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Synthesis and antitumor activity of novel pyrazolo[3,4-*d*]pyrimidines and related heterocycles

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ABSTRACT

The reaction between 5-amino-4-imino-3-methyl-1-phenyl-1,4-dihydro-pyrazolo[3,4-d]pyrimidine ***(2a)*** *or (3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)- hydrazine* ***(3)*** *and several available reactants afforded new heterocycles with pyrazolo[3,4-d]pyrimidine nucleus. Some of the newly synthesized compounds were screened against MCF-7 cell line, compounds* ***4b, 5a, 10c*** *and* ***12c*** *showed the highest activity among the tested compounds with* IC_{50} *between 0.013 and 0.018 μM.*

Key words: Pyrazolo[3,4-*d*]pyrimidine derivatives, Imino, MCF-7, *Z* and *E* Geometrical isomers.

INTRODUCTION

Cancer still remains one of the most feared diseases in the world. When normal cells lose their regulatory mechanisms that control the growth and multiplication, cancer cells are formed. Cancer is caused by gene mutations or interfering with normal cell differentiation, initiated by chemicals, viruses, smoking or diet [1].

There are three traditional approaches for the treatment of cancer: surgery, radiotherapy and chemotherapy. As combination therapy is more effective than using a single drug, chemotherapy is usually used alongside surgery and radiotherapy [2]. Now, cancer chemotherapy is entering a new era by using molecular target therapeutics (highly selective agents which target specific molecular targets that are abnormal or over expressed in the cancer cells) [3]. So, pharmaceutical attention has been focused on the more selective antineoplastic agents with the minimum side effects.

Several pyrazole derivatives received great attention due to their biological and pharmacological activities not only as potential inhibitors of HIV-1 [4] , pesticides [5] fungicides [6], analgesic drugs [4], antihypertensive agents [7] and anticancer activity [8], but they are also important and useful as starting materials for the synthesis of other fused heterocyclic systems, among these, pyrazolo[3,4-*d*]pyrimidine derivatives [9] ,which have a considerable chemical and pharmacological importance as purine analogues [10-12]. Furthermore, compounds containing the triazolo[1,5-*c*] pyrimidine moiety were reported to exhibit remarkable adenosine receptor affinity [13]. Particularly, the 5- amino-9-chloro-2-(2-furyl)-1,2,4-triazolo[1,5-*c*]quinazoloine **(1)** was found to be a highly potent adenosine antagonist [14]. Also, the tricyclic heterocyclic pyrazolo[1,5-*c*]-1,2,4-triazolo[4,3-*e*]pyrimidines **2**, **3** were planned as human A_{2a} and A_3 adenosine receptor subtype antagonists respectively [15,16].

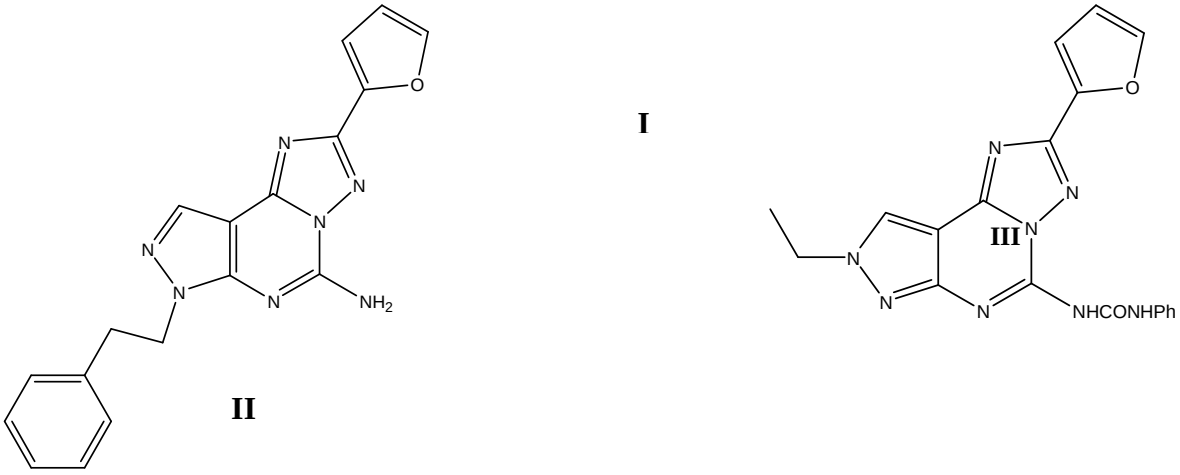


Fig. 1: Structure of previously synthesized compounds containing pyrazolopyrimidine core in tricyclic structure as potent antitumor agents.

Based on the above observation, a pyrazole nucleus was used as a starting material for the preparation of pyrazolo[3,4-*d*]pyrimidine with imino **2a** or hydrazine **3** groups at position 4 of the pyrimidine ring. From these latter compounds, new targets - chemically related to adenosine antagonist - were synthesized, maintaining the pyrazolo[3,4-*d*]pyrimidine nucleus and replaced the 2-(2-furyl)-triazole moiety with five memberd rings [pyrazole, triazole and tetrazole] or six membered ring fused to pyrazolo[3,4-*d*]pyrimidine ring system and functionalized by the introduction of several functions as ester, cyano and substituted amino groups. In addition, new pyrazolo[3,4-*d*]pyrimidine derivatives bearing either acyclic or heterocyclic pyrazole moiety at position 4 of the pyrimidine ring were also synthesized. Some of the newly synthesized compounds were tested against breast cancer cell line (MCF-7).

MATERIALS AND METHODS

Experimental

Melting points were determined on a Graffin apparatus and were uncorrected. Element analyses (C, H, N) were carried out on Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA) at the Micro analytical unit of Cairo University, Egypt. All compounds were within $\pm$ 0.4% of the theoretical values. IR spectra were determined as KBr discs on Shimadzu IR 435 Spectrophotometer and values were represented in cm^{-1}. ^{1}HNMR and ^{13}CNMR spectra were carried out on a Bruker 300 MHz NMR Spectrophotometer in Cairo University, Egypt, using (Bruker, Munich, Germany) in DMSO-d_6 or $CDCl_3$-d_6 as a solvent, TMS as internal standard and chemical shifts were recorded in ppm on δ scale. Mass spectra were run on Hewlett Packard 5988 Spectrometer, Micro analytical center, Cairo University, Egypt. Progress of the reactions was monitored by TLC using TLC sheets precoated with UV fluorescent silica gel MERCK 60 F 254 that were visualized by UV lamp.

General procedure for synthesis of compounds 2b&c.

To a solution of compound **1** (2.54 g, 0.01 mol) in absolute ethanol (20 ml), the appropriate amine (0.01 mol) was added. The mixture was stirred at room temperature for 1 h. The formed precipitate was filtered, dried washed with ethanol to afford **2a&b**. Spectroscopic data for all these compounds are given below.

3,5-Dimethyl-4-Imino-1-phenyl-5-methylamino-1H-4,5-dihydro-pyrazolo[3,4d]pyrimidines (2b).

Isolated as white solid, Yield: 48%, m.p.: 145-147^{0}C (recrystallized from EtOH 95%); IR (KBr, cm^{-1}), 3433 (NH), 3042 (CH arom.), 2922 (CH aliph.), 1681 (C=N).; ^{1}H NMR (300 MHz, DMSO-d6) δ ppm 2.53 (s, 3H, CH_3), 3.40 (s, 3H, N-CH_3), 7.30 (s, 1H, NH, D_2O exchangeable), 7.33 (t, 1H, *J*= 6.3 Hz, A-H), 7.50 (t, 2H, *J*= 6.3 Hz, Ar-H), 7.97 (d, 2H, *J*= 6.3 Hz, A-H) and 8.12 (s, 1H, CH of pyrimidine); EIMS (m/z) (relative abundance %), 240 [M+1 $^{+\cdot}$, 17.12], 239 [M $^{+\cdot}$, 100], 238 [M-H $^{+\cdot}$, 37.50], 209 [$C_{11}H_7N_5$ $^+$, 23.07], and 77 [C_6H_5 $^+$, 21.50]; Anal. Calcd for $C_{13}H_{13}N_5$: C, 65.25; H, 5.48; N, 29.27. Found: C, 65.51; H, 5.00; N, 29.84.

4-Imino-3-methyl-1-phenyl-5-phenylamino-1H-4,5-dihydro-pyrazolo[3,4d]pyrimidines (2c).

Isolated as white solid, Yield: 50%, m.p.: 220-222^{0}C (recrystallized from EtOH 95%); IR (KBr, cm^{-1}), 3441, 3269 (2NH), 3049 (CH arom.), 2916 (CH aliph.), 1622 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.62 (s, 3H, CH_3),

3.27 (s, 1H, NH, D_2O exchangeable), 6.74 (t, 1H, *J*= 7.2 Hz, Ar-H), 6.83 (d, 2H, *J*= 7.8 Hz, Ar-H), 7.17 (t, 2H, *J*= 7.2 Hz, Ar-H), 7.30 (t, 1H, *J*= 6.3 Hz, Ar-H), 7.52 (t, 2H, *J*= 8.4 Hz, Ar-H), 8.14 (d, 2H, *J*= 7.5 Hz, Ar-H), 8.33 (s, 1H, CH of pyrimidine) and 9.48 (s, 1H, NH, D_2O exchangeable).; EIMS (*m/z*) (relative abundance %), 317 [M+1 ⁻⁺·, 13.72], 316 [M ⁻⁺·, 61.70], 224 [$C_{12}H_{10}N_5$ ⁻⁺, 17.83], 209 [$C_{11}H_7N_5$ ⁻⁺·,18.33] and 77 [C_6H_5 ⁻⁺, 100]; Anal. Calcd for $C_{18}H_{16}N_6$: C, 68.34; H, 5.10; N, 26.56. Found: C, 68.17; H, 5.05; N, 26.40.

General procedure for synthesis of (3-Methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)-hydrazine (3)
To a solution of compound **1** (2.54 g, 0.01 mol) in absolute ethanol (20 ml), hydrazine hydrate (16.51 ml, 99%) was added and the mixture was heated under reflux for 1 h. The precipitate formed was filtered while hot, dried and washed with hot ethanol to furnish compound **3**. Isolated as yellow crystals, Yield: 42%, mp: 221-223^{0}C (recrystallized from dioxane); IR (KBr, cm^{-1}), 3271, 3101(NH and NH_2), 3028 (CH arom.), 2924 (CH aliph.), 1578 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.63 (s, 3H, CH_3), 4.74 (s, 2H, NH_2, D_2O exchangeable), 7.28 (t, 1H, *J*= 6.9 Hz, A-H), 7.50 (m, 2H, Ar-H), 8.15 (d, 2H, *J*= 7.8 Hz, A-H) and 8.35 (s, 1H, CH of pyrimidine) and 8.79 (s, 1H, NH, D_2O exchangeable); EIMS (*m/z*) (relative abundance %), 240 [M ⁻⁺·, 2.66], 198 [$C_{11}H_{10}N_4$ ⁻⁺·, 100], 154 [$C_{10}H_6N_2$ ⁻⁺·,21.11], 80 [$C_4H_4N_2$ ⁻⁺·, 54.94] and 77 [C_4HN_2 ⁻⁺, 62.45]; Anal. Calcd for $C_{12}H_{12}N_6$: C, 59.99; H, 5.03; N, 34.98%. Found: C, 60.16; H, 4.85; N, 34.60%.

General procedure for synthesis of compounds 4a&b.
A suspension of compound **2a** (0.24 g, 0.001 mol) and the appropriate isothiocyanate derivative (0.001 mol) in absolute ethanol (10 ml) was heated under reflux for 8 h. The solid that formed on hot was filtered, to afford compounds **4a&b**.

2-Ethylamino-9-methyl- 7-phenyl- 7H- pyrazolo[4,3-e] [1,2,4] triazolo[1,5-c]pyrimidine (4a) Isolated as white solid, Yield: 55%, mp: 165-167^{0}C (recrystallized from DMF/EtOH); IR (KBr, cm^{-1}), 3415 (NH), 3069 (CH arom.), 2969, 2924, 2878 (CH aliph.), 1658 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.20 (t, 3H, *J*= 7.5 Hz, $CH_2\underline{CH_3}$), 2.67 (s, 3H, CH_3), 3.31 (m, 2H, $NH\underline{CH_2}CH_3$), 7.03 (t, 1H, *J*= 5.7 Hz, NH, D_2O exchangeable), 7.37 (t, 1H, *J*= 8.4 Hz, Ar-H), 7.56 (t, 2H, *J*= 6.6 Hz, Ar-H), 8.12 (d, 2H, *J*= 6.6 Hz, Ar-H) and 9.24 (s, 1H, CH of pyrimidine); EIMS (*m/z*) (relative abundance %), 294 [M+1 ⁻⁺·, 16.69], 293 [M ⁻⁺·, 82.34], 279 [$C_{14}H_{13}N_7$ ⁻⁺·, 19.57], 278 [$C_{14}H_{12}N_7$ ⁻⁺, 100], 265 [$C_{13}H_{11}N_7$ ⁻⁺·, 18.99] and 77 [C_6H_5 ⁻⁺, 43.39];; Anal. Calcd for $C_{15}H_{15}N_7$: C, 61.42; H, 5.15; N, 33.43%. Found: C, 61.00; H, 4.89; N, 33.61%.

9-Methyl-2-phenylamino-7-phenyl-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine (4b) Isolated as white solid, Yield: 63%, mp: 286-288^{0}C (recrystallized from DMF/EtOH); IR (KBr, cm^{-1}), 3251 (NH), 3138, 3073 (CH arom.), 2925 (CH aliph.), 1658 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.75 (s, 3H, CH_3), 6.94 (t, 1H, *J*= 7.5 Hz, Ar-H), 7.36 (m, 4H, Ar-H), 7.59 (t, 1H, *J*= 8.4 Hz, Ar-H), 7.74 (d, 2H, *J*= 7.5 Hz, Ar-H), 8.13 (d, 2H, *J*= 7.2 Hz, Ar-H), 9.48 (s, 1H, CH of pyrimidine) and 10.15 (s, 1H, NH, D_2O exchangeable); EIMS (*m/z*) (relative abundance %), 342 [M+1 ⁻⁺·, 22.90], 341 [M ⁻⁺·, 100], 340 [M-1 ⁻⁺·, 17.96], and 77 [C_6H_5 ⁻⁺, 34.90]; Anal. Calcd for $C_{19}H_{15}N_7$: C, 66.85; H, 4.43; N, 28.72%. Found: C, 66.72; H, 4.41; N, 29.08%.

General procedure for synthesis of compounds 5a&b.
A mixture of **2a** (0.24 g, 0.001 mol) and diethyl oxalate or diethyl malonate (5 ml) was heated under reflux for 10 h. The reaction mixture was concentrated and left to cool. The solid separated was filtered, to give compounds **5a&b.**

Ethyl(3-methyl-1-phenyl-1H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-yl)carboxylate (5a).
Isolated as yellow solid, Yield: 81%, mp: 197-199^{0}C (recrystallized from dioxane); IR (KBr, cm^{-1}), 3063 (CH arom.), 2986, 2928 (CH aliph.), 1739 (C=O), 1648 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.38 (t, 3H, *J*= 7.2 Hz, $CH_2\underline{CH_3}$), 2.77 (s, 3H, CH_3), 4.46 (q, 2H, *J*= 7.2 Hz, $\underline{CH_2}CH_3$), 7.44 (t, 1H, *J*= 7.2 Hz, Ar-H), 7.60 (t, 2H, *J*= 7.2 Hz, Ar-H), 8.08 (d, 2H, *J*= 8.7 Hz, Ar-H) and 9.78 (s, 1H, CH of pyrimidine); EIMS (*m/z*) (relative abundance %), 323 [M+1 ⁻⁺·, 21.12], 322 [M ⁻⁺·, 100], 250 [$C_{13}H_{10}N_6$ ⁻⁺·, 45.46], 224 [$C_{12}H_{10}N_5$ ⁻⁺, 21.79], and 77 [C_6H_5 ⁻⁺, 52.53]; Anal. Calcd for $C_{16}H_{14}N_6O_2$: C, 59.62; H, 4.38; N, 26.07%. Found: C, 59.57; H, 4.21; N, 29.08%.

Ethyl(3-methyl-1-phenyl-1H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-yl)methylcarboxylate (5b)
IR (KBr, cm^{-1}), 3092 (CH arom.), 2979, 2929 (CH aliph.), 1735 (C=O), 1647 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.22 (t, 3H, *J*= 7.2 Hz, $CH_2\underline{CH_3}$), 2.73 (s, 3H, CH_3), 4.07 (s, 2H, CH_2), 4.16 (q, 2H, *J*= 7.2 Hz, $\underline{CH_2}CH_3$), 7.40 (t, 1H, *J*= 7.8 Hz, Ar-H), 7.59 (t, 2H, J_1= 7.8 Hz, Ar-H), 8.10 (d, 2H, *J*= 8.4 Hz, Ar-H) and 9.63 (s, 1H, CH of pyrimidine); EIMS (*m/z*) (relative abundance %), 337 [M+1 ⁻⁺·, 21.46], 336 [M ⁻⁺·, 100], 264 [$C_{14}H_{12}N_6$ ⁻⁺·,

95.43], 263 [$C_{14}H_{11}N_6$ ⌉$^+$, 32.92], and 77 [C_6H_5 ⌉$^+$, 43.61]; Anal. Calcd for $C_{17}H_{16}N_6O_2$: C, 60.71; H, 4.79; N, 24.99%. Found: C, 61.00; H, 4.89; N, 25.25%.

General procedure for synthesis of compounds 6a&b.
A mixture of **2a** (2.4 g, 0.01 mol) and chloroacetyl chloride or ethyl cyanoacetate (1.12 g, 0.01 mol) was refluxed in dioxane (20 ml) for 6 h. The reaction mixture was filtered while hot and the filtrate was evaporated to dryness to give compounds **6a&b**.

2-Chloromethyl-9-methyl-7-phenyl-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine (6a)
Isolated as white solid, Yield: 63%, mp: 194-196^{0}C (recrystallized from DMF/ethanol 95%); IR (KBr, cm^{-1}), 3091 (CH arom.), 2923 (CH aliph.), 1648 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.74 (s, 3H, CH_3), 5.03 (s, 2H, CH_2), 7.43 (t, 1H, *J*= 7.2 Hz, Ar-H), 7.60 (t, 2H, *J*= 8.1 Hz, Ar-H), 8.09 (d, 2H, *J*= 5.4 Hz, Ar-H) and 9.66 (s, 1H, CH of pyrimidine); EIMS (*m/z*) (relative abundance %), 300 [M+2 ⌉$^{+\cdot}$, 16.34], 299 [M+1 ⌉$^{+\cdot}$, 12.50], 398 [M ⌉$^{+\cdot}$, 51.94], 263 [$C_{14}H_{11}N_6$ ⌉$^{\cdot}$, 8.16] and 77 [C_6H_5 ⌉$^+$, 100]; Anal. Calcd for $C_{17}H_{16}N_6O_2$: C, 56.29; H, 3.71; N, 28.13%. Found: C, 56.08; H, 3.91; N, 28.45%.

(9-Methyl-7-phenyl-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-acetonitrile (6b)
The solid separated was filtered, dried to yield compound **6b**. Isolated as white solid, Yield: 76%, mp: 232-234^{0}C (recrystallized from dioxane); IR (KBr, cm^{-1}), 3072 (CH arom.), 2922 (CH aliph.), 2254 (C≡N), 1651 (C=N);^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.73 (s, 3H, CH_3), 4.56 (s, 2H, CH_2), 7.40 (t, 1H, *J*= 7.8 Hz, Ar-H), 7.60 (t, 2H, *J*= 8.1 Hz, Ar-H), 8.09 (d, 2H, *J*= 8.4 Hz, Ar-H) and 9.69 (s, 1H, CH of pyrimidine); EIMS (*m/z*) (relative abundance %), 290 [M+1 ⌉$^{+\cdot}$, 19.72], 289 [M ⌉$^{+\cdot}$, 100], 288 [$C_{15}H_{10}N_7$ ⌉$^+$, 30.38] and 77 [C_6H_5 ⌉$^+$, 48.56]; Anal. Calcd for $C_{15}H_{11}N_7$: C, 62.28; H, 3.83; N, 33.89%. Found: C, 62.48; H, 3.80; N, 33.68 %.

General procedure for synthesis of compounds 7a-d.
A mixture of compound **2a** (2.40 g, 0.01 mol) and the appropriate aromatic aldehyde (0.01 mol) in absolute ethanol (30 ml), piperidine (0.5mL) was added and the mixture was heated under reflux for 3-5 h. The precipitate that formed on hot was filtered off and washed with ethanol to yield **7a-d**.

9-Methyl-7-phenyl-2-(3-hydroxyphenyl)-7H-pyrazolo[4,3-e][1,2,4] triazolo[1,5-c]pyrimidine (7a).
Isolated as yellow solid, Yield: 53%, mp: 286-288^{0}C (recrystallized from Acetone); IR (KBr, cm^{-1}), 3495 (OH), 3041 (CH arom.), 2927 (CH aliph.), 1649 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.81 (s, 3H, CH_3), 6.94 (d, 1H, *J*= 8.4 Hz, Ar-H), 7.41 (m, 2H, Ar-H), 7.61 (t, 2H, *J*= 7.5 Hz, Ar-H), 7.72 (m, 2H, Ar-H), 8.13 (d, 2H, *J*= 8.4 Hz, Ar-H), 9.72 (s, 1H, CH of pyrimidine) and 9.80 (s, 1H, OH, D_2O exchangeable); EIMS (*m/z*) (relative abundance %), 342 [M ⌉$^{+\cdot}$, 3.66], 149 [$C_6H_5N_4O$ ⌉$^+$, 15.19], 95 [$C_4H_5N_3$ ⌉$^+$, 28.35], 81[$C_3H_3N_3$ ⌉$^+$, 36.33] and 69 [$C_3H_5N_2$ ⌉$^{+\cdot}$, 100]; Anal. Calcd for $C_{19}H_{14}N_6O$: C, 66.66; H, 4.12; N, 24.55%. Found: C, 66.42; H, 4.06; N, 24.38%.

9-Methyl-7-phenyl-2-(3-methoxyphenyl)-7H-pyrazolo[4,3-e][1,2,4] triazolo[1,5-c]pyrimidine (7b).
Isolated as yellow solid, Yield: 33%, mp: 239-241^{0}C (recrystallized from Acetone); IR (KBr, cm^{-1}), 3085, 3000 (CH arom.), 2932, 2835 (CH aliph.), 1652 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.80 (s, 3H, CH_3), 3.87 (s, 3H, CH_3), 7.15 (d, 1H, *J*= 8.1 Hz, Ar-H), 7.48 (m, 3H, Ar-H), 7.60 (t, 1H, *J*= 8.1 Hz, Ar-H), 7.76 (s, 1H, Ar-H), 7.86 (d, 1H, *J*= 7.8 Hz, Ar-H), 8.12 (d, 2H, *J*= 7.8 Hz, Ar-H) and 9.69 (s, 1H, CH of pyrimidine); EIMS (*m/z*) (relative abundance %), 357 [M+1 ⌉$^{+\cdot}$, 23.53], 356 [M ⌉$^{+\cdot}$, 100], 355 [M-1 ⌉$^{+\cdot}$, 37.37], 326 [$C_{19}H_{14}N_6$ ⌉$^+$, 15.31], and 77 [C_6H_5 ⌉$^+$, 78.71]; Anal. Calcd for $C_{20}H_{16}N_6O$: C, 67.40; H, 4.53; N, 23.58%. Found: C, 67.44; H, 4.51; N, 23.83%.

9-Methyl-7-phenyl-2-(4-chlorophenyl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine (7c). *Isolated as yellow solid, Yield: 50%, mp: 243-245^{0}C (recrystallized from propanol);* IR (KBr, cm^{-1}), 3067, 3002 (CH arom.), 2924 (CH aliph.), 1650 (C=N); ^{1}H NMR (300 MHz, DMSO- d_6) δ ppm 2.79 (s, 3H, CH_3), 7.39 (t, 1H, J= 8.4 Hz, A-H), 7.59 (t, 2H, J= 8.4 Hz, Ar-H), 8.08 (d, 2H, J= 7.2 Hz, A-H), 8.38 (d, 2H, J= 8.7 Hz, Ar-H), 8.45 (d, 2H, J=8.7 Hz, Ar-H) and 9.75 (s, 1H, CH of pyrimidine); EIMS (*m/z*) (relative abundance %), 362 [M+2 ⌉$^{+\cdot}$, 30.57], 361 [M+1 ⌉$^{+\cdot}$, 31.85], 360 [M ⌉$^{+\cdot}$, 100], 118 [$C_5H_2N_4$ ⌉$^+$, 8.11], 103 [$C_6H_3N_2$ ⌉$^{+\cdot}$, 9.27] and 77 [C_6H_5 ⌉$^+$, 33.40]; Anal. Calcd for $C_{19}H_{13}ClN_6$: C, 63.25; H, 3.63; N, 23.29%. Found: C, 63.19 H, 3.80; N, 23.16%.

9-Methyl-7-phenyl-2-(4-nitrophenyl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine (7d). Isolated as orange solid, Yield: 35%, mp: 290-292^{0}C (recrystallized from propanol); IR (KBr, cm^{-1}), 3067, 3002 (CH arom.), 3067 (CH arom.), 2924 (CH aliph.), 1646 (C=N); ^{1}H NMR (300 MHz, DMSO- d_6) δ ppm 2.79 (s, 3H, CH_3), 7.42 (t,

1H, *J*= 6.6 Hz, Ar-H), 7.58 (t, 2H, *J*= 8.1 Hz, Ar-H), 8.08 (d, 2H, *J*= 6.6 Hz, Ar-H), 8.43 (m, 4H, Ar-H) and 9.74 (s, 1H, CH of pyrimidine); EIMS (*m/z*) (relative abundance %), 372 [M+1 ⁻⁺·, 11.92], 371 [M ⁻⁺·, 47.80], 370 [M-1 ⁻⁺·, 9.41], 80 [$C_3H_2N3_5$ ⁻⁺, 100] and 64 [C_3N_2 ⁻⁺, 48.43]; Anal. Calcd for $C_{19}H_{13}N_7O_2$: C, 61.45; H, 3.53; N, 26.40%. Found: C, 61.74 H, 3.67; N, 26.14%.

General procedure for synthesis of 9-Methyl-7-phenyl-7H-pyrazolo[4,3-e] tetrazolo[1,5-c]pyrimidine (8).
An ice-cold solution of sodium nitrite (0.21 g/5 ml H_2O, 0.003 mol) was added with stirring during five minutes to a cold solution of **2a** (0.24 g, 0.001 mol) in acetic acid (10 ml). Stirring was then continued for 2 h. The reaction mixture was poured into water (≈30 ml). The separated solid was filtered off and washed with water to afford compound **8**. Isolated as white solid, Yield: 50%, mp: 157-159^{0}C (recrystallized from ethanol 95%); IR (KBr, cm^{-1}), 3187 (CH arom.), 2925 (CH aliph.), 1649 (C=N);^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.62 (s, 3H, CH_3), 7.28 (t, 1H, *J*= 8.4 Hz, Ar-H), 7.50 (t, 2H, *J*= 8.4 Hz, Ar-H), 8.16 (d, 2H, *J*= 7.5 Hz, Ar-H) and 8.26 (s, 1H, CH of pyrimidine); EIMS (*m/z*) (relative abundance %), 251 [M ⁻⁺·, 0.28], 225 [$C_{11}H_9N_6$ ⁻⁺, 100], 224 [$C_{11}H_8N_6$ ⁻⁺·, 28.50] and 77 [C_6H_5 ⁻⁺, 47.95] ; Anal. Calcd for $C_{12}H_9N_7$: C, 57.37; H, 3.61; N, 39.02%. Found: C, 57.21; H, 3.54; N, 38.89 %.

General procedure for synthesis of 3-methyl-1-phenyl-1H,7H-5,6-dioxo-dihydro pyrazolo [3',4':4,5] pyrimido[1,6-b][1,2,4]triazine (9).
A mixture of **2a** (0.24 g, 0.001 mol) and oxalyl chloride (0.126 g, 0.001 mol) in dry benzene (10 mL) was heated under reflux for 8 h. The reaction mixture was evaporated to dryness, washed with 10% Na_2CO_3 solution (5 mL). The separated solid was filtered, dried to yield compound **9**. Isolated as green solid, Yield: 76%, mp: 258-260^{0}C (recrystallized from mixture of ethanol/benzene (1:1); IR (KBr, cm^{-1}), 3251 (NH), 3073 (CH arom.), 2925 (CH aliph.), 1726, 1658 (2C=O);^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.50 (s, 3H, CH_3), 7.36 (t, 1H, *J*= 7.5 Hz, Ar-H), 7.53 (t, 2H, *J*= 7.8 Hz, Ar-H), 8.02 (d, 2H, *J*= 7.8 Hz, Ar-H), 8.13 (s, 1H, CH of pyrimidine), and 12.31 (s, 1H, NH, D_2O exchangeable); EIMS (*m/z*) (relative abundance %), 295 [M+1 ⁻⁺·, 1.68], 294 [M ⁻⁺·, 9.13], 252 [$C_{12}H_8N_6O$ ⁻⁺·, 13.50], 251 [$C_{13}H_9N_5O$ ⁻⁺·, 100], 250 [$C_{12}H_6N_6O$ ⁻⁺·, 29.93], 236 [$C_{12}H_8N_6$ ⁻⁺·, 13.22], 182 [$C_{10}H_6N_4$ ⁻⁺·, 14.88] and 77 [C_6H_5 ⁻⁺, 37.18]; Anal. Calcd for $C_{14}H_{10}N_6O_2$: C, 57.14; H, 3.43; N, 28.56%. Found: C, 56.86; H, 3.40; N, 28.49 %.

General procedure for synthesis of compounds 10a-d.
To a solution of **3** (2.40 g, 0.01 mol) in absolute ethanol (20 ml), the appropriate ethoxy derivative of malononitrile or cyanoacetate (0.01 mol) and TEA (0.5 mL) were added. The mixture was heated under reflux for 2-5 h. The mixture allowed to cool then poured into ice-cold water (≈30 ml). The solid separated was filtered off and washed with ethanol to give **10a-d**.

5-Amino-1-(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)-1H-pyrazole-4-carbonitrile (10a).
Isolated as brown solid, Yield: 70%, mp: 245-247^{0}C (recrystallized from EtOH 95%); IR (KBr, cm^{-1}), 3377, 3262 (NH_2), 2927 (CH aliph.), 2216 (C≡N), 1625 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.76 (s, 3H, CH_3), 7.40 (t, 1H, *J*= 8.4 Hz, Ar-H), 7.58 (t, 2H, *J*= 8.4 Hz, Ar-H), 8.12 (m, 3H, Ar-H and CH of pyrazole ring), 8.36 (s, 2H, NH_2, D_2O exchangeable) and 8.89 (s, 1H, CH of pyrimidine); EIMS (*m/z*) (relative abundance %), 317 [M+1 ⁻⁺·, 21.71], 316 [M ⁻⁺·, 100], 315 [M-1 ⁻⁺·, 10.96], 300 [$C_{16}H_{10}N_7$ ⁻⁺, 68.70], 288 [$C_{15}H_{10}N_7$ ⁻⁺, 13.94] and 77 [C_6H_5 ⁻⁺, 23.31] ; Anal. Calcd for $C_{16}H_{12}N_8$: C, 60.75; H, 3.82; N, 35.42%. Found: C, 60.74; H, 3.69; N, 35.56%.

5-amino-3-methyl-1-(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)-1H-pyrazole-4-carbonitrile (10b).
Isolated as brown solid, Yield: 82%, mp: 290-292^{0}C (recrystallized from EtOH 95%); IR (KBr, cm^{-1}), 3366, 3270 (NH_2), 3057 (CH arom.), 2993, 2924 9CH aliph.), 2213 (C≡N), 1641 (C=N).; ^{1}H NMR (300 MHz, DMSO- d_6) δ ppm 2.27 (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 7.41(t, 1H, *J*= 7.8 Hz, Ar-H), 7.57 (t, 2H, *J*= 7.8 Hz , Ar-H), 8.12 (d, 2H, *J*= 7.8 Hz, Ar-H), 8.36 (s, 2H, NH_2, D_2O exchangeable) and 8.83 (s, 1H, CH of pyrimidine); EIMS (*m/z*) (relative abundance %), 331 [M+1 ⁻⁺·, 9.43], 330 [M ⁻⁺·, 24.61], 209 [$C_{12}H_9N_4$ ⁻⁺, 28.73], 207 [$C_{12}H_7N_4$ ⁻⁺, 100] and 77 [C_4HN_2 ⁻⁺, 90.80] ; Anal. Calcd for $C_{17}H_{14}N_8$: C, 61.81; H, 4.27; N, 33.92%. Found: C, 61.72; H, 4.42; N, 33.75%.

5-Amino-4-ethyl-1-(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (10c).
Isolated as white off solid, Yield: 71%, mp: 143-145^{0}C (recrystallized from EtOH 95%); IR (KBr, cm^{-1}), 3446, 3326 (NH_2), 3060 (CH arom.), 2937 (CH aliph.), 1688 (C=O), 1608 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$- d_6) δ ppm 1.39 (t, 3H, *J*= 7.5 Hz, $CH_2\underline{CH_3}$), 2.93 (s, 3H, CH_3), 4.34 (q, 2H, *J*= 7.5 Hz, $\underline{CH_2}CH_3$), 7.35 (t, 1H, *J*= 8.1 Hz, Ar-H), 7.55 (t, 2H, *J*= 8.1 Hz, Ar-H), 7.60 (s, 2H,NH_2, D_2O exchangeable), 7.91 (s, 1H, CH pyrazole), 8.13 (d, 2H, *J*= 8.1

Hz, Ar-H), and 8.78 (s, 1H, CH of pyrimidine); EIMS (*m/z*) (relative abundance %), 364 [M+1 ⌉$^{+\cdot}$, 25.55], 363 [M ⌉$^{+\cdot}$, 100], 334 [$C_{16}H_{12}N_7O_2$ ⌉$^{+}$, 17.67], 316 [$C_{16}H_{10}N_7O$ ⌉$^{+}$, 29.90], 289 [$C_{15}H_{11}N_7$ ⌉$^{+\cdot}$, 23.73] and 226 [$C_{11}H_{10}N_6$ ⌉$^{+\cdot}$, 92.91]; Anal. Calcd for $C_{18}H_{17}N_7O_2$: C, 59.50; H, 4.72; N, 26.98%. Found: C, 59.19; H, 5.10 ; N, 26.92%.

5-Amino-4-ethyl-3-methyl-1-(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)-1H-pyrazole-4-carboxylate (10d).

Isolated as white off solid, Yield: 72%, mp: 151-153^{0}C (recrystallized from EtOH 95%); IR (KBr, cm^{-1}), 3422, 3303 (NH_2), 3055 (CH arom.), 2929 (CH aliph.), 1678 (C=O), 1609 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$- d_6) δ ppm 1.40 (t, 3H, *J*= 14.4 Hz, $CH_2\underline{CH_3}$), 2.47 (s, 3H, CH_3), 2.97 (s, 3H, CH_3), 4.34 (q, 2H, *J*= 14.4 Hz, $\underline{CH_2}CH_3$), 7.36 (t, 1H, *J*= 7.2 Hz, Ar-H), 7.54 (t, 2H, *J*= 8.1 Hz, Ar-H), 7.68 (s, 2H,NH_2, D_2O exchangeable), 8.14 (d, 2H, *J*= 7.5 Hz, Ar-H), and 8.73 (s, 1H, CH of pyrimidine); EIMS (*m/z*) (relative abundance %), 378 [M+1 ⌉$^{+\cdot}$, 26.66], 377 [M ⌉$^{+\cdot}$, 100], 330 [$C_{17}H_{10}N_6O_2$ ⌉$^{+\cdot}$, 23.98], 303 [$C_{16}H_{11}N_6O$ ⌉$^{+}$, 24.08] and 209 [$C_{11}H_7N_5$ ⌉$^{+\cdot}$, 14.68]; Anal. Calcd for $C_{19}H_{19}N_7O_2$: C, 60.47; H, 5.07; N, 25.98%. Found: C, 60.70; H, 5.00 ; N, 25.58%.

General procedure for synthesis of 3,5-dimethyl-1-(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)-1H-pyrazole (11).

To a suspention of **3** (2.4 g, 0.01 mol) in absolute ethanol (20 ml), acetyl acetone (1 g, 0.01 mol) was added, the mixture was heated under reflux for 3 hrs. The solid separated on hot was filtered off and washed with ethanol to afford compound **11**. Isolated as yellow solid, Yield: 42%, mp: 115-117^{0}C (recrystallized from dioxane); IR (KBr, cm^{-1}), 3049 (CH arom.), 2981, 2925 (CH aliph.), 1548 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.28 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 6.28 (s, 1H, CH), 7.38 (t, 1H, *J*= 11.1 Hz, Ar-H), 7.57 (t, 2H, *J*= 12 Hz, Ar-H), 8.15 (d, 2H, *J*= 11.4 Hz, Ar-H) and 8.88 (s, 1H, CH of pyrimidine); EIMS (*m/z*) (relative abundance %), 304 [M ⌉$^{+\cdot}$, 100], 303 [$C_{17}H_{15}N_6$ ⌉$^{+}$, 17.90], 289 [$C_{16}H_{13}N_6$ ⌉$^{+}$, 100], 95 [$C_5H_7N_2$ ⌉$^{+}$, 35.70] and 77 [C_4HN_2 ⌉$^{+}$, 96.40]; Anal. Calcd for $C_{17}H_{16}N_6$: C, 67.09; H, 5.30; N, 27.61%. Found: C, 66.93; H, 5.28 ; N, 27.40%.

General procedure for synthesis of 3-[(3-Methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)-hydrazono]-(ZE)-butyric acid ethyl ester (12).

Method A:

To a suspension of **3** (2.40 g, 0.01 mol) in absolute ethanol (20 ml), ethyl acetoacetate (1.30 g, 0.01 mol) was added; the mixture was heated under reflux for 5 h. The solid separated on hot was filtered off and washed with water to afford compound **12**.

Method B:

A mixture of **3** (2.40 g, 0.01 mol) and ethyl acetoacetate (1.30 g, 0.01 mol) was heated without solvent for 0.5 h. After cooling, absolute ethanol (30 ml) was added and the mixture was heated under reflux for additional 3 h. The solid separated on hot was filtered off and washed with water to yield **12**. Isolated as yellow solid, Yield: 58%, mp: 142-144^{0}C (recrystallized from chloroform /methanol (8:2)); IR (KBr, cm^{-1}), 3333 (NH), 3066 (CH arom.), 2975, 2920 (CH aliph.), 1714 (C=O), 1654 (C=N); ^{1}H NMR (300 MHz, DMSO- d_6) δ ppm 1.17, 1.21 (2t, 3H, *J*= 14.1 Hz, -$CH_2\underline{CH_3}$, (*Z and E* isomers)), 2.12 (s, 3H, CH_3), 2.53, 2.69 (s, 3H, CH_3 , (*Z and E* isomers)), 3.44, 3.60 (2s, 2H, CH_2, (*Z and E* isomers)), 4.07, 4.16 (2q, 2H, *J*= 14.1 Hz, -$\underline{CH_2}CH_3$, (*Z and E* isomers)), 7.33 (t, 1H, *J*= 7.8 Hz, Ar-H), 7.53 (t, 2H, *J*= 7.8 Hz, Ar-H), 7.81 (s, 1H, CH of pyrimidine), 7.98 (m, 2H, Ar-H) and 11.66 (s, 1H, NH, D_2O exchangeable); EIMS (m/z) (relative abundance %), 353 [M+1 ⌉$^{+\cdot}$, 5.69], 352 [M ⌉$^{+\cdot}$, 19.74], 266 [$C_{14}H_{14}N_6$ ⌉$^{+\cdot}$, 23.60], 265 [$C_{14}H_{13}N_6$ ⌉$^{+}$, 100] and 77 [C_4HN_2 ⌉$^{+}$, 42.32]; Anal. Calcd for $C_{18}H_{20}N_6O_2$: C, 61.35; H, 5.72; N, 23.85%. Found: C, 61.19; H, 5.68; N, 23.73%.

General procedure for synthesis of compounds 13a-d.

A mixture of compound **3** (2.40 g, 0.01 mol), the appropriate aromatic aldehyde (0.01 mol) and catalytic amount of piperidine (0.5 ml) was heated under reflux in absolute ethanol (30 ml) for 3-5 h. The precipitate that formed on hot was filtered off and washed with ethanol to yield **13a-d**.

N-(Benzylidene)-N'-(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)-hydrazine (13a). Isolated as yellow solid, Yield: 62%, mp: 170-172^{0}C (recrystallized from dioxane); IR (KBr, cm^{-1}), 3438 (NH), 3127, 3051 (CH arom.), 2982, 2927 (CH aliph.), 1576 (C=N); ^{1}H NMR (300 MHz, DMSO- d_6) δ ppm 2.56, 2.81 (2s, 3H, CH_3, *Z and E* isomers), 7.44 (m, 6H, Ar-H), 7.50 (m, 4H, Ar-H), 7.95 (m, 5H, 4Ar-H and N=CH), 8.38, 8.45 (2s, 1H, CH of pyrimidine, *Z and E* isomers) and 11.81, 11.98 (2s, 1H, NH, D_2O exchangeable, *Z and E* isomers); EIMS (*m/z*) (relative abundance %), 329 [M+1 ⌉$^{+\cdot}$, 8.21], 328 [M ⌉$^{+\cdot}$, 35.53], 327 [$C_{19}H_{15}N_6$ ⌉$^{+}$, 12.51], 251 [$C_{13}H_{11}N_6$ ⌉$^{+}$,

34.26], 225 [$C_{12}H_{11}N_5$ ⌉$^{+\cdot}$, 61.55], 209 [$C_{12}H_9N_4$ ⌉$^+$, 14.83] and 77 [C_4HN_2 ⌉$^+$, 100]; Anal. Calcd for $C_{19}H_{16}N_6$: C, 69.50; H, 4.91; N, 25.59%. Found: C, 69.69; H, 5.00; N, 25.60%.

N-(3-Fluorobenzylidene)-N'-(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)-hydrazine (13b).
Isolated as yellow solid, Yield: 62%, mp: 170-172^{0}C (recrystallized from dioxane); IR (KBr, cm^{-1}), 3206 (NH), 3059 (CH arom.), 2924 (CH aliph.), 1582 (C=N); ^{1}H NMR (300 MHz, DMSO- d_6) δ ppm 2.55, 2.79 (2s, 3H, CH_3, *Z and E* isomers), 7.32 (m, 3H, Ar-H), 7.52 (t, 2H, *J*= 8.4 Hz, Ar-H), 7.94 (s, 1H, N=CH), 8.03 (m, 4H, Ar-H), 8.06, 8.45 (2s, 1H, CH of pyrimidine, *Z and E* isomers) and 11.84, 12.00 (2s, 1H, NH, D_2O exchangeable, *Z and E* isomers); EIMS (*m/z*) (relative abundance %), 347 [M+1 ⌉$^{+\cdot}$, 15.05], 346 [M ⌉$^{+\cdot}$, 60.59], 251 [$C_{13}H_{11}N_6$ ⌉$^+$, 37.49], 225 [$C_{12}H_{11}N_5$ ⌉$^{+\cdot}$, 100], 209 [$C_{12}H_9N_4$ ⌉$^+$, 25.02] and 77 [C_6H_5 ⌉$^+$, 77.56]; Anal. Calcd for $C_{19}H_{15}FN_6$: C, 65.89; H, 4.37; N, 24.26%. Found: C, 65.88; H, 4.71; N, 24.61%.

N-(3-Methoxybenzylidene)-N'-(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)-hydrazine (13c).
Isolated as yellow solid, Yield: 56%, mp: 268-270^{0}C (recrystallized from dioxane); IR (KBr, cm^{-1}), 3438 (NH), 3127, 3051 (CH arom.), 2982, (CH aliph.), 1576 (C=N); ^{1}H NMR (300 MHz, DMSO- d_6) δ ppm 2.56, 2.82 (2s, 3H, CH_3, *Z and E* isomers), 3.82 (s, 3H, -OCH_3), 6.99 (d, 1H, *J*= 8.4 Hz, Ar-H), 7.33 (m, 2H, Ar-H), 7.51 (t, 3H, *J*= 7.8 Hz, Ar-H), 7.58 (s, 1H, Ar-H), 7.92 (s, 1H, N=CH), 8.02 (d, 2H, *J*= 7.8 Hz, Ar-H), 8.32, 8.41 (2s, 1H, CH of pyrimidine, *Z and E* isomers) and 11.77, 11.90 (2s, 1H, NH, D_2O exchangeable, *Z and E* isomers);

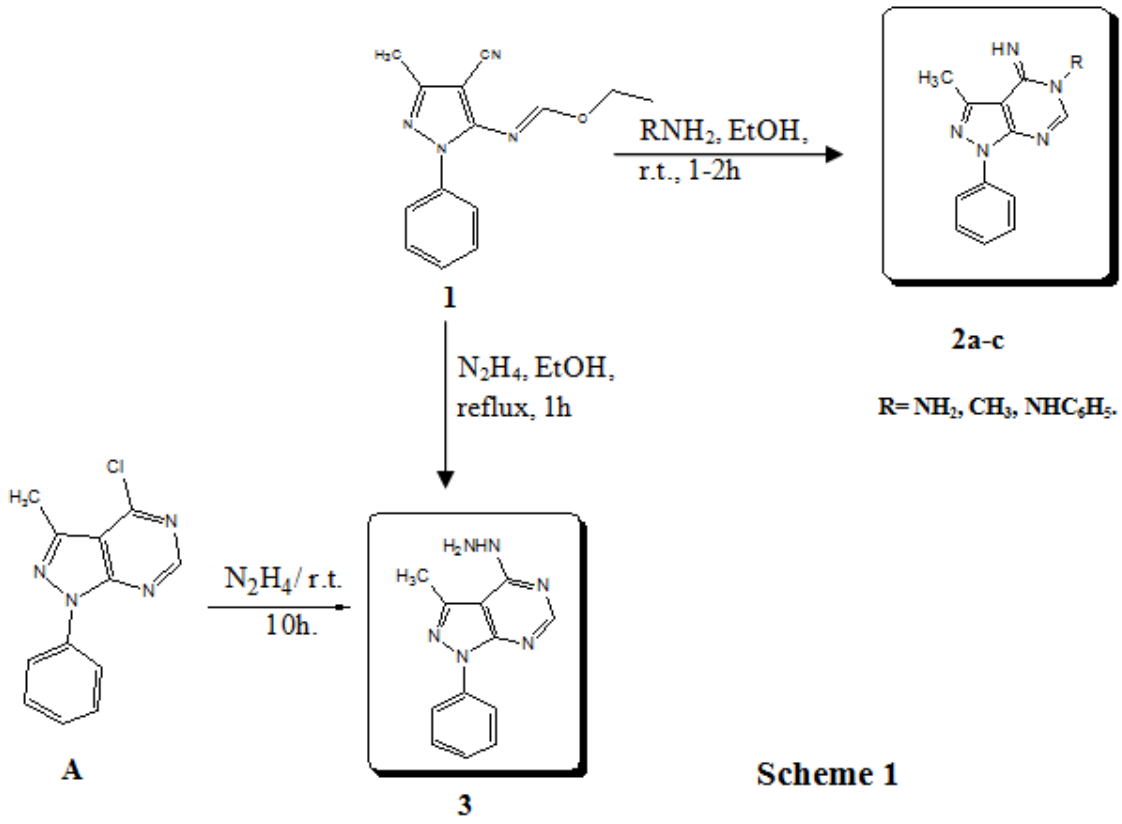
^{13}C NMR (300 MHz, DMSO- d_6) δ ppm 14.07, 18.32 (CH_3), 55.14 (OCH_3), 102.16 (C-3a), 110.98, 112.70 (C-6''), 115.35, 115.96 (C-4''), 120.67, 120.82 (C-2''), 125.72, 126.24 (C-3''), 128.87 (C-2', C-6'), 129.45 (C-4'), 129.89 (C-7a), 135.44, 136.63 (C-1''), 138.32 (C-3', C-5'), 143.28 (C-1'), 144.93, 146.37 (C-6), 147.66, 147.98 (N=CH), 149.45 (C-3), 153.14, 154.82 (C-5''), 156.28, 159.44 (C-4); EIMS (*m/z*) (relative abundance %), 388 [M ⌉$^{+\cdot}$, 0.03], 251 [$C_{13}H_{11}N_6$ ⌉$^+$, 50.35], 225 [$C_{12}H_{11}N_5$ ⌉$^{+\cdot}$, 100] and 209 [$C_{12}H_9N_4$ ⌉$^+$, 30.11]; Anal. Calcd for $C_{20}H_{18}N_6O$: C, 67.02; H, 5.06; N, 23.45%. Found: C, 67.00; H, 4.99; N, 23.72%.

N-(ZE)-(4-Chlorobenzylidene)-N'-(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)-hydrazine (13d).
Isolated as white solid, Yield: 49%, mp: 265-267^{0}C (recrystallized from dioxane); IR (KBr, cm^{-1}), 3433 (NH), 3071 (CH arom.), 2986, 2922 (CH aliph.), 1587 (C=N); ^{1}H NMR (300 MHz, DMSO- d_6) δ ppm 2.55, 2.77 (2s, 3H, CH_3, *Z and E* isomers), 7.34 (t, 1H, *J*= 7.5 Hz, Ar-H), 7.52 (m, 4H, Ar-H), 7.94(s, 1H, N=CH), 8.00 (m, 4H, Ar-H), 8.43 (s, 1H, CH of pyrimidine) and 11.83, 12.02 (2s, 1H, NH, D_2O exchangeable, *Z and E* isomers); EIMS (m/z) (relative abundance %), 363 [M+1 ⌉$^{+\cdot}$, 20.00], 362 [M ⌉$^{+\cdot}$, 24.07], 336 [$C_{18}H_{15}ClN_5$ ⌉$^+$, 23.33], 310 [$C_{18}H_{10}N_6$ ⌉$^{+\cdot}$, 20.00], 294 [$C_{18}H_8N_5$ ⌉$^+$, 26.30], 283 [$C_{17}H_9N_5$ ⌉$^{+\cdot}$, 28.89], 198 [$C_{11}H_{10}N_4$ ⌉$^{+\cdot}$, 23.33] and 80 [$C_4H_4N_2$ ⌉$^{+\cdot}$, 100]; Anal. Calcd for $C_{19}H_{15}ClN_6$: C, 62.90; H, 4.17; N, 23.16%. Found: C, 63.20; H, 4.36; N, 23.17%.

RESULTS AND DISCUSSION

Chemistry

Compound **1** was synthesized as reported.[17] Compound **2a** as the starting material used in Scheme **1** was prepared from the reaction of formamidic acid ethyl ester **1** and excess of hydrazine hydrate in methanol at 0^{o}C [18]. Moreover, cyclization of formimidic acid ethyl ester **1** to the corresponding iminopyrimidine was occurred in ethanol using different basic reagents such as aliphatic amines (namely, methyl amine) or aromatic amines (namely, phenyl hydrazine) to afford **2b&c**, respectively in good yield. The formation of compound **2b&c** was confirmed by ^{1}HNMR which revealed the presence of =NH group at δ 7.30 and 3.20, sequentially.

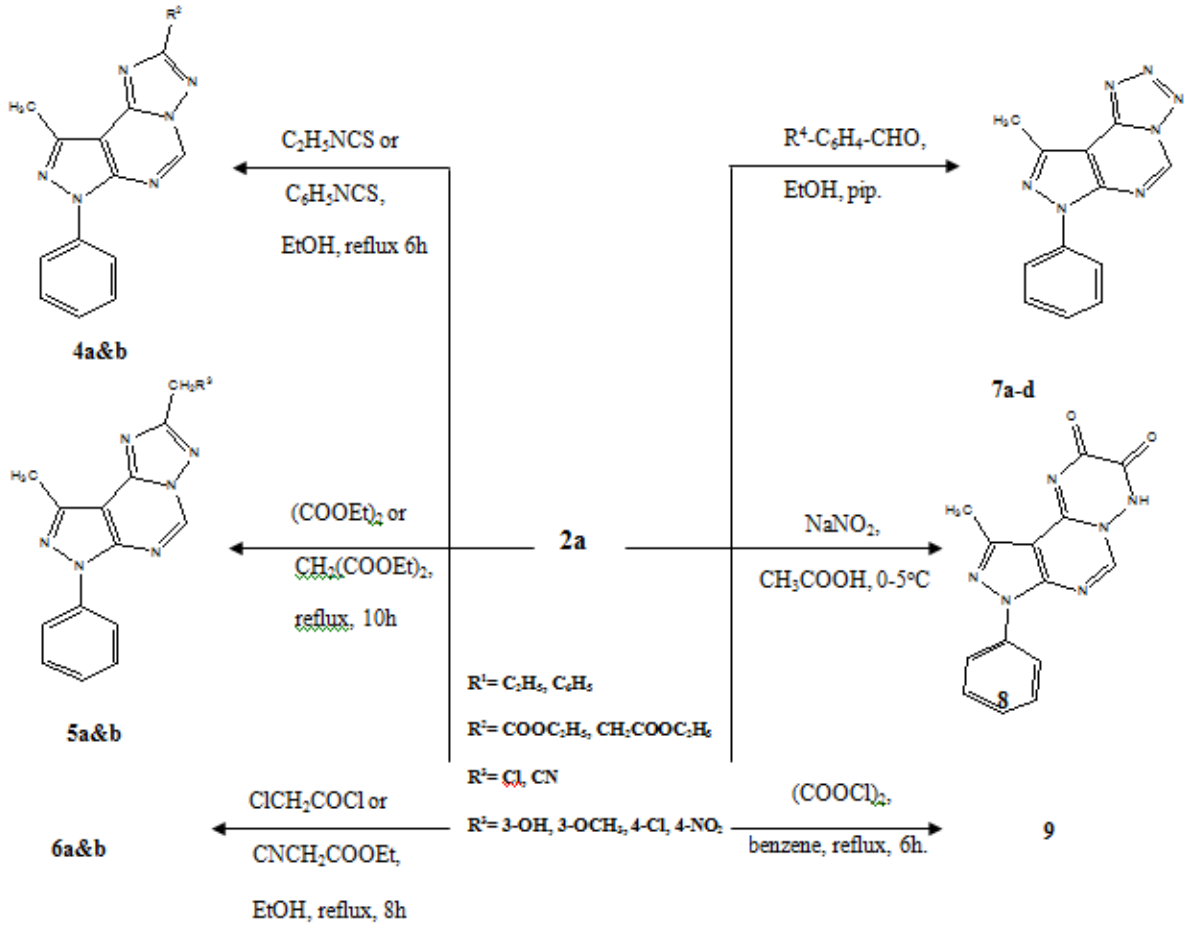


Scheme 1

Moreover, the mass spectrum of **2b&c** wed that compound **3** was synthesized by Davoodnia *et al*. [19] from the reaction of 4-chloro-1*H*-pdisplayed molecular ion peak at *m/z* 239 and 316 ($M^{+\cdot}$**)**, respectively. Searching in literature shoyrazolo[3,4-*d*]pyrimidine **A** and hydrazine hydrate at room temperature for 10 h. In this work, a new method for the preparation of compound **3** was depicted from compound **1** depending on Dimorth rearrangement in basic conditions and under refux in ethanol as in similar reactions [20-21]. The ^{1}HNMR spectrum of **3** showed the characteristic singlet signals at δ 4.74 ppm and at δ 8.79 ppm for the protons of NH_2 and NH, sequentially, which were disappeared upon addition of D_2O. Also, mass spectrum of compound **3** showed the corresponding molecular ion peak at *m/z* 240 **(Scheme 1)**.

The 2-ethyl or phenyl amino derivatives **4a&b** were obtained when **2a** was treated with ethyl or phenyl isothiocyanate. ^{1}HNMR spectrum of **4a** revealed the presence of the NH signal at δ 7.03 ppm as triplet. Besides, a multiplet signal appeared at δ 3.31 ppm corresponding to CH_2 protons. While CH_3 of the ester group appeared at δ 1.20 as triplet signal.

Heating compound **2a** with diethyl oxalate or diethyl malonate under reflux conditions gave compounds **5a&b**, sequentially. IR spectra of **5a&b** showed absorption bands at 1739 and 1735 cm^{-1}, respectively, attributed to C=O of ester group. Also, the reaction of **2a** with chloroacetyl chloride and ethyl cyanoacetate in refluxing ethanol yielded the cyclized form of chloromethyl triazole **6a** and methyl carbonitrile **6b**. The IR spectrum of **6b** showed the presence of cyano group at 2254 cm^{-1}. Furthermore, ^{1}HNMR spectrum for compound **6b** revealed a singlet signal at δ 4.56 ppm attributed to two protons of CH_2CN. Reacting compound **2a** with different aromatic aldehydes, the cyclized triazole derivatives were obtained **7a-d**. The mass spectrum of compound **7a** exhibited a molecular ion peak at *m/z* 342. Tetrazolo derivative **8** was obtained when compound **2a** was subjected to the diazotization reaction conditions. Moreover, the reaction of **2a** and oxalyl chloride afforded tricyclic structure **9** with the construction of six member ring system. IR spectrum of compound **9** showed two C=O groups at 1726, 1658 cm^{-1}. ^{1}HNMR spectrum of **9** revealed the presence of an exchangeable singlet peak at δ 12.31 ppm corresponding to NH proton **(Scheme 2)**.



Scheme 2

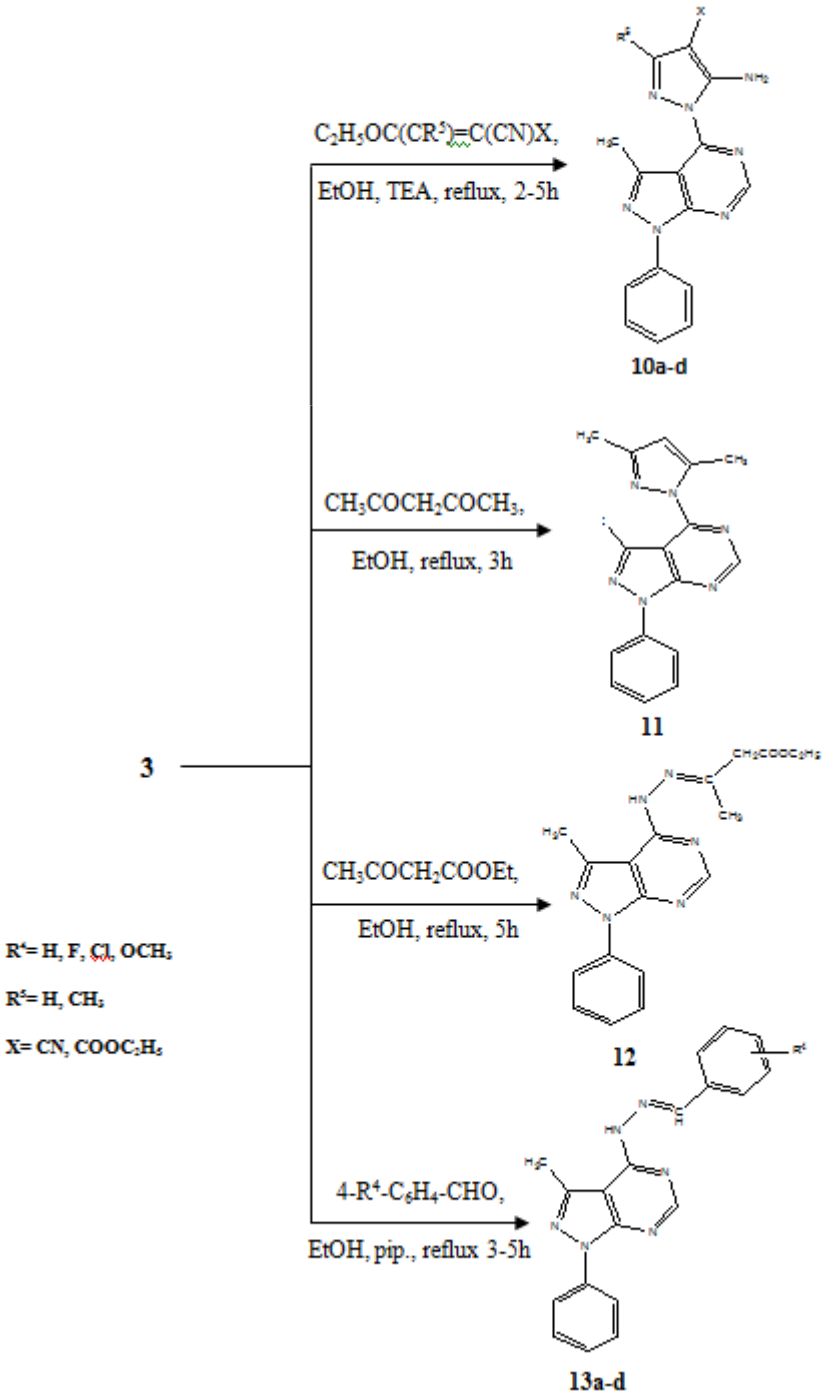
The second key compound **3** reacted with the appropriate ethoxymethylene or ethoxyethylidene malononitrile or cyanoacetate giving new pyrazole ring bearing *ortho* amino- cyano or amino ester groups **10a-d**. The IR spectra of compounds **10a&b** revealed the C≡N group at 2213 and 2216 cm^{-1}, sequentially.[1]HNMR showed a single signal at δ 8.36 ppm corresponding to two protons of NH_2 which were D_2O exchangeable. The C=O group of the ester moiety in compounds **10c&d** appeared in IR spectra at 1688 and 1678 cm^{-1}, respectively. Besides, the NH_2 group of **10c&d** appeared at δ 7.60 and 7.68 ppm, sequentially. Reaction of **3** with acetyl acetone gave another pyrazole ring with two methyl groups **11**. In contrast, when compound **3** was allowed to react with ethyl acetoacetate in refluxing ethanol, or fused with ethylacetoacetate then refluxed with ethanol, the pyrazole ring was not formed and we got an open chain product with IR spectrum has C=O group at 1714 cm^{-1}. [1]HNMR spectrum showed ethyl group of the ester moiety, two singlet peaks at δ 1.17, 1.21 ppm for CH_3 (*Z* and *E* isomeric forms) and at δ 3.44 and 3.60 ppm for CH_2 (*Z* and *E* isomeric forms). Also, the NH appeared as singlet signal at δ 11.66 ppm disappeared by D_2O. Mass spectrum of **11** showed a molecular ion peak at *m/z* 325 and a base peak at *m/z* 265. This confirmed the open acyclic structure of compound **11**.

Different arylidene derivatives **12a-d** were also obtained from compound **3** and different aromatic aldehydes. As arylidene-hydrazide structure was reported to exist in two geometrical isomers (*E* and *Z*) about –C=N double bond,[22,23] the structure of compounds **12a-d** was confirmed with IR, [1]HNMR and mass spectra for their presence as *Z* and *E* geometrical isomers. [1]HNMR of compound **12a** showed two sets of signals at δ 2.56 and 2.81 ppm attributed to CH_3 protons. Also, CH proton of pyrimidine ring appeared at δ 8.38 and 8.45 ppm. Moreover, *Z* and *E* isomers of NH proton appeared at δ 11.81 and 11.98 ppm. [13]CNMR of compound **12c** revealed the presence of two peaks at δ 14.07 and 18.32 ppm corresponding to protons of CH_3 group. Also –N=CH- appeared as two sets of peaks at δ 147.66 and 147.98 ppm **(Scheme 3).**

BIOLOGICAL TESTING

Materials and methods

The human breast tumor cell line (MCF-7) was obtained from NCI, Cairo, Egypt.



Measurement of potential cytotoxicity

The effects of compounds on the growth of tumor cell lines (MCF-7), were evaluated according to the procedure adopted by the National Cancer Institute, Cairo, Egypt for the *in vitro* anticancer drug screening that use the protein-binding dye sulforhodamine B (SRB) to assess growth inhibition.[24] Cell were routinely maintained as adherent cell cultures in RPMI- 1640 medium supplemented with 10% heat-inactived fetal bovine serum (FBS) and 1% penicillin/ streptomycin at 37^{o}C in humidified atmosphere containing 5% CO_2. The cell line was regularly subcultured to be maintained in the expotential growth phase. Cells were exposed for 48 h to five concentrations of compounds (0, 5, 12.5, 25and 50 ug/ml). Compounds were prepared in dimethylsulphoxide (DMSO), were freshly diluted with cell

culture medium just prior the assays. Methotrexate was used as positive control. For each test compound and the cell line a dose-response curve was generated and the growth inhibition of 50% (IC_{50}), corresponding to the concentration of compound that inhibits 50% of the net cell growth was determined. The results of *in vitro* cytotoxic activity experiments are presented in **(Table 1)**.

Table 1: Results of *in vitro* cytotoxic activity of some of the synthesized compounds on human breast adenocarcinoma cell line (MCF-7)

Compound no.	IC_{50} in μM[a]
2a	0.057
4a	0.073
4b	**0.013**
5a	**0.015**
5b	0.049
6a	**0.025**
6b	0.051
7b	**0.042**
10c	**0.018**
12c	**0.015**
Methotrexate	0.046

[a] *The values given are means of three experiments.*

RESULTS AND DISCUSSION

Six of the ten newly test compounds exhibited antitumor activity against MCF-7 with IC_{50} between 0.013 to 0.042 μM. On the other hand, compound **4a** gave the highst result than all the other compounds. Furthermore, the result obtained from compounds **5a, 6a, 7b, 10c** and **12c** showed good antitumor activity (IC_{50} between 0.015 to 0.042 μM) than that of the other test compounds and the standard methotrexate.

CONCLUSION

New heterocycles were obtained from the reaction between compounds **2a** and **3** with several commercially available reactants. All compounds obtained retain the pyrazolo [3,4-*d*] pyrimidine core. Some of the newly synthesized compounds were tested *in vitro* on human breast cancer cell line (MCF-7). Some of the test compounds showed potent antitumor activity, especially compound **4b** with IC_{50} equal to 0.013 μM. Compounds **5a**, **6a**, **7b**, **10c** and **12c** showed also good antitumor activity with IC_{50} between 0.015 and 0.042 μM. While, compounds **2a**, **4a**, **5b** and **6b** showed weak antitumor activity with IC_{50} between 0.049 and 0.073 μM compared to methotrexate the positive control which has IC_{50} equal to 0.046 μM. Thus, introducing substituted amino group or ester group at the triazole moiety or pyrazole ring resulted in higher cytotoxic activity.

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