

Electronic Supplementary Material (ESI)

Microscale Purification in Support of High-Throughput Medicinal Chemistry

Chandan L. Barhate*^a, Andrew F. Donnell^b, Merrill Davies^a, Ling Li^b, Yong Zhang^c, Fukang Yang^c,
Regina Black^d, Greg Zipp^a, Yingru Zhang^a, Cullen Cavallaro^b, E. Scott Priestley^b, and Harold N. Weller^a

^aSeparation and Analytical Technology Team, Bristol Myers Squibb, Lawrenceville, NJ, USA.

^bChemotype Discovery and Optimization, Bristol Myers Squibb, Lawrenceville, NJ, USA

^cNovel Drug Modalities, Bristol Myers Squibb, Lawrenceville, NJ, USA

^dAgilent Technologies, Incorporated, Wilmington, Delaware 19808, United States

* Corresponding author, email: chandan.barhate@bms.com

Page	Contents
S2	Experimental section
S5	Microscale UHPLC-MS purification system components
S6	Delay calibration
S7	UHPLC-MS microscale fraction collection performance evaluation
S8	Ten compound test mixture
S9	Column loading studies using ten compound test mixture
S10	Microscale purification workflow
S11	Synthesis and microscale purification of CCR1 library
S15	Representative microscale purification chromatograms from CCR1 library
S16	Representative microscale purification chromatograms from amide library
S17	High-resolution mass spectrometry (HRMS) data
S19	Representative ¹ H NMR data of four compounds

Experimental Section

Microscale purification instrument: All purifications were performed in reverse phase mode on an UHPLC-MS instrument. A commercially available Agilent 1290 Infinity Series UHPLC system (Agilent Technologies, Palo Alto, CA) equipped with a binary pump (G7120A), a multisampler with injector multiwash (G7167B), column compartment (G7116B) diode array detector (DAD), single quadrupole mass spectrometer (G6130B) and analytical fraction collector (G5664B) was used for all liquid chromatography separations and purifications. The instrument was supported by OpenLab CDS™ software [Rev.C.01.10 (236)] in Microsoft Windows 10. The extra-column effects were minimized by using narrow diameter tubing (75 µm and 127 µm). Fraction collection was carried out based on the extracted ion chromatogram for the target mass, and the additional delay volume between the MS detector and the fraction collector was provided by adding a knitted delay coil (1 m X 0.5 mm, 0.2 mL, Agilent p/n 5067-6181). The fractions were collected in Matrix™ 1.4 mL 2D barcoded polypropylene tubes. The LC-MS was operated in positive ion mode with the drying gas temperature of 350 °C, nebulizer pressure of 35 psig, and a drying gas flow 3.0 and 12 L/min. The microscale purification analysis were carried on Waters XTerra™ C8 column (10 cm X 0.46 cm ID, 3.5 µm, 12 % Carbon Load and 125Å pore size) and/ Waters XBridge™ C8 column (10 cm X 0.46 cm ID, 2.5 µm, 13 % Carbon Load and 130Å pore size), flow rate 2 mL/min, LC eluent A: Water (0.1 % TFA), LC eluent B: Acetonitrile (0.1% TFA), linear gradient from 5 % to 95 % B in 4 mins, column temperature 45 °C.

Chemicals and reagents: Water (HPLC-Grade), trifluoroacetic acid (TFA) , acetonitrile (CH₃CN) (HPLC-Grade), isopropanol (IPA), and dimethyl sulfoxide (DMSO) were obtained from Sigma Aldrich (St. Louis, MO, USA). Agilent Delay calibrant (p/n 5190-8223) consisted of Patent Blue VF sodium salt , Sunset Yellow FCF and Sudan Orange G purchased from Agilent Technologies (Santa Clara, California).

Pre-and Post-purification analysis: All pre- and post-purification analyses were performed on a Shimadzu UHPLC-MS system (Shimadzu Scientific Instruments, Columbia, MD). The Shimadzu Nexera 30 Series UHPLC-MS system consisted of (2) LC-30AD pumps with X2 LPGE-30 solvent selector valves (binary gradient mode), (2) DGU-20A5R solvent degassers, a SIL-30ACMP autosampler configured for 96- and 384-well microtiter plates, a CTO-20AC column oven equipped with (2) IDEX 6/7 18k psi column valves plus (2) post-column 7-port analytical manifolds, an SPD-30AM multi-wavelength UV detector, and an LCMS-2020 single quadrupole MS equipped with a DUIS probe

(simultaneous ESI and APCI). The instrument was controlled by LabSolutions CDS™ and NexLab™ open-access software (versions 5.89 and 1.1.21.23, respectively) on Windows 10 platform. The pre-purification UHPLC-MS method employed a Waters Cortecs™ C8 column (1.6mm, 2.1 mm ID x 30 mm L) with 0.05% TFA in CH₃CN /Water gradient (5% to 95% CH₃CN over 0.75 minutes with a 0.25 minute hold at 95% CH₃CN) at 1.7 mL/min. The column temperature was 50 °C. The LCMS-2020 detector was operated in DUIS mode with desolvation line and heat block temperatures of 300 and 400 °C, respectively, and nebulizing gas and drying gas flows of 1.5 and 15.0 L/min, respectively. For post-purification analysis, the same TFA-CH₃CN method was used, and an additional orthogonal method was added. The orthogonal method consisted of a Waters Acquity BEH Phenyl™ column (1.7 μm particle size, 30 mm X 2.1 mm ID), flow rate: 1.5 mL/min, LC eluent A: 5:95 Methanol:water (10mM ammonium acetate), LC eluent B: 95:5 MeOH:water (10 mM ammonium acetate), linear gradient from 5% to 95% B in 0.65 min, hold at 100% B for 0.35 min. Column temperature 50 °C. All other analysis conditions were the same as the TFA-CH₃CN method.

Calculation of resolution: The resolution (Rs) referred to in this study was determined using the half-height method: where t_1 and t_2 are the retention times of the two peaks of interest, and $w_{0.5,1}$ and $w_{0.5,2}$ are the peak widths measured at half height,
$$Rs = \frac{2(t_2 - t_1)}{1.7(w_{0.5,1} + w_{0.5,2})}$$
.

Columns: Waters XTerra™ C8 column (10 cm X 0.46 cm ID, 3.5 μm, 12 % Carbon Load and 125Å pore size), Waters XBridge™ C8 column (10 cm X 0.46 cm ID, 2.5 μm, 13 % Carbon Load and 130Å pore size), and Waters Cortecs C18 1.6um 2.1 x 30 mm were purchased from Waters Co. (Milford, MA).

Purity of collected target compounds contained in each fraction was determined by area normalization:

Percentage peak area of analyte X = [(peak area of analyte X) / (sum of all relevant peak areas)] x 100

Fraction collection, weighing and evaporation: Fractions were collected in Matrix™ 1.4 mL 2D-barcode polypropylene tubes (C/N 3790) purchased from ThermoFisher Scientific (Waltham, MA). These tubes were prepared using a MicroTasker™ robot (Sirius Automation Group, Inc.) equipped with a barcode reader and a 5-place semi-microbalance. Fractions were evaporated with a Genevac EZ-2™ Plus. The final weights were acquired using the MicroTasker™ robot.

Liquid handlers and reaction wellplates: Reactions were carried out in 384-well polypropylene microplates purchased from Analytical Sales and Service, Inc. (Flanders, NJ) (cat. # 384280). Reaction mixtures were filtered through 0.7 μm 384-well filter plates purchased from Agilent Technologies (Santa Clara, CA) (cat. # 201027-100). Liquid handling operations were performed using either an Opentrons OT-2™ equipped with an 8-channel pipette (Opentrons, Brooklyn, NY), an Agilent Bravo equipped with a 384-channel head (Agilent Technologies, Santa Clara, CA), or an SPT LabTech Mosquito with a 4.5 mm pitch pipette spool (SPT Labtech, Inc., Boston, MA).

^1H NMR: NMR spectra were measured on a Bruker 400 MHz spectrometer. Chemical shifts are reported in ppm downfield from TMS using residual nondeuterated solvent as an internal standard (DMSO, 2.50 ppm). Data for ^1H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz) and integration.

High-resolution mass spectrometry (HRMS): “The compounds were dissolved in approximately 1mM DMSO, diluted 20X with water/acetonitrile/isopropanol and analyzed by LC-HRAM (high-resolution, accurate-mass). The LC was a Waters Acquity UPLC system. 2 μL sample injection were made onto a Waters Acquity BEH C18 2x50mm column running water/acetonitrile (0.1% formic acid) mobile phases into the mass spectrometer. The mass spectrometer was a Thermo Q Exactive Orbitrap set to ESI pos/neg switching mode and a resolution of 17,500. For data processing, the LC peak apex scan was used to determine the observed mass of the molecular ion

Microscale UHPLC-MS purification system components and optimization



Figure S1. Microgram-scale UHPLC-MS purification system components. (© Agilent Technologies, Inc. Reproduced with permission.)

Table S1. Preparative HPLC-MS and Microscale UHPLC-MS instrument parameter comparison

	Microscale UHPLC-MS system parameters	Preparative HPLC-MS system parameters
Column Dimensions	100 X 0.46 mm ID	100 X 21 mm ID
Particle Size (μm)	2.5 and 3.5	5
Flow rate (mL)	2-5	15-45
System tubing (μm ID)	75 and 127	300
Backpressure (Bar)	1300	400
Injection Loop (μL)	100	5000
Detector flow cell volume (μL)	1	13
Mass load per reaction (mg)	0.5-2.5	5 -50
Gradient time per sample (min)	3-4	8-10
Knitted delay coil	1 m X 0.5 mm, 0.2 mL	5m X 0.5 mm, 1.0 mL
Fraction collection tubes	1.4 mL 2D barcoded tubes	16 X 150 mm tubes

Delay calibration

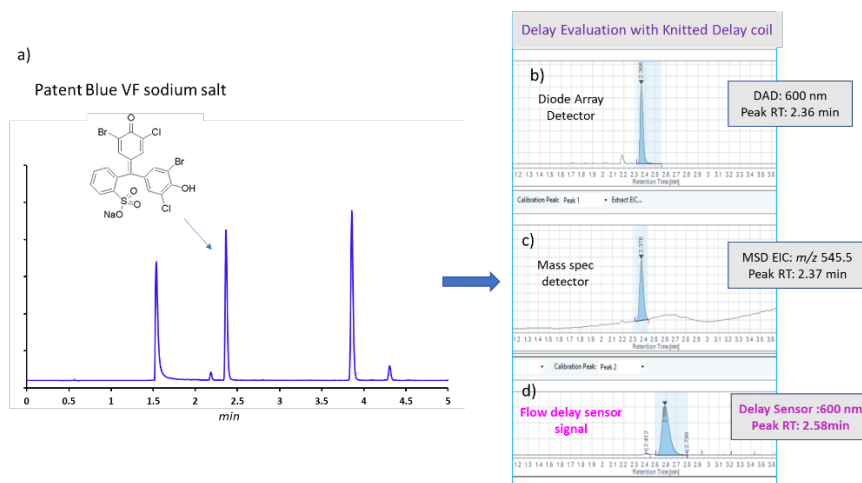


Figure S2. Experiment to determine delay time and/volume between diode array detector, mass spectrometry detector and fraction collector. a) Column: 100 mm X 4.6 mm ID Waters XTerra™ C8 column, Flow rate: 2 mL/min. The LC eluents were A: Water (0.1 % TFA) B: Acetonitrile (0.1% TFA) , linear gradient from 5 % to 95 % B in 4 mins, column temperature 45 °C. b) Patent Blue VF sodium salt DAD signal at 600 nm c) Extracted ion chromatogram (EIC) of Patent Blue VF sodium salt. d) FDS signal of Patent Blue VF sodium salt at 600 nm.

Table S2. Delay Evaluation

Diode array detector (DAD)		
Calculated delay time (min)	0.07 ^a	0.22 ^b
Calculated delay volume (mL)	0.14 ^a	0.44 ^b
Mass spectrometry detector (MSD) to fraction collector delay		
Delay sensor correction time (min)	0.06 ^a	0.21 ^b
Delay sensor Correction time (min)	-0.01 ^a	-0.01 ^b
Effective delay time (min)	0.05 ^a	0.20 ^b

^a without knitted delay coil

^b with knitted delay coil dimensions: 1 m X 0.5 mm, 0.2 mL

UHPLC-MS microscale fraction collection performance evaluation

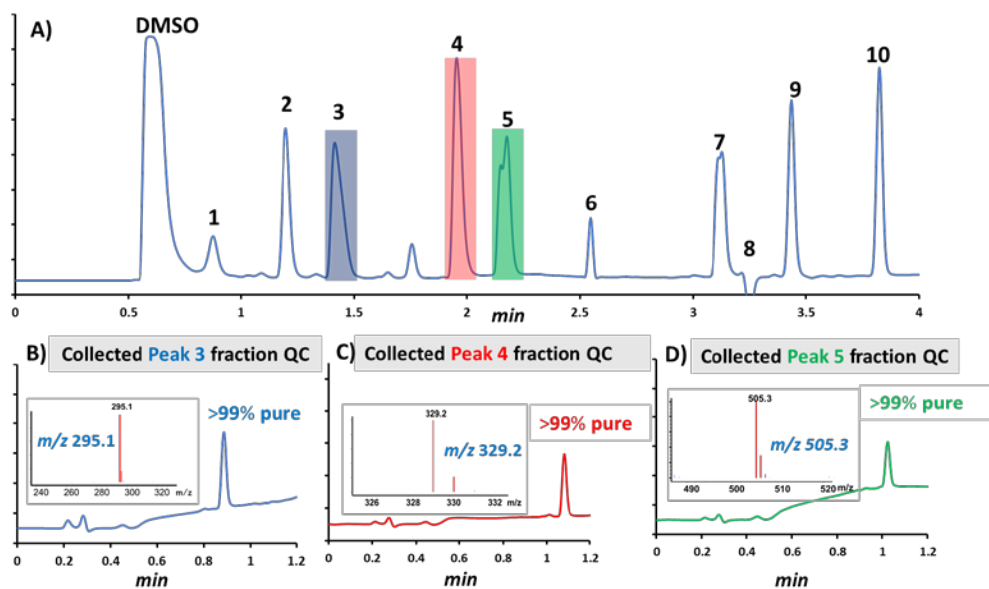


Figure S3. Optimized UHPLC-MS fraction collection performance evaluation (A) standard ten compound test mixture separation on 100 mm X 4.6 mm ID Waters XTerra™ C8 column, flow rate: 2 mL/min, LC eluents were A: Water (0.1% TFA) B: Acetonitrile (0.1% TFA), linear gradient from 5 % to 95 % B in 4 mins, column temperature 45 °C (B) (C) (D) Collected Peak 3, Peak 5 and Peak 6 purity analysis using UHPLC-MS, 50 mm X 2.1 mm ID Zorbax Eclipse Plus™ C18 column, Water (0.1 % TFA) B: Acetonitrile (0.1% TFA) , linear gradient from 5 % to 95 % B in 2.0 mins, flow rate: 0.6 mL/min, DAD: 220 nm

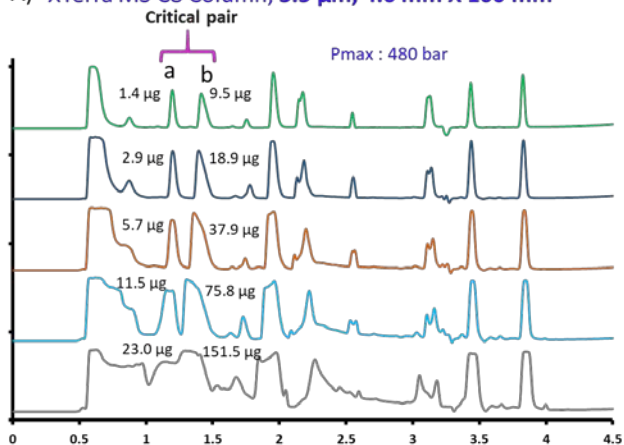
Table S3: Ten compound test mixture retention times

Peak	Test mix compound	Structure	Exact Mass	^a Retention Time (min)	^b Retention Time (min)
1	Metronidazole		171.1	0.87	1.19
2	Hydroxybenzotriazole monohydrate		135	1.19	1.40
3	Aspartame		294.1	1.42	1.77
4	Labetalol HCl		328.2	1.95	2.16
5	Dipyridamole		504.3	2.16	2.30
6	4-Aminobenzophenone		197.1	2.54	2.58
7	Chrysin		254.1	3.13	2.93
8	Disperse Yellow 3		269.1	3.27	3.25
9	Beclomethasone dipropionate		520.2	3.41	3.66
10	Dipentyl phthalate		306.2	3.82	4.26

^a Waters XTerra™ MS C8, 3.5 μm column, ^b Waters Xbridge™ C8 Column 2.5 μm column

Column loading studies with ten compound test mixture

A) XTerra MS C8 Column, 3.5 μm , 4.6 mm X 100 mm



B) XBridge C8 Column 2.5 μm , 4.6 mm X 100 mm

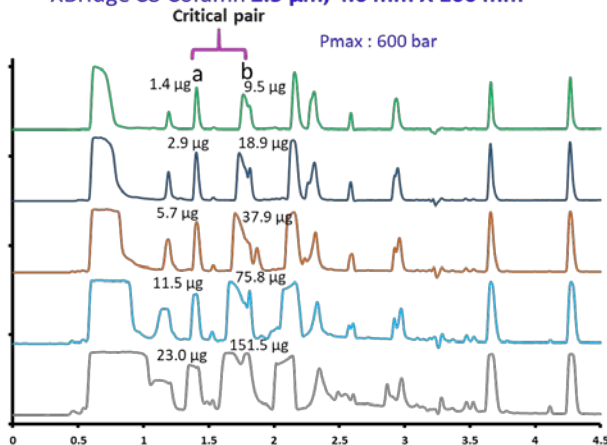


Figure S4: Column loading studies for microgram scale library purifications. A) Sample loading effect using 10 compound test mixture using 100 mm X 4.6 mm id Waters XTerra™ C8 column, flow rate: 2 mL/min, LC eluents were A: Water (0.1% TFA) B: Acetonitrile (0.1% TFA), linear gradient from 5% to 95% B in 4 mins. Column temperature 45 °C B) Sample loading effect using 10 compound test mixture using Waters XBridge™ C8 Column 2.5 μm , 100 mm X 4.6 mm id. Flow rate: 2 mL/min, LC eluents were A: Water (0.1% TFA) B: Acetonitrile (0.1% TFA) , linear gradient from 5% to 95% B in 4 mins, column temperature 45 °C.

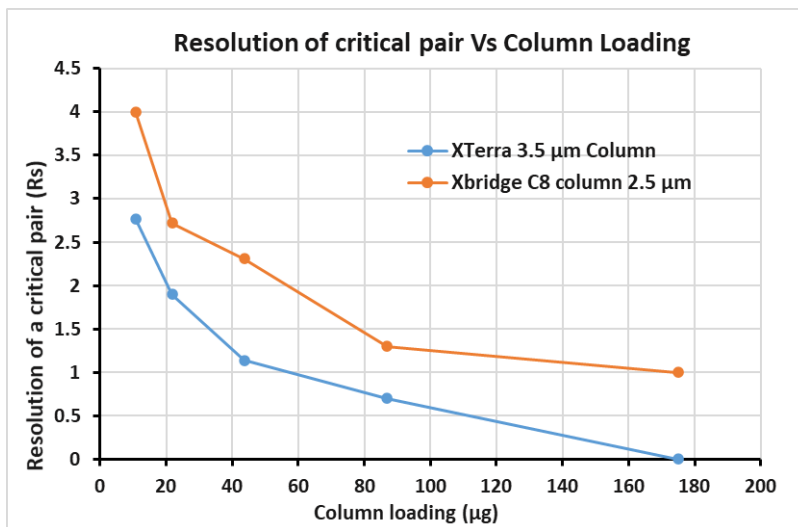


Figure S5: Resolution of critical pair marked in Fig. S3 vs. column loading

Microscale purification workflow

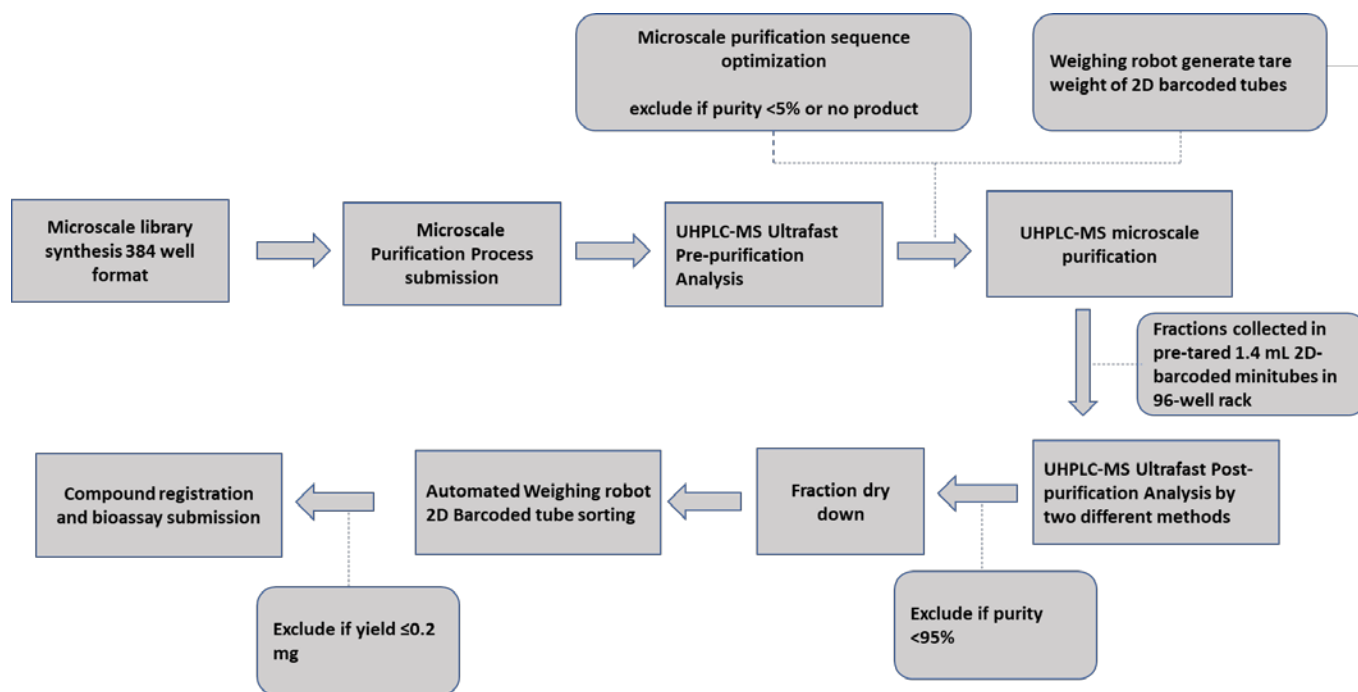
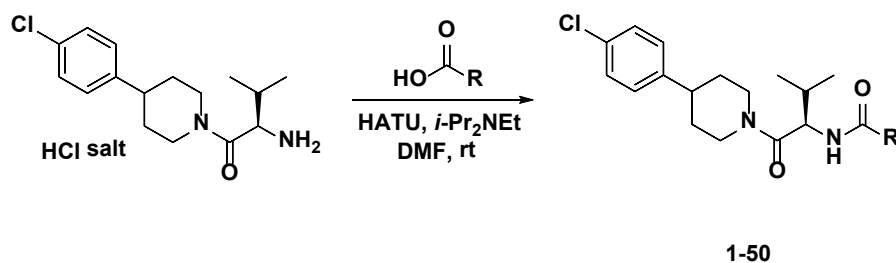


Figure S6: Schematic representation of the high-throughput microscale purification workflow

Scheme S1. Synthesis of CCR1 library compounds 1-50



all R groups are
displayed in Table S4

Stock solutions were prepared for each carboxylic acid reagent (250 mM in DMF), the starting amine (R)-2-amino-1-(4-(4-chlorophenyl)piperidin-1-yl)-3-methylbutan-1-one hydrochloride (400 mM in DMF), HATU (600 mM in DMF), and *i*-Pr₂NEt (1200 mM in DMF).

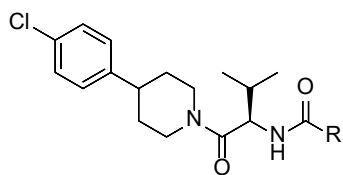
The carboxylic acid stock solutions (14 μ L, 3.5 μ mol) were dosed to the appropriate wells of a 384-well polypropylene microplate using an automated liquid handler, then the starting amine stock solution (5.0 μ L, 2.0 μ mol) was added, followed by HATU (5.0 μ L, 3.0 μ mol) and *i*-Pr₂NEt (10 μ L, 12 μ mol), and the contents of each well was mixed (3 x 20 μ L). The plate was centrifuged, and then sealed with a 384-well mat and shaken at rt for 1 h.

The contents of each reaction well were transferred to the corresponding wells of a 0.7 μ m 384-well polypropylene filter plate stacked on a 384-well polypropylene microplate. The stacked plates were centrifuged to filter the reaction mixtures. Each well of the original reaction plate was rinsed with DMF (26 μ L), which was transferred to the filter plate, and the stacked plates were centrifuged to filter the rinses into the same receiving plate.

Ultra-fast pre-purification UHPLC-MS analysis samples were prepared by transferring a 0.5 μ L aliquot from each well to a separate 384-well polypropylene microplate that had been pre-loaded with DMSO (30 μ L). The wells were mixed (4 x 0.8 μ L) and the plate was centrifuged, and the purity was assessed via UHPLC-MS. The receiving plate was sealed using a PlateLoc thermal microplate sealer and purified by the microscale purification workflow. For compounds containing a basic nitrogen, the percent yield was calculated based on the molecular weight of the compound as a mono-TFA salt.


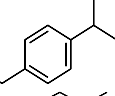





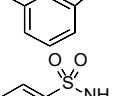

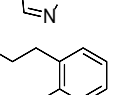




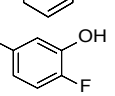




All the compounds (1-50) mentioned in the manuscript have been previously reported and characterized.^{1,2}

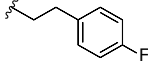
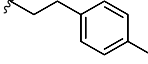
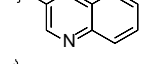
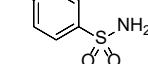
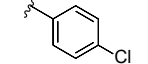
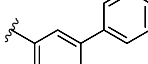
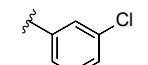
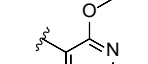
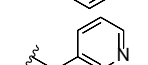
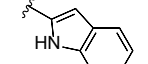
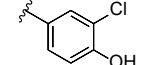
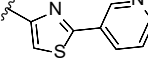
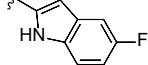
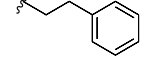
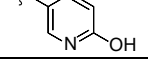
Table S4. Microscale purification of CCR1 library



1-50

Cmpd	R	clogP	Fraction Volume (μL)	Yield (mg)	% yield	m/z [M+H] ⁺	^a Ultrafast Post-purification analysis		^b Ultrafast Post-purification analysis	
							Rt (min)	% purity	Rt (min)	% purity
1		4.74	497	0.35	30	466.1	0.55	>99	0.72	>99
2		3.61	533	0.32	42	377.1	0.58	91	0.72	98
3		5.51	441	0.47	52	455.0	0.66	>99	0.76	>99
4		4.50	533	0.50	62	405.1	0.63	>99	0.75	>99
5		4.64	386	0.49	59	417.1	0.62	>99	0.74	>99
6		4.95	533	0.50	60	419.1	0.65	>99	0.76	>99
7		4.64	497	0.50	60	417.1	0.62	>99	0.74	>99
8		4.62	515	0.49	57	431.1	0.61	>99	0.73	>99
9		5.01	478	0.49	59	413.1	0.62	>99	0.74	>99
10		5.08	405	0.47	53	447.0	0.63	>99	0.75	>99
11		5.01	496	0.46	56	413.1	0.63	>99	0.75	>99
12		5.08	404	0.44	49	447.0	0.63	>99	0.75	>99
13		5.02	478	0.45	39	456.0	0.67	>99	0.77	>99
14		4.52	552	0.41	52	393.1	0.63	>99	0.74	>99
15		4.31	515	0.47	60	391.1	0.61	>99	0.74	>99
16		4.78	460	0.44	51	435.1	0.63	>99	0.75	>99

17		5.52	515	0.55	64	427.1	0.63	>99	0.74	>99
18		5.72	497	0.54	59	455.1	0.68	>99	0.77	>99
19		4.99	515	0.53	62	427.1	0.63	>99	0.75	>99
20		4.68	478	0.56	61	457.0	0.64	>99	0.76	>99
21		5.44	460	0.52	59	441.1	0.65	>99	0.76	>99
22		4.62	496	0.50	58	431.1	0.61	>99	0.74	>99
23		4.71	460	0.44	51	429.1	0.59	>99	0.73	>99
24		3.96	736	0.60	52	466.1	0.60	>99	0.75	>99
25		4.60	313	0.35	40	438.1	0.64	>99	0.75	98
26		3.71	441	0.48	47	512.0	0.57	>99	0.71	>99
27		4.03	515	0.49	44	439.1	0.57	95	0.73	>99
28		5.44	441	0.48	54	441.1	0.64	>99	0.76	>99
29		4.71	478	0.41	48	429.1	0.56	>99	0.71	>99
30		2.89	533	0.48	57	418.1	0.56	>99	0.70	>99
31		5.91	479	0.53	53	497.0	0.66	>99	0.75	>99
32		5.06	478	0.49	55	445.1	0.63	>99	0.75	>99
33		5.52	386	0.45	53	427.1	0.64	>99	0.75	>99
34		4.34	478	0.44	51	433.1	0.57	>99	0.71	>99
35		4.60	184	c	c	c	c	c	c	c

36		5.06	460	0.61	69	445.1	0.63	>99	0.75	>99
37		5.44	497	0.62	70	441.1	0.64	>99	0.76	98
38		4.66	625	0.63	56	450.1	0.54	>99	0.74	>99
39		3.10	478	0.53	55	478.0	0.54	93	0.70	92
40		5.10	368	0.52	60	433.0	0.64	91	0.76	>93
41		6.14	313	0.45	47	475.1	0.68	>99	0.78	>99
42		5.10	386	0.56	65	433.0	0.65	>99	0.75	>99
43		3.72	515	0.52	48	430.1	0.60	>99	0.74	>99
44		3.26	644	0.67	63	414.1	0.46	>99	0.70	>99
45		4.52	368	0.46	53	438.1	0.63	>99	0.74	>99
46		4.80	331	0.35	39	449.0	0.59	96	0.73	93
47		4.35	552	0.63	53	483.1	0.53	>99	0.75	>99
48		4.66	313	0.39	43	456.0	0.63	>99	0.75	>99
49		3.26	570	0.58	70	414.1	0.46	>99	0.70	>99
50		3.57	460	0.42	40	416.0	0.50	>99	0.69	>99

^a Waters Cortecs™ UPLC C8 1.6 μm 50 x 2.1 mm ID column, flow rate: 1.7 mL/min, LC eluent A: 5:95 CH₃CN:water (0.05% TFA), LC eluent B: 95:5 CH₃CN:water (0.05% TFA), linear gradient from 0% to 100% B in 0.75 min, hold at 100% B for 0.25 min. Column temperature 50 °C.

^b Waters Acquity BEH Phenyl™ column (1.7 μm particle size, 30 mm X 2.1 mm ID), flow rate: 1.5 mL/min, LC eluent A: 5:95 MeOH:water (10mM ammonium acetate), LC eluent B: 95:5 MeOH:water (10 mM ammonium acetate), linear gradient from 5% to 95% B in 0.65 min, hold at 100% B for 0.35 min. Column temperature 50 °C.

^c This analog did not meet the pre-purification purity criteria and was not submitted for microscale purification.

Representative microscale purification, ultrafast pre-and post-purification analysis

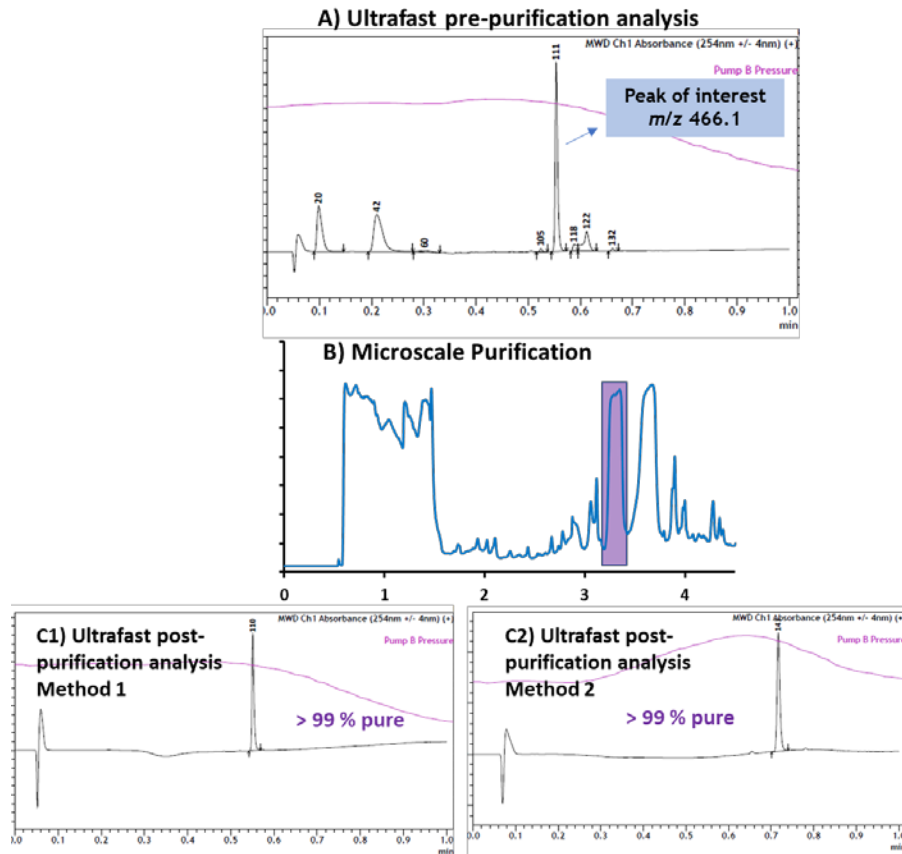


Figure S7. Representative microscale purification and ultrafast pre-and post-purification analysis of compound **1**. A) Ultrafast pre-purification analysis using Waters Cortecs™ UPLC C8 1.6 μm 50 x 2.1 mm ID column, flow rate: 1.7 mL/min, LC eluent A: 5:95 CH_3CN :water (0.05% TFA), LC eluent B: 95:5 CH_3CN :water (0.05% TFA), linear gradient from 0% to 100% B in 0.75 min, hold at 100% B for 0.25 min. Column temperature 50 °C. B) Microscale Purification analysis using Waters XBridge™ MS C8 Column, 2.5 μm , 4.6 mm X 100 mm column, flow rate: 2 mL/min, LC eluent A: water (0.1% TFA), LC eluent B: CH_3CN (0.1% TFA), linear gradient from 5% to 95% B in 4 min, column temperature 45 °C. Highlighted chromatogram portion shows the collected fraction. C1) Ultrafast post-purification analysis Method 1: Waters Cortecs UPLC C8 1.6 μm 2.1 x 50 mm column, flow rate 1.7 mL/min, LC eluent A: 5:95 CH_3CN :water (0.05% TFA), LC eluent B: 95:5 CH_3CN :water (0.05% TFA), linear gradient from 0% to 100% B in 0.75 min, hold at 100% B for 0.40 min. Column temperature 50 °C. C2) Fast post-purification analysis Method 2: Waters Acquity BEH Phenyl™ column (1.7 μm particle size, 30 mm X 2.1 mm ID), flow rate: 1.5 mL/min, LC eluent A: 5:95 MeOH:water (10mM ammonium acetate), LC eluent B: 95:5 MeOH:water (10 mM ammonium acetate), linear gradient from 5% to 95% B in 0.65 min, hold at 100% B for 0.35 min, column temperature 50 °C.

Representative microscale purification chromatograms from additional amide library

Compound structures and synthesis information are not disclosed due to proprietary reasons.

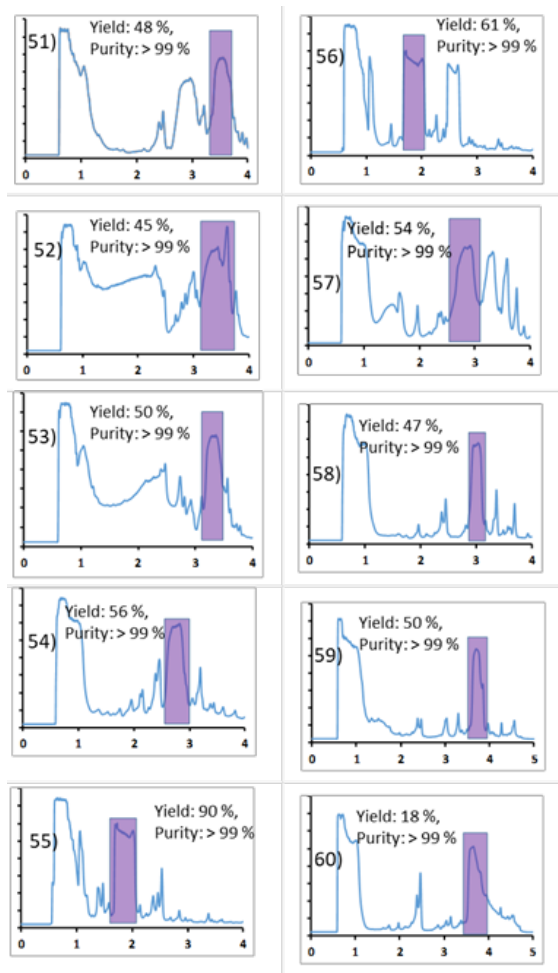


Figure S8. Representative microscale amide library purifications. Highlighted chromatogram portion shows the collected fractions. Waters XBridge™ MS C8, 2.5 μ m, 100 x 4.6 mm ID column, flow rate: 2 mL/min. The LC eluents were A: Water (0.1% TFA), B: Acetonitrile (0.1% TFA), linear gradient from 5% to 95% B in 4 min, column temperature 45 °C.

Table S5. High-resolution mass spectrometry (HRMS) data of compounds 1–50.

Compound	Molecular formula	Calculated <i>m/z</i>	Experimental <i>m/z</i>	Δ (ppm)
1	C ₂₆ H ₂₈ ClN ₃ O ₃	466.1892	466.1893	0.12
2	C ₂₁ H ₂₉ ClN ₂ O ₂	377.1990	377.1989	-0.3
3	C ₂₅ H ₂₇ ClN ₂ O ₂ S	455.1555	455.1558	0.7
4	C ₂₃ H ₃₃ ClN ₂ O ₂	405.2303	405.2306	0.71
5	C ₂₃ H ₂₆ ClFN ₂ O ₂	417.1740	417.1741	0.36
6	C ₂₄ H ₃₅ ClN ₂ O ₂	419.2460	419.2463	0.8
7	C ₂₃ H ₂₆ ClFN ₂ O ₂	417.1740	417.1740	0.17
8	C ₂₄ H ₂₈ ClFN ₂ O ₂	431.1896	431.1898	0.53
9	C ₂₄ H ₂₉ ClN ₂ O ₂	413.1990	413.1989	-0.32
10	C ₂₄ H ₂₈ Cl ₂ N ₂ O ₂	447.1601	447.1600	-0.13
11	C ₂₄ H ₂₉ ClN ₂ O ₂	413.1990	413.1994	0.89
12	C ₂₄ H ₂₈ Cl ₂ N ₂ O ₂	447.1601	447.1600	-0.09
13	C ₂₄ H ₂₆ ClN ₃ O ₂ S	456.1507	456.1511	0.85
14	C ₂₂ H ₃₃ ClN ₂ O ₂	393.2303	393.2306	0.65
15	C ₂₂ H ₃₁ ClN ₂ O ₂	391.2147	391.2147	0.07
16	C ₂₃ H ₂₅ ClF ₂ N ₂ O ₂	435.1645	435.165	1.15
17	C ₂₅ H ₃₁ ClN ₂ O ₂	427.2147	427.2149	0.58
18	C ₂₇ H ₃₅ ClN ₂ O ₂	455.2460	455.2466	1.44
19	C ₂₅ H ₃₁ ClN ₂ O ₂	427.2147	427.2150	0.84
20	C ₂₆ H ₃₃ ClN ₂ O ₃	457.2252	457.2253	0.09
21	C ₂₆ H ₃₃ ClN ₂ O ₂	441.2303	441.2308	0.95
22	C ₂₄ H ₂₈ ClFN ₂ O ₂	431.1896	431.1902	1.44
23	C ₂₄ H ₂₉ ClN ₂ O ₃	429.1939	429.1944	0.99
24	C ₂₅ H ₂₈ ClN ₅ O ₂	466.2004	466.2010	1.2

25	C25H28CIN3O2	438.1943	438.1944	0.25
26	C23H27Cl2N3O4S	512.1172	512.1174	0.33
27	C24H27CIN4O2	439.1895	439.1901	1.23
28	C26H33CIN2O2	441.2303	441.2304	0.18
29	C24H29CIN2O3	429.1939	429.1939	-0.23
30	C22H28CIN3O3	418.1892	418.1896	0.87
31	C25H28ClF3N2O3	497.1813	497.1818	0.88
32	C25H30ClFN2O2	445.2053	445.2059	1.55
33	C25H31CIN2O2	421.2147	427.2148	0.18
34	C23H26ClFN2O3	433.1689	433.1691	0.47
35	C25H28CIN3O2	438.1943	438.1951	1.82
36	C25H30ClFN2O2	445.2053	445.2058	1.17
37	C26H33CIN2O2	441.2303	441.2305	0.38
38	C26H28CIN3O2	450.1943	450.1945	0.49
39	C23H28CIN3O4S	478.1562	478.1561	-0.09
40	C23H26Cl2N2O2	433.1444	433.1449	1.09
41	C29H31CIN2O2	475.2147	475.2145	-0.49
42	C23H26Cl2N2O2	433.1444	433.1444	0
43	C23H28CIN3O3	430.1892	430.1894	0.57
44	C23H28CIN3O2	414.1943	414.1945	0.5
45	C25H28CIN3O2	438.1943	438.1939	-0.85
46	C23H26Cl2N2O3	449.1393	449.1391	-0.61
47	C25H27CIN4O2S	483.1616	483.1620	0.78
48	C25H27ClFN3O2	456.1849	456.1854	1.14
49	C23H28CIN3O2	414.1943	414.1945	0.62
50	C22H26CIN3O3	416.1735	416.1737	0.25

A representative selection of four compounds from the library was re-synthesized and subjected to the microscale purification. The isolated products were analyzed by ^1H NMR and gave spectra consistent with those previously reported in the literature.

Compound **2**. ^1H NMR (400 MHz, DMSO- d_6) δ 7.95 - 7.84 (m, 1H), 7.38 - 7.32 (m, 2H), 7.29 - 7.21 (m, 2H), 4.65 - 4.51 (m, 2H), 4.24 - 4.12 (m, 1H), 3.17 - 3.05 (m, 1H), 2.87 - 2.77 (m, 1H), 2.71 - 2.60 (m, 1H), 2.10 - 1.94 (m, 3H), 1.90 - 1.72 (m, 2H), 1.56 - 1.32 (m, 2H), 0.99 - 0.91 (m, 1H), 0.91 - 0.82 (m, 6H), 0.44 - 0.34 (m, 2H), 0.17 - 0.06 (m, 2H).

Compound **3**. ^1H NMR (400 MHz, DMSO- d_6) δ 8.86 - 8.80 (m, 1H), 8.38 - 8.32 (m, 1H), 8.04 - 7.98 (m, 1H), 7.98 - 7.91 (m, 1H), 7.50 - 7.41 (m, 2H), 7.39 - 7.33 (m, 1H), 7.32 - 7.25 (m, 2H), 7.23 - 7.17 (m, 1H), 4.79 - 4.72 (m, 1H), 4.63 - 4.55 (m, 1H), 4.40 - 4.19 (m, 1H), 3.23 - 3.11 (m, 1H), 2.89 - 2.78 (m, 1H), 2.76 - 2.62 (m, 1H), 2.30 - 2.16 (m, 1H), 1.96 - 1.71 (m, 2H), 1.60 - 1.35 (m, 2H), 1.01 - 0.91 (m, 6H).

Compound **5**. ^1H NMR (400 MHz, DMSO- d_6) δ 8.65 - 8.58 (m, 1H), 7.79 - 7.69 (m, 2H), 7.55 - 7.48 (m, 1H), 7.42 - 7.30 (m, 3H), 7.29 - 7.25 (m, 1H), 7.24 - 7.18 (m, 1H), 4.79 - 4.74 (m, 1H), 4.61 - 4.53 (m, 1H), 4.39 - 4.22 (m, 1H), 3.21 - 3.10 (m, 1H), 2.89 - 2.78 (m, 1H), 2.73 - 2.61 (m, 1H), 2.26 - 2.13 (m, 1H), 1.93 - 1.72 (m, 2H), 1.58 - 1.34 (m, 2H), 0.98 - 0.88 (m, 6H).

Compound **7**. ^1H NMR (400 MHz, DMSO- d_6) δ 8.56 - 8.49 (m, 1H), 8.02 - 7.95 (m, 2H), 7.38 - 7.25 (m, 5H), 7.23 - 7.19 (m, 1H), 4.79 - 4.72 (m, 1H), 4.61 - 4.53 (m, 1H), 4.39 - 4.22 (m, 1H), 3.21 - 3.09 (m, 1H), 2.89 - 2.77 (m, 1H), 2.72 - 2.61 (m, 1H), 2.25 - 2.13 (m, 1H), 1.95 - 1.72 (m, 2H), 1.58 - 1.33 (m, 2H), 0.98 - 0.88 (m, 6H).

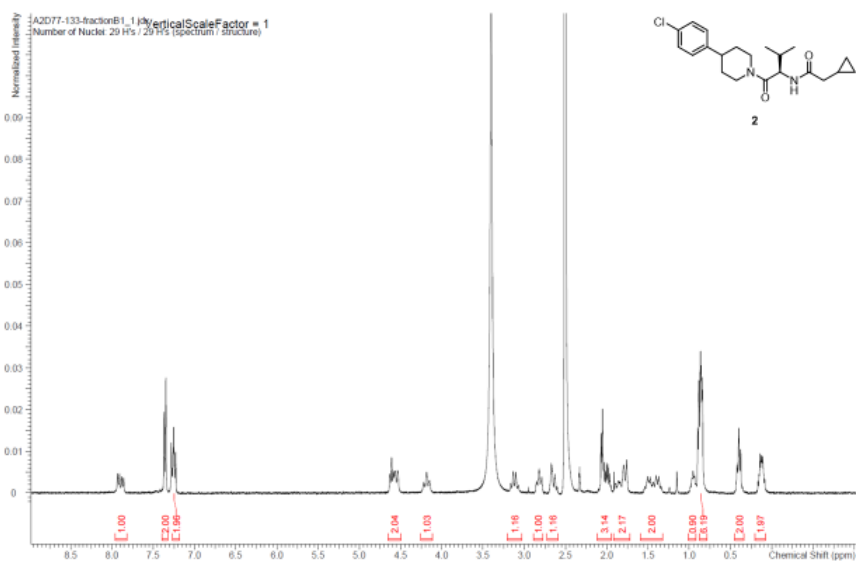


Figure S9.1 ¹H NMR (400 MHz, DMSO-d₆) of compound 2.

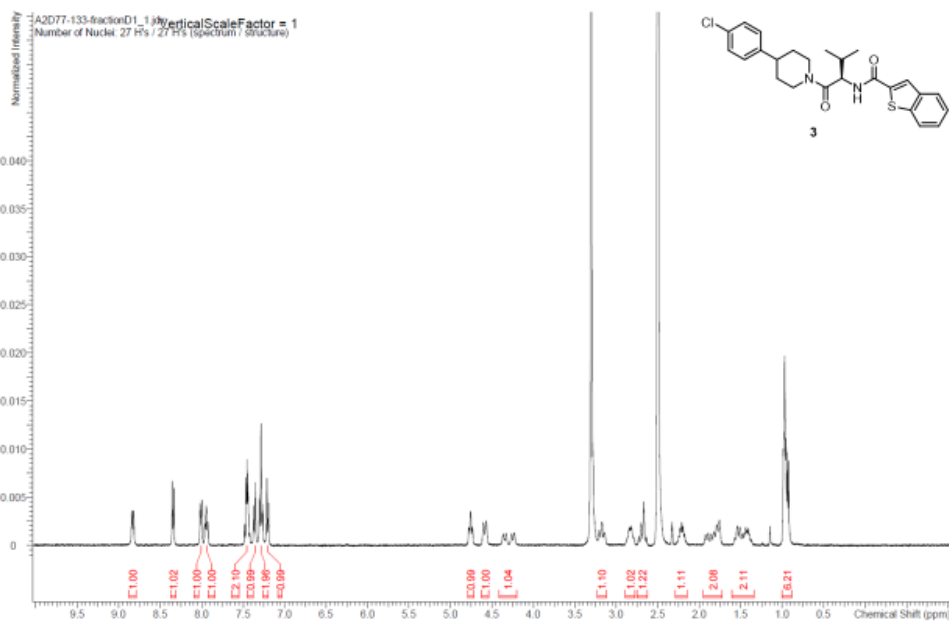


Figure S9.2 ¹H NMR (400 MHz, DMSO-d₆) of compound 3.

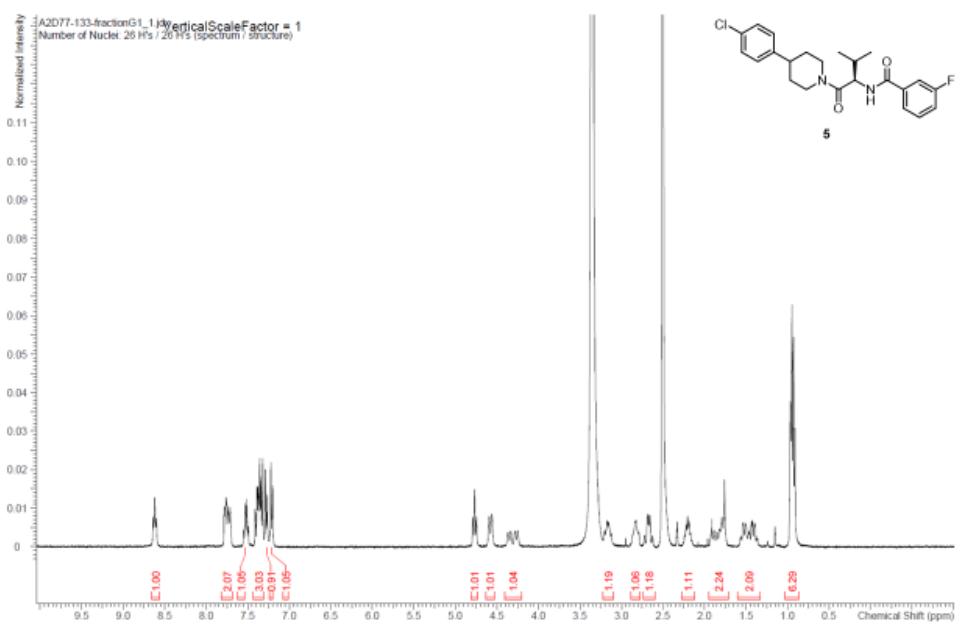


Figure S9.3 ¹H NMR (400 MHz, DMSO-d₆) of compound 5.

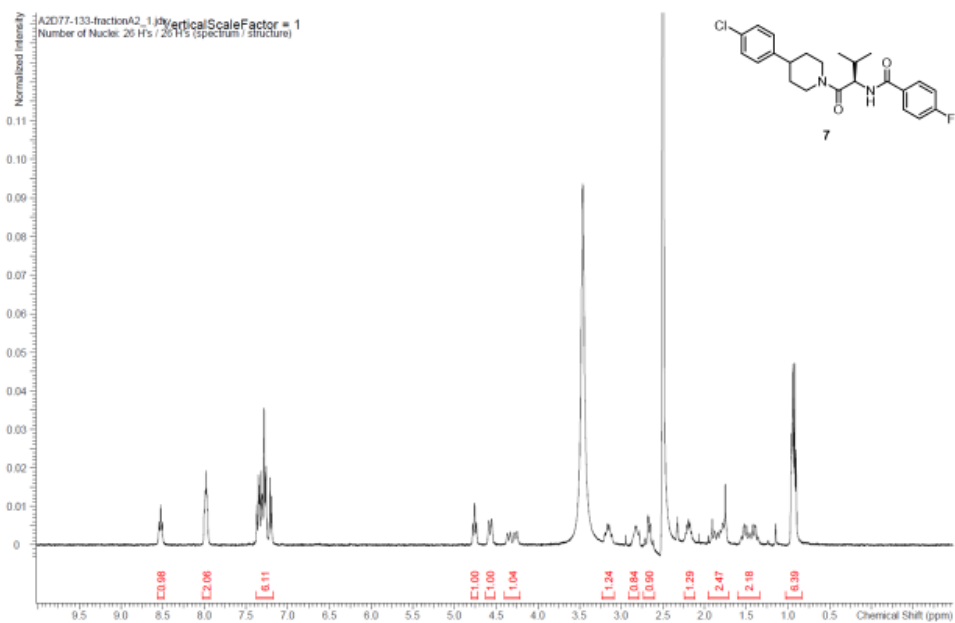


Figure S9.3 ¹H NMR (400 MHz, DMSO-d₆) of compound 7.

References:

1. C. L. Cavallaro, S. Briceno, J. Chen, M. E. Cvijic, P. Davies, J. Hynes Jr, R.-Q. Liu, S. Mandlekar, A. V. Rose and A. J. Tebben, *Journal of medicinal chemistry*, 2012, **55**, 9643-9653.
2. *US Pat.*, US7601844, 2009.

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