Bioorganic & Medicinal Chemistry xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Design, synthesis and pharmacological evaluation of new acyl sulfonamides as potent and selective Bcl-2 inhibitors

Xiaohua Liu [a,d], Yu Zhang [b,d], Wenjing Huang [b], Wenfu Tan [b,*], Ao Zhang [a,c,*]

[a] CAS Key Laboratory of Receptor Research, and the State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica (SIMM), University of Chinese Academy of Sciences, Shanghai 201203, China
[b] Department of Pharmacology, School of Pharmacy, Fudan University, Shanghai 201203, China
[c] School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China

ARTICLE INFO

Article history:
Received 16 October 2017
Revised 30 November 2017
Accepted 1 December 2017
Available online xxxx

Keywords:
Bcl-2
Bcl-xL
Sulfonamide
Navitoclax
Apoptosis

ABSTRACT

The antiapoptotic protein Bcl-2, overexpressed in many tumor cells, is an attractive target for potential small molecule anticancer drug discovery. Herein, we report a different structural modification approach on ABT-263 by merging the piperazinyl-phenyl fragment into a bicyclic framework leading to a series of novel analogues, among which tetrahydroisoquinoline **13** was nearly equally potent against Bcl-2 as ABT-263. Further SAR in the P4-interaction pocket afforded the difluoroazetidine substituted analogue **55,** which retained good Bcl-2 activity with improved Bcl-2/Bcl-xL selectivity.

1. Introduction

The B-cell lymphoma-2 (Bcl-2) gene family encodes at least 20 proapoptotic and antiapoptotic proteins that are the key regulators of apoptosis in the mitochondria-mediated death pathway.[1] The antiapoptotic protein family includes Bcl-2, Bcl-xL, Bcl-w, Mcl-1 and A1 bearing four Bcl homology (BH) domains (BH1-4) and a transmembrane domain. The proapoptotic proteins include Bax and Bak and act as the essential apoptotic effectors containing three BH domains (BH1-3). Interplay of the proapoptotic and the antiapoptotic proteins modulates cell survival and death.[2] In normal healthy cell, antiapoptotic proteins sequester their apoptotic counterparts through BH3 domain binding. To evade apoptosis, tumor cells can upregulate antiapoptotic protein Bcl-2 and block the normal apoptotic pathway.[3] Therefore, a small molecule Bcl-2 inhibitor designed to bind to the BH3 binding groove in the antiapoptotic proteins may restore the normal apoptotic signaling and overcome the apoptosis resistance of cancer cells.[4–6]

The binding site on Bcl-2/Bcl-xL is a narrow and long groove comprised by two large nearby pockets (P2 and P4) and three small hydrophobic pockets (P1, P3 and P5).[7] It is a challenge to design Bcl-2 inhibitors by targeting the interaction of Bcl-2/Bcl-xL proteins with their proapoptotic binding partners including BAD and BIM proteins. Nevertheless, during recent decades, a number of small-molecule inhibitors targeting Bcl-2 proteins have been developed and a few of them have been clinically investigated either as single agents or as combinations with conventional anticancer drugs to treat Bcl-2 dependent cancers.[8] Among which, only compound **3**[9] (ABT-199, venetoclax, Fig. 1) from AbbVie was succeeded in the clinic and awarded the FDA's approval in 2016 for the treatment of relapsed or refractory chronic lymphoid leukemia (CLL) with 17p deletion. Different from the dual Bcl-2/Bcl-xL non-selective profile of the earlier inhibitors **1**[10,11] (ABT-737) and **2**[12,13] (ABT-263), **3** represents the first-in-class selective Bcl-2 inhibitor sparing the on-target thrombocytopenia caused by Bcl-xL inhibition.

The reported co-crystal structure of **2** (ABT-263, navitoclax) with Bcl-2 (Fig. 2) indicated that this compound well occupied the BH3 binding grove, especially the two high affinity interaction P2 and P4 hot spots.[14] The chlorophenyl cyclohexene component was located in the P2 pocket and contributed to the activity of both Bcl-2 and Bcl-xL.[15] The phenyl thioether moiety was folded back under the tri-substituted phenyl moiety to form π-π interaction and occupied the P4 pocket. The central *N*-(4-piperazinyl-phenyla-

* Corresponding authors at: CAS Key Laboratory of Receptor Research, and the State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica (SIMM), University of Chinese Academy of Sciences, Shanghai 201203, China.
E-mail addresses: wftan@fudan.edu.cn (W. Tan), aozhang@simm.ac.cn (A. Zhang).
[d] These authors contributed equally to this work.

https://doi.org/10.1016/j.bmc.2017.12.001
0968-0896/

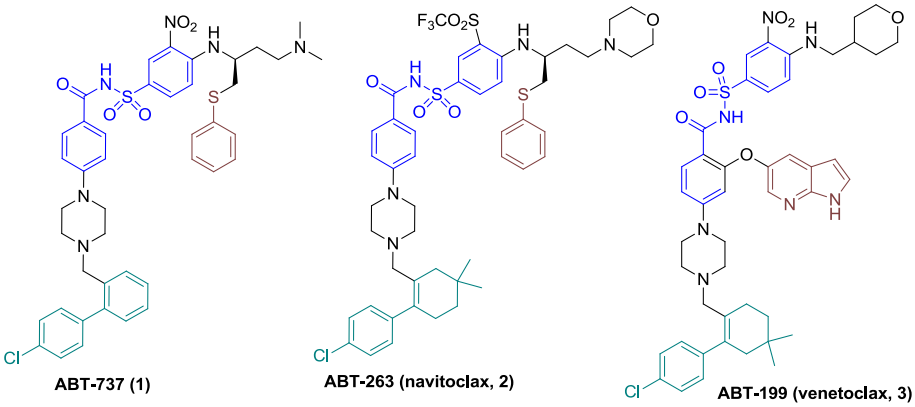


Fig. 1. Representative Bcl-2 family protein inhibitors.

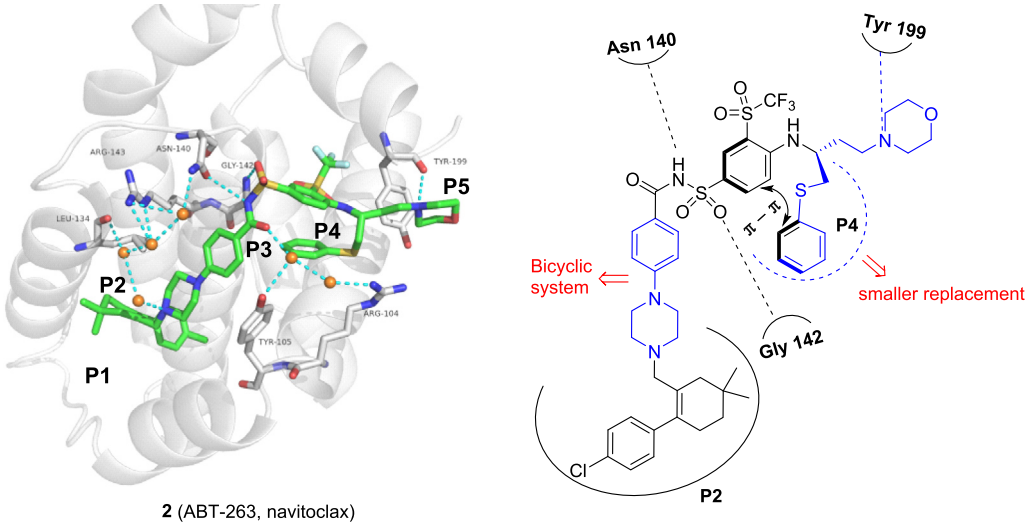


Fig. 2. Key interactions of **2** (ABT-263, navitoclax) with Bcl-2 (PDB code: 4LVT)8 and our design (in red).

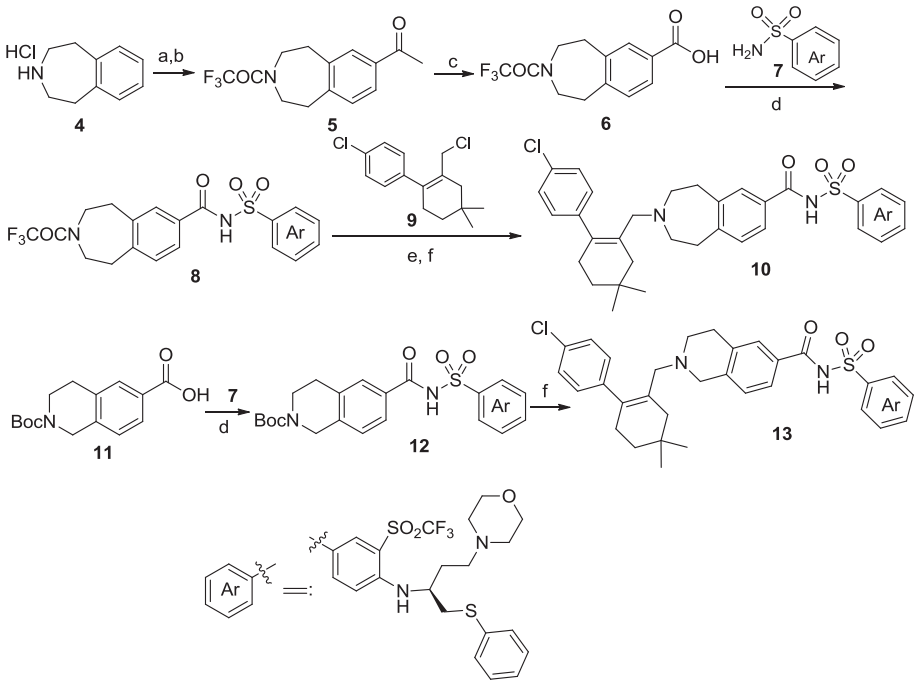
cyl) benzenesulfonamide component accreted the two components and provided several key H-bonding interactions as well. With the introduction of an azaindole as the new P4-binding moiety to capture the electrostatic interaction with Bcl-2 selective Asp103 moiety, along with structural change in the P2-binding portion, compound **3** showed high selectivity against Bcl-2 over Bcl-xL. To gain more insights on the structural determinants of Bcl-2 selectivity in the acylsulfonamide class, here we report a different structural modification approach on compound **2** by merging the piperazinyl-phenyl fragment into a bicyclic framework. Efforts to downsize the P4 interaction moiety together with its connecting bis(sulfonyl)aniline component were also conducted (Fig. 2).

2. Chemistry

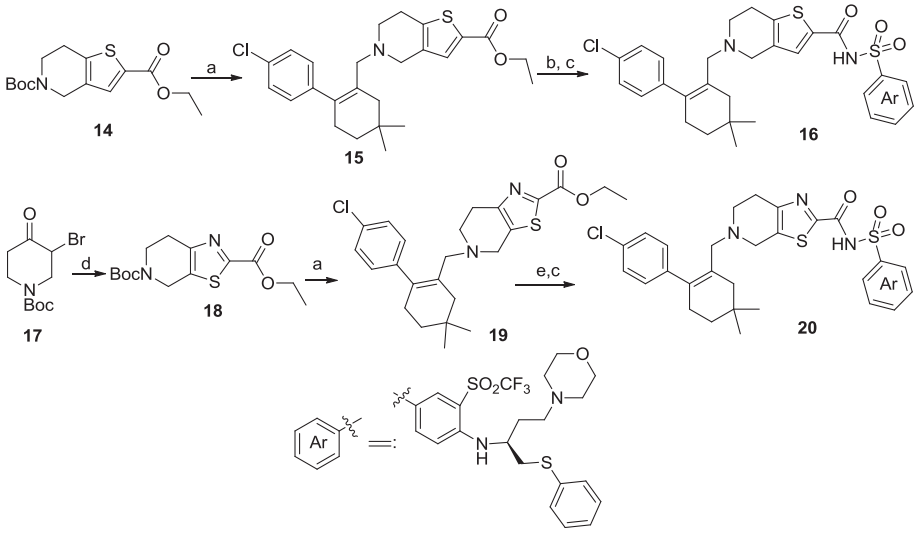
As shown in Scheme 1, we first synthesized a small series of analogues of **2** by replacement of the phenylpiperazine component with heterocycle-fused bicyclic frameworks. The benzazepine **10** was prepared from commercially available benzazepine **4**, which was first treated with TFAA followed by Friedel-Crafts acylation with acetyl chloride to give **5**.[18,19] Oxidation of **5** with OXONE in the presence of TFA delivered benzoic acid **6**.[20] Compound **7**[16] was prepared via several steps according to literature procedures, and then condensed with **6** in the presence of EDCI and a catalytic amount of DMAP to yield acyl sulfonamide **8**. *N*-Deprotection of **8** followed by nucleophilic substitution with **9**[17] afforded the final compound **10**. Tetrahydroisoquinoline **13** was prepared by following similar reaction procedures as that for preparation of **10** from tetrahydroisoquinoline-6-carboxylic acid **11**.

Thiophene and thiazole-fused bicyclic analogues **16** and **20** were prepared as shown in Scheme 2. 2-Ethyl 6,7-dihydrothieno [3,2-*c*]pyridine-2,5(4H)-dicarboxylate **14**[21] was deprotected and treated with **9** to give ester **15**. Hydrolysis of **15** followed by condensation with **7** provided acyl sulfonamide **16**. 2-Ethyl 6,7-dihydrothiazolo[5,4-c] pyridine-2,5(4H)-dicarboxylate **18**[22] was obtained by cyclization of 3-bromo-4-oxopiperidine-1-carboxylate **17** with ethyl 2-amino-2-thioxoacetate. By following similar reaction procedures as that for preparation of **15**, precursor **19** was produced in 31% yield. Hydrolysis of **19** with aqueous Na_2CO_3 followed by condensation with **7** provided acyl sulfonamide **20**.

Preparation of benzimidazoles **28** and **29** is outlined in Scheme 3. *tert*-Butyl (1-(hydroxymethyl)cyclopropyl)carbamate (**21**) was mesylated, and then substituted with NaI, followed by treating with methyl 1H-benzo[*d*]imidazole-5-carboxylate led to **22** as a pair of 6- and 7-isomers in about 1:1 ratio.[23] Intermediate **24**[12] was obtained via *N*-deprotection of **22** followed by reductive amination with aldehyde **23**.[12] Suzuki cross-coupling of **24** with 4-chlorophenylboronic acid afforded **25**.[12] Hydrolysis of **25** generated two separable isomers **26** and **27**. Subsequent condensation



Scheme 1. Synthesis of compounds **10** and **13**. Reagents and conditions: (a) TFAA, Et_3N, CH_2Cl_2, overnight; (b) acetyl chloride, $AlCl_3$, CH_2Cl_2, 0 °C – rt, 2 h, 81%; (c) Oxone, TFA, 1,4-dioxane, 100 °C, 30%; (d) **7**, EDCI, DMAP, CH_2Cl_2, rt, overnight, 60–73%; (e) K_2CO_3, MeOH/H_2O (2:1), rt, 3 h; (f) (i) 2 M HCl in MeOH, MeOH, rt, 2 h; (ii) **9**, DIPEA, MeCN, reflux, 10 h, 57–64%.



Scheme 2. Synthesis of compounds **16** and **20**. Reagents and conditions: (a) (i) TFA, CH_2Cl_2, rt, 2 h; (ii) **9**, DIPEA, MeCN, rt, 5 h, 31–55%; (b) LiOH, EtOH/H_2O (3:2), rt, overnight; (c) **7**, EDCI, DMAP, CH_2Cl_2, rt, overnight, 14–33%; (d) ethyl 2-amino-2-thioxoacetate, EtOH, reflux, overnight, 38%; (e) Na_2CO_3, DME/H_2O/EtOH (7:3:2), 85 °C.

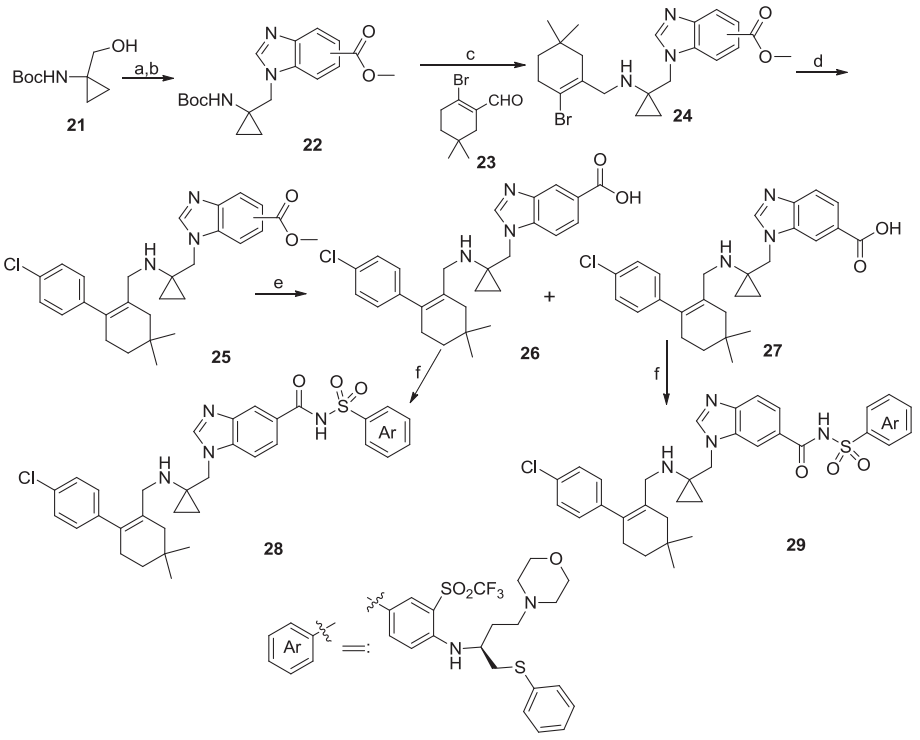
of **26** and **27** with **7** provided **28** and **29** in 40% and 36% yields, respectively.

As shown in Scheme 4, ammonization of sulfonyl chlorides **30a–c** afforded sulfonamides **31a–c**.[24] Aromatic nucleophilic substitution of fluorobenzene **32** by appropriate amines gave corresponding sulfonamides **33a–c**.[24] Condensation of benzoic acid **34**[4] with sulfonamides **31a–c** or **33a–c** provided acyl sulfonamides **35–40** under aforementioned standard condensation conditions.

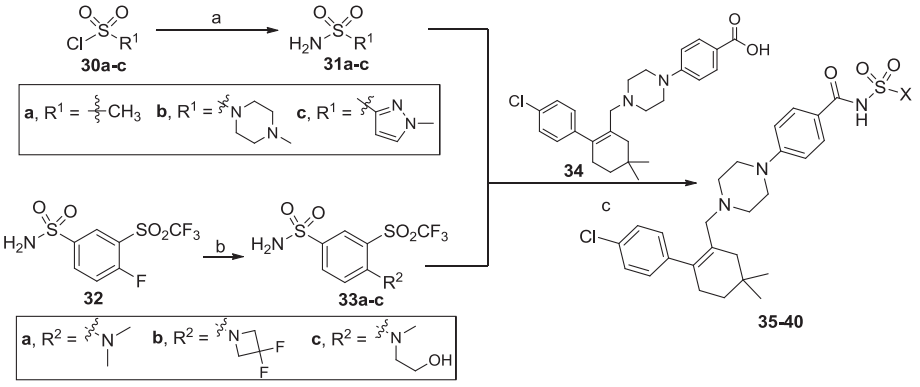
Similarly, Substitution of cyclopropyl methanol **21** with morpholine or 3,3-difluoroazetidine hydrochloride produced **41a–b** (Scheme 5). Subsequent *N*-deprotection followed by aromatic nucleophilic substitution provided intermediates **42a–b**.[25] Amination of carboxylic acid **43** with morpholine followed by similar aromatic nucleophilic substitution provided intermediate **45**. Reduction of amide **45** with BH_3.DMS delivered sulfonamide **46**.[16] In addition, etheric sulfonamides **49a–b** were prepared from ammonization of (3-(bromomethyl) oxetan-3-yl)methanol followed by substitution with fluorobenzene **32** in the presence of NaH. Treatment of acid **34** with precursor **42a–b**, **45**, **46**, or **49a–b** provided corresponding acyl sulfonamides **50–55** in 35–50% yields.

3. Results and discussion

All the newly synthesized analogues of **2** were evaluated in a fluorescence polarization assay (FPA) for their ability to displace



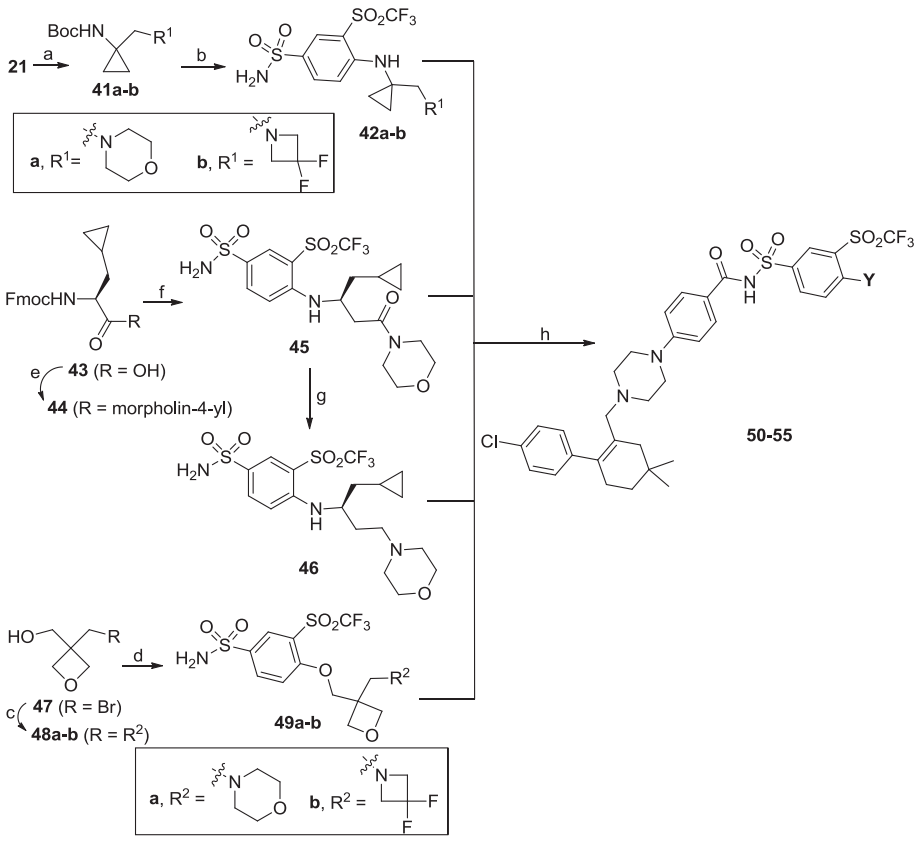
Scheme 3. Synthesis of compounds **28** and **29**. Reagents and conditions: (a) (i) MsCl, Et_3N, CH_2Cl_2, 0 °C, 0.5 h, (ii) NaI, Me_2CO, reflux, 1 h, 87% (two steps); (b) methyl 1H-benzo[*d*]imidazole-5-carboxylate, Cs_2CO_3, MeCN, 50 °C, 1 h, 61%; (c) (i) TFA, CH_2Cl_2, rt, 0.5 h, (ii) **23**, $NaBH_3CN$, HOAc, EtOH, overnight, 96%; (d) 4-chlorophenylboronic acid, 2 M Na_2CO_3 aq, $PdCl_2(PPh_3)_2$, DME/H_2O/EtOH (7:3:2), 85 °C, 3 h, 84%; (e) LiOH, EtOH/H_2O (3:2), overnight; (f) **7**, EDCI, DMAP, CH_2Cl_2, rt, overnight, 36–40%.



Scheme 4. Synthesis of compounds 35–40. Reagents and conditions: (a) NH_4OH, THF, rt, 5 h, 95–98%; (b) DIPEA, DMSO, 60 °C, 72–90%; (c) 34, EDCI, DMAP, DIPEA, CH_2Cl_2, rt, overnight, 18–57%.

a Bax-derived peptide from Bcl-2, and a Bad-derived peptide from Bcl-xL. Compound **2** (ABT-263, navitoclax) was used as a reference for comparison, which showed nearly equal potency against Bcl-2 and Bcl-xL with IC_{50} values of 10.3 and 8.2 nM, respectively in our assay (Table 1). Meanwhile, the approved drug **3** (ABT-199, venetoclax) was also tested and showed IC_{50} values of 7.39 and 52.7 nM against Bcl-2 and Bcl-xL, respectively, confirming its high selectivity of Bcl-2/Bcl-xL. As shown in Table 1, the bicyclic subseries of analogues generally retained moderate to good activity against both Bcl-2 and Bcl-xL, with moderate preference to Bcl-xL. The benzazepine **10** was 19-fold less potent than reference **2**, whereas the tetrahydroisoquinoline **13** was nearly equally potent to compound **2** with IC_{50} values of 12.6 and 14.6 nM against Bcl-2 and Bcl-xL, respectively. To explore the potential binding mode of **13** with Bcl-2, we used a surrogate ligand **13B** (Fig. 3A) to avoid the influence of F atom in Autodock. As expect, the binding mode of **13B** (Fig. 3B) revealed it mimicked the interactions of inhibitor **2** with Bcl-2, and the two structures of **2** and **13B** can be overlaid to a large extent (Fig. 3C). The thiophen- and thiazole-fused bicycles **16** and **20** retained good potency and showed increased selectivity for Bcl-xL. The selectivity for Bcl-xL is further elevated in benzoimidazole **28** that showed IC_{50} values of 107 and 29 nM respectively against Bcl-2 and Bcl-xL. Interestingly, the regio-isomer **29** was inactive against both Bcl-2 and Bcl-xL, indicating that the steric repulsion caused by the regiochemistry in **29** likely interferes the inhibitor-target interaction.

To validate the importance of the P4-interaction component, we first evaluated a subseries of simplified analogues. As shown in Table 2, removal of the P4-interaction moiety-connected aniline with a simple alkyl or heterocycles led to compounds **35**–**37**. These



Scheme 5. Synthesis of compounds **50**–**55**. Reagents and conditions: (a) morpholine or 3,3-difluoroazetidine hydrochloride, K_2CO_3, MeCN, 50 °C, 3 h, 48–58%; (b) (i) TFA, CH_2Cl_2, 2 h, (ii) 4-fluoro-3-((trifluoromethyl)sulfonyl) benzenesulfonamide, DIPEA, DMSO, 60 °C, 45–69%; (c) morpholine or 3,3-difluoroazetidine hydrochloride, K_2CO_3, KI, MeCN, 75 °C, 6 h, 46–70%; (d) **32**, NaH, THF, -10 °C, 0.5 h, 35–36%; (e) morpholine, PyBop, DIPEA, DMF, rt, 10 h; (f) **32**, DIPEA, DMF, rt, 3d, 48% (two steps); (g) Borane (2M in methyl sulfide), 50 °C, 75%; (h) **34**, EDCI, DMAP, DIPEA, CH_2Cl_2, rt, overnight, 35–53%.

compounds were inactive against Bcl-xL, whereas though reduced but moderate potency was retained against Bcl-2. In this subseries, pyrazole **37** showed high potency against Bcl-2 with an IC_{50} value of 67 nM and the Bcl-2/Bcl-xL selectivity is >15-fold. This result indicated that a simple heterocycle like the pyrazole moiety in **37** might mimic the role of the azaindole in the clinical drug **3** to interact in the P4-pocket. Moderate potency was observed for compounds **38**–**40** bearing downsized P4-interaction moieties. These three compounds showed similar potency against both Bcl-2 (46–65 nM) and Bcl-xL (116–217 nM), in spite of the different amino substituents on the phenyl ring.

Next, we replaced the chiral dialkylamino moiety in reference **2**, with a small series of heterocycle-substituted alkylamino groups. As shown in Table 3, the cyclopropane-substituted diamines **50** and **51** retained moderate potency against Bcl-2 with IC_{50} values of 40.7 and 34.8 nM, respectively. The difluoroazetidine **51** also displayed a 10-foldselectivity for Bcl-2 over Bcl-xL. Reducing the basicity of the amino side chain by introducing an amido moiety led to compound **52**, showing slightly lower potency and reduced selectivity with IC_{50} values of 68 and 59 nM respectively against Bcl-2 and Bcl-xL. High potency was observed by reduction of amide **52**, and the resulting compound **53** showed IC_{50} values of 20.7 nM against Bcl-2. Greater potency was obtained by introducing an oxetane moiety in the linker, affording compounds **54** and **55**. Both compounds showed high Bcl-2 potency with IC_{50} values of 14.5 and 17.3 nM, respectively, only slightly less potency than reference **2**. The latter compound, though slightly less potent than **2**, showed an improved Bcl-2/Bcl-xL selectivity of 6-fold, which is much improved compared with that of **2**.

4. Summary

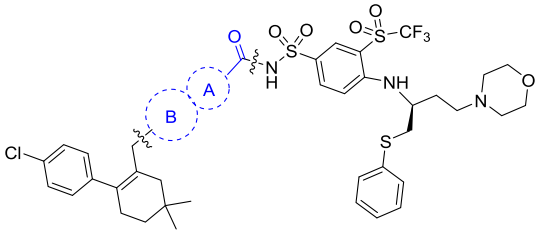
In conclusion, we conducted a structural modification on acyl sulfonamide **2** (navitoclax), the precedent of the first-in-class Bcl-2 inhibitor **3** (venetoclax) and evaluated the potency and selectivity of the resulting analogues against Bcl-2 and Bcl-xL. Merging the arylpiperazine fragment of **2** into various bicyclic frameworks generally retained moderate to good activity against both Bcl-2 and Bcl-xL, with moderate preference to Bcl-xL. Tetrahydroisoquinoline **13** is nearly equally potent against Bcl-2 as compound **2**. Further SAR in the P4-interaction pocket indicated that simply substituted arylsulfonamide lacking the phenyl thioether-connected chiral amino group retained good Bcl-2 activity with improved Bcl-2/Bcl-xL selectivity. Compared to reference **2**, the difluoroazetidine substituted analogue **55** showed slightly less Bcl-2 potency (17.3 vs 10.3 nM), but the selectivity over Bcl-xL was improved (SF, 6.0 vs 0.8). Since the inhibition of Bcl-xL was relevant to the dose-limiting toxicity of **2**, the current result will provide useful insights to design more potent and selective Bcl-2 inhibitors.

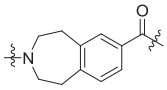
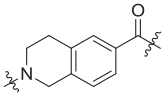
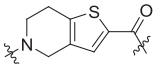
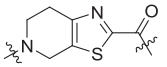
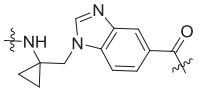
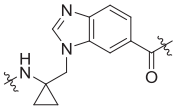
5. Experimental section

5.1. General

All solvents and chemical reagents were obtained from commercial sources and used without further purifications. ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury 300, 400 or 500 NMR spectrometer. Low- and high-resolution mass spectra

Table 1
Inhibition of bicyclic analogues against Bcl-2 and Bcl-xL.



Compound		IC_{50}, nM		SF[a]
		Bcl-2	Bcl-xL	
10		193 ± 96.3	25.6 ± 9.3	0.13
13		12.6 ± 6.0	14.6 ± 3.3	1.15
16		57.9 ± 11.1	26.6 ± 3.0	0.46
20		38.1 ± 4.0	13.2 ± 5.0	0.35
28		108 ± 32.5	29.3 ± 4.7	0.27
29		>1000	>1000	–
2 (ABT-263)	–	10.3 ± 1.7	8.2 ± 2.1	0.80
3 (ABT-199)	–	7.39 ± 3.5	52.7 ± 14.4	7.13

[a] Selectivity Factor = IC_{50} (Bcl-xL)/IC_{50} (Bcl-2).

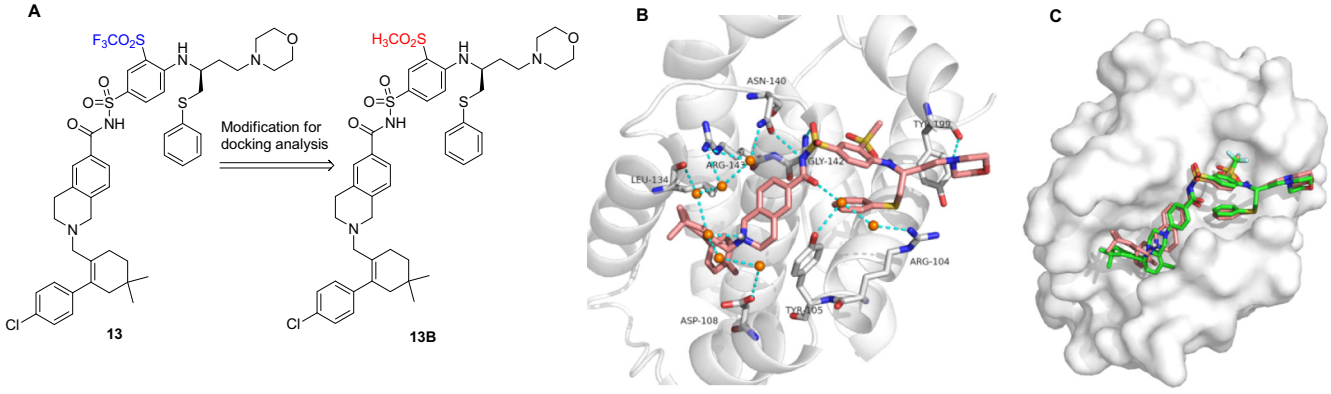


Fig. 3. (A) Structural modifications used to create surrogate ligand **13B** for rigid protein docking with Bcl-2. (B) Binding mode of **13B** (pink) with Bcl-2. (C) Overlay of binding geometry of compound **2** (green) and **13B** (pink) with Bcl-2.

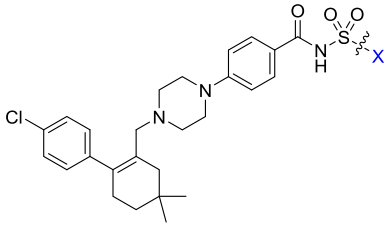
was recorded in the ESI mode. Flash column chromatography on silica gel (200–300 mesh) was used for the routine purification of reaction products. The column output was monitored by TLC on silica gel (200–300 mesh) precoated on glass plates (15 mm × 50 mm), and spots were visualized by UV light at 254 or 365 nm. NOE was used in the structural confirmation.

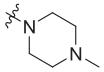
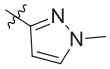
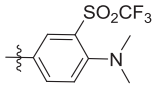
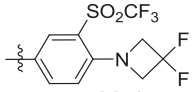
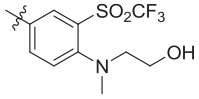
5.2. Synthetic procedures

5.2.1. 3-(2,2,2-Trifluoroacetyl)-2,3,4,5-tetrahydro-1H-benzo[d] azepine-7-carboxylic acid (**6**)

To a solution of **5**[17,18](1.1 g, 3.8 mmol) in dioxane (30 mL) was added OXONE (7 g, 11.4 mmol) and TFA (0.57 mL, 7.7 mmol).[19]

Table 2
Inhibition of new acyl sulfonamides against Bcl-2 and Bcl-xL.



Compound	X =	IC_{50}, nM		SF[a]
		Bcl-2	Bcl-xL	
35		495 ± 409	>1000	>2.02
36		168 ± 63.7	>1000	>5.93
37		67.4 ± 31.5	>1000	>14.84
38		65.1 ± 26.1	218 ± 9.9	3.34
39		46.6 ± 8.3	162 ± 72.4	3.48
40		46.0 ± 15.3	116 ± 13.6	2.53
2 (ABT-263)	–	10.3 ± 1.7	8.2 ± 2.1	0.80

[a] Selectivity Factor = IC_{50} (Bcl-xL)/IC_{50} (Bcl-2).

The mixture was heated to reflux for 24 h and then cooled to room temperature. H_2O was added and the mixture was extracted with EtOAc. The combined organic layers were treated with aqueous $NaHCO_3$ solution and the aqueous layer was poured onto crushed ice and treated with 2 M HCl; a white solid precipitated out. The precipitate was filtered off and dried in vacuo to give **6** (330 mg, 30%). ^{1}H NMR (300 MHz, CD_3OD) δ 7.83 (d, J = 9.1 Hz, 2H), 7.29 (d, J = 9.2 Hz, 1H), 3.77 (t, J = 9.5 Hz, 4H), 3.08 (t, J = 9.7 Hz, 4H).

*5.2.2. (R)-N-((4-((4-morpholino-1-(phenylthio)butan-2-yl)amino) -3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)-3-(2,2,2-trifluoroacetyl)-2,3,4,5-tetrahydro-1H-benzo[d]azepine-7-carboxamide (**8**)*

To a solution of **6** (60 mg, 0.2 mmol) in CH_2Cl_2 (4 mL) were added EDCI (58 mg, 0.3 mmol) and DMAP (4 mg, 0.03 mmol). The solution was stirred at room temperature for 0.5 h and compound **7**[15] (97 mg, 0.18 mmol) was added. The resulting mixture was stirred for another 8 h and water was added. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated under vacuo. The residue was prified by chromatography (CH_2Cl_2/CH_3OH 40:1) to provide **8** as white solid (108 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.04 (d, J = 8.9 Hz, 1H), 7.77–7.67 (m, 2H), 7.41–7.35 (m, 2H), 7.33–7.22 (m, 3H), 7.17 (t, J = 8.7 Hz, 1H), 7.01 (d, J = 8.5 Hz, 1H), 6.73 (d, J = 9.3 Hz, 1H), 4.01–3.99 (m, 1H), 3.82 (t, J = 4.7 Hz, 4H), 3.78–3.61 (d, J = 28.5 Hz, 4H), 3.18–3.04 (m, 2H), 3.00–2.98 (m, 4H), 2.77–2.65 (m, 6H), 2.27–2.15 (m, 1H), 1.92–1.86 (m, 1H).

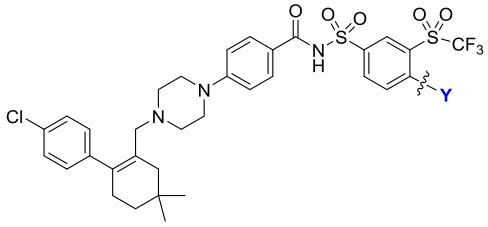
*5.2.3. (R)-3-((4′-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)-N-((4-((4-morpholino-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)-2,3,4,5-tetrahydro-1H-benzo[d]azepine-7-carboxamide (**10**)*

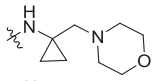
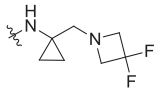
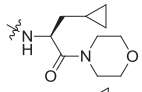
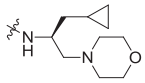
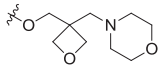
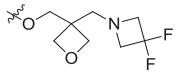
Compound **8** (100 mg, 0.12 mmol) was dissolved in MeOH (3 mL) and H_2O (1 mL), and K_2CO_3 (84 mg, 0.6 mmol) was added. The reaction was stirred at room temperature for 2 h and then concentrated to give the amine crude intermediate as pale oil (134 mg). Without purification, the amine intermediate (56 mg) was dissolved in MeCN (1 mL), and compound **9**[16] (14 mg, 0.05 mmol), DIPEA (21 μL, 0.65 mmol) was added. The mixture was stirred at reflux for 10 h, then washed with water and extracted with EtOAc. The combined organics were dried over Na_2SO_4, filtered, and concentrated under vacuo. The residue was prified by chromatography (CH_2Cl_2/CH_3OH 40:1) to provide **10** (30 mg, 64%) as white solid. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 8.33 (d, J = 2.2 Hz, 1H), 8.06 (dd, J = 9.2, 2.2 Hz, 1H), 7.70 (d, J = 8.2 Hz, 2H), 7.42–7.37 (m, 2H), 7.34–7.26 (m, 4H), 7.21 (t, J = 7.3 Hz, 1H), 7.17–7.11 (m, 3H), 7.06 – 7.01 (m, 2H), 4.24–4.22 (mf, 1H), 3.59 (td, J = 6.0, 3.4 Hz, 4H), 3.36 (qd, J = 14.0, 6.1 Hz, 2H), 3.15 (s, 2H), 3.02 (s, 4H), 2.67 (s, 4H), 2.53–2.49 (m, 4H), 2.40–2.26 (m, 4H), 2.14 (s, 3H), 1.93–1.85 (m, 1H), 1.47 (t, J = 6.5 Hz, 2H), 0.96 (s, 6H). HRMS (ESI) calcd for $C_{47}H_{55}ClF_3N_4O_6S_3$, 959.2919; found, 959.291.

*5.2.4. (R)-Tert-butyl 6-(((4-((4-morpholino-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)carbamoyl)-3,4-dihydroisoquinoline-2(1H)-carboxylate (**12**)*

Compound **12** was obtained according to the procedure of preparing **8** using compound **7** and 2-(*tert*-butoxycarbonyl)-

Table 3
Inhibition of new acyl sulfonamides against Bcl-2 and Bcl-xL.



Compound	Y =	IC_{50}, nM Bcl-2	Bcl-xL	SF[a]
50		40.7 ± 7.2	55.3 ± 12.1	1.36
51		34.8 ± 5.9	342 ± 73.1	9.83
52		68.2 ± 12.3	59.3 ± 18.1	0.87
53		20.7 ± 3.4	33.5 ± 5.6	1.62
54		14.5 ± 5.4	49.3 ± 7.2	3.40
55		17.3 ± 4.8	105 ± 11.9	6.06
2 (ABT-263)	–	10.3 ± 1.7	8.2 ± 2.1	0.80

[a] Selectivity Factor = IC_{50} (Bcl-xL)/IC_{50} (Bcl-2).

1234-tetrahydroisoquinoline-6-carboxylic acid. White solid; 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.05 (d, J = 9.1 Hz, 1H), 7.71–7.64 (s, 2H), 7.39 (d, J = 7.4 Hz, 2H), 7.34–7.28 (m, 3H), 7.17 (d, J = 8.1 Hz, 1H), 7.07 (d, J = 8.6 Hz, 1H), 6.68 (d, J = 9.3 Hz, 1H), 4.60 (s, 2H), 4.06–3.95 (m, 1H), 3.81 (s, 4H), 3.65 (t, J = 6.0 Hz, 2H), 3.11 (qd, J = 13.9, 5.9 Hz, 2H), 2.86 (t, J = 5.8 Hz, 2H), 2.68–2.61 (m, 6H), 2.30–2.16 (m, 1H), 1.88–1.82 (m, 1H), 1.51 (s, 9H).

*5.2.5. (R)-2-((4′-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)-N-((4-((4-morpholino-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)-1,2,3,4-tetrahydroisoquinoline-6-carboxamide (**13**)*

To a solution of compound **12** (30 mg, 0.04 mmol) dissolved in MeOH (1 mL) was added 2 M HCl in MeOH (0.5 mL). The mixture was allowed to stir at room temperature until the starting material was consumed completely. The reaction was concertrated to give crude precursor which was used for the next step without further purification.

Compound **13** was obtained according to the procedure of preparing **10** using the crude precursor and compound **9**. White solid; 59%; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 8.32 (s, 1H), 8.03 (d, J = 9.2 Hz, 1H), 7.71–7.61 (m, 2H), 7.40 (d, J = 7.8 Hz, 2H), 7.35 (d, J = 6.6 Hz, 2H), 7.32–7.26 (m, 2H), 7.25–7.19 (m, 3H), 7.16–7.02 (m, 3H), 4.29–4.25 (m, 1H), 3.73–3.66 (m, 6H), 3.39 (qd, J = 13.9, 5.8 Hz, 2H), 3.23 (s, 2H), 2.92 (s, 2H), 2.80–2.53 (m, 8H), 2.34 (t, J = 7.2 Hz, 2H), 2.23 (m, 1H), 2.12 (s, 2H), 1.97 (m, 1H), 1.50 (t, J = 7.2 Hz, 2H), 0.98 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.39, 139.93, 136.25, 134.45, 133.01, 132.73, 130.79, 128.96, 128.89, 128.78, 128.47, 126.99, 126.15, 125.21, 121.14, 118.54, 112.51, 107.91, 66.45, 59.36, 54.20, 53.97, 53.28, 50.11, 49.73, 40.69, 38.77, 34.83, 31.00, 29.86, 28.79, 27.51, 26.61. HRMS (ESI) calcd for $C_{46}H_{51}ClF_3N_4O_6S_3$, 943.2617; found, 943.2617.

*5.2.6. Ethyl 5-((4′-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine-2-carboxylate (**15**)*

Compound **15** was obtained according to the procedure of preparing **13** using compound **9** and 5-*tert*-butyl 2-ethyl 6,7-dihydrothieno[3,2-*c*]pyridine-2,5(4H)-dicarboxylate. White solid; 55%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (s, 1H), 7.24–7.18 (m, 2H), 6.98–6.91 (m, 2H), 4.24 (q, J = 7.1 Hz, 2H), 3.26 (s, 2H), 2.89 (s, 2H), 2.75 (t, J = 5.7 Hz, 2H), 2.50 (t, J = 5.7 Hz, 2H), 2.20 (d, J = 6.9 Hz, 2H), 1.96 (s, 2H), 1.41 (t, J = 6.5 Hz, 2H), 1.28 (t, J = 7.1 Hz, 4H), 0.92 (s, 6H).

*5.2.7. (R)-5-((4′-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)-N-((4-((4-morpholino-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine-2-carboxamide (**16**)*

To a solution of compound **15** (22 mg, 0.05 mmol) dissolved in EtOH (1.5 mL) and H_2O (1 mL) was added LiOH (84 mg, 0.6 mmol). The reaction was stirred at room temperature for 2 h and then concentrated. The residue was dissolved in water and treated with 1 M HCl; a white solid precipitated out. The precipitate was filtered off and dried in vacuo to give crude acid.

Compound **16** was obtained according to the procedure of preparing **8** using the crude acid and compound **7**. White solid; 33%; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 8.22 (d, J = 2.2 Hz, 1H), 7.90 (d, J = 9.1 Hz, 1H), 7.39–7.35 (m, 5H), 7.29–7.25 (m, 2H), 7.22–7.19 (m, 3H), 6.94 (dd, J = 15.6, 9.1 Hz, 2H), 4.31–4.17 (m, 1H), 3.69–3.62 (m, 6H), 3.47–3.20 (m, 4H), 2.94–2.85 (m, 4H), 2.68–2.56 (m, 6H), 2.32 (t, J = 6.5 Hz, 2H), 2.24–2.16 (m, 1H), 2.11 (s, 2H), 2.00–1.88 (m, 1H), 1.48 (t, J = 6.5 Hz, 2H), 0.96 (d, J = 4.6 Hz, 6H). HRMS (ESI) calcd for $C_{44}H_{49}ClF_3N_4O_6S_4$, 949.2181; found, 949.2205.

*5.2.8. Ethyl 5-((4′-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxylate (**19**)*

Compound **19** was obtained according to the procedure of preparing **15**. White solid; 31%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (d, J = 8.6 Hz, 2H), 6.99 (d, J = 8.6 Hz, 2H), 4.44 (q, J = 7.3 Hz, 2H), 3.56 (s, 2H), 2.99 (s, 2H), 2.88 (t, J = 6.0 Hz, 2H), 2.66 (t, J = 6.0 Hz, 2H), 2.26 (t, J = 6.8 Hz, 2H), 2.02 (s, 2H), 1.52–1.44 (m, 2H), 1.41 (t, J = 6.8 Hz, 3H), 0.98 (s, 6H).

*5.2.9. (R)-5-((4′-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)-N-((4-((4-morpholino-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine-2-carboxamide (**20**)*

Compound **19** (40 mg, 0.09 mmol) was dissolved in DME (0.7 mL), H_2O (0.3 mL), and EtOH (0.2 mL), and 2 M aqueous Na_2CO_3 (90 μL) was added. The reaction was stirred at 85 °C for 2 h and then concentrated. The residue was dissolved in water and treated with 1 M HCl; a white solid precipitated out. The precipitate was filtered off and dried in vacuo to give crude acid.

Compound **20** was obtained according to the procedure of preparing **8** using the crude acid and compound **7**. White solid; 14%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 8.00 (d, J = 8.7 Hz, 1H), 7.37 (d, J = 7.3 Hz, 2H), 7.33–7.27 (m, 4H), 7.25 (m, 1H), 7.07 (d, J = 9.1 Hz, 1H), 6.99 (d, J = 7.3 Hz, 2H), 6.60 (d, J = 8.7 Hz, 1H), 3.92 (s, 1H), 3.69 (s, 4H), 3.58 (s, 2H), 3.14–3.01 (m, 4H), 2.80 (s, 2H), 2.69 (s, 2H), 2.58–2.36 (m, 6H), 2.27 (t, J = 7.3 Hz, 2H), 2.16–2.13 (m, 1H), 2.01 (s, 2H), 1.75–1.71 (m, 1H), 1.47 (t, J = 7.3 Hz, 2H), 0.97 (s, 6H). HRMS (ESI) calcd for $C_{43}H_{48}ClF_3N_5O_6S_4$, 950.2134; found, 950.2119.

*5.2.10. Methyl 1-((1-(((2-bromo-5,5-dimethylcyclohex-1-en-1-yl) methyl)-amino)cyclopropyl)methyl)-1H-benzo[d]imidazole-5 and 6-carboxylates (**24**)*

To a solution of isomers **22**[22] (535 mg, 1.55 mmol) dissolved in CH_2Cl_2 (15 mL) was added TFA (2.3 mL). The mixture was allowed to stir at room temperature until the starting material was consumed completely. The reaction was concertrated to give crude amide salt.

Without further purification, the crude amide salt was dissolved in EtOH, and compound **23**[11] (293 mg, 1.35 mmol) was added. The mixture was allowed to stir at room temperature until the imine intermediate was observed completely. $NaBH_3CN$ (110 mg, 1.76 mmol) was added and the mixture was stirred at rt overnight. The reaction was concentrated under vacuo and prified by chromatography (petroleum ether/EtOAc 2:1) to provide **24** (600 mg, 96%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (s, 0.5H), 8.34–8.16 (m, 1.5H), 8.00 (dd, J = 13.0, 8.6 Hz, 1H), 7.81 (d, J = 8.6 Hz, 0.5H), 7.47 (d, J = 8.6 Hz, 0.5H), 4.22 (d, J = 7.8 Hz, 2H), 3.94 (d, J = 2.6 Hz, 3H), 3.31 (d, J = 3.1 Hz, 2H), 2.41 (s, 2H), 1.78 (s, 2H), 1.33 (t, J = 5.6 Hz, 2H), 0.88–0.74 (m, 10*H*).

*5.2.11. 1-((1-(((4′-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)amino)cyclopropyl)methyl)-1H-benzo[d]imidazole-5-carboxylic acid (**26**) and 1-((1-(((4′-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)amino)cyclopropyl)methyl)-1H-benzo[d]imidazole-6-carboxylic acid (**27**)*

Compounds **25**[11] were prepared according to corresponding literature procedures by Suzuki cross-coupling of **24** with 4-chlorophenylboronic acid. The hydrolysis of **25** provided compound **26** and **27** according to the above method using LiOH. The two isomers were separated by chromatography (CH_2Cl_2/CH_3OH 50:1) and diagnosed by NOE. Compound **26**, white solid; 42%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.74 (s, 1H), 8.20 (s, 1H), 8.12 (d, J = 8.5 Hz, 1H), 7.31 (d, J = 8.5 Hz, 1H), 7.23 (d, J = 8.4 Hz, 2H), 6.98 (d, J = 8.4 Hz, 2H), 4.02 (s, 2H), 3.05 (s, 2H), 2.17 (t, J = 6.6 Hz, 2H), 1.79 (s, 2H), 1.37 (t, J = 6.6 Hz, 2H), 0.89 (s, 6H), 0.77–0.66 (m, 4H). Compound **27**, white solid; 35%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (s, 1H), 8.18 (s, 1H), 8.13 (dd, J = 8.5, 1.3 Hz, 1H), 7.94 (d, J = 8.5 Hz, 1H), 7.21 (d, J = 8.4 Hz, 2H), 6.97 (d, J = 8.4 Hz, 2H), 4.07 (s, 2H), 3.03 (s, 2H), 2.21–2.13 (m, 2H), 1.78 (t, J = 2.2 Hz, 2H), 1.36 (t, J = 6.4 Hz, 2H), 0.88 (s, 6H), 0.72 (s, 4H).

*5.2.12. (R)-1-((1-(((4′-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)amino)cyclopropyl)methyl)-N-((4-((4-morpholino-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)-1H-benzo[d]imidazole-5-carboxamide (**28**)*

Compound **28** were obtained according to the procedure of preparing **8** from compound **26** and **7**. White solid; 40%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.42 (d, J = 2.2 Hz, 1H), 8.40 (s, 1H), 8.21 (d, J = 9.1 Hz, 1H), 7.89 (dd, J = 9.0, 2.2 Hz, 1H), 7.41–7.36 (m, 2H), 7.33–7.28 (m, 3H), 7.25–7.24 (m, 1H), 7.08–7.06 (m, 3H), 6.87 (d, J = 8.3 Hz, 2H), 6.66 (d, J = 9.0 Hz, 1H), 3.96–3.91 (m, 3H), 3.73–3.61 (m, 4H), 3.08 (qd, J = 13.9, 5.8 Hz, 2H), 2.98 (s, 4H), 2.52–2.31 (m, 6H), 2.20–2.08 (m, 3H), 1.77 (s, 2H), 1.71–1.68 (m, 1H), 1.34 (t, J = 6.4 Hz, 2H), 0.84 (s, 6H), 0.69 (d, J = 13.4 Hz, 4H). HRMS (ESI) calcd for $C_{48}H_{55}ClF_3N_6O_6S_3$, 999.298; found, 999.2962.

*5.2.13. (R)-1-((1-(((4′-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)amino)cyclopropyl)methyl)-N-((4-((4-morpholino-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)-1H-benzo[d]imidazole-6-carboxamide (**29**)*

Compound **29** were obtained according to the procedure of preparing **8** from compound **27** and **7**. White solid; 36%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 1H), 8.07–7.97 (m, 3H), 7.78–7.68 (m, 2H), 7.38–7.36 (m, 2H), 7.32–7.28 (m, 2H), 7.25–7.23 (m, 1H), 7.08 (d, J = 8.2 Hz, 3H), 6.90 (d, J = 8.2 Hz, 2H), 6.63 (d, J = 9.3 Hz, 1H), 3.97–3.94 (m, 3H), 3.71 (s, 4H), 3.17–2.94 (m, 4H), 2.54–2.46 (m, 6H), 2.14–2.13 (m, 3H), 1.78–1.74 (m, 3H), 1.36 (t, J = 6.4 Hz, 2H), 0.87 (s, 6H), 0.68–0.67 (d, J = 5.2 Hz, 4H). HRMS (ESI) calcd for $C_{48}H_{55}ClF_3N_6O_6S_3$, 999.298; found, 999.2964.

*5.2.14. 4-(4-((4′-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)piperazin-1-yl)-N-(methylsulfonyl)benzamide (**35**)*

Compound **31a** was prepared according to literature procedures.[23] Compound **35** were obtained according to the procedure of preparing **8** using compound **31a** and **34**. White solid; 57%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (d, J = 8.7 Hz, 2H), 7.28 (d, J = 8.0 Hz, 2H), 6.99 (d, J = 8.0 Hz, 2H), 6.80 (d, J = 8.7 Hz, 2H), 3.43–3.23 (m, 7H), 2.87–2.84 (m, 2H), 2.39 (s, 4H), 2.26 (t, J = 6.5 Hz, 2H), 2.04 (s, 2H), 1.47 (t, J = 6.5 Hz, 2H), 1.00 (s, 6H). HRMS (ESI) calcd for $C_{27}H_{35}ClN_3O_3S$, 516.2082; found, 516.2093.

*5.2.15. 4-(4-((4′-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)piperazin-1-yl)-N-((4-methylpiperazin-1-yl)sulfonyl)benzamide (**36**)*

Compound **31b** was prepared according to literature procedures.[23] Compound **36** were obtained according to the procedure of preparing **8** using compound **31b** and **34**. White solid; 18%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 8.5 Hz, 2H), 7.28 (d, J = 8.4 Hz, 2H), 7.02 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 8.5 Hz, 2H), 3.51 (t, J = 5.2 Hz, 4H), 3.27 (t, J = 5.2 Hz, 4H), 2.85 (s, 2H), 2.59 (s, 4H), 2.39–2.36 (m, 7H), 2.27 (t, J = 6.5 Hz, 2H), 2.04 (s, 2H), 1.49 (t, J = 6.5 Hz, 2H), 1.01 (s, 6H). HRMS (ESI) calcd for $C_{31}H_{41}ClN_5O_3S$, 598.2624; found, 598.2637.

*5.2.16. 4-(4-((4′-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)piperazin-1-yl)-N-((1-methyl-1H-pyrazol-3-yl)sulfonyl) benzamide (**37**)*

Compound **31c** was prepared according to literature procedures.[23] Compound **37** were obtained according to the procedure of preparing **8** using compound **31c** and **34**. White solid; 36%; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 7.90 (d, J = 8.6 Hz, 2H), 7.72 (d, J = 2.3 Hz, 1H), 7.37 (d, J = 8.4 Hz, 2H), 7.18 (d, J = 8.4 Hz, 2H), 6.83 (d, J = 8.6 Hz, 2H), 6.65 (s, 1H), 3.81 (s, 3H), 3.31–3.19 (m, 4H), 2.85 (s, 2H), 2.40 (t, J = 4.9 Hz, 4H), 2.31 (t, J = 6.5 Hz, 2H), 2.09 (s, 2H), 1.50 (t, J = 6.5 Hz, 2H), 1.01 (s, 6H). HRMS (ESI) calcd for $C_{30}H_{35}ClN_5O_3S$, 580.2155; found, 580.217.

*5.2.17. 4-(4-((4′-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)piperazin-1-yl)-N-((4-(dimethylamino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (**38**)*

Compound **33a** was prepared according to literature procedures.[24] Compound **38** were obtained according to the procedure of preparing **8** using compound **33a** and **34**. White solid; 39%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (d, J = 2.2 Hz, 1H), 8.36 (dd, J = 8.7, 2.2 Hz, 1H), 7.70 (d, J = 8.4 Hz, 2H), 7.34–7.29 (m, 3H), 7.00 (d, J = 8.2 Hz, 2H), 6.61 (d, J = 8.4 Hz, 2H), 4.54 (s, 1H), 3.43–3.17 (m, 6H), 2.89 (s, 6H), 2.72 (s, 4H), 2.28 (t, J = 6.4 Hz, 2H), 2.09 (s, 2H), 1.46 (t, J = 6.4 Hz, 2H), 0.93 (s, 6H). HRMS (ESI) calcd for $C_{35}H_{41}ClF_3N_4O_5S_2$, 753.2154; found, 753.2132.

*5.2.18. 4-(4-((4′-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)piperazin-1-yl)-N-((4-(3,3-difluoroazetidin-1-yl)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (**39**)*

Compound **33b** was prepared according to literature procedures.[24] Compound **39** were obtained according to the procedure of preparing **8** using compound **33b** and **34**. White solid; 42%; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 8.54 (s, 1H), 8.23 (d, J = 8.8 Hz, 1H), 7.82 (d, J = 8.3 Hz, 2H), 7.37 (d, J = 8.1 Hz, 2H), 7.20 (d, J = 8.1 Hz, 2H), 6.96–6.89 (m, 3H), 4.85 (t, J = 12.2 Hz, 4H), 3.48 (s, 4H), 3.12 (s, 2H), 2.65 (s, 4H), 2.33 (t, J = 6.4 Hz, 2H), 2.20 (s, 2H), 1.51 (t, J = 6.4 Hz, 2H), 1.02 (s, 6H). HRMS (ESI) calcd for $C_{36}H_{39}ClF_5N_4O_5S_2$, 801.1965; found, 801.1972.

*5.2.19. 4-(4-((4′-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)piperazin-1-yl)-N-((4-((2-hydroxyethyl)(methyl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (**40**)*

Compound **33c** was prepared according to literature procedures.[24] Compound **40** were obtained according to the procedure of preparing **8** using compound **33c** and **34**. White solid; 43%; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 8.61 (d, J = 2.2 Hz, 1H), 8.35 (dd, J = 8.9, 2.2 Hz, 1H), 7.83 (d, J = 8.7 Hz, 2H), 7.67 (d, J = 8.9 Hz, 1H), 7.37 (d, J = 8.1 Hz, 2H), 7.18 (d, J = 8.1 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 3.81 (t, J = 5.8 Hz, 2H), 3.47 (t, J = 5.8 Hz, 2H), 3.37 (t, J = 5.0 Hz, 4H), 3.06 (s, 3H), 2.98 (s, 2H), 2.52 (s, 4H), 2.32 (t, J = 6.5 Hz, 2H), 2.11 (s, 2H), 1.50 (t, J = 6.5 Hz, 2H), 1.00 (s, 6H). HRMS (ESI) calcd for $C_{36}H_{41}ClF_3N_4O_6S_2$, 781.2114; found, 781.2121.

*5.2.20. 4-((1-(Morpholinomethyl)cyclopropyl)amino)-3-((trifluoromethyl)sulfonyl)benzenesulfonamide (**42a**)*

Compounds **42a** were prepared according to corresponding literature procedures.[23] Colorless oil; 69%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, J = 2.2 Hz, 1H), 8.03 (dd, J = 9.2, 2.2 Hz, 1H), 7.31 (d, J = 9.2 Hz, 1H), 7.26 (s, 1H), 4.89 (s, 2H), 3.64 (t, J = 11.9 Hz, 4H), 2.76 (s, 2H), 1.60 (s, 3H), 1.01–0.85 (m, 4H).

*5.2.21. 4-((1-((3,3-Difluoroazetidin-1-yl)methyl)cyclopropyl) amino)-3-((trifluoromethyl)sulfonyl)benzenesulfonamide (**42b**)*

Compounds **42b** were prepared according to corresponding literature procedures.[23] White foam; 45%; ^{1}H NMR (400 MHz, Chloroform-d) δ 8.29 (d, J = 2.2 Hz, 1H), 8.00 (dd, J = 9.2, 2.2 Hz, 1H), 7.29–7.26 (m, 2H), 5.02 (s, 2H), 3.67 (s, 4H), 2.51 (s, 6H), 0.91 (s, 4H).

*5.2.22. 4-((3-(Morpholinomethyl)oxetan-3-yl)methoxy)-3-((trifluoromethyl)sulfonyl)benzenesulfonamide (**49a**)*

To a stirred solution of **48a** (110 g, 0.57 mmol)in anhydrous THF (50 mL), NaH (30 mg, 0.76 mmol) was added under N_2 at −10 °C and stirred for 10 min. Compound **32** was added and stirred for another 1 h. Water was added to quenching the reaction and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated under vacuo. The residue was prified by chromatography (CH_2Cl_2/CH_3OH 50:1) to provide **49a** as colorless oil (66 mg, 36%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, J = 2.4 Hz, 1H), 8.34 (dd, J = 8.9, 2.4 Hz, 1H), 7.40 (d, J = 8.9 Hz, 1H), 5.13 (s, 2H), 4.62 (d, J = 6.7 Hz, 4H), 4.51 (s, 2H), 3.63 (s, 4H), 2.87 (s, 2H), 2.37 (s, 4H).

*5.2.23. 4-((3-((3,3-Difluoroazetidin-1-yl)methyl)oxetan-3-yl) methoxy)-3-((trifluoromethyl)sulfonyl)benzenesulfonamide (**49b**)*

Compound **49b** was obtained according to the procedure of preparing **49a** using the crude acid and compound **7**. Colorless oil; 35%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, J = 2.4 Hz, 1H), 8.32 (dd, J = 9.0, 2.4 Hz, 1H), 7.32 (d, J = 9.0 Hz, 1H), 5.05 (s, 2H), 4.54–4.52 (m, 4H), 4.46 (d, J = 6.7 Hz, 2H), 3.64 (t, J = 11.8 Hz, 4H), 3.13 (s, 2H).

*5.2.24. 4-(4-((4′-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)piperazin-1-yl)-N-((4-((1-(morpholinomethyl)cyclopropyl) amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (**50**)*

Compound **50** were obtained according to the procedure of preparing **8** from compound **42a** and **34**. White solid; 35%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.29 (d, J = 8.9 Hz, 1H), 7.65 (d, J = 8.6 Hz, 2H), 7.30 (d, J = 14.8 Hz, 2H), 7.24 (d, J = 8.9 Hz, 1H), 6.99 (d, J = 8.1 Hz, 2H), 6.77 (d, J = 8.6 Hz, 2H), 3.65 (t, J = 4.6 Hz, 4H), 3.37–3.25 (m, 4H), 2.88 (s, 2H), 2.50–2.41 (m, 10*H*), 2.26 (t, J = 6.4 Hz, 2H), 2.03 (s, 2H), 1.46 (t, J = 6.4 Hz, 2H), 0.98 (s, 6H), 0.89 (s, 4H). HRMS (ESI) calcd for $C_{41}H_{48}ClF_3N_5O_6S_2$, 862.2692; found, 862.2668.

*5.2.25. 4-(4-((4′-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)piperazin-1-yl)-N-((4-((1-((3,3-difluoroazetidin-1-yl)methyl) cyclopropyl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl) benzamide (**51**)*

Compound **51** were obtained according to the procedure of preparing **8** from compound **42b** and **34**. White solid; 45%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, J = 2.2 Hz, 1H), 8.31 (dd, J = 9.2, 2.2 Hz, 1H), 7.65 (d, J = 8.6 Hz, 2H), 7.28 (d, J = 8.3 Hz, 2H), 7.22 (d, J = 9.2 Hz, 1H), 6.99 (d, J = 8.3 Hz, 2H), 6.76 (d, J = 8.6 Hz, 2H), 3.61 (t, J = 11.9 Hz, 4H), 3.30 (s, 4H), 2.89 (s, 2H), 2.72 (s, 2H), 2.41 (s, 4H), 2.25 (t, J = 6.5 Hz, 2H), 2.03 (s, 2H), 1.46 (t, J = 6.5 Hz, 2H), 0.97 (s, 6H), 0.94–0.89 (m, 2H), 0.88–0.82 (m, 2H). HRMS (ESI) calcd for $C_{40}H_{44}ClF_5N_5O_5S_2$, 868.2398; found, 868.2401.

*5.2.26. (S)-4-(4-((4′-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)piperazin-1-yl)-N-((4-((3-cyclopropyl-1-morpholino-1-oxopropan-2-yl)amino)-3-((trifluoromethyl)sulfonyl) phenyl)sulfonyl)benzamide (**52**)*

Compound **52** were obtained according to the procedure of preparing **8** from compound **45**[15] and **34**. White solid; 36%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, J = 2.2 Hz, 1H), 8.24 (d, J = 9.1 Hz, 1H), 8.02 (d, J = 6.7 Hz, 1H), 7.64 (d, J = 8.5 Hz, 2H), 7.28 (d, J = 8.4 Hz, 2H), 6.99 (d, J = 8.4 Hz, 2H), 6.78 (d, J = 9.1 Hz, 1H), 6.67 (d, J = 8.5 Hz, 2H), 4.65–4.55 (m, 1H), 3.75 (q, J = 6.4 Hz, 1H), 3.61–3.53 (m, 4H), 3.28 (s, 4H), 2.97 (s, 2H), 2.48 (s, 4H), 2.26 (t, J = 6.4 Hz, 2H), 2.03 (s, 2H), 1.81–1.63 (m, 2H), 1.46 (t, J = 6.4 Hz, 2H), 0.96 (s, 6H), 0.74–0.65 (m, 1H), 0.51 (q, J = 5.1 Hz, 2H), 0.09 (q, J = 5.1 Hz, 2H). HRMS (ESI) calcd for $C_{43}H_{52}ClF_3N_5O_7S_2$, 906.2943; found, 906.2968.

*5.2.27. (S)-4-(4-((4′-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)piperazin-1-yl)-N-((4-((1-cyclopropyl-3-morpholinopropan-2-yl)amino)-3-((trifluoromethyl)sulfonyl) phenyl)sulfonyl)benzamide (**53**)*

Compound **53** were obtained according to the procedure of preparing **8** from compound **46**[15] and **34**. White solid; 53%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, J = 2.2 Hz, 1H), 8.25 (dd, J = 9.2, 2.2 Hz, 1H), 7.67 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 6.8 Hz, 1H), 7.27 (d, J = 8.4 Hz, 2H), 6.99 (d, J = 8.4 Hz, 2H), 6.87 (d, J = 9.2 Hz, 1H), 6.76 (d, J = 8.7 Hz, 2H), 3.75 (q, J = 6.4 Hz, 1H), 3.67 (t, J = 4.4 Hz, 4H), 3.30 (t, J = 5.1 Hz, 4H), 2.90 (s, 2H), 2.59 (d, J = 6.4 Hz, 2H), 2.53–2.37 (m, 8H), 2.26 (t, J = 6.4 Hz, 2H), 2.02 (s, 2H), 1.54 (td, J = 6.6, 4.3 Hz, 2H), 1.46 (t, J = 6.4 Hz, 2H), 0.97 (s, 6H), 0.67 (dt, J = 13.3, 7.1 Hz, 1H), 0.51 (d, J = 7.7 Hz, 2H), 0.11 (dq, J = 9.3, 4.8, 4.1 Hz, 2H). HRMS (ESI) calcd for $C_{43}H_{54}ClF_3N_5O_7S_2$, 892.3151; found, 892.3129.

*5.2.28. 4-(4-((4′-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)piperazin-1-yl)-N-((4-((3-(morpholinomethyl) oxetan-3-yl)methoxy)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl) benzamide (**54**)*

Compound **54** were obtained according to the procedure of preparing **8** from compound **49a** and **34**. White solid; 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.55–8.46 (m, 1H), 7.71 (d, J = 8.2 Hz, 2H), 7.31 (d, J = 8.1 Hz, 2H), 7.00 (d, J = 8.0 Hz, 2H), 6.60 (s, 2H), 4.62–4.45 (m, 6H), 3.59 (t, J = 4.6 Hz, 4H), 3.33–3.23 (m, 6H), 2.81–2.68 (m, 6H), 2.36–2.29 (m, 6H), 2.07 (s, 2H), 1.46 (t, J = 6.6 Hz, 2H), 0.93 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.19, 152.93, 140.56, 136.50, 135.26, 132.40, 132.34, 129.91, 129.17, 128.39, 120.73, 119.27, 118.13, 114.05, 113.51, 77.38, 71.50, 71.30, 66.44, 60.04, 53.65, 51.92, 45.92, 42.69, 40.87, 34.98, 30.76, 28.64, 27.60. HRMS (ESI) calcd for $C_{42}H_{51}ClF_3N_4O_8S_2$, 895.2783; found, 895.2783.

*5.2.29. 4-(4-((4′-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1′-biphenyl]-2-yl)methyl)piperazin-1-yl)-N-((4-((3-((3,3-difluoroazetidin-1-yl)methyl) oxetan-3-yl)methoxy)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl) benzamide (**55**)*

Compound **55** were obtained according to the procedure of preparing **8** from compound **49b** and **34**. White solid; 51%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 1H), 8.48 (d, J = 8.5 Hz, 1H), 7.71 (d, J = 8.2 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.13 (d, J = 8.5 Hz, 1H), 7.00 (d, J = 8.0 Hz, 2H), 6.55 (s, 2H), 4.56–4.29 (m, 6H), 3.60 (t, J = 11.9 Hz, 4H), 3.33 (s, 6H), 3.07 (s, 2H), 2.75 (s, 4H), 2.29 (t, J = 6.4 Hz, 2H), 2.06 (s, 2H), 1.45 (t, J = 6.5 Hz, 2H), 0.91 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.86, 152.96, 140.75, 138.29, 133.06, 132.80, 130.33, 129.47, 128.76, 123.64, 121.04, 119.51, 118.61, 118.44, 116.42, 114.23, 113.87, 113.65, 77.28, 76.77, 71.46, 66.31 (t, J = 23.0 Hz), 60.65, 60.28, 52.20, 46.10, 43.58, 41.12, 35.24, 31.10, 28.94, 27.88. HRMS (ESI) calcd for $C_{41}H_{45}ClF_5N_4O_7S_2$, 899.2344; found, 899.2353.

Acknowledgments

This work was supported by grants from Chinese NSF (Grants 81773565, 81773767, 81430080). Supporting grants from the National Program on Key Basic Research Project of China (Grant 2015CB910603-004), the International Cooperative Program (Grant GJHZ1622) and Key Program of the Frontier Science (Grant 160621) of the Chinese Academy of Sciences, the Shanghai Commission of Science and Technology (Grants 16XD1404600, 14431900400), and CAS Key Laboratory of Receptor Research of SIMM (Grants SIMM1606YKF-08, SIMM1606YZZ-06) are also highly appreciated.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmc.2017.12.001.

References

1. Danial NN, Korsmeyer SJ. Cell death: critical control points. *Cell.*. 2004;116:2005–2019.
2. Opferman JT. Attacking cancer's achilles heel: antagonism of antiapoptotic BCL-2 family members. *FEBS J.*. 2016;283:2661–2675.
3. Wang S, Yang D, Lippman ME. Targeting Bcl-2 and Bcl-xL with nonpeptidic small-molecule antagonists. *Semin Oncol.*. 2003;30:133–142.
4. Delbridge AR, Grabow S, Strasser A, et al. Thirty years of BCL-2: translating cell death discoveries into novel cancer therapies. *Nat Rev Cancer.*. 2016;16:99–109.
5. Hennessy EJ. Selective inhibitors of Bcl-2 and Bcl-xL: balancing antitumor activity with on-target toxicity. *Bioorg Med Chem Lett.*. 2016;26:2015–2114.
6. Ashkenazi A, Fairbrother WJ, Leverson JD, et al. From basic apoptosis discoveries to advanced selective BCL-2 family inhibitors. *Nat Rev Drug Discov.*. 2017;16:273–284.
7. Zheng CH, Zhou YJ, Zhu J, et al. Construction of a three-dimensional pharmacophore for Bcl-2 inhibitors by flexible docking and the multiple copy simultaneous search method. *Bioorg Med Chem.*. 2007;15:6407–6417.
8. Fletcher S, Yap JL, Chen L, et al. Expanding the cancer arsenal with targeted therapies: disarmament of the antiapoptotic Bcl-2 proteins by small molecules. *J Med Chem.*. 2017;60:821–838.
9. Elmore SW, Souers AJ, Leverson JD, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat Med.*. 2013;19:2020–2208.
10. Bruncko M, Oost TK, Elmore SW, et al. Studies leading to potent, dual inhibitors of Bcl-2 and Bcl-xL. *J Med Chem.*. 2007;50:641–662.
11. Oltersdorf T, Elmore SW, Rosenberg SH, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature.*. 2005;435:677–681.
12. Elmore SW, Park CM, Bruncko M, et al. Discovery of an orally bioavailable small molecule inhibitor of prosurvival B-cell lymphoma 2 proteins. *J Med Chem.*. 2008;51:6902–6915.
13. Elmore SW, Tse C, Shoemaker AR, et al. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. *Cancer Res.*. 2008;68:3421–3428.
14. Petros AM, Olejniczak ET, Fesik SW. Structural biology of the Bcl-2 family of proteins. *Biochim Biophys Acta.*. 2004;1644:83–94.
15. Lee EF, Czabotar PE, Smith BJ, et al. Crystal structure of ABT-737 complexed with Bcl-xL: implications for selectivity of antagonists of the Bcl-2 family. *Cell Death Differ.*. 2007;14:1711–1713.
16. Ghosh I, Porter JR, Helmers MR, et al. Profiling small molecule inhibitors against helix–receptor interactions: the Bcl-2 family inhibitor BH3I-1 potently inhibits p53/hDM2. *Chem Commun.*. 2010;46:8020–8022.
17. Weissleder R, Giedt RJ, Sprachman MM, et al. Imaging cellular distribution of Bcl inhibitors using small molecule drug conjugates. *Bioconjugate Chem.*. 2014;25:2081–2085.
18. Kasai S, Kamaura M, Kamata M, et al. Melanin-concentrating hormone receptor 1 antagonists: synthesis, structure-activity relationship, docking studies, and biological evaluation of 2,3,4,5-tetrahydro-1H-3-benzazepine derivatives. *Bioorg Med Chem.*. 2011;19:6261–6273.
19. Drakulić BJ, Supuran CT, Cvijetić IN, et al. 5-Aryl-1H-pyrazole-3- carboxylic acids as selective inhibitors of human carbonic anhydrases IX and XII. *Bioorg Med Chem.*. 2015;23:4649–4659.
20. Sawant SD, Kumar KAA, Venkateswarlu V, et al. A metal-free approach to carboxylic acids by oxidation of alkyl, aryl, or heteroaryl alkyl ketones or arylalkynes. *Synthesis.*. 2015;47:3161–3168.
21. Venkatesan AM, Agarwal A, Abe T, et al. Structure-activity relationship of 6-methylidene penems bearing 6,5 bicyclic heterocycles as broad-spectrum β-lactamase inhibitors: evidence for 1,4-thiazepine intermediates with C7 R stereochemistry by computational methods. *J Med Chem.*. 2006;49:4623–4637.

22. Staben ST, Castanedo GM, Blaquiere N, et al. Structure-based design of tricyclic NF-κB inducing kinase (NIK) inhibitors that have high selectivity over phosphoinositide-3-kinase (PI3K). *J Med Chem.*. 2017;60:627–640.
23. Waldmann H, Zimmermann G, Schultz-Fademrecht C, et al. Structure guided design and kinetic analysis of highly potent benzimidazole inhibitors targeting the PDEδ prenyl binding site. *J Med Chem.*. 2014;57:5435–5448.
24. Vivas JM, Díaz JA, Morante ME, et al. Synthesis and anti-inflammatory evaluation of new sulfamoylheterocarboxylic derivatives. *Arch Pharm.*. 1996;329:229–238.
25. Lawhorn BG, Philp J, Zhao Y, et al. Identification of purines and 7-deazapurines as potent and selective Type I inhibitors of troponin I-interacting kinase (TNNI3K). *J Med Chem.*. 2015;58:7431–7448.

Please cite this article in press as: Liu X., et al. Bioorg. Med. Chem. (2017), https://doi.org/10.1016/j.bmc.2017.12.001