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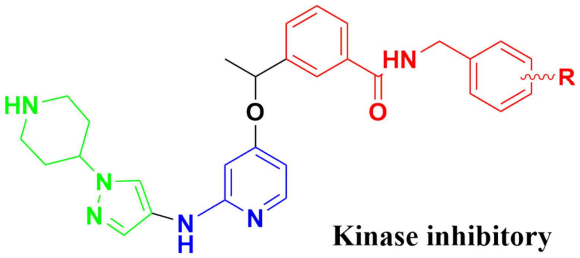
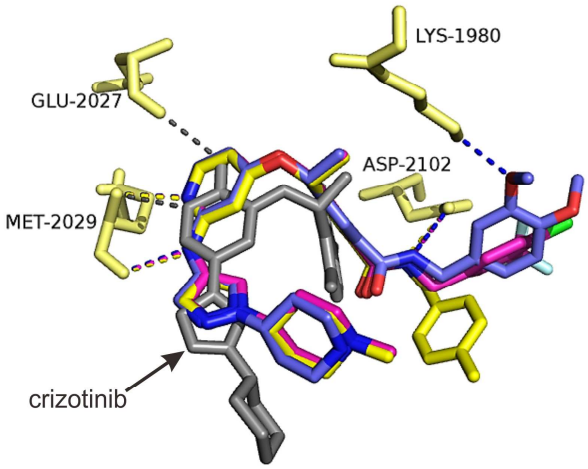


		Kinase inhibitory ROS1	ALK
14c	R=3,4-OCH_3	0.365μM	4.45μM
13d	R=4-Me	0.44μM	3.1μM
13e	R=3-CF_3,4-Cl	2.30μM	9.2μM



Design, synthesis, biological evaluation and molecular modeling of novel 2-amino-4-(1-phenylethoxy) pyridine derivatives as potential ROS1 inhibitors

Yuanxin Tian[1], Tingting Zhang[1], Lifan Long, Zhonghuang Li, Shanhe Wan, Guangfa Wang, Yonghuan Yu, Ju Hou, Xiaoyun Wu[*], Jiajie Zhang[*]

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1 These authors contributed equally to this work.

Abstract

With the aim of discovering potential and selective inhibitors targeting ROS1 kinase, we rationally designed synthesized and evaluated two series of novel 2-amino-pyridine derivatives with 1-phenylethoxy at C-3 and C-4 position. The enzymic assays results indicated that six of the new compounds **13b**-**13d** and **14a**-**14c** showed remarkably higher inhibitory activities against ROS1 kinase. The most promising compounds, **13d** and **14c** displayed the most desired ROS1 inhibitory activity with IC_{50} values of 440 nM and 370 nM respectively. Furthermore, **13d** and **14c** displayed ROS1 inhibitory selectivity of about 7-fold and 12-fold, relative to that of ALK sharing about 49% amino acid sequence homology in the kinase domains. They also showed good anti-proliferative effects against ROS1-addicted HCC78 cell lines with the IC_{50} values of 8.1 μM and 65.3 μM, respectively. Moreover, molecular docking and molecular dynamics simulation studies disclosed that compound **14c** and **13d** shared similar binding poses with Crizotinib except the selective binding site of ROS1. It also gave a probable molecular explanation for their activity and selectivity, which the methoxyl group in benzene ring was the crucial to the selectivity to ROS1 versus ALK.

Keywords: ROS1 inhibitor; Pyridine derivatives; Molecular docking; Molecular dynamics simulation.

1. Introduction

Receptor tyrosine kinases (RTKs) are critical players in cellular communication network and function, including cell proliferation, migration, metabolism, differentiation, and survival. They also are pivotal regulators of normal cellular processes, in which their activities are normally tightly controlled and regulated [1,2]. As one of the last two orphan human RTKs and the sole member of the ROS1 family, Proto-oncogene tyrosine-protein kinase ROS (ROS1) and its mechanism of activation of human kinase have not been fully identified in different body tissues so far [3]. However, the ectopic expression of ROS1, as a results of gene fusion, has been identified as another clinically actionable oncogenic driver mutation in several cancerous diseases, including colorectal cancer [4], inflammatory myofibroblastic tumor (IMT) [5], gastric adenocarcinoma [6], non small cell lung cancer (NSCLC) [7-9] and so on [10]. Accumulating evidence advocates that ROS1 has become a drugable target [11,12]. Many researchers are driving efforts to develop effective ROS1 inhibitors [13]. Until now, most of ROS1 inhibitors with excellent in vitro inhibitory activities, including the first macrocyclic 3(rd)-generation inhibitor lorlatinib [14], are the clinical/preclinical success of ALK inhibitors, such as Crizotinib [15-17], Entrectinib [18] and Brigatinib [19] (their chemical structures were listed in Figure. 1(A)), since ROS1 shares about 49% amino acid sequence homology with ALK in the kinase domain and up to 77% of the adenosine triphosphate(ATP)-binding region[20]. Few compounds are obtained by rational design with active against ROS1 but inactive against ALK except a series of pyrazole derivatives such as KIST301072 and their modification 7c (Figure. 1(A)), which developed by S. H. Lee's group [8,21,22]. Crizotinib, granted accelerated approval for the first-generation ALK inhibitor by FDA on 2011, recently expanded its new indication to the treatment of ROS1 rearranged NSCLC in March 2016 [13]. However, the insurgence of their mutation that confer resistance [23] to the current therapy

raises the need to identify new structures. Hence, it's absolutely important for the medicinal chemistry researchers to further explore and produce selective and potent ROS1 inhibitors as therapeutic strategy for different human malignancies [24].

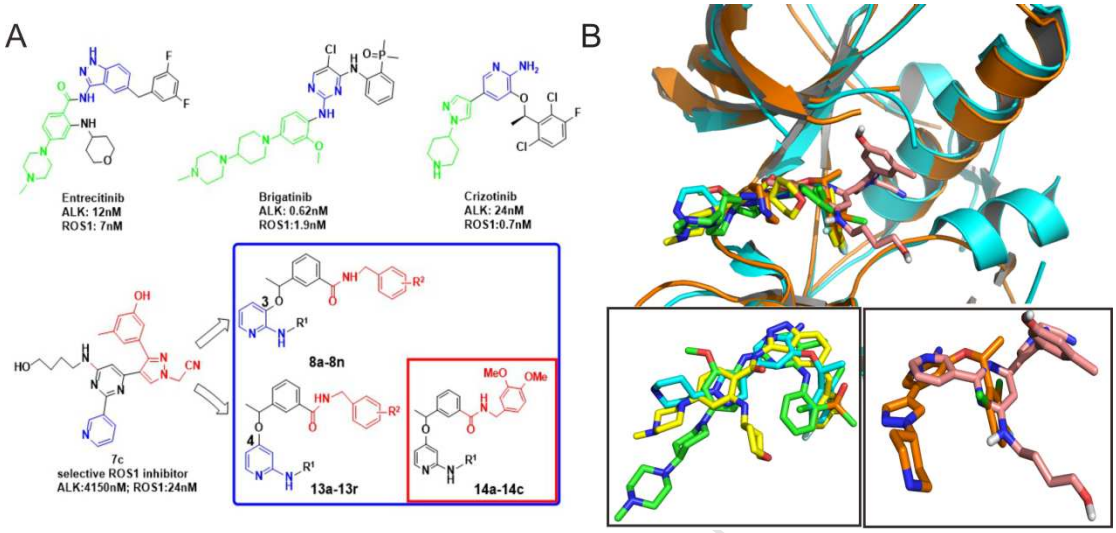


Figure 1.(A) The structures of clinical/preclinical ALK/ROS1 inhibitors and our designed new compounds (framed by blue rectangle); (B) their binding poses comparison (ALK+Crizotinib (2xp2, cyan); ALK+Entrectinib (5fto, yellow); ALK+Brigatinib (5j7h, green); ROS1+Crizotinib (3zbf, orange); ROS1+7c (docking structure, pink)).

As most clinical successful ALK inhibitors showed promising ROS1 activity, it was an efficient method to explore the ROS1 inhibitor based on the ALK inhibitor [25]. The co-crystal structures of ALK (cyan) and ROS1 (orange) complexes and the docking structure of ROS1 and selective inhibitor 7c (pink) were compared to find their subtle structure difference. As shown in **Figure 1**, the blue fragments can interact with hinge region; and the green fragment can occupy the solvent area near the hinge region. The active site occupied by red regions of 7c, which was composed of P-loop and DFG-motif in ALK/ROS1 seems not typical interaction. Hence the Crizotinib, Entrectinib and Brigatinib showed the dual ALK/ROS1 activities. However, for selective inhibitor ROS1:**7c** complex, the red fragment in **7c** displayed additional interaction with the active site mentioned above. The interaction may contribute to the selectivity for ROS1 versus ALK, which is in accord with the conclusion from S. H. Lee [8]. Our results based on molecule simulation also proved the opinion [26]. Combining our previous studies [27], the

2-amino-3-(1-phenylethoxy)pyridine was considered as a molecule's skeleton of potent ALK/ROS1 inhibitors. If the interactions between ligand and the P-loop and/or DFG-motif (corresponding to the red region in **7c**) are enhanced, the selectivity of ligand to ROS1 versus ALK may be improved. Corresponding to the pyrimidinyl in **7c**, the benzene ring of 2-amino-3-(1-phenylethoxy) pyridine skeleton would increase hydrophobic interactions with the hydrophobic pocket. Furthermore, as a hydrogen bond acceptor and donator, the acylamide moiety acted as a linker because of its flexibility that had been widely applied in drug design.

Based on the structure comparisons and binding modes analysis above, two series of derivatives at C-3, C-4 substituted 2-amino-3-(1-phenylethoxy) pyridine and 2-amino-4-(1-phenylethoxy) pyridine (structures shown in **Figure 1(A)**) were designed and synthesized in order to improve the ROS1 inhibitory selectivity. The substitutes in 2-NH_2 position (R^1 group) were changed to investigate the influence from the solvent area near the hinge region. Then the new compounds were evaluated the enzymic activities against ALK/ROS1 kinases and in vitro anti-proliferative activities in their corresponding cell lines. Based on the results, two compounds were selected for further selectivity study. Their binding modes and the selective mechanism were disclosed by docking and MD simulation, which verified our design strategy and displayed the structure-activity relationship.

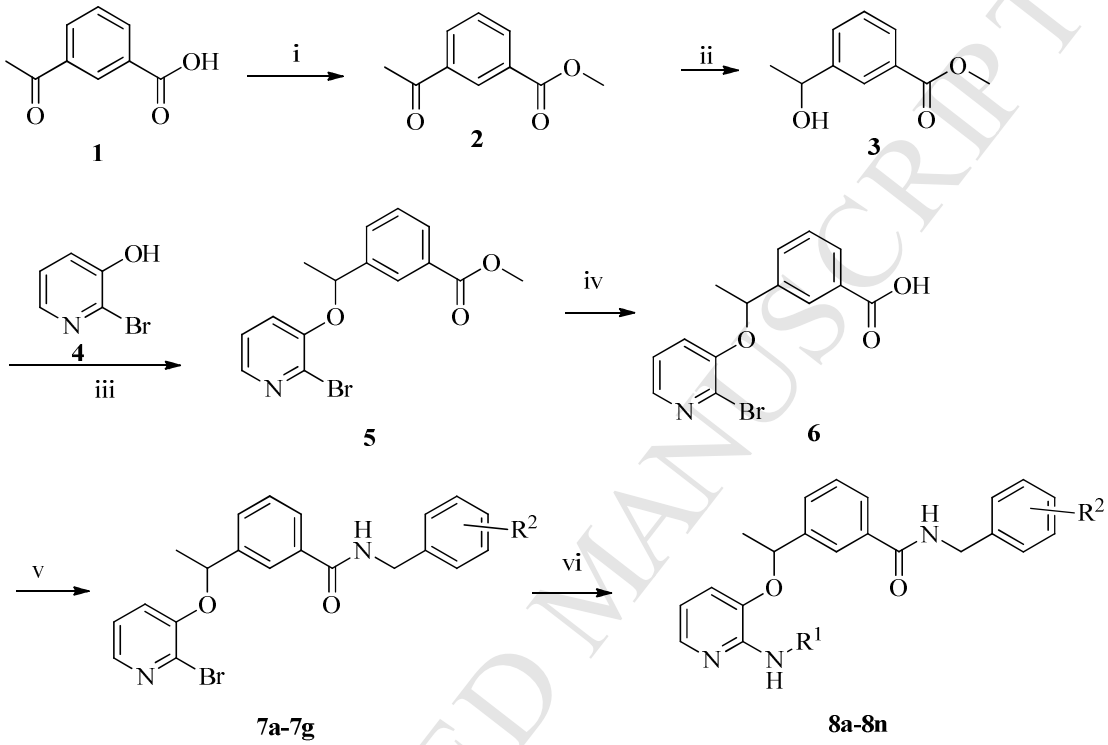
2. **Results and discussion**

2.1. Chemistry

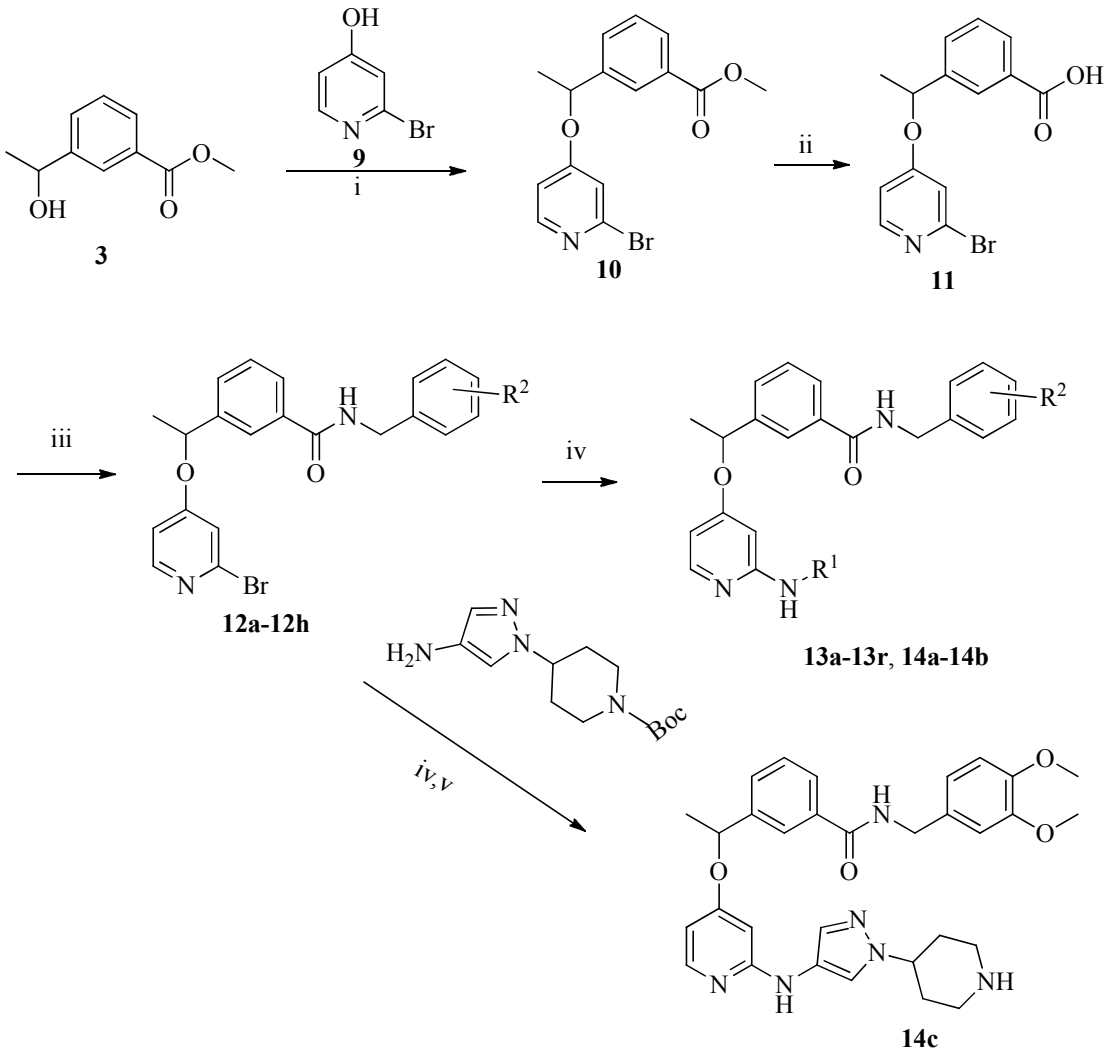
The target compounds were obtained by the following synthesized route with benzoic acid derivatives illustrated in **Scheme 1.** Esterification of commercially available 3-acetylbenzoic acid with concentrated sulfuric acid provided intermediate **2**, which was then subjected to reduction by sodium cyanoborohydride to offer compound **3** as colorless liquid. Compound **5** was prepared via Mitsunobu coupling of compound **3** with 2-bromopyridin-3-ol, treating with triphenylphosphine and diethyl azodicarboxylate. The intermediate compound **5** followed by hydrolysis allowed readily access to compound **6**. Compound **6** was condensed with a variety of benzylamines to acquire intermediates **7a-7g**.The resulting intermediates **7a-7g** was transformed to target compounds **8a-8n** of series-**I** with different reagents by

Buchwald-Hartwig cross coupling reaction as described in **Scheme 1**. Target compounds **13a-13r** and **14a-14c** of series-II were similarly obtained of series-I as shown in **Scheme 2**.

The chemical structures of all target compounds were confirmed by ^{1}H NMR, ^{13}C NMR, ESI-MS, and HRMS.



Scheme 1. Reagents and conditions: (i) H_2SO_4, methanol, reflux; (ii) Sodium cyanoborohydride, methanol, HCl, r.t.; (iii) DEAD, PPh_3, THF, r.t.; (iv) methanol, NaOH, H_2O, rt; (v) CDI, CH_2Cl_2, r.t.; (vi) Cs_2CO_3, $Pd_2(dba)_3$, BINAP, DMF, 90℃.



Scheme 2. Reagents and conditions: (i) DEAD, PPh_3, THF, r.t.; (ii) methanol, NaOH, H_2O, r.t.; (iii) CDI, CH_2Cl_2, r.t.; (iv) Cs_2CO_3, $Pd_2(dba)_3$, BINAP, DMF, 90℃; (v) acetate/CH_2Cl_2 (8%-100%), r.t..

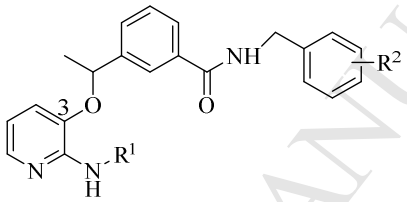
2.2. Biological Activity

2.2.1Kinase inhibitory activity

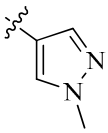
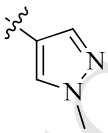
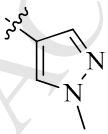
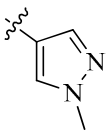
The kinase inhibitory activities of the target compounds were tested in duplicates with 10 doses in 3-fold serial dilution starting at 10 mM and their inhibitory activities were summarized in **Table 1** and **Table 2**. As shown in this two tables, compounds **8e-8h, 8j-8k, 8n, 13a, 13e-13j, 13m-13o** and **13r** exhibited moderate inhibitory activities against ROS1 with IC_{50} values ranging from 1.4-9.6 μM. Notably, compound **13b-13d** and **14a-14c** displayed significant potency against ROS1 with

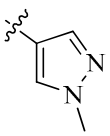
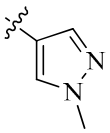
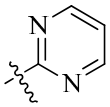
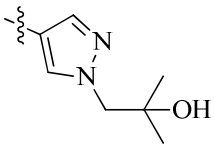
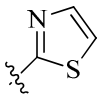
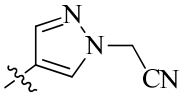
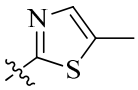
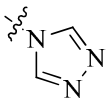
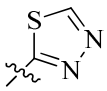
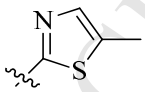
IC_{50} values ranging from 370-640 nM, and others had no significant potency against ROS1 activities (IC_{50} > 10μM). Based on the results mentioned above, we can conclude that the substitution of C-4 position of 1-phenylethoxy is more favorable for ROS1 inhibitory activity. In addition, it's noteworthy that **13d** and **14c** stood out as the most potent ROS1 inhibitors among the two series compounds with IC_{50} values of 440 nM and 370 nM, respectively. Furthermore, **13d** and **14c** showed ROS1 inhibitory selectivity of approximately 7-fold and 12-fold relative to that of ALK.

Table 1 The kinase inhibitory activities and in vitro anti-proliferative effects of synthesized compounds (at C-3 substituted).



8a-8n

Compd.	R^1	R^2	kinase inhibitory activities IC_{50}(μM)[a]		In vitro antiproliferative effects (IC_{50}, μM)[b]	
			ALK	ROS1	H3122	HCC78
8a		4-F	8.1	>10	43.8	68.5
8b		3-OMe	8.6	>10	49.4	78.6
8c		4-OCF_3	8.9	>10	8.3	73.9
8d		3-Cl,4-F	9.5	>10	35.7	7.8

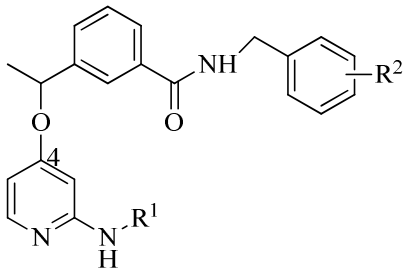
8e		3-CF_3,4-Cl	5.9	6.3	50.7	48.6
8f		2-isopropyl sulfonyl	>10	8.7	>100	63.1
8g		4-Me	>10	6.2	>100	>100
8h		4-Me	>10	9.4	9.1	20.5
8i		4-Me	8.0	>10	15.3	27.1
8j		4-Me	>10	8.7	38.4	57.4
8k		4-Me	>10	9.4	8.5	14.8
8l		4-Me	8.7	>10	23.7	9.4
8m		4-Me	7.6	>10	20.8	17.6
8n		3-Cl,4-F	8.6	9.6	8.2	34.7

[a] Values are the average of two independent experiments.

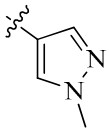
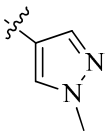
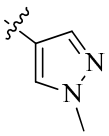
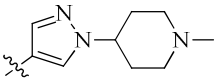
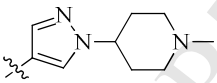
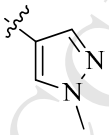
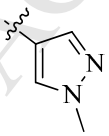
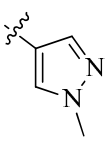
[b] The date were means from at least three independent experiments.

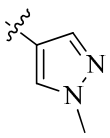
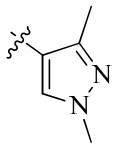
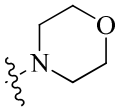
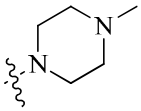
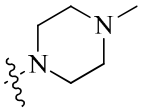
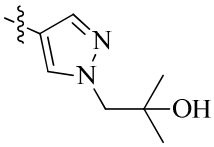
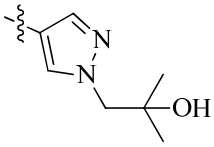
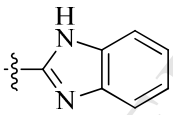
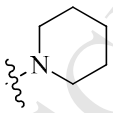
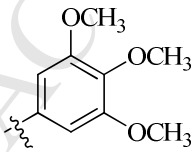
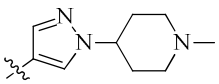
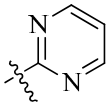
[c] N.D, not determined.

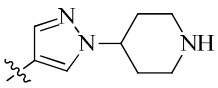
Table 2. The kinase inhibitory activities and in vitro anti-proliferative effects of synthesized compounds (at C-4 substituted).



13a-13r,14a-14c

Compd.	R^1	R^2	kinase inhibitory activities IC_{50} (μM)[a]		*In vitro* antiproliferative effects (IC_{50}, μM)[b]	
			ALK	ROS1	H3122	HCC78
13a		3-OMe	6.1	1.8	>100	64.4
13b		3,4-di-OMe	3.6	0.64	38.5	76.6
13c		3,4,5-tri-OMe	2.3	0.41	29.0	73.9
13d		4-Me	3.1	0.44	8.9	8.1
13e		3-CF_3,4-Cl	9.2	2.3	8.0	7.3
13f		3,5- di-F	4.7	2.4	7.6	13.2
13g		3,5- di-CF_3	>10	7.4	24.7	9.3
13h		4-CN	6.7	1.5	35.1	8.7

13i		4-Me	6.7	1.8	7.7	8.9
13j		4-Me	3.5	1.4	13.4	17.5
13k		4-Me	6.8	>10	>100	68.3
13l		4-Me	7.0	>10	8.1	26.2
13m		3-CF_3,4-Cl	9.7	9.1	21.7	23.5
13n		4-Me	>10	2.4	>100	49.7
13o		3-CF_3,4-Cl	9.6	8.4	38.4	>100
13p		4-Me	>10	>10	9.4	>100
13q		4-Me	4.7	>10	37.4	24.5
13r		4-Me	8.7	2.4	7.8	15.7
14a		3,4-di-OMe	1.8	0.57	14.8	9.0
14b		3,4-di-OMe	2.8	0.44	>100	>100

14c		3,4-di-OMe	4.5	0.37	47.2	65.3
Crizotinib			N.D	N.D	0.8	2.0

[a] Values are the average of two independent experiments.

[b] The date were means from at least three independent experiments.

[c] N.D, not determined.

Afterwards, to further investigate the kinase selectivity of **13d** and **14c**, we evaluated the inhibitory activity against a panel of 12 RTKs associated with tumorigenesis [22,28,29] at a concentration of 1 μM. As shown in the **Table 3**, **13d** and **14c** didn't display significant potency against the 12 RTKs (percent inhibition values ranging from 0% to 16%).Consequently, **13d** and **14c** are considered as two highly selective ROS1 inhibitors.

Table 3. Kinase selectivity profiles of compound **13d** and **14c**.

Kinase	inhibition rate at 1 μM (%)[a]		**Kinase**	inhibition rate at 1 μM (%))[a]	
	13d	**14c**		**13d**	**14c**
ABL1	9	11	JAK2	12	10
BRAF	15	13	KIT	2	3
CDK2/cyclin A	1	2	LTK(TYK1)	9	15
EGFR/(ErbB1)	1	0	MAPK3	8	7
FGFR1	11	16	MET(c-Met)	3	5
IGF1R	7	9	PDGFR alpha	6	1

[a] Values are the average of two independent experiments.

2.2.2. In vitro anti-proliferative activity

The synthesized compounds were set to research the inhibitory effects on cell proliferation in ALK-addicted H3122 cell and ROS1-addicted HCC78 cell by the standard MTT colorimetric assay in vitro[30]. Crizotinib was used as positive control. The results summarized in **Table 1** and **Table 2**, which were derived from at least three independent experiments. It can be noted that most of compounds displayed moderate to high potency against HCC78 and H3122 cell, some of the compounds showed parallel anti-proliferative activities against HCC78 cell line as Crizotinib.

Eight compounds (**8d, 8l, 13d-13e, 13g-13i** and **14a**) displayed significant potency with IC_{50} values of 7.3-9.4 μM, which turned out to be similar to that of Crizotinib (IC_{50}=2.00 μM). Nevertheless, the cellular responses of some synthesized compounds were not consistent with the enzymatic assays absolutely. **13b-13c** and **14b-14c** exhibited good potency against ROS1 kinase, however, gave the weak potency against HCC78 cell line, which expressed the SLC34A2-ROS1 fusion protein. **8d, 8h-8i, 8k, 8l-8m, 13e-13f, 13g, 13i, 13l-13m** and **13q-13r** showed poor activities against ROS1 kinase, but they displayed anti-proliferative activities against HCC78 cell line at IC_{50}＜10μM. We supposed that the different sensitivity to ROS1 fusion genes induced by synthesized compounds led to different response in HCC78 cell line, and some compounds could induce cell apoptosis, or accelerate cell metabolism, or have some other mechanism [31].

2.3. Preliminary structure-activity relationships

Based on the biological activities listed in **Table 1** and **Table 2**, the preliminary structure-activity relationships of the synthesized compounds against ROS1 were summarized in **Figure 2**. In general, the 1-phenylethoxy substitution at C-4 position of the 2-amino-pyridine ring displayed better activity than that at C-3 position. For R^1 group, one of hydrogen atoms of 2-NH_2, replaced by hydrophilic group, would be in favor of activity. Among the substitution groups, 1-(piperidin-4-yl)-1*H*-pyrazol-4-yl was the optimal group, then followed by 1-(1-methylpiperidin-4-yl)-1*H*-pyrazol-4-yl, pyrimidin-2-yl, 1,3-dimethyl-1*H*-pyrazol-4-yl and 1-methyl-1*H*-pyrazol-4-yl. ROS1 activities were relatively poor when R^1 was other heterocyclics and benzenes. For R^2 group, the methoxyl groups on the benzene ring of the synthesized compounds were in favor of the activity. Among these groups, 3,4,5-trimethoxyl was the optimal group, then followed by 3,4-dimethoxyl, 3-methoxyl, 4-methyl and 4-CN. For example, compound **13c** (IC_{50}=410 nM), possessing three favorable methoxyl groups, maintained significant potency against ROS1 kinase than the relevant two and mono-substituted analogs **13b** (IC_{50}=640 nM) and **13a** (IC_{50}=1.8 μM)**.** Furthermore**,** the compounds **14a-14c** with methoxyl in benzene ring also showed promising

activities against ROS1 kinase. The results demonstrated that the methoxyl may be crucial to the selectivity of ROS1 versus ALK.

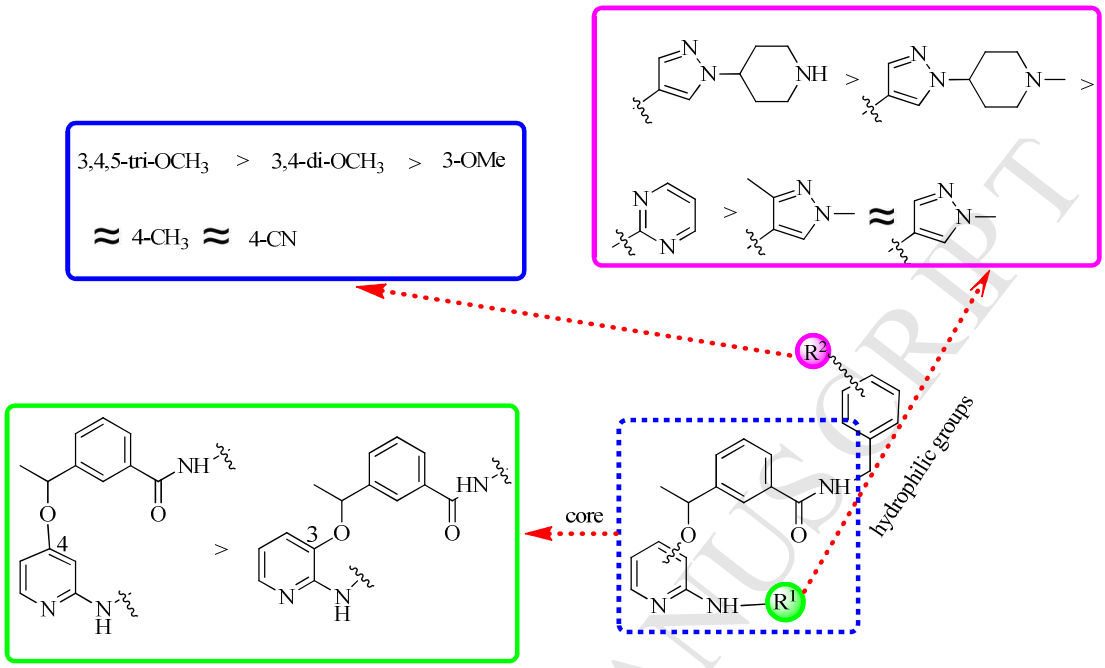


Figure 2. Preliminary structure-activity relationships of ROS1 kinase inhibitory

2.4 Molecular modeling:

In order to investigate the probable binding conformations of the new compounds, docking experiments were performed with the Surflex-Dock program from Sybyl7.3. The crystal structure of ROS1 (PDB entry: 3zbf) and the compounds are pretreated according to our previous work [32]. The docking results indicated that the **13a-13r** and **14a-14c** adopted a similar binding mode to the Crizotinib. However, the compounds **8a-8n** couldn't fit the binding site of ROS1 well because of the steric hindrance effect. Due to the same R^1 group that extended to the solvent region near the hinge region, the compounds **13d**, **13e** and **14c** were chosen as examples to illustrate the binding modes (displayed in **Figure 3**).

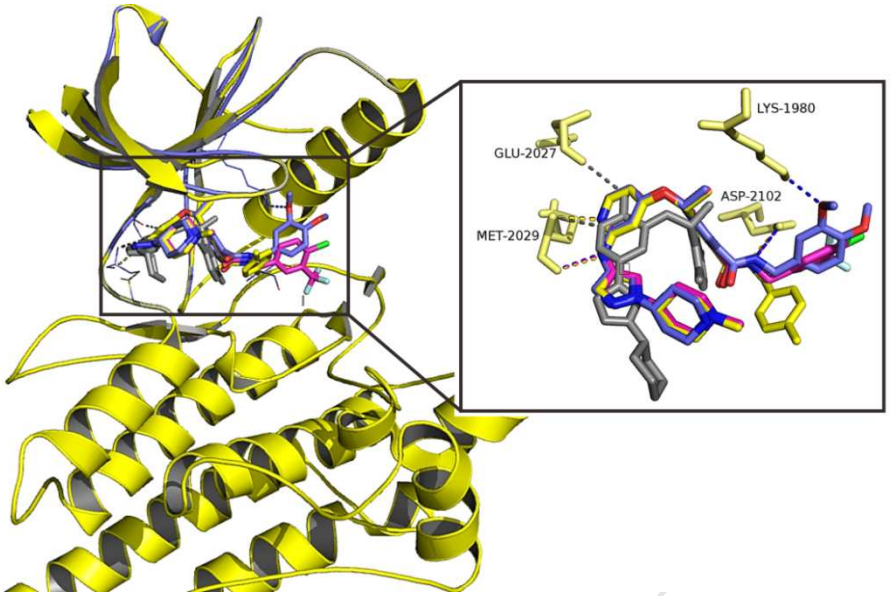


Figure 3. Superimposition of docking poses for the new compound **14c** (blue), **13e** (magenta), **13d** (yellow) and Crizotinib (gray) based on docking.

As we expected, the 2-amine-pyridine moiety can formed double H-bonds with the Met2029 from hinge region. Comparing Crizotinib, the difference between the two binding mode was that Crizotinib could form double H-bonds with Glu2027 and Met2029. The interaction with hinge regions is a typical characteristic of the dual ALK/ROS1 inhibitors. Our designed group (red group in **Figure 1**) extended to another active site composed of P-loop and DFG-motif. The NH from acylamino made H-bond to Asp2102 from DFG-motif. The **14c** with -OCH_3 group formed an additional H-bond with Lys1980 from beta-sheet, which could explain that the compounds with -OCH_3 group (**13a-13c**, **14a-14c**) displayed the lower inhibitory activity against ROS1 and better selectivity to ALK. Moreover, the benzamide (ph-CO-NH) linker could match the hydrophobic pocket and increased the hydrophobic interactions, which was favorable for the activity and selectivity.

Although compound **14c** showed the relative selectivity and activity as we expected, it was not desired. Moreover, the difference between **13e** and **14c** is only the R^2 group, while the activities showed great difference. We noticed that the compounds with 3-CF_3-4-Cl group have poorer selectivity, such as **8e, 13e, 13m** and **13o**. In order to further explore the contribution of R^2 group in details, molecular dynamic (MD)

simulations were carried out on the active compound **14c**. The compound **13e** with the same R^1 group and less activity was chosen as a comparison.

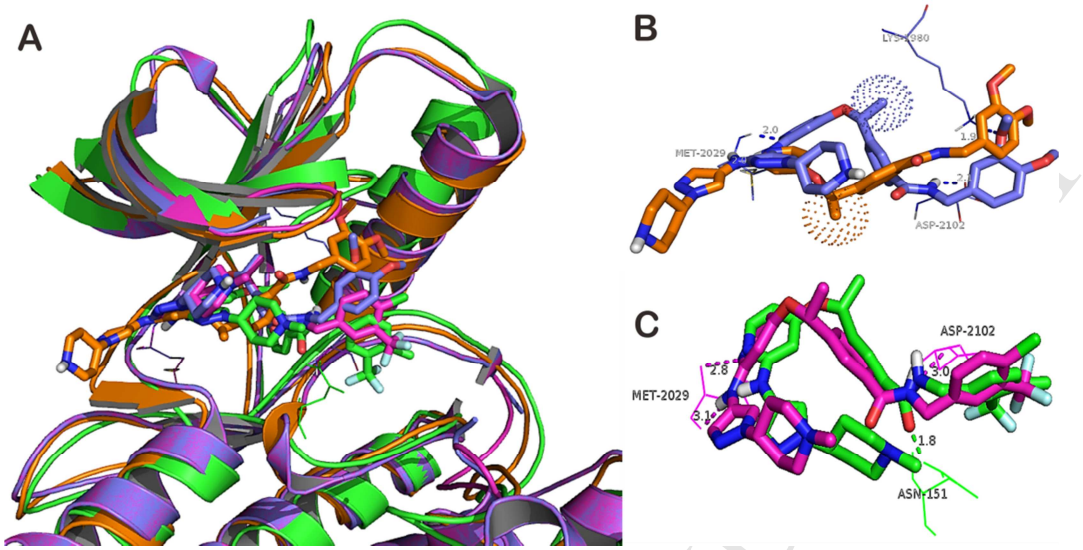


Figure 4. (A)The conformation changes of **14c** (initial: blue; after MD: orange) and **13e** (initial: magenta; after MD: green) after 20 ns Molecular Dynamic simulation; (B) View of superimposed conformational change of the ligand **14c**. The OCH(CH_3) group turned from inner to outer of the ATP-binding site (shown in ball); (C) View of superimposed conformational change of the ligand **13e.**

The docking structures as the initial structures were processed in explicit aqueous solution. After 20 ns MD simulation, the stability of each system is evaluated by the root-mean-square deviation (RMSD) of the backbone atoms against their initial structures (shown in Figure S1 of supporting information). From the Figure S1, we could see the **14c** had larger RMSD values than **13e** although the RMSD values of protein backbone atoms were relatively stable, indicating there was larger conformation changes of **14c** than that of **13e**. In fact, compound **14c** really displayed larger conformation changes (shown in **Figure 4)**. In initial docking structure, the amino-pyridine of two compounds can interact with Met2029 from the hinge region by double H-bonds and the acylamide linker formed H-bonds with Asp2102 from DFG-motif. Compound **14c** has an additional H-bond between Lys1980 and -OCH_3 group. During 20ns MD simulation, the H-bonds mentioned above in **13e** were

weakened and gradually disappeared because of another H-bond between C=O and Asn2084. Although the conformation had little changes, the key interaction for activities was disappeared and there were no other interactions in selective active site between the ligand and protein, which directly led to the low activity of ROS1. For compound **14c**, because of the H-bonds made by -OCH_3 and Lys1980, the conformation displayed larger changes to fit the selective active site composed by the DFG-motif and P-loop. The ethoxyl group was almost displayed 180 degree turned from the inner to outer of hydrophobic pocket (shown in **Figure 4B**). This conformation change directly led to the disappearance of hydrogen bonds in the hinge region. The details of the hydrogen bonds of **14c** and **13e** are listed in **Table 4**.

Table 4. Hydrogen bonds analysis of 14c and 13e during the 20ns MD simulation.

13e (2.3μM)			14c (0.37μM)		
donor	acceptor	occupancy	donor	acceptor	occupancy
ASN151-Side-ND2	MOL298-Side-O2	**89.85%**	MOL298-Side-N2	**MET96-Main-O**	**32.95%**
MET96-Main-N	MOL298-Side-N1	**12.28%**	**MET96-Main-N**	MOL298-Side-N6	**22.51%**
MOL298-Side-N6	**ASP169-Side-OD1**	4.87%	MOL298-Side-N1	**ASP169-Side-OD1**	18.67%
HIE144-Side-NE2	MOL298-Side-F1	2.12%	MOL298-Side-N1	**ASP169-Side-OD2**	4.57%
HIE144-Side-NE2	MOL298-Side-F2	1.54%	LYS47-Side-NZ	MOL298-Side-O2	0.07%
HIE144-Side-NE2	MOL298-Side-F3	1.08%	LYS47-Side-NZ	MOL298-Side-N1	0.02%

From the **Table 4**, we could see the occupancy of H-bonds from the hinge region and DFG-motif in **14c** were 55.46% and 23.24%, which is corresponding in **13e** were 12.28% and 4.87%, while another H-bonds between Asn151(Asn2084) and C=O showed the high occupancy 89.85% in **13e**. Moreover, because of the H-bond from Lys1980, the 3,4-dimethoxypheny group could match the hydrophilic pocket made by P-loop and DFG-motif, which benefited the lower activity to ROS1. This phenomenon happened to in accord with the interaction of compound **7c,** one of the selective ROS1 from S. H. Lee's group. Therefore, the **14c** displayed the relative selective to ROS1 versus ALK. On account of the reduction of key interaction in the hinge region and the selective site, **14c** didn't show promising activity. Further modification of **14c** is underway based on our simulation results.

3. Conclusion

In summary, in order to explore selective ROS1 inhibitor, two series of new 2-amino-pyridine derivatives with 1-phenylethoxy at C-3 and C-4 position processing amide linker were designed, synthesized and evaluated for their biological activities against ROS1 and ALK. The results indicated that most compounds, especially C4-substituted derivatives, exhibited moderate enzyme activities of ROS1 and ALK (with the IC_{50} below 10 μM). Especially, the compounds **13d** and **14c** stood out the promising ROS1 inhibitory activities with the IC_{50} values of 440 nM and 370 nM. Moreover, they also displayed the ROS1 inhibitory selectivity of about 10-fold relative to that of ALK in enzyme domain. The molecular docking demonstrated that the binding poses of **13d** and **14c** with ROS1 were similar to Crizotinib except the selective binding site. Molecular dynamic simulation revealed that the distinct conformation changes of **14c** lead to the better selectivity and the unsatisfactory activity to ROS1. The 2-amino-pyridine derivatives with 1-phenylethoxy at C-3 and C-4 position of our studies had not been reported, which had broken the limits of several existing ROS1 inhibitors. The further modifications of **13d** and **14c** to improve the selectivity and activity to ROS1 are underway based on our results.

4. Experimental

4.1. Chemistry

All chemicals were purchased from commercial vendors and were used without further purification unless otherwise stated. The ^{1}H and ^{13}C NMR spectra were generated on a BRUKER AVIII 400 MHz and 101 MHz spectrometer with tetramethylsilane (TMS) as the internal standard. Mass spectra were performed on WATERS ZQ4000 with electrospray ionization source. High resolution mass spectra (HRMS) were recorded on a Thermo Fisher Scientific Orbitrap Fusion Tribrid (Q-OT-qIT) mass spectrometer (Thermo Fisher Scientific, Bremen, German). The CHN elemental analyses of all the target compounds were recorded by LECO

TRUSPEC CHN analyzer. All reactions were assayed by thin-layer chromatography (TLC). Flash column chromatography separations were performed on silica gel (200-300 mesh, Qingdao Ocean Chemicals, China) by using Yamazen AI-580 flash chromatography. Melting points were obtained on an X-4 Melting-point Apparatus with Microscope (Gongyi City Yuhua Instrument Co., Ltd., Henan, China) and were uncorrected.

*4.1.1. methyl 3-acetylbenzoate (**2**)*

To a solution of 3-acetylbenzoic acid (**1**) (5.00 g, 30.7 mmol) in MeOH (147 mL) added into 10 mL concentrated H_2SO_4. The reaction mixture was stirred at reflux for 24h. After removal of the solvent, the residue was poured into H_2O (80 mL) and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. Filtration and concentration in vacuo to give **2** as light yellow liquid (4.54 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, ArH, 1H), 8.24 (t, *J* = 7.5 Hz, ArH, 1H), 8.17 (d, *J* = 7.5 Hz, ArH, 1H), 7.57 (t, *J* = 7.7 Hz, ArH, 1H), 3.96 (s, OCH_3, 3H), 2.66 (s, CH_3, 3H). ESI-MS m/z: 179 $[M+H]^+$.

*4.1.2. methyl 3-(1-hydroxyethyl)benzoate (**3**)*

To a solution of methyl 3-acetylbenzoate **2** (4.00 g, 22.5 mmol) in MeOH (104 mL) was added sodium cyanoborohydride slowly (2.84 g, 45.0 mmol). The mixture was adjusted pH to 3, and stirred at ambient temperature for overnight. After removal of the solvent, the residue was poured into H_2O (65 mL) and extracted with EtOAc. The organic layer was washed with H_2O and brine and dried over $MgSO_4$, The concentrates was purified by flash chromatography on silica gel eluting with EtOAc/ petroleum ether (1%-10%) to give **3** as pale yellow liquid (3.24 g, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (s, ArH, 1H), 7.82 (d, *J* = 7.6 Hz, ArH, 1H), 7.61 (d, *J* = 7.6 Hz, ArH, 1H), 7.47 (t, *J* = 7.6 Hz, ArH, 1H), 5.31 (d, *J* = 4.4 Hz, OH, 1H), 4.80 (q, *J* = 6.0 Hz, CH, 1H), 3.86 (s, OCH_3, 3H), 1.34 (d, *J* = 6.4 Hz, CH_3, 3H). ESI-MS m/z: 181$[M+H]^+$.

*4.1.3. methyl 3-(1-((2-bromopyridin-3-yl)oxy)ethyl)benzoate (**5**)*

To a stirred solution of triphenylphosphine (6.31 g, 24.1 mmol) and DEAD (4.49,

25.8 mmol) in THF (30 mL) at 0 ℃was added a solution of **3** (3.10 g, 17.2 mmol) and 2-bromopyridin-3-ol **4** (3.44 g, 19.8 mmol) in THF (20 mL). The resulting luminous yellow solution was stirred under a nitrogen atmosphere at ambient temperature for 24 h at which all starting materials had been consumed. The solvent was removed, the residue was purified by flash chromatography on silica gel eluting with EtOAc/ petroleum ether (1%-20%) to give **5** as light yellow liquid (4.97 g, 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06 (s, ArH, 1H), 7.92 (dd, *J* = 1.2, ArH, 4.4 Hz, 1H), 7.88 (d, *J* = 7.6 Hz, ArH, 1H), 7.72 (d, *J* = 7.6 Hz, ArH, 1H), 7.54 (t, *J* = 7.8 Hz, ArH, 1H), 7.45 (dd, *J* = 1.2, 8.0 Hz, ArH, 1H), 7.29 (dd, *J* = 4.8, 8.0 Hz, ArH, 1H), 5.84 (q, *J* = 6.4 Hz, CH, 1H), 3.86 (s, OCH_3, 3H), 1.62 (d, *J* = 6.4 Hz, CH_3, 3H). ESI-MS m/z: 336.6 and 338.5 $[M+H]^+$.

*4.1.4. 3-(1-((2-bromopyridin-3-yl)oxy)ethyl)benzoic acid (**6**)*

To a solution of **5** (4.80 g, 14.29 mmol) in MeOH (60mL) was added sodium hydroxide (1.42 g, 35.5mmol) in water (21mL), the mixture was stirred at ambient temperature for 1.5h, after the complete conversion of the starting material, the solution was acidified to pH=5 with 1M hydrochloric acid. The mixture was filtered and condensed to give **6** as white solid (4.14 g, 90%).^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 (s, ArH, 1H), 7.92 (dd, *J* = 1.2, 4.4 Hz, ArH, 1H), 7.86 (d, *J* = 7.6 Hz, ArH, 1H), 7.68 (d, *J* = 8.0 Hz, ArH, 1H), 7.51 (t, *J* = 7.6 Hz, ArH, 1H), 7.44 (dd, *J* = 0.8, 8.0 Hz, ArH, 1H), 7.29 (dd, *J* = 4.8, 8.8 Hz, ArH, 1H), 5.82 (q, *J* = 6.4 Hz, CH, 1H), 1.62 (d, *J* = 6.4 Hz, CH_3, 3H). ESI-MS m/z: 320.2 and 322.3$[M-H]^-$.

*4.1.5. 3-(1-((2-bromopyridin-3-yl)oxy)ethyl)-N-(4-fluorobenzyl)benzamide (**7a**)*

CDI (112 mg, 0.69 mmol) was added under nitrogen to a solution of **6** (200 mg, 0.62 mmol) in dry methylene chloride (15 mL). The solution was stirred at room temperature for 1 h, then (4-fluorophenyl)methanamine (116 mg, 0.93 mmol) was added, and stirred at room temperature under nitrogen or 24 h. After the complete conversion of the starting material, the reaction was quenched with 1 M HCl. The aqueous phase was extracted with methylene chloride (3 × 50 mL). The combined organic phases were washed with brine until neutral pH, dried on Na_2SO_4, filtered,

and the solvent was eliminated under reduced pressure to give **7a** (240 mg, 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.07 (t, *J* = 5.8 Hz, NH, 1H), 7.96 (s, ArH, 1H), 7.91 (d, *J* = 4.0 Hz, ArH, 1H), 7.80 (d, *J* = 8.0 Hz, ArH, 1H), 7.58 (d, *J* = 7.6 Hz, ArH, 1H), 7.47 (t, *J* = 7.6 Hz, ArH, 1H), 7.42 (d, *J* = 7.6 Hz, ArH, 1H), 7.38 – 7.35 (m, ArH, 2H), 7.29 (dd, *J* = 4.4, 8.0 Hz, ArH, 1H), 7.16 (t, *J* = 8.6 Hz, ArH, 2H), 5.75 (q, *J* = 6.4 Hz, CH, 1H), 4.46 (d, *J* = 5.6 Hz, CH_2, 2H), 1.63 (d, *J* = 6.0 Hz, CH_3, 3H). ESI-MS m/z: 427.4 and 429.5$[M-H]^-$.

*4.1.6. 3-(1-((2-bromopyridin-3-yl)oxy)ethyl)-N-(3-methoxybenzyl)benzamide (**7b**)*

Compound **6** (200 mg, 0.62 mmol) and (3-methoxyphenyl)methanamine (127 mg, 0.93 mmol) following the similar procedure described for the preparation of **7a** afforded **7b** (251 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8.0 Hz, ArH, 2H), 7.71 (d, *J* = 8.0 Hz, ArH, 1H), 7.53 (d, *J* = 7.6 Hz, ArH, 1H), 7.39 (t, *J* = 7.6 Hz, ArH, 1H), 7.24 (t, *J* = 7.8 Hz, ArH, 1H), 7.03 – 7.00 (m, ArH, 1H), 6.96 – 6.88 (m, ArH, 4H), 6.82 (d, *J* = 8.0 Hz, ArH, 1H), 5.36 (q, *J* = 6.2 Hz, CH, 1H), 4.59 (d, *J* = 5.6 Hz, CH_2, 2H), 3.77 (s, OCH_3, 3H), 1.69 (d, *J* = 6.4 Hz, CH_3, 3H). ESI-MS m/z: 441.5 and 443.6$[M+H]^+$.

*4.1.7. 3-(1-((2-bromopyridin-3-yl)oxy)ethyl)-N-(4-(trifluoromethoxy)benzyl)benzamide (**7c**)*

Compound **6** (200 mg, 0.62 mmol) and (4-(trifluoromethoxy)phenyl) methane mine (177 mg, 0.93 mmol) following the similar procedure described for the preparation of **7a** afforded **7c** (264 mg, 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (t, *J* = 5.6 Hz, NH, 1H), 7.97 (s, ArH, 1H), 7.91 (d, *J* = 4.4 Hz, ArH, 1H), 7.81 (d, *J* = 7.6 Hz, ArH, 1H), 7.59 (d, *J* = 7.6 Hz, ArH, 1H), 7.50 – 7.38 (m, ArH, 4H), 7.36 – 7.27 (m, ArH, 3H), 5.76 (q, *J* = 6.2 Hz, CH, 1H), 4.50 (d, *J* = 5.6 Hz, CH_2, 2H), 1.64 (d, *J* = 6.4 Hz, CH_3, 3H). ESI-MS m/z: 493.3 and 495.4$[M-H]^-$.

*4.1.8. 3-(1-((2-bromopyridin-3-yl)oxy)ethyl)-N-(3-chloro-4-fluorobenzyl)benzamide (**7d**)*

Compound **6** (200 mg, 0.62 mmol) and (3-chloro-4-fluorophenyl) methanamine (100 mg, 0.93 mmol) following the similar procedure described for the preparation of

7a afforded **7d** (261 mg, 91%).^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (t, *J* = 6.0 Hz, NH, 1H), 7.96 (s, ArH, 1H), 7.91 (dd, *J* = 1.6, 4.4 Hz, ArH, 1H), 7.80 (d, *J* = 7.8 Hz, ArH, 1H), 7.59 (d, *J* = 8.0 Hz, ArH, 1H), 7.53 (dd, *J* = 2.0, 7.2 Hz, ArH, 1H), 7.48 (t, *J* = 7.8 Hz, ArH, 1H), 7.42 (dd, *J* = 1.6, 8.0 Hz, ArH, 1H), 7.36 – 7.33 (m, ArH, 2H), 7.29 (dd, *J* = 4.4, 8.0 Hz, ArH, 1H), 5.76 (q, *J* = 6.2 Hz, CH, 1H), 4.46 (d, *J* = 6.0 Hz, CH_2, 2H), 1.63 (d, *J* = 6.4 Hz, CH_3, 3H). ESI-MS m/z: 463.5 and 465.6$[M+H]^+$.

*4.1.9. 3-(1-((2-bromopyridin-3-yl)oxy)ethyl)-N-(4-chloro-3-(trifluoromethyl)benzyl)benzamide (**7e**)*

Compound **6** (200 mg, 0.62 mmol) and (4-chloro-3-(trifluoromethyl)phenyl) methanamine (194 mg, 0.93 mmol) following the similar procedure described for the preparation of **7a** afforded **7e** (276 mg, 87%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (t, *J* = 5.8 Hz, NH, 1H), 7.96 (s, ArH, 1H), 7.91 (dd, *J* = 1.2, 4.4 Hz, ArH, 1H), 7.82 – 7.79 (m, ArH, 2H), 7.70 (d, *J* = 8.4 Hz, ArH, 1H), 7.65 – 7.59 (m, ArH, 2H), 7.49 (t, *J* = 7.6 Hz, ArH, 1H), 7.42 (dd, *J* = 1.6, 8.4 Hz, ArH, 1H), 7.28 (dd, *J* = 4.8, 8.4 Hz, ArH, 1H), 5.76 (q, *J* = 6.4 Hz, CH, 1H), 4.54 (d, *J* = 6.0 Hz, CH_2, 2H), 1.63 (d, *J* = 6.0 Hz, CH_3, 3H). ESI-MS m/z: 511.8 and 513.7$[M-H]^-$.

*4.1.10. 3-(1-((2-bromopyridin-3-yl)oxy)ethyl)-N-(2-(isopropylsulfonyl) benzyl)benzamide (**7f**)*

Compound **6** (200 mg, 0.62 mmol) and (2-(isopropylsulfonyl)phenyl) methanamine (198 mg, 0.93 mmol) following the similar procedure described for the preparation of **7a** afforded **7f** (278 mg, 87%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (t, *J* = 5.6 Hz, NH, 1H), 7.99 (s, ArH, 1H), 7.92 (d, *J* = 3.6 Hz, ArH, 1H), 7.87 (dd, *J* = 8.0, 14.0 Hz, ArH, 2H), 7.72 (t, *J* = 7.6 Hz, ArH, 1H), 7.61 (d, *J* = 4.4 Hz, ArH, 2H), 7.57 – 7.49 (m, ArH, 2H), 7.43 (d, *J* = 8.0 Hz, ArH, 1H), 7.32 (dd, *J* = 4.8, 8.4 Hz, ArH, 1H), 5.78 (q, *J* = 6.4 Hz, CH, 1H), 4.85 (m, CH_2, 2H), 3.75 (m, SCH, 2H), 1.65 (d, *J* = 6.4 Hz, OCHCH$_3$, 3H), 1.23 (d, *J* = 6.8 Hz, CH_3, 3H), 1.22 (d, *J* = 6.8 Hz, CH_3, 3H). ESI-MS m/z: 517.5 and 519.6$[M+H]^+$.

*4.1.11. 3-(1-((2-bromopyridin-3-yl)oxy)ethyl)-N-(4-methylbenzyl)benzamide (**7g**)*

Compound **6** (200 mg, 0.62 mmol) and *p*-tolylmethanamine (111 mg, 0.93 mmol)

following the similar procedure described for the preparation of **7a** afforded **7g** (244 mg, 93%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (t, *J* = 6.0 Hz, NH, 1H), 7.96 (s, ArH, 1H), 7.91 (dd, *J* = 1.6, 4.4 Hz, ArH, 1H), 7.79 (d, *J* = 7.6 Hz, ArH, 1H), 7.57 (d, *J* = 8.0 Hz, ArH, 1H), 7.47 (t, *J* = 7.8 Hz, ArH, 1H), 7.42 (dd, *J* = 1.6, 8.4 Hz, ArH, 1H), 7.28 (dd, *J* = 4.8, 8.4 Hz, ArH, 1H), 7.21 (d, *J* = 8.0 Hz, ArH, 2H), 7.15 – 7.13 (m, ArH, 2H), 5.75 (q, *J* = 6.2 Hz, CH, 1H), 4.43 (d, *J* = 6.0 Hz, CH_2, 2H), 2.28 (s, $ArCH_3$, 3H), 1.63 (d, *J* = 6.4 Hz, CH_3, 3H). ESI-MS m/z: 425.4 and 427.5$[M+H]^+$.

4.1.12. methyl 3-(1-((2-bromopyridin-4-yl)oxy)ethyl)benzoate ***(10)***

Compound **3** (3.10 g, 17.2 mmol) and 2-bromopyridin-4-ol **9** (3.44 g, 19.8 mmol) following the similar procedure described for the preparation of **5** afforded **10** (4.80 g, 83%). ^{1}H NMR (400 MHz, DMSO) δ 8.14 (d, *J* = 5.6 Hz, ArH, 1H), 8.03 (s, ArH, 1H), 7.90 (d, *J* = 7.6 Hz, ArH, 1H), 7.72 (d, *J* = 7.6 Hz, ArH, 1H), 7.55 (t, *J* = 7.6 Hz, ArH, 1H), 7.23 (d, *J* = 1.6 Hz, ArH, 1H), 7.03 (dd, *J* = 2.0, 6.0 Hz, ArH, 1H), 5.87 (q, *J* = 6.2 Hz, CH, 1H), 3.86 (s, OCH_3, 3H), 1.58 (d, *J* = 6.0 Hz, CH_3, 3H),ESI-MS m/z: 336.6 and 338.5 $[M+H]^+$.

4.1.13. 3-(1-((2-bromopyridin-4-yl)oxy)ethyl)benzoic acid ***(11)***

Compound **10** (4.80 g, 14.29 mmol) and sodium hydroxide (1.42 g, 35.5mmol) following the similar procedure described for the preparation of **6** afforded **11** (3.6 g, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.06 (s, COOH, 1H), 8.14 (d, *J* = 5.6 Hz, ArH, 1H), 8.01 (s, ArH, 1H), 7.88 (d, *J* = 7.6 Hz, ArH, 1H), 7.68 (d, *J* = 7.6 Hz, ArH, 1H), 7.52 (t, *J* = 7.6 Hz, ArH, 1H), 7.23 (s, ArH, 1H), 7.03 (d, *J* = 5.6 Hz, ArH, 1H), 5.86 (q, *J* = 6.4 Hz, CH, 1H), 1.58 (d, *J* = 6.0 Hz, CH_3, 3H), ESI-MS m/z: 320.2 and 322.3 $[M-H]^-$.

4.1.14. 3-(1-((2-bromopyridin-4-yl)oxy)ethyl)-N-(3-methoxybenzyl)benzamide ***(12a)***

Compound **11** (200 mg, 0.62 mmol) and (3-methoxyphenyl)methanamine (125 mg, 0.93 mmol) following the similar procedure described for the preparation of **7a** afforded **12a** (224 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 5.6 Hz, ArH, 1H),7.86 (d, *J* = 8.0 Hz, ArH, 1H), 7.71 (d, *J* = 8.0 Hz, ArH, 1H), 7.46 (d, *J* = 7.6 Hz, ArH, 1H), 7.39 (t, *J* = 7.6 Hz, ArH, 1H), 7.24 (t, *J* = 7.8 Hz, ArH, 1H), 6.96 – 6.88 (m, ArH, 3H), 6.82 (d, *J* = 8.0 Hz, ArH, 1H), 6.68 (d, *J* = 8.0 Hz, ArH, 1H), 5.36 (q, *J* =

6.2 Hz, CH, 1H), 4.59 (d, *J* = 5.6 Hz, CH_2, 2H), 3.77 (s, OCH_3, 3H), 1.69 (d, *J* = 6.4 Hz, CH_3, 3H). ESI-MS m/z: 441.5 and 443.6$[M+H]^+$.

4.1.15. 3-(1-((2-bromopyridin-4-yl)oxy)ethyl)-N-(3,4-dimethoxybenzyl)benzamide ***(12b)***

Compound **11** (200 mg, 0.62 mmol) and (3,4-dimethoxyphenyl)methanamine (155 mg, 0.93 mmol) following the similar procedure described for the preparation of **7a** afforded **12b** (251 mg, 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.97 (t, *J* = 5.8 Hz, NH, 1H), 8.13 (d, *J* = 6.0 Hz, ArH, 1H), 7.94 (s, ArH, 1H), 7.81 (d, *J* = 7.6 Hz, ArH, 1H), 7.58 (d, *J* = 8.0 Hz, ArH, 1H), 7.48 (t, *J* = 7.6 Hz, ArH, 1H), 7.20 (d, *J* = 2.4 Hz, ArH, 1H), 7.01 (dd, *J* = 2.4, 5.6 Hz, ArH, 1H), 6.95 (d, *J* = 1.6 Hz, ArH, 1H), 6.90 (d, *J* = 8.0 Hz, ArH, 1H), 6.84 (dd, *J* = 2.0, 8.4 Hz, ArH, 1H), 5.79 (q, *J* = 6.4 Hz, CH, 1H), 4.41 (d, *J* = 6.0 Hz, CH_2, 2H), 3.74 (s, OCH_3, 3H), 3.72 (s, OCH_3, 3H), 1.60 (d, *J* = 6.4 Hz, CH_3, 3H). ESI-MS m/z: 471.3 and 473.2$[M+H]^+$.

4.1.16. 3-(1-((2-bromopyridin-4-yl)oxy)ethyl)-N-(3,4,5-trimethoxybenzyl)benzamide ***(12c)***

Compound **11** (200 mg, 0.62 mmol) and (3,4,5-trimethoxyphenyl) meth anamine (183 mg, 0.93 mmol) following the similar procedure described for the preparation of **7a** afforded **12c** (276 mg, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (t, *J* = 5.8 Hz, NH, 1H), 8.13 (d, *J* = 5.6 Hz, ArH, 1H), 7.94 (s, ArH, 1H), 7.82 (d, *J* = 7.6 Hz, ArH, 1H), 7.59 (d, *J* = 7.6 Hz, ArH, 1H), 7.49 (t, *J* = 7.6 Hz, ArH, 1H), 7.20 (d, *J* = 2.0 Hz, ArH, 1H), 7.01 (dd, *J* = 2.4, 5.6 Hz, ArH, 1H), 6.65 (s, ArH, 2H), 5.80 (q, *J* = 6.2 Hz, CH, 1H), 4.42 (d, *J* = 6.0 Hz, CH_2, 2H), 3.75 (s, 2×OCH_3, 6H), 3.64 (s, OCH_3, 3H), 1.60 (d, *J*= 6.4 Hz, CH_3, 3H). ESI-MS m/z: 500.4 and 502.5$[M+H]^+$.

4.1.17. 3-(1-((2-bromopyridin-4-yl)oxy)ethyl)-N-(4-methylbenzyl)benzamide ***(12d)***

Compound **11** (200 mg, 0.62 mmol) and *p*-tolylmethanamine (111 mg, 0.93 mmol) following the similar procedure described for the preparation of **7a** afforded **12d** (231 mg, 88%).^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (s, NH, 1H), 8.13 (d, *J* = 5.2 Hz, ArH, 1H), 7.95 (s, ArH, 1H), 7.82 (d, *J* = 7.2 Hz, ArH, 1H), 7.58 (d, *J* = 7.6 Hz, ArH, 1H), 7.48 (t, *J* = 7.6 Hz, ArH, 1H), 7.20 (s, ArH, 3H), 7.14 (d, *J* = 7.2 Hz, ArH, 2H),

7.02 (d, *J* = 5.2 Hz, ArH, 1H), 5.79 (q, *J* = 6.0 Hz, CH, 1H), 4.44 (d, *J* = 4.8 Hz, CH_2, 2H), 2.28 (s, $ArCH_3$, 3H), 1.60 (d, *J* = 5.6 Hz, CH_3, 3H). ESI-MS m/z: 425.4 and 427.5$[M+H]^+$.

*4.1.18. 3-(1-((2-bromopyridin-4-yl)oxy)ethyl)-N-(4-chloro-3-(trifluoromethyl)benzyl) benzamide (**12e**)*

Compound **11** (200 mg, 0.62 mmol) and (4-chloro-3-(trifluoromethyl)phenyl) methanamine (194 mg, 0.93 mmol) following the similar procedure described for the preparation of **7a** afforded **12e** (265 mg, 82%).^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (t, *J* = 5.8 Hz, NH, 1H), 7.97 (s, ArH, 1H), 7.92 (d, *J* = 4.4 Hz, ArH, 1H), 7.81 (d, *J* = 9.2 Hz, ArH, 2H), 7.70 (d, *J* = 8.4 Hz, ArH, 1H), 7.62 (dd, *J* = 8.4, 16.0 Hz, ArH, 2H), 7.49 (t, *J* = 7.8 Hz, ArH, 1H), 7.42 (d, *J* = 8.0 Hz, ArH, 1H), 7.28 (dd, *J* = 4.4, 8.0 Hz, ArH, 1H), 5.76 (q, *J* = 6.4 Hz, CH, 1H), 4.54 (d, *J* = 6.0 Hz, CH_2, 2H), 1.64 (d, *J* = 6.4 Hz, CH_3, 3H). ESI-MS m/z: 511.8 and 513.7$[M-H]^-$.

*4.1.19. 3-(1-((2-bromopyridin-4-yl)oxy)ethyl)-N-(3,5-difluorobenzyl)benzamide (**12f**)*

Compound **11** (200 mg, 0.62 mmol) and (3,5-difluorophenyl)methanamine (132 mg, 0.93 mmol) following the similar procedure described for the preparation of **7a** afforded **12f** (234 mg, 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (t, *J* = 5.8 Hz, NH, 1H), 8.13 (d, *J* = 5.6 Hz, ArH, 1H), 7.97 (s, ArH, 1H), 7.85 (d, *J* = 7.6 Hz, ArH, 1H), 7.61 (d, *J* = 7.6 Hz, ArH, 1H), 7.50 (t, *J* = 7.6 Hz, ArH, 1H), 7.21 (s, ArH, 1H), 7.10 (t, *J* = 9.2 Hz, ArH, 1H), 7.03 (t, *J* = 7.2 Hz, ArH, 3H), 5.81 (q, *J* = 6.0 Hz, CH, 1H), 4.51 (d, *J* = 5.6 Hz, CH_2, 2H), 1.60 (d, *J* = 6.4 Hz, CH_3, 3H). ESI-MS m/z: 447.5 and 449.6$[M+H]^+$.

*4.1.20. N-(3,5-bis(trifluoromethyl)benzyl)-3-(1-((2-bromopyridin-4-yl)oxy)ethyl)benzamide (**12g**)*

Compound **11** (200 mg, 0.62 mmol) and (3,5-bis(trifluoromethyl)-phenyl) methanamine (226 mg, 0.93 mmol) following the similar procedure described for the preparation of **7a** afforded **12f** (288 mg, 85%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (t, *J* = 5.8 Hz, NH, 1H), 8.13 (d, *J* = 6.0 Hz, ArH, 1H), 8.02 (d, *J* = 7.2 Hz, ArH, 3H),

7.95 (s, ArH, 1H), 7.83 (d, *J* = 7.6 Hz, ArH, 1H), 7.62 (d, *J* = 7.6 Hz, ArH, 1H), 7.51 (t, *J* = 7.8 Hz, ArH, 1H), 7.20 (d, *J* = 2.4 Hz, ArH, 1H), 7.01 (dd, *J* = 2.4, 6.0 Hz, ArH, 1H), 5.81 (q, *J* = 6.4 Hz, CH, 1H), 4.66 (d, *J* = 6.0 Hz, CH_2, 2H), 1.60 (d, *J* = 6.4 Hz, CH_3, 3H).ESI-MS m/z: 547.5 and 549.6$[M+H]^+$.

4.1.21. 3-(1-((2-bromopyridin-4-yl)oxy)ethyl)-N-(4-cyanobenzyl)benzamide ***(12h)***

Compound **11** (200 mg, 0.62 mmol) and 4-(aminomethyl)benzonitrile (122 mg, 0.93 mmol) following the similar procedure described for the preparation of **7a** afforded **12h** (240 mg, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.19 (t, *J* = 5.6 Hz, NH, 1H), 8.14 (d, *J* = 5.6 Hz, ArH, 1H), 7.97 (s, ArH, 1H), 7.84(d, *J* = 7.6, ArH, 1H), 7.81 (d, *J* = 7.6, ArH, 2H), 7.61 (d, *J* = 7.6 Hz, ArH, 1H), 7.52 (d, *J* = 7.6 Hz, ArH, 3H), 7.21 (s, ArH, 1H), 7.02 (d, *J* = 5.6 Hz, ArH, 1H), 5.81 (q, *J* = 6.0 Hz, CH, 1H), 4.56 (d, *J* = 5.6 Hz, CH_2, 2H), 1.60 (d, *J* = 6.0 Hz, CH_3, 3H). ESI-MS m/z: 436.5 and 438.5$[M+H]^+$.

4.1.22.

N-(4-fluorobenzyl)-3-(1-((2-((1-methyl-1H-pyrazol-4-yl)amino)pyridin-3-yl)oxy)ethyl) benzamide ***(8a)***

A mixture of compound **7a** (212 mg, 0.50 mmol), 1-methyl-1*H*-pyrazol-4-amine (97 mg, 1.00 mmol), Pd_2 (dpa)$_3$ (30 mg, 0.03 mmol), BINAP (60 mg, 0.10 mmol) and Cs_2CO_3 (446 mg, 1.40 mmol) in DMF (15 mL) was degassed and charged with nitrogen for three times and then heated in an oil bath at 90 ℃ for 7 h under the protection of nitrogen. The reaction mixture was diluted with water (30 mL) and extracted with EtOAc (30 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. The concentrates was purified by flash chromatography on silica gel eluting with EtOAc/CH_2Cl_2 (8%-100%) to give **8a** (90 mg, 45%). mp 105-107 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, ArH, 1H), 7.87 (s, ArH, 1H), 7.76 (dd, *J* = 1.2, 4.8 Hz, ArH, 1H), 7.68 (d, *J* = 7.6 Hz, ArH, 1H), 7.53 – 7.51 (m, ArH, 2H), 7.44 (t, *J* = 7.6 Hz, ArH, 1H), 7.36 – 7.32 (m, ArH, 2H), 7.05 (t, *J* = 8.8 Hz, ArH, 2H), 6.85 (s, ArNH, 1H), 6.73 (dd, *J* = 1.2, 8.0 Hz, ArH, 1H), 6.49 – 6.45 (m, ArH, 1H), 5.39 (q, *J* = 6.4 Hz, CH, 1H), 4.62 (d, *J* = 5.6 Hz, CH_2, 2H), 3.91

(s, NCH_3, 3H), 1.75 (d, *J* = 6.4 Hz, CH_3, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 163.4, 146.7, 142.9, 140.0, 138.7, 134.9, 133.9, 130.6, 129.6, 129.5, 129.2, 128.5, 126.1, 124.4, 123.2, 121.2, 117.3, 115.7, 115.5, 112.9, 76.5, 43.4, 39.1, 24.3. ESI-MS m/z: 446.6$[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{25}H_{24}FN_5O_2$ $[M+H]^+$, 446.1990; found, 446.1992. Anal. Calcd. for $C_{25}H_{24}FN_5O_2$: C, 67.40; H, 5.43; N, 15.72; found: C, 67.14; H, 5.57; N, 15.89.

4.1.23.
N-(3-methoxybenzyl)-3-(1-((2-((1-methyl-1H-pyrazol-4-yl)amino)pyridin-3-yl)oxy)ethyl)benzamide ***(8b)***

Compound **7b** (225 mg, 0.50 mmol) and 1-methyl-1*H*-pyrazol-4-amine (97 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **8b** (118 mg, 52%). mp 90-92 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (t, *J* = 6.0 Hz, NH, 1H), 8.20 (s, ArH, 1H), 8.08 (s, ArH, 1H), 8.01 (s, ArH, 1H), 7.78 (d, *J* = 7.6 Hz, ArH, 1H),7.65 – 7.62 (m, ArH, 3H), 7.45 (t, *J* = 7.8 Hz, ArH, 1H), 7.24 (t, *J* = 8.0 Hz, ArH, 1H), 6.98 (dd, *J* =1.2, 8.0 Hz, ArH, 1H), 6.89 (d, *J* = 8.0 Hz, ArNH, 1H), 6.88 (s, ArH, 1H), 6.83 – 6.80 (m, ArH, 1H), 6.47 (dd, *J* = 5.2, 8.0 Hz, ArNH, 1H), 5.63 (q, *J* = 6.4 Hz, CH, 1H), 4.45 (d, *J* = 6.0 Hz, CH_2, 2H), 3.81 (s, CH_3, 3H), 3.73 (s, CH_3, 3H), 1.67 (d, *J* = 6.4 Hz, $CHCH_3$, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.5, 159.7, 147.0, 143.2, 141.6, 140.1, 138.5, 135.0, 130.6, 129.8, 129.0, 128.9, 126.8, 125.5, 124.2, 120.8, 119.8, 117.7, 113.4, 112.7, 112.5, 75.5, 55.4, 43.0, 39.0, 24.3. ESI-MS m/z: 458.6 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{26}H_{27}N_5O_3$$[M+H]^+$, 458.2185; found, 458.2190. Anal. Calcd. for $C_{26}H_{27}N_5O_3$: C, 68.25; H, 5.95; N, 15.31; found: C, 68.08; H, 6.12; N, 15.13.

4.1.24.
3-(1-((2-((1-methyl-1H-pyrazol-4-yl)amino)pyridin-3-yl)oxy)ethyl)-N-(3-(trifluoromethoxy)benzyl)benzamide ***(8c)***

Compound **7c** (248 mg, 0.50 mmol) and 1-methyl-1*H*-pyrazol-4-amine (97 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **8c** (122 mg, 48%). mp 102-105 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, ArH, 1H), 7.89 (s, ArH, 1H), 7.76 (d, *J* = 5.2 Hz, ArH, 1H), 7.69 (d, *J* = 7.6 Hz, ArH,

1H), 7.53 (d, *J* = 4.8 Hz, ArH, 2H), 7.45 (t, *J* = 7.6 Hz, ArH, 1H), 7.4 (d, *J* = 8.0 Hz, ArH, 3H), 7.21 (d, *J* = 8.0 Hz, ArH, 2H), 6.90 (s, ArH, 1H), 6.74 (d, *J* = 7.6 Hz, ArH, 1H), 6.59 (s, ArNH, 1H), 6.47 (dd, *J* = 5.2, 7.6 Hz, ArH, 1H), 5.40 (q, *J* = 6.2 Hz, CH, 1H), 4.66 (d, *J* = 5.6 Hz, CH_2, 2H), 3.91 (s, NCH_3, 3H), 1.76 (d, *J* = 6.4 Hz, CH_3, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 148.5, 146.7, 142.9, 140.0, 138.8, 136.9, 134.7, 130.6, 129.3, 129.2, 128.5, 126.2, 124.6, 124.4, 123.3, 121.2, 117.3, 112.9, 76.5, 43.3, 39.1, 24.4. ESI-MS m/z: 512.6 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{26}H_{24}F_3N_5O_3[M+H]^+$, 512.1906; found, 512.1909. Anal. Calcd. for $C_{26}H_{24}F_3N_5O_3$: C, 61.05; H, 4.73; N, 13.69; found: C, 61.40; H, 4.56; N, 13.37.

4.1.25.

N-(3-chloro-4-fluorobenzyl)-3-(1-((2-((1-methyl-1H-pyrazol-4-yl)amino)pyridin-3-yl) oxy)ethyl)benzamide ***(8d)***

Compound **7d** (232 mg, 0.50 mmol) and 1-methyl-1*H*-pyrazol-4-amine (97 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **8d** (151 mg, 63%). mp 99-101 ℃. ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, ArH, 1H), 7.88 (s, ArH, 1H), 7.76 (dd, *J* = 1.2, 5.2 Hz, ArH, 1H), 7.70 (d, J = 7.6 Hz ArH, 1H), 7.53 (d, *J* = 7.6 Hz, ArH, 1H), 7.52 (s, ArH, 1H), 7.46 – 7.39 (m, ArH, 2H), 7.23 (m, ArH, 1H), 7.11 (t, *J* = 8.6 Hz, ArH, 1H), 6.85 (s, ArH, 1H), 6.73 (dd, *J* = 7.6, 1.2 Hz, ArH, 1H), 6.61 (s, ArNH, 1H), 6.47 (dd, *J* = 5.2, 7.6 Hz, ArH, 1H), 5.40 (q, *J* = 6.4 Hz, CH, 1H), 4.59 (d, *J* = 6.0 Hz, CH_2, 2H), 3.90 (s, NCH_3, 3H), 1.76 (d, *J* = 6.4 Hz, CH_3, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 158.7, 156.2, 146.7, 142.9, 140.0, 138.8, 135.3, 134.6, 130.6, 130.0, 129.2, 128.6, 127.6, 126.2, 124.4, 121.2, 117.3, 116.8, 116.6, 112.9, 76.5, 42.9, 39.1, 24.4. ESI-MS m/z: 480.9 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{25}H_{23}ClFN_5O_2[M+H]^+$, 480.1600; found, 480.1603. Anal. Calcd. for $C_{25}H_{23}ClFN_5O_2$: C, 62.56; H, 4.83; N, 14.59; found: C, 62.25; H, 4.58; N, 14.35.

4.1.26.

N-(4-chloro-3-(trifluoromethyl)benzyl)-3-(1-((2-((1-methyl-1H-pyrazol-4-yl)amino)py ridin-3-yl)oxy)ethyl)benzamide ***(8e)***

Compound **7e** (257 mg, 0.50 mmol) and 1-methyl-1*H*-pyrazol-4-amine (97 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **8e** (111 mg, 42%). mp 123-125 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (t, *J* = 5.8 Hz, CONH, 1H), 8.20 (s, ArH, 1H), 8.08 (s, ArH, 1H), 8.00 (s, ArH, 1H), 7.79 (dd, *J* = 1.2, 10.8 Hz, ArH, 2H), 7.69 – 7.61 (m, ArH, 4H), 7.46 (t, *J* = 7.8 Hz, ArH, 1H), 6.97 (dd, *J* = 1.2, 8.0 Hz, ArH, 1H), 6.47 (dd, *J* = 4.8, 7.6 Hz, ArH, 1H), 5.63 (q, *J* = 6.2 Hz, CH, 1H), 4.53 (d, *J* = 6.0 Hz, CH_2, 2H), 3.80 (s, NCH_3, 3H), 1.67 (d, *J* = 6.4 Hz, CH_3, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.7, 147.0, 146.0, 143.3, 140.2, 140.1, 138.5, 138.3, 135.4, 134.7, 133.5, 132.0, 130.6, 129.4, 129.1, 126.8, 125.5, 124.2, 123.8, 120.8, 117.7, 112.7, 75.5, 42.2, 39.0, 24.3. ESI-MS m/z: 531.0 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{26}H_{23}ClF_3N_5O_2[M+H]^+$, 530.1573; found, 530.1570. Anal. Calcd. for $C_{26}H_{23}ClF_3N_5O_2$: C, 58.93; H, 4.37; N, 13.22; found: C, 58.75; H, 4.13; N, 13.07.

4.1.27. N-(2-(isopropylsulfonyl)benzyl)-3-(1-((2-((1-methyl-1H-pyrazol-4-yl)amino)pyridin-3-yl)oxy)ethyl)benzamide ***(8f)***

Compound **7f** (259 mg, 0.50 mmol) and 1-methyl-1*H*-pyrazol-4-amine (97 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **8f** (123 mg, 46%). mp 156-158 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, ArH, 1H), 7.98 (dd, *J* = 1.2, 8.0 Hz, ArH, 1H), 7.85 (s, ArH, 1H), 7.81 (dd, *J* = 0.8, 7.6 Hz, ArH, 1H), 7.74 (dd, *J* = 1.6, 5.2 Hz, ArH, 1H), 7.67 – 7.63 (m, ArH, 2H), 7.54 – 7.47 (m, ArH, 3H), 7.40 (t, *J* = 7.6 Hz, ArH, 1H), 6.85 (s, ArNH, 1H), 6.71 (dd, *J* = 0.8, 7.6 Hz, ArH, 1H), 6.46 (dd, *J* = 5.2, 8.0 Hz, ArH, 1H), 5.37 (q, *J* = 6.4 Hz, CH, 1H), 4.88 (d, *J* = 6.4 Hz, CH_2, 2H), 3.92 (s, NCH_3, 3H), 3.38 – 3.23 (m, $\underline{CH}(CH_3)_2$, 1H), 1.72 (d, *J* = 6.4 Hz, $OCH\underline{CH_3}$, 3H), 1.38 (d, *J* = 6.8 Hz, $CH(\underline{CH_3})_2$, 3H) , 1.37 (d, *J* = 6.8 Hz, $CH(\underline{CH_3})_2$, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.9, 147.0, 143.3, 142.4, 140.9, 140.6, 140.1, 134.9, 134.6, 134.4, 131.1, 130.7, 129.2, 129.1, 129.1, 127.8, 126.9, 125.6, 120.9, 117.8, 112.7, 75.5, 54.4, 39.0, 24.3, 15.4, 15.3. ESI-MS m/z: 534.5 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{28}H_{31}N_5O_4S$ $[M+H]^+$, 534.2169;

found, 534.2172. Anal. Calcd. for $C_{28}H_{31}N_5O_4S$: C, 63.02; H, 5.86; N, 13.12; found: C, 63.30; H, 5.57; N, 13.02.

4.1.28.
N-(4-methylbenzyl)-3-(1-((2-(pyrimidin-2-ylamino)pyridin-3-yl)oxy)ethyl)benzamide ***(8g)***

Compound **7g** (212 mg, 0.50 mmol) and pyrimidin-2-amine (95 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **8g** (123 mg, 56%). mp 114-116 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, *J* = 4.8 Hz, ArH, 2H), 8.05 (dd, *J* = 1.6, 5.2 Hz, ArH, 1H), 7.87 (s, ArH, 1H), 7.69 (d, *J* =7.6 Hz, ArH, 1H), 7.49 (d, *J* = 7.6 Hz, ArH, 1H), 7.41 (t, *J* = 7.6 Hz, ArH, 1H), 7.26 (d, *J* = 8.0 Hz, ArH, 2H), 7.17 (d, *J* = 8.0 Hz, ArH, 2H), 6.94 (dd, *J* = 1.2, 8.0 Hz, ArH, 1H), 6.86 (t, *J* = 4.8 Hz, ArH, 1H), 6.77 (dd, *J* = 4.8, 8.0 Hz, ArH, 1H), 6.55 (s, ArNH, 1H), 5.41 (q, *J* = 6.4 Hz, CH, 1H), 4.62 (m, CH_2, 2H), 2.36 (s, $ArCH_3$, 3H), 1.75 (d, *J* = 6.4 Hz, CH_3, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 166.8, 158.9, 158.3, 143.6, 142.4, 141.9, 139.5, 137.3, 135.2, 135.0, 129.4, 129.2, 128.3, 127.9, 126.5, 124.3, 119.6, 117.4, 113.9, 77.2, 43.9, 24.3, 21.0. ESI-MS m/z: 440.5 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{26}H_{24}N_5O_2[M+H]^+$, 440.2082; found, 440.2084. Anal. Calcd. for $C_{26}H_{24}N_5O_2$: C, 71.05; H, 5.73; N, 15.93 ; found : C, 70.89; H, 5.94; N, 15.76.;

4.1.29.
3-(1-((2-((1-(2-hydroxy-2-methylpropyl)-1H-pyrazol-4-yl)amino)pyridin-3-yl)oxy)ethyl)-N-(4-methylbenzyl)benzamide ***(8h)***

Compound **7g** (259 mg, 0.50 mmol) and 1-(4-amino-1*H*-pyrazol-1-yl)-2-methylpropan-2-ol (155 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **8h** (120 mg, 48%). mp 103-105 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.97 (t, *J* = 5.8 Hz, CONH, 1H), 8.22 (s, ArH, 1H), 8.12 (s, ArH, 1H), 8.01 (s, ArH, 1H), 7.77 (d, *J* = 7.6 Hz, ArH, 1H), 7.68 – 7.62(m, ArH, 2H), 7.44 (t, *J* = 7.8 Hz, ArH, 1H), 7.21 (d, *J* = 8.0 Hz, ArH, 2H), 7.12 (d, *J* = 8.0 Hz, ArH, 2H), 6.97 (dd, *J* = 1.2, 8.0 Hz, ArH, 1H), 6.46 (dd, *J* = 4.8, 7.6 Hz, ArH, 1H), 5.63 (q, *J* = 6.2Hz, CH, 1H), 4.67 (s, OH, 1H), 4.43 (d, *J* = 6.0 Hz, $NHCH_2$, 2H), 3.97 (s, CH_2, 2H), 2.27 (s, $ArCH_3$, 3H), 1.67 (d, *J* = 6.4 Hz, $CHCH_3$, 3H), 1.07 (s, $C(CH_3)_2$,6H).^{13}C

NMR (101 MHz, DMSO-d_6) δ 166.4, 147.0, 143.2, 140.1, 138.5, 137.0, 136.2, 135.1, 130.4, 129.2, 129.0, 128.8, 127.7, 126.8, 125.5, 123.9, 121.2, 117.6, 112.6, 75.4, 69.9, 62.6, 42.8, 27.6, 24.3, 21.1. ESI-MS m/z: 500.7 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{29}H_{33}N_5O_3[M+H]^+$, 500.2661; found, 500.2657. Anal. Calcd. for $C_{29}H_{33}N_5O_3$: C, 69.72; H, 6.66; N, 14.02; found: C, 69.94; H, 6.87; N, 13.79.

4.1.30.
N-(4-methylbenzyl)-3-(1-((2-(thiazol-2-ylamino)pyridin-3-yl)oxy)ethyl)benzamide ***(8i)***

Compound **7g** (212 mg, 0.50 mmol) and thiazol-2-amine (100 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **8i** (91 mg, 41%). mp 105-107 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, CONH, 1H), 7.91 (d, *J* = 4.8 Hz, ArH, 1H), 7.88 (s, ArH, 1H),7.69 (d, *J* = 7.6 Hz, ArH, 1H), 7.49 (d, *J* = 7.6 Hz, ArH, 1H), 7.42 (m, ArH, 2H), 7.27 (s, ArH, 1H), 7.16 (m, ArH, 3H), 6.90 – 6.86 (m, ArH, 2H), 6.71 (dd, *J* = 5.2, 7.6 Hz, ArH, 1H), 6.58 (s, ArNH, 1H), 5.43 (q, *J* = 6.4 Hz, CH, 1H), 4.62 (d, *J* = 5.6 Hz, CH_2, 2H), 2.36 (s, $ArCH_3$, 3H), 1.75 (d, *J* = 6.4 Hz, CH_3, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 166.8, 160.0, 142.6, 142.3, 140.1, 137.9, 137.3, 137.0, 135.2, 135.0, 129.4, 129.3, 129.2, 128.2, 128.0, 127.4, 126.5, 124.4, 118.5, 115.8, 111.3, 44.4, 43.9, 24.3, 21.1. ESI-MS m/z: 445.6 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{25}H_{24}N_4O_2S$ $[M+H]^+$, 445.1691; found, 445.1696. Anal. Calcd. for $C_{25}H_{24}N_4O_2S$: C, 67.54; H, 5.44; N, 12.60; found: C, 67.16; H, 5.68; N, 12.32.

4.1.31.
3-(1-((2-((1-(cyanomethyl)-1H-pyrazol-4-yl)amino)pyridin-3-yl)oxy)ethyl)-N-(4-methylbenzyl)benzamide ***(8j)***

Compound **7g** (212 mg, 0.50 mmol) and 2-(4-amino-1*H*-pyrazol-1-yl)acetonitrile (122 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **8j** (122 mg, 52%). mp 96-99 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.97 (t, *J* = 6.0 Hz, CONH, 1H), 8.44 (br s, NH, 1H), 8.30 (s, ArH, 1H), 8.01 (s, ArH, 1H), 7.80 (s, ArH, 1H), 7.77 (d, *J* = 8.0 Hz, ArH, 1H), 7.65 – 7.63 (m, ArH, 2H), 7.44 (t, *J* = 7.6 Hz, ArH, 1H), 7.21 (d, *J* = 8.0 Hz, ArH, 2H), 7.12 (d, *J* =

8.0 Hz, ArH, 2H), 7.03 (d, *J* = 7.6 Hz, ArH, 1H), 6.53 (dd, *J* = 4.8, 7.6 Hz, ArH, 1H), 5.65 (q, *J* = 6.2 Hz, CH, 1H), 5.47 (s, CH_2CN, 2H), 4.43 (d, *J* = 6.0 Hz, CH_2, 2H), 2.27 (s, $ArCH_3$, 3H), 1.68 (d, *J* = 6.0 Hz, CH_3, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.4, 146.7, 143.1, 140.3, 138.4, 136.9, 136.2, 135.1, 133.1, 129.2, 129.0, 128.8, 127.7, 126.8, 125.5, 125.2, 120.4, 118.0, 116.7, 113.2, 75.6, 42.8, 39.6, 24.3, 21.1. ESI-MS m/z: 467.7 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{27}H_{26}N_6O_2[M+H]^+$, 467.2191; found, 467.2193. Anal. Calcd. for $C_{27}H_{26}N_6O_2$:C, 69.51; H, 5.62; N, 18.01; found: C, 69.22; H, 5.78; N, 17.86.

4.1.32.
N-(4-methylbenzyl)-3-(1-((2-((5-methylthiazol-2-yl)amino)pyridin-3-yl)oxy)ethyl)benzamide ***(8k)***

Compound **7g** (212 mg, 0.50 mmol) and 5-methylthiazol-2-amine (114 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **8k** (130 mg, 57%). mp 100-103 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.96 (s, ArNH, 1H), 8.96 (t, *J* = 6.0 Hz, CONH, 1H), 8.06 (s, ArH, 1H), 7.77 – 7.75 (m, ArH, 2H), 7.71 (d, *J* = 8.0 Hz, ArH, 1H), 7.44 (t, *J* = 7.6 Hz, ArH, 1H), 7.18 (t, *J* = 8.4 Hz, ArH, 3H), 7.11 – 7.09 (m, ArH, 3H), 6.76 (dd, *J* = 5.2, 8.0 Hz, ArH, 1H), 5.69 (q, *J* = 6.2 Hz, CH, 1H), 4.42 (d, *J* = 6.0 Hz, CH_2, 2H), 2.35 (d, *J* = 1.2 Hz, $ArCH_3$, 3H), 2.27 (s, $ArCH_3$, 3H), 1.69 (d, *J* = 6.4 Hz, CH_3, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 166.6, 158.0, 142.5, 141.8, 139.9, 137.5, 136.6, 135.6, 135.1, 133.7, 129.0, 128.8, 127.9, 127.6, 126.9, 124.8, 124.7, 118.3, 115.5, 76.8, 43.3, 24.1, 20.9, 11.4. ESI-MS m/z: 459.4 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{26}H_{26}N_4O_2S$ $[M+H]^+$, 459.1851; found, 459.1854. Anal. Calcd. for $C_{26}H_{26}N_4O_2S$: C, 68.10; H, 5.71; N, 12.22; found: C, 68.42; H, 5.56; N, 12.35.

4.1.33.
3-(1-((2-((4H-1,2,4-triazol-4-yl)amino)pyridin-3-yl)oxy)ethyl)-N-(4-methylbenzyl)benzamide ***(8l)***

Compound **7g** (212 mg, 0.50 mmol) and 4*H*-1,2,4-triazol-4-amine (84 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **8l** (92 mg, 43%). mp 234-237 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.92 (s,

ArNH, 1H), 8.99 (t, *J* = 6.2 Hz, CONH, 1H), 8.64 (s, ArH, 2H), 8.02 (s, ArH, 1H), 7.80 (d, *J* = 7.6 Hz, ArH, 1H), 7.67 (d, *J* = 7.6 Hz, ArH, 1H), 7.53 (dd, *J* = 0.8, 4.8 Hz, ArH, 1H), 7.47 (t, *J* = 7.6 Hz, ArH, 1H), 7.21 – 7.17 (m, ArH, 3H), 7.14 (d, *J* = 8.0 Hz, ArH, 2H), 6.73 (dd, *J* = 5.2, 8.0 Hz, ArH, 1H), 5.70 (q, *J* = 6.2 Hz, CH, 1H), 4.45 (d, *J* = 5.6 Hz, CH_2, 2H), 2.28 (s, $ArCH_3$, 3H), 1.65 (d, *J* = 6.4 Hz, CH_3, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.5, 148.3, 145.1, 142.8, 140.3, 138.7, 136.9, 136.2, 135.2, 129.2, 129.0, 127.7, 126.9, 125.6, 120.0, 117.0, 75.8, 42.8, 24.1, 21.1. ESI-MS m/z: 429.5 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{24}H_{24}N_6O_2[M+H]^+$, 429.2045; found, 429.2041. Anal. Calcd. for $C_{24}H_{24}N_6O_2$: C, 67.27; H, 5.65; N, 19.61; found: C, 67.07; H, 5.89; N, 19.84.

4.1.34.
*3-(1-((2-((1,3,4-thiadiazol-2-yl)amino)pyridin-3-yl)oxy)ethyl)-N-(4-methylbenzyl)benzamide (**8m**)*

Compound **7g** (212 mg, 0.50 mmol) and 1,3,4-thiadiazol-2-amine (101 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **8m** (89 mg, 40%). mp 103-105 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93 (s, ArNH, 1H), 9.03 (s, ArH, 1H), 8.95 (t, *J* = 5.8 Hz, CONH, 1H), 8.07 (s, ArH, 1H), 7.81 (d, *J* = 4.8 Hz, ArH, 1H), 7.75 (t, *J* = 8.0 Hz, ArH, 2H), 7.44 (t, *J* = 7.6 Hz, ArH, 1H), 7.26 (d, *J* = 8.0 Hz, ArH, 1H), 7.20 (d, *J* = 8.0 Hz, ArH, 2H), 7.11 (d, *J* = 7.9 Hz, ArH, 2H), 6.85 (dd, *J* = 4.8, 7.6 Hz, ArH, 1H), 5.72 (q, *J* = 6.2 Hz, CH, 1H), 4.42 (d, *J* = 6.0 Hz, CH_2, 2H), 2.27 (s, $ArCH_3$, 3H), 1.71 (d, *J* = 6.0 Hz, CH_3, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.5, 160.2, 147.2, 142.8, 142.3, 141.2, 137.2, 136.9, 136.2, 135.2, 129.2, 129.0, 127.7, 127.4, 126.9, 125.8, 120.0, 117.3, 75.9, 42.8, 24.1, 21.1. ESI-MS m/z: 446.5 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{24}H_{23}N_5O_2S$ $[M+H]^+$, 446.1643; found, 446.1647. Anal. Calcd. for $C_{24}H_{23}N_5O_2S$: C, 64.70; H, 5.20; N, 15.72; found: C, 64.46; H, 5.36; N, 15.98.

4.1.35.
*N-(3-chloro-4-fluorobenzyl)-3-(1-((2-((5-methylthiazol-2-yl)amino)pyridin-3-yl)oxy)ethyl)benzamide (**8n**)*

Compound **7d** (232 mg, 0.50 mmol) and 5-methylthiazol-2-amine (114 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **8n** (111 mg, 45%). mp 112-114 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (br s, ArNH, 1H), 7.89 (m, ArH, 2H), 7.74 (m, ArH, 1H), 7.50 – 7.40 (m, ArH, 3H), 7.24 (m, ArH, 1H), 7.10 (t, *J* = 8.8 Hz, ArH, 1H), 7.03 (d, *J* = 1.2 Hz, ArH, 1H), 6.90 (dd, *J* = 1.2, 8.0 Hz, ArH, 1H), 6.70 (dd, *J* = 4.8, 7.6 Hz, ArH, 1H), 5.42 (q, *J* = 6.4 Hz, CH, 1H), 4.60 (d, *J* = 5.6 Hz, CH_2, 2H), 2.42 (d, *J* = 1.2 Hz, $ArCH_3$, 3H), 1.74 (d, *J* = 6.4 Hz, CH_3, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 167.0, 158.4, 156.2, 142.8, 142.3, 139.9, 138.2, 134.8, 133.6, 123.0, 129.3, 128.8, 127.6, 127.5, 126.9, 125.2, 124.0, 118.6, 116.8, 116.6, 115.7, 77.0, 42.9, 24.7, 11.5. ESI-MS m/z: 497.3 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{26}H_{26}N_4O_2S$ $[M+H]^+$, 497.1210; found, 497.1212. Anal. Calcd. for $C_{26}H_{26}N_4O_2S$: C, 60.42; H, 4.46; N, 11.27; found: C, 60.28; H, 4.59; N, 11.45.

*4.1.36. N-(3-methoxybenzyl)-3-(1-((2-((1-methyl-1H-pyrazol-4-yl)amino)pyridin-4-yl)oxy)ethyl)benzamide (**13a**)*

Compound **12a** (220 mg, 0.50 mmol) and 1-methyl-1*H*-pyrazol-4-amine (97 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **13a** (126 mg, 55%). mp 90-93 ℃.^{1}H NMR (400 MHz, DMSO-d_6) δ 9.05 (t, *J* = 5.8 Hz, CONH, 1H), 8.53 (s, ArNH, 1H), 7.94 (s, ArH, 1H), 7.88 – 7.81 (m, ArH, 3H), 7.55 (d, *J* = 7.6 Hz, ArH, 1H), 7.47 (t, *J* = 7.8 Hz, ArH, 1H), 7.31 (s, ArH, 1H), 7.25 (t, *J* = 8.0 Hz, ArH, 1H), 6.91 (d, *J* = 6.8 Hz, ArH, 1H), 6.90 (s, ArH, 1H), 6.84 – 6.81 (m, ArH, 1H), 6.28 (dd, *J* = 2.0, 6.0 Hz, ArH, 1H), 6.07 (d, *J* = 1.6 Hz, ArH, 1H), 5.55 (q, *J* = 6.0 Hz, CH, 1H), 4.47 (d, *J* = 6.0 Hz, CH_2, 2H), 3.77 (s, NCH_3, 3H), 3.74 (s, OCH_3, 3H), 1.58 (d, *J* = 6.0 Hz, $CHCH_3$, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.4, 164.6, 159.7, 157.8, 149.2, 143.1, 141.6, 135.1, 130.4, 129.8, 129.1, 128.7, 126.8, 125.1, 124.4, 121.0, 119.9, 113.4, 112.5, 103.4, 94.1, 74.9, 55.4, 43.0, 39.0, 24.3. ESI-MS m/z: 458.6 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{26}H_{27}N_5O_3$ $[M+H]^+$, 458.2195; found, 458.2191. Anal. Calcd. for $C_{26}H_{27}N_5O_3$: C, 68.25; H, 5.95; N, 15.31; found: C, 68.49; H, 6.11; N, 15.67.

4.1.37.
*N-(3,4-dimethoxybenzyl)-3-(1-((2-((1-methyl-1H-pyrazol-4-yl)amino)pyridin-4-yl)oxy)ethyl)benzamide (**13b**)*

Compound **12b** (236 mg, 0.50 mmol) and 1-methyl-1*H*-pyrazol-4-amine (97 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **13b** (112 mg, 46%). mp 118-120 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.97 (s, CONH, 1H), 8.52 (s, ArNH, 1H), 7.92 (s, ArH, 1H), 7.85 (d, *J* = 6.0 Hz, ArH, 1H), 7.80 (d, *J* = 6.8 Hz, ArH, 2H), 7.53 (d, *J* = 7.6 Hz, ArH, 1H), 7.46 (t, *J* = 7.6 Hz, ArH, 1H), 7.30 (s, ArH, 1H), 6.95 (s, ArH, 1H), 6.90 (d, *J* = 8.0 Hz, ArH, 1H), 6.85 (d, *J* = 8.4 Hz, ArH, 1H), 6.27 (d, *J* = 5.6 Hz, ArH, 1H), 6.06 (s, ArH, 1H), 5.55 (q, *J* = 6.4 Hz, CH, 1H), 4.41 (d, *J* = 5.6 Hz, CH_2, 2H), 3.77 (s, NCH_3, 3H), 3.73 (s, OCH_3, 6H), 1.58 (d, *J* = 6.0 Hz, CHCH_3, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.3, 164.6, 157.8, 149.2, 149.0, 148.2, 143.1, 135.3, 132.5, 130.4, 129.0, 128.7, 126.8, 125.0, 124.4, 121.0, 119.9, 112.2, 112.0, 103.4, 94.1, 74.9, 56.0, 55.9, 42.9, 39.0, 24.3. ESI-MS m/z: 488.5 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{27}H_{29}N_5O_4[M+H]^+$, 488.2296; found, 488.2300. Anal. Calcd. for $C_{27}H_{29}N_5O_4$: C, 66.51; H, 6.00; N, 14.36; found: C, 66.37; H, 5.83; N, 14.12.

4.1.38.
*3-(1-((2-((1-methyl-1H-pyrazol-4-yl)amino)pyridin-4-yl)oxy)ethyl)-N-(3,4,5-trimethoxybenzyl)benzamide (**13c**)*

Compound **12c** (250 mg, 0.50 mmol) and 1-methyl-1*H*-pyrazol-4-amine (97 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **13c** (134 mg, 52%). mp 108-110 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (s, CONH, 1H), 8.52 (s, ArNH, 1H), 7.92 – 7.80 (m, ArH, 4H), 7.54 – 7.30 (m, ArH, 2H), 7.30 (s, ArH, 1H), 6.65 (s, ArH, 2H), 6.27 (s, ArH, 1H), 6.05 (s, ArH, 1H), 5.55 (q, *J* = 4.0 Hz, CH, 1H), 4.42 (s, CH_2, 2H), 3.76 (s, NCH_3, 3H), 3.74 (s, OCH_3, 6H), 3.64 (s, OCH_3, 3H), 1.57 (d, *J* = 3.6 Hz, CHCH_3, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.5, 164.6, 157.8, 153.2, 149.2, 143.1, 136.8, 135.6, 135.3, 130.4, 129.1, 128.8, 126.8, 125.0, 124.4, 121.0, 105.2, 103.3, 99.9, 94.1, 74.9, 60.4, 56.2, 43.4, 39.0, 24.3. ESI-MS m/z: 518.5 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{28}H_{31}N_5O_5[M+H]^+$,

518.2402; found, 518.2400. Anal. Calcd. for $C_{28}H_{31}N_5O_5$: C, 64.98; H, 6.04; N, 13.53; found: C, 64.79; H, 6.38; N, 13.38.

4.1.39.
N-(4-methylbenzyl)-3-(1-((2-((1-(1-methylpiperidin-4-yl)-1H-pyrazol-4-yl)amino)pyridin-4-yl)oxy)ethyl)benzamide ***(13d)***

Compound **12d** (212 mg, 0.50 mmol) and 1-(1-methylpiperidin-4-yl)-1*H*-pyrazol-4-amine (180 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **13d** (105 mg, 40%). mp 92-94 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (t, *J* = 5.8 Hz, CONH, 1H), 8.51 (s, ArNH, 1H), 7.92 (s, ArH, 1H), 7.86 (d, *J* = 5.6 Hz, ArH, 1H), 7.85 (s, ArH, 1H), 7.81 (d, *J* = 7.6 Hz, ArH, 1H), 7.53 (d, *J* = 8.0 Hz, ArH, 1H), 7.46 (t, *J* = 7.6 Hz, ArH, 1H), 7.35 (s, ArH, 1H), 7.21 (d, *J* = 8.0 Hz, ArH, 2H), 7.13 (d, *J* = 7.6 Hz, ArH, 2H), 6.27 (dd, *J* = 6.0, 2.0 Hz, ArH, 1H), 6.06 (d, *J* = 2.0 Hz, ArH, 1H), 5.55 (q, *J* = 6.2 Hz, CH, 1H), 4.44 (d, *J* = 5.6 Hz, NCH_2, 2H), 4.06 – 3.99 (m, NCH, 1H), 2.84 (d, *J* = 11.2 Hz, CH_2, 2H), 2.27 (s, CH_3, 3H), 2.20 (s, CH_3, 3H), 2.03 – 2.00 (m, CH_2, 2H), 1.94 – 1.89 (m, 2 × CH_2, 4H), 1.57 (d, *J* = 6.4 Hz, $CHCH_3$, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.3, 164.6, 158.0, 149.2, 143.1, 137.0, 136.2, 135.2, 130.3, 129.2, 129.0, 128.7, 127.7, 126.8, 125.0, 124.0, 118.3, 103.3, 94.1, 74.9, 58.3, 54.6, 46.1, 42.8, 32.4, 24.3, 21.1. ESI-MS m/z: 525.6 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{31}H_{36}N_6O_2[M+H]^+$, 525.2971; found, 525.2975. Anal. Calcd. for $C_{31}H_{36}N_6O_2$: C, 70.97; H, 6.92; N, 16.02; found: C, 70.69; H, 7.13; N, 16.23.

4.1.40.
N-(4-chloro-3-(trifluoromethyl)benzyl)-3-(1-((2-((1-(1-methylpiperidin-4-yl)-1H-pyrazol-4-yl)amino)pyridin-4-yl)oxy)ethyl)benzamide ***(13e)***

Compound **12e** (217 mg, 0.50 mmol) and 1-(1-methylpiperidin-4-yl)-1*H*-pyrazol-4-amine (180 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **13e** (242 mg, 79%). mp 113-116 ℃.^{1}H NMR (400 MHz, DMSO-d_6) δ 9.17 (t, *J* = 5.2 Hz, CONH, 1H), 8.50 (s, ArNH, 1H), 7.92 (s, ArH, 1H), 7.83 (m, ArH, 4H), 7.67 (m, ArH, 2H), 7.56 (d, *J* = 7.2 Hz, ArH, 1H), 7.48 (t, *J* = 7.4 Hz, ArH, 1H), 7.35 (s, ArH, 1H), 6.27 (d, *J* = 4.8 Hz, ArH, 1H), 6.06 (s,

ArH, 1H), 5.56 (q, *J* = 6.0 Hz, CH, 1H), 4.54 (d, *J* = 5.2 Hz, NCH_2, 2H), 4.05 – 3.98 (m, NCH,1H), 2.83 (d, *J* = 10.4 Hz, CH_2, 2H), 2.19 (s, NCH_3, 3H), 2.01 – 1.91 (m, 3 × CH_2, 6H), 1.57 (d, *J* = 6.0 Hz, $CHCH_3$, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.6, 164.6, 158.0, 149.2, 143.2, 140.3, 134.7, 133.5, 132.0, 130.4, 129.4, 129.1, 129.0, 127.3, 127.2, 126.8, 125.0, 124.0, 118.3, 103.3, 94.0, 74.8, 58.4, 54.6, 46.2, 42.3, 32.5, 24.3. ESI-MS m/z: 613.4 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{31}H_{32}ClF_3N_6O_2[M+H]^+$, 613.2302; found, 613.2306. Anal. Calcd. for $C_{31}H_{32}ClF_3N_6O_2$: C, 60.73; H, 5.26; N, 13.71; found: C, 60.58; H, 5.49; N, 13.56.

4.1.41.
*N-(3,5-difluorobenzyl)-3-(1-((2-((1-methyl-1H-pyrazol-4-yl)amino)pyridin-4-yl)oxy)ethyl)benzamide (**13f**)*

Compound **12f** (223 mg, 0.50 mmol) and 1-methyl-1*H*-pyrazol-4-amine (97 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **13f** (106 mg, 46%). mp 91-96 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (t, *J* = 5.8 Hz, CONH, 1H), 8.53 (s, ArNH, 1H), 7.94 (s, ArH, 1H), 7.88 – 7.80 (m, ArH, 3H), 7.56 (d, *J* = 8.0 Hz, ArH, 1H), 7.49 (t, *J* = 7.6 Hz, ArH, 1H), 7.31 (s, ArH, 1H), 7.13 – 7.04 (m, ArH, 3H), 6.28 (dd, *J* = 2.0, 6.0 Hz, ArH, 1H), 6.07 (d, *J* = 2.0 Hz, ArH, 1H), 5.56 (q, *J* = 6.0 Hz, CH, 1H), 4.51 (d, *J* = 5.6 Hz, CH_2, 2H), 3.77 (s, NCH_3, 3H), 1.58 (d, *J* = 6.4 Hz, $CHCH_3$, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.6, 164.6, 161.6, 157.9, 149.2, 144.9, 143.2, 134.8, 130.4, 129.1, 128.9, 126.8, 125.1, 124.4, 121.0, 110.8, 110.5, 103.4, 94.1, 74.9, 42.5, 39.0, 24.3. ESI-MS m/z: 464.5 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{25}H_{23}F_2N_5O_2[M+H]^+$, 464.1894; found, 464.1897. Anal. Calcd. for $C_{25}H_{23}F_2N_5O_2$: C, 64.79; H, 5.00; N, 15.11; found: C, 64.63; H, 4.86; N, 15.37.

4.1.42.
*N-(3,5-bis(trifluoromethyl)benzyl)-3-(1-((2-((1-methyl-1H-pyrazol-4-yl)amino)pyridin-4-yl)oxy)ethyl)benzamide (**13g**)*

Compound **12g** (273 mg, 0.50 mmol) and 1-methyl-1*H*-pyrazol-4-amine (97 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **13g** (146 mg, 52%). Mp 94-97 ℃ ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24 (t, *J*

= 5.8 Hz, CONH, 1H), 8.56 (s, ArNH, 1H), 8.04 (s, ArH, 1H), 8.02 (d, *J* = 10.0 Hz, ArH, 2H), 7.92 (s, ArH, 1H), 7.86 – 7.79 (m, ArH, 3H), 7.57 (d, *J* = 7.6 Hz, ArH, 1H), 7.50 (t, *J* = 7.6 Hz, ArH, 1H), 7.30 (s, ArH, 1H), 6.28 (dd, *J* = 2.4, 6.0 Hz, ArH, 1H), 6.07 (d, *J* = 2.0 Hz, ArH, 1H), 5.56 (q, *J* = 6.2 Hz, CH, 1H), 4.66 (d, *J* = 5.6 Hz, CH_2, 2H), 3.76 (s, NCH_3, 3H), 1.58 (d, *J* = 6.4 Hz, $CHCH_3$, 3H).^{13}C NMR (101 MHz, , DMSO-d_6) δ 166.8, 164.7, 157.8, 148.9, 143.6, 143.2, 134.7, 130.8, 130.5, 130.4, 129.2, 129.1, 128.7, 126.8, 125.1, 124.2, 122.4, 121.2, 103.4, 94.1, 74.9, 55.3, 42.5, 39.0, 24.3. ESI-MS m/z: 564.4 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{27}H_{23}F_6N_5O_2[M+H]^+$, 564.1829; found, 564.1831. Anal. Calcd. for $C_{27}H_{23}F_6N_5O_2$: C, 57.55; H, 4.11; N, 12.43; found: C, 57.29; H, 4.32; N, 12.65;

4.1.43.
*N-(4-cyanobenzyl)-3-(1-((2-((1-methyl-1H-pyrazol-4-yl)amino)pyridin-4-yl)oxy)ethyl) benzamide (**13h**)*

Compound **12h** (218 mg, 0.50 mmol) and 1-methyl-1*H*-pyrazol-4-amine (97 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **13h** (99 mg, 44%). mp 96-99 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (t, *J* = 5.8 Hz, CONH, 1H), 8.53 (s, ArNH, 1H), 7.94 (s, ArH, 1H), 7.87 – 7.79 (m, ArH, 5H), 7.57 – 7.47 (m, ArH, 4H), 7.30 (s, ArH, 1H), 6.28 (dd, *J* = 2.0, 6.0 Hz, ArH, 1H), 6.06 (d, *J* = 1.6 Hz, ArH, 1H), 5.56 (q, *J* = 6.0 Hz, CH, 1H), 4.56 (d, *J* = 6.0 Hz, CH_2, 2H), 3.77 (s, NCH_3, 3H), 1.58 (d, *J* = 6.0 Hz, $CHCH_3$, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.6, 164.6, 157.8, 149.2, 145.9, 143.2, 134.8, 132.7, 130.4, 129.1, 129.0, 128.5, 126.8, 125.0, 124.4, 121.0, 119.3, 110.0, 103.4, 94.1, 74.9, 42.9, 39.0, 24.3. ESI-MS m/z: 453.6 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{26}H_{24}N_6O_2[M+H]^+$, 453.2029; found, 453.2034. Anal. Calcd. for $C_{26}H_{24}N_6O_2$: C, 69.01; H, 5.35; N, 18.57; found: C, 68.89; H, 5.49; N, 18.36.

4.1.44.
*3-(1-((2-((1-methyl-1H-pyrazol-4-yl)amino)pyridin-4-yl)oxy)ethyl)-N-(4-methylbenzyl)benzamide (**13i**)*

Compound **12d** (212 mg, 0.50 mmol) and 1-methyl-1*H*-pyrazol-4-amine (97 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a**

afforded **13i** (110 mg, 50%). mp 88-90 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (t, J = 5.6 Hz, CONH, 1H), 8.52 (s, ArNH,1H), 7.92 (s, ArH, 1H), 7.85 (d, J = 5.6 Hz, ArH, 1H), 7.80 (d, J = 7.2 Hz, ArH, 1H), 7.79 (s, ArH, 1H),7.53 (d, J = 7.6 Hz, ArH, 1H), 7.46 (t, J = 7.6 Hz, ArH, 1H), 7.29 (s, ArH, 1H), 7.21 (d, J = 7.6 Hz, ArH, 2H), 7.13 (d, J = 7.6 Hz, ArH, 2H), 6.27 (d, J = 4.8 Hz, ArH, 1H), 6.05 (s, ArH, 1H), 5.54 (q, J = 5.8 Hz, CH, 1H), 4.43 (d, J = 5.6 Hz, CH_2, 2H), 3.76 (s, NCH_3, 3H), 2.28 (s, $ArCH_3$, 3H), 1.57 (d, J = 6.4 Hz, $CHCH_3$, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.3, 164.6, 157.8, 149.2, 143.1, 137.0, 136.2, 135.2, 130.3, 129.2, 129.0, 128.7, 127.7, 126.8, 125.0, 124.4, 121.0, 103.4, 94.1, 74.9, 42.8, 39.0, 24.3, 21.1. ESI-MS m/z: 442.5 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{26}H_{27}N_5O_2[M+H]^+$, 442.2239; found, 442.2242. Anal. Calcd. for $C_{26}H_{27}N_5O_2$: C, 70.73; H, 6.16; N, 15.86; found: C, 70.56; H, 6.43; N, 15.59.

*4.1.45. 3-(1-((2-((1,3-dimethyl-1H-pyrazol-4-yl)amino)pyridin-4-yl)oxy)ethyl)-N-(4-methylbenzyl)benzamide (**13j**)*

Compound **12d** (212 mg, 0.50 mmol) and 1,3-dimethyl-1*H*-pyrazol-4-amine (111 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **13j** (132 mg, 58%). mp 89-91 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (t, J = 5.6 Hz, CONH, 1H), 7.91 (s, ArH, 1H), 7.83 – 7.80 (m, ArH, 2H), 7.69 (s, ArH, 1H), 7.52 – 7.44 (m, ArH, 2H), 7.21 (d, J = 7.6 Hz, ArH, 2H), 7.13 (d, J = 8.0 Hz, ArH, 2H), 6.25 (d, J = 5.6 Hz, ArH, 1H), 6.06 (s, ArH, 1H), 5.51 (q, J = 6.0 Hz, CH, 1H), 4.44 (d, J = 5.6 Hz, CH_2, 2H), 3.70 (s, NCH_3, 3H), 2.28 (s, CH_3,3H), 2.00 (s, CH_3, 3H), 1.56 (d, J = 6.0 Hz, $CHCH_3$, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.3, 164.7, 159.1, 149.2, 143.1, 137.0, 136.2, 135.1, 129.2, 129.0, 128.7, 127.7, 126.8, 125.0, 124.4, 120.9, 103.1, 93.6, 74.8, 42.8, 38.8, 24.2, 21.1, 11.5. ESI-MS m/z: 456.5 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{27}H_{29}N_5O_2[M+H]^+$, 456.2028; found, 456.2030. Anal. Calcd. for $C_{27}H_{29}N_5O_2$: C, 71.19; H, 6.42; N, 15.37; found: C, 71.01; H, 6.56; N, 15.25.

4.1.46.
*N-(4-methylbenzyl)-3-(1-((2-(morpholinoamino)pyridin-4-yl)oxy)ethyl)benzamide (**13k**)*

Compound **12d** (212 mg, 0.50 mmol) and morpholin-4-amine (102 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **13k** (136 mg, 61%). mp 103-106 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (t, *J* = 5.8 Hz, CONH, 1H), 7.94 (s, ArH, 1H), 7.80 (d, *J* = 8.0 Hz, ArH, 1H), 7.75 (d, *J* = 6.0 Hz, ArH, 1H), 7.55 (d, *J* = 7.6 Hz, ArH, 1H), 7.45 (t, *J* = 7.8 Hz, ArH, 1H), 7.20 (d, *J* = 8.0 Hz, ArH, 2H), 7.13 (d, *J* = 8.0 Hz, ArH, 2H), 6.32 (d, *J* = 2.4 Hz, ArH, 1H), 6.21 (dd, *J* = 2.4, 6.0 Hz, ArH, 1H), 5.56 (q, *J* = 6.2 Hz, CH,1H), 4.43 (d, *J* = 6.0 Hz, NHCH_2, 2H), 3.59 (s, CH_2, 4H), 2.61 – 2.54 (m, CH_2, 4H), 2.28 (s, ArCH_3, 3H), 1.58 (d, *J* = 6.4 Hz, CHCH_3, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.2, 165.5, 161.4, 149.5, 143.3, 137.0, 136.2, 135.1, 129.2, 129.0, 128.8, 127.7, 126.7, 125.1, 103.2, 92.5, 75.1, 66.7, 55.2, 42.8, 24.3, 21.1. ESI-MS m/z: 447.6 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{26}H_{30}N_4O_3[M+H]^+$, 447.2393; found, 447.2395. Anal. Calcd. for $C_{26}H_{30}N_4O_3$: C, 69.93; H, 6.77; N, 12.55; found: C, 69.69; H, 6.95; N, 12.36.

4.1.47.
*N-(4-methylbenzyl)-3-(1-((2-((4-methylpiperazin-1-yl)amino)pyridin-4-yl)oxy)ethyl)benzamide (**13l**)*

Compound **12d** (212 mg, 0.50 mmol) and 4-methylpiperazin-1-amine (115 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **13l** (83 mg, 36%). mp 124-127 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (s, ArH, 1H), 7.82 (d, *J* = 6.0 Hz, ArH, 1H), 7.66 (d, *J* = 8.0 Hz, ArH, 1H), 7.53 (d, *J* = 7.6 Hz, ArH, 1H), 7.42 (t, *J* = 7.6 Hz, ArH, 1H), 7.27 (d, *J* = 7.6 Hz, ArH, 2H), 7.20 (d, *J* = 7.6 Hz, ArH, 2H), 6.46 (s, ArH, 1H), 6.36 (br s, ArNH, 1H), 6.24 – 6.22 (m, ArH, 1H), 5.43 (q, *J* = 6.0 Hz, CH, 1H), 4.63 (d, *J* = 5.6 Hz, NHCH_2, 2H), 2.71 (s, CH_2, 4H), 2.38 (s, CH_3, 3H), 2.35 (m, CH_2, 4H), 1.67 (d, *J* = 6.4 Hz, CHCH_3, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.2, 166.1, 164.2, 142.0, 137.0, 136.2, 135.4, 129.2, 127.8, 125.5, 116.9, 114.2, 98.3, 95.3, 92.7, 77.5, 74.1, 66.2, 52.8, 51.5, 42.8, 23.8, 21.1. ESI-MS m/z: 460.7 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for

$C_{27}H_{33}N_5O_2[M+H]^+$, 460.2708; found, 460.2713. Anal. Calcd. for $C_{27}H_{33}N_5O_2$: C, 70.56; H, 7.24; N, 15.24; found: C, 70.37; H, 7.03; N, 15.11.

4.1.48.
N-(4-chloro-3-(trifluoromethyl)benzyl)-3-(1-((2-((4-methylpiperazin-1-yl)amino)pyridin-4-yl)oxy)ethyl)benzamide ***(13m)***

Compound **12e** (217 mg, 0.50 mmol) and 4-methylpiperazin-1-amine (115 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **13m** (107 mg, 39%). mp 92-94 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.17 (t, *J* = 5.8 Hz, CONH, 1H), 7.94 (s, ArH, 1H), 7.81 (d, *J* = 8.0 Hz, ArH, 2H), 7.73 (d, *J* = 6.0 Hz, ArH, 1H), 7.70 (d, *J* = 8.4 Hz, ArH, 1H), 7.64 (d, *J* = 8.4 Hz, ArH, 1H), 7.56 (d, *J* = 8.0 Hz, ArH, 1H), 7.47 (t, *J* = 7.6 Hz, ArH, 1H), 7.10 (s, ArH, 1H), 6.26 (d, *J* = 2.0 Hz, ArH, 1H), 6.20 (dd, *J* = 2.4, 5.6 Hz, ArH, 1H), 5.55 (q, *J* = 6.2 Hz, CH, 1H), 4.54 (d, *J* = 6.0 Hz, NHCH_2, 2H), 2.62 (s, CH_2, 4H), 2.36 (s, CH_2, 4H), 2.16 (s, CH_3, 3H), 1.58 (d, *J* = 6.4 Hz, CHCH_3, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.5, 165.4, 161.6, 149.4, 143.4, 140.3, 134.7, 133.5, 132.0, 129.4, 129.1, 129.0, 127.3, 127.2, 126.7, 125.1, 103.1, 99.9, 92.4, 75.1, 70.2, 55.0, 54.2, 45.6, 42.3, 24.3. ESI-MS m/z: 548.5 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{27}H_{29}ClF_3N_5O_2[M+H]^+$, 548.2045; found, 548.2041. Anal. Calcd. for $C_{27}H_{29}ClF_3N_5O_2$: C, 59.18; H, 5.33; N, 12.78; found: C, 59.02; H, 5.09; N, 12.91.

4.1.49.
3-(1-((2-((1-(2-hydroxy-2-methylpropyl)-1H-pyrazol-4-yl)amino)pyridin-4-yl)oxy)ethyl)-N-(4-methylbenzyl)benzamide ***(13n)***

Compound **12d** (212 mg, 0.50 mmol) and 1-(4-amino-1*H*-pyrazol-1-yl)-2-methylpropan-2-ol (155 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **13n** (102 mg, 41%). mp 115-118 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (s, CONH, 1H), 8.54 (s, ArNH, 1H), 7.92 – 7.79 (m, ArH, 4H), 7.54 – 7.34 (m, ArH, 3H), 7.21 (d, *J* = 7.2, ArH, 2H), 7.13 (d, *J* = 7.2, ArH, 2H), 6.27 (d, *J* = 4.4 Hz, ArH, 1H), 6.06 (s, ArH, 1H), 5.54 (q, *J* = 5.4 Hz, CH, 1H), 4.64 (s, OH, 1H), 4.43 (d, *J* = 4.4 Hz, $ArCH_2$, 2H), 3.93 (s, NCH_2, 2H), 2.27 (s, $ArCH_3$, 3H), 1.57 (d, *J* = 4.8 Hz, CHCH_3, 3H), 1.04 (s, $C(CH_3)_2$, 6H). ^{13}C NMR (101

MHz, DMSO-d_6) δ 166.3, 164.5, 157.8, 149.2, 143.0, 137.0, 136.2, 135.2, 130.1, 129.2, 129.0, 128.7, 127.7, 126.8, 125.0, 124.1, 121.3, 103.3, 94.1, 74.8, 69.9, 62.6, 42.8, 27.6, 24.3, 21.1. ESI-MS m/z: 500.5 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{29}H_{33}N_5O_3[M+H]^+$, 500.2656; found, 500.2659. Anal. Calcd. for $C_{29}H_{33}N_5O_3$: C, 69.72; H, 6.66; N, 14.02; found: C, 69.89; H, 6.78; N, 13.87.

4.1.50.
N-(4-chloro-3-(trifluoromethyl)benzyl)-3-(1-((2-((1-(2-hydroxy-2-methylpropyl)-1H-pyrazol-4-yl)amino)pyridin-4-yl)oxy)ethyl)benzamide ***(13o)***

Compound **12e** (217 mg, 0.50 mmol) and 1-(4-amino-1*H*-pyrazol-1-yl)-2-methylpropan-2-ol (155 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **13o** (126 mg, 43%). mp 106-108 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (t, *J* = 5.8 Hz, CONH, 1H), 8.53 (s, ArNH, 1H), 7.92 (s, ArH, 1H), 7.87 – 7.80 (m, ArH, 4H), 7.70 (d, *J* = 8.4 Hz, ArH, 1H), 7.64 (d, *J* = 8.4 Hz, ArH, 1H), 7.56 (d, *J* = 7.6 Hz, ArH, 1H), 7.48 (t, *J* = 7.6 Hz, ArH, 1H), 7.34 (s, ArH, 1H), 6.27 (dd, *J* = 2.0, 5.6 Hz, ArH, 1H), 6.07 (d, *J* = 1.6 Hz, ArH, 1H), 5.55 (q, *J* = 6.0 Hz, CH, 1H), 4.63 (s, OH, 1H), 4.54 (d, *J* = 6.0 Hz, $ArCH_2$, 2H), 3.94 (s, NCH_2, 2H), 1.58 (d, *J* = 6.4 Hz, $CHCH_3$, 3H), 1.04 (s, $C(CH_3)_2$, 6H).^{13}C NMR (101 MHz, , DMSO-d_6) δ 166.6, 164.5, 157.9, 149.2, 143.2, 140.2, 134.7, 133.5, 132.0, 130.2, 129.4, 129.1, 129.0, 127.3, 127.2, 126.8, 126.6, 125.0, 124.0, 121.3, 103.3, 94.1, 74.8, 69.9, 62.6, 42.3, 27.6, 24.3. ESI-MS m/z: 588.5 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{29}H_{29}ClF_3N_5O_3[M+H]^+$, 588.1990; found, 588.1991. Anal. Calcd. for $C_{29}H_{29}ClF_3N_5O3$: C, 59.23; H, 4.97; N, 11.91; found: C, 59.01; H, 5.21; N, 11.65.

4.1.51.
3-(1-((2-((1H-benzo[d]imidazol-2-yl)amino)pyridin-4-yl)oxy)ethyl)-N-(4-methylbenzyl)benzamide ***(13p)***

Compound **12d** (212 mg, 0.50 mmol) and 1*H*-benzo[d]imidazol-2-amine (133 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **13p** (107 mg, 45%). mp 158-160 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.89 (s, NH, 1H), 10.40 (s, NH, 1H), 9.02 (s, NH, 1H), 8.07 (d, *J* = 5.2 Hz, ArH, 1H), 7.97 (s, ArH, 1H), 7.82 (d, *J* = 7.2 Hz, ArH, 1H), 7.60 (d, *J* = 6.8 Hz, ArH, 1H), 7.48

(t, *J* = 7.6 Hz, ArH, 1H), 7.39 (s, ArH, 2H), 7.21 (d, *J* = 7.6 Hz, ArH, 2H), 7.13 (d, *J* = 6.8 Hz, ArH, 2H), 7.01 (s, ArH, 2H), 6.83 (s, ArH, 1H), 6.57 (d, *J* = 4.8 Hz, ArH, 1H), 5.61 (q, *J* = 5.6 Hz, CH, 1H), 4.44 (d, *J* = 4.0 Hz, NCH_2, 2H), 2.27 (s, $ArCH_3$, 3H), 1.63 (d, *J* = 5.2 Hz, $CHCH_3$, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.3, 165.2, 155.5, 150.0, 148.7, 142.6, 137.0, 136.2, 135.2, 129.2, 129.1, 128.9, 128.9, 128.5, 128.4, 127.7, 127.0, 125.2, 105.8, 97.1, 75.5, 42.8, 24.2, 21.1. ESI-MS m/z: 478.5 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{29}H_{27}N_5O_2[M+H]^+$, 478.2241; found, 478.2242. Anal. Calcd. for $C_{29}H_{27}N_5O_2$: C, 72.94; H, 5.70; N, 14.66; found: C, 72.76; H, 5.96; N, 14.49.

4.1.52.
N-(4-methylbenzyl)-3-(1-((2-(piperidin-1-ylamino)pyridin-4-yl)oxy)ethyl)benzamide ***(13q)***

Compound **12d** (212 mg, 0.50 mmol) and piperidin-1-amine (100 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **13q** (142 mg, 64%).mp 93-95 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, ArH, 1H), 7.80 (d, *J* = 5.6 Hz, ArH, 1H), 7.68 (d, *J* = 7.2 Hz, ArH, 1H), 7.53 (d, *J* = 7.6 Hz, ArH, 1H), 7.42 (t, *J* = 7.6 Hz, ArH, 1H), 7.29 (s, ArH, 1H), 7.28 (d, *J* = 7.6 Hz, ArH, 2H), 7.19 (d, *J* = 7.6 Hz, ArH, 2H), 6.48 (s, ArH, 1H), 6.39 (br s, NH, 1H), 6.20 (d, *J* = 6.0 Hz, ArH, 1H), 5.44 (q, *J* = 6.4 Hz, CH, 1H), 5.32 (br s, NH, 1H), 4.63 (d, *J* = 5.6 Hz, $ArCH_2$, 2H), 2.57 (s, 2H), 2.37 (s, $ArCH_3$, 3H), 1.76 (s, 2H), 1.65 (m, $CHCH_3$, CH_2, 7H), 1.41 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.2, 165.4, 161.7, 149.3, 143.3, 137.0, 136.2, 135.1, 129.2, 128.9, 128.8, 127.7, 126.7, 125.1, 102.9, 92.3, 75.0, 56.0, 42.8, 26.1, 24.3, 23.6, 21.1. ESI-MS m/z: 445.6 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{27}H_{32}N_4O_2[M+H]^+$, 445.2594; found, 445.2600. Anal. Calcd. for $C_{27}H_{32}N_4O_2$: C, 72.94; H, 7.26; N, 12.60; found: C, 72.58; H, 7.49; N, 12.27.

4.1.53.
N-(4-methylbenzyl)-3-(1-((2-((3,4,5-trimethoxyphenyl)amino)pyridin-4-yl)oxy)ethyl)benzamide ***(13r)***

Compound **12d** (212 mg, 0.50 mmol) and 3,4,5-trimethoxyaniline (183 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a**

afforded **13r** (134 mg, 51%). mp 94-96 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (t, *J* = 5.8 Hz, CONH, 1H), 8.74 (s, ArNH, 1H), 7.94 – 7.91 (m, ArH, 2H), 7.82 (d, *J* = 7.6 Hz, ArH, 1H), 7.55 (d, *J* = 8.4 Hz, ArH, 1H), 7.48 (d, *J* = 7.6 Hz, ArH, 1H), 7.21 (d, *J* = 7.6 Hz, ArH, 2H), 7.13 (d, *J* = 7.6 Hz, ArH, 2H), 6.98 (s, ArH, 2H), 6.38 (dd, *J* = 2.0, 6.0 Hz, ArH, 1H), 6.24 (d, *J* = 2.0 Hz, ArH, 1H), 5.57 (q, *J* = 6.2 Hz, CH, 1H), 4.44 (d, *J* = 6.0 Hz, $ArCH_2$, 2H), 3.73 (s, 2 × OCH_3, 6H), 3.60 (s, OCH_3, 3H), 2.27 (s, $ArCH_3$, 3H), 1.59 (d, *J* = 6.4 Hz, $CHCH_3$, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.3, 164.6, 158.0, 153.1, 148.9, 142.91, 138.4, 137.0, 136.2, 135.2, 132.0, 131.9, 131.8, 129.2, 129.1, 128.9, 128.7, 127.7, 126.8, 125.0, 104.6, 96.6, 95.9, 75.0, 60.5, 56.1, 42.8, 24.3, 21.1. ESI-MS m/z: 528.6 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{31}H_{33}N_3O_5[M+H]^+$, 528.2494; found, 528.2496. Anal. Calcd. for $C_{31}H_{33}N_3O_5$: C, 70.57; H, 6.30; N, 7.96; found: C, 70.31; H, 6.01; N, 8.21.

4.1.54. N-(3,4-dimethoxybenzyl)-3-(1-((2-((1-(1-methylpiperidin-4-yl)-1H-pyrazol-4-yl)amino)pyridin-4-yl)oxy)ethyl)benzamide ***(14a)***

Compound **12b** (236 mg, 0.50 mmol) and 1-(1-methylpiperidin-4-yl)-1*H*-pyrazol-4-amine (180 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **14a** (131 mg, 46%). mp 138-140 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, *J* = 6.0 Hz, ArH, 1H), 7.74 (s, ArH, 1H), 7.66 (d, *J* = 7.2 Hz, ArH, 1H), 7.48 (s, ArH, 1H), 7.44 – 7.37 (m, ArH, 2H), 7.26 (s, ArH, 1H), 6.92 (d, *J* = 6.8 Hz, ArH, 2H), 6.84 (d, *J* = 8.0 Hz, ArH, 1H), 6.54 (br s, NH, 1H), 6.22 (dd, *J* = 2.0, 5.6 Hz, ArH, 1H), 5.95 (d, *J* = 2.0 Hz, ArH, 1H), 5.29 (q, *J* = 5.9 Hz, CH, 1H), 4.59 (d, *J* = 5.6 Hz, $ArCH_2$, 2H), 4.07 – 4.01 (m, NCH, 1H), 3.88 (s, OCH_3, 3H), 3.86 (s, OCH_3, 3H), 2.97 (d, *J* = 11.2 Hz, CH_2, 2H), 2.34 (s, NCH_3, 3H), 2.13 (t, *J* =10.8 Hz, 2 × CH_2, 4H), 2.05 – 1.97 (m, CH_2, 2H), 1.60 (d, *J* = 6.4 Hz, $CHCH_3$, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 167.1, 165.5, 159.0, 149.2, 148.7, 148.5, 142.9, 135.2, 133.8, 130.8, 129.1, 128.2, 126.1, 124.2, 122.4, 120.8, 120.2, 111.4, 111.3, 103.6, 92.9, 75.5, 59.1, 56.0, 55.9, 54.5, 45.9, 43.9, 32.4, 32.3, 24.1. ESI-MS m/z: 571.5 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{32}H_{38}N_6O_4[M+H]^+$, 571.3023; found, 571.3027. Anal. Calcd. for $C_{32}H_{38}N_6O_4$: C, 67.35; H, 6.71; N, 14.73; found: C, 67.09;

H, 6.52; N, 14.95.

4.1.55.
*N-(3,4-dimethoxybenzyl)-3-(1-((2-(pyrimidin-2-ylamino)pyridin-4-yl)oxy)ethyl)benzamide (**14b**)*

Compound **12b** (236 mg, 0.50 mmol) and pyrimidin-2-amine (95 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded **14b** (155 mg, 64%). mp 99-101 ℃.^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, ArNH, 1H), 8.98 (t, *J* = 5.8 Hz, CONH, 1H), 8.56 (d, *J* = 4.8 Hz, ArH, 2H), 8.07 (d, *J* = 6.0 Hz, ArH, 1H), 8.00 (s, ArH, 2H), 7.80 (d, *J* = 8.0 Hz, ArH, 1H), 7.61 (d, *J* = 7.6 Hz, ArH, 1H), 7.48 (t, *J* = 7.8 Hz, ArH, 1H), 6.95 – 6.85 (m, ArH, 4H), 6.58 (dd, *J* = 2.0, 5.6 Hz, ArH, 1H), 5.64 (q, *J* = 6.2 Hz, CH, 1H), 4.41 (d, *J* = 5.6 Hz, CH_2, 2H), 3.72 (s, OCH_3, 3H), 3.71 (s, OCH_3, 3H), 1.63 (d, *J* = 6.4 Hz, $CHCH_3$, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 166.3, 165.2, 159.4, 158.4, 154.8, 149.2, 149.0, 148.2, 142.9, 135.2, 132.5, 129.0, 128.9, 126.9, 125.3, 119.9, 113.9, 112.2, 112.0, 106.0, 99.7, 75.6, 56.0, 55.8, 42.9, 24.3. ESI-MS m/z: 486.5 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{27}H_{27}N_5O_4[M+H]^+$, 486.2137; found, 486.2140. Anal. Calcd. for $C_{27}H_{27}N_5O_4$: C, 66.79; H, 5.61; N, 14.42; found: C, 66.98; H, 5.40; N, 14.27.

4.1.56.
*N-(3,4-dimethoxybenzyl)-3-(1-((2-((1-(piperidin-4-yl)-1H-pyrazol-4-yl)amino)pyridin-4-yl)oxy)ethyl)benzamide (**14c**)*

Compound **12b** (236 mg, 0.50 mmol) and *tert*-butyl 4-(4-amino-1*H*-pyrazol-1-yl) piperidine-1-carboxylate (266 mg, 1.00 mmol) following the similar procedure described above for the preparation of **8a** afforded the Boc protected **14c**. ESI-MS m/z: 657.4 $[M+H]^+$. The Boc protected **14c** was dissolved in a mixed solvent system (CH_2Cl_2/TFA = 4:1, 5 mL), then the reaction mixture was stirred at room temperature 1h, and concentrated. The residue was added into saturated Na_2CO_3 solution (40 mL), and extracted with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. The concentrates was purified by flash chromatography on silica gel eluting with MeOH/CH_2Cl_2 (5% - 60%) to give **14c** (152 mg, 55%). mp 145-147 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 – 7.75 (m, ArH, 2H), 7.67 (d, *J* = 7.2 Hz, ArH, 1H), 7.48 (s, ArH, 1H), 7.43 – 7.35 (m, ArH, 2H), 7.10 (s, ArH, 1H), 6.91 (d, *J* = 6.0 Hz, ArH, 2H), 6.82 (d, *J* = 8.8 Hz, ArH, 1H), 6.22 (d, *J* =

5.6 Hz, ArH, 1H), 6.00 (s, ArH, 1H), 5.30 (q, *J* = 5.4 Hz, CH, 1H), 4.57 (d, *J* = 5.6 Hz, CH_2, 2H), 3.85 (s, OCH_3, 3H), 3.83 (s, OCH_3, 3H), 3.30 (m, CH_2, 2H), 2.85 (m, CH_2, 2H), 2.11 (m, CH_2, 5H), 1.58 (d, *J* = 5.6 Hz, $CHCH_3$, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.2, 165.5, 158.6, 154.7, 149.1, 148.4, 148.1, 142.9, 135.0, 133.4, 130.8, 129.1, 128.3, 126.2, 124.5, 122.7, 120.6, 120.1, 111.4, 111.2, 103.8, 75.5, 57.9, 55.9, 44.2, 43.9, 31.5, 24.1. ESI-MS m/z: 557.5 $[M+H]^+$. HRMS, ESI^+, m/z: Calcd. for $C_{31}H_{36}N_6O_4[M+H]^+$, 557.2870; found, 557.2873. Anal. Calcd. for $C_{31}H_{36}N_6O_4$: C, 66.89; H, 6.52; N, 15.10; found: C, 67.13; H, 6.39; N, 15.40.

4.2. Enzyme assays

The kinase inhibitory activity was evaluated by using the Z'-LYTE technology platform (Life Technologies).The experiments were performed according to the instructions of the manufacturer, and the compounds in duplicates with 10 doses in 3-fold serial dilution starting at 10μM. The IC_{50} values were calculated from the inhibition curves from two separate experiments, and single point concentration testing (1μM) with two independent data points (n=2) for kinase selectivity profile of compound **13d** and **14c**.

4.3. HCC78 and H3122 cell lines proliferation inhibition assays

The antiproliferative activities of the new synthesized compounds were evaluated against HCC78 and H3122 cell lines by the standard MTT assay in vitro, with Crizotinib as the positive control. The two cell lines were purchased from Cell Bank of China Science Academy (Shanghai, China). All chemicals and solvents were purchased from Sigma-Aldrich or Gibco. RPMI 1640 medium containing 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin were used for cell growth medium.

The cell lines were suspended in medium to adjust their concentration to approximate 5 × 10^3 cells/mL. The suspension was dispensed at 100μL/well to a 96-well plate and at 37℃ in a humidified atmosphere with 5% CO_2 for 24h. The tested compounds at the indicated final concentrations were added to the culture

medium and incubated for 48 h. Fresh MTT was added to each well at the terminal concentration of 5 mg/mL in PBS, and incubated with cells at 37℃ for 4h. The formazan crystals in each well were dissolved in 150 μL DMSO, and the absorbency at 570 nm was measured with an enzyme-linked immunosorbent assay plate reader. All of the compounds were tested three times in each of the cell lines. The IC_{50} values were calculated by curve fitting using GraphPad Prism Version 4.

4.4. Molecular modeling

Molecular docking was performed with Surflex-Dock program that is interfaced with Sybyl 7.3 [33] according to our previously published protocol [34]. The crystal structure of ROS1 kinase in complex with Crizotinib (ID: 3zbf) was obtained from protein data bank (PDB) [35]. All the water and ligands were removed and the random hydrogen atoms were added. The structures of the synthesized compounds were generated and minimized using tripos force fields. All the other default parameters were used except the bloat value was set to 1 when the protomol was generated. The highest-scored conformation based on the Surflex-Dock scoring functions, was selected as the final docking conformation.

The MD simulations were performed using AMBER 12 software package [36]. The missing residues of 3zbf were modeled by sybyl7.3 programs. The atomic partial charges for **14c** and **13e** were obtained by using the restrained electrostatic potential (RESP) fitting technique [37] implemented in AmberTools [38] at the HF/6-31G* level of the Gaussian09 suite [39]. The force field parameters of small molecules were generated by the general amber force field (GAFF) [40] and AMBER ff99SB force field[41] was used to simulate the protein structure. The ionization state of amino acid residue was set according to the standard protocol. Each complex was immersed in a periodic truncated octahedron box of TIP3P [42] water molecules with a margin distance of 10.0 Å. Then Cl^- or Na^+ ions were added to neutralize the systems using LeaP module in AMBER12.

After the systems were prepared fully, structural optimizations were first performed on the relaxed water molecules and counter ions in two steps with the

harmonic constraint potential of 2.0 kcal·(mol·Å^2)$^{-1}$ on all heavy atoms of both protein and ligands. Afterward, the whole system was minimized without any restraint. The above steps were all executed by 5000 cycles of steepest descent minimization followed by 5000 cycles of conjugate gradient minimization. After system optimization, running MD simulations was started on the systems by gradually heating each system in the NVT ensemble from 0 to 300 K in 50 ps using a Langevin thermostat with a coupling coefficient of 1.0/ps and a force constant 2.0 kcal·(mol·Å^2)$^{-1}$ on the complex. Then 500 ps of density equilibration with a force constant 2.0 kcal·(mol·Å^2)$^{-1}$ on the complex were performed. Subsequently the systems were again equilibrated for 500 ps by releasing all the restrains. Finally, 20 ns production MD runs were performed under the constant temperature of 300 K in the NPT ensemble with periodic boundary conditions for each system. During MD procedure, the long-range Coulombic interactions were handled using the Particle Mesh Ewald (PME) method [43]. The cutoff distance for the long-range vdw energy term was 12.0 Å. The SHAKE algorithm [44]was employed on all atoms covalently bond to hydrogen atoms, allowing for an integration time step of 2 fs. The hydrogen bonds from the equilibrium trajectories of the systems were calculated by VMD1.9 software with a distance cutoff value of 3.5Å and an angle cutoff value of 35°.

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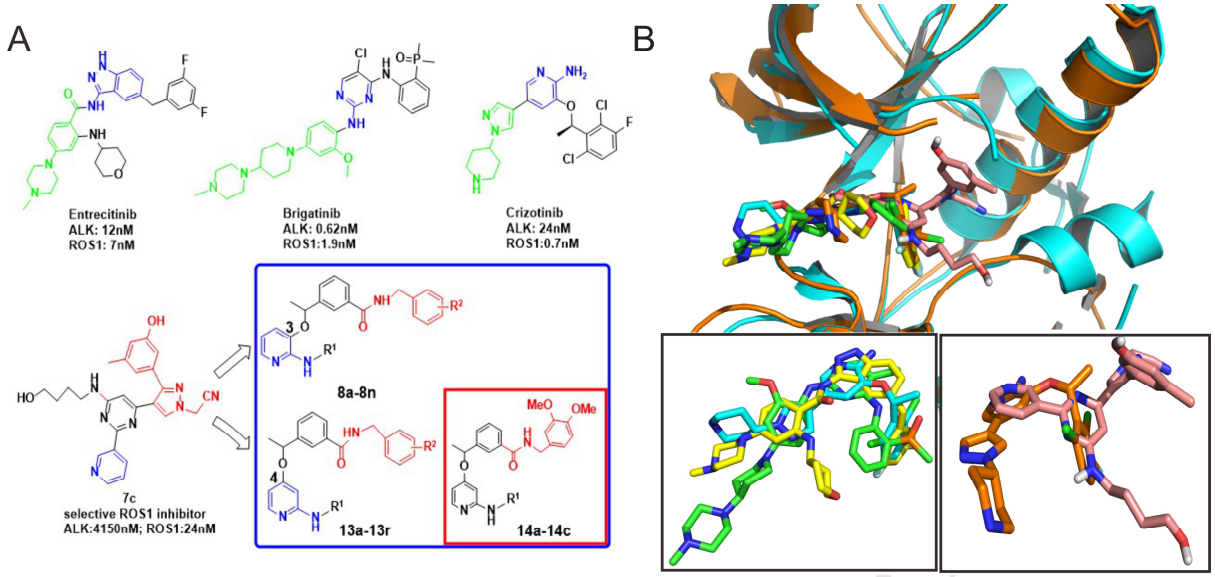

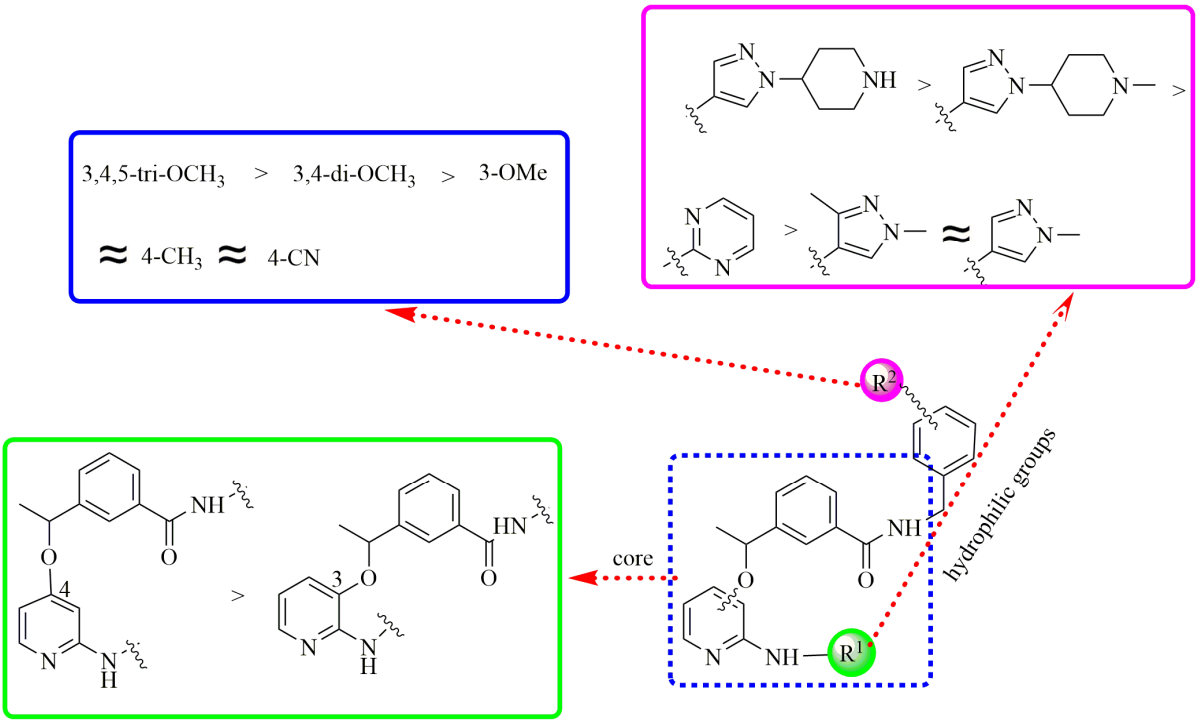

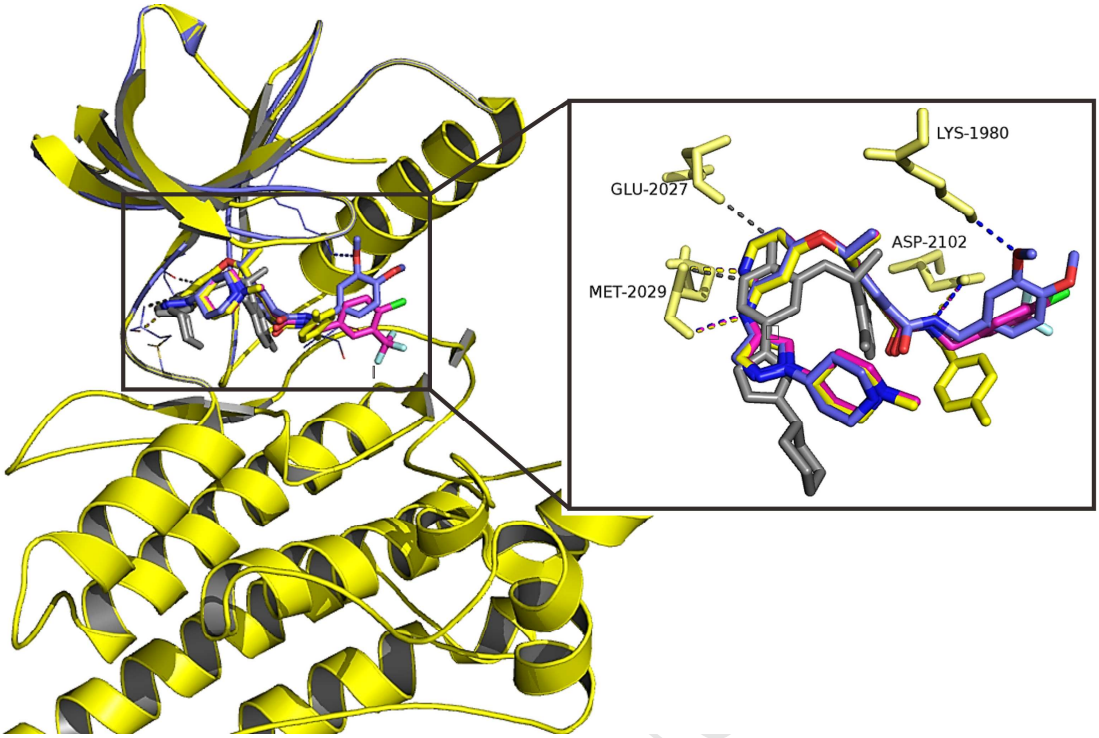

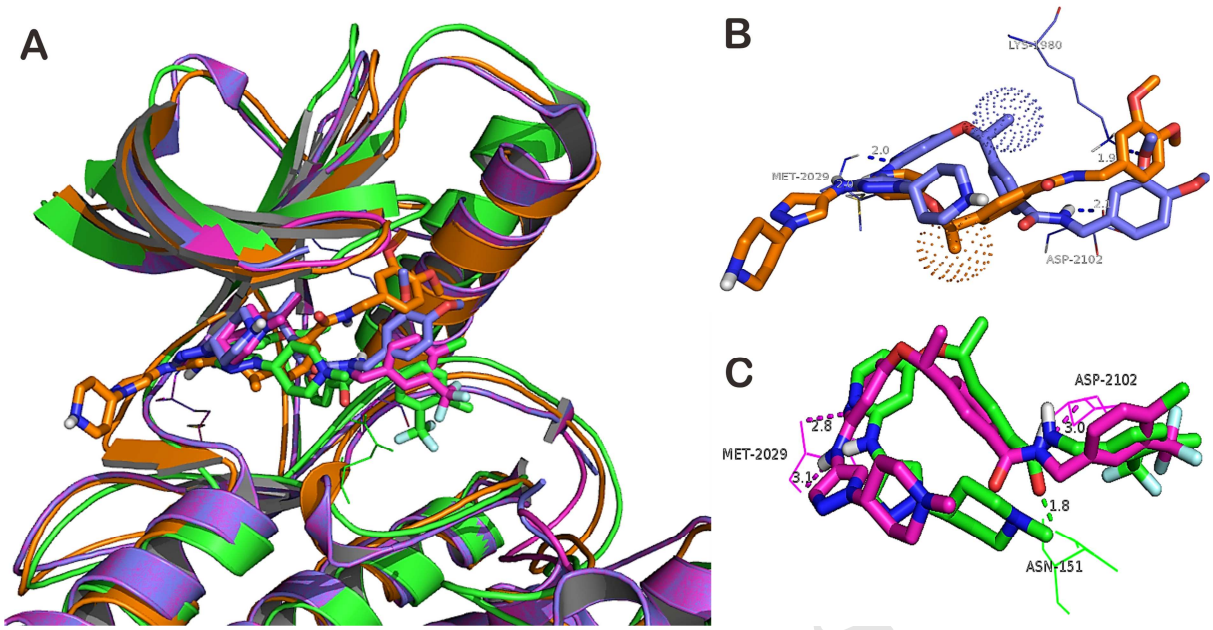

Highlight

- Two novel series of 2-amino-pyridine derivatives with 1-phenylethoxy at C-3 and C-4 position were rationally designed and synthesized for ROS1 inhibitors.
- The compounds 14c and 13d exhibited potential inhibitory activities against ROS1 and HCC78 cell line.
- Molecular docking and molecular dynamics simulation disclosed that the binding mode of the potential compounds as we expected and also gave a probable molecular explanation for their activity and selectivity.
- The structures of compounds are novel and expand the chemical diversity of selective ROS1 inhibitors.