

Synthesis and Biological Activities of Some New Pyrazolo [3,4-d] Pyrimidine Derivatives

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Synthesis of 3-amino-6-methyl-4-phenyl-1H-pyrazolo [3,4-d] pyrimidine (II), which underwent cycloaddition on heating with some vicinal chlorocyanoheterocyclic systems, diethyl malonate, phenacyl bromide and diethyl oxalate. On the other hand, the reactions of (II) with aromatic aldehydes, α -dicarbonyl compounds, α -keto acid and different acylating agents have been investigated.

INTRODUCTION

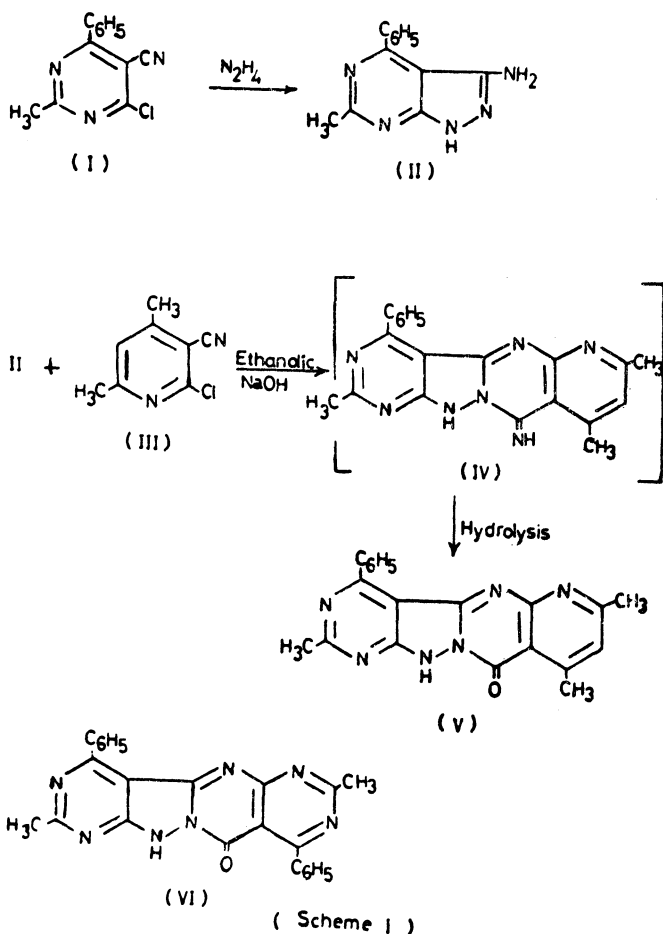
Pyrazolopyrimidines have been found to possess antispermatogenic and anticancer activities.^{1,2} In view of the significant biological activities of these compounds the present investigation describes the synthesis of some pyrazolo [3,4-d] pyrimidines as potential antibacterial or antifungal activity.

RESULTS AND DISCUSSION

A vicinal chlorocyanopyrimidine is a suitable starting material for the synthesis of fused pyrimidine heterocycles. Thus, treatment of 4-chloro-5-cyano-2-methyl-6-phenylpyrimidine (I) with hydrazine hydrate in ethanol readily accomplished ring closure to provide 3-amino-6-methyl-4-phenyl-1H-pyrazolo [3,4-d] pyrimidine (II).

Refluxing of II with 2-chloro-3-cyano-4,6-dimethylpyridine³ (III) in ethanolic sodium hydroxide gave 2,7,9-trimethyl-4-phenyl-10-oxo-12H-pyrimido [3',5':3,4] pyrazolo [1,5:1',2'] pyrimido [4',5'-b] pyridine (V). The IR spectrum of (V) exhibited a strong absorption band at 1640 cm^{-1} (C=O) with the absence of the band which is characteristic of $\text{C}\equiv\text{N}$ function. This proves that the formation of (V) was furnished probably through the intermediate imino (IV) as shown in Scheme (I). The PMR spectrum (DMSO- d_6) of (V) showed absorption centered at δ 2.65 (s, 3H, $\text{C}_9\text{-CH}_3$), δ 3.3 (s, 3H, $\text{C}_7\text{-CH}_3$), δ 4.8 (s, 3H, $\text{C}_2\text{-CH}_3$), δ 7.79 (m, 6H, aromatic protons) and δ 13.0 ppm (s, 1H, NH). Similarly, the reaction of (II) with (I) in ethanolic sodium hydroxide yielded 2,7-dimethyl-4,9-diphenyl-10-oxo-12H-pyrimido [4',5':3,4] pyrazolo [1,5:1',2'] pyrimido [4',5'-d] pyrimidine (VI) (Scheme I).

On the other hand, treatment of (II) with diethyl malonate, phenacyl bromide and diethyl oxalate afforded 2-methyl-7,10-dihydro-4-phenyl-

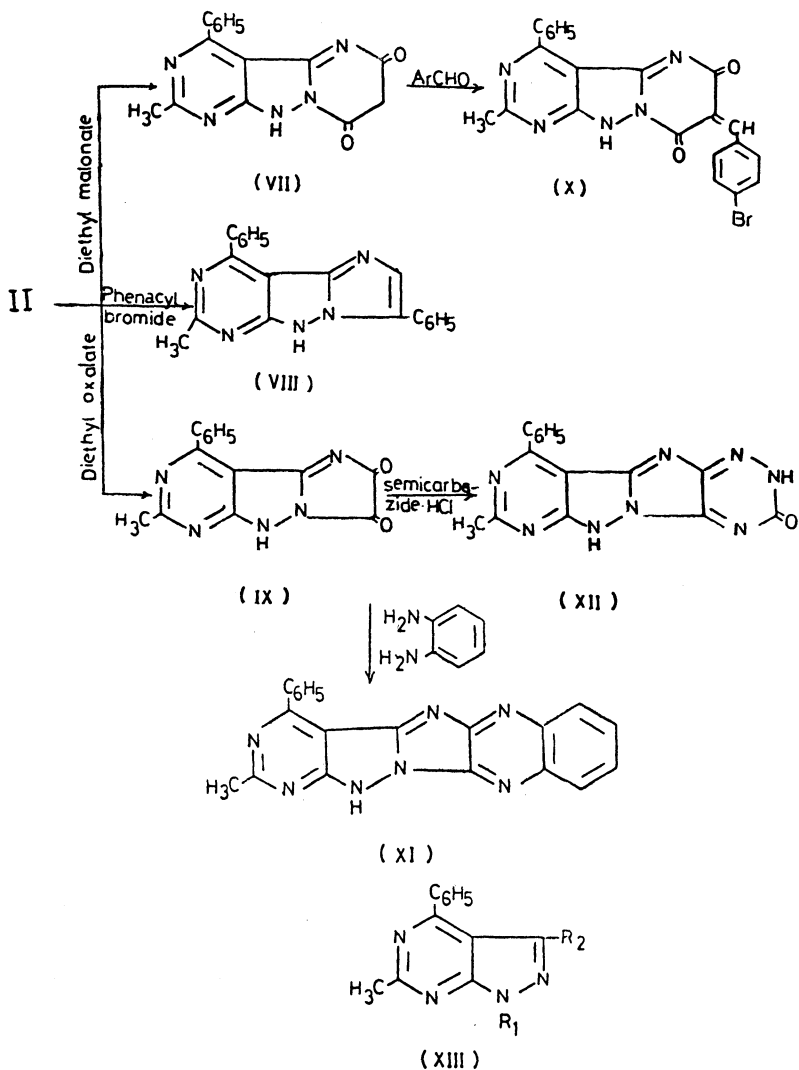


pyrimido [4',5':3,4] pyrazolo [1,5-a] pyrimidin-6,8-dione (VII), 2-methyl-9H-4,7-diphenylpyrimido [4',5':3,4] pyrazolo [1,5-a] imidazole (VIII) and 2-methyl-9H-4-phenylpyrimido [4',5':3,4] pyrazolo [1,5-a] imidazol-6,7-dione (IX). The IR spectrum of (VII) displayed absorption bands around 3270 (NH), 1640 (C=O) and 1420 cm^{-1} (def. CH₂). The PMR spectrum (DMSO-d₆) of (VII) showed signals at δ 3.6 (s, 3H, CH₃), δ 4.8 (s, 2H, CH₂) and δ 7.8 ppm (m, 6H, aromatic protons and NH).

Condensation of (VII) with *p*-bromobenzaldehyde gave 7-(*p*-bromobenzylidene)-2-methyl-10H-4-phenylpyrimido [4',5':3,4] pyrazolo [1,5-a] pyrimidin-6,8-dione (X).

Treatment of (IX) with *o*-phenylene diamine and semicarbazide hydrochloride yielded 2-methyl-13H-4-phenylpyrimido [4',5':3,4] pyrazolo [1,5:1',2'] imidazo [4',5'-b] benzopyrazine (XI) and 8-methyl-6-hydro-

10-phenylpyrimido [4',5' : 3,4] pyrazolo [1,5 : 1',2'] imidazo [4',5'-e] (1,2,4)-triazin-3 (2H) one (XII) (Scheme II)



- a, $R_1 = H$; $R_2 = N=CHC_6H_5$; f, $R_1 = COCH_3$; $R_2 = NHCOCH_3$.
 b, $R_1 = H$; $R_2 = N=CHC_6H_4OCH_3-p$; g, $R_1 = H$; $R_2 = NHCOC_6H_4COOH-o$
 c, $R_1 = H$; $R_2 = N=C(CH_3)COCH_3$; h, $R_1 = H$; $R_2 = NHCSNHC_6H_5$.
 d, $R_1 = H$; $R_2 = N=C(C_6H_5)COC_6H_5$; i, $R_1 = H$; $R_2 = NHSO_2C_6H_4CH_3-p$.
 e, $R_1 = H$; $R_2 = NHCHO$; j, $R_1 = H$; $R_2 = N=C(CH_3)COOH$.

(Scheme II)

Similar to 3,6-diamino-4 (*p*-anisyl)-5-cyanopyrazolo [3,4-*b*] pyridine⁴ and 3-amino-4,5-diphenylpyrazolo [3,4-*c*] pyridazine⁵, the amino group at position 3 in the pyrazole ring of 3-amino-6-methyl-4-phenyl-1*H*-pyrazolo [3,4-*d*] pyrimidine (II) exhibits the normal characteristic reactions of this group. Thus, compound (II) condenses with aromatic aldehydes and α -dicarbonyl compounds yielding (XIIIa-*b*) and (XIIIc-*d*) respectively, while it reacts with formic acid to give 3-(*N*-formamido)-6-methyl-4-phenyl-1*H*-pyrazolo [3,4-*d*] pyrimidine (XIIIe). The acylation of (II) with different acylating agents namely, acetyl chloride, phthalic anhydride, phenyl isothiocyanate and *p*-toluenesulphonyl chloride afforded (XIIIf-*i*) respectively. Treatment of (II) with pyruvic acid gave 3-(α -carboxyethylideneamino)-6-methyl-4-phenyl-1*H*-pyrazolo [3,4-*d*] pyrimidine (XIIIj) (Scheme II).

Antibacterial and Antifungal Activities

The biological activities of some new synthesized compounds were tested against gram positive bacteria (*Bacillus punilus* and *Sarcina lutea*), gram negative bacteria (*Escherichia coli* and *Salmonella wild*), acid fast bacterium (*Mycobacterium phlei*), an yeast (*Candida albicans*) and a filamentous fungus (*Aspergillus niger*).

TABLE I
ANTIMICROBIAL ACTIVITY MIC μ gm/mL

Com- pound	Gram +ve ^a		Gram -ve ^b		Acid fast ^c	Fungi ^d	
	<i>B.p.</i>	<i>S.l.</i>	<i>E.c.</i>	<i>S.w.</i>	<i>M.p.</i>	<i>C.a.</i>	<i>A.n.</i>
II	50	> 100	> 100	50	> 100	> 100	> 100
V	> 100	> 100	> 100	> 100	> 100	> 100	> 100
VI	> 100	> 100	> 100	> 100	> 100	> 100	> 100
VII	> 100	> 100	100	> 100	> 100	> 100	> 100
VIII	> 100	> 100	> 100	> 100	> 100	> 100	> 100
IX	> 100	> 100	> 100	100	100	> 100	> 100
X	> 100	> 100	> 100	> 100	> 100	> 100	> 100
XI	> 100	> 100	> 100	> 100	> 100	> 100	> 100
XIIIh	> 100	> 100	> 100	100	> 100	> 100	> 100
XIIIi	> 100	> 100	> 100	> 100	> 100	> 100	> 100

^a *B.p.* = *Bacillus punilus*,

S.l. = *Sarcina lutea*.

^b *E.c.* = *Escherichia coli*,

S.w. = *Salmonella wild*.

^c *M.p.* = *Mycobacterium phlei*.

^d *C.a.* = *Candida albicans*,

A.n. = *Aspergillus niger*.

According to filter paper disc method⁶, small discs of filter paper of uniform thickness and size containing graded amount of the agent to be tested were dipped into the seeded plates containing the antimicrobial solutions to be assayed, left for diffusion and incubated at 37°C for 24 hrs. for the bacterial growth and at 24°C for 4 days for the fungal growth. The agent diffuses into the agar and prevents the growth of the microorganism in a clear zone around the disc.

Table I of previous page shows that compounds (II), (IX) and (XIIIh) have activities against *S.w.*, compound (II) has activity against *B.p.*, compound (VII) has activity against *E.c.* and (IX) has activity against *M.p.* No compounds showed antifungal activity.

EXPERIMENTAL

All melting points reported are uncorrected, the IR spectra (KBr) were recorded on Perkin-Elmer 598 spectrophotometer and the H¹ nmr spectra were determined with Perkin-Elmer R12A instrument.

3-Amino-6-Methyl-4-Phenyl-1H-Pyrazolo [3,4-d] Pyrimidine (II)

A mixture of (I) (0.01 mole) and hydrazine hydrate (5 mL) in ethanol (20 mL) was refluxed for 4 hrs. The solid obtained was filtered off and recrystallized from ethanol to give (II) as yellow crystals, m.pt. 285–6°C (yield 87%). (Found: C, 64.10; H, 4.80; N, 30.90; C₁₂H₁₁N₅ requires C, 64.00; H, 4.88; N, 31.11.) IR: 3400–3140 (NH₂ and NH), 1610 cm⁻¹ (C=N and C=C).

Formation of (V) and (VI)

A mixture of (II) (0.01 mole) and (III) or (I) (0.012 mole) in ethanolic NaOH (5%, 30 mL) was refluxed for 6 hrs. The solid obtained on neutralization with dil. HCl was filtered off and recrystallized from DMF to give (V) and (VI) as yellow crystals respectively.

Compound (V). (yield 65%), m.pt. 280–1°C, mixed m.pt. 210°C. (Found: C, 67.50; H, 4.60; N, 23.50; C₂₀H₁₆N₆O requires C, 67.41; H, 4.49; N, 23.59.)

Compound (VI). (yield 60%), m.pt. > 290°. (Found: C, 68.40; H, 4.00; N, 23.30; C₂₄H₁₇N₇O requires C, 68.73; H, 4.05; N, 23.38.) IR: 3340–3240 (NH) and 1640 cm⁻¹ (C=O).

Reaction of (II) with Diethyl Malonate

A mixture of (II) (0.01 mole) and diethyl malonate (0.015 mole) in ethanol (30 mL) was treated with acetic acid (1 mL) and refluxed for 3 hrs. The solid obtained was filtered off and recrystallized from DMF

to give (VII) as yellow crystals (yield 87%), m.pt. $> 290^\circ$. (Found: C, 61.50; H, 3.80; N, 23.80; $C_{15}H_{11}N_5O_2$ requires C, 61.43; H, 3.75; N, 23.89.)

Formation of (VIII)

This compound was prepared in the manner described for (VII) in 55% yield and recrystallized from DMF to give (VIII) as orange crystals, m.pt. $> 290^\circ$. (Found: C, 74.00; H, 4.60; N, 21.50; $C_{20}H_{15}N_5$ requires C, 73.84; H, 4.61; N, 21.53.) IR: 3340–3220 (NH) and 1580 cm^{-1} (C=N and C=C).

Formation of (IX)

This compound was prepared in the manner described for (VII) in 85% yield and recrystallized from DMF to give (IX) as yellow crystals, m.pt. $280\text{--}1^\circ\text{C}$, mixed m.pt. $221\text{--}2^\circ\text{C}$. (Found: C, 60.50; H, 3.30; N, 25.10; $C_{14}H_9N_5O_2$ requires C, 60.21; H, 3.22; N, 25.08). IR: 3260 (NH) and 1640 cm^{-1} (C=O).

7-(*p*-Bromobenzylidene)-2-Methyl-10H-4-Phenylpyrimido [4',5':3,4] Pyrazolo [1,5-a] Pyrimidin-6,8-dione (X)

A mixture of (VII) (0.01 mole) and *p*-bromobenzaldehyde (0.015 mole) in DMF (20 mL) was refluxed for 3 hrs., poured onto cold water. The solid obtained was filtered off and recrystallized from DMF to give (X) as pale yellow crystals, m.pt. $> 290^\circ\text{C}$ (mixed m.pt. $210\text{--}11^\circ\text{C}$ (yield 80%). (Found: C, 57.10; H, 3.40; N, 15.00; $C_{22}H_{14}N_5O_2\text{Br}$ requires C, 57.39; H, 3.04; N, 15.21.) IR: 3320 (NH), 1660 (C=O) and 1580 cm^{-1} (C=N and C=C).

Reaction of (IX) with *o*-phenylene Diamine

A mixture of (IX) (0.01 mole) and *o*-phenylene diamine (0.012 mole) in DMF (30 mL) was refluxed for 3 hrs. The solid obtained was filtered off and recrystallized from ethanol-DMF to give (XI) as yellow crystals, m.pt. $> 290^\circ\text{C}$ (yield 53%). (Found: C, 68.30; H, 4.00; N, 28.00; $C_{20}H_{13}N_7$ requires C, 68.37; H, 3.70; N, 27.92). IR: 3320 (NH), 1580 cm^{-1} (C=N and C=C) and absence of C=O absorption band.

Formation of (XII)

A mixture of (IX) (0.01 mole) in DMF (20 mL) and semicarbazide hydrochloride (0.01 mole) in water (5 mL) was refluxed for 4 hrs. The solid obtained was filtered off and recrystallized from DMF to give (XII) as yellow crystals, m.pt. $> 290^\circ\text{C}$ (yield 55%). (Found: C, 56.50; H, 3.00; N, 35.00; $C_{15}H_{10}N_8O$ requires C, 56.60; H, 3.14; N, 35.22). IR: 3320 (NH) and 1640 cm^{-1} (C=O).

3-(Arylideneamino)-4-Phenyl-6-Methyl-1H-Pyrazolo [3,4-d] Pyrimidine (XIIIa,b)

A mixture of (II) (0.01 mole) and aromatic aldehyde (benzaldehyde or *p*-methoxybenzaldehyde) (0.012 mole) in absolute ethanol (30 mL) was refluxed for 1 hr. The solid obtained was filtered off and recrystallized from the proper solvent to give (XIIIa, b) as yellow crystals respectively.

Compound XIIIa. m.pt. 225–6°C (DMF) (yield 60%). (Found: C, 72.60; H, 4.60; N, 22.10; C₁₉H₁₅N₅ requires C, 72.84; H, 4.79; N, 22.36). IR: 3220 (NH), 1580 (C=N and C=C) and 2900 cm⁻¹ (CH aliphatic).

Compound XIIIb. m.pt. 232–3°C (ethanol) (yield 78%). (Found: C, 69.60; H, 5.10; N, 20.10; C₂₀H₁₀N₅O requires C, 69.97; H, 4.95; N, 20.40.) IR: 3330–3150 (NH), 1610 (C=N and C=C) and 2920 cm⁻¹ (CH aliphatic).

3-(α -Acetylethylideneamino)- and 3-(α -Benzoylbenzylideneamino)-4-Phenyl-6-Methyl-1H-Pyrazolo [3,4-d] Pyrimidine (XIIIc,d)

A mixture of (II) (0.01 mole) and butan-2,3-dione or 1,2-diphenyl-ethanedione (0.015 mole) in absolute ethanol (30 mL) was refluxed for 3 hrs. The solid obtained was filtered off and recrystallized from the proper solvent to give (XIIIc, d) as yellow crystals respectively.

Compound XIIIc. m.pt. 274–5°C (DMF) (yield 70%). (Found: C, 65.60; H, 5.10; N, 23.80; C₁₆H₁₅N₅O requires C, 65.52; H, 5.11; N, 23.89.) IR: 3390 (NH), 1640 (C=O) and 1580 cm⁻¹ (C=N and C=C).

Compound XIIId. m.pt. 196–7°C (acetic acid) (yield 75%). (Found: C, 74.60; H, 4.50; N, 16.80; C₂₆H₁₉N₅O requires C, 74.82; H, 4.55; N, 16.78.) IR: 3260–3220 (NH), 1660 (C=O) and 1600 cm⁻¹ (C=N and C=C).

3-(N-Formamido)-6-Methyl-4-Phenyl-1H-Pyrazolo [3,4-d] Pyrimidine (XIIIe)

A mixture of (II) (0.01 mole) and formic acid (25 mL) was refluxed for 3 hrs. The solid obtained was filtered off and recrystallized from ethanol-DMF to give (XIIIe) as yellow crystals, m.pt. 268–9°C (yield 62%). Found: C, 61.70; H, 4.50; N, 26.60; C₁₃H₁₁N₅O requires C, 61.66; H, 4.34; N, 27.66.) IR: 3280 (NH), 1720 (C=O) and 2800 cm⁻¹ (CH aldehydic).

3-Acetylamino-6-Methyl-4-Phenyl-1-Acetylpyrazolo [3,4-d] Pyrimidine (XIII f)

A mixture of (II) (0.01 mole) and acetic anhydride (5 mL) in glacial acetic acid (30 mL) was refluxed for 30 min., poured onto cold water and neutralized with NH₄OH. The solid obtained was filtered off and recrystallized from ethanol to give (XIII f) as yellow crystals m.pt. 210–212°C (yield 82%). (Found: C, 67.60; H, 4.00; N, 19.90; C₁₆H₁₅N₅O₂ requires C, 67.72; H, 4.20; N, 19.60.) IR: 3360–3180 (NH), 1720 (C=O) and 1600 cm⁻¹ (C=N) and C=C). PMR (DMSO-d₆) δ 2.8 (s, 3H, C₆, CH₃), δ 2.9 (s, 3H, CH₃, 3-acetyl amino), δ 3.7 (s, 3H, N₁COCH₃), δ 5.5 (s, 1H, NH) and δ 7.6 ppm (m, 5H, aromatic protons).

3-(*o*-Carboxybenzoylamino)-6-Methyl-4-Phenyl-1H-Pyrazolo [3,4-d] Pyrimidine (XIIIg)

A mixture of (II) (0.01 mole) and phthalic anhydride (0.015 mole) in glacial acetic acid (30 mL) was refluxed for 1 hr. The solid obtained was filtered off and recrystallized from ethanol acetic acid to give (XIIIg) as colourless crystals, m.pt. 256–8°C (yield 72%). (Found: C, 64.30; H, 4.30; N, 18.80; C₂₀H₁₅N₅O₃ requires C, 64.34; H, 4.02; N, 18.76.) IR: 3260 (NH), 1780 (C=O amide) and 1720 cm⁻¹ (C=O of COOH group).

3-(*N*-Phenyl) thiuredo-6-Methyl-4-Phenyl-1H-Pyrazolo [3,4-d] Pyrimidine (XIIIh)

A mixture of (II) (0.01 mole) and phenyl isothiocyanate (0.012 mole) in methanol (30 mL) was refluxed for 3 hrs. (steam-bath). The solid obtained was filtered off and recrystallized from ethanol to give (XIIIh) as orange crystals, m.pt. 149–150°C (yield 92%). (Found: C, 63.40; H, 4.50; N, 23.20; C₁₉H₁₆N₅S requires C, 63.33; H, 4.44; N, 23.33.) IR: 3240 (NH) and 1220 cm⁻¹ (C=S).

3-(*p*-Toluenesulphonamido)-6-Methyl-4-Phenyl-1H-Pyrazolo [3,4-d] Pyrimidine (XIIIi)

A mixture of (II) (0.01 mole) and *p*-toluenesulphonyl chloride (0.012 mole) in dry pyridine (20 mL) was refluxed for 1 hr. The solid obtained was filtered off and recrystallized from DMF to give (XIIIi) as pale yellow crystals, m.pt. 222–3°C (yield 85%). (Found: C, 59.80; H, 4.50; N, 18.40; C₁₉H₁₇N₅SO₂ requires C, 60.15; H, 4.48; N, 18.46.) IR: 3280 (NH) and 1320, 1110 cm⁻¹ (S=O).

3-(α -Carboxyethylideneamino)-6-Methyl-4-phenyl-1H-Pyrazolo [3,4-d] Pyrimidine (XIIIj)

A mixture of (II) (0.01 mole) and pyruvic acid (0.015 mole) in absolute ethanol (30 mL) was stirred at room temperature for 3 hrs., diluted with water. The solid obtained was filtered off and recrystallized from ethanol to give (XIIIj); as pale yellow crystals, m.pt. 210–11°C (yield 68%). Found: C, 61.20; H, 4.50; N, 23.50). C₁₅H₁₃N₅O₂ requires C, 61.01; H, 4.40; N, 23.72. IR: 3200 (NH), 1680 (C=O) and 1600 cm⁻¹ (C=N and C=C).

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Mycotoxins and Phycotoxins

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