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Effect of the Structural Modification of 2-Benzylamino-4-(4-iodophenyl)amino-6-methylpyrimidine on the Biological Activity of Its Derivatives

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Abstract—Modification of the aliphatic-aromatic moiety in the 2-benzylmino-4-(4-iodophenyl)amino-6-methylpyrimidine leads to a change in the site of biological action of the formed structural analogs.

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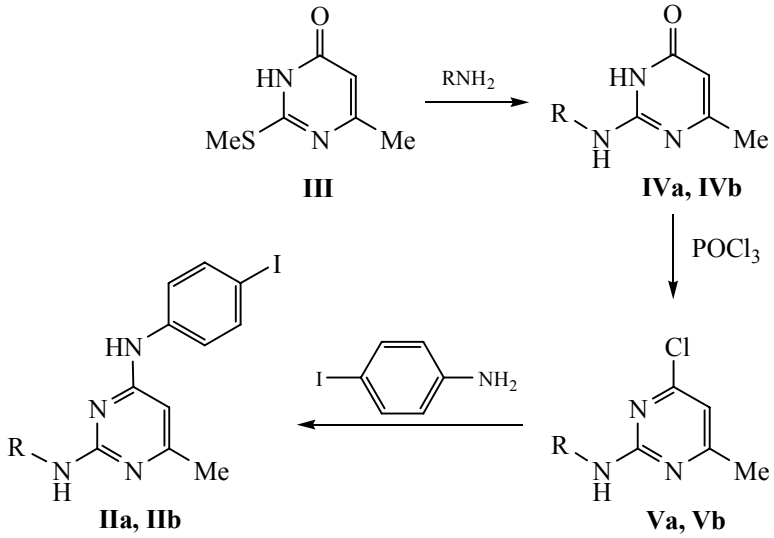
To illustrate the importance of the presence of aliphatic-aromatic fragments in the 2-benzylmino-4-(4-iodophenyl)amino-6-methylpyrimidine hydrochloride molecule (**I**) for maintaining the capability to inhibit the growth of *Mycobacterium smegmatis* cell culture at low concentrations [1], we synthesized the hydrochlorides of 4-(4-iodophenyl)amino-6-methyl-2-phenylaminopyrimidine (**IIa**), 4-(4-iodophenyl)amino-6-methyl-2-[(furan-2-yl)methyl]aminopyrimidine (**IIb**), and 2-amino-4-(4-iodophenyl)amino-6-methylpyrimidine (**IIc**). Compounds **IIa** and **IIb** were prepared by us along the unified scheme, which included amination of 6-methyl-2-methylthiopyrimidin-4(3*H*)-one (**III**) with phenylamine and (furan-2-yl)methylamine, exchange chlorination with phosphorus oxychloride of the formed 2-amino-6-methylpyrimidin-4 (3*H*)-ones (**IVa, IVb**), and finally the amination of the obtained 2-amino-6-methyl-4-chloropyrimidines (**Va, Vb**) with 4-iodophenylamine in the absence of a solvent at 90–100ºC at 1:1 ratio of substrate and reagent. However, we were confronted with a considerable tarring at the exchange chloroination of compounds **IVa** and **IVb**. In the case of aminopyrimidinone **IVb** we succeeded to minimize this undesirable process by reducing the



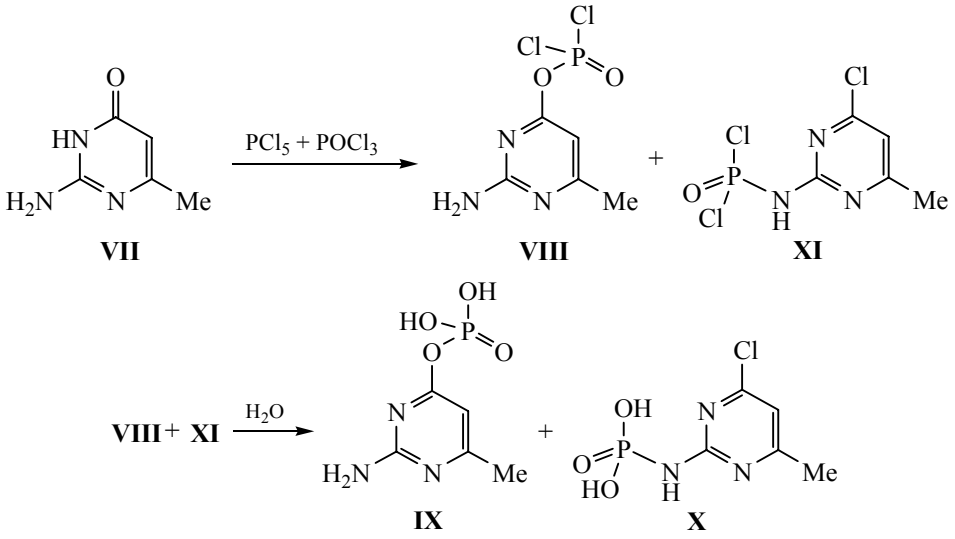
R = Ph (**a**), (furan-2-yl)methyl (**b**)

contact time of the substances, while such a change in the conditions of synthesis of aminochloropyrimidine **Va** did not result in a marked increase in its yield.

Structures of arylaminopyrimidines **IIa, IIb** are confirmed by ^{1}H NMR spectra, which contain the characteristic signals of protons of the exocyclic amino group at 10.4 (N^2H), 11.0 (N^4H), and 8.4 (N^2H), 11.0 (N^4H) ppm, respectively. Integral intensities of the signals of aromatic protons correspond to their total number.

According to literature data, compound **IIc** can be obtained by amination of 2-amino-6-methyl-4-chloropyrimidine (**VI**) with 4-iodophenylamine [2], and substrate **VI**, by exchange chlorination of 2-amino-6-methylpyrimidin-4(3*H*)-one (**VII**) with phosphorus oxychloride either without a solvent [3], or in 1,2-dichlorobenzene [4], or with a mixture of phosphorus oxychloride and pentachloride [5]. While carrying out the exchange chlorination of pyrimidinone **VII** we noted that the procedures described in the cited papers are irreproducible. Refluxing compound **VII** with an excess of phosphorus oxychloride until the end of the release of hydrogen chloride followed by the treatment of the reaction mixture with ice and alkalinizing it with 25% aqueous ammonia solution to pH ~ 8–9 (method *a*) leads to the formation of small amounts of a mixture of substances inseparable by fractional crystallization. The same result was achieved by performing the reaction in 1,2-dichlorobenzene. Heating pyrimidinone **VII** with a mixture of phosphorus oxychloride and pentachloride followed by processing the reaction mixture along the method *a* we obtained a solution from which after some time a colorless precipitate separated, which was readily soluble in polar protic solvents (water, ethanol), and sparingly soluble in non-polar aprotic solvents (benzene, cyclohexane) even at elevated temperature. The available data on the solubility of chloropyrimidine **VI** in various solvents [6] are not consistent with the above noted that points to the discrepancy between the expected structure and that of the isolated compound.

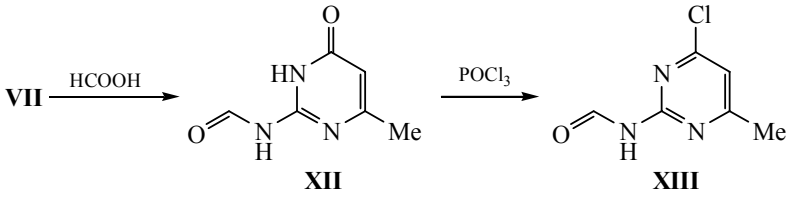
High solubility of the isolated product in water indicated the presence in its structure of the hydrophilic fragment of phosphoric acid, whose appearance might be due to O-phosphorylation of substrate **VII** with the subsequent hydrolysis of intermediate (2-amino-6-methylpyrimidin-4-yl)dichlorophosphate **VIII**. Later on, we recorded the ^{1}H and ^{31}P NMR spectra and got an opportunity to partially verify the correctness of our assumptions and to formulate a conclusion on the preferential direction of the reaction course in the of exchange chlorination of compound **VII**. The ^{1}H NMR spectrum contains as singlets the paired signals of the protons of the methyl and methine groups near 2.2 and 2.4 ppm and about 5.7 and 6.9 ppm, respectively. The ^{31}P NMR spectrum contains the signals of phosphorus nuclei with chemical shifts –0.5 and –12.6 ppm attributable to monosubstituted phosphoric acid and its amide [7], respectively. Less shielded singlets of protons occupied the position close to the signals characteristic of the protons of methyl groups and connected with the C^5 atom in various 2-amino-6-methylpyrimidin-4(3*H*)-ones, the positions of signals more downfield were close to those of analogs protons in 2-amino-6-methyl-4-chloropyrimidines [1, 8]. These spectral characteristics should be interpreted as corresponding to a mixture of (2-amino-6-methylpyrimidin-4-yl)phosphoric acid (**IX**) and phosphoric



N-(6-methyl-4-chloropyrimidin-2-yl)amide (**X**) formed at the hydrolysis of the dichlorophosphate **VIII** and *N*-(6-methyl-4-chloropyrimidin-2-yl)dichlorophosphoramide **XI**, the primary compounds arising in the reaction of pyrimidinone **VII** with a mixture of phosphorus oxychloride and pentachloride.

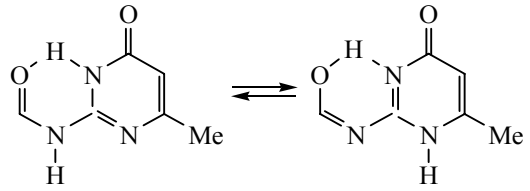
The ratio of integral intensities of proton signals of methyl or methine groups in compounds **IX** and **X** is 5:1 suggesting a prevalence of O-phosphorylation over the replacement of the oxygen atom by chlorine in the pyrimidinone **VII**.

In order to influence the reactivity of the compound **VII** we performed formylation of the substrate with formic acid and thus prepared 6-methyl-2-formylaminopyrimidin-4(3*H*)-one **XII**, which at the treatment with phosphorus oxychloride was converted to 6-methyl-2-formylamino-4-chloropyrimidine (**XIII**), free from (6-methyl-2-formylaminopyrimidin-4-yl)phosphoric acid as a potential impurity. The ^{1}H NMR spectrum of the formylaminochloropyrimidine **XIII** is characterized by the presence of singlets of protons at the C^5 at 6.4 ppm and secondary amino group at about 6.8 ppm, shifted downfield as compared with the signals of the same groups in the parent compound **XII** due to the electron-withdrawing effect of halogen substituent, and by the absence of the proton signal of the cyclic amino group, existing in the region of 10.7 ppm in the spectrum of formylaminopyrimidinone **XII**.



XII **XIII**

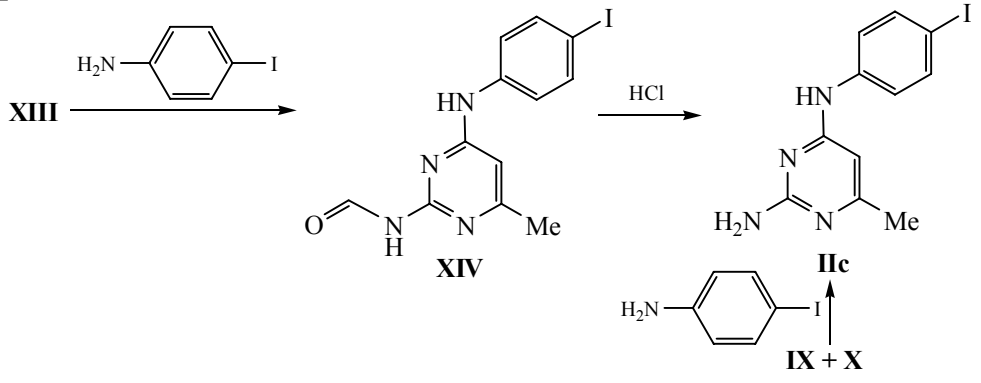
In contrast to the IR spectrum of compound **XII** containing superimposed band of stretching vibrations of C=O groups located near 1660 cm^{-1} the absorption band of the carbonyl group of formylaminochloropyrimidine **XIII** appears in the region of the stretching vibrations of carbonyl groups of secondary amides (1647 cm^{-1}). The displacement of the band "amide-II" of this compound to higher frequencies (1548 cm^{-1}) compared with a similar band in the spectrum of formylaminopyrimidinone **XII** (1498 cm^{-1}) points to the disappearance of the potential intramolecular hydrogen bond in the substrate molecule.



Finally, the appearance in the spectrum of compound **XIII** of the absorption band near 1475 cm^{-1} confirms aromatic character of this substance.

The assumed scheme of the formation of an intramolecular hydrogen bond was illustrated by comparing the intensities of the coincident frequencies of stretching vibrations of C=O groups of formylaminopyrimidinone **XII** and its precursor **VII** (1660 cm^{-1}), whose ratio was 2.5:1.

The condensation of formylaminochloropyrimidine **XIII** with 4-iodophenylamine taken in equimolar ratio in the absence of solvent at 100ºC resulted in 4-(4-iodophenyl)amino-6-methyl-2-formylaminopyrimidine hydrochloride (**XIV**). This compound was subjected to deformylation by heating with concentrated hydrochloric acid at 80–90°C to give diaminopyrimidine **IIc** .



XIV

We isolated the same compound **IIc** at the amination of a mixture of pyrimidylphosphoric acid **IX** and phosphoric chloropyrimidylamide **X** with 4-iodophenylamine in the absence of solvent at 100ºC, with subsequent processing of the reaction product with concentrated hydrochloric acid (method *b*).

Due to the high expenditure of the solvent at the recrystallization of the formamide **XIV**, we subsequently restricted ourselves to a thorough washing the substrate prior to introduction into the deformylation reaction and thus increased the total yield of compound **IIc** from 10 to 50% calculated on the original formylaminochloropyrimidine **XIII** (method *c*).

It should be noted that in order to prevent contamination of compound **XIV** with tarry substances of unknown nature it is necessary to control strictly the conditions of interaction of formylaminochloropyrimidine **XIII** with 4-iodophenylamine and to keep the process temperature no higher than it is specified.

While the ^{1}H NMR spectrum of formamide **XIV** differs from the spectrum of compound **XIII** by the presence of singlets of aromatic protons and protons at the atom N^4 in the region of 7.6 and 10.8 ppm respectively, the spectrum of diaminopyrimidine **IIc** practically coincides with the spectrum of compound **XIV** with the exception of the region of 7.5–8.0 ppm, where the proton signals of primary amino group and aromatic protons form a pseudo-singlet with an integral intensity corresponding to six protons.

The removal of the methylene group or the benzyl fragment from the structure of benzylaminopyrimidine **I** resulted in a significant change in the biological action of respective compounds **IIa** and **IIc**, which acquired the ability to inhibit the cell growth of the related culture, *Mycobacterium tuberculosis*, in concentrations of 0.05 and 0.0125 g l^{-1}, respectively. The replacement of the aromatic ring of benzylaminopyrimidine **I** by a heterocycle led to a loss of the antimycobacterial activity by the furylaminopyrimidine **IIb** at the concentrations up to 0.1 g l^{-1}.

EXPERIMENTAL

^{1}H and ^{31}P NMR spectra were recorded on a Bruker WM-400 (operating frequency 400.13 MHz) and a Bruker AC-200 (operating frequency 81.01 MHz) spectrometers, respectively, solvent DMSO-d_6, as internal reference were utilized the residual proton signals of the solvent. IR spectra were obtained on a FTIR FSM-1201 spectrometer from KBr tablets. The individuality of the compounds was monitored by TLC on the Silufol UV-254 plates in the systems: 1-butanol–acetic acid–water 1:1:1 (A), tetrachlorometane–2-propanol, 4:1 (B), acetone–hexane, 1:1 (C), acetone–heptane, 1:1 (D) tetrachlorometane–2-propanol, 5:1 (E). The spots were developed by UV light. Elemental analysis was performed on a Hewlett Packard B-185 analyzer. Aqueous or aqueous-ethanol solutions of compounds **IIa–IIc** and **XIV** gave a positive test for the presence of chloride ion with an aqueous solution of silver nitrate. 6-Methyl-2-methylthiopyrimidin-4(3*H*)-one **III** was prepared by the method [9], 2-amino-6-methylpyrimidin-4(3*H*)-one **VII**, according to [10]. A modification of the method [11] for the synthesis of 4-iodophenylamine consisting in an additional extraction of the product with hexane allowed us to prepare this amine as colorless needles in 59% yield, mp 64ºC (published [12]: mp 63–65ºC).

4-(4-Iodophenyl)amino-6-methyl-2-phenylaminopyrimidine (IIa). A mixture of 80 mg of phenylaminochloropyrimidine **VIa** and 80 mg of 4-iodophenylamine was kept at 95ºC to solidification. The crushed solid was crystallized from ethanol–DMF (10:1), washed with ethanol, and after drying at 60ºC for 4 h 51 mg (32%) of compound **IIa** was isolated as hydrochloride, mp 285ºC (dec.), R_f 0.82 (A). ^{1}H NMR spectrum, δ, ppm: 2.38 s (3H, Me), 6.34 s (1H, CH), 7.19–7.60 m (9H, Ar), 10.37 s (1H, NH), 10.92 s (1H, NH), 13.24 br.s (1H, N^+H). Found, %: C 46.13, H 3.52, N 12.46. $C_{17}H_{15}IN_4O\cdot HCl$. Calculated, %: C 46.54, H 3.68, N 12.77.

4-(4-Iodophenyl)amino-6-methyl-2-[(furan-2-yl)-methyl]aminopyrimidine (IIb). A mixture of 0.4 g of furfurylaminochloropyrimidine **VIb** and 0.39 g of 4-iodophenylamine was heated to 95ºC, kept at this temperature till the turbidity of the melt, and then at 110–115ºC till hardening. The crushed solid was crystallized from ethanol. After drying at 60ºC for 4 h we obtained 0.57 g (72%) of compound **IIb** as hydrochloride, mp 267ºC, R_f 0.80 (A). ^{1}H NMR spectrum, δ, ppm: 2.31 s (3H, Me), 4.58 s (2H, CH_2), 6.29 m (3H, Ar), 7.62 m (5H, Ar), 8.43 s (1H, NH) , 11.08 s (1H, NH), 13.34 br.s (1H, N^+H). Found, %: C 43.07, H 3.41, N 12.21. $C_{16}H_{15}IN_4O\cdot HCl$. Calculated, %: C 43.41, H 3.64, N 12.66.

2-Amino-4-(4-iodophenyl)amino-6-methylpyrimidine (IIc). *a*. A mixture of 0.6 g of formylaminopyrimidine **XIV** and 10 ml of concentrated

hydrochloric acid was heated at 85–90°C for 30 min, then the suspension was cooled, filtered, and the filtrate evaporated in vacuo to dryness. The combined residue was recrystallized from water and dried at 60ºC for 6 h. 0.28 g (51%) of compound **IIc** was isolated as hydrochloride, mp 294ºC (decomp.), R_f 0.76 (A). ^{1}H NMR spectrum, δ, ppm: 2.29 s (3H, Me), 6.27 s (1H, CH), 7.63 s (6H, NH_2, Ar), 10.90 s (1H, NH), 13.13 s (1H, N^+H). Found, %: C 35.92, H 3.13, N 15.09. $C_{11}H_{11}IN_4$·HCl. Calculated, %: C 36.44, H 3.34, N 15.45.

b. 0.2 g of a mixture of acids **IX** and **X** and 0.21 g of 4-iodophenylamine was heated at 90°C to solidification. The solid was crushed and suspended in 5 ml of concentrated hydrochloric acid, the precipitate was filtered off, washed with a small amount of cold water, and recrystallized from 2-propanol with the addition of DMF to create a homogenous solution. After washing with 2-propanol, the product was dried at 60ºC for 6 h. 67 mg (20%, calculated on 4-iodophenylamine) of compound **IIc** was isolated as hydrochloride.

c. A mixture of 1 g of formylaminochloropyrimidine **XIII** and 1.27 g of 4-iodophenylamine was heated at 100ºC till solidification. The reaction product was crushed and heated in 30 ml of concentrated hydrochloric acid at 85–90°C for 30 min, then cooled, the precipitate was filtered off, and the filtrate was evaporated in vacuo to dryness. The combined residue was recrystallized from water and dried at 60ºC. We obtained 0.93 g (51%, calculated on the original formylaminochloropyrimidine) of compound **IIc** as hydrochloride.

6-Methyl-2-phenylaminopyrimidin-4(3*H*)-one (IVa). A mixture of 1.56 g of thioester **III** and 0.93 g of freshly distilled phenylamine was heated to 160ºC and maintained at this temperature until complete release of methanethiol. The cooled solid was crushed and recrystallized from 60% aqueous acetic acid. After washing with water and drying at 60ºC for 6 h 0.66 g (33%) of compound **IVa** was isolated, mp 252ºC, R_f 0.69 (B). ^{1}H NMR spectrum, δ, ppm: 2.13 s (3H, Me), 5.63 s (1H, CH), 6.96–7.62 s (5H, Ph), 8.70 br.s (1H, NH_e), 10.52 br.s (1H, NH). Found, %: C 65.13, H 5.12, N 20.89. $C_{11}H_{11}N_3O$. Calculated, %: C 65.66, H 5.51, N 20.88.

6-Methyl-2-[(furan-2-yl)methyl]aminopyrimidin-4(3*H*)-one (IVb). A mixture of 4.68 g of thioester **III** and 5.82 g of freshly distilled (furan-2-yl)methylamine was heated to 150ºC and maintained at this temperature until complete release of methanethiol. After distilling off the excess (furan-2-yl)methylamine in a vacuum the oily residue formed was crystallized by trituration with 30 ml of acetonitrile, the precipitate was filtered off, recrystallized from acetonitrile and after drying at 60ºC for 4 h 4.60 g (75%) of compound **IVb** was isolated, mp 159ºC, R_f 0.25 (B). ^{1}H NMR spectrum, δ, ppm: 2.04 s (3H, Me), 4.46 s (2H, CH_2), 5.38 s (1H, CH), 6.25, 6.34 m (2H, Ht), 6.65 s (1H, NH_e), 7.49 d (1H, Ht), 10.41 s (1H, NH). Found, %: C 58.17, H 5.64, N 20.12. $C_{10}H_{11}N_3O_2$. Calculated, %: C 58.53, H 5.40, N 20.48.

6-Methyl-2-phenylamino-4-chloropyrimidine (Va). A mixture of 0.6 g of phenylaminopyrimidinone **IVa** and 25 g of freshly distilled phosphorus oxychloride was refluxed for 10 min. The phosphorus oxychloride excess was distilled off in vacuo, the residue was mixed with a thoroughly crushed ice and alkalinized with 25% aqueous ammonia to pH 9. The aqueous layer was decanted, the oily residue was crystallized by adding 50% aqueous ethanol. The resulting precipitate was filtered off and recrystallized from a mixture of the same solvent and washed with water. After drying in a vacuum over phosphorus pentoxide 90 mg (13%) of compound **Va** was isolated, mp 89ºC, R_f 0.53 (D). ^{1}H NMR spectrum, δ, ppm: 2.37 s (3H, Me), 6.76 s (1H, CH), 6.92–6.72 m (5H, Ph), 9.85 s (1H, NH). Found, %: C 59.76, H 4.77, N 18.96. $C_{11}H_{10}ClN_3$. Calculated, %: C 60.14, H 4.59, N 19.13.

6-Methyl-2-[(furan-2-yl)methyl]amino-4-chloropyrimidine (Vb). A mixture of 2 g of furfurylaminopyrimidinone **IVb** and 25 g of freshly distilled phosphorus oxychloride was refluxed for 20 min. After distilling off the excess phosphorus oxychloride in a vacuum, the residual oil was mixed with finely crushed ice, alkalinized with 25% aqueous ammonia solution to pH 9–10, and triturated to obtain a suspension. The precipitate was filtered off, washed with water, and recrystallized twice from 50% aqueous ethanol with decanting the solvent from the insoluble oily residue. After drying in a vacuum over phosphorus pentoxide 0.79 g (36%) of compound **Vb** was obtained, mp 94ºC, R_f 0.75 (D). ^{1}H NMR spectrum, δ, ppm: 2.26 s (3H, Me), 4.45, 4.46 d (2H, CH_2), 6.19–6.49 m (3H, Ar), 7.42 s (1H, Ar), 7.77 br.s (1H, NH). Found, %: C 53.21%, H 4.39, N 18.56. $C_{10}H_{10}ClN_3O$. Calculated, %: C 53.70%, H 4.51, N 18.79. When the time of contact of the reagents was increased to 1 h the yield of the compound **Vb** decreased to 0.17 g (7.6%).

6-Methyl-2-formylaminopyrimidin-4(3*H*)-one (XII). A mixture of 6.36 g pyrimidinone **VII** and 90 ml of formic acid was refluxed for 8 h. After the complete removal of excess acid in vacuo, the residue was refluxed with 40 ml of ethanol for 30 min, on cooling the precipitate was filtered off, washed with ethanol, and dried at 60ºC for 6 h. 6.95 g (89%) of compound **XII** was isolated, mp > 300ºC, R_f 0.59 (A). To obtain an analytical sample, the product was recrystallized from ethanol–DMF (4:1), washed with ethanol, and dried in a vacuum over phosphorus pentoxide. ^{1}H NMR spectrum, δ, ppm: 1.98 s (3H, Me), 5.36 s (1H, CH), 6.51 br.s (2H, NH_e, CHO), 10.69 br.s (1H, NH). Found, %: C 46.51%, H 4.35, N 27.73. $C_6H_7N_3O_2$. Calculated, %: C 47.06%, H 4.61, N 27.44.

6-Methyl-2-formylamino-4-chloropyrimidine (XIII). A mixture of 6.95 g of formylaminopyrimidinone **XII** and 60 ml of freshly distilled phosphorus oxychloride was refluxed for 1.5 h. The excess phosphorus oxychloride was completely removed in vacuo, the residue was mixed with finely crushed ice and neutralized with 25% aqueous ammonia. The suspension was left at room temperature for three days, the precipitate was filtered off, washed with a small amount of cold water, and dried in a stream of warm air to constant weight. The dry product was extracted with benzene in a Soxlet apparatus, and the precipitate separated from the extract was dried at 60ºC for 6 h. 3.33 g (43%) of compound **XIII** was obtained, mp 184ºC, R_f 0.82 (A). ^{1}H NMR spectrum, δ, ppm: 2.22 s (3H, Me), 6.45 s (1H, CH), 6.84 s (2H, NH_e, CHO). Found, %: C 41.76%, H 3.60, N 24.39. $C_6H_6ClN_3O$. Calculated, %: C 42.00%, H 3.52, N 24.49.

4-(4-Iodophenyl)amino-6-methyl-2-formylaminopyrimidine (XIV). A mixture of 0.5 g formylaminochloropyrimidine **XIII** and 0.64 g of 4-iodophenylamine was heated at 100ºC to solidification. The solid was crushed and recrystallized from ethanol, washed with ethanol, and dried at 60ºC for 4 h to obtain 0.6 g (52%) of compound **XIV** as the hydrochloride, mp 292ºC (decomp.), R_f 0.89 (A). ^{1}H NMR spectrum, δ, ppm: 2.29 s (3H, Me), 6.25 s (1H, CH), 7.62 s (4H, Ar), 8.09 br.s (2H, NH_e, CHO), 10.85 s (1H, N^4H), 13.12 br.s (1H, N^+H). Found, %: C 36.45%, H 2.84, N 14.05. $C_{12}H_{11}IN_4O\cdot HCl$. Calculated, %: C 36.90%, H 3.10, N 14.34.

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