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Synthesis, Characterization and *in vitro* Antitumor Evaluation of New Pyrazolo[3,4-*d*]Pyrimidine Derivatives

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Abstract

A new series of 3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine derivatives was synthesized. The antitumor activity of this series against human breast adenocarcinoma cell line MCF7 was evaluated. Out of twenty new derivatives, ten were revealed mild to moderate activity compared with doxorubicin as a reference antitumor. Among this new series *N*-(2-chlorophenyl)-2-(3-(methylthio)-4-oxo-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-5(4*H*)-yl)acetamide (**13 a**) was found the most active one with IC_{50} equal to 23 µM.

Keywords: Pyrazolo[3,4-*d*]pyrimidine; Antitumor; Human breast adenocarcinoma cell line MCF7

Introduction

Cancer is the most serious health problem and the second major cause of death in the developing countries [1,2]. In spite of significant process in the development of novel chemotherapeutic agents in the last seven decades, success in developing targeted non-toxic drugs with minor side effects has only achieved in the last one [3]. Therefore, the discovery of new selective, potent and safe antitumor agents is a must. Pyrazolo[3,4-*d*] pyrimidine nucleus is the bio-isostere of purine [4,5]. Hence exhibits promising activity as antitumor by competitive inhibition for ATP kinase enzymes. Many pyrazolo[3,4-*d*]pyrimidine derivatives were reported as antitumor agents [6-8]. The cytotoxic activity of such compound may attributed to inhibition of several enzymes such as tyrosine kinase [9], Src kinase [10], cyclin dependent kinase (CDK) [11], mammalian target of rapamycin (mTOR) [12] and glycogen synthase kinase (GSK) [13,14]. In addition, the presence of methylsulphanyl group at the 3 position of pyrazolo[3,4-*d*]pyrimidine nucleus was reported to potentiate the antitumor activity of such nucleus [11,15]. For example, compound **1** and **2** (Figure 1) were exhibited excellent antitumor activity against breast adenocarcinoma cell line MCF 7 with an IC_{50} values of 12.0 and 7.50 µM respectively [16]. Also, compound 3 displayed superior activity as cytotoxic against A549 cell line with IC_{50} K_b value of 5.28 µM [4].

Based on these scientific facts and for further exploration of novel antitumor agents, we supposed that incorporation of these structural features together may results in potent antitumor agents that act on breast adenocarcinoma cell line. In this work, new 3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine derivatives **10-16** were synthesized, incorporating the methylsulphanyl group at the 3 position of pyrazolo[3,4-*d*]pyrimidine ring system and varying the substituents at the 4 and 5 positions of such ring in order to study the effect of these varying substitutions on the antitumor activity of pyrazolo[3,4-*d*] pyrimidine nucleus against human breast adenocarcinoma cell line MCF7.

Results and Discussion

Chemistry

Scheme 1 shows the synthetic pathway of the starting pyrazolo[3,4-*d*] pyrimidin-4(5*H*)-one derivatives **8** and 4-chloro-3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**9**) which were accomplished via reaction of malononitrile with carbon disulfide in the presence of sodium ethoxide followed by methylation of the product with dimethyl sulphate. The resulting 2(bis(methylthio)methylene)malononitrile was then treated with phenyl hydrazine in absolute ethanol [17]. Cyclization of the 5-amino-3-(methylthio)-1-phenyl-1*H*pyrazole-4-carbonitrile (**7**) by the action of formic acid afforded 3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one [18]. Structure of the latter was confirmed by the disappearance of the C≡N and NH characteristic absorption bands in the IR spectrum of the starting 5-amino-1*H*pyrazole-4-carbonitrile **7**. Chlorination of compound **8** with phosphorus oxychloride yielded the 4-chloro derivative **9** [19,20]. The latter was allowed to react with different aliphatic and aromatic amines to afford the target pyrazolo[3,4-*d*]pyrimidin-4-amine derivatives $\mathbf{10}_{a\text{-}e}$, **11** and **12** (Scheme 2).

Formation of compounds **10-12** was confirmed by spectral data and elemental analyses. The ^{1}HNMR spectra of these derivatives demonstrated the appearance of a new D_2O exchangeable singlet signals at δ 8.48-8.60 ppm corresponding to the NH protons. Mass spectra of these compounds showed distinctive molecular ion peaks at the right m/z values.

Scheme 3 shows the synthetic pathway of the target compounds $\mathbf{13}_{a\text{-}h}$ through reaction of 3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*] pyrimidin-4(5*H*)-one (**8**) with 2-chloro-*N*-phenylacetamide or 3-chloro-*N*-phenylpropanamide derivatives. Structures of these amides were confirmed depending on spectral data and elemental analyses. The IR spectra of these compounds showed the characteristic NH stretching bands at the range of 3223-3317 cm^{-1}. In addition, the ^{1}HNMR spectra of the same derivatives showed singlet signals corresponding to NH protons at δ 9.72-10.78 ppm.

Ester derivatives $\mathbf{14}_{a,b}$ were prepared via condensation of compound **8** with alkyl chloroacetate in the presence of potassium

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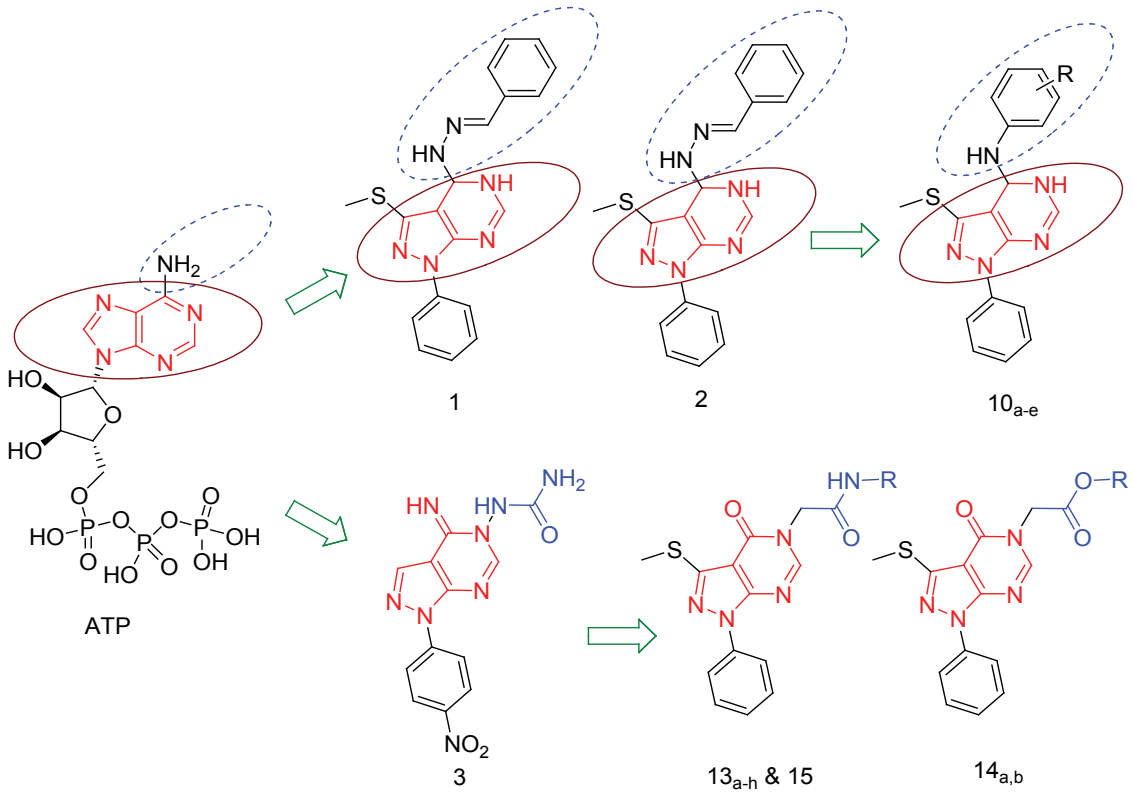
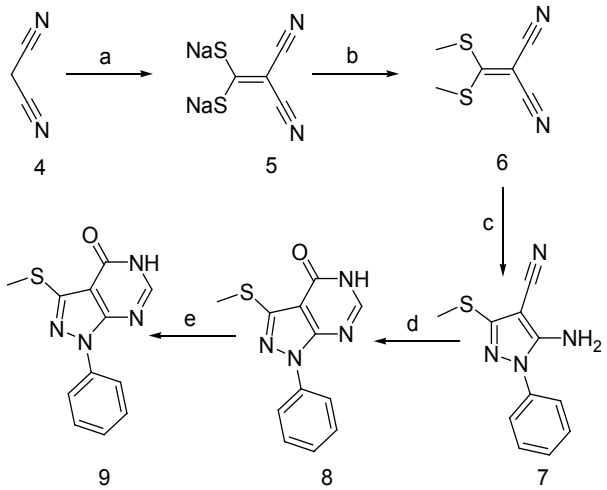
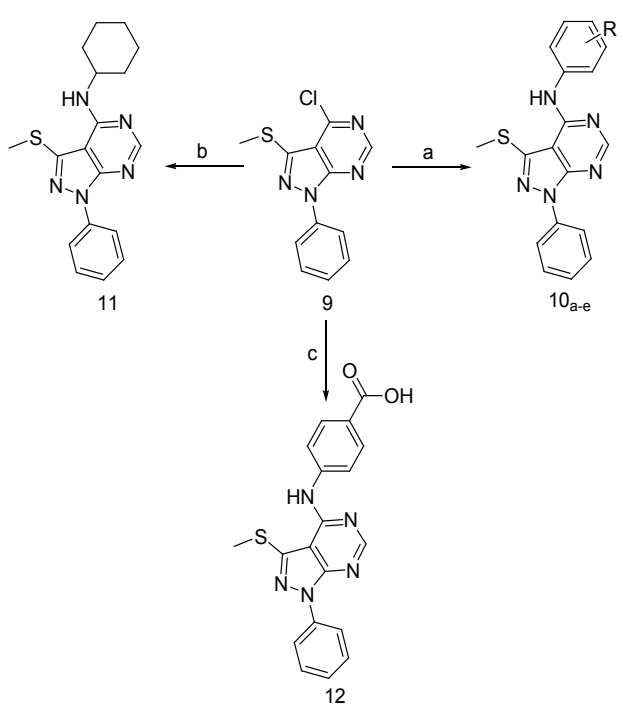


Figure 1: ATP, potent antitumor pyrazolo[3,4-*d*]pyrimidines and designed compounds.

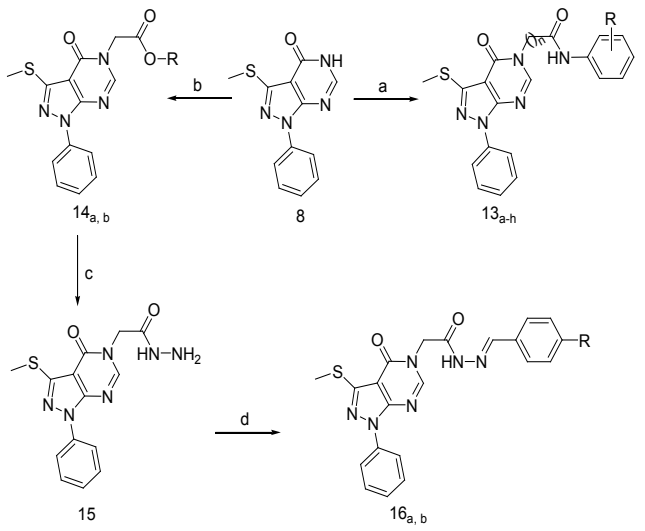


Scheme 1: Synthetic pathway of starting pyrazolo[3,4-*d*]pyrimidine.



Scheme 2: Synthetic pathway of target compounds 10-12. Reagents: a) $RC_6H_4NH_2$, $C_2H_5OH/(C_2H_5)_3N$; b) Cyclohexylamine, $C_2H_5OH/(C_2H_5)_3N$; c) 4-(Aminomethyl)benzoic acid, $C_2H_5OH/(C_2H_5)_3N$

carbonate. Structures of these two ester derivatives were confirmed on the basis of their spectral data and elemental analyses. The IR spectra of these esters showed sharp elevation of the wavenumber of the C=O absorption compared to that of the starting amide. Condensation of **14**$_b$ with hydrazine hydrate produced the hydrazide **15**. The ^{1}HNMR spectrum of this new compound revealed two D_2O exchangeable signals of the NH and NH_2 protons at 9.41 and 4.30 ppm respectively. Disappearance of the



Scheme 3: Synthetic pathway of target compounds **13-16.** Reagents: For **13**$_{a-d}$: a) $ClCH_2CONHC_6H_4R$, K_2CO_3, C_2H_5OH; For **13**$_{e-h}$: a) $ClCH_2CH_2CONHC_6H_4R$, K_2CO_3, C_2H_5OH; b) $ClCH_2CH_2COOR$, K_2CO_3, C_2H_5OH; c) NH_2NH_2; d) RC_6H_4CHO, C_2H_5OH

NH_2 signal in the ^{1}HNMR spectra of compounds **16**$_{a,b}$ confirms their structures.

For **13**$_{a-d}$ n=1, R=H, 2-Cl, 3-CH_3, 4-$COOC_2H_5$; For **13**$_{e-h}$: n=2, R=H, 2-Cl, 3-CH_3, 4-$COOC_2H_5$; For **14**$_{a-b}$: R=CH_3, C_2H_5; For **16**$_{a-b}$: R=H, OH

Reagents: For **13**$_{a-d}$: a) $ClCH_2CONHC_6H_4R$, K_2CO_3, C_2H_5OH; For **13**$_{e-h}$: a) $ClCH_2CH_2CONHC_6H_4R$, K_2CO_3, C_2H_5OH; b) $ClCH_2CH_2COOR$, K_2CO_3, C_2H_5OH; c) NH_2NH_2; d) RC_6H_4CHO, C_2H_5OH

In vitro antitumor screening

All of the newly synthesized derivatives were evaluated for antitumor activity by measuring the inhibitory effect of such compounds against human breast adenocarcinoma cell line MCF7 using MITT technique [21,22]. The MTT Cell Proliferation Assay measures the reduction in cancer cell viability due to apoptosis or necrosis as a response to external factor. The yellow colored tetrazolium salt of MTT is reducible by the action of metabolically active cells, through dehydrogenase enzymes that leads to generation of NADH and NADPH reducing equivalents. The produced intracellular purple formazan can be solubilized and spectrophotometrically quantified [23]. The results of *in vitro* antitumor activity were compared with doxorubicin as a reference antitumor agent. The parameter used herein is the IC_{50}, which represents the concentration needed for 50% inhibition of the cell viability. A relation between the IC_{50} values of the new compounds that showed more than 50% inhibition against MCF-7 and that of the reference antitumor agent are shown in Table 1 and represented graphically in Figure 2.

Experimental

General

All melting points were taken on electro thermal (LA9000 SERIS) digital melting point apparatus and are uncorrected. IR spectra were recorded on PyeUnicam Sp 1000 spectrophotometer and were carried out at the Pharmaceutical Analytical Unit, Faculty of Pharmacy, Al-Azhar University, Egypt. The ^{1}HNMR and ^{13}CNMR spectra were recorded in DMSO-D_6 either on Varian Mercury VXR-300 NMR spectrophotometer at the Microanalytical Unit of Cairo University or BURKER 400 MHZ spectrophotometer at the Nuclear Magnetic Resonance Lab, Faculty of Pharmacy, Zagazig University, Egypt. Chemical shifts were related to that of the solvent. TMS was used a standard. Mass spectra were recorded on Hewlett Packard 5988 spectrometer at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Progress of the reactions were monitored by TLC pre-coated with UV fluorescent silica gel and was visualized using UV lamp and different solvent systems as mobile phases. 5-Amino-3-(methylthio)-1-phenyl-1*H*-pyrazole-4-carbonitrile (**7**) and 3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**8**) were prepared according to published method[17]. Compound 9 was obtained following reported procedure [19]. 2-chloro-*N*-arylactamide and 3-chloro-*N*-arylpropanamide derivatives were prepared as reported [24].

General procedure for synthesis of 3-(methylthio)-1-phenyl-*N*-aryl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amines 10$_{a-e}$**:** A mixture of compound **9** (10 mmol) and the appropriate aniline derivative (10 mmol) in absolute ethanol (35 ml) containing trimethylamine (15 mmol) was heated under reflux for 6 hours. The reaction mixture was cooled, and the separated solid was filtered, dried and finally recrystallized from ethanol.

3-(Methylthio)-1-phenyl-N-o-tolyl-1H-pyrazolo[3,4-d] pyrimidin-4-amine (10a): White solid; Yield: 85%; m. p. 130-131°C. IR (KBr) cm^{-1}: 3372 (NH), 3047 (CH aromatic), 2924 (CH aliphatic). ^{1}HNMR (DMSO-*d6*) δ ppm: 8.45 (s, 1H, NH, D_2O exchangeable), 8.42 (s, 1H, pyrimidine-H2), 8.19 (d, 2H, *J*=1.80 Hz, phenylpyrazole-H2, H6), 7.80 (t, 2H, *J*=7.80 Hz, phenylpyrazole-H3, H5), 7.59 (t, 1H, *J*=2.10

Comp. No.	IC_{50} (in μM)
10$_a$	288
10$_e$	128
11	169
12	270
13$_a$	23
13$_b$	49
13$_d$	97
14$_a$	276
14$_b$	326
16$_b$	61
Doxorubicin	4.27

Table 1: Results of *in vitro* cytotoxic activity of compounds showed more than 50% inhibition of MCF 7 adenocarcinoma cell line.

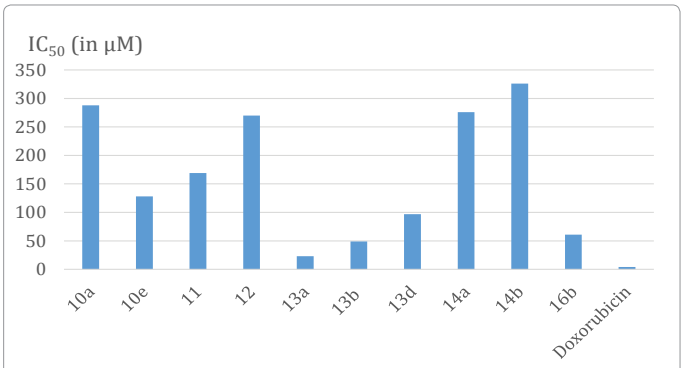


Figure 2: IC_{50} in μM of the synthesized compounds and doxorubicin against MCF7 adenocarcinoma cell line.

Hz, phenylpyrazole-H4), 7.56 (t, 1H, *J*=6.90 Hz, phenyl-H5), 7.38 (d, 1H, *J*=1.50 Hz, phenyl-H3),7.35 (d, 1H, *J*=1.50 Hz, phenyl-H6), 7.29 (t, 1H, *J*=6.00 Hz, phenyl-H4), 2.50 (s, 3H, SCH_3), 2.30 (s, 3H, Ar-CH_3). *MS* (m/z): 347 ($C_{19}H_{17}N_5S$, 53.48%, M^+), 332 ($C_{18}H_{15}N_5S$, M-CH_3, 74.57%), 77 (C_6H_5, 100%). Analytical Calculated for: ($C_{19}H_{17}N_5S$) (M.W.=347): C, 65.68; H, 4.39; N, 20.16%; Found: C, 65.81; H, 4.89; N, 20.31%.

2-(3-(Methylthio)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-ylamino)benzenethiol (10_b): White solid; Yield: 75%; m. p. 141-142°C. IR (KBr) cm^{-1}: 3291 (NH), 3053 (CH aromatic), 2918 (CH aliphatic). ^{1}HNMR (DMSO-*d6*) δ ppm: 12.42 (s, 1H, SH, D_2O exchangeable), 8.07 (s, 1H, pyrimidine-H2), 8.06 (d, 2H, *J*=4.80 Hz, phenylpyrazole-H2, H6), 7.93 (t, 2H, *J*=10.00 Hz, phenylpyrazole-H3, H5), 7.58 (t, 1H, *J*=3.00 HZ, phenylpyrazole-H4), 7.49 (t, 1H, *J*=10.00 HZ, phenyl-H5), 7.44 (d, 1H, *J*=2.80 Hz, phenyl-H3),7.35 (d, 1H, *J*=4.00 Hz, phenyl-H6), 7.32 (t, 1H, *J*=3.60 Hz, phenyl-H4), 2.60 (s, 3H, SCH_3). *MS* (m/z): 365 ($C_{18}H_{15}N_5S_2$, 2.33%, M^+). Analytical Calculated for: ($C_{18}H_{15}N_5S_2$) (M.W.=365): C, 59.16; H, 4.14; N, 19.16%; Found: C, 59.21; H, 3.83; N, 19.47%.

N-(2,6-dichlorophenyl)-3-(methylthio)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (10_c): White solid; Yield: 63%; m. p. 231-232°C. IR (KBr) cm^{-1}: 3455 (NH), 3050 (CH aromatic), 2938 (CH aliphatic). ^{1}HNMR (DMSO-*d6*) δ ppm: 10.40 (s, 1H, NH, D_2O exchangeable), 8.66 (s, 1H, pyrimidine-H2), 8.18 (d, 2H, *J*=4.80 Hz, phenylpyrazole-H2, H6), 8.04 (t, 2H, *J*=8.10 Hz, phenylpyrazole-H3, H5), 7.59 (t, 1H, *J*=6.90 Hz, phenylpyrazole-H4), 7.54 (d, 2H, *J*=7.20 Hz, phenyl-H3,H5), 7.39 (t, 1H, *J*=6.60 Hz, phenyl-H4), 2.60 (s, 3H, SCH_3). *MS* (m/z): 403 ($C_{18}H_{13}Cl_2N_5S$, 0.41%, M+2), 401 ($C_{18}H_{13}Cl_2N_5S$, 1.46%, M^+), 366 ($C_{18}H_{13}ClN_5S$, 2.42%), 331 ($C_{18}H_{13}N_5S$, 1.47%), 256 ($C_{12}H_{10}N_5S$, 3.01%), 241 ($C_{12}H_9N_4S$, 4.62%). Analytical Calculated for: ($C_{18}H_{13}Cl_2N_5S$) (M.W.=401): C, 53.74; H, 3.26; N, 17.41%; Found: C, 53.92; H, 3.23; N, 17.68%.

4-(3-(Methylthio)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-ylamino)benzoic acid (10_d): White solid; Yield: 85%; m. p. 162-164°C. IR (KBr) cm^{-1}: 3397 (OH), 3360 (NH), 3047 (CH aromatic), 2924 (CH aliphatic), 1689 (C=O). ^{1}HNMR (DMSO-*d6*) δ ppm: 13.80 (s, 1H, OH, D_2O exchangeable), 11.25 (s, 1H, NH, D_2O exchangeable), 8.52 (s, 1H, pyrimidine-H2), 8.50-7.30 (m, 9H, Ar-H), 2.62 (s, 3H, SCH_3). *MS* (m/z): 377 ($C_{19}H_{15}N_5O_2S$, 1.19%, M^+), 333 ($C_{18}H_{15}N_5S$, 2.4%). Analytical Calculated for: ($C_{19}H_{15}N_5O_2S$) (M.W.=377): C, 60.47; H, 4.01; N, 18.56%; Found: C, 60.64; H, 4.09; N, 18.73%.

Ethyl-4-((3-(methylthio)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)amino)benzoate (10_e): White solid; Yield: 85%; m. p. 123-124°C. IR (KBr) cm^{-1}: 3372 (NH), 3033 (CH aromatic), 2940 (CH aliphatic), 1710 (C=O). ^{1}HNMR (DMSO-*d6*) δ ppm: 8.79 (s, 1H, NH, D_2O exchangeable), 8.60 (s, 1H, pyrimidine-H2), 8.17 (d, 2H, *J*=1.80 Hz, phenylpyrazole-H2, H6), 7.97 (t, 2H, *J*=8.70 Hz, phenylpyrazole-H3, H5), 7.56 (d, 2H, *J*=8.10 Hz, phenyl-H2,H6), 7.37 (t, 1H, *J*=6.00 Hz, phenylpyrazole-H4), 7.33 (t, 2H, *J*=6.00 Hz, phenylH2,H6), 4.23 (q, 2H, *J*=7.20 Hz, CH_2CH_3), 2.62 (s, 3H, S-CH_3), 1.3 (t, 3H, *J*=6.6 Hz, CH_2CH_3). *MS* (m/z): 405 ($C_{21}H_{19}N_5O_2S$, 6.85%, M^+), 376 ($C_{19}H_{14}N_5O_2S$, 3.72%), 256 ($C_{12}H_{10}N_5S$, 2.27%), 241 ($C_{12}H_9N_4S$, 12.52%). Analytical Calculated for: ($C_{21}H_{19}N_5O_2S$) (M.W.=405): C, 62.21; H, 4.72; N, 17.27%; Found: C, 62.47; H, 4.81; N, 17.49%.

N-Cyclohexyl-3-(methylthio)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (11): Into a solution of equimolar amounts of compound **9** and cyclohexylamine (10 mmol each) in ethanol (30 ml), trimethylamine (15 mmol) was added. The reaction mixture was heated under reflux for 6 hours then allowed to cool. The crude product was filtered out, dried and finally recrystallized from ethanol. White solid; Yield: 74%; m. p. 114-116 °C. IR (KBr) cm^{-1}: 3397 (NH), 3031 (CH aromatic), 2925 (CH aliphatic). ^{1}HNMR (DMSO-*d6*) δ ppm: 8.36 (s, 1H, pyrimidine-H2), 8.16 (d, 2H, H6, *J*=8.10, phenyl-H2), 7.55 (t, 2H, H5, *J*=8.40, phenyl-H3), 7.33 (t, 1H, *J*=7.20, phenyl-H4), 6.32 (s, 1H, NH, D_2Oexchangeable), 2.71 (s, 3H, SCH_3) 1.97-1.36 (m, 11H, cyclohexyl). *MS* (m/z): 339 ($C_{18}H_{21}N_5S$, 23.10%, M^+), 257 ($C_{12}H_{11}N_5S$, M-C_6H_{11}, 100%). Analytical Calculated for: ($C_{18}H_{21}N_5S$) (M.W.=339): C, 63.69; H, 6.24; N, 20.63%; Found: C, 63.85; H, 6.32; N, 20.86%.

4-((3-(Methylthio)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-ylamino)methyl)benzoic acid (12): Into a solution of equimolar amounts of compound **9** and 4-(aminomethyl)benzoic acid (10 mmol each) in ethanol (30 ml), trimethylamine (15 mmol) was added. The reaction mixture was heated under reflux for 6 hours then allowed to cool. The crude product was filtered out, dried and finally recrystallized from ethanol. White solid; Yield: 70%; m. p. 170-171°C. IR (KBr) cm^{-1}: 3382 (broad OH), 3027 (CH aromatic), 2985 (CH aliphatic), 1699 (C=O). ^{1}HNMR (DMSO-*d6*) δ ppm: 10.82 (s, 1H, OH), 8.39 (s, 1H, pyrimidine-H2), 7.88 (d, 2H, phenyl-H2, H6, *J*=7.2), 7.30 (m, 5H, phenylpyrazole), 6.87 (d, 2H, phenyl-H3, H5, *J*=7.2), 6.28 (s, 1H, NH), 4.32 (s, 2H, CH_2), 2.68 (s, 3H, S-CH_3). *MS* (m/z): 391 ($C_{20}H_{17}N_5O_2S$, M^+, 100%), 270 ($C_{19}H_{14}N_5O_2S$, M-CH_3, 46.20%), 256 ($C_{12}H_{11}N_5S$, M-COOH-$C_6H_4CH_2$, 43.23%). Analytical Calculated for: ($C_{20}H_{17}N_5O_2S$) (M.W.=391): C, 61.37; H, 4.38; N, 17.89%; Found: C, 61.59; H, 4.43; N, 18.15%.

General procedure for synthesis of 2-(3-(methylthio)-4-oxo-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-5(4H)-yl)-*N*-arylacetamide and *N*-arylpropanamide 13_{a-h}: Into a solution of compound **8** (10 mmol each) in DMF (30 ml) containing potassium carbonate (0.5 g), the appropriate 2-chloro *N*-arylacetamide or 3-chloro-*N*-arylpropanamide (10 mmol) was added. The reaction mixture was heated under reflux for 3 hours. After complete reaction, the reaction mixture was filtered while hot, concentrated, cooled and the resulting solid product was dried and finally recrystallized from ethanol.

2-(3-(Methylthio)-4-oxo-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-5(4H)-yl)-N-phenylacetamide (13_a): White solid; Yield: 85%; m. p. 164-165°C. IR (KBr) cm^{-1}: 3307 (NH), 3053 (CH aromatic), 2925 (CH aliphatic), 1672 (C=O). ^{1}HNMR (DMSO-*d6*) δ ppm: 10.42 (s, 1H, NH, D_2O exchangeable), 8.47 (s, 1H, pyrimidine-H2), 8.04 (t, 2H, *J*=7.20 Hz, Aniline-H3,H5), 7.59 (d, 2H, *J*=2.00 Hz, Aniline-H2,H6), 7.57 (d, 2H, *J*=8.40 Hz, phenylpyrazole-H3,H5), 7.41 (t, 1H, *J*=7.20 Hz, 7.34 (t, 2H, *J*=7.60 Hz, phenylpyrazole-H2,H6), 7.07 (t, 1H, *J*=7.20 Hz, phenylpyrazole-H4), 4.87 (s, 2H, CH_2C=O), 2.63 (s, 3H, S-CH_3). ^{13}CNMR (DMSO-*d6* 400 MHz) δ ppm: 13.26,48.59, (Aliphatic CH_3 and CH_2), 104.94, 119.53, 121.76, 124.07, 127.39, 129.36, 129.72, 138.45, 139.04, 146.08, 152.96, 153.18, 156.51 (Aromatic carbons), 165.74 (C=O). *MS* (m/z): 391 ($C_{20}H_{17}N_5O_2S$, M, 42.33%), 299 ($C_{14}H_{11}N_4O_2S$, M-NHC_6H_5, 100%), 271 ($C_{13}H_{11}N_4OS$, 51.75%), 257 ($C_{12}H_9N_4OS$, 8.2%). Analytical Calculated for: ($C_{20}H_{17}N_5O_2S$) (M.W.=391): C, 61.37; H, 4.38; N, 17.89%; Found: C, 61.48; H, 4.43; N, 18.12%.

N-(2-chlorophenyl)-2-(3-(methylthio)-4-oxo-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-5(4H)-yl)acetamide (13_b): White solid; Yield: 78%; m. p. 146-147°C. IR (KBr) cm^{-1}: 3258 (NH), 3072 (CH aromatic), 2932 (CH aliphatic), 1695 (C=O). ^{1}HNMR (DMSO-*d6*) δ ppm: 10.09 (s, 1H, NH, D_2O exchangeable), 8.48 (s, 1H, pyrimidine-H2), 8.04 (d, 2H, *J*=7.80 Hz, Phenylpyrazole-H2, H6), 7.75 (d, 1H, *J*=8.10 Hz, Aniline-H6), 7.57 (d, 1H, *J*=8.10 Hz, Aniline-H3), 7.55 (t, 2H, *J*=8.10 Hz, Phenylpyrazole-H3,H5), 7.53(t, 1H, *J*=8.10 Hz, Phenylpyrazole-H4), 7.4 (t, 1H, J=7.60 Hz, Aniline-H5), 7.2

(t, 1H, *J*=7.60 Hz, Aniline-H4), 4.87 (s, 2H, CH_2C=O), 2.63 (s, 3H, S-CH_3). *MS* (m/z): 427 ($C_{20}H_{16}ClN_5O_2S$, M+2, 1.52%), 4.82%,425 ($C_{20}H_{16}ClN_5O_2S$, M^+, 4.82%), 299 ($C_{14}H_{11}N_4O_2S$, M-NHC_6H_4Cl, 84.7%), 271 ($C_{13}H_{11}N_4OS$, 100%). Analytical Calculated for: ($C_{20}H_{16}ClN_5O_2S$) (M.W.=425): C, 56.40; H, 3.79; N, 16.44%; Found: C, 56.61; H, 3.76; N, 16.58%.

2-(3-(Methylthio)-4-oxo-1-phenyl-1H-pyrazolo[3,4-d] pyrimidin-5(4H)-yl)-N-m-tolylacetamide (13_c): White solid; Yield: 82%; m. p. 152-153°C. IR (KBr) cm^{-1}: 3299 (NH), 3038 (CH aromatic), 2925 (CH aliphatic), 1672 (C=O). ^{1}HNMR (DMSO-*d6*) δ ppm: 10.37 (s,1H, NH, D_2O exchangeable), 8.46 (s, 1H, pyrimidine-H2), 8.06 (d, 2H, *J*=7.60 Hz, Phenylpyrazole-H2H6), 7.59 (d, 1H, *J*=7.60 Hz, Aniline-H6), 7.43 (t, 2H, *J*=5.60 Hz, phenylpyrazole-H3,H5), 7.39 (t, 1H, *J*=7.60 Hz, Phenylpyrazole-H4), 7.36 (s, 1H, Aniline-H2), 7.22 (t, 1H, *J*=8.00 Hz, Aniline-H5), 6.90 (d, 1H, *J*=7.60 Hz, Aniline-H4), 4.87 (s, 2H, CH_2C=O), 2.63 (s, 3H, S-CH_3), 2.27 (s, 3H, Ar-CH_3). *MS* (m/z): 405 ($C_{21}H_{19}N_5O_2S$, M, 52.08%), 299 ($C_{14}H_{11}N_4O_2S$, M-$NHC_6H_4CH_3$, 100%), 271 ($C_{13}H_{11}N_4OS$, 67.81%). Analytical Calculated for: ($C_{21}H_{19}N_5O_2S$) (M.W.=405): C, 62.21; H, 4.72; N, 17.27%; Found: C, 62.38; H, 4.76; N, 17.49%.

Ethyl 4-(2-(3-(methylthio)-4-oxo-1-phenyl-1H-pyrazolo[3,4-d] pyrimidin-5(4H)-yl)acetamido)benzoate (13_d): White solid; Yield: 65%; m. p. 72-73°C. IR (KBr) cm^{-1}: 3287 (NH), 3058 (CH aromatic), 2988 (CH aliphatic), 1695 (C=O). ^{1}HNMR (DMSO-*d6*) δ ppm: 10.78 (s, 1H, NH), 8.4 (s, 1H, pyrimidine-H2), 8.70 (d, 2H, *J*=8.80 Hz, Aniline-H3,H5), 7.80 (d, 2H, *J*=6.80 Hz, Aniline-H2,H6), 7.80 (t, 2H, *J*=8.40 Hz, Phenylpyrazole-H3,H5), 7.51 (t, 2H, *J*=8.10 Hz, Phenylpyrazole-H2,H6), 7.42 (t, 1H, *J*=7.20 Hz, Phenylpyrazole-H4), 4.90 (s, 2H, CH_2C=O), 4.30 (q, 2H, *J*=6.80 Hz, CH_2CH_3), 2.60 (s, 3H, S-CH_3), 1.29 (t, 3H, *J*=7.20 Hz, CH_2CH_3). *MS* (m/z): 463 ($C_{23}H_{21}N_5O_4S$, M, 5.65%), 299 ($C_{14}H_{11}N_4O_2S$, M-$NHC_6H4COOC_2H_5$, 19.63%), 271 ($C_{13}H_{11}N_4OS$, 18.52%), 174 ($C_9H_5N_2S$, 100%). Analytical Calculated for: ($C_{23}H_{21}N_5O_4S$) (M.W.=463): C, 59.60; H, 4.57; N, 15.11%; Found: C, 59.70; H, 4.63; N, 15.15%.

3-(3-(Methylthio)-4-oxo-1-phenyl-1H-pyrazolo[3,4-d] pyrimidin-5(4H)-yl)-N-phenylpropanamide (13_e): White solid; Yield: 80%; m. p. 154-155°C. IR (KBr) cm^{-1}: 3317 (NH), 3010 (CH aromatic), 2924 (CH aliphatic), 1674 (C=O). ^{1}HNMR (DMSO-*d6*) δ ppm: 12.44 (s, 1H, NH, D_2O exchangeable), 8.44 (s, 1H, pyrimidine-H2), 8.02 (t, 2H, *J*=7.20 Hz, Phenylpyrazole-H2,H6), 7.54 (d, 2H, *J*=2.00 Hz, Aniline-H2,H6), 7.50 (d, 2H, *J*=8.40 Hz, phenylpyrazole-H3,H5), 7.37 (t, 1H, *J*=7.20 Hz), 7.33 (t, 2H, *J*=7.60 Hz, phenylpyrazole-H4), 7.28 (t, 1H, *J*=7.20 Hz, Aniline-H3,H5), 7.25 (t, 1H, *J*=7.20 Hz, Aniline-H4), 4.26 (t, 2H, *J*=7.20 Hz NCH_2), 2.87 (t, 2H, *J*=7.20 Hz CH_2C=O), 2.61 (s, 3H, S-CH_3). ^{13}CNMR (DMSO-*d6* 400 MHz) δ ppm: 13.27, 31.12, 31.22 (Aliphatic carbons), 105.86, 119.62, 121.66, 122,79, 129.74, 138.60, 145.89, 150.40, 152.79, 153.48, 156.58, 157.42, 162.74 (Aromatic carbons), 169.09 (C=O). *MS* (m/z): 405 ($C_{21}H_{19}N_5O_2S$, M, 45.17%), 313 ($C_{15}H_{13}N_4O_2S$, M- NHC_6H_5, 48.33%), 285 ($C_{14}H_{13}N_4OS$, 7.07%), 259 ($C_{12}H_9N_4OS$, 100%). Analytical Calculated for: ($C_{21}H_{19}N_5O_2S$) (M.W.=405): C, 62.21; H, 4.72; N, 17.27%; Found: C, 62.45; H, 4.75; N, 17.53%.

N-(2-chlorophenyl)-3-(3-(methylthio)-4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)propanamide (13_f): White solid; Yield: 70%; m. p. 149-150°C. IR (KBr) cm^{-1}: 3301 (NH), 3083(CH aromatic), 2931 (CH aliphatic), 1680 (C=O). ^{1}HNMR (DMSO-*d6*) δ ppm: 9.72 (s, 1H, NH D_2O exchangeable), 8.44 (s, 1H, pyrimidine-H2), 8.03 (d, 2H, *J*=7.80 Hz, Phenylpyrazole-H2,H6), 7.61 (d, 1H, *J*=8.10 Hz, Aniline-H6), 7.57 (d, 1H, *J*=8.10 Hz, Aniline-H3), 7.46 (t, 2H, *J*=8.10 Hz, Phenylpyrazole-H3H5), 7.39 (t, 1H, *J*=8.10 Hz, Phenylpyrazole-H4), 7.34 (t, 1H, *J*=7.50 Hz, Aniline-H5), 7.20 (t, 1H, *J*=7.60 Hz, Aniline-H4), 4.29 (t, 2H, *J*=80 Hz, CH_2CH_2), 2.8 (t, 2H, *J*=10.00 Hz, CH_2-C=O), 2.50 (s, 3H, S-CH_3). *MS* (m/z): 441 ($C_{21}H_{18}ClN_5O_2S$, M+2, 3.78%, M^+), 439 ($C_{21}H_{18}ClN_5O_2S$, M, 11.49%), 313 ($C_{15}H_{13}N_4O_2S$, 35.54%), 285 ($C_{14}H_{13}N_4OS$, 5.89%), 257 ($C_{12}H_9N_4OS$, 8.2%). Analytical Calculated for: ($C_{21}H_{18}ClN_5O_2S$) (M.W.=439): C, 57.34; H, 4.12; N, 15.92%; Found: C, 57.49; H, 4.19; N, 16.08%.

3-(3-(methylthio)-4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)-N-(m-tolyl)propanamide (13_g): White solid. Yield: 55%; m. p. 138-141°C. IR (KBr) cm^{-1}: 3223 (NH), 3049(CH aromatic), 2980 (CH aliphatic), 1693 (C=O). ^{1}H NMR (DMSO-d_6) δ ppm: 9.92 (s, 1H, NH D_2O exchangeable), 8.45 (s, 1H, pyrimidine-H2), 8.02 (d, 2H, *J*=7.8 Hz, Phenylpyrazole-H2H6), 7.56 (d, 1H, *J*=7.80 Hz, Aniline-H6), 7.38 (t, 2H, *J*=5.70 Hz, phenylpyrazole-H3,H5), 7.32 (t, 1H, *J*=8.40 Hz, Phenylpyrazole-H4), 7.17 (s, 1H, Aniline-H2), 7.14 (t, 1H, *J*=8 Hz, Aniline-H5), 6.85 (d, 1H, *J*=7.50 Hz, Aniline-H4), 4.2 (t, 2H, SCH_2), 2.8 (t, 2H, CH_2-C=O), 2.6 (s, 3H, Ar-CH_3), 2.2 (s, 3H, S-CH_3). MS (m/z): 419 ($C_{22}H_{21}N_5O_2S$, M, 2.06%,), 314 ($C_{15}H_{14}N_4O_2S$, 10.23%), 257 ($C_{12}H_9N_4OS$, 8.2%), 105 (C_7H_6N, 100%). Analytical Calculated for: ($C_{22}H_{21}N_5O_2S$) (M.W.=419): C, 62.99; H, 5.05; N, 16.69%; Found: C, 63.21; H, 5.11; N, 16.87%.

Ethyl 4-(3-(3-(methylthio)-4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)propanamido)benzoate (13_h): White solid. Yield; 85%; m. p. 160 °C. IR (KBr) cm^{-1}: 3287 (NH), 3058 (CH aromatic), 2988 (CH aliphatic), 1695 (C=O). ^{1}HNMR (DMSO-d_6) δ ppm: 10.78 (s, 1H, NH), 8.40 (s, 1H, pyrimidine-H2), 8.71 (d, 2H, *J*=8.80 Hz, Aniline-H3,H5), 7.81 (d, 2H, *J*=6.80 Hz, Aniline-H2H6), 7.81 (t, 2H, *J*=8.40 Hz, Phenylpyrazole-H3H5), 7.53 (t, 2H, *J*=8.10 Hz, Phenylpyrazole-H2H6), 7.42 (t, 1H, *J*=7.20 Hz, Phenylpyrazole-H4), 3.90 (t, 2H, *J*=7.20, SCH_2), 4.35 (t, 2H, *J*=7.20, CH_2C=O), 4.3 (q, 2H, *J*=6.80 Hz, CH_2CH_3), 2.6 (s, 3H, S-CH_3), 1.29 (t, 3H, *J*=7.20 Hz, CH_2CH_3). MS (m/z): 477 ($C_{24}H_{23}N_5O_4S$, 27.54%, M^+), 432 ($C_{22}H_{18}N_5O_3S$, 2.51%), 313 ($C_{15}H_{13}N_4O_2S$, 55.20%), 285 ($C_{14}H_{13}N_4OS$, 8.39%). Analytical Calculated for: ($C_{24}H_{23}N_5O_4S$) (M.W.=477): C, 60.36; H, 4.85; N, 14.65%; Found: C, 60.64; H, 4.93; N, 14.85%.

General procedure for synthesis of alkyl 2-(3-(methylthio)-4-oxo-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-5(4*H*)-yl)acetate 14_{a-b}: Into a solution of compound **9** (10 mmol) in DMF (30 ml) containing potassium carbonate (0.5 gm), the appropriate alkyl-2-chloroacetate (10 mmol) was added. The reaction mixture was heated under reflux for 4 hours. After complete reaction (as indicated by TLC), the reaction mixture was filtered while hot, concentrated, cooled and the resulting solid product was recrystallized from ethanol.

Methyl -2-(3-(methylthio)-4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)acetate (14_a): White solid; Yield: 70%; m. p. 78-79°C. IR (KBr) cm^{-1}: 3044 (CH aromatic), 2948 (CH aliphatic), 1747 (C=O). ^{1}HNMR (DMSO-d_6) δ ppm: 8.47 (s, 1H, pyrimidine-H2), 8.02 (d, 2H, *J*=7.80 Hz, phenyl-H2,H6), 7.57 (t, 2H, *J*=8.10 Hz phenyl-H3, H5), 7.41 (t, 1H, *J*=7.20 Hz, phenyl-H4), 4.85 (s, 2H, CH_2C=O), 3.73 (s, 3H, O-CH_3), 2.63 (s, 3H, S-CH_3). ^{13}C NMR (DMSO-d_6. 400 MHz) δ (ppm): 13.25,47.03,52.95 (Aliphatic carbons), 104.77, 121.85, 127.46, 129.68, 138.33, 146.12, 152.52, 152.79, 156.30 (Aromatic carbons), 168.81 (C=O). MS (m/z): 330 ($C_{15}H_{14}N_4O_3S$, M, 100%), 315 ($C_{14}H_{11}N_4O_3S$, M-CH_3, 1.07%). Analytical Calculated for: ($C_{15}H_{14}N_4O_3S$) (M.W.=330): C, 54.54; H, 4.27; N, 16.96%; Found: C, 54.71; H, 4.36; N, 17.21%.

Ethyl 2-(3-(methylthio)-4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)acetate (14_b): White solid; Yield: 85%; m. p. 82-83°C. IR (KBr) cm^{-1}: IR (KBr)cm^{-1}: 3044 (CH aromatic), 2948 (CH aliphatic), 1747 (C=O). ^{1}HNMR (DMSO-d_6) δ ppm: 8.47 (s, 1H, pyrimidine-H2), 8.02 (d, 2H, *J*=7.80 Hz, phenyl-H2,H6), 7.57 (t, 2H, *J*=8.00 Hz phenyl-H3, H5), 7.41 (t, 1H, *J*=7.20 Hz, phenyl-H4), 4.85 (s, 2H, CH_2C=O), 3.73 (s, 3H, O-CH_3), 2.63 (s, 3H, S-CH_3). ^{13}C NMR (DMSO-d_6. 400 MHz) δ (ppm): 13.25,47.03,52.95 (Aliphatic carbons), 104.77, 121.85, 127.46, 129.68, 138.33, 146.12, 152.52, 156.30 (Aromatic carbons), 168.81 (C=O). MS (m/z): 330 ($C_{15}H_{14}N_4O_3S$, M, 100%), 314 ($C_{14}H_{10}N_4O_3S$, M-CH_3, 100%), 299 ($C_{14}H_{11}N_4O_2S$, 4.76%), 257 ($C_{12}H_9N_4OS$, 2.35%). Analytical Calculated for: ($C_{15}H_{14}N_4O_3S$) (M.W.=330): C, 54.54; H, 4.27; N, 16.96%; Found: C, 54.71; H, 4.36; N, 17.21%.

Synthesis of 2-(3-(methylthio)-4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)acetohydrazide (15): Into a solution of **14_b** (10 mmol) in ethanol (30 ml), hydrazine- hydrate (20 mmol) was added. The reaction mixture was heated under reflux for 6 hours. After complete reaction, the reaction allowed to cool. The separated solid was and filtered out, recrystallized from ethanol. White solid; Yield: 65%; m. p. 122-123°C. IR (KBr) cm^{-1}: 3307 ($NHNH_2$), 3017 (CH aromatic), 2984 (CH aliphatic), 1673 (C=O). ^{1}HNMR (DMSO-d_6) δ ppm: 9.41 (s, 1H, NH D_2O exchangeable), 8.40 (s, 1H, pyrimidine-H2), 8.05 (d, 2H, *J*=8.10 Hz, phenyl-H2, H6), 7.58 (t, 2H, *J*=7.20 Hz, phenyl-H3, H5), 7.39 (t, 1H, *J*=1.20 Hz, phenyl-H4), 4.6 (s, 2H, CH_2C=O), 4.30 (s, 2H, NH_2 D_2O exchangeable), 2.5 (s, 3H, S-CH_3). MS (m/z): 330 ($C_{14}H_{14}N_6O_2S$, 13.32%, M^+), 299 ($C_{14}H_{11}N_4O_2S$, 100%), 271 ($C_{13}H_{11}N_4OS$, 65.87%), 257 ($C_{12}H_9N_4OS$, 1.79%). Analytical Calculated for: ($C_{14}H_{14}N_6O_2S$ (M.W.=330): C, 50.90; H, 4.27; N, 25.44%; Found: C, 51.23; H, 4.34; N, 25.61%.

General procedure for synthesis of *N'*-benzylidene derivatives-2-(3-(methylthio)-4-oxo-1-phenyl-1,4-dihydro-5*H*-pyrazolo[3,4-*d*] pyrimidin-5-yl)acetohydrazide 16_{a-b}: Into a solution of **15** (10 mmol) in glacial acetic acid (20 ml), benzaldehyde derivatives (10 mmol) was added. The mixture was then heated under reflux for 5 hours. The reaction mixture was concentrated and allowed to cool. The separated solid was filtered and finally recrystallized from ethanol.

(E)-N'-benzylidene-2-(3-(methylthio)-4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)acetohydrazide (16_a): White solid; Yield: 73%; m. p. 189-190°C. IR (KBr) cm^{-1}: 3196 (NH), 3044 (CH aromatic), 2929 (CH aliphatic), 1680 (C=O). ^{1}HNMR (DMSO-d_6) δ ppm: 11.88 (s, 1H, NH D_2O exchangeable), 8.49 (s, 1H, pyrimidine-H2), 8.07 (t, 3H, *J*=8.00 Hz, phenyl-H3,H4,H5), 7.75 (d, 2H, *J*=8.00 Hz, phenyl-H2,H6), 7.58 (d, 2H, *J*=8.00 Hz, phenylpyrazole-H2,H6), 7.46 (t, 2H, *J*=6.00 Hz, Phenylpyrazole-H3,H5), 7.44 (t, 1H, *J*=7.60 Hz, Phenylpyrazole-H4), 7.39 (s,1H, CH=N), 5.24 (s, 2H, CH_2C=O), 2.63 (s, 3H, S-CH_3). ^{13}C NMR (DMSO-d_6. 400 MHZ) δ (ppm): 13.26, 46.88, 47.44 (Aliphatic carbons), 104.96 121.73, 127.63, 129.93, 130.67, 134.46, 138.46, 146.06, 147.88, 152.94, 153.12, 156.51, 163.76, (Aromatic carbons), 168.64 (C=O). MS (m/z): 418 ($C_{21}H_{18}N_6O_2S$, 2.30%, M^+), 299 ($C_{14}H_{11}N_4O_2S$, 100%), 271 ($C_{13}H_{11}N_4OS$, 65.87%). Analytical Calculated for: ($C_{21}H_{18}N_6O_2S$ (M.W.=418): C, 60.27; H, 4.34; N, 20.08%; Found: C, 51.23; H, 4.34; N, 25.61%.

(E)-N'-(4-hydroxybenzylidene)-2-(3-(methylthio)-4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl) acetohydrazide (16_b): White solid; Yield: 78%; m. p. 205-206 C. IR (KBr) cm^{-1}: 3017 (CH aromatic), 2984 (CH aliphatic), 1673 (C=O), 3307 (NH). ^{1}HNMR (DMSO-d_6) δ ppm: 11.67 (s, 1H, NH D_2O exchangeable), 9.97 (s, 1H, OH D_2O exchangeable), 8.47 (s, 1H, pyrimidine-H2), 8.05 (d, 2H, *J*=8.00 Hz, phenyl-H2,H6), 7.97 (s, 2H, CH=N), 7.58 (t, 4H, *J*=8.00 Hz, phenylpyrazole-H3,H5, Phenyl-H3,H5), 7.40 (t, 1H, *J*=8.00 Hz, Phenylpyrazole-H4), 6.86 (d, 2H, *J*=8.00 Hz, Phenylpyrazole-H2,H6), 5.19 (s, 2H, CH_2C=O), 2.63 (s, 3H, S-CH_3). ^{13}C NMR (DMSO-d_6. 400 MHZ) δ (ppm): 13.26,20.89,47.37 (Aliphatic carbons), 104.96, 116.20, 121.72, 125.42, 127.33, 129.68, 138.47, 145.13, 148.16, 152.94, 156.52, 159.90, 163.33, (Aromatic carbons), 168.24 (C=O). MS (m/z): 434 ($C_{21}H_{18}N_6O_3S$, 2.66%, M^+), 328 ($C_{14}H_{12}N_6O_2S$, 19.44%), 271 ($C_{13}H_{11}N_4OS$, 4.40%), 257 ($C_{12}H_9N_4OS$, 1.79%). Analytical Calculated for: ($C_{21}H_{18}N_6O_3S$ (M.W.=434): C, 58.08; H, 4.18; N, 19.34%; Found: C, 51.23; H, 4.34; N, 25.61%.

Biological Testing

Materials and methods

Human breast adenocarcinoma cell line MCF7, were purchased from the American Type Cell Culture Collection (ATCC, Manassas, USA) and grown on Roswell Park Memorial Institute Medium (RPMI 1640) supplemented with 100 g/ml of streptomycin, 100 units/ml of penicillin and 10% of heat inactivated fetal bovine serum in a humidified, 5% (v/v) CO_2 atmosphere at 37°C.

Measurement of potential antitumor

The antitumor activity of newly synthesized pyrazolo[3,4-*d*] pyrimidines were measured *in vitro* on human breast adenocarcinoma cell line MCF7 using SulfoRhodamine-B stain (SRB) assay applying the method of 3-[4,5-dimethylthiazole-2-yl]-2,5-dimethyltetrazolium bromide (MTT) technique [21,22]. Exponentially grown cells from the selected cancer cell line were trypsinized, counted and seeded at the appropriate densities (2000-1000 cells/0.33 cm^2). Cells were then incubated in a humidified atmosphere at 37°C. for 24 hours. Then, cells were exposed to different concentrations of the test compounds (0.1, 1, 10, 100, 1000 πM) for 72 hours. After that, the viability of treated cells was determined according to MTT technique. The viability of cells was expressed as percentage of control and the concentration that induces 50% inhibition of cell proliferation (IC_{50}). The relation between the surviving fraction and the compound concentration was plotted and the IC_{50} was calculated for each compound. Results are given in Table 1.

Conclusion

A series of novel 1-phenyl-3-methylsulphanylpyrazolo[3,4-*d*] pyrimidines **10-16** was synthesized. The antitumor activity of this new series was investigated against human breast adenocarcinoma cell line MCF7. Ten of the test compounds showed moderate activity relative to that of doxorubicin. The *N*-arylacetamide derivatives (13_{a-h}) exhibited better antitumor activity than all other series. Among this series, compound 13_a displayed the highest activity with IC_{50} equal to 23 μM. As it obvious from the results in Table 1 and Figure 2, increasing the linker length by one more CH_2 unit in compounds 13_{a-h} results in dramatic fall in the activity. Presence of hydrogen bond donor at the **para** position of the aromatic ring in the new derivatives $16_{a, b}$ is essential for the activity. This becomes clear upon comparing the MIC values of 16_a (above 326 μM) with that of 16_b which is only 61 μM. Further studies are required in order to determine the mechanism of the antitumor action and to identify the SAR of other positions of pyrazolo[3,4-*d*]pyrimidine nucleus.

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References

1. Gao X, Lu Y, Fang L, Fang X, Xing Y, et al. (2013) Synthesis and anticancer activity of some novel 2-phenazinamine derivatives. Eur J Med Chem 69: 1-9.
2. Seffrin JR, Hill D, Burkart W, Magrath I, Badwe RA, et al. (2009) It Is Time to Include Cancer and Other Noncommunicable Diseases in the Millennium Development Goals. Cancer J Clin 59: 282-284.
3. Narang AS, Desai DS (2009) In: Pharmaceutical Perspectives of Cancer Therapeutics. Springer US: New York, USA.
4. Mishra CB, Mongre RK, Kumari S, Jeong DK, Tiwari M (2017) Synthesis, in vitro and in vivo anticancer activity of novel 1-(4-imino-1-substituted-1H-pyrazolo[3,4-d]pyrimidin-5(4H)-yl)urea derivatives. RSC Adv 6: 24491-24500.
5. Jorda R, Havlíček L, McNae IW, Walkinshaw MD, Voller J (2011) Pyrazolo[4,3-d] pyrimidine bioisostere of roscovitine: evaluation of a novel selective inhibitor of cyclin-dependent kinases with antiproliferative activity. J Med Chem 54: 2980-2993.
6. Ismail NSM, Ali EMH, Ibrahim DA, Serya RAT, Abou EE (2017) Pyrazolo [3, 4-d] pyrimidine based scaffold derivatives targeting kinases as anticancer agents. A Futur J Pharm Sci.
7. Kandeel MM, Mohamed LM, Hamid MAKE, Negmeldin AT (2012) Design, Synthesis, and Antitumor Evaluation of Novel Pyrazolo[3,4-d]pyrimidine Derivatives. Sci Pharm 80: 531-545.
8. Abdellatif K, Abdelall E, Abdelgawad M, Ahmed R, Bakr R (2014) Synthesis and Anticancer Activity of Some New Pyrazolo[3,4-d]pyrimidin-4-one Derivatives. Molecules 19: 3297-3309.
9. Ducray R, Ballard P, Barlaam BC, Hickinson MD, Kettle JG, et al. (2008) Novel 3-alkoxy-1H-pyrazolo[3,4-d]pyrimidines as EGFR and erbB2 receptor tyrosine kinase inhibitors. Bioorg Med Chem Lett 18: 959-962.
10. Kumar A, Ahmad I, Chhikara BS, Tiwari R, Mandal D, et al. (2011) Synthesis of 3-phenylpyrazolopyrimidine-1, 2, 3-triazole conjugates and evaluation of their Src kinase inhibitory and anticancer activities. Bioorg Med Chem Lett 21: 1342-1346.
11. Markwalder JA, Arnone MR, Benfield PA, Boisclair M, Burton CR (2004) Synthesis and Biological Evaluation of 1-Aryl-4,5-dihydro-1H-pyrazolo[3,4-d] pyrimidin-4-one Inhibitors of Cyclin-Dependent Kinases. J Med Chem 47: 5894-5911.
12. Curran KJ, Verheijen JC, Kaplan J, Richard DJ, Toral BL, et al. (2010) Pyrazolopyrimidines as highly potent and selective, ATP-competitive inhibitors of the mammalian target of rapamycin (mTOR): Optimization of the 1-substituent. Bioorg Med Chem Lett 20: 1440-1444.
13. Meijer L, Flajolet M, Greengard P (2004) Pharmacological inhibitors of glycogen synthase kinase 3. Trends Pharmacol Sci 25: 471-480.
14. Peat AJ, Boucheron JA, Dickerson SH, Garrido D, Mills W, et al. (2004) Novel pyrazolopyrimidine derivatives as GSK-3 inhibitors. Bioorg Med Chem Lett 14: 2121-2125.
15. El-Enany MM, Kamel MM, Khalil OM, El-Nassan HB (2010) Synthesis and antitumor activity of novel 6-aryl and 6-alkylpyrazolo[3,4-d]pyrimidin-4-one derivatives. Eur J Med Chem 45: 5286-5291.
16. Hamid AEMK, Mihovilovic MD, El-Nassan HB (2012) Synthesis of novel pyrazolo [3, 4-d] pyrimidine derivatives as potential anti-breast cancer agents. Eur J Med Chem 57: 323-328.
17. Tominaga Y, Honkawa Y, Hara M, Hosomi AJ (1990) Heterocycl Chem 27: 775-783.
18. Traxler P, Bold G, Frei J, Lang M, Lydon N (1997) Use of a Pharmacophore Model for the Design of EGF-R Tyrosine Kinase Inhibitors: 4-(Phenylamino) pyrazolo[3,4-d]pyrimidines. J Med Chem 40: 3601-3616.
19. Davoodnia A, Zhiani R, Tavakoli HN, Monatshefte F (2008) Chemie Chem Mon 139: 1405-1407.
20. Abdou NS, Serya RAT, Esmat A, Tolba MF, Ismail NSM, et al. (2015) Synthesis and in vitro antiproliferative activity of novel pyrazolo[3,4-d]pyrimidine derivatives. Med Chem Commun 6: 1518-1534.
21. Sharma A, Chakravarti B, Gupt MP, Siddiqui JA, Konwar R, et al. (2010) Synthesis and anti-breast cancer activity of biphenyl based chalcones. Bioorg Med Chem 18: 4711-4720.
22. Freimoser FM, Jakob CA, Aebi M, Tuor U (1999) The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay is a fast and reliable method for colorimetric determination of fungal cell densities. Appl Environ Microbiol 65: 3727-3729.
23. Ferrari M, Fornasiero MC, Isetta AM (1990) MTT colorimetric assay for testing macrophage cytotoxic activity in vitro. J Immunol Methods 131: 165-172.
24. Sahu NP, Pal C, Mandal NB, Banerjee S, Raha M, et al. (2002) Synthesis of a novel quinoline derivative, 2-(2-methylquinolin-4-ylamino)-N-phenylacetamide - a potential antileishmanial agent. Bioorganic Med Chem 10: 1687-1693.

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