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

Ahmed M. El-Morsy[1], Mohamed S. El-Sayed[1], Hamada S. Abulkhair[1,2*]

[1]Department of Organic Chemistry, College of Pharmacy, Al-Azhar University, Cairo, Egypt
[2]Material and Biomedical Sciences, Zewail City of Science and Technology, Cairo, Egypt
Email: *hamadaorganic@azhar.edu.eg

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Abstract

A new series of 3-(methylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine derivatives was synthesized. The structures of the new derivatives were confirmed by the spectral data and elemental analyses. 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

1. 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 bioisostere of purine [4] [5], which 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] [7] [8]. The cytotoxic activity of such compound may attribute 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 methyl sulphonyl 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 MCF7 with 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} 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 result 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 methyl sulphonyl 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.

2. Results and Discussion

2.1. Chemistry

Scheme 1 shows the synthetic pathway of the starting pyrazolo[3,4-*d*]pyrimidin-

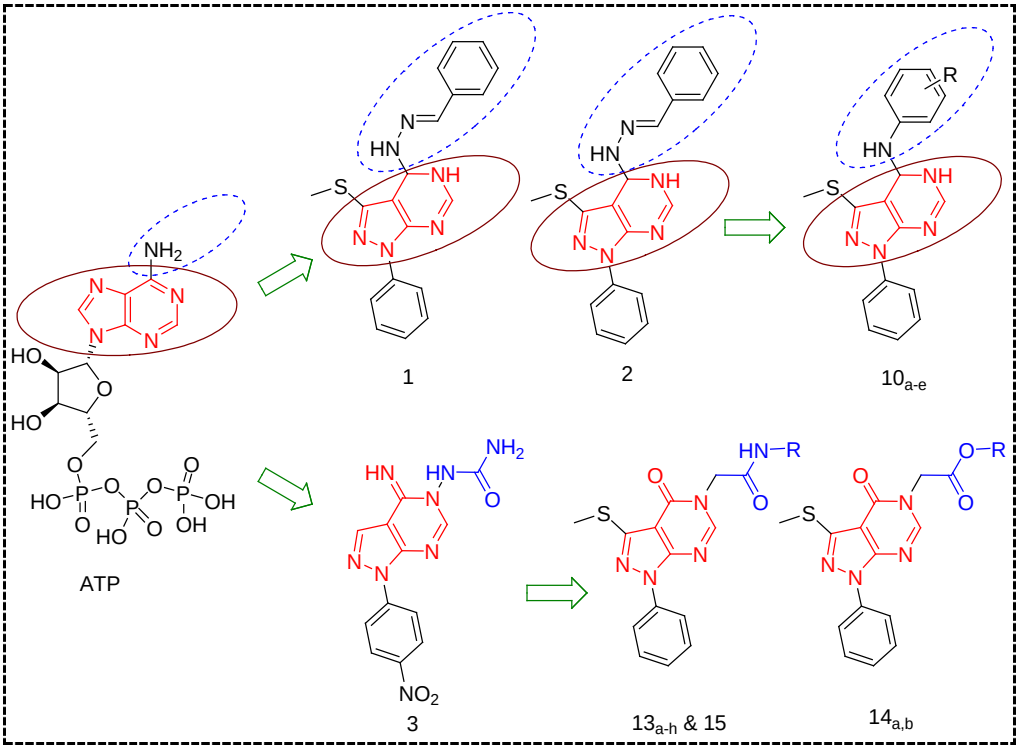
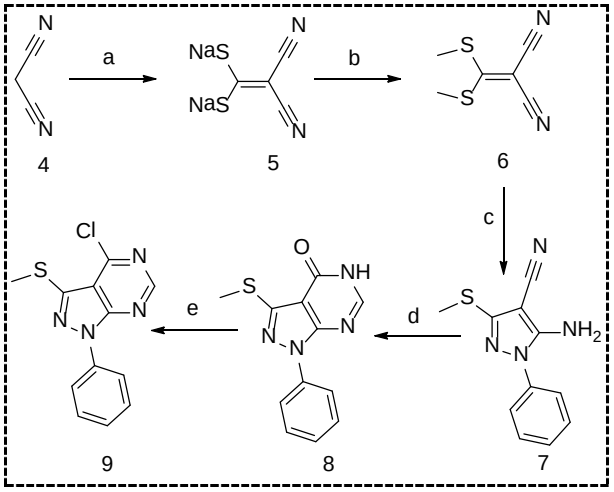


Figure 1. Structural similarities and pharmacophoric features of ATP, reported potent antitumor pyrazolo[3,4-*d*]pyrimidines and new designed compounds.



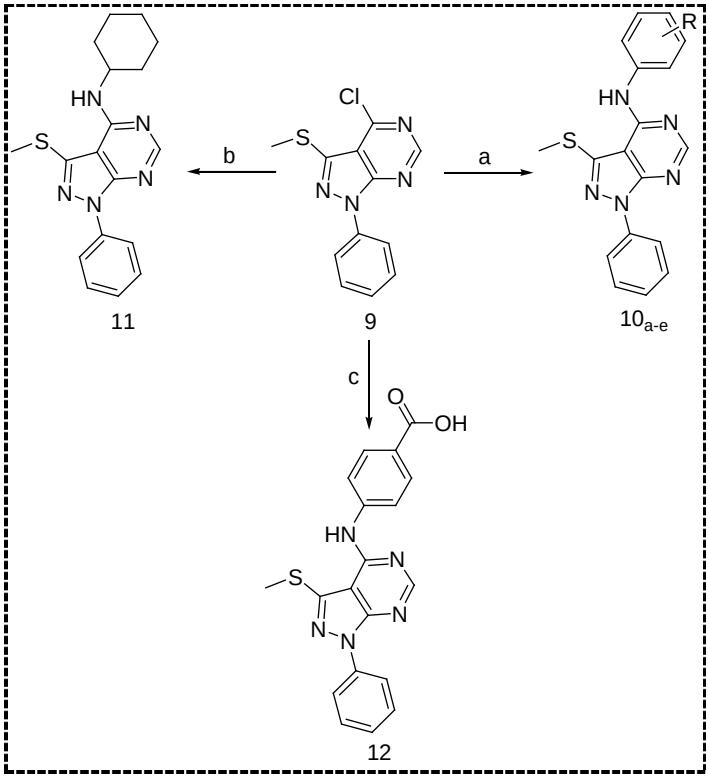
Scheme 1. Synthetic pathway of starting pyrazolo[3,4-*d*]pyrimidine. Reagents: (a) CS_2, C_2H_5OH; (b) $(CH_3)_2SO_4$, C_2H_5OH; (c) NH_2NH_2, C_2H_5OH; d) HCOOH; e) $POCl_3$.

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 $10_{a\text{-}e}$, 11 and 12 (Scheme 2).

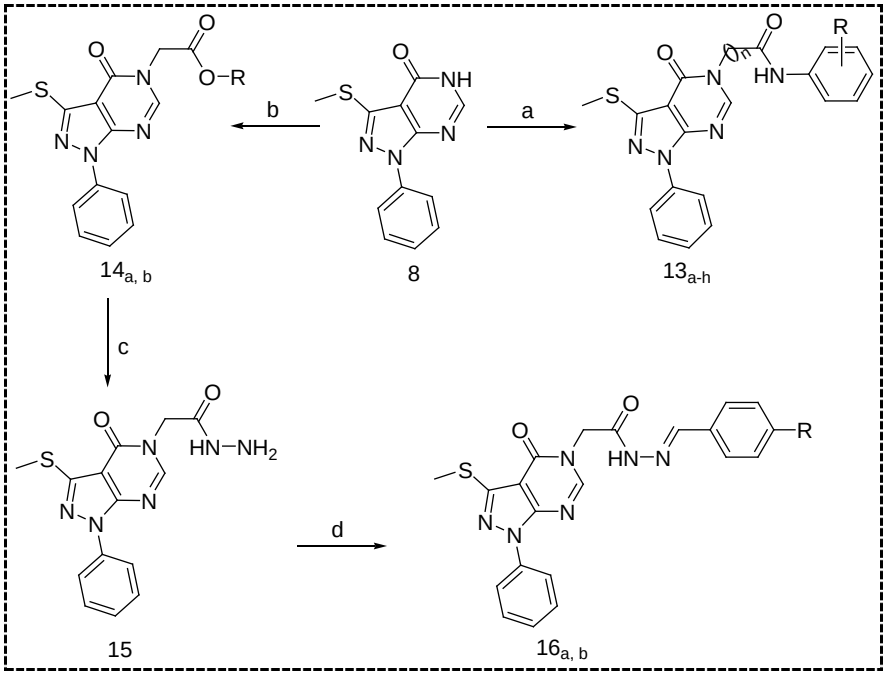
Formation of compounds 10 - 12 was confirmed by spectral data and elemental analyses. The ^{1}HNMR spectra of these derivatives demonstrated the appearance of a new D_2O exchangeable singlet signals at δ 8.48 - 8.60 ppm corresponding to the NH protons. Mass spectra of these compounds showed distinctive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

Scheme 3 shows the synthetic pathway of the target compounds $13_{a\text{-}h}$ 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 $14_{a,b}$ were prepared via condensation of compound 8 with alkyl chloroacetate in the presence of potassium carbonate. Structures of these



Scheme 2. Synthetic pathway of target compounds 10 - 12. R = 2-CH_3, 2-SH, 2,6-Cl_2, 2-COOH, 4-$COOC_2H_5$. 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, $C_2H_5OH/(C_2H_5)_3N$.



Scheme 3. Synthetic pathway of target compounds 13 - 16. For $13_{a\text{-}d}$ n = 1, R = H, 2-Cl, 3-CH_3, 4-$COOC_2H_5$; For $13_{e\text{-}h}$: n = 2, R = H, 2-Cl, 3-CH_3, 4-$COOC_2H_5$; For $14_{a\text{-}b}$: R = CH_3, C_2H_5; For $16_{a\text{-}b}$: R = H, OH. Reagents: For $13_{a\text{-}d}$: (a) $ClCH_2CONHC_6H_4R$, K_2CO_3, C_2H_5OH; For $13_{e\text{-}h}$: (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.

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

2.2. *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 is shown in **Table 1** and represented graphically in **Figure 2**.

3. Experimental

3.1. 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 & Biotechnology, Al-Azhar University, Cairo, Egypt. Progress of the reactions was 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

Table 1. Results of in vitro cytotoxic activity of compounds showed more than 50% inhibition of MCF7 adenocarcinoma cell line.

Comp. No.	Conc. (μM)	Average Absorbance (%)	Viable Cells (%)	Standard Deviation	IC_{50} (μM)
10_a	0.00	0.221	100.000	0.799	128
	0.10	0.201	90.937	0.804	
	1.00	0.190	86.254	0.919	
	10.00	0.181	82.175	0.841	
	100.00	0.124	56.193	1.047	
	1000.00	0.021	9.366	0.604	
10_e	0.00	0.4220	100.000	2.052	42
	0.10	0.3893	92.259	1.241	
	1.00	0.3723	88.231	0.285	
	10.00	0.3573	84.676	0.440	
	100.00	0.3167	75.039	1.226	
	1000.00	0.1450	34.360	0.410	
11	0.00	0.221	100.000	0.799	169.8
	0.10	0.198	89.728	0.884	
	1.00	0.196	88.822	1.140	
	10.00	0.177	80.363	0.841	
	100.00	0.134	60.725	0.453	
	1000.00	0.050	22.659	0.523	
12	0.00	0.221	100.000	0.799	257
	0.10	0.194	88.066	0.218	
	1.00	0.185	83.686	0.400	
	10.00	0.156	70.846	1.180	
	100.00	0.148	67.070	0.785	
	1000.00	0.053	23.867	0.799	
13_a	0.00	0.4937	100.000	0.761	23
	0.10	0.4593	93.045	0.389	
	1.00	0.4423	89.602	0.411	
	10.00	0.3830	77.583	0.510	
	100.00	0.0417	8.440	0.294	
	1000.00	0.0187	3.781	0.243	
13_b	0.00	0.221	100.000	0.799	49
	0.10	0.188	85.045	0.371	
	1.00	0.169	76.586	1.199	
	10.00	0.167	75.529	0.545	
	100.00	0.085	38.369	0.302	
	1000.00	0.067	30.211	1.702	
13_d	0.00	0.4220	100.000	2.052	61.7
	0.10	0.3993	94.629	1.507	
	1.00	0.3777	89.494	0.553	

Continued

	10.00	0.3437	81.438	0.480	
	100.00	0.1750	41.469	0.684	
	1000.00	0.1153	27.330	0.890	
	0.00	0.221	100.000	0.799	
	0.10	0.194	88.066	1.627	
14_a	1.00	0.188	85.196	0.785	288.4
	10.00	0.180	81.722	0.658	
	100.00	0.150	67.976	1.457	
	1000.00	0.067	30.363	0.692	
	0.00	0.221	100.000	0.799	
	0.10	0.190	86.254	0.996	
14_b	1.00	0.180	81.420	0.919	776
	10.00	0.171	77.341	1.089	
	100.00	0.151	68.278	1.343	
	1000.00	0.109	49.245	2.634	
	0.00	0.4220	100.000	2.052	
	0.10	0.3907	92.575	1.139	
16_b	1.00	0.3767	89.258	0.617	47.8
	10.00	0.3540	83.886	0.137	
	100.00	0.1343	31.833	0.754	
	1000.00	0.0253	6.003	0.418	
	0.00	0.221	100.000	0.799	
	0.10	0.177	80.212	0.746	
Doxorubicin	1.00	0.150	67.976	1.199	4.27
	10.00	0.138	62.689	0.658	
	100.00	0.093	41.994	0.658	
	1000.00	0.056	25.227	1.057	

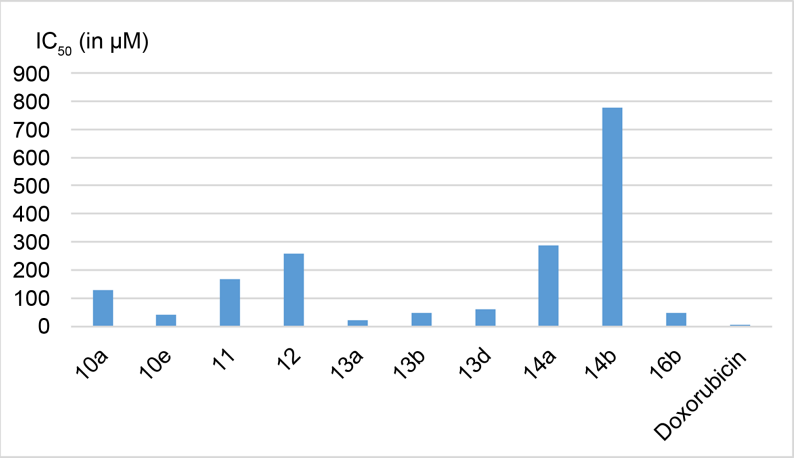


Figure 2. IC_{50} in μM of the synthesized compounds and doxorubicin against MCF7 adenocarcinoma cell line.

procedure [19]. 2-Chloro-*N*-arylactamide and 3-chloro-*N*-arylpropanamide derivatives were prepared as reported [24].

3.2. 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.

3.2.1. 3-(Methylthio)-1-Phenyl-*N*-o-Tolyl-1*H*-Pyrazolo[3,4-*d*]Pyrimidin-4-Amine (10a)

White solid; Yield: 85%; m. p. 130˚C - 131˚C. IR (KBr) cm^{-1}: 3372 (NH), 3047 (CH aromatic), 2924 (CH aliphatic). ^{1}HNMR (DMSO-*d*6) 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 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%). Anal. Calc. 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%.

3.2.2. 2-(3-(Methylthio)-1-Phenyl-1*H*-Pyrazolo[3,4-*d*]Pyrimidin-4-Ylamino)Benzenethiol (10_b)

White solid; Yield: 75%; m. p. 141˚C - 142˚C. IR (KBr) cm^{-1}: 3291 (NH), 3053 (CH aromatic), 2918 (CH aliphatic). ^{1}HNMR (DMSO-*d*6) 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^+). Anal. Calc. 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%.

3.2.3. *N*-(2,6-Dichlorophenyl)-3-(Methylthio)-1-Phenyl-1*H*-Pyrazolo [3,4-*d*]Pyrimidin-4-Amine (10_c)

White solid; Yield: 63%; m. p. 231˚C - 232˚C. IR (KBr) cm^{-1}: 3455 (NH), 3050 (CH aromatic), 2938 (CH aliphatic). ^{1}HNMR (DMSO-*d*6) 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%). Anal. Calc. 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%.

3.2.4. 4-(3-(Methylthio)-1-Phenyl-1*H*-Pyrazolo[3,4-*d*]Pyrimidin-4-Ylamino)Benzoic Acid (10_d)

White solid; Yield: 85%; m. p. 162˚C - 164 ˚C. IR (KBr) cm^{-1}: 3397 (OH), 3360 (NH), 3047 (CH aromatic), 2924 (CH aliphatic), 1689 (C=O). ^{1}HNMR (DMSO-*d*6) 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%). Anal. Calc. 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%.

3.2.5. Ethyl-4-((3-(Methylthio)-1-Phenyl-1*H*-Pyrazolo[3,4-*d*]Pyrimidin-4-yl)Amino)Benzoate (10_e)

White solid; Yield: 85%; m. p. 123˚C - 124˚C. IR (KBr) cm^{-1}: 3372 (NH), 3033 (CH aromatic), 2940 (CH aliphatic), 1710 (C=O). ^{1}HNMR (DMSO-*d*6) 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, phenyl-pyrazole-H4), 7.33 (t, 2H, J = 6.00 Hz, phenyl H2, 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%). Anal. Calc. 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%.

3.3. *N*-Cyclohexyl-3-(Methylthio)-1-Phenyl-1*H*-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˚C - 116˚C. IR (KBr) cm^{-1}: 3397 (NH), 3031 (CH aromatic), 2925 (CH aliphatic). ^{1}HNMR (DMSO-*d*6) 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_2O exchangeable), 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%). Anal. Calc. 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%.

3.4. 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˚C - 171˚C. IR (KBr) cm^{-1}: 3382

(broad OH), 3027 (CH aromatic), 2985 (CH aliphatic), 1699 (C=O). ^{1}HNMR (DMSO-*d*6) 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%). Anal. Calc. 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%.

3.5. 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.

3.5.1. 2-(3-(Methylthio)-4-Oxo-1-Phenyl-1*H*-Pyrazolo[3,4-*d*]Pyrimidin-5(4*H*)-yl)-*N*-Phenylacetamide (13_a)

White solid; Yield: 85%; m. p. 164˚C - 165˚C. IR (KBr) cm^{-1}: 3307 (NH), 3053 (CH aromatic), 2925 (CH aliphatic), 1672 (C=O). ^{1}HNMR (DMSO-*d*6) 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_2 C=O), 2.63 (s, 3H, S-CH_3). ^{13}CNMR (DMSO-*d*6 400 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%). Anal. Calc. 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%.

3.5.2. *N*-(2-Chlorophenyl)-2-(3-(Methylthio)-4-Oxo-1-Phenyl-1*H*-Pyrazolo[3,4-*d*]Pyrimidin-5(4*H*)-yl)Acetamide (13_b)

White solid; Yield: 78%; m. p. 146˚C - 147˚C. IR (KBr) cm^{-1}: 3258 (NH), 3072 (CH aromatic), 2932 (CH aliphatic), 1695 (C=O). ^{1}HNMR (DMSO-*d*6) 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_2 C=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%). Anal. Calc. 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%.

3.5.3. 2-(3-(Methylthio)-4-Oxo-1-Phenyl-1*H*-Pyrazolo[3,4-*d*]Pyrimidin-5(4*H*)-yl)-*N*-*m*-Tolylacetamide (13_c)

White solid; Yield: 82%; m. p. 152˚C - 153˚C. IR (KBr) cm^{-1}: 3299 (NH), 3038 (CH aromatic), 2925 (CH aliphatic), 1672 (C=O). ^{1}HNMR (DMSO-*d*6) 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-H2, H6), 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_2 C=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%). Anal. Calc. 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%.

3.5.4. Ethyl 4-(2-(3-(Methylthio)-4-Oxo-1-Phenyl-1*H*-Pyrazolo[3,4-*d*] Pyrimidin-5(4*H*)-yl)Acetamido)Benzoate (13_d)

White solid; Yield: 65%; m. p. 72˚C - 73˚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), 8.4 (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_2 C=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-$NHC_6H_4COOC_2H_5$, 19.63%), 271 ($C_{13}H_{11}N_4OS$, 18.52%), 174 ($C_9H_5N_2S$, 100%). Anal. Calc. 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%.

3.5.5. 3-(3-(Methylthio)-4-Oxo-1-Phenyl-1*H*-Pyrazolo[3,4-*d*]Pyrimidin-5(4*H*)-yl)-*N*-Phenylpropanamide (13_e)

White solid; Yield: 80%; m. p. 154˚C - 155˚C. IR (KBr) cm^{-1}: 3317 (NH), 3010 (CH aromatic), 2924 (CH aliphatic), 1674 (C=O). ^{1}HNMR (DMSO-*d*6) 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_2 C=O), 2.61 (s, 3H, S-CH_3). ^{13}CNMR (DMSO-*d*6 400 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%). Anal. Calc. 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%.

3.5.6. *N*-(2-Chlorophenyl)-3-(3-(Methylthio)-4-Oxo-1-Phenyl-1,4-Dihydro-5*H*-Pyrazolo[3,4-*d*]Pyrimidin-5-yl)Propanamide (13_f)

White solid; Yield: 70%; m. p. 149˚C - 150˚C. IR (KBr) cm^{-1}: 3301 (NH), 3083 (CH aromatic), 2931 (CH aliphatic), 1680 (C=O). ^{1}HNMR (DMSO-*d*6) 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-H3, H5), 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%). Anal. Calc. 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%.

3.5.7. 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˚C - 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-H2, H6), 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%). Anal. Calc. 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%.

3.5.8. Ethyl 4-(3-(3-(Methylthio)-4-Oxo-1-Phenyl-1,4-Dihydro-5*H*-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-H2, H6), 7.81 (t, 2H, *J* = 8.40 Hz, Phenylpyrazole-H3, H5), 7.53 (t, 2H, *J* = 8.10 Hz, Phenylpyrazole-H2, H6), 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_2 C=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%). Anal. Calc. 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%.

3.6. 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 $14_{a\text{-}b}$

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.

3.6.1. Methyl -2-(3-(Methylthio)-4-Oxo-1-Phenyl-1,4-Dihydro-5*H*-Pyrazolo[3,4-*d*]Pyrimidin-5-yl)Acetate (14_a)

White solid; Yield: 70%; m. p. 78˚C - 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_2 C=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%). Anal. Calc. 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%.

3.6.2. Ethyl 2-(3-(Methylthio)-4-Oxo-1-Phenyl-1,4-Dihydro-5*H*-Pyrazolo [3,4-*d*]Pyrimidin-5-yl)Acetate (14_b)

White solid; Yield: 85%; m. p. 82˚C - 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_2 C=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%). Anal. Calc. 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%.

3.7. Synthesis of 2-(3-(Methylthio)-4-Oxo-1-Phenyl-1,4-Dihydro-5*H*-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˚C - 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%). Anal. Calc. 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%.

3.8. 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 $16_{a\text{-}b}$

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.

3.8.1. (*E*)-*N*'-Benzylidene-2-(3-(Methylthio)-4-Oxo-1-Phenyl-1,4-Dihydro-5*H*-Pyrazolo[3,4-*d*]Pyrimidin-5-yl)Acetohydrazide (16_a)

White solid; Yield: 73%; m. p. 189˚C - 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_2 C=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%). Anal. Calc. 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%.

3.8.2. (*E*)-*N*'-(4-Hydroxybenzylidene)-2-(3-(Methylthio)-4-Oxo-1-Phenyl-1,4-Dihydro-5*H*-Pyrazolo[3,4-*d*]Pyrimidin-5-yl) Acetohydrazide (16_b)

White solid; Yield: 78%; m. p. 205˚C - 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_2 C=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%). Anal. Calc. 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%.

4. Biological Testing

4.1. Materials and Method

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.

4.2. 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 IC50 was calculated for each compound. Results are given in Table 1.

5. Conclusion

A series of novel 1-phenyl-3-methyl sulphonyl pyrazolo[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. It is 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 comparing the high IC_{50} value of 16_a with that of 16_b which is only 47.8 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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