Library Synthesis and Screening: 2,4-Diphenylthiazoles and 2,4-Diphenyloxazoles as Potential Novel Prion Disease Therapeutics

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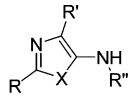
Transmissible spongiform encephalopathies (TSEs) are a family of invariably fatal neurodegenerative disorders for which no effective therapeutics are currently available. In this paper, we report on the synthesis and screening of a small library of 2,4-diphenylthiazol-5-ylamine and 2,4-diphenyloxazol-5-ylamine derivatives as potential novel prion disease therapeutics. Various synthetic strategies were investigated, including a novel phosgene-mediated cyclization of 2-*N*-benzoylphenylglycinonitrile, and a total of 45 compounds were synthesized. Library members were tested for both binding to prion protein (PrP^C) using the surface plasmon resonance technique and for inhibition of PrP^{Sc} formation in persistently infected SMB cells. Of the compounds prepared, 15 were found to bind to human PrP^C and six showed inhibition of PrP^{Sc} formation, displaying EC_{50}s between 1.5 and 20 μM.

Introduction

Prion diseases, or transmissible spongiform encephalopathies (TSEs[a]), are invariably fatal neurodegenerative disorders affecting humans and animals. As yet, no effective curative or prophylactic therapy exists.[1] Prominent examples of prion diseases include bovine spongiform encephalopathy (BSE, cattle), scrapie (sheep), chronic wasting disorder (CWD, deer and elk), and transmissible mink encephalopathy (TME).[2] Since a new variant of the human TSE Creutzfeldt−Jacob disease (vCJD) was discovered, thought to have been triggered by the consumption of contaminated beef products, prion diseases have been the focus of much research effort.[3−5] They represent a highly significant risk to public health due to transmission both to and between humans—iatrogenic transmission having been reported via contact with contaminated neurosurgical instruments, grafts, and blood/tissue products[6]—and as such continue to receive high profile media attention. TSEs are associated with a post-translational conversion of the cell-surface glycosylphosphatidylinositol (GPI)-anchored protein PrP^C (or PrP^{sen}) to a partially protease resistant isoform denoted PrP^{Sc} (or PrP^{res}). As part of a TSE-oriented medicinal chemistry program, we are seeking small drug-like molecules that interact with human PrP^C ($huPrP^C$) and/or reduce levels of PrP^{Sc} in persistently infected cells, with the aim of identifying novel prion disease therapeutics.

From a vHTS study,[7] followed by the screening of sourced compounds by surface plasmon resonance (SPR),[8] structure **1a** (Figure 1) was identified as binding to $huPrP^C$. As this compound was a singleton and no suitable analogues were found to be available commercially, a convenient synthetic route to such structures was sought.

Specifically, we intended to first prepare a library of amides at the 5-position through derivatization of the free 5-amino compound **1b** (Figure 1). 5-Aminothiazoles have received attention ranging from antibiotics[9] to photosensitisers;[10] however, searching the literature for a general route allowing for structural variation and based on readily available starting materials gave limited results. Published routes to these compounds are restricted with regard to substitution at the 2- and 4-positions, sometimes involving many synthetic steps.[11−13] To address this deficit we recently published a route to 5-aminothiazoles[14] offering control over substitution at positions 2 and 4 in which we also reported the isolation of 5-aminooxazoles as a side product in some cases. When subjected to an SPR binding assay, compounds **1c** and **2a** (Figure 1) both showed binding to $huPrP^C$.



1a, R = 3-Pyridyl, R' = Thiophenyl, R" = 2-Furoyl, X = S
1b, R = 3-Pyridyl, R' = Thiophenyl, R" = H, X = S
1c, R = R' = Phenyl, R" = $COCF_3$, X = S
1d, R = R' = Phenyl, R" = H, X = S
2a, R = R' = Phenyl, R" = $COCF_3$, X = O
2b, R = R' = Phenyl, R" = H, X = O

Figure 1. Hit compound **1a** and early lead compounds.

Synthesis. Our initial goal was to prepare a library of analogues of **1a** with a range of amide functionalities at the 5-position. As such, synthesis of 2-nicotinyl-5-(2-thienyl)-thiazol-5-ylamine **1b** was pursued as a key intermediate for further derivatization by a range of carboxylic acids. Access to this compound proved difficult, however, and after approaching it unsuccessfully by a number of routes, it was decided instead to prepare the 2,4-diphenyl analogue **1d** for synthetic expediency. This intermediate was prepared as reported previously[14] and was purified further by flash column chromatography where necessary prior to library synthesis.

5-Amino compound **1d** proved unreactive toward carboxylic acids under standard DCC−HOBT coupling conditions. Addition of an amine base (4-ethylmorpholine, NEM) to drive the reaction resulted in formation of the bis-amide product. Use of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate as coupling agent in the presence of DIPEA also resulted in bis-amide formation. Nonetheless, amide formation was found to proceed smoothly by reaction of **1d** with acid chlorides in pyridine. A library of 18 such derivatives (**1e**−**x**)

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[a] Abbreviations: PrP^C, normal cellular prion protein or PrP-sen; PrP^{Sc}, disease-causing isoform or PrP-res; $huPrP^C$, human PrP^C; $moPrP^C$, murine PrP^C; SMB, scrapie-infected mouse brain; SPR, surface plasmon resonance; TSE, transmissible spongiform encephalopathies; vCJD, variant Creutzfeldt−Jakob disease; vHTS, virtual high-throughput screening.

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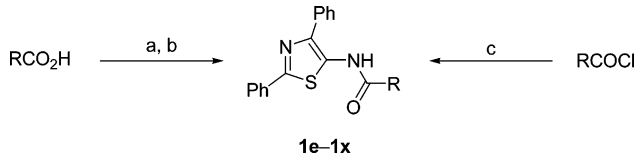
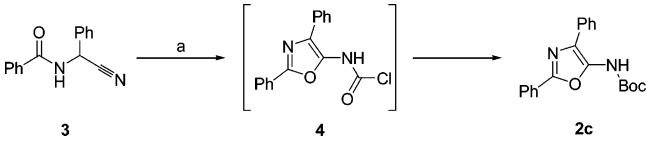
Scheme 1. Synthesis of Thiazole Library by Amide Formation[a]



1e–1x

[a] Reagents and conditions: (a) $(COCl)_2$, DCM, room temp., 80 min; (b) **1d**, pyridine, room temp., 18 h; (c) pyridine, DMAP, **1d**, room temp., 18 h.

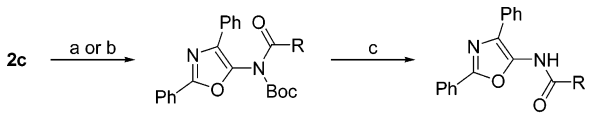
Scheme 2. Synthesis of (2,4-Diphenyloxazol-5-yl)-carbamic Acid *tert*-Butyl Ester **2c**[a]



3 **4** **2c**

[a] Reagents and conditions: (a) Triphosgene, DCM, room temp, 15 min, then ^{t}BuOH, DCM, room temp., 5 min.

Scheme 3. Synthesis of Oxazole Library by Bis-amide Formation and Deprotection[a]



2d–2w

[a] Reagents and conditions: (a) NaH, THF, room temp., 5 min, then RCOCl, THF, room temp., 10 min; (b) RCOCl, DMAP, DIPEA, room temp., 2 h; (c) TFA (20%) in DCM, room temp, 18 h.

was prepared by this method, with the exception of two cases (**1g**, **1r**) where the relevant acid chloride was not available commercially. These amide derivatives were prepared from the carboxylic acids, through *in situ* formation of the acid chloride followed by reaction with amine **1d** in pyridine (Scheme 1).

Since both *N*-(2,4-diphenylthiazol-5-yl)-2,2,2-trifluoroacetamide **1c** and the analogous structure *N*-(2,4-diphenyloxazol-5-yl)-2,2,2-trifluoroacetamide **2a** had displayed binding to PrP^C in our SPR assay, a series of 5-amidooxazoles analogous to the existing library of thiazole derivatives was desired to widen the scope of this comparison.

2,4-Diphenyloxazol-5-ylamine, analogous to 5-aminothiazole **1b**, has been reported in the literature.[15] In our hands, however, this compound could not be isolated, being found to exist in equilibrium with its ring-opened form. A different approach to the required 5-amidooxazoles was therefore necessary. We confronted the problem by looking to trap the 2,4-diphenyloxazol-5-ylamine as a suitably protected derivative as it was formed. The solution to this problem was inspired by the work of Verschave et al., in which oxalyl chloride is used in the formation of oxazoyl-5-oxylamic acid esters[16], which led us to develop a novel phosgene-mediated cyclization of 2-*N*-benzoylphenylglycinonitrile **3**. Subsequent quenching of the chlorocarbamate intermediate **4** with 2-methyl-2-propanol provided the *N*-Boc-protected amine **2c** in an acceptable overall yield (Scheme 2). Preparation of **2c** from 2-phenylglycinonitrile hydrochloride and benzoyl chloride via a related one-pot procedure was also found to be possible without the need for isolation of the open-chain intermediate **3**.

The desired library of 5-amidooxazoles was accessed from *N*-Boc protected intermediate **2c** through acylation at N-5 with the same series of acid chlorides detailed above followed by TFA-mediated removal of the *N*-Boc group (Scheme 3). At first, N-acylation reactions were carried out by deprotonation of **2c** with sodium hydride in dry THF followed by treatment with the acid chloride, though it was later found that milder conditions were adequate.

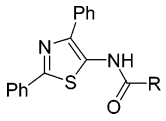
N-Acylation of **2c** in DCM, catalyzed by DMAP and in the presence of DIPEA, was typically complete in 2 h at room temperature. Removal of the *N*-Boc protecting group was accomplished by addition of TFA to the reaction mixture to a final dilution of 1:4 TFA−DCM followed by stirring for 18 h. This method proved a particularly convenient protocol for parallel synthesis of 5-amidooxazoles **2d**−**2w**, which were purified by recrystallization or column chromatography as necessary.

Some of the 5-amidooxazoles prepared appeared to be unstable, however; repeated HPLC analysis revealed that the amide products containing a pyridine (**2j** and **2k**), 5-nitrofuryl (**2n**), or isoxazolyl (**2o**) ring all showed slow decomposition over a period of days or weeks, even on storage at −20 °C. These results naturally preclude the incorporation of such substituents into any future libraries, though none of the unstable compounds displayed any activity in the cell line assay.

Screening (SPR) Methodology. Surface plasmon resonance (SPR) was carried out using a BIAcore 3000 (BIAcore, Uppsala, Sweden) equipped with a CM5 sensor chip (carboxymethylated dextran). The methodology was as reported previously.[8] Interactions were measured with two forms of prion protein, full length human ($huPrP^C$) and full length murine ($moPrP^C$). Compounds were screened at 40 μM in running buffer (10 mM sodium phosphate, pH 7.4, 150 mM NaCl, 3.4 mM EDTA, 0.005% (v/v) surfactant P20) containing 6.5% DMSO. DMSO calibration using buffer samples containing 5.5−7.5% DMSO was carried out to correct for solvent effects. Compounds producing a response of more than 2.5 response units (RU) were considered to be binders. Binding affinities are expressed as $\%RU_{max}$, that is, as a percentage of the theoretical maximum response for 1:1 protein−ligand binding. We have previously observed that the numerical binding data obtained during the SPR assay varies considerably with the age of the chip;[8] therefore, direct quantitative comparison is only relevant between compounds included in the same screening run. Attempts to include all of the compounds in this study within the same run proved unsuccessful, however. Satisfactory results were obtained when the compounds were split between runs: most were ultimately screened together, but the rest of the structures were evaluated separately as indicated in Tables 1 and 2. Direct comparison of numerical binding data between all the compounds is immaterial because of this, though the data presented does serve to indicate a relative degree of binding for the various compounds. No data is presented for compounds either seen to interfere with the chip surface, or for those that could not be washed off the protein (Tables 1 and 2).

Screening (SMB Cells) Methodology. Compounds were screened for inhibition of PrP^{Sc} formation in SMB cells of mesodermal origin, the procedure used being based upon that reported by Rudyk et al.[17] A persistently infected mouse cell line (SMB), cloned originally from scrapie infected mouse brain but of non-neuronal origin,[18] was used. Cells were grown in tissue culture-treated plastic dishes in Medium 199 (phenol red free), supplemented with 10% newborn calf serum (heat inactivated), 5% fetal calf serum (heat inactivated), and penicillin−streptomycin at 10 mg L^{-1} at 37 °C in an atmosphere of 5% CO_2 in air at 95% relative humidity. Medium was changed every third or fourth day, and every 7 days confluent cells were passaged using 0.05% trypsin and 0.002% EDTA at a split ratio of 4. To assess the effects of compounds, cells were distributed

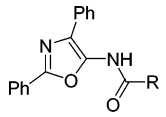
Table 1. Yields and Screening Results for the Thiazole Library



compd no.	R	yield [%]	%RU_{max} ($huPrP^C$)	%RU_{max} ($moPrP^C$)	PrP^{Sc} rel to control [%] (± std dev)	concn [μM]
1c	CF_3	−	9.5	18.7	13.7 (± 7.6)	2.5
1e	Ph	74	0	0	97.7 (± 3.7)	10
1f	4-F-Ph	86	−[a]	−[a]	109.3 (± 2.7)	10
1g	4-OMe-Ph	12	−[a]	−[a]	69.4 (± 0.8)	10
1h	2-CF_3-Ph	83	0	0	102.0 (± 1.3)	10
1i	3-CF_3-Ph	83	65.0	0	105.1 (± 4.4)	10
1j	4-CF_3-Ph	79	0	0	112.5 (± 2.8)	10
1k	3-pyridyl	69	0	0	93.9 (± 3.7)	10
1l	4-pyridyl	75	5.0[b]	8.5[b]	99.8 (± 1.0)	10
1m	2-quinoyl	65	−[a]	−[a]	93.4 (± 10.8)	10
1n	2-furyl	51	6.5	8.4	98.7 (± 7.6)	10
1o	5-NO_2-2-furyl	9	0	0	95.2 (± 3.8)	10
1p	5-isoxazoyl	74	0	0	86.2 (± 4.3)	1
1q	thiophen-2-yl	82	−[a]	−[a]	86.3 (± 5.1)	1
1r	5-Me-thiophen-2-yl	67	19.8[b]	0[b]	83.6 (± 2.4)	10
1s	benzo[*b*]thiophen-2 -yl	83	21.1	0	101.3 (± 15.5)	10
1t	benzofuran-2-yl	86	−[a,b]	−[a,b]	88.2 (± 2.1)	10
1u	benzothiazol-2-yl	27	40.1[b]	0[b]	60.2 (± 2.9)	1
1v	benzo[*b*]thiophen-5 -yl	59	−[a,b]	−[a,b]	87.4 (± 0.0)	10
1w	cyclopropyl	61	0	0	74.6 (± 3.2)	1
1x	CH(*n*-propyl)$_2$	74	0	0	76.8 (± 4.5)	1

[a] Compound interacted with the chip surface or could not be washed off the protein. [b] Binding data obtained from a different screening run.

Table 2. Yields and Screening Results for the Oxazole Library



compd no.	R	yield [%]	%RU_{max} ($huPrP^C$)	%RU_{max} ($moPrP^C$)	PrP^{Sc} rel to control [%] (std dev [%])	concn [μM]
2a	CF_3	−	13.7	22	35.1 (± 11.2)	2.5
2c	*O*-*tert*-butyl	22	11.3	10.6	46.1 (± 7.0)	1
2d	Ph	72	−[a]	−[a]	72.8 (± 26.5)	1
2e	4-F-Ph	81	0	0	80.4 (± 14.3)	1
2f	4-OMe-Ph	69	0[b]	0[b]	84.9 (± 7.8)	10
2g	2-CF_3-Ph	71	−[a]	−[a]	51.7 (± 1.6)	1
2h	3-CF_3-Ph	54	−[a]	−[a]	80.9 (± 1.9)	1
2i	4-CF_3-Ph	31	0	0	80.2 (± 1.2)	10
2j	3-pyridyl	14	37.3	40.0	85.6 (± 7.4)	1
2k	4-pyridyl	59	0[b]	0[b]	93.2 (± 1.2)	10
2l	2-quinoyl	27	0	0	106.8 (± 8.7)	10
2m	2-furyl	77	3.8[b]	0[b]	92.9 (± 2.1)	10
2n	5-NO_2-2-furyl	19	0	0	84.2 (± 7.9)	10
2o	5-isoxazoyl	23	18.3	21.7	77.1 (± 1.0)	10
2p	thiophen-2-yl	79	27.5	37.4	90.5 (± 12.8)	1
2q	5-Me-thiophen-2-yl	56	−[a,b]	−[a,b]	79.3 (± 1.8)	10
2r	benzo[*b*]thiophen-2 -yl	18	48.0	59.6	83.2 (± 10.8)	10
2s	benzofuran-2-yl	55	48.9[b]	58.0[b]	86.6 (± 4.2)	10
2t	benzothiazol-2-yl	47	−[a]	−[a]	87.8 (± 2.5)	10
2u	benzo[*b*]thiophen-5 -yl	65	−[a]	−[a]	84.8 (± 3.8)	10
2v	cyclopropyl	54	0	0	87.8 (± 0.2)	10
2w	CH(*n*-propyl)$_2$	38	−[a,b]	−[a,b]	95.8 (± 0.4)	10

[a] Compound interacted with the chip surface or could not be washed off the protein. [b] Binding data obtained from a different screening run.

into 96-well cluster plates at 3×10^4 cells per well and incubated for 24 h to allow for cell attachment. The compounds were diluted to 400 times the required concentration in DMSO as stock solutions then transferred, at a 20-fold dilution, into Hank's balanced salt solution. This solution was then transferred at a further 20-fold dilution into the cell medium. The cells were incubated with the compound-containing medium for 5 days.

After 5 days, cell viability was assessed by the MTT assay following the standard protocol supplied with the reagent (Sigma). For dot blot analyses, cells were extracted using lysis buffer (10 mM Tris−HCl [pH 7.6], 100 mM NaCl, 10 mM EDTA, 0.5% v/v NP40, and 0.5% w/v sodium deoxycholate), and the content of the well was loaded onto a nitrocellulose membrane (0.45 μm) under gentle vacuum at a total cellular protein concentration of approximately 30−40 μg/well (determined by the Bradford assay following the protocol supplied with the reagent, Sigma). The membrane was air-dried and subjected to 75 μg mL^{-1} proteinase K digestion for 1 h at

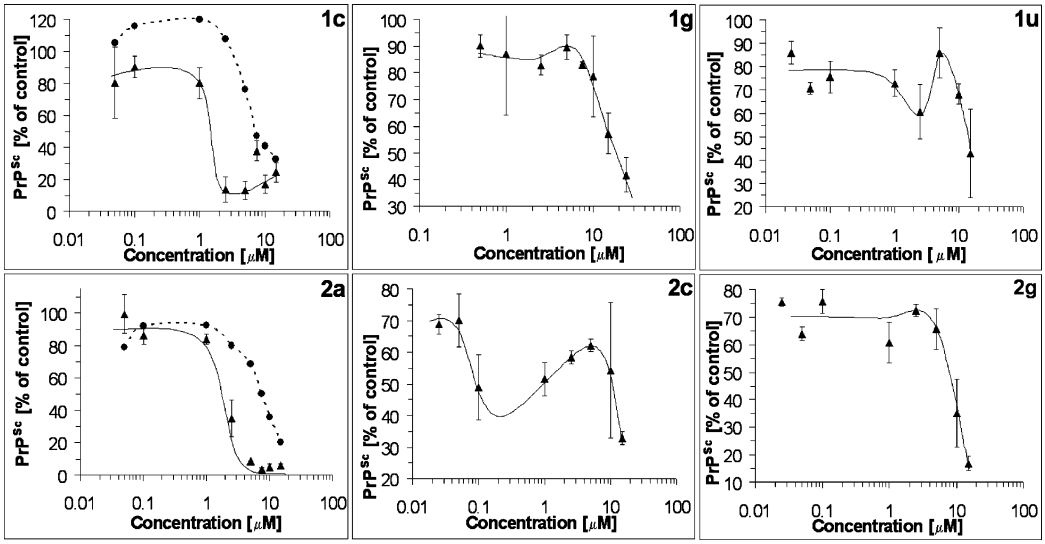


Figure 2. IC_{50} curves for compounds **1c**, **1g**, **1u**, **2a**, **2c**, and **2g**. Circles (graphs **1c** and **2a**) represent cell viability; other compounds not toxic at concentrations shown.

37 °C. The reaction was stopped with 1 mM phenylmethylsulfonyl fluoride (PMSF) in 20 mM Tris−HCl-buffered saline (TBS) and the membrane washed extensively with TBS and immersed in 1.8 M guanidine thiocyanate in TBS for 10 min at room temperature. After further washing with TBS, the membrane was blocked using 5% fat-free milk powder in phosphate-buffered saline (PBS), processed with 0.2 μg mL^{-1} mouse monoclonal anti-PrP 6H4 (Prionics), and developed using an ECL kit (Amersham Pharmacia Biotech).

Each experiment was carried out in triplicate and an average value for PrP^{Sc} concentration calculated, relative to an untreated control, together with a standard deviation. Compounds were initially screened at 1 and 10 μM and were considered to be active if PrP^{Sc} levels were reduced to less than 70% of that of the untreated control after 5 days' exposure. The amounts of PrP^{Sc} remaining as a percentage relative to the control are given in Tables 1 and 2. Where an acceptable dose−response curve was observed, EC_{50} values were determined. However, where the data was inconclusive, the lowest active concentration supplanted the initial screening result in Tables 1 and 2.

Results and Discussion

SPR Screening. Among the thiazole library (Table 1), it can be seen that seven compounds bound to $huPrP^{C}$ and three to $moPrP^{C}$, a further six compounds being found to interact with the chip surface. The oxazole library included eight binders to $huPrP^{C}$ and seven to $moPrP^{C}$, with seven compounds found to interact with the chip surface (Table 2). Most compounds bound to both or neither form of PrP^{C} except for five compounds which bound to $huPrP^{C}$ only, these mostly coming from the thiazole library (**1i**, **1r**, **1s**, and **1u**). Perhaps the most interesting observation was the difference in behavior between the two libraries. There were several examples where binding was effected or lost by changing the azole heteroatom between sulfur and oxygen, notably **1k** and **2j**; **1l** and **2k**; **1p** and **2o**. In other cases the comparison between O and S was hampered by either of both of the compounds interacting with the carboxymethylated dextran chip surface.

Cell Line Assay. The results from the remaining compounds are shown in Tables 1 and 2. It was interesting to note that synthetic intermediates **1c**, **2a**, and **2c** were the most potent compounds found in this investigation, with **1u** and **2g** also found to be active at 1 μM. Compound **1g** was a borderline active at 10 μM but demonstrated more effective clearance of PrP^{Sc} at higher concentrations. The remainder of the compounds were largely inactive, rendering a comprehensive SAR elusive at this stage. In a similar manner to the binding behavior, cell line activity was highly influenced by the presence of either O or S in the azole ring. With the exception of the trifluoroacetates **1c** and **2a**, there is no common R group between the actives of the thiazole and the oxazole libraries. Looking at the oxazole library, the activity of 2-trifluoromethylphenyl compound **2g** when compared to the 3- and 4-trifluoromethyl derivatives **2h** and **2i** suggests an interesting effect. If the role of the CF_3 group were purely to activate the amide carbonyl as a hydrogen bond acceptor, the 4-substituted analogue **2i** would be expected to show some activity also. The activity of **1g** suggests that this is not a steric effect: the trifluoromethyl group of **2g** may therefore be involved in a protein−ligand interaction. However, as none of the corresponding thiazoles (**1h**−**j**) were active, it cannot be determined whether this is a general trend. Cyclopropyl compound **1w** reduced PrP^{Sc} levels to 74.6% of the control at 1 μM, which could be considered as borderline activity, whereas the corresponding oxazole **2v** showed no activity at 10 μM. Conversely, phenyl compound **2d** was a similar borderline active at 1 μM (although with high standard deviation in this instance), while the thiazole analogue **1e** showed no activity at the higher concentration. The primary amine **1d** and the bis-amide compounds formed as described earlier were not found to be active in our cell-line assays.

Compounds **1c** and **2a** were both found to have EC_{50}s of ~1.5 μM, **1g** ~20 μM, **1u** ~13 μM, **2c** ~11 μM, and **2g** ~8 μM (Figure 2). Compounds **1u** and **2c** displayed an intriguing biphasic dose−response. Known as hormesis, this phenomenon is not fully understood,[19] although it has been encountered in some of our other screening subjects[20] and may be indicative of metabolism occurring at higher concentrations. In particular, examination of the dose−response profile of *N*-Boc compound **2c** reveals that this compound displays a possible initial EC_{50} in the range of 100 nM before PrP^{Sc} levels recover as drug concentration increases. This apparent activity, coupled with that of the trifluoroacetates **1c** and **2a** and the borderline activity of cyclopropyl derivative **1w**, suggests that related compounds bearing small, lipophilic, aliphatic groups at the 5-position merit further investigation in the search for more potent inhibitors of PrP^{Sc} accumulation.

Although it has not been possible thus far to establish any direct correlation between PrP^C binding affinity and cell line activity, it is encouraging to note that all of the active compounds reported here for which data could be obtained showed binding to the full-length human PrP^C, and all but **1u** to the murine protein. It was not possible to establish whether actives **1g** and **2g** were binders to PrP^C using the SPR method. From the results reported here it is evident that factors other than PrP^C binding alone are responsible for cell line activity, though given that the mechanisms underlying prion disease are not yet fully understood, any alternative explanation for these compounds' mode of action would merely be speculative in nature. Our goal was primarily to identify new lead compounds in the search for effective therapeutics against a presently untreatable class of fatal diseases, and this goal has been realized in the present work.

Conclusions

Thiazoles and oxazoles are frequently encountered in compounds of biological interest. However, we present here the first reported instance of antiprion activity in compounds of this type. Out of 45 compounds synthesized, 15 were found to bind to PrP^C. Six of the 45 compounds showed potent inhibition of PrP^{Sc} formation with EC_{50}s in the range 1.5−20 μM. These structures are therefore of considerable interest for further development, and as such, variation of the groups at positions 2 and 4 of the active azoles is under investigation, with a view to both improving activity and establishing a more defined SAR. Consequently, it is anticipated that new compounds such as these, displaying significant antiprion activity, may eventually address the lack of clinical effect found with compounds such as quinacrine in treating the invariably fatal TSEs. To this end, the results reported herein represent a useful advance in addressing this pressing medical need, presenting compounds with low-micromolar activity as valuable leads for further development.

Experimental Section

General Procedures. Melting points were measured using a Bibby-Sterilin SMP10 melting point apparatus and are uncorrected. Accurate mass and nominal mass measurements were measured using a Waters-Micromass LCT electrospray mass spectrometer. Flash column chromatography was carried out using Fluorochem silica gel 60 Å. All compounds were isolated in >95% purity as determined by HPLC, unless otherwise stated. All reagents were purchased directly from commercial sources and used as supplied. Compounds **1c**, **1d**, and **2a** have been reported previously.[14] Full characterization data for each new compound is provided in the Supporting Information.

General Procedure for the Synthesis of Thiazoles. Acid chloride (0.33 mmol) was added to a solution of **1d** (76 mg, 0.30 mmol) and DMAP (10 mg) in pyridine (3 mL). The reaction mixture was stirred at ambient temperature for 18 h. All volatiles were removed under reduced pressure, and the residue was taken up in DCM (30 mL). This solution was washed thoroughly with 1 M HCl (4 × 30 mL) and then sat. $NaHCO_3$ (2 × 30 mL), dried over $MgSO_4$, filtered, and evaporated to dryness to provide the product. Where necessary, further purification was carried out by flash column chromatography on silica gel.

***N*-(2,4-Diphenylthiazol-5-yl)-benzamide (1e):** *m/z* (ES); HRMS, 357 ($[M + H]^+$); HRMS, found 357.1047 ($C_{22}H_{17}N_2OS$, $[M + H]^+$, requires 357.1062).

***N*-(2,4-Diphenylthiazol-5-yl)-4-fluorobenzamide (1f):** *m/z* (ES), 375 ($[M + H]^+$); HRMS, found 375.0961 ($C_{22}H_{16}FN_2OS$, $[M + H]^+$, requires 375.0967).

***N*-(2,4-Diphenylthiazol-5-yl)-2-trifluoromethylbenzamide (1h):** *m/z* (ES), 425 ($[M + H]^+$); HRMS, found 425.0938 ($C_{23}H_{16}F_3N_2OS$, $[M + H]^+$, requires 425.0935).

***N*-(2,4-Diphenylthiazol-5-yl)-3-trifluoromethylbenzamide (1i):** *m/z* (ES), 425 ($[M + H]^+$); HRMS, found 425.0926 ($C_{23}H_{16}F_3N_2OS$, $[M + H]^+$, requires 425.0935).

***N*-(2,4-Diphenylthiazol-5-yl)-4-trifluoromethylbenzamide (1j):** *m/z* (ES), 425 ($[M + H]^+$); HRMS, found 425.0949 ($C_{23}H_{16}F_3N_2OS$, $[M + H]^+$, requires 425.0935).

***N*-(2,4-Diphenylthiazol-5-yl)nicotinamide (1k):** *m/z* (ES), 358 ($[M + H]^+$); HRMS, found 358.1000 ($C_{21}H_{16}N_3OS$, $[M + H]^+$, requires 358.1014).

***N*-(2,4-Diphenylthiazol-5-yl)isonicotinamide (1l):** *m/z* (EI), 357 (M^+); HRMS, found 357.0922 ($C_{21}H_{15}N_3OS$, M^+, requires 357.0936).

Quinoline-2-carboxylic acid (2,4-diphenylthiazol-5-yl)amide (1m): *m/z* (ES), 408 ($[M + H]^+$); HRMS, found 408.1155 ($C_{25}H_{18}N_3OS$, $[M + H]^+$, requires 408.1171).

Furan-2-carboxylic acid (2,4-diphenylthiazol-5-yl)amide (1n): *m/z* (ES), 346 ($[M + H]^+$); HRMS, found 347.0868 ($C_{20}H_{15}N_2O_2S$, $[M + H]^+$, requires 347.0854).

5-Nitrofuran-2-carboxylic acid (2,4-diphenylthiazol-5-yl)amide (1o): *m/z* (ES), 392 ($[M + H]^+$); HRMS, found 392.0710 ($C_{20}H_{14}N_3O_4S$, $[M + H]^+$, requires 392.0705).

Isoxazole-5-carboxylic acid (2,4-diphenylthiazol-5-yl)amide (1p): *m/z* (ES), 348 ($[M + H]^+$); HRMS, found 348.0790 ($C_{19}H_{14}N_3O_2S$, $[M + H]^+$, requires 348.0807).

Thiophene-2-carboxylic acid (2,4-diphenylthiazol-5-yl)amide (1q): *m/z* (ES), 363 ($[M + H]^+$); HRMS, found 363.0615 ($C_{20}H_{15}N_2OS_2$, $[M + H]^+$, requires 363.0626).

Benzo[*β*]thiophene-2-carboxylic acid (2,4-diphenylthiazol-5-yl)amide (1s): *m/z* (ES), 413 ($[M + H]^+$); HRMS, found 413.0780 ($C_{24}H_{17}N_2OS_2$, $[M + H]^+$, requires 413.0782).

Benzofuran-2-carboxylic acid (2,4-diphenylthiazol-5-yl)amide (1t): *m/z* (ES), 397 ($[M + H]^+$); HRMS, found 397.1005 ($C_{24}H_{17}N_2O_2S$, $[M + H]^+$, requires 397.1011).

Benzothiazole-2-carboxylic acid (2,4-diphenylthiazol-5-yl)-amide (1u): *m/z* (ES), 414 ($[M + H]^+$); HRMS, found 414.0748 ($C_{23}H_{16}N_3OS_2$, $[M + H]^+$, requires 414.0735).

Benzo[*β*]thiophene-5-carboxylic acid (2,4-diphenylthiazol-5-yl)amide (1v): *m/z* (ES), 413 ($[M + H]^+$); HRMS, found 413.0788 ($C_{24}H_{17}N_2OS_2$, $[M + H]^+$, requires 413.0782).

Cyclopropanecarboxylic acid (2,4-diphenylthiazol-5-yl)amide (1w): *m/z* (ES), 343 ($[M + Na]^+$); HRMS, found 343.0893 ($C_{19}H_{16}NaN_2OS$, $[M + Na]^+$, requires 343.0881).

***N*-(2,4-Diphenylthiazol-5-yl)-4-methoxybenzamide (1g).** A stirred suspension of *p*-anisic acid (44 mg, 0.29 mmol) in DCM (3.0 mL) was treated with oxalyl chloride (26.2 μL, 0.30 mmol) and DMF (40 μL), at which point effervescence was observed. A homogeneous solution was gradually obtained as the reaction took place. After 80 min, the reaction mixture was concentrated under reduced pressure and dried further under high vacuum (with periodical warming) to yield the crystalline, crude acid chloride. 2,4-Diphenyl-5-aminothiazole (70 mg, 0.28 mmol), pyridine (3.0 mL), and DMAP (10 mg) were added, and the resultant orange solution was stirred at ambient temperature for 18 h. The pyridine was removed under vacuum and the residue taken up in DCM (40 mL) and then washed with 1 M HCl (4 × 50 mL) followed by sat. $NaHCO_3$ (2 × 40 mL). The organic layer was evaporated and the residue purified by flash column chromatography on silica gel, eluted with 60−75−90% DCM−hexane, to provide **1e** as a yellowish solid (13 mg, 12%). *m/z* (ES), 409 ($[M + Na]^+$); HRMS, found 409.1006 ($C_{23}H_{18}N_2NaO_2S$, $[M + Na]^+$, requires 409.0987).

5-Methylthiophene-2-carboxylic Acid (2,4-diphenylthiazol-5-yl)amide (1r). 5-Methylthiophene-2-carboxylic acid (213 mg, 1.50 mmol) was suspended in DCM (5.0 mL), and then oxalyl chloride (131 μL, 1.50 mmol) and DMF−DCM (1:9, 20 μL) were added. After the mixture was stirred at ambient temperature for 1 h, a solution of 2,4-diphenyl-5-aminothiazole (252 mg, 1.00 mmol) in pyridine (5.0 mL) was added and stirring of the resultant mixture was continued at ambient temperature for 18 h. The reaction mixture was diluted with DCM (60 mL) and washed successively with 1 M HCl (4 × 40 mL) and sat. $NaHCO_3$ (2 × 40 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated giving crude material, which was purified by flash column chromatography on

silica gel, eluting with 60−75% DCM−hexane and yielding **1r** as an off-white solid (252 mg, 67%). *m/z* (EI), 376 (M^+); HRMS, found 376.0695 ($C_{21}H_{16}N_2OS_2$, M^+, requires 376.0704).

2-Propylpentanoic Acid (2,4-diphenylthiazol-5-yl)amide (1x). A stirred solution of 2-propylpentanoic acid (218 μL, 200 mg, 1.39 mmol) in DCM (3 mL) was treated with oxalyl chloride (122 μL, 177 mg, 1.39 mmol) and DMF−DCM (1:4, 20 μL). After being stirred for 1 h, the reaction mixture was evaporated to dryness and further dried under high vacuum to yield the crude acid chloride. **1d** (70 mg, 0.28 mmol) in pyridine (3 mL) was added, followed by DMAP (10 mg), and the solution stirred at ambient temperature for 18 h. All volatiles were removed under reduced pressure, and the residue was taken up in DCM (40 mL). This solution was washed thoroughly with 1 M HCl (3 × 40 mL) and then sat. $NaHCO_3$ (3 × 40 mL) and evaporated. Purification by flash column chromatography on silica gel, eluting with 40−50% DCM−hexane, afforded the bis-amide product, 2,4-diphenyl-5-[*N*,*N*-bis(2-propylpentanoyl)amino]thiazole (57 mg, 41%), which crystallized as a pale orange solid upon scratching. A portion of this material (47 mg) was suspended in 2-propanol (2.0 mL), and then tetraethylammonium hydroxide, 20% w/v aqueous solution (146 μL, 2.0 mmol), was added. Dissolution of the starting material was observed as hydrolysis took place. After 2.5 h, the mixture was diluted with 1 M HCl (50 mL) and DCM (50 mL). The organic layer was separated, dried over $MgSO_4$, filtered, and evaporated. Purification by flash column chromatography on silica gel, eluted with 50−65% DCM−hexane, yielded **1x** as a white, crystalline solid (26 mg, 74%). *m/z* (ES), 379 ($[M + H]^+$); HRMS, found 379.1841 ($C_{23}H_{27}N_2OS$, $[M + H]^+$, requires 379.1844).

(2,4-Diphenyloxazol-5-yl)-carbamic Acid *tert*-Butyl Ester (2c). 2-(Benzoylamino)-2-phenylglycinonitrile hydrochloride (3.89 g, 14.3 mmol) was partitioned between DCM (70 mL) and water (50 mL), and sodium carbonate was added portionwise, with thorough mixing, until the aqueous layer was basic to universal indicator paper (pH 10). The organic layer was separated, dried over $MgSO_4$, and filtered. The filtrate (∼100 mL) was added slowly to a solution of triphosgene (4.23 g, 14.3 mmol) in DCM (30 mL). A precipitate began to appear during this addition. After stirring for 15 min, *tert*-butyl alcohol (30 mL) was added cautiously. A homogeneous solution resulted after 2−3 min, at which point stirring was continued for an additional 5 min. The reaction was quenched by addition of 0.1 M K_2CO_3 (200 mL), and the organic layer was separated, washed with further 0.1 M K_2CO_3 (2 × 150 mL), and dried over $MgSO_4$. The crude material was purified by flash column chromatography on silica gel, eluted with 60−90−100% DCM−hexane, giving **2c** as an off-white foam (1.07 g, 22%). *m/z* (EI^-), 335 (M^-); HRMS, found 335.1396 ($C_{20}H_{20}N_2O_3$, M^-, requires 335.1396).

General Procedure for the Synthesis of 5-Amidooxazoles (NaH procedure). To a suspension of sodium hydride (60% dispersion in mineral oil, 0.65 mmol) in dry THF (3.0 mL) was added a solution of **2c** (0.59 mmol) in dry THF (4.0 mL). Then after 5 min, the reaction mixture was clear and a solution of acid chloride (0.65 mmol) was added. The reaction was deemed complete by TLC after 10 min. The reaction was quenched by the cautious addition of water followed by the removal of all volatiles under reduced pressure. The aqueous mixture remaining was extracted thoroughly with DCM. The combined organic phases were combined, dried over $MgSO_4$, and concentrated to a volume of 50 mL under reduced pressure. TFA was added to a concentration of 20% and the mixture stirred at ambient temperature for 18 h. Removal of all volatiles under reduced pressure gave the crude product, which was recrystallized from hot n-hexane−ethyl acetate.

***N*-(2,4-Diphenyloxazol-5-yl)benzamide (2d):** *m/z* (ES), 341 ($[M + H]^+$); HRMS, found 341.1289 ($C_{22}H_{17}N_2O_2$, $[M + H]^+$, requires 341.1290).

***N*-(2,4-Diphenyloxazol-5-yl)-4-fluorobenzamide (2e):** *m/z* (ES), 359 ($[M + H]^+$); HRMS, found 359.1188 ($C_{22}H_{16}N_2O_2F$, $[M + H]^+$, requires 359.1196).

***N*-(2,4-Diphenyloxazol-5-yl)-2-trifluoromethylbenzamide (2g):** *m/z* (ES), 409 ($[M + H]^+$); HRMS, found 409.1151 ($C_{23}H_{16}F_3N_2O_2$, $[M + H]^+$, requires 409.1164).

***N*-(2,4-Diphenyloxazol-5-yl)-3-trifluoromethylbenzamide (2h):** *m/z* (ES), 409 ($[M + H]^+$); HRMS, found 409.1180 ($C_{23}H_{16}F_3N_2O_2$, $[M + H]^+$, requires 409.1164).

***N*-(2,4-Diphenyloxazol-5-yl)-4-trifluoromethylbenzamide (2i):** *m/z* (ES), 409 ($[M + H]^+$); HRMS, found 409.1149 ($C_{23}H_{16}F_3N_2O_2$, $[M + H]^+$, requires 409.1164).

***N*-(2,4-Diphenyloxazol-5-yl)nicotinamide (2j):** *m/z* (ES), 342 ($[M + H]^+$); HRMS, found 342.1235 ($C_{21}H_{16}N_3O_2$, $[M + H]^+$, requires 342.1234).

Quinoline-2-carboxylic acid (2,4-diphenyloxazol-5-yl)amide (2l): *m/z* (ES), 392 ($[M + H]^+$); HRMS, found 392.1384 ($C_{25}H_{18}N_3O_2$, $[M + H]^+$, requires 392.1399).

5-Nitrofuran-2-carboxylic acid (2,4-diphenyloxazol-5-yl)amide (2n): *m/z* (ES), 376 ($[M + H]^+$); HRMS, found 376.0934 ($C_{20}H_{14}N_3O_5$, $[M + H]^+$, requires 376.0933).

Isoxazole-5-carboxylic Acid (2,4-diphenyloxazol-5-yl)amide (2o): Purification by flash column chromatography (n-hexane−ethyl acetate, 4:1) gave **2o** (58.2 mg, 23%); *m/z* (ES), 332 ($[M + H]^+$); HRMS, found 332.1023 ($C_{19}H_{14}N_3O_3$, $[M + H]^+$, requires 332.1035).

Thiophene-2-carboxylic acid (2,4-diphenyloxazol-5-yl)amide (2p): *m/z* (ES), 347 ($[M + H]^+$); HRMS, found 347.0851 ($C_{20}H_{15}N_2O_2S$, $[M + H]^+$, requires 347.0854).

Benzo[*β*]thiophene-2-carboxylic acid (2,4-diphenyloxazol-5-yl)amide (2r): *m/z* (ES), 397 ($[M + H]^+$); HRMS, found 397.1001 ($C_{24}H_{17}N_2O_2S$, $[M + H]^+$, requires 397.1011).

Cyclopropanecarboxylic acid (2,4-diphenyloxazol-5-yl)amide (2v): *m/z* (ES), 305 ($[M + H]^+$); HRMS, found 305.1278 ($C_{19}H_{17}N_2O_2$, $[M + H]^+$, requires 305.1290).

***N*-(2,4-Diphenyloxazol-5-yl)-4-methoxybenzamide (2f) (DMAP procedure).** 2,4-Diphenyl-5-*N*-Boc-aminooxazole **2c** (100 mg, 0.30 mmol) was dissolved in dry DCM (3.0 mL), and then *N*,*N*-diisopropylethylamine (58 μL, 0.33 mmol) and DMAP (∼7 mg, 20 mol %) were added followed by 4-methoxybenzoyl chloride (45 μL, 0.33 mmol). The reaction was deemed complete by TLC after 150 min of stirring at ambient temperature. TFA (0.75 mL) was then added and stirring continued at ambient temperature for 18 h. DCM (40 mL) was added and the solution washed with water (2 × 40 mL) and then dried over $MgSO_4$. Further purification was achieved by flash column chromatography on silica gel, eluted with 0−1% MeOH−DCM, to provide **2f** as an off-white foam (76 mg, 69%); *m/z* (ES), 371 ($[M + H]^+$); HRMS, found 371.1383 ($C_{23}H_{19}N_2O_3$, $[M + H]^+$, requires 371.1396).

***N*-(2,4-Diphenyloxazol-5-yl)isonicotinamide (2k):** *m/z* (ES), 342 ($[M + H]^+$); HRMS, found 342.1245 ($C_{21}H_{16}N_3O_2$, $[M + H]^+$, requires 342.1243).

Furan-2-carboxylic acid (2,4-diphenyloxazol-5-yl)amide (2m): *m/z* (ES), 331 ($[M + H]^+$); HRMS, found 331.1093 ($C_{20}H_{15}N_2O_3$, $[M + H]^+$, requires 331.1083).

5-Methylthiophene-2-carboxylic acid (2,4-diphenyloxazol-5-yl)amide (2q): *m/z* (ES), 361 ($[M + H]^+$); HRMS, found 361.1007 ($C_{21}H_{17}N_2O_2S$, $[M + H]^+$, requires 361.1011).

Benzofuran-2-carboxylic acid (2,4-diphenyloxazol-5-yl)amide (2s): *m/z* (ES), 381 ($[M + H]^+$); HRMS, found 381.1250 ($C_{24}H_{17}N_2O_3$, $[M + H]^+$, requires 381.1239).

Benzothiazole-2-carboxylic acid (2,4-diphenyloxazol-5-yl)-amide (2t): *m/z* (ES), 398 ($[M + H]^+$); HRMS, found 398.0977 ($C_{23}H_{16}N_3O_2S$, $[M + H]^+$, requires 398.0963).

Benzo[*β*]thiophene-5-carboxylic acid (2,4-diphenyloxazol-5-yl)amide (2u): *m/z* (ES), 397 ($[M + H]^+$); HRMS, found 397.1029 ($C_{24}H_{17}N_2O_2S$, $[M + H]^+$, requires 397.1011).

2-Propylpentanoic acid (2,4-diphenyloxazol-5-yl)amide (2w): *m/z* (ES), 363 ($[M + H]^+$); HRMS, found 363.2066 ($C_{23}H_{27}N_2O_2$, $[M + H]^+$, requires 363.2073).

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Supporting Information Available: Spectroscopic data for all new compounds **1e**−**x**, **2c**−**w** (IR, ^{1}H NMR, ^{13}C NMR), and analysis of compound purity by HPLC. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Weissmann, C.; Aguzzi, A. Approaches to Therapy of Prion Diseases. *Annu. Rev. Med.* **2005**, *56*, 321−344.

(2) Prusiner, S. B. Prions. *Proc. Nat. Acad. Sci. U.S.A.* **1998**, *95*, 13363−13383.

(3) Will, R. G.; Ironside, J. W.; Zeidler, M.; Cousens, S. N.; Estibeiro, K.; Alperovitch, A.; Poser, S.; Pocchiari, M.; Hofman, A.; Smith, P. G. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* **1996**, *347*, 921−925.

(4) Collinge, J.; Sidle, K. C. L.; Meads, J.; Ironside, J.; Hill, A. F. Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. *Nature* **1996**, *383*, 685−690.

(5) Anderson, R. M.; Donnelly, C. A.; Ferguson, N. M.; Woolhouse, M. E. J.; Watt, C. J.; Udy, H. J.; MaWhinney, S.; Dunstan, S. P.; Southwood, T. R. E.; Wilesmith, J. W.; Ryan, J. B. M.; Hoinville, L. J.; Hillerton, J. E.; Austin, A. R.; Wells, G. A. H. Transmission dynamics and epidemiology of BSE in British cattle. *Nature* **1996**, *382*, 779−788.

(6) Brown, P.; Preece, M.; Brandel, J. P.; Sato, T.; McShane, L.; Zerr, I.; Fletcher, A.; Will, R. G.; Pocchiari, M.; Cashman, N. R.; d'Aignaux, J. H.; Cervenakova, L.; Fradkin, J.; Schonberger, L. B.; Collins, S. J. Iatrogenic Creutzfeldt-Jakob disease at the millennium. *Neurology* **2000**, *55*, 1075−1081.

(7) Mpamhanga, C.; Chen, B.; Mutter, R.; Willett, P.; McClay, I. Virtual high-throughput screening for the discovery of potential prion disease therapeutics. Manuscript in preparation.

(8) Touil, F.; Pratt, S.; Mutter, R.; Chen, B. Screening a library of potential prion disease therapeutics against cellular prion proteins and studying their mode of biological activities by surface plasmon resonance. *J. Pharm. Biomed. Anal.* **2006**, *40*, 822−832.

(9) Hoshino, K.; Ishida, H.; Omovskaya, O.; Dudley, M.; Rleger, R.; Watkins, W. J.; Zhang, J. Z.; Renau, T. E.; Lee, V. J.; Ota, T.; Nakayama, K.; Ishida, Y.; Otsuka, M.; Kawato, H., Patent JP 2002322054, 2002.

(10) Shimada, Y., Patent JP 02113070, 1990.

(11) Corrao, S. L.; Macielag, M. J.; Turchi, I. J. Rearrangement of 4-(Aminothiocarbonyl)oxazoles to 5-Aminothiazoles. Synthetic and MINDO/3 MO Studies. *J. Org. Chem.* **1990**, *55*, 4484−4487.

(12) Burger, K.; Hoss, E.; Geith, K. Introduction of Amino-Groups Adjacent to Trifluoromethyl Groups in Heterocyclic-Compounds − Synthesis of 5-Amino-4-trifluoromethyl-thiazoles. *Synthesis* **1990**, 357−360.

(13) Golankiewicz, B.; Januszczyk, P.; Gdaniec, M.; Kosturkiewicz, Z. Reaction of Acylaminocyanoesters with 2,4-Bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-Disulfide Leading to Substituted Aminothiazoles. Crystal Structure of 5-Aminothiazole-4-carboxylic Acid Ethyl Ester. *Tetrahedron* **1985**, *41*, 5989−5994.

(14) Thompson, M. J.; Heal, W.; Chen, B. Synthesis of 5-aminothiazoles as building blocks for library synthesis. *Tetrahedron Lett.* **2006**, *47*, 2361−2364.

(15) (a) Lichtenberger, J.; Fleury, J-P. Formation, constitution, and properties of some derivatives of 2,4-disubstituted 5-aminooxazoles. II. 2-Aryl(alkyl)-4-phenyl-5-arylideneaminooxazoles. *Bull. Soc. Chim. Fr.* **1956**, 1184−1192; (b) Kille, G.; Fleury, J-P. Heterocyclisation of α-acylaminonitriles. II. Structure of 5-aminooxazoles and of their salts. *Bull. Soc. Chim. Fr.* **1967**, 4619−4624.

(16) Verschave, P.; Vekemans, J.; Hoornaert, G. *N*-Acylated Alpha-Aminonitriles and their Conversion into 5-Aminooxazole, 5(4*H*)-Oxazolone and 4(5*H*)-Imidazolone Derivatives. *Tetrahedron* **1984**, *40*, 2395−2404.

(17) Rudyk, H.; Vasiljevic, S.; Hennion, R. M.; Birkett, C. R.; Hope, J.; Gilbert, I. H. Screening Congo Red and its analogues for their ability to prevent the formation of PrP-res in scrapie-infected cells. *J. Gen. Virol.* **2000**, *81*, 1155−1164.

(18) Clarke, M. C.; Haig, D. A. Evidence for the multiplication of scrapie agent in cell culture. *Nature* **1970**, *225*, 100−101.

(19) Calabrese, E. J.; Baldwin, L. A. Hormesis: The dose-response revolution. *Annu. Rev. Pharmacol. Toxicol.* **2003**, *43*, 175−197.

(20) Guo, K.; Mutter, R.; Heal, W.; Reddy, T. R. K.; Pratt, S.; Cope, H.; Thompson, M. J.; Chen, B. Synthesis and evaluation of a focused library of pyridine dicarbonitriles against prion disease. *Eur. J. Med. Chem.*, submitted.

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