SUPPORTING INFORMATION

Selective inhibitor of platelet-activating factor acetylhydrolases 1b2 and 1b3 that impairs cancer cell survival

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SUPPORTING FIGURES AND TABLES

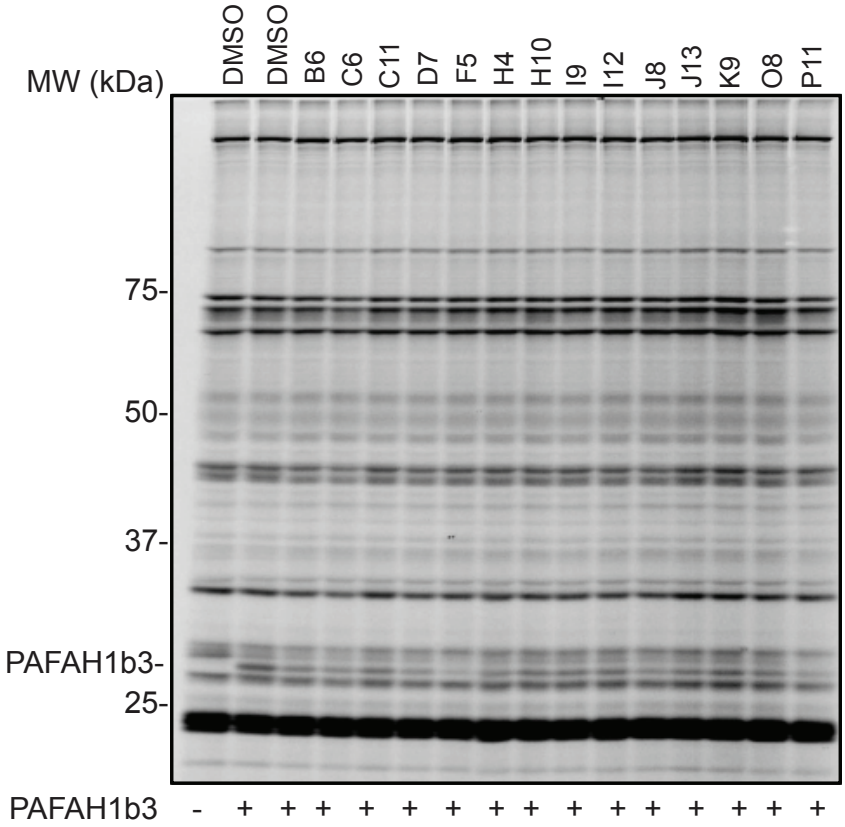


Figure S1. Gel-based ABPP analysis of functionalized tetrahydropyridines as inhibitors of PAFAH1b3. Mouse brain soluble proteomes doped with recombinant mouse PAFAH1b3 (1 μM) were treated with test compounds (10 μM, 30 min at 37 °C) followed by FP-alkyne (1 μM, 10 min at 25 °C). Fluorescent gel shown in grayscale.

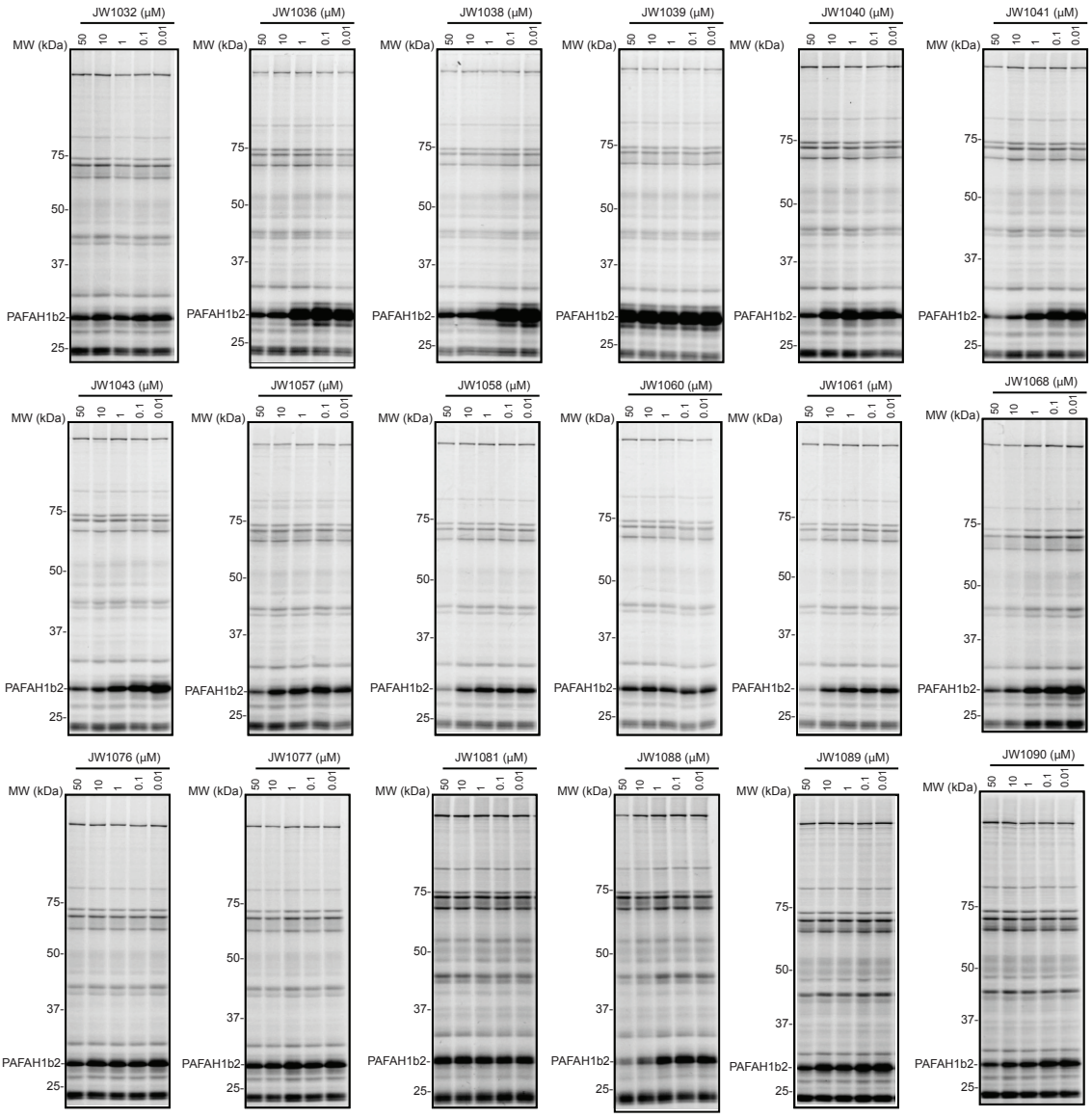


Figure S2. Representative gel-based ABPP results for functionalized tetrahydropyridines analyzed in mouse brain soluble proteomes doped with recombinant mouse PAFAH1b2 (1 μM).

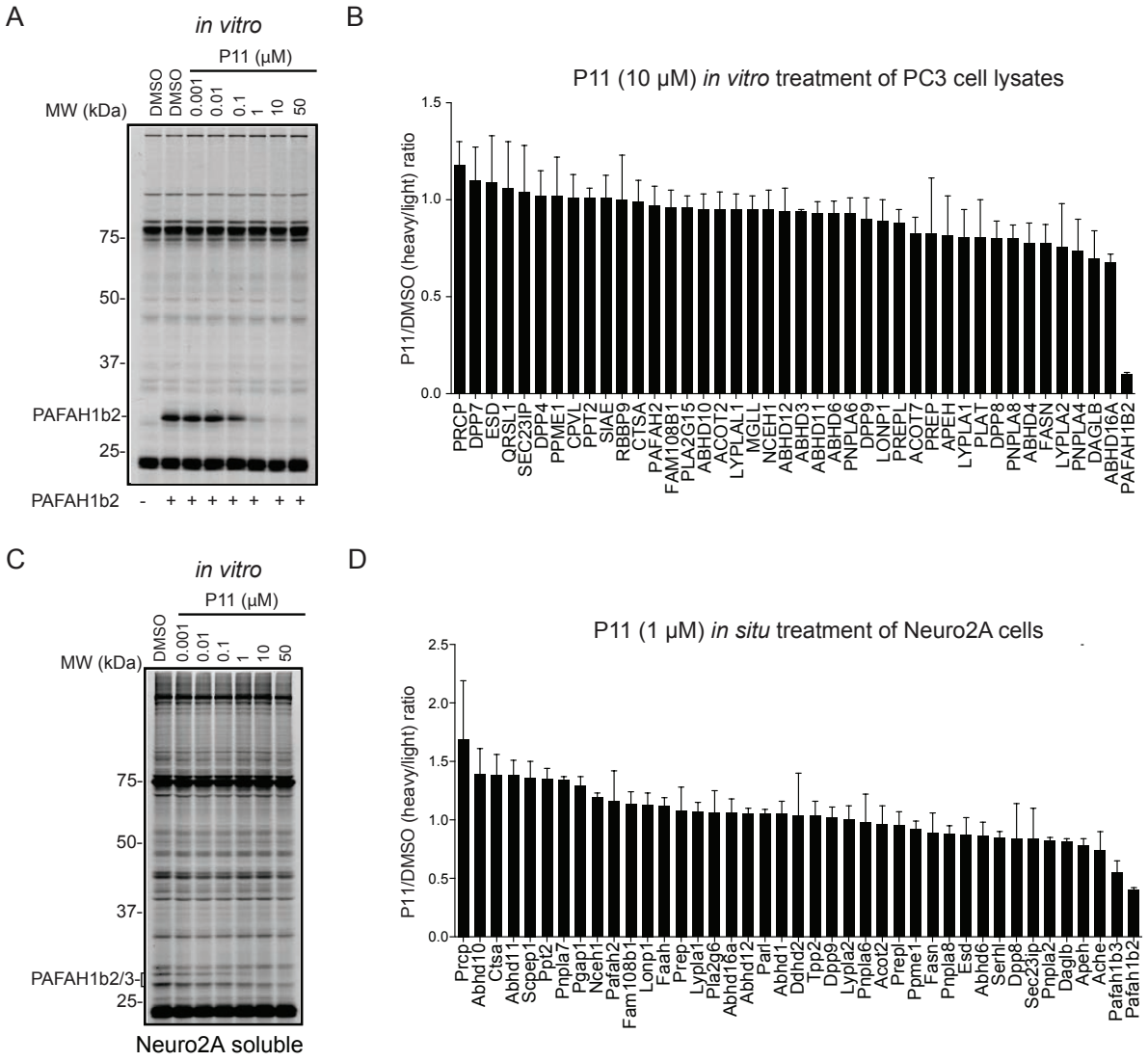


Figure S3. Gel- and MS-based ABPP analysis of PAFAH1b2/3 inhibitor P11. (A) Analysis of P11 activity in the presence of detergent. Competitive gel-based ABPP assays were performed as described in **Figure 2A**, with the addition of 0.01% Triton X-100 into the reactions. The IC_{50} value for inhibition of PAFAH1b2 by P11 was unaffected by the detergent (IC_{50} (without Triton) = 0.83 μM; IC_{50} (with Triton) = 0.88 μM). (B) *In vitro* activity of P11 against human PAFAH1b2 in PC3 proteomes was measured by ABPP-SILAC analysis. Heavy and light proteomes from PC3 cells cultured in isotopically labeled amino acids were treated with P11 (10 μM) or DMSO, respectively, for 30 min at 37 °C. Serine hydrolase activities were then enriched with FP-biotin (1 μM, 30 min, 37 °C) and activities detected by LC-MS/MS, demonstrating that P11 blocked PAFAH1b2 activity while not cross-reacting with other serine hydrolases detected in the PC3 proteome. Note that PAFAH1b3 was not detected in PC3 proteomes in agreement with previous reports.[1] Data are reported as mean values ± SEM for two independent experiments (C) Gel-based ABPP of soluble proteomes from Neuro2A cells treated with P11 at the indicated concentrations (30 min), followed by FP-alkyne (1 μM, 10 min). Fluorescent gel shown in grayscale. (D) ABPP-SILAC analysis of inhibitor-treated Neuro2A cells. Light and heavy-labeled cells were treated *in situ* with DMSO or P11 (1 μM), respectively, for 4 h, followed by FP-alkyne (1 μM, 30 min).

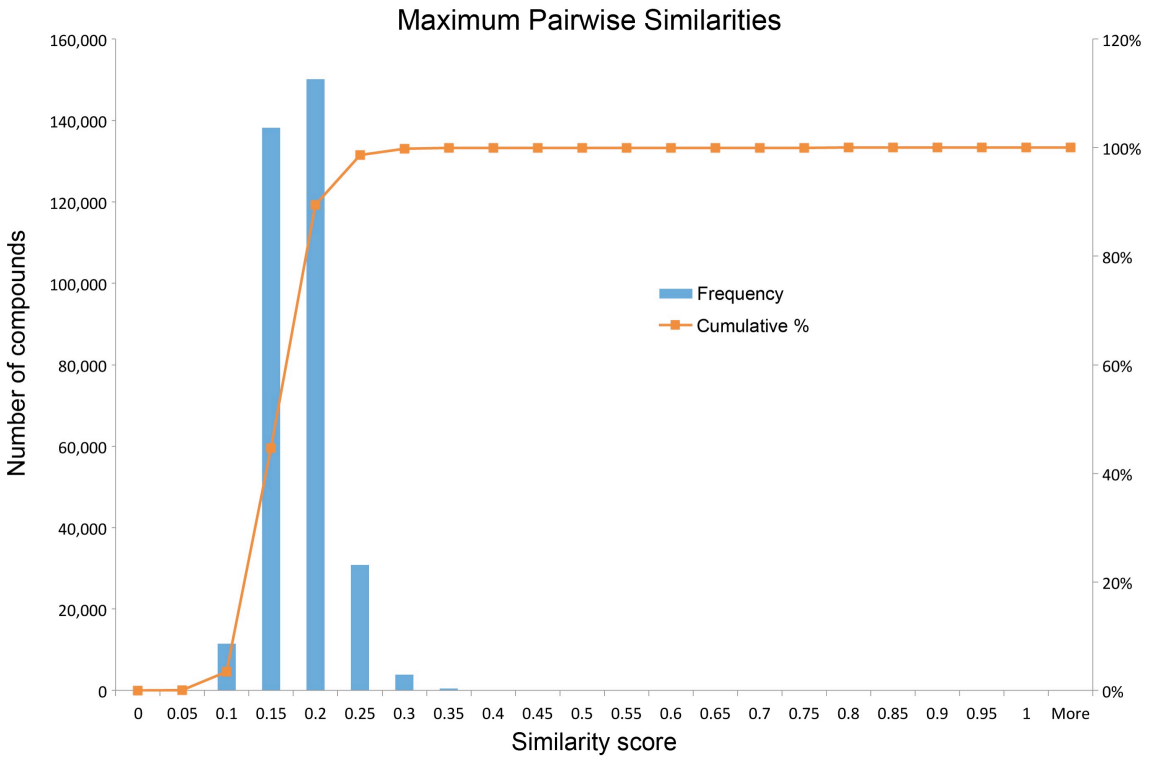
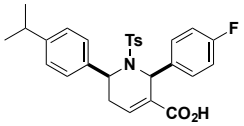
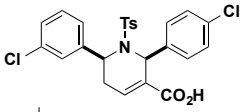
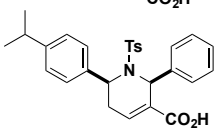
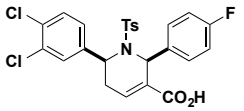
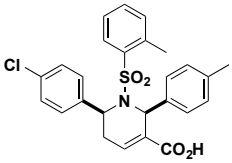
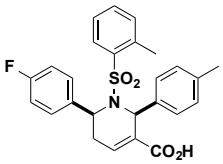
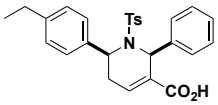
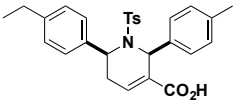
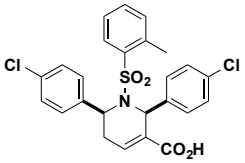
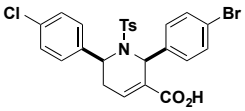
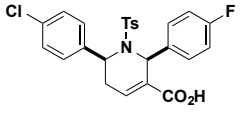
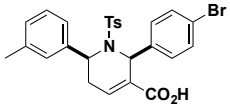


Figure S4. Histogram of the closest pairwise similarities (via ECFP-6 fingerprints and the Tanimoto metric) between the MLSMR library and any of the tetrahydropyridine active compounds. These neighborhood statistics reveal that 98% of MLSMR compounds have a similarity to the tetrahydropyridines of 0.25 or less and 99.9% of the MLSMR have a similarity value of 0.4 or less.

Compound	Structure	% inhibition *in vitro* 20 μM
B6		92
B8		80
B10		85
C6		85
C7		61
C11		65
D7		62
F5		83
G7		48
H4		92
H10		88
I5		89

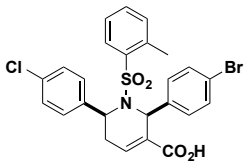
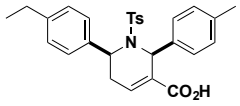
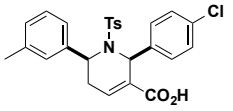
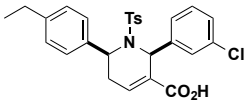
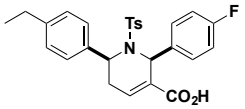
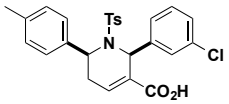
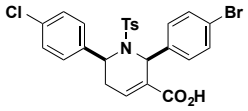
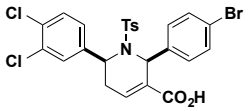
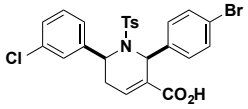
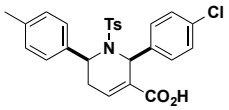
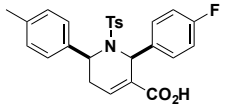
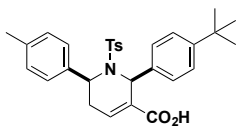
Compound	Structure	% inhibition *in vitro* 20 μM
I8		71
I9		?
I12		34
J8		52
J11		81
J13		65
K7		80
K9		92
L12		72
N9		84
O8		88
P11		99

Table S1. Structures and percentage inhibition values for various functionalized tetrahydropyridines tested against recombinant mouse PAFAH1b2. Inhibitors were screened at 20 μM by gel-based ABPP in mouse brain soluble proteome doped with recombinant mouse PAFAH1b2 (1 μM). % inhibition values were determined using gel-based competitive ABPP by quantifying reductions of FP-alkyne labeling from gel images following preincubation of mouse brain soluble proteomes with each inhibitor.

	PAFAH1b2	PAFAH2	PLA2G7	SIAE	PREPL	DAGLB	ABHD4	HTRA1	PAD4	PRMT1
% inhibition	86%, 93%	-1%	15%	-12%	-7%	14%	9%	-95%	2%	5%

Table S2. Inhibitory values for P11 screened against PAFAH1b2 and nine other enzymes (seven serine hydrolases) by fluopol-ABPP as part of the MLPCN program. Note that P11 only showed activity against PAFAH1b2 and did not appreciably inhibit the other screened enzymes, including two additional PAF hydrolases (PAFAH2 and PLA2G7)

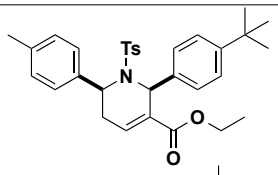
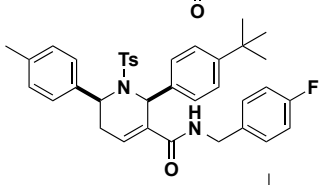
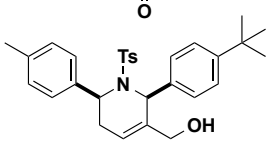
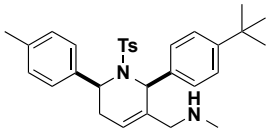
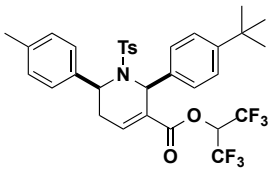
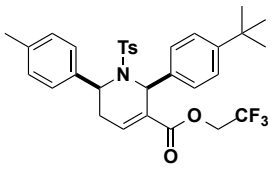
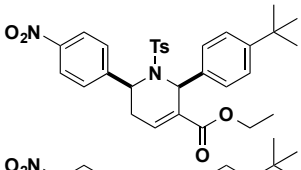
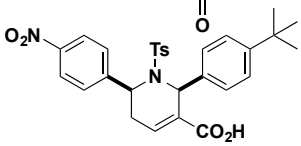
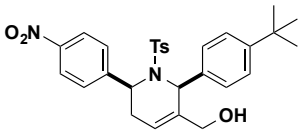
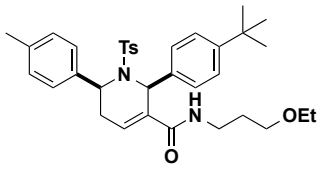
Compound	Structure	% inhibition *in vitro* 10 μM
JW1032		3
JW1040		5
JW1057		7
JW1060		6
JW1076		4
JW1077		5
JW1081		1
JW1088		1
JW1089		8
JW1090		11

Table S3. Structures and percentage inhibition values for P11 analogues tested against recombinant PAFAH1b2. Inhibitors were screened at 10 μM by gel-based ABPP in mouse brain soluble proteome doped with recombinant mouse PAFAH1b2 (1 μM). % inhibition values were determined using gel-based competitive ABPP by quantifying reductions of FP-alkyne labeling from gel images following preincubation of mouse brain soluble proteomes with each inhibitor.

Table S4. Proteomic data for ABPP-MudPIT experiments.

See accompanying Excel spreadsheet.

Table S5. Proteomic data for ABPP-SILAC experiments.

See accompanying Excel spreadsheet.

SUPPORTING MATERIALS AND METHODS

Materials. FP-alkyne[2], and FP-Biotin[3], and FP-Rh[4], were synthesized as described previously. Chemical reagents were obtained from Sigma-Aldrich or ThermoFisher unless otherwise indicated. Cell culture media and supplements were obtained from CellGro and Omega Scientific.

Analysis of primary and secondary screening data. Primary screening data for P11 are available online at PubChem (https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?cid=16656440). In collaboration with the Molecular Libraries Probe Production Centers Network (MLPCN), we screened the 300,000+ National Institutes of Health (NIH) compound library for PAFAH1b2 inhibition using fluopol-ABPP (**Figure 1A**, **B**). In brief, the well fluorescence polarization median value (mP) was obtained using the PerkinElmer Viewlux software to calculate the percent inhibition for each compound: Percent Inhibition = $(mP^{\text{Test Compound}} - mP^{\text{Negative Control}}) / (mP^{\text{Positive Control}} - mP^{\text{Negative Control}}) * 100$. The test compound was defined as wells containing PAFAH1b2 in the presence of test compound, negative controls were defined as wells containing PAFAH1b2 and DMSO, and positive controls were defined as wells containing no PAFAH1b2 protein. These data were used to calculate 1) the average percent inhibition of all compounds tested, and 2) three times their standard deviation. The sum of these two values was used as a cutoff parameter. Any compound that showed greater inhibition than the cutoff parameter (24.1%) was declared active. The reported PubChem Activity Score (https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=492953) has been normalized to 100% observed primary inhibition. Negative % inhibition values are reported as activity score zero. The activity score range for active compounds is 100-13, and for inactive compounds 13-0. From the initial 4,161 active compound list, we chose only those showing >50% inhibition of the PAFAH1b2 fluopol signal resulting in 1,118 compounds (0.37% hit rate). From this filtered list, we chose compounds for follow-up studies that had < 4% hit rate in all other bioassays reported and were not active in previous screens performed against other serine hydrolases (http://pubchem.ncbi.nlm.nih.gov/). The additional filter step yielded 172 candidate PAFAH1b2 inhibitors, which were subjected to secondary screening by gel-based competitive ABPP assays.

Preparation of Mouse Tissue Proteomes. Mouse brains were Dounce-homogenized on ice in PBS (pH 7.5) followed by a low-speed spin (1,400 x *g*, 5 min) to remove debris. After sonication, the supernatant was then centrifuged (100,000 x *g*, 45 min) to provide the cytosolic fraction in the supernatant and the membrane fraction as a pellet. The pellet was washed and resuspended in PBS by sonication. Protein concentrations were determined using a protein assay kit (Bio-Rad). Samples were stored at -80 °C until use.

Cell Culture and Preparation of Neuro2A and PC3 Proteomes. Neuro2A murine neuroblastoma and human PC3 cells were grown in DMEM and F12K media supplemented with 10% fetal bovine serum and 2 mM L-glutamine in a humidified 5% CO_2 incubator at 37 °C, respectively. Cells were harvested and separated into membrane and soluble fractions as described for mouse tissue proteomes.

Competitive ABPP Assays in Proteomes with FP-alkyne. The following procedure was adapted from a previously reported method[5]. For *in vitro* experiments, proteomes were diluted to 1 mg/mL in PBS (pH 7.5, 50 µL total reaction volume), doped with 1 µM recombinant PAFAH1b2 and PAFAH1b3, and incubated with compound at the indicated concentrations (1 µL of a 50x stock in DMSO) for 30 min at 37 °C, followed by labeling with 1 µM FP-alkyne (1 µL of a 50x stock in DMSO) for 10 min at 25 °C. Total protein concentrations for each fraction were adjusted to 1 mg/mL in PBS (50 µL total reaction volume). Samples were then subjected to click chemistry by the addition of 1 mM $CuSO_4$ (1 µL of a 50x stock in DMSO), followed by TBTA (3 µL of a 17x stock in 4:1 *t*-

butanol:DMSO), then 25 μM Rh-N_3 (1 μL of a 50x stock in DMSO) and 1 mM TCEP (1 μL of a 50x stock in DMSO). Reactions were vortexed, incubated for 1 h at 25 °C, and quenched with 4x SDS-PAGE loading buffer and analyzed by in-gel fluorescence scanning. Percent inhibition values were calculated by quantifying reductions of FP-alkyne labeling from gel images following preincubation of proteomes with each inhibitor. IC_{50} values were determined from dose-response curves from three independent replicates for each inhibitor concentration using GraphPad Prism software.

Gel filtration analysis of inhibition. Recombinant PAFAH1b2 (250 nM) in PBS was incubated with DMSO, P11 (10 μM), or FP-alkyne (5 μM) for 30 min at 37 °C, and each reaction was split into two fractions. One fraction was reacted directly with FP-Rh (1 μM), and the other was passaged over a Sephadex G-25 M column (GE Healthcare) and then reacted with FP-Rh at a final concentration of 1 μM in 50 μL total reaction volume. The reaction was incubated for 15 min at 25 °C, quenched with 2x SDS-PAGE loading buffer, separated by SDS-PAGE, and visualized by in-gel fluorescence scanning. After fluorescence scanning, the gel was stained with Coomassie for protein load comparison.

PAF hydrolysis assays. Recombinant, purified PAFAH1b2 or PAFAH1b3 (5 nM enzyme) in Tris buffer (50 mM Tris, 150 mM NaCl, 0.01% Pluronic F-127, 1 mM EDTA, 100 μL final reaction volume) was incubated with P11 at the indicated concentrations (1 μL of a 100 x stock in DMSO) or DMSO for 30 min at 25 °C. 100 μM PAF (1 μL of a 100x stock in DMSO) was then added for 30 min at 25 °C. Reactions were quenched by the addition of 500 μL 2:1 v/v $CHCl_3$:MeOH, doped with 1 nmol d4-lysoPAF, vortexed, then centrifuged (1,400 x *g*, 3 min) to separate the phases. 30 μl of the resultant organic phase was injected onto an Agilent 1100 series LC-MSD SL instrument. LC separation was achieved with a Gemini reverse-phase C18 column (5 μm, 4.6 mm x 50 mm, Phenomenex). Mobile phase A was composed of 95:5 (v/v) H2O:MeOH, and mobile phase B was composed of 60:35:5 (v/v/v) *i*-PrOH:MeOH:H2O. 0.1% ammonium hydroxide and 0.1% formic acid was included to assist in negative and positive ion formation, respectively. The flow rate was 0.75 mL/min and the gradient consisted of 1.5 min 0% B, a linear increase to 100% B over 5 min, followed by an isocratic gradient of 100% B for 3.5 min before equilibrating for 2 min at 0% B. MS analysis was performed with an electrospray ionization (ESI) source. The capillary voltage was set to 3.0 kV and the fragmentor voltage was set to 100 V. The drying gas temperature was 350 °C, the drying gas flow rate was 10 mL/min, and the nebulizer pressure was 35 psi. Hydrolysis products were quantified by measuring the area under the peak in comparison to a d_4-lysoPAF standard (Cayman).

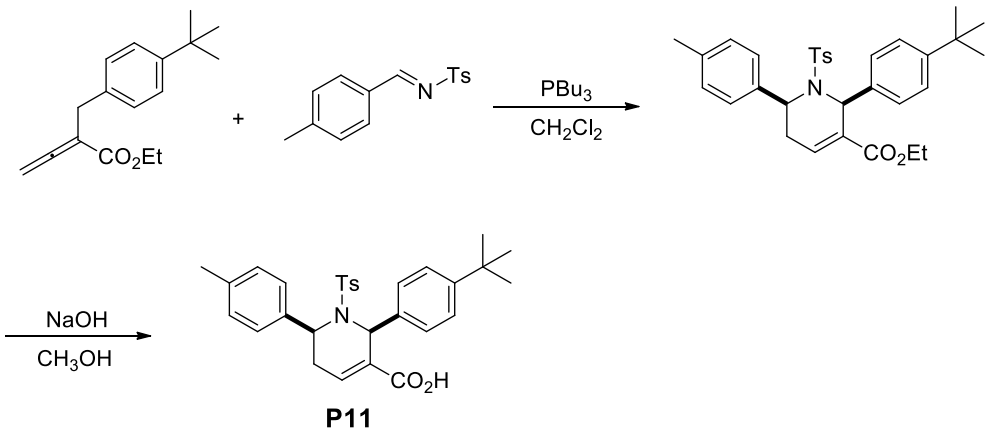
Competitive ABPP-MudPIT of Mouse Brain Proteomes. Mouse brain soluble and membrane proteomes (1 mg/mL in 50 mM Tris pH 8.0) was treated with 5 μM P11 (20 μL of a 50x DMSO stock) or DMSO (20 μL) for 30 min at 25 °C, followed by 1 μM FP-biotin for 30 min at 25 °C (1 mL total reaction volume). Proteomes were then solubilized with 1% Triton X-100 and rotated at 4 °C for 1 h, desalted over PD-10 desalting columns (GE Healthcare), and FP-labeled proteins were enriched with streptavidin beads as previously described[6]. The enrichments were washed with 1% SDS, 6M urea, and Tris followed by resuspension in 6 M urea in Tris, reduced with 10 mM tris(2-carboxyethyl)phosphine (TCEP) for 30 min at 25 °C, and alkylated with 40 mM iodoacetamide for 30 min at 25 °C in the dark. On-bead digestions were performed for 12 h at 37 °C with sequence-grade modified trypsin (Promega; 2 μg) in 2M urea in the presence of 2 mM $CaCl_2$. Peptide samples were acidified to a final concentration of 5% (v/v) formic acid, pressure-loaded on to a biphasic (strong cation exchange/reversed phase) capillary column. MudPIT analysis of eluted peptides was carried out as previously described[7] on a coupled Agilent 1200 LC-Thermo Scientific LTQ XL MS instrument. The MS2 spectra data were extracted from the raw file using RAW Xtractor (version 1.9.9.2; publicly available at http://fields.scripps.edu/downloads.php). MS2 spectra data were searched using the Prolucid algorithm using a mouse reverse-concatenated nonredundant (gene-centric) FASTA database that was assembled from the Uniprot

database (http://www.uniprot.org/). Prolucid searches allowed for static modification of cysteine residues (+57.0215 m/z; iodoacetamide alkylation) and methionine oxidation (+15.9949 *m/z*) and required at least one tryptic end. The resulting MS2 spectra matches were assembled into protein identifications and filtered using DTASelect (version 2.0.47) using the --trypstat and modstat options (applies different statistical models for the analysis of peptide tryptic and modification state) allowing a FDR less than 1% at the peptide level. Data are representative of mean values ± SEM for 3 independent replicates.

Cell Survival assays. Cell survival assays were performed using the Cell Proliferation Reagent WST-1 (Roche). Cells were treated with DMSO or compounds (10 μM) in serum free media for 4 h, seeded into a 96-well plate and cell survival assay performed according to the manufacturer's instructions.

SYNTHETIC METHODS

Synthesis of tetrahydropyridine P11



P11

General Information: All commercially available chemicals were obtained from Aldrich, Acros, Fisher, Fluka, or Maybridge and were used without further purification, except where noted. Dry solvents were obtained by passing through activated alumina columns. All reactions were carried out under inert nitrogen atmosphere using oven-baked glassware unless otherwise noted. Flash chromatography was performed using 230-400 mesh silica gel 60. NMR spectra were generated on a Varian 400 MHz instrument. Chemical shifts were recorded in ppm relative to tetramethylsilane (TMS) with multiplicities given as s (singlet), bs (broad singlet), d (doublet), t (triplet), dt (double of triplets), q (quadruplet), qd (quadruplet of doublets), m (multiplet).

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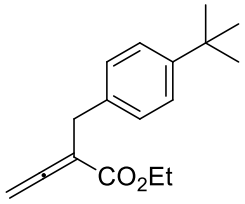
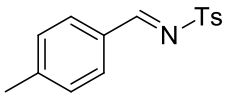
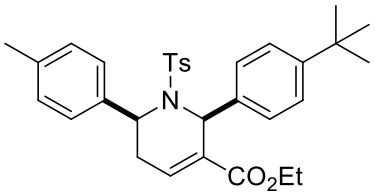


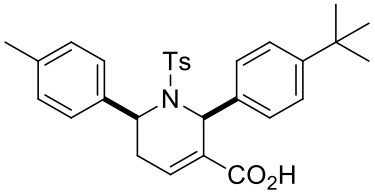
Ethyl-2-(4-(*tert*-butyl)benzyl)buta-2,3-dienoate. Ethyl 2-(triphenylphosphoranylidene)acetate (2.4 g, 6.8 mmol) was added to a solution of 1-(bromomethyl)-4-(*tert*-butyl)benzene (1.7 g, 7.6 mmol) in dry CH_2Cl_2 (50 mL) and allowed to stir for 4 d at 60 °C at room temperature under N_2. The reaction was concentrated. The resultant crude was dissolved in CH_2Cl_2 (2 x 50 mL) and Et_3N (1.9 mL, 13.8 mmol) was added, followed by stirring for 30 min. AcCl (0.5 mL, 6.8 mmol) was then slowly added over 30 min with vigorous stirring. The resultant suspension was stirred for 16 h and concentrated. The crude mixture was stirred with Et_2O (100 mL) for 2 h and then filtered over a pad of silica gel. The residue on the filter was washed with Et_2O and organic fractions were combined and concentrated. The mixture was purified by SiO_2 flash chromatography (5−15% EtOAc/hexanes) to give Ethyl-2-(4-(tert-butyl)benzyl)buta-2,3-dienoate as a yellow oil (1.1 g, 61 %).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, *J* = 8.3 Hz, 2H), 7.18 (d, *J* = 8.2 Hz, 2H), 5.09 (t, *J* = 2.2 Hz, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.51 (t, *J* = 2.2 Hz, 2H), 1.29 (s, 9H), 1.23 (t, *J* = 2.2 Hz, 3H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{17}H_{22}O_2$: 259.3538, found 259.3541.



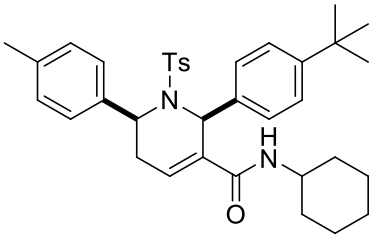
4-methyl-*N*-(4-methylbenzylidene)benzenesulfonamide. Boron trifluoride diethyl ester complex (0.2 mL, 1.8 mmol) was slowly added to a mixture of *p*-toluenesulfonamide (3.7 g, 21.6 mmol) and 4-methylbenzaldehyde (2.6 g, 21.6 mmol) in dry toluene (150 mL) at reflux under N_2 and stirred for 12 h. After cooling to room temperature, the mixture was quenched with 1 N NaOH (10 mL) and extracted with EtOAc three times. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to provide the crude product, which was used in the next step without further purification.



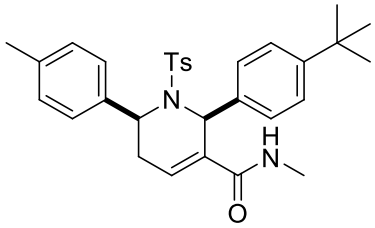
Ethyl-2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxylate (JW1032). Ethyl-2-(4-(*tert*-butyl)benzyl)buta-2,3-dienoate (4.8 mg, 18.3 mmol) was slowly added to a solution of *N*-tosylimine (4.2 g, 15.3 mmol) and PBu_3 (0.8 mL, 3.1 mmol) in dry CH_2Cl_2 (50 mL) at room temperature under N_2. The mixture was then stirred at room temperature for 24 h. After complete consumption of starting material as assessed by TLC, the mixture was concentrated and purified by SiO_2 flash chromatography (5−15% EtOAc/hexanes) to give Ethyl-2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxylate as a yellow solid (4.2 g, 52 %).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.2 Hz, 2H), 7.26 (d, *J* = 8.1 Hz, 2H), 7.11 (t, *J* = 8.0 Hz, 1H), 6.90 - 6.86 (m, 3H), 6.82 - 6.78 (m, 3H), 6.65 (d, *J* = 8.1 Hz, 2H), 6.12 (s, 1H), 5.12 (d, *J* = 4.0 Hz, 1H), 4.07 (q, *J* = 7.0 Hz, 2H), 2.70 -2.68 (m, 1H), 2.41 (s, 3H), 2.18 - 2.16 (m, 1H), 2.11 (s, 3H), 1.26 (s, 9H), 1.22 (t, *J* = 7.0 Hz, 3H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{32}H_{37}NO_4S$: 532.7134, found 532.7137.



2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxylic acid (P11). 2N aqueous NaOH (1.1 mL, 2.2 mmol) was added to a solution of Ethyl-2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxylate (580 mg, 1.1 mmol) in dry EtOH (5.0 mL) and stirred briefly at room temperature under N_2. The mixture was then stirred for 8 h at 65 °C followed by acidification to pH 1 using 6 N HCl. The product was then extracted with Et_2O (3 x 5.0 mL). The combined extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The mixture was purified by SiO_2 flash chromatography (30% EtOAc/hexanes) to give 2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxylic acid as a white solid (410 mg, 75 %).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.0 Hz, 2H), 7.30 – 7.24 (m, 3H), 6.92 (d, *J* = 8.0 Hz, 2H), 6.86 (d, *J* = 8.0 Hz, 2H), 6.82 (d, *J* = 8.0 Hz, 2H), 6.69 (d, *J* = 8.0 Hz, 2H), 6.09 (s, 1H), 5.16 (d, *J* = 4.0 Hz, 1H), 2.72 -2.66 (m, 1H), 2.42 (s, 3H), 2.20 - 2.17 (m, 1H), 2.13 (s, 3H), 1.20 (s, 9H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{30}H_{33}NO_4S$: 504.2203, found 504.2204.

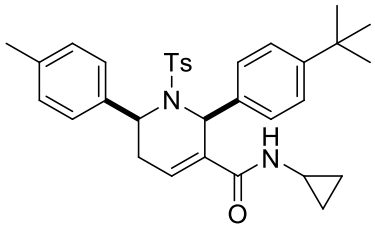


2-(4-(*tert*-butyl)phenyl)-*N*-cyclohexyl-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxamide (JW1039). General Procedure A. Cyclohexanamine (15 mg, 0.1 mmol), EDC (19 mg, 0.1 mmol), and DMAP were added to a solution of 2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxylic acid (25 mg, 0.05 mmol) in dry CH_2Cl_2 (1.0 mL) and stirred for 12 h at room temperature under N_2. The reaction was quenched with saturated aqueous $NaHCO_3$ (3.0 mL) and the aqueous layer extracted with CH_2Cl_2 (3 x 3.0 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The mixture was purified by SiO_2 flash chromatography (25% EtOAc/hexanes) to give 2-(4-(*tert*-butyl)phenyl)-*N*-cyclohexyl-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxamide as a yellow solid (21 mg, 72 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 8 Hz, 2H), 7.00 - 6.85 (m, 7H), 6.70 (d, *J* = 8.1 Hz, 2H), 6.01 (s, 1H), 5.25 (d, *J* = 8.0 Hz, 1H), 5.14 – 5.13 (d, *J* = 4.2 Hz, 1H), 3.73 -3.71 (m, 1H), 2.70 - 2.64 (m, 1H), 2.44 (s, 3H), 2.14 (s, 3H), 2.05 - 1.99 (m, 1H), 1.72 (m, 1H), 1.46 - 1.25 (m, 6H), 1.22 - 1.20 (m, 10H), 1.07 - 1.04 (m, 1H), 0.88 (m, 1H); HRMS (ESI+) *m/z* calculated for $[M-H_2O]^+$ $C_{36}H_{44}N_2O_3S$: 585.3145, found 585.3151.



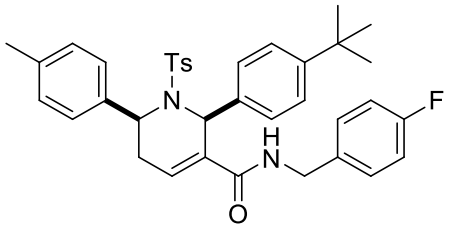
2-(4-(*tert*-butyl)phenyl)-*N*-methyl-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxamide (JW1036). The title compound was prepared from P11 (25 mg, 0.05 mmol) and methylamine (70 mL, 0.15 mmol) according to general procedure A. The crude product was purified by SiO_2 flash chromatography (25% EtOAc/hexanes) to provide 2-(4-(*tert*-butyl)phenyl)-*N*-methyl-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxamide (18 mg, 72 %) as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.0 Hz, 2H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.02 - 6.88 (m, 6H), 6.68 (d, *J* = 8.0 Hz, 2H), 6.04 (s, 1H), 5.22 – 5.14 (m, 2H), 2.92 (s, 3H), 2.71 - 2.68 (m, 1H), 2.40 (s, 3H), 2.12 (s, 3H), 2.02 - 1.97 (m, 1H), 1.20 (m, 9H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{31}H_{36}N_2O_3S$: 517.2531, found 517.2529.

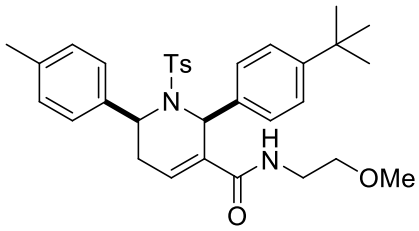


2-(4-(*tert*-butyl)phenyl)-*N*-cyclopropyl-6-(p-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxamide (JW1038). The title compound was prepared from P11 (25 mg, 0.05 mmol) and cyclopropanamine (9 mg, 0.15 mmol) according to general procedure A. The crude product was purified by SiO_2 flash chromatography (25% EtOAc/hexanes) to provide 2-(4-(*tert*-butyl)phenyl)-*N*-cyclopropyl-6-(p-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxamide (17 mg, 63 %) as a white solid.

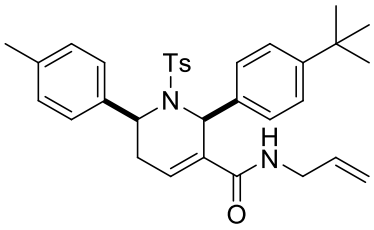
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.1 Hz, 2H), 7.33 (d, *J* = 8.1 Hz, 2H), 7.12 (d, J = 8.0 Hz, 2H), 6.92- 6.88 (m, 4H), 6.08 (d, *J* = 8.0 Hz, 2H), 6.10 (s, 1H), 5.20 – 5.16 (m, 2H), 3.02 (m, 1H), 2.70 - 2.66 (m, 1H), 2.38 (s, 3H), 2.10 (s, 3H), 2.12 - 2.06 (m, 1H), 1.22 (m, 9H), 1.01 - 0.96 (m, 4H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{33}H_{38}N_2O_3S$: 543.7388, found 543.7391.



2-(4-(*tert*-butyl)phenyl)-*N*-(4-fluorobenzyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxamide (JW1040). The title compound was prepared from P11 (19 mg, 0.04 mmol) and (4-fluorophenyl)methanamine (14 mg, 0.12 mmol) according to general procedure A. The crude product was purified by SiO_2 flash chromatography (25% EtOAc/hexanes) to provide 2-(4-(*tert*-butyl)phenyl)-*N*-(4-fluorobenzyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxamide (19 mg, 83 %) as a colorless solid:
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.0 Hz, 2H), 7.41 - 7.39 (m, 4H), 7.14 - 7.08 (m, 4H), 6.90- 6.86 (m, 4H), 6.13 (d, *J* = 8.1 Hz, 2H), 6.06 (s, 1H), 5.10 – 5.08 (m, 2H), 4.20 (s, 2H), 2.70 - 2.68 (m, 1H), 2.34 (s, 3H), 2.10 (s, 3H), 2.11 - 2.08 (m, 1H), 1.22 (m, 9H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{37}H_{39}FN_2O_3S$: 611.2736, found 611.2735.

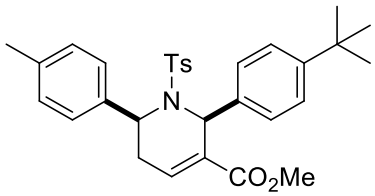


2-(4-(*tert*-butyl)phenyl)-*N*-(2-methoxyethyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxamide (JW1041). The title compound was prepared from P11 (19 mg, 0.4 mmol) and 2-methoxyethanamine (9 mg, 0.12 mmol) according to general procedure A. The crude product was purified by SiO_2 flash chromatography (25% EtOAc/hexanes) to provide 2-(4-(*tert*-butyl)phenyl)-*N*-(2-methoxyethyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxamide (15 mg, 71 %) as a yellow solid.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, *J* = 8.0 Hz, 2H), 7.41 (d, *J* = 8.1 Hz, 2H), 7.08 (d, *J* = 8.0 Hz, 2H), 6.94- 6.89 (m, 4H), 6.13 (d, *J* = 8.0 Hz, 2H), 6.08 (s, 1H), 5.11 – 5.08 (m, 2H), 3.88 (t, *J* = 5.6 Hz, 2H), 3.64 (s, 3H), 3.08 (t, *J* = 5.6 Hz, 2H), 2.72 - 2.68 (m, 1H), 2.36 (s, 3H), 2.14 (s, 3H), 2.08 - 2.05 (m, 1H), 1.20 (m, 9H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{33}H_{40}N_2O_4S$: 561.2776, found 561.2279.

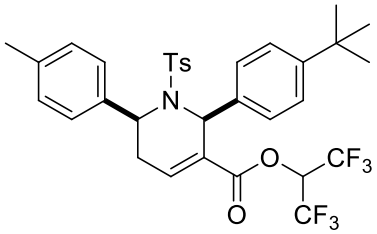


***N*-allyl-2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxamide (JW1043).** The title compound was prepared from P11 (19 mg, 0.04 mmol) and prop-2-en-1-amine (7 mg, 0.12 mmol) according to general procedure A. The crude product was purified by SiO_2 flash chromatography (25% EtOAc/hexanes) to provide *N*-allyl-2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxamide (14 mg, 62 %) as a white solid:
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.0 Hz, 2H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.12 (d, *J* = 8.0 Hz, 2H), 6.96- 6.90 (m, 4H), 6.11 (d, *J* = 8.0 Hz, 2H), 6.10 (s, 1H), 5.61 (m, 1H), 5.21 – 5.10 (m, 4H), 4.12 (d, *J* = 4.2 Hz, 2H), 2.70 - 2.68 (m, 1H), 2.34 (s, 3H), 2.12 (s, 3H), 2.10 -

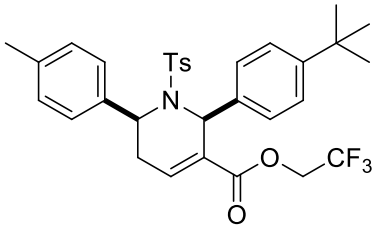
2.08 (m, 1H), 1.21 (m, 9H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{33}H_{38}N_2O_3S$: 543.2671, found 543.2668.



Methyl-2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxylate (JW1068). The title compound was prepared from P11 (29 mg, 0.05 mmol) and methanol (excess) according to general procedure A. The crude product was purified by SiO_2 flash chromatography (15% EtOAc/hexanes) to provide Methyl-2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxylate (15 mg, 68 %) as a yellow oil.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.1 Hz, 2H), 7.34 (d, *J* = 8.1 Hz, 2H), 7.08 (t, *J* = 8.0 Hz, 1H), 6.94 - 6.86 (m, 3H), 6.80 - 6.76 (m, 3H), 6.60 (d, *J* = 8.1 Hz, 2H), 6.01 (s, 1H), 5.22 (d, *J* = 4.0 Hz, 1H), 3.62 (s, 3H), 2.72 -2.68 (m, 1H), 2.44 (s, 3H), 2.08 - 2.04 (m, 1H), 2.02 (s, 3H), 1.24 (s, 9H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{31}H_{35}NO_4S$: 518.6858, found 518.6859..

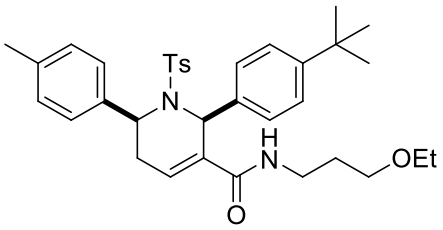


1,1,1,3,3,3-hexafluoropropan-2-yl 2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxylate (JW1076). The title compound was prepared from P11 (25 mg, 0.05 mmol) and hexafluoroisopropanol (23 mg, 0.15 mmol) according to general procedure A. The crude product was purified by SiO_2 flash chromatography (15% EtOAc/hexanes) to provide 1,1,1,3,3,3-hexafluoropropan-2-yl 2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxylate (21 mg, 65 %) as a white solid.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, *J* = 8.2 Hz, 2H), 7.41 (d, *J* = 8.1 Hz, 2H), 7.14 (t, *J* = 8.0 Hz, 1H), 6.88 (d, *J* = 8.0 Hz, 2H), 6.80 (d, *J* = 8.0 Hz, 2H), 6.78 (d, *J* = 8.0 Hz, 2H), 6.71 (d, *J* = 8.0 Hz, 2H), 6.21 (s, 1H), 5.89 - 5.72 (m, 1H), 5.16 (d, *J* = 4.0 Hz, 1H), 2.70 - 2.66 (m, 1H), 2.44 (s, 3H), 2.22 - 2.17 (m, 1H), 2.10 (s, 3H), 1.21 (s, 9H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{33}H_{33}F_6NO_4S$: 654.2109, found 654.2112.

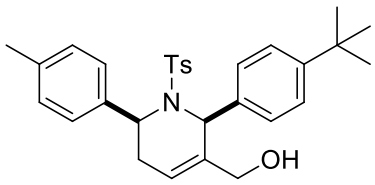


2,2,2-trifluoroethyl 2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxylate (JW1077). The title compound was prepared from P11 (25 mg, 0.05 mmol) and 2,2,2-trifluoroethanol (6 mg, 0.15 mmol) according to general procedure A. The crude product was purified by SiO_2 flash chromatography (15% EtOAc/hexanes) to provide 2,2,2-trifluoroethyl 2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxylate (15 mg, 52 %) as a white solid.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.0 Hz, 2H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.08 (t, *J* = 8.0 Hz, 1H), 6.88 - 6.83 (m, 4H), 6.68 (d, *J* = 8.0 Hz, 2H), 6.58 (d, *J* = 8.0 Hz, 2H), 6.18 (s, 1H), 5.18 - 5.08 (m, 3H), 2.72 - 2.68 (m, 1H), 2.42 (s, 3H), 2.20 - 2.16 (m, 1H), 2.14 (s, 3H),

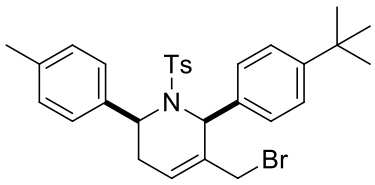
1.24 (s, 9H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{32}H_{34}F_3NO_4S$: 586.2228, found 586.2227.



2-(4-(*tert*-butyl)phenyl)-*N*-(3-ethoxypropyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxamide (JW1090). The title compound was prepared from P11 (25 mg, 0.05 mmol) and 3-ethoxypropan-1-amine (15 mg, 0.15 mmol) according to general procedure A. The crude product was purified by SiO_2 flash chromatography (25% EtOAc/hexanes) to provide 2-(4-(*tert*-butyl)phenyl)-*N*-(3-ethoxypropyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxamide (21 mg, 72 %) as a colorless solid.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.1 Hz, 2H), 7.13 (t, *J* = 7.4 Hz, 1H), 6.91 - 6.87 (m, 3H), 6.80 - 6.76 (m, 3H), 6.53 (d, *J* = 8.1 Hz, 2H), 6.08 (s, 1H), 5.21 (d, *J* = 4.0 Hz, 1H), 3.77 (q, *J* = 6.4 Hz, 2H), 3.37 - 3.32 (m, 2H), 3.02 - 3.00 (m, 2H), 2.74 -2.70 (m, 1H), 2.43 (s, 3H), 2.22 - 2.18 (m, 1H), 2.14 (s, 3H),1.77 (m, 2H), 1.22 (s, 9H), 1.12 (t, *J* = 6.4 Hz, 3H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{35}H_{44}N_2O_4S$: 589.3009, found 589.3012.

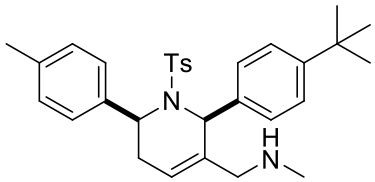


2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridin-3-yl)methanol (JW1057). $LiAlH_4$ (8 mg, 0.21 mmol) was added to a solution of **JW1032** (100 mg, 0.19 mmol) in dry Et_2O (3.0 mL) and allowed to stir for 30 min at 0 °C under N_2. The reaction was quenched with saturated aqueous $NaHCO_3$ (3.0 mL) and the aqueous layer extracted with Et_2O (3 x 3.0 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The mixture was purified by SiO_2 flash chromatography (20% EtOAc/hexanes) to give 2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridin-3-yl)methanol as a white solid (74 mg, 78 %).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 8.2 Hz, 2H), 7.28 – 7.22 (m, 3H), 7.01 (d, *J* = 8.1 Hz, 2H), 6.92 (d, *J* = 8.0 Hz, 2H), 6.84 (d, *J* = 8.0 Hz, 2H), 6.74 (d, *J* = 8.0 Hz, 2H), 6.05 (s, 1H), 5.04 (d, *J* = 4.0 Hz, 1H), 3.64 (s, 2H), 2.68 -2.64 (m, 1H), 2.38 (s, 3H), 2.16 - 2.12 (m, 1H), 2.08 (s, 3H), 1.16 (s, 9H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{30}H_{35}NO_3S$: 490.2408, found 490.2412.

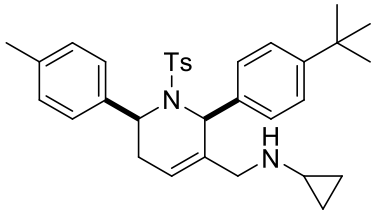


5-(bromomethyl)-6-(4-(*tert*-butyl)phenyl)-2-(*p*-tolyl)-1-tosyl-1,2,3,6-tetrahydropyridine (JW1058). CBr_4 (61 mg, 0.18 mmol) and PPh_3 (38 mg, 0.14 mmol) was added to a solution of **JW1057** (60 mg, 0.12 mmol) in dry CH_2Cl_2 (2.0 mL) and allowed to stir at room temperature under N_2 for 12 h. The reaction was quenched with saturated aqueous $NaHCO_3$ (3.0 mL) and the aqueous layer extracted with CH_2Cl_2 (3 x 3.0 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The mixture was

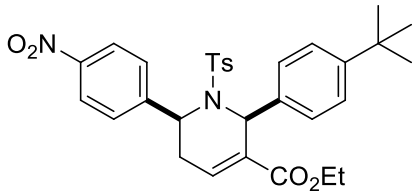
purified by SiO_2 flash chromatography (10% EtOAc/hexanes) to give 5-(bromomethyl)-6-(4-(*tert*-butyl)phenyl)-2-(*p*-tolyl)-1-tosyl-1,2,3,6-tetrahydropyridine as a yellow solid (52 mg, 79 %).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.0 Hz, 2H), 7.30 – 7.22 (m, 5H), 7.12 - 7.04 (m, 6H), 6.10 (s, 1H), 5.21 (d, *J* = 4.0 Hz, 1H), 3.20 (s, 2H), 2.60 -2.56 (m, 1H), 2.30 (s, 3H), 2.12 - 2.08 (m, 1H), 2.10 (s, 3H), 1.21 (s, 9H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{30}H_{34}BrNO_2S$: 552.1564, found 552.1568.



1-(2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridin-3-yl)-*N*-methylmethanamine (JW1060). Methylamine (90 mL, 0.2 mmol) was added to a stirring solution of **JW1058** (20 mg, 0.04 mmol) in dry CH_2Cl_2 (1.0 mL) and allowed to stir for 12 h at 50 °C under N_2. The reaction was quenched with saturated aqueous $NaHCO_3$ (3.0 mL) and the aqueous layer extracted with CH_2Cl_2 (3 x 3.0 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The mixture was purified by flash chromatography (25% EtOAc/hexanes) to give 1-(2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridin-3-yl)-*N*-methylmethanamine as a yellow oil (14 mg, 70 %).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.8 Hz, 2H), 7.32 – 7.28 (m, 3H), 7.11 (d, *J* = 8.0 Hz, 2H), 6.82 (d, *J* = 7.6 Hz, 2H), 6.78 - 6.68 (m, 4H), 5.98 (s, 1H), 5.12 (m, 1H), 3.52 (s, 3H), 3.08 (d, *J* = 4.2 Hz, 2H), 2.60 -2.54 (m, 1H), 2.20 (s, 3H), 2.06 - 2.02 (m, 1H), 2.02 (s, 3H), 1.22 (s, 9H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{31}H_{38}N_2O_2S$: 503.2720, found 503.2716.

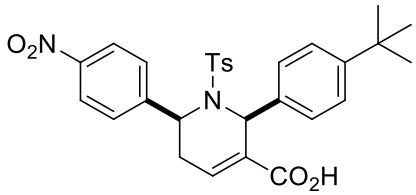


***N*-((2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)cyclopropanamine (JW1061).** The title compound was prepared from **JW1058** (20 mg, 0.04 mmol) and cyclopropanamine (12 mg, 0.2 mmol) according to synthetic procedures described for preparation of **JW1060**. The crude product was purified by SiO_2 flash chromatography (25% EtOAc/hexanes) to provide *N*-((2-(4-(*tert*-butyl)phenyl)-6-(*p*-tolyl)-1-tosyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)cyclopropanamine (15 mg, 71 %) as a colorless oil.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 7.8 Hz, 2H), 7.28 - 7.14 (m, 4H), 6.76- 6.70 (m, 4H), 6.20 (m, 2H), 6.08 (s, 1H), 5.06 – 5.02 (m, 2H), 3.22 (s, 2H), 2.58 - 2.54 (m, 1H), 2.30 (s, 3H), 2.12 (s, 3H), 2.08 - 2.04 (m, 2H), 1.24 (m, 9H), 1.02 - 0.97 (m, 4H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{33}H_{40}N_2O_2S$: 529.2872, found 529.2871.

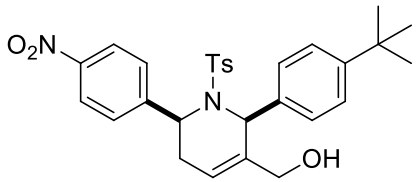


Ethyl-2-(4-(*tert*-butyl)phenyl)-6-(4-nitrophenyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxylate (JW1081). The title compound was prepared from ethyl 2-(4-(*tert*-butyl)benzyl)buta-2,3-dienoate (400 mg, 1.54 mmol) and (*E*)-4-methyl-*N*-(4-nitrobenzylidene)benzenesulfonamide (392 mg, 1.29 mmol) according to synthetic

procedures described for preparation of **JW1032**. The crude product was purified by SiO_2 flash chromatography (15% EtOAc/hexanes) to provide ethyl-2-(4-(*tert*-butyl)phenyl)-6-(4-nitrophenyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxylate (342 mg, 47 %) as a yellow solid.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 - 7.73 (m, 4H), 7.22 (d, *J* = 8.1 Hz, 2H), 7.14 - 7.10 (m, 3H), 6.94 - 6.88 (m, 4H), 6.16 (s, 1H), 5.17 (d, *J* = 4.2 Hz, 1H), 4.15 (q, *J* = 6.8 Hz, 2H), 2.63 -2.60 (m, 1H), 2.52 (s, 3H), 2.32 - 2.26 (m, 1H), 1.21 (s, 9H), 1.08 (t, *J* = 6.8 Hz, 3H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{31}H_{34}N_2O_6S$: 563.2211, found 563.2207.

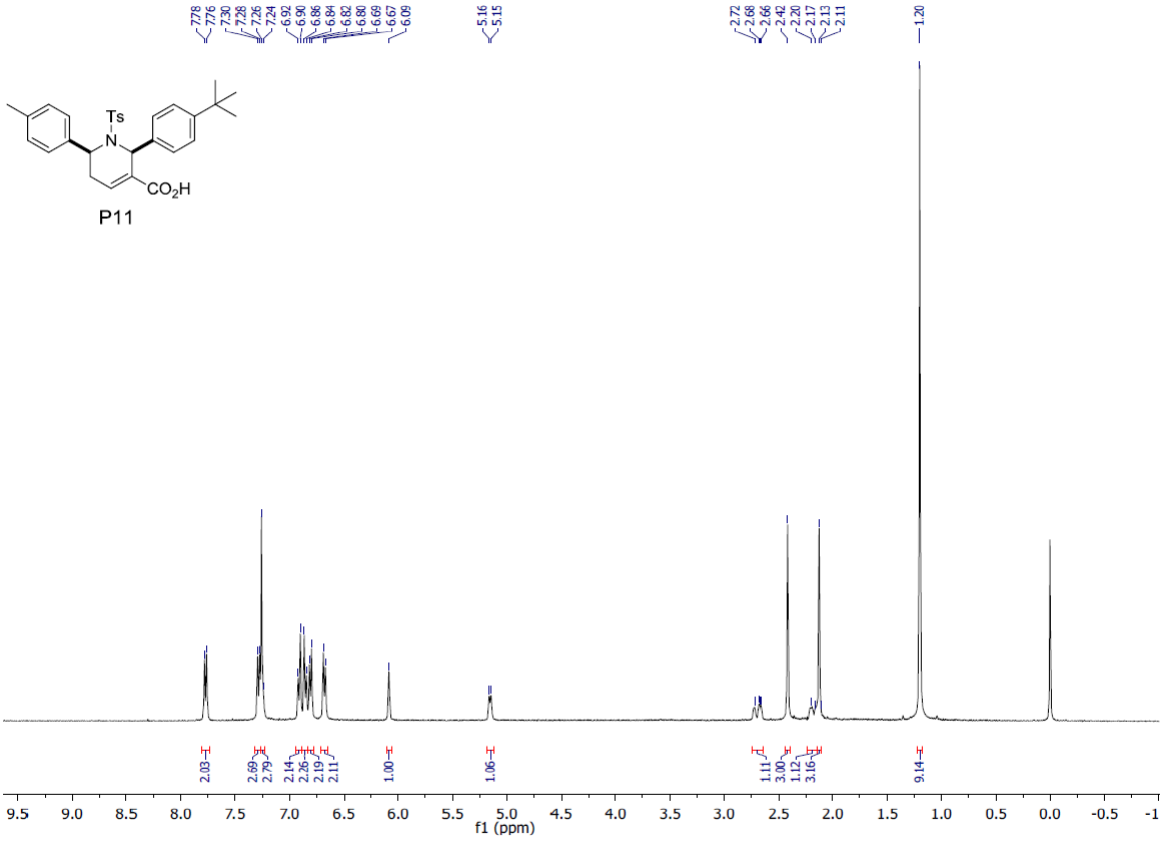


2-(4-(*tert*-butyl)phenyl)-6-(4-nitrophenyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxylic acid (JW1088). The title compound was prepared from **JW1081** (150 mg, 0.27 mmol) and aqueous 2N NaOH (1.3 mL, 2.7 mmol) according to synthetic procedures described for preparation of **JW1088**. The crude product was purified by SiO_2 flash chromatography (25% EtOAc/hexanes) to provide 2-(4-(*tert*-butyl)phenyl)-6-(4-nitrophenyl)-1-tosyl-1,2,5,6-tetrahydropyridine-3-carboxylic acid (82 mg, 57 %) as a yellow solid.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 - 7.72 (m, 4H), 7.32 (d, *J* = 7.8 Hz, 2H), 7.10 - 7.06 (m, 3H), 6.90 - 6.86 (m, 4H), 6.08 (s, 1H), 5.10 (d, *J* = 4.0 Hz, 1H), 2.50 -2.46 (m, 1H), 2.32 (s, 3H), 2.21 - 2.18 (m, 1H), 1.22 (s, 9H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{29}H_{30}N_2O_6S$: 535.1907, found 535.1905.



(2-(4-(*tert*-butyl)phenyl)-6-(4-nitrophenyl)-1-tosyl-1,2,5,6-tetrahydropyridin-3-yl)methanol (JW1089). NaBH4 (8 mg, 0.2 mmol) was added to a stirring solution of **JW1081** (25 mg, 0.04 mmol) in dry EtOH (1.0 mL) at 0°C under N_2 and allowed to stir for 12 h at 50 °C. The reaction was quenched with saturated aqueous $NaHCO_3$ (3.0 mL) and the aqueous layer extracted with Et_2O (3 x 3.0 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The mixture was purified by SiO_2 flash chromatography (20% EtOAc/hexanes) to give (2-(4-(*tert*-butyl)phenyl)-6-(4-nitrophenyl)-1-tosyl-1,2,5,6-tetrahydropyridin-3-yl)methanol as a yellow solid (12 mg, 57 %).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 - 7.76 (m, 4H), 7.36 (d, *J* = 7.8 Hz, 2H), 7.21 - 7.18 (m, 3H), 6.92 - 6.85 (m, 4H), 6.00 (s, 1H), 5.14 (d, *J* = 4.2 Hz, 1H), 3.90 (s, 2H), 2.54 -2.50 (m, 1H), 2.34 (s, 3H), 2.20 - 2.16 (m, 1H), 1.21 (s, 9H); HRMS (ESI+) *m/z* calculated for $[M+H]^+$ $C_{29}H_{32}N_2O_5S$: 521.2098, found 521.2087.

NMR SPECTRA


7.78
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6.09
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5.15
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2.17
2.13
2.11
1.20
Ts
N
CO2H
P11
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2.69
2.79
2.14
2.26
2.19
2.11
1.00
1.06
1.11
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3.16
9.14
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1
f1 (ppm)

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