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ARTICLE

Novel 5-aryloxypyrimidine SEN1576 as a candidate for the treatment of Alzheimer's disease

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Abstract

Prefibrillar assembly of amyloid-β (Aβ) is a major event underlying the development of neuropathology and dementia in Alzheimer's disease (AD). This study determined the neuroprotective properties of an orally bioavailable Aβ synaptotoxicity inhibitor, SEN1576. Binding of SEN1576 to monomeric A$\beta_{1–42}$ was measured using surface plasmon resonance. Thioflavin-T and MTT assays determined the ability of SEN1576 to block A$\beta_{1–42}$-induced aggregation and reduction in cell viability, respectively. *In vivo* long-term potentiation (LTP) determined effects on synaptic toxicity induced by intracerebroventricular (i.c.v.) injection of cell-derived Aβ oligomers. An operant behavioural schedule measured effects of oral administration following i.c.v. injection of Aβ oligomers in normal rats. SEN1576 bound to monomeric A$\beta_{1–42}$, protected neuronal cells exposed to A$\beta_{1–42}$, reduced deficits in *in vivo* LTP and behaviour. SEN1576 exhibits the necessary features of a drug candidate for further development as a disease modifying treatment for the early stages of AD-like dementia.

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Introduction

Cognitive deficits leading to the dementia syndrome seen in Alzheimer's disease (AD) have recently been argued to begin with a disruption of synaptic transmission due to the formation of amyloid-beta (Aβ) oligomers (Haass and Selkoe, 2007; Klyubin *et al.*, 2008; Shankar *et al.*, 2008; Ondrejcak *et al.*, 2010; Pharm *et al.*, 2010). From a theoretical perspective, targeting the effects of Aβ oligomerization and the oligomer assembly mechanism should form a logical basis for early pharmacological intervention, slowing or preventing dementia in AD (Tanzi *et al.*, 2007; Walsh and Selkoe, 2007). The contention that initial accumulation of extracellular oligomeric Aβ has detrimental effects in AD has been inferred from the work of several research groups (Walsh *et al.*, 2005a). For example, cultured cells (7PA2 CM) that express human amyloid precursor protein, and secrete Aβ oligomers, dimers, trimers and tetramers of Aβ, composed of N- and C-terminally heterogeneous human Aβ peptides, including the A$\beta_{1–40}$ and A$\beta_{1–42}$ species found in human brain and extracellular fluids (Podlisny *et al.*, 1995, 1998; Walsh *et al.*, 2002, 2005a), impair long-term potentiation (LTP) and memory in experimental animals. Using LTP, a model of synaptic plasticity, which provides an investigational representation of memory processes at the cellular level (Ondrejcak *et al.*, 2010), it has been established that the exposure of hippocampal cells to Aβ oligomers reduces memory ability at the cellular level in normal rats and mice, both *in vitro* (Wang *et al.*, 2004) and *in vivo* (Walsh *et al.*, 2002). Behavioural studies using normal freely moving rats have also found that 7PA2 CM produces detrimental effects. It has been established that intracerebroventricular (i.c.v.) injection of 7PA2 CM disrupts memory for learned behaviour in the rat (Cleary *et al.*, 2005), and this original finding has been consistently confirmed (e.g., Townsend *et al.*, 2006; Poling *et al.*, 2008). This work has also been expanded to demonstrate the protective effects of putative pharmacotherapies against 7PA2 CM-induced behavioural deterioration (Townsend *et al.*, 2006; O'Hare *et al.*, 2010). Consequently, the Aβ oligomer toxicity theory of fundamental processes relative to the development of dementia

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in AD has substantial support from the contemporary literature, and pharmacotherapies targeting these underlying mechanisms are of considerable scientific interest.

With respect to pharmacotherapies for AD dementia, acetyl cholinesterase inhibitors (AChEIs) and an antagonist of the *N*-methyl-D-aspartate (NMDA) receptor (memantine) have been approved for clinical treatment (Farlow, 2002; Shah and Reichman, 2006). AChEIs were designed to promote acetylcholine (ACh) neurotransmission by preventing the degradation of ACh into choline and choline transferase. However, the effect of AChEIs in the mild cognitive impairment, which precedes AD is not translated into an effect that prevents decline into the full AD syndrome (Raschetti *et al.*, 2007). The NMDA receptor antagonist, memantine, blocks NMDA ion channels during pathological over-activation associated with moderate concentrations of glutamate, thus reducing excitotoxicity (Parsons *et al.*, 1999). While memantine has been reported to be of clinical, albeit limited, benefit in slowing behavioural deterioration in AD (Reisberg *et al.*, 2003; Tariot *et al.*, 2004; Areosa *et al.*, 2005), it has been reported to be of no clinical benefit in mild to moderate AD (Schneider *et al.*, 2011).

From the contemporary literature, specifically targeting synaptotoxic Aβ oligomers as a strategy for the treatment of dementia in AD is desirable and, unlike other pharmacotherapies, this approach is likely to offer a better safety profile (Amijee and Scopes, 2009). Non-peptide small molecule inhibitors of Aβ aggregation have proven difficult to develop and, of the few available compounds of this type, many are unsuitable for clinical application because of their biological profile, poor oral bioavailability, or short half-life (Amijee and Scopes, 2009). However, Nakagami *et al.*, (2002) reported that the small molecule RS-0406 inhibited Aβ toxicity, RS-0406 was then shown to protect hippocampal neurons against A$\beta_{1–42}$-induced cytotoxicity, to alleviate A$\beta_{1–42}$-impaired LTP (Walsh *et al.*, 2005b), and to arrest 7PA2 CM-induced behavioural deterioration (O'Hare *et al.*, 2010). Unfortunately, RS-0406 is not orally bioavailable, and as such is not a realistic candidate for the clinical treatment of dementia in AD.

Our group modified the RS-0406 *N N*′-bis (3-hydroxyphenyl)pyridazine-3,6-diamine structure to improve its potency and oral bioavailability. This work resulted in a pharmacologically active, non-peptide small molecular weight compound, SEN1269 that bound to Aβ and demonstrated more significant neuroprotection than RS-0406 in biochemical, electrophysiological and behavioural models of dementia in AD (Scopes *et al.*, 2012). However, SEN1269, like RS-0406, was only effective following i.c.v. administration, and was, thus, similarly not a viable candidate for the therapeutic treatment of dementia in AD. We then modified the chemical structure of SEN1269 to produce an orally bioavailable analogue, SEN1500, which demonstrated biochemical neuroprotection against Aβ insults, and against the synaptotoxicity and behavioural deterioration following i.c.v. injection of Aβ oligomers (O'Hare *et al.*, 2013). We report the effects of a derivative of SEN1500 with greater brain penetration, SEN1576 (Fig. 1), on binding to A$\beta_{1–42}$ monomer and oligomers, inhibition of A$\beta_{1–42}$ aggregation, protection of a neuronal cell line exposed to A$\beta_{1–42}$, and on *in vivo* LTP and oral dosing of SEN1576 relative to 7PA2 CM-induced behavioural deficits in freely moving intact rats. Also, genotoxic liabilities using the Ames test and cardiovascular safety profiles *in vitro* and *in vivo* are presented.

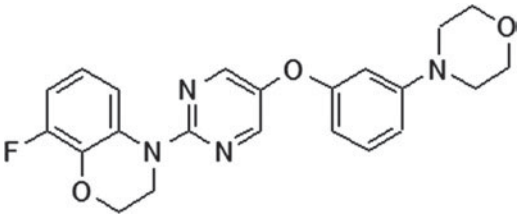

Fig. 1. Chemical structure of SEN1576.

Method

Compound

SEN1576 (8-Fluoro-4-[5-(3-morpholin-4-ylphenoxy) pyrimidin-2-yl]-3,4-dihydro-2H-benzo[1,4]oxazine), 99.6% purity, was synthesized by Oxygen Healthcare Ltd (India) and Aptuit Ltd (UK).

Aβ binding, aggregation and cell viability assays

Full details of the methods used have previously been reported (Scopes *et al.*, 2012; O'Hare *et al.*, 2013). Briefly, surface plasmon resonance was used to measure binding of SEN1576 to A$\beta_{1–42}$. *N*-terminal biotinylated A$\beta_{1–42}$ (rPeptide, Bogart, USA) was prepared in 50% dimethyl sulfoxide (DMSO) and pulse injected into a streptavidin (SA) chip surface until the immobilization level was established (Biocore T-100 biosensor), 1 mM biotin was injected into a second flow cell to allow saturation of the SA chip as control. Binding of SEN1576 in the flow phase onto immobilized monomeric A$\beta_{1–42}$ was established and K_D data relating to SEN1576 according to a 1:1 model were determined. A thioflavin-T assay (LeVine, 1999) was used to quantify Aβ fibrillogenesis and to measure SEN1576 inhibition of A$\beta_{1–42}$ aggregations. A$\beta_{1–42}$ HCL salt (rPeptide, Bogart, USA) was dissolved in hexafluoroisopropanol, with brief sonication and vortexing. This solution was freeze-dried, then dissolved in DMSO. The activity of SEN1576 in inhibiting A$\beta_{1–42}$ aggregation was assessed by incubation with 10 μM A$\beta_{1–42}$ at 37 °C for 24 h in phosphate buffered saline. At 24 h a 50 μl aliquot was dispensed into a black 96 well plate and 50 μl thioflavin-T (40 μM in 50 mM glycine buffer) was added to each well. The plate was shaken and fluorescence was recorded using excitation and emission filters

of 440 (±15) and 485 (±10) nm, respectively. An MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay (Shearman *et al.*, 1995) was used to determine the effect of SEN1576 on the deficit in SH-SY5Y cell viability caused by A$\beta_{1–42}$. Cell wells were prepared with 3 μl of SEN1576 in DMSO (mixed with 294 μl opti-MEM) then 3 μl of A$\beta_{1–42}$ (2 mM) was added and mixed thoroughly. 50 μl of this solution was aspirated and dispensed into wells containing 50 μl of opti-MEM media+SH-SY5Y cells (A$\beta_{1–42}$ final concentration 10 μM) which were then incubated for 24 h. 15 μl of MTT was added to the cell wells, which were then incubated in 5% CO_2 at 37 °C for 4 h. 100 μl of stop/solubilisation solution was added and the wells were placed in a humidified box at room temperature overnight before reading.

7PA2 cells

7PA2 cells are stably transfected Chinese hamster ovary (CHO) cells incorporating cDNA for APP (APP751) specific for the familial AD mutation Val717Phe (Podlisny *et al.*, 1998; Shankar *et al.*, 2011). 7PA2 cells secrete the commonly implicated A$\beta_{1–40}$ and A$\beta_{1–42}$ fragments and more than 90 Aβ sequence-containing peptides and proteins (Portelius *et al.*, 2013). The cells were grown to just below confluence in DMEM, containing 10% FBS and 200 μg/ml G418, briefly washed in DPS and incubated at 37 °C with 5% CO_2 for 18 h with sufficient volume of DMEM to just cover the cells. After incubation the medium was centrifuged at 3000 *g* for 15 min and either used directly or snap frozen and stored at −20 °C. Using ELISA, the concentration of total Aβ in the 7PA2 CM used in the LTP and behavioural studies was in the range of 2–5 nM.

In vivo LTP

Male Sprague-Dawley rats weighing 310–480 g were anaesthetized using intraperitoneal (i.p.) urethane injection (1 ml/100 g) with i.v. supplements of 0.2 ml/100 g (12% solution) as required. Body temperature was maintained at 37 °C using a homoeothermic blanket, and polyethylene catheters were inserted into the right femoral artery and vein for monitoring arterial blood pressure and for anaesthetic drug administration, respectively. The head was mounted in a stereotaxic frame before lowering a concentric bipolar stimulating electrode and carbon fibre-recording electrode vertically into the CA1 area of the hippocampus. Using the atlas of Paxinos and Watson (1998), the stimulating electrode coordinates were; bregma −3.5 mm, lateral 2.5 mm and 2.0–2.5 mm below the pial surface, the recording electrode coordinates were; bregma −4.5 mm, lateral 2.1 mm and 2.0–2.5 mm below the pial surface. Subsequently, a preloaded 30-gauge stainless steel i.c.v. cannula was lowered into the lateral ventricle: bregma +0.5 mm, lateral 1.5 and 3.6 mm below the pial surface with a 15–17° rostro-caudal angle. Field excitatory post-synaptic potentials (fEPSPs) with superimposed population spikes (PSs) were then recorded. Electrical stimulation (0.1 ms pulse width, 10–100 V, 0.14 Hz) was used to identify and optimize fEPSPs with superimposed PSs, and an input/output curve was created to determine maximal PS amplitude and the voltage required to generate a PS of 30–40% of the maximum amplitude. Stimulation parameters were maintained at 0.033 Hz and, after stabilization, recording commenced of PS activity for 2 min before SEN1576 (0.3 or 1 mg/kg) or vehicle was given i.v. followed 6 min later by i.c.v. administration of 10 μl 7PA2 CM or CHO CM (wild-type control) at a rate of 2.5 μl/min. A second treatment with SEN1576 (at the matching dose) or vehicle was given 24 min later, this was 8 min prior to high frequency stimulation (HFS) of the hippocampal Schaffer-collateral pathway. This was comprised of 3 periods (inter-period interval 30 s) of HFS (10 trains of 10 stimuli; 200 Hz; inter-train interval 50 ms). PSs were recorded for 90 min after HFS and the final 10 min of recording (20 sweeps) were analysed for group comparisons using one-way analysis of variance (ANOVA), with a Bonferroni *post-hoc* test for multiple pair-wise comparisons.

Operant behaviour in intact rats

Male Sprague-Dawley rats (n=98, weighing 220–250 g at the beginning of the experiment) were maintained at 90% of their free-feeding body weights (on a sliding scale to allow for maturation) and housed individually, with water available *ad libitum* in the home cage. The temperature in the *vivarium* was maintained at 23 °C under a 12 h light/12 h dark cycle (lights on 08:00 h). The rats were trained and tested in two-lever rat test chambers (Med Associates Inc., USA) enclosed in sound attenuating compartments. Reinforcers were 45 mg sucrose pellets (BioServ, USA), which were delivered into a tray, situated midway between the operant levers. A training procedure similar to that previously reported (Cleary *et al.*, 2005; Reed *et al.*, 2011) was employed. Behavioural sessions were conducted 7 d a wk and the rats were trained to press both levers for food reinforcement. Over approximately 20–30 training sessions, an alternating-lever cyclic-ratio (ALCR) schedule of reinforcement was introduced. Under this assay, rats must alternate to the other lever after pressing the currently correct lever a sufficient number of times to obtain a reinforcer. The number of lever presses required for each reinforcer changes, increased from 2 responses per food pellet up to 56 responses per food pellet, and then decreased back to 2 responses per food pellet over 6 cycles, one complete cycle requiring alternating responses of 2, 6, 12, 20, 30, 42, 56, 56, 42, 30, 20, 12, 6 and 2. This provides a measure of short-term memory (readily available information for a short period of time), and generates data on 2 types of errors on the operant schedule. Lever-switching errors occur when a subject fails to

alternate levers after being reinforced (i.e. continues to press the lever that produced the immediate reinforcer – short-term memory error), and incorrect lever perseveration errors occur when a subject continues to press the incorrect lever after making a lever-switching error (thus compounding the short-term memory error). This type of error indicates disruption of well-learned behaviours, or reference memory (this includes reasoning and goal-oriented manipulation of information). When responding under the ALCR schedule reflected no change in trends among subjects, all rats were anaesthetized with fentanyl citrate (0.4 ml/kg), placed in a stereotaxic frame, and fitted with a permanent indwelling cannula (23-gauge) aimed at the lateral ventricle. Half of the rats received left lateral ventricle cannula implants and the other half received right lateral ventricle cannula implants. With the incisor bar set 3 mm below the interaural line, stereotaxic co-ordinates for implantation were; bregma −1.0 mm, −1.5 mm lateral to midline and 3 mm below the surface of the skull (Paxinos and Watson, 1998). All rats were allowed 7 d for recovery before experimental testing, and cannula placement was verified by observation of vigorous drinking (>5 ml/20 min) following i.c.v. injection of 5 μl angiotensin II (0.5 μg/ml). Following recovery from surgery, rats were randomly assigned to one of six groups. Group 1 received oral dosing with the vehicle (maple syrup) used for suspension of SEN1576 plus i.c.v. CHO CM, to establish overall baseline performance; group 2 received oral dosing with 20 mg/kg SEN1576 plus i.c.v. CHO CM, to control for any adverse effect of the drug; group 3 received oral dosing with vehicle plus i.c.v. 7PA2 CM, to establish the geodetic datum against which drug effects were measured; groups 4, 5 and 6 received oral dosing with 1, 5 or 20 mg/kg SEN1576, respectively, plus i.c.v. 7PA2 CM, to investigate dose-response effects. Oral dosing was carried out 1.5 h prior to, and again 30 min prior to i.c.v. injections. Subjects were placed in the operant chambers for collection of ALCR data 2 h after i.c.v. injections (Cleary *et al.*, 2005). Oral treatments were performed by suspending SEN1576 in maple syrup, which the rats drank readily from a syringe. Data were analysed by one-way analysis of variance, followed by Fisher's least significant differences *post-hoc* test.

Ames test

As part of the toxicological testing, the potential mutagenicity of SEN1576 was assessed in a bacterial reverse mutation test (Ames test; Sequani Ltd.). SEN1576 was tested *in vitro* for its ability to induce mutations in 4 histidine dependent auxotrophic mutants of *Salmonella typhimurium*, strains TA1535, TA1537, TA98 and TA100, and 1 tryptophan dependent auxotrophic mutant of *Escheria coli*, WP2 *uvr*A. A Range Finder experiment was performed to determine the cytotoxic range of the test item under plate incorporation conditions in TA98 and WP2 *uvr*A, in the presence and absence of S-9 mix (a liver post-microsomal fraction derived from the livers of Aroclor 1254-treated rats). The main mutation experiments were then conducted using both plate incorporation and pre-incubation methods. Two independent mutation tests were performed, each in both the presence and absence of S-9 mix. The bacteria were exposed to SEN1576 dissolved in dimethylsulphoxide; dimethylsulphoxide also acted as the negative control. The positive control chemicals were Sodium Azide (TA1535 and TA100), 9-Aminoacridine (TA1535), 2-Nitroflourene (TA98) and 4-Nitroquinoline-N-Oxide (WP2 *uvr*A) in the absence of S-9 mix, and 2-Aminoanthracene (all strains) in the presence of S-9 mix.

Cardiovascular safety pharmacology

Conscious, freely moving male guinea pigs (Dunkin-Hartley) were used to assess the effects of oral administration of SEN1576 at doses of 30, 100 and 300 mg/kg, on arterial blood pressure, heart rate and lead II electrocardiogram (ECG), in compliance with work suitable for worldwide regulatory submission (e.g. EEC Council Directive 75/318/EEC). Eight guinea pigs were surgically implanted with telemetry transducers (type TL11M2-C50-PXT; Data Sciences International) for the measurement of arterial blood pressure, heart rate, core body temperature and lead II ECG. The animals were anaesthetized with isoflurane, the telemetry transducers were placed intraperitoneally and a blood pressure catheter was inserted into the femoral artery, ECG electrodes were tunnelled subcutaneously and were fixed on the right shoulder and left lower thorax.

All experiments using animals were performed under Home Office License (UK) and with the approval of individual institutional ethics committees.

Results

Biochemistry

Surface plasmon resonance analysis verified the binding of SEN1576 to A$\beta_{1–42}$ monomer and oligomers. Immobilized A$\beta_{1–42}$ was characterized using 6E10, 4G8, A11 and OC antibodies. For the A$\beta_{1–42}$ monomer surface, 6E10 and 4G8 showed strong binding responses, whereas A11 and OC (Aβ oligomer and Aβ fibril specific, respectively) showed no binding. In contrast, for the A$\beta_{1–42}$ oligomer surface A11 and OC showed strong binding responses and only weak signals were apparent with 6E10 and 4G8. SEN1576 bound to monomeric A$\beta_{1–42}$ in a concentration related manner, with a K_D of 3.8 μM, which suggests that its mode of action involved direct interaction with A$\beta_{1–42}$. SEN1576 also bound to A$\beta_{1–42}$ oligomers in a concentration related manner (Fig. 2). Incubation of SEN1576 (10 μM) with A$\beta_{1–42}$ (10 μM) gave a 46% reduction in thioflavin-T fluorescence, demonstrating inhibition of the A$\beta_{1–42}$ aggregation process. In the MTT

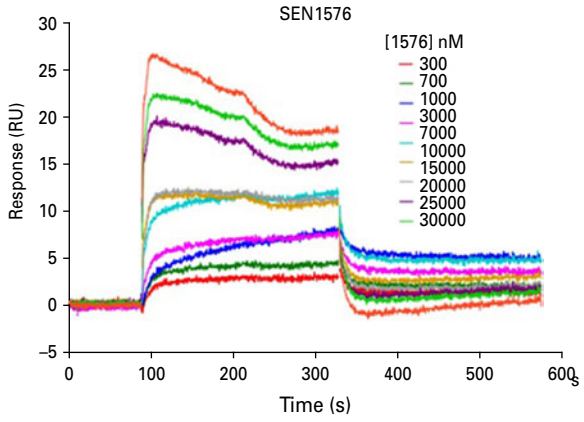

Fig. 2. Surface plasmon resonance curves elicited by binding of SEN1576 (300–30000 nM) to monomeric Aβ_{1-42}.

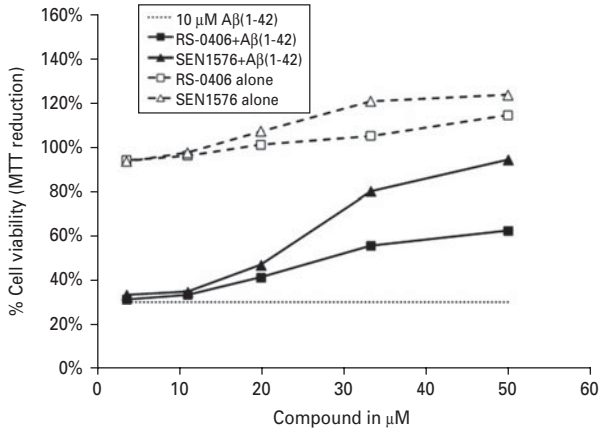

Fig. 3. Concentration response curve of RS-0406 and SEN1576 incubated with 10 μM Aβ_{1-42} on SHSY5Y cells.

cell viability assay, SEN1576 protected the SH-SY5Y neuronal cell line exposed to Aβ_{1-42} (10 μM) insult; IC_{50}= 23 μM (Fig. 3). With respect to effects on liver microsomal enzymes and/or the metabolite profiles, half-lives in the presence of human and rat liver microsomes were >60 min and the half-life in hepatocytes was >120 min.

Pharmacokinetics

Key pharmacokinetic parameters for SEN1576 in the rat are summarized in Table 1. SEN1576 was well-absorbed following oral administration and penetrated the brain. Oral bioavailability was 56%, and following a 2 mg/kg i.v. dose the mean brain:plasma ratio over the first 3 h was 1.65:1. SEN1576 had a low clearance rate (CL= 15 ml/min/kg) and a Cmax of 4.5 μM, indicating a suitable profile for oral administration. Based upon the per cent plasma protein (91.9) and brain homogenate binding (96.4) of SEN1576, it was predicted that a 20 mg/kg oral dose would provide ~350 and ~250 nM free drug in the plasma and brain, respectively, at the Tmax. A 60 mg/kg/day oral dose of SEN1576 administered to mice (n=6) for 14 days resulted in a mean plasma concentration of 0.97 μM and a mean brain concentration of 7.7 μM. These data indicated that SEN1576 had a suitable profile for evaluation in *in vivo* efficacy studies using oral administration.

Electrophysiology

In the control conditions, i.c.v. administration of CHO CM, followed by i.v. injections of SEN1576 vehicle, at the end of the recording period and 90 min after induction, produced LTP with PS amplitude of 180±8% of baseline (n=7, Fig. 4*a*). In rats injected i.c.v. with 7PA2 CM and treated with i.v. vehicle, the LTP of PS amplitude was reduced to 118±9% of baseline (n=7, Fig. 4*b*). In rats injected i.c.v. with 7PA2 CM and treated i.v. with SEN1576 at 0.3 mg/kg, the LTP of PS amplitude was 164±10% of baseline (n=6, Fig. 4*b, d*); which, following i.v. treatment with 1.0 mg/kg SEN1576, the LTP of PS amplitude was 175±1% of baseline (n=6, Fig. 4*c, d*). SEN1576 treatment at 1.0 mg/kg had no effect on LTP in rats administered i.c.v. with CHO CM, with LTP of the PS amounting to 176±11% of baseline (n=8, Fig. 4*a, d*).

Operant behaviour

There was a statistically significant overall treatment effect on lever switching errors ($F_{5,978}$=4.83, p=0.0007), this can be seen in Fig. 5*a*. The i.c.v. 7PA2 CM-injected group exhibited significantly more lever switching errors as compared to the group injected i.c.v. with CHO CM and the group pre-dosed with 20 mg/kg SEN1576 and injected i.c.v. with CHO CM (p's<0.001). The i.c.v. 7PA2 CM injected groups pre-dosed with 5 and 20 mg/kg SEN1576 exhibited significantly fewer lever-switching errors (p=0.0017 and 0.0061, respectively) but the reduction in lever switching errors was marginally beyond significance in the group pre-dosed with 1 mg/kg SEN1576 (p=0.051). There was also a statistically significant overall treatment effect on incorrect lever perseverations ($F_{5,78}$= 5.73, p<0.0001), this can be seen in Fig. 5*b*. The i.c.v. 7PA2 CM-injected group exhibited significantly more incorrect lever perseverations as compared to the group injected i.c.v. with CHO CM and the group pre-dosed with 20 mg/kg SEN1576 and injected i.c.v. with CHO CM (p's<0.001). The i.c.v. 7PA2-CM injected groups pre-dosed with 5 and 20 mg/kg SEN1576 exhibited significantly fewer incorrect lever perseverations (p=0.001 and 0.036, respectively) but the reduction in incorrect lever perseverations was not significant in the group pre-dosed with 1 mg/kg SEN1576 (p=0.33). The ALCR schedule also provides data on actual rates of responding at each schedule value. That is, the running response rate (RRR), which is calculated as the response rate minus the post-reinforcement pause duration indicates the actual rate of lever pressing. In the current study, there were no significant differences (p's<0.05) in RRRs between the control and experimental groups at any drug dose, suggesting

Table 1. Key pharmacokinetic parameters for SEN1576

Dose	$t_{½}$ (min)	CL (ml/min/kg)	V_d (ml/kg)	AUCINF (min.mg/ml)
2 mg/kg, i.v.	377	17	9181	122 807
Dose	$t_{½}$ (min)	C_{max} (ng/ml)	T_{max} (min)	AUCINF (min.mg/ml)
20 mg/kg, p.o.	424	1855	50	709 190

that SEN1576 did not induce sedation or cause hyperactivity.

Ames test

SEN1576 was tested up to the limit of solubility of 1500 μg/plate and did not show any significant effect (p's>0.05, Dunnett's t-statistic calculated for each group) against the four *Salmonella typhimurium* strains or the *Escherichia coli* strain in the presence or absence of the S-9 mix. These results indicate that SEN1576 was not mutagenic.

Cardiovascular safety pharmacology

During the cardiovascular assessment in conscious telemetered guinea pigs, SEN1576 (30, 100 and 300 mg/kg orally) had no significant effect (p's>0.05) on blood pressure (systolic, diastolic and mean), heart rate, or any other electrophysiological parameters, such as corrected QT interval, at any of the doses tested. Analysis of change from baseline data relative to respective vehicle treatment was conducted using Dunnett's *post-hoc* test following ANOVA with repeated measures for all parameters.

Discussion

The development of pharmacotherapies for treatment of the dementia syndrome seen in AD has been a considerable challenge. Approved pharmacological interventions provide only limited mitigation of symptomatology, however revision of the amyloid cascade hypothesis to incorporate contemporary findings relative to the role of prefibrillar Aβ in AD (Walsh and Selkoe, 2007), has opened avenues for the development of new compounds that target mechanisms prior to the complete fibrillation of Aβ. Synthetic Aβ oligomers and cell-derived 7PA2 CM oligomers of Aβ have been shown to produce behavioural toxicity (Reed *et al.*, 2011), hence pharmacotherapeutic targeting of the Aβ fibrillation mechanism at the oligomer level makes sound scientific sense. Our group modified the non-peptide small molecule, RS-0406, previously shown to inhibit Aβ toxicity and A$\beta_{1–42}$ aggregation (Nakagami *et al.*, 2002), to protect primary hippocampal neurons against A$\beta_{1–42}$-induced cytotoxicity, to rescue A$\beta_{1–42}$-impaired LTP (Walsh *et al.*, 2005b), and to arrest 7PA2 CM-induced behavioural deterioration (Poling *et al.*, 2008). Early modifications produced a pharmacologically active non-peptide small molecule, SEN1269, that when administered i.c.v. was protective against Aβ-induced cell death, *in vitro* and *in vivo* synaptotoxicity and behavioural deterioration induced by 7PA2 CM (Scopes *et al.*, 2012). Subsequent modifications produced an orally bioavailable CNS-penetrating non-peptide small molecule, SEN1500, which bound to A$\beta_{1–42}$ monomer and 7PA2 CM, reduced thioflavin-T fluorescence and protected neuronal cells and primary cortical neurons exposed to A$\beta_{1–42}$, alleviated *in vitro* depression of LTP induced by synthetic A$\beta_{1–42}$ and 7PA2 CM, *in vivo* depression of LTP induced by 7PA2 CM, and oral administration of SEN1500 reduced behavioural deterioration induced by 7PA2 CM (O'Hare *et al.*, 2013).

The current study investigated the effects of SEN1576 relative to pharmacokinetic, biochemical, electrophysiological, behavioural, mutagenic and physiological factors relevant to the development of a compound for the treatment of AD in humans. These findings indicate that SEN1576 is not mutagenic, and does not induce adverse physiological consequences in normal animals, such as effects on cardiological function. The pharmacokinetic data from the rat and mouse studies indicate that SEN1576 had greater brain penetration than its parent compound SEN1500. In the current study, SEN1576 bound to monomeric A$\beta_{1–42}$ in a concentration related manner, the K_D of 3.8 μM suggested a mode of action involving direct interaction with A$\beta_{1–42}$ [K_D for SEN1500=3.4 μM (O'Hare *et al.*, 2013)], and also bound to A$\beta_{1–42}$ oligomers. When SEN1576 (10 μM) was incubated with A$\beta_{1–42}$, there was a 46% reduction in thioflavin-T fluorescence [41% for SEN1500 (O'Hare *et al.*, 2013)], thus demonstrating inhibition of the A$\beta_{1–42}$ aggregation process. SEN1576 also protected the SH-SY5Y neuronal cell line following exposure to A$\beta_{1–42}$, IC_{50}=23 μM [IC_{50}=26 μM for SEN1500 (O'Hare *et al.*, 2013)]. Experimental parameters for the parent compound, RS-0406, have been described previously (O'Hare *et al.*, 2010), showing ~35% inhibition at 10 μM in the thioflavin-T assay and ~40% protection at 40 μM in the MTT assay. The pharmacokinetic data obtained in rats (Table 1) show that following oral administration, SEN1576 was well absorbed and penetrated the brain. Oral bioavailability was 56% [42% for SEN1500 (O'Hare *et al.*, 2013)], and after a 2 mg/kg i.v. injection the

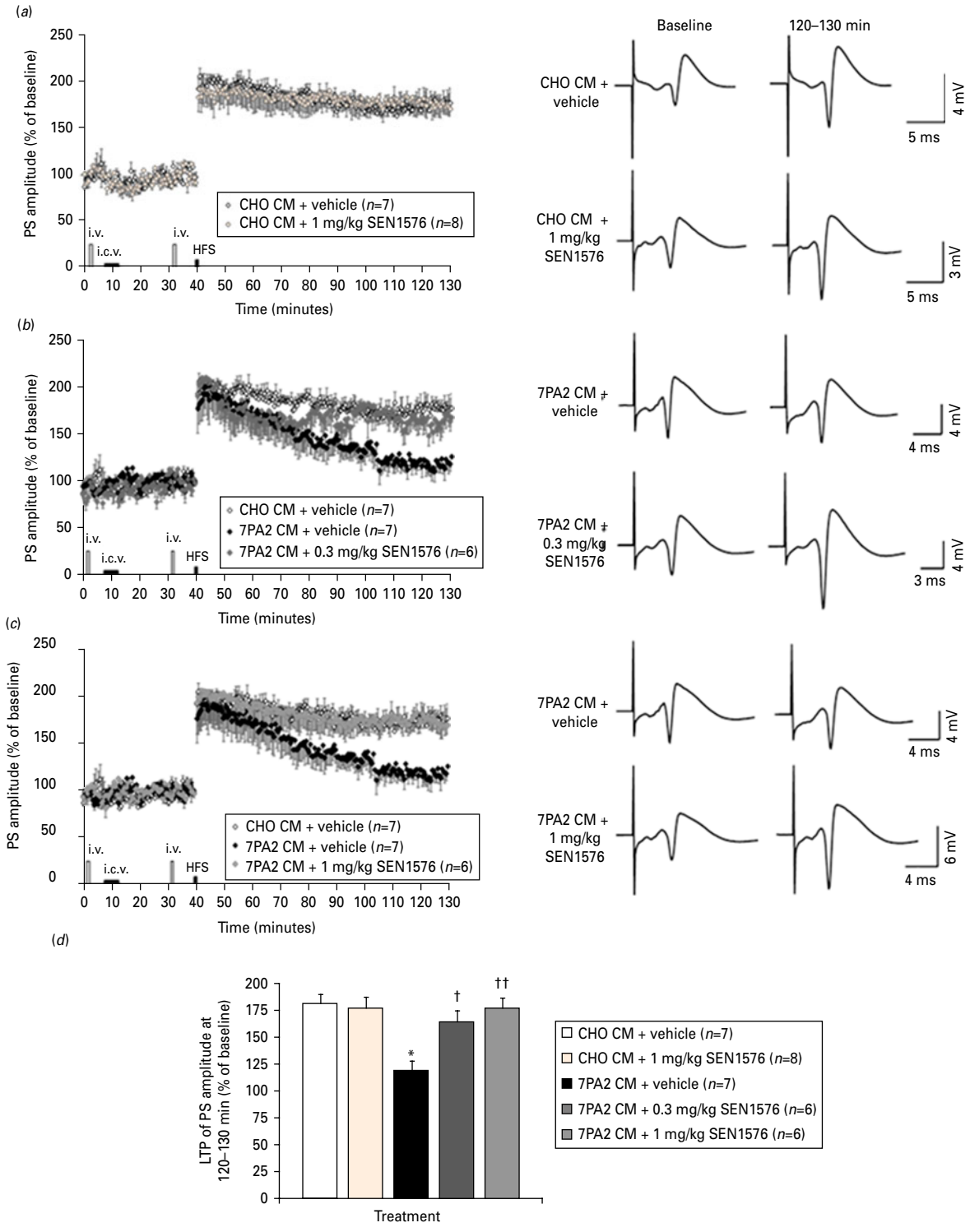

Fig. 4. (*a*) Scatter plot and typical PS traces demonstrating LTP of PS amplitude after CHO CM injection i.c.v. with vehicle or 1 mg/kg SEN1576 i.v. administration in the CA1 region of anaesthetized rats. (HFS: high-frequency stimulation). (*b*) Scatter plot and typical PS traces demonstrating LTP of PS amplitude after CHO CM and 7PA2 CM injection i.c.v. with vehicle or 0.3 mg/kg SEN1576 i.v. administration in the CA1 region of anaesthetized rats. (*c*) Scatter plot and typical PS traces demonstrating LTP of PS amplitude after CHO CM and 7PA2 CM injection i.c.v. with vehicle or 1 mg/kg SEN1576 i.v. administration in the CA1 region of anaesthetized rats. (*d*) Effect of SEN1576 (0.3 mg/kg and 1 mg/kg i.v.) treatment on i.c.v. 7PA2 CM-induced suppression of LTP. Data presented as mean±S.E.M. *$p<0.01$ indicates significant difference between the i.c.v. 7PA2 CM-injected group as compared to the i.c.v. CHO CM-injected group and the i.c.v. CHO CM-injected group treated with 1 mg/kg SEN1576. †$p<0.05$ and ††$p<0.01$ indicates significant differences between the i.c.v. 7PA2 CM-injected vehicle treated group as compared to the i.c.v. 7PA2 CM-injected groups treated with 0.3 mg/kg SEN1576 and 1 mg/kg SEN1576 i.v.

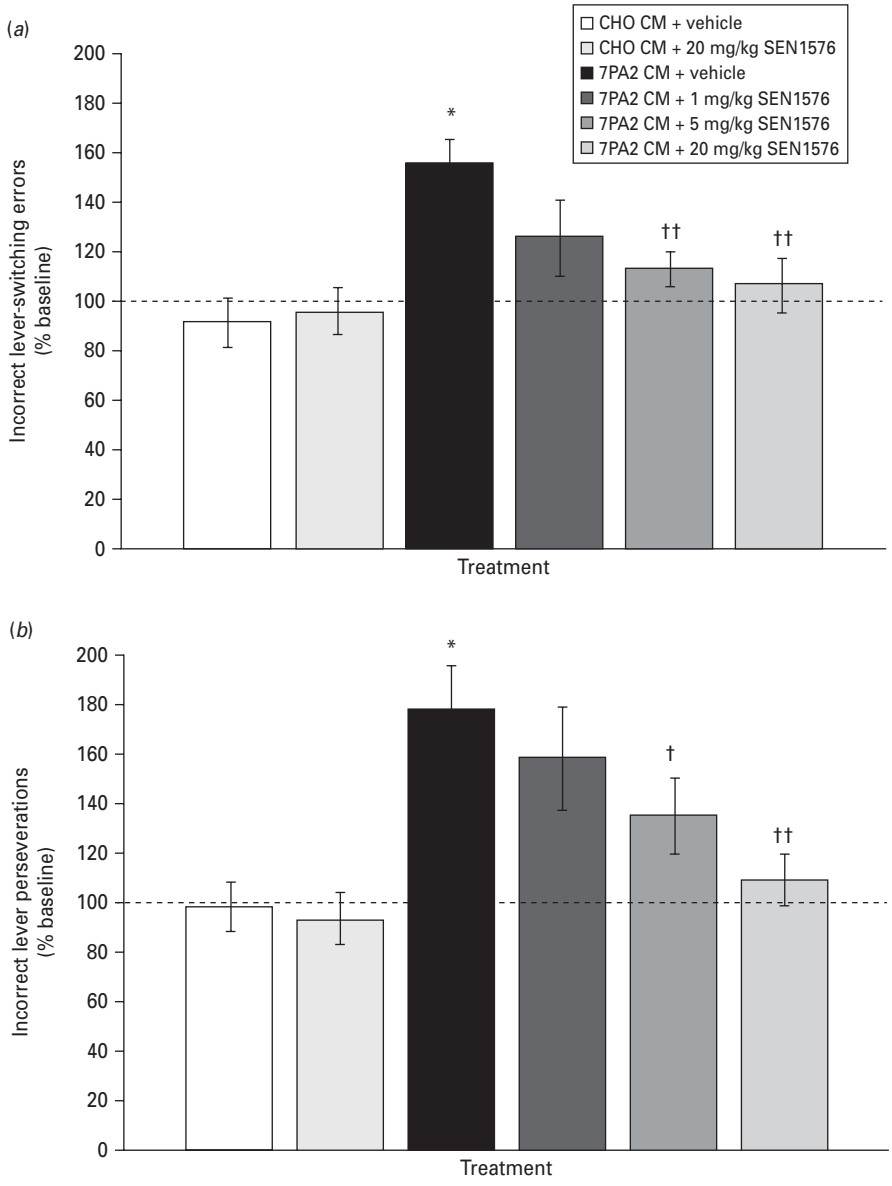

Fig. 5. Effect of SEN1576 (1, 5 and 20 mg/kg, oral) treatment on i.c.v. 7PA2 CM-induced lever-switching errors (*a*) and incorrect lever perseverations (*b*). Data presented as mean±S.E.M. *$p<0.001$ indicates significant difference between the i.c.v. 7PA2 CM-injected group as compared to the i.c.v. CHO CM-injected group and the i.c.v. CHO CM-injected group treated with 20 mg/kg SEN1576. †$p<0.05$ and ††$p<0.01$ indicates significant differences between the i.c.v. 7PA2 CM-injected group as compared to the i.c.v. 7PA2 CM-injected groups treated with 5 mg/kg SEN1576 and 20 mg/kg SEN1576.

mean brain:plasma ratio over the first 3 h was 1.65:1; the corresponding brain:plasma ratio for SEN1500 was 0.77:1 (O'Hare *et al.*, 2013), showing superiority of SEN1576 as a potential treatment for accumulation of Aβ in the brain. Although clearance of SEN1576 (15 ml/min/kg) was higher than that for SEN1500 (6 ml/min/kg), its higher volume of distribution results in a longer half-life (see Supplementary Materials). It is of note that in the study conducted using mice, SEN1576 administered orally over a 14 d period produced a mean plasma concentration of 0.97 μM and a mean brain concentration of 7.7 μM, a brain to plasma ratio of 7.9. Corresponding data for SEN1500 demonstrated a mean plasma concentration of 11.2 μM and a mean brain concentration of 6.4 μM, a brain to plasma ratio of 0.57. While SEN1576 binds to serum proteins this does not preclude CNS exposure of the compound. SEN1576 was submitted for biochemical profiling against 69 non-peptide, peptide and nuclear receptors, ion channels and amine transporters, and 23 enzymes, the findings from these studies confirmed the selectivity of SEN1576 for interacting with Aβ.

The *in vivo* LTP experiment demonstrated that SEN1576 treatment at 0.3 and 1 mg/kg i.v. significantly rescued the depression of LTP in the CA1 region of the hippocampus induced following i.c.v. administration

of 7PA2 CM. This finding indicates that, using the LTP model of synaptic activity, indicating cellular mechanisms underlying learning and memory, SEN1576 protected against 7PA2 CM-induced synaptic toxicity. SEN1576 treatment held the depression of LTP following 7PA2 CM administration to 164±10% of baseline (180±8% in the control condition and 118±9% in the 7PA2 CM condition) following treatment with 0.3 mg/kg SEN1576, and to 175±10% of baseline following treatment with 1 mg/kg SEN1576. These data show that SEN1576 was effective in protecting against the synaptotoxicity induced following i.c.v. 7PA2 CM administration. Taken in conjunction with the results of the biochemical assays, these findings indicate that SEN1576 should provide a useful pharmacotherapeutic intervention for the control of dementia in AD.

Investigation of the effects of SEN1576 relative to memory in freely moving intact animals employed the ALCR operant schedule of food reinforcement. This procedure has been shown to be highly sensitive to adverse effects on memory induced by psychopharmacological manipulations (Weldon *et al.*, 1996), and has been used extensively in contemporary models of dementia research involving experimental animals (e.g., Cleary *et al.*, 2005; Townsend *et al.*, 2006; Poling *et al.*, 2008; O'Hare *et al.*, 2010), we investigated the effects of orally administered SEN1576 treatment following i.c.v. 7PA2 CM injections in the rat. The results of these studies indicated that oral administration of SEN1576 significantly and dose-dependently reduced lever-switching errors and incorrect lever perseverations under the ALCR schedule; this finding denotes a protective effect on memory deterioration following an i.c.v. injection administered 7PA2 CM insult. SEN1576 did not alter RRRs, thus indicating that it did not induce sedation or cause hyperactivity in the rat, and at doses of 100 and 300 mg/kg it did not affect blood pressure in the guinea pig, thus suggesting no effects on anxiety. SEN1576 was also found to have an encouraging pharmacokinetic, toxicology and safe pharmacological profile for progression as a dementia-modifying drug. Future studies, using transgenic animals will employ imaging techniques to evaluate the chronic effect of SEN1576 on brain Aβ oligomer proliferation and Aβ aggregate burden.

Supplementary material

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/1461145713000886.

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Statement of interest

D.I.C.S., H.A., E.N. and J.M.T. were employees of Senexis Limited when these studies were conducted, D.S and R.J. are employees of Neurosolutions Limited.

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