a side effect [19], and PPS administration risks brain hemorrhage due to its anticoagulant activity.

## 5. Polycationic compounds

Polycationic compounds, including branched polymers of polyamidoamide (PAMAM), polypropyleneimine (PPI) and polyethyleneimine (PEI) [64], cationic phosphorus-containing dendrimers (P-dendrimers) [65], cationic lipopolyamine DOSPA [66], and cationic polysaccharides [67], have also been reported to be antiprion agents, reducing $PrP^{Sc}$ levels in infected N2a cells.

Supattapone *et al.* showed that PAMAM, PPI and PEI dendrimers decreased $PrP^{Sc}$ and prion infectivity in infected N2a cells in a dose-dependent manner [64,68]. PAMAM generation 4.0, PPI generation 4.0 and high-molecular weight PEI had a half maximal inhibitory concentration ($IC_{50}$) value of 80 ng/ml [68]. Structure–activity studies revealed that a high surface density of primary amino groups is required for activity [68]. The authors also showed that PPI labelled with fluorescein isothiocyanate accumulated in lysosomes in N2a cells [64] and that the antiprion activity was attenuated by chloroquine [68], suggesting that lysosomes might be the site of action for the agents. Moreover, incubation of purified $PrP^{Sc}$ with PPI dendrimer rendered the $PrP^{Sc}$ susceptible to protease K digestion [64]. However, PPI-mediated denaturation activity was strain-specific, being effective against $PrP^{Sc}$ from BSE-infected brains, but not from natural sheep scrapie-infected brains [64]. Taken together, these results indicate that the antiprion activity of the polycationic dendrimers might be attributable to their effects on $PrP^{Sc}$ degradation in lysosomes.

P-dendrimer generation 3 (pd-G3), pd-G4 and pd-G5 are synthesised cationic branching molecules containing phosphorus atoms at each branching and protonated terminal tertiary amines [65]. These molecules are less toxic and more stable, compared with PAMAM, PPI and PEI dendrimers [65]. Solassol *et al.* showed that the $IC_{50}$ values for pd-G3, pd-G4 and pd-G5 were 10, 1.5 and 5 µg/ml, respectively, in N2a subclone cells (N2a#58) infected with 22L prion [65]. They also showed that pd-G4 (50 or 100 µg/mouse) reduced $PrP^{Sc}$ in the spleens of mice infected with C506M3 scrapie prions when intraperitoneally administered every 2 days from day 2 post infection to day 30 [65]. The mechanism for the antiprion activity of P-dendrimers might be the same as or similar to that of PAMAM, PPI and PEI dendrimers. Pd-G4 also rendered pre-existing $PrP^{Sc}$ in the brain homogenates from different animals infected with 263K, BSE, Chandler and 22L prion strains susceptible to protease K treatment [65].

Winklhofer and Tatzelt showed that DOSPA, a cationic lipopolyamine, decreased $PrP^{Sc}$ in infected N2a cells by stimulating degradation of pre-existing $PrP^{Sc}$ and by blocking *de novo* formation of $PrP^{Sc}$ [66]. Structure–activity analysis revealed that lipids with a headgroup composed of the polyamine spermine and a quarternary ammonium ion between the headgroup and the lipophylic tail may effectively reduce $PrP^{Sc}$ in cells [66].

Cationic polysaccharides were synthesised by conjugation of various oligoamines (propane-1,3-diamine, spermidine, *N,N-bis*(2-aminoethyl)-1,3-propanediamine, *N,N-bis*(2-aminopropyl)-1,2-ethylenediamine or spermine) to polysaccharides (dextran, pullulan or arabinogalactan) and investigated for their antiprion activity in infected N2a cells [67]. These compounds are water-soluble, relatively non-toxic, biodegradable and biocompatible [67]. Dextran-spermine was the most potent, reducing $PrP^{Sc}$ to undetectable levels in cells at a concentration of as low as 31 ng/ml after 4 days of exposure [67].

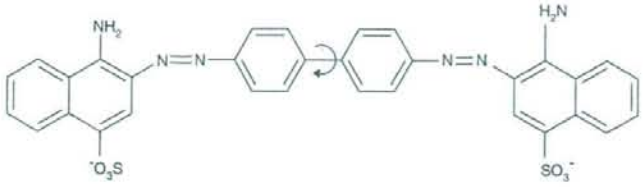
## 6. Amyloid-binding compounds

### 6.1 Congo red and other amyloid-binding dyes

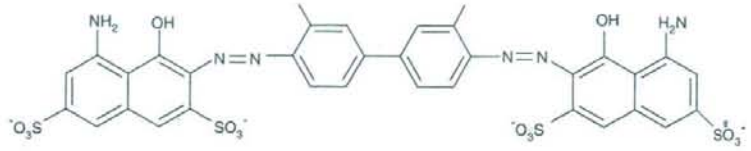
Caughey and Race examined the effects of the amyloid-binding dye, Congo red (**Figure 3A**), on $PrP^{Sc}$ levels in infected N2a cells, showing that Congo red could reduce $PrP^{Sc}$ levels [69]. Other amyloid-binding dyes, including Trypan Blue (**Figure 3B**), Evans Blue (**Figure 3C**), Sirius Red F3B (**Figure 3D**), Primuline (**Figure 3E**) and Thioflavin-S, were subsequently investigated using the cells [70,71]. Sirius Red F3B decreased $PrP^{Sc}$ to a similar extent as Congo red, whereas the others were less effective [71]. The antiprion effects of Congo red were then investigated using experimental animals [72,73]. Hamsters intracerebrally infected with 263K prion were intraperitoneally inoculated with Congo red [72]. The treated hamsters developed the disease significantly later than non-treated animals when the treatment was started by 2 weeks, but not from 3 weeks post infection [72]. These results indicate no therapeutic effects of peripherally infused Congo red against clinically advanced diseases.

Congo red is toxic and does not cross the BBB [74]. Moreover, its benzidine spacer is potentially carcinogenic [75]. To improve these undesirable properties and to enhance the antiprion activity of Congo red, several groups carried out structure–activity relationship studies using various Congo red analogues [73-77]. An analogue comprising a single naphthalene group markedly reduced the antiprion activity in infected N2a cells [75-77], suggesting that the whole molecule is required for full antiprion activity. Analogues containing either a spacer replaced with less toxic moieties or the sulphonate groups with carboxylic acids, which might improve the BBB permeability of the compounds, or the amino groups with hydroxyl groups or its acylation with trifluoroactamide, were synthesised and investigated for their antiprion activities in infected N2a cells [75-77]. None increased the activity [75-77] although some compounds inhibited $PrP^{Sc}$ accumulation in the cells at high concentrations (100 µM) [75,76]. However, at low concentrations (1 µM), they increased $PrP^{Sc}$ levels above those of untreated cells [75,76]. To successfully synthesise lower toxic and higher

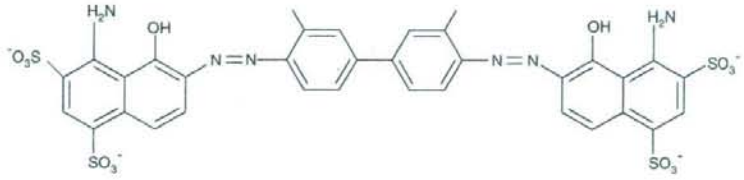
**A**: Congo red



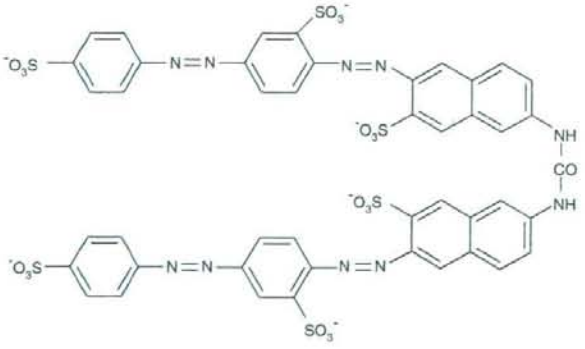
**B**: Trypan blue



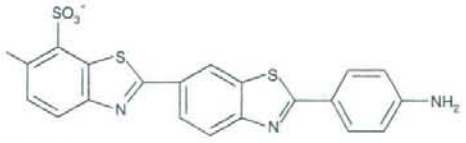
**C**: Evans blue



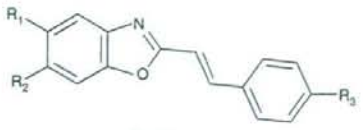
**D**: Sirius red F3B



**E**: Primuline



**F**: BF-168



$R_1$: H
$R_2$: $O(CH_2)_2F$
$R_3$: $NH(CH_3)$

**Figure 3. Chemical structures of amyloid-binding compounds: Congo red (A), Trypan blue (B), Evans blue (C), Sirius red (D), Primuline (E) and BF-168 (F).**

BBB-permeable analogues of Congo red with similar or even higher antiprion activity, further structure–activity studies might be needed.

Mechanisms for the antiprion activity of Congo red or other related compounds are not fully understood. It was shown that $PrP^{Sc}$ bound with Congo red was highly resistant to chemical and physical denaturation treatments [78], therefore Congo red might overstabilise the conformation of $PrP^{Sc}$, thereby impairing the activity that might be essential to convert $PrP^{c}$ to $PrP^{Sc}$. It is also suggested that, like polyanionic compounds, Congo red may inhibit $PrP^{Sc}$ formation by interfering with interaction between PrPs and endogenous GAGs because Congo red was reported to block their interaction [47].

### 6.2 Blood–brain barrier permeable amyloid-binding compounds

Ishikawa *et al.* synthesised the unique antiprion compound BF-168 (Figure 3F), which is a styrylbenzoxazole derivative [79]. BF-168 is structurally related to Congo red, but showed higher permeability through the BBB without apparent toxicity in mice when intravenously administrated with doses of ~ 10 mg/kg [79]. BF-168 accumulated at amyloid plaques of $PrP^{Sc}$ in the brains of infected mice [79], giving rise to the possibility of *in vivo* $PrP^{Sc}$ imaging. In addition, intravenous administration of BF-168 (4 mg/kg body weight of mouse) once a week from 4 weeks post infection slightly, but significantly, extended incubation times by 5.6 days in $PrP^{c}$-overexpressing Tga20 mice that had been infected with RML prions [79]. However, the effects were marginal and specific to prion strains, effective against RML, but not 22L or Fukuoka-1 prions [79].

The same group also developed another amyloidophilic compound, cpd-B, and showed that it decreased $PrP^{Sc}$ levels in infected N2a cells at $IC_{50}$ values of ~ 60 pM [80]. They also showed that cpd-B could effectively enter the brain and significantly extended incubation times in infected mice even when orally administrated [80]. Tga20 mice intracerebrally infected with RML prion were orally administered cpd-B (0.2% weight in the feed) through the infection and the incubation times were much extended in the treated mice by 85.8 days [80]. However, similarly to BF-168, the effect of cpd-B was strain-specific, most effective for RML prion, less effective for 22L or Fukuoka-1 prion and marginally effective for 263K prion [80]. It remains to be investigated whether this compound could also be therapeutically effective in animal models.

### 6.3 Tetracyclic compounds

Anthracycline 4′-iodo-4′-deoxyrubicin (IDX, Figure 4A) is a derivative of doxyrubicin, an anticancer drug [81], and binds to various types of amyloid, including $PrP^{Sc}$ amyloid [82]. IDX belongs to a family of tetracyclic compounds, such as tetracycline and doxycycline (Figure 4B). This family of compounds contains a hydrophobic core with hydrophilic substituents, the structure commonly observed in other amyloid-binding compounds, such as Congo red, suggesting that this IDX structure might be involved in the interaction with amyloid.

IDX, tetracycline and doxycycline showed a unique antiprion activity. Preincubation of homogenates from the 263K prion-affected brains with IDX (2.9 mM), tetracycline (1 mM) or doxycycline (1 mM) reduced infectivity of the homogenates [83]. Teteracycline or doxycycline could revert $PrP^{Sc}$ purified from vCJD patients, BSE cattle and 263K prion-affected hamsters to the protease-sensitive PrP [83]. Moreover, tetracycline disrupted amyloid formed by the PrP-derived peptide, PrP106-126 [84]. Therefore, it has been suggested that the antiprion activity of these tetracyclic compounds might be attributable to their amyloid destabilisation activity. Their therapeutic effects still remain to be investigated in animal models.

## 7. Suramin

Suramin (Figure 5) is an organic sulfated polyanion like PPS and DS500 and has some structural homology to Congo red. Suramin decreased $PrP^{Sc}$ in infected N2a cells [34,45,85]. Moreover, peripherally administrated suramin exhibited small, but significant, benefit in hamsters, which have been intraperitoneally, but not intracerebrally infected with 263K prion [62]. Treatment with suramin three times a week either just before or just after infection extended incubation times by 8.3 or 10.0 days, respectively [62]. In contrast, no effect was reported in intraperitoneally infected hamsters when suramin was administrated four times every week, starting one week post infection [62]. These results indicate that peripheral administration of suramin seems to have no therapeutic effect.

Suramin was reported to induce aggregation of $PrP^{c}$ in a postER/Golgi compartment and block further trafficking of $PrP^{c}$ to the cell membrane [85]. Aggregates of the misfolded PrP were instead transported to lysosomes, where the aggregates were degraded [85]. It was also shown that suramin induced misfolding of $PrP^{c}$ on the cell surface, stimulating internalisation and subsequent intracellular degradation of the misfolded $PrP^{c}$ [86]. Therefore, this suggests that the antiprion activity of suramin might be due to misfolding of $PrP^{c}$ in a postER/Golgi compartment and/or at the cell surface, followed by enhanced degradation of $PrP^{c}$.

## 8. Tetrapyrrolic antiprion compounds

Tetrapyrroles, such as porphyrins (Figure 6A) and phthalocyanins (Figure 6B), belong to a large family of compounds that include the biologically important haems and chlorophylls [87]. Caughey and colleagues first showed that porphyrins and phthalocyanins have an antiprion activity [88], reducing $PrP^{Sc}$ levels in infected N2a cells [89,90].

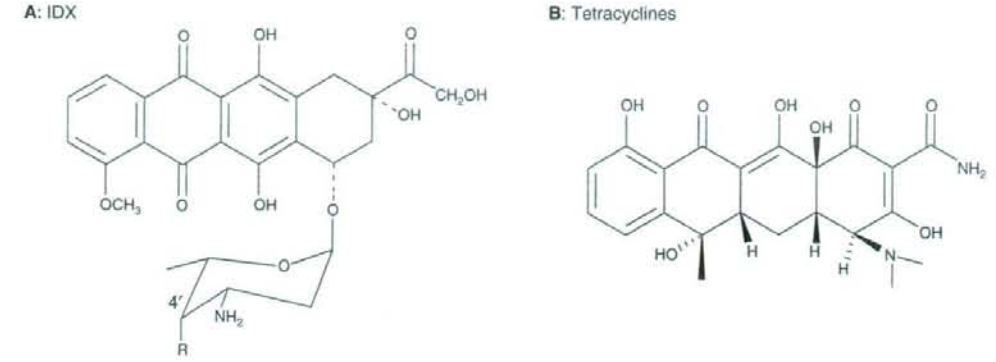



**Figure 4. Chemical structures of tetracyclic compounds of anthracycline 4'-iodo-4'-deoxy-doxorubicin (A) and tetracyclines (B).**

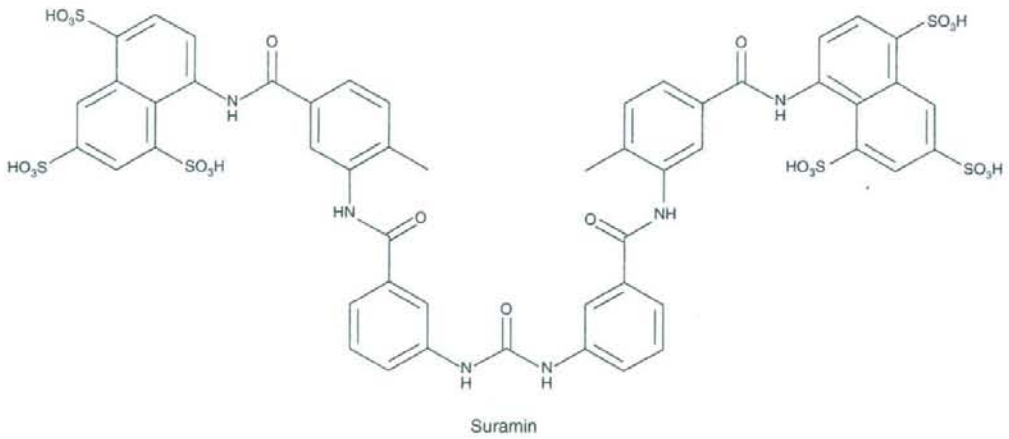



**Figure 5. Chemical structure of suramin.**

Among > 30 different porphyrins and phthalocyanins tested, the authors found that about two-thirds were able to inhibit $PrP^{Sc}$ formation in the cells [88]. Some porphyrins and phthalocyanins were examined for their therapeutic or prophylactic effects on prion diseases using Tg7 mice infected with 263K prion [91-93]. Phthalocyanine tetrasulfonate (PcTS, Figure 6C), when intraperitoneally inoculated on the day of infection followed by further inoculation 3 times a week for 4 weeks, prolonged incubation times by 135.0 days in the mice [91]. However, late treatment with PcTS, starting from 56 days after infection, showed no effects [91]. TMPP-$Fe^{3+}$ (meso-tetra[4-*N*-methylpyridyl]porphine iron[III]) and DPG2- $Fe^{3+}$ (deuteroporphyrin IX 2,4-*bis*-[ethylene glycol] iron[III]) showed similar antiprion activity to PcTS [91]. TSP-$Fe^{3+}$ (meso-tetra[4-sulfonylphenyl]porphyrin iron[III]), but not TSP-$Mn^{3+}$, slightly prolonged incubation times in Tg7 mice intracerebrally inoculated with 263K prions [94]. These results indicate that tetrapyrrolic compounds might be prophylactically, but not therapeutically, applicable to prion diseases. However, tetrapyrrolic compounds have advantages over other antiprion compounds with respect to a large number of presently available derivatives and easy introduction of a wide variety of modifications, suggesting possible development of therapeutically effective compounds [87].

Porphyrins and phthalocyanins strongly and selectively bind to proteins and induce conformational changes in them [87]. Therefore, it is conceivable that these compounds could bind to $PrP^{Sc}$ and change its conformation, reducing prion infectivity. Consistent with this, preincubation of prion inocula with PcTS diminished the infectivity of the inocula [91]. Caughey *et al.* also reported that antiprion activity of the compounds could be correlated with their tendency to oligomerise [94].

## 9. Antiprion compounds related to cholesterol metabolism

### 9.1 Polyene antibiotics

Amphotericin B (Figure 7), an antifungal polyene antibiotic, significantly prolonged incubation times in infected hamsters and mice when orally or intraperitoneally administered throughout the incubation period [95-97]. Demaimay *et al.* also showed that intraperitoneal administration of amphotericin B (1 or 2.5 mg/kg) even at middle (80 days after infection) or late stages (110 days after infection) of the disease slightly, but still significantly, extended incubation times by 22 days in C506M3 scrapie prion-infected mice [98]. However, later treatment (140 days after infection) had no effect on the infected mice [98]. Amphotericin B was also effective in infected cells, deceasing $PrP^{Sc}$ levels [99].

It has been reported that amphotericin B interacts with cholesterols [100]. Therefore, it is conceivable that amphotericin B might change the properties of lipid raft domains, which are considered to be the sites involved in the $PrP^{Sc}$ formation [101], and that the alteration of raft microenvironment might be associated with the antiprion activity of amphotericin B.

Amphotericin B has many undesirable side effects, in particular renal dysfunction, limiting its clinical usage [102]. MS-8209 (Figure 7) is a much more soluble and less toxic derivative of amphotericin B, encompassing antifungal activities comparable to amphotericin B [95]. Treatment with MS-8209 prolonged the incubation times of hamsters or Tg7 mice infected with 263K prions more than with amphotericin B [95,103]. Mepartricin and filipin are other ampotericin B-related compounds with antiprion activities [104,105]. Mepartricin (1 mg/kg) could lengthen the incubation times by 21.6 days in hamsters inoculated with 263K prions intraperitoneally, but not intracerebrally [104]. Filipin decreased $PrP^{Sc}$ levels in infected N2a cells, with an $IC_{50}$ value of 1.25 μg/ml [105], disturbed copper-stimulated endocytosis of $PrP^{c}$ and induced release of $PrP^{c}$ from the cell membrane [105]. Therefore, such alteration of $PrP^{c}$ trafficking or location might be associated with the antiprion activity of filipin. No animal data using filipin has become available.

### 9.2 Statins

Statins are inhibitors of the cholesterol synthetic pathway and decrease cellular levels of cholesterol [106,107]. Cholesterol is an important component of rafts and is considered to be important for $PrP^{Sc}$ production. It has been shown that lovastatin or squalestatin decreased $PrP^{Sc}$ levels in infected N2a cells [30,33]. Moreover, the lipophilic cholesterol-depleting inhibitor, simvastatin, which can passes the BBB effectively, exhibited very weak therapeutic effects in mice infected 139A or ME7 prion [108,109]. 139A prion-infected mice orally treated with simvastatin 100 days after infection, a time point when infection of the CNS is established, developed the disease with small, but significantly longer, incubation times than non-treated mice (194 versus 178 days) [108]. However, accumulation of $PrP^{Sc}$ and pathological changes in the brains were indistinguishable between treated and non-treated mice [108]. Thus, it remains to be elucidated whether or not the prolongation of incubation times is due to the antiprion activity or other activities of simvastatin.

## 10. Antiprion tricyclic and related compounds

### 10.1 Lysosomotropic compounds and lysosomal protease inhibitors

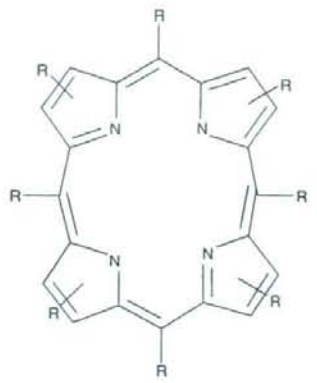
Lysosomotropic compounds including quinacrine (Figure 8A) [110,111], tilorone (Figure 8B), chloroquine (Figure 8C) and suramin (Figure 5) were shown to have antiprion activity in infected N2a cells, reducing $PrP^{Sc}$ levels in a dose-dependent manner [34]. Inhibitors of lysosomal cysteine proteases, such as E-64d, E-64 and leupeptin, also decreased $PrP^{Sc}$ in cells [34]. As quinacrine has been clinically used as an antimalarial drug, there is a high expectation that quinacrine may also be clinically effective for prion diseases. However, neither prophylactic nor therapeutic effects of quinacrine and E-64d were observed in Tg7 mice infected with 263K prion when they were administered via either the intraperitoneal or intraventricular routes [63,112]. Quinacrine also showed no therapeutic effects in mice infected with mouse-adapted BSE 6PB1 prion [112].

### 10.2 Quinacrine in clinical trials

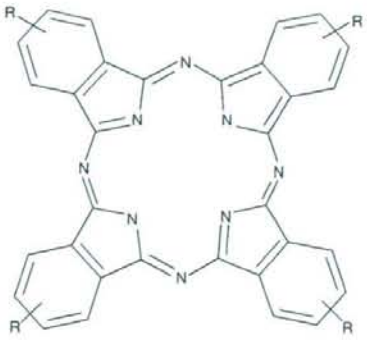
A large scale randomised controlled clinical trial of therapeutic quinacrine in many types of human prion diseases is being undertaken in the United Kingdom as the PRION-1 study [41]. In this study, eligible patients aged > 12 years are orally given 1 g quinacrine on the first day of treatment followed by 100 mg 3 times daily. The patients are followed up by several assessments including medical history, physical examination, blood examinations, neurological examinations and a series of neurological assessments.

Nakajima *et al.* enterally administered quinacrine (300 mg/day) for three months to three patients with sporadic CJD and one with possible iatrogenic CJD [17]. They reported that transient and modest improvement in mood or cognitive function, lasting 2 – 8 weeks during the quinacrine treatment, was invariably observed in all patients and that two patients with sporadic CJD eventually died 2 months later, 1 patient with sporadic CJD 11 months later and 1 patient with iatrogenic CJD 6 years after the onset of disease [17]. They also reported that quinacrine was well tolerated in these patients, except for liver dysfunction and yellowish pigmentation [17]. Moreover, Benito-Leon reported a clinical trial of combined quinacrine and chlorpromazine therapy in two patients with FFI [16]. However, no clinical benefits of the therapy were observed in these patients [16].

A: Porphyrins



B: Phthalocyanins



C: Phthalocyanine tetrasulfonate

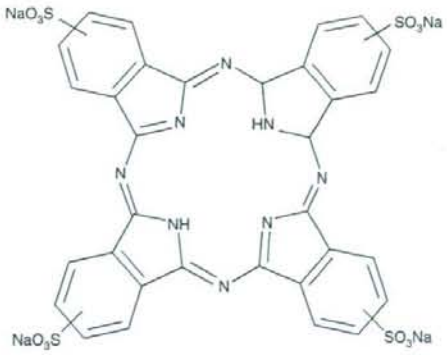


**Figure 6. Chemical structures of metal-free porphyrins (A), metal-free phthalocyanins (B) and phthalocyanine tetrasulfonate (C).**

### 10.3 Other quinacrine-related compounds

Phenothiazine and acridine derivatives, which are other tricyclic compounds structurally related to quinacrine, have been identified as antiprion agents using infected cells. The phenothiazine derivative, chlorpromazine (Figure 8D), decreased $PrP^{Sc}$ levels in infected N2a cells [113]. However, no antiprion effects of chlorpromazine were detected in mice [114]. *Bis*-acridines (Figure 8E) [115,116], which comprise two acridine heterocycles connected by a linker, showed very strong antiprion activity in the cells [117]. Some of them markedly decreased $PrP^{Sc}$ levels with an $IC_{50}$ value of 25 – 40 nM [117]. Length and structure of the linker were important for their antiprion activity [117]. However, there have been no data using animal models with these compounds.

Quinoline derivatives are other non-tricyclic quinacrine-related compounds with antiprion activity [118], which decreased $PrP^{Sc}$ levels in infected N2a cells. 2,2′-Biquinolin (Figure 8F) was the most potent compound with an $IC_{50}$ value of 3 nM and showed small, but significant, therapeutic effects in 263K prion-infected Tg7 mice [119]. Intraperitoneal or intraventricular administration of 2,2′-biquinolin extended the incubation times by 3.8 or 5.3 days, respectively [119]. Mefloquine (Figure 8G), a quinoline antimalarial drug, effectively reduced $PrP^{Sc}$ levels in N2a cells infected with RML or 22L prion, but did not extend the incubation times in Tg7 mice infected with 263K prion [120].

## 11. Cell signalling inhibitors

### 11.1 Tyrosine kinase inhibitor

Pharmacological approaches were performed to identify antiprion compounds using already known inhibitors of tyrosine kinase enzymes. Ertmer *et al.* found that, among ~ 50 inhibitors, STI571, also known as imatinib mesilate, could reduce $PrP^{Sc}$ levels in infected N2a cells with an $IC_{50}$ value of ≤ 1 μM [121]. $PrP^{Sc}$ degradation in lysosomes was markedly stimulated by STI571 in the cells [121]. STI571 is an inhibitor of tyrosine kinases, such as c-Abl and c-Kit. The authors showed that c-Abl is a very likely target molecule for the antiprion activity of STI571 [121]. Moreover, STI571 is already clinically used as an anticancer drug for chronic myeloid leukaemia [122] and ~ 2.8% of orally administered STI571 can be detected in the cerebrospinal fluid [123]. These facts highly suggest that STI571 might be effective as a therapeutic agent for prion diseases. However, Yun *et al.* recently reported that early peripheral treatment (from 7 days after infection for 1 month) with imatinib mesilate only very slightly prolonged the incubation times by 17 days in mice infected with RML prion [124]. Moreover, no significant extension of the incubation times could be detected in infected mice when the agent was administered from 128 days after infection even via the intraventricular route [124]. Therefore, these results indicate that the agent may not be therapeutically effective for prion diseases.

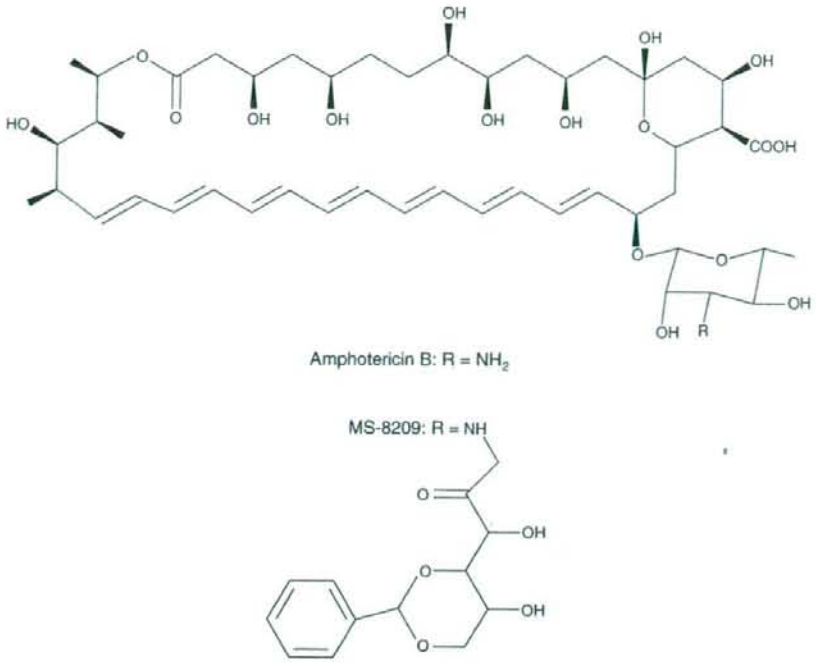



**Figure 7. Chemical structures of amphotericin B and its derivative, MS-8209 [103].**

### 11.2 Phospholipase $A_2$ inhibitors

Phospholipase $A_2$ inhibitors, including cytidine-5-diphosphocholine, bromoenol lactone, aristolochic acid and arachidonyl trifluoromethyl ketone, showed antiprion activity, decreasing $PrP^{Sc}$ levels in three different infected cell lines [125]. Phospholipase $A_2$ specifically cleaves the acyl ester bond of membrane phospholipids to produce a free fatty acid and lysophospholipid [126]. It was therefore suggested that phospholipase $A_2$ is involved in the maintenance of cell membrane integrity and that the antiprion activity of phospholipase $A_2$ inhibitors might be attributable to alteration of cell membrane integrity. Interestingly, the enzymatic activity of phospholipase $A_2$ is strongly inhibited by antimalarial drugs, such as chloroquine, quinacrine and quinine [126]. It is therefore possible that phospholipase $A_2$ inhibitors and antimalarial drugs might execute their antiprion activities via the same or similar mechanisms.

### 11.3 Mitogen-activated protein kinase kinase (MEK) 1/2 inhibitors

Nordstrom *et al.* found that MEK1/2 inhibitors showed antiprion activity, decreasing $PrP^{Sc}$ levels in RML prion-infected GT1-1 cells [127]. The inhibitors stimulated degradation of $PrP^{Sc}$ or prevented the *de novo* synthesis of $PrP^{Sc}$ [127]. The authors also found that the MEK 1/2 inhibitor, SL327, which was designed to pass the BBB, could effectively reduce $PrP^{Sc}$ levels in infected cells [127]. However, no animal data have become available with these inhibitors to date.

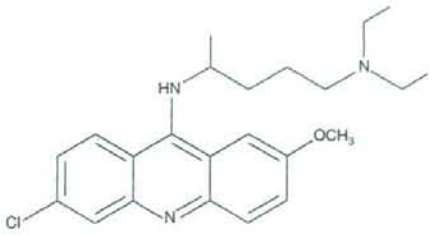
## 12. Gene silencing therapy for prion diseases

### 12.1 Prion protein-specific gene silencing

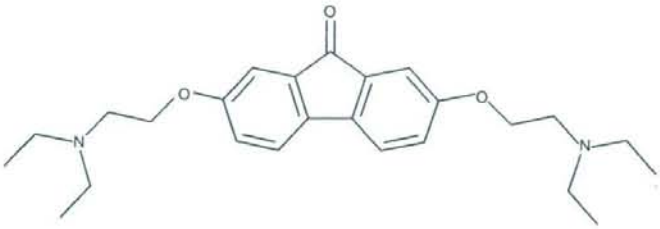
The author and others previously showed that mice devoid of $PrP^{c}$ (PrP-/-) are resistant to prion diseases, neither developing the disease nor accumulating $PrP^{Sc}$ in the brains [128-131]. Moreover, it was reported that depletion of $PrP^{c}$ from neurons in mice after establishment of the CNS infection of RML prion reversed pathological changes, such as spongiosis and neuronal cell death, and recovered cognitive deficits and neurophysiological dysfunction of synapses in spite of marked accumulation of $PrP^{Sc}$ surrounding the neurons [132,133]. These results indicate that depletion of $PrP^{c}$ from brains, and in particular from neurons, might be therapeutic.

Small interfering RNAs (siRNAs) are known to post-transcriptionally downregulate a gene of interest. Therefore, siRNA-mediated downregulation of $PrP^{c}$ might be therapeutic against prion diseases [134-137]. It has been reported that PrP-specific siRNAs reduced $PrP^{c}$ and concomitantly $PrP^{Sc}$ levels in infected N2a cells [138,139]. Moreover, Pfeifer *et al.*

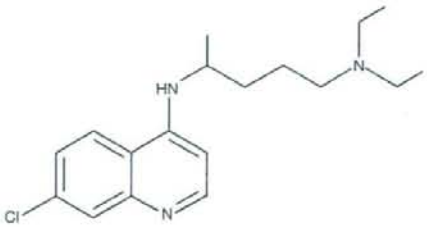
**A**: Quinacrine



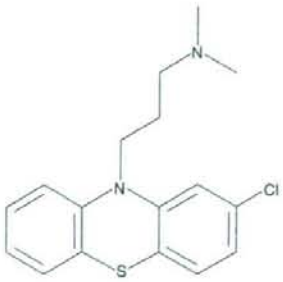
**B**: Tilorone



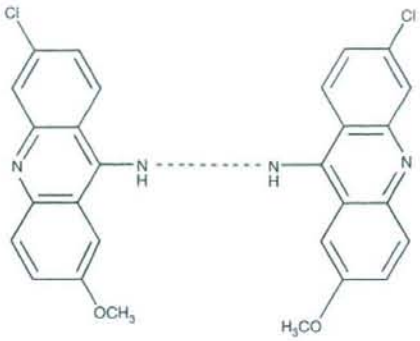
**C**: Chloroquine



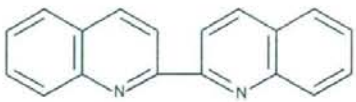
**D**: Chlorpromazine



**E**: *Bis*-acridines



**F**: 2,2′-Biquinoline



**G**: Mefloquine

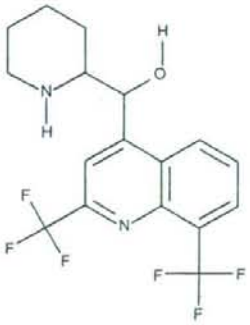


**Figure 8. Chemical structures of quinacrine (A) and its related compounds of tilorone (B), chloroquine (C), chlorpromazine (D), *bis*-acridines (E), 2,2′-biquinoline (F) and mefloquine (G).**

generated lentiviral vector expressing PrP-specific short hairpin RNAs (shRNAs) and showed that the intracranially-injected vector effectively downregulated $PrP^c$ in the brains of mice [140]. However, the effect was very restricted to the injection site of the vector and its surrounding area [140], indicating a limitation of the vector-mediated downregulation of $PrP^c$ as a therapy for prion diseases.

PrP-/- mice spontaneously developed neurological abnormalities, such as impaired long-term potentiation, altered circadian rhythm and sleep and demyelination in spinal cords and the peripheral nervous system [141-143]. In addition, the author and others demonstrated that PrP-/- mice were highly susceptible to ischaemic brain damage, showing marked neuronal cell death in the affected area [144,145]. Therefore, great cautions should be paid when depletion of $PrP^c$ is considered as a therapy for prion diseases.

### 12.2 LRP/LR-specific gene silencing

Leucht *et al.* reported that downregulation of LRP/LR by siRNA could effectively reduce $PrP^{Sc}$ formation in infected N2a cells [27]. LRP/LR was shown to bind to $PrP^c$ and $PrP^{Sc}$ and was actively involved in $PrP^{Sc}$ formation [25-27,146]. However, it remains to be elucidated how the interaction between LRP/LR and $PrP^c$ and/or $PrP^{Sc}$ is exactly involved in $PrP^{Sc}$ formation, although it is speculated that LRP/LR might act as a co-receptor for $PrP^{Sc}$ together with $PrP^c$ [25].

## 13. Immunotherapy for prion diseases

### 13.1 Antiprion protein antibodies

Certain anti-PrP antibodies have antiprion activities, clearing both $PrP^{Sc}$ and prion infectivity from persistently infected cultured cells [147,148], giving rise to the possibility of immunotherapy for prion diseases. However, the mechanisms for the antiprion activities of these antibodies are not fully understood. It was shown that the Fab portion alone was enough to execute antiprion activity [148]. Moreover, Donofrio *et al.* successfully generated recombinant single chain antibody (scFv) fragments derived from anti-PrP monoclonal antibody 6H4, designated sc4.1 and sc4.5, in mammalian RD-4 rhabdomyosarcoma cells [149] and showed that sc4.1 secreted from the cells could effectively reduce $PrP^{Sc}$ levels in infected N2a cells [149]. These results indicate that the Fc portion is dispensable for antibody mediated antiprion activity. R72 Fab, which binds to PrP molecules coated on a plate, but not to PrP molecules expressed on the cell surface, did not decrease $PrP^{Sc}$ levels in the cells [148]. This suggests that binding to native PrP molecules, particularly $PrP^c$, might be crucial for anti-PrP antibodies to execute the antiprion activities [150]. 15B3 monoclonal antibody was established as a $PrP^{Sc}$-specific antibody [151]. It is therefore interesting to investigate whether or not the antibody exhibits antiprion activity, reducing $PrP^{Sc}$ levels in infected cells. D13, D18, R1 and R2 Fabs, which bind to residues 95 – 103, 132 – 156, 220 – 231 and 225 – 231, respectively, showed antiprion activities in the cells [148]. However, E123 and E149 Fabs, which bind to residues 29 – 37and 72 – 86, respectively, exhibited no effects on $PrP^{Sc}$ levels [148]. Moreover, other investigators reported that 6H4, SAF34, SAF61, ICSM35 [152], ICSM 18 [152], 3S9 and 2H9 anti-PrP monoclonal antibodies binding to residues 144 – 152, 59 – 89, 144 – 152, 91 – 110, 146 – 159, 141 – 161 and 151 – 221, respectively, showed antiprion activities [147,153-155]. These indicate that the antiprion activity might be independently mediated via multiple sites on $PrP^c$. Kim *et al.* showed that anti-PrP antibodies with antiprion activities disturbed $PrP^c$ internalisation [156]. Therefore, this suggested that the antibody-induced impairment of subcellular localisation of $PrP^c$ could be involved in the antiprion activities of the antibodies. It is also conceivable that the antibodies might disturb the interaction of $PrP^c$ and $PrP^{Sc}$ and/or a co-factor(s), essential for $PrP^{Sc}$ formation.

### 13.2 Anti-LRP/LR antibodies

Leucht *et al.* showed that the LRP/LR-specific antibody, W3, could reduce $PrP^{Sc}$ levels in infected N2a cells [27]. LRP/LR interacts with $PrP^c$ either directly with residues 149 – 179 or indirectly to the N-terminus of $PrP^c$ via endogenous GAGs [24]. W3 was shown to compete with recombinant PrP (GST:PrP) for binding to LRP/LR expressed on the cell surface [27]. This therefore suggested that the disturbance of interaction between $PrP^c$ and LRP/LR might be crucial for W3 to prevent $PrP^{Sc}$ formation. Alternatively, W3 also reduced $PrP^c$ levels in the cells [27], suggesting that the interaction of LRP/LR with $PrP^c$ might be involved in regulation of $PrP^c$ metabolism or that LRP/LR-$PrP^c$-W3 complexes might stimulate internalisation of $PrP^c$ into lysosomes for degradation.

Large amounts of recombinant single chain antibodies, scFv S18 and N3 [157], both of which could interfere with the interaction of LRP/LR and PrP, were successfully produced [158]. They also showed that intraperitoneal injection of S18 (1 mg) once a week for 8 weeks from 1 day prior to the infection reduced $PrP^{Sc}$ levels by ~ 40% in the spleen of mice infected with RML prions [158]. However, no significant prolongation in the incubation and survival times could be detected in those mice [158]. These results indicate that peripheral administration of scFv might be prophylactic, but not therapeutic for the diseases.

### 13.3 PrP-$Fc_2$

Meier *et al.* generated tg mice expressing PrP-$Fc_2$, a fusion protein consisting of full-length mouse PrP fused to the Fcγ tail of human $IgG_1$, and showed that these tg mice developed the disease with much longer incubation times after infection with RML prions [159]. Pathological changes and accumulation of $PrP^{Sc}$ in the brains were indistinguishable between

terminally ill tg and non-tg mice [159]. The authors also showed that the tg mice on the PrP-null background never succumbed to the disease without detectable $PrP^{Sc}$ [159], indicating that PrP-$Fc_2$ itself could not be converted into the pathogenic form. PrP-$Fc_2$ has the potential to bind to $PrP^{Sc}$; therefore binding of PrP-Fc2 to $PrP^{Sc}$ might disturb conversion of $PrP^{c}$ to $PrP^{Sc}$ [160,161].

### 13.4 Blood–brain barrier for antiprion macromolecules

It has been documented that antibody-mediated immunotherapy is clinically very effective in other disorders including autoimmune diseases and cancers [162-165]. Therefore, immunotherapy using antibodies against PrP [147,148] or LRP/LR [27], PrP- or LRP/LR-specific scFvs [158], or PrP-$Fc_2$ [159], seems promising for prion diseases. However, these are macromolecules unable to cross the BBB. Therefore, it is necessary to develop effective delivery systems of macromolecules into brains. Ludewigs *et al.* recently published a comprehensive review for potential delivery systems for antiprion components, including lentivirus and adenovirus vector systems for antiprion antibodies or PrP-specific siRNA [166].

### 13.5 Active and passive immunisation against prion proteins

White *et al.* demonstrated that intraperitoneal administration of two anti-PrP monoclonal antibodies, ICSM 18 and 35, could protect mice from peripheral infection by RML prions, but were ineffective on prions directly infecting the brains of mice [153]. These results clearly indicate that passive immunisation with anti-PrP antibodies is effective against the prion infection in peripheral tissues, but not in the CNS probably due to inability of antibodies to across the BBB.

Sigurdsson *et al.* reported that subcutaneous immunisation of mice with recombinant mouse PrP induces anti-PrP autoantibodies and extended the survival times by 16 days after inoculation with 139A prion [167]. In contrast, we found that bovine and sheep, but not mouse, recombinant PrPs stimulated anti-PrP autoantibody responses in mice and that the incubation times were extended by 31 days in the bovine PrP-immunised mice and ~ 70% of the sheep PrP-immunised mice developed the disease with prolonged onsets [168]. Goñi *et al.* orally immunised mice with an attenuated *Salmonella typhimurium* LVR01 lipopolysaccharide vaccine strain expressing mouse PrP fused with non-toxic fragment C of tetanus toxin and showed that the orally immunised mice elicited higher IgG and IgA antibody responses against PrP and ~ 30% of the immunised mice were alive without any clinical signs up to 500 days post oral infection with 139A mouse prions [169]. However, it is conceivable that active immunisation against PrP might be prophylactic, but not therapeutic for prion diseases for the same reasons that passive immunisation with anti-PrP antibodies is prophylactic, but not therapeutic for prion diseases.

## 14. Neuroprotective compounds

### 14.1 Flupirtine in a clinical trial

Flupirtine is a centrally acting, non-opiate analgesic compound [170] and also an antiapoptotic agent for neurons. It acts partly by increasing levels of Bcl-2, an antiapoptotic molecule, and intracellular glutathione (GSH), an antioxidant [171]. Perovfic *et al.* reported that flupirtine (1 – 3 µg/ml) prevented rat primary cortical cells from apoptosis induced by the PrP106-126 peptide [172], which is considered to mediate some aspects of the $PrP^{Sc}$-related neurotoxicity [173]. This result suggests that flupirtine-mediated neuroprotection might be therapeutic for prion diseases.

Otto *et al.* recently reported a double-blind placebo-controlled study of flupirtine in patients with CJD [15]. Twenty-eight patients with CJD were randomized for oral treatment with either flupirtine ($n_s$ = 13) or a placebo (n = 15) [15]. One hundred mg of each compound was given to the patients on the first day and then the dosage increased up to 300 to 400 mg/day within 3 days [15]. No significant prolongation in the survival times could be detected between the groups [15]. However, flupirtine-treated patients performed several different dementia tests better than patients treated with placebo [15]. These results suggest that flupirtine has beneficial effects on cognitive function in CJD patients, although it remains to be investigated whether or not flupirtine protects neurons from the neurotoxicity of $PrP^{Sc}$ in the treated patients or whether or not the beneficial effects are attributable to the neuroprotective function or other functions of flupirtine.

### 14.2 A case report of antioxidants

Levels of nitric oxide and superoxide were significantly elevated in the brains of mice infected with scrapie ME7 and 139A prions [174]. Consistently, oxidative damage to lipids, proteins or DNA was detected in the brains of infected mice or hamsters and patients with sporadic and familial CJD [175-178]. These results suggest that oxidative stress might be involved in neuronal cell death in these diseases and that antioxidants might be effective for protecting neurons from $PrP^{Sc}$-mediated cell death in prion diseases. It was reported that, in one case of CJD tested, apnoeic episodes and myoclonus and rigidity were reduced after administration of a mixture of antioxidant supplements, including coenzyme Q-10, NADH, vitamin C, vitamin E, B complex, a multivitamin-mineral mixture, L-glutamine, omega 3 fatty acids, α lipoic acid and magnesium, together with parenteral administration of glutathione and ascorbate [179]. However, this was a preliminary report for assessment of effects of antioxidants on prion diseases. Further studies, including use of animal models, are needed.

### 14.3 Endoplasmic reticulum stress in neuronal cell death

It is suggested that endoplasmic reticulum (ER) stress might be involved in neuronal cell death induced by $PrP^{Sc}$ [180].

Hetz *et al.* reported that $PrP^{Sc}$, which was purified from 139A prion-infected mouse brains, induced marked apoptosis in normal N2a cells and that ER stress markers, including ER-resident caspase 12, ER chaperones, such as Grp94, Grp78 and Grp58, were concomitantly induced in the cells [181]. The authors also showed that some of the ER stress markers were elevated in the brains of mice infected with prions and of patients with sporadic and variant CJD [181]. ER stress is controlled by the unfolded protein response (UPR) and the ER-associated degradation (ERAD) system [182-184]. UPR is mediated by shutdown of protein synthesis and stimulation of expression of chaperone proteins. ERAD involves the ubiquitin/proteasome-dependent protein degradation system. Therefore, augmentation of these responses might be an alternative therapy against prion diseases.

## 15. Screening methods of antiprion compounds

### 15.1 Cell-based high-throughput *in vitro* assay

Kocisko *et al.* developed a high-throughput screening system using infected N2a cells in combination with a dot blot assay for $PrP^{Sc}$ [185]. Cells in a 96-well plate were incubated with μmolar concentrations of various compounds for 5 days, lysed in buffer and subjected to a dot blot assay for $PrP^{Sc}$. To distinguish $PrP^{Sc}$ from $PrP^{c}$, the lysates were treated with protease K. The authors screened a library of 2000 drugs and natural products (The Spectrum Collection, MicroSource Discovery, Inc.) for their antiprion activities to decrease $PrP^{Sc}$ levels and found 17 potent compounds, including the 2 already known compounds, quinacrine and lovastatin [185]. The remaining 15 compounds were novel inhibitors, including polyphenols (tannic acid, katacine, bisepigallocatechin digallate, Figure 9A), antimalarial drugs (bebeerine, tetrandrine, amodiaquine, Figure 9B), antihistamines (astemizole, terfenadine, Figure 9C), phenothiazine analogues (tiotixene, prochlorperazine, thioridazine, trifluoperazine, Figure 9D) and steroid-type compounds (budesonide, clomifene, chrysanthellin A, Figure 9E). The authors subsequently tested the therapeutic effects of some of the identified compounds in mice by oral or intrapritoneal administration starting one or two weeks after infection [186]. However, none of them showed significant therapeutic effects [186]. The treated mice died with similar time courses to non-treated control mice [186].

### 15.2 High-throughput, solid-phase assay based on cell-free conversion

Maxson *et al.* developed a high-throughput, solid-phase assay for screening antiprion compounds [187]. In this assay, $PrP^{Sc}$ immobilised onto a 96-well plate is incubated with $^{35}S$-labelled $PrP^{c}$ with or without various compounds. Thereafter, the reaction mixtures are treated with protease K and subjected to liquid scintillation counting to calculate efficiencies of the conversion of the $S^{35}$-labelled $PrP^{c}$ into $PrP^{Sc}$-like protease K-resistant PrP. If compounds have the potential to inhibit $PrP^{Sc}$ formation, the calculated percentage conversion should be low compared to that without compounds. Indeed, the authors showed that PcTS, a known inhibitor of conversion, prevented the conversion of $^{35}S$-labelled $PrP^{c}$ into protease K-resistant PrP in this system, lowering the percentage conversion [187]. Therefore, this high-throughput, solid-phase assay might be useful for large-scale screening of antiprion compounds.

### 15.3 Yeast-based screening assay

Bach *et al.* developed a rapid, yeast-based, two-step assay for screening antiprion compounds [188]. In this assay, a yeast prion responsible for the phenotype [$PSI^{+}$] was used. [$PSI^{+}$] yeasts growing on an agar plate containing yeast extract-peptone-dextrose (YPD)-rich medium forms white colonies whereas [psi-], a cured or prion-free phenotype, develops red colonies on the plate. Thus, cured or prion-free yeasts can be easily differentiates from [$PSI^{+}$] yeasts by their colony color. When filters containing antiprion compounds are spotted on the [$PSI^{+}$] yeast-growing agar plate, the yeasts are cured, forming a halo of red colonies around the filters. Using this assay, the authors identified 6 antiprion compounds from 2500 agents, as finally judged using infected N2a cells. One of these compounds is the already known antiprion agent, phenanthridine, and the others belong to a new class of molecules, the kastellpaolitines (Figure 10A) [188]. No animal data for these compounds are available.

### 15.4 High-throughput screening based on scanning for intensely fluorescent targets

Bertsch *et al.* developed a high-throughput screening system for identification of antiprion compounds that could inhibit interaction between $PrP^{c}$ and $PrP^{Sc}$ [189,190]. In this system, green fluorophore-labelled recombinant mouse PrP (instead of $PrP^{c}$), purified human $PrP^{Sc}$ and red fluorophore-labelled L42 monoclonal antibodies, which recognise human, but not mouse PrP, were incubated with or without various compounds and then subjected to analysis by the SIFT method in two-dimensional fluorescence intensity histograms [191]. If a compound prevents the interaction between the recombinant PrP and $PrP^{Sc}$, green fluorescent intensities of the complexes are decreased and, thus, the colour distribution is shifted towards the red sectors of the histograms. The authors identified 256 compounds from a library of 10,000 drug-like agents (ChemBridge DIVERSet1) and further confirmed 80 compounds by dose-response curves with half-maximal inhibition of the interaction from 0.3 – 60 μM using this system [191]. Out of 80 compounds, 6 showed antiprion activities in infected N2a cells, decreasing $PrP^{Sc}$ levels [191]. Four compounds share the *N'*-benzylidene-benzohydrazide core structure (Figure 10B) and showed significant reduction of $PrP^{Sc}$ levels in the cells [191].

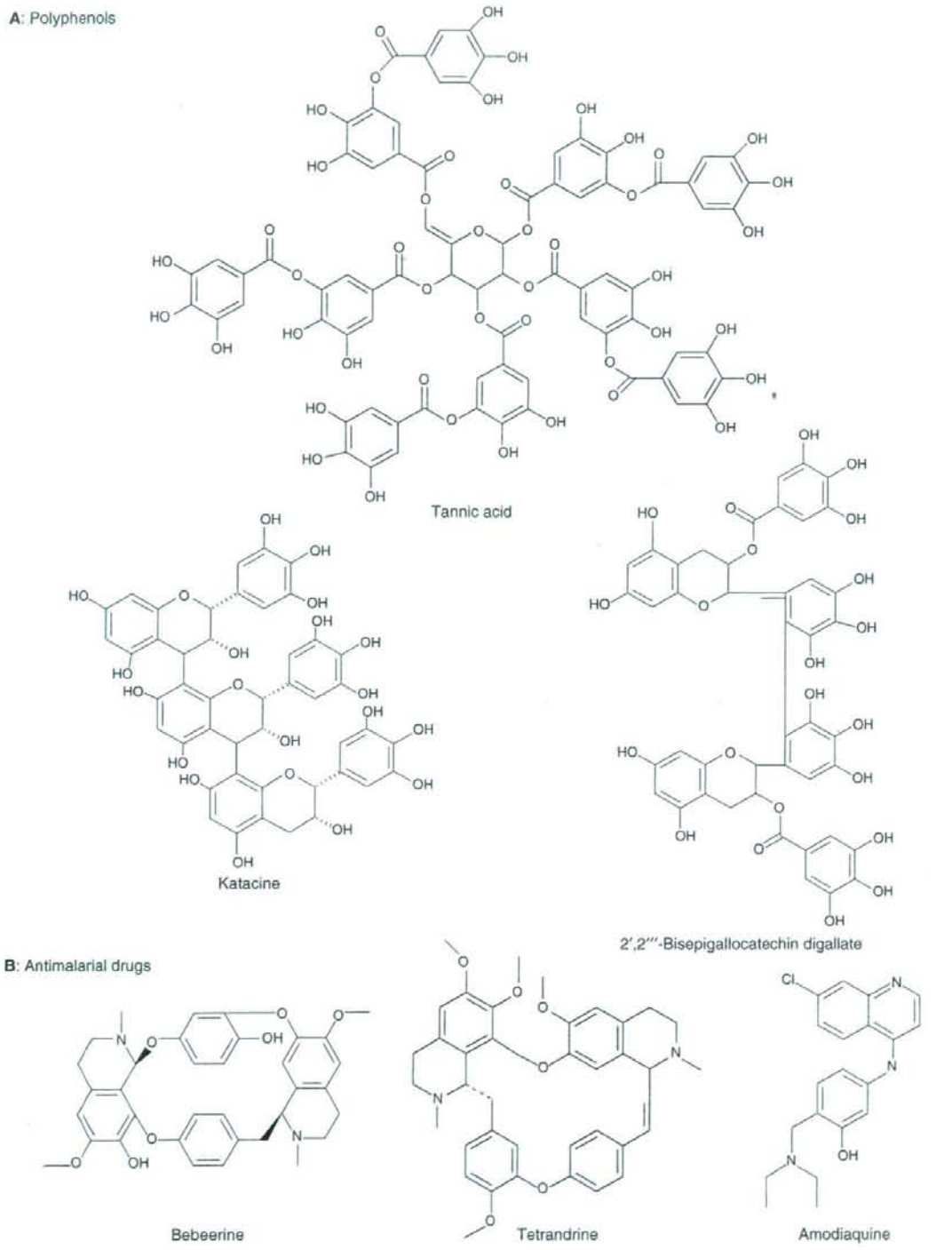



**Figure 9. Chemical structures of 15 compounds identified by Kocisco *et al.* [185] (continued).**

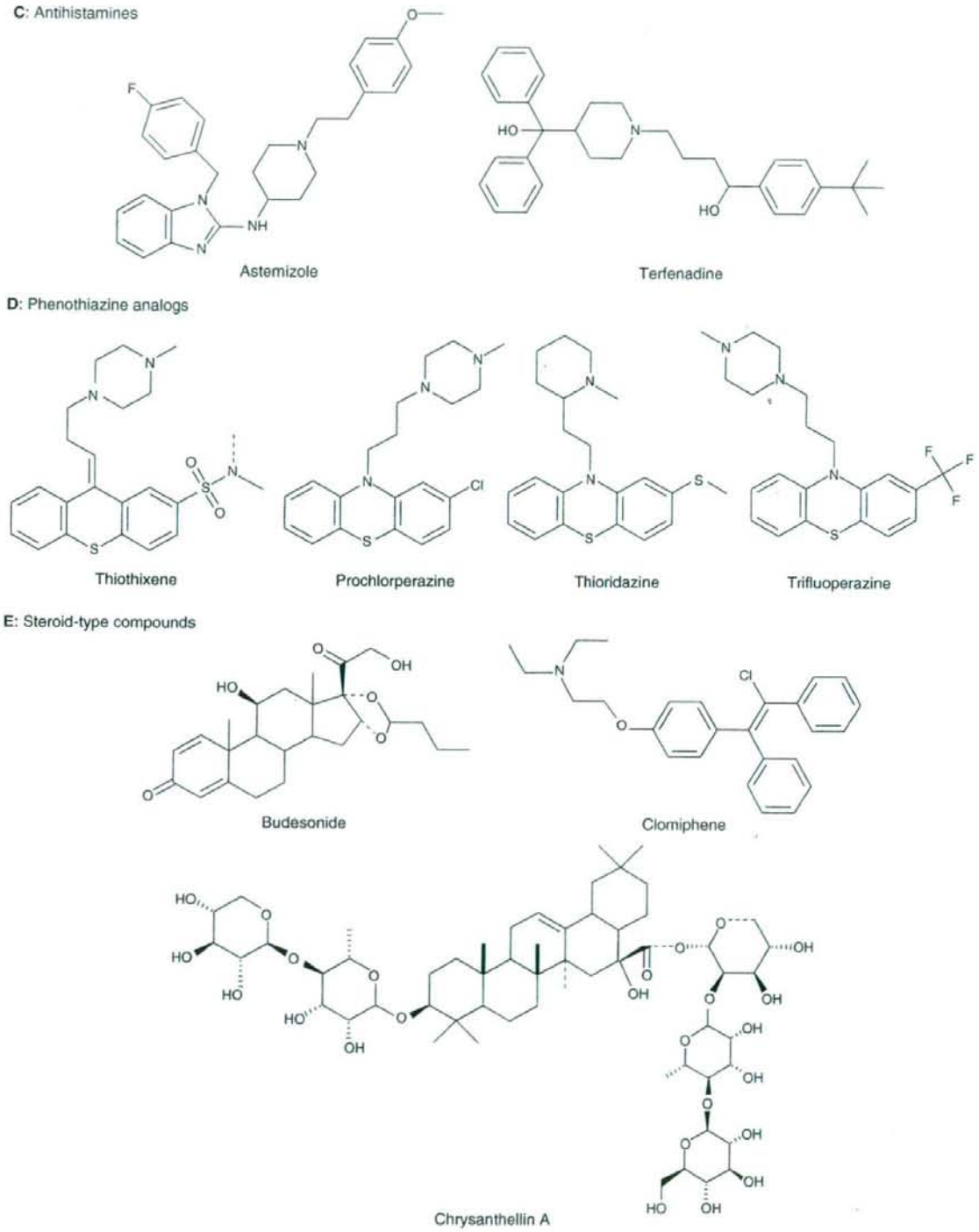
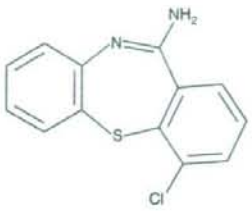
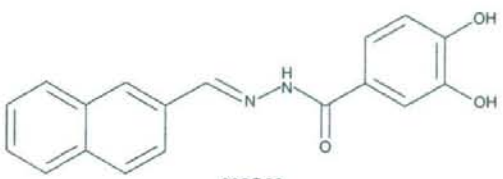



**Figure 9. Chemical structures of 15 compounds identified by Kocisco *et al.* [185].**

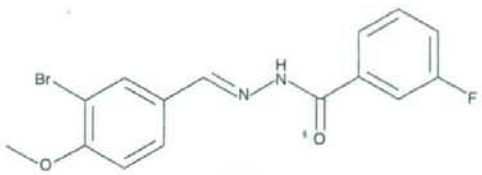
**A**: Kastellpaolitine 1



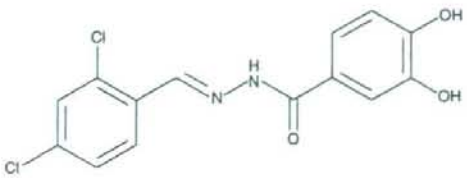
**B**: *N'*-Benzylidene-benzohydrazides



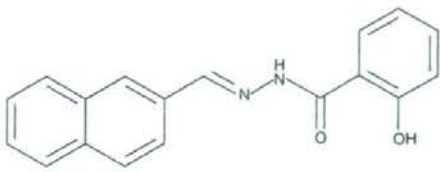
293G02



309F02



305E04



297F03

**C**: 2-Pyrrolidin-1-yl-*N*-[4-[4-(2-pyrrolidin-1-yl-acetylamino)-benzyl]-phenyl]-acetamide

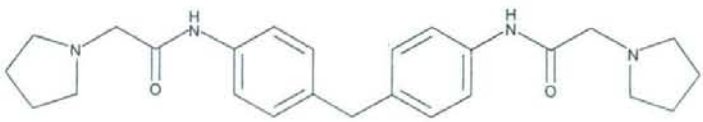


**Figure 10. Chemical structures of kastellpaolitine 1 (A; [188]), *N'*-benzylidene-benzohydrazides (B; [191]) and 2-pyrrolidin-1-yl-*N*-[4-[4-(2-pyrrolidin-1-yl-acetylamino)-benzyl]-phenyl]-acetamide (C; [193]).**

### 15.5 Antiprion compound screening based on surface plasmon resonance

Kawatake *et al.* reported a new screening system for antiprion compounds using surface plasmon resonance [192]. The authors immobilised recombinant mouse PrP121-231 on a sensor chip of an optical biosensor and investigated the binding affinity of already known antiprion compounds to recombinant PrP121 – 231 [192]. The binding affinities of the compounds were well correlated with their inhibitory activities on $PrP^{Sc}$ formation in infected cells [192], validating the system as useful for screening antiprion compounds.

### 15.6 Dynamics-based drug screening of antiprion compounds

Kuwata *et al.* conducted *in silico* screening of antiprion compounds [193]. The authors noticed that, according to the thermodynamical stability profile of recombinant $PrP^{c}$, residues in two distant helices forming a major cavity are less stable and slowly fluctuating. The conversion of $PrP^{c}$ to $PrP^{Sc}$ requires conformational changes in $PrP^{c}$. Therefore, the authors thought that compounds that bind to and stabilise the cavity might disturb the conformational change of $PrP^{c}$ into $PrP^{Sc}$. They performed

*in silico* screening of 320,000 candidate compounds for their binding ability to the cavity [193]. The authors successfully selected 59 compounds by *in silico* screening and subjected 44 compounds to a secondary screening using infected N2a cells, resulting in identification of the new antiprion compound, 2-pyrrolidin-1-yl-*N*-[4-[4-(2-pyrrolidin-1-yl-acetylamino)-benzyl]-phenyl]-acetamide (**Figure 10C**) [194], termed GN8 [193]. Interestingly, GN8 was therapeutically effective in mice infected with Fukuoka-1 prion, prolonging incubation times of the disease, when administrated via intraventicular or subcutaneous routes [193]. Intracerebral treatment with GN8 at a dose of 250 μg/kg per day for 42 – 70 and 70 – 98 days post infection extended the survival times by 8.5 and 17.7 days, respectively [193]. Moreover, the infected mice subcutaneously treated with GN8 at a dose of 8.9 mg/kg per day for 67 – 95 and 67 – 123 days after infection survived the disease up to 148.6 and 151.4 days after infection, respectively, both of which were significantly longer than the 133.0 days of non-treated mice [193].

## 16. Expert opinion

The so far identified antiprion compounds have no or low ability to cross the BBB and, therefore, being unable to effectively access brains they have no or very little therapeutic effect, even in animals. Doh-Ura *et al.* showed that bypassing the BBB by direct injection of PPS into the brain via an intraventricular route could augment its effectiveness, prolonging incubation times in mice [63]. However, the effectiveness was inversely correlated to interval length between the times of infection and administration of PPS [63] and none of the treated animals survived the disease [63], indicating that such mechanical bypassing might have limitation for therapy of prion diseases. Therefore, isolation of antiprion compounds capable of crossing the BBB effectively enough to exert their therapeutic effect against the disease are now awaited by screening of the BBB-permeable compounds for antiprion activity or by synthesis of such compounds using the already identified compounds as reference points. On the other hand, Dohgu *et al.* reported that inhibitors of the multi-drug resistant molecule P-glycoprotein, cyclosporin and verapamil, increased permeability of quinacrine through a monolayer of mouse brain endothelial cells [195], suggesting that such inhibitors of multi-drug resistant proteins might be useful to enhance the therapeutic effects of antiprion compounds.

The earlier the infected animals were treated with antiprion compounds, the more the treatment was effective, prolonging the incubation times in the animals, indicating that early treatment of the disease is important. However, identification of infected individuals among human populations is very difficult unless they have developed specific symptoms. Thus, the treatments are presently applied to clinically advanced patients, reducing their therapeutic effectiveness. Therefore, development of techniques for preclinical diagnosis of the disease is important. Soto and colleagues reported that, using PMCA technology [196,197], they successfully detected prions in the blood of presymptomatic hamsters experimentally infected with a scrapie prion [198]. This result suggests that this system might be useful for preclinical diagnosis. However, there might be ethical problems in this assay because $PrP^{c}$ used in this assay should be extracted from normal brain tissues. Atarashi *et al.* recently showed that, instead of $PrP^{c}$ from brains, recombinant PrP also could be used in this assay [199]. Therefore, the elucidation of whether this assay system could be applicable to human samples is awaited.

## Acknowledgement

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## Declaration of interest

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## Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.


1. Prusiner SB. Prions. Proc Natl Acad Sci USA 1998;95(23):13363-83
2. Dearmond SJ, Prusiner SB. Etiology and pathogenesis of prion diseases. Am J Pathol 1995;146(4):785-811
3. Duffy P, Wolf J, Collins G, et al. Possible person-to-person transmission of Creutzfeldt-Jakob disease [letter]. N Engl J Med 1974;290(12):692-3
4. Bernoulli C, Siegfried J, Baumgartner G, et al. Danger of accidental person-to-person transmission of Creutzfeldt-Jakob disease by surgery. Lancet 1977;1(8009):478-9
5. Koch TK, Berg BO, De Armond SJ, Gravina RF. Creutzfeldt-Jakob disease in a young adult with idiopathic hypopituitarism. Possible relation to the administration of cadaveric human growth hormone. N Engl J Med 1985;313(12):731-3
6. Thadani V, Penar PL, Partington J, et al. Creutzfeldt-Jakob disease probably acquired from a cadaveric dura mater graft. Case report. J Neurosurg 1988;69(5):766-9
7. Gajdusek DC. Unconventional viruses and the origin and disappearance of kuru. Science 1977;197(4307):943-60
8. Bruce ME, Will RG, Ironside JW, et al. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. Nature 1997;389(6650):498-501
9. Hill AF, Desbruslais M, Joiner S, et al. The same prion strain causes vCJD and BSE. Nature 1997;389(6650):448-50, 526
10. Collee JG, Bradley R, Liberski PP. Variant CJD (vCJD) and bovine spongiform encephalopathy (BSE): 10 and 20 years on: part 2. Folia Neuropathol 2006;44(2):102-10
11. Peden AH, Ritchie DL, Ironside JW. Risks of transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Folia Neuropathol 2005;43(4):271-8
12. Llewelyn CA, Hewitt PE, Knight RS, et al. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Lancet 2004;363(9407):417-21
13. Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet 2004;364(9433):527-9
14. Trevitt CR, Collinge J. A systematic review of prion therapeutics in experimental models. Brain 2006;129(Pt 9):2241-65
15. Otto M, Cepek L, Ratzka P, et al. Efficacy of flupirtine on cognitive function in patients with CJD: a double-blind study. Neurology 2004;62(5):714-8
16. Benito-Leon J. Combined quinacrine and chlorpromazine therapy in fatal familial insomnia. Clin Neuropharmacol 2004;27(4):201-3
17. Nakajima M, Yamada T, Kusuhara T, et al. Results of quinacrine administration to patients with Creutzfeldt-Jakob disease. Dement Geriatr Cogn Disord 2004;17(3):158-63
18. Todd NV, Morrow J, Doh-Ura K, et al. Cerebroventricular infusion of pentosan polysulphate in human variant Creutzfeldt-Jakob disease. J Infect 2005;50(5):394-6
19. Rainov NG, Tsuboi Y, Krolak-Salmon P, Vighetto A, Doh-Ura K. Experimental treatments for human transmissible spongiform encephalopathies: is there a role for pentosan polysulfate? Expert Opin Biol Ther 2007;7(5):713-26
20. Prusiner SB. Prions: novel infectious pathogens. Adv Virus Res 1984;29:1-56
21. Weissmann C, Enari M, Klohn PC, Rossi D, Flechsig E. Molecular biology of prions. Acta Neurobiol Exp (Wars) 2002;62(3):153-66
22. Legname G, Baskakov IV, Nguyen HO, et al. Synthetic mammalian prions. Science 2004;305(5684):673-6
23. Silveira JR, Raymond GJ, Hughson AG, et al. The most infectious prion protein particles. Nature 2005;437(7056):257-61
24. Hundt C, Peyrin JM, Haik S, et al. Identification of interaction domains of the prion protein with its 37-kDa/67-kDa laminin receptor. EMBO J 2001;20(21):5876-86
25. Gauczynski S, Nikles D, El-Gogo S, et al. The 37-kDa/67-kDa laminin receptor acts as a receptor for infectious prions and is inhibited by polysulfated glycanes. J Infect Dis 2006;194(5):702-9
26. Vana K, Weiss S. A trans-dominant negative 37kDa/67kDa laminin receptor mutant impairs PrP(Sc) propagation in scrapie-infected neuronal cells. J Mol Biol 2006;358(1):57-66
27. Leucht C, Simoneau S, Rey C, et al. The 37 kDa/67 kDa laminin receptor is required for PrP(Sc) propagation in scrapie-infected neuronal cells. EMBO Rep 2003;4(3):290-5
28. Harris DA. Cellular biology of prion diseases. Clin Microbiol Rev 1999;12(3):429-44
29. Gorodinsky A, Harris DA. Glycolipid-anchored proteins in neuroblastoma cells form detergent-resistant complexes without caveolin. J Cell Biol 1995;129(3):619-27
30. Taraboulos A, Scott M, Semenov A, et al. Cholesterol depletion and modification of COOH-terminal targeting sequence of the prion protein inhibit formation of the scrapie isoform. J Cell Biol 1995;129(1):121-32
31. Vey M, Pilkuhn S, Wille H, et al. Subcellular colocalization of the cellular and scrapie prion proteins in caveolae-like membranous domains. Proc Natl Acad Sci USA 1996;93(25):14945-9
32. Sarnataro D, Campana V, Paladino S, et al. PrP(C) association with lipid rafts in the early secretory pathway stabilizes its cellular conformation. Mol Biol Cell 2004;15(9):4031-42
33. Bate C, Salmona M, Diomede L, Williams A. Squalestatin cures prion-infected neurons and protects against prion neurotoxicity. J Biol Chem 2004;279(15):14983-90
34. Doh-Ura K, Iwaki T, Caughey B. Lysosomotropic agents and cysteine protease inhibitors inhibit scrapie-associated prion protein accumulation. J Virol 2000;74(10):4894-7
35. Kocisko DA, Come JH, Priola SA, et al. Cell-free formation of protease-resistant prion protein. Nature 1994;370(6489):471-4
36. Saborio GP, Permanne B, Soto C. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. Nature 2001;411(6839):810-3
37. Castilla J, Saa P, Hetz C, Soto C. In vitro generation of infectious scrapie prions. Cell 2005;121(2):195-206
38. Race RE, Fadness LH, Chesebro B. Characterization of scrapie infection in mouse neuroblastoma cells. J Gen Virol 1987;68(Pt 5):1391-9

39. Nishida N, Harris DA, Vilette D, et al. Successful transmission of three mouse-adapted scrapie strains to murine neuroblastoma cell lines overexpressing wild-type mouse prion protein. J Virol 2000;74(1):320-5

40. Schatzl HM, Laszlo L, Holtzman DM, et al. A hypothalamic neuronal cell line persistently infected with scrapie prions exhibits apoptosis. J Virol 1997;71(11):8821-31

41. MRC Clinical Trials Unit. PRION-1: Randomised trial of quinacrine in human prion disease. Available at: URL:www.ctu.mrc.ac.uk/studies/cjd.asp [Accessed January 2008]

42. Kimberlin RH, Walker CA. The antiviral compound HPA-23 can prevent scrapie when administered at the time of infection. Arch Virol 1983;78(1-2):9-18

43. Dealler Stephen Francis (GB). GB2334211 (1999)
- **Describes antiprion activities of polyanionic compounds.**

44. Sangaku Renkei Kiko Kyushu KK. JP2003040778; 2003

45. Gabizon R, Meiner Z, Halimi M, Ben-Sasson SA. Heparin-like molecules bind differentially to prion-proteins and change their intracellular metabolic fate. J Cell Physiol 1993;157(2):319-25

46. Caughey B, Raymond GJ. Sulfated polyanion inhibition of scrapie-associated PrP accumulation in cultured cells. J Virol 1993;67(2):643-50

47. Caughey B, Brown K, Raymond GJ, Katzenstein GE, Thresher W. Binding of the protease-sensitive form of PrP (prion protein) to sulfated glycosaminoglycan and congo red [corrected]. J Virol 1994;68(4):2135-41

48. Adjou KT, Simoneau S, Sales N, et al. A novel generation of heparan sulfate mimetics for the treatment of prion diseases. J Gen Virol 2003;84(Pt 9):2595-603

49. Schonberger O, Horonchik L, Gabizon R, et al. Novel heparan mimetics potently inhibit the scrapie prion protein and its endocytosis. Biochem Biophys Res Commun 2003;312(2):473-9

50. Replicor, Inc. (CA). CA2538245 (2005)
- **Describes antiprion activities of phosphorothioate oligonucleotides.**

51. Replicor, Inc. (CA); Juteau Jean-MARC (CA); Vaillant Andrew (CA): WO2006002540 (2006)
- **Describes antiprion activities of phosphorothioate oligonucleotides.**

52. Replicor, Inc. (CA). US2007123480 (2007)
- **Describes antiprion activities of phosphorothioate oligonucleotides.**

53. Kocisko DA, Vaillant A, Lee KS, et al. Potent antiscrapie activities of degenerate phosphorothioate oligonucleotides. Antimicrob Agents Chemother 2006;50(3):1034-44

54. Karpuj MV, Giles K, Gelibter-Niv S, et al. Phosphorothioate oligonucleotides reduce PrP levels and prion infectivity in cultured cells. Mol Med (Cambridge, Mass) 2007;13(3-4):190-8

55. Nat Inst of Asv Ind & Technol; Nat Veterinary Assay Lab Minis; Nat Agriculture & Food Res Org. JP2006320289 (2006)

56. Proske D, Gilch S, Wopfner F, et al. Prion-protein-specific aptamer reduces PrPSc formation. Chembiochem 2002;3(8):717-25

57. Rhie A, Kirby L, Sayer N, et al. Characterization of 2'-fluoro-RNA aptamers that bind preferentially to disease-associated conformations of prion protein and inhibit conversion. J Biol Chem 2003;278(41):39697-705

58. Shyng SL, Lehmann S, Moulder KL, Harris DA. Sulfated glycans stimulate endocytosis of the cellular isoform of the prion protein, PrPC, in cultured cells. J Biol Chem 1995;270(50):30221-9

59. Kimberlin RH, Walker CA. Suppression of scrapie infection in mice by heteropolyanion 23, dextran sulfate, and some other polyanions. Antimicrob Agents Chemother 1986;30(3):409-13

60. Ehlers B, Diringer H. Dextran sulphate 500 delays and prevents mouse scrapie by impairment of agent replication in spleen. J Gen Virol 1984;65(Pt 8):1325-30

61. Farquhar CF, Dickinson AG. Prolongation of scrapie incubation period by an injection of dextran sulphate 500 within the month before or after infection. J Gen Virol 1986;67(Pt 3):463-73

62. Ladogana A, Casaccia P, Ingrosso L, et al. Sulphate polyanions prolong the incubation period of scrapie-infected hamsters. J Gen Virol 1992;73(Pt 3):661-5

63. Doh-Ura K, Ishikawa K, Murakami-Kubo I, et al. Treatment of transmissible spongiform encephalopathy by intraventricular drug infusion in animal models. J Virol 2004;78(10):4999-5006

64. Supattapone S, Wille H, Uyechi L, et al. Branched polyamines cure prion-infected neuroblastoma cells. J Virol 2001;75(7):3453-61

65. Solassol J, Crozet C, Perrier V, et al. Cationic phosphorus-containing dendrimers reduce prion replication both in cell culture and in mice infected with scrapie. J Gen Virol 2004;85(Pt 6):1791-9

66. Winklhofer KF, Tatzelt J. Cationic lipopolyamines induce degradation of PrPSc in scrapie-infected mouse neuroblastoma cells. Biol Chem 2000;381(5-6):463-9

67. Yudovin-Farber I, Azzam T, Metzer E, Taraboulos A, Domb AJ. Cationic polysaccharides as antiprion agents J Med Chem 2005;48(5):1414-20

68. Supattapone S, Nguyen HO, Cohen FE, Prusiner SB, Scott MR. Elimination of prions by branched polyamines and implications for therapeutics. Proc Natl Acad Sci USA 1999;96(25):14529-34

69. US Health (US). WO9401116 (1994)

70. Caughey B, Race RE. Potent inhibition of scrapie-associated PrP accumulation by congo red. J Neurochem 1992;59(2):768-71

71. Demaimay R, Chesebro B, Caughey B. Inhibition of formation of protease-resistant prion protein by Trypan Blue, Sirius Red and other Congo Red analogs. Arch Virol Suppl 2000;(16):277-83

72. Ingrosso L, Ladogana A, Pocchiari M. Congo red prolongs the incubation period in scrapie-infected hamsters. J Virol 1995;69(1):506-8

73. Poli G, Martino PA, Villa S, et al. Evaluation of antiprion activity of congo red and its derivatives in experimentally infected hamsters. Arzneimittelforschung 2004;54(7):406-15

74. Gray LE Jr, Ostby JS. The effects of prenatal administration of azo dyes on testicular development in the mouse: a structure activity profile of dyes derived from benzidine, dimethylbenzidine, or dimethoxybenzidine. Fundam Appl Toxicol 1993;20(2):177-83

75. Rudyk H, Vasiljevic S, Hennion RM, et al. Screening Congo Red and its analogues for their ability to prevent the formation of PrP-res in scrapie-infected cells. J Gen Virol 2000;81(Pt 4):1155-64

76. Rudyk H, Knaggs MH, Vasiljevic S, et al. Synthesis and evaluation of analogues of Congo red as potential compounds against transmissible spongiform encephalopathies. Eur J Med Chem 2003;38(6):567-79

77. Demaimay R, Harper J, Gordon H, et al. Structural aspects of Congo red as an inhibitor of protease-resistant prion protein formation. J Neurochem 1998;71(6):2534-41

78. Caspi S, Halimi M, Yanai A, et al. The antiprion activity of Congo red. Putative mechanism. J Biol Chem 1998;273(6):3484-9

79. Ishikawa K, Kudo Y, Nishida N, et al. Styrylbenzoazole derivatives for imaging of prion plaques and treatment of transmissible spongiform encephalopathies. J Neurochem 2006;99(1):198-205

80. Kawasaki Y, Kawagoe K, Chen CJ, et al. Orally administered amyloidophilic compound is effective in prolonging the incubation periods of animals cerebrally infected with prion diseases in a prion strain-dependent manner. J Virol 2007;81(23):12889-98

81. Barbieri B, Giuliani FC, Bordoni T, et al. Chemical and biological characterization of 4'-iodo-4'-deoxydoxorubicin. Cancer Res 1987;47(15):4001-6

82. Tagliavini F, McArthur RA, Canciani B, et al. Effectiveness of anthracycline against experimental prion disease in Syrian hamsters. Science 1997;276(5315):1119-22

83. Forloni G, Iussich S, Awan T, et al. Tetracyclines affect prion infectivity. Proc Natl Acad Sci USA 2002;99(16):10849-54

84. Tagliavini F, Forloni G, Colombo L, et al. Tetracycline affects abnormal properties of synthetic PrP peptides and PrP(Sc) in vitro. J Mol Biol 2000;300(5):1309-22

85. Gilch S, Winklhofer KF, Groschup MH, et al. Intracellular re-routing of prion protein prevents propagation of PrP(Sc) and delays onset of prion disease. EMBO J 2001;20(15):3957-66

86. Nunziante M, Kehler C, Maas E, et al. Charged bipolar suramin derivatives induce aggregation of the prion protein at the cell surface and inhibit PrPSc replication. J Cell Sci 2005;118(Pt 21):4959-73

87. Priola SA, Caughey B, Caughey WS. Novel therapeutic uses for porphyrins and phthalocyanines in the transmissible spongiform encephalopathies. Curr Opin Microbiol 1999;2(5):563-6

88. Caughey WS, Raymond LD, Horiuchi M, Caughey B. Inhibition of protease-resistant prion protein formation by porphyrins and phthalocyanines. Proc Natl Acad Sci USA 1998;95(21):12117-22

89. US Health (US); Caughey Winslow S (US); Caughey Byron (US). WO0009111 (2000)

90. US Health (US). US6632808 (2003)

91. Priola SA, Raines A, Caughey WS. Porphyrin and phthalocyanine antiscrapie compounds. Science 2000;287(5457):1503-6

92. Priola SA, Raines A, Caughey W. Prophylactic and therapeutic effects of phthalocyanine tetrasulfonate in scrapie-infected mice. J Infect Dis 2003;188(5):699-705

93. Kocisko DA, Caughey WS, Race RE, et al. A porphyrin increases survival time of mice after intracerebral prion infection. Antimicrob Agents Chemother 2006;50(2):759-61

94. Caughey WS, Priola SA, Kocisko DA, et al. Cyclic tetrapyrrole sulfonation, metals, and oligomerization in antiprion activity. Antimicrob Agents Chemother 2007;51(11):3887-94

95. Adjou KT, Demaimay R, Deslys JP, et al. MS-8209, a water-soluble amphotericin B derivative, affects both scrapie agent replication and PrPres accumulation in Syrian hamster scrapie. J Gen Virol 1999;80(Pt 4):1079-85

96. Demaimay R, Race R, Chesebro B. Effectiveness of polyene antibiotics in treatment of transmissible spongiform encephalopathy in transgenic mice expressing Syrian hamster PrP only in neurons. J Virol 1999;73(4):3511-3

97. Amyx H, Salazar DC, Gajdusek DC, Gibbs CJ Jr. Chemotherapeutic trials in experimental slow virus diseases. Neurology 1984;34(Suppl 1):149

98. Demaimay R, Adjou KT, Beringue V, et al. Late treatment with polyene antibiotics can prolong the survival time of scrapie-infected animals. J Virol 1997;71(12):9685-9

99. Mange A, Nishida N, Milhavet O, et al. Amphotericin B inhibits the generation of the scrapie isoform of the prion protein in infected cultures. J Virol 2000;74(7):3135-40

100. Brajtburg J, Elberg S, Bolard J, et al. Interaction of plasma proteins and lipoproteins with amphotericin B. J Infect Dis 1984;149(6):986-97

101. Caughey B, Baron GS. Prions and their partners in crime. Nature 2006;443(7113):803-10

102. Saint-Julien L, Joly V, Seman M, Carbon C, Yeni P. Activity of MS-8209, a nonester amphotericin B derivative, in treatment of experimental systemic mycoses. Antimicrob Agents Chemother 1992;36(12):2722-8

103. Adjou KT, Demaimay R, Lasmezas C, et al. MS-8209, a new amphotericin B derivative, provides enhanced efficacy in delaying hamster scrapie. Antimicrob Agents Chemother 1995;39(12):2810-2

104. Pocchiari M, Casaccia P, Ladogana A. Amphotericin B: a novel class of antiscrapie drugs. J Infect Dis 1989;160(5):795-802

105. Marella M, Lehmann S, Grassi J, Chabry J. Filipin prevents pathological prion protein accumulation by reducing endocytosis and inducing cellular PrP release. J Biol Chem 2002;277(28):25457-64

106. Sviridov D, Nestel P, Watts G. Statins and metabolism of high density lipoprotein. Cardiovasc Hematol Agents Med Chem 2007;5(3):215-21

107. Glassberg H, Rader DJ. Management of lipids in the prevention of cardiovascular events. Ann Rev Med 2007; In Press

108. Mok SW, Thelen KM, Riemer C, et al. Simvastatin prolongs survival times in prion infections of the central nervous system. Biochem Biophys Res Commun 2006;348(2):697-702

109. Kempster S, Bate C, Williams A. Simvastatin treatment prolongs the survival of scrapie-infected mice. Neuroreport 2007;18(5):479-82

110. Univ California (US); Prusiner Stanley B (US); Korth Carsten (US); May Barnaby CH (US). WO02096431 (2002)

111. Touchsensor Tech LLC (US). US2005020582 (2005)

112. Barret A, Tagliavini F, Forloni G, et al. Evaluation of quinacrine treatment for prion diseases. J Virol 2003;77(15):8462-9

113. Korth C, May BC, Cohen FE, Prusiner SB. Acridine and phenothiazine derivatives as pharmacotherapeutics for prion disease. Proc Natl Acad Sci USA 2001;98(17):9836-41

114. Roikhel VM, Fokina GI, Pogodina VV. Influence of aminasine on experimental scrapie in mice. Acta Virol 1984;28(4):321-4

115. Univ California (US). US2004229898 (2004)

116. Univ California (US); Prusiner Stanley B (US); Korth Carsten (US); May Barnaby CH (US). WO2005027824 (2005)

117. May BC, Fafarman AT, Hong SB, et al. Potent inhibition of scrapie prion replication in cultured cells by bis-acridines. Proc Natl Acad Sci USA 2003;100(6):3416-21

118. Sangaku Renkei Kiko Kyushu KK. JP2004099553 (2004)

119. Murakami-Kubo I, Doh-Ura K, Ishikawa K, et al. Quinoline derivatives are therapeutic candidates for transmissible spongiform encephalopathies. J Virol 2004;78(3):1281-8

120. Kocisko DA, Caughey B. Mefloquine, an antimalaria drug with antiprion activity in vitro, lacks activity in vivo. J Virol 2006;80(2):1044-6

121. Ertmer A, Gilch S, Yun SW, et al. The tyrosine kinase inhibitor STI571 induces cellular clearance of PrPSc in prion-infected cells. J Biol Chem 2004;279(40):41918-27

122. Jabbour E, Cortes J, Giles F, Kantarjian H. Current perspectives on the treatment of patients with chronic myeloid leukemia: an individualized approach to treatment. Cancer J 2007;13(6):357-65

123. Petzer AL, Gunsilius E, Hayes M, et al. Low concentrations of STI571 in the cerebrospinal fluid: a case report. B J Haematol 2002;117(3):623-5

124. Yun SW, Ertmer A, Flechsig E, et al. The tyrosine kinase inhibitor imatinib mesylate delays prion neuroinvasion by inhibiting prion propagation in the periphery. J Neurovirol 2007;13(4):328-37

125. Bate C, Reid S, Williams A. Phospholipase A2 inhibitors or platelet-activating factor antagonists prevent prion replication. J Biol Chem 2004;279(35):36405-11

126. Farooqui AA, Ong WY, Horrocks LA. Inhibitors of brain phospholipase A2 activity: their neuropharmacological effects and therapeutic importance for the treatment of neurologic disorders. Pharmacol Rev 2006;58(3):591-620

127. Nordstrom EK, Luhr KM, Ibanez C, Kristensson K. Inhibitors of the mitogen-activated protein kinase kinase 1/2 signaling pathway clear prion-infected cells from PrPSc. J Neurosci 2005;25(37):8451-6

128. Bueler H, Aguzzi A, Sailer A, et al. Mice devoid of PrP are resistant to scrapie. Cell 1993;73(7):1339-47

129. Prusiner SB, Groth D, Serban A, et al. Ablation of the prion protein (PrP) gene in mice prevents scrapie and facilitates production of anti-PrP antibodies. Proc Natl Acad Sci USA 1993;90(22):10608-12

130. Sakaguchi S, Katamine S, Shigematsu K, et al. Accumulation of proteinase K-resistant prion protein (PrP) is restricted by the expression level of normal PrP in mice inoculated with a mouse-adapted strain of the Creutzfeldt-Jakob disease agent. J Virol 1995;69(12):7586-92

131. Manson JC, Clarke AR, McBride PA, McConnell I, Hope J. PrP gene dosage determines the timing but not the final intensity or distribution of lesions in scrapie pathology. Neurodegeneration 1994;3(4):331-40

132. Mallucci GR, White MD, Farmer M, et al. Targeting cellular prion protein reverses early cognitive deficits and neurophysiological dysfunction in prion-infected mice. Neuron 2007;53(3):325-35

133. Mallucci G, Dickinson A, Linehan J, et al. Depleting neuronal PrP in prion infection prevents disease and reverses spongiosis. Science 2003;302(5646):871-4

134. Alnylam Europ AG (DE). EP1550719 (2005)

135. Alnylam Europ AG (DE). JP2007031443 (2007)

136. Alnylam Europ AG (DE). EP1798285 (2007)

137. Japan Health Sciences Foundation (JP); Hohjoh Hirohiko (JP). WO2005078093 (2005)

138. Tilly G, Chapuis J, Vilette D, Laude H, Vilotte JL. Efficient and specific down-regulation of prion protein expression by RNAi. Biochem Biophys Res Commun 2003;305(3):548-51

139. Daude N, Marella M, Chabry J. Specific inhibition of pathological prion protein accumulation by small interfering RNAs. J Cell Sci 2003;116(Pt 13):2775-9

140. Pfeifer A, Eigenbrod S, Al-Khadra S, et al. Lentivector-mediated RNAi efficiently suppresses prion protein and prolongs survival of scrapie-infected mice. J Clin Invest 2006;116(12):3204-10

141. Collinge J, Whittington MA, Sidle KC, et al. Prion protein is necessary for normal synaptic function. Nature 1994;370(6487):295-7

142. Tobler I, Gaus SE, Deboer T, et al. Altered circadian activity rhythms and sleep in mice devoid of prion protein. Nature 1996;380(6575):639-42

143. Nishida N, Tremblay P, Sugimoto T, et al. A mouse prion protein transgene rescues mice deficient for the prion protein gene from purkinje cell degeneration and demyelination. Lab Invest 1999;79(6):689-97

144. McLennan NF, Brennan PM, McNeill A, et al. Prion protein accumulation and neuroprotection in hypoxic brain damage. Am J Pathol 2004;165(1):227-35

145. Sakurai-Yamashita Y, Sakaguchi S, Yoshikawa D, et al. Female-specific neuroprotection against transient brain ischemia observed in mice devoid of prion protein is abolished by ectopic expression of prion protein-like protein. Neuroscience 2005;136(1):281-7

146. Gauczynski S, Peyrin JM, Haik S, et al. The 37-kDa/67-kDa laminin receptor acts as the cell-surface receptor for the cellular prion protein. EMBO J 2001;20(21):5863-75

147. Enari M, Flechsig E, Weissmann C. Scrapie prion protein accumulation by scrapie-infected neuroblastoma cells abrogated by exposure to a prion protein antibody. Proc Natl Acad Sci USA 2001;98(16):9295-9

148. Peretz D, Williamson RA, Kaneko K, et al. Antibodies inhibit prion propagation and clear cell cultures of prion infectivity. Nature 2001;412(6848):739-43

149. Donofrio G, Heppner FL, Polymenidou M, Musahl C, Aguzzi A. Paracrine inhibition of prion propagation by anti-PrP

single-chain Fv miniantibodies. J Virol 2005;79(13):8330-8

150. Polymenidou M, Heppner FL, Pellicioli EC, et al. Humoral immune response to native eukaryotic prion protein correlates with antiprion protection. Proc Natl Acad Sci USA 2004;101(Suppl 2):14670-6
151. Korth C, Stierli B, Streit P, et al. Prion (PrPSc)-specific epitope defined by a monoclonal antibody. Nature 1997;390(6655):74-7
152. Medical Res Council (GB). US2006280745 (2006)
153. White AR, Enever P, Tayebi M, et al. Monoclonal antibodies inhibit prion replication and delay the development of prion disease. Nature 2003;422(6927):80-3
154. Perrier V, Solassol J, Crozet C, et al. Anti-PrP antibodies block PrPSc replication in prion-infected cell cultures by accelerating PrPC degradation. J Neurochem 2004;89(2):454-63
155. Miyamoto K, Nakamura N, Aosasa M, et al. Inhibition of prion propagation in scrapie-infected mouse neuroblastoma cell lines using mouse monoclonal antibodies against prion protein. Biochem Biophys Res Commun 2005;335(1):197-204
156. Kim CL, Karino A, Ishiguro N, et al. Cell-surface retention of PrPC by anti-PrP antibody prevents protease-resistant PrP formation. J Gen Virol 2004;85(Pt 11):3473-82
157. Stefan Weiss (DE/DE); Melvyn Little (GB/DE); Stefan Knackmuss (DE/DE); Clemence Rey (FR/DE); Peter Rottgen (DE/DE); Claudia Buttner (DE/DE); UWE Reusch (DE/DE). WO2005/035580 (2005)
158. Zuber C, Knackmuss S, Rey C, et al. Single chain Fv antibodies directed against the 37 kDa/67 kDa laminin receptor as therapeutic tools in prion diseases. Mol Immunol 2008;45(1):144-51
159. Meier P, Genoud N, Prinz M, et al. Soluble dimeric prion protein binds PrP(Sc) in vivo and antagonizes prion disease. Cell 2003;113(1):49-60
160. Univ Zuerich (CH). CA2518549 (2004)
161. Univ Zuerich (CH). EP1603951 (2005)
162. Waldmann TA. Daclizumab (anti-Tac, Zenapax) in the treatment of leukemia/lymphoma. Oncogene 2007;26(25):3699-703
163. Stinchcombe TE, Socinski MA. Bevacizumab in the treatment of non-small-cell lung cancer. Oncogene 2007;26(25):3691-8
164. Kavanaugh A. Interleukin-6 inhibition and clinical efficacy in rheumatoid arthritis treatment--data from randomized clinical trials. Bull NYU Hosp Joint Dis 2007;65(Suppl 1):S16-20
165. Pescovitz MD. Rituximab, an anti-cd20 monoclonal antibody: history and mechanism of action. Am J Transplant 2006;6(5 Pt 1):859-66
166. Ludewigs H, Zuber C, Vana K, et al. Therapeutic approaches for prion disorders. Expert Rev Antiinfect Ther 2007;5(4):613-30
167. Sigurdsson EM, Brown DR, Daniels M, et al. Immunization delays the onset of prion disease in mice. Am J Pathol 2002;161(1):13-7
168. Ishibashi D, Yamanaka H, Yamaguchi N, et al. Immunization with recombinant bovine but not mouse prion protein delays the onset of disease in mice inoculated with a mouse-adapted prion. Vaccine 2007;25(6):985-92
169. Goni F, Knudsen E, Schreiber F, et al. Mucosal vaccination delays or prevents prion infection via an oral route. Neuroscience 2005;133(2):413-21
170. Szelenyi I, Nickel B, Borbe HO, Brune K. Mode of antinociceptive action of flupirtine in the rat. B J Pharmacol 1989;97(3):835-42
171. Dhar S, Bitting RL, Rylova SN, et al. Flupirtine blocks apoptosis in batten patient lymphoblasts and in human postmitotic CLN3- and CLN2-deficient neurons. Ann Neurol 2002;51(4):448-66
172. Perovic S, Schroder HC, Pergande G, Ushijima H, Muller WE. Effect of flupirtine on Bcl-2 and glutathione level in neuronal cells treated in vitro with the prion protein fragment (PrP106-126). Exp Neurol 1997;147(2):518-24
173. Forloni G, Angeretti N, Chiesa R, et al. Neurotoxicity of a prion protein fragment. Nature 1993;362(6420):543-6
174. Wong BS, Brown DR, Pan T, et al. Oxidative impairment in scrapie-infected mice is associated with brain metals perturbations and altered antioxidant activities. J Neurochem 2001;79(3):689-98
175. Choi SI, Ju WK, Choi EK, et al. Mitochondrial dysfunction induced by oxidative stress in the brains of hamsters infected with the 263 K scrapie agent. Acta Neuropathol (Berl) 1998;96(3):279-86
176. Milhavet O, McMahon HE, Rachidi W, et al. Prion infection impairs the cellular response to oxidative stress. Proc Natl Acad Sci USA 2000;97(25):13937-42
177. Guentchev M, Voigtlander T, Haberler C, Groschup MH, Budka H. Evidence for oxidative stress in experimental prion disease. Neurobiol Dis 2000;7(4):270-3
178. Guentchev M, Siedlak SL, Jarius C, et al. Oxidative damage to nucleic acids in human prion disease. Neurobiol Dis 2002;9(3):275-81
179. Drisko JA. The use of antioxidants in transmissible spongiform encephalopathies: a case report. J Am Coll Nutr 2002;21(1):22-5
180. Castilla J, Hetz C, Soto C. Molecular mechanisms of neurotoxicity of pathological prion protein. Curr Mol Med 2004;4(4):397-403
181. Hetz C, Russelakis-Carneiro M, Maundrell K, Castilla J, Soto C. Caspase-12 and endoplasmic reticulum stress mediate neurotoxicity of pathological prion protein. EMBO J 2003;22(20):5435-45
182. Lindholm D, Wootz H, Korhonen L. ER stress and neurodegenerative diseases. Cell Death Differ 2006;13(3):385-92
183. Paschen W. Endoplasmic reticulum: a primary target in various acute disorders and degenerative diseases of the brain. Cell Calcium 2003;34(4-5):365-83
184. Zhao L, Ackerman SL. Endoplasmic reticulum stress in health and disease. Curr Opin Cell Biol 2006;18(4):444-52
185. Kocisko DA, Baron GS, Rubenstein R, et al. New inhibitors of scrapie-associated prion protein formation in a library of 2000 drugs and natural products. J Virol 2003;77(19):10288-94
186. Kocisko DA, Morrey JD, Race RE, Chen J, Caughey B. Evaluation of new cell culture inhibitors of protease-resistant prion protein against scrapie infection in mice. J Gen Virol 2004;85(Pt 8):2479-83
187. Maxson L, Wong C, Herrmann LM, Caughey B, Baron GS. A solid-phase assay for identification of modulators of prion