SUPPLEMENTAL INFORMATION:
Different 2-aminothiazole therapeutics produce distinct patterns of scrapie prion neuropathology in mouse brains

Kurt Giles, David B. Berry, Carlo Condello, Ronald C. Hawley,
Alejandra Gallardo-Godoy, Clifford Bryant, Abby Oehler, Manuel Elepano,
Sumita Bhardwaj, Smita Patel, B. Michael Silber, Shenheng Guan,
Stephen J. DeArmond, Adam R. Renslo, and Stanley B. Prusiner

SUPPLEMENTAL METHODS

Synthesis and characterization of IND124, IND125, and IND126

General

Reagents and solvents were purchased from Aldrich Chemical, Acros Organics, Alfa Aesar, AK Scientific, or TCI America and used as received. 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. Analytical TLC plates from EM Science (Silica Gel 60 F254) were employed for TLC analyses. ^{1}H NMR spectra were recorded on a Varian INOVA-400 400 MHz spectrometer. 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, and mass to charge ratio. All analogues submitted for testing were judged to be of 95% or higher purity based on analytical LC/MS analysis. LC/MS analyses were performed on a Waters Micromass ZQ/ Waters 2795 separation module/Waters 2996 photodiode arraydetector system controlled by MassLynx 4.0 software. Separations were carried out on an XTerra MS C18 5 μm 4.6 mm × 50 mm column at ambient temperature using a mobile phase of water-acetonitrile (ACN) containing 0.05% trifluoroacetic acid. Gradient elution was employed wherein the ACN-water ratio was increased linearly from 5 to 95% ACN over 2.5 min, then maintained at 95% ACN for

1.5 min, and then decreased to 5% ACN over 0.5 min and maintained at 5% ACN for 0.5 min. Compound purity was determined by integrating peak areas of the liquid chromatogram, monitored at 254 nm.

[4-(1,3-oxazol-5-yl)phenyl]thiourea

To a stirred solution of 4-(1,3-oxazol-5-yl)aniline (1.00 g, 6.2 mmol) in acetone (10 mL) at 25 °C, benzoyl isothiocyanate (1.12 g, 0.93 ml, 6.9 mmol) was added slowly. The mixture was heated at reflux for 3 h and then cooled. The solvent was evaporated, and the crude product (1-benzoyl-3-[4-(1,3-oxazol-5-yl)phenyl]thiourea) was taken up in 5N NaOH (10 ml) and stirred with heating at 100 °C for 30 min or until debenzoylation was judged complete by TLC. The reaction mixture was then poured into 5N HCl (10 mL) and ice (20 g) and stirred for 30 min, after which time, it was carefully treated with saturated aqueous Na_2CO_3 to reach pH ~8. The product precipitated and was filtered, washed with water, and dried *in vacuo*. Re-crystallization from MeOH afforded the title compound (0.44 g, 32%): ^{1}H NMR (400 MHz, DMSO-*d*6) d 7.55 (m, *J* = 8.42 Hz, 2 H) 7.61 (s, 1 H) 7.66 (m, *J* = 8.42 Hz, 2 H) 8.40 (s, 1 H) 9.82 (s, 1 H); MS (ESI) m/z 219.83 (MH+).

N-[4-(1,3-oxazol-5-yl)phenyl]-4-(pyridin-4-yl)-1,3-thiazol-2-amine [IND124]

A flask was charged with [4-(1,3-oxazol-5-yl)phenyl]thiourea (0.310 g, 1.41 mmol) and 2-bromo-1-(pyridine-4-yl)ethan-1-one hydrobromide (0.397 g, 1.41 mmol) in EtOH (40 mL) and stirred at room temperature for 18 h. The reaction mixture was then poured into 150 ml ice-water, and 5N NaOH was added carefully to reach pH ~8. The solid precipitate was collected by filtration, washed with water, and dried to afford the crude

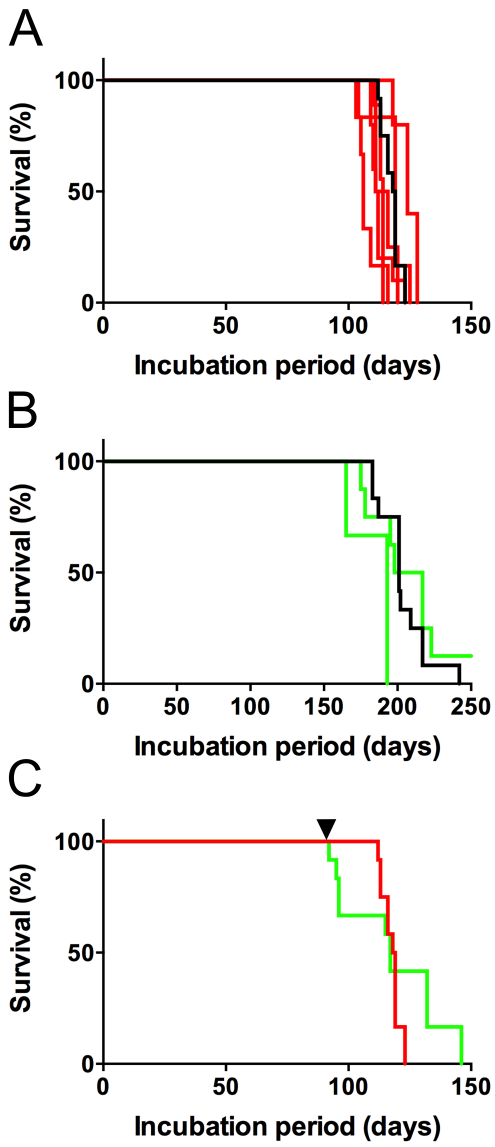
product. This material was then triturated with hot ethyl acetate to afford the title compound (292 mg, 65%): ^{1}H NMR (400 MHz, DMSO-*d*6) d 7.56 (s, 1 H) 7.73 (d, *J* = 8.06 Hz, 2 H) 7.77 (s, 1 H) 7.85 (d, *J* = 8.24 Hz, 2 H) 7.87–7.91 (m, 2 H) 8.34–8.43 (m, 1 H) 8.63 (d, *J* = 4.58 Hz, 2 H) 10.60 (s, 1 H); MS (ESI) m/z 321.02 (MH+).

N-[4-(1,3-oxazol-5-yl)phenyl]-4-(3-phenyl-1,2-oxazol-5-yl)-1,3-thiazol-2-amine [IND125]

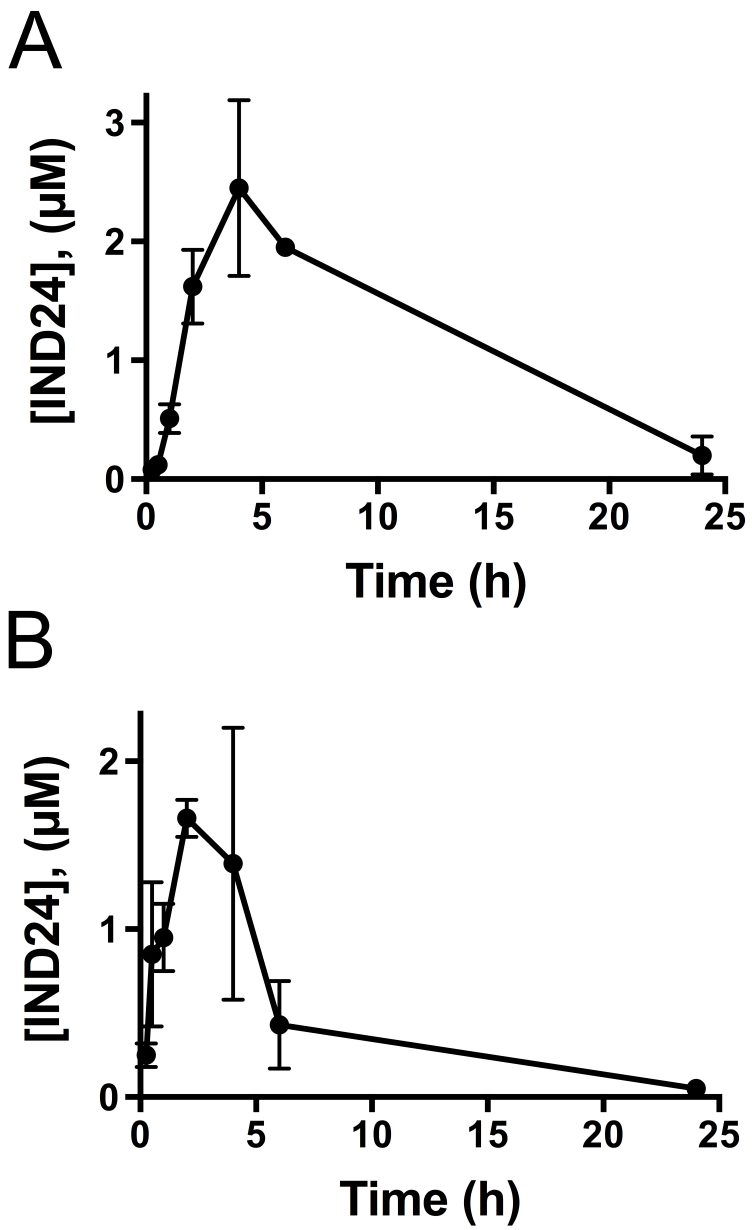
A flask was charged with [4-(1,3-oxazol-5-yl)phenyl]thiourea (0.310 g, 1.41 mmol) and 2-bromo-1-(3-phenyl-1,2-oxazole-5-yl)ethan-1-one (0.376 g, 1.41 mmol) in EtOH (40 mL) and stirred at room temperature for 18 h. The reaction mixture was then poured into ice-water, and 5M NaOH was added carefully to reach pH ~8. The solid precipitate was collected by filtration, washed with water, and dried to afford the title compound (0.470 g, 86%): ^{1}H NMR (400 MHz, DMSO-*d*6) d 7.40 (s, 1 H) 7.55 (m, 4 H) 7.64 (s, 1 H) 7.73 (d, *J* = 8.42 Hz, 2 H) 7.86 (d, *J* = 8.61 Hz, 2 H) 7.98 (m, 2 H) 8.40 (s, 1 H) 10.69 (s, 1 H); MS (ESI) m/z 386.97 (MH+).

4-(1-benzofuran-2-yl)-N-[4-(1,3-oxazol-5-yl)phenyl]-1,3-thiazol-2-amine [IND126]

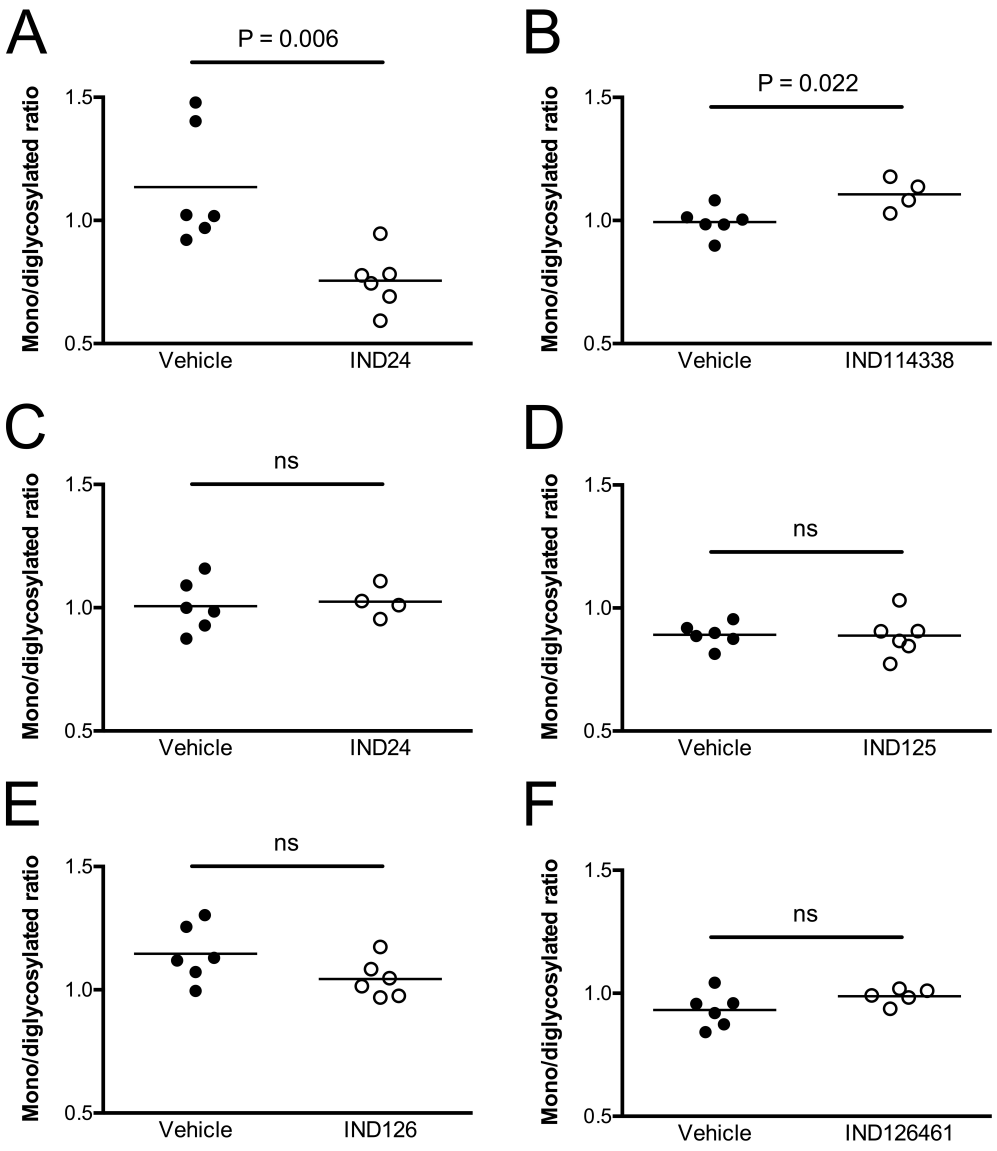
A flask was charged with [4-(1,3-oxazol-5-yl)phenyl]thiourea (0.050 g, 0.23 mmol) and 1-(1-benzofuran-2-yl)-2-bromoethan-1-one (0.055 g, 0.23 mmol) in EtOH (5 mL) and heated with stirring at 60 °C for 5 h. The reaction mixture was allowed to cool, poured into ice-water, and saturated aqueous Na_2CO_3 was added to reach pH ~8. The solid precipitate was collected by filtration, washed with water, and dried to afford the title compound (0.078 g, 95%): ^{1}H NMR (400 MHz, DMSO-*d*6) d 7.20 (s, 1 H) 7.20–7.32 (m, 2 H) 7.36 (s, 1 H) 7.52 (s, 1 H) 7.57 (d, *J* = 7.87 Hz, 1 H) 7.62–7.72 (m, 3 H) 7.81 (d, *J* = 8.79 Hz, 2 H) 8.35 (s, 1 H) 10.58 (s, 1 H); MS (ESI) m/z 359.95 (MH+).



Supplemental Fig. 1. Kaplan-Meier survival curves of RML-inoculated wild-type mice treated with vehicle (red) or IND24 (green) at 210 mg/kg/d. Multiple independent repeats of (**A**) vehicle-treated and (**B**) IND24-treated experiments in comparison to previously reported experiments (black curves) shows the high reproducibility of the assay. (**C**) Initiating IND24 treatment at 90 dpi (arrowhead) led to greater variation in disease onset compared to vehicle-treated controls.



Supplemental Fig. 2. Concentration of IND24 in the brain (**A**) and plasma (**B**) of wild-type FVB mice following a single 10 mg/kg oral gavage dose. Points represent means, and error bars range (*n*=2 per point).



Supplemental Fig. 3. Mono- to diglycosylated glycoform ratios of PK-resistant PrP from vehicle- (solid symbols) and compound-treated (open symbols) mice. (**A**, **B**) Wild-type mice treated with: IND24 (**A**) or IND114338 (**B**). (**C–F**) Tg4053 mice overexpressing PrP treated with IND24 (**C**), IND125 (**D**), or IND126 (**E**), or IND126461 (**F**). Significantly different glycoform ratios were only observed in wild-type mice. ns, not significant.

Supplemental Table 1. Reproducibility of incubation periods in RML-infected mice treated with vehicle or IND24 at 210 mg/kg/d.

Dosing intervention (dpi) [a]	Mean incubation period ± sem (days)	n/n_0 [b]	Survival index ± SEM
none	118 ± 1 [c]	12/12	100 [d]
none	113 ± 0	9/9	
none	113 ± 1	10/10	
none	108 ± 2	6/6	
none	118 ± 3	6/6	
none	124 ± 2	5/5	
1>	198 ± 16	3/3	168 ± 14
1>	200 ± 7	7/8	169 ± 6

[a] Day treatment started, ">" indicates dosing continued until end of experiment.
[b] n, number of ill mice; n_0, number of inoculated mice.
[c] Data previously reported in Berry DB, Lu D, Geva M, Watts JC, Bhardwaj S, Oehler A, Renslo AR, DeArmond SJ, Prusiner SB and Giles K (2013) Drug resistance confounding prion therapeutics. *Proc. Natl. Acad. Sci. U.S.A.* **110**:E4160–E4169.
[d] Vehicle-treated control used to calculate survival index.

Supplemental Table 2. Combined effect of excipient and sex on time to disease onset in wt mice.

Inoculum	Treatment	Dose (mg/kg/d)	Excipient	Male		Female	
				Clinical onset ± SEM (days)	n/n_0 [a]	Clinical onset ± SEM (days)	n/n_0 [a]
none	none	—	1.25% PEG400	104 ± 12	6/6	> 280	0/6
none	none	—	0.125% PEG400	121 ± 9	6/6	n.d.	n.d.
none	none	—	0.5% CMC	> 220	0/3	> 220	0/4
RML	none	—	0.5% CMC	96 ± 5	4/4	115 ± 2	4/4
RML	IND114338	25	0.5% CMC	111 ± 6	3/3	140 ± 12	4/4
RML	IND114338	200	0.5% CMC	167 ± 15	3/3	227 ± 19	4/4

[a] n, number of ill mice; n_0, number of inoculated mice; n.d., not determined.