

Supplementary information

Establishing drug discovery and identification of hit series for the anti-apoptotic proteins, Bcl-2 and Mcl-1

‡James B. Murray¹, ‡James Davidson¹, Ijen Chen¹, Ben Davis¹, Pawel Dokurno¹, Christopher J Graham¹, Richard Harris¹, Allan Jordan¹, †, Natalia Matassova¹, Christopher Pedder¹, Stuart Ray¹, Stephen D. Roughley¹, Julia Smith¹, Claire Walmsley¹, Yikang Wang¹, Neil Whitehead¹, Douglas S Williamson¹, Patrick Casara², Thierry Le Diguarher², John Hickman², Jerome Stark², ‡András Kotschy³, ‡Olivier Geneste², Roderick E Hubbard^{1,4,}*

¹ Vernalis (R&D) Ltd, Granta Park, Abington, Cambridge, CB21 6GB, UK

² Institut de Recherches Servier Oncology R&D Unit, Croissy Sur Seine 78290, France

³ Servier Research Institute of Medicinal Chemistry, Budapest 1031, Hungary

⁴ YSBL, University of York, Heslington, York, YO10 5DD, UK

* corresponding author r.hubbard@vernalis.com

Details of protein constructs

The tag sequence is underlined, the cleavage sequence is in italics

Bcl-2#2 sequence:

N-terminal His tag - thrombin cleavage site

MGSSHHHHHHSSGLVPRGSHMAHAGRTGYDNREIVMKYIHYKLSQRGYEWDAAGDDVE
ENRTEAPEGTESEVVHLTLRQAGDDFSRRYRRDFAEMSSQLHLTPFTARGRFATVVEEL
FRDGVNWGRIVAFFEFGGVMCVESVNREMSPLVDNIALWMTEYLNRLHTWIQDNGG
WDAFVELYGPSMR

Bcl-2#3 sequence:

C-terminal His tag

MSQDNREIVMKYISYKLSQRGYEWDAAGDVEENRTEAPEGTESEVVHQTLRQAGDDFSL
RYRRDFAEMSSQLHLTPGTAYASFATVVEELFRDGVNWGRIVAFFEFGGVMCVESVNR
EMSVLVDNIAAWMATYLNHLHTWIQDNGGWDAFVELYGNNAEAESRKGGQERFLEH
HHHHH

Bcl-2#3' sequence:

C-terminal His tag

MSQDNREIVMKYISYKLSQRGYEWDAAGDVEENRTEAPEGTASAVVHQTLRQAGDDFSL
RYRRDFAQMSSQLHLTPGTAYASFATVVEALFRDGVNWGRIVAFFEFGGVMCVESVNR
AMSVLVDNIAAWMATYLNHLHTWIQDNGGWDAFVELYGNNAEAESRKGGQERFLEH
HHHHH

hBcl-2 sequence

N-terminal His tag – thrombin cleavage site

MGHHHHHHHHSAGLVPRGSMAHAGRTGYDNREIVMKYIHYKLSQRGYEWDAAGDVGA
APPGAAPAPGIFSSQPGHTPHPAASRDVPARTSPLQTPAAPGAAAGPALSPVPPVVHLTL
RQAGDDFSRRYRRDFAEMSSQLHLTPFTARGRFATVVEELFRDGVNWGRIVAFFEFGG
VMCVESVNREMSPLVDNIALWMTEYLNRLHTWIQDNGGWDAFVELYGPSMR

GST-3C-hMcl-1 sequence

N-terminal GST, 3C protease cleavage site, hMcl-1 - Used for initial crystallography

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYI
DGDVKLTQSMARIYIADKHNMLGGCPKERAISMLEGAVLDIRYGVSRIAYSKDFETLK
VDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL
VCFKKRIEAIPQIDKYLKSSKYIAWPLOGWQATFGGGDHPPKSD*LEVLFQGPLGSEDELY*
RQSLEIISRYLREQATGAKDTKPMGRSGATSRKALETLRRVGDGVQRNHETAFQGMLR
KLDIKNEDDVKSLSRVMIHVFSDBGVTNWGRIVTLISFGAFVAKHLKTINQESCIEPLAESI
TDVLVRTKRDWLKQRGWDGFVEFFHVEDLEGG

GST-3C-hMcl-1#1 sequence

N-terminal GST, 3C protease cleavage site, hMcl-1 with mutations – used for some crystallography

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYI
DGDVKLTQSMAIIRYIADKHNMLGGCPKERAISMLEGAVLDIRYGVSRIAYSKDFETLK
VDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL
VCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD*LEVLFQGPLGSEDDLY*
RQSLEIISRYLREQATGAKDTKPMGRSGATSRKALETLRVGDGVQRNHETAFQGMLR
KLDIKNEGDVKSFSRVMVHVFKDGVTNWGRIVTLISFGAFVAKHLKSVNQESFIEPLAET
ITDVLVRTKRDWLVKQRGWDGDFVEFFHVQDLEGG

His-TEV-hMcl-1 sequence

N terminal hexahis tag, TEV cleavage site, hMcl-1 – used for fragment screen and FP assay

MAHHHHHHSS*ENLYFQGPLGSEDELYRQSLEIISRYLREQATGAKDTKPMGRSGATSRK*
*ALETLRVGDGVQRNHETAFQGMLRKLDIKNEDDVKSLSRVMHVFS**SDGVTNWGRIVT*
LISFGAFVAKHLKTINQESCIEPLAESITDVLVRTKRDWLVKQRGWDGDFVEFFHVEDLEG
G

Double His-TEV-hMcl-1 sequence

MHHHHHHHSSGLVPRGSGMKETAAAKFERQHMDSPDLGTDDDDKMAHHHHHHHSS*EN*
LYFQGPLGSEDELYRQSLEIISRYLREQATGAKDTKPMGRSGATSRKALETLRVGDGV
*QRNHETAFQGMLRKLDIKNEDDVKSLSRVMHVFS**SDGVTNWGRIVTLISFGAFVAKHLK*
TINQESCIEPLAESITDVLVRTKRDWLVKQRGWDGDFVEFFHVEDLEGG

His-Thrombin-hMcl-1 sequence

N terminal hexahis tag Thrombin cleavage site, hMcl-1 – used for crystallography

MHHHHHHHLVPRGSEDELYRQSLEIISRYLREQATGAKDTKPMGRSGATSRKALETLRV
GDGVQRNHETAFQGMLRKLDIKNEDDVKSLSRVMHVFS*SDGVTNWGRIVTLISFGAFV*
AKHLKTINQESCIEPLAESITDVLVRTKRDWLVKQRGWDGDFVEFFHVEDLEGG

MBP-hMcl-1 sequence

Maltose-binding protein N-terminal fusion, used for later crystallography

MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGP
DIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIY
NKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYAAGK
YDIKDVGVDNAGAKAGLTFLVDLIKHKHMNADTDYSIAEAAFNKGETAMTINGPAWAW
SNIDTSAVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEA
VNKDKPLGAVALKSYYYEELAKDPRIAATMENAQKGEIMPNIQMSAFWYAVRTAVINA
ASGRQTVDEALKDAQTGSELYRQSLEIISRYLREQATGAADTAPMGASGATSRKALET
LRVGDGVQRNHETAFQGMLRKLDIKNEDDVKSLSRVMHVFS*SDGVTNWGRIVTLISFG*
AFVAKHLKTINQESCIEPLAESITDVLVRTKRDWLVKQRGWDGDFVEFFHV

This protein was used for crystallography undertaken during late lead optimisation.

His-TEV mMcl-1 sequence

N terminal hexa his tag, TEV cleavage site, mouse Mcl-1 – used for 2D NMR experiments

MAHHHHHSS*ENLYFQG*PLGSEDDLYRQSLEIISRYLREQATGSKDSKPLGEAGAAGRR
ALETLLRRVGDGVQRNHETAFQGMLRKLDIKNEGDVKSFSRVMVHVFKDGVTNWGRIV
TLISFGAFVAKHLKSVNQESFIEPLAETITDVLVRTKRDWLVKQRGWDGFVEFFHVQDL
EGG

Compound synthesis

Chemistry. General Methods. All solvents and reagents were used as obtained from commercial vendors. All tested compounds were determined to be >95% pure by LC-MS (Method A).

LC-MS Method A

Instruments: Agilent HP1100 with DAD and MSD G1946 D (positive and negative ionization, scanning range: 150–1000 Da) or Agilent HP1200 with DAD and MSD 6140 (positive and negative ionization, scanning range: 150–1000 Da). UV detection was at 230 nm, 254 nm and 270 nm. The conditions and methods are identical for both instruments.

Column: Gemini NX 5 μ m C18, 30 \times 2.1 mm, from Phenomenex.

Temperature: 40 °C.

Mobile phase: Solvent A: Water/ 10 mM ammonium formate/ 0.08% (v/v) formic acid pH = 3.5.

Solvent B: Acetonitrile/ 5.3 % (v/v) Solvent A/ 0.08% (v/v) formic acid.

Injection volume: 1 μ L

Gradient:

Time (min)	Solvent A (%)	Solvent B (%)	Flow Rate (ml min ⁻¹)
0.00	95	5	1.0
0.25	95	5	1.0
2.50	5	95	1.0
2.55	5	95	1.7
3.60	5	95	1.7
3.65	5	95	1.0
3.70	95	5	1.0
3.75	95	5	1.0

LC-MS Method B

Instrument: Agilent 1290 Infinity II series instrument connected to an Agilent TOF 6230 single quadrupole with an ESI source.

Column: Gemini NX 5 μ m C18, 30 \times 2.1 mm, from Phenomenex.

Temperature: 40 °C.

Mobile phase: Solvent A: Water/ 10 mM ammonium formate/ 0.08% (v/v) formic acid
pH = 3.5.

Solvent B: Acetonitrile/ 5.3% (v/v) Solvent A/ 0.08% (v/v) formic acid.

Injection volume: 1 μ L

Gradient:

Time (min)	Solvent A (%)	Solvent B (%)	Flow Rate (ml min ⁻¹)
0.00	95	5	1.3
0.12	95	5	1.3
1.30	5	95	1.3
1.35	5	95	1.6
1.85	5	95	1.6
1.90	5	95	1.3
1.95	95	5	1.3

¹H (400 MHz) NMR analyses were performed using a Bruker DPX-400 spectrometer. The spectral reference was the known chemical shift of the sample solvent (chloroform; 7.25 ppm or DMSO-d₆; 2.50 ppm). ¹H NMR data are reported indicating the chemical shift (δ) as parts per million (ppm), the integration (e.g. 1 H), and the multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; sept, septet; m, multiplet; br, broad; dd, doublet of doublets). Coupling constants are reported in hertz.

Method for Preparative HPLC compound purification

Preparative HPLC purifications were performed on a Waters FractionLynx MS autopurification system with a Gemini 5 μ m C18(2), 100 mm \times 20 mm i.d. column from Phenomenex, running at a flow rate of 20 mL min⁻¹ with UV diode array detection (210–400 nm) and mass-directed collection. The mass spectrometer was a Waters Micromass ZQ2000 spectrometer operating in positive or negative ion electrospray ionization modes, with a molecular weight scan range of 150 to 1000.

Solvent A: HPLC grade Water + 10 mM ammonium acetate + 0.08% v/v formic acid.

Solvent B: 95% v/v HPLC grade acetonitrile + 5% v/v Solvent A + 0.08% v/v formic acid.

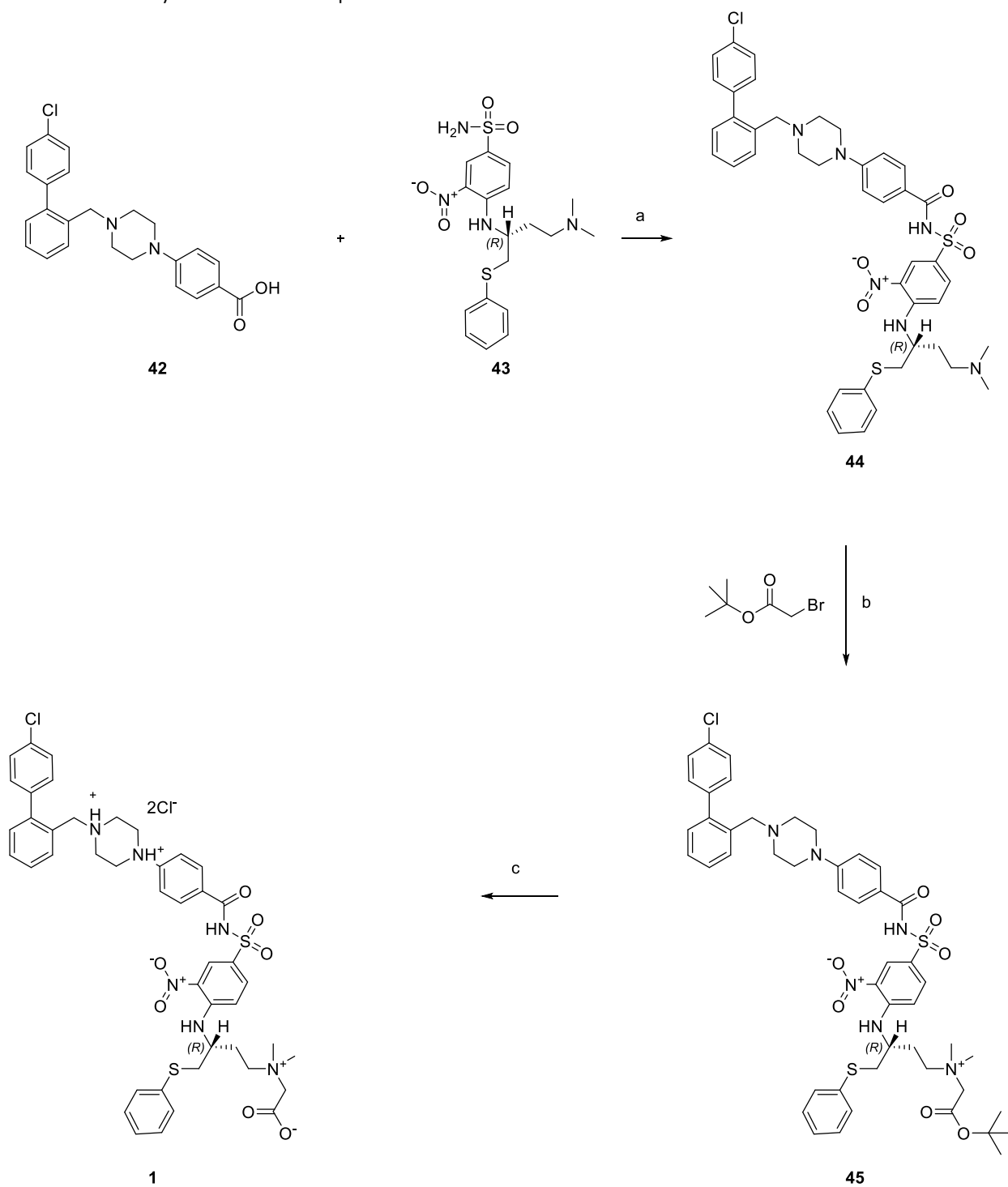
Gradient:

Time (min)	Solvent A (%)	Solvent B (%)
0.00	95	5
1.50	77	23
7.50	67	33
10.00	5	95

PAINS analysis of compounds reported in this paper

The 41 compounds were assessed against the 480 PAINS filters in the RDKit software (<http://rdkit.blogspot.com/2015/08/curating-pains-filters.html>). The SMILES representation of three compounds (**3**, **11** and **36**) match a PAINS filter. All three compounds were confirmed as binding by NMR and in the FP assay. The crystal structure of **3** was determined bound to hMcl-1 (Figure 4) and **6** is part of the hybrid compound for which a crystal structure was determined bound to Bcl-2 (Figure 6). This confirms that these compounds are binding to the targets and this is not an artefact.

Scheme S1. Synthesis of compound **1**.^a



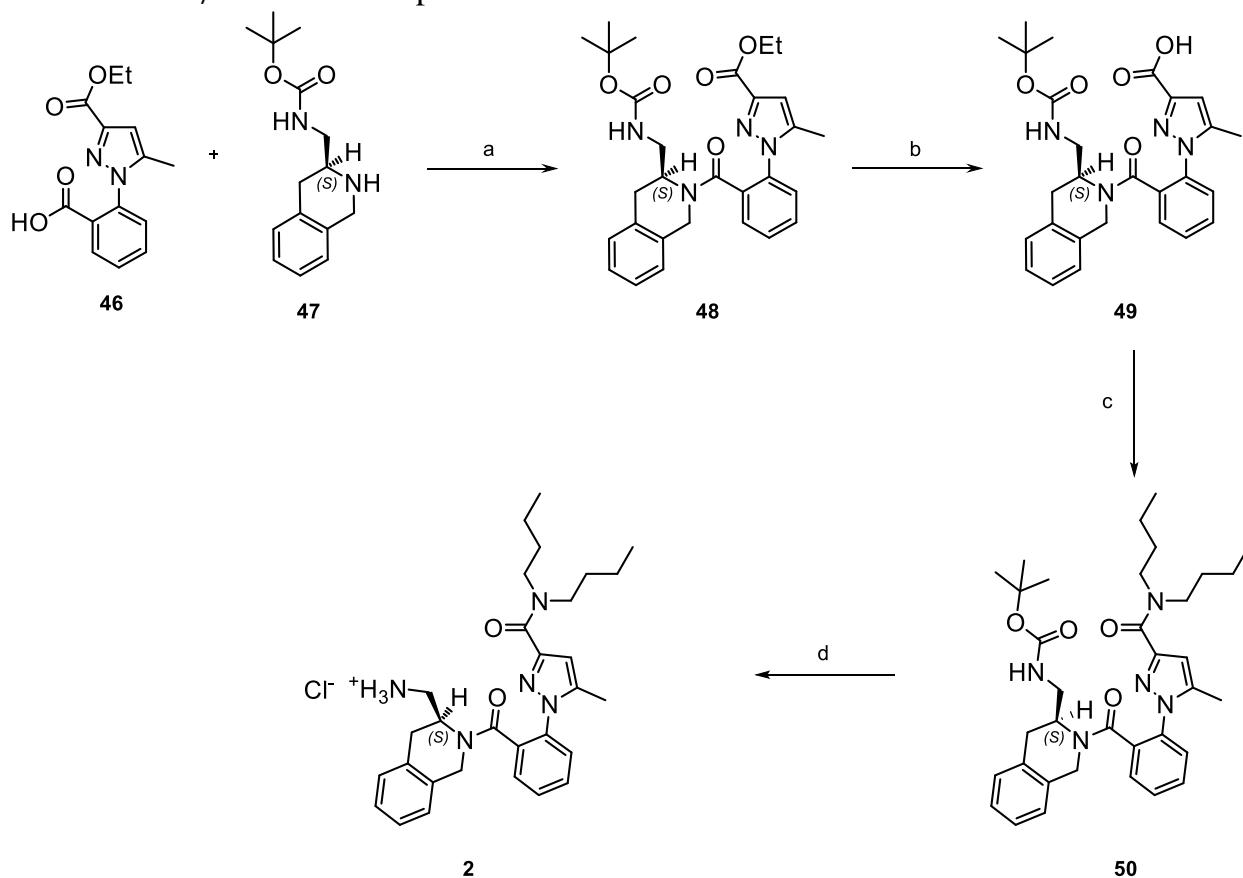
^aReagents and conditions: (a) EDC, DIPEA, DMAP, THF, rt; (b) DIPEA, KI, DMF, rt; (c) 5N HCl, dioxane, rt.

2-[[[(3R)-3-[4-[[4-[4-[[2-(4-Chlorophenyl)phenyl]methyl]piperazin-1-yl]benzoyl]sulfamoyl]-2-nitro-anilino]-4-phenylsulfanyl-butyl]-dimethyl-ammonio]acetate dihydrochloride (1) *Step 1.* To a suspension of 4-[4-[[2-(4-chlorophenyl)phenyl]methyl]piperazin-1-yl]benzoic acid dichloride (**42**) (99 mg, 0.207 mmol) in THF (8 mL) was added 4-[[[(1R)-3-(dimethylamino)-1-(phenylsulfanylmethyl)propyl]amino]-3-nitro-benzenesulfonamide (**43**)¹ (80 mg, 0.188 mmol), *N,N*-diisopropylethylamine (0.1 mL, 0.564 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (50 mg, 0.263 mmol) and 4-dimethylaminopyridine (32 mg, 0.263 mmol). The solution was stirred at room temperature overnight, poured into water and extracted with ethyl acetate. The combined organic layers were dried over MgSO₄, filtered and evaporated to dryness. The residue was purified by reverse phase chromatography (acetonitrile/ water + 0.5% TFA as eluent) to give 4-[4-[[2-(4-chlorophenyl)phenyl]methyl]piperazin-1-yl]-*N*-[4-[[[(1R)-3-(dimethylamino)-1-(phenylsulfanylmethyl)propyl]amino]-3-nitro-phenyl]sulfonyl-benzamide (**44**) (68 mg, 35%) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 9.50 (1H, m), 8.55 (1 H, br s), 8.20 (1 H, d), 7.90 (1 H, dd), 7.75 (2 H, d), 7.65 (1 H, m), 7.45 (4 H, m), 7.40 (2 H, d), 7.30 (3 H, m), 7.15 (4 H, m), 6.90 (2 H, d), 4.20 (1 H, m), 3.90 (2 H, m), 3.35 (6 H, m), 3.15 (2H, m), 2.75 (10 H, m), 2.15 (2 H, m). ESI/FIA/HR: [M+H]⁺ = 813.2657 measured (813.260 theoretical).

Step 2. To a solution of 4-[4-[[2-(4-chlorophenyl)phenyl]methyl]piperazin-1-yl]-*N*-[4-[[[(1R)-3-(dimethylamino)-1-(phenylsulfanylmethyl)propyl]amino]-3-nitro-phenyl]sulfonyl-benzamide (**44**) (200 mg, 0.245 mmol) in *N,N*-dimethylformamide (10 mL) was added *tert*-butyl 2-bromoacetate (40 μL, 0.26 mmol), *N,N*-diisopropylethylamine (90 μL, 0.49mmol) and potassium iodide (41 mg, 0.245mmol). The reaction mixture was stirred for 2 h at room temperature and then diluted with a mixture water/ ethyl acetate. The combined organic layers were washed two times with a saturated lithium chloride solution, dried over MgSO₄, filtered and evaporated to dryness. The residue was purified by flash chromatography (heptane/ methanol gradient) to give (2-*tert*-butoxy-2-oxo-ethyl)-[(3R)-3-[4-[[4-[4-[[2-(4-chlorophenyl)phenyl]methyl]piperazin-1-yl]benzoyl]sulfamoyl]-2-nitro-anilino]-4-phenylsulfanyl-butyl]-dimethyl-ammonium (**45**) (125 mg, 51%) as a white powder. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.46 (1 H, d), 8.10 (1 H, d), 7.81 (1 H, dd), 7.72 (2 H, d), 7.51 (1 H, d), 7.48 (4 H, s), 7.37 (2 H, m), 7.29 (2 H, d), 7.24 (3 H, m), 7.17 (1 H, t), 6.88 (1 H, d), 6.78 (2 H, d), 4.28 (2 H, s), 4.08 (1 H, m), 3.62 (1 H, m), 3.52 (1 H, m), 3.38 (2 H, s), 3.34 (2 H, d), 3.15 (6 H, s), 3.12 (4 H, m), 2.39 (4 H, dd), 2.22 (2 H, q), 1.42 (9 H, s). ESI/FIA/HR: [M]⁺ = 927.337 measured 927.334.

Step 3. To a suspension of 4-(2-*tert*-butoxy-2-oxo-ethyl)-[(3R)-3-[4-[[4-[4-[[2-(4-chlorophenyl)phenyl]methyl]piperazin-1-yl]benzoyl]sulfamoyl]-2-nitro-anilino]-4-phenylsulfanylbutyl]-dimethylammonium (**45**) (119 mg, 0.128 mmol) in dioxane (1.3 mL) was added dropwise a 6N HCl solution in water (1 mL, 5.77 mmol). The reaction mixture was stirred for 60 h at room temperature, and then concentrated to dryness. The residue was purified by reverse phase chromatography (acetonitrile/ water) to give 2-[[[(3R)-3-[4-[[4-[4-[[2-(4-chlorophenyl)phenyl]methyl]piperazin-1-yl]benzoyl]sulfamoyl]-2-nitro-anilino]-4-phenylsulfanyl-butyl]-dimethyl-ammonio]acetate dihydrochloride (**1**) (80 mg, 74%) as a white powder. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.58 (1 H, d), 8.20 (1 H, d), 8.00 (1 H, m), 7.89 (1 H, dd), 7.77 (2 H, d), 7.48 (4 H, m), 7.37 (2 H, d), 7.29 (1 H, m), 7.26 (2 H, d), 7.15 (4 H, m), 6.90 (2 H, d), 4.27 (2 H, s), 4.18 (1 H, m), 4.11 (2 H, m), 3.63 (2 H, m), 3.53 (4 H, m), 3.37 (2 H, m), 3.19 (6 H, s), 2.90 (4 H, m), 2.27 (2 H, q). ESI/FIA/HR: [M + H]⁺ = 871.2714 measured 871.2760.

Scheme S2. Synthesis of compound **2**.^a



^aReagents and conditions: (a) EDC, HOBT, Et₃N, DCM, rt; (b) LiOH.H₂O, dioxane, H₂O, rt; (c) NHBu₂, EDC, HOBT, Et₃N, DCM, rt; (d) HCl, dioxane, DCM, rt.

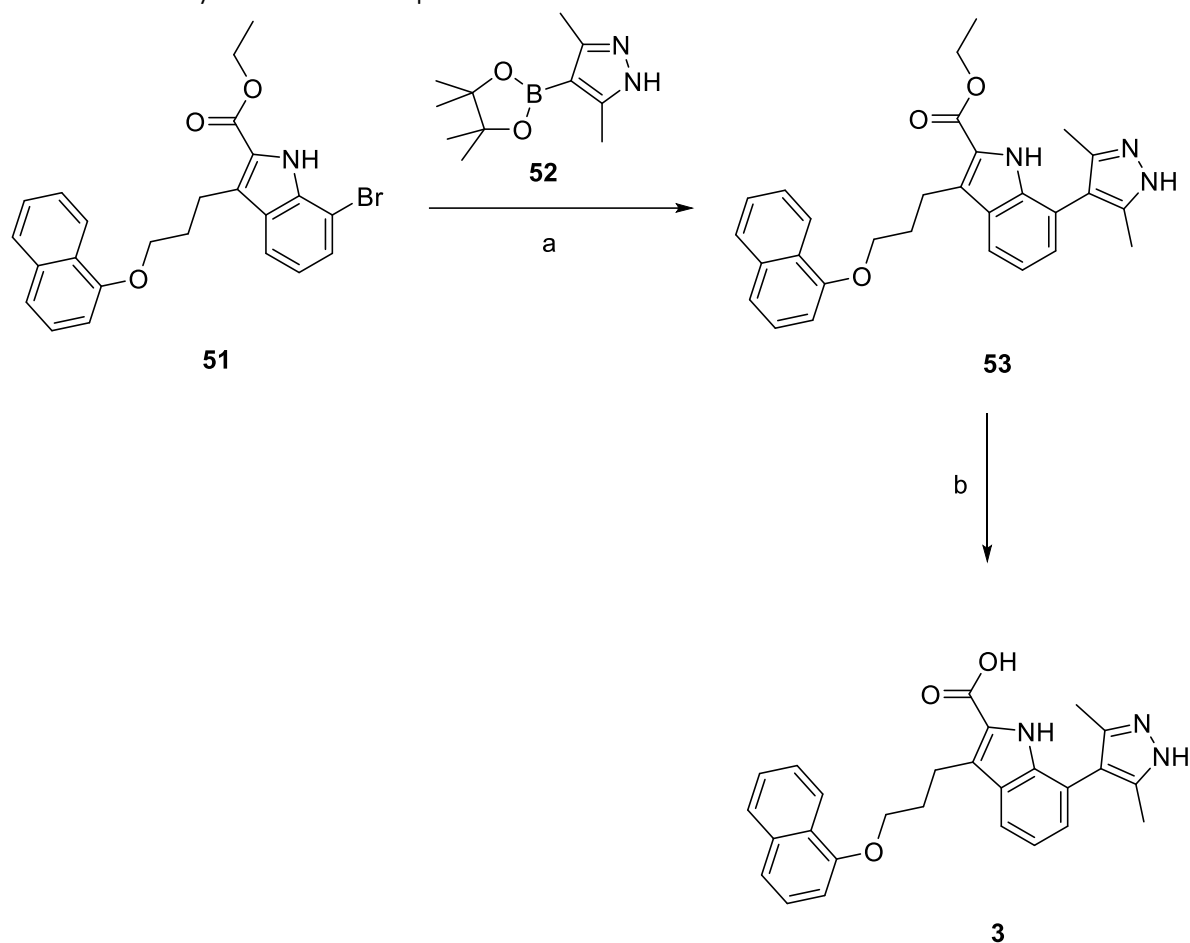
1-[2-[(3S)-3-(Aminomethyl)-3,4-dihydro-1H-isoquinoline-2-carbonyl]phenyl]-N,N-dibutyl-5-methyl-pyrazole-3-carboxamide (2) *Step 1*. To a solution of 2-(3-ethoxycarbonyl-5-methyl-pyrazol-1-yl)benzoic acid (**46**)² (0.3 g, 1.09 mmol) in dichloromethane (20 mL) was added *tert*-butyl *N*-[(3*S*)-1,2,3,4-tetrahydroisoquinolin-3-yl]methylcarbamate (**47**)³ (0.3 g, 1.15 mmol), *N,N,N*-triethylamine (310 μ L, 2.2 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (0.25 g, 1.31 mmol) and hydroxybenzotriazole (0.18 mg, 1.31 mmol). The reaction mixture was stirred at room temperature overnight, poured into water and extracted with ethyl acetate. The combined organic layers were washed with saturated aqueous NaHCO₃ solution, brine, dried over MgSO₄, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica (petroleum ether/ ethyl acetate as eluent) to give ethyl 1-[2-[(3*S*)-3-[(*tert*-butoxycarbonylamino)methyl]-3,4-dihydro-1*H*-isoquinoline-2-carbonyl]phenyl]-5-methyl-pyrazole-3-carboxylate (**48**) (0.4 g, 71%) as a colourless oil. ¹H NMR (400 MHz, DMSO-*d*₆) δ : 7.3 (4 H, m), 7.2 (4 H, m), 7.05 (1 H, t), 6.55 (1 H, s), 4.9/4.25 (2 H, 2d), 4.1 (2 H, m), 3.9 (1 H, m), 3.35/2.5 (2 H, m), 2.9 (2 H, m), 2.15 (3 H, s), 1.4 (9 H, s), 1.15 (3 H, t). MS (ESI): *m/z* 519 [M + H]⁺.

Step 2. To a solution of ethyl 1-[2-[(3*S*)-3-[(*tert*-butoxycarbonylamino)methyl]-3,4-dihydro-1*H*-isoquinoline-2-carbonyl]phenyl]-5-methyl-pyrazole-3-carboxylate (**48**) (300 mg, 0.578 mmol) in dioxane (8 mL) and water (2 mL) was added lithium hydroxide monohydrate (120 mg, 2.81 mmol). The reaction mixture was stirred at room temperature overnight and evaporated to dryness. The residue was suspended in HCl 1N aqueous solution, and the resulting white precipitate was filtered, washed with water and dried under vacuum in presence of P₂O₅ at 40 °C overnight, affording 1-[2-[(3*S*)-3-[(*tert*-butoxycarbonylamino)methyl]-3,4-dihydro-1*H*-isoquinoline-2-carbonyl]phenyl]-5-methyl-pyrazole-3-carboxylic acid (**49**) (215 mg, 71%) used in the next chemical step without purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 12.166 (1 H, m), 7.65–7.4 (4 H, m), 7.2–6.85 (4 H, m), 6.65–6.3 (1 H, m), 6.48 (1 H, s), 5.00–4.15 (2 H, m), 4.8–3.8 (1 H, m), 3.25–2.5 (4 H, m), 2.15 (3 H, s), 1.34 (9 H, s). MS (ESI): *m/z* 491 [M + H]⁺.

Step 3. To a solution of 1-[2-[(3*S*)-3-[(*tert*-butoxycarbonylamino)methyl]-3,4-dihydro-1*H*-isoquinoline-2-carbonyl]phenyl]-5-methyl-pyrazole-3-carboxylic acid (**49**) (200 mg, 0.407 mmol) in dichloromethane (15 mL) was added dibutylamine (75 μL, 0.428 mmol), *N,N,N*-triethylamine (110 μL, 0.815 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (94 mg, 0.489 mmol) and hydroxybenzotriazole (66 mg, 0.489 mmol). The reaction mixture was stirred at room temperature for 48 h, poured into water and extracted with dichloromethane. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica (dichloromethane/ methanol as eluent) to give *tert*-butyl *N*-{[(3*S*)-2-[2-[3-(dibutylcarbamoyl)-5-methylpyrazol-1-yl]benzoyl]-3,4-dihydro-1*H*-isoquinolin-3-yl]methyl}carbamate (**50**) (193 mg, 79%) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 7.65–7.3 (4 H, m), 7.2–6.85 (4H, m), 6.52 (1 H, m), 6.31 (1 H, s), 5–4.2 (2 H, m), 4.85–3.8 (1 H, m), 3.5–2.5 (8 H, m), 2.16 (3 H, s), 1.5–0.9 (8 H, m), 1.35 (9 H, m), 0.79 (6 H, m). MS (ESI): *m/z* 602 [M + H]⁺.

Step 4. To a solution of *tert*-butyl *N*-{[(3*S*)-2-[2-[3-(dibutylcarbamoyl)-5-methyl-1*H*-pyrazol-1-yl]benzoyl]-1,2,3,4-tetrahydroisoquinolin-3-yl]methyl}carbamate (**50**) (120 mg, 0.199 mmol) in dichloromethane (10 mL) was added a 4N HCl solution in dioxane (150 μL, 0.6 mmol). The reaction mixture was stirred at room temperature overnight and evaporated to dryness. The residue was suspended in diisopropyl ether, then the resulting white precipitate was filtered, washed with diisopropylether and dried under vacuum in presence of P₂O₅ at 40 °C overnight, affording 1-[2-[(3*S*)-3-(aminomethyl)-3,4-dihydro-1*H*-isoquinoline-2-carbonyl]phenyl]-*N,N*-dibutyl-5-methyl-pyrazole-3-carboxamide (**2**) (76mg, 75%) as a white crystalline powder. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 7.99 (3 H, m), 7.63 (4 H, m), 7.15 (4 H, m), 6.33 (1 H, s), 4.87 (1 H, m), 4.43 (2 H, m), 3.4–2.6 (8 H, m), 2.16 (3 H, s), 1.5-0.6 (14 H, m). Presence of conformers observed at room temperature. IR (ATR): ν: NH₃⁺ : 3100–220 cm⁻¹; ν: >C=O : 1613 cm⁻¹. HRMS (ESI): *m/z* calculated for C₃₀H₄₀N₅O₂ [M + H]⁺ 502.3182. Found: 502.3193. Elemental analysis: calculated (%) for C₃₀H₄₀N₅O₂.HCl: C 66.96, H 7.49, N 13.01, Cl 6.59 found: C 67.25, H 7.32, N 12.95, Cl 6.62.

Scheme S3. Synthesis of compound **3**.^a

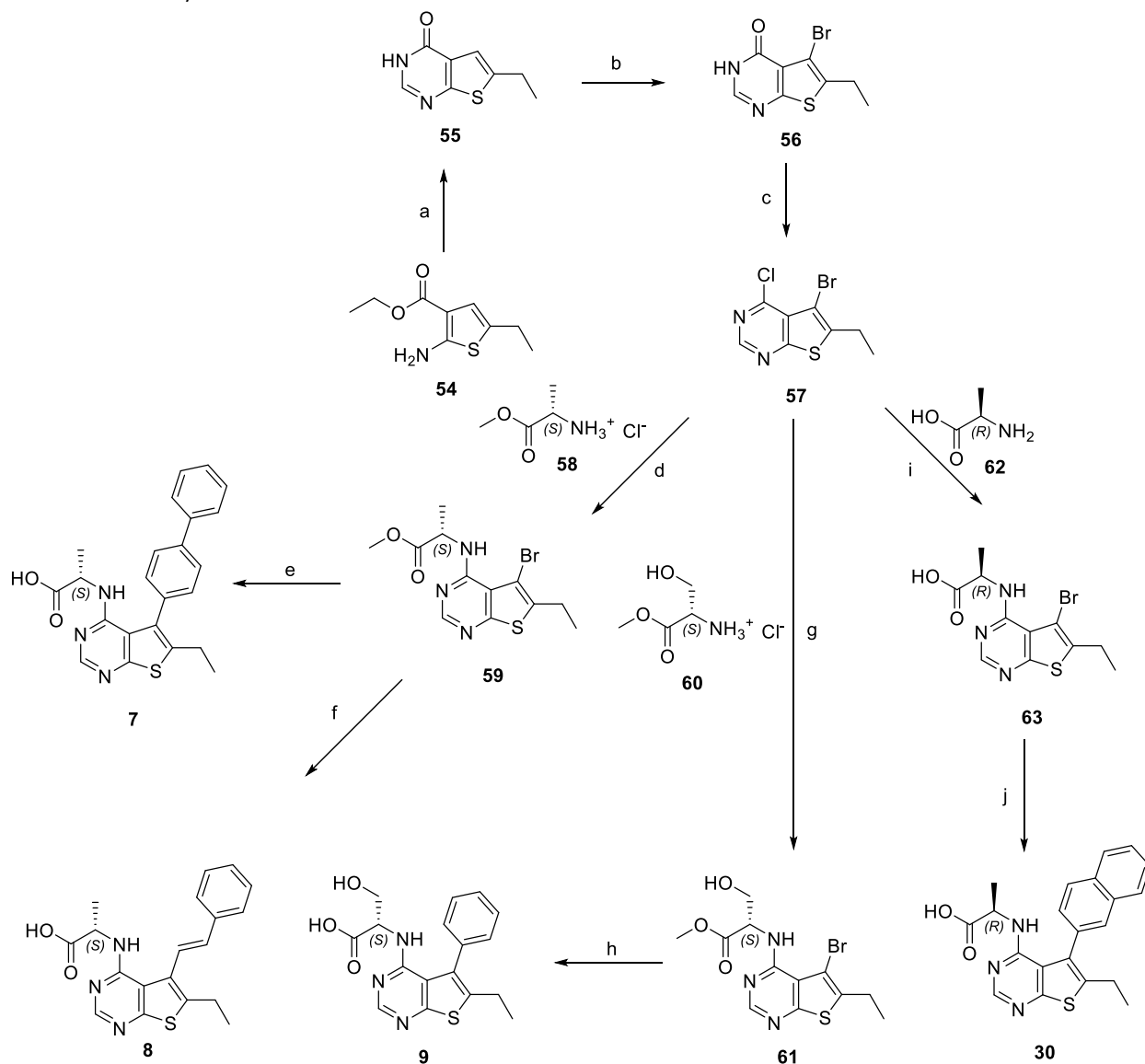


^aReagents and conditions: (a) PdCl₂(PPh₃)₂, 2M K₃PO_{4(aq)}, 1,4-dioxane, 90 then 140 °C, μ wave; (b) 2M NaOH_(aq), THF, MeOH, 40 °C, 49%.

7-(3,5-Dimethyl-1H-pyrazol-4-yl)-3-[3-(naphthalen-1-yloxy)propyl]-1H-indole-2-carboxylic acid (3**)** *Step 1.* Ethyl 7-bromo-3-[3-(naphthalen-1-yloxy)propyl]-1H-indole-2-carboxylate (**51**)⁴ (150 mg, 0.33 mmol) and 3,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (**52**) (369 mg, 1.66 mmol) were stirred in a mixture of dioxane (2 mL) and 2M aqueous potassium phosphate (1.16 mL) under nitrogen. The reaction mixture was then treated with bis(triphenylphosphine)palladium dichloride (23 mg, 33 μ mol), and heated at 90 °C for 96 h under nitrogen. The reaction mixture was then heated with microwaves at 140 °C for a further 0.5 h and then cooled, diluted with saturated brine (30 mL) and extracted with ethyl acetate (2 \times 30 mL). The combined organic extracts were then dried over magnesium sulfate, concentrated in vacuo, loaded onto a 20 g silica column and eluted with hexane-ethyl acetate (0 to 100%). Ethyl 7-(3,5-dimethyl-1H-pyrazol-4-yl)-3-[3-(naphthalen-1-yloxy)propyl]-1H-indole-2-carboxylate (**53**) was isolated as a yellow solid (151 mg, 97%). LC-MS (Method A) m/z 468 [M + H]⁺; t_R = 2.82 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ : 12.32 (1 H, s), 12.02 (1H, s), 10.73 (1 H, s), 8.29–8.21 (1 H, m), 7.93–7.84 (1 H, m), 7.68–7.51 (17 H, m), 7.53–7.44 (1 H, m), 7.44–7.36 (1 H, m), 7.11–7.01 (2 H, m), 6.93 (1 H, d, J 7.1 Hz), 4.32–4.18 (4 H, m), 3.37–3.31 (2 H, m), 2.29–.18 (2 H, m), 2.12 (8 H, s), 2.05 (6 H, s), 1.28 (3 H, t, J 7.1 Hz).

Step 2. Ethyl 7-(3,5-dimethyl-1*H*-pyrazol-4-yl)-3-[3-(naphthalen-1-yloxy)propyl]-1*H*-indole-2-carboxylate (**53**) (151 mg, 0.32 mmol) was dissolved in THF (1.5 mL) and methanol (1.5 mL), treated with 2M aqueous sodium hydroxide solution (1.5 mL) and then heated at 40 °C for 3 h. The volatile components were then removed in vacuo and the residue was diluted with saturated brine (30 mL), adjusted to pH 1 with 2M hydrochloric acid then extracted with ethyl acetate (2 × 30 mL). The combined organic extracts were washed with saturated brine (20 mL), dried over magnesium sulfate and concentrated in vacuo. The residue was dissolved in DCM, loaded onto a 5 g Isolute PE-AX anion exchange cartridge, washed with DCM, methanol and then further DCM to remove impurities. The product was eluted from the cartridge with 10% formic acid in DCM, and the eluent was concentrated in vacuo, redissolved in ethyl acetate (50 mL), washed with saturated brine (2 × 20mL), dried over magnesium sulfate and concentrated in vacuo. 7-(3,5-Dimethyl-1*H*-pyrazol-4-yl)-3-[3-(naphthalen-1-yloxy)propyl]-1*H*-indole-2-carboxylic acid (**3**) was isolated as a white foam (69.4 mg, 49%). LC-MS (Method A) *m/z* 440 [M + H]⁺; *t*_R = 2.64 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 12.61 (1 H, s), 10.53 (1 H, s), 8.26 (1 H, dt, *J* 6.5, 3.4 Hz), 7.92–7.84 (1 H, m), 7.63 (1 H, dd, *J* 7.3, 2.0 Hz), 7.58–7.48 (2 H, m), 7.47 (1 H, d, *J* 8.3 Hz), 7.44–7.36 (1 H, m), 7.09–6.99 (2 H, m), 6.92 (1 H, d, *J* 7.4 Hz), 4.21 (2 H, t, *J* 6.1 Hz), 3.35 (1 H d, *J* 15.1 Hz), 2.24 (2 H, q, *J* 6.7 Hz), 2.04 (6 H, s).

Scheme S4. Synthesis of **7–9** and **30**^a



^aReagents and conditions: (a) HC(O)NH_2 , 230 °C, 75%; (b) MeCO_2H , Br_2 , rt, 100%; (c) POCl_3 , PhNMe_2 , 120 °C, 76%; (d) Et_3N , DMF, 60 °C, 53%; (e) PhPhB(OH)_2 , PS- PPh_3 -Pd, Na_2CO_3 , DME, EtOH, H_2O , rt to 150 °C, μwave , 28%; (f) [(*E*)-2-phenylethenyl]boronic acid, PS- PPh_3 -Pd, Na_2CO_3 , DME, EtOH, H_2O , rt to 150 °C, μwave , 5%; (g) Me_3N , DMF, 60 °C, 35%; (h) PhB(OH)_2 , PS- PPh_3 -Pd, Na_2CO_3 , DME, EtOH, H_2O , 160 °C, μwave , 60%; (i) K_2CO_3 , H_2O , 100 °C then 2M $\text{HCl}_{(\text{aq})}$, 84%; (j) 2-naphthyl- B(OH)_2 , K_2CO_3 , Pd(OAc)_2 , PPh_3 , DME, EtOH, H_2O , 100 °C then 2M $\text{HCl}_{(\text{aq})}$, 23%.

(2*S*)-2-[(5{[1,1'-Biphenyl]-4-yl}-6-ethylhieno[2,3-*d*]pyrimidin-4-yl)amino]propanoic acid (7). *Step 1.* Ethyl 2-amino-5-ethylthiophene-3-carboxylate (**54**) (10 g, 50.18 mmol) in formamide (50 mL) was heated to reflux (230 °C) for 1.5 h. The reaction mixture was cooled to room temperature, poured into water (200 mL) with stirring and stirred for 1 h. The resulting precipitate was filtered, and the residue washed with water (3 × 100 mL) and then dried under

reduced pressure to give 6-ethyl-3*H*,4*H*-thieno[2,3-*d*]pyrimidine (**55**) as a brown powder (6.79 g, 75%). LC-MS (Method A) *m/z* 181 [*M* + *H*]⁺; *R* = 1.67 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 12.44 (1 H, s, br), 8.07 (1 H, s), 7.12 (1 H, m), 2.83–2.89 (2 H, m), 1.27 (3 H, t, *J* 7.60 Hz).

Step 2. To a 100 mL round bottom flask was added 6-ethyl-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-4-one (**55**) (1.33 g, 7.4 mmol) and glacial acetic acid (15 mL) followed by bromine (1.35 mL, 63 mmol) drop-wise over 1 min. After the addition, the reaction mixture was stirred at room temperature for 4 h. The mixture was then diluted with water (15 mL). The resulting precipitate was collected by filtration, the residue washed thoroughly with water then dried under reduced pressure to a beige powder (1.91g, 100%), identified as 5-bromo-6-ethyl-3*H*,4*H*-thieno[2,3-*d*]pyrimidine (**56**). LC-MS (Method A) *m/z* 259 [*M* + *H*]⁺; *R* = 1.91 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 12.59 (1 H, br s), 8.13 (1 H, s), 2.84 (2 H, q, *J* 7.60 Hz), 1.23 (3 H, t, *J* 7.6 Hz).

Step 3. 5-Bromo-6-ethyl-3*H*,4*H*-thieno[2,3-*d*]pyrimidin-4-one (**56**) (1 g, 3.86 mmol) was stirred in phosphoryl trichloride (14 mL, 150 mmol). *N,N*-dimethylaniline (0.49 mL, 3.86 mmol) was added and the reaction mixture was heated at reflux (~120 °C) for 4 h. Excess phosphoryl trichloride was removed under reduced pressure and the residue quenched by addition of ice-water (300 mL) with vigorous stirring. The resulting precipitate was filtered and the residue washed with water (3 × 30 mL) and then dried under reduced pressure for 16 h to give a beige solid (0.81 g, 76%), identified as 5-bromo-4-chloro-6-ethylthieno[2,3-*d*]pyrimidine (**57**). LC-MS (Method A) *m/z* 228 [*M* + *H*]⁺; *R* = 1.42 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.94 (1 H, s), 7.12 (1 H, s), 3.01 (2 H, q, *J* 7.60 Hz), 1.30 (3 H, t, *J* 7.60Hz).

Step 4. 5-Bromo-4-chloro-6-ethylthieno[2,3-*d*]pyrimidine (**57**) was suspended in DMF (10 mL), and then methyl (2*S*)-2-aminopropanoate hydrochloride (**58**) (230 mg, 1.62 mmol) was added followed by triethylamine (0.458 mL, 3.30 mmol). The reaction mixture was heated to 60 °C for 4 h, cooled to room temperature, diluted with water (20 mL) and then extracted with ethyl acetate (3 × 50 mL). The organic extracts were combined, dried (MgSO₄) and evaporated under reduced pressure. The residue was purified on a silica gel column using hexane to 50% ethyl acetate in hexane to give methyl (2*S*)-2-((5-bromo-6-ethylthieno[2,3-*d*]pyrimidin-4-yl)amino)propanoate (**59**) as a yellow gum (201 mg, 53%). LC-MS (Method A) *m/z* 345 [*M* + *H*]⁺; *R* = 1.37 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.39 (1 H, s), 7.34 (1 H, d, *J* 6.60 Hz), 4.78–4.85 (1 H, m), 3.70 (3 H, s), 2.89 (2 H, q, *J* 7.60 Hz), 1.53 (3 H, d, *J* 7.20 Hz), 1.26 (3 H, t, *J* 7.60 Hz).

Step 5. To a 5 mL microwave vial were added methyl (2*S*)-2-((5-bromo-6-ethylthieno[2,3-*d*]pyrimidin-4-yl)amino)propanoate (**59**) (50 mg, 0.146 mmol), 4-biphenylboronic acid (58 mg, 0.291 mmol), polystyrene-supported tetrakis(triphenylphosphine) palladium(0) (PS-PPh₃-Pd) (0.11 mmol/ g, 146 mg), sodium carbonate (46 mg, 0.41 mmol), followed by 1,2-dimethoxyethane (0.75 mL), ethanol (0.75 mL) and water (0.5 mL). The reaction mixture was stirred at room temperature for 5 min under a stream of nitrogen, heated with microwaves at 150 °C for 60 min and then cooled to room temperature. The reaction mixture was then filtered through Celite and the eluted material was concentrated under reduced pressure. The residue was purified by preparative HPLC to give (2*S*)-2-[(5{[1,1'-biphenyl]-4-yl}-6-ethylthieno[2,3-*d*]pyrimidin-4-yl)amino]propanoic acid (**7**) as a white solid (16.5 mg, 28%). LC-MS (Method A) *m/z* 404 [*M* + *H*]⁺; *R* = 1.40 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 12.79 (1 H, br s), 8.38 (1 H, s), 7.87–7.89 (2 H, m), 7.76–7.79 (2 H, m), 7.50–7.54 (4 H, m), 7.40–7.44 (1 H, m), 5.26 (1

H, d J 6.6 Hz), 4.50–4.42 (1 H, m), 2.73 (2 H, q, J 7.6 Hz), 1.21 (3 H, t, J 7.6 Hz), 1.14 (3 H, d, J 7.1 Hz).

(2*S*)-2-({6-Ethyl-5-[(*E*)-2-phenylethenyl]thieno[2,3-*d*]pyrimidin-4-yl}amino)propanoic acid (8). To a 5 mL microwave vial was added methyl (2*S*)-2-({5-bromo-6-ethylthieno[2,3-*d*]pyrimidin-4-yl}amino)propanoate (**59**) (50 mg, 0.146 mmol), [(*E*)-2-phenylethenyl]boronic acid (43 mg, 0.291 mmol), PS-PPh₃-Pd (0.11 mmol/g, 146 mg) and sodium carbonate (46 mg, 0.41 mmol), followed by 1,2-dimethoxyethane (0.75 mL), ethanol (0.75 mL) and water (0.5 mL). The reaction mixture was stirred at room temperature for 5 min under a stream of nitrogen, heated with microwaves at 150 °C for 60 min and then cooled to room temperature. The reaction mixture was then filtered through Celite and the eluted material was concentrated under reduced pressure. The residue was purified by preparative HPLC to give (2*S*)-2-({6-ethyl-5-[(*E*)-2-phenylethenyl]thieno[2,3-*d*]pyrimidin-4-yl}amino)propanoic acid (**8**) as an off-white solid (2.8 mg, 5%). LC-MS (Method A) m/z 354 [$M + H$]⁺; t_R = 1.33 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ : 12.86 (1 H, br s), 8.33 (1 H, s), 7.64–7.67 (2 H, m), 7.27–7.48 (4 H, m), 6.80 (1 H, d, J 16.1 Hz), 6.58 (1 H, d, J 6.6 Hz), 2.93 (2 H, q, J 7.6 Hz), 1.35 (3 H, d, J 7.2 Hz), 1.25 (3 H, t, J 7.6 Hz).

(2*S*)-2-({6-Ethyl-5-phenylthieno[2,3-*d*]pyrimidin-4-yl}amino)-3-hydroxypropanoic acid (9). *Step 1.* A mixture of 5-bromo-4-chloro-6-ethylthieno[2,3-*d*]pyrimidine (**57**) (100 mg, 0.36 mmol), (L)-serine methyl ester hydrochloride (**60**) (84 mg, 0.54 mmol), and trimethylamine (0.15 mL, 1.08 mmol) in dry DMF (5 mL) was heated at 60 °C under nitrogen for 6 h, and was then allowed to cool to ambient temperature. Dilution with water afforded a precipitate that was collected by filtration, washed with water, and dried in vacuo. Purification by flash column chromatography, eluting with 10–75% ethyl acetate in hexane, afforded methyl (2*S*)-2-({5-bromo-6-ethylthieno[2,3-*d*]pyrimidin-4-yl}amino)-3-hydroxypropanoate (**61**) as an off-white solid 45.6 mg, 35%). LC-MS (Method A) m/z 360 [$M + H$]⁺; t_R = 2.12 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ : 8.38 (1 H, s), 7.61 (1 H, d, J 7.5 Hz), 5.43 (1 H, s), 4.87 (1 H, dt, J 7.0, 3.3 Hz), 4.02–3.80 (2 H, m), 3.69 (3 H, s), 2.89 (2 H, q, J 7.5 Hz), 1.26 (3 H, t, J 7.5 Hz).

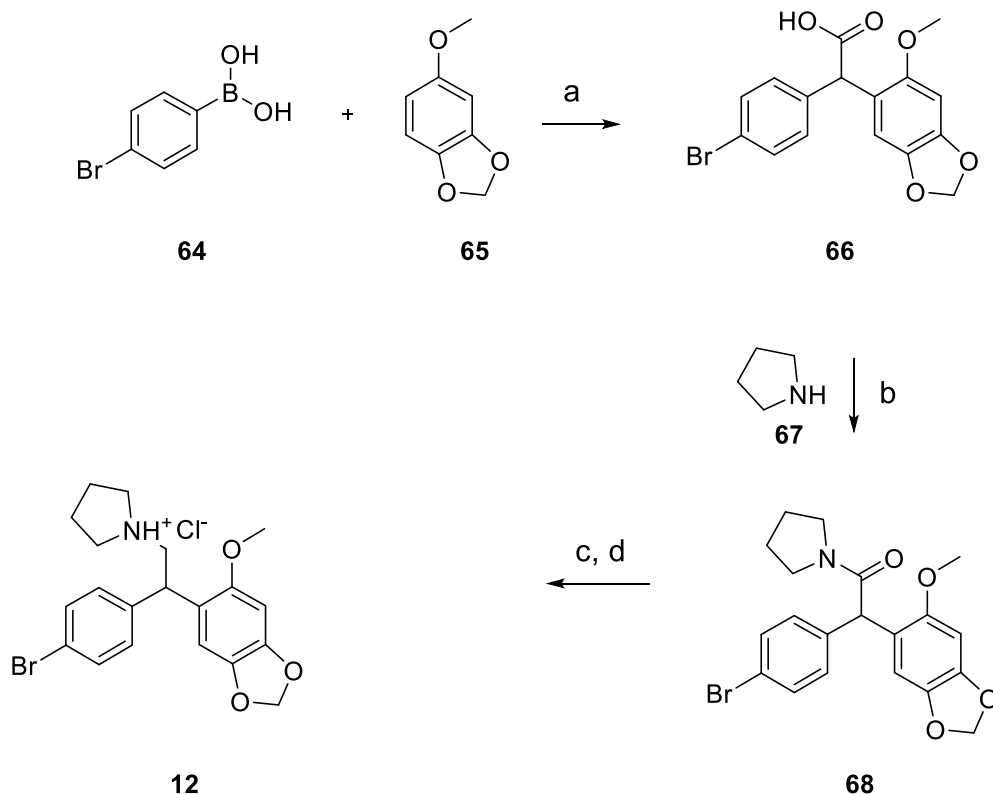
Step 2. A mixture of methyl (2*S*)-2-({5-bromo-6-ethylthieno[2,3-*d*]pyrimidin-4-yl}amino)-3-hydroxypropanoate (**61**) (41.9 mg, 0.12 mmol), phenylboronic acid (28 mg, 0.24 mmol), sodium carbonate (37 mg, 0.36 mmol), and PS-PPh₃-Pd (0.11 mmol/g, 205 mg, 10 mol%) in DME (0.7 mL)/ ethanol (0.7 mL)/ water (0.45 mL) was evacuated and flushed with nitrogen (4 ×) then heated at 160 °C for 2 h under microwave irradiation. The reaction mixture was then filtered through Celite, eluting with a mixture of methanol and aqueous sodium carbonate, and the volatile organics were removed by rotary evaporation. The resulting aqueous phase was washed with dichloromethane, filtered, and then acidified with 2N aqueous hydrochloric acid to pH 3. The aqueous phase was extracted with dichloromethane (3 ×), dried (MgSO₄), and evaporated to afford (2*S*)-2-({6-ethyl-5-phenylthieno[2,3-*d*]pyrimidin-4-yl}amino)-3-hydroxypropanoic acid (**9**) as a light-beige solid (24.7 mg, 60%). LC-MS (Method A) m/z 344 [$M + H$]⁺; t_R = 1.97 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ : 8.34 (1 H, s), 7.57–7.44 (3 H, m), 7.46–7.34 (2 H, m), 5.61 (1 H, d, J 7.4 Hz), 4.55 (1H, dt, J 7.3, 3.6 Hz), 3.66–3.52 (2H, m), 2.65 (2 H, q, J 7.5 Hz), 1.17 (3 H, td, J 7.4, 4.5 Hz).

(2*R*)-2-{{6-Ethyl-5-(naphthalen-2-yl)thieno[2,3-*d*]pyrimidin-4-yl}amino}propanoic acid (30). *Step 1.* 5-Bromo-4-chloro-6-ethylthieno[2,3-*d*]pyrimidine (**57**) (1.85 g, 6.67 mmol), D-alanine (**62**) (2.97 g, 33.3 mmol) and potassium carbonate (4.60 g, 33.3 mmol) were placed in a

flask, to which water (20 mL) was added. The reaction mixture was stirred at 100 °C for 4 h under N₂. The reaction mixture was then filtered through Celite, and the pad washed with water. The pH of the filtrate was adjusted to 1 with 2 M aqueous HCl. The formed precipitate was filtered, washed with water and then dried *in vacuo* to yield (2*R*)-2-({5-bromo-6-ethylthieno[2,3-*d*]pyrimidin-4-yl}amino)propanoic acid (**63**) (1.84 g, 5.58 mmol, 84%); ¹H NMR (500 MHz, DMSO-*d*₆) δ: 13.07 (1 H, br s), 8.39 (1 H, s), 7.39 (1 H, d, *J* 6.5 Hz), 4.72 (1 H, m), 2.88 (2 H, q, *J* 7.5 Hz), 1.51 (3 H, d, *J* 7.0 Hz), 1.25 (3 H, t, *J* 7.5 Hz). ¹³C NMR (125 MHz, DMSO-*d*₆) δ: 174.3, 163.4, 155.0, 153.7, 138.3, 113.5, 99.0, 49.6, 23.1, 18.0, 14.7. HRMS calculated for C₁₁H₁₂BrN₃O₂S: 328.9834; found 329.9901 (M+H).

Step 2. (2*R*)-2-({5-Bromo-6-ethylthieno[2,3-*d*]pyrimidin-4-yl}amino)propanoic acid (**63**) (165 mg, 0.50 mmol), 2-naphthylboronic acid (258 mg, 1.50 mmol), potassium carbonate (346 mg, 2.50 mmol), palladium(II) acetate (11 mg, 0.05 mmol) and triphenylphosphine (26 mg, 0.10 mmol) were mixed with a mixture of DME (1.5 mL), ethanol (1.5 mL) and water (3 mL). The reaction mixture was stirred under nitrogen at 100 °C for 18 h and then cooled, filtered through Celite, and washed with water. The filtrate was washed with Et₂O, and then the pH of the aqueous phase was set to 1 with 2 M aqueous HCl. The formed precipitate was filtered, washed with water and dried *in vacuo*. The residue was purified *via* preparative reverse phase chromatography using 0.1% aqueous TFA solution and MeCN as eluent to obtain (2*R*)-2-{{6-ethyl-5-(naphthalen-2-yl)thieno[2,3-*d*]pyrimidin-4-yl}amino}propanoic acid (**30**) (44 mg, 0.12 mmol, 23%) ¹H NMR (500 MHz, DMSO-*d*₆) δ: 12.68 (1 H, br s), 8.40 (1 H, s), 8.11 (1 H, dd, *J* 8.3, 3.5 Hz), 8.06–7.97 (3 H, m), 7.61 (2 H, m), 7.54 (1 H, t, *J* 7.2 Hz), 5.28 (1 H, m), 4.43 (1 H, m), 2.71 (2 H, m), 1.19 (3 H, t, *J* 7.5 Hz), 1.03/0.97 (3 H, d, *J* 7.0 Hz). ¹³C NMR (125 MHz, DMSO-*d*₆) δ: 153.0, 129.5, 129.3, 128.5, 128.1, 127.5, 127.2, 127.2, 48.9, 21.8, 17.9, 16.4. HRMS calculated for C₂₁H₁₉N₃O₂S: 377.1198; found 378.1268 (M+H).

Scheme S5. Synthesis of compound **12**.^a



^aReagents and conditions: (a) HC(O)CO₂H.H₂O, dioxane, Δ; (b) EDC, HOBT, Et₃N, DCM, rt; (c) BH₃.THF, THF, Δ; (d) HCl, Et₂O.

1-[2-(4-Bromophenyl)-2-(6-methoxy-1,3-benzodioxol-5-yl)ethyl]pyrrolidine hydrochloride (12) *Step 1*.⁵ To a solution of 5-methoxy-1,3-benzodioxole (**64**) (1 g, 6.57 mmol) in dioxane (20 mL) was added (4-bromophenyl)boronic acid (**65**) (1.3 g, 6.57 mmol) and oxoacetic acid monohydrate (605 mg, 6.57 mmol). The reaction mixture was refluxed for 20 h and then evaporated to dryness. The residue was purified by flash chromatography on silica (dichloromethane/ methanol as eluent) to afford 2-(4-bromophenyl)-2-(6-methoxy-1,3-benzodioxol-5-yl)acetic acid (**66**) (1.65 g, 71%) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 12.6 (1 H, m), 7.5 (2 H, d), 7.2 (2 H, d), 6.8 (1 H, s), 6.6 (1 H, s), 5.95 (2 H, m), 5.1 (1 H, s), 3.7 (3 H, s). MS (ESI): *m/z* 364.9 [M + H]⁺.

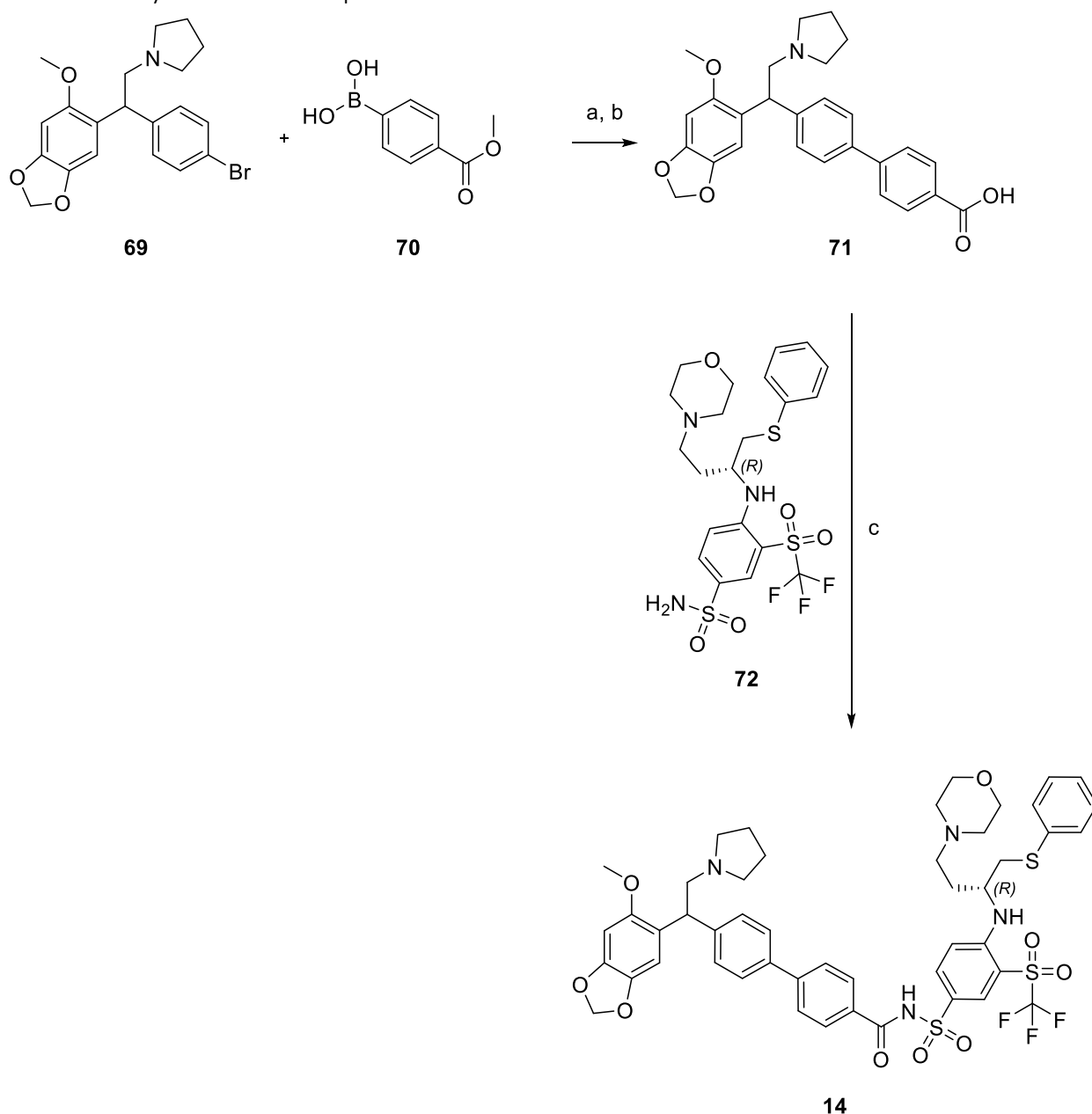
Step 2. To a solution of 2-(4-bromophenyl)-2-(6-methoxy-1,3-benzodioxol-5-yl)acetic acid (**66**) (1.65 g, 4.52 mmol) in dichloromethane (90 mL) was added pyrrolidine (**67**) (0.41 mL, 4.97 mmol), *N,N,N*-triethylamine (1.2 mL, 9.04 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (1 g, 5.42 mmol) and hydroxybenzotriazole (0.733 g, 5.42 mmol). The reaction mixture was stirred at room temperature overnight, poured into aqueous HCl 0.1N, and extracted with dichloromethane. The combined organic layers were washed with water, a saturated aqueous solution of NaHCO₃ followed by brine, then dried over MgSO₄, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica (petroleum ether/ ethyl acetate) to afford 2-(4-bromophenyl)-2-(6-methoxy-1,3-benzodioxol-5-yl)-1-pyrrolidin-1-yl-ethanone (**68**) (1.6 g, 84%) as a white solid. ¹H NMR (400 MHz, DMSO-

d_6) δ : 7.42 (2 H, d), 7.18 (2 H, d), 6.7 (1 H, s), 6.5 (1 H, s), 5.89 (2 H, s), 5.38 (1 H, s), 3.74 (3 H, s), 3.48 (4 H, m), 1.85 (4 H, m). HRMS (DEI): calculated for $C_{20}H_{20}BrNO_4$ $[M + H]^+$ 417.0570. Found: 407.0572.

Step 3. To a suspension of 2-(4-bromophenyl)-2-(6-methoxy-1,3-benzodioxol-5-yl)-1-pyrrolidin-1-yl-ethanone (**68**) (600 mg, 1.43 mmol) in THF (29 mL) was added a 1M solution of borane tetrahydrofuran complex solution in THF (1.7 mL, 1.7 mmol). The reaction mixture was refluxed for 4 h and another portion of borane tetrahydrofuran complex solution in THF was added (2 mL, 2 mmol). After 15 h of reflux, another portion was added (2 mL, 2 mmol) and the reaction mixture was refluxed for 6 h, cooled down and evaporated to dryness. The residue was diluted with methanol (20 mL), HCl 5N was added (0.6 mL) and the reaction mixture was heated to reflux for 1 h and stirred for 50 h at room temperature. After evaporation to dryness, the residue was neutralized until pH 8 with saturated aqueous solution of $NaHCO_3$, and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and evaporated to dryness. The residue (1-[2-(4-bromophenyl)-2-(6-methoxy-1,3-benzodioxol-5-yl)ethyl]pyrrolidine as free base, 524 mg, 91%) appeared pure enough to undergo salt formation in the next synthetic step. 1H NMR (400 MHz, $DMSO-d_6$) δ : 7.37 (2 H, d), 7.13 (2 H, d), 6.88 (2 H, m), 6.69 (1 H, s), 6.49 (1 H, s), 4.53 (1 H, m), 3.7 (3 H, s), 2.92 (2 H, m), 2.5 (4 H, m), 1.7 (4 H, m).

Step 4. To a suspension of 1-[2-(4-bromophenyl)-2-(6-methoxy-1,3-benzodioxol-5-yl)ethyl]pyrrolidine (100 mg, 0.248 mmol) in dichloromethane (5 mL) was added 1M hydrochloric acid solution in diethyl ether (0.3 mL, 0.3 mmol). The reaction mixture was allowed to stir at room temperature for 15 min and was then evaporated to dryness. The residue was suspended in diisopropyl ether, then the resulting white precipitate was filtered, washed with diisopropylether (2 \times) and dried under vacuum in presence of P_2O_5 at 40 $^{\circ}C$ overnight, affording 1-[2-(4-bromophenyl)-2-(6-methoxy-1,3-benzodioxol-5-yl)ethyl]pyrrolidine hydrochloride (**12**) as white crystalline powder (88 mg, 81%). 1H NMR (400 MHz, $DMSO-d_6$) δ : (1 H, br s), 7.55 (2 H, d), 7.3 (2 H, d), 7.05 (1 H, s), 6.8 (1 H, s), 5.95 (2 H, dd), 4.75 (1 H, t), 3.85 (2 H m), 3.75 (3 H, s), 3.4–3.0 (4 H, m), 1.95–1.8 (4 H, m). IR (ATR): ν : $>NH^+$: 2440 cm^{-1} ; ν Ar: 1625 cm^{-1} ; $\nu >C-O-C<$: 1031 cm^{-1} . ESI/FIA/HR: $[M + H]^+ = 404.0880$ measured (404.0861 theoretical). Elemental analysis: calculated (%) for $C_{20}H_{22}BrNO_3 \cdot HCl$: C 54.50, H 5.26, N 3.18, Cl 8.04 Found: C 54.44, H 5.22, N 3.14, Cl 8.00.

Scheme S6. Synthesis of compound **14**.



“Reagents and conditions: (a) K_2CO_3 , $\text{Pd}(\text{PPh}_3)_4$, PhMe , Δ ; (b) 5N $\text{HCl}_{(\text{aq})}$, dioxane; (c) EDC, DMAP, DCM, rt; C18 purification.

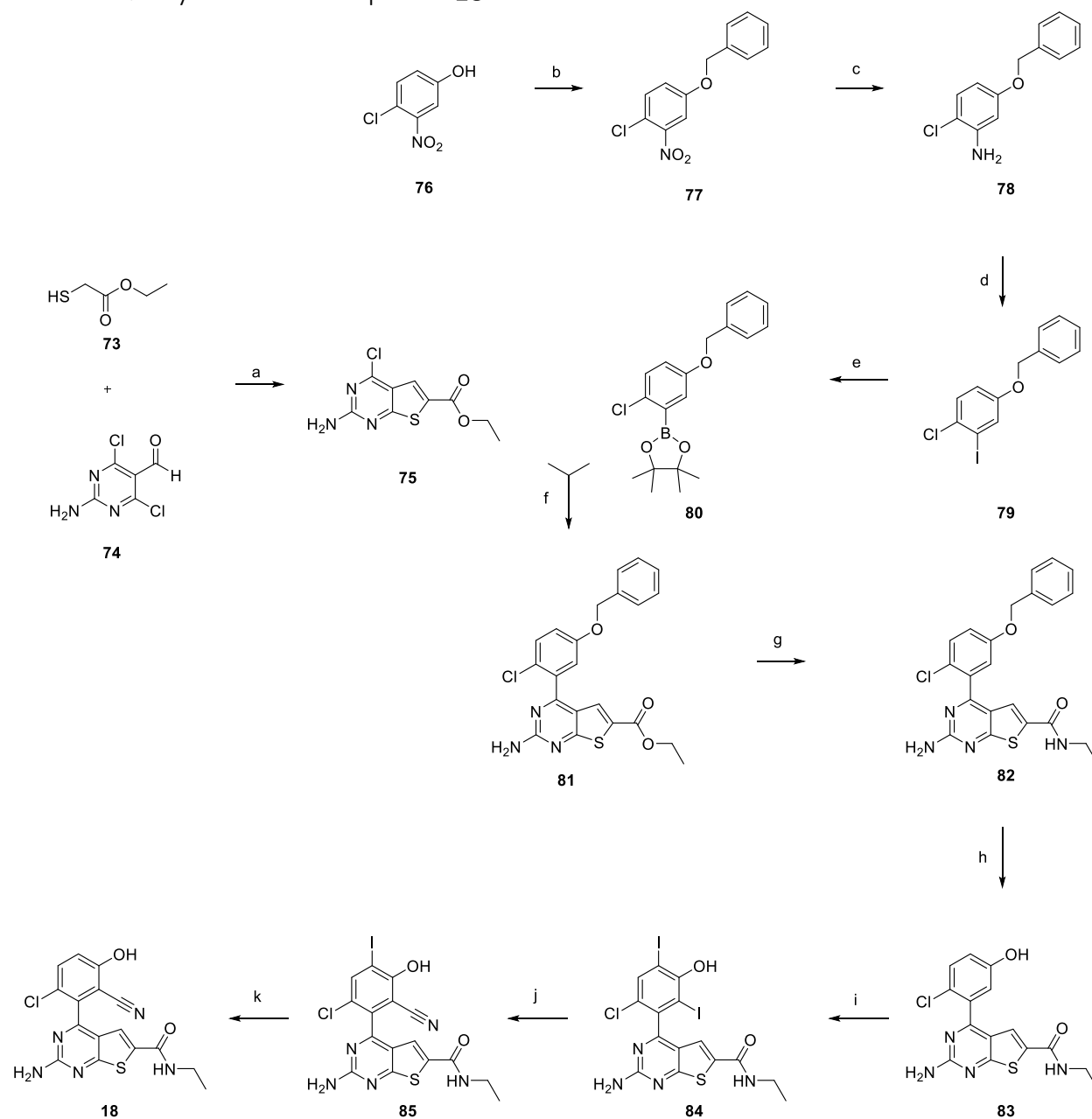
4-[4-[1-(6-Methoxy-1,3-benzodioxol-5-yl)-2-pyrrolidin-1-yl-ethyl]phenyl]-N-[[4-[(1R)-3-morpholino-1-(phenylsulfanylmethyl)propyl]amino]-3-(trifluoromethylsulfonyl)phenyl]sulfonyl-benzamide (14**)** *Step 1*. To a solution of 1-[2-(4-bromophenyl)-2-(6-methoxy-1,3-benzodioxol-5-yl)ethyl]pyrrolidine (**69**) (5 g, 12.4 mmol) in a mixture of dioxane (124 mL) and water (41 mL) was added (4-methoxycarbonylphenyl)boronic acid (**70**) (2.7 g, 14.8 mmol) and potassium carbonate (3.4 g, 24.8 mmol). The suspension was degassed over 15 min by bubbling a stream of argon. Tetrakis(triphenylphosphine)palladium(0) (716 mg, 0.62 mmol) was added

and the reaction mixture was refluxed for 5 h, then cooled down, poured into a mixture water/ethyl acetate and extracted with ethyl acetate. The combined organic layers were washed with a saturated aqueous solution of ammonium chloride and then brine, dried over MgSO₄, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica (dichloromethane/ methanol) to afford methyl 4-[4-[1-(6-methoxy-1,3-benzodioxol-5-yl)-2-pyrrolidin-1-yl-ethyl]phenyl]benzoate (3.36 g, 59%) as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ: 8 (2 H, d), 7.8 (2 H, d), 7.6 (2 H, d), 7.4 (2 H, d), 6.9 (1 H, s), 6.7 (1 H, s), 5.95 (2 H, dd), 4.55 (1 H, t), 3.9 (3 H, s), 3.7 (3 H, s), 3.0 (2 H, m), 2.45 (4 H, m), 1.6 (4 H, m).

Step 2. To a solution of methyl 4-[4-[1-(6-methoxy-1,3-benzodioxol-5-yl)-2-pyrrolidin-1-yl-ethyl]phenyl]benzoate (0.5 g, 1.09 mmol) in dioxane (11 mL) was added an aqueous 5N hydrochloric acid solution (10.9 mL, 54.5 mmol). The reaction mixture was refluxed for 4 h and then evaporated to dryness. The residue was triturated with ethyl acetate and the precipitated was filtered and purified by C18 chromatography (acetonitrile/ water + 0.1% TFA) to afford 4-[4-[1-(6-methoxy-1,3-benzodioxol-5-yl)-2-pyrrolidin-1-yl-ethyl]phenyl]benzoic acid (**71**) (255mg, 53%) as a slightly beige solid. ¹H NMR (400 MHz, DMSO-d₆) δ: 13 (1 H, br s), 9.2 (1 H, br s), 8.0 (2 H, d), 7.8 (2 H, d), 7.7 (2 H, d), 7.45 (2 H, d), 7.1 (1 H, s), 6.85 (1 H, s), 5.95 (2 H, dd), 4.75 (1 H, t), 3.95 (2 H, dd), 3.75 (3 H, s), 3.6–3.0 (4 H, m), 2.1–1.8 (4 H, m). ESI/FIA/HR: [M+H]⁺ = 446.1974 measured (446.1967 theoretical).

Step 3. To a solution of 4-[4-[1-(6-methoxy-1,3-benzodioxol-5-yl)-2-pyrrolidin-1-yl-ethyl]phenyl]benzoic acid (**71**) (395 mg, 0.706 mmol) in dichloromethane (10 mL) was added 4-[[[(1*R*)-3-morpholino-1-(phenylsulfanylmethyl)propyl]amino]-3-(trifluoromethylsulfonyl)benzenesulfonamide (**72**)⁶ (469 mg, 0.847 mmol), *N,N*-diisopropylethylamine (0.49 mL, 2.82 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (189 mg, 0.988 mmol) and 4-dimethylaminopyridine (121 mg, 0.988 mmol). The reaction mixture was stirred at room temperature for 50 h, poured into aqueous saturated solution of ammonium chloride and extracted with dichloromethane. The combined organic layers were washed with water, then brine, dried over MgSO₄, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica (dichloromethane/ 7N NH₃ in methanol solution) to afford 4-[4-[1-(6-methoxy-1,3-benzodioxol-5-yl)-2-pyrrolidin-1-yl-ethyl]phenyl]-*N*-[4-[[[(1*R*)-3-morpholino-1-(phenylsulfanylmethyl)propyl]amino]-3-(trifluoromethylsulfonyl)phenyl]sulfonyl-benzamide (**14**) (529 mg, 72%) as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ: 10 (1 H, br s), 9.2 (1 H, br s), 8.2 (1 H, s), 2.0–1.8 (4 H, m), 8.05 (1 H, dd), 7.95 (2 H, d), 7.8 (2 H, d), 3.95–3.9 (2 H, m), 7.7 (2 H, d), 7.45 (2 H, d), 7.3 (2 H, d), 7.25 (2 H, m), 7.15 (2 H, m), 7.1 (1 H, s), 7.0 (1 H, d), 6.8 (1 H, s), 6 (2H, s), 4.75 (1 H, dd), 4.15 (1 H, m), 3.8 (3 H, s), 3.75–2.8 (16 H, m), 2.15 (2 H, m). ¹⁹F NMR (376 MHz, DMSO-d₆) δ: -73.7 (TFA), -78.7 (CF₃). IR (ATR): ν > NH : 3371 cm⁻¹; ν > NH⁺ : 2800–2380 cm⁻¹; ν > C=O : 1671 cm⁻¹; ν C=C: 1600 cm⁻¹; ν NO₂<: 1510/1349 cm⁻¹; ν SO₂: 1349/1167 cm⁻¹. ESI/FIA/HR: [M+H]⁺ = 981.2832 measured (981.2848 theoretical). Elemental analysis: calculated (%) for C₄₈H₅₁F₃N₄O₉S₃.2TFA: C 51.65, H 4.42, N 4.63, S 7.96 Found: C 50.81, H 4.32, N 4.51, S 7.74.

Scheme S7. Synthesis of compound **18**^a



^aReagents and conditions: (a) K₂CO₃, MeCN, 85 °C, 82%; (b) PhCH₂Br, K₂CO₃, Me₂CO, 60 °C, 100%; (c) Fe, MeCO₂H, H₂O, 85°C, 93%; (d) 6M HCl, MeCO₂H, NaNO₂, H₂O, then KI, I₂, H₂O, 86%; (e) KOAc, B₂pin₂, DMF, Pd(OAc)₂, 90 °C, (f) PdCl₂(PPh₃)₂, K₃PO₄(aq), 1,4-dioxane, 100 °C, 45% over 2 steps; (g) EtNH₂, MeOH, 85°C, 93%; (h) BCl₃, CH₂Cl₂, 87%; (i) NIS, MeCO₂H, 61%; (j) NaCN, CuI, Pd(Ph₃P)₄, MeCN, 75 °C; 84%; (k) PdCl₂, Et₃SiH, MeCN, 110 °C, 33%.

2-Amino-4-(6-chloro-2-cyano-3-hydroxyphenyl)-N-ethylthieno[2,3-d]pyrimidine-6-carboxamide (18) *Step 1.* Ethyl-2-mercaptoacetate (**73**) (2.86 mL, 26 mmol) was added dropwise to a suspension of 2-amino-4,6-dichloro-5-pyrimidine carbaldehyde (**74**) (5.0 g, 26 mmol) and potassium carbonate (9.0 g, 65.2 mmol) in acetonitrile (160 mL). The reaction mixture was heated to 85 °C for 5 h and then allowed to cool to ambient temperature. The reaction mixture was then filtered, and the residue was washed with acetonitrile (250 mL) followed by water (250 mL) and dried in vacuo at 60°C to afford 2-amino-4-chloro-thieno[2,3-*d*]pyrimidine-6-carboxylic acid ethyl ester (**75**) (5.52 g, 82%) as a yellow solid: LCMS (Method A) *m/z* 260 [*M* + *H*]⁺; *t*_R = 2.37 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 2.09 (3 H, t, *J* 7.0 Hz), 4.31 (2 H, q, *J* 7.0 Hz), 7.70 (2 H, brs), 7.78 (1 H, s).

Step 2. Benzyl bromide (6.54 mL, 55 mmol) was added to a stirred suspension of 4-chloro-3-nitrophenol (**76**) (8.0 g, 46 mmol) and potassium carbonate (7.6 g, 55 mmol) in acetone (50 mL). The mixture was heated to 60 °C for 3 h and then allowed to cool to ambient temperature. Water was added to the suspension (150 mL) and this was extracted twice with dichloromethane (100 mL). The combined extracts were washed successively with aqueous sodium hydroxide (2.0 M), water and saturated sodium chloride solution. The combined organic extracts were dried over MgSO₄ and concentrated in vacuo to afford 4-(benzyloxy)-1-chloro-2-nitrobenzene (**77**) (13 g, 100%) as a yellow oil. ¹H NMR (400 MHz, CDCl₃) δ: 7.40 (1 H, d, *J* 3.0 Hz), 7.36–7.21 (6 H, m), 7.04 (1 H, dd, *J* 8.9, 3.0 Hz), 5.02 (2 H, s).

Step 3. Iron powder (12.83 g, 230 mmol) was added to a suspension of (4-(benzyloxy)-1-chloro-2-nitrobenzene (**77**) (12.12 g, 46 mmol) in a mixture of acetic acid (150 mL) and water (75 mL). This mixture was heated at 85°C for 1.5 h. The resulting suspension was filtered through Celite, and the hot filtrate was allowed to cool. Water (200 mL) was added and the mixture was extracted with dichloromethane (3 × 175 mL). The combined organics were washed with 2M sodium hydroxide solution followed by saturated sodium chloride solution and then dried over MgSO₄ and concentrated in vacuo to afford 5-(benzyloxy)-2-chloroaniline (**78**) (9.95 g, 93%) as a light tan powder. LCMS (Method A) *m/z* 234, 236 [*M* + *H*]⁺; *t*_R = 2.67 min. ¹H NMR (400 MHz, CDCl₃) δ: 7.40–7.20 (7 H, m), 7.05 (1 H, d, *J* 8.8 Hz), 6.34 (1 H, d, *J* 2.8 Hz), 6.28 (1 H, dd, *J* 8.7, 2.8 Hz), 4.93 (2 H, s).

Step 4. Hydrochloric acid (6.0 M, 18 mL) was added to a solution of 5-(benzyloxy)-2-chloroaniline (**78**) (5.0 g, 21.4 mmol) in acetic acid (70 mL) and the resulting suspension cooled (ice-water salt bath). A solution of sodium nitrite (1.77 g, 25.7 mmol, 1.2 eq) in water (10 mL) was added slowly (keeping the internal temperature <5 °C). On complete addition, the resulting solution was stirred for 30 min, then poured into a solution of potassium iodide (7.1 g, 42.79 mmol) and iodine (1.63 g, 6.42 mmol) in water (40 mL), and the mixture stirred for 90 min. Water (100 mL) was added and the mixture extracted with dichloromethane (3 × 75 mL). The combined organic extracts were washed with 10% (w/v) aqueous sodium thiosulfate solution (2 × 50 mL), aqueous sodium hydroxide (2.0 M; 50 mL), water (2 × 50 mL) and saturated aqueous sodium chloride solution (100 mL). The solution was dried over anhydrous Na₂SO₄ and concentrated in vacuo to afford 4-(benzyloxy)-1-chloro-2-iodobenzene (**79**) (6.22 g, 86%) as a dark brown oil. ¹H NMR (400 MHz, CDCl₃) δ: 7.39 (1 H, d, *J* 2.9 Hz), 7.35–7.20 (6 H, m), 6.82 (1 H, dd, *J* 8.8, 2.8 Hz), 4.94 (2 H, s).

Step 5. Potassium acetate (5.3 g, 53.98 mmol) was added to a solution of 4-(benzyloxy)-1-chloro-2-iodobenzene (**79**) (6.2 g, 18 mmol, 1 eq) and bis(pinacolato)diboron (**80**) (4.8 g, 18.9

mmol) in DMF (30 mL) under a nitrogen atmosphere. Palladium acetate (202 mg, 0.9 mmol) was added and the mixture heated at 90 °C for 18 h. The resulting solution was concentrated in vacuo, and the residue taken up in ethyl acetate (150 mL), washed with water (3 × 100 mL) followed by saturated aqueous sodium chloride solution (100 mL) and then dried over anhydrous Na₂SO₄ and concentrated in vacuo to a pale-brown gum. The residue was taken up in 1,4-dioxane (60 mL) and ethyl 2-amino-4-chlorothieno[2,3-*d*]pyrimidine-6-carboxylate (**75**) (4.17 g, 16.2 mmol) and aqueous potassium phosphate (tribasic; 2.0 M; 15 mL) were added under a nitrogen atmosphere. Dichlorobis(triphenylphosphine)palladium(II) (180 mg) was then added and the mixture heated at 100 °C for 3 h. The mixture was allowed to cool and ethyl acetate (100 mL) was added. This mixture was washed with saturated aqueous sodium chloride solution (50 mL), dried over anhydrous Na₂SO₄ and concentrated to a pale yellow solid, which was washed with diethyl ether/ hexane (1:1) then dried in vacuo at 40 °C to afford ethyl 4-[5-(benzyloxy)-2-chlorophenyl]thieno[2,3-*d*]pyrimidine-6-carboxylate (**81**) (3.17 g, 45%) as a tan powder. LCMS (Method A) *m/z* 440, 442 [*M* + *H*]⁺; *t*_R = 2.84 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 7.59–7.30 (9 H, m), 7.22 (2 H m), 5.15 (2 H, s), 4.28 (2 H, q, *J* 7.2 Hz), 1.27 (3 H, t, *J* 7.1 Hz).

Step 6. Ethyl 2-amino-4-[5-(benzyloxy)-2-chlorophenyl]thieno[2,3-*d*]pyrimidine-6-carboxylate (**81**) (3.17 g, 7.2 mmol) was suspended in ethylamine in methanol solution (21.6 mL, 2 M, 43.2 mmol) and the mixture was heated in a sealed vial (Caution) at 85 °C for 18 h. The reaction mixture was cooled to 0 °C, the vial was opened, and the solvents were removed in vacuo. The solid residue was triturated with diethyl ether, and the residue filtered off and washed with diethyl ether/hexane (1:1) to afford 2-amino-4-[5-(benzyloxy)-2-chlorophenyl]-*N*-ethylthieno[2,3-*d*]pyrimidine-6-carboxamide (**82**) (2.95g, 93%) as a tan powder. This powder was suspended in DCM (30 mL) and cooled to -78 °C under a nitrogen atmosphere. Boron trichloride solution (1M in DCM, 33.6 mL, 1 M, 33.6 mmol) was added dropwise. The resulting suspension was allowed to warm to ambient temperature and stirred for 3 h. The reaction mixture was then cooled in an ice/water bath. Methanol (20 mL) was added cautiously, and the resulting mixture was stirred for 1 h and then concentrated to a yellow/green solid. The residue was suspended in aqueous sodium acetate (10% w/v; 100 mL) and extracted with ethyl acetate (200 mL). The extracts were washed with water (2 × 200 mL) and then saturated aqueous sodium chloride solution (100 mL). The organic solution was dried over anhydrous Na₂SO₄ and concentrated to a pale-brown solid in vacuo, which was washed with hexane, filtered, and dried in vacuo to afford 2-amino-4-(2-chloro-5-hydroxyphenyl)-*N*-ethylthieno[2,3-*d*]pyrimidine-6-carboxamide (**83**) (2.07 g, 87%) as a pale-brown solid. LCMS (Method A) *m/z* 349, 351 [*M* + *H*]⁺; *t*_R = 2.00 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 10.05 (1 H, s), 8.63 (1 H, t, *J* 5.5 Hz), 7.56 (1 H, s), 7.42 (1 H, d, *J* 8.7 Hz), 7.21 (2 H, s), 6.94 (1 H, dd, *J* 8.7, 2.9 Hz), 6.86 (1 H, d, *J* 2.9 Hz), 3.27–3.16 (2 H, m), 1.07 (3 H, t, *J* 7.2 Hz).

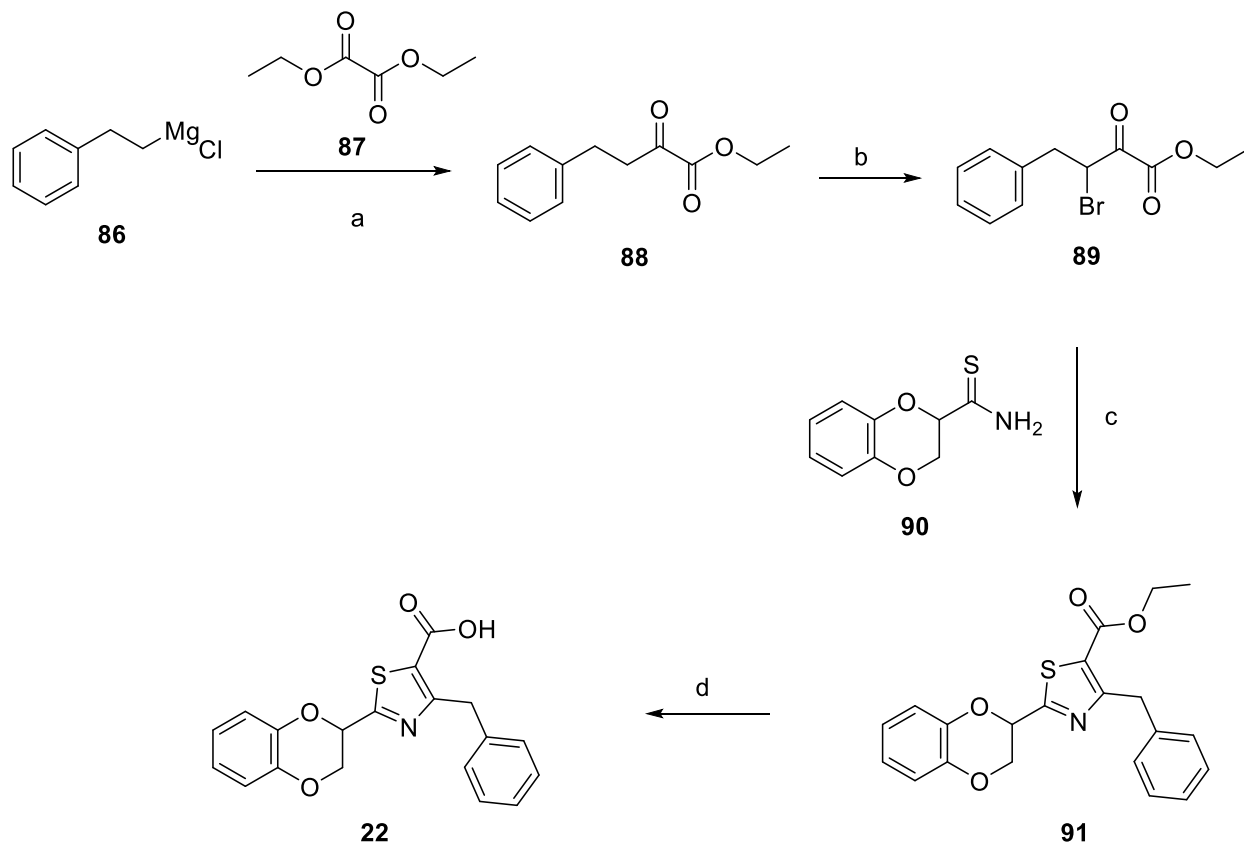
Step 7. *N*-Iodosuccinimide (178 mg, 0.79 mmol) was added to a stirred solution of 2-amino-4-(2-chloro-5-hydroxyphenyl)-*N*-ethylthieno[2,3-*d*]pyrimidine-6-carboxamide (**83**) (230 mg, 0.7 mmol) in acetic acid (5 mL). The reaction mixture was stirred at ambient temperature for 2 h. The solvents were then evaporated; and the residue was partitioned between water and ethyl acetate. The organic extracts were washed with saturated aqueous sodium chloride solution, then dried over anhydrous Na₂SO₄ and concentrated to a pale-brown solid in vacuo. The residue was washed with diethyl ether/hexane (1:1) to afford 2-amino-4-(6-chloro-3-hydroxy-2,4-diiodophenyl)-*N*-ethylthieno[2,3-*d*]pyrimidine-6-carboxamide (**84**) (242 mg, 61%) as a beige powder. LCMS (Method A) *m/z* 601, 603 [*M* + *H*]⁺; *t*_R = 2.31 min. ¹H NMR (400 MHz,

DMSO-*d*₆) δ : 10.05 (1 H, s), 8.55 (1 H, t, *J* 5.5 Hz), 8.04 (1 H, s), 7.42 (1 H, s), 7.29 (2 H, s), 3.25–3.16 (2 H, m), 1.10 (3H, t, *J* 7.1 Hz).

Step 8. To a degassed solution of 2-amino-4-(6-chloro-3-hydroxy-2,4-diiodophenyl)-*N*-ethylthieno[2,3-*d*]pyrimidine-6-carboxamide (**84**) (190 mg, 0.32 mmol) and sodium cyanide (31 mg, 0.63 mmol) in acetonitrile (10 mL) was added copper(I) iodide (6 mg, 0.03 mmol) and tetrakis(triphenylphosphine)palladium (18.3 mg, 0.02 mmol). The reaction mixture was heated at 75 °C for 12 h under nitrogen. The mixture was then cooled to ambient temperature, diluted with ethyl acetate (20 mL) and filtered through celite. The filtrate was washed with water followed by saturated sodium chloride solution. The organic extracts were dried over MgSO₄ and concentrated in vacuo to afford 2-amino-4-(6-chloro-2-cyano-3-hydroxy-4-iodophenyl)-*N*-ethylthieno[2,3-*d*]pyrimidine-6-carboxamide (**85**) (133 mg, 84%) as a brown powder, which was carried forward without further purification. LCMS (Method A) *m/z* 500, 502 [M + H]⁺; *t*R = 2.18 min.

Step 9. Palladium chloride (4.72 mg, 0.03 mmol, 0.1 eq) was added to a degassed solution of 2-amino-4-(6-chloro-2-cyano-3-hydroxy-4-iodophenyl)-*N*-ethylthieno[2,3-*d*]pyrimidine-6-carboxamide (**85**) (133 mg, 0.27 mmol, 1 eq) and triethylsilane (63.8 μ L, 0.4 mmol) in acetonitrile (4 mL). The reaction mixture was heated in a microwave at 110 °C for 11 mins. The mixture was then absorbed onto celite and purified via flash column chromatography eluting in a gradient of DCM to 10% methanol in DCM to afford 2-amino-4-(6-chloro-2-cyano-3-hydroxyphenyl)-*N*-ethylthieno[2,3-*d*]pyrimidine-6-carboxamide (**18**) as a white powder (33 mg, 33%). ¹H NMR (400 MHz, DMSO-*d*₆) δ : 8.59 (1 H, d, *J* 5.3 Hz), 7.79 (1 H, d, *J* 9.0 Hz), 7.51 (1 H, s), 7.38 (2 H, s), 7.21 (1 H, d, *J* 9.0 Hz), 3.27–3.18 (2 H, m), 1.08 (3 H, t, *J* 7.2 Hz).

Scheme S8. Synthesis of compound **22**.^a



^aReagents and conditions: (a) Et₂O, -78 °C to rt, 77%; (b) CuBr₂, CHCl₃, EtOAc, Δ, 84%; (c) EtOH, 140 °C, μwave, 46%; (d) 2M NaOH_(aq), EtOH, rt, 80%.

5-Benzyl-2-(2,3-dihydro-1,4-benzodioxin-2-yl)-1,3-thiazole-4-carboxylic acid (22). *Step 1.* Diethyl oxalate (**87**) (2.0 g, 13.7 mmol) was dissolved in dry diethyl ether (20 mL) and cooled to -78 °C under N₂. To this was then slowly added a solution of 1.0M phenethylmagnesium chloride (**86**) in THF (15.06 mL, 15.06 mmol). The reaction mixture was stirred at -78 °C for 15 min, warmed to room temperature and stirred for 30 mins, and then quenched with dropwise addition of saturated aqueous ammonium chloride solution. The reaction mixture was then diluted with saturated aqueous ammonium chloride solution (50 mL) and extracted with diethyl ether (2 × 50 mL). The combined organics were dried over magnesium sulfate, concentrated in vacuo, dry-loaded onto a 50 g silica column and eluted with a gradient of hexane to 9:1 hexane-diethyl ether. Ethyl 2-oxo-4-phenylbutanoate (**88**) was isolated as a clear oil (2.168 g, 77%). ¹H NMR (400 MHz, DMSO-*d*₆) δ: 7.16–6.92 (5 H, m), 4.15–4.00 (2 H, m), 2.99 (2 H, dd, *J* 8.0, 7.0 Hz), 2.66 (2 H t, *J* 7.5 Hz), 1.15–1.00 (3 H, m).

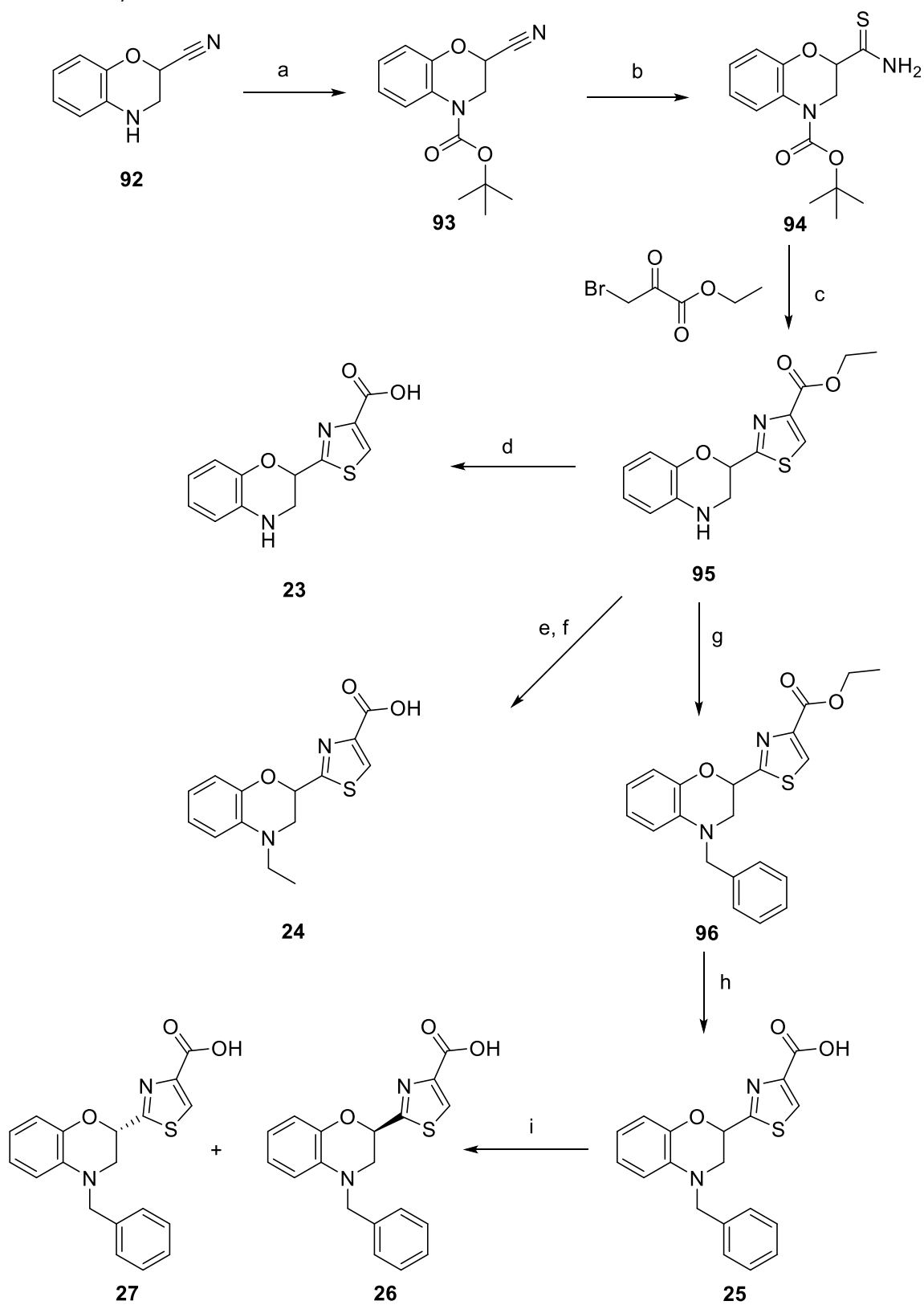
Step 2. Ethyl 2-oxo-4-phenylbutanoate (**88**) (2.168 g, 10.5 mmol) was stirred in a mixture of chloroform (50 mL) and ethyl acetate (100 mL). The reaction mixture was then treated with copper(II) bromide (7.04 g, 31.5 mmol) and refluxed for 16 h under nitrogen. The reaction mixture was then cooled to room temperature, and filtered through a Celite pad. The residue was

washed with further ethyl acetate (2×100 mL) and the combined organic extracts were concentrated in vacuo and loaded onto a 50g silica column. The column was eluted with a gradient of hexane to 9:1 hexane-diethyl ether. Ethyl 3-bromo-2-oxo-4-phenylbutanoate (**89**) was isolated as a yellow oil (2.516 g, 84%). ^1H NMR (400 MHz, DMSO- d_6) δ : 7.39–7.25 (6 H, m), 5.36 (1 H dd, J 10.1, 4.5 Hz), 4.38–4.24 (3 H, m), 3.48 (1 H, dd, J 14.7, 4.4 Hz), 3.09 (1 H, dd, J 14.8, 10.1 Hz), 1.28 (3 H, dt, J 9.6, 7.1 Hz).

Step 3. Ethyl 3-bromo-2-oxo-4-phenylbutanoate (**89**) (1.50 g, 5.26 mmol) and (2,3-dihydro-1,4-benzodioxine-2-carbothioamide (**90**) (1.03 g, 5.26 mmol) were combined in dry ethanol (20 mL). The reaction mixture was then sealed and heated with microwaves at 140 °C for 1.5 h. After cooling to ambient temperature, the reaction mixture was diluted with saturated aqueous sodium bicarbonate solution (50 mL) and extracted with ethyl acetate (2×70 mL). The combined organic extracts were then washed with saturated brine (20 mL), dried over magnesium sulfate and concentrated in vacuo. The residue was loaded onto a 50 g silica column and eluted with a gradient of hexane to 4:1 hexane-diethyl ether. Ethyl 4-benzyl-2-(2,3-dihydro-1,4-benzodioxin-2-yl)-1,3-thiazole-5-carboxylate (**91**) (931 mg, 2.42 mmol, 46%) was isolated as a yellow solid. LC-MS (Method A) m/z 382 $[\text{M} + \text{H}]^+$; $t_R = 2.64$ min. ^1H NMR (400 MHz, DMSO- d_6) δ : 7.37–7.21 (5 H, m), 7.02–6.94 (1 H, m), 6.94–6.84 (3 H, m), 5.70 (1 H, dd, J 5.9, 2.6 Hz), 4.62–4.47 (3 H, m), 4.42–4.26 (3 H, m), 1.31 (3 H, t, J 7.1 Hz).

Step 4. Ethyl 4-benzyl-2-(2,3-dihydro-1,4-benzodioxin-2-yl)-1,3-thiazole-5-carboxylate (**91**) (904 mg, 2.37 mmol) was stirred in ethanol (10 mL) at room temperature. 2M Aqueous sodium hydroxide (10 mL) was added and the reaction mixture was stirred for 3 h at ambient temperature. The ethanol was removed in vacuo and the residual aqueous solution was acidified to pH 1 with 2M aqueous hydrogen chloride, diluted with water (50 mL) and extracted with ethyl acetate (2×100 mL). The combined organics were washed with saturated brine (20 mL), dried over magnesium sulfate and concentrated in vacuo. The residual solid was triturated with hexane-diethyl ether (5 mL) and then dried in vacuo. 4-Benzyl-2-(2,3-dihydro-1,4-benzodioxin-2-yl)-1,3-thiazole-5-carboxylic acid (**22**) was isolated as a cream solid (673 mg, 80%). LC-MS (Method A) m/z 354 $[\text{M} + \text{H}]^+$; $t_R = 2.33$ min. ^1H NMR (400 MHz, DMSO- d_6) δ : 13.18 (1 H, s), 7.37–7.20 (5 H, m), 7.02–6.94 (1 H, m), 6.94–6.84 (3 H, m), 5.68 (1 H, dd, J 5.9, 2.6 Hz), 4.62–4.47 (3 H, m), 4.36 (1 H, dd, J 11.7, 5.9 Hz).

Scheme S9. Synthesis of **23-27**.^a



“Reagents and conditions: (a) Boc_2O , NEt_3 , DMAP, THF, 76%; (b) 40% $(\text{NH}_4)_2\text{S}_{(\text{aq})}$, MeOH, rt, 99%; (c) EtOH, 140 °C, μwave , 36%; (d) 2M $\text{NaOH}_{(\text{aq})}$, EtOH, THF, rt, 67%; (e) $\text{NaBH}(\text{OAc})_3$, 1,4-dioxane, 100 °C, μwave ; (f) 2M $\text{NaOH}_{(\text{aq})}$, EtOH, 14% (over 2 steps); (g) BnBr , NaH, DMF, 48%; (h) 2M $\text{NaOH}_{(\text{aq})}$, EtOH, 93%; (i) Daicel chiralpak AD.

2-(3,4-Dihydro-2H-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylic acid (23). *Step 1.* 3,4-Dihydro-2H-1,4-benzoxazine-2-carbonitrile (**92**) (1.02 g, 6.37 mmol) was dissolved in dry THF (20 mL) under N_2 . Triethylamine (2.13 mL, 15.29 mmol) and 4-dimethylaminopyridine (10 mg, 0.082 mmol) were added, followed by di-*tert*-butyl dicarbonate (1.53 g, 7.0 mmol) and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture then underwent dropwise addition of 2M aqueous sodium hydroxide, and was then diluted with saturated aqueous sodium bicarbonate solution (50 mL) and extracted with ethyl acetate (2×70 mL). The organic extracts were then washed with saturated brine (20 mL), dried over magnesium sulfate and concentrated in vacuo. The crude residue was then loaded onto a 50 g silica column and eluted with a gradient of hexane to hexane-ethyl acetate (13:2). *tert*-Butyl 2-cyano-3,4-dihydro-2H-1,4-benzoxazine-4-carboxylate (**93**) was isolated as an off-white solid (1.27g, 76%). LC-MS (Method A) m/z 205 $[\text{M-Boc} + \text{H}]^+$; $t_R = 2.35$ min. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ : 7.69 (1 H, d, J 8.3 Hz), 7.13–7.05 (1 H, m), 7.05–6.96 (2 H, m), 5.72 (1 H, t, J 2.6 Hz), 4.61 (1 H, dd, J 14.3, 2.5 Hz), 3.63–3.55 (1 H, m), 1.50 (9 H, s).

Step 2. *tert*-Butyl 2-cyano-3,4-dihydro-2H-1,4-benzoxazine-4-carboxylate (**93**) (1.27 g, 4.84 mmol) was stirred in methanol (50 mL) at room temperature. 40% Aqueous ammonium sulfide solution (1.2 mL) was then added and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated in vacuo and the crude residue was diluted with saturated ammonium bicarbonate solution (100 mL) and extracted with ethyl acetate (2×100 mL). The organic extracts were then washed with saturated brine (20 mL), dried over magnesium sulfate and concentrated in vacuo. *tert*-Butyl 2-carbamothioyl-3,4-dihydro-2H-1,4-benzoxazine-4-carboxylate (**94**) was isolated as a yellow solid (1.43 g, 99%). LC-MS (Method A) m/z 239 $[\text{M} + \text{H}]^+$; $t_R = 2.28$ min. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ : 10.14 (1 H, s), 9.35 (1 H, s), 7.63 (1 H, d, J 8.2 Hz), 7.08–6.99 (2 H, m), 6.91 (1 H, ddd, J 8.6, 5.4, 3.4 Hz), 4.91 (1 H, dd, J 7.4, 3.2 Hz), 4.43 (1 H, d, J 12.6 Hz), 3.61 (1 H, s), 1.49 (9 H, s).

Step 3. *tert*-Butyl 2-carbamothioyl-3,4-dihydro-2H-1,4-benzoxazine-4-carboxylate (**94**) (1.43 g, 4.79 mmol) and ethyl bromopyruvate (0.61 mL, 4.79 mmol) were combined in dry ethanol (20 mL) and then sealed and heated with microwaves at 140 °C for 1.5 h. The reaction mixture was then cooled, diluted with saturated aqueous sodium bicarbonate solution (50 mL) and extracted with ethyl acetate (2×70 mL). The combined organic extracts were then washed with saturated brine (20 mL), dried over magnesium sulfate and concentrated in vacuo. The crude residue was loaded onto a 50 g silica column and eluted with a gradient of hexane to 3:1 hexane-ethyl acetate. Ethyl 2-(3,4-dihydro-2H-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylate (**95**) was isolated as a yellow solid (501 mg, 36%). LC-MS (Method A) m/z 291 $[\text{M} + \text{H}]^+$; $t_R = 2.15$ min. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ : 8.54 (1 H, s), 6.82 (1 H, dd, J 7.8, 1.4 Hz), 6.75 (1 H, td, J 7.5, 1.4 Hz), 6.64 (1 H, dd, J 7.9, 1.6 Hz), 6.56 (1 H, td, J 7.6, 1.7 Hz), 6.10 (1 H, t, J 2.6 Hz), 5.59 (1 H, dd, J 5.4, 2.9 Hz), 4.31 (2 H, qd, J 7.1, 1.8 Hz), 3.65 (1 H, dt, J 12.2, 2.8 Hz), 3.46 (1H, ddd, J 12.3, 5.4, 2.8 Hz), 1.31 (3 H, t, J 7.1 Hz).

Step 4. Ethyl 2-(3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylate (**95**) (200 mg, 0.69 mmol) was stirred in ethanol (2 mL) and THF (2 mL) at room temperature, treated with 2M aqueous sodium hydroxide solution (4 mL) and then stirred for 2 h. The organic solvents were then removed in vacuo and the remaining aqueous residue was diluted with 0.1M aqueous hydrochloric acid (20 mL) and extracted with ethyl acetate (2 × 50 mL). The organic extracts were then dried over magnesium sulfate and concentrated in vacuo. The residual solid was then triturated with minimal diethyl ether (2 ×). 2-(3,4-Dihydro-2*H*-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylic acid (**23**) was isolated as an off-white solid (121 mg, 67%). LC-MS (Method A) *m/z* 263 [M + H]⁺; *t*_R = 1.74 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.10 (1 H, s), 8.45 (1 H, s), 6.82 (1 H, dd, *J* 8.0, 1.4 Hz), 6.75 (1 H, td, *J* 7.6, 1.5 Hz), 6.64 (1 H, dd, *J* 7.9, 1.6 Hz), 6.56 (1 H, td, *J* 7.6, 1.6 Hz), 6.09 (1 H, s), 5.56 (1 H, dd, *J* 5.5, 3.0 Hz), 3.66 (1 H, dt, *J* 12.3, 2.8 Hz), 3.46 (1 H, ddd, *J* 12.2, 5.6, 2.7 Hz).

2-(4-Ethyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylic acid (24). Ethyl 2-(3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylate (**95**) (75 mg, 0.258 mmol) was dissolved in dry dioxane (3 mL), treated with sodium triacetoxyborohydride (164 mg, 0.775 mol) then sealed and heated with microwaves at 100 °C for 5 min. The reaction was then cooled, poured onto saturated brine (20 mL) and extracted with ethyl acetate (2 × 20 mL). The organic extracts were dried over magnesium sulfate and concentrated in vacuo. The crude residual materials were then dissolved in ethanol (2 mL), treated with 2M aqueous sodium hydroxide solution (1 mL) and then stirred for 2 h. The reaction mixture was concentrated in vacuo and the residual aqueous solution was diluted with water (20 mL), adjusted to pH 1 with 2M aqueous hydrochloric acid and then extracted with ethyl acetate (2 × 20mL). The combined organic extracts were then dried over magnesium sulfate, concentrated in vacuo and then purified by reverse phase preparative HPLC. 2-(4-Ethyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylic acid (**24**) was isolated as an off-white solid (10.6 mg, 14%). LC-MS (Method A) *m/z* 291 [M + H]⁺; *t*_R = 2.03 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.11 (1 H, s), 8.45 (1 H, s), 6.89–6.76 (3 H, m), 6.66–6.57 (1 H, m), 5.66 (1 H, dd, *J* 5.7, 2.9 Hz), 3.67 (1 H, dd, *J* 12.2, 3.0 Hz), 3.50–3.36 (3 H, m), 1.07 (3 H, t, *J* 7.0 Hz).

2-(4-Benzyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylic acid (25). *Step 1.* Ethyl 2-(3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylate (250 mg, 0.86 mmol) and benzyl bromide (176 mg, 1.03 mmol) was dissolved in dry DMF (5 mL) under nitrogen. 60% Sodium hydride in mineral oil was added and the reaction mixture was stirred at room temperature for 72 h. The mixture was then quenched with dropwise addition of acetic acid, and then adjusted to pH 10 with aqueous ammonium hydroxide. The reaction mixture was then diluted with saturated ammonium bicarbonate solution (30 mL) and extracted with ethyl acetate (2 × 40 mL). The combined organic extracts were then washed with saturated brine (20 mL), dried over magnesium sulfate and concentrated in vacuo. The crude residue was loaded onto a 20 g silica column and eluted with a gradient of hexane to 4:1 hexane-ethyl acetate. Ethyl 2-(4-benzyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylate (**96**) was isolated as a yellow oil (157 mg, 48%). LC-MS (Method A) *m/z* 381 [M + H]⁺; *t*_R = 2.61 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.56 (1 H, s), 7.34–7.17 (6 H, m), 6.89 (1 H, dd, *J* 7.9, 1.5 Hz), 6.82–6.69 (2 H, m), 6.71–6.54 (1 H, m), 5.75 (1 H, dd, *J* 5.7, 2.9 Hz), 4.58–4.45 (2 H, m), 4.31 (2 H, qd, *J* 7.1, 2.3 Hz), 3.84–3.71 (1 H, m), 3.61–3.52 (1 H, m), 1.30 (3 H, t, *J* 7.1 Hz).

Step 2. Ethyl 2-(4-benzyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylate (157 mg, 0.41 mmol) was dissolved in ethanol (1.3 mL) and treated with 2M aqueous sodium hydroxide solution (1.3 mL) and then stirred for 3 h. The reaction mixture was then diluted with water (20 mL), acidified to pH 1 with 2M aqueous hydrochloric acid and then extracted with ethyl acetate (2 × 30 mL). The combined organic extracts were then dried over magnesium sulfate and concentrated in vacuo. 2-(4-Benzyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylic acid (**25**) was isolated as a yellow solid (136 mg, 93%). LC-MS (Method A) *m/z* 353 [M + H]⁺; *t*_R = 2.27 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.10 (1 H, s), 8.48 (1 H, s), 7.35–7.19 (5 H, m), 6.89 (1 H, dd, *J* 7.9, 1.5 Hz), 6.78 (1 H, ddd, *J* 8.6, 7.0, 1.5 Hz), 6.73 (1 H, dd, *J* 8.1, 1.7 Hz), 6.63 (1 H, td, *J* 7.5, 1.7 Hz), 5.73 (1 H, dd, *J* 5.7, 2.9 Hz), 4.59–4.46 (2 H, m), 3.78 (1 H, dd, *J* 12.3, 2.9 Hz), 3.60 (1 H, dd, *J* 12.3, 5.8 Hz).

2-[(2*R*)-4-Benzyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]-1,3-thiazole-4-carboxylic acid (26**)**

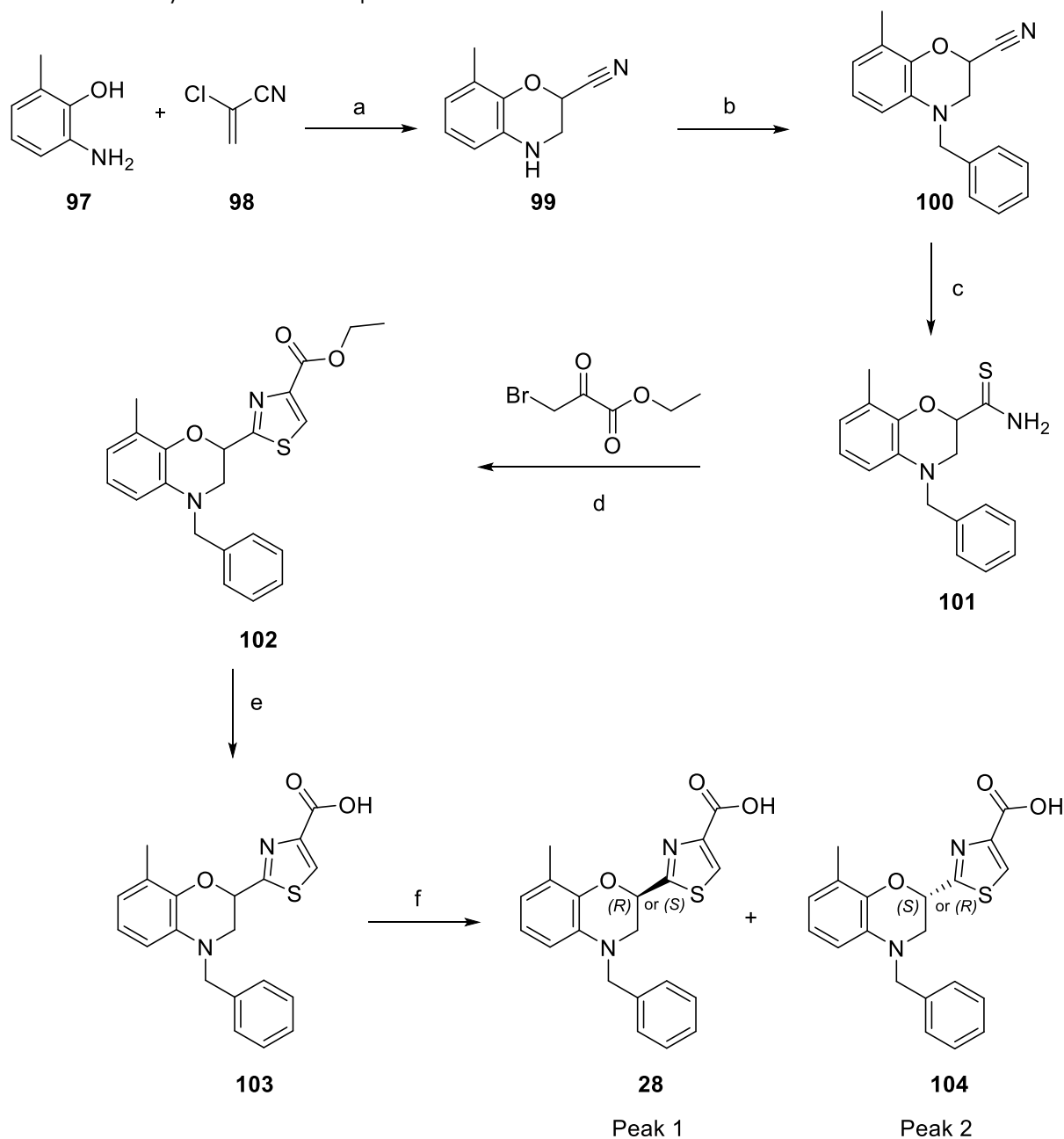
2-[(2*S*)-4-Benzyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]-1,3-thiazole-4-carboxylic acid (27**)**

2-(4-Benzyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylic acid (**25**), 20 mg, 0.057 mmol) was separated by chiral chromatography using Daicel chiralpak AD-10 μM column. Mobile phase was 85:15 hexane-isopropanol with 0.2% TFA as modifier.

2-[(2*R*)-4-Benzyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]-1,3-thiazole-4-carboxylic acid (**26**) was isolated as a brown solid (7.5 mg). LC-MS (Method B) *m/z* 353 [M + H]⁺; *t*_R = 1.34 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.10 (1 H, s), 8.47 (1 H, s), 7.34–7.20 (5 H, m), 6.89 (1 H, dd, *J* 8.0, 1.5 Hz), 6.81–6.71 (2 H, m), 6.63 (1 H, td, *J* 7.4, 1.7 Hz), 5.73 (1 H, dd, *J* 5.7, 2.9 Hz), 4.53 (2 H, d, *J* 2.8 Hz), 3.78 (1 H, dd, *J* 12.3, 3.0 Hz), 3.60 (1 H, dd, *J* 12.3, 5.8 Hz).

2-[(2*S*)-4-Benzyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]-1,3-thiazole-4-carboxylic acid (**27**) was isolated as a brown solid (6.7 mg). LC-MS (Method B) *m/z* 353 [M + H]⁺; *t*_R = 1.34 min. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.12 (1 H, s), 8.47 (1 H, s), 7.34–7.23 (5 H, m), 6.89 (1 H, dd, *J* 8.1, 1.4 Hz), 6.82–6.71 (2 H, m), 6.63 (1 H, td, *J* 7.5, 1.7 Hz), 5.73 (1 H, dd, *J* 5.7, 2.9 Hz), 4.53 (2 H, d, *J* 3.0 Hz), 3.78 (1 H, dd, *J* 12.3, 3.0 Hz), 3.60 (1 H, dd, *J* 12.4, 5.8 Hz).

Scheme S10. Synthesis of compound **28**.^a



^aReagents and conditions: (a) K_2CO_3 , MeCN, Δ , 54%; (b) NaH, DMF, PhCH_2Br , 61%; (c) $\text{S}(\text{NH}_4)_2(\text{Aq})$, MeOH, 87%; (d) EtOH, Δ , 29%; (e) $\text{LiOH}\cdot\text{H}_2\text{O}$, MeOH, 79%; (f) Chiral HPLC.

2-[(2R or S)-4-Benzyl-8-methyl-3,4-dihydro-2H-1,4-benzoxazin-2-yl]-1,3-thiazole-4-carboxylic acid (28**)** *Step 1*. A stirred mixture of 6-amino-2-methylphenol (**97**) (5.00 g, 40.61 mmol), 2-chloroacrylate (**98**) (3.57 mL, 44.66 mmol), potassium carbonate (16.84 g, 121.83 mmol) and acetonitrile (200 mL) was heated at reflux for 21 h. The reaction mixture was then cooled and evaporated. The residue was subjected to silica-gel column chromatography [hexane then hexane-EtOAc (4:1) as eluent]. The eluted material, obtained as a brown solid (3.83 g, 54%), was identified as 8-methyl-3,4-dihydro-2H-1,4-benzoxazine-2-carbonitrile (**99**); LC-MS

(Method B) m/z 175 $[M + H]^+$; $t_R = 1.11$ min. 1H NMR (400 MHz, DMSO- d_6) δ : 6.68 (1 H, t, J 7.6 Hz), 6.51 (1 H, dd, J 8.0, 1.7 Hz), 6.45 (1 H, dt, J 7.4, 1.1 Hz), 6.10 (1 H, d, J 3.3 Hz), 5.59 (1 H, td, J 2.9, 0.9 Hz), 3.56 (1 H, ddd, J 12.9, 4.0, 2.7 Hz), 3.44 (1 H, ddd, J 13.0, 3.2, 2.0 Hz), 2.09 (3 H, d, J 0.9 Hz).

Step 2. To a solution of 8-methyl-3,4-dihydro-2*H*-1,4-benzoxazine-2-carbonitrile (**99**) (0.500 g, 2.87 mmol) in DMF (5 mL) was added sodium hydride (60% dispersion in mineral oil, 0.10 g), followed by benzyl bromide (0.34 mL, 2.87 mmol). The reaction mixture was stirred at rt for 18 h, and then quenched with dropwise addition of water and evaporated. The residue was subjected to silica-gel column chromatography [hexane then hexane-EtOAc (4:1) as eluent]. The eluted material (0.462 g, 61%) was identified as 4-benzyl-8-methyl-3,4-dihydro-2*H*-1,4-benzoxazine-2-carbonitrile (**100**); LC-MS (Method B) m/z 265 $[M + H]^+$; $t_R = 1.38$ min. 1H NMR (400 MHz, DMSO- d_6) δ : 7.40–7.14 (5 H, m), 6.70 (1 H, dd, J 8.2, 7.4 Hz), 6.61–6.49 (2 H, m), 4.58 (1 H, d, J 16.3 Hz), 4.49 (1 H, d, J 16.3 Hz), 3.70 (1 H, dd, J 12.9, 2.6 Hz), 3.63 (1 H, dd, J 12.9, 3.1 Hz), 2.13 (3 H, s).

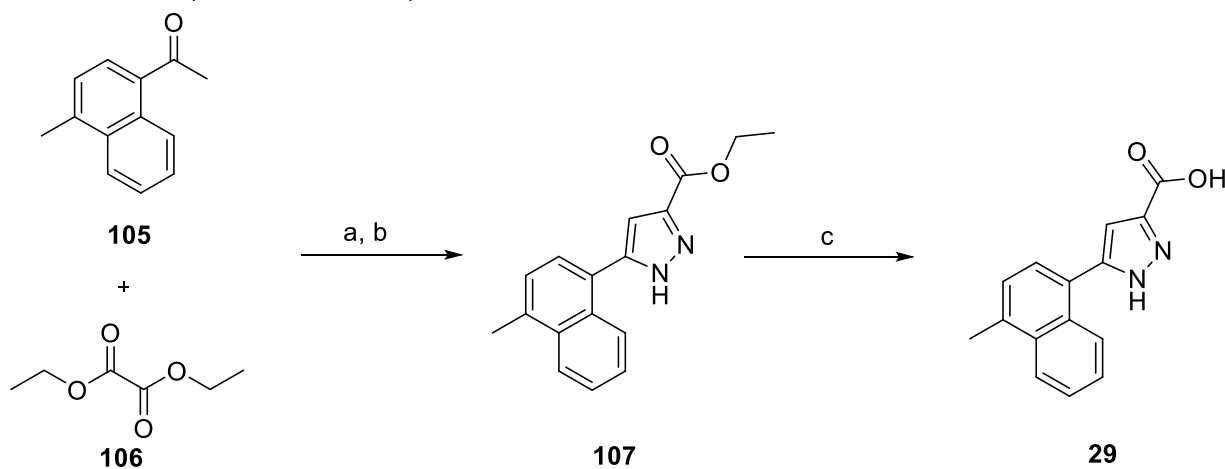
Step 3. To a solution of 4-benzyl-8-methyl-3,4-dihydro-2*H*-1,4-benzoxazine-2-carbonitrile (**100**) (0.430 g, 1.627 mmol) in methanol (20 mL) was added ammonium sulfide (20% aqueous solution, 5 mL). After 72 g stirring at rt, the reaction mixture was evaporated, and the residue dried in vacuo. The resulting yellow solid (0.420 g, 87%), was identified as 4-benzyl-8-methyl-3,4-dihydro-2*H*-1,4-benzoxazine-2-carbothioamide (**101**); LC-MS (Method B) m/z 299 $[M + H]^+$; $t_R = 1.27$ min. 1H NMR (400 MHz, DMSO- d_6) δ : 10.16 (1 H, br s), 9.17 (1 H, br s), 7.38–7.20 (5 H, m), 6.65 (1 H, dd, J 8.2, 7.2 Hz), 6.58 (1 H, dd, J 8.1, 1.7 Hz), 6.53–6.45 (1 H, m), 4.83 (1 H, dd, J 8.0, 2.6 Hz), 4.53 (1 H, d, J 16.2 Hz), 4.44 (1 H, d, J 16.1 Hz), 3.73 (1 H, dd, J 12.0, 2.7 Hz), 3.29 (1 H, dd, J 11.9, 8.1 Hz), 2.20 (3 H, s).

Step 4. A stirred mixture of 4-benzyl-8-methyl-3,4-dihydro-2*H*-1,4-benzoxazine-2-carbothioamide (**101**) (0.42 g, 1.78 mmol) and ethyl bromopyruvate (0.32 mL, 1.78 mmol) in ethanol (10 mL) was heated at reflux for 18 h. The reaction mixture was then cool and evaporated. The residue was subjected to silica-gel column chromatography [hexane then hexane-EtOAc (4:1) as eluent]. The eluted material, obtained as a yellow solid (0.20 g, 29%), was identified as ethyl 2-(4-benzyl-8-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylate (**102**); LC-MS (Method B) m/z 395 $[M + H]^+$; $t_R = 1.46$ min. 1H NMR (400 MHz, DMSO- d_6) δ : 8.55 (1 H, s), 7.36–7.18 (5 H, m), 6.67 (1 H, dd, J 8.2, 7.3 Hz), 6.58–6.51 (2 H, m), 5.77 (1 H, q, J 2.8 Hz), 4.56–4.48 (2 H, m), 4.36–4.28 (2 H, m), 3.80 (1 H, dd, J 12.3, 3.0 Hz), 3.59 (1 H, dd, J 12.3, 5.7 Hz), 2.20 (3 H, s), 1.31 (3 H, t, J 7.1 Hz).

Step 5. A mixture of ethyl 2-(4-benzyl-8-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylate (**102**) (0.190 g, 0.482 mmol), lithium hydroxide monohydrate (0.101 g, 2.41 mmol) and methanol (3 mL) was stirred at rt for 72 h. The reaction mixture was evaporated, and the residue partitioned between water and ethyl acetate. The aqueous phase was separated, and acidified to pH 1 with 2M HCl_(aq). The mixture was extracted with ethyl acetate (2 \times), and the combined organic extracts were dried (MgSO₄) and evaporated to a white solid (0.140 g, 79%), identified as 2-(4-benzyl-8-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylic acid (**103**); LC-MS (Method B) m/z 367 $[M + H]^+$; $t_R = 1.36$ min. 1H NMR (400 MHz, DMSO- d_6) δ : 13.10 (1 H, s), 8.48 (1 H, s), 7.34–7.18 (5 H, m), 6.71–6.62 (1 H, m), 6.57–6.50 (2 H, m), 5.75 (1 H, dd, J 5.7, 3.0 Hz), 4.58–4.43 (2 H, m), 3.88–3.75 (1 H, m), 3.60 (1 H, dd, J 12.2, 5.7 Hz), 2.20 (3 H, s).

Step 6. 2-(4-Benzyl-8-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)-1,3-thiazole-4-carboxylic acid was separated by chiral chromatography using a Daicel chiralpak AD-10 μ M column. Mobile phase was 85:15 hexane-isopropanol with 0.2% TFA as modifier. The first eluted material, obtained as a pale brown solid (0.004 g), was identified as 2-[(2*R* or *S*)-4-benzyl-8-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]-1,3-thiazole-4-carboxylic acid (**28**); LC-MS (Method B) m/z 367 $[M + H]^+$; t_R = 1.36 min. The second eluted material, obtained as a pale brown solid (0.006 g), was identified as 2-[(2*S* or *R*)-4-benzyl-8-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-2-yl]-1,3-thiazole-4-carboxylic acid (**104**); LC-MS (Method B) m/z 367 $[M + H]^+$; t_R = 1.36 min.

Scheme S11. Synthesis of compound **29**.^a



^aReagents and conditions: (a) EtONa, -78 °C to rt, (b) MeCO₂H, H₂NNH₂·H₂O, 58% (c) 2M NaOH(aq), MeOH, 32%.

5-(4-Methylnaphthalen-1-yl)-1*H*-pyrazole-3-carboxylic acid (29**).** *Step 1.* Sodium (49.91 mg, 2.17 mmol) was dissolved in anhydrous ethanol (2 mL) under nitrogen and then cooled to -78 °C. A solution of 1-(4-methylnaphthalen-1-yl)ethanone (**105**) (200 mg, 1.09 mmol) in ethanol (5 mL) was added and the reaction mixture was stirred at -78 °C for 30 min, followed by the addition of diethyl oxalate (**106**) (162 μ L, 1.19 mmol). The reaction mixture was slowly allowed to warm to rt and stirred overnight, upon which a pale yellow precipitate had formed. Acetic acid (124 μ L, 2.17 mmol) followed by hydrazine hydrate (58 μ L, 1.19 mmol) was added and the reaction mixture continued to stir at rt. On completion, the reaction was diluted with water and acidified with aqueous 2M HCl. The reaction mixture was extracted with ethyl acetate, and the combined organic extracts were washed with dilute aqueous HCl followed by saturated sodium bicarbonate solution. The organic extracts were dried (MgSO₄) and evaporated under reduced pressure. The residue was purified by column chromatography on an Isolute pre-packed silica column in a gradient of isohexane to 1:1 ethyl acetate/isohexane to afford ethyl 5-(4-methylnaphthalen-1-yl)-1*H*-pyrazole-3-carboxylate (**107**) as a yellow oil (177 mg, 58%). LC-MS (Method A) m/z 561 $[2M + H]^+$; t_R = 2.45 min. ¹H NMR (400 MHz, CDCl₃) δ : 8.21 (1 H, d,

J 8.3 Hz), 8.09 (1 H, d, *J* 8.1 Hz), 7.63–7.48 (3 H, m), 7.38 (1 H, d, *J* 7.2 Hz), 7.12 (1 H, s), 4.46 (2 H, q, *J* 7.1 Hz), 2.77 (3 H, s), 1.45 (3 H, t, *J* 7.1 Hz).

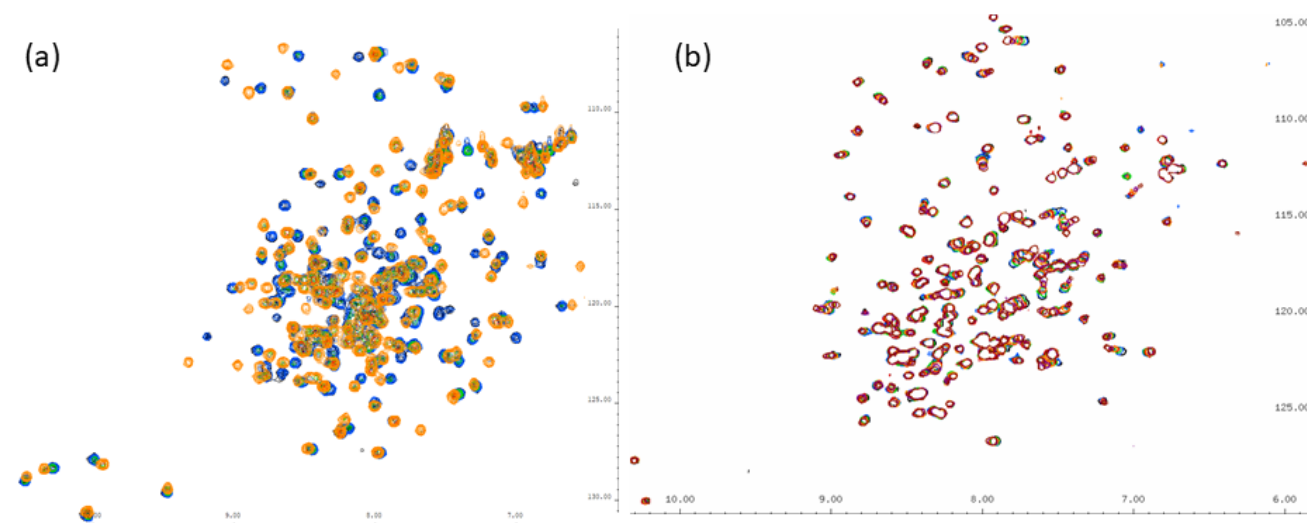
Step 2. To a solution of ethyl 5-(4-methylnaphthalen-1-yl)-1*H*-pyrazole-3-carboxylate (**107**) (177 mg, 0.63 mmol) in methanol (2 mL) was added 2M NaOH (1 mL) and the reaction mixture heated to 40 °C for 2 h. The reaction was then cooled to rt, diluted with water and acidified to pH 4 with 2M HCl. The reaction mixture was extracted with ethyl acetate, and the combined organic extracts were dried (MgSO₄) and evaporated under reduced pressure. The residual material was triturated with ether and collected by filtration to afford 5-(4-methylnaphthalen-1-yl)-1*H*-pyrazole-3-carboxylic acid (**29**) as a white powder (53 mg, 0.21 mmol, 32%). LC-MS (Method A) *m/z* 253 [M + H]⁺; *t*_R = 2.06 min. ¹H NMR (400 MHz, DMSO-*d*₆ + TFA) δ: 8.31–8.25 (1 H, m), 8.11–8.05 (1 H, m), 7.62–7.53 (3 H, m), 7.45–7.40 (1 H, m), 7.01 (1 H, s), 2.69 (3 H, s).

REFERENCES FOR COMPOUND SYNTHESIS

1. McClellan, W.; Oost, T.; Bruncko, M.; Wang, X.; Augeri, D. J.; Baumeister, S. A.; Dickman, D. A.; Ding, H.; Dinges, J.; Fesik, S. W.; Hajduk, P. J.; Kunzer, A. R.; Nettesheim, D. G.; Petros, A. M.; Rosenberg, S. H.; Shen, W.; Thomas, S. A.; Wendt, M. D. PCT Patent Application WO 2002024636, 2002.
2. Porter, J.; Payne, A.; de Candole, B.; Ford, D.; Hutchinson, B.; Trevitt, G.; Turner, J.; Edwards, C.; Watkins, C.; Whitcombe, I.; Davis, J.; Stubberfield, C. Tetrahydroisoquinoline amide substituted phenyl pyrazoles as selective Bcl-2 inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 230–233.
3. Casara, P.; Le Diguarher, T.; Henlin, J.-M.; Starck, J.-B.; Le Tiran, A.; De Nanteuil, G.; Geneste, O.; Davidson, J. E. P.; Murray, J. B.; Chen, I.; Walmsley, C.; Graham, C. J.; Ray, S.; Maddox, D.; Bedford, S. PCT Patent Application WO2015011396, 2015.
4. Elmore, S. W.; Souers, A. J.; Bruncko, M.; Song, X.; Wang, X.; Hasvold, L. A. Wang, L.; Kunzer, A. R.; Park, C.; Wendt, M. D.; Tao, Z.; Madar, D. PCT Patent Application WO2008131000, 2008.
5. Naskara, D.; Roy, A.; Seibel, W. L.; Portlock, D. E. Novel Petasis boronic acid-Mannich reactions with tertiary aromatic amines. *Tetrahedron Lett.* **2003**, *44*, 5819–5821.
6. Park, C.-M.; Bruncko, M.; Adickes, J.; Bauch, J.; Ding, H.; Kunzer, A.; Marsh, K. C.; Nimmer, P.; Shoemaker, A. R.; Song, X.; Tahir, S. K.; Tse, C.; Wang, X.; Wendt, M. D.; Yang, X.; Zhang, H.; Fesik, S. W.; Rosenberg, S. H.; Elmore, S. W. Discovery of an orally bioavailable small molecule inhibitor of prosurvival B-cell lymphoma 2 proteins. *J. Med. Chem.* **2008**, *51*, 6902–6915.

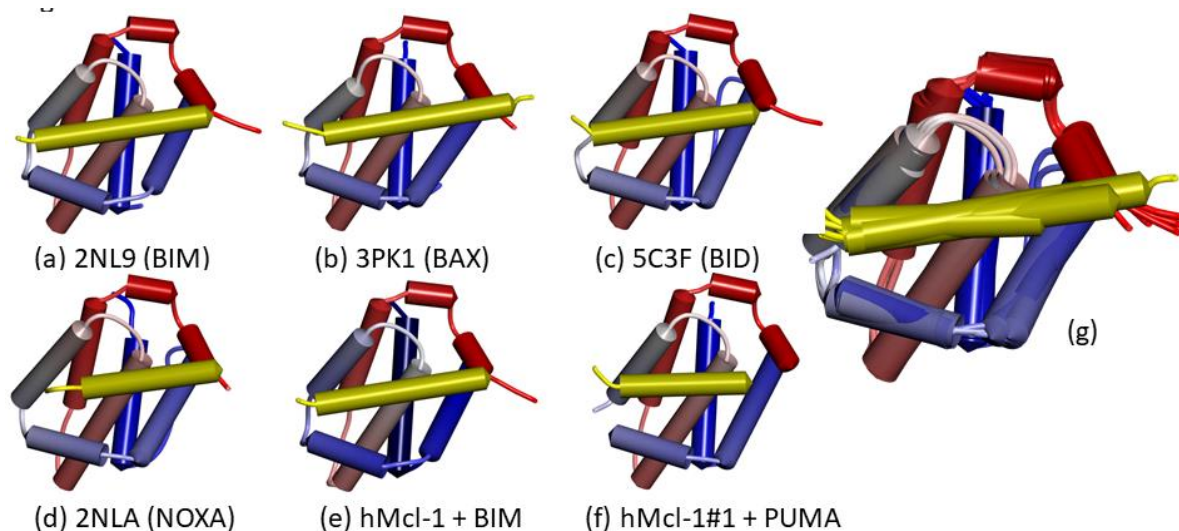
Supplementary Figures

Figure S1 – ^{13}C - ^{15}N HSQC spectrum for compound binding



(a). Titration of compound **1** (0 – black, 50- blue, 100-green, 200-orange μM) onto 200 μM Bcl-2#3 (b). Titration of compound **21** (0 – black, 10 – blue, 30 – green, 60 – red, 100 – orange, 200 – cyan, 400 – crimson μM) onto 40 μM mMcl-1

Figure S2 – comparison of structures of Mcl-1 bound to various peptides



Cartoon representations of literature and Vernalis structures of different forms of Mcl-1 (coloured blue to red for N to C terminal secondary structure) bound to different BH3-only peptides (yellow) as: (a). pdb code 2NL9 - BIM peptide bound; (b). pdb code 3PK1 - BAX peptide bound (Mcl-1 in 3PK1 has the same sequence as in 2NL9 with methionine in place of seleno-methionine and residues SGAAGRR replaced by the human sequence AKATSRK, and with D173S); (c). pdb code 5C3F - BID peptide bound (Mcl-1 in 5C3F has the same sequence as in 3PK1 with S173E); (d). pdb code 2NLA with BIM peptide bound (Mcl-1 in 2NLA has the same sequence as in 2NL9 with methionine in place of seleno-methionine). (e) and (f). structures reported here for (e) hMcl-1 with BIM peptide bound and (f) hMcl-1#1 with PUMA peptide bound

Figure S3 – ITC and SPR measurements

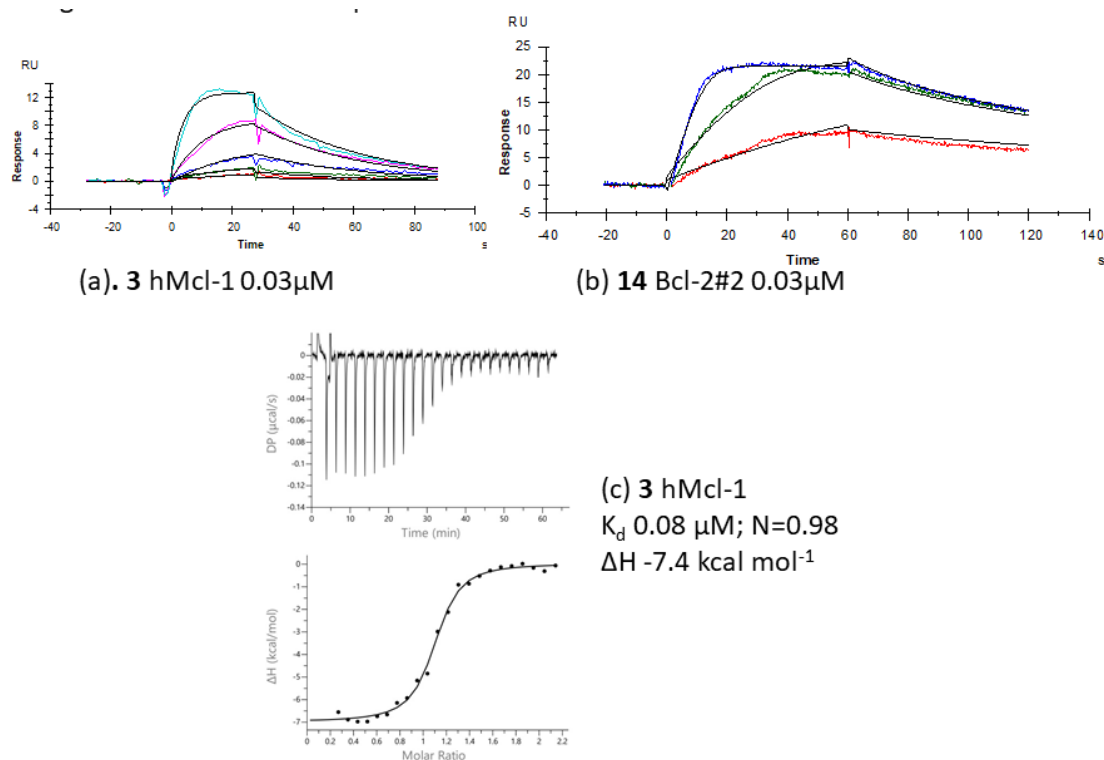
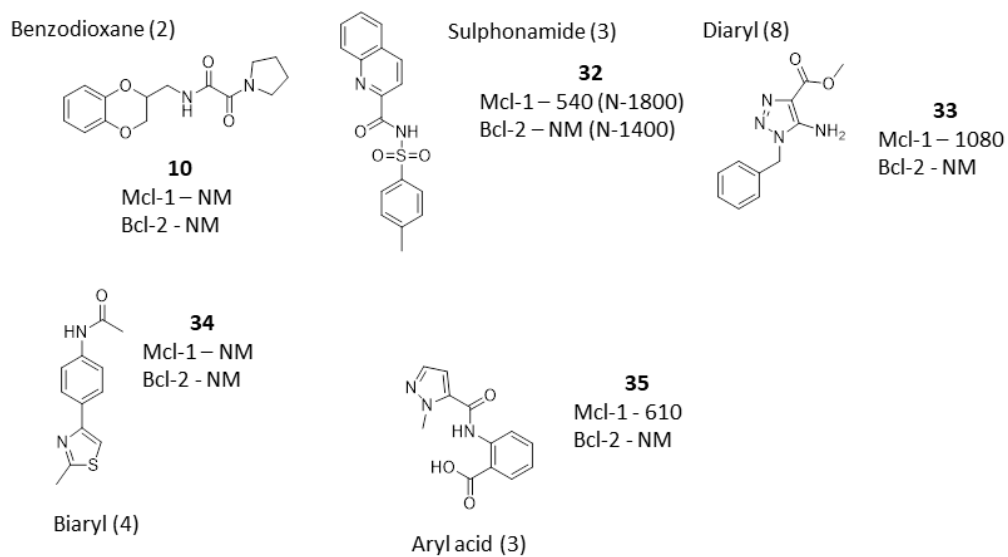


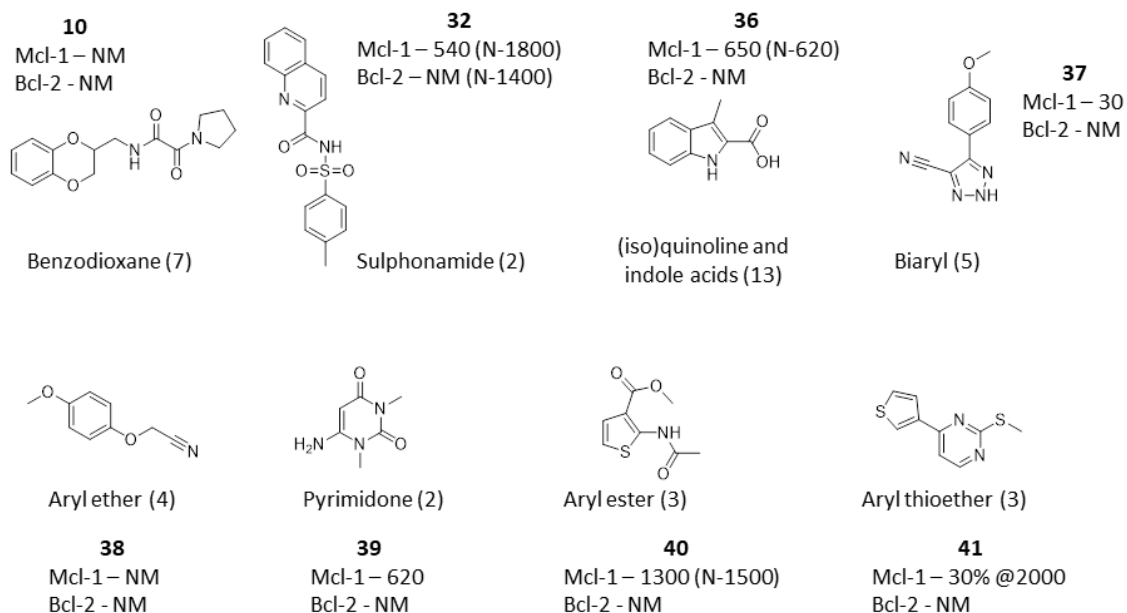
Figure S3. Measurement of direct binding of (a) **3** to hMcl-1 and (b) **14** to Bcl-2 by SPR and (c) **3** to hMcl-1 by ITC. The SPR measurements were made in a multiple cycle kinetics mode, with the raw sensorgrams coloured and the calculated curves fitted to 1:1 binding model in black. The measurements were made on double his-tagged hMcl-1 and single-his tagged Bcl-2#2

Figure S4 – Hits from a fragment screen against Bcl-2



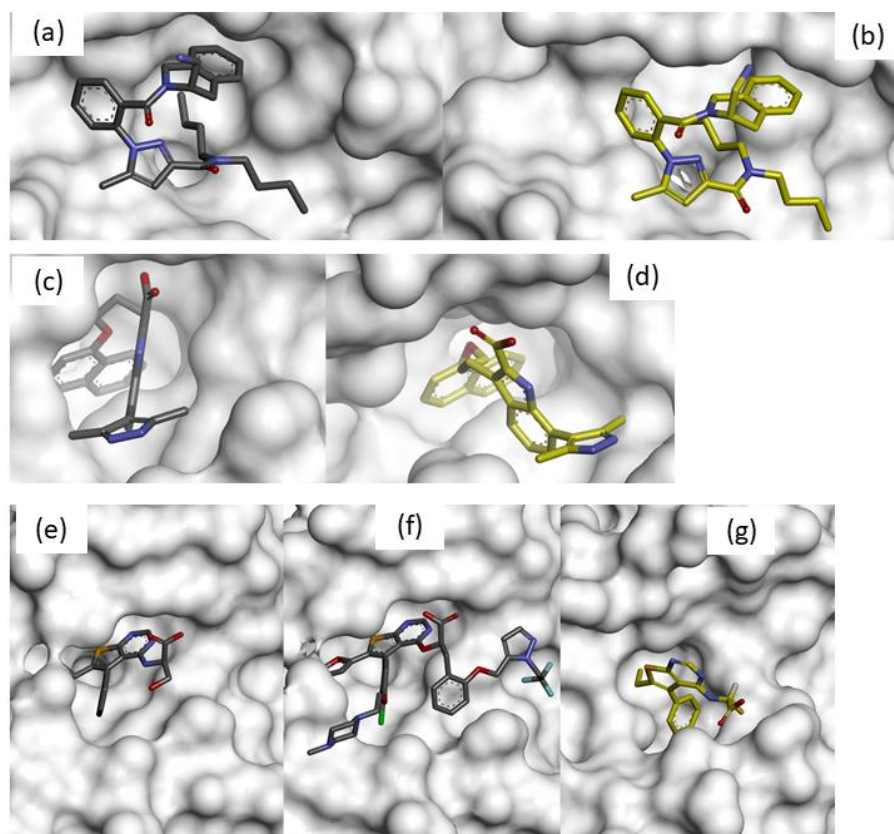
Data shown are IC₅₀ for FP assay (and K_d for ¹H,¹⁵N HSQC) for that target in μM. See the legend to Figure 3 for further details

Figure S5 – Hits from a fragment screen against Mcl-1



Data shown are IC₅₀ for FP assay (and K_d for ¹H, ¹⁵N HSQC) for that target in μM. See legend to Figure 3 for more details.

Figure S6 – Comparison of X-ray and NGM generated structures



Details of the binding site of the structure of **2** binding to Bcl-2 as determined in (a) crystal structure of Bcl-2#3 and (b) NGM using the Bcl-2 structure of **1** bound to bcl-2#3; details of the binding site of the structure of **3** binding to Mcl-1 as determined in (c) crystal structure of hMcl-1 and (d) NGM using the Mcl-1 structure of pdb code 2NL9; details of the binding site of the crystal structure of MBP-hMcl-1 bound to (e) **9** and (f) S 63845¹ compared to (g) NGM of **30** using a homology model of hMcl-1 generated as described in methods. The solvent accessible surface of the protein is shown together with compounds in stick with grey (for X-ray) and yellow (for NGM) carbon atoms

Supplementary Tables

Table S1 – BH3-only peptides bind Bcl2#3 with the same selectivity vs Bcl-x_L as Bcl-2

Protein	F-Puma	BAK CF	F-BIM L	F-BAD L	F-HRK	F-BMF
GST-Bcl-x _L	103	16	15	10	200	15
Bcl-2	54	89	9	8	No binding	97
Bcl-2#3	24	19	3	4	No binding	53

Data are the K_d (nM) measured in an FP assay (for conditions see above) for each of three proteins (Bcl-x_L, Bcl-2 and Bcl-2#3) binding to 6 different fluorescein labelled BH3 peptides.

Table S2 – crystallographic data collection

Compound (PDB code)	Data collecti on	Space group, (Unit cell dimensions)	Resolution (Å) ^a	Comple teness (%) ^a	Unique reflectio ns	Redund ancy	Rmerge _{a, b}	<I/σ(I) > _{a, c}
Mcl-1								
Bim (6QFI)	ESRF ID29	I222, (a=52.8, b=71.7, c=117.7Å)	2.3 -50.0 (2.30 - 2.38)	79.4 (17.2)	8265	3.7	0.083 (0.342)	15.0 (1.5)
Puma (6QFM)	ESRF ID29	P212121 (a=36.0, b=58.3, c=76.9)	2.0 - 25.0 (2.00 - 2.07)	84.8 (40.4)	9678	3.2	0.079 (0.355)	13.3 (1.9)
3 (6QFQ)	DLS i03	P41212 (a=b=48.1, c=148.5)	1.6 - 50.0 (1.60 - 1.66)	99.0 (98.8)	43693	4.6	0.078 (>1)	15.3 (1.3)
9 MBP- Mcl1 (6QGD)	DLS i03	P21212 (a=100.5, b=135.8, c=38.0)	1.76 - 34.4 (1.76 - 1.81)	99.4 (99.2)	52298	4.0	0.052 (0.895)	12.9 (1.4)
Bcl-2								
Puma (6QG8)	ESRF ID29	P212121 (a=40.1, b=55.0, c=84.7)	1.8 - 50.0 (1.80 - 1.86)	75.0 (16.7)	13577	3.5	0.111 (0.534)	10.7 (1.3)
1 (6QGG)	DLS i03	P41 (a=b=55.3, c=58.6)	1.49 - 50.0 (1.49 - 1.54)	94.7 (94.9)	27361	4.1	0.059 (0.196)	19.8 (7.1)
2 (6QGK)	DLS i04	P212121 (a=47.6, b=50.1, c=65.4)	1.7 - 25.0 (1.70 - 1.76)	82.8 (45.2)	14748	4.4	0.071 (0.360)	17.4 (2.6)
13 (6QGH)	R-axis	P212121 (a=44.4, b=55.1, c=64.7)	1.90 - 27.9 (1.90 - 1.97)	98.0 (90.8)	12847	4.6	0.103 (0.471)	8.1 (2.8)
14 (6QGJ)	R-axis	P212121 (a=45.0, b=56.2, c=64.4)	1.75 - 30.0 (1.75 - 1.81)	99.1 (98.0)	16972	3.9	0.034 (0.539)	35.6 (2.3)

DLS – Diamond Light Source, Harwell, UK; ESRF – European Synchrotron Facility, Grenoble, France; R-axis - rotating anode RUH300 coupled with R-axis IV++ image plate (Rigaku MSC instrument installed at Vernalis (R&D) Ltd, Cambridge, UK).

^a Values in parentheses are for highest resolution shells

^b $R_{\text{merge}} = \sum(|I - \langle I \rangle|) / \sum I$

^c Signal to noise ratio of intensities.

Table S3 - Refinement statistics

Compound	Resolution (Å) ^a	No. of reflections used in refinement ^a	Completeness (%) ^a	Rwork ^{a, d}	Rfree ^{a, d, e}	Average B-factor (Å ²)	RMS deviations from ideal f Bonds (Å) / Angles (°)	Ramachandran plot favoured / Allowed / Outliers (%)
Mcl-1								
Bim (6QFI)	2.4 - 20.0 (2.40 - 2.53)	7623 (561)	88.1 (44.8)	0.186 (0.269)	0.236 (0.315)	46.7	0.010 / 1.33	96.3 / 3.0 / 0.6
Puma (6QFM)	2.0 - 10.0 (2.00 - 2.11)	9105 (679)	83.9 (43.3)	0.202 (0.270)	0.261 (0.286)	35.2	0.009 / 1.28	98.0 / 2.0 / 0.0
3 (6QFQ)	1.6 - 20.0 (1.60 - 1.69)	22548 (3344)	99.3 (99.7)	0.189 (0.297)	0.223 (0.311)	26.9	0.015 / 1.84	100 / 0 / 0
9 MBP-Mcl1 (6QGD)	1.8 - 20.0 (1.80 - 1.90)	46429 (6989)	99.2 (99.6)	0.177 (0.318)	0.208 (0.337)	38.0	0.021 / 1.88	99.0 / 1.0 / 0.0
Bcl-2								
Puma (6QG8)	1.90 - 20.0 (1.80 - 1.85)	12321 (950)	84.4 (43.6)	0.169 (0.251)	0.216 (0.267)	29.3	0.011 / 1.49	99.3 / 0.7 / 0.0
1 (6QGG)	1.50 - 15.00 (1.50 - 1.58)	25595 (3948)	94.6 (95.8)	0.151 (0.171)	0.173 (0.210)	16.8	0.019 / 1.91	99.3 / 0.7 / 0.0
2 (6QGK)	1.80 - 15.00 (1.80 - 1.90)	12725 (1519)	88.7 (71.8)	0.166 (0.228)	0.202 (0.233)	29.5	0.012 / 1.45	99.3 / 0.7 / 0.0
13 (6QGH)	2.00 - 20.0 (2.00 - 2.11)	10574 (1555)	98.8 (97.9)	0.177 (0.267)	0.206 (0.314)	29.6	0.012 / 1.47	96.7 / 3.3 / 0.0
14 (6QGJ)	1.90 - 10.0 (1.90 - 2.00)	12582 (1831)	98.6 (100)	0.201 (0.247)	0.225 (0.283)	36.6	0.014 / 1.90	98.6 / 1.4 / 0.0

^a Values in parentheses are for highest resolution shells

^d $R = \sum(F_{\text{obs}} - F_{\text{calc}}) / \sum F_{\text{obs}}$

^e Five percent of reflections were randomly chosen for calculation of R_{free} .

^f Root mean square deviations from ideal geometry²

^g As defined in³

References for supplementary figures and tables

1. Kotschy, A.; Szlavik, Z.; Murray, J.; Davidson, J.; Maragno, A. L.; Le Toumelin-Braizat, G.; Chanrion, M.; Kelly, G. L.; Gong, J. N.; Moujalled, D. M.; Bruno, A.; Csekei, M.; Paczal, A.; Szabo, Z. B.; Sipos, S.; Radics, G.; Proszenyak, A.; Balint, B.; Ondi, L.; Blasko, G.; Robertson, A.; Surgenor, A.; Dokurno, P.; Chen, I.; Matassova, N.; Smith, J.; Pedder, C.; Graham, C.; Studeny, A.; Lysiak-Auvity, G.; Girard, A. M.; Grave, F.; Segal, D.; Riffkin, C. D.; Pomilio, G.; Galbraith, L. C.; Aubrey, B. J.; Brennan, M. S.; Herold, M. J.; Chang, C.; Guasconi, G.; Cauquil, N.; Melchior, F.; Guigal-Stephan, N.; Lockhart, B.; Colland, F.; Hickman, J. A.; Roberts, A. W.; Huang, D. C.; Wei, A. H.; Strasser, A.; Lessene, G.; Geneste, O. The MCL1 inhibitor S63845 is tolerable and effective in diverse cancer models. *Nature* **2016**, 538, 477-482.
2. Engh, R. A.; Huber, R. Accurate bond and angle parameters for X-ray protein structure refinement. *Acta Crystallographica Section A* **1991**, 47, 392-400.
3. Lovell, S. C.; Davis, I. W.; Arendall, W. B., 3rd; de Bakker, P. I.; Word, J. M.; Prisant, M. G.; Richardson, J. S.; Richardson, D. C. Structure validation by C α geometry: phi,psi and C β deviation. *Proteins* **2003**, 50, 437-450.