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Synthesis and Biological Activity of the 2-Amino-4-(4-iodophenyl)amino-6-methylpyrimidine Isosteric Analogs

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Abstract—Transformation of the structure of 6-methylisocytosine results in the isosteric analogs of 2-amino-4-(4-iodophenyl)amino-6-methylpyrimidine exhibiting tuberculocidal properties. The level of biological activity of the synthesized compounds depends on the site of location of the halogen atom.

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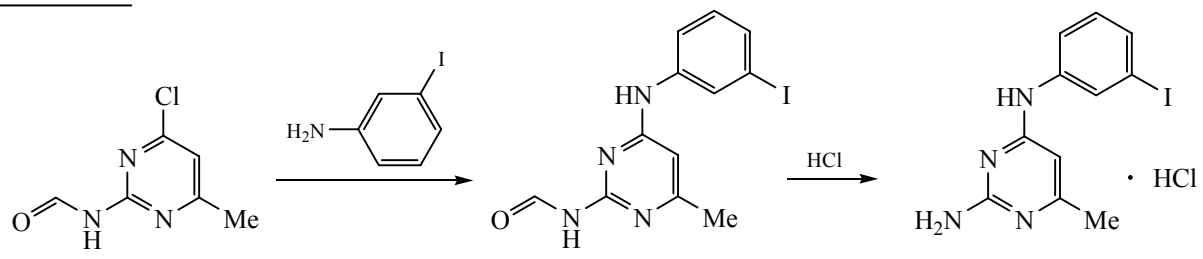
A factor determining the ability of 2-amino-4-arylamino-6-methylpyrimidines (**I**) to inhibit the growth of *Mycobacterium smegmatis* cell culture at low concentrations is the presence of halogen atoms in meta- or para-position of the aromatic ring [1, 2]. The migration of the halogen to the hetero-ring causes the loss of antimycobacterial activity due to the formation of respective inactive 2-amino-5-halo-6-methyl-4-phenylaminopyrimidine (**II**) [3]. In order to find out whether this trend occurs in the series of 2-amino-4-(4-iodophenyl)amino-6-methyl-pyrimidine hydrochloride (**III**) and its isosteric analogs inhibiting the cell growth of the related culture, *Mycobacterium tuberculosis* [4], we synthesized 2-amino-4-(3-iodophenyl)amino-6-methyl-pyrimidine (**IV**) and 2-amino-5-iodo-6-methyl-4-phenylaminopyrimidine (**V**) hydrochlorides and invest-tigated the tuberculocidal properties of these compounds.

Iodophenylaminopirimidine **IV** formed at the acidic hydrolysis of the intermediate 4-(3-iodophenyl)amino-6-methyl-2-formylaminopyrimidine **VI**, which, in turn, resulted from the condensation of 6-methyl-2-formyl-amino-4-chloropyrimidine **VII** with 3-iodophenyl-amine at elevated temperature in the absence of a solvent and was used without further purification in the deformylation step.

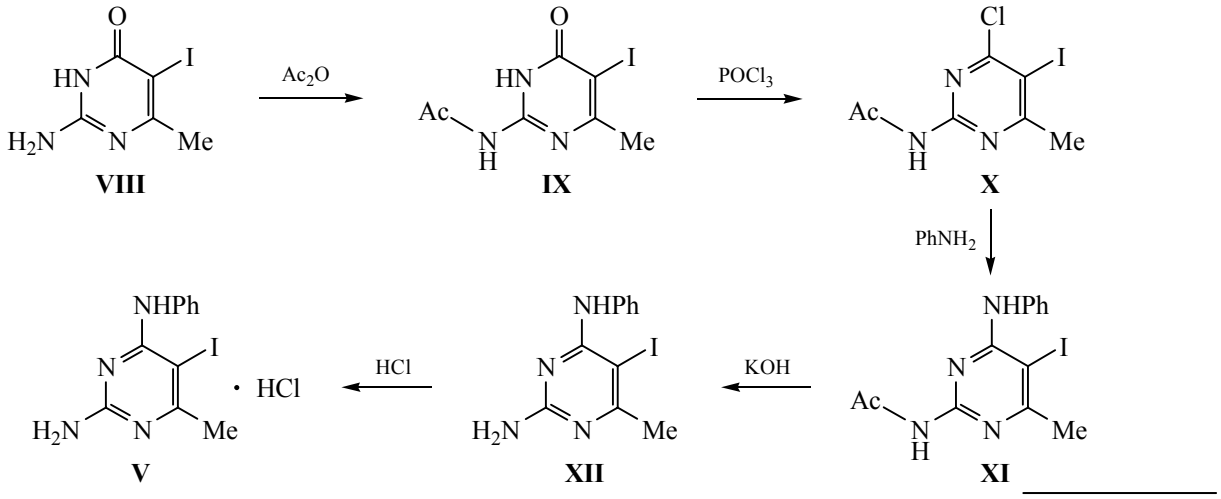


VII **VI** **IV**

In the ^{1}H NMR spectrum of compound **IV** there are the characteristic proton signals of primary and secondary amino groups near 8.0 and 10.9 ppm respectively, and the signal of the proton H^5 is shifted upfield compared with similar parameter of the substrate **VII**.

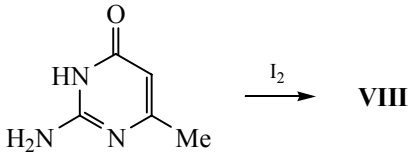
To obtain iodopyrimidine **V** we performed se-quential modification of the 2-amino-5-iodo-6-methyl-pyrimidin-4(3*H*)-one **VIII**.

The acetylation of iodoisocytosine **VIII** with excess of acetic anhydride led to 2-acetylamino-5-iodo-6-



methyl-pyrimidin-4(3*H*)-one **IX**, which at treating with phosphorus oxychloride was converted into 2-acetylamino-5-iodo-6-methyl-4-chloropyrimidine **X**. The protection of amino group of compound **VIII** was carried out in order to avoid its possible [4] interaction with the chlorinating agent. The condensation of dihalopyrimidine **X** with phenylamine in ethanol gave 2-acetylamino-5-iodo-6-methyl-4-phenyl-aminopyrimidine **XI**. The latter underwent deacetylation under alkaline hydrolysis to 2-amino-5-iodo-6-methyl-4-phenylaminopyrimidine **XII**. The quaternization of the free base **XII** with hydrogen chloride in an inert solvent caused the formation of the target iodopyrimidine **V**.

In contrast to the proposed [5] method of iodination of 2-amino-6-methyl-pyrimidin-4(3*H*)-one **XIII** with iodine in the presence of hydrogen peroxide under the influence of infrared irradiation, we used a less laborious method, based on the processing compound **XIII** with iodine in aqueous alkaline solution that ensured a 60% yield of reaction product **VIII**.



XIII

Attempted formylation of iodoisocytosine **VIII** by heating the substrate with 85% formic acid resulted in formation of 5-iodo-6-methyl-2-formyl-aminopyrimidin-4(3*H*)-one **XIV** in a yield not exceeding 10%: the compound **VIII** mostly underwent deiodination according to the redox reactions:

$$2RI + 2H_3O^+ + 2e^- \rightarrow 2RH + I_2 + H_2O,$$
$$HCOO^- - 2e^- \rightarrow CO_2 + H^+,$$

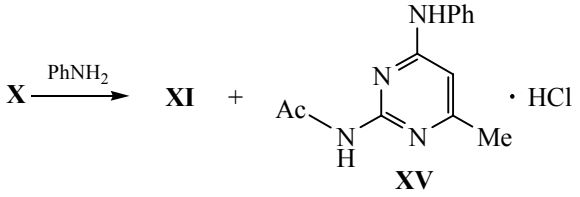
where R = 2-amino-6-methyl-4-oxo-3,4-dihydropyrimidin-5-yl.

^{1}H NMR spectrum of formylaminopyrimidine **XIV** contains the characteristic signals of the protons of the methyl group around 2.3 ppm and formamide group around 6.9 ppm, with integral intensities ratio 3:2.

We succeeded to perform exchange chlorination of compounds **IX** limiting the contact of the reactant by the period of homogenization of the initially formed suspension. At more prolonged heating a significant tarring of the reaction mixture occurred, and it was difficult to purify the isolated dihalopyrimidine **X** from the low-melting impurities of unknown origin.

The amination of compound **X** proceeded rather difficultly. In the course of heating the equimolar mixture with phenylamine in ethanol in the absence of hydrogen chloride acceptor in the amount needed for the obtaining N^2-acetyl-5-iododiaminopyrimidine **XI** directly as the hydrochloride, we observed the formation of a new compound, a measurable amount of which was extracted from the reaction mixture at the complete removal of the solvent. The double crystallization of the combined product from 30% aqueous ethanol resulted in a chromatographically pure compound **XI** as a free base. ^{1}H NMR spectrum of N^2-acetyl-5-iododiaminopyrimidine **XI** contained the signals of the protons of methyl groups around 2.1 and 2.5 ppm, aromatic ring at 7.0–7.9 ppm, and secondary amino groups at about 8.0 and 10.1 ppm After concentration of the mother liquor in vacuo, the residue showed a positive test for the presence of chloride ion with the aqueous solution of silver nitrate. The crystallization from 30% ethanol did not increase the chromatographic purity of the substance, although the

character of its melting corresponded to a purified product. ^{1}H NMR spectrum showed that the substance was a mixture of N^2-acetyl-5-iododiaminopyrimidine **XI** and 2-acetylamino-6-methyl-4-phenylaminopyrimidine **XV**, identified by the proton signals of the methyl groups about 2.3 and 2.5 ppm, methine group at 6.2 ppm, the secondary amino groups near 8.2 and 6.10 ppm, and the quaternized nitrogen atom in the region of 12–13 ppm.



XV

We succeeded to avoid deiodination of compound **XI** *in situ* by performing reaction of dihalopyrimidine **X** and phenylamine in the presence of triethylamine. In this case we met the difficulty at the purification the resulting N^2-acetyl-5-iododiaminopyrimidine **XI** from the impurities of unknown nature, and therefore we carried out alkaline hydrolysis of the crude substrate and obtained 5-iododiaminopyrimidine **XII** in a yield of about 40%. Despite the simplification of the synthesis procedure, compound **XII** isolated from the reaction mixture required repeated crystallization to reach the chromatographic purity and narrow temperature range of melting. In the ^{1}H NMR spectrum of the purified 5-iododiaminopyrimidine **XII** there were the signals of the protons of methyl group around 2.4 ppm, of the primary and secondary amino groups at about 6.2 and 7.6 ppm, respectively, and of the aromatic fragment at 7.0–7.7 ppm.

The results of the biological screening of the isosteric analogs of diaminopyrimidine **III** indicate that its tuberculocidal effect is due to, among other potential factors, the strict localization of the halogen atom in the *para*-position of the aromatic ring. When the iodine is in the *meta*-position of the ring the respective compound **IV** does not exhibit the ability to inhibit *Mycobacterium tuberculosis*, but inhibits to 100% the cell growth of the related culture *Mycobacterium smegmatis*, at a concentration of 25 mg ml^{-1}. The migration of the halogen from the para-positions of the aromatic ring of diaminopyrimidine **III** in the hetero-ring results in the loss of inhibiting properties of the arising iodopyrimidine **V** with respect to the cells of both cultures even at increasing its concentrations to 100 μg ml^{-1}.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a spectrometer Bruker WM-400 (operating frequency 400.13 MHz) in DMSO-d_6, internal reference the residual proton signals of the solvent. Individuality of compounds was monitored by TLC on Silufol UV-254 plates in the systems: 1-butanol–acetic acid–water, 1:1:1 (eluent A) and acetone–heptane, 2:1 (eluent B). The development of spots was performed under UV irradiation. Elemental analyzes were carried out on a Hewlett Packard B-185 analyzer. Water or water-ethanol solutions of compounds **IV** and **V** gave a positive test for the presence of chloride ion with an aqueous solution of silver nitrate. 6-Methyl-2-formylamino-4-chloropyrimidine **VII** was prepared by the method [4], 2-amino-6-methyl-pyrimidin-4(3*H*)-one **XIII,** by the method [6].

2-Amino-4-(3-iodophenyl)amino-6-methylpyrimidine (IV). A mixture of 0.41 g of formylaminochloropyrimidine **VII** and 0.53 g of 3-iodophenylamine was kept at 100°C until solidification. The melt was mechanically ground and dispersed in 10 ml of concentrated hydrochloric acid. The mixture was heated to homogenization, kept in a boiling water bath for 30 min, then cooled, the precipitate formed was filtered off, and the filtrate was evaporated to dryness in a vacuum. The combined products were recrystallized from ethanol and dried at 60°C for 6 h. 0.33 g (42%) of compound **IV** was obtained as the hydrochloride, mp 260°C (decomp.), R_f 0.78 (A). ^{1}H NMR spectrum, δ, ppm: 2.31 s (3H, Me), 6.29 s (1H, CH), 7.11–8.09 m (6H, Ar, NH_2), 10.91 s (1H, NH), 13.15 s (1H, N^+H). Found, %: C 36.45, H 3.17, N 15.06. $C_{11}H_{11}IN_4$·HCl. Calculated, %: C 36.44, H 3.34, N 15.45.

2-Amino-5-iodo-6-methyl-4-phenylaminopyrimidine (V) hydrochloride. Through the solution of 117 mg of compound **XII** in 15 ml of anhydrous benzene was passed a flow of dry hydrogen chloride until saturation. The resulting suspension was filtered off, the solid was washed with water-free benzene and dried at 60°C for 6 h to afford 112 mg (86%) of compound **V**, mp 181°C (decomp.), R_f 0.74 (A). Found, %: C 36.18%, H 3.21, N 15.23. $C_{11}H_{11}IN_4$·HCl. Calculated, %: C 36.44%, H 3.34; N 15.45.

2-Amino-5-iodo-6-methyl-pyrimidin-4(3*H*)-one (VIII). To a solution of 4 g of isocytosine **XIII** in 10% aqueous potassium hydroxide prepared from 6 g of alkali and 54 ml of water was added dropwise at vigorous stirring a solution of 8.13 g of iodine in

aqueous potassium hydroxide of the same concentration. The mixture was stirred for 1 h and after 24 h neutralized with concentrated hydrochloric acid. The precipitate formed was filtered off, washed with water, and recrystallized from 60% DMF. After thorough washing with water and drying at 80°C for 8 h 5.17 g (64%) of compound **VIII** was obtained, mp. 203°C (decomp.) [published data [5]: mp 225–226°C (decomp.)], R_f 0.76 (A). ^{1}H NMR spectrum, δ, ppm: 2.19 s (3H, Me), 6.60 br.s (1H, NH_2), 11.12 br.s (1H, NH). Found, %: C 23.63%, H 2.57, N 16.56. $C_5H_6IN_3O$. Calculated, %: C 23.92%, H 2.41; N 16.74.

2-Acetylamino-5-iodo-6-methylpyrimidin-4(3*H*)-one (IX). A mixture of 2.43 g of iodoisocytosine **VIII** and 36 ml of freshly distilled acetic anhydride was boiled for 1 h, the formed precipitate was filtered off and recrystallized from 30% acetic acid. After thorough washing with water and drying at 60°C for 6 h, 1.2 g (42%) of compound **IX** was isolated, mp 215°C (decomp.), R_f 0.88 (A). ^{1}H NMR spectrum, δ, ppm: 2.16 s (3H, Me), 2.44 s (3H, Ac), 11.72 s (1H, NH), 11.93 s (1H, NH). Found, %: C 28.76%, H 2.43, N 13.96. $C_7H_8IN_3O_2$. Calculated, %: C 28.69%, H 2.75, N 14.34.

2-Acetylamino-5-iodo-6-methyl-4-chloropyrimidine (X). A mixture of 1.2 g of acetylaminopyrimidinone **IX** and 15 ml of freshly distilled phosphorus oxychloride was heated at a temperature not exceeding 115°C until dissolution. Excess of phosphorus oxychloride was com-pletely removed in vacuo, the residue was mixed with finely crushed ice and ground to form a slurry. The precipitate was filtered off, washed with water, and recrystallized from 40% ethanol. After washing with water and drying at 60°C for 6 h 0.76 g (59%) of compound **X** was isolated, mp 167°C, R_f 0.59 (B). Found, %: C 26.87%, H 2.37, N 13.61. $C_7H_7ClIN_3O$. Calculated, %: C 26.99%, H 2.27, N 13.49.

2-Amino-5-iodo-6-methyl-4-phenylaminopyrimidine (XII), free base. A mixture of 1.49 g of dihalopyrimidine **X**, 0.44 g of phenylamine, and 0.48 g of triethylamine in 20 ml of ethanol was refluxed for 1 h. After removal of ethanol in vacuo, the residue was triturated with 15 ml of ethyl acetate to form a slurry, and left to stand for 2 days. The precipitate formed was filtered off, washed with ethyl acetate, and dried at 60°C for 2 h. 0.93 g (53%) of crude 2-acetylamino-5-iodo-6-methyl-4-phenylamino-pyrimidine **XI** was obtained. It was dissolved in 20 ml of 60% ethanol containing 0.51 g of potassium hydroxide. The mixture was kept in a boiling water bath for 1 h, then evaporated in a vacuum to dryness, the residue was mixed with 10 ml of water, insoluble part was filtered off, washed with water, and dried at 60°C for 6 h. The dry product was recrystallized three times from cyclohexane to achieve chromatographic purity and narrow melting temperature range. After drying in a high vacuum 117 mg (7.5% based on the original dihalopyrimidine **X**) of compound **XII** was obtained, mp 143°C, R_f 0.31 (B). ^{1}H NMR spectrum, δ, ppm: 2.39 s (3H, Me), 6.16 s (2H, NH_2), 7.01 m (1H, Ph), 7.28 m (2H, Ph), 7.57 s (1H, NH) , 7.65 d (2H, Ph). Found, %: C 40.29%, H 3.35, N 16.89. $C_{11}H_{11}IN_4$. Calculated, %: C 40.51%, H 3.40, N 17.18.

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