M).

EXPERIMENTAL

IR spectra were obtained on a Perkin-Elmer 457, and PMR spectra on a Varian XL-100, internal standard TMS.

4-Hydroxy-7-bromopyrrolo[3,2-d]pyrimidine (IIa). To a suspension of 1.6 g (0.012 mole) of (Ia) [6] in 20 ml of acetic acid was added a solution of 2.1 g (0.013 mole) of bromine in 5 ml of acetic acid. Ten ml of water was then added, and the mixture stirred at 45°C until the color of the bromine had disappeared (∿1.5 h). The mixture was then cooled, and the solid which separated was filtered off and washed with water and ethanol to give 2 g of (IIa).

Compounds (IIb) and (IIc) were obtained similarly from (Ib) [1] and (Ic) [9].

4-Hydroxy-7-nitropyrrolo[3,2-d]pyrimidine (IIIa). To 6 ml of concentrated nitric acid (d = 1.5), cooled to 0°C, was added portionwise with stirring 2 g of (Ia), the temperature of the mixture being kept at 0-5°C. The mixture was stirred at this temperature for 30 min, then at 20-25°C for 30 min. The solution was poured onto ice, and kept for 16 h at 5-10°C. The solid which separated was filtered off and washed with water to give 2.1 g of (IIIa).

Compound (IIIb) was obtained similarly.

4-Hydroxy-7-aminopyrrolo[3,2-d]pyrimidine (IVa). To a suspension of 0.19 g of (IIIa) in 20 ml of isoamyl alcohol was added 1.5 ml of hydrazine hydrate and 1 g of Raney nickel. The mixture was heated at 75-85°C with stirring for 3 h, and filtered from the catalyst while still hot. The filtrate was cooled, and the precipitate filtered off to give 0.07 g of (IVa). IR spectrum, ν_{max}, cm^{-1}: 3360 (NH_2).

Similarly obtained was (IVb) (reaction time 10 min). IR spectrum, ν_{max}, cm^{-1}: 3350 cm^{-1} (NH_2).

2-Phenyl-4-mercaptopyrrolo[3,2-d]pyrimidine-7-aldehyde (VIb). A mixture of 6.5 g (0.025 mole) of (Vb) [5], 2 g (0.026 mole) of thiourea, and 150 ml of ethanol was boiled with stirring for 6 h. The solution was evaporated *in vacuo*, and the residue was dissolved in 150 ml of 1 N NaOH. The resulting solution was neutralized to pH 6.0, and the solid which separated was filtered off and washed with water to give 5.5 g of (VIb).

Similarly obtained from (Va) [8] was (VIa).

4-Ethylthiopyrrolo[3,2-d]pyrimidine (VIIa). To a solution of 0.3 g (0.002 mole) of (VIa) in 6 ml of 2% NaOH was added 0.27 g (0.002 mole) of EtBr. The mixture was heated at 40-46°C for 10 h, and the solid which separated on cooling was filtered off and washed with water to give 0.2 g of (VIIa).

Similarly obtained were (VIIb-e). Reaction times for (IIIa, d), 3 and 1.5 h. PMR spectrum of (VIIc) (C_5D_5N), δ, ppm: 1.45 and 3.48 (C_2H_5); 8.52 (6-H); 7.4-9.0 (C_6H_5); 10.77 (CHO). PMR spectrum of (VIId) (C_5D_5N), δ, ppm: 4.9 (CH_2); 8.6 (6-H); 7.1-9.0 (C_6H_5); 10.5 (CHO).

Reaction of (VIb) with an excess of EtBr (3 mole) gave a mixture of (VIIc) and (VIII) (by TLC on Silufol UV-254 in chloroform).

2-Phenyl-4-ethylthio-5-ethylpyrrolo[3,2-d]pyrimidine-7-aldehyde (VIII). To a solution of 1.5 g (0.006 mole) of (VIc) in 20 ml of 4% NaOH was added 2.2 g (0.014 mole) of EtI, and the mixture was heated with stirring at 70°C for 18 h. The solid which separated on cooling was filtered off and washed with water, giving 1 g of (VIII). PMR spectrum ($CDCl_3$), δ, ppm: 1.57, 1.54 (CH_3CH_2); 3.54, 4.50 (CH_3CH_2); 7.92 (6-H); 7.4-8.6 (C_6H_5); 10.44 (CHO).

2-Phenyl-4-mercapto-7-n-butylaminomethylenepyrrolo[3,2-d]pyrimidine (IXa). A solution of 2 g (0.008 mole) of (VIb) and 1.2 g (0.016 mole) of n-butylamine in 60 ml of dry dioxane was heated with stirring for 12 h at 80°C. The solvent was distilled off *in vacuo*, and the residue crystallized to give 1.4 g of (IXa).

Similarly obtained was (IXb), heating being continued for 3 h at 80°C.

2-Phenyl-4-mercapto-7-n-butylaminomethylpyrrolo[3,2-d]pyrimidine (Xa). To a suspension of 1.3 g (0.004 mole) of (IXa) in 30 ml of dry methanol was added gradually 0.2 g (0.005 mole) of $NaBH_4$. The mixture was boiled for 2 h, and the solid which separated on cooling was filtered off and washed with water to give 0.8 g of (Xa).

Similarly obtained was (Xb), the mixture being boiled for 15 min. The compound (Xb) was isolated by diluting the reaction mixture with water.

LITERATURE CITED

1. N. E. Britikova and K. Yu. Novitskii, Khim. Geterotsikl. Soedin., No. 12, 1612-1674 (1977).
2. A. G. Gordon and R. Ford, The Chemists's Companion [in Russian], Moscow (1976), p. 288.
3. G. N. Pershin (ed.), Methods of Experimental Chemotherapy (2nd edn.) [in Russian], Moscow (1971).
4. G. A. Modnikova, O. S. Sizova, K. Yu. Novitskii, et al., Khim.-farm. Zh., No. 5, 36-40 (1982).
5. O. S. Sizova, N. E. Britikova, K. Yu. Novitskii, et al., ibid., No. 7, 63-66 (1980).
6. O. S. Sizova, N. E. Britikova, K. Yu. Novitskii, et al., ibid., No. 11, 58-63 (1982).
7. V. N. Sokolova, G. A. Modnikova, K. Yu. Novitskii, et al., ibid., No. 9, 17-22 (1979).
8. Kin-ichi Imal. Chem. Pharm. Bull., 12, 1030-1040 (1964).
9. Kuiyoshi Tanaka, Toshio Sugawa, Ritsuo Nakamori, et al., ibid., 1024-1030.