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Structure–activity relationship study of 9-aminoacridine compounds in scrapie-infected neuroblastoma cells

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Abstract—A focused library of variously substituted 9-aminoacridine compounds was screened for bioactivity against accumulation of the infectious prion protein isoform, denoted PrP^{Sc}, in a cell model of prion replication. The efficacy of compounds against PrP^{Sc} accumulation was influenced by both substituents of the distal tertiary amine and acridine heterocycle, while cellular cytotoxicity was encoded in the acridine heterocycle substituents.

In the prion diseases, the cellular prion protein, denoted PrP^{C}, undergoes a conformational conversion to a pathogenic isoform, designated PrP^{Sc}.[1] PrP^{Sc} is characterized structurally by substantial beta-sheet content and a tendency to exist as higher-order oligomeric species.[2]

A search for potential therapeutic compounds by phenotypic screening in PrP^{Sc}-infected cells identified 9-aminoacridine-based compounds, including quinacrine (Fig. 1, **1**), that were effective at reducing PrP^{Sc} in these cell-based models of prion replication.[3–5] Quinacrine has since been evaluated in animal models of prion disease[6,7] and is the focus of clinical studies to evaluate its usefulness in the treatment of human prion diseases.[8] Our medicinal chemistry efforts have focused on defining a relevant structure–activity relationship (SAR) for 9-aminoacridine compounds, with a goal of identifying more potent compounds for therapeutic application. Recent ADMET studies highlighted the undesirable toxicity of quinacrine, a relatively high rate of P-glycoprotein–mediated efflux that reduces effective CNS concentrations, and P-450 instability.[9–12] Thus, it is imperative to identify not only potent 9-aminoacridine compounds, but also analogs that may overcome some of the pharmacokinetic limitations of quinacrine.

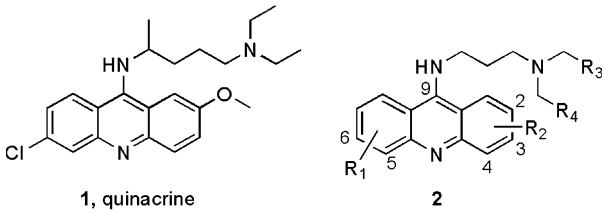

Figure 1. The 9-aminoacridine compound, quinacrine, **1**. 9-Aminoacridine numbering, **2**.

Keywords: Prion; PrP; PrP^{Sc}; PrP^{C}; 9-Aminoacridine; Quinacrine; ScN2a.
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To begin to define in more detail the structural requirements of bioactive 9-aminoacridine compounds, a library of variously substituted compounds was prepared and screened for bioactivity in mouse neuroblastoma cells persistently infected with PrP^{Sc} (ScN2a).[13] The synthesis of the acridine compound library has been reported in detail elsewhere.[14] The compound library of 144 unique compounds was synthesized in parallel, and in such a way as to allow diverse substituents to be incorporated into the acridine heterocycle as well as into the distal dibasic side chain at C-9 (see Fig. 1, **2** for acridine numbering).

The purity and identity of library components was confirmed by LC–MS prior to screening in culture. Acridine compounds were assayed for both cellular cytotoxicity and efficacy against PrP^{Sc} accumulation using a duplex screening assay that has been described previously.[15]

Briefly, ScN2a cells are incubated on microtiter plates in the presence of the test compound for five days, at which time, cell viability is determined using the fluorescent probe, Calcein-AM. Subsequently, cells were lysed, digested with proteinase K (PK), and the PK-resistant fragment of PrP^{Sc}, designated PrP 27-30,[16] was precipitated using sodium phosphotungstic acid (PTA).[17] PrP 27-30 is quantified by ELISA using high-affinity antibodies directed against PrP.[18] The acridine library was initially screened at 1 μM concentration, then certain 9-aminoacridine compounds were selected for further characterization over a concentration range of 0.01 to 25 μM. The duplex screening assay quantifies both cellular cytotoxicity, expressed as the concentration required to reduce viable cells to 50% (Table 1, LD_{50}), and bioactivity (Table 1, EC_{50}), expressed as the compound concentration needed to reduce PrP^{Sc} to 50%, relative to untreated control ScN2a cells.

Certain 9-aminoacridine compounds are cytotoxic due to intercalation of the acridine heterocycle between adjacent nucleic acid bases.[19] This feature has made acridine compounds useful nuclear imaging agents,[20] and targets for cancer chemotherapies.[21] However, the resulting cytotoxicity must be mitigated when considering the potential therapeutic applications of this class. The substituents of the acridine heterocycle appeared to be the dominant factor in determining cytotoxicity of the library components. Acridine heterocycles substituted with 3-fluoro-6-methoxy-4-methylacridine (e.g., **11**, $LD_{50} = 0.6 \pm 0.1$ μM and **12**, $LD_{50} = 0.6 \pm 0.1$ μM) and 3-methoxy-5-methylacridine (e.g., **13**, $LD_{50} = 1.3 \pm 0.1$ μM and **14**, $LD_{50} = 1.4 \pm 0.1$ μM) demonstrated potent toxicity, with LD_{50} values approximately 10-fold lower than quinacrine (**1**, $LD_{50} = 9.6 \pm 0.7$ μM). The ScN2a cell cytotoxicity of 9-aminoacridine compounds was validated by screening select compounds for cellular cytotoxicity in human kidney (HEK293) and liver (HEPG2) cells.[22] LD_{50} values (Table 1) derived from HEK293 and HEPG2 cell lines generally paralleled cytotoxicity SAR derived from ScN2a cells. Notably, compound **7** was approximately 2-fold less cytotoxic than quinacrine toward both HEK293 and HEPG2 cell lines. The potent toxicity resulting from 3-fluoro-6-methoxy-4-methyl- (e.g., **11**) and 3-methoxy-5-methyl- (e.g., **13**) substitution patterns has not previously been described. The resultant toxicity of these substitution patterns would exclude their use in future studies directed toward developing prion therapeutics, but may have an application in the development of potent cancer chemotherapeutics.

Previous SAR studies on 9-aminoacridine compounds revealed a dependence of bioactivity on the steric and electronic features of the acridine C-9 substituent, with potent compounds bearing alkyl diamine substituents.[4] In addition to the bioactive C-9 substituent of quinacrine (e.g., **1**, $EC_{50} = 0.5 \pm 0.1$ μM and **6**, $EC_{50} = 0.7 \pm 0.1$ μM), the current acridine library explored a variety of additional diamine substituents, where diversity was incorporated at the distal tertiary amine. The chemistry allowed for the synthesis of both symmetric (Fig. 1, **2**, $R_3 = R_4$) and non-symmetric (**2**, $R_3 \neq R_4$) distal tertiary amines. Compounds with smaller alkyl-substituted distal amines (e.g., **7**, $EC_{50} = 0.4 \pm 0.1$ μM and **8**, $EC_{50} = 0.8 \pm 0.1$ μM) had greater bioactivity over those with larger aryl substituents (e.g., **9**, $EC_{50} = 7.1 \pm 0.4$ μM and **10**, $EC_{50} = 3.9 \pm 0.3$ μM). A variety of acridine heterocycle substituents were tolerated (Table 1). The 6-chloro-2-methoxyacridine substitution (e.g., **3**, $EC_{50} = 0.6 \pm 0.1$ μM) and regiomeric pattern, 2-choloro-6-methoxyacridine (e.g., **5**, $EC_{50} = 0.4 \pm 0.1$ μM), both performed well. Of note were 2-(trifluoromethyl)-6-methoxyacridine–substituted compounds (e.g., **7**, $EC_{50} = 0.4 \pm 0.1$ μM and **8**, $EC_{50} = 0.8 \pm 0.1$ μM), which were typically bioactive at nanomolar concentrations when substituted with appropriate distal amines.

Given the existing toxicity of the acridine class, it is useful to consider a therapeutic index [LD_{50}/EC_{50}] when evaluating acridine compounds for overall efficacy in a cell-based assay of prion accumulation. For clinical application, an ideal therapeutic index would be large. However, given the uniformly fatal progression of the prion diseases, therapeutic indices of lower values may be tolerated. Acridine compounds **6** ($LD_{50}/EC_{50} = 23.3 \pm 6.3$) and **7** ($LD_{50}/EC_{50} = 28.6 \pm 6.8$) had therapeutic indices equivalent to quinacrine (**1**, $LD_{50}/EC_{50} = 18.5 \pm 2.9$). Additionally, compound **7** had reduced cytotoxicity toward liver and kidney cells, relative to quinacrine. As such, these compounds may warrant further ADMET characterization and evaluation in animal models of prion disease.

In summary, we have further defined the SAR of anti-prion 9-aminoacridine compounds using a focused library of substituted acridine analogs. Our findings demonstrate the importance of the acridine heterocycle in determining compound cytotoxicity and the involvement of both the heterocyclic scaffold and C-9 substituent in determining bioactivity against PrP^{Sc} accumulation.

Table 1. Bioactivity and cytotoxicity of 9-aminoacridine compounds

Compound	ScN2a EC_{50} ± SE[a] (μM)	ScN2a LD_{50} ± SE[b] (μM)	Therapeutic index ± SE[c]	HEK293 LD_{50} ± SE[b] (μM)	HEPG2 LD_{50} ± SE[b] (μM)
1 (quinacrine)	0.5 ± 0.1	9.6 ± 0.7	18.5 ± 2.9	7.6 ± 0.8	7.9 ± 0.8
3	0.6 ± 0.1	6.4 ± 0.1	10.9 ± 1.3	ND[d]	ND
4	0.6 ± 0.1	8.5 ± 1.8	13.6 ± 4.4	4.4 ± 0.6	6.9 ± 1.7
5	0.4 ± 0.1	6.0 ± 0.3	15.8 ± 2.1	10.7 ± 1.1	13.8 ± 0.1
6	0.7 ± 0.1	17.4 ± 1.7	23.3 ± 6.3	ND[d]	ND
7	0.4 ± 0.1	11.7 ± 1.3	28.6 ± 6.8	16.4 ± 2.3	18.1 ± 3.5
8	0.8 ± 0.1	7.6 ± 1.0	9.8 ± 1.8	14.6 ± 1.4	16.5 ± 3.8
9	7.1 ± 0.4	18.3 ± 4.0	2.6 ± 0.7	22.4 ± 2.4	>25
10	3.9 ± 0.3	13.0 ± 1.8	2.6 ± 0.7	23.1 ± 1.9	>25
11	ND[d]	0.6 ± 0.1	ND	0.8 ± 0.1	0.7 ± 0.1
12	ND[d]	0.6 ± 0.1	ND	0.7 ± 0.1	0.6 ± 0.1
13	1.0 ± 0.2	1.3 ± 0.1	1.3 ± 0.3	1.4 ± 0.4	0.7 ± 0.1
14	1.0 ± 0.4	1.4 ± 0.1	1.3 ± 0.5	0.7 ± 0.1	0.7 ± 0.1

[a] Compound concentration required to reduce PrP^{Sc} to 50%, relative to untreated ScN2a cells.

[b] Compound concentration required to reduce the number of viable cells to 50%, relative to untreated control cells, either ScN2a, HEK293 or HEPG2 cells.

[c] Ratio LD_{50}/EC_{50} for ScN2a cells.

[d] Not determined; insufficient data to derive full dose–response curves. Dose–response curves were performed in triplicate and derived from three independent experiments. Standard errors (SE) are rounded to two significant figures.

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22. Human HEK293 and HEPG2 cell lines (CCF-UCSF) were plated at approximately 100,000 cells/well onto black 96-well cell culture plates (Greiner Bio-One) in 190 μL DMEM (CCF-UCSF), supplemented with 10% fetal calf serum (Hyclone). 9-Aminoacridine compounds were added to give a final concentration range of 0.01–25 μM. DMSO concentrations did not exceed 0.25% v/v. Cells were incubated for 5 days, at which time media were removed, and cells were washed with warmed PBS (CCF-UCSF) (2× 200 μL). Calcein-AM (Molecular Probes) was added as a solution in PBS (final concentration 2.5 μM, 100 μL), and cells were incubated at 37 °C for 30 min. Cell viability was quantified by fluorescein excitation/emission using a JL Biosystems Analyst AD plate reader. DMSO-treated cells, no cells, and quinacrine served as controls. Dose–response curves were plotted using SigmaPlot, and LD_{50} values were determined from these curves.