

Discovery of BNC375, a Potent, Selective, and Orally Available Type I Positive Allosteric Modulator of $\alpha 7$ nAChRs

Andrew J. Harvey,^{#,‡} Thomas D. Avery,^{#,◆} Laurent Schaeffer,[§] Christophe Joseph,[§] Belinda C. Huff,[#] Rajinder Singh,^{#,||} Christophe Morice,[§] Bruno Giethlen,[§] Anton A. Grishin,^{#,⊥} Carolyn J. Coles,[#] Peter Kolesik,[#] Stéphanie Wagner,[†] Emile Andriambeloson,[†] Bertrand Huyard,[†] Etienne Poiraud,[†] Dharam Paul,^{*,#} and Susan M. O'Connor[#]

[#]Bionomics Limited, 31 Dagleish Street, Thebarton, SA-5031, Australia

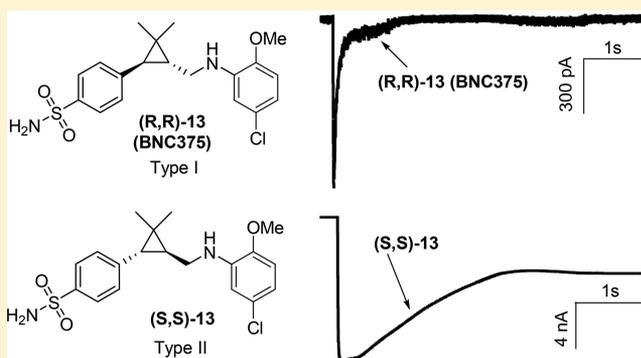
[†]Neurofit, 850 Boulevard Sébastien Brant, Bioparc 1, Parc d'Innovation, 67400 Illkirch, France

[§]Prestwick Chemicals, 220 Boulevard Gonthier d'Andernach, Parc d'Innovation, 67400 Illkirch, France

Supporting Information

ABSTRACT: Positive allosteric modulators (PAMs) of $\alpha 7$ nAChRs can have different properties with respect to their effects on channel kinetics. Type I PAMs amplify peak channel response to acetylcholine but do not appear to influence channel desensitization kinetics, whereas Type II PAMs both increase channel response and delay receptor desensitization. Both Type I and Type II PAMs are reported in literature, but there are limited reports describing their structure–kinetic profile relationships. Here, we report a novel class of compounds with either Type I or Type II behavior that can be tuned by the relative stereochemistry around the central cyclopropyl ring: for example, (R,R)-13 (BNC375) and its analogues with RR stereochemistry around the central cyclopropyl ring are Type I PAMs, whereas compounds in the same series with SS stereochemistry (e.g., (S,S)-13) are Type II PAMs as measured using patch-clamp electrophysiology. Further fine control over the kinetics has been achieved by changing the substitutions on the aniline ring: generally the substitution with strong electron withdrawing groups reduces the Type II character of these compounds. Our structure–activity optimization efforts have led to the discovery of BNC375, a small molecule with good CNS-drug like properties and clinical candidate potential.

KEYWORDS: Alpha 7 nicotinic acetylcholine receptor, positive allosteric modulators, memory, T-maze, attention



Activation of alpha 7 nicotinic acetylcholine receptors ($\alpha 7$ nAChRs) improves cognitive function both in humans¹ and rodents^{1,2} and thus represents a promising therapeutic approach for cognitive impairment in Alzheimer's disease³ and schizophrenia.^{3,4} The activity of $\alpha 7$ nAChR can be enhanced through either orthosteric agonists or positive allosteric modulators (PAMs). In contrast to $\alpha 7$ agonists, $\alpha 7$ PAMs do not activate $\alpha 7$ nAChRs by themselves and work by amplification of responses induced by endogenous agonists, hence preserving spatiotemporal signaling patterns. Furthermore, $\alpha 7$ nAChR PAMs may allow for greater selectivity to be achieved as compared to orthosteric agonists, providing for a potentially cleaner side effect profile.^{1,4} Based on the functional property of modulation, $\alpha 7$ PAMs can be broadly classified into two categories: Type I and Type II.⁵ Type I $\alpha 7$ PAMs, such as AVL-3288⁶ (1), NS1738⁷ (2), and Lu AF58801⁸ (3, Figure 1), increase channel response to acetylcholine without affecting receptor desensitization, whereas Type II $\alpha 7$ PAMs, such as TQS⁹ (4), RO5126946¹⁰ (5), JNJ-1930942¹¹ (6), PNU-120596¹² (7), and A-867744¹³ (8), not only increase

channel response but also delay receptor desensitization. The PAMs reported in the literature are structurally quite diverse, and small structural changes have been reported to produce profound effects on the pharmacological profile. For example, in the work reported by Gill-Thind,¹⁴ the *cis*–*cis* diastereomers were all classified as allosteric agonists or Type II PAMs. In contrast, all of the *cis*–*trans* diastereomers were Type I PAMs, negative allosteric modulators, or silent allosteric modulators. Here, we report a new class of compounds that can be tuned either to a Type I or Type II $\alpha 7$ PAM, depending upon the stereochemistry around the central cyclopropyl ring. Our synthetic methods have allowed us to access both enantiomers from the *trans*-racemates, and the structure–activity optimization of these enantiomers has led to the identification of a potential candidate for clinical development.

Received: January 3, 2019

Accepted: March 25, 2019

Published: March 25, 2019



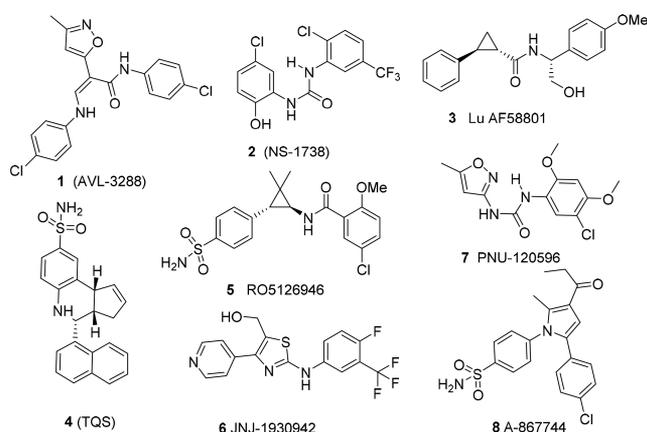


Figure 1. Representative examples of reported $\alpha 7$ nAChRs PAMS.

During the early days of the program, the knowledge about the structural requirements for $\alpha 7$ PAM activity was ambiguous because literature examples covered many diverse structural classes containing two terminal rings joined through linear and more constrained cyclic linkers. Some examples contained one ring decorated with a highly polar sulfamyl group, whereas others lacked such polar substituents. Therefore, the lack of understanding in this area persuaded us to design a small focused library of compounds belonging to different chemical classes to discover novel $\alpha 7$ PAMs with identifiable structural features crucial for biological activity. The primary *in vitro* screening assay was performed by electrophysiology using the Patchliner (Nanion), an automatic planar patch clamp instrument. The potentiation of an EC₂₀ acetylcholine (ACh) response by 3 μ M of each PAM was determined. Secondary screening and more detailed characterization of promising compounds were performed with conventional manual patch-clamp recordings using a fast-application system (Dynaflow, Cellectricon, Sweden). All experiments were performed in stable cell lines expressing human or rat $\alpha 7$ nAChRs in rat GH4C1 cells. The percent change in peak current produced by 3 μ M test compound plus acetylcholine versus acetylcholine alone was called P₃ (percent potentiation at 3 μ M). *In vivo* efficacy was measured using the mouse T-maze Continuous Alternation Task (T-maze) as the primary model and was reported as the doses that significantly reversed the scopolamine induced impairment of spontaneous alternation (for details see SI: *in vitro* and *in vivo* evaluation).

Nine hits were identified during the primary screen of a small focused library. Three of them (9, 10, and 11; Figure 2)

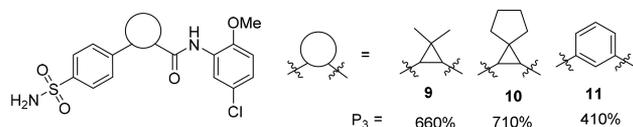


Figure 2. Key compounds from the hit identification phase.

were of particular interest,¹⁵ exhibiting P₃ values of 660, 710, and 410%, respectively. The activity of 9 and 10 was confirmed in the secondary screening assay where they exhibited P₃ values of 420% and 360%, respectively (Table 1).

The hits 9 and 10 significantly reversed scopolamine induced impairment of spontaneous alternation in the mouse T-maze at *intraperitoneal* (*ip*) doses of 3 and 30 mg/kg. However, compounds 10 and 11 were found to be inactive in

Table 1. Profile of Hit Compounds 9, 10, and 11^a

compound	9	10	11
primary screening P ₃ (%)	660	710	410
secondary screening P ₃ (%)	420	360	NT
secondary screening AUC/P ₃	5.6	5.6	NT
logD _{7.4}	3.7	4.2	NT
Sol _{6.5} (μ g/mL)	3.1–6.3	1.6–3.1	NT
primary <i>in vivo</i> assay: mouse T-maze	<i>ip</i> 3, 30	3, 30	NT
significant dose (mg/kg)	<i>po</i> NT	NS (3, 30)	NS (3, 30)

^aNT: not tested. NS: not significant.

T-maze after oral doses of 3 and 30 mg/kg, and compound 9 was not tested orally. In order to achieve oral efficacy, further modification of these hits to find a lead compound was carried out.

As shown in Figure 2, the hit molecules 9–11 have a modular structure, composed of three distinctive regions: right-hand side (RHS) ring, left-hand side (LHS) ring, and central linker. So, we planned to explore SAR for these regions (a representative set of synthesized compounds is shown in Figure 3). As found in the primary hits, the sulfamyl group on

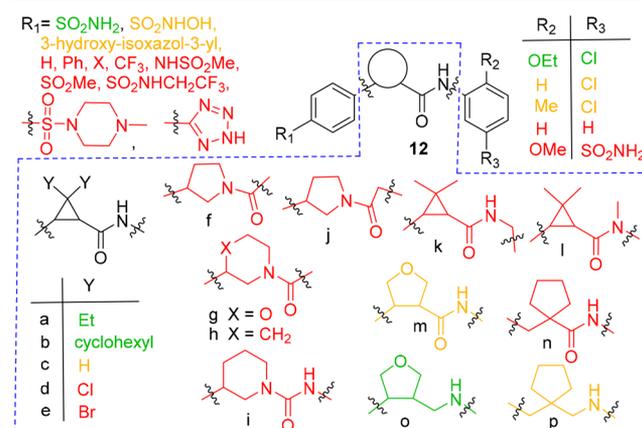
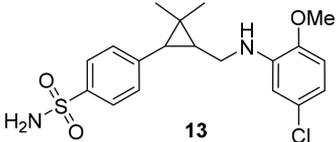


Figure 3. Representative examples exploring the hit to lead SAR (green, active; orange, weakly active; red, inactive).

the *para* position of LHS phenyl ring and 2-alkoxy-5-halo group on RHS aniline ring were essential features for *in vitro* $\alpha 7$ PAM activity. The central linker was modified in a variety of ways and we found that compounds containing the secondary amides with a 3,3-dialkyl substituted 1,2-cyclopropyl (12a, 12b) and 3,4-tetrahydrofuryl (12m) moieties between the carbonyl carbon and LHS phenyl ring exhibited good *in vitro* activity. Removal of dimethyl group from cyclopropyl (12c) or its replacement with dihalo (12d, 12e) rendered these compounds inactive. Interestingly, the modest gain in activity, compared to the amide molecules 12m and 12n, was seen in the corresponding amines 12o and 12p, respectively. Hence, we reduced the amide functionality in the most active compounds 9 and 10 to their corresponding amines 13 and 14 and observed a significant enhancement in $\alpha 7$ nAChR potentiation with P₃ values of 7900% and 9890%, respectively. In contrast, the corresponding amine of compound 11 was found to be inactive. The racemic compound 13 was selected for further evaluation due to its lower lipophilicity and the easy accessibility of its key intermediate. Compound 13 showed *in*

in vivo activity (T-maze) at the doses of 3 and 10 mg/kg when administered orally. To confirm the allosteric nature of compound **13**, it was evaluated in the manual patch clamp assay in the absence of acetylcholine and failed to evoke currents in stable cell lines expressing $\alpha 7$ channels. However, **13** exhibited typical allosteric behavior by potentiating acetylcholine-evoked currents in a concentration-dependent manner. In the manual patch clamp, **13** showed an EC₅₀ of 25 nM and peak current potentiation P_{\max} of 650% relative to the peak current evoked by ACh at an EC₂₀ concentration. Overall, racemate **13** exhibited a good lead profile (Table 2) and had a

Table 2. Profile of Lead Compound **13**



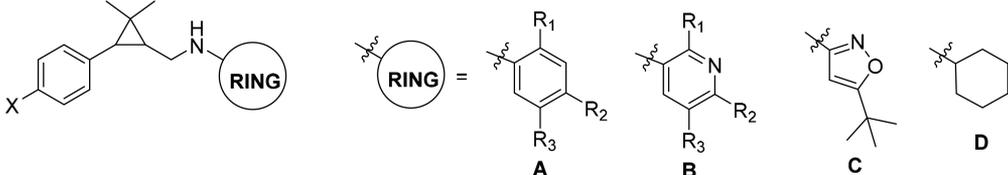
primary screening P_3 (%)		7900
secondary screening P_{\max} (%)		650
secondary screening EC ₅₀ (nM)		25
solubility ($\mu\text{g/mL}$)	pH 2.0	1.6–3.1
	pH 6.5	>100
microsomal stability (E_H) ^a	human	0.64
	mouse	0.82
primary <i>in vivo</i> mouse T-maze		3, 10
oral significant dose (mg/kg)		

^aMethod to calculate the predicted *in vivo* hepatic extraction ratio (E_H) is described in SI

modular structure that enabled further lead optimization. The main aim of lead optimization was to improve the ADME properties of the compound. We focused on decreasing

lipophilicity to improve the solubility and clearance while maintaining the potency. The initial attempts to reduce lipophilicity began with removal of the dimethyl group on the central cyclopropyl ring.¹⁶ The obtained des-dimethylcyclopropyl analogue had reduced activity. Also, the replacement of the 1,2-cyclopropyl ring with more polar 3,4-tetrahydrofuryl ring reduced the activity. Synthetic attempts to replace LHS *p*-sulfamoylphenyl ring with *p*-sulfamoylpyridyl proved unsuccessful. Replacement of *p*-sulfamoyl group on LHS phenyl ring with more polar groups such as *p*-sulfonohydrazide (**32**) and *N*-hydroxysulfonamide (**33**) rendered the analogs inactive or very weakly active. Additionally, we attempted to replace the *p*-sulfamoyl group with comparable or slightly more lipophilic groups, such as *N*-alkylsulfonamides, saturated heterocycles, heteroaryl, and aryl, to identify a more active LHS that could be combined later with a polar RHS to balance the lipophilicity of the molecule. However, all the attempts proved unsuccessful. The only change leading to the retention of activity was the attachment of methanesulfonamide group through nitrogen to the aryl ring rather than the sulfur atom. However, the original lead compound **13** with a *p*-sulfamoyl group was still the most active analog, indicating that the sulfamoyl group was a critical feature of potent $\alpha 7$ PAMs in the series (see the data in SI: SAR analysis tables). Having learned that the central part and the LHS phenyl ring could tolerate only limited modifications, we then sought to explore the RHS of the molecule and synthesized a set of 32 analogs (see the SI: SAR analysis tables). Representative compounds and their PAM activities are shown in Table 3. Some analogs with lower lipophilicity were achieved by replacing the RHS 5-chloro-2-methoxyphenyl ring in **13** with 5-*tert*-butyl-3-oxazolyl (**72**), 2-methyl-3-pyridyl (**69**), 2-methoxy-3-pyridyl (**21**), 5-fluoro-3-pyridyl (**22**), or hexyl (**74**). These changes were found to be either inactive or relatively less active. Substitution on the

Table 3. Representative Examples for SAR on the RHS Modifications

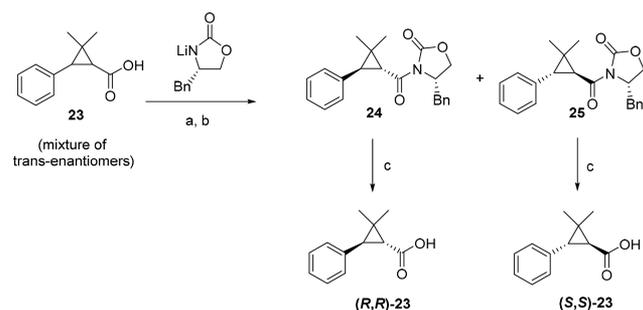


	X	ring	R ₁	R ₂	R ₃	P_3 (%)	SEM (<i>n</i>)
13	SO ₂ NH ₂	A	OMe	H	Cl	7900	3560 (2)
14	SO ₂ NH ₂	A	H	H	H	2570	660 (3)
15	SO ₂ NH ₂	A	Me	H	Cl	12310	3870 (3)
16	SO ₂ NH ₂	A	Me	F	H	8660	4280 (3)
17	SO ₂ NH ₂	A	Me	F	Cl	9230	900 (3)
18	SO ₂ NH ₂	A	OMe	H	CF ₃	8170	2060 (4)
19	SO ₂ NH ₂	A	OCHF ₂	F	H	3360	1190 (3)
20	SO ₂ NH ₂	A	OCF ₃	H	F	2440	660 (2)
21	SO ₂ NH ₂	B	OMe	H	H	2420	550 (4)
22	SO ₂ NH ₂	B	H	H	F	3000	400 (4)
32	SO ₂ NHNH ₂	A	OMe	H	Cl	130	100 (3)
33	SO ₂ NHOH	A	OMe	H	Cl	720	260 (6)
58	SO ₂ NH ₂	A	OMe	H	SO ₂ NH ₂	<100	12 (2)
66	SO ₂ NH ₂	A	H	morpholino	H	<100	16 (3)
67	SO ₂ NH ₂	A	H	H	morpholino	460	12 (4)
69	SO ₂ NH ₂	B	Me	H	H	130	50 (2)
72	SO ₂ NH ₂	C				<100	8 (2)
74	SO ₂ NH ₂	D				<100	30 (6)

phenyl with polar groups such as sulfamyl (**58**) or morpholino (**66** or **67**) also reduced the activity. In general, decreasing lipophilicity to improve solubility and clearance failed to give an active compound. Efforts devoted to explore the alternatives for metabolically labile $-\text{OCH}_3$ and Cl groups and to protect the *para*-position on the RHS ring provided some active compounds (**19**, **26**, **27**, **28**). This effort revealed that a 2,4- or 2,5-disubstitution pattern on the RHS phenyl ring retained good levels of activity.

All the compounds with chiral centers were prepared using racemic building blocks and were a mixture of more than one diastereoisomer or enantiomer. Compound **13** and related active analogs (shown in Table 3) that featured a cyclopropyl ring in the linker were prepared from *trans* building block **23** and were a mixture of two *trans* enantiomers with *R,R* stereochemistry in one and *S,S* in the other around the cyclopropane ring. In order to profile both enantiomers, we prepared an enantiopure building block by developing a method to separate the *trans* enantiomeric pair of acid **23** (Scheme 1). The racemic mixture of acid **23** was reacted with a

Scheme 1. Synthesis of Enantiopure Building Block **23**^a



^aReagents and conditions: a) SOCl_2 , DCM; b) (*S*)-4-benzyl-2-oxazolidnone, $n\text{BuLi}$, THF; c) $\text{H}_2\text{O}/\text{THF}$, H_2O_2 , LiOH .

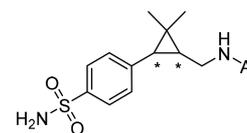
chiral auxiliary (*S*)-4-isopropyl-2-oxazolidinone to prepare the mixture of two diastereoisomers, **24** and **25**. The resulting diastereoisomers were separated by repeated flash column chromatography.

The absolute stereochemistry of **24**, the first eluting diastereoisomer, was determined by X-ray crystallography to be *R,R,S* (cyclopropyl chiral centers were found to be *R,R*), and the stereochemistry of the other, **25**, was assigned to be *S,S,S*. These two diastereoisomers were then separately hydrolyzed to corresponding enantiopure *trans* acids *(R,R)*-**23** and *(S,S)*-**23**. The enantiopure acids were used to produce the enantiopure final compounds, and the enantiomeric-excess of *trans* enantiomers of compound **13** was measured by analytical HPLC and found to be >98%, demonstrating no racemization in subsequent synthetic steps. The synthetic procedures and the analytical data of the representative compounds are presented in the Supporting Information.

Both *trans* enantiomers of compound **13** were found to be active in the primary screening assay at $3\ \mu\text{M}$. On Patchliner, the peak current potentiation by the enantiomer *(R,R)*-**13** was 1160%, which was considerably lower than the parent *trans* racemic mixture; however, the peak potentiation of other enantiomer, *(S,S)*-**13**, was found to be 11840%, which was consistent with the parent *trans* racemic mixture. The shape of the current trace of *(R,R)*-**13** was sharp and resembled a Type I PAM, whereas *(S,S)*-**13** was like a Type II PAM (Patchliner

current traces are shown in Table 4). It appeared that the *R,R* enantiomer was only moderately active compared to the parent

Table 4. Effect of RHS Substituents on Kinetics of $\alpha 7$ nAChR Modulation^a



No.	-Ar	Current Trace ^a	
		<i>P</i> ₃ (%)	
		<i>R,R</i>	<i>S,S</i>
13		 1160%	 11840%
16		 470%	 17580%
26		 460%	 17430%
27		 420%	 8930%
19		 170%	 8170%
28		 410%	 2860%

^aAll current traces are plotted on same time scale (*x*-axis), but peak current amplitude (*y*-axis) is not drawn to scale.

racemic mixture, and much less active than the *S,S*-enantiomer. Next, we confirmed the PAM activity by performing full dose responses using manual electrophysiology. Similar to the primary screening results, the enantiopure *(R,R)*-**13** and *(S,S)*-**13** exhibited dramatically different kinetics of desensitization on $\alpha 7$ nAChRs. Like the prototypical Type I PAM, AVL-3288 (**1**), *(R,R)*-**13** significantly potentiated the acetylcholine signal without changing the rapid receptor desensitization. This was reflected in the $\text{AUC}/P_{\text{max}}$ value of 1.7. In contrast, *(S,S)*-**13**, not only potentiated the signal but also significantly delayed the desensitization ($\text{AUC}/P_{\text{max}} = 25$). This kinetic profile resembled that of PNU-120596 (**7**), a typical Type II PAM. This profound difference in channel modulation was further investigated by comparing the activity profile of enantiomers of related active compounds. The enantiomeric pairs of a range of analogues of **13** (Table 4) are shown in Table 4 with P_3 values and current traces measured at $3\ \mu\text{M}$ using Patchliner. In

general, the *S,S*-enantiomers had a greater effect on receptor desensitization (i.e., more Type II in nature) than their *R,R*-enantiomer partners. Interestingly, it was possible to tune the compounds toward more Type I-like PAM activity by changing the substituents on the RHS aryl ring, as indicated by their current traces (Table 4). The electron withdrawing substituents on the RHS ring directed the enantiomers more toward Type I PAM character. The greatest impact in shifting the *S,S*-enantiomer toward Type I activity appeared to come from the nature of the *ortho*-substituents, where electron-withdrawing substituents (CF₃ and OCF₂H) pushed the kinetics more toward Type I; however, electron-donating substituents (methoxy and methyl) yielded compounds that had a greater effect on delaying desensitization (Type II). These results suggest that the electron density on the aniline ring may have a pivotal role in tuning desensitization kinetics. The discovery of different kinetics of the *trans*-enantiomers provided an opportunity to make a comparison between the efficacy and safety of Type I and II PAMs. The *in vitro* ADME and pharmacokinetics properties of the enantiomers of compound 13 (Table 5) showed that both (*R,R*)-13 and

Table 5. Profiling of Enantiomers of Compound 13

compounds	(<i>R,R</i>)-13	(<i>S,S</i>)-13
primary screening (<i>α7</i> GH4C1 planar patch clamp)		
P ₃ (%)	1160	11840
AUC/P ₃	3.1	57
secondary screen: <i>α7</i> GH4C1 manual patch clamp		
EC ₅₀	1.9 μM	0.063 μM
P _{max}	1570%	1630%
AUC/P _{max}	1.7	25
physicochemistry and <i>in vitro</i> ADME		
solubility at pH 6.5	1.6–3.1 μg/mL	
solubility at pH 2.0	>100 μg/mL	
logD7.4	4.2	
P _{app} (x10 ⁻⁶ cm/s) CACO-2	11	14
microsomal stability (E _H)		
h	0.84	0.59
m	0.79	0.71
rat PK		
Cl _p (mL/min/kg)	35	43
V _{ss} (L/kg)	1.8	5.1
BA (%)	62	77
AUC _{0-inf} /dose (h·μM/mg)	1.2	1.0
plasma half-life (h)	1.2	3.9

(*S,S*)-13 exhibited similar permeability in *in vitro* Caco-2 assay. In a human liver microsome stability assay, compound (*S,S*)-13 was slightly more stable (E_H = 0.59) than compound (*R,R*)-13 (E_H = 0.84), whereas the stability in mouse liver microsomes was very similar for both enantiomers (E_H = 0.79 and 0.71 for (*R,R*)-13 and (*S,S*)-13, respectively). These results were further supported by comparable *in vivo* plasma clearance and bioavailability in rats. Single dose IV (bolus, 4.0 mg/kg) PK in male Sprague–Dawley rats of each compound, formulated as 1.1 mg/mL in saline-based vehicle containing 0.1 M hydroxypropyl-β-cyclodextrin and 10% (v/v) DMSO, showed moderate plasma clearance and good exposure with dose normalized AUC of 1.2 and 1.0 h·μM, respectively. The oral bioavailability (BA) of these enantiomers in rat was found to be 62% for the *R,R*-enantiomer and 77% for the *S,S*-enantiomer. The *in vivo* efficacy of (*R,R*)-13 and (*S,S*)-13 was tested in mouse T-maze model at a range of 0.003–10.0 mg/

kg dose (Table 6). The compounds were formulated in saline-based vehicle containing 25% Cremophor ELP and were

Table 6. *In Vivo* Efficacy of Selected Compound in Mouse T-maze

compound	dose (mg/kg)	significance of reversal ^a	reversion (%)	n
<i>(R,R)</i> -13	0.003	ns	−9	10
	0.03	<i>d</i>	51	10
	0.3	<i>d</i>	62	10
	1.0	<i>d</i>	78	10
	3.0	<i>d</i>	78	10
<i>(S,S)</i> -13	0.003	ns	−3	10
	0.03	ns	11	9
	0.3	<i>d</i>	65	10
<i>(R,R)</i> -19	3.0	<i>d</i>	90	10
	1.0	ns	28	10
	3.0	ns	8	10
<i>(S,S)</i> -19	10.0	<i>c</i>	41	10
	1.0	<i>b</i>	46	10
	3.0	ns	16	10
<i>(R,R)</i> -26	10.0	<i>c</i>	51	10
	1.0	ns	−9	10
	3.0	ns	0	10
<i>(R,R)</i> -27	10.0	ns	10	10
	1.0	ns	8	10
	3.0	ns	31	10
<i>(S,S)</i> -27	10.0	ns	15	10
	1.0	ns	−13	10
	3.0	ns	−15	10
<i>(R,R)</i> -28	10.0	ns	−5	10
	1.0	ns	−18	9
	3.0	ns	38	10
<i>(S,S)</i> -28	10.0	ns	−9	10
	1.0	<i>b</i>	40	10
	3.0	<i>b</i>	39	9
	10.0	<i>d</i>	79	10

^ans: not significant. ^b**p* < 0.05. ^c***p* < 0.01. ^d****p* < 0.001.

administered orally. The significant difference between the group means was assessed by one-way analysis of variance (ANOVA) followed by Fisher's Protected Least Significant Difference using GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, CA). A *p* value < 0.05 was considered statistically significant. (*R,R*)-13 exhibited the MED of 0.03 mg/kg, and full reversal of the scopolamine-induced impairment was achieved at 1.0 mg/kg. (*S,S*)-13 was found to be less potent with MED of 0.3 mg/kg, and full reversal of the scopolamine-induced impairment was achieved at 3.0 mg/kg. Despite (*S,S*)-13 being more potent *in vitro*, the *in vivo* potency was lower, and thus, higher *in vitro* potency did not offer any clear advantage in this case.

Considering that a Type I PAM with an EC₅₀ ≈ 1 μM can achieve comparable or even better *in vivo* efficacy to a Type II PAM with an EC₅₀ < 100 nM, we compared the *in vivo* efficacy of modulators exhibiting similar potentiation profiles, but lower *in vitro* microsomal clearance. Compounds (*R,R*)-19, (*S,S*)-19, (*R,R*)-26, (*R,R*)-27, (*S,S*)-27, (*R,R*)-28, and (*S,S*)-28 were chosen because they all showed improved intrinsic clearance in mouse and human microsomes relative to (*R,R*)-13 and (*S,S*)-13 (data not shown).

Upon evaluation in the mouse T-maze at 1, 3, and 10 mg/kg doses, these compounds were found to be less potent than

(*R,R*)-13 and (*S,S*)-13 (Table 6). Only (*S,S*)-28 exhibited comparable efficacy but at higher dose of 10 mg/kg. The lower *in vivo* potency/efficacy of these compounds may have been due to other DMPK properties, which were not fully profiled for these compounds. Based upon its overall profile and superior potency in the mouse T-maze assay, we selected (*R,R*)-13 for further evaluation and profiling. In addition to being highly potent *in vivo*, (*R,R*)-13 possessed an excellent safety profile: no change in the spatial and temporal response of the receptor to endogenous ligand ACh, no activity on other related channels (data not disclosed), and no inhibition of major drug metabolizing enzymes such as CYP3A4, CYP2C9, CYP2D6, CYP1A2, and CYP2C19 ($IC_{50} > 10 \mu M$). It is worth mentioning that, although some of the related analogs have similar or improved solubility and *in vitro* microsomal clearance, the overall combination of properties of (*R,R*)-13 (BNC 375), including percent potentiation of the acetylcholine response, oral bioavailability, half-life, and brain exposure, has provided a molecule with efficacy across a broad range of oral doses *in vivo* that warrants further evaluation as a potential clinical candidate.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acsmchemlett.9b00001](https://doi.org/10.1021/acsmchemlett.9b00001).

Synthetic procedures and analytical characterization data for key compounds, detailed SAR analysis tables, biological assay protocols, and crystallography experimental (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: dpaul@bionomics.com.au.

ORCID

Dharam Paul: [0000-0001-9540-9455](https://orcid.org/0000-0001-9540-9455)

Present Addresses

[‡](A.J.H.) UniQuest Pty Ltd., Level 7, General Purpose South Building, Staff House Road, Brisbane, QLD-4072, Australia.

^{||}(R.S.) Department of Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, 381 Royal Parade, Parkville, VIC-3052, Australia.

[†](A.A.G.) Bigtincan, Level 20, 320 Pitt Street, Sydney, NSW 2000, Australia.

[◆](T.D.A.) Centre for Nanoscale BioPhotonics, The University of Adelaide, North Terrace, Adelaide, SA-5000, Australia.

Author Contributions

The manuscript was written by D.P. with editing support from S.O.C., A.J.H., T.D.A., C.M., and R.S. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): The authors are employees of Bionomics and its subsidiaries (Prestwick and Neurofit), and Bionomics has a commercial interest in positive allosteric modulation of nicotine acetylcholine receptors.

■ ACKNOWLEDGMENTS

The authors gratefully thank Ian Bell, Jason Uslaner, and Xiaohai Wang of MSD for their review and constructive comments, and Ben Harvey of Bionomics for providing the publication quality Patchliner current trace figures.

■ ABBREVIATIONS

PAM, positive allosteric modulator; nAChRs, nicotinic acetylcholine receptors; ACh, acetylcholine; SAR, structure–activity relationship; ADME, absorption, distribution, metabolism, and excretion; E_H , hepatic extraction ratio; BA, bioavailability; DMPK, drug metabolism and pharmacokinetics; CYP, cytochrome P450

■ REFERENCES

- (1) Lewis, A. S.; Schalkwyk, G. I.; Bloch, M. H. Alpha-7 nicotinic agonists for cognitive deficits in neuropsychiatric disorders: A translational meta-analysis of rodent and human studies. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2017**, *75*, 45–53.
- (2) Potasiewicz, A.; Nikiforuk, A.; Holuj, M.; Popik, P. Stimulation of nicotinic acetylcholine alpha7 receptors rescue schizophrenia-like cognitive impairments in rats. *J. Psychopharmacol.* **2017**, *31* (2), 260–271.
- (3) Lendvai, B.; Kassai, F.; Szájlí, Á.; Némethy, Z. $\alpha 7$ Nicotinic acetylcholine receptors and their role in cognition. *Brain Res. Bull.* **2013**, *93*, 86–96.
- (4) Yang, T.; Xiao, T.; Sun, Q.; Wang, K. The current agonists and positive allosteric modulators of $\alpha 7$ nAChR for CNS indications in clinical trials. *Acta Pharm. Sin. B* **2017**, *7* (6), 611–622.
- (5) Uteshev, V. V. The therapeutic promise of positive allosteric modulation of nicotinic receptors. *Eur. J. Pharmacol.* **2014**, *727*, 181–185.
- (6) Ng, H. J.; Whittemore, E. R.; Tran, M. B.; Hogenkamp, D. J.; Broide, R. S.; Johnstone, T. B.; Zheng, L.; Stevens, K. E.; Gee, K. W. Nootropic $\alpha 7$ nicotinic receptor allosteric modulator derived from GABA_A receptor modulators. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104* (19), 8059–8064.
- (7) Timmermann, D. B.; Grønlien, J. H.; Kohlhaas, K. L.; Nielsen, E. Ø.; Dam, E.; Jørgensen, T. D.; Ahring, P. K.; Peters, D.; Holst, D.; Chrsitensen, J. K.; Malysz, J.; Briggs, C. A.; Gopalakrishnan, M.; Olsen, G. M. An allosteric modulator of the $\alpha 7$ nicotinic acetylcholine receptor possessing cognition-enhancing properties *in vivo*. *J. Pharmacol. Exp. Ther.* **2007**, *323* (1), 294–307.
- (8) Eskildsen, J.; Redrobe, J. P.; Sams, A. G.; Dekermendjian, K.; Laursen, M.; Boll, J. B.; Papke, R. L.; Bundgaard, C.; Frederiksen, K.; Bastlund, J. F. 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. *Bioorg. Med. Chem. Lett.* **2014**, *24* (1), 288–93.
- (9) Halvard Grønlien, J.; HÅkerud, M.; Ween, H.; Thorin-Hagene, K.; Briggs, C. A.; Gopalakrishnan, M.; Malysz, J. Distinct profiles of 7 nAChR positive allosteric modulation revealed by structurally diverse chemotypes. *Mol. Pharmacol.* **2007**, *72*, 715–724.
- (10) Sahdeo, S.; Wallace, T.; Hirakawa, R.; Knoflach, F.; Bertrand, D.; Maag, H.; Misner, D.; Tombaugh, G. C.; Santarelli, L.; Brameld, K.; Milla, M. E.; Button, D. C. Characterization of RO5126946, a novel $\alpha 7$ nicotinic acetylcholine receptor-positive allosteric modulator. *J. Pharmacol. Exp. Ther.* **2014**, *350* (2), 455–68.
- (11) Dinklo, T.; Shaban, H.; Thuring, J. W.; Lavreysen, H.; Stevens, K. E.; Zheng, L.; Mackie, C.; Grantham, C.; Vandenberg, I.; Meuldere, G.; Peeters, L.; Verachttert, H.; Prins, E. D.; Lesage, A. S. J. Characterization of 2-[[4-fluoro-3-(trifluoromethyl)phenyl]amino]-4-(4-pyridinyl)-5-thiazolemethanol (JNJ-1930942), a novel positive allosteric modulator of the $\alpha 7$ nicotinic acetylcholine receptor. *J. Pharmacol. Exp. Ther.* **2011**, *336* (2), 560–74.

(12) Hurst, R.; Hajós, M.; Raggenbass, M.; Wall, T.; Higdon, N.; Lawson, J.; Rutherford-Root, K.; Berkenpas, M.; Hoffmann, W.; Piotrowski, D.; Groppi, V. E.; Allaman, G.; Ogier, R.; Bertrand, S.; Bertrand, D.; Arneric, S. P. A novel positive allosteric modulator of the $\alpha 7$ neuronal nicotinic acetylcholine receptor: in vitro and in vivo characterization. *J. Neurosci.* **2005**, *25* (17), 4396–4405.

(13) Malysz, J.; Grønlien, J. H.; Anderson, D. J.; HÅkerud, M.; Thorin-Hagene, K.; Ween, H.; Wetterstrand, C.; Briggs, C. A.; Faghhi, R.; Bunnelle, W. H.; Gopalakrishnan, M. In vitro pharmacological characterization of a novel allosteric modulator of $\alpha 7$ neuronal acetylcholine receptor, 4-(5-(4-chlorophenyl)-2-methyl-3-propionyl-1H-pyrrol-1-yl)benzenesulfonamide (A-867744), exhibiting unique pharmacological profile. *J. Pharmacol. Exp. Ther.* **2009**, *330* (1), 257–267.

(14) Gill-Thind, J. K.; Dhankher, P.; D'Oyley, J. M.; Sheppard, T. D.; Millar, N. S. Structurally similar allosteric modulators of $\alpha 7$ nicotinic acetylcholine receptors exhibit five distinct pharmacological effects. *J. Biol. Chem.* **2015**, *290* (6), 3552–3562.

(15) Harvey, A.; Fluck, A.; Giethlen, B.; Paul, D.; Schaeffer, L. Positive allosteric modulators of the alpha 7 nicotine acetylcholine receptors and uses thereof. WO 2012103583.

(16) Harvey, A.; Avery, T.; Paul, D.; Ripper, J.; Huff, B.; Singh, R.; Schaeffer, L.; Joseph, C.; Morice, C.; Giethlen, B. $\alpha 7$ Nicotine acetylcholine receptor modulators and uses thereof. WO 2014019023.