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Original article

Discovery of 6-substituted indole-3-glyoxylamides as lead antiprion agents with enhanced cell line activity, improved microsomal stability and low toxicity

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ABSTRACT

A series of highly potent indole-3-glyoxylamide based antiprion agents was previously characterized, focusing on optimization of structure–activity relationship (SAR) at positions 1–3 of the indole system. New libraries interrogating the SAR at indole C-4 to C-7 now demonstrate that introducing electron-withdrawing substituents at C-6 may improve biological activity by up to an order of magnitude, and additionally confer higher metabolic stability. For the present screening libraries, both the degree of potency and trends in SAR were consistent across two cell line models of prion disease, and the large majority of compounds showed no evidence of toxic effects in zebrafish. The foregoing observations thus make the indole-3-glyoxylamides an attractive lead series for continuing development as potential therapeutic agents against prion disease.

1. Introduction

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are a group of rare but invariably fatal neurodegenerative disorders afflicting both humans and animals. The most common such condition affecting humans is Creutzfeldt–Jakob Disease (CJD), and other examples include familial fatal insomnia (FFI) and Gerstmann–Sträussler–Scheinker syndrome (GSS). Animal prion diseases include scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease (CWD) in deer, elk and moose. In recent years, the latter of these has been recognized as a significant epidemic amongst wildlife populations across certain regions of North America [1].

The fundamental molecular event common to all TSEs is post-translational refolding of normal cellular prion protein, PrP^{C}, into its disease-associated isoform, PrP^{Sc}. This β-sheet rich form of the protein readily forms insoluble aggregates, deposition of which is thought to be central to pathogenesis and disease progression. Despite in vitro characterization of a large number of antiprion compounds capable of clearing PrP^{Sc} from infected cell lines, little therapeutic efficacy in vivo has been achieved to date, even in the face of considerable research effort [2]. An effective treatment to arrest or reverse progression of clinical prion disease therefore remains an important goal.

We recently characterized a series of indole-3-glyoxylamides **1** as potent antiprion agents (Fig. 1) [3], many of which demonstrated low nanomolar EC_{50} values against a prion-infected cell line (SMB.s15) [4]. This cellular system is one of several models [5] for prion disease that persistently produce PrP^{Sc} and is therefore used in screening for potential therapeutics. Previous efforts were focused around developing the structure–activity relationship

Abbreviations: BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt–Jakob disease; CWD, chronic wasting disease; FFI, familial fatal insomnia; GSS, Gerstmann–Sträussler–Scheinker syndrome; hpf, hours post fertilization; MAPK, mitogen-activated protein kinase; PrP^{C}, normal cellular prion protein (or PrP-sen); PrP^{Sc}, disease-causing isoform (or PrP-res); SAR, structure–activity relationship; ScN2a, scrapie-infected neuroblastoma cells; SMB, scrapie-infected mouse brain; TBST, tris-buffered saline (pH 7.6) containing 0.05% Tween 20; TSE, transmissible spongiform encephalopathy; vCJD, variant Creutzfeldt-Jakob disease.

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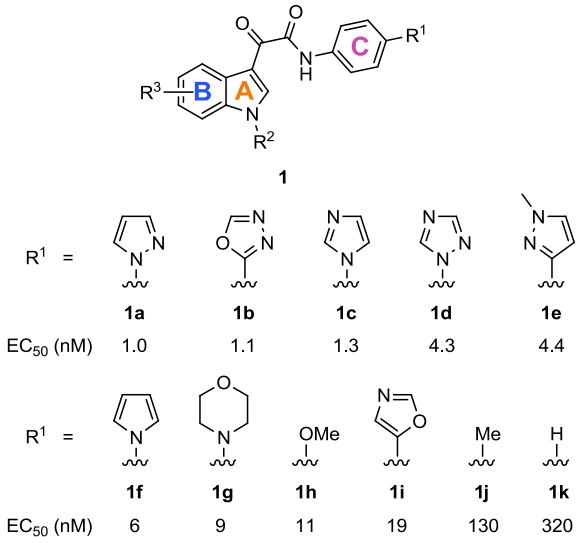

Fig. 1. Representative members of the indole-3-glyoxylamide lead series previously identified as potent inhibitors of PrP^{Sc} accumulation. In all of the examples **1a**–**1k**, $R^2 = R^3 = H$.

(SAR) at positions 1–3 of the indole ring (ring A, Fig. 1), including investigation of the most suitable glyoxylamide substituent, which was found optimally to consist of a *para*-substituted aniline moiety (ring C). The effect of substitution at indole C-4 to C-7 (ring B) is one important aspect of the SAR which was not considered in this earlier work, and assessment of such modifications is therefore a focus of the current study.

In addition to their highly potent in vitro antiprion activity, compounds of general structure **1** constitute an encouraging lead series given the wide variety of drug candidates containing the indole-3-glyoxylamide moiety (Fig. 2). Indeed, this substructure could be thought of as a 'privileged scaffold' [6] for drug discovery, considering the significant number of such compounds in various stages of clinical or preclinical development, across a range of biological activities (Table 1). Some attrition has been inevitable, but generally seems not to be due to adverse events in the case of this compound class. Thus, given the range of related examples which have already progressed to clinical studies for other disease indications (Table 1), we were encouraged that the antiprion indole-3-glyoxylamides are certainly amenable to tuning toward acceptable physical and pharmacokinetic properties. The structures in lead series **1** may thus be considered a promising starting point, with good potential for the development of clinically useful antiprion agents.

We therefore undertook to establish the effect of introducing substituents around 'ring B' (Fig. 1) upon cell line activity, in order to complete a basic understanding of the antiprion SAR across the whole of the central indole-3-glyoxylamide framework. In addition to this extension to the known SAR, it was also considered important to identify compounds with the best preclinical potential by consideration of other relevant properties. The criteria chosen were in vitro metabolic stability, and in vivo evaluation in zebrafish as an initial toxicity screen.

2. Chemistry

Synthesis of the new screening compounds substituted in 'ring B' was carried out using a protocol similar to that reported previously [3] (Scheme 1). In the presence of electron-withdrawing substituents (R^3 = F, Cl), it was found necessary to extend the duration of the first step (reaction with oxalyl chloride) to 24 h to achieve complete conversion to the intermediate glyoxylyl chorides. In terms of final yield, this approach actually proved advantageous for all of the substituted indoles, and was therefore adopted as a general method during the preparation of screening libraries. Initially, compounds derived from either methyl- or chloro-substituted indoles were synthesized. All such products were obtained successfully, with the exception of those derived from 4-chloroindole. In this case, it appeared that the extent of reaction with oxalyl chloride had only been very small.

3. Results and discussion

3.1. Investigation of antiprion structure–activity relationship

Methyl- and chloro-substituted series derived from parent structures **1h**, **1j**, **1g** and **1k** all displayed varying antiprion potency in the SMB cell line (Table 2). This range of R^1 groups (OMe, Me, morpholine and H, respectively, for the parent compounds listed) was chosen firstly because it represents a range of activities within the existing lead series (EC_{50} values between 11 and 320 nM), and secondly for ease of synthesis. Whereas compounds containing a five-membered aromatic heterocycle in the R^1 position are the

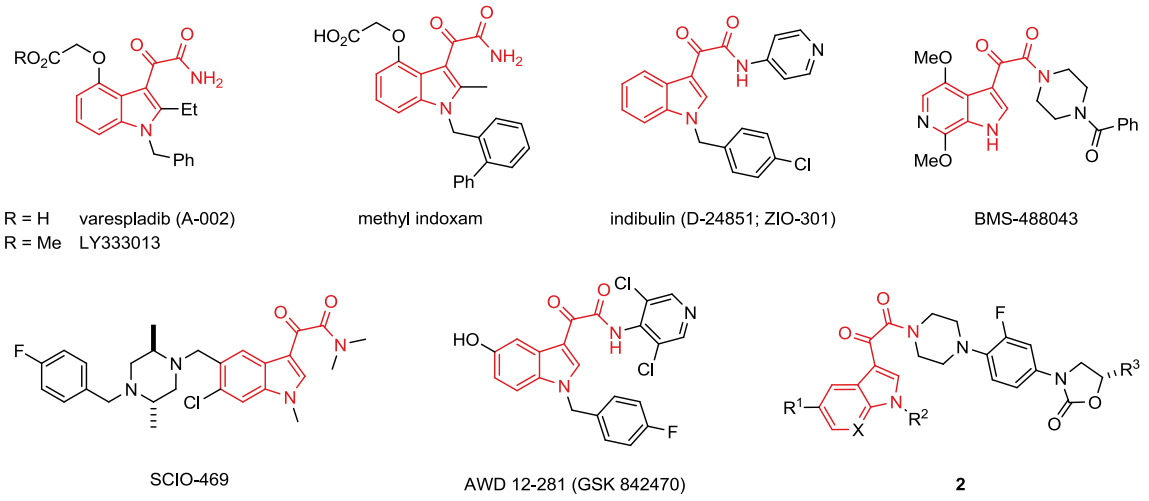

Fig. 2. Several compounds containing an indole-3-glyoxylamide (or closely related) substructure have progressed to preclinical or clinical evaluation.