European Journal of Medicinal Chemistry 82 (2014) 363–371

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Evaluation of the antiprion activity of 6-aminophenanthridines and related heterocycles

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ARTICLE INFO

Article history:
Received 15 February 2014
Received in revised form
21 May 2014
Accepted 31 May 2014
Available online 3 June 2014

Keywords:
Neurodegenerative diseases
Protein misfolding
Prions
Phenanthridine

ABSTRACT

Series of 6-aminophenanthridines and related heterocyclic compounds such as benzonaphtyridines were prepared. Reduction of one of the three aromatic rings was also performed. The compounds were first tested for their antiprion activity in a previously described yeast-based colourimetric prion assay. The most potent derivatives were then assayed *ex vivo* against the mammalian prion PrP^{Sc} in a cell-based assay. Several of the new compounds were found more potent than the parent lead 6-aminophenanthridine. The most promising compounds against yeast and mammalian prions were 8-azido-6-aminophenanthridine (**3m**), and 7,10-dihydrophenanthridin-6-amine (**14**). In the mammalian cell-based assay, the IC_{50} of these two compounds were around 5 μM and 1.8 μM, respectively.

1. Introduction

Transmissible spongiform encephalopathies (TSE) are a group of fatal neurodegenerative disorders affecting mammals which notably include Creutzfeldt-Jakob disease as well as Gerstmann–Straüssler–Scheinker syndrome, fatal familial insomnia and Kuru. These neurodegenerative diseases are all characterized by the accumulation of an abnormal form of the prion protein PrP^{Sc} which assembles into amyloid aggregates in the brain [1]. There is currently no treatment available for this type of disorders.

Several series of compounds have been reported to possess antiprion activity, among which diphenylmethane derivatives such as GN8 [2], 2-aminothiazoles [3], indole-3-glyoxylamides [4], 2,4-diarylthiazoles [5], diketopiperazines [6], acridines [7], fluphenazine [8] and trimipramine [9]. Immunological methods to treat prion diseases have also been described [10]. In rare cases, the mechanism of the antiprion activity was studied by the authors [11].

We have developed a simple, economic, safe and rapid yeast-based method to screen for anti-prion drugs [12]. This assay is based on the successive evaluation of the antiprion activity of compounds against two unrelated yeast prions [PSI^{+}] and [URE3] using *in vivo* yeast-based assays and then against the mammalian prion using PrP^{Sc} chronically infected-cells. Using this multi-step screening method, several potent antiprion compounds such as 6-aminophenanthridine (6AP) [12], guanabenz (GA) [13] and imiquimod (IQ) [14] were discovered. The antiprion activity of these three compounds was further confirmed *in vivo* in a murine model for prion diseases [12–14]. 6AP, GA and IQ has been shown to specifically inhibit the protein folding activity of the ribosome (termed PFAR, a protein chaperone activity which is borne by the domain V of the large ribosomal RNA of the large subunit of the ribosome), suggesting that PFAR is a conserved cellular activity important for prion propagation [15,16]. The aim of this piece of work was to identify new potent antiprion compounds by performing a structure–activity relationship study on the basis of the antiprion 6AP compound, and to further validate the correlation between the yeast-based and the mammalian cell-based assays.

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http://dx.doi.org/10.1016/j.ejmech.2014.05.083
0223-5234/

2. Results and discussion

2.1. Chemistry

A series of 6-aminophenanthridines was prepared by Suzuki Miyaura coupling of either a 2 aminoboronic ester with a 2-halobenzonitrile or a 2-cyanophenylboronic ester with a 2 haloaniline (Scheme 1).

Diamino-6,7-phenanthridine (**3k**) and 6,8-phenanthridine (**3l**) were obtained starting from the corresponding nitro derivatives (Scheme 1).

The azido derivative **3m** was prepared in a simple four steps procedure starting from **3g** (Scheme 2). The protection of the 6-amino group was first achieved via phthalimidation followed by reduction of the nitro group into the primary amine. The conversion to the azide was conducted by diazotization. Removal of the phthalimide was finally achieved by hydrazinolysis.

The Suzuki coupling could also be extended to the preparation of several other structurally related heterocyclic systems such as amino benzonaphthyridines (**8a,b**, **10a**–**c**, Scheme 3).

We have also experimented the reduction of 6-aminophenanthridine under Birch conditions, thereby affording dihydrophenanthridine **14** (Scheme 4). The structure of **14** was confirmed by crystal structure analysis (Fig. 1). Reduction of **14** by catalytic hydrogenation afforded the tetrahydroderivative **15** (Scheme 4).

Another family corresponds to compounds that were substituted on the amino group. These secondary amines **17** were obtained from 6-chlorophenanthrine **16** (Scheme 5).

2.2. Biological evaluation of the antiprion activity of 6-AP derivatives, benzonaphtyridines, hydrogenated compounds and 6-alkylphenanthridines

The antiprion activity of the prepared compounds was evaluated against the two yeast prions [PSI^+] and [URE3] and against the PrP^{Sc} mammalian prion in cell culture.

2.2.1. Determination of synthesized compounds activity against yeast prions [PSI^+] and [URE3]

The compounds were tested against the two yeast prions [PSI^+] and [URE3], as described previously [12,17,18]. Guanidine hydrochloride (GdnHCl) was used as positive control [12]. Compounds **3a**, **3b**, **3c**, **3e**, **3f**, **3g**, **3k**, **3m**, **3n**, **3p**, **14** and **15** were active against both [PSI^+] and [URE3] to various degrees (Fig. 2, Tables 1 and 2). Compounds **3d**, **3h**, **3j**, **3l** and **3o** were slightly active against [PSI^+] only (Fig. 2B, Table 1).

2.2.2. PrP^{Sc} clearance assay in MovS6 cells

The prepared compounds were then tested for their ability to clear PrP^{Sc} using the previously described mammalian MovS6 cell-based assay [19]. MovS6 cells correspond to a murine peripheral neuroglial cell line issued from transgenic mice (TgOv, [20]) in which the murine PRNP gene encoding murine PrP has been replaced by the ovine gene (VRQ allele). PrP expression is under the control of its endogenous promoter. These cells are chronically infected with the 127S sheep scrapie agent [19]. When the PrP protein is under its cellular conformation (PrP^C), it is sensitive to proteinase K (PK) digestion. On the contrary, when PrP is under its PrP^{Sc} prion conformation, it forms amyloid fibres and is partially resistant to PK digestion. The presence of PrP^{Sc} was thus detected on the basis of its PK resistance. This *ex vivo* assay showed that compounds **3a**, **3b**, **3c**, **3g**, **3h**, **3k**, **3m**, **3n**, **3o**, **3p**, **14** and **15** were active against PrP^{Sc} (Tables 1 and 2). Only compounds **3a** [12], **3h**, **3m**, **3n** [12], **3o**, **3p**, **14** and **15** presented an IC_{50} equal or lower than 10 μM (Fig. 2C).

2.3. Structure activity relationships

It can be first observed that introduction of groups on the 6-amino moiety completely abolished the antiprion activity of compounds as it can be noticed with compounds **17a**–**b**. Introduction of electrowithdrawing groups in positions 7 and 8 enhanced the antiprion activity (compounds **3b**, **3g**, **3n**, **3p**). Conversely, most of changes made in position 2 were detrimental to the antiprion activity (**3d** and **3j**). The only exception was observed with **3o**, one of the most potent compound against mammalian prion.

Regarding the heterocyclic scaffold, the replacement of one of the phenyl ring by a pyridine did not lead to active compounds (compounds **8** and **10**).

The reduction of one of the phenyl ring was more fruitful as it can be seen from the evaluation of the dihydroderivative (compound **14**), but also in the case of the tetrahydroderivative (compounds **15**). These two hydrogenated compounds **14** and **15** displayed approximately equal potency against yeast prions, **14** being slightly more potent than **15** against mammalian the prion PrP^{Sc}.

2.4. Correlation between the yeast-based and the mammalian cell-based assays

As shown in Tables 1 and 2, compounds found positive in the yeast-based tests also displayed a potent antiprion activity against the mammalian PrP^{Sc} prion in cell culture. This fair correlation between the yeast-based and the mammalian cell-based assays is observed for most compounds (Tables 1 and 2), and was expected since 6AP has been shown to act on a target conserved from yeast to mammals.

3. Conclusion

The results of the yeast-based assay correlated nicely with the mammalian cell-based assay, showing the potency of the simple yeast-based method to isolate compounds active against PrP^{Sc} mammalian prion. Several highly potent compounds were isolated during this SAR study. The dihydroderivative (**14**) and 6-amino-8-azidophenanthridine (**3m**) will be selected for further *in vivo* tests. Conversely, due to its low solubility and the potential toxicity of nitroaromatic derivatives, further study of 8-nitro-6-AP (**3o**) was not envisioned.

Finally, it must be emphasized that the interest of compounds active in antiprion tests goes far beyond the therapy of prion diseases. There are evidences that other much more common protein aggregation-based diseases like Alzheimer, Parkinson or Huntington diseases share partially overlapping aetiology [21–23]. In line, we recently observed that 6AP presents noticeable activity in models for other amyloid-based diseases that share similarities with prion diseases. For example, 6AP has also recently been shown to have a beneficial effect on oculopharyngeal muscular dystrophy (OPMD) *in vivo* in a drosophila model [24]. OPMD is a syndrome characterized by progressive degeneration of specific muscles caused by extension of a polyalanine tract in the PABPN1 protein that hence accumulates as amyloid fibres within the nuclei of muscular cells, thus leading to muscle degeneration and disorganization. 6AP led to suppression of all OPMD typical phenotypes *in vivo* in drosophila when given in the diet of larvae as well as adults [24]. These results support the general interest of 6AP series.