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Synthesis and biological evaluation of new pyrazol-4-ylpyrimidine derivatives as potential ROS1 kinase inhibitors

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ABSTRACT

With the aim of discovering potent and selective kinase inhibitors targeting ROS1 kinase, we designed, synthesized and screened a series of new pyrazol-4-ylpyrimidine derivatives based on our previously discovered lead compound **KIST301072**. Compounds **6a**–**e** and **7a**–**e** showed good to excellent activities against ROS1 kinase, and seven out of tested compounds were more potent than **KIST301072**. Compound **7c** was the most potent with IC_{50} of 24 nM. Moreover, compound **7c** showed ROS1 inhibitory selectivity of about 170-fold, relative to that of ALK sharing about 49% amino acid sequence homology with ROS1 kinase in the kinase domain. *In silico* modeling of **7c** at ROS1 active site revealed some essential features for ROS1 inhibitory activity. Based on this study as well as the previous studies, we could build a hypothetical model predicting the required essential features for ROS1 inhibitory activity. The model validity has been tested through a second set of compounds.

1. Introduction

Receptor tyrosine kinases (RTKs) are important players in the process of signal transduction and cellular communication. They act as the cell surface receptors for a number of important growth factors and hormones [1]. In addition to the vital role of these RTKs as regulators for normal cellular processes, they also have another dark side presented by their key roles in the initiation and progression of a number of cancers. In these types of cancers, gene translocations resulting in kinase fusion proteins with constitutive and uncontrolled activity are common [2,3].

The normal functions of human ROS1 kinase in different body tissues have not been fully identified. However, the ectopic expression as well as the production of variable mutant forms of ROS1 kinase have been reported in a number of cancers, such as glioblastoma multiforme, cholangiocarcinoma, gastric adenocarcinoma and non-small cell lung cancer, suggesting a role for ROS1 kinase in such tumors. It is thought also that c-ROS gene may have a role in some cardiovascular diseases, and the fact that homozygous male mice targeted against c-ROS gene are healthy but infertile, has inspired researchers to think about ROS inhibition as a method for development of new male contraceptives [4].

AP26113, TAE684 and Foretinib as well as Crizotinib have been developed as ALK inhibitors, and most of the compounds have simultaneously demonstrated excellent *in vitro* inhibitory activities against ROS1 kinase (Fig. 1). The reason has been inferred because ROS1 kinase shares 49% amino acid sequence homology with ALK in the kinase domain [5–7]. Crizotinib, which has been launched as ALK inhibitor by FDA on 2011, was executed for a Phase I trial of ROS1-positive lung cancer patient [8].

Few compounds that are active against ROS1 kinase but inactive against ALK have been reported. The first selective inhibitors for ROS1 RTK versus 45 kinases have been recently developed by our research group, where **KIST301072** with an IC_{50} of 199 nM was initially discovered [9], followed by the development of a simplified and equipotent derivative with an IC_{50} value of 209 nM [10]. These two compounds represented promising new leads for the development of more potent and more selective ROS1 kinase inhibitors, and following this discovery, it was important to explore the new chemical scaffold, in order to have a clear picture about the structure activity relationship (SAR) of the new group of ROS1 kinase inhibitors. The first study emphasized the importance of the

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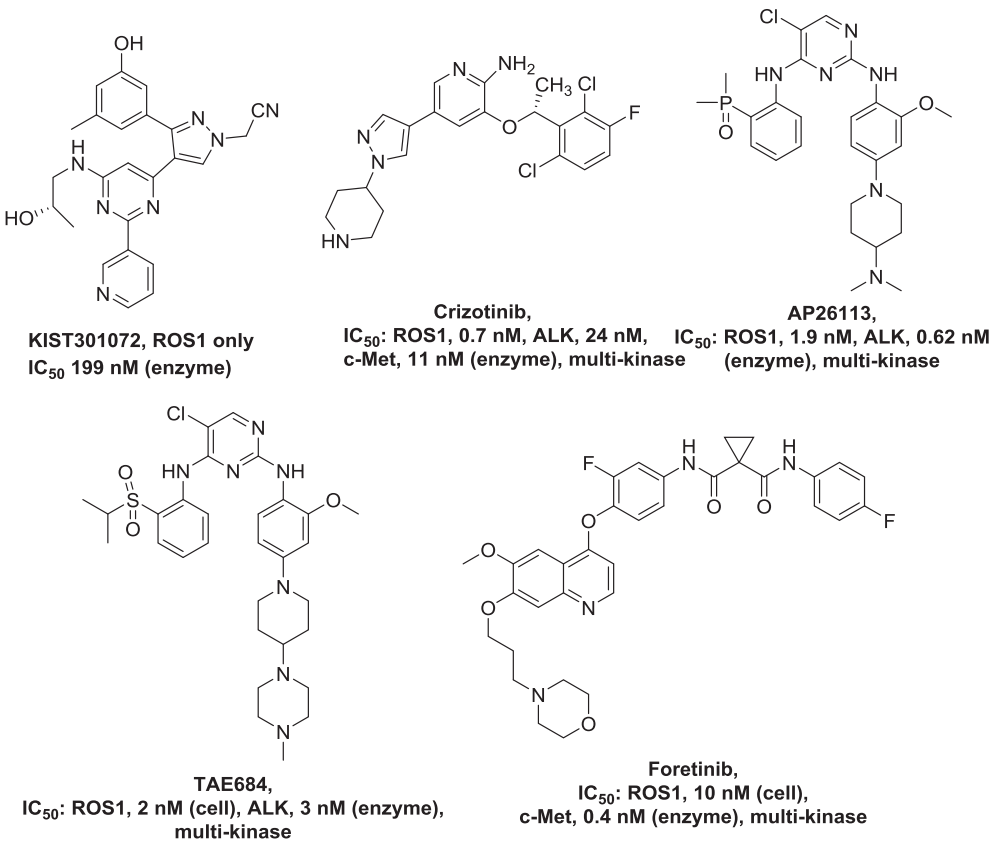


Fig. 1. Structure of the potential ROS1 kinase inhibitors.

acetonitrile group at the pyrazole ring and also the importance of having a hydrogen bond donor on the distal phenyl ring linked to the pyridine moiety [11]. The other study has showed that pyrazole moiety and the size of the substituent carried by the distal phenylpyridine moiety is critical for activity [12].

In order to study the effect of the polar side chain in this new group of compounds, a first set of compounds was synthesized and screened *in-vitro* against ROS1 kinase. Also, to study their selectivity on ROS1 kinase, the synthesized derivatives **6a**–**e** and **7a**–**e** were screened *in-vitro* against ALK and c-Met, and screened against HCC78 cell line, which expresses the SLC34A2–ROS1 fusion [13].

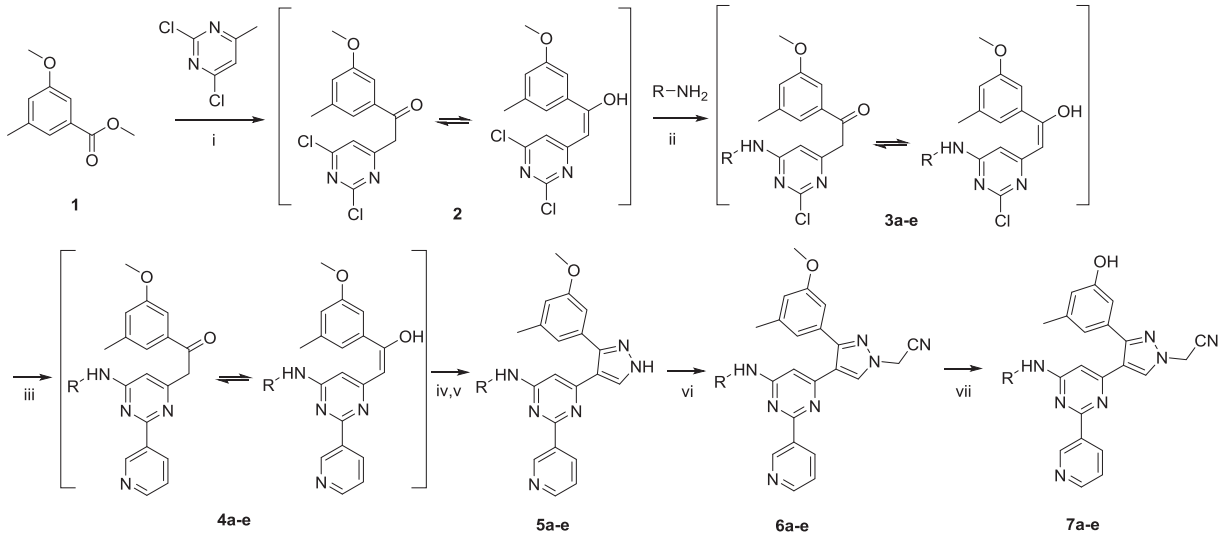
Based on this set of compounds as well as our previous studies, we could develop a hypothetical model predicting the required essential features for ROS1 inhibitory activity. To test the model validity, a second set of compounds has been designed, synthesized and screened against ROS1 kinase.

2. Results and discussion

2.1. Chemistry

The compounds were synthesized following the approach reported previously (Scheme 1) [9–12]. Methyl 3-methoxy-5-methylbenzoate (**1**) was synthesized by condensation of diethyl oxalate with acetone in the presence of sodium ethoxide in absolute ethanol [14–16]. The benzoate ester **1** underwent a nucleophilic attack at its carboxylic carbon by lithiated 2,4-dichloro-6-methylpyrimidine, obtained by dropwise addition of lithium bis(-trimethylsilyl)amide (LHMDS) in dry THF at 0 °C. The resulting tautomeric α,β-unsaturated ketone **2** was then subjected to a nucleophilic substitution reaction with the amino group of the appropriate amine by heating at 80 °C in dry THF for 3 h. Compounds **4a**–**e** were prepared by Suzuki coupling of compounds **3a**–**e** with 3-pyridineboronic acid, in the presence of dichloro bis(triphenylphosphine)Pd(II) and potassium carbonate, in a mixed solvent of THF, acetonitrile and water (2:1:1, v/v/v). The conversion of the resulted tautomeric products **4a**–**e** to the pyrazole derivatives **5a**–**e** was achieved through two successive steps. In the first step, compounds **4a**–**e** were heated with excess *N,N*-dimethylformamide dimethylacetal for 2 h, and the resulting product was taken to the next step without further purification, where it was cyclized with hydrazine monohydrate in absolute ethanol into the pyrazole derivatives **5a**–**e**. The reaction of the resulting pyrazoles **5a**–**e** with iodoacetonitrile in the presence of excess cesium carbonate produced two different regioisomers. The intended *1H*-pyrazole isomers **6a**–**e** were the major products of the reaction with lower R_f values, while the other *2H*-pyrazole isomers were produced as minor products with higher R_f values. The required isomers **6a**–**e** were separated in a pure form by column chromatography. This was proved by observing the 2D-NOESY NMR spectrum of compound **6b** and its *2H*-pyrazole isomer (Supplementary data), as representatives of the all regioisomers. In 2D-NOESY NMR spectrum of compound **6b**, there is no cross peak that might indicate through space interaction between the $-CH_2-$ protons of the acetonitrile group and any of the aromatic protons of the 3-methoxy-5-methylphenyl group.

2D-NOESY NMR spectrum of *2H*-pyrazole isomer shows the presence of such cross-peaks, since the acetonitrile group is close enough to the aromatic protons of 3-methoxy-5-methylphenyl group to exhibit NOE effect as clarified in Fig. 2. Furthermore, the expected higher shielding of the two protons of the acetonitrile $-CH_2-$ in the *2H*-pyrazole isomer caused by the anisotropic effect

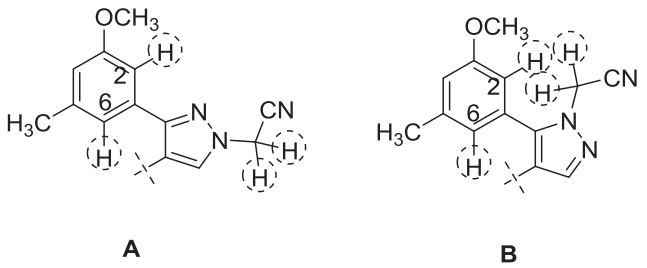


Scheme 1. Reaction conditions and reagents: (i) LHMDS, THF, N_2, 0 °C - rt, 24 h; (ii) THF, 80 °C, 3 h; (iii) 3-pyridineboronic acid, $Pd(PPh_3)_2Cl_2$, K_2CO_3, N_2,THF/CH_3CN/H_2O (2:1:1), 90 °C, 3 h; (iv) DMF-DMA, 90 °C, 2 h; (v) hydrazine hydrate, abs. EtOH, rt, 2 h; (vi) Cs_2CO_3, iodoacetonitrile, DMF, rt,16 h; (vii) $BF_3 \cdot S(CH_3)_2$, dichloromethane, N_2, rt, 12 h.

of the nearby aromatic electron cloud, was proved in the selected representative isomers as shown in the Supplementary data. In the *2H*-pyrazole isomers, such protons appeared in around 4.95 ppm, while in the *1H*-pyrazole isomers, these protons were more shifted downfield and appeared in at 5.20 ppm. The final hydroxyl products **7a**–**e** was obtained by demethylation of the methoxy group of the corresponding compounds **6a**–**e** using 10 equivalent of borontrifluoride–dimethylsulfide complex in dichloromethane [9].

Scheme 2 presents the synthesis of compounds **19**–**22**. First, 2,4-dichloro-6-methylpyrimidine underwent a nucleophilic substitution reaction with the amino group of 3-methoxypropylamine. Two regioisomers, the 2- and 4- substituted pyrimidine, were produced from this step. The major isomer was the 2-substiuted pyrimidine (**8**). Compound **8** was then subjected to Suzuki coupling with either 3-pyridineboronic acid or phenylboronic acid to give compounds **13** and **18**, respectively. Compound **13** was activated at its methyl group using LHMDS to react with the benzoic acid esters **11** and **12** resulting mainly in the enol isomers **14** and **15**, respectively. Similarly, compound **18** reacted with the benzoic acid esters **11** and **12** to give compounds **19** and **20**, respectively. In two steps, compounds **14**, **15** and **19**, **20** were cyclized to the target pyrimidines. In the first step, compounds **14**, **15** and **19**, **20** were heated with excess *N,N*-dimethylformamide dimethylacetal for 2 h, and the resulted product was taken to the next step without further purification, where it was cyclized according to the reported procedure [17] with acetamidine and sodium ethoxide in absolute ethanol into the pyrimidine derivatives **16**, **17** and **21**, **22**, respectively.

Scheme 3 presents the synthesis of the target compounds **28** and **29**. First, 2-chloro-4-methylpyridine was activated at its methyl group using LHMDS to react with the benzoic acid esters **11** and **12** resulting mainly in the ketone isomers **24** and **25**, respectively. Compounds **24** and **25** were subjected to cyclization in two successive steps as it was done in Scheme 2 to give **26** and **27**, respectively. Finally, through Suzuki coupling, compounds **26** and **27** were coupled with 3-pyridineboronic acid to give compounds **28** and **29**, respectively.



A

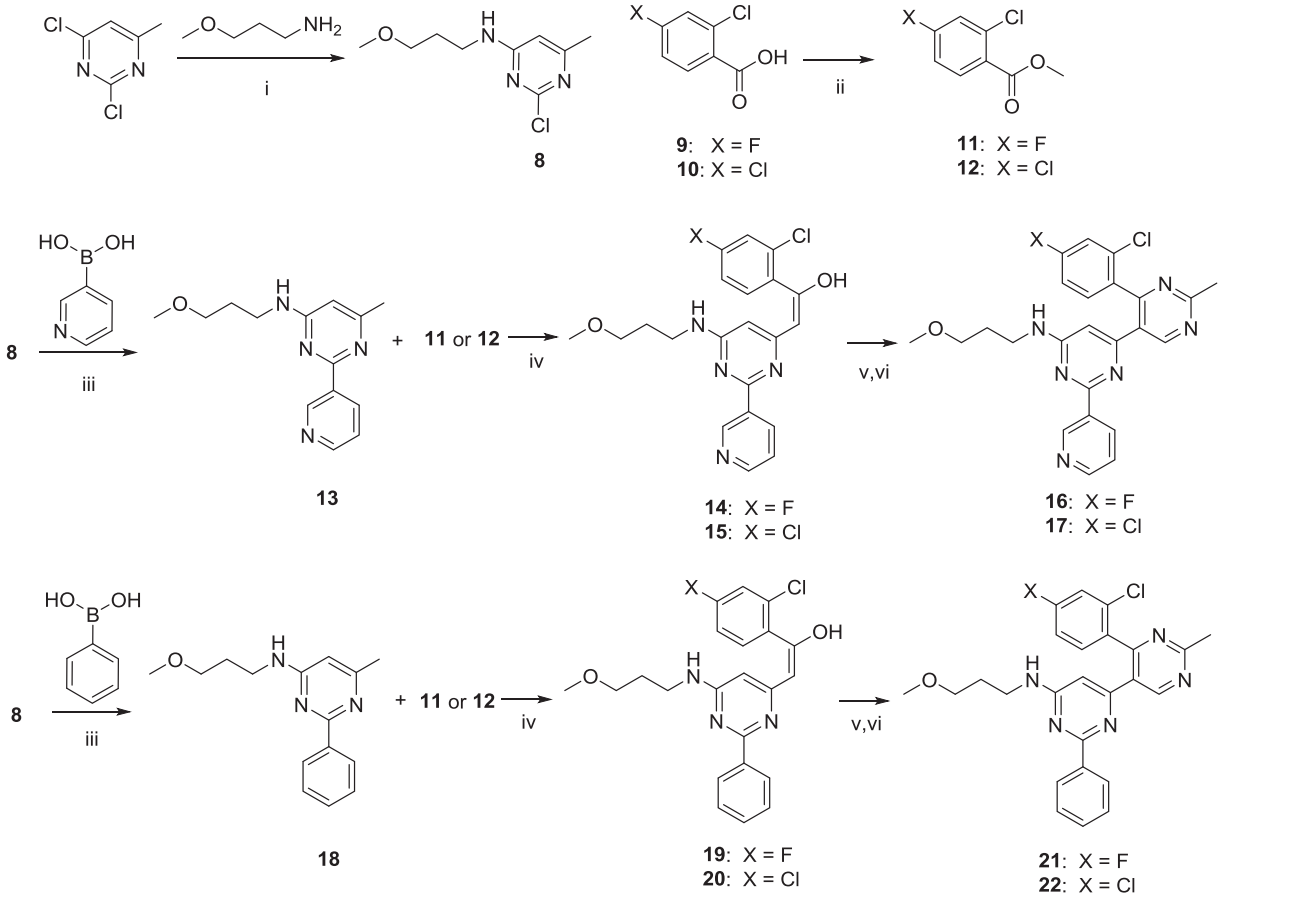
B

A: The distance between acetonitrile -CH_2- and the aromatic protons doesn't allow for NOE interaction in compound **6b**; B: NOE effect between acetonitrile -CH_2- and the aromatic protons in the *2H*-pyrazole isomer of compound **6b**.

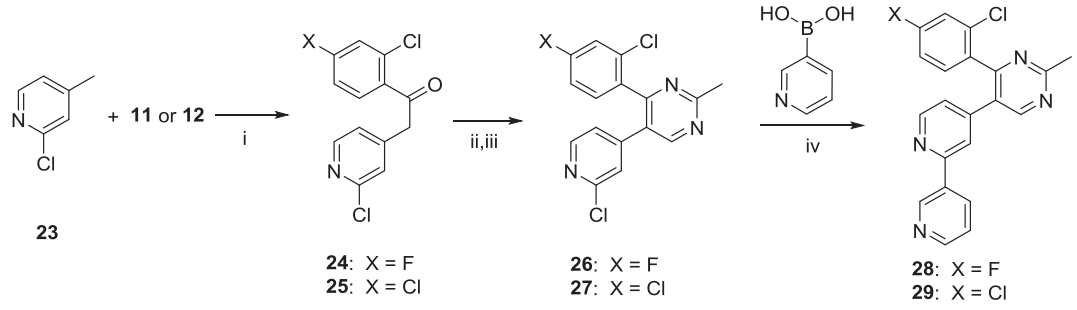
Fig. 2. NOE interactions in compound **6b** and its *2H*-pyrazole isomer.

2.2. Biological activity of pyrazole compounds *6a–e* and *7a–e*

By referring to the IC_{50}s of the tested compounds summarized in Table 1, and correlating between structural variations and the resulted ROS1 inhibitory activity, we can conclude that a polar substitution at position 6 of the pyrimidine ring gives higher activity. These polar groups might act as solvent exposed moieties within ROS1 kinase. Among the compounds tested, the eight compounds with the polar substituent were most active, and seven of them are more active than the lead compound. There are five compounds have a hydroxyl group at position 3 of the phenyl group, while the other five have a methoxy group. The hydroxyl forms of compounds **7a**, 7**b**, and 7**c** were found to have higher activity than their methoxy analogs **6a**, **6b** and **6c**. While in compounds **6d**, **6e** and their corresponding hydroxyl analogs **7d** and 7**e**, the activity of methoxy derivatives was found to be higher than that of the hydroxyl derivatives. It is also worthy to mention that one methylene group homologation in each couple of these compounds **6b** and **6c**, **6d** and **6e**, **7b** and **7c**, and **7d** and **7e**, respectively, lead to significant increase in ROS1 inhibitory activity. To test the selectivity of the synthesized compounds, we screened them against two kinases ALK and c-Met as shown in Table 1. The compounds did not show any inhibitory activity against c-Met and showed very low



Scheme 2. Reaction conditions and reagents: (i) THF, TEA, 80 °C, 3 h; (ii) CH_3OH, H_2SO_4, reflux, 10 h; (iii) $Pd(PPh_3)_2Cl_2$, K_2CO_3, N_2, $THF/CH_3CN/H_2O$ (2:1:1), 90 °C, 5 h; (iv) LHMDS, THF, N_2, 0 °C to rt, 12 h; (v) DMF-DMA, 90 °C, 2 h; (vi) acetamidine, NaOEt, EtOH, rt, 6 h.



Scheme 3. Reaction conditions and reagents: (i) LHMDS, THF, N_2, rt, 3 h; (ii) DMF-DMA, 90 °C, 2 h; (iii) acetamidine, NaOEt, EtOH, rt, 6 h; (iv) $Pd(PPh_3)_2Cl_2$, K_2CO_3, N_2, $THF/CH_3CN/H_2O$ (2:1:1), 90 °C, 5 h.

activity on ALK. Also the compounds **6a**–**e** and **7a**–**e** were screened against HCC78 cell line, which expresses the SLC34A2–ROS1 fusion, and showed cell-based activities between 13.6 and 90.9 μM.

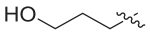
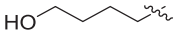
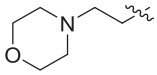
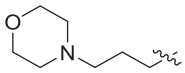
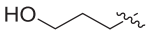
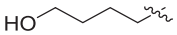
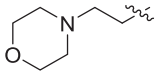
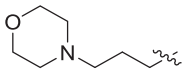
We can conclude that a small change in structure of the polar side chain has led to significant improvement in ROS1 inhibitory activity in this series of compounds. These polar side chains might have a role as solvent exposure moieties.

2.3. Docking studies

The 2.2 Å cocrystal structure of crizotinib with the phosphorylated ROS1-kinase domain describes how the inhibitor binds in the ATP-binding site [18]. ROS1 binds crizotinib at the ATP-binding site in the cleft between the N-terminal and C-terminal domains of the kinase. The G2032 residue sits at the solvent front in the distal end of the kinase hinge and creates a turn, putting the G2032 α-carbon in position to engage in a Van der Waals interaction with the pyrazole ring of crizotinib [18]. The hinge interactions include a hydrogen bond between the pyridine and of crizotinib and the backbone nitrogen of Met2029 (Fig. 3). Remarkably, this is the only direct polar interaction observed between ROS1-kinase domain and crizotinib [18].

In order to gain some insights into the interaction between the synthesized inhibitors and ROS1 kinase, we performed docking simulation in the active site of ROS1 kinase using MOE.2008.10 software.

Table 1
The IC_{50} values of the target compounds **6a–e** and **7a–e** against ROS1, ALK and c-Met kinases.

Compd no.	R	ROS1 (IC_{50}, μM)	ALK (IC_{50}, μM)	c-Met (IC_{50}, μM)	HCC78 cell (IC_{50}, μM)
6a		0.920	Not tested	No inhibition	not tested
6b		0.119	4.32	No inhibition	50.9
6c		0.056	1.92	No inhibition	33.9
6d		0.148	10.2	No inhibition	57.6
6e		0.088	6.22	No inhibition	28.4
7a		0.780	Not tested	No inhibition	not tested
7b		0.108	9.72	No inhibition	73.4
7c		0.024	4.15	No inhibition	13.6
7d		0.478	20<	No inhibition	90.9
7e		0.183	17.6	No inhibition	55.5
KIST301072	–	0.199	No inhibition	No inhibition	Not tested
Crizotinib	–	0.0017	0.0006	0.011	0.775

Using the crystal structure ROS1 kinase enzyme co-crystallized with crizotinib (PDB code 3zbf), we found that compound 7**c**, which showed the highest ROS1 kinase inhibitory activity, exhibits similar binding behavior to that of crizotinib. The most stable docking pose of compound **7c** aligned with crizotinib in the binding pocket is showed in Fig. 4.

Compound **7c** occupies a comparable space in the binding pocket, and is overlaid over the main skeleton of crizotinib and in the same orientation of its binding. It is also showing how well it fits into the hinge region through hydrogen bonding (HB) interaction with backbone nitrogen of Met-2029. Moreover, the polar side chain takes a similar orientation to that of piperidine ring in crizotinib. The overall binding interaction of compound **7c** is shown in Fig. 5. It can be observed that, the phenolic hydroxyl group occupies the hydrophobic pocket and makes hydrogen bond with CO backbone of Asp-2102. This interaction has a role such as compound **6c** is twofold less potent than **7c**.

Based on this modeling study, we think that the essential features for ROS1 inhibitory activity in the pyrazol-4-ylpyrimidine scaffold are; hydrogen bond acceptor for hinge interaction, aryl and/or heteroaryl system for hydrophobic interaction, and polar chain to occupy solvent exposure region. These elements might be linked together in the way that appears in this proposed model (Fig. 6).

As an attempt to use the proposed model to explain the relative potencies in Table 1, we record the following points. First; in each couple of these compounds **6b** and **6c**, **6d** and **6e**, **7b** and **7c**, and **7d** and **7e**, respectively, one methylene group homologation lead to increase in activity. This extra methylene group may allow the terminal polar group to be in a better position as solvent exposure. This observation might also be supported by the observation that, compounds **7b** and **7c** are more active than the lead compound. These compounds are homologs to the lead compound that differ only in increasing the length of the side chain, in other words, making their side chain hydroxyl group more terminal than its location in the lead compound. Second; the higher activity of some hydroxyl analogs over their methoxy analogs might be due to extra hydrogen bonding through this hydroxyl group. This may be proved by the binding mode of compound **7c** (Fig. 5).

2.4. Testing of model validity

2.4.1. Testing of model validity on a new set of compounds

To test the model validity and the contribution of each of its features, a second set of six target compounds has been designed, synthesized and screened against ROS1 kinase. All the six compounds share the hydrophobic region, in which the substituted pyrazole was replaced with an isosteric pyrimidine ring, and the phenyl ring has halogen substituents. Three pairs of compounds have been synthesized. The first pair (compounds **16** and **17**) fulfills all the postulated essential features. While the second pair (compounds **21** and **22**) is missing the hydrogen bond acceptor to the

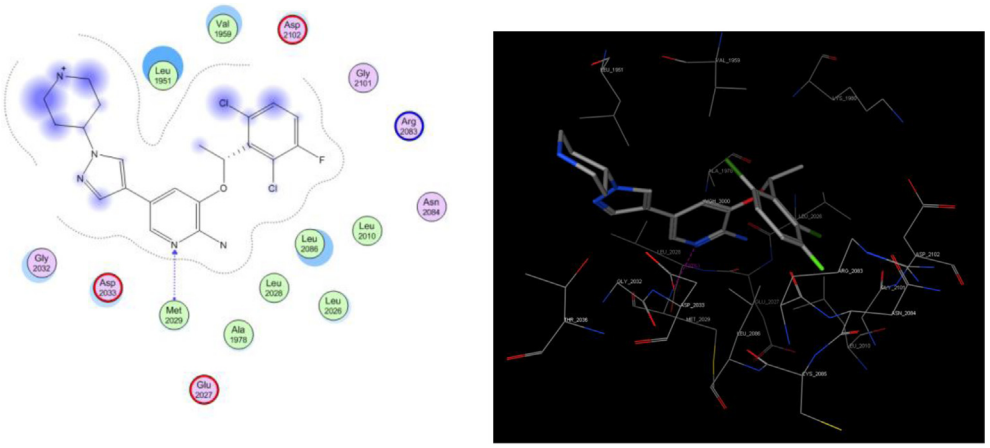
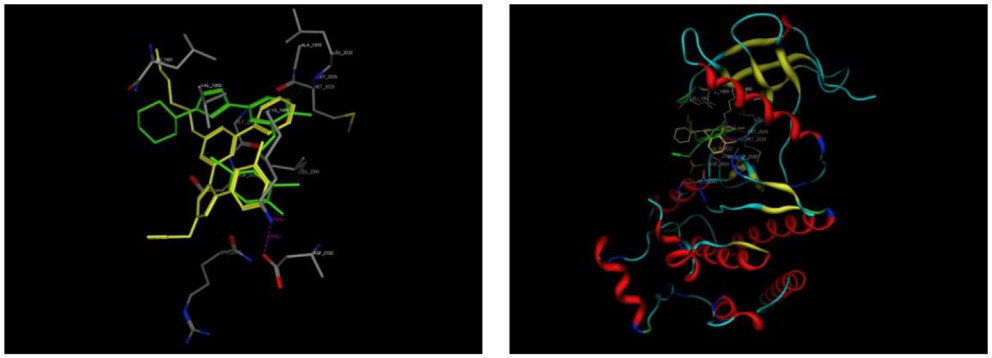


Fig. 3. Binding mode of crizotinib in ROS1 active pocket in 2D and 3D, respectively.



a b

Fig. 4. Overlay of crizotinib (green stick) and compound **7c** (yellow stick) bound to ROS1 active site, close and distant view, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hinge region. Also the third pair (compounds **28** and **29**) is missing the polar side chain.

By referring to the IC_{50}s of the second set of tested compounds summarized in Table 2, it is clear that the absence of the hinge-binding component (compounds **21** and **22**) lead to totally loss of activity. Also, it can be observed that; compound **17**, which fulfills all the proposed features, has a comparable activity to compound **7d** from the first set of tested compounds. But the comparable activity of compound **17** (fulfilling all features) and compounds **28** and **29** (missing polar side chain) makes the role of polar side chain in the potency somewhat unclear. It is also worthy to mention that the four active compounds in this second set (**16**, **17**, **28**, and **29**) are far less active than most active compounds in the first set of compounds (**6c**, **6e**, and **7c**). It is also worth mentioning that comparing the IC_{50}s of compounds **KIST301072**, **7c** and **17** shows the importance of the phenolic group together with the 4-hydroxyl butyl amino side chain.

2.4.2. Testing of model validity on selected compounds from our previous studies

In order to evaluate our studies in a collective way, we used this model to interpret the variation in ROS1 inhibitory activity in the previous studies. The most active compounds from the previous studies were selected for evaluation (Table 3). It is clear that compounds **30** and compound **KIST301072** which fulfills the all proposed features are the most active, while **KIST301080** which lacks the polar side chain still has comparable activity to **KIST301072**. Compounds **31** and **32** are far less active. The apparent reason for that is the absence of hinge-binding moiety.

Testing model validity on both the new compound as well as the compounds from previous studies highlighted some important points. The hinge component and the hydrophobic region seem very essential for ROS1 inhibitory activity within this scaffold. Despite the great improvement in activity of the first set of compounds is due to the polar side chain, yet the presence of other active ROS1 inhibitors lacking this polar chain shows the complex nature of the interactions of this part of the molecule.

3. Conclusion

This study has investigated the effect of polar side chain introduced into the pyrazol-4-ylpyrimidine scaffold of ROS1 inhibitors. Exploring different chains has led to the discovery of potent inhibitors that showed excellent selectivity to ROS1 versus the closely related kinase ALK. Molecular modeling studies showed a proposed binding mode of compound **7c** in ROS1 kinase domain. Based on that, we put a model showing the essential features for the pyrazol-4-ylpyrimidine ROS1 inhibitors. The model validity has been tested through a second set of compounds as well as the most active compounds from the previous studies. Model validity testing showed the essential role of the hinge component and the

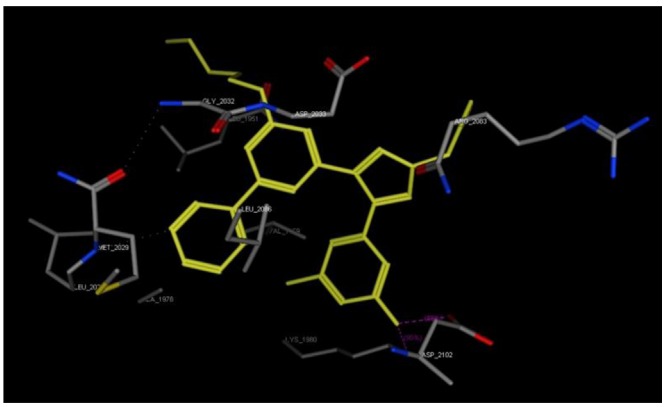


Fig. 5. Binding mode of compound **7c**.

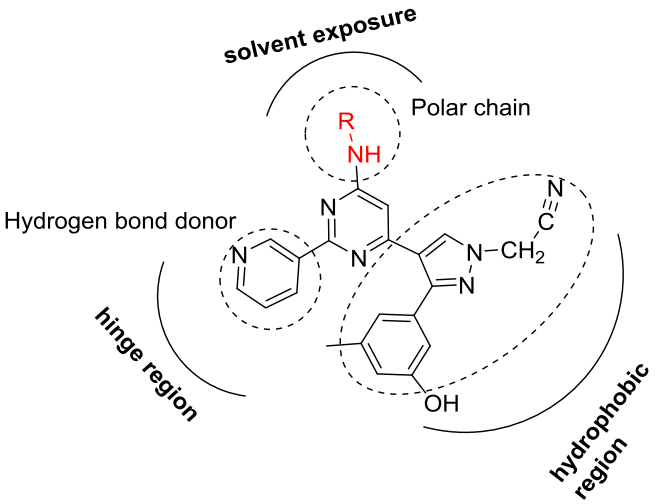


Fig. 6. Proposed model for pyrazol-4-ylpyrimidine ROS1 inhibitors.

Table 2
The IC_{50} values of the compounds **16**, **17**, **21**, **22**, **28**, and **29** against ROS1 kinase.

Compd no.	ROS1 IC_{50} (μM)
16	1.330
17	0.357
21	No inhibition
22	No inhibition
28	0.570
29	0.330

hydrophobic region. The polar side chain in presence of the other essential features has some role in enhancing the ROS1 inhibitory activity.

4. Experimental

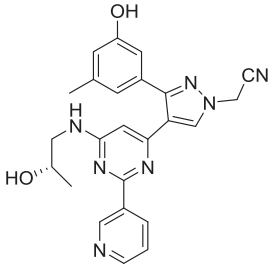
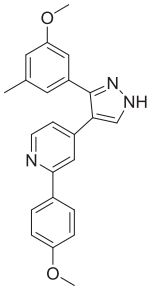
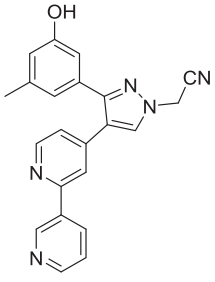
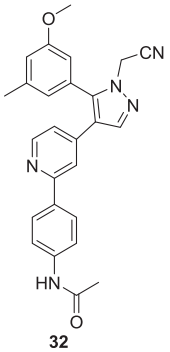
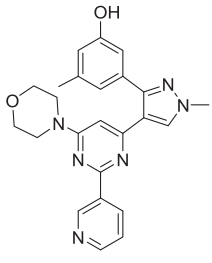
4.1. General

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded on a Bruker Avance 400 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 F_{254} prepared by E. Merck. All the commercially available reagents were obtained from Aldrich, Tokyo Chemicals Industry and generally used without further purification.

4.2. 2-(2,6-Dichloropyrimidin-4-yl)-1-(3-methoxy-5-methylphenyl)ethanone (**2**)

To a solution of methyl 3-methoxy-5-methylbenzoate (5.0 g, 27.7 mmol) and 2,4-dichloro-6-methylpyrimidine (5.4 g, 33.1 mmol) in 75 mL of dry THF was added a 1.0 M solution of lithium bis(trimethylsilyl)amide in THF (55.6 mL, 55.6 mmol) at 0 °C dropwise using dropping funnel, and reaction mixture was left for stirring at 0 °C to ambient temperature for 12 h. The reaction was quenched with 1 N aqueous HCl solution for neutralization, and then extracted with ethyl acetate (2 × 150 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography using the mixed solvent of hexane and dichloromethane (4:1, v/v) to give the title compound as a mixture of keto/enol tautomers (1:1.5) (7 g, 81%). mp 263–264 °C; ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H), 3.86 (s, 3H), 6.01 (s, 1H), 6.88 (s, 1H), 7.01 (s, 1H), 7.21 (s, 1H), 7.26 (s, 1H), 13.54 (s, 1H);

Table 3
Most active ROS1 inhibitor pyrazole compounds from our previous studies.

Compound	ROS1 IC_{50}(μM)	Reference	Compound	ROS1 IC_{50}(μM)	Reference
 KIST301072	0.199	[9]	 31	1.250	[12]
 KIST301080	0.209	[10]	 32	6.250	[11]
 30	0.0136	[19]			

^{13}C NMR ($CDCl_3$), δ 21.47, 21.60, 46.81, 55.39, 55.49, 93.09, 108.70, 110.03, 114.72, 118.12, 119.52, 120.81,121.28, 122.00, 135.24, 136.99, 139.83, 140.23, 157.82, 159.84, 160.00, 160.48, 161.10, 162.55, 167.65, 168.11, 169.46, 193.85.

4.3. General procedure for the synthesis of compounds **3a**–**e**

A mixture of compound **2** (1 g, 3.23 mmol) and the appropriate amine in THF was heated to 80 °C in flask, in an oil bath for 3 h. The reaction mixture was left to cool at room temperature and then concentrated *in vacuo*. The residue was partitioned between water and ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography using the proper mobile phase.

4.3.1. 2-(2-Chloro-6-(isobutylamino)pyrimidin-4-yl)-1-(3-methoxy-5-methylphenyl)ethanone (**3a**)

Flash column chromatography was carried out using (ethyl acetate–hexane, 1:10, v/v) as a mixture of keto/enol tautomers (1:2). Yield: (640 mg, 58%); mp 114–115 °C; ^{1}H NMR ($CDCl_3$) δ 1.00 (d, J = 6.8 Hz, 6H), 1.97 (m, 1H), 2.41 (s, 3H), 3.27 (t, J = 6.4 Hz, 2H), 3.87 (s, 3H), 5.87 (s, 1H), 6.32 (s, 1H), 6.83 (s, 1H), 7.18 (s, 1H), 7.25 (s, 1H), 14.96 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 20.11, 20.21, 21.46, 21.58, 28.28, 48.97, 49.30, 55.35, 55.44, 93.61, 105.50, 108.26, 117.27, 119.22, 119.53, 122.31, 136.36, 139.56.

4.3.2. 2-(2-Chloro-6-(3-hydroxypropylamino)pyrimidin-4-yl)-1-(3-methoxy-5-methylphenyl)ethanone (**3b**)

Flash column chromatography was carried out using (ethyl acetate-hexane, 2:1, v/v) to give the title compound as a mixture of keto/enol tautomers (1:1.6). Yield: (550 mg, 49%); mp 105–106 °C; ^{1}H NMR ($CDCl_3$) δ 1.90 (m, 2H), 2.40 (s, 3H), 3.59–3.65 (m 4H), 3.87 (s, 3H), 5.86 (s, 1H), 6.33 (s, 1H), 6.83 (s, 1H), 7.16 (s, 1H), 7.23 (s, 1H), 15.33 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.46, 21.59, 32.60, 38.07, 55.36, 55.47, 58.85, 93.38, 105.64, 108.33, 110.10, 110.15, 117.33, 119.26, 120.94, 122.21, 136.36, 139.60, 140.03, 159.17, 159.75, 159.89, 160.40, 165.79, 194.62.

4.3.3. 2-(2-Chloro-6-(4-hydroxybutylamino)pyrimidin-4-yl)-1-(3-methoxy-5-methylphenyl)ethanone (**3c**)

Flash column chromatography was carried out using (ethyl acetate-hexane, 2:1, v/v) to give the title compound as a mixture of keto/enol tautomers (1:1.5). Yield: (660 mg, 56%); mp 115–117 °C; ^{1}H NMR ($CDCl_3$) δ 1.70–1.76 (m, 4H), 2.41 (s, 3H), 3.48–3.50 (m, 2H), 3.76–3.78 (m, 2H), 3.86 (s, 3H), 5.88 (s, 1H), 6.33 (s, 1H), 6.84 (s, 1H), 7.17 (s, 1H), 7.24 (s, 1H), 15.12 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.47, 21.58, 25.75, 25.96, 29.63, 29.81, 41.20, 41.53, 55.36, 55.46, 62.24, 62.39, 93.44, 105.45, 108.30, 109.79, 110.31, 117.30, 119.24, 121.80, 122.27, 136.47, 139.57, 139.95, 159.76, 159.86, 160.53, 165.67, 168.13, 196.04.

4.3.4. 2-(2-Chloro-6-(2-morpholinoethylamino)pyrimidin-4-yl)-1-(3-methoxy-5-methylphenyl)ethanone (**3d**)

Flash column chromatography was carried out using (ethyl acetate-hexane, 2:1, v/v) to give the title compound as a mixture of keto/enol tautomers (1:2.3). Yield: (630 mg, 48%); mp 114–116 °C; ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H), 2.54 (m, 4H), 2.68 (m, 2H), 3.54 (m, 2H), 3.76 (m, 4H), 3.89 (s, 3H), 5.87 (s, 1H), 6.33 (s, 1H), 6.83 (s, 1H), 7.17 (s, 1H), 7.23 (s, 1H), 15.03 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.48, 21.58, 37.57, 37.69, 47.58, 53.24, 53.28, 55.34, 55.44, 66.69, 66.78, 93.51, 105.58, 108.22, 109.84, 110.33, 117.30, 119.19, 120.63, 122.23, 136.34, 137.58, 139.58, 139.89, 158.99, 159.78, 159.85, 160.67, 165.85, 166.19, 168.18, 195.23.

4.3.5. 2-(2-Chloro-6-(3-morpholinopropylamino)pyrimidin-4-yl)-1-(3-methoxy-5-methylphenyl) ethanone (**3e**)

Flash column chromatography was carried out using (ethyl acetate-hexane, 2:1, v/v) to give the title compound as a mixture of keto/enol tautomers (1:2.3). Yield: (620 mg, 46%); mp 96–99 °C; ^{1}H NMR ($CDCl_3$) δ 1.86–1.89 (m, 2H), 2.41 (s, 3H), 2.55–2.57 (m, 6H), 3.55–3.58 (m, 2H), 3.81 (m, 4H), 3.87 (s, 3H), 5.86 (s, 1H), 6.31 (s, 1H), 6.83 (s, 1H), 7.17 (s, 1H), 7.24 (s, 1H), 15.00 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.49, 21.59, 25.10, 46.55, 53.57, 53.70, 55.35, 55.44, 66.24, 66.90, 93.47, 105.42, 108.30, 109.62, 110.37, 117.20, 119.21, 120.59, 122.26, 136.43, 137.62, 139.55, 139.87, 159.75, 159.85, 160.71, 165.79, 166.17, 168.17, 195.31.

4.4. General procedure for the synthesis of compounds **4a**–**e**

To a glass vial containing a magnetic stir bar was added a mixture of the starting compound **3a**–**e** (1 mmol), 3-pyridineboroic acid (1.5 mmol), bis(triphenylphosphine)palladium(II) dichloride (0.05 mmol) and potassium carbonate (2 mmol) and the vial was purged with nitrogen gas for 10 min. Then a mixed solvent of THF/acetonitrile/water (8 mL, 2:1:1, v/v/v) was injected, and the vial was once again bubbled with nitrogen for 10 min, and then capped. The reaction mixture was stirred at 90 °C under nitrogen. After 3 h, the reaction mixture was left to cool to ambient temperature. The reaction mixture was filtered through celite, and then the filtrate was concentrated *in vacuo*, and the residue was purified by column chromatography using the proper mobile phase.

4.4.1. 2-(6-Isobutylamino-2-(pyridin-3-yl)pyrimidin-4-yl)-1-(3-methoxy-5-methylphenyl)ethanone (**4a**)

Flash column chromatography was carried out using (hexane-ethyl acetate, 4:1, v/v) to give the title compound as a mixture of keto/enol tautomers (1:2.3). Yield: (316 mg, 81%); mp 51–53 °C; ^{1}H NMR ($CDCl_3$) δ 1.05 (d, J = 6.8 Hz, 6H), 1.99–2.04 (m, 1H), 2.40 (s, 3H), 3.33 (t, J = 6.4 Hz, 2H), 3.87 (s, 3H), 5.63 (s, 1H), 6.01 (S, 1H), 6.71 (s, 1H), 6.82 (s, 1H), 7.23 (s, 1H), 7.28 (s, 1H), 7.37–7.42 (m, 1H), 8.32 (d, J = 1.6 Hz, 1H), 8.71 (dd, J = 1.6, 4.8 Hz, 1H), 9.23 (s, 1H), 15.33 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 20.27, 20.32, 21.45, 21.58, 28.46, 29.69, 48.26, 49.06, 49.30, 55.33, 55.40, 93.85, 102.80, 106.14, 108.25, 110.34, 117.15, 119.28, 120.62, 122.39, 123.41, 123.47, 133.07, 133.13, 134.35, 134.45, 135.56, 137.12, 137.73, 139.46, 139.79, 148.45, 148.60, 149.93, 151.06, 159.77, 159.82, 161.45, 162.95, 164.75, 165.46, 195.92.

4.4.2. 2-(6-(3-Hydroxypropylamino)-2-(pyridin-3-yl)pyrimidin-4-yl)-1-(3-methoxy-5-methylphenyl) ethanone (**4b**)

Flash column chromatography was carried out using (ethyl acetate-methanol, 30:1, v/v) to give the title compound as a mixture of keto/enol tautomers (1: 1.7). Yield: (137 mg, 35%); mp 99–100 °C; ^{1}H NMR ($CDCl_3$) δ 1.88–1.92 (m, 2H), 2.38 (s, 3H), 3.64–3.71 (m, 4H), 3.82–3.85 (m. 2H), 3.87 (s. 3H), 5.97 (s, 1H), 6.04 (t, J = 6.4 Hz, 1H), 6.66 (s, 1H), 6.81 (s, 1H), 7.19 (s, 1H), 7.25 (s, 1H), 7.38–7.42 (m, 1H), 8.27 (dd, J = 1.6, 8.0 Hz,1H), 8.67 (dd, J = 1.6, 5.2 Hz, 1H), 9.19 (d, J = 1.6, 1H), 15.39 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.46, 21.60, 32.20, 32.93, 37.81, 47.94, 55.37, 55.46, 59.07, 59.90, 93.67, 103.10, 106.77, 108.39, 110.25, 117.26, 119.35, 120.79, 122.31, 123.57, 123.63, 132.89, 134.41, 134.55, 137.12, 137.61, 139.54, 139.96, 148.32, 148.54, 151.12, 151.27, 158.59, 159.79, 159.89, 161.03, 163.14, 164.70, 195.62.

4.4.3. 2-(6-(4-Hydroxybutylamino)-2-(pyridin-3-yl)pyrimidin-4-yl)-1-(3-methoxy-5-methylphenyl) ethanone (**4c**)

Flash column chromatography was carried out using (ethyl acetate-methanol, 30:1, v/v) to give the title compound as a mixture of keto/enol tautomers (1: 1.9). Yield: (264 mg, 65%); mp

52–54 °C; ^{1}H NMR ($CDCl_3$) δ 1.70–1.76 (m, 4H), 2.39 (s, 3H), 3.51–3.54 (m, 2H), 3.74 (t, J = 6 Hz, 2H), 3.86 (s, 3H), 5.82 (s, 1H), 5.99 (s, 1H), 6.68 (s, 1H), 6.81 (s, 1H), 7.20 (s, 1H), 7.26 (s, 1H), 7.37–7.42 (m, 1H), 8.28 (d, J = 8.0 Hz, 1H), 8.69 (d, J = 1.2 Hz, 1H), 9.21 (s, 1H) 15.49 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.48, 21.60, 25.97, 26.18, 29.90, 30.03, 41.22, 41.51, 48.12, 55.35, 55.44, 62.15, 62.41, 93.52, 102.66, 106.33, 108.33, 110.41, 117.16, 119.32, 120.61, 122.36, 123.51, 133.09, 134.41, 134.53, 137.34, 137.72, 139.49, 139.87, 148.37, 148.54, 150.99, 151.07, 159.76, 161.34, 162.75, 164.32, 165.52, 195.95.

4.4.4. 2-(6-(2-Morpholinoethylamino)-1-(3-methoxy-5-methylphenyl)-2-(pyridin-3-yl)pyrimidin-4-yl)ethanone (**4d**)

Flash column chromatography was carried out using (ethyl acetate-methanol, 10:1, v/v) to give the title compound as a mixture of keto/enol tautomers. Yield: (317 mg, 71%); mp 72–74 °C; ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H), 2.50 (m 4H), 2.65 (m 2H), 3.59 (m 2H), 3.74 (m, 4H), 3.82 (s, 3H), 5.88 (s, 1H), 5.99 (s, 1H), 6.70 (s, 1H), 6.80 (s, 1H), 7.20 (s, 1H), 7.39–7.40 (m, 1H), 8.29 (d, J = 8.0 Hz, 1H), 8.67 (d, J = 3.6 Hz, 1H), 9.21 (s, 1H), 15.38 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.48, 21.58, 22.96, 23.83, 28.79, 30.52, 37.70, 37.74, 38.73, 48.06, 53.37, 53.40, 55.33, 55.41, 57.33, 66.75, 66.80, 66.97, 93.71, 102.98, 106.41, 108.16, 110.51, 117.14, 119.22, 120.40, 122.30, 123.43, 123.46, 128.76, 130.34, 133.24, 134.34, 134.5, 137.23, 137.84, 139.48, 139.82, 148.44, 148.57, 151.06, 151.14, 159.77, 162.73, 165.47, 196.09.

4.4.5. 2-(6-(3-Morpholinopropylamino)-1-(3-methoxy-5-methylphenyl)-2-(pyridin-3-yl)pyrimidin-4-yl)ethanone (**4e**)

Flash column chromatography was carried out using (ethyl acetate-methanol, 3:1, v/v) to give the title compound as a mixture of keto/enol tautomers (1:2.2). Yield: (244 mg, 53%); mp 79–80 °C; ^{1}H NMR ($CDCl_3$) δ 1.70–1.87 (m, J = 6.8, 13.2 Hz, 2H), 2.41 (s, 3H), 2.51–2.62 (m, 6H), 3.62–3.66 (m, 2H), 3.82–3.83 (m, 4H), 3.88 (s, 3H), 6.02 (s, 1H), 6.48 (s, 1H), 6.73 (s, 1H), 6.83 (s, 1H), 7.22 (s, 1H), 7.41–7.43 (m, 1H), 8.32 (d, J = 1.6 Hz, 1H), 8.71 (dd, J = 1.6, 4.8 Hz, 1H), 9.24 (s, 1H) 15.48 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.50, 21.61, 25.14, 25.73, 29.70, 30.94, 40.43, 41.13, 53.66, 53.74, 55.36, 55.43, 57.11, 57.47, 66.85, 67.01, 93.69, 102.63, 106.22, 108.20, 110.39, 117.08, 119.25, 120.57, 122.38, 123.44, 123.48, 133.26, 134.31, 134.44, 137.23, 137.72, 139.49, 139.83, 148.45, 148.58, 151.04, 151.12, 159.75, 159.82, 162.82, 164.64, 165.59, 195.98.

4.5. General procedure for the synthesis of compounds **5a–e**

A mixture of compound **4a–e** (0.5 mmol) and dimethylformamide dimethyl acetal (3.5 mmol) was heated to 90 °C for 2 h and allowed to cool to ambient temperature. The excess reagent was evaporated and the residue was dissolved in 10 mL of absolute ethanol, then hydrazine hydrate (3.5 mmol) was added. The mixture was stirred at ambient temperature for 12 h. The solution was concentrated *in vacuo*. The residue was diluted with water and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography using the proper mobile phase.

4.5.1. N-isobutyl-6-(3-(3-methoxy-5-methylphenyl)-1H-pyrazol-4-yl)-2-(pyridin-3-yl)pyrimidin-4-amine (**5a**)

Flash column chromatography was carried out using (dichloromethane-methanol, 20:1, v/v). Yield (122 mg, 59%); mp 67–68 °C; ^{1}H NMR ($CDCl_3$) δ 0.99 (d, J = 6.8 Hz, 6H), 1.92–1.95 (m, 1H), 2.34 (s, 3H), 3.31 (m, 2H), 3.74 (s, 3H), 6.81 (s, 1H), 6.93 (s, 1H), 6.95 (s, 1H), 6.99 (s, 1H), 7.33–7.36 (m, 1H), 8.14 (s, 1H), 8.17 (s, 1H), 8.63 (dd, J = 1.6, 4.8 Hz, 1H), 8.98 (d, J = 1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 20.32, 21.51, 28.51, 49.07, 55.32, 103.77, 111.63, 115.67, 122.10, 123.49, 131.99, 133.44, 134.37, 139.93, 148.32, 150.89, 159.75, 162.12, 162.72.

4.5.2. 3-(6-(3-(3-Methoxy-5-methylphenyl)-1H-pyrazol-4-yl)-2-(pyridin-3-yl)pyrimidin-4-yl)amino) propan-1-ol (**5b**)

Flash column chromatography was carried out using (ethyl acetate-methanol, 10:1, v/v). Yield (148 mg, 71%); mp 70–72 °C; ^{1}H NMR ($CDCl_3$) δ 1.80–1.84 (m, 2H), 2.33 (s, 3H), 3.67–3.74 (m 7H), 5.72 (s, 1H), 6.81 (s, 1H), 6.91 (s, 1H), 6.92 (s, 1H), 6.96 (s, 1H), 7.33–7.36 (dd, J = 4.8, 7.6 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H), 8.19 (s, 1H), 8.64 (d, J = 1.6, 4.8 Hz, 1H), 8.89 (d, J = 1.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 21.49, 32.86, 37.66, 55.31, 58.89, 104.29, 111.71, 115.53, 117.78, 122.14, 123.59, 133.13, 134.37, 139.91, 148.25, 151.03, 159.72, 160.99, 162.35, 162.93.

4.5.3. 4-(6-(3-(3-Methoxy-5-methylphenyl)-1H-pyrazol-4-yl)-2-(pyridin-3-yl)pyrimidin-4-yl)amino) butan-1-ol (**5c**)

Flash column chromatography was carried out using (dichloromethane-methanol, 20:1, v/v). Yield (159 mg, 74%); mp 79–81 °C; ^{1}H NMR ($CDCl_3$) δ 1.71–1.78 (m, 4H), 2.40 (s, 3H), 3.57–3.59 (m 2H), 3.75 (t, J = 6 Hz, 2H), 3.81 (s, 3H), 6.87 (s, 1H), 6.94 (s, 1H), 6.97 (s, 1H), 7.00 (s, 1H), 7.39 (ddd, J = 0.8, 4.8, 8.0 Hz, 1H), 8.17 (dt, J = 1.6, 8.0 Hz, 1H), 8.36 (s, 1H), 8.68 (dd, J = 1.6, 4.8 Hz, 1H), 8.97 (d, J = 2.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 21.53, 26.14, 29.94, 41.21, 55.40, 62.52, 103.64, 111.70, 115.77, 122.09, 123.56, 131.87, 132.94, 134.47, 140.13, 148.40, 151.24, 159.83, 160.78, 163.54.

4.5.4. 6-(3-(3-Methoxy-5-methylphenyl)-1H-pyrazol-4-yl)-N-(2-morpholinoethyl)-2-(pyridin-3-yl) pyrimidin-4-amine (**5d**)

Flash column chromatography was carried out using (ethyl acetate-methanol, 2:1, v/v). Yield (146 mg, 62%); mp 87–88 °C; ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H), 2.63 (m, 4H), 2.72 (m, 2H), 3.66 (m, 2H), 3.76 (s, 3H), 3.80 (t, J = 4.4 Hz, 4H), 5.90 (s, 1H), 6.82 (s, 1H), 6.93 (s, 1H), 6.97 (s, 1H), 6.99 (s, 1H), 7.35 (ddd, J = 0.8, 4.8, 8.0 Hz, 1H), 8.15 (dt, J = 1.6, 8.0 Hz, 2H), 8.20 (s, 1H), 8.65 (dd, J = 1.6, 4.8 Hz, 1H), 8.97 (d, J = 1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 21.15, 37.67, 53.74, 55.31, 57.03, 67.34, 104.08, 111.76, 115.51, 118.01, 122.14, 123.47, 132.05, 133.51, 134.90, 139.86, 148.28, 150.84, 159.72, 161.19, 162.69.

4.5.5. 6-(3-(3-Methoxy-5-methylphenyl)-1H-pyrazol-4-yl)-N-(3-morpholinopropyl)-2-(pyridin-3-yl)pyrimidin-4-amine (**5e**)

Flash column chromatography was carried out using (ethyl acetate-methanol, 2:1, v/v). Yield (167 mg, 69%); mp 62–64 °C; ^{1}H NMR ($CDCl_3$) δ 1.86–1.89 (m, 2H), 2.37 (s, 3H), 2.54–2.56 (m, 6H), 3.58 (m, 2H), 3.78–3.80 (m, 7H), 5.99 (s, 1H), 6.84 (s, 1H), 6.94 (s, 1H), 6.95 (s, 1H), 7.00 (s, 1H), 7.36 (ddd, J = 0.8, 4.8, 8.0 Hz, 1H), 8.16 (dt, J = 1.6, 8.0 Hz, 1H), 8.19 (s, 1H), 8.65 (dd, J = 1.6, 4.8 Hz, 1H), 8.97 (d, J = 1.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 21.53, 25.87, 40.29, 53.64, 55.34, 57.08, 66.78, 103.92, 111.69, 115.59, 118.54, 122.12, 123.48, 132.05, 133.51, 134.29, 139.93, 148.27, 150.82, 153.98, 159.74, 161.08, 162.89.

4.6. General procedure for the synthesis of compounds **6a–e**

In a vial capped with a rubber septum, a mixture of compound **5a–e** (0.35 mmol) and cesium carbonate (1.05 mmol) was purged with nitrogen gas with 5 min. Then 5 mL anhydrous dimethylformamide was injected into the vial, and the vial was once again bubbled with nitrogen for 5 min and left under nitrogen using nitrogen balloon. Then iodoacetonitrile (0.7 mmol) was injected and the reaction mixture was left for stirring at ambient temperature for 16 h. The reaction mixture was quenched by water (10 mL), and then extracted with ethyl acetate (2 × 10 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was

purified by column chromatography using the proper mobile phase.

4.6.1. 2-(4-(6-(Isobutylamino)-2-(pyridin-3-yl)pyrimidin-4-yl)-3-(3-methoxy-5-methylphenyl)-1H-pyrazol-1-yl)acetonitrile (**6a**)

The residue was purified by column chromatography using (ethyl acetate-hexane, 1:2, v/v). Yield (48 mg, 30%); mp 57–58 °C; ^{1}H NMR ($CDCl_3$) δ 1.04 (d, J = 6.8 Hz, 6H), 1.98 (m, J = 6.8, 13.6 Hz, 1H), 2.39 (s, 3H), 3.37 (m, 2H), 3.81 (s, 3H), 5.20 (s, 2H), 5.46 (s, 1H), 6.86 (s, 1H), 6.91 (s, 1H), 6.96 (s, 1H), 7.04 (s, 1H), 7.37 (ddd, = 0.8, 4.8, 8.0 Hz, 1H), 8.13 (dt, J = 2.0, 8.0 Hz, 1H), 8.27 (s, 1H), 8.67 (dd, J = 1.6, 8 Hz, 1H), 8.96 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 20.14, 21.52, 28.53, 29.70, 39.39, 49.09, 55.34, 104.13, 111.57, 113.32, 115.72, 122.24, 123.48, 131.95, 133.20, 133.29, 134.31, 139.84, 148.39, 151.08, 152.63, 159.73, 162.44.

4.6.2. 2-(4-(6-(3-Hydroxypropylamino)-2-(pyridin-3-yl)pyrimidin-4-yl)-3-(3-methoxy-5-methylphenyl)-1H-pyrazol-1-yl)acetonitrile (**6b**)

The residue was purified by column chromatography using (dichloromethane-methanol, 30:1, v/v). Yield (53 mg, 33%); mp 78–79 °C; ^{1}H NMR ($CDCl_3$) δ 1.87–1.89 (m, 2H), 2.40 (s, 3H), 3.74–3.78 (m, 4H), 3.82 (s, 3H), 5.20 (s, 2H), 6.88 (s, 1H), 6.92 (s, 1H), 6.94 (s, 1H), 7.02 (s, 1H), 7.38 (dd, J = 4.8, 8.0 Hz, 1H), 8.12 (dt, J = 2.0, 8.0 Hz, 1H), 8.38 (s, 1H), 8.69 (dd, J = 1.2, 4.8 Hz, 1H), 8.89 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.52, 32.78, 37.81, 40.01, 55.39, 59.22, 104.52, 11.74, 113.22, 115.79, 122.24, 123.60, 132.58, 133.07, 134.39, 140.01, 148.42, 151.51, 152.89, 159.83.

4.6.3. 2-(4-(6-(4-Hydroxybutylamino)-2-(pyridin-3-yl)pyrimidin-4-yl)-3-(3-methoxy-5-methylphenyl)-1H-pyrazol-1-yl)acetonitrile (**6c**)

The residue was purified by column chromatography using (dichloromethane-methanol, 30:1, v/v). Yield (55 mg, 34%) mp 48–50 °C; ^{1}H NMR ($CDCl_3$) δ 1.71–1.81 (m, 4H), 2.39 (s, 3H), 3.56–3.61 (m, 2H), 3.75 (t, J = 6 Hz, 2H), 3.81 (s, 3H), 5.20 (s, 2H), 5.61 (s, 1H), 6.86 (s, 1H), 6.91 (s, 1H), 6.95 (s, 1H), 7.03 (s, 1H), 7.36 (dd, J = 4.8, 7.6 Hz, 1H), 8.12 (dt, J = 1.6, 8.0 Hz,1H), 8.32 (s, 1H), 8.67 (d, J = 3.2 Hz, 1H), 8.85 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.51, 26.19, 29.93, 39.95, 41.25, 55.37, 62.49, 104.13, 111.65, 113.35, 115.72, 122.26, 123.51, 132.18, 133.02, 133.27, 134.33, 139.88, 148.40, 151.19, 152.66, 159.74, 162.61.

4.6.4. 2-(3-(3-Methoxy-5-methylphenyl)-4-(6-((2-morpholinoethyl)amino)-2-(pyridin-3-yl)pyrimidin-4-yl)-1H-pyrazol-1-yl)acetonitrile (**6d**)

The residue was purified by column chromatography using (dichloromethane-methanol, 20:1, v/v). Yield (52 mg, 29%); mp 58–59 °C; ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3H), 2.61 (m, 4H), 2.72 (m, 2H), 3.67 (m, 2H), 3.77–3.80 (m, 7H), 5.19 (s, 2H), 5.78 (s, 1H), 6.86 (s, 1H), 6.94 (s, 1H), 6.96 (s, 1H), 7.04 (s, 1H), 7.36 (dd, J = 4.8, 8.0 Hz, 1H), 8.14 (dt, J = 2.0, 8.0 Hz, 1H), 8.29 (s, 1H), 8.67 (dd, J = 1.6, 4.8 Hz, 1H), 8.96 (d, J = 1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 21.52, 37.64, 39.92, 53.42, 55.35, 57.40, 66.71, 104.43, 111.67, 113.35, 115.64, 121.49, 122.25, 123.44, 131.97, 133.19, 133.36, 134.23, 139.80, 148.40, 151.13, 152.56, 159.72, 160.09, 162.65.

4.6.5. 2-(3-(3-Methoxy-5-methylphenyl)-4-(6-((3-morpholinopropyl)amino)-2-(pyridin-3-yl)pyrimidin-4-yl)-1H-pyrazol-1-yl)acetonitrile (**6e**)

The residue was purified by column chromatography using (dichloromethane-methanol, 20:1, v/v). Yield (64 mg, 35%); mp 51–53 °C; ^{1}H NMR ($CDCl_3$) δ 1.87–1.93 (m, J = 6.4, 12.8 Hz, 2H), 2.38 (s, 3H), 2.58–2.62 (m, 6H), 3.61–3.63 (m, 2H), 3.77–3.85 (m, 7H), 5.20 (s, 2H), 5.95 (s, 1H), 6.86 (s, 1H), 6.92 (s, 1H), 6.96 (s, 1H), 7.04 (s, 1H), 7.35 (dd, J = 4.8, 7.6 Hz, 1H), 8.10 (d, J = 2.0 Hz, 1H), 8.26 (s, 1H), 8.66 (d, J = 3.6 Hz, 1H), 8.96 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.52, 25.75, 39.92, 40.45, 53.65, 55.35, 57.16, 66.77, 104.17, 111.64, 113.41, 115.65, 121.54, 122.26, 123.43, 131.94, 133.21, 133.39, 134.17, 139.79, 148.39, 151.07, 152.55, 159.71, 160.03, 162.88.

4.7. General procedure for the synthesis of compounds **7a–e**

To a solution of compound **6a–e** (0.01 mmol) in 5 mL of dry dichloromethane was added boron trifluoride-dimethylsulfide complex (1 mmol) dropwise. The resulting suspension was stirred at room temperature for 12 h. The mixture was concentrated and the residue was partitioned between ethyl acetate and sodium bicarbonate solution. The layers were separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography using the proper mobile phase.

4.7.1. 2-(3-(3-Hydroxy-5-methylphenyl)-4-(6-(isobutylamino)-2-(pyridin-3-yl)pyrimidin-4-yl)-1H-pyrazol-1-yl)acetonitrile (**7a**)

The residue was purified by column chromatography using (ethyl acetate-hexane, 1:2, v/v). Yield (20 mg, 45%); mp 99–100 °C; ^{1}H NMR (CD_3OD) δ 0.97 (d, J = 6.4 Hz, 6H), 1.92 (m, 1H), 2.39 (s, 3H), 3.24 (m, 2H), 5.44 (s, 2H), 6.76 (s, 1H), 6.82 (s, 1H), 6.88 (s, 1H), 6.93 (s, 1H), 7.49 (dd, J = 4.8, 8.0 Hz, 1H), 8.23 (dt, J = 1.6, 8.0 Hz, 1H), 8.36 (s, 1H), 8.59 (dd, J = 1.6, 4.8 Hz, 1H), 8.96 (d, J = 1.6 Hz, 1H); ^{13}C NMR (CD_3OD) δ 19.27, 20.12, 28.18, 38.90, 60.14, 103.22, 113.06, 114.32, 116.02, 120.46, 120.84, 123.85, 132.83, 133.76, 133.88, 134.84, 139.40, 147.16, 149.93, 152.56, 157.16, 160.52, 163.11.

4.7.2. 2-(3-(3-Hydroxy-5-methylphenyl)-4-(6-(isobutylamino)-2-(pyridin-3-yl)pyrimidin-4-yl)-1H-pyrazol-1-yl)acetonitrile (**7b**)

The residue was purified by column chromatography using (dichloromethane-methanol, 15:1, v/v). Yield (23 mg, 52%); mp 50–51 °C; ^{1}H NMR (CD_3OD) δ 1.84–1.87 (m, 2H), 2.34 (s, 3H), 3.56 (m, 2H), 3.69 (m, 2H), 5.45 (s, 2H), 6.77 (s, 1H), 6.82 (s, 1H), 6.89 (s, 1H), 6.95 (s, 1H), 7.52 (ddd, J = 4.8, 4.8, 8.0 Hz, 1H), 8.26 (dt, J = 1.6, 8.0 Hz, 1H), 8.40 (s, 1H), 8.61 (d, J = 3.6 Hz, 1H), 8.94 (s, 1H); ^{13}C NMR (CD_3OD) δ 20.07, 32.10, 37.77, 38.89, 59.27, 103.43, 113.07, 114.27, 116.04, 120.84, 123.87, 132.89, 133.73, 133.85, 134.89, 139.49, 147.18, 150.01, 152.57, 157.20, 160.57, 160.57, 163.08.

4.7.3. 2-(3-(3-Hydroxy-5-methylphenyl)-4-(6-((4-hydroxybutyl)amino)-2-(pyridin-3-yl)pyrimidin-4-yl)-1H-pyrazol-1-yl)acetonitrile (**7c**)

The residue was purified by column chromatography using (dichloromethane-methanol, 15:1, v/v). Yield (21 mg, 46%); mp 85–87 °C; ^{1}H NMR (CD_3OD) δ 1.63–1.70 (m, 4H), 2.33 (s, 3H), 3.46 (m, 2H), 3.63 (t, J = 6.4 Hz, 2H), 5.44 (s, 2H), 6.77 (s, 1H), 6.82 (s, 1H), 6.88 (s, 1H), 6.92 (s, 1H), 7.50 (ddd, J = 0.8, 5.2, 8.0 Hz, 1H), 8.24 (dt, J = 1.6, 8.0 Hz, 1H), 8.37 (s, 1H), 8.59 (dd, J = 1.6, 4.8 Hz, 1H), 8.79 (d, J = 1.6 Hz, 1H). ^{13}C NMR (CD_3OD) δ 20.11, 25.75, 29.27, 29.66, 38.90, 40.66, 48.45, 61.40, 103.85, 113.09, 114.31, 116.03, 120.47, 120.85, 123.85, 132.88, 133.77, 133.88, 134.86, 139.44, 147.15, 149.93, 152.54, 157.17, 160.52, 162.99.

4.7.4. 2-(3-(3-Hydroxy-5-methylphenyl)-4-(6-((2-morpholinoethyl)amino)-2-(pyridin-3-yl)pyrimidin-4-yl)-1H-pyrazol-1-yl)acetonitrile (**7d**)

The residue was purified by column chromatography using (dichloromethane-methanol, 10:1, v/v). Yield (20 mg, 40%); mp 94–96 °C; ^{1}H NMR (CD_3OD) δ 2.34 (s, 3H), 2.75–2.81 (m, 6H), 3.62–3.64 (m 2H), 3.75–3.77 (m, 4H), 5.45 (s, 2H), 6.77 (s, 1H), 6.83 (s, 1H), 6.91 (s, 1H), 7.10 (s, 1H), 7.54 (dd, J = 4.8, 8.0 Hz, 1H), 8.32 (d,

J = 8.0 Hz, 1H), 8.43 (s, 1H), 8.64 (s, 1H), 9.02 (s, 1H); ^{13}C NMR (CD_3OD) δ 20.09, 29.33, 36.93, 38.92, 53.04, 57.37, 65.62, 103.86, 113.11, 114.28, 116.01, 120.30, 120.82, 123.91, 132.98, 133.74, 134.92, 139.40, 147.24, 150.12, 151.36, 152.48, 157.14, 160.79, 162.88.

4.7.5. 2-(3-(3-Hydroxy-5-methylphenyl)-4-(6-((3-morpholinopropyl)amino)-2-(pyridin-3-yl)pyrimidin-4-yl)-1H-pyrazol-1-yl)acetonitrile (**7e**)

The residue was purified by column chromatography using (dichloromethane-methanol, 10:1, v/v). Yield (18 mg, 35%); mp 73–74 °C; ^{1}H NMR (CD_3OD) δ 1.87–1.89 (m, 2H), 2.34 (s, 3H), 2.59–2.65 (m 6H), 3.61 (m, 2H), 3.50–3.51 (m, 2H), 3.75 (m, 4H), 5.45 (s, 2H), 6.77 (s, 1H), 6.83 (s, 1H), 6.90 (s, 1H), 7.01 (s, 1H), 7.53 (ddd, J = 0.8, 5.2, 8.0 Hz, 1H), 8.28 (dd, J = 2.0, 8.0 Hz, 1H), 8.41 (s, 1H), 8.62 (dd, J = 1.6, 5.2 Hz, 1H), 8.98 (s, 1H); ^{13}C NMR (CD_3OD) δ 20.10, 25.42, 38.90, 38.98, 53.11, 56.25, 65.80, 113.12, 114.29, 115.99, 120.43, 120.88, 123.88, 132.87, 133.86, 134.87, 139.38, 147.20, 147.58, 150.04, 152.56, 157.15, 160.67, 162.98, 163.59.

4.8. 2-Chloro-N-(3-methoxypropyl)-6-methylpyrimidin-4-amine (**8**)

To a solution of 2,4-dichloro-6-methylpyrimidine (3 g, 18.4 mmol) in THF, was added 3-methoxypropylamine (2.0 mL, 2.0 mmol) and triethylamine (2.8 mL, 2.0 mmol), and the mixture was refluxed for three hours. The reaction mixture was left to cool at room temperature and then concentrated *in vacuo*. The residue was partitioned between water and ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography using (ethyl acetate-hexane, 1:5, v/v) to give the title compound as oil. Yield (1.9 g, 48%); ^{1}H NMR ($CDCl_3$) δ 1.80 (p, J = 6.4, 12.8 Hz, 2H), 2.22 (s, 3H), 3.25 (s, 3H), 3.38–3.47 (m, 4H), 6.01 (brs, 1H), 6.34 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 23.77, 29.20, 39.12, 58.58, 70.81, 108.71, 162.15, 196.39.

4.9. General procedure for the synthesis of compounds **11** and **12**

To a mixture of the appropriate substituted benzoic acid (8.6 mmol) in methanol (10 mL) was added sulfuric acid (0.5 mL), and the mixture was heated overnight at 60 °C. The reaction was then allowed to cool to room temperature, the solvent was reduced by evaporation, and the residue was diluted in cold water and neutralized with sodium hydrogen carbonate solution. The aqueous solution was extracted with ethyl acetate. The organic phase was washed with water and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to give the title compound as oil.

4.9.1. 2-Chloro-4-fluorobenzoic acid (**11**)

Yield (1.5 g, 92%); ^{1}H NMR ($CDCl_3$) δ 3.91 (s, 3H), 7.01 (ddd, J = 2.4, 7.6, 10.0 Hz, 1H), 7.17 (dd, J = 2.4, 8.4 Hz, 1H), 7.89 (dd, J = 6.4, 8.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 52.39, 113.99, 118.55, 125.97, 133.52, 135.75, 162.78, 165.17.

4.9.2. Methyl 2,4-dichlorobenzoate (**12**)

Yield (1.52 g, 87%); ^{1}H NMR ($CDCl_3$) δ 3.93 (s, 3H), 7.29 (dd, J = 2.0, 8.4 Hz, 1H), 7.47 (d, J = 2.0 Hz, 1H), 7.81 (d, J = 8.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 52.54, 126.98, 128.18, 130.99, 132.52, 134.83, 138.32, 165.15.

4.10. General procedure for the synthesis of compounds **13** and **18**

In a two neck flask was added a mixture of compound **8**, the appropriate boronic acid (1.1 mmol), bis(triphenylphosphine)palladium(II) dichloride (0.05 mmol) and potassium carbonate (2 mmol) and the flask was fixed to a condenser and purged with nitrogen gas for 10 min. Then a mixed solvent of THF/acetonitrile/water (20 mL, 2:1:1, v/v/v) was injected, and the flask was once again bubbled with nitrogen for 10 min. The reaction mixture was stirred at 90 °C under nitrogen. After 3 h, the reaction mixture was left to cool to ambient temperature. The reaction mixture was filtered through celite, and then the filtrate was concentrated *in vacuo*, and the residue was purified by column chromatography using the proper mobile phase.

4.10.1. N-(3-methoxypropyl)-6-methyl-2-(pyridin-3-yl)pyrimidin-4-amine (**13**)

Flash column chromatography was carried out using ethyl acetate to give the title compound as viscous liquid. Yield (1.80 g, 79%); ^{1}H NMR ($CDCl_3$) δ 1.85 (p, J = 6.4, 12.8 Hz, 2H), 2.34 (s, 3H), 3.28 (s, 3H), 3.44–3.57 (m, 4H), 5.78 (brs, 1H), 6.79 (s, 1H), 7.32 (ddd, J = 0.8, 4.8, 8.0 Hz, 1H), 8.26 (d, J = 8.0 Hz, 1H), 8.61 (dd, J = 1.6, 4.8 Hz, 1H), 9.17 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.20, 29.43, 38.95, 58.54, 70.76, 105.51, 123.32, 133.23, 134.25, 148.36, 150.79, 161.79, 162.65, 168.59.

4.10.2. N-(3-methoxypropyl)-6-methyl-2-phenylpyrimidin-4-amine (**18**)

Flash column chromatography was carried out using (hexane-ethyl acetate, 3:1, v/v) to give the title compound as viscous yellow liquid. Yield (0.41 g, 35%); ^{1}H NMR ($CDCl_3$) δ 1.95 (p, J = 6.4, 12.8 Hz, 2H), 2.41 (s, 3H), 3.37 (s, 3H), 3. 53 (t, J = 6.0 Hz, 2H), 3.64 (q, J = 6.4 Hz, 2H), 5.59 (brs, 1H), 6.87 (s, 1H), 7.47 (m, 3H), 8.05 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 24.16, 29.60, 39.00, 58.62, 70.87, 105.75, 126.99, 128.55, 130.19, 137.78, 162.69, 164.49, 168.12.

4.11. General procedure for the synthesis of compounds **14**, **15**, **19**, and **20**

To a solution of the appropriate methyl benzoic ester derivative **11** and **12** (1 equivalent) and either compound **13** or **18** (1 equivalent) in dry THF under nitrogen atmosphere, was added a 1.0 M solution of lithium bis(trimethylsilyl)amide in THF (1.5 equivalent) at 0 °C dropwise, and the reaction mixture was left for stirring at 0 °C to ambient temperature for 12 h. The reaction was quenched with a saturated ammonium chloride solution, and then extracted with ethyl acetate. The organic layer was washed with brine, dried anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was then purified using the appropriate mobile phase.

4.11.1. 1-(2-Chloro-4-fluorophenyl)-2-(6-((3-methoxypropyl)amino)-2-(pyridin-3-yl)pyrimidin-4-yl)ethan-1-one (**14**)

Flash column chromatography was carried out using ethyl acetate to give the title compound in the enol form. Yield: (0.6 g, 93%); mp 125–126 °C; ^{1}H NMR ($CDCl_3$) δ 2.00 (p, J = 6.0, 12.4 Hz, 2H), 3.42 (s, 3H), 3. 59 (t, J = 6.0 Hz, 2H), 3.66 (q, J = 6.0 Hz, 2H), 5.82 (s, 1H), 5.99 (brs, 1H), 6.69 (s, 1H), 7.07 (ddd, J = 2.4, 7.6, 10.4 Hz, 1H), 7.22 (dd, J = 2.8, 8.8 Hz, 1H), 7.45 (dd, J = 4.4, 8.0 Hz, 1H), 7.64 (dd, J = 6.0, 8.8, Hz, 1H), 8.36 (dt, J = 1.6, 3.6 Hz, 1H), 8.73 (dd, J = 1.6, 4.8 Hz, 1H), 9.24 (s, 1H), 15.33 (brs, 1H); ^{13}C NMR ($CDCl_3$) δ 29.71, 39.85, 58.89, 71.02, 102.44, 114.16, 117.78, 123.62, 131.40, 132.92, 134.68, 148.25, 151.03, 161.29, 163.90.

4.11.2. 1-(2,4-Dichlorophenyl)-2-(6-((3-methoxypropyl)amino)-2-(pyridin-3-yl)pyrimidin-4-yl)ethan-1-one (**15**)

Flash column chromatography was carried out using ethyl acetate to give the title compound in the enol form. Yield: (0.34 g, 72%); mp 165–166 °C; ^{1}H NMR (DMSO-d_6) δ 1.86 (p, J = 6.4, 12.8 Hz, 2H), 3.26 (s, 3H), 3.44–3.47 (m, 4H), 5.65 (s, 1H), 6.69 (s, 1H), 7.49–7.55 (m, 2H), 7.59 (d, J = 8.0 Hz, 1H), 7.67 (d, J = 1.6 Hz, 1H), 8.25 (brs, 1H), 8.41 (d, J = 8.0 Hz, 1H), 8.68 (dd, J = 1.2, 4.8 Hz, 1H),

9.26 (d, $J = 1.6$ Hz, 1H), 14.57 (brs, 1H); ^{13}C NMR (DMSO-d_6) δ 29.33, 38.27, 58.41, 69.96, 94.40, 101.08, 124.16, 125.37, 128.50, 130.03, 131.34, 131.89, 132.72, 134.57, 134.66, 139.65, 148.48, 151.65, 159.76, 165.54.

4.11.3. 1-(2-Chloro-4-fluorophenyl)-2-(6-((3-methoxypropyl) amino)-2-phenylpyrimidin-4-yl)ethen-1-ol (**19**)

Flash column chromatography was carried out using (hexane-ethyl acetate, 3:1, v/v) to give the title compound in the enol form. Yield: (0.15 g, 47%); mp 128–129 °C; ^{1}H NMR ($CDCl_3$) δ 1.98 (p, $J = 6.4$, 12.4 Hz, 2H), 3.40 (s, 3H), 3.56–3.64 (m, 4H), 5.77 (s, 1H), 5.93 (brs, 1H), 6.67 (s, 1H), 7.04–7.07 (m, 1H), 7.20–7.23 (m, 1H), 7.48–7.51 (m, H), 7.62–7.66 (m, 1H), 8.02–8.04 (m, 2H), 15.38 (brs, 1H); ^{13}C NMR ($CDCl_3$) δ 29.23, 39.66, 58.85, 71.01, 102.28, 111.62, 113.09, 116.70, 117.67, 126.97, 127.05, 127.10, 128.67, 128.70, 130.18, 130.56, 131.05, 132.82, 137.34, 161.25, 163.75, 164.09.

4.11.4. 1-(2,4-Dichlorophenyl)-2-(6-((3-methoxypropyl)amino)-2-phenylpyrimidin-4-yl)ethen-1-ol (**20**)

Flash column chromatography was carried out using (hexane-ethyl acetate, 3:1, v/v) to give the title compound in the enol form. Yield: (0.15 g, 45%); mp 145–146 °C; ^{1}H NMR (DMSO-d_6) δ 1.86 (p, $J = 6.8$, 13.2 Hz, 2H), 3.35 (s, 3H), 3.44–3.52 (m, 4H), 5.66 (s, 1H), 6.88 (s, 1H), 7.50–7.52 (m, 4H), 7.61 (m, 1H), 7.679 (brs, 1H), 8.09–8.11 (m, 2H), 14.65 (brs, 1H); ^{13}C NMR (DMSO-d_6) δ 30.89, 34.85, 58.41, 69.98, 125.38, 127.21, 127.93, 128.51, 129.13, 130.02, 131.12, 131.38, 131.93, 134.58, 137.22, 139.66, 146.12, 151.93.

4.12. General procedure for the synthesis of compounds **16**, **17**, **21**, and **22**

A mixture of any of compounds **14**, **15**, **19**, and **20** (1 equivalent) and dimethylformamide dimethyl acetal (7 equivalents) was heated to 90 °C for 2 h and allowed to cool to ambient temperature. The excess reagent was evaporated and the residue was dissolved in absolute ethanol (10 mL), then acetamidine hydrochloride (1.2 equivalents) and sodium ethoxide were added. The mixture was stirred at ambient temperature for 6 h. The solution was concentrated *in vacuo*. The residue was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography using the proper mobile phase.

4.12.1. 4′-(2-Chloro-4-fluorophenyl)-N-(3-methoxypropyl)-2′-methyl-2-(pyridin-3-yl)-[4,5′-bipyrimidin]-6-amine (**16**)

Flash column chromatography was carried out using (hexane-ethyl acetate, 1:4, v/v) to give the title compound as sticky solid. Yield: (0.31 g, 45%); ^{1}H NMR ($CDCl_3$) δ 1.87 (m, 2H), 2.90 (s, 3H), 3.39 (s, 3H), 3.55–3.53 (m, 4H), 5.65 (brs, 1H), 6.75 (brs, 1H), 7.12–7.19 (m, 1H), 7.39 (ddd, $J = 0.4$, 4.8, 8.0 Hz, 1H), 7.48 (dd, $J = 6.0$, 8.4 Hz, 1H), 8.15 (dt, $J = 2.0$, 4.0 Hz, 1H), 8.68 (dd, $J = 1.2$, 4.8 Hz, 1H), 8.97 (d, $J = 1.2$ Hz, 1H), 9.15 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.11, 29.34, 58.81, 71.07, 105.21, 114.66, 117.18, 123.63, 129.01, 132.12, 132.89, 133.45, 134.52, 148.15, 151.12, 158.00, 161.58,162.22, 162.63, 163.12, 164.09, 168.64.

4.12.2. 4′-(2,4-Dichlorophenyl)-N-(3-methoxypropyl)-2′-methyl-2-(pyridin-3-yl)-[4,5′-bipyrimidin]-6-amine (**17**)

Flash column chromatography was carried out using (hexane-ethyl acetate, 1:4, v/v) to give the title compound as sticky solid. Yield: (0.13 g, 52%); ^{1}H NMR ($CDCl_3$) δ 1.86 (m, 2H), 2.90 (s, 3H), 3.40 (s, 3H), 3.51–3.54 (m, 4H), 5.70 (brs, 1H), 6.79 (brs, 1H), 7.40–7.46 (m, 4H), 8.16 (d, $J = 7.6$ Hz, 1H), 8.71 (dd, $J = 1.2$, 4.8 Hz, 1H), 9.02 (d, $J = 1.2$ Hz, 1H), 9.15 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.12, 29.33, 39.41, 58.83, 71.10, 105.14, 123.70, 127.56, 128.89, 129.57, 131.70, 132.93, 133.21, 134.59, 148.08, 151.01, 157.93, 162.18, 162.55, 163.05, 168.73.

4.12.3. 4′-(2-Chloro-4-fluorophenyl)-N-(3-methoxypropyl)-2′-methyl-2-phenyl-[4,5′-bipyrimidin]-6-amine (**21**)

Flash column chromatography was carried out using (hexane-ethyl acetate, 2:1, v/v) to give the title compound as sticky solid. Yield: (0.044 g, 26%); ^{1}H NMR ($CDCl_3$) δ 1.86 (m, 2H), 2.90 (s, 3H), 3.38 (s, 3H), 3.50–3.53 (m, 4H), 5.53 (t, $J = 5.6$ Hz, 1H), 6.76 (brs, 1H), 7.11–7.16 (m, 2H), 7.43–7.49 (m, 4H), 7.80–7.82 (m, 2H), 9.16 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.10, 29.44, 58.78, 71.00, 105.51, 114.54, 117.14, 126.92, 128.74, 129.28, 130.68, 132.10, 133.48, 133.99, 137.13, 158.02, 161.55, 162.25, 162.56, 164.05, 165.25, 168.41.

4.12.4. 4′-(2,4-Dichlorophenyl)-N-(3-methoxypropyl)-2′-methyl-2-phenyl-[4,5′-bipyrimidin]-6-amine (**22**)

Flash column chromatography was carried out using (hexane-ethyl acetate, 2:1, v/v) to give the title compound as sticky solid. Yield: (0.07 g, 52%); ^{1}H NMR ($CDCl_3$) δ 1.84 (m, 2H), 2.90 (s, 3H), 3.38 (s, 3H), 3.49–3.52 (m, 4H), 5.59 (brs, 1H), 6.78 (brs, 1H), 7.39–7.47 (m, 6H), 7.79–7.81 (m, 2H), 9.17 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.10, 29.43, 39.22, 58.78, 71.01, 105.43, 126.97, 127.47, 128.76, 129.15, 129.54, 130.67, 131.70, 133.27, 135.60, 136.38, 137.18, 157.98, 162.17, 162.36, 162.59,165.36, 168.46.

4.13. General procedure for the synthesis of compounds **24** and **25**

To a solution of 2-chloro-4-methylpyridine (1 equivalent) and the appropriate methyl benzoic ester derivative **11** or **12** (1 equivalent) in dry THF under nitrogen atmosphere, was added a 1.0 M solution of lithium bis(trimethylsilyl)amide in THF (1.5 equivalent) at 0 °C dropwise. The reaction mixture was stirred at 0 °C for 30 min and to room temperature for 3 h. The reaction was quenched with a saturated ammonium chloride solution, and then extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was then purified using the appropriate mobile phase.

4.13.1. 1-(2-Chloro-4-fluorophenyl)-2-(2-chloropyridin-4-yl)ethen-1-ol (**24**)

Flash column chromatography was carried out using (hexane-ethyl acetate, 3:1, v/v) to give the title compound as the ketone isomer. Yield: (0.40 g, 83%); ^{1}H NMR ($CDCl_3$) δ 4.29 (s, 2H), 7.09–7.11 (m, 1H), 7.14 (dd, $J = 1.6$, 5.2 Hz, 1H), 7.21 (dd, $J = 2.4$, 8.4 Hz, 1H), 7.26 (d, $J = 0.8$ Hz, 1H), 7.58 (dd, $J = 6.0$, 8.4 Hz, 1H), 8.34 (d, $J = 5.2$ Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 47.85, 114.79, 118.37, 123.76, 125.41, 131.68, 133.07, 134.31, 145.81, 151.78, 162.74, 165.29, 196.51.

4.13.2. 2-(2-Chloropyridin-4-yl)-1-(2,4-dichlorophenyl)ethen-1-ol (**25**)

Flash column chromatography was carried out using (hexane-ethyl acetate, 3:1, v/v) to give the title compound as the ketone isomer. Yield: (0.35 g, 87%); ^{1}H NMR ($CDCl_3$) δ 4.26 (s, 2H), 7.12 (dd, $J = 1.2$, 5.2 Hz, 1H), 7.25 (s, 1H), 7.34 (dd, $J = 2.0$, 8.4 Hz, 1H), 7.46–7.48 (m, 2H), 8.33 (d, $J = 5.2$ Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 47.85, 123.76, 125.40, 127.69, 130.56, 130.68, 132.22, 136.38, 138.31, 145.63, 149.69, 151.78, 196.79.

4.14. General procedure for the synthesis of compounds **26** and **27**

The same procedure used in synthesis of compounds **16**, **17**, **21**, and **22**.

4.14.1. 4-(2-Chloro-4-fluorophenyl)-5-(2-chloropyridin-4-yl)-2-methylpyrimidine (**26**)

Flash column chromatography was carried out using (hexane-ethyl acetate, 1:1, v/v) to give the title compound. Yield: (0.28 g, 57%); ^{1}H NMR ($CDCl_3$) δ 2.87 (s, 3H), 6.93 (dd, J = 1.6, 5.2 Hz, 1H), 7.09–7.12 (m, 2H), 7.14–7.15 (m, 1H), 7.35–7.39 (m, 1H), 8.28 (dd, J = 0.8, 5.2 Hz, 1H), 8.73 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.98, 114.90, 117.55, 122.13, 123.82, 124.14, 125.99, 128.25, 132.17, 132.34, 133.19, 146.52, 149.76, 151.92, 157.13, 161.77, 162.01, 164.30, 168.69.

4.14.2. 5-(2-Chloropyridin-4-yl)-4-(2,4-dichlorophenyl)-2-methylpyrimidine (**27**)

Flash column chromatography was carried out using (hexane-ethyl acetate, 1:1, v/v) to give the title compound. Yield: (0.245 g, 64%); ^{1}H NMR ($CDCl_3$) δ 2.80 (s, 3H), 6.89 (dd, J = 1.6, 4.8 Hz, 1H), 7.12–7.13 (m, 1H), 7.29 (m, 2H), 7.31 (m. 1H), 8.21–8.23 (m, 1H), 8.69 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 25.95, 122.11, 123.77, 127.70, 128.09, 129.87, 131.70, 132.85, 134.57, 136.30, 146.38, 149.75, 151.89, 157.17, 161.78, 168.65.

4.15. General procedure for the synthesis of compounds **28** and **29**

The same procedure used in synthesis of compounds **4a**–**e**.

4.15.1. 4-(4-(2-Chloro-4-fluorophenyl)-2-methylpyrimidin-5-yl)-2,3′-bipyridine (**28**)

Flash column chromatography was carried out using gradient elution with ethyl acetate followed by a mixture of (dichloromethane–methanol 40:1, v/v) to give the title compound as sticky solid. Yield: (0.15 g, 30%); ^{1}H NMR ($CDCl_3$) δ 2.81 (s, 3H), 7.02–7.53 (m, 3H), 7.30–7.39 (m, 2H), 7.45 (m, 1H), 8.12–8.15 (m, 1H), 8.56–8.59 (m, 2H), 8.75 (s, 1H), 8.92 (dd, J = 0.8, 2.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 25.93, 114.77, 117.42, 120.26, 122.45, 123.65, 129.21, 132.19, 132.74, 133.25, 134.23, 134.35, 144.44, 147.93, 150.14, 154.99, 157.24, 161.60, 161.99, 164.12, 168.31.

4.15.2. 4-(4-(2,4-Dichlorophenyl)-2-methylpyrimidin-5-yl)-2,3′-bipyridine (**29**)

Flash column chromatography was carried out using gradient elution with ethyl acetate followed by a mixture of (dichloromethane–ethanol 40:1, v/v) to give the title compound as sticky solid. Yield: (0.116 g, 26%); ^{1}H NMR ($CDCl_3$) δ 2.8 (s, 3H), 7.08 (dd, J = 1.6, 5.2 Hz, 1H), 7.37–7.40 (m, 4H), 7.48 (q, J = 0.8 Hz, 1H), 8.16 (ddd, J = 1.6, 2.4, 8.0 Hz, 1H), 8.64–8.6 (m, 2H), 8.61, (s, 1H), 8.97 (d, J = 2.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 26.00, 120.29, 122.42, 123.63, 127.67, 129.16, 129.89, 131.74, 133.06, 134.22, 134.33, 135.02, 136.24, 144.34, 148.12, 149.33, 150.33, 155.21, 157.31, 161.93, 168.43.

4.16. Enzyme screening

Kinase assays were performed at Reaction Biology Corporation using the 'HotSpot' assay platform [20]. Briefly, specific kinase/substrate pairs along with required cofactors were prepared in 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. Compounds were delivered into the reaction, followed ~20 min later by addition of a mixture of ATP (Sigma) and ^{33}P ATP (Perkin Elmer) to a final concentration of 10 μM. Reactions were carried out at 25 °C for 120 min, followed by spotting of the reactions onto P81 ion exchange filter paper (Whatman # 3698-915). Unbound phosphate was removed by extensive washing of filters in 0.1% phosphoric acid.

After subtraction of background derived from control reactions containing inactive enzyme, kinase activity data were expressed as the percent remaining kinase activity in test samples compared to vehicle (dimethyl sulfoxide) reactions. IC_{50} values and curve fits were obtained using Prism (GraphPad Software). Compounds were tested against both ROS1 kinase, ALK and c-Met kinase in a 10-dose IC_{50} mode with 3 fold serial dilutions starting at 20 μM, using Staurosporine as a positive control.

4.17. HCC78 cell screening

Cell line assays was performed at Reaction Biology Corporation using the 'Cell Viability Assay' [20]. RPMI 1640 medium containing 10% FBS was used for cell growth medium and assay medium for the screening of the synthesized compounds **6a**–**e**, and **7a**–**e**. HCC78 cells (non-small cell lung carcinoma) were treated with each the synthesized compounds in duplicates with 10 doses in 3-fold serial dilution starting at 100 μM. Cells were cultured in 37 °C and 5% CO_2 humidified incubator for 48 h. Then the cell availability was measured using CellTiter-Glo assay kit following the protocol from the manufacture (Promega). Also HCC-78 cells were treated with Staurosporine as control compound in duplicates with 10 doses in 3-fold serial dilution starting at 10 μM.

4.18. Docking studies

Docking studies were performed using 'Molecular Operating Environment' (MOE version 2008.10; Chemical Computing Group Canada). The program operated under 'Windows XP' operating system installed on an Intel Pentium IV PC with a 2.8 MHz processor and 512 RAM. Compounds to be studied were built using the builder interface of the MOE program and subjected to energy minimization tool using the included MOPAC 7.0. The produced model was subjected to Systematic Conformational Search where all items were set as default with RMS gradient of 0.01 kcal/mol and RMS distance of 0.1 A. The X-ray crystallographic structure of ROS1 kinase enzyme co-crystallized with crizotinib (PDB code 3zbf) was obtained from the Protein Data Bank [18,21]. The enzyme was prepared for docking studies where: (i) the ligand molecule with any existing solvent molecules were removed from the enzyme active site. (ii) hydrogen atoms were added to the structure with their standard geometry. (iii) MOE Alpha Site Finder was used for the active sites search in the enzyme structure and dummy atoms were created from the obtained alpha spheres. The obtained ligand–enzyme complex model was then used in calculating the energy parameters using MMFF94x force field energy calculation and predicting the ligand–enzyme interactions at the active site.

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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.2014.11.023.

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