Similar Structure−Activity Relationships of Quinoline Derivatives for Antiprion and Antimalarial Effects

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Prion diseases are invariably fatal neurodegenerative diseases, in which the infectious agent consists of PrP^{Sc}, a pathogenic misfolded isoform of the normal cellular prion protein (PrP^{C}). Until now, no pharmacological options exist for these novel pathogens. Here we describe the screening of a series of polyquinolines and quinolines linked to a large variety of terminal groups for their ability to cure a persistently prion infected cell line (ScN2a). Several compounds showed antiprion activity in the nanomolar range. The most active molecule, named **42**, had a half-effective concentration (EC_{50}) for antiprion activity of 50 nM. In a library of quinoline derivatives we were able to identify several structure−activity relationships (SAR). Remarkably, antiprion SAR in ScN2a cells were similar to antimalarial SAR in a cell model of malaria, particularly for the sulfonamide quinoline derivatives, suggesting that some molecular targets of antiprion and antimalarial substances overlap.

Introduction

Transmissible spongiform encephalopathies (TSEs) are invariably fatal neurodegenerative diseases that manifest as spontaneous, inherited, or infectious maladies. In humans, they occur as Creutzfeld-Jakob disease (CJD[a]), Gerstmann-Sträussler-Scheinker disease (GSS), familial fatal insomnia (FFI), or Kuru. These disorders are caused by prions, unique pathogens consisting of a disease-specific conformational isoform of the prion protein, PrP^{Sc}, that replicate by converting the normal host prion protein PrP^{C}.[1] PrP^{C} is a GPI-anchored membrane glycoprotein of yet unknown function. Once converted into PrP^{Sc}, due to the conformational rearrangement, PrP acquires novel biophysical and biochemical characteristics such as partial protease resistance which allows detection of prions in affected cells.

Prion diseases are rare in humans but endemic in sheep (scrapie) or even epidemic in cattle (bovine spongiform encephalopathy; BSE) and mule deer (chronic wasting disease; CWD). Transmission of prions from cattle infected with BSE to humans caused variant CJD (vCJD).[2,3] A survey of tonsillectomies and appendectomies in Great Britain, the foremost BSE-affected country, has led to the estimation that about 50 to 700 people per million could be infected with vCJD.[4] Furthermore, transmission of human prions through contaminated blood has been reported recently,[5] raising fears on unrecognized spread of the disease and therefore demanding pharmacological remedies.

In search for antiprion pharmaceuticals, several substances have been identified to possess antiprion potency both in cell models and animal models of prion disease. Pharmacological studies in prion inoculated animals are time-consuming, laborious, and costly due to the need of performing these experiments under particular biosafety conditions, which has limited these in vivo experiments to established lead compounds. Drug screening in permanently scrapie-infected mouse neuroblastoma cells (ScN2a) has been very successful since candidate antiprion compounds can easily be evaluated by adding them into the cell culture medium and measuring the presence of protease-resistant PrP immunoreactivity by Western blotting of cell lysates after a defined period of time. Employing this assay has led to the discovery of antiprion compounds Congo red and analogues,[6−8] polyamine dendrimers,[9] cationic polysaccharides,[10] statins,[11] and heterocyclic compounds[12−15] including heterocyclic bis-acridines.[14] Many of these and other compounds, when tested in prion-infected animals did not show antiprion activity when administered shortly before onset of neurologic symptoms. A major requirement for a successful antiprion drug, therefore, is activity at time points late in the incubation period, and a pharmacokinetic profile with low toxicity and excellent blood−brain barrier (BBB) permeability.

In this study, we focused on quinoline derivatives with chloroquine as the derivatized compound. Chloroquine has been used as an antimalarial drug with low toxicity for decades and is known to cross the BBB.[16,17] Its antiprion activity has been reported in several studies.[12,15,18] We investigated the antiprion potency of a library of 50 quinoline derivatives consisting of sulfonamide-, amide-, and amine-containing polyquinolines with different linkers. Most of these compounds showed antiprion activity in ScN2a cells in a low micromolar range, but several of them with a 10 to 100-fold higher potency than chloroquine. When we compared the structure−activity relationship (SAR) of the antiprion effects with the antimalarial effects, we identified remarkable similarities suggesting that development of even more potent antiprion polyquinoline derivatives may take advantage from the huge libraries of antimalarial substances available.

Results

We previously reported the synthesis of polyquinolines from 4,7-dichloroquinoline and adequate polyaminolinkers,[19] sul-

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[a] Abbrevations: CJD, Creutzfeldt-Jakob disease; PrP, prion protein; GPI, glycosylphosphatidylinositol; BBB, blood−brain barrier; FAA, full antiprion activity; CQ, chloroquine; DIEA, diisopropylethylamine; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, *N*-hydroxybenzotriazole; P_{HPLC}, purity determined by HPLC; MALDI-MS, matrix-assisted laser desorption ionization mass spectrometry.

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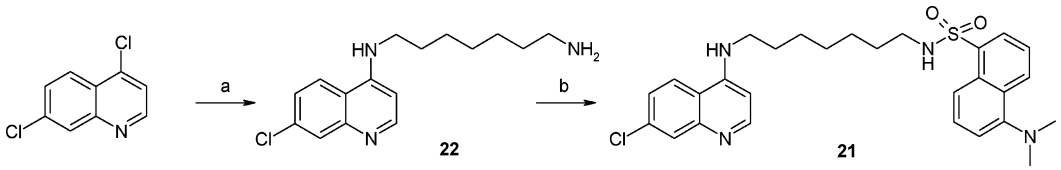
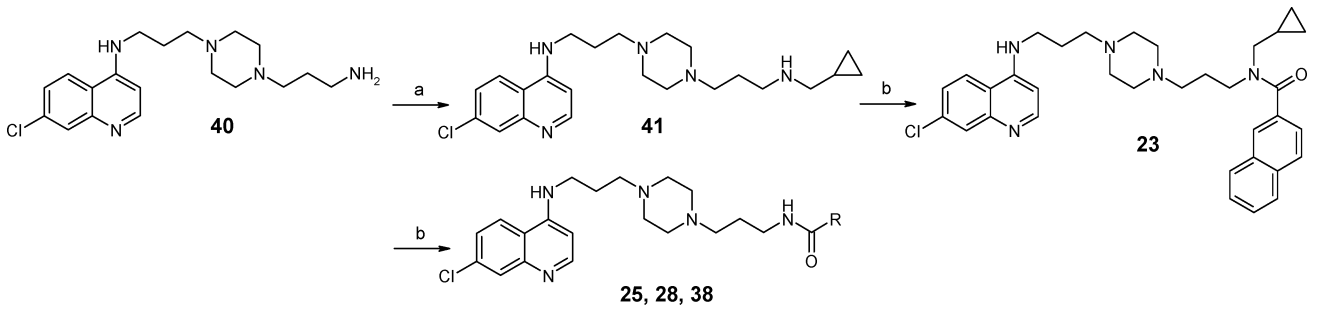
Scheme 1. Synthesis of Sulfonamide **21**[a]



[a] Reagents: (a) 1,7-diaminoheptane, 1-pentanol; (b) dansyl chloride, CH_2Cl_2.

Scheme 2. Synthesis of Amides **23**, **25**, **28**, and **38**[a]



[a] Reagents: (a) cyclopentane carboxaldehyde, 3 Å molecular sieves, MeOH then $NaBH_4$, MeOH; (b) adequate carboxylic acid, HBTU, HOBt, DIEA, CH_2Cl_2.

fonamides,[20] amides, and amines.[21] Compound **21** was easily synthesized according to Scheme 1. Starting from a common amino precursor **40**, [21,22] secondary amide **23** was synthesized in two steps and amides **25**, **28**, and **38** in one step using HOBt and HBTU as a coupling reagent (Scheme 2).

These compounds had been tested for their antimalarial properties and their cytotoxic effects upon human MRC-5 cells (diploid embryonic lung cell line).[19−22] These data were integrated into Tables 1−4 to allow an extensive comparison with their antiprion effects.

All quinoline derivatives were tested for antiprion activity in a permanently scrapie-infected mouse neuroblastoma (ScN2a) cell line, an established cellular model for prion disease [23,24] that is commonly used for that purpose.[6,12] For quantification of antiprion activity, Western blotting of protease-digested ScN2a cell lysates for PrP immunoreactivity (PrP^{Sc}) was used (Figure 5). This method is precise and has been cross-validated with ELISA methods and bioassays.[9,13,25]

The particular concentration where no PrP^{Sc} could be detected by Western Blot was named "Full Antiprion Activity" (FAA), and EC_{50} was the concentration needed to reduce the amount of cellular PrP^{Sc} to 50% of the corresponding amount present in untreated cells. A concentration was defined toxic when cells did not reach 80% confluency after one week of treatment (see Tables 1−4). All substances were used in concentrations of up to 10 μM because this was the FAA of the chemical lead chloroquine. Cells were treated for one week with the compound dissolved from a 1000× stock solution into the cell culture medium. Cell culture medium was exchanged every other day. In each single assay, activity was compared to untreated control cells and cells treated with 1 μM quinacrine as a positive control for antiprion activity.[12] Each compound was tested at least three times in independently set up experiments.

On the basis of their chemical structures, we grouped our quinoline derivatives in four different classes of substances: 1. Polyquinolines (Series Q; Figures 1−3, Table 1) consisting of different linked bis-, tris-, and tetraquinolines. 2. Sulfonamides (Series S; Figure 4, Scheme 1, Table 2) consisting of N^1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazinesulfonamide derivatives except **21**. 3. Amides (Series A; Figure 4, Scheme 2, Table 3) consisting of N^1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine-amide derivatives, and 4. Amines (Series B and C, Figure 4, Table 4) consisting of N^1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine-amine derivatives.

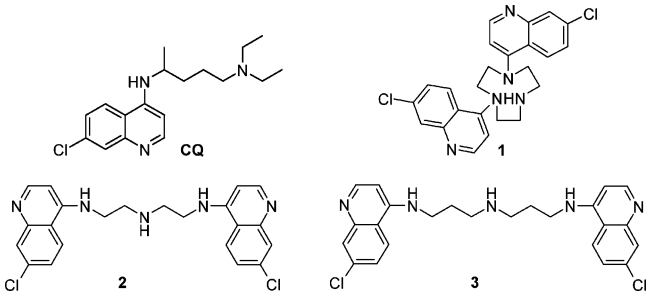


Figure 1. Chloroquine (CQ) and bisquinolines.

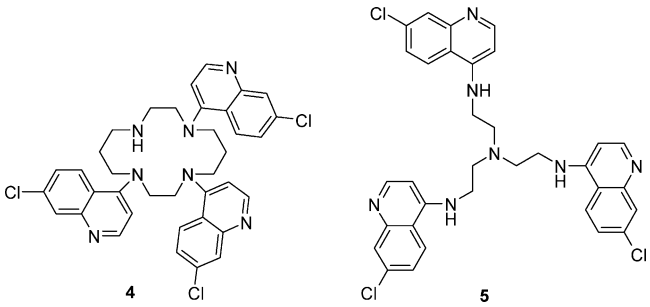


Figure 2. Trisquinolines.

Structure−Activity Relationships on the Inhibition of PrP^{Sc} Formation. The activity of the compounds depended on the chemical family (polyquinolines, amides, amines, or sulfonamides) and the nature of the substituent. Half effective concentrations (EC_{50}) varied within a large range from 50 nM to more than 10 μM.

Series Q: Polyquinolines. Antiprion activities varied within a large interval, from an EC_{50} of 0.5 μM to more than 10 μM (Figures 1−3; Table 1). Starting from chloroquine, the addition of a second chloroquinoline moiety did not modify antiprion activity. To the contrary, addition of a third chloroquinoline moiety led to a decrease in antiprion activity. Regarding bisquinolines (**1**, **2**) or trisquinolines (**4**, **5**), the structure of the linker (linear or cyclic) did not influence antiprion activity.

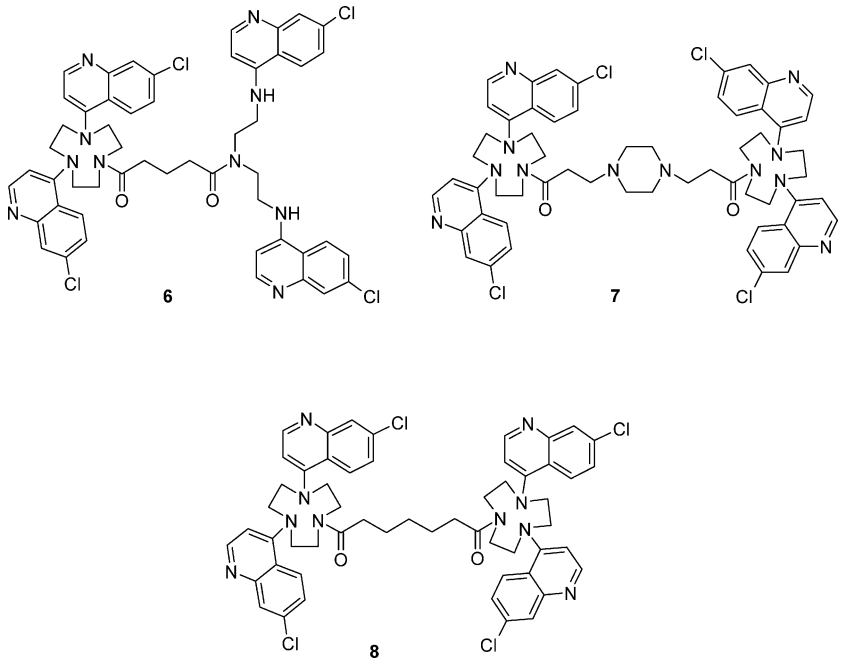


Figure 3. Tetraquinolines.

Table 1. Polyquinolines: Inhibition on PrP^{Sc} Formation in ScN2a Cells

	antiprion effect			selectivity:	MRC-5 toxicity:[19]	selectivity:	antimalarial effect:[19]
compound	activity[a] (nM)	EC_{50}[b] (nM)	toxicity[c] (nM)	SI_1 = tox/EC_{50}	CC_{50}[f] (nM)	SI_2= CC_{50}/EC_{50}	IC_{50}[g] (nM)
CQ	10.000	4.000	n.d.[d]	>2.5	50.000	12.5	110 ± 26
1	10.000	5.000	n.d.	>2	>32.000	>6.4	112.8 ± 24.9
2	5.000	3.000	n.d.	>3.3	25.000	>8.4	142.1 ± 10.2
3	n.d.[e]	n.d.[e]	1.000	-	700	-	75.4 ± 22.6
4	n.d.	n.d.	n.d.	-	>32.000	-	>1000
5	n.d.	n.d.	n.d.	-	>32.000	-	>1000
6	5.000	3.000	10.000	3.3	>32.000	>10.7	152.2 ± 9.2
7	1.000	600	7.500	12.5	>32.000	>53.4	76.9 ± 9.4
8	1.000	500	n.d.	>20	>32.000	>64	32.7 ± 13.5

[a] Approximate concentration of compound required for complete eradication of PrP^{Sc} from ScN2a-cells. [b] Approximate concentration of compound required to reduce PrP^{Sc} content to 50% of untreated ScN2a cells. [c] Concentration at which ScN2a cells did not reach 80% confluency after a 6 day treatment. [d] No toxicity detected at a concentration of 10μM. [e] No activity detected in nontoxic concentrations or at 10μM. [f] Concentration at which 50% of cells did not survive after a 7 day treatment. [g] Concentration required to inhibit *P. falciparum* growth using CQ-resistant strain FcB1 to 50%.

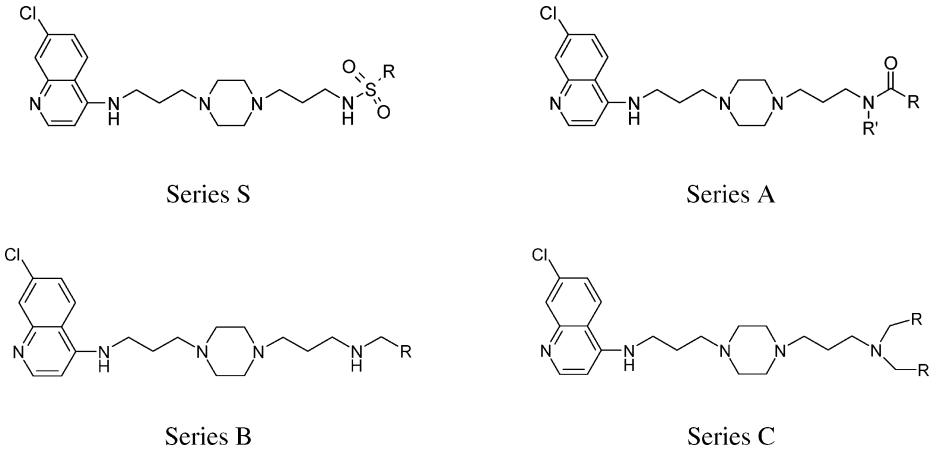
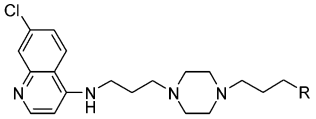


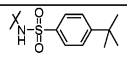
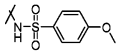
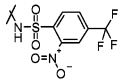
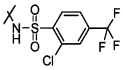
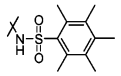
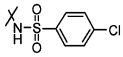
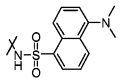
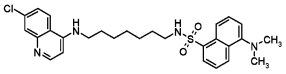
Figure 4. Quinoline derivatives.

Interestingly, the association of two bisquinoline-cyclams increased antiprion activity 10 times; however, the way the bisquinoline-cyclams were linked did not influence antiprion activity (**7**, **8**) even though the cyclam itself proved important (compare **6** and **8**).

Series S: Sulfonamides. Antiprion activities varied within a large range of EC_{50} values between 75 nM to more than 1 μM (Table 2). Best activities were obtained for aromatic substituents, but only one aliphatic substituent was evaluated (**19**) which exhibited no significant antiprion activity. Para substitution on the phenyl ring led to higher activities as compared to the nonsubstituted phenyl group. Bulky substituents such as *tert*-butyl (**9**) or an electron-donating group such as a methoxy group (**10**) considerably enhanced their antiprion activities. In contrast, a chlorine substituent (**16**) possessed less antiprion activity.

Table 2. Sulfonamides: Inhibition on PrP^{Sc} Formation in ScN2a Cells



cpd	R	antiprion effect			selectivity	MRC-5 toxicity[20]	selectivity	antimalarial effect[20]
		activity[a] (nM)	EC_{50}[b] (nM)	toxicity[c] (nM)	SI_1 = tox/EC_{50}	CC_{50}[f] (nM)	SI_2 = CC_{50}/EC_{50}	IC_{50}[g] (nM)
9		100	75	1.000	13.35	1.000	13.35	11.2 ± 2.7
10		250	75	2.000	26.67	3.900	52	43.2 ± 1.9
11		500	80	2.000	25	2.000	25	26.0 ± 2.9
12		500	100	5.000	50	6.000	60	69.0 ± 0.7
13		250	100	500	5	1.000	10	8.5 ± 0.7
14		200	150	2.500	16.67	<3.000	<20	15 ± 0.5
15		250	200	1.000	5	1.000	5	16.9 ± 1.2
16		500	250	2.000	8	-	-	26% [h]
17		500	250	2.500	10	1.000	4	16.7 ± 2.0
18		1000	500	3.000	6	5.800	11.6	112 ± 17
19		10.000	6.000	n.d.[d]	-	> 32.000	-	187 ± 28
20		n.d.[e]	n.d.[e]	n.d.[d]	-	4.000	-	167 ± 36
21		750	250	5.000	20	4.000	16	204.7±15.6

[a–g] See notes in Table 1. [h] Inhibition percentage of parasite growth at a concentration of 10 nM.

Polysubstitution on the phenyl group was allowed, depending on the position and the substituents. The presence of a bulky substituent in para-position on the phenyl group such as trifluoromethoxy (**11**) maintained antiprion activity if there was no chlorine substituent present. Increasing the phenyl substitutions yielded a less active compound (**15**).

The presence of more a hydrophobic aromatic substituent such as naphthyl (**13**) increased antiprion activity and addition of a polar group (**18**) decreased antiprion activity. The phenyl ring could favorably be replaced by a thiophene (**12**).

Regarding dansyl derivatives of quinoline, the structure of the linker (linear or cyclic) between the two aromatic moieties did not influence antiprion activity (compare **21** and **18**).

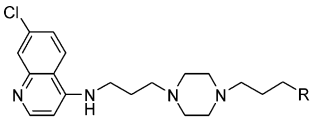
Series A: Amides. Antiprion activities varied within a large range of EC_{50} values between 75 nM to more than 1 μM (Table 3). Best antiprion activities were obtained for aromatic substituents bearing bulky or hydrophobic groups. Except in the case of a chlorine substituent, the SAR followed the same rules as for Series S. Para-substitution led to higher activities when compared with meta or nonsubstituted phenyl ring. The thiophene derivative (**35**) was more active than the phenyl derivative (**36**). Introduction of a polar substituent led to a dramatic loss of activity (compare **36** and **38**, **24** and **32**).

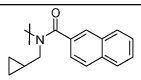
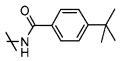
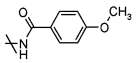
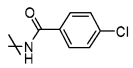
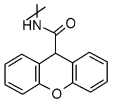
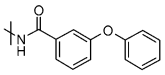
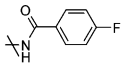
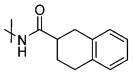
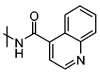
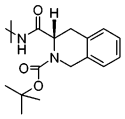
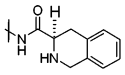
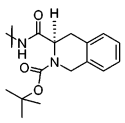
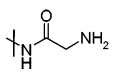
The antiprion activity of aliphatic derivatives strongly depended on the nature of the substituents. They were generally less active than the aromatic compounds but the presence of an aromatic moiety within the aliphatic substituent maintained antiprion activity (**28**). A small and polar substituent yielded poor antiprion activity (**39**). Interestingly, the stereochemistry of the substituent was important, a tetrahydroisoquinoline substituent effected antiprion activity only in the (*R*)-configuration. In this case, the substitution of the nitrogen atom did not influence antiprion activity (**34**, **33**). Introduction of a small aliphatic substituent on the amide nitrogen maintained antiprion activity (**23**).

Series B and C: Secondary and Tertiary Amines. Antiprion activity of these compounds again clearly depended on the nature of the substituent. In the case of small substituents, tertiary amines seemed to be more active than secondary amines (**43**, **45**) whereas bulky substituents on tertiary amines seemed detrimental for activity (**44**, **46**) (Table 4).

Except in the case of chlorine substituents, compounds of series S or A provided about the same results (Table 5). The important point was in each case the aromatic and hydrophobic nature of the substituent. We observed that compounds of series S were often more toxic than their amide analogues.

Table 3. Amides: Inhibition on PrP^{Sc} Formation in ScN2a Cells

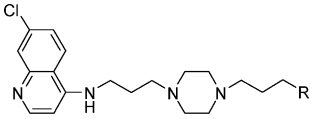


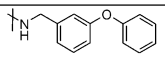
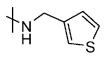
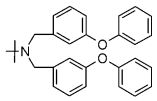
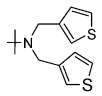
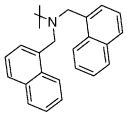
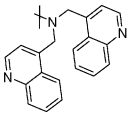
cpd	R	anti prion effect			selectivity	MRC-5 toxicity[21]	selectivity	antimalarial effect[21]
		activity[a] (nM)	EC_{50}[b] (nM)	toxicity[c] (nM)	SI_1 = tox / EC_{50}	CC_{50}[f] (nM)	SI_2 = CC_{50}/EC_{50}	IC_{50}[g] (nM)
23		300	75	2.000	26.67	-	-	23%[h]
24		100	80	1.000	12.5	4.000	50	155.0 ± 17
25		200	100	1.000	10	-	-	54.8 ± 7.8
26		300	100	3.000	30	4.000	40	20.2 ± 1.3
27		500	100	2.500	25	1.000	10	16.7 ± 5.2
28		300	100	750	7.5	-	-	11%[g]
29		300	200	750	3.75	5.000	25	20.8 ± 5.9
30		300	200	3.000	15	6.800	34	48.9 ± 11.5
31		400	200	5.000	25	5.400	21.6	38.2 ± 7.5
32		500	250	2.000	8	16.400	66	20.1 ± 2.3
33		500	250	2.000	8	4.000	16	390.9 ± 41.8
34		500	300	5.000	16.7	22.000	73.3	27.7 ± 4.5
35		750	500	5.000	10	20.500	41	112.1 ± 36.0
36		1.000	800	7.500	9.38	15.600	22.3	80.6 ± 5.8
37		n.d.[e]	1.000	3.000	-	4.000	4	395.7 ± 95.5
38		5.000	3.000	n.d.[d]	-	-	-	1%[h]
39		n.d.[e]	n.d.[e]	n.d.[d]	-	25.000	-	318.0 ± 52.0

[a–g] See notes in Table 1. [h] Inhibition percentage of parasite growth at a concentration of 100 nM.

Considering favorable substituents, activity could be improved by the replacement of an amide bond by secondary or tertiary amines. In the case of bulky substituents, secondary amines seemed to exhibit the highest antiprion activity, but these compounds were generally more toxic than their amide counterparts.

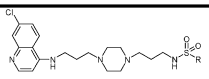
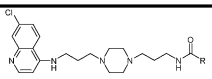
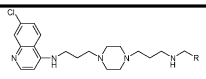
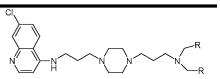
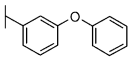
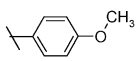
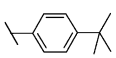
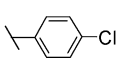
Table 4. Amines: Inhibition on PrP^{Sc} Formation in ScN2a Cells



cpd	R	antiprion effect			selectivity	MRC-5 toxicity[21]	selectivity	antimalarial effect[21]
		activity[a] (nM)	EC_{50}[b] (nM)	toxicity[c] (nM)	SI_1 = tox / EC_{50}	CC_{50}[f] (nM)	SI_2 = CC_{50}/EC_{50}	IC_{50}[g] (nM)
42		150	50	500	10	1.000	20	19.8 ± 1.3
43		250	90	2500	27.8	1.000	11	11.4 ± 0.9
44		500	250	5000	20	4.000	16	260.5 ± 16.2
45		250	175	1000	5.7	1.000	5.7	62.8 ± 8.4
46		750	300	2000	6.7	4.000	13.5	215.0 ± 46.4
47		1000	750	5000	6.7	5.600	7.5	142.1 ± 8.5

[a−g] See notes in Table 1.

Table 5. Antiprion Activity Comparison between Series S, A, B, and C (in nM). SI_1 and SI_2 Are Selectivity Indices Calculated as the Ratio between Toxicity and Activity (see Tables 1−4)

	Series S				Series A				Series B				Series C			
R																
	ref	EC_{50}	SI_1	SI_2	ref	EC_{50}	SI_1	SI_2	ref	EC_{50}	SI_1	SI_2	ref	EC_{50}	SI_1	SI_2
					**32**	250	8	66					**47**	750	6.7	7.5
					**35**	500	10	41	**43**	90	27.8	11	**45**	100	5.7	5.7
	**13**	100	5	10	**24**	80	12.5	50					**46**	300	6.7	13.5
					**29**	200	-	25	**42**	50	10	20	**44**	250	20	16
	**10**	75	-	52	**26**	100	-	40								
	**9**	75	13.35	13.35	**25**	100	10	-								
	**16**	250	-	-	**27**	100	-	10								

Following this reasoning, a schematic view of the construction of maximal antiprion potency is given in Figure 6 for amine-, amide-, and sulfonamide-containing quinoline derivatives.

Toxicity is an important parameter to take into account for the selection of the most promising compounds. For that purpose, the selectivity index SI was calculated as the ratio

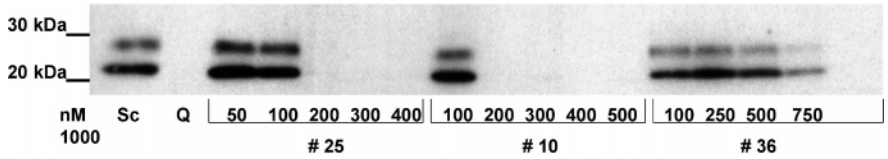


Figure 5. Western blot of protease-digested ScN2a cell lysates depicting the presence or absence of prions (PrP^{Sc}) after treatment with different compounds. Treatment of ScN2a cells with different concentrations of **25**, **10**, and **36** (as indicated) for 7 days and then left without treatment and weekly splitting for 3 weeks. Control cells were untreated (Sc, negative control) or treated with an antiprion effective concentration of quinacrine (Q, 1μM; positive control). Antibody: affinity-purified chicken α-mouse PrP. Lack of PrP^{Sc} after treatment demonstrated cure of cells from prions. **25** and **10** cure the cells at a concentration of 200 nM whereas the concentration of **36** needs to be 1000 nM.

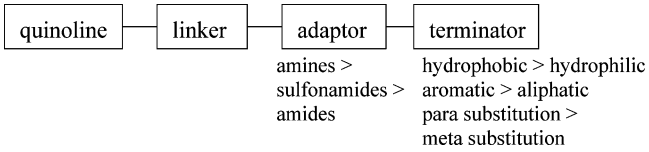


Figure 6. Antiprion-SAR of quinoline derivatives.

between toxicity and activity for each compound. A high SI indicates a high therapeutic potential and compounds exhibiting a SI higher than 10 may generally be considered good candidate compounds. For the present data, we calculated two selectivity indexes, one comparing antiprion activity with toxicity in ScN2a cells (SI_1), the other with toxicity in MRC-5 cells (SI_2). Compounds with a SI_1 superior than 20 were compounds **8**, **12**, **43**, and **44** and compounds with a SI_2 superior than 20 were compounds **7**, **8**, **10**, **11**, **12**, **24**, **26**, **29**, **30**, **32**, and **42**. The most interesting compounds were **7**, **8**, **10**, **12**, **24**, **32**, and **34** ($SI_2 > 50$). For comparison, SI_1 (CQ) was higher than 2.5 and SI_2 (CQ) was 12.5.

Discussion

We have identified new antiprion lead compounds after screening a limited library of polyquinoline and quinoline compounds. These compounds have a 10 to 100 fold increase in antiprion activity compared to the starting compound chloroquine that was selected due to its favorable BBB permeability and relative lack of serious side effects. Several of these compounds have an EC_{50} below 100 nM and an SI above 20 making them useful as new antiprion pharmaceutical lead compounds. By comparison, quinacrine, a previous antiprion lead compound currently undergoing evaluation in clinical trials, showed an EC_{50} of 300 nM and an SI of 20.[12]

Chloroquine had originally been synthesized as an antimalaria drug during World War II[26] and since then has been derivatized to more active compounds with less side effects. The occurrence of chloroquin-resistant *Plasmodium* strains has urged the development of novel antimalarial compounds. The present derivatized quinoline library had also been screened for antimalarial activity by their ability to inhibit parasite growth at 100 nM using a semiautomated microdilution technique; for cell-infection in vitro, the CQ-resistant strain FcB1 was chosen that also infects red blood cells.[27,28] Compounds displaying an inhibition percentage above 80% were selected for further pharmacological characterization. Using this assay and selecting candidate compounds by their favorable selectivity indices, we later on also demonstrated in vivo antimalarial activity of these compounds.[21]

CQ is believed to exert its antimalarial activity by inhibiting hemozoin formation in the acidic digestive vacuole of the parasite *Plasmodium*. CQ resistance likely involves several mechanisms, among others mutations of the vacuolar *Plasmodium falciparum* chloroquine-resistance transporter (PfCRT). However, reversal of CQ resistance by molecules such as verapamil and chlorpromazine suggests that an active CQ efflux by multidrug resistant mechanism may significantly contribute to CQ resistance. This is why we originally designed and synthesized the polyquinoline derivatives (series Q) as bulky derivatives of CQ that would hamper active efflux by PfCRT. In fact, compound **8** was demonstrated to be four times more active than CQ upon FcB1 strain.[19]

The introduction of a piperazine linker in the structure led to compounds with similar high antimalarial activity irrespective of the degree of CQ resistance of *Plasmodium falciparum* strains. The compounds of series S, A, B, and C were designed to accumulate in higher concentrations in the *Plasmodium's* food vacuoles than CQ. Although some compounds displayed a CQ-type mechanism of action, some of them were shown to have a cytosolic site of action being different to that of CQ (fluorescent assays[20]).

The antiprion activity of antimalarial compounds was dicovered by pure coincidence. For instance, quinacrine was initially chosen as a candidate drug due to its historical relation to the phenothiazines,[12] and to its known lysosomotropic effects.[15] When comparing the antiprion activity with the antimalarial activity of the present quinoline derivatives, we were surprised to see similar SAR for the quinoline derivatives even though the EC_{50} for the antimalarial effects was in average 5−10 times stronger than the EC_{50} for the antiprion effects. While there were clear similarities of half-maximum activities with statistical trends of a correlation for series A, B, and C, a true positive correlation could only be calculated for the sulfonamides ($\rho_S = 0.60$, $P = 0.038$; Spearman's rank correlation test). Here, the overlap between the most active compounds regarding their activities against both malaria and prions in vitro (e.g. **1** and **5**) and the overlap of inactive compounds (e.g. **11** and **13**) was statistically significant.

At this stage, there is no single molecular mechanism known or likely to mediate both the antimalarial and antiprion effects of the polyquinoline derivatives. The molecular target for the antimalarial effects of the 4-aminoquinoline family is supposed to be at least partially a CQ-type mechanism, i.e., a mechanism involving inhibiting hemozoin formation in the acidic digestive vacuole of *Plasmodium*. The corresponding cell compartment in mammals, the lysosome, is also the target of the quinacrine and other heterocyclic antiprion compounds.[12,15] A specific molecular target for the antiprion effects of the heterocyclic compounds is not known; however, their antiprion potency can be sufficiently explained by their cellular cholesterol-redistributing effects causing destabilization of conversion-mandatory detergent-resistant membrane domains, or "lipid rafts".[29] Cholesterol redistribution induced by the heterocyclic compounds was mainly from plasma membrane into endosomal/lysosomal cell compartments. Of note, lipid rafts on *Plasmodium*-infected erythrocytes have also been demonstrated to be essential for *Plasmodium* infection,[30] indicating that disturbance of cellular cholesterol distribution may also lead to impaired *Plasmodium* infection of erythrocytes. However, it is currently not established

in how far an effect of quinolines on cholesterol metabolism in erythrocytes contributes to their antimalarial effects.

In summary, this study shows that there are interesting and unexpected common SARs between antimalarial and antiprion activities of the 4-aminoquinoline family of pharmaceuticals. Among the compounds evaluated for antiprion activity, 25 compounds were more active than quinacrine, the previous lead compound, and at least four of them exhibited a SI superior to that of quinacrine, making them candidates for further in vivo studies. A possible overlap of subcellular targets of action of the antiprion and antimalarial mechanisms could be narrowed down to the acidic organelles or endosomal/lysosomal compartments and, eventually, downstream molecular targets of lipid metabolism. It may be useful to determine if the two bioassays offer some predictability on each other's outcome, at least concerning particular derivatives of chloroquine, i.e., if antimalarial activity in vitro could also predict antiprion activity in vitro, or vice versa. This might entail practical consequences for drug development that could take advantage of SARs in each field.

Experimental Section

Chemistry. All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) using UV light as a visualizing agent. Thick-layer chromatography (TLC) was performed using silica gel from Merck, from which the compounds were extracted by the following solvent system: CH_2Cl_2/MeOH/NH_4OH, 75:25:4. All melting points were determined on a Büchi melting point apparatus and were uncorrected. ^1H and ^{13}C NMR spectra were obtained using a Bruker 300 MHz spectrometer, and chemical shifts (δ) were expressed in ppm relative to TMS used as an internal standard. Mass spectra were recorded on a MALDI-TOF Voyager-DE-STR spectrometer. The purity of final compounds was verified by two types of high-pressure liquid chromatography (HPLC) columns: C18 Deltapak (C18N) and C4 Interchrom UP5WC4−25QS (C4). Analytical HPLC was performed on a Shimadzu system equipped with a UV detector set at 254 nm. Compounds were dissolved in MeOH/water or ethyl acetate and injected through a 50 μL loop. The following eluent systems were used: A (H_2O/TFA, 100:0.05) and B (CH_3CN/H_2O/TFA, 80:20:0.05). HPLC retention times (HPLC t_R) were obtained, at flow rates of 1 mL/min, using the following conditions: a gradient run from 100% eluent A during 1 min, then to 100% eluent B over the next 30 min. 4,7-dichloroquinoline was obtained from Acros, HBTU and HOBt from Novabiochem, and other reagents from Acros, Aldrich, Lancaster, and Avocado.

5-Dimethylamino-naphthalene-1-sulfonic Acid [7-(7-Chloro-quinolin-4-ylamino)-heptyl]-amide (21). A solution of 4,7-dichloroquinoline (1.0 g, 5.05 mmol), 1,7-diaminoheptane (1.97 g, 15.1 mmol), and DIEA (2.8 mL, 15.1 mmol) in 5 mL of 1-pentanol was refluxed for 18 h and then cooled to room temperature. Reaction media was diluted with 50 mL of CH_2Cl_2. Then the mixture was washed with 10% NaOH (3 × 50 mL). The organic layer was separated and dried over $MgSO_4$, the solvent evaporated, and the residue purified by flash chromatography (CH_2Cl_2/MeOH/NH_4OH: 80/20/0.5) to yield the amino intermediate 22 (1.17 g, 80% yield) which was characterized by HPLC, NMR, and MALDI-MS.

To a solution of **22** (150 mg, 0.51 mmol) in 4 mL of dry CH_2Cl_2 were added DIEA (0.22 mL, 1.24 mmol) and dansyl chloride (223 mg, 0.82 mmol). The mixture was stirred at room temperature for 4 h, diluted with 15 mL of CH_2Cl_2, and washed and dried over $MgSO_4$, the solvent evaporated, and the residue purified by TLC (CH_2Cl_2/MeOH/NH_4OH: 90/10/0.9) to yield the desired product (120 mg, 55% yield). R_f 0.7 (CH_2Cl_2/MeOH/NH_4OH: 90/10/0.9); MALDI-MS *m*/*z* 525.2 (MH^+).

Naphthalene-2-carboxylic Acid [7-(7-Chloro-quinolin-4-ylamino)-heptyl]-cyclopropyl methyl-amide (23). To a solution of 4121 (170 mg, 0.41 mmol) in 3 mL of dry CH_2Cl_2 were added the 2-naphthoic acid (107 mg, 0.62 mmol), HBTU (314 mg, 0.82 mmol), HOBt (112 mg, 0.82 mmol), and DIEA (361 mL, 2.05 mmol). After stirring the mixture at room temperature for 4 h, 15 mL of CH_2Cl_2 was added. Then the mixture was washed with aqueous 1 M $NaHCO_3$ (2 × 25 mL). The organic layer was separated and dried over $MgSO_4$, the solvent evaporated, and the residue purified by TLC (CH_2Cl_2/MeOH: 90/10) to yield the desired product (132 mg, 60%). R_f 0.15 (CH_2Cl_2/MeOH: 90/10); MALDI-MS *m*/*z* 570.3−572.3 (MH^+).

General Procedure for the Synthesis of Compounds 25, 28, 38. To a solution of **40**[21] (150 mg, 0.41 mmol) in 3 mL of dry CH_2Cl_2 were added the appropriate carboxylic acid (0.62 mmol), HBTU (314 mg, 0.82 mmol), HOBt (112 mg, 0.82 mmol), and DIEA (361 μL, 2.05 mmol). After stirring the mixture at room temperature for 4 h, 15 mL of CH_2Cl_2 was added. Then the mixture was washed with aqueous 1 M $NaHCO_3$ (2 × 25 mL). The organic layer was separated and dried over $MgSO_4$, the solvent evaporated, and the residue purified by TLC to yield the desired product.

4-*tert*-Butyl-*N*-(3-{4-[3-(7-chloro-quinolin-4-ylamino)-propyl]-piperazin-1-yl}-propyl)-benzamide (25). Prepared from 4-tert-butylbenzoic acid (108 mg, 0.62 mmol) and obtained after TLC (CH_2Cl_2/MeOH/NH_4OH: 90/10/0.9) as a white solid (139 mg, 65% yield). R_f 0.35 (CH_2Cl_2/MeOH/NH_4OH: 90/10/0.9); MALDI-MS *m*/*z* 522.5−524.4 (MH^+).

9*H*-Xanthene-9-carboxylic Acid (3-{4-[3-(7-Chloro-quinolin-4-ylamino)-propyl]-piperazin-1-yl}-propyl)-amide (28). Prepared from xanthene-9-carboxylic acid (140 mg, 0.62 mmol) and obtained after TLC (CH_2Cl_2/MeOH/NH_4OH: 90/10/0.9) as a yellow solid (136 mg, 58% yield). R_f 0.20 (CH_2Cl_2/MeOH/NH_4OH: 90/10/0.9); MALDI-MS *m*/*z* 570.3−572.3 (MH^+).

***N*-(3-{4-[3-(7-Chloro-quinolin-4-ylamino)-propyl]-piperazin-1-yl}-propyl)-isonicotinamide (38).** Prepared from isonicotinic acid (51 mg, 0.62 mmol) and obtained after two two successive TLC (CH_2Cl_2/MeOH/NH_4OH: 90/10/0.9) as a white powder (86 mg, 25% yield). R_f 0.10 (CH_2Cl_2/MeOH/NH_4OH: 90/10/0.9); MALDI-MS *m*/*z* 467.2−469.2 (MH^+).

PrPSc Inhibition Assay in ScN2a Cells. Neuroblastoma cells were infected with the RML strain of mouse-adapted scrapie prions and subcloned.[23] 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 L-glutamine. Medium was exchanged every second day, together with the drug. Cells were lysed (lysis buffer: 10 mM Tris, pH 8.0,150 mM NaCl, 0.5% Triton X-100, 0.5% deoxycholate) on the seventh day, having achieved 80−100% confluency.

Western Blotting. Cell lysates were digested with proteinase K at 20 μg/mL for 30 min at 37 °C. The reaction was stopped with 2 mM PMSF, and the lysates were centrifuged for 45 min at 100 000*g*. Pellets were resuspended in sample buffer and SDS/PAGE/immunoblotting was performed according to standard techniques. Immunoblots were incubated with chicken α-PrP-antibody at 1:2000 dilution (see below). Horseradish peroxidase (HRP)-conjugated anti-chicken secondary antibody (Pierce) was used at 1:25000 dilution, and the blots were developed by chemoluminescence (ECL, Amersham).

Isolation and Affinity Purification of Chicken α-MousePrP Polyclonal Antibody. IgY isolation: Eggs were collected from chickens immunized with recombinant mouse PrP (recMoPrP). RecMoPrP had been produced as previously described.[31] Yolks were diluted 1:5 in cold 20 mM NaAc pH 5.2 and left overnight at 4 °C. After removing insoluble material (20.000 × *g*, 20 min), IgY was salted out by addition of 20% (w/v) $(NH_4)_2SO_4$ and pelleted after 2 h at 4 °C (20.000*g*, 20 min). IgY was then resolubilized in 20 mM Tris pH 8, 150 mM NaCl, 0.1% Tween-20, 1 mM EDTA.

Affinity purification: recMoPrP was diluted to 0.5 mg/mL in a suspension of NHS-activated Sepharose (Amersham Pharmacia) prewashed according to manufacturer's protocol) in cold 50 mM $NaHCO_3$ pH 8.3, 1% Triton X-100, 20% DMSO, at 3 mg protein per mL resin. This suspension was stirred overnight at 4 °C, and free NHS groups were subsequently blocked with 50 mM glycine

(1 h, room temperature). The resin was then washed consecutively with 50 mM Tris pH 8, 50 mM glycine pH 3, and finally 20 mM Tris pH 8, 150 NaCl, 0.2% Triton X-100, 0.2% Tween-20, 2 mM EDTA. In this buffer, immobilized recMoPrP was mixed with crude α-MoPrP IgY isolate (approximately 200 mg IgY per mg MoPrP) and stirred overnight at 4 °C. The resin was then collected and washed extensively, followed by elution with 100 mM glycine pH 3, 1 M NaCl, 1% Triton X-100 (pH adjusted to 8 immediately afterward). This antibody recognized the un- and monoglycosylated form of PrP better than the diglycosylated one.

Statistical Computing. EC_{50}s of antiprion and antimalarial activities of substance subgroups were correlated using Standard package statistical software 11.0.2 (SPSS Inc, Chicago, IL). Since for noneffective concentration ranges, no precise values were experimentally obtained, and antiprion and antimalarial EC_{50}s within and between groups were not equally distributed, Spearman's rank correlation test rather than Pearson's correlation test was used. Rho (ρ_S) denominates the degree of correlation (from 0 [no correlation] to 1 [maximal correlation]), whereas the *P*-value (*P*) indicates the probability that the correlation was achieved by chance (*t*-test; a *P*-value <0.05 is considered statistically significant). Spearman's rank correlation formula can be found in the Supporting Information.

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Supporting Information Available: Purity criteria, NMR data, and statistical procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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