Synthesis of Analogues of Congo Red and Evaluation of Their Anti-Prion Activity

Shane Sellarajah,[†] Tamuna Lekishvili,[‡] Claire Bowring,[‡] Andrew R. Thompsett,[‡] Helene Rudyk,[†] Christopher R. Birkett,[§] David R. Brown,[‡] and Ian H. Gilbert*,[†]

Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF10 3XF, U.K., Department of Biology and Biochemistry, University of Bath, Bath, BA2 7AY, U.K., and Institute for Animal Health, Compton Laboratory, Compton, Newbury, Berkshire RG20 7NN, U.K.

Received January 28, 2004

No cure as of yet exists for any of the transmissible spongiform encephalopathies. In this paper, we describe the synthesis of analogues of Congo red and evaluation against a cellular model of infection, the SMB (scrapie mouse brain) persistently infected cell line, for their ability to inhibit the infectivity of the abnormal form of prion protein (PrP-res). The compounds have also been tested for their ability to inhibit the polymerization of PrP^{C} by PrP-res. A number of analogues showed inhibition of PrP-res infectivity at nanomolar concentrations. Several analogues show promise; the most active compound, **2a**, inhibits the formation of PrP-res in SMB cells with an EC_{50} of 25–50 nM.

Introduction

Transmissible spongiform encephalopathies (TSE) are a group of rare neurological degenerative disorders.[1] The diseases can arise spontaneously, have a genetic origin, or can occur by infection. In humans, the TSEs are Creutzfeldt Jakob Disease (CJD), Gertmann–Straussler–Scheinker disease (GSS), Fatal Familial Insomnia (FFI), Kuru, and more recently, a new form of the disease called variant CJD.[2,3] TSEs affect other organisms; diseases of note include bovine spongiform encephalopathy (BSE) in cattle and scrapie in sheep. There is no cure currently available for these diseases, which are fatal.

The causative agent of these diseases is thought to be or to contain an abnormally folded protein, the prion protein. During the course of the disease, there is an accumulation of the misfolded prion protein form (PrP-res).[4] The normal cellular prion protein (PrP^{C}) is a glycolipid-anchored membrane protein and is easily degraded by proteases, whereas PrP-res is highly resistant to degradation.

A number of compounds have shown anti-prion activity in models of TSE infection.[5–8] Compounds shown to have anti-prion activity include sulfated polysaccharides such as pentosan polysulfate,[9] Congo red and other diazo dyes, amphotericin B and analogues, anthracyclines, phthalocyanines and porphyrins, inorganic ions, branched polyamines, and antagonists of the *N*-methyl-D-aspartate (NMDA) receptor such as memantine.[6] Recently, there has been interest in quinacrine and other acridine derivatives.[10,11] In addition, further screening methodologies have recently been reported to allow screening of larger numbers of compounds in vitro.[12]

We were particularly interested in the diazo dye Congo red, which has been shown to have anti-prion activity in a cell free polymerization assay,[13] in cellular assays,[14,15] and in vivo activity against scrapie-infected golden Syrian hamsters.[16]

The molecule itself has a number of shortfalls:[17] (i) it has a lack of specificity, (ii) it does not have significant permeability through the blood–brain barrier[18,19] (presumably because of its charged nature, primarily caused by the presence of two sulfonate groups), and (iii) the diazo bonds in Congo red can be cleaved by enzymes present in the mammalian gut and intestines,[20,21] giving rise to benzidine (Figure 1), a highly carcinogenic compound strongly associated with urinary bladder cancer.[22] However, unlike many of the other compounds reported to have activity in various models of TSEs, Congo red is a small molecule, which should be amenable to chemical synthesis and structure–activity relationship studies, with the aim of producing a low-molecular-weight therapeutic agent. We have previously reported some structure–activity relationship studies with Congo red,[13–15] which taken together with other work[17,23] have allowed us to come to the following principal conclusions:

- Although Congo red is a symmetrical molecule, both "halves" of the molecule are required for activity.
- It is possible to exchange the sulfonate groups for carboxylic acid groups without a significant effect on the activity.
- It also should be possible to vary the location of the acidic group on the aromatic ring.
- The amino group may not be necessary for activity.
- Some variation in the linker (biphenyl) is possible.

In this paper, we address the following:

- Replacement of the diazo group with amide bonds and sulfonamide bonds. These are bioisoteres of the diazo group and will prevent the molecule from being metabolized to benzidine.
- To enable blood–brain barrier penetration, we will choose nonpolar terminator groups or, alternatively,

* Author to whom correspondence should be addressed. Tel: +44 29 2087 5800. Fax: +44 29 2087 4149. E-mail: gilbertih@cf.ac.uk.
† Cardiff University.
‡ University of Bath.
§ Institure for Animal Health.

10.1021/jm049922t CCC: $27.50 © 2004 American Chemical Society
Published on Web 09/29/2004

Congo Red

Benzidine

terminator connector linker

Figure 1. Structures of Congo red and benzidine.

Figure 2. Active mixture.

Added hydroxy groups to enhance aqueous solubility

Figure 3. Structure of compound **2a**.

ester-masked polar carboxylic acids, which should be hydrolyzed by esterases present in the brain.

Preliminary results from our group were obtained from a mixture of two compounds, **A** and **B** (Figure 2). In an assay conducted in persistently scrapie-infected SMB cells, the mixture reduced levels of PrP-res to 55% of control levels at 10 μM.

Results and Discussion

Chemistry. The active mixture of **A** and **B** (Figure 2) proved to be difficult to separate by normal purification techniques such as recrystallization or column chromatography. This was mainly due to the low solubility of compounds **A** and **B** in any organic solvent. Thus, the initial molecule envisaged for synthesis was very similar to diamide **A**, with the added solubilizing effect of two extra hydroxy groups (compound **2a**, Figure 3).

As discussed below, **2a** eventually proved to be our most active compound in the SMB cellular model. Therefore, different molecules based on the molecular architecture of **2a** were designed for synthesis and testing (Figure 4). Essentially, in these compounds, the linker was kept as either biphenyl or phenyl; the diazo connector was replaced by the sulfonamide, amide, imine, aminomethylene, and alkene connectors; the structure of the terminator was varied. The replacements for the diazo bond are all isosteres of this bond (Figure 4).

Sulfonamide analogues (series 1) could be synthesized from the readily available 4,4′-biphenyldisulfonyl chloride **8** and the appropriate amine utilizing DMAP as a catalyst and pyridine as the solvent.

The amines utilized in the reactions were either readily commercially available or synthesized via one-step esterifications (for example, see Scheme 2).

The biphenyl amide analogues (series 2) were synthesized either by the reaction of the corresponding acid chloride with the appropriate aniline derivative in the presence of triethylamine or by using TBTU, a uronium-based peptide-coupling reagent (Scheme 3).

The monophenyl analogues (series 3) were synthesized using terephthaloyl chloride and the appropriate aniline.

The imine analogues (series 4 and 5) were made by the condensation of the corresponding aldehyde with the appropriate amine. The corresponding imines were generally insoluble in ethanol and precipitated from solution; filtration and washing generally provided compounds in good yields. The biphenyl analogues proved not only to be less soluble but also had longer reaction times. The monophenyl imines (series 5) were synthesized from commerically available terephthalaldehyde, whereas the biphenyl analogues (series 4) required 4,4′-biphenyldialdehyde to be made first (Scheme 5).

The amine analogues (series 6) were synthesized by the reduction of the imines with sodium borohydride. The only limiting factor was the initial solubility of the imines, many of which proved to be insoluble in any solvent once formed. Generally, the reduction was favorable in a methanol/dichloromethane mixture.

The alkenes (series 7) were prepared using the Heck reaction, which gave trans stereochemistry (Scheme 7). The reagent 1,4-divinylbenzene could be purchased commercially but only as a mixture with 1,2-divinylbenzene; therefore, this compound was prepared from terephthaldehyde using a Wittig reaction.

Biological Assays. Cellular Assays. The synthesized compounds were tested in the SMB cell line assay. The SMB cells are persistently scrapie-infected mouse cells cloned from a scrapie-infected mouse brain but of non-neuronal origin.[14,15,24,25] These cells are highly phagocytic and may mimic cells involved in the initial uptake and replication of the agent in peripheral infection.[14] In addition, these cells show stable persistent scrapie infection over many passages, suggesting that they are suitable for drug screening.[25] We have never observed the SMB cells to lose their infection or "self-cure". Indeed, we have successfully used this cell line to screen many derivatives of Congo red.[14,15] Compounds were initially screened at 10 μM to see the effect at this concentration. Those for which there was no detectable PrP-res at this concentration are marked as "+ve" in the tables. Several of the compounds were also evaluated at 50 μM. The percentage of PrP-res compared to control is indicated in the tables; the lower this value, the more potent the compounds. The results

Series 1 Series 2

Series 3 Series 4

Series 5 Series 6

Series 7

R_1, R_2, R_3, R_4, R_5 = H, OH, OMe, CO_2H, CO_2Me, CO_2Ph, NO_2, CN

Figure 4. General structures of the target compounds.

Scheme 1. Sulfonamide Coupling

8 + pyridine DMAP

Scheme 3. Amide Coupling

(i) $SOCl_2$, $ClCH_2CH_2Cl$; (ii) Et_3N, THF, Ar-NH_2 or TBTU, DMA

Scheme 2. Synthesis of Amines

H^+, MeOH → 9

$SOCl_2$, MeOH → 10

from these assays are shown in Tables 1–7 for the seven series of compounds: the sulfonamides (Table 1); amides with a biphenyl linker (Table 2); amides with a phenyl linker (Table 3); imines with a biphenyl linker (Table 4); imines with a phenyl linker (Table 5); amines with a phenyl linker (Table 6); and alkenes with a phenyl linker (Table 7). An example of a Western Blot is given in Figure 5.

For the sulfonamides (Table 1), none of the compounds showed inhibition of PrP-res at 10 μM, although some of the compounds (**1a** and **1c**) showed some activity at 50 μM. Interestingly, compound **1a**, which has the same terminator as the lead compound, **2a**, showed some activity at this higher concentration.

Several of the amides with a biphenyl core (Table 2) showed good activity. In particular, compounds **2a**, **2b**, and **2h** showed the prevention of PrP-res formation. In **2a** and **2h**, there is a free hydroxyl group, and in compound **2b**, a methoxy group attached to the phenyl ring; the hydroxyl group of **2a** and **2h** could act as an H-bond donor or acceptor, whereas the methoxy group of **2b** could act as an H-bond acceptor. Compound **2g**, which is a regioisomer of **2a** and **2h**, was inactive. This suggests that the relative orientations of the ester and the hydroxyl group are important in the terminator. Compound **2e**, which is the analogue of **2b** with the free carboxylate, did not show activity, indicating that the ester has a role. Often esters are subject to cleavage by

Scheme 4. Imine Formation

Scheme 5. Synthesis of Biphenyl Dialdehyde[a]

[a] (i) BH_3SMe_2, THF; (ii) Pyridinium Dichromate, Dichloromethane

Scheme 6. Reduction of Imines

Scheme 7. Alkene Coupling

esterases; if this is the case here, then the ester may be acting as a prodrug to allow the delivery of compounds. Other compounds with the methyl carboxylate removed or replaced (**2c**, **2d**, **2e**, **2f**, and **2i**) were inactive in the cell culture assay, further indicating that the ester may have a role in the activity of the molecule; by altering the structure of the ester it may be possible to modify the potency and physicochemical properties of the compounds.

The compounds of series 3 were the amides with a phenyl core (Table 3). Compounds **3b** and **3h** showed activity, and following a fuller dose–response experiment, the ED_{50} values were found to be 250 and 5000 nM, respectively. None of the other compounds in this series showed activity in the cellular assays. This may be due to the replacement of the biphenyl core with a smaller core. Compound **3h** is the analogue of **2a**, which has maintained some but not all of the activity of **2a**, suggesting not only that the biphenyl moiety plays an important part in the activity of **2a** but also that the terminator functionality is important. So far, we have been unable to prepare the analogues of **2b** or **2h** in sufficient purity, which would provide a more interesting comparison with the data for the biphenyl analogues.

For the biphenyl series of imines (series 4, Table 4), two of the compounds showed an inhibition of PrP-res formation in the cellular assay (**4c** and **4e**). In the terminator of **4c**, there is a free carboxylic acid and a hydroxyl group, whereas **4e** contains only a hydroxyl group. Interestingly, amides or sulfonamides with these terminator groups were inactive. Compound **4b**, which has the same terminator group as **2a**, was inactive at 10 μM but showed some effect at 50 μM.

In Table 5, we present the data for the series of compounds with a phenyl linker and an imine (series 5). None of these compounds showed any significant activity in the cellular assay, with the exception of **5s**. In this compound, there is a nitrile substituent on the terminator group, which can act as a hydrogen-bond acceptor if it is not charged.[26]

The aminomethylene compounds of series 6 (Table 6) all showed activity except **6c**. Compound **6b** contains the same terminator group as **2a**.

Finally, a series of compounds were prepared in which the diazo bond was replaced by an alkene (series 7, Table 7). Compound **7a** is named X-34, a compound used by Klunk et al. for diagnosing Alzheimer's disease.[27,28] This compound showed no activity at 10 μM but weak activity at 50 μM. Analogue **7b**, in which the carboxylic acid functionality has been removed from the terminator, shows potent activity. Compound **7b** showed activity at 2 μM; however, the mechanism of action is unknown, but it is possibly different from that of the other molecules in this paper. This molecule is an interesting lead and could be acting through an antioxidant mode of action.

For compounds showing activity at 10 μM, a full dose–response study was undertaken to get a more accurate indication of the potency (Table 8). The most active compound, **2a**, showed an ED_{50} of 25–50 nM. A number of other compounds showed potent inhibition, including **2b**, **3b**, **4e**, **5s**, **6a**, and **6b**, which all showed an ED_{50} of <500 nM. The two compounds that showed the most potent activity, **2a** and **6b**, have the same terminator group. Compound **3h** has the same terminator group but has lower activity.

Table 1. Sulfonamides: Synthetic Yield, Inhibition of PrP-res Formation in SMB Cells at 10 μM, and Inhibition of Polymerization at 100 μM

R–NH–SO$_2$–C$_6$H$_4$–C$_6$H$_4$–SO$_2$–NH–R

Entry	R	MW	Yield	Inhibition of PrP-res formation in SMB cells at 10μM	Polymerization (% of control)
1a	MeO_2C–(aryl, OH)	612.64	28	-ve (77% of PrP-res at 50μM)	30
1b	MeO_2C–(aryl, OMe)	640.69	50	-ve (-ve 50μM)	77
1c	HO_2C–(aryl, OMe)	612.64	49	-ve (33% of PrP-res at 50μM)	62
1d	HO_2C–(aryl, OH)	584.58	67	-ve (-ve 50μM)	12 precipitation
1e	HO_2C, HO_2C–(aryl)	640.61	48	-ve (-ve 50μM)	2 after pre-centrifugation
1f	PhO_2C–(aryl, HO)	736.78	4	-ve (-ve 50μM)	90

Polymerization Assays. To try to understand the mode of action of these compounds, we carried out investigations to determine if these compounds could inhibit the polymerization of PrP^C by PrP-res. In this assay, the seed for polymerization was recombinant mouse PrP that was refolded and aggregated using Mn^{2+} ions. This was then used to seed the aggregation of recombinant mouse PrP (which was refolded in the absence of metal ions).[29] The aggregation was monitored by looking at the scattering of UV light. The effect of compounds on inhibiting aggregation was determined. It was discovered that some of the compounds degraded when kept in the UV beam, so the UV beam was not left on continually. Some of the compounds caused precipitation prior to the addition of the seed. This means that the interpretation of the data related to these compounds is unreliable. Where this is the case, it is marked in the tables.

For the sulfonamides (Table 1), several compounds showed an inhibition of polymerization. In particular, compounds **1a** and **1c**, which showed weak activity in the cellular assays, also showed an inhibition of polymerization. Compound **1b**, which was not active in the cellular assay, showed some activity in the polymerization assay. Compound **1a**, which showed the greatest inhibition of polymerization, contains the same terminator groups as **2a**, the lead compound.

For the amides with a biphenyl core (Table 2), compounds **2a** and **2b** showed an inhibition of polymerization. These compounds also showed activity in the cellular assays. However, compound **2h**, which showed an inhibition of PrP-res formation in the cellular assays, actually seemed to stimulate aggregation in the polymerization assay. Similarly, the other compounds assayed in this series appeared to stimulate aggregation.

In the third series of compounds investigated, the amides with a phenyl core (Table 3), compounds **3e**, **3g**, and **3h** appeared to cause the prevention of polymerization. The other compounds assayed were either inactive or caused precipitation.

In series 4, where compounds contained a biphenyl linker and an imine bond (Table 4), **4b** and **4c** showed some inhibition of polymerization. None of the other compounds investigated showed inhibition. The two compounds that inhibited polymerization are the two compounds that also have some effect on cell culture assays, indicating that the mode of action of these compounds may include the inhibition of polymerization. The terminator group on **4b** is the same as that on **2a**, and that on **4c** differs only in the carboxylate ester being replaced by a free carboxylate group.

The compounds of series 5 are those with a phenyl linker and an imine bond (Table 5). A number of these showed an inhibition of PrP^C polymerization: **5c**, **5d**, **5f**, **5g**, **5h**, and **5q**. The only compound in this series that was active in the cellular assay (**5s**) had a very small effect on polymerization. Also, the terminator group found in **2a** is not active in this series of

Table 2. Amides: Synthetic Yield, Inhibition of PrP-res Formation in SMB Cells at 10 μM, and Inhibition of Polymerization at 100 μM[a]

R–NH–C(O)–C$_6$H$_4$–C$_6$H$_4$–C(O)–NH–R

Entry	R	MW	Yield	Inhibition of PrP-res formation in SMB cells at 10μM	Polymerization (% of control)
2a	MeO$_2$C, OH	540.52	10-14	+ve	17-50
2b	MeO$_2$C, MeO	568.57	46	+ve	70
2c	OH	424.45	14	-ve	430 precipitated
2d		392.45	48	-ve	171
2e	HO$_2$C, MeO	540.52	32	-ve	472
2f	O$_2$N, OH	514.44	74	-ve	160
2g	MeO$_2$C, HO	540.52	60	-ve	NR precipitated
2h	HO, MeO$_2$C	540.52	51	+ve	249
2i	NC	442.47	63	-ve	196

[a] Inhibition of polymerization at 100 μM.

compounds (compound **5a**). The presence of a carboxylate group (either as the free acid or as an ester) seemed to be important for activity. Compounds not containing these functionalities were not active in this assay. Compound **5e** is a direct homologue of **5d** (one of the most active compounds), except that it has an extra methylene between the terminator and the imine but is inactive, suggesting that conjugation with the terminator is an important feature.

For the amine-linked compounds (series 6, Table 6), several of the compounds showed the inhibition of PrP-res formation, **6a** and **6c**. However, the other two compounds show no activity. Of particular note here is **6b**, which stimulated polymerization but was one of the most potent compounds in the cellular assay. This may indicate that there is more than one mechanism of action for these compounds. Finally, **7a** (Table 7) shows some inhibition of polymerization.

Detachment Assays. Another possible mode of action of the compounds would be detaching PrP^C from the surface of the cells. Therefore, the supernatant was examined to determined if there were increased levels of PrP^C in treated cells compared to control levels. However, none was detected, suggesting that the primary mode of action of **2a** is not the removal of the PrP^C from the surface of the cell.

Toxicity Assays. It is important that the compounds are not toxic to neuronal cells. Therefore, a toxicity assay was done using cultures of neurons isolated from mice with the lead compound (**2a**) (Figure 6). The compound was found to be nontoxic at 0.1 μM. There was a very low level of toxicity at 0.5 μM and an IC_{50} of around 30–40 μM. This indicates that **2a** causes selective action against TSE-infected cells. For comparison, the toxicity of compound **3g** was investigated. This compound had no effect on levels of PrP-res in the cell

Table 3. Phenyl Amides: Synthetic Yield, Inhibition of PrP-res Formation in SMB Cells at 10 μM, and Inhibition of Polymerization at 100 μM

R–NH–C(O)–C$_6$H$_4$–C(O)–NH–R

Entry	R	MW	Yield	Inhibition of PrP-res formation in SMB cells at 10μM	Polymerization (% of control)
3a	MeO$_2$C, OMe	492.48	83	-ve	70 precipitated
3b	MeO$_2$C, MeO$_2$C	548.50	94	+ve	147
3c	HO$_2$C, HO$_2$C	492.39	77	-ve	1 precipitated
3d	MeO$_2$C–	432.13	47	-ve	100
3e		316.35	92	-ve	54
3f	O$_2$N–, OH	438.08	90	-ve	15 precipitated
3g	NC–	366.37	65	-ve	68
3h	MeO$_2$C–, OH	464.42	56	+ve	62

culture assays and also showed a higher toxicity to neurons compared to **2a**.

Discussion

As outlined in the Introduction, the scientific literature contains reports on a number of compounds that have been investigated for the treatment of prion diseases. Some of these have been tested in animal models of infection. These include the sulfated polysaccharides (pentosan polysulfate being the most studied example), which can protect mice from scrapie infection when injected intraperitoneally immediately after infection but has poor blood–brain barrier permeability.[9] Amphotericin B has shown some promise in animal studies,[30] being able to extend the incubation time for the disease, even given some time post-infection, but was disappointing when used in a human case.[31] In addition, the drug is very toxic. Recently, quinacrine and chlorpromazine have been highlighted as potential agents for the treatment of these diseases.[10] However, quinacrine suffers from toxicity problems and had no significant effects in an animal model of infection.[32] Work from Prusiner's group has led to the development of some bis-acridines, which show very good activity in cellular models, although no in vivo data has been reported on these to date.[11] The anthracycline IDX has also been shown to have activity in animal models of infection; however, it has poor blood–brain barrier permeability, which would limit its therapeutic value.[33] Porphyrins and phthalocyanines have also been investigated and have shown the ability to increase the incubation period of scrapie-infected mice.[34] Congo red and other azo dyes have been investigated as potential agents against prion diseases. Congo red has been shown to increase the incubation time of the disease in mouse models of infection,[16] but it has very low blood–brain barrier permeability and is also unsuitable as a drug as previously described.

Unfortunately, of those tested in animal models of disease, all of the compounds have limitations, and there is an urgent need for the development of therapeutic agents for these diseases.[35] This lack of therapeutic

Table 4. Biphenyl Imines: Synthetic Yield, Inhibition of PrP-res Formation in SMB Cells at 10 μM, and Inhibition of Polymerization at 100 μM

R–N=CH–C6H4–C6H4–CH=N–R

Entry	Terminator	MW	Yield	Inhibition of PrP-res formation in SMB cells at 10μM	Polymerization (% of control)
4a	MeO2C, OMe	536.6	14	-ve	
4b	MeO2C, OH	508.52	45	-ve (53% of PrP-res at 50μM)	49
4c	HO2C, OH	480.47	28	+ve	62
4d	OH	392.45	70	-ve	281
4e	HO	392.45	69	+ve	104
4f		360.45	74	-ve	315

agents has lead us to explore Congo red further as a drug lead.

The compounds described in this paper can be categorized in four ways as a result of these assays:

(i) Compounds that are active in both the cellular and polymerization assay. These compounds may be preventing PrP-res formation in cells by preventing polymerization of PrP^C (**2a**, **2b**, **3h**, **4c**, **5s**, and **6a**).

(ii) Compounds that are active in the polymerization assay but not in the cellular assay. Either these compounds are too weak at inhibiting polymerization to be active in cellular assays, or they are inactivated in a cellular system, or they are unable to access the molecular target. It is possible that some structural modification can be used to give activity in cellular models (**1a**, **1b**, **1c**, **3e**, **3g**, **4b**, **5d**, **5f**, **5g**, **5h**, **5q**, **6c**, and **7a**).

(iii) Compounds that are active in the cellular assay but not in the polymerization assay. These compounds probably are preventing PrP-res formation in the SMB cells by a mode of action other than the inhibition of polymerization. Further investigation is required to determine their mode of action (**2h**, **3b**, **4e**, **6b**, and **6d**).

(iv) Compounds that are active in neither assay. They are unsuitable for further investigation (**1d**, **1e**, **1f**, **2c**, **2d**, **2e**, **2f**, **2g**, **2i**, **3a**, **3c**, **3d**, **3f**, **4d**, **4f**, **5a**, **5b**, **5c**, **5e**, **5i**, **5j**, **5l**, **5m**, **5n**, **5o**, and **5r**).

Both the cellular and polymerization assays provide useful information in the context of a drug discovery program. The polymerization assay provides information on the possible modes of action of compounds and can identify most, but not all, molecules that reduce PrP-res formation in cellular assays. On its own, however, it is not a useful screen to select out compounds for cellular assays. In addition, the cellular assay employed on its own also has limitations. The assay does not identify all compounds that prevent polymerization. These latter compounds may be a useful source of information for future rounds of drug design.

Effect of the Connector Group. A number of different connector groups have been investigated: sulfonamide, amide, imine, aminomethylene, and alkene. There appears to be some room for variation of the connector. The most promising results in the cellular assay were obtained with amide or aminomethylene connectors. Some of the imines were also active in these assays. The sulfonamide compounds were inactive in a cellular model of infection. There is too little data on the alkene linker to make conclusions.

The sulfonamides (series 1) appeared less active than the biphenyl amides (series 2). In general, the sulfonamide is a bioisostere of the amide group. However, in the case of the sulfonamides, the NH is ionizable and may well be ionized at physiological pH. Although ionization may well increase the solubility of these compounds, interaction with the prion protein may be also be prevented. However, some of the sulfonamides showed weak activity in a cellular model and an inhibition of polymerization.

The biphenyl amides showed particular promise, with compounds **2a**, **2b** and **2h** being active in the cellular assay. Compound **2a** is of great interest. For the biphenyl amides, there seems to be some correlation between the prevention of PrP-res formation in the cell culture and the prevention of the polymerization of PrP^C. This suggests that the anti-TSE activity of at least some of the compounds is related to their ability to prevent polymerization.

Imines are normally readily subject to hydrolysis. However in this case, the imines are highly conjugated,

Table 5. Phenyl Imines: Synthetic Yield, Inhibition of PrP-res Formation in SMB Cells at 10 μM, and Inhibition of Polymerization at 100 μM

R–N ... N–R

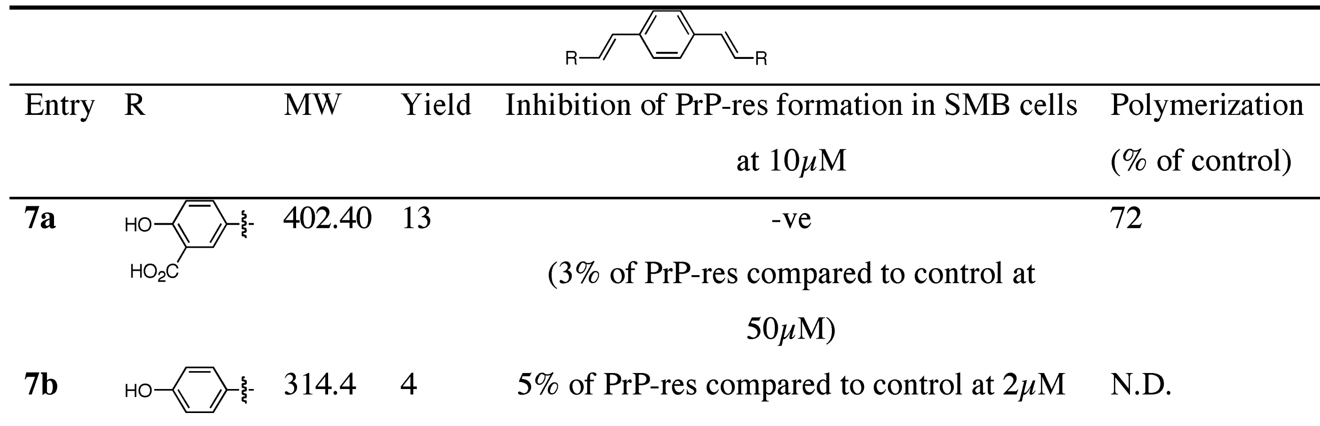
Entry	Terminator	MW	Yield	Inhibition of PrP-res formation in SMB cells at 10μM	Polymerization (% of control)
5a	MeO_2C, OH	432.44	76	-ve (-ve at 50μM)	140
5b	PhO_2C, HO	556.58	14	-ve (-ve at 50μM)	113
5c	MeO_2C, OMe	460.49	45	-ve (-ve at 50μM)	47
5d	HO_2C	372.38	78	-ve	12
5e	HO_2C	400.44	64	-ve	117
5f	HO_2C, OMe	432.44	84	-ve	11
5g	HO, HO_2C	404.38	66	-ve	53
5h	HO_2C, OH	404.38	74	-ve (–ve 50μM)	35
5i	OH	316.35	55	-ve	85
5j	HO	316.35	33	-ve	240
5k		284.35	74	-ve	
5l	CN	434.15	12	-ve	148
5m	O_2N, OH	406.09	64	-ve	109
5n	MeO_2C, HO	432.13	41	-ve	113
5o	EtO_2C, HO	460.48	30	-ve	92
5p	HO, MeO_2C	432.13	22	-ve	
5q	HO, EtO_2C	460.48	69	-ve	60
5r	OH	344.41	86	-ve	230
5s	NC	334.37	42	+ve	81

Table 6. Phenyl Amines: Synthetic Yield, Inhibition of PrP-res Formation in SMB cells at 10 μM, and Inhibition of Polymerization at 100 μM

R–NH–CH_2–C_6H_4–CH_2–HN–R

Entry	R	MW	Yield	Inhibition of PrP-res formation in SMB cells at 10μM	Polymerization (% of control)
6a	MeO_2C, OMe	464.52	23	+ve	20
6b	MeO_2C, OH	436.47	49	+ve	166
6c	HO_2C, OH	408.40	21	-ve	79
6d	OH	320.39	10	+ve	240

Table 7. Phenyl Alkenes: Synthetic Yield, Inhibition of PrP-res Formation in SMB Cells at 10 μM, and Inhibition of Polymerization at 100 μM



Entry	R	MW	Yield	Inhibition of PrP-res formation in SMB cells at 10μM	Polymerization (% of control)
7a	HO, HO_2C	402.40	13	-ve (3% of PrP-res compared to control at 50μM)	72
7b	HO	314.4	4	5% of PrP-res compared to control at 2μM	N.D.

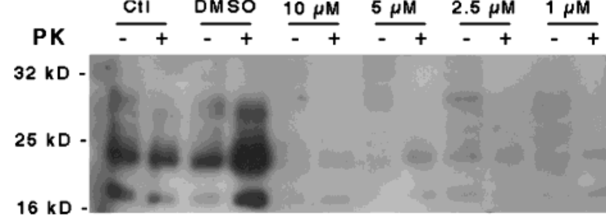


Figure 5. Western blot showing the effects of varying concentrations of compound **2a** on PrP-res formation in SMB Cells. Ctl = untreated control. DMSO = treated with the DMSO at the concentration used with **2a**. PK = proteinase K.

which stablizes them to hydrolysis. They should represent a very good mimic of the diazo bond found in Congo red. By ensuring that the nitrogen of the imine is attached to the terminator rather than the linker, any hydrolysis should not give rise to benzidene or its analogues. The biphenyl imines (series 4) showed higher activity than the corresponding monophenyl imines (series 5) in the cellular assay, although several of the monophenyl imines showed a potent inhibition of polymerization. These latter compounds tended to be compounds with carboxylates on the terminator: **5d**, and **5f**, **5g**, **5h**.

The aminomethylene compounds (series 6) show some very promising activity not only in the cellular assay but also in the polymerization assay.

Effect of the Terminator Group. Structure–activity relationships indicate that the most active compounds in cellular assays have a methyl carboxylate attached to the terminator and either a hydroxy or methoxy group. Clearly, the terminator found in compound **2a** is active in both the cellular and polymerization assays. This terminator group is generally active for other series of compounds in either the cellular or polymerization assays (**1a**, **3h**, **4b**, and **6b** but not **5a**). However, other regioisomers are also active (**2b** and **2h**). Some compounds containing a carboxylic acid on the terminator are active in the inhibition of polymerization but not in the cellular assay. Conversely, compounds that are active in the cellular assay and contain a methyl ester are not active in the polymerization assay (**2h**, **3b**, and **6b**). This could imply, assuming that the mechanism of action of these compounds is the inhibi-

Table 8. ED_{50} Values of Compounds in SMB Cells Showing Activity <10 μM

Entry	Structure	ED_{50} nM
2a		25-50
2b		250
2h		5000
3b		250
3h		5000
4e		250
5s		500
6a		250
6b		75
6d		2500

Figure 6. Cell viability of cerebellar neurones when incubated with **2a** and **3g**.

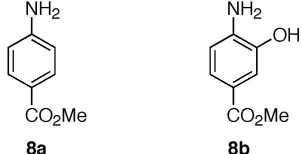


Figure 7. Terminator groups investigated for activity.

tion of polymerization, that the methyl esters are metabolized to the active form, the carboxylic acid, by the cells and then inhibit polymerization. Some evidence exists for this: **6c**, the demethylated analogue of **6b**, is active in the polymerization assay. The compounds that contain free carboxylic acids may not be active in the cellular assays because they cannot access the site of action. In addition, there are several compounds that are active in cellular and/or polymerization assays that do not have a carboxylate substituent, implying that this is not an essential substituent for activity.

To investigate the effect of the terminator group, we carried out assays with two of the terminator groups not attached to anything else. The terminator groups investigated were methyl-4-aminobenzoate (**8a**) and methyl-4-amino-3-hydroxybenzoate (**8b**), the terminator group of **2a** (Figure 7). Both of these compounds showed ED_{50} values of about 1 μM in the cellular assay, suggesting that the terminator has an important role in the anti-prion activity of the compounds.

Effect of the Linker. The linkers we used were the biphenyl and phenyl. In general, where it is possible to compare, the biphenyl compounds were more active than the phenyl compounds in cellular assays. This could be due to some kind of intermolecular interactions between the linker (the biphenyl group) and the molecular target or the linker maintaining the terminators at the correct distance.

Conclusions

In this paper, we have described the preparation of a number of compounds in which we have replaced the diazo bond of Congo red with a variety of other bonds, including imine, aminomethylene, and alkene. It is important to replace this bond because metabolism of this bond gives rise to benzidine in the gastrointestinal

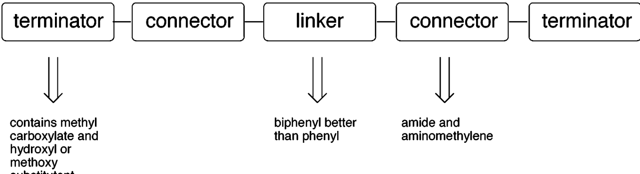


Figure 8. Most potent compounds in the cellular assays.

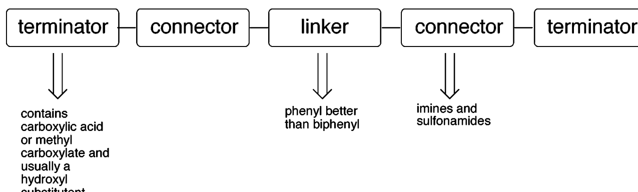


Figure 9. Most potent compounds in the polymerization assays.

tract. In addition, we have carried out a series of structure−activity studies with the terminator group and have prepared analogues with a much lower charge than is found in Congo red, which should facilitate delivery of the compounds across biological barriers. Compounds were assayed in a persistently infected scrapie cell line, the SMB cell line, and also in a cellular polymerization assay. The structure−activity relationships for the cellular model are shown in Figure 8, and those for the polymerization assay, in Figure 9.

Our work has yielded a number of compounds showing potent activity in a cellular model of prion diseases. The most potent compound is **2a**, which shows strong inhibition of PrP-res formation, with an ED_{50} in the range of 25−50 nM in the SMB cells. The compound appears to have a good selectivity index against neuronal cells, with an IC_{50} of 30−40 μM and a selectivity index of approximately 1000-fold, indicating that it could have a therapeutic window. In addition to compound **2a**, there are a variety of other analogues that show potent activity, with six other compounds showing an ED_{50} of <0.5 μM. These compounds represent exciting leads in this area and are significantly more potent than the starting material for this project, Congo red. They are low-molecular-weight compounds that probably have sufficient lipophilicity to cross the blood−brain barrier. They are also amenable to further structure−activity relationship studies.

The mode or modes of action of these compounds are not fully established but are probably due to preventing the formation of PrP-res. Detachment of PrP from the cellular surface has been discounted.

The approach that we adopted has yielded some interesting lead molecules that are worthy of further study. Even though the mechanisms of action of any of these compounds have not been elucidated, the compilation presents an array of compounds with structure−activity relationships. This work gives us a sound platform from which to launch further investigations.

Experimental Section

General Procedures. ^{1}H and ^{13}C NMR spectra were recorded for solutions (in specified solvents) on a Bruker Advance DPX300 spectrometer at 300 and 75 MHz, respectively. Chemical shifts are quoted in ppm downfield with respect to tetramethylsilane (δ = 0.00 ppm). Coupling constants (*J*) are quoted in Hertz to the nearest 0.5 Hz. Low-

Phenyl Compounds

Biphenyl Compounds

Figure 10. Numbering system used for assigning NMR spectra.

resolution ES mass spectrometry was performed on a Fisons VG platform II electrospray mass spectrometer at the Welsh School of Pharmacy, Cardiff University. Low-resolution EI, CI, and FAB and all high-resolution mass spectra were recorded on a VG ZAB spectrometer at the Engineering and Physical Sciences Research Council (EPSRC) Mass Spectrometry Center at the University College of Wales, Swansea. IR spectra were recorded on an Perkin-Elmer 1600 series spectrophotometer using sodium chloride plates for liquid samples and potassium bromide for solid samples. Elemental analyses were obtained from MEDAC Analytical and Chemical Consultancy Services, Ltd. Melting points are uncorrected values and were determined using a Gallenkamp melting-point apparatus.

All reactions were performed in an oven-dried apparatus under an atmosphere of nitrogen. All solvents were of reagent grade. Anhydrous diethyl ether, methanol, and tetrahydrofuran were purchased from Fluka. Anhydrous *N,N*-dimethylacetamide, *N,N*-dimethylformamide, and 1,4-dioxane were purchased from Aldrich Chemical Company. Reaction temperatures of −78 and 0 °C were achieved by the use of acetone/dry ice baths and water/ice baths, respectively. Thin-layer chromatography (TLC) was performed on Fluka aluminum-backed silica plates. Compounds were visualized under a UV lamp with aqueous potassium permanganate solution and/or phosphomolybdic acid in ethanolic solution. Column chromatography was performed on Fluka silica gel (with the solvent mixtures specified).

The assignment of spectra was done according to the numbering system shown in Figure 10. The numbering of terminators was done according to the normal IUPAC rules except using the symbol ″ to differentiate from the numbering of the core.

Synthesis of Sulfonamide Analogues. Bis-methyl 4-({[4′-(Aminosulfonyl)-1,1′-biphenyl-4-yl]sulfonyl}amino)-3-hydroxybenzoate (1a). To DMAP (0.012 g, 0.099 mmol) and methyl-4-amino-3-hydroxybenzoate (0.366 g, 2.2 mmol) in anhydrous pyridine (10 mL) at 0 °C was added 4,4′-biphenyldisulfonyl chloride (0.350 g, 0.99 mmol). The reaction was stirred at room temperature overnight (14 h). HCl (50 mL, 2 N) was added, and the organic phase was extracted with ethyl acetate (2 × 25 mL). The organic layer was separated, dried ($MgSO_4$), and evaporated in vacuo. Column chromatography in a 4:1 ethyl acetate/petroleum ether mixture yielded product **1a** as a white solid (0.169 g, 0.276 mmol, 28%); mp 289−290 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 3.77 (6H, s, OMe), 7.35 (6H, m, $C_{2''}$−H + $C_{5''}$−H +$C_{6''}$−H), 7.89 (8H, br, s, C_2−H + C_3−H), 9.97 (4H, br, s, NH + OH); ^{13}C NMR (75 MHz, pyridine-d_5) δ 54.2 (OMe), 119.2 (COH), 123.9 ($C_{6''}$), 125.0 ($C_{1''}$), 129.7 ($C_{2''}$), 130.3 (C_3), 130.5 (C_2), 133.7 (CNH), 143.4 (C_4), 145.5 (C_1), 152.4 ($C_{5''}$), 168.9 (*C*OO); ES$^-$ *m*/*z* 610.9 (M$^-$); high-resolution ES$^-$ *m*/*z* found 611.0787 $C_{28}H_{23}N_2O_{10}S_2$ (M − H) requires 611.0794. Anal. ($C_{28}H_{24}N_2O_{10}S_2 \cdot 0.2C_5H_5N$) C, H, N.

Bis-methyl 3-({[4′-(Aminosulfonyl)-1,1′-biphenyl-4-yl]sulfonyl}amino)-4-methoxybenzoate (1b). An identical procedure to the one employed for **1a** was used. DMAP (0.017 g, 0.142 mmol) and methyl-3-amino-4-methoxybenzoate (0.567 g, 3.13 mmol) were reacted with 4−4′-biphenyldisulfonyl chloride (0.350 g, 0.99 mmol) in anhydrous pyridine (10 mL). Product **1b** was isolated as an off-white solid (0.458 g, 0.71 mmol, 50%); mp 265−268 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 3.57 (6H, s,OMe), 3.80 (6H, s, COOMe), 7.01 (2H, d, J = 8.7 Hz, $C_{5''}$−H), 7.78−7.90 (12H, m, $C_{2''}$−H + $C_{6''}$−H + C_3−H + C_2−H), 9.89 (2H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 52.3 (COOMe), 56.1 (OMe), 112.0 ($C_{5''}$), 122.0 ($C_{2''}$), 125.6 ($C_{6''}$), 126.4 ($C_{1''}$), 127.7 (C_3), 127.9 (C_2), 128.8 (CNH), 140.3 (C_4), 142.7 (C_1), 156.5 ($C_{4''}$), 165.8 (*C*OO); ES$^-$ *m*/*z* 638.8 (M$^-$); high-resolution ES$^+$ *m*/*z* found 658.1531 $C_{30}H_{32}N_3O_{10}S_2$ (M + NH_4) requires 658.1529. Anal. ($C_{30}H_{28}N_2O_{10}S_2$) C, H, N.

Bis-3-({[4′-(aminosulfonyl)-1,1′-biphenyl-4-yl]sulfonyl}amino)-4-methoxybenzoic acid (1c). To compound **1b** (0.100 g, 0.156 mmol) was added dropwise an aqueous solution of NaOH (4 mL of a 0.15 M solution, 0.625 mmol). The mixture was left stirring at room temperature for 2 h. HCl (5 mL, 2 N) was added dropwise, and a white precipitate was formed. The solid was filtered and dried in vacuo. Product **1c** was isolated as a white solid (0.030 g, 0.076 mmol, 49%); mp >360 °C (decomp.); ^1H NMR (300 MHz, DMSO-d_6) δ 3.53 (6H, s, OMe), 7.00 (2H, d, J = 8.6 Hz, $C_{5''}$−H), 7.72−7.90 (12H, m, $C_{2''}$−H + $C_{6''}$−H + C_3−H + C_2−H), 9.84 (2H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.1 (OMe), 111.8 ($C_{5''}$), 123.2 ($C_{2''}$), 125.4 ($C_{6''}$), 126.7 ($C_{1''}$), 127.7 (C_3), 127.9 (C_2), 128.9 (CNH), 140.4 (C_4), 142.7 (C_1), 156.3 ($C_{4''}$), 166.9 (COOH); ES$^-$ *m*/*z* 610.8 (M − H)$^-$, 305.0 ({M − 2H}$^-$/2); high-resolution ES$^-$ *m*/*z* found 633.0606 $C_{28}H_{22}N_2O_{10}S_2Na$ requires 633.0614.

Bis-4-({[4′-(aminosulfonyl)-1,1′-biphenyl-4-yl]sulfonyl}amino)-3-hydroxybenzoic acid (1d). An identical procedure to the one employed for **1c** was used. **1a** (0.086 g, 0.14 mmol) was reacted with aqueous NaOH (4 mL of a 1.4 M solution, 0.023 g, 0.56 mmol), and product **1d** was isolated as a light-brown solid (0.045 g,0.094 mmol, 55%); mp >360 °C (decomp.); ^1H NMR (300 MHz, DMSO-d_6) δ 7.32 (6H, br, s, $C_{2''}$−H + $C_{5''}$−H + $C_{6''}$−H), 7.89−8.04 (8H, br, m, C_2−H + C_3−H), 9.76 (2H, s, OH), 10.14 (2H, s, NH), 12.77 (2H, br, s, CO_2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 116.5 ($C_{5''}$), 118.5 ($C_{2''}$), 122.7 ($C_{1''}$), 124.1 ($C_{6''}$), 129.6 (C_3), 129.9 (C_2), 130.9 (C_4), 142.2 (CNH), 144.5 (C_1), 150.9 (COH), 168.9 (COOH); ES$^-$ *m*/*z* 582.5 (M − H)$^-$, 291.1 ({M − 2H}$^-$/2); high-resolution ES$^-$ *m*/*z* found 583.0494 $C_{26}H_{19}N_2O_{10}S_2$ (M − H) requires 583.0487; Anal. ($C_{26}H_{16}N_2Na_4O_{10}S_2 \cdot 1.5H_2O$) C, H, N.

Bis-dimethyl 5-({[4′-(Aminosulfonyl)-1,1′-biphenyl-4-yl]sulfonyl}amino)isophthalate (1e). An identical procedure to the one employed for **1a** was used. DMAP (0.024 g, 0.199 mmol) and dimethyl-5-aminoisophthalate (0.917 g, 4.38 mmol) were reacted with 4,4′-biphenyldisulfonyl chloride (0.700 g, 1.99 mmol) in anhydrous pyridine (15 mL). The required product (the tetramethylester) was not formed; instead, it was hydrolyzed to **1e** during the workup. Product **1e** was isolated as an off-white solid (0.0618 g, 0.96 mmol, 48%); mp 278−281 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 7.83−7.95 (12H, m, $C_{4''}$−H + $C_{6''}$−H + C_2−H + C_3−H), 8.12 (2H, s, $C_{2''}$−H), 10.89 (2H, s, NH), 13.34 (4H, br, s, COOH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 124.3 ($C_{2''}$), 125.7 ($C_{4''}$), 127.6 (C_3), 128.6 (C_2), 132.7 ($C_{3''}$), 138.8 (C_4), 139.2 (C_1), 142.9 (CNH), 166.3 (COOH); ES$^-$ *m*/*z* 638.5(M$^-$), 319.0 (m/2z); high-resolution ES$^-$ *m*/*z* found 639.0373 $C_{28}H_{19}N_2O_{12}S_2$ (M − H) requires 639.0379; Anal. ($C_{28}H_{20}N_2O_{12}S_2 \cdot 2.5H_2O$) C, H, N.

Bis-phenyl 4-({[4′-(Aminosulfonyl)-1,1′-biphenyl-4-yl]sulfonyl}amino)-2-hydroxybenzoate (1f). An identical procedure to the one employed for **1a** was used. DMAP (0.026 g, 0.216 mmol) and phenyl-4-aminosalicylate (1.09 g, 4.76 mmol) were reacted with 4,4′-biphenyldisulfonyl chloride (0.760 g, 2.16 mmol) in anhydrous pyridine (15 mL). Product **1f** was isolated as a white solid (0.065 g, 0.088 mmol, 4%); mp 266−270 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 6.77−6.81 (4H, m, $C_{3''}$−H + $C_{5''}$−H), 7.21−7.46 (10H, m, Ar−H), 7.87 (2H, d, J = 9.3 Hz, $C_{6''}$−H), 7.96 (8H, s, C_2−H + C_3−H), 10.39 (2H, s, NH), 11.28 (2H, br, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 103.9 ($C_{3''}$), 106.4 ($C_{5''}$), 108.1 ($C_{1''}$), 120.4 (Ar−C), 124.5 (Ar−C), 125.9 (C_3), 126.7 (C_2), 127.9 (Ar−C), 130.8 ($C_{6''}$), 137.5 (C_4), 141.2 (C_1), 148.5 (Ar−C + CNH), 159.7 (COH), 164.7 (COO); ES$^-$ *m*/*z* 734.7 (M$^-$); high-resolution ES$^-$ *m*/*z* found 735.1100 $C_{38}H_{27}N_2O_{10}S_2$ (M − H) requires 735.1107; Anal. ($C_{38}H_{28}N_2O_{10}S_2 \cdot 0.2H_2O$) C, H, N.

Synthesis of Amide Analogues. Bis-methyl 4-({[4′-(Aminocarbonyl)-1,1′-biphenyl-4-yl]carbonyl}amino)-3-hydroxybenzoate (2a). To a mixture of biphenyl 4,4′ dicarboxylic acid (0.300 g, 1.24 mmol) and TBTU (0.875 g, 2.72 mmol) in anhydrous DMA (15 mL) was added DIPEA (0.7 mL, 3.71 mmol). After stirring at room temperature for 15 min, methyl-4-amino-3-hydroxybenzoate (0.455 g, 2.72 mmol) was dissolved in DMA (5 mL) in a separate flask and added dropwise to the reaction flask. The reaction mixture was left stirring for 48 h at room temperature. HCl (40 mL, 2 N) was added, and the organic product was extracted with ethyl acetate (2 × 40 mL). The organic phase was dried ($MgSO_4$) and then evaporated in vacuo to leave the crude product as an off-white solid. Pure compound **2a** was obtained after recrystallization from ethyl acetate (0.070 g, 0.13 mmol, 10%); mp 286−288 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.75 (6H, br, s, OMe), 6.08 (4H, br s, NH + OH), 6.80 (2H, br, s, $C_{2''}$−H), 7.58 (4H, br, s, $C_{5''}$−H + $C_{6''}$−H), 8.00 (4H, br, s, C_2−H), 8.27 (4H, br, s, C_3−H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 52.2 (OMe), 115.4 ($C_{2''}$), 116.6 ($C_{5''}$), 124.8 ($C_{6''}$), 127.9 ($C_{1''}$), 129.1 (C_2), 129.6 (C_3), 131.6 (CNH), 136.1 (C_4), 144.4 (C_1), 146.5 (COH), 164.9 (CONH), 166.5 (COO); ES$^-$ *m/z* 538.9 (M^-); high-resolution ES$^+$ *m/z* found 558.1878 $C_{30}H_{28}N_3O_8$ (M + NH_4) requires 558.1876; Anal. ($C_{30}H_{24}N_2O_8$) C, H, N.

Bis-methyl 4-({[4′-(Aminocarbonyl)-1,1′-biphenyl-4-yl]-carbonyl}amino)-3-hydroxybenzoate (2a) (Alternative Procedure). A mixture of methyl-4-amino-3-hydroxybenzoate (0.438 g, 2.15 mmol) and triethylamine (0.45 mL, 3.22 mmol) in anhydrous THF (15 mL) was cooled to 0 °C. After 10 min at 0 °C, 4,4′-biphenyldicarboxyl chloride (0.300 g, 1.07 mmol) was added to the reaction mixture, and a solid formed immediately. The mixture was left stirring at room temperature for 18 h. Water (50 mL) was added, and the organic product was extracted with ethyl acetate (2 × 40 mL). The organic phase was washed with 2 N HCl (50 mL), $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer was then dried ($MgSO_4$) and concentrated in vacuo. Column chromatography (1:1 ethyl acetate/hexane) afforded product **2a** as a slightly off-white solid (0.080 g, 0.15 mmol, 14%). Characterization data was identical to that obtained with the initial experimental procedure.

Bis-methyl 4-({[4′-(Aminocarbonyl)-1,1′-biphenyl-4-yl]-carbonyl}amino)-2-methoxybenzoate (2b). Compound **2b** was made identically to **2a** (alternative procedure). Methyl-4-amino-2-methoxybenzoate (0.260 g, 1.43 mmol) and triethylamine (0.2 mL, 1.42 mmol) were reacted with 4,4′-biphenyldicarboxyl chloride (0.200 g, 0.71 mmol) in anhydrous THF (10 mL). An identical workup procedure was employed, and product **2b** was isolated as an off-white solid (0.186 g, 0.33 mmol, 46%); mp 280−283 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.77 (6H, s, OMe), 3.84 (6H, s, COO*Me*), 7.55 (2H, d, J = 6.0 Hz, $C_{5''}$−H), 7.74 (4H, m, $C_{3''}$−H + $C_{6''}$−H), 7.98 (4H, d, J = 6.9 Hz, C_2−H), 8.13 (4H, d, J = 6.9 Hz, C_3−H), 10.58 (2H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 51.9 (COO*Me*), 55.9 (OMe), 103.9 ($C_{3''}$), 111.6 ($C_{1''}$), 114.5 ($C_{5''}$), 127.3 (C_2), 128.9 (C_3), 130.3 ($C_{6''}$), 132.4 (C_4), 134.2 (C_1), 142.5 (CNH), 144.6 ($C_{2''}$), 159.6 (CONH), 165.7 (COO); EI *m/z* 568.3 (M^+); high-resolution EI *m/z* found 568.1842 $C_{32}H_{28}N_2O_8$ requires 568.1840; Anal. ($C_{32}H_{28}N_2O_8$·0.5H_2O) C, H, N.

Bis-*N,N*′-(2-hydroxyphenyl)-1,1′-biphenyl-4,4′-dicarboxamide (2c). Compound **2c** was made identically to **2a** (alternative procedure). 2-Aminophenol (0.234 g, 2.15 mmol) and triethylamine (0.45 mL, 3.21 mmol) were reacted with 4,4′-biphenyldicarboxyl chloride (0.300 g, 1.07 mmol) in anhydrous THF (10 mL). An identical workup procedure was employed, and product **2c** was isolated as an off-white solid (0.065 g, 0.153 mmol, 14%); mp 260−263 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.87 (2H, t, J = 7.5 Hz, $C_{6''}$−H), 6.95 (2H, d, J = 7.9 Hz, $C_{5''}$−H), 7.07 (2H, t, J = 7.5 Hz, $C_{4''}$−H), 7.71 (2H, d, J = 7.9 Hz, $C_{3''}$pH), 7.95 (4H, d, J = 8.2 Hz, C_3−H), 8.12 (4H, d, J = 8.2 Hz, C_2−H), 9.64 (2H, s, NH), 9.78 (2H, br, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 116.3 ($C_{6''}$), 119.4 ($C_{4''}$), 124.7 ($C_{3''}$), 126.2 (CNH), 127.3 ($C_{5''}$), 128.7 (C_2), 134.1 (C_3), 142.3 (C_4), 149.9 (C_1), 159.9 ($C_{1''}$), 165.1 (CONH); ES$^+$ *m/z* 447.2 (M + Na); high-resolution ES$^+$ *m/z* found 447.1319 $C_{26}H_{20}N_2O_4$-Na requires 447.1321; Anal. ($C_{26}H_{20}N_2O_4$.0.09HCl) C, H; N calcd 6.55%, N found 6.11%.

***N,N*′-Diphenyl-1,1′-biphenyl-4,4′-dicarboxamide (2d).** 4,4′-Biphenyldicarboxyl chloride (0.300 g, 1.07 mmol) and aniline (0.2 mL, 2.15 mmol) in anhydrous THF (15 mL) were stirred for 1 h at room temperature, and a solid precipitated, which was filtered and washed with water (30 mL) and hexane (20 mL) to leave product **2d** as a white solid. Yield 0.202 g (0.51 mmol, 48%); mp >360 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.12 (2H, m, $C_{4''}$−H), 7.35 (4H, m, $C_{3'',5''}$−H), 7.80 (4H, d, J = 7.7 Hz, $C_{2'',6''}$−H), 7.93 (4H, d, J = 8.3 Hz, C_2−H), 8.09 (4H, d, J = 8.3 Hz, C_3−H), 10.34 (2H, s, NH). ^{13}C NMR could not be done because of insolubility. Anal. ($C_{26}H_{20}N_2O_2$·0.5HCl) C, H, N.

Bis-4-({[4′-(aminocarbonyl)-1,1′-biphenyl-4-yl]carbonyl}-amino)-2-methoxybenzoic Acid (2e). An identical procedure to the one employed for **1c** was used. **2b** (0.100 g, 0.176 mmol) was reacted with aqueous NaOH (0.070 g, 1.76 mmol) in 1 mL of water and THF (5 mL). Product **2e** was isolated as a brown solid (0.030 g, 0.05 mmol, 32%); mp 280 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.84 (6H, s, OMe), 7.54 (2H, d, J = 8.6 Hz, $C_{5''}$−H), 7.86−8.16 (12H, m, $C_{3''}$−H + $C_{6''}$−H + C_2−H + C_3−H), 10.60 (2H, s, NH), 12.70 (2H, br, s, COOH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 55.9 (OMe), 104.0 ($C_{3''}$), 111.6 ($C_{1''}$), 115.6 ($C_{5''}$), 127.3 (C_2), 127.5 ($C_{3''}$), 130.6 ($C_{6''}$), 132.5 (C_4), 142.5 (C_1), 144.3 (CNH), 159.6 ($C_{2''}$), 165.7 ($CONH_9$), 167.4 (COO); ES$^+$ *m/z* 563.3 (M + Na); high-resolution ES$^+$ *m/z* found 563.1407 $C_{30}H_{24}N_2O_8Na$ requires 563.1430.

Bis-*N,N*′-(2-hydroxy-4-nitrophenyl)-1,1′-biphenyl-4,4′-dicarboxamide (2f). This compound was made identically to **2a** (alternative procedure). 2-Amino-5-nitrophenol (0.331 g, 2.15 mmol) and triethylamine (0.45 mL, 3.21 mmol) were reacted with 4,4′-biphenyldicarboxyl chloride (0.300 g, 1.07 mmol) in anhydrous THF (10 mL). An identical workup procedure was employed, and product **2f** was isolated as an orange/red solid (0.406 g, 0.79 mmol, 74%); mp 302−304 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.81 (2H, s, $C_{6''}$−H), 6.87 (2H, d, J = 9.0 Hz, $C_{3''}$−H), 7.98 (2H, d, J = 9.0 Hz, $C_{4''}$−H), 8.05 (4H, d, J = 8.2 Hz, C_2−H), 8.30 (4H, d, J = 8.2 Hz, C_3−H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 119.9 ($C_{6''}$), 114.3 ($C_{4''}$), 120.2 (C_3), 127.6 (C_2), 128.9 (C_3), 131.4 (CNH), 134.9 (C_4), 135.4 (C_1), 144.1 ($C_{5''}$), 148.7 (COH), 164.4 (CONH); Anal. ($C_{26}H_{18}N_4O_8$·0.3H_2O) C, H, N.

Bis-methyl 4-({[4′-(Aminocarbonyl)-1,1′-biphenyl-4-yl]-carbonyl}amino)-2-hydroxybenzoate (2g). This compound was made identically to **2a** (alternative procedure). Methyl-4-amino-2-hydroxybenzoate (0.238 g, 1.43 mmol) and triethylamine (0.30 mL, 2.13 mmol) were reacted with 4,4′-biphenyldicarboxyl chloride (0.200 g, 0.71 mmol) in anhydrous THF (10 mL). An identical workup procedure was employed, and product **2g** was isolated as a white solid (0.230 g, 0.43 mmol, 60%); mp 246−250 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.85 (6H, s, OMe), 7.39 (2H, d, J = 8.3 Hz, $C_{5''}$−H), 7.87−8.35 (12H, m, $C_{3''}$−H + $C_{6''}$−H + C_2−H + C_3−H); 10.75 (2H, s, NH), 10.90 (2H, br, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 51.9 (OMe), 106.9 ($C_{3''}$), 111.8 ($C_{5''}$), 115.0 ($C_{1''}$), 127.3 (C_2), 127.6 (C_3), 130.3 ($C_{6''}$), 131.4 (C_4), 144.9 (C_1), 151.3 (CNH), 156.4 (COH), 163.2 (CONH), 166.0 (COO); Anal. ($C_{30}H_{24}N_2O_8$·0.4H_2O) C, H, N.

Bis-methyl 5-({[4′-(Aminocarbonyl)-1,1′-biphenyl-4-yl]-carbonyl}amino)-2-hydroxybenzoate (2h). This compound was made identically to **2a** (alternative procedure). Methyl-3-amino-5-hydroxybenzoate (0.357 g, 2.15 mmol) and triethylamine (0.45 mL, 3.21 mmol) were reacted with 4,4′-biphenyldicarboxyl chloride (0.300 g, 1.07 mmol) in anhydrous THF (10 mL). An identical workup procedure was employed, and product **2h** was isolated as a white solid (0.294 g, 0.54 mmol, 51%); mp 290−292 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.93 (6H, s, OMe), 7.03 (2H, d, J = 8.9 Hz, $C_{3''}$−H), 8.11 (4H, d, J = 7.4 Hz, C_3−H), 7.94 (6H, m, $C_{4''}$−H + C_2−H), 8.35 (2H, s, $C_{6''}$−H), 10.37 (4H, s, OH + NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 52.9 (OMe), 112.8 ($C_{3''}$), 117.8 ($C_{1''}$), 121.8 ($C_{6''}$), 127.2 ($C_{4''}$), 128.7 (C_2), 128.9 (C_3), 131.4 (CNH), 134.8 (C_4), 142.3 (C_1), 156.7 (COH), 165.1 (CONH), 169.4 (COO); EI *m/z* 540.2 $(M + H)^+$;

high-resolution EI *m/z* found 541.1605 $C_{30}H_{25}N_2O_8$ (M + H) requires 541.1604; Anal. ($C_{30}H_{24}N_2O_8$·0.3H_2O) C, H, N.

Bis-*N,N'*-(4-cyanophenyl)-1,1'-biphenyl-4,4'-dicarboxamide (2i). This compound was made identically to **2a** (alternative procedure). 4-Aminobenzonitrile (0.254 g, 2.15 mmol) and triethylamine (0.5 mL, 3.21 mmol) were reacted with 4,4'-biphenyldicarboxyl chloride (0.300 g, 1.07 mmol) in anhydrous THF (20 mL). An identical workup procedure was employed, and product **2i** was isolated as a white solid (0.300 g, 0.68 mmol, 63%); mp 335−338 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.84−8.13 (16H, m, $C_{2'',6''}$−H + $C_{3'',5''}$−H + C_2−H + C_3−H), 10.74 (2H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 105.8 ($C_{1''}$), 120.6 (CN), 127.5 ($C_{3''}$), 129.0 (C_2), 130.4 (C_3), 133.5 ($C_{2''}$), 134.1 (C_4), 142.7 (C_1), 143.8 (CNH), 166.1 (CONH); ES$^-$ *m/z* 441.1 (M − H$^-$); high-resolution ES$^-$ *m/z* found 441.1343 $C_{28}H_{17}N_4O_2$ requires 441.1352; Anal. ($C_{28}H_{18}N_4O_2$·0.6H_2O) C, H; N calcd 12.36%, N found 10.60%.

Methyl 3-[(4-{[(2-Methoxy-5-(methoxycarbonyl)phenyl)-amino]carbonyl}benzoyl)amino]-4-methoxybenzoate (3a). This compound was made identically to **2a** (alternative procedure). Methyl-3-amino-4-methoxybenzoate (0.892 g, 4.92 mmol) and triethylamine (1.1 mL, 7.38 mmol) were reacted with terephthaloyl chloride (0.500 g, 2.46 mmol) in anhydrous THF (10 mL). An identical workup procedure was employed, and product **3a** was isolated as an off-white solid (1.006 g, 2.04 mmol, 83%); mp 266−268 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.89 (6H, s, OMe), 3.98 (6H, s, COO*Me*), 7.27 (2H, d, J = 8.6 Hz, $C_{5''}$−H), 7.88 (2H, d, J = 8.7 Hz, $C_{6''}$−H), 8.15 (4H, s, C_2−H), 8.48 (2H, s, $C_{2''}$−H), 9.85 (2H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 52.3 (COO*Me*), 56.5 (OMe), 111.7 ($C_{5''}$), 121.8 ($C_{2''}$), 125.7 (CNH), 128.1 (C_2), 129.9 ($C_{6''}$), 137.2 (C_1), 155.8 ($C_{4''}$), 164.9 (CONH), 166.1 (COO); Anal. ($C_{26}H_{24}N_2O_8$·0.2H_2O) C, H, N.

Methyl 3-[(4-{[(3-(Methoxycarbonyl)-5-(methoxycarbonyl)phenyl)amino]carbonyl}benzoyl)amino]-5-(methoxycarbonyl)benzoate (3b). This compound was made identically to **2a** (alternative procedure). Dimethyl-5-amino-isophthalate (1.030 g, 4.92 mmol) and triethylamine (1.1 mL, 7.38 mmol) were reacted with terephthaloyl chloride (0.500 g, 2.46 mmol) in anhydrous THF (15 mL). An identical workup procedure was employed, and product **3b** was isolated as a white solid (1.266 g, 2.31 mmol, 94%); mp 303−306 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.92 (12H, s, OMe), 8.15 (4H, s, C_2−H), 8.22 (2H, s, $C_{2''}$−H), 8.74 (4H, s, $C_{4'',6''}$−H), 10.80 (2H, s, NH). ^{13}C NMR could not be done because of the insolubility of the compound. Anal. ($C_{28}H_{24}N_2O_{10}$·1H_2O) C, H, N.

3-[(4-{[(3-Carboxy-5-carboxyphenyl)amino]carbonyl}-benzoyl)amino]-5-carboxybenzoic Acid (3c). An identical procedure to the one employed for **1c** was used. **3b** (0.250 g, 0.456 mmol) was reacted with aqueous NaOH (0.291 g, 0.729 mmol) in water (4 mL), and product **3c** was isolated as a light-gray solid (0.172 g, 0.35 mmol, 77%); mp 320 °C (decomp.); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (4H, s, C_2−H), 8.24 (2H, s, $C_{2''}$−H), 8.68 (4H, s, $C_{4'',6''}$−H), 10.79 (2H, s, NH), 13.31 (4H, br, s, COOH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 125.1 ($C_{4'',6''}$), 125.5 ($C_{2''}$), 128.2 (C_2), 132.0 ($C_{3''}$), 137.4 (C_1), 140.0 (CNH), 165.4 (COOH), 166.8 (CONH); Anal. ($C_{24}H_{16}N_2O_{10}$.2HCl·2H_2O) C, H, N.

Methyl 4-[(4-{[(4-(Methoxycarbonyl)phenyl)amino]-carbonyl}benzoyl)amino]benzoate (3d). This compound was made identically to **2a** (alternative procedure). Methyl-4-aminobenzoate (0.551 g, 2.95 mmol) and triethylamine (1.1 mL, 7.38 mmol) were reacted with terephthaloyl chloride (0.300 g, 1.48 mmol) in anhydrous THF (10 mL). An identical workup procedure was employed, and product **3d** was isolated as a white solid (0.303 g, 0.70 mmol, 47%); mp 340−343 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.85 (3H, s, OMe), 7.99 (8H, m, $C_{3'',5''}$−H + $C_{2'',6''}$−H), 8.12 (4H, s, C_2−H), 10.74 (2H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 52.3 (OMe), 120.0 ($C_{3',5''}$), 124.8 ($C_{1''}$), 128. ($C_{2'',6''}$), 130.5 (C_2), 137.6 (C_1), 143.7 (CNH), 165.6 (CONH), 166.1 (COO); Anal. ($C_{24}H_{20}N_2O_6$) C, H, N.

***N,N'*-Diphenylterephthalamide (3e).** An identical procedure to the one employed for **2d** was carried out. Aniline (0.5 mL, 4.92 mmol) and terephthaloyl chloride (0.500 g, 2.46 mmol) were reacted in anhydrous THF (20 mL). Product **3e** was isolated as a white solid (0.719 g, 2.27 mmol, 92%); mp 340−342 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.14 (2H, t, J = 7.4 Hz, $C_{4''}$−H), 7.39 (4H, t, J = 7.9 Hz, $C_{3'',5''}$−H), 7.81 (4H, d, J = 7.9 Hz, $C_{2'',6''}$−H), 8.10 (4H, s, C_2−H), 10.42 (2H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 120.8 ($C_{2'',6''}$), 124.2 ($C_{4''}$), 128.1 (C_2), 129.0 ($C_{3'',5''}$), 137.8 (C_1), 139.3 (CNH), 165.2 (CONH); Anal. ($C_{20}H_{16}N_2O_2$) C, H, N.

***N,N'*-Bis-(2-hydroxy-4-nitrophenyl)terephthalamide (3f).** This compound was made identically to **2a** (alternative procedure), with the exception of adding DMAP as a catalyst (0.009 g, 0.689 mmol). 2-Amino-5-nitrophenol (1.062 g, 6.89 mmol) and triethylamine (0.45 mL, 3.21 mmol) were reacted with terephthaloyl chloride (0.700 g, 3.44 mmol) in anhydrous THF (15 mL). An identical workup procedure was employed, and product **3f** was isolated as an orange solid (1.362 g, 3.1 mmol, 90%); mp 283−286 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.71 (2H, s, $C_{3''}$−H), 7.73 (2H, d, J = 9.4 Hz, $C_{6''}$−H), 8.08 (4H, s, C_2−H), 8.26 (2H, d, J = 9.4 Hz, $C_{5''}$−H), 9.86 (2H, br, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 109.8 ($C_{3''}$), 114.0 ($C_{5''}$), 121.4 ($C_{6''}$), 128.2 (C_2), 133.6 (CNH), 137.2 (C_1), 143.9 ($C_{4''}$), 150.4 (COH), 164.7 (CONH); Anal. ($C_{20}H_{14}N_4O_8$·0.4H_2O0.4Et_3N) C, H, N.

***N,N'*-Bis-(4-cyanophenyl)terephthalamide (3g).** Compound **3g** was made identically to **2a** (alternative procedure). 4-Aminobenzonitrile (0.349 g, 2.95 mmol) and triethylamine (0.6 mL, 4.44 mmol) were reacted with terephthaloyl chloride (0.300 g, 1.48 mmol) in anhydrous THF (20 mL). An identical workup procedure was employed, and product **3g** was isolated as a white solid (0.351 g, 0.96 mmol, 65%); mp 343−346 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.86 (4H, d, J = 8.8 Hz, $C_{2'',6''}$−H), 8.03 (4H, d, J = 8.8 Hz, $C_{3'',5''}$−H), 8.13 (4H, s, C_2−H), 10.82 (2H, s, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 106.0 ($C_{1''}$), 119.4 (CN), 120.7 ($C_{3'',5''}$), 128.4 (C_2), 133.6 ($C_{2'',6''}$), 137.6 (C_1), 143.6 (CNH), 165.8 (CONH); Anal. ($C_{22}H_{14}N_4O_2$.0.2Et_3N·0.1H_2O) C, H, N.

Methyl 4-{[4-({[2-Hydroxy-4-(methoxycarbonyl)phenyl]-amino}carbonyl)benzoyl]amino}3-hydroxybenzoate (3h). Compound **3h** was made identically to **2a** (alternative procedure). Methyl-4-amino-4-hydroxybenzoate hydrogen sulfate salt (0.522 g, 1.97 mmol) and triethylamine (1.4 mL, 9.85 mmol) were reacted with terephthaloyl chloride (0.200 g, 0.98 mmol) in anhydrous THF (3 mL). An identical workup procedure was employed, and product **3h** was isolated as a light-brown solid (0.258 g, 0.56 mmol, 56%); mp 287−290 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.82 (6H, s, OMe), 7.48 (2H, d, J = 8.3 Hz, $C_{5''}$−H), 7.51 (2H, s, $C_{2''}$−H), 8.01 (2H, d, J = 8.3 Hz, $C_{6''}$−H), 8.09 (4H, s, C_2−H), 9.66 (2H, s, NH), 10.45 (2H, br, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 52.4 (OMe), 116.1 ($C_{2''}$), 120.8 ($C_{5''}$), 123.0 ($C_{6''}$), 126.5 (C_2), 128.2 ($C_{1''}$), 130.9 (CNH), 137.3 (C_1), 148.9 (COH), 164.9 (CONH), 166.3 (COO); Anal. ($C_{24}H_{20}N_2O_8$·0.5H_2O) C, H, N.

Synthesis of Imines. Bis-methyl 3-{[(1*Z*)-1,1'-Biphenyl-4-ylmethylene]amino}-4-methoxybenzoate (4a). Compound **4a** was prepared with an identical procedure to the one employed for **5a**. 4,4'-Biphenyldialdehyde (0.100 g, 0.47 mmol) and methyl-3-amino-4-methoxybenzoate (0.181 g, 0.99 mmol) were reacted in ethanol (5 mL). Product **4a** was isolated as a yellow solid (0.035 g, 0.065 mmol, 14%); mp 188−190 °C;^{1}H NMR (300 MHz, DMSO-d_6) δ 3.85 (6H, s, OMe), 3.91 (6H, s, COO*Me*−H), 7.22 (2H, d, J = 8.6 Hz, $C_{5''}$−H), 7.62 (2H, d, J = 2.0 Hz, $C_{2''}$−H), 7.87 (2H, dd, J = 2.0 Hz and 8.6 Hz, $C_{6''}$−H), 7.96 (4H, d, J = 8.3 Hz, C_2−H), 8.08 (4H, d, J = 8.3 Hz, C_3−H), 8.67 (2H, s, CH=N); ^{13}C NMR (75 MHz, DMSO-d_6) δ 52.3 (COO*Me*), 56.3 (OMe), 112.1 ($C_{5''}$), 120.9 ($C_{1''}$), 122.4 ($C_{2''}$), 127.6 (C_2), 128.7 ($C_{6''}$), 129.9 (C_3), 135.9 (C_4), 141.6 ($C_{3''}$), 142.4 (C_1), 156.3 ($C_{4''}$), 162.1 (CH=N), 166.3 (COO); Anal. ($C_{32}H_{28}N_2O_6$) C, H, N.

Bis-methyl 4-{[(1*Z*)-1,1'-Biphenyl-4-ylmethylene]amino}-3-hydroxybenzoate (4b). An identical procedure to the one employed for **5a** was used. 4,4'-Biphenyldialdehyde (0.044 g, 0.209 mmol) and methyl-4-amino-3-hydroxybenzoate (0.070 g, 0.418 mmol) were reacted in ethanol (10 mL). Product **4b** was isolated as a yellow solid (0.048 g, 0.094 mmol, 45%); mp 221°C

(decomp.);^{1}H NMR (300 MHz, DMSO-d_6) δ 3.85 (6H, s, OMe), 7.27 (2H, d, *J* = 7.9 Hz, $C_{5''}$−H), 7.48−7.51 (4H, m, $C_{2''}$−H + $C_{6''}$−H), 7.98 (4H, d, *J* = 8.1 Hz, C_2−H), 8.15 (4H, d, *J* = 8.1 Hz, C_3−H), 8.78 (2H, s, CH=N), 9.63 (2H, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 51.6 (OMe), 112.8 ($C_{2''}$), 114.8 ($C_{5''}$), 116.5 ($C_{6''}$), 122.9 (C_2), 128.3 (C_3), 130.6 (C_4), 136.2 ($C_{1''}$), 142.7 (C_1), 143.1 ($C_{4''}$), 144.7 (COH), 160.1 (CH=N), 166.8 (COO); ES$^-$ *m*/*z* 507.2 (M − H$^-$); high-resolution ES$^-$ *m*/*z* found 507.1560 $C_{30}H_{23}N_2O_6$ requires 507.1556; Anal. ($C_{30}H_{24}N_2O_6$·0.25H_2O) C, H; (N calcd 5.46%, N found 4.74%).

Bis-4-{[(1*Z*)-1,1′-biphenyl-4-ylmethylene]amino}-3-hydroxybenzoic Acid (4c). An identical procedure to the one employed for **5a** was used. 4,4′-Biphenyldialdehyde (0.060 g, 0.285 mmol) and 4-amino-3-hydroxybenzoic acid (0.087 g, 0.570 mmol) were reacted in ethanol (10 mL). The product **4c** was isolated as a yellow solid (0.039 g, 0.081 mmol, 28%); mp >360 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.25 (2H, d, *J* = 8.0 Hz, $C_{5''}$−H), 7.45−7.50 (4H, m, $C_{2''}$−H + $C_{6''}$−H), 7.97 (4H, d, *J* = 7.9 Hz, C_2−H), 8.16 (4H, d, *J* = 7.9 Hz, C_3−H), 8.78 (2H, s, CH=N), 9.53 (2H, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 112.8 ($C_{2''}$), 115.1 ($C_{5''}$), 117.8 ($C_{6''}$), 122.9 (C_2), 128.3 (C_3), 130.6 (C_4), 136.1 ($C_{1''}$), 142.3 (C_1), 143.1 ($C_{4''}$), 144.7 (COH), 160.1 (CH=N), 168.0 (COO); ES$^-$ *m*/*z* 479.2 (M − H$^-$); high-resolution ES$^-$ *m*/*z* found 479.1250 $C_{28}H_{19}N_2O_6$ requires 479.1243; Anal. ($C_{28}H_{20}N_2O_6$) C, H; N calcd 5.83%, N found 5.23%.

2-{[(1*Z*)-(4′-{(*Z*)-[(2-Hydroxyphenyl)imino]methyl}-1,1′-biphenyl-4-yl)methylene]amino}phenol (4d). An identical procedure to the one employed for **5a** was used. 4,4′-Biphenyldialdehyde (0.100 g, 0.47 mmol) and 2-aminophenol (0.103 g, 0.95 mmol) were reacted in ethanol (15 mL). Product **4d** was isolated as a yellow solid (0.129 g, 0.33 mmol, 70%); mp 226−230 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.84−6.94 (4H, m, $C_{5''}$−H + $C_{6''}$−H), 7.10 (2H, t, *J* = 7.1 Hz, $C_{4''}$−H), 7.26 (2H, d, *J* = 7.8 Hz, $C_{3''}$−H), 7.95 (4H, d, *J* = 8.3 Hz, C_2−H), 8.16 (4H, d, *J* = 8.3 Hz, C_3−H), 8.79 (2H, s, CH=N), 9.07 (2H, br, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 116.4 ($C_{6''}$), 119.4 ($C_{4''}$), 119.8 ($C_{3''}$), 127.4 (C_2), 127.9 ($C_{5''}$), 129.9 (C_3), 136.3 (C_4), 138.1 (C_1), 142.0 ($C_{2''}$), 151.7 (COH), 158.9 (CH=N); Anal. ($C_{26}H_{20}N_2O_2$) C, H, N.

4-{[(1*Z*)-(4′-{(*Z*)-[(4-Hydroxyphenyl)imino]methyl}-1,1′-biphenyl-4-yl)methylene]amino}phenol (4e). An identical procedure to the one employed for **5a** was used. 4,4′-Biphenyldialdehyde (0.100 g, 0.47 mmol) and 4-aminophenol (0.103 g, 0.95 mmol) were reacted in ethanol (15 mL). Product **4e** was isolated as a yellow solid (0.127 g, 0.32 mmol, 69%); mp >360 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.82 (4H, d, *J* = 8.6 Hz, $C_{2'',6''}$−H), 7.25 (4H, d, *J* = 8.6 Hz, $C_{3'',5''}$−H), 7.90 (4H, d, *J* = 8.2 Hz, C_2−H), 8.01 (4H, d, *J* = 8.2 Hz, C_3−H). 8.69 (2H, s, CH=N), 9.56 (2H, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 116.1 ($C_{2'',6''}$), 122.9 ($C_{3'',5''}$), 127.4 (C_2), 129.3 (C_3), 136.3 (C_4), 141.7 (C_1), 142.9 ($C_{4''}$), 156.8 (COH), 156.9 (CH=N); Anal. $C_{26}H_{20}N_2O_2$·0.3H_2O) C, H, N.

***N*-((1*Z*)-{4′-[(*Z*)-(Phenylimino)methyl]-1,1′-biphenyl-4-yl}methylene)aniline (4f).** An identical procedure to the one employed for **5a** was used. 4,4′-Biphenyldialdehyde (0.200 g, 0.95 mmol) and aniline (0.17 mL, 1.90 mmol) were reacted in ethanol (20 mL). Product **4f** was isolated as a yellow solid (0.252 g, 0.69 mmol, 74%); mp 237−238 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.26−7.33 (6H, m, $C_{2'',6''}$−H + $C_{4'}$−H), 7.45 (4H, t, *J* = 7.5 Hz, $C_{3'',5'}$−H), 7.96 (4H, d, *J* = 8.3 Hz, C_2−H), 8.08 (4H, d, *J* = 8.3 Hz, C_3−H), 8.71 (2H, s, CH=N); ^{13}C NMR (75 MHz, DMSO-d_6) δ 121.4 ($C_{2'',6''}$), 126.0 ($C_{4''}$), 127.6 (C_2), 129.6 (C_3), 129.7 ($C_{3'',5''}$), 135.9 (C_4), 142.2 (C_1), 151.7 ($C_{1''}$), 160.5 (CH=N); Anal. ($C_{26}H_{20}N_2$) C, H, N.

Methyl 3-Hydroxy-4-({(1*Z*)-[4-((*Z*)-{[2-hydroxy-4-(methoxycarbonyl)phenyl]imino}methyl)phenyl]methylene}-amino)benzoate (5a). Terephthalaldehyde (0.200 g, 1.49 mmol) and methyl-4-amino-3-hydroxy-benzoate (0.498 g, 2.98 mmol) were added to ethanol (15 mL), and the reaction mixture was heated to 55 °C for 5 h. The precipitate that formed was filtered and washed with ethanol (40 mL). Product **5a** was isolated as a yellow powder. No further purification was necessary (0.491 g, 1.13 mmol, 76%); mp 263−266 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.84 (6H, s, OMe), 7.26 (2H, d, *J* = 8.1 Hz, $C_{5''}$−H), 7.50 (4H, m, $C_{2''}$−H + $C_{6''}$−H), 8.16 (4H, s, C_2−H), 8.78 (2H, s, CH=N), 9.68 (2H, br, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 52.4 (OMe), 116.9 ($C_{2''}$), 120.4 ($C_{5''}$), 121.1 ($C_{6''}$), 128.3 (C_2), 129.6 ($C_{1''}$), 138.9 (C_1), 143.1 ($C_{4''}$), 150.9 (COH), 161.6 (CH=N), 166.2 (COO); Anal. ($C_{24}H_{20}N_2O_6$·0.1H_2O) C, H, N.

Phenyl 2-Hydroxy-4-({(1*Z*)-[4-((*Z*)-{[3-hydroxy-4-(phenyloxycarbonyl)phenyl]imino}methyl)phenyl]methylidene}amino)benzoate (5b). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.500 g, 3.72 mmol) and phenyl-4-aminosalicylate (1.708 g, 7.45 mmol) were reacted in ethanol (25 mL). Product **5b** was isolated as a yellow solid (0.291 g, 0.52 mmol, 14%); mp 185−186 °C;^{1}H NMR (300 MHz, DMSO-d_6) δ 6.92 (4H, m, $C_{3''}$−H + $C_{5''}$−H), 7.35 (6H, m, Ar−H), 7.50 (4H, m, Ar−H), 8.06 (2H, d, *J* = 8.3 Hz, $C_{6''}$−H), 8.14 (4H, s, C_2−H), 8.76 (2H, s, CH=N), 10.5 (2H, br s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 109.4 ($C_{3''}$), 110.7 ($C_{5''}$), 113.4 ($C_{1''}$), 122.3 (Ar−H), 126.5 (Ar−H), 129.8 (Ar−H), 129.9 (C_2), 132.3 ($C_{6''}$), 138.7 (C_1), 150.5 (Ar−H), 158.5 ($C_{4''}$), 161.7 (COH), 162.7 (CH=N), 167.0 (COO); Anal. ($C_{34}H_{24}N_2O_6$) C, H, N.

Methyl 4-Methoxy-3-({(1*Z*)-[4-((*Z*)-{[2-methoxy-5-(methoxycarbonyl)phenyl]imino}methyl)phenyl]methylene}-amino)benzoate (5c). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.500 g, 3.72 mmol) and methyl-3-amino-4-methoxybenzoate (1.350 g, 7.45 mmol) were reacted in ethanol (25 mL). Product **5c** was isolated as a yellow solid (0.764 g, 1.65 mmol, 45%); mp 182−183 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.97 (6H, s, OMe), 4.03 (6H, s, COO*Me*), 7.03 (2H, d, *J* = 8.6 Hz, $C_{5''}$−H), 7.78 (2H, d, *J* = 2.1 Hz, $C_{2''}$−H), 7.99 (2H, dd, *J* = 2.1 Hz and 8.6 Hz, $C_{6''}$−H), 8.10 (4H, s, C_2−H), 8.63 (2H, s, CH=N). ^{13}C could not be carried out because of solubility problems. Anal. ($C_{26}H_{24}N_2O_6$·0.2H_2O) C, H, N.

4-{[(1*Z*)-(4-{(*Z*)-[(4-Carboxyphenyl)imino]methyl}-phenyl)methylene]amino}benzoic acid (5d). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.300 g, 2.23 mmol) and 4-aminobenzoic acid (0.628 g, 4.58 mmol) were reacted in ethanol (20 mL). Product **5d** was isolated as a yellow solid (0.647 g, 1.73 mmol, 78%); mp >360 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.37 (4H, d, *J* = 8.5 Hz, $C_{3'',5''}$−H), 8.00 (4H, d, *J* = 8.5 Hz, $C_{2'',6''}$−H), 8.12 (4H, s, C_2−H), 8.74 (2H, s, CH=N), 12.93 (2H, br, s, COOH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 121.5 ($C_{3'',5''}$), 128.6 (C_2), 129.7 ($C_{1''}$), 130.9 ($C_{2'',6''}$), 138.8 (C_1), 155.5 ($C_{4''}$), 162.2 (CH=N), 167.3 (COOH); Anal. ($C_{22}H_{16}N_2O_4$·0.1H_2O) C, H, N.

4-({[(1*Z*)-(4-{(*Z*)-[(4-Carboxybenzyl)imino]methyl}-phenyl)methylene]amino}methyl)benzoic Acid (5e). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.250 g, 1.86 mmol) and 4-(aminomethyl)-benzoic acid (0.563 g, 3.72 mmol) were reacted in ethanol (15 mL). Product **5e** was isolated as a white solid (0.475 g, 1.19 mmol, 64%); mp >235 °C (decomp.);^{1}H NMR (300 MHz, DMSO-d_6) δ 4.88 (4H, s, CH_2), 7.46 (4H, d, *J* = 8.2 Hz, $C_{3'',5''}$−H), 7.89 (4H, s, C_2−H), 7.92 (4H, d, *J* = 8.2 Hz, $C_{2'',6''}$−H), 8.59 (2H, s, CH=N), 12.95 (2H, br, s, COOH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 63.8 (CH_2), 128.2 ($C_{1''}$), 128.7 ($C_{3'',5''}$), 129.7 (C_2), 129.8 ($C_{2'',6''}$), 138.2 (C_1), 144.8 ($C_{4''}$), 162.4 (CH=N), 167.6 (COOH); Anal. ($C_{24}H_{20}N_2O_4$·0.2H_2O) C, H, N.

3-{[(1*Z*)−(4-{(*Z*)-[(5-Carboxy-2-methoxyphenyl)imino]-methyl}phenyl)methylene]amino}-4-methoxybenzoic Acid (5f). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.080 g, 0.596 mmol) and 3-amino-4-methoxybenzoic acid (0.192 g, 1.19 mmol) were reacted in ethanol (10 mL). Product **5f** was isolated as a yellow solid (0.216 g, 0.50 mmol, 84%); mp >335 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.90 (6H, s, OMe), 7.20 (2H, d, *J* = 8.6 Hz, $C_{5''}$−H), 7.62 (2H, d, *J* = 2.0 Hz, $C_{2''}$−H), 7.85 (2H, dd, *J* = 2.0 Hz and 8.6 Hz, $C_{6''}$−H), 8.09 (4H, s, C_2−H), 8.69 (2H, s, CH=N), 12.76 (2H, br, s, COOH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.3 (OMe), 112.0 ($C_{5''}$), 121.2 ($C_{1''}$), 123.6 ($C_{2''}$), 129.5 (C_2), 138.8 ($C_{6''}$), 141.2 (C_1), 156.0 ($C_{3''}$), 161.8 ($C_{4''}$), 167.3 (CH=N), 172.3 (COOH); Anal. ($C_{24}H_{20}N_2O_6$·1H_2O) C, H, N.

5-{[(1*Z*)-(4-{(*Z*)-[(3-Carboxy-4-hydroxyphenyl)imino]methyl}phenyl)methylene]amino}-2-hydroxybenzoic Acid (5g). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.200 g, 1.49 mmol) and 5-aminosalicylic acid (0.456 g, 2.98 mmol) were reacted in ethanol (10 mL). Product **5g** was isolated as a gray solid (0.400 g, 0.99 mmol, 66%); mp >360 °C;^{1}H NMR (300 MHz, DMSO-d_6) δ 7.03 (2H, d, J = 8.8 Hz, $C_{3''}$−H), 7.60 (2H, dd, J = 2.7 Hz and 8.8 Hz, $C_{4''}$−H), 7.78 (2H, d, J = 2.7 Hz, $C_{6''}$−H), 8.06 (4H, s, C_2−H), 8.78 (2H, s, CH=N); ^{13}C NMR (75 MHz, DMSO-d_6) δ 113.7 ($C_{3''}$), 118.3 ($C_{1''}$), 122.8 ($C_{6''}$), 129.1 ($C_{4''}$), 129.4 (C_2), 138.7 (C_1), 142.6 ($C_{5''}$), 158.7 ($C_{2''}$), 160.3 (CH=N), 172.0 (COOH); Anal. ($C_{22}H_{16}N_2O_6{\cdot}0.5H_2O$) C, H, N.

4-{[(1*Z*)-(4-{(*Z*)-[(4-Carboxy-2-hydroxyphenyl)imino]methyl}phenyl)methylene]amino}-3-hydroxybenzoic Acid (5h). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.100 g, 0.74 mmol) and 4-amino-3-hydroxybenzoic acid (0.239 g, 1.56 mmol) were reacted in ethanol (10 mL). Product **5h** was isolated as a brown solid (0.220 g, 0.54 mmol, 74%); mp >360 °C;^{1}H NMR (300 MHz, DMSO-d_6) δ 7.24 (2H, d, J = 8.1 Hz, $C_{5''}$−H), 7.44 (4H, m, $C_{2''}$−H + $C_{6''}$−H), 8.17 (4H, s, C_2−H), 8.79 (2H, s, CH=N), 9.57 (2H, br, s, OH), 12.79 (2H, br, s, COOH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 117.1 ($C_{2''}$), 120.2 ($C_{5''}$), 121.2 ($C_{6''}$), 129.6 (C_2), 129.8 ($C_{1''}$), 138.9 (C_1), 142.7 ($C_{4''}$), 150.8 (COH), 161.3 (CH=N), 167.4 (COOH); Anal. ($C_{22}H_{16}N_2O_6{\cdot}0.2H_2O$) C, H, N.

2-{[(1*Z*)-(4-{(*Z*)-[(2-Hydroxyphenyl)imino]methyl}phenyl)methylene]amino}phenol (5i). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.300 g, 2.23 mmol) and 2-aminophenol (0.488 g, 4.47 mmol) were reacted in ethanol (15 mL). Product **5i** was isolated as a yellow solid (0.387 g, 1.22 mmol, 55%); mp 188−190 °C;^{1}H NMR (300 MHz, DMSO-d_6) δ 6.84−6.93 (4H, m, $C_{5''}$−H + $C_{6''}$−H), 7.12 (2H, t, J = 6.8 Hz, $C_{4''}$−H), 7.27 (2H, d, J = 6.8 Hz, $C_{3''}$−H), 8.17 (4H, s, C_2−H), 8.81 (2H, s, CH=N), 9.11 (2H, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 116.5 ($C_{6''}$), 119.5 ($C_{4''}$), 119.9 ($C_{3''}$), 128.1 ($C_{5''}$), 129.4 (C_2), 137.9 (C_1), 138.9 ($C_{2''}$), 151.8 (COH), 160.0 (CH=N); Anal. ($C_{20}H_{16}N_2O_2$) C, H, N.

4-{[(1*Z*)-(4-{(*Z*)-[(4-Hydroxyphenyl)imino]methyl}phenyl)methylene]amino}phenol (5j). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.300 g, 2.33 mmol) and 4-aminophenol (0.496 g, 4.47 mmol) were reacted in ethanol (15 mL). Product **5j** was isolated as a yellow solid (0.244 g, 0.77 mmol, 33%); mp 260−262 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.81 (4H, d, J = 8.6 Hz, $C_{2'',6''}$−H), 7.25 (4H, d, J = 8.6 Hz, $C_{3'',5''}$−H), 8.01 (4H, s, C_2−H), 8.69 (2H, s, CH=N), 9.58 (2H, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 116.1 ($C_{2'',6''}$), 123.0 ($C_{3'',5''}$), 128.9 (C_2), 138.7 (C_1), 142.7 ($C_{4''}$), 156.7 ($C_{1''}$), 160.2 (CH=N); Anal. ($C_{20}H_{16}N_2O_2{\cdot}0.1H_2O$) C, H, N.

***N*-((1*Z*)-{4-[(*Z*)-(Phenylimino)methyl]phenyl}methylene)aniline (5k).** An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.200 g, 1.49 mmol) and aniline (0.27 mL, 2.98 mmol) were reacted in ethanol (15 mL). Product **5k** was isolated as a yellow solid (0.313 g, 1.1 mmol, 74%); mp 158−160 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.25−7.34 (6H, m, $C_{2',6''}$−H + $C_{4''}$−H), 7.45 (4H, t, J = 8.1 Hz, $C_{3'',5''}$−H), 8.09 (4H, s, C_2−H), 8.72 (2H, s, CH=N); ^{13}C NMR (75 MHz, DMSO-d_6) δ 121.5 ($C_{2'',6''}$), 126.7 ($C_{4''}$), 129.4 (C_2), 129.6 ($C_{3'',5''}$), 138.8 (C_1), 151.5 ($C_{1''}$), 160.4 (CH=N); Anal. ($C_{20}H_{16}N_2$) C, H, N.

4-{[(1*Z*)-(4-{(*Z*)-[(4-Cyanophenyl)imino]methyl}naphthyl)methylene]amino}naphthonitrile (5l). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.050 g, 0.372 mmol) and 4-amino-1-naphthalene carbonitrile (0.125 g, 0.745 mmol) were reacted in ethanol (6 mL). Product **5l** was isolated as a yellow solid (0.020 g, 0.046 mmol, 12%); ^{1}H NMR (300 MHz, DMSO-d_6, 313K) δ 7.46 (2H, d, J = 7.8 Hz, $C_{3''}$−H), 7.80 (2H, t, J = 7.3 Hz, $C_{7''}$−H), 7.91 (2H, t, J = 7.3 Hz, $C_{6''}$−H), 8.20 (2H, d, J = 8.5 Hz, $C_{5''}$−H), 8.26 (2H, d, J = 7.6 Hz, $C_{8''}$−H), 8.34 (4H, s, C_2−H), 8.47 (2H, d, J = 7.8 Hz, $C_{2''}$−H), 8.92 (2H, s, CH=N). ^{13}C could not be done because of a lack of solubility.

2-{[(1*Z*)-(4-{(*Z*)-[(2-Hydroxy-4-nitrophenyl)imino]methyl}phenyl)methylene]amino}-5-nitrophenol (5m). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.300 g, 2.2 mmol) and 2-amino-5-nitrophenol (0.690 g, 4.4 mmol) were reacted in ethanol (15 mL). Product **5m** was isolated as a dark-yellow solid (0.573 g, 1.41 mmol, 64%); mp 268−270 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.35 (2H, d, J = 8.4 Hz, $C_{3''}$−H), 7.76 (4H, m, $C_{4''}$−H + $C_{6''}$−H), 8.18 (4H, s, C_2−H), 8.78 (2H, s, CH=N), 10.32 (2H, br, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 110.9 ($C_{6''}$), 115.5 ($C_{4''}$), 121.0 ($C_{3''}$), 129.9 (C_2), 138.9 (C_1), 145.6 ($C_{2''}$), 145.9 ($C_{5''}$), 151.0 (COH), 163.0 (CH=N); Anal. ($C_{20}H_{14}N_4O_6{\cdot}2H_2O{\cdot}0.1Et_3N$) C, H, N.

Methyl 2-Hydroxy-4-({(1*Z*)-[4-((*Z*)-{[3-hydroxy-4-(methoxycarbonyl)phenyl]imino}methyl)phenyl]methylene}amino)benzoate (5n). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.200 g, 1.49 mmol) and methyl-4-amino-2-hydroxybenzoate (0.49 g, 2.98 mmol) were reacted in ethanol (13 mL). Product **5n** was isolated as a yellow solid (0.267 g, 0.62 mmol, 41%); mp 198−201 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.84 (6H, br, s, OMe), 6.78 (4H, br, s, $C_{3''}$−H + $C_{5''}$−H), 7.77 (2H, br, s, $C_{6''}$−H), 8.03 (4H, br, s, C_2−H), 8.65 (2H, br, s, CH=N), 10.63 (2H, br, s, OH). ^{13}C could not be done because of a problem with solubility. Anal. ($C_{24}H_{20}N_2O_6{\cdot}0.5H_2O$) C, H, N.

Ethyl 2-Hydroxy-4-({(1*Z*)-[4-((*Z*)-{[3-hydroxy-4-(ethoxycarbonyl)phenyl]imino}methyl)phenyl]methylene}amino)benzoate (5o). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.060 g, 0.44 mmol) and ethyl-4-amino-2-hydroxybenzoate (0.161 g, 0.88 mmol) were reacted in ethanol (5 mL). Product **5o** was isolated as a yellow solid (0.060 g, 0.13 mmol, 30%); mp 190−191 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37 (6H, t, J = 7.1 Hz, CH_3−H), 4.39 (4H, q, J = 7.1 Hz, CH_2−H), 6.85 (2H, s, $C_{3''}$−H), 6.88 (2H, d, J = 8.1 Hz, $C_{5''}$−H), 7.86 (2H, d, J = 8.1 Hz, $C_{6''}$−H), 8.11 (4H, s, C_2−H), 8.73 (2H, s, CH=N), 10.78 (2H, s, OH). ^{13}C could not be done because of a problem with solubility. Anal. ($C_{26}H_{24}N_2O_6{\cdot}0.4H_2O$) C, H, N.

Methyl 2-Hydroxy-5-({(1*Z*)-[4-((*Z*)-{[4-hydroxy-3-(methoxycarbonyl)phenyl]imino}methyl)phenyl]methylene}amino)benzoate (5p). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.100 g, 0.75 mmol) and methyl-5-amino-2-hydroxybenzoate (0.250 g, 1.49 mmol) were reacted in ethanol (7 mL). Product **5p** was isolated as a yellow solid (0.070 g, 0.16 mmol, 22%); mp 207−210 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.88 (6H, s, OMe), 7.04 (2H, dd, J = 1.2 Hz and 8.8 Hz, $C_{4''}$−H), 7.59 (2H, d, J = 8.8 Hz, $C_{3''}$−H), 7.72 (2H, s, $C_{6''}$−H), 8.02 (4H, s, C_2−H), 8.74 (2H, s, CH=N), 10.47 (2H, s, OH). ^{13}C could not be done because of a lack of solubility. Anal. $C_{24}H_{20}N_2O_6$) C, H, N.

Ethyl 2-Hydroxy-5-({(1*Z*)-[4-((*Z*)-{[4-hydroxy-3-(ethoxycarbonyl)phenyl]imino}methyl)phenyl]methylene}amino)benzoate (5q). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.050 g, 0.37 mmol) and ethyl-5-amino-2-hydroxybenzoate (0.134 g, 0.74 mmol) were reacted in ethanol (7 mL). Product **5q** was isolated as a yellow solid (0.117 g, 0.25 mmol, 69%); mp 171−173 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.33 (6H, t, J = 7.1 Hz, CH_3), 4.36 (4H, q, J = 7.1 Hz, CH_2), 7.04 (2H, d, J = 8.8 Hz, $C_{3''}$−H), 7.60 (2H, d, J = 8.8 Hz, $C_{4''}$−H), 7.72 (2H, s, $C_{6''}$−H), 8.03 (4H, s, C_2−H), 8.74 (2H, s, CH=N), 10.55 (2H, s, OH). ^{13}C could not be carried out because of the insolubility of the compound. Anal. ($C_{26}H_{24}N_2O_6{\cdot}0.2H_2O$) C, H, N.

2-{[(1*Z*)-(4-{(*Z*)-[(2-Hydroxy-4-methylphenyl)imino]methyl}phenyl)methylene]amino}-5-methylphenol (5r). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.300 g, 2.2 mmol) and 2-amino-5-methylphenol (0.550 g, 4.4 mmol) were reacted in ethanol (15 mL). Product **5r** was isolated as a yellow solid (0.649 g, 1.88 mmol, 86%); mp 205−207 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.26 (6H, s, CH_3), 6.68 (2H, d, J = 8.1 Hz, $C_{4''}$−H), 6.75 (2H, s, $C_{6''}$−H), 7.23 (2H, d, J = 8.1 Hz, $C_{3''}$−H), 8.15 (4H, s, C_2−H), 8.81 (2H, s, CH=N), 8.99 (2H, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.3 (CH_3), 117.0 ($C_{6''}$), 118.9 ($C_{4''}$), 120.6 ($C_{3''}$), 129.3 (C_2),

135.1 (C_1), 138.0 ($C_{2''}$), 138.9 ($C_{5''}$), 151.9 (COH), 157.4 (CH=N); Anal. ($C_{22}H_{20}N_2O_2$·0.2H_2O) C, H, N.

4-{[(1*Z*)-(4-{(*Z*)-[(4-Cyanophenyl)imino]methyl}phenyl)-methylene]amino}benzonitrile (5s). An identical procedure to the one employed for **5a** was used. Terephthalaldehyde (0.200 g, 1.49 mmol) and 4-amino-benzonitrile (0.352 g, 2.98 mmol) were reacted in ethanol (10 mL). Product **5s** was isolated as a yellow solid (0.207 g, 0.62 mmol, 42%); mp 209−210 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.46 (4H, d, J = 8.2 Hz, $C_{3'',5''}$−H), 7.92 (4H, d, J = 8.2 Hz, $C_{2'',6''}$−H), 8.13 (4H, s, C_2−H), 8.74 (2H, s, CH=N); ^{13}C NMR (75 MHz, DMSO-d_6) δ 95.9 ($C_{1''}$), 113.8 (CN), 122.5 ($C_{3'',5'}$), 130.4 (C_2), 133.8 ($C_{2'',6''}$), 133.9 (C_1), 140.1 (C_4), 153.4 (CH=N); Anal. ($C_{22}H_{14}N_4$·0.2H_2O) C, H, N.

Synthesis of Amines. Bis-methyl 3-{[4-(Aminomethyl)-benzyl]amino}-4-methoxybenzoate (6a). Anhydrous methanol (8 mL) was added slowly dropwise to sodium borohydride (0.100 g, 2.6 mmol); effervescence occured. In a separate flask, **5c** (0.200 g, 0.42 mmol) was dissolved in anhydrous dichloromethane (8 mL) and added slowly dropwise to the main reaction vessel. After stirring at room temperature for 12 h, a precipitate formed, which was filtered and washed with 2 N HCl (10 mL), water (10 mL), methanol (20 mL), and dichloromethane (20 mL). Product **6a** was obtained as a white solid without the need for further purification (0.044 g, 0.095 mmol, 23%); mp 218−220 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.71 (6H, s, OMe), 3.88 (6H, s, COO*Me*), 4.30 (4H, d, J = 5.9 Hz, CH_2), 5.77 (2H, t, J = 5.9 Hz, NH), 6.89 (4H, m, $C_{2''}$−H + $C_{5''}$−H), 7.22 (6H, m, $C_{6''}$−H + C_2−H). ^{13}C could not be done becaouse of a lack of solubility. ES$^+$ m/z 462.9 (M$^+$); Anal. ($C_{26}H_{28}N_2O_6$.0.7HCl) C, H, N.

Bis-methyl 4-{[4-(Aminomethyl)benzyl]amino}-3-hydroxybenzoate (6b). An identical procedure to the one employed for **6a** was used. **5a** (0.300 g, 0.693 mmol) in dichloromethane (10 mL) was reacted with sodium borohydride (0.158 g, 4.16 mmol) in methanol (5 mL). Water (20 mL) was added to precipitate the product, which was washed further with water (20 mL) and dichloromethane (20 mL). Product **6b** was isolated as a light-brown solid (0.148 g, 0.34 mmol, 49%); mp 239−240 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.71 (6H, s, OMe), 4.33 (4H, d, J = 6.2 Hz, CH_2), 6.15 (2H, t, J = 6.2 Hz, NH), 6.36 (2H, d, J = 8.7 Hz, $C_{5''}$−H), 7.23 (8H, m, C_2−H + $C_{2''}$−H + $C_{6''}$−H), 9.74 (2H, br, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 45.7 (OMe), 51.5 (CH_2), 108.6 ($C_{5''}$), 113.5 ($C_{2''}$), 116.1 ($C_{1''}$), 123.0 ($C_{6'}$), 127.3 (C_2), 138.5 ($C_{4''}$), 142.2 (C_1), 143.5 ($C_{3''}$), 166.7 (COO); ES$^+$ m/z 459.5 (100%, M + Na$^+$); high-resolution ES$^+$ m/z found 459.1538 $C_{24}H_{24}N_2O_6$Na (M + Na$^+$) requires 459.1532; Anal. ($C_{24}H_{24}N_2O_6$·0.7H_2O) C, H, N.

4-[(4-{[(4-Carboxy-2-hydroxyphenyl)amino]methyl}-benzyl)amino]-3-hydroxybenzoic Acid (6c). A solution of NaOH (0.070 g, 1.71 mmol) in water (6 mL) was added dropwise to **6b** (0.075 g, 0.171 mmol), and the reaction mixture was stirred at room temperature for 45 min. HCl (8 mL, 2 N) was added dropwise to the reaction mixture until a neutral pH was achieved. A solid precipitated, which was filtered and washed with water (20 mL). Product **6c** was obtained as a white solid without further need for purification (0.015 g, 0.037 mmol, 21%); mp >360 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.34 (4H, br, s, CH_2), 6.08 (2H, br, s, NH), 6.38 (2H, br, s, $C_{5''}$−H), 7.21−7.30 (8H, m, C_2−H + $C_{2'}$−H + $C_{6'}$−H), 9.67 (2H, br, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 53.6 (CH_2), 115.9 ($C_{5''}$), 118.1 ($C_{2''}$), 119.6 ($C_{1''}$), 122.4 ($C_{6''}$), 129.8 (C_2), 132.1 ($C_{4''}$), 140.6 (C_1), 143.6 (COH), 169.9 (COOH); Anal. ($C_{22}H_{20}N_2O_6$·0.8HCl) C, H, N.

2-[(4-{[(2-Hydroxyphenyl)amino]methyl}benzyl)amino]-phenol (6d). An identical procedure to the one employed for **6a** was used. **5i** (0.200 g, 0.63 mmol) was dissolved in dichloromethane (10 mL) and reacted with sodium borohydride (0.158 g, 4.16 mmol) in methanol (10 mL). After stirring for 12 h, dichloromethane (20 mL) was added, and the solution was washed with 2 N HCl (20 mL) and saturated sodium bicarbonate solution (20 mL). The organic phase was separated, dried ($MgSO_4$), and evaporated in vacuo. The crude product was then purified by column chromatography (1:1 ethyl acetate/hexane) to leave **6d** as a light-orange solid (0.020 g, 0.062 mmol, 10%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.25 (4H, d, J = 6.0 Hz, CH_2); 5.19 (2H, t, J = 6.0 Hz, NH), 6.36 (4H, m, $C_{3''}$−H + $C_{5''}$−H), 6.51 (2H, t, J = 7.3 Hz, $C_{4''}$−H), 6.63 (2H, d, J = 7.8 Hz, $C_{6''}$−H), 7.28 (4H, s, C_2−H), 9.25 (2H, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 46.6 (CH_2), 110.4 ($C_{3''}$), 113.7 ($C_{6''}$), 116.0 ($C_{5''}$), 119.8 ($C_{4''}$), 127.3 (C_2), 137.5 ($C_{2''}$), 139.1 (C_{81}), 144.3 (COH); EI$^+$ m/z 320.1 (M$^+$); high-resolution EI$^+$ m/z found 320.1535 $C_{20}H_{20}N_2O_2$ requires 320.1525; Anal. ($C_{20}H_{20}N_2O_2$·0.2HCl) C, H: N calcd 8.55%, N found 7.95%.

Synthesis of Alkene Analogues. 5-((*E*)-2-{4-[(*E*)-2-(3-Carboxy-4-hydroxyphenyl)vinyl]phenyl}vinyl)-2-hydroxybenzoic Acid (7a). A mixture of DMF (17 mL) and water (3 mL) was added to 1,4-divinylbenzene (0.200 g, 1.53 mmol), 5-iodosalicylic acid (0.811 g, 3.07 mmol), 10% palladium acetate (0.034 g, 0.153 mmol), and potassium carbonate (0.424 g, 3.07 mmol). The reaction mixture was heated to 100 °C. After 18 h, water (75 mL) was added followed by 2 M HCl (10 mL). Upon addition of the acid, a solid precipitated, which was filtered. The solid was recrystallized from ethyl acetate (80 mL), filtered, and washed with petroleum ether (20 mL). Product **7a** was isolated as a light-green crystalline solid (0.080 g, 0.19 mmol, 13%); mp >300 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.98 (1H, d, J = 8.6 Hz, $C_{3''}$−H), 7.11 (1H, d, J = 16.4 Hz, CH=CH), 7.25 (1H, d, J = 16.4 Hz, CH=CH), 7.59 (2H, s, C_2−H), 7.81 (1H, dd, J = 1.9 Hz and 8.7 Hz, $C_{4''}$−H), 7.99 (1H, d, J = 1.9 Hz, $C_{6''}$−H), 11.44 (1H, br, s, COOH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 113.5 ($C_{3''}$), 118.0 ($C_{1''}$), 126.8 (CH=CH), 126.9 (CH=CH), 127.4 (C_2), 128.8 ($C_{5'}$), 128.9 ($C_{6''}$), 133.4 ($C_{4''}$), 136.6 (C_1), 161.0 (COH), 172.1 (COOH); Anal. ($C_{24}H_{18}O_6$) C, H.

4-((*E*)-2-{4-[(*E*)-2-(4-Hydroxyphenyl)vinyl]phenyl}vinyl)-phenol (7b). An identical procedure to the one employed for **7a** was used. 1,4-Divinylbenzene (0.240 g, 1.84 mmol), 4-iodophenol (0.811 g, 3.68 mmol), 10% palladium acetate (0.042 g, 0.184 mmol), and potassium carbonate (0.509 g, 3.68 mmol) were reacted together in DMF (15 mL) and water (5 mL). After work up, the crude was columned in a 1:1 ethyl acetate/hexane mixture. Product **7b** was isolated as a gray-green solid (0.025 g, 0.079 mmol, 4%); ^{1}H NMR (300 MHz, MeOD-d_4) δ 6.81 (4H, d, J = 8.6 Hz, $C_{2'',6''}$−H), 7.08 (2H, d, J = 16.4 Hz, C*H*=CH), 7.33 (2H, d, J = 16.4 Hz, CH=C*H*), 7.47 (4H, C_2−H), 7.88 (4H, d, J = 8.6 Hz, $C_{3'',5''}$−H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 117.0 ($C_{2'',5''}$), 125.7 (*C*H=CH), 128.0 (CH=CH), 129.9 (C_2), 130.1 ($C_{4''}$), 131.6 ($C_{3'',5''}$), 133.9 (C_1), 159.7 (COH); ES$^-$ m/z 312.7 (M$^-$); high-resolution ES$^+$ m/z found 315.1382 $C_{22}H_{18}O_2$ (M + H$^+$) requires 315.1380.

Methyl 4-Aminobenzoate (8a). Concentrated sulfuric acid (7 mL) was added dropwise slowly to 4-aminobenzoic acid (10 g, 72.90 mmol) in methanol (150 mL). The reaction mixture was heated to reflux for 36 h. The solution remaining was evaporated in vacuo to dryness. Water (100 mL) was added, and the pH of the solution was adjusted to 3 using a 2 M solution of NaOH. The precipitate formed, was filtered, and was washed with water (50 mL). Pure product **8a** was isolatedas an off-white solid (7.980 g, 52.80 mmol, 72%); mp 107−110 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.77 (3H, s, OMe), 6.60 (2H, d, J = 8.7 Hz, $C_{3'',5''}$−H), 7.68 (2H, d, J = 8.7 Hz, $C_{2'',6''}$−H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 51.5 (OMe), 113.1 ($C_{3'',5''}$), 116.2 ($C_{1''}$), 131.4 ($C_{2'',6''}$), 153.8 ($C_{4''}$), 166.7 (COO); ES$^+$ m/z 174.0 (M + Na$^+$), 325.1 (2M + Na$^+$).

Methyl 4-Amino-3-hydroxybenzoate (8b). Concentrated sulfuric acid (3.5 mL) was added dropwise slowly to 4-amino-3-hydroxybenzoic acid (5.0 g, 32.65 mmol) in methanol (140 mL). The reaction mixture was heated to reflux for 36 h. The solution remaining was evaporated in vacuo to dryness. Ice water (125 mL) was added, and the pH of the solution was adjusted to 5 using a 2 M NaOH solution. The brown precipitate that was formed was filtered and washed with water (50 mL). Product **8b** was isolated as a brown solid (3.360 g, 20.10 mmol, 62%); mp 116−117 °C; ^{1}H NMR (300 MHz, MeOD-d_4) δ 3.82 (3H, s, OMe), 6.67 (1H, d, J = 8.20 Hz, $C_{6''}$−H), 7.32 (1H, d, J = 1.89 Hz, $C_{2''}$−H), 7.36 (1H, d, J = 1.89 and 8.20 Hz, $C_{5''}$−H); ^{13}C NMR (75 MHz, MeOD-d_4) δ 52.4

(OMe), 114.9 ($C_{5''}$), 116.3 ($C_{2''}$), 119.8 ($C_{6''}$), 124.6 ($C_{1''}$), 143.7 (COH), 145.2 (CNH_2), 169.8 (COO); ES^+ *m/z* 167.5 (M^+), 189.9 ($M + Na^+$).

Cell Culture Assays. Cell Culture. SMB cells were grown in tissue culture-treated flasks in 199 medium (Gibco) supplemented with 10%(v/v) fetal calf serum, 5%(v/v) newborn calf serum (Gibco), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C and 5% CO_2.

Drug Treatment. Drugs were resuspended in DMSO at a stock concentration of 10 mM. To assess the effects of a drug, we plated SMB cells at 50% confluency per well in 6-well plates. Cells were left for 24 h to allow for attachment. The media were then replaced with fresh media containing the appropriate dilution of the 10 mM drug stock. Four days after the addition of the drug, the media were removed and the protein was extracted.

Protein Extraction. Cells were lysed in PBS containing 1% Triton-X 100 and 1% Igepal CA-630 for 20 min at 37 °C. Cell lysates were either placed on ice or treated with 80 μg/mL proteinase k for 1 h at 37 °C. Proteins were concentrated from the total cell lysate by methanol precipitation, and the protein pellet was resuspended and denatured by boiling for 5 min in 8 M urea.

Western Blotting. Samples were electrophoresed on a 12% acrylamide gel and transferred electrophoretically to the PVDF membrane (Immobilon-P, Millipore). PrP was detected using the primary antibody DR1 as previously described[36] and an HRP conjugated secondary antibody (Dako). Specific protein bands were visualized using ECL Plus chemiluminescent reagent (Amersham Pharmacia Biotech) followed by autoradiography. Autoradiographs were analyzed using Scion Image densitometric software (Scion Corporation).

In Vitro Aggregation Assay. All measurements were performed on a Cary 100Bio UV−vis spectrophotometer at 325 nm using a quartz cuvette of 5-mm path length. The substrate recombinant mouse PrP (rPrP) was refolded in the absence of metal ions, and the seed for aggregation was aged manganese-refolded recombinant mouse PrP (MnPrP) prepared as previously described.[29,37] Briefly, a seed of MnPrP induces the immediate aggregation of substrate rPrP, which is observed as an increase in solution turbidity. The resultant scattering of UV light at 325 nm results in an increased absorbance measurement. The abilities of the potential anti-TSE compounds to prevent this turbidity increase were measured, and the results were expressed as a percentage of the turbidity observed with a DMSO control. rPrP (50 μg) and anti-TSE compound (100 μM) were preincubated in 500 μL of H_2O at pH 6.5 for 30 min to provide a zero for the measurement. MnPrP seed (10 μg) from a 400 μg mL^{-1} stock was added to the drug/rPrP mixture, and an initial reading was obtained immediately. A second reading was measured after 5 min, and the increase in absorbance over 5 min was recorded. Time in the spectrophotometer beam was minimized due to the sensitivity to UV light of some Congo red derivatives.

Toxicity Assay. Cerebellar neuronal cultures were prepared from 6-day-old mice 129SV. Cultures were prepared as described previously.[38] Briefly, the cerebella were dissociated in Hanks' media (Sigma) containing 0.5% trypsin (Sigma) and plated at (1−2) × 10^6 cells/cm^2 in 24-well trays (Falcon) coated with poly-D-lysine (50 μg/mL, Sigma). Cultures were maintained in Dulbecco's minimal essential media (Sigma) supplemented with 10% fetal calf serum, 2 mM glutamine, and 1% antibiotics (penicillin, streptomycin) (Sigma). The cultures were maintained at 37 °C with 5% CO_2. Compounds were prepared in DMSO (Sigma). After 4 days of treatment, the survival of the cerebellar cells was determined. MTT (3,[4,5 dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide, Sigma) was diluted to 200 μM in Hanks' solution and added to cultures for 30 min at 37 °C. The MTT formazan product was released from cells by the addition of dimethyl sulfoxide (Sigma) and measured at 570 nm in a spectrophotometer (Bio50, Cary). Relative survival in comparison to the control treated with the DMSO vehicle could then be determined.

Acknowledgment. We acknowledge the Medical Research Council for funding and the EPSRC National Mass Spectrometry Centre, Swansea for accurate mass spectrometry.

Supporting Information Available: Elemental analysis data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM049922T