

1-1-2014

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Recommended Citation

RAHMOUNI, AMEUR; ROMDHANE, ANIS; SAID, ABDERRAHIM BEN; MAJOULI, KAOUTHER; and JANNET, HICHEM BEN (2014) "Synthesis of new pyrazole and antibacterial pyrazolopyrimidine derivatives," *Turkish Journal of Chemistry*. Vol. 38: No. 2, Article 5. <https://doi.org/10.3906/kim-1303-20>
Available at: <https://journals.tubitak.gov.tr/chem/vol38/iss2/5>

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Synthesis of new pyrazole and antibacterial pyrazolopyrimidine derivatives

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Received: 09.03.2013 • Accepted: 18.08.2013 • Published Online: 14.03.2014 • Printed: 11.04.2014

Abstract: 3-Substituted-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amines **2a–c** were synthesized by treating 5-aminopyrazole-4-carbonitriles **1a–c** with formamide. The reactivity of compounds **1a–c** towards some cyclic anhydrides was studied. The condensation of 5-aminopyrazole-4-carbonitrile **1b** with triethylorthoformate gives imidate **7b**, which reacts with a series of primary amines and leads to pyrazolo[3,4-*d*]pyrimidine-4-amines **9** and **10**. The reaction of imidate **7b** with ammonia and hydroxylamine afforded pyrazolopyrimidine **2b** and pyrazolo[3,4-*d*]pyrimidin-5-(4*H*)-ol **11**, respectively. The synthesized compounds were completely characterized by ¹H NMR, ¹³C NMR, IR, and HRMS. The antibacterial activity of some new synthesized compounds was evaluated and appeared to be significant.

Key words: Aminopyrazoles, pyrimidines, maleic anhydride, pyrazolo[3,4-*d*]pyrimidines, Dimroth rearrangement, antibacterial activity

1. Introduction

Examples of natural products containing a pyrazole nucleus are very scarce, but many synthetic pyrazoles are biologically active,¹ and some have shown pharmacological utility as antipyretic, analgesic, and anti-inflammatory agents,^{1–3} as well as for their antimicrobial properties, especially antibacterial⁴ and antifungal activities.⁵ Moreover, this heterocyclic moiety is present within the core structure of numerous drugs, including Celebrex, Viagra, and Zaleplon.⁶

Our literature survey showed that the chemistry of fused pyrazolo[3,4-*d*]pyrimidine derivatives has drawn great attention due to their pharmacological importance^{7,8} and their structural resemblance to purines.⁹ In fact, several pyrazolo[3,4-*d*]pyrimidine derivatives demonstrated significant antimicrobial^{10,11} and cytotoxic activities.¹²

On the other hand, the literature^{13–15} reveals that several methods have been described for the elaboration of substituted pyrazolo[3,4-*d*]pyrimidines. Among the already known routes to the fused pyrazolopyrimidine scaffold, the most commonly used strategy involves a preliminary transformation of aminopyrazole-carbonitrile derivatives into the corresponding imidates followed by a subsequent ring-closure into pyrazolo[3,4-*d*]pyrimidines upon treatment with hydrazine.¹⁶

Therefore, taking into account all these above-described data, we report here our recent work on the

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synthesis of a new family of fused heterocyclic compounds using a nucleophilic addition-cyclization reaction on 5-aminopyrazole-4-carbonitriles with formamide and a series of cyclic anhydrides.

Prompted by the varied biological activities of pyrazolo[3,4-*d*]pyrimidine derivatives, we also described the synthesis of pyrazolo[3,4-*d*]pyrimidine-4-amines by condensation of imidate **7b** with various aromatic primary amines. Some new synthesized compounds were evaluated for their antibacterial activity using microdilution tests against some strains of bacteria.

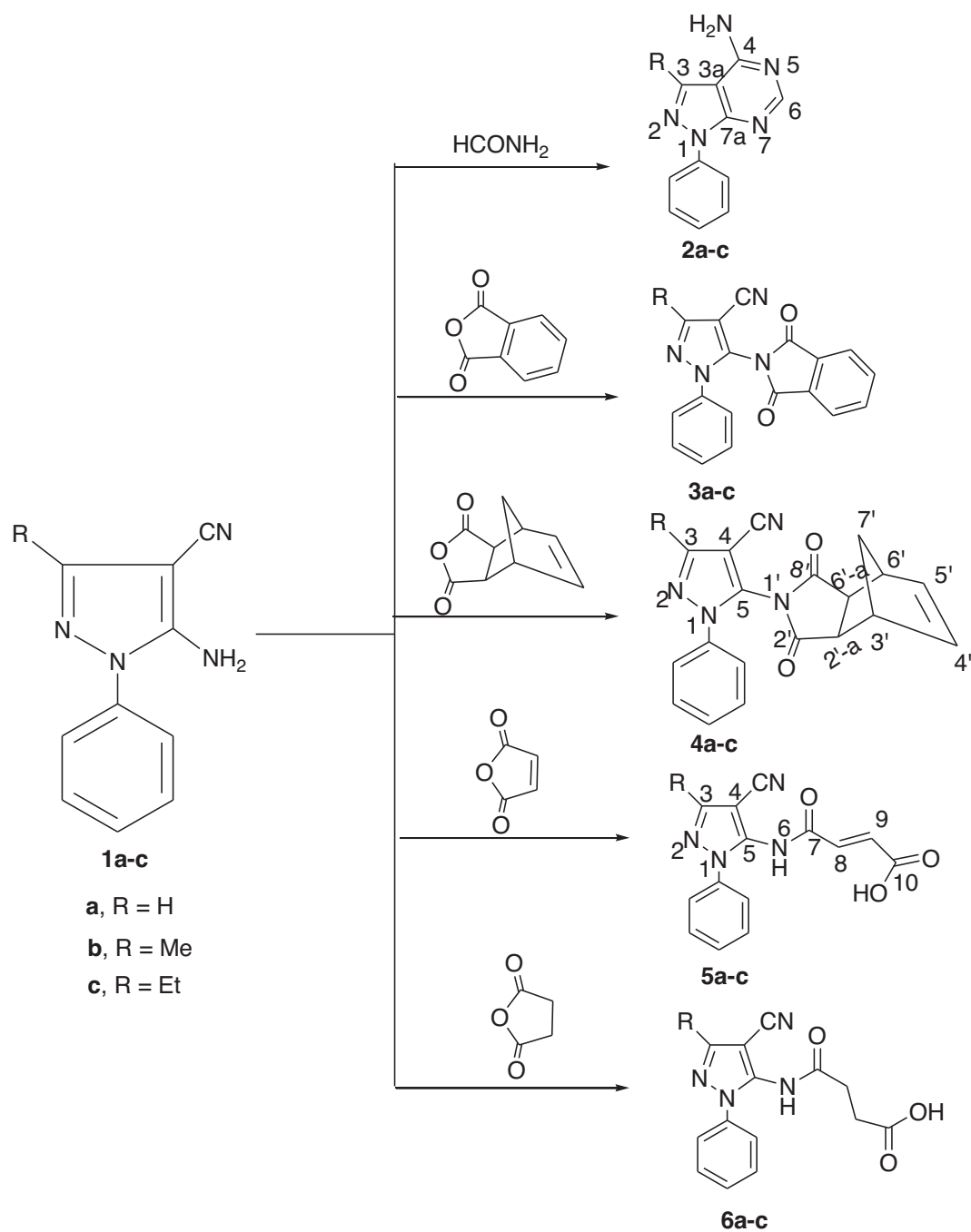
2. Results and discussion

According to the previously reported method,¹⁷ we synthesized the starting material 5-aminopyrazole-4-carbonitriles **1a-c** and we subjected them to a reaction with formamide^{18,19} to give pyrazolo[3,4-*d*]pyrimidines **2a-c** (Scheme 1). The structure of these compounds was confirmed based on their spectral data. The IR spectra of obtained products **2a-c** showed large bands between 3200 and 3400 cm⁻¹, assignable to NH₂, but no absorption frequency in the CN region. ¹H NMR spectra showed that NH₂-protons appeared as broad singlets in the 3.34–8.20 ppm region, while H₆ resonated in the 8.26–8.35 ppm region. The ¹³C NMR spectra of these compounds revealed essentially the appearance of the signals of C₄ and C₆ carbons at δ 156.4–157.2 and 158.1–158.9 ppm, respectively. Moreover, we note the disappearance of the signal due to the carbon of the –CN function.

The literature^{20–23} shows that aminopyrazole type **1a-c** is often used as synthon for preparing some novel fused pyrazoles. Therefore, it was considered of interest to synthesize some new heterocyclic systems incorporating pyrazole fragments. In this context, we reacted the intermediates **1a-c** with a series of cyclic anhydrides in refluxing glacial acetic acid to produce the corresponding imides **3a-c** and **4a-c** (Scheme 1). When we used phthalic and tricyclic anhydrides we did not observe any side product and the reaction led to the corresponding imides **3a-c** and **4a-c**, respectively. In the case of the use of the maleic and succinic anhydrides in the same conditions the reaction afforded only the corresponding imides **5a-c** and **6a-c**. The cyclic side chain in the phthalic and tricyclic anhydrides may favor intramolecular cyclization in the case of **3a-c** and **4a-c**, while free rotation at the C₇–C₈ band in compounds **5a-c** and **6a-c** may not favor this intramolecular cyclization.

The IR spectra of the obtained imides revealed the presence of characteristic absorption bands at ν 2210–2220 and at 1695–1710 cm⁻¹ assignable to cyano and carbonyl groups, respectively. In addition, ¹H NMR spectra of these compounds showed the absence of signals related to the mobile protons. The formation of these products was also confirmed by ¹³C NMR data with the observation of signals of carbons introduced by cyclic anhydrides. Furthermore, the mass spectra (ES+) of compounds **3a-c** and **4a-c** showed molecular ion peaks in good agreement with the assigned structures. Under the same conditions, maleic and succinic anhydrides reacted with the appropriate aminopyrazoles **1a-c** to generate the corresponding carboxy-amides **5a-c** and **6a-c**, respectively, in good yields (Scheme 1). The structure of the obtained carboxy-amides was appropriately established by spectroscopic NMR and IR data. The IR spectra of compounds **5a-c** showed 2 strong absorption bands at ν 1650–1660 and 1730–1745 cm⁻¹ assignable to amide and acid carbonyl groups, respectively, and other important bands revealed at 3244 cm⁻¹ and 3334 cm⁻¹ were attributed to the amide NH and OH carboxylic acid, respectively. The ¹H NMR spectrum of compound **5c** as an example exhibits 2 doublets at δ 6.65 ppm ($J = 16$ Hz) assigned to the olefinic hydrogens and a broad singlet at 12.70 ppm corresponding to the acidic proton.

Furthermore, the 5-amino pyrazole-4-carbonitrile **1b** was reacted with triethyl orthoformate to give the corresponding ethoxymethylene amino derivative **7b** (Scheme 2), often used as a synthon key to access



Scheme 1. Synthetic route of compounds **2-6**.

pyrazolo[3,4-*d*]pyrimidines.²⁴ It seemed of interest to react the intermediate **7b** with a series of amines (Scheme 2). In this case, we considered that the presence of amidine moiety may ensure the possibility of closure of the pyrimidine ring, resulting in novel derivatives of pyrazolo[3,4-*d*]pyrimidine of significant biological interest since such compounds are substituted analogues of the well-known drug allopurinol.²⁵ We selected some aromatic and aliphatic primary amines, the more basic ammonia and hydroxylamine hydrochloride, to study their reactions with the imidate **7b**.

The imidate **7b** reacted with both their electrophilic sites with aliphatic amines to yield the new pyrazolopyrimidines type **9a–c** in 2 steps. In the first step, the condensation of **7b** with aliphatic amines in ethanol in the presence of a catalytic amount of acetic acid led to the intermediate **8a–c** by the nucleophilic attack of the NH₂ motif on imidic carbon. In the second step, the isolable amidine **8a–c** was heated in toluene in the presence of a few drops of piperidine to provide the novel pyrazolopyrimidines **9a–c** via Dimroth rearrangement.^{26,27} The isomerization of **8'a–c** into thermodynamically more stable pyrazolopyrimidines derivative **9a–c** (Scheme 2) seems to occur through base-catalyzed tandem ring opening and ring closure. This rearrangement is consistent with those reported in some earlier reports.^{16,27}

The alternative structure **8a** was excluded based on NMR data. The ¹H NMR spectrum showed the disappearance of signals related to the ethoxy group and the appearance of signals introduced by the amine used (R = Ph), and a characteristic signal at δ 8.38 ppm, assignable to the proton –N=CH. The analysis of the ¹³C NMR spectrum revealed that the signal of the CN group appeared at δ 117.4 ppm.

In addition, a whole set of linkages confirming the molecular structure of compounds **8a–c** was deduced from 2D NMR experiments. As an example, the spreading of the ¹H-¹H COSY spectrum of compound **8a** shows the correlation of both methylenic and ethylenic protons with that of the –NH– function.

The ¹H NMR spectra of compounds **9a–c** showed the presence of a singlet at δ 8.34–8.50 ppm attributable to the aromatic proton H₆. The disappearance of the cyano signal in the ¹³C NMR spectra of compounds **9a–c** was in favor of an intramolecular cyclization but it was not sufficient to differentiate between structures of the nonconversion products **8'a–c** and the rearranged ones **9a–c** of which the structures were confirmed by 2D NMR experiments.

The ¹H-¹H COSY spectrum of compound **9a** as an example showed a clear correlation peak between –NH– (br s, 5.70 ppm) and the 2 methylenic protons (d, 4.90 ppm, $J = 5.7$ Hz), which was in favor of the Dimroth rearrangement of the nonisolable intermediate **8'a**, which leads to **9a**. The reaction of **7b** with some aromatic amines led to the *N*-aryl-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-4-amines **10a–d** via the Dimroth rearrangement of the intermediates **7'a–d**.

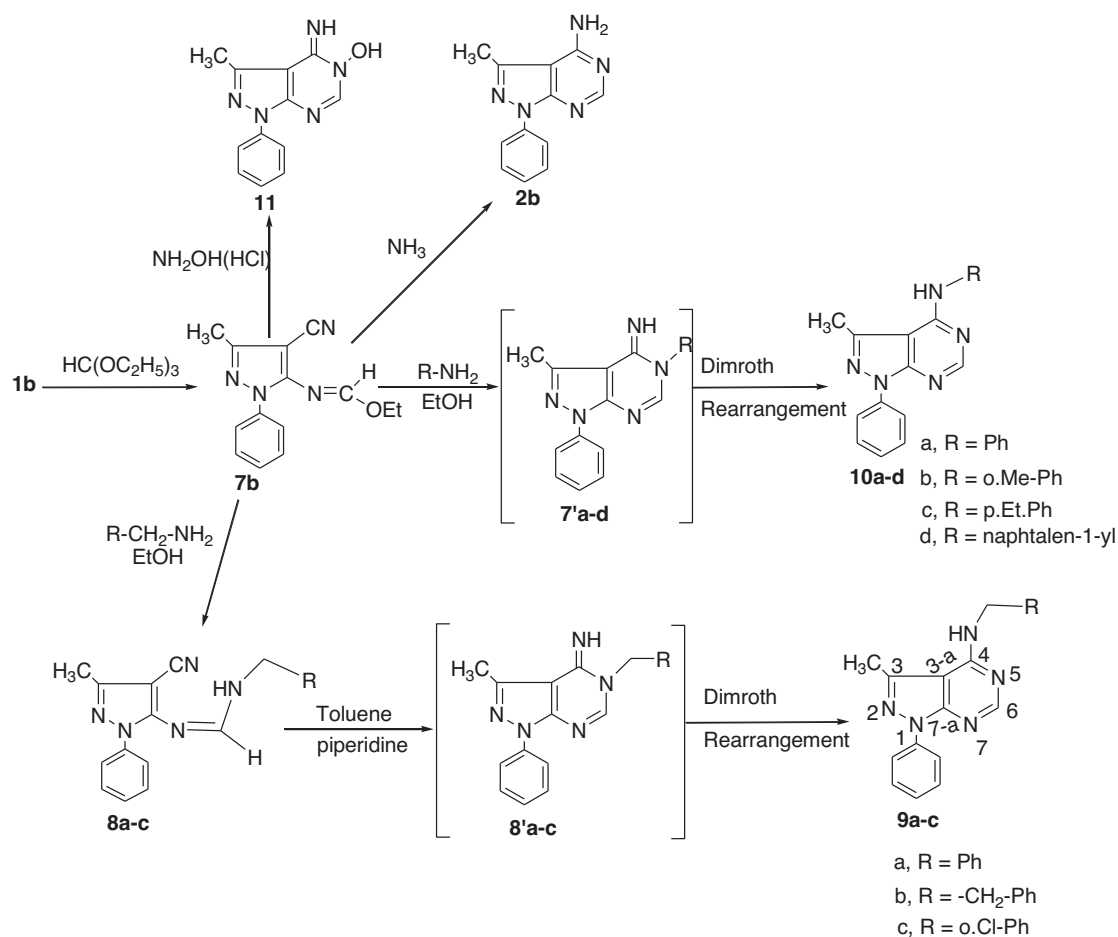
The structure of compounds **10a–d** was confirmed by 2D NMR experiments.

The HMBC spectrum of compound **10b** as an example showed a correlation peak between the –NH– proton (br s, 8.01 ppm) and the quaternary (δ c 116.0 ppm) and tertiary (δ c 118.4 ppm) aromatic carbons, reinforcing the structure of **10b**.

Hydroxyiminolysis of iminoether **7b** in ethanol in the presence of triethylamine provides pyrazolo[3,4-*d*]pyrimidine **11** (Scheme 2). The formation of compound **11** was established by the presence of vibration bands corresponding to the OH and NH functions near 3500 and 3400 cm⁻¹, respectively. In the ¹H NMR spectrum of compound **11** measured in dimethyl-*d*₆ sulfoxide, we observed the signal of a CH₃ group at δ 2.78 ppm, the signal of an OH group at δ 4.08 ppm, and aromatic proton signals and NH at 7.28–8.19 ppm.

Finally the imidate **7b** was added to methanol saturated with ammonia at 0 °C for 1 h (Scheme 2). The reaction mixture was then warmed to room temperature and stirred for 6 h to produce pyrazolo[3,4-*d*]pyrimidine **2b**, which was already synthesized by the addition of formamide to 5-aminopyrazole-4-carbonitrile **1b** (Scheme 1). The ¹H NMR spectrum of compound **2b** exhibited 2 singlets at δ 7.30 and 8.40 ppm, representing the protons of pyrimidine and NH₂, respectively.

The mass spectra of all prepared compounds were compatible with the proposed structures.



Scheme 2. Synthetic route of compounds **8–11** and **2b**.

3. Antibacterial activity

The incorporation of another heterocyclic moiety in pyrazole, in the form of a substituent or as a fused component, changes its properties and converts it into an altogether new and important heterocyclic derivative. Pyrimidines have attracted particular interest over the last few decades due to the use of such a ring system as the core nucleus in various drugs.²⁸ They are well known for their popular pharmacological activities.^{29,30} Considering the importance of pyrazolopyrimidine derivatives for their biological activity, it was thought worthwhile to test most of our prepared compounds (**3a–c**, **4a–c**, **5a–c**, **6a–c**, **9a–c**, **10a–c**, **11**, and **2b**) for their antibacterial activity against some bacteria, namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*.

The minimum inhibitory concentrations (MICs) were ascertained by the broth dilution method (microdilution using 96-well microplates).³¹ The results presented in the Table showed that **9a** is the most active towards *Pseudomonas aeruginosa*. We also noted that adding a CH₂ to the fragment R decreases this activity.

Compounds **10a** and **10b** were the most active against *E. coli*. The presence of the chlorine atom in the ortho position in **10c** significantly reduced this activity. The remaining compounds were found to have slight or moderate activity against the tested organisms and some of the compounds were found to be inactive (MIC > 4).

Table. Antibacterial activity of some synthesized compounds: **2b**, **9a-c**, **10a-c**, and **11**.

Compounds	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Enterococcus faecalis	Acinetobacter
	MIC (mg/mL)				
2b	4	2	2	1	4
9a	2	4	0.5	2	> 4
9b	> 4	> 4	2	4	> 4
9c	2	> 4	2	2	> 4
10a	4	0.5	1	> 4	2
10b	1	0.5	1	> 4	2
10c	2	> 4	> 4	2	> 4
11	1	> 4	2	2	> 4
Ampicillin	0.62	1.25	1.25	-	-

Compounds **3a-c**, **4a-c**, **5a-c**, and **6a-c** did not show any significant antibacterial activity against the strains used (MIC > 100).

4. Experimental section

General. All melting points were determined on a Kofler-type microscope and are uncorrected. IR spectra were recorded on a PerkinElmer FT-IR spectrophotometer (4000–400 cm^{-1}) using KBr pellets. ^1H NMR and ^{13}C NMR spectra were recorded at room temperature (rt) in CDCl_3 and dimethylsulfoxide ($\text{DMSO}-d_6$) at 300 MHz and at 75 MHz, respectively, using residual nondeuterated solvent peaks as internal reference. Coupling constants are given in hertz. HRMS spectra were acquired with an electrospray-time-of-flight (ESI-TOF, LCT Premier XE, Waters) mass spectrometer in positive ion mode.

General procedure for 3-substituted-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4 amines **2a-c**

A mixture of compound **1** (10 mmol) and formamide (10 mmol) was heated under reflux for 2 h, and then left to cool overnight at ambient temperature. The solid formed was filtered, dried, and recrystallized from ethanol.

1-Phenyl -1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**2a**):

Gray solid, yield: 80%, mp: 215–217 °C (EtOH); IR (KBr, cm^{-1}) ν : 3200–3400 (NH_2). ^1H NMR ($\text{DMSO}-d_6$, 300 MHz): δ (ppm) = 7.28–8.19 (m, 5H, H-arom), 7.81 (s, 2H, NH_2), 8.29 (s, 1H, H-3), 8.35 (s, 1H, H-6). ^{13}C NMR ($\text{DMSO}-d_6$, 75 MHz): δ (ppm) = 101.9 (C-3a), 121.0, 126.5, 129.6, 134.5, (C-arom), 140.7 (C-3), 153.7 (C-7a), 157.2 (C-4), 158.8 (C-6). HRMS $[\text{M} + \text{H}]^+$ calcd for $(\text{C}_{11}\text{H}_{10}\text{N}_5)^+$ 212.0936, found 212.0945.

3-Methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**2b**):

Gray solid, yield: 85%, mp: 217–218 °C (EtOH); IR (KBr, cm^{-1}) ν : 3250–3450 (NH_2), ^1H NMR ($\text{DMSO}-d_6$, 300 MHz): δ (ppm) = 2.60 (s, 3H, CH_3), 7.28–7.80 (m, 5H, H-arom), 8.20 (s, 2H, NH_2), 8.35 (s, 1H, H-6). ^{13}C NMR ($\text{DMSO}-d_6$, 75 MHz): δ (ppm) = 14.5 (CH_3), 100.9 (C-3a), 120.1, 126.1, 129.4, 139.0 (C-arom), 141.7 (C-3), 153.5 (C-7a), 156.8 (C-4) 158.1 (C-6). HRMS $[\text{M} + \text{H}]^+$ calcd for $(\text{C}_{12}\text{H}_{12}\text{N}_5)^+$ 226.1011, found 226.1027.

3-Ethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**2c**):

Gray solid, yield: 80%, mp: 222–224 °C (EtOH); IR (KBr, cm^{-1}) ν : 3200–3490 (NH_2), ^1H NMR

(DMSO-*d*₆, 300 MHz): δ (ppm) = 1.28 (t, 3H, $J = 7.5$ Hz, $-\text{CH}_2-\underline{\text{C}}\text{H}_3$), 3.05 (q, 2H, $J = 7.5$ Hz, $-\underline{\text{C}}\text{H}_2-\text{CH}_3$), 3.34 (s, 2H, NH₂) 7.23–8.18 (m, 5H, H-arom), 8.26 (s, 1H, H-6). ¹³C NMR (DMSO-*d*₆, 75 MHz): δ (ppm) = 13.5 ($-\text{CH}_2-\underline{\text{C}}\text{H}_3$), 21.9 ($-\underline{\text{C}}\text{H}_2-\text{CH}_3$), 100.0 (C-3a), 120.8, 126.0, 129.4, 139.5 (C-arom), 144.5 (C-3), 154.8 (C-7a), 156.4 (C-4) 158.9 (C-6). HRMS [M + H]⁺ calcd for (C₁₃H₁₄N₅)⁺ 240.1145, found 240.1150.

General procedure for 5-(1,3-dioxoisindolin-2-yl)-3-substituted-1-phenyl-1H-pyrazole-4-carbonitriles 3a–c and 4a–c

Phthalic or bicyclic anhydride (0.1 mol) was completely dissolved at room temperature in glacial acetic acid (20 mL) or THF, and then aminopyrazoles **1a–c** (0.1 mol) were added to the solution; the resulting mixture was stirred under reflux for 3 h. The reaction mixture was allowed to cool to room temperature, and then was poured into water; the precipitate formed was filtered off, washed with water, dried, and crystallized from ethanol to give the imides **3** and **4**.

5-(1,3-Dioxoisindolin-2-yl)-1-phenyl-1H-pyrazole-4-carbonitrile (3a):

Green solid, yield: 75%, mp: 157–159 °C (EtOH); IR (KBr, cm⁻¹) ν : 1680 (C=O, imide), 2210 (CN). ¹H NMR (CDCl₃, 300 MHz): δ (ppm) = 7.30–8.01 (m, 9H, H-arom), 8.30 (s, 1H, H-3). ¹³C NMR (CDCl₃, 75 MHz): δ (ppm) = 78.1 (C-4), 116.8 (CN), 120.8, 126.4, 127.7, 129.2, 132.3, 133.3, 139.1 (C-arom), 152.8 (C-3), 156.3 (C-5), 168.6 (C=O). HRMS [M + H]⁺ calcd for (C₁₈H₁₁N₄O₂)⁺ 315.0810, found 315.0813.

5-(1,3-Dioxoisindolin-2-yl)-3-methyl-1-phenyl-1H-pyrazole-4-carbonitrile (3b):

Green solid, yield: 80%, mp: 160–162 °C (EtOH); IR (KBr, cm⁻¹) ν : 1695 (C=O, imide), 2220 (CN). ¹H NMR (CDCl₃, 300 MHz): δ (ppm) = 2.69 (CH₃), 7.39–8.10 (m, 9H, H-arom). ¹³C NMR (CDCl₃, 75 MHz): δ (ppm) = 13.6 (CH₃), 78.7 (C-4), 117.1 (CN), 120.7, 125.9, 128.1, 129.2, 132.2, 132.8, 139.1 (C-arom), 153.0 (C-3), 158.3 (C-5), 167.6 (C=O). HRMS [M + H]⁺ calcd for (C₁₉H₁₃N₄O₂)⁺ 329.1035, found 329.1039.

5-(1,3-Dioxoisindolin-2-yl)-3-ethyl-1-phenyl-1H-pyrazole-4-carbonitrile (3c):

Green solid, yield: 80%, mp: 164–166 °C (EtOH); IR (KBr, cm⁻¹) ν : 1710 (C=O, imide), 2217 (CN). ¹H NMR (CDCl₃, 300 MHz): δ (ppm) = 1.32 (t, 3H, $J = 7.1$ Hz, $-\text{CH}_2-\underline{\text{C}}\text{H}_3$), 2.90 (q, 2H, $J = 7.2$ Hz, $-\underline{\text{C}}\text{H}_2-\text{CH}_3$), 7.20–8.05 (m, 9H, H-arom). ¹³C NMR (CDCl₃, 75 MHz): δ (ppm) = 14.5 ($-\text{CH}_2-\underline{\text{C}}\text{H}_3$), 21.9 ($-\underline{\text{C}}\text{H}_2-\text{CH}_3$), 79.1 (C-4), 117.0 (CN), 120.2, 126.4, 128.1, 129.2, 132.2, 132.0, 138.7 (C-arom), 153.5 (C-3), 157.6 (C-5), 167.7 (C=O). HRMS [M + H]⁺ calcd for (C₂₀H₁₅N₄O₂)⁺ 343.1017, found 343.1024.

5-(1,3-Dioxopyrrolidino[3,4-*d*]hept[2.2.1]-5-en-2-yl)-1-phenyl-1H-pyrazole-4-carbonitrile (4a):

White solid, yield: 70%, mp: 170–172 °C (EtOH); IR (KBr, cm⁻¹) ν : 1700 (C=O, imide), 2210 (CN). ¹H NMR (CDCl₃, 300 MHz): δ (ppm) = 1.50–1.68 (m, 2H, H-7'), 3.36 (m, 2H, H-3', H-6'), 3.48 (m, 2H, H-6'a, H-2'a), 5.40 (s, 2H, H-4', H-5'), 7.50–7.55 (m, 5H, H-arom), 8.10 (s, 1H, H-3). ¹³C NMR (CDCl₃, 75 MHz): δ (ppm) = 45.1 (C-6'a, C-2'a), 46.7 (C-7'), 52.6 (C-3', C-6'), 82.1 (C-4), 114.4 (CN), 124.1, 126.9, 127.7, 129.5 (C-arom), 135.3 (C-4', C-5'), 150.0 (C-5), 152.1 (C-3), 174.1 (C=O). HRMS [M + H]⁺ calcd for (C₁₉H₁₅N₄O₂)⁺ 331.1138, found 331.1143.

5-(1,3-Dioxopyrrolidino[3,4-*b*]hept[2.2.1]-5-en-2-yl)-3-methyl-1-phenyl-1H-pyrazole-4-carbonitrile (4b):

White solid, yield: 77%, mp: 174–176 °C (EtOH); IR (KBr, cm⁻¹) ν : 1710 (C=O, imide), 2215 (CN). ¹H NMR (CDCl₃, 300 MHz): δ (ppm) = 1.53–1.70 (m, 2H, H-7'), 2.45 (CH₃), 3.32 (m, 2H, H-3', H-6'), 3.50 (m, 2H, H-6'a, H-2'a), 5.43 (s, 2H, H-4', H-5'), 7.48–7.54 (m, 5H, H-arom). ¹³C NMR (CDCl₃, 75 MHz): δ

(ppm) = 12.5 (CH₃), 44.6 (C-6'-a, C-2'a), 46.2 (C-7'), 51.8 (C-3', C-6'), 82.0 (C-4), 114.1 (CN), 124.1, 126.9, 127.7, 129.5 (C-arom), 134.7 (C-4', C-5'), 150.1 (C-5), 152.0 (C-3), 173.9 (C=O). HRMS [M + H]⁺ calcd for (C₂₀H₁₇N₄O₂)⁺ 345.1350, found 345.1356.

5-(1,3-Dioxopyrrolidino[3,4-*d*]hept[2.2.1]-5-en-2-yl)-3-ethyl-1-phenyl-1*H*-pyrazole-4-carbonitrile (4c):

White solid, yield: 75%, mp: 181–183 °C (EtOH); IR (KBr, cm⁻¹) ν : 1705 (C=O, imide), 2220 (CN). ¹H NMR (CDCl₃, 300 MHz): δ (ppm) = 1.25 (t, 3H, *J* = 7.1 Hz, -CH₂-CH₃), 3.05 (q, 2H, *J* = 7.4 Hz, -CH₂-CH₃), 1.51–1.67 (m, 2H, H-7'), 3.33 (m, 2H, H-3', H-6'), 3.53 (m, 2H, H-6'a, H-2'a), 5.46 (s, 2H, H-4', H-5'), 7.39–7.45 (m, 5H, H-arom). ¹³C NMR (CDCl₃, 75 MHz): δ (ppm) = 13.5 (-CH₂-CH₃), 21.4 (-CH₂-CH₃), 45.3 (C-6'a, C-2'a), 46.7 (C-7'), 52.7 (C-3', C-6'), 81.5 (C-4), 114.6 (CN), 124.1, 126.9, 127.7, 129.5 (C-arom), 134.5 (C-4', C-5'), 150.1 (C-5), 151.7 (C-3), 174.5 (C=O). HRMS [M + H]⁺ calcd for (C₂₁H₁₉N₄O₂)⁺ 359.1458, found 359.1462.

General procedure for 4-(4-cyano-3-substituted-1-phenyl-1*H*-pyrazol-5-ylamino)-4-oxocarboxylic-acids 5a–c and 6a–c

Maleic or succinic anhydride (0.1 mol) was completely dissolved at room temperature in glacial acetic (20 mL), and then aminopyrazoles **1a–c** (0.1 mol) were added to the solution; the resulting mixture was stirred under reflux for 3 h. The reaction mixture was allowed to cool to room temperature and then was poured into water; the precipitate formed was filtered off, washed with water, dried, and crystallized from ethanol to afford the *N*-cyclic maleamic or succinic acids.

(E)-4-(4-Cyano-1-phenyl-1*H*-pyrazol-5-ylamino)-4-oxobut-2-enoic acid (5a):

Yellow solid, yield: 75%, mp: 260–262 °C (EtOH); IR (KBr, cm⁻¹) ν : 1650 (C=O, amide), 1730 (C=O, acid), 3244 (-NH-), 3334 (OH). ¹H NMR (DMSO-*d*₆, 300 MHz): δ (ppm) = 6.81 (d, 1H, *J* = 16 Hz, H-9), 7.20 (d, 1H, *J* = 16 Hz, H-8), 7.21–8.10 (m, 6H, NH + H-arom), 8.40 (s, 1H, H-3), 12.87 (s, 1H, OH). ¹³C NMR (DMSO-*d*₆, 75 MHz): δ (ppm) = 79.1 (C-4), 117.1 (CN), 121.7, 126.5, 129.6, 139.5 (C-arom), 132.8 (C-9), 138.7, (C-8), 152.3 (C-3), 153.2 (C-5), 159.9 (C-7), 168.6 (C-10). HRMS [M + H]⁺ calcd for (C₁₄H₁₁N₄O₃)⁺ 283.0853, found 283.0855.

(E)-4-(4-Cyano-3-methyl-1-phenyl-1*H*-pyrazol-5-ylamino)-4-oxobut-2-enoic acid (5b):

Yellow solid, yield: 80%, mp: 265–267 °C (EtOH); IR (KBr, cm⁻¹) ν : 1660 (C=O, amide), 1735 (C=O, acid), 3240 (-NH-), 3330 (OH). ¹H NMR (DMSO-*d*₆, 300 MHz): δ (ppm) = 3.07 (CH₃), 6.86 (d, 1H, *J* = 16 Hz, H-9), 7.38 (d, 1H, *J* = 16 Hz, H-8), 7.20–8.05 (m, 6H, NH + H-arom), 12.97 (s, 1H, OH). ¹³C NMR (DMSO-*d*₆, 75 MHz): δ (ppm) = 14.5 (CH₃), 80.1 (C-4), 116.0 (CN), 122.7, 127.4, 130.6, 139.7 (C-arom), 130.1 (C-9), 136.1 (C-8), 151.8 (C-3), 154.0 (C-5), 159.9 (C-7), 169.7 (C-10). HRMS [M + H]⁺ calcd for (C₁₅H₁₃N₄O₃)⁺ 297.0988, found 297.0988.

(E)-4-(4-Cyano-3-ethyl-1-phenyl-1*H*-pyrazol-5-ylamino)-4-oxobut-2-enoic acid (5c):

Yellow solid, yield: 85%, mp: 267–269 °C (EtOH); IR (KBr, cm⁻¹) ν : 1650 (C=O, amide) 1730 (C=O, acid), 3235 (-NH-), 3330 (OH). ¹H NMR (DMSO-*d*₆, 300 MHz): δ (ppm) = 1.32 (t, 3H, *J* = 7.0 Hz, -CH₂-CH₃), 2.91 (q, 2H, *J* = 7.0 Hz, -CH₂-CH₃), 6.65 (d, 1H, *J* = 16 Hz, H-9), 7.02 (d, 1H, *J* = 16 Hz, H-8), 7.20–8.05 (m, 6H, NH + H-arom), 12.70 (s, 1H, OH). ¹³C NMR (DMSO-*d*₆, 75 MHz): δ (ppm) = 13.3 (-CH₂-CH₃), 21.6 (-CH₂-CH₃), 78.9 (C-4), 114.0 (CN), 121.8, 126.8, 129.6, 139.7 (C-arom), 130.0 (C-9),

135.7 (C-8), 151.9 (C-3), 153.5 (C-5), 158.3 (C-7), 167.7 (C-10). HRMS $[M + H]^+$ calcd for $(C_{16}H_{15}N_4O_3)^+$ 311.1144, found 311.1150.

4-(4-Cyano-1-phenyl-1*H*-pyrazol-5-ylamino)-4-oxobutanoic acid (6a):

White solid, yield: 65%, mp: 230–232 °C (EtOH); IR (KBr, cm^{-1}) ν : 1660 (C=O, amide), 1740 (C=O, acid), 3240 (–NH–), 3330 (OH). 1H NMR (DMSO- d_6 , 300 MHz): 2.30 (t, 2H, $J = 6.01$ Hz, H-8), 2.47 (t, 2H, $J = 6.01$ Hz, H-9), 7.21–7.50 (m, 5H, H-arom), 8.29 (s, 1H, H-3), 8.40 (s, 1H, –NH–), 12.55 (s, 1H, OH). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ (ppm) = 27.4 (C-8), 34.9 (C-9), 83.8 (C-4), 117.1 (CN), 120.7, 126.3, 129.6, 139.8 (C-arom), 152.3 (C-3), 152.5 (C-5), 169.9 (C-7), 171.9 (C-10). HRMS $[M + H]^+$ calcd for $(C_{14}H_{13}N_4O_3)^+$ 285.1001, found 285.1003.

4-(4-Cyano-3-methyl-1-phenyl-1*H*-pyrazol-5-ylamino)-4-oxobutanoic acid (6b):

White solid, yield: 68%, mp: 237–239 °C (EtOH); IR (KBr, cm^{-1}) ν : 1650 (C=O, amide), 1740 (C=O, acid), 3235 (–NH–), 3330 (OH). 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) = 2.40 (t, 2H, $J = 5.9$ Hz, H-8), 2.46 (t, 2H, $J = 5.9$ Hz, H-9), 2.58 (s, 3H, CH_3), 7.30–7.62 (m, 5H, H-arom), 8.60 (s, 1H, –NH–), 12.40 (s, 1H, OH). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ (ppm) = 14.7 (CH_3), 28.5 (C-8), 32.0 (C-9), 79.2 (C-4), 116.6 (CN), 120.2, 126.3, 129.6, 139.4 (C-arom), 152.2 (C-3), 153.8 (C-5), 170.8 (C-7), 173.7 (C-10). HRMS $[M + H]^+$ calcd for $(C_{15}H_{15}N_4O_3)^+$ 299.1053, found 299.1055.

4-(4-Cyano-3-ethyl-1-phenyl-1*H*-pyrazol-5-ylamino)-4-oxobutanoic acid (6c):

White solid, yield: 68%, mp: 240–242 °C (EtOH); IR (KBr, cm^{-1}) ν : 1655 (C=O, amide), 1735 (C=O, acid), 3230 (–NH–), 3334 (OH). 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) = 1.30 (t, 3H, $J = 7.0$ Hz, $-CH_2-CH_3$), 2.37 (t, 2H, $J = 5.9$ Hz, H-8), 2.46 (t, 2H, $J = 5.9$ Hz, H-9), 2.60 (q, 2H, $J = 7.1$ Hz, $-CH_2-CH_3$), 7.20–7.52 (m, 5H, H-arom), 8.35 (s, 1H, –NH–), 12.34 (s, 1H, OH). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ (ppm) = 13.5 ($-CH_2-CH_3$), 21.7 ($-CH_2-CH_3$), 29.9 (C-8), 31.6 (C-9), 81.0 (C-4), 116.0 (CN), 121.2, 126.2, 129.3, 138.3 (C-arom), 152.0 (C-3), 153.3 (C-5), 173.8 (C-7), 177.7 (C-10). HRMS $[M + H]^+$ calcd for $(C_{16}H_{17}N_4O_3)^+$ 313.1220, found 313.1235.

General procedure for 3-methyl-*N*-benzyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amines 9a–c

Firstly to a solution of imidate **7** (0.1 mol) in ethanol (20 mL) in the presence of a few drops of acetic acid was added aliphatic amines (0.01 mol). The reaction was heated under reflux for 4 h. After cooling, the product was collected and recrystallized from ethanol to afford **8a–c** as colorless needles.

Secondly a solution of amidines **8a–c** in toluene in the presence of a few drops of piperidine was heated under reflux for 2 h. The residue obtained in each case after removing the solvent in vacuo was chromatographed on silica gel using chloroform as a mobile phase to yield compounds **9a–c**.

***N*-Benzyl-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (9a):**

Yellow solid, yield: 70%, mp: 180–182 °C (EtOH); IR (KBr, cm^{-1}) ν : 3450 (NH). 1H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 2.68 (s, 3H, CH_3), 4.90 (d, 2H, $J = 5.7$ Hz, $-CH_2-Ph$), 5.70 (br s, 1H, –NH–), 7.28–8.13 (m, 10H, H-arom), 8.50 (s, 1H, H-6). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) = 15.4 (CH_3), 45.2 ($-CH_2-Ph$), 103.8 (C-3-a), 121.8, 124.4, 126.7, 128.2, 129.3, 137.3, 139.1, 141.7 (C-arom), 150.3 (C-3), 154.3 (C-7-a), 156.6 (C-6) 157.5 (C-4). HRMS $[M + H]^+$ calcd for $(C_{19}H_{18}N_5)^+$ 316.1480, found 316.1492.

3-Methyl-*N*-phenethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (9b):

Yellow solid, yield: 68%, mp: 183–185 °C (EtOH); IR (KBr, cm^{-1}) ν : 3420 (NH). 1H NMR ($CDCl_3$,

300 MHz): δ 2.29 (s, 3H, CH₃), 2.92 (t, 2H, $J = 7.1$ Hz, -NH-CH₂-CH₂-Ph), 3.88 (q, 2H, $J = 7.0$ Hz, -NH-CH₂-CH₂-Ph), 5.16 (br s, 1H, -NH-), 7.00–7.93 (m, 10H, H-arom), 8.34 (s, 1H, H-6). ¹³C NMR (CDCl₃, 75 MHz): δ (ppm) = 14.5 (CH₃), 35.3 (-NH-CH₂-CH₂-Ph), 45.2 (-CH₂-CH₂-Ph), 104.1 (C-3-a), 121.9, 126.9, 127.4, 129.0, 129.3, 138.5, 139.1, 139.7 (C-arom), 144.7 (C-3), 150.5 (C-7-a), 156.1 (C-6), 157.1 (C-4). HRMS [M + H]⁺ calcd for (C₂₀H₂₀N₅)⁺ 330.1624, found 330.1632.

3-Methyl-N-(2-chlorobenzyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (9c):

Yellow solid, yield: 70%, mp: 179–181 °C (EtOH); IR (KBr, cm⁻¹) ν : 3445 (NH). ¹H NMR (CDCl₃, 300 MHz): δ (ppm) = 2.60 (s, 3H, CH₃), 4.01 (d, 2H, $J = 7.0$ Hz, -NH-CH₂-2.Cl.Ph), 5.80 (br s, 1H, -NH-), 7.19–8.35 (m, 9H, H-arom), 8.41 (s, 1H, H-6). ¹³C NMR (CDCl₃, 75 MHz): δ (ppm) = 14.4 (CH₃), 39.7 (-NH-CH₂-2.Cl.Ph), 103.2 (C-3-a), 121.1, 125.3, 126.9, 128.5, 128.3, 129.3, 133.0, 137.3, 139.1, 141.3 (C-arom), 144.1 (C-3), 153.2 (C-7-a), 156.8 (C-6) 157.5 (C-4). HRMS [M + H]⁺ calcd for (C₁₉H₁₇ClN₅)⁺ 350.8773, found 350.8780.

General procedure for the synthesis of 3-methyl-N-aryl-1-phenyl-1H-pyrazolo[3, 4-d]pyrimidin-4-amines 10a–d

The appropriate primary aromatic amine (0.001 mol) was added to the suitable imidate **7b** (0.01 mol), and the mixture was stirred at reflux in toluene (20 mL) for 6 h. After cooling, the precipitated solid was filtered, washed with cold ether and dried, and then recrystallized from ethanol to give compounds **10a–d**.

3-Methyl-N,1-diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (10a):

Yellow solid, yield: 60%, mp: 171–173 °C (EtOH); IR (KBr, cm⁻¹) ν : 3440 (NH). ¹H NMR (CDCl₃, 300 MHz): δ (ppm) = 2.37 (s, 3H, CH₃), 6.99–7.66 (m, 11H, NH + H-arom), 8.85 (s, 1H, H-6). ¹³C NMR (CDCl₃, 75 MHz): δ (ppm) = 13.5 (CH₃), 101.9 (C-3-a), 116.0, 119.1, 123.1, 127.1, 129.3, 139.1, 140.1 (C-arom), 141.8 (C-3), 150.5 (C-7a), 155.8 (C-4), 156.0 (C-6). HRMS [M + H]⁺ calcd for (C₁₈H₁₆N₅)⁺ 302.1405, found 302.1410.

3-Methyl-1-phenyl-N-o-tolyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (10b):

Yellow solid, yield: 65%, mp: 190–192 °C (EtOH); IR (KBr, cm⁻¹) ν : 3450 (NH). ¹H NMR (CDCl₃, 300 MHz): δ (ppm) = 2.33 (s, 3H, Ph-CH₃), 2.46 (s, 3H, CH₃), 6.82–7.79 (m, 9H, H-arom), 8.01 (br s, 1H, NH), 8.93 (s, 1H, H-6). ¹³C NMR (CDCl₃, 75 MHz): δ (ppm) = 13.6 (CH₃), 21.8 (Ph-CH₃), 103.9 (C-3a), 116.1, 118.4, 121.1, 126.1, 127.1, 128.0, 129.4, 129.7, 139.1, 141.4 (C-arom), 143.4 (C-3), 150.0 (C-7a), 155.1 (C-4), 156.1 (C-6). HRMS [M + H]⁺ calcd for (C₁₉H₁₈N₅)⁺ 316.1562, found 316.1567.

N-(4-ethylphenyl) 3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (10c):

Yellow solid, yield: 75%, mp: 187–189 °C (EtOH); IR (KBr, cm⁻¹) ν : 3450 (NH). ¹H NMR (CDCl₃, 300 MHz): δ (ppm) = 1.27 (t, 3H, $J = 6.9$ Hz, Ph-CH₂-CH₃), 2.60 (s, 3H, CH₃), 2.67 (q, 2H, $J = 7.0$ Hz, Ph-CH₂-CH₃), 6.82–8.01 (m, 10H, NH + H-arom), 8.10 (s, 1H, H-6). ¹³C NMR (CDCl₃, 75 MHz): δ (ppm) = 15.5 (Ph-CH₂-CH₃), 16.0 (CH₃), 21.7 (Ph-CH₂-CH₃), 103.2 (C-3a), 116.1, 121.2, 126.2, 127.1, 128.8, 129.5, 129.5, 138.1, 139.7 (C-arom), 142.5 (C-3), 151.0 (C-7a), 154.3 (C-4), 156.6 (C-6). HRMS [M + H]⁺ calcd for (C₂₀H₂₀N₅)⁺ 330.1718, found 330.1720.

3-Methyl-N-(naphthalen-1-yl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (10d):

Yellow solid, yield: 75%, mp: 190–192 °C (EtOH); IR (KBr, cm⁻¹) ν : 3430 (NH). ¹H NMR (CDCl₃, 300 MHz): δ (ppm) = 2.79 (s, 3H, CH₃), 6.82–8.04 (m, 12H, H-arom + NH + H-6). ¹³C NMR (CDCl₃, 75 MHz): δ (ppm) = 13.5 (CH₃), 104.2 (C-3a), 110.1, 117.9, 120.1, 120.8, 123.3, 124.1, 125.1, 126.1, 126.8, 127.8,

127.3, 129.5, 132.1, 138.6, 140.2 (C-arom), 143.8 (C-3), 150.2 (C-7a), 153.7 (C-4), 157.0 (C-6). HRMS $[M + H]^+$ calcd for $(C_{22}H_{18}N_5)^+$ 352.2219, found 352.2221.

General procedure for the synthesis of 4-imino-3-methyl-1-phenyl-1*H*,4*H* pyrazolo[3,4-*d*]pyrimidin-5-ol 11

A mixture of **7** (10 mmol) and hydroxylamine hydrochloride (10 mmol) in ethanol (20 mL) containing triethylamine (5 mL) was refluxed for 5 h. The reaction mixture was then cooled and poured into cold water. The formed precipitate was filtered off, washed with water, dried, and recrystallized from ethanol.

4-Imino-3-methyl-1-phenyl-1*H*,4*H*-pyrazolo[3,4-*d*]pyrimidin-5-ol (11):

Yellow solid, yield: 88%, mp: 215–217 °C (EtOH); IR (KBr, cm^{-1}): 3400 (NH), 3500 (OH). 1H NMR (DMSO- d_6 , 300 MHz): δ (ppm) = 2.78 (s, 3H, CH_3), 4.08 (s, 1H, NH), 7.48–8.18(m, 5H, H-arom), 8.92 (s, 1H, H-6), 9.00 (s, 1H, OH). ^{13}C NMR (DMSO- d_6 , 75 MHz): δ (ppm) = 14.5 (CH_3), 103.1 (C-3a), 121.2, 126.5, 129.6, 139.6 (C-arom), 143.6 (C-7a), 146.9 (C-3), 147.4 (C-6), 159.5 (C-4). HRMS $[M + H]^+$ calcd for $(C_{12}H_{12}N_5O)^+$ 242.1042, found 242.1046.

5. Antibacterial activity

5.1. Microorganisms

The antibacterial activity was tested against 5 microorganisms, including reference strains consisting of gram-negative rods: *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853); gram-positive cocci: *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212); and clinical strains: *Acinetobacter* sp. The bacterial strains were cultured overnight at 37 °C in Mueller–Hinton agar.

5.2. Micro-well dilution assay

The MIC was defined as the lowest concentration able to inhibit any visible bacterial growth. MIC values were determined by a microtiter plate dilution method dissolving the sample in 10% DMSO solution. Sterile 10% DMSO solution (100 μ L) was pipetted into all wells of the microtiter plate before transferring 100 μ L of stock solution to the microplate. Serial dilutions were made to obtain concentrations ranging from 10 to 0.0775 mg/mL. Finally, 50 μ L of 10^6 colony forming units (cfu/mL) (according to McFarland turbidity standards) of standard microorganism suspensions were inoculated onto microplates and incubated at 37 °C for 24 h. At the end of the incubation period, the plates were evaluated for the presence or absence of growth. MIC values were determined as the lowest concentration of the sample at which the absence of growth was recorded. All the samples were screened 3 times against each microorganism. Ampicillin was used as antibacterial positive. The final concentration of DMSO in the well had no effect on bacterial growth.

References

1. Stefani, H. A.; Pereira, C. M. P.; Almeida, R. B.; Braga, R. C.; Guzen, K. P.; Cella, R. *Tetrahedron Lett.* **2005**, *46*, 6833–6837.
2. Kamal El-Dean, A. M.; Geies, A. A. *J. Chem. Res. (S)* **1997**, *10*, 352–353, (*M*), 2255–2269.
3. Keche, A. P.; Hatnapure, G. D.; Tale, R. H.; Rodge, A. H.; Kamble, V. M. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6611–6615.
4. Desai, N. C.; Joshi, V. V.; Rajpara, K. M.; Vaghani, H. V.; Satodiya, H. M. *J. Fluorine Chem.* **2012**, *142*, 67–78.

5. Youssef, A. M.; Neeland, E. G.; Villanueva, E. B. White, M. S.; El-Ashmawy, I. M.; Patrick, B.; Klegeris, A.; Abd-El-Aziz, A. S. *Bioorg. Med. Chem.* **2010**, *18*, 5685–5696.
6. Ramadan, A. M.; Eltaib, A. A.; Kamal, U. S. *Tetrahedron* **2012**, *68*, 1637–1667.
7. Elkhawaga, A. M.; Kamal El-Dean, A. M.; Radwan, S. M.; Ahmed, M. M. *Bull. Korean Chem. Soc.* **2009**, *30*, 561–566.
8. Dreassi, E.; Zizzari, A. T.; Mori, M.; Filippi, I.; Belfiore, A.; Naldini, A.; Carraro, F.; Santucci, A.; Schenone, S.; Botta, M. *Eur. J. Med. Chem.* **2010**, *45*, 5958–5964.
9. El-Enany, M. M.; Kamel, M. M.; Khalil, O. M.; El-Nassan, H. B. *Eur. J. Med. Chem.* **2010**, *45*, 5286–5291.
10. Holla, B. S.; Mahalinga, M.; Karthikeyan, M. S.; Akberalib, P. M.; Shetty, N. S. *Bioorg. Med. Chem.* **2006**, *14*, 2040–2047.
11. Dawood, K. M.; Farag, A. M.; Khedr, N. A. *ARKIVOC*. **2008**, *15*, 166–175.
12. Jiang, M. X.; Warshakoon, N. C.; Miller, M. J. *J. Org. Chem.* **2005**, *70*, 2824–2826.
13. Kim, D. C.; Lee, Y. R.; Yang, B. S.; Shin, K. J.; Kim, D. J.; Chung, B. Y.; Yoo, K. H. *Eur. J. Med. Chem.* **2003**, *38*, 525–532.
14. Majid, M. H.; Ghadir, R.; Fatemeh, F. B.; Nasim, S. *J. Mol. Catal. A-Chem.* **2006**, *256*, 238–241.
15. He, H. Y.; Zhao, J. N.; Jia, R.; Zhao, Y. L.; Yang, S. Y.; Yu, L. T.; Yang, L. *Molecules* **2011**, *16*, 10685–10649.
16. Oliveira-Campos, A. M. F.; Salaheldin, A. M.; Rodrigues, L. M. *ARKIVOC*. **2007**, *16*, 92–100.
17. Rashad, A. E.; Shamroukh, A. H.; Hegab, M. I.; Awad, H. M. *Acta Chim. Slov.* **2005**, *52*, 429–434.
18. Israr, A.; Anil, K.; Bhupender, S. C.; Rakesh, T.; Deendayal, M.; Keykavous, P. *Bioorg. Med. Chem.* **2011**, *21*, 1342–1346.
19. Khulud, M. A.; Hassan, M. A. A.; Shar, S. A. *Molecules*. **2010**, *15*, 3932–3957.
20. Baraldi, P. G.; El-Kashef, H.; Farghaly, A. R.; Vanellec, P.; Fruttarolo, F. *Tetrahedron* **2004**, *60*, 5093–5104.
21. Colomer, J. P.; Moyano, E. L. *Tetrahedron Lett.* **2011**, *52*, 1561–1565.
22. Trujillo-Ferrara, J.; Vázquez, I.; Espinosa, J.; Santillan, R.; Farfán, N.; Höpfl, H. *Eur. J. Pharmac. Sci.* **2003**, *18*, 313–322.
23. Nascimento-Júnior, N. M.; Mendes, T. C. F.; Leal, D. M.; Corrêa, C. M. N.; Sudo, R. T.; Zapata-Sudo, G.; Barreiro, E. J. Fraga, C. A. M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 74–77.
24. Eljazi, I. A.; Samar, A. A. *Molecules* **2001**, *6*, 621–638.
25. Biagi, G.; Costantini, A.; Costantino, L.; Giorgi, I.; Livi, O.; Pecorari, P.; Rinaldi, M.; Scartoni, V. *J. Med. Chem.* **1996**, *39*, 2529–2535.
26. Ducray, R.; Ballard, P. Barlaam, B. C.; Hickinson, M. D.; Kettle, J. G.; Ogilvie, D. J.; Trigwell, C. B. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 959–962.
27. Shawali, A. S.; Hassaneen, H. M.; Shurrab, N. Kh. *Tetrahedron* **2008**, *64*, 10339–10343.
28. Abunada, N. M.; Hassaneen, H. M.; Kandile, N. G.; Miqdad, O. A. *Molecules* **2008**, *13*, 1501–1517.
29. El-Sayed, T. *Eur. J. Med. Chem.* **2009**, *44*, 4385–4392.
30. Ghorab, M. M.; Ragab, F. A.; Alqasoumi, S. I.; Alafeefy, A. M.; Aboulmagd, S. A. *Eur. J. Med. Chem.* **2010**, *45*, 171–178.
31. Jabrane, A.; Ben Jannet, H.; Mastouri, M.; Mighri, Z., Casanova, J. *Nat. Prod. Res.* **2010**, *24*, 491–499.