SUPPORTING INFORMATION

Discovery and Optimization of Piperidyl-1,2,3-Triazole Ureas as Potent, Selective, and in Vivo-Active Inhibitors of *α/β*-Hydrolase Domain Containing 6 (ABHD6)

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I. Experimental Section

II. Supplementary Figures

I. EXPERIMENTAL SECTION

Synthetic Methods. Commercially-available chemicals and reagents were purchased from the following vendors: Sigma-Aldrich, Acros, Fisher, Fluka, Maybridge, Combi-Blocks, BioBlocks, and Matrix Scientific and used without further purification unless noted otherwise. Dry solvents were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Reactions were performed under a nitrogen atmosphere using oven-baked glassware unless otherwise noted. Flash chromatography was performed using 230-400 mesh silica gel. Reactions were monitored by analytical thin-layer chromatography (TLC) on precoated, glass backed silica gel 60 F_{254} plates. Reactions were purified either by pTLC, also on silica gel 60 F_{254} plates or by flash chromatography on 40-60 MYM mesh silica gel as specified. ^{1}H-NMR and spectra were recorded in $CDCl_3$ on a Varian Mercury-300 spectrometer, a Varian Inova-400 or a Bruker DRX-600 spectrometer, and were referenced to trimethylsilane (TMS). Chemical shifts were reported in ppm relative to TMS and *J* values were reported in Hz. High resolution mass spectrometry (HRMS) experiments were performed at The Scripps Research Institute Mass Spectrometry Core on an Agilent mass spectrometer using electrospray ionization-time of flight (ESI-TOF)**.**

LC/MS Analysis of Compound Purity. The purity of compounds was determined by LC/MS on an Agilent 1100 series LC-MSD SL instrument with UV detection at 254 nm. Chromatographic separation was performed using a Phenomenex Gemini C18 column (5 µm, 50 mm x 4.6 mm). Mobile phases A and B were composed of H_2O (0.1% formic acid) and CH_3CN (0.1% formic acid), respectively. Using a constant flow rate of 0.5 mL/min, the mobile phase was as follows: 1.0 min, 10% B; 2.0 min, 10-98% B (linear gradient); 5.0 min, 98% B; 2.0 min, 10% B. All final compounds were determined to be $\geq$95% pure by this method.

LC-MS/MS Analysis of SILAC samples. Multidimensional liquid chromatography tandem mass spectrometry (MudPIT) analysis of samples was performed using an Agilent 1200-series quaternary pump and Thermo Scientific LTQ-Orbitrap ion trap mass spectrometer. Peptides were eluted in a 5-step MudPIT experiment using 0%, 25%, 50%, 80%, and 100% salt bumps of 500 mM aqueous ammonium acetate and data were collected in data-dependent acquisition mode with dynamic exclusion turned on (20 s, repeat of 1). Specifically, one full MS (MS1) scan (400-1800 m/z) was followed by 30 MS2 scans of the most abundant ions. 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 (available through IP2-Integrated Proteomics Pipeline http://goldfish.scripps.edu/ip2.jsp) against the latest version of the mouse UniProt database concatenated with the reversed database for assessment of false-discovery rates. ProLucid searches allowed for static modification of cysteine residues (+57.02146 due to alkylation), methionine oxidation (+15.9949), mass shifts of labeled amino acids (+10.0083 R, +8.0142 K) and no enzyme specificity. The resulting MS2 spectra matches were assembled into protein identifications and filtered using DTASelect (version 2.0) using the --modstat, --mass, and --trypstat options (applies different statistical models for the analysis of high resolution masses, peptide digestion state, and methionine oxidation state respectively). The ratios of heavy/light (test compound/DMSO) peaks were calculated using in-house software (CIMAGE) and normalized at the peptide level to the average ratio of all non-serine hydrolase peptides. Reported

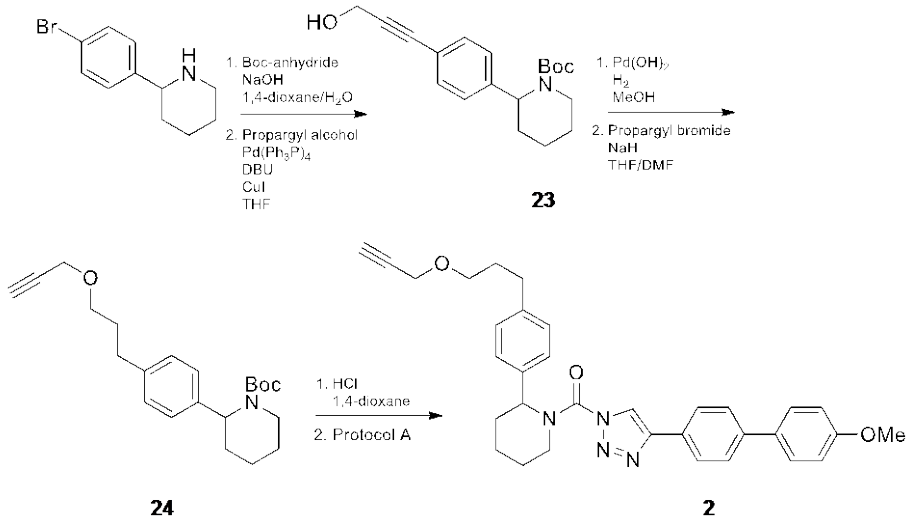
ratios represent the mean of all unique, quantified peptides per protein and do not include peptides that were >3 standard deviations from the median peptide value. Proteins with less than three peptides per protein ID were not included in the analysis.

Cycloaddition reactions with clickable probe 2. Click chemistry was performed following previously described protocols.[1]

General Procedure A for Synthesis of 1,2,3-Triazole Ureas. A solution of urea (0.024 mmol) in dioxane (1 mL) and H_2O (0.1 mL) was treated with a substituted boronic acid (0.039 mmol), K_2CO_3 (10 mg, 0.072 mmol) and $PdCl_2$(dppf) (4 mg, 0.0049 mmol), and the reaction mixture was stirred for 2 h at 80°C under N_2. The mixture was poured into H_2O and extracted with ethyl acetate. The organic layer was washed with H_2O and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude oil was purified by chromatography or pTLC to give the pure 1,2,3-triazole urea. All compounds were purified as the racemate, which was suitable for biological studies.[1]

(4-(4'-Methoxy-[1,1'-biphenyl]-4-yl)-1H-1,2,3-triazol-1-yl)(2-phenylpiperidin-1-yl)methanone (1).
1 was prepared and characterized as previously described.[2]

(4-(4'-Methoxy-[1,1'-biphenyl]-4-yl)-1*H*-1,2,3-triazol-1-yl)(2-(4-(3-(prop-2-yn-1-yloxy)propyl)phenyl)piperidin-1-yl)methanone (2).



2-(4-bromophenyl)piperidine (500 mg, 2.1 mmol) was dissolved in H_2O (5 mL) and 1,4-dioxane (5 mL) and treated with 1 N NaOH (2.1 mL). The reaction was brought to 0 °C and treated with Boc-anhydride (454 mg, 2.1 mmol) and was allowed to stir for 3 hr at 25 °C. The reaction was then diluted with 1 N aqueous HCl and extracted with ethyl acetate. The organics were dried over Na_2SO_4, and the solvent was evaporated. The product was taken to the next step without purification. The tert-butyl 2-(4-bromophenyl)piperidine-1-carboxylate (330 mg, 0.97 mmol) was dissolved in THF (3 mL) and treated with propargyl alcohol (3.9 mmol, 217 mg), 1,8-Diazabicyclo[5.4.0]undec-7-ene (2 mmol, 298 mg), $Pd(PPh_3)_4$ (112 mg, 0.1 mmol) and CuI (0.03 mmol, 6 mg). The reaction vial was capped and the reaction stirred at 90 °C for 2 hr. The

reaction was cooled to room temperature and then diluted with ethyl acetate and washed with 1 N HCl, followed by saturated aqueous $NaHCO_3$. The organics were dried over Na_2SO_4, and the solvent was evaporated. The product was purified by silica column (3:1 hexanes/ethyl acetate) to yield **23** (282 mg, 0.89 mmols, 92% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40 (d, *J* = 8.3 Hz, 2H), 7.16 (d, *J* = 8.3 Hz, 2H), 5.39 (s, 1H), 4.49 (d, *J* = 5.9 Hz, 2H), 4.09 – 4.00 (m, 1H), 2.74 (ddd, *J* = 13.4, 11.8, 3.8 Hz, 1H), 2.26 (ddd, *J* = 14.3, 2.9, 1.4 Hz, 1H), 2.10 (t, *J* = 6.1 Hz, 1H), 1.88 (tdd, *J* = 13.6, 5.6, 3.6 Hz, 1H), 1.66 – 1.32 (m, 13H). MS calculated for $C_{19}H_{26}NO_3$ $[M+H]^+$ 316.2, found 316.3.

Compound **23** (150 mg, 0.48 mmol) was dissolved in methanol (5 mL) and treated with $Pd(OH)_2$ on carbon (50 mg), followed by treatment with H_2 gas (balloon). The reaction was stirred for 12 hr and then filtered over celite and the solvent removed. The crude product was then dissolved in anhydrous THF (0.8 mL) and DMF (0.2 mL) and was brought to 0 °C. The reaction was treated with NaH (22 mg of 60% dispersion in mineral oil, 0.55 mmol). The reaction was stirred for 30 min at 0 °C and was treated with propargyl bromide (110 mg of 80% solution in toluene, 0.74 mmol). The reaction was stirred for 3 hr, diluted with ethyl acetate and washed with H_2O. The organics were dried over Na_2SO_4, and the solvent was evaporated. The product was purified by pTLC (6:1 hexanes/ethyl acetate) to yield **24** (74 mg, 0.2 mmols, 43% yield over two steps). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.19 – 7.10 (m, 4H), 5.39 (br s, 1H), 4.14 (d, *J* = 2.4 Hz, 2H), 4.01 and 4.05 (2 br s, rotamers, 1H), 3.53 (t, *J* = 6.4 Hz, 2H), 2.75 (m, 1H), 2.69 (dd, *J* = 8.7, 6.8 Hz, 2H), 2.42 (t, *J* = 2.4 Hz, 1H), 2.33 – 2.25 (m, 1H), 1.96 – 1.80 (m, 3H), 1.63 – 1.49 (m, 3H), 1.46 (s, 9H). MS calculated for $C_{17}H_{24}NO$ $[M+H]^+$ 258.2, found 258.3.

Compound **24** (74 mg, 0.2 mmols) was treated with 4 N HCl in 1,4-dioxane (3 mL) and stirred for 2 hr at 25 °C. The solvent was evaporated using a stream of N_2 to yield 60 mg (0.2 mmols) of crude 2-(4-(3-(prop-2-yn-1-yloxy)propyl)phenyl)piperidine hydrochloride, which was then reacted with 4-(4'-methoxy-[1,1'-biphenyl]-4-yl)-1H-1,2,3-triazole (51 mg, 0.2 mmols) according to general procedure A to yield **2**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.42 (s, 1H), 7.93 (d, *J* = 8.4 Hz, 2H), 7.65 (d, *J* = 8.6 Hz, 2H), 7.58 (d, *J* = 8.9 Hz, 2H), 7.29 (d, *J* = 8.1 Hz, 2H), 7.23 (d, *J* = 8.3 Hz, 2H), 7.00 (d, *J* = 8.9 Hz, 2H), 5.90 (s, 1H), 4.36 (d, *J* = 13.5 Hz, 1H), 4.15 (d, *J* = 2.4 Hz, 2H), 3.86 (s, 3H), 3.54 (t, *J* = 6.3 Hz, 2H), 3.18 (t, *J* = 12.6 Hz, 1H), 2.77 – 2.66 (m, 2H), 2.51 (d, *J* = 13.6 Hz, 1H), 2.43 (t, *J* = 2.4 Hz, 1H), 2.18 – 2.07 (m, 1H), 2.00 – 1.88 (m, 2H), 1.88 – 1.57 (m, 4H). HRMS calculated for $C_{33}H_{35}N_4O_3$ $[M+H]^+$ 535.2704, found 535.2702.

(2-Benzylpiperidin-1-yl)(4-(2'-methoxy-[1,1'-biphenyl]-4-yl)-1H-1,2,3-triazol-1-yl)methanone (3).
3 was prepared and characterized as previously described.[2]

(2-Benzylpiperidin-1-yl)(4-(3'-(hydroxymethyl)-[1,1'-biphenyl]-4-yl)-1H-1,2,3-triazol-1-yl)methanone (4).
4 was prepared as described.[1]

(2-Benzylpiperidin-1-yl)(4-(4'-(piperidine-1-carbonyl)-[1,1'-biphenyl]-4-yl)-1H-1,2,3-triazol-1-yl)methanone (5).
5 was prepared as described.[1]

(4-(2'-Methoxy-[1,1'-biphenyl]-4-yl)-1H-1,2,3-triazol-1-yl)(2-phenylpiperidin-1-yl)methanone (6).
6 was prepared as described.[1]

(4-(4-Bromophenyl)-1H-1,2,3-triazol-1-yl)(2-phenylpiperidin-1-yl)methanone (7).
7 was prepared and characterized as previously reported.[2]

(4-(2'-(Hydroxymethyl)-[1,1'-biphenyl]-4-yl)-1*H*-1,2,3-triazol-1-yl)(2-phenylpiperidin-1-yl)methanone (8). A solution of **7** (10 mg, 0.024 mmol) in dioxane (1 mL) and H_2O (0.1 mL) was treated with (2-(hydroxymethyl)phenyl)boronic acid (6 mg, 0.039 mmol, 1.5 equiv), K_2CO_3 (10 mg, 0.072 mmol, 3.0 equiv) and $PdCl_2$(dppf) (4 mg, 0.0049 mmol, 0.2 equiv), and the reaction mixture was stirred for 2 hr at 80°C under N_2. The mixture was poured into H_2O and extracted with ethyl acetate. The organic layer was washed with H_2O and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude oil was purified by pTLC to give **8** (6 mg, 0.014 mmol, 56%).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.44 (s, 1H), 7.94 (d, 2H, *J* = 8.3 Hz), 7.58 (m, 1H), 7.47 (d, 2H, *J* = 8.3 Hz), 7.45-7.26 (m, 8H), 5.94 (br, 1H), 4.66 (d, 2H, *J* = 5.2 Hz), 4.38 (brd, 1H, *J* = 13.7 Hz), 3.19 (m, 1H), 2.54 (brd, 1H, *J* = 14.2 Hz), 2.16 (m, 1H), 1.90-1.55 (m, 4H). HRMS calculated for $C_{27}H_{27}N_4O_2$ $[M+H]^+$ 439.2128, found 439.2129.

(4-(4'-(Hydroxymethyl)-[1,1'-biphenyl]-4-yl)-1*H*-1,2,3-triazol-1-yl)(2-phenylpiperidin-1-yl)methanone (10). Prepared as described for **8** using **7** (10 mg, 0.024 mmol)**,** (4-(hydroxymethyl)phenyl)boronic acid (6 mg, 0.036 mmol, 1.5 equiv), K_2CO_3 (10 mg, 0.072 mmol, 3.0 equiv) and $PdCl_2$(dppf) (4 mg, 0.0049 mmol, 0.2 equiv) in dioxane (1 mL) and H_2O (0.1 mL) yielding **10** (5 mg, 0.011 mmol, 38%).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.44 (s, 1H), 7.69 (d, 2H, *J* = 8.4 Hz), 7.64 (d, 2H, *J* = 8.2 Hz), 7.47 (d, 2H, *J* = 8.2 Hz), 7.43-7.25 (m, 5H), 5.93 (br, 1H), 4.76 (d, 2H, *J* = 4.6 Hz), 4.38 (brd, 1H, *J* = 12.2 Hz), 3.19 (m, 1H), 2.47 (brd, 1H, *J* = 14.0 Hz), 2.16 (m, 1H), 1.92-1.63 (m, 4H). HRMS calculated for $C_{27}H_{27}N_4O_2$ $[M+H]^+$ 439.2128, found 439.2126.

(2-Phenylpiperidin-1-yl)(4-(4'-(piperidine-1-carbonyl)-[1,1'-biphenyl]-4-yl)-1*H*-1,2,3-triazol-1-yl)methanone (12). Prepared as described for **8** using **7** (10 mg, 0.024 mmol)**,** (4-(piperidine-1-carbonyl)phenyl)boronic acid (8 mg, 0.036 mmol, 1.5 equiv), K_2CO_3 (10 mg, 0.072 mmol, 3.0 equiv) and $PdCl_2$(dppf) (4 mg, 0.0049 mmol, 0.2 equiv) in dioxane (1 mL) and H_2O (0.1 mL) yielding **12** (5 mg, 0.010 mmol, 40%).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.45 (s, 1H), 7.97 (d, 2H, *J* = 8.3 Hz), 7.70 (d, 2H, *J* = 8.3 Hz), 7.66 (d, 2H, *J* = 8.3 Hz), 7.49 (d, 2H, *J* = 8.3 Hz), 7.45-7.25 (m, 5H), 5.93 (br, 1H), 4.38 (brd, 1H, *J* = 13.3 Hz), 3.80-3.15 (m, 5H), 2.54 (d, 1H, *J* = 13.5 Hz), 2.15 (m, 1H), 1.90-1.50 (m, 10H). HRMS calculated for $C_{32}H_{34}N_5O_2$ $[M+H]^+$ 520.2707, found 520.2713.

4'-(1-(2-Phenylpiperidine-1-carbonyl)-1*H*-1,2,3-triazol-4-yl)-[1,1'-biphenyl]-4-carboxamide (13). Prepared as described for **8** using **7** (10 mg, 0.024 mmol)**,** (4-carbamoylphenyl)boronic acid (6 mg, 0.036 mmol, 1.5 equiv), K_2CO_3 (10 mg, 0.072 mmol, 3.0 equiv) and $PdCl_2$(dppf) (4 mg, 0.0049 mmol, 0.2 equiv) in dioxane (1 mL) and H_2O (0.1 mL) yielding **13** (3 mg, 0.007 mmol, 27%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.46 (s, 1H), 7.99 (d, 2H, *J* = 8.1 Hz), 7.92 (d, 2H, *J* = 8.1 Hz), 7.85-7.70 (m, 4H), 5.93 (br, 1H), 7.45-7.25 (m, 5H), 4.38 (m, 1H), 3.20 (m, 1H), 2.53 (m, 1H), 2.18 (m, 1H), 1.90-1.63 (m, 4H). HRMS calculated for $C_{27}H_{26}N_5O_2$ $[M+H]^+$ 452.2081, found 452.2082.

(2-Benzylpiperidin-1-yl)(4-(4-bromophenyl)-1*H*-1,2,3-triazol-1-yl)methanone (14). **14** was prepared and characterized as previously reported.[2]

(4-(4-Bromophenyl)-1*H*-1,2,3-triazol-1-yl)(2-phenethylpiperidin-1-yl)methanone (15). **15** was prepared as described.[1]

Benzyl 4'-(1-(2-phenylpiperidine-1-carbonyl)-1*H*-1,2,3-triazol-4-yl)-[1,1'-biphenyl]-3-carboxylate (18). Prepared as described for **8** using **7** (25 mg, 0.061 mmol)**,** (3-((benzyloxy)carbonyl)phenyl)boronic acid (16 mg, 0.063 mmol, 1.0 equiv), K_2CO_3 (25 mg, 0.18 mmol, 3.0 equiv) and $PdCl_2$(dppf) (8 mg, 0.01 mmol, 0.2 equiv) in dioxane (2 mL) and H_2O (0.2 mL) yielding **18** (18 mg, 0.032 mmol, 55%).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.45 (s, 1H), 8.35 (s, 1H), 8.08 (d, 1H, *J* = 7.8 Hz), 7.97 (d, 2H, *J* = 8.3 Hz), 7.83 (d, 1H, *J* = 8.2 Hz), 7.72 (d, 2H, *J* = 8.3 Hz), 7.56-7.26 (m, 11H), 5.94 (br, 1H), 5.41 (s, 2H), 4.38 (d, 1H, *J* = 13.7 Hz), 3.19 (m, 1H), 2.54 (d, 1H, *J* = 14.1 Hz), 2.16 (m, 1H), 1.91-1.64 (m, 4H). HRMS calculated for $C_{34}H_{31}N_4O_3$ $[M+H]^+$ 543.2391, found 543.2379.

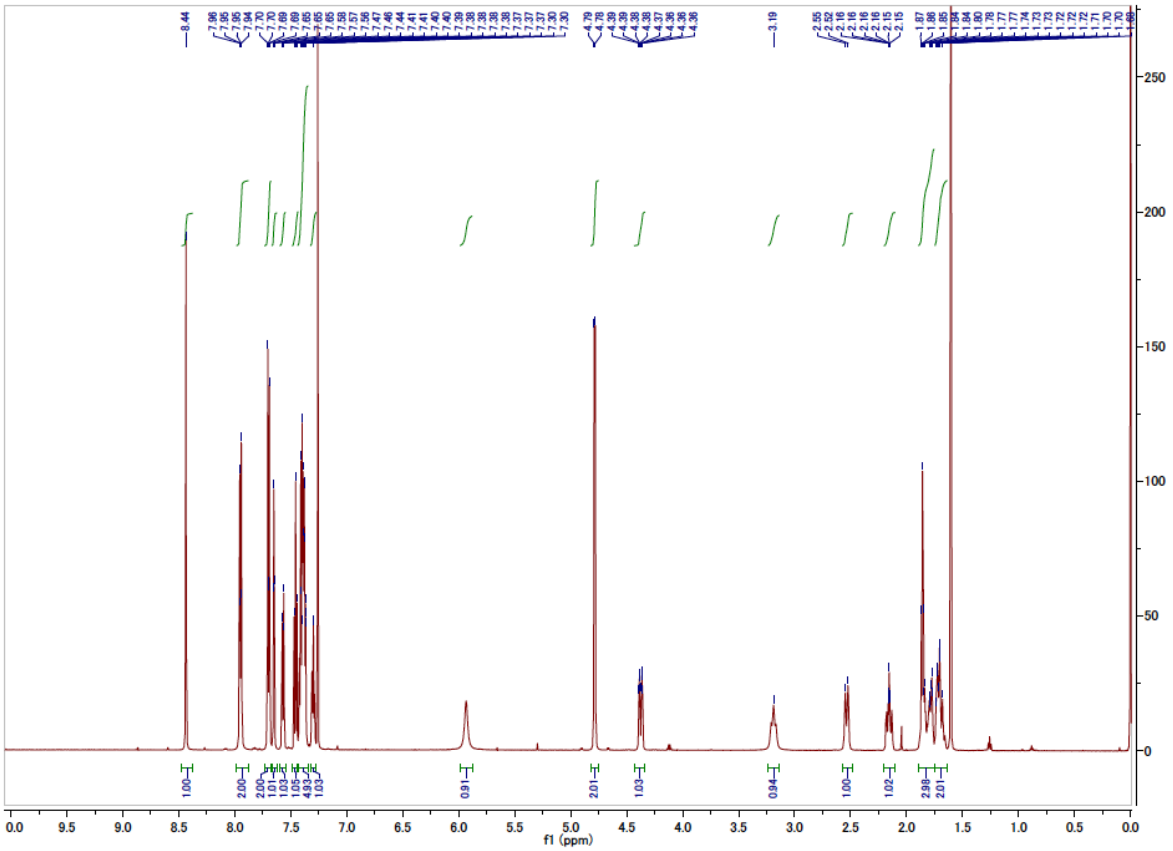
Benzyl 4'-(1-(2-phenethylpiperidine-1-carbonyl)-1*H*-1,2,3-triazol-4-yl)-[1,1'-biphenyl]-3-carboxylate (19). Prepared as described for **8** using **15** (25 mg, 0.061 mmol)**,** (3-((benzyloxy)carbonyl)phenyl)boronic acid (16 mg, 0.063 mmol, 1.0 equiv), K_2CO_3 (25 mg, 0.18 mmol, 3.0 equiv) and $PdCl_2$(dppf) (8 mg, 0.01 mmol, 0.2 equiv) in dioxane (2 mL) and H_2O (0.2 mL) yielding **19** (20 mg, 0.035 mmol, 57%).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.35 (s, 1H), 8.32 (br, 1H), 8.08 (d, 1H, *J* = 7.8 Hz), 7.97 (d, 2H, *J* = 8.2 Hz), 7.84 (d, 1H, *J* = 7.8 Hz), 7.72 (d, 2H, *J* = 8.2 Hz), 7.56-7.08 (m, 11H), 5.41 (s, 2H), 4.75 (m, 1H), 4.34 (d, 1H, *J* = 14.1 Hz), 3.41-2.45 (m, 3H), 2.29 (m, 1H), 2.00-1.75 (m, 7H). HRMS calculated for $C_{36}H_{35}N_4O_3$ $[M+H]^+$ 571.2704, found 571.2705.

4'-(1-(2-Phenylpiperidine-1-carbonyl)-1*H*-1,2,3-triazol-4-yl)-[1,1'-biphenyl]-3-carboxylic acid (21). A solution of **18** (12 mg, 0.022 mmol) in THF (2 ml) was treated with 10% Pd-C (10 mg) and the mixture was stirred overnight at room temperature under N_2. The mixture was passed through Celite and the filtrate was concentrated under reduced pressure. pTLC (ethyl acetate:hexane:acetic acid=75:25:1) afforded **21** (2 mg, 20%).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.47 (s, 1H), 8.39 (s, 1H), 8.11 (d, 1H, *J* = 7.8 Hz), 8.00 (d, 2H, *J* = 8.3 Hz), 7.89 (d, 1H, *J* = 7.8 Hz), 7.75 (d, 2H, *J* = 8.3 Hz), 7.58 (t, 1H, *J* = 7.8 Hz), 7.44-7.25 (m, 5H), 4.38 (brd, 1H, *J* = 13.8 Hz), 3.20 (m, 1H), 2.54 (brd, 1H, *J* = 14.8 Hz), 2.13 (m, 1H), 1.90-1.63 (m, 4H). HRMS calculated for $C_{27}H_{25}N_4O_3$ $[M+H]^+$ 453.1921, found 453.1910.

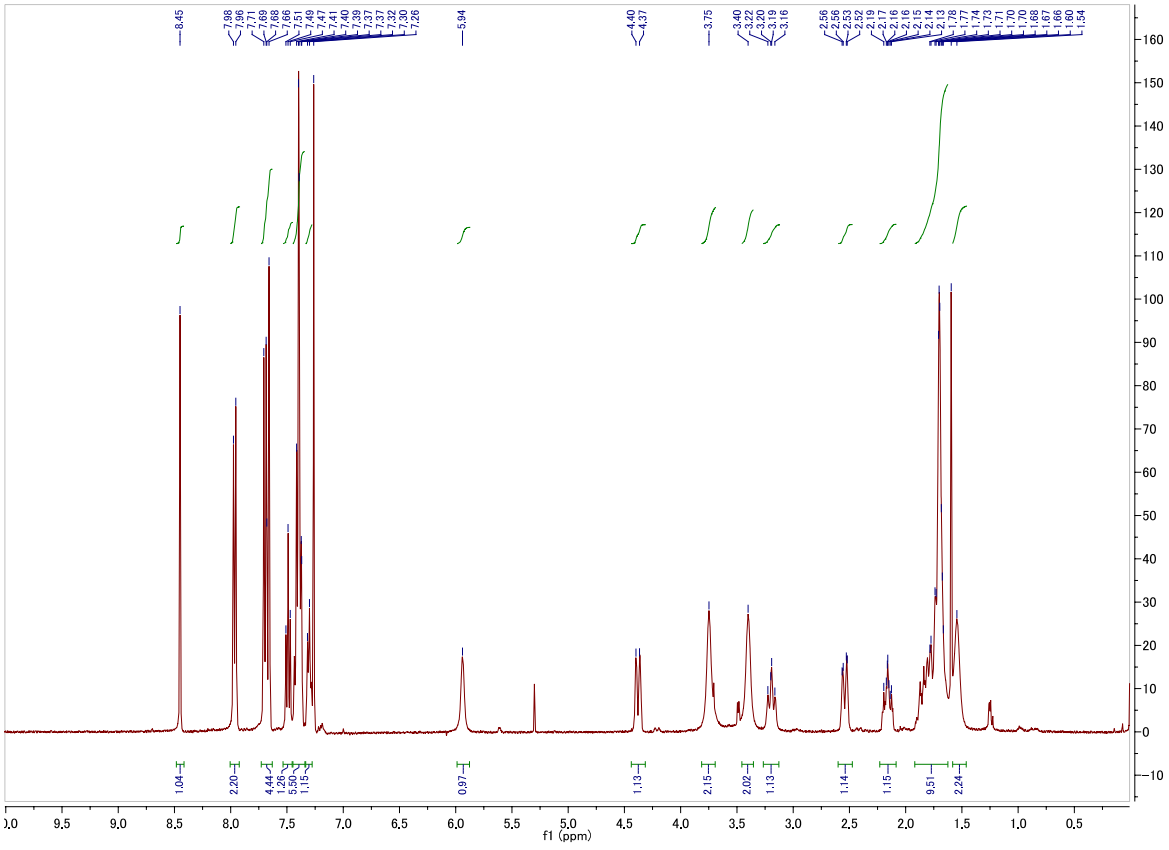
4'-(1-(2-Phenethylpiperidine-1-carbonyl)-1*H*-1,2,3-triazol-4-yl)-[1,1'-biphenyl]-3-carboxylic acid (22). A solution of **19** (12 mg, 0.021 mmol) in THF (2 ml) was treated with 10% Pd-C (10 mg) and the mixture was stirred overnight at room temperature under N_2. The mixture was passed through Celite and the filtrate was concentrated under reduced pressure. pTLC (ethyl acetate:hexane:acetic acid=75:25:1) afforded **22** (8 mg, 79%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.41 (s, 1H), 8.35 (br, 1H), 8.12 (d, 1H, *J* = 7.8 Hz), 7.99 (d, 2H, *J* = 8.4 Hz), 7.90 (d, 1H, *J* = 7.8 Hz), 7.75 (d, 2H, *J* = 8.4 Hz), 7.59 (t, 1H, *J* = 7.8 Hz), 7.30-7.15 (m, 5H), 4.76 (m, 1H), 4.34 (brd, 1H, *J* = 13.8 Hz), 3.38-2.48 (m, 3H), 2.28 (m, 1H), 2.00-1.63 (m, 7H). HRMS calculated for $C_{29}H_{29}N_4O_3$ $[M+H]^+$ 481.2234, found 481.2234.

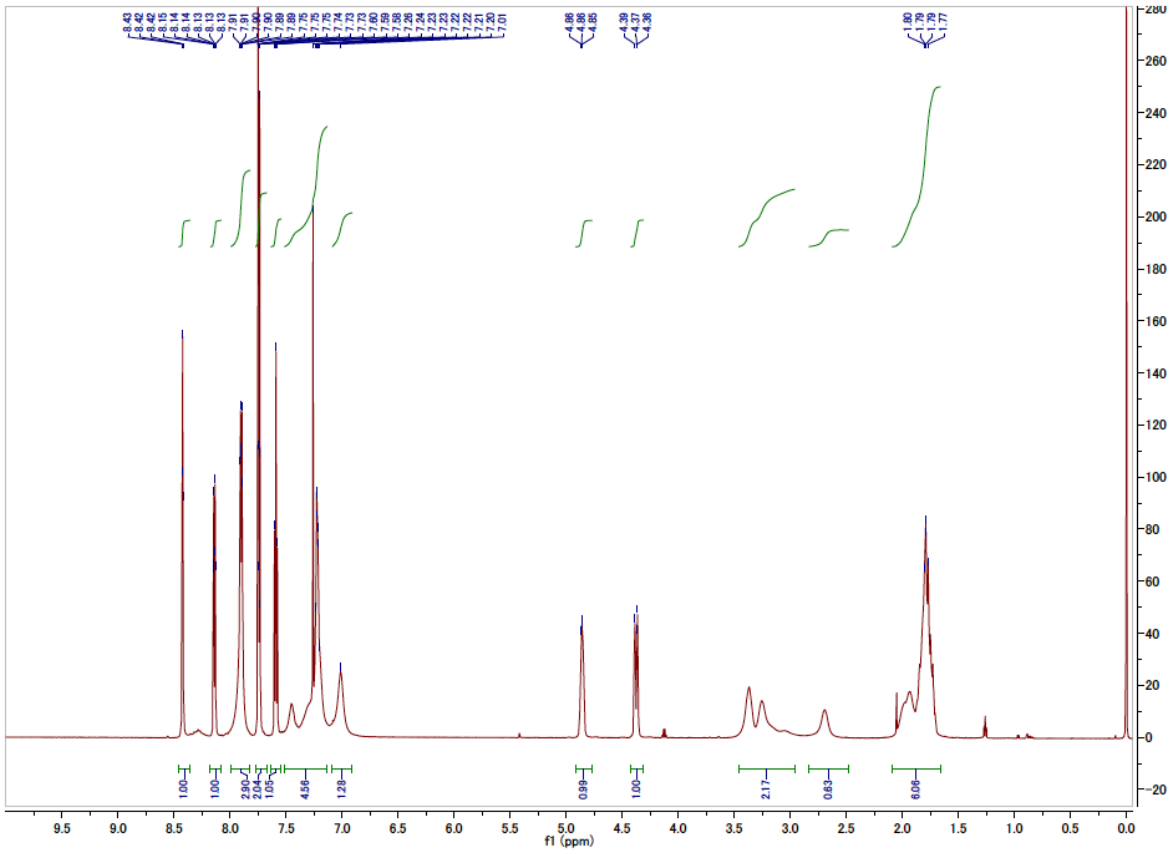
KT182 ^{1}H NMR SPECTRA



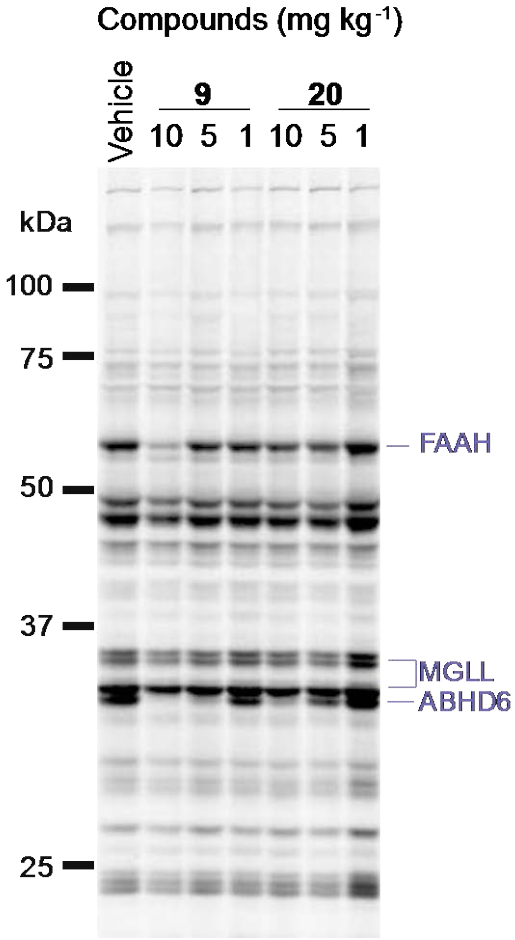
KT185 ^{1}H NMR SPECTRA



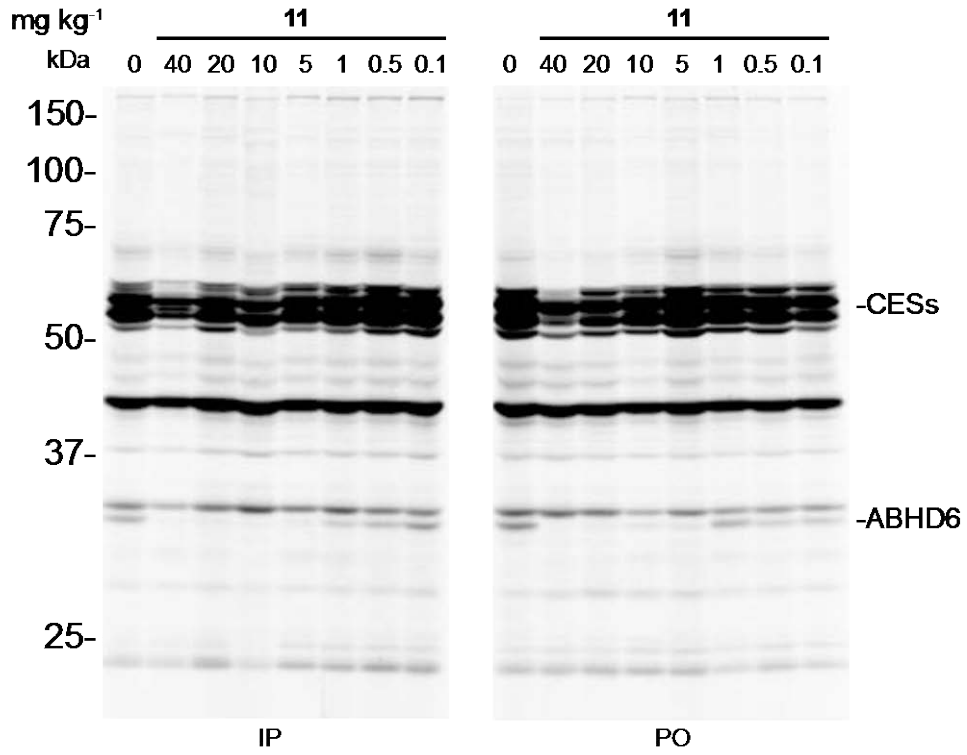
KT203 ^{1}H NMR SPECTRA



II. SUPPLEMENTARY FIGURES



Supplementary Figure 1. Potency and selectivity of **9** (KT182) and **20** (KT203) in the mouse brain of animals treated by oral gavage. Mice were administered vehicle or test compounds (10, 5, or 1 mg kg^{-1}, p.o.), and, after 4 hr, sacrificed and their brain tissues harvested, the membrane fractions isolated, and subjected to gel-based competitive ABPP with FP-Rh (1 µM, 30 min, 37 °C). Fluorescent gels shown in gray scale. Assignment of serine hydrolase enzyme activities in competitive ABPP gels are based on gel migration patterns consistent with past studies.[1-3]



Supplementary Figure 2. Potency and selectivity of **11** (KT185) in the mouse liver *in vivo*. (A) Mice were administered vehicle or test compounds (1-40 mg kg^{-1}, i.p. or p.o.), and, after 4 hr, sacrificed and brain tissue harvested, the membrane fractions subjected to gel-based competitive ABPP with FP-Rh (1 µM, 30 min, 37 °C). Compound **11** produced near-complete inactivation of ABHD6 at 5-10 mg kg^{-1} and showed good selectivity against other liver SHs in mice treated by both i.p. and p.o. routes of administration. For gel-based ABPP experiments, proteomes were labeled with FP-Rh (1 µM probe) for 30 min at 37 °C. Serine hydrolase activities in gels were assigned as described in **Figure 1**.

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