

A Chimeric Ligand Approach Leading to Potent Antiprion Active Acridine Derivatives: Design, Synthesis, and Biological Investigations

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Human transmissible neurodegenerations including Creutzfeldt–Jakob disease are unique, since they are caused by prions, an infectious agent that replicates without nucleic acids but instead by inducing conversion of a host-resident normal prion protein to a misfolded conformational isoform. For pharmacotherapy of these unusual diseases, tricyclic heterocyclic compounds such as quinacrine have been considered, but with ambiguous success *in vivo*, so far. On the basis of the synergistic antiprion effects of quinacrine and iminodibenzyl derivatives, we introduce a novel class of potential pharmaceuticals representing structural chimeras of quinacrine and imipramine analogues. We describe the chemical synthesis and bioassays of a focused library of these compounds. The most potent target compound **2a** revealed an EC₅₀ value of 20 nM determined with a cell model of prion disease, thus substantially improving the antiprion efficacy of quinacrine.

Introduction

Prion diseases are transmissible neurodegenerative diseases of humans and animals.¹ The prion agent replicates without a nucleic acid-encoded genome by converting host-resident normal prion protein (PrP^C)^a to a pathogenic and infectious conformational isoform PrP^{Sc}. Human prion diseases such as the most prevalent Creutzfeldt–Jakob disease (CJD) are rare diseases that can—uniquely—be of sporadic, genetic, or infectious origin. Animal prion diseases are almost exclusively infectious in origin in the form of scrapie of sheep, bovine spongiform encephalopathy (BSE) or mad cow disease of cattle, or chronic wasting disease of American mule deer or elk. The emergence of a new strain of human prions causing variant CJD (vCJD) through the consumption of BSE-contaminated material² led to 160 deaths, primarily in Great Britain, and to concerns of an epidemic of vCJD which, so far, has neither happened nor been completely excluded.³ The new vCJD strain has different characteristics, such as the occurrence of infectivity in blood and peripheral organs, which increases the risk of horizontal transmission, for example, during blood transfusions.⁴ These issues have led to a focus on pharmacotherapeutic options of prion diseases both for symptomatic, curative, and prophylactic purposes or for decontamination of blood and transplanted organs.

Although screening for antiprion compounds in scrapie-infected mouse neuroblastoma cells (ScN2a) as a cell culture model of prion disease has led to the identification of many antiprion compounds, most of them proved to be ineffective when applied to experimentally prion-infected rodents owing to the lack of blood–brain barrier (BBB) permeability or toxicity.⁵ The identification of BBB-permeable heterocyclic

compounds sparked a tremendous effort to enter clinical trials applying the approved drug quinacrine to patients with CJD.⁶ Since animal experiments with quinacrine gave only modest or ambiguous results,⁷ quinacrine pharmacotherapy could be improved by introducing a combination of antiprion drugs.⁸ Structural modifications employing quinacrine as a lead compound revealed that bis-acridines augmented antiprion activity around 10-fold *in vitro*,⁹ but poor solubility is likely to limit their *in vivo* use. The synergistic antiprion effects of quinacrine- and iminodibenzyl-derived antidepressants have prompted us to synthesize heterodimers incorporating both recognition elements with a piperazine unit,¹⁰ which is known as a privileged substructure in a great number of bioactive agents, as the linking element.

On the basis of preliminary investigations on the hybrid **1** (Figure 1) displaying a 5-fold improved antiprion potency over quinacrine,⁸ we herein describe a novel class of antiprion agents of type **2** defined by covalently linked acridine and iminodibenzyl moieties and variations of the particular molecular subunits. SAR studies led to the discovery of the iminodibenzyl derivative **2a** as the most active antiprion compound yet described.

Chemistry. Following our strategy to investigate the structure–activity relationships of quinacrine–imipramine chimeras, we intended to realize modifications within the different pharmacophoric substructures. Thus, the iminodibenzyl, the acridine, and the spacer unit should be an object of diversification, leading to a focused set of test compounds.

The synthesis of target compounds was performed starting from iminodibenzyl (**3a**), phenothiazine (**3b**), and diphenylamine (**3c**) (Scheme 1). The conversions into the respective *N*-chloropropyl or *N*-bromopropyl derivatives were accomplished by direct alkylation with 1-bromo-3-chloropropane or via palladium-catalyzed allylation¹¹ followed by hydroboration and subsequent Appel reaction. The latter was established as an alternative due to the fact that alkylation with the 1,3-biselectrophile resulted in a mixture of *N*-chloropropyl and *N*-allyl substituted products, which is obviously due to β -elimination. The halopropyl derivatives **4a–c**^{12–15} were converted into the respective cyanoalkylpiperazines **5a–i**^{16–18} by nucleo-

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^a Abbreviations: BBB, blood–brain barrier; CJD, Creutzfeldt–Jakob disease; FCS, fetal calf serum; MEM, minimal essential medium; PrP^C, host-resident normal prion protein; PrP^{Sc}, infectious conformational isoform of prion protein; RML, Rocky Mountain Laboratory; Tris, tris[(hydroxymethyl)amino]methane; vCJD, variant Creutzfeldt–Jakob disease.

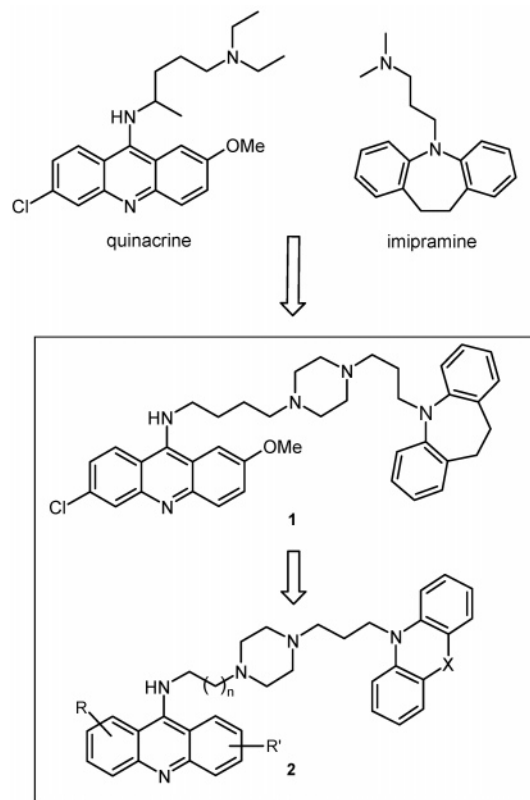
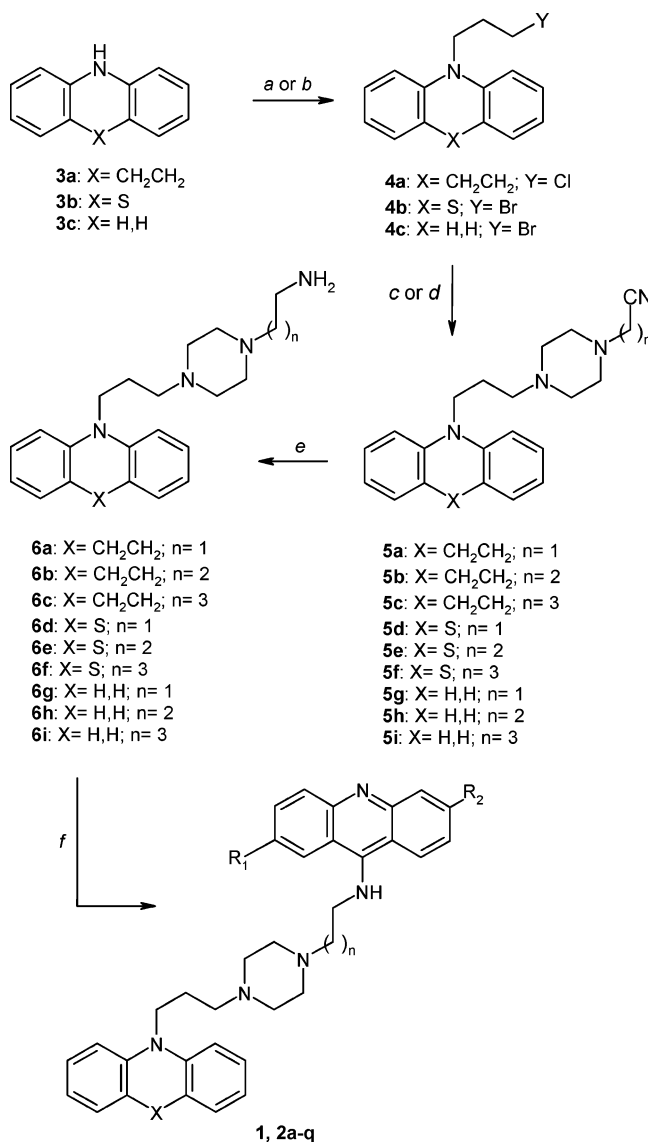


Figure 1. Development of structural chimeras based on quinacrine and imipramine.

philic substitution. Subsequent reduction with LiAlH_4 gave the primary amines **6a–i**, which underwent S_N reaction with 9-chloroacridine or 6,9-dichloro-2-methoxyacridine, yielding the desired antiprion chimeras **1** and **2a–q**.

Biological Investigations. The acridine derived chimeras **1** and **2a–q** were investigated for their ability to inhibit the pathogenic prion protein PrP^{Sc} in a cell-based assay employing quinacrine and imipramine as reference agents (Table 1). All test compounds revealed significant dose-dependent antiprion activity. Our initial observations were directed to the test compound **1** displaying the closest structural similarity to the reference building blocks. In fact, the EC_{50} value of 80 nM indicated a substantially higher antiprion activity when compared to quinacrine (300 nM) and imipramine (5000 nM). Thus, the dimerization concept presented by May et al.⁹ proved to be fruitful also for heterodimeric agents. The methoxy- and chloro-substituents, which were adopted from the quinacrine structure, proved to be beneficial for antiprion activity, because the unsubstituted analogue **2c** showed reduced potency ($\text{EC}_{50} = 150$ nM). To evaluate the relationship between the distance of the heterocyclic core units and the biological activity, the shortened ethylene and propylene analogues **2a,d** and **2b,e**, respectively, were also evaluated for antiprion activity. Interestingly, shortening of the original four-carbon spacer between the aminoacridine and the piperazine unit was associated by a reduction of biological activity for **2b,d,e**. However, the ethylene unit proved to be superior for the unsubstituted acridines **2a–c**, resulting in an EC_{50} value of 20 nM for the test compound **2a** (Figure 2). The same SAR pattern could be observed for the diphenylamine-derived bioisosteres **2l–q** when the ethylene-bridged agent **2l** incorporating an unsubstituted acridine moiety revealed an EC_{50} value of 75 nM. Replacement of the iminodibenzyl moiety by the phenothiazine scaffold provoked a reduction of antiprion activity, as indicated by EC_{50} values between 140 and

Scheme 1^a



^a Reagents and conditions: (a) for **4a**, 1-bromo-3-chloropropane, NaNH_2 , toluene, rt, 21 h (crude 100%). For **4b**, 1-bromo-3-chloropropane, NaH, DMF, rt, 4 h (90%). (b) (1) allylmethyl carbonate, $\text{Pd}(\text{PPh}_3)_4$, THF, rt, 5 h (90%); (2) 9-BBN, THF, 2 N NaOH, H_2O_2 30%, rt, 16 h (71%); (3) PPh_3 , CBr_4 , acetonitrile, rt, 17 h (80%). (c) For **5a**, (1) piperazine, K_2CO_3 , acetonitrile, 16 h; (2) bromoacetonitrile, K_2CO_3 , acetonitrile, 16 h (57%). For **5b**, (1) piperazine, K_2CO_3 , acetonitrile, 16 h; (2) bromopropionitrile, K_2CO_3 , acetonitrile, 16 h (74%). For **5c**, (1) piperazine, K_2CO_3 , acetonitrile, 16 h; (2) bromobutyronitrile, K_2CO_3 , acetonitrile, 16 h (31%). (d) For **5d** and **5g**, piperazinoacetonitrile, diisopropylethylamine, NaI, DMF, reflux, 18 h (54–70%). For **5e** and **5h**, piperazinoacetonitrile, diisopropylethylamine, NaI, DMF, reflux, 18 h (75–77%). For **5f** and **5i**, piperazinoacetonitrile, diisopropylethylamine, NaI, DMF, reflux, 18 h (50–72%). (e) LiAlH_4 , diethyl ether, 0 °C, 30 min (21–91%). (f) 6,9-Dichloro-2-methoxyacridine and 9-chloroacridine (1.0 mmol), respectively, phenol (10.0 mmol), 100 °C, 18 h (30–64%).

830 nM for the test compounds **2f–k**. Thus, taking all derivatives into consideration, the iminodibenzyl framework proved to be the most valuable bioisostere.

In addition to the recently reported hybrid **1** showing an EC_{50} value of 80 nM, the analogue **2a** bearing an unsubstituted acridine moiety in combination with an ethylene spacer to the piperazine turned out to exhibit the strongest potency, with a hitherto unprecedented EC_{50} value of 20 nM and a full antiprion activity (FAA) of 75 nM. When compared to the lead structure quinacrine, the construction of chimeric derivatives in most of

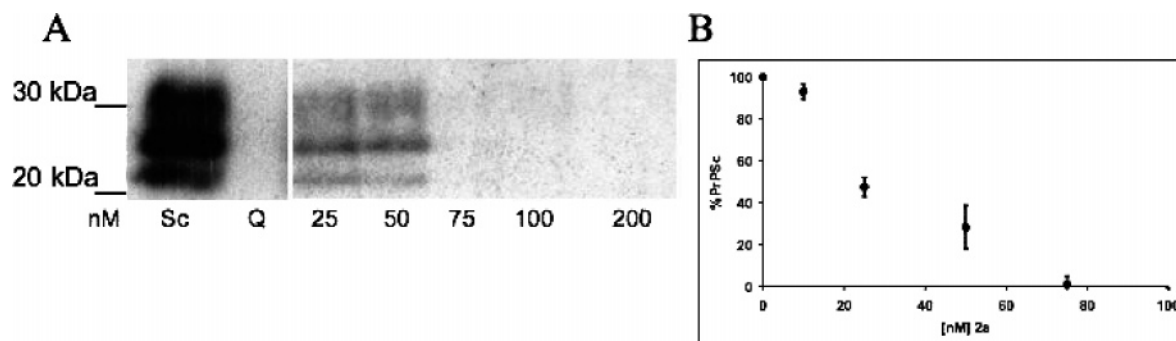
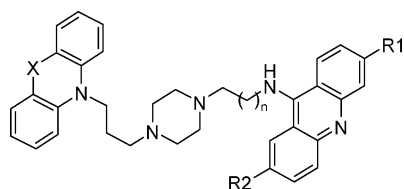


Figure 2. Western blot (A) and densitometry quantitation (B) of compound **2a**. (A) Nontreated (Sc) or quinacrine-treated (Q; 1 mM) ScN2a cells were used as controls; increasing concentrations of compound **2a** show the disappearance of protease-resistant PrP immunoreactivity (a monoclonal antibody against mouse PrP was used). (B) Three independent experiments as in part A were quantified by densitometry. The EC₅₀ can be extrapolated to 20 nM. Bars represent standard deviations.

Table 1. Antiprion Activity of Test Compounds **2a–r**^a



compd	R1	R2	X	n	EC ₅₀ (nM)	FAA (nM) ^b	toxicity (nM) ^c
2a	H	H	CH ₂ CH ₂	1	20 ± 6	75	500
2b	H	H	CH ₂ CH ₂	2	230 ± 30	500	1000
2c	H	H	CH ₂ CH ₂	3	150 ± 30	400	750
2d	Cl	OMe	CH ₂ CH ₂	1	570 ± 60	1000	2000
2e	Cl	OMe	CH ₂ CH ₂	2	260 ± 30	500	1500
1	Cl	OMe	CH ₂ CH ₂	3	80 ± 5	100	400
2f	H	H	S	1	370 ± 70	600	3000
2g	H	H	S	2	140 ± 40	500	1000
2h	H	H	S	3	300 ± 50	750	4000
2i	Cl	OMe	S	1	830 ± 140	2000	4000
2j	Cl	OMe	S	2	200 ± 80	500	2500
2k	Cl	OMe	S	3	350 ± 50	1000	2000
2l	H	H	H, H	1	75 ± 5	200	1000
2m	H	H	H, H	2	120 ± 20	500	1500
2n	H	H	H, H	3	230 ± 30	400	1000
2o	Cl	OMe	H, H	1	290 ± 20	500	1500
2p	Cl	OMe	H, H	2	280 ± 60	500	2000
2q	Cl	OMe	H, H	3	250 ± 50	500	>1000
quinacrine					300 ± 30	500	
imipramine					5000 ± 540	10000	

^a EC₅₀ is defined as the concentration of the test compound leading to 50% reduction of protease-resistant PrP. ^b FAA (full antiprion activity) is defined as the concentration of the test compound leading to complete reduction of protease-resistant PrP. ^c Toxicity was defined as less than 80% confluency of ScN2a cells after 1 week of treatment.

the cases resulted in retention or even increase of potency, clearly proving that the tricyclic or diphenylamine serves as an additional pharmacophoric element, presumably addressing an accessory binding site of the—yet still unknown—molecular target of these antiprion active agents.

Conclusions

We have introduced a novel family of structural hybrids including the iminodibenzyl derivative **2a** demonstrating an unprecedented antiprion potency. SARs support the specific action of this class of compounds, even though the exact mechanism of antiprion action remains unclear. Redistribution of cellular cholesterol from conversion-mandatory lipid rafts on the plasma membrane has been proposed to account for the antiprion actions of the heterocyclic compounds, including test compound **1**. Of note, May et al. synthesized quinacrine-based

bis-acridines in order to improve antiprion potency, assuming that quinacrine binds to the prion protein and linking two acridine moieties would increase affinity for PrP.⁹ Although high-affinity binding of quinacrine or its derivatives has not been demonstrated, bis-acridines exhibit up to 10-fold increase in antiprion potency compared to quinacrine.

Our reasoning of synthesizing acridine and iminodibenzyl moieties into structural chimeras started from a completely different background: the observation of the additive antiprion effects of quinacrine and iminodibenzyl derivatives in ScN2a cells led us to merge both antiprion-active compounds into a single molecule. The favorable BBB-permeability profile of the iminodibenzyl derivatives such as the tricyclic antidepressant imipramine added to the future in vivo potential of this class of compounds. The best compound of this class so far, **2a**, has a 15-fold increased antiprion potency when compared to quinacrine, making it a new lead compound. Given the effects of these compounds on cellular lipid metabolism and the intactness of lipid raft compartments,⁸ we expect that this novel class of compounds might also be applicable to diseases where these cellular compartments play a decisive role.

Experimental Section

Chemistry. All reactions were carried out under nitrogen atmosphere unless otherwise stated. Solvents were purified and dried by standard procedures. All reagents were of commercial quality and used as purchased. MS were run on a Finnegan MAT TSQ 70 spectrometer by EI (70 eV) with solid inlet and a Bruker Esquire 2000 spectrometer applying APC ionization. [α]_D²⁰ values were measured on a Perkin-Elmer 241 instrument. The ¹H NMR spectra were obtained on a Bruker AM 360 (360 MHz) and Bruker AVANCE (600 MHz) spectrometer in CDCl₃ relative to TMS (*J* values in Hz). IR spectra were performed on a Jasco FT/IR 410 spectrometer. Purification by chromatography was performed using silica gel 60, and TLC analyses were performed using Merck 60 F₂₅₄ aluminum sheets and analyzed by UV light (254 nm) or in the presence of iodine. Preparative and analytical HPLC was performed on an Agilent 1100 preparative HPLC system employing a VWL detector. C, H, N elementary analyses were performed at the Department of Organic Chemistry of the Friedrich Alexander University or at the microanalytical laboratory Ilse Beetz (Kronach, Germany).

General Procedure for the Preparation of *N*-(3-Cyanoalkyl)-piperazines **5a–c.** A suspension of **4a** (30.5 mmol), piperazine (61.0 mmol), Na₂CO₃ (30.5 mmol), and NaI (6.1 mmol) in acetonitrile (250 mL) was heated at reflux temperature for 16 h. Addition of diethyl ether (250 mL) and Na₂SO₄ was followed by filtration and evaporation of the solvent. The residue was resolved in acetonitrile (10 mL), treated with the respective bromoalkylnitrile (2 equiv), Na₂CO₃ (1 equiv), and NaI (0.2 equiv), and stirred at reflux temperature (for bromopropionitrile and bromobutyronitrile)

or at ambient temperature (for bromoacetonitrile) for 16 h. After addition of diethyl ether (30 mL) and Na₂SO₄, filtration, and evaporation, the crude product was purified by flash chromatography.

[3-(10,11-Dihydrodibenzo[*b,f*]azepin-5-yl)propyl]piperazin-1-ylacetoneitrile (5a). The title compound was prepared according to the general procedure when the crude product was purified by flash chromatography (hexane/ethyl acetate/methanol 7/2/1) to give **5a** (57%) as a yellow oil. ¹H NMR (360 MHz): δ 1.73 (quint, *J* = 7.0 Hz, 2H), 2.32–2.60 (m, 10H), 3.15 (s, 4H), 3.45 (s, 2H), 3.76 (t, *J* = 7.0 Hz, 2H), 6.90 (ddd, *J* = 7.3 Hz, 7.2 Hz, 1.3 Hz, 2H), 7.05–7.15 (m, 6H). EIMS: *m/z* 360 (M⁺).

4-{4-[3-(10,11-Dihydrodibenzo[*b,f*]azepin-5-yl)propyl]piperazin-1-yl}butyronitrile (5c). The title compound was prepared according to the general procedure when the crude product was purified by flash chromatography (hexane/ethyl acetate/methanol 7/2/1 + 0.5% triethylamine) to give **5c** (31%) as a yellow oil. ¹H NMR (600 MHz): δ 1.75 (quint, *J* = 6.9 Hz, 2H), 1.78 (quint, *J* = 6.9 Hz, 2H), 2.36–2.46 (m, 12H), 2.57 (t, *J* = 6.9 Hz, 2H), 3.15 (s, 4H), 3.76 (t, *J* = 6.9 Hz, 2H), 6.90 (ddd, *J* = 7.4 Hz, 7.2 Hz, 1.1 Hz, 2H), 7.06 (dd, *J* = 8.1 Hz, 1.1 Hz, 2H), 7.08 (dd, *J* = 7.4 Hz, 1.3 Hz, 2H), 7.11 (ddd, *J* = 8.1 Hz, 7.2 Hz, 1.3 Hz, 2H). EIMS: *m/z* 388 (M⁺).

General Procedure for the Preparation of *N*-(3-Cyanoalkyl)piperazines 5d–i. A solution of *N*-(3-chloropropyl)phenothiazine or *N*-(3-bromopropyl)diphenylamine (5.0 mmol), the respective piperazin-1-ylalkyl nitrile (20.0 mmol), diisopropylethylamine (5.0 mmol), and NaI (1.0 mmol) was refluxed in DMF (10 mL) for 18 h. In the case of piperazinylpropionitrile, side reactions (retro-Michael reaction and *N*-formylation) were observed that could be avoided by shortening the reaction time to 2 h. After adding brine (100 mL), the mixture was extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with brine and water. After drying (Na₂SO₄) and evaporation, the crude product was purified by flash chromatography.

3-[4-(3-Phenothiazin-10-ylpropyl)piperazin-1-yl]propionitrile (5e).²⁰ The title compound was prepared according to the general procedure. The crude product was purified by flash chromatography (hexane/ethyl acetate 7/3 + 0.5% TEA) to give **5e** (75%) as a yellow oil. ¹H NMR (360 MHz): δ 1.94 (quint, *J* = 6.9 Hz, 2H), 2.33–2.58 (m, 12H), 2.67 (t, *J* = 6.8 Hz, 2H), 3.92 (t, *J* = 6.9 Hz, 2H), 6.85–6.94 (m, 4H), 7.10–7.16 (m, 4H).

[4-(3-Diphenylaminopropyl)piperazin-1-yl]acetoneitrile (5g). The title compound was prepared according to the general procedure. The crude product was purified by flash chromatography (hexane/ethyl acetate 7/3 + 0.5% triethylamine) to give **5g** (70%) as a yellow solid. ¹H NMR (600 MHz): δ 1.81 (quin, *J* = 7.2 Hz, 2H), 2.33–2.67 (m, 10H), 3.49 (s, 2H), 3.77 (t, *J* = 7.2 Hz, 2H), 6.93 (dd, *J* = 7.6 Hz, 7.6 Hz, 2H), 7.00 (d, *J* = 7.8 Hz, 4H), 7.25 (dd, *J* = 7.8 Hz, 7.6 Hz, 4H). EIMS: *m/z* 334 (M⁺).

General Procedure for Preparation of Aminoalkylpiperazines 6a–i. The respective nitrile (1.0 mmol) was dissolved in dry diethyl ether (10 mL) and cooled to 0 °C. LiAlH₄ (1 M in diethyl ether; 2.5 mmol) was added dropwise and stirring was continued for 30 min at room temperature. In the case of the piperazinylpropionitriles, 0.5 mmol of LiAlH₄ was used to avoid the formation of a retro-Michael side product. The reaction mixture was cooled and quenched with Na₂CO₃ solution. After addition of ethyl acetate (30 mL), Na₂SO₄, and Celite the mixture was filtered. Evaporation of the solvent gave the respective primary amines. No further purification was necessary.

2-{4-[3-(10,11-Dihydrodibenzo[*b,f*]azepin-5-yl)propyl]piperazin-1-yl}ethylamine (6a). The title compound was prepared according to the general procedure, yielding 21% of **6a** as a yellow oil. ¹H NMR (360 MHz): δ: 1.66–1.79 (m, 2H), 2.07–2.23 (bs, 2H), 2.24–2.59 (m, 14H), 3.15 (s, 4H), 3.76 (t, *J* = 7.0 Hz, 2H), 6.86–6.94 (m, 2H), 7.05–7.15 (m, 6H). EIMS: *m/z* 364 (M⁺).

4-{4-[3-(10,11-Dihydrodibenzo[*b,f*]azepin-5-yl)propyl]piperazin-1-yl}butylamine (6c). The title compound was prepared according to the general procedure, yielding 89% of **6c** as a yellow oil. ¹H NMR (600 MHz): δ: 1.41–1.55 (m, 4H), 1.74 (quint, *J* =

7.0 Hz, 2H), 2.23–2.62 (m, 16H), 3.14 (s, 4H), 3.75 (t, *J* = 7.0 Hz, 2H), 6.87–6.93 (m, 2H), 7.04–7.14 (m, 6H). EIMS: *m/z* 392 (M⁺).

3-[4-(3-Phenothiazin-10-ylpropyl)piperazin-1-yl]propylamine (6e).²⁰ The title compound was prepared according to the general procedure to give 73% of **6e** as a solid. ¹H NMR (360 MHz): δ 1.63 (quint, *J* = 6.9 Hz, 2H), 1.80 (bs, 2H), 1.95 (quint, *J* = 7.0 Hz, 2H), 2.33–2.53 (m, 10H), 2.74 (t, *J* = 6.9 Hz, 2H), 3.91 (t, *J* = 7.0 Hz, 2H), 6.85–6.93 (m, 4H), 7.09–7.16 (m, 4H).

2-[4-(3-Diphenylaminopropyl)piperazin-1-yl]ethylamine (6g). The title compound was prepared according to the general procedure, yielding 65% of **6g** as a yellow oil. ¹H NMR (600 MHz): δ 1.75 (bs, 2H), 1.82 (quint, *J* = 7.2 Hz, 2H), 2.29–2.59 (m, 12H), 2.78 (t, *J* = 6.2 Hz, 2H), 3.76 (t, *J* = 7.2 Hz, 2H), 6.92 (dd, *J* = 7.7 Hz, 7.7 Hz, 2H), 7.00 (d, *J* = 7.9 Hz, 4H), 7.24 (dd, *J* = 7.9 Hz, 7.7 Hz, 4H). EIMS: *m/z* 338 (M⁺).

General Procedure for Preparation of 9-Aminoacridines 1 and 2a–q. The respective primary amine (1.0 equiv) and commercially available 6,9-dichloro-2-methoxyacridine or 9-chloroacridine (1.0 equiv) were stirred with phenol (10.0 equiv) at 100 °C for 18 h. The mixture was basified with 2 N NaOH (50 mL) and extracted with ethyl acetate (3 × 50 mL). After drying (Na₂SO₄) and evaporation, the crude product was purified by gravitation column chromatography.

***N*-Acridin-9-yl-*N*-(2-{4-[3-(10,11-dihydrodibenzo[*b,f*]azepin-5-yl)propyl]piperazin-1-yl}ethyl)amine (2a).** The title compound was prepared according to the general procedure starting from **6a** (19.6 mg, 0.054 mmol) and 9-chloroacridine (11.5 mg, 0.054 mmol). The crude product was purified by gravitation column chromatography (hexane/ethyl acetate/methanol 8/1.5/0.5 + 0.5% triethylamine) to give **2a** (16.9 mg, 58%) as yellow crystals. Mp: 120–122 °C. ¹H NMR (600 MHz): δ 1.78 (quint, *J* = 6.9 Hz, 2H), 2.41–2.65 (m, 10H), 2.69 (t, *J* = 5.8 Hz, 2H), 3.17 (s, 4H), 3.76 (t, *J* = 6.4 Hz, 2H), 3.79 (t, *J* = 6.9 Hz, 2H), 3.86–3.95 (m, 2H), 6.88–6.94 (m, 2H), 7.06–7.16 (m, 6H), 7.36 (ddd, *J* = 8.5 Hz, 6.8 Hz, 1.1 Hz, 2H), 7.69 (ddd, *J* = 8.5 Hz, 6.8 Hz, 1.1 Hz, 2H), 8.11–8.20 (m, 4H). EIMS: *m/z* 541 (M⁺). Anal. (C₃₆H₃₉N₅) HRMS purity HPLC.

***N*-(2-Chloro-6-methoxyacridin-9-yl)-*N*-(3-{4-[3-(10,11-dihydrodibenzo[*b,f*]azepin-5-yl)propyl]piperazin-1-yl}butyl)amine (1).** The title compound was prepared according to the general procedure from **6c** (73.6 mg, 0.187 mmol) and 6,9-dichloro-2-methoxyacridine (104.3 mg, 0.375 mmol). The crude product was purified by flash chromatography (ethyl acetate/methanol 95/5 + 0.5% TEA) to give **1** (71.2 mg, 60%) as yellow crystals. Mp: 56–58 °C. ¹H NMR (360 MHz): δ 1.55 (quint, *J* = 7.2 Hz, 2H), 1.75 (quint, *J* = 7.2 Hz, 2H), 1.79 (quint, *J* = 7.2 Hz, 2H), 2.29–2.50 (m, 12 H), 3.16 (s, 4H), 3.73 (t, *J* = 7.1 Hz, 2H), 3.77 (t, *J* = 7.1 Hz, 2H), 3.97 (s, 3H), 6.92 (ddd, *J* = 7.3 Hz, 7.3 Hz, 1.2 Hz, 2H), 7.04–7.13 (m, 6H), 7.23 (d, *J* = 2.7 Hz, 2H), 7.30 (dd, *J* = 9.1 Hz, 2.0 Hz, 2H), 7.99–8.05 (m, 3H), 8.07 (d, *J* = 2.0 Hz, 2H). APCI-MS: *m/z* 634 (M⁺ + 1). Anal. (C₃₉H₄₄ClN₅O) C, H, N.

***N*-Acridin-9-yl-*N*-(3-[4-(3-phenothiazin-10-ylpropyl)piperazin-1-yl]propyl)amine (2g).** The title compound was prepared according to the general procedure from **6e** (68.2 mg, 0.178 mmol) and 9-chloroacridine (38.1 mg, 0.178 mmol). The crude product was purified by gravitation column chromatography (hexane/ethyl acetate/methanol 8/1.5/0.5) and crystallized in methanol to afford **2g** (49.9 mg, 50%) as yellow needles. Mp: 115 °C. ¹H NMR (600 MHz): δ 1.94 (quint, *J* = 5.9 Hz, 2H), 1.99 (quint, *J* = 7.0 Hz, 2H), 2.48–2.69 (m, 12H), 3.95 (t, *J* = 7.0 Hz, 2H), 4.05 (t, *J* = 5.9 Hz, 2H), 6.87–6.95 (m, 4H), 7.11–7.17 (m, 4H), 7.22 (ddd, *J* = 8.7 Hz, 6.7 Hz, 1.0 Hz, 2H), 7.59 (ddd, *J* = 8.7 Hz, 6.7 Hz, 1.0 Hz, 2H), 8.10 (dd, *J* = 8.7 Hz, 1.0 Hz, 2H), 8.19 (dd, *J* = 8.7 Hz, 1.0 Hz, 2H). EIMS: *m/z* 559 (M⁺). Anal. (C₃₅H₃₇N₅S) C, H, N.

***N*-Acridin-9-yl-*N*-(2-[4-(3-diphenylaminopropyl)piperazin-1-yl]ethyl)amine (2i).** The title compound was prepared according to the general procedure from **6g** (46.6 mg, 0.138 mmol) and 9-chloroacridine (29.4 mg, 0.138 mmol). The crude product was purified by gravitation column chromatography (hexane/ethyl acetate/methanol 7/2/1 + 0.5% triethylamine) to give **2i** (21.2 mg,

30%) as yellow crystal. Mp: 110 °C. ¹H NMR (360 MHz): δ 1.86 (quint, *J* = 7.2 Hz, 2H), 2.39–2.75 (m, 12H), 3.79 (t, *J* = 7.2 Hz, 2H), 3.88 (t, *J* = 5.6 Hz, 2H), 6.55 (bs, NH), 6.94 (dddd, *J* = 7.4 Hz, 7.3 Hz, 1.0 Hz, 0.9 Hz, 2H), 7.02 (dd, *J* = 8.6 Hz, 1.1 Hz, 4H), 7.21–7.29 (m, 4H), 7.36 (ddd, *J* = 8.9 Hz, 6.6 Hz, 1.0 Hz, 2H), 7.67 (ddd, *J* = 8.7 Hz, 6.6 Hz, 1.1 Hz, 2H), 8.09 (dd, *J* = 8.9 Hz, 1.1 Hz, 2H), 8.18 (dd, *J* = 8.7 Hz, 1.0 Hz, 2H). EIMS: *m/z* 515 (M⁺). Anal. (C₃₄H₃₇N₅) C, H, N.

PrP^{Sc} Inhibition Assay in ScN2a Cells. ScN2a cells are an established cell model of prion disease. Mouse neuroblastoma cells (ATCC CCL131) that express PrP^C on their cell surface can be infected with mouse-adapted scrapie strains. In this case, the RML strain was used to infect N2a cells that subsequently were subcloned for selecting highest PrP^{Sc}-bearing cells.²¹ The advantage of this model is that candidate substances can be directly assayed by adding them into the cell culture medium. A confluent 10-cm dish was split and a drop of cells (ca 100 μL) was pipetted into a 60-mm dish of 4 mL of MEM containing 10% (vol/vol) FCS, penicillin–streptomycin, and L-glutamine. Medium was exchanged every second day, together with the drug. Cells were lysed (lysis buffer: 10 mM Tris, pH 8.0, 150 mM NaCl, 0.5% Triton X-100, 0.5% deoxycholate) on the seventh day, having achieved about 80% confluency. All experiments were repeated three times. Cell lysates were digested with proteinase K at 20 μg/mL for 30 min at 37 °C. The reaction was stopped with 2 mM PMSF, and the lysates were centrifuged for 45 min at 100 000g in an ultracentrifuge (Beckman-Coulter). Pellets were resuspended in sample buffer, and SDS–PAGE/immunoblotting was performed according to standard techniques. Immunoblots were incubated with an α-PrP-antibody and developed with enhanced chemiluminescence (Amersham Pharmacia). Effective concentrations where half the antiprion activity was achieved (EC₅₀) were determined by densitometry of five concentration points measured from immunoreactive bands on Western blot using NIH image software.

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Supporting Information Available: Experimental procedures and analytical data for compounds **2b–f,i–k,m–q**, **4a–c**, **5b,d,f,h,i**, and **6b,d,f,h,i**, elemental analysis results, high-resolution mass spectrometry data, and HPLC purities. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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