Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2015, **13**, 7050

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

Xiaoyan Pan, Jinyun Dong, Yaling Shi, Ruili Shao, Fen Wei, Jinfeng Wang and Jie Zhang*

Received 3rd March 2015,
Accepted 20th May 2015

DOI: 10.1039/c5ob00430f

www.rsc.org/obc

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**, and **10u** were potent Bcr-Abl inhibitors with IC_{50} values at the 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) resulting from the reciprocal translocation between chromosomes 9 and 22.[1] This translocation creates a 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]

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 the 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 a DFG (Asp-Phe-Gly)-out conformation.[10,11] According to pharmacophore features, binding sites of Imatinib could be divided into three subregions (Fig. 1),[12] two hydrophobicity regions, the adenine pocket (including the hinge region) where the adenine ring of ATP occupied the DFG-out pocket (also known as the "allosteric site") and the linker that allowed the inhibitor to access to the allosteric site (surrounded by the DFG motif and gatekeeper residue). Based on the structural analysis of Imatinib, we initiated the development of potent Bcr-Abl inhibitors with new chemotypes.

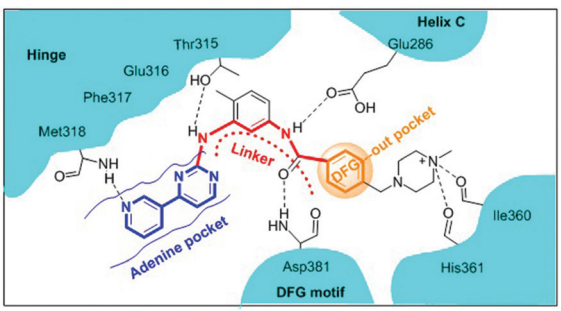


Fig. 1 Interactions between Imatinib and Bc-Abl tyrosine kinase.

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 (Fig. 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 the hinge region, heterocyclic and phenyl rings were introduced to replace pyridine and pyrimidine rings of Imati-

School of Pharmacy, Health Science Center, Xi'an Jiaotong University, No. 76, Yanta West Road, Xi'an, Shaanxi Province 710061, P.R. China.
E-mail: zhj8623@mail.xjtu.edu.cn

†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/c5ob00430f

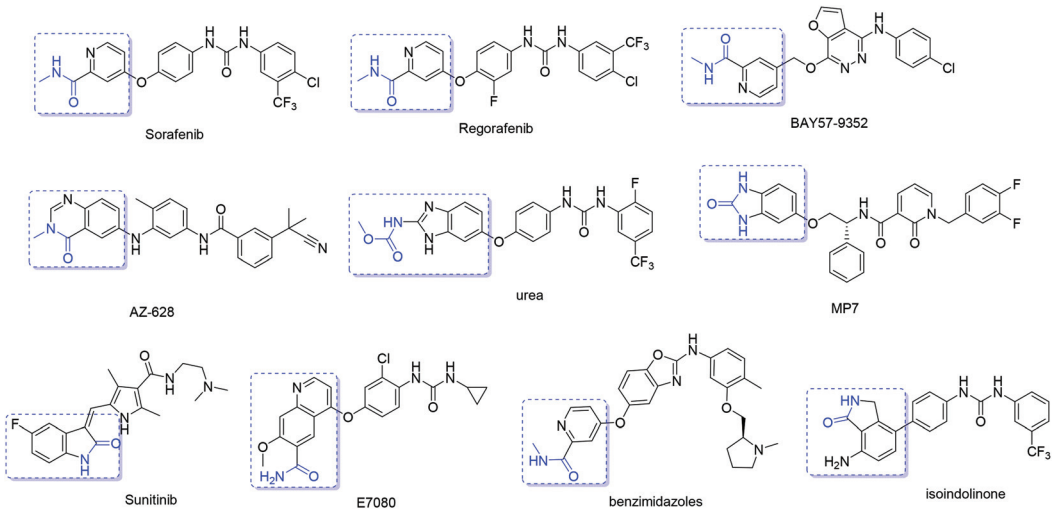


Fig. 2 Tyrosine kinase inhibitors containing an amide group.

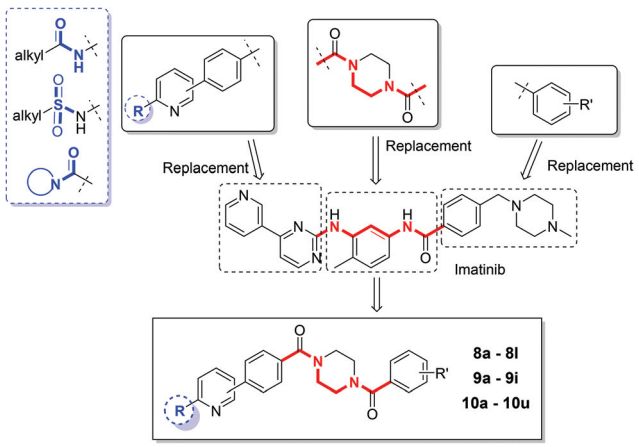


Fig. 3 Design strategy and structures of title compounds.

nib (Fig. 3). For the 'linker', it was found that there was a six-atom linker of Imatinib (colored in red) between the adenine pocket and the DFG-out pocket. Based on 'six-atom regulation', diacylated piperazine was introduced as a flexible linker (Fig. 3). Firstly, diacylated piperazine can retain the amide forming hydrogen bonds with the DFG-motif. Secondly, the highly flexible piperazine might avoid space clash with the bulky group of gatekeeper residue. Moreover, various substituted 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.

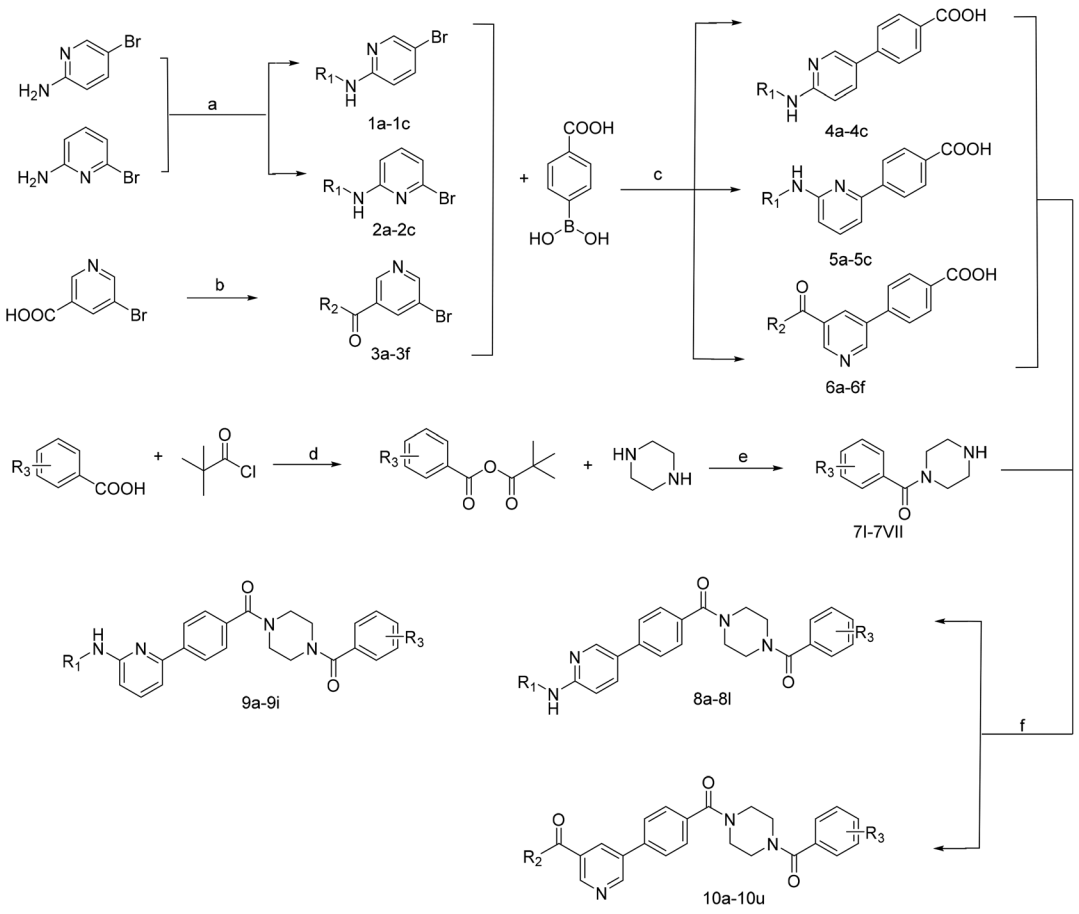
Results and discussion

Chemistry

The synthetic route of title compounds (series **8**, **9** and **10**) is 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, the 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 the 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 the 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.

In vitro enzymatic assays

The biological evaluation of all the title compounds was preliminarily performed with Imatinib as the positive control.[32,33] The results are summarized in Tables 1 and 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



1a: R_1=acetyl
1b: R_1=pivaloyl
1c: R_1=methanesulfonyl

2a: R_1=acetyl
2b: R_1=pivaloyl
2c: R_1=methanesulfonyl

3a: R_2=morpholine
3b: R_2=N,N-diisopropylamino
3c: R_2=N-cyclopropylamino
3d: R_2=N,N- diethylamino
3e: R_2=N-isopropylamino
3f: R_2=N-[2-(dimethylamino)ethyl]amino

7I: R_3=3-CF_3
7II: R_3=3-CF_3-4-Cl
7III: R_3=3,4-*di*-F
7IV: R_3= 3-OCH_3
7V: R_3=4-$C(CH_3)_3$
7VI: R_3=3-$N(CH_3)_2$
7VII: R_3=2,4-*di*-Cl

8a: R_1=acetyl , R_3=3-CF_3
8b: R_1=acetyl , R_3=3-CF_3-4-Cl
8c: R_1=acetyl , R_3=3,4-*di*-F
8d: R_1=acetyl , R_3=3-OCH_3
8e: R_1=pivaloyl , R_3=3-CF_3
8f: R_1=pivaloyl , R_3=3-CF_3-4-Cl
8g: R_1=pivaloyl , R_3=3,4-*di*-F
8h: R_1=pivaloyl , R_3=3-OCH_3
8i: R_1=methanesulfonyl , R_3=3-CF_3
8j: R_1=methanesulfonyl , R_3=3-CF_3-4-Cl
8k: R_1=methanesulfonyl , R_3=3,4-*di*-F
8l: R_1=methanesulfonyl , R_3=3-OCH_3

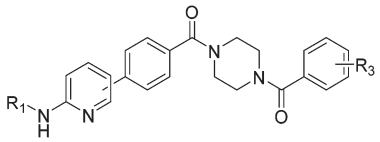
9a: R_1=acetyl , R_3=3-CF_3
9b: R_1=acetyl , R_3=3-CF_3-4-Cl
9c: R_1=acetyl , R_3=4-$C(CH_3)_3$
9d: R_1=pivaloyl , R_3=3-CF_3
9e: R_1=pivaloyl , R_3=3-CF_3-4-Cl
9f: R_1=pivaloyl , R_3=4-$C(CH_3)_3$
9g: R_1=methanesulfonyl , R_3=3-CF_3
9h: R_1=methanesulfonyl , R_3=3-CF_3-4-Cl
9i: R_1=methanesulfonyl , R_3=4-$C(CH_3)_3$

10a: R_2=morpholine , R_3=3-CF_3
10b: R_2=morpholine , R_3=3,4-*di*-F
10c: R_2=morpholine , R_3=3-$N(CH_3)_2$
10d: R_2=N,N-diisopropylamino , R_3=3-CF_3
10e: R_2=N,N-diisopropylamino , R_3=3,4-*di*-F
10f: R_2=N,N-diisopropylamino , R_3=3-$N(CH_3)_2$
10g: R_2=N,N-diisopropylamino , R_3=2,4-*di*-Cl
10h: R_2=N-cyclopropylamino , R_3=3-CF_3
10i: R_2=N-cyclopropylamino , R_3=3,4-*di*-F
10j: R_2=N-cyclopropylamino , R_3=3-$N(CH_3)_2$
10k: R_2=N-cyclopropylamino , R_3=2,4-*di*-Cl
10l: R_2=N,N- diethylamino , R_3=3-CF_3
10m: R_2=N,N- diethylamino , R_3=3-CF_3-4-Cl
10n: R_2=N,N- diethylamino , R_3=3-$N(CH_3)_2$
10o: R_2=N-isopropylamino , R_3=3-CF_3
10p: R_2=N-isopropylamino , R_3=3-CF_3-4-Cl
10q: R_2=N-isopropylamino , R_3=3-$N(CH_3)_2$
10r: R_2=N-isopropylamino , R_3=2,4-*di*-Cl
10s: R_2=N-[2-(dimethylamino)ethyl]amino , R_3=3-CF_3
10t: R_2=N-[2-(dimethylamino)ethyl]amino , R_3=3-CF_3-4-Cl
10u: R_2=N-[2-(dimethylamino)ethyl]amino , R_3=2,4-*di*-Cl

Scheme 1 Reagents and conditions: (a) acyl chloride, Et_3N, CH_2Cl_2, 0 °C → r.t.; (b) $SOCl_2$, reflux; amine, CH_2Cl_2, 0 °C → r.t.; (c) $Pd(PPh_3)_4$, Cs_2CO_3, CH_3CN/H_2O(v : v = 3 : 2), 100 °C; (d) Et_3N, CH_2Cl_2, r.t.; (e) EtOH, r.t.; (f) ClCOO-iBu, NMM, THF, 0 °C; **7I–7VII**, r.t.

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 the sub-micromolar level.

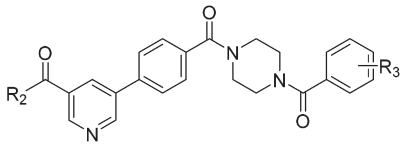
Table 1 Structures and activities of **8a–8l** and **9a–9i** towards Bcr-Abl and K562 cells *in vitro*



	Pyridine	R_1	R_3	Abl IC_{50} [a] (μM)	K562 IC_{50} [b] (μM)
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%.

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



	R_2	R_3	Abl IC_{50} [a] (μM)	K562 IC_{50} [b] (μM)
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%.

As illustrated in Table 1, the majority of series **8** were more potent than series **9**. The results indicated that the amide group was better at the *para*-position than at the *meta*-position for Bcr-Abl inhibition. Compounds **8d**, **8h** and **8l** displayed the highest activity which suggested that the *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** are summarized in Table 2. Most of them exhibited decreased Bcr-Abl inhibition compared with series **8** and **9**. Compounds with a bulky group (**10a**, **10b**, **10e**, **10f**, **10i** and **10j**) showed less enzymatic inhibitory potency compared to those (**10l–10r**) with small groups. The results indicated that a bulky substituent was unfavourable for the activity. Compounds **10s–10u** were more potent than the others which suggested that the incorporation of a slender side chain was in favor of improving the potency. In addition, the substituents at the 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 the 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. Their inhibitory ratios 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.

In vitro antiproliferative activity assays

All the title compounds were evaluated for their antiproliferative activity against Bcr-Abl positive leukemia cells (K562) with Imatinib as the positive control.[34] The bioactivity data are presented in Tables 1 and 2. All the compounds showed moderate to significant activity against K562 cells with IC_{50} values

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

	(% Inhibition at 1.2 μM)	
	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

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. 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 the pivaloyl group was more beneficial for bioactivity than acetyl or methanesulfonyl. We speculated that the introduction of a 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 be attributed to their poor capability to pass through the cell membrane. In addition, it was found that the substituents on the terminal phenyl ring had a slight influence on activity.

The bioactivity data of series **10** are summarized in Table 2. Most compounds showed decreased 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 a slender side chain was favourable. In addition, the substituents at the terminal phenyl ring also affected the activity. Compounds **10f**, **10j** and **10n** with dimethylamino substituents were less potent than those with halogen substituents (**10a**, **10d**, **10g**, **10k**, **10p**, **10t**).

Table 4 Kinase selectivity profile of select compounds

	(% Inhibition at 1.2 μM)			
	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

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 are 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 in **9e** and **9f**.

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) were imported,[35] and its ligand Imatinib was used to define the binding cavity and generate the promotal. The binding modes are shown in Fig. 4 and 5.

As shown in Fig. 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 mole-

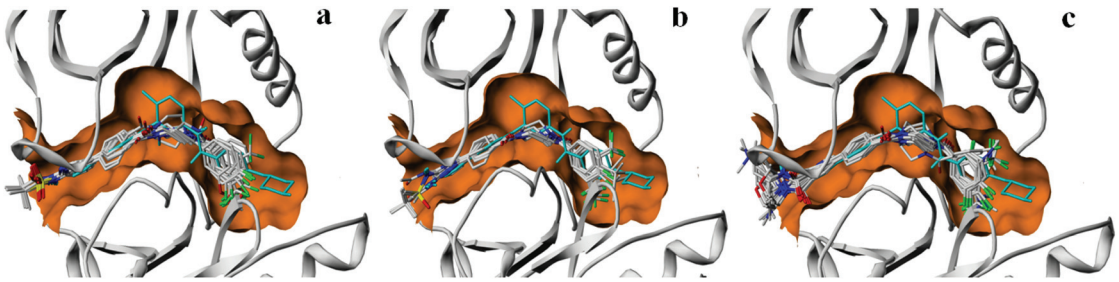


Fig. 4 The predicted binding modes of series **8** (a), **9** (b), **10** (c) with Bcr-Abl. Imatinib is colored in cyan.

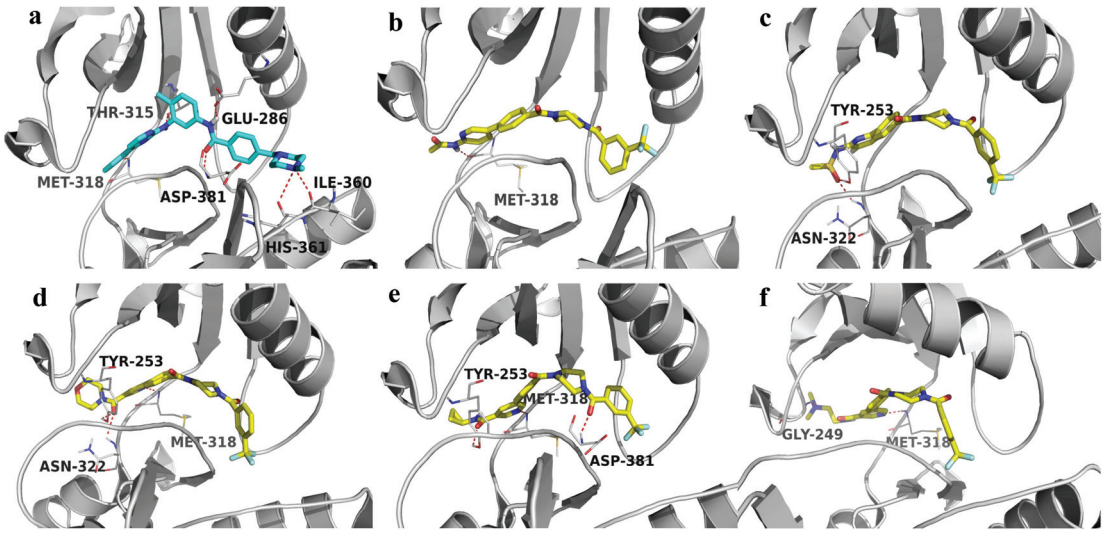


Fig. 5 The predicted binding modes of Imatinib (a), **8a** (b), **9a** (c), **10a** (d), **10h** (e) and **10s** (f) with Bcr-Abl.

cule 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 mode similar to that of Imatinib. Their pyridine and phenyl rings overlap well with the pyridine and pyrimidine rings of Imatinib, as well as the terminal phenyl ring. Diacylated piperazine, the six-atom linker, smoothly passes through the narrow cavity surrounded by the DFG motif and gatekeeper residue. Compared with rigid methyl benzene, highly flexible piperazine avoids the clash with gatekeeper residue which often occurs in kinase resistance mutations.

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

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 Fig. 6, Pantinib fitted well in the ATP pocket of T315I-Abl. However, the increased bulk of the Ile 315 side chain caused steric repulsion, thereby blocking 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 the receptor. The

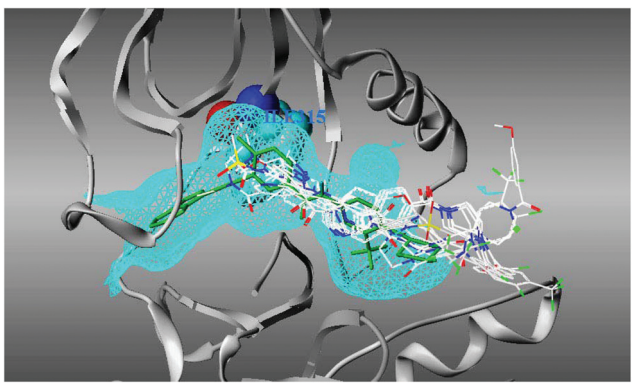


Fig. 6 The predicted binding modes of select compounds with Bcr-Abl (T315I). Pantinib is colored in green. Residue Ile315 is shown in space fill model.

docking results were consistent with the inhibitory activity against Bcr-Abl^{T315I}.

QSAR studies. 3D-QSAR studies were performed with the 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: $pIC_{50} = -\log IC_{50}$. The conformations of training sets generated from the docking study were used. Based on the docking results, the template molecule **8a** was taken and the others were aligned to it by the DATABASE ALIGNMENT method (Fig. 7).

The steric and electrostatic fields were calculated at each lattice intersection of a regularly spaced grid of 2.0 Å in all three dimensions within a defined region. An sp^3 carbon atom with +1.00 charge was used as a probe atom. The steric and

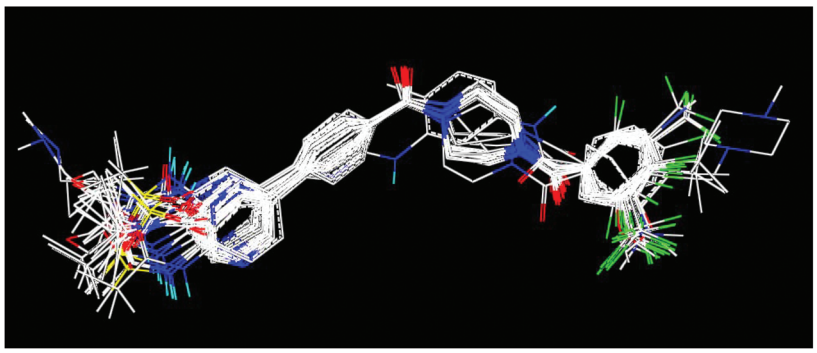


Fig. 7 Superposition of 34 Bcr-Abl inhibitors for CoMFA construction.

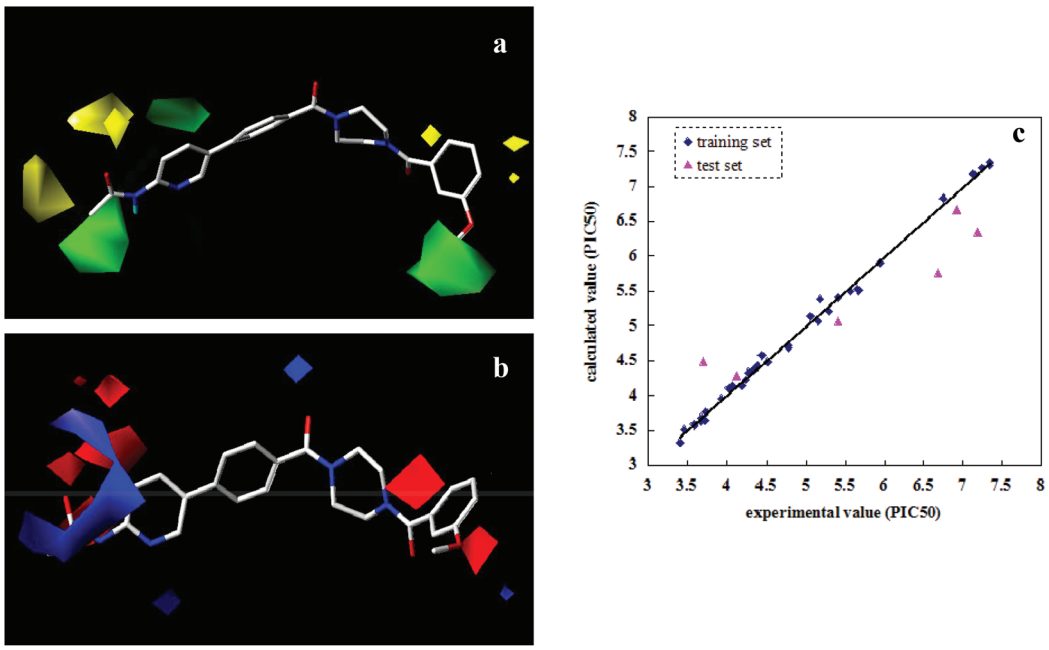


Fig. 8 Contour plot and the predictability of the CoMFA model. (a) and (b) show the steric and electrostatic contour maps of the CoMFA model. Green contours indicate regions where bulky groups increase activity, whereas yellow contours indicate regions where bulky groups decrease activity. Blue contours indicate regions where positive groups increase activity, whereas red contours indicate regions where negative charge increases activity. (c) shows the correlations between the experimental and the predicted activities for the training and test sets for the optimal CoMFA.

electrostatic fields were truncated at +30.00 kcal mol^{-1}, and the electrostatic fields were ignored at the lattice points with maximal steric interactions. The PLS method was used to linearly correlate CoMFA fields with activity values.[37–39] The cross-validation analysis was performed using the leave-one-out (LOO) method.[40] The cross-validated q^2 that resulted in optimum number of components and lowest standard error of prediction was considered for further analysis. We have evaluated different filter values σ at least selected σ as 2.00 kcal mol^{-1} 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 fields is 61.8% and 38.2%, respectively.

To visualize the information content of the derived 3D-QSAR model, CoMFA contour maps were generated. Fig. 8a and b show the steric and electrostatic contour maps of the CoMFA models. The steric plots indicate that it is feasible and beneficial to introduce a bulky side chain into the pyridine ring, and the introduction of a bulky group on position 3 of the terminal phenyl ring may favor the inhibition activity of these compounds. The electrostatic plot suggests that the electronic plot is consistent with the structure occupying the

adenine pocket of the target compounds. N atoms are near the blue moieties, and O atoms are near the red moieties. The introduction of a 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 Fig. 8c, the CoMFA model can predict test set compounds well.

Conclusion

In summary, we have developed forty-two compounds with flexible diacylated piperazine linkers 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** was comparable with that of Imatinib, and compounds **8e**, **10q**, **10s**, **10u** were also potent with IC_{50} values at the 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 a similar binding mode with Imatinib. The six-atom linker, diacylated piperazine, smoothly passes through the narrow cavity surrounded by the DFG motif and gatekeeper residue, and the side chain containing an amide group can form H-bond interactions with the hinge region. The 3D-QSAR research is instructive for further research. It is feasible and beneficial to introduce a side chain containing N and O atoms into the pyridine ring, as well as to introduce a bulky group on position 3 of the terminal 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 correction. ^{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 for those in aqueous media were carried out by standard techniques for moisture exclusion. Anhydrous reactions were carried out in dried glassware under a nitrogen atmosphere. The boiling range for petroleum ether is 60–90 °C.

General procedure: compounds (1a–1c and 2a–2c)

5(6)-Bromopyridin-2-amine (5.19 g, 30 mmol) and Et_3N (20 mL) were dissolved in anhydrous CH_2Cl_2 (100 mL), cooled to 0 °C, followed by dropwise addition of the appropriate acyl chloride (36 mmol). The resulting mixture was warmed to room temperature and stirred overnight. The reaction mixture was diluted with CH_2Cl_2, and the organic layer was washed with water (30 mL × 3), $NaHCO_3$ (30 mL × 3), and NaCl solution (30 mL). Then the organic phase was dried using Na_2SO_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 2.54 mL acetyl chloride. Elution with petroleum ether/ethyl acetate (5/1, v/v) gave a white solid (5.65 g, 88%). Mp 78–81 °C; EI-MS (*m*/*z*) 214[M]$^+$.

***N*-(5-Bromopyridin-2-yl)-2,2-dimethylpropanamide (1b).** The procedure described above was used with 4.43 mL pivaloyl chloride. Elution with petroleum ether gave a white solid (7.37g, 96%). Mp 42–44 °C; EI-MS (*m*/*z*) 256[M]$^+$.

***N*-(5-Bromopyridin-2-yl)methanesulfonamide (1c).** The procedure described above was used with 2.78 mL methanesulfonylchloride. Elution with petroleum ether/ethyl acetate (1/1, v/v) gave a white solid (6.75 g, 90%). Mp 161–163 °C; EI-MS (*m*/*z*) 250[M]$^+$.

***N*-(6-Bromopyridin-2-yl)acetamide (2a).** The procedure described above was used with 2.54 mL acetyl chloride. Elution with petroleum ether/ethyl acetate (5/1, v/v) gave a white solid (5.52 g, 86%). Mp 159–160 °C; EI-MS (*m*/*z*) 214[M]$^+$.

***N*-(6-Bromopyridin-2-yl)-2,2-dimethylpropanamide (2b).** The procedure described above was used with 4.43 mL pivaloyl chloride. Elution with petroleum ether gave a white solid (7.29 g, 95%). Mp 90–91 °C; EI-MS (*m*/*z*) 256[M]$^+$.

***N*-(6-Bromopyridin-2-yl)methanesulfonamide (2c).** The procedure described above was used with 2.78 mL methanesulfonylchloride. Elution with petroleum ether/ethyl acetate (1/1, v/v) gave a white solid (6.30 g, 84%). Mp 179–180 °C; EI-MS (*m*/*z*) 250[M]$^+$.

General procedure: compounds (3a–3f)

Sulfoxide chloride (36 mL, 494 mmol) was added dropwise at room temperature under N_2 to solid 5-bromonicotinic acid (5.00 g, 24.7 mmol). The resulting mixture was refluxed for 2 h and the volatiles were removed *in vacuo*. The crude acid chloride was dissolved in anhydrous CH_2Cl_2 (30 mL) and the solution was added slowly at 0 °C to a solution of the corresponding amine (54.4 mmol) in CH_2Cl_2 (30 mL). Stirring was continued overnight. Aqueous K_2CO_3 (2 M, 20 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (15 mL × 3). The combined organic extract was dried using Na_2SO_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 4.74 mL morpholine. Elution with petroleum ether/ethyl acetate (1/1, v/v) gave a white solid (5.55 g, 83%). Mp 76–78 °C; EI-MS (*m*/*z*) 271[M]$^+$.

5-Bromo-*N*,*N*-diisopropylnicotinamide(3b). The procedure described above was used with 7.67 mL diisopropylamine. Elution with petroleum ether/ethyl acetate (1/1, v/v) gave a white solid (6.03 g, 86%). Mp 90–91 °C; EI-MS (*m*/*z*) 284[M]$^+$.

5-Bromo-*N*-cyclopropylnicotinamide(3c). The procedure described above was used with 3.77 mL cyclopropylamine. Elution with petroleum ether/ethyl acetate (1/1, v/v) gave a white solid (5.27 g, 89%). Mp 140–142 °C; EI-MS (*m*/*z*) 240[M]$^+$.

5-Bromo-*N*,*N*-diethylnicotinamide(3d). The procedure described above was used with 5.60 mL diethylamine. Elution with petroleum ether/ethyl acetate (1/1, v/v) gave an oil (5.50 g, 87%). EI-MS (*m*/*z*) 256[M]$^+$.

5-Bromo-*N*-isopropylnicotinamide(3e). The procedure described above was used with 4.67 mL isopropylamine. Elution with petroleum ether/ethyl acetate (1/1, v/v) gave a white solid (5.26 g, 88%). Mp 105–106 °C; EI-MS (*m*/*z*) 242[M]$^+$.

5-Bromo-*N*-[2-(dimethylamino)ethyl]nicotinamide(3f). The procedure described above was used with 4.80 mL *N*,*N*-dimethyl-1,2-ethanediamine. Elution with ethyl acetate gave an oil (5.35 g, 80%). EI-MS (*m*/*z*) 271[M]$^+$.

General procedure: compounds 4a–4c, 5a–5c, and 6a–6f

Bromo-substituted aromatic heterocyclic amide (20 mmol), 4-carboxyphenylboronic acid (**2**) (3.66 g, 22 mmol), Cs_2CO_3 (13.0 g, 40 mmol), and $Pd(PPh_3)_4$ (1.2 g, 1 mmol) were suspended in a mixture of CH_3CN/H_2O (200 mL, v : v = 3 : 2) under N_2. The mixture was heated in an oil bath at 90 °C for 48 h. The mixture was filtered immediately after the reaction finished. The filtrate was adjusted to about pH 4 using HCl solution (6 mol L^{-1}). The precipitate was filtered out and dried under vacuum overnight to give a white solid.

4-[6-(Acetylamino)pyridin-3-yl]benzoic acid (4a). The procedure described above was used with 4.30 g *N*-(5-bromopyridin-2-yl)acetamide (**1a**), giving a solid (3.89 g, 76%). Mp 156–158 °C; EI-MS (*m*/*z*) 256[M]$^+$; ^{1}H NMR (400 MHz, 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 5.14 g *N*-(5-bromopyridin-2-yl)-2,2-dimethylpropanamide, giving a solid (4.77 g, 80%). Mp 276–277 °C; EI-MS (*m*/*z*) 298[M]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) 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 5.02 g *N*-(5-bromopyridin-2-yl)methanesulfonamide (**1c**), giving a solid (4.32 g, 74%). Mp 294–295 °C; EI-MS (*m*/*z*) 292[M]$^+$; ^{1}H NMR (400 MHz, 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 4.30 g *N*-(6-bromopyridin-2-yl)acetamide (**2a**), giving a solid (3.99 g, 78%). Mp 317–318 °C; EI-MS (*m*/*z*) 256[M]$^+$; ^{1}H NMR (400 MHz, 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 5.14 g *N*-(6-bromopyridin-2-yl)-2,2-dimethylpropanamide (**2b**), giving a solid (4.95 g, 83%). Mp 266–268 °C; EI-MS (*m*/*z*) 298[M]$^+$; ^{1}H NMR (400 MHz, 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 5.02 g *N*-(6-bromopyridin-2-yl)methanesulfonamide (**2c**), giving a solid (4.20 g, 72%). Mp 280–281 °C; EI-MS (*m*/*z*) 292[M]$^+$; ^{1}H NMR (400 MHz, 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 5.42 g 4-[(5-bromopyridin-3-yl)carbonyl]morpholine (**3a**), giving a solid (4.99 g, 80%). Mp 216–218 °C; EI-MS (*m*/*z*) 312[M]$^+$; ^{1}H NMR (400 MHz, 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 5.14 g 5-bromo-*N*,*N*-diisopropylnicotinamide (**3b**), giving a solid (5.28 g, 81%). Mp 74–76 °C; EI-MS (*m*/*z*) 326[M]$^+$; ^{1}H NMR (400 MHz, 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 4.82 g 5-bromo-*N*-cyclopropylnicotinamide (**3c**), giving a solid (4.79 g, 85%). Mp 258–260 °C; EI-MS (*m*/*z*) 282[M]$^+$; ^{1}H NMR (400 MHz, 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 5.12 g 5-bromo-*N*,*N*-diethylnicotinamide (**3d**), giving a solid (4.53 g, 76%). Mp 235–236 °C; EI-MS (*m*/*z*) 298[M]$^+$; ^{1}H NMR (400 MHz, 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 4.86 g 5-bromo-*N*-isopropylnicotinamide (**3e**), giving a solid (4.43 g, 78%). Mp 290–291 °C; EI-MS (*m*/*z*) 284[M]$^+$; ^{1}H NMR (400 MHz, 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 5.44 g 5-bromo-*N*-[2-(dimethylamino)ethyl]nicotinamide (**3f**), giving a solid (4.38 g, 70%). Mp 160–162 °C; EI-MS (*m*/*z*) 313.95[M + H]$^+$; ^{1}H NMR (400 MHz, 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 (2.42 mL, 20 mmol) and Et_3N (4.60 mL, 30 mmol) were added to a mixture of the corresponding benzoic acid (20 mmol) in CH_2Cl_2 (80 mL). The resulting mixture was stirred at r.t. until a clear solution was observed. A solution of piperazine (3.44 g, 40 mmol) in EtOH (80 mL) was added, and the mixture was further stirred for 3–4 h. Concentrated HCl (4 mL) was added and the resulting mixture was extracted with CH_2Cl_2 (30 mL × 2). The CH_2Cl_2 layer was discarded. NaOH (8 g) was added to the aqueous solution and then extracted with CH_2Cl_2 (150 mL). The organic extract was further washed with H_2O (50 mL) and then dried using Na_2SO_4. 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 100 mL flask, compounds **4a–4c**, **5a–5c**, or **6a–6f** (3.5 mmol) and 4-methylmorpholine (1.2 mL, 10.5 mmol) were dissolved in THF (20 mL). Under 0 °C, the THF (8 mL) solution of isobutyl chloroformate (0.7 mL, 5.25 mmol) was dropped slowly into the above suspension. Then the mixture was reacted under 0 °C for 1 h.

After that, the THF (15 mL) solution of compounds **7I–7VII** (5.25 mmol) and 4-methylmorpholine (1.2 mL, 10.5 mmol) 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 CH_2Cl_2 (50 mL). The organic phase was washed with water (15 mL × 3), saturated $NaHCO_3$ solution (10 mL × 2), and NaCl solution (10 mL). Then the organic phase was dried using Na_2SO_4, 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 0.90 g 4-[6-(acetylamino)pyridin-3-yl]-benzoic acid (**4a**) and 1.35 g 1-[3-(trifluoromethyl)benzoyl]-piperazine (**7I**). Elution with petroleum ether/ethyl acetate (1/3, v/v) gave a solid (0.49 g, 28%). Mp 224–226 °C; EI-MS (*m*/*z*) 496 [M]$^+$; ^{1}H NMR (400 MHz, 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); ^{13}C 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 0.90 g 4-[6-(acetylamino)-pyridin-3-yl]benzoic acid (**4a**) and 1.50 g 1-[4-chloro-3-(trifluoromethyl)benzoyl]piperazine (**7II**). Elution with petroleum ether/ethyl acetate (1/3, v/v) gave a solid (0.54 g, 29%). Mp 241–242 °C; EI-MS (*m*/*z*) 530[M]$^+$; ^{1}H NMR (400 MHz, 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); ^{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; ^{13}C NMR (101 MHz, DMSO-*d*6) 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 0.90 g 4-[6-(acetylamino)pyridin-3-yl]benzoic acid (**4a**) and 1.18 g 1-(3,4-difluorobenzoyl)piperazine (**7III**). Elution with petroleum ether/ethyl acetate (1/3, v/v) gave a solid (0.36 g, 22%). Mp 263–266 °C; EI-MS (*m*/*z*) 464[M]$^+$; ^{1}H NMR (400 MHz, 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 0.90 g 4-[6-(acetylamino)pyridin-3-yl]benzoic acid (**4a**) and 1.16 g 1-(3-methoxybenzoyl)piperazine (**7IV**). Elution with petroleum ether/ethyl acetate (1/3, v/v) gave a solid (0.40 g, 25%). Mp 207–209 °C; EI-MS (*m*/*z*) 458[M]$^+$; ^{1}H NMR (400 MHz, 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]$^+$ $C_{26}H_{27}N_4O_4$: 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 1.04 g 4-{6-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl}benzoic acid (**4b**) and 1.35 g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate (1/1, v/v) gave a solid (0.51 g, 27%). Mp 190–193 °C; EI-MS (*m*/*z*) 538[M]$^+$; ^{1}H NMR (400 MHz, 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 1.04 g 4-{6-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl}benzoic acid (**4b**) and 1.50 g 1-[4-chloro-3-(trifluoromethyl)benzoyl]piperazine (**7II**). Elution with petroleum ether/ethyl acetate (1/1, v/v) gave a solid (0.60 g, 30%). Mp 194–196 °C; EI-MS (*m*/*z*) 572[M]$^+$; ^{1}H NMR (400 MHz, 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 1.04 g 4-{6-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl}benzoic acid (**4b**) and 1.18 g 1-(3,4-difluorobenzoyl)piperazine (**7III**). Elution with petroleum ether/ethyl acetate (1/1, v/v) gave a solid (0.42 g, 24%). Mp 191–193 °C; EI-MS (*m*/*z*) 506[M]$^+$; ^{1}H NMR (400 MHz, 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 1.04 g 4-{6-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl}benzoic acid (**4b**) and 1.16 g 1-(3-methoxybenzoyl)piperazine (**7IV**). Elution with petroleum ether/ethyl acetate (1/1, v/v) gave a solid (0.45 g, 26%). Mp 176–177 °C; EI-MS (*m*/*z*) 500[M]$^+$; ^{1}H NMR (400 MHz, 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]$^+$ $C_{29}H_{33}N_4O_4$: 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 1.02 g 4-{6-[(methylsulfonyl)-amino]pyridin-3-yl}benzoic acid (**4c**) and 1.35 g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate (1/7, v/v) gave a solid (0.43 g, 23%). Mp 261–263 °C; EI-MS (*m*/*z*) 532[M]$^+$; ^{1}H NMR (400 MHz, 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 1.02 g 4-{6-[(methylsulfonyl)amino]pyridin-3-yl}benzoic acid (**4c**) and 1.50 g 1-[4-chloro-3-(trifluoromethyl)benzoyl]piperazine (**7II**). Elution with petroleum ether/ethyl acetate (1/7, v/v) gave a solid (0.42 g, 21%). Mp 247–250 °C; EI-MS (*m*/*z*) 566[M]$^+$; ^{1}H NMR (400 MHz, 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 1.02 g 4-{6-[(methylsulfonyl)-amino]pyridin-3-yl}benzoic acid (**4c**) and 1.18 g 1-(3,4-difluorobenzoyl)piperazine (**7III**). Elution with petroleum ether/ethyl acetate (1/1, v/v) gave a solid (0.38 g, 22%). Mp 270–272 °C; EI-MS (*m*/*z*) 499.10[M − 1]$^+$; ^{1}H NMR (400 MHz, 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); ^{13}C 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 1.02 g 4-{6-[(methylsulfonyl)-amino]pyridin-3-yl}benzoic acid (**4c**) and 1.16 g 1-(3-methoxybenzoyl)piperazine (**7IV**). Elution with petroleum ether/ethyl acetate (1/7, v/v) gave a solid (0.41 g, 24%). Mp 270–272 °C; EI-MS (*m*/*z*) 493.20[M − 1]$^+$; ^{1}H NMR (400 MHz, 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); ^{13}C 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 0.90 g 4-[6-(acetylamino)pyridin-2-yl]-benzoic acid (**5a**) and 1.35 g 1-[3-(trifluoromethyl)benzoyl]-piperazine (**7I**). Elution with petroleum ether/ethyl acetate (1/3, v/v) gave a solid (0.47 g, 27%). Mp 214–216 °C; EI-MS (*m*/*z*) 496 [M]$^+$; ^{1}H NMR (400 MHz, 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); ^{13}C 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 0.90 g 4-[6-(acetylamino)-pyridin-2-yl]benzoic acid (**5a**) and 1.50 g 1-[4-chloro-3-(trifluoromethyl)benzoyl]piperazine (**7II**). Elution with petroleum ether/ethyl acetate (1/3, v/v) gave a solid (0.48 g, 26%). Mp 226–228 °C; EI-MS (*m*/*z*) 530[M]$^+$; ^{1}H NMR (400 MHz, 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); ^{13}C 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 0.90 g 4-[6-(acetylamino)pyridin-2-yl]benzoic acid (**5a**) and 1.29 g 1-(4-*tert*-butylbenzoyl)piperazine (**7V**). Elution with petroleum ether/ethyl acetate (1/1, v/v) gave a solid (0.50 g, 30%). Mp 217–220 °C; EI-MS (*m*/*z*) 484[M]$^+$; ^{1}H NMR (400 MHz, 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); ^{13}C 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 1.04 g 4-{6-[(2,2-dimethylpropanoyl)amino]pyridin-2-yl}benzoic acid (**5b**) and 1.35 g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate (1/1, v/v) gave a solid (0.58 g, 31%). Mp 171–174 °C; EI-MS (*m*/*z*) 538[M]$^+$; ^{1}H NMR (400 MHz, 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); ^{13}C 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 1.04 g 4-{6-[(2,2-dimethylpropanoyl)amino]pyridin-2-yl}benzoic acid (**5b**) and 1.50 g 1-[4-chloro-3-(trifluoromethyl)benzoyl]piperazine (**7II**). Elution with petroleum ether/ethyl acetate (1/1, v/v) gave a solid (0.58 g, 29%). Mp 187–190 °C; EI-MS (*m*/*z*) 572[M]$^+$; ^{1}H NMR (400 MHz, 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); ^{13}C 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 1.04 g 4-{6-[(2,2-dimethylpropanoyl)amino]pyridin-2-yl}benzoic acid (**5b**) and 1.29 g 1-(4-*tert*-butylbenzoyl)piperazine (**7V**). Elution with petroleum ether/ethyl acetate (1/1, v/v) gave a solid (0.61 g, 33%). Mp 237–240 °C; EI-MS (*m*/*z*) 526[M]$^+$; ^{1}H NMR (400 MHz, 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); ^{13}C 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 1.02 g 4-{6-[(methylsulfonyl)-amino]pyridin-2-yl}benzoic acid (**5c**) and 1.35 g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate (1/7, v/v) gave a solid (0.41 g, 22%). Mp 243–244 °C; EI-MS (*m*/*z*) 532[M]$^+$; ^{1}H NMR (400 MHz, 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); ^{13}C 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 1.02 g 4-{6-[(methylsulfonyl)amino]pyridin-2-yl}benzoic acid (**5c**) and 1.50 g 1-[4-chloro-3-(trifluoromethyl)benzoyl]piperazine (**7II**). Elution with petroleum ether/ethyl acetate (1/7, v/v) gave a solid (0.40 g, 20%). Mp 120–122 °C; EI-MS (*m*/*z*) 566[M]$^+$; ^{1}H NMR (400 MHz, 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); ^{13}C 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]$^+$ $C_{25}H_{22}ClF_3N_4O_4SNa$: 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 1.02 g 4-{6-[(methylsulfonyl)-amino]pyridin-2-yl}benzoic acid (**5c**) and 1.29 g 1-(4-*tert*-butylbenzoyl)piperazine (**7V**). Elution with petroleum ether/ethyl acetate (1/1, v/v) gave a solid (0.44 g, 24%). Mp 225–228 °C; EI-MS (*m*/*z*) 520[M]$^+$; ^{1}H NMR (400 MHz, 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 1.09 g 4-[5-(morpholin-4-ylcarbonyl)pyridin-3-yl]benzoic acid (**6a**) and 1.35 g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate (1/5, v/v) gave a solid (0.44 g, 23%). Mp 179–181 °C; EI-MS (m/z) 552[M]$^+$; ^{1}H NMR (400 MHz, 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 1.09 g 4-[5-(morpholin-4-ylcarbonyl)pyridin-3-yl]benzoic acid (**6a**) and 1.18 g 1-(3,4-difluorobenzoyl)piperazine (**7III**). Elution with petroleum ether/ethyl acetate (1/5, v/v) gave a solid (0.38 g, 21%). Mp 212–213 °C; EI-MS (m/z) 520[M]$^+$; ^{1}H NMR (400 MHz, 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 1.09 g 4-[5-(morpholin-4-ylcarbonyl)pyridin-3-yl]benzoic acid (**6a**) and 1.22 g dimethyl[3-(piperazin-1-ylcarbonyl)phenyl]amine (**7VI**). Elution with petroleum ether/ethyl acetate (1/7, v/v) gave a solid (0.39 g, 21%). Mp 185–187 °C; EI-MS (m/z) 527[M]$^+$; ^{1}H NMR (400 MHz, 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 1.04 g 4-{5-[(diisopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6b**) and 1.35 g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate (1/5, v/v) gave a solid (0.51 g, 26%). Mp 161–163 °C; EI-MS (m/z) 566[M]$^+$; ^{1}H NMR (400 MHz, 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 = 4.0 Hz, 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 1.04 g 4-{5-[(diisopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6b**) and 1.18 g 1-(3,4-difluorobenzoyl)piperazine (**7III**). Elution with petroleum ether/ethyl acetate (1/5, v/v) gave a solid (0.41 g, 22%). Mp 194–197 °C; EI-MS (m/z) 534[M]$^+$; ^{1}H NMR (400 MHz, 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 1.04 g 4-{5-[(diisopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6b**) and 1.22 g dimethyl[3-(piperazin-1-ylcarbonyl)phenyl]amine (**7VI**). Elution with petroleum ether/ethyl acetate (1/7, v/v) gave a solid (0.38 g, 20%). Mp 197–199 °C; EI-MS (m/z) 541[M]$^+$; ^{1}H NMR (400 MHz, 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 1.04 g 4-{5-[(diisopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6b**) and 1.36 g 1-(2,4-dichlorobenzoyl)piperazine (**7VII**). Elution with petroleum ether/ethyl acetate (1/5, v/v) gave a solid (0.47 g, 24%). Mp 214–215 °C; EI-MS (m/z) 566[M]$^+$; ^{1}H NMR (400 MHz, 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 0.99 g 4-{5-[(cyclopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6c**) and 1.35 g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate (1/5, v/v) gave a solid (0.46 g, 25%). Mp

180–182 °C; EI-MS (*m*/*z*) 522[M]$^+$; ^{1}H NMR (400 MHz, 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 0.99 g 4-{5-[(cyclopropylamino)carbonyl]-pyridin-3-yl}benzoic acid (**6c**) and 1.18 g 1-(3,4-difluorobenzoyl)piperazine (**7III**). Elution with petroleum ether/ethyl acetate (1/5, v/v) gave a solid (0.41 g, 24%). Mp 221–222 °C; EI-MS (*m*/*z*) 490[M]$^+$; ^{1}H NMR (400 MHz, 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 0.99 g 4-{5-[(cyclopropylamino)-carbonyl]pyridin-3-yl}benzoic acid (**6c**) and 1.22 g dimethyl-[3-(piperazin-1-ylcarbonyl)phenyl]amine (**7VI**). Elution with petroleum ether/ethyl acetate (1/7, v/v) gave a solid (0.36 g, 21%). Mp 184–186 °C; EI-MS (*m*/*z*) 497[M]$^+$; ^{1}H NMR (400 MHz, 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, 23.54, 6.24.

***N*-Cyclopropyl-5-(4-{[4-(2,4-dichlorobenzoyl)piperazin-1-yl]carbonyl}phenyl)nicotinamide (10k).** The procedure described above was used with 0.99 g 4-{5-[(cyclopropylamino)carbonyl]-pyridin-3-yl}benzoic acid (**6c**) and 1.36 g 1-(2,4-dichlorobenzoyl)piperazine (**7VII**). Elution with petroleum ether/ethyl acetate (1/5, v/v) gave a solid (0.44 g, 24%). Mp 183–185 °C; EI-MS (*m*/*z*) 522[M]$^+$; ^{1}H NMR (400 MHz, 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 1.04 g 4-{5-[(diethylamino)carbonyl]pyridin-3-yl}benzoic acid (**6d**) and 1.35 g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate (1/5, v/v) gave a solid (0.49 g, 26%). Mp 125–127 °C; EI-MS (*m*/*z*) 538[M]$^+$; ^{1}H NMR (400 MHz, 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 = 4.0 Hz, 2H), 3.24 (d, J = 8.0 Hz, 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 1.04 g 4-{5-[(diethylamino)carbonyl]pyridin-3-yl}benzoic acid (**6d**) and 1.50 g 1-[4-chloro-3-(trifluoromethyl)benzoyl]piperazine (**7II**). Elution with petroleum ether/ethyl acetate (1/5, v/v) gave a solid (0.46 g, 23%). Mp 149–151 °C; EI-MS (*m*/*z*) 572[M]$^+$; ^{1}H NMR (400 MHz, 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]$^+$ $C_{29}H_{28}ClF_3N_4O_3Na$: 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 1.04 g 4-{5-[(diethylamino)carbonyl]pyridin-3-yl}benzoic acid (**6d**) and 1.22 g dimethyl-[3-(piperazin-1-ylcarbonyl)phenyl]amine (**7VI**). Elution with petroleum ether/ethyl acetate (1/7, v/v) gave a solid (0.39 g, 22%). Mp 157–158 °C; EI-MS (*m*/*z*) 513[M]$^+$; ^{1}H NMR (400 MHz, 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 = 4.0 Hz, 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 1.00 g 4-{5-[(isopropylamino)-carbonyl]pyridin-3-yl}benzoic acid (**6e**) and 1.35 g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with petroleum ether/ethyl acetate (1/5, v/v) gave a solid (0.49 g, 27%). Mp 225–227 °C; EI-MS (*m*/*z*) 524[M]$^+$; ^{1}H NMR (400 MHz, 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 1.00 g 4-{5-[(isopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6e**) and 1.50 g 1-[4-chloro-3-(trifluoromethyl)benzoyl]piperazine (**7II**). Elution with petroleum ether/ethyl acetate (1/5, v/v) gave a solid (0.50 g, 26%). Mp 162–164 °C; EI-MS (*m*/*z*) 558[M]$^+$; ^{1}H NMR (400 MHz, 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]$^+$ $C_{28}H_{27}ClF_3N_4O_3$: 559.1724, found 559.1728; calcd for [M + Na]$^+$ $C_{28}H_{26}ClF_3N_4O_3Na$: 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 1.00 g 4-{5-[(isopropylamino)carbonyl]pyridin-3-yl}benzoic acid (**6e**) and 1.22 g dimethyl[3-(piperazin-1-ylcarbonyl)phenyl]amine (**7VI**). Elution with petroleum ether/ethyl acetate (1/7, v/v) gave a solid (0.37 g, 21%). Mp 216–218 °C; EI-MS (*m*/*z*) 499[M]$^+$; ^{1}H NMR (400 MHz, 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 1.00 g 4-{5-[(isopropylamino)carbonyl]-pyridin-3-yl}benzoic acid (**6e**) and 1.36 g 1-(2,4-dichlorobenzoyl)piperazine (**7VII**). Elution with petroleum ether/ethyl acetate (1/5, v/v) gave a solid (0.42 g, 23%). Mp 225–227 °C; EI-MS (*m*/*z*) 524[M]$^+$; ^{1}H NMR (400 MHz, 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 1.09 g 4-[5-({[2-(dimethylamino)ethyl]amino}carbonyl)pyridin-3-yl]benzoic acid (**6f**) and 1.35 g 1-[3-(trifluoromethyl)benzoyl]piperazine (**7I**). Elution with ethyl acetate gave a solid (0.44 g, 23%). Mp 109–111 °C; EI-MS (*m*/*z*) 554.20[M + 1]$^+$; ^{1}H NMR (400 MHz, 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 1.09 g 4-[5-({[2-(dimethylamino)ethyl]amino}carbonyl)pyridin-3-yl]-benzoic acid (**6f**) and 1.50 g 1-[4-chloro-3-(trifluoro methyl)-benzoyl]piperazine (**7II**). Elution with ethyl acetate gave a solid (0.49 g, 24%). Mp 133–135 °C; EI-MS (*m*/*z*) 588.10[M + 1]$^+$; ^{1}H NMR (400 MHz, 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 1.09 g 4-[5-({[2-(dimethylamino)-ethyl]amino}carbonyl)pyridin-3-yl]benzoic acid (**6f**) and 1.36 g 1-(2,4-dichlorobenzoyl)piperazine (**7VII**). Elution with ethyl acetate gave a solid (0.42 g, 22%). Mp 114–116 °C; EI-MS (*m*/*z*) 554.10[M + 1]$^+$; ^{1}H NMR (400 MHz, 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** was evaluated against K562 cell lines by the standard MTT assay *in vitro*, with Imatinib as the positive control. The cancer cell lines were cultured in RPMI 1640 medium with 10% fetal bovine serum (FBS). Approximately 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 °C 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^{-1}, and incubated with cells at 37 °C 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 control. General procedures are as follows: Kinases (4 ng μl^{-1}) were incubated with substrates (0.2 μg μl^{-1}), 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^{-1}, and DTT 1 mM in a 384-well plate with a total volume of 5 μL. The assay plate was incubated at 30 °C 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 a 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

This work was supported by the National Natural Science Foundation (NNSF) of China (Grant No. 81302641 and 81302737) and the Fundamental Research Funds for the Central Universities.

Notes and references

1 L. Charles and M. D. Sawyers, *N. Engl. J. Med.*, 1999, **340**, 1330–1340.
2 C. A. Schiffer, *N. Engl. J. Med.*, 2007, **357**, 258–265.
3 M. W. Deininger, J. M. Goldman and J. V. Melo, *Blood*, 2000, **96**, 3343–3356.
4 K. Miyazawa and K. Toyama, *Nihon Naika Gakkai Zasshi*, 1995, **84**, 965–971.
5 S. Nakamura, D. Yokota, L. Tan, Y. Nagata, T. Takemura, I. Hirano, K. Shigeno, K. Shibata, S. Fujisawa and K. Ohnishi, *Int. J. Cancer*, 2012, **30**, 1046–1059.
6 M. Deininger, E. Buchdunger and B. J. Druker, *Blood*, 2005, **105**, 2640–2653.
7 T. Schindler, W. Bornmann, P. Pellicena, W. T. Miller, B. Clarkson and J. Kuriyan, *Science*, 2000, **289**, 1938–1942.
8 B. J. Druker, C. L. Sawyers, H. Kantarjian, D. J. Resta, S. F. Reese, J. M. Ford, R. Capdeville and M. Talpaz, *N. Engl. J. Med.*, 2001, **344**, 1038–1042.
9 M. J. Eck and P. W. Manley, *Curr. Opin. Cell Biol.*, 2009, **21**, 288–295.
10 Y. Liu and N. S. Gray, *Nat. Chem. Biol.*, 2006, **7**, 358–364.
11 B. Nagar, W. G. Bornmann, P. Pellicena, T. Schindler, D. R. Veach, W. T. Miller, B. Clarkson and J. Kuriyan, *Cancer Res.*, 2002, **62**, 4236–4243.
12 J. Dietrich, C. Hulme and L. H. Hurley, *Bioorg. Med. Chem.*, 2010, **18**, 5738–5748.
13 T. Ahmad and T. Eisen, *Clin. Cancer Res.*, 2004, **10**, 6388S–6392S.
14 S. M. Wilhelm, J. Dumas, L. Adnane, M. Lynch, C. A. Carter, G. Schütz, K. H. Thierauch and D. Zopf, *Int. J. Cancer*, 2011, **129**, 245–255.
15 S. J. Boyer, *Curr. Top. Med. Chem.*, 2002, **2**, 973–1000.
16 V. Khazak, I. Astsaturov, I. G. Serebriiskii and E. A. Golemis, *Expert Opin. Ther. Targets*, 2007, **11**, 1587–1609.
17 M. Hasegawa, N. Nishigaki, Y. Washio, K. Kano, P. A. Harris, H. Sato, I. Mori, R. I. West, M. Shibahara, H. Toyoda, L. Wang, R. T. Nolte, J. M. Veal and M. Cheung, *J. Med. Chem.*, 2007, **50**, 4453–4470.
18 K. Nagashima, S. D. Shumway, S. Sathyanarayanan, A. H. Chen, B. Dolinski, Y. Xu, H. Keilhack, T. Nguyen, M. Wiznerowicz, L. Li, B. A. Lutterbach, A. Chi, C. Paweletz, T. Allison, Y. Yan, S. K. Munshi, A. Klippel, M. Kraus, E. V. Bobkova, S. Deshmukh, Z. Xu, U. Mueller, A. A. Szewczak, B. S. Pan, V. Richon, R. Pollock, P. Blume-Jensen, A. Northrup and J. N. Andersen, *J. Biol. Chem.*, 2011, **286**, 6433–6448.
19 K. M. Sakamoto, *Curr. Opin. Invest. Drugs*, 2004, **5**, 1329–1339.
20 J. Matsui, Y. Yamamoto, Y. Funahashi, A. Tsuruoka, T. Watanabe, T. Wakabayashi, T. Uenaka and M. Asada, *Int. J. Cancer*, 2008, **122**, 664–671.
21 B. L. Hodous, S. D. Geuns-Meyer, P. E. Hughes, B. K. Albrecht, S. Bellon, J. Bready, S. Caenepeel, V. J. Cee, S. C. Chaffee, A. Coxon, M. Emery, J. Fretland, P. Gallant, Y. Gu, D. Hoffman, R. E. Johnson, R. Kendall, J. L. Kim, A. M. Long, M. Morrison, P. R. Olivieri, V. F. Patel, A. Polverino, P. Rose, P. Tempest, L. Wang, D. A. Whittington and H. Zhao, *J. Med. Chem.*, 2007, **50**, 611–626.
22 M. L. Curtin, R. R. Frey, H. R. Heyman, K. A. Sarris, D. H. Steinman, J. H. Holmes, P. F. Bousquet, G. A. Cunha, M. D. Moskey, A. A. Ahmed, L. J. Pease, K. B. Glaser, K. D. Stewart, S. K. Davidsen and M. R. Michaelides, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 4505–4509.
23 J. Zhou, B. Li, F. Hu and B. F. Shi, *Org. Lett.*, 2013, **15**, 3460–3463.
24 M. Karramkam, F. Hinnen, M. Berrehouma, C. Hlavacek, F. Vaufrey, C. Halldin, J. A. McCarron, V. W. Pike and F. Dollé, *Bioorg. Med. Chem.*, 2003, **11**, 2769–2782.
25 N. Miyaura and A. Suzuki, *Chem. Rev.*, 1995, **95**, 2457–2483.
26 S. Hadia-Ruah, M. Hamilton, M. Miller, P. D. J. Grootenhuis, B. Bear, J. McCartney, J. Zhou and F. Goor, *US* 20080019915-A1, 2008.
27 N. Robert, A. L. Bonneau, C. Hoarau and F. Marsais, *Org. Lett.*, 2006, **8**, 6071–6074.
28 S. Röver, M. Andjelkovic, A. Bénardeau, E. Chaput, W. Guba, P. Hebeisen, S. Mohr, M. Nettekoven, U. Obst,

W. F. Richter, C. Ullmer, P. Waldmeier and M. B. Wright, *J. Med. Chem.*, 2013, **56**, 9874–9896.
29 L. Lai, E. Wang and B. J. Luh, *Synthesis*, 2001, 361–363.
30 P. L. Beaulieu, P. Lavallee, A. Abraham, P. C. Anderson, C. Boucher, Y. Bousquet, J. S. Duceppe, J. Gillard, V. Gorys, C. Grand-Maıtre, L. Grenier, Y. Guindon, I. Guse, L. Plamondon, F. Soucy, S. Valois, D. Wernic and C. Yoakim, *J. Org. Chem.*, 1997, **62**, 3440–3448.
31 X. Pan, J. Dong, H. Gao, F. Wang, Y. Zhang, S. Wang and J. Zhang, *Chem. Biol. Drug Des.*, 2014, **83**, 592–599.
32 C. Zhang, C. Tan, X. Zu, X. Zhai, F. Liu, B. Chu, X. Ma, Y. Chen, P. Gong and Y. Jiang, *Eur. J. Med. Chem.*, 2011, **46**, 1404–1414.
33 H. Li, R. D. Totoritis, L. A. Lor, B. Schwartz, P. Caprioli, A. J. Jurewicz and G. Zhang, *Assay Drug Dev. Technol.*, 2009, **7**, 598–605.
34 X. Pan, F. Wang, Y. Zhang, H. Gao, Z. Hu, S. Wang and J. Zhang, *Bioorg. Med. Chem.*, 2013, **21**, 2527–2534.
35 B. Nagar, W. G. Bornmann, P. Pellicena, T. Schindler, D. R. Veach, W. T. Miller, B. Clarkson and J. Kuriyan, *Cancer Res.*, 2002, **62**, 4236–4243.
36 T. O'Hare, W. C. Shakespeare, X. Zhu, C. A. Eide, V. M. Rivera, F. Wang, L. T. Adrian, T. Zhou, W. S. Huang, Q. Xu, C. A. Metcalf 3rd, J. W. Tyner, M. M. Loriaux, A. S. Corbin, S. Wardwell, Y. Ning, J. A. Keats, Y. Wang, R. Sundaramoorthi, M. Thomas, D. Zhou, J. Snodgrass, L. Commodore, T. K. Sawyer, D. C. Dalgarno, M. W. Deininger, B. J. Druker and T. Clackson, *Cancer Cell*, 2009, **16**, 401–412.
37 S. Wold, A. Ruhe, H. Wold and W. Dunn III, *Siam J. Sci. Stat. Comput.*, 1984, **5**, 735–743.
38 W. J. Dunn, S. Wold, U. Edlund, S. Hellberg and J. Gasteiger, *Quant. Struct. Act. Relat.*, 1984, **3**, 31–137.
39 P. Geladi, *J. Chemom.*, 1988, **2**, 231–246.
40 R. D. Cramer III, J. D. Bunce and D. E. Patterson, *Quant. Struct. Act. Relat.*, 1988, **7**, 18–25.