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Design and synthesis of new potent anticancer pyrazoles with high FLT3 kinase inhibitory selectivity

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ABSTRACT

A new series of 1*H*- and 2*H*-pyrazole derivatives (35 final compounds) has been designed and synthesized in this study. A selected group (13 compounds) was then tested over a panel of 60 cancer cell lines at a single dose concentration of 10 μM. At this concentration, six compounds have showed moderate to strong mean inhibitions, and were further tested at five-dose testing mode to determine their IC_{50} over the 60 cell lines. The IC_{50} values of the tested compounds indicated high potency (as for compound **10f**) as well as high efficacy (as for compound **11e**). Accordingly, compound **10f** was then tested at a single dose concentration of 10 μM over a panel of 54 kinases to determine its kinase inhibitory profile. The compound has showed good selectivity towards FLT3 kinase, associated with a moderate potency, with an IC_{50} value of 1.74 μM.

1. Introduction

Cancer has been the leading cause of death worldwide, and it accounted for 7.4 million deaths (around 13% of all deaths) in 2004 according to WHO reports.[1] The conventional cancer treatments such as surgery, radiation and cytotoxic chemotherapy seem to be no longer effective neither for the complete eradication of disease nor for the improvement of cancer patients' life. This has prompted the development of a variety of new therapies that are capable of selectively targeting the substantial cause of cancer, resulting in a better and more complete response and at the same time, minimal side effects, and more improvement of patient's life.

The deregulated cell growth in cancer occurs as a result of perturbed signal transduction defined, in its broadest sense, as all cellular signals that modulate or alter cellular behavior or function.[2,3] Consequently, cancers do not necessarily arise as a result of an increased rate of cell proliferation. Rather, it is the critical balance between the rate of cell-cycle progression (cell division) and cell growth (cell mass) on one hand, and programmed cell death (apoptosis) on the other.[4] During normal embryonic development and in adult life, signaling needs to be precisely coordinated and integrated at all times, and properly regulated differentiation signals are critical for preventing oncogenesis. Protein kinases are important players in the process of cell signaling and signal transduction, and the dysregulation of such signals is most probably caused by mutations or abnormalities in the substantial controlling kinases.[5,6] Hence, the targeting of oncogenic kinases has recently become one of the new effective strategies for the development of selective therapies for specific cancers.[7]

The hematopoietic class III receptor tyrosine kinase (RTK) FLT3 (FLK2, STK1) has recently received much attention as a potential drug target in cancer treatment.[8–10] The activation of FLT3 by different types of mutations plays an important role in proliferation, resistance to apoptosis, and prevention of differentiation of leukemia blasts in acute myeloid leukemia (AML). The signal transduction of FLT3 involves activation of several conserved pathways, including the RAS/MAP-kinase and the phosphoinositied-3-kinase/Akt signaling cascades. A number of potent FLT3 inhibitors, such as Lestaurtinib (CEP-701),[11] ABT-869,[12] MLN-518,[13] and Fostamatinib,[14] which differ in their selectivity profiles, both with respect to other kinases and among wild-type FLT3, are currently under preclinical and clinical investigations as potential drug candidates for AML.

In this study, a number of 1*H*- and 2*H*-pyrazole derivatives (35 final compounds) have been designed in rationale to the potent B-Raf inhibitor 'GDC-0879'.[15] As showed in Figure 1, the compounds share a general structure at which, the *N*-(dihydroinden-3-ylidene)hydroxylamine group of GDC-0879 has been replaced with a 3-(methoxy/hydroxyl)-5-methylphenyl group attached to position 3 of the pyrazole nucleus (instead of position 4 in GDC-0879), and an acetonitrile group (instead of the 2-hydroxyethyl group in GDC-0879) is attached either to position 1 (in 1*H*-pyraoles) or position 2 (in 2*H*-pyrazoles). In position 4 of the pyrazole ring, a pyridin-4-yl group (similar to that in GDC-0879) or a pyridin-3-yl group was attached, to which, a series of variable substi-

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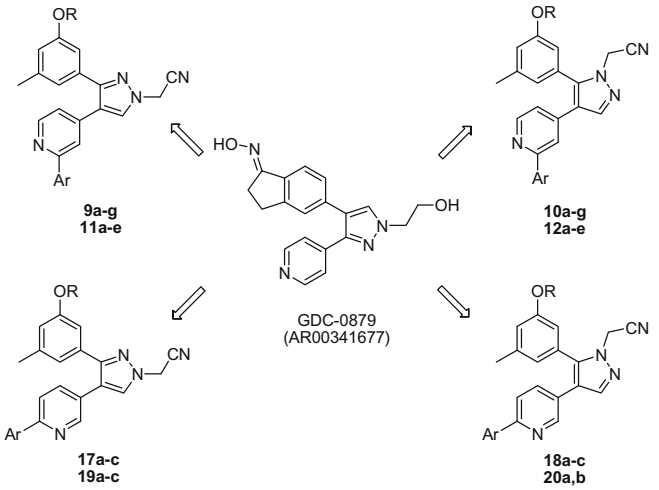


Figure 1. Structure of GDC-0879 and the new rationally designed compounds.

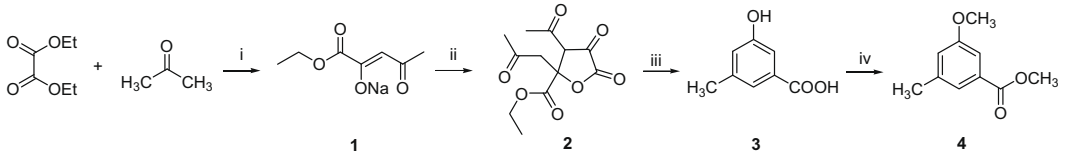
tuted aryl moieties were linked through a group of Suzuki couplings. A selected group (13 compounds) from the target compounds was then tested over a panel of 60 cancer cell lines at a single dose concentration of 10 μM. At this concentration, six compounds have showed very good activity and were accordingly further tested at five-dose testing mode to determine their IC_{50} over the 60 cell lines. The IC_{50} values of the tested compounds indicated high potency (as for compound **10f**) as well as high efficacy (as for compound **11e**). Accordingly, compound **10f** was then tested at a single dose concentration of 10 μM over a panel of 54 kinases to determine its kinase inhibitory profile. The compound has showed good selectivity towards FLT3 kinase, associated with a moderate potency, with an IC_{50} value of 1.74 μM.

2. Results and discussion

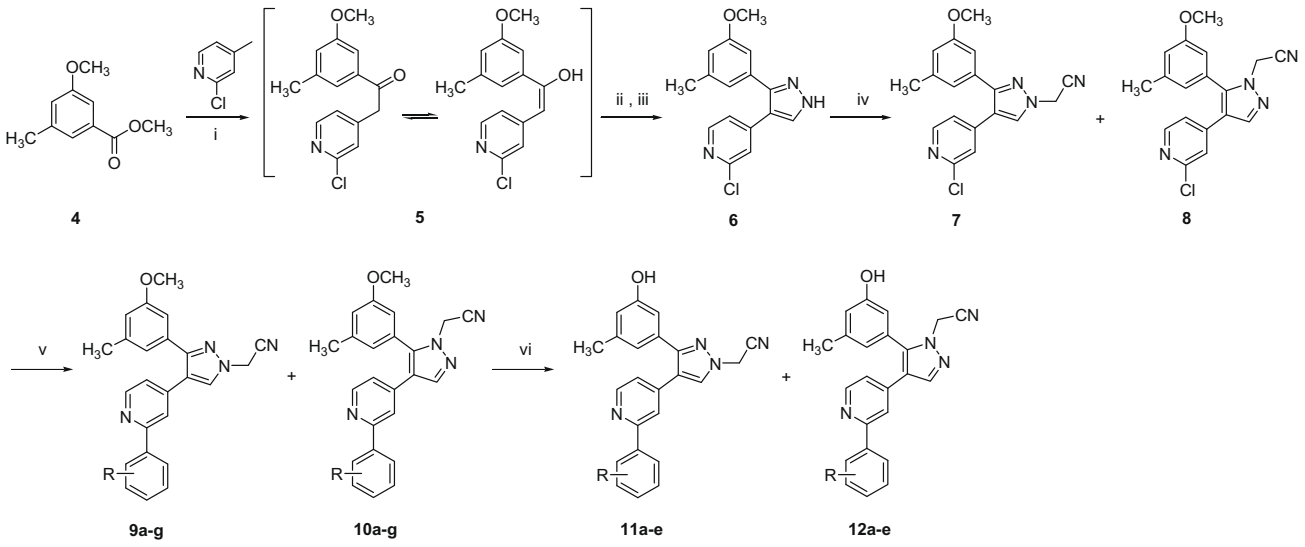
2.1. Chemistry

For the preparation of the target compounds, the key ester, methyl 3-methoxy-5-methylbenzoate (**4**), was first prepared as illustrated in Scheme 1. The synthesis started with the preparation of the sodium salt of ethyl 2-hydroxy-4-oxopent-2-enoate (**1**) according to the literature procedure, through the condensation of diethyl oxalate with acetone in the presence of sodium ethoxide in absolute ethanol.[16] The resulted salt **1** was then cyclized into Claisen furan derivative **2** by heating in 50% acetic acid followed by acidification with sulfuric acid.[17] The resulted Claisen compound underwent rearrangement and aromatization into 3-hydroxy-5-methylbenzoic acid (**3**) within less than one hour by heating with magnesium oxide in boiling water, followed by acidification with hydrochloric acid to precipitate the product.[17] Methyl esterification and O-methylation of the resulted phenolic acid **3** were achieved in a single step and in high yield (94%) to give compound **4** through a little modification of the literature procedure,[18] where the acid **3** was refluxed with excess potassium carbonate and iodomethane in acetone, and in the presence of a catalytic amount of DMAP.

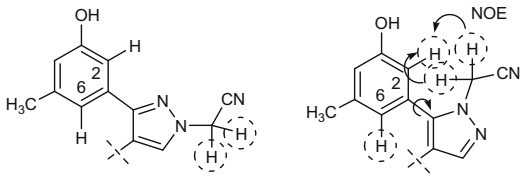
In Scheme 2, the benzoate ester **4** underwent a nucleophilic attack at its carboxylic carbon by the activated methylene group of 2-chloro-4-methylpyrimidine. The activation of this methyl group into an active methylene was achieved by dropwise addition of lithium bis(trimethylsilyl)amide (LHMDS) in dry THF at room temperature. The resulted tautomeric α,β-unsaturated ketone **5** was



Scheme 1. Reagents and conditions: (i) NaOEt, abs EtOH, rt, 4 h, 87%; (ii) acetic acid/H_2O (1:1), rt, 2 h, 50%; (iii) MgO, H_2O, reflux, 45 min, 42%; (iv) K_2CO_3, CH_3I, DMAP, acetone, 65 °C, 12 h, 94%.



Scheme 2. Reagents and conditions: (i) LHMDS, THF, N_2, rt, 18 h, 72%; (ii) DMF–DMA, reflux, 12 h; (iii) hydrazine hydrate, abs EtOH, rt, 2 h, 81%; (iv) K_2CO_3, iodoacetonitrile, acetone, reflux, 4 h, 92%; (v) arylboronic acid, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, N_2, THF/H_2O; (4:1), 70 °C, 12 h; (vi) $BF_3{\cdot}S(CH_3)_2$, dichloromethane, N_2, rt, 24 h.



a: The distance between acetonitrile -CH_2- and the aromatic protons doesn't allow for NOE interaction in compound **9e**; **b**: NOE effect between acetonitrile -CH_2- and the aromatic protons in compound **10e**.

Figure 2. NOE interactions in compounds **9e** and **10e**.

then converted to the required pyrazole derivative **6** through two successive steps. In the first step, compound **5** was heated with excess *N*,*N*-dimethylformamide dimethylacetal for 12 h, and the resulted product was taken to the next step without further purification, where it was cyclized with hydrazine monohydrate in absolute ethanol into the pyrazole derivative **6**. The reaction of the resulted pyrazole **6** with iodoacetonitrile in the presence of excess potassium carbonate produced two different regioisomers; a major isomer **7** with R_f value of 0.74 (EtOAc–hexane, 1:1, v/v), and a minor isomer **8** with R_f value of 0.84 (EtOAc–hexane, 1:1, v/v). A mixture of these two isomers was taken to the next step without separation, where it underwent a series of Suzuki coupling reactions with a group of arylboronic acids, in the presence of dichloro bis(triphenylphosphine)Pd(II) and potassium carbonate, in a mixed solvent of THF and water in a (4:1) ratio. These series of Suzuki coupling reactions produced two isomers in every reaction 9a–**g** and **10a–g**, which were separated in a pure form by preparative TLC. In all of these reactions, the 1*H*-pyrazole isomer (isomer **9**) was the product with the lower R_f while the 2*H*-pyrazole isomer (isomer **10**) was the product with the higher R_f. This was proved by the 2D-NOESY NMR spectrum of compounds **9e** and **10e** as a model for the whole series. The absence of any cross peak between the acetonitrile –CH_2– and any of the aromatic protons of 3-methoxy-5-methylphenyl group in the 2D-NOESY NMR spectrum of compound **9e** while the presence of such peaks in the 2D-NOESY NMR spectrum of compound **10e** has confirmed the assigned structures (Fig. 2). Furthermore, the expected higher shielding of the two protons of the acetonitrile –CH_2– in all of the 2*H*-pyrazole isomers caused by the anisotropic effect of the nearby aromatic electron cloud, was proved in the assigned structures as showed in Section 4 and in the ^{1}H NMR spectra in Supplementary data. In the 2*H*-isomers, such protons appeared at a δ value of around 4.95–4.97, while in the 1*H*-isomers, these protons were more downfield shifted and appeared in the range of 5.10–5.20 ppm.

The final hydroxyl derivatives **11a–e** and **12a–e** were obtained by demethylation of the methoxy group of the corresponding methoxy compound using 10 equiv of borontrifluoride–dimethylsulfide complex in dichloromethane.

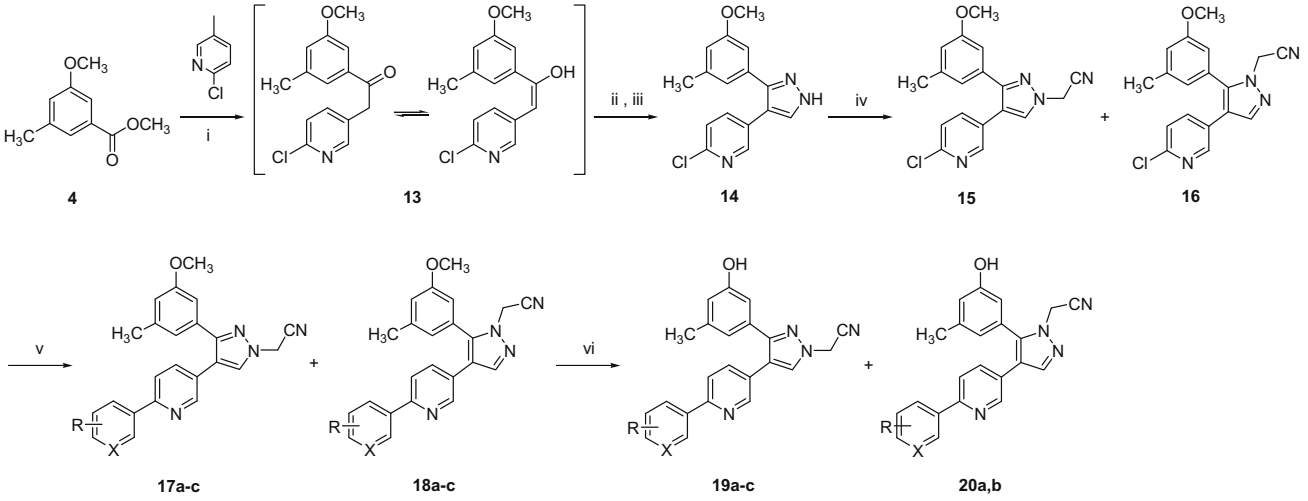
Typical procedures to those used in Scheme 2 have been applied in Scheme 3. The only difference was in the starting amine, where in Scheme 3, 2-chloro-5-methylpyridine was used instead of 2-chloro-4-methylpyridine in Scheme 2. All compounds synthesized were listed in Table 1.

2.2. Biological Screening

2.2.1. In vitro anticancer screening

The structures of the final products were submitted to National Cancer Institute (NCI),[19] Bethesda, Maryland, USA, and the 13 compounds showed in Table 2 were selected on the basis of degree of structural variation and computer modeling techniques for evaluation of their antineoplastic activity. The selected compounds were subjected to in vitro anticancer assay against tumor cells in a full panel of 60 cell lines taken from nine different tissues (blood, lung, colon, CNS, skin, ovary, kidney, prostate and breast). The compounds were tested at a single dose concentration of 10 μM, and the percentages of growth inhibition over the 60 tested cell lines were determined. The mean inhibition percentages of all of the tested compounds over the full panel of cell lines are illustrated in Table 2. As showed in Table 2, the mean inhibition was strong in compounds **9f**, **10f**, **11d** and **17c**, moderate in compounds **10c** and **11e**, while weak in compounds **9c**, **9d**, **9e**, **9g**, **10d**, **11a** and **11c**. The figures showing the multiple inhibitions exerted by the tested compounds are illustrated in Supplementary data.

By referring to the inhibitory effects of the tested compounds summarized in Table 2, and correlating between the structural variations and the resulted anticancer activities, we can conclude that the best aryl substitution at the terminal pyridyl moiety in Scheme 2 compounds is that using 4-(dimethylamino)phenyl group. The derivatives containing such group were found to possess the highest anticancer activity over the 60 tested cell lines. It was found that both the 1*H* (**9f**) and 2*H* (**10f**) isomers containing such group have comparable activity, which means that the relative position of the acetonitrile group has a little effect on activity, relative to



Scheme 3. Reagents and conditions: (i) LHMDS, THF, N_2, rt, 18 h, 55%; (ii) DMF–DMA, reflux, 12 h; (iii) hydrazine hydrate, abs EtOH, rt, 4 h, 62%; (iv) K_2CO_3, iodoacetonitrile, acetone, reflux, 4 h, 80%; (v) arylboronic acid, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, N_2, THF/H_2O (4:1), 70 °C, 12 h; (vi) $BF_3{\cdot}S(CH_3)_2$, dichloromethane, N_2, rt, 24 h.

Table 1
Synthesized derivatives from Schemes 2 and 3 and their corresponding yields

Compd	X	R	Yield%
9a	—	H	74
9b	—	3-Ac	79
9c	—	4-Ac	68
9d	—	3-NH–Ac	75
9e	—	4-CN	82
9f	—	4-$N(CH_3)_2$	79
9g	—	4-OPh	72
10a	—	H	62
10b	—	3-Ac	77
10c	—	4-Ac	75
10d	—	3-NH–Ac	63
10e	—	4-CN	77
10f	—	4-$N(CH_3)_2$	50
10g	—	4-OPh	69
11a	—	H	70
11b	—	3-Ac	61
11c	—	4-CN	81
11d	—	4-$N(CH_3)_2$	75
11e	—	4-OPh	62
12a	—	3-Ac	65
12b	—	4-Ac	42
12c	—	3-NH-Ac	55
12d	—	4-CN	72
12e	—	4-OPh	61
17a	N	H	55
17b	H	2-Ac	48
17c	H	3-Ac	76
18a	N	H	61
18b	H	2-Ac	77
18c	H	3-Ac	80
19a	N	H	61
19b	H	2-Ac	77
19c	H	3-Ac	75
20a	H	2-Ac	48
20b	H	3-Ac	72

Table 2
Compounds selected for single dose cancer cell-line screening and their mean inhibitory percentages

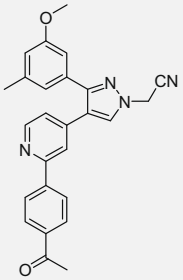
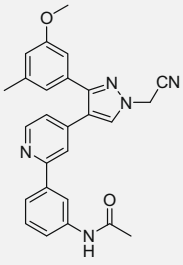
Compd	Structure	Inhibition %[a]
9c		18.23
9d		1.18

Table 2 (*continued*)

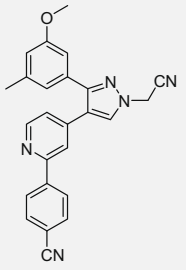
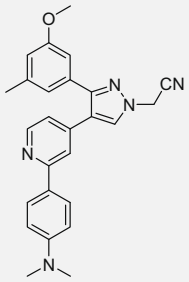
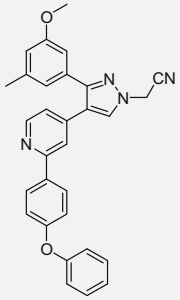
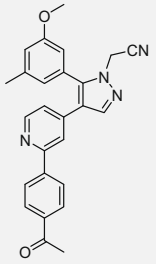
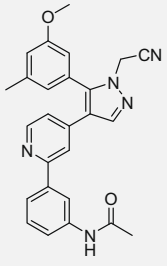
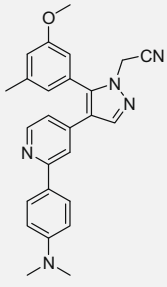
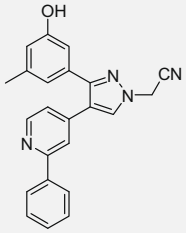
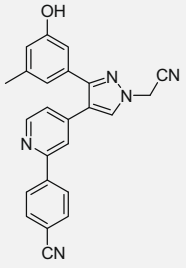
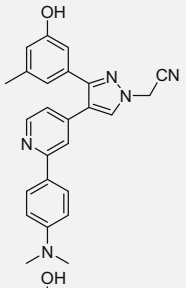
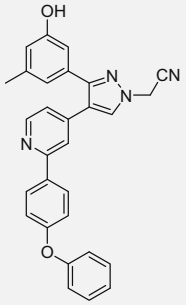
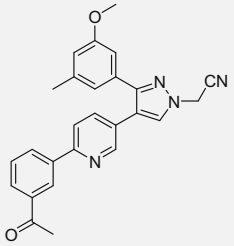
Compd	Structure	Inhibition %[a]
9e		14.38
9f		92.30
9g		13.48
10c		33.36
10d		9.40
10f		88.36

Table 2 (*continued*)

Compd	Structure	Inhibition %[a]
11a		16.41
11c		20.75
11d		65.35
11e		46.59
17c		78.65

[a] % Inhibition represents the mean inhibition percentages over the 60 cell lines. The inhibition percentages are calculated by subtracting the growth percentages from 100.

the effect exerted by the aromatic ring substituted at the terminal pyridyl group. Furthermore, the hydroxy-isomer of compound **9f** (compound **11d**) which contains the same 4-(dimethylamino)phenyl group was found also to have good activity, but slightly lower than that of compound **9f**. This means that the methoxy group is more favored than the hydroxy for good anticancer activity within this group of compounds. This was however contradicted by the relative activities of compounds **9g** and **11e**. Both of these two compounds are substituted with 4-phenoxyphenyl moiety at the terminal pyridine group, and the only difference between them lies in the methoxy group of compound **9g** which was demethylated to the corresponding hydroxy group in compound **11e**. In this case, the activity of the hydroxyl derivative **11e** was found to be higher than that of the methoxy derivative **9g**. A possible reason for this inversion in activity between the methoxy and hydroxyl analogs could be owed to the possible variation in binding modes between the 4-phenoxyphenyl derivatives and the relatively smaller 4-(dimethylamino)phenyl derivatives to their target site, since the relatively larger size of the 4-phenoxyphenyl derivatives may force the compounds to bind differently to their target site. By comparing the activities of compounds **9c** and **10c**, both with a 4-acetylphenyl group at the terminal pyridine, it was found that the activity of **10c** (the 2*H*-isomer) is higher than that of **9c** (the 1*H*-isomer), and this may suggest (along with the IC_{50} data of compounds **9f** and **10f**) that the presence of the acetonitrile group at position 2 in the 2*H*-isomer is more favored for activity. The only screened compound from Scheme 3 (compound **17c**) has showed also good activity, but unfortunately, no other compounds were selected for screening from the same scheme, so that a reliable SAR could be made to this group of compounds.

After this initial single dose screening of the 13 selected compounds, the compounds showing the highest activity at the single dose were further tested in a five-dose testing mode, in order to determine their IC_{50} values over the 60 tumor cell lines. These compounds are **9f**, **10c**, **10f**, **11d**, **11e** and **17c**. For each of these compounds, the IC_{50} (the concentration producing 50% inhibition), TGI (the concentration producing 100% inhibition) and LC_{50} (the concentration causing 50% lethality or 50% tumor regression) were recorded. The five-dose testing results of these six compounds are showed in Table 3.

As showed in Table 3, the six tested compounds have showed good potency over almost all of the 60 tested cell lines. However, the five-dose testing data were so much interesting in compounds **9f**, **10f** and **11e**. In compounds **9f** and **10f**, a very high potency was observed over almost the whole cell-lines panel, with IC_{50} values in the nM scale. The potency was however higher in compound **10f**, with IC_{50} values as small as of 23 nM at the melanoma cell-line MDA-MB-435. The two compounds (**9f** and **10f**) have showed also high efficacies, but only at a limited range of cell lines. For examples, the TGI and LC_{50} values of compound **9f** over the NSCLC cell line NCI-H522 were 0.492 μM and 3.24 μM, respectively, while for compound **10f**, the values were 0.085 μM and 3.22 μM, respectively, at the same cell line. Among the six tested compounds, compound **11e** has showed the greatest efficacies over a very broad range of cell lines. The compound was capable of inducing total growth inhibition (TGI) and 50% lethality (LC_{50}) at almost all of the 60 cell lines with TGI and LC_{50} values as small as of 2.9 μM and 5.38 μM, respectively, at the colon cancer cell line HCT-116.

2.2.2. In vitro kinase screening

In order to investigate the mechanism of action and the kinase inhibitory profile of this new class of compounds, compound **10f** with the highest potency at the cancer cell lines was tested at a single dose concentration of 10 μM over a panel of 54 kinases at Reaction Biology Corporation.[20] It worth also to mention that a selected group from these compounds was previously screened against ROS receptor tyrosine kinase (RTK), where the discovery of a potent ROS RTK inhibitor has been reported.[21] As showed in Figure 3, compound **10f** has showed very good selectivity for FLT3 kinase with an inhibition percentage of 82.3% at the testing dose. Accordingly, the compound was further tested against FLT3 kinase in a 10-dose IC_{50} testing mode with threefold serial dilutions starting at 20 μM concentration using hot-spot technique. The compound has showed a moderate potency with an IC_{50} of 1.74 μM. This means that this compound can be used as a good new lead for the development of selective FLT3 kinase inhibitors. However, the moderate potency of the compound over FLT3 kinase cannot justify for its strong and broad spectrum anticancer activity, and

Table 3
IC_{50}, TGI and LC_{50} values in μM of compounds **9f**, **10c**, **10f**, **11d**, **11e**, and **17c** over 60 cancer cell lines

	Cell line	9f			10c			10f			11d			11e			17c		
		IC_{50}[a]	TGI[b]	LC_{50}[c]	IC_{50}[a]	TGI[b]	LC_{50}[c]	IC_{50}[a]	TGI[b]	LC_{50}[c]	IC_{50}[a]	TGI[b]	LC_{50}[c]	IC_{50}[a]	TGI[b]	LC_{50}[c]	IC_{50}[a]	TGI[b]	LC_{50}[c]
Leukemia	CCRF-CEM	0.454	>100	>100	2.97	>100	>100	0.237	>100	>100	3.59	>100	>100	2.85	>100	>100	2.61	>100	>100
	HL-60(TB)	0.293	0.923	>100	2.26	6.50	>100	0.105	0.465	>100	3.14	12.9	>100	2.25	5.94	>100	1.78	15.1	>100
	K-562	0.161	>100	>100	0.795	>100	>100	0.058	3.82	>100	2.55	32.8	>100	0.049	6.02	>100	0.365	>100	>100
	MOLT-4	0.626	>100	>100	2.63	>100	>100	0.271	NA	>100	3.17	46.0	>100	2.03	6.82	>100	3.35	>100	>100
	RPMI-8226	1.36	10.7	>100	2.59	9.02	>100	0.266	1.96	>100	3.71	22.2	>100	2.25	6.52	>100	3.30	18.6	>100
	SR	0.461	>100	>100	2.87	>100	>100	NA	NA	NA	2.64	>100	>100	3.77	37.2	>100	1.62	>100	>100
Non-small cell lung cancer	A549/ATCC	0.583	48.3	>100	3.68	>100	>100	0.155	>100	>100	4.18	54.5	>100	2.00	5.07	24.7	3.87	30.8	>100
	EKVX	0.696	>100	>100	5.80	>100	>100	0.290	>100	>100	3.92	92.8	>100	2.16	8.95	>100	3.98	50.7	>100
	HOP-62	0.390	18.7	>100	3.35	>100	>100	0.263	>100	>100	2.82	23.4	>100	11.7	24.7	52.3	3.07	36.9	>100
	HOP-92	0.557	26.6	>100	1.46	7.56	>100	<0.010	0.055	>100	3.03	46.8	>100	1.59	5.24	39.5	2.63	9.09	>100
	NCI-H226	0.968	12.7	>100	5.47	>100	>100	NA	NA	NA	4.18	21.8	>100	3.22	12.2	64.5	4.29	82.3	>100
	NCI-H23	0.514	>100	>100	3.90	>100	>100	0.276	>100	>100	4.48	24.8	>100	1.94	3.97	8.13	3.45	84.0	>100
	NCI-H322M	NA	>100	>100	>100	>100	>100	0.331	>100	>100	6.08	59.8	>100	5.68	19.2	48.6	4.36	50.8	>100
	NCI-H460	0.371	1.68	94.7	2.41	NA	>100	0.058	0.282	4.99	2.79	10.2	70.5	1.87	3.58	6.85	2.04	5.72	26.6
	NCI-H522	0.196	0.492	3.24	1.93	6.96	>100	0.027	0.085	3.22	1.93	7.14	68.1	2.72	10.4	35.1	1.64	6.72	>100
Colon cancer	COLO 205	0.320	0.769	3.91	1.95	3.70	7.02	0.220	>100	>100	1.95	4.13	8.78	1.80	3.26	5.90	1.85	4.13	9.20
	HCC-2998	0.455	1.93	9.69	3.41	>100	>100	NA	NA	NA	3.19	13.2	37.2	1.91	3.39	6.02	2.08	6.77	54.4
	HCT-116	0.353	1.64	>100	1.70	5.40	>100	0.050	1.03	>100	2.71	10.4	41.9	1.56	2.90	5.38	2.33	10.6	87.4
	HCT-15	0.350	8.79	>100	3.14	>100	>100	0.077	>100	>100	3.36	18.2	>100	2.14	5.38	26.1	2.14	>100	>100
	HT29	0.481	2.88	37.2	3.21	>100	>100	0.038	0.211	NA	4.65	20.8	93.9	1.83	3.84	8.06	3.28	18.7	>100
	KM12	0.310	1.62	25.5	2.87	>100	>100	0.048	0.264	>100	1.63	7.66	29.2	1.92	4.87	15.3	2.23	13.9	>100
	SW-620	0.332	>100	>100	2.27	>100	>100	0.039	>100	>100	2.83	17.5	>100	1.94	4.33	9.63	1.30	19.3	>100
CNS cancer	SF-268	0.478	8.90	>100	3.14	>100	>100	0.302	>100	>100	3.55	16.9	77.9	3.96	15.5	41.4	4.07	81.4	>100
	SF-295	0.304	1.91	>100	5.84	>100	>100	0.053	0.315	>100	2.18	8.71	84.1	4.42	21.2	75.2	2.63	>100	>100
	SF-539	0.259	0.596	>100	2.02	4.66	>100	0.127	NA	>100	2.12	5.33	27.3	3.38	13.5	44.0	1.73	4.65	>100
	SNB-19	0.567	>100	>100	>100	>100	>100	0.362	>100	>100	4.54	43.9	>100	10.7	23.4	51.1	6.62	>100	>100
	SNB-75	0.286	0.848	>100	2.08	NA	>100	0.069	>100	>100	1.86	5.94	>100	3.82	17.1	42.0	1.96	6.24	>100
	U251	0.415	5.86	>100	2.89	>100	>100	0.178	>100	>100	3.14	12.9	61.3	1.61	2.99	5.56	3.17	13.5	>100
Melanoma	LOX IMVI	0.681	14.8	>100	3.39	>100	>100	0.067	>100	>100	5.13	17.8	47.7	1.87	3.39	6.13	3.52	>100	>100
	MALME-3M	NA	>100	>100	5.82	>100	>100	0.060	>100	>100	4.56	39.2	>100	1.81	3.58	7.12	3.76	96.4	>100
	M14	0.258	0.769	>100	1.97	NA	>100	0.054	0.483	>100	1.83	4.76	20.4	1.70	3.12	5.73	1.17	3.30	NA
	MDA-MB-435	0.235	0.613	>100	1.93	>100	>100	0.023	0.067	>100	1.01	3.21	>100	2.32	6.84	60.6	0.341	1.60	>100
	SK-MEL-2	1.01	45.5	>100	5.88	>100	>100	0.204	>100	>100	4.15	15.8	>100	3.44	11.7	44.2	3.73	41.3	>100
	SK-MEL-28	0.776	>100	>100	7.66	>100	>100	NA	NA	NA	5.22	32.4	>100	4.92	17.0	41.4	3.63	93.6	>100
	SK-MEL-5	0.269	0.672	24.0	1.94	4.96	>100	0.039	0.309	>100	1.99	5.75	22.9	2.93	9.90	31.5	1.54	6.79	39.8
	UACC-257	0.448	>100	>100	5.24	>100	>100	0.036	>100	>100	4.35	35.6	>100	2.38	8.38	31.7	3.61	>100	>100
	UACC-62	0.337	40.7	>100	4.05	>100	>100	0.062	>100	>100	3.05	14.0	72.1	4.67	18.1	44.4	4.36	>100	>100
Ovarian cancer	IGROV1	0.402	30.1	>100	3.57	>100	>100	0.061	40.9	>100	3.91	24.8	>100	1.74	3.75	8.07	2.73	10.4	>100
	OVCAR-3	0.204	0.448	NA	2.61	8.05	>100	0.030	NA	>100	1.91	4.78	21.2	1.88	3.95	8.29	1.34	3.30	8.13
	OVCAR-4	0.549	>100	>100	5.13	>100	>100	0.270	>100	>100	4.85	>100	>100	4.56	16.0	48.6	3.76	>100	>100
	OVCAR-5	3.98	>100	>100	>100	>100	>100	>100	>100	>100	14.9	60.4	>100	4.84	19.2	48.3	2.43	33.9	>100
	OVCAR-8	0.522	41.6	>100	2.86	>100	>100	0.236	>100	>100	4.20	69.4	>100	2.08	5.54	21.9	3.16	35.2	>100
	NCI/ADR-RES	0.253	0.655	>100	2.87	>100	>100	0.048	>100	>100	2.54	7.93	>100	2.06	4.56	15.8	1.31	6.06	>100
	SK-OV-3	0.304	0.882	>100	4.22	>100	>100	0.504	>100	>100	2.85	8.03	>100	12.0	24.4	49.6	3.13	9.10	>100
Renal cancer	786-0	0.420	5.43	>100	3.11	>100	>100	0.085	>100	>100	3.41	16.6	>100	2.14	5.38	18.9	3.52	93.0	>100
	A498	0.246	0.818	>100	2.03	>100	>100	0.132	>100	>100	3.03	13.4	>100	3.87	16.7	44.1	1.08	5.14	72.5
	ACHN	0.499	12.4	>100	3.76	>100	>100	0.068	>100	>100	3.73	23.7	>100	2.84	9.14	33.8	4.19	>100	>100
	CAKI-1	0.345	18.2	>100	4.24	>100	>100	0.037	0.089	>100	2.89	16.4	>100	1.80	5.78	41.0	1.91	10.5	>100
	RXF 393	0.258	0.633	>100	2.32	6.02	NA	NA	NA	NA	2.87	6.14	>100	2.08	4.31	8.92	1.49	3.59	8.62
	SN12C	0.730	33.1	>100	4.49	>100	>100	0.398	>100	>100	4.28	30.0	>100	1.80	4.02	9.01	6.08	>100	>100
	TK-10	1.01	78.9	>100	NA	>100	>100	0.636	>100	>100	8.40	57.8	>100	4.25	15.1	43.6	3.28	11.5	>100

	UO-31	0.619	98.7	>100	2.61	>100	>100	0.508	>100	>100	3.23	32.5	>100	2.18	6.54	31.7	3.18	58.2	>100
Prost.	PC-3	0.363	>100	>100	3.59	>100	>100	0.052	>100	>100	3.77	47.2	>100	2.15	7.37	62.0	3.45	>100	>100
	DU-145	0.319	1.11	>100	3.42	>100	>100	0.113	0.334	NA	2.75	8.57	53.2	2.28	7.18	26.1	1.98	4.95	>100
Breast cancer	MCF7	0.066	22.7	>100	3.25	>100	>100	NA	NA	NA	0.975	13.9	69.9	2.23	5.48	21.9	2.05	15.9	>100
	MDA-MB-231	1.09	NA	>100	3.63	>100	>100	0.256	NA	>100	2.94	22.6	>100	2.28	7.33	57.7	3.04	>100	>100
	HS 578T	0.264	0.695	>100	1.68	5.68	>100	0.166	>100	>100	2.13	7.00	>100	2.02	6.08	>100	1.28	3.87	31.2
	BT-549	1.62	7.33	>100	3.12	>100	>100	0.075	0.514	>100	2.77	12.1	>100	2.66	11.2	43.1	2.73	15.1	>100
	T-47D	0.475	17.3	>100	2.42	8.02	>100	0.056	>100	>100	2.59	12.4	>100	2.04	5.81	39.1	2.23	8.23	>100
	MDA-MB-468	0.181	0.635	7.24	1.43	3.87	21.3	0.026	0.080	1.04	1.63	4.02	9.94	1.79	3.85	8.28	0.275	1.49	52.3

[a] IC_{50} is the concentration producing 50% inhibition.
[b] TGI is the concentration producing 100% inhibition.
[c] LC_{50} is the concentration causing 50% lethality (50% tumor regression), NA means that the data is not available.

this suggests the presence of other underlying mechanisms that (in addition to the moderate FLT3 kinase inhibition) controls the activity of these new compounds against cancer.

3. Conclusions

A new series of highly potent and efficient anticancer pyrazoles has been designed and synthesized in this study. A structure–activity relationship (SAR) has been made to correlate between compounds structures and activity. The SAR made has revealed that for best activity the compounds should be substituted with a 4-(dimethylamino)phenyl moiety at the 2-position of the terminal pyridyl group. In the second place for good activity comes the 4-phenoxyphenyl group, and then the 4-acetyl group. The presence of the acetonitrile group at the position 2 of the pyrazole ring (2*H*-isomers) was found also to be favored for activity rather than in position 1. The effect of demethylation of the methoxy group to produce the hydroxyl analog was found not to be constant within the derivatives, which suggests the possibility of variable binding modes for such derivatives at their target's binding site, or the presence of different targets, the binding mode at each of which would likely be different. The screening of compound **10f** over 54 kinases showed high selectivity of the compound over FLT3 kinase, but unfortunately with moderate potency, which means that the compounds may have also another target that may contribute, together with the FLT3 inhibition to the anticancer effect of these new compounds. Currently compounds **9f**, **10f** and **11e** are being further tested in vivo in NCI, USA. The compounds would be also screened by our group over a large number of potential molecular targets (such as tubulin, phosphatases and other cell-cycle specific targets) in order to further investigate their possible mechanism of action.

4. Experimental

4.1. General

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were recorded on a Bruker Avance 300 spectrometer with TMS as an internal standard. The IR spectra were recorded on Perkin Elmer Spectrum GX spectrometer. Melting points were taken on a Thomas-Hoover capillary melting apparatus and were uncorrected. Column chromatography was performed on Merck Silica Gel 60 (230–400 mesh). TLC was carried out using glass sheets precoated with Silica Gel 60 F254 prepared by E. Merck. All the commercially available reagents were obtained from Aldrich and Tokyo Kasei Chemicals and generally used without further purification.

4.2. Synthesis

4.2.1. 2-(2-Chloropyridin-4-yl)-1-(3-methoxy-5-methylphenyl) ethanone (5)

To a solution of the ester **4** (9.0 g, 50 mmol) and 2-chloro-4-methyllpyridine (7.0 g, 55 mmol) in THF (90 mL) was added dropwise lithium bis(trimethylsilyl)amide 1 M/THF (75 mL, 75 mmol) at 0 °C under N_2 atmosphere. The mixture was warmed to room temperature and stirred for 18 h. Saturated aqueous ammonium chloride solution (150 mL) was added to the mixture, followed by extraction with ethyl acetate (300 mL × 2). The combined organic layers were washed with saturated NaCl solution, dried over anhydrous $MgSO_4$ and evaporated under vacuum to yield crude **5** (9.92 g, 72%) which was used for the next step without further purification; mp 88–89 °C; IR ν/cm^{-1}: 3432, 3055, 2954, 2919, 2834, 1683, 1598, 1466, 1346, 1323, 1288, 1181, 1155, 1041, 847, 679, 579; ^{1}H NMR ($CDCl_3$): δ 2.41 (s, 3H), 3.84 (s, 3H), 4.26

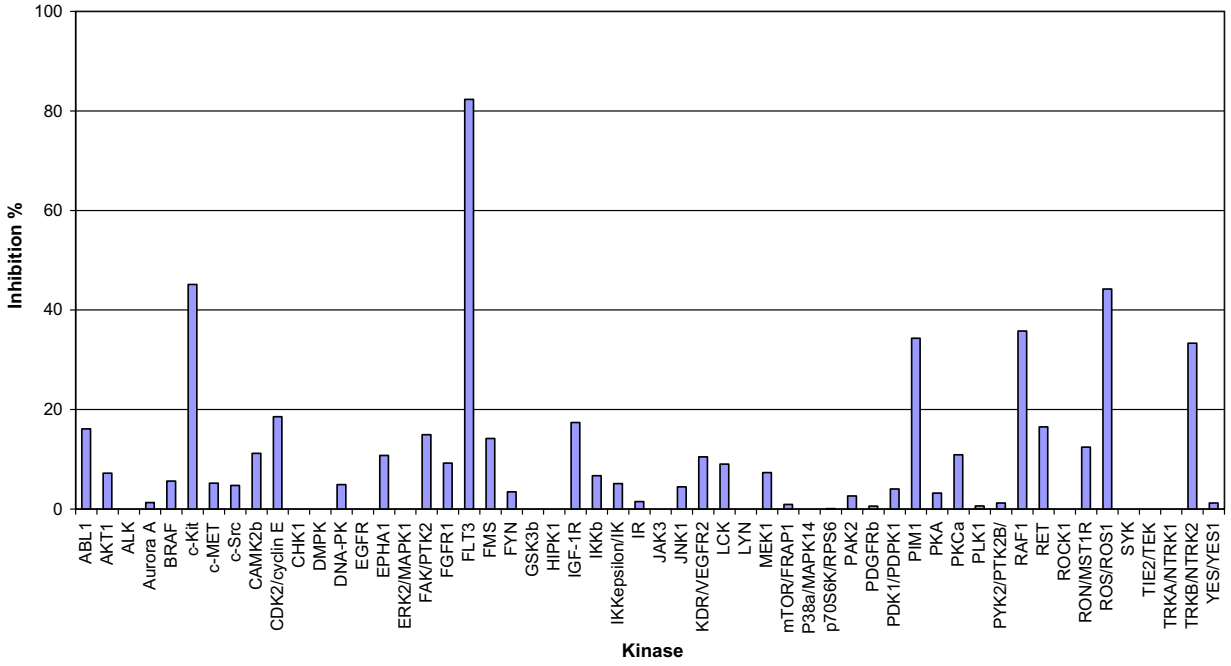


Figure 3. Inhibition percentages of compound **10f** at a single dose concentration of 10 μM over 54 kinases.

(s, 2H), 6.98 (s, 1H), 7.13 (d, J = 4.5 Hz, 1H), 7.25 (s, 1H), 7.30 (s, 1H), 7.38 (s, 1H), 8.34 (d, J = 4.6 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.36, 21.52, 44.14, 55.41, 55.47, 110.12, 120.79, 121.82, 123.82, 125.45, 137.23, 140.16, 146.86, 149.57, 151.69, 159.97, 195.14.

4.2.2. 2-Chloro-4-(3-(3-methoxy-5-methylphenyl)-1*H*-pyrazol-4-yl)pyridine (6)

A mixture of compound **5** (9.1 g, 33 mmol) and *N,N*-dimethylformamide dimethylacetal (30 mL, 255 mmol) was heated under reflux in an oil bath for 12 h. Excess *N,N*-dimethylformamide dimethylacetal was removed under vacuum, and then the crude product was dissolved in absolute ethanol (150 mL). Hydrazine monohydrate (3.3 g, 66 mmol) was added and the mixture was stirred at room temperature for 2 h. The solvent was then removed under vacuum and the crude product was crystallized from ethyl acetate/hexane to yield pure **6** as buff to brown powder (8.0 g, 81%); mp 131–132; IR ν/cm^{-1}: 3442, 3112, 2915, 2848, 1596, 1064, 866, 832, 769; ^{1}H NMR ($CDCl_3$): δ 2.33 (s, 3H), 3.75 (s, 3H), 6.78 (s, 1H), 6.80 (s, 1H), 6.85 (s, 1H), 7.13 (dd, J = 1.1, 4.2 Hz, 1H), 7.32 (s, 1H), 7.76 (s, 1H), 8.25 (d, J = 5.3 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.54, 55.30, 111.11, 115.63, 115.89, 121.03, 121.49, 122.27, 140.41, 144.28, 149.61, 151.84, 159.91.

4.2.3. General procedure for the synthesis of compounds 7 and 8

A mixture of compound **6** (4.6 g, 15.35 mmol) and ground K_2CO_3 (10.6 g, 76.73 mmol) in acetone (100 mL) was stirred under reflux for 2 h. To the reaction mixture was added iodoacetonitrile (1.34 mL, 18.42 mmol) dropwise. Heating and stirring were maintained for two more hours, after which acetone was removed under vacuum, and the residue was partitioned between water (200 mL) and ethyl acetate (200 mL). The aqueous layer was further extracted with ethyl acetate (100 mL), and then the combined organics were dried over anhydrous $MgSO_4$ and evaporated under vacuum. The crude product was purified by column chromatography (silica gel, ethyl acetate–hexane 2:3 v/v) to give a pale yellow oily mixture of compounds **7** and **8** in an approximate ration of (2:1), respectively, that has been used in the next step without separation of the two isomers (4.78 g, 92%).

4.2.3.1. 2-(4-(2-Chloropyridin-4-yl)-3-(3-methoxy-5-methylphenyl)-1*H*-pyrazol-1-yl)acetonitrile (7).

IR ν/cm^{-1}: 3432, 2938, 1596, 1532, 1379, 1168, 1130, 1063, 850, 778, 731; ^{1}H NMR ($CDCl_3$): δ 2.29 (s, 3H), 3.71 (s, 3H), 5.19 (s, 2H), 6.73 (s, 1H), 6.75 (s, 1H), 6.85 (s, 1H), 7.06 (d, J = 4.4 Hz, 1H), 7.26 (s, 1H), 7.79 (s, 1H), 8.24 (d, J = 4.8 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.53, 39.97, 55.29, 110.91, 111.12, 113.65, 115.67, 118.75, 121.59, 121.65, 122.78, 130.58, 132.37, 140.16, 143.28, 149.68, 151.45, 151.79, 159.71, 162.33.

4.2.3.2. 2-(4-(2-Chloropyridin-4-yl)-5-(3-methoxy-5-methylphenyl)-1*H*-pyrazol-1-yl)acetonitrile (8).

IR ν/cm^{-1}: 3429, 3937, 1596, 1464, 1372, 1156, 844, 803; ^{1}H NMR ($CDCl_3$): δ 2.39 (s, 3H), 3.83 (s, 3H), 4.94 (s, 2H), 6.63 (s, 1H), 6.74 (s, 1H), 6.92–6.96 (m, 2H), 7.19 (s, 1H), 7.93 (s, 1H), 8.17 (d, J = 5.2 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.56, 37.78, 55.48, 112.15, 113.91, 117.08, 118.16, 119.88, 121.36, 122.48, 128.46, 139.57, 141.60, 142.19, 142.85, 149.78, 151.95, 160.48.

4.2.4. General procedure for the synthesis of compounds 9a–g and 10a–g

A mixture of starting material **7** and **8** mixture (300 mg, 0.886 mmol), the appropriate aryl boronic acid (0.974 mmol), dichlorobis(triphenylphosphine)Pd(II) (31 mg, 0.0443 mmol) and K_2CO_3 (130 mg, 0.886 mmol) was placed in a mixed solvent of THF and water (4:1, 10 mL). N_2 gas was bubbled into this mixture for 10 min, and then the mixture was heated at 70 °C while stirring under N_2 atmosphere for 12 h. The reaction mixture was left to cool at room temperature, and then poured into ice water (100 mL) and extracted with ethyl acetate (100 mL × 3). The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated under vacuum. The target products were separated in pure form by preparative TLC on silica plates using the proper ratio of ethyl acetate/hexane. After four runs using the specified mobile phase; the products were extracted from silica using ethyl acetate (100 mL × 2) followed by methanol (100 mL). For calculation of yields, it was roughly assumed that the amount of compounds **7** and **8** in every starting 300 mg of starting mixture are 200 and 100 mg, respectively.

4.2.4.1. 2-(3-(3-Methoxy-5-methylphenyl)-4-(2-phenylpyridin-4-yl)-1*H*-pyrazol-1-yl)acetonitrile (9a). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:2 v/v). Yield: (166 mg, 74%); mp 55–56 °C; IR ν/cm^{-1}: 3428, 2924, 1596, 1447, 1418, 1166, 1154, 1060, 850, 777, 697; ^{1}H NMR ($CDCl_3$): δ 2.32 (s, 3H), 3.71 (s, 3H), 5.14 (s, 2H), 6.79 (s, 1H), 6.84 (s, 1H), 6.96 (s, 1H), 7.13 (d, J = 4.9 Hz, 1H), 7.41–7.48 (m, 3H), 7.65 (s, 1H), 7.79 (s, 1H), 7.88 (d, J = 6.9 Hz, 2H), 8.61 (d, J = 5.1 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.51, 39.86, 55.25, 110.90, 113.61, 115.61, 119.78, 120.42, 121.24, 121.76, 126.89, 128.78, 129.11, 130.05, 132.90, 139.19, 139.98, 140.77, 149.89, 151.46, 157.75, 159.75.

4.2.4.2. 2-(4-(2-(3-Acetylphenyl)pyridin-4-yl)-3-(3-methoxy-5-methylphenyl)-1*H*-pyrazol-1-yl)acetonitrile (9b). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:1 v/v). Yield: (197 mg, 79%); mp 73–74 °C; IR ν/cm^{-1}: 3436, 2937, 1683, 1607, 1423, 1247, 1167, 1061, 804, 695, 590; ^{1}H NMR ($CDCl_3$): δ 2.27 (s, 3H), 2.60 (s, 3H), 3.67 (s, 3H), 5.19 (s, 2H), 6.76 (s, 1H), 6.80 (s, 1H), 6.91 (s, 1H), 7.13 (s, 1H), 7.49 (br s, 1H), 7.64 (s, 1H), 7.85 (s, 1H), 7.95 (d, J = 5.4 Hz, 1H), 8.04 (d, J = 5.7 Hz, 1H), 8.39 (s, 1H), 8.56 (s, 1H); ^{13}C NMR ($CDCl_3$): 21.51, 26.78, 39.89, 55.23, 111.03, 113.93, 115.51, 119.76, 119.90, 121.63, 121.77, 126.71, 128.60, 128.76, 129.11, 130.54, 131.45, 132.06, 132.92, 137.52, 139.61, 140.00, 141.09, 149.91, 151.38, 156.47, 159.69, 198.16.

4.2.4.3. 2-(4-(2-(4-Acetylphenyl)pyridin-4-yl)-3-(3-methoxy-5-methylphenyl)-1*H*-pyrazol-1-yl)acetonitrile (9c). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:2 v/v). Yield: (170 mg, 68%); mp 99–100 °C; IR ν/cm^{-1}: 3463, 2924, 1678, 1667, 1603, 1270, 1166, 1070, 849, 467; ^{1}H NMR ($CDCl_3$): δ 2.32 (s, 3H), 2.65 (s, 3H), 3.72 (s, 3H), 5.20 (s, 2H), 6.79 (s, 1H), 6.82 (s, 1H), 6.94 (s, 1H), 7.20 (d, J = 5.1 Hz, 1H), 7.69 (s, 1H), 7.88 (s, 1H), 7.95 (d, J = 8.4 Hz, 2H), 8.02 (d, J = 8.4 Hz, 2H), 8.63 (d, J = 5.1 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.54, 26.80, 39.95, 55.27, 110.97, 113.53, 115.58, 120.20, 121.74, 121.88, 127.00, 128.82, 130.02, 132.77, 137.19, 140.06, 140.95, 143.39, 150.15, 151.54, 156.35, 159.77, 197.91.

4.2.4.4. *N*-(3-(4-(1-(Cyanomethyl)-3-(3-methoxy-5-methylphenyl)-1*H*-pyrazol-4-yl)pyridin-2-yl)phenyl)acetamide (9d). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:2 v/v). Yield: (194 mg, 75%); mp 103–105 °C; IR ν/cm^{-1}: 3281, 3083, 2937, 1674, 1595, 1549, 1453, 1321, 1167, 1062, 848, 797, 699; ^{1}H NMR ($CDCl_3$): δ 2.13 (s, 3H), 2.47 (s, 3H), 3.68 (s, 3H), 5.14 (s, 2H), 6.74 (s, 1H), 6.78 (s, 1H), 6.89 (s, 1H), 7.03 (d, J = 4.7 Hz, 1H), 7.28–7.34 (m, 1H), 7.51–7.57 (m, 2H), 7.62 (d, J = 7.8 Hz, 1H), 7.71 (s, 1H), 8.09 (s, 1H), 8.27 (s, 1H), 8.47 (d, J = 4.8 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.48, 24.44, 39.80, 55.27, 110.92, 113.82, 115.56, 118.46, 119.72, 120.02, 120.70, 121.51, 121.82, 122.60, 129.42, 130.37, 132.93, 138.68, 139.83, 139.99, 140.96, 149.59, 151.33, 157.11, 159.65, 169.06.

4.2.4.5. 4-(4-(1-(Cyanomethyl)-3-(3-methoxy-5-methylphenyl)-1*H*-pyrazol-4-yl)pyridin-2-yl)benzonitrile (9e). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:2 v/v). Yield: (196 mg, 82%); mp 83–84 °C; IR ν/cm^{-1}: 3421, 2938, 2227, 1604, 1546, 1451, 1167, 1059, 842; ^{1}H NMR ($CDCl_3$): δ 2.31 (s, 3H), 3.71 (s, 3H), 5.19 (s, 2H), 6.79 (s, 1H), 6.82 (s, 1H), 6.93 (s, 1H), 7.22 (d, J = 4.6 Hz, 1H), 7.66 (s, 1H), 7.71 (d, J = 8.3 Hz, 2H), 7.87 (s, 1H), 7.97 (d, J = 8.3 Hz, 2H), 8.63 (d, J = 5.1 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.50, 39.96, 55.27, 111.11, 112.47, 113.57, 115.51, 118.78, 119.97, 120.10, 121.75, 122.22, 127.38, 130.09, 132.53, 132.80, 140.06, 141.17, 143.30, 150.26, 151.51, 155.44, 159.81.

4.2.4.6. 2-(4-(2-(4-(Dimethylamino)phenyl)pyridin-4-yl)-3-(3-methoxy-5-methylphenyl)-1*H*-pyrazol-1-yl)acetonitrile (9f). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:3 v/v). Yield: (197 mg, 79%); mp 98–99 °C; IR ν/cm^{-1}: 3409, 2934, 1606, 1531, 1441, 1194, 1167, 1060, 820, 462; ^{1}H NMR ($CDCl_3$): δ 2.31 (s, 3H), 3.01 (s, 6H), 3.70 (s, 3H), 5.10 (s, 2H), 6.75–6.78 (m, 3H), 6.84 (s, 1H), 6.97–7.00 (m, 2H), 7.56 (s, 1H), 7.72 (s, 1H), 7.80 (d, J = 8.8 Hz, 2H), 8.52 (d, J = 5.0 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.53, 39.79, 40.34, 55.25, 110.68, 112.16, 113.70, 115.67, 118.33, 119.88, 120.69, 121.74, 126.77, 127.72, 130.10, 132.99, 139.89, 140.46, 149.55, 151.13, 151.35, 157.78, 159.68.

4.2.4.7. 2-(3-(3-Methoxy-5-methylphenyl)-4-(2-(4-phenoxyphenyl)pyridin-4-yl)-1*H*-pyrazol-1-yl)acetonitrile (9g). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:3 v/v). Yield: (201 mg, 72%); mp 80–81 °C; IR ν/cm^{-1}: 3419, 3064, 2937, 2835, 1596, 1510, 1490, 1238, 1167, 1059, 871, 840, 692; ^{1}H NMR ($CDCl_3$): δ 2.31 (s, 3H), 3.72 (s, 3H), 5.18 (s, 2H), 6.79 (s, 1H), 6.83 (s, 1H), 6.94 (s, 1H), 7.04 (d, 8.0 Hz, 4H), 7.13–7.22 (m, 2H), 7.37 (t, J = 7.3 Hz, 2H), 7.64 (s, 1H), 7.79 (d, J = 8.3 Hz, 2H), 7.94 (s, 1H), 8.57 (d, J = 5.0 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.53, 39.89, 55.28, 111.05, 113.70, 115.65, 118.72, 119.42, 119.67, 119.74, 121.03, 121.80, 123.92, 128.57, 129.81, 129.94, 130.86, 132.40, 132.82, 140.09, 142.48, 148.36, 151.65, 156.10, 156.43, 158.98, 159.81.

4.2.4.8. 2-(5-(3-Methoxy-5-methylphenyl)-4-(2-phenylpyridin-4-yl)-1*H*-pyrazol-1-yl)acetonitrile (10a). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:2 v/v). Yield: (70 mg, 62%); mp 51–52 °C; IR ν/cm^{-1}: 3428, 2964, 1596, 1445, 1414, 1386, 1263, 1155, 1072, 1037, 843, 801, 777, 694; ^{1}H NMR ($CDCl_3$): δ 2.41 (s, 3H), 3.81 (s, 3H), 4.95 (s, 2H), 6.76 (s, 1H), 6.82 (s, 1H), 6.95 (s, 1H), 7.06 (d, J = 4.5 Hz, 1H), 7.41–7.44 (m, 3H), 7.58 (s, 1H), 7.80 (d, J = 6.3 Hz, 2H), 8.01 (s, 1H), 8.53 (d, J = 4.8 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.57, 37.76, 55.49, 112.31, 114.07, 116.90, 118.29, 119.60, 119.70, 122.75, 126.83, 128.71, 129.03, 129.11, 139.21, 139.61, 140.45, 141.47, 141.89, 149.88, 157.70, 160.51.

4.2.4.9. 2-(4-(2-(3-Acetylphenyl)pyridin-4-yl)-5-(3-methoxy-5-methylphenyl)-1*H*-pyrazol-1-yl)acetonitrile (10b). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:2 v/v). Yield: (96 mg, 77%); mp 65–66 °C; IR ν/cm^{-1}: 3440, 2938, 1684, 1606, 1420, 1358, 1248, 1230, 1156, 846, 804, 689, 589; ^{1}H NMR ($CDCl_3$): δ 2.39 (s, 3H), 2.62 (s, 3H), 3.79 (s, 3H), 4.96 (s, 2H), 6.74 (s, 1H), 6.80 (s, 1H), 6.95 (s, 1H), 7.10 (br s, 1H), 7.50 (t, J = 7.5 Hz, 1H), 7.56 (s, 1H), 7.94–8.00 (m, 3H), 8.29 (s, 1H), 8.52 (d, J = 4.2 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.59, 26.78, 37.77, 55.78, 112.33, 114.14, 116.93, 118.18, 119.32, 120.14, 122.64, 126.60, 128.68, 129.01, 131.39, 137.53, 139.59, 139.72, 140.66, 141.54, 141.93, 150.05, 156.54, 160.45, 197.89.

4.2.4.10. 2-(4-(2-(4-Acetylphenyl)pyridin-4-yl)-5-(3-methoxy-5-methylphenyl)-1*H*-pyrazol-1-yl)acetonitrile (10c). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:2 v/v). Yield: (93 mg, 75%); mp 156–157 °C; IR ν/cm^{-1}: 3473, 2939, 1676, 1604, 1594, 1423, 1267, 1172, 839, 471; ^{1}H NMR ($CDCl_3$): δ 2.42 (s, 3H), 2.65 (s, 3H), 3.82 (s, 3H), 4.97 (s, 2H), 6.75 (s, 1H), 6.82 (s, 1H), 6.96 (s, 1H), 7.12 (d, J = 4.9 Hz, 1H), 7.60 (s, 1H), 7.87 (d, J = 8.1 Hz, 2H), 7.99–8.02 (m, 3H), 8.56 (d, J = 5.1 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.60, 26.78, 37.78, 55.52, 112.34, 114.02, 116.86, 118.62, 119.33, 120.38, 122.67, 126.90, 128.76, 129.01, 137.12, 139.56, 140.61, 141.58, 141.96, 143.49, 150.20, 156.29, 160.54, 197.88.

4.2.4.11. N-(3-(4-(1-(Cyanomethyl)-5-(3-methoxy-5-methylphenyl)-1*H*-pyrazol-4-yl)pyridin-2-yl)phenyl)acetamide (10d). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:1 v/v). Yield: (81 mg, 63%); mp 97–98 °C; IR ν/cm^{-1}: 3302, 3080, 2936, 1674, 1595, 1551, 1465, 1374, 1322, 846, 794, 698; ^{1}H NMR ($CDCl_3$): δ 2.15 (s, 3H), 2.39 (s, 3H), 3.81 (s, 3H), 4.95 (s, 2H), 6.74 (s, 1H), 6.79 (s, 1H), 6.93 (s, 1H), 7.01 (d, J = 4.7 Hz, 1H), 7.32–7.37 (m, 1H), 7.43 (d, J = 7.7 Hz, 1H), 7.57 (s, 1H), 7.75–7.77 (m, 2H), 7.98 (s, 1H), 7.99 (s, 1H), 8.46 (d, J = 4.8 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.56, 24.51, 37.76, 55.52, 112.24, 114.07, 117.00, 118.36, 119.50, 119.89, 120.67, 122.39, 122.78, 129.08, 129.39, 138.57, 139.60, 139.92, 140.49, 141.52, 141.88, 149.81, 157.12, 160.45, 168.55.

4.2.4.12. 4-(4-(1-(Cyanomethyl)-5-(3-methoxy-5-methylphenyl)-1*H*-pyrazol-4-yl)pyridin-2-yl)benzonitrile (10e). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:2 v/v). Yield: (92 mg, 77%); mp 161–162 °C; IR ν/cm^{-1}: 3420, 2941, 2218, 1605, 1467, 1386, 1155, 855, 834; ^{1}H NMR ($CDCl_3$): δ 2.42 (s, 3H), 3.85 (s, 3H), 4.96 (s, 2H), 6.75 (s, 1H), 6.82 (s, 1H), 6.96 (s, 1H), 7.13 (d, J = 4.9 Hz, 1H), 7.58 (s, 1H), 7.71 (d, J = 8.1 Hz, 2H), 7.91 (d, J = 8.1 Hz, 2H), 8.02 (s, 1H), 8.56 (d, J = 5.1 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.58, 37.80, 55.51, 112.40, 112.47, 113.95, 116.85, 118.53, 118.73, 119.20, 120.71, 122.65, 127.30, 129.01, 132.48, 139.51, 140.82, 141.60, 142.00, 143.39, 150.32, 155.46, 160.59.

4.2.4.13. 2-(4-(2-(4-(Dimethylamino)phenyl)pyridin-4-yl)-5-(3-methoxy-5-methylphenyl)-1*H*-pyrazol-1-yl)aceto-nitrile (10f). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:3 v/v). Yield: (62 mg, 50%); mp 150–151 °C; IR ν/cm^{-1}: 3443, 2920, 1600, 1531, 1417, 1371, 1200, 1172, 820, 805; ^{1}H NMR ($CDCl_3$): δ 2.41 (s, 3H), 3.02 (s, 6H), 3.81 (s, 3H), 4.95 (s, 2H), 6.76–6.81 (m, 4H), 6.91–6.93 (m, 2H), 7.50 (s, 1H), 7.73 (d, J = 8.6 Hz, 2H), 8.00 (s, 1H), 8.46 (d, J = 5.1 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.58, 37.74, 40.38, 55.48, 112.12, 114.12, 116.82, 116.95, 118.33, 119.98, 122.82, 127.04, 127.63, 129.23, 139.68, 139.94, 141.33, 141.70, 149.68, 151.05, 157.84, 160.42.

4.2.4.14. 2-(5-(3-Methoxy-5-methylphenyl)-4-(2-(4-phenoxyphenyl)pyridin-4-yl)-1*H*-pyrazol-1-yl)acetonitrile (10g). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:3 v/v). Yield: (96 mg, 69%); mp 69–70 °C; IR ν/cm^{-1}: 3037, 2937, 2835, 1595, 1489, 1423, 1237, 1167, 838, 691; ^{1}H NMR ($CDCl_3$): δ 2.41 (s, 3H), 3.81 (s, 3H), 4.96 (s, 2H), 6.76 (s, 1H), 6.82 (s, 1H), 6.95 (s, 1H), 7.05 (d, 7.3 Hz, 5H), 7.14 (t, J = 7.1 Hz, 1H), 7.36 (t, J = 7.1 Hz, 2H), 7.56 (s, 1H), 7.78 (d, J = 8.0 Hz, 2H), 8.01 (s, 1H), 8.51 (d, J = 3.8 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.59, 37.78, 55.50, 112.34, 114.14, 116.90, 117.87, 118.75, 119.23, 119.46, 122.73, 123.69, 128.38, 129.07, 129.88, 133.77, 139.63, 140.88, 141.50, 141.99, 149.50, 156.74, 156.77, 158.46, 160.43.

4.2.5. General procedure for the synthesis of compounds 11a–e and 12a–e

To a solution of the starting methoxy compound **9a–g** or **10a–g** (0.12 mmol) in dichloromethane (4 mL) was added borontrifluoride–methyl sulfide complex (0.126 mL, 1.2 mmol) dropwise at room temperature and under N_2 atmosphere. The resulting suspension was stirred for 24 h, and then the mixture was concentrated under vacuum. The residue was partitioned between ethyl acetate (100 mL) and brine (50 mL). The organic layer was separated and dried over anhydrous $MgSO_4$, then evaporated under vacuum. The residue was then purified by column chromatography to yield the pure hydroxyl product.

4.2.5.1. 2-(3-(3-Hydroxy-5-methylphenyl)-4-(2-phenylpyridin-4-yl)-1*H*-pyrazol-1-yl)acetonitrile (11a). Column chromatography (silica gel, ethyl acetate–hexane 1:1 v/v). Yield: (31 mg, 70%); mp 110–111 °C; IR ν/cm^{-1}: 3433, 2922, 1609, 1547, 1448, 1168, 852, 777, 699; ^{1}H NMR ($CDCl_3$): δ 2.31 (s, 3H), 5.09 (s, 2H), 6.54 (s, 1H), 6.73 (s, 1H), 6.98–7.03 (m, 2H), 7.28–7.32 (m, 3H), 7.51 (s, 1H), 7.62–7.65 (m, 3H), 8.30 (s, 1H); ^{13}C NMR ($CDCl_3$): 21.51, 39.82, 112.71, 113.60, 117.21, 119.73, 120.33, 120.77, 121.35, 127.14, 128.79, 129.29, 130.37, 132.21, 138.30, 140.62, 141.52, 148.83, 149.41, 150.24, 151.30, 156.81, 157.68.

4.2.5.2. 2-(4-(2-(3-Acetylphenyl)pyridin-4-yl)-3-(3-hydroxy-5-methylphenyl)-1*H*-pyrazol-1-yl)acetonitrile (11b). Column chromatography (silica gel, ethyl acetate–hexane 1:1 v/v). Yield: (30 mg, 61%); mp 84–85 °C; IR ν/cm^{-1}: 3408, 3059, 2924, 1683, 1607, 1425, 1358, 1299, 1250, 1166, 804, 725, 694, 542; ^{1}H NMR ($CDCl_3$): δ 2.29 (s, 3H), 2.49 (s, 3H), 5.16 (s, 2H), 6.59 (s, 1H), 6.73 (s, 1H), 6.97 (s, 1H), 7.11 (d, J = 4.8 Hz, 1H), 7.36 (t, J = 7.7 Hz, 1H), 7.53 (s, 1H), 7.77 (s, 1H), 7.83 (t, J = 7.2 Hz, 2H), 8.05 (s, 1H), 8.35 (d, J = 5.1 Hz, 1H).

4.2.5.3. 4-(4-(1-(Cyanomethyl)-3-(3-hydroxy-5-methylphenyl)-1*H*-pyrazol-4-yl)pyridin-2-yl)benzonitrile (11c). Column chromatography (silica gel, ethyl acetate–hexane 1:1 v/v). Yield: (38 mg, 81%); mp 147–149 °C; IR ν/cm^{-1}: 3401, 2229, 1605, 1547, 1449, 1300, 1167, 843; ^{1}H NMR ($CDCl_3$): δ 2.27 (s, 3H), 5.18 (s, 2H), 6.66 (s, 1H), 6.71 (s, 1H), 6.92 (s, 1H), 7.19 (d, J = 4.9 Hz, 1H), 7.59–7.64 (m, 3H), 7.80–7.84 (m, 3H), 8.51 (d, J = 5.1 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.37, 39.94, 112.47, 112.71, 113.50, 117.05, 118.58, 119.64, 120.60, 121.23, 122.22, 127.53, 130.27, 132.51, 132.59, 140.57, 141.52, 142.90, 149.56, 151.42, 155.48, 156.43.

4.2.5.4. 2-(4-(2-(4-(Dimethylamino)phenyl)pyridin-4-yl)-3-(3-hydroxy-5-methylphenyl)-1*H*-pyrazol-1-yl)acetonitrile (11d). Column chromatography (silica gel, ethyl acetate–hexane 1:2 v/v). Yield: (37 mg, 75%); mp 138–139 °C; IR ν/cm^{-1}: 3420, 2922, 1607, 1531, 1444, 1167, 819; ^{1}H NMR ($CDCl_3$): δ 2.29 (s, 3H), 2.92 (s, 6H), 4.99 (s, 2H), 6.60–6.62 (m, 3H), 6.73 (s, 1H), 6.83 (d, J = 4.3 Hz, 1H), 6.96 (s, 1H), 7.41 (s, 1H), 7.54–7.57 (m, 3H), 8.11 (d, J = 4.8 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.45, 39.58, 40.22, 112.14, 112.87, 113.69, 117.15, 119.17, 119.94, 120.39, 125.73, 128.03, 130.51, 132.53, 140.40, 141.31, 148.21, 151.12, 151.27, 156.95, 157.46.

4.2.5.5. 2-(3-(3-Hydroxy-5-methylphenyl)-4-(2-(4-phenoxyphenyl)pyridin-4-yl)-1*H*-pyrazol-1-yl)acetonitrile (11e). Column chromatography (silica gel, ethyl acetate–hexane 1:2 v/v). Yield: (34 mg, 62%); mp 104–105 °C; IR ν/cm^{-1}: 3041, 2919, 1608, 1490, 1448, 1237, 1167, 845, 762, 691; ^{1}H NMR ($CDCl_3$): δ 2.29 (s, 3H), 5.09 (s, 2H), 6.62 (s, 1H), 6.71 (s, 1H), 6.90–7.01 (m, 6H), 7.13 (t, 7.2 Hz, 1H), 7.34 (t, J = 7.7 Hz, 2H), 7.49 (s, 1H), 7.58 (d, J = 8.4 Hz, 2H), 7.68 (s, 1H), 8.28 (d, J = 4.8 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.47, 39.81, 112.66, 117.23, 118.49, 119.43, 119.83, 120.32, 120.52, 121.02, 123.84, 128.67, 129.90, 130.37, 132.36, 133.15, 140.57, 141.54, 148.89, 151.23, 156.42, 156.72, 157.08, 158.56.

4.2.5.6. 2-(4-(2-(3-Acetylphenyl)pyridin-4-yl)-5-(3-hydroxy-5-methylphenyl)-1*H*-pyrazol-1-yl)acetonitrile (12a). Column chromatography (silica gel, ethyl acetate–hexane 1:1 v/v). Yield: (32 mg, 65%); mp 123–125 °C; IR ν/cm^{-1}: 3429, 2923, 1684, 1608, 1422, 1360, 1307, 1234, 851, 692, 594; ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H), 2.58 (s, 3H), 4.95 (s, 2H), 6.37 (s, 1H), 6.69 (s, 1H), 6.87 (s, 1H), 7.00 (d, J = 5.0 Hz, 1H), 7.44 (t, J = 7.5 Hz, 1H), 7.53 (s, 1H), 7.89–7.95 (m, 3H), 8.19 (s, 1H), 8.37 (d, J = 5.1 Hz, 1H), 9.51 (s, 1H); ^{13}C

NMR ($CDCl_3$): 21.51, 26.80, 37.66, 114.02, 114.09, 118.73, 118.95, 119.21, 120.55, 121.27, 126.96, 128.32, 129.03, 129.23, 131.81, 137.26, 139.15, 139.69, 141.50, 141.72, 142.24, 149.21, 156.63, 157.89, 198.40.

4.2.5.7. 2-(4-(2-(4-Acetylphenyl)pyridin-4-yl)-5-(3-hydroxy-5-methylphenyl)-1*H*-pyrazol-1-yl)acetonitrile (12b). Column chromatography (silica gel, ethyl acetate–hexane 1:1 v/v). Yield: (21 mg, 42%); mp 215–216 °C; IR ν/cm^{-1}: 3107, 2993, 2926, 1672, 1604, 1404, 1302, 1268, 1166, 836; ^1H NMR ($CDCl_3$): δ 2.37 (s, 3H), 2.61 (s, 3H), 4.95 (s, 2H), 6.56 (s, 1H), 6.73 (s, 1H), 6.91 (s, 1H), 7.11 (s, 1H), 7.51 (s, 1H), 7.75 (d, J = 7.6 Hz, 2H), 7.92–7.98 (m, 3H), 8.46 (s, 1H).

4.2.5.8. *N*-(3-(4-(1-(Cyanomethyl)-5-(3-hydroxy-5-methylphenyl)-1*H*-pyrazol-4-yl)pyridin-2-yl)phenyl)acetamide (12c). Column chromatography (silica gel, ethyl acetate). Yield: (28 mg, 55%); mp 142–143 °C; IR ν/cm^{-1}: 3380, 2927, 2857, 1671, 1605, 1550, 1452, 1311, 1164, 850, 795, 699; ^1H NMR (CD_3OD): δ 2.16 (s, 3H), 2.36 (s, 3H), 5.14 (s, 2H), 6.66 (s, 1H), 6.75 (s, 1H), 6.89 (s, 1H), 7.23 (d, J = 5.1 Hz, 1H), 7.36 (d, J = 3.9 Hz, 2H), 7.65 (br s, 2H), 7.97 (s, 1H), 8.17 (s, 1H), 8.41 (d, J = 5.2 Hz, 1H); ^{13}C NMR (CD_3OD): 20.07, 22.46, 37.03, 113.48, 114.37, 117.72, 118.52, 118.69, 118.76, 119.85, 120.61, 121.12, 122.30, 128.80, 129.11, 139.00, 139.26, 139.62, 141.44, 141.61, 142.38, 149.04, 157.39, 158.40, 170.35.

4.2.5.9. 4-(4-(1-(Cyanomethyl)-5-(3-hydroxy-5-methylphenyl)-1*H*-pyrazol-4-yl)pyridin-2-yl)benzonitrile (12d). Column chromatography (silica gel, ethyl acetate–hexane 1:1 v/v). Yield: (34 mg, 72%); mp 225–227 °C; IR ν/cm^{-1}: 3065, 2993, 2219, 1607, 1460, 1390, 1305, 1166, 1105, 839; ^1H NMR ($CDCl_3$): δ 2.37 (s, 3H), 4.96 (s, 2H), 6.61 (s, 1H), 6.75 (s, 1H), 6.90 (s, 1H), 7.14 (d, J = 5.1 Hz, 1H), 7.54 (s, 1H), 7.66 (d, J = 8.1 Hz, 2H), 7.83 (d, J = 8.4 Hz, 2H), 8.00 (s, 1H), 8.50 (d, J = 5.2 Hz, 1H).

4.2.5.10. 2-(5-(3-Hydroxy-5-methylphenyl)-4-(2-(4-phenoxyphenyl)pyridin-4-yl)-1*H*-pyrazol-1-yl)acetonitrile (12e). Column chromatography (silica gel, ethyl acetate–hexane 1:2 v/v). Yield: (34 mg, 61%); mp 101–102 °C; IR ν/cm^{-1}: 3041, 2923, 1607, 1490, 1425, 1238, 1167, 870, 836, 691, 461; ^1H NMR ($CDCl_3$): δ 2.36 (s, 3H), 4.91 (s, 2H), 6.45 (s, 1H), 6.72 (s, 1H), 6.86 (s, 1H), 6.94–7.03 (m, 5H), 7.15 (t, 7.4 Hz, 1H), 7.36 (t, J = 8.0 Hz, 2H), 7.49 (s, 1H), 7.65 (d, J = 8.6 Hz, 2H), 7.95 (s, 1H), 8.32 (d, J = 5.3 Hz, 1H), 9.11 (s, 1H); ^{13}C NMR ($CDCl_3$): 21.47, 37.67, 113.97, 114.03, 118.68, 118.88, 118.98, 119.28, 119.58, 121.62, 123.80, 128.62, 129.90, 133.57, 139.59, 141.19, 141.71, 142.15, 149.05, 156.53, 157.20, 157.79, 158.47.

4.2.6. 2-(2-Chloropyridin-4-yl)-1-(3-methoxy-5-methylphenyl) ethanone (13)

To a solution of the ester **4** (9 g, 50 mmol) and 2-chloro-5-methylpyridine (7.0 g, 55 mmol) in THF (90 mL) was added dropwise lithium bis(trimethylsilyl)amide 1 M/THF (75 mL, 75 mmol) at 0 °C under N_2 atmosphere. The mixture was warmed to room temperature and stirred for 18 h. Saturated aqueous ammonium chloride solution (150 mL) was added to the mixture, followed by extraction with ethyl acetate (300 mL × 2). The combined organic layers were washed with saturated NaCl, dried over anhydrous $MgSO_4$ and evaporated under vacuum to yield crude **13** (7.58 g, 55%) as a sticky solid which was used for the next step without further purification; mp <40 °C; IR ν/cm^{-1}: 3390, 2964, 1660, 1596, 1263, 1103, 1064, 802; ^1H NMR ($CDCl_3$): δ 2.29 (s, 3H), 3.74 (s, 3H), 3.83 (s, 2H), 6.84 (s, 1H), 7.14 (s, 1H), 7.29 (s, 1H), 7.35–7.39 (m, 2H), 8.12 (s, 1H); ^{13}C NMR ($CDCl_3$): 17.55, 21.23, 52.00, 55.26, 111.11, 120.06, 122.73, 123.65, 131.09, 132.09, 139.48, 139.62, 148.43, 149.70, 159.49, 167.06.

4.2.7. 2-Chloro-5-(3-(3-methoxy-5-methylphenyl)-1*H*-pyrazol-4-yl)pyridine (14)

A mixture of compound **13** (7.28 g, 26.5 mmol) and *N,N*-dimethylformamide dimethylacetal (24 mL, 204 mmol) was heated under reflux in an oil bath for 12 h. The excess unreacted *N,N*-dimethylformamide dimethylacetal was removed under vacuum, and the residue was dissolved in absolute ethanol (120 mL). Hydrazine monohydrate (2.65 g, 53 mmol) was added to the ethanolic solution, and the mixture was stirred at room temperature for 4 h, then the solvent was removed under vacuum. The crude product was purified by column chromatography (silica gel, ethyl acetate–hexane 1:2 v/v) to yield pure **14** (4.92 g, 62%) as red sticky oil; IR ν/cm^{-1}: 3161, 2938, 1595, 1454, 1285, 1155, 1113, 1062, 837; ^1H NMR ($CDCl_3$): δ 2.29 (s, 3H), 3.71 (s, 3H), 6.74 (s, 2H), 6.82 (s, 1H), 7.25 (d, J = 8.3 Hz, 1H), 7.55 (d, J = 8.3 Hz, 1H), 7.68 (s, 1H), 8.38 (s, 1H), 12.25 (s, 1H); ^{13}C NMR ($CDCl_3$): 21.53, 55.50, 110.99, 114.96, 115.19, 121.30, 123.93, 128.18, 131.31, 138.10, 140.29, 148.56, 149.33, 159.90.

4.2.8. General procedure for the synthesis of compounds 15 and 16

A mixture of compound **14** (4.6 g, 15.35 mmol) and ground K_2CO_3 (10.6 g, 76.73 mmol) in acetone (100 mL) was stirred under reflux for 2 h. To the reaction mixture was added iodoacetonitrile (1.34 mL, 18.42 mmol) dropwise. Heating and stirring were maintained for two more hours, after which acetone was removed under vacuum, and the residue was partitioned between water (200 mL) and ethyl acetate (200 mL). The aqueous layer was further extracted with ethyl acetate (100 mL), and then the combined organics were dried over anhydrous $MgSO_4$ and evaporated under vacuum. The crude product was purified by column chromatography (silica gel, ethyl acetate–hexane 2:3 v/v) to give an oily mixture of compounds **15** and **16** in an approximate ration of (2:1), respectively, that has been used in the next step without separation of the two isomers (Yield: 4.16 g, 80%).

4.2.8.1. 2-(4-(6-Chloropyridin-3-yl)-3-(3-methoxy-5-methylphenyl)-1H-pyrazol-1-yl)acetonitrile (15). IR ν/cm^{-1}: 2964, 2835, 1595, 1544, 1464, 1434, 1262, 1166, 1100, 1061, 1031, 803; ^1H NMR ($CDCl_3$): δ 2.28 (s, 3H), 3.66 (s, 3H), 5.16 (s, 2H), 6.71 (s, 2H), 6.84 (s, 1H), 7.24 (d, J = 8.3 Hz, 1H), 7.49 (dd, J = 2.3, 5.9 Hz, 1H), 7.70 (s, 1H), 8.32 (s, 1H); ^{13}C NMR ($CDCl_3$): 21.57, 39.89, 55.23, 110.80, 113.76, 115.28, 117.76, 121.47, 124.02, 127.24, 129.92, 132.57, 138.58, 140.12, 148.71, 149.92, 151.17, 159.69, 162.33.

4.2.9. General procedure for the synthesis of compounds 17a–c and 18a–c

The same procedure used for the preparation of compounds **9a–g** and **10a–g** was applied here starting with an un-isolated mixture of compounds **15** and **16**.

4.2.9.1. 2-(3-(3-Methoxy-5-methylphenyl)-4-(6-(pyridin-3-yl)pyridin-3-yl)-1H-pyrazol-1-yl)acetonitrile (17a). Preparative TLC separation was carried out using (ethyl acetate–hexane 3:1 v/v). Yield: (124 mg, 55%); mp 66–67 °C; IR ν/cm^{-1}: 2936, 2835, 1594, 1562, 1464, 1440 1416, 1292, 1166, 1062, 848, 808, 707; ^1H NMR ($CDCl_3$): δ 2.27 (s, 3H), 3.67 (s, 3H), 5.16 (s, 2H), 6.71 (s, 1H), 6.76 (s, 1H), 6.90 (s, 1H), 7.36–7.40 (m, 1H), 7.62–7.69 (m, 2H), 7.73 (s, 1H), 8.29 (d, J = 7.7 Hz, 1H), 8.62–8.65 (m, 2H), 9.19 (s, 1H); ^{13}C NMR ($CDCl_3$): 21.51, 39.85, 55.18, 110.91, 113.73, 115.30, 118.78, 119.96, 121.57,

123.62, 127.41, 129.76, 132.92, 134.10, 134.35, 136.58, 139.97, 148.09, 149.28, 149.93, 151.30, 153.13, 159.71.

4.2.9.2. 2-(4-(6-(2-Acetylphenyl)pyridin-3-yl)-3-(3-methoxy-5-methylphenyl)-1H-pyrazol-1-yl)acetonitrile (17b). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:2 v/v). Yield: (120 mg, 48%); mp 176–177 °C; IR ν/cm^{-1}: 3128, 2977, 2922, 1681, 1594, 1551, 1436, 1418, 1170, 1154, 1066, 843, 762, 594; ^{1}H NMR ($CDCl_3$): δ 2.25 (s, 3H), 2.28 (s, 3H), 3.70 (s, 3H), 5.14 (s, 2H), 6.72 (s, 1H), 6.76 (s, 1H), 6.89 (s, 1H), 7.44–7.65 (m, 6H), 7.70 (s, 1H), 8.54 (s, 1H); ^{13}C NMR ($CDCl_3$): 21.51, 30.58, 39.79, 55.19, 110.80, 113.82, 115.30, 118.66, 121.54, 121.89, 127.06, 127.63, 128.80, 128.96, 130.02, 130.36, 132.92, 136.56, 138.15, 139.93, 141.68, 148.55, 151.23, 155.76, 159.71, 204.39.

4.2.9.3. 2-(4-(6-(3-Acetylphenyl)pyridin-3-yl)-3-(3-methoxy-5-methylphenyl)-1H-pyrazol-1-yl)acetonitrile (17c). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:2 v/v). Yield: (190 mg, 76%); mp 73–74 °C; IR ν/cm^{-1}: 2937, 1683, 1595, 1564, 1423, 1293, 1242, 1166, 1065, 847, 802, 692; ^{1}H NMR ($CDCl_3$): δ 2.27 (s, 3H), 2.65 (s, 3H), 3.66 (s, 3H), 5.16 (s, 2H), 6.71 (s, 1H), 6.77 (s, 1H), 6.91 (s, 1H), 7.54 (t, J = 7.7 Hz, 1H), 7.60–7.72 (m, 3H), 8.97 (d, J = 7.5 Hz, 1H), 8.19 (d, J = 7.5 Hz, 1H), 8.58 (s, 1H), 8.63 (s, 1H); ^{13}C NMR ($CDCl_3$): 21.55, 26.83, 39.84, 55.19, 110.91, 113.92, 115.23, 118.77, 120.03, 121.58, 126.53, 127.18, 128.77, 129.14, 129.93, 131.22, 133.02, 136.57, 137.63, 139.31, 139.97, 149.01, 151.22, 154.56, 159.68, 198.12.

4.2.9.4. 2-(5-(3-Methoxy-5-methylphenyl)-4-(6-(pyridin-3-yl) pyridin-3-yl)-1H-pyrazol-1-yl)acetonitrile (18a). Preparative TLC separation was carried out using (ethyl acetate–hexane 3:1 v/v). Yield: (69 mg, 61%); mp 64–65 °C; IR ν/cm^{-1}: 2923, 2835, 1594, 1465, 1418, 1196, 1168, 1156, 849, 811, 704; ^{1}H NMR ($CDCl_3$): δ 2.37 (s, 3H), 3.79 (s, 3H), 4.94 (s, 2H), 6.71 (s, 1H), 6.77 (s, 1H), 6.88 (s, 1H), 7.36–7.40 (m, 1H), 7.60–7.67 (m, 2H), 7.92 (s, 1H), 8.27 (d, J = 7.7 Hz, 1H), 8.60–8.62 (m, 2H), 9.15 (s, 1H); ^{13}C NMR ($CDCl_3$): 21.60, 37.81, 55.41, 112.42, 114.14, 116.71, 118.30, 120.26, 122.69, 123.62, 127.25, 128.99, 134.09, 134.43, 135.01, 139.48, 141.21, 141.39, 147.98, 148.25, 149.80, 152.64, 160.40.

4.2.9.5. 2-(4-(6-(2-Acetylphenyl)pyridin-3-yl)-5-(3-methoxy-5-methylphenyl)-1H-pyrazol-1-yl)acetonitrile (18b). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:2 v/v). Yield: (96 mg, 77%); mp 72–73 °C; IR ν/cm^{-1}: 2924, 1689, 1594, 1355, 1243, 1168, 1155, 847, 760, 700, 593; ^{1}H NMR ($CDCl_3$): δ 2.18 (s, 3H), 2.36 (s, 3H), 3.78 (s, 3H), 4.95 (s, 2H), 6.68 (s, 1H), 6.75 (s, 1H), 6.86 (s, 1H), 7.43–7.59 (m, 6H), 7.91 (s, 1H), 8.49 (s, 1H); ^{13}C NMR ($CDCl_3$): 21.55, 30.49, 37.81, 55.38, 112.37, 114.78, 116.72, 118.33, 122.13, 122.67, 126.78, 127.62, 128.64, 128.92, 128.99, 130.26, 134.89, 138.22, 139.49, 141.23, 141.33, 141.58, 147.50, 155.33, 160.40, 204.23.

4.2.9.6. 2-(4-(6-(3-Acetylphenyl)pyridin-3-yl)-5-(3-methoxy-5-methylphenyl)-1H-pyrazol-1-yl)acetonitrile (18c). Preparative TLC separation was carried out using (ethyl acetate–hexane 1:2 v/v). Yield: (100 mg, 80%); mp 149–150 °C; IR ν/cm^{-1}: 3436, 2943, 1686, 1595, 1463, 1355, 1245, 1173, 1028, 852, 810, 701; ^{1}H NMR ($CDCl_3$): δ 2.37 (s, 3H), 2.67 (s, 3H), 3.78 (s, 3H), 4.95 (s, 2H), 6.71 (s, 1H), 6.77 (s, 1H), 6.87 (s, 1H), 7.51–7.70 (m, 3H), 7.92 (s, 1H), 7.97 (d, J = 7.6 Hz, 1H), 8.16 (d, J = 7.8 Hz, 1H), 8.53 (s, 1H), 8.57 (d, J = 1.2 Hz, 1H); ^{13}C NMR ($CDCl_3$): 21.61, 26.82, 37.80, 55.41, 112.41, 114.22, 116.66, 118.39, 120.33, 122.71, 126.53, 126.96, 128.67, 129.04, 129.09, 131.18, 135.01, 137.58, 139.36, 139.48, 141.15, 141.36, 147.96, 154.21, 160.37, 198.06.

4.2.10. General procedure for the synthesis of compounds 19a–c and 20a,b

The same procedure used for the preparation of compounds **11a–e** and **11a–e** was applied here starting with the appropriate methoxy compounds **17a–c** and **18a–c**.

4.2.10.1. 2-(3-(3-Hydroxy-5-methylphenyl)-4-(6-(pyridin-3-yl)pyridin-3-yl)-1H-pyrazol-1-yl)acetonitrile (19a). Column chromatography (silica, acetone–ethyl acetate 1:50 v/v). Yield: (27 mg, 61%); mp 258–259 °C; IR ν/cm^{-1}: 3429, 3121, 2993, 2948, 2646, 1596, 1567, 1419, 1178, 859, 806, 698; ^{1}H NMR (DMSO-d_6): δ 2.31 (s, 3H), 5.60 (s, 2H), 6.59 (s, 2H), 6.73 (s, 1H), 7.49–7.54 (m, 1H), 7.74 (d, J = 7.5 Hz, 1H), 8.06 (d, J = 8.2 Hz, 1H), 8.25 (s, 1H), 8.45 (d, J = 7.8 Hz, 1H), 8.61 (s, 2H), 9.29 (s, 1H), 9.39 (s, 1H); ^{13}C NMR (DMSO-d_6): 21.53, 112.66, 116.34, 116.37, 117.27, 120.01, 120.71, 124.29, 128.23, 132.36, 133.80, 134.03, 134.16, 136.89, 139.61, 148.07, 149.12, 150.28, 150.32, 152.24, 157.74.

4.2.10.2. 2-(4-(6-(2-Acetylphenyl)pyridin-3-yl)-3-(3-hydroxy-5-methylphenyl)-1H-pyrazol-1-yl)acetonitrile (19b). Column chromatography (silica, ethyl acetate–hexane 1:1 v/v). Yield: (38 mg, 77%); mp 108–109 °C; IR ν/cm^{-1}: 3116, 3059, 2924, 1684, 1596, 1436, 1298, 1165, 849, 761, 735; ^{1}H NMR ($CDCl_3$): δ 2.24 (s, 3H), 2.39 (s, 3H), 5.13 (s, 2H), 6.39 (s, 1H), 6.58 (s, 1H), 6.99 (s, 1H), 7.46–7.66 (m, 6H), 7.70 (s, 1H), 8.46 (s, 1H); ^{13}C NMR ($CDCl_3$): 21.38, 29.89, 39.77, 112.53, 113.80, 116.70, 118.53, 120.26, 122.31, 127.24, 128.01, 128.85, 129.05, 129.83, 130.97, 132.38, 137.25, 138.70, 140.20, 140.65, 148.32, 151.07, 156.37.

4.2.10.3. 2-(4-(6-(3-Acetylphenyl)pyridin-3-yl)-5-(3-hydroxy-5-methylphenyl)-1H-pyrazol-1-yl)acetonitrile (19c). Column chromatography (silica, ethyl acetate–hexane 1:1 v/v). Yield: (37 mg, 75%); mp 100–101 °C; IR ν/cm^{-1}: 3418, 3121, 1682, 1598, 1425, 1299, 1244, 1166, 850, 803, 693; ^{1}H NMR ($CDCl_3$): δ 2.31 (s, 3H), 2.65 (s, 3H), 4.96 (s, 2H), 6.55 (s, 1H), 6.67 (s, 1H), 6.79 (s, 1H), 7.55–7.74 (m, 3H), 7.90 (s, 1H), 7.98 (d, J = 7.5 Hz, 1H), 8.10 (d, J = 7.3 Hz, 1H), 8.49 (br s, 2H); ^{13}C NMR ($CDCl_3$): 21.45, 26.81, 37.75, 113.82, 114.03, 117.46, 118.45, 121.49, 121.74, 126.91, 127.88, 128.48, 129.38, 131.48, 136.59, 137.63, 139.40, 141.53, 141.68, 146.30, 153.28, 157.69, 198.13.

4.2.10.4. 2-(4-(6-(2-Acetylphenyl)pyridin-3-yl)-5-(3-hydroxy-5-methylphenyl)-1H-pyrazol-1-yl)acetonitrile (20a). Column chromatography (silica, ethyl acetate–hexane 1:1 v/v). Yield: (21 mg, 48%); mp 142–143 °C; IR ν/cm^{-1}: 3099, 3064, 2925, 2853, 1684, 1597, 1356, 1303, 1250, 1160, 860, 765, 732; ^{1}H NMR ($CDCl_3$): δ 2.23 (s, 3H), 2.27 (s, 3H), 4.92 (s, 2H), 6.45 (s, 1H), 6.62 (s, 1H), 6.71 (s, 1H), 7.46–7.63 (m, 6H), 7.88 (s, 1H), 8.41 (s, 1H); ^{13}C NMR ($CDCl_3$): 21.37, 29.95, 37.78, 113.96, 114.07, 117.74, 118.48, 121.59, 122.25, 127.46, 127.52, 128.32, 128.62, 129.22, 130.69, 136.18, 137.35, 139.38, 141.22, 141.39, 141.60, 146.14, 154.62, 157.66.

4.2.10.5. 2-(4-(6-(3-Acetylphenyl)pyridin-3-yl)-3-(3-hydroxy-5-methylphenyl)-1H-pyrazol-1-yl)acetonitrile (20b). Column chromatography (silica, ethyl acetate–hexane 1:1 v/v). Yield: (35 mg, 72%); mp 103–105 °C; IR ν/cm^{-1}: 3429, 2958, 2926, 1683, 1596, 1426, 1359, 1301, 1244, 850, 804, 700; ^{1}H NMR ($CDCl_3$): δ 2.30 (s, 3H), 2.60 (s, 3H), 5.12 (s, 2H), 6.60 (s, 1H), 6.69 (s, 1H), 7.03 (s, 1H), 7.39–7.48 (m, 2H), 7.61–7.64 (m, 2H), 7.79 (d, J = 7.6 Hz, 1H), 7.95 (d, J = 7.5 Hz, 1H), 8.30 (s, 1H), 8.50 (s, 1H), 8.84 (s, 1H); ^{13}C NMR ($CDCl_3$): 21.48, 26.78, 39.82, 112.17, 113.66, 117.03, 118.13, 120.49, 120.77, 126.74, 127.72, 129.11, 130.06, 131.26, 132.48, 137.49, 137.58, 138.19, 140.74, 147.90, 151.07, 154.30, 156.70, 198.22.

4.3. Cell-line screening

Cell-line screening was applied at the National Cancer Institute (NCI), Bethesda, Maryland, USA,[19] applying the following procedure. The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96-well microtiter plates in 100 μL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/mL gentamicin. Additional four, 10-fold or 1/2 log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 μL of these different drug dilutions are added to the appropriate microtiter wells already containing 100 μL of medium, resulting in the required final drug concentrations. Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μL of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

$[(Ti - Tz)/(C - Tz)] \times 100$ for concentrations for which $Ti \geqslant Tz$

$[(Ti - Tz)/Tz] \times 100$ for concentrations for which $Ti < Tz$.

Three dose–response parameters are calculated for each experimental agent. Growth inhibition of 50% (IC_{50}) is calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from $Ti = Tz$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(Ti - Tz)/Tz] \times 100 = -50$. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested.

4.4. Enzyme screening

Kinase assays were performed at Reaction Biology Corporation using the 'HotSpot' assay platform.[20,21] Kinase Assay Protocol. Reaction Buffer: base Reaction buffer; 20 mM Hepes (pH 7.5), 10 mM $MgCl_2$, 1 mM EGTA, 0.02% Brij35, 0.02 mg/mL BSA, 0.1 mM Na_3VO_4, 2 mM DTT, 1% DMSO. Reaction procedure: To a freshly prepared buffer solution was added any required cofactor for the enzymatic reaction, followed by the addition of the selected kinase at a concentration of 20 μM. The contents were mixed gently, and then the compound under test (compound **10f**) dissolved in DMSO was added to the reaction mixture in the 10 μM concentration. 339-ATP (specific activity 500 μCi/μL) was added to the mixture in order to initiate the reaction, and the mixture was incubated at room temperature for 2 h. Staurosporine was used as a control compound in a five-dose IC_{50} mode with 10-fold serial dilutions starting at 20 μM, and the reaction was carried out at 10 μM ATP concentration.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.04.029.

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