Letter

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Discovery of BNC375, a Potent, Selective, and Orally Available Type I Positive Allosteric Modulator of α7 nAChRs.

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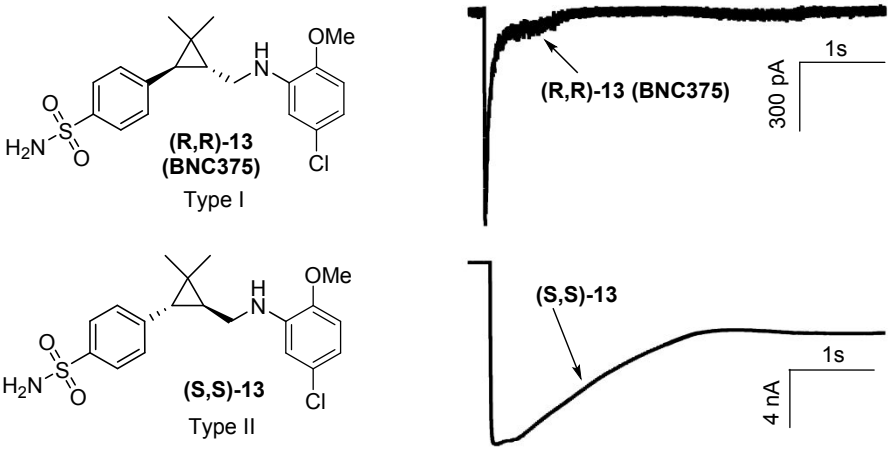
Alpha 7 nicotinic acetylcholine receptor, positive allosteric modulators, memory, T-maze, Attention

ABSTRACT: Positive allosteric modulators (PAMs) of α7 nAChRs can have different properties with respect to their effects on channel kinetics. Type I PAMs amplify peak channel response to acetycholine 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 of aniline with strong electron withdrawing groups reduces the Type II character of these compounds. Our structure-activity optimization efforts has led to the discovery of **BNC375**, a small molecule with good CNS-drug like properties and clinical candidate potential.



Activation of alpha 7 nicotinic acetylcholine receptors (α7 nAChRs) improves cognitive function both in humans[1] and rodents[1,2], and thus represents a promising therapeutic approach for cognitive impairment in Alzheimer's disease[3] and schizophrenia[3,4]. The activity of α7 nAChR can be enhanced through either orthosteric agonists or positive allosteric modulators (PAMs). In contrast to α7 agonists, α7 PAMs do not activate α7 nAChRs by themselves and work by amplification of responses induced by endogenous agonists, hence preserving spatiotemporal signaling patterns. Furthermore, α7 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, α7 PAMs can be broadly classified into two categories: Type I and Type II[5]. Type I α7 PAMs, such as AVL-3288[6] (**1**), NS1738[7] (**2**) and Lu AF58801[8] (**3**, Figure 1), increase channel responses to acetylcholine without affecting receptor desensitization, whereas Type II α7 PAMs, such as TQS[9] (**4**), RO5126946[10] (**5**), JNJ-1930942[11] (**6**), PNU-120596[12] (**7**) and A-867744[13] (**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,

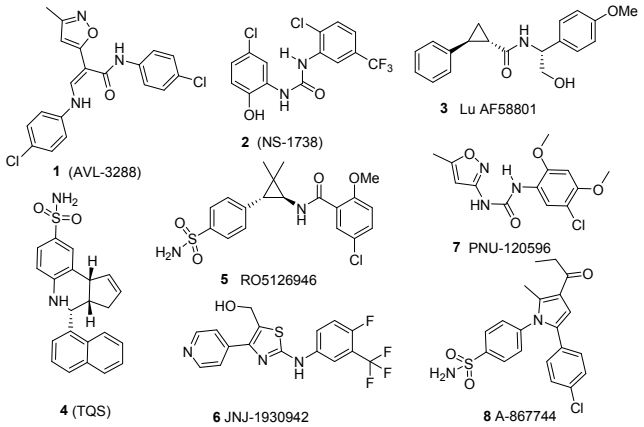


Figure 1. Representative examples of reported α7 nAChRs PAMS.

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 α7 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.

During the early days of the program, the knowledge about the structural requirements for α7 PAM activity was ambiguous because literature examples covered many diverse structural classes containing two terminal rings joined through linear, as well as 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 α7 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 EC_{20} acetylcholine (ACh) response by 3μM of each PAM was determined. Secondary screening and more detailed characterisation of promising compounds was 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 α7 nAChR in rat GH4C1 cells. The percent change in peak current produced by 3 μM of test compound plus acetylcholine versus acetylcholine alone was called P_3 (percent potentiation at 3 μM). *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) were of particular interest[15], exhibiting P_3 values of 660, 710 and 410 %, respectively. The activity of **9** and **10** was confirmed in the secondary screening assay where they exhibited P_3 values of 420% and 360%, respectively (Table 1).

The hits **9** & **10** significantly reversed scopolamine induced impairment of spontaneous alternation in the mouse T-maze at

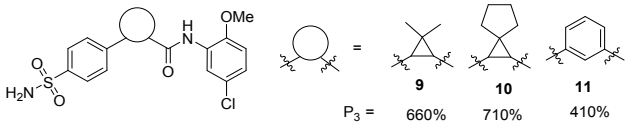


Figure 2. Key compounds from the hit identification phase.

intraperitoneal (*ip*) doses of 3 mg/kg and 30 mg/kg. However, compounds **10** and **11** were found to be inactive in 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.

Table 1. Profile of hit compounds 9, 10 and 11

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
$Sol_{6.5}$ (μg/mL)		3.1-6.3	1.6-3.1	NT
Primary *in vivo* Assay: mouse T-maze Significant dose (mg/kg)	*ip*	3, 30	3, 30	NT
	po	NT	NS (3, 30)	NS (3, 30)

NT: Not tested; NS: Not significant

As shown in Figure 2, the hit molecules **9-11** have a modular structure, composed of three distinctive regions: RHS ring, 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 the *para* position of LHS phenyl ring and 2-alkoxy-5-halo group on RHS aniline ring were essential features for in vitro α7 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

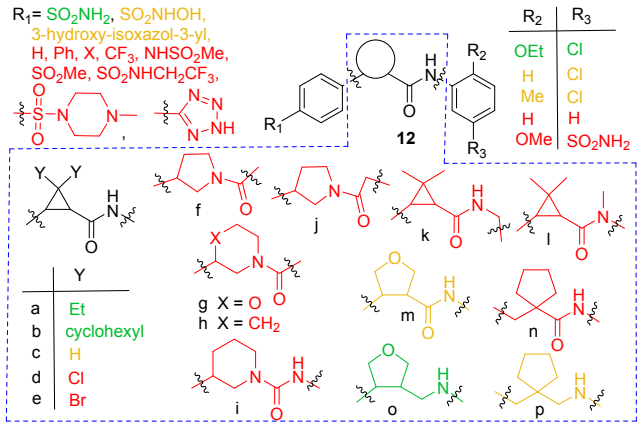
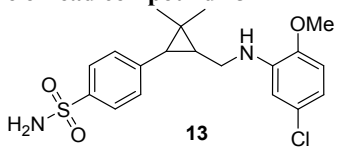


Figure 3. Representative examples exploring the hit to lead SAR (green: active; orange: weakly active and red: inactive)

Table 2. Profile of lead compound 13



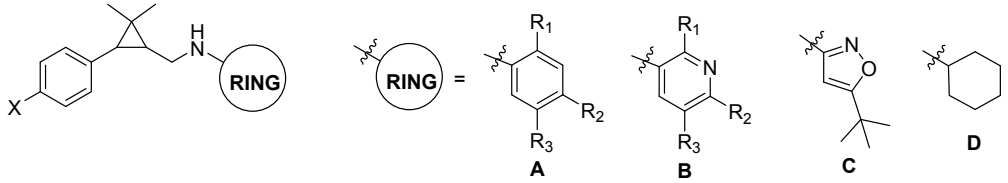
13

Primary Screening P_3 (%)		7900
Secondary Screening P_{max} (%)		650
Secondary Screening EC_{50} (nM)		25
Solubility (μg/ml)	pH 2.0	1.6-3.1
	pH 6.5	>100
Microsomal stability (E_H)§	human	0.64
	mouse	0.82
Primary in vivo Mouse T-maze Oral significant dose (mg/kg)		3, 10

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

dihalo (**12d**, **12e**) rendered the compared to the amide molecules **12m** and **12n**, was seen in the corresponding amines **12o** and **12p**, respectively. Hence, we compounds inactive. Interestingly, the modest gain in activity, 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 α7 nAChR potentiation with P_3 values of 7900%, 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 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 α7 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 EC_{50} of 25 nM and peak current potentiation P_{max} of 650% relative to the peak current evoked by ACh at an EC_{20} concentration. Overall, racemate **13** exhibited a good lead profile (Table 2) and had a 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

Table 3. Representative examples for SAR on the RHS modifications



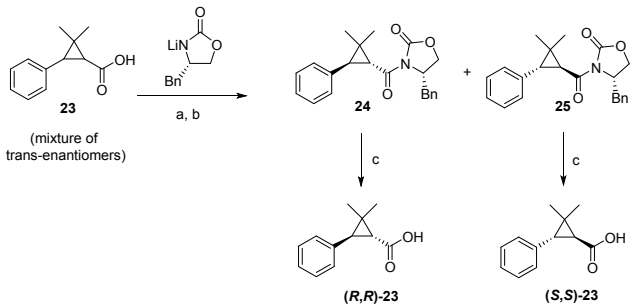
	X	RING	R_1	R_2	R_3	P_3 (%)	SEM (n)
13	SO_2NH_2	A	OMe	H	Cl	7900	3560 (2)
14	SO_2NH_2	A	H	H	H	2570	660 (3)
15	SO_2NH_2	A	Me	H	Cl	12310	3870 (3)
16	SO_2NH_2	A	Me	F	H	8660	4280 (3)
17	SO_2NH_2	A	Me	F	Cl	9230	900 (3)
18	SO_2NH_2	A	OMe	H	CF_3	8170	2060 (4)
19	SO_2NH_2	A	$OCHF_2$	F	H	3360	1190 (3)
20	SO_2NH_2	A	OCF_3	H	F	2440	660 (2)
21	SO_2NH_2	B	OMe	H	H	2420	550 (4)
22	SO_2NH_2	B	H	H	F	3000	400 (4)
32	SO_2NHNH_2	A	OMe	H	Cl	130	100 (3)
33	SO_2NHOH	A	OMe	H	Cl	720	260 (6)
58	SO_2NH_2	A	OMe	H	SO_2NH_2	<100	12 (2)
66	SO_2NH_2	A	H	morpholino	H	<100	16 (3)
67	SO_2NH_2	A	H	H	morpholino	460	12 (4)
69	SO_2NH_2	B	Me	H	H	130	50 (2)
72	SO_2NH_2	C	-	-	-	<100	8 (2)
74	SO_2NH_2	D	-	-	-	<100	30 (6)

to replace LHS p-sulfamoylphenyl ring with p-sulfamoylpyridyl proved unsuccessful. Replacement of p-sulfamyl 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-sulfamyl group with comparable or slightly more lithophilic 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 attachment of the methanesulfonamide group through the nitrogen to the aryl ring rather than the sulfur atom. However, the original lead compound **13** with a p-sulfamyl group was still the most active analog, indicating that the sulfamyl group was a critical feature of potent α7 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 2-methoxy-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 phenyl with polar groups such as sulfamyl (**58**) or morpholino (**66** or **67**) also reduced the activity. In general, decreasing lipholicity to improve solubility and clearance failed to give an active compound. Efforts devoted to explore the alternatives for metabolically labile $–OCH_3$ 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 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 supplementary information.

Scheme 1. Synthesis of enantiopure building block 23

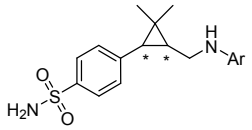


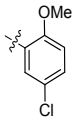
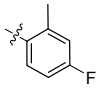
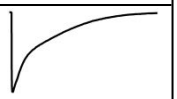
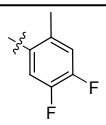
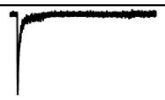
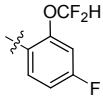
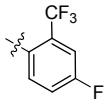
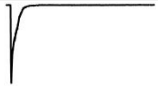
Reagents and conditions: a) $SOCl_2$, DCM ; b) (S)-4-benzyl-2-oxazolidinone, nBuLi, THF; c) H_2O/THF, H_2O_2, LiOH

Both *trans* enantiomers of compound **13** were found to be active in the primary screening assay at 3 μM. 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 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 α7 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 AUC/P_{max} value of 1.7. In contrast, (***S*,*S*)-13**, not only potentiated the signal but also significantly delayed the desensitization ($AUC/P_{max} = 25$). 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 μM 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*-enantiomeric 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 towards Type I PAM character. The greatest impact in shifting the *S,S*-enantiomer towards Type I activity appeared to come from the nature of the ortho substituents, where electron-withdrawing substituents (CF_3 and OCF_2H) pushed the kinetics more towards 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

Table 4. Effect of RHS substituents on kinetics of α7 nAChR modulation



No.	-Ar	Current Trace^ P_3 (%)	
		R,R	S,S
13		 1160%	 11840%
16		 470%	 17580%
26		 460%	 17430%
27		 420%	 8930%
19		 170%	 8170%
28		 410%	 2860%

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

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 (***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 0.1M 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

Table 5. Profiling of enantiomers of compound 13

Compounds		**(*R*,*R*)-13**	**(*S*,*S*)-13**
Primary Screening (rα7 GH4C1 planar patch clamp)			
P_3 (%)		1160	11840
AUC/P_3		3.1	57
Secondary screen: rα7 GH4C1 manual patch clamp			
EC_{50}		1.9 μM	0.063 μM
P_{max}		1570%	1630%
AUC/P_{max}		1.7	25
Physicochemistry & in vitro 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
$AUC_{0\text{-}inf}$ /Dose (h*μM)/mg		1.2	1.0
Plasma half-life (hr)		1.2	3.9

a range of 0.003-10.0 mg/kg dose (Table 6). The compounds were formulated in saline based vehicle containing 25% cremephor ELP and were administered orally. The significant difference between the group means were 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 $EC_{50} \approx 1$ μM can achieve comparable or even better *in vivo* efficacy to a Type II PAM with an $EC_{50} < 100$ 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).

Table 6. *In vivo* efficacy of selected compound in mouse T-maze

Comp.	Dose (mg/kg)	Significance of reversal*	Reversion (%)	n
(*R,R*)-13	0.003	ns	-9	10
	0.03	***	51	10
	0.3	***	62	10
	1.0	***	78	10
	3.0	***	78	10
(*S,S*)-13	0.003	ns	-3	10
	0.03	ns	11	9
	0.3	***	65	10
	3.0	***	90	10
(*R,R*)-19	1.0	ns	28	10
	3.0	ns	8	10
	10.0	**	41	10
(*S,S*)-19	1.0	*	46	10
	3.0	ns	16	10
	10.0	**	51	10
(*R,R*)-26	1.0	ns	-9	10
	3.0	ns	0	10
	10.0	ns	10	10
(*R,R*)-27	1.0	ns	8	10
	3.0	ns	31	10
	10.0	ns	15	10
(*S,S*)-27	1.0	ns	-13	10
	3.0	ns	-15	10
	10.0	ns	-5	10
(*R,R*)-28	1.0	ns	-18	9
	3.0	ns	38	10
	10.0	ns	-9	10
(*S,S*)-28	1.0	*	40	10
	3.0	*	39	9
	10.0	***	79	10

* ns: not significant; * p <0.05; ** p <0.01; *** p <0.001

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 μM). 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.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

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

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Note: 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.

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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;

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