Bioorg. Med. Chem. 40 (2021) 116186

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and biological evaluation of imidazopyridazine derivatives containing isoquinoline group as potent MNK1/2 inhibitors

Hong Bu [a,1], Xinrui Yuan [a,1], Hanshu Wu [b], Jinpei Zhou [b,*], Huibin Zhang [a,*]

[a] Center of Drug Discovery, State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China
[b] Department of Medicinal Chemistry, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China

ARTICLE INFO

Keywords:
MNK1/2 kinases
eIF4E
ETC-206
MNKs inhibitor

ABSTRACT

Mitogen-activated protein kinase (MAPK)-interacting kinases (MNKs) are located at the meeting-point of ERK and p38 MAPK signaling pathways, which can phosphorylate eukaryotic translation initiation factor 4E (eIF4E) at the conserved serine 209 exclusively. MNKs modulate the translation of mRNA involved in tumor-associated signaling pathways. Consequently, selective inhibitors of MNK1/2 could reduce the level of phosphorylated eIF4E. Series of imidazopyrazines, imidazopyridazines and imidazopyridines derivatives were synthesized and evaluated as MNK1/2 inhibitors. Several compounds exhibited great inhibitory activity against MNK1/2 and selected compounds showed moderate to excellent anti-proliferative potency against diffuse large B-cell lymphoma (DLBCL) cell lines. In particular, compound **II-5** (MNK1 IC50 = 2.3 nM; MNK2 IC50 = 3.4 nM) exhibited excellent enzymatic inhibitory potency and proved to be the most potent compound against TMD-8 and DOHH-2 cell lines with IC_{50} value of 0.3896 μM and 0.4092 μM respectively. These results demonstrated that compound **II-5** could be considered as a potential MNK1/2 inhibitor for further investigation.

1. Introduction

Mitogen-activated protein kinase interacting protein kinases (MNKs) are serine/threonine kinases, which belong to the Ca^{2+}/calmodulin-dependent kinases (CaMK) family.[1] In human cells, MNKs encoded by two genes (MKNK1 and MKNK2) are present in two isoforms: MNK1 and MNK2, which derive four variants by alternative splicing: MNK1a/MNK2a (full length) and MNK1b/MNK2b (lacking the short C terminal fragment).[2,3] The amino acid sequences of these four variants are approximately 80% similar, and the sequences of their core catalytic domains are highly consistent. Besides, they have similar *N*-terminal regions, and both contain the *N*-terminal nuclear localization signal sequence (NLS), which allows MNKs to enter into the nucleus. MNK1a and MNK2a contain a MAPK binding domain at the C-terminus, which enables them to be activated by phosphorylation of ERK and P38 MAPK. MNK1a can be phosphorylated by both ERKs and p38α/β.[4]

MNKs are located at the downstream intersection of Ras/Raf/ERK and p38 MAPK signaling pathways. Once stimulated by extracellular factors, ERK and p38 MAPK are respectively responsible for the phosphorylation of Thr209/Thr214 (MNK1a) and Thr244/Thr249 (MNK2a) at the activating cyclic peptide chains, thereby activating MNKs.[5,6] Unlike MNK1, which has a high affinity with ERK and p38 MAPK, MNK2 mainly binds to ERK. MNKs are mainly responsible for the phosphorylation of multiple downstream substrates, including eIF4E, hnRNPA1, Sprouty2, PSF, and cPLA2. The eukaryotic translation initiation factor 4E (eIF4E) is the most prominent and well-characterized substrate of MNKs, which is phosphorylated exclusively by MNKs at the conserved Ser209.[7,8] MNKs/eIF4E signaling pathway can affect the synthesis of various chemokines, cytokines and immune checkpoint proteins, thereby regulating the initiation of innate immunity, the activation of acquired immunity, and the response to immune-related cytokine.[9,10] In recent years, MNKs has attracted the attention and investment of many scientific research institutions and pharmaceutical companies as a tumor treatment target. Furthermore, with the development of tumor immunotherapy, multiple studies have also demonstrated the potential role of MNKs in immune system. [11,12]

ETC-206 (MNK IC_{50} = 64 nM, MNK2 IC_{50} = 86 nM), a selective MNK1/2 inhibitor, was approved to enter into phase I clinical trials for the treatment of BC-CML patients in combination with dasatinib.[13] From November 2016 to June 2017, ETC-206 has been in clinical trials in Singapore, which showed good bioavailability, safety and tolerance.[14] The binding mode of ETC-206 and MNK2 exhibited that

* Corresponding authors.
E-mail addresses: jpzhou668@163.com (J. Zhou), zhanghb80@cpu.edu.cn (H. Zhang).
[1] Authors contributed equally.

https://doi.org/10.1016/j.bmc.2021.116186
Received 10 February 2021; Received in revised form 20 April 2021; Accepted 26 April 2021
Available online 2 May 2021

imidazopyridine formed a hydrogen bond with Met162 of hinge region of MNK2. Benzonitrile group extended into hydrophobic pocket and had cation-π interaction with gatekeeper residue Phe159. Importantly, benzonitrile group interacted with the side chain of Lys113 by hydrogen bond. This hydrophobic pocket surrounded by the residues Val98, Lys113, Phe227 and Phe159 has more space for bicyclic group (Fig. 1). Base on this, a series of novel **ETC-206** derivatives have been designed, synthesized and evaluated *in vitro* (Fig. 2).

2. Results and discussions

2.1. Chemistry

The compounds **I-1∼I-12** were prepared as shown in Scheme 1. The synthesis was started from commercially available 5-bromopyridin-2-amine **2** with 2-bromo-1,1-diethoxyethane **3** to afford the intermediate **4**. Suzuki coupling of compound **4** introduced a *para*-benzoyl ester to give **5**, which was halogenated with NBS to afford the intermediate **6**. The ester was hydrolyzed to carboxylic acid **7**, and the carboxylic acid was converted to amides **8a∼8d** by amide formation. Arylation using a second Suzuki cross coupling afforded compounds **I-1∼I-9** and **9a∼9c**. Removal of *N*-Boc protecting group of 9a∼9c with trifluoroacetic acid afforded target compounds **I-10∼I-12**.

Compounds **II-1∼II-3**, as shown in Scheme 2, were prepared in 3 steps starting from intermediate **7**. Compound **7** was converted to amides **10a∼10c** followed by a Suzuki cross coupling reaction to give **11a∼11c**. Formation of pyridone **II-1∼II-3** was carried out by the reaction of compounds **11a∼11c** with trifluoroacetic acid to remove *N*-Boc protecting group.

The general synthetic route toward **II-4∼II-9** was depicted in Scheme 3. Compounds **12a∼12b** were reacted with 2-bromo-1,1-diethoxyethane **3** to afford intermediates **13a-13b**, which were converted to **16a∼16b** by 3 steps comprising a Suzuki coupling, bromination reaction and ester hydrolysis. Compounds **16a∼16b** were converted to **17a-17f** by amide formation. Suzuki coupling between **17a** and **17f** and 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline provided compounds **18a-18f**. Removal of *N*-Boc protecting group of 18a∼18f in the presence of trifluoroacetic acid delivered the corresponding target compounds **II-4∼II-9**.

2.2. In vitro activity against MNK1 and MNK2

The *in vitro* inhibitory activity of these synthesized compounds was evaluated by ADP-Glo^{TM} kinase assay. Initially, 4-cyanophenyl group of **ETC-206** was replaced with quinoline or isoquinoline to afford compounds **I-1∼I-3**. Three compounds showed acceptable inhibitory activity against MNK1 at the concentration of 500 nM. Interestingly, only isoquinoline derivative **I-1** exhibited significant potency against MNK2. Furtherly, we replaced morpholinyl group by 4-morpholino-piperidyl, 4-piperidino-piperidyl and piperazinyl group (**I-4∼I-12**). Similar with compound **I-1**, isoquinoline derivatives **I-4**, **I-7** and **I-10** displayed better inhibitory activity than quinoline analogues.

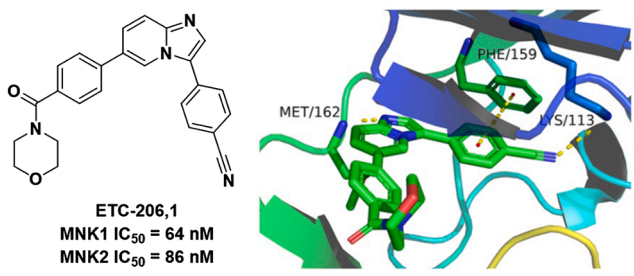


Fig. 1. Chemical structures of **ETC-206** and cocrystal structure of **ETC-206** in MNK2 (PDBcode: 6AC3).

The IC_{50} values were determined for the compounds that exhibited more 50% inhibitory activity at the concentration of 500 nM. In general, isoquinoline derivatives showed excellent potency with IC_{50} values ranging from 1.5 to 26 nM. **I-1** and **ETC-206** were similar potency toward MNK1 inhibition, but for MNK2, **ETC-206** was 2.7-fold more potent than **I-1**. Compared with quinoline, isoquinoline group led to 8-fold∼50-fold improvement (e.g. **I-1** vs **I-2** and **I-3**). Based on isoquinoline group, replacement of morpholinyl by 4-morpholino-piperidyl (**I-4**), 4-piperidino-piperidyl (**I-7**) and piperazinyl group (**I-10**) still displayed excellent potency and compound **I-7** was about 5-fold potent than **ETC-206** (Table 1).

These results indicated that the isoquinoline group contributed to promising affinity to MNK1 and MNK2 and was selected for further modification. In comparison to imidazopyridine derivatives, imidazopyrazine derivatives (**II-7∼II-9**) were less potent, and imidazopyridazine derivatives (**II-4∼II-6**) were equipotent to **II-1∼II-3**. Among these aminopiperidine analogues, (*S*)-3-aminopiperidyl analogues (**II-2** and **II-5**) exhibited 5-fold improvement in potency for MNK1 and MNK2 (Table 2).

2.3. Anti-proliferative activity of selective compounds against TMD-8 and DOHH-2 cell lines

MNK1/2 inhibitors showed significant anti-proliferative effects against diffuse large B-cell lymphoma (DLBCL) cell lines *in vitro*. The anti-proliferative activity of selective compounds was evaluated *in vitro*, using DLBCL cell lines TMD-8 and DOHH-2. The IC_{50} values of these compounds were in general in the micromolar range, similar to **ETC-206**. Compound **II-5** appeared to be more potent than **ETC-206** with 5.5-fold improvement for TMD-8 and 4-fold improvement for DOHH-2 (Table 3).

2.4. Kinase inhibitory selectivity profiling of compound *II-5*

In order to further evaluate the inhibitory selectivity, compound **II-5** was tested against several kinases to determine its kinase selectivity. As summarized in Table 4, **II-5** displayed high selectivity over other kinases, such as BTK, CDK1 and JAK1.

2.5. Compound *II-5* inducedapoptosis in TMD-8 cells

Subsequently, in order to investigate whether compound **II-5** could cause TMD-8 cell apoptosis, annexin V-FITC apoptosis detection assay was performed. The result showed compound **II-5** led to significant TMD-8 cells apoptosis in a concentration-dependent manner comparing with the negative control.Thus, compound **II-5** had a significant ability to induce TMD-8 cell apoptosis which might be the reason for its anti-proliferative effect (Fig. 3).

3. Conclusions

In this study, series of imidazopyridine, imidazopyridazine and imidazopyrazine derivatives were designed and synthesized as selective and potent MNK1/2 inhibitors. All the compounds were evaluated for enzymatic inhibitory activity against MNK1/2 *in vitro*. And the anti-proliferation of some selected compounds against DLBCL cell lines were also tested in this paper. Among these compounds, isoquinoline derivatives, such as **I-4**, **I-7** and **I-10**, displayed excellent inhibitory activity, which indicated the isoquinoline group contributed to promising affinity to MNK1 and MNK2. And the further modification showed **II-2** and **II-5** exhibited high enzymatic inhibitory potency and anti-proliferative activity against DLBCL cell lines. Remarkably, compound **II-5** (MNK1 IC_{50} = 2.3 nM; MNK2 IC_{50} = 3.4 nM) was found to possess the stronger anti-proliferative activity against TMD-8 and DOHH-2 cell lines than **ETC-206**. Compound **II-5** displayed high inhibitory selectivity over several kinases and had strong ability to induce cell apoptosis.

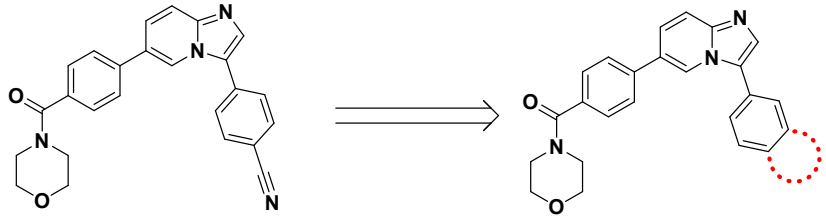
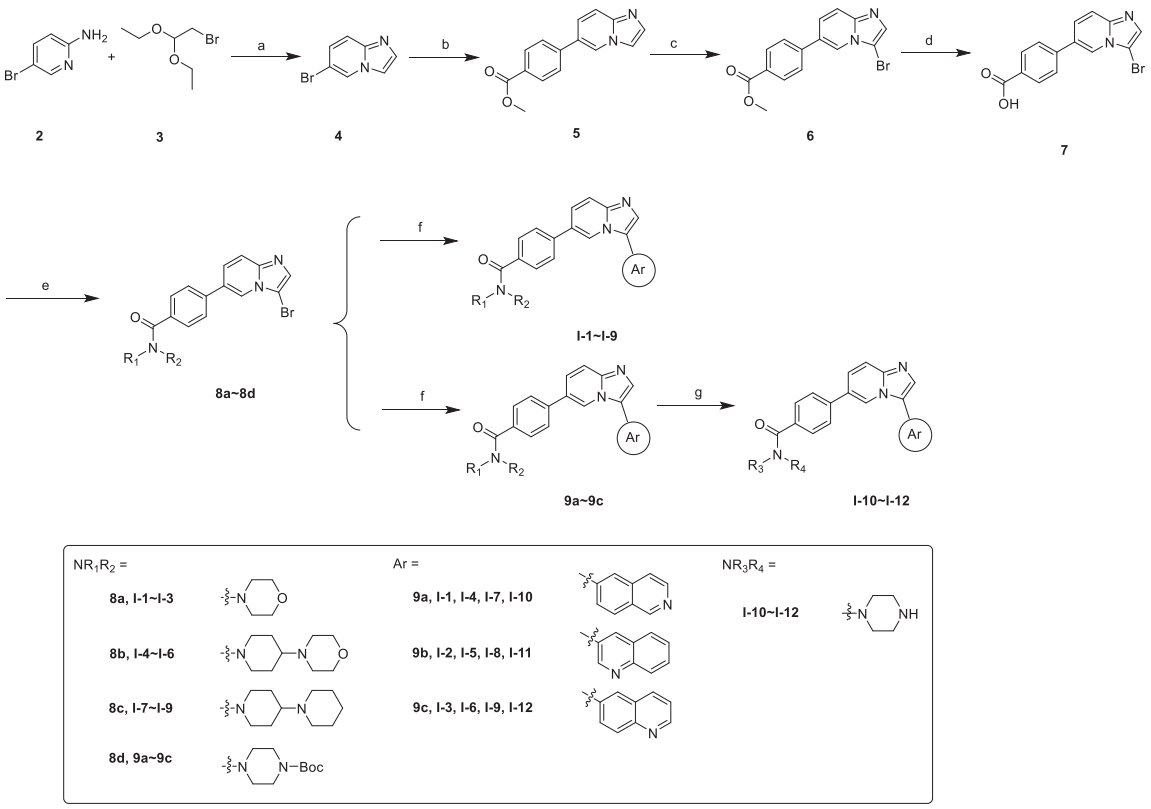


Fig. 2. Design of imidazopyridazine derivatives containing isoquinoline group as MNKs inhibitors.



Scheme 1. Reagents and conditions: (a) HBr, C_2H_5OH/H_2O, 100℃; (b) (4-(methoxycarbonyl)phenyl) boronic acid, $Pd(PPh_3)_4$, $K_3PO_4{\cdot}3H_2O$, 1,4-dioxane/H_2O, N_2, r.t. to 95 °C; (c) NBS, DCM/CH_3CN, r.t.; (d) LiOH, THF/H_2O, r.t.; (e) R_1R_2NH, EDCl, HOBt, TEA, DMAP, DMF, 40 °C; (f) Boric acid, $Pd(PPh_3)_4$, $K_3PO_4{\cdot}3H_2O$, 1,4-dioxane/H_2O, N_2, r.t. to 95 °C; (g) TFA, DCM, r.t., 6 h.

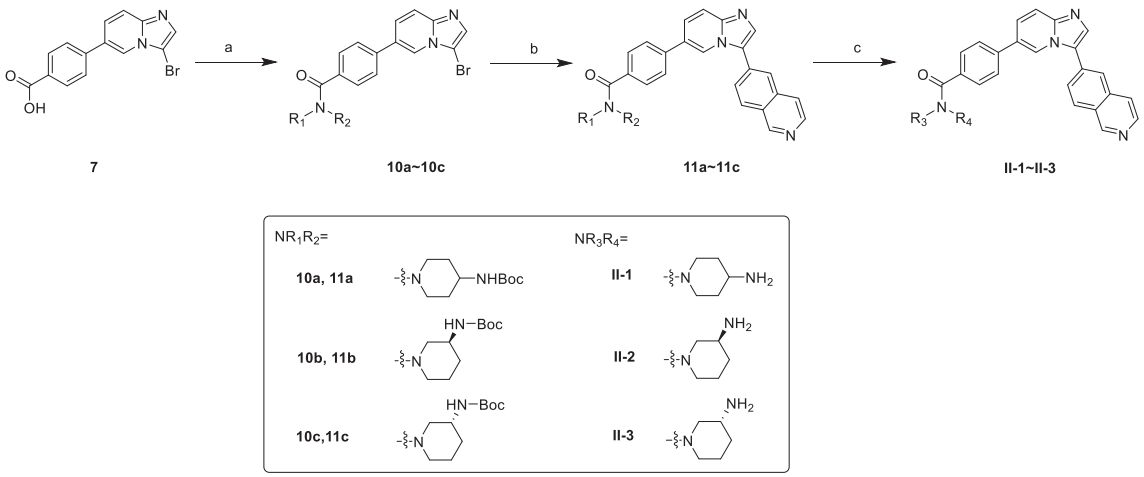
Primary SAR studies exhibited that introducing isoquinoline to replace 4-cyanophenyl group of **ETC-206** led to significant increase in potency, which may be due to the hydrogen bond between N-1 of isoquinoline and Lys113 residue was still retained. Furthermore, the bicyclic fragment of isoquinoline could better occupy the hydrophobic area. Further modification indicated that compared to imidazopyridine derivatives, imidazopyrazine derivatives were less potent, and imidazopyridazine derivatives were equally effective with imidazopyridine derivatives at increasing potency for MNK1/2 inhibition.

More and more studies support that MNKs/eIF4E pathway is closely related to the development, metastasis, drug resistance and poor prognosis of various cancers.[15,16] Besides, MNKs/eIF4E also can regulate various biological processes, including inflammation, autophagy and apoptosis.[17,18] Hence, there is a growing interest in development and characterization of novel selective MNK1/2 inhibitors. Our further effort will be devoted on exploiting the function and mechanism of these inhibitors and designing more highly potent MNK1/2 inhibitors with satisfactory druggability and safety profiles.

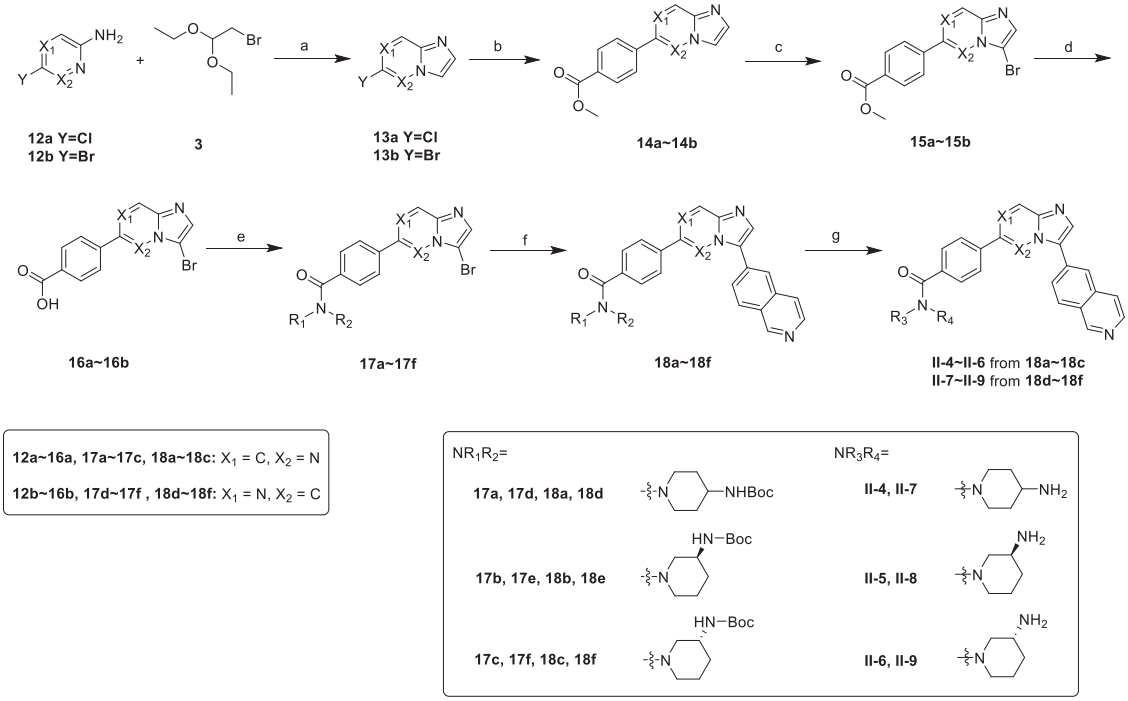
4. Experimental section

4.1. Chemistry

Reagents were purchased from commercial sources and all commercial materials and solvents were reagent grade which used without further purification unless expressly stated. All the reactions were monitored by thin-layer chromatography (TLC) performed on 0.25 mm silicagel plates (GF254 Tsingtao Haiyang Chemicals, China) and visualized under ultraviolet lamp at 254 nm. Column chromatography was conducted on silica gel (200–300 mesh, Tsingtao Haiyang Chemicals, China). Melting points of the synthesized compounds were determined with capillary apparatus and were not corrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker ARX-400 spectrometer at 400 MHz or Bruker ARX-300 spectrometer at 300 MHz with TMS as the internal standard in CDCl3, CH3OD or DMSO-d_6 unless otherwise indicated. Samples were prepared as solutions in a deuterated solvent and referenced to the appropriate internal nondeuterated solvent peak. Mass spectrometry (MS) were performed with Hewlett-Packard 1100 LC/MSD



Scheme 2. Reagents and conditions: (a) R_1R_2NH, EDCl, HOBt, TEA, DMAP, DMF, 40 °C; (b) 6-(4,4,5,5-tetramethyl-1,3,2- dioxaborolan-2-yl)isoquinoline, $Pd(PPh_3)_4$, $K_3PO_4{\cdot}3H_2O$, 1,4-dioxane/H_2O, N_2, r.t. to 95 °C; (c) TFA, DCM, r.t., 6 h.



Scheme 3. Reagents and conditions: (a) HBr, C_2H_5OH/H_2O, 100℃; (b) (4-(methoxycarbonyl)phenyl) boronic acid, $Pd(PPh_3)_4$, $K_3PO_4{\cdot}3H_2O$, 1,4-dioxane/H_2O, N_2, r. t. to 95 °C; (c) NBS, DCM/CH_3CN, r.t.; (d) LiOH, THF/H_2O, r.t.; (e) R_1R_2NH, EDCl, HOBt, TEA, DMAP, DMF, 40 °C; (f) 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline, $Pd(PPh_3)_4$, $K_3PO_4{\cdot}3H_2O$, 1,4-dioxane/H_2O, N_2, r.t. to 95 °C; (g) TFA, DCM, r.t., 6 h.

spectrometer by electrospray ionization.

4.1.1. General synthetic procedures

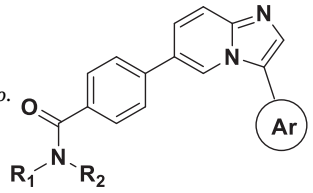
General Procedure A: Suzuki Cross Coupling Using $Pd(PPh_3)_4$. To a slurry of the halide derivative (1.0 eq) and boronic acid (1.2 eq) in a mixture of 1,4-dioxane (10 mL/mmol) and H_2O (2 mL/mmol) was added $K_3PO_4{\cdot}3H_2O$ (2.0 eq). The mixture was stirred at room temperature for 15 min under nitrogen atmosphere. To the mixture was added $Pd(PPh_3)_4$ (0.1 eq) and then heated at 95 °C for 8 h under nitrogen. The mixture was colded to room temperature and filtered through a short pad of celite to remove insoluble solids. The filtrate was concentrated to dryness in vacuo and diluted with H_2O. After extraction with CH_2Cl_2, the combined organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography.

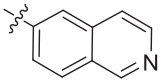
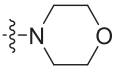
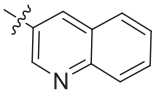
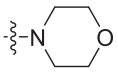
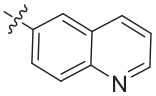
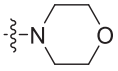
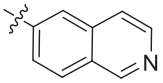
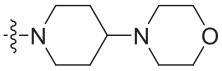
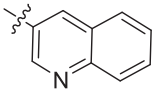
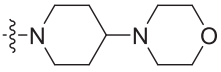
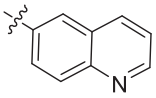
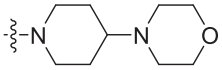
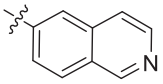
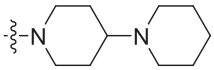
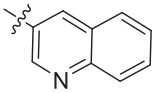
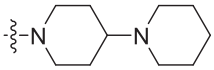
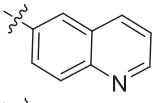
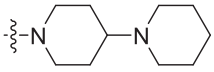
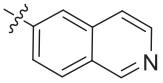
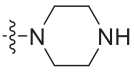
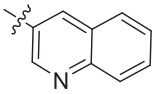
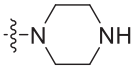
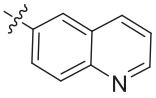
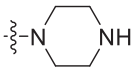
General Procedure B: Amide Coupling. To a solution of carboxylic acid derivative (1.0 eq) in DMF (2.8 mL/mmol) was added amine (1.2 eq), followed by the addition of EDCI (1.2 eq), HOBt (1.2 eq), DMAP (0.1 eq) and TEA (1.5 eq). The reaction mixture was heated at 40 °C for 8 h. After dilution with CH_2Cl_2, the mixture was washed with saturated sodium bicarbonate solution and water. The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography.

General Procedure C: Aromatic Bromination. A solution of bicyclic derivative (imidazopyridine, imidazopyridazine or imidazopyrazine derivatives) and *N*-bromosuccinimide (1.2 eq) in CH_3CN (5 mL/mmol) and CH_2Cl_2 (1.6 mL/mmol) was stirred at room temperature for 30 min then was diluted with H_2O. After extraction with CH_2Cl_2, the combined organic phase was washed with saturated sodium bicarbonate solution, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was

Table 1

Chemical structure of target compounds **I-1**~**I-12** and their enzymatic inhibitory activities *in vitro*.



Cpd.	Ar	NR_1R_2	Inh% at 500 nM		IC_{50} (nM)[a]	
			MNK1	MNK2	MNK1	MNK2
I-1			93.7%	106%	7.0	25.7
I-2			71%	21%	153.7	635.7
I-3			74.7%	64%	370.8	215.8
I-4			87.78%	77.71%	4.0	3.2
I-5			40.59%	18.67%	–	–
I-6			49.75%	34.94%	–	–
I-7			92.92%	74.7%	2.4	1.5
I-8			71.04%	48.8%	–	–
I-9			62.77%	2.41%	–	–
I-10			98.72%	77.71%	9.4	2.9
I-11			70.71%	68.07%	67.6	51.2
I-12			73.54%	49.89%	–	–
ETC-206					10.6	9.4

[a] Three repeated experiments were performed to calculate the average IC_{50}.

purified by flash column chromatography.

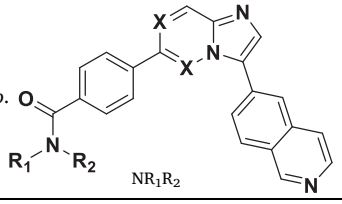
General Procedure D: Ester Hydrolysis. To a solution of carboxylic ester in THF (4 mL/mmol) was added LiOH (8.0 eq) and and water (1.28 mL/mmol). The mixture was stirred at room temperature for 8 h, then, solvent was concentrated in vacuo, diluted with water (2.56 mL/mmol) and acidified with 1 M HCl aqueous solution till pH 2. After stirred at room temperature for additional 20 min, the precipitate was isolated by filtration and dried to afford the desired product.

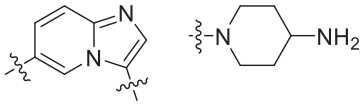
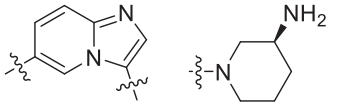
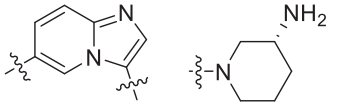
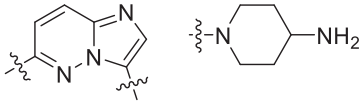
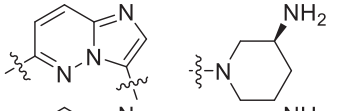
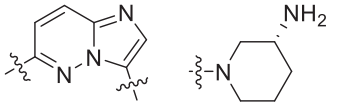
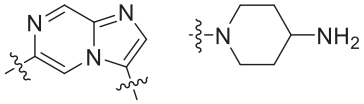
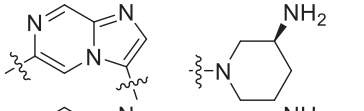
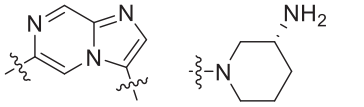
General Procedure E: To a slurry of aromatic amine protected by Boc group in CH_2Cl_2 (20 mL/mmol) was added trifluoroacetic acid (10 eq). The mixture was stirred at room temperature for 4 h. The mixture was concentrated in vacuo, and the residue was diluted with CH_2Cl_2 (20 mL/mmol). To the mixture was then added trimethylamine (1.2 eq). After stirred at room temperature for additional 30 min, the mixture was concentrated in vacuo. The residue was purified by flash column chromatography

4.1.2. Synthesis of 4-(3-bromoimidazo[1,2-a]pyridin-6-yl)benzoic acid (7)

To a solution of 5-bromopyridin-2-amine (**2**, 5 g, 28.90 mmol, 1.0 eq) in C_2H_5OH (25 mL) and H_2O (30 mL) was added 2-bromo-1,1-diethoxyethane (**3**, 8.54 g, 43.35 mmol, 1.5 eq) and 40% HBr aqueous solution (5 mL) at room temperature, respectively. The mixture was heated at 100 °C for 8 h. Solvent was concentrated in vacuo and the pH of residue was adjusted to 7 with $NaHCO_3$ aqueous solution. The mixture was diluted with H_2O (50 mL) and extracted with EtOAc (150 mL × 3). The

Table 2
Chemical structure of target compounds **II-1**~**II-9** and their enzymatic inhibitory activities *in vitro*.



Cpd.		IC$_{50}$ (nM)[a]	
		MNK1	MNK2
II-1		16.8	5.2
II-2		2.5	2.5
II-3		7.7	8.9
II-4		14.5	15.7
II-5		2.3	3.4
II-6		9.4	6.6
II-7		32.1	18.5
II-8		27.3	19.4
II-9		42.0	26.7
ETC-206		10.6	9.4

[a] Three repeated experiments were performed to calculate the average IC$_{50}$.

Table 3
The anti-proliferative effects of selected compounds against cancer cell lines.

Cpd.	Anti-proliferative activity/IC$_{50}$ (μM)[a]	
	TMD-8	DOHH-2
I-4	4.261	7.546
I-7	4.010	3.823
I-10	6.641	8.682
II-1	2.063	8.302
II-2	3.465	6.734
II-3	2.301	6.278
II-4	2.085	0.5411
II-5	0.3896	0.4092
II-6	2.419	0.3998
II-7	7.568	12.00
II-8	2.555	5.190
II-9	3.096	3.252
ETC-206	2.138	1.624

[a] Three repeated experiments were performed to calculate the average IC$_{50}$.

Table 4
Selectivity profiling of compound **II-5**.

Rank	Kinase Inh% at 500 nM	
1	MNK1	102.63%
2	MNK2	97.31%
3	BTK	4.1%
4	CDK1	5.01%
5	IRAK4	22.27%
6	AMPK	10.22%
7	JAK1	6.5%
8	CSF1R	11.64%

combined organic phase was washed with saturated sodium bicarbonate solution (75 mL × 3), dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, petroleum ether/EtOAc = 5/1) to afford 6-bromoimidazo[1,2-*a*] pyridine (**4,** 4.71 g, 83%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.92 (s, 1H), 7.93 (s, 1H), 7.61 (s, 1H), 7.56 (d, J = 9.6 Hz, 1H), 7.33 (dd, J = 9.5, 1.8 Hz, 1H).

To a solution of compound **4** (2 g, 10.15 mmol, 1.0 eq) in 1,4-dioxane (30 mL) and H_2O (3 mL) was added (4-(methoxycarbonyl)phenyl) boronic acid (2.19 g, 12.18 mmol, 1.2 eq) and $K_3PO_4{\cdot}3H_2O$ (6.76 g, 25.38 mmol, 2.5 eq). The mixture was stirred at room temperature for 15 min under nitrogen. To the mixture was then added $Pd(PPh_3)_4$ (562 mg, 0.51 mmol, 0.05 eq). The mixture was heated at 95 °C for 8 h under nitrogen. The mixture was colded to room temperature and filtered with celite to remove insoluble solids. The filtrate was concentrated in vacuo and diluted with H_2O (100 mL). After extraction with CH_2Cl_2 (100 mL × 3), the combined organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, petroleum ether/EtOAc = 1/1) to afford methyl 4-(imidazo[1,2-*a*]pyridin-6-yl)benzoate (**5,** 2.2 g, 85.91%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.08 (s, 1H), 8.07 (d, J = 8.4 Hz, 2H), 7.99 (s, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.69–7.62 (m, 3H), 3.89 (s, 3H).

To a solution of compound **5** (2 g, 7.93 mmol, 1.0 eq) in CH_3CN (39 mL) and CH_2Cl_2 (13 mL) was added NBS (1.69 g, 9.51 mmol, 1.2 eq). The mixture was stirred at room temperature for 30 min and diluted with H_2O (75 mL). After extraction with CH_2Cl_2 (100 mL × 3), the combined organic phase was washed with saturated sodium bicarbonate solution (50 mL × 3), dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, petroleum ether/EtOAc = 2/1) to afford methyl 4-(3-bromoimidazo [1,2-*a*]pyridin-6-yl)benzoate (**6,** 2.57 g, 97.89%) as yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.34 (s, 1H), 8.24–8.11 (m, 2H), 7.75 (d, J = 9.4 Hz, 1H), 7.68 (dd, J = 5.4, 2.8 Hz, 3H), 7.54 (dd, J = 9.4, 1.8 Hz, 1H), 3.96 (s, 3H).

To a solution of compound **6** (969 mg, 2.93 mmol, 1.0 eq) in THF (12 mL) was added LiOH aqueous solution (561 mg, 23.41 mmol, 8.0 eq). The mixture was stirred at room temperature for 8 h. Solvent was concentrated in vacuo and the pH of residue was adjusted to 2 with 1 M HCl aqueous solution. After stirred at room temperature for additional 20 min, the mixture was filtered. Then the filter cake was collected and dried. The crude product **7** (904 mg, 97.42%) was given as yellow solid and could be used in the next step without further purification.

*4.1.3. Synthesis of (4-(3-bromoimidazo[1,2-a]pyridin-6-yl)phenyl) (morpholino)methanone (**8a**)*

To a solution of compound **7** (904 mg, 2.85 mmol, 1.0 eq) in DMF (5 mL) was added morpholine (298 mg, 3.42 mmol, 1.2 eq), followed by the addition of EDCI (601 mg, 3.42 mmol, 1.2 eq), HOBt (462 mg, 3.42 mmol, 1.2 eq), DMAP (35 mg, 0.29 mmol, 0.1 eq) and TEA (433 mg, 4.28 mmol, 1.5 eq). The mixture was heated at 40 °C for 8 h. After

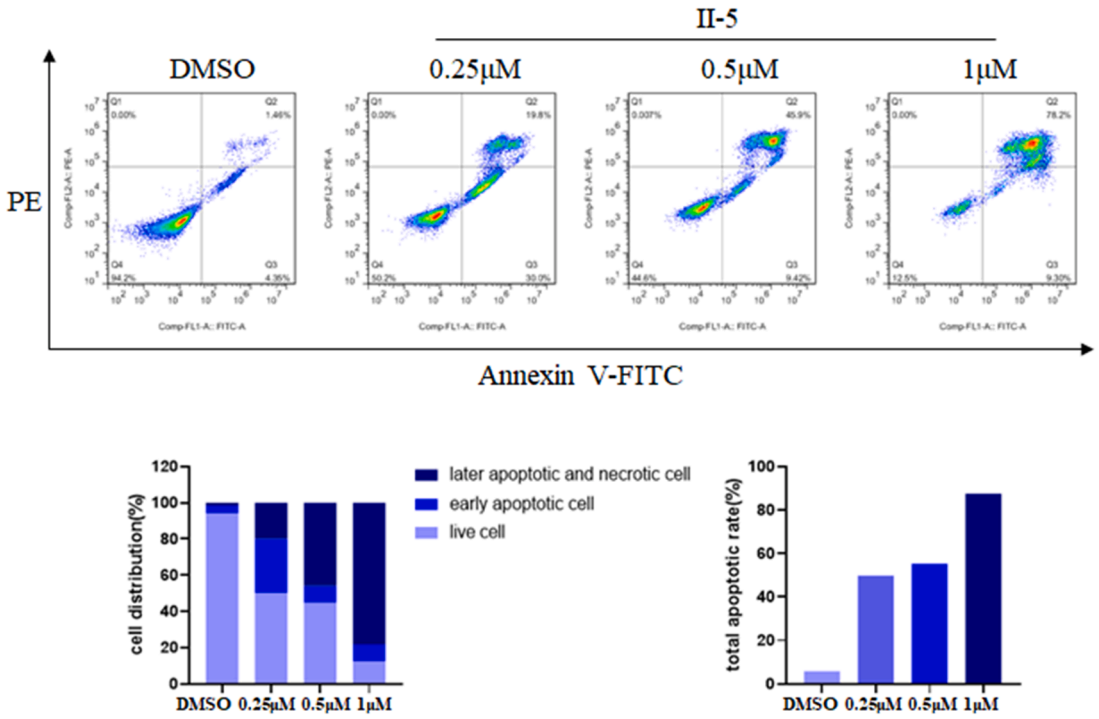


Fig. 3. Compound II-5 induced apoptosis in TMD-8 cells. TMD-8 cells were treated with compound II-5 at 0.25, 0.5 and 1 μM for 24 h, and apoptotic cells were determined by flow cytometry.

dilution with CH_2Cl_2 (200 mL), the mixture was washed with saturated sodium bicarbonate solution (50 mL × 3) and water (50 mL × 3). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, CH_2Cl_2/ CH_3OH = 100/1) to afford (4-(3-bromoimidazo[1,2-*a*] pyridin-6-yl)phenyl)(morpholino)methanone (**8a,** 953 mg, 86.78%) as yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.32 (s, 1H), 7.72 (dd, *J* = 21.1, 8.4 Hz, 4H), 7.55 (dd, *J* = 13.8, 9.4 Hz, 3H), 3.67 (d, *J* = 47.9 Hz, 8H).

*4.1.4. Synthesis of (4-(3-(isoquinolin-6-yl)imidazo[1,2-a]pyridin-6-yl) phenyl)(morpholino)methanone (**I-1**)*

To a solution of compound **8a** (100 mg, 0.26 mmol, 1.0 eq) in 1,4-dioxane (2 mL) and H_2O (0.2 mL) was added 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline (79 mg, 0.31 mmol, 1.2 eq) and $K_3PO_4{\cdot}3H_2O$ (138 mg, 0.52 mmol, 2.0 eq). The mixture was stirred at room temperature for 15 min under nitrogen. To the mixture was added $Pd(PPh_3)_4$ (30 mg, 0.03 mmol, 0.1 eq) and then heated at 95 °C for 8 h under nitrogen. The mixture was colded to room temperature and filtered with celite to remove insoluble solids. The filtrate was concentrated in vacuo and diluted with H_2O (50 mL). After extraction with CH_2Cl_2 (75 mL × 3), the combined organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, CH_2Cl_2/ CH_3OH = 100/1) to afford (4-(3-(isoquinolin-6-yl)imidazo[1,2-*a*]pyridin-6-yl)phenyl)(morpholino)methanone (**I-1,** 71 mg, 63.1%) as white solid. m.p.: 168-172℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.36 (s, 1H), 8.64 (d, *J* = 4.7 Hz, 2H), 8.18 (d, *J* = 8.4 Hz, 1H), 8.08 (s, 1H), 7.98–7.84 (m, 3H), 7.76 (d, *J* = 5.5 Hz, 1H), 7.69–7.50 (m, 5H), 3.68 (d, *J* = 69.2 Hz, 8H); MS: found *m/z* $[M+H]^+$ 435.1, calcd. *m/z* [M] 434.2.

*4.1.5. Synthesis of (4-(3-(quinolin-3-yl)imidazo[1,2-a]pyridin-6-yl) phenyl)(morpholino)methanone (**I-2**)*

Following general procedure A, compound **8a** (100 mg, 0.26 mmol, 1.0 eq), quinolin-3-ylboronic acid (54 mg, 0.31 mmol, 1.2 eq), $K_3PO_4{\cdot}3H_2O$ (138 mg, 0.52 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (30 mg, 0.03 mmol, 0.1 eq) provided **I-2** (70 mg, 62.2%) as white solid. m.p.: 125-130℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.20 (s, 1H), 8.53 (s, 1H), 8.38 (s, 1H) , 8.21 (d, *J* = 6.0 Hz, 1H), 7.96–7.81 (m, 4H), 7.69–7.51 (m, 6H), 3.65 (d, *J* = 75 Hz, 8H); MS: found *m/z* $[M+H]^+$ 435.2, calcd. *m/z* [M] 434.2.

*4.1.6. Synthesis of (4-(3-(quinolin-6-yl)imidazo[1,2-a]pyridin-6-yl) phenyl)(morpholino)methanone (**I-3**)*

Following general procedure A, compound **8a** (100 mg, 0.26 mmol, 1.0 eq), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline (79 mg, 0.31 mmol, 1.2 eq), $K_3PO_4{\cdot}3H_2O$ (138 mg, 0.52 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (30 mg, 0.03 mmol, 0.1 eq) provided **I-3** (66 mg, 58.8%) as white solid. m.p.: 125-130℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.00 (d, *J* = 3.7 Hz, 1H), 8.58 (s, 1H), 8.28 (dd, *J* = 13.6, 9.0 Hz, 2H), 8.06 (s, 1H), 7.97 (d, *J* = 7.4 Hz, 1H), 7.92–7.79 (m, 2H), 7.61 (d, *J* = 7.9 Hz, 2H), 7.52 (d, *J* = 7.9 Hz, 4H), 3.64 (d, *J* = 48.1 Hz, 8H); MS: found *m/z* $[M+H]^+$ 435.3, calcd. *m/z* [M] 434.2.

*4.1.7. Synthesis of (4-(3-bromoimidazo[1,2-a]pyridin-6-yl)phenyl)(4-morpholinopiperidin-1-yl)methanone (**8b**)*

Following general procedure B, compound **7** (500 mg, 1.58 mmol, 1.0 eq), 4-(piperidin-4-yl)morpholine (322 mg, 1.89 mmol, 1.2 eq), EDCI (322 mg, 1.89 mmol, 1.2 eq), HOBt (256 mg, 1.89 mmol, 1.2 eq), DMAP (20 mg, 0.16 mmol, 0.1 eq) and TEA (479 mg, 4.73 mmol, 3.0 eq) provided **8b** (520 mg, 70.27%) as yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.54–8.51 (m, 1H), 7.86 (d, *J* = 8.3 Hz, 2H), 7.81–7.79 (m, 1H), 7.77 (d, *J* = 0.8 Hz, 1H), 7.75 (d, *J* = 1.7 Hz, 1H), 7.53 (d, *J* = 8.3 Hz, 2H), 4.47 (s, 1H), 3.66 (s, 1H), 3.61–3.54 (m, 4H), 3.08 (s, 1H), 2.83 (s, 1H), 2.50–2.42 (m, 5H), 1.82 (d, *J* = 46.9 Hz, 2H), 1.37 (d, *J* = 18.8 Hz, 2H).

*4.1.8. Synthesis of (4-(3-(isoquinolin-6-yl)imidazo[1,2-a]pyridin-6-yl) phenyl)(4-morpholinopiperidin-1-yl)methanone (**I-4**)*

Following general procedure A, compound **8b** (100 mg, 0.21 mmol, 1.0 eq), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline (82 mg, 0.32 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (114 mg, 0.42 mmol, 2.0 eq)

and $Pd(PPh_3)_4$ (24 mg, 0.02 mmol, 0.1 eq) provided **I-4** (57 mg, 48%) as white solid. m.p.: 207-218℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.39 (d, J = 7.7 Hz, 1H), 8.94 (d, J = 7.1 Hz, 1H), 8.57 (dd, J = 7.7, 5.8 Hz, 1H), 8.43 (d, J = 7.3 Hz, 1H), 8.30 (t, J = 8.1 Hz, 1H), 8.16–8.04 (m, 2H), 8.03–7.94 (m, 1H), 7.85 (dd, J = 10.1, 5.7 Hz, 3H), 7.76 (dd, J = 9.3, 7.9 Hz, 1H), 7.51 (t, J = 8.0 Hz, 2H), 4.48 (s, 1H), 3.47 (d, J = 90.0 Hz, 10*H*), 2.95 (d, J = 95.3 Hz, 2H), 1.82 (d, J = 39.6 Hz, 2H), 1.48 (d, J = 5.4 Hz, 2H); MS: found m/z $[M+H]^+$ 518.3, calcd. m/z [M] 517.3.

*4.1.9. Synthesis of (4-morpholinopiperidin-1-yl)(4-(3-(quinolin-3-yl) imidazo[1,2-a]pyridin-6-yl)phenyl)methanone (**I-5**)*

Following general procedure A, compound **8b** (100 mg, 0.21 mmol, 1.0 eq), quinolin-3-ylboronic acid (56 mg, 0.32 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (114 mg, 0.42 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (24 mg, 0.02 mmol, 0.1 eq) provided **I-5** (28 mg, 25%) as white solid. m.p.: 202-207℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.22 (t, J = 11.3 Hz, 1H), 8.56 (d, J = 21.7 Hz, 1H), 8.46–8.35 (m, 1H), 8.21 (d, J = 8.4 Hz, 1H), 7.95 (dd, J = 15.5, 7.2 Hz, 2H), 7.84 (t, J = 10.3 Hz, 2H), 7.73–7.63 (m, 2H), 7.62–7.56 (m, 2H), 7.50 (d, J = 8.1 Hz, 2H), 4.77 (s, 1H), 3.77 (d, J = 3.6 Hz, 5H), 2.98 (d, J = 77.0 Hz, 2H), 2.61 (s, 6H), 1.90 (s, 4H); MS: found m/z $[M+H]^+$ 518.3, calcd. m/z [M] 517.3.

*4.1.10. Synthesis of (4-morpholinopiperidin-1-yl)(4-(3-(quinolin-6-yl) imidazo[1,2-a]pyridin-6-yl)phenyl)methanone (**I-6**)*

Following general procedure A, compound **8b** (100 mg, 0.21 mmol, 1.0 eq), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline (82 mg, 0.32 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (114 mg, 0.42 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (24 mg, 0.02 mmol, 0.1 eq) provided **I-6** (48 mg, 44%) as white solid. m.p.: 176-178℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.96 (dd, J = 4.2, 1.7 Hz, 1H), 8.89 (s, 1H), 8.51 (dd, J = 8.3, 1.4 Hz, 1H), 8.43 (d, J = 1.7 Hz, 1H), 8.16 (dt, J = 8.7, 5.3 Hz, 2H), 7.99 (s, 1H), 7.84 (dd, J = 9.0, 3.1 Hz, 3H), 7.73 (dd, J = 9.4, 1.7 Hz, 1H), 7.61 (dd, J = 8.3, 4.2 Hz, 1H), 7.50 (d, J = 8.3 Hz, 2H), 3.62 (s, 1H), 3.58 (s, 4H), 2.95 (d, J = 82.6 Hz, 2H), 2.50 (s, 6H), 1.80 (s, 2H), 1.36 (d, J = 8.3 Hz, 2H); MS: found m/z $[M+H]^+$ 518.3, calcd. m/z [M] 517.3.

*4.1.11. Synthesis of [1,4′-bipiperidin]-1′-yl(4-(3-bromoimidazo[1,2-a] pyridin-6-yl)phenyl)methanone (**8c**)*

Following general procedure B, compound **7** (500 mg, 1.58 mmol, 1.0 eq), 1,4′-bipiperidine (319 mg, 1.89 mmol, 1.2 eq), EDCI (322 mg, 1.89 mmol, 1.2 eq), HOBt (256 mg, 1.89 mmol, 1.2 eq), DMAP (20 mg, 0.16 mmol, 0.1 eq) and TEA (479 mg, 4.73 mmol, 3.0 eq) provided **8c** (670 mg, 90.92%) as yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.53 (s, 1H), 7.86 (d, J = 8.2 Hz, 2H), 7.77 (dt, J = 9.3, 5.0 Hz, 4H), 7.54 (d, J = 8.2 Hz, 2H), 4.56 (s, 1H), 3.71 (s, 1H), 3.05 (d, J = 22.6 Hz, 1H), 2.72 (d, J = 41.9 Hz, 6H), 1.87 (d, J = 44.9 Hz, 2H), 1.58 (s, 4H), 1.54–1.39 (m, 4H).

*4.1.12. Synthesis of [1,4′-bipiperidin]-1′-yl(4-(3-(isoquinolin-6-yl)imidazo [1,2-a]pyridin-6-yl)phenyl)methanone (**I-7**)*

Following general procedure A, compound **8c** (100 mg, 0.21 mmol, 1.0 eq), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline (77 mg, 0.30 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (114 mg, 0.42 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (24 mg, 0.02 mmol, 0.1 eq) provided **I-7** (40 mg, 36%) as white solid. m.p.: 158-165℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.39 (d, J = 5.9 Hz, 1H), 8.94 (d, J = 5.4 Hz, 1H), 8.57 (t, J = 5.9 Hz, 1H), 8.43 (d, J = 5.7 Hz, 1H), 8.36–8.26 (m, 1H), 8.09 (ddd, J = 16.6, 9.1, 3.9 Hz, 2H), 7.97 (t, J = 5.9 Hz, 1H), 7.90–7.83 (m, 3H), 7.77 (dd, J = 7.8, 3.2 Hz, 1H), 7.51 (dd, J = 8.0, 6.2 Hz, 2H), 4.55 (s, 1H), 3.70 (s, 1H), 3.07 (s, 1H), 2.75 (s, 1H), 2.61 (s, 3H), 1.77 (s, 2H), 1.56 (s, 4H), 1.50–1.40 (m, 4H); MS: found m/z $[M+H]^+$ 516.3, calcd. m/z [M] 515.3.

*4.1.13. Synthesis of [1,4′-bipiperidin]-1′-yl(4-(3-(quinolin-3-yl)imidazo [1,2-a]pyridin-6-yl)phenyl)methanone (**I-8**)*

Following general procedure A, compound **8c** (100 mg, 0.21 mmol, 1.0 eq), quinolin-3-ylboronic acid (56 mg, 0.32 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (114 mg, 0.42 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (24 mg, 0.02 mmol, 0.1 eq) provided **I-8** (52 mg, 47%) as white solid. m.p.: 168-172℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.27 (d, J = 2.1 Hz, 1H), 8.92 (s, 1H), 8.85 (s, 1H), 8.17–8.07 (m, 3H), 7.88–7.84 (m, 3H), 7.82 (d, J = 9.4 Hz, 1H), 7.78–7.74 (m, 1H), 7.70 (dd, J = 13.1, 5.7 Hz, 1H), 7.51 (t, J = 7.1 Hz, 2H), 4.54 (s, 1H), 3.70 (s, 1H), 3.04 (d, J = 19.7 Hz, 1H), 2.88–2.56 (m, 5H), 2.00 (s, 1H), 1.76 (s, 2H), 1.56 (s, 5H), 1.43 (s, 4H); MS: found m/z $[M+H]^+$ 516.3, calcd. m/z [M] 515.3.

*4.1.14. Synthesis of [1,4′-bipiperidin]-1′-yl(4-(3-(quinolin-6-yl)imidazo [1,2-a]pyridin-6-yl)phenyl)methanone (**I-9**)*

Following general procedure A, compound **8c** (100 mg, 0.21 mmol, 1.0 eq), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline (77 mg, 0.30 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (114 mg, 0.42 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (24 mg, 0.02 mmol, 0.1 eq) provided **I-9** (56 mg, 51%) as white solid. m.p.: 147-156℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.96 (dd, J = 4.2, 1.7 Hz, 1H), 8.90 (s, 1H), 8.52 (d, J = 8.2 Hz, 1H), 8.44 (s, 1H), 8.22–8.12 (m, 2H), 8.01 (s, 1H), 7.84 (dd, J = 8.8, 4.5 Hz, 3H), 7.74 (dd, J = 9.4, 1.7 Hz, 1H), 7.62 (dd, J = 8.3, 4.2 Hz, 1H), 7.51 (t, J = 8.4 Hz, 2H), 4.51 (s, 1H), 3.66 (s, 1H), 3.01 (d, J = 25.2 Hz, 1H), 2.72 (d, J = 27.6 Hz, 1H), 2.52 (d, J = 1.7 Hz, 5H), 1.76 (d, J = 51.4 Hz, 2H), 1.49 (s, 4H), 1.42–1.34 (m, 4H); MS: found m/z $[M+H]^+$ 516.3, calcd. m/z [M] 515.3.

*4.1.15. Synthesis of tert-butyl 4-(4-(3-bromoimidazo[1,2-a]pyridin-6-yl) benzoyl)piperazine-1-carboxylate (**8d**)*

Following general procedure B, compound **7** (500 mg, 1.58 mmol, 1.0 eq), *tert*-butyl piperazine-1-carboxylate (353 mg, 1.89 mmol, 1.2 eq), EDCI (322 mg, 1.89 mmol, 1.2 eq), HOBt (256 mg, 1.89 mmol, 1.2 eq), DMAP (20 mg, 0.16 mmol, 0.1 eq) and TEA (479 mg, 4.73 mmol, 3.0 eq) provided **8c** (663 mg, 86.73%) as yellow solid.

Synthesis of *Tert*-butyl-4-(4-(3-(isoquinolin-6-yl)imidazo[1,2-*a*]pyridine-6-yl)benzoyl)piperazine-1-carboxylate (**9a**)

Following general procedure A, compound **8d** (100 mg, 0.21 mmol, 1.0 eq), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline (79 mg, 0.31 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (114 mg, 0.42 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (24 mg, 0.02 mmol, 0.1 eq) provided **9a** (78 mg, 71%) as white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.36 (s, 1H), 8.64 (d, J = 5.8 Hz, 2H), 8.20 (d, J = 8.5 Hz, 1H), 8.08 (s, 1H), 7.96–7.84 (m, 3H), 7.77 (d, J = 5.9 Hz, 1H), 7.64 (s, 1H), 7.60 (dd, J = 6.5, 4.7 Hz, 2H), 7.53 (d, J = 8.2 Hz, 2H), 3.84–3.38 (m, 9H), 1.49 (s, 9H).

*4.1.16. Synthesis of (4-(3-(isoquinolin-6-yl)imidazo[1,2-a]pyridin-6-yl) phenyl)(piperazin-1-yl)methanone (**I-10**)*

To a solution of compound **9a** (78 mg, 0.15 mmol, 1.0 eq) in CH_2Cl_2 (3 mL) was added TFA (167 mg, 1.46 mmol, 10 eq) dropwise. The mixture was stirred at room temperature for 4 h. The mixture was concentrated in vacuo, and the residue was diluted with CH_2Cl_2 (3 mL). To the mixture was then added TEA (18 mg, 0.18 mmol, 1.2 eq). After stirred at room temperature for additional 30 min, the mixture was concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, CH_2Cl_2/ CH_3OH = 20/1) to afford (4-(3-(isoquinolin-6-yl)imidazo[1,2-*a*]pyridin-6-yl)phenyl)(piperazin-1-yl)methanone (**I-10,** 41 mg, 65%) as yellow solid. m.p.: 189-196℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.38 (s, 1H), 8.93 (s, 1H), 8.57 (d, J = 5.7 Hz, 1H), 8.42 (s, 1H), 8.30 (d, J = 8.5 Hz, 1H), 8.09 (dd, J = 8.5, 1.4 Hz, 1H), 8.06 (s, 1H), 7.96 (d, J = 5.7 Hz, 1H), 7.91–7.83 (m, 3H), 7.75 (dd, J = 9.4, 1.4 Hz, 1H), 7.55 (d, J = 8.2 Hz, 2H), 3.60 (d, J = 20.4 Hz, 4H), 2.99 (d, J = 24.9 Hz, 4H); MS: found m/z $[M+H]^+$ 434.3, calcd. m/z [M] 433.2.

*4.1.17. Synthesis of Tert-butyl-4-(4-(3-(quinolin-3-yl)imidazo[1,2-a] pyridine-6-yl)benzoyl)piperazine-1-carboxylate (**9b**)*

Following general procedure A, compound **8d** (100 mg, 0.21 mmol, 1.0 eq), quinolin-3-ylboronic acid (53 mg, 0.31 mmol, 1.5 eq),

$K_3PO_4{\cdot}3H_2O$ (114 mg, 0.42 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (24 mg, 0.02 mmol, 0.1 eq) provided **9b** (82 mg, 75%) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.27 (s, 1H), 8.93 (s, 1H), 8.84 (s, 1H), 8.12 (dd, J = 12.5, 9.6 Hz, 3H), 7.93–7.65 (m, 6H), 7.53 (d, J = 7.8 Hz, 2H), 3.48 (d, J = 79.7 Hz, 8H), 1.42 (s, 9H).

*4.1.18. Synthesis of Piperazin-1-yl(4-(3-(quinolin-3-yl)imidazo[1,2-a] pyridin-6-yl)phenyl)methanone (**I-11**)*

Following general procedure E, compound **9b** (82 mg, 0.15 mmol, 1.0 eq), TFA (167 mg, 1.46 mmol, 10 eq) and TEA (18 mg, 0.18 mmol, 1.2 eq) provided **I-11** (45 mg, 68%) as white solid. m.p.: 139-145℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.27 (d, J = 1.6 Hz, 1H), 9.05 (s, 1H), 8.92 (s, 1H), 8.84 (s, 1H), 8.20–8.06 (m, 3H), 7.96–7.65 (m, 6H), 7.56 (dd, J = 21.6, 8.1 Hz, 2H), 3.67 (d, J = 36.1 Hz, 4H), 3.11 (d, J = 7.0 Hz, 4H); MS: found m/z $[M+H]^+$ 434.3, calcd. m/z [M] 433.2.

*4.1.19. Synthesis of Tert-butyl-4-(4-(3-(quinolin–6-yl) imidazo [1,2-a] pyridine-6-yl) benzoyl) piperazine-1-carboxylate (**9c**)*

Following general procedure A, compound **8d** (100 mg, 0.21 mmol, 1.0 eq), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline (79 mg, 0.31 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (114 mg, 0.42 mmol, 2.0 eq) and $Pd(PPh_3)_4$ (24 mg, 0.02 mmol, 0.1 eq) provided **9c** (70 mg, 64%) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.96 (d, J = 2.0 Hz, 1H), 8.89 (s, 1H), 8.51 (d, J = 8.4 Hz, 1H), 8.43 (s, 1H), 8.17 (dd, J = 16.5, 7.7 Hz, 2H), 8.01 (s, 1H), 7.89–7.82 (m, 3H), 7.74 (d, J = 9.3 Hz, 1H), 7.62 (ddd, J = 8.2, 4.1, 2.6 Hz, 1H), 7.57–7.50 (m, 2H), 3.49 (d, J = 91.7 Hz, 9H), 1.42 (d, J = 2.2 Hz, 9H).

*4.1.20. Synthesis of Piperazin-1-yl(4-(3-(quinolin-6-yl)imidazo[1,2-a] pyridin-6-yl)phenyl)methanone (**I-12**)*

Following general procedure E, compound **9c** (70 mg, 0.13 mmol, 1.0 eq), TFA (127 mg, 1.31 mmol, 10 eq) and TEA (16 mg, 0.16 mmol, 1.2 eq) provided **I-12** (33 mg, 58%) as white solid. m.p.: 194-200℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.00 (s, 1H), 8.97 (dd, J = 4.2, 1.7 Hz, 1H), 8.89 (s, 1H), 8.52 (d, J = 7.4 Hz, 1H), 8.44 (d, J = 1.6 Hz, 1H), 8.17 (dt, J = 8.8, 5.3 Hz, 2H), 8.05 (s, 1H), 7.89 (d, J = 5.3 Hz, 1H), 7.87 (d, J = 4.4 Hz, 2H), 7.79 (dd, J = 9.4, 1.5 Hz, 1H), 7.62 (dd, J = 8.3, 4.2 Hz, 1H), 7.59 (d, J = 8.3 Hz, 2H), 3.82–3.60 (m, 4H), 3.19 (d, J = 3.8 Hz, 4H); MS: found m/z $[M+H]^+$ 434.3, calcd. m/z [M] 433.2.

*4.1.21. Synthesis of Tert-butyl (1-(4-(3-bromoimidazo[1,2-a]pyridin-6-yl) benzoyl)piperidin-4-yl)carbamate (**10a**)*

Following general procedure B, compound **7** (300 mg, 0.95 mmol, 1.0 eq), 4-(Boc-amino)piperidine (227 mg, 1.14 mmol, 1.2 eq), EDCI (199 mg, 1.14 mmol, 1.2 eq), HOBt (153 mg, 1.14 mmol, 1.2 eq), DMAP (12 mg, 0.09 mmol, 0.1 eq) and TEA (191 mg, 1.89 mmol, 2.0 eq) provided **10a** (430 mg, 91%) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.53 (s, 1H), 7.87 (s, 2H), 7.82–7.79 (m, 1H), 7.79–7.72 (m, 2H), 7.51 (s, 2H), 6.93 (s, 1H), 4.34 (s, 1H), 3.56 (s, 2H), 3.06 (d, J = 70.2 Hz, 2H), 2.81 (ddd, J = 63.2, 17.4, 14.3 Hz, 2H), 1.76 (s, 2H), 1.40 (s, 9H).

*4.1.22. Synthesis of Tert-butyl (1-(4-(3-(isoquinolin-6-yl)imidazo[1,2-a] pyridin-6-yl)benzoyl)piperidin-4-yl)carbamate (**11a**)*

Following general procedure A, compound **10a** (100 mg, 0.21 mmol, 1.0 eq), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline (78 mg, 0.31 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (137 mg, 0.52 mmol, 2.5 eq) and $Pd(PPh_3)_4$ (23 mg, 0.02 mmol, 0.1 eq) provided **11a** (66 mg, 60%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.37 (s, 1H), 8.64 (d, J = 8.4 Hz, 2H), 8.20 (d, J = 8.5 Hz, 1H), 8.09 (s, 1H), 7.91 (dd, J = 17.5, 8.2 Hz, 3H), 7.78 (d, J = 5.7 Hz, 1H), 7.64–7.57 (m, 3H), 7.52 (d, J = 8.1 Hz, 2H), 4.57 (d, J = 48.8 Hz, 2H), 3.76 (s, 2H), 3.08 (d, J = 56.4 Hz, 2H), 2.09 (t, J = 17.9 Hz, 3H), 1.47 (s, 9H).

*4.1.23. Synthesis of (4-aminopiperidin-1-yl)(4-(3-(isoquinolin-6-yl) imidazo[1,2-a]pyridin-6-yl)phenyl)methanone (**II-1**)*

Following general procedure E, compound **11a** (66 mg, 0.12 mmol, 1.0 eq), TFA (137 mg, 1.21 mmol, 10 eq) and TEA (15 mg, 0.14 mmol, 1.2 eq) provided **II-1** (25 mg, 46%) as yellow solid. m.p.: 178-183℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.51 (s, 1H), 9.00 (s, 1H), 8.62 (d, J = 5.6 Hz, 1H), 8.51 (s, 1H), 8.40 (d, J = 8.6 Hz, 1H), 8.25 (s, 1H), 8.17 (d, J = 8.5 Hz, 1H), 8.10 (d, J = 6.0 Hz, 3H), 7.96 (t, J = 9.2 Hz, 2H), 7.89 (d, J = 8.1 Hz, 2H), 7.51 (d, J = 8.1 Hz, 2H), 4.46 (d, J = 23.2 Hz, 1H), 4.27 (dt, J = 17.5, 8.8 Hz, 1H), 3.33 (s, 2H), 2.91 (s, 1H), 1.96 (d, J = 14.4 Hz, 2H), 1.48 (s, 2H); MS: found m/z $[M+H]^+$ 448.2, calcd. m/z [M] 447.2.

*4.1.24. Synthesis of (S)-tert-butyl (1-(4-(3-bromoimidazo[1,2-a]pyridin-6-yl)benzoyl)piperidin-4-yl)carbamate (**10b**)*

Following general procedure B, compound **7** (290 mg, 0.91 mmol, 1.0 eq), (S)-3-(Boc-amino)piperidine (220 mg, 1.10 mmol, 1.2 eq), EDCI (191 mg, 1.09 mmol, 1.2 eq), HOBt (146 mg, 1.09 mmol, 1.2 eq), DMAP (12 mg, 0.09 mmol, 0.1 eq) and TEA (184 mg, 1.82 mmol, 2.0 eq) provided **10b** (403 mg, 88%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.52 (s, 1H), 7.91–7.70 (m, 5H), 7.54 (d, J = 7.8 Hz, 2H), 6.99 (s, 1H), 3.37 (s, 2H), 3.02 (s, 1H), 2.90 (s, 1H), 2.74 (s, 1H), 1.75 (d, J = 60.4 Hz, 2H), 1.54–1.26 (m, 11H).

*4.1.25. Synthesis of Tert-butyl (S)-(1-(4-(3-(isoquinolin-6-yl)imidazo[1,2-a]pyridin-6-yl)benzoyl)piperidin-3-yl) carbamate (**11b**)*

Following general procedure A, compound **10b** (100 mg, 0.21 mmol, 1.0 eq), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline (77 mg, 0.31 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (137 mg, 0.52 mmol, 2.5 eq) and $Pd(PPh_3)_4$ (23 mg, 0.02 mmol, 0.1 eq) provided **11b** (76 mg, 69%) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.38 (s, 1H), 8.92 (s, 1H), 8.56 (d, J = 5.5 Hz, 1H), 8.42 (s, 1H), 8.30 (d, J = 8.4 Hz, 1H), 8.14–8.04 (m, 2H), 7.97 (d, J = 4.9 Hz, 1H), 7.86 (d, J = 9.2 Hz, 3H), 7.76 (d, J = 9.4 Hz, 1H), 7.51 (d, J = 7.1 Hz, 2H), 6.99 (s, 1H), 4.15 (d, J = 149.1 Hz, 2H), 2.85 (d, J = 132.5 Hz, 3H), 1.84 (t, J = 67.1 Hz, 3H), 1.42 (s, 9H).

*4.1.26. Synthesis of (S)-(3-aminopiperidin-1-yl)(4-(3-(isoquinolin-6-yl) imidazo[1,2-a]pyridin-6-yl)pheny)methanone (**II-2**)*

Following general procedure E, compound **11b** (76 mg, 0.14 mmol, 1.0 eq), TFA (161 mg, 1.41 mmol, 10 eq) and TEA (17 mg, 0.17 mmol, 1.2 eq) provided **II-2** (30 mg, 48%) as yellow solid. m.p.: 181-189℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.38 (s, 1H), 8.93 (s, 1H), 8.57 (d, J = 5.7 Hz, 1H), 8.42 (s, 1H), 8.30 (d, J = 8.6 Hz, 1H), 8.09 (dd, J = 8.5, 1.6 Hz, 1H), 8.05 (s, 1H), 7.96 (d, J = 5.8 Hz, 1H), 7.86 (d, J = 8.2 Hz, 3H), 7.75 (dd, J = 9.4, 1.6 Hz, 1H), 7.52 (d, J = 8.2 Hz, 2H), 3.39 (dd, J = 14.0, 7.0 Hz, 2H), 3.18 (s, 1H), 2.96–2.85 (m, 2H), 2.00–1.83 (m, 1H), 1.68 (s, 1H), 1.41 (d, J = 10.1 Hz, 2H); MS: found m/z $[M+H]^+$ 448.2, calcd. m/z [M] 447.2.

*4.1.27. Synthesis of (R)-tert-butyl (1-(4-(3-bromoimidazo[1,2-a]pyridin-6-yl)benzoyl)piperidin-4-yl)carbamate (**10c**)*

Following general procedure B, compound **7** (300 mg, 0.95 mmol, 1.0 eq), (R)-3-(Boc-amino)piperidine (227 mg, 1.14 mmol, 1.2 eq), EDCI (199 mg, 1.14 mmol, 1.2 eq), HOBt (153 mg, 1.14 mmol, 1.2 eq), DMAP (12 mg, 0.09 mmol, 0.1 eq) and TEA (191 mg, 1.89 mmol, 2.0 eq) provided **10c** (422 mg, 89%) as white solid.

*4.1.28. Synthesis of Tert-butyl (R)-(1-(4-(3-(isoquinolin-6-yl)imidazo [1,2-a]pyridin-6-yl)benzoyl)piperidin-3-yl)carbamate (**11c**)*

Following general procedure A, compound **10c** (100 mg, 0.21 mmol, 1.0 eq), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline (77 mg, 0.31 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (137 mg, 0.52 mmol, 2.5 eq) and $Pd(PPh_3)_4$ (23 mg, 0.02 mmol, 0.1 eq) provided **11c** (88 mg, 80%) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.39 (d, J = 9.3 Hz, 1H), 8.92 (s, 1H), 8.57 (t, J = 7.6 Hz, 1H), 8.42 (s, 1H), 8.30 (d, J =

8.6 Hz, 1H), 8.13–8.05 (m, 2H), 7.97 (s, 1H), 7.86 (d, J = 9.3 Hz, 3H), 7.76 (s, 1H), 7.52 (s, 2H), 6.99 (s, 1H), 4.40–3.90 (m, 2H), 3.70 (s, 1H), 3.10 (d, J = 64.3 Hz, 3H), 2.68 (s, 1H), 1.84 (t, J = 66.6 Hz, 3H), 1.42 (s, 9H).

*4.1.29. Synthesis of (R)-(3-aminopiperidin-1-yl)(4-(3-(isoquinolin-6-yl) imidazo[1,2-a]pyridin-6-yl)phenyl)methanone (**II-3**)*

Following general procedure E, compound **11c** (88 mg, 0.16 mmol, 1.0 eq), TFA (182 mg, 1.61 mmol, 10 eq) and TEA (20 mg, 0.19 mmol, 1.2 eq) provided **II-3** (49 mg, 68%) as yellow solid. m.p.: 182-192℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.38 (s, 1H), 8.93 (s, 1H), 8.56 (d, J = 5.7 Hz, 1H), 8.42 (s, 1H), 8.30 (d, J = 8.7 Hz, 1H), 8.09 (d, J = 8.6 Hz, 1H), 8.05 (s, 1H), 7.96 (d, J = 5.5 Hz, 1H), 7.85 (d, J = 7.3 Hz, 3H), 7.76 (d, J = 9.5 Hz, 1H), 7.51 (d, J = 7.8 Hz, 2H), 4.26 (d, J = 48.1 Hz, 1H), 3.64 (d, J = 19.4 Hz, 1H), 3.02 (s, 1H), 2.84 (s, 2H), 1.98–1.64 (m, 2H), 1.38 (s, 2H), 1.34 (s, 2H); MS: found *m/z* $[M+H]^+$ 448.2, calcd. *m/z* [M] 447.2.

*4.1.30. Synthesis of 4-(3-bromoimidazo[1,2-b]pyridazin-6-yl)benzoic acid (**16a**)*

To a solution of 6-chloropyridazin-3-amine (**12a**, 5 g, 38.60 mmol, 1.0 eq) in C_2H_5OH (50 mL) and H_2O (30 mL) was added 2-bromo-1,1-diethoxyethane (**3**, 11.41 g, 57.89 mmol, 1.5 eq) and 40% HBr aqueous solution (5 mL) at room temperature, respectively. The mixture was heated at 100 °C for 8 h. Solvent was concentrated in vacuo and the pH of residue was adjusted to 7 with $NaHCO_3$ aqueous solution. The mixture was diluted with H_2O (50 mL) and extracted with EtOAc (150 mL × 3). The combined organic phase was washed with saturated sodium bicarbonate solution (75 mL × 3), dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, petroleum ether/EtOAc = 5/1) to afford 6-chloroimidazo[1,2-*b*]pyridazine (**13a,** 5.38 g, 90.77%) as white solid.

Following general procedure A, compound **13a** (2 g, 13.02 mmol, 1.0 eq), (4-(methoxycarbonyl)phenyl)boronic acid (2.81 g, 15.63 mmol, 1.2 eq), Na_2CO_3 (4.14 g, 39.07 mmol, 3.0 eq) and $Pd(PPh_3)_4$ (752.49 mg, 0.65 mmol, 0.05 eq) provided **14a** (2.51 g, 76.10%) as white solid.

Following general procedure C, compound **14a** (2 g, 7.90 mmol, 1.0 eq) and NBS (1.69 g, 9.48 mmol, 1.2 eq) provided **15a** (2.23 g, 85.02%) as yellow solid.

Following general procedure D, compound **15a** (1 g, 3.01 mmol, 1.0 eq) and LiOH aqueous solution (576.76 mg, 24.08 mmol, 8.0 eq) provided **16a** (935.21 mg, 97.64%) as yellow solid.

*4.1.31. Synthesis of 4-(3-bromoimidazo[1,2-a]pyrazin-6-yl)benzoic acid (**16b**)*

To a solution of 5-bromopyrazin-2-amine (**12b**, 5 g, 28.74 mmol, 1.0 eq) in C_2H_5OH (50 mL) and H_2O (30 mL) was added 2-bromo-1,1-diethoxyethane (**2**, 8.49 g, 43.10 mmol, 1.5 eq) and 40% HBr aqueous solution (5 mL) at room temperature, respectively. The mixture was heated at 100 °C for 8 h. Solvent was concentrated in vacuo and the pH of residue was adjusted to 7 with $NaHCO_3$ aqueous solution. The mixture was diluted with H_2O (50 mL) and extracted with EtOAc (150 mL × 3). The combined organic phase was washed with saturated sodium bicarbonate solution (75 mL × 3), dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, petroleum ether/EtOAc = 5/1) to afford 6-bromoimidazo[1,2-*a*]pyrazine (**13b,** 5.09 g, 89.45%) as white solid.

Following general procedure A, compound **13b** (2 g, 10.10 mmol, 1.0 eq), (4-(methoxycarbonyl)phenyl)boronic acid (2.18 g, 12.12 mmol, 1.2 eq), $K_3PO_4{\cdot}3H_2O$ (6.72 g, 25.25 mmol, 2.5 eq) and $Pd(PPh_3)_4$ (583.56 mg, 0.50 mmol, 0.05 eq) provided **14b** (1.98 g, 77.41%) as white solid.

Following general procedure C, compound **14b** (2 g, 7.90 mmol, 1.0 eq) and NBS (1.69 g, 9.48 mmol, 1.2 eq) provided **15b** (2.32 g, 88.45%) as yellow solid.

Following general procedure D, compound **15b** (1 g, 3.01 mmol, 1.0 eq) and LiOH aqueous solution (576.76 mg, 24.08 mmol, 8.0 eq) provided **16b** (922.35 mg, 96.30%) as yellow solid.

*4.1.32. Synthesis of (4-aminopiperidin-1-yl)(4-(3-(isoquinolin-6-yl) imidazo[1,2-a]pyridazin-6-yl)phenyl)methanone (**II-4**)*

Following general procedure B, compound **16a** (400 mg, 1.26 mmol, 1.0 eq), 4-(Boc-amino)piperidine (302 mg, 1.51 mmol, 1.2 eq), EDCI (264 mg, 1.51 mmol, 1.2 eq), HOBt (203 mg, 2.52 mmol, 1.2 eq), DMAP (17 mg, 0.13 mmol, 0.1 equiv) and TEA (339 mg, 1.89 mmol, 2.0 eq) provided **17a** (330 mg, 53%) as white solid.

Following general procedure A, compound **17a** (100 mg, 0.20 mmol, 1.0 eq), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline (76 mg, 0.30 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (132 mg, 0.50 mmol, 2.5 eq) and $Pd(PPh_3)_4$ (23 mg, 0.02 mmol, 0.1 eq) provided **18a** (89 mg, 71%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.33 (d, J = 7.3 Hz, 1H), 8.86 (d, J = 6.8 Hz, 1H), 8.66–8.57 (m, 1H), 8.40–8.30 (m, 2H), 8.17 (dt, J = 19.5, 8.1 Hz, 4H), 7.83–7.76 (m, 1H), 7.64 (dd, J = 11.7, 5.3 Hz, 3H), 4.62 (d, J = 50.1 Hz, 2H), 3.79 (s, 2H), 3.12 (d, J = 65.0 Hz, 2H), 2.04 (s, 3H), 1.47 (t, J = 12.4 Hz, 9H).

Following general procedure E, compound **18a** (89 mg, 0.16 mmol, 1.0 eq), TFA (184 mg, 1.61 mmol, 10 eq) and TEA (21 mg, 0.19 mmol, 1.2 eq) provided **II-4** (44 mg, 60%) as yellow solid. m.p.: 195-199℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.36 (s, 1H), 9.02 (s, 1H), 8.58 (dd, J = 8.8, 2.7 Hz, 2H), 8.49 (d, J = 8.4 Hz, 1H), 8.42 (dd, J = 9.4, 2.6 Hz, 1H), 8.29 (s, 3H), 8.07–8.00 (m, 1H), 7.96 (s, 1H), 7.70–7.60 (m, 2H), 4.53–4.24 (m, 2H), 3.18 (d, J = 2.4 Hz, 2H), 2.96 (s, 1H), 1.97 (d, J = 12.7 Hz, 2H), 1.40 (s, 2H); MS: found *m/z* $[M+H]^+$ 449.2, calcd. *m/z* [M] 448.2.

*4.1.33. Synthesis of (S)-(3-aminopiperidin-1-yl)(4-(3-(isoquinolin-6-yl) imidazo[1,2-a]pyridazin-6-yl)phenyl)methanone (**II-5**)*

Following general procedure B, compound **16a** (400 mg, 1.26 mmol, 1.0 eq), (*S*)-3-(Boc-amino)piperidine (302 mg, 1.51 mmol, 1.2 eq), EDCI (264 mg, 1.51 mmol, 1.2 eq), HOBt (203 mg, 2.52 mmol, 1.2 eq), DMAP (17 mg, 0.13 mmol, 0.1 equiv) and TEA (339 mg, 1.89 mmol, 2.0 eq) provided **17b** (529 mg, 84%) as white solid.

Following general procedure A, compound **17b** (100 mg, 0.21 mmol, 1.0 eq), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline (76 mg, 0.30 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (137 mg, 0.52 mmol, 2.5 eq) and $Pd(PPh_3)_4$ (23 mg, 0.02 mmol, 0.1 eq) provided **18b** (92 mg, 84%) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.35 (s, 1H), 9.02 (s, 1H), 8.60–8.54 (m, 2H), 8.49 (d, J = 8.7 Hz, 1H), 8.41 (d, J = 9.5 Hz, 1H), 8.28 (d, J = 8.3 Hz, 3H), 8.02 (d, J = 9.4 Hz, 1H), 7.96 (d, J = 5.2 Hz, 1H), 7.66 (d, J = 6.2 Hz, 2H), 7.01 (d, J = 23.1 Hz, 1H), 4.15 (d, J = 156.6 Hz, 1H), 3.46 (s, 2H), 3.14 (d, J = 60.6 Hz, 2H), 2.72 (d, J = 28.7 Hz, 1H), 1.87 (s, 2H), 1.68 (s, 1H), 1.43 (s, 9H).

Following general procedure E, compound **18b** (92 mg, 0.17 mmol, 1.0 eq), TFA (195 mg, 1.71 mmol, 10 eq) and TEA (21 mg, 0.19 mmol, 1.2 eq) provided **II-5** (38 mg, 51%) as yellow solid. m.p.: 193-199℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.42 (s, 1H), 9.06 (s, 1H), 8.62 (s, 1H), 8.59 (d, J = 6.2 Hz, 1H), 8.53 (d, J = 8.6 Hz, 1H), 8.44 (d, J = 9.4 Hz, 1H), 8.35–8.29 (m, 2H), 8.10–7.95 (m, 3H), 7.71 (d, J = 7.7 Hz, 2H), 4.47–4.05 (m, 1H), 3.86 (s, 1H), 3.25 (s, 3H), 2.04 (s, 1H), 1.77 (s, 1H), 1.60 (s, 2H); MS: found *m/z* $[M+H]^+$ 449.2, calcd. *m/z* [M] 448.2.

*4.1.34. Synthesis of (R)-(3-aminopiperidin-1-yl)(4-(3-(isoquinolin-6-yl) imidazo[1,2-a]pyridazin-6-yl)phenyl)methanone (**II-6**)*

Following general procedure B, compound **16a** (400 mg, 1.26 mmol, 1.0 eq), (R)-3-(Boc-amino)piperidine (302 mg, 1.51 mmol, 1.2 eq), EDCI (264 mg, 1.51 mmol, 1.2 eq), HOBt (203 mg, 2.52 mmol, 1.2 eq), DMAP (17 mg, 0.13 mmol, 0.1 equiv) and TEA (339 mg, 1.89 mmol, 2.0 eq) provided **17c** (460 mg, 73%) as white solid.

Following general procedure A, compound **17c** (100 mg, 0.21 mmol, 1.0 eq), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline (76 mg, 0.30 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (137 mg, 0.52 mmol, 2.5 eq) and $Pd(PPh_3)_4$ (23 mg, 0.02 mmol, 0.1 eq) provided **18c** (83 mg, 76%)

as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.32 (s, 1H), 8.87 (s, 1H), 8.60 (d, J = 5.8 Hz, 1H), 8.38–8.31 (m, 2H), 8.22–8.09 (m, 4H), 7.82 (d, J = 5.5 Hz, 1H), 7.66 (dd, J = 9.0, 2.6 Hz, 3H), 4.62 (s, 1H), 4.39 (q, J = 7.2 Hz, 1H), 3.87 (dd, J = 42.1, 37.4 Hz, 2H), 3.30 (s, 2H), 2.03 (s, 3H), 1.40 (d, J = 7.1 Hz, 9H).

Following general procedure E, compound **18c** (83 mg, 0.15 mmol, 1.0 eq), TFA (172 mg, 1.51 mmol, 10 eq) and TEA (20 mg, 0.18 mmol, 1.2 eq) provided **II-6** (51 mg, 75%) as yellow solid. m.p.: 187-205℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.41 (s, 1H), 9.06 (s, 1H), 8.65–8.57 (m, 2H), 8.53 (d, J = 9.9 Hz, 1H), 8.45 (d, J = 9.5 Hz, 1H), 8.32 (dd, J = 8.4, 4.7 Hz, 3H), 8.04 (dd, J = 13.6, 7.7 Hz, 2H), 7.72 (d, J = 8.2 Hz, 2H), 4.29 (dd, J = 14.4, 7.2 Hz, 2H), 3.28 (s, 2H), 3.18 (s, 1H), 2.00 (d, J = 37.9 Hz, 1H), 1.69 (d, J = 65.6 Hz, 3H); MS: found m/z $[M+H]^+$ 449.2, calcd. m/z [M] 448.2.

4.1.35. Synthesis of (4-aminopiperidin-1-yl)(4-(3-(isoquinolin-6-yl) imidazo[1,2-a]pyrazin-6-yl)phenyl)methanone (II-7)

Following general procedure B, compound **16b** (400 mg, 1.26 mmol, 1.0 eq), 4-(Boc-amino)piperidine (302 mg, 1.51 mmol, 1.2 eq), EDCI (264 mg, 1.51 mmol, 1.2 eq), HOBt (203 mg, 2.52 mmol, 1.2 eq), DMAP (17 mg, 0.13 mmol, 0.1 equiv) and TEA (339 mg, 1.89 mmol, 2.0 eq) provided **17d** (440 mg, 70%) as white solid.

Following general procedure A, compound **17d** (100 mg, 0.21 mmol, 1.0 eq), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline (76 mg, 0.30 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (137 mg, 0.52 mmol, 2.5 eq) and $Pd(PPh_3)_4$ (23 mg, 0.02 mmol, 0.1 eq) provided **18d** (44 mg, 40%) as white solid.

Following general procedure E, compound **18d** (44 mg, 0.08 mmol, 1.0 eq), TFA (92 mg, 0.81 mmol, 10 eq) and TEA (11 mg, 0.10 mmol, 1.2 eq) provided **II-7** (20 mg, 56%) as yellow solid. m.p.: 211-214℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.43 (s, 1H), 9.35 (s, 1H), 9.18 (s, 1H), 8.60 (d, J = 5.6 Hz, 1H), 8.51 (s, 1H), 8.39–8.28 (m, 2H), 8.19 (d, J = 8.1 Hz, 2H), 8.14 (d, J = 8.6 Hz, 1H), 8.00 (d, J = 5.5 Hz, 1H), 7.50 (d, J = 7.9 Hz, 2H), 4.40 (s, 1H), 3.64 (s, 2H), 3.06 (s, 2H), 2.93 (s, 1H), 1.83 (d, J = 27.8 Hz, 3H); MS: found m/z $[M+H]^+$ 449.2, calcd. m/z [M] 448.2.

4.1.36. Synthesis of (S)-(3-aminopiperidin-1-yl)(4-(3-(isoquinolin-6-yl) imidazo[1,2-a]pyrazin-6-yl)phenyl)methanone (II-8)

Following general procedure B, compound **16b** (400 mg, 1.26 mmol, 1.0 eq), (S)-3-(Boc-amino)piperidine (302 mg, 1.51 mmol, 1.2 eq), EDCI (264 mg, 1.51 mmol, 1.2 eq), HOBt (203 mg, 2.52 mmol, 1.2 eq), DMAP (17 mg, 0.13 mmol, 0.1 equiv) and TEA (339 mg, 1.89 mmol, 2.0 eq) provided **17e** (506 mg, 81%) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.23 (t, J = 16.7 Hz, 1H), 8.86 (s, 1H), 8.23 (s, 2H), 8.04 (dd, J = 18.5, 15.9 Hz, 1H), 7.55 (s, 2H), 7.01 (s, 1H), 4.04 (d, J = 7.0 Hz, 1H), 3.44 (s, 2H), 2.91 (dd, J = 72.7, 51.1 Hz, 3H), 2.00 (dd, J = 18.5, 15.9 Hz, 1H), 1.77 (d, J = 76.4 Hz, 2H), 1.42 (s, 9H).

Following general procedure A, compound **17e** (100 mg, 0.21 mmol, 1.0 eq), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline (76 mg, 0.30 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (137 mg, 0.52 mmol, 2.5 eq) and $Pd(PPh_3)_4$ (23 mg, 0.02 mmol, 0.1 eq) provided **18e** (76 mg, 69%) as white solid.

Following general procedure E, compound **18e** (76 mg, 0.14 mmol, 1.0 eq), TFA (161 mg, 1.41 mmol, 10 eq) and TEA (19 mg, 0.17 mmol, 1.2 eq) provided **II-8** (32 mg, 52%) as white solid. m.p.: 178-179℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.42 (s, 1H), 9.33 (d, J = 1.2 Hz, 1H), 9.17 (s, 1H), 8.61 (t, J = 5.1 Hz, 1H), 8.50 (s, 1H), 8.34 (d, J = 8.5 Hz, 1H), 8.30 (d, J = 4.7 Hz, 1H), 8.18 (d, J = 8.3 Hz, 2H), 8.15–8.11 (m, 1H), 7.99 (d, J = 5.6 Hz, 1H), 7.52 (d, J = 8.3 Hz, 2H), 4.45–4.12 (m, 2H), 2.81 (d, J = 28.6 Hz, 3H), 1.92 (s, 1H), 1.70 (d, J = 32.2 Hz, 1H), 1.47 (s, 2H); MS: found m/z $[M+H]^+$ 449.2, calcd. m/z [M] 448.2.

4.1.37. Synthesis of (R)-(3-aminopiperidin-1-yl)(4-(3-(isoquinolin-6-yl) imidazo[1,2-a]pyrazin-6-yl)phenyl)methanone (II-9)

Following general procedure B, compound **16b** (400 mg, 1.26 mmol, 1.0 eq), (R)-3-(Boc-amino)piperidine (302 mg, 1.51 mmol, 1.2 eq), EDCI (264 mg, 1.51 mmol, 1.2 eq), HOBt (203 mg, 2.52 mmol, 1.2 eq), DMAP (17 mg, 0.13 mmol, 0.1 equiv) and TEA (339 mg, 1.89 mmol, 2.0 eq) provided **17f** (583 mg, 93%) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.25 (d, J = 19.4 Hz, 1H), 8.86 (s, 1H), 8.22 (d, J = 4.6 Hz, 2H), 8.06 (d, J = 20.7 Hz, 1H), 7.55 (s, 2H), 7.01 (s, 1H), 4.17 (d, J = 141.6 Hz, 1H), 3.44 (s, 2H), 3.25–2.63 (m, 3H), 1.78 (d, J = 72.3 Hz, 3H), 1.42 (s, 9H).

Following general procedure A, compound **17f** (100 mg, 0.21 mmol, 1.0 eq), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline (76 mg, 0.30 mmol, 1.5 eq), $K_3PO_4{\cdot}3H_2O$ (137 mg, 0.52 mmol, 2.5 eq) and $Pd(PPh_3)_4$ (23 mg, 0.02 mmol, 0.1 eq) provided **18f** (101 mg, 92%) as white solid.

Following general procedure E, compound **18f** (101 mg, 0.18 mmol, 1.0 eq), TFA (206 mg, 1.81 mmol, 10 eq) and TEA (24 mg, 0.22 mmol, 1.2 eq) provided **II-9** (56 mg, 68%) as white solid. m.p.: 182-188℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.42 (s, 1H), 9.34 (s, 1H), 9.17 (s, 1H), 8.65–8.58 (m, 1H), 8.50 (s, 1H), 8.35 (d, J = 8.4 Hz, 1H), 8.30 (d, J = 1.5 Hz, 1H), 8.16 (dd, J = 18.2, 7.6 Hz, 3H), 8.00 (d, J = 5.6 Hz, 1H), 7.53 (d, J = 7.0 Hz, 2H), 4.39–4.16 (m, 1H), 3.64 (s, 1H), 2.87 (s, 3H), 2.03–1.86 (m, 1H), 1.68 (s, 1H), 1.44 (d, J = 10.5 Hz, 2H); MS: found m/z $[M+H]^+$ 449.2, calcd. m/z [M] 448.2.

4.2. Enzyme activity using ADP-Glo™ kinase assay kit

MNK1/2 were obtained from Carna Biosciences (MNK1 catalog No. 02–145 and MNK2 catalog No.02–146) and assay was performed using ADP-Glo™ kinase assay kit (Promega, catalog No. V6930). The enzyme reaction was performed in reaction buffer, which consisted of 15 mM HEPES (pH7.4), 20 mM NaCl, 1 mM EGTA, 10 mM $MgCl_2$, 0.1 mg/mL BGG and 0.02% Tween-20. The assay was carried out in 384-well plate in a final volume of 25 μL. The end concentration of enzyme, substrate peptide (TATKSGSTTKNR, Genscript) and ATP was 10 nM, 100 μM and 300 μM for MNK1, 3 nM, 50 μM and 10 μM for MNK1, respectively. Compounds were screened at serial diluted concentration in the presence of 1% DMSO. MNK1 or MNK2 enzyme was pre-incubated with compound and peptide substrate for 10 min at room temperature before the addition of ATP. After the addition of ATP, the enzyme reaction was incubated at room temperature for 40 min. Reaction was subsequently quenched by the addition of ADP-Glo Reagent and incubating for 40 min. The final luminescent signal is produced by the addition of Kinase Detection Reagent and incubating 40 min. The luminescent signal was detected using Synergy H1 microplate reader (BioTek) and the concentration of compound necessary to achieve inhibition of enzyme activity by 50% (IC_{50}) is calculated using signals from 6-point compound dilution series.

4.3. MTT assay

The cell proliferation assay was assessed by MTT assay (Beyotime, catalog No. ST316). Briefly, the lymphoma cells were seeded in 96-well plates and grown for 12 h. The cells were then treated with various concentrations of medium containing test compounds and 1‰ DMSO, with each concentration tested in triplicate, and then cells were cultured for an additional 72 h. DMSO solution was set to the control group. Cell viability was measured using MTT according to the instructions. The data were normalized to the control groups (DMSO). IC_{50} values were obtained by using the Logit method and reported as the mean ± SD from three independent determinations.

4.4. Annexin V-FITC apoptosis detection assay

After staining with annexin V-FITC and PE using the Annexin V-FITC apoptosis detection kit (KeyGEN BioTECH, China), cells were detected by flow cytometry to assess the membrane and nuclear events during apoptosis. TMD-8 cells (1 × 105/2mL per well) were seeded in six-well

plates and treated with compound **II-5** at 0.25, 0.5 and 1 μM, respectively. After 24 h, cells were collected, washed twice with cold PBS, and resuspended in 500 μL of a binding buffer which was then added to 5 μL of annexin V-FITC and incubated at 37 °C in the dark for 15 min. Subsequently, the buffer was added to 5 μL of PI and incubated at 37 °C in the dark for 5 min. The samples were analyzed by a FACScan flow cytometer (MACSQuant® X, Germany).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2021.116186.

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