Supporting Information

GSK973 Is An Inhibitor of the Second Bromodomains (BD2s) of the Bromodomain and Extra-Terminal (BET) Family

Alex Preston*[1], Stephen J. Atkinson[1], Paul Bamborough[2], Chun-wa Chung[2], Laurie J. Gordon[2], Paola Grandi[3], James R. J. Gray[1], Lee A. Harrison[1], Antonia J. Lewis[2], David Lugo[1], Cassie Messenger[2], Anne-Marie Michon[3], Darren J. Mitchell[1], Rab K. Prinjha[1], Inmaculada Rioja[1], Jon Seal[1], Simon Taylor[1†], Pierre Thesmar[1], Ian D. Wall[2], Robert J. Watson[1],James M. Woolven[2], Emmanuel H. Demont*[1]

[1]Epigenetics Discovery Performance Unit and [2]Platform Technology and Science, GlaxoSmithKline Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, U.K. [3]Cellzome GmbH, GlaxoSmithKline, Meyerhofstrasse 1, 69117 Heidelberg, Germany.

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In vitro assays protocols

Proteins expression: These were carried out as described previously.[1]

BET assays: BET BRD4 BD1 and BD2 TR-FRET Assays. Tandem bromodomains of 6His-Thr-BRD4(1−477) were expressed, with an appropriate mutation in BD2(Y390A) to monitor compound binding to BD1, or in BD1(97A) to monitor compound binding to BD2. Analogous Y→A mutants were used to measure binding to the other BET bromodomains: 6His-Thr-BRD2(1−473 Y386A or Y113A), 6His-Thr-BRD3(1−435 Y348A or Y73A), 6His-FLAG-Tev-BRDT(1−397 Y309A or Y66A). The AlexaFluor 647 labeled BET bromodomain ligand was prepared as follows: To a solution of Alexa Fluor 647 hydroxysuccinimide ester in DMF was added a 1.8-fold excess of N-(5-aminopentyl)-2-((4S)-6-(4-chlorophenyl)-8-methoxy-1-methyl-4H-benzo[f][1,2,4]triazolo[4,3-a][1,4]-diazepin-4-yl)acetamide, also in DMF, and when thoroughly mixed, the solution was basified by the addition of a 3-fold excess of diisopropylethylamine. Reaction progress was followed by electrospray LC/MS, and when judged complete, the product was isolated and purified by reversed-phase C18 HPLC. The final compound was characterized by mass spectroscopy and analytical reversed-phase HPLC.

Compounds were titrated from 10 mM in 100% DMSO and 50 nL transferred to a low volume black 384 well micro titre plate using a Labcyte Echo 555. A Thermo Scientific Multidrop Combi was used to dispense 5 μL of 20 nM protein in an assay buffer of 50 mM HEPES, 150 mM NaCl, 5% glycerol, 1 mM DTT and 1 mM CHAPS, pH 7.4, and in the presence of 100 nM fluorescent ligand (~*Kd* concentration for the interaction between BRD4 BD1 and ligand). After equilibrating for 30 mins in the dark at rt, the bromodomain protein:fluorescent ligand interaction was detected using TR-FRET following a 5 μL addition of 3 nM europium chelate labelled anti-6His antibody (Perkin Elmer, W1024, AD0111) in assay buffer. Time resolved fluorescence (TRF) was then detected on a TRF laser equipped Perkin Elmer Envision multimode plate reader (excitation = 337 nm; emission 1 = 615 nm; emission 2 = 665 nm; dual wavelength bias dichroic = 400 nm, 630 nm). TR-FRET ratio was calculated using the following equation: Ratio = ((Acceptor fluorescence at 665 nm) / (Donor fluorescence at 615 nm)) *

1000. TR-FRET ratio data was normalised to high (DMSO) and low (compound control derivative of I-BET762) controls and IC_{50} values determined for each of the compounds tested by fitting the fluorescence ratio data to a four parameter model: y = a + ((b – a) / (1 + (10 ^ x / 10 ^ c) ^ d) where 'a' is the minimum, 'b' is the Hill slope, 'c' is the IC50 and 'd' is the maximum.

hWB MCP-1 Assay: Compounds to be tested were diluted in 100% DMSO to give a range of appropriate concentrations at 140x the required final assay concentration, of which 1 µL was added to a 96 well tissue culture plate. 130 µL of human whole blood, collected into sodium heparin anticoagulant, (1 unit/mL final) was added to each well and plates were incubated at 37°C (5% C02) for 30 min before the addition of 10 µL of 2.8 µg/mL LPS (Salmonella Typhosa), diluted in complete RPMI 1640 (final concentration 200 ng/mL), to give a total volume of 140 µL per well. After further incubation for 24 h at 37 °C, 140 µL of PBS was added to each well. The plates were sealed, shaken for 10 min and then centrifuged (2500 rpm x 10 min). 100 µL of the supernatant was removed and MCP-1 levels assayed immediately by immunoassay (MesoScale Discovery technology).

BROMOscan® Bromodomain Profiling: BROMOscan® bromodomain profiling was provided by Eurofins DiscoverX Corp. (Fremont, CA, USA, http://www.discoverx.com). Determination of the KD between test compounds and DNA tagged bromodomains was achieved through binding competition against a proprietary reference immobilized ligand.

SPR Methods: Surface plasmon resonance (SPR)-based ligand binding assays were performed using a Biacore S200 instrument (GE Healthcare) on CM5 chips (GE Healthcare) at 25 °C. Proteins were immobilised using standard amine coupling chemistry (7 minutes activation with EDC/NHS, protein injection, then quenching with 1 M ethanolamine pH 8.5 for 7 min), with a running buffer of 0.01 M HEPES pH 7.4, 0.15 M NaCl, 3mM EDTA,0.005% v/v Tween 20. Two proteins (1) 6His-Thr-Brd4(1-477) Y390A, which tests for binding to the N-terminal bromodomain of BRD4, BRD4-BD1 and (2) His-tagged cleaved BRD4 (347-463), which tests for the binding to the C-terminal bromodomain of BRD4, BRD4-BD2, were immobilised to typically ~7-10kRU for 6His-Thr-Brd4(1-477) Y390A and ~2-3kRU for BRD4 (347-463) using injections of 50-60 µg/ml of protein in 10 mM Na acetate pH 5.5

and 5.0 respectively in the presence of 50μM of a nanomolar inhibitor. These two proteins were always immobilised onto the same CM5 to allow simultaneously affinity data to be obtained for BRD4-BD1 and BRD4-BD2 binding.

Binding assays were performed in 10mM HEPES,150mM NaCl, 3mM EDTA, 0.005% Tween 20, 1% DMSO, pH 7.4, with a 30 μg/min flow-rate. Ligands were injected in a dose-response series (11-point 3-fold serial dilutions), with solvent correction and positive/negative control cycles being used throughout assays to control for differences in response to bulk refractive index changes and loss of binding activity from the surface respectively. The positive control was 5μM iBET-151 and the negative control was 1.5% DMSO without any inhibitor present. An injection time of 90s was used with dissociation time of 270s and temperature of 25°C. Dose-response data (from reference- subtracted curves) were fitted to a steady-state 1:1 binding model to determine K_D with the BIAevaluation software.

Dose response titrations were run from two top concentrations of 100μM and 300nM to allow characterisation of the high affinity to the BD2 domain and provide an estimate for the low affinity to the BD1 domain. K_d against BRD4 BD2 and BD1 of 34 nM and >3000 nM.

ChromLogD$_{7.4}$

Chromatographic hydrophobicity index (ChiLogD$_{7.4}$) was determined using fast gradient HPLC, according to literature procedures,[2-3] using a Waters Aquity UPLC System, Phenomenex Gemini NX 50x2 mm, 3 um HPLC column, 0-100% pH 7.40 ammonium acetate buffer/acetonitrile gradient. Retention time was compared to standards of known pH to derive Chromatographic Hydrophobicity Index (CHI). ChromLogD = 0.0857CHI – 2.

Artificial membrane permeability

Permeability across a lipid membrane was measured using the published protocol.[4]

Xray Structure Determination: All the statistics for the data collection and refined co-ordinates are given in Table S1. The final crystal structures are deposited in the Protein Data Bank under the accession codes shown in Figure 4.

BRD2-BD2 crystals were grown by streak seeding into hanging drops of 500ul protein solution at 11.5mg/mL and 500uL well solution of 30% PEG 300, 0.1M MES buffer pH6.5 at 20°C. Crystals appeared within 24hrs and were transferred into a fresh solution of the same well solution with 2% DMSO and compound at nominally 10mM. Soaked crystals were briefly transferred into a solution consisting of 30%w/v PEG300, 0.1M MES buffer, pH 6.5 supplemented with 10% ethylene glycol prior to flash freezing in liquid nitrogen. Data from a single crystal was collected 100K on an in-house RIGAKU FR-E^+ SUPERBRIGHT/Saturn A200 detector/ACTOR robotic system and processed using XDS and AutoPROC and scaled using AIMLESS. The $P2_12_12$ cell has a single molecule in the ASU. Manual model building was performed using COOT and refined using REFMAC within the CCP4 software suite. Clear difference density for the ligand in the acetylated lysine binding site was observed and the ligand was unambiguously modelled.

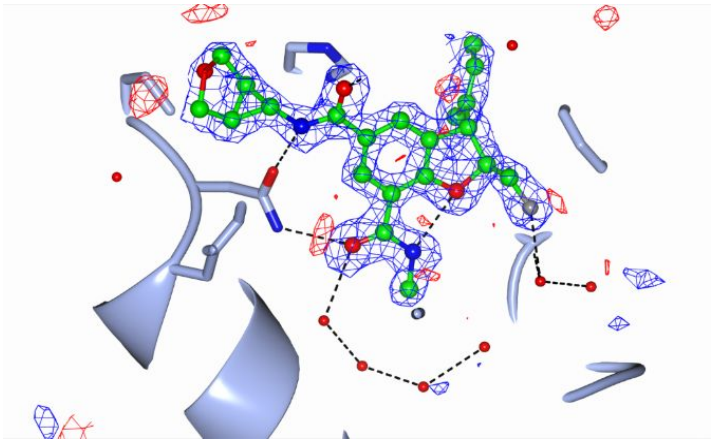
Table S1: Data collection and refinement statistics for BRD2-BD2 X-ray structures.

(collection on a single crystal)	**BRD2-BD1 / GSK973**
Data collection	
Space group	$P2_12_12$
Cell dimensions	
a, *b*, *c* (Å)	71.877, 52.473, 32.070
α, β, γ (°)	90.000, 90.000, 90.000
Resolution (Å)	42.38-1.55 (1.64-1.55)
R_{merge}	0.024 (0.186)
$I/\sigma I$	31.6 (4.7)
Completeness (%)	89.2.0 (52.5)
Redundancy	3.4 (2.0)
No. reflections	54967 (2907)
No. uniq reflections	16200 (1479)
Refinement	
Resolution (Å)	42.38-1.55
No. uniq reflections	15359
No of atoms	
Protein	952
Ligand/ion	30 / 26
Water	228
B-factors	
Protein	18.3
Ligand/ion	19.3 / 40.0
Water	40.1
R.m.s deviations	
Bond lengths (Å)	0.003
Bond angles (º)	1.163

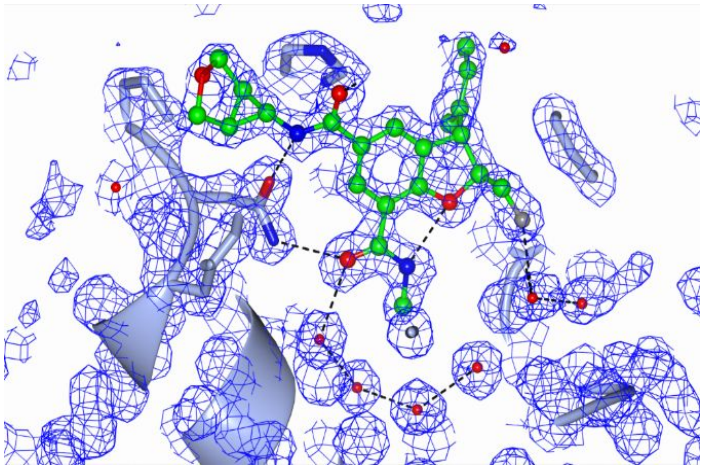
*Highest resolution shell is shown in parenthesis

Figure S1: OMIT density maps for ligands

OMIT (Fo-Fc) map around the ligand contoured at ±3.0sigma (blue/red)



OMIT (2Fo-Fc)map around the ligand contoured at +1.0 sigma (blue)



DMPK

In Vitro Clearance in Hepatocytes. The metabolic stability of selected compounds was tested in male Wistar Han rat, male Beagle dog and mixed gender pooled human hepatocytes. Suspensions of cryopreserved hepatocytes from each species were used. Incubations were performed at a test or control compoundconcentration of 0.5μM at 37°C, at a cell density of 0.5million viable cells/mL. Control incubations were also performed in lysed cells to reveal any non-enzymatic degradation. For all Rat incubations and Dog incubations for compounds **28**, **29** & **36** (n=1), samples (50 μL) were removed from the incubation mixture at 0, 5, 10, 20, 40 and 60min. For all Human incubations and Dog incubations for compounds **31**, **34**, **45** & **36** (n=2), samples (50 μL) were removed from the incubation mixture at 0, 10, 20, 40, 60 and 120min. The samples were added to methanol, containing internal standard, (100μL) to stop the reaction. Following protein precipitation, the compound remaining in the supernatants was measured using specific LC-MS/MS methods as a ratio to the internal standard in the absence of a calibration curve. Peak area ratios (Compound to IS) were fitted to an unweighted logarithmic decline in substrate. Using the first order rate constant, clearance was calculated by adjustment for protein concentration, volume of the incubation and hepatic scaling factor.

In-vivo DMPK Studies. All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals. For all in vivo studies, the temperature and humidity were nominally maintained at 21 °C ± 2 °C and 55% ± 10%, respectively. The diet for rodents was 5LF2 Eurodent Diet 14% (PMI Labdiet, Richmond, IN) and for dogs was Harlan Teklad 2021C (HarlanTeklad, Madison, WI). There were no known contaminants in the diet or water at concentrations that could interfere with the outcome of the studies.

Rat surgical preparation for IV infusion studies. Male Wistar Han rats (supplied by Charles River UK Ltd.) were surgically prepared at GSK with implanted cannulae in the femoral vein (for drug administration) and jugular vein (for blood sampling). Each rat received Cefuroxime (116 mg/kg sc)

and carprofen (7.5 mg/kg sc) as a preoperative antibiotic and analgesic, respectively. Each rat was allowed to recover for at least 2 days prior to dosing. Rats had free access to food and water throughout.

Rat IV n=1 PK studies. Surgically prepared male Wistar Han Rats each received a 1 h intravenous (iv) infusion of compound of interest, as a discrete dose, formulated in DMSO and 10% (w/v) Kleptose HPB in saline aq (2%:98% (v/v)) at a concentration of 0.2 mg/mL to achieve a target dose of 1 mg/kg. Serial blood samples (~50 μL) were collected predose and up to 7 h after the start of the iv infusion. Urine was collected via metacage from start of infusion to 7 h timepoint. Diluted blood and urine samples were analyzed for parent compound using a specific LC−MS/MS assay (LLQ = 1-10 ng/mL). At the end of the study the rats were euthanized by a Schedule 1 technique.

Rat PO n=3 PK studies. Male Wistar Han Rats (no surgical preparation) received an oral gavage administration of compound of interest, as a discrete dose, suspended in 1% (w/v) methylcellulose aq at a target dose of 3 mg/kg. Serial blood samples (~20 μL) were collected via temporary tail vein cannulation up to 7 h after oral dosing (all) and additional tail vein venepuncture sampling up to 24 h (compounds 31 & 35 only) after oral dosing. Diluted blood samples were analyzed for parent compound using a specific LC−MS/MS assay (LLQ = 1-5 ng/mL).

Benchmark Rat PK. Rat PK studies were conducted as a crossover design over two dosing occasions, with 3 days between dose administrations in surgically prepared male Wistar Han Rats. On the first dosing occasion, rats each received a 1 h intravenous (iv) infusion of compound 35 formulated in DMSO and 10% (w/v) Kleptose HPB in saline aq (2%:98% (v/v)) at a concentration of 0.2 mg/mL to achieve a target dose of 1 mg/kg. On the second dosing occasion, the same three rats were administered compound 35 suspended in 1% (w/v) methylcellulose 400 aq at a concentration of 0.6 mg/mL orally, at a target dose of 3 mg/kg. Serial blood samples (~60 μL) were collected predose and up to 24 h after the start of the iv infusion and after oral dosing. Urine was collected via individually housed metacages at 0-7, 7-12 and 12-24 h after start of infusion. Diluted blood and urine samples were analyzed using a specific LC−MS/MS assay (LLQ = 1 & 2 ng/mL respectively). At the end of the study the rats were euthanized by a Schedule 1 technique.

Dog PK Study. Three healthy, laboratory-bred, male Beagle dogs (supplied by Harlan Laboratories, U.K.) were used. The dogs were fasted overnight prior to each dose administration and fed approximately 4 h after the start of dosing. The dogs had free access to water throughout the study. This study was conducted as a crossover design, with more than 7 days between dose administrations. On the first dosing occasion, three dogs each received a 1 h intravenous (iv) infusion of compound 35 formulated in DMSO and 10% (w/v) Kleptose HPB in saline aq (2%:98% (v/v)), at a concentration of 0.2 mg/mL to achieve a target dose of 1 mg/kg. On a subsequent dosing occasion, two of the same three dogs were administered compound 35 as a wet bead-milled spray-dried suspended in 1.5% (w/v) HPMC Pharmacoat 603 with 0.15% (w/v) SLS (aq) at a concentration of 0.4 mg/mL orally, at a target dose of 2 mg/kg. A temporary cannula was inserted into the cephalic vein from which serial blood samples (∼100 μL) were collected predose and up to 26 h after the start of dosing. After collection of the 2 h time point the cannula was removed and later time points were taken via direct venepuncture of the jugular vein. Urine was collected via individual metcages following IV infusion over the time period 2-8 and 8-26 h. Diluted blood and urine samples were analyzed for parent drug concentration using a specific LC−MS/MS assay (LLQ = 2 & 5 ng/mL respectively). At the end of each study the dogs were returned to the colony.

Blood Sample Analysis. Diluted blood samples (1:1 with water) were extracted using protein precipitation with acetonitrile containing an analytical internal standard. An aliquot of the supernatant was analyzed by reverse phase LC−MS/MS using a heat assisted electrospray interface in positive ion mode. Samples were assayed against calibration standards prepared in control blood.

PK Data Analysis from PK Studies. PK parameters were obtained from the blood concentration−time profiles using noncompartmental analysis with WinNonlin Professional 6.3 (Pharsight, Mountain View, CA).

Table S2: Full assay data from tables 1-3, including mean pIC_{50}, standard deviation, and the number of independent test occasions in BRD4 BD1 and BD2 FRET assays

Cpd	BRD4 pIC_{50} (n)		Selectivity	ChromLogD
	BRD4 BD2	BRD4 BD1	BD2/BD1	@ pH 7.4
4	5.6 ± 0.05 (2)	4.6 ± 0.18 (2)	10	5.23
5	6.1 ± 0.12 (2)	4.7 ± 0.16 (2)	25	5.4
6	5 ± 0.02 (2)	< 4.3 (2)	5	5.48
7	5.9 ± 0.09 (9)	5.1 ± 0.11 (9)	6	2.99
14	6.5 ± 0.1 (4)	5.1 ± 0.11 (6)	25	2.03
15	7 ± 0.1 (2)	4.8 (1)	150	3.28
16	6.9 ± 0.22 (3)	5.1 ± 0.2 (3)	60	2.38
17	7.5 ± 0.1 (8)	4.9 ± 0.1 (8)	400	3.55
18	6.9 ± 0.09 (5)	4.8 ± 0.15 (4/5)*	126	2.99
19	7.4 ± 0.1 (4)	4.4 (3/6)*	1000	4.11
20	7.1 ± 0.19 (16)	4.8 ± 0.16 (14)	200	3.07
21	7.9 ± 0.2 (7)	4.7 ± 0.2 (7)	1600	4.24
22	6.2 ± 0.16 (4)	4.7 ± 0.22 (3)	30	1.9
23	6.8 ± 0.1 (6)	< 4.3 (6)	300	3.12
24	6.2 ± 0.08 (4)	5.1 ± 0.1 (3)	13	2.74
25	6.7 ± 0.1 (2)	4.4 (1/2)*	200	3.81
26	7.4 ± 0.2 (7)	4.5 ± 0.2 (7)	800	2.53
27	6.9 ± 0.1 (2)	4.7 (2)	150	2.89
28	7.5 ± 0.1 (3)	4.9 (2)	400	3.34
29	7.6 ± 0.1 (3)	< 3.3 (6)	> 20000	3.94
30	8 ± 0.2 (3)	5 (1/3)*	1000	4.53
31	7.7 ± 0.2 (7)	4.8 ± 0.5 (7)	800	3
32	7.1 ± 0.1 (3)	4.5 ± 0.1 (3)	400	2.92
33	6.8 ± 0.1 (3)	< 4.3 (3)	> 300	3.51
34	7.4 ± 0.1 (5)	4.3 (1/5)*	1200	2.18
35	7.6 ± 0.1 (2)	4.8 ± 0.1 (3)	600	2.94
36	7.8 ± 0.2 (16)	4.6 ± 0.1 (19/20)*	1600	3.56

* Some pIC_{50} results were below the curve-fitting threshold (pIC_{50} < 4.3) so can not be included in mean and SD. In these cases, the number included in mean / number of times tested are both shown.

Table S3: Comparison of activities at BD1 for BRD4, BRD3, BRD2 and BRDT for a set of pan-BD2 selective BET inhibitors.

Cpd	BD1 pIC_{50}s				BD2 pIC_{50}s			
	BRD2	BRD3	BRD4	BRDT	BRD2	BRD3	BRD4	BRDT
7	5.1	4.9	4,8	5.0	5.9	6.3	5.7	5.7
15	5.1	4.4	4.8	4.5	7	7.1	7	6.6
17	4.7	4.8	4.9	4.5	7.1	7.5	7.5	6.9
18	4,8	4,7	4,9	4,7	6.9	6.9	6.2	6.4
19	< 4.3	< 4.3	4.4	< 4.3	6.7	7.2	7.4	6.8
20	4.8	4.6	4.6	4.7	7.1	7.2	6.6	6.8
21	< 4.3	4.4	4.7	4.5	7.3	7.8	7.9	7.4
26	< 4.3	< 4.3	4.5	< 4.3	6.9	7.2	7.4	6.9
28	4.8	4.7	4.9	4.5	7	7.3	7.5	6.9
29	< 4.3	< 4.3	< 3.3	< 4.3	6.6	7.1	7.6	7.2
30	< 4.3	< 4.3	5	< 4.3	7.5	7.7	8	7.5
31	4.5	< 4.3	4.8	< 4.3	7.1	7.7	7.7	7.3
32	< 4.3	< 4.3	4.5	< 4.3	6.5	7	7.1	6.6
33	< 4.3	< 4.3	< 4.3	< 4.3	6.2	6.6	6.8	6.1
34	< 4.3	< 4.3	4.3	< 4.3	6.8	7.3	7.4	6.7
36	4.4	4.5	4.6	4.5	7.5	7.8	7.8	7.4

Table S4: Bromoscan Profile of compound **36** for BET proteins

Protein	BD2 pKd	BD1 pKd	Log(selectivity)
BRD2	8.3	5.3	3
BRD3	8.5	5.2	3.3
BRD4	8.7	5.6	3.1
BRDT	8.3	5.4	2.9

Table S5: Bromoscan Profile of compound **36** outside the BET proteins and comparison with BRD4 BD2 pIC_{50}

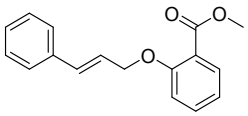
BRD	pKd	Log(selectivity)
BRD4 (2)	8.7	-
ATAD2A	< 4.5	> 4.2
ATAD2B	< 4.5	> 4.2
BAZ2A	5.5	3.2
BAZ2B	5.1	3.6
BRD1	< 4.5	> 4.2
BRD7	< 4.5	> 4.2
BRD8 (1)	< 4.5	> 4.2
BRD8 (2)	< 4.5	> 4.2
BRD9	5.7	3
BRPF1	< 4.5	> 4.2
BRPF3	< 4.5	> 4.2
CECR2	5.5	3.2
CREBBP	4.6	4.1
EP300	< 4.5	> 4.2
FALZ	< 4.5	> 4.2
GCN5L2	4.7	4
PBRM1 (2)	4.7	4
PBRM1 (5)	5	3.7
PCAF	< 4.5	> 4.2
SMARCA2	4.8	3.9
SMARCA4	4.8	3.9
TAF1 (2)	6.4	2.3
TAF1L (2)	5.7	3
TRIM24 (PHD, BD)	4.5	4.2
TRIM33 (PHD,BD)	< 4.5	> 4.2
WDR9 (2)	< 4.5	> 4.2

General Experimental Information

Unless otherwise stated, all reactions were carried out under an atmosphere of nitrogen in heat or oven dried glassware and anhydrous solvent. Solvents and reagents were purchased from commercial suppliers and used as received. Reactions were monitored by thin layer chromatography (TLC) or LCMS. TLC was carried out on glass or aluminium-backed 60 silica plates coated with UV_{254} fluorescent indicator. Spots were visualised using UV light (254 or 365 nm) or alkaline $KMnO_4$ solution, followed by gentle heating. LCMS analysis was carried out on a Waters Acquity UPLC instrument equipped with a BEH column (50 mm x 2.1 mm, 1.7 μm packing diameter) and Waters micromass ZQ MS using alternate-scan positive and negative electrospray. Analytes were detected as a summed UV wavelength of 210 – 350 nm. Flash column chromatography was carried out using Biotage SP4 or Isolera One apparatus with SNAP silica cartridges. Mass directed automatic purification (MDAP) was carried out using a Waters ZQ MS using alternate-scan positive and negative electrospray and a summed UV wavelength of 210 – 350 nm. NMR spectra were recorded at ambient temperature (unless otherwise stated) using standard pulse methods on any of the following spectrometers and signal frequencies: Bruker AV-400 (^1H = 400 MHz, ^{13}C = 101 MHz,), Bruker AV-600 (^1H = 600 MHz, ^{13}C = 150 MHz,), Bruker DPX-250 spectrometer at 250 MHz, Varian INOVA spectrometer at 300 MHz. Chemical shifts are referenced to trimethylsilane (TMS) or the residual solvent peak, and are reported in ppm. Coupling constants are quoted to the nearest 0.1 Hz and multiplicities are given by the following abbreviations and combinations thereof: s (singlet), δ (doublet), t (triplet), q (quartet), quin (quintet), sxt (sextet), m (multiplet), br. (broad). IR spectra were obtained on a Perkin Elmer Spectrum 1 machine. Optical rotation of chiral products was measured using a Jasco P1030 polarimeter. Melting point analysis was carried out using a Stuart SMP40 melting point apparatus. Liquid chromatography high resolution mass spectra (HRMS) were recorded on a Micromass Q-Tof Ultima hybrid quadrupole time-of-flight mass spectrometer, with analytes separated on an Agilent 1100 Liquid Chromatograph equipped with a Phenomenex Luna C18(2) reversed phase column (100 mm x 2.1 mm, 3 μm packing diameter).

Compound 3

Step 1: Methyl 2-(cinnamyloxy)benzoate:



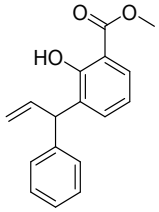
Chemical Formula: $C_{17}H_{16}O_3$
Molecular Weight: 268.31

A solution of methyl 2-hydroxybenzoate (3.8 mL, 30 mmol) in acetone (100 mL) was treated with K_2CO_3 (8.29 g, 60.0 mmol), KI (0.1 g, 0.6 mmol) and (E)-(3-chloroprop-1-en-1-yl)benzene (3.47 mL, 36.0 mmol) and the resulting mixture was stirred at reflux for 11 h then was cooled to room temperature. The insolubles were filtered off, rinsed with EtOAc and the combined organics were concentrated *in vacuo*. The residue was partitioned between Et_2O and water and the layers were separated. The aqueous phase was extracted twice with Et_2O and the combined organics were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give methyl 2-(cinnamyloxy)benzoate (7.12 g, 88%) as a pale yellow solid which was used in the next step without further purification.

LCMS (method high pH): Retention time 1.29 min, $[M+H]^+ = 269$

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.82-7.88 (m, 1H), 7.41-7.49 (m, 3H), 7.32-7.38 (m, 2H), 7.24-7.31 (m, 1H), 6.98-7.08 (m, 2H), 6.80-6.87 (m, 1H), 6.40-6.50 (m, 1H), 4.83 (dd, *J* = 5.4, 1.5 Hz, 2H), 3.95 (s, 3H).

Step 2: Methyl 2-hydroxy-3-(1-phenylallyl)benzoate



Chemical Formula: $C_{17}H_{16}O_3$
Molecular Weight: 268.31

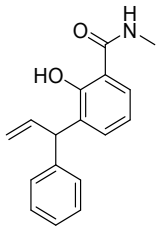
A solution of methyl 2-(cinnamyloxy)benzoate (0.8 g, 3 mmol) in N,N-dimethylaniline (7 mL) was refluxed for 2 h then was cooled to room temperature and partitioned between Et_2O and a 24-26% w/w HCl aqueous solution. The layers were separated and the aqueous phase was extracted with Et_2O. The combined organics were washed with a 2N NaOH aqueous solution, dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue obtained by flash chromatography on silica gel (100

g column, 0-15% EtOAc in cyclohexane) gave methyl 2-hydroxy-3-(1-phenylallyl)benzoate (440 mg, 55%) as a colourless oil.

LCMS (method high pH): Retention time 1.46 min, $[M+H]^+ = 269$

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 11.16 (s, 1H), 7.75-7.82 (m, 1H), 7.19-7.39 (m, 6H), 6.88 (t, J = 7.8 Hz, 1H), 6.26-6.39 (m, 1H), 5.22-5.34 (m, 2H), 4.93-5.01 (m, 1H), 3.95 (s, 3H).

Step 3: 2-Hydroxy-N-methyl-3-(1-phenylallyl)benzamide



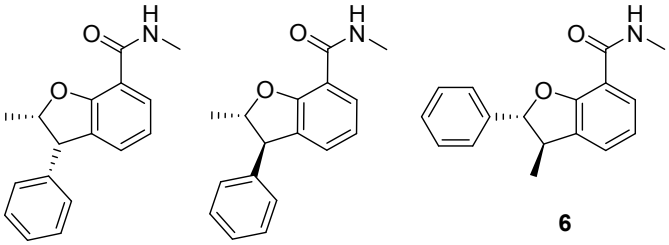
Chemical Formula: $C_{17}H_{17}NO_2$
Molecular Weight: 267.33

A solution of methyl 2-hydroxy-3-(1-phenylallyl)benzoate (440 mg, 1.64 mmol) in THF (3 mL) at room temperature was treated with methylamine (48% w/w in water, 1 mL) and the resulting mixture was stirred at this temperature for 8 h then was concentrated *in vacuo.* The residue was partitioned between CH_2Cl_2 and water and the layers were separated. The organic phase was dried using a phase separator and concentrated in vacuo to give 2-hydroxy-N-methyl-3-(1-phenylallyl)benzamide (400 mg, 91%) as a yellow solid which was used in the next step without further purification.

LCMS (method high pH): Retention time 1.25 min, $[M+H]^+ = 268.3$

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 12.72 (s, 1H) 7.18-7.36 (m, 8H), 6.82 (t, J = 7.8 Hz, 1H), 6.24-6.38 (m, 2H), 5.22-5.27 (m, 1H), 4.97 (dt, J = 17.1, 1.5 Hz, 1H), 3.02 (d, J = 4.9 Hz, 3H).

Compounds 4,5 and 6



4 **5** **6**

Chemical Formula: $C_{17}H_{17}NO_2$
Molecular Weight: 267.33

A solution of 2-hydroxy-N-methyl-3-(1-phenylallyl)benzamide (273 mg, 1.02 mmol) in trifluoroacetic acid (5 mL) was stirred at 80°C for 5 h then was cooled to room temperature and concentrated *in vacuo*. The residue obtained was purified by MDAP to give (2S,3S)-N,2-dimethyl-3-phenyl-2,3-dihydrobenzofuran-7-carboxamide (44 mg, 16%), (2S,3R)-N,2-dimethyl-3-phenyl-2,3-dihydrobenzofuran-7-carboxamide (16 mg, 6%) and (2R,3R)-N,3-dimethyl-2-phenyl-2,3-dihydrobenzofuran-7-carboxamide (30 mg, 11%) as white foams.

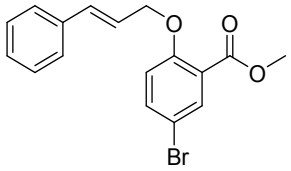
Cpd 4: 1H NMR (600 MHz, $CDCl_3$) δ ppm 7.99 (dd, *J* = 7.9, 0.7 Hz, 1H), 7.67 (br d, *J* = 4.2 Hz, 1H), 7.25 - 7.29 (m, 2H), 7.21 - 7.25 (m, 1H), 7.16 (dt, *J* = 7.3, 1.1 Hz, 1H), 6.97 (t, *J* = 7.5 Hz, 1H), 6.92 - 6.95 (m, 2H), 5.21 (dq, *J* = 8.6, 6.6 Hz, 1H), 4.58 (d, *J* = 8.6 Hz, 1H), 3.02 (d, *J* = 5.0 Hz, 3H), 1.09 (d, *J* = 6.6 Hz, 3H).
LCMS (method high pH): Retention time 1.11 min, $[M+H]^+$ = 268.2

Cpd 5: 1H NMR (600 MHz, $CDCl_3$) δ ppm 8.07 (dt, *J* = 7.9, 1.1 Hz, 1H), 7.68 (br s, 1H), 7.40 - 7.44 (m, 2H), 7.35 -7.39 (m, 1H), 7.24 - 7.29 (m, 2H), 7.12 (dt, *J* = 7.3, 1.3 Hz, H), 7.01 - 7.05 (m, 1H), 4.90 (dq, *J* = 8.3, 6.2 Hz, 1H), 4.29 (d, *J* = 8.4 Hz, 1H), 3.11 (d, *J* = 5.0 Hz, 3H), .69 (d, *J* = 6.2 Hz, 3H).
LCMS (method high pH): Retention time 1.13 min, $[M+H]^+$ = 268.2

Cpd 6: 1H NMR (600 MHz, $CDCl_3$) δ ppm 8.03 (d, *J* = 8.1 Hz, 1H), 7.57 - 7.63 (m, 1H), 7.42 (d, *J* = 2.0 Hz, 2H), 7.42 - 7.44 (m, 2H), 7.39 - 7.42 (m, 1H), 7.25 - 7.28 (m, 1H), 7.05 (t, *J* = 7.5 Hz, 1H), 5.30 (d, *J* = 8.6 Hz, 1H), 3.48 (dq, *J* = 8.1, 7.2 Hz, 1H), 2.98 (d, *J* = 5.0 Hz, 3H), 1.46 (d, *J* = 7.0 Hz, 3H).
LCMS (method high pH): Retention time 1.14 min, $[M+H]^+$ = 268.2

Compound 9

Step 1: Methyl 5-bromo-2-(cinnamyloxy)benzoate



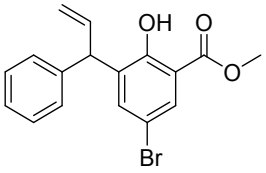
Chemical Formula: $C_{17}H_{15}BrO_3$
Exact Mass: 346.02

A flask was charged with methyl 5-bromo-2-hydroxybenzoate (52.3 g, 226 mmol), K_2CO_3 (50.1 g, 362 mmol), KI (1.88 g, 11.3 mmol) and then was filled with acetone (500 mL). The resulting suspension was treated with (E)-(3-chloroprop-1-en-1-yl)benzene (26.2 mL, 272 mmol) before being stirred at reflux for 6 h. At this stage, K_2CO_3 (20.0 g, 145 mmol) and (E)-(3-chloroprop-1-en-1-yl)benzene (12.0 mL, 124 mmol) were added and the mixture was refluxed for 8 h then was cooled to room temperature. The solid residue was filtered off and washed with acetone. The combined organics were concentrated *in vacuo*.

The solid residue filtered off was partitioned between water and EtOAc and the layers were separated. EtOAc obtained was used to dissolve the residue from the mother liquors (acetone). This organic phase was washed with water then brine, dried over $MgSO_4$ and concentrated *in vacuo* to give a solid residue which was dried under house vacuum at 40^0C for 16 h to give methyl 5-bromo-2-(cinnamyloxy)benzoate (80 g, 102%) as a yellow solid which was used in the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.95 (d, *J* = 2.7 Hz, 1H), 7.56 (dd, *J* = 2.4, 8.8 Hz, 1H), 7.39-7.46 (m, 2H), 7.25-7.38 (m, 3H), 6.94 (d, *J* = 9.0 Hz, 1H), 6.81 (d, *J* = 16.1 Hz, 1H), 6.41 (td, *J* = 5.5, 16.1 Hz, 1H), 4.80 (d, *J* = 5.5 Hz, 2H), 3.93 (s, 3H).
LCMS (method high pH): Retention time 1.42 min, $[M+H]^+$ = 346 and 348 (1 Br)

Step 2: Methyl 5-bromo-2-hydroxy-3-(1-phenylallyl)benzoate



Chemical Formula: $C_{17}H_{15}BrO_3$
Exact Mass: 346.02

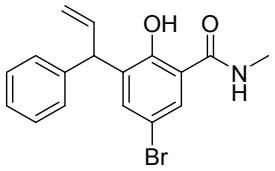
Two flasks were charged each with methyl 5-bromo-2-(cinnamyloxy)benzoate (39.5 g, 114 mmol) then filled with N,N-dimethylaniline (250 mL). The resulting solutions were stirred at reflux for 3 h then were cooled to room temperature and combined. The resulting mixture was added to a ice cold mixture of Et_2O (1000 mL) and 24-26% w/w HCl aqueous solution (900 mL). Once the addition was complete, EtOAc (500 mL) was added and the layers were separated. The aqueous phase was extracted twice with EtOAc. The combined organics were washed consecutively with a 2N HCl aqueous solution, water then brine, and then were dried over $MgSO_4$ and concentrated *in vacuo* to give crude methyl 5-bromo-2-

hydroxy-3-(1-phenylallyl)benzoate (72 g, 90%) as a yellow oil which was used in the next step without purification.

LCMS (method high pH): Retention time 1.57 min, $[M+H]^+$ = 346 and 348 (1 Br)

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 11.07 (s, 1H), 7.89 (d, *J* = 2.4 Hz, 1H), 7.44 (d, *J* = 2.4Hz, 1H), 7.30-7.36 (m, 2H), 7.19-7.27 (m, 3H), 6.28 (ddd, *J* = 17.1, 10.3, 6.8 Hz, 1H,) 5.29 (dt, *J* = 10.0, 1.3 Hz, 1H), 5.19 (d, *J* = 6.4 Hz, 1H), 4.98 (dt, *J* = 17.2, 1.7 Hz, 1H), 3.96 (s, 3H).

Step 3: 5-Bromo-2-hydroxy-N-methyl-3-(1-phenylallyl)benzamide



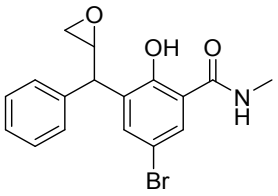
Chemical Formula: $C_{17}H_{16}BrNO_2$
Exact Mass: 345.04

A solution of methyl 5-bromo-2-hydroxy-3-(1-phenylallyl)benzoate (72.0 g, 207 mmol) in THF (250 mL) at room temperature was treated with methanamine (40% w/w in water, 90 mL, 1.0 mol) and the resulting mixture was stirred at this temperature for 16 h then was concentrated *in vacuo*. The residue was partitioned between CH_2Cl_2 and water and the layers were separated. The aqueous phase was extracted twice with CH_2Cl_2 and the combined organics were dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (780 g column, gradient (EtOAc in hexanes): 5% (2 CV), 5 to 35% (over 10 CV), 35% (5 CV)) gave 5-bromo-2-hydroxy-N-methyl-3-(1-phenylallyl)benzamide (44.6 g, 62%) as a brown foam.

LCMS (method high pH): Retention time 1.26 min, $[M+H]^+$ = 345 and 347 (1 Br)

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 12.64 (s, 1H), 7.29-7.37 (m, 4H), 7.20-7.27 (m, 3H), 6.19-6.33 (m, 2H), 5.28 (dt, *J* = 10.1, 1.5 Hz, 1H), 5.22 (d, *J* = 6.5 Hz, 1H), 4.98 (dt, *J* = 17.1, 1.5 Hz, 1H), 3.01 (d, *J* = 5.0 Hz, 3H).

Step 4: 5-Bromo-2-hydroxy-N-methyl-3-(oxiran-2-yl(phenyl)methyl)benzamide

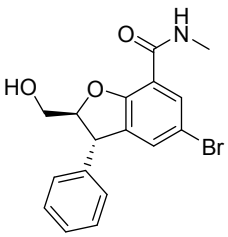


Chemical Formula: $C_{17}H_{16}BrNO_3$
Exact Mass: 361.03

A solution of 5-bromo-2-hydroxy-N-methyl-3-(1-phenylallyl)benzamide (36.6 g, 106 mmol) in CH_2Cl_2 (300 mL) at room temperature was treated with m-CPBA (< 77% w/w, 52.1 g, 211 mmol) and the resulting mixture was stirred at this temperature for 48 h. The mixture was then partitioned between CH_2Cl_2 and a mixture of saturated $NaHCO_3$ aqueous solution (200 mL) and sodium thiosulfate pentahydrate (52.5 g, 211 mmol) in water (100 mL). The biphasic mixture was vigorously stirred for 20 min then the layers were separated. The aqueous phase was extracted twice with CH_2Cl_2 and the combined organics were washed three times with a saturated $NaHCO_3$ aqueous solution, then with water, and then were dried over $MgSO_4$ and concentrated *in vacuo* to give 5-bromo-2-hydroxy-N-methyl-3-(oxiran-2-yl(phenyl)methyl)benzamide (1:1 mixture of diastereoisomers, 37.4 g, 98%) as a pale yellow foam which was used in the next step without purification.

LCMS (method high pH): Retention time 1.02 and 1.04 min, $[M+H]^+ = 362$ and 364 (1 Br)

Compound 10: (2S*,3S*)-5-Bromo-2-(hydroxymethyl)-N-methyl-3-phenyl-2,3-dihydrobenzofuran-7-carboxamide



Chemical Formula: $C_{17}H_{16}BrNO_3$
Exact Mass: 361.03

A solution of 5-bromo-2-hydroxy-N-methyl-3-(oxiran-2-yl(phenyl)methyl)benzamide (37.4 g, 103 mmol) in DMSO (150 mL) and water (40 mL) was cooled to 0°C using an ice bath then was treated with an ice-cooled solution of potassium hydroxide (11.6 g, 207 mmol) in water (40 mL). The resulting black solution was stirred at this temperature for 7 h then was left still in a freezer (-20°C) for 16 h. The mixture was then warmed to 0°C and stirred for a further 2 h before being treated with acetic acid (13.6 mL, 237 mmol). The mixture was then diluted with water and EtOAc and the layers were separated.

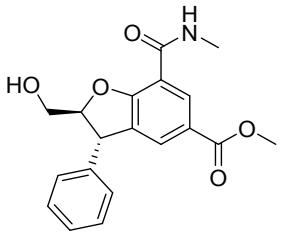
The aqueous phase was extracted three times with EtOAc and the combined organics were washed with water then brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue obtained was triturated with Et_2O and the precipitate formed was filtered off, rinsed with Et_2O and dried at 40°C under house vacuum for 2 h to give (2S*,3S*)-5-bromo-2-(hydroxymethyl)-N-methyl-3-phenyl-2,3-dihydrobenzofuran-7-carboxamide (25 g, 67%) as a white solid.

LCMS (method high pH): Retention time 1.04 min, $[M+H]^+$ = 362 and 364 (1 Br)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.96 (q, *J* = 4.6 Hz, 1H), 7.73 (dd, *J* = 0.7, 2.2 Hz, 1H), 7.15-7.42 (m, 6H), 5.50 (br. s., 1H), 4.82 (ddd, *J* = 3.5, 4.9, 7.1 Hz, 1H), 4.61 (d, *J* = 7.1 Hz, 1H), 3.63-3.82 (m, 2H), 2.86 (d, *J* = 4.6 Hz, 3H).

It was possible to separate the two enantiomers of compound **10**: **10** (2.4 g) was purified by chiral chromatography on a 30 mm x 25 cm Chiralcel OD-H (5um) column using 250 mg injections dissolved in 3 mL EtOH, eluting with 25% EtOH (+0.2% isopropylamine) / heptane (+0.2 %isopropylamine), with a flow rate of 30 mL/min, monitoring at 215 nm. Product-containing fractions were evaporated *in vacuo* to give the fast eluting enantiomer (2*R*, *R*)-5-bromo-2-(hydroxymethyl)-N-methyl-3-phenyl-2,3-dihydrobenzofuran-7-carboxamide (retention time: 5.49 min; 1.04 g, 43%) as a colourless solid and the slowest eluting enantiomer (2*S*,3*S*)-5-bromo-2-(hydroxymethyl)-N-methyl-3-phenyl-2,3-dihydrobenzofuran-7-carboxamide (retention time: 8.96 min, 1.00 g, 42%) as a colourless solid.

Compound 11: (2S,3S)-Methyl 2-(hydroxymethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylat



Chemical Formula: $C_{19}H_{19}NO_5$
Exact Mass: 341.13

(2S*,3S*)-5-bromo-2-(hydroxymethyl)-N-methyl-3-phenyl-2,3-dihydrobenzofuran-7-carboxamide (22.5 g, 62.1 mmol) was dissolved in a mixture of DMF (200 mL) and MeOH (100 mL), then Xantphos (3.6 g, 6.2 mmol) was added. The solution was degassed with nitrogen and $Pd(OAc)_2$ (1.4 g, 6.2 mmol)

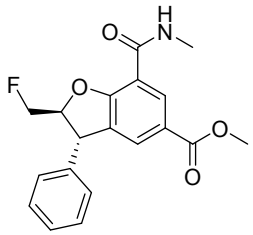
and Et_3N (26.0 mL, 186 mmol) were added. The mixture was purged with carbon monoxide, then a balloon full of carbon monoxide was fitted and the mixture heated at 70°C for 16 h. The mixture was cooled to room temperature, diluted with water (600 mL) and then was extracted with EtOAc (2 x 300 mL). The combined organics were washed with water (2 x 200 mL), dried over $MgSO_4$ and concentrated *in vacuo.* Purification of the residue by chromatography on silica gel (340 g column, gradient:0-25% EtOH in EtOAc) gave (2*S**,3*S**)-methyl 2-(hydroxymethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate (10.4 g, 49%) as a pale yellow solid.

Chiral purification could be performed also at this stage: (2*S**,3*S**)-methyl 2-(hydroxymethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate was purified using SFC: 3 g of material were dissolved in MeOH (60 mL) with heating. Injection: 1.0 mL via syringe pump, cycletime = 8 min. Runtime = 12 min. Column – Lux iCellulose-5 [Chiralpak IC5](250 x 21.2 mm, 5 micron. Solvent: MeOH with 30%(70%CO_2) cosolvent mixture. Flowrate of 75 ml/min. Detection UV at 220 nm. Column temp = 40°C. This gave methyl (2S,3S)-2-(hydroxymethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate (1.34 g, 45%).

LCMS (method formic): Retention time 0.89 min, $[M+H]^+ = 342$

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.34 (d, *J* = 1.2 Hz, 1H), 7.91 (q, *J* = 4.6 Hz, 1H), 7.54-7.58 (m, 1H), 7.24-7.41 (m, 5H), 5.26 (t, *J* = 6.1 Hz, 1H), 4.93 (ddd, *J* = 3.4, 4.6, 7.6 Hz, 1H), 4.65 (d, *J* = 7.6 Hz, 1H), 3.80-3.88 (m, 1H), 3.78 (s, 3H), 3.68-3.76 (m, 1H), 2.89 (d, *J* = 4.6 Hz, 3H).

Compound 12: (2S,3S)-Methyl 2-(fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate



Chemical Formula: $C_{19}H_{18}FNO_4$
Exact Mass: 343.12

A solution of (2S*,3S*)-methyl 2-(hydroxymethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate (3.10 g, 9.08 mmol) in CH_2Cl_2 (50 mL) was cooled under nitrogen

using an ice bath, then was treated with deoxofluor (8.40 mL, 22.7 mmol) dropwise. The resulting mixture was stirred at 0°C for 2 h, at 40°C for 18 h, then was cooled to room temperature. The mixture was diluted with CH_2Cl_2 (50 mL) and then cautiously added to a saturated $NaHCO_3$ aqueous solution (200 mL). The biphasic mixture was stirred for 30 min at room temperature and the layers were separated. The aqueous phase was extracted with CH_2Cl_2 (50 mL) and the combined organics were dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by chromatography on silica gel (50 g column, gradient: 0 to 100% EtOAc in hexane) gave (2S*,3S*)-methyl 2-(fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate (2.2 g, 71%) as a colourless solid.

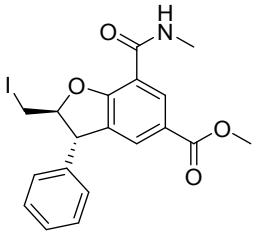
Chiral purification could be performed also at this stage: (2S*,3S*)-methyl 2-(fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate (6.5 g) was dissolved in 50% EtOH / heptane (96 mL) with heating. Injection: 4 mL of the solution was injected onto the column (50% EtOH / heptane, flow rate = 30 mL/min, detection wavelength = 215 nm, 4. Ref 550, 100, Column 30 mm x 25 cm Chiralcel OJ-H (5 μm). Total number of injections = 24. Fractions from 16.5-26 min were bulked and concentrated *in vacuo* to afford methyl (2S,3S)-2-(fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate (2.5 g, 38.5%).

LCMS (method high pH): Retention time 1.07 min, $[M+H]^+ = 344$

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.32 (d, *J* = 2.0 Hz, 1H), 7.93 (q, *J* = 4.6 Hz, 1H), 7.57-7.61 (m, 1H), 7.27-7.42 (m, 5H), 5.13-5.27 (m, 1H), 4.97 (dd, *J* = 2.4, 11.0 Hz, 1H), 4.80-4.89 (m, 1H), 4.66-4.76 (m, 1H), 3.79 (s, 3H), 2.88 (d, *J* = 4.6 Hz, 3H).

Compound 13:

Step 1: (2S,3S)-Methyl 2-(iodomethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate



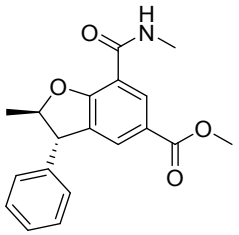
Chemical Formula: $C_{19}H_{18}INO_4$
Exact Mass: 451.03

A solution of iodine (1.84 g, 7.24 mmol) in CH_2Cl_2 (50 mL) under nitrogen was treated with triphenylphosphine (2.06 g, 7.85 mmol) and 1H-imidazole (0.534 g, 7.85 mmol). The resulting suspension was stirred 10 min at room temperature, then was treated with (2S,3S)-methyl 2-(hydroxymethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate (2.06 g, 6.03 mmol). The resulting suspension was stirred at room temperature for 2 h then was washed with water (100 mL), dried using a phase separator and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (40 g column, gradient: 0 to 100% EtOAc in hexane) gave (2S,3S)-methyl 2-(iodomethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate (2.2 g, 81%) as a colourless solid.

LCMS (method high pH): Retention time 1.23 min, $[M+H]^+ = 452$

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.77 (d, *J* = 1.2 Hz, 1H), 7.75-7.81 (m, 1H), 7.45 (d, *J* = 3.9 Hz, 1H), 7.31-7.41 (m, 3H), 7.16-7.22 (m, 2H), 4.88 (q, *J* = 5.9 Hz, 1H), 4.47 (d, *J* = 6.6 Hz, 1H), 3.86-3.89 (m, 3H), 3.52-3.63 (m, 2H), 3.09 (d, *J* = 4.6 Hz, 3H).

Step 2: (2R,3S)-Methyl 2-methyl-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate



Chemical Formula: $C_{19}H_{19}NO_4$
Exact Mass: 325.13

A solution of (2*S*,3*S*)-methyl 2-(iodomethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate (1.64 g, 3.63 mmol) in MeOH (50 mL), was treated with DIPEA (0.635 mL, 3.63 mmol) then with palladium on charcoal (10%w/w, 50% wet, 0.164 g) and the resulting mixture was stirred at

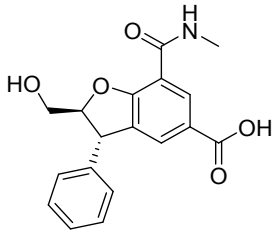
this temperature under a H_2 atmosphere (1 bar) for 24 h. The mixture was further treated with palladium on charcoal (10%w/w, 50% wet, 0.164 g) and stirred for 24 h under H_2 (1 bar). The catalyst was filtered off using a pad of celite (2.5 g) and rinsed with MeOH. The combined organics were concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (40 g column, gradient: 0 to 70% EtOAc in hexane) gave (2R,3S)-methyl 2-methyl-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate (1.06 g, 89%) as a white solid.

LCMS (method high pH): Retention time 1.15 min, $[M+H]^+ = 326$

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.34 (d, J = 2.0 Hz, 1H), 7.90 (d, J = 4.6 Hz, 1H), 7.53-7.56 (m, 1H), 7.36-7.42 (m, 2H), 7.31-7.35 (m, 1H), 7.25-7.29 (m, 2H), 5.02 (dd, J = 7.8, 6.1 Hz, 1H), 4.44 (d, J = 7.8 Hz, 1H), 3.79 (s, 3H), 2.87 (d, J = 4.6 Hz, 3H), 1.58 (d, J = 6.1 Hz, 3H).

Formation of the acid precursors:

(2S,3S)-2-(Hydroxymethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid



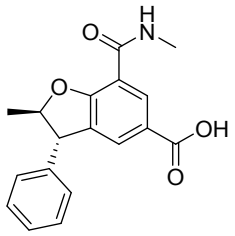
Chemical Formula: $C_{18}H_{17}NO_5$
Exact Mass: 327.11

A solution of (2S,3S)-methyl 2-(hydroxymethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate (1.34 g, 3.93 mmol) in MeOH (20 mL) at room temperature was treated with a 2N NaOH aqueous solution and the resulting mixture was stirred at this temperature for 24 h then most of the solvent was removed *in vacuo*. The solid residue was dissolved in a minimum amount of water (10 mL), and the solution was treated with a 2N HCl aqueous solution to pH 4, forming a thick white suspension. The solid was filtrated, washed with water and dried under house vacuum to give (2S,3S)-2-(hydroxymethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (1.21 g, 94%) which was used in the next step without further purification.

LCMS (method formic): Retention time 0.75 min, $[M+H]^+ = 328$

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.76 (s, 1H), 8.32 (d, J = 1.5 Hz, 1H), 7.89 (d, J = 4.6 Hz, 1H), 7.52-7.55 (m, 1H), 7.35-7.41 (m, 2H), 7.29-7.33 (m, 1H), 7.24-7.28 (m, 2H), 5.25 (t, J = 6.0 Hz, 1H), 4.92 (ddd, J = 7.6, 4.6, 3.4 Hz, 1H), 4.63 (d, J = 7.3 Hz, 1H), 3.79-3.86 (m, 1H), 3.69-3.77 (m, 1H), 2.88 (d, J = 4.6 Hz, 3H).

(2R,3S)-2-Methyl-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid



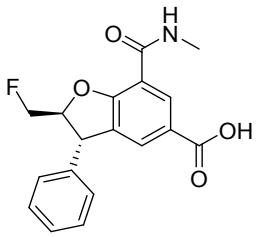
Chemical Formula: $C_{18}H_{17}NO_4$
Exact Mass: 311.12

A solution of (2*R*,3*S*)-methyl 2-methyl-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate (1.4 g, 4.3 mmol) in EtOH (20 mL) was treated at room temperature with a 2N NaOH aqueous solution (10 mL, 20 mmol) and the resulting mixture was stirred at this temperature for 16 h. The solvent was then evaporated to half volume, and the mixture was treated with a 2N HCl aqueous solution (11 mL, 22 mmol) and then was extracted with CH_2Cl_2 (2 x 30 mL). The combined organics were dried using a phase separator and concentrated *in vacuo* to give (2*R*,3*S*)-2-methyl-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (1.3 g, 97%) as a colourless solid.

LCMS (method high pH): Retention time 0.69 min, $[M+H]^+ = 312$

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.83 (d, J = 2.0 Hz, 1H), 7.78-7.82 (m, 1H), 7.50-7.57 (m, 1H), 7.30-7.42 (m, 3H), 7.17-7.23 (m, 2H), 4.97 (dq, J = 8.3, 6.2 Hz, 1H), 4.26 (d, J = 8.3 Hz, 1H), 3.75 (q, J = 7.0 Hz, 1H), 3.08 (d, J = 4.9 Hz, 3H), 1.67 (d, J = 6.1 Hz, 3H).

(2S,3S)-2-(Fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid



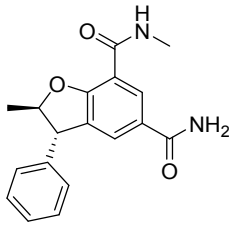
Chemical Formula: $C_{18}H_{16}FNO_4$
Exact Mass: 329.11

A solution of (2S,3S)-methyl 2-(fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylate (2.2 g, 6.4 mmol) in EtOH (20 mL) and THF (20 mL) at room temperature was treated with a 2N NaOH aqueous solution (10 mL, 20 mmol) and the resulting mixture was stirred at this temperature for 18 h, then was concentrated *in vacuo*. The residue was dissolved in water (50 mL), and the solution was then acidified with a 2N HCl aqueous solution to pH 2. The resulting mixture was extracted with CH_2Cl_2 (2 x 50 mL) and the combined organics were dried using a phase separator and concentrated *in vacuo* to give (2*S*,3*S*)-2-(fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (2.1 g, 100%) as a colourless solid, which was used in the next step without further purification.

LCMS (method formic): Retention time 0.91 min, $[M+H]^+ = 330$

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (q, *J* = 14.6 Hz, 1H), 7.82-7.85 (m, 1H), 7.31-7.48 (m, 4H), 7.17-7.24 (m, 2H), 5.01-5.12 (m, 1H), 4.87-4.93 (m, 0.5H), 4.73-4.81 (m, 1H), 4.65 (dd, *J* = 5.0, 10.9 Hz, 0.5H), 4.58 (d, *J* = 8.1 Hz, 1H), 3.09 (d, *J* = 4.6 Hz, 3H), OH not seen.

Compound 15: (2R,3S)-N^7,2-dimethyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide



Chemical Formula: $C_{18}H_{18}N_2O_3$
Exact Mass: 310.13

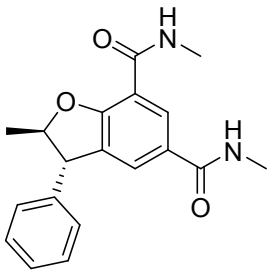
A solution of (2*R*,3*S*)-2-methyl-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (50 mg, 0.16 mmol) in CH_2Cl_2 (5 mL) at room temperature was treated with HATU (92 mg, 0.24 mmol) and Et_3N (0.045 mL, 0.32 mmol). The resulting mixture was stirred at this temperature for 1 h,

then was treated with 0.88 SG aqueous ammonium hydroxide (0.2 mL, 5.1 mmol). The resulting solution was stirred for a further 2 h then was concentrated *in vacuo*. Purification of the residue obtained by MDAP (method high pH) gave (2R,3S)-N^7,2-dimethyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (13 mg, 26%) as a colourless solid.

LCMS (method HpH): Retention time 0.89 min, $[M+H]^+ = 311$

^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.41 (d, *J* = 1.7 Hz, 1H), 7.63 (d, *J* = 1.2 Hz, 1H), 7.35 - 7.41 (m, 2H), 7.29 - 7.35 (m, 1H), 7.27 (d, *J* = 7.3 Hz, 2H), 4.97 - 5.06 (m, 1H), 4.37 (d, *J* = 8.1 Hz, 1H), 3.02 (s, 3H), 1.65 (d, *J* = 6.1 Hz, 3H).

Compound 17: (2R,3S)-N^5,N^7,2-Trimethyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide



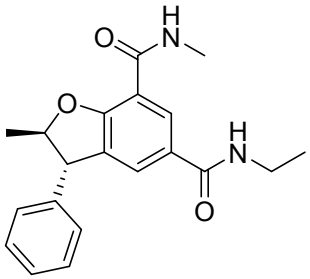
Chemical Formula: $C_{19}H_{20}N_2O_3$
Exact Mass: 324.15

A solution of (2*R*,3*S*)-2-methyl-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (50 mg, 0.16 mmol) in DMF (2 mL) at room temperature was treated with DIPEA (0.034 mL, 0.19 mmol), HATU (67.2 mg, 0.177 mmol) and methanamine (2N in THF, 0.2 mL, 0.4 mmol) and the resulting mixture was stirred at this temperature for 15 min then was treated with a 2N HCl aqueous solution (5 mL). The aqueous phase was extracted with EtOAc (20 mL) and the organic phase was washed with water (4 * 10 mL) then with a saturated LiCl aqueous solution followed by brine. The organic phase was then dried using a phase separator and concentrated *in vacuo* to give (2R,3S)-N^5,N^7,2-trimethyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (40 mg, 77%).

LCMS (method formic): Retention time 0.90 min, $[M+H]^+ = 325$

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.30 (d, *J* = 1.5 Hz, 1H), 7.73-7.76 (m, 1H), 7.60 (d, *J* = 4.2 Hz, 1H), 7.29-7.40 (m, 3H), 7.17-7.22 (m, 2H), 6.31 (d, *J* = 3.4 Hz, 1H), 4.96 (dq, *J* = 8.3, 6.3 Hz, 1H), 4.25 (d, *J* = 8.3 Hz, 1H), 3.08 (d, *J* = 4.6 Hz, 3H), 2.98 (d, *J* = 4.9 Hz, 3H), 1.66 (d, *J* = 6.1 Hz, 3H).

Compound 19: (2R,3S)-N^5-Ethyl-N^7,2-dimethyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide



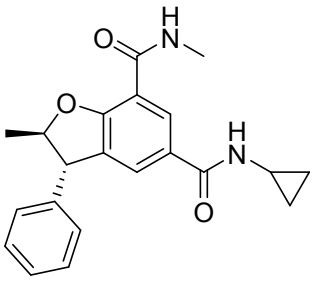
Chemical Formula: $C_{20}H_{22}N_2O_3$
Exact Mass: 338.16

A solution of (2S,3R)-2-methyl-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (50 mg, 0.16 mmol) in DMF (0.5 mL) at room temperature was treated with DIPEA (0.034 mL, 0.19 mmol), HATU (67.2 mg, 0.177 mmol) and ethanamine (2N in THF, 0.080 mL, 0.16 mmol) and the resulting mixture was stirred at this temperature for 15 min then was treated with a 2N HCl aqueous solution (5 mL). The aqueous phase was extracted with EtOAc (20 mL) and the organic phase was washed with water (4 * 10 mL) then with a saturated LiCl aqueous solution followed by brine. The organic phase was then dried using a phase separator and concentrated *in vacuo* to give (2S,3R)-N^5-ethyl-N^7,2-dimethyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (50 mg, 92%).

LCMS (method formic): Retention time 0.97 min, $[M+H]^+$ = 339

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.31 (d, *J* = 1.7 Hz, 1H), 7.74-7.77 (m, 1H), 7.59 (d, *J* = 4.2 Hz, 1H), 7.30-7.40 (m, 3H), 7.16-7.22 (m, 2H), 6.26 (br. s., 1H), 4.91-5.01 (m, 1H), 4.25 (d, *J* = 8.3 Hz, 1H), 3.42-3.50 (m, 2H), 3.08 (d, *J* = 4.6 Hz, 3H), 1.66 (d, *J* = 6.1 Hz, 3H), 1.21 - 1.27 (m, 3H).

Compound 21: (2R,3S)-N^5-Cyclopropyl-N^7,2-dimethyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide



Chemical Formula: $C_{21}H_{22}N_2O_3$
Exact Mass: 350.16

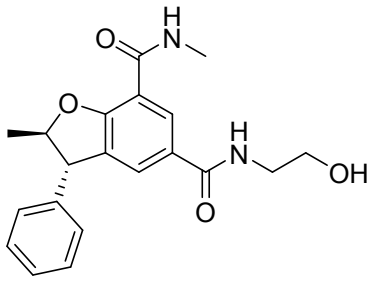
DIPEA (0.128 mL, 0.732 mmol), 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (278 mg, 0.732 mmol) and cyclopropylamine (0.052 mL, 0.73 mmol) were successively added to a solution of (2R*,3S*)-2-methyl-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (190 mg, 0.610 mmol) in DMF (2 mL). The mixture was concentrated *in vacuo* after 15 min. Purification of the residue obtained by flash chromatography on silica gel (10 g column, gradient: 0-50% EtOAc in cyclohexane) gave (2R*,3S*)-N^5-cyclopropyl-N^7,2-dimethyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (400 mg) contaminated with HATU. This material was then purified by chiral chromatography:

- Analytical method: Approximatively 0.5 mg of material was dissolved in 50% EtOH/heptane (1 mL), 20 uL injected on column. Elution: 10% EtOH in heptane, f = 1.0 mL/min,wavelength 215 nm. Column 4.6 mmid x 25cm Chiralpak IA.
- Preparative method: Approximatively 400 mg of material was dissolved in EtOH (4 mL). Injections (2 in total): 2 mL of the solution was injected onto the column. Elution:10% EtOH in heptane, f = 30 mL/min,wavelength, 215 nm. Column 30 mm x 25 cm Chiralpak IA (5 um). This gave (2R,3S)-N^5-Cyclopropyl-N^7,2-dimethyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide as the fastest eluting enantiomer (retention time: 16.25 min versus 28.10 min for slowest isomer; 91 mg, 23%).

LCMS (method high pH): Retention time 0.99 min, [M+H]+ = 351.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.24 (d, *J* = 1.5 Hz, 1H), 7.72 (s, 1H), 7.59 (d, *J* = 3.9 Hz, 1H), 7.29-7.40 (m, 3H), 7.16-7.21 (m, 2H), 6.40 (br. s., 1H), 4.96 (dd, *J* = 8.2, 6.2 Hz, 1H), 4.25 (d, *J* = 8.3 Hz, 1H), 3.07 (d, *J* = 4.9 Hz, 3H), 2.87 (td, *J* = 7.1, 3.2 Hz, 1H), 1.65 (d, *J* = 6.1 Hz, 3H), 0.80-0.88 (m, 2H), 0.58-0.65 (m, 2H).

Compound 23: (2R,3S)-N^5-(2-Hydroxyethyl)-N^7,2-dimethyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide



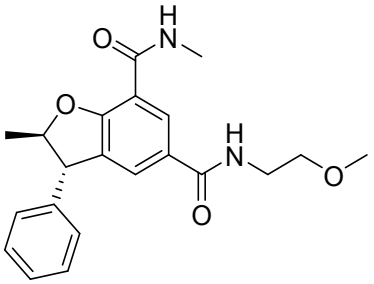
Chemical Formula: $C_{20}H_{22}N_2O_4$
Exact Mass: 354.16

A solution of (2R,3S)-2-methyl-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (30 mg, 0.096 mmol) in CH_2Cl_2 (5 mL) at room temperature was treated withHATU (55.0 mg, 0.145 mmol) and Et_3N (0.054 mL, 0.38 mmol) and the resulting mixture was stirred at this temperature for 1 h before being treated with 2-aminoethanol (11.8 mg, 0.193 mmol). The resulting mixture was stirred at this temperature for 2 h then was concentrated *in vacuo*. Purification of the residue obtained by MDAP (method high pH) gave (2R,3S)-N^5-(2-hydroxyethyl)-N^7,2-dimethyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (25 mg, 73%) as a colourless solid.

LCMS (method high pH): Retention time 0.87 min, $[M+H]^+ = 355$

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.37 (t, J = 5.5 Hz, 1H), 8.27 (d, J = 1.5 Hz, 1H), 7.86 (d, J = 4.6 Hz, 1H), 7.59-7.56 (m, 1H), 7.36-7.42 (m, 2H), 7.31-7.34 (m, 1H), 7.24-7.29 (m, 2H), 4.94 (dd, J = 7.8, 6.1 Hz, 1H), 4.64 (t, J = 5.6 Hz, 1H), 4.40 (d, J = 7.8 Hz, 1H), 3.46 (q, J = 6.1 Hz, 2H), 3.26 (q, J = 6.2 Hz, 2H), 2.87 (d, J = 4.6 Hz, 3H), 1.56 (d, J = 6.4 Hz, 3H).

Compound 25: (2R,3S)-N^5-(2-Methoxyethyl)-N^7,2-dimethyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide



Chemical Formula: $C_{21}H_{24}N_2O_4$
Exact Mass: 368.17

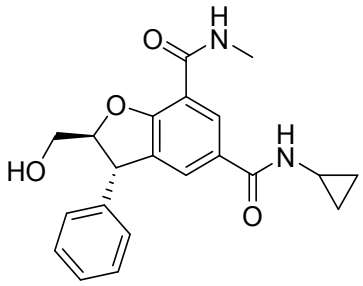
A solution of (2R,3S)-2-methyl-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (50 mg, 0.16 mmol) in CH_2Cl_2 (5 mL) at room temperature was treated withHATU (92 mg, 0.24 mmol) and Et_3N (0.045 mL, 0.32 mmol) and the resulting mixture was stirred at this temperature for 1 h before being treated with 2-methoxyethanamine (12.1 mg, 0.161 mmol). The resulting mixture was stirred at this temperature for 2 h then was concentrated *in vacuo*. Purification of the residue obtained by flash chromatography on silica gel (10 g column, 0-100% EtOAc/cyclohexane) gave (2R,3S)-N^5-(2-

methoxyethyl)-N^7,2-dimethyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (51 mg, 86%) as a colourless solid.

LCMS (method high pH): Retention time 0.97 min, $[M+H]^+ = 369$

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.34 (d, *J* = 1.5 Hz, 1H), 7.72-7.75 (m, 1H), 7.55-7.58 (m, 1H), 7.57 (d, *J* = 3.9 Hz, 1H), 7.29-7.40 (m, 2H), 7.17-7.22 (m, 2H), 6.56 (br. s., 1H), 4.96 (dd, *J* = 8.2, 6.2 Hz, 1H), 4.25 (d, *J* = 8.1 Hz, 1H), 3.59-3.64 (m, 2H), 3.52-3.56 (m, 2H), 3.08 (d, *J* = 4.9 Hz, 3H), 2.82 (s, 3H), 1.66 (d, *J* = 6.4 Hz, 3H).

Compound 26: (2S,3S)-N^5-Cyclopropyl-2-(hydroxymethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide



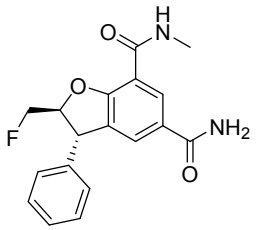
Chemical Formula: $C_{21}H_{22}N_2O_4$
Exact Mass: 366.16

A solution of (2*R*,3*R*)-2-(hydroxymethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (100 mg, 0.306 mmol) in DMF (2 mL) at room temperature was treated with DIPEA (0.064 mL, 0.37 mmol), HATU (139 mg, 0.367 mmol) and cyclopropylamine (0.043 mL, 0.61 mmol) and the resulting mixture was stirred at this temperature for 15 min then was concentrated *in vacuo*. Purification of the residue obtained by flash chromatography on silica gel (10 g column, gradient: 0-25% EtOH in EtOAc) gave the expected product contaminated with HATU. Further purification of this residue by flash chromatography on silica gel (10 g column, gradient: 0-25% EtOH in EtOAc) gave (2R,3R)-N^5-cyclopropyl-2-(hydroxymethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (14 mg, 13%).

LCMS (method formic): Retention time 0.77 min, $[M+H]^+ = 367$

^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.34 (d, *J* = 1.2 Hz, 1H), 7.56-7.58 (m, 1H), 7.35-7.41 (m, 2H), 7.29-7.34 (m, 1H), 7.24-7.28 (m, 2H), 4.94 (ddd, *J* = 8.2, 5.5, 2.9 Hz, 1H), 4.60 (d, *J* = 7.8 Hz, 1H), 4.00 (dd, *J* = 12.7, 2.9 Hz, 1H), 3.86 (dd, *J* = 12.6, 5.7 Hz, 1H), 3.01 (s, 3H), 2.76-2.82 (m, 1H), 0.73-0.79 (m, 2H), 0.57-0.63 (m, 2H).

Compound 27: (2S,3S)-2-(Fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide



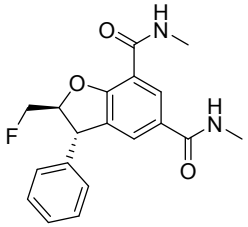
Chemical Formula: $C_{18}H_{17}FN_2O_3$
Exact Mass: 328.12

(2S*,3S*)2-(fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (200 mg, 0.607 mmol), HATU (254 mg, 0.668 mmol) and DIPEA (0.318 mL, 1.82 mmol) were stirred in DMF (4 mL) at room temperature for 5 min, NH_4Cl (97 mg, 1.8 mmol) was added and the reaction stirred at room temperature for 5 min. The reaction was diluted with 10% w/w citric acid aqueous solution and was extracted with EtOAc. The organic phase was washed with 10% w/w LiCl aqueous solution, dried using a hydrophobic frit and concentrated *in vacuo* to give a yellow solid. This solid was purified using silica gel column chromatography eluting with gradient of 0-12% EtOH:EtOAc to give (2S*,3S*))2-(fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (125 mg, 0.381 mmol, 63 % yield) as a white solid.

(2S*,3S*))-2-(fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (90 mg) was purified by chiral HPLC. The racemate was dissolved in EtOH (4 mL). Injection: 2 mL of the solution was injected onto the column; isocratic method 30% EtOH:heptane; flow rate = 30 mL/min, detection wavelength = 215 nm. Ref 550 nm, 100 nm, Column 30 mm x 25 cm Chiralpak AD-H (5 μm). Total number of injections = 2. Fractions from 12.5-14.5 min were bulked and concentrated *in vacuo* to afford (2S,3S)-2-(fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (27 mg, 30%) as a white solid.
LCMS (method formic): Rt = 0.80 min, [MH]+ = 329

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.26 (d, J = 1.7 Hz, 1H), 7.85-7.94 (m, 2H), 7.59-7.64 (m, 1H), 7.36-7.43 (m, 2H), 7.26-7.35 (m, 3H), 7.16 (br. s., 1H), 5.06-5.17 (m, 1H), 4.78-4.85 (m, 1H), 4.84 (m, J = 100 Hz, 1H[F]), 4.68 (d, J = 7.3 Hz, 1H), 2.87 (d, J = 4.6 Hz, 3H).

Compound 28: (2S,3S)-2-(Fluoromethyl)-N^5,N^7-dimethyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide



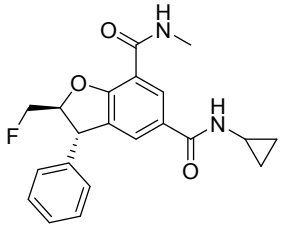
Chemical Formula: $C_{19}H_{19}FN_2O_3$
Exact Mass: 342.14

A solution of (2*S*,3*S*)-2-(fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (55 mg, 0.17 mmol) in DMF (3 mL) at room temperature was treated with Et_3N (0.047 mL, 0.33 mmol) and HATU (83 mg, 0.22 mmol) The resulting solution was stirred at this temperature for 30 min, then methanamine (2N in THF, 0.109 mL, 0.217 mmol) was added and the resulting mixture was stirred at room temperature for 2 h then was concentrated *in vacuo*. Purification of the residue by MDAP (method high pH) gave (2S,3S)-2-(fluoromethyl)-N^5,N^7-dimethyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (16 mg, 28%).

LCMS (method formic): Retention time 0.85 min, $[M+H]^+ = 343$

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.37 (d, J = 4.4 Hz, 1H), 8.23 (d, J = 1.5 Hz, 1H), 7.88 (d, J = 4.6 Hz, 1H), 7.58 (d, J = 1.2 Hz, 1H), 7.36-7.42 (m, 2H), 7.32-7.35 (m, 1H), 7.27-7.31 (m, 2H), 5.05-5.17 (m, 1H), 4.79-4.86 (m, 1H), 4.87 (m, J = 100 Hz, 1H[F]), 4.69 (d, J = 7.1 Hz, 1H), 2.88 (d, J = 4.6 Hz, 3H), 2.71 (d, J = 4.4 Hz, 3H).

Compound 29: (2S,3S)-N^5-Cyclopropyl-2-(fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide



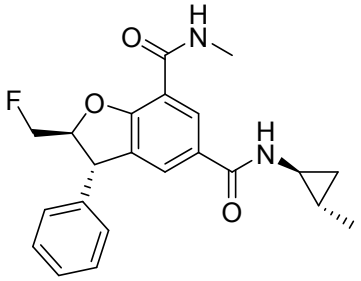
Chemical Formula: $C_{21}H_{21}FN_2O_3$
Exact Mass: 368.15

A solution of (2*S*,3*S*)-2-(fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (100 mg, 0.304 mmol) in DMF (3 mL) at room temperature was treated with Et_3N (0.085 mL, 0.61 mmol) and HATU (150 mg, 0.395 mmol). The mixture was stirred at this temperature for 30 min, then cyclopropylamine (0.028 mL, 0.40 mmol) was added and the resulting mixture was stirred at room temperature for 2 h then was concentrated *in vacuo*. Purification of the residue by MDAP (high pH method) gave (2S,3S)-N^5-cyclopropyl-2-(fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (30 mg, 27%) as a colourless solid.

LCMS (method formic): Retention time 0.94 min, $[M+H]^+ = 369$

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.26 (d, *J* = 1.5 Hz, 1H), 7.76-7.80 (m, 1H), 7.48 (d, *J* = 4.2 Hz, 1H), 7.31-7.42 (m, 2H), 7.18-7.23 (m, 2H), 6.37 (br. s., 1H), 5.00-5.11 (m, 1H), 4.88 (dd, *J* = 10.9, 2.6 Hz, 1H), 4.72-4.79 (m, 1H), 4.64 (dd, *J* = 10.8, 5.1 Hz, 1H), 4.55 (d, *J* = 8.1 Hz, 1H), 3.08 (d, *J* = 4.6 Hz, 3H), 2.89 (td, *J* = 7.1, 3.2 Hz, 1H), 0.82-0.89 (m, 2H), 0.58-0.65 (m, 2H).

Compound 30: (2S,3S)-2-(Fluoromethyl)-N^7-methyl-N^5-((1S,2S)-2-methylcyclopropyl)-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide



Chemical Formula: $C_{22}H_{23}FN_2O_3$
Exact Mass: 382.17

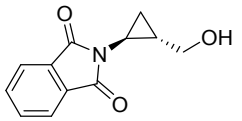
A solution of (2*S*,3*S*)-2-(fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (100 mg, 0.304 mmol) in DMF (3 mL) at room temperature was treated with Et_3N (0.085 mL, 0.61 mmol) and HATU (150 mg, 0.395 mmol). The resulting solution was stirred at this temperature for 30 min, then (1S,2S)-2-methylcyclopropanamine (commercially available from for example Enamine, 28.1 mg, 0.395 mmol) was added and the resulting mixture was stirred at room temperature for 2 h then was concentrated *in vacuo*. Purification of the residue by MDAP (method high pH) gave (2S,3S)-2-(fluoromethyl)-N^7-methyl-N^5-((1S,2S)-2-methylcyclopropyl)-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (39 mg, 34%) as a colourless solid.

LCMS (method formic): Retention time 1.00 min, $[M+H]^+$ = 383

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.36 (d, *J* = 4.2 Hz, 1H), 8.21 (d, *J* = 1.5 Hz, 1H), 7.88 (d, *J* = 4.4 Hz, 1H), 7.57 (s, 1H), 7.36-7.42 (m, 2H), 7.33 (d, *J* = 7.3 Hz, 1H), 7.26-7.31 (m, 2H), 5.04-5.16 (m, 1H), 4.78-4.85 (m, 1H), 4.85 (m, *J* = 100 Hz, 1H[F]), 4.67 (d, *J* = 7.1 Hz, 1H), 3.18 (d, *J* = 4.6 Hz, 1H), 2.87 (d, *J* = 4.6 Hz, 3H), 1.03 (d, *J* = 6.1 Hz, 3H), 0.89 (dtd, *J* = 9.0, 6.0, 6.0, 3.4 Hz, 1H), 0.68 (dt, *J* = 8.7, 4.6 Hz, 1H), 0.39 - 0.46 (m, 1H).

Compound 31:

Step 1: 2-((1S,2S)-2-(Hydroxymethyl)cyclopropyl)isoindoline-1,3-dione



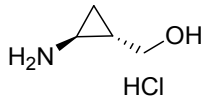
Chemical Formula: $C_{12}H_{11}NO_3$
Exact Mass: 217.07

((1S*,2S*)-2-Aminocyclopropyl)methanol (10 g, 115 mmol, commercially available from, for example, Enamine) was dissolved in toluene (150 mL), phthalic anhydride (22 g, 149 mmol) was added and the reaction heated at 110°C under nitrogen. The reaction was stirred for 5 h. The solution was then cooled to room temperature and partitioned between EtOAc (50 mL) and water (50 mL), and the layers were separated. The aqueous phase was extracted with EtOAc (2 x 50 mL), and the combined organics were washed with brine (60 mL), dried over a hydrophobic frit and concentrated *in vacuo* to give 34.0 g as a black oil. This was purified by chromatography on SiO_2 (Biotage SNAP 750 g, eluting with 0-100% ethyl acetate/cyclohexane). The desired fractions were concentrated *in vacuo* to give 26 g of a colourless oil. This was further purified by chromatography on SiO_2 (Biotage SNAP 750 g, eluting with 10-60% CH_2Cl_2/Et_2O). The desired fractions were concentrated *in vacuo* to give 19.5 g as a colourless oil. This was suspended in Et_2O (600 mL) and filtered under vacuum. The filtrate was concentrated to give 2-((1S*,2S*)-2-(hydroxymethyl)cyclopropyl)isoindoline-1,3-dione (16.4 g, 42%) as a colourless oil.

LCMS (method formic): Retention time 1.07 min, $[M+H]^+ = 218.2$

2-((1S*,2S*)-2-(hydroxymethyl)cyclopropyl)isoindoline-1,3-dione (16.4 g) was purified by chiral HPLC. The racemate was dissolved in EtOH (100 mL). Injection: 2.5 mL of the solution was injected onto the column (50% EtOH/Heptane, flow rate = 30 mL/min, detection wavelength = 215 nm, 4. Ref 550, 100, Column 30 mm x 25 cm Chiralpak AD-H (5 µm) Lot No ADH12143-01). Total number of injections = 40. Fractions from 12-14.5 min were bulked and labelled peak 1. Fractions from 19.5-26 min were bulked and labelled peak 2. The bulked fractions were concentrated *in vacuo* and then transferred to weighed flasks. The final compounds were recovered from CH_2Cl_2 and heptane in order to obtain a solid. The fractions corresponding to peak 1 (fastest eluant) were collected to afford 2-((1S,2S)-2-(hydroxymethyl)cyclopropyl)isoindoline-1,3-dione (5.74 g, 35%). The fractions corresponding to peak 2 were collected to afford the enantiomeric product (7.24 g, 44%).

Step 2: ((1S,2S)-2-Aminocyclopropyl)methanol hydrochloride



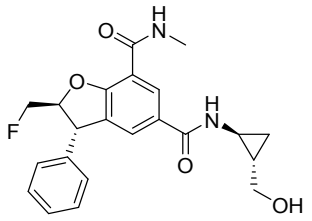
Chemical Formula: C_4H_9NO
Exact Mass: 87.07

Hydrazine hydrate (0.466 mL, 9.65 mmol, 65% wt.) was added slowly to a suspension of 2-((1*S*,2*S*)-2-(hydroxymethyl)cyclopropyl)isoindoline-1,3-dione (2.0 g, 9.21 mmol) in EtOH (46 mL). The reaction mixture was stirred at 50°C under nitrogen for 16 h then was cooled to room temperature. The resulting

white precipitate was filtered under vacuum. The filtrate was acidified with HCl (4N in dioxane, 57.5 mL, 230 mmol) and evaporated *in vacuo* to give the crude product. The residue was suspended in MeOH and purified by SPE on sulphonic acid (SCX) column using sequential solvents: MeOH followed by 2N ammonia in MeOH. The appropriate fractions were combined and acidified with HCl (4N in dioxane, 6 mL, 24 mmol), before evaporating *in vacuo* to yield a white slurry. Concerned that salt formation had not completed successfully, the residue was taken up in EtOH (30 mL) and treated with aqueous 2N HCl aqueous solution(10 mL) and evaporated *in vacuo* once more to yield a white slurry (1540 mg). The sample was dried *in vacuo* over 3 days to yield a white paste ((1*S*,2*S*)-2-aminocyclopropyl)methanol hydrochloride (1035 mg, 73%).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.40 (br. s., 3 H), 4.07-6.59 (obs., 1H), 3.36 (dd, J = 11.2, 5.9 Hz, 1H), 3.27 (dd, J = 10.8, 5.9 Hz, 1H), 2.37 (dsxt, J = 7.9, 4.2, 4.2, 4.2, 4.2, 4.2 Hz, 1H), 1.34-1.46 (m, 1H), 0.88 (ddd, J = 9.7, 5.6, 4.0 Hz, 1H), 0.65 (dt, J = 7.6, 6.0 Hz, 1H).

Step 3: (2S,3S)-2-(Fluoromethyl)-N^5-((1S,2S)-2-(hydroxymethyl)cyclopropyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide



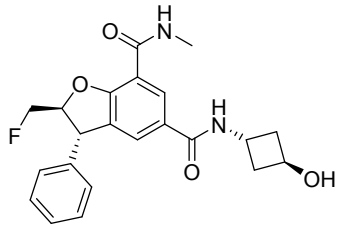
Chemical Formula: $C_{22}H_{23}FN_2O_4$
Exact Mass: 398.16

A solution of (2S,3S)-2-(fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (80 mg, 0.24 mmol) in DMF (5 mL) at room temperature was treated with ((1S,2S)-2-aminocyclopropyl)methanol hydrochloride (45.0 mg, 0.36 mmol), HATU (139 mg, 0.364 mmol) and DIPEA (0.127 mL, 0.729 mmol) and the resulting mixture was stirred at this temperature for 2 h then was concentrated *in vacuo*. The residue was taken up in EtOAc (10 mL) and the organic phase was washed with water then brine. The organic phase was dried over sodium sulphate, filtered through a hydrophobic frit and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (10 g column, gradient 5-100 % (3:1 EtOAc:EtOH) in cyclohexane) gave (2S,3S)-2-(fluoromethyl)-N^5-((1S,2S)-2-(hydroxymethyl)cyclopropyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (70 mg, 72%). The sample was purified by MDAP (formic method) to give (2S,3S)-2-(fluoromethyl)-N^5-((1S,2S)-2-(hydroxymethyl)cyclopropyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (49 mg, 45%) as a cream solid.

LCMS (method high pH): Rt = 0.83 min, $[MH]^+$ = 399

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.36 (d, *J* = 1.5 Hz, 1H), 7.78 (s, 1H), 7.53 (d, *J* = 4.6 Hz, 1H), 7.30-7.40 (m, 3H), 7.17-7.21 (m, 2H), 7.16 (br. s., 1H), 4.99-5.10 (m, 1H), 4.88 (dd, *J* = 10.8, 2.4 Hz, 1H), 4.71-4 .78 (m, 1H), 4.63 (dd, *J* = 10.8, 5.1 Hz, 1H), 4.54 (d, *J* = 8.1 Hz, 1H), 3.91 (dd, *J* = 11.0, 4.9 Hz, 1H), 3.08-3.14 (m, 1H), 3.06 (d, *J* = 4.9 Hz, 3H), 2.54-2.60 (m, 1H), 1.22-1.32 (m, 1H), 0.98 (ddd, *J* = 9.5, 5.4, 4.6 Hz, 1H), 0.80 - 0.87 (m, 1H).

Compound 32: (2S,3S)-2-(Fluoromethyl)-N^5-((1r,3S)-3-hydroxycyclobutyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide

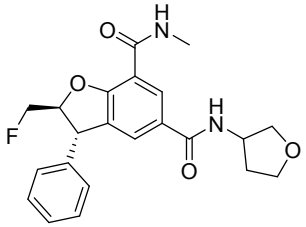


Chemical Formula: $C_{22}H_{23}FN_2O_4$
Exact Mass: 398.16

(2S,3S)-2-(Fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (50 mg, 0.15 mmol), HATU (69.3 mg, 0.182 mmol) and DIPEA (0.080 mL, 0.45 mmol) were stirred in DMF (2 mL) at room temperature for 5 min, (1r,3r)-3-aminocyclobutanol hydrochloride (22.5 mg, 0.182 mmol) was added and the reaction was stirred at room temperature for 30 min. The reaction was diluted with water and extracted with EtOAc, the organic phase was washed with 10% w/w LiCl aqueous solution, dried using a hydrophobic frit and concentrated *in vacuo* to give a white solid. This solid was purified using silica gel column chromatography eluting with a gradient of 0-100% (25% EtOH:EtOAc):CH_2Cl_2 to give (2S,3S)-2-(fluoromethyl)-N^5-((1r,3S)-3-hydroxycyclobutyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (52 mg, 86%) as a white solid.
LCMS (formic method): Retention time 0.82 min, $[M+H]^+$ = 399

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.60 (d, *J* = 6.8 Hz, 1H), 8.25 (d, *J* = 1.7 Hz, 1H), 7.95 (d, *J* = 4.9 Hz, 1H), 7.59-7.61 (m, 1H), 7.36-7.42 (m, 2H), 7.26-7.35 (m, 3H), 5.04-5.15 (m, 1H), 5.00 (d, *J* = 5.4 Hz, 1H), 4.84 (m, *J* = 100 Hz, 1H[F]), 4.66-4.69 (m, 1H), 4.34-4.40 (m, 1H), 4.24-4.30 (m, 1H), 3.39-3.48 (m, 1H), 2.87 (d, *J*=4.6 Hz, 3H),2.18-2.28 (m, 2H), 2.09 (ddd, *J* = 12.8, 8.2, 4.6 Hz, 2H).

Compound 33: (2S,3S)-2-(Fluoromethyl)-N^7-methyl-3-phenyl-N^5-(tetrahydrofuran-3-yl)-2,3-dihydrobenzofuran-5,7-dicarboxamide



Chemical Formula: $C_{22}H_{23}FN_2O_4$
Exact Mass: 398.16

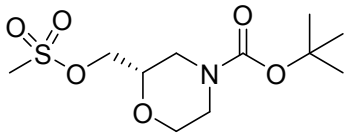
(2S,3S)-2-(Fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (50 mg, 0.15 mmol), HATU (69.3 mg, 0.182 mmol) and DIPEA (0.080 mL, 0.45 mmol) were dissolved in DMF (4 mL) with stirring at room temperature for 5 min. Tetrahydrofuran-3-amine (17.2 mg, 0.197 mmol) was dissolved in DMF (1mL) and added to the reaction mixture, which was then stirred at room temperature for 1 h. The reaction mixture was diluted with water, extracted with CH_2Cl_2 and brine was added. The organics were washed with 10% w/w LiCl aqueous solution and brine was added. The organic layers were dried via a hydrophobic frit and concentrated *in vacuo*. The residue was purified using silica gel column chromatography eluting with a gradient of 0-20% 2N NH_3 in MeOH: CH_2Cl_2 to give crude title compound. This was further purified using a MDAP (high pH method) to give (2S,3S)-2-(fluoromethyl)-N^7-methyl-3-phenyl-N^5-(tetrahydrofuran-3-yl)-2,3-dihydrobenzofuran-5,7-dicarboxamide (1:1 mix of diastereoisomers) (30 mg, 50%), as a white solid.

LCMS (2 min High pH): Retention time 0.90 min, [MH]+ = 399.

^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.38 (d, *J* = 1.5 Hz, 1H), 7.62-7.64 (m, 1H), 7.37-7.43 (m, 2H), 7.33-7.36 (m, 1H), 7.27-7.31 (m, 2H), 5.15 (td, *J* = 4.9, 2.2 Hz, 1H), 5.10 (td, *J* = 4.9, 2.2 Hz, 1H), 4.95 (dd, *J* = 10.9, 2.3 Hz, 1H), 4.76-4.86 (m, 1H), 4.65-4.73 (m, 2H), 4.53 (ddd, *J* = 7.9, 4.0, 2.0 Hz, 1H), 3.90-3.99 (m, 2H), 3.81 (td, *J* = 8.3, 5.9 Hz, 1H), 3.69 (dd, *J* = 9.2, 4.0 Hz, 1H), 3.03 (s, 3H), 2.21-2.31 (m, 1H), 1.92 - 2.01 (m, 1H).

compound 34:

Step 1: (S)-*tert*-Butyl 2-(((methylsulfonyl)oxy)methyl)morpholine-4-carboxylate



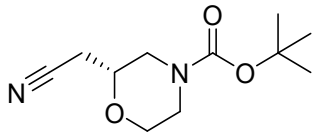
Chemical Formula: $C_{11}H_{21}NO_6S$
Exact Mass: 295.11

(*S*)-*tert*-Butyl 2-(hydroxymethyl)morpholine-4-carboxylate (commercially available from, for example, Activate Scientific) (3.00 g, 13.8 mmol) and NEt_3 (3.85 mL, 27.6 mmol) were stirred in CH_2Cl_2 (30 mL) at 0°C. Mesyl-Cl (1.614 mL, 20.71 mmol) was added portionwise over 5 min and the reaction was stirred at room temperature for 4 h. The reaction was then diluted with further CH_2Cl_2 and

was washed with a 1N HCl aqueous solution, a saturated $NaHCO_3$ aqueous solution and water, dried using a hydrophobic frit and concentrated *in vacuo* to give (S)-*tert*-butyl 2-(((methylsulfonyl)oxy)methyl)morpholine-4-carboxylate (4.24 g, 104%) as a yellow oil which was used in next step without further purification.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.13 - 4.35 (m, 2 H), 3.76 - 3.95 (m, 2 H), 3.71 (br. d, J = 13.2 Hz, 1 H), 3.62 (br. ddt, J = 10.6, 5.9, 3.1, 3.1 Hz, 1 H), 3.43 (td, J = 11.6, 2.7 Hz, 1 H), 3.14 - 3.31 (m, 3 H), 2.62 - 2.99 (m, 2 H), 1.31 - 1.52 (m, 9 H).

Step 2: (R)-*tert*-Butyl 2-(cyanomethyl)morpholine-4-carboxylate

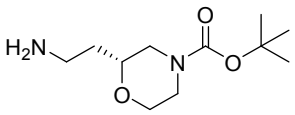


Chemical Formula: $C_{11}H_{18}N_2O_3$
Exact Mass: 226.13

(*S*)-*tert*-Butyl 2-(((methylsulfonyl)oxy)methyl)morpholine-4-carboxylate (4.2 g, 14 mmol), KCN (0.972 g, 14.9 mmol) and KI (3.54 g, 21.3 mmol) were stirred at 100^{o}C in DMSO (30 mL) for 4 h. The reaction was then cooled to room temperature, diluted with water and extracted with EtOAc. The organic layer was washed with water and brine, dried using a hydrophobic frit and concentrated *in vacuo* to give a yellow oil. This oil was purified by flash chromatography on silica gel (50 g column, gradient: 0-50% EtOAc:cyclohexane) to give (*R*)-*tert*-butyl 2-(cyanomethyl)morpholine-4-carboxylate (2.393 g, 74%) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.85 (br. dd, J = 11.5, 2.2 Hz, 2H), 3.70 (br. d, J = 13.2 Hz, 1H), 3.52-3.63 (m, 1H), 3.44 (td, J = 11.6, 2.9 Hz, 1H), 2.79-2.93 (m, 2H), 2.67-2.79 (m, 1H), 2.57 - 2.67 (m, 1H), 1.41 (s, 9H).

Step 3: (R)-*tert*-Butyl 2-(2-aminoethyl)morpholine-4-carboxylate



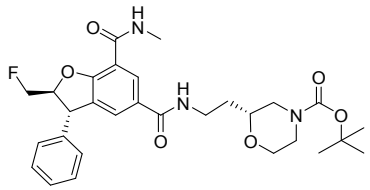
Chemical Formula: $C_{11}H_{22}N_2O_3$
Exact Mass: 230.16

(*R*)-*tert*-Butyl 2-(cyanomethyl)morpholine-4-carboxylate (2.39 g, 10.6 mmol) was taken up in THF (20 mL) and stirred at room temperature, borane tetrahydrofuran complex (1M in THF, 15.84 mL, 15.84 mmol) was added over 10 min and the reaction stirred at room temperature for 2 h. The reaction was quenched by the careful addition of MeOH until all effervesence had stopped. The reaction was concentrated *in vacuo* and the residue was dissolved in MeOH and the resulting solution was treated with 1M NaOH (50 mL) and stirred at room temperature for 2 h, a precipitate resulted. The reaction

was concentrated *in vacuo* to remove MeOH and was diluted with water and extracted with EtOAc. The combined organics were washed with water, dried using a hydrophobic frit and concentrated *in vacuo* to give the crude product as a colourless oil. This was further purified by flash chromatography on silica gel (50 g column, gradient: 0-8% 2N NH_3 in MeOH in CH_2Cl_2) to give (*R*)-*tert*-butyl 2-(2-aminoethyl)morpholine-4-carboxylate (965 mg, 40%) as a colourless oil.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.56 - 3.90 (m, 3H), 3.23 - 3.46 (m, 2H), 2.01 - 3.11 (obs m, 6H), 1.28 - 1.62 (m, 11H).

Step 4: (R)-*tert*-Butyl 2-(2-((2S,3S)-2-(fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxamido)ethyl)morpholine-4-carboxylate

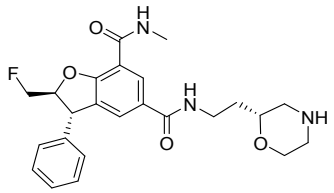


Chemical Formula: $C_{29}H_{36}FN_3O_6$
Exact Mass: 541.26

(2*S*,3*S*)-2-(Fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (55 mg, 0.17 mmol), HATU (76 mg, 0.20 mmol) and DIPEA (0.088 mL, 0.50 mmol) were dissolved in DMF (4 mL) and the resulting mixture was stirred at room temperature for 10 min before being treated with (R)-*tert*-butyl 2-(2-aminoethyl)morpholine-4-carboxylate (42.3 mg, 0.184 mmol) in DMF (1 mL). The resulting solution was stirred at this temperature for 1 h then was diluted with water (10 mL). The aqueous phase was extracted with EtOAc (3 x 30 mL) and the combined organics were washed twice with a 10% w/w LiCl aqueous solution, dried using a hydrophobic frit and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (10 g column, gradient: 0-100% EtOAc in cyclohexane) gave (R)-*ter*t-butyl 2-(2-((2S,3S)-2-(fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxamido)ethyl)morpholine-4-carboxylate (21 mg, 23%) as a colourless oil.

LCMS (method formic): Retention time 1.12 min, $[M+H]^+ = 542$

Step 5: (2S,3S)-2-(Fluoromethyl)-N^7-methyl-N^5-(2-((R)-morpholin-2-yl)ethyl)-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide



Chemical Formula: $C_{24}H_{28}FN_3O_4$
Exact Mass: 441.21

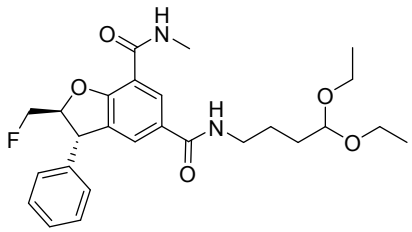
A solution of (R)-*tert*-butyl 2-(2-((2*S*,3*S*)-2-(fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxamido)ethyl)morpholine-4-carboxylate (21 mg, 0.039 mmol) in CH_2Cl_2 (5 mL) at room temperature was treated with trifluoroacetic acid (0.5 mL) and the resulting solution was stirred at this temperature for 1 h then was treated with a saturated $NaHCO_3$ aqueous solution (10 mL). The biphasic mixture was stirred 20 min at room temperature then was diluted with water and extracted with CH_2Cl_2 (3 x 20 mL). The combined organics were washed twice with a 10% w/w LiCl aqueous solution, dried via a hydrophobic frit and concentrated *in vacuo*. The residue was taken up in MeOH (3 mL) and eluted through a 500 mg NH_2 isolute column with MeOH (the column was prewashed with MeOH (~10 mL)). The relevant fractions were combined and concentrated *in vacuo* to give (2*S*,3*S*)-2-(fluoromethyl)-N^7-methyl-N^5-(2-((R)-morpholin-2-yl)ethyl)-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (6 mg, 35%) as an off white gum.

LCMS (method high pH): Retention time 0.84 min, $[M+H]^+ = 442$

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.33 (d, *J* = 1.5 Hz, 1H), 7.74-7.78 (m, 1H), 7.51 (d, *J* = 4.6 Hz, 1H), 7.30-7.41 (m, 3H), 7.26 (d, *J* = 4.6 Hz, 1H), 7.20 (dd, *J* = 8.1, 1.2 Hz, 2H), 4.97-5.10 (m, 1H), 4.72-4.79 (m, 1H), 4.76 (m, J = 100 Hz, 1H[F]), 4.54 (d, *J* = 8.3 Hz, 1H), 4.00 (dd, *J* = 11.5, 2.0 Hz, 1H), 3.55-3.72 (m, 3H), 3.36-3.46 (m, 1H), 3.07 (d, *J* = 4.9 Hz, 3H), 2.79-2.94 (m, 3H), 2.63 (dd, *J* = 12.1, 10.1 Hz, 1H), 1.62-1.77 (m, 2H). NH not seen.

Compound 35:

Step 1: (2S,3S)-N^5-(4,4-Diethoxybutyl)-2-(fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide

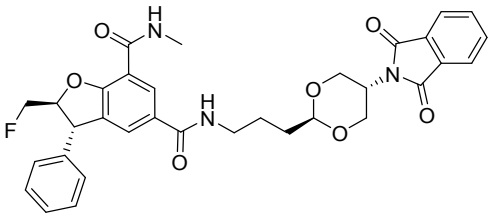


Chemical Formula: $C_{26}H_{33}FN_2O_5$
Exact Mass: 472.24

(2*S*,3*S*)-2-(Fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (200 mg, 0.607 mmol), HATU (277 mg, 0.729 mmol) and DIPEA (0.318 mL, 1.82 mmol) were dissolved in DMF (1 mL) and the resulting mixture was stirred at room temperature for 5 min. 4,4-Diethoxybutan-1-amine (0.109 mL, 0.607 mmol) was added and the resulting mixture was stirred at room temperature for 1.5 h. Further 4,4-diethoxybutan-1-amine (0.109 mL, 0.607 mmol) was added and the reaction was stirred for 5 min then left to stand overnight. It was then diluted in EtOAc and the

organic phase was washed with a 2% w/w citric acid aqueous solution, brine, and then with a saturated $NaHCO_3$ aqueous solution and concentrated *in vacuo*. The residue obtained was dissolved in CH_2Cl_2 (5 mL). The insolubles were filtered off, dissolved in MeOH (5 mL) and blown down overnight to give a first fraction of product. The CH_2Cl_2 filtrate was loaded onto a 25 g silica cartridge. Purification by flash chromatography on silica gel (20-100% EtOAc in cyclohexane) gave a second fraction of product. Both fractions were combined to give (2*S*,3*S*)-N^5-(4,4-diethoxybutyl)-2-(fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (158 mg, 55%) as a white solid.
LCMS (2 min high pH): Retention time 1.08 min, $[M-H]^-$ = 471

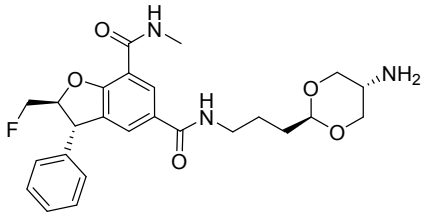
Step 2: (2S,3S)-N^5-(3-((2r,5S)-5-(1,3-Dioxoisoindolin-2-yl)-1,3-dioxan-2-yl)propyl)-2-(fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide



Chemical Formula: $C_{33}H_{32}FN_3O_7$
Exact Mass: 601.22

A suspension of (2*S*,3*S*)-N^5-(4,4-diethoxybutyl)-2-(fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (158 mg, 0.318 mmol), 2-(1,3-dihydroxypropan-2-yl)isoindoline-1,3-dione (70.3 mg, 0.318 mmol) and *p*-toluenesulfonic acid monohydrate (60.4 mg, 0.318 mmol) in toluene (6 mL) was stirred at 40°C for 1.5 h then at 70°C under N_2 for a further 4 h before being allowed to cool to room temperature and left to stand overnight. The solvent was then removed *in vacuo*. The residue obtained was partitioned between EtOAc and a 1N Na_2CO_3 aqueous solution and the layers were separated. The aqueous phase was extracted with EtOAc and the combined organics were dried using a hydrophobic frit. The filtrate was concentrated *in vacuo.* Purification of the residue by flash chromatography on silica gel (50 g column, gradient 70-100% EtOAc in cyclohexane) gave (2*S*,3*S*)-N^5-(3-(5-(1,3-dioxoisoindolin-2-yl)-1,3-dioxan-2-yl)propyl)-2-(fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (48 mg, 25%) as a yellow solid.
LCMS (method formic): Retention time 1.12 min, $[M+H]^+$ = 602

Step 3: (2S,3S)-N^5-(3-((2r,5S)-5-Amino-1,3-dioxan-2-yl)propyl)-2-(fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide



Chemical Formula: $C_{25}H_{30}FN_3O_5$
Exact Mass: 471.22

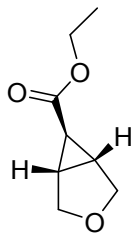
To a suspension of (2S,3S)-N^5-(3-((2r,5S)-5-(1,3-dioxoisoindolin-2-yl)-1,3-dioxan-2-yl)propyl)-2-(fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (48 mg, 0.080 mmol) in EtOH (2 mL) was added hydrazine hydrate (3.9 μL, 0.080 mmol) and the resulting suspension was stirred at 50°C for 23 h. Further EtOH (1 mL) was added and the reaction was left to stir at 50°C for a further 24 h. Hydrazine hydrate (3.9 μL, 0.080 mmol) was added and the reaction left to stir at 50°C over the weekend. Further hydrazine hydrate (39 μL, 0.80 mmol) were added to the reaction mixture and the temperature lowered to 40°C. The reaction mixture was stirred for 8 h then was allowed to cool to room temperature and left to stand overnight. The volatiles were evaporated under a stream of nitrogen. The residue was purified by MDAP (high pH) to give (2S,3S)-N^5-(3-((2r,5S)-5-Amino-1,3-dioxan-2-yl)propyl)-2-(fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (21.8 mg, 58%) as a beige solid.

LCMS (method formic): Retention time 0.63 min, $[M+H]^+ = 472$

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.39-8.43 (m, 1H), 8.23 (d, J = 1.7 Hz, 1H), 7.88 (d, J = 4.9 Hz, 1H), 7.59 (d, J = 1.2 Hz, 1H), 7.37-7.42 (m, 2H), 7.27-7.35 (m, 3H), 5.06-5.16 (m, 1H), 4.79-4.85 (m, 1H), 4.84 (d, J = 98 Hz, 1H[F]), 4.68 (d, J = 6.8 Hz, 1H), 4.35-4.39 (m, 1H), 3.90 (dd, J = 10.9, 4.8 Hz, 1H), 3.82-3.88 (m, 1H), 3.62 (t, J = 10.8 Hz, 1H), 3.10-3.23 (m, 4H), 2.88 (d, J = 4.6 Hz, 3H), 2.67 (t, J = 2.0 Hz, 1H), 2.31-2.35 (m, 1H), 1.54 (br. s., 1H), 1.24 (s, 1H).

Compound 36: (2S,3S)-N^5-((1R,5S,6r)-3-oxabicyclo[3.1.0]hexan-6-yl)-2-(fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide

Step 1: Ethyl (1R,5S,6r)-3-oxabicyclo[3.1.0]hexane-6-carboxylate



Chemical Formula: $C_8H_{12}O_3$
Exact Mass: 156.08

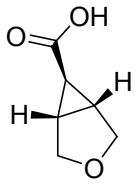
Ethyl diazoacetate (8.6 g, 76 mmol) in CH_2Cl_2 (200 mL) was added at room temperature dropwise over 4 h to a solution of 2,5-dihydrofuran (5.3 g, 76 mmol) and rhodium(II) acetate dimer (1.67 g, 3.78

mmol) in CH_2Cl_2 (400 mL) under nitrogen. Once the addition was complete, the mixture was stirred at this temperature for 18 h then was filtered through celite and concentrated *in vacuo* to give a dark green oil. Purification of this residue by flash chromatography on silica gel (110 g column, gradient: 0 to 30% EtOAc in hexanes) gave ethyl (1R,5S,6r)-3-oxabicyclo[3.1.0]hexane-6-carboxylate (3.2 g, 27%) as a colourless liquid.

LCMS (method formic): Retention time 0.75 min, $[M+H]^+ = 157$

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.14 (q, J = 7.3 Hz, 2H), 3.93 (d, J = 8.8 Hz, 2H), 3.75 (d, J = 8.3 Hz, 2H), 2.14-2.18 (m, 2H), 1.58-1.63 (m, 1H), 1.25-1.29 (m, 3H).

Step 2: (1R,5S,6r)-3-oxabicyclo[3.1.0]hexane-6-carboxylic acid

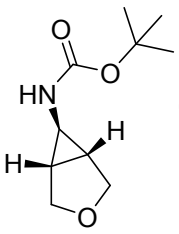


Chemical Formula: $C_6H_8O_3$
Exact Mass: 128.05

A solution of (1R,5S,6r)-ethyl 3-oxabicyclo[3.1.0]hexane-6-carboxylate (5.0 g, 32 mmol) in EtOH (50 mL) was treated at room temperature with a 2N NaOH aqueous solution (48 mL, 96 mmol) and the resulting mixture was stirred at this temperature for 18 h, then concentrated to half volume *in vacuo*. The resulting solution was washed with Et_2O (50 mL), then acidified with a 2N HCl aqueous solution to pH 4 and extracted with EtOAc (3 x 100 mL). The combined organics were dried over $MgSO_4$ and concentrated *in vacuo* to give (1R,5S,6r)-3-oxabicyclo[3.1.0]hexane-6-carboxylic acid (3.9 g, 95%) as a colourless solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.19 (s, 1H), 3.81 (d, J = 8.8 Hz, 2H), 3.62 (d, J = 8.8 Hz, 2H), 2.08 (dd, J = 2.7, 1.7 Hz, 2H), 1.32 (t, J = 3.2 Hz, 1H).

Step 3: *tert*-Butyl (1R,5S,6r)-3-oxabicyclo[3.1.0]hexan-6-ylcarbamate

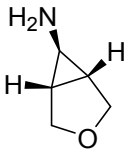


Chemical Formula: $C_{10}H_{17}NO_3$
Exact Mass: 199.12

A suspension of (1R,5S,6r)-3-oxabicyclo[3.1.0]hexane-6-carboxylic acid (3.90 g, 30.4 mmol) in toluene (20 mL) at room temperature was treated with NEt_3 (12.7 mL, 91.0 mmol) and diphenyl phosphorazidate (7.85 mL, 36.5 mmol) and the resulting mixture was stirred for 20 min at this temperature then *tert*-butanol (20.0 mL, 209 mmol) was added. The solution was then heated at reflux for 5 h before being cooled to room temperature. The mixture was diluted with EtOAc (50 mL) and the organic phase was washed with water (50 mL), then with a saturated $NaHCO_3$ solution (50 mL) before being dried over $MgSO_4$ and concentrated *in vacuo* to give *tert*-butyl (1R,5S,6r)-3-oxabicyclo[3.1.0]hexan-6-ylcarbamate (3.4g, 56%) as a beige crystalline solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 6.99 (br s, 1H), 3.77 (d, J = 8.3 Hz, 2H), 3.58 (d, J = 8.3 Hz, 2H), 2.18 (br s, 1H), 1.70 (td, J = 2.1, 1.3 Hz, 2H), 1.39 (s, 9H),

Step 4: (1R,5S,6r)-3-Oxabicyclo[3.1.0]hexan-6-amine

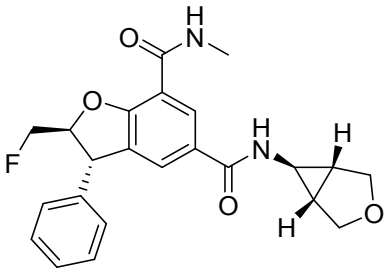


Chemical Formula: C_5H_9NO
Exact Mass: 99.07

A solution of *tert*-butyl (1R,5S,6r)-3-oxabicyclo[3.1.0]hexan-6-ylcarbamate (3.40 g, 17.1 mmol) in CH_2Cl_2 (20 mL) at room temperature was treated with a 4N HCl solution in dioxane (12.8 mL, 51.2 mmol) and the resulting mixture was stirred for 2 h at this temperature then the solvent was evaporated *in vacuo*. The residue was dissolved in MeOH and loaded onto a 50 g SCX cartridge, which was washed with MeOH (100 mL), then eluted with 2N NH_3 in MeOH. The eluant was evaporated *in vacuo* to give (1R,5S,6r)-3-oxabicyclo[3.1.0]hexan-6-amine (1.6 g, 95%) as a pale yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.86 (d, *J* = 8.3 Hz, 2H), 3.68 (d, *J* = 8.3 Hz, 2H), 2.22 (t, *J* = 2.0 Hz, 1H), 1.56 - 1.64 (m, 2H), 1.48 (br s, 2H).

Step 5: (2S,3S)-N^5-((1R,5S,6r)-3-oxabicyclo[3.1.0]hexan-6-yl)-2-(fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide



Chemical Formula: $C_{23}H_{23}FN_2O_4$
Exact Mass: 410.16

(2*S*,3*S*)-2-(Fluoromethyl)-7-(methylcarbamoyl)-3-phenyl-2,3-dihydrobenzofuran-5-carboxylic acid (1.9 g, 5.8 mmol) (1R,5S,6r)-3-oxabicyclo[3.1.0]hexan-6-amine hydrochloride (1.02 g, 7.50 mmol), HATU (2.85 g, 7.50 mmol) and Et_3N (2.010 mL, 14.42 mmol) were disolved in CH_2Cl_2 (20 mL) and the resulting mixture was stirred at room temperature for 16 h. The organic phase was then washed successively with a 0.5N HCl aqueous solution (20 mL), a 1N NaOH aqueous solution (20 mL) and brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo* to give a pale yellow gum. Purification of this residue by flash chromatography on silica gel (column 100 g, gradient : 0 to 80% (25%EtOH/EtOAc) in cyclohexane) gave (2S,3S)-N^5-((1R,5S,6r)-3-oxabicyclo[3.1.0]hexan-6-yl)-2-(fluoromethyl)-N^7-methyl-3-phenyl-2,3-dihydrobenzofuran-5,7-dicarboxamide (1.90 g, 80%) as a colourless solid.

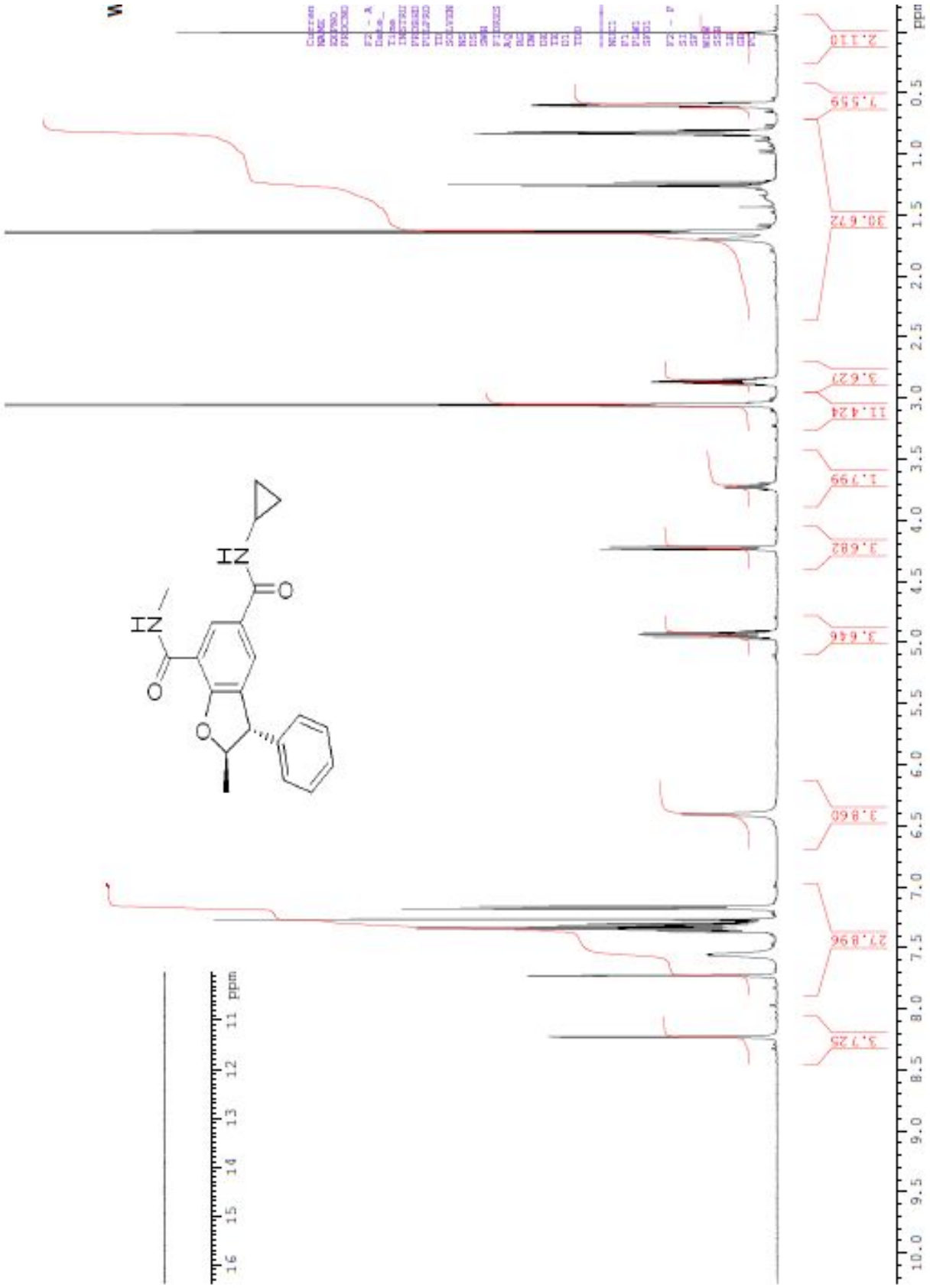
LCMS (method formic): Retention time 0.88 min, $[M+H]^+ = 411$

HRMS: calculated monoisotopic mass for $C_{23}H_{24}FN_2O_4$: calculated: 411.1720; measured: 411.17171

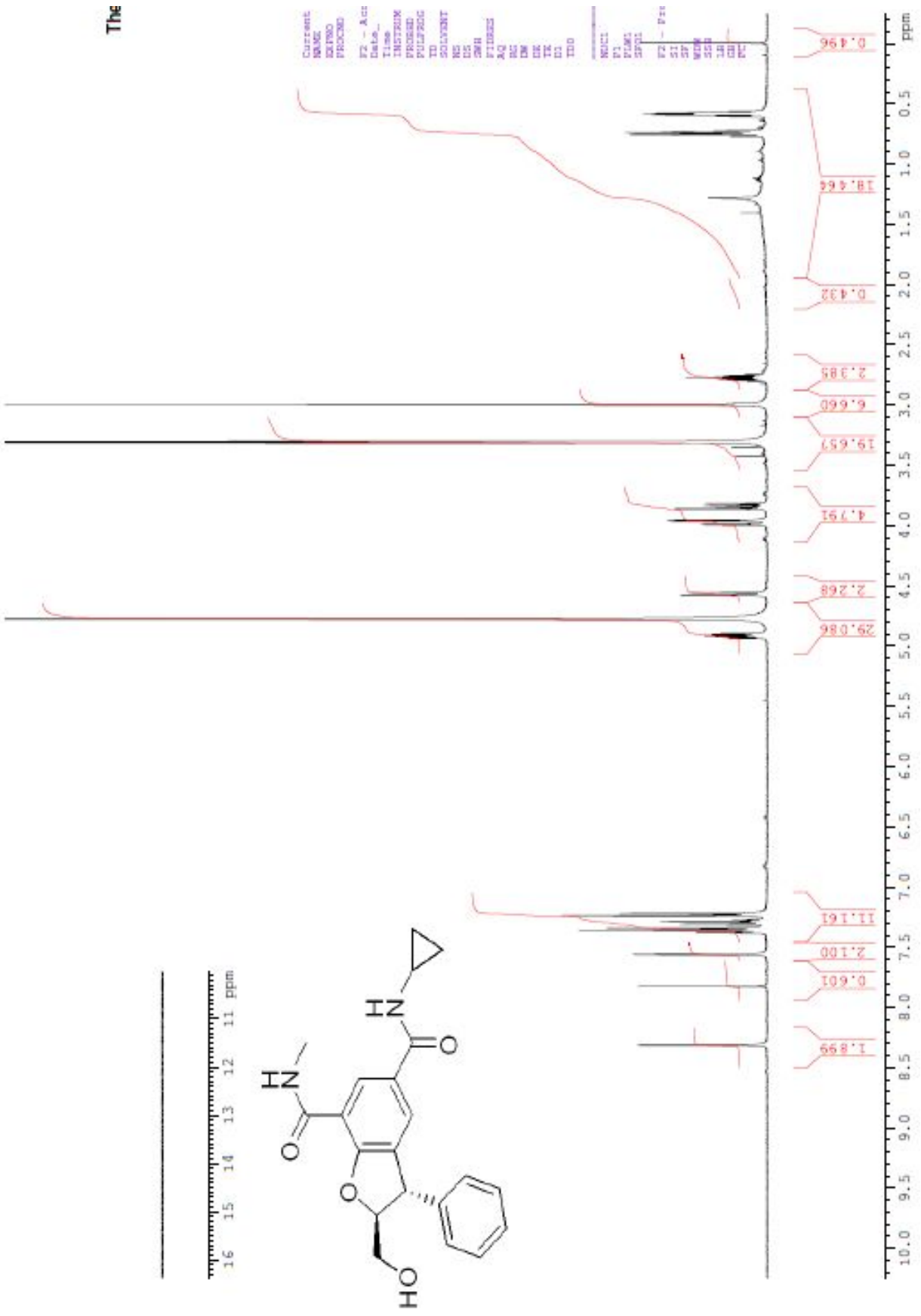
^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.44 (d, *J* = 4.0 Hz, 1H), 8.22 (d, *J* = 2.0 Hz, 1H), 7.89 (q, *J* = 4.5 Hz, 1H), 7.59-7.57 (m, 1H), 7.42-7.36 (m, 2H), 7.35-7.30 (m, 1H), 7.30-7.26 (m, 2H), 5.17-5.04 (m, 1H), 4.97-4.80 (m, 1H), 4.85-4.67 (m, 1H), 4.68 (d, *J* = 7.5 Hz, 1H), 3.82 (d, *J* = 8.5 Hz, 2H), 3.61 (dd, *J* = 3.0, 8.5 Hz, 2H), 2.87 (d, *J* = 4.5 Hz, 3H), 2.58-2.53 (m, 1H), 1.89-1.80 (m, 2H).

^{13}C (151 MHz, DMSO-d_6) δ ppm 23.9 (2C), 26.3 (1C), 30.9 (1C), 47.2 (d, J = 6.4 Hz, 1C), 68.6 (m, 2C), 82.9 (d, J = 171.4 Hz, 1C), 90.7 (d, J = 17.7 Hz, 1C), 116.1 (1C), 126.6 (1 C), 127.4 (1C), 127.5 (1C), 127.9 (2C), 128.7 (1C), 129.0 (2C), 132.0 (1C), 141.3 (1C), 158.4 (1C), 163.7 (1C), 165.7 (1C).

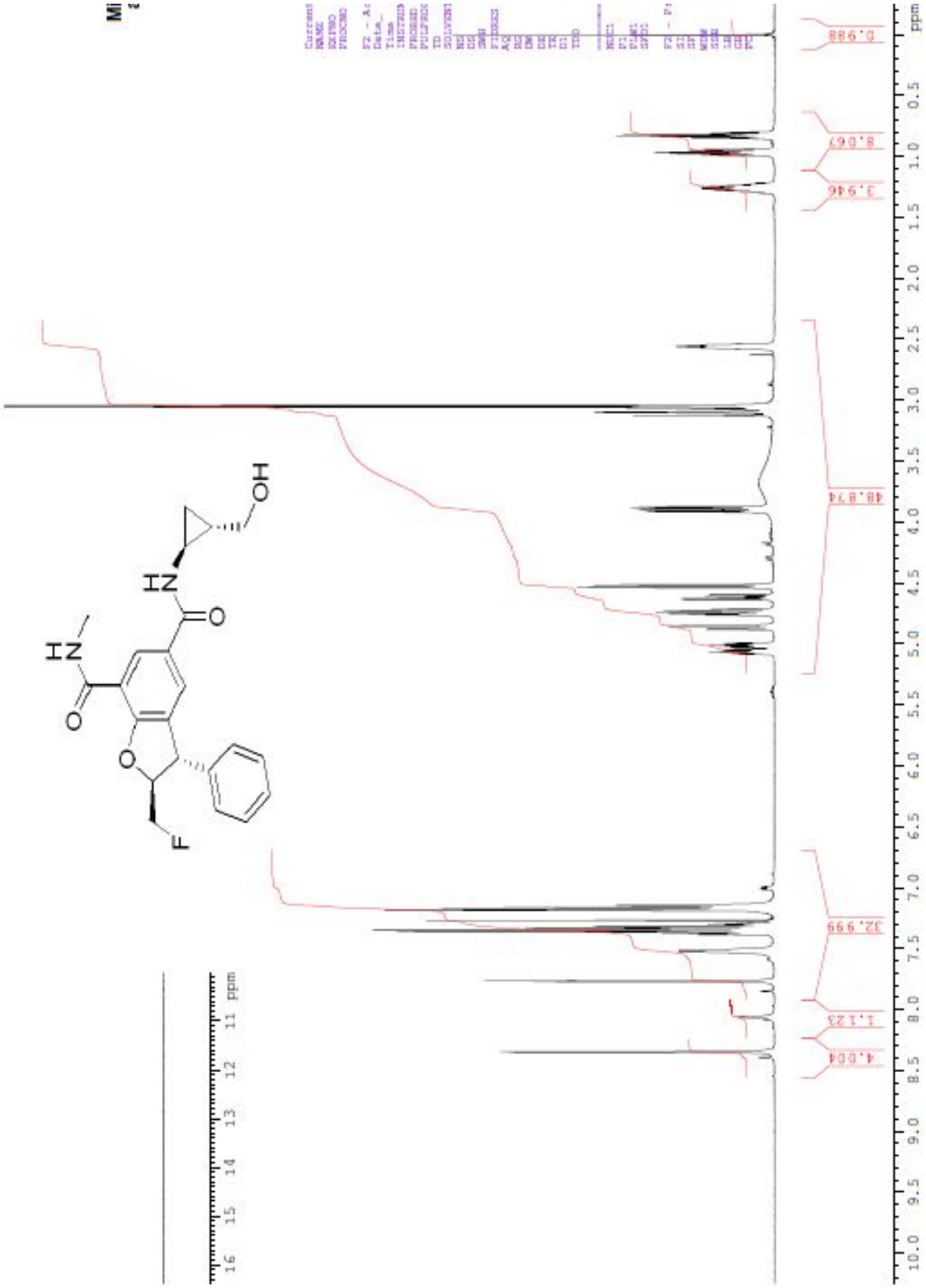
^{1}H NMR Compound **21** in $CDCl_3$ (400 MHz):



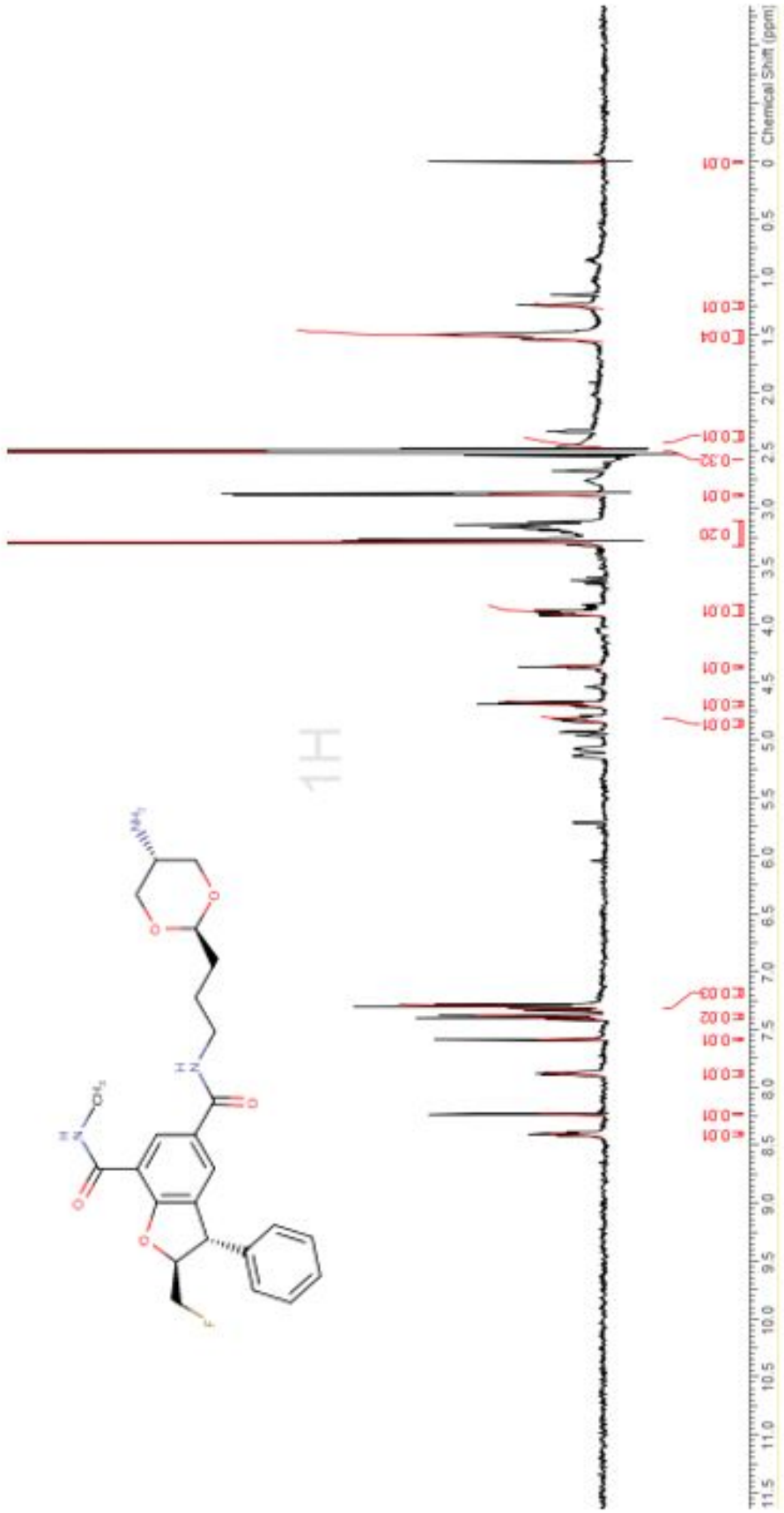
^{1}H NMR Compound **26** in CD_3OD (400 MHz):



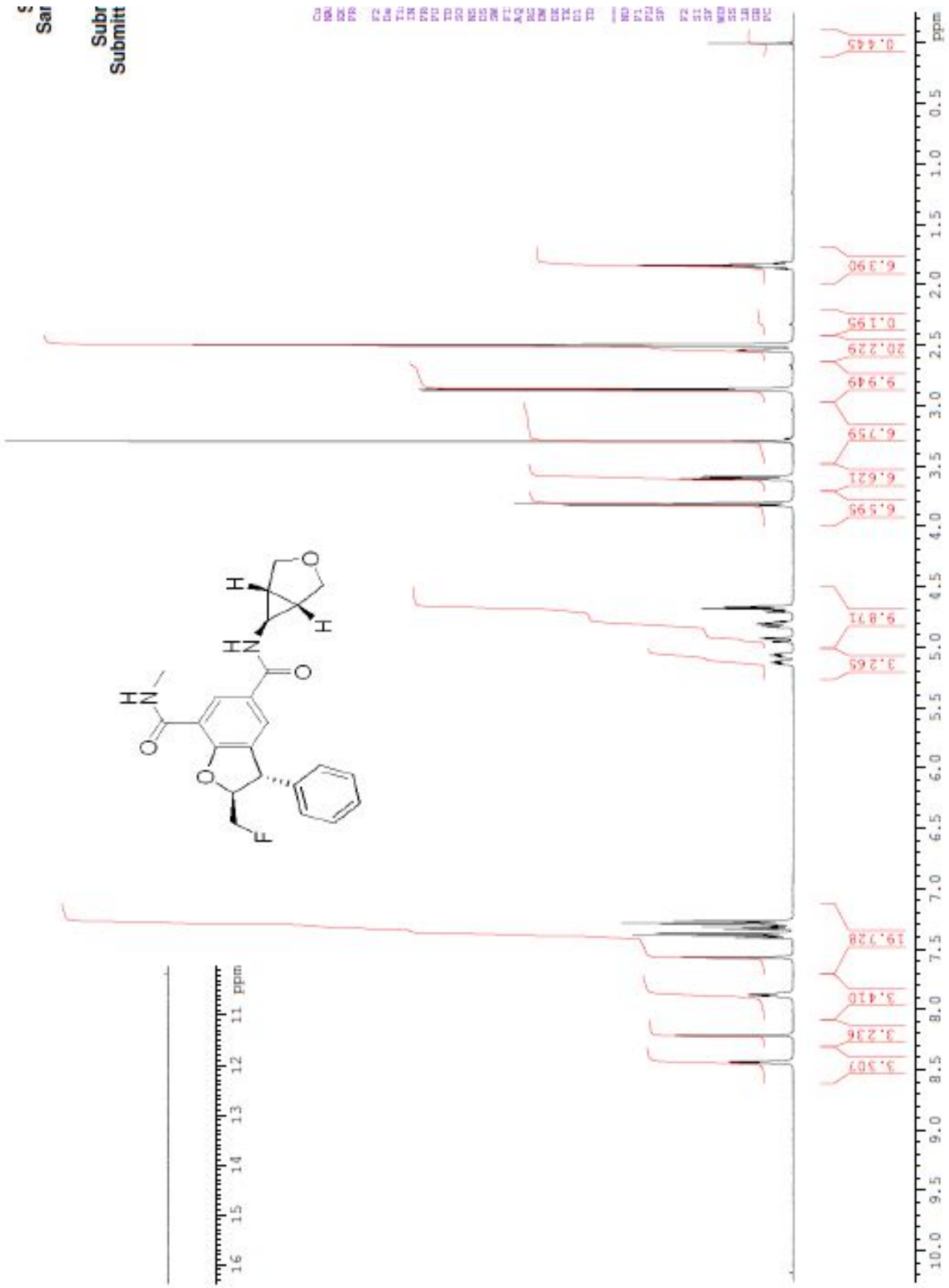
^{1}H NMR Compound **31** in $CDCl_3$ (400 MHz):



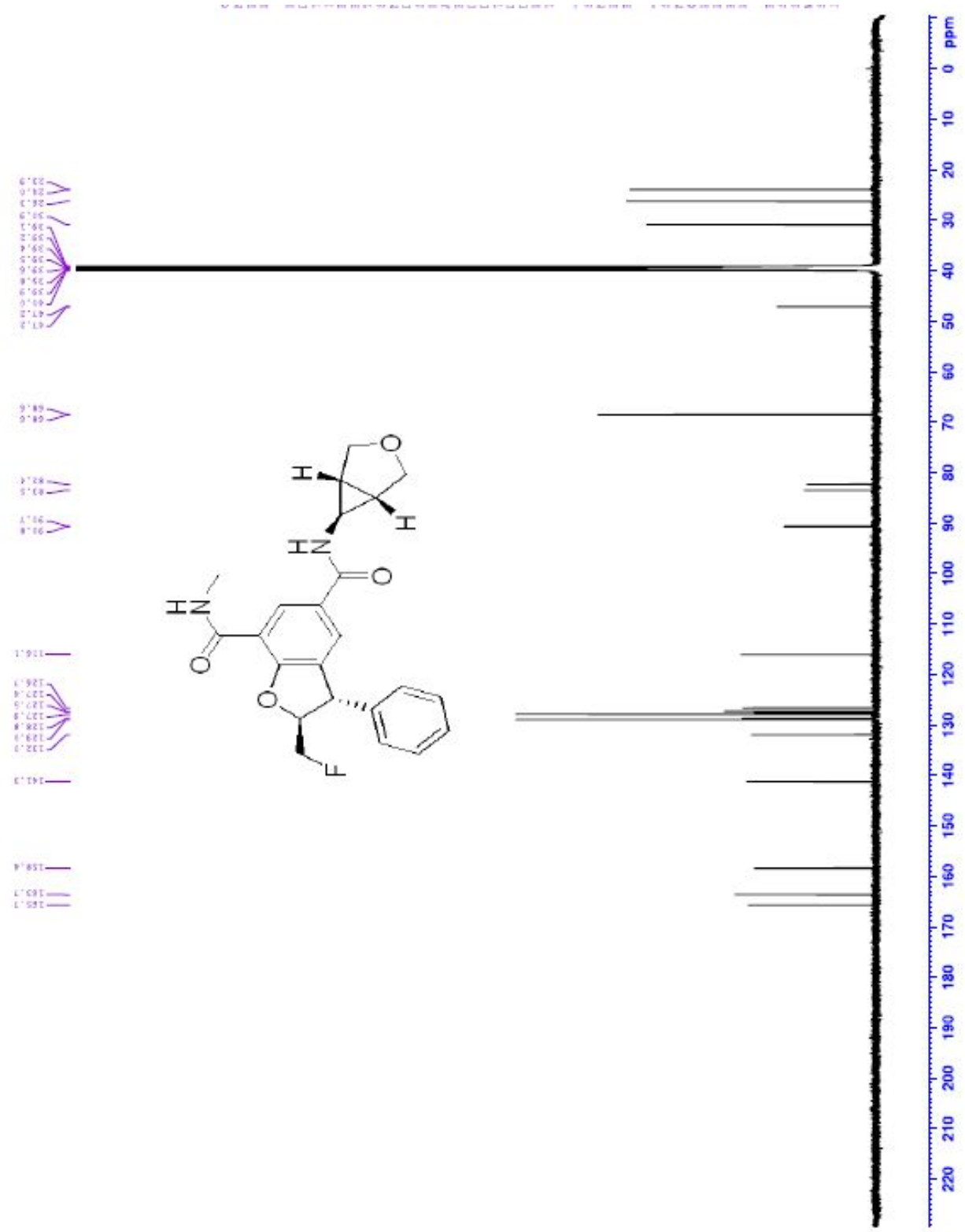
[1]H NMR Compound **35** in D6-DMSO (400 MHz):



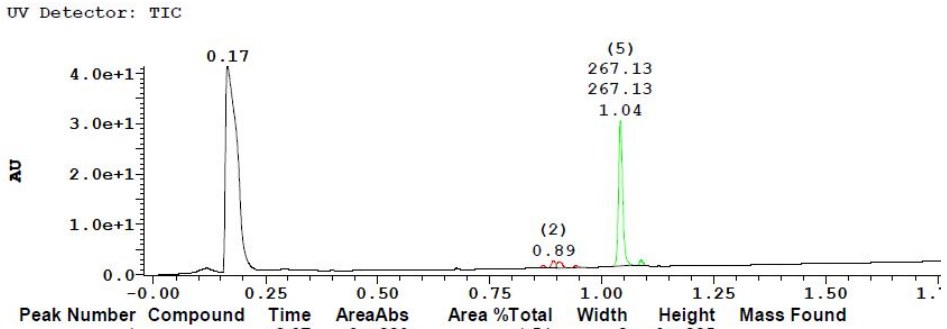
[1]H NMR of compound **36 (**GSK973) in DMSO (400 MHz):



^{13}C NMR of compound **36** (GSK973) in DMSO (151 MHz):

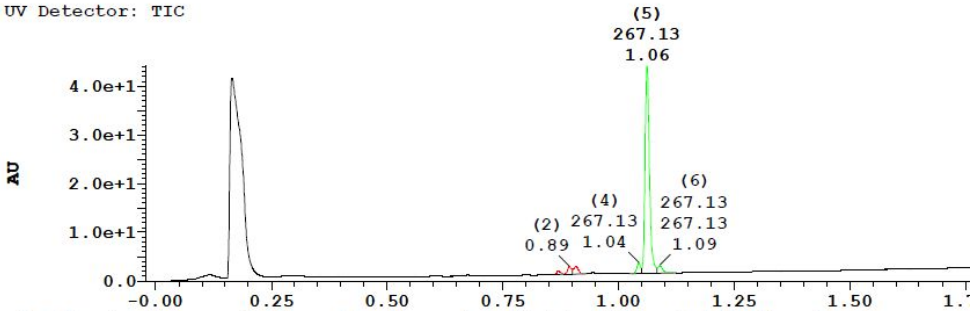


LCMS Cpd 4:



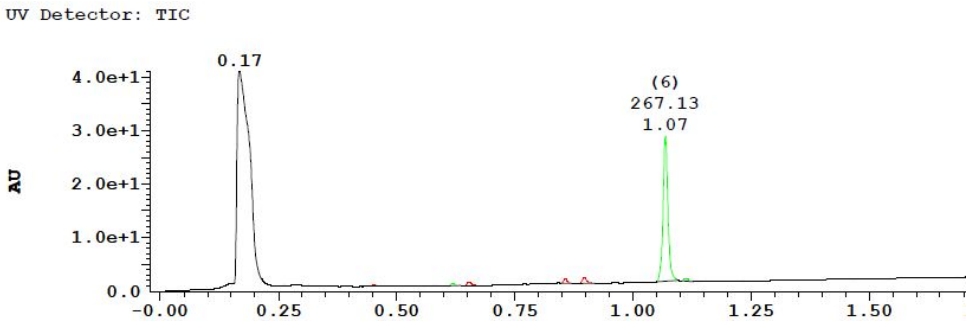
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1		0.87	6e+003	1.54	0	6e+005	
2		0.89	2e+004	4.26	0	1e+006	
3		0.91	2e+004	4.78	0	1e+006	
4		0.94	4e+003	1.18	0	4e+005	
5	Found	1.04	3e+005	85.25	0	3e+007	267.13, 267.13
7	Found	1.09	1e+004	2.99	0	1e+006	267.13, 267.13

LCMS Cpd 5:



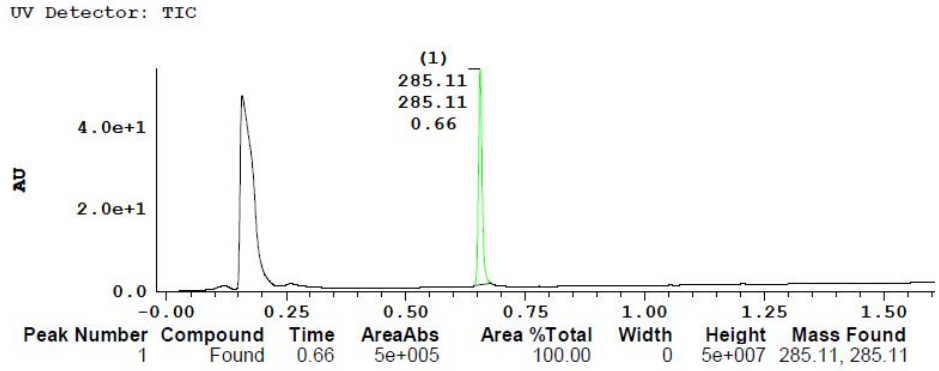
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1		0.87	8e+003	1.38	0	8e+005	
2		0.89	1e+004	2.68	0	2e+006	
3		0.91	2e+004	3.62	0	1e+006	
4	Found	1.04	2e+004	3.73	0	2e+006	267.13
5	Found	1.06	5e+005	85.03	0	4e+007	267.13

LCMS Cpd 6:



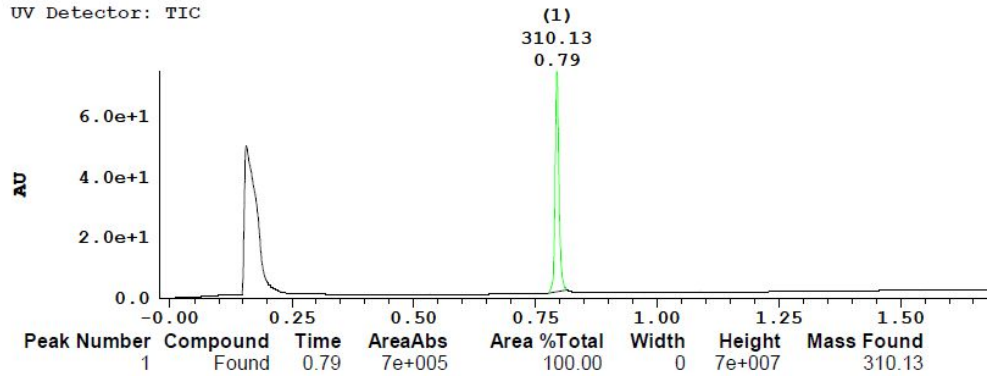
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1		0.45	5e+003	1.46	0	3e+005	
2	Found	0.62	4e+003	1.15	0	5e+005	267.13
3		0.65	7e+003	1.87	0	6e+005	
4		0.86	8e+003	2.15	0	8e+005	
5		0.90	1e+004	3.34	0	1e+006	
6	Found	1.07	3e+005	88.68	0	3e+007	267.13

LCMS Cpd 14:



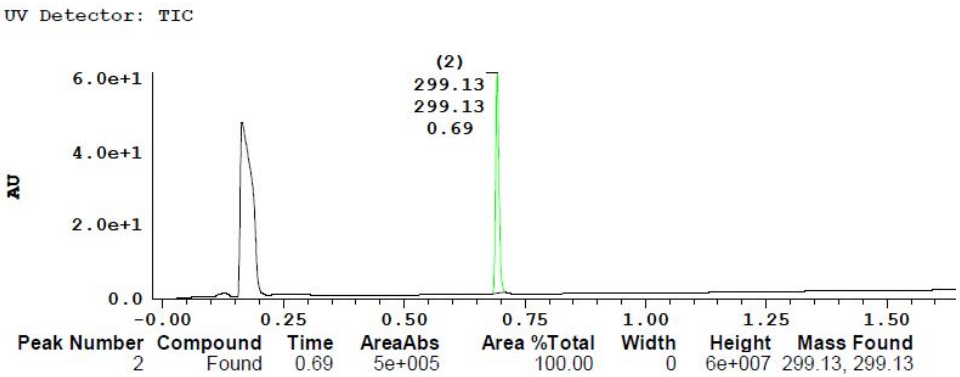
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.66	5e+005	100.00	0	5e+007	285.11, 285.11

LCMS Cpd 15:



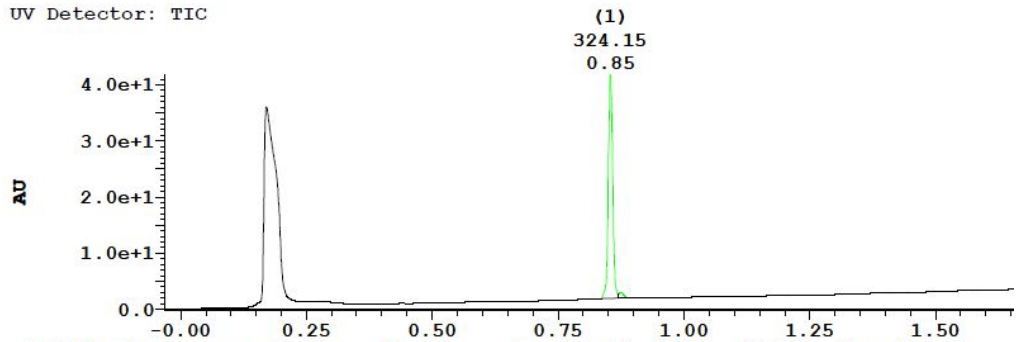
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.79	7e+005	100.00	0	7e+007	310.13

LCMS Cpd 16:



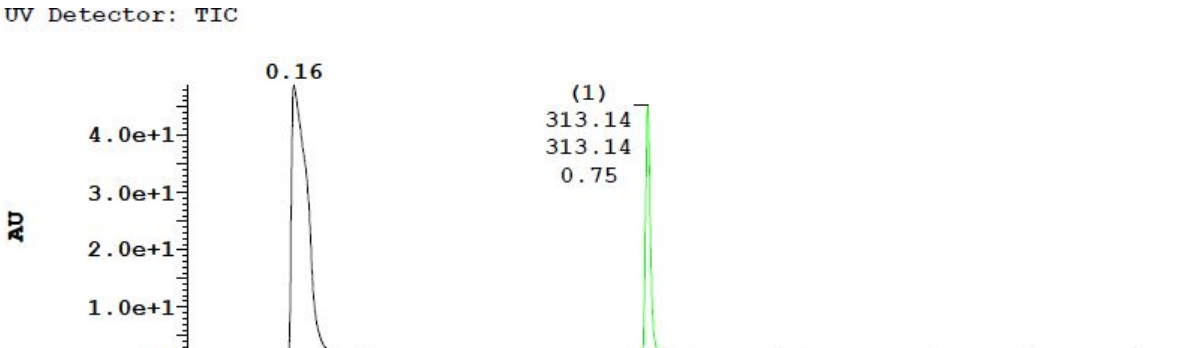
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
2	Found	0.69	5e+005	100.00	0	6e+007	299.13, 299.13

LCMS Cpd 17:



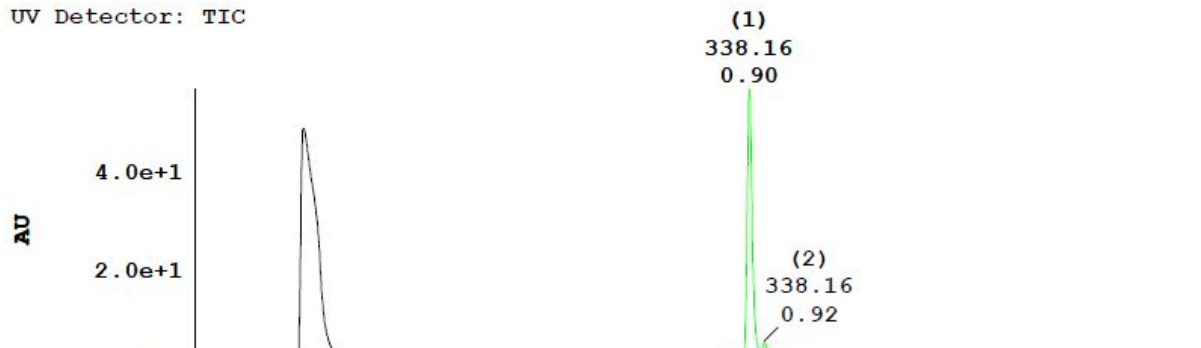
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.85	4e+005	97.36	0	4e+007	324.15
2	Found	0.88	1e+004	2.64	0	1e+006	324.15

LCMS Cpd 18:



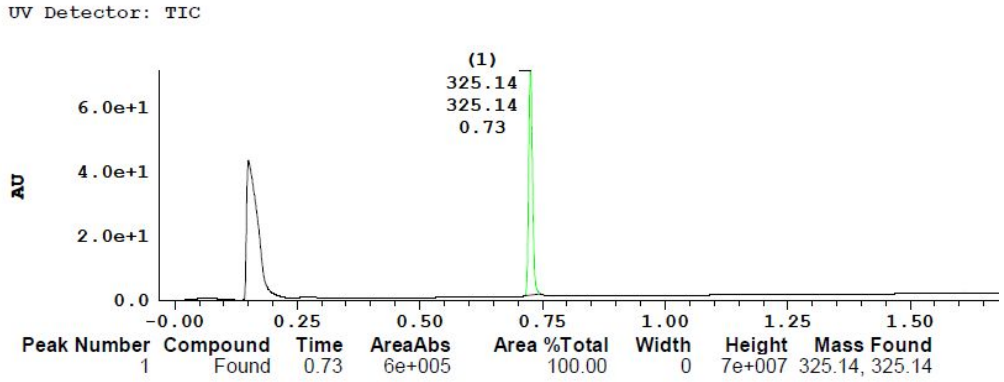
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.75	4e+005	100.00	0	4e+007	313.14, 313.14

LCMS Cpd 19:



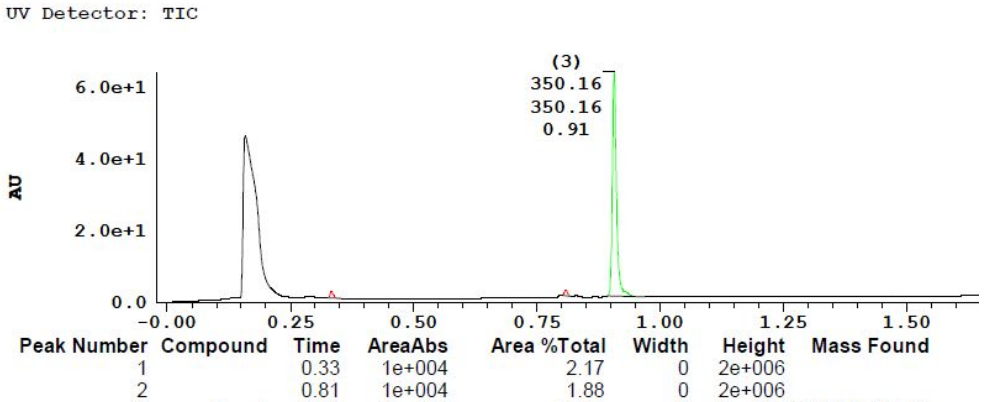
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.90	6e+005	92.09	0	6e+007	338.16
2	Found	0.92	5e+004	7.91	0	4e+006	338.16

LCMS Cpd 20:



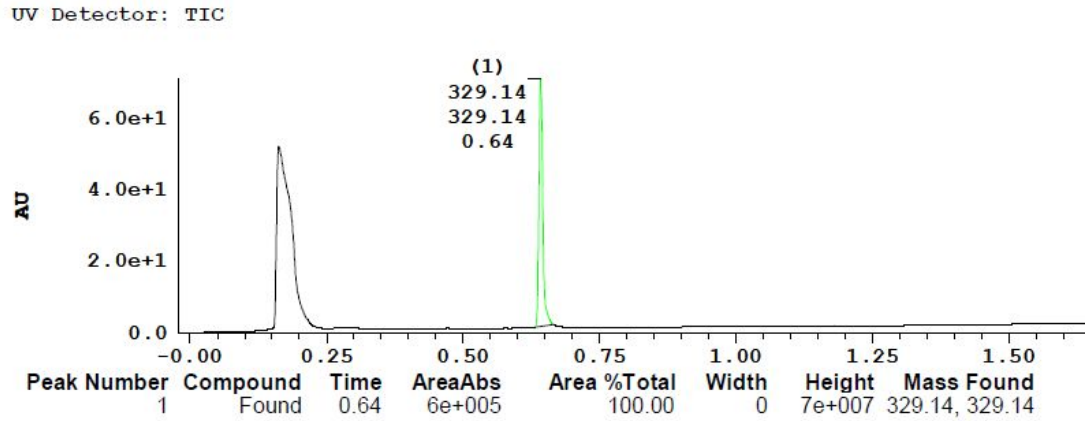
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.73	6e+005	100.00	0	7e+007	325.14, 325.14

LCMS Cpd 21:



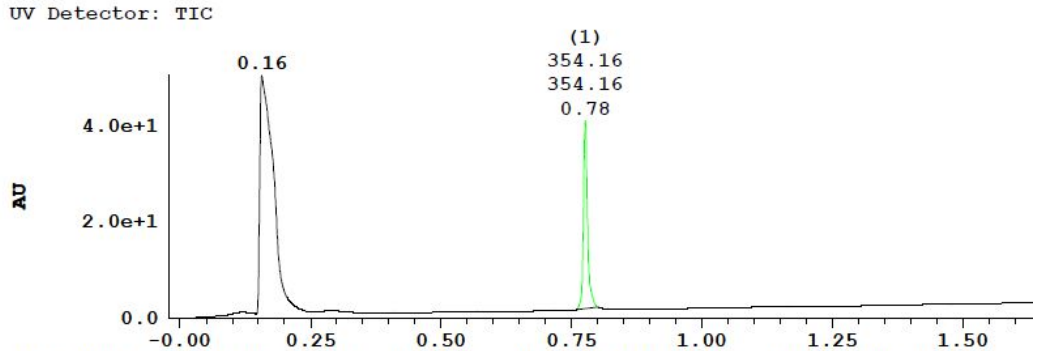
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1		0.33	1e+004	2.17	0	2e+006	
2		0.81	1e+004	1.88	0	2e+006	
3	Found	0.91	6e+005	95.96	0	6e+007	350.16, 350.16

LCMS Cpd 22:



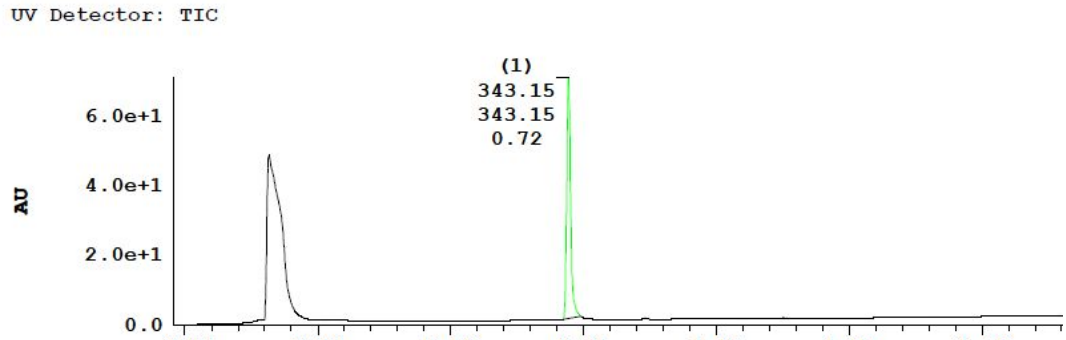
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.64	6e+005	100.00	0	7e+007	329.14, 329.14

LCMS Cpd 23:



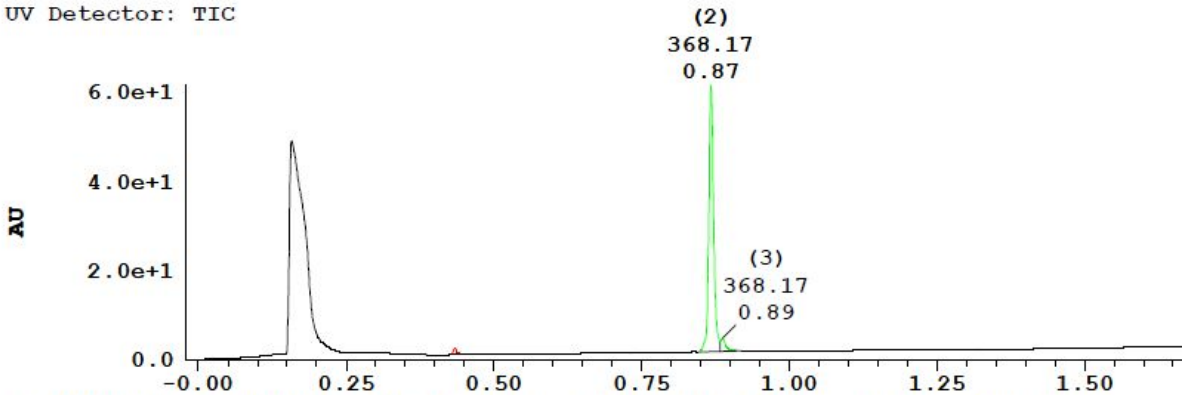
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.78	4e+005	100.00	0	4e+007	354.16, 354.16

LCMS Cpd 24:



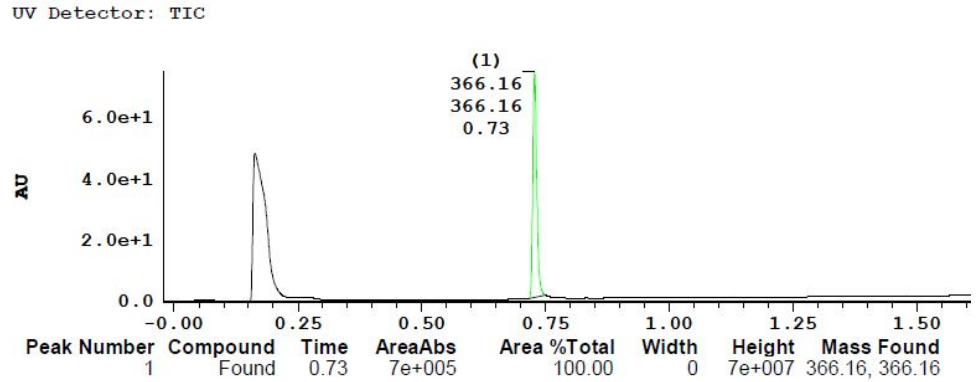
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.72	6e+005	100.00	0	7e+007	343.15, 343.15

LCMS Cpd 25:



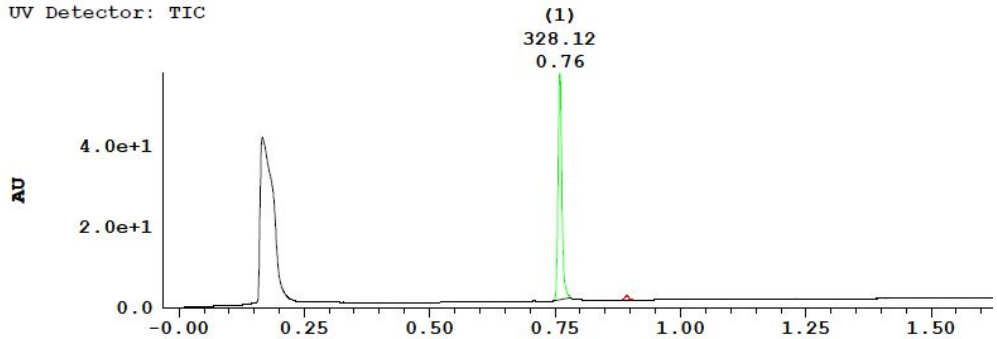
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1		0.43	1e+004	1.75	0	1e+006	
2	Found	0.87	6e+005	93.26	0	6e+007	368.17
3	Found	0.89	3e+004	4.99	0	3e+006	368.17

LCMS Cpd 26:



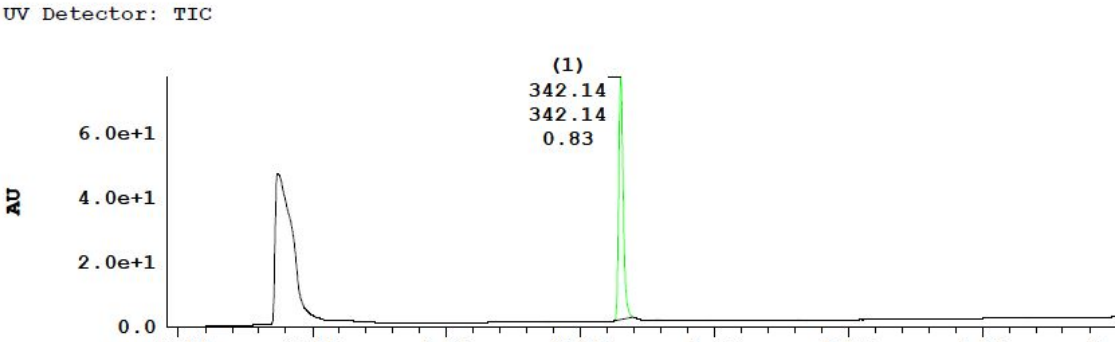
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.73	7e+005	100.00	0	7e+007	366.16, 366.16

LCMS Cpd 27:



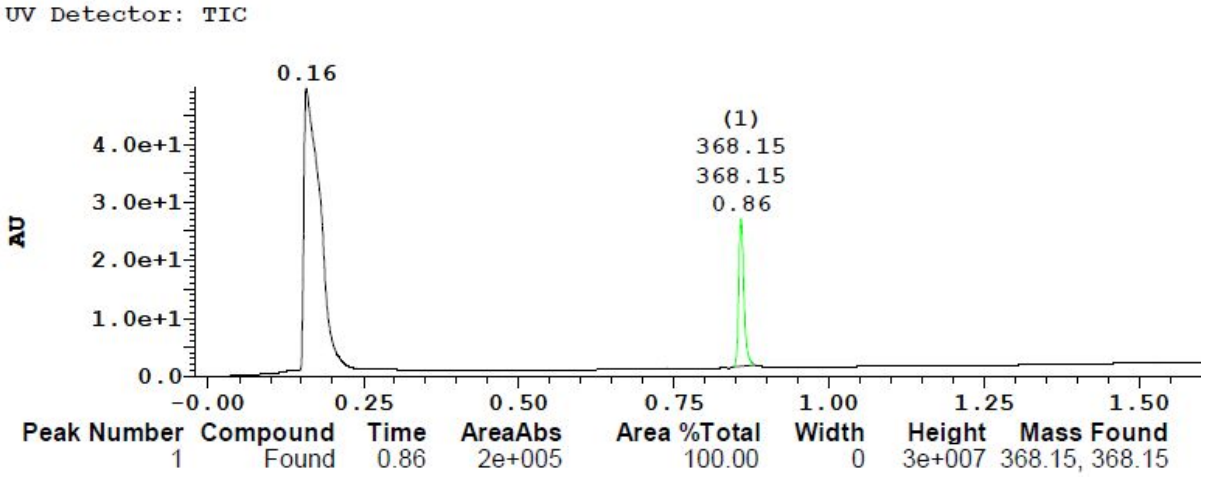
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.76	5e+005	97.97	0	6e+007	328.12
2		0.89	1e+004	2.03	0	1e+006	

LCMS Cpd 28:



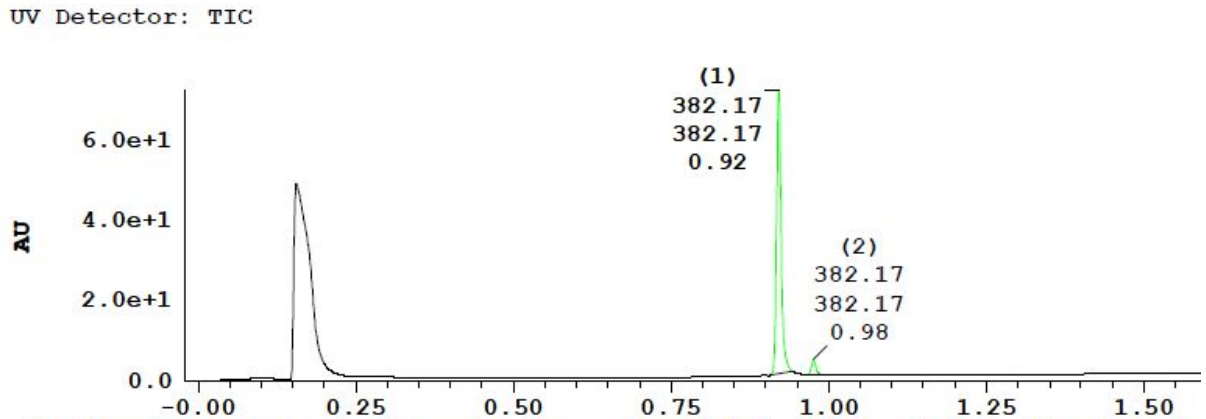
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.82	7e+005	100.00	0	8e+007	342.14, 342.14

LCMS Cpd 29:



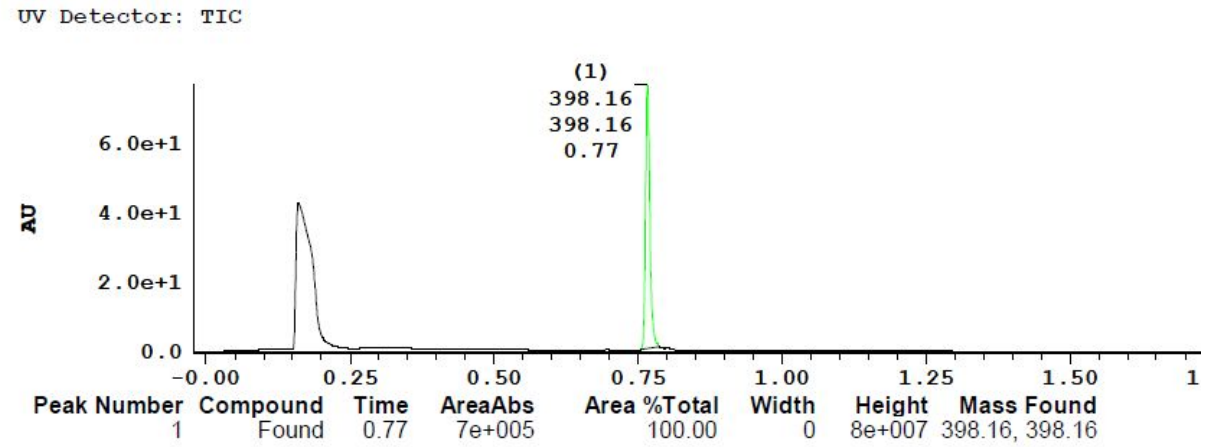
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.86	2e+005	100.00	0	3e+007	368.15, 368.15

LCMS Cpd 30:



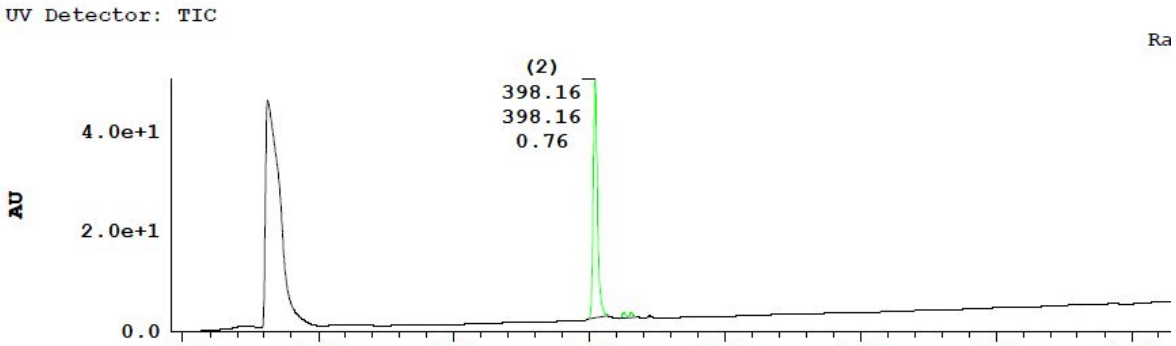
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.92	6e+005	95.24	0	7e+007	382.17, 382.17
2	Found	0.98	3e+004	4.76	0	4e+006	382.17, 382.17

LCMS Cpd 31:



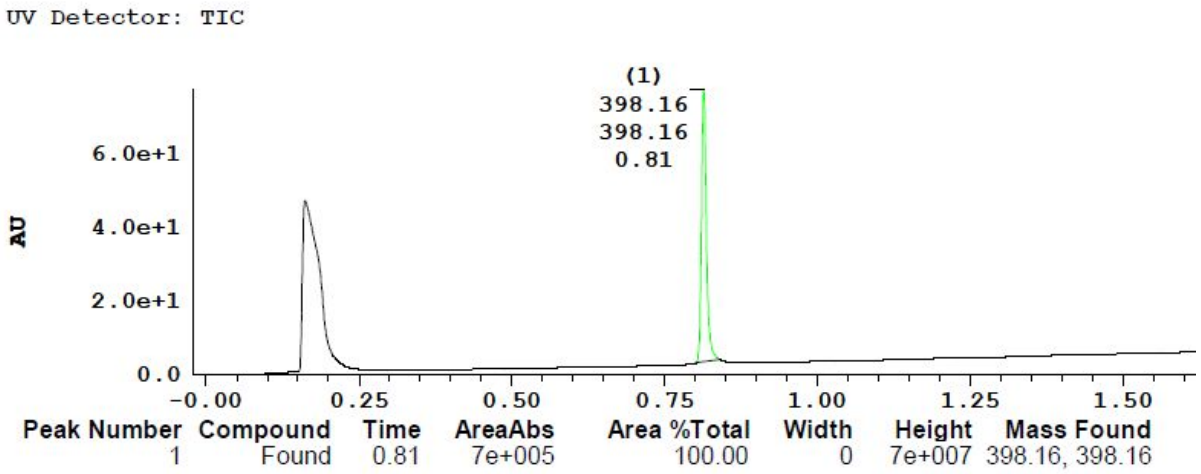
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.77	7e+005	100.00	0	8e+007	398.16, 398.16

LCMS Cpd 32:



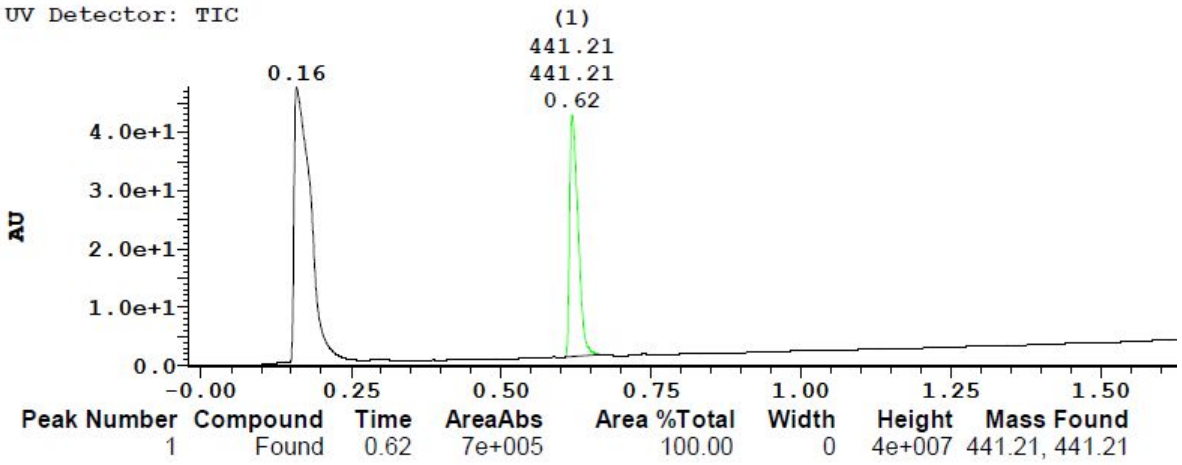
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
2	Found	0.76	4e+005	96.70	0	5e+007	398.16, 398.16
3	Found	0.81	7e+003	1.64	0	1e+006	398.16, 398.16
4	Found	0.83	7e+003	1.66	0	9e+005	398.16

LCMS Cpd 33:



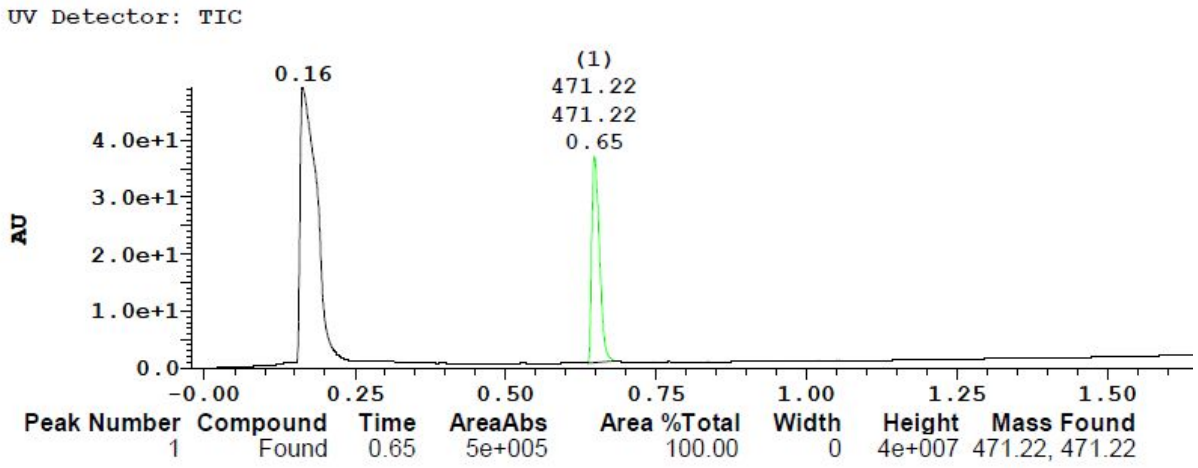
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.81	7e+005	100.00	0	7e+007	398.16, 398.16

LCMS Cpd 34:



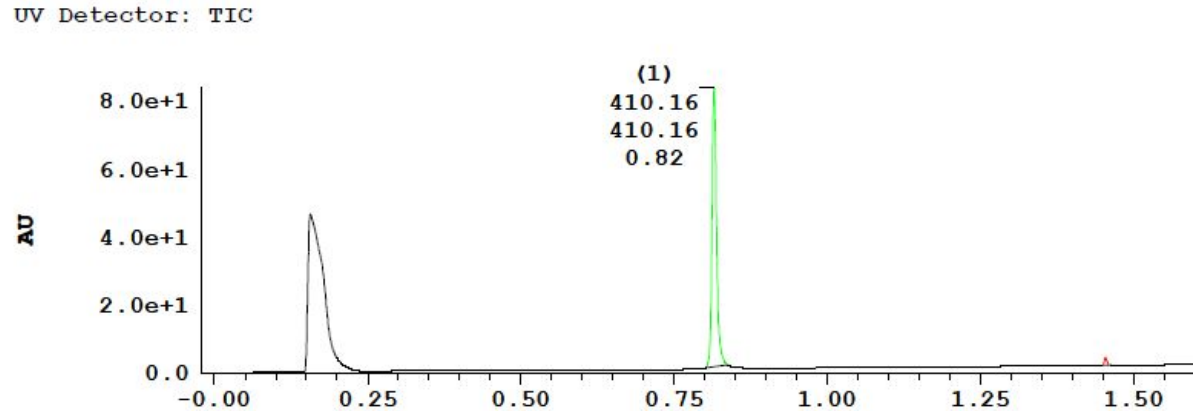
Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.62	7e+005	100.00	0	4e+007	441.21, 441.21

LCMS Cpd 35:



Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.65	5e+005	100.00	0	4e+007	471.22, 471.22

LCMS Cpd 36:



Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1	Found	0.82	7e+005	98.50	0	8e+007	410.16, 410.16
2		1.45	1e+004	1.50	0	2e+006	

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2. Valko, K.; Nunhuck, S.; Bevan, C.; Abraham, M. H.; Reynolds, D. P., Fast gradient HPLC method to determine compounds binding to human serum albumin. Relationships with octanol/water and immobilized artificial membrane lipophilicity. *J Pharm Sci* **2003,** *92* (11), 2236-48.

3. Camurri, G.; Zaramella, A., High-throughput liquid chromatography/mass spectrometry method for the determination of the chromatographic hydrophobicity index. *Anal Chem* **2001,** *73* (15), 3716-22.

4. Ballell, L.; Bates, R. H.; Young, R. J.; Alvarez-Gomez, D.; Alvarez-Ruiz, E.; Barroso, V.; Blanco, D.; Crespo, B.; Escribano, J.; Gonzalez, R.; Lozano, S.; Huss, S.; Santos-Villarejo, A.; Martin-Plaza, J. J.; Mendoza, A.; Rebollo-Lopez, M. J.; Remuinan-Blanco, M.; Lavandera, J. L.; Perez-Herran, E.; Gamo-

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