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Mediterranean Journal of Chemistry **2014**, 2(6), 679-690

Synthesis of novel pyrazolo[3,4-*d*]pyrimidinone derivatives as cytotoxic inhibitors

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Abstract: Various α-fonctionalized iminoethers **2** were easily prepared from ethyl 5-amino-3-substituted-1-phenyl-1*H*-pyrazole-4-carboxylate **1**. The reaction of iminoethers **2** with ammonia afforded 3-substitued-1-phenyl-1*H*-pyrazolo[3,4-*d*] pyrimidin-4(5H)-ones **3** which were also synthesized by the addition of formamide to ethyl 5-amino-3-substituted-1-phenyl-1*H*-pyrazole-4-carboxylate **1.** The 5-amino-3-substitued-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5H)-ones **4** were obtained from hydrazonolysis of iminoethers **2**. Otherwise, the condensation of these intermediates **2** with a series of some primary amines and hydroxylamine led respectively, to the corresponding 3,5-disubstitued-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5H)-ones **5** and the 3-substitued-5-hydroxy-1-phenyl-1*H*-pyrazolo[3,4-*d*] pyrimidin-4-(5H)-ones **6**. The synthesized compounds **1-6** were completely characterized by ^{1}H NMR, ^{13}C NMR, IR and HRMS. Some synthesized compounds were evaluated for their cytotoxic effect using the Human cervical adenocarcinoma Hela cell line.

Keywords: aminopyrazoles; condensation reaction; α-functionalized iminoethers; pyrazolo[3,4-*d*]pyrimidinones; cytotoxic activity.

Introduction

Heterocyclic chemistry constitutes an essential branch of organic chemistry and widely known to display an array of biological properties[1]. Indeed simple nitrogen-containing heterocyclic receives a large amount of attention in the literature, as a consequence of its exciting biological properties and its role as pharmacophores of considerable historical importance[2].

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DOI: http://dx.doi.org/

Pyrazoles represent key structural motifs in heterocyclic chemistry and occupy a significant position in medicinal and pesticide chemistry because of their capability to exhibit a wide range of bioactivities, including their antimicrobial activity[3,4]. Moreover, this heterocyclic moiety constitutes the structure of numerous drugs, including Celebrex, Viagra and Zaleplon[5,6]. Hence, the synthesis and study of pyrazolo-fused compounds took a lot of interest due to their wide variety of biological and pharmacological properties[7]. Our literature survey showed that the chemistry of fused pyrazolo[3,4-*d*]pyrimidine derivatives has also drawn great attention due to its pharmacological importance and also its structural resemblance to purines[8]. Several substituted pyrazolo[3,4-*d*]pyrimidine derivatives demonstrated significant antifungal[9], antitumoral activity[10].

Different mechanisms accounted for the cytotoxic effects of this class of compounds; in fact, they have been reported to act as cycline-dependant kinase inhibitors[11], tyrosine kinase inhibitors[12] and potent xanthine oxidase inhibitors[13]. Keeping this in mind, it was aimed in this report to synthesize a ethyl 5-amino-3-substitued-1-phenyl-1*H*-pyrazole-4-carboxylate **1** and to use it as building blocks in the synthesis of novel fused pyrazolo[3,4-*d*]pyrimidinones **3-6**.

Finally, the cytotoxicity activities of some of them were evaluated using the Human cervical adenocarcinoma Hela cell line.

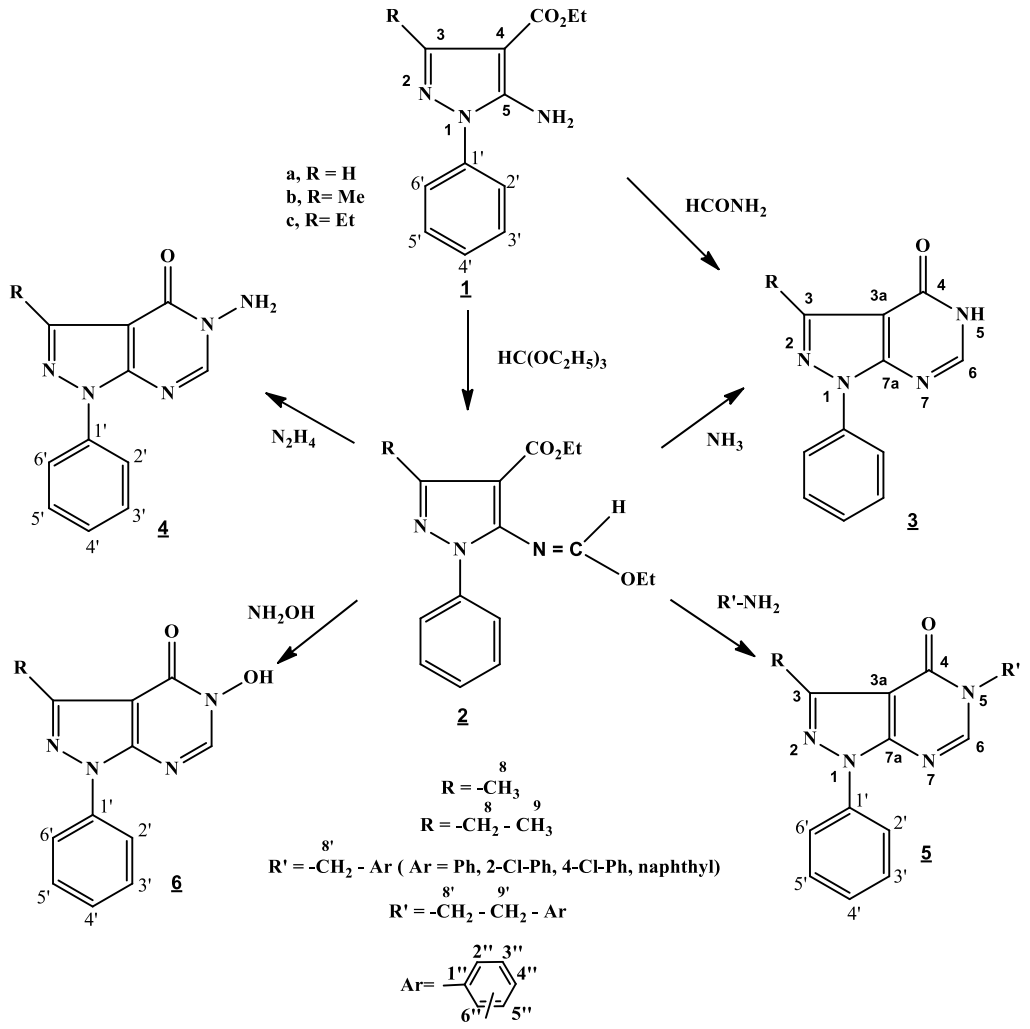
Results and Discussion

According to the previously reported method[14] we have synthesized the starting material, Ethyl 5-amino-3-substituted-1-phenyl-1*H*-pyrazole-4-carboxylates **1a-c**. α-Functionalized iminoethers **2a-c** were prepared in excellent yield by treating **1a-c** with triethylorthoformate in acetic anhydride at refluxing temperature. The structure of **2a-c** was assigned by the absence of ν_{N-H} in IR and the presence of a triplet at δ 1.30 ppm and a quartet at 3.36 ppm corresponding to protons of the ethoxy group belonging to the iminoether moiety and a singlet around δ 8.01 ppm due to N=CH in the ^{1}H NMR spectrum, along with the other expected signals.

The reaction of ethyl 5-amino-3-substituted-1-phenyl-1*H*-pyrazole-4-carboxylates **1a-c** with formamide[15,16] afforded 3-substitued-1-phenyl-1*H*-pyrazolo[3,4-*d*] pyrimidin-4(5H)-ones **3a-c** which was already synthesized by the addition of cold aqueous alcoholic ammonia to α-functionalized iminoethers **2** (Scheme 1). The structure of compound **3b** as an example was confirmed according to its spectral data, where its IR spectrum showed vibration bands at (υ, cm^{-1}): 3200 (NH), 1674 (C=O) and ^{1}H NMR spectrum showed signals at δ 8.40 and 12.38 ppm for H-6 and NH, respectively. The ^{13}C NMR spectrum revealed that the signal of the C-4 (C=O) appeared at δ 157.4 ppm.

5-amino-3-substitued-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-4(5H)-ones (**4a-c**) was also prepared by the reaction of iminoethers **2a-c** with hydrazine hydrate in ethanol at 40 °C for one hour[17] (Scheme1). Mechanistically, the reaction begins with a first nucleophilic attack of the NH_2 group on the imidic carbon, then an intramolecular cyclization via a second nucleophilic attack on the carbon of the ester function to generate **4a-c**.

The structural assignments of these products were based on the spectral data. Their IR spectra showed large bands between 3200 and 3400 cm^{-1}, assignable to the NH_2 stretching vibration and a characteristic band near υ = 1670 cm^{-1}corresponding to a C=O linkage. Their ^{1}H NMR spectra showed a characteristic signal near δ 5.10 ppm, assignable to the NH_2 protons.



Scheme 1. Synthetic pathway for the formation of compounds **2-6**.

The reaction of iminoethers **2a-c** with an excess of the suitable primary amine[18], under refluxing toluene afforded the 3,5-disubstitued-1-phenyl-1*H*-pyrazolo[3,4-*d*] pyrimidin-4(5H)-one **5** (Scheme 1), and the corresponding products were obtained in good to excellent yields (Table1). The structures of these compounds were confirmed according to their spectral data, where the IR spectra of compounds **5** indicated the existence of characteristic absorption bands at 1574-1587 and at 1670-1677 cm^{-1} assignable to –C=N and carbonyl groups, respectively. In addition, ^{1}HNMR spectra of these compounds showed the disappearance of signals related to the two ethoxy groups and the observation of signals introduced by amine and the appearance of a new singlet at 7.84-8.85 ppm characteristic of the iminic proton H-6.

The formation of these products was also confirmed by ^{13}C NMR data with the observation of signals of carbons introduced by amine and the signal of the C=O group appeared at δ 1670-1677 ppm. Furthermore, the mass spectra (ES+) of compounds **5** showed pseudo-molecular ion peaks which are in good agreement with the assigned structures.

3-Substitued-5-hydroxy-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-(5H)-one **6a-c** were prepared by condensation of the iminoethers **2a-c** with an equimolar amount of hydroxylamine hydrochloride in ethanol in the presence of triethylamine. Formation of the

products was established by the presence of vibration bands corresponding to the OH and C=O functions near 3400 and 1670 cm^{-1}, respectively.

Table 1: Preparation of 3,5-disubstitued-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one **5**

Product	R	R'	Yield %
5a	H	$-CH_2$-Ph	75
5b	H	$-CH_2-CH_2$-Ph	80
5c	H	(2Cl)-CH_2-Ph	65
5d	H	(4Cl)-CH_2-Ph	70
5e	H	Naphthyl	80
5f	CH_3	$-CH_2$-Ph	54
5g	CH_3	$-CH_2-CH_2$-Ph	56
5h	CH_3	(2Cl)-CH_2-Ph	60
5i	CH_3	(4Cl)-CH_2-Ph	70
5j	CH_3	Naphthyl	85
5k	C_2H_5	$-CH_2$-Ph	58
5l	C_2H_5	$-CH_2-CH_2$-Ph	56
5m	C_2H_5	(2Cl)-CH_2-Ph	58
5n	C_2H_5	(4Cl)-CH_2-Ph	58
5o	C_2H_5	Naphthyl	65

The ^{1}H NMR spectrum of compound **6a** as an example exhibited a singlets at δ 8.74 ppm, assigned to the proton of the pyrimidinone moiety, the signal of OH group resonates in the 7.36-8.39 ppm region. The mass spectra of all prepared compounds were compatible with the proposed structures.

Cytotoxic activity

The compounds **3a, 3b**, **3c, 4a, 4b, 4c, 5b, 5c, 5f** and **5j**, were tested for their cytotoxic activity using the Human cervical adenocarcinoma Hela cell line. The results are presented in Table 2. The results showed that the compound **3b** having a methyl group in C-3 is definitely more active (IC_{50} = 13.10^{-2} µmol/mL) than **3c** having an ethyl group at the same position (IC_{50} = 87.10^{-2} µmol/mL). These results compared to that of **3a** with a tertiary carbon C-3 (IC_{50} = 1 µmol/mL) showed that alkylation in this position improved the activity of the compound. The same observation was noted with series **4**. The activity decreased in the same direction as the effect of the groups (CH_3, C_2H_5, H) fixed at C-3 of the pyrazole system. It was noted that the introduction of the NH_2 group at N-5 of the pyrimidinone moiety decreased the cytotoxic activity compared to that of compounds **3** (Table 2).

The presence of the naphtyl group at N-5 in the compound **5j** improved its activity (IC_{50} = 66.10^{-2} µmol/ mL) by comparison with that of its analog **5f** carrying at the same position a benzyl group (IC_{50} =1.5 µmol/mL). The presence of chlorine atom in ortho position of the benzyl group practically does not involve a considerable effect on the activity of **5c** (IC_{50} = 160 µmol/mL) by comparison with that of its analogue **5f**.

To conclude, we notice that the presence of the CH_3 group at C-3 of the pyrazole system and the non-substitution of the nitrogen atom N-5 of the pyrimidinone moiety could be the origin of the cytotoxic activity of the tested pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-ones. The presence of the pyrazole and the pyrimidine moieties in these compounds may contribute to

the inhibition of the tumour HeLa cells. Our results are accompanied by the potent inhibitory of the growth of several human tumour cell lines by some pyrazolo[3,4-*d*]pyrimidines, structurally related with allopurinol a well-known xanthine oxidase inhibitor, clinically used in the therapy of gout[19].

Table 2: Cytotoxic activity of certain pyrazolo[3,4-*d*]pyrimidine-4(5*H*)-ones.

Product	R	R'	IC_{50} (µmol/mL)[a]
3a	H	H	1.0 ± 0.021
3b	CH_3	H	0.13 ± 0.011
3c	C_2H_5	H	0.87 ± 0.012
4a	H	NH_2	1.40 ± 0.012
4b	CH_3	NH_2	0.82 ± 0.011
4c	C_2H_5	NH_2	3.70 ± 0.021
5b	H	CH_2-CH_2-Ph	1.50 ± 0.021
5c	H	(2Cl)-CH_2-Ph	1.60 ± 0.012
5f	CH_3	-CH_2-Ph	1.50 ± 0.021
5j	CH_3	Naphthyl	0.66 ± 0.011

[a]Results are expressed as means ± SEM of 3 independent observations performed in triplicate. Doxorubicin was used as positive control (IC_{50} = 0.0015 ± 0.0001 µmol/mL).

Conclusion

In this paper, we report the synthesis of pyrazolo[3,4-*d*]pyrimidinone derivatives and the cytotoxic evaluation of some of the novel compounds. Most of the tested compounds showed cytotoxic activity, especially compound **3b** which displayed the highest activity with IC_{50} equal to 13.10^{-2} µmol/mL.

Experimental Section

All melting points were determined on a Kofler-type microscope and are uncorrected. IR spectra were recorded on a Perkin-Elmer Fourier transform FT–IR spectrophotometer (4000–400 cm^{-1}) using KBr pellets. ^1H NMR and ^{13}C NMR spectra were recorded at room temperature (rt) in $CDCl_3$, dimethylsulfoxide (DMSO-d_6) and CD_3OD at 300 MHz and at 75MHz, respectively, using residual non deuterated solvent peaks as internal reference coupling constant are given in Hz. HRMS spectra were acquired with an electrospray-time-of-flight (ESI-TOF, LCT Premier XE, Waters) mass spectrometer in the positive ion mode.

General procedure for ethyl 5-amino-3-substitued-1-phenyl-1*H*-pyrazole-4-carboxylates 1a-c.

To a cold solution of phenylhydrazine (2.0 g, 10 mmol) in ethanol (50 mL), the appropriate *α*-cyanocinnamonitriles (10 mmol) were added. The reaction mixture was then stirred at room temperature for 6h. After cooling, the resulting precipitate was refluxed in ethanol in the presence of a catalytic amount of acetic acid afforded pyrazoles **1a-c**. The solid product that formed was filtered, washed with cold ethanol, dried and recrystallized from a suitable solvent.

Ethyl 5-amino-1-phenyl-1H-pyrazole-4-carboxylate 1a.

Yellow crystals; Yield: 75%; m.p.: 127-129 °C; IR (KBr, cm^{-1}) ν: 1670 (C=O); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.36 (t, ^3J=7.1 Hz, 3H, O-CH_2-C$\underline{H}_3$), 4.39 (q, ^3J=7.1Hz, 2H,O-C$\underline{H}_2$-CH_3), 6.51 (s, 2H, NH_2), 7.28-8.04 (m, 5H, Ar-H), 8.79 (s, 1H, H-3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2 (O-CH_2-$\underline{C}H_3$), 60.5 (O-$\underline{C}H_2$-CH_3), 108.1(C-4), 121.1 (C-2',6'), 126.1 (C-4')129.7 (C-3',5'), 139.4 (C-1'), 141.7 (C-3), 151.5 (C-5), 157.3 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{12}H_{14}N_3O_2)^+$ 232.1001 found 232. 1011.

Ethyl5-amino-3-methyl-1-phenyl-1H-pyrazole-4-carboxylate 1b.

Yellow crystals; Yield: 80%; m.p.: 126-128 °C; IR (KBr, cm^{-1}) ν: 1670 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.34 (t, ^3J=7.1 Hz, 3H, O-CH_2-C$\underline{H}_3$), 2.57 (s, 3H, CH_3), 4.37 (q, ^3J= 7.1 Hz, 2H, O-C$\underline{H}_2$-CH_3), 6.50 (s, 2H, NH_2), 7.26-8.13 (m, 5H, Ar-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.1 (CH_3), 15.1 (O-CH_2-$\underline{C}H_3$), 60.5 (O-$\underline{C}H_2$-CH_3), 109.1 (C-4), 121.1 (C-2',6'), 126.1 (C-4'), 129.7 (C-3',5'), 139.4 (C-1'), 141.9 (C-3), 152.2 (C-5), 157.2 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{13}H_{16}N_3O_2)^+$ 246.1160 found 246.1172.

Ethyl 5-amino-3-ethyl-1-phenyl-1H-pyrazole-4-carboxylate 1c.

Yellow crystals; Yield 70%; m.p.: 127-129 °C; IR (KBr, cm^{-1}) ν: 1670 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.25 (*t*, *J*=7.1 Hz, 3H, -CH_2-C$\underline{H}_3$), 1.34 (t, ^3J=7.1 Hz, 3H, O-CH_2-C$\underline{H}_3$), 2.49 (q, ^3J= 7.2 Hz, 2H, -C$\underline{H}_2$-CH_3), 3.89 (q, ^3J= 7.2 Hz, 2H, O-C$\underline{H}_2$-CH_3), 6.52 (s, 2H, NH_2), 7.26-8.13 (m, 5H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1 (O-CH_2-$\underline{C}H_3$), 14.2 (-CH_2-$\underline{C}H_3$), 24.1 (-$\underline{C}H_2$-CH_3), 60.6 (O-$\underline{C}H_2$-CH_3), 107.9 (C-4), 120.1 (C-2', C-6'), 126.3 (C-4'), 129.7 (C-3', C-5'), 139.4 (C-1'), 141.7 (C-3), 152.1 (C-5), 157.1 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{14}H_{18}N_3O_2)^+$ 260.1307, found 260.1317.

General procedure for ethyl 5-(ethoxymethyleneamino)-3-substitued-1-phenyl-1*H*-pyrazole-4-carboxylate 2a-c.

The mixture of **1**(a-c) (0.01 mmol) and triethylorthoformate (0.01 mmol) in acetic anhydride (30 mL) was refluxed for 3h. The solvent was then removed under reduced pressure. The remaining solid was recrystallized from ethanol to give compounds **2a-c**.

Ethyl 5-(ethoxymethyleneamino)-1-phenyl-1H-pyrazole-4-carboxylate 2a.

Yellow crystals; Yield: 70%; m.p.: 120-122 °C; IR (KBr, cm^{-1}) ν: 1670 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.30 (t, ^3J=7.2 Hz, 3H, O-$CH_2C\underline{H}_3$), 1.36 (t, ^3J=7.1 Hz, 3H, O-CH_2-C$\underline{H}_3$, ester), 4.30 (q, ^3J=7.2 Hz, 2H, O-C$\underline{H}_2CH_3$), 4.39 (q, ^3J= 7.1Hz, 2H, O-C$\underline{H}_2$-CH_3, ester), 7.28-8.04 (m, 5H, Ar-H), 8.06 (s, 1H, H-3), 8.29 (s, 1H, N=CH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.2 (O-CH_2-$\underline{C}H_3$, ester), 14.9 (O-$CH_2\underline{C}H_3$), 60.5(O-$\underline{C}H_2$-CH_3, ester), 61.0 (O-CH_2CH_3), 106.1 (C-4), 121.2 (C-2', C-6'), 126.3 (C-4'), 129.7 (C-3', C-5'), 139.4 (C-1'), 141.9 (C-3), 146.1 (C-5), 151.5 (N=C), 157.3 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{15}H_{18}N_3O_3)^+$ 288. 1300, found 288. 1307.

Ethyl 5-(ethoxymethyleneamino)-3-methyl-1-phenyl-1H-pyrazole-4-carboxylate 2b.

Yellow crystals; Yield: 70%; m.p.: 118-120 °C; IR (KBr, cm^{-1}) ν: 1670 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.20 (t, ^3J=7.1 Hz, 3H, O-$CH_2C\underline{H}_3$) 1.31 (t, ^3J=6.9 Hz, 3H,O-$CH_2C\underline{H}_3$, ester), 2.51(s, 3H, CH_3), 4.30 (q, ^3J=7.0 Hz, 2H, O-C$\underline{H}_2CH_3$), 4.39 (q, ^3J=7.2 Hz, 2H,O-C$\underline{H}_2CH_3$, ester), 7.21-8.01 (m, 5H, Ar-H), 8.30 (s, 1H, N=CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2 (O-CH_2-$\underline{C}H_3$, ester), 14.9 (CH_3), 15.1 (O-$CH_2\underline{C}H_3$), 60.8 (O-$\underline{C}H_2$-CH_3, ester), 61.0 (O-$\underline{C}H_2CH_3$), 109.7 (C-4), 121.2 (C-2', C-6'), 126.3 (C-4'), 129.7 (C-3', C-5'), 139.4 (C-1'), 141.8 (C-3), 147.1 (C-5), 151.5 (N=C), 158.2 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{16}H_{20}N_3O_3)^+$ 302.1458, found 302.1464.

***Ethyl 5-(ethoxymethyleneamino)-3-ethyl-1-phenyl-1H-pyrazole-4-carboxylate 2c*.**
Yellow crystals; Yield: 70%; m.p.: 110-112 °C; IR (KBr, cm^{-1}) ν: 1670 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.19 (t, ^3J=7.0, 3H, O-CH_2-C<u>H</u>$_3$), 1.27 (t, ^3J=7.0 Hz, 3H, CH_2C<u>H</u>$_3$), 1.30 (t, ^3J=7.1 Hz, 3H, O-CH_2C<u>H</u>$_3$, ester), 2.61(q, ^3J=7.0, 2H, C<u>H</u>$_2$$CH_3$), 3.55 (q, ^3J=7.1 Hz, 2H, C<u>H</u>$_2$$CH_3$), 4.39 (q, ^3J=6.90 Hz, 2H, O-C<u>H</u>$_2$$CH_3$, ester), 7.24-8.11 (m, 5H, Ar-H), 8.33 (s, 1H, N=CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.0 (O-CH_2-<u>C</u>H_3, ester), 14.2 (CH_2<u>C</u>H_3), 15.1 (O-CH_2<u>C</u>H_3), 23.3 (<u>C</u>H_2CH_3), 60.9 (O-<u>C</u>H_2-CH_3, ester), 61.0 (O-<u>C</u>H_2CH_3), 110.1 (C-4), 121.2 (C-2', C-6'), 126.3 (C-4'), 129.7 (C-3', C-5'), 139.7 (C-1'), 142.9 (C-3), 148.8 (C-5), 151.3 (N=C), 158.1 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{17}H_{22}N_3O_3)^+$ 316.1614, found 316.1622.

General procedure for the synthesis of 3-substitued-1-phenyl-1H-pyrazolo[3,4-*d*] pyrimidin-4(5*H*)ones 3a-c.

Method (a): A mixture of **1**(a-c) (2 mmol) and formamide (20 mL) was heated under reflux for 1.5 h, then left to cool to temperature overnight. The solid was filtered, washed with water, dried and recristallized from methanol, in yields (85%).
Method (b)[20]: Imidate**2** (a-c) (3 mmol) were added to methanol (20 mL) saturated with ammonia at 0°C for 1h, warmed to room temperature and the reaction mixture stirred for 6h. The solid which precipitated was collected and recrystallized from an appropriate solvent.

***1-phenyl-1H-pyrazolo[3,4-d] pyrimidin-4(5H)-one 3a*.**
Creamy white crystals; Yield: 75%; m.p.: 264-266 °C; IR (KBr, cm^{-1}) ν: 3240 (NH), 1672 (C=O), 1587 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.31-8.03 (m, 5H, Ar-H), 8.21 (s, 1H, H-3), 8.30 (s, 1H, H-6), 12.6 (s, 1H, -NH-). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 108.1 (C-3a), 122.1 (C-2', C-6'), 127.3 (C-4'), 129.1 (C-3', C-5'), 136.1 (C-1'), 148.3 (C-7a), 149.1 (C-3), 152.1 (C-6), 157.7 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{11}H_9N_4O)^+$ 213.0771, found 213.0776.
***3-Methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 3b*.**
Creamy white crystals; Yield: 85%; m.p.: 273-275 °C; IR (KBr, cm^{-1}) ν: 3247 (NH), 1670 (C=O), 1577 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.50 (s, 3H, CH_3), 7.31-8.01 (m, 5H, Ar-H), 8.10 (s, 1H, H-6), 12.40 (s, 1H, -NH-). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.1 (CH_3), 105.1 (C-3a), 121.1 (C-2', C-6'), 126.7 (C-4'), 129.5 (C-3', C-5'), 136.5 (C-1'), 148.4 (C-7a), 149.2 (C-3), 152.2 (C-6), 157.7 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{12}H_{11}N_4O)^+$ 227.0929, found 227.0933.
3-Ethyl-1-phenyl-1H-pyrazolo[3,4-d] pyrimidin-4(5H)-one 3c.
Creamy white crystals; Yield: 85%; m.p.: 284-286 °C; IR (KBr, cm^{-1}) ν: 3245 (NH), 1671 (C=O), 1575 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.30 (t, ^3J =7.2 Hz, 3H, -CH_2-C<u>H</u>$_3$), 3.06 (q, ^3J =7.5 Hz, 2H, -C<u>H</u>$_2$-CH_3), 7.31–8.06 (m, 5H, Ar-H), 8.11 (s, 1H, H-6), 12.39 (s, 1H, -NH-). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.1 (-CH_2-<u>C</u>H_3), 22.0,(-<u>C</u>H_2-CH_3), 105.1 (C-3a), 120.6 (C-2', C-6'), 126.1 (C-4'), 129.3 (C-3', C-5'), 137.7 (C-1'), 149.5 (C-7a), 150.2 (C-3), 152.5 (C-6), 157.8 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{13}H_{13}N_4O)^+$ 241.1085, found 241.1089.

General procedure for the synthesis of 5-amino-3-substitued-1-phenyl-1*H*-pyrazolo[3,4-*d*] pyrimidin-4(5*H*)-ones 4a-c.

A mixture of **2a-c** (3 mmol), hydrazine hydrate (5 mL) and acetic acid (2 mL) in ethanol (20 mL) was heated under reflux for 1h. The precipitate was collected and recrystallized from the proper solvent to afford **4a-c** as colorless needles.

5-Amino-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 4a.

White crystals; Yield: 75%; m.p.: 236-238 °C; IR (KBr, cm^{-1}) ν: 3300-3205 (NH_2), 1674 (C=O), 1590 (C=N). ^{1}H NMR (DMSO-d_6,300 MHz): δ 5.78 (s, 2H, NH_2), 7.38–8.05 (m, 5H, Ar-H), 8.40 (s, 1H, H-3), 8.51 (s, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6): δ 106.5 (C-3a), 121.3 (C-2', C-6'), 127.1 (C-4'), 129.2 (C-3', C-5'), 136.9 (C-1'), 148.0 (C-7a), 150.8 (C-3), 151.4 (C-6), 156.5 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{11}H_{10}N_5O)^+$ 228.0874, found 228.0885.

5-Amino-3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 4b.

White crystals; Yield: 80%; m.p.: 234-237 °C; IR (KBr, cm^{-1}) ν: 3350-3290 (NH_2), 1680 (C=O), 1593 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.51 (s, 3H, CH_3), 5.76 (s, 2H, NH_2), 7.30–8.00 (m, 5H, Ar-H), 8.42 (s, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6): δ 15.3 (CH_3), 105.1 (C-3a), 121.4 (C-2', C-6'), 127.1 (C-4'), 129.2 (C-3', C-5'), 136.7 (C-1'), 148.1 (C-7a), 150.8 (C-3), 151.6 (C-6), 157.6 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{12}H_{12}N_5O)^+$ 242.1038, found 242.1042.

5-Amino-3-ethyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 4c.

White crystals; Yield: 70%; m.p.: 236 -238 °C; IR (KBr, cm^{-1}) ν: 3400-3360 (NH_2), 1710 (C=O), 1590 (C=N). ^{1}H NMR (DMSO-d_6,300 MHz): δ 1.30 (t, ^3J =7.2 Hz, 3H, -CH_2-C$\underline{H}_3$), 3.01 (q, ^3J=7.4 Hz, 2H, -C$\underline{H}_2$-CH_3), 5.06 (s, 2H, NH_2), 7.04–8.24 (m, 5H, Ar-H), 8.48 (s, 1H, CH_{-6}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 15.3 (-CH_2-$\underline{C}H_3$), 23.1 (-$\underline{C}H_2$-CH_3), 106.1 (C-3a), 120.9 (C-2', C-6'), 127.0 (C-4'), 129.4 (C-3', C-5'), 136.7 (C-1'), 148.1 (C-7a), 150.7 (C-3), 151.7 (C-6), 157.9 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{13}H_{14}N_5O)^+$ 256.1198, found 256.1204.

General procedure for the synthesis of 3,5-disubstitued-1-phenyl-1*H*-pyrazolo[3,4-*d*] pyrimidin-4(5*H*)-ones 5a-o.

The appropriate primary amine (0.001 mol) was added to the suitable imidate **2**(a-c) (0.001 mol), and the mixture was stirred at reflux of toluene (20 mL) for 6h. After cooling, the precipitated solid was filtered, washed with cold ether, and dried to obtain compounds **5**.

5-Benzyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 5a.

White crystals; m.p.: 170-172 °C; IR (KBr, cm^{-1}) ν: 1674 (C=O), 1574 (C=N). ^{1}H NMR (DMSO-d_6,300 MHz): δ 5.18 (s, 2H, H-8'), 7.25–8.01 (m, 11H, H-3 + Ar-H), 8.66 (s, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6): δ 44.6 (C-8'), 106.8 (C-3a), 121.4 (C-2', C-6'), 126.5 (C-4'), 127.1 (C-4''), 128.5 (C-2'', C-6''), 128.8 (C-3'', C-5''), 129.2 (C-3', C-5'), 136.0 (C-1''), 137.7(C-1'), 148.0 (C-7a), 151.1 (C-3), 151.2 (C-6), 156.2 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{18}H_{15}N_4O)^+$ 303.3105, found 303.3108

5-Phenylethyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 5b.

White crystals; m.p.: 176-178 °C; IR (KBr, cm^{-1}) ν: 1676 (C=O), 1575 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.06 (t, ^3J = 6.9 Hz, 2H, H-9'), 4.24 (t, ^3J=7.2 Hz, 2H, H-8'), 7.18-8.20 (m, 11H, H-3 + Ar-H), 8.62 (s, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.5 (C-9'), 46.8 (C-8'), 106.7 (C-3a), 121.4 (C-2', C-6'), 126.5 (C-4''), 127.1 (C-4'), 128.5 (C-2'', C-

6''), 128.5 (C-3'', C-5''), 128.8 (C-3', C-5'), 137.7 (C-1'), 139.6 (C-1''), 148.1 (C-7a), 151.0 (C-3), 151.1 (C-6), 157.4 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{19}H_{17}N_4O)^+$ 317.1402, found 317.1402.

***5-(2-Chlorobenzyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 5c*.**

White crystals; m.p.: 179-182 °C; IR (KBr, cm^{-1}) ν: 1677 (C=O), 1581 (C=N). ^1H NMR (DMSO-d_6,300 MHz): δ 5.31 (s, 2H, H-8'), 7.26–8.05 (m, 9H, Ar-H + H-6), 8.22 (s, 1H, H-3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 44.1 (C-8'), 105.4 (C-3a), 121.8 (C-2''), 126.6 (C-6''), 127.8 (C-2', C-6'), 127.9 (C-4''), 128.5 (C-4'), 129.6 (C-3''), 129.8 (C-3', C-5'), 132.2 (C-5''), 134.3 (C-1'),146.5 (C-1''), 148.5 (C-7a), 152.1 (C-3), 152.3 (C-6), 157.4 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{18}H_{14}N_4OCl)^+$ 337.0856, found 337.0861.

***5-(4-Chlorobenzyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 5d*.**

White crystals; m.p.: 180-182 °C; IR (KBr, cm^{-1}) ν: 1670 (C=O), 1578 (C=N). ^1H NMR (DMSO-d_6, 300 MHz): δ 5.10 (s, 2H, H-8'), 7.26–8.05 (m, 9H, Ar-H), 7.36 (s, 1H, H-3), 8.32 (s, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6): δ 47.13 (C-8'), 108.2 (C-3a), 121.2 (C-2', C-6'), 126.8 (C-4'), 128.6 (C-3'', C-5''), 128.8 (C-3', C-5'), 129.4 (C-2'', C-6''), 132.1 (C-4''), 139.2 (C-1''), 139.5 (C-1'), 146.5 (C-7a), 152.1 (C-3), 152.3 (C-6), 157.4 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{18}H_{14}N_4OCl)^+$ 337.0855, found 337.0861.

***5-(Naphtalen-1-yl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 5e*.**

Creamy white crystals; m.p.: 180-184 °C; IR (KBr, cm^{-1}) ν: 1672 (C=O), 1579 (C=N). ^1H NMR (DMSO-d_6, 300 MHz): δ 7.36–8.15 (m, 13H, H-3 + Ar-H), 8.49 (s, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6): δ 105.1 (C-3a), 121.5, 122.3, 125.7, 126.7, 126.8, 126.9, 127.5, 128.3, 129.2, 129.7, 129.7, 133.6, 133.7, 138.0 ($C_{\text{-arom}}$), 146.4 (C-7a), 151.5 (C-3), 151.6 (C-6), 157.3 (C=O). HRMS-ES $[M+H]^+$calcd for $(C_{21}H_{15}N_4O)^+$ 339.0199, found 339.1001.

***5-Benzyl-3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 5f*.**

White crystals; m.p.: 173-176 °C; IR (KBr, cm^{-1}) ν: 1674 (C=O), 1574 (C=N). ^1H NMR (DMSO-d_6, 300 MHz): δ 2.51 (s, 3H, H-8), 5.20 ppm (s, 2H, H-8'), 7.25–8.17 (m, 10H, Ar-H), 8.64 (s, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.7 (C-8), 43.6 (C-8'), 105.5 (C-3a), 121.8 (C-2', C-6'), 127.2 (C-4'), 127.9 (C-4''), 128.5 (C-2'', C-6''), 126.8 (C-3'', C-5''), 129.8 (C-3', C-5'), 132.2 (C-1''), 134.3 (C-1'), 146.5 (C-7a), 152.1 (C-3), 152.3 (C-6), 157.2 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{19}H_{17}N_4O)^+$ 317.1402, found 317.1406.

***3-Methyl-5-phenylethyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 5g*.**

White crystals; m.p.: 189-192 °C; IR (KBr, cm^{-1}) ν: 1676 (C=O), 1575 (C=N); ^1H NMR (CD_3OD, 300 MHz): δ 2.62 (s, 3H, H-8), 3.03 (t, ^3J =7.1 Hz, 2H, H-9'), 4.23 (t, ^3J=7.2 Hz, 2H, H-8') 7.21–7.84 (m, 11H, Ar-H + H-6). ^{13}C NMR (75 MHz, CD_3OD): δ 13.7 (C-8), 34.5 (C-9'), 46.8 (C-8'), 106.7 (C-3a), 121.4 (C-2', C-6'), 126.5 (C-4''), 127.1 (C-4'), 128.5 (C-2'', C-6''), 128.8 (C-3', C-5'), 129.2 (C-3'', C-5''), 136.1 (C-1'), 137.7 (C-1''), 146.1 (C-7a), 151.9 (C-3), 152.1 (C-6), 157.5 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{20}H_{19}N_4O)^+$ 331.1401, found 331.1406.

***5-(2-Chlorobenzyl)-3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 5h*.**

White crystals; m.p.: 188-192 °C; IR (KBr, cm^{-1}) ν: 1677 (C=O), 1581 (C=N). ^1H NMR (DMSO-d_6, 300 MHz): δ 2.49 (s, 3H, H-8), 5.24 (s, 2H, H-8'), 7.03–8.02 (m, 9H, Ar-H), 8.57 (s, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.7 (C-8), 44.1 (C-8'), 105.5 (C-3a), 121.8 (C-2''), 127.2 (C-6''), 127.9 (C-2', C-6'), 128.7 (C-4''), 128.8 (C-4'), 129.6 (C-3''), 129.9 (C-3', C-5'), 132.2 (C-5''), 134.3 (C-1'), 138.6 (C-1''), 146.9 (C-7a), 152.1 (C-3), 152.3 (C-6), 157.5 (C=O).HRMS-ES $[M+H]^+$ calcd for $(C_{19}H_{16}N_4OCl)^+$ 351.1006, found 351.1013.

5-(4-Chlorobenzyl)-3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 5i.

White crystals; m.p.: 190-192 °C; IR (KBr, cm^{-1}) ν: 1675 (C=O), 1587 [1] (C=N). ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 2.79 (s, 3H, H-8), 5.20 (s, 2H, H-8'), 7.13–8.02 (m, 9H, Ar-H), 8.55 (s, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-*d*$_6$): δ 13.6 (C-8), 47.1 (C-8'), 105.4 (C-3a), 120.6 (C-2', C-6'), 126.2 (C-4'), 128.6 (C-3'', C-5''), 128.7 (C-3', C-5'), 129.5 (C-2'', C-6''), 131.1 (C-4''), 137.3 (C-1''), 138.6 (C-1'), 146.8 (C-7a), 152.2 (C-3), 152.4 (C-6), 157.5 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{19}H_{16}N_4OCl)^+$ 351.1008, found 351.1013.

3-Methyl-5-(naphtalen-1-yl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 5j.

Creamy white crystals; m.p.: 195-196 °C; IR (KBr, cm^{-1}) ν: 1677 (C=O), 1577 (C=N). ^{1}H NMR (DMSO-*d*$_6$, 300 MHz): δ 2.54 (s, 3H, H-8), 7.36–8.15 (m, 12H, Ar-H), 8.48 (s, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-*d*$_6$): δ 14.1(C-8), 105.1 (C-3a), 121.5, 122.3, 125.6, 126.7, 126.8, 126.9, 127.5, 128.3, 129.2, 129.7, 129.8, 133.6, 133.7, 138.1 ($C_{\text{-arom}}$), 146.4 (C-7a), 151.5 (C-3), 151.6 (C-6), 157.5 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{22}H_{17}N_4O)^+$ 353.1393, found 353.1402.

5-benzyl-3-ethyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 5k.

White crystals; m.p.: 189-191 °C; IR (KBr, cm^{-1}) ν: 1672 (C=O), 1579 (C=N). ^{1}H NMR (DMSO-*d*$_6$, 300 MHz): δ 1.61 (t, ^3J =7.2 Hz, 3H, H-9), 3.31 (q, ^3J=7.4 Hz, 2H, H-8), 5.20 (s, 2H, H-8'), 7.43–8.80 (m, 10H, Ar-H), 8.85 (s, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-*d*$_6$): δ 13.7 (C-9), 22.1 (C-8), 47.1 (C-8'), 106.1 (C-3a), 121.1 (C-2', C-6'), 126.3 (C-4'), 126.8 (C-4''), 126.9 (C-2'', C-6''), 128.4 (C-3'', C-5''), 129.7 (C-3', C-5'), 139.4 (C-1''), 142.7 (C-1'), 146.1 (C-7a), 151.3 (C-3), 151.5 (C-6), 157.3 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{20}H_{19}N_4O)^+$ 331.1486, found 331.1490.

3-Ethyl-5-phenylethyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 5l.

White crystals; m.p.: 189-192 °C; IR (KBr, cm^{-1}) ν: 1676 (C=O), 1575 (C=N). ^{1}H NMR (DMSO-*d*$_6$, 300 MHz) : δ 1.61 (t, ^3J =7.2 Hz, 3H, H-9), 2.61 (q, ^3J=7.4 Hz, 3H, H-8), 2.80 (t, ^3J =7.2 Hz, H-9'), 3.30 (t, ^3J=7.2 Hz, H-8'), 7.43–8.60 (m, 10H, Ar-H), 8.79 (s, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-*d*$_6$): δ 13.8 (C-9), 21.6 (C-8), 33.2 (C-9'), 47.1 (C-8'), 105.9 (C-3a), 120.9 (C-2', C-6'), 126.3 (C-4''), 127.9 (C-4'), 128.4 (C-2'', C-6''), 129.1 (C-3'', C-5'') 129.7 (C-3', C-5'),139.7 (C-1'), 139.8 (C-1''), 146.1 (C-7a), 151.3 (C-3), 151.6 (C-6), 157.5 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{21}H_{21}N_4O)^+$ 345.1587, found 345.1593.

5-(2-Chlorobenzyl)-3-ethyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 5m.

White crystals; m.p.: 189-192 °C; IR (KBr, cm^{-1}) ν: 1674 (C=O), 1586 (C=N). ^{1}H NMR (DMSO-*d*$_6$, 300 MHz): δ 1.27 (t, ^3J =7.2 Hz, 3H, H-9), 2.58 (q, ^3J =7.4 Hz, 2H, H-8), 4.89 (s, 2H, H-8'), 7.00–8.15 (m, 9H, Ar-H), 8.65 (s, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-*d*$_6$): δ 14.1(C-9), 21.6 (C-8), 39.5 (C-8'), 105.1 (C-3a), 120.6 (C-2''), 126.3 (C-6''), 126.4 (C-2', C-6'), 128.3 (C-4''), 128.7 (C-4'), 129.6 (C-3''), 129.8 (C-3', C-5'), 132.6 (C-5''), 134.5 (C-1'), 138.7 (C-1''), 146.5 (C-7a), 152.1 (C-3), 152.3 (C-6), 157.1 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{20}H_{18}N_4OCl)^+$ 365.1103, found 365.1108.

5-(4-Chlorobenzyl)-3-ethyl-1-phenyl-1H-pyrazolo [3,4-d]pyrimidin-4(5H)-one 5n.

White crystals; m.p.: 191-192 °C; IR (KBr, cm^{-1}) ν: 1674 (C=O), 1586 (C=N). ^{1}H NMR (DMSO-*d*$_6$, 300 MHz): δ 1.33 (t, ^3J =7.2 Hz, 3H, H-9), 2.58 (q, ^3J=7.4 Hz, 2H, H-8), 5.27 (s, 2H, H-8'), 7.24–8.15 (m, 9H, Ar-H), 8.67 (s, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-*d*$_6$): δ 14.1 (C-9), 22.4 (C-8), 47.2 (C-8'), 106.4 (C-3a), 121.6 (C-2'', C-6''), 126.2 (C-4'), 128.6 (C-3'', C-5''), 128.7 (C-3', C-5'), 129.5 (C-2'', C-6''), 131.1 (C-4''), 137.4 (C-1''), 138.5 (C-1') ,146.8 (C-7a), 152.2 (C-3), 152.5 (C-6), 157.6 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{20}H_{18}N_4OCl)^+$ 365.1103, found 365.1108.

***3-Ethyl-5-(naphtalen-1-yl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 5o*.**
Creamy white crystals; m.p.: 199-200 °C; IR (KBr, cm^{-1}) ν: 1674 (C=O), 1586 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.27 (t, ^3J =7.2 Hz, 3H, H-9), 2.61 (q, ^3J =7.4 Hz, 2H, H-8), 7.27–8.01 (m, 12H, Ar-H), 8.61 (s, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.1(C-9), 23.1(C-8), 105.1 (C-3a), 121.6, 122.3, 125.6, 126.7, 126.8, 126.9, 127.5, 128.3, 129.2, 129.7, 129.8, 132.9, 134.7, 138.1 ($C_{\text{-arom}}$), 146.5 (C-7a), 151.5 (C-3), 151.6 (C-6), 157.5 (C=O). HRMS-ES $[M+H]^+$ calcd for $(C_{23}H_{19}N_4O)^+$ 367.1502, found 367.1508.

General procedure for the synthesis of 3-substitued-5-hydroxy-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-(5H)-ones 6a-c.

A mixture of **2**(a-c) (10 mmol) and hydroxylamine hydrochloride (10 mmol) in ethanol (20 mL) containing triethylamine (5 mL) was boiled under reflux for 5h. The reaction mixture was then cooled and poured into cold water. The formed precipitate was filtred off washed with water, dried and recristallized from methanol.

***5-Hydroxy-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 6a*.**
White crystals; 70%; m.p.: 240-244 °C; IR (KBr, cm^{-1}) ν: 3500-3300 (OH), 1672 (C=O), 1579 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.36-8.39 (m, 7H, H-3 + Ar-H+ OH), 8.74 (s, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6): δ 105.1 (C-3a), 121.1 (C-2', C-6'), 126.9 (C-4'), 129.7 (C-3', C-5'), 140.1 (C-1'), 144.7 (C-7a), 146.1 (C-3), 151.5 (C-6), 157.3 (C=O). HRMS-ES $[M-H]^-$ calcd for $(C_{11}H_7N_4O_2)^-$ 227.0558, found 227.0569.

***5-Hydroxy-3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 6b*.**
White crystals; Yield: 68%; m.p.: 242-145 °C; IR (KBr, cm^{-1}) ν:3530-3300 (OH), 1676 (C=O), 1580 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.51 (s, 3H, CH_3), 7.30–8.65 (m, 7H, H-6+ Ar-H+ OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 13.1 (CH_3), 105.1 (C-3a), 121.5 (C-2', C-6'), 126.3 (C-4'), 129.4 (C-3', C-5'), 140.9 (C-1'), 144.7 (C-7a), 146.1 (C-3), 151.5 (C-6), 157.3 (C=O). HRMS-ES $[M+H]^-$ calcd for $(C_{12}H_9N_4O_2)^-$ 241.0801, found 241.0803.

***3-Ethyl-5-hydroxy-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one 6c*.**
White crystals; Yield: 70%; m.p.: 247-249 °C; IR (KBr, cm^{-1}) ν: 3500-3300 (OH), 1672 (C=O), 1579 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.30 (t, ^3J =7.2 Hz, 3H, -CH_2-C<u>H</u>$_3$), 3.01 (q, ^3J=7.5 Hz, 2H, -C<u>H</u>$_2$-CH_3), 7.36–8.39 (m, 7H, Ar-H+ H-6 + OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.7 (-CH_2-<u>C</u>H_3), 22.1 (-<u>C</u>H_2-CH_3), 105.1 (C-3a), 121.1 (C-2', C-6'), 126.9 (C-4'), 129.7 (C-3', C-5'), 140.1 (C-1'), 144.5 (C-7a), 146.2 (C-3), 150.9 (C-6), 157.2 (C=O). HRMS-ES $[M-H]^-$ calcd for $(C_{13}H_{11}N_4O_2)^-$ 255.1010, found 255.1015.

Cytotoxic activity

Cell culture:

The Human cervical adenocarcinoma, HeLa cell line was obtained from American Type Culture Collection (ATCC, Rockville, MD, USA) and cells were cultured in a humidified atmosphere of 5% CO_2 in air at 37 °C, in RPMI1640 medium containing 10% (v/v) fetal calf serum, 2 mM glutamine, and antibiotics (200 U of penicillin and 50 mg of streptomycin per liter).

Cell viability assay:

Cytotoxicity was measured using MTT test with slight modifications[21]. Cells were seeded at 5.10^3 cells/well in 200 µL of growth medium and incubated at 37 °C during 24h for cell adheson. The microplates were treated by the tested compounds and incubated for three times (24h, 48h and 72h). Then, 10 µL of MTT were added in each well (5 mg/mL) and the incubation was continued for 2 h.

100 µL of DMSO was added to each well. The absorbance (A) was measured at 570 nm by Multiskan Ascent (Ascent Software version 2.6) microplate reader. This assay was realized in triplicate as a cell viability index with doxorubicin as positive control. The percentages of cell growth were calculated as follow:
Cell growth (%) = [A (sample)/A (control)] x 100. Cytotoxicity is expressed as the concentration of the tested compound inhibiting cell growth by 50% (IC_{50}).

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