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Short communication

Synthesis and anti-prion activity evaluation of aminoquinoline analogues

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ABSTRACT

Transmissible spongiform encephalopathies form a group of neurodegenerative diseases that affect humans and other mammals. They occur when the native prion protein is converted into an infectious isoform, the scrapie PrP, which aggregates, leading to neurodegeneration. Although several compounds were evaluated for their ability to inhibit this conversion, there is no effective therapy for such diseases. Previous studies have shown that antimalarial compounds, such as quinolines, possess anti-scrapie activity. Here, we report the synthesis and evaluate the effect of aminoquinoline derivatives on the aggregation of a prion peptide. Our results show that 4-amino-7-chloroquinoline and *N*-(7-chloro-4-quinolinyl)-1,2-ethanediamine inhibit the aggregation significantly. Therefore, such aminoquinolines might be considered as candidates for the further development of therapeutics to prevent the development of prion diseases.

1. Introduction

The prion protein (PrP) is an unusual infectious pathogen that is responsible for neurodegenerative diseases that affect humans and other animals – the transmissible spongiform encephalopathies [1]. Conversion of the innocuous, native PrP (PrP^C, which is rich in α-helical structures) into an abnormal isoform, the PrP scrapie (PrP^{Sc}, which is rich in β-sheet structures) is the most important step leading to the development of these diseases [1]. PrP^C is soluble and does not aggregate. In contrast, PrP^{Sc} is protease-resistant, highly insoluble in aqueous solution and aggregates readily [2]. *In vitro*, *ex vivo* and transgenic animal studies suggest that the conversion is aided by other biological molecules that lower the energetic barrier preventing the spontaneous conversion of PrP^C to PrP^{Sc}, acting as cofactors [3–6]. Among such cofactors, nucleic acids have been shown to interact with PrP^C, presenting both catalytic and protective effects on the conversion of PrP into a scrapie-like conformation (depending on the PrP:NA molar ratio) [4,6]. Despite huge efforts from the scientific community, there is no effective therapy to prevent or reduce the progression of the disease to date.

A great variety of compounds and also synthetic peptides have been evaluated for their ability to prevent the conversion of PrP^C to PrP^{Sc} and to inhibit PrP aggregation [7–9]. Polyamines [10], sulfated glycans [11,12] and cyclic tetrapyrroles, such as porphyrins [13], are effective *in vitro*. However, not all of these presented prophylactic activity *in vivo* [14]. Several antimalarial compounds, such as acridines and quinolines, were also investigated as effective inhibitors of proteinase-resistant PrP forms in neuroblastoma cells (N2a) that had been infected with PrP^{Sc} [8,15–19]. One antimalarial drug, mefloquine (a quinoline compound) showed high activity against scrapie agent-infected cells [18]. However, when administered to mice that had been inoculated with a scrapie strain, it did not delay the onset of clinical symptoms [18]. Quinacrine, an important inhibitor [16], did not increase survival in a murine model of prion disease [20,21], and high doses of this compound lead to liver damage without therapeutic efficacy in humans affected with Creutzfeldt-Jakob disease [22]. A recent observational study reported that 300 mg per day of quinacrine was tolerated reasonably, but it did not affect the clinical course of the prion disease significantly [23]. Other antimalarials that have been proposed for prion therapy include the 4- and 8-aminoquinolines. The results presented so far indicate that at least two fused aromatic rings are necessary for PrP binding and anti-scrapie activity [16,17,24]. In a recent high-throughput assay that evaluated PrP^{Sc} reduction in prion-infected neuroblastoma cell lines (ScN2a), 2-aminothiazoles were identified

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as potent anti-prion compounds [25]. However, the mechanism of action of all the cited compounds has not yet been understood fully.

In view of these prior results, we studied the effect of novel 4-aminoquinoline derivatives (Table 1) on the aggregation of PrP. The selected compounds act as antimalarials, and inhibit the spontaneous formation of hemozoin (a malarial pigment that is generated upon heme polymerization in the *Plasmodium*) [26]. The mechanism of antimalarial action of such compounds is believed to operate via direct interaction with heme, blocking the formation of hemozoin and leading to free heme accumulation in the food vacuole of *Plasmodium*. This results in the death of the parasite, as free heme is toxic to these infectious agents [27]. We investigated compounds that contain a chlorine atom at position 7 on the quinoline ring in this work, as it was verified previously that this substitution plays an important role in the inhibition of hemozoin crystal formation. The substitution is, however, not a determinant for the binding of the compound to heme [28].

2. Chemistry

The compounds 4-amino-7-chloroquinoline (**1**), 7-chloro-4-methylaminoquinoline (**2**), 2-(7-chloro-4-quinolinyl)-aminoethanol (**3**), *N*-(7-chloro-4-quinolinyl)-1,2-ethanediamine (**4**), N^2-(7-chloro-4-quinolinyl)-N^1,N^1-dimethyl-1,2-ethanediamine (**5**) and 4-(cyclopentylamino)-7-chloro-quinoline (**7**) were synthesized as reported previously [28,29]. Compound **6**, N^2-(7-trifluoromethylthio-4-quinolinyl)-N^1,N^1-diethyl-1,2-ethanediamine, is a new aminoquinoline derivative and was prepared using a six-step synthesis, as described in the Experimental section.

3. Results

3.1. ShaPrP peptide aggregation is inhibited by 4-aminoquinolines

To verify whether the aminoquinolines investigated in this work would modulate the aggregation of prion peptides, we evaluated the aggregation of the peptide in the presence of the selected compounds. To perform the aggregation kinetic assays, we chose a model peptide encompassing the Syrian hamster PrP (ShaPrP) residues 109–149 ($PrP^{109-149}$). This domain corresponds to a loop and the first α-helix of the N-terminal region of PrP, which is implicated in the β-sheet conversion [30,31]. It was previously shown that this PrP domain promptly aggregates when diluted in aqueous solutions under denaturing conditions [4,32]. Briefly, we monitored the aggregation of the peptide by the increase in light scattering at 450 nm as a function of time after dilution in MES buffer at pH 5.0. On dilution in aqueous buffer, aggregation occurs within a few seconds (Fig. 1) and is dependent on the concentration of the peptide [4,32]. We tested whether the compounds could inhibit aggregation of the prion peptide. The peptide is maintained in a 6 M urea stock solution at pH 5.0; in this condition, it is partially unfolded and does not aggregate [4]. To study the aggregation kinetics, we diluted the peptide in buffer containing increasing concentrations of the compounds and monitored the aggregation by following the changes in light scattering as a function of time.

Our results show that 4-amino-7-chloroquinoline (**1**) and *N*-(7-chloro-4-quinolinyl)-1,2-ethanediamine (**4**) inhibit the aggregation of $ShaPrP^{109-149}$ significantly (Table 1). Both compounds lead to ~90% inhibition at 1.0 μM. At the same concentration, 7-chloro-4-methylaminoquinoline (**2**) and N^2-(7-trifluoromethylthio-4-quinolinyl)-N^1,N^1-diethyl-1,2-ethanediamine (**6**) inhibited the aggregation by 60% and 50%, respectively. No significant inhibition of aggregation was mediated by the other 4-aminoquinolines at the same concentration (1.0 μM) (Fig. 2). Compounds **1** and **4** were the most active compounds against prion peptide aggregation, and these inhibited the aggregation by 50% at approximately 0.2 and 0.1 μM, respectively (Table 1). These values are somewhat better than those obtained using the antimalarial drug, mefloquine, which inhibits the aggregation by 50% at ~1 μM [18]. However, the activity of mefloquine was evaluated in cells that expressed PrP scrapie, and the compounds that we tested might also have different inhibitory concentrations in this model. Using fluorescence anisotropy measurements (Fig. 3), we observed that compounds **1** and **4** were able to bind to the prion domain at the concentrations that we tested.

3.2. Inhibitory 4-aminoquinolines reduce the aggregation of the ShaPrP peptide into amyloid-like structures

We also evaluated the binding of the fluorescent probe thioflavin T (Thio-T) to the peptide in the presence or absence of the aminoquinolines. Thioflavin T is a benzothiazole dye that is commonly used to diagnose amyloid fibrils [33,34]. When free in aqueous solution, its fluorescence emission is negligible. However, on binding to highly ordered, beta-sheet-rich structures, such as amyloid fibrils, its fluorescence emission at 482 nm is increased markedly [33,34]. We observed that Thio-T binds to the peptide (in the absence of aminoquinolines or urea) which aggregates, forming amyloid-like structures. The binding of Congo red was also evaluated, confirming the results obtained with Thio-T (not shown).

To monitor whether the inhibition of aggregation that is mediated by compounds **1** and **4** was related to a decrease in the formation of such structured aggregates, we incubated Thio-T with $ShaPrP^{109-149}$ in the presence of the selected aminoquinolines and measured its fluorescence emission (Fig. 4). Thio-T binding was reduced significantly in the presence of compounds (**1**), (**2**), (**3**), (**4**) and (**7**) (Fig. 4). However, Thio-T binding was reduced more for compounds **1** and **4**, which were the only aminoquinolines that inhibited $ShaPrP^{109-149}$ aggregation significantly at 1.0 μM. All of the data was corrected for the emission of Thio-T in buffer alone, and in the presence of the aminoquinolines but not the peptide.

4. Discussion

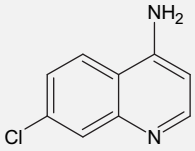
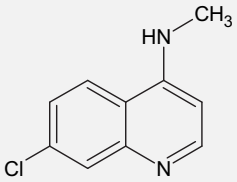
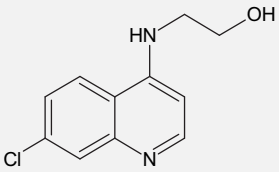
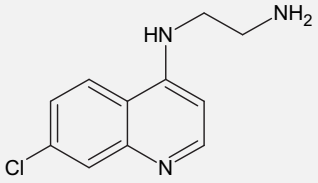
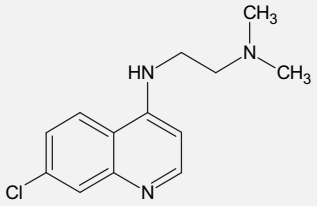
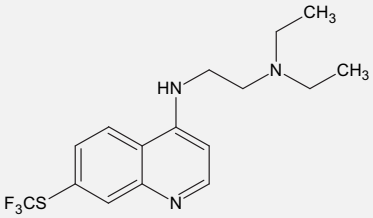
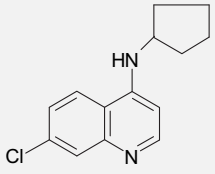
The search for compounds that stabilize cellular PrP or prevent its conversion into PrP^{Sc} remains important for the control of such devastating diseases, where the formation of PrP^{Sc} is characteristic of the transmissible spongiform encephalopathies. Therefore, the identification of compounds that inhibit PrP aggregation *in vitro* is an initial step in testing for *in vivo* and *ex vivo* anti-scrapie activity.

Here, we have investigated the effect of novel 4-aminoquinolines on the aggregation profile of a prion hydrophobic domain. Evaluating the aggregation kinetics of this peptide (which corresponds to part of the N-terminal PrP domain and to the first alpha-helix of the Syrian hamster prion protein) [30,31] is a valuable tool for the study of anti-aggregating compounds.

Several approaches to developing new drugs for prion disease therapy involve screening drugs that are already marketed for other applications. The advantage is that this can bypass some clinical stages in drug approval. Most methods for such screening are based on the "curative" effect on cell lines that are naturally scrapie-infected [8,10,11,16–19] i.e., by evaluation of the loss of proteinase-K resistance of PrP extracted from the cells when treated with an effective compound.

Some antimalarial compounds have been evaluated for anti-scrapie activity and were found to be effective inhibitors of proteinase-resistant forms of PrP in neuroblastoma cells (N2a) that were infected with PrP^{Sc} [8,16–19]. However, when tested *in vivo*, the results were not as satisfactory as hoped. One promising compound, quinacrine [16], showed no efficacy in a murine model of prion disease [20,21]. When administered to humans suffering from

Table 1
The anti-aggregating activity of 4-aminoquinolines.

	Compound		% Aggregation with 1 μM	$C_{50\%}$[a]
(**1**)		4-amino-7-chloroquinoline	8.4%	~0.2 μM
(**2**)		7-chloro-4-methylaminoquinoline	38.6%	>0.9 μM
(**3**)		2-(7-chloro-4-quinolinyl)-aminoethanol	97%	>1 μM
(**4**)		*N*-(7-chloro-4-quinolinyl)-1,2-ethanediamine	7.9%	~0.1 μM
(**5**)		N^2-(7-chloro-4-quinolinyl)-N^1,N^1-dimethyl-1,2-ethanediamine	82%	>1 μM
(**6**)		N^2-(7-trifluoromethylthio-4-quinolinyl)-N^1,N^1-diethyl-1,2-ethanediamine	54%	>1 μM
(**7**)		4-(cyclopentylamino)-7-chloro-quinoline	88%	>1 μM

[a] The concentration of the compound where 50% of aggregation was achieved for the peptide at 0.5 μM final concentration.

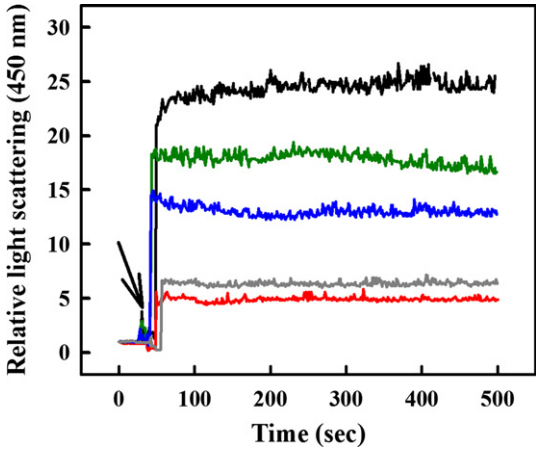


Fig. 1. The inhibition of PrP peptide aggregation by 4-amine-7-chloroquinoline (**1**), as monitored by changes in the light scattering (LS). The aggregation was followed by measuring the LS (excitation 450 nm, emission 450 nm). We diluted ShaPrP$^{109-149}$ that had been previously unfolded in a 6 M urea solution at pH 5.0 to a final concentration of 0.5 μM in 50 mM MES buffer, in the absence or presence of increasing concentrations of compound **1**. The light scattering of the solutions was monitored as a function of time. The graph shows the aggregation of the peptide in the absence of compound **1** (black trace); in 6 M urea (red trace/dashed black trace); with 0.1 μM (**1**) (green trace/dark gray trace), with 0.25 μM (**1**) (blue trace/light gray trace) and with 1.0 μM (**1**) (gray trace/dashed gray trace). The arrow indicates the moment at which the peptide was diluted in the buffer, and this was ~50 s after initiating acquisition of the baseline. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Creutzfeldt-Jakob disease, no therapeutic efficacy was obtained – even when the subjects were treated with high doses [22]. Therefore, identifying and developing new anti-prion disease drugs that would be more effective and less toxic is important. Although the mechanism of action of most anti-prion compounds has not yet been understood, an interesting report showed that two structurally unrelated anti-prion drugs, 6-aminophenanthridine and guanabenz, inhibit the RNA-mediated ribosomal protein folding activity, without affecting protein synthesis [35]. Both compounds interact with ribosome in an RNA-dependent manner [35], evidencing the possible role of RNA in prion physiopathology [6].

We have identified two novel 4-aminoquinolines (compounds **1** and **4**) that inhibited the aggregation of a ShaPrP peptide

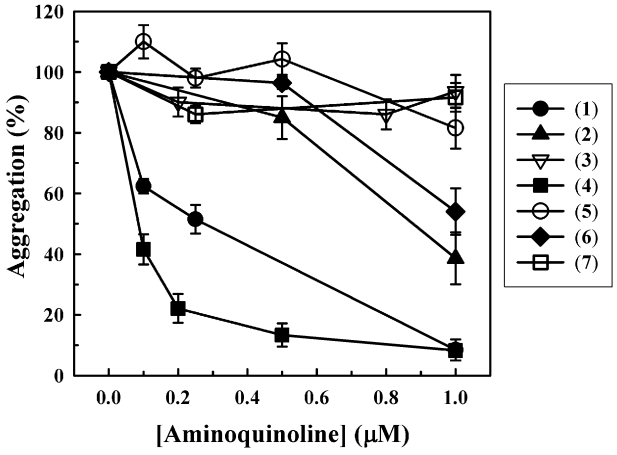


Fig. 2. The extent of inhibition of the aggregation is shown for compounds (**1**), (**2**), (**3**), (**4**), (**5**), (**6**) and (**7**). The maximum light scattering (at 450 nm) obtained from each kinetic trace was measured and normalized relative to the control (peptide aggregation in the absence of urea or the compounds), which was taken to represent 100% aggregation (0% inhibition). This was taken as the highest LS value when the peptide was diluted in 6 M urea. Each data point is the mean of three independent measurements (the means ± S.E.M.).

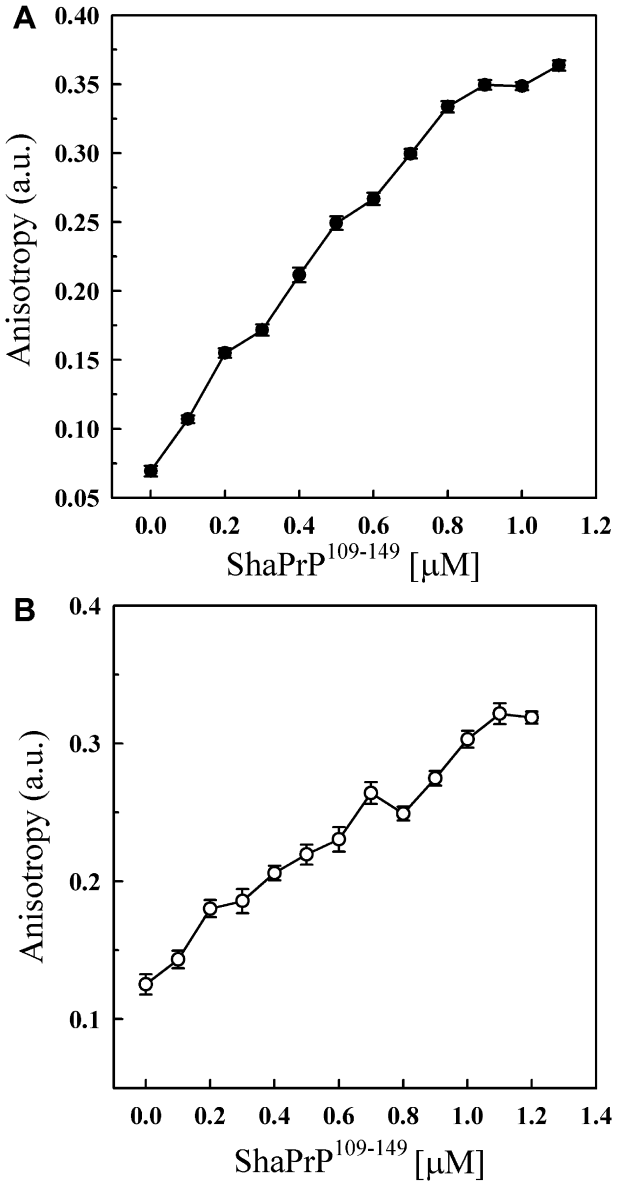


Fig. 3. Evaluation of the binding of ShaPrP$^{109-149}$ to the quinoline compounds using fluorescence anisotropy. ShaPrP$^{109-149}$ was titrated into a solution containing the quinoline compounds (**1**) or (**4**) at pH 5.0, and the fluorescence anisotropy of the compounds was recorded. A) 4-amino-7-chloroquinoline (**1**) at 0.5 μM; excitation: 360 nm, emission: 410 nm; B) *N*-(7-chloro-4-quinolinyl)-1,2-ethanediamine (**4**) at 0.5 μM; excitation: 390 nm, emission: 450 nm.

significantly. This raises the possibility that they might serve as lead compounds with anti-prion activity.

A detailed structure-activity analysis is not possible, given the small number of quinolines that were investigated in this study. However, several features are worth noting. The most active compounds (**1** and **4**) contain primary amino groups. Interestingly, the activity does not appear to relate to the basicity of the amine, as the primary alkyl amine **4** is much more basic than the aromatic primary amine **1**. While this might suggest that the hydrogen bonding ability of the group is required for its activity, the effect appears to be more complex, as compound **3** (which contains an –OH group that can act as either a hydrogen bond donor or acceptor) is almost inactive. Attaching a methyl group to the aromatic amino function results in intermediate activity, whereas the much more bulky cyclopentyl group almost abolishes the activity. Finally, the intermediate activity of **6** (relative to

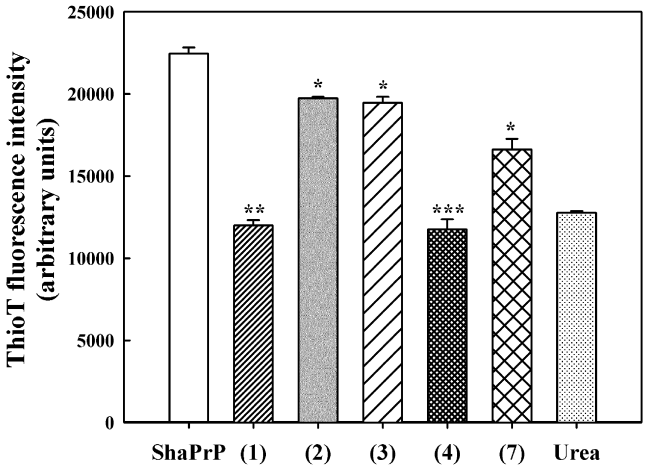


Fig. 4. The binding of thioflavin T to ShaPrP$^{109-149}$ is reduced in the presence of compounds (**1**) and (**4**). ShaPrP$^{109-149}$ (1.0 μM) was incubated with the selected aminoquinolines (10 μM) and Thio-T was added after 10 min at a final concentration of 20 μM. The excitation was set at 450 nm and the emission was recorded from 470 to 520 nm. The plot shows the area of the Thio-T fluorescence emission spectra (in the presence of ShaPrP$^{109-149}$) after subtraction of the probe emission at the same conditions and without the addition of peptide. Each data point is the mean of three experiments (the means ± S.E.M.). *, ** and ***, denote significant differences ($^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, respectively).

the virtual inactivity of **5**) hints that changes at the 7-position on the quinoline ring may affect the activity. It is noteworthy that the $-SCF_3$ group in **6** is considerably more electron-withdrawing than the −Cl group present in **5**. However, the investigation of a substantially larger set of compounds would be needed to confirm these apparent trends.

In conclusion, we believe that this new class of compounds shows promise for the future development of anti-scrapie compounds, and that assays in neuroblastoma cells that have been infected with PrP^{Sc} should be undertaken to address the pharmacology of such therapeutic candidates.

5. Experimental

5.1. Reagents and the peptide model

All the reagents used were of analytical grade. Thioflavin T was purchased from Sigma–Aldrich (St. Louis, MO, USA). The Syrian hamster prion protein peptide (109–149) was acquired from Genemed Synthesis, Inc. (San Antonio, TX, USA), where it was made using solid phase synthesis and purified by RP-HPLC (>90% purity). The identity of the peptide was confirmed using mass spectrometry, according to the Genemed Quality Control Certificate. The peptide was solubilized just before use in 50 mM MES (4-Morpholinoethanesulfonic acid) buffer at pH 5.0 with 6 M urea and 10 mM sodium dodecyl sulfate (SDS). The peptide stock solution was centrifuged at 10,000 rpm for 10 min, and the concentration of the supernatant was measured using a molar extinction coefficient of 12,490 M^{-1} cm^{-1} at 280 nm. This value was calculated from the amino acid sequence using the software ProtParam (http://us.expasy.org/tools/protparam.html). The sequence of the prion peptide was: 109 MKHMAGAAAAGAVVGGLGGWMLGSAMSRPMMH FGNDWEDRY 149.

5.2. The source or synthesis methods of the compounds

The synthesis of compounds 4-amino-7-chloroquinoline (**1**), 7-chloro-4-methylaminoquinoline (**2**), 2-(7-chloro-4-quinolinyl)-aminoethanol (**3**), *N*-(7-chloro-4-quinolinyl)-1,2-ethanediamine (**4**), N^2-(7-chloro-4-quinolinyl)-N^1,N^1-dimethyl-1,2-ethanediamine (**5**) has been reported in detail previously (in a description of their antimalarial activities) [28]. The synthesis of 4-(cyclopentylamino)-7-chloro-quinoline (**7**) has also been reported recently (in connection with its antischistosomal activity) [29]. Compound **6**, N^2-(7-trifluoromethylthio-4-quinolinyl)-N^1,N^1-diethyl-1,2-ethanediamine, is a new compound and was prepared using a six-step synthesis:

A mixture of 3-trifluoromethylthioaniline (1.0 g, 5.3 mmol) and diethyl methoxymethylene malonate (1.3 g, 5.8 mmol) was refluxed in ethanol (100 ml) for 3 h, after which the solvent was removed *in vacuo*. The resulting solid was recrystallized from ethanol and dried to give ethyl 2-ethoxycarbonyl-3-(3-trifluoromethylthioanilino)propenoate (**6a**), (1.9 g, 99%), mp 69 °C; δ_H (400 MHz, $CDCl_3$) 1.29 (3H, t, $J = 7.0$ Hz, CH_3), 1.32 (3H, t, $J = 7.0$ Hz, CH_3), 4.22 (2H, q, $J = 7.0$ Hz, CH_2), 7.15 (1H, m, Ar-H), 7.32 (3H, m, Ar-H), 8.38 (1H, d, $J = 13.6$ Hz, vinylic H), 10.97 (1H, bd, $J = 13.2$ Hz, NH); δ_C (100.6 MHz, $CDCl_3$) 14.2 (CH_3), 60.3 (CH_2), 60.6 (CH_2), 95.1 (C=CH), 119.2 (C-6), 124.4 (C-4), 126.4 (C-3), 129.3 (q, $J_{C\text{-}F} = 308.2$ Hz, SCF_3), 130.8 (C-5), 132.0 (C-2), 140.4 (C-1), 151.2 (C=CH), 165.5 (C=O), 168.9 (C=O); MS (HRMS), Found: *m/z* 363.07429, requires M^+: 363.07521; CHNS, Found: C, 49.40; H, 4.82; N, 3.87; S, 8.8%. Requires for $C_{15}H_{16}F_3NO_4S$: C, 49.5; H, 4.4; N, 3.8; S, 8.8%.

6a (1.0 g, 2.7 mmol) was refluxed in diphenyl ether (10 ml) for 30 min. The flask was opened during the first 10 min of the reaction to allow the ethanol that was formed to escape. When the reaction mixture was cooled to room temperature, a white precipitate formed. This was filtered and washed with petroleum ether, ethyl acetate and methanol to remove all diphenyl ether and other impurities to give ethyl 1,4-dihydro-4-oxo-7-(trifluoromethylthio) quinoline-3-carboxylate (**6b**) (0.6 g, 75%) after drying, mp 265–266 °C; ν_{max}/cm^{-1} (KBr) 3150, 1637 (CO); δ_H (200 MHz, d_6-DMSO) 1.30 (3H, t, $J = 7.2$ Hz, CH_3), 4.25 (2H, q, $J = 7.2$ Hz, CH_2), 7.60 (1H, d, $J = 4.6$ Hz, H-6), 7.98 (1H, s, H-8), 8.25 (1H, d, $J = 4.6$ Hz, H-5), 8.67 (1H, s, H-2); MS, Found: *m/z* 317, requires M^+: 317; CHNS, Found: C, 49.1; H, 3.1; N, 4.4; S, 9.7%. Requires for $C_{13}H_{10}F_3NO_3S$: C, 49.2; H, 3.1; N, 4.4; S, 10.1%.

A mixture of **6b** (0.9 g, 2.8 mmol) and 10% NaOH (10 ml) was heated at 120 °C for 2 h. During this time, all of the solid dissolved. The aqueous phase was then carefully neutralized with 10% H_2SO_4 to avoid the formation of the water-soluble quinolinium ion. A white solid precipitated, and this was filtered off and washed with cold water. The solid was dried over P_2O_5 under vacuum to yield 1,4-dihydro-4-oxo-7-(trifluoromethylthio)quinoline-3-carboxylic acid (**6c**) (0.5 g, 70%), mp 233–236 °C; ν_{max}/cm^{-1} (KBr) 2900, 1620; δ_H (300 MHz, d_6-DMSO) 7.80 (1H, dd, $J = 8.4$, 1.5 Hz, H-6), 8.16 (1H, d, $J = 1.5$ Hz, H-8), 8.37 (1H, d, $J = 8.4$ Hz, H-5), 8.98 (1H, s, H-2), 13.44 (1H, bs, COOH), 14.85 (1H, bs, Ar-OH); δ_C (75.5 MHz, d_6-DMSO) 108.5 (C-3), 125.6 (Ar-CH), 126.3 (C-5), 126.8 (C-8), 129.3 (q, $J_{C\text{-}F} = 308.3$ Hz, SCF_3), 129.5 (C-4a), 131.3 (C-6), 139.5 (C-8a), 146.3 (C-2), 165.7 (COOH), 177.8 (C-4); MS (HRMS), Found: *m/z* 289.00182; Requires for $C_{11}H_6F_3NO_3S$: 289.00205.

6c (0.8 g, 2.9 mmol) was added to diphenyl ether (8 ml) and the mixture was refluxed in a preheated oil bath. After 30 min, when the solid had entirely dissolved, the reaction mixture was allowed to cool. A precipitate formed, and this was filtered, and washed with petroleum ether and cold ethyl acetate to remove all diphenyl ether to yield 7-(trifluoromethylthio)quinoline-4-one (**6d**) (0.4 g, 68%), which was recrystallized from methanol and ethyl acetate, mp 214–215 °C; ν_{max}/cm^{-1} (KBr) 2943, 1630; δ_H (300 MHz, d_6-DMSO) 6.12 (1H, $J = 7.2$ Hz, H-3), 7.52 (1H, dd, $J = 8.4$, 1.2 Hz, H-6), 7.92 (1H, d, $J = 1.2$ Hz, H-8), 7.99 (1H, d, $J = 7.2$ Hz, H-2), 8.19 (1H, d, $J = 8.4$ Hz, H-5), 11.89 (1H, bs, Ar-OH); δ_C (100.6 MHz, d_6-DMSO) 109.6 (C-3), 125.6 (C-6), 126.6 (C-7/C-4a), 126.6 (C-7/C-4a), 126.7 (C-5), 128.7 (C-8), 129.4 (q, $J_{C\text{-}F} = 308.2$ Hz, SCF_3), 140.1 (C-8a), 140.2 (C-2), 176.1 (C-4); MS (HRMS), Found: *m/z* 245.01228; Requires for $C_{10}H_6F_3NOS$: 245.01222.

A mixture of **6d** (0.4 g, 1.4 mmol), phosphorous oxychloride (1 ml, 11.4 mmol) and phosphorous pentachloride (0.3 g, 1.6 mmol) was refluxed under N_2 at 120 °C for 30 min. The reaction mixture was then poured into cold aq. NaOH (1 M, 50 ml) and the white precipitate that formed as a result was filtered and washed with water. The solid was then dissolved in ethyl acetate (100 ml), dried ($MgSO_4$) and the solvent removed *in vacuo*. Recrystallization from petroleum ether gave 4-chloro-7-trifluoromethylthioquinoline (**6e**) (0.3 g, 88%), mp 69 °C; δ_H (400 MHz, $CDCl_3$) 7.57 (1H, d, $J = 4.7$ Hz, H-3), 7.83 (1H, dd, $J = 9.0$, 1.7 Hz, H-6), 8.28 (1H, d, $J = 9.0$ Hz, H-5), 8.48 (1H, d, $J = 1.7$ Hz, H-8), 8.85 (1H, d, $J = 4.7$ Hz, H-2); δ_C (75.5 MHz, $CDCl_3$) 122.8 (C-3), 122.9 (C-7), 125.7 (C-5), 127.6 (C-4a), 129.6 (q, $J_{C\text{-}F} = 308.6$ Hz, SCF_3), 133.2 (C-6), 138 (C-8), 142.9 (C-4), 149.0 (C-8a), 151.3 (C-2); MS (HRMS), Found: *m/z* 262.97808; Requires for $C_{10}H_5ClF_3NS$: 262.97833.

6e (0.2 g, 1.0 mmol) and *N,N*-diethylethylenediamine (3 ml) were heated together in a sealed tube under N_2 at 140 °C for 4 h. Aq. 10% NaOH (2 ml) was added to the product and the resulting yellow precipitate was then extracted into dichloromethane (3 × 100 ml). The solvent was dried ($MgSO_4$) and removed under reduced pressure to furnish a residue which was chromatographed on silica gel using methanol and dichloromethane (2:8) as the eluent to give **6** (0.4 g, 80%), mp 110–111 °C; δ_H (400 MHz, $CDCl_3$) 1.08 (6H, t, $J = 7.3$ Hz, 2× CH_3), 2.61 (4H, q, $J = 7.3$ Hz, 2× CH_2), 2.83 (2H, t, $J = 6.2$ Hz, CH_2 β), 3.28 (2H, q, $J = 6.2$ Hz, CH_2 α), 6.21 (1H, bs, NH), 6.43 (1H, d, $J = 5.1$ Hz, H-3), 7.61 (1H, dd, $J = 9.0$, 1.8 Hz, H-6), 7.72 (1H, d, $J = 9.0$ Hz, H-5), 8.30 (1H, d, $J = 1.8$ Hz, H-8), 8.58 (1H, d, $J = 5.1$ Hz, H-2); δ_C (100.6 MHz, $CDCl_3$) 12.3 ($CH_2\underline{C}H_3$), 39.9 (CH_2-α), 46.7 ($\underline{C}H_2CH_3$), 50.8 (CH_2-β), 100.3 (C-3), 120.3 (C-7), 121.2 (C-5), 125.2 (C-4a), 129.8 (q, $J_{C\text{-}F} = 308.1$ Hz, SCF_3), 130.3 (C-6), 138.5 (C-8), 148.7 (C-4), 149.9 (C-8a), 152.5 (C-2); MS (HRMS), Found: *m/z* 344.14095; Requires for $C_{16}H_{21}F_3N_3S$ (M + H): 344.14082.

5.3. Spectroscopic measurements

Light scattering and fluorescence anisotropy measurements were recorded on a PC1 spectrofluorometer (ISS, Champaign, IL) or on a Jasco FP 6300 (Jasco Corp., Tokyo, Japan) in the "L" geometry (at 90° in relation to the excitation light). The aggregation was monitored by measuring the light scattering (excitation 450 nm, emission 450 nm). For the aggregation kinetic assays, the peptide, which had previously been unfolded in 6 M urea supplemented with 10 mM SDS at pH 5.0, was diluted in buffer and the light scattering was monitored as a function of time. The maximum light scattering obtained from each kinetic trace was measured and normalized, considering the control to represent 100% aggregation. Zero percent aggregation was assumed for the LS value obtained when the peptide was diluted in 6 M urea, where it does not aggregate. For the aggregation kinetic assays, we diluted the peptide at least 100-fold in the test buffer. The binding to thioflavin T was evaluated by exciting the probe at 450 nm and recording the fluorescence emission from 470 to 520 nm.

5.4. Statistics

Statistical analyses were carried out using the program Sigma Plot (version 10.0). For the data presented in Figs. 2–4, we present the mean of triplicates, and the error bars indicate the standard error (SEM). For the thioflavin T binding assay (Fig. 4), we used a two-sample t-Test to determine the statistical differences between the control (100% aggregation) and the results that were obtained in the presence of the compounds ($^{*}p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.001$).

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References

[1] S.B. Prusiner, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 13363–13383.
[2] A. Aguzzi, M. Polymenidou, Cell 116 (2004) 313–327.
[3] G.C. Telling, M. Scott, J. Mastrianni, R. Gabizon, M. Torchia, F.E. Cohen, S.J. DeArmond, S.B. Prusiner, Cell 83 (1995) 79–90.
[4] Y. Cordeiro, F. Machado, L. Juliano, M.A. Juliano, R.R. Brentani, D. Foguel, J.L. Silva, J. Biol. Chem. 276 (2001) 49400–49409.
[5] S. Supattapone, J. Mol. Med. 82 (2004) 348–356.
[6] J.L. Silva, L.M.T.R. Lima, D. Foguel, Y. Cordeiro, Trends Biochem. Sci. 33 (2008) 132–140.
[7] S.A. Priola, A. Raines, W.S. Caughey, Science 287 (2000) 1503–1506.
[8] D.A. Kocisko, G.S. Baron, R. Rubenstein, J. Chen, S. Kuizon, B. Caughey, J. Virol. 77 (2003) 10288–10294.
[9] S. Sellarajah, C. Boussard, T. Lekishvili, D.R. Brown, I.H. Gilbert, Eur. J. Med. Chem. 43 (2008) 2418–2427.
[10] S. Supattapone, H.O. Nguyen, F.E. Cohen, S.B. Prusiner, M.R. Scott, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 14529–14534.
[11] B. Caughey, G.J. Raymond, J. Virol. 67 (1993) 643–650.
[12] R. Gabizon, Z. Meiner, M. Halimi, S.A. Ben-Sasson, J. Cell. Physiol. 157 (1993) 319–325.
[13] W.S. Caughey, L.D. Raymond, M. Horiuchi, B. Caughey, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 12117–12122.
[14] N.R. Cashman, B. Caughey, Nat. Rev. Drug Discov. 3 (2004) 874–884.
[15] K. Doh-ura, T. Iwaki, B. Caughey, J. Virol. 74 (2000) 4894–4897.
[16] C. Korth, B.C. May, F.E. Cohen, S.B. Prusiner, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 9836–9841.
[17] I. Murakami-Kubo, K. Doh-ura, K. Ishikawa, S. Kawatake, K. Sasaki, J. Kira, S. Ohta, T. Iwaki, J. Virol. 78 (2004) 1281–1288.
[18] D.A. Kocisko, B. Caughey, J. Virol. 80 (2006) 1044–1046.
[19] R. Klingenstein, P. Melnyk, S.R. Leliveld, A. Ryckebusch, C. Korth, J. Med. Chem. 49 (2006) 5300–5308.
[20] S.J. Collins, V. Lewis, M. Brazier, A.F. Hill, A. Fletcher, C.L. Masters, Ann. Neurol. 52 (2002) 503–506.
[21] A. Barret, F. Tagliavini, G. Forloni, C. Bate, M. Salmona, L. Colombo, L.A. De, L. Limido, S. Suardi, G. Rossi, F. Auvre, K.T. Adjou, N. Sales, A. Williams, C. Lasmezas, J.P. Deslys, J. Virol. 77 (2003) 8462–8469.
[22] P. Follette, Science 299 (2003) 191–192.
[23] J. Collinge, M. Gorham, F. Hudson, A. Kennedy, G. Keogh, S. Pal, M. Rossor, P. Rudge, D. Siddique, M. Spyer, D. Thomas, S. Walker, T. Webb, S. Wroe, J. Darbyshire, Lancet Neurol. 8 (2009) 334–344.
[24] H. Cope, R. Mutter, W. Heal, C. Pascoe, P. Brown, S. Pratt, B. Chen, Eur. J. Med. Chem. 41 (2006) 1124–1143.
[25] S. Ghaemmaghami, B.C.H. May, A.R. Renslo, S.B. Prusiner, J. Virol. 84 (2010) 3408–3412.
[26] T.J. Egan, J. Inorg. Biochem. 91 (2002) 19–26.
[27] T.J. Egan, D.C. Ross, P.A. Adams, FEBS Lett. 352 (1994) 54–57.
[28] T.J. Egan, R. Hunter, C.H. Kaschula, H.M. Marques, A. Misplon, J. Walden, J. Med. Chem. 43 (2000) 283–291.
[29] J.B. Correa Soares, D. Menezes, M.A. Vannier-Santos, A. Ferreira-Pereira, G.T. Almeida, T.M. Venancio, S. Verjovski-Almeida, V.K. Zishiri, D. Kuter, R. Hunter, T.J. . Egan, M.F. Oliveira, PLoS Negl. Trop. Dis. 3 (2009) e477.
[30] Z. Huang, S.B. Prusiner, F.E. Cohen, Fold. Des. 1 (1996) 13–19.
[31] C. Govaerts, H. Wille, S.B. Prusiner, F.E. Cohen, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 8342–8347.
[32] Y. Cordeiro, L.M.T.R. Lima, M.P. Gomes, D. Foguel, J.L. Silva, J. Biol. Chem. 279 (2004) 5346–5352.
[33] H. Naiki, K. Higuchi, M. Hosokawa, T. Takeda, Anal. Biochem. 177 (1989) 244–249.
[34] H. LeVine III, Meth. Enzymol. 309 (1999) 274–284.
[35] D. Tribouillard-Tanvier, S. Dos Reis, F. Gug, C. Voisset, V. Béringue, R. Sabate, E. Kikovska, N. Talarek, S. Bach, C. Huang, N. Desban, S.J. Saupe, S. Supattapone, J.Y. Thuret, S. Chédin, D. Vilette, H. Galons, S. Sanyal, M. Blondel, PLoS One. 3 (2008) e2174.