Bioorganic & Medicinal Chemistry Letters 24 (2014) 288–293

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery and optimization of Lu AF58801, a novel, selective and brain penetrant positive allosteric modulator of alpha-7 nicotinic acetylcholine receptors: Attenuation of subchronic phencyclidine (PCP)-induced cognitive deficits in rats following oral administration

Jørgen Eskildsen [a,*], John P. Redrobe [a], Anette G. Sams [a], Kim Dekermendjian [a], Morten Laursen [a], Jette B. Boll [a], Roger L. Papke [b], Christoffer Bundgaard [a], Kristen Frederiksen [a], Jesper F. Bastlund [a]

[a] Neuroscience Research DK, H. Lundbeck A/S, Ottiliavej 9, 2500 Valby, Denmark
[b] Department of Pharmacology, University of Florida College of Medicine, Gainesville, FL, USA

ARTICLE INFO

Article history:
Received 6 September 2013
Revised 7 November 2013
Accepted 10 November 2013
Available online 20 November 2013

Keywords:
Nicotinic acetylcholine receptors
Positive allosteric modulator
Electrophysiology
Lead optimization
Novel object recognition

ABSTRACT

In this Letter, we describe a chemical lead optimization campaign starting from a novel, weak α7 nicotinic acetylcholine receptor positive allosteric modulator (PAM) hit from a HTS screen. Exploration of the structure–activity relationships for α7 PAM potency, intrinsic hepatic clearance, the structure–property relationships for lipophilicity, and thermodynamic solubility, led to the identification of Lu AF58801: a potent, orally available, brain penetrant PAM of the α7 nicotinic acetylcholine receptor, showing efficacy in a novel object recognition task in rats treated subchronically with phencyclidine (PCP).

There is general consensus, that the release of acetylecholine (Ach) is a key factor in mnemonic mechanisms in humans[1] and rodents.[2] Nicotine is known to enhance attention and cognitive performance by a nonselective activation of multiple nicotinic acetylcholine receptors (nAChRs). nAChRs belong to the super family of ligand gated ionic channels, and gate the flow of cations including calcium. Seventeen subunits of nAChRs have been reported to date, which are identified as α2–α10, β1–β4, γ, δ, and ε. Many functionally distinct nAChR complexes exist, for example five alpha7 subunits can form homomeric α7 receptors (nAChRα7), which are some of the most abundant nicotinic receptors, along with heteromeric α4β2 receptors, in the brain (for review see[3]).

nAChRα7 are expressed in brain areas involved in cognitive processing such as the hippocampus, thalamic nuclei, and frontal cortex, and their physiological role in the modulation of neurotransmitter release, particularly glutamate and GABA, has focused attention on this subtype.[4] In addition to a physiological role, nAChRα7 have been implicated in certain pathological conditions in that decreased nAChRα7 expression have been reported in diseases associated with memory impairment, including schizophrenia and Alzheimer's disease.[5,6] Taken together, this evidence suggests that activation of nAChRα7's may lead to improvement of cognitive processing in human diseases. To this end, compounds like EVP6124 and TC5619, acting as agonists of the nAChRα7's, have recently been shown to alleviate cognitive disturbances associated with schizophrenia and Alzheimer's disease in small clinical phaseII studies.[7–9] Despite the proposed beneficial effects of nAChRα7 agonism, it remains uncertain whether chronic treatment with agonists provide suboptimal benefit due to sustained activation and subsequent desensitization of nAChRα7's.

In contrast to agonists, nAChRα7 PAMs can reinforce endogenous cholinergic transmission without directly stimulating the target receptor.[10] Thus, PAMs can selectively modulate the activity of ACh at nAChRα7's, preserving the activation and deactivation kinetics of the receptor, without leading to desensitization after prolonged binding to the receptor. Accordingly, nAChRα7 selective PAMs have emerged as potential drug candidates.[11] Here we report on a new series of nAChRα7 selective PAMs.[12]

High throughput screening of the Lundbeck compound collection identified a series of relatively lipophilic secondary amides (Fig. 1) compounds **1**–**3**.

* Corresponding author. Tel.: +45 36301311; fax: +45 36438293.
E-mail address: JORE@lundbeck.com (J. Eskildsen).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.11.022

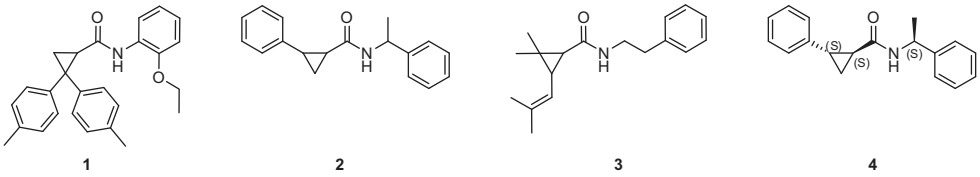
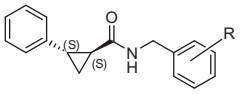


Figure 1. HTS hits **1**–**3** and pure enantiomer **4**.

Table 1
Profile of HTS hit **2**

Flux EC_{50}	3.3 μM
Flux Max*E*	37%
MW	265.35 amu
*c*log*P*[14]	3.4
tPSA	29 Å^2
$CL_{int,\ human}$	2.9 L/min
$CL_{int,\ rat}$	49 mL/min
CYP inhibition IC_{50} (μM)	2C9 (28) 2C19 (>40) 2D6 (19) 3A4 (12) 1A2 (>40)
Thermodynamic solubility pH 7.4	12 μg/mL

Table 2
SAR exploration of east side phenyl



Compd	R	EC_{50} (μM)	E_{max} (%)	$Cl_{int,human}$ (L/min)
5	H	4.2	11	4.8
6	2-Me	3.3	10	6.4
7	3-Me	1.9	4	8.1
8	4-Me	3.6	10	5.7
9	2-OMe	>20	1	6.9
10	3-OMe	3.6	9	5.8
11	4-OMe	4.5	13	2.8
12	2-F	3.7	15	6.6
13	3-F	2.4	12	6.2
14	4-F	2.7	8	5.4

A common feature was the 2-phenyl-cyclopropyl moeity on the carbonyl side and a phenyl group on the amino-side of the amide connected either via a single bond or via 1 or 2 carbon atoms. The most active compound, **2**, was profiled as shown in Table 1. The absolute configuration of **2** was not available during the initial HTS triage of primary hits, but analysis of the NMR spectra of **2** established the configuration of the cyclopropane moeity to be *trans*. Compound **2** showed an EC_{50} of 3.3 μM and E_{max} = 37% relative to an E_{max} for PNU120596. Incubation of **2** in human liver microsomes suggested a relatively high intrinsic clearance ($Cl_{int,human}$ = 2.9 L/min, liver blood flow 1.3 L/min). With respect to physicochemical properties, **2** showed a thermodynamic aqueous solubility at pH 7.4 of 12 μg/mL.[13] The combined observations suggested to us that an α7 PAM tool compound may be derived from these initial hits and we decided to invest in a hit-to-lead campaign with the aim of indentifying a brain penetrant α7 PAM tool compound. Key objectives were improvement in physicochemical and pharmacokinetic parameters, and to establish which degree of in-vitro modulation was necessary to achieve in-vivo effects at relevant plasma and brain exposures.

Separation of commercially available racemic *trans* 2-phenylcyclopropane carboxylic acid by Supercritical Fluid Chromatography (SFC) gave *R*,*R* and *S*,*Strans* compounds.[15] All α7 PAM activity was displayed by compounds derived from *S*,*Strans* 2-phenylcyclopropyl carboxylic acid. As both enantiomers of 1-phenylethanamines were commercially available, we were able to establish that compounds derived from *S*-1-phenylethanamine were dramatically more active than compounds derived from the R isomer. Thus we could conclude that practically all α7 PAM activity resided in compound **4** (Fig. 1).

The initial chemistry focus was the synthesis of amides derived from achiral benzylamines and *S*,*S*-*trans*cyclopropyl carboxylic acid to explore the SAR without significantly increasing the lipophilicity. Both the measured log*P* and the *c*log*P* of **4** was 3.4. Representative examples are shown in Table 2. The SAR, as with other allosteric ligands, was shallow and showed little variability in the EC_{50} values. Introduction of methyl-, methoxy- or fluoro in the *meta*- and *para*-portion of the benzylamine had little effect on the EC_{50} or E_{max} values. While the fluoro substituent was allowed in the *ortho*-, *meta*- and *para*-position (**12**–**14**) there was a tendency for intolerance of bigger substituents like methoxy (**9**) in the *ortho* position. Disappointingly, compounds **5** thru **14** also showed lower E_{max} values and high intrinsic microsomal clearance. The *c*log*P* neutral introduction of a 4-MeO substituent to give **11** demonstrated the best stabilization in human liver microsomes ($Cl_{int,human}$ = 2.8 L/min) combined with a relatively high E_{max} for this series of compounds. This general drop in E_{max} relative to **4** prompted us to investigate compounds containing the *S* methyl in the benzylic position. It was clear that a critical issue for the series would be optimization with respect to potency, microsomal stability and aqueous solubility.

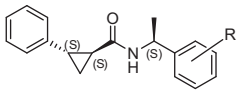
Based on experience from earlier projects, involving structurally related compounds, we knew there was a propensity for potassium channel modulation. In particular for lipophilic compounds bearing *meta* substituents. To monitor and mitigate this undesired cross-reactivity we therefore decided to include a human KCNQ2 screen in our SAR-driving screening cascade.[16] Representative examples illustrating the α7 SAR along with KCNQ2 data are shown in Table 3.

As shown in Table 3, high intrinsic human clearance values were observed for all these compounds, which may in part be the result of the relatively high lipophilicity of compounds **4**–**21**. The *c*log*P* for the compounds in Table 3 were between 3.3 and 3.9. Relatively high KCNQ2 potency was observed for **15**, **16** and **21**. Of particular interest to us was the 4-MeO substituted compound **18**, which offered an attractive balance between the α7 PAM potency, especially the high E_{max} of 65%, and medium to low activity in the KCNQ2 flux assay. However, the high intrinsic human clearance and low aqueous solubility of 1.8 μg/mL was unacceptable. Next, our exploration focused on the linker region with the specific aim to optimize the physicochemical and metabolic properties.

Representative examples of these efforts are shown in Table 4. The first analog **22** demonstrated, as also suggested from the initial HTS hits, that a two carbon linker was also allowed.

However, this resulted in a dramatic increase in intrinsic human clearance ($Cl_{int,human}$ = 7.5 L/min). A breakthrough was achieved with the identification of the *R* hydroxymethyl group as the optimal replacement of the *S* methyl linker substituent. The *R*

Table 3
Exploration of (*S*)-Me analogs



Compd	R	α7		*c*log*P*	$Cl_{int,\ human}$ (L/min)	hKCNQ2	
		EC_{50} (μM)	E_{max} (%)			EC_{50} (μM)	E_{max} (%)
4	H	3.2	37	3.4	4.1	8.4	67
15	4-Me	2.6	31	3.9	3.4	0.5	85
16	2-OMe	>20	0	3.3	7.2	1.2	84
17	3-OMe	3.1	11	3.3	5.7	4.5	72
18	4-OMe	1.5	65	3.3	4.6	4.9	57
19	2-F	2.0	50	3.5	3.6	4.1	58
20	3-F	3.1	41	3.5	5.4	3.2	80
21	4-F	2.2	60	3.5	3.7	1.8	67

alcohol **23** had a measured log*P* of 2.8, it displayed good α7 PAM potency, and had an aqueous solubility of 12.5 μg/mL. Most importantly, **23** showed a substantial decrease in intrinsic clearance to values below human liver blood flow. Compound **23** did not inhibit cytochrome P450 enzymes and exhibited high membrane permeability with no efflux liability. Compound **23** retained the KCNQ2 activity that was observed in **4** (EC_{50} = 3.8 μM/E_{max} = 87%). The absolute configuration of the hydroxymethyl group was an important SAR element for α7 PAM activity, as was the case for the methyl group in **4**. The *S* hydroxymethyl epimer **24** was devoid of any α7 PAM activity. Extending the linker substituent by one methylene, as in **25**, resulted in a drop in α7 PAM activity, and a significant increase in microsomal clearance.

In the final stage of the lead optimization campaign, we sought to combine our phenyl group SAR knowledge, as outlined in Table 3, with the linker SAR understanding outlined in Table 4. Representative examples are shown in Table 5.

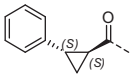
The first target molecule was the introduction of a 4-MeO substituent to give **26**, which from now on will be referred to as Lu AF58801. This modification led to an increase in α7 PAM activity, an improved selectivity against hKCNQ2, and a slight improvement in metabolic stability. The measured log*P* of 2.7 and aqueous solubility of 8 μg/mL was within our target profile for a α7 PAM tool compound. Introduction of a 4-CF_3O substituent, to give **27**, led to a substantial drop in aqueous solubility (<1 μg/mL), and an increase in intrinsic clearance. Extending the *para* substituent to 4-OEt, to give **28**, led to decreased metabolic stability. Fine tuning around compound Lu AF58801, by introduction of fluorines in compounds **29** thru **31**, did not result in compounds with an improved profile.

On the basis of the combined data, Lu AF58801 showed the best overall profile and was selected to be progressed for further studies.

The compounds were synthesized following the routes outlined in Schemes 1–3. Racemic *trans*-2-phenylcyclopropane carboxylic acids were separated into pure enantiomers using chiral SFC.[17]

The synthesis of racemic *trans*-2-phenylcyclopropane carboxylic acids is well described in the literature, for example, as outlined in Scheme 1.[18] Substituted cinnamic acids were reacted with *N*,*O*-dimethylhydroxylamine to give Weinreb type amides. Reaction with trimethylsulfonium iodide gave racemic *trans* 2-phenylcyclopropane carboxamides that could be hydrolyzed to racemic *trans* 2-phenylcyclopropyl carboxylic acids. The racemic compounds were separated into their *R*,*R* and *S*,*S* isomers by chiral SFC.[19] Chiral methylbenzylamines were either commercially available or were prepared by methods described by Ellman.[20]

Table 4
Exploration of linker analogs



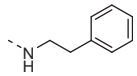
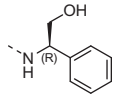
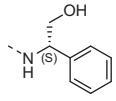
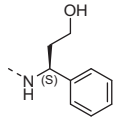
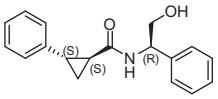
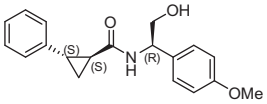
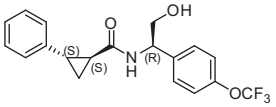
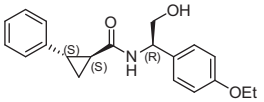
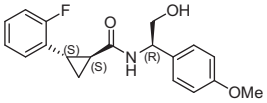
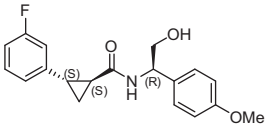
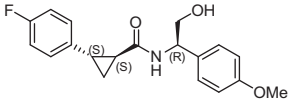
Compd	R	α7		Solubility (μg/mL)	*c*log*P*	$Cl_{int,\ human}$ (L/min)	hKCNQ2	
		EC_{50} (μM)	E_{max} (%)				EC_{50} (μM)	E_{max} (%)
22		2.8	21	—	3.2	7.5	17	15
23		2.0	65	12.5	2.4	0.9	3.8	87
24		>20	3	—	2.4	0.9	>10	—
25		4.3	21	19	2.7	4	25	72

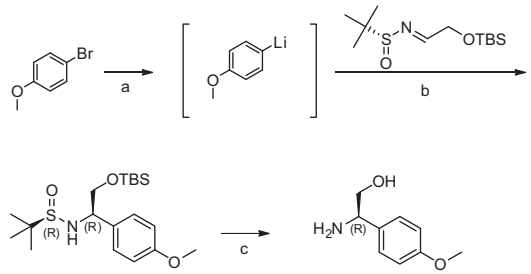
Table 5
Connecting the pieces

Compd		α7		Solubility (μg/mL)	*c*log*P*	$Cl_{int,\ human}$ (L/min)	hKNCQ2	
		EC_{50} (μM)	E_{max} (%)				EC_{50} (μM)	E_{max} (%)
23		2.0	65	12.5	2.4	0.9	3.8	87
26		0.9	64	8	2.3	0.7	6.7	87
27		2.5	26	<1	3.5	2.6	0.8	60
28		0.3	69	9	2.9	1.4	4.3	90
29		1.3	59	—[a]	2.5	1.7	2.8	89
30		0.6	60	3.5	2.5	1.2	6.0	86
31		0.6	70	—[a]	2.5	0.4	6.8	90

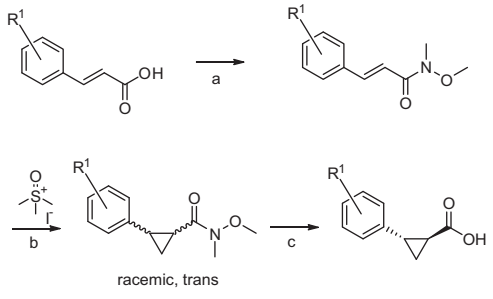
[a] Crystalline sample could not be obtained.

Chiral 2-amino-2-phenylethanols were either commercially available or were prepared as outlined for *R*-2-amino-2-(4-methoxyphenyl)ethanolin Scheme 2, using methods described by Ellman.[21] Halogen–lithium exchange on 4-bromo-anisol gave a lithio-intermediate that was reacted with $R_{(S)}$-2-methylpropane-2-sulfinic acid [2-(*tert*-butyldimethylsilanyloxy)ethylidene]amide to give a diastereomeric addition product with 80–90% de favoring the *R* diastereomer. The diastereomers were smoothly separated by flash chromatography. Global deprotection using HCl in dioxane gave the desired *R* aminoalcohol building block.

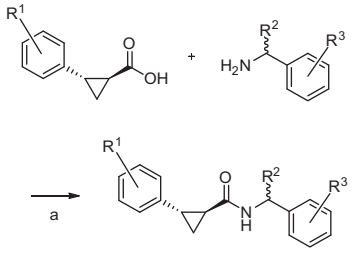
The desired α7 PAM compounds were prepared by standard coupling reactions between the *trans* 2-phenylcyclopropane carboxylic acids and the amines as outlined in Scheme 3.[22]



Scheme 2. Reagents and conditions: (a) *tert*-BuLi, MTBE, −78 °C, 2 h; (b) (R_S)-2-methylpropane-2-sulfinic acid [2-(*tert*-butyldimethylsilanyloxy)ethylidene]amide, −78 °C, 3 h (85% overall yield from steps a and b); (c) 2 M HCl in dioxane, room temperature, 16 h (quant).



Scheme 1. Reagents and conditions: (a) *N*,*O*-dimethylhydroxylamine hydrochloride, EDCI, DMAP, Et_3N, DCM, room temperature overnight (70–88%); (b) NaH, DMF, 0–25 °C, 2 h (71–90%); (c) (i) NaOH, MeOH/H_2O, reflux 3 h; (ii) SFC separation (25–30%).



Scheme 3. Reagents and conditions: (a) HATU, DMF, Et_3N, 25 °C, 0.5–5 h.

Electrophysiological effects of Lu AF58801 were evaluated on human-α7 and rat-α7 receptors expressed in *Xenopus* oocytes. α7 receptors were activated by 30 μM ACh for 30 s, and the activity of Lu AF58801 was evaluated by testing Ach ± compound with a preincubation time of 1 min (Fig. 2).

Lu AF58801 showed concentration-dependent PAM activity on both human and rat α7 receptors, with corresponding EC_{50} values on both peak response and on AUC response, while the fold maximum modulation of control ACh response was much higher on rat receptors than on human receptors (Table 6). 30 μM Ach was used as standard. The effect of Lu AF58801 was also investigated on heteromeric nicotinic receptors expressed in *Xenopus* oocytes. No significant effects were observed at 10 or 30 μM on α3β4, α4β2, or muscle α1β1γδ receptors (data not shown). The selectivity of Lu AF58801 was further examined using a panel of more than 108 molecular targets (panel affinity screening at Cerep, France). No appreciable interaction was found with any of the examined targets in this panel, other than at the 5-HT_2 receptor subtype. Lu AF58801 was shown to inhibit the 5-HT_{2a} receptor by 51% at 10 μM.

Exposure assessment of Lu AF58801 was performed in Lister Hooded rats following an oral dose of 10 mg/kg (dose volume: 5 ml/kg, in 100% PEG400). The compound was shown to be rapidly absorbed systemically with high plasma concentrations, followed by a first-order elimination profile with a half-life round 1 h (Fig. 3). Lu AF58801 showed high oral bioavailability of 70% in rats relative to an intravenous dose and measurement of brain homogenate concentrations at the different time-points resulted in a brain-to-plasma ratio of 0.15, based on AUCs of the respective matrices. From the total brain exposure measured at 0.5 h (305 ng/g), an unbound concentration of around 70 nM was estimated based on a free fraction of 7% in rat brain homogenate.

Lu AF58801 was also broadly profiled in our standard battery of in vitro safety pharmacology and toxicology assays. Briefly, Lu AF58801 was not cytotoxic and did not lead to formation of reactive oxygen species up to 100 μM. The compound had no effect on heterologously expressed hERG, hNav1.5, hKv4.3/KChIP2, hKCNQ1/mink or hHCN4 when tested at 10 μM in automated patch clamp. However, at 10 μM, there was a tendency towards an increase in hKir2.1 (33 ± 22%, mean ± SE), and a decrease in hCav1.2 currents (35 ± 8%, mean ± SE), however these effects at high concentrations are not judged relevant for the actions of Lu AF58801

Table 6
Effect of Lu AF58801 on human and rat α7 receptors expressed in *Xenopus* oocytes

	Human alpha7		Rat alpha7	
	EC_{50} (nM)	Max fold mod	EC_{50} (nM)	Max fold mod
Peak	1400	43	1000	250
AUC	1600	44	1000	2200

in-vivo. The compound was not mutagenic in the AMES test either with or without liver enzyme metabolic activation, and did not induce micronuclei up to 100 μM.

The novel object recognition (NOR) task[23] is a test paradigm thought to reflect visual learning and recognition processing.[24] The subchronic administration of phencyclidine (subPCP) induces a behavioural and neurobiological syndrome in rodents, upon cessation of treatment, which bears a remarkable similarity to some of the core symptoms in schizophrenic patients, including cognitive disruption.[25]

NOR testing was performed as previously described.[26,27] As shown in Figure 4, subchronic vehicle-treated rats spent significantly more time exploring a novel object during the 3 min test trial (as evidenced by a high discrimination index score). Vehicle-treated animals, that had previously received PCP for 1 week 7 days prior, spent approximately an equal amount of time exploring both a familiar and a novel object (reflected in a discrimination index score close to 0). Acute administration of Lu AF58801 (10–30 mg/kg, po) dose-dependently attenuated PCP-induced deficits in novel object exploration, an effect that reached statistical significance for the 30 mg/kg treatment group ($P < 0.05$). Lu AF58801 did not affect total object exploration time of objects, or distance travelled in the test arena at any dose tested (data not shown). Analysis of brain samples from rats treated with 10 or 30 mg/kg Lu AF58801, harvested at the end of behavioural testing (i.e., 90 min post-administration), revealed free brain concentrations corresponding to 56 and 145 nM, respectively ($n = 6$). At this low drug concentration, AF58801 is only expected to modulate α7 receptors, with no or very little contribution from other targets, such as KCNQ channels. Taken together with the in-vitro potency on alpha7 receptors this suggest that only low fold modulation of the ACh response (2–3 fold) on the alpha7 receptor is needed to obtain in-vivo efficacy in the NOR assay.

In summary, we have developed a potent, brain penetrant, positive allosteric modulator of the α7 nicotinic acetylcholine receptor, Lu AF58801, based on a hit-to-lead optimization campaign of an initial HTS hit. The optimization of hit **2** involved the identification of a hydroxymethyl group in the linker region which dramatically improved the microsomal stability of the

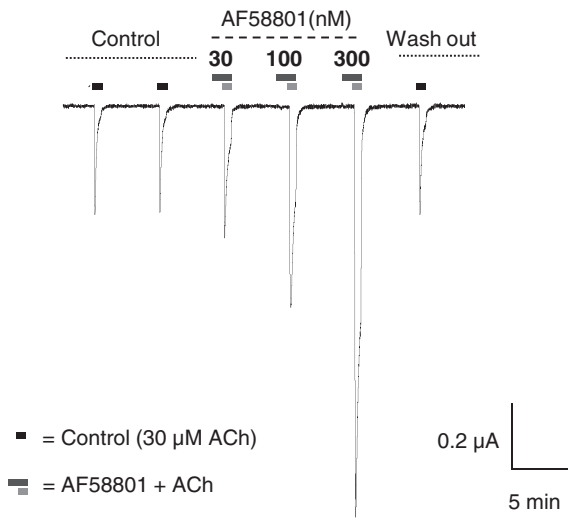


Figure 2. Lu AF58801 showed potentiation of the control response to ACh (30 μM) even at low concentrations (30, 100 and 300 nM) at human α7 receptors expressed in oocytes.

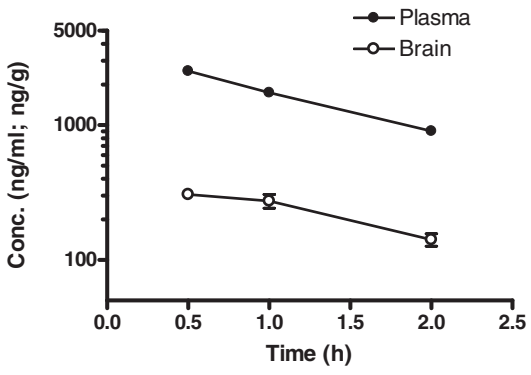


Figure 3. Plasma (ng/ml) and brain (ng/g) concentration–time courses of AF58801 following oral administration of 10 mg/kg to Lister Hooded rats. Each data point represents an average of $n = 3 \pm$ SEM.

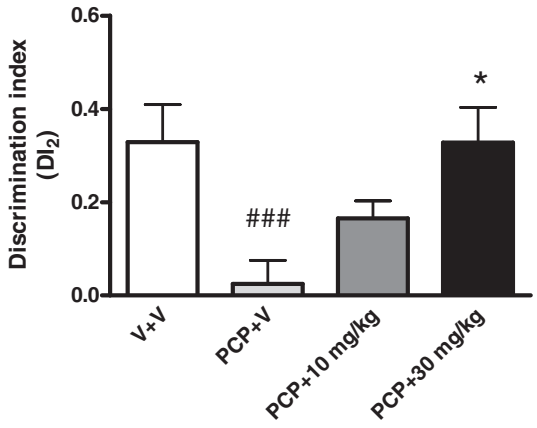


Figure 4. The effects of Lu AF58801 in the subPCP NOR task in rats.[28] ###$P < 0.001$ versus vehicle + vehicle-treated group; *$P < 0.05$ versus PCP + vehicle-treated group ($n = 12$).

compounds. Lu AF58801 was active in the subchronic PCP novel object recognition model in rats, a paradigm with some relevance to cognitive deficits associated with schizophrenia. Lu AF58801 is considered an attractive tool compound for α7 nicotinic receptor modulation in-vivo. Further evaluation of this probe is underway.

Acknowledgments

Dr. Jane Wang and Miao Wei from WuXi Apptec Shanghai are acknowledged for expert organic synthesis assistance. We thank Sascha Bull for excellent technical assistance with in vivo behavioural experiments.

References and notes

1. Green, A.; Ellis, K. A.; Ellis, J.; Bartholomeusz, C. F.; Ilic, S.; Croft, R. J.; Phan, K. L.; Nathan, P. J. *Pharmacol. Biochem. Behav.* **2005**, *81*, 575.
2. Rogers, J. L.; Kesner, R. P. *Neurobiol. Learn. Mem.* **2003**, *80*, 332.
3. Gotti, C.; Clementi, F.; Fornari, A.; Gaimarri, A.; Guiducci, S.; Manfredi, I.; Moretti, M.; Pedrazzi, P.; Pucci, L.; Zoli, M. *Biochem. Pharmacol.* **2009**, *78*, 703.
4. Broad, L. M.; Sher, E.; Astles, P. C.; Zwart, R.; O'Neill, M. J. *Drugs Future* **2007**, *32*, 161.
5. Leonard, S.; Breese, C.; Adams, C.; Benhammou, K.; Gault, J.; Stevens, K.; Lee, M.; Adler, L.; Olincy, A.; Ros, S. R.; Freedman, R. *Eur. J. Pharmacol.* **2000**, *393*, 237.
6. Perry, E.; Martin-Ruiz, C.; Lee, M.; Griffiths, M.; Johnson, M.; Piggott, M.; Haroutunian, V.; Buxbaum, J. D.; Näsland, J.; Davis, K.; Gotti, C.; Clementi, F.; Tzartos, S.; Cohen, O.; Soreq, H.; Jaros, E.; Perry, R.; Ballard, C.; McKeith, I.; Court, J. *Eur. J. Pharmacol.* **2000**, *393*, 215.
7. Bencherif, M.; Dunbar, G. C.; Hosford, D. A.; Gatto, G. J.; Hauser, T.; Jordan, K. G.; Segreti, A.C. Treatment of Cognitive Dysfunction in Schizophrenia, WO2012099836 A1 (PCT), 2012.
8. Hilt, D. *EVP-6124, A Selective Alpha-7 Partial Agonist, has Positive Effects on Cognition and Clinical Function in Mild to Moderate Alzheimers Disease Patients. Oral Presentation at Alzheimer's Association International Conference 2012 (AAIC)*; Vancouver: Canada, 2012.
9. Hilt, D. EVP-6124 Shows Statistically Significant Improvement in Cognition, Function and Impact on Negative Symptoms. Oral Presentation at American College of Neuropsychopharmacology Annual Meeting 2011, USA.
10. Hurst, R. S.; Hajós, M.; Raggenbass, M.; Wall, T. M.; Higdon, N. R.; Lawson, J. A.; Rutherford-Root, K. L.; Berkenpas, M. B.; Hoffmann, W. E.; Piotrowski, D. W.; Groppi, V. E.; Allaman, G.; Ogier, R.; Bertrand, S.; Bertrand, D.; Arneric, S. P. *J. Neurosci.* **2005**, *25*, 4396.
11. Faghih, R.; Gfesser, G. A.; Gopalakrishnan, M. *Recent Patents CNS Drug Disc.* **2007**, *2*, 99.
12. As the nAChα7 receptor is a calcium permeable ion channel, the activity of this receptor can be measured in cell lines expressing the target using calcium sensitive dyes. In this paper, we used a rat GH4C1 cell line with stable expression of nAChα7 receptors. The cells were seeded in 384-well plates and loaded with a calcium sensitive dye (calcium-4). The α7 receptor was activated with 20 μM ACh and the activity of new compounds was measured as a change in fluorescence between control ± compounds, measured on a FDSS 700 reader. PNU-120596 was used as positive control and the max stimulation of new compounds was calculated relative to PNU-120596 defined as 100%.
13. All aqueous solubilities were determined in phosphate buffer pH 7.4 based on crystalline material. For **2** the aqueous solubility measurement was based on crystalline or partly crystalline material.
14. *c*log*P* was calculated using Daylight Version 4.9 from Daylight Chemical Information Systems, Inc.
15. The absolute configuration was performed by comparing optical rotary power with literature values.
16. A CHO cell line with stable expression of human KCNQ2 receptors was used in a 86 Rb efflux assay to determine the activity of new compounds on the KCNQ2 receptor. KCNQ2 cells were seeded in 96 well plates and loaded with 86 Rb. KCNQ2 channels were activated by adding 30 mM KCl to the external buffer, and the activity of new compounds was measured by calculating the change in efflux of 86 Rb in control ± compounds. EC_{50} values and maximum stimulation was calculated for the new compounds. Retigabine was used as standard positive control.
17. Commercially available, racemic *trans* 2-phenyl-cyclopropanecarboxylic acid (Sigma–Aldrich, catalog no P22354) was subjected to chiral SFC separation. The separation was performed on a Berger Multigram II machine, operating at 50 mL/min at 35 °C and 100 bar backpressure using stacked injections. The column was a ChiralpakAD 5u, 250 × 21 mm. The eluent was CO_2 (70%) and ethanol (30%). (1*S*,2*S*)-2-Phenyl-cyclopropanecarboxylic acid was obtained as an oil that slowly solidified upon standing. $[\alpha]_D^{20} = +300.9°$ (*c* 1% EtOH). (Lit: $[\alpha]_D^{20} = +389°$ (*c* 0.61, $CHCl_3$) Kozikowski et al., *J. Med. Chem.* **2009**, *52*, 1885; Lit: $[\alpha]_D^{20} = +311.7°$ (*c* 1.776, EtOH) Walborsky et al., *Tetrahedron* **1964**, *20*, 1695).
18. Rodriques, K. E. *Tetrahedron Lett.* **1991**, *32*, 1275.
19. In all cases, the dextrorotary *trans* 2-phenyl-cyclopropanecarboxylic acids were used for further synthesis.
20. (a) Datta, G. K.; Ellman, J. A. *J. Org. Chem.* **2010**, *75*, 6283; (b) Tanuwidjaja, J.; Peltier, H. M.; Ellman, J. A. *J. Org. Chem.* **2007**, *72*, 626; (c) Robak, M. T.; Herbage, M. A.; Ellman, J. A. *Chem. Rev.* **2010**, *110*, 3600.
21. Tang, T. P.; Volkman, S. K.; Ellman, J. A. *J. Org. Chem.* **2001**, *66*, 8772.
22. The a solution of 4-bromo-anisol (7.0 g, 37.4 mmol) in 70 mL of MTBE was added 1.3 M of *tert*-butyllithium (60.5 mL) dropwise at −78 °C. The resulting mixture was stirred at −78 °C for 2 h under Ar. To this solution was added dropwise a solution of $R_{(S)}$-2-methylpropane-2-sulfinic acid [2-(*tert*-butyl-dimethyl-silanyl-oxy)ethylidene]amide (11.0 g, 41.2 mmol) in dry MTBE (100 mL). The resulting mixture was stirring at −78 °C for 3 h and then at room temperature overnight. The mixture was quenched with saturated aqueous NH_4Cl solution and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated to dryness. Purified by HPLC (Gilson GX281, Gemini column, gradient 55–85% acetonitrile in water over 25 min at 25 mL/min flowrate) gave (*R*)-*N*-((*R*)-2-((*tert*-butyldimethylsilyl)oxy)-1-(4-methoxyphenyl)ethyl)-2-methylpropane-2-sulfinamide as an oil (1.22 g, 85%). HNMR ($CDCl_3$ 400 MHz) δ 7.28 (d, $J = 1.2$ Hz, 1H), 6.90 (s, 1H), 6.88 (s, 1H), 4.48–4.51 (m, 1H), 4.28 (d, $J = 0.8$ Hz, 1H), 3.82 (s, 3H), 3.74–3.77 (m, 1H), 3.60 (t, $J = 9.6$ Hz, 1H), 1.24 (s, 9H), 0.93 (s, 9H), 0.08 (d, $J = 5.2$ Hz, 1H). $[\alpha]_D^{20} = -90.6°$ (*c* 0.106, MeOH). To a solution of (*R*)-*N*-((*R*)-2-((*tert*-butyldimethylsilyl)oxy)-1-(4-methoxyphenyl) ethyl)-2-methylpropane-2-sulfinamide (2.2 g, mmol) in anhydrous dioxane (20 mL) was added HCl (2 M in dioxane, 2 mL) at room temperature. The mixture was stirred at room temperature for 16 h and the mixture was evaporated to dryness. The material was used without further purification. To a mixture of (*R*)-2-amino-2-(4-methoxyphenyl)ethanol hydrochloride (2.15 g, 13.2 mmol) and HATU (5.47 g, 14.4 mmol) in DMF (20 mL) was added Et_3N (2.42 g, 24 mmol). The resulting mixture was stirred at room temperature for 30 min. To this mixture was added (1*S*,2*S*)-2-phenyl-cyclopropanecarboxylic acid (2.0 g, 12 mmol) and the mixture was stirred at room temperature for 5 h. The mixture was evaporated to dryness and purified by preparative HPLC (Gilson GX281, Gemini column, gradient 35–60% acetonitrile in water over 25 min at 80 mL/min flowrate) to give compound AF58801 as a colorless solid (1.5 g, 47%). HNMR ($CDCl_3$ 400 MHz) δ 7.16–7.28 (m, 5H), 7.06–7.08 (m, 2H), 6.86–6.90 (m, 2H), 6.25 (d, $J = 6.8$ Hz, 1H), 5.02–5.06 (m, 1H), 3.84–3.92 (m, 2H), 3.79 (s, 3H), 2.89 (d, $J = 0.8$ Hz, 1H), 2.48–2.53 (m, 1H), 1.63–1.69 (m, 2H), 1.25–1.32 (m, 2H). CNMR (DMSO-d_6, 150 MHz) δ 170.9, 158.6, 141.7, 133.7, 128.8, 128.5, 126.4, 126.2, 114.0, 65.2, 55.5, 55.0, 26.1, 24.2, 16.0. LC–MS *m*/*z* = 312.1 $[M+H]^+$. $[\alpha]_D^{20} = -219.6°$ (*c* 0.1175, MeOH).
23. Ennaceur, A.; Delacour, J. *Behav. Brain Res.* **1988**, *31*, 47.
24. Young, J. W.; Powell, S. B.; Risbrough, V.; Marston, H. M.; Geyer, M. A. *Pharmacol. Ther.* **2009**, *122*, 150.
25. Jentsch, J. D.; Roth, R. H. *Neuropsychopharmacology* **1999**, *20*, 201.
26. Redrobe, J. P.; Bull, S.; Plath, N. *Front. Psychiatry* **2010**, *11*, 146.
27. Redrobe, J. P.; Elster, L.; Frederiksen, K.; Bundgaard, C.; de Jong, I. E.; Smith, G. P.; Bruun, A. T.; Larsen, P. H.; Didriksen, M. *Psychopharmacology* **2012**, *221*, 451.
28. Male Lister Hooded rats (Charles River, Crl:LH, Germany; 220–240 g) were administered subPCP (synthesized at H. Lundbeck A/S, dissolved in 0.9% saline; 5 mg/kg, 1 ml/kg, ip bid for 7 days) or saline (1 ml/kg ip bid for 7 days) at 7 am and 7 pm, followed by a 7-day washout period prior to behavioural testing. Lu AF58801 (dissolved in 100% PEG400) was administered 30 min prior to the acquisition trial of the NOR procedure via the po route. Data is presented as the discrimination index, calculated as the novel object exploration time (Tn) minus the familiar object exploration time (Tf) divided by the total exploration time (Tn + Tf). One-way ANOVA, followed by appropriate All-Pairwise Multiple Comparison post-hoc analysis (Bonferroni's *t*-test) was used to investigate statistical differences between test groups ($P < 0.05$).