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Research paper

Design, synthesis, broad-spectrum antiproliferative activity, and kinase inhibitory effect of triarylpyrazole derivatives possessing arylamides or arylureas moieties

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ABSTRACT

A novel series of 1,3,4-triarylpyrazole derivatives possessing terminal arylamide or arylurea terminal moieties has been designed and synthesized. Their *in vitro* antiproliferative activities were investigated against a panel of 58 cell lines of nine different cancer types at the NCI, USA. The urea analogues **2b**, **2c**, and **2f** as well as the amide derivatives **3e** and **3f** exerted the highest mean % inhibition values over the 58 cell line panel at 10 μM, and thus were further tested in 5-dose testing mode to determine their GI_{50}, TGI, and LC_{50} values. The above mentioned compounds have shown stronger antiproliferative activities in terms of potency and efficacy upon comparing their results with Sorafenib as a reference compound. Among them, compounds **2c** and **2f** possessing 3,4-dichlorophenylurea terminal moiety showed the highest mean %inhibition value of about 99.85 and 104.15% respectively over the 58-cell line panel at 10 μM concentration. Also compounds **2b**, **3e**, and **3f** exhibited mean % inhibition over 80% at 10 μM concentration. The GI_{50} value of compound **3e** over K-562 cancer cell line was 0.75 μM. Accordingly, compound **2f** was screened over seven kinases at a single-dose concentration of 10 μM to profile its kinase inhibitory activity. Interestingly, the compound showed highly inhibitory activities (90.44% and 87.71%) against BRAF (V600E) and RAF1 kinases, respectively. Its IC_{50} value against BRAF (V600E) was 0.77 μM. Compounds **2b**, **2c**, **2f**, **3e**, and **3f** exerted high selectivity towards cancer cell lines than L132 normal lung cells.

1. Introduction

Cancer is one of the serious public health problems in the world. The statistics show that its incidence and mortality is growing in the developing as well as developed countries. Despite significant advances in the diagnostic and therapeutic techniques nowadays, cancer is considered the second most frequent cause of death after cardiovascular diseases [1,2]. According to the World Health Organization (WHO) report [3], more than 13 million deaths from cancer worldwide are expected to occur in 2030. Considering numerous reports and publications on the synthesis of anticancer agents, there is no drug with 100% efficacy. Therefore, there is still instant demand for more drug discovery leading to efficient anticancer compounds with specific mechanism of action to overcome the side effects associated with current chemotherapeutics in cancer treatment, such as toxicity and drug resistance.

Many research articles have recently reported the potential antiproliferative activity of arylureas and arylamides against a variety of cancer cell lines [4–24]. Sorafenib (Fig. 1) possessing arylurea terminal moiety is an example of anticancer that has been approved by the U.S. Food and Drug Administration (FDA) for

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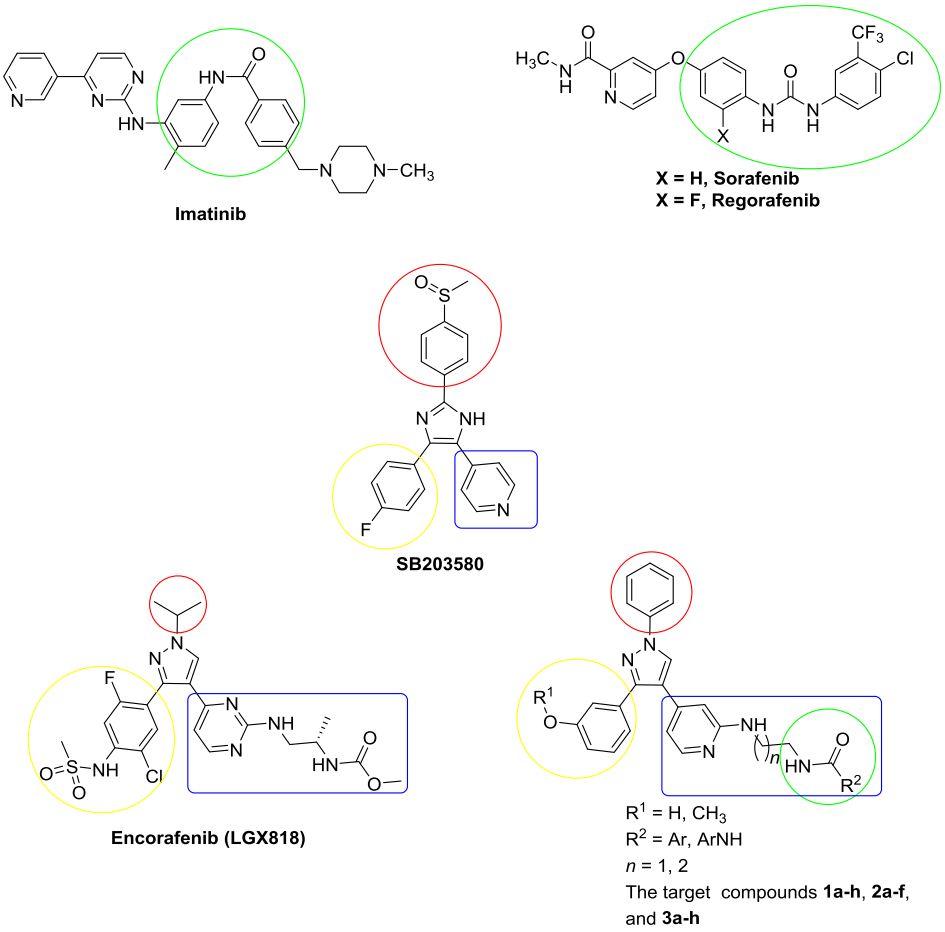


Fig. 1. Structures of Imatinib, Sorafenib, Regorafenib, SB203580, Encorafenib (LGX818), and the target compounds **1a**–**h**, **2a**–**f**, and **3a**–**h**.

treatment of advanced renal cancer, and also currently being in clinical trials for treatment of many other cancer types as metastatic colorectal, ovarian, brain, esophageal/gastroesophageal, leukemia, glioblastoma, Hodgkin's lymphoma, metastatic breast, advanced gastric, hepatocellular carcinoma (HCC), thyroid, non-small cell lung cancer (NSCLC), pancreatic, prostate, bladder, skin/ocular melanoma and neuroendocrine cancers [25,26]. Imatinib (Fig. 1) is an example of anticancer agents having arylamides terminal moiety that is used for treatment of chronic myeloid leukemia (CML) with diminished side effects [27]. It has been studied in clinical trials for treatment of gastrointestinal stromal tumor (GIST), thyroid cancer, breast cancer, meningioma, ovarian cancer, and non-small cell lung cancer (NSCLC) in combination with other drugs [28].

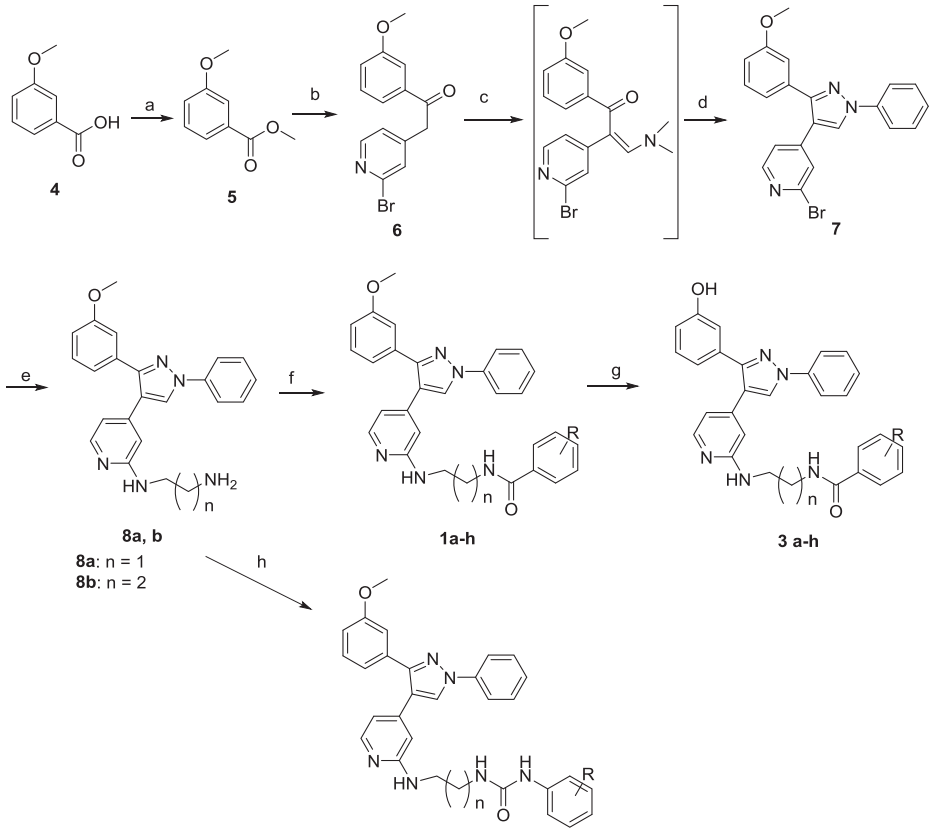
In addition, much attention has been paid to the chemistry and biological activities of 1,3,4-triarylpyrazole scaffold. Several compounds possessing 1,3,4-triarylpyrazole scaffold have been recently reported as potential antiproliferative agents [7,10,14,17,29,30].

In the present study, a new series of 1,3,4-triarylpyrazole derivatives possessing terminal arylamide or arylurea moieties were designed with similarity to SB203580 and Encorafenib (LGX818) (Fig. 1). SB203580 was reported to bind to P38/MAP kinase through hydrophobic/hydrogen bonding interactions of the fluorophenyl ring with a hydrophobic region, hydrogen bonding of the pyridyl and imidazole rings with the kinase hinge region, and the methylsulfinylphenyl ring with the phosphate binding region [32,33]. There is another hydrophobic region below the pyridyl ring which was not occupied by SB203580 [32]. That's why the terminal chain on the pyridyl ring was important. Our target compounds were also designed similar to Encorafenib through ligand-based design approach. The *N*-phenyl ring on the pyrazole ring mimics the *N*-isopropyl group of Encorafenib, and estimated to undergo similar interaction. The two aryl rings at positions 3 and 4 of the pyrazole ring mimic the two aryl rings at the same positions on the Encorafenib structure. And the side chain on the pyridyl ring mimics that of Encorafenib with replacement of the terminal methoxy group of the carbamate moiety of Encorafenib with arylamino or aryl moiety. The designed target compounds were synthesized and tested for *in vitro* antiproliferative activities against NCI-58 cancer cell line panel of nine different cancer types. Kinase inhibitory activities of the most active compounds against wild-type BRAF, V600E mutated BRAF, RAF1, EGFR, P38α/MAPK14, ABL1 and ABL1 (T315I) were also examined in order to test their possible mechanism of action.

2. Results and discussion

2.1. Chemistry

Synthesis of the target compounds **1a**–**h** & **2a**–**f and 3a**–**h** was achieved through the pathway illustrated in Scheme 1. Refluxing the 3-methoxybenzoic acid with methanol in the presence of few drops of sulfuric acid afforded the corresponding methyl ester **5** [34]. Reacting the ester with 4-picoline in the presence of lithium bis(trimethylsilyl)amide (LiHMDS) led to formation of the ketide intermediate **6**. Cyclization to the pyrazole compound **7** was carried



Scheme 1. Reagents and conditions: a) MeOH, dps H_2SO_4, reflux, overnight; b) 2-bromopicoline, THF, LiHMDS, dpwise, −78 °C, then rt, 6 h; c) DMF-DMA, 80 °C, 3 h; d) phenylhydrazine, EtOH, rt, overnight; e) 1,2-ethylenediamine or 1,3-propylenediamine, pyridine, reflux, 8 h; f) appropriate benzoyl chloride, Et_3N, CH_2Cl_2, 0 °C, overnight; g) BBr_3, CH_2Cl_2, −78 °C, then rt, 3 h; h) appropriate aryl isocyanate, THF, rt, overnight.

out by treatment of compound **6** with dimethylformamide dimethylacetal (DMF-DMA) followed by reaction with phenylhydrazine [17]. 1,3,4-Triarylpyrazole possessing pyridylaminoethylamine **8a** or pyridylaminopropylamine **8b** were carried out through refluxing compound **6** with 1,2-diaminoethane or 1,3-diaminopropane in presence of pyridine [35]. Reaction of the terminal amino group of **8a** or **8b** with the appropriate benzoyl chloride in the presence of triethylamine afforded the corresponding compounds **1a-h** with amide moiety as a linker. Synthesis of the methoxy compounds **2a**–**f** possessing urea linker was carried out by interaction of the amino compound **8a** or **8b** with the appropriate arylisocyanate derivatives to give the required products. Demethylation of the methoxy group of **1a**–**h** using boron tribromide afforded the corresponding hydroxyl derivatives **3a**–**h** while in case of urea linker, the demethylation process did not work upon using the same procedure with amide linker due to its insolubility in methylene chloride or THF or even heating with hydrobromic acid to overcome the insolubility issues which resulted in destruction of the compounds. Structures of the target compounds, their yield percentages, and melting points are illustrated in Table 1.

2.2. In vitro antiproliferative activity

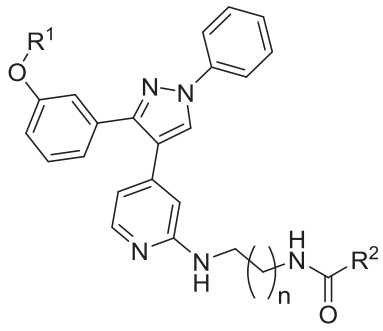
2.2.1. Single-dose testing

Structures of the synthesized target compounds were submitted to National Cancer Institute (NCI), Bethesda, Maryland, USA, and the eighteen compounds shown in Fig. 2 were selected for evaluation of their antineoplastic activity. The selected compounds were tested for *in vitro* anticancer assay against a full panel of 58 human cancer cell lines of nine different cancer types (leukemia, NSCLC, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancers). The compounds were tested at a single-dose concentration of 10 μM, and the percentages of growth inhibition over the 58 tested cell lines were determined. The mean % inhibitions of the NCI-58 cancer cell line panel after treatment with each of the tested compounds are illustrated in Fig. 2.

The results showed that the 1,3,4-triarylpyrazole derivatives bearing urea linker were more active than the corresponding analogues with amide moiety. They have shown higher mean % inhibition values than those of amides. This could be rationalized that the longer linker length might allow more appropriate fitting at the receptor site and/or the terminal NH moiety of the urea spacer might form additional hydrogen bond(s) at the receptor site. Any or both of those effects would enable optimum drug affinity with the receptor site, and hence stronger antiproliferative activity.

Regarding the amide series, it was found that increasing the length in the spacer as propylene gave better mean % inhibition results than those derivatives with ethylene moiety leading to more compound fitting inside the receptor or by even increasing its lipophilicity leading to more cell penetration and hence more cytotoxic activity (compounds **1e, 1f** and **1g** gave higher mean inhibition percentages than **1a**, **1b** and **1c**). It was also found for both amide and urea linker that compounds with substituted aromatic tail are generally more active than unsubstituted phenyl ones (compounds **1f**, **3f**, with trifluormethylphenyl terminal moieties gave higher mean %inhibition than **1e** and **3e**, respectively in amide series and in urea series compounds **2b**, **2c** and **2f** with trifluormethylphenyl and dichlorophenyl terminal moieties in the urea series have shown better results than compound **2a** with phenyl terminal moiety only). Those hydrophobic substituents

Table 1
Structures of the target amide compounds **1a**–**h**, **2a**–**f**, **3a**–**h**, their yield%, and melting points.



1a-h, 2a-f, 3a-h

Compound no.	n	R^1	R^2	Yield%	Melting point (°C)
1a	1	CH_3	C_6H_5	55	163–5
1b	1	CH_3	4-F-C_6H_4	46	180–2
1c	1	CH_3	3-CF_3–C_6H_4	48	164–6
1d	1	CH_3	4-OCH_3–C_6H_4	49	167–9
1e	2	CH_3	C_6H_5	60	170–2
1f	2	CH_3	4-F-C_6H_4	65	171–3
1g	2	CH_3	3-CF_3–C_6H_4	62	133–5
1h	2	CH_3	4-OCH_3–C_6H_4	58	174–6
2a	1	CH_3	C_6H_5–NH	56	133–5
2b	1	CH_3	3-CF_3–C6H4–NH	61	200–2
2c	1	CH_3	3,4-Cl_2–C_6H_4–NH	67	187–9
2d	2	CH_3	C_6H_5–NH	71	148–50
2e	2	CH_3	3-CF_3–C_6H_4–NH	68	160–2
2f	2	CH_3	3,4-Cl_2–C_6H_4–NH	75	167–9
3a	1	H	C_6H_5	32	122–4
3b	1	H	4-F-C_6H_4	35	118–20
3c	1	H	3-CF_3–C_6H_4	40	135–7
3d	1	H	4-OH–C_6H_4	26	146–8
3e	2	H	C_6H_5	34	126–8
3f	2	H	4-F-C_6H_4	41	182–4
3g	2	H	3-CF_3–C_6H_4	45	164–6
3h	2	H	4-OH–C_6H_4	25	136–8

because of the differences in steric and/or electronic properties between methoxy and hydroxyl groups. This reveals that the hydroxyl group on position 3 of the phenyl ring at pyrazole ring is optimal for the activity due to hydrogen bond formation at the receptor site. So it can be concluded that propylene spacer, substituted terminal phenyl derivatives, and the hydroxyl group at position 3 of the phenyl ring on the pyrazole are the optimum for activity. And as shown in Fig. 2, it was found that compound **2f** possessing 3,4-dichlorophenyl urea and propylene moieties exhibited mean % inhibition of 104.1% at 10 μM concentration. Compound **2f** not only stopped the cell proliferation, but also started showing lethal effect. Compounds **2b**, **2c**, **3e**, and **3f** showed over 80% mean inhibition percentages. They also showed % inhibition of more than 100% inhibition over many cancer cell lines. For example, compound **2f** exhibited 192.15%, 161.84%, and 144.74% against LOX IMVI melanoma cell line, and HCC-2998 and COLO 205 colon cancer cell lines. Other compounds showed also %inhibition more than 100% inhibition against different cancer cell lines (as shown in Fig. I in the supplementary file).

2.2.2. Five dose testing

Compounds **2b**, **2c**, **2f**, **3e**, and **3f** with promising results in single-dose testing were further tested in a five-dose testing mode, in order to determine their GI_{50}, TGI, and LC_{50} values over the 58 cancer cell lines. The mean GI_{50} values of these five compounds over the nine cancer types are shown in Table 2.

Most of the compounds exhibited high potency (in micromolar and in submicromolar scales) over the most sensitive cancer cell lines of the nine cancer types. Most of the mean GI_{50} data were less than 10 μM. Compounds **2b**, **2f**, and **3e** results have almost better mean GI_{50} in comparison to Sorafenib over many subpanels. Of special interest, compounds **2b**, **2f**, **3e**, and **3f** showed slightly higher potencies against most of the most sensitive cell lines than Sorafenib over all the nine subpanels of nine different cancer types. Compound **3e** also exhibits submicromolar of 0.75 μM against K-562 leukemia cell line. The GI_{50} values of the five compounds tested in five-dose mode over the most sensitive cell line of each subpanel

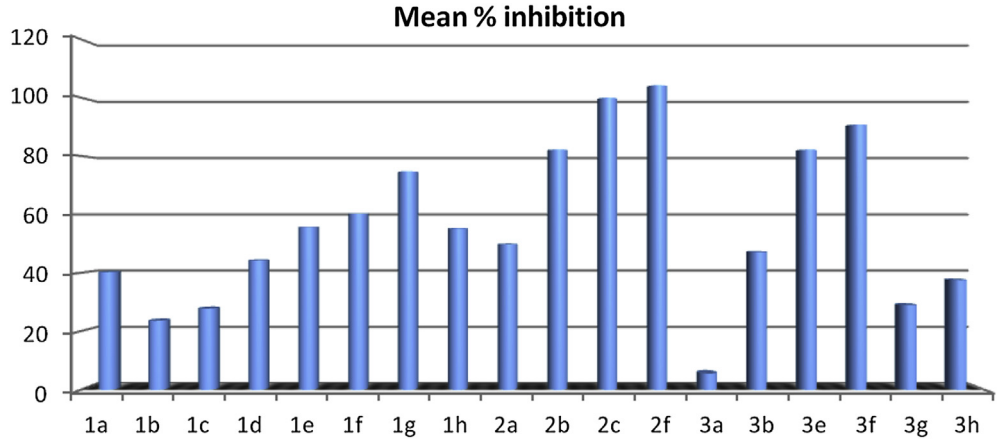


Fig. 2. Mean inhibition percentages observed with the final compounds in single-dose (10 μM) 58-cancer cell line screening. Mean % inhibition represents the mean inhibition percentages over all the 58 tested cancer cell lines. The inhibition percentages were calculated by subtracting the growth percentages from 100.

might enhance the strength of hydrophobic interactions at the receptor site and/or enhance the overall hydrophobicity of the molecule leading to higher penetration inside the cells.

The hydroxyl compounds **3b**, **3e** and **3f** were more active than the corresponding methoxy analogues **1b**, **1e** and **1f** which could be are summarized in Table 3. From Table 3, it could be found that compounds **2b**, **2f** and **3e** were the most potent compounds from our target compounds over the most sensitive cell lines as compared with Sorafenib. The results of Sorafenib were obtained from NCI datawarehouse index [35]. Compounds **2b**, **2f**, **3e**, and **3f**

Table 2
Mean GI_{50} values (μM) of the tested compounds over each cancer subpanel.[a]

Subpanel cancer cell lines[b]										
No. of cell lines in each subpanel		I	II	III	IV	V	VI	VII	VIII	IX
		5	8	7	6	9	7	8	2	6
Compound no.	**2b**	2.04	2.03	1.98	2.07	1.91	2.54	2.14	3.09	2.23
	2c	3.40	3.02	3.73	7.19	4.15	4.72	4.63	5.95	3.35
	2f	2.31	3.51	2.44	2.93	1.89	3.74	3.14	4.71	2.69
	3e	1.62	3.40	2.30	3.60	2.30	4.17	3.63	3.69	2.13
	3f	1.98	3.12	2.16	3.29	1.91	3.46	2.84	3.72	1.89
	Sorafenib	2.43	2.25	2.19	2.33	1.87	2.88	2.94	2.58	2.17

[a] Mean GI_{50} values were calculated by dividing the summation of GI_{50} values of the compound over cell lines of the same cancer type by the number of cell lines in the subpanel.
[b] I: Leukemia; II: Non-Small Cell Lung Cancer; III: Colon Cancer; IV: CNS Cancer; V: Melanoma VII: Ovarian Cancer; VII: Renal Cancer; VIII: Prostate Cancer; IX: Breast Cancer.

Table 3
GI_{50} values (μM) of the tested compounds over the most sensitive cell line of each cancer subpanel.

	Cancer cell line									
Compound no.	K-562[a]	HOP-92[b]	Colo-205[c]	HCT-15[c]	SNB-75[d]	SK-MEL-5[e]	NCI/ADR-RES[f]	RXF-393[g]	PC-3[h]	MDA-MB-468[i]
1g	3.69	2.48	3.33	3.40	17.0	1.54	3.54	2.20	3.18	1.95
2b	2.13	1.46	1.88	1.70	1.62	1.73	2.22	1.53	2.02	1.70
2f	2.38	2.32	1.87	2.40	2.52	1.75	2.19	1.80	3.96	1.92
3e	0.75	1.70	1.71	1.51	1.68	1.49	2.67	1.97	2.10	1.38
3f	1.93	2.13	1.60	1.77	5.19	1.61	2.20	1.63	2.81	1.59
Sorafenib	3.16	1.58	1.99	2.51	3.16	1.58	2.51	2.51	1.99	1.99

[a]Leukemia cell line
[b]non-small cell lung cancer cell line
[c]colon cancer cell line
[d]CNS cancer cell line
[e]melanoma cell line
[f]ovarian cancer cell line
[g]renal cancer cell line
[h]prostate cancer cell line
[i]breast cancer cell line

were more active antiproliferative agents than the corresponding imidazothiazole analogues [31].

In order to study the selectivity of the most potent compounds towards cancer cells over normal cells, the cytotoxicity of compounds **1g**, **2b**, **2f**, **3e** and **3f** were tested against L132 human lung normal cell line. These compounds showed high IC_{50} values over L132 cell line, indicating high selectivity towards cancer cells than normal cells. The high selectivity indices of those potent compounds reveal their high safety.

The TGI and LC_{50} values of compounds **2b**, **2f**, **3e**, and **3f** against the most sensitive cell line of each cancer subpanel are summarized in Table 4, and compared with those of Sorafenib as a reference standard. It is noticed that the four compounds were more efficacious than Sorafenib against the colon, ovarian, and renal cancer cell lines. All the four compounds showed one-digit micromolar GI_{50} values against most of the tested cell line. So they showed 100% inhibition over most of those cell lines at less than 10 μM concentration. Both compounds **2f** and **3f** showed less values of TGI than Sorafenib (more efficacy) against seven different cell lines. Upon comparing their TGI results with their IC_{50} values against L132 normal cells, they inhibited 100% of the growth of many cancer cell lines at less than 7 μM concentration, but they could inhibit only 50% of L132 normal lung cell growth at 100 and 31.3 μM concentrations, respectively. This means very high selectivity towards cancer cells than normal cells. Regarding LC_{50}, compound **3f** exerted the highest efficacy. Its LC_{50} values were in one-digit micromolar effect against six different cancer cell lines.

Table 4
TGI and LC_{50} data (μM) of compounds **2b**, **2f**, **3e**, **3f**, and Sorafenib.

Cell line	Comp. **2b**		Comp. **2f**		Comp. **3e**		Comp. **3f**		Sorafenib	
	TGI	LC_{50}	TGI	LC_{50}	TGI	LC_{50}	TGI	LC_{50}	TGI	LC_{50}
K-562	100	100	5.85	100	ND[a]	100	4.83	100	100	100
HOP-92	7.65	100	7.68	71.0	24.5	100	3.28	7.38	5.01	63.10
Colo-205	3.34	6.95	3.82	7.80	3.67	7.88	3.78	7.60	5.01	31.6
HCT-15	4.70	100	6.66	29.10	5.12	100	3.71	8.09	10.0	50.1
SNB-75	100	100	19.2	100	46.9	100	12.5	74.0	3.98	7.94
SK-MEL-5	3.55	7.82	3.32	6.3	3.12	6.54	3.15	5.72	2.51	5.01
NCI/ADR-RES	5.75	100	5.39	86.90	9.79	100	5.82	29.70	15.8	100
RXF-393	4.63	25.0	4.87	23.0	6.20	100	3.03	5.99	10.00	50.1
PC-3	15.7	100	22.4	100	15.20	100	5.24	44.30	5.01	100
MDA-MB-468	3.50	ND[a]	4.34	9.82	3.36	ND[a]	3.43	6.92	5.01	50.12

[a] Not determined.

2.3. In vitro kinase screening

In order to investigate the mechanism of action of this series of compounds at molecular level, the most potent compounds **2b**, **2f**, **3e** and **3f** were selected to be tested at a single-dose concentration of 10 μM over seven kinases; BRAF (wild-type), BRAF (V600E), RAF1, EGFR, P38a/MAPK14, ABL1, and ABL1 (T315I). The target compounds were designed based on combination or merging of pyridyl pyrazole scaffold with arylamide moieties like Imatinib or arylurea moieties like Sorafenib. Since Imatinib targets Bcr-ABL and Sorafenib target other selected enzymes (B-RAF, V600E-B-RAF, C-RAF, and P38α), so we tested our potential compounds against these kinases. In addition, the compounds were active against some cell lines such as COLO 205 and SK-MEL-5 which contain over-expressed RAF kinases as growth factors. So we wanted to study the inhibitory effects of the most potential compounds against these kinases. As illustrated in Table 5, compound **2f** possessing terminal 3,4-dichlorophenyl urea, propylene spacer, and methoxyphenyl moieties has been the most active of the series showing the strongest inhibitory effect over two kinases at 10 μM. It exerted 90.44% and 87.71% inhibitions against BRAF (V600E) and RAF1 kinases, respectively. It has also shown moderate activity of 57.57% and 45.45% against both BRAF (wild-type), and P38α/MAPK14, respectively. It was further tested in a 10-dose testing mode in order to determine its IC_{50} values over V600E mutated BRAF and RAF1. The IC_{50} values were 0.77 μM and 1.50 μM, respectively. So the affinity of compound **2f** was found to be two-fold higher towards BRAF (V600E) than C-RAF, and much more active than the other tested kinases. Compound **2f** exerted high potency against cell lines with over-expressed V600E-B-RAF such as COLO 205, HT29 (Supplementary file) colon cancer cell lines, and SK-MEL-5 melanoma cell line [36–39]. As explained in the introduction section, V600E-BRAF and C-RAF are over-expressed in a variety of cancer types. So we can conclude that the inhibitory effect of compound **2f** on RAF kinases is, at least in part, a potential mechanism of its antiproliferative effect.

Several pyrazole derivatives have been reported in the literature as kinase inhibitors. They differ in both chemical structure and kinase selectivity. For example, some diarylpyrazole derivatives possessing substituted aminopyrimidinyl pyrazole derivatives were reported as JNK kinase inhibitors [40]. The aminopyridyl or aminopyrimidinyl moieties mimic the aminopurine moiety of the ATP molecule. So those compounds could act as ATP competitive kinase inhibitors. In addition, the binding mode of the similar imidazole compound SB203580 was explained in the Introduction section. The pyridyl and imidazole moieties were reported to bind to the hinge region similar to the aminopurine moiety of the ATP molecule [32,33].

3. Conclusion

In this study, a series of new 1,3,4-triarylpyrazole derivatives possessing arylamide or arylurea terminal moieties was designed and synthesized. Eighteen target compounds were selected for single-dose *in vitro* antiproliferative test over NCI-58 cancer cell line panel of nine different cancer types and five compounds were selected for five-dose testing mode for determination of GI_{50}, TGI, and LC_{50} values. These five compounds **2b**, **2c**, **2f**, **3e**, and **3f** with amide and urea linker showed higher mean % inhibition values of 82.12%, 90.68%, 82.14%, 99.85%, and 104.09% respectively, and compound **2f** possessing 3′,4'-dichlorophenylurea terminal moiety showed the most promising results at five-dose testing. It exerted high potency, efficacy, and broad-spectrum antiproliferative activities over many cancer cell lines of different cancer types with superiority to Sorafenib. It has also showed good inhibitory value against V600E mutated BRAF and RAF1 kinases with IC_{50} values of 0.77 and 1.50 μM, respectively. Due to the high potency of compound **2f** against cancer cell lines containing over-expressed BRAF (V600E) kinase, it could be concluded that BRAF (V600E) kinase inhibition might be, at least in part, a mechanism of antiproliferative activity of this compound at molecular level. Compounds **2b**, **2c**, **2f**, **3e**, and **3f** exerted superior selectivity towards cancer cells than normal cells. So they can be utilized as promising lead compounds for future development of potent, efficacious, and selective anticancer agents. Further modifications of this series in order to optimize the scaffold and to improve their anticancer activities are currently in progress.

4. Experimental

4.1. General

The target compounds were purified by column chromatography using silica gel (0.040–0.063 mm, 230–400 mesh) and technical grade solvents. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 or 300 spectrometer using tetramethylsilane as an internal standard. Melting points were obtained on a Walden Precision Apparatus Electrothermal 9300 apparatus and are uncorrected. Solvents and liquid reagents were transferred using hypodermic syringes. Purity percentages of the target compounds were determined by LC-MS and found to be more than 95%. All solvents and reagents were purchased from commercial sources and used without further purification.

4.2. Methyl 3-methoxybenzoate (**5**)

A mixture of 3-methoxybenzoic (**4**, 2.12 g, 0.001 mol) and methanol (20 mL) were heated under reflux until the benzoic acid

Table 5
Kinase inhibitory effects (%inhibition) of the most active compounds.[a]

Kinases	Compound no.				Sorafenib (IC_{50}, nM)
	2b	**2f**	**3e**	**3f**	
B-RAF	13.99 ± 0.61	56.87 ± 0.70	10.76 ± 1.00	22.50 ± 0.79	38 ± 9
B-RAF (V600E)	36.21 ± 1.48	90.44 ± 0.18 (0.77 μM)[b]	34.40 ± 0.09	40.06 ± 0.71	22 ± 6
EGFR	−2.09	3.38 ± 0.62	3.72 ± 0.34	15.70 ± 0.34	–
P38α/MAPK14	−20.33	45.83 ± 0.38	74.50 ± 0.20	66.59 ± 0.10	38 ± 3
RAF1	45.08 ± 0.41	87.71 ± 0.19 (1.50 μM)[b]	39.60 ± 2.01	36.41 ± 0.53	6 ± 3
ABL	13.45 ± 0.09	7.70 ± 1.51	15.99 ± 1.62	16.08 ± 1.00	–
ABL(T135I)	2.92 ± 0.37	6.38 ± 0.84	9.61 ± 0.49	14.58 ± 0.96	–

[a] % inhibition of all the compounds were tested at 10 μM concentration, duplicate testing. The results are expressed as mean %inhibition ± S.E.M.
[b] IC50 values.

was dissolved in methanol then few drops of concentrated sulfuric acid was added to the mixture and refluxed for 8 h. The resulting mixture was cooled to room temperature, diluted with water and a saturated solution of sodium bicarbonate was added to the mixture to neutralize the benzoic acid, extracted with ethyl acetate, dried and evaporated to get the required ester compound **5**.

4.3. 2-(2-Bromopyridin-4-yl)-1-(3-methoxyphenyl)ethan-1-one (**6**)

A solution of compound **5** (1.0 g, 5.0 mmol) and 2-bromo-4-picoline (0.5 mL, 5.6 mmol) in THF (5 mL) was cooled to −25 °C, and LiHMDS (3.7 mL,1.0 M solution in THF, 19.9 mmol) was slowly added thereto to maintain the temperature at −25 °C. The resulting mixture was stirred overnight at room temperature. The mixture was quenched with saturated aqueous NH_4Cl (15 mL), and ethyl acetate (20 mL) was added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic layer was washed with saline and dried over anhydrous sodium sulfate. The organic solvent was evaporated under vacuum and the residue was purified by flash column chromatography (silica gel, hexane ethyl acetate 1:1 v/v then switching to hexane-ethyl acetate 1:5 v/v) to yield the title compound (0.58 g, 45%). mp: 85–88 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (d, 1H, J = 4.8 Hz), 7.55 (d, 1H, J = 7.6 Hz), 7.56–7.48 (m, 1H), 7.40 (t, 2H, J = 8.0 Hz), 7.15 (m, 2H), 4.25 (s, 2H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.0, 150.0, 146.5, 137.3, 129.9, 129.2, 124.2, 121.0, 120.2, 112.8, 55.5, 44.0.

4.4. 2-Bromo-4-(3-(3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridine (**7**)

Compound **6** (1.0 g, 3.8 mmol) was added to dimethylformamide dimethylacetal (5.14 mL, 38.2 mmol) and the mixture was stirred at room temperature for 18 h. The resulting solution was concentrated to dryness to get an oil to be used in the next step as such. To a solution of that oil (0.137 g, 0.457 mmol) in EtOH (3 mL), phenylhydrazine (1 mL) was added, and the reaction mixture was stirred at room temperature overnight. Water (5 mL) was added to the reaction mixture and extracted with ethyl acetate (3 × 5 mL). The combined organic layer was washed with saline and dried over anhydrous Na_2SO_4. After evaporation of the organic solvent, the residue was purified by column chromatography (silica gel, hexane-ethyl acetate 1:1 v/v then switching to hexane-ethyl acetate 1:5 v/v) to yield the title compound (50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, 1H, J = 5.2 Hz), 7.98 (s, 1H), 7.38–7.37 (m, 1H), 7.31–7.23 (m, 5H), 7.00 (dd, 1H, J = 1.2, 5.2 Hz), 6.94–6.91 (m, 1H), 6.77–6.75 (m, 1H), 6.68–6.66 (m, 1H), 3.66 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.9, 150.0, 143.6, 142.6, 140.7, 139.3, 130.3, 128.9, 127.9, 125.7, 125.1, 122.5, 120.9, 118.3, 115.5, 115.2, 55.3.

4.5. N1-(4-(3-(3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridin-2-yl)ethane1,2-diamine (**8a**) and N1-(4-(3-(3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyridin-2-yl)propane-1,3-diamine (**8b**)

A mixture of compound **7** (10.0 g, 42.2 mmol) and pyridine (4.24 g, 53.6 mmol) in 1,2-diaminoethane or 1,3-diaminopropane (43 mL) was refluxed under nitrogen for 8 h. When the reaction completed, the reaction mixture was evaporated under reduced pressure and cooled, and the resulting residue was treated with water (150 mL) and methylene chloride. The organic layer was collected and washed with additional water (100 mL) then dried over sodium sulfate and evaporated to get the required product as grayish white solid compound **8a** and **8b**, respectively which was dried and used in the next step without further purification.

Compound **8a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94–7.90 (m, 2H), 7.28–7.24 (m, 6H), 6.89 (d, 1H, J = 8.0 Hz), 6.77 (d, 1H, J = 7.6 Hz), 6.69 (s, 1H), 6.45 (d, 1H, J = 4.8 Hz), 6.22 (s, 1H), 3.65 (s, 3H), 3.21–3.18 (m, 2H), 2.82–2.80 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.6, 159.1, 148.1, 141.8, 139.9, 139.5, 131.2, 129.9, 128.8, 127.5, 125.1, 122.8, 120.4, 115.6, 114.8, 111.9, 104.6, 55.3, 39.9, 39.6, 32.4.

Compound **8b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94–7.90 (m, 2H), 7.29–7.20 (m, 6H), 6.88 (d, 1H, J = 8.0 Hz), 6.77 (d, 1H, J = 7.6 Hz), 6.70 (s, 1H), 6.43 (d, 1H, J = 4.8 Hz), 6.25 (s, 1H), 3.65 (s, 3H), 3.20–3.17 (m, 2H), 2.80–2.77 (m, 2H), 1.68–1.65 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.6, 159.1, 148.1, 141.8, 139.9, 139.5, 131.2, 129.9, 128.8, 127.5, 125.1, 122.8, 120.4, 115.6, 114.8, 111.9, 104.6, 55.3, 39.9, 39.6, 32.4.

4.6. N-(2-((4-(3-(3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridin-2-yl)amino)ethyl)-benzamide (**1a**)

To a solution of compound **8a** (50 mg, 0.175 mmol) in anhydrous dichloromethane (3 mL), TEA (35.4 mg, 0.35 mmol) was added at 0 °C, stirred for 15 min, then benzoyl chloride (29 mg, 0.21 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 24 h. When the reaction completed, the solvent was removed and the residue was partitioned between ethyl acetate and water. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic layer was washed with brine two times and the organic solvent was evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, hexane-ethyl acetate 4:1 v/v) to give the required product as solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (s, 1H), 8.10 (d, J = 6.4 Hz, 1H), 7.85–7.84 (m, 3H), 7.53–7.45 (m, 3H), 7.38–7.27 (m, 5H), 6.98–6.95 (m, 1H), 6.81–6.78 (m, 2H), 6.59–6.56 (m, 1H), 6.44 (d, J = 4.8 Hz, 1H), 6.31 (t, J = 4.8 Hz, 1H), 3.63 (s, 3H), 3.38–3.34 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.9, 159.6, 148.1, 141.1, 140.3, 139.9, 139.6, 135.0, 131.6, 131.4, 129.3, 128.7, 128.5, 127.6, 125.8, 123.0, 120.4, 116.4, 115.0, 111.1, 105.7, 55.6; MS m/z: 490.0 [M+1].

Compounds (**1b**–**d**) were prepared from **8a** and appropriate acid chloride as reported in the synthesis of **1a**.

4.6.1. 4-Fluoro-N-(2-((4-(3-(3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyridin-2-yl)-amino)ethyl)benzamide (**1b**)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (s, 1H), 8.09 (s, 1H), 7.93–7.90 (m, 2H), 7.83 (d, J = 4.8 Hz, 1H), 7.36–7.25 (m, 8H), 6.96 (d, J = 7.6 Hz, 1H), 6.80–6.78 (m, 2H), 6.57 (s, 1H), 6.41 (s, 1H), 6.29 (d, J = 6.0 Hz, 1H), 3.62 (s, 3H), 3.36–3.34 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.8, 159.6, 148.1, 141.0, 140.3, 139.9, 139.6, 135.0, 131.5, 131.4, 130.4, 130.2, 129.3, 128.2, 125.8, 123.0, 120.4, 116.4, 115.7, 115.5, 115.0, 111.1, 105.7, 55.6; MS m/z: 508.0 [M+1].

4.6.2. N-(2-((4-(3-(3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridin-2-yl)amino)ethyl)-3-(trifluoromethyl)benzamide (**1c**)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (s, 1H), 8.20–8.13 (m, 3H), 7.91 (d, J = 7.6 Hz, 1H), 7.84 (d, J = 5.2 Hz, 1H), 7.74 (t, J = 7.6 Hz, 1H), 7.39–7.35 (m, 5H), 6.98–6.95 (m, 1H), 6.81–6.79 (m, 3H), 6.48 (s, 1H), 6.34 (d, J = 5.2 Hz, 1H), 3.63 (s, 3H), 3.42–3.38 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.4, 159.6, 158.9, 146.9, 141.7, 140.5, 139.8, 139.7, 135.8, 131.8, 131.3, 130.5, 130.1, 129.7, 129.4, 128.2, 125.8, 124.3, 124.2, 123.1, 122.9, 120.1, 116.4, 115.1,111.1, 105.7, 55.6; MS m/z: 558.0 [M+1].

4.6.3. 3-Methoxy-N-(2-((4-(3-(3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyridin-2-yl)amino)ethyl)benzamide (**1d**)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (t, J = 5.2 Hz, 1H), 8.10 (s, 1H), 7.83 (d, J = 5.2 Hz, 1H), 7.45–7.25 (m, 9H), 7.09–7.07 (m, 1H),

6.97–6.95 (m, 1H), 6.80–6.78 (m, 2H), 6.59 (t, J = 5.2 Hz, 1H), 6.43 (s, 1H), 6.30 (d, J = 5.2 Hz, 1H), 3.79 (s, 3H), 3.63 (s, 3H), 3.34–3.12 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6, 159.6, 159.5, 148.1, 141.0, 140.2, 139.9, 139.6, 136.4, 131.4, 130.4, 129.9, 129.3, 128.2, 125.8, 123.0, 120.4, 119.9, 117.4, 116.4, 115.0, 112.8, 111.1, 105.7, 55.7, 55.5; MS m/z: 520.0 [M+1].

Compounds (**1e**–**h**) were prepared from **8b** and appropriate acid chloride as reported in the synthesis of **1a**.

*4.6.4. N-(3-((4-(3-(3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridin-2-yl)amino)-propyl)benzamide (**1e**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (t, J = 4.8 Hz, 1H), 8.09 (s, 1H), 7.51–7.44 (m, 3H), 7.36–7.25 (m, 6H), 6.97–6.94 (m, 1H), 6.81–6.78 (m, 2H), 6.44 (t, J = 7.2 Hz, 1H), 6.34–6,30 (m, 2H), 3.63 (s, 3H), 3.31–3.29 (m, 2H), 3.29–3.25 (m, 2H), 1.69 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6, 159.7, 159.6, 148.2, 140.9, 140.2, 139.9, 139.6, 135.1, 131.5, 131.4, 130.4, 129.3, 128.7, 128.2, 127.6, 125.8, 122.9, 120.5, 116.4, 115.0, 110.8, 105.4, 55.5, 39.2, 37.6, 29.5; MS m/z: 504.0 [M+1].

*4.6.5. 4-Fluoro-N-(3-((4-(3-(3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyridin-2-yl)-amino)propyl)benzamide (**1f**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 (s, 1H), 8.09 (s, 1H), 7.93–7.90 (m, 2H), 7.83 (d, J = 4.8 Hz, 1H), 7.41–7.25 (m, 8H), 6.95 (d, J = 7.2 Hz, 1H), 6.81–6.78 (m, 2H), 6.43 (s, 1H), 6.34–6.30 (m, 2H), 3.62 (s, 3H), 3.33–3.31 (m, 2H), 3.17–3.15 (m, 2H), 1.69 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.6, 163.0, 159.6, 148.2, 140.9,140.2, 139.9, 139.6, 131.6, 131.4, 130.4, 130.3, 130.2, 129.3, 128.2, 125.8, 122.9, 120.4, 116.4, 115.7, 115.5, 114.9, 110.8, 55.6, 39.0, 37.6, 29.4; MS m/z: 522.0 [M+1].

*4.6.6. N-(3-((4-(3-(3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridin-2-yl)amino)-propyl)-3-(trifluoromethyl)benzamide (**1g**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (s, 1H), 8.20–8.16 (m, 2H), 8.10 (s, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.83 (d, J = 5.6 Hz, 1H), 7.73 (t, J = 8.0 Hz, 1H), 7.37–7.25 (m, 6H), 6.95 (dd, J = 2.0, 8.0 Hz, 1H), 6.81–6.79 (m, 2H), 6.46 (t, J = 5.6 Hz, 1H), 6.35–6.31 (m, 2H), 3.63 (s, 3H), 3.37–3.33 (m, 2H), 3.20–3.18 (m, 2H), 1.73 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.1, 159.6, 148.2, 140.9, 140.2, 139.9, 139.6, 135.9, 131.7, 131.4, 130.4, 130.1, 129.8, 129.4, 129.3, 128.2, 125.8, 124.2, 123.2, 123.0, 120.4, 116.4, 115.0, 110.8, 105.4, 55.5, 39.0, 37.8, 29.3; MS m/z: 572.0 [M+1].

*4.6.7. 3-Methoxy-N-(3-((4-(3-(3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyridin-2-yl)amino)propyl)benzamide (**1h**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (t, J = 4.8 Hz, 1H), 8.09 (s, 1H), 7.82 (d, J = 5.6 Hz, 1H), 7.44–7.25 (m, 9H), 7.09–7.06 (m, 1H), 6.97–6.95 (m, 1H), 6.80–6.79 (m, 2H), 6.42 (t, J = 5.6 Hz, 1H), 6.33–6.30 (m, 2H), 3.79 (s, 3H), 3.63 (s, 3H), 3.29–3.28 (m, 2H), 3.17–3.15 (m, 2H), 1.69 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6, 159.6, 148.2, 140.9, 140.2, 139.9, 139.6, 136.6, 131.4, 130.4, 129.9, 129.3, 128.2, 125.8, 123.0, 120.5, 119.8, 117.3, 116.4, 115.0, 112.8, 110.8, 105.4, 55.7, 55.6, 39.0, 37.6, 31.2, 29.5; MS m/z: 534.0 [M+1].

*4.7. 1-(2-((4-(3-(3-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridin-2-yl)amino)ethyl)-3-phenylurea (**2a**)*

To a solution of **8a** (50 mg, 0.175 mmol) in anhydrous THF (3 mL), phenyl isocyanate (53 mg, 0.23 mmol) was added to the solution and the mixture was stirred overnight till completion of the reaction. After evaporation of the solvent under reduced pressure the residue was purified by column chromatography using the appropriate ratio of ethyl acetate: methanol to yield the product as solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (s, 1H), 8.13 (s, 1H), 7.87 (d, J = 5.2 Hz, 1H), 7.44–7.25 (m, 10H), 7.00 (dd, J = 1.2, 8.0 Hz, 1H), 6.92 (t, J = 7.6 Hz, 1H), 6.85–6.83 (m, 2H), 6.54 (brs, 1H), 6.44 (s, 1H), 6.34 (d, J = 5.2 Hz, 1H), 6.27 (brs, 1H), 3.66 (s, 3H), 3.42 (brs, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.7, 159.6, 155.6, 148.2, 140.9, 140.3, 139.9, 139.6, 131.4, 130.4, 129.3, 129.1, 128.2, 125.8, 123.0, 121.5, 120.4, 118.2, 116.4, 115.0, 111.1, 105.5, 55.6, 41.9; MS m/z: 505.0 [M+1].

Compounds **2b,c** were prepared from **8a** and appropriate isocyanate derivative as reported in the synthesis of **2a**.

*4.7.1. 1-(2-((4-(3-(3-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridin-2-yl)amino)ethyl)-3-(3-(trifluoromethyl)phenyl)urea (**2b**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.98 (s, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.49–7.20 (m, 9H), 6.97–6.94 (m, 1H), 6.80–6.78 (m, 2H), 6.51 (brs, 1H), 6.38–6.37 (m, 2H), 6.20 (dd, J = 1.2, 8.0 Hz, 1H), 3.63 (s, 3H), 3.24–3.23 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.6, 159.5, 155.6, 148.2, 141.9, 141.0, 140.2, 139.9, 139.6, 131.4, 130.4, 130.2, 129.7, 129.3, 128.2, 125.8, 123.0, 121.6, 120.4, 117.7, 116.4, 115.0, 114.0, 111.1, 105.5, 55.5, 41.8; MS m/z: 573.0 [M+1].

*4.7.2. 1-(3,4-Dichlorophenyl)-3-(2-((4-(3-(3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyridin-2-yl)amino)ethyl)urea (**2c**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (s, 1H), 8.13 (s, 1H), 7.87 (d, J = 5.2 Hz, 1H), 7.44–7.25 (m, 10H), 7.05 (dd, J = 1.2, 8.0 Hz, 1H), 6.91 (t, J = 8.0 Hz, 1H), 6.85–6.83 (m, 2H), 6.54 (s, 1H), 6.44 (s, 1H), 6.34 (d, J = 5.2 Hz,1H), 6.27 (s, 1H), 3.66 (s, 3H), 3.42 (br s, 4H); MS m/z: 572.9 [M+1].

Compounds (**2d**–**f**) were prepared from **8b** and appropriate isocyanate derivative as reported in the synthesis of **2a**.

*4.7.3. 1-(3-((4-(3-(3-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridin-2-yl)amino)propyl) -3-phenylurea (**2d**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (s, 1H), 8.09 (s, 1H), 7.83 (d, J = 5.2 Hz, 1H), 7.41–7.21 (m, 10H), 6.98–6.95 (m, 1H), 6.87 (t, J = 8.0 Hz, 1H), 6.80–6.78 (m, 2H), 6.43 (t, J = 5.6 Hz, 1H), 6.34–6.30 (m, 2H), 6.17 (t, J = 5.6 Hz, 1H), 3.63 (s, 3H), 3.16–3.11 (m, 4H), 1.60 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.7, 159.6, 155.8, 148.3, 141.1, 140.9, 140.2, 139.9, 139.6, 131.4, 130.4, 129.1, 129.1, 128.2, 125.8, 123.0, 121.4, 120.5, 118.1, 116.4, 115.0, 110.8, 105.4, 55.6, 38.9, 37.4, 30.2; MS m/z: 519.1 [M+1].

*4.7.4. 1-(3-((4-(3-(3-Methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridin-2-yl)amino)propyl) -3-(3-(trifluoromethyl)phenyl)urea (**2e**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.87 (s, 1H), 8.09 (s, 1H), 7.98 (s, 1H), 7.83 (d, J = 5.6 Hz, 1H), 7.44–7.20 (m, 9H), 6.98–6.95 (m, 1H), 6.80–6.78 (m, 2H), 6.43 (d, J = 5.6 Hz, 1H), 6.33–6.29 (m, 3H), 3.63 (s, 3H), 3.16–3.11 (m, 4H), 1.60 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.7, 159.6, 155.6, 148.2, 141.9, 140.9, 140.2, 139.9, 139.6, 131.4, 130.4, 130.2, 129.3, 128.2, 125.8, 123.0, 121.6, 120.5, 117.6, 116.4, 115.0, 114.0, 110.8, 105.4, 55.6, 38.9, 37.4, 30.2; MS m/z: 587.0 [M+1].

*4.7.5. 1-(3,4-Dichlorophenyl)-3-(3-((4-(3-(3-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridine-2-yl)amino)propyl)urea (**2f**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (s, 1H), 8.09 (s, 1H), 7.86–7.82 (m, 2H), 7.45–7.24 (m, 7H), 6.98–6.95 (m, 1H), 6.80–6.78 (m, 2H), 6.42 (t, J = 5.6 Hz, 1H), 6.33–6.29 (m, 3H), 3.63 (s, 3H), 3.15–3.12 (m, 4H), 1.61 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.7, 159.6, 155.4, 148.2, 141.3, 140.9, 140.2, 139.9, 139.6, 131.4, 130.9, 130.4, 129.3, 128.2, 125.8, 122.9, 122.6, 120.5, 119.1, 118.1, 116.4, 115.0, 110.8, 105.6, 55.6, 38.8, 37.5, 30.0; MS m/z: 589.6 [M+1].

*4.8. N-(2-((4-(3-(3-Hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridin-2-yl)amino) ethyl)benzamide (**3a**)*

To a solution of compound **1a** (150 mg, 0.3 mmol) in methylene

chloride (3 mL), BBr_3 (3 mL of a 1 M solution in methylene chloride) was added dropwise at −78 °C under N_2. The reaction mixture was stirred at the same temperature for 1 h, and then allowed to warm to room temperature and stirred for another 4 h. The mixture was quenched with saturated aqueous Na_2CO_3. Ethyl acetate (5 mL) was added and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (3 × 2 mL). The combined organic layer extracts were washed with brine, and dried over anhydrous Na_2SO_4. After evaporation of the organic solvent, the residue was purified by short column chromatography (silica gel, using ethyl acetate then switching to ethyl acetate-methanol 4:1 v/v) to yield the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.80 (d, J = 5.6 Hz, 1H), 7.73 (brs, 1H), 7.62 (d, J = 5.6 Hz, 1H), 7.37 (t, J = 7.6 Hz, 1H), 7.28 (t, J = 7.6 Hz, 2H), 7.21–7.14 (m, 6H), 7.05 (t, J = 8.0 Hz, 1H), 6.80 (dd, J = 1.6, 8.0 Hz, 1H), 6.79–6.76 (m, 1H), 6.51–6.47 (m, 2H), 6.23 (s, 1H), 4.84 (s, 1H), 3.41–3.37 (m, 2H), 3.35–3.32 (m, 2H); MS m/z: 476.0 [M+1].

Compounds **3b**–**h** were synthesized by the same procedure as described for synthesis of compound **3a**.

4.8.1. 4-Fluoro-N-(2-((4-(3-(3-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyridin-2-yl) amino)ethyl)benzamide (**3b**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.97 (s, 1H), 7.66 (d, J = 5.2 Hz, 1H), 7.62–7.58 (m, 2H), 7.18–7.11 (m, 5H), 7.02 (t, J = 8.0 Hz, 1H), 6.88 (t, J = 8.4 Hz, 2H), 6.77 (dd, J = 1.6, 8.0 Hz, 1H), 6.69 (s, 1H), 6.48 (d, J = 7.6 Hz, 1H), 6.40 (d, J = 4.8 Hz, 1H), 6.21 (s, 1H), 3.28–3.25 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 164.9, 162.4, 157.8, 156.5, 145.9, 141.4, 139.5, 138.3, 138.2, 129.8, 129.2, 129.0, 128.4, 128.3, 127.8, 126.6, 123.9, 120.6, 118.6, 115.7, 114.6, 114.3, 111.5, 104.8, 40.7, 40.3, 28.7; MS m/z: 494.0 [M+1].

4.8.2. N-(2-((4-(3-(3-Hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridin-2-yl)amino)ethyl)-3-(trifluoromethyl)benzamide (**3c**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.92 (s, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.82 (s, 1H), 7.74 (d, J = 7.6 Hz, 1H), 7.62 (d, J = 7.6 Hz, 1H), 7.41 (t, J = 8.0 Hz, 1H), 7.18–7.12 (m, 5H), 7.06 (t, J = 8.0 Hz, 1H), 6.81–6.78 (m, 1H), 6.71–6.69 (m, 1H), 6.53 (dd, J = 2.4, 8.0 Hz, 1H), 6.47 (dd, 1.6, 8.0 Hz, 1H), 6.22 (s, 1H), 3.40–3.31 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 157.8, 156.1, 145.7, 141.6, 139.4, 138.4, 138.3, 133.9, 130.0, 129.8, 129.6, 129.2, 128.1, 127.8, 127.0, 126.6, 123.9, 122.9, 121.3, 120.9, 118.6, 116.4, 115.7, 111.6, 104.9, 40.2, 31.8; MS m/z: 544.0 [M+1].

4.8.3. N-(2-((4-(3-(3-Hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridin-2-yl)amino)ethyl)-3-methoxybenzamide (**3d**)

^{1}H NMR (400 MHz, MeOD) δ 8.09 (s, 1H), 7.92(d, J = 5.6 Hz, 1H), 7.45 (s, 3H), 7.30–7.25 (m, 5H), 7.18(t, J = 7.2, 1H), 6.95–6.89 (m, 1H), 6.87–6.85 (m, 1H), 6.78–6.75 (m, 2H), 6.49 (d, J = 5.2, 1H), 6.33 (s, 1H), 3.30–3.26 (m, 4H); ^{13}C NMR (100 MHz, MeOD-d_6) δ 169.0, 157.9, 155.7, 139.6, 139.3, 132.4, 130.9, 130.2, 129.3, 128.7, 128.6, 128.1, 127.9, 126.8, 125.3, 124.1, 121.1, 118.4, 117.7, 116.7, 116.4, 114.7, 113.9, 111.2, 35.7, 34.1, 29.8; MS m/z: 492.0 [M+1].

4.8.4. N-(3-((4-(3-(3-Hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridin-2-yl)amino) propyl)benzamide (**3e**)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (s, 1H), 8.07 (s, 1H), 7.84–7.81 (m, 3H), 7.51–7.43 (m, 3H), 7.38–7.16 (m, 6H), 6.78 (d, J = 8.0 Hz, 1H), 6.64 (d, J = 8.0 Hz, 1H), 6.59 (s, 1H), 6.43 (brs, 1H), 6.33 (s, 1H), 6.28 (m, 1H), 3.30–3.28 (m, 2H), 3.18–3.17 (m, 2H), 1.71–1.69 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7, 159.7, 157.9, 148.2, 140.9, 140.4, 139.9, 139.6, 138.4, 135.1, 131.5, 131.3, 130.4, 129.3, 128.7, 128.0, 125.5, 121.4, 120.3, 117.3, 116.6, 110.8, 105.4, 39.2, 37.6, 29.5; MS m/z: 490.0 [M+1].

4.8.5. 4-Fluoro-N-(3-((4-(3-(3-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)pyridin-2-yl) amino)propyl)benzamide (**3f**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.82–7.80 (m, 3H), 7.61 (d, J = 6.0 Hz, 1H), 7.26–7.24 (m, 5H), 7.13 (t, J = 8.0 Hz, 1H), 7.01 (t, J = 8.4 Hz, 2H), 6.91–6.88 (m, 1H), 6.84 (s, 1H), 6.58–6.53 (m, 2H), 6.27 (s, 1H), 3.27–3.25 (m, 2H), 3.12–3.10 (m, 2H), 1.66–1.63 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 166.0, 163.5, 157.6, 156.9, 144.2, 143.4, 141.0, 139.3, 130.6, 130.4, 130.2, 129.6, 129.5, 128.9, 127.8, 125.0, 121.6, 119.0, 117.5, 117.1, 115.6, 115.4, 111.5, 105.3, 39.4, 37.4, 28.8; MS m/z: 508.0 [M+1].

4.8.6. N-(3-((4-(3-(3-Hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridin-2-yl)amino) propyl)-3-(trifluoromethyl)benzamide (**3g**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.87 (brs, 2H), 7.64 (d, J = 8.0 Hz, 1H), 7.57 (d, J = 6.8 Hz, 1H), 7.45 (t, J = 8.0 Hz, 1H), 7.22–7.16 (m, 6H), 7.07 (t, J = 8.0 Hz, 1H), 6.83 (dd, J = 1.6, 8.0 Hz, 1H), 6.78–6.76 (m, 1H), 6.49 (t, J = 6.0 Hz, 2H), 6.18 (s, 1H), 6.01 (brs, 1H), 3.36–3.33 (m, 2H), 3.10–3.05 (m, 2H), 1.63–1.60 (m, 2H); MS m/z: 558.0 [M+1].

4.8.7. N-(3-((4-(3-(3-Hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl) pyridin-2-yl)amino) propyl)-3-methoxybenzamide (**3h**)

^{1}H NMR (400 MHz, MeOD) δ 7.96 (s, 1H), 7.72 (d, J = 5.6 Hz, 1H), 7.27 (s, 3H), 7.20–7.17 (m, 5H), 7.10 (t, J = 7.2 Hz, 1H), 6.88–6.85 (m, 1H), 6.75–6.73 (m, 1H), 6.59–6.57 (m, 2H), 6.42 (d, J = 5.6 Hz, 1H), 6.33 (s, 1H), 3.34–3.32 (m, 2H), 3.17–3.14 (m, 2H), 1.71–1.68 (m, 2H); ^{13}C NMR (100 MHz, MeOD) δ 169.0, 158.9, 157.7, 157.5, 146.3, 142.2, 141.0, 139.4, 138.9, 135.8, 130.8, 129.8, 129.2, 128.6, 127.7, 125.3, 121.3, 119.8, 118.1, 117.7, 116.9, 115.9, 113.9, 110.9, 105.7, 38.7, 37.1, 28.8; MS m/z: 506.0 [M+1].

4.9. Cancer cell line screening at the NCI

Screening against the cancer cell lines was carried out at the National Cancer Institute (NCI), Bethesda, Maryland, USA, applying the standard protocol of the NCI [41].

4.10. Kinase profiling

Reaction Biology Corp. Kinase HotSpot^{SM} service [42] was used for screening of compounds **2b**, **2f**, **3e**, and **3f**. Assay protocol: In a final reaction volume of 25 μL, kinase (5–10 mU) is incubated with 25 mM Tris pH 7.5, 0.02 mM EGTA, 0.66 mg/mL myelin basic protein, 10 mM magnesium acetate and [γ^{33}P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the Mg-ATP mix. After incubation for 40 min at room temperature, the reaction is stopped by the addition of 5 μL of a 3% phosphoric acid solution. 10 μL of the reaction is then spotted onto a P30 filtermat and washed three times for 5 min in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.04.048.

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