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2-Aminothiazoles as Therapeutic Leads for Prion Diseases

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2-Aminothiazoles are a new class of small molecules with antiprion activity in prion-infected neuroblastoma cell lines (*J. Virol.* **2010**, *84*, 3408). We report here structure−activity studies undertaken to improve the potency and physiochemical properties of 2-aminothiazoles, with a particular emphasis on achieving and sustaining high drug concentrations in the brain. The results of this effort include the generation of informative structure−activity relationships (SAR) and the identification of lead compounds that are orally absorbed and achieve high brain concentrations in animals. The new aminothiazole analogue (5-methylpyridin-2-yl)-[4-(3-phenylisoxazol-5-yl)-thiazol-2-yl]-amine (**27**), for example, exhibited an EC_{50} of 0.94 μM in prion-infected neuroblastoma cells (ScN2a-cl3) and reached a concentration of ~25 μM in the brains of mice following three days of oral administration in a rodent liquid diet. The studies described herein suggest 2-aminothiazoles as promising new leads in the search for effective therapeutics for prion diseases.

Introduction

The protein misfolding diseases are a family of debilitating neurological disorders associated with the misprocessing of cellular proteins into alternate non-native isoforms that confer cellular toxicity, often associated with oligomeric deposits derived from misfolded protein. Prominent examples of these disorders include Alzheimer's disease, Huntington's disease, Parkinson's disease, frontotemporal dementias, and the prion diseases: Creutzfeldt−Jakob disease in humans, chronic wasting disease in deer, and scrapie in sheep.[2,3] In prion disease, the endogenous prion protein (PrPC)[a] is converted by an unknown mechanism into a protease-resistant and β-sheet-rich form denoted PrP^{Sc}. This conversion can occur spontaneously, result from inherited mutations in the PrP^{C} gene, or be triggered by infection with exogenous PrP^{Sc}. Prion diseases are invariably fatal, and no viable treatments for these devastating disorders are currently available.

While the mechanisms of protein misfolding and subsequent disease progression remain unclear, it is well-known that infectious forms of animal prions can be propagated in cell culture, notably in prion-infected, murine neuroblastoma (ScN2a) cell lines.[4,5] Various immunological methods are available to measure prion load in these cell lines, and so they have provided a valuable means for evaluating the antiprion properties of large and small molecules alike. Among small molecules that have been reported to possess antiprion properties are the acridines[6,7] (e.g., quinacrine, (**1**) and structurally related tricyclic antidepressants, dimeric[8] and chimeric[9] analogues of **1**, statins,[10] 2,4-diphenylthiazole and 2,4-diphenyloxazole amides,[11] pyrazolones,[12,13] indole-3-glyoxamides,[14] and pyridyl hydrazones[15] (e.g., "compound B", **2**). In addition, larger molecules of a polyanionic chemotype (suramin, pentosan polysulfate) or polycationic chemotype (dendritic polyamines, cationic polysaccharides[16]) have been reported to exhibit antiprion activity in cells, although it seems unlikely such species could be used therapeutically. In fact, no small molecule has yet been shown to be broadly effective against a range of prion strains in an animal model of disease[17] and only hydrazone **2** has been reported to significantly extend survival in animals (albeit strain-dependent and at high doses).[15] One possible explanation for this poor track record is that the majority of small molecules investigated to date were originally designed for other purposes (e.g., malaria, hyperlipidemia) and not optimized for either antiprion effects or for the ability to cross the blood−brain barrier (BBB).

We recently described the discovery of antiprion small molecules containing the 2-aminothiazole ring system (e.g., **3**, Figure 1).[1] Preliminary mechanistic profiling of aminothiazole analogues indicated that they neither diminish the expression of PrP^{C} nor denature PrP^{Sc}, thus suggesting that a mechanism influencing PrP^{Sc} formation or clearance is more likely.[1] For example, the compounds might inhibit as-yet unidentified auxiliary macromolecules[18] that promote prion replication or enhance the activity of proteins that facilitate the clearance of PrP^{Sc}. Here we report structure−activity studies aimed at improving the potency of 2-aminothiazoles in vitro and altering the physiochemical properties of these molecules so as to

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[a]Abbreviations: SAR, structure−activity relationships; ScN2a-cl3, neuroblastoma cells infected with "clone-3" Rocky Mountain Laboratory prions; PrP^{C}, endogenous form of the prion protein; PrP^{Sc}, misfolded and pathogenic form of the prion protein; BBB, blood−brain barrier; clogP, calculated octonal/water partition coefficient; P-gp, P-glycoprotein; MDR1-MDCK, multidrug resistance-1 Madin−Darby canine kidney cell line; FVB mice, mice inbred for the Friend leukemia virus B (*Fv1b*) allele.

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increase their likelihood of accessing the brain in vivo. The SAR studies reveal those positions in 2-aminothiazole structure that are sensitive or insensitive to modification (Figure 2). Similarly, structural modifications that improve metabolic stability and permeability are reported, as are the brain concentrations of a representative analogue in mice. Together, these data suggest that 2-aminothiazoles represent a promising new class of drug leads for prion diseases.

Results and Discussion

The antiprion action of new aminothiazole analogues was evaluated using a new "clone-3" cell line (denoted ScN2a-cl3[19]) that expresses a higher level of PrP^{Sc} as compared to the ScN2a cell line used in the original screen. In general, we have found that EC_{50} values for antiprion compounds tend to be ~10-fold higher in the high-expressing cells, and thus the ScN2a-cl3 cell line represents a more stringent test of antiprion action. The EC_{50} values presented in the discussion below represent mean values from three separate determinations using ScN2a-cl3 cells. The precision of the assay is high; the coefficient of variance in mean pEC_{50} values is generally less than 5% (see Supporting Information). The high quality of the assay data allowed even subtle SAR trends to be assigned with some confidence. An evaluation of compound toxicity toward ScN2a-cl3 cells was carried out using the fluorescent probe calcein-AM as we have reported previously.[20] Almost without exception we found that 2-aminothiazole analogues are not toxic to ScN2a-cl3 cells (Supporting Information), indicating that 2-aminothiazoles reduce PrP^{Sc} load in ScN2a-cl3 cells by a drug-like mechanism (i.e., not simply by killing cells). Notably, 2-aminothiazoles do not reduce PrP^{Sc} load in *nondividing* ScN2a-cl3 cells that have been arrested in cell division by treatment with sodium butyrate. It should be noted, however, that lack of activity in nondividing cells does not necessarily preclude antiprion efficacy in animals, as we found that hydrazone **2**, like 2-aminothiazoles, has no effect on nondividing ScN2a-cl3 cells (data not shown).

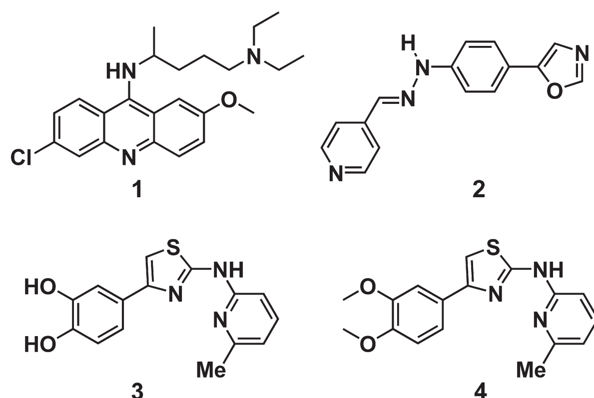
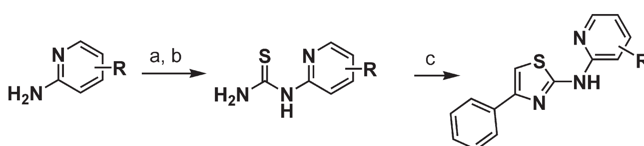


Figure 1. Structures of small molecules with antiprion properties.

The SAR studies described herein were undertaken with the dual objectives of expanding upon nascent SAR from the primary screen[1] and identifying improved aminothiazole analogues with a higher likelihood of penetrating the brain in animals. To help achieve the latter objective, we applied recently advanced[21] guidelines for assessing the potential CNS activity of small molecules. These "rules of thumb" advise special attention to properties such as molecular weight (< 500 Da, preferred), polar surface area (< 90 $Å^2$), clogP (2−5), and the number of hydrogen bond donors (< 3). The synthesis of new 2-aminothiazole analogues was carried out in both serial and parallel formats using the Hantzsch-type condensation of bromomethyl ketones with thioureas (Scheme 1 and Supporting Information). An early objective of the SAR study was to modify the catechol ring present in early screening hits like **3** because such functionality would likely limit brain exposure in vivo and might also present metabolic and/or toxicological liabilities. Evaluation of the corresponding dimethoxyphenyl analogue **4** (Figure 1) showed it to be equipotent to **3**, thereby alleviating concerns that a catechol A-ring might be required for antiprion activity. With this potential liability eliminated, a systematic exploration of aminothiazole SAR was initiated.

Preliminary SAR gleaned from the initial screen[1] suggested a preference for 2-pyridyl type C-rings over simple aryl congeners. To evaluate more fully the C-ring SAR, a series of analogues were synthesized with alkyl, aryl, or heteroaryl groups at this position (Chart 1). The *N*-alkyl analogue (**5**) was without significant activity, while among regioisomeric pyridyl analogues, 2-pyridyl analogue **7** was indeed more potent than the 3-pyridyl or 4-pyridyl congeners (**8** and **9**). Hence, analogue **7** had an EC_{50} value (defined as the effective concentration for reducing PrP^{Sc} load in ScN2a-cl3 cells by 50%) of 1.22 μM, roughly 10-fold lower than the original[1] screening hits. Replacement of the 2-pyridyl ring in **7** with 2-pyrimidyl or 2-pyrazinyl rings produced analogues of comparable (**10**) and reduced (**11**) potency, respectively. Next, we examined ring-substitution effects in the favored 2-pyridyl

Scheme 1. Hantzsch-Type Synthesis of 2-Aminothiazole Analogues from Amines via Thiourea Intermediates[a]



[a] Reagents and conditions: (a) PhSCN, acetone, reflux; (b) NaOH, MeOH, reflux; (c) bromoacetophenone, EtOH, reflux.

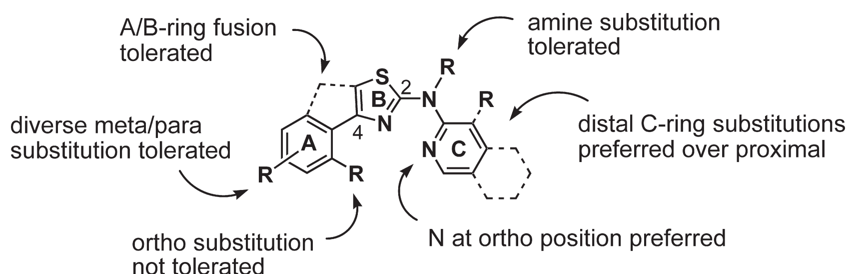


Figure 2. Summary of structure−antiprion activity relationships for 2-aminothiazole analogues. The three rings are arbitrarily denoted A, B, and C for convenience.

Chart 1

R =

Compound	5	6	7	8	9	10	11
ScN2a-cl3 EC_{50} (μM)	>32	8.2	1.22	28	6.38	3.94	15.6

Compound	12	13	4	14	15	16	17	18	19	20
ScN2a-cl3 EC_{50} (μM)	2.53	1.00	3.01	0.79	7.29	1.00	0.11	0.43	0.39	>32

Chart 2

R^1 =

Compound	21	22	23	24	25	26	27	28	29
ScN2a-cl3 EC_{50} (μM)	3.03	>29	1.57	0.86	7.88	1.23	0.94	>26	>32

R^2 =

Compound	30	31	32	33	34	35	36	37
ScN2a-cl3 EC_{50} (μM)	0.34	>32	0.31	3.08	2.01	0.80	>32	8.66

C-ring type. In general, ring substitution was most favorable in positions distal from the B−C ring connection, as in methyl substituted analogues **14** and **16**, and especially in the more extended bicyclic C-ring analogues **17**−**19** (EC_{50} = 0.11 μM for **17**). In contrast, analogues substituted proximally to the B−C ring connection (e.g., **15**, **20**) were notably less potent than their unsubstituted comparators. Electronic effects in the C-ring appear to be of comparably smaller importance; analogues with electron rich (**13**) or electron deficient (**12**) 2-pyridyl rings showed similar activities. The most potent analogues identified from this series were those with more extended bicyclic C-ring systems. Hence, quinoline, isoquinoline, and naphthalene analogues (**17**−**19**) were approximately 10-fold more potent than comparable monocyclic analogues and at least 100-fold more potent than the original screening hits.

Having identified several viable new C-ring subtypes, we next explored SAR of the "A-ring" positioned at C-4 of the aminothiazole ring (Figure 2). As noted above, bis-methylation of the catechol function in **3** to afford **4** was a tolerated modification. A more systematic exploration of A-ring preferences was carried out in the context of the favored 2-pyridyl and isoquinoline C-ring types. Various aromatic and heteroaromatic ring systems could be tolerated (Chart 2), but analogues bearing small alkyl groups at this position were either inactive (**29**, **36**) or less potent (**37**). Analogues **25** and **33** bearing unsubstituted phenyl A-rings were between 2- and 10-fold less potent than pyridyl (**23**, **24**, **32**) or *para*-methoxyphenyl A-ring analogues (**21**, **30**). Whereas *para*-methoxyphenyl analogue **30** was among the most potent analogues examined (EC_{50} = 0.34 μM), the analogous trifluoromethoxyphenyl analogue **31** was surprisingly inactive. Other favorable A-rings conferring low or submicromolar potencies included phenyl-substituted isoxazoles as found in analogues **27** and **35** and pyridyl-substituted thiophenes as in analogues **26** and **34**.

The general tolerance of para or meta substitution on the A-ring can be contrasted with an apparent intolerance for substitution at the ortho position. This effect was evident in both six-membered (compare **21** and **22**) and five-membered (compare **27** and **28**) A-rings and was true regardless of C-ring chemotype. These findings suggest perhaps that a coplanar arrangement of the A- and B-ring is important for activity, the presence of an ortho substituent as in **22** and **28** disfavoring such a conformation. To test this supposition, we prepared fused tricyclic analogues **38** and **39** (Figure 3) in which a coplanar conformation is enforced by A-B ring fusion. In both cases, the A−B ring fusion was tolerated, being somewhat favored in the case of **38** (as compared to **25**) and somewhat disfavored in the case of **39** (as compared to **14**). This observation that ortho substitution is tolerated *only* in the context of A−B ring fusion supports the notion that a coplanar conformation of these ring systems is important for activity.

Among the new A-ring variants examined (Chart 2), phenylisoxazole **27** was notable for its submicromolar potency and improved stability to rat liver microsomes as compared to phenyl (**25**) and pyridyl (**23**) congeners (Table 1). This finding led to a reinvestigation of favored C-ring types in the context

of the phenylisoxazole A-ring (Chart 3). Perhaps not surprisingly, the SAR of phenylisoxazole A-ring analogues was not completely reconcilable with SAR in the original dimethoxyphenyl A-ring series. Thus, whereas extended bicyclic C-rings (isoquinoline, quinoline) were optimal in combination with the dimethoxyphenyl A-ring (Chart 1), the most potent phenylisoxazole analogues were those bearing methoxypyridine C-rings, as in analogues **40** (EC_{50} = 0.23 μM) and **41** (EC_{50} = 0.25 μM). By comparison, other pyridine (**27**, **42**−**45**) and quinoline (**35**) C-ring analogues were between 4- and 20-fold less potent.

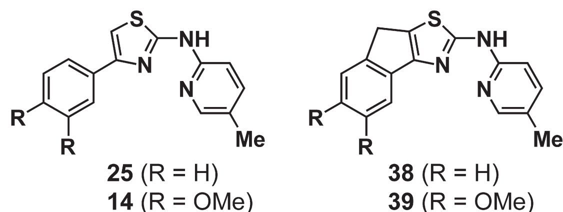


Figure 3. Chemical structures and antiprion activity of analogues **38** and **39**, in which ring fusion enforces a coplanar A/B-ring conformation. Activities of the corresponding unconstrained analogues **25** and **14** are shown for comparison.

We also examined SAR relating to the nature of connection between the A-, B-, and C-rings. For example, insertion of an amide function between the A- and B-ring in analogue **14** produced analogue **46** of comparable potency (Figure 4). However, the consequent introduction of an additional hydrogen bond donor in **46** was judged undesirable with respect to CNS properties, and so amide-linked analogues like **46** were not pursued further. With respect to the B−C ring connection, methylation (as in **49**) or acylation (as in **50**) of the amine linkage in analogue **17** was well tolerated. In contrast, replacement of the amine linkage in **17** with amide linkages (as in **47** and **48**) led to a ∼100-fold loss of potency (Figure 4). Overall, activity data derived from analogues **47**−**50** suggest that proper spacing of the B- and C-rings is important for antiprion activity, whereas the presence of a hydrogen bond donor in the B−C ring linkage is not. This latter finding is significant because the complete elimination of hydrogen bond donors in analogues like **49** and **50** would predict for better permeability across the BBB in animals.

Table 1. In Vitro Microsome Stability, Permeability Data, and Calculated Physiochemical Properties for Select 2-Aminothiazole Analogues

	antiprion activity	microsome stability[a]	permeability[b] (10^{-6} cm/s)		calcd properties[c]			
compd	ScN2a-cl3 EC_{50} (μM)	$T_{1/2}$ (min)	$P_{A \to B}$	$P_{B \to A}$	MW	PSA (Å^2)	ClogP	HBD
25	7.88	10	nd	nd	267.4	66.0	4.5	1
23	1.57	20	32.6	42.3	268.3	78.9	3.2	1
27	0.94	151	6.8	8.3	334.4	92.1	4.9	1
28	> 26	19	11.2	8.6	348.4	92.1	5.1	1
12	2.53	150	9.4	8.7	381.4	84.5	4.4	1
13	1.00	45	20.1	24.4	343.4	93.7	3.3	1
17	0.11	83	13.2	10.5	363.4	84.5	4.5	1
18	0.43	69	9.2	8.9	363.4	84.5	4.9	1

[a] Rat liver microsomes. [b] Permeability across MDR1-MDCK cell monolayers in the apical to basal (absorptive) and basal to apical (secretory) directions. [c] Molecular weight (MW), polar surface area (PSA), and calculated *n*-octanol−water partition coefficient (ClogP) values calculated using MarvinSketch 4.1.8. HBD = number of hydrogen bond donors. nd = not determined.

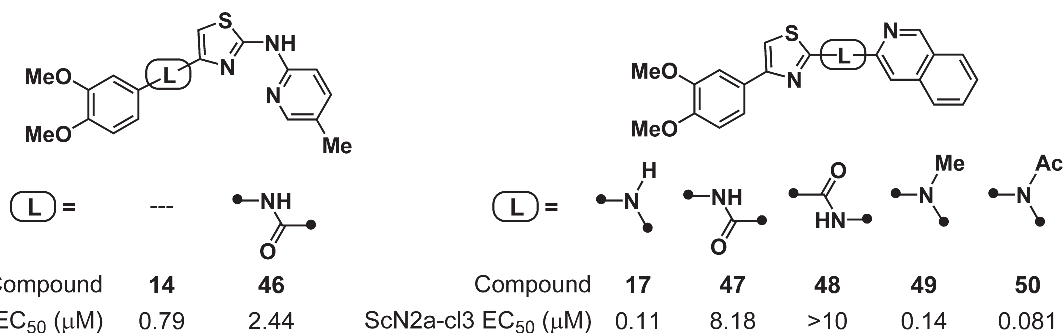
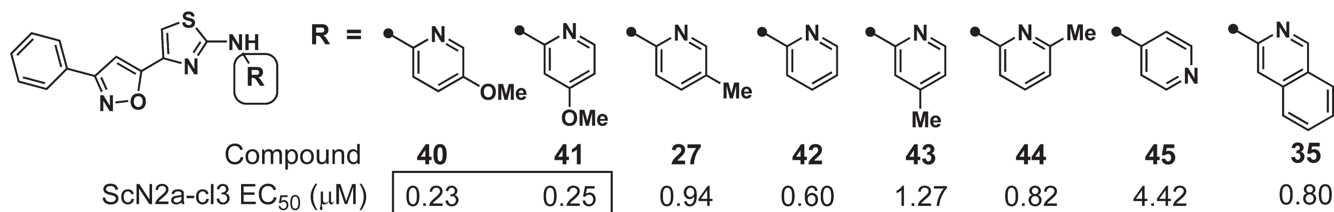


Figure 4. Chemical structures and antiprion activity of analogues with modified A−B ring linkages (**46**) or B−C ring linkages (**47**−**50**). The introduction of an amide linkage was better tolerated at the A−B ring connection (**46**) than at the B−C ring connection (**47** and **48**). Alkylation or acylation of the amino B−C ring linkage was tolerated (**17** vs **49** and **50**), demonstrating that a hydrogen bond donor is not required in this position.

Chart 3



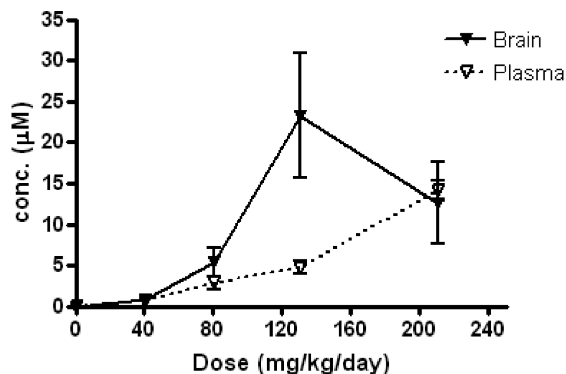


Figure 5. Brain and plasma concentrations (μM) of aminothiazole **27** in mice after three days of feeding. Compound **27** was administered at the indicated doses as part of a rodent liquid diet (n = 3 per dosing group).

The SAR studies described above have revealed a number of structural determinants in the antiprion activities of 2-aminothiazoles. Just as importantly, many of the new analogues possess physiochemical properties that predict permeability across the BBB. In fact, most small molecules do not readily traverse the BBB and/or are subject to active efflux mediated by drug resistance transporters (e.g., P-glycoprotein transporter; P-gp) expressed in the endothelial cells that constitute the BBB.[22] Efflux by P-gp is much more difficult to predict than is passive permeation of the BBB. To address the potential for efflux, a subset of aminothiazole analogues were evaluated for permeability in P-gp-expressing Multidrug resistance-1 Madin−Darby canine kidney (MDR1-MDCK) cell monolayers, an assay that has been utilized as an in vitro predictor of in vivo BBB permeability.[23] All eight analogues evaluated showed good permeability in this assay, and more importantly, none appeared likely to be P-gp substrates based on absorptive (apical to basalateral) and secretory (basalateral to apical) permeability values (Table 1). In fact, analogues **28**, **12**, **17**, and **18** showed greater permeability in the absorptive direction. Furthermore, analogues **17** and **18**, as well as **12** and **27**, displayed excellent stability to rat liver microsomes in vitro. On the basis of antiprion potency, metabolic stability, and permeability, optimized 2-aminothiazole analogues like **27**, **13**, **17**, and **18** were considered as candidates for further study in animals.

While a full account of the pharmacokinetic optimization and pharmacological evaluation of 2-aminothiazoles will appear in a subsequent communication, we present here the results of a representative feeding experiment in which compound **27** was administered at escalating doses (0, 40, 80, 130, or 210 mg/kg/day) to wild-type FVB mice for three days as part of a rodent liquid diet (Figure 5). This protocol is suitable for subsequent animal efficacy trials, where daily dosing for well over 100 days is required (administration by oral gavage is not practical for such long-term experiments). Brain and plasma concentrations of compound **27** were measured after the 3-day administration period. Increasing doses of **27** resulted in a linear increase in plasma concentrations (Figure 5). Doses up to 130 mg/kg/day also resulted in linear increases in brain concentrations. While significant variability between animals was seen at the two highest doses, concentrations of **27** in brain generally exceeded those in plasma. Mean brain concentrations of **27** were in excess of the compound's in vitro activity (EC_{50} = 0.94 μM), surpassing it by as much as 25-fold at the higher doses. Because the reported concentrations were determined at an arbitrary time point following three days of feeding, they represent pseudo steady-state rather than peak concentrations. Full pharmacokinetic parameters were not determined as part of this study, as the intent was to evaluate drug concentrations in brain and plasma at pseudo steady-state. Differences in feeding behavior among individual animals may partially explain the observed variability within certain animal cohorts. Overall, the excellent brain concentrations achieved in these studies confirm that 2-aminothiazole analogues such as **27** are absorbed following oral administration and achieve and maintain high concentrations in the brains of animals. These results prompted us to select several 2-aminothiazole analogues as candidates for further investigation in mouse models of prion disease.

Conclusion

In conclusion, we have identified improved 2-aminothiazole analogues that possess EC_{50} values as low as 81 nM in ScN2a-cl3 cells. The SAR revealed in this study suggests action at one or more defined molecular targets, the identification of which remains to be established. The physiochemical properties of many 2-aminothiazole analogues are favorable for possible therapeutic use in prion diseases. Preliminary animal studies demonstrate that members of the 2-aminothiazole class are orally absorbed when formulated appropriately in liquid rodent diet and can achieve steady-state brain concentrations well in excess of their in vitro potencies. Whether any of the 2-aminothiazoles described herein can extend the lives of humans or experimental animals with prion disease is unknown but will be of considerable interest.

Experimental Section

General. 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 ca. 10−50 Torr. Automated 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. Melting points were determined with an electrothermal capillary melting point apparatus and are uncorrected. ^{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 (**3**−**50**) 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 array detector system controlled by MassLynx 4.0 software. Separations were carried out on an XTerra MS C_{18} 5 μm 4.6 mm × 50 mm column at ambient temperature using a mobile phase of water−acetonitrile containing 0.05% trifluoroacetic acid. Gradient elution was employed wherein the acetonitrile−water ratio was increased linearly from 5 to 95% acetonitrile over 2.5 min, then maintained at 95% acetonitrile for 1.5 min, and 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 Preparing Thiourea Intermediates from Amines. Neat phenyl isothiocyanate (1.1 mmol, 1.1 equiv) was added dropwise to a stirred solution of the aniline, aminopyridine, or other amine building block (1 mmol) in acetone (10 mL) at room temperature. The reaction mixture was heated to reflux for 1−3 h until judged complete (LC/MS) and then cooled, poured into water−ice, and stirred for an additional 30 min. The benzoyl thiourea precipitate was collected by filtration and washed with more water. This crude material was dissolved in methanol (20 mL) and treated with 5 mL of aqueous 1N NaOH. The reaction mixture was heated to 80 °C until hydrolysis was judged complete (LC/MS). After cooling, the reaction mixture was poured into water−ice and sufficient aqueous 1N HCl was added to produce a neutral (pH ~ 7) solution. The thiourea intermediate typically precipitates from the neutral solution and was collected by filtration and dried. This two-step procedure provides thiourea intermediates in 50−95% overall yield, with purities generally > 90% as determined by ^{1}H NMR. These intermediates were used in the next step without further purification.

General Procedure for Preparing 2-Aminothiazole Analogues. An ethanolic solution (~10 mL) of the desired thiourea (1 mmol) and the requisite bromoacetophenone (1 mmol) was heated at reflux for 3−5 h or until the reaction was judged complete (LC/MS). The reaction mixture was then poured into water−ice (20 mL) and stirred for another 30 min. A solution of aqueous 1N Na_2CO_3 was then added to produce a solution of pH ~ 8. The aminothiazole product typically precipitated from this solution and was collected by filtration and washed with water. Crude aminothiazoles were purified by column chromatography on silica gel (~40−80% ethyl acetate−hexanes). Relevant fractions were collected and concentrated to afford the desired product in 60−95% yields, with purity of > 95% as determined by ^{1}H NMR.

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-(6-methylpyridin-2-yl)-amine (4). Intermediate **51** was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 92% yield; mp 264−266 °C. ^{1}H NMR (DMSO-d_6) δ 11.41 (br s, 1H, NH), 7.63 (t, *J* = 7.78 Hz, 1H), 7.42−7.50 (m, 2H), 7.32 (s, 1H), 7.00 (d, *J* = 8.42 Hz, 1H), 6.91 (d, *J* = 8.24 Hz, 1H), 6.82 (d, *J* = 7.33 Hz, 1H), 3.82 (s, 3H), 3.79 (s, 3H), 2.49 (s, 3H). LCMS (ESI) *m*/*z* 328 (MH$^+$).

4-(3,4-Dimethoxyphenyl)-*N*-methyl-1,3-thiazol-2-amine (5). Methyl thiourea (Aldrich Chemical) was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 60% yield; mp 120−123 °C. ^{1}H NMR (DMSO-d_6) δ 7.53 (d, *J* = 4.76 Hz, 1H), 7.35−7.41 (m, 2H), 6.91−6.97 (m, 2H), 3.79 (s, 3H), 3.76 (s, 2H), 2.86 (d, *J* = 4.76 Hz, 3H). LCMS (ESI) *m*/*z* 251 (MH$^+$).

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-pyridin-2-yl-amine (6). Intermediate **62** was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 62% yield; mp 211−214 °C. ^{1}H NMR (DMSO-d_6) δ 11.37 (s, 1H, NH), 8.27−8.33 (m, 1H), 7.66−7.74 (m, 1H), 7.43−7.50 (m, 2H), 7.31 (s, 1H), 7.09 (d, *J* = 8.24 Hz, 1H), 6.99 (d, *J* = 8.42 Hz, 1H), 6.92 (ddd, *J* = 0.92, 5.08, 7.19 Hz, 1H), 3.82 (s, 3H), 3.78 (s, 3H), LCMS (ESI) *m*/*z* 314 (MH$^+$).

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-pyridin-3-yl-amine (7). Intermediate **57** was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 80% yield; mp 251−254 °C. ^{1}H NMR (DMSO-d_6) δ 11.25 (s, 1H, NH), 9.47 (s, 1H), 8.39−8.55 (m, 2H), 7.95 (s, 1H), 7.48−7.55 (m, 2H), 7.47 (s, 1H), 6.95−7.06 (m, 1H), 3.87 (s, 3H), 3.81 (s, 3H). LCMS (ESI) *m*/*z* 314 (MH$^+$).

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-pyridin-4-yl-amine (8). Pyridin-4-yl-thiourea (Alfa Aesar) was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 56% yield; mp 205−207 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (s, 1H, NH), 8.41 (d, *J* = 6.23 Hz, 2H), 7.62−7.69 (m, 2H), 7.52 (dd, *J* = 2.01, 8.24 Hz, 1H), 7.48 (d, *J* = 2.01 Hz, 1H), 7.39 (s, 1H), 7.03 (d, *J* = 8.42 Hz, 1H), 3.85 (s, 3H), 3.79 (s, 3H). LCMS (ESI) *m*/*z* 314 (MH$^+$).

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-phenyl-amine (9). *N*-Phenylthiourea (AK Scientific) was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 67% yield; mp 170−174 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.23 (s, 1H, NH), 7.71 (dd, *J* = 0.92, 8.61 Hz, 2H), 7.49−7.53 (m, 1H), 7.47 (t, *J* = 1.92 Hz, 1H), 7.30−7.39 (m, 2H), 7.22 (s, 1H), 6.99−7.05 (m, 1H), 6.93−6.99 (m, 1H), 3.82 (s, 3H), 3.78 (s, 3H). LCMS (ESI) *m*/*z* 313 (MH$^+$).

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-pyrimidin-2-yl-amine (10). Intermediate **61** was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 62% yield; mp 225−228 °C. ^{1}H NMR (DMSO-d_6) δ 11.80 (br s, 1H, NH), 8.65 (s, 1H), 8.64 (s, 1H), 7.49 (s, 1H), 7.46 (d, *J* = 1.28 Hz, 1H), 7.43 (s, 1H), 7.04 (td, *J* = 0.73, 4.85 Hz, 1H), 7.00 (d, *J* = 8.06 Hz, 1H), 3.82 (s, 3H), 3.78 (s, 3H). LCMS (ESI) *m*/*z* 315 (MH$^+$).

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-pyrazin-2-yl-amine (11). Intermediate **58** was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 66% yield; mp 205−207 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.83 (s, 1H, NH), 8.50 (d, *J* = 1.46 Hz, 1H), 8.32 (dd, *J* = 1.46, 2.75 Hz, 1H), 8.13 (d, *J* = 2.93 Hz, 1H), 7.47−7.51 (m, 1H), 7.46 (d, *J* = 2.01 Hz, 1H), 7.43 (s, 1H), 7.01 (d, *J* = 8.24 Hz, 1H), 3.83 (s, 3H), 3.79 (s, 3H). LCMS (ESI) *m*/*z* 315 (MH$^+$).

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-(5-trifluoromethylpyridin-2-yl)-amine (12). Intermediate **55** was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 68% yield; mp 244−247 °C. ^{1}H NMR (DMSO-d_6) δ 11.90 (br s, 1H, NH), 8.68 (s, 1H), 8.05 (dd, *J* = 2.56, 8.79 Hz, 1H), 7.47−7.51 (m, 1H), 7.46 (d, *J* = 2.01 Hz, 1H), 7.44 (s, 1H), 7.24 (d, *J* = 8.79 Hz, 1H), 7.00 (d, *J* = 8.24 Hz, 1H), 3.82 (s, 3H), 3.79 (s, 3H). LCMS (ESI) *m*/*z* 382 (MH$^+$).

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-(5-methoxypyridin-2-yl)-amine (13). Intermediate **56** was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 56% yield; mp 222−225 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (br s, 1H, NH), 8.05 (d, *J* = 2.93 Hz, 1H), 7.42−7.49 (m, 3H), 7.27 (s, 1H), 7.11 (d, *J* = 8.97 Hz, 1H), 7.00 (d, *J* = 8.42 Hz, 1H), 3.82 (s, 3H), 3.81 (s, 3H), 3.78 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.5, 150.8, 149.4, 149.2, 149.0, 146.9, 131.9, 128.7, 126.5, 118.7, 112.5, 112.1, 110.0, 103.8, 56.6, 56.2, 56.1. LCMS (ESI) *m*/*z* 344 (MH$^+$).

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-(5-methylpyridin-2-yl)-amine (14). Intermediate **52** was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 89% yield; mp 190−192 °C. ^{1}H NMR (DMSO-d_6) δ 11.38 (br s, 1H, NH), 8.15 (s, 1H), 7.60 (d, *J* = 8.24 Hz, 1H), 7.43−7.49 (m, 2H), 7.30 (s, 1H), 7.05 (d, *J* = 8.24 Hz, 1H), 6.99 (d, *J* = 8.24 Hz, 1H), 3.82 (s, 3H), 3.78 (s, 3H), 2.24 (s, 3H). LCMS (ESI) *m*/*z* 328 (MH$^+$).

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-(3-methylpyridin-2-yl)-amine (15). Intermediate **54** was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 92% yield; mp 264−266 °C. ^{1}H NMR (DMSO-d_6) δ 10.65 (bs, 1H, NH), 8.21 (d, *J* = 5.0 Hz, 1H), 7.67 (d, *J* = 6.8 Hz, 1H), 7.51 (s, 1H), 7.50 (dd, *J* = 10.4, 1.5, 1H), 7.39 (s, 1H), 7.00 (s, 1H), 6.98 (s, 1H), 3.82 (s, 3H), 3.77 (s, 3H), 2.37 (s, 3H). LCMS (ESI) *m*/*z* 328 (MH$^+$).

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-(4-methylpyridin-2-yl)-amine (16). Intermediate **53** was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 68% yield; mp 211−213 °C. ^{1}H NMR (DMSO-d_6) δ 11.46 (br s, 1H, NH), 8.19 (d, J = 5.49 Hz, 1H), 7.48 (s, 1H), 7.46 (d, J = 2.01 Hz, 1H), 7.33 (s, 1H), 6.99 (d, J = 8.24 Hz, 1H), 6.93 (s, 1H), 6.83 (d, J = 4.94 Hz, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 2.31 (s, 3H). LCMS (ESI) m/z 328 (MH$^+$).

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-isoquinolin-3-yl-amine (17). Intermediate **59** was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 78% yield; mp 200−203 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H, NH), 9.19 (s, 1H), 8.03 (d, J = 8.24 Hz, 1H), 7.80 (d, J = 8.06 Hz, 1H), 7.65 (td, J = 1.19, 7.55 Hz, 1H), 7.47−7.55 (m, 3H), 7.39−7.45 (m, 1H), 7.29 (s, 1H), 7.01 (d, J = 8.24 Hz, 1H), 3.84 (s, 3H), 3.79 (s, 3H). ^{13}C NMR (100 MHz, chloroform-d) δ 161.4, 150.1, 149.6, 149.0, 148.7, 148.4, 138.3, 130.6, 128.6, 127.8, 125.7, 124.7, 124.3, 118.6, 111.4, 109.6, 103.1, 103.0, 56.0, 56.0. LCMS (ESI) m/z 364 (MH$^+$).

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-quinolin-2-yl-amine (18). Intermediate **60** was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 80% yield; mp 257−259 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (br s, 1H, NH), 8.23 (d, J = 8.97 Hz, 1H), 7.83−7.88 (m, 2H), 7.69 (td, J = 1.37, 7.65 Hz, 1H), 7.47−7.53 (m, 2H), 7.44 (s, 1H), 7.39−7.44 (m, 1H), 7.28 (d, J = 8.79 Hz, 1H), 7.02 (d, J = 8.24 Hz, 1H), 3.84 (s, 3H), 3.79 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.7, 151.2, 149.5, 149.4, 149.1, 146.6, 138.5, 130.6, 128.6, 128.5, 126.6, 124.7, 124.4, 118.7, 113.5, 112.6, 110.0, 105.8, 56.2, 56.2. LCMS (ESI) m/z 364 (MH$^+$).

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-naphthalen-2-yl-amine (19). Intermediate **64** was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 68% yield; mp 221−224 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.49 (s, 1H, NH), 8.54 (s, 1H), 7.87 (d, J = 8.97 Hz, 1H), 7.81 (t, J = 9.16 Hz, 2H), 7.53−7.61 (m, 3H), 7.47 (t, J = 7.42 Hz, 1H), 7.31−7.38 (m, 1H), 7.29 (s, 1H), 7.05 (d, J = 8.79 Hz, 1H), 3.89 (s, 3H), 3.81 (s, 3H). LCMS (ESI) m/z 363 (MH$^+$).

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-naphthalen-1-yl-amine (20). Naphthalen-1-yl-thiourea (TCI America) was reacted with commercially available 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 59% yield; mp 244−246 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.24 (br s, 1H, NH), 8.27−8.33 (m, 1H), 8.23 (dd, J = 2.93, 7.51 Hz, 1H), 7.96 (dt, J = 2.38, 4.76 Hz, 1H), 7.70 (d, J = 8.06 Hz, 1H), 7.51−7.61 (m, 3H), 7.41−7.48 (m, 2H), 7.21 (s, 1H), 7.01 (d, J = 8.97 Hz, 1H), 3.83 (s, 3H), 3.79 (s, 3H). LCMS (ESI) m/z 363 (MH$^+$).

[4-(4-Methoxyphenyl)-thiazol-2-yl]-(5-methylpyridin-2-yl)-amine (21). Intermediate **52** was reacted with commercially available 2-bromo-1-(4-methoxyphenyl)-ethanone according to the general procedure to afford the title compound in 73% yield; mp 246−248 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.60 (br s, 1H, NH), 8.21 (s, 1H), 7.79−7.94 (m, J = 8.61 Hz, 2H), 7.69 (br s, 1H), 7.32 (br s, 1H), 7.13 (br s, 1H), 6.97−7.04 (m, J = 7.87 Hz, 2H), 3.81 (s, 3H), 2.28 (s, 3H). LCMS (ESI) m/z 298 (MH$^+$).

[4-(2-Methoxyphenyl)-thiazol-2-yl]-(5-methylpyridin-2-yl)-amine (22). Intermediate **52** was reacted with commercially available 2-bromo-1-(2-methoxyphenyl)-ethanone according to the general procedure to afford the title compound in 78% yield; mp 228−230 °C. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.34 (s, 1H), 8.00 (dd, J = 1.92, 8.70 Hz, 1H), 7.94 (d, J = 7.87 Hz, 1H), 7.62 (s, 1H), 7.40−7.49 (m, 1H), 7.25 (d, J = 8.79 Hz, 1H), 7.20 (d, J = 8.42 Hz, 1H), 7.08−7.16 (m, 1H), 4.02 (s, 3H), 2.41 (s, 3H). LCMS (ESI) m/z 298 (MH$^+$).

(5-Methylpyridin-2-yl)-[4-pyridin-4-yl)-thiazol-2-yl]-amine (23). Intermediate **52** was reacted with commercially available 2-bromo-1-pyridin-4-yl-ethanone according to the general procedure to afford the title compound in 72% yield; mp decomposition at 291 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.58 (br s, 1H, NH), 8.90−8.95 (m, 2H), 8.42−8.46 (m, 2H), 8.40 (s, 1H), 8.16−8.20 (m, 1H), 7.62 (dd, J = 2.38, 8.42 Hz, 1H), 7.07 (d, J = 8.24 Hz, 1H), 2.25 (s, 3H). LCMS (ESI) m/z 269 (MH$^+$).

(5-Methylpyridin-2-yl)-(4-pyridin-2-yl-thiazol-2-yl)-amine (24). Intermediate **52** was reacted with commercially available 2-bromo-1-pyridin-2-yl-ethanone according to the general procedure to afford the title compound in 62% yield; mp 184−190 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H, NH), 8.59 (dt, J = 0.92, 4.76 Hz, 1H), 8.13−8.17 (m, 1H), 7.93−7.98 (m, 1H), 7.87 (td, J = 1.28, 7.69 Hz, 1H), 7.61 (s, 1H), 7.56 (dd, J = 2.20, 8.42 Hz, 1H), 7.27−7.34 (m, 1H), 7.03 (d, J = 8.42 Hz, 1H), 2.23 (s, 3H). LCMS (ESI) m/z 269 (MH$^+$).

(5-Methylpyridin-2-yl)-(4-phenylthiazol-2-yl)-amine (25). Intermediate **52** was reacted with commercially available 2-bromo-1-phenyl-ethanone according to the general procedure to afford the title compound in 82% yield; mp 260−264 °C. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.32−8.37 (m, 1H), 8.17 (dd, J = 2.01, 8.97 Hz, 1H), 7.93−8.01 (m, 2H), 7.54 (s, 1H), 7.45−7.52 (m, 2H), 7.33−7.45 (m, 2H), 2.43 (s, 3H). LCMS (ESI) m/z 268 (MH$^+$).

(5-Methylpyridin-2-yl)-[4-(5-pyridin-2-yl-thiophen-2-yl)-thiazol-2-yl]-amine (26). Intermediate **52** was reacted with commercially available 2-bromo-1-(5-pyridin-2-yl-thiophen-2-yl)-ethanone according to the general procedure to afford the title compound in 73% yield; mp 197−199 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37 (s, 1H, NH), 8.52 (d, J = 5.86 Hz, 1H), 8.13 (s, 1H), 7.88−7.92 (m, 1H), 7.77−7.86 (m, 1H), 7.73−7.77 (m, 1H), 7.55 (dd, J = 2.20, 8.42 Hz, 1H), 7.50 (d, J = 3.85 Hz, 1H), 7.33 (s, 1H), 7.25 (dd, J = 4.76, 7.33 Hz, 1H), 7.02 (d, J = 8.42 Hz, 1H), 2.22 (s, 3H). LCMS (ESI) m/z 351 (MH$^+$).

(5-Methylpyridin-2-yl)-[4-(3-phenylisoxazol-5-yl)-thiazol-2-yl]-amine (27). Intermediate **52** was reacted with commercially available 2-bromo-1-(3-phenylisoxazol-5-yl)-ethanone according to the general procedure to afford the title compound in 63% yield; mp 224−227 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.56 (s, 1H, NH), 8.17 (s, 1H), 7.88−7.99 (m, 2H), 7.65 (s, 1H), 7.51−7.61 (m, 4H), 7.19 (s, 1H), 7.02 (d, J = 8.42 Hz, 1H), 2.24 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.9, 162.9, 161.3, 150.1, 146.4, 139.7, 137.9, 131.0, 129.8 (2), 129.2, 127.4 (2), 125.8, 112.1, 111.2, 99.0, 17.9. LCMS (ESI) m/z 335 (MH$^+$).

[4-(5-Methyl-3-phenylisoxazol-4-yl)-thiazol-2-yl]-(5-methylpyridin-2-yl)-amine (28). Intermediate **52** was reacted with commercially available 2-bromo-1-(5-methyl-3-phenylisoxazol-4-yl)-ethanone according to the general procedure to afford the title compound in 54% yield; mp 267−269 °C. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.12 (dd, J = 2.11, 8.88 Hz, 1H), 7.71 (s, 1H), 7.51−7.57 (m, 2H), 7.40−7.48 (m, 3H), 7.31 (d, J = 8.97 Hz, 1H), 7.22 (s, 1H), 2.64 (s, 3H), 2.37 (s, 3H). LCMS (ESI) m/z 349 (MH$^+$).

(5-Methylpyridin-2-yl)-(4-methylthiazol-2-yl)-amine (29). Intermediate **52** was reacted with commercially available 1-bromo-propan-2-one according to the general procedure to afford the title compound in 64% yield; mp 212−214 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ d 11.01 (br s, 1H, NH), 8.09 (s, 1H), 7.51 (dd, J = 2.01, 8.42 Hz, 1H), 6.94 (d, J = 8.42 Hz, 1H), 6.48 (s, 1H), 2.21 (s, 3H), 2.20 (s, 3H). LCMS (ESI) m/z 206 (MH$^+$).

Isoquinolin-3-yl-[4-(4-methoxyphenyl)-thiazol-2-yl]-amine (30). Intermediate **59** was reacted with commercially available 2-bromo-1-(4-methoxyphenyl)-ethanone according to the general procedure to afford the title compound in 57% yield; mp 224−228 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37 (s, 1H, NH), 9.19 (s, 1H), 8.02 (d, J = 8.06 Hz, 1H), 7.84−7.92 (m, 2H), 7.81 (d, J = 8.24 Hz, 1H), 7.61−7.71 (m, 1H), 7.54 (br s, 1H), 7.38−7.47 (m, 1H), 7.19−7.28 (m, 1H), 6.93−7.05 (m, 2H), 3.79 (s, 3H). LCMS (ESI) m/z 334 (MH$^+$).

Isoquinolin-3-yl-[4-(4-trifluoromethoxyphenyl)-thiazol-2-yl]-amine (31). Intermediate **59** was reacted with commercially available 2-bromo-1-(4-trifluoromethoxyphenyl)-ethanone according to the general procedure to afford the title compound in 67% yield; mp 269−271 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.46 (s, 1H), 9.20 (s, 1H), 8.00−8.09 (m, 3H), 7.83 (d, *J* = 8.42 Hz, 1H), 7.66 (t, *J* = 7.60 Hz, 1H), 7.54 (s, 1H), 7.50 (s, 1H), 7.37−7.47 (m, 3H). LCMS (ESI) *m*/*z* 388 (MH$^+$).

Isoquinolin-3-yl-[4-pyridin-4-yl)-thiazol-2-yl]-amine (32). Intermediate **59** was reacted with commercially available 2-bromo-1-pyridin-4-yl-ethanone according to the general procedure to afford the title compound in 52% yield; mp 259−260 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.54 (s, 1H, NH), 9.22 (s, 1H), 8.58−8.70 (m, 2H), 8.05 (d, *J* = 8.24 Hz, 1H), 7.87−7.91 (m, 2H), 7.85 (d, *J* = 8.24 Hz, 1H), 7.80 (s, 1H), 7.66 (ddd, *J* = 1.10, 6.91, 8.29 Hz, 1H), 7.55 (s, 1H), 7.41−7.49 (m, 1H). LCMS (ESI) *m*/*z* 305 (MH$^+$).

Isoquinolin-3-yl-(4-phenylthiazol-2-yl)-amine (33). Intermediate **59** was reacted with commercially available 2-bromo-1-phenyl-ethanone according to the general procedure to afford the title compound in 72% yield; mp 265−267 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.44 (br s, 1H, NH), 9.21 (s, 1H), 8.04 (d, *J* = 8.42 Hz, 1H), 7.91−7.97 (m, 2H), 7.83 (d, *J* = 8.61 Hz, 1H), 7.66 (dd, *J* = 7.05, 8.15 Hz, 1H), 7.55 (s, 1H), 7.39−7.48 (m, 4H), 7.28−7.35 (m, 1H). LCMS (ESI) *m*/*z* 304 (MH$^+$).

Isoquinolin-3-yl-[4-(5-pyridin-2-yl-thiophen-2-yl)-thiazol-2-yl]-amine (34). Intermediate **59** was reacted with commercially available 2-bromo-1-(5-pyridin-2-yl-thiophen-2-yl)-ethanone according to the general procedure to afford the title compound in 63% yield; mp 246−248 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.54 (br s, 1H, NH), 9.20 (s, 1H), 8.52−8.57 (m, 1H), 8.03 (d, *J* = 8.24 Hz, 1H), 7.90−7.95 (m, 1H), 7.79−7.87 (m, 2H), 7.78 (d, *J* = 4.03 Hz, 1H), 7.66 (ddd, *J* = 1.10, 6.91, 8.29 Hz, 1H), 7.54 (d, *J* = 3.85 Hz, 1H), 7.49 (s, 1H), 7.43 (ddd, *J* = 0.92, 6.91, 8.10 Hz, 1H), 7.36 (s, 1H), 7.27 (ddd, *J* = 1.10, 4.90, 7.37 Hz, 1H). LCMS (ESI) *m*/*z* 387 (MH$^+$).

Isoquinolin-3-yl-[4-(3-phenylisoxazol-5-yl)-thiazol-2-yl]-amine (35). Intermediate **59** was reacted with commercially available 2-bromo-1-(3-phenylisoxazol-5-yl)-ethanone according to the general procedure to afford the title compound in 59% yield; mp decomposition 260 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.68 (s, 1H, NH), 9.21 (s, 1H), 8.04 (d, *J* = 8.42 Hz, 1H), 7.94 (dd, *J* = 1.74, 6.32 Hz, 2H), 7.85 (d, *J* = 8.24 Hz, 1H), 7.62−7.70 (m, 2H), 7.48−7.58 (m, 4H), 7.39−7.48 (m, 1H), 7.24 (s, 1H). LCMS (ESI) *m*/*z* 371 (MH$^+$).

Isoquinolin-3-yl-(4-methylthiazol-2-yl)-amine (36). Intermediate **59** was reacted with commercially available 1-bromo-propan-2-one according to the general procedure to afford the title compound in 73% yield; mp 212−215 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.16 (br s, 1H, NH), 9.15 (s, 1H), 8.00 (d, *J* = 7.87 Hz, 1H), 7.77 (d, *J* = 8.24 Hz, 1H), 7.57−7.70 (m, 1H), 7.47 (s, 1H), 7.34−7.45 (m, 1H), 6.51 (s, 1H), 2.26 (s, 3H). LCMS (ESI) *m*/*z* 242 (MH$^+$).

Isoquinolin-3-yl-(4-trifluoromethylthiazol-2-yl)-amine (37). Intermediate **59** was reacted with commercially available 3-bromo-1,1,1-trifluoro-propan-2-one according to the general procedure to afford the title compound in 67% yield; mp 200−203 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.81 (s, 1H), 9.23 (s, 1H), 8.02−8.09 (m, 1H), 7.80−7.87 (m, 1H), 7.64−7.72 (m, 2H), 7.46 (ddd, *J* = 1.01, 6.96, 8.15 Hz, 1H), 7.37 (s, 1H). LCMS (ESI) *m*/*z* 296 (MH$^+$).

(8*H*-Indeno[1,2-*d*]thiazol-2-yl)-(5-methylpyridin-2-yl)-amine (38). A solution of intermediate **52** (1 mmol) and commercially available 2-bromoindan-1-one (1 mmol, 1 equiv) in EtOH (10 mL) was heated to 60 °C for 3 h, after which time the reaction was judged complete. The reaction mixture was then poured into water−ice (20 mL) and stirred for 30 min. Aqueous 1N Na_2CO_3 was then added to this solution until a pH ∼ 8 was reached. The product precipitated from this solution and was collected by filtration and washed with water. The crude product was purified by column chromatography (40−80% ethyl acetate−hexane). Relevant fractions were collected and evaporated to afford the product in 69% yield; mp decomposition 282 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.85 (s, 1H), 8.18−8.23 (m, 1H), 7.74 (d, *J* = 8.24 Hz, 1H), 7.63 (d, *J* = 7.33 Hz, 1H), 7.54 (d, *J* = 7.51 Hz, 1H), 7.37 (t, *J* = 7.42 Hz, 1H), 7.20−7.27 (m, 1H), 7.13 (d, *J* = 8.42 Hz, 1H), 3.85 (s, 2H), 2.28 (s, 3H). LCMS (ESI) *m*/*z* 280 (MH$^+$).

(5,6-Dimethoxy-8*H*-indeno[1,2-*d*]thiazol-2-yl)-(5-methylpyridin-2-yl)-amine (39). A solution of intermediate **52** (1 mmol) and bromo-5,6-dimethoxy-indan-1-one (**65**, 1 mmol, 1 equiv) in EtOH (10 mL) was heated to 60 °C for 3 h, after which time the reaction was judged complete. The reaction mixture was then poured into water−ice (20 mL) and stirred for 30 min. Aqueous 1N Na_2CO_3 was then added to this solution until a pH ∼ 8 was reached. The product precipitated from this solution and was collected by filtration and washed with water. The crude product was purified by column chromatography (40−80% ethyl acetate−hexane). Relevant fractions were collected and evaporated to afford the product in 72% yield; mp decomposition 265 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.60 (s, 1H), 8.18 (s, 1H), 7.68 (d, *J* = 8.06 Hz, 1H), 7.23 (s, 1H), 7.18 (s, 1H), 7.10 (d, *J* = 8.79 Hz, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 3.74 (s, 2H), 2.26 (s, 3H). LCMS (ESI) *m*/*z* 340 (MH$^+$).

(5-Methoxypyridin-2-yl)-[4-(3-phenylisoxazol-5-yl)-thiazol-2-yl]-amine (40). Intermediate **56** was reacted with commercially available 2-bromo-1-(3-phenylisoxazol-5-yl)-ethanone according to the general procedure to afford the title compound in 53% yield; mp 203−205 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.49 (s, 1H, NH), 8.06 (d, *J* = 3.11 Hz, 1H), 7.88−7.97 (m, 2H), 7.62 (s, 1H), 7.50−7.59 (m, 3H), 7.47 (dd, *J* = 2.93, 8.97 Hz, 1H), 7.19 (s, 1H), 7.09 (d, *J* = 8.97 Hz, 1H), 3.81 (s, 3H). LCMS (ESI) *m*/*z* 351 (MH$^+$).

(4-Methoxypyridin-2-yl)-[4-(3-phenylisoxazol-5-yl)-thiazol-2-yl]-amine (41). Intermediate **63** was reacted with commercially available 2-bromo-1-(3-phenylisoxazol-5-yl)-ethanone according to the general procedure to afford the title compound in 58% yield; mp 177−179 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.54 (s, 1H), 8.16 (d, *J* = 6.23 Hz, 1H), 7.88−8.01 (m, 2H), 7.67 (s, 1H), 7.47−7.59 (m, 3H), 7.20 (s, 1H), 6.57−6.66 (m, 2H), 3.82 (s, 3H). LCMS (ESI) *m*/*z* 351 (MH$^+$).

[4-(3-Phenylisoxazol-5-yl)-thiazol-2-yl]-pyridin-2-yl-amine (42). Intermediate **62** was reacted with commercially available 2-bromo-1-(3-phenylisoxazol-5-yl)-ethanone according to the general procedure to afford the title compound in 52% yield; mp 211−215 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.68 (s, 1H, NH), 8.34 (dd, *J* = 0.92, 5.13 Hz, 1H), 7.90−7.99 (m, 2H), 7.75 (ddd, *J* = 1.83, 7.05, 8.52 Hz, 1H), 7.69 (s, 1H), 7.51−7.59 (m, 3H), 7.21 (s, 1H), 7.10 (d, *J* = 8.24 Hz, 1H), 6.98 (ddd, *J* = 0.92, 5.63, 6.64 Hz, 1H). LCMS (ESI) *m*/*z* 321 (MH$^+$).

(4-Methylpyridin-2-yl)-[4-(3-phenylisoxazol-5-yl)-thiazol-2-yl]-amine (43). Intermediate **53** was reacted with commercially available 2-bromo-1-(3-phenylisoxazol-5-yl)-ethanone according to the general procedure to afford the title compound in 62% yield; mp decomposition 296 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (br s, 1H, NH), 8.22 (d, *J* = 5.31 Hz, 1H), 7.88−7.98 (m, 2H), 7.70 (s, 1H), 7.49−7.60 (m, 3H), 7.25 (s, 1H), 6.93 (s, 1H), 6.86 (d, *J* = 5.31 Hz, 1H), 2.32 (s, 3H). LCMS (ESI) *m*/*z* 335 (MH$^+$).

(6-Methylpyridin-2-yl)-[4-(3-phenylisoxazol-5-yl)-thiazol-2-yl]-amine (44). Intermediate **51** was reacted with commercially available 2-bromo-1-(3-phenylisoxazol-5-yl)-ethanone according to the general procedure to afford the title compound in 58% yield; mp 291−293 °C. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.17−8.26 (m, 1H), 7.86−7.95 (m, 3H), 7.48−7.55 (m, 3H), 7.26−7.34 (m, 3H), 2.85 (s, 3H). LCMS (ESI) *m*/*z* 335 (MH$^+$).

[4-(3-Phenylisoxazol-5-yl)-thiazol-2-yl]-pyridin-4-yl-amine (45). Pyridin-4-yl-thiourea (Alfa Aesar) was reacted with commercially available 2-bromo-1-(3-phenylisoxazol-5-yl)-ethanone according to the general procedure to afford the title compound

in 57% yield; mp 269−270 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (br s, 1H), 8.39−8.46 (m, 2H), 7.95−8.04 (m, 2H), 7.66−7.73 (m, 3H), 7.51−7.61 (m, 3H), 7.45 (s, 1H). LCMS (ESI) m/z 321 (MH$^+$).

2-(5-Methylpyridin-2-ylamino)-thiazole-4-carboxylic Acid (3,4-Dimethoxyphenyl)-amide (46). A solution of commercially available 3,4-dimethoxyphenylamine (0.13 mmol, 1 equiv) and triethylamine (0.65 mmol, 5 equiv) in THF (2 mL) and was added to a solution of **67** (0.13 mmol) and HATU (0.13 mmol) in THF (2 mL). The reaction mixture was stirred for 3 h at 60 °C. After cooling, the mixture was concentrated and the crude product taken into 100 mL of ethyl acetate and washed with water. The organic phase was dried ($MgSO_4$), filtered, and concentrated. The crude residue was purified by column chromatography on silica gel (30−60% ethyl acetate−hexane) and relevant fractions collected and concentrated to afford the desired product as a white powder in 76% yield; mp 212−214 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.36 (s, 1H), 9.55 (s, 1H), 8.15−8.17 (m, 1H), 7.69 (s, 1H), 7.58 (dd, J = 2.20, 8.61 Hz, 1H), 7.45 (d, J = 2.38 Hz, 1H), 7.30 (dd, J = 2.38, 8.61 Hz, 1H), 7.05 (d, J = 8.42 Hz, 1H), 6.93 (d, J = 8.79 Hz, 1H), 3.76 (s, 3H), 3.74 (s, 3H), 2.24 (s, 3H). LCMS (ESI) m/z 371 (MH$^+$).

Isoquinoline-3-carboxylic Acid [4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-amide (47). A solution of **70** (30 mg, 0.13 mmol, 1 equiv) and triethylamine (0.65 mmol, 5 equiv) in THF (2 mL) was added to a solution of commercially available isoquinoline-3-carboxylic acid (22 mg, 0.13 mmol) and HATU (50 mg, 0.13 mmol) in THF (2 mL). The reaction was stirred for 3 h at 60 °C and then cooled to room temperature and poured into water−ice. The product was extracted with EtOAc, dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by column chromatography on silica gel (ethyl acetate−hexanes) to afford the product as a white powder in 39% yield; mp 228−230 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (s, 2H), 9.51 (s, 1H), 8.77 (s, 1H), 8.34 (d, J = 7.33 Hz, 1H), 8.29 (d, J = 8.06 Hz, 1H), 7.87−7.99 (m, 2H), 7.66 (s, 1H), 7.56 (d, J = 2.01 Hz, 1H), 7.53 (dd, J = 1.92, 8.33 Hz, 1H), 7.03 (d, J = 8.42 Hz, 1H), 3.85 (s, 3H), 3.80 (s, 3H). LCMS (ESI) m/z 392 (MH$^+$).

4-(3,4-Dimethoxyphenyl)-thiazole-2-carboxylic Acid Isoquinolin-3-ylamide (48). A solution of commercially available isoquinolin-3-ylamine (0.06 g, 0.19 mmol, 1 equiv) and triethylamine (0.95 mmol, 5 equiv) in THF (3 mL) was added to a mixture of **69** (0.10 g, 0.19 mmol) and HATU (0.15 g, 0.19 mmol) in THF (3 mL). The reaction mixture was stirred for 3 h at 60 °C and then cooled to room temperature and poured into water−ice. This solution was extracted with EtOAc and the organic phase dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by column chromatography on silica gel (ethyl acetate−hexanes) to afford the product as a white powder in 55% yield; mp 196−202 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 9.27 (s, 1H), 8.59 (s, 1H), 8.46 (s, 1H), 8.14 (d, J = 8.06 Hz, 1H), 8.01 (d, J = 8.24 Hz, 1H), 7.78 (ddd, J = 1.19, 6.91, 8.20 Hz, 1H), 7.68−7.75 (m, 2H), 7.56−7.66 (m, 1H), 7.07 (d, J = 8.24 Hz, 1H), 3.90 (s, 3H), 3.83 (s, 3H). LCMS (ESI) m/z 392 (MH$^+$).

[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-isoquinolin-3-yl-methyl-amine (49). To a solution of **17** (50 mg, 0.138 mmol) in THF (3 mL) was added NaH (60% in mineral oil; 8 mg, 0.21 mmol, 1.5 equiv) at 0 °C. After stirring for 15 min at 0 °C, the reaction mixture was treated with methyl iodide (39 mg, 17.2 μL, 0.28 mmol, 2 equiv) and stirred for 2 h at room temperature. The reaction was quenched by the addition of aqueous NH_4Cl, and the product was extracted with ether (2 × 50 mL). Combined organic phases were dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by column chromatography (10−50% ethyl acetate−hexane) to provide the product in 77% yield; mp 165−166 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.27 (s, 1H), 8.09 (d, J = 8.06 Hz, 1H), 7.94 (d, J = 8.42 Hz, 1H), 7.69−7.78 (m, 1H), 7.65 (s, 1H), 7.43−7.58 (m, 3H), 7.35 (s, 1H), 7.00 (d, J = 8.97 Hz, 1H), 3.94 (s, 3H), 3.84 (s, 3H), 3.78 (s, 3H). ^{13}C NMR (100 MHz, chloroform-d) δ 162.9, 150.4, 149.5, 149.2, 148.9, 148.9, 138.5, 131.0, 128.9, 127.9, 126.3, 125.0, 125.0, 118.7, 111.5, 109.7, 105.0, 102.8, 56.2, 56.1, 36.3. LCMS (ESI) m/z 378 (MH$^+$).

***N*-[4-(3,4-Dimethoxyphenyl)-thiazol-2-yl]-*N*-isoquinolin-3-yl-acetamide (50).** Acetic anhydride (5 mL) was added to **17** (50 mg, 0.138 mmol) and the reaction mixture heated to 100 °C for 4 h. The solution was allowed to cool to room temperature and then poured into water−ice. The product was extracted with EtOAc and the organic phase dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by column chromatography (30−60% ethyl acetate−hexane) to provide the product as a white powder in 81% yield; mp 164−165 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.46 (s, 1H), 8.32 (d, J = 8.24 Hz, 1H), 8.25 (s, 1H), 8.13 (d, J = 7.69 Hz, 1H), 7.88−7.95 (m, 1H), 7.84 (ddd, J = 1.19, 6.91, 8.19 Hz, 1H), 7.64 (s, 1H), 7.17 (d, J = 2.01 Hz, 1H), 7.02 (dd, J = 1.92, 8.33 Hz, 1H), 6.82 (d, J = 8.61 Hz, 1H), 3.67 (s, 3H), 3.59 (s, 3H), 2.15 (s, 3H); ^{13}C NMR (100 MHz, chloroform-d) δ 170.0, 160.1, 153.2, 149.4, 149.0, 148.9, 147.8, 137.6, 131.4, 128.7, 128.6, 128.0, 127.9, 127.2, 121.0, 118.7, 111.3, 109.6, 107.4, 56.1, 55.7, 24.0. LCMS (ESI) m/z 406 (MH$^+$).

(6-Methylpyridin-2-yl)-thiourea (51). Commercially available 6-methylpyridin-2-ylamine was reacted according to the general procedure to afford the product in 58% yield; mp 188−190 °C. ^{1}H NMR (DMSO-d_6) δ 10.59 (s, 1H), 10.38 (s, 1H), 8.78 (s, 1H), 7.59 (t, J = 8.0 Hz, 1H), 6.90 (d, J = 8.3 Hz, 1H), 6.84 (d, J = 7.6 Hz, 1H), 2.35 (s, 3H). LCMS (ESI) m/z 168 (MH$^+$).

(5-Methylpyridin-2-yl)-thiourea (52). Commercially available 5-methylpyridin-2-ylamine was reacted according to the general procedure to afford the product in 60% yield; mp 178−181 °C. ^{1}H NMR (DMSO-d_6) δ 10.46 (s, 1H), 10.38 (s, 1H), 8.73 (s, 1H), 8.00 (d, J = 2.2 Hz, 1H), 7.54 (dd, J = 8.45, 2.3 Hz, 1H), 7.02 (d, J = 8.6 Hz, 1H), 2.17 (s, 3H). LCMS (ESI) m/z 168 (MH$^+$).

(4-Methylpyridin-2-yl)-thiourea (53). Commercially available 4-methylpyridin-2-ylamine was reacted according to the general procedure to afford the product in 60% yield; mp 210−212 °C. ^{1}H NMR (DMSO-d_6) δ 10.57 (s, 1H), 10.38 (s, 1H), 8.77 (s, 1H), 8.03 (d, J = 5.3 Hz, 1H), 6.91 (s, 1H), 6.83 (d, J = 4.75 Hz, 1H), 2.21 (s, 3H). LCMS (ESI) m/z 168 (MH$^+$).

(3-Methylpyridin-2-yl)-thiourea (54). Commercially available 3-methylpyridin-2-ylamine was reacted according to the general procedure to afford the product in 52% yield; mp 152−154 °C. ^{1}H NMR (DMSO-d_6) δ 10.33 (s, 1H), 8.87 (s, 1H), 8.73 (s, 1H), 8.08 (d, J = 4.9 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.00 (dd, J = 7.5, 5.0 Hz, 1H), 2.25 (s, 3H). LCMS (ESI) m/z 168 (MH$^+$).

(5-Trifluoromethylpyridin-2-yl)-thiourea (55). Commercially available 5-trifluoromethylpyridin-2-ylamine was reacted according to the general procedure to afford the product in 62% yield; mp 203−205 °C. ^{1}H NMR (DMSO-d_6) δ 10.88 (s, 1H), 10.36 (s, 1H), 9.16 (s, 1H), 8.60 (s, 1H), 8.11 (dd, J = 9.0, 2.4 Hz, 1H), 7.31 (d, J = 8.8 Hz, 1H). LCMS (ESI) m/z 222 (MH$^+$).

(5-Methoxypyridin-2-yl)-thiourea (56). Commercially available 5-methoxypyridin-2-ylamine was reacted according to the general procedure to afford the product in 58% yield; mp 131−135 °C. ^{1}H NMR (DMSO-d_6) δ 10.40 (s, 1H), 10.29 (s, 1H), 8.68 (s, 1H), 7.94−7.96 (m, 1H), 7.46 (dd, J = 3.11, 9.16 Hz, 1H), 7.15 (d, J = 8.97 Hz, 1H), 3.79 (s, 3H). LCMS (ESI) m/z 184 (MH$^+$).

Pyridin-3-yl-thiourea (57). Commercially available pyridin-3-ylamine was reacted according to the general procedure to afford the product in 64% yield; mp 165−167 °C. ^{1}H NMR (DMSO-d_6) δ 9.77 (s, 1H), 8.55 (d, J = 2.56 Hz, 1H), 8.30 (dd, J = 1.46, 4.76 Hz, 1H), 7.91−7.99 (m, 2H), 7.31−7.39 (m, 2H). LCMS (ESI) m/z 154 (MH$^+$).

Pyrazin-2-yl-thiourea (58). Commercially available pyrazin-2-ylamine was reacted according to the general procedure to afford the product in 64% yield; mp 238−240 °C. ^{1}H NMR (DMSO-d_6) δ 10.79 (s, 1H), 9.90 (s, 1H), 9.04 (s, 1H), 8.49 (s, 1H), 8.19 (d, J = 0.7 Hz, 2H). LCMS (ESI) m/z 155 (MH$^+$).

Isoquinolin-3-yl-thiourea (59). Commercially available isoquinolin-3-ylamine was reacted according to the general procedure to afford the product in 74% yield; mp 225−227 °C. ^{1}H NMR (DMSO-d_6) d 10.60 (s, 1H), 10.25 (s, 1H), 9.13 (s, 1H), 8.72 (s, 1H), 8.07 (d, J = 8.24 Hz, 1H), 7.81 (d, J = 8.42 Hz, 1H), 7.70 (ddd, J = 1.28, 6.87, 8.33 Hz, 1H), 7.56 (s, 1H), 7.52 (ddd, J = 1.10, 6.96, 8.24 Hz, 1H). LCMS (ESI) m/z 204 (MH^+).

Quinolin-2-yl-thiourea (60). Commercially available quinolin-2-ylamine was reacted according to the general procedure to afford the product in 68% yield; mp 179−180 °C. ^{1}H NMR (DMSO-d_6) d 11.10 (s, 1H), 10.80 (s, 1H), 9.17 (s, 1H), 8.29 (d, J = 8.97 Hz, 1H), 7.85 (dt, J = 1.56, 8.06 Hz, 2H), 7.64−7.73 (m, 1H), 7.44−7.52 (m, 1H), 7.34 (d, J = 8.79 Hz, 1H). LCMS (ESI) m/z 204 (MH^+).

Pyrimidin-2-yl-thiourea (61). Commercially available pyrimidin-2-ylamine was reacted according to the general procedure to afford the product in 77% yield; mp 262−264 °C. ^{1}H NMR (DMSO-d_6) δ 10.53 (s, 1H), 10.16 (s, 1H), 9.09 (s, 1H), 8.60 (d, J = 5.0 Hz, 2H), 7.11 (t, J = 5.0 Hz, 1H). LCMS (ESI) m/z 155 (MH^+).

Pyridin-2-yl-thiourea (62). Commercially available pyridin-2-ylamine was reacted according to the general procedure to afford the product in 67% yield; mp 144−146 °C. ^{1}H NMR (DMSO-d_6) d 10.57 (br s, 1H), 10.52 (s, 1H), 8.87 (br s, 1H), 8.23 (dd, J = 1.28, 5.13 Hz, 1H), 7.69−7.82 (m, 1H), 7.16 (d, J = 8.42 Hz, 1H), 7.04 (ddd, J = 0.73, 5.17, 7.28 Hz, 1H). LCMS (ESI) m/z 154 (MH^+).

(4-Methoxypyridin-2-yl)-thiourea (63). Commercially available 4-methoxypyridin-2-ylamine was reacted according to the general procedure to afford the product in 52% yield; mp 214−217 °C. ^{1}H NMR (DMSO-d_6) d 10.66 (br s, 1H), 10.34 (s, 1H), 8.83 (br s, 1H), 8.06 (d, J = 6.04 Hz, 1H), 6.75 (d, J = 2.20 Hz, 1H), 6.67 (dd, J = 2.38, 6.04 Hz, 1H), 3.78 (s, 3H). LCMS (ESI) m/z 184 (MH^+).

Naphthalen-2-yl-thiourea (64). Commercially available naphthalen-2-ylamine was reacted according to the general procedure to afford the product in 65% yield; mp 190−192 °C. ^{1}H NMR (DMSO-d_6) d 9.88 (s, 1H), 7.95 (s, 1H), 7.82−7.89 (m, 3H), 7.38−7.56 (m, 4H). LCMS (ESI) m/z 203 (MH^+).

Bromo-5,6-dimethoxy-indan-1-one (65). A solution of bromine (1.0 g, 330 μL, 6.30 mmol, 1.2 equiv) in Et_2O (5 mL) was added dropwise to a solution of commercially available 5,6-dimethoxy-indan-1-one (1.0 g, 5.2 mmol) in Et_2O (30 mL). The reaction mixture was stirred at room temperature overnight, solvent evaporated, and the resulting crude product recrystallized from MeOH (10 mL) to afford 0.65 g (46%) of the desired intermediate **65** a yellow solid; mp 162−163 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.15 (s, 1H), 7.13 (s, 1H), 4.96 (ddd, J = 0.92, 2.75, 7.14 Hz, 1H), 3.88−3.90 (m, 3H), 3.81−3.83 (m, 3H), 3.75−3.81 (m, 1H), 3.21 (dd, J = 2.75, 17.95 Hz, 1H). LCMS (ESI) m/z 272 (MH^+).

2-(5-Methylpyridin-2-ylamino)-thiazole-4-carboxylic Acid Ethyl Ester (66). A solution of ethyl bromopyruvate (0.5 g, 0.32 mL, 2.6 mmol) and intermediate **52** (2.6 mmol, 1 equiv) in EtOH (10 mL) were stirred at 60 °C for 1 h. The solvent was then evaporated to afford crude **66**, which was used directly in the next step; mp 198−200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (s, 1H), 7.92 (s, 1H), 7.56 (d, J = 8.25 Hz, 1H), 7.17 (d, J = 8.25 Hz, 1H), 4.33 (q, J = 6.95 Hz, 2H), 2.24 (s, 3H), 1.37 (t, J = 6.95 Hz, 3H). LCMS (ESI) m/z 264 (MH^+).

2-(5-Methylpyridin-2-ylamino)-thiazole-4-carboxylic Acid (67). Crude **66** (0.7 g, 2.66 mmol) and aqueous 5N HCl (3 mL) were heated in a sealed tube in a CEM microwave for 10 min at 130 °C. After cooling, the precipitate was filtered and washed with water and acetone to afford **67** as a white powder in 83% yield. This material was used in the next step without further purification; mp decomposition 280 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1H), 7.78−7.81 (m, 1H), 7.61−7.68 (m, 1H), 7.02−7.08 (m, 1H), 2.24 (s, 3H). LCMS (ESI) m/z 236 (MH^+).

4-(3,4-Dimethoxyphenyl)-thiazole-2-carboxylic Acid Ethyl Ester (68). A solution of 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone (0.5 g, 0.96 mmol) and ethyl thiooxamate (0.25 g, 0.96 mmol) in EtOH (3 mL) was stirred at room temperature overnight. The solvent was then evaporated and the crude product purified by column chromatography on silica (ethyl acetate−hexanes) to afford the product as an orange powder in 97% yield; mp 100−102 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (s, 1H), 7.52−7.58 (m, 2H), 7.05 (d, J = 8.42 Hz, 1H), 4.41 (q, J = 7.14 Hz, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 1.35 (t, J = 7.14 Hz, 3H). LCMS (ESI) m/z 294 (MH^+).

4-(3,4-Dimethoxyphenyl)-thiazole-2-carboxylic Acid (69). To a solution of **68** (0.55 g, 1.87 mmol) in MeOH was added 5N NaOH (1 mL). The reaction was stirred at room temperature overnight, and then the mixture was poured into water−ice and 1N HCl added until the solution reached pH ~ 2. This solution was extracted with EtOAc, and the organic phase dried ($MgSO_4$), filtered, and concentrated to afford the desired product as an orange solid in ~90% yield. This material could be used in the next step without further purification; mp 105−106 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (s, 1H), 7.57−7.59 (m, 1H), 7.56 (s, 1H), 7.06 (d, J = 8.42 Hz, 1H), 3.86 (s, 3H), 3.81 (s, 3H). LCMS (ESI) m/z 266 (MH^+).

4-(3,4-Dimethoxyphenyl)-thiazol-2-ylamine (70). A solution of 2-bromo-1-(3,4-dimethoxyphenyl)-ethanone (0.10 g, 0.39 mmol) and thiourea (0.03 g, 0.39 mmol) in EtOH was stirred at 80 °C overnight. The solvent was evaporated, and the residue was partitioned between EtOAc and satd $NaHCO_3$. The organic layer was washed with water, dried ($MgSO_4$), filtered, and concentrated. The solid residue was crystallized from ethyl acetate−hexanes to afford the product as a light-yellow powder in 60% yield; mp 198−201 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.30−7.38 (m, 2H), 7.00 (s, 2H), 6.93 (d, J = 8.42 Hz, 1H), 6.87 (s, 1H), 3.78 (s, 3H), 3.76 (s, 3H). LCMS (ESI) m/z 237 (MH^+).

Assays of Antiprion Activity and Cell Viability. The methods employed to evaluate the effects of compounds on PrP^{Sc} levels and cell viability were similar to previously published protocols[20] with the following modifications. ScN2a cells (N2a cells infected with the Rocky Mountain Laboratory prion strain) were seeded into black wall, clear bottom, tissue culture treated plates (Greiner) at either 40000 cells/well (in 100 μL of assay medium: MEM supplemented with 10% FBS, GlutaMax and 500 μg/mL Geneticin) for dividing cell assays or 150000 cells/well (in assay medium + 7 mM sodium butyrate to arrest cell division) for nondividing cell assays. Compounds were dissolved in 100% DMSO and diluted in assay medium at 2× final concentration before addition to the assay plates (0.5% final DMSO concentration). Compound addition occurred 4 h (dividing cells) or 24 h (nondividing cells) after cell seeding into the assay plates. After 5 days incubation at 37 °C in a humidified and 5% CO_2-enriched environment, lysates were generated as previously described[20] and transferred to high binding ELISA plates (Greiner) coated with D18 primary antibody for overnight incubation at 4 °C. The next day, the plates were washed three times with TBST before addition of 100 μL of a 1:1000 dilution of HRP-conjugated D13 antibody in 1% BSA/PBS for a 1 h incubation at room temperature. After incubation with the D13 antibody, the plates were washed seven times with TBST, 100 μL of ABTS was added to each well for 10 min, and absorbance at 405 nm was read using a SpectraMax M5 plate reader (Molecular Devices, Sunnyvale, CA). Calcein cell viability assays were run on separately seeded 96-well black wall plates as previously described.[1]

In Vitro ADME Studies. Permeability (MDR1-MDCK) and microsome stability data was generated at ADMETRx, Inc. (Kalamazoo, MI) as described below.

Bidirectional MDR1-MDCK Cell Permeability. MDR1-MDCK cells were grown to confluence for 5−10 days on 1 μm filters in 24-well plates. Aliquots of DMSO solute stocks were diluted into Hank's balanced salt solution (HBSS) pH 7.4 containing 25 mM + 0.05% PS80 to give 10 μM solute concentration. The solute containing donor solutions were transferred to either the

apical or basolateral chamber of the permeability diffusion apparatus. Receiver solutions consisted of Hank's balanced salt solution (HBSS) pH 7.4 containing 25 mM HEPES + 0.05% PS80. Sequential samples of transported solute were taken at 20 min intervals using an automated liquid handling platform. The concentration of transported solute during each sampling interval was determined by HPLC/UV/MS. Permeability coefficients were calculated for each sampling interval. The average and standard deviation from the intervals are reported. Mass balance in the system was ascertained by comparing the sum of total transported solute and remaining donor solute with the starting mass of solute and is expressed as a percentage of donor solute at time zero. Significant deviations from 100% (generally less than 70%) suggest solute adsorption to the apparatus or monolayer or chemical or metabolic instability during the course of the experiment. Mass balance values for compounds reported herein were between 67% and 86%. In the event of mass balance, less than 70%, the cell monolayers were extracted with acetonitrile and analyzed for the solute of interest. Determinates were conducted in duplicate.

Hepatic Microsome Stability. Aliquots of DMSO solute stock were diluted into acetonitrile and then into assay buffer. Assay buffer was pH 7.4 phosphate buffered saline (PBS). Final experimental solute concentrations were 1 μM (0.6% acetonitrile, 0.01% DMSO). Commercially available rat human hepatic microsomes (approx 0.3 mg/mL final concentration) and NADPH (1 mM) with 4 mM UDPGA or PBS were added. The resulting mixture was incubated at 37 °C with aliquots removed at 0, 1, 10, 30, and 60 min and quenched with acetonitrile containing 2 μM carbamazepine (internal standard), centrifuged, and supernatant analyzed by LCMS for remaining starting material. Duplicate incubations were run at each time point. Control incubations were conducted with midazolam ($T_{1/2}$ = 11 min) and 4-nitrophenol ($T_{1/2}$ = 20 min).

In Vivo Studies. Aminothiazole analogue (e.g., 4.5 g of **27**) was added to 15 mL of pure PEG400, vortexed, and sonicated to ensure dissolution, then stored at +4 °C until needed. This highly concentrated PEG400 solution was subsequently diluted to final dosing concentrations, where the volume added was composed of homogenized solid rodent feed, cocoa (taste masking agent), and water. Wild-type FVB mice weigh ~25 g and typically drink 20 mL of liquid diet per day, allowing an estimate of daily drug consumption. A single dosing cohort consisted of three mice in a shared cage, and liquid diet was provided at the start of the study in sufficient volume to last for the entire three-day trial (~200 mL). At the end of the three-day dosing period, animals were euthanized by CO_2, followed by collection of plasma (cardiac puncture) and removal of whole brain. Heparinized blood was centrifuged to separate plasma and both plasma, and brain samples were stored at −80 °C prior to analysis. Plasma samples were prepared for analysis by precipitating proteins and reconstituting the remaining fraction with HPLC mobile phase. Brain samples were typically prepared by 4-fold dilution with water after weighing and then homogenized using bead-beater or Polytron, resulting in a highly concentrated solution that was further diluted with mobile phase as appropriate in preparation for bioanalytical analysis using LC-MS. Analysis was by LC-MS (Shimadzu dual-HPLC pumps, C18 analytical column, with detection using an Applied Biosystems API-4000 triple quadrupole mass spectrometer). Specific LC-MS methods were developed for each compound analyzed, and the stability of the compounds in brain and plasma were demonstrated for the time period of sample handling, workup, and LC-MS analysis.

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Supporting Information Available: Synthetic schemes for new 2-aminothiazole analogues **4**−**50** and synthetic intermediates **51**−**70**. Triplicate data set for compounds **3**−**50** in the ScN2a-cl3 dividing cell assay (EC_{50} and pEC_{50} values), standard deviation (SD), and percent coefficient of variation (CV) values. Mean values (three determinations) for **3**−**50** in the ScN2a-cl3 nondividing cell assay and calcein-AM cell viability assay. 1D NOESY data that supports the assigned site of methylation and acylation for compounds **49** and **50**, respectively. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ghaemmaghami, S.; May, B. C.; Renslo, A. R.; Prusiner, S. B. Discovery of 2-aminothiazoles as potent antiprion compounds. *J. Virol.* **2010**, *84*, 3408–3412.
(2) Prusiner, S. B. Prions. *Proc. Natl. Acad. Sci. U.S.A* **1998**, *95*, 13363–13383.
(3) Miller, G. Neurodegeneration. Could they all be prion diseases? *Science* **2009**, *326*, 1337–1339.
(4) Race, R. E.; Fadness, L. H.; Chesebro, B. Characterization of scrapie infection in mouse neuroblastoma cells. *J. Gen. Virol.* **1987**, *68*, 1391–1399.
(5) Butler, D. A.; Scott, M. R.; Bockman, J. M.; Borchelt, D. R.; Taraboulos, A.; Hsiao, K. K.; Kingsbury, D. T.; Prusiner, S. B. Scrapie-infected murine neuroblastoma cells produce protease-resistant prion proteins. *J. Virol.* **1988**, *62*, 1558–1564.
(6) Korth, C.; May, B. C.; Cohen, F. E.; Prusiner, S. B. Acridine and phenothiazine derivatives as pharmacotherapeutics for prion disease. *Proc. Natl. Acad. Sci. U.S.A* **2001**, *98*, 9836–9841.
(7) May, B. C.; Witkop, J.; Sherrill, J.; Anderson, M. O.; Madrid, P. B.; Zorn, J. A.; Prusiner, S. B.; Cohen, F. E.; Guy, R. K. Structure−activity relationship study of 9-aminoacridine compounds in scrapie-infected neuroblastoma cells. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4913–4916.
(8) May, B. C.; Fafarman, A. T.; Hong, S. B.; Rogers, M.; Deady, L. W.; Prusiner, S. B.; Cohen, F. E. Potent inhibition of scrapie prion replication in cultured cells by bis-acridines. *Proc. Natl. Acad. Sci. U.S.A* **2003**, *100*, 3416–3421.
(9) Dollinger, S.; Lober, S.; Klingenstein, R.; Korth, C.; Gmeiner, P. A chimeric ligand approach leading to potent antiprion active acridine derivatives: design, synthesis, and biological investigations. *J. Med. Chem.* **2006**, *49*, 6591–6595.
(10) Kempster, S.; Bate, C.; Williams, A. Simvastatin treatment prolongs the survival of scrapie-infected mice. *NeuroReport* **2007**, *18*, 479–482.
(11) Heal, W.; Thompson, M. J.; Mutter, R.; Cope, H.; Louth, J. C.; Chen, B. Library Synthesis and Screening: 2,4-Diphenylthiazoles and 2,4-Diphenyloxazoles as Potential Novel Prion Disease Therapeutics. *J. Med. Chem.* **2007**, *50*, 1347–1353.
(12) Kimata, A.; Nakagawa, H.; Ohyama, R.; Fukuuchi, T.; Ohta, S.; Suzuki, T.; Miyata, N. New Series of Antiprion Compounds: Pyrazolone Derivatives Have the Potent Activity of Inhibiting Protease-Resistant Prion Protein Accumulation. *J. Med. Chem.* **2007**, *50*, 5053–5056.
(13) Kimata, A.; Nakagawa, H.; Ohyama, R.; Fukuuchi, T.; Ohta, S.; Doh-ura, K.; Suzuki, T.; Miyata, N. Additions and Corrections to New Series of Antiprion Compounds: Pyrazolone Derivatives Have the Potent Activity of Inhibiting Protease-Resistant Prion Protein Accumulation. *J. Med. Chem.* **2008**, *51*, 1503.
(14) Thompson, M. J.; Borsenberger, V.; Louth, J. C.; Judd, K. E.; Chen, B. Design, Synthesis, and Structure−Activity Relationship of Indole-3-glyoxylamide Libraries Possessing Highly Potent Activity in a Cell Line Model of Prion Disease. *J. Med. Chem.* **2009**, *52*, 7503–7511.
(15) Kawasaki, Y.; Kawagoe, K.; Chen, C. J.; Teruya, K.; Sakasegawa, Y.; Doh-ura, K. Orally administered amyloidophilic compound is effective in prolonging the incubation periods of animals cerebrally infected with prion diseases in a prion strain-dependent manner. *J. Virol.* **2007**, *81*, 12889–12898.
(16) Yudovin-Farber, I.; Azzam, T.; Metzer, E.; Taraboulos, A.; Domb, A. J. Cationic polysaccharides as antiprion agents. *J. Med. Chem.* **2005**, *48*, 1414–1420.
(17) Trevitt, C. R.; Collinge, J. A systematic review of prion therapeutics in experimental models. *Brain* **2006**, *129*, 2241–2265.
(18) Perrier, V.; Wallace, A. C.; Kaneko, K.; Safar, J.; Prusiner, S. B.; Cohen, F. E. Mimicking dominant negative inhibition of prion

replication through structure-based drug design. *Proc. Natl. Acad. Sci. U.S.A* **2000**, *97*, 6073–6078.

(19) Ghaemmaghami, S.; Ullman, J.; Ahn, M.; St Martin, S.; Prusiner, S. B. Chemical induction of misfolded prion protein conformers in cell culture. *J. Biol. Chem.* **2010**, *285*, 10415–10423.

(20) May, B. C.; Zorn, J. A.; Witkop, J.; Sherrill, J.; Wallace, A. C.; Legname, G.; Prusiner, S. B.; Cohen, F. E. Structure−activity relationship study of prion inhibition by 2-aminopyridine-3,5-dicarbonitrile-based compounds: parallel synthesis, bioactivity, and in vitro pharmacokinetics. *J. Med. Chem.* **2007**, *50*, 65–73.

(21) Hitchcock, S. A.; Pennington, L. D. Structure−brain exposure relationships. *J. Med. Chem.* **2006**, *49*, 7559–7583.

(22) Aller, S. G.; Yu, J.; Ward, A.; Weng, Y.; Chittaboina, S.; Zhuo, R.; Harrell, P. M.; Trinh, Y. T.; Zhang, Q.; Urbatsch, I. L.; Chang, G. Structure of P-glycoprotein reveals a molecular basis for polyspecific drug binding. *Science* **2009**, *323*, 1718–1722.

(23) Braun, A.; Hammerle, S.; Suda, K.; Rothen-Rutishauser, B.; Gunthert, M.; Kramer, S. D.; Wunderli-Allenspach, H. Cell cultures as tools in biopharmacy. *Eur. J. Pharm. Sci.* **2000**, *11* (Suppl 2), S51–S60.