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Journal Name

ARTICLE

Discovery of novel Bcr-Abl inhibitors with diacylated piperazine as flexible linker

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Abstract: Forty-two compounds (series **8**, **9** and **10**) incorporated with diacylated piperazine have been synthesized and evaluated as novel Bcr-Abl inhibitors based on 'six-atom linker'. Five of them, **8d**, **8h**, **8l**, **10m** and **10p**, displayed potent Bcr-Abl inhibitory activity comparable with Imatinib. Moreover, compounds **8e, 10q, 10s, 10u** were potent Bcr-Abl inhibitors with IC_{50} values at sub-micromolecular level. Most compounds exhibited moderate to high antiproliferative activity against K562 cells. In particular, compound **9e** was the most promising Bcr-Abl inhibitor. Docking studies revealed that the binding modes of these compounds were similar with Imatinib. These compounds could be considered as promising lead compounds for further optimization.

Introduction

Chronic Myeloid Leukemia (CML) is a hematological malignancy characterized by Philadelphia chromosome (Ph) resulted from the reciprocal translocation between chromosomes 9 and 22 [1]. This translocation creates breakpoint cluster region (BCR) abelson tyrosine kinase (ABL) fusion gene complex, and this gene complex encodes a constitutively active form of the Bcr-Abl fusion tyrosine kinase [2,3]. Bcr-Abl plays a critical role in the signal transduction pathways and transformation related to CML [4]. It can phosphorylate a series of downstream substrates, leading to the unlimited proliferation of mature granulocytes [5]. Due to non-expression of Bcr-Abl kianse in the normal hemopoietic stem cell, it is a well validated target for the treatment of CML [6].
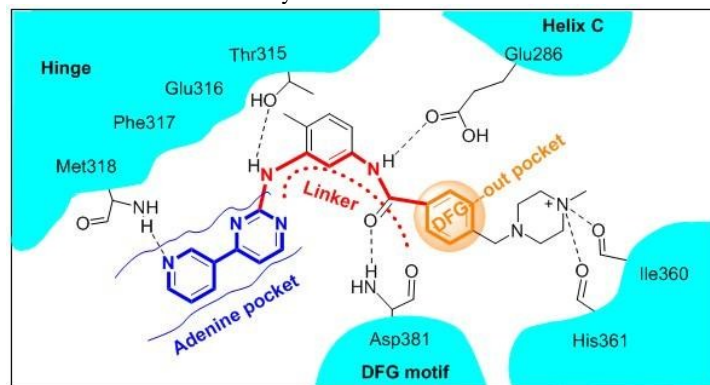
Figure 1. Interactions between Imatinib and Bc-Abl tyrosine kinase

Imatinib (STI571, Gleevec) is the first approved Bcr-Abl inhibitor [7]. It is now considered as the first-line therapy for CML cases due to its high efficacy and relatively mild side effects [8]. It can specifically bind to ATP-binding site of Bcr-Abl to prevent substrate accessing [9]. The blocking of the active site limits the repetitive growth and proliferation of CML cells. Crystallographic studies reveal that Imatinib binds to the inactive conformation of Bcr-Abl as DFG(Asp-Phe-Gly)-out conformation [10, 11]. According to pharmacophore features, binding sites of Imatinib could be divided into three subregions (**Figure 1**) [12], two hydrophobicity regions,

the adenine pocket (including hinge region) where the adenine ring of ATP occupied, the DFG-out pocket (also known as "allosteric site") and the linker that allowed inhibitor to access to the allosteric site (surrounded by DFG motif and gatekeeper residue). Based on the structural analysis of Imatinib, we initiated to develop potent Bcr-Abl inhibitors with new chemotypes.
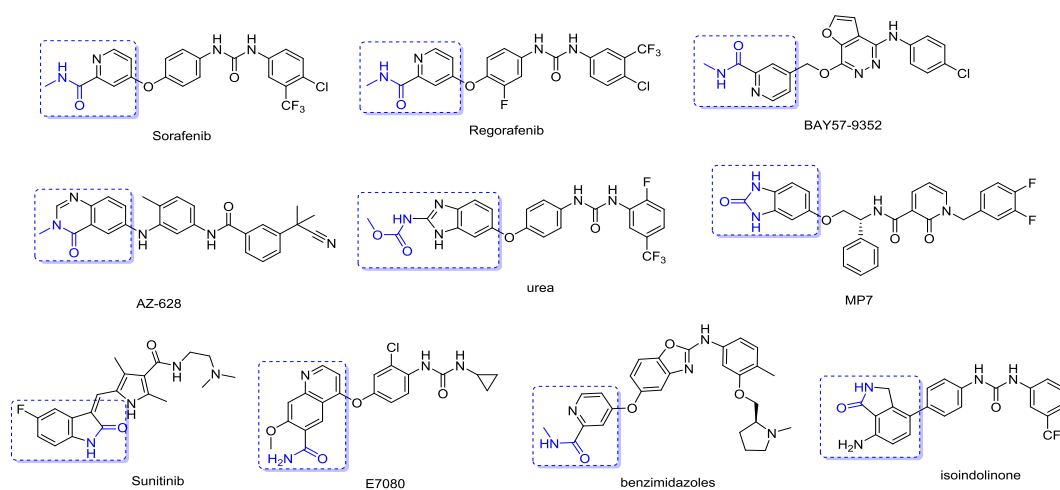
Figure 2. Tyrosine kinase inhibitors containing amide group

The structural modification of Imatinib was focused on two parts, the adenine pocket and the linker. It is well known that tyrosine kianses typically share a conserved adenine pocket. To date, many efforts have been made to find novel Bcr-Abl inhibitors (**Figure 2**) [13-22]. Many of them possess the heterocyclic amide fragment (colored in blue) binding to the hinge region. Since the amide group can provide more H-bond interactions with hinge region, heterocyclic and phenyl were introduced to replace pyridine and pyrimidine rings of Imatinib (**Figure 3**). For the 'linker', it was found that there was six-atom linker of Imatinib (colored in red) between adenine pocket and DFG-out pocket. Based on 'six-atom regulation', diacylated piperazine was introduced as flexible linker (**Figure 3**). Firstly, diacylated piperazine can retain the amide forming hydrogen bonds with DFG-motif. Secondly, the high flexible piperazine might avoid space clash with the bulky group of gatekeeper residue. Moreover, various substituented phenyl rings were introduced into the DFG-out pocket. Above all, we have designed and synthesized forty-two novel Bcr-Abl inhibitors with new chemotypes.
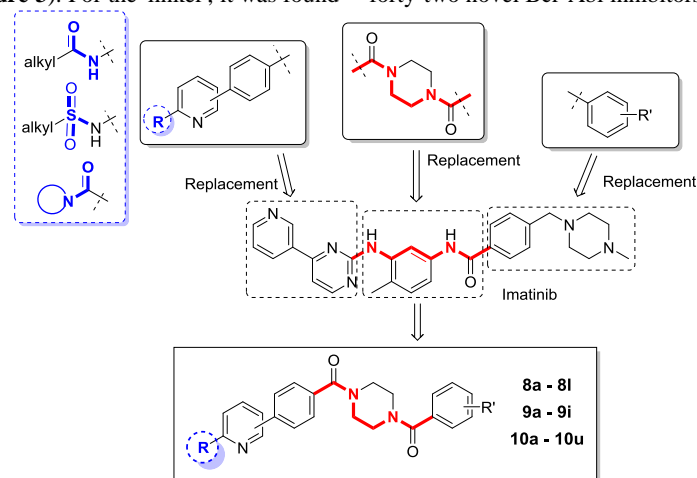

Figure 3. Design strategy and structures of title compounds

Results and discussion
Chemistry

The synthetic route of title compounds **(series 8, 9 and 10)** was illustrated in **Scheme 1**. Two types of key intermediates were employed to afford the title compounds. One was heterocyclic biphenyl carboxylic acids (**4a**-**4c**, **5a**-**5c**, and **6a**-**6f**). Another key intermediate was monoacylated piperazines (**7I**-**7VII**). The key intermediates **4a**-**4c** and **5a**-**5c** were prepared in two steps. Firstly, amino group of bromo-pyridin-2-amine was acylated to yield **1a**-**1c** and **2a**-**2c** [23, 24]. Then **1a**-**1c** or **2a**-**2c** were coupled with 4-carboxyphenylboronic acid in the presence of Pd(PPh$_3$)$_4$ to provide key intermediates **4a**-**4c** and **5a**-**5c** [25, 26]. For the synthesis of **6a**-**6f**, 5-bromonicotinic acid was chosen as starting material. Reaction of 5-bromonicotinic acid with thionyl chloride gave the corresponding benzoyl chloride. Then the activated benzoyl chloride was reacted with various amines to afford **3a**-**3f** [27]. Coupling of **3a**-**3f** with 4-carboxyphenylboronic acid yielded **6a**-**6f** [25, 28]. Monoacylation of piperazine was carried out using trimethylacetic arylcarboxylic anhydride [29]. Various benzoic acids reacted with trimethylacetyl chloride in the presence of triethylamine to give trimethylacetic arylcarboxylic anhydride. Then the generated mixed anhydrides were further treated with piperazine in ethanol to provide monoacylated piperazine derivatives **7I**-**7VII**. The title compounds **8a**-**8l**, **9a**-**9i**, and **10a**-**10u** were prepared using mixed anhydride approach [30, 31]. Compounds **4a**-**4c**, **5a**-**5c** or **6a**-**6f** were allowed to react with isobutyl chloroformate in tetrahydrofuran in the presence of *N*-methylmorpholine, forming isobutyric anhydride. Then, the generated isobutyric anhydride was treated with **7I**-**7VII** in anhydrous tetrahydrofuran to give the target compounds **8a**-**8l**, **9a**-**9i**, **10a**-**10u** in moderate yield.
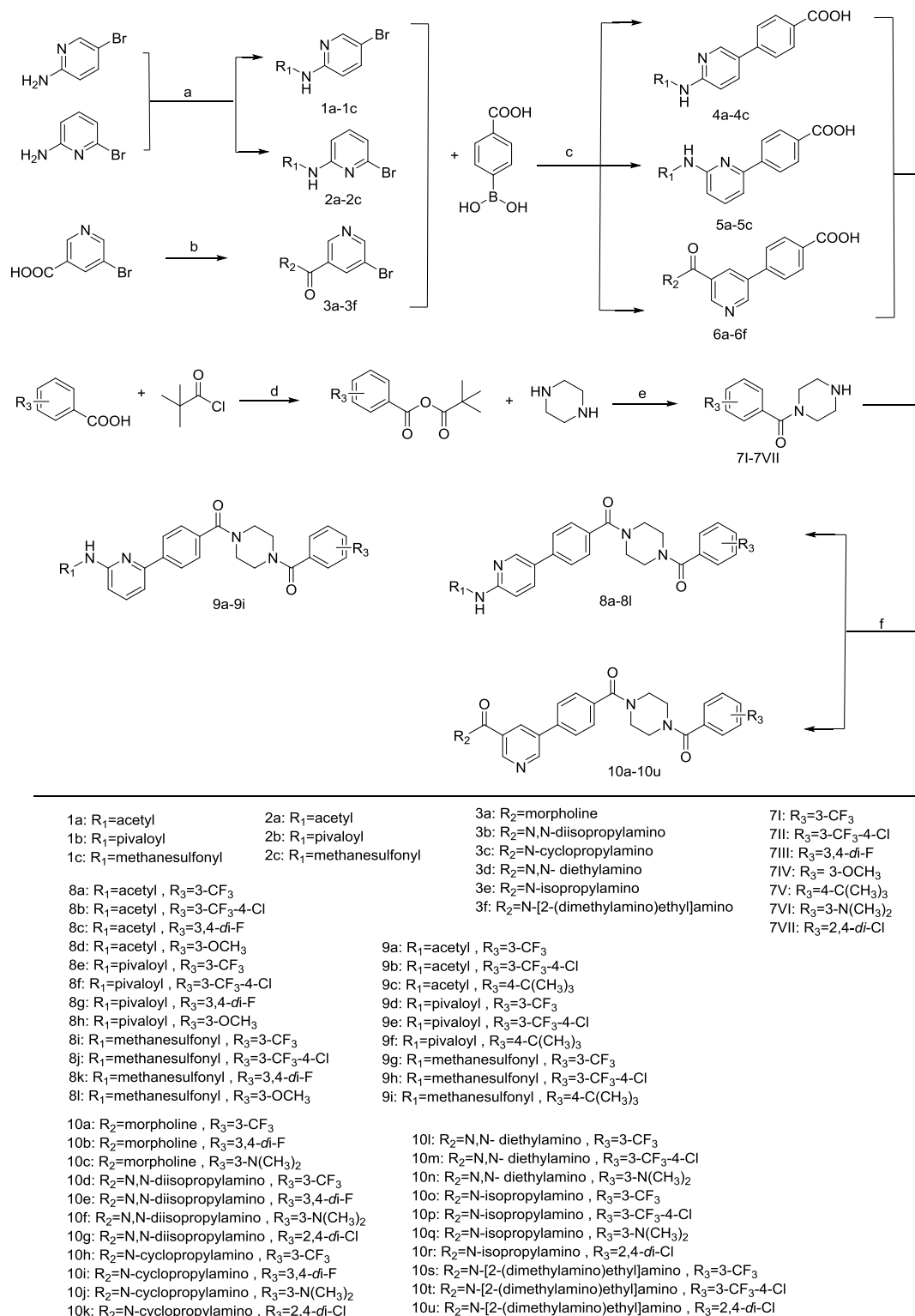
Scheme 1. *Reagents and conditions*: a. Acyl chloride, Et$_3$N, CH$_2$Cl$_2$, 0 °C→r.t.; b. SOCl$_2$, reflux; Amine, CH$_2$Cl$_2$, 0 °C→r.t.; c. Pd(PPh$_3$)$_4$, Cs$_2$CO$_3$, CH$_3$CN/H$_2$O(V:V=3:2), 100 °C; d. Et$_3$N, CH$_2$Cl$_2$, r.t.; e. EtOH, r.t.; f. ClCOO-iBu, NMM, THF, 0 °C; **7I-7VII**, r.t.

In vitro enzymatic assays

The biological evaluation of all the title compounds was preliminarily performed with Imatinib as positive control [32, 33].

The results were summarized in **Table 1** and **Table 2**. Most of them exhibited moderate to significant potency with IC$_{50}$ values ranging from 0.046 μM to 395.59 μM. The activities of compounds **8d**

(0.057 μM), **8h** (0.046 μM), **8l** (0.046 μM), **10m** (0.065 μM) and **10p** (0.049 μM) were comparable with Imatinib (0.074 μM). Meanwhile, compounds **9e** (0.21 μM), **10q** (0.12 μM), **10s** (0.18 μM) and **10u** (0.74 μM) also displayed potent Bcr-Abl inhibitory activities with IC_{50} values at sub-micromolar level.

As illustrated in **Table 1**, the majority of series **8** were more potent than series **9**. The results indicated that amide group was better at *para*-position than *meta*-position for Bcr-Abl inhibition. Compounds **8d**, **8h** and **8l** displayed the highest activity which suggested that *m*-methoxy group was beneficial for the potency. It was also concluded that 4-Cl-3-CF_3 was in favor of Bcr-Abl inhibition. For example, compounds **9e** and **9h** displayed higher activity than the others.

The activities of series **10** were summarized in **Table 2**. Most of them exhibited decreased Bcr-Abl inhibition compared with series **8** and **9**. Compounds with bulky group (**10a**, **10b**, **10e**, **10f**, **10i** and **10j**) showed less enzymatic inhibitory potency compared to that (**10l**-**10r**) with small groups. The results indicated that bulky substituent was unfavourable for the activity. Compounds **10s**-**10u** were more potent than the others which suggested that the incorporation of slender side chain was in favor of improving the potency. In addition, the

substituents at terminal phenyl ring also affected the inhibitory activity. Most compounds with halogen substituents, especially 2,4-*di*-chlorine, displayed potent Bcr-Abl inhibition. This revealed that the halogen atoms on terminal phenyl ring played an important role in the biological activities.

The emergence of resistance caused by mutations in Bcr-Abl has become a major challenge for clinical management of CML. The resistance to Imatinib is often associated with a point mutation (T315I) in the gatekeeper region. Herein, twelve potent compounds were selected to evaluate their inhibitory potency against T315I mutant. The inhibitory ratios of them were determined against wild-type and T315I-Abl (**Table 3**). These compounds showed higher potency against wild-type Bcr-Abl with inhibition ratios ranging from 40% to 68%. However, they displayed less potency against Bcr-Abl[T315I]. The T315I point mutation could alter the geometry of the ATP site to interrupt the binding of inhibitors with Bcr-Abl. Since acquired resistance is a major challenge in clinical treatment, the development of inhibitors against T315I-Abl will be taken into consideration in our further study.

Table 1. Structures and activities of **8a-8l** and **9a-9i** towards Bcr-Abl and K562 cells *in vitro*

	Pyridine	R_1	R_3	Abl $IC_{50}(μM)^a$	K562 $IC_{50}(μM)^b$
8a	5	acetyl	3-CF_3	6.70	49.85
8b	5	acetyl	3-CF_3-4-Cl	75.04	50.38
8c	5	acetyl	3,4-*di*-F	2.15	47.34
8d	5	acetyl	3-OCH_3	0.057	56.73
8e	5	pivaloyl	3-CF_3	31.28	21.98
8f	5	pivaloyl	3-CF_3-4-Cl	57.10	31.38
8g	5	pivaloyl	3,4-*di*-F	2.75	8.12
8h	5	pivaloyl	3-OCH_3	0.046	59.03
8i	5	methanesulfonyl	3-CF_3	49.18	22.52
8j	5	methanesulfonyl	3-CF_3-4-Cl	16.80	72.35
8k	5	methanesulfonyl	3,4-*di*-F	36.80	147.47
8l	5	methanesulfonyl	3-OCH_3	0.046	122.28
9a	6	acetyl	3-CF_3	64.59	160.00
9b	6	acetyl	3-CF_3-4-Cl	85.58	136.66
9c	6	acetyl	4-$C(CH_3)_3$	192.61	21.43
9d	6	pivaloyl	3-CF_3	7.14	2.09
9e	6	pivaloyl	3-CF_3-4-Cl	0.21	1.93
9f	6	pivaloyl	4-$C(CH_3)_3$	94.95	1.10
9g	6	methanesulfonyl	3-CF_3	265.46	60.64
9h	6	methanesulfonyl	3-CF_3-4-Cl	3.96	3.21
9i	6	methanesulfonyl	4-$C(CH_3)_3$	119.03	45.69
Imatinib				0.074	4.12

[a]Values were the average of two experiments, SD<10%.
[b]Values were the average of three experiments, SD<10%.

In vitro antiproliferative activity assays

All the title compounds were evaluated for their antiproliferative activity against Bcr-Abl positive leukemia cell (K562) with Imatinib as positive control [34]. The bioactivity data was presented in **Table** 1 and **Table** 2. All the compounds showed moderate to significant activity against K562 cells with IC_{50} values ranging from 1.10 μM to 481.11 μM. Among them, compounds **9d**-**9f** and **9h** were more potent than Imatinib. Among series **8**, compounds **8e**-**8h** exhibited the highest potency, while **8i**-**8l** showed the lowest activity. The similar results were also observed in series **9**, and the order of their activities was **9d**-**9f** > **9g**-**9i** > **9a**-**9c**. The results suggested that pivaloyl group was more beneficial for the bioactivity than acetyl or
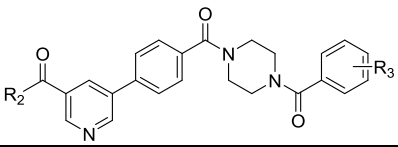
methanesulfonyl. We speculated that the introduction of pivaloyl group could improve the liposolubility which led to the high permeability. Compounds **8d**, **8h**, and **8l** exhibited low cellular growth inhibition but potent Bcr-Abl inhibition. It may attribute to their poor capability to pass through the cell membrane. In addition, it was found that the substituents on terminal phenyl ring had a slight influence on activity.

The bioactivity data of series **10** was summarized in **Table 2**. Most compounds showed decrease efficiency against K562 cells compared with series **8** and **9**. Compounds with bulky substituents (**10b**, **10c**, **10e**, **10f**, **10i** and **10j**) exhibited less antiproliferative activity. While compounds with small groups (**10l**, **10m** and **10p**-**10r**) were more

potent. Compounds **10s-10u** were more potent than others, which suggested that the incorporation of slender side chain was favourable. In addition, the substituents at terminal phenyl ring also affected the

activity. Compounds **10f**, **10j** and **10n** with dimethylamino substituents were less potent than that with halogen substituents (**10a**, **10d**, **10g**, **10k**, **10p**, **10t**).

Table 2. Structures and activities of **10a-10u** towards Bcr-Abl and K562 cells *in vitro*

	R$_2$	R$_3$	Abl IC$_{50}$(μM)[a]	K562 IC$_{50}$(μM)[b]
10a	morpholine	3-CF$_3$	261.73	64.56
10b	morpholine	3,4-*di*-F	212.35	199.70
10c	morpholine	3-N(CH$_3$)$_2$	2.23	214.26
10d	*N,N*-diisopropylamino	3-CF$_3$	41.79	47.63
10e	*N,N*-diisopropylamino	3,4-*di*-F	350.78	115.07
10f	*N,N*-diisopropylamino	3-N(CH$_3$)$_2$	215.13	306.30
10g	*N,N*-diisopropylamino	2,4-*di*-Cl	16.83	18.21
10h	*N*-cyclopropylamino	3-CF$_3$	45.94	53.82
10i	*N*-cyclopropylamino	3,4-*di*-F	395.59	101.08
10j	*N*-cyclopropylamino	3-N(CH$_3$)$_2$	200.64	198.60
10k	*N*-cyclopropylamino	2,4-*di*-Cl	53.50	17.76
10l	*N,N*-diethylamino	3-CF$_3$	3.93	78.91
10m	*N,N*-diethylamino	3-CF$_3$-4-Cl	0.065	16.24
10n	*N,N*-diethylamino	3-N(CH$_3$)$_2$	1.15	190.99
10o	*N*-isopropylamino	3-CF$_3$	185.51	481.11
10p	*N*-isopropylamino	3-CF$_3$-4-Cl	0.049	13.36
10q	*N*-isopropylamino	3-N(CH$_3$)$_2$	0.12	72.38
10r	*N*-isopropylamino	2,4-*di*-Cl	5.17	27.82
10s	*N*-[2-(dimethylamino)ethyl]amino	3-CF$_3$	0.18	23.66
10t	*N*-[2-(dimethylamino)ethyl]amino	3-CF$_3$-4-Cl	8.90	17.07
10u	*N*-[2-(dimethylamino)ethyl]amino	2,4-*di*-Cl	0.74	19.69
	Imatinib		0.074	4.12

[a]Values were the average of two experiments, SD<10%.

[b]Values were the average of three experiments, SD<10%.

Kinase Selectivity assays

We investigated the kinase selectivity of ten potent compounds against three kinases including VEGFR-2, Src and B-Raf. Herein, **8d**, **8h**, **8l**, **9e**, **9h**, **10m**, **10p**, **10q**, **10s**, **10u** were evaluated for their selective profile. The results were summarized in **Table 4**. The results revealed that these compounds showed less potency against VEGFR2, Src, B-Raf compared with Bcr-Abl. It was demonstrated that they exhibited high selectivity for Bcr-Abl relative to kinases including VEGFR2, Src, and B-Raf. In addition, several compounds exhibited moderate inhibitory activity against VEGFR2 which might cause the inconsistency of the Abl inhibition and K562 cell growth inhibition, such as **9e** and **9f**.

Table 3. Potency profiles on wild-type and T315I mutant for ten selected compounds.

	(% inhibition at 1.2μM)	
	Abl	Abl(T315I)
8c	40	1
8d	54	1
8h	65	3
8j	42	10
8l	61	2
9e	57	2
9h	45	1
10m	68	1
10p	67	0
10q	54	8
10s	56	0
10u	52	0

Table 4. kinase selectivity profile of select compounds

	(% inhibition at 1.2μM)			
	Abl	VEGFR-2	Src	B-Raf
8d	54	5	2	2
8h	65	8	6	4
8l	61	14	7	5
9e	57	14	7	6
9h	45	21	9	7
10m	68	20	7	5
10p	67	20	6	6
10q	54	20	3	4
10s	56	18	0	2
10u	52	8	0	0

## Molecular Docking and SAR studies

Molecular docking

To investigate the interactions between inhibitors and Bcr-Abl, docking studies were performed using Surflex-Dock Module of Sybyl-X 2.0. The molecules were drawn with Sketch and minimized under Tripos Force field with Gasteiger-Huckel charge. Crystal structures of Abl (PDB code 1IEP) was imported [35], and its ligand Imatinib was used to define the binding cavity and generate the promotal. The binding modes were shown in **Figure 4** and **Figure 5**. As shown in **Figure 4**, Imatinib penetrates through the central region of Bcr-Abl from one side to the other. The pyridine and pyrimidine rings occlude the region where the adenine ring of ATP normally binds (Adenine pocket), and the rest of the molecule stretches into the hydrophobic region (DFG-out pocket) to freeze the kinase

conformation [11]. Series **8**, **9** and **10** bound to the active site with a similar mode to that of Imatinib. The pyridine and phenyl ring of them overlap well with the pyridine and pyrimidine rings of Imatinib, as well as the terminal phenyl ring. Diacylated piperazine, six-atom linker, smoothly passes through the narrow cavity surrounded by DFG motif and gatekeeper residue. Compared with rigid methyl benzene, higher flexible piperazine avoid the clash with gatekeeper residue which is often occurred in kinase resistance mutations.
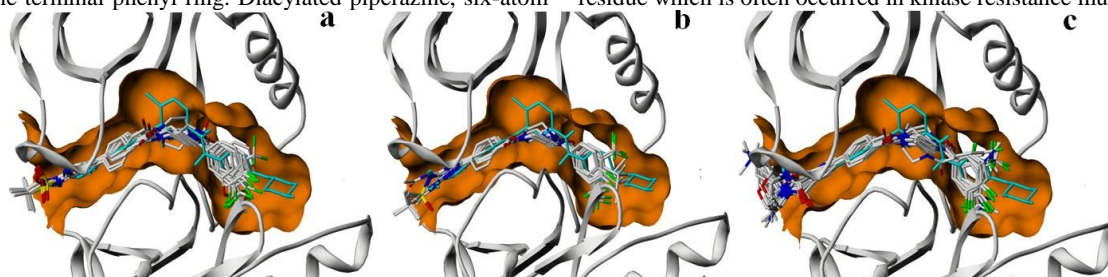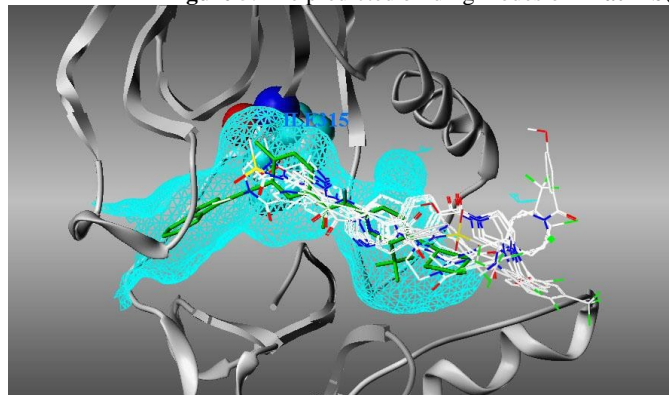

Figure 4. The predicted binding modes of series **8** (a), **9** (b), **10** (c) with Bcr-Abl. Imatinib is colored in cyan.

To further analysis the interactions between the inhibitors and Bcr-Abl kinase, more specific docking details are given in **Figure 5**. The docking studies of five representative compounds (**8a**, **9a**, **10a**, **10h**, **10s**) and Imatinib were carried out. As shown in **Figure 5a**, Imatinib forms five H-bonds with Glu286, Thr315, Met318, Asp381, His361 and Ile360 [11]. From **Figure 5b-5f**, it is revealed that the amide group of the title compounds always has H-bond interactions with amino acid Met318 included in hinge region. In addition, amide group also formed H-bonds with amino acids Gly249, Tyr253, and Asn322. The docking results suggested that the introduction of amide group is successful in the design of novel Bcr-Abl inhibitors. Unfortunately, it is found that these compounds rarely make H-bond with amino acids Asp381 and Glu286. More efforts are needed in further structural optimization of this part.
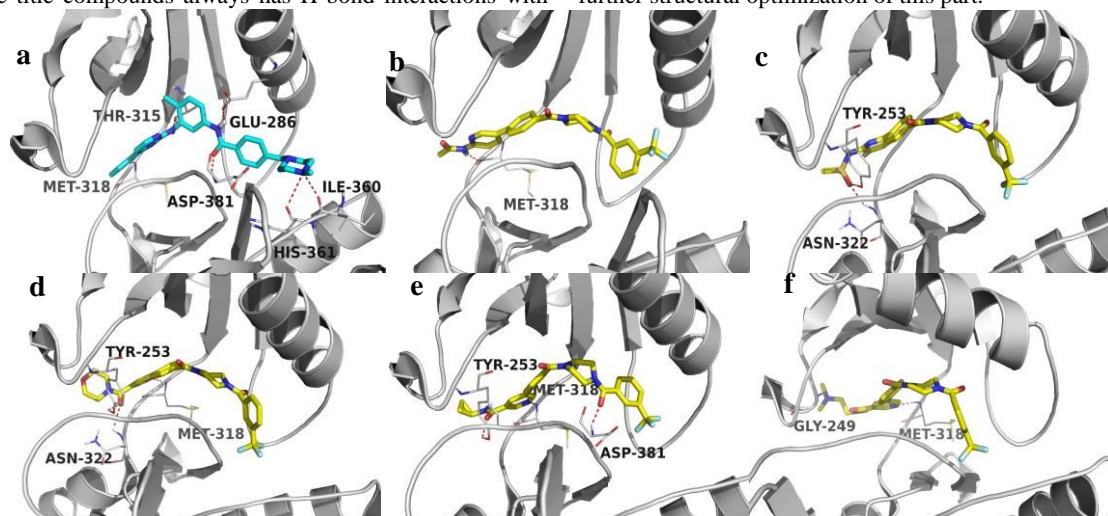
Figure 5. The predicted binding modes of **Imatinib**(a), **8a**(b), **9a**(c), **10a**(d), **10h**(e) and **10s**(f) with Bcr-Abl.


Figure 6. The predicted binding modes of select compounds with Bcr-Abl (T315I). Pantinib is colored in green. Residue Ile315 is shown in space fill model.

Docking of designed compounds (**8c**, **8d**, **8h**, **8j**, **8l**, **9e**, **9h**, **10m**, **10p**, **10q**, **10s**, **10u**) with T315I Bcr-Abl (PDB code 3IK3) was also performed, with Pantinib (AP24534) as the control [36]. As shown in **Figure 6**, Pantinib fit well in the ATP pocket of T315I-Abl. However, the increased bulk of the Ile 315 side chain caused steric repulsion, thereby blocking the access of the title compounds to the hydrophobic pocket near the gatekeeper residue. Therefore, the designed compounds had no interaction with the hinge region of Bcr-Abl (T315I), which was fatal for the binding of inhibitors with receptor. The docking results were consistent with the inhibitory activity against Bcr-Abl[T315I].

QSAR studies

3D-QSAR studies were performed with CoMFA module of Sybyl-X 2.0. The test set consisted of 8 compounds (**8b**, **9e**, **9h**, **10j**, **10m**, **10q**), while the other 36 compounds (Imatinib, **8a**, **8c-8h**, **8j-8l**, **9a-9d**, **9f**, **9g**, **9i**, **10a-10i**, **10k**, **10l**, **10n**, **10o**, **10r-10t**) composed of the training set. The IC_{50} values were converted into pIC_{50} according to the formula: $pIC_{50} = -logIC_{50}$. The conformations of training set generated from docking study were used. Based on the docking results, the template molecule **8a** was taken and the others were aligned to it by DATABASE ALIGNMENT method (**Figure 7**).
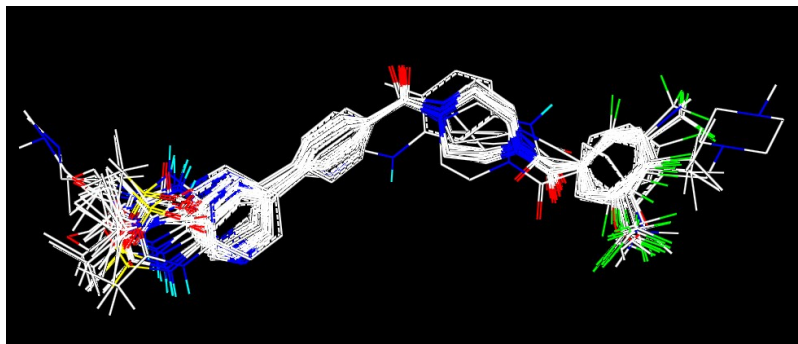
Figure 7. Superposition of 34 Bcr-Abl inhibitors for CoMFA construction

The steric and electrostatic fields were calculated at each lattice intersection of regularly spaced grid of 2.0 Å in all three dimensions within defined region. An sp^3 carbon atom with +1.00 charge was used as a probe atom. The steric and electrostatic fields were truncated at +30.00 kcal/mol, and the electrostatic fields were ignored at the lattice points with maximal steric interactions. PLS method was used to linearly correlate CoMFA fields with activity values [37-39]. The cross-validation analysis was performed using leave-one-out (LOO) method [40]. The cross-validated q^2 that resulted in optimum number of components and lowest standard error of prediction were considered for further analysis. We have evaluated different filter value σ and at least selected σ as 2.00 kcal/mol to speed up the analysis and reduce noise. The LOO cross-validated q^2 of the CoMFA model is 0.568, and the non-cross-validated r^2 for the model established is 0.996. The value of the variance ratio F (n_1 = 9, n_2 = 24) is 671.934, and standard error of

the estimate (SEE) is 0.088. The contribution of electrostatic and steric is 61.8% and 38.2%, respectively.

To visualize the information content of the derived 3D-QSAR model, CoMFA contour maps were generated. **Figure 8a** and **8b** show the steric and electrostatic contour maps of the CoMFA models. The steric plots indicate that it is feasible and beneficial to introduce bulky side chain into pyridine ring, and the introduction of bulky group on position 3 of terminal phenyl ring may favored the inhibition activity for these compounds. The electrostatic plot suggests that the electronic plot is consistent with the structure occupying in adenine pocket of the target compounds. N atoms are near the blue moieties, and O atoms are near the red moieties. The introduction of side chain containing N and O into the adenine pocket is beneficial for the inhibitory potency. The activities of the training and test sets were also predicted. As shown in **Figure 8c**, the CoMFA model can predict test set compounds well.
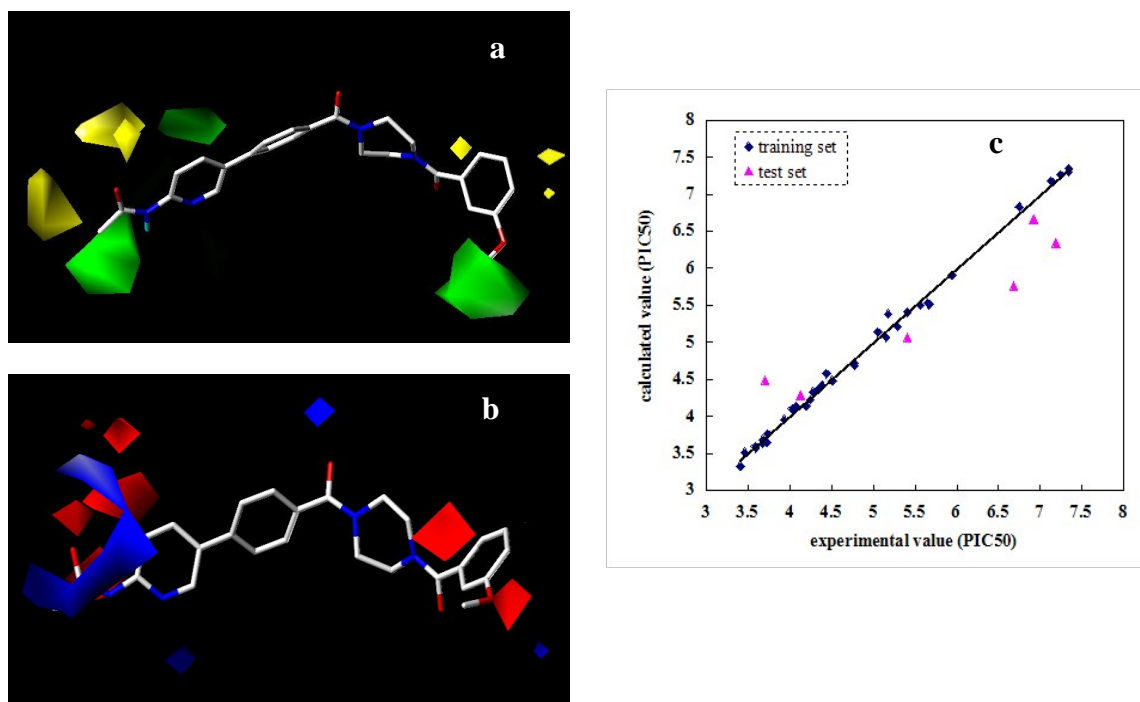
Figure 8. Contour plot and the predictability of the CoMFA model. Figures **a** and **b** show the steric and electrostatic counter maps of the CoMFA model. Green contours indicate regions where bulky groups increase activity, whereas yellow contours indicate regions where bulky groups decrease activity. Blue contours indicate regions where positive groups increase activity, whereas red contours indicate regions where negative charge increases activity. Figure **c** shows the correlations between the experimental and the predicted activities for the training and test sets for the optimal CoMFA.

Conclusion

In summary, we have developed forty-two compounds with flexible diacylated piperazine linker as novel Bcr-Abl inhibitors. All the title compounds were investigated for their Abl kinase inhibition *in vitro*, and most of them exhibited potent inhibitory activity. The activity of compounds **8d**, **8h**, **8l**, **10m** and **10p** were comparable with that of Imatinib, and compounds **8e**, **10q**, **10s**, **10u** were also potent with IC_{50} values at sub-micromolecular level. Compounds (**9d-9f** and **9h**) displayed higher antiproliferative activity than Imatinib. In particular, compound **9e** exhibited potent enzymatic inhibitory activity as well as excellent antiproliferative activity.

The docking results showed that all the title compounds exhibited the similar binding mode with Imatinib. The six-atom linker, diacylated piperazine, smoothly passes through the narrow cavity surrounded by DFG motif and gatekeeper residue, and the side chain containing amide group can form H-bond interactions with hinge region. The 3D-QSAR research is instructive for further research. It is feasible and beneficial to introduce side chain containing N and O atoms into pyridine ring, as well as introducing bulky group on position 3 of teriminal phenyl ring. These results provide future research directions to develop novel and potent Bcr-Abl inhibitors.

Experimental

General procedures

Starting materials and other reagents were purchased from commercial suppliers and were used without further purification unless otherwise indicated. Melting points were determined on an X-4 micro-melting apparatus without corrected. ^{1}H NMR spectra was measured on a Bruker Advance 400 MHz spectrometer, using TMS as an internal standard. ^{1}H NMR and ^{13}C NMR spectra were obtained as DMSO-d_6 solutions as indicated (reported in ppm). MS were obtained using gas chromatography mass spectrometry (GC-MS) on a Shimadzu GC-MS-QP2010 instrument with an ESI interface. HMRS were obtained on a Bruker microTOF-Q II spectrometer. Thin-layer chromatography (TLC) used silica gel GF$_{254}$. All reactions except those in aqueous media were carried out by standard techniques for moisture exclusion. Anhydrous reactions were carried out over dried glassware under nitrogen atmosphere. The boiling range for petroleum ether is 60-90 ℃.

General procedure: Compounds (1a-1c and 2a-2c)

5(6)-bromopyridin-2-amine (5.19g，30mmol) and Et$_3$N (20mL) were dissolved in anhydrous CH$_2$Cl$_2$ (100mL), cooled to 0 ℃, followed by dropwise addition of the appropriate acyl chloride (36mmol). The resulting mixture was warmed to room temperature and stirred overnight. The reaction mixture was diluted with CH$_2$Cl$_2$, and the organic layer was washed with water (30mL×3), NaHCO$_3$ (30mL×3), NaCl solution (30mL). Then the organic phase was dried over Na$_2$SO$_4$, filtered and concentrated. The residue was purified by chromatography on silica gel.

N-(5-bromopyridin-2-yl)acetamide (1a)

The procedure described above was used with 2.54mL acetyl chloride. Elution with petroleum ether/ethyl acetate(5/1, v/v) gave a white solid (5.65g, 88%). Mp 78-81 ℃; EI-MS (m/z) 214[M]$^+$.

N-(5-bromopyridin-2-yl)-2,2-dimethylpropanamide (1b)

The procedure described above was used with 4.43mL pivaloyl chloride. Elution with petroleum ether gave a white solid (7.37g, 96%). Mp 42-44 ℃; EI-MS (m/z) 256[M]$^+$.

N-(5-bromopyridin-2-yl)methanesulfonamide (1c)

The procedure described above was used with 2.78mL methanesulfonylchloride. Elution with petroleum ether/ethyl acetate(1/1, v/v) gave a white solid (6.75g, 90%). Mp 161-163 ℃; EI-MS (m/z) 250[M]$^+$.

N-(6-bromopyridin-2-yl)acetamide (2a)

The procedure described above was used with 2.54mL acetyl chloride. Elution with petroleum ether/ethyl acetate(5/1, v/v) gave a white solid (5.52g, 86%). Mp 159-160 ℃; EI-MS (m/z) 214[M]$^+$.

N-(6-bromopyridin-2-yl)-2,2-dimethylpropanamide (2b)

The procedure described above was used with 4.43mL pivaloyl chloride. Elution with petroleum ether gave a white solid (7.29g, 95%). Mp 90-91 ℃; EI-MS (m/z) 256[M]$^+$.

N-(6-bromopyridin-2-yl)methanesulfonamide (2c)

The procedure described above was used with 2.78mL methanesulfonylchloride. Elution with petroleum ether/ethyl acetate(1/1, v/v) gave a white solid (6.30g, 84%). Mp 179-180 ℃; EI-MS (m/z) 250[M]$^+$.

General procedure: Compounds (3a-3f)

Sulfoxide chloride (36mL, 494mmol) was added dropwise at room temperature under N$_2$ to solid 5-bromonicotinic acid (5.00g, 24.7mmol). The resulting mixture was refluxed for 2h and the volatiles were removed *in vacuo*. The crude acid chloride was dissolved in anhydrous CH$_2$Cl$_2$ (30mL) and the solution was added slowly at 0 ℃ to a solution of corresponding amine (54.4mmol) in CH$_2$Cl$_2$ (30mL). Stirring was continued overnight. Aqueous K$_2$CO$_3$ (2M, 20mL) was added and aqueous layer was extracted with CH$_2$Cl$_2$ (15mL×3). The combined organic extract was dried over Na$_2$SO$_4$, concentrated *in vacuo* and the crude product was purified by chromatography on silica gel.

4-[(5-bromopyridin-3-yl)carbonyl]morpholine(3a)

The procedure described above was used with 4.74mL morpholine. Elution with petroleum ether/ethyl acetate(1/1, v/v) gave a white solid (5.55g, 83%). Mp 76-78 ℃; EI-MS (m/z) 271[M]$^+$.

5-bromo-*N*,*N*-diisopropylnicotinamide(3b)

The procedure described above was used with 7.67mL diisopropylamine. Elution with petroleum ether/ethyl acetate(1/1, v/v) gave a white solid (6.03g, 86%). Mp 90-91 ℃; EI-MS (m/z) 284[M]$^+$.

5-bromo-*N*-cyclopropylnicotinamide(3c)

The procedure described above was used with 3.77mL cyclopropylamine. Elution with petroleum ether/ethyl acetate(1/1, v/v) gave a white solid (5.27g, 89%). Mp 140-142 ℃; EI-MS (m/z) 240[M]$^+$.

5-bromo-*N*,*N*-diethylnicotinamide(3d)

The procedure described above was used with 5.60mL diethylamine. Elution with petroleum ether/ethyl acetate(1/1, v/v) gave an oil (5.50g, 87%). EI-MS (m/z) 256[M]$^+$.

5-bromo-*N*-isopropylnicotinamide(3e)

The procedure described above was used with 4.67mL isopropylamine. Elution with petroleum ether/ethyl acetate(1/1, v/v) gave a white solid (5.26g, 88%). Mp 105-106 ℃; EI-MS (m/z) 242[M]$^+$.

5-bromo-*N*-[2-(dimethylamino)ethyl]nicotinamide(3f)

The procedure described above was used with 4.80mL *N*,*N*-dimethyl-1,2-ethanediamine. Elution with ethyl acetate gave an oil (5.35g, 80%). EI-MS (m/z) 271[M]$^+$.

General procedure: Compounds 4a-4c, 5a-5c, and 6a-6f

Bromo-substitued aromatic heterocyclic amide (20mmol), 4-carboxyphenylboronic acid (**2**) (3.66g, 22mmol), Cs$_2$CO$_3$ (13.0g, 40mmol), and Pd(PPh$_3$)$_4$ (1.2g, 1mmol) were suspended in a mixture of CH$_3$CN/H$_2$O (200mL, V:V=3:2) under N$_2$. The mixture was heated in oil bath at 90 ℃ for 48h. The mixture was filtered immediately after the reaction finished. The filtrate was adjusted to about pH 4 using HCl solution (6mol/L). Precipitate was filtered out and dried under vacuum overnight to give white solid.

4-[6-(acetylamino)pyridin-3-yl]benzoic acid (4a)

The procedure described above was used with 4.30g *N*-(5-bromopyridin-2-yl)acetamide (**1a**), giving a solid (3.89g, 76%). Mp

156-158 ℃; EI-MS (m/z) 256[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 8.74-8.71 (m, 1H), 8.41 (d, J = 2.3 Hz, 1H), 8.18 (d, J = 1.9 Hz, 2H), 8.04 (d, J = 1.8 Hz, 1H), 8.02 (d, J = 1.7 Hz, 1H), 7.12 (d, J = 9.3 Hz, 1H), 2.13 (s, 3H).

4-{6-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl} benzoic acid (4b)

The procedure described above was used with 5.14g N-(5-bromopyridin-2-yl)-2,2-dimethylpropanamide, giving a solid (4.77g, 80%). Mp 276-277 ℃; EI-MS (m/z) 298[M]⁺; ¹H NMR (400MHz, DMSO-d₆) δ = 8.74 (t, J = 1.6 Hz, 1H), 8.19 (d, J = 1.6 Hz, 2H), 8.04 (d, J = 8.5 Hz, 2H), 7.87 (d, J = 8.5 Hz, 2H), 1.26 (s, 9H).

4-{6-[(methylsulfonyl)amino]pyridin-3-yl}benzoic acid (4c)

The procedure described above was used with 5.02g N-(5-bromopyridin-2-yl)methanesulfonamide (1c), giving a solid (4.32g, 74%). Mp 294-295 ℃; EI-MS (m/z) 292[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 8.66 (d, J = 2.3 Hz, 1H), 8.14 (dd, J = 8.7, 2.5 Hz, 1H), 8.06 (d, J = 8.5 Hz, 1H), 8.03 (d, J = 8.5 Hz, 1H), 7.88 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 8.5 Hz, 1H), 7.10 (d, J = 8.6 Hz, 1H), 3.34 (s, 3H).

4-[6-(acetylamino)pyridin-2-yl]benzoic acid (5a)

The procedure described above was used with 4.30g N-(6-bromopyridin-2-yl)acetamide (2a), giving a solid (3.99g, 78%). Mp 317-318 ℃; EI-MS (m/z) 256[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 10.58 (s, 1H), 8.20 (d, J = 8.5 Hz, 2H), 8.06 (dd, J = 4.8, 3.7 Hz, 3H), 7.90 (t, J = 7.9 Hz, 1H), 7.74 (d, J = 7.6 Hz, 1H), 2.15 (s, 3H).

4-{6-[(2,2-dimethylpropanoyl)amino]pyridin-2-yl}benzoic acid (5b)

The procedure described above was used with 5.14g N-(6-bromopyridin-2-yl)-2,2-dimethylpropanamide (2b), giving a solid (4.95g, 83%). Mp 266-268 ℃; EI-MS (m/z) 298[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 9.73 (s, 1H), 8.25 (d, J = 8.4 Hz, 2H), 8.06 (dd, J = 8.3, 1.9 Hz, 3H), 7.90 (t, J = 7.9 Hz, 1H), 7.76 (d, J = 7.6 Hz, 1H), 1.29 (s, 9H).

4-{6-[(methylsulfonyl)amino]pyridin-2-yl}benzoic acid (5c)

The procedure described above was used with 5.02g N-(6-bromopyridin-2-yl)methanesulfonamide (2c), giving a solid (4.20g, 72%). Mp 280-281 ℃; EI-MS (m/z) 292[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 8.18 (d, J = 8.5 Hz, 2H), 8.06 (d, J = 8.5 Hz, 2H), 7.87 (t, J = 7.9 Hz, 1H), 7.71 (d, J = 7.6 Hz, 1H), 6.96 (d, J = 8.1 Hz, 1H), 3.43 (s, 3H).

4-[5-(morpholin-4-ylcarbonyl)pyridin-3-yl]benzoic acid (6a)

The procedure described above was used with 5.42g 4-[(5-bromopyridin-3-yl)carbonyl]morpholine(3a), giving a solid (4.99g, 80%). Mp 216-218 ℃; EI-MS (m/z) 312[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 9.05 (d, J = 2.2 Hz, 1H), 8.67 (d, J = 1.9 Hz, 1H), 8.21 (t, J = 2.1 Hz, 1H), 8.07 (dd, J = 8.4, 4.0 Hz, 2H), 7.94 (t, J = 7.0 Hz, 2H), 3.78-3.38 (m, 8H).

4-{5-[(diisopropylamino)carbonyl]pyridin-3-yl}benzoic acid (6b)

The procedure described above was used with 5.14g 5-bromo-N,N-diisopropylnicotinamide(3b), giving a solid (5.28g, 81%). Mp 74-76 ℃; EI-MS (m/z) 326[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 9.01 (d, J = 2.2 Hz, 1H), 8.56 (d, J = 1.9 Hz, 1H), 8.09 (t, J = 2.1 Hz, 1H), 8.06 (d, J = 8.4 Hz, 2H), 7.93 (d, J = 8.4 Hz, 2H), 1.63-1.03 (m, 14H).

4-{5-[(cyclopropylamino)carbonyl]pyridin-3-yl}benzoic acid (6c)

The procedure described above was used with 4.82g 5-bromo-N-cyclopropylnicotinamide(3c), giving a solid (4.79g, 85%). Mp 258-260 ℃; EI-MS (m/z) 282[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 9.08 (d, J = 2.2 Hz, 1H), 9.00 (d, J = 2.0 Hz, 1H), 8.49 (t, J = 2.1 Hz, 1H), 8.08 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 8.4 Hz, 2H), 2.97-2.85 (m, 1H), 0.73-0.78 (m, 2H), 0.67-0.58 (m, 2H).

4-{5-[(diethylamino)carbonyl]pyridin-3-yl}benzoic acid (6d)

The procedure described above was used with 5.12g 5-bromo-N,N-diethylnicotinamide(3d), giving a solid (4.53g, 76%). Mp 235-236 ℃; EI-MS (m/z) 298[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 9.03 (d, J = 2.2 Hz, 1H), 8.61 (d, J = 1.9 Hz, 1H), 8.16 (t, J = 2.1 Hz, 1H), 8.06 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 8.4 Hz, 2H), 3.49 (q, J = 6.6 Hz, 2H), 3.24 (q, J = 6.5 Hz, 2H), 1.19 (t, J = 6.1 Hz, 3H), 1.09 (t, J = 6.0 Hz, 3H).

4-{5-[(isopropylamino)carbonyl]pyridin-3-yl}benzoic acid (6e)

The procedure described above was used with 4.86g 5-bromo-N-isopropylnicotinamide(3e), giving a solid (4.43g, 78%). Mp 290-291 ℃; EI-MS (m/z) 284[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 9.08 (d, J = 2.1 Hz, 1H), 9.02 (d, J = 2.0 Hz, 1H), 8.52 (t, J = 2.1 Hz, 1H), 8.09 (d, J = 8.4 Hz, 2H), 7.95 (d, J = 8.3 Hz, 2H), 4.11-4.19 (m, 1H), 1.21 (d, J = 6.6 Hz, 6H).

4-[5-({[2-(dimethylamino)ethyl]amino}carbonyl)pyridin-3-yl] benzoic acid (6f)

The procedure described above was used with 5.44g 5-bromo-N-[2-(dimethylamino)ethyl]nicotinamide(3f), giving a solid (4.38g, 70%). Mp 160-162 ℃; EI-MS (m/z) 313.95[M+H]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 9.03 (d, J = 2.2 Hz, 1H), 8.61 (d, J = 1.9 Hz, 1H), 8.16 (t, J = 2.1 Hz, 1H), 8.06 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 8.5 Hz, 2H), 3.49 (d, J = 6.9 Hz, 2H), 3.24 (d, J = 6.9 Hz, 2H), 1.19 (t, J = 6.2 Hz, 3H), 1.09 (t, J = 6.5 Hz, 3H).

General procedure: Compounds 7I-7VII

Trimethylacetyl chloride (2.42mL, 20mmol) and Et₃N (4.60mL, 30mmol) were added to a mixture of corresponding benzoic acid (20mmol) in CH₂Cl₂ (80mL). The resulting mixture was stirred at r.t. until a clear solution was observed. A solution of piperazine (3.44 g, 40mmol) in EtOH (80mL) was added, and the mixture was further stirred for 3-4 h. Concentrated HCl (4mL) was added and the resulting mixture was extracted with CH₂Cl₂ (30mL×2). The CH₂Cl₂ layer was discarded. NaOH (8g) was added to the aqueous solution and then extracted with CH₂Cl₂ (150mL). The organic extract was further washed with H₂O (50mL) and then dried by Na₂SO₄. Evaporation of the solvent at reduced pressure yielded the crude product reserved for the next step.

General procedure: target compounds 8a-8l, 9a-9i, 10a-10u.

In a 100mL flask, compounds 4a-4c, 5a-5c, or 6a-6f (3.5mmol) and 4-methylmorpholine (1.2mL, 10.5mmol) were dissloved in THF (20mL). Under 0 ℃, the THF (8mL) solution of isobutyl chloroformate (0.7mL, 5.25mmol) was dropped slowly into the above suspension. Then the mixture was reacted under 0 ℃ for 1h. After that, the THF (15mL) solution of compounds 7I-7VII (5.25mmol) and 4-methylmorpholine (1.2mL, 10.5mmol) was dropped slowly into the above solution. Then the ice bath was removed and the mixture was reacted at r.t. overnight. THF was removed *in vacuo,* and the residue was diluted with CH₂Cl₂ (50mL). The organic phase was washed with water (15mL×3), saturated NaHCO₃ solution (10mL×2), NaCl solution (10mL). Then the organic phase was dried by Na₂SO₄, and filtered, giving the crude product. The crude product was purified by chromatography, giving the target compounds.

N-{5-[4-({4-[3-(trifluoromethyl)benzoyl]piperazin-1-yl}carbonyl) phenyl]pyridin-2-yl}acetamide (8a)

The procedure described above was used with 0.90g 4-[6-(acetylamino)pyridin-3-yl]benzoic acid (4a) and 1.35g 1-[3-(trifluoromethyl)benzoyl]piperazine (7I). Elution with petroleum ether/ethyl acetate(1/3, v/v) gave a solid (0.49g, 28%). Mp 224-226 ℃; EI-MS (m/z) 496[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 8.69 (s, 1H), 8.16 (d, J = 11.7 Hz, 2H), 7.85 (d, J = 6.7 Hz, 1H), 7.80 (s, 3H), 7.78 − 7.68 (m, 2H), 7.54 (d, J = 7.4 Hz, 2H), 3.84-3.40 (m, 8H), 2.13 (s, 3H); ¹³C NMR (101 MHz, DMSO-d_6) δ 169.85, 169.46, 168.18, 152.22, 146.34, 138.57, 137.20, 136.71, 135.04, 131.48, 130.50, 130.16, 128.39, 126.76, 125.69, 124.30, 124.27, 122.98, 113.66, 47.47, 42.25, 24.40.

N-{5-[4-({4-[4-chloro-3-(trifluoromethyl)benzoyl]piperazin-1-yl}carbonyl)phenyl]pyridin-2-yl}acetamide (8b)

The procedure described above was used with 0.90g 4-[6-(acetylamino)pyridin-3-yl]benzoic acid (**4a**) and 1.50g 1-[4-chloro-3-(trifluoromethyl)benzoyl]piperazine (**7II**). Elution with petroleum ether/ethyl acetate(1/3, v/v) gave a solid (0.54g, 29%). Mp 241-242 ℃; EI-MS (m/z) 530[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.69 (s, 1H), 8.16 (d, J = 10.7 Hz, 2H), 7.91 (s, 1H), 7.81 (d, J = 8.0 Hz, 4H), 7.54 (d, J = 7.7 Hz, 2H), 3.80-3.38 (m, 8H), 2.13 (s, 3H); 13C NMR (101 MHz, DMSO-d6) δ 169.85, 169.45, 167.27, 152.21, 146.35, 138.57, 136.72, 135.69, 135.05, 133.16, 132.37, 130.50, 128.38, 126.77, 124.37, 121.66, 113.67, 47.45, 42.27, 24.41; ^{13}C NMR (101 MHz, DMSO-$d6$) δ 169.85, 169.45, 167.27, 152.21, 146.35, 138.57, 136.72, 135.69, 135.05, 133.16, 132.37, 130.50, 128.38, 126.77, 124.37, 121.66, 113.67, 47.45, 42.27, 24.41.

N-{5-(4-{[4-(3,4-difluorobenzoyl)piperazin-1-yl]carbonyl}phenyl)pyridin-2-yl]acetamide (8c)

The procedure described above was used with 0.90g 4-[6-(acetylamino)pyridin-3-yl]benzoic acid (**4a**) and 1.18g 1-(3,4-difluorobenzoyl)piperazine (**7III**). Elution with petroleum ether/ethyl acetate(1/3, v/v) gave a solid (0.36g, 22%). Mp 263-266 ℃; EI-MS (m/z) 464[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.69 (s, 1H), 8.16 (d, J = 10.1 Hz, 2H), 7.84 (d, J = 8.5 Hz, 1H), 7.81 (d, J = 7.7 Hz, 2H), 7.54 (d, J = 7.1 Hz, 3H), 7.33 (s, 1H), 3.63 - 3.34 (m, 8H), 2.13 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.85, 169.43, 167.51, 152.22, 146.35, 138.57, 136.72, 135.04, 133.44, 130.49, 128.39, 126.75, 124.87, 118.39, 118.21, 117.36, 117.18, 113.66, 47.43, 42.38, 24.33.

N-[5-(4-{[4-(3-methoxybenzoyl)piperazin-1-yl]carbonyl}phenyl)pyridin-2-yl]acetamide (8d)

The procedure described above was used with 0.90g 4-[6-(acetylamino)pyridin-3-yl]benzoic acid (**4a**) and 1.16g 1-(3-methoxybenzoyl)piperazine (**7IV**). Elution with petroleum ether/ethyl acetate(1/3, v/v) gave a solid (0.40g, 25%). Mp 207-209 ℃; EI-MS (m/z) 458[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.69 (s, 1H), 8.16 (d, J = 10.8 Hz, 2H), 7.80 (d, J = 7.8 Hz, 2H), 7.53 (d, J = 7.9 Hz, 2H), 7.37 (t, J = 7.6 Hz, 1H), 7.03 (d, J = 7.7 Hz, 1H), 6.98 (d, J = 7.9 Hz, 2H), 3.79 (s, 3H), 3.67 - 3.44 (m, 8H), 2.13 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.86, 169.43, 169.40, 159.59, 152.22, 146.35, 138.55, 137.47, 136.71, 135.07, 130.50, 130.16, 128.40, 126.75, 119.42, 115.79, 113.66, 112.79, 55.69, 47.48, 42.24, 24.45; HRMS (ESI): Calcd. for [M+H]$^+$ C$_{26}$H$_{27}$N$_4$O$_4$: 459.2032, found 459.2022.

2,2-dimethyl-N-{5-[4-({4-[3-(trifluoromethyl)benzoyl]piperazin-1-yl}carbonyl)phenyl]pyridin-2-yl} propanamide (8e)

The procedure described above was used with 1.04g 4-{6-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl}benzoic acid (**4b**) and 1.35g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate(1/1, v/v) gave a solid (0.51g, 27%). Mp 190-193 ℃; EI-MS (m/z) 538[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.71 (s, 1H), 8.17 (s, 2H), 7.85 (d, J = 7.7 Hz, 1H), 7.82 (d, J = 8.4 Hz, 3H), 7.79-7.74 (m, 1H), 7.74-7.70 (m, 1H), 7.54 (d, J = 6.8 Hz, 2H), 3.75-3.41 (m, 8H), 1.26 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 177.74, 169.45, 168.17, 152.46, 146.08, 138.53, 137.20, 136.58, 135.09, 131.48, 130.62, 130.16, 128.39, 126.78, 125.69, 124.26, 124.22, 122.98, 114.40, 47.32, 42.11, 39.89, 27.33.

N-{5-[4-({4-[4-chloro-3-(trifluoromethyl)benzoyl]piperazin-1-yl}carbonyl)phenyl]pyridin-2-yl}-2,2-dimethylpropanamide (8f)

The procedure described above was used with 1.04g 4-{6-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl}benzoic acid (**4b**) and 1.50g 1-[4-chloro-3-(trifluoromethyl)benzoyl]piperazine (**7II**). Elution with petroleum ether/ethyl acetate(1/1, v/v) gave a solid (0.60g, 30%). Mp 194-196 ℃; EI-MS (m/z) 572[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.71 (s, 1H), 8.16 (s, 1H), 7.91 (s, 1H), 7.88-7.75 (m, 4H), 7.67-7.59 (m, 1H), 7.54 (d, J = 7.9 Hz, 2H), 3.43-3.69 (m, 8H), 1.26 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 177.75, 169.46, 167.27, 152.45, 146.09, 138.53, 136.59, 135.67, 135.07, 133.15, 132.36, 132.00, 128.39, 126.79, 124.37, 121.65, 114.41, 47.52, 42.23, 39.89, 27.34.

N-[5-(4-{[4-(3,4-difluorobenzoyl)piperazin-1-yl]carbonyl}phenyl)pyridin-2-yl]-2,2-dimethylpropanamide(8g)

The procedure described above was used with 1.04g 4-{6-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl}benzoic acid (**4b**) and 1.18g 1-(3,4-difluorobenzoyl)piperazine (**7III**). Elution with petroleum ether/ethyl acetate(1/1, v/v) gave a solid (0.42g, 24%). Mp 191-193 ℃; EI-MS (m/z) 506[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.71 (s, 1H), 8.17 (s, 2H), 7.82 (d, J = 7.9 Hz, 2H), 7.59 (d, J = 9.5 Hz, 1H), 7.54 (d, J = 8.0 Hz, 3H), 7.33 (s, 1H), 3.67- 3.46 (m, 8H), 1.26 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 177.76, 169.44, 167.52, 152.46, 146.09, 138.53, 136.60, 135.08, 133.53, 130.62, 128.40, 126.79, 124.94, 118.39, 118.21, 117.36, 117.18, 114.41, 47.26, 42.08, 39.90, 27.34.

N-[5-(4-{[4-(3-methoxybenzoyl)piperazin-1-yl]carbonyl}phenyl)pyridin-2-yl]-2,2-dimethylpropanamide (8h)

The procedure described above was used with 1.04g 4-{6-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl}benzoic acid (**4b**) and 1.16g 1-(3-methoxybenzoyl)piperazine (**7IV**). Elution with petroleum ether/ethyl acetate(1/1, v/v) gave a solid (0.45g, 26%). Mp 176-177 ℃; EI-MS (m/z) 500[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.71 (s, 1H), 8.21-8.12 (m, 2H), 7.82 (d, J = 7.7 Hz, 2H), 7.54 (d, J = 7.9 Hz, 2H), 7.38 (t, J = 7.4 Hz, 1H), 7.03 (d, J = 7.7 Hz, 1H), 6.98 (d, J = 7.9 Hz, 2H), 3.79 (s, 3H), 3.77-3.36 (m, 8H), 1.27 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 177.76, 169.42, 169.39, 161.27, 159.58, 152.44, 146.10, 138.49, 137.48, 136.61, 135.13, 130.62, 130.17, 128.40, 126.79, 119.42, 115.82, 114.41, 112.78, 55.71, 47.66, 42.12, 39.90, 27.35; HRMS (ESI): Calcd. for [M+H]$^+$ C$_{29}$H$_{33}$N$_4$O$_4$: 501.2502, found 501.2495.

N-{5-[4-({4-[3-(trifluoromethyl)benzoyl]piperazin-1-yl}carbonyl)phenyl]pyridin-2-yl}methanesulfonamide (8i)

The procedure described above was used with 1.02g 4-{6-[(methylsulfonyl)amino]pyridin-3-yl}benzoic acid (**4c**) and 1.35g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate(1/7, v/v) gave a solid (0.43g, 23%). Mp 261-263 ℃; EI-MS (m/z) 532[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.63 (s, 1H), 8.11 (d, J = 8.4 Hz, 1H), 7.85 (d, J = 6.7 Hz, 1H), 7.79 (d, J = 4.9 Hz, 2H), 7.76 (s, 2H), 7.72 (d, J = 7.4 Hz, 1H), 7.54 (d, J = 7.1 Hz, 2H), 7.09 (d, J = 8.7 Hz, 1H), 3.86 – 3.37 (m, 11H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.44, 168.19, 152.45, 145.60, 138.40, 137.52, 137.20, 135.10, 131.48, 130.17, 128.40, 126.75, 125.69, 124.30, 124.26, 122.98, 112.76, 47.43, 42.24, 42.04.

N-{5-[4-({4-[4-chloro-3-(trifluoromethyl)benzoyl]piperazin-1-yl}carbonyl)phenyl]pyridin-2-yl}methanesulfonamide (8j)

The procedure described above was used with 1.02g 4-{6-[(methylsulfonyl)amino]pyridin-3-yl}benzoic acid (**4c**) and 1.50g 1-[4-chloro-3-(trifluoromethyl)benzoyl]piperazine (**7II**). Elution with petroleum ether/ethyl acetate(1/7, v/v) gave a solid (0.42g, 21%). Mp 247-250 ℃; EI-MS (m/z) 566[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.63 (s, 1H), 8.11 (d, J = 8.4 Hz, 1H), 7.91 (s, 1H), 7.83 (s, 1H), 7.77 (d, J = 6.9 Hz, 3H), 7.53 (d, J = 7.2 Hz, 2H), 7.09 (d, J = 8.6 Hz, 1H), 3.61 (s, 3H), 3.36-3.69 (m, 8H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.43, 167.26, 152.43, 145.58, 138.41, 137.53, 135.68, 135.09, 133.15, 132.36, 130.57, 128.39, 127.80, 126.76, 125.64, 124.37, 121.66, 118.94, 112.75, 47.52, 42.25, 41.99.

N-[5-(4-{[4-(3,4-difluorobenzoyl)piperazin-1-yl]carbonyl}phenyl)pyridin-2-yl]methanesulfonamide (8k)

The procedure described above was used with 1.02g 4-{6-[(methylsulfonyl)amino]pyridin-3-yl}benzoic acid (**4c**) and 1.18g 1-(3,4-difluorobenzoyl)piperazine (**7III**). Elution with petroleum

ether/ethyl acetate(1/1, v/v) gave a solid (0.38g, 22%). Mp 270-272 ℃; EI-MS (m/z) 499.10[M-1]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 8.53 (s, 1H), 7.94 (d, J = 8.4 Hz, 1H), 7.73 (d, J = 7.8 Hz, 2H), 7.61-7.52 (m, 2H), 7.50 (d, J = 7.6 Hz, 2H), 7.36-7.28 (m, 1H), 6.89 (d, J = 8.4 Hz, 1H), 3.40-3.78 (m, 8H), 3.15 (s, 3H); ¹³C NMR (101 MHz, DMSO-d_6) δ 175.79, 169.32, 152.43, 145.63, 138.41, 138.32, 137.53, 135.27, 135.09, 129.27, 128.37, 126.74, 124.91, 118.38, 118.21, 117.36, 117.18, 112.75, 44.75, 42.25, 42.00.

N-[5-(4-{[4-(3-methoxybenzoyl)piperazin-1-yl]carbonyl}phenyl) pyridin-2-yl]methanesulfonamide (8l)

The procedure described above was used with 1.02g 4-{6-[(methylsulfonyl)amino]pyridin-3-yl}benzoic acid (**4c**) and 1.16g 1-(3-methoxybenzoyl)piperazine (**7IV**). Elution with petroleum ether/ethyl acetate(1/7, v/v) gave a solid (0.41g, 24%). Mp 270-272 ℃; EI-MS (m/z) 493.20[M-1]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 8.63 (s, 1H), 8.11 (d, J = 8.8 Hz, 1H), 7.77 (d, J = 7.7 Hz, 2H), 7.53 (d, J = 7.8 Hz, 2H), 7.38 (s, 1H), 7.09 (d, J = 8.7 Hz, 1H), 7.03 (d, J = 8.5 Hz, 1H), 6.98 (d, J = 8.2 Hz, 2H), 3.79 (s, 3H), 3.67 - 3.43 (m, 11H); ¹³C NMR (101 MHz, DMSO-d_6) δ 169.39, 159.59, 152.44, 138.37, 137.53, 137.48, 135.13, 130.17, 128.40, 126.75, 119.42, 115.81, 112.78, 55.71, 47.50, 42.25, 42.08; HRMS (ESI): Calcd. for [M+Na]⁺ $C_{25}H_{26}N_4O_5SNa$: 517.1522, found 517.1514.

N-{6-[4-({4-[3-(trifluoromethyl)benzoyl]piperazin-1-yl}carbonyl) phenyl]pyridin-2-yl}acetamide (9a)

The procedure described above was used with 0.90g 4-[6-(acetylamino)pyridin-2-yl]benzoic acid (**5a**) and 1.35g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate(1/3, v/v) gave a solid (0.47g, 27%). Mp 214-216 ℃; EI-MS (m/z) 496 [M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 8.16 (d, J = 8.1 Hz, 2H), 8.06 (d, J = 8.1 Hz, 1H), 7.87 (dd, J = 15.7, 7.6 Hz, 2H), 7.80 (s, 1H), 7.76 (d, J = 7.4 Hz, 1H), 7.71 (d, J = 7.3 Hz, 2H), 7.56 (d, J = 7.1 Hz, 2H), 3.37-3.71 (m, 8H), 2.14 (s, 3H); ¹³C NMR (101 MHz, DMSO-d_6) δ 169.94, 169.46, 168.19, 154.10, 152.42, 139.81, 139.79, 137.20, 136.44, 131.49, 130.17, 127.97, 127.01, 126.82, 126.78, 125.69, 124.30, 124.26, 122.98, 116.18, 113.04, 47.54, 42.13, 24.46.

N-{6-[4-({4-[4-chloro-3-(trifluoromethyl)benzoyl]piperazin-1-yl} carbonyl)phenyl]pyridin-2-yl}acetamide (9b)

The procedure described above was used with 0.90g 4-[6-(acetylamino)pyridin-2-yl]benzoic acid (**5a**) and 1.50g 1-[4-chloro-3-(trifluoromethyl)benzoyl]piperazine (**7II**). Elution with petroleum ether/ethyl acetate(1/3, v/v) gave a solid (0.48g, 26%). Mp 226-228 ℃; EI-MS (m/z) 530[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 8.16 (d, J = 8.1 Hz, 2H), 8.07 (d, J = 8.0 Hz, 1H), 7.90 (s, 1H), 7.87 (d, J = 8.1 Hz, 1H), 7.84 (d, J = 7.9 Hz, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.71 (d, J = 7.6 Hz, 1H), 7.56 (d, J = 7.9 Hz, 2H), 3.78-3.40 (m, 8H), 2.14 (s, 3H); ¹³C NMR (101 MHz, DMSO-d_6) δ 169.94, 169.45, 167.27, 154.09, 152.41, 139.80, 136.43, 135.68, 133.16, 132.37, 127.97, 127.22, 127.01, 116.19, 113.04, 47.57, 42.27, 24.47.

N-[6-(4-{[4-(4-tert-butylbenzoyl)piperazin-1-yl]carbonyl}phenyl) pyridin-2-yl]acetamide (9c)

The procedure described above was used with 0.90g 4-[6-(acetylamino)pyridin-2-yl]benzoic acid (**5a**) and 1.29g 1-(4-tert-butylbenzoyl)piperazine (**7V**). Elution with petroleum ether/ethyl acetate(1/1, v/v) gave a solid (0.50g, 30%). Mp 217-220 ℃; EI-MS (m/z) 484[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 8.15 (d, J = 8.0 Hz, 2H), 7.89 (d, J = 7.8 Hz, 2H), 7.71 (d, J = 7.7 Hz, 1H), 7.56 (d, J = 8.0 Hz, 2H), 7.48 (d, J = 7.8 Hz, 2H), 7.38 (d, J = 8.1 Hz, 2H), 3.47-3.63 (m, 8H), 3.34 (s, 3H), 1.30 (s, 9H); ¹³C NMR (101 MHz, DMSO-d_6) δ 169.41, 167.55, 153.86, 152.48, 152.41, 139.83, 139.78, 133.14, 131.52, 130.16, 127.50, 125.63, 116.57, 113.39, 47.58, 42.46, 34.97, 31.41, 24.45.

2,2-dimethyl-N-{6-4-({4-[3-(trifluoromethyl)benzoyl]piperazin-1-yl}carbonyl)phenyl]pyridin-2-yl}propanamide (9d)

The procedure described above was used with 1.04g 4-{6-[(2,2-dimethylpropanoyl)amino]pyridin-2-yl}benzoic acid (**5b**) and 1.35g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate(1/1, v/v) gave a solid (0.58g, 31%). Mp 171-174 ℃; EI-MS (m/z) 538[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 8.20 (d, J = 8.0 Hz, 2H), 8.03 (d, J = 8.2 Hz, 1H), 7.90 (d, J = 7.8 Hz, 1H), 7.86 (d, J = 9.9 Hz, 1H), 7.80 (s, 1H), 7.76 (d, J = 7.5 Hz, 1H), 7.73 (d, J = 7.5 Hz, 2H), 7.55 (d, J = 7.5 Hz, 2H), 3.83-3.39 (m, 8H), 1.28 (s, 9H); ¹³C NMR (101 MHz, DMSO-d_6) δ 177.68, 169.49, 168.19, 154.07, 152.51, 139.74, 139.61, 137.20, 136.43, 131.48, 130.17, 127.90, 127.14, 126.82, 126.78, 125.69, 124.30, 124.27, 122.98, 116.29, 113.88, 47.45, 42.10, 39.86, 27.40.

N-[6-4-({4-[4-chloro-3-(trifluoromethyl)benzoyl]piperazin-1-yl} carbonyl)phenyl]pyridin-2-yl]-2,2-dimethylpropanamide (9e)

The procedure described above was used with 1.04g 4-{6-[(2,2-dimethylpropanoyl)amino]pyridin-2-yl}benzoic acid (**5b**) and 1.50g 1-[4-chloro-3-(trifluoromethyl)benzoyl]piperazine (**7II**). Elution with petroleum ether/ethyl acetate(1/1, v/v) gave a solid (0.58g, 29%). Mp 187-190 ℃; EI-MS (m/z) 572[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 8.21 (d, J = 7.9 Hz, 2H), 8.03 (d, J = 8.2 Hz, 1H), 7.90 (d, J = 7.7 Hz, 2H), 7.88-7.81 (m, 1H), 7.77 (d, J = 7.8 Hz, 1H), 7.73 (d, J = 7.6 Hz, 1H), 7.55 (d, J = 7.8 Hz, 2H), 3.43-3.69 (m, 8H), 1.28 (s, 9H); ¹³C NMR (101 MHz, DMSO-d_6) δ 177.68, 169.49, 167.28, 154.07, 152.50, 139.75, 139.61, 136.43, 135.68, 133.15, 132.42, 132.37, 127.89, 127.22, 127.14, 116.29, 113.89, 47.46, 42.23, 39.86, 27.41; HRMS (ESI): Calcd. for [M+H]⁺ $C_{29}H_{29}ClF_3N_4O_3$: 573.1880, found 573.1886.

N-{6-(4-{[4-(4-tert-butylbenzoyl)piperazin-1-yl]carbonyl}phenyl) pyridin-2-yl]-2,2-dimethyl propanamide (9f)

The procedure described above was used with 1.04g 4-{6-[(2,2-dimethylpropanoyl)amino]pyridin-2-yl}benzoic acid (**5b**) and 1.29g 1-(4-tert-butylbenzoyl)piperazine (**7V**). Elution with petroleum ether/ethyl acetate(1/1, v/v) gave a solid (0.61g, 33%). Mp 237-240 ℃; EI-MS (m/z) 526[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 8.20 (d, J = 7.9 Hz, 2H), 8.03 (d, J = 8.2 Hz, 1H), 7.89 (t, J = 8.0 Hz, 1H), 7.73 (d, J = 7.6 Hz, 1H), 7.55 (d, J = 8.1 Hz, 2H), 7.48 (d, J = 7.8 Hz, 2H), 7.38 (d, J = 8.2 Hz, 2H), 3.78-3.42 (m, 8H), 1.30 (s, 9H), 1.28 (s, 9H); ¹³C NMR (101 MHz, DMSO-d_6) δ 177.68, 169.79, 169.44, 154.08, 152.81, 152.51, 139.71, 139.62, 136.48, 133.16, 127.90, 127.50, 127.12, 125.64, 116.30, 113.88, 47.55, 42.07, 39.87, 34.99, 31.43, 27.42.

N-{6-[4-({4-[3-(trifluoromethyl)benzoyl]piperazin-1-yl}carbonyl) phenyl]pyridin-2-yl}methanesulfonamide (9g)

The procedure described above was used with 1.02g 4-{6-[(methylsulfonyl)amino]pyridin-2-yl}benzoic acid (**5c**) and 1.35g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate(1/7, v/v) gave a solid (0.41g, 22%). Mp 243-244 ℃; EI-MS (m/z) 532[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 8.13 (d, J = 8.1 Hz, 2H), 7.85 (t, J = 7.8 Hz, 2H), 7.80 (s, 1H), 7.79-7.70 (m, 2H), 7.67 (d, J = 7.8 Hz, 1H), 7.56 (d, J = 7.1 Hz, 2H), 6.91 (d, J = 8.1 Hz, 1H), 3.43 (s, 3H), 3.44-3.71(m, 8H); ¹³C NMR (101 MHz, DMSO-d_6) δ 169.39, 168.18, 154.02, 152.50, 140.09, 139.78, 137.21, 136.58, 131.48, 130.17, 128.11, 126.95, 126.81, 126.77, 125.69, 124.30, 124.26, 122.98, 114.98, 111.50, 47.40, 42.43, 41.98.

N-{6-[4-({4-[4-chloro-3-(trifluoromethyl)benzoyl]piperazin-1-yl} carbonyl)phenyl]pyridin-2-yl}methane sulfonamide (9h)

The procedure described above was used with 1.02g 4-{6-[(methylsulfonyl)amino]pyridin-2-yl}benzoic acid (**5c**) and 1.50g 1-[4-chloro-3-(trifluoromethyl)benzoyl]piperazine (**7II**). Elution with petroleum ether/ethyl acetate(1/7, v/v) gave a solid (0.40g, 20%). Mp 120-122 ℃; EI-MS (m/z) 566[M]⁺; ¹H NMR (400MHz, DMSO-d_6) δ 8.13 (d, J = 8.0 Hz, 2H), 7.91 (s, 1H), 7.84 (d, J = 7.9 Hz, 2H), 7.78 (s, 1H), 7.68 (d, J = 7.6 Hz, 1H), 7.56 (d, J = 7.6 Hz, 2H), 6.92 (d, J = 8.1 Hz, 1H), 3.44 (s, 3H), 3.79-3.39 (m, 8H); ¹³C NMR (101

MHz, DMSO-d_6) δ 169.39, 167.27, 154.02, 152.50, 140.09, 139.79, 136.57, 135.68, 133.14, 132.39, 132.35, 128.11, 127.22, 126.95, 114.98, 111.51, 47.48, 42.43, 42.18; HRMS (ESI): Calcd. for [M+Na]$^+$ C$_{25}$H$_{22}$ClF$_3$N$_4$O$_4$SNa: 589.0900, found 589.0909.

N-[6-(4-{[4-(4-tert-butylbenzoyl)piperazin-1-yl]carbonyl}phenyl) pyridin-2-yl]methanesulfonamide(9i)

The procedure described above was used with 1.02g 4-{6-[(methylsulfonyl)amino]pyridin-2-yl}benzoic acid (**5c**) and 1.29g 1-(4-tert-butylbenzoyl)piperazine (**7V**). Elution with petroleum ether/ethyl acetate(1/1, v/v) gave a solid (0.44g, 24%). Mp 225-228 ℃; EI-MS (m/z) 520[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.13 (d, *J* = 8.0 Hz, 2H), 7.85 (t, *J* = 7.9 Hz, 1H), 7.68 (d, *J* = 7.7 Hz, 1H), 7.56 (d, *J* = 8.1 Hz, 2H), 7.48 (d, *J* = 7.9 Hz, 2H), 7.38 (d, *J* = 8.2 Hz, 2H), 6.92 (d, *J* = 8.1 Hz, 1H), 3.44 (s, 3H), 3.33-3.67(m, 8H), 1.30 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.79, 169.34, 154.04, 152.80, 152.49, 140.10, 139.75, 136.63, 133.17, 128.12, 127.50, 126.93, 125.64, 114.99, 111.49, 47.62, 42.44, 42.13, 34.99, 31.43.

4-({5-[4-({4-[3-(trifluoromethyl)benzoyl]piperazin-1-yl}carbonyl) phenyl]pyridin-3-yl}carbonyl)morpholine (10a)

The procedure described above was used with 1.09g 4-[5-(morpholin-4-ylcarbonyl)pyridin-3-yl]benzoic acid (**6a**) and 1.35g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate(1/5, v/v) gave a solid (0.44g, 23%). Mp 179-181 ℃; EI-MS (m/z) 552[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.02 (s, 1H), 8.65 (s, 1H), 8.18 (s, 1H), 7.89 (d, *J* = 8.1 Hz, 2H), 7.85 (s, 1H), 7.80 (s, 1H), 7.79-7.68 (m, 2H), 7.58 (d, *J* = 6.8 Hz, 2H), 3.54 (m, 16H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.29, 168.18, 167.07, 148.94, 147.37, 137.93, 137.20, 136.00, 134.85, 133.15, 132.16, 131.49, 130.18, 128.40, 127.65, 126.83, 126.79, 125.69, 124.30, 124.26, 122.98, 66.48, 66.44, 48.16, 47.38, 42.53, 42.27.

4-{[5-(4-{[4-(3,4-difluorobenzoyl)piperazin-1-yl]carbonyl}phenyl) pyridin-3-yl]carbonyl}morpholine (10b)

The procedure described above was used with 1.09g 4-[5-(morpholin-4-ylcarbonyl)pyridin-3-yl]benzoic acid (**6a**) and 1.18g 1-(3,4-difluorobenzoyl)piperazine (**7III**). Elution with petroleum ether/ethyl acetate(1/5, v/v) gave a solid (0.38g, 21%). Mp 212-213 ℃; EI-MS (m/z) 520[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.04 (dd, J= 8.5, 1.8 Hz, 1H), 8.66 (dd, *J* = 7.2, 1.8 Hz, 1H), 8.21 (t, *J* = 2.1 Hz, 1H), 8.18 (s, 1H), 8.07 (d, *J* = 8.4 Hz, 1H), 7.93 (d, *J* = 8.5 Hz, 1H), 7.89 (d, *J* = 7.7 Hz, 1H), 7.58 (d, *J* = 7.3 Hz, 2H), 3.81-3.38 (m, 16H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.27, 167.45, 167.01, 149.06, 148.94, 147.36, 140.88, 137.94, 135.98, 134.85, 133.36, 133.16, 132.19, 130.51, 127.76, 124.94, 118.38, 118.21, 117.36, 117.18, 66.48, 48.19, 47.59, 42.53, 42.18.

Dimethyl{3-[(4-{4-[5-(morpholin-4-ylcarbonyl)pyridin-3-yl] benzoyl}piperazin-1-yl)carbonyl]phenyl}amine (10c)

The procedure described above was used with 1.09g 4-[5-(morpholin-4-ylcarbonyl)pyridin-3-yl]benzoic acid (**6a**) and 1.22g dimethyl[3-(piperazin-1-ylcarbonyl)phenyl]amine (**7VI**). Elution with petroleum ether/ethyl acetate(1/7, v/v) gave a solid (0.39g, 21%). Mp 185-187 ℃; EI-MS (m/z) 527[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.04 (d, *J* = 9.9 Hz, 1H), 8.66 (d, *J* = 11.3 Hz, 1H), 8.19 (d, *J* = 12.8 Hz, 1H), 8.07 (d, *J* = 7.8 Hz, 1H), 7.93 (d, *J* = 7.3 Hz, 1H), 7.89 (d, *J* = 7.7 Hz, 1H), 7.58 (d, *J* = 7.4 Hz, 1H), 7.24 (s, 1H), 6.79 (d, *J* = 7.0 Hz, 1H), 6.69 (s, 1H), 6.66 (d, *J* = 6.5 Hz, 1H), 3.90-3.33 (m, 16H), 2.92 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.42, 169.27, 167.22, 150.66, 149.13, 147.63, 140.82, 136.82, 133.37, 133.15, 132.25, 130.50, 129.44, 128.41, 127.76, 114.58, 113.71, 110.81, 66.49, 48.09, 47.53, 42.53, 42.11, 40.41.

N,N-diisopropyl-5-[4-({4-[3-(trifluoromethyl)benzoyl]piperazin-1-yl}carbonyl)phenyl]nicotinamide (10d)

The procedure described above was used with 1.04g 4-{5-[(diisopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6b**) and 1.35g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with

petroleum ether/ethyl acetate(1/5, v/v) gave a solid (0.51g, 26%). Mp 161-163 ℃; EI-MS (m/z) 566[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.98 (s, 1H), 8.54 (s, 1H), 8.07 (s, 1H), 7.89 (d, *J* = 7.9 Hz, 2H), 7.85 (d, *J* = 4.0Hz, 1H), 7.80 (s, 1H), 7.75 (s, 1H), 7.72 (d, *J* = 7.9 Hz, 1H), 7.57 (d, *J* = 7.1 Hz, 2H), 3.43-3.67 (m, 10H), 1.47 (s, 6H), 1.16 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.31, 168.18, 167.59, 148.10, 145.79, 138.00, 137.20, 135.95, 135.15, 134.97, 131.49, 130.18, 128.40, 127.63, 126.81, 126.79, 125.69, 124.33, 124.26, 122.98, 51.46, 47.47, 45.58, 42.07, 20.79.

5-(4-{[4-(3,4-difluorobenzoyl)piperazin-1-yl]carbonyl}phenyl)-N,N-diisopropylnicotinamide (10e)

The procedure described above was used with 1.04g 4-{5-[(diisopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6b**) and 1.18g 1-(3,4-difluorobenzoyl)piperazine (**7III**). Elution with petroleum ether/ethyl acetate(1/5, v/v) gave a solid (0.41g, 22%). Mp 194-197 ℃; EI-MS (m/z) 534[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.98 (s, 1H), 8.54 (d, *J* = 1.8 Hz, 1H), 8.06 (s, 1H), 7.89 (d, *J* = 7.8 Hz, 2H), 7.57 (d, *J* = 8.1 Hz, 4H), 7.31-7.33 (m, 1H), 3.46-3.68 (m, 8H), 1.48 (s, 6H), 1.16 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.29, 167.59, 167.53, 148.10, 145.79, 138.01, 135.94, 135.15, 134.97, 131.50, 128.40, 127.63, 124.94, 118.38, 118.21, 117.36, 117.18, 51.57, 47.45, 45.61, 42.41, 20.79.

5-[4-({4-[3-(dimethylamino)benzoyl]piperazin-1-yl}carbonyl) phenyl]-N,N-diisopropylnicotinamide (10f)

The procedure described above was used with 1.04g 4-{5-[(diisopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6b**) and 1.22g dimethyl[3-(piperazin-1-ylcarbonyl)phenyl]amine (**7VI**). Elution with petroleum ether/ethyl acetate(1/7, v/v) gave a solid (0.38g, 20%). Mp 197-199 ℃; EI-MS (m/z) 541[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.99 (s, 1H), 8.54 (d, *J* = 1.7 Hz, 1H), 8.06 (s, 1H), 7.89 (d, *J* = 7.8 Hz, 2H), 7.57 (d, *J* = 8.0 Hz, 2H), 7.24 (t, *J* = 7.7 Hz, 1H), 6.79 (d, *J* = 7.6 Hz, 1H), 6.69 (s, 1H), 6.66 (d, *J* = 7.3 Hz, 1H), 3.79-3.37 (m, 10H), 2.92 (s, 6H), 1.47 (s, 6H), 1.16 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.37, 169.27, 167.59, 150.65, 148.10, 145.78, 137.96, 136.83, 136.01, 135.15, 134.98, 131.49, 129.43, 128.40, 127.62, 114.59, 113.70, 110.84, 51.45, 47.51, 45.59, 42.15, 40.46, 20.81.

5-(4-{[4-(2,4-dichlorobenzoyl)piperazin-1-yl]carbonyl}phenyl)-N,N-diisopropylnicotinamide (10g)

The procedure described above was used with 1.04g 4-{5-[(diisopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6b**) and 1.36g 1-(2,4-dichlorobenzoyl)piperazine (**7VII**). Elution with petroleum ether/ethyl acetate(1/5, v/v) gave a solid (0.47g, 24%). Mp 214-215 ℃; EI-MS (m/z) 566[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.98 (s, 1H), 8.54 (d, *J* = 1.8 Hz, 1H), 8.06 (s, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.76 (s, 1H), 7.58 (d, *J* = 7.6 Hz, 2H), 7.54 (d, *J* = 3.7 Hz, 1H), 7.47 (d, *J* = 8.2 Hz, 1H), 3.83-3.40 (m, 8H), 3.25 (s, 2H), 1.47 (s, 6H), 1.19 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.31, 167.59, 165.41, 148.10, 145.79, 138.00, 135.90, 135.15, 134.96, 134.88, 134.84, 131.50, 130.86, 129.90, 129.58, 128.42, 127.62, 51.49, 46.51, 45.61, 41.67, 20.80.

N-cyclopropyl-5-[4-({4-[3-(trifluoromethyl)benzoyl]piperazin-1-yl}carbonyl)phenyl]nicotinamide (10h)

The procedure described above was used with 0.99g 4-{5-[(cyclopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6c**) and 1.35g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate(1/5, v/v) gave a solid (0.46g, 25%). Mp 180-182 ℃; EI-MS (m/z) 522[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.06 (s, 1H), 8.98 (d, *J* = 1.5 Hz, 1H), 8.74 (d, *J* = 3.8 Hz, 1H), 8.45 (d, *J* = 2.0 Hz, 1H), 7.90 (d, *J* = 8.2 Hz, 2H), 7.86 (d, *J* = 7.6 Hz, 1H), 7.80 (s, 1H), 7.74 (dd, *J* = 15.9, 7.1 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 2H), 3.84-3.38 (m, 8H), 2.90 (dd, *J* = 7.3, 3.4 Hz, 1H), 0.80-0.72 (m, 2H), 0.66-0.58 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.31, 168.19, 166.24, 150.20, 148.22, 138.10, 137.20, 135.98, 134.70,

133.19, 131.49, 130.33, 130.18, 128.41, 127.61, 126.82, 126.79, 125.69, 124.30, 124.26, 122.98, 47.43, 42.19, 23.53, 6.23.

N-cyclopropyl-5-(4-{[4-(3,4-difluorobenzoyl)piperazin-1-yl] carbonyl}phenyl)nicotinamide (10i)

The procedure described above was used with 0.99g 4-{5-[(cyclopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6c**) and 1.18g 1-(3,4-difluorobenzoyl)piperazine (**7III**). Elution with petroleum ether/ethyl acetate(1/5, v/v) gave a solid (0.41g, 24%). Mp 221-222℃; EI-MS (m/z) 490[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.98 (s, 1H), 8.75 (d, J = 3.1 Hz, 1H), 8.45 (s, 1H), 7.90 (d, J = 7.7 Hz, 2H), 7.59 (d, J = 7.6 Hz, 4H), 7.39-7.28 (m, 1H), 3.44-3.66 (m, 8H), 1.21 (s, 1H), 0.75 (d, J = 6.3 Hz, 2H), 0.61 (d, J = 4.0 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.29, 167.52, 166.24, 150.20, 148.22, 138.11, 135.96, 134.69, 133.20, 130.33, 128.41, 127.61, 124.87, 118.39, 118.22, 117.36, 117.18, 47.63, 42.35, 23.53, 6.23.

N-cyclopropyl-5-[4-({4-[3-(dimethylamino)benzoyl]piperazin-1-yl}carbonyl)phenyl]nicotinamide (10j)

The procedure described above was used with 0.99g 4-{5-[(cyclopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6c**) and 1.22g dimethyl[3-(piperazin-1-ylcarbonyl)phenyl]amine (**7VI**). Elution with petroleum ether/ethyl acetate(1/7, v/v) gave a solid (0.36g, 21%). Mp 184-186℃; EI-MS (m/z) 497[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.06 (s, 1H), 8.98 (s, 1H), 8.74 (d, J = 3.8 Hz, 1H), 8.45 (s, 1H), 7.90 (d, J = 7.6 Hz, 2H), 7.59 (d, J = 8.0 Hz, 2H), 7.24 (t, J = 7.8 Hz, 1H), 6.79 (d, J = 7.4 Hz, 1H), 6.69 (s, 1H), 6.66 (d, J = 7.3 Hz, 1H), 3.46-3.66 (m, 8H), 2.92 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.39, 169.28, 166.25, 150.65, 150.20, 148.21, 138.05, 136.82, 136.03, 134.71, 133.18, 130.33, 129.43, 128.42, 127.60, 114.59, 113.70, 110.83, 47.56, 42.19, 40.45, 6.24.

N-cyclopropyl-5-(4-{[4-(2,4-dichlorobenzoyl)piperazin-1-yl] carbonyl}phenyl)nicotinamide (10k)

The procedure described above was used with 0.99g 4-{5-[(cyclopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6c**) and 1.36g 1-(2,4-dichlorobenzoyl)piperazine (**7VII**). Elution with petroleum ether/ethyl acetate(1/5, v/v) gave a solid (0.44g, 24%). Mp 183-185℃; EI-MS (m/z) 522[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 8.98 (s, 1H), 8.74 (d, J = 3.5 Hz, 1H), 8.45 (s, 1H), 7.89 (d, J = 6.3 Hz, 2H), 7.75 (s, 1H), 7.60 (d, J = 7.5 Hz, 2H), 7.58-7.51 (m, 1H), 7.47 (d, J = 8.0 Hz, 1H), 3.26-3.73 (m, 8H), 2.96-2.85 (m, 1H), 0.75 (q, J = 6.6 Hz, 2H), 0.62 (d, J = 2.4 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.31, 166.24, 165.42, 150.20, 148.23, 138.11, 135.91, 134.86, 134.69, 133.19, 130.86, 130.32, 129.89, 129.58, 128.44, 127.59, 46.65, 41.74, 23.54, 6.24.

N,N-diethyl-5-[4-({4-[3-(trifluoromethyl)benzoyl]piperazin-1-yl}carbonyl)phenyl]nicotinamide (10l)

The procedure described above was used with 1.04g 4-{5-[(diethylamino)carbonyl]pyridin-3-yl}benzoic acid (**6d**) and 1.35g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate(1/5, v/v) gave a solid (0.49g, 26%). Mp 125-127℃; EI-MS (m/z) 538[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.59 (s, 1H), 8.13 (s, 1H), 7.90 (d, J = 7.7 Hz, 2H), 7.86 (d, J = 7.8 Hz, 1H), 7.80 (s, 1H), 7.75 (s, 1H), 7.72 (d, J = 7.8 Hz, 1H), 7.57 (d, J = 7.1 Hz, 2H), 3.42-3.71 (m, 8H), 3.49 (d, J = 4.0Hz, 2H), 3.24 (d, J = 8.0Hz, 2H), 1.15 (d, J = 47.4 Hz, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.30, 168.18, 167.84, 148.50, 146.44, 137.95, 137.20, 135.97, 134.83, 133.70, 132.26, 131.49, 130.18, 128.41, 127.62, 126.82, 126.78, 125.69, 124.30, 124.26, 122.98, 47.64, 43.49, 42.13, 14.53, 13.29.

5-[4-({4-[4-chloro-3-(trifluoromethyl)benzoyl]piperazin-1-yl] carbonyl)phenyl]-*N,N*-diethylnicotinamide (10m)

The procedure described above was used with 1.04g 4-{5-[(diethylamino)carbonyl]pyridin-3-yl}benzoic acid (**6d**) and 1.50g 1-[4-chloro-3-(trifluoromethyl)benzoyl]piperazine (**7II**). Elution with petroleum ether/ethyl acetate(1/5, v/v) gave a solid (0.46g, 23%). Mp 149-151℃; EI-MS (m/z) 572[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.59 (d, J = 1.8 Hz, 1H), 8.13 (d, J = 1.6 Hz, 1H), 7.91 (s, 2H), 7.89 (s, 1H), 7.81 (dd, J = 25.5, 7.8 Hz, 2H), 7.57 (d, J = 7.9 Hz, 2H), 3.43-3.68 (m, 8H), 3.49 (d, J = 6.5 Hz, 2H), 3.24 (d, J = 6.5 Hz, 2H), 1.19 (s, 3H), 1.09 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.30, 167.84, 167.27, 148.50, 146.44, 137.96, 135.96, 135.67, 134.82, 133.70, 133.16, 132.36, 132.26, 128.40, 127.62, 127.22, 126.90, 124.37, 121.69, 118.94, 47.41, 43.48, 42.13, 41.70, 14.52, 13.27; HRMS (ESI): Calcd. for [M+Na]$^+$ C$_{29}$H$_{28}$ClF$_3$N$_4$O$_3$Na: 595.1700, found 595.1690.

5-[4-({4-[4-(dimethylamino)benzoyl]piperazin-1-yl}carbonyl) phenyl]-*N,N*-diethylnicotinamide (10n)

The procedure described above was used with 1.04g 4-{5-[(diethylamino)carbonyl]pyridin-3-yl}benzoic acid (**6d**) and 1.22g dimethyl[3-(piperazin-1-ylcarbonyl)phenyl]amine (**7VI**). Elution with petroleum ether/ethyl acetate(1/7, v/v) gave a solid (0.39g, 22%). Mp 157-158℃; EI-MS (m/z) 513[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.59 (d, J = 1.4 Hz, 1H), 8.13 (s, 1H), 7.89 (d, J = 7.7 Hz, 2H), 7.57 (d, J = 7.9 Hz, 2H), 7.24 (t, J = 7.5 Hz, 1H), 6.79 (d, J = 8.0 Hz, 1H), 6.69 (s, 1H), 6.66 (d, J = 7.3 Hz, 1H), 3.36-3.66 (m, 8H), 3.49 (d, J = 5.9 Hz, 2H), 3.24 (d, J = 5.9 Hz, 2H), 2.92 (s, 6H), 1.19 (t, J = 4.0Hz, 3H), 1.09 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.38, 169.27, 167.85, 150.65, 148.50, 146.44, 137.92, 136.84, 136.03, 134.84, 133.70, 132.27, 129.42, 128.43, 127.60, 114.61, 113.69, 110.85, 47.52, 43.50, 42.26, 40.44, 14.54, 13.29.

N-isopropyl-5-[4-({4-[3-(trifluoromethyl)benzoyl]piperazin-1-yl] carbonyl)phenyl]nicotinamide (10o)

The procedure described above was used with 1.00g 4-{5-[(isopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6e**) and 1.35g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate(1/5, v/v) gave a solid (0.49g, 27%). Mp 225-227℃; EI-MS (m/z) 524[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.06 (s, 1H), 9.01 (d, J = 1.6 Hz, 1H), 8.54 (d, J = 7.6 Hz, 1H), 8.48 (d, J = 2.0 Hz, 1H), 7.91 (d, J = 8.1 Hz, 2H), 7.86 (d, J = 7.0 Hz, 1H), 7.80 (s, 1H), 7.74 (dd, J = 16.3, 7.3 Hz, 2H), 7.60 (d, J = 7.9 Hz, 2H), 4.15 (dd, J = 13.7, 6.8 Hz, 1H), 3.84-3.39 (m, 8H), 1.22 (s, 3H), 1.20 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.33, 168.19, 164.12, 150.09, 148.38, 138.17, 137.20, 135.96, 134.68, 133.22, 131.49, 130.67, 130.18, 128.40, 127.64, 126.81, 126.78, 125.69, 124.30, 124.27, 122.98, 47.43, 42.23, 41.70, 22.76.

5-[4-({4-[4-chloro-3-(trifluoromethyl)benzoyl]piperazin-1-yl] carbonyl)phenyl]-*N*-isopropylnicotinamide (10p)

The procedure described above was used with 1.00g 4-{5-[(isopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6e**) and 1.50g 1-[4-chloro-3-(trifluoromethyl)benzoyl]piperazine (**7II**). Elution with petroleum ether/ethyl acetate(1/5, v/v) gave a solid (0.50g, 26%). Mp 162-164℃; EI-MS (m/z) 558[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.54 (d, J = 7.5 Hz, 1H), 8.48 (d, J = 1.8 Hz, 1H), 7.91 (s, 3H), 7.81 (dd, J = 24.7, 7.7 Hz, 2H), 7.60 (d, J = 8.0 Hz, 2H), 4.15 (dd, J = 13.7, 6.8 Hz, 1H), 3.44-3.69 (m, 8H), 1.22 (s, 3H), 1.20 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.33, 167.28, 164.12, 150.09, 148.38, 138.18, 135.94, 135.66, 134.67, 133.22, 133.15, 132.35, 130.66, 128.39, 127.63, 127.22, 126.92, 124.37, 121.65, 118.93, 47.45, 42.20, 41.70, 22.75; HRMS (ESI): Calcd. for [M+H]$^+$ C$_{28}$H$_{27}$ClF$_3$N$_4$O$_3$: 559.1724, found 559.1728; Calcd. for [M+Na]$^+$ C$_{28}$H$_{26}$ClF$_3$N$_4$O$_3$Na: 581.1543, found 581.1549.

5-[4-({4-[3-(dimethylamino)benzoyl]piperazin-1-yl}carbonyl) phenyl]-*N*-isopropylnicotinamide (10q)

The procedure described above was used with 1.00g 4-{5-[(isopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6e**) and 1.22g dimethyl[3-(piperazin-1-ylcarbonyl)phenyl]amine (**7VI**). Elution

with petroleum ether/ethyl acetate(1/7, v/v) gave a solid (0.37g, 21%). Mp 216-218 ℃; EI-MS (m/z) 499[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.01 (d, J = 1.7 Hz, 1H), 8.54 (d, J = 7.6 Hz, 1H), 8.48 (s, 1H), 7.91 (d, J = 7.7 Hz, 2H), 7.60 (d, J = 8.0 Hz, 2H), 7.24 (t, J = 7.5 Hz, 1H), 6.79 (d, J = 7.7 Hz, 1H), 6.69 (s, 1H), 6.66 (d, J = 7.4 Hz, 1H), 4.15 (dd, J = 13.6, 6.8 Hz, 1H), 3.79-3.40 (m, 8H), 2.92 (s, 6H), 1.22 (s, 3H), 1.20 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.39, 169.29, 164.12, 150.65, 150.10, 148.38, 138.12, 136.82, 136.01, 134.69, 133.21, 130.66, 129.43, 128.41, 127.63, 114.60, 113.70, 110.84, 47.55, 42.23, 41.70, 40.45, 22.78.

5-(4-{[4-(2,4-dichlorobenzoyl)piperazin-1-yl]carbonyl}phenyl)-*N*-isopropylnicotinamide (10r)

The procedure described above was used with 1.00g 4-{5-[(isopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6e**) and 1.36g 1-(2,4-dichlorobenzoyl)piperazine (**7VII**). Elution with petroleum ether/ethyl acetate(1/5, v/v) gave a solid (0.42g, 23%). Mp 225-227 ℃; EI-MS (m/z) 524[M]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.00 (d, J = 1.7 Hz, 1H), 8.54 (d, J = 7.6 Hz, 1H), 8.48 (s, 1H), 7.90 (d, J = 6.4 Hz, 2H), 7.75 (s, 1H), 7.60 (d, J = 7.7 Hz, 2H), 7.58-7.51 (m, 1H), 7.47 (d, J = 8.0 Hz, 1H), 4.15 (dd, J = 13.6, 6.8 Hz, 1H), 3.26-3.73 (m, 8H), 1.22 (s, 3H), 1.20 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.32, 165.42, 164.11, 150.10, 148.38, 138.17, 135.90, 134.87, 134.67, 133.22, 130.86, 130.65, 129.89, 129.58, 128.43, 127.63, 46.59, 41.70, 41.60, 22.78.

N-[2-(dimethylamino)ethyl]-5-[4-({4-[3-(trifluoromethyl)benzoyl]piperazin-1-yl}carbonyl)phenyl]nicotinamide (10s)

The procedure described above was used with 1.09g 4-[5-({[2-(dimethylamino)ethyl]amino}carbonyl)pyridin-3-yl]benzoic acid (**6f**) and 1.35g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with ethyl acetate gave a solid (0.44g, 23%). Mp 109-111 ℃; EI-MS (m/z) 554.20[M+1]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.01 (d, J = 1.8 Hz, 1H), 8.77 (s, 1H), 8.49 (s, 1H), 7.91 (d, J = 7.8 Hz, 2H), 7.86 (d, J = 7.3 Hz, 1H), 7.80 (s, 1H), 7.74 (dd, J = 16.0, 7.3 Hz, 2H), 7.60 (d, J = 7.5 Hz, 2H), 3.80-3.42 (m, 12H), 2.23 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.33, 168.20, 164.98, 150.20, 148.31, 138.10, 137.19, 135.97, 134.71, 133.23, 131.48, 130.40, 130.17, 128.41, 127.61, 126.82, 126.78, 125.69, 124.30, 124.26, 122.98, 58.42, 47.45, 45.49, 42.23, 37.73.

5-[4-({4-[4-chloro-3-(trifluoromethyl)benzoyl]piperazin-1-yl}carbonyl)phenyl]-*N*-[2-(dimethylamino)ethyl]nicotinamide (10t)

The procedure described above was used with 1.09g 4-[5-({[2-(dimethylamino)ethyl]amino}carbonyl)pyridin-3-yl]benzoic acid (**6f**) and 1.50g 1-[4-chloro-3-(trifluoro methyl)benzoyl]piperazine (**7II**). Elution with ethyl acetate gave a solid (0.49g, 24%). Mp 133-135 ℃; EI-MS (m/z) 588.10[M+1]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.01 (s, 1H), 8.81 (s, 1H), 8.50 (s, 1H), 7.91 (s, 3H), 7.81 (dd, J = 24.5, 7.0 Hz, 2H), 7.60 (d, J = 7.3 Hz, 2H), 3.44-3.70 (m, 8H), 3.44 (t, J = 6.0 Hz, 4H), 2.26 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.32, 167.28, 165.00, 150.20, 148.33, 138.11, 135.95, 135.65, 134.68, 133.25, 133.15, 132.36, 130.36, 128.40, 127.61, 127.22, 126.91, 124.36, 121.65, 118.92, 58.31, 47.42, 45.35, 42.13, 37.60.

5-(4-{[4-(2,4-dichlorobenzoyl)piperazin-1-yl]carbonyl}phenyl)-*N*-[2-(dimethylamino)ethyl]nicotinamide (10u)

The procedure described above was used with 1.09g 4-[5-({[2-(dimethylamino)ethyl]amino}carbonyl)pyridin-3-yl]benzoic acid (**6f**) and 1.36g 1-(2,4-dichlorobenzoyl)piperazine (**7VII**). Elution with ethyl acetate gave a solid (0.42g, 22%). Mp 114-116 ℃; EI-MS (m/z) 554.10[M+1]$^+$; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.01 (d, J = 1.6 Hz, 1H), 8.76 (d, J = 5.1 Hz, 1H), 8.49 (s, 1H), 7.90 (d, J = 6.3 Hz, 2H), 7.75 (s, 1H), 7.60 (d, J = 7.7 Hz, 2H), 7.58-7.52 (m, 1H), 7.47 (d, J = 8.1 Hz, 1H), 3.57-3.73 (m, 8H), 3.26 (s, 4H), 2.22 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.33, 165.43, 165.04, 150.21, 148.35, 138.10, 135.89, 134.85, 134.68, 133.28, 130.86, 130.32, 129.89, 129.56, 128.44, 127.60, 58.18, 46.58, 45.20, 41.69, 37.44.

MTT assay *in vitro*

The cytotoxic activity of compounds **8a-8l**, **9a-9i**, and **10a-10u** were evaluated against K562 cell lines by the standard MTT assay *in vitro*, with Imatinib as the positive controls. The cancer cell lines were cultured in RPMI 1640 medium with 10% fetal bovine serum (FBS). Approximate 2.5×10^3 cells, suspended in RPMI 1640 medium, were plated into each well of a 96-well plate and incubated in 5% CO$_2$ at 37 ℃ for 24 h. 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 0.5 mg/mL, and incubated with cells at 37 ℃ for 4 h. After the supernatant was discarded, 150 μL DMSO was added to each well, and the absorbance values were determined by a microplate reader (Bio-Rad, Hercules, CA, USA) at 490 nm.

In vitro enzymatic assays

The *in vitro* Bcr-Abl (wild-type and T315I) inhibition assays of compounds **8a-8l**, **9a-9i**, and **10a-10u** and selective profile assays were evaluated by the ADP-Glo™ kinase assay (Promega, Madison, WI), with Imatinib as the positive controls. General procedures are as the following: Kinases (4ng/μl) were incubated with substrates (0.2 μg/μl), compounds (1.2×10^{-4} - 12 μM) and ATP (25 μM) in a final buffer of Tris 40 mM, MgCl$_2$ 10 mM, BSA 0.1 mg/mL, DTT 1 mM in 384-well plate with the total volume of 5 μL. The assay plate was incubated at 30 ℃ for 1 h. After the plate cooled for 5 min at room temperature, 5 μL of ADP-Glo reagent was added into each well to stop the reaction and consume the remaining ADP within 40 min. At the end, 10 μL of kinase detection reagent was added into the well and incubated for 30 min to produce a luminescence signal. The luminescence was read by VICTOR X multilabel plate reader. The signal was correlated with the amount of ATP present in the reaction and was inversely correlated with the kinase activity.

Acknowledgements

Notes and references

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Electronic Supplementary Information (ESI) available: Melting points, Mass spectrometry, ^{1}H NMR spectrum, ^{13}C NMR spectrum and HRMS of key Intermediates and title Compounds **8a-8l, 9a-9i,** and **10a-10u**. See DOI: 10.1039/b000000x/

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