SUPPORTING INFORMATION: Biaryl amides and hydrozones as therapeutics for scrapie in transgenic mice

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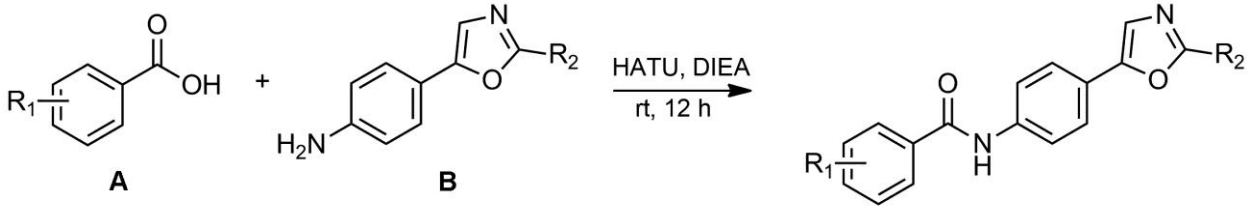
Synthesis and characterization of Compd B analogs

Reagents and solvents were purchased from Aldrich Chemical, Acros Organics, Alfa Aesar, AK Scientific, or TCI America and used as received unless otherwise indicated. Air- and/or moisture-sensitive reactions were carried out under an argon atmosphere in oven-dried glassware using anhydrous solvents from commercial suppliers. Air- and/or moisture-sensitive reagents were transferred via syringe or cannula and were introduced into reaction vessels through rubber septa. Solvent removal was accomplished with a rotary evaporator at ~10–50 Torr. Microwave irradiation was carried out with a CEM Intellivent Explorer system. Automated silica gel column chromatography was carried out using a Biotage SP1 system and silica gel cartridges from Biotage. Analytical TLC plates from EM Science (Silica Gel 60 F_{254}) were employed for TLC analyses. ^{1}H NMR spectra were recorded on a Bruker Avance III plus 400 MHz. Chemical shifts are reported in δ units (ppm) relative to TMS as an internal standard. Coupling constants (*J*) are reported in hertz (Hz). Characterization data are reported as follows: chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, br=broad, m=multiplet), coupling constants, number of protons, mass-to-charge ratio.

All analogs submitted for testing were judged to be of 95% or higher purity based on analytical LC/MS. Analysis of compounds was performed on an Agilent LC/MSD 1200 Series, a quadruple mass spectrometer equipped with a Welchrom XB-C18 column (50 × 4.6 mm, 5 µm) at ambient temperature using a mobile phase of water-acetonitrile containing 0.05% trifluoroacetic acid with a flow rate of 1.5 mL/min. Gradient elution was employed wherein the acetonitrile-water ratio was increased linearly from 5 to 95% acetonitrile over 2.5 min, maintained at 95% acetonitrile for 1.5 min, then decreased to 5% acetonitrile over 0.5 min and maintained at 5% acetonitrile for 0.5 min. Compound purity was determined by integrating peak areas of the liquid chromatogram, monitored at 254 nm.

General procedure for amide coupling with 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU).



All aryl or heteroaryl carboxylic acids and the corresponding 4-(2-alkyl/cyclopropyloxazol-5-yl) anilines cited in this study are commercially available. To a solution of appropriate benzoic acid **A** (1.0 mmol) and 2-substituted oxazol-5-yl aniline **B** (1.0 mmol), HATU (570 mg, 1.5 mmol) in anhydrous DMF (3.5 mL) and diisopropylethylamine (DIEA) (387 mg, 3.0 mmol) were added. The reaction mixture was stirred at room temperature for 12 h and then poured into 5 mL of ice-cold water. The resulting precipitates were filtered and washed with water to give to the crude

product, which was further purified by preparative HPLC to give the substituted N-(4-(oxazol-5-yl)phenyl)benzamide in good yield.

***N*-[4-(1,3-oxazol-5-yl)phenyl]pyridine-4-carboxamide (1).** The title compound was prepared from pyridine-4-carboxylic acid and 4-(oxazol-5-yl)aniline according to the above procedure to afford the title compound as a yellow solid (22 mg) in 15% yield. Pyridine-4-carboxylic acid (68.6 mg, 0.567 mmol) and 4-(1,3-oxazol-5-yl)aniline (100 mg, 0.624 mmol) were dissolved in 2 ml DMF. HATU (474 mg, 1.25 mmol) was added followed by diisopropylethylamine (402 mg, 0.542 ml, 3.12 mmol). The reaction was stirred at room temperature for 72 h. The reaction mixture was diluted with ethyl acetate, shaken with 50% saturated $NaHCO_3$ then brine. The organic layer was further dried with $MgSO_4$. Solvent was removed to afford a crude product that was triturated with ethyl acetate to afford a product of intermediate purity which was then recrystallized from ethyl acetate to afford a final pure product (21.9 mg, 15%). ^{1}H NMR (400 MHz, DMSO-d6) d 10.65 (s, 1H), 8.77–8.83 (m, 2H), 8.43 (s, 1H), 7.84–7.93 (m, 4H), 7.72–7.78 (m, 2H), 7.64 (s, 1H) MS [m+H$^+$] = 266.

4-Methoxy-*N*-[4-(1,3-oxazol-5-yl)phenyl]benzamide (2). The title compound was prepared from 4-methoxybenzoic acid and 4-(oxazol-5-yl) aniline according to the above procedure to afford the title compound as a yellow solid (65 mg) in 39% yield. 4-methoxybenzoic acid (86.2 mg, 0.567 mmol) and 4-(1,3-oxazol-5-yl)aniline (100 mg, 0.624 mmol) were dissolved in 2 ml DMF. HATU (474 mg, 1.25 mmol) was added followed by diisopropylethylamine (402 mg, 0.542 ml, 3.12 mmol). The reaction was stirred at room temperature for 72 h. The reaction mixture was diluted with ethyl acetate, shaken with 50% saturated $NaHCO_3$ then brine. The pure product precipitated in the

course of the workup. After the brine wash, the 2-phase mixture was filtered on paper (1.5-cm disc), washed twice with water, then twice with ether to afford the product (65.3 mg, 39%). ^{1}H NMR (400 MHz, DMSO-d6) d 10.24 (s, 1H), 8.41 (s, 1H), 7.93–8.02 (m, 2H), 7.87–7.93 (m, 2H), 7.68–7.74 (m, 2H), 7.61 (s, 1H), 7.04–7.12 (m, 2H), 3.85 (s, 3H) MS [m+H+] = 295.

4-Cyano-*N*-(4-(2-methyloxazol-5-yl)phenyl)benzamide (7). The title compound was prepared from 4-cyanobenzoic acid and 4-(2-methyloxazol-5-yl)aniline according to the above procedure to afford the title compound as a yellow solid (55 mg) in 10% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.53 (3H, s), 7.19 (1H, s), 7.62–7.64 (2H, m), 7.66–7.69 (2H, m), 7.80–7.83 (2H, m), 7.98–8.00 (2H, m); LCMS (ESI) m/z 304.1 (MH+).

4-Chloro-*N*-(4-(2-cyclopropyloxazol-5-yl)phenyl)benzamide (14). The title compound was prepared from 4-chlorobenzoic acid and 4-(2-cyclopropyloxazol-5-yl)aniline according to the above procedure to afford the title compound as a yellow solid (124 mg) in 32% yield. ^{1}H NMR (400 MHz, CDCl3) δ 1.07–1.14 (4H, m), 2.11–2.14 (1H, m), 7.14 (1H, s), 7.47–7.48 (2H, m), 7.57–7.59 (2H, m), 7.67–7.69 (2H, m), 7.81–7.83 (3H, m); LCMS (ESI) m/z 338.7 (MH+).

***N*-(4-(2-cyclopropyloxazol-5-yl)phenyl)-4-(2-methoxyethoxy)benzamide (15).** The title compound was prepared from commercial 4-(2-methoxy-ethoxy)-benzoic acid and 4-(2-cyclopropyloxazol-5-yl)aniline according to the above procedure to afford the title compound as a yellow solid (69 mg) in 45% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06–1.14 (4H, m), 2.11–2.13 (1H, m), 3.47 (3H, s), 3.77–3.79 (2H, m), 4.18–4.20 (2H,

m), 7.02–7.03 (2H, m), 7.13 (1H, s), 7.56–7.58 (2H, m), 7.67–7.69 (2H, m), 7.76 (1H, s), 7.83–7.85 (2H, m); LCMS (ESI) m/z 379.1 (MH+).

4-Cyano-*N*-(4-(2-cyclopropyloxazol-5-yl)phenyl)benzamide (17). The title compound was prepared from 4-cyanobenzoic acid and 4-(2-cyclopropyloxazol-5-yl)aniline according to the above procedure to afford the title compound as a yellow solid (120 mg) in 48% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07–1.13 (4H, m), 2.11–2.12 (1H, m), 7.16 (1H, s), 7.59–7.61 (2H, m), 7.70–7.71 (2H, m), 7.80–7.82 (3H, m), 7.97–8.00 (2H, m); LCMS (ESI) m/z 329.8 (MH+).

Supporting Table 1. Brain concentrations of Compd B after long-term dosing.

Timepoint (days)	Concentration (μM)	
	Group 1	Group 2
7	8.2, 12.2, 10.8	0.7, 0.5, 0.3
14	0.03, 0.04, 0.06	0.06, 0.11, 0.09
21	0.02, 0.02, 0.03	0.11, 0.09, 1.4
28	1.5, 2.0, 3.2	1.5, 13.7, 0.3

Sets of three mice were dosed with Compd B at 110 mg/kg/day for 7, 14, 21, or 28 days, after which they were euthanized and their brains removed for analysis. This was performed in two independent replicate groups; values given for individual mice.