

Acridine and phenothiazine derivatives as pharmacotherapeutics for prion disease

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Prion diseases in humans and animals are invariably fatal. Prions are composed of a disease-causing isoform (PrP^{Sc}) of the normal host prion protein (PrP^C) and replicate by stimulating the conversion of PrP^C into nascent PrP^{Sc}. We report here that tricyclic derivatives of acridine and phenothiazine exhibit half-maximal inhibition of PrP^{Sc} formation at effective concentrations (EC₅₀) between 0.3 μ M and 3 μ M in cultured cells chronically infected with prions. The EC₅₀ for chlorpromazine was 3 μ M, whereas quinacrine was 10 times more potent. A variety of 9-substituted, acridine-based analogues of quinacrine were synthesized, which demonstrated variable antiprion potencies similar to those of chlorpromazine and emphasized the importance of the side chain in mediating the inhibition of PrP^{Sc} formation. Thus, our studies show that tricyclic compounds with an aliphatic side chain at the middle ring moiety constitute a new class of antiprion reagents. Because quinacrine and chlorpromazine have been used in humans for many years as antimalarial and antipsychotic drugs, respectively, and are known to pass the blood–brain barrier, we suggest that they are immediate candidates for the treatment of Creutzfeldt–Jakob disease and other prion diseases.

Prion diseases are uniquely manifest as spontaneous, inherited, and infectious maladies. These diseases include Gerstmann–Sträussler–Scheinker (GSS) disease, fatal insomnia, and Creutzfeldt–Jakob disease (CJD). Most cases of CJD are sporadic with 10–15% being inherited (1). Although the infectious human prion diseases are most notorious, they account for less than 1% of all prion disorders (2). Concern about these infectious disorders has been heightened by the identification of more than 100 young adults and teenagers who have developed new variant CJD (nvCJD) in Europe after exposure to bovine prions from cattle with bovine spongiform encephalopathy (BSE; refs. 3 and 4). Other infectious prion diseases include kuru, which is found among New Guinea natives and is caused by ritualistic cannibalism, and iatrogenic CJD, which is caused by prion-contaminated cadaveric growth hormone and dura mater grafts (2, 5, 6).

A wealth of experimental data indicates that prions are composed solely of a misfolded prion protein (PrP) isoform (PrP^{Sc}) of a glycolipid-anchored host protein (PrP^C; refs. 7 and 8). Unlike all other infectious agents, prions are devoid of nucleic acid (9, 10). For years, the existence of prion strains caused many investigators to argue that a small nucleic acid encodes prion diversity (11). Eventually, convincing data accumulated arguing that prion diversity is enciphered in the conformation of PrP^{Sc} (3, 12, 13).

Patients with CJD and other prion diseases develop progressive neurologic dysfunction. Prion diseases are invariably fatal, and death frequently occurs in less than 1 year after the first symptoms appear (2). No effective therapy exists for prion diseases in humans or animals (14).

Many compounds have been identified that inhibit prion propagation when administered at the time of inoculation in rodents (15–18). Treatment with these same compounds administered immediately before or during the onset of neurologic dysfunction has proven ineffective. Other compounds that in-

hibit PrP^{Sc} formation, including Congo red, have been identified by using scrapie-infected cultured cells (19–21). Some of these compounds have been examined in rodents but none have been effective when given around the time that neurologic signs appear (22).

In a search for compounds that might prove effective in treating prion diseases, we have used scrapie-infected neuroblastoma (ScN2a) cells to screen for inhibition of nascent PrP^{Sc} formation as well as the clearance of preexisting PrP^{Sc}. Based on the demonstration that PrP^{Sc} formation occurs in cholesterol-rich microdomains, inhibitors of cholesterol biosynthesis were examined for their ability to inhibit the conversion of PrP^C into PrP^{Sc} (23, 24). Although statin drugs were found to inhibit PrP^{Sc} formation in cultured cells, the level of cholesterol depletion required does not permit such an approach to be used in animals. In contrast, dominant negative inhibition of PrP^{Sc} formation has been found in sheep and humans (25, 26). This approach was the basis of rational drug design strategy that identified several lead compounds, which mimic a discontinuous epitope on the surface of PrP^C (27). In another study, we identified antibody fragments (Fabs) that bind to PrP^C on the surface of cells and inhibit PrP^{Sc} formation (D. Peretz, R. A. Williamson, K. Kaneko, J. Vergara, E. Leclerc, I. R. Mehlhorn, G. Legname, M. R. Wormald, P. M. Rudd, R. A. Dwek, *et al.*, unpublished data). Treatment of cells with these Fab fragments also resulted in the clearance of preexisting PrP^{Sc}. Branched polyamines also were found to promote the clearance of PrP^{Sc} from scrapie-infected cultured cells (28).

Because the blood–brain barrier (BBB) restricts the access of many molecules to the central nervous system, we decided to screen a variety of drugs that are known to penetrate the BBB for their ability to inhibit PrP^{Sc} formation. We report here on tricyclic acridine and phenothiazine derivatives. Phenothiazines are compounds with a tricyclic scaffold and a side chain extending from the middle ring moiety that have been used to treat psychoses for nearly 50 years (29). Effective concentrations for half-maximal inhibition (EC₅₀) of PrP^{Sc} formation were determined in ScN2a cells. Chlorpromazine inhibited PrP^{Sc} formation at 3 μ M, whereas quinacrine, a structural antecedent of the phenothiazines, was 10 times more potent. A variety of quinacrine derivatives were synthesized and demonstrated potencies similar to chlorpromazine, emphasizing the importance of the aliphatic side chain in mediating the inhibition of PrP^{Sc} formation. Our findings define a new class of antiprion compounds consisting of a tricyclic scaffold with an aliphatic side chain extending from the middle ring moiety.

Abbreviations: PrP, prion protein; PrP^C, normal cellular isoform; PrP^{Sc}, disease-causing isoform; CJD, Creutzfeldt–Jakob disease; BBB, blood–brain barrier; ScN2a, scrapie-infected neuroblastoma.

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While preparing our manuscript, we learned of three earlier reports describing the potent inhibition of PrP^{Sc} formation by acridine and phenothiazine derivatives. Chlorpromazine administered repeatedly to mice s.c. before and after intracerebral inoculation of scrapie prions resulted in a prolongation of the incubation period by 1 month, but no change in the incubation period was seen if the prions were inoculated i.p. (30). In another study, 50 μ M of chlorpromazine was added to hamster brain fractions enriched for membranes and the suspension was exposed to UV irradiation (31). The prion titers of the nonirradiated controls (exposed only to chlorpromazine) were reduced by \approx 10-fold, whereas the titers of the irradiated samples were reduced $>$ 100-fold. In a third study, quinacrine and other lysotrophic reagents added to ScN2a cells resulted in the clearance of PrP^{Sc} (32).

Because quinacrine has been used in humans as an antimalarial drug for over 60 years and is known to cross the BBB, it is an immediate candidate for the treatment of patients dying of CJD and other prion diseases. Because humans can tolerate 100–1,000 mg of quinacrine administered orally on a daily schedule, such doses are likely to find an immediate place in attempts to treat patients with clinical signs of prion disease. As an alternative or even an adjunct to quinacrine therapy, phenothiazine derivatives effective in PrP^{Sc} inhibition may be used to treat humans, despite their weaker antiprion potency in our ScN2a cell assay, because they may cross the BBB more effectively. It will be important to treat patients dying of prion diseases within the context of well designed clinical trials to establish as rapidly as possible whether quinacrine or phenothiazine derivatives can provide effective treatment.

Materials and Methods

Compounds were purchased from Sigma and Aldrich. Drugs were dissolved in PBS whenever possible (chlorpromazine and quinacrine are soluble in PBS), otherwise in 100% DMSO and then diluted into a stock solution of 10% (vol/vol) DMSO/PBS. Final DMSO concentration was, in no case, greater than 0.1%, and most often less than 0.01%. Stock solutions were filtered although a 0.2- μ m syringe filter, kept at 4°C during the time of the experiment, and made freshly for each separate experiment.

Synthesis of 9-Substituted Acridines. The appropriate amine (0.35 mM) was reacted with 6,9-dichloro-2-methoxyacridine (0.35 mM) in phenol (500 mg) by heating at 120°C for 2 h. The reaction mixture was cooled to room temperature, diluted with 5 M aqueous sodium hydroxide (50 ml), and extracted with ether (3 \times 75 ml). The combined organics were extracted with 10% (vol/vol) aqueous HCl (3 \times 75 ml), and the pooled aqueous phases made alkaline to pH \approx 11 with 10% (vol/vol) aqueous potassium hydroxide. The aqueous phase then was extracted with ether (3 \times 75 ml), and the pooled organics were dried (MgSO₄), filtered, and evaporated to give the desired 9-substituted acridine in 53–78% yield. The structure of the acridine analogs was confirmed by ¹H, ¹³C NMR (CD₃OD) and high-resolution electrospray MS.

PrP^{Sc} Inhibition Assay in ScN2a Cells. Neuroblastoma cells were infected with the RML strain of mouse-adapted scrapie prions and subcloned (33). A confluent 10-cm dish was split and a drop of cells was pipetted into a 60-mm dish of 4 ml of MEM containing 10% (vol/vol) FCS, penicillin-streptomycin, and nonessential amino acids. 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% Nonidet P-40/0.5% deoxycholate) on the 7th day, having achieved 80% confluency.

Western Blotting and Densitometry. Cell lysates were digested with proteinase K at 20 μ g/ml for 30 min at 37°C. The reaction was

stopped with 2 mM PMSF, and the lysates were centrifuged for 45 min at 100,000 \times g. Pellets were resuspended in sample buffer and SDS/PAGE/immunoblotting was performed according to standard techniques. Immunoblots were incubated with the recombinant Fab fragment D13 (34), a secondary horseradish peroxidase-labeled Ab, and developed with the enhanced chemiluminescence (ECL) system (Amersham Pharmacia). Densitometry was performed with National Institutes of Health IMAGE software, computing at least three independent experiments.

Results

At concentrations in the range of 2–10 μ M, treatment with promazine, chlorpromazine, and acepromazine, but not with phenothiazine, 2-chlorphenothiazine, or phenazine, led to the disappearance of PrP^{Sc} in ScN2a cells after 6-day treatments (Fig. 1). These effects were already visible 2 days after the beginning of treatment (data not shown).

Because the structurally unrelated high potency antipsychotic drugs haloperidol and clozapine did not inhibit PrP^{Sc} formation, it is likely that inhibition of PrP^{Sc} formation by phenothiazine derivatives is not mediated through dopamine antagonism and thus, the antipsychotic and antiprion effects of these substances are dissociable. Moreover, addition of dopamine to cultures of ScN2a cells grown in the presence of chlorpromazine did not reverse the inhibition of PrP^{Sc} production (data not shown).

For chlorpromazine, a clear relationship could be established between dose and PrP^{Sc} inhibition after 7 days of treatment of ScN2a cells (Fig. 2a). The EC₅₀ concentration, at which 50% of PrP^{Sc} production is eliminated, was 2 μ M and the EC₉₀ was 5 μ M (Fig. 2a Bottom). At 10 μ M, chlorpromazine was cytotoxic; the total cell number was decreased by 50%, and tubulin in the cell lysate was reduced compared with controls (Fig. 2a).

We observed cytotoxicity with many phenothiazines, at doses between 8 and 50 μ M or higher. For the perazines and phenazines, however, cytotoxicity at concentrations $>$ 6 μ M made reliable measurements of dose-response relationships for these drugs questionable, although we did observe PrP^{Sc} inhibition. It is noteworthy that methylene blue did not inhibit PrP^{Sc} formation, even at cytotoxic concentrations (Fig. 2c).

Our data suggest that the tricyclic scaffold alone is not sufficient for PrP^{Sc} inhibition because phenazine, phenothiazine, and 2-chlorphenothiazine do not inhibit PrP^{Sc} formation (Fig. 1). A side-chain substituent on the central ring was necessary for inhibition of PrP^{Sc}: promazine, chlorpromazine, and acepromazine, all of which have a dimethylaminopropyl side chain extending from the ring nitrogen at the 9 position of the tricyclic scaffold, displayed EC₅₀ concentrations of 5 μ M. Promethazine, with a dimethylamino-2-methylpropyl side chain, was slightly less active with an EC₅₀ of \approx 8 μ M.

The nitrogen at the 9 position of the tricyclic scaffold increased the antiprion potency. Chlorprothixene, with a carbon substituted for the nitrogen and a dimethylaminopropylidene side chain, had an EC₅₀ of \approx 10 μ M, demonstrating that it is not as efficient in inhibiting PrP^{Sc} formation as its chlorpromazine counterpart. The tricyclic antidepressant imipramine, with a dibenzoazepine scaffold and the same side chain as promazine, inhibited PrP^{Sc} formation at an EC₅₀ concentration of \approx 10 μ M. Our findings argue that the tricyclic scaffold containing a nitrogen at the 9 position as well as the length and composition of the side chain mediate the antiprion effects of the phenothiazines.

To extend the structure–activity relationships described above, we examined the tricyclic compounds quinacrine and methylene blue. Quinacrine was 10-fold more potent than chlorpromazine, completely inhibiting PrP^{Sc} formation at 400 nM (Fig. 2b). Inhibition of PrP^{Sc} formation by quinacrine was evident at 200 nM, whereas cytotoxic effects were not observed below 5 μ M (Fig. 2b). An EC₅₀ value of 300 nM for quinacrine (Table 1) is similar to that observed by others investigators (32).

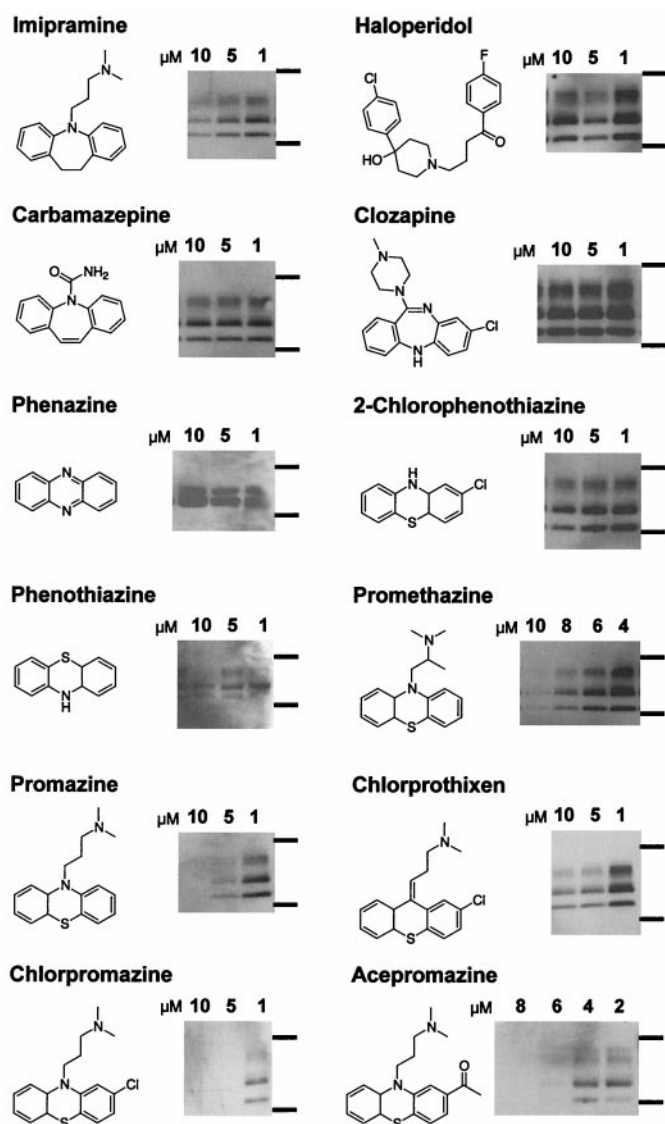


Fig. 1. PrP^{Sc}-inhibiting effects of phenothiazine derivatives and other psychopharmacological substances. Anti-PrP immunoblots of protease-digested ScN2a cell lysates treated with the respective substance for 6 days at the concentration indicated. Molecular mass markers at the right of the immunoblot correspond to 37 kDa (upper bands) and 20 kDa (lower bands). The characteristic PrP immunostain depicted corresponds to the amount of PrP^{Sc} and prion infectivity present in the neuroblastoma cells after treatment with the concentration indicated above. The schematic molecular structure of the compound used is depicted on the left side of each immunoblot.

Neither methylene blue nor other structurally related nontoxic dyes inhibited PrP^{Sc} formation (Fig. 2c).

At a cellular level, the range between efficacious and toxic quinacrine concentrations defines a promising therapeutic index. These results suggest that quinacrine may be an even more potent antiprion substance than the phenothiazines and may represent a new lead compound for the development of such drugs. The specificity of the PrP^{Sc}-inhibiting effect was emphasized when structural relatives of quinacrine (quinacrine mustard, amsacrine, and 9-aminoacridine) as well as some synthesized derivatives (BM no. 49, BM no. 48, and BM no. 52) failed to demonstrate PrP^{Sc} inhibition at the same concentrations, and others had weaker antiprion efficacy (BM no. 47, BM no. 51, pamaquine, chloroquine; Table 1).

In contrast to the phenothiazines, quinacrine does not have a sulfur atom in the middle ring moiety of the tricyclic scaffold. Instead, it contains a carbon at the 9 position, to which the aliphatic side chain is attached (Table 1). Quinacrine has been used widely as an antimalarial drug and thus numerous analogs have been synthesized. Pamaquine and chloroquine contain the same side chain found in quinacrine but are anchored to a bicyclic quinoline scaffold. These two drugs inhibit PrP^{Sc} formation with an EC₅₀ of $\approx 4 \mu\text{M}$ (Table 1).

Similar to the phenothiazine derivatives, the aliphatic side chain of quinacrine was found to modify antiprion activity. Neither 9-aminoacridine, lacking an aliphatic side chain, nor quinacrine mustard, with a bis-(2-chloroethyl) amine-substituted side chain, inhibited PrP^{Sc} formation as effectively as quinacrine. Of note, 9-aminoacridine resembles tacrine, an acridine proposed to be effective in the treatment of Alzheimer's disease (35); however, because it lacks the necessary side-chain, it was not an effective antiprion drug in the ScN2a cell assay (data not shown). To test further the effects of the side-chain substitution, we synthesized analogues of quinacrine to contain variations in the side chain linked to the acridine scaffold (Table 1). The 9-substituted acridines, BM no. 47, BM no. 48, BM no. 49, BM no. 51, and BM no. 52, were prepared by heating 6,9-dichloro-2-methoxyacridine with the corresponding amine in phenol (36). Compounds BM no. 48, BM no. 49, and BM no. 52, all containing major variations of the side chain by introducing a ring structure and/or oxygen atoms, were not effective inhibitors of PrP^{Sc} formation. Compounds BM no. 47 and BM no. 51 contained minor variations in the side chain and were 10 times less effective than quinacrine as inhibitors of PrP^{Sc} formation; these compounds exhibited EC₅₀ values of ≈ 3 to $4 \mu\text{M}$ (Table 1). Our results argue that the aliphatic side chain carries major determinants that modify the potency of PrP^{Sc} inhibition. By comparing the side-chain variations and the EC₅₀ values, we found that both the number of carbon atoms and the diethyl-amino bifurcation determine the antiprion activity of acridine derivatives.

Quinacrine treatment seems to cure the cells of prion infection. ScN2a cells were treated with 0.2–0.8 μM quinacrine for 6 days. After removal of the quinacrine, the cells were cultured for an additional 3 weeks. As judged by Western blotting, PrP^{Sc} did not reappear during the 3 weeks after discontinuation of quinacrine when the concentration of quinacrine was initially $>0.4 \mu\text{M}$ (Fig. 3).

Discussion

In the studies reported here, we have identified a class of compounds with a tricyclic ring structure and an aliphatic chain extending from the 9 position that inhibits PrP^{Sc} formation and possibly enhances clearance from ScN2a cells. Because some of these compounds have been used for the chronic treatment of diseases in humans, they are candidates for immediate treatment of CJD and other prion diseases, all of which are invariably fatal.

Some of the earliest studies of tricyclic drugs in humans are the investigations of Guttmann and Ehrlich (37), who pioneered the use of methylene blue as an antimalarial. Quinacrine was derived from methylene blue and was introduced in the 1930s as a drug with improved antimalarial properties (38, 39). The phenothiazines were discovered as a by-product of a search for antimalarial substances less toxic than quinacrine (40).

Quinacrine, commonly referred to as atabrine or mepacrine, was used widely during World War II as an antimalarial agent (41). Although newer antimalarial drugs have replaced quinacrine, it is approved for the treatment of giardiasis, producing cure rates of greater than 90%. Quinacrine is known to exert its antitrypanosomal effects by inhibiting trypanothione reductase from *Trypanosoma cruzi* (36), but no mammalian reductase has been shown to bind to quinacrine.

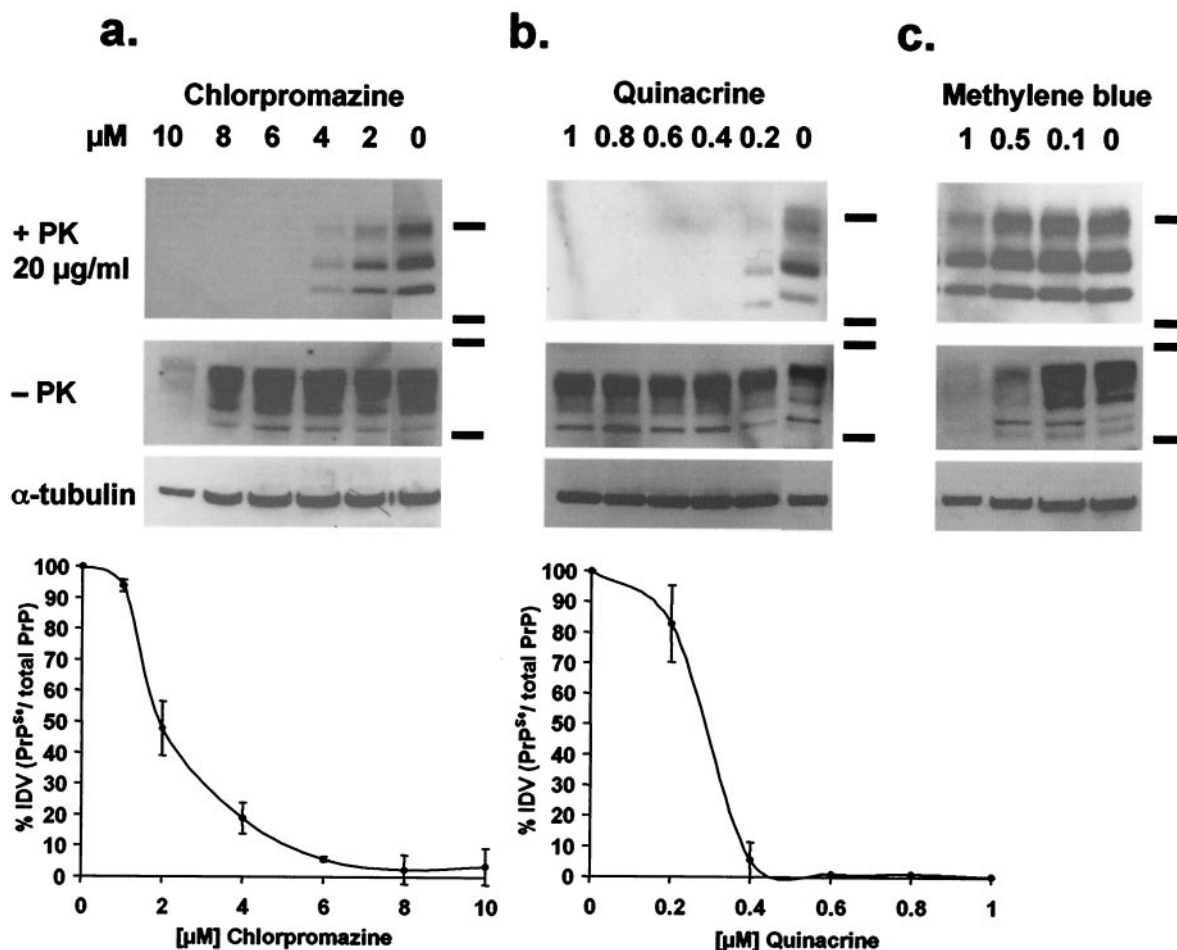


Fig. 2. Dose-response relationship of ScN2a cells treated with chlorpromazine, quinacrine, and methylene blue. Anti-PrP immunoblots of the same ScN2a cell lysates treated with proteinase K (1st row), without proteinase K (2nd row), anti-tubulin immunoblot of the same lysate (3rd row), and average immunoblot densitometry values (IDV) of three independent immunoblots (*Bottom*; bars represent standard error). ScN2a cells were treated for 6 days with chlorpromazine (a), quinacrine (b), or methylene blue (c). EC₅₀ levels are $\approx 2 \mu\text{M}$ for chlorpromazine and $\approx 250 \text{ nM}$ for quinacrine. Methylene blue does not inhibit PrP^{Sc} formation and is cytotoxic at concentrations $> 500 \text{ nM}$ (see text for discussion).

Dosage of quinacrine is recommended to be 100 mg orally for a few days for the treatment of giardiasis, whereas the drug has been administered orally for treatment of malaria in daily doses from 100 mg to 1,000 mg for up to many months (42–44). Quinacrine distributes slowly and achieves steady-state levels only 4 weeks after the start of therapy. Some of the lowest concentrations in the body are measured in the central nervous system (CNS). In low oral doses, few side effects are described; higher oral doses and parenteral administration increase the occurrence of serious side effects in the cardiovascular system, skin, gastrointestinal system, and CNS. A toxic psychosis from quinacrine has been reported in as many as 0.5% of recipients (42, 44).

Chlorpromazine has been used in the treatment of schizophrenia and other psychotic conditions since the 1950s (29). Daily oral dosage is usually between 100–600 mg. Side effects of chlorpromazine at doses used clinically include the tardive dyskinesias and occasionally agranulocytosis.

For the inhibition of PrP^{Sc} formation, the preferred effective composition of the aliphatic side chain for phenothiazine and acridine derivatives includes at least three or four carbon atoms, respectively, between two nitrogen atoms. The last nitrogen atom of the aliphatic side chain for phenothiazine and acridine derivatives has two methyl and alkyl side chains, respectively. The structure–activity relationships for inhibition of PrP^{Sc} for-

mation described here should be useful in developing even more potent tricyclic compounds for treating prion diseases. The structures of chlorpromazine and quinacrine are quite different from those of the small molecules that were designed for dominant negative inhibition of prion formation (27). These tricyclic compounds are also quite different from other known inhibitors of PrP^{Sc} formation including Congo red, heparins, polysulfated glucosaminoglycans, branched polyamines, and lovastatin. Congo red is a multicyclic anionic molecule that binds to PrP amyloid plaques (45) and, more generally, to β -sheet structures of amyloidogenic proteins. It has been suggested that Congo red binds to PrP^{Sc} and thereby sequesters the infectious template (19, 20). Other polyanionic molecules, such as the sulfated polyanions, also are thought to act nonspecifically on the PrP^{Sc} template (18, 46, 47). We have no evidence that phenothiazine derivatives or quinacrine bind to PrP^{Sc} because in applying an assay (28) in which purified mouse prion rods were preincubated with these substances for 2 hours, no decrease in the amount of protease-resistant PrP^{Sc} was observed (data not shown). Lovastatin is thought to act by depleting cells from cholesterol and thereby destroying the raft compartments where the conversion of PrP^C into PrP^{Sc} occurs (24). The inability of highly charged polyanions to cross the BBB and reach target cells, as well as the toxicity of lovastatin, make these substances unlikely therapeutics.

Table 1. Structure–activity relationship of quinacrine analogues on PrP^{Sc} inhibition

Compound	Structure	EC ₅₀ ,* μM	Compound	Structure	EC ₅₀ ,* μM
Quinacrine		~0.3	Quinacrine mustard		>5
BM no. 51		~3	BM no. 49		>5
BM no. 47		~4	BM no. 52		>5
Pamaquine		~4	Amsacrine		>5
Chloroquine		~4	BM no. 48		>5
9-Amino-acridine		>5	Methylene blue [†]		>1

*EC₅₀ measured in a dose-response curve for quinacrine as depicted in Fig. 2a and estimated semiquantitatively from visual inspection of Western blots for all other compounds.

[†]Because of toxicity, no EC₅₀ > 1 μM could be determined.

The mechanism by which quinacrine and chlorpromazine inhibit PrP^{Sc} formation and possibly enhance clearance is unknown. In contrast to the branched polyamines, both quinacrine and chlorpromazine require a few days of treatment before PrP^{Sc}

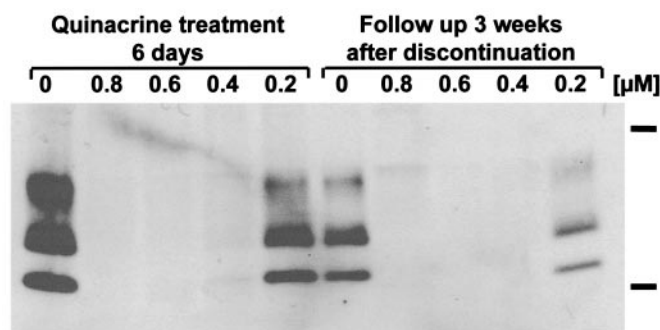


Fig. 3. Quinacrine treatment permanently cures ScN2a cells. Anti-PrP immunoblots of protease-digested ScN2a cell lysates treated with 0.2–0.8 μM quinacrine for 6 days (left) and then left without treatment and weekly splitting for 3 weeks. The lack of reappearance of protease-resistant PrP^{Sc} after discontinuation of treatment demonstrates that quinacrine permanently cures ScN2a cells. Molecular mass markers at the right of the immunoblot correspond to 37 kDa (upper band) and 20 kDa (lower band).

in ScN2a cells disappear. The branched polyamines require only a few hours of treatment before the levels of preexisting PrP^{Sc} decline (28).

The enormous experience with the chronic administration of quinacrine as an antimalarial presents a situation in which this drug can be used immediately to treat humans dying of prion disease. Determining the best regimen will undoubtedly be performed in humans but such studies need to be carefully controlled so that the results will be meaningful. For example, it will be critical to determine whether the candidate patients actually have prion disease before receiving quinacrine. Because no reliable blood or cerebrospinal fluid test for prion disease in humans has been developed, brain biopsies will be essential unless the electroencephalograms and MRIs show patterns characteristic of prion disease (2). If quinacrine can be shown to cure prion disease in symptomatic humans, then prophylactic treatment for carriers of PrP gene mutations and for people previously exposed to prions should be considered. At the same time, it will be reasonable to consider treating animals exposed to prions; such a possibility might provide a much-needed rational approach to managing the problems currently plaguing European livestock industries.

Because the treatment of people with prion disease requires the drug to cross the BBB, it is unclear whether quinacrine or chlorpromazine will be more effective. It is possible that chlor-

promazine, with a higher EC₅₀ in the ScN2a cells than quina-
crine, may prove more efficacious clinically if it crosses the BBB
much more readily. It is well known that the phenothiazines
exhibit large differences in their distribution between plasma and
brain (48), arguing that drugs, like the perazines or thioridazine,
which could not be reliably examined because of their cytotoxicity
to ScN2a cells, might be more potent antiprion drugs than
chlorpromazine or even quinacrine. Alternatively, very high
doses of phenothiazines assuring high concentrations in the brain
might be given to produce a state described at the beginning of
the chlorpromazine therapy era as “artificial hibernation” (49).
Still another possibility is the use of chlorpromazine in combi-
nation with quinacrine. Whether or not the two drugs act
synergistically remains to be established.

The structure–activity relationships reported here provide a
platform from which to synthesize many new compounds, some
of which will undoubtedly have superior antiprion potencies as
well as higher penetration of the BBB. Whether these new
compounds will be useful in dissecting the etiologies of such
psychotic disorders as the schizophrenias remains unclear. It has
been suggested that the schizophrenias might be disorders of
protein processing and thus are etiologically similar to many
other neurodegenerative diseases (14, 50).

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