SYNTHESIS AND ANTIVIRAL ACTIVITY OF PYRAZOLO[3,4-d]-1,3,2-DIAZAPHOSPHORINS

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Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 32, No. 7, pp. 16 – 19, July, 1998.

Original article submitted December 29, 1997.

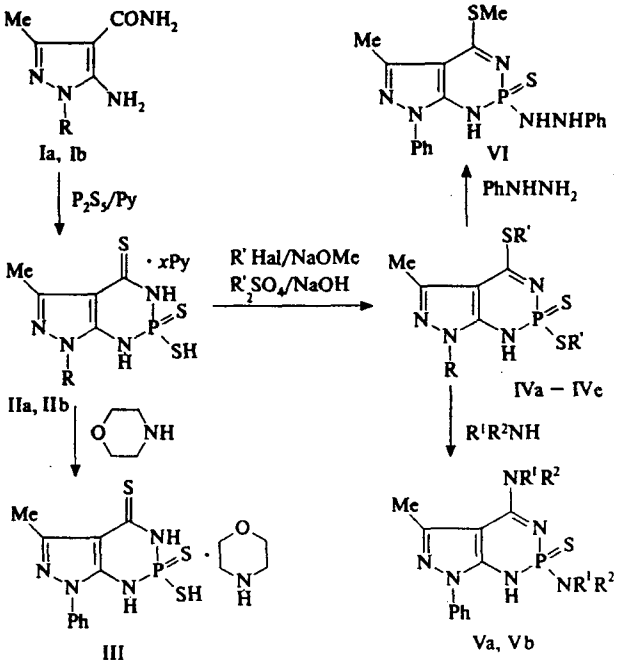
The interaction between compounds containing amino and carbamoyl (or thiocarbamoyl) groups in the neighboring positions, on the one hand, and the P_2S_5 – pyridine system, on the other hand, may readily close the 1,3,2-diazaphosphorin cycle [1 – 4]. This approach was used for the synthesis of phosphorus-containing analogs of purines [3] and pyrimidines [4]. The group of pyrazolo-[3,4-d]pyrimidine derivatives was reported to include compounds possessing pronounced antitumor activity [5 – 7], while the group of 1,3,2-diazaphosphorins contains compounds exhibiting the pharmacological effects of some other types as well [1, 8, 9].

The purpose of this work was to develop the synthesis of phosphorus-containing analogs of pyrazolo-[3,4-d]pyrimidine and study their antiviral properties.

The initial compounds were 3-methyl-4-carbamoyl-5-aminopyrazole (Ia) and 1-phenyl-3-methyl-4-carbamoyl-5-aminopyrazole (Ib) obtained as mentioned above by the interaction of 1-cyano-2-dimethylaminocrotonamide with hydrazine or phenylhydrazine [5]. Unlike the method used in [5, 6], where the pyrimidine cycle closure was achieved by the reactions of compounds Ia and Ib with formamide and thiourea, we have used the reaction of compounds Ia and Ib with a P_2S_5 – pyridine system. As a result, we isolated diazaphosphorins (IIa, IIb) in the form of pyridine-containing solvates having variable composition, which could be converted into stable salts by using amines of higher basicity (e.g., morpholine) [4]. In the case of initial compound IIb, the proposed structure of the resulting salt (III) was confirmed by the data of spectroscopic methods and the results of elemental analyses.

Diazaphosphorins IIa and IIb in the form of sodium salts readily enter the S-alkylation reaction to form 2,4-bisalkyl derivatives (IVa – IVe). It is interesting to note that, in contrast to diazaphosphorins described previously [3, 4], reactions of these derivatives with dimethyl sulfate do not lead to N-alkylation.

The dimethylthio derivative IVb was brought into contact with various primary and secondary amines in the absence of solvents, the reactions being conducted at a boiling temperature of each particular amine. In the case of reactions with piperidine and benzylamine (T_b = 106 and 185°C, respectively), a 30-min boiling of the mixture yielded 2,4-diamino substituted products (Va, Vb). A monohydrazide (VI) was isolated from the reaction mixture by 2 – 3-min boiling with phenylhydrazine (T_b = 241°C).



I, II: R = H (a), Ph (b);
IV: R = H, R′ = Me (a); R = Ph, R′ = Me (b); R = Ph, R′ = CH_2Ph (c); R = H, R′ = CH_2Ph (d); R = H, R′ = CH_2CO_2Me (e);
V: R^1 = H, R^2 = CH_2Ph (a); R^1R^2 = $-(CH_2)_4-$ (b)

The ^1H NMR spectrum of compound VI measured in DMSO-d_6 displays the following signals (δ, ppm): 2.35 and 2.41 (2s, 3H, Me, SMe), 6.67 (1H), 6.90 (2H), 7.10 (2H, aro-

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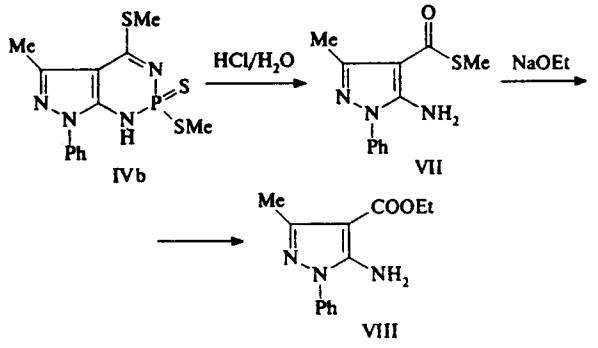
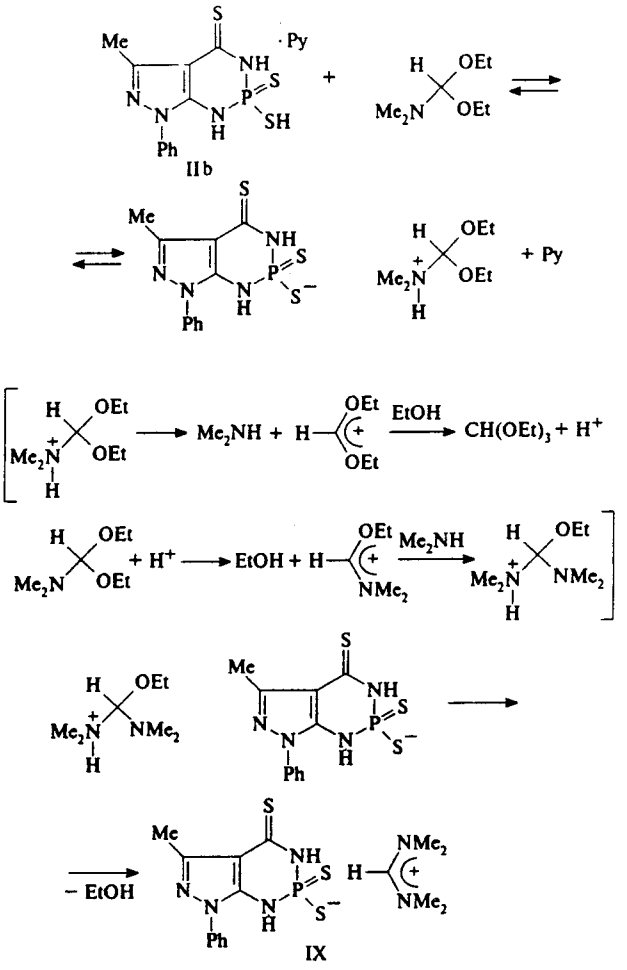
0091-150X/98/3207-0358$20.00

matic protons of phenylhydrazine fragments), 7.22 – 7.48 (m, 5H, phenyl cycle in position 7). The signals of NH protons are observed at 7.19 ppm ($^2J_{NH,P}$ 3.2 Hz), 7.50 ppm, and 9.66 ppm ($^2J_{NH,P}$ 5.3 Hz, 1-NH). The mass spectrum of compound VI contains peaks of the following ions (*m/z*): 414 $[M]^+$, 307 $[M–PhNHNH]^+$, 108 $[PhNHNH_2]^+$. Because prolonged boiling led to gumming of the reaction mass, we failed to isolate a disubstituted product.

We have also attempted to perform hydrolysis of the 2,4-dimethylthio derivative IVb with diluted hydrochloric acid. The reaction led to opening of the phosphorus-containing cycle with the formation of methyl ester of 1-phenyl-3-methyl-5-aminopyrazole-4-thiolcarboxylic acid (VII). This was evidenced by the data of mass spectrometry and IR spectrophotometry; mass spectrum (*m/z*): 247 (I_{rel} = 22) $[M]^+$, 200 (100) [M–SMe], 77 (14) $[PH]^+$; IR spectrum (λ_{max}, cm^{-1}): 3320, 3490 (NH_2), 1670 (CO). The structure of compound VII was additionally confirmed by the subsequent transetherification with sodium ethylate leading to the previously reported 1-phenyl-3-methyl-4-ethoxycarbonyl-5-aminopyrazole (VIII).



Finally, we have attempted to alkylate compound IIb with DMF diacetal. However, the system featured displacement of the pyridine component of the solvate by tetramethylformamidine instead of alkylation. This resulted in the formation of an amidinium salt IX, as confirmed by the results of elemental analyses and the data of ^1H NMR spectroscopy. A characteristic feature of the ^1H NMR spectrum of compound IX measured in DMSO-d_6 is the presence of signals corresponding to the amidinium cation fragment (δ, ppm): 3.13, 3.24 (2s, 6H, $2NMe_2$), 7.90 (s, 1H, CH^+). The anion part of salt IX corresponds to the following signals (δ, ppm): 2.48 (s, 3H, 5-CH_3), 7.32 – 7.55 (m, 5H, 7-Ph), 8.90 (bs, 1H, NH), 9.38 (d, 1H, $^2J_{NH,P}$ 16.5 Hz, NH). The formation of similar salts during the reactions of acetals with acids having analogous mechanisms was discussed in [10].



EXPERIMENTAL CHEMICAL PART

The ^1H NMR spectra were measured on a Unity+400 spectrometer (Varian, USA) using DMSO-d_6 as the solvent and TMS as the internal standard. The mass spectra were obtained with an SSQ-710 spectrometer (Finnigan, USA) with direct introduction of the samples into the ion source operated at an ionization chamber temperature of 180°C and an electron impact energy of 70 eV. The IR absorption spectra were recorded with a Perkin-Elmer Model 599 spectrophotometer using samples prepared as nujol mulls. The course of the reactions was monitored and the purity of synthesized compounds was checked by TLC on Silufol UV-254 plates developed under UV illumination. The melting temperatures were determined with the aid of a Boetius heating stage. Some physicochemical characteristics of the synthesized compounds are listed in Table 1. The data of elemental analysis (C, H, N, S) of compounds III – VII and IX agree with the results of analytical calculations according to empirical formulas.

2-Mercapto-5-methyl-7*H*-pyrazolo[3,4-d]-1,3,2-diazaphosphorin-2,4(1*H*,3*H*)-dithione · *x*Py (IIa). To a thoroughly triturated mixture of 1.5 g (6.8 mmole) of phosphorus pentasulfide and 1.5 g (10.7 mmole) of pyrazole Ia was added 8 ml of dry pyridine, and the mixture was boiled with stirring

for 1 h. Then was added 40 ml of benzene, and the mixture was additionally boiled for 5 min and cooled to 20°C. After benzene was decanted from the oily product, 50 ml of water was added and the mixture was stirred for 2 – 3 h until complete conversion of the oil into a light-yellow precipitate. The precipitate was filtered and washed with large volumes of water and methanol. Mass spectrum of compound IIa (*m/z*): 249 $[M]^+$.

A similar procedure was used to obtain **2-mercapto-5-methyl-7-phenylpyrazolo[3,4-d]-1,3,2-diazaphosphorin-2,4(1*H*,3*H*)-dithione · *x*Py (IIb).**

A Salt of 2-mercapto-5-methyl-7-phenylpyrazolo[3,4-d]-1,3,2-diazaphosphorin-2,4(1*H*,3*H*)-dithione with morpholine (III). To a suspension of 0.55 g (1.6 mmole) of compound IIb in 5 ml of methanol was added 0.44 ml (5 mmole) of morpholine, after which the initial salt dissolved. The solution was heated to boiling, boiled for 5 min, and cooled. To this solution was added diluted hydrochloric acid so as to obtain pH 6 – 7. The precipitate was separated by filtration and washed with water. The mass spectrum of compound III (*m/z*): 326 $[M]^+$, 293 $[M–SH]^+$, 77 $[Ph]^+$.

2,4-Dimethylthio-5-methyl-7H-pyrazolo[3,4-d]-1,3,2-diazaphosphorin-2(1*H*)-thione (IVa). To a suspension of 1.5 g (6 mmole) of compound IIa in 20 ml of water was added dropwise with stirring 10 % aqueous NaOH until complete dissolution of the precipitate of compound IIa. After double washing of the resulting solution with chloroform, dimethyl sulfate (2.3 g, 18 mmole) was added and the mixture was stirred for 20 min. The precipitate was separated by filtration and washed with water and 2-propanol. The ^{1}H NMR spectrum of compound IVa at room-temperature (δ, ppm): 2.03 (d, 3H, $^3J_{Me,P}$ 15.8 Hz, PSMe), 2.44 (s, 3H, 5-Me), 2.48 (bs, 3H, 4-SMe), 9.70 (broad signal, 1H, 1-NH), 12.68 (strongly broadened signal, 1H, 7-NH). The observed broadening of the 4-SMe group singlet is probably related to a particular conformational state of P atom, rather than to the spin – spin coupling, because the spectrum measured at an elevated temperature (60°C) showed significant narrowing of this singlet. The subsequent cooling of the sample was accompanied by reversible broadening of the signal. The mass spectrum of compound IVa (*m/z*): 278 $[M]^+$, 231 $[M–SMe]^+$, 199 $[M–SMe–S]^+$, 151 $[M–SMe–SMe–SH]^+$; IR spectrum (λ_{max}, cm^{-1}): 3100, 3240 (NH), 1590 (C=C).

TABLE 1. Characteristics of the Synthesized Compounds

Compound	Empirical formula	M.p., °C (solvent)	Yield, %
IIa	$C_5H_7N_4PS_3 \cdot xC_5H_5N$	~ 150	~ 48
IIb	$C_{11}H_{11}N_4PS_3 \cdot xC_5H_5N$	~ 170	~ 72
III	$C_{11}H_{11}N_4PS_3 \cdot C_4H_9NO$	225 – 228 (DMF – water, 1 : 2)	80
IVa	$C_7H_{11}N_4PS_3$	220 – 223 (methanol)	38
IVb	$C_{13}H_{15}N_4PS_3$	203 – 206 (methanol)	68
IVc	$C_{25}H_{23}N_4PS_3$	148 – 150 (methanol – DMF, 3 : 1)	55
IVd	$C_{19}H_{19}N_4PS_3$	198 – 202 (methanol – DMF, 2 : 1)	30
IVe	$C_{11}H_{15}N_4O_4PS_3$	190 – 192 (methanol)	55
Va	$C_{25}H_{25}N_6PS$	197 – 199 (DMF – water, 3 : 1)	64
Vb	$C_{21}H_{29}N_6PS$	181 – 184 (acetone)	56
VI	$C_{18}H_{19}N_6PS$	195 – 198 (DMF – water, 2 : 1)	66
VII	$C_{12}H_{13}N_3OS$	126 – 128 (2-propanol)	66
IX	$C_{16}H_{23}N_6PS_3$	177 – 179 (ethanol)	85

2,4-Dimethylthio-5-methyl-7-phenylpyrazolo[3, 4-d]-1,3,2-diazaphosphorin-2(1*H*)-thione (IVb). Compound IVb was obtained similarly to IVa proceeding from solvate IIb. The ^{1}H NMR spectrum of compound IVb (δ, ppm): 2.13 (d, 3H, $^3J_{Me,P}$ 15.8 Hz, PSMe), 2.42, 2.52 (2s, 3H, 4-SMe, 5-Me), 7.40 – 7.58 (m, 5H, Ph), 10.39 (bs, 1H, NH); mass spectrum (*m/z*): 354 $[M]^+$, 307 $[M–SMe]^+$, 275 $[M–SMe–S]^+$, 227 $[M–SMe–SMe–SH]^+$.

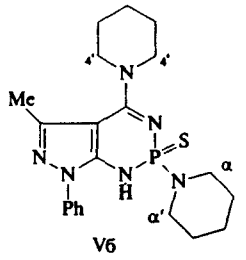
2,4-Dibenzylthio-5-methyl-7-phenylpyrazolo[3,4-d]-1,3,2-diazaphosphorin-2(1*H*)-thione (IVc). To a solution of sodium methylate, prepared from 0.14 g (6 mmole) Na and 10 ml methanol, was added 0.81 g (2 mmole) of solvate IIb and 0.51 g (4 mmole) of benzyl chloride. The solution was heated to boiling, boiled for 5 min, and cooled. To this solution was added diluted hydrochloric acid so as to obtain pH 6 – 7. The oil product was doubly washed with water, mixed with 10 ml of 2-propanol, heated to boiling, and cooled. The precipitate of compound IVc was separated by filtration.

2,4-Dibenzylthio-5-methyl-7H-pyrazolo[3,4-d]-1,3,2-diazaphosphorin-2(1*H*)-thione (IVd). Compound IVd was obtained similarly to IVc proceeding from solvate IIa and benzyl chloride.

2,4-Di(methoxycarbonylmethyl)thio-5-methyl-7H-pyrazolo[3,4-d]-1,3,2-diazaphosphorin-2(1H)-thione (IVe). Compound IVe was obtained similarly to IVc proceeding from solvate IIa and bromoacetic acid methyl ether.

2,4-Dibenzylamino-5-methyl-7-phenylpyrazolo[3,4-d]-1,3,2-diazaphosphorin-2(1*H*)-thione (Va). A mixture of 0.71 g (2 mmole) of dimethylthio derivative IVb and 1.07 g (10 mmole) of benzylamine was heated to boiling, boiled for 30 min, cooled, and triturated with 2-propanol. The ^{1}H NMR spectrum of compound Va (δ, ppm): 2.38 (s, 3H, Me), 3.79 – 3.91 (m, 2H, $^2J_{CH^a,CH^b}$ 13.7 Hz, $^3J_{NH^a,P}$ 17.0 Hz, $^3J_{CH^b,P}$ 17.4 Hz, PS$\underline{CH_2}$Ph), 4.32, 4.39 (2d, 1H, $^2J_{CH^a,CH^b}$ 13.5 Hz, 4-S$\underline{CH_2}$Ph), 7.15 – 7.53 (m, 15H, 3Ph), 10.43 (bs, 1H, NH).

2,4-Dipiperidino-5-methyl-7-phenylpyrazolo[3,4-d]-1,3,2-diazaphosphorin-2(1H)-thione (Vb). Compound Vb was obtained similarly to Va proceeding from compound IVb and piperidine. The ^{1}H NMR spectrum of compound Vb (δ, ppm): 2.29 (s, 3H, Me), 1.38 – 1.70 (m, 12H, $6CH_2$), 3.16 (m, 4H, $2(4'\text{-}CH_2)$), 3.30, 3.48 (2m, 2H, α-CH_2, α'-CH_2), 7.40 – 7.58 (m, 5H, Ph), 8.74 (bs, 1H, NH).



V6

5-Methyl-4-methylthio-7-phenyl-2-phenylhydrazino pyrazolo[3,4-d]-1,3,2-diazaphosphorin-2(1H)-thione (VI). Compound VI was obtained similarly to Va proceeding from compound IVb and phenylhydrazine; the boiling time decreases to 2 – 3 min.

5-Amino-3-methyl-7-methoxythiocarbonyl-1-phenyl pyrazole (VII). To 2.2 g (6.7 mmole) of dimethylthio derivative IVb was added 10 ml of 20% HCl, and the mixture was heated to boiling, boiled for 10 – 15 min (until the initial compound IVb is completely dissolved) and cooled. This solution was adjusted with an aqueous NaOH solution so as to obtain pH 4 – 5. The precipitate was separated by filtration and washed with water.

5-Amino-3-methyl-1-phenyl-4-ethoxycarbonylpyrazole (VIII). To 0.7 g (2.8 mmole) of aminopyrazole VII was added a solution of sodium ethylate prepared from 0.1 g (4.3 mmole) Na and 8 ml ethanol. The mixture was heated to boiling (after which the initial compound VII dissolves), boiled for 1 h, cooled, diluted with 50 ml water, and adjusted with diluted hydrochloric acid so as to obtain pH 6 – 7. The precipitate of compound VIII was separated by filtration and washed with water.

Tetramethylformamidinium salt of 2-mercapto-5-methyl-7-phenylpyrazolo[3,4-d]-1,3,2-diazaphosphorin-2,4-(1*H*,3*H*)-dithione (IX). To a suspension of 1 g (2.5 mmole) of compound IIb in 5 ml of ethanol was added with stirring 0.75 ml (5 mmole) of DMF diacetal, after which the initial compound IIb dissolved. The precipitate of compound IX formed within 1 min was separated by filtration and washed with ethanol.

EXPERIMENTAL BIOLOGICAL PART

The antiviral activity of the synthesized 7-methylpyrazolo[3,4-d]-1,3,2-diazaphosphorin derivatives was characterized by their effect on the influenza virus A/Aichi (H_3N_2) using the model of influenzal pneumonia in mice. The model disorder was reproduced by intranasal inoculation of the virus at a dose of 10 LD_{50}, which leads to the loss of 90 – 100% control animals within 5 – 7 days after infection. The test compounds were introduced over 5 days at a daily a dose of 60 mg/kg (p.o.). The protective effect of the compounds studied was evaluated as the percentage decrease in the lethality level against the control.

It was established that five of the nine compounds studied exhibited a pronounced protective effect on the influenzal pneumonia model, as evidenced by a 40 – 50% reduction in the lethality of test animals (Table 2). Compounds III and VI showed low activity, while compounds Va and Vb were virtually inactive. An analysis of the relationship between the chemical structure and antiviral effect of the compounds tested shows that the maximum activity is observed for compounds (IVa and IVd) containing alkyl or aryl groups in positions 2 and 4 and hydrogen atoms in position 1. Substitution of a phenyl radical for the latter hydrogen in position 1 leads to a decrease in the antiviral activity. Introduction of amino groups at position 2 and 4 completely eliminates the activity.

The experimental results obtained in this work are indicative of good prospects in searching for new antiviral agents in the series of pyrazolo[3,4-d]diazaphosphorin derivatives.

TABLE 2. Antiviral Activity of Methylpyrazolo[3,4-d]-1,3,2-diazaphosphorin Derivatives in Mice

Compound	Decrease in lethality, % of control	Confidence level p (according to [11])
IVa	50	< 0.001
IVd	50	< 0.001
IVb	40	< 0.001
IVc	40	< 0.001
Va	0	–
Vb	0	–
IVe	40	< 0.001
III	20	< 0.05
IV	20	< 0.05

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