Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Scaffold hopping enables direct access to more potent PROTACs with *in vivo* activity

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SUPPLEMENTAL FIGURES

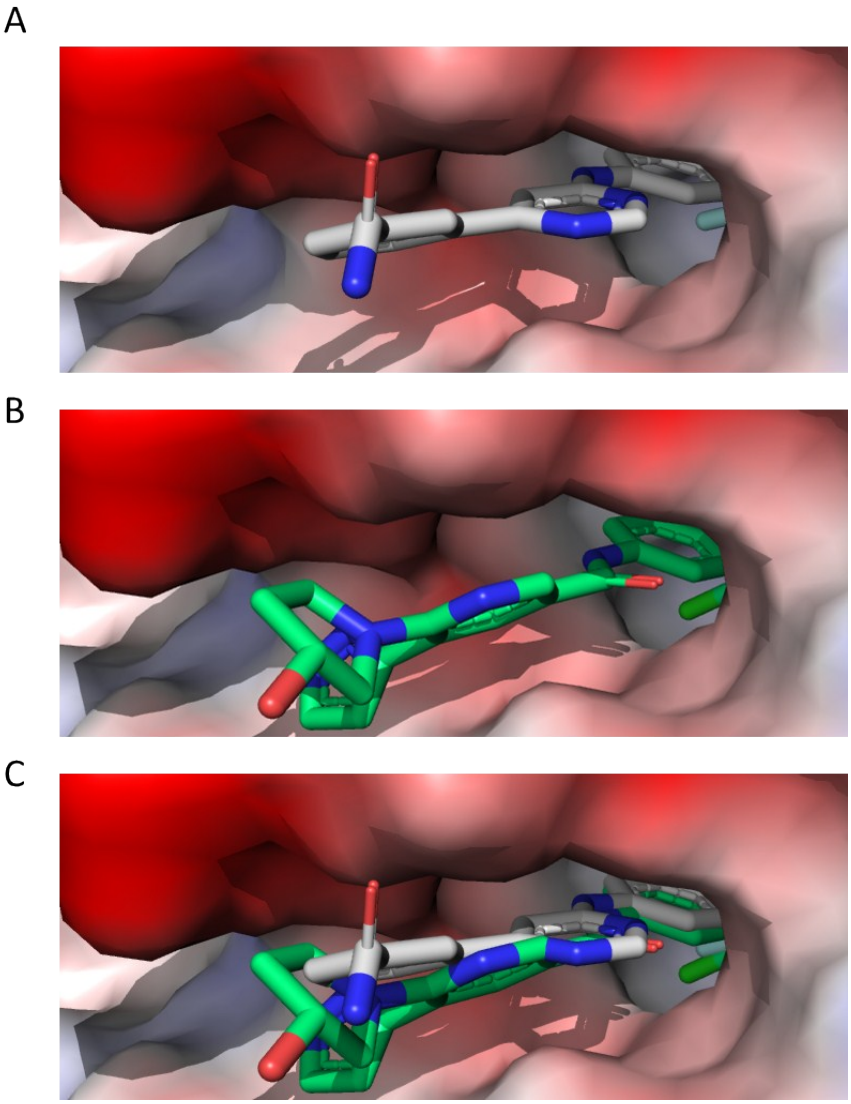


Figure S1 – Co-Crystal structures of Abl with GNF-2 [PDB ID: 3K5V[1]] (a) Abl001 [PDB ID: 5MO4[2]] (b) or overlay (c).

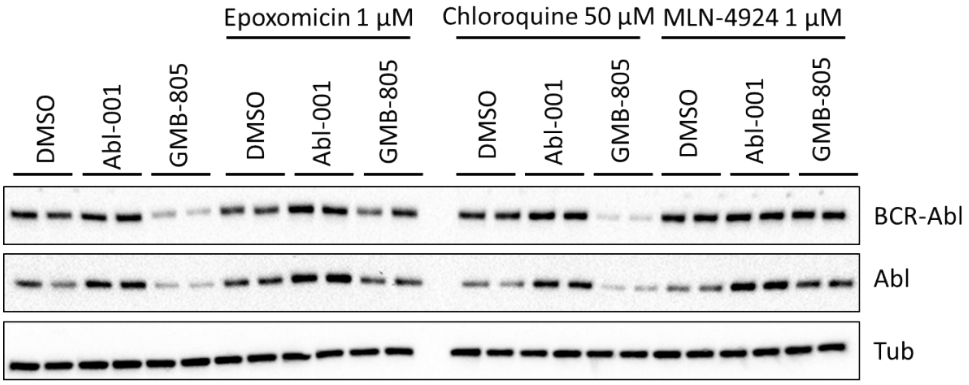


Figure S2 – Co-Treatment Experiments to confirm PROTAC Mechanism. K562 Cells were treated with 1 µM Abl-001 or GMB-805 for 8 hours in the presence of the indicated compounds.

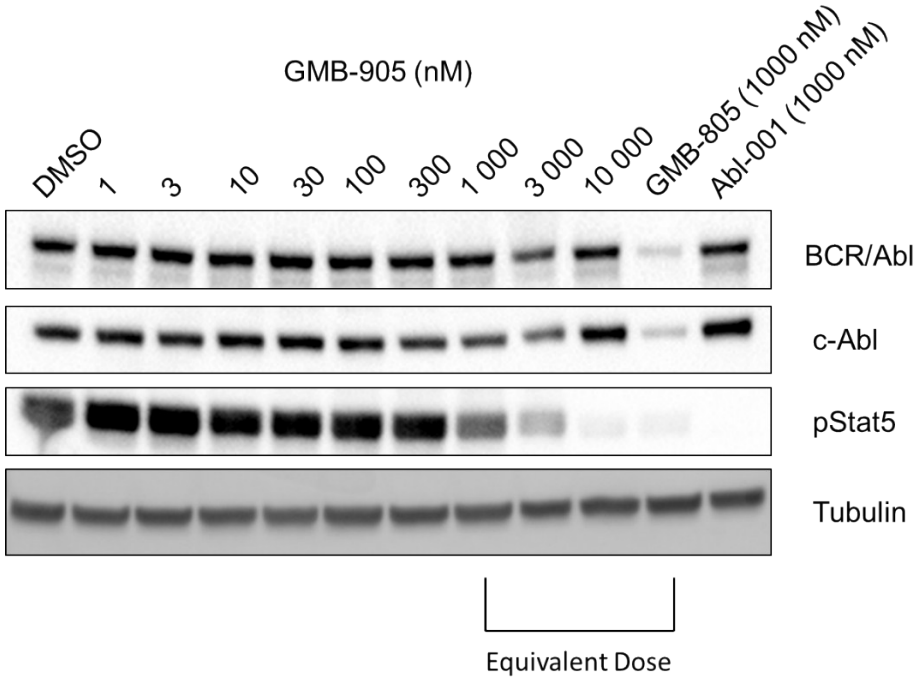
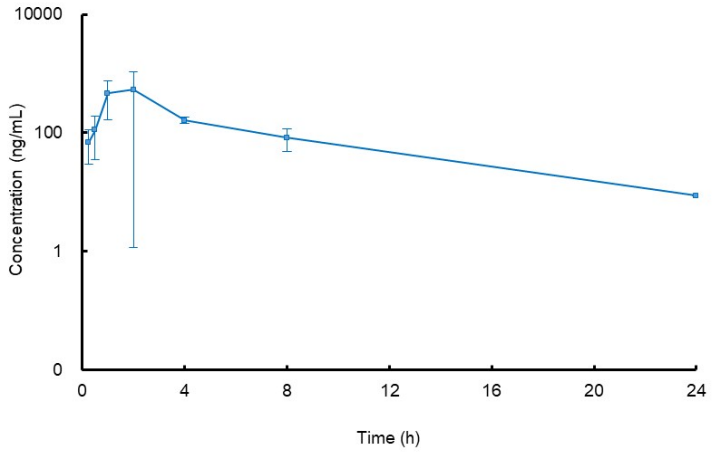


Figure S3 Characterisation of GMB-905 (Diastereomer of GMB-805). K562 cells were treated with the indicated compounds for 24 hours.



$t_{1/2}$ (h)	t_{max} (h)	C_{max} (ng/mL)	AUC_{last} (h*ng/mL)
3.67	2.00	536	2419

Figure S4 Pharmacokinetic profile of GMB-805 in mouse

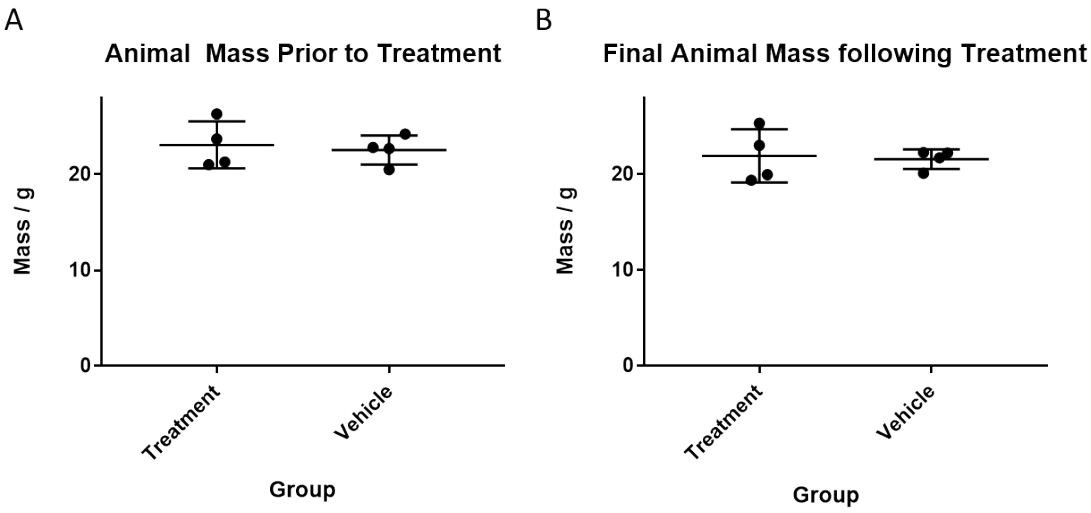


Figure S5 Treatment with GMB-805 induced no weight loss

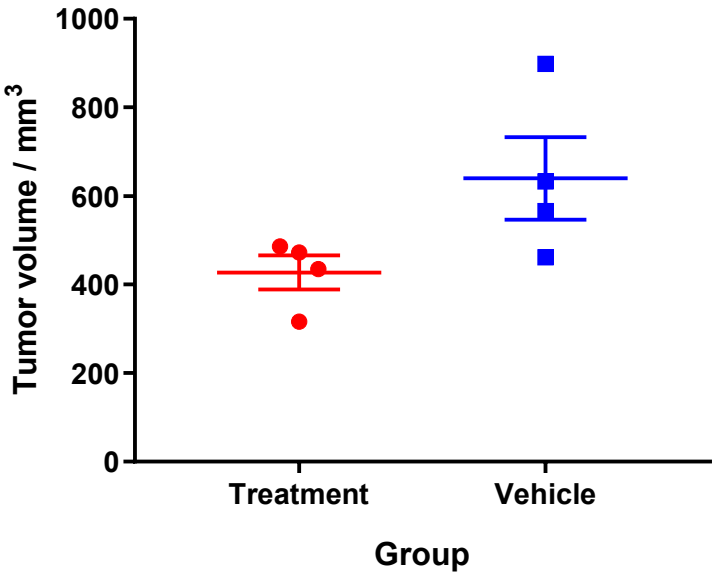


Figure S6 – Final tumor volumes from GMB-805 treated (red) or vehicle (blue) treated animals

MATERIALS AND METHODS

Cell lines

The K562 cell line was purchased from ATCC and cultured in IMDM (Gibco) supplemented with 10% FBS and penicillin/streptomycin (Invitrogen). All cell lines were confirmed mycoplasma negative prior to experimental use.

Immunoblot analysis

Cells were treated with the indicated concentrations of compound for 24 hours, washed with PBS and then harvested in lysis buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 0.5% NP-40, phosphatase and protease inhibitors). Following centrifugation at 13,000 x g for 15 min at 4 °C to pellet insoluble materials, the protein concentrations of the supernatants were quantitated by BCA assay (Thermo Fisher Scientific). Protein samples were resolved by SDS-PAGE, transferred to nitrocellulose, blocked with 5% non-fat milk and probed with the indicated antibodies (c-ABL1, SantaCruz #24-11 anti-mouse 1:1000; tubulin, Millipore #05-66—MI anti-mouse 1:5000; pSTAT5, #CS9351S anti-rabbit 1:1000). Immunoblots were developed using enhanced chemiluminescence and visualized using a Bio-Rad Chemi-Doc MP Imaging System with Image Lab v.5.2.1 software (Bio-Rad Laboratories). DC_{50} values were calculated from band density of BCR-Abl normalized to tubulin and non-linear regression in Prism 6 software (GraphPad).

Cell viability assay

Cells were exposed to dose ranges of compounds and incubated for 3 days at 37°C, 5% CO_2 and subjected to a CellTiter 96 AQueous One solution cell proliferation assay (Promega). The values were normalized to the controls. IC_{50} values were calculated using Prism 6 software (GraphPad) employing the "[Inhibitor] vs. normalized response -- Variable slope" method of non-linear regression.

Pharmacokinetics

The pharmacokinetic properties were determined at Pharmaron, China. The PROTAC was formulated (in 5% EtOH & 5% Solutol HS15 in D5W(ESD-2)) and administered by I.P. injection at a dosing level of 10 mg/kg (n=3). Plasma samples were collected at 0.25h, 0.5h, 1h, 2h, 4h, 8h, 24h following dosing. Acetonitrile was added to precipitate protein and the samples were vortexed for 30 s, centrifuged (4000 rpm, 15 minutes), diluted with water and the compound concentration determined by LC/MS/MS according to a standard curve. $T_{1/2}$ was calculated using non-compartmental analysis. [3]

In vivo Xenograft model

All in vivo experiments were conducted in accordance with institutional guidelines and were approved by Yale Institutional Animal Care & Use Committee. Mice were housed in pathogen-free animal

facilities and with access to food and water ad libitum. K562 cells in Matrigel (Corning Life Sciences) were injected subcutaneously into the flank of athymic mice (Charles River) and allowed to proliferate until tumours reached ~200 mm^3 at which point mice were randomized into treatment groups and treated I.P. with vehicle or PROTAC once a day at 200 mg/kg for 3 days. Tumour were measured via caliper and volumes calculated following equation 1.

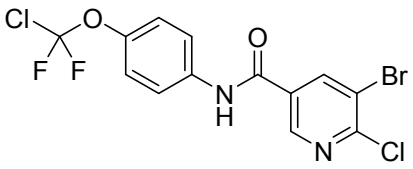
Equation 1

$$V = 0.5 \; x \; L \; x \; W^2$$

Where V = volume, L = Length and W = Width

PROTAC synthesis

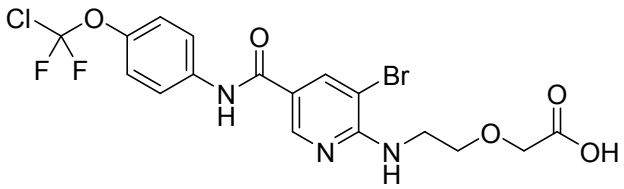
5-bromo-6-chloro-N-(4-(chlorodifluoromethoxy)phenyl)nicotinamide



Following a literature procedure,[2] 5-bromo-6-chloronicotinic acid (300 mg, 1.27 mmol) was suspended in toluene and treated with DMF (30 µl, 30 mol%) followed by thionyl chloride (278 µl, 3.81 mmol). The reaction mixture was then heated to 80 °C for 1 hour with stirring before being concentrated *in vacuo* and resuspended in anhydrous THF. The acid chloride solution was treated with triethylamine (442 µl, 3.17 mmol) followed by a solution of 4-(chlorodifluoromethoxy)aniline (258 mg, 1.33 mmol) in THF. The reaction mixture was stirred at r.t. for 1 hour, concentrated *in vacuo* and purified by column chromatography eluting with 0-50% ethyl acetate/hexane to yield the title compound (395 mg, 76%).

Data matches literature reports.[2]

2-(2-((3-bromo-5-((4-(chlorodifluoromethoxy)phenyl)carbamoyl)pyridin-2-yl)amino)ethoxy)acetic acid

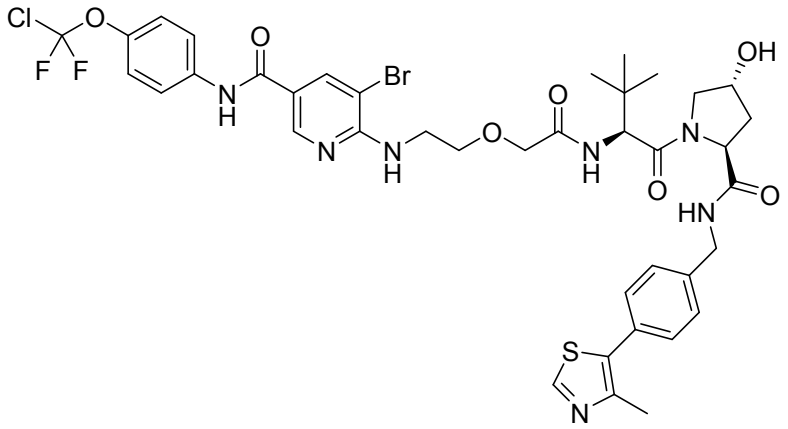


5-bromo-6-chloro-N-(4-(chlorodifluoromethoxy)phenyl)nicotinamide (50 mg, 0.121 mmol) and 2-(2-aminoethoxy)acetic acid (17 mg, 0.146 mmol) were suspended in isopropanol and treated with triethylamine (37 µl, 0.267 mmol) and heated to 140 °C under microwave conditions for 6 hours. The reaction mixture was concentrated *in vacuo* and purified by column chromatography eluting with 0-15% methanol/DCM to yield the title compound (35 mg).

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.61 (d, J = 2.1 Hz, 1H), 8.22 (d, J = 2.1 Hz, 1H), 7.74 (d, J = 9.1 Hz, 2H), 7.22 (d, J = 8.7 Hz, 2H), 4.02 (s, 2H), 3.18 (q, J = 7.3 Hz, 1H), 1.29 (t, J = 7.3 Hz, 3H).

LC-MS (ESI) m/z: 494.496

5-bromo-N-(4-(chlorodifluoromethoxy)phenyl)-6-((2-(2-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)ethyl)amino)nicotinamide

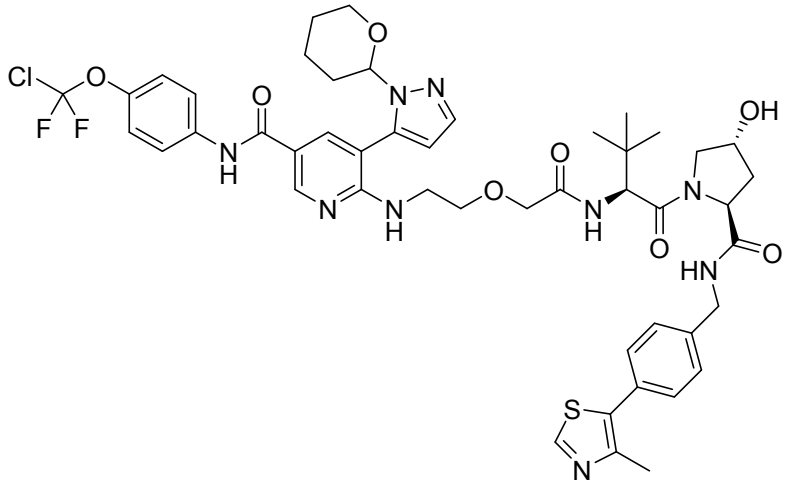


2-[2-[[3-bromo-5-[[4-[chloro(difluoro)methoxy]phenyl]carbamoyl]-2-pyridyl]amino]ethoxy]acetic acid (35 mg, 0.072 mmol) dissolved in DMF (10 ml) and treated sequentially with HATU (27 mg, 0.072 mmol), triethylamine (30 µl, 0.216 mmol) and (2S,4R)-1-[(2S)-2-amino-3,3-dimethyl-butanoyl]-4-hydroxy-N-[[4-(4-methylthiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide (31 mg, 0.072 mmol). The reaction mixture was stirred overnight at r.t., diluted with ethyl acetate (20 ml), washed with water (10 ml) and brine (10 ml), dried over $MgSO_4$ and concentrated *in vacuo*. Purified by column chromatography eluting with 0-10% methanol/DCM to yield the title compound (18 mg, 28%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H), 8.94 (s, 1H), 8.63 (d, J = 2.1 Hz, 1H), 8.55 (t, J = 6.0 Hz, 1H), 8.27 (d, J = 2.1 Hz, 1H), 7.81 (d, J = 9.1 Hz, 2H), 7.44 (d, J = 9.5 Hz, 1H), 7.36 (s, 2H), 7.30 (d, J = 8.7 Hz, 2H), 7.09 – 7.00 (m, 1H), 5.12 (d, J = 3.5 Hz, 1H), 4.52 (d, J = 9.5 Hz, 1H), 4.45 – 4.26 (m, 3H), 4.21 (dd, J = 15.8, 5.7 Hz, 1H), 3.98 (d, J = 2.3 Hz, 2H), 3.73 – 3.53 (m, 4H), 3.06 (s, 1H), 2.40 (s, 3H), 2.00 (d, J = 8.4 Hz, 1H), 1.94 – 1.79 (m, 1H), 1.13 (s, 2H), 0.89 (s, 9H).

LC-MS (ESI) m/z: 906.908

N-(4-(chlorodifluoromethoxy)phenyl)-6-((2-(2-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methyl-1,2,3-thiadiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)ethyl)amino)-5-(1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazol-5-yl)nicotinamide



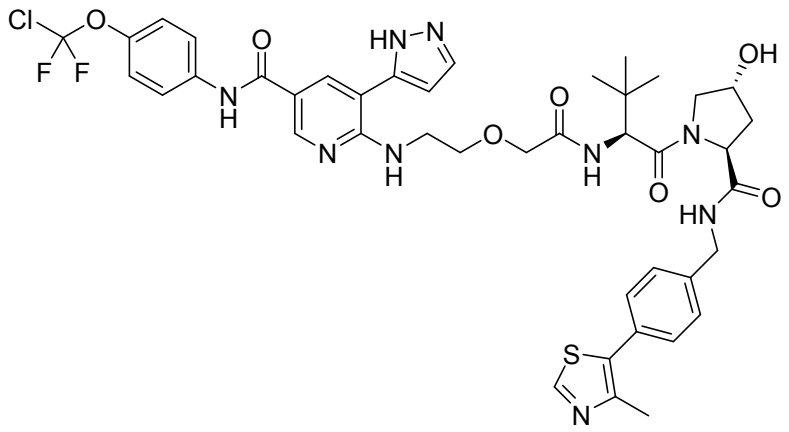
5-bromo-N-(4-(chlorodifluoromethoxy)phenyl)-6-((2-(2-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl) benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy) ethyl)amino)nicotinamide (18 mg, 0.026 mmol), 1-tetrahydropyran-2-yl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazole (7.2 mg, 0.026 mmol), tetrakis(triphenylphosphine)palladium(0) (2.3 mg, 10 mol%) and tripotassium phosphate (13 mg, 0.060 mmol) were suspended in toluene (5 ml), placed under a nitrogen atmosphere and heated to 110°C overnight. Concentrated *in vacuo* and purified by preparative TLC eluting with 10% methanol/DCM (14 mg)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.85 (d, *J* = 16.4 Hz, 1H), 8.76 – 8.40 (m, 2H), 7.90 (t, *J* = 2.9 Hz, 1H), 7.76 – 7.65 (m, 2H), 7.63 (d, *J* = 1.7 Hz, 1H), 7.28 (t, *J* = 7.3 Hz, 3H), 7.16 (d, *J* = 8.6 Hz, 2H), 7.07 (dd, *J* = 9.2, 5.7 Hz, 1H), 6.33 (dd, *J* = 7.2, 1.8 Hz, 1H), 5.46 (d, *J* = 6.0 Hz, 1H), 4.97 (ddd, *J* = 10.6, 5.1, 2.4 Hz, 1H), 4.74 – 4.24 (m, 5H), 4.17 – 3.85 (m, 4H), 3.80 – 3.35 (m, 7H), 2.44 (d, *J* = 1.7 Hz, 4H), 2.33 – 2.21 (m, 1H), 2.21 – 1.90 (m, 5H), 1.80 (d, *J* = 13.3 Hz, 1H), 1.74 – 1.41 (m, 3H), 1.18 (t, *J* = 7.0 Hz, 2H), 0.91 (d, *J* = 4.8 Hz, 9H).

HRMS: calc. $[M+H]^+$ for $C_{47}H_{54}ClF_2N_9O_8S$ = 978.3545; found = 978.3815$[M+H]^+$.

GMB-805

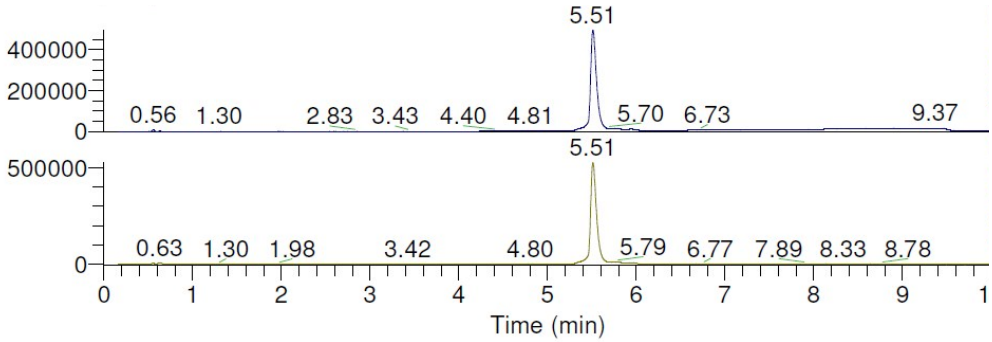
N-(4-(chlorodifluoromethoxy)phenyl)-6-((2-(2-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methyl-1,2,3-thiadiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)ethyl)amino)-5-(1H-pyrazol-5-yl)nicotinamide,



N-(4-(chlorodifluoromethoxy)phenyl)-6-((2-(2-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methyl-1,2,3-thiadiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)ethyl)amino)-5-(1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazol-5-yl)nicotinamide (14 mg, 0.014 mmol) was dissolved in 20% TFA/DCM and stirred for 2 hours. Concentrated *in vacuo* and purified by preparative TLC eluting with 10% methanol/DCM.

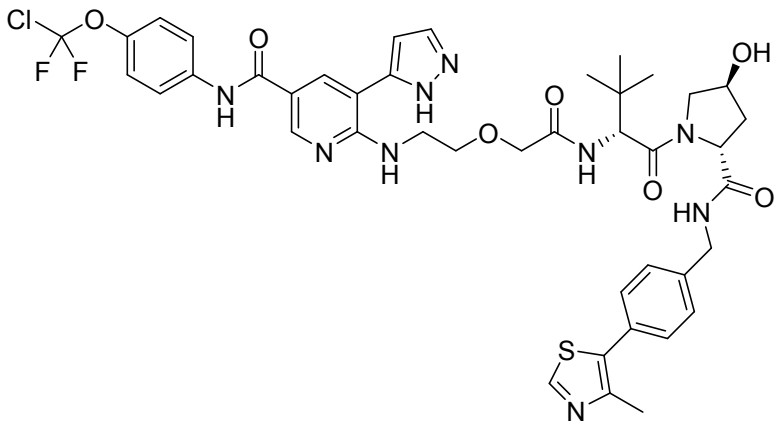
^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.72 (s, 1H), 9.40 (s, 1H), 8.66 (d, J = 1.9 Hz, 1H), 8.51 (d, J = 2.2 Hz, 1H), 8.34 (s, 1H), 8.17 (d, J = 2.3 Hz, 1H), 7.79 – 7.71 (m, 2H), 7.37 (d, J = 8.1 Hz, 2H), 7.32 (d, J = 8.2 Hz, 2H), 7.22 (s, 1H), 7.07 (d, J = 2.4 Hz, 1H), 6.83 (d, J = 7.0 Hz, 1H), 6.55 (d, J = 2.4 Hz, 1H), 4.74 – 4.47 (m, 4H), 4.38 – 4.28 (m, 2H), 4.01 (s, 2H), 3.79 – 3.69 (m, 2H), 2.40 (s, 3H), 1.23 (s, 4H), 0.99 (s, 9H), 0.92 (d, J = 3.3 Hz, 2H).

HRMS: calc. $[M+H]^+$ for $C_{42}H_{46}ClF_2N_9O_7S$ = 894.2970; found = 894.3103$[M+H]^+$.



GMB-905

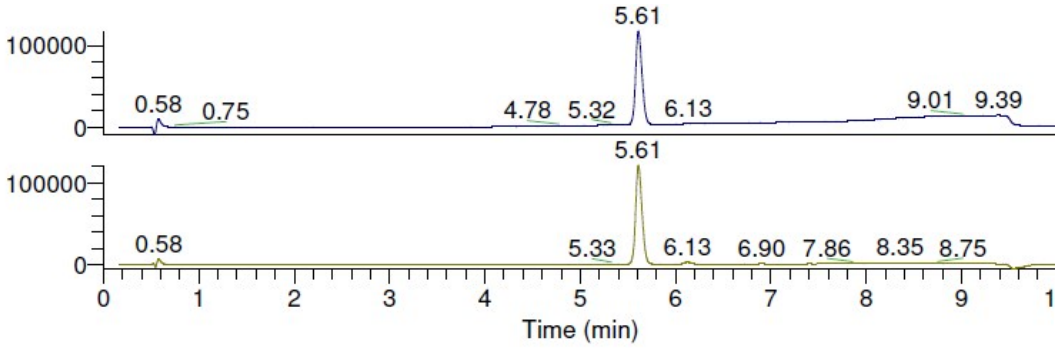
N-(4-(chlorodifluoromethoxy)phenyl)-6-((2-(2-(((R)-1-((2R,4S)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)ethyl)amino)-5-(1H-pyrazol-5-yl)nicotinamide



Prepared as described above but using the diastereomeric VHL ligand.

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.13 (s, 1H), 10.19 (s, 1H), 8.94 (s, 1H), 8.65 – 8.59 (m, 2H), 8.28 (s, 1H), 7.85 (dd, J = 9.9, 2.7 Hz, 2H), 7.32 (d, J = 8.7 Hz, 2H), 6.91 (s, 1H), 6.25 (s, 1H), 5.42 (s, 1H), 4.42 – 4.13 (m, 4H), 3.99 (s, 2H), 3.91 – 3.67 (m, 4H), 2.63 (d, J = 2.0 Hz, 2H), 2.39 (s, 3H), 2.29 (t, J = 1.9 Hz, 2H), 1.71 (dt, J = 12.3, 6.1 Hz, 1H), 1.20 (s, 2H), 0.88 (s, 9H).

HRMS: calc. $[M+H]^+$ for $C_{42}H_{46}ClF_2N_9O_7S$ = 894.2970; found = 894.3213 $[M+H]^+$.



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