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Design, Synthesis, and Structure−Activity Relationship of Indole-3-glyoxylamide Libraries Possessing Highly Potent Activity in a Cell Line Model of Prion Disease

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Transmissible spongiform encephalopathies (TSEs) are a family of invariably fatal neurodegenerative disorders for which no effective curative therapy currently exists. We report here the synthesis of a library of indole-3-glyoxylamides and their evaluation as potential antiprion agents. A number of compounds demonstrated submicromolar activity in a cell line model of prion disease together with a defined structure−activity relationship, permitting the design of more potent compounds that effected clearance of scrapie in the low nanomolar range. Thus, the indole-3-glyoxylamides described herein constitute ideal candidates to progress to further development as potential therapeutics for the family of human prion disorders.

Introduction

Prion diseases, or transmissible spongiform encephalopathies (TSEs[a]), are a group of rare neurodegenerative disorders affecting both humans and animals. They may occur as sporadic, inherited, or iatrogenic diseases, which progress rapidly after onset of symptoms and are invariably fatal. The most common such condition affecting humans is Creutzfeldt−Jakob Disease (CJD),[1] though other human prion disorders have been characterized, most notably variant CJD[2] (vCJD), Gerstmann−Sträussler−Scheinker syndrome[3] (GSS), and familial fatal insomnia[4] (FFI). A number of TSEs also affect various animal species, including scrapie[5] in sheep, bovine spongiform encephalopathy[6] (BSE) in cattle, and chronic wasting disease[7] (CWD) in deer and elk.

A common feature of TSEs is the deposition of insoluble aggregates of disease-associated prion protein (PrP^{Sc}), the post-translationally refolded and partially protease-resistant isoform of normal cellular prion protein (PrP^{C}), a glycosylphosphatidylinositol (GPI)-anchored cell surface protein. These observed deposits of PrP^{Sc} are thought, either directly or indirectly, to be the cause of neuronal cell death in TSEs, a process that leads to formation of vacuoles and ultimately results in the characteristic spongiform degeneration of brain tissue. The normal biological function of PrP^{C} remains incompletely defined, but it is expressed predominantly in neurons and is highly conserved across all mammalian species.[8] Evidence for multifaceted normal function is emerging, with apparent roles for PrP^{C} in neuroprotection,[8] cell adhesion,[9] and iron metabolism,[10] among others.

Whereas exact mechanisms of infectivity and pathogenesis in prion disease are far from fully elucidated, significant work by Mallucci et al.[11] confirmed the neurotoxicity of PrP^{Sc} and demonstrated that expression of PrP^{C} in host neurons is required for PrP^{Sc} replication and, hence, disease progression. As such, persistently infected cell lines acting as a host for PrP^{Sc} are frequently used as an in vitro model of prion disease.[12] In these cells, active replication of the scrapie protein occurs, leading to its accumulation in readily detectable amounts. An infected mouse neuroblastoma-derived cell line, ScN2a,[13] has gained widespread use for in vitro screening of potential antiprion agents, as has a scrapie mouse brain (SMB) cell line, cloned from culture of murine brain infected with the Chandler scrapie strain.[14]

A handful of compounds showing potent in vitro clearance of PrP^{Sc} have been evaluated clinically for treatment of TSEs (Figure 1) but with little success to date. The antimalarial quinacrine failed to demonstrate any clear beneficial effect when administered compassionately to patients with CJD,[15] a finding confirmed by the more recent patient-preference trial termed "PRION-1",[16] wherein it was also concluded that quinacrine did not significantly affect the clinical course of prion disease. These failings in the clinic have hypothetically been attributed to an unfavorable pharmacokinetic profile of the drug.[17]

In other studies, a double-blind, placebo-controlled trial of flupirtine suggested this compound slowed cognitive decline, but it did not markedly extend survival.[18] Preliminary results with pentosan polysulfate (PPS) showed evidence for slowing of disease progression,[19] though further studies are necessary to confirm these findings. Treatment with doxycycline was found to reduce prion infectivity and offer the prospect of significantly longer survival of CJD patients,[20] though a formal clinical trial has not yet concluded.[21] As is apparent,

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[a] Abbreviations: TSE, transmissible spongiform encephalopathy; CJD, Creutzfeldt−Jakob disease; vCJD, variant Creutzfeldt−Jakob disease; GSS, Gerstmann−Sträussler−Scheinker syndrome; FFI, familial fatal insomnia; BSE, bovine spongiform encephalopathy; CWD, chronic wasting disease; PrP^{C} (or PrP-sen), normal cellular prion protein; PrP^{Sc} (or PrP-res), disease-causing isoform; GPI, glycosylphosphatidylinositol; SMB, scrapie-infected mouse brain; ScNB, scrapie agent-infected neuroblastoma cells; PPS, pentosan polysulfate; SAR, structure−activity relationship; SPR, surface plasmon resonance.

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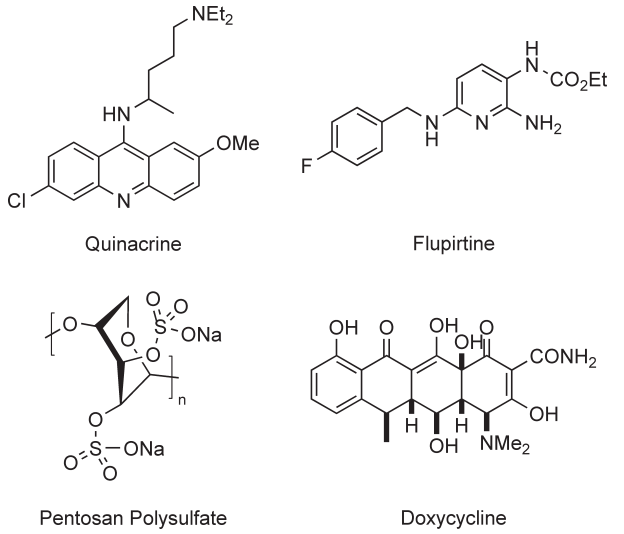


Figure 1. Existing drugs that have been investigated as potential therapeutics in human prion disease.

disappointingly little progress has been made in the therapy of TSEs despite considerable effort.[22] There is still a pressing need for potent antiprion agents that retain their efficacy in vivo and consequently exert an obvious clinical benefit.

Many classes of compound have exhibited in vitro activity, including 9-aminoacridine derivatives related to quinacrine,[23] 2-aminopyridine-3,5-dicarbonitriles,[24] azobenzene and its analogues,[25] *N*′-benzylidenebenzohydrazides,[26] 2,4-diphenylthiazoles and oxazoles,[27] and a small series of pyrazolone derivatives,[28] among others. Despite the relatively large number of potential therapeutics characterized by cell line screening, very few have been progressed to animal studies, and those that were failed to retain any remedial efficacy in vivo.[29] Indeed, the groups of compounds listed above are generally not ideal as drug candidates, since they have insufficient potency for in vivo activity (typically low-micromolar EC_{50} values in vitro), lack a discernible structure−activity relationship (SAR), or exert cytotoxic effects at relatively low concentrations, narrowing the therapeutic window. A requirement evidently exists for access to potently active compounds that are noncytotoxic, are suitably "druglike" in nature,[30] and exhibit a well-defined SAR, this being particularly desirable because it would suggest a single, definite mode of action that may be deduced by further study.

We herein present preliminary results regarding a library of compounds with obvious potential to satisfy the above criteria, in addition to comprising a novel structural class of antiprion agents. During evaluation of a library of commercially sourced screening compounds in the SMB cell line assay, two indole-3-glyoxylamides **1** and **2** (Figure 2a) were confirmed as active inhibitors of PrP^{Sc} accumulation, displaying EC_{50} values of 1.5 and 6.4 μM, respectively. The indole-3-glyoxylamides are in fact a medicinally significant class of compounds, represented clinically by GW 842470 (reportedly under investigation as a treatment for atopic dermatitis[31]) and indibulin (D-24851), a potent tubulin polymerization inhibitor[32] that recently completed a phase I clinical trial in patients with solid tumors[33] (Figure 2b).

Considering the structures of **1** and **2**, we chose to focus initial library design around varying substitution at positions 1−3 of the indole system, particularly with respect to glyoxylamides derived from a range of amines at the 3-position.

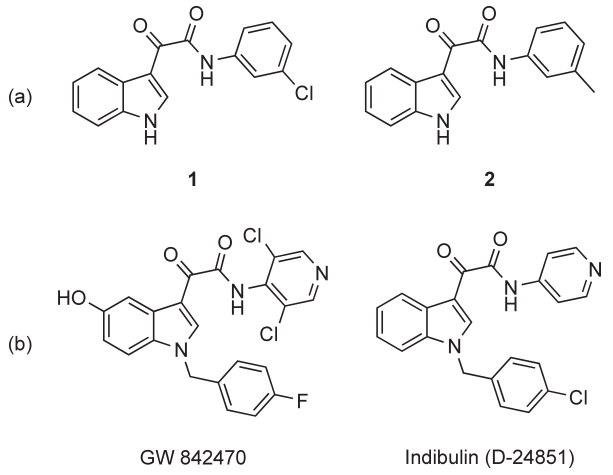
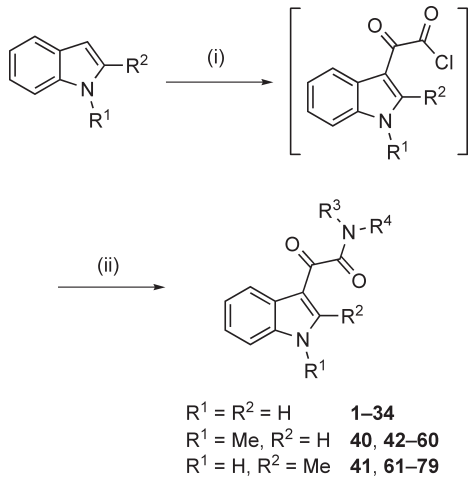


Figure 2. (a) Indole-3-glyoxylamides that displayed antiprion activity during cell-line screening of a commercially sourced compound library. (b) Related indole-3-glyoxylamide derivatives in ongoing clinical evaluation.

Scheme 1. Preparation of Indole-3-glyoxylamide Library Members[a]



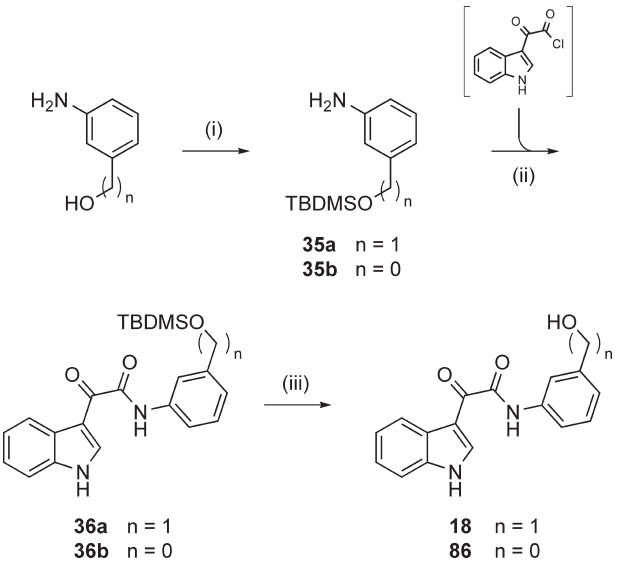
[a] Reagents and conditions: (i) oxalyl chloride, Et_2O or THF, room temp, 1 h; (ii) R^3R^4NH, $^iPr_2NEt$, room temp, 3−18 h.

Chemistry

Synthesis of the library members was based upon known protocols[34] and proved relatively straightforward (Scheme 1). Both acylation of the 3-position of indole by oxalyl chloride and subsequent reaction of the glyoxylyl chloride intermediate with various amines were carried out in a one-pot format in parallel with few problems. During the course of library synthesis from unsubstituted indole ($R^1 = R^2 = H$), THF was found to offer advantage over ether as solvent owing to increased solubility of the amines and glyoxylamide products. Recently, another group independently reported similar results during synthesis of antibacterials containing indole-3-glyoxylamide substituents;[35] thus, most of the compounds in the present study were synthesized using THF as solvent. After workup, library members were purified as necessary by recrystallization, column chromatography, or a combination of both. Some of the products required two rounds of recrystallization from different solvent systems in order to achieve a good degree of purity.

Synthesis of **18**, derived from 3-(hydroxymethyl)aniline, required protection of the more nucleophilic alcohol function;

Scheme 2. Preparation of Hydroxy-Containing Compounds Required a Suitable Protection Strategy for the Alcohol[a]



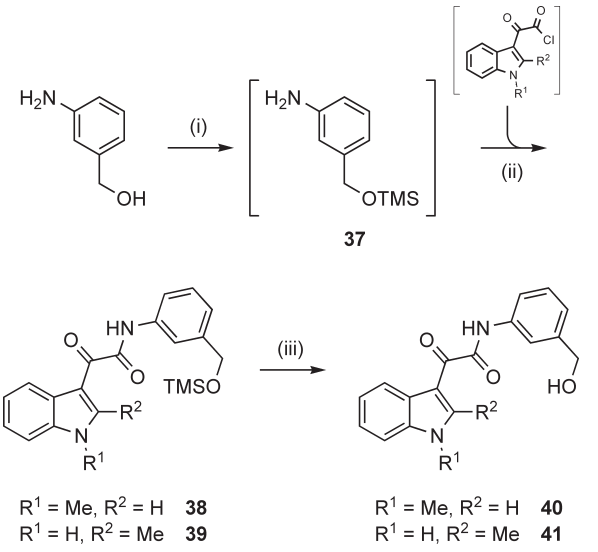
[a] Reagents and conditions: (i) TBDMS-Cl, imidazole, DMF, 0 °C, 18 h, 70% (n = 1), or TBDMS-Cl, imidazole, THF, room temp, 30 min, 56% (n = 0); (ii) $^{i}Pr_2NEt$, THF, room temp, 3 h, 19% (n = 1) or 23% (n = 0); (iii) TBAF, THF, room temp, 1 h, 63% (n = 1) or 80% (n = 0, as tetrabutylammonium salt).

thus, *O*-TBDMS derivative **35a** was prepared and utilized in the glyoxylamide formation step (Scheme 2, n = 1). Removal of the silyl protecting group from **36a** proceeded smoothly giving the desired hydroxymethyl compound **18**.

In order to investigate the effect of substitution at N-1 and C-2, libraries derived from 1-methylindole (R^1 = Me, R^2 = H) and 2-methylindole (R^1 = H, R^2 = Me) were also prepared, using a subset of the amines employed in the synthesis of the initial library. Compounds containing a 2-methyl group were typically isolated in relatively low yield (perhaps due to increased steric demand on the amide coupling step), but nonetheless, they were obtained in sufficient quantities for screening.

Structures derived from 3-(hydroxymethyl)aniline were again included among the library members synthesized using 1- and 2-methylindole, though this time a transient protection approach was employed. The *O*-TMS protected aniline building block **37** was generated in situ and reacted immediately with an indole-3-glyoxylyl chloride to afford either **38** or **39** (Scheme 3). Acidic workup cleaved the silyl group, providing controlled access to screening compounds **39** and **40** without the need for isolation of any synthetic intermediates.

Scheme 3. A More Expedient, Transient TMS Protection Strategy for Additional Library Members Derived from 3-(Hydroxymethyl)aniline[a]



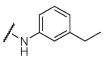
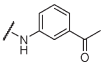
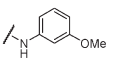
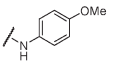
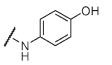
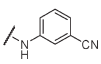
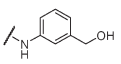
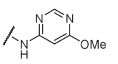
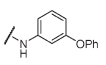
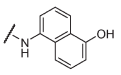
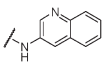
[a] Reagents and conditions: (i) TMS-Cl, THF, room temp, 1 h; (ii) $^{i}Pr_2NEt$, THF, room temp, 3 h; (iii) 1 M HCl.

Screening (SMB Cells) Methodology

Compounds were screened for inhibition of PrP^{Sc} formation in SMB cells of mesodermal origin essentially as described previously.[27] The procedure was based upon that reported by Rudyk et al.[36] A persistently infected mouse cell line (SMB), cloned originally from scrapie infected mouse brain but of non-neuronal origin,[14a] was used. Cells were grown in tissue culture-treated plastic dishes in medium 199 (phenol red free), supplemented with 10% newborn calf serum (heat inactivated), 5% fetal calf serum (heat inactivated), and penicillin−streptomycin at 10 mg L^{-1} at 37 °C in an atmosphere of 5% CO_2 in air at 95% relative humidity. Medium was changed every third or fourth day, and every 7 days confluent cells were passaged using 0.05% trypsin and 0.002% EDTA at a split ratio of 4. To assess the effects of compounds, cells were distributed into 96-well cluster plates at 3×10^4 cells per well and incubated for 24 h to allow for cell attachment. The compounds were prepared at 400 times the required concentration in DMSO as stock solutions and then transferred, at a 20-fold dilution, into Hank's balanced salt solution. This solution was then transferred at a further 20-fold dilution into the cell medium. The cells were incubated with the compound-containing medium for 5 days. After this time, cell viability was assessed by the MTT assay following the standard protocol supplied with the reagent (Sigma). For dot blot analyses, cells were extracted using lysis buffer (10 mM Tris-HCl [pH 7.6], 100 mM NaCl, 10 mM EDTA, 0.5% v/v NP40, and 0.5% w/v sodium deoxycholate), and the content of the well was loaded onto a nitrocellulose membrane (0.45 μm) under gentle vacuum at a total cellular protein concentration of approximately 30−40 μg/well (determined by the Bradford assay following the protocol supplied with the reagent; Sigma). The membrane was air-dried and subjected to 75 μg mL^{-1} proteinase K digestion for 1 h at 37 °C. The reaction was stopped with 1 mM phenylmethylsulfonyl fluoride (PMSF) in 20 mM Tris-HCl-buffered saline (TBS) and the membrane washed extensively with TBS and immersed in 1.8 M guanidine thiocyanate in TBS for 10 min at room temperature. After further washing with TBS, the membrane was blocked using 5% fat-free milk powder in phosphate-buffered saline (PBS), processed with 0.2 μg mL^{-1} mouse monoclonal anti-PrP 6H4 (Prionics), and developed using an ECL kit (Amersham Pharmacia Biotech).

Every experiment was carried out in triplicate and an average value for PrP^{Sc} concentration calculated, relative to an untreated control (DMSO only), together with a standard deviation. Curcumin was employed as a positive control and effected essentially complete clearance of PrP^{Sc} at the concentration used (10 μM). Test compounds were initially screened at 1, 10, and 20 μM and were considered to be active if PrP^{Sc} levels were reduced to less than 70% of that of the untreated control after 5 days' exposure. The amounts of

Table 1. Initial Screening Results and EC_{50} Values for Indole-3-glyoxylamides **1**−**34** in the SMB Cell Line Assay

						
Compound No.	**3**	**4**	**2**	**6**	**7**	**8**
Yield (%)	95	21	31	21	82	61
%PrP^{Sc} (conc)[a]	19.5±13.0 (1)[b]	93.8±29.7 (20)	21.7±15.5 (10)	4.1±1.7 (1)	37.9±23 (10)	98.8±22 (10)
EC_{50} (μM)	**0.32**[c]	–	**6.35**	**0.13**	**12.1**	–
						
9	**1**	**10**	**11**	**12**	**13**	**14**
7	14	11	13	72	47	51
85.4±20.0 (20)	40.0±10.5 (10)	5.9±1.2 (1)	140±0.1 (10)[c]	17.5±7.0 (10)	6.7±8.2 (1)	17.8±8.6 (1)
–	**1.51**	**0.065**	–	**0.23**	**0.011**	**0.24**
						
15	**16**	**17**	**18**	**19**	**20**	**21**
70	15	86	12[d]	25	19	63
27.1±8.9 (1)	10.3±13.2 (1)	70.1±28.9 (20)	17.6±10.1 (10)	23.0±16.5 (1)	90.0±24.9 (20)	107±18.9 (20)
0.64	**0.064**	–	**14.8**	**0.68**	–	–
						
22	**23**	**24**	**25**	**26**	**27**	**28**
45	69	70	81	81	88	13
95.2±18.2 (20)	98.3±15.7 (20)	135±31.5 (20)	101±42.3 (20)	115±21.2 (20)	41.3±14.8 (1)	80.8±14.3 (20)
–	–	–	–	–	**0.17**	–
						
29	**30**	**31**	**32**	**33**	**34**	
29	13	52	63	50	55	
74.9±8.2 (20)	158±17.3 (20)	83.1±16.1 (20)	127±9.9 (24)	112±18.6 (20)	80.3±13.7(20)	
–	–	–	–	–	–	

[a] %PrP^{Sc} remaining, relative to an untreated control, after 5 days of exposure to the test compound at the concentration specified (μM). [b] Results are listed for the lowest concentration showing activity (< 70% of control). For inactive compounds, the result at the highest nontoxic concentration tested is given. [c] Toxic at 20 μM. [d] Yield over two steps (see Scheme 2). [e] Each EC_{50} determination was carried out in triplicate and each compound assessed in at least two independent experiments. Full details for each active compound presented in Tables 1−3 are included in the Supporting Information, including individual dose−reponse curves and standard deviation of the reported EC_{50}.

PrP^{Sc} remaining as a percentage relative to the control are given in Tables 1−3. Compounds showing activity were rescreened over a range of concentrations to determine an EC_{50} value, such experiments being repeated at least twice (in triplicate) to validate the results so obtained. Two well documented antiprion agents were also investigated as positive controls. Observed EC_{50} values of 0.95 ± 0.07 and 0.42 ± 0.12 μM were obtained for curcumin and quinacrine, respectively, in the SMB cells. Reported values are 0.01 μM for curcumin (in ScNB cells)[37] and range from 0.3 to 0.5 μM for quinacrine (in ScN2a cells).[38] As such, in the SMB cell model, quinacrine displays the same degree of efficacy as in other prion-infected cell lines whereas curcumin is 100-fold less potent than described elsewhere.

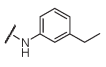
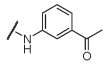
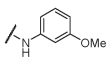
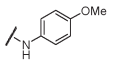
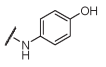
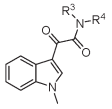
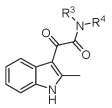
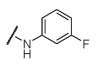
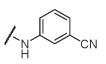
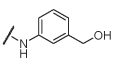
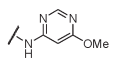
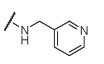
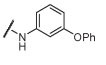
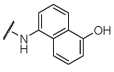
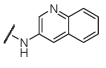
Results and Discussion

Elucidation of Structure−Activity Relationship. Screening results for the initial compound library derived from unsubstituted indole are shown in Table 1. Examination of the data quickly reveals some obvious trends. Only glyoxylamides derived from aromatic amines displayed any cell line activity; compounds prepared from aliphatic (**28**−**31**) or benzylic (**20**−**22**) amines were all inactive. Furthermore, library members synthesized from secondary amines (**32**−**34**) were also ineffective. The potent activity of **3**, derived from aniline, was completely abolished by introducing a methyl group at the glyoxylamide position (**32**).

Indeed, perhaps the most unexpected result of all was the potent activity of unsubstituted phenyl compound **3**, observed to have an EC_{50} of 0.32 μM. Meta-substituted derivatives, including parent compounds **1** and **2**, possessed comparable or lower efficacy; only substitution at the para-position improved upon the activity of phenyl compound **3**. Specifically, *p*-chloro analogue **10** (EC_{50} = 65 nM), *p*-methoxy derivative **13** (EC_{50} = 11 nM), and *p*-fluoro derivative **16** (EC_{50} = 64 nM) were the most potent candidates identified within this initial screening set. Ortho-substitution was clearly not tolerated, as compounds **4** (*o*-Me), **9** (*o*-Cl), and **11** (*o*-OMe) all proved ineffective.

Aspects of a clear SAR were thus emerging from this first set of test compounds. Glyoxylamides derived from primary aromatic amines and bearing a para-substituent were revealed as requirements for optimum potency in the SMB cell line assay. The only exception to this trend was

Table 2. Bioactivity of Indole-3-glyoxylamides with an Additional Methyl Group Introduced at Either the 1- or 2-Position

Compound No. Yield (%) %PrP^{Sc} (conc)[a] **EC_{50} (μM)**						
	**42** 86 81.4±23.3(20)[b] **6.90**	**43** 38 132±38.5 (20) –	**44** 6 157±58.8 (20) –	**45** 58 61.8±8.0 (20) **4.73**[f]	**46** 73 11.6±1.4 (1) **0.068**	**47** 42 47.3±16.1 (10) **3.77**
	**61** 83 41.6±15.0(10)[c] –	**62** 30 78.8±22.5 (20) –	**63** 24 84.4±13.2 (20) –	**64** 52 133±11.7(20) –	**65** 60 72.4±16.0(20) –	**66** 18 83.1±13.7 (20) –
						
48 59 114±20.7 (20) –	**49** 82 91.2±13.2 (20) –	**40** 25 137±59.5 (20) –	**50** 76 86.4±11.5(10)[d] –	**51** 79 148±57.1 (10)[d] –	**52** 44 117±36.1 (10)[d] –	**53** 71 109±1.7 (20) –
67 17 64.0±18.2 (20) –	**68** 21 78.5±24.6 (20) –	**41** 11 80.8±10.7 (20) –	**69** 38 69.7±31.4(20)[e] –	**70** 72 76.7±25.4(10)[d] –	**71** 27 73.7±10.0 (20) –	**72** 61 83.0±8.7 (20) –
						
54 55 169±77.6 (10)[d] –	**55** 49 121±28.8 (20) –	**56** 37 114±33.5 (20) –	**57** 23 102±25.2 (10)[d] –	**58** 11 124±46.2 (20) –	**59** 30 100±14.9 (20) –	**60** 14 131±62.5 (10)[d] –
73 49 103±10.3 (10)[d] –	**74** 72 96.3±18.0 (20) –	**75** 0[e] – –	**76** 18 87.6±14.1 (20) –	**77** 22 129±36.7 (10)[d] –	**78** 25 81.9±25.4 (20) –	**79** 47 102±14.7 (20) –

[a] %PrP^{Sc} remaining, relative to an untreated control, after 5 days of exposure to the test compound at the concentration specified (μM). [b] Results are listed for the lowest concentration showing activity (< 70% of control). For inactive compounds, the result at the highest nontoxic concentration tested is given. [c] Some compounds showed activity in the initial screen, but meaningful dose−response curves could not be obtained (i.e., false positives). [d] Toxic at 20 μM. [e] Synthesis of this compound failed. [f] Very inconsistent results were obtained with **45**, making an accurate EC_{50} measurement problematic.

3-aminoquinolyl compound **27**, which was the only bicyclic compound (**24**−**27**) displaying activity. Comparison of the isosteric 2-naphthylamine derived analogue was excluded during initial library design, however, owing to the well established carcinogenicity of the parent amine.[39]

Having established some basic requirements for activity regarding the glyoxylamide portion of the molecule, we were keen to examine the effects of substitution at positions 1 and 2 of the indole system. To this end, a second library was synthesized, derived from 1-methylindole and 2-methylindole in combination with a subset of the amines depicted in Table 1 (Scheme 1, Table 2).

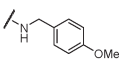
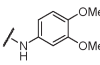
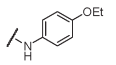
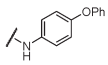
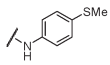
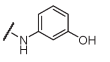
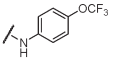
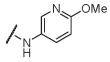
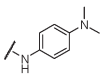
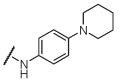
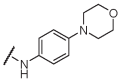
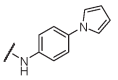
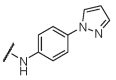
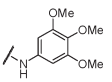
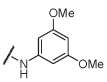
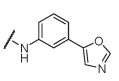
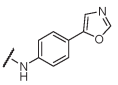
All of the 2-methylindole-3-glyoxylamides (**41**, **61**−**79**) were devoid of cell line activity, including those where the analogous 2-unsubstituted compounds had displayed submicromolar EC_{50} values. Thus, substitution at the 2-position of the indole ring is clearly not tolerated in terms of antiprion activity. Incorporation of a methyl group at N-1 also produced a marked effect. 1-Methyl derivatives of the most active indole-3-glyoxylamides retained some activity, but EC_{50} values were about an order of magnitude higher (compare **3**, 0.32 μM, with **42**, 6.90 μM; **13**, 11 nM, with **46**, 68 nM; **14**, 0.24 μM, with **47**, 3.77 μM). Changing the indole N−H to N−Me resulted in a drop in activity, suggesting that this N−H group makes a significant, but not critical, interaction with the target at the site of action.

Additional requirements for antiprion activity had therefore been deduced from this second library. Indole-3-glyoxylamides unsubstituted at N-1 are preferred, and there must be no substituent at C-2. Together with the requirements already identified (i.e., glyoxylamides derived from para-substituted anilines), these observations constitute a very clearly defined SAR for antiprion activity of indole-3-glyoxylamides. In addition, the highly potent activity of **13** (*p*-OMe) and **16** (*p*-F) strongly suggested an advantage in having a hydrogen-bond acceptor at this position.

In order to further refine the above conclusions, an additional set of compounds was prepared, derived from unsubstituted indole and again using the conditions detailed in Scheme 1 ($R^1 = R^2 = H$). In the design of this final library (Table 3), our intention was to further probe the effect of varied para-substituents upon cell line activity, paying particular attention to differing hydrogen-bond acceptor character at this position. Library member **86** was prepared using a protection strategy similar to that for some of the hydroxy-containing compounds already described (Scheme 2, $n = 0$).

Screening results from this final library (Table 3) revealed that analogues of active compounds **3**, **13**, and **16** with a nitrogen included at the 3-position of the aromatic ring

Table 3. Screening Results for Additional Indole-3-glyoxylamides Designed To Improve Understanding of Antiprion SAR

						
Compound No.	**80**	**81**	**82**	**83**	**84**	**85**
Yield (%)	46	20	22	41	48	17
%PrP^{Sc} (conc)[a]	104±15.3 (20)[b]	104±3.7 (20)	19.3±12.2 (1)	6.5±3.8 (1)	17.5±3.3 (10)	5.6±1.5 (1)
EC_{50} (μM)	–	–	**0.52**	**0.060**	**1.03**	**0.040**
						
86	**87**	**88**	**89**	**90**	**91**	**92**
15[d]	15	27	23	25	13	15
52.4±12.3 (1)	1.4±2.0 (1)	48.7±2.0 (1)	6.1± 0.8 (1)[c]	6.7±0.2 (1)	8.0±3.2 (10)	4.9±0.6 (1)
0.82	**0.32**	**1.20**	**0.29**	**0.38**	**1.66**	**0.12**
						
93	**94**	**95**	**96**	**97**	**98**	**99**
20	4	46	19	6	35	3
52.3±3.2 (1)	27.0±7.9 (1)	7.2±1.0 (1)	6.5±2.7 (1)	4.6±1.5 (1)	17.1±4.9 (1)	7.0±3.2 (1)
1.21	**0.79**	**0.026**	**0.072**	**0.009**	**0.006**	**0.001**
						
100	**101**	**102**	**103**	**104**	**105**	
27	34	12	8	2	21	
62.5±11.4 (20)	143±10.6 (20)	75.4±10.9 (20)	16.8±8.1 (10)	16.8±8.2 (1)	105±2.6 (10)	
–	–	–	**0.53**	**0.019**	–	

[a] %PrP^{Sc} remaining, relative to an untreated control, after 5 days of exposure to the test compound at the concentration specified (μM). [b] Results are listed for the lowest concentration showing activity (< 70% of control). For inactive compounds, the result at the highest nontoxic concentration tested is given. [c] Toxic at 10 and 20 μM. [d] Yield over two steps.

(**91**−**93**) all displayed a reduction in potency by approximately an order of magnitude. The detrimental effect of incorporating a ring nitrogen was also evident from the results for 3- and 4-pyridyl compounds **91** (EC_{50} = 1.66 μM) and **94** (EC_{50} = 0.79 μM), though of these isomers, the 4-pyridyl derivative **94** was the more effective, reflecting the general requirement for para- rather than meta-substitution observed in the initial screening set (Table 1).

Indeed, within this final library, the previously observed preference for para-substitution gains added support. Comparison of **86** with **14** (*m*-OH, EC_{50} = 0.82 μM; *p*-OH, EC_{50} = 0.24 μM) and of **103** with **104** (*m*-oxazol-5-yl, EC_{50} = 0.53 μM; *p*-oxazol-5-yl, EC_{50} = 19 nM) further underscores the trend already identified for improved potency of para- over meta-substituted compounds. In addition, library members derived from a number of 3,4-disubstituted anilines were prepared (3,4-dimethoxy-, **82**; 3,4-difluoro-, **87**; 3,4-dimethyl-, **88**). In all cases, these compounds displayed EC_{50} values closer to those of the corresponding meta-substituted rather than para-substituted analogue; note, for example, the activity of 3,4-difluorophenyl derivative **87** (EC_{50} = 0.32 μM) compared to its *m*- and *p*-fluoro analogues **15** (EC_{50} = 0.64 μM) and **16** (EC_{50} = 64 nM). Clearly, there is no complementary, additive effect between the activities of the individually substituted compounds, emphasizing a requirement for para-substitution only; and as further evidence of this, 3,4,5-trisubstituted screening subjects (**100**, trifluoro-; **101**, trimethoxy-) showed a complete loss of activity.

Cell line activity was also abolished upon insertion of a methylene bridge between the amide nitrogen and phenyl ring; i.e., the potent activity of **12** (EC_{50} = 0.23 μM) and **13** (EC_{50} = 11 nM) was completely eradicated in compounds **80** and **81**, prepared from the analogous benzylamines.

Clear trends were apparent in the potencies of several indole-3-glyoxylamides derived from additional subsets of para-substituted anilines. The sequence of EC_{50} values exhibited by *p*-OMe **13** (11 nM) < *p*-SMe **85** (40 nM) < *p*-OEt **83** (60 nM) < *p*-OCF_3 **90** (0.38 μM) < *p*-OPh **84** (1.03 μM) does suggest an important role for the H-bonding character of the para-substituent but also a binding pocket of limited size or shape. The most effective group of compounds by far was those possessing a *p*-amino substituent: *p*-piperidino **96** (72 nM) > *p*-NMe_2 **95** (26 nM) > *p*-morpholino **97** (9 nM) > *p*-pyrrolyl **98** (6 nM) > *p*-1*H*-pyrazolyl **99** (1 nM). The last three compounds, all showing an observed EC_{50} < 10 nM, contain an N-linked heterocycle at the key para-position of the phenyl ring. In combination with the similarly potent activity of *p*-oxazol-5-yl derivative **104** (EC_{50} = 19 nM), it is apparent that a (preferably aromatic) heterocycle, containing at least one hydrogen-bond acceptor, should be present at the para-position of the aromatic ring for optimal antiprion activity.

Mode of Action. Given the potent activity of many of the indole-3-glyoxylamides above, it is evidently important to understand their mode of action in the antiprion assay. They do not appear to exert their effect through direct interaction with PrP^{C}, however, since in the surface plasmon resonance (SPR) binding assay we have documented previously,[24c,27,40] evaluation of a selection of the active compounds (**3**, **12**−**15**, and **27**, all with EC_{50} < 1 μM) gave no evidence for discernible binding to either the human or murine protein at concentrations up to 40 μM. In addition, it is worth noting

that the clearly defined SAR described above does not appear to correlate with those deduced for other modes of action of related indole-3-glyoxylamides, for example, as tubulin polymerization inhibitors[41] or as ligands for the benzodiazepine binding site of the $GABA_A$ receptor.[34b,34c] Although it presents considerable challenges, elucidating the cellular target of the presently described lead series is an obvious priority and may offer some insight into the as yet poorly understood mechanisms operating within prion disease.

Conclusions and Future Perspective

Indole-3-glyoxylamides prepared from a variety of arylamines have been established as a novel lead series against prion disease, possessing in vitro activity down to nanomolar concentrations with a tightly defined SAR. A survey of the available literature reveals no class of antiprion compounds has yet been reported with both this degree of potency and a clear SAR, and as such, discovery of the indole-3-glyoxylamide libraries detailed above may revive promise that small-molecule therapeutics against prion disease can be found. It would be desirable to further validate the most active indole compounds in other cell models of prion infection, and the results of such work will be reported in due course. Additional investigations (to refine pharmacokinetic properties, gain further understanding of the SAR, and elucidate possible modes of action of the compounds) are similarly ongoing in order to identify the best possible candidates for progression into in vivo studies.

Experimental Section

General Procedures. Accurate mass and nominal mass measurements were obtained using a Waters-Micromass LCT electrospray mass spectrometer. Anhydrous grade THF was obtained from an in-house "Grubbs" solvent purification system; all other solvents and reagents were purchased from commercial sources and used as supplied. All reactions were performed under N_2, and parallel synthesis was carried out on a Radleys 12-position carousel. Evaporation of solvent from reaction tubes was performed using a Büchi Multivapor P-12 system. Parallel extractions were carried out with liquid−liquid extraction columns, 20 mL sample loading capacity, in conjunction with a carousel workup station. Where required, flash column chromatography was carried out using prepacked silica columns (20 g/70 mL). Purity of screening compounds was assessed by HPLC (Waters SymmetryShield 3.5 μm C-18 column, 150 mm × 4.6 mm, 30−95% MeCN in water over 12 min at 1.0 mL min^{-1}, held 10 min; 15 μL injection; UV detection at 254 nm; run time 22 min). The large majority of compounds were isolated in >95% purity; a complete list of HPLC purities, along with full characterization data for each screening compound, is provided in the Supporting Information.

Indole-3-glyoxylamides 1−34 and 40−79. General Procedure. A dry carousel reaction tube was charged with indole (234 mg, 2 mmol), 1-methylindole (250 μL, 262 mg, 2 mmol), or 2-methylindole (262 mg, 2 mmol) as appropriate, and this starting material was dissolved in dry THF (12 mL). Oxalyl chloride (192 μL, 279 mg, 2.2 mmol) was added and the mixture stirred at room temperature. After 1 h, *N*,*N*-diisopropylethylamine (785 μL, 582 mg, 4.5 mmol) was introduced to the mixture, followed by the relevant amine (2.4 mmol). The temperature was raised to 45 °C, and heating continued for 18 h. The solvent was evaporated, then the residue resuspended in a mixture of ethyl acetate (20 mL) and brine (10 mL) and stirred vigorously for 30 min. After the sample was passed through a liquid−liquid extraction column, ensuring washing through of the column with additional ethyl acetate, a second extraction was carried out with saturated NH_4Cl (10 mL) in the same manner. Product solutions were then evaporated to dryness giving the crude indole-3-glyoxylamides, which were purified as necessary (see Supporting Information for individual compound details). Typically, two sequential recrystallizations, from ethyl acetate−hexane followed by 2-propanol−water, afforded final products of good purity.

For products derived from poorly nucleophilic anilines (e.g., **89**−**91**, **93**, **99**, **100**), a further modification was found helpful. Immediately following introduction of the arylamine to the reaction mixture, DMAP (24 mg, 0.2 mmol, 10 mol %) was added to assist the final step, as its use was found necessary to promote any meaningful conversion to product in such cases.

3-(*tert*-Butyldimethylsilyloxymethyl)aniline 35a. *tert*-Butyldimethylsilyl chloride (3.01 g, 20 mmol) was added to a solution of 3-aminobenzyl alcohol (2.46 g, 20 mmol) and imidazole (3.40 g, 50 mmol) in anhydrous DMF (25 mL) at 0 °C. After 2 h, cooling was removed and the mixture allowed to warm to room temperature overnight. The mixture was poured into water (150 mL) and extracted into ether (3 × 100 mL), following which the combined extracts were washed with brine, dried over $MgSO_4$, and evaporated to dryness. Flash column chromatography on silica gel, eluted with ethyl acetate−hexane (1:4), provided the title compound as a pale-yellow oil (3.37 g, 70%). δ_H (250 MHz, $CDCl_3$) 7.01 (t, 1H, J = 8.0), 6.64−6.56 (m, 2H), 6.50−6.44 (m, 1H), 4.56 (s, 2H), 3.51 (br s, 2H), 0.84 (s, 9H), 0.00 (s, 6H); δ_C (62.8 MHz, $CDCl_3$) 146.3, 142.8, 129.1, 116.4, 113.7, 112.8, 64.9, 26.0, 18.4, −5.3; *m*/*z* (ES) 238 ($[M + H]^+$); HRMS, found 238.1625 ($C_{13}H_{24}NOSi$ requires 238.1627).

***N*-(3-(Hydroxymethyl)phenyl)-2-(1*H*-indol-3-yl)-2-oxoacetamide 18.** The TBDMS-protected intermediate **36a** (Scheme 2) was prepared from indole and amine **35a** according to the general procedure above. δ_H (400 MHz, $CDCl_3$) 9.41 (s, 1H), 9.18 (br s, 2H), 8.48 (d, 1H, J = 7.5), 7.70−7.64 (m, 2H), 7.47 (d, 1H, J = 7.5), 7.43−7.32 (m, 3H), 7.19 (d, 1H, J = 7.5), 4.80 (s, 2H), 0.99 (s, 9H), 0.15 (s, 6H); δ_C (125 MHz, $CDCl_3$) 180.5, 160.0, 142.8, 138.5, 136.8, 135.7, 129.1, 126.7, 124.4, 123.6, 122.7, 122.5, 118.5, 117.5, 113.2, 111.7, 64.7, 26.0, 18.5, −5.2; *m*/*z* (ES) 409 ($[M + H]^+$); HRMS, found 409.1934 ($C_{23}H_{29}N_2O_3Si$ requires 409.1947). Compound **36a** (166 mg, 0.41 mmol) was dissolved in THF (2.5 mL), followed by addition of tetrabutylammonium fluoride (1.0 M in THF, 0.45 mL, 0.45 mmol). After 1 h the reaction mixture was evaporated and the residue purified by flash column chromatography on silica gel, eluting with methanol−DCM (0:100, then 2:98, then 1:19), affording the title compound as pale-yellow needles (73 mg, 61%).

***N*-(3-(Hydroxymethyl)phenyl)-2-(1-methyl-1*H*-indol-3-yl)-2-oxoacetamide 40 and *N*-(3-(Hydroxymethyl)phenyl)-2-(2-methyl-1*H*-indol-3-yl)-2-oxoacetamide 41.** Trimethylsilyl chloride (253 μL, 217 mg, 2 mmol) was added to a solution of 3-aminobenzyl alcohol (205 mg, 1.67 mmol) in dry THF (5 mL). After 1 h, this solution was introduced directly into a reaction between the appropriate substituted indole (1 mmol) and oxalyl chloride (100 μL, 146 mg, 1.2 mmol) in the same solvent (5 mL) (which had already been stirring at room temperature for 1 h) together with *N*,*N*-diisopropylethylamine (610 μL, 452 mg, 3.5 mmol). After a further 3 h, the reaction was quenched by addition of 1 M HCl (10 mL), and after the mixture was stirred for 10 min, it was evaporated to dryness. The crude product was taken up in ethyl acetate and washed successively with 0.4 M K_2CO_3 (2 × 25 mL), saturated NH_4Cl (2 × 25 mL) and brine, then dried over $MgSO_4$ and evaporated once more. Purification by flash column chromatography on silica gel, eluted with ethyl acetate−cyclohexane (1:1), gave either compound **40** (79 mg, 25%; HPLC purity 99%) or **41** (36 mg, 11%; HPLC purity 88%) as required.

3-(*tert*-Butyldimethylsilyloxy)aniline 35b. 3-Aminophenol (2.00 g, 18.3 mmol) and imidazole (2.00 g, 29.4 mmol) were

dissolved in anhydrous THF (50 mL). Then *tert*-butyldimethylsilyl chloride (3.60 g, 23.4 mmol) was added with vigorous stirring. After 30 min, the reaction mixture was poured into water (200 mL) and extracted into ether (3 × 50 mL). The combined extracts were dried over $MgSO_4$ and evaporated to dryness giving the crude product as a viscous, colorless oil which was used in the subsequent step without further purification. δ_H (400 MHz, $CDCl_3$) 7.02 (t, 1H, $J = 8.0$), 6.35−6.27 (m, 2H), 6.23 (t, 1H, $J = 2.0$), 3.17 (br s, 2H), 1.00 (s, 9H), 0.21 (s, 6H).

***N*-(3-Hydroxyphenyl)-2-(1*H*-indol-3-yl)-2-oxoacetamide 86.** Protected intermediate **36b** (Scheme 2) was prepared from indole and crude 3-(*tert*-butyldimethylsilyloxy)aniline **35b** (0.80 g, 3.6 mmol) according to the general procedure for indole-3-glyoxylamides above. δ_H (400 MHz, $CDCl_3$) 9.35 (s, 1H), 9.19 (d, 1H, $J = 3.0$), 9.02 (br s, 1H), 8.51−8.47 (m, 1H), 7.50−7.46 (m, 1H), 7.43−7.34 (m, 3H), 7.31−7.24 (m, 2H), 6.71 (ddd, 1H, $J = 1.5, 2.5, 7.5$), 1.03 (s, 9H), 0.26 (s, 6H); δ_C (62.8 MHz, $CDCl_3$) 180.5, 159.9, 156.4, 138.4, 137.9, 135.7, 129.9, 126.7, 124.4, 123.6, 122.5, 116.8, 113.2, 112.9, 111.9, 111.7, 25.7, 18.2, −4.4; *m*/*z* (ES) 395 ($[M + H]^+$); HRMS, found 395.1807 ($C_{22}H_{27}N_2O_3Si$ requires 395.1791). Compound **36b** (132 mg, 0.34 mmol) was then dissolved in THF (1.5 mL), and tetrabutylammonium fluoride (1.0 M in THF, 0.37 mL, 0.37 mmol) was added. A thick precipitate had appeared within a few minutes. After 30 min, the product was isolated by filtration, washed twice each with THF, DCM, and water, and dried thoroughly. The title compound **86** was thereby obtained as its tetrabutylammonium salt (140 mg, 80%).

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Supporting Information Available: Spectroscopic data for all library members (HRMS, IR, ^{1}H NMR, ^{13}C NMR); individual compound purification details; compound purities by HPLC; and dose−response curves for all cell line active compounds (including the positive controls curcumin and quinacrine). This material is available free of charge via the Internet at http://pubs.acs.org.

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