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Short communication

Investigations of amide bond variation and biaryl modification in analogues of α7 nAChR agonist SEN12333

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ABSTRACT

Several lines of experimental evidence support the involvement of the α_7 nAChR in schizophrenia and Alzheimer's disease. Modulators of the α7 nAChR have been extensively reviewed for the treatment of the cognitive deficits associated with these pathologies. SEN12333 represents a novel α7 nAChR agonist chemotype with potential for reduced side effects but requiring further SAR exploration. The present work investigates the amide bond of SEN12333, specifically its connectivity and replacement with the tetrazole functionality, a known *cis* amide isostere. The results reveal the original amide bond connectivity of SEN12333 to be favorable for binding affinity and agonist activity at α7 nAChRs. The use of a tetrazole isostere completely abolishes affinity and functional activity and suggests that SEN12333 binds in a linear conformation. Results reported herein also suggest the pyridine nitrogen within the terminal aromatic ring of SEN12333 is not essential for binding affinity or functional activity. Further SAR investigations involving manipulation of other moieties contained within SEN12333 are warranted.

1. Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs) belong to the superfamily of ligand-gated cation channels and have recently generated extensive interest as potential targets of therapeutics in the treatment of cognitive disorders [1–3]. These receptors exist as pentameric transmembrane ion channels where subtype is formed from either a homo- or heteropentameric combination of twelve possible subunits: α2–α10 and β2–β4. The homomeric α7 nAChR is one of the most commonly expressed nicotinic receptors in the human brain, found in high levels in regions associated with learning and memory, such as the cerebral cortex and the hippocampus [4]. Experimental evidence supports the involvement of the α7 nAChR in schizophrenia and Alzheimer's disease (AD) [5]. Modulators of α7 nAChRs have been extensively reviewed for the treatment of the cognitive deficits associated with these pathologies [6–8].

Multiple α7 agonists have entered clinical trials. Unfortunately, due to unfavorable side effects and pharmacokinetic issues the majority of these studies have been discontinued [9]. The known α7 ligands currently and previously in clinical trials display relatively little structural diversity and are primarily centered on anabaseine, quinuclidine, or diazabicyclic scaffolds [10]. Consequently, the majority of them possess the same cross-reactivity profiles with other sites and similar unwanted side effects. There is a strong demand for structurally differentiated α7 nAChR agonists with more desirable drug-like properties and improved cross-reactivity profiles for research in this field to further progress.

High-throughput screening by Siena Biotech and Wyeth has identified piperazine **1** (Fig. 1) as a novel chemotype of weak, partial agonist activity at α7 nAChRs. Preliminary SAR investigation

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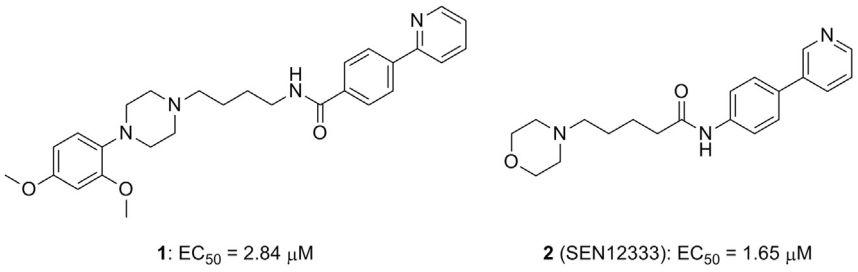


Fig. 1. Agonists at α_7 nAChRs identified by Siena Biotech and Wyeth.

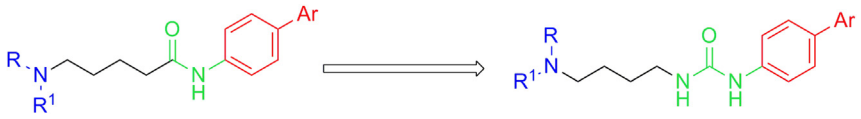


Fig. 2. The incorporation of the urea functionality with pharmacophoric elements: basic center (blue), carbon chain (black), central linker (green) and biaryl system (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

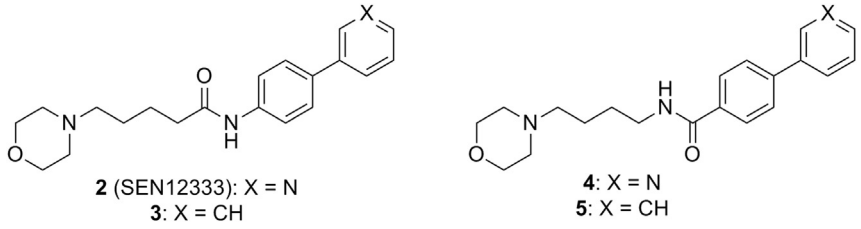


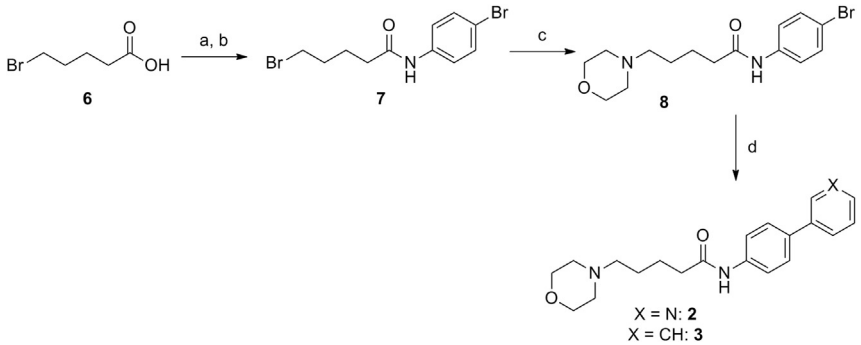
Fig. 3. Amido-varied SEN12333 analogues.

of the piperazine, biaryl and amide regions of compound **1** resulted in the development of SEN12333 (**2**) [11]. SEN12333 is an α7 nAChR agonist, with selectivity over other nAChR subtypes, 5-HT3 receptors, and hERG channels [11,12]. Additionally, it displayed favorable bioavailability and brain permeation *in vivo* [12]. Initial investigations of SEN12333 (**2**) in animal models of episodic memory confirmed its ability to reverse both scopolamine- and MK-801-induced amnesia [11,12].

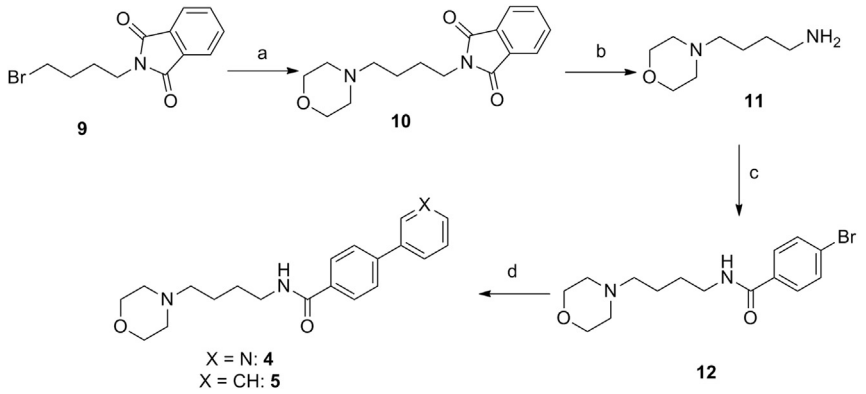
Limited SAR data is reported for this unique class of α7 nAChR ligands, with brief studies conducted into modification of the morpholine ring and variation of the arylanilide groups [11]. Previous work within our laboratory has identified the 4-carbon chain linker of compound **2** to be optimal for binding and functional activity at α7 nAChRs [13]. Minimal SAR investigation is available for the amide bond of SEN12333 (**2**). Haydaar and co-workers have described replacement of the amide moiety with the urea functionality (Fig. 2) [14]. Though these compounds generally exhibited enhanced potency at α7 nAChRs, they also demonstrated low selectivity, particularly against receptors containing α3 subunit, unfavorable hERG activity and substantial CYP450 inhibition [14].

The present investigations were aimed at exploring the connectivity of the amide bond. The transposition of amide to reverse-amide is a concept well-known to medicinal chemistry [15–17]. Additionally, the effect of the pyridine nitrogen within the terminal aromatic ring of SEN12333 (**2**) was simultaneously investigated. The lack of a clear rationale why the pyridine nitrogen was moved from the 2-position in original hit **1** to the 3-position in SEN12333 (**2**), prompted us to question if it was required at all. Accordingly, compounds **3**–**5** were designed to address this question (Fig. 3).

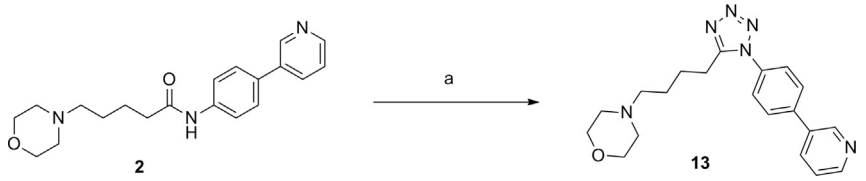
Aside from investigating the amide connectivity, the amide linker was also replaced with a tetrazole group. The selection of a tetrazole linker is based upon the knowledge that tetrazoles are *cis*-amide isosteres [18–21]. It was envisioned that this would provide us further mechanistic detail into the binding mode of SEN12333 (**2**)



Scheme 1. Reagents and conditions: (a) $(COCl)_2$, rt, 45 min; (b) 4-bromoaniline, Et_3N, CH_2Cl_2, −78 °C to rt, 4 h, 91% yield over 2 steps; (c) morpholine, NaI, Et_3N, DMF, reflux, 16 h, 85%; (d) $Pd(PPh_3)_4$, appropriate boronic acid, MeCN/sat. aq. Na_2CO_3 (1:1), reflux, 18 h, 68 or 83%.



Scheme 2. Reagents and conditions: (a) Morpholine, NaI, Et_3N, THF, reflux, 16 h, 66%; (b) $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 1 h; (c) 4-bromobenzoyl chloride, Et_3N, CH_2CH_2, −78 °C to rt, 2 h, 64% over two steps; (d) $Pd(PPh_3)_4$, appropriate boronic acid, MeCN/sat. aq. Na_2CO_3 (1:1), reflux, 18 h, 70 or 91%.



Scheme 3. Reagents and conditions. (a) (i) PCl_5, CH_2Cl_2, rt, 2 h; (ii) $TMSN_3$, 0 °C, 16 h, 40–71%.

at α_7 nAChRs and potentially reduce the number of rotatable bonds within the molecule. Tetrazoles are resistant to certain metabolic pathways, and their incorporation can often result in increased bioavailability of a drug [18]. There are two regioisomeric tetrazoles to be studied. One series would be accessed from SEN12333 (**2**) and analogues containing this original amide bond connectivity (**3**); whereas the other tetrazole series could be accessed from the related 'reverse-amide' bond analogues (**4** and **5**).

2. Chemistry

The syntheses of analogues **2** and **3** are depicted in Scheme 1 and involved the initial conversion of 5-bromovaleric acid (**6**) to the corresponding acid chloride. Subsequent treatment with 4-bromoaniline in the presence of triethylamine gave desired anilide **7** (91%). Alkylation of morpholine with bromide **7** in the presence of catalytic iodide afford compound **8** in 85% yield. Subjecting bromoarene **8** to Suzuki cross-coupling with either 3-pyridylboronic acid or phenylboronic acid gave the desired ligands SEN12333 (**2**) and **3** (83 or 68%) respectively. SEN12333 (**2**) was synthesized for direct comparison with the new analogues.

An alternative synthesis was proposed for reverse-amide analogues **4** and **5** (Scheme 2). Alkylation of morpholine with bromide **9** gave amine **10** (66%), subsequent hydrazinolysis and amide bond formation yielded compound **12** (64% over two steps). Final Suzuki cross-coupling with either 3-pyridylboronic acid or phenylboronic acid gave the desired retro-amide ligands **4** and **5** (70 or 91%) respectively.

The corresponding tetrazole analogues **13**–**16** were readily synthesized from amides **2**–**5**, a representative example is shown in Scheme 3. The amides **2**–**5** were treated with phosphorus pentachloride to form the presumed corresponding imidoyl chloride. However this was not isolated as the addition of trimethylsilylazide furnished the desired tetrazoles **13**–**16** following basic workup (40–71%).

Single crystals of SEN12333 (**2**) and tetrazole **13** were grown by slow recrystallization in CH_2Cl_2 and *n*-hexane and subjected to X-Ray crystal analysis. In addition to structure confirmation, it was thought that by identifying their solid-state conformations it would help to further predict the mode of binding of the amide and tetrazole analogues. The Oak Ridge Thermal Ellipsoid Plots (ORTEP) of SEN12333 (**2**) and tetrazole **13** are shown in Figs. 4 and 5 respectively. SEN12333 (**2**, Fig. 4) has a large degree of conformational flexibility and it is immediately apparent that its lowest energy configuration state adopts a *trans* amide conformation to linearize the structure and minimize steric interactions. In contrast, the tetrazole ring present within compound **13** locks the amide bond

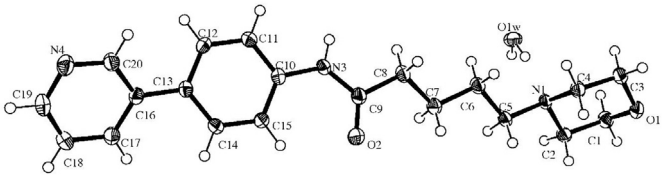


Fig. 4. ORTEP representation of SEN12333 (**2**).

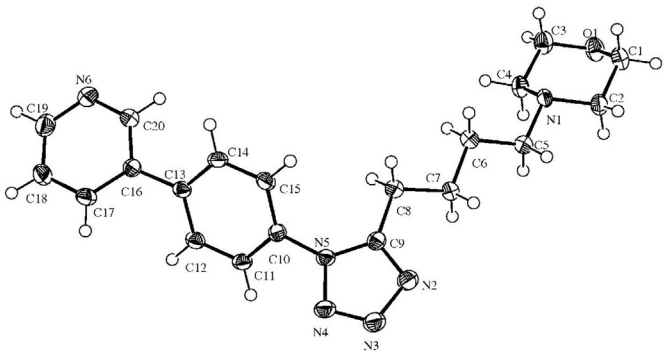
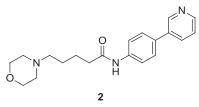
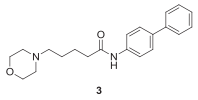
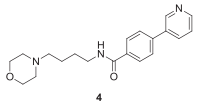
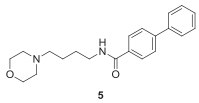
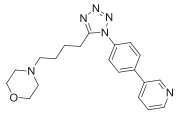
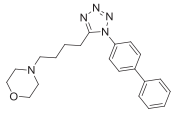
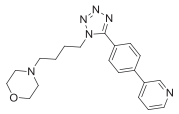
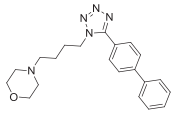


Fig. 5. ORTREP representation of tetrazole **13**.

Table 1
Binding affinities of newly synthesized compounds.

Compound	K_i (nM)[a]			Physicochemical properties		
	α7	α4β2	α3β4	CLogP[b]	PSA[b]	LLE[c]
 2	670 ± 119	>10,000	>10,000	2.25	53.93	3.92
 3	2300 ± 1000	>10,000	>10,000	3.58	41.57	2.06
 4	11,000 ± 4000	>10,000	>10,000	1.93	53.93	3.02
 5	1800 ± 200	>10,000	>10,000	3.28	41.57	2.46
 13	216,000	DNC	DNC	2.08	65.15	1.59
 14	236,000	DNC	>1,000,000	3.47	52.79	0.16
 15	165,000	DNC	>1,000,000	2.81	65.15	0.97
 16	156,000	DNC	>100,000	4.31	52.79	−0.5

[a] Competition curves were constructed in which 10 concentrations of a sample were included. DNC = the fitting does not converge due to extremely low affinities. K_i values are the Mean ± SEM of three to six independent measurements. For low binding affinity compounds, estimated K_i values or K_i value ranges were provided.
[b] Calculated using ChemDraw12.0.
[c] Calculated according to equation LLE = pIC_{50} (or pK_i) − cLogP.

into a *cis* conformation and as illustrated in Fig. 5. It is evident that the compound **13** resides in a bent form. If the binding conformation of SEN12333 (2) is similar to the low energy, *trans* amide conformation depicted in Fig. 4, these studies suggest the mode of binding of SEN12333 (2) and tetrazole 13 would be vastly different. It was envisioned that this would additionally provide greater insight into the binding mode of SEN12333 (2), specifically, if the amide bond was residing in either a *cis* or *trans* conformation. It was thought that reverse-amide **4** would have a similar conformation to SEN12333 (**2**) while the tetrazole **15** would mimic analogue **13**, however, X-Ray crystal analysis was only obtained for a representative example of each class of compounds.

3. Pharmacology

All newly synthesized compounds and SEN12333 (**2**) as a reference were evaluated in competitive binding assays to determine their affinity and selectivity for the α7 nAChR. The results are summarized in Table 1. All compounds were selective for the α7 nAChR subtype; however, a large range of affinities was evident. Removal of the pyridine nitrogen from reference SEN12333 (**2**) to give biphenyl analogue **3** resulted in a decrease in affinity. Reversal of the amide bond order of SEN12333 (**2**) to give compound **4** led to a marked decrease in affinity, however, this decrease in affinity was restored partially in retro-amide biphenyl analogue **5**. These results suggest the original amide bond order of SEN12333 (**2**) to be favorable and that the pyridine nitrogen is not essential for binding or selectivity at α7 receptors. Conversion of compounds to their corresponding tetrazole resulted in a loss of affinity at α7 receptors with K_i values over 150 μM for all tetrazole analogues. Considering the high concentrations required for the tetrazole series, a clear determination of the effect of the pyridine or biphenyl compounds in this series was not possible. In contrast to compounds **2**–**5**, tetrazoles synthesized from the original amide

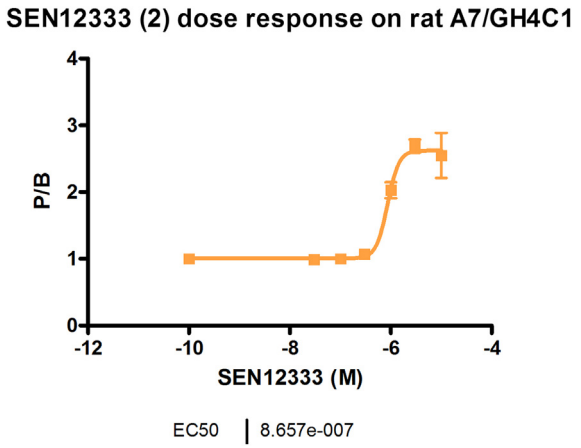


Fig. 6. Dose response curve of SEN12333 (**2**) on rat α_7 GH4C1 cells; EC_{50} = 0.86 μM.

bond order exhibited decreased affinity compared to tetrazoles synthesized from analogues containing the reversed amide bond order (K_i values **13** and **14** approximately 220 μM vs. those of **15** and **16** approximately 130 μM). These findings illustrate that the tetrazole motif is not tolerated as an amide isostere in this series of compounds. This could be attributed to one or more of a combination of effects including the electronics of the tetrazole ring, increased steric bulk, or the *cis* conformation of the tetrazole isostere as illustrated in Fig. 5.

Table 1 also describes the physicochemical properties cLogP, polar surface area (PSA) and ligand lipophilicity efficiency (LLE) for all newly synthesized compounds. It has been recognized for numerous years that the success of a lead in drug development is largely affected by its lipophilicity and the influence of LogP on drug potency, pharmacokinetics and toxicity [22]. Lipophilic bases can potentially trigger cardiovascular toxicological effects through interaction with hERG channels and tissue toxicity through promotion of cellular phospholipidosis [23]. In terms of absorption and permeability, the 'rule of five' guidelines define the optimum for cLogP to be less than 5. In line with this, the average clogP value of marketed CNS drugs is 3.43 [24–27]. PSA is commonly utilized in drug design in the optimization of a drug's capability to permeate cell membranes such as the BBB [28]. PSA of approximately 120 $Å^2$ is deemed necessary to permeate cell membranes and 40–70 $Å^2$ is required to cross the BBB [28,29]. The development of a novel index known as ligand lipophilicity efficiency (LLE) has served as an

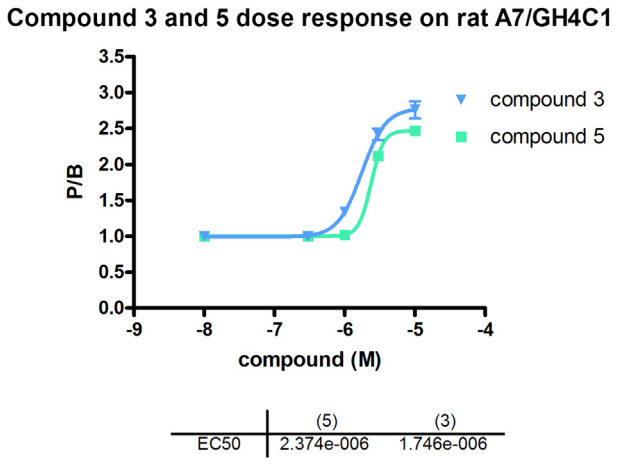


Fig. 7. Dose response curve of compound **3** and **5** on rat α_7 GH4C1 cells; **3**: EC_{50} = 1.7 μM, **5**: EC_{50} = 2 μM.

important guide in the process of lead optimization, and also as a flag for selection of preclinical candidates [30]. A typical oral drug with cLogP of 2.5 and potency within the range of 1–10 nM suggests a target LLE of 5–7 or greater, a value >5 is considered highly optimized for drug discovery. The advantages of high *in vivo* potency are low total doses in humans and decreased adventitious compound related toxicity [31].

All compounds display acceptable PSA values and cLogP < 5. Whilst biphenyl compound **5** displays a binding affinity similar to that of SEN12333 (**2**) (K_i values as 670 vs 1800 nM, respectively), its decreased LLE value of 2.46 suggests that it may not be as successful *in vivo*. While the cLogP and PSA values of compounds **4** and **5** are acceptable, their LLE values (3.02 and 2.46 respectively) also suggest their limited potential *in vivo*. Due to the extremely low binding affinities of tetrazole containing analogues **13**–**16**, they all display unacceptable LLE values, further confirming the limited use of tetrazoles as an amide isostere in SEN12333 (**2**) and related analogues.

To determine their agonistic activity at α7 nAChRs, SEN12333 and all amide-varied and tetrazole analogues were evaluated in a fluorescent calcium assay using a GH4C1 cell line expressing rat α7 receptors. Compounds were tested at concentrations of 0.3, 1, 3 and 10 μM ($n = 3$). Fluo4 Direct Dye from Molecular Probes was used and the assay was performed on a Perkin Elmer CellLux high throughput fluorescent reader. Peak/Base ratios were calculated to show activity where a peak/base values >1.00 was indicative of agonist activity.

Unfortunately, aside from SEN12333 (**2**), only compounds **3** and **5** showed agonism at α7 nAChRs (Figs. 6 and 7). This was to be expected given the concentration range tested and the K_i values from the binding studies (Table 1). The EC_{50} of SEN12333 (**2**) was observed to be 0.86 μM. The biphenyl analogue **3**, retained agonistic activity at α7 receptors with an EC_{50} of 1.7 μM. Retro-amide biphenyl analogue **5** similarly maintained agonistic activity with an EC_{50} of 2.0 μM. The lack of activity of the tetrazole containing compounds **13**–**16** highlights incorporation of this moiety is also detrimental for agonistic activity at α7 nAChRs.

4. Conclusions

In summary, seven new analogues of SEN12333 (**2**) have been synthesized to examine the effects of amide bond orientation, the use of a tetrazole as an amide bond isostere and the importance of the pyridine nitrogen within the biaryl system. The original amide bond orientation of SEN12333 (**2**) was found to be favored; reversal of the amide bond resulted in a significant loss of binding and agonistic activity at α7 nAChRs (**2** vs **4**). Biphenyl analogues of the reverse amide series were found to be more potent than their corresponding phenyl-pyridine analogues (**5** vs **4**) and importantly maintained agonistic activity at α7 nAChRs. Furthermore, the biphenyl motif of compound **5** was found to restore the potency lost in the reversed amide pyridine compound **4**. The use of a tetrazole functionality as an amide bond isostere resulted in a dramatic loss of potency and a complete loss of agonist activity for all types of analogues. The most potent compound of the series was still SEN12333 (**2**, K_i = 670 nM) where this amide bond orientation appears to be optimal for binding and activity at α7 nAChRs.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.07.029.

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