Accepted Manuscript

Design, synthesis and cytotoxicity studies of novel pyrazolo[1, 5-a]pyridines derivatives

Chitrakar Ravi, Arem Qayum, Darapaneni Chandramohan, Shashank K. Singh, Subbarayappa Adimurthy

PII: S0223-5234(16)30975-8

DOI: 10.1016/j.ejmech.2016.11.037

Reference: EJMECH 9070

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 8 August 2016

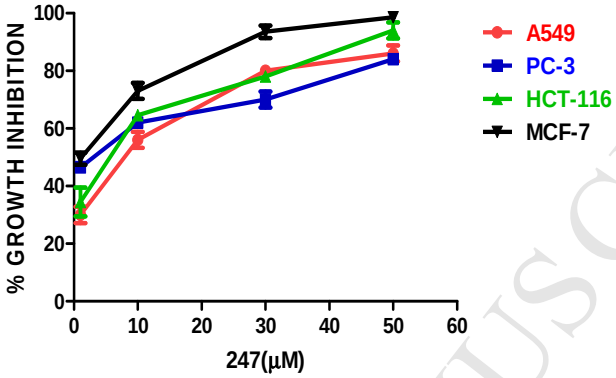
Revised Date: 20 October 2016

Accepted Date: 16 November 2016

Please cite this article as: C. Ravi, A. Qayum, D. Chandramohan, S.K. Singh, S. Adimurthy, Design, synthesis and cytotoxicity studies of novel pyrazolo[1, 5-a]pyridines derivatives, *European Journal of Medicinal Chemistry* (2016), doi: 10.1016/j.ejmech.2016.11.037.

Design, Synthesis and cytotoxicity studies of Novel pyrazolo[1, 5-a]pyridines derivatives

Chitrakar Ravi,[a#] Arem Qayum,[b#] Darapaneni Chandramohan,[a] Shashank K. Singh*[b] and Subbarayappa Adimurthy*[a]



Against various cancer cell lines (A549, PC-3, HCT-116, MCF-7) determined by SRB assay.

Design, Synthesis and cytotoxicity studies of Novel pyrazolo[1, 5-a]pyridines derivatives

Chitrakar Ravi, [a#] Arem Qayum,[b#] Darapaneni Chandramohan, [a]Shashank K. Singh*[b] and Subbarayappa Adimurthy*[a]

[a]Academy of Scientific & Innovative Research, Natural products and green chemistry division, CSIR–Central Salt & Marine Chemicals Research Institute, G.B. Marg, Bhavnagar-364 002. Gujarat (INDIA).
E-mail:adimurthy@csmcri.org
[b]CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu- 180001 India.
E-mail: sksingh@iiim.ac.in
[#]Authors contributed equally to the manuscript.

Abstract:
Copper–mediated synthesis of various pyrazolo[1, 5–a]pyridine-3-carboxylates has been described. The biological activities of these molecules have been evaluated against various human cancer cell lines A549 (Lung adenocarcinoma cell line), MCF-7 (Breast carcinoma cell line), HCT-116 (Colon cancer cell line), and PC-3 (Prostate cancer cell line) through SRB assay. Compound 247 led to accumulation MCF-7 cells in G1-phase and revealed its important role in mitotic cell cycle progression.

Keywords: Pyrazolo[1, 5–a]pyridines, cytotoxicity, MCF-7 cells, Growth inhibition, SRB assay.

* Corresponding authors.
SubbarayappaAdimurthy, E-mail address: adimurthy@csmcri.org
Shashank K. Singh, E-mail address: sksingh@iiim.ac.in

1. Introduction:

With the advent of new emerging fields of medicinal chemistry, the search for new anticancer chemotherapeutic agents continues to be an active area of research in order to develop novel therapies in particular to cancer [1,2]. In the last several decades, pyrazolo[1, 5-a]pyridines derivatives have received considerable attention and reported to confine as anti- inflammatory[3], anti-tumor[4], anti-microbial[5], anti-fungal[6] and anti-viral activities[7]. Pyrazolopyridines are also originating in many systems that affect the central nervous system and found to be an active D4 antagonist [8]. The dopamine D3

receptor subtype is associated with several neuropathologies such as schizophrenia, attention-deficit disorder, unipolar major depression, and Parkinson's disease [9]. The D3 receptor controls dopamine synthesis, release, neuronal firing and also inhibits cocaine-seeking behavior without proving rewarding effects. Several pyrazolo[1,5-a]pyridines derivatives used for treating psycho-stimulant addiction [10]. Pyrazolopyrimidine derivatives which are heterocyclic systems containing the pyrazole fused ring to pyrimidine rings confers unique class of compounds which makes these derivatives as constructional structure in anti-cancer research and drug discovery [11]. Cells respond to DNA damage by instigating complex signaling pathways that activate various cellular responses, including DNA repair and arrest of cell cycle progression. Cell cycle arrests at the G1, S and G2/M phases are crucial steps in the proper DNA damage response as they permit cells to repair the damaged DNA in sufficient time and thereby prevent the accumulation of mutations [12]. DNA damage response signaling pathway ATM/ATR-Chk1/Chk2 plays a central role in the induction of cell cycle arrest in the G1, S or G2/M phases by regulating important cell cycle regulators [13]. Atypical regulation of cell cycle upon DNA damage is a hallmark of various cancer cells, which often causes the failure of DNA repair, damage, thereby resulting in genomic instability [14]. Thus, the identification of physiological targets and the exploration of underlying regulatory mechanisms for DNA damage-induced cell cycle arrest are critical issues as well as in the implication of mitosis control [15]. In view of these studies, we have synthesized new derivatives of pyrazolo[1,5-a]pyridine for evaluation of anti-cancer properties. Also, we tested the cytotoxicity of pyrazolo[1,5-a]pyridine derivatives on a series of human cancer cell lines which showed effective cytotoxicity potential against several human cancer cell lines as well as its effect on cell cycle progression contributes an important parameter of anti-tumor drug action. Thus, we analyzed the effects of pyrazolo[1,5-a]pyridine on cell cycle, and the results showed that treatment with pyrazolo[1,5-a]pyridine derivative **247** led to accumulation of MCF-7 cells in G1-phase and revealed its important role in mitotic cell cycle progression. Some pyrazolo pyridine derivatives have been shown to be highly successful biological activity. Therefore, the structural modification of pyrazolo pyridine by introducing ester groups and aromatic or hetero aromatic groups should be the target of topic for the cytotoxicity studies (Figure 1).

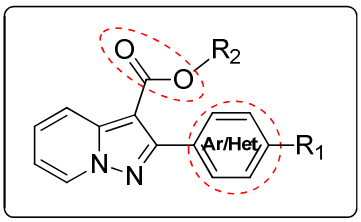
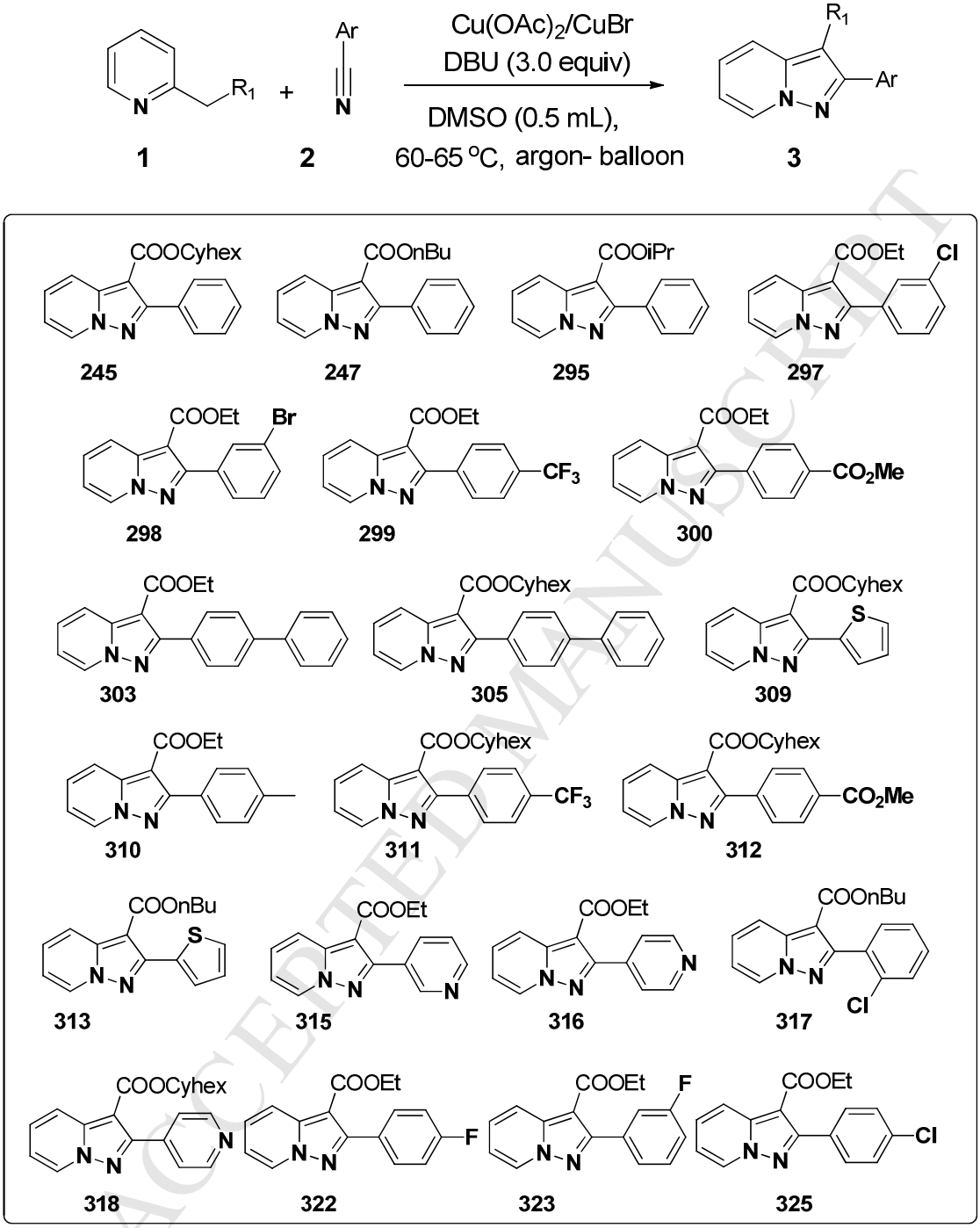


Figure 1. Design of novel 2-aryl or hetero aryl pyrazolo[1,5-a]pyridine-3-carboxylates

2. Results and Discussion:

2.1 Synthesis of functionalized pyrazolo[1, 5-a]pyridine:

As demonstrated in Scheme 1, functionalized pyrazolo[1, 5-a]pyridine **3** derivatives via copper promoted [3+2] cyclisation of pyridyl acetates **1** and benzonitriles **2** in DMSO under argon atmosphere, converted to corresponding pyrazolo[1, 5-a]pyridines **3** from commercially available aromatic nitriles and various pyridyl acetates [16]. Some of the pyridyl acetates were prepared according to literature procedure [17], pyridylacetic acid hydrochloride and alcohol in presence of triethylamine and dicyclohexylcarbodiimide with catalytic amount of 4-(dimethyl amino) pyridine in DCM solvent. All the pyridyl acetate derivatives was isolated after column chromatography and characterized by ^{1}H NMR, ^{13}C NMR and Mass spectrometry. All the pyrazolo[1, 5-a]pyridine derivatives reported in the present manuscript were well characterized by ^{1}H, ^{13}C NMR, FT-IR and Mass spectrometry.



Scheme 1. Synthesis of functionalized pyrazolo[1, 5-a]pyridine derivatives via (3+2) Cyclisation of pyridyl acetates and benzonitriles.

2.2 Biological Analysis:

To evaluate the anti-tumor cytotoxicity of the various synthesized compounds by SRB

assay (Table 1) [18], four different human cancer cell lines were used: A549 (Lung adenocarcinoma cell line), MCF-7 (Breast carcinoma cell line), HCT-116 (Colon cancer cell line), and PC-3 (Prostate cancer cell line). A total of 21 compounds were derived from the pyrazolo[1,5-a]pyridine. Out of 21, 247 showed potent anti-tumor toxicity against human breast cancer cell line (MCF-7). The survival fraction was gradually decreased as the concentration was increased. Cytotoxicity data of pyrazolo[1,5-a] pyridine derivatives against above mentioned human cancer cell lines is shown in Table 1.

Table 1. Preliminary *in vitro* screening of pyrazolo[1, 5-a]pyridines derivatives against various human cancer cell lines.

TISSUE			LUNG	PROSTATE	COLON	BREAST
CELL LINE			A549	PC-3	HCT-116	MCF-7
No.	Sample code	Conc. (μM)	% Growth inhibition			
1	245	50	81	75	83	89
2	247	50	83	85	83	97
3	295	50	72	82	79	88
4	297	50	79	59	69	72
5	298	50	75	64	77	74
6	299	50	19	36	42	62
7	300	50	72	58	84	83
8	303	50	72	58	71	66
9	305	50	40	29	23	38
10	309	50	73	72	78	83
11	310	50	33	27	57	61
12	311	50	63	69	81	78
13	312	50	21	25	54	57
14	313	50	52	40	63	73
15	315	50	07	00	00	25
16	316	50	08	00	00	32
17	317	50	76	61	77	70
18	318	50	77	74	65	82
19	322	50	39	18	27	34
20	323	50	02	16	12	18
21	325	50	05	25	22	33
Paclitaxel		1	78	72	--	--
5-FU		20	--	--	58	--
Doxorubicin		1	--	--	--	62

The *in vitro* cytotoxicity studies indicated the greater sensitivity of MCF-7 cells towards cytotoxicity of synthetic analogues than the other cancer cell lines, as evidenced by the degree of cytotoxicity (IC_{50} values) after 48 h of treatment [19].

Table 2- Determination of IC_{50} against various human cancer cell lines.

TISSUE		LUNG	PROSTATE	COLON	BREAST
CELL LINE		A549	PC-3	HCT-116	MCF-7
No.	Sample code	IC_{50}(μM)			
1	245	9.3	13.6	4.8	13
2	**247**	**6.5**	**2.6**	**4.5**	**1.54**
3	295	16.6	14.1	12.7	16.9
4	297	17.1	29.3	18.9	24.2
5	298	23.7	30.1	24.3	32.4
6	300	21.4	28.4	18.5	23.1
7	303	33.5	35.1	19.1	38.07
8	309	14.9	23.4	16.7	6.9
9	311	34.9	21.9	12.9	17.1
10	313	47.5	>50	22.1	33.2
11	317	11.8	25.9	11.8	22.4
12	318	5.7	31.9	8.9	12.7

The cytotoxicity profiles of all the derivatives clearly identified the compound 247 as a potential candidate, displaying IC_{50} values in lower concentrations against MCF-7 cells (Table2). So, compound 247 seemed to be more potent in inducing cytotoxicity against breast carcinoma cell line (MCF-7) with IC_{50} of 1.54μM as compared to other derivatives (figure 2).

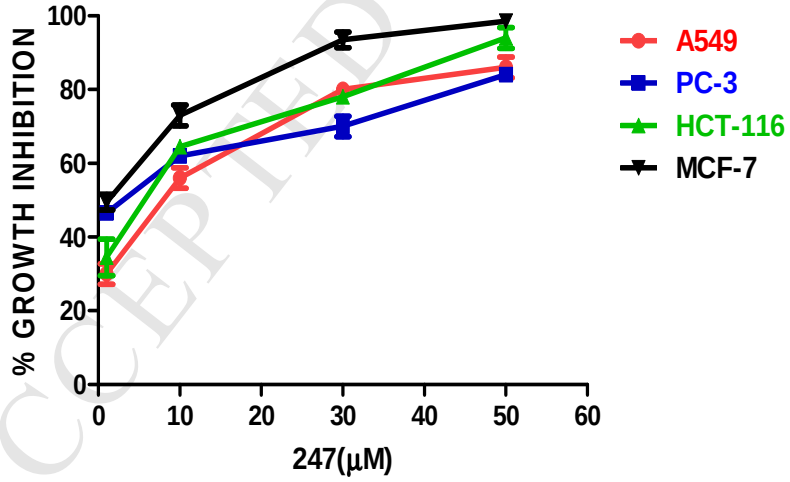
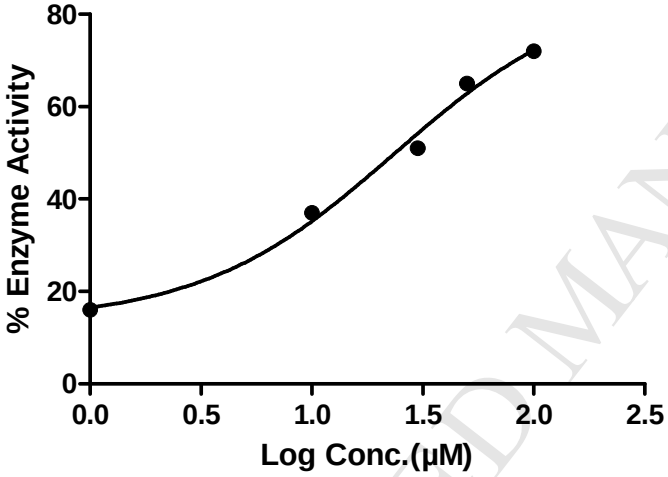


Figure 2. Growth inhibition curve on different concentrations of compound 247 against various cancer cell lines (A549, PC-3, HCT-116, MCF-7) determined by SRB assay. The values were calculated by using Graph PAD Prism software version 5.0. The results represent mean ± SD of three experiments.

The kinases modulate several physiological mechanisms such as cell proliferation, differentiation, migration and metabolism by transferring the ATP terminal phosphate to

tyrosine residues of protein substrates. It has been showed that kinase transfiguration (especially hyperactivation, hyperproduction, or mutations) leading to the disruption of cell signaling cascades play important roles in several diseases, including diabetes, inflammation, neurological disorders and cancer. Therefore, kinases represent important targets for anticancer drug development. Compound 247 was tested through enzyme assay for inhibition of the tyrosine kinase activity of a recombinant EGF-R using Poly (Glu_4, Tyr) substrate. The enzymatic assay revealed that compound 247 has an effect on potency towards binding to the active site (figure 3).



Conc.(µM)	%Enzyme Activity
1	16
10	37
30	51
50	65
100	72
IC_{50}	23.7

Figure 3. Graphical representation of % enzyme activity and IC_{50} of compound 247 on EGFR using GraphPad Prism.

For the analysis of nuclear morphology, MCF-7 cells were stained with DAPI to study nuclear alterations and apoptotic body formation. Cells treated with compound 247 exhibited no characteristic morphological changes which were marked by nuclear shrinkage, chromatin condensation and apoptotic but homogeneously stained with DAPI (figure 4).

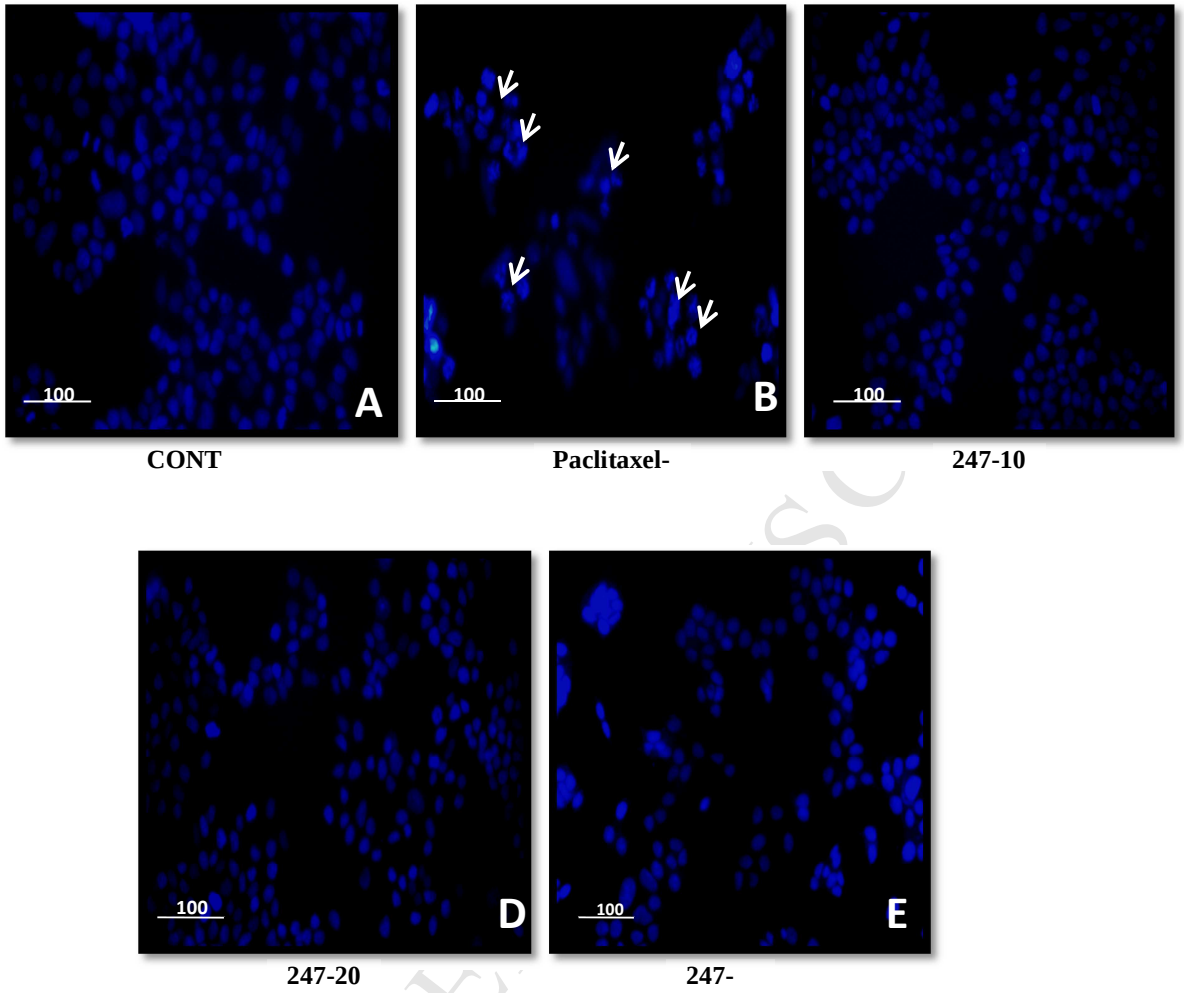


Figure 4: Cells were treated with compound 247 at 10, 20 and 30µM for 24 h, then stained with DAPI in order to analyze the nuclear morphological changes. Untreated MCF-7 cells showed normal nuclear morphology whereas Paclitaxel showed typical apoptotic bodies. Cells treated with 247 showed concentration dependent effect to arrest the growth of the cells.

Reactive oxygen species (ROS) are chemically reactive oxygen molecules which are formed as a natural byproduct of the normal metabolism of oxygen and comprise of important roles in cell signaling and homeostasis. As cancer cells exhibit greater ROS stress than normal cells and facilitate cancer cell survival. Progression of cell cycle which was driven by various growth factors and receptor tyrosine kinases (RTK) require ROS for activation. In contrast, a high level of ROS can suppress tumor growth through the

sustained activation of cell-cycle inhibitors. The antitumor effect of compound 247 is enhanced through ROS stress by production of H_2O_2 in a concentration dependent manner (figure 5).

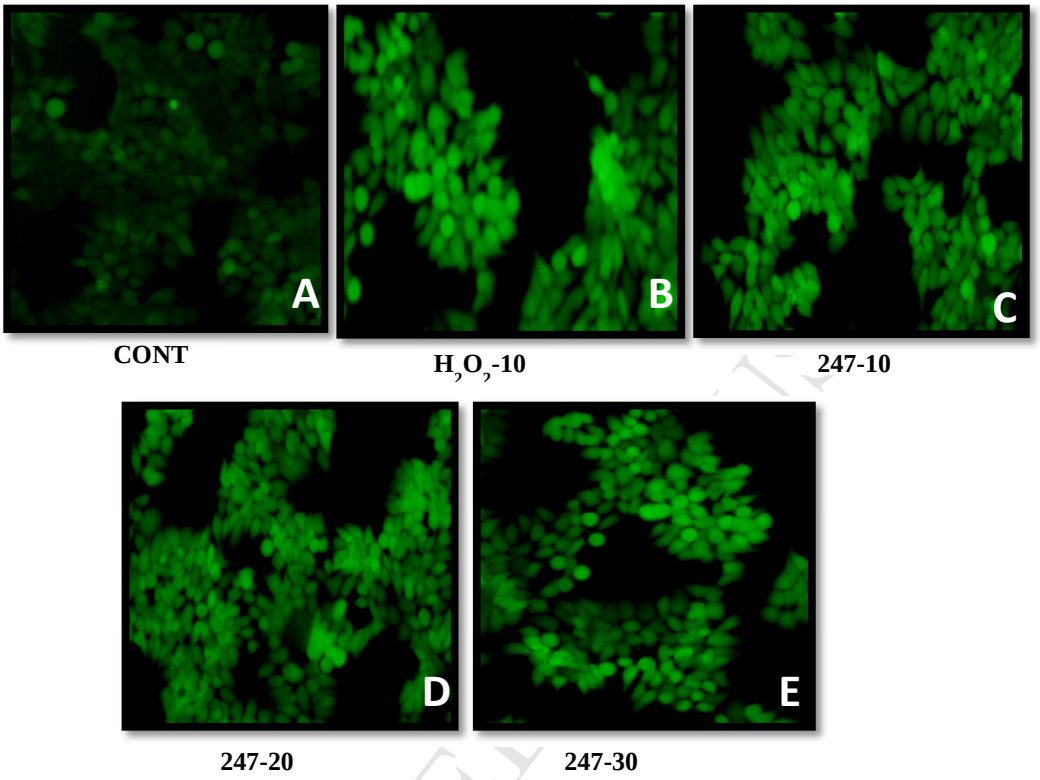
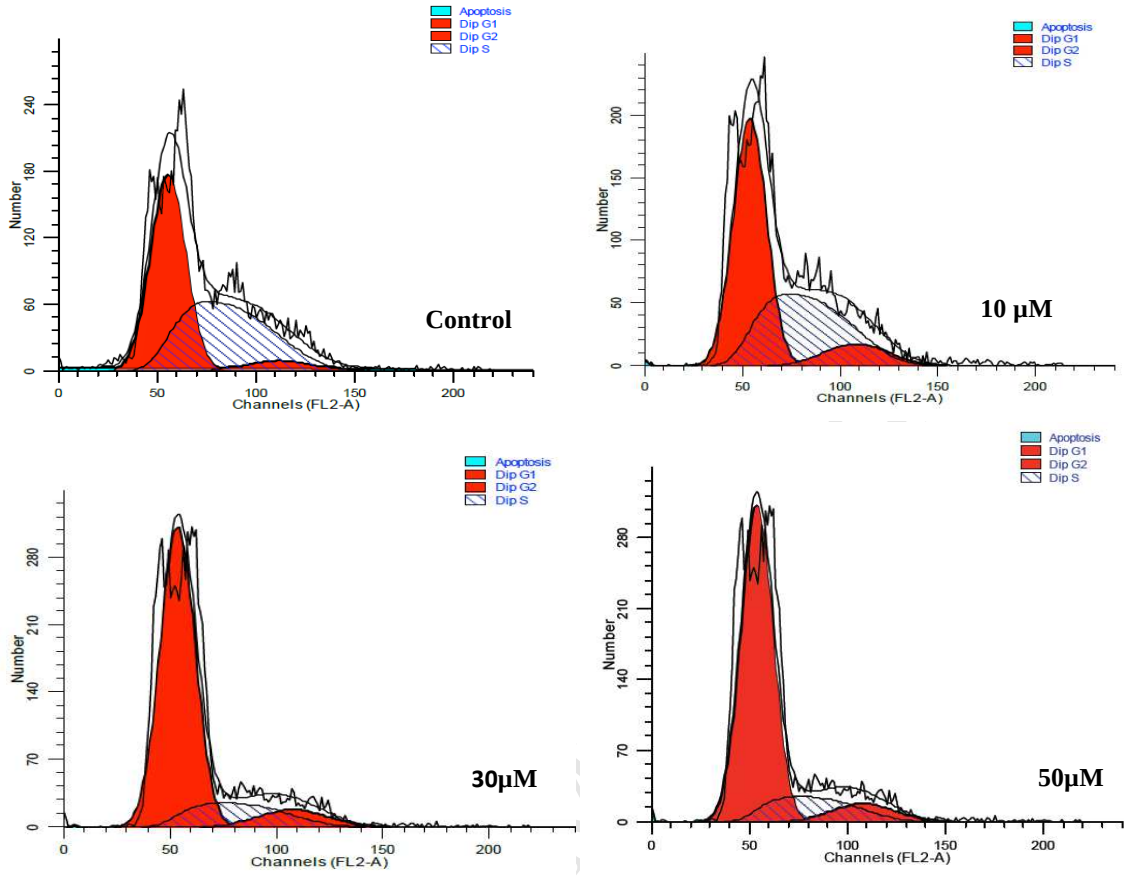


Figure 5. MCF-7 cells were treated with the indicated concentrations of 247 for 24h and H_2O_2 taken as a positive control. The fluorescence intensity of DCF was increased in a dose dependent manner after 24h exposure to the test compound.

To further investigate whether 247 could induce an alterations in cell cycle phases in breast cancer cells, flow cytometric analyses of propidium iodide stained nuclei cells were performed in which 247 induced G1 arrest of 52%, 74.94% and 88.63% in opposition to 49% of its negative control at IC_{50} value as well as at higher concentrations in MCF-7 cells. (Figure 6) It was concluded from these results that 247 is a strong inhibitor of cell cycle progression at the G_1 phase in a concentration independent manner [20].



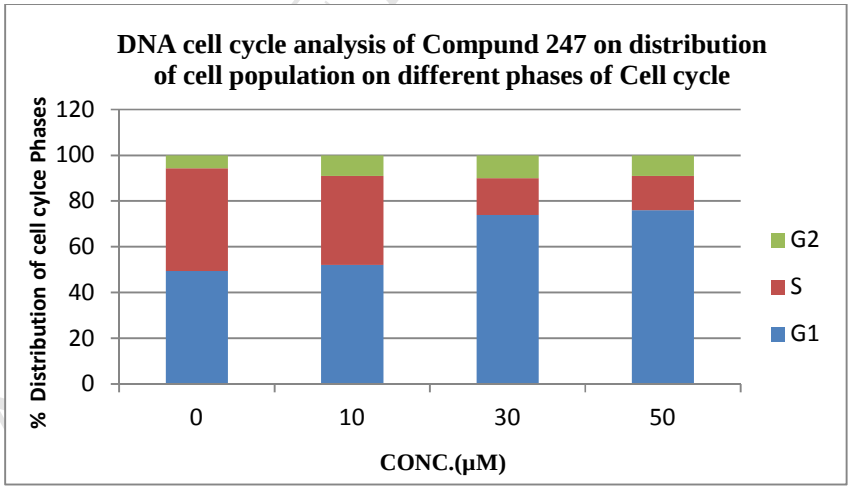


Figure 6: Effect of compound 247 on cell cycle progression. MCF-7 cells were treated with the indicated concentrations of 247 for 24 h. Cells were stained with PI to determine DNA fluorescence and cell cycle phase distribution by flow cytometry as described in Methods. Data are representative of one of two similar experiments.

3. Conclusion

Some novel pyrazolo[1, 5-a]pyridines were synthesized through facile procedures and showed significant in vitro cytotoxic potential on various human cancer cell lines. They revealed various cancer cell lines (A549, PC-3, HCT-116, MCF-7) determined by SRB assay. Compound 247 led to accumulation MCF-7 cells in G1-phase and revealed its important role in mitotic cell cycle progression.

4. Experimental

4.1 General Experimental protocol:

All commercially available chemicals and reagents were used without any further purification unless otherwise indicated. ^{1}H and ^{13}C NMR spectra were recorded at 500, and 125 MHz, respectively. The spectra were recorded in $CDCl_3$ as solvent. Multiplicity was indicated as follows: s (singlet); d (doublet); t (triplet); m (multiplet); dd (doublet of doublets), etc. and coupling constants (J) was given in Hz. Chemical shifts are reported in ppm relative to TMS as an internal standard. The peaks around delta values of ^{1}H NMR (7.2), and ^{13}C NMR (77.0) are correspond to deuterated solvent chloroform respectively. Mass spectra were obtained using electron impact (EI) ionization method. Progress of the reactions was monitored by thin layer chromatography (TLC). All products were purified through column chromatography using silica gel 100-200 mesh size with hexane/ethyl acetate as eluent unless otherwise indicated.

*4.1.1 Typical procedure for the synthesis of cyclohexyl 2-phenylpyrazolo[1,5-a]pyridine-3-carboxylate (**245**):*

65.7 mg (0.3 mmol) of cyclohexyl 2-(pyridin-2-yl)acetate **1**, 463.5 mg (15 mmol) of benzonitrile (**2**), 54.6 mg $Cu(OAc)_2$ (0.3 mmol), 42.9 mg CuBr (0.3 mmol), 45.6 mg DBU (0.9 mmol) and DMSO (0.5 mL) were placed in a 25-mL double-necked round-bottomed flask. The mixture was heated in an oil bath at 60-65 ^{o}C for 6 h under an argon atmosphere (balloon). After completion of the reaction, it was allowed to room temperature and then the mixture was purified through column chromatography using silica gel (5 % EtOAc/hexane) to afford **245**; in 60% yield (58.0 mg).

(Eluent: 5% EtOAc/hexane); 60% yield (58 mg); colour less liquid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (d, J = 6.5 Hz, 1H), 8.23 (d, J = 8.5 Hz, 1H), 7.78- 7.76 (m, 2H), 7.46-7.38 (m, 4H), 6.94 (t, J = 7.0 Hz, 1H), 5.00 (sept, J = 4.0 Hz, 1H), 1.93-1.89 (m, 2H), 1.65-1.63 (m, 2H), 1.53-1.23 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.1, 156.9, 142.6, 132.7, 129.7, 128.79, 128.74, 127.7, 127.1, 119.7, 113.7, 101.2, 72.2, 31.7, 25.4, 23.6. IR (nujol) 3406, 3070, 2942, 2865, 2365, 1712, 1510, 1139, 1051, 764, 692, 445. HRMS calcd for $C_{20}H_{21}O_2N_2$: 321.1603. Found: 321.1590.

*4.1.2 Butyl 2-phenylpyrazolo[1,5-a]pyridine-3-carboxylate (**247**)*

Eluent: 5% EtOAc/hexane);52% yield (46 mg); White solid; M.p.62-67 °C;^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (d, J = 6.5 Hz, 1H), 8.22 (d, J = 9.0 Hz, 1H), 7.77-7.75 (m, 2H), 7.47-7.39 (m, 4H), 6.95 (t, J = 7.0 Hz, 1H), 4.25 (t, J = 7.0 Hz, 2H), 1.63 (quin, J = 7.0 Hz, 2H), 1.30 (quin, J = 7.5 Hz, 2H), 0.89 (t, J = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.7, 156.9, 142.7, 132.6, 129.9, 128.8, 128.7, 127.7, 127.2, 119.6, 113.8, 100.8, 63.8, 30.7, 19.2, 13.6. IR (KBr) 3406, 3070, 2942, 2865, 1629, 1510, 1465, 1206, 1139, 1051, 764, 692, 445. HRMS calcd for $C_{18}H_{19}O_2N_2$: 295.1447. Found: 295.1451.

*4.1.3 Tert-butyl-2-phenylpyrazolo[1,5-a]pyridine-3-carboxylate (**295**)*

(Eluent: 5% EtOAc/hexane);62% yield (54.6 mg); White solid; M.p.88-93°C;^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (d, J= 6.5 Hz, 1H), 8.20 (d, J = 9.0 Hz, 1H), 7.74 (d, J = 8.0 Hz, 2H), 7.46-7.36 (m, 4H), 6.92 (t, J = 6.5 Hz, 1H), 1.46 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.9, 156.7, 142.5, 132.9, 129.9, 128.6, 128.5, 127.6, 126.8, 119.6, 113.5, 102.2, 80.4, 28.3. IR (KBr) 3424, 2975, 2367, 1631, 1502, 1132, 784, 503. HRMS calcd for $C_{18}H_{18}O_2N_2Na$: 317.1266. Found: 317.1270.

*4.1.4 Ethyl-2-(3-chlorophenyl)pyrazolo[1,5-a]pyridine-3-carboxylate (**297**)*

(Eluent: 5% EtOAc/hexane);58% yield (52.6 mg); White solid; M.p.128-133°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (d, J = 6.5 Hz, 1H), 8.24 (d, J = 9.0 Hz, 1H), 7.806-7.802 (m 1H), 7.70-7.68 (m, 1H), 7.45-7.37 (m, 3), 7.00-6.98 (m, 1H), 4.34 (q, J = 7.0 Hz, 2H), 1.32 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.3, 155.3, 142.7, 134.3, 133.5, 130.1, 128.9, 128.88, 128.84, 128.2, 127.4, 119.8, 114.1, 100.9, 60.0, 14.2. IR (KBr) 3449, 2365, 1710, 1144. HRMS calcd for $C_{16}H_{14}ClN_2O_2$: 301.0744. Found: 301.0747.

*4.1.5 Ethyl-2-(3-bromophenyl)pyrazolo[1,5-a]pyridine-3-carboxylate (**298**)*

(Eluent: 5% EtOAc/hexane);51% yield (52.5 mg); White solid; M.p.120-125°C;^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (d, J = 7.0 Hz, 1H), 8.24 (d, J = 9.0 Hz, 1H), 7.96-7.95 (m, 1H), 7.74 (d, J = 8.0 Hz, 1H), 7.57-7.56 (m, 1H), 7.45 (d, J = 6.5 Hz, 1H), 7.34 (t, J = 7.0 Hz, 1H), 7.00-6.95 (m, 1H), 4.34 (q, J = 7.0 Hz, 2H), 1.34 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.3, 155.2, 142.7, 134.6, 133.0, 131.8, 129.2, 128.8, 128.7, 127.4, 121.7, 119.8, 114.1, 100.9, 60.0, 14.2. IR (KBr) 3397, 3031, 2918, 2368, 1628, 1509, 1466, 1216, 1134, 1052, 752, 688, 498. HRMS calcd for $C_{16}H_{14}O_2N_2Br$: 345.0239. Found: 345.0243.

*4.1.6 Ethyl-2-(4-(trifluoromethyl)phenyl)pyrazolo[1,5-a]pyridine-3-carboxylate(**299**)*

(Eluent: 5% EtOAc/hexane);70% yield (70.2 mg); White solid; M.p.97-102°C ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (d, J = 7.0 Hz, 1H), 8.23 (d, J = 9.0 Hz, 1H), 7.93 (d, J = 7.5 Hz, 2H), 7.71 (d, J = 7.5 Hz, 2H), 7.44 (t, J = 7.5 Hz, 1H), 6.99 (t, J = 6.5 Hz, 1H), 4.34 (q, J = 7.5 Hz, 2H), 1.32 (t, J = 7.5 Hz, 3H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.2, 155.4, 142.5, 136.2, 130.7 (d J = 111 Hz), 128.8, 127.5(d J = 51.5 Hz), 124.8 (d J = 81 Hz), 119.8, 114.2, 100.9, 60.0, 14.2. IR (KBr) 3407, 3030, 2365, 1632, 1511, 1210, 1143, 784, 522. HRMS calcd for $C_{17}H_{14}O_2N_2F_3$: 335.1007. Found: 335.1011.

*4.1.7 Ethyl-2-(4-(methoxycarbonyl)phenyl)pyrazolo[1,5-a]pyridine-3-carboxylate (**300**)*

(Eluent: 5% EtOAc/hexane);62% yield (60.5 mg); White solid; M.p.128-133°C ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 (d, J = 7.5 Hz, 1H), 8.25 (d, J = 9.0 Hz, 1H), 8.13 (d, J = 9.0 Hz, 2H), 7.88 (d, J = 8.5 Hz, 2H), 7.45 (t, J = 7.0 Hz, 1H), 7.00 (t, J = 6.5 Hz, 1H), 4.34 (q, J= 7.0 Hz, 2H), 3.95 (s, 3H), 1.30 (t, J = 7.0 Hz, 3H) ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.0, 163.3, 155.8, 142.7, 137.2, 130.2, 130.1, 129.0, 128.9, 127.4, 119.8, 114.1, 101.1, 60.0, 52.1, 14.2. IR (KBr) 3430, 2954, 2366, 1723, 1514, 1219,1053, 771. HRMS calcd for $C_{18}H_{17}O_4N_2$: 325.1188. Found: 325.1191.

*4.1.8 Ethyl-2-([1,1'-biphenyl]-4-yl)pyrazolo[1,5-a]pyridine-3-carboxylate (**303**)*

Eluent: 5% EtOAc/hexane);52% yield (53 mg); White solid; M.p.106-111°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (d, J = 7.5 Hz, 1H), 8.24 (d, J = 9.0 Hz, 1H), 7.92 (d, J = 8.0 Hz, 2H), 7.71-7.61 (m, 4H), 7.49-7.37 (m, 4H), 6.98-6.96 (m, 1H), 4.36 (q, J = 7.5 Hz, 2H), 1.36(t, J = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.5, 156.6, 142.6, 141.5, 140.8, 131.4, 130.3, 128.8, 128.7, 127.3, 127.18, 127.13, 126.4, 119.7, 113.8, 100.7, 59.9,

14.3. IR (KBr) 3381, 2979, 2367, 1702, 1508, 1135, 790, 464. HRMS calcd for C_{22} H_{19} O_2 N_2: 343.1447. Found: 343.1448.

*4.1.9 Cyclohexyl-2-([1,1'-biphenyl]-4-yl)pyrazolo[1,5-a]pyridine-3-carboxylate (**305**)*

Eluent: 5% EtOAc/hexane);59% yield (70 mg); White solid; M.p.143-148°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 (d, J = 7.0 Hz, 1H), 8.24 (d, J = 9.0 Hz, 1H), 7.89 (d, J = 8.0 Hz, 2H), 7.69-7.65 (m, 4H), 7.47-7.35 (m, 4H), 6.95 (t, J = 6.5 Hz, 1H), 5.03 (sept, J = 4.0 Hz, 1H), 1.95-1.92 (m, 2H), 1.68-1.65 (m, 2H), 1.55-1.50 (m, 3H), 1.41-1.35 (m, 2H), 1.28-1.25 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.1, 142.7, 141.5, 140.9, 131.7, 130.4, 128.8, 128.7, 127.4, 127.2, 126.5, 119.8, 113.8, 101.2, 72.3, 31.8, 25.4, 23.7. IR (KBr) 3430, 2934, 2365, 1631, 1512, 1162, 1048, 762. HRMS calcd for C_{26} H_{25} O_2 N_2: 397.1916. Found: 397.1922.

4.1.10 cyclohexyl 2-(thiophen-2-yl)pyrazolo[1,5-a]pyridine-3-carboxylate (309)

Eluent: 5% EtOAc/hexane); 34% yield (33 mg); White solid; M.p.124-127°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (d, J = 7.0 Hz, 1H), 8.20 (q, J = 3.5 Hz, 2H), 7.44 (t, J = 6.0 Hz, 1H), 7.41-7.37 (m, 1H), 7.16 (m, 1H), 6.97-6.93 (m, 1H), 4.57 (q, J = 7.0 Hz,1H), 2.10-2.04 (m, 2H), 1.82-1.77 (m, 2H), 1.64-1.57 (m, 3H), 1.50-1.41 (m, 3H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.5, 150.5, 142.8, 133.8, 130.5, 128.6, 127.5, 127.4, 127.2, 127.1, 126.5, 119.8, 113.9, 113.8, 72.7, 60.1, 32.0, 25.4, 24.0. IR (KBr) 3449, 2935, 2368, 1704, 1509, 1210, 1118, 1011, 787, 712, 444. HRMS calcd for $C_{18}H_{19}O_2N_2S$: 327.1167. Found: 327.1153.

*4.1.11 Ethyl-2-(p-tolyl)pyrazolo[1,5-a]pyridine-3-carboxylate (**310**)*

(Eluent: 5% EtOAc/hexane);32% yield (32 mg); White solid; M.p.90-95°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (d, J = 7.0 Hz, 1H), 8.20 (d, J = 9.0 Hz, 1H), 7.70 (d, J = 8.0 Hz, 2H), 7.40 (t, J = 8.0 Hz, 2H), 7.27-7.25 (m, 2H), 6.94 (t, J = 7.0 Hz, 1H), 4.34 (q, J= 7.0 Hz, 2H), 2.41 (s, 3H), 1.33 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.4, 155.8, 141.4, 137.5, 128.6, 128.3, 127.5, 127.2, 125.8, 118.4, 112.4, 99.4, 58.6, 20.1, 13.1. IR (KBr) 3449, 2367, 1511, 1476, 1437, 1210, 1129, 1044, 784, 508. HRMS calcd for C_{17} H_{17} O_2 N_2: 281.1290. Found: 281.1297.

*4.1.12 Cyclohexyl-2-(4-(trifluoromethyl)phenyl)pyrazolo[1,5-a]pyridine-3-carboxylate (**311**)*

(Eluent: 5% EtOAc/hexane);68% yield (79.5 mg); White solid; M.p.66-71°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (d, *J* = 7.0 Hz, 1H), 8.24 (d, *J* = 8.5 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.72 (d, *J* = 8.5 Hz, 2H), 7.46 (t, *J* = 7.0 Hz, 1H), 7.01 (t, *J* = 6.5 Hz, 1H), 5.00 (sept, *J*= 5.0 Hz, 1H), 1.94-1.90 (m, 2H), 1.65-1.63 (m, 2H), 1.52-1.26 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.8, 155.4, 142.5, 136.4, 130.5, 130.5(d *J* = 72.5 Hz), 128.8, 127.4, 124.8(d *J* = 86.5 Hz), 119.9, 114.1, 101.5, 72.5, 31.7, 25.3, 23.6. IR (KBr) 3449, 2942, 2368, 1699, 1510, 1324, 1162, 1119, 1067, 849,788, 603, 507. HRMS calcd for $C_{21}H_{20}O_2N_2F_3$: 389.1477. Found: 389.1489.

*4.1.13 Cyclohexyl-2-(4-(methoxycarbonyl)phenyl)pyrazolo[1,5-a]pyridine-3-carboxylate (**312**)*

(Eluent: 5% EtOAc/hexane); 72% yield (80.5 mg); White solid; M.p.121-126°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (d, *J* = 7.0 Hz, 1H), 8.23 (d, *J* = 9.0 Hz, 1H), 8.12 (d, *J* = 8.5 Hz, 2H), 7.86 (d, *J* = 8.5 Hz, 2H),.7.41 (t, *J* = 7.5 Hz, 1H), 6.96 (t, *J* = 7.0 Hz, 1H), 4.97 (sept, *J* = 5.0 Hz, 1H), 3.90 (s, 3H), 1.91-1.89 (m, 2H), 1.65-1.62 (m, 2H), 1.52-1.21(m, 6H).^{13}C NMR (125 MHz, $CDCl_3$) δ 165.7, 161.8, 154.4, 141.4, 136.1, 128.9, 128.8, 127.7, 127.5, 126.5, 118.6, 112.8, 100.2, 71.3, 50.9, 30.5, 24.1, 22.5. IR (KBr) 3421, 2936, 2367, 1724, 1696. 1439, 1284, 1114, 771, 489. HRMS calcd for $C_{22}H_{23}N_2O_4$: 379.1658. Found: 379.1650.

*4.1.14 Butyl-2-(thiophen-2-yl)pyrazolo[1,5-a]pyridine-3-carboxylate (**313**)*

(Eluent: 5% EtOAc/hexane); 37% yield (32 mg); White solid; M.p.68-73°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (d, *J* = 7.0 Hz, 1H), 8.18-8.15 (m, 2H), 7.43-7.37 (m, 2H), 7.15 (t, *J* = 5.5 Hz, 1H), 6.94 (t, J = 7.0 Hz, 1H), 4.37 (t, *J* = 7.0 Hz, 2H), 1.79 (quin, *J* = 7.0 Hz 2H), 1.51-1.41 (m, 2H), 0.98 (t, *J* = 7.0 Hz, 3H).. ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.5, 150.4, 142.8, 133.8, 130.4, 128.6, 127.47, 127.42, 119.7, 113.9, 100.0, 64.0, 30.8.19.3, 13.7. IR (KBr) 3449, 2963, 2371, 1702, 1504, 1206, 1115, 697, 451. HRMS calcd for $C_{16}H_{17}O_2N_2S$: 301.1011. Found: 301.1001.

*4.1.15 Ethyl-2-(pyridin-3-yl)pyrazolo[1,5-a]pyridine-3-carboxylate (**315**)*

(Eluent: EtOAc); 72% yield (57.5 mg); White solid; M.p.138-143°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.01-9.00 (m, 1H), 8.68-8.66 (m,1H), 8.55 (d, *J* = 7.0 Hz, 1H), 8.25 (d, *J* = 7.0 Hz, 1H), 8.15-8.12 (m, 1H), 7.45 (t, *J* = 7.0 Hz, 1H), 7.40-7.38 (m,1H), 7.01 (t, *J* = 7.0 Hz, 1H), 4.33 (quar, *J* = 7.0 Hz, 2H), 1.31 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125

MHz, $CDCl_3$) δ 163.2, 153.7, 150.5, 149.6, 142.7, 137.4, 128.8, 128.7, 127.5, 122.5, 119.8, 114.2, 101.2, 60.0, 14.2. IR (KBr) 3449, 3034, 2977, 2368, 1633, 1575, 1511, 1211, 1144, 783, 563. HRMS calcd for $C_{15}H_{14}O_2N_3$: 268.1086. Found: 268.1081.

*4.1.16 Ethyl-2-(pyridin-4-yl)pyrazolo[1,5-a]pyridine-3-carboxylate (**316**)*

(Eluent: EtOAc);81% yield (66 mg); White solid; M.p.154-159°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.72 (d, *J* = 5.5 Hz, 2H), 8.55 (d, *J* = 7.0 Hz, 1H), 8.25 (d, *J* = 9.0 Hz, 1H), 7.75 (d, *J* = 5.5 Hz, 2H), 7.46 (t, *J* = 7.5 Hz, 1H), 7.02 (t, *J* = 6.5 Hz, 1H), 4.35 (q, *J* = 7.0 Hz, 2H), 1.33 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.1, 154.1, 149.3, 142.6, 140.5, 128.5, 127.6, 124.4, 119.9, 114.2, 101.2, 60.2, 14.2. IR (KBr) 3449, 3069, 3032, 2973, 2369, 1632, 1511, 1211, 1153, 1058, 784, 508. HRMS calcd for $C_{15}H_{14}O_2N_3$: 268.1086. Found: 268.1075.

*4.1.17 Butyl-2-(2-chlorophenyl)pyrazolo[1,5-a]pyridine-3-carboxylate (**317**)*

(Eluent: 5% EtOAc/hexane);60% yield (52.0 mg); White solid; M.p.78-83°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 (d, *J* = 7.0 Hz, 1H), 8.25 (d, *J* = 9.0 Hz, 1H), 7.49-7.43 (m, 3H), 7.40-7.33 (m, 2H), 6.99 (t, *J* = 7.0 Hz, 1H), 4.15 (t, *J* = 7.5 Hz, 2H), 1.46 (quin, *J* = 6,5 Hz 2H), 1.13 (sextet, *J* = 7.5 Hz, 2H), 0.82 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.3, 154.2, 141.9, 134.1, 132.8, 131.1, 129.8, 129.1, 128.9, 127.3, 126.2, 119.4, 113.9, 102.7, 63.7, 30.5, 19.0, 13.6. IR (KBr) 3432, 2960, 2366, 1637, 1244, 1025, 755, 430. HRMS calcd for $C_{18}H_{18}O_2N_2Cl$: 329.1057. Found: 329.10565.

*4.1.18 Cyclohexyl-2-(pyridin-4-yl)pyrazolo[1,5-a]pyridine-3-carboxylate (**318**)*

(Eluent: EtOAc);84% yield (81 mg); White solid; M.p.98-103°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.76 (d, *J* = 5.0 Hz, 2H), 8.56 (d, *J* = 7.0 Hz, 1H), 8.28 (d, *J* = 9.0 Hz, 1H), 7.76 (d, *J* = 5.5 Hz, 2H), 7.48 (t, *J* = 7.5 Hz, 1H), 7.03 (t, *J* = 7.0 Hz, 1H), 5.03 (sept, *J* = 5.0 Hz, 1H), 1.97-1.96 (m, 2H), 1.70-1.68 (m, 2H), 1.58-1.27 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.6, 154.0, 149.2, 142.6, 140.6, 128.8, 127.4, 124.4, 119.9, 114.3, 101.6, 72.7, 31.7, 25.2, 23.6. IR (KBr) 3393, 2943, 2367, 1705, 1507, 1205, 1145, 1051, 786, 446. HRMS calcd for $C_{19}H_{20}O_2N_3$: 322.1556. Found: 322.1555.

*4.1.19 Ethyl-2-(4-fluorophenyl)pyrazolo[1,5-a]pyridine-3-carboxylate (**322**)*

(Eluent: 5% EtOAc/hexane);62% yield(53 mg); White solid; M.p.90-95°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (d, *J* = 7.0 Hz, 1H), 8.20 (d, *J* = 9.0 Hz, 1H), 7.79 (s, 2H), 7.40 (t, *J* = 7.5 Hz, 1H), 7.13 (t, *J* = 8.0 Hz, 2H), 6.96 (d, *J* = 6.5 Hz, 1H), 4.23 (q, *J*= 7.0 Hz,

2H), 1.32 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.2 (d, *J* = 246.2 Hz),163.4, 155.9, 142.6, 131.8, (d, *J* =8.1 Hz), 128.7, (d, *J* = 28.5 Hz), 127.2, 119.7, 114.7, (d, *J* = 21.3 Hz), 113.8, 100.6, 59.9, 14.2. IR (KBr) 3450, 3032, 2370, 1713, 1512, 1142, 786, 511. HRMS calcd for $C_{16}H_{14}O_2N_2F$: 285.1039. Found: 285.1028.

*4.1.20 Ethyl-2-(3-fluorophenyl)pyrazolo[1,5-a]pyridine-3-carboxylate (**323**)*

(Eluent: 5% EtOAc/hexane);61% yield (52 mg); White solid; M.p.116-121°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (d, *J* = 7.0 Hz, 1H), 8.23 (d, *J* = 9.0 Hz, 1H), 7.60 (d, *J* = 7.5 Hz, 1H), 7.54 (d, *J* = 10,0 Hz, 1H),7.44-7.39 (m, 2H), 7.15-7.11 (m, 1H), 6.99 (t, *J* = 6.5 Hz, 1H), 4.35 (q, *J* = 7.0 Hz, 2H), 1.33 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.3, (d, *J* = 20.6 Hz),161.2, 155.5, 142.7, 134.6, (d, *J* = 8.1 Hz), 129.2, (d, *J* = 8.1 Hz), 128.8, 127.3, 125.7, 119.8, 117.2, (d, *J* = 22.8 Hz), 115.7, (d, *J* = 21.0 Hz), 114.0, 100.8, 59.9, 14.1. IR (KBr) 3449, 3033, 2979, 1715, 1513, 1203, 775, 467. HRMS calcd for $C_{16}H_{14}O_2N_2F$: 285.1039. Found: 285.1032.

*4.1.21 Ethyl-2-(4-chlorophenyl)pyrazolo[1,5-a]pyridine-3-carboxylate (**325**)*

(Eluent: 5% EtOAc/hexane);63% yield (57 mg); White solid; M.p.124-129°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (d, *J* = 6.5 Hz, 1H), 8.20 (d, *J* = 9.0 Hz, 1H), 7.76 (d, *J* = 8.5 Hz, 2H), 7.43-7.35 (m, 3H), 6.95 (t, *J* = 6.5 Hz, 1H), 4.34 (q, *J* = 7.5 Hz, 2H), 1.33 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.6, 155.6, 142.6, 134.9, 131.3, 130.9, 128.7, 127.9, 127.3, 119.7, 113.9, 100.7, 59.9, 14.2. IR (KBr) 3430, 3030, 2365, 1713, 1511, 1218, 1143, 784, 500. HRMS calcd for $C_{16}H_{14}ClN_2O_2$: 301.0744. Found: 301.0744.

5. Methodologies:

5.1 Cell Culture and Growth Conditions:

The Human cancer cell lines (A549,PC-3,HCT-116 and MCF-7) were acquired from National Cancer Institute, Frederick, U.S.A. Cell lines were grown in tissue culture flasks in complete growth medium (RPMI-1640 medium with 2mM glutamine, pH 7.4, supplemented with 10% fetal bovine serum, 100µg/ml streptomycin and 100 units/ml penicillin in carbon dioxide incubator (New Brunswick, Galaxy 170R, Eppendorf) at 37°C, 5% CO_2 and 98% RH.

5.2 In vitro Cytotoxicity by SRB assay:

The SRB assay was performed in which 100µl of cell suspension was added to each well of the 96-well tissue culture plate. The cells were allowed to grow in carbon dioxide incubator (37°C, 5% CO_2, 90% RH) for 24 hours. Test materials in complete growth medium (100µl) along with known cytotoxic agents: Paclitaxel, 5-FU and Doxorubicin as positive controls were added after 24 hours of incubation to the wells containing cell suspension. The plates were further incubated for 48 hours in a carbon dioxide incubator. The cells were then immobilized with ice-cold trichloroacetic acid for 1 hour at 4°C. After that, the plates were washed three times with water and air-dried. Plates were then stained with Sulforhodamine B dye (0.4 % in 1% acetic acid, 100µl) for 30 minutes. Again, the plates were washed three times with 1% (vol/vol) acetic acid to remove the unbound dye. The adsorbed dye was dissolved in 10mM Tris Buffer (pH 10.4) and plates were gently stirred for 10 minutes on a mechanical stirrer. The optical density (OD) was recorded on ELISA reader at 540 nm. The cell growth was determined by subtracting mean OD value of respective blank from the mean OD value of experimental set. Percent growth in presence of test material was calculated considering the growth in absence of any test material as 100% and in turn percent growth inhibition in presence of test material was calculated. IC_{50} was determined by plotting OD against concentration by Graph Pad Prism version 5.0 (1, 2).

5.3 DAPI staining:

Human Breast cancer MCF-7 cells were seeded at a density of $1.5x10^5$/ml in six well plates. The compound 247 was added at concentrations of 10, 20 and 30µM for 24h; Paclitaxel was taken as a positive control. After 24h incubation, cells were trypsinized, washed and resuspended in PBS. Slides were fixed in chilled methanol for 30 min and stained with 4', 6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) 1µg/ml and incubated at 37^0C for 30 min in the dark. After PBS washing, the cells were mounted in glycerol: PBS (9:1) and covered with glass cover slip. The slides were then observed for nuclear morphological alterations under fluorescence microscope (Olympus) using UV filter at 40X magnification.

5.4 Reactive Oxygen Species (ROS):

MCF-7 breast cancer cells ($1x10^5$) were seeded into six well plate and treated with 10, 20 and 30µM of compound 247 for 24h. H_2O_2 was added to the medium 2h prior to the termination. The cells were further incubated with 10µM DCFDA for 30 min at 37^oC in dark. Then, cells were washed with PBS and analyzed under fluorescence microscope (Nikon D3100) at magnification of 20X.The fluorescence intensity was measured with a Tecan microplate reader (Infinite M200 PRO) at an excitation and emission wavelength of 480/530nm.

5.5 Analysis of cell cycle phases by flow cytometry:

MCF-7 cells (1×10^6 cells/2 mL/well) were treated with 247 at different concentration of 10, 30, 50 µM/mL for 24 h. After 24 h, cells were harvested, washed twice in PBS, and fixed in 70% cold ethanol overnight. Cells were again washed with PBS and subjected to RNase digestion (0.1 mg/ml) at 37^0C for 90 min and finally cells were incubated with PI (10 mg/ml). Cells are then analyzed immediately on FACS Calibur Flow Cytometer (Becton Dickinson, Franklin Lakes, NJ). The results were analyzed by Mod fit (Verity Software House Inc., Topsham.

5.6 Bioluminescent EGFR Kinase Assay:

EGFR kinase activity was measured by using ADP^{TM} Glo™ EGFR Kinase Assay (Promega Corporation). It generates luminescent signal which is proportional to ADP concentration produced by the kinase reaction. Assay was carried out according to manufacturer's protocol. Dose response of test material was assessed in duplicates by using different dilutions of the compound. Tyrosine kinase buffer diluted enzyme (10µl), substrate-ATP (10µl) and compound (5µl of each dilution) were transferred to white 96-well microtiter plate (Costar # 3912, Corning, NY) and incubated at ambient temperature for 60 min. In all wells 30ng EGFR kinase, 5µM ATP, 0.2µg/µl Poly (Glu4, Tyr) substrate was present. In blank except enzyme all components were present. ADP Glo reagent (25µl) was added to stop the reaction and the plate was again incubated for 40 min at ambient temperature and finally 50 µl of kinase detection reagent was added to each well. By using BioTek Synergy Mx microplate reader luminescent signal was

detected after 30 min. The percent enzyme activity was calculated by subtracting the luminescent values for blank from the values of all other reactions. Luminescent values of each reaction were then divided by the 0μM inhibitor reaction and multiplied 100 times to determine the % enzyme activity. To calculate the IC_{50}, Prism, version 5.04, from GraphPad Software (La Jolla, CA), was used.

Acknowledgements

CSIR-CSMCRI Communication No.117/2016. C.R. is thankful to AcSIR for the Ph.D. enrolment and the "Analytical Discipline and Centralized Instrumental Facilities" for providing instrumentation facilities. We thank DST, Government of India (SR/S1/OC-13/2011) as well as CSIR 12thfive-year project (BSC-0205) for the financial support.

References:

[1] J. J. Mousseau, J. A. Bull, C. L. Ladd, A. Fortier, D. S. Roman and A. B. CharetteJ. Org. Chem. 76 (2011) 8243–8261.

[2] J. J.Mousseau, A. Fortier and A. B. Charette Org. Lett.12 (2010) 516-519.

[3]A.Kojima, S.Takita, T.Sumiya, K.Ochiai, K.Iwase, T.Kishi, A.Ohinata, Y.Yageta, T.Y asue, Y.Kohno,Bioorg Med ChemLett. 19 (2013) 5311-5316.

[4] B.Burja, Z. T.Cimbora, S.Tomic. Bio. Med. Chem. 18 (2010) 2375-2387.

[5] A. H. Moustafa, H. A. Saad, W. S. Shehab and M. M. El-Mobayed, Antimicrobial Activity 1 (2008) 115-135.

[6] M.G. Mamolo, D. Zampieri, V. Falagiani, L. Vio, M. Fermeglia, M. Ferrone, S. Pricl, E. Banfi and G. Scialino. ARKIVOC. 5 (2004) 231–250.

[7] A.E. Rashad, M.I. Hegab, R.E. Abdel-Megeid, J.A. Micky, F.M.E. Abdel-Megeid.16 (2008) 7102–7106.

[8] S. Lober, H. Hubner, P. Gmeiner. 9 (1999) 97–102.

[9] S. Lober, H. Hubner, P. Gmeiner. Bioorg. Med. Chem. Lett. 12 (2002)2377-2380.

[10] L. Bettinetti, K. Schlotter, H. Hubner, P. Gmeiner. J. Med. Chem. 45 (2002)4594-4597.

[11] S. R. Roopan, and R. Sompalle, Synthetic Communications. 8 (2016) 645-672.

[12] G. I. Evan and K. H. Vousden, Nature.411 (2001) 342-348.

[13] M. B. Kastan and J.Bartek, Nature. 432 (2004) 316-323.

[14] S.Negrini, V.Gorgoulisand T.D.Halazonetis, Nature Reviews Molecular Cell Biology 11(2010) 220–228.

[15] J. Luo, N. L. Solimini and S. J. Elledge, Cell. 136 (2009)823–837.

[16] D. C. Mohan, C.Ravi,S. N. Rao, S. Adimurthy, Org. Biomol. Chem. 13(2015) 3556–3560.

[17] H.Y.Kim, D. A.Lantrip, P. L.Fuchs, Org. Lett. 3(2001) 14-18.

[18] V. Vichai and K. Kirtikara, Nature Protocols 1(2006) 1112 – 1116.

[19] A.Monks, D.Scudiero, P.Skehan, R. Shoemaker, K.Paull, D.Vistica, C.Hose, J. Langley, P.Cronise, A.Vaigro-Wolff, M.Gray-Goodrich, H.Campbell, J.Mayo and M. Boyd, J. Natl. Cancer Inst. 83(1991) 757-766.

[20] N.Gao, D.C. Flynn, Z. Zhang, X. Zhong, V. Walker, K. J.Liu,X. Shi and B. Jiang, Am J Physiol Cell Physiol. 287(2004) C281–C291.

Highlights

- This work highlights the synthesis of substituted pyrazolo[1,5-a]pyridine derivatives as anticancer agents.
- *In vitro* screening of target pyrazolo[1, 5-a]pyridines against various human cancer cell lines.
- Determination of IC_{50} against various human cancer cell lines.
- Pyrazolo[1, 5-a]pyridines are active anticancer, antimicrobial, antiviral, antipsychotic, etc.