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Improved 2,4-Diarylthiazole-Based Antiprion Agents: Switching the Sense of the Amide Group at C5 Leads to an Increase in Potency

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Amide derivatives of 2,4-diarylthiazole-5-carboxylic acids were synthesised and tested for efficacy in a cell line model of prion disease. A number of compounds demonstrating antiprion activity were thereby identified from the screening libraries, showing improved potency and reproducibility of results relative to amide derivatives of the related 2,4-diphenyl-5-aminothiazole, which have been documented previously. Thus, 'switching' the sense of the amide bond at thiazole C5 revealed a more promising lead series of potential prion disease therapeutics. Furthermore, 3,5-diaryl-1,2,4-thiadiazoles isolated as by-products during library synthesis provided a handful of additional examples possessing an antiprion effect, thereby augmenting the set of newly identified active compounds. Evaluation of binding to cellular prion protein (PrP^C) showed only weak affinities at best, suggesting that the newly identified antiprion agents do not mediate their biological effect through direct interaction with PrP^C.

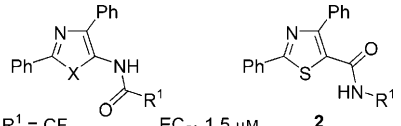
Introduction

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are a group of invariably fatal disorders that afflict both humans and animals for which no effective therapy presently exists. Although rare, their inevitable lethality renders the discovery of suitable treatments a pressing clinical need.

The principal such human condition is Creutzfeldt–Jakob disease (CJD), which accounts for a mortality rate of approximately one person per million per year across all populations studied to date.[1] The disease presents in sporadic, inherited, and iatrogenic forms, and whereas the aetiology of sporadic CJD remains uncertain, the familial form is associated with specific pathogenic mutations in *prnp*, the gene that encodes normal cellular prion protein (PrP^C).[2] Similarly, the less prevalent human prion diseases, Gerstmann–Sträussler–Scheinker syndrome (GSS) and familial fatal insomnia (FFI), are associated with other defined *prnp* mutations.[3]

Examples of prion disease in animals include scrapie in sheep and goats, chronic wasting disease (CWD) in cervids, and bovine spongiform encephalopathy (BSE) in cattle. The latter of these is thought to have given rise to the emergence of variant CJD (vCJD)[4] in humans as a result of consumption of contaminated meat products, and consequently triggered much research into the mechanisms of, and potential treatments for, human prion diseases.

The central molecular event common to all TSEs is conversion of native PrP^C into a refolded, pathogenic isoform denoted PrP^{Sc}. Thus, stably infected cell lines acting as a host for PrP^{Sc} are routinely employed for in vitro screening of potential antiprion agents.[5] We previously reported the use of a scrapie mouse brain (SMB) cell line,[6,7] cloned from murine brain infected with the Chandler scrapie strain, in the identification of a number of active inhibitors of PrP^{Sc} accumulation across four distinct structural classes: 9-aminoacridines and related compounds,[8] pyridine-3,5-dicarbonitriles,[9] indole-3-glyoxylamides,[10] and 2,4-diphenyl- thiazoles and oxazoles **1** (Figure 1).[11] Within the latter class, six moderately active compounds were identified, with EC_{50} values ranging from 1.5–20 μM.



1a X = O, R^1 = CF_3 EC_{50} 1.5 μM
1b X = S, R^1 = CF_3 EC_{50} 1.5 μM
1c X = S, R^1 = *p*-MeO-C_6H_4 EC_{50} 20 μM
1d X = O, R^1 = *o*-CF_3-C_6H_4 EC_{50} 8 μM

2

Figure 1. General structure of existing 2,4-diphenylazole-based antiprion agents and their "reverse amide" analogues.

Owing to poor reproducibility of these compounds' antiprion effect across differing passage numbers, and also to clear cytotoxicity of trifluoroacetyl compounds **1a** and **1b** close to their active concentrations (EC_{50} ~ 1.5 μM; LD_{50} ~ 5 μM),[11] analogues with improved potency and therapeutic window were sought. Considering possible modifications to **1** that might

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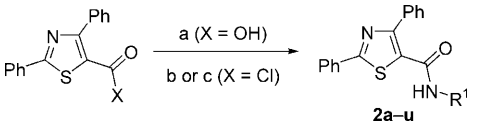
achieve these aims, we devised a library of 'reverse amide' thiazole analogues **2** (Figure 1), and set out to explore the influence of this structural change upon antiprion activity.

Results and Discussion

Synthesis and screening

A range of 2,4-diphenylthiazole-5-carboxamides **2a**–**u** was prepared via straightforward amide coupling reactions from either the requisite carboxylic acid or acyl chloride (Scheme 1). These



Scheme 1. Preparation of thiazole-5-carboxamide library members. Reagents and conditions: a) R^1NH_2, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, *i*Pr_2NEt, $CHCl_3$, RT, 3 h; b) R^1NH_2, *i*Pr_2NEt, CH_2Cl_2, RT, 3 h; c) R^1NH_2 (6 equiv), CH_2Cl_2, RT, 2 h.

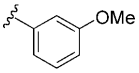
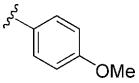
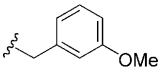
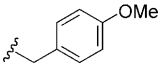
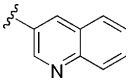
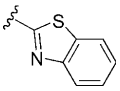
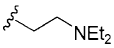
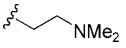
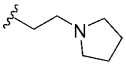
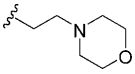
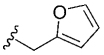
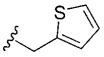
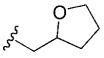
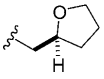
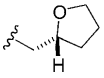
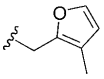
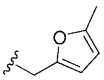
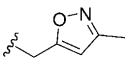
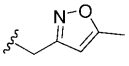
compounds were screened for antiprion activity in the SMB cell line as described,[10] and LD_{50} determination was carried out where cytotoxicity was observed during the initial screen (Table 1).

Two active library members were initially identified, including 2-(diethylamino)ethyl derivative **2i**, with an EC_{50} value of 4.9 μM (Figure 2a), which inspired the synthesis of analogues **2j**–**2l**. As with the parent compound, dimethylamino derivative **2j** showed borderline toxicity at higher concentrations (>10 μM; Figure 2b), but similar potency with respect to PrP^{Sc} clearance. Pyrrolidino analogue **2k** was marginal in terms of activity (EC_{50} = 10.7 μM) and still showed some cytotoxicity, whilst the final compound in the series—morpholino analogue **2l**—displayed no discernible antiprion activity. Therefore, in this 2-(dialkylamino)ethyl set of compounds **2i**–**2l**, smaller substituents on nitrogen are evidently more favourable for activity.

Although an antiprion effect is evident in the case of **2i**–**2k**, ambiguous results were obtained for cell viability (Figure 2a–c). Whereas clear-cut toxicity was not observed at higher concentrations—as was observed for compounds **1a** and **1b**[11]—some decrease in cell number was apparent, and a distinct change in morphology of the remaining cells was evident (Figure 2d–h). Cells exposed to **2j** or **2k** at 1 μM showed no difference in appearance from the untreated control, but at a test compound concentration of 30 μM, a definite morphological change became clear, with apparently increased cell adhesion in these wells. The observed changes were noticeable gradually with increasing compound concentration, becoming evident at ~7.5 μM for **2j** or **2k** (data not shown). Compound **2i** did show some such effect, but not as consistently, and proved less toxic than its related analogues **2j** and **2k** (Figure 2a–c); thus, of the 2-aminoalkyl series, **2i** was selected for further optimisation.

In terms of potency, however, the most promising lead from the initial series of amides was furfurylamine-derived compound **2m**, by far the most active antiprion agent of the 2,4-diphenylthiazole class identified to date (EC_{50} = 0.43 μM). Through preparation and screening of a range of closely relat-

Table 1. Antiprion screening and cytotoxicity results for compounds **2a**–**u**.

Compd	R^1	Yield [%] (Method)	EC_{50} [μM]	LD_{50} [μM]
2a		25 (B)	–[a]	>20
2b		24 (B)	–	>20
2c		80 (B)	–	>20
2d		74 (B)	–	>20
2e		64 (B)	–	>20
2f		84 (B)	–	>20
2g		46 (B)	–	>20
2h		65 (B)	–	>20
2i		59 (B)	4.9 ± 1.1	ambiguous[b]
2j		59 (C)	4.8 ± 1.4	ambiguous[b]
2k		43 (C)	10.7 ± 0.5	ambiguous[b]
2l		88 (B)	–	>15
2m		79 (B)	0.43 ± 0.19	>20
2n		53 (A)	–	>20
2o		63 (A)	–	>20
2p		78 (A)	–	>20
2q		77 (A)	–	>20
2r		57 (B)	–	>20
2s		42 (A)	–	>20
2t		53 (A)	–	>20
2u		35 (B)	–	>20

[a] No antiprion effect observed up to 20 μM. [b] Compounds **2i**–**2k** showed borderline toxicity at higher concentrations (cell viability ~50–70%), but conclusive LD_{50} curves could not be obtained.

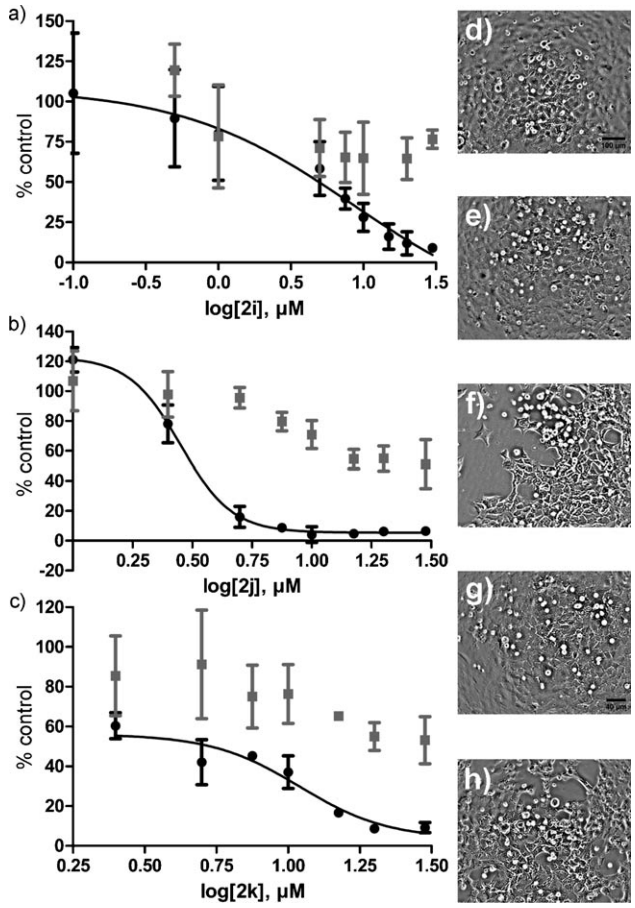
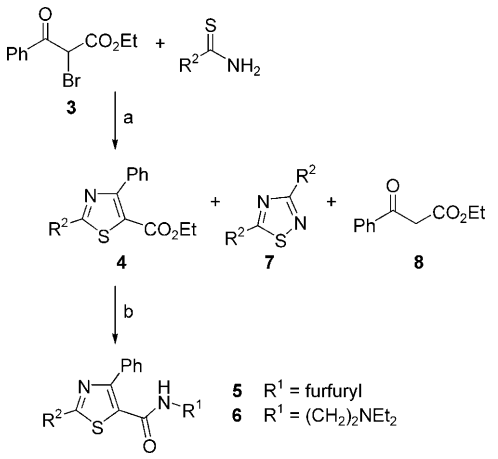


Figure 2. Typical results for assessment of antiprion activity (black line) versus cell viability (square symbols, as determined by MTT assay) for test compounds a) **2i**, b) **2j**, and c) **2k**. Cell images illustrate the changes in morphology observed at higher concentrations of **2j** and **2k**: d) untreated control, e) **2j** at 1 μM, f) **2j** at 30 μM, g) **2k** at 1 μM, and h) **2k** at 30 μM. Scale bar in image d) represents 100 μm; all panels are to the same scale, with the exception of image g), in which the scale bar represents 40 μm. Images were captured after exposure to the test compound for five days.

ed analogues of this structure, **2n**–**2u**, it became disappointingly clear that variation of the furfuryl group is not tolerated, as activity was abolished in all of the associated compounds.

Variation at the 2-position of the thiazole ring was then explored, whilst maintaining the best substituents at C5 identified above, in an attempt to improve activity. Hantzsch thiazole synthesis[12] from ethyl 2-benzoyl-2-bromoacetate **3**[13] and a range of thioamides provided 4-phenylthiazole-5-carboxylic esters **4a**–**c**, which, following aminolysis mediated by 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD),[14] afforded a range of new test compounds, **5a**–**c** and **6a**–**c** (Scheme 2), analogous to the existing lead compounds **2i** and **2m**.

During the Hantzsch thiazole step, two other products were unexpectedly detected in varying quantities, in addition to the desired thiazole-5-carboxylic esters **4**. Most intriguing was the isolation of 1,2,4-thiadiazoles **7** in significant amounts (up to 41% yield). Formation of these species via dimerisation of thioamides is well established,[15] but normally requires the use of an oxidising agent; thus, in line with expectations, no reaction was detected upon holding a thioamide component alone in



Scheme 2. Preparation of analogues incorporating variations at the 2-position of the thiazole ring. Reagents and conditions: a) EtOH, reflux, 90 min; b) R^1NH_2, TBD (30 mol%), 80 °C, 18 h.

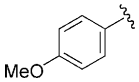
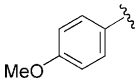
ethanol at reflux. Identification of ethyl benzoylacetate **8** as an additional side-product during thiazole synthesis offered a plausible explanation: the α-bromoketone **3** functions as an oxidant to promote dimerisation of the thioamide (in turn being reduced to **8**), in addition to acting as an electrophile en route to desired product **4**.

Only one previous report exists of 1,2,4-thiadiazole formation during the Hantzsch thiazole synthesis,[16] but interestingly, a relatively hindered α-bromoketone (ethyl 2-bromo-2-(*o*-nitrobenzoyl)acetate) was also employed in this case. As such, we propose that the extent of formation of 1,2,4-thiadiazoles **7** during the Hantzsch reaction may generally be significant where sterically demanding α-bromoketones are employed. With such electrophiles of lower reactivity, the extent to which they act as oxidising agents in a competing process to form **7** appears to become important, though as is evident from the variable product distribution in the present examples, the nature of the thioamide also plays a role.

Screening of the new thiazole sets **5** and **6** (Table 2) again revealed that any modification to **2m** is not tolerated, as activity was lost in all of its analogues **5a**–**c**. Variation to (diethylamino)ethyl lead **2i** gave more productive results, with *p*-methoxy-substituted analogue **6b** displaying similar activity (EC_{50} = 4.0 μM, versus 4.9 μM for its parent structure), but lower toxicity towards the SMB cells (Figure 3a), that is, with no induction of the morphological changes observed on exposure to **2i**–**2k**. Replacing the phenyl ring at C2 by a heterocycle, as in **6a** or **6c**, compromised activity to a significant extent. Considering the full set of screening results thus far, it would appear that analogues of **2i** or **2j** bearing substituted phenyl rings at thiazole C2 (that is, the R^2 position of **6**) offer a promising avenue of exploration towards finding new compounds that possess both improved antiprion activity and decreased cytotoxicity.

Synthetic intermediates **4a**–**c** and 1,2,4-thiadiazole by-products **7a**–**c** were also screened for any antiprion effect, revealing **7b** (R^2 = *p*-methoxyphenyl) as a novel lead structure with an EC_{50} value of 1.62 μM (Figure 3b; Table 3). In a similar trend

Table 2. Antiprion screening and cytotoxicity results for compounds **4a–f** and **5a-f**.

Compd	R^2	Yield [%][a]	EC_{50} [μM]	LD_{50} [μM]
5a		38	–[b]	>20
5b		12	–	>20
5c		14	–	>20
6a		37	~20	>30
6b		18	4.0±2.0	>10[c]
6c		17	6.7±0.4	~30

[a] Overall yield for the two-step procedure presented in Scheme 2. [b] No antiprion effect observed up to 20 μM. [c] Partial decrease in cell viability at higher concentrations, but not to the same extent observed for **2i–2k**.

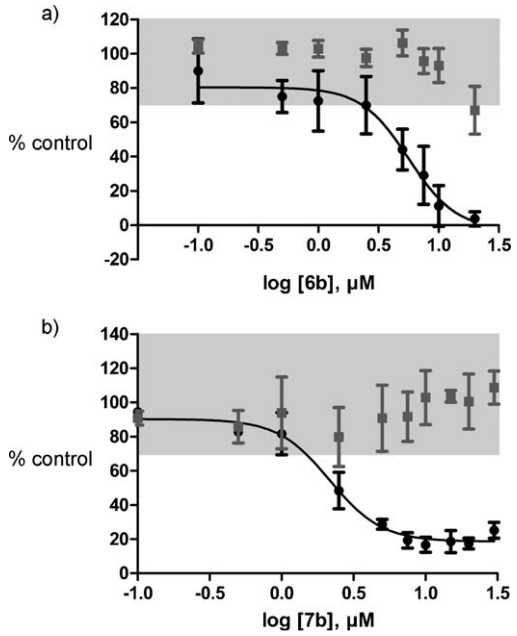
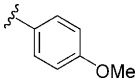
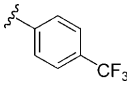
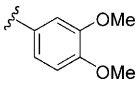
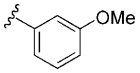
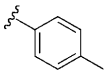


Figure 3. Dose–response curves for a) compound **6b** and b) compound **7b**, showing clear antiprion activity (solid line shows PrP^{Sc} concentration relative to untreated control), but lower toxicity than compounds **2i–k**. Points denoted by square symbols show cell viability, with the shaded area representing "nontoxic" (viability >70% of untreated control). The data above are fused from multiple experiments; results for each individual experiment are presented in the Supporting Information.

to the thiazole series **6a–c**, analogues **7a** and **7c** bearing heterocyclic substituents proved inactive, suggesting that a series of 1,2,4-thiadiazoles **7** incorporating substituted phenyl groups at the R^2 positions would likewise be the best candidates to

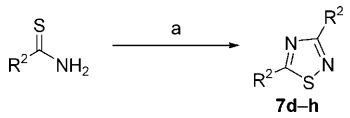
Table 3. Results for additional 1,2,4-thiadiazole compounds **7d–h**.

Compd	R^2	Yield [%][a]	EC_{50} [μM]	LD_{50} [μM]
7a		25[b]	–[e]	>20
7b		12[b]	1.62±0.77	>20
7c		39[b]	–	>20
7d		9[c]	–	>20
7e		63	2.94±0.02	>20
7f		71	n/a[f]	0.67
7g		78	borderline[g]	>20
7h		8[d]	–	>20

[a] Yield for dimerisation of thioamide R^2CSNH_2 after column chromatography; except: [b] Isolated during synthesis of **4a–c**. [c] Synthesised in the absence of $Et_4N^+Br^-$, resulting in poor conversion. [d] Low yield is seemingly due to retention/decomposition of this compound on the silica column during chromatography. [e] No antiprion effect observed up to 20 μM. [f] Compound is too toxic to the cells to assess activity. [g] Borderline antiprion activity observed towards the higher end of the concentration range tested (PrP^{Sc} ~70% of control at 20 μM), but not sufficiently potent to be considered a positive result.

offer insight into any potential structure–activity relationships (SAR).

This premise was tested by preparing a small additional set of 1,2,4-thiadiazoles **7d–h** by oxidative dimerisation of appropriate thioamides (Scheme 3). Although the combination of *o*-



Scheme 3. Extra 1,2,4-thiadiazole derivatives prepared by thioamide dimerisation. Reagents and conditions: a) $PhI(OAc)_2$, $Et_4N^+Br^-$, CH_2Cl_2, reflux, 2 h (8–78%).

iodoxybenzoic acid (IBX) and tetraethylammonium bromide has recently been described as an efficient method for this process,[15] the considerable expense of IBX led us to substitute an alternative hypervalent iodine reagent in its place; iodobenzene diacetate (IBDA) gave satisfactory results, and all reactions were complete within 2 h at reflux in dichloromethane. IBDA has been reported as an effective promoter of thioamide dimerisation before, albeit using a polymer-supported reagent[17] or reaction in an ionic liquid[18] rather than a conventional organic solvent.

Upon screening of the new thiadiazoles, it was pleasing to discover that two compounds possessed notable antiprion activity (Table 3), although toxicity towards the SMB cells was observed in one case (*m*-methoxy analogue **7f**). Whereas *p*-tolyl derivative **7g** only displayed a very weak effect, the 3,4-dimethoxyphenyl analogue **7e** was more potent, demonstrating an EC_{50} value close to that of **7b**. Coupled with the ineffectiveness of *p*-trifluoromethyl compound **7d**, these results suggest that 3,5-diaryl-1,2,4-thiadiazoles bearing electron-donating groups on the phenyl rings may constitute a worthwhile course of future study, to refine the antiprion properties of this family of compounds.

The active leads identified in the present work—all being structures of type **2**, **6**, and **7**—each incorporate a central five-membered heterocycle bearing two aromatic substituents arranged in a 1,3-sense. Interestingly, other recent studies have reported antiprion agents that contain very similar substructural motifs, namely, 1,3- or 1,4-diphenyl-1*H*-pyrazol-5(4*H*)-one derivatives[19] and 2-(*N*-pyridin-2-ylamino)-4-arylthiazoles.[20] In addition, a series of pyridine-3,5-dicarbonitrile antiprion compounds bearing (2-aminoalkyl)thio groups[21] displayed marked similarities to present analogues **2i**–**2l** in terms of the relationship between the nature of the amine side chain and antiprion activity. Whereas no direct conclusions should be drawn immediately, the possibility of a shared mechanism of action between the related compound classes detailed above is perhaps worthy of further scrutiny.

Further characterisation of active leads

The 2,4-diarylthiazole-based compounds were originally identified as potential ligands for normal cellular prion protein (PrP^C) in a virtual screening programme, with the earlier effective antiprion examples **1a**–**d** all being found to bind to PrP^C to some extent.[11] Thus, the cell-line-active compounds described above (and some of their closest structural analogues) were also assessed for any interaction with PrP^C, using the same surface plasmon resonance (SPR)-based assay employed previously.[22]

Briefly, a solution of each test compound is passed over the protein of interest immobilised on a sensor chip surface. The SPR response, measured in the instrument's response units (RU), increases in proportion to any ligand binding that takes place. Results are expressed in a normalised form—percent RU_{max}—that is, as a percentage relative to the calculated value of SPR response corresponding to formation of a 1:1 protein–ligand complex. Compounds of interest are further characterised by screening over a range of concentrations to assess the dose dependence of the observed binding. Interaction with both human (hu-PrP^C) and murine (mo-PrP^C) forms of the protein was measured.

A number of members of the present test set showed weak binding to both forms of the protein (full results are included in table S2 in the Supporting Information), with three compounds displaying a significant interaction (**5b**, **6a**, and **6b**; Figure 4). The recorded response for **5b** can be seen to approach a limiting value as concentration increases (Figure 4a),

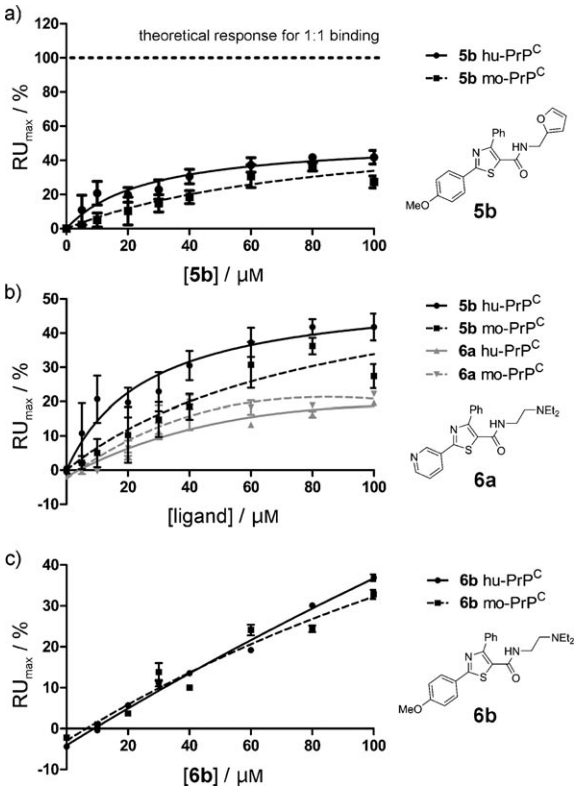


Figure 4. Interaction of compounds **5b**, **6a**, and **6b** with immobilised human (hu-PrP^C) and murine (mo-PrP^C) forms of recombinant prion protein, over a range of concentrations, as measured by surface plasmon resonance (SPR). a) Binding of **5b** approaches a limiting value, suggesting formation of a specific 1:1 ligand–protein complex, although the measured SPR response (RU) falls short of the calculated value, which would correspond to an equimolar ratio of ligand and immobilised protein (RU_{max}). b) Compounds **5b** and **6a** each show evidence for specific binding to both forms of PrP^C. c) **6b** shows similar affinity for the proteins, but appears to interact with them in a nonspecific manner, that is, no approach towards a limiting response is observed in this case.

giving a curve characteristic of 1:1 complex formation. The response at saturation is considerably lower than expected, however, at only 40% RU_{max}. If this were a genuine binding event, it is plausible that the ordering of the protein on the chip surface is such that the ligand binding site is not accessible for all of the immobilised PrP^C, which would explain this apparent discrepancy. Variation of binding response over time has already been noted during this assay,[22] and dynamic reordering of the protein bound to the chip surface could certainly explain these changes in observed binding over the lifetime of the chip.

Considering compound **5b**, fitting to a saturation binding model gave apparent affinities (K_D) of 27 and 87 μM against hu-PrP^C and mo-PrP^C, respectively. Possible specific binding was also observed for **6a** (Figure 4b), although the interaction seems considerably weaker, such that K_D values could not be computed. Lastly, a linear dose–response relationship was observed during assessment of **6b** (Figure 4c), suggesting nonspecific interaction with both forms of PrP^C.

Of the three ligands identified by SPR, however, only **6b** displayed any significant activity during cell line screening. Also

considering the weak binding affinities observed, it appears likely that the antiprion effect of all cell-line-active compounds identified above (**2i**–**2k**, **2m**, **6a**–**c**, and **7b**) is not mediated through interaction with PrP^C. To reach a more firm conclusion, binding of the three most potent leads (**2m**, **6b**, and **7b**) to the human form (hu-PrP^C) was further investigated by measurement of fluorescence quenching, an approach that has been applied successfully to quantify binding of Cu^{2+} to the prion protein.[23]

Compound **6b** did indeed show interaction with hu-PrP^C by this method (observed $K_D = 3.8 \pm 0.8$ μM; Figure 5a), suggesting stronger binding of this ligand than was evident by SPR.

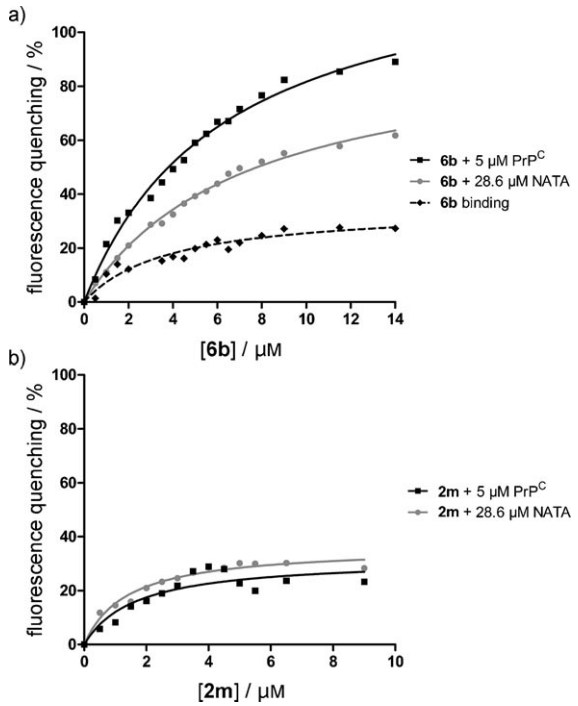


Figure 5. Assessment of binding of **6b** and **2m** to PrP^C by the fluorescence quenching technique. Quenching of fluorescence of *N*-acetyl-L-tryptophanamide (NATA), at a concentration equivalent to the tryptophan residues in the protein,[22a] was also measured to correct for collisional effects. It can be seen that a) **6b** shows some affinity for the protein, as was observed by SPR, and b) **2m** does not bind to PrP^C, again reflecting the results obtained by the alternative assay.

The SPR results were also reflected for **2m**, with no discernible binding evident from the fluorescence quenching data (Figure 2b); results for **7b** were almost identical. Thus, there is good correlation between assessment of binding to PrP^C between the two assays, with the two most potent leads (**2m** and **7b**) emerging as clear non-binders, and **6b** apparently showing weak affinity for the protein.

The three ligands identified by SPR (**5b**, **6a**, and **6b**) were also screened for their tendency to form colloid-like aggregates in aqueous solution using the dynamic light scattering (DLS) technique. Awareness of aggregation-based effects in protein inhibition and binding assays has gained a high profile in recent years,[24] and of particular relevance in the present context, several small-molecule inhibitors of prion amyloid formation were found to operate through an aggregation mechanism.[25] Testing for aggregation behaviour is therefore an especially valuable tool in eliminating 'false positive' hits from typical screening programmes, such that computational methods for predicting such effects have recently been developed.[26] Results for the present compound set (Figure 6), including the known aggregator[24b] benzyl benzoate (BB) as a positive control, revealed **5b** is a significant aggregator over the concentration range examined in the SPR experiment. This tendency precludes reliable assessment of the compound in any direct binding assay, rendering the above SPR results (Figure 4a) inconclusive. Related structure **2m** showed less tendency to aggregate, apparently forming small particles (158 ± 29 nm diameter) at 10 μM, although it could not be assessed at higher concentrations due to insolubility, which had not been encountered for the *p*-methoxy-substituted analogue **5b**. In contrast to the preceding *N*-furfurylamides, 2-(diethylamino)ethyl-containing compounds **6a** and **6b** both showed very low aggregation tendency, together with full aqueous solubility up to 200 μM, as might be expected given the presence of this more hydrophilic side chain. The interaction of **6b** with PrP^C observed in both the SPR and fluorescence quenching assays therefore appears to reflect genuine binding to the protein, as defined particles were only detectable by DLS at 200 μM; similarly, analogue **6a** also appears to function as a ligand based on the SPR results (Figure 4b), albeit with noticeably weaker affinity.

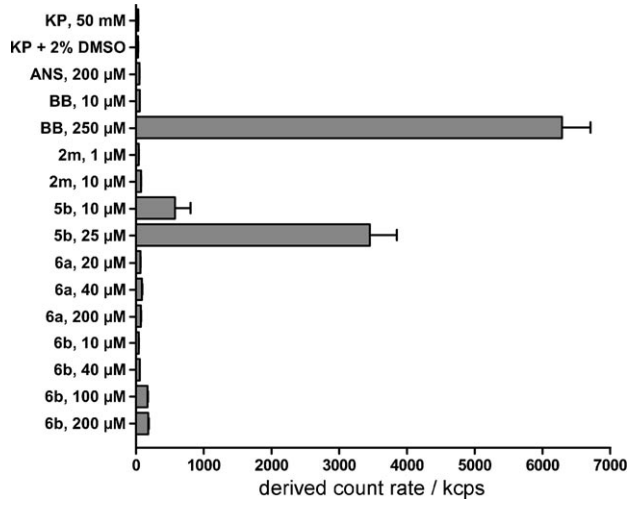


Figure 6. Assessment of the aggregation tendency of screening compounds in aqueous solution. A high count rate indicates the presence of light-scattering particles in solution. Compound **5b** shows clear evidence for formation of aggregates under these conditions. KP: potassium phosphate buffer (pH 7.4); ANS: 8-anilino-1-naphthalenesulfonic acid, a known non-aggregator;[23e] BB: benzyl benzoate.

When considering the suitability of a compound as a potential drug, it is advantageous to measure stability towards microsomal metabolism. In this way, compounds that are likely to be metabolically labile may be excluded or modified at an early stage of the discovery process. The cell-line-active antiprion agents in the present study were assessed by incubation

with mouse liver microsome (MLM) preparation, with mixed results (Table 4). The most potent compound, 2,4-diphenylthiazole derivative **2m**, showed significant depletion after 30 min; 1,2,4-thiadiazole series **7** fared better, though both analogues

Table 4. Microsomal stability assessment of cell-line-active compounds.

Compd	EC_{50} [μM]	MLM Stability [%][a]
2m	0.43 ± 0.19	55.5 ± 0.8
6b	4.0 ± 2.0	–[b]
7b	1.62 ± 0.77	76.0 ± 1.3
7e	2.94 ± 0.02	90.4 ± 1.6
testosterone[c]	n.d.	53.1 ± 0.1
diclofenac[c]	n.d.	81.9 ± 1.4

[a] Percentage of compound remaining after 30 min incubation with mouse liver microsome (MLM) preparation. [b] No result could be obtained for compound **6b** and related structures containing the 2-(diethylamino)ethyl group due to unusual interaction with the C_{18} HPLC column; these difficulties are addressed in greater detail in the main text discussion. [c] Testosterone[27] and diclofenac[28] were used as positive controls, as both compounds are known to be metabolised by cytochromes present in microsomal preparations; n.d.: not determined.

were metabolised to some degree. The challenge for future work in this area will therefore be to develop compounds that retain or improve cell line activity, whilst proving more robust towards likely first-pass clearance.

Regrettably, data could not be obtained for the most active member of the *N*,*N*-(dialkylamino)ethyl-containing thiazoles, **6b**, and related analogues **2i**–**k**, **6a**, and **6c**. For each of these compounds, unsuitable or erratic behaviour was observed under the HPLC conditions used for the assay, precluding accurate determination of any extent of metabolism.[29]

Conclusions

A more coherent lead series of 2,4-diarylthiazole-based antiprion agents has been identified by "switching" the sense of the amide bond at C5. Thiazole-5-carboxamides such as those of type **2** and **6** showed more potent and reproducible cell line activity than related amide structures **1** (X = S; Figure 1), derived from 5-amino-2,4-diphenylthiazole. Of the present screening set, compounds **2m** and **6b** offer the best promise for further development as potential prion disease therapeutics.

Binding of the biologically active library members to the prion protein was largely found to be weak or negligible, which, with the possible exception of **6b** (K_D = 3.8 μM, EC_{50} = 4.0 μM), suggests that the antiprion activity of the present compounds is not effected through interaction with PrP^C.

Screening of a small number of 3,5-diaryl-1,2,4-thiadiazoles, initially isolated as by-products during preparation of the thiazole libraries, revealed two members of this series (**7b** and **7e**) as novel inhibitors of PrP^{Sc} accumulation. Preliminary aspects of a SAR with respect to antiprion activity have been deduced for both the thiazole and thiadiazole series of compounds, particularly suggesting the importance of electron-donating groups attached to the aromatic substituents.

Experimental Section

Materials and general methods: 2,4-Diphenylthiazole-5-carboxylic acid and 2,4-diphenylthiazole-5-carbonyl chloride were purchased from Maybridge (UK) and ABCR (Germany), respectively. Anhydrous CH_2Cl_2 was obtained from an in-house 'Grubbs' apparatus. 'Petroleum ether' refers to light petroleum, bp: 40–60 °C. Reagents for SPR screening: *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N*′-(3-diethylaminopropyl)carbodiimide hydrochloride (EDC), 1 M ethanolamine, HBS-EP buffer, surfactant P20, regeneration solution (10 mM glycine·HCl, pH 3.0) and CM-dextran (M_r = 13 kDa), were all purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Recombinant full-length human prion protein (hu-PrP^C) and full-length mouse prion protein (mo-PrP^C) were generous gifts from Dr. Andrew Gill (Roslin Institute, University of Edinburgh, UK). All other reagents and solvents were obtained from appropriate commercial sources and used as supplied. Melting points were determined using a Gallenkamp melting point apparatus in capillary tubes, and are uncorrected. ^1H and ^{13}C NMR spectra were recorded at 400 and 100 Hz, respectively, on a Bruker AV-1400 spectrometer, or at 250 and 62.8 MHz, respectively, on a Bruker AV-1250 model. Mass spectra were acquired using a Micromass LCT Premier XE system. Infrared spectra were recorded on a PerkinElmer Spectrum RX instrument fitted with a SensIR Technologies Durasampl*IR*™ II device. HPLC analysis of compound purity was carried out using a Genesis 4 μm C_{18} column, 4.6 × 150 mm, eluted with a gradient of MeCN/H_2O (30 → 100% over 12 min; hold at 100% MeCN for 10 min), flow rate: 1 mL min^{-1}, with UV detection at λ 254 nm. Optical rotation values were measured with an Optical Activity Ltd AA-10 polarimeter. Fluorescence spectra were recorded at the steady state using a PerkinElmer LS 50B Spectrometer, and test samples contained within quartz cuvettes. Dynamic light scattering (DLS) measurements were carried out using a Malvern Instruments Zetasizer Nano ZS system, equipped with a 4 mW He–Ne laser at λ 633 nm.

Assessment of antiprion activity in SMB cells: Compounds were screened for inhibition of PrP^{Sc} formation in the SMB.s15 cell line[7] according to the protocol described previously.[9,10] 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. The medium was changed every third or fourth day, and every seven 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 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 prepared at 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 five days. After this time, 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; 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 ~30–40 μg per 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 37 °C. The reaction was stopped with 1 mM phenylmethylsulfonyl fluoride (PMSF) in

20 mM Tris·HCl-buffered saline (TBS) and the membrane was 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). Every experiment was carried out in triplicate, and an average value for PrP^{Sc} concentration was calculated, relative to an untreated control (DMSO only), together with a standard deviation. Curcumin was employed as a positive control, and effected complete clearance of PrP^{Sc} at the concentration used (10 μM; EC_{50} = 0.95 μM[10]). Test compounds were initially screened at 1, 10, and 20 μM and were considered to be active if PrP^{Sc} levels were decreased to < 70% of that of the untreated control after five days' exposure. Compounds showing activity were re-screened over a range of concentrations to determine an EC_{50} value; such experiments were repeated at least twice (in triplicate) to validate the results so obtained. LD_{50} values were assessed from a plot of cell viability (determined by the MTT assay), after five days' exposure, against test compound concentration.

Assessment of binding to PrP^C by SPR: The method used was essentially as reported previously.[22] Experiments were carried out using a Biacore 3000 instrument equipped with a CM5 sensor chip, containing a CM-dextran surface. Prior to screening, PrP^C was immobilised on the chip surface according to the following protocol. A 1:1 mixture of 100 mM NHS and 400 mM EDC was passed over the sensor chip for 7 min at a flow rate of 5 μL min^{-1}. A solution of PrP^C, at a concentration of 2 μg mL^{-1} in HBS-EP buffer, was then passed over the chip surface for 7 min. Unreacted sites were blocked by injection of 1 M ethanolamine (pH 8.5) for 7 min, after which the chip surface was prepared for use by three consecutive injections (5 μL at 30 μL min^{-1}) of 25 mM NaOH/1 M NaCl solution. Finally, the sensor chip surface was equilibrated with running buffer (10 mM Na_3PO_4, pH 7.4; 150 mM NaCl; 3.4 mM EDTA; 0.005% *v/v* surfactant P20) overnight prior to screening. The four flow cells of the Biacore instrument were employed as follows: flow cell 1 was used as a reference surface (no immobilised protein); flow cell 2 contained hu-PrP^C; flow cell 3 contained mo-PrP^C; and flow cell 4 was not used. Test compounds were dissolved in DMSO at 800 μM and diluted to the required concentration (40 μM for routine screening; variable concentrations for K_D determination) with running buffer prior to injection. Assays were performed at 25 °C with a flow rate of 30 μL min^{-1}. To correct for solvent effects, 6.5% DMSO was added to the running buffer, and DMSO calibration sequences using buffer samples containing 5.5–7.5% DMSO (in 0.5% intervals) were carried out at the start of each run, and after every 10 test compounds. Each analytical cycle consisted of running buffer for 1 min (stabilisation phase), sample injection for 1 min (association phase), and running buffer for 3 min (dissociation phase). Between cycles, surface regeneration was carried out at 35 μL min^{-1} by injecting 25 mM NaOH/1 M NaCl/0.0005% SDS pH 8.5 (for 30 s), then by 10 mM glycine·HCl pH 3.0 (for 35 s), followed by a re-equilibration phase in running buffer for 1 min. Each test compound was injected in triplicate in order to establish an average response (recorded in resonance units, RU) and a standard deviation. Binding affinities are expressed as percent RU_{max}, where RU_{max} is the theoretical response for a 1:1 binding interaction between the ligand and PrP^C, calculated according to the following Equation (1), in which $RU_{immobilised\ protein}$ is the response (in RU) observed at the end of the immobilisation procedure.

$$RU_{max} = (RU_{immobilised\ protein}/M_{r\ protein}) \times M_{r\ ligand} \quad (1)$$

Assessment of binding to PrP^C by fluorescence quenching: Recombinant human PrP^c was prepared at a protein concentration of 5 μM in 25 mM NaOAc buffer (pH 5.5) then its initial fluorescence spectrum was recorded (λ_{ex} 290 nm; λ_{em} 310–500 nm; 5 nm slit; 1200 nm min^{-1}; eight scans). Additional samples were then prepared in the same buffer, which in addition to PrP^C, contained the test compound of interest at various concentrations (for example, 17 concentrations ranging between 1–50 μM in the case of **6b**), added from a stock solution in DMSO (10 mM). After equilibration at ambient temperature for 2 min, the fluorescence spectrum of each test solution was recorded in the same manner as above. Correction for collisional quenching effects was applied by repeating the measurements with 5 μM PrP^C replaced by 28.6 μM *N*-acetyl-L-tryptophanamide, equivalent to the concentration of Trp residues in the protein,[23a] and subtracting the collisional fluorescence quenching readings from those observed during assessment of ligand binding. Raw data were collected using Winlab software, converted into ASCII format, then imported into GraphPad Prism 5.02 for curve fitting and nonlinear regression (one-site binding model), to determine observed K_D values.

Microsomal stability assay: The procedure was modified from that reported in a recent study of quinacrine metabolism.[30] A mixture of test compound (5.0 mM stock solution in DMSO, 0.8 μL), isocitrate dehydrogenase (31.4 μL, 2 U), 0.2 M potassium phosphate buffer (pH 7.0, containing 10 mM D,L-isocitric acid trisodium salt and 10 mM $MgCl_2$; 228 μL), and microsome preparation from mouse liver (0.5 mg mL^{-1} in the same buffer; 100 μL) was incubated in an Eppendorf tube for 5 min at 37 °C. NADPH (10 mM stock solution; 40 μL) was then added to initiate the reaction. Contents of the tube were mixed using aspiration/dispensation five times, then a t = 0 aliquot (150 μL) was drawn immediately and quenched with ice-cold acetonitrile (300 μL), containing 8 μM 1-(2-methyl-1*H*-indol-3-yl)-2-morpholinoethane-1,2-dione[10] as internal reference standard. The remaining reaction mixture was maintained at 37 °C for 30 min, then a second aliquot removed and diluted as above. The quenched solutions were each vortexed for 30 s and centrifuged at 4000 rpm (45° fixed-angle rotor F-45-12-11) for 10 min, then the supernatant analysed by HPLC: Genesis 4 μm C_{18} column, 4.6 × 150 mm; 5–95% MeCN/H_2O over 4 min, hold 6 min at 95% MeCN; flow rate 1 mL min^{-1}; UV detection at λ 267 nm. Each analysis was performed in triplicate, and metabolism results after 30 min were compared with those at t = 0. The percentage of drug remaining, together with the standard deviation, is reported for each test compound. During assessment of testosterone, *N*-(4-(1*H*-pyrrol-1-yl)phenyl)-2-(1*H*-indol-3-yl)-2-oxoacetamide[10] was used as internal standard, due to the appearance of metabolite peaks overlapping the signal for the reference described above.

Assessment of aggregation tendency by dynamic light scattering (DLS): Compounds were diluted from a 10 mM DMSO stock solution to the required concentration in 50 mM potassium phosphate buffer, pH 7.4, at a final DMSO concentration of 2%. Three measurements of the derived count rate (in kilocounts per second; kcps) were taken, and a mean value is reported for each sample assessed, together with the standard deviation over the three results.

Synthesis of 2,4-diphenylthiazole-5-carboxamides (2a–u). Method A: The relevant amine (R^1NH_2, 0.44 mmol) was added to a mixture of 2,4-diphenylthiazole-5-carboxylic acid (125 mg, 0.44 mmol) and Hünig's base (155 μL, 115 mg, 0.89 mmol) in $CHCl_3$ (4 mL). Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (231 mg, 0.44 mmol) was introduced to the reaction mixture, which was then stirred at room temperature for 3 h. After this time, the solution was applied directly to a silica gel column and

eluted as indicated for each individual example to provide the pure product.

Method B: The amine (R^1NH_2, 0.44 mmol) was dissolved in anhydrous CH_2Cl_2 (3 mL) under N_2, then Hünig's base (77 μL, 57 mg, 0.44 mmol) and 2,4-diphenylthiazole-5-carbonyl chloride (133 mg, 0.44 mmol) were added, and the reaction mixture stirred at room temperature for 3 h. The solution was applied directly to a silica gel column and eluted as indicated for each individual example to provide the pure product.

Method C: Excess amine (R^1NH_2, 2.64 mmol, 6 equiv) was added to a solution of 2,4-diphenylthiazole-5-carbonyl chloride (133 mg, 0.44 mmol) in anhydrous CH_2Cl_2 (3 mL) under N_2, then the reaction mixture stirred at room temperature for 2 h. The solution was applied directly to a silica gel column and eluted as indicated for each individual example to provide the pure product.

2,4-Diphenylthiazole-5-carboxylic acid *tert*-butylamide (2a): After elution with CH_2Cl_2, obtained as a white solid (37 mg, 25%): ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.97–7.88 (m, 2H), 7.66–7.57 (m, 2H), 7.52–7.33 (m, 6H), 5.49 (s, 1H), 1.14 ppm (s, 9H); ^{13}C NMR (62.8 MHz, $CDCl_3$): δ = 28.4, 51.9, 126.8, 128.9, 129.0, 129.5, 129.6, 130.7, 131.8, 133.1, 134.1, 153.9, 160.4, 168.5 ppm; IR (solid): $\tilde{\nu}$ = 3264, 3063, 2966, 1630, 1555, 1482, 1346, 1225, 978, 761, 684 cm^{-1}; MS (ES): *m/z* (%): 337 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{20}H_{21}N_2OS$: 337.1375, found: 337.1378.

2,4-Diphenylthiazole-5-carboxylic acid (2-chlorophenyl)amide (2b): After elution with 7.5% EtOAc/hexane, obtained as a white solid (41 mg, 24%): ^{1}H NMR (250 MHz, $CDCl_3$): δ = 8.49 (d, *J* = 8.0 Hz, 1H), 8.16–7.98 (m, 3H), 7.78–7.69 (m, 2H), 7.59–7.42 (m, 6H), 7.31–7.18 (m, 2H), 6.99 ppm (t, *J* = 8.0 Hz, 1H); ^{13}C NMR (62.8 MHz, $CDCl_3$): δ = 107.4, 121.3, 122.4, 124.8, 126.9, 127.7, 129.0, 129.1, 129.5, 129.7, 130.1, 131.1, 132.9, 133.7, 134.4, 155.5, 159.2, 169.9 ppm; IR (solid): $\tilde{\nu}$ = 3360, 1656, 1590, 1525, 1438, 1421, 1328, 1313, 756, 747, 694, 681, 585, 572 cm^{-1}; MS (ES): *m/z* (%): 391 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{22}H_{15}ClN_2OS$: 391.0672, found: 391.0671.

2,4-Diphenylthiazole-5-carboxylic acid (3-methoxyphenyl)amide (2c): After elution with 75→100% CH_2Cl_2/hexane, obtained as a white solid (136 mg, 80%): ^{1}H NMR (250 MHz, $CDCl_3$): δ = 8.06–7.98 (m, 2H), 7.79–7.72 (m, 2H), 7.62–7.54 (m, 3H), 7.52–7.41 (m, 4H), 7.13 (t, *J* = 8.0 Hz, 1H), 7.07 (t, *J* = 2.5 Hz, 1H), 6.67–6.53 (m, 2H), 3.77 ppm (s, 3H); ^{13}C NMR (62.8 MHz, $CDCl_3$): δ = 55.3, 105.1, 110.5, 111.5, 126.9, 129.1, 129.4, 129.7, 130.1, 131.1, 132.9, 133.9, 138.4, 154.7, 159.0, 160.1, 169.8 ppm; IR (solid): $\tilde{\nu}$ = 3298, 1638, 1600, 1493, 1412, 1258, 1162, 1040, 836, 779, 682 cm^{-1}; MS (ES): *m/z* (%): 387 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{23}H_{19}N_2O_2S$: 387.1167, found: 387.1160.

2,4-Diphenylthiazole-5-carboxylic acid (4-methoxyphenyl)amide (2d): After elution with CH_2Cl_2, obtained as a pale-yellow solid (126 mg, 74%): ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 10.47 (s, 1H), 8.10–8.00 (m, 2H), 7.88–7.78 (m, 2H), 7.61–7.36 (m, 8H), 6.92 (d, *J* = 8.9 Hz, 2H), 3.73 ppm (s, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 55.2, 114.0, 121.5, 126.4, 127.4, 128.3, 128.5, 128.7, 129.4, 131.0, 131.5, 132.3, 133.6, 153.2, 155.9, 159.5, 165.7 ppm; IR (solid): $\tilde{\nu}$ = 3309, 1630, 1599, 1527, 1516, 1480, 1414, 1336, 1237, 1032, 810, 760, 684 cm^{-1}; MS (ES): *m/z* (%): 409 (100) $[M+Na]^+$; HRMS-ES: *m/z* $[M+Na]^+$ calcd for $C_{23}H_{18}N_2O_2SNa$: 409.0987, found: 409.0995.

2,4-Diphenylthiazole-5-carboxylic acid 3-methoxybenzylamide (2e): After elution with 0→1% MeOH/CH_2Cl_2, obtained as a pale-yellow solid (113 mg, 64%): ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.94–7.83 (m, 2H), 7.60–7.52 (m, 2H), 7.41–7.27 (m, 6H), 7.11 (t, *J* = 8.0 Hz, 1H), 6.75–6.68 (m, 1H), 6.64–6.59 (m, 2H), 6.04 (t, *J* = 5.0 Hz, 1H), 4.33 (d, *J* = 6.0 Hz, 2H), 3.69 ppm (s, 3H); ^{13}C NMR (62.8 MHz, $CDCl_3$): δ = 44.1, 55.3, 113.1, 113.4, 120.0, 126.8, 128.95, 129.04, 129.4, 129.5, 129.7, 130.9, 132.9, 133.9, 138.7, 154.6, 159.8, 161.1, 168.9 ppm; IR (solid): $\tilde{\nu}$ = 3291, 1635, 1597, 1534, 1483, 1435, 1258, 1167, 1044, 760, 686 cm^{-1}; MS (ES): *m/z* (%): 401 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{24}H_{21}N_2O_2S$: 401.1324, found: 401.1309.

2,4-Diphenylthiazole-5-carboxylic acid 4-methoxybenzylamide (2f): After elution with 0→1% MeOH/CH_2Cl_2, obtained as a white solid (148 mg, 84%): ^{1}H NMR (250 MHz, $CDCl_3$): δ = 8.02–7.95 (m, 2H), 7.66–7.59 (m, 2H), 7.49–7.35 (m, 6H), 7.07–7.00 (m, 2H), 6.83–6.77 (m, 2H), 6.00 (br s, 1H), 4.37 (d, *J* = 5.5 Hz, 2H), 3.78 ppm (s, 3H); ^{13}C NMR (62.8 MHz, $CDCl_3$): δ = 43.6, 55.3, 114.0, 126.8, 128.9, 129.0, 129.2, 129.3, 129.4, 129.5, 129.7, 130.8, 133.0, 133.9, 154.5, 159.1, 161.0, 168.9 ppm; IR (solid): $\tilde{\nu}$ = 3316, 1617, 1532, 1509, 1478, 1250, 1234, 1023, 817, 760, 699, 681, 654 cm^{-1}; MS (ES): *m/z* (%): 401 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{24}H_{21}N_2O_2S$: 401.1324, found: 401.1306.

2,4-Diphenylthiazole-5-carboxylic acid quinolin-3-ylamide (2g): After elution with CH_2Cl_2/hexane (3:1) then 0→1→2.5% MeOH/CH_2Cl_2, obtained as an off-white foam (82 mg, 46%): ^{1}H NMR (250 MHz, $CDCl_3$): δ = 8.73 (d, *J* = 2.0 Hz, 1H), 8.11–7.95 (m, 4H), 7.84–7.43 ppm (m, 12H); ^{13}C NMR (62.8 MHz, $CDCl_3$): δ = 123.5, 127.0, 127.4, 127.8, 128.1, 128.4, 129.07, 129.14, 129.6, 129.7, 130.0, 130.4, 130.7, 131.3, 132.8, 133.8, 143.3, 145.4, 155.5, 159.5, 170.4 ppm; IR (solid): $\tilde{\nu}$ = 3293, 1668, 1641, 1533, 1482, 1364, 1312, 1256, 748, 726, 685, 639, 605 cm^{-1}; MS (ES): *m/z* (%): 408 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{25}H_{18}N_3OS$: 408.1171, found: 408.1181.

2,4-Diphenylthiazole-5-carboxylic acid benzothiazol-2-ylamide (2h): After elution with CH_2Cl_2, obtained as a white, crystalline solid (118 mg, 65%): ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 13.28 (s, 1H), 8.09–8.01 (m, 2H), 7.95 (d, *J* = 7.3 Hz, 1H), 7.86–7.79 (m, 2H), 7.66–7.41 (m, 8H), 7.36–7.28 ppm (m, 1H); ^{13}C NMR (62.8 MHz, [D$_6$]DMSO): δ = 122.2, 123.8, 126.5, 126.7, 128.1, 128.9, 129.3, 129.4, 131.2, 132.3, 134.0, 156.6, 167.4 ppm; IR (solid): $\tilde{\nu}$ = 2734, 1677, 1658, 1557, 1442, 1432, 1331, 1302, 1272, 859, 754, 741, 727, 681, 666 cm^{-1}; MS (ES): *m/z* (%): 414 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{23}H_{16}N_3OS_2$: 414.0735, found: 414.0731.

2,4-Diphenylthiazole-5-carboxylic acid (2-diethylaminoethyl)amide (2i): After elution with 2.5→5% MeOH/CH_2Cl_2, obtained as a thick, viscous oil (98 mg, 59%): ^{1}H NMR (250 MHz, $CDCl_3$): δ = 8.02–7.93 (m, 2H), 7.74–7.66 (m, 2H), 7.51–7.38 (m, 6H), 6.62 (br s, 1H), 3.34 (q, *J* = 5.5 Hz, 2H), 2.43 (t, *J* = 6.0 Hz, 2H), 2.33 (q, *J* = 7.0 Hz, 4H), 0.80 ppm (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (62.8 MHz, $CDCl_3$): δ = 11.1, 37.4, 46.1, 50.6, 126.8, 128.7, 128.8, 129.0, 129.2, 129.4, 130.7, 133.1, 134.2, 154.5, 161.3, 168.4 ppm; IR (neat): $\tilde{\nu}$ = 3276, 2967, 2926, 2810, 1634, 1542, 760, 729, 687 cm^{-1}; MS (ES): *m/z* (%): 380 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{22}H_{25}N_3OS$: 380.1797, found: 380.1786.

***N*-[2-(Dimethylamino)ethyl]-2,4-diphenylthiazole-5-carboxamide (2j):** After elution with 0→2.5→5→7.5% MeOH/CH_2Cl_2, obtained as a thick, colourless oil (91 mg, 59%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04–7.99 (m, 2H), 7.72–7.68 (m, 2H), 7.54–7.43 (m, 6H), 6.46 (br m, 1H), 3.35 (q, *J* = 6.0 Hz, 2H), 2.26 (t, *J* = 6.0 Hz, 2H), 2.01 ppm (s, 6H); ^{13}C NMR (62.8 MHz, $CDCl_3$): δ = 37.2, 44.8, 56.8, 126.8, 128.8, 129.0, 129.2, 129.4, 130.1, 130.7, 133.1, 134.3, 154.6, 161.2, 168.6 ppm; IR (neat): $\tilde{\nu}$ = 3308, 2938, 2818, 2767, 1632, 1536, 760, 686 cm^{-1}; MS (ES): *m/z* (%): 352 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{20}H_{22}N_3OS$: 352.1484, found: 352.1478.

2,4-Diphenyl-*N*-[2-(pyrrolidin-1-yl)ethyl]thiazole-5-carboxamide (2k): After elution with 0→2.5→5→7.5% MeOH/CH_2Cl_2, obtained as a thick, colourless oil (71 mg, 43%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.04–7.99 (m, 2H), 7.73–7.69 (m, 2H), 7.53–7.43 (m, 6H), 6.52 (br m, 1H), 3.40 (q, J=5.5 Hz, 2H), 2.47 (t, J=6.0 Hz, 2H), 2.35–2.27 (m, 4H), 1.67–1.59 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 23.4, 38.4, 53.4, 53.6, 126.8, 128.8, 129.0, 129.3, 129.4, 130.1, 130.7, 133.1, 134.2, 154.5, 161.2, 168.6 ppm; IR (neat): $\tilde{\nu}$=3304, 2965, 2796, 1627, 1532, 1480, 1434, 1310, 1266, 1144, 840, 760, 695, 684 cm^{-1}; MS (ES): *m/z* (%): 378 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{22}H_{24}N_3OS$: 378.1640, found: 378.1649.

2,4-Diphenylthiazole-5-carboxylic acid (2-morpholin-4-ylethyl)amide (2l): After elution with 1→2.5→5 MeOH/CH_2Cl_2, obtained as a white, crystalline solid (152 mg, 88%): ^{1}H NMR (250 MHz, $CDCl_3$): δ=8.05–7.93 (m, 2H), 7.77–7.66 (m, 2H), 7.55–7.37 (m, 6H), 6.39 (br s, 1H), 3.51–3.32 (m, 6H), 2.31 (t, J=6.0 Hz, 2H), 2.27–2.15 ppm (m, 4H); ^{13}C NMR (62.8 MHz, $CDCl_3$): δ=36.0, 52.9, 56.2, 66.7, 126.8, 128.8, 129.0, 129.4, 129.5, 129.9, 130.8, 133.0, 134.3, 154.4, 161.2, 168.8 ppm; IR (solid): $\tilde{\nu}$=3292, 2964, 2860, 2808, 1636, 1535, 1308, 1116, 866, 762, 693, 683 cm^{-1}; MS (ES): *m/z* (%): 809 (20) $[2M+Na]^+$, 394 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{22}H_{24}N_3O_2S$: 394.1589, found: 394.1598.

2,4-Diphenylthiazole-5-carboxylic acid (furan-2-ylmethyl)amide (2m): After elution with CH_2Cl_2, obtained as an off-white, crystalline solid (125 mg, 79%): ^{1}H NMR (250 MHz, $CDCl_3$): δ=7.99 (dd, J= 2.8 Hz, 6.1 Hz, 2H), 7.64 (dd, J=2.9 Hz, 6.3 Hz, 2H), 7.48–7.38 (m, 6H), 7.33–7.28 (m, 1H), 6.29 (dd, J=2.0 Hz, 3.2 Hz, 1H), 6.15–6.04 (m, 2H), 4.44 ppm (d, J=5.5 Hz, 2H); ^{13}C NMR (62.8 MHz, $CDCl_3$): δ=36.8, 107.7, 110.5, 126.8, 129.0, 129.1, 129.3, 129.4, 129.5, 130.9, 133.0, 133.8, 142.2, 150.4, 154.7, 161.0, 169.1 ppm; IR (solid): $\tilde{\nu}$= 3294, 1637, 1512, 1477, 1433, 1331, 1256, 1193, 1148, 1073, 767, 744, 692, 599 cm^{-1}; MS (ES): *m/z* (%): 361 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{21}H_{17}N_2O_2S$: 361.1011, found: 361.1021.

2,4-Diphenyl-*N*-(thiophen-2-ylmethyl)thiazole-5-carboxamide (2n): After elution with CH_2Cl_2, obtained as a white solid (88 mg, 53%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.05–7.99 (m, 2H), 7.69–7.63 (m, 2H), 7.52–7.39 (m, 6H), 7.24 (dd, J=1.0 Hz, 5.0 Hz, 1H), 6.95 (dd, J=3.5 Hz, 5.0 Hz, 1H), 6.88–6.86 (m, 1H), 6.12 (t, J=5.5 Hz, 1H), 4.64 ppm (d, J=5.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 38.6, 125.4, 126.3, 126.8, 126.9, 128.99, 129.04, 129.4, 129.5, 130.9, 133.0, 133.8, 139.5, 154.8, 160.9, 169.1 ppm; IR (solid): $\tilde{\nu}$=3230, 3049, 1621, 1545, 756, 692, 688 cm^{-1}; MS (ES): *m/z* (%): 377 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{21}H_{17}N_2OS_2$: 377.0782, found: 377.0787.

2,4-Diphenyl-*N*-[(tetrahydrofuran-2-yl)methyl]thiazole-5-carboxamide (2o): After elution with 50→70→90% EtOAc/hexane, obtained as a white solid (101 mg, 63%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.04–7.99 (m, 2H), 7.75–7.70 (m, 2H), 7.55–7.43 (m, 6H), 6.15 (t, J=5.0 Hz, 1H), 3.91–3.84 (m, 1H), 3.62 (t, J=7.0 Hz, 2H), 3.53 (ddd, J=4.0 Hz, 5.5 Hz, 14.0 Hz, 1H), 3.30 (dt, J=6.0 Hz, 14.0 Hz, 1H), 1.95–1.71 (m, 3H), 1.50–1.39 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=25.7, 28.4, 43.3, 67.9, 77.0, 126.8, 128.99, 129.02, 129.38, 129.43, 129.8, 130.8, 133.0, 134.2, 154.6, 161.4, 168.8 ppm; IR (solid): $\tilde{\nu}$=3268, 3054, 2945, 2868, 1644, 1074, 970, 762, 689 cm^{-1}; MS (ES): *m/z* (%): 365 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{21}H_{21}N_2O_2S$: 365.1324, found: 365.1322.

(*S*)-2,4-Diphenyl-*N*-[(tetrahydrofuran-2-yl)methyl]thiazole-5-carboxamide (2p): After elution with 20→30→40% EtOAc/isohexane, obtained as a thick, colourless gum (126 mg, 78%): $[\alpha]_D^{20}=+14.0^\circ$ (c=0.5 in acetone); ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.05–7.99 (m, 2H), 7.75–7.70 (m, 2H), 7.56–7.44 (m, 6H), 6.15 (br s, 1H), 3.92–3.84 (m, 1H), 3.62 (t, J=7.0 Hz, 2H), 3.54 (ddd, J=4.0 Hz, 5.5 Hz, 14.0 Hz, 1H), 3.30 (dt, J=6.0 Hz, 14.0 Hz, 1H), 1.95–1.71 (m, 3H), 1.51–1.41 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=25.7, 28.4, 43.3, 67.9, 77.0, 126.8, 128.98, 129.01, 129.38, 129.43, 129.8, 130.8, 133.0, 134.2, 154.6, 161.4, 168.8 ppm; IR (solid): $\tilde{\nu}$=3288, 2950, 2860, 1637, 1534, 1062, 761, 686 cm^{-1}; MS (ES): *m/z* (%): 365 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{21}H_{21}N_2O_2S$: 365.1324, found: 365.1325.

(*R*)-2,4-Diphenyl-*N*-[(tetrahydrofuran-2-yl)methyl]thiazole-5-carboxamide (2q): After elution with 20→30→40% EtOAc/isohexane, obtained as a thick, colourless gum (124 mg, 77%): $[\alpha]_D^{20}=-12.0^\circ$ (c=0.5 in acetone); ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.06–7.99 (m, 2H), 7.75–7.70 (m, 2H), 7.56–7.44 (m, 6H), 6.15 (br s, 1H), 3.91–3.84 (m, 1H), 3.62 (t, J=7.0 Hz, 2H), 3.54 (ddd, J=4.0 Hz, 5.5 Hz, 14.0 Hz, 1H), 3.30 (dt, J=6.0 Hz, 14.0 Hz, 1H), 1.95–1.71 (m, 3H), 1.51–1.41 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=25.7, 28.4, 43.3, 67.9, 77.0, 126.8, 128.98, 129.01, 129.38, 129.43, 129.8, 130.8, 133.0, 134.2, 154.6, 161.4, 168.8 ppm; IR (solid): $\tilde{\nu}$=3286, 2941, 2860, 1632, 1532, 1069, 761, 686 cm^{-1}; MS (ES): *m/z* (%): 365 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{21}H_{21}N_2O_2S$: 365.1324, found: 365.1335.

***N*-[(3-Methylfuran-2-yl)methyl]-2,4-diphenylthiazole-5-carboxamide (2r):** After elution with 10→15→20→25% EtOAc/hexane, obtained as a white solid (94 mg, 57%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.04–7.99 (m, 2H), 7.68–7.63 (m, 2H), 7.50–7.40 (m, 6H), 7.23 (d, J=2.0 Hz, 1H), 6.20 (d, J=1.5 Hz, 1H), 6.06 (t, J=5.0 Hz, 1H), 4.42 (d, J=5.5 Hz, 2H), 2.01 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=10.1, 35.2, 113.5, 117.7, 127.2, 129.3, 129.4, 129.71, 129.77, 129.83, 131.3, 133.4, 134.2, 141.7, 146.0, 155.1, 161.3, 169.4 ppm; IR (solid): $\tilde{\nu}$=3222, 1629, 1550, 1483, 1438, 1270, 1107, 756, 730, 694, 681 cm^{-1}; MS (ES): *m/z* (%): 375 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{22}H_{19}N_2O_2S$: 375.1167, found: 375.1159.

***N*-[(5-Methylfuran-2-yl)methyl]-2,4-diphenylthiazole-5-carboxamide (2s):** After elution with 0→1% MeOH/CH_2Cl_2, obtained as a white solid (69 mg, 42%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.05–7.99 (m, 2H), 7.71–7.66 (m, 2H), 7.50–7.41 (m, 6H), 6.10 (t, J=6.0 Hz, 1H), 6.03 (d, J=3.0 Hz, 1H), 5.89 (dd, J=1.0 Hz, 3.0 Hz, 1H), 4.42 (d, J=5.5 Hz, 2H), 2.26 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 13.9, 37.4, 106.7, 108.9, 127.2, 129.3, 129.4, 129.8, 129.9, 131.3, 133.4, 134.2, 148.8, 152.4, 155.0, 161.3, 169.4 ppm; IR (solid): $\tilde{\nu}$= 3238, 3061, 1619, 1543, 1480, 1020, 979, 772, 758, 683 cm^{-1}; MS (ES): *m/z* (%): 375 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{22}H_{19}N_2O_2S$: 375.1167, found: 375.1176.

***N*-[(3-Methylisoxazol-5-yl)methyl]-2,4-diphenylthiazole-5-carboxamide (2t):** After elution with 0→1% MeOH/CH_2Cl_2, obtained as a white solid (87 mg, 53%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.01–7.97 (m, 2H), 7.70–7.64 (m, 2H), 7.52–7.42 (m, 6H), 6.35 (t, J=6.0 Hz, 1H), 5.95 (s, 1H), 4.51 (d, J=6.0 Hz, 2H), 2.28 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=11.4, 35.5, 103.1, 126.8, 128.6, 129.07, 129.12, 129.3, 129.8, 131.0, 132.8, 133.8, 155.2, 159.9, 161.3, 167.6, 169.4 ppm; IR (solid): $\tilde{\nu}$=3272, 1632, 1533, 1481, 1427, 1335, 1212, 760, 685 cm^{-1}; MS (ES): *m/z* (%): 375 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{21}H_{18}N_3O_2S$: 375.1120, found: 375.1107.

***N*-[(5-Methylisoxazol-3-yl)methyl]-2,4-diphenylthiazole-5-carboxamide (2u):** After elution with 0→1% MeOH/CH_2Cl_2, obtained as a white solid (58 mg, 35%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.04–7.99 (m, 2H), 7.71–7.66 (m, 2H), 7.52–7.44 (m, 6H), 6.31 (t, J=5.5 Hz, 1H), 5.95 (s, 1H), 4.48 (d, J=6.0 Hz, 2H), 2.41 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=12.2, 35.8, 101.0, 126.8, 128.9, 129.1, 129.4, 129.7, 131.0, 132.9, 133.8, 155.1, 160.4, 161.4, 169.2, 170.1 ppm; IR

(solid): $\tilde{\nu}$ = 3308, 1631, 1606, 1536, 1478, 1435, 1266, 982, 817, 768, 686, 608 cm^{-1}; MS (ES): *m/z* (%): 375 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{21}H_{18}N_3O_2S$: 375.1120, found: 375.1106.

Ethyl 2-bromo-2-benzoylacetate (3):[13] A solution of ethyl benzoylacetate (6.93 mL, 7.69 g, 40 mmol) in CH_2Cl_2 (180 mL) was cooled to 0 °C, then bromine (1.84 mL, 5.75 g, 36 mmol) in the same solvent (30 mL) was added dropwise over 15 min. The reaction mixture was stirred for an additional 1 h then transferred to a separating funnel, washed with 10% K_2CO_3 (150 mL), dried over $MgSO_4$ and evaporated giving a thick oily residue. The title compound was obtained after flash column chromatography on silica, eluted with 30→40→50→60% CH_2Cl_2/hexane, and obtained as a pale-yellow oil (8.14 g, 75%): R_f = 0.25 (hexane/CH_2Cl_2, 3:2); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.05–7.99 (m, 2H), 7.69–7.63 (m, 1H), 7.57–7.50 (m, 2H), 5.69 (s, 1H), 4.32 (q, *J* = 7.0 Hz, 2H), 1.28 ppm (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.3, 46.8, 63.7, 129.3, 129.6, 133.8, 134.7, 165.6, 188.5 ppm; IR (oil): $\tilde{\nu}$ = 2981, 1758, 1736, 1684, 1448, 1300, 1256, 1183, 1138, 1023, 1000, 686 cm^{-1}; MS (ES^-): *m/z* (%): 269 (100) $[M-H]^-$; HRMS-ES: *m/z* $[M-H]^-$ calcd for $C_{11}H_{10}BrO_3$: 268.9813, found: 268.9824.

Thiazole-5-carboxylic esters (4a–c). General procedure: Ethyl 2-bromo-2-benzoylacetate **3** (0.95 g, 3.5 mmol) and a thioamide (3.5 mmol) were combined in EtOH (20 mL), and the mixture was heated at reflux until TLC analysis showed complete consumption of the bromide starting material (typically 90 min). The solvent was evaporated, then the residue was taken up in CH_2Cl_2 and washed with saturated $NaHCO_3$, then dried over $MgSO_4$ and evaporated. Pure product was obtained after flash column chromatography on silica, eluted with the solvent system detailed below for each individual case.

Ethyl 4-phenyl-2-(pyridin-3-yl)thiazole-5-carboxylate (4a): After elution with 10→20→30→40→50% EtOAc/hexane, obtained as a pale-beige solid (0.63 g, 58%): R_f = 0.30 (CH_2Cl_2/MeOH, 39:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.26 (d, *J* = 2.0 Hz, 1H), 8.74 (dd, *J* = 1.5 Hz, 4.5 Hz, 1H), 8.36 (dt, *J* = 4.0 Hz, 8.0 Hz, 1H), 7.88–7.82 (m, 2H), 7.53–7.47 (m, 3H), 6.90 (ddd, *J* = 0.5 Hz, 5.0 Hz, 8.0 Hz, 1H), 4.34 (q, *J* = 7.5 Hz, 2H), 1.34 ppm (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.1, 61.8, 123.3, 123.9, 127.9, 129.0, 129.4, 129.9, 133.8, 134.1, 148.0, 151.7, 161.0, 161.3, 166.3 ppm; IR (solid): $\tilde{\nu}$ = 2976, 1722, 1520, 1479, 1408, 1246, 1233, 1138, 1084, 1020, 814, 774, 753, 688 cm^{-1}; MS (ES): *m/z* (%): 311 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{17}H_{15}N_2O_2S$: 311.0854, found: 311.0847.

Ethyl 2-(4-methoxyphenyl)-4-phenylthiazole-5-carboxylate (4b): Due to the close R_f values of **4b** and **7b**, some overlap occurred during chromatography (elution with 5→7.5→10→12.5% EtOAc/hexane); these 'mixed fractions' were concentrated and subjected to further purification using a second silica column. The pure title compound was finally obtained as a microcrystalline, white solid (0.33 g, 27%): R_f = 0.43 (hexane/EtOAc, 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.01 (dt, *J* = 2.5 Hz, 9.0 Hz, 2H), 7.86–7.82 (m, 2H), 7.52–7.44 (m, 3H), 6.99 (dt, *J* = 2.5 Hz, 9.0 Hz, 2H), 4.31 (q, *J* = 7.0 Hz, 2H), 3.89 (s, 3H), 1.32 ppm (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.2, 55.5, 61.4, 114.4, 121.5, 125.8, 127.8, 128.6, 129.1, 129.9, 134.4, 160.8, 161.7, 162.1, 169.8 ppm; IR (solid): $\tilde{\nu}$ = 3003, 2980, 2934, 2836, 1717, 1606, 1520, 1444, 1327, 1254, 1234, 1171, 1140, 1084, 1037, 1020, 828, 771, 752, 700, 686, 604 cm^{-1}; MS (ES): *m/z* (%): 340 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{19}H_{18}NO_3S$: 340.1007, found: 340.0996. Ethyl benzoylacetate **8** was also isolated from this reaction, as the highest-running spot, and obtained as a colourless oil (258 mg, 38%): R_f ~ 0.55 (hexane/EtOAc, 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.00–7.95 (m, 2H), 7.63 (t, *J* = 7.5 Hz, 1H), 7.51 (t, 2H, *J* = 8.0 Hz), 4.24 (q, *J* = 7.0 Hz, 2H), 4.02 (s, 2H), 1.28 ppm (t, 3H, *J* = 7.0 Hz); MS (ES): *m/z* (%): 193 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{11}H_{13}O_3$: 193.0865, found: 193.0864.

Ethyl 2-(thiophen-2-yl)-4-phenylthiazole-5-carboxylate (4c): After elution with toluene, obtained as a white solid (0.31 g, 28%): R_f = 0.30 (hexane/EtOAc, 92:8); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.86–7.81 (m, 2H), 7.67 (dd, *J* = 1.0 Hz, 4.0 Hz, 1H), 7.51 (dd, *J* = 1.0 Hz, 5.0 Hz, 1H), 7.50–7.45 (m, 3H), 7.15 (dd, *J* = 4.0 Hz, 5.0 Hz, 1H), 4.32 (q, *J* = 7.0 Hz, 2H), 1.33 ppm (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.6, 62.0, 121.9, 128.2, 128.5, 128.6, 129.7, 129.9, 130.4, 134.3, 137.1, 161.1, 161.8, 163.9 ppm; IR (solid): $\tilde{\nu}$ = 2976, 1720, 1442, 1249, 1227, 1136, 1083, 752, 685 cm^{-1}; MS (ES): *m/z* (%): 316 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{16}H_{14}NO_2S_2$: 316.0466, found: 316.0477. Ethyl benzoylacetate **8** was also isolated from this reaction, as the last compound to elute from the column, and obtained as a pale-yellow oil (162 mg, 24%).

Thiazole-5-carboxamides (5a-c, 6a-c). General procedure: The thiazole-5-carboxylic ester **4a-c** (0.5 mmol) and TBD (21 mg, 0.15 mmol) were mixed thoroughly in a 50 mL size Radleys carousel reaction tube, then a small stirrer bar was introduced to ensure good mixing over the course of the reaction. Either furfurylamine (53 μL, 58 mg, 0.6 mmol) or *N,N*-diethylethylenediamine (84 μL, 70 mg, 0.6 mmol) was added, then the tube heated under N_2 at 80 °C for 18 h with stirring of the reaction mixture. The resultant material was allowed to cool back to ambient temperature, then dissolved in a small volume of CH_2Cl_2, loaded directly to a silica column, and eluted as described for each individual case to provide the pure amide product.

***N*-(Furan-2-ylmethyl)-4-phenyl-2-(pyridin-3-yl)thiazole-5-carboxamide (5a):** After elution with 50→70→90→100% EtOAc/hexane, obtained as a pale-yellow, amorphous solid (119 mg, 66%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.22 (s, 1H), 8.70 (d, *J* = 4.0 Hz, 1H), 8.29 (dt, *J* = 2.0 Hz, 8.0 Hz, 1H), 7.69–7.64 (m, 2H), 7.49–7.40 (m, 4H), 7.34 (dd, *J* = 0.5 Hz, 2.0 Hz, 1H), 6.33 (dd, *J* = 2.0 Hz, 3.0 Hz, 1H), 6.20–6.15 (m, 2H), 4.49 ppm (d, *J* = 5.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 36.8, 107.8, 110.5, 123.8, 129.0, 129.3, 129.7, 130.2, 133.4, 134.0, 142.3, 147.8, 150.2, 151.5, 154.9, 160.6, 165.6 ppm; IR (solid): $\tilde{\nu}$ = 3288, 1623, 1536, 977, 744, 691 cm^{-1}; MS (ES): *m/z* (%): 362 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{20}H_{16}N_3O_2S$: 362.0963, found: 362.0957.

***N*-(Furan-2-ylmethyl)-2-(4-methoxyphenyl)-4-phenylthiazole-5-carboxamide (5b):** After elution with 10→20→30% EtOAc/hexane, obtained as a white solid (86 mg, 45%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.95 (d, *J* = 9.0 Hz, 2H), 7.68–7.62 (m, 2H), 7.48–7.40 (m, 3H), 7.35–7.32 (m, 1H), 6.97 (d, *J* = 9.0 Hz, 2H), 6.32 (dd, *J* = 2.0 Hz, 3.0 Hz, 1H), 6.15 (d, *J* = 3.0 Hz, 1H), 6.07 (t, *J* = 5.5 Hz, 1H), 4.46 (d, *J* = 5.5 Hz, 2H), 3.88 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 36.8, 55.5, 107.6, 110.4, 114.4, 125.9, 128.37, 128.42, 128.9, 129.35, 129.44, 133.9, 142.2, 150.4, 154.6, 161.1, 161.9, 169.0 ppm; IR (solid): $\tilde{\nu}$ = 3224, 3058, 1620, 1607, 1547, 1479, 1306, 1257, 1150, 1029, 828, 759, 739, 698, 645, 600 cm^{-1}; MS (ES): *m/z* (%): 391 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{22}H_{19}N_2O_3S$: 391.1116, found: 391.1130.

***N*-(Furan-2-ylmethyl)-4-phenyl-2-(thiophen-2-yl)thiazole-5-carboxamide (5c):** After elution with 5→10→15→20→25% EtOAc/hexane, obtained as a greasy, pale-orange solid (92 mg, 50%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.67–7.63 (m, 3H), 7.50–7.42 (m, 4H), 7.34 (dd, *J* = 1.0 Hz, 2.0 Hz, 1H), 7.14 (dd, *J* = 3.5 Hz, 5.0 Hz, 1H), 6.33 (dd, *J* = 2.0 Hz, 3.0 Hz, 1H), 6.15 (dd, *J* = 0.5 Hz, 3.0 Hz, 1H),

6.04 (t, $J=5.0$ Hz, 1H), 4.48 ppm (d, $J=5.5$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=36.8$, 107.6, 110.4, 128.0, 128.2, 128.6, 129.0, 129.1, 129.4, 129.6, 133.5, 136.6, 142.2, 150.3, 154.5, 160.7, 162.9 ppm; IR (solid): $\tilde{\nu}=3324$, 3107, 2927, 1640, 1524, 1502, 1147, 920, 754, 740, 692, 598 cm^{-1}; MS (ES): *m/z* (%): 367 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{19}H_{15}N_2O_2S_2$: 367.0575, found: 367.0588.

***N*-(2-(Diethylamino)ethyl)-4-phenyl-2-(pyridin-3-yl)thiazole-5-carboxamide (6a):** After elution with 0→5→10→15→20% MeOH/CH_2Cl_2, obtained as a thick, sticky gum with a pale reddish-brown tint (104 mg, 55%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=9.24$ (dd, $J=$ 0.5 Hz, 2.5 Hz, 1H), 8.71 (dd, $J=1.5$ Hz, 5.0 Hz, 1H), 8.30 (dt, $J=$ 2.0 Hz, 8.0 Hz, 1H), 7.75–7.70 (m, 2H), 7.55–7.46 (m, 3H), 7.42 (ddd, $J=0.5$ Hz, 5.0 Hz, 8.0 Hz, 1H), 6.62 (br s, 1H), 2.57–2.26 (m, 6H), 0.84 ppm (t, $J=7.0$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=11.1$, 37.4, 46.2, 50.6, 123.7, 128.9, 129.1, 129.3, 129.5, 133.8, 147.9, 151.4, 154.7, 160.9, 165.1 ppm; IR (neat): $\tilde{\nu}=3276$, 3057, 2968, 2811, 1639, 1525, 1504, 1475, 732, 697 cm^{-1}; MS (ES): *m/z* (%): 381 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{21}H_{25}N_4OS$: 381.1749, found: 381.1744.

***N*-[2-(Diethylamino)ethyl]-2-(4-methoxyphenyl)-4-phenylthiazole-5-carboxamide (6b):** After elution with 2→4→6→8% MeOH/CH_2Cl_2, obtained as a thick, colourless gum (132 mg, 65%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.92$ (d, $J=9.0$ Hz, 2H), 7.71 (dd, $J=$ 2.0 Hz, 8.0 Hz, 2H), 7.50–7.43 (m, 2H), 6.93 (d, $J=9.0$ Hz, 2H), 6.78 (br s, 1H), 3.85 (s, 3H), 3.40 (q, $J=5.5$ Hz, 2H), 2.53 (t, $J=5.5$ Hz, 2H), 2.48–2.39 (m, 4H), 0.88 ppm (t, $J=7.0$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=11.2$, 37.5, 46.6, 51.1, 55.8, 114.7, 126.4, 128.8, 129.2, 129.6, 129.8, 134.8, 155.1, 162.0, 162.1, 168.7 ppm; IR (neat): $\tilde{\nu}=3269$, 2967, 2931, 2832, 1634, 1605, 1256, 1172, 1031, 828, 728, 698 cm^{-1}; MS (ES): *m/z* (%): 410 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{23}H_{28}N_3O_2S$: 410.1902, found: 410.1915.

***N*-[2-(Diethylamino)ethyl]-4-phenyl-2-(thiophen-2-yl)thiazole-5-carboxamide (6c):** After elution with 0→2.5→5→7.5% MeOH/CH_2Cl_2, obtained as a thick, pale-yellow gum (113 mg, 59%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.72$–7.66 (m, 1H), 7.60 (d, $J=3.5$ Hz, 1H), 7.52–7.42 (m, 4H), 7.28 (s, 1H), 7.11 (dd, $J=4.0$ Hz, 5.0 Hz, 1H), 6.57 (br s, 1H), 3.37 (q, $J=5.5$ Hz, 2H), 2.51–2.30 (m, 6H), 0.84 ppm (t, $J=6.5$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=11.1$, 37.3, 46.1, 50.6, 127.7, 128.0, 128.78, 128.84, 129.3, 129.4, 133.9, 136.8, 154.2, 161.1, 162.3 ppm; IR (neat): $\tilde{\nu}=2970$, 1641, 1527, 1480, 1447, 1332, 919, 844, 722, 698 cm^{-1}; MS (ES): *m/z* (%): 386 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{20}H_{24}N_3OS_2$: 386.1361, found: 386.1370.

3,5-Bis(pyridin-3-yl)-1,2,4-thiadiazole (7a): Isolated during the synthesis of **4a** as a lower-running spot, eluted with 3% MeOH/CH_2Cl_2 after **4a** had been recovered from the column, and obtained as a pale-yellow powder (104 mg, 25%): $R_f=0.15$ (CH_2Cl_2/MeOH, 39:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=9.62$ (d, $J=1.5$ Hz, 1H), 9.28 (d, $J=1.5$ Hz, 1H), 8.81 (dd, $J=1.5$ Hz, 5.0 Hz, 1H), 8.75 (dd, $J=1.5$ Hz, 4.5 Hz, 1H), 8.66 (dt, $J=2.0$ Hz, 8.0 Hz, 1H), 8.37 (dt, $J=$ 2.0 Hz, 8.0 Hz, 1H), 7.52 (ddd, $J=0.5$ Hz, 5.0 Hz, 8.0 Hz, 1H), 7.48 ppm (ddd, $J=0.5$ Hz, 5.0 Hz, 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=123.7$, 124.1, 126.6, 128.4, 134.6, 135.6, 148.5, 149.7, 151.2, 152.9, 171.6, 185.6 ppm; IR (solid): $\tilde{\nu}=3046$, 1589, 1574, 1477, 1399, 1338, 1297, 1126, 1023, 988, 899, 811, 726, 697, 616 cm^{-1}; MS (ES): *m/z* (%): 241 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{12}H_9N_4S$: 241.0548, found: 241.0556.

3,5-Bis(4-methoxyphenyl)-1,2,4-thiadiazole (7b): Isolated during the synthesis of **4b** (lower-running spot), then further purified by preparative HPLC (Alltima HP C_{18} HL 5 μm column; isocratic conditions, 85:15 MeCN/H_2O; flow rate 20 mL min^{-1}; UV detection at λ 254 nm) to remove remaining traces of **4b**. The title compound was obtained as white solid (63 mg, 12%): $R_f=0.36$ (hexane/EtOAc, 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.34$ (d, $J=9.0$ Hz, 2H), 8.00 (d, $J=9.0$ Hz, 2H), 7.02 (dd, $J=2.0$ Hz, 9.0 Hz, 4H), 3.90 ppm (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=55.4$, 55.5, 114.0, 114.5, 123.7, 126.0, 129.2, 129.9, 161.3, 162.5, 173.4, 187.4 ppm; IR (solid): $\tilde{\nu}=2963$, 2940, 2842, 1465, 1412, 1235, 1170, 1105, 1029, 1013, 987, 832, 748, 702 cm^{-1}; MS (ES): *m/z* (%): 299 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{16}H_{15}N_2O_2S$: 299.0854, found: 299.0854.

3,5-Bis(thiophen-2-yl)-1,2,4-thiadiazole (7c): Isolated during the synthesis of **4c** (higher-running spot) as a beige powder (171 mg, 39%): $R_f=0.40$ (hexane/EtOAc, 92:8); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=$ 7.96 (dd, $J=1.0$ Hz, 3.5 Hz, 1H), 7.72 (dd, $J=1.0$ Hz, 3.5 Hz, 1H), 7.61 (dd, $J=1.0$ Hz, 5.0 Hz, 1H), 7.48 (dd, $J=1.0$ Hz, 5.0 Hz, 1H), 7.21–7.16 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=127.9$, 128.5, 128.9, 129.3, 129.9, 130.6, 133.1, 136.2, 168.4, 180.7 ppm; IR (solid): $\tilde{\nu}=3097$, 1540, 1462, 1413, 1312, 1285, 1221, 1091, 1070, 1036, 835, 705 cm^{-1}; MS (ES): *m/z* (%): 251 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{10}H_7N_2S_3$: 251.9771, found: 251.9777.

1,2,4-Thiadiazoles by dimerisation of thioamides (7d–h). General procedure: The thioamide (2.0 mmol), iodobenzene diacetate (644 mg, 2.0 mmol) and tetraethylammonium bromide (420 mg, 2.0 mmol) were combined in CH_2Cl_2 (10 mL). The mixture was heated at reflux; any solids initially apparent in suspension had dissolved within a few minutes of beginning heating. After 2 h at reflux, the reaction mixture was diluted with additional CH_2Cl_2, washed with saturated sodium bisulfite solution, then separated and evaporated. The crude product was purified by flash column chromatography on silica gel, eluted as indicated for each individual case.

3,5-Bis(4-(trifluoromethyl)phenyl)-1,2,4-thiadiazole (7d): After column elution with 2→4→6→8% EtOAc/petroleum ether, obtained as an off-white, microcrystalline solid (32 mg, 9%): $R_f=0.50$ (hexane/EtOAc, 9:1); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=8.54$ (d, $J=$ 8.0 Hz, 2H), 8.20 (d, $J=8.0$ Hz, 2H), 7.86–7.77 ppm (m, 4H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=125.8$ (q, $J=3.5$ Hz), 126.4 (q, $J=$ 3.5 Hz), 127.9, 128.7, 132.2 (q, $J=32.5$ Hz), 133.5, 133.7 (q, $J=$ 32.5 Hz), 135.6, 172.7, 187.0 ppm; IR (solid): $\tilde{\nu}=1473$, 1409, 1319, 1188, 1132, 1120, 1103, 1066, 1015, 990, 848, 712, 659 cm^{-1}; MS (ES): *m/z* (%): 375 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{16}H_9F_6N_2S$: 375.0391, found: 375.0383.

3,5-Bis(3,4-dimethoxyphenyl)-1,2,4-thiadiazole (7e): After column elution with 20→30→40% EtOAc/petroleum ether, obtained as a pale-pink solid (225 mg, 63%): $R_f=0.15$ (petroleum ether/EtOAc, 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.01$ (dd, $J=$ 2.0 Hz, 8.5 Hz, 1H), 7.88 (d, $J=1.5$ Hz, 1H), 7.60–7.55 (m, 2H), 6.99–6.92 (m, 2H), 4.02 (s, 3H), 4.01 (s, 3H), 3.95 ppm (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=55.95$, 56.03, 56.07, 56.14, 109.8, 110.8, 111.2, 121.2, 121.8, 123.7, 126.1, 149.0, 149.4, 150.9, 152.2, 173.3, 187.6 ppm; IR (solid): $\tilde{\nu}=2962$, 2837, 1600, 1521, 1454, 1422, 1264, 1244, 1228, 1171, 1138, 1108, 1020, 870, 834, 805, 739, 720 cm^{-1}; MS (ES): *m/z* (%): 359 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{18}H_{19}N_2O_4S$: 359.1066, found: 359.1064.

3,5-Bis(3-methoxyphenyl)-1,2,4-thiadiazole (7f): After column elution with 2.5→5→7.5% EtOAc/petroleum ether, obtained as a thick, colourless gum which crystallised on standing to give a pale-yellow solid (212 mg, 71%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.02$ (dt, $J=1.0$ Hz, 7.5 Hz, 1H), 7.97–7.94 (m, 1H), 7.64–7.59 (m, 2H), 7.44 (t, $J=8.0$ Hz, 2H), 7.11 (ddd, $J=1.0$ Hz, 2.5 Hz, 8.5 Hz, 1H), 7.06 (ddd, $J=1.0$ Hz, 2.5 Hz, 8.5 Hz, 1H), 3.94 (s, 3H), 3.93 ppm (s, 3H);

^{13}C NMR (100 MHz, $CDCl_3$): δ = 55.5, 55.6, 112.2, 113.0, 116.8, 118.0, 120.1, 121.0, 129.8, 130.4, 131.8, 134.1, 159.9, 160.2, 173.6, 188.0 ppm; IR (solid): $\tilde{\nu}$ = 3000, 2942, 2833, 1594, 1504, 1474, 1458, 1430, 1396, 1312, 1276, 1260, 1230, 1219, 1181, 1164, 1048, 1010, 866, 817, 789, 775, 733, 679 cm^{-1}; MS (ES): *m/z* (%): 299 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{16}H_{15}N_2O_2S$: 299.0854, found: 299.0848.

3,5-Bis(*p*-tolyl)-1,2,4-thiadiazole (7 g): After column elution with 2→4→6% EtOAc/petroleum ether, obtained as a fine, off-white solid (207 mg, 78%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.31 (d, *J* = 8.5 Hz, 2H), 7.96 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 7.5 Hz, 4H), 2.45 ppm (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.0, 22.1, 127.8, 128.6, 128.7, 129.8, 130.3, 130.8, 140.9, 142.9, 174.2, 188.4 ppm; IR (solid): $\tilde{\nu}$ = 3036, 2971, 2917, 1608, 1473, 1404, 1320, 1174, 1106, 984, 898, 827, 813, 736, 698 cm^{-1}; MS (ES): *m/z* (%): 267 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{16}H_{15}N_2S$: 267.0956, found: 267.0954.

3,5-Bis(pyridin-4-yl)-1,2,4-thiadiazole (7 h): After column elution with 50→75→100% EtOAc/petroleum ether, then 2.5→5% EtOH/EtOAc, obtained the title compound as a pale-pink solid (19 mg, 8%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.90–8.81 (m, 4H), 8.26–8.23 (m, 2H), 7.93–7.90 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 121.4, 122.5, 137.2, 139.4, 151.2, 151.7, 172.6, 187.1 ppm; IR (solid): $\tilde{\nu}$ = 1600, 1465, 1407, 1344, 1291, 823, 732, 710, 676, 634 cm^{-1}; MS (ES): *m/z* (%): 241 (100) $[M+H]^+$; HRMS-ES: *m/z* $[M+H]^+$ calcd for $C_{12}H_9N_4S$: 241.0548, found: 241.0541.

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[1] a) R. C. Holman, E. D. Belay, K. Y. Christensen, R. A. Maddox, A. M. Minino, A. M. Folkema, D. L. Haberling, T. A. Hammett, K. D. Kochanek, J. J. Sejvar, L. B. Schonberger, *PLoS ONE* **2010**, *5*, e8521, DOI: 10.1371/journal.pone.0008521; b) A. Ladogana, M. Puopolo, E. A. Croes, H. Budka, C. Jarius, S. Collins, G. M. Klug, T. Sutcliffe, A. Giulivi, A. Alperovitch, N. Delasnerie-Laupretre, J. P. Brandel, S. Poser, H. Kretzschmar, I. Rietveld, E. Mitrova, J. D. Cuesta, P. Martinez-Martin, M. Glatzel, A. Aguzzi, R. Knight, H. Ward, M. Pocchiari, C. M. van Duijn, R. G. Will, I. Zerr, *Neurology* **2005**, *64*, 1586–1591.

[2] a) G. G. Kovács, G. Trabattoni, J. A. Hainfeller, J. W. Ironside, R. S. G. Knight, H. Budka, *J. Neurol.* **2002**, *249*, 1567–1582; b) J. D. F. Wadsworth, A. F. Hill, J. A. Beck, J. Collinge, *Br. Med. Bull.* **2003**, *66*, 241–254.

[3] a) M. W. van der Kamp, V. Daggett, *Protein Eng. Des. Sel.* **2009**, *22*, 461–468; b) S. Collins, C. A. McLean, C. L. Masters, *J. Clin. Neurosci.* **2001**, *8*, 387–397.

[4] a) R. G. Will, J. W. Ironside, M. Zeidler, S. N. Cousens, K. Estibeiro, A. Alperovitch, S. Poser, M. Pocchiari, A. Hofman, P. G. Smith, *Lancet* **1996**, *347*, 921–925; b) J. Collinge, K. C. L. Sidle, J. Meads, J. Ironside, A. F. Hill, *Nature* **1996**, *382*, 779–788.

[5] D. Vilette, *Vet. Res.* **2008**, *39*, 10–29, DOI: 10.1051/vetres:2007049.

[6] a) M. C. Clarke, D. A. Haig, *Nature* **1970**, *225*, 100–101; b) D. A. Haig, M. C. Clare, *Nature* **1971**, *234*, 106–107; c) C. R. Birkett, R. M. Hennion, D. A. Bembridge, M. C. Clarke, A. Chree, M. E. Bruce, C. J. Bostock, *EMBO J.* **2001**, *20*, 3351–3358.

[7] The SMB cell line used in this study (SMB.s15) was provided by the TSE Resource Centre at the Roslin Institute, University of Edinburgh (UK); http://www.roslin.ed.ac.uk/tseresourcecentre/cell_lines.html (accessed June 17, 2010).

[8] H. Cope, R. Mutter, W. Heal, C. Pascoe, P. Brown, S. Pratt, B. Chen, *Eur. J. Med. Chem.* **2006**, *41*, 1124–1143.

[9] K. Guo, R. Mutter, W. Heal, T. R. K. Reddy, H. Cope, S. Pratt, M. J. Thompson, B. Chen, *Eur. J. Med. Chem.* **2008**, *43*, 93–106.

[10] M. J. Thompson, V. Borsenberger, J. C. Louth, K. E. Judd, B. Chen, *J. Med. Chem.* **2009**, *52*, 7503–7511.

[11] W. Heal, M. J. Thompson, R. Mutter, H. Cope, J. C. Louth, B. Chen, *J. Med. Chem.* **2007**, *50*, 1347–1353.

[12] A. Hantzsch, J. H. Weber, *Ber. Dtsch. Chem. Ges.* **1887**, *20*, 3118.

[13] E. J. Freyne, J. F. Lacrampe, F. Deroose, G. M. Boeckx, M. Willems, W. Embrechts, E. Coesemans, J. J. Willems, J. M. Fortin, Y. Ligney, L. L. Dillen, W. F. Cools, J. Goossens, D. Corens, A. de Groot, J. P. van Wauwe, *J. Med. Chem.* **2005**, *48*, 2167–2175.

[14] C. Sabot, K. A. Kumar, S. Meunier, C. Mioskowski, *Tetrahedron Lett.* **2007**, *48*, 3863–3866.

[15] P. C. Patil, D. S. Bhalerao, P. S. Dangate, K. G. Akamanchi, *Tetrahedron Lett.* **2009**, *50*, 5820–5822, and references cited therein.

[16] K. T. Potts, J. L. Marshall, *J. Org. Chem.* **1976**, *41*, 129–133.

[17] D.-P. Cheng, Z.-C. Chen, *Synth. Commun.* **2002**, *32*, 2155–2159.

[18] M. Yan, Z.-C. Chen, Q.-G. Zheng, *J. Chem. Res. Synop.* **2003**, 618–619.

[19] A. Kimata, H. Nakagawa, R. Ohyama, T. Fukuuchi, S. Ohta, T. Suzuki, N. Miyata, *J. Med. Chem.* **2007**, *50*, 5053–5056.

[20] S. Ghaemmaghami, B. C. H. May, A. R. Renslo, S. B. Prusiner, *J. Virol.* **2010**, *84*, 3408–3412.

[21] B. C. H. May, J. A. Zorn, J. Witkop, J. Sherrill, A. C. Wallace, G. Legname, S. B. Prusiner, F. E. Cohen, *J. Med. Chem.* **2007**, *50*, 65–73.

[22] F. Touil, S. Pratt, R. Mutter, B. Chen, *J. Pharm. Biomed. Anal.* **2006**, *40*, 822–832.

[23] a) M. A. Wells, G. S. Jackson, S. Jones, L. L. P. Hosszu, C. J. Craven, A. R. Clarke, J. Collinge, J. P. Waltho, *Biochem. J.* **2006**, *399*, 435–444; b) E. D. Walter, M. Chattopadhyay, G. L. Millhauser, *Biochemistry* **2006**, *45*, 13083–13092.

[24] a) K. E. D. Coan, B. K. Shoichet, *J. Am. Chem. Soc.* **2008**, *130*, 9606–9612; b) J. Seidler, S. L. McGovern, T. N. Doman, B. K. Shoichet, *J. Med. Chem.* **2003**, *46*, 4477–4486; c) S. L. McGovern, B. T. Helfand, B. Feng, B. K. Shoichet, *J. Med. Chem.* **2003**, *46*, 4265–4272; d) S. L. McGovern, B. K. Shoichet, *J. Med. Chem.* **2003**, *46*, 1478–1483; e) S. L. McGovern, E. Caselli, N. Grigorieff, B. K. Shoichet, *J. Med. Chem.* **2002**, *45*, 1712–1722.

[25] B. Y. Feng, B. H. Toyama, H. Wille, D. W. Colby, S. R. Collins, B. C. H. May, S. B. Prusiner, J. Weissman, B. K. Shoichet, *Nat. Chem. Biol.* **2008**, *4*, 197–199.

[26] a) H. Rao, Z. Li, X. Li, X. Ma, C. Ung, H. Li, X. Liu, Y. Chen, *J. Comput. Chem.* **2010**, *31*, 752–763; b) J. B. Baell, G. A. Holloway, *J. Med. Chem.* **2010**, *53*, 2719–2740.

[27] a) J.-X. Duan, H. Jiao, J. Kaizerman, T. Stanton, J. W. Evans, L. Lan, G. Lorente, M. Banica, D. Jung, J. Wang, H. Ma, X. Li, Z. Yang, R. M. Hoffman, W. S. Ammons, C. P. Hart, M. Matteucci, *J. Med. Chem.* **2008**, *51*, 2412–2420; b) K.-C. Cheng, J. B. Schenkman, *J. Biol. Chem.* **1983**, *258*, 11738–11744.

[28] a) W. Tang, *Curr. Drug Metab.* **2003**, *4*, 319–329; b) S. Kumar, K. Samuel, R. Subramanian, M. P. Braun, R. A. Stearns, S.-H. L. Chiu, D. C. Evans, T. A. Baillie, *J. Pharmacol. Exp. Ther.* **2002**, *303*, 969–978.

[29] Further information on this point is provided in the Supporting Information.

[30] Y. Huang, H. Okochi, B. C. H. May, G. Legname, S. B. Prusiner, L. Z. Benet, B. J. Guglielmo, E. T. Lin, *Drug Metab. Dispos.* **2006**, *34*, 1136–1144.

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