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Discovery of novel 2-aminopyridine-3-carboxamides as c-Met kinase inhibitors

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ABSTRACT

A series of 2-aminopyridine-3-carboxamide derivatives against c-Met were designed and synthesized by employing bioisosteric replacement of heterocyclic moieties with the amide bond. The structure–activity relationship (SAR) at various positions of the scaffold was explored. In this study, a promising compound (***S***)-**24o** with a c-Met IC_{50} of 0.022 μM was identified. The compound exhibited dose-dependent inhibition of the phosphorylation of c-Met as well as downstream signaling in EBC-1 cells. Furthermore, the interactive binding model of (***S***)-**24o** with c-Met was elucidated by virtue of a molecular modeling study.

1. Introduction

c-Met is a receptor tyrosine kinase (RTK), consisting of a disulfide linked α-chain and β-chain. c-Met is known to be a pleiotropic cytokine owing to its regulation of widespread effectors. Activation of c-Met occurs upon binding with hepatocyte growth factor (HGF) and interaction with other membrane receptors.[1] Deregulation of c-Met, frequently associated with Met gene amplification, c-Met overexpression or mutations, has been documented in most solid tumors and hematological malignancies.[2,3] Furthermore, c-Met signaling might be responsible for the emergence of the resistance mechanism in chemotherapy, radiotherapy and other RTK based target therapy.[4] Therefore, c-Met kinase is a promising therapeutic target for cancer.

To date, the main strategies employed to interrupt the c-Met signaling pathway involve blocking the interaction between c-Met and HGF via antibodies[5–16] or antagonists[17–21] and interfering with the active site of the kinase domain with small -molecule kinase inhibitors.[22–41] Recently, among the various strategies, the use of small molecules modulating c-Met has captured extensive attention, resulting in a respectable number of compounds reaching the clinical evaluation.[42]

2-Aminopyridine based c-Met inhibitors (Fig. 1A) have been widely explored in recent years.[26,28,43–46] Generally, these structures feature an aminopyridine core responsible for H-bonding with the hinge region, an aryl fragment (P1 part) extending into the inside pocket and a substituent (P2 part) facing to the solvent accessible region. Crizotinib (Fig. 1A, 1) developed by Pfizer scientists displayed the c-Met inhibition with a K_i value of 0.002 μM.[26] The co-crystal structure of crizotinib (Fig. 1B) bound to c-Met revealed that the 2,6-dichloro-3-fluorobenzyloxy fragment was able to adopt an ideal conformation for involvement in a π–π interaction with Y1230, indicating that an aryl fragment linked with a flexible linker at the C-3 position of the aminopyridine core was tolerated. Lee et al.[28] reported a series of aminopyridines substituted with benzoxazole, among which compound **4** showed highly potent c-Met inhibition (IC_{50} = 0.003 μM). Besides, aminopyridine analogs substituted with benzimidazole (e.g., compound **5**)[45,46] or tetrazole[43] linked with the aryl fragments (e.g., compound **3**) have also been presented. Hence, a rigid substituent or linker was compatible as well at the C-3 position of the aminopyridine core.

Bioisosteric replacement is a fundamental tactical approach widely used in medicinal chemistry.[47] Replacement of heterocyclic moieties with the amide bond is a common means deployed in analogs design. Since the aminopyridine analogs with the imidazole (**5**), oxazole (**4**) or tetrazole (**3**) at the C-3 position displayed potency against c-Met, we surmised the replacement the imidazole, oxazole or tetrazole with the amide bond might give rise to novel c-Met kinase inhibitors (Fig. 2). Meanwhile, according to the co-crystal structural information (Fig. 1B), the introduction of suitable aryl fragments on the amide bond, engaging in a π–π interaction with Y1230, would be appropriate. Herein, we disclose our efforts

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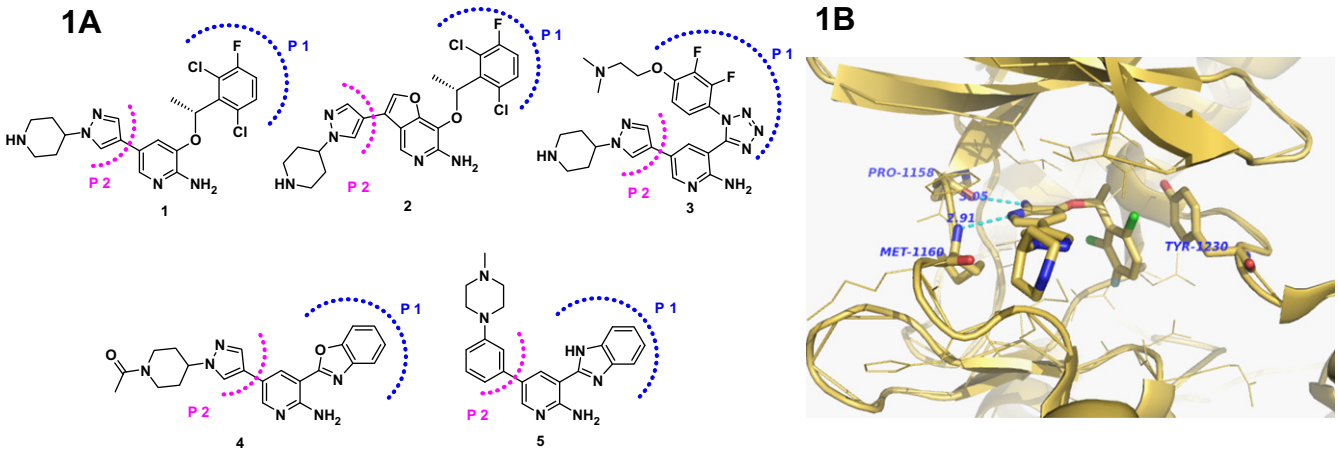


Figure 1. A. Selected examples of amino-pyridine based c-Met kinase inhibitors. B. Cocyrstal structure of crizotinib (**1**) bound to c-Met (2-WGJ).

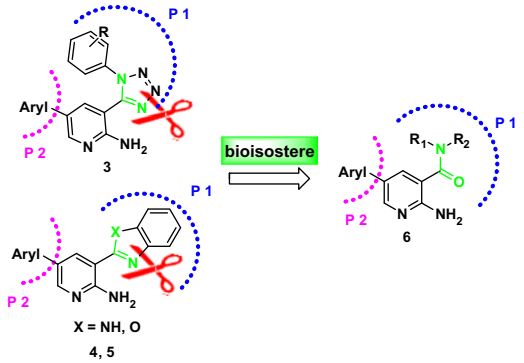
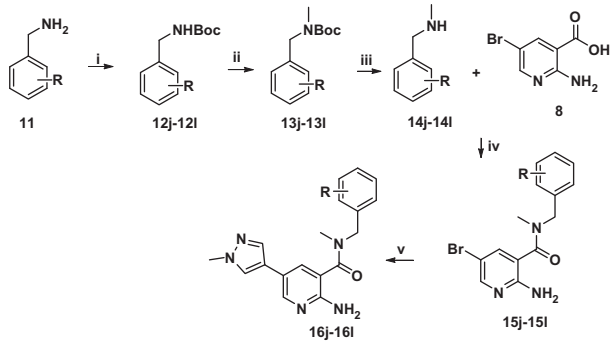


Figure 2. Design of novel 2-aminopyridine-3-carboxamides based on known c-Met kinase inhibitors.



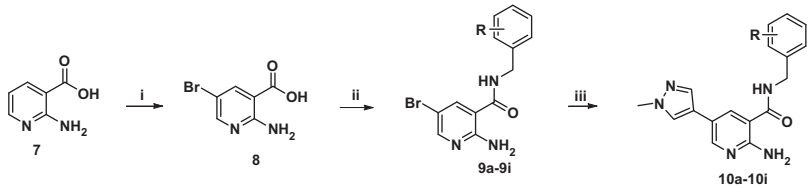
Scheme 2. Reagents and conditions: (**i**) $(Boc)_2O$, THF, rt; (ii) NaH, MeI, DMF, 0 °C-rt; (iii) CH_2Cl_2/TFA (4:1), rt; (iv) HATU, DIPEA, DMF, 0 °C-rt; (**v**) 1-methyl-4-(4,4,5,5-tetramethyl-1,3, 2-dioxaborolan-2-yl)-1*H*-pyrazole, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, DME/H_2O (4:1), 80 °C.

to discover novel 2-aminopyridine-3-carboxamides as c-Met inhibitors.

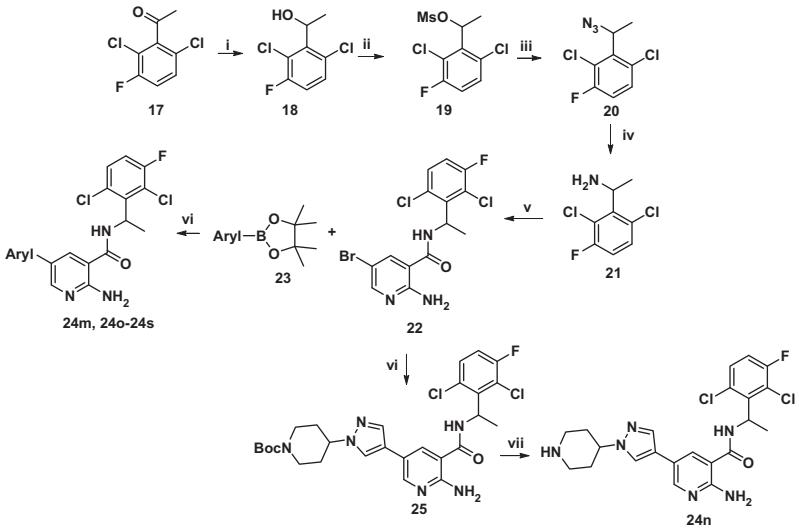
2. Chemistry

Our synthesis commenced with bromination of 2-aminonicotinic acid, followed by coupling with various commercial available benzylamines, to give the intermediates **9a–9i** as shown in Scheme 1. Suzuki coupling of 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole with **9** smoothly afforded compounds **10a–10i**. The preparation of compounds (**16j–16l**) was outlined in Scheme 2. Protection of benzylamines **11**, followed by sequential methylation and deprotection, provided *N*-methyl benzylamines **14**, which were condensed with **8** to give 2-amino-*N*-benzyl-5-bromo-*N*-methylpyridine-3-carboxamides **15j–l**. Treatment of **15j–l** with 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole under Suzuki coupling conditions furnished the desired products (**16j–16l**).

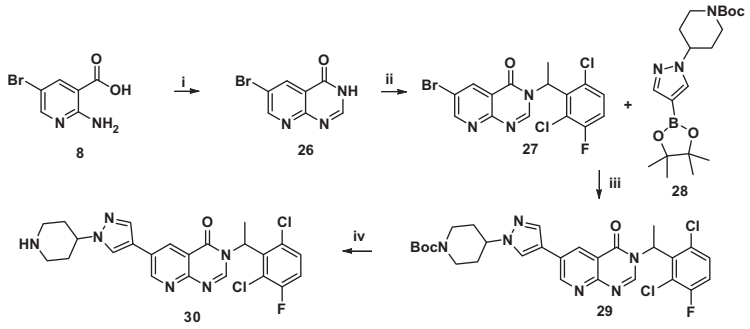
The synthesis of compounds (**24m**, **24o–24s**) was described in Scheme 3. Reduction of 2,6-dichloro-3-fluoroacetophenone with $NaBH_4$ afforded the benzyl alcohol **18**, which was converted into the mesylate **19**. Substitution of **19** with sodium azide, followed by reduction of the resulting benzyl azide using zinc and ammonium chloride (Zn/NH_4Cl), delivered intermediate **21**. Compounds **24m**, **24o–24s**, **25** were similarly obtained as shown in Scheme 1. The removal of Boc group of **25** gave compound **24n**. Compounds **29** and **30** were prepared according to Scheme 4. 2-Amino-5-bromonicotinic acid **8** was smoothly converted into the intermediate **26** in the presence of $HCONH_2$ under microwave irradiation. Alkylation of **26**, followed by Suzuki-coupling, furnished compound **29**. Treatment of **29** with trifluoroacetic acid (TFA) in dichloromethane (CH_2Cl_2) afforded compound **30**.



Scheme 1. Reagents and conditions: (**i**) Br_2, HAc, rt; (ii) benzylamines, HATU, DIPEA, DMF, 0 °C-rt; (iii) 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, DME/H_2O (4:1), 80 °C.



Scheme 3. Reagents and conditions: (i) $NaBH_4$, MeOH, rt; (ii) MsCl, Et_3N, 0 °C-rt; (iii) NaN_3, DMF, 50 °C; (iv) Zn, NH_4Cl, EtOH/H_2O (3:1), rt; (v) **8**, HATU, DIPEA, DMF, 0 °C-rt; (vi) $Pd(PPh_3)_4$, KF·$2H_2O$, DME/H_2O/EtOH (7:3:2), 110 °C, Mw; (vii) CH_2Cl_2/TFA (4:1), rt.



Scheme 4. Reagents and conditions: (i) $HCONH_2$, Mw, 150 °C; (ii) **19**, NaH, DMF, 0–80 °C; (iii) $Pd(PPh_3)_4$, KF.$2H_2O$, DME/H_2O/EtOH (7:3:2), 110 °C, Mw; (iv) CH_2Cl_2/TFA (4:1), rt.

3. Biology

3.1. ELISA kinase assay

The tyrosine kinase activities were evaluated using enzyme-linked-immunosorbent assay (ELISA). Briefly, 96-well plates were precoated with 20 μg/ml Poly (Glu,Tyr) 4:1 (Sigma). 50 μL of 10 μM ATP solution diluted in kinase reaction buffer (50 mM HEPES pH 7.4, 50 mM $MgCl_2$, 0.5 mM $MnCl_2$, 0.2 mM Na_3VO_4, 1 mM DTT) was added to each well. Various concentrations of compounds diluted in 10 μL of 1% DMSO (v/v) were added to each reaction well, with 1% DMSO (v/v) used as the negative control. The kinase reaction was initiated by the addition of purified tyrosine kinase proteins diluted in 40 μL of kinase reaction buffer solution. After incubation for 60 min at 37 °C, the plate was washed three times with phosphate buffered saline (PBS) containing 0.1% Tween 20 (T-PBS). Next, 100 μL of anti-phosphotyrosine (PY99) antibody (1:500 diluted in 5 mg/mL BSA T-PBS) was added. After 30 min incubation at 37 °C, the plate was washed three times. A solution of 100 μL horseradish peroxidase-conjugated goat anti-mouse IgG (1:1000 diluted in 5 mg/mL BSA T-PBS) was added. The plate was reincubated at 37 °C for 30 min, and washed as before. Finally, 100 μL of a solution containing 0.03% H_2O_2 and 2 mg/mL *o*-phenylenediamine in 0.1 mM citrate buffer, pH 5.5, was added and samples were incubated at room temperature until color emerged. The reaction was terminated by the addition of 50 μL of 2 M H_2SO_4, and the plate was read using a multiwell spectrophotometer (VERSAmax™, Molecular Devices, Sunnyvale, CA,USA) at 490 nm. The inhibition rate (%) was calculated using the following equation: [1-(A490/A490 control)] × 100%. IC_{50} values were calculated from the inhibition curves.

3.2. Cell proliferation assay

Cells were seeded in 96-well tissue culture plates. The following day, cells were exposed to compounds at various concentrations and further cultured for 72 h. Finally, cell proliferation was determined using sulforhodamine B (SRB; Sigma) assay. Briefly, cells were fixed by addition of cold 10% trichloroacetic acid (TCA, 4 °C) and incubated at 4 °C for 1 h. After washing to remove TCA, drying in air, 0.4% SRB dissolved in 1% acetic acid was added to each well for staining at room temperature. Then, the plate was washed with 1% acetic acid to remove unbound SRB and dried again. Finally, 10 mM Tris base was added into each well to solubilize the cell-bound dye. The plate was shaken followed by reading the optical density (OD) at 515 nm using a microplate spectrophotometer.

3.3. Western blot analysis

Cells were cultured under regular growth conditions to the exponential growth phase. The cells were then treated with appropriate concentrations of the corresponding compounds for 4 h at

37 °C and lysed in 1 × SDS sample buffer. The lysates were subsequently resolved on 10% SDS-PAGE, and transferred to nitrocellulose membranes. Membranes were probed with, phosphor-c-Met and c-Met, phospho-ERK1/2 and ERK1/2, phospho-AKT and AKT (All from Cell Signaling Technology, Beverly, MA) and GAPDH (KangChen Bio-tech) antibody then subsequently with anti-rabbit or anti-mouse IgG horseradish peroxidase. Immunoreactive proteins were detected using an enhanced chemiluminescence detection reagent.

4. Results and discussion

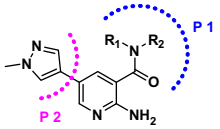
1-Methylpyrazole is usually used to modulate physical chemical properties in active c-Met inhibitors, presumably due to its lower *c*Log *P* and higher PSA.[42] In order to identify potent c-Met inhibitors efficiently, we initially selected 1-methylpyrazole as a substituent at the C-5 position (P2 part) while varying the amide bond (P1 part) (Table 1). The initial biochemical assay found that compounds **10a**–**10i** exhibited moderate activity against c-Met with IC_{50} ranging from 7.00 to 0.41 μM. Clearly, derivatives bearing 2, 5-difluoro (**10c**) and 3,4-difluoro (**10e**) benzyl groups displayed more potent c-Met inhibition than other difluoro substituted analogs (**10a**, **10b**, **10d**). The activity did not significantly change with the introduction of *o,o′*-disubstituents (**10f**–**10i**). Methylation of the amide bond (**16j-16l**) resulted in the complete loss of activity, suggesting that the *N,N′*- disubstituted amide was detrimental to the binding affinity. Although the introduction of various benzyl substituents on the amide bond gave no substantial change of the binding affinity, the primary structure-activity relationship (SAR) in Table 1 revealed that compounds (**10c**, **10e**, **10h**, **10i**) displayed moderate enzymatic and EBC-1 cellular potency, warranting the further modification of the 2-aminopyridine-3-carboxamide scaffold against c-Met kinase.

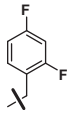
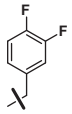
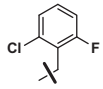
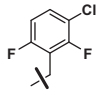
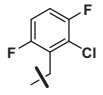
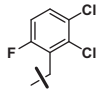
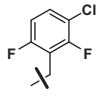
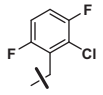
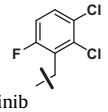
We envisaged that the aryl fragments which had been introduced on the amide bond (Table 1) lacked the effective π–π interaction with Y1230, which led us to install the 2,6-dichloro-3-fluorobenzyl group (a key structural feature of crizotinib involved in π–π interaction with Y1230) with the expectation of an improvement in potency. As anticipated, compound **24m** (Table 2) with the incorporation of this functionality exhibited an improved IC_{50} of 0.28 μM, albeit suffering from a loss of cellular potency. The encouraging result prompted us to optimize the substituents at the C-5 position. Appending the polar piperidine (**24n**) onto the pyrazole retained the comparable emzymatic inhibition (IC_{50} = 0.32 μM), and had a remarkable improvement in cellular potency. With the incorporation of a tetrahydropyranyl substituent, compound **24o** exhibited a three-fold improvement of the enzymatic potency compared with **24m**, and also shown EBC-1 inhibition. Replacement of the pyrazole group with other hetero (**24p**–**24r**) and aryl (**24s**) functionalities resulted in a sharp loss of cellular potency. These observations indicated that modification at the C-5 position had a significant influence on enzymatic and cellular activity.

Next, we wondered whether the amide bond might be too flexible to enable the aryl groups linked with the amide bond efficiently involved in π–π interaction with Y1230. To test this hypothesis, compounds **29** and **30** in which the amide bond is locked into the quinazolinone ring were prepared according to Scheme 4. However, this strategy proved to be unsuccessful, probably due to the loss of an important H-bond between the amino group and the hinge region (Table 3).

Given the promising enzymatic and cellular potency, compound **24o** was selected for subsequent evaluation. In consideration of configuration affecting the c-Met activity, the two enantiomers of compound **24o** were prepared. Biological evaluation found that

Table 1
SAR of substituents on the amide bond

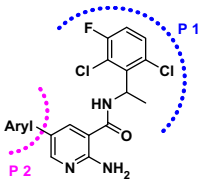


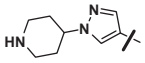
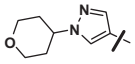
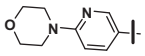
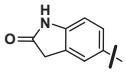
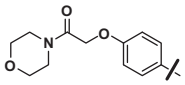
Compound	R_1	R_2	c-Met IC_{50} (μM)	EBC-1[a] IC_{50} (μM)
10a	H		1.20 ± 0.19	ND[b]
10b	H		7.00 ± 1.06	ND[b]
10c	H		0.60 ± 0.03	15.00 ± 1.05
10d	H		4.11 ± 0.04	ND[b]
10e	H		0.48 ± 0.06	14.58 ± 2.35
10f	H		1.30 ± 0.10	ND[b]
10g	H		1.14 ± 0.06	ND[b]
10h	H		0.41 ± 0.10	6.90 ± 0.82
10i	H		0.56 ± 0.06	12.71 ± 1.82
16j	Me		10.9%@1 μM	ND[b]
16k	Me		18.1%@1 μM	ND[b]
16l	Me		13.1%@1 μM	ND[b]
Control	Crizotinib		0.0016 ± 0.0002	0.053 ± 0.011

[a] EBC-1: human non-small-cell lung cancer cell line that expresses elevated levels of constitutively active c-Met
[b] ND: no data.

(***S***)-**24o** was more potent, with an enzymatic IC_{50} of 0.022 μM, and an EBC-1 cell IC_{50} of 1.22 μM, respectively (Fig. 3). The effects of the compound (***S***)-**24o** on c-Met phosphorylation and downstream signaling pathway were further evaluated in EBC-1 human non-small-cell lung cancer cell line which expresses elevated levels of constitutively active c-Met. We found that compound (***S***)-**24o** inhibited the phosphorylation of c-Met in a dose-dependent manner in EBC-1 cells. In addition, Erk1/2 and AKT, the key downstream molecules of c-Met that play important roles in

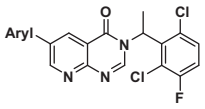
Table 2
SAR about the aryl/heroaryl derivatives at the C-5 position



compound	Aryl	c-Met IC_{50} (μM)	EBC-1[a] IC_{50} (μM)
24m		0.28 ± 0.04	> 50
24n		0.32 ± 0.08	1.46 ± 0.07
24o		0.099 ± 0.02	3.49 ± 1.35
24p		0.51 ± 0.10	ND[b]
24q		0.21 ± 0.03	17.80 ± 1.34
24r		1.14 ± 0.03	ND[b]
24s		0.34 ± 0.01	6.05 ± 0.74
Control	Crizotinib	0.0016 ± 0.0002	0.053 ± 0.011

[a] EBC-1: human non-small-cell lung cancer cell line that expresses elevated levels of constitutively active c-Met
[b] ND: no data.

Table 3
Effects of 3*H*,4*H*-pyrido[2,3-d]pyrimidin-4-ones on inhibitory activity against c-Met



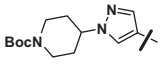
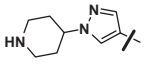
Compound	Aryl	c-Met IC_{50} (μM)
29		28.9%@10 μM
30		34.1%@10 μM

Table 4
Inhibition of tyrosine kinases by compound (***S***)-**24o**.

Kinase	Enzyme IC_{50} (μM)
RON	8.26 ± 0.56
Mer	>10
TYRO3	>10
AXL	>10
KDR	>10
EGFR	>10
FGFR1	3.48 ± 0.73
PDGFR	>10
Flt-1	8.16 ± 0.38
ABL	4.99 ± 3.95
RET	0.21 ± 0.09
EPH-A2	6.18 ± 2.86
ErbB2	>10
ErbB4	>10

c-Met function, were also significantly inhibited upon treatment with (***S***)-**24o**. These data support the fact that (***S***)-**24o** inhibits c-Met activity as well as subsequent c-Met downstream signaling at a cellular level.

To examine whether compound (***S***)-**24o** is a selective c-Met inhibitor, (***S***)-**24o** was screened against another c-Met family member Ron and other 13 tyrosine kinases (Table 4). Compared to its high potency against c-Met (IC_{50} = 0.022 ± 0.003 μM), (***S***)-**24o** also exhibited inhibitory effects against RON, FGFR1 ABL, EPH-A2, and Flt-1 although the potency was 2- to 9-fold lower than that against c-Met. Moreover, compound (***S***)-**24o** showed little or no kinase inhibition activity against other 7 tyrosine kinases (IC_{50} > 10 μM) (Table 4). These data suggest that compound (***S***)-**24o** is a promising selective multitarget kinase inhibitor.

5. Binding model analysis

Although the aminopyridine scaffold is frequently present in c-Met inhibitors, the 2-aminopyridine-3-carboxamide scaffold against c-Met has not been explored yet. To further elucidate the binding mode of the novel 2-aminopyridine-3-carboxamide scaffold with c-Met, a detailed docking analysis was performed. In our study, the co-crystal structure of PF-02341066 with c-Met was selected as the docking model (PDB ID code: 2WGJ). The conformation analysis was conducted using AutoDock4.2 due to its powerful conformational search capabilities.[48] For each compound, 50 conformations provided by the Lamarckian Genetic Algorithm were obtained to efficiently probe the conformational binding space (Fig. 4A). The binding model was exemplified by the interaction of compound **24o** with c-Met (Fig. 4C). Similar to crizotinib (Fig. 4B), the amine and the nitrogen atom of the pyridine ring in compound **24o** H-bond well with the hinge protein residues Pro1158 and Met1160. The pyrazole substituent extends into the solvent-exposed region, indicating that the introduction of

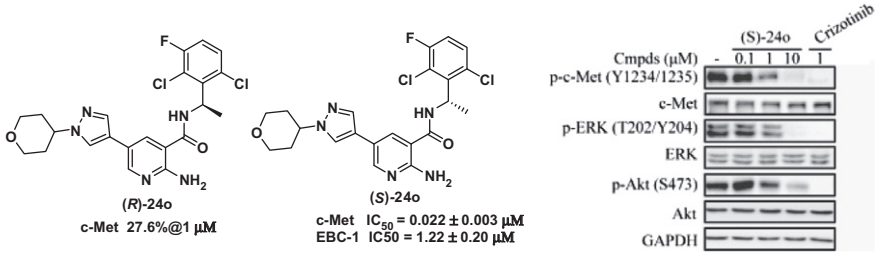


Figure 3. Effects of configuration of analog **24o** on inhibitory activity and its downstream signaling pathways in EBC-1 cells.

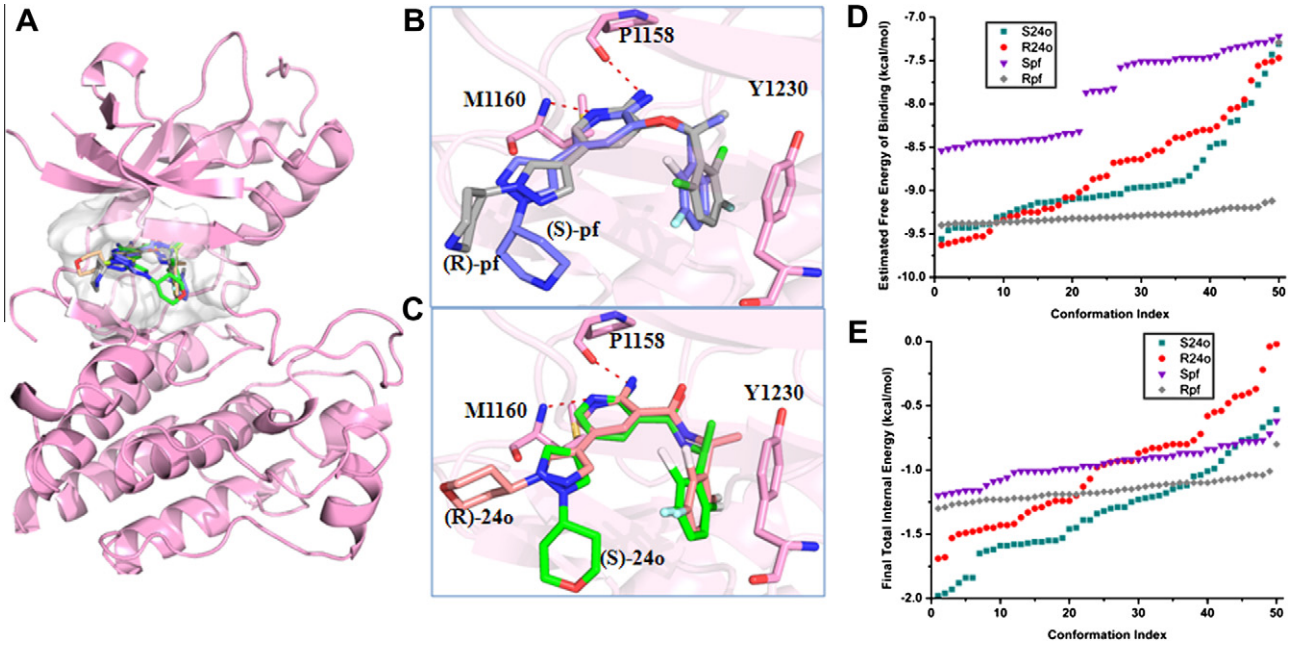


Figure 4. (a) The structure of c-Met kinase was represented as cartoon, while compounds were shown as sticks in the pocket, with surrounding residues shown as gray surface. (b) Binding poses of compounds (*R*)-pf-02341066 (gray stick) and (*S*)-pf-02341066 (purple stick). The surrounding key residues were shown in pink stick. (C) Binding poses of compound (***S***)-**24o** (green stick) and (***R***)-**24o** (light red stick). (d) The estimated free energy of 50 conformations of compounds (***S***)-**24o**, (***R***)-**24o**, (*S*)-pf, (*R*)-pf. To be clarity, the energy data was ranked. (e) The final total internal energy of the four compounds.

hydrophilic substituents here probably is beneficial for potency. In addition, the pyridine and pyrazole rings are in a coplanar conformation, stabilized by resonance, potentially increasing the strength of the H-bond at the hinge region. Furthermore, the 2,6-dichloro-3-fluorobenzyl group orientated by the amide function is involved in an excellent π-π interaction with residue Tyr1230.

In our study, our attention was drawn to the fact that the S enantiomer of **24o** is more potent than the R, while the situation for PF-02341066 is vice versa. In addition to the optimized binding poses, AutoDock4.2 has a free-energy scoring function based on a linear regression analysis of a set of diverse protein-ligand complexes with known inhibition constants. This scoring function uses the AMBER force field to estimate the free energy of binding and final total internal energy. Since the kinase protein was set rigid in the docking simulation, the discrepancy on the total internal energy could be theoretically viewed as the index of the internal energy of compounds only, which may afford the clues for differentiated potency here. Based on the estimated free energy of binding (Fig. 4D), it is obvious that compound (*R*)-pf-02341066 is more potent than (*S*)-pf-02341066, which is consistent with the observed value, indicating that the energetic scoring function is reasonable for this system. In this model, compound (***S***)**-24o** does not have a significant advantage compared with its enantiomer. Regarding the final total internal energy data (Fig. 4E), the 50 conformations of each compound suggest that, in the binding pocket, (***S***)-**24o** has favorable internal energy compared with (***R***)-**24o**, while (*R*)-pf-02341066 is more advantageous than the S configuration. To be more specific, the binding pose of **(*S*)-24o** is in a low energy conformation, which is also the case for (*R*)-pf-02341066. In conclusion, owing to the accessibility of the binding pose, different configurations in **24o** and pf-02341066 displayed different potency against the kinase.

6. Conclusions

In conclusion, a series of 2-aminopyridine-3-carboxamide derivatives were designed synthesized and evaluated as novel c-Met inhibitors. The SAR exploration led to the identification of a potent compound (***S***)-**24o** with a c-Met IC_{50} of 0.022 μM. This compound exhibited dose-dependent inhibition of the phosphorylation of c-Met as well as c-Met downstream signaling in EBC-1 cells. Initial biochemical selectivity screening revealed that (***S***)-**24o** is a multitarget kinase inhibitor. The interactive binding model of (***S***)-**24o** was elucidated using AutoDock4.2. Further optimization of the 2-aminopyridine-3-carboxamide scaffold in order to identify compounds with improved enzymatic and cellular potency is currently under investigation.

7. Experimental

Unless otherwise noted, the reagents (chemicals) were purchased from commercial sources, and used without further purification. Analytical thin layer chromatography (TLC) was HSGF 254 (0.15-0.2 mm thickness). Compound spots were visualized by UV light (254 nm). Column chromatography was performed on silica gel FCP 200–300. NMR spectra were run on 300 or 400 MHz instrument. Chemical shifts were reported in parts per million (ppm, δ) downfield from tetramethylsilane. Proton coupling patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Low- and high-resolution mass spectra (LRMS and HRMS) were measured on spectrometer. Chiral HPLC analysis was performed on a Chiral-Park IA column.

7.0.1. 2-Amino-5-bromonicotinic acid (8)

2-Aminonicotinic acid (10.00 g, 72.40 mmol) was suspended in 40 ml of glacial acetic acid. To this suspension was added dropwise a solution of 4.82 ml (94.12 mmol) of bromine in 20 ml of glacial acetic acid. The mixture was stirred vigorouly at room temperature for 30 min. The formed precipitate was filtered off and washed with glacial acetic acid. The filter cake was collected and crystallized from the boiling methol to afford compound **8** as a white solid

(14.5 g, 92% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.30 (d, J = 2.4 Hz, 1H), 8.16 (d, J = 2.4 Hz, 1H); MS (ESI, m/z): 216.8 $[M+H]^+$.

7.0.2. 2-Amino-5-bromo-*N*-[(2,3-difluorophenyl)methyl] pyridine-3-carboxamide (9a). General experimental procedure for 9b-9i, 15j-15l and 22

2-Amino-5-bromonicotinic acid (250 mg, 1.15 mmol) was dissolved in 4 ml of DMF. The solution was cooled in an ice bath. Then to the solution were successively added HATU (460 mg, 1.21 mmol), DIPEA (210.68 μl, 1.21 mmol), (2,3-difluorophenyl)methanamine (173 mg, 1.21 mmol). The resulting solution was stirred at room temperature for 2 h and then the saturated sodium bicarbonate solution was added. The mixture was extracted with ethyl acetate (EA). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was used in the next step without purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (t, J = 5.6 Hz, 1H), 8.16 (s, 2H), 7.38-7.09 (m, 5H), 4.49 (d, J = 5.6 Hz, 2H); MS (ESI, m/z): 341.8 $[M+H]^+$.

7.0.3. 2-Amino-5-bromo-*N*-[(2,4-difluorophenyl)methyl] pyridine-3-carboxamide (9b)

Compound **9b** was prepared in a similar manner as described for compound **9a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (t, J = 5.6 Hz, 1H), 8.16 (s, 2H), 7.46–7.40 (m, 1H), 7.35–7.15 (m, 3H), 7.09–7.01 (m, 1H), 4.42 (d, J = 5.6 Hz, 2H); MS (ESI, m/z): 341.9 $[M+H]^+$.

7.0.4. 2-Amino-5-bromo-*N*-[(2,5-difluorophenyl)methyl] pyridine-3-carboxamide (9c)

Compound **9c** was prepared in a similar manner as described for compound **9a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.08 (t, J = 5.6 Hz, 1H), 8.18 (d, J = 2.4 Hz, 1H), 8.17 (d, J = 2.4 Hz, 1H), 7.30–7.09 (m, 5H), 4.44 (d, J = 5.6 Hz, 2H); MS (ESI, m/z): 342.0 $[M+H]^+$.

7.0.5. 2-Amino-5-bromo-*N*-[(3,5-difluorophenyl)methyl] pyridine-3-carboxamide (9d)

Compound **9d** was prepared in a similar manner as described for compound **9a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (t, J = 5.6 Hz, 1H), 8.18 (d, J = 2.4 Hz, 1H), 8.17 (d, J = 2.4 Hz, 1H), 7.27 (s, 2H), 7.14–7.01 (m, 3H), 4.44 (d, J = 5.6 Hz, 2H); MS (ESI, m/z): 341.9 $[M+H]^+$.

7.0.6. 2-Amino-5-bromo-*N*-[(3,4-difluorophenyl)methyl] pyridine-3-carboxamide (9e)

Compound **9e** was prepared in a similar manner as described for compound **9a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (t, J = 5.6 Hz, 1H), 8.15 (s, 1H), 7.41–7.33 (m, 1H), 7.25 (s, 2H), 7.17–7.06 (m, 1H), 4.40 (d, J = 5.6 Hz, 2H); MS (ESI, m/z): 341.7 $[M+H]^+$.

7.0.7. 2-Amino-5-bromo-*N*-[(2-chloro-6-fluorophenyl)methyl] pyridine-3-carboxamide (9f)

Compound **9f** was prepared in a similar manner as described for compound **9a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.81 (t, J = 4.4 Hz, 1H), 8.14 (d, J = 2.4 Hz, 1H), 8.08 (d, J = 2.4 Hz, 1H), 7.46–7.22 (m, 5H), 4.54 (d, J = 3.6 Hz, 2H); MS (ESI, m/z): 357.9 $[M+H]^+$.

7.0.8. 2-Amino-5-bromo-*N*-[(3-chloro-2,6-difluorophenyl) methyl]pyridine-3-carboxamide (9g)

Compound **9g** was prepared in a similar manner as described for compound **9a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.96 (t, J = 4.8 Hz, 1H), 8.14 (d, J = 2.4 Hz, 1H), 8.09 (d, J = 2.4 Hz, 1H), 7.64–7.58 (m, 1H), 7.26 (s, 2H), 7.23–7.15 (m, 1H), 4.49 (d, J = 4.8 Hz, 2H); MS (ESI, m/z): 375.9 $[M+H]^+$.

7.0.9. 2-Amino-5-bromo-*N*-[(2-chloro-3,6-difluorophenyl) methyl]pyridine-3-carboxamide (9h)

Compound **9h** was prepared in a similar manner as described for compound **9a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (t, J = 4.4 Hz, 1H), 8.14 (d, J = 2.4 Hz, 1H), 8.07 (d, J = 2.4 Hz, 1H), 7.50–7.44 (m, 1H), 7.36–7.30 (m, 1H), 7.26 (s, 2H), 4.56 (d, J = 3.6 Hz, 2H); MS (ESI, m/z): 375.8 $[M+H]^+$.

7.0.10. 2-Amino-5-bromo-*N*-[(2,3-dichloro-6-fluorophenyl) methyl]pyridine-3-carboxamide (9i)

Compound **9i** was prepared in a similar manner as described for compound **9a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (t, J = 4.4 Hz, 1H), 8.14 (d, J = 2.4 Hz, 1H), 8.07 (d, J = 2.4 Hz, 1H), 7.69 (dd, J = 5.6, 9.2 Hz, 1H), 7.35 (t, J = 9.2 Hz, 1H), 7.26 (s, 2H), 4.57 (d, J = 3.2 Hz, 2H); MS (ESI, m/z): 391.9 $[M+H]^+$.

7.0.11. 2-Amino-*N*-[(2,3-difluorophenyl)methyl]-5-(1-methyl-1*H*-pyrazol-4-yl)pyridine-3-carboxamide (10a). General experimental procedure for 10a–10i and 16j–16l

To a solution of 2-amino-5-bromo-*N*-[(2,3-difluorophenyl)methyl]pyridine-3-carboxamide **9a** (250 mg, 0.73 mmol) and 1-methyl-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (160 mg, 1.05 mmol) was added sodium carbonate (232 mg, 2.19 mmol) solution in 1 ml of water, followed by $Pd(PPh_3)Cl_2$ (25.64 mg, 0.05 mmol). The resulting reaction medium was evacuated and refilled with argon three times. Then the reaction medium was heated at 80 °C for 16 h and was extracted with EA. After washing with brine, the solution was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$, 1/50) to afford compound **10a** as a white solid in 81% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J = 2.4 Hz, 1H), 7.69 (d, J = 2.4 Hz, 1H), 7.61 (s, 1H), 7.52 (s, 1H), 7.20–7.06 (m, 3H), 6.83 (t, J = 5.6 Hz, 1H), 6.36 (s, 2H), 4.69 (d, J = 5.6 Hz, 2H), 3.90 (s, 3H); MS (ESI, m/z): 344.0 $[M+H]^+$; HRMS (ESI) calcd for $C_{17}H_{16}F_2N_5O$ ($[M+H]^+$): 344.1323; found : 344.1328.

7.0.12. 2-Amino-*N*-[(2,4-difluorophenyl)methyl]-5-(1-methyl-1*H*-pyrazol-4-yl)pyridine-3-carboxamide (10b)

Compound **10b** was prepared as a white solid in a similar manner as described for compound **10a**. Yield: 47%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J = 2.0 Hz, 1H), 7.69 (d, J = 2.0 Hz, 1H), 7.62 (s, 1H), 7.53 (s, 1H), 7.44–7.38 (m, 1H), 6.89–6.81 (m, 2H), 6.77 (br, 1H), 6.41 (s, 2H), 4.62 (d, J = 5.6 Hz, 2H), 3.90 (s, 3H). EI–MS m/z 343 $[M]^+$; HRMS (EI) m/z calcd for $[M]^+$ 343.1245, found 343.1238.

7.0.13. 2-Amino-*N*-[(2,5-difluorophenyl)methyl]-5-(1-methyl-1*H*-pyrazol-4-yl)pyridine-3-carboxamide (10c)

Compound **10c** was prepared as a white solid in a similar manner as described for compound **10a**. Yield: 83%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 (t, J = 5.6 Hz, 1H), 8.35 (d, J = 2.4 Hz, 1H), 8.18 (d,

J = 2.4 Hz, 1H), 8.01 (s, 1H), 7.79 (s, 1H), 7.30–7.24 (m, 1H), 7.22–7.14 (m, 2H), 7.06 (s, 2H), 4.49 (d, *J* = 6.0 Hz, 2H), 3.85 (s, 3H); EI–MS *m*/*z* 343 $[M]^+$; HRMS (EI) *m*/*z* calcd for $[M]^+$ 343.1245, found 343.1250.

7.0.14. 2-Amino-*N*-[(3,5-difluorophenyl)methyl]-5-(1-methyl-1*H*-pyrazol-4-yl)pyridine-3-carboxamide (10d)

Compound **10d** was prepared as a white solid in a similar manner as described for compound **10a**. Yield: 55%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, *J* = 2.0 Hz, 1H), 7.69 (d, *J* = 2.0 Hz, 1H), 7.61 (s, 1H), 7.52 (s, 1H), 6.90–6.86 (m, 2H), 6.81 (t, *J* = 5.6 Hz, 1H), 6.76–6.70 (m, 1H), 6.34 (s, 2H), 4.60 (d, *J* = 5.6 Hz, 2H), 3.90 (s, 3H); MS (ESI, *m*/*z*): 344.0 $[M+H]^+$; HRMS (ESI) calcd for $C_{17}H_{16}F_2N_5O$ ($[M+H]^+$): 344.1323; found : 344.1320.

7.0.15. 2-Amino-*N*-[(3,4-difluorophenyl)methyl]-5-(1-methyl-1*H*-pyrazol-4-yl)pyridine-3-carboxamide (10e)

Compound **10e** was prepared as a white solid in a similar manner as described for compound **10a**. Yield: 77%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, *J* = 2.0 Hz, 1H), 7.71 (d, *J* = 2.0 Hz, 1H), 7.61 (s, 1H), 7.52 (s, 1H), 7.20–7.06 (m, 3H), 6.85 (t, *J* = 5.6 Hz, 1H), 6.38 (s, 2H), 4.56 (d, *J* = 5.6 Hz, 2H), 3.89 (s, 3H); MS (ESI, *m*/*z*): 344.0 $[M+H]^+$; HRMS (ESI) calcd for $C_{17}H_{15}F_2N_5O$ ($[M+H]^+$): 344.1323; found : 344.1315.

7.0.16. 2-Amino-*N*-[(2-chloro-6-fluorophenyl)methyl]-5-(1-methyl-1*H*-pyrazol-4-yl)pyridine-3-carboxamide (10f)

Compound **10f** was prepared as a white solid in a similar manner as described for compound **10a**. Yield: 53%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, *J* = 2.4 Hz, 1H), 7.65 (d, *J* = 2.4 Hz, 1H), 7.61 (d, *J* = 1.6 Hz, 1H), 7.52 (s, 1H), 7.29–7.22 (m, 2H), 7.08–7.03 (m, 1H), 6.64 (t, *J* = 5.6 Hz, 1H), 6.39 (s, 2H), 4.80 (d, *J* = 1.6, 5.6 Hz, 2H), 3.92 (s, 3H); MS (ESI, *m*/*z*): 359.9 $[M+H]^+$; HRMS (ESI) calcd for $C_{17}H_{16}ClFN_5O$ ($[M+H]^+$): 360.1027; found : 360.1034.

7.0.17. 2-Amino-*N*-[(3-chloro-2,6-difluorophenyl)methyl]-5-(1-methyl-1*H*-pyrazol-4-yl)pyridine-3-carboxamide (10g)

Compound **10g** was prepared as a white solid in a similar manner as described for compound **10a**. Yield: 72%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, *J* = 2.0 Hz, 1H), 7.64 (d, *J* = 2.0 Hz, 1H), 7.61 (s, 1H), 7.52 (s, 1H), 7.37–7.31 (m, 1H), 6.93–6.88 (m, 1H), 6.68 (t, *J* = 5.6 Hz, 1H), 6.33 (s, 2H), 4.73 (d, *J* = 5.6 Hz, 2H), 3.91 (s, 3H); EI–MS *m*/*z* 377 $[M]^+$; HRMS (EI) *m*/*z* calcd for $[M]^+$ 377.0855, found 377.0850.

7.0.18. 2-Amino-*N*-[(2-chloro-3,6-difluorophenyl)methyl]-5-(1-methyl-1*H*-pyrazol-4-yl)pyridine-3-carboxamide (10h)

Compound **10h** was prepared as a white solid in a similar manner as described for compound **10a**. Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, *J* = 2.4 Hz, 1H), 7.62 (s, 1H), 7.59 (d, *J* = 2.4 Hz, 1H), 7.51 (s, 1H), 7.16–7.10 (m, 1H), 7.06–7.01 (m, 1H), 6.52 (t, *J* = 5.6 Hz, 1H), 6.25 (s, 2H), 4.81 (dd, *J* = 1.2, 5.6 Hz, 2H), 3.92 (s, 3H); EI–MS *m*/*z* 377 $[M]^+$; HRMS (EI) *m*/*z* calcd for $[M]^+$ 377.0855, found 377.0848.

7.0.19. 2-Amino-*N*-[(2,3-dichloro-6-fluorophenyl)methyl]-5-(1-methyl-1*H*-pyrazol-4-yl)pyridine-3-carboxamide (10i)

Compound **10i** was prepared as a white solid in a similar manner as described for compound **10a**. Yield: 64%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, *J* = 2.4 Hz, 1H), 7.63 (d, *J* = 2.4 Hz, 1H), 7.61 (s, 1H), 7.52 (s, 1H), 7.43 (dd, *J* = 5.6, 8.8 Hz, 1H), 7.05–7.01 (m, 1H), 6.68 (t, *J* = 5.6 Hz, 1H), 6.33 (s, 2H), 4.82 (dd, *J* = 1.6, 5.6 Hz, 2H), 3.91 (s, 3H); EI–MS *m*/*z* 393 $[M]^+$; HRMS (EI) *m*/*z* calcd for $[M]^+$ 393.0559, found 393.0559.

7.1. *tert*-butyl *N*-[(3-chloro-2,6-difluorophenyl)methyl] carbamate (12j). General experimental procedure for 12k–12l

Di-*tert*-butyl dicarbonate (385 mg, 1.76 mmol) was slowly added to an ice-cooled stirred THF (4 ml) solution of (3-chloro-2,6-difluorophenyl)methanamine (298 mg, 1.68 mmol). The resulting reaction mixture was stirred at 0 °C for 1 h. The reaction solution was concentrated under reduced pressure and the residue was extracted with CH_2Cl_2. After washing with saturated NaCl, the organic layer was dried over anhydrous Na_2SO_4. After the removal of the solvent by evaporation under vacuum, the crude product was pure enough for use in the next step. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.60–7.54 (m, 1H), 7.36 (t, *J* = 5.6 Hz, 1H), 7.19–7.11 (m, 1H), 4.20 (d, *J* = 5.6 Hz, 2H), 1.36 (s, 9H).

7.2. *tert*-butyl *N*-[(2-chloro-3,6-difluorophenyl)methyl] carbamate (12k)

Compound **12k** was prepared in a similar manner as described for compound **12j**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45–7.39 (m, 1H), 7.30–7.25 (m, 2H), 4.27 (d, *J* = 4.8 Hz, 2H), 1.36 (s, 9H).

7.3. *tert*-butyl *N*-[(2,3-dichloro-6-fluorophenyl)methyl] carbamate (12l)

Compound **12l** was prepared in a similar manner as described for compound **12j**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.64 (dd, *J* = 5.2, 8.8 Hz, 1H), 7.30–7.24 (m, 1H), 4.29 (d, *J* = 4.0 Hz, 2H), 1.36 (s, 9H).

7.4. *tert*-butyl *N*-[(3-chloro-2,6-difluorophenyl)methyl]-*N*-methylcarbamate (13j). General experimental procedure for 13k–13l

69 mg (1.73 mmol) of sodium hydride (60 percent in oil) was added portionwise to an ice-cooled solution of 400 mg (1.44 mmol) of *tert*-butyl *N*-[(3-chloro-2,6-difluorophenyl)methyl] carbamate in 4 ml of DMF, and the reaction medium was stirred for 30 min. Then 183 µl (3.60 mmol) of methyl iodide was added dropwise and stirring is continued for 18 h. The ice-cold water was added to the reaction medium. The mixture was extracted with ethyl acetate. The organic phase was washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified by silica gel column chromatography (EA/PE, 1/50) to afford compound **13j** in 87% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.28 (m, 1H), 6.88–6.83 (m, 1H), 4.53 (s, 2H), 2.85 (s, 3H), 1.46 (s, 9H).

7.5. *tert*-butyl *N*-[(2-chloro-3,6-difluorophenyl)methyl]-*N*-methylcarbamate (13k)

Compound **13k** was prepared in a similar manner as described for compound **13j**. Yield: 94%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11–7.05 (m, 1H), 7.00–6.94 (m, 1H), 4.67 (s, 2H), 2.75 (s, 3H), 1.47 (s, 9H).

7.6. *tert*-butyl *N*-[(2,3-dichloro-6-fluorophenyl)methyl]-*N*-methylcarbamate (13l)

Compound **13l** was prepared in a similar manner as described for compound **13j**. Yield: 90%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40

(dd, J = 5.6, 8.8 Hz, 1H), 6.98 (t, J = 8.8 Hz, 1H), 4.69 (s, 2H), 2.74 (s, 3H), 1.47 (s, 9H).

7.6.1. [(3-Chloro-2,6-difluorophenyl)methyl](methyl)amine (14j). General experimental procedure for 14k-14l, 24n and 30

To a solution of *tert*-butyl *N*-[(3-chloro-2,6-difluorophenyl)methyl]-*N*-methylcarbamate **13j** (379 mg, 1.30 mmol) in 2 ml of CH_2Cl_2 was added 0.5 ml of TFA. The resulting solution was stirred at room temperature for 2 h and concentrated under vacuum. The residue was diluted with CH_2Cl_2 and washed with saturated sodium carbonate, followed by brine. The organic layer was dried over anhydrous Na_2SO_4. After the removal of the solvent by evaporation under vacuum, the crude product was pure enough for use in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32–7.25 (m, 1H), 6.87–6.82 (m, 1H), 3.90 (s, 2H), 3.75 (s, 1H), 2.42 (s, 3H).

7.6.2. [(2-chloro-3,6-difluorophenyl)methyl](methyl)amine (14k)

Compound **14k** was prepared in a similar manner as described for compound **14j**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24–7.18 (m, 1H), 7.10–7.03 (m, 1H), 4.24 (d, J = 2.0 Hz, 2H), 2.62 (s, 3H).

7.6.3. [(2,3-dichloro-6-fluorophenyl)methyl](methyl)amine (14l)

Compound **14l** was prepared in a similar manner as described for compound **14j**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (dd, J = 5.2, 8.8 Hz, 1H), 6.95 (t, J = 8.8 Hz, 1H), 3.95 (d, J = 2.4 Hz, 2H), 2.38 (s, 3H).

7.6.4. 2-Amino-5-bromo-*N*-[(3-chloro-2,6-difluorophenyl)methyl]-*N*-methylpyridine-3-carboxamide (15j)

Compound **15j** was prepared in a similar manner as described for compound **9a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.49 (s, 1H), 7.40–7.35 (m, 1H), 6.94–6.89 (m, 1H), 5.25 (s, 2H), 4.82 (s, 2H), 2.95 (s, 3H); MS (ESI, m/z): 389.9 $[M+H]^+$.

7.6.5. 2-Amino-5-bromo-*N*-[(2-chloro-3,6-difluorophenyl)methyl]-*N*-methylpyridine-3-carboxamide (15k)

Compound **15k** was prepared in a similar manner as described for compound **9a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, J = 2.4 Hz, 1H), 7.48 (d, J = 2.0 Hz, 1H), 7.17–7.11 (m, 1H), 7.06–7.00 (m, 1H), 5.26 (s, 2H), 4.90 (s, 2H), 2.86 (s, 3H); MS (ESI, m/z): 389.8 $[M+H]^+$.

7.6.6. 2-Amino-5-bromo-*N*-[(2,3-dichloro-6-fluorophenyl)methyl]-*N*-methylpyridine-3-carboxamide (15l)

Compound **15l** was prepared in a similar manner as described for compound **9a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, J = 2.4 Hz, 1H), 7.50–7.43 (m, 2H), 7.03 (t, J = 8.8 Hz, 1H), 5.27 (s, 2H), 4.91 (s, 2H), 2.85 (s, 3H); MS (ESI, m/z): 405.8 $[M+H]^+$.

7.6.7. 2-Amino-*N*-[(3-chloro-2,6-difluorophenyl)methyl]-*N*-methyl-5-(1-methyl-1*H*-pyrazol-4-yl)pyridine-3-carboxamide (16j)

Compound **16j** was prepared as a white solid in a similar manner as described for compound **10a**. Yield: 42%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J = 2.4 Hz, 1H), 7.64 (s, 1H), 7.52 (s, 1H), 7.49 (d, J = 2.4 Hz, 1H), 7.37–7.33 (m, 1H), 6.93–6.88 (m, 1H), 5.12 (s, 2H), 4.84 (s, 2H), 3.93 (s, 3H), 2.97 (s, 3H); EI–MS m/z 391 $[M]^+$; HRMS (EI) m/z calcd for $[M]^+$ 391.1011, found 391.1012.

7.6.8. 2-Amino-*N*-[(2-chloro-3,6-difluorophenyl)methyl]-*N*-methyl-5-(1-methyl-1*H*-pyrazol-4-yl)pyridine-3-carboxamide (16k)

Compound **16k** was prepared as a white solid in a similar manner as described for compound **10a**. Yield: 79%; (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.64 (s, 1H), 7.61 (s, 1H), 7.55 (s, 1H), 7.19–7.14 (m, 1H), 7.08–7.03 (m, 1H), 5.90 (s, 2H), 4.94 (s, 2H), 3.95 (s, 3H), 2.91 (s, 3H); EI–MS m/z 391 $[M]^+$; HRMS (EI) m/z calcd for $[M]^+$ 391.1011, found 391.1012.

7.6.9. 2-Amino-*N*-[(2,3-dichloro-6-fluorophenyl)methyl]-*N*-methyl-5-(1-methyl-1*H*-pyrazol-4-yl)pyridine-3-carboxamide (16l)

Compound **16l** was prepared as a white solid in a similar manner as described for compound **10a**. Yield: 66%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, J = 2.0 Hz, 1H), 7.62 (d, J = 1.2 Hz, 1H), 7.51 (d, J = 1.2 Hz ,1H), 7.48 (d, J = 2.4 Hz, 1H), 7.44 (dd, J = 5.6, 8.8 Hz, 1H), 7.04–7.01 (m, 1H), 5.19 (s, 2H), 4.94 (s, 2H), 3.92 (s, 3H), 2.87 (s, 3H); EI–MS m/z 407 $[M]^+$; HRMS (EI) m/z calcd for $[M]^+$ 407.0716, found 407.0718.

7.6.10. 1-(2,6-Dichloro-3-fluorophenyl)ethan-1-ol (18)

To a solution of 1-(2,6-dichloro-3-fluorophenyl)ethan-1-one **17** (20.00 g, 96.60 mmol) in 100 ml of MeOH was added portionwise $NaBH_4$ (7.31 g, 193.21 mmol). The resulting mixture was stirred at room temperature for 2 h and then quenched with 20 ml of water. After the removal of MeOH under vacuum, the residue was extracted with EA and washed with brine. The organic layer was dried over anhydrous Na_2SO_4. After the removal of the solvent by evaporation under vacuum, the crude product was pure enough for use in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (dd, J = 4.8, 8.8 Hz, 1H), 7.02 (dd, J = 8.0, 8.8 Hz, 1H), 5.57 (q, J = 6.8 Hz, 1H), 2.86 (br, 1H), 1.64 (d, J = 6.8 Hz, 3H).

7.6.11. 1-(2,6-Dichloro-3-fluorophenyl)ethyl methanesulfonate (19)

To a solution of 1-(2,6-dichloro-3-fluorophenyl)ethan-1-ol **18** (19.00 g, 90.89 mmol) in 150 ml of CH_2Cl_2 was added Et_3N (13.27 ml, 95.43 mmol) and catalytic amount of DMAP. The resulting solution was cooled in an ice bath and added dropwise MsCl (7.39 ml, 95.43 mmol). After complete addition of MsCl, the reaction mixture was maintained in the ice bath for 1 h and then 30 ml of water was added to the reaction mixture. The organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was pure enough for use in the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (dd, J = 4.8, 9.0 Hz, 1H), 7.12 (dd, J = 8.1, 9.0 Hz, 1H), 6.45 (q, J = 6.9 Hz, 1H), 2.91 (s, 3H), 1.84 (d, J = 6.9 Hz, 3H).

7.6.12. 2-(1-Azidoethyl)-1,3-dichloro-4-fluorobenzene (20)

To a solution of 1-(2,6-Dichloro-3-fluorophenyl)ethyl methanesulfonate 19 (500 mg, 1.74 mmol) in 4 ml of DMF was added NaN_3 (226 mg, 3.48 mmol). The resulting mixture was stirred at 50 °C for 1 h and diluted with 20 ml of water. The mixture was extracted with EA (2 × 10 ml). The organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. After the removal of the solvent by evaporation under vacuum, the crude product was pure enough for use in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (dd, J = 4.8, 8.8 Hz, 1H), 7.08 (dd, J = 8.0, 8.8 Hz, 1H), 5.55 (q, J = 6.8 Hz, 1H), 1.65 (d, J = 6.8 Hz, 3H).

7.6.13. 1-(2,6-Dichloro-3-fluorophenyl)ethan-1-amine (21)

2-(1-Azidoethyl)-1,3-dichloro-4-fluorobenzene **20** (300 mg, 1.28 mmol) was suspended in 4 ml of EtOH/H_2O (v/v, 3/1). To this suspension was added Zn powder (109 mg, 1.67 mmol) and NH_4Cl (171.40 mg, 3.20 mmol). The reaction mixture was heated at 80 °C for 1 h. Then the reaction mixture was filtered off and concentrated under vacuum. The residue was partitioned between ethyl acetate and water. The organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was pure enough for use in the next step. Yield: 71%; ^{1}H

NMR (400 MHz, $CDCl_3$) δ 7.25 (dd, J = 4.8, 8.8 Hz, 1H), 6.99 (dd, J = 8.0, 8.8 Hz, 1H), 4.90 (q, J = 6.8 Hz, 1H), 2.32 (s, 2H), 1.56 (d, J = 6.8 Hz, 3H); MS (ESI, m/z): 208.0 $[M+H]^+$.

7.7. 2-Amino-5-bromo-*N*-[1-(2,6-dichloro-3-fluorophenyl) ethyl]pyridine-3-carboxamide (22)

Compound **22** was prepared in a similar manner as described for compound **9a**. ^1H NMR (300 MHz, DMSO-d_6) δ 9.12 (d, J = 5.4 Hz, 1H), 8.27 (d, J = 2.4 Hz, 1H), 8.15 (d, J = 2.4 Hz, 1H), 7.46 (dd, J = 5.1, 8.7 Hz, 1H), 7.36 (t, J = 8.7 Hz, 1H), 7.12 (s, 2H), 5.61–5.54 (m, 1H), 1.56 (d, J = 7.2 Hz, 3H); MS (ESI, m/z): 405.9 $[M+H]^+$.

7.8. 2-Amino-*N*-[1-(2,6-dichloro-3-fluorophenyl)ethyl]-5-(1-methyl-1*H*-pyrazol-4-yl)pyridine-3-carboxamide (24m). General experimental procedure for 24o–24s, 25 and 29

A vial was charged with 2-amino-5-bromo-*N*-[1-(2,6-dichloro-3-fluorophenyl)ethyl]pyridine-3-carboxamide **22** (350 mg, 0.86 mmol), 1-methyl-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (179 mg, 0.86 mmol), KF.2H_2O (243 mg, 2.58 mmol), $Pd(PPh_3)_4$ (50 mg, 0.43 mmol) and 5 ml of DME/H_2O/EtOH (v/v/v, 7/3/2). Then the vial was capped and heated at 110 °C for 50 min under microwave irradiation. The reaction mixture was extracted with EA. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1/50) to afford compound **24m** as a white solid. Yield: 39%; ^1H NMR (300 MHz, DMSO-d_6) δ 9.02 (d, J = 5.7 Hz, 1H), 8.34(d, J = 2.1 Hz, 1H), 8.22 (d, J = 2.1 Hz, 1H), 8.04 (s, 1H), 7.82 (s, 1H), 7.49 (dd, J = 5.1, 8.7 Hz, 1H), 7.40–7.34 (m, 1H), 6.89 (s, 2H), 5.69–5.60 (m, 1H), 3.39 (s, 3H), 1.62 (d, J = 7.2 Hz, 3H); MS (ESI, m/z): 407.9 $[M+H]^+$; HRMS (ESI) calcd for $C_{18}H_{17}Cl_2FN_5O$ $[M+H]^+$: 408.0794, found: 408.0782.

7.9. *tert*-butyl 4-[4-(6-amino-5-{[1-(2,6-dichloro-3-fluorophenyl)ethyl]carbamoyl}pyridin-3-yl)-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (25)

Compound **25** was prepared in a similar manner as described for compound **24m**. Yield: 38%; ^1H NMR (300 MHz, $CDCl_3$) δ 8.29 (d, J = 2.1 Hz, 1H), 7.72 (d, J = 2.1 Hz, 1H), 7.69 (s, 1H), 7.59 (s, 1H), 7.30 (dd, J = 5.1, 9.0 Hz, 1H), 7.15 (d, J = 8.1 Hz, 1H), 7.08–7.03 (m, 1H), 6.24 (s, 2H), 6.16–6.11 (m, 1H), 4.35–4.25 (m, 3H), 2.96–2.87 (m, 2H), 2.18–2.15 (m, 2H), 2.03–1.89 (m, 2H), 1.69 (d, J = 7.2 Hz, 3H), 1.49 (s, 9H); MS (ESI, m/z): 577.2 $[M+H]^+$.

7.10. 2-Amino-*N*-[1-(2,6-dichloro-3-fluorophenyl)ethyl]-5-[1-(piperidin-4-yl)-1*H*-pyrazol-4-yl]pyridine-3-carboxamide (24n).

Compound **24n** was prepared in a similar manner as described for compound **14j**. Yield: 93%; ^1H NMR (300 MHz, $CDCl_3$) δ 8.29 (d, J = 2.1 Hz, 1H), 7.72 (d, J = 2.1 Hz, 1H), 7.69 (s, 1H), 7.61 (s, 1H), 7.30 (dd, J = 5.1, 9.0 Hz, 1H), 7.17 (d, J = 8.4 Hz, 1H), 7.04 (dd, J = 8.1, 8.7 Hz, 1H), 6.24 (s, 2H), 6.19–6.08 (m, 1H), 4.31–4.23 (m, 1H), 3.31–3.27 (m, 2H), 2.86–2.77 (m, 2H), 2.23–2.19 (m, 2H), 2.03–1.94 (m, 2H), 1.68 (d, J = 6.9 Hz, 3H); MS (ESI, m/z): 477.3 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{24}Cl_2FN_6O$ ($[M+H]^+$): 477.1373; found : 477.1361.

7.11. 2-Amino-*N*-[1-(2,6-dichloro-3-fluorophenyl)ethyl]-5-[1-(oxan-4-yl)-1*H*-pyrazol-4-yl]pyridine-3-carboxamide (24o).

Compound **24o** was prepared as a white solid in a similar manner as described for compound **24m**. Yield: 59%; ^1H NMR (300 MHz, $CDCl_3$) δ 8.28 (d, J = 1.8 Hz, 1H), 7.75 (d, J = 1.8 Hz, 1H), 7.69 (s, 1H), 7.60 (s, 1H), 7.32–7.25 (m, 1H), 7.17 (d, J = 8.4 Hz, 1H), 7.08–7.02 (m, 1H), 6.32 (s, 2H), 6.16–6.07 (m, 1H), 4.42–4.32 (m, 1H), 4.14–4.09 (m, 2H), 3.59–3.51 (m, 2H), 2.14–2.07 (m, 4H), 1.68 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.76, 158.53, 156.55, 156.09, 139.09, 136.11, 135.27, 129.96, 123.50, 118.44, 118.31, 116.22, 116.04, 111.68, 66.95, 58.67, 46.84, 33.48, 18.48; MS (ESI, m/z): 477.9 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{23}Cl_2FN_5O_2$ ($[M+H]^+$): 478.1213; found : 478.1213. The enantiomers of **24o** were prepared by chiral resolution of the racemic mixture on a chiral park IA column (1 cm × 25 cm, EtOH/hexane = 1/3, flow rate: 5 mL/min, t_{R1} = 18.94 min, t_{R2} = 34.77 min,). The absolute stereochemistry of enantiomers was determined by comparsion of HPLC retention times to (*R*)-24o, which was prepared from the known (1*S*)-1-(2,6-dichloro-3-fluorophenyl)ethan-1-ol as described in the Supplementary data content.

7.12. 2-Amino-*N*-[1-(2,6-dichloro-3-fluorophenyl)ethyl]-5-[6-(morpholin-4-yl)pyridin-3-yl]pyridine-3-carboxamide (24p)

Compound **24p** was prepared as a white solid in a similar manner as described for compound **24m**. Yield: 40%; ^1H NMR (400 MHz, $CDCl_3$) δ 8.34 (dd, J = 0.8, 2.4 Hz, 1H), 8.26 (d, J = 2.4 Hz, 1H), 7.83 (d, J = 2.4 Hz, 1H), 7.65 (dd, J = 2.8, 8.8 Hz, 1H) 7.32–7.28 (m, 1H), 7.23 (d, J = 9.6 Hz, 1H), 7.05 (dd, J = 8.0, 8.8 Hz, 1H), 6.74 (dd, J = 0.4, 8.8 Hz, 1H), 6.60 (s, 2H), 6.19–6.09 (m, 1H), 3.86 (t, J = 8.8 Hz, 4H), 3.57 (t, J = 8.8 Hz, 4H), 1.69 (d, J = 7.2 Hz, 3H); MS (ESI, m/z): 489.9 $[M+H]^+$; HRMS (ESI) calcd for $C_{23}H_{23}Cl_2FN_5O_2$ ($[M+H]^+$): 490.1213; found : 490.1225.

7.13. 2-Amino-*N*-[1-(2,6-dichloro-3-fluorophenyl)ethyl]-5-(2-oxo-2,3-dihydro-1H-indol-5-yl)pyridine-3-carboxamide (24q)

Compound **24q** was prepared as a light red solid in a similar manner as described for compound **24m**. Yield: 20%; ^1H NMR (300 MHz, DMSO-d_6) δ 10.45 (s, 1H), 9.15 (d, J = 5.1 Hz, 1H), 8.35 (d, J = 2.1 Hz, 1H), 8.27 (d, J = 2.1 Hz, 1H), 7.55 (s, 1H), 7.50–7.45 (m, 2H), 7.36 (t, J = 8.7 Hz, 1H), 6.96 (s, 2H), 6.90 (d, J = 8.1 Hz, 1H), 5.68–5.59 (m, 1H), 3.56 (s, 2H), 1.60 (d, J = 7.2 Hz, 3H); MS (ESI, m/z): 458.8 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{18}Cl_2FN_4O_2$ ($[M+H]^+$): 459.0791; found : 459.0778.

7.14. 2-Amino-*N*-[1-(2,6-dichloro-3-fluorophenyl)ethyl]-5-(pyrimidin-5-yl)pyridine-3-carboxamide (24r)

Compound **24r** was prepared as a white solid in a similar manner as described for compound **24m**. Yield: 37%; ^1H NMR (400 MHz, $CDCl_3$) δ 9.21 (s, 1H), 8.90 (s, 1H), 8.40 (d, J = 2.4 Hz, 1H), 7.84 (d, J = 2.4 Hz, 1H), 7.32–7.29 (m, 1H), 7.17 (d, J = 8.4 Hz, 1H), 7.06 (dd, J = 8.0, 8.8 Hz, 1H), 6.57 (s, 2H), 6.18–6.10 (m, 1H), 1.69 (d, J = 6.8 Hz, 3H); MS (ESI, m/z): 405.9 $[M+H]^+$; HRMS (ESI) calcd for $C_{18}H_{15}Cl_2FN_5O$ ($[M+H]^+$): 406.0638; found: 406.0645.

7.15. 2-Amino-*N*-[1-(2,6-dichloro-3-fluorophenyl)ethyl]-5-{4-[2-(morpholin-4-yl)-2-oxoethoxy]phenyl}pyridine-3-carboxamide (24s).

Compound **24s** was prepared as a white solid in a similar manner as described for compound **24m**. Yield: 46%; ^1H NMR (400 MHz, $CDCl_3$) δ 8.34 (d, J = 2.4 Hz, 1H), 7.79 (d, J = 2.4 Hz, 1H), 7.41 (d, J = 8.4 Hz, 2H), 7.31–7.28 (m, 1H), 7.19 (d, J = 8.8 Hz, 1H), 7.06–7.01 (m, 3H), 6.32 (s, 2H), 6.15–6.08 (m, 1H), 4.73 (s, 2H), 3.70–3.66 (m, 4H), 3.63–3.61 (m, 4H), 1.66 (d, J = 6.8 Hz, 3H); MS (ESI, m/z): 546.9 $[M+H]^+$; HRMS (ESI) calcd for $C_{26}H_{26}Cl_2FN_4O_4$ ($[M+H]^+$): 547.1315; found : 547.1296.

7.16. 6-Bromo-3*H*,4*H*-pyrido[2,3-d]pyrimidin-4-one (26)

To a vial was charged with 2-amino-5-bromonicotinic acid **8** (1 g, 4.61 mmol) and 1.1 ml of formamide. The vial was capped and heated at 150 °C for 30 min under microwave irradiation. To the reaction mixture was added water. The precipitate was filtered off and recrystallized from water to afford brown solid in 36% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.72 (s, 1H), 9.03 (d, J = 2.4 Hz, 1H), 8.61 (d, J = 2.4 Hz, 1H), 8.35 (s, 1H); MS (ESI, m/z): 225.8 $[M+H]^+$.

7.17. 6-Bromo-3-[1-(2,6-dichloro-3-fluorophenyl)ethyl]-3*H*,4*H*-pyrido[2,3-d]pyrimidin-4-one (27)

38.9 mg (0.97 mmol) of sodium hydride (60 percent in oil) was added to an ice-cooled solution of 200 mg (0.88 mmol) of **6**-bromo-3*H*,4*H*-pyrido[2,3-d]pyrimidin-4-one 27 in 4 ml of DMF, and the reaction medium is stirred for 10 min. Then 304.88 mg (1.06 mmol) of 1-(2,6-dichloro-3-fluorophenyl)ethyl methanesulfonate **19** was added. The resulting mixture was heated at 80 °C for 4 h. The ice-cold water was added to the reaction medium. The mixture was extracted with ethyl acetate. The organic phase was washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified by silica gel column chromatography (EA/PE, 1/5-1/2) to afford compound **27** in 50% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.99 (d, J = 2.4 Hz, 1H), 8.69 (s, 1H), 8.61 (d, J = 2.4 Hz, 1H), 7.29–7.25 (m, 1H), 7.09–7.04 (m, 1H), 6.31 (q, J = 7.2 Hz, 1H), 2.01 (d, J = 7.2 Hz, 3H); MS (ESI, m/z): 415.7 $[M+H]^+$.

7.18. *tert*-butyl 4-(4-{3-[1-(2,6-dichloro-3-fluorophenyl)ethyl]-4-oxo-3*H*,4*H*-pyrido[2,3-d]pyrimidin-6-yl}-1*H*-pyrazol-1-yl)piperidine-1-carboxylate (29)

Compound **29** was prepared in a similar manner as described for compound **24m**. Yield: 39.4%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.12 (d, J = 2.4 Hz, 1H), 8.65 (s, 1H), 8.52 (d, J = 2.4 Hz, 1H), 7.85 (s, 1H), 7.78 (s, 1H), 7.29–7.25 (m, 1H), 7.09–7.03 (m, 1H), 6.34 (q, J = 7.5 Hz, 1H), 4.34–4.22 (m, 3H), 2.95–2.84 (m, 2H), 2.18–2.13 (m, 2H), 2.03–1.86 (m, 5H), 1.47 (s, 9H); MS (ESI, m/z): 586.7 $[M+H]^+$.

7.19. 3-[1-(2,6-Dichloro-3-fluorophenyl)ethyl]-6-[1-(piperidin-yl)-1*H*-pyrazol-4-yl]-3*H*,4*H*-pyrido[2,3-d]pyrimidin-4-one (30)

Compound **30** was prepared as a white solid in a similar as described for compound **14j**. Yield: 85%; ^{1}H NMR (400 MHz, CD_3OD) δ 9.19–9.18 (m, 1H), 8.82 (s, 1H), 8.58–8.56 (m, 1H), 8.33 (s, 1H), 8.05 (s, 1H), 7.41-7.36 (m, 1H), 7.25–7.20 (m, 1H), 6.33–6.28 (m, 1H), 4.66–4.59 (m, 1H), 3.60–3.57 (m, 2H), 3.28–3.20 (m, 2H), 2.39–2.26 (m, 4H), 2.01 (s, J = 7.6 Hz, 3H); MS (ESI, m/z): 487.3 $[M+H]^+$; HRMS (ESI) calcd for $C_{23}H_{22}Cl_2FN_6O$ ($[M+H]^+$): 487.1216; found : 487.1211.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.07.007.

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