Discovery and Structure−Activity Relationship of Quinuclidine Benzamides as Agonists of α7 Nicotinic Acetylcholine Receptors

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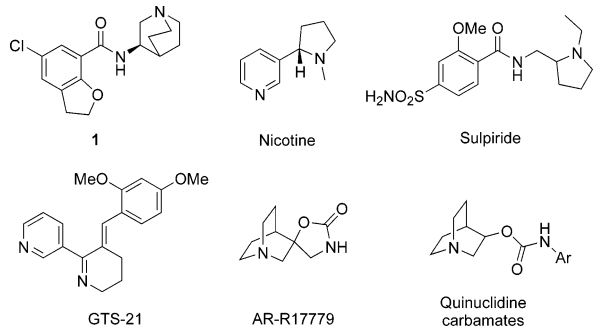
Abstract: A library of benzamides was tested for α7 nicotinic acetylcholine receptor (nAChR) agonist activity using a chimeric receptor in a functional, cell-based, high-throughput assay. From this library, quinuclidine benzamides were found to have α7 nAChR agonist activity. The SAR diverged from the activity of this compound class verses the 5-HT_3 receptor, a structural homologue of the α7 nAChR. PNU-282987, the most potent compound from this series, was also shown to open native α7 nAChRs in cultured rat neurons and to reverse an amphetamine-induced gating deficit in rats.

Nicotinic acetylcholine receptors (nAChRs) are found throughout the central and peripheral nervous systems and in the neuromuscular junction.[1] Numerous studies have established the importance of nAChRs within the CNS, in particular their link to higher processes such as memory, cognition, reward, and sensory processing.[1] Neuronal nAChRs are pentameric ligand-gated ion channels that are formed by combinations of α and β subunits or as homopentamers in the cases of α7, α8, and α9. To date, 10 α and 4 β isoforms have been discovered, resulting in a huge diversity of possible compositions, though only a small subset of combinations were shown to give rise to functionally and physiologically relevant channels.[2]

The homomeric α7 subtype is highly permeable to calcium and was proposed to modulate a variety of attentional and cognitive processes.[3] These receptors are prevalent in the hippocampus where they modulate inhibitory GABAergic synaptic transmission involved in sensory processing. Deficits in auditory sensory processing are thought to lead to a state of sensory overload and are hypothesized to contribute to the attentional and cognitive problems in a variety of CNS diseases, most convincingly in schizophrenia. It was shown that schizophrenics have decreased levels of α7 nAChRs and sensory gating deficits that can be corrected with nicotine; analogous deficits in rodent models can also be restored with the α7 nAChR partial agonist GTS-21.[4] Improvements in sensory gating were shown to correlate with improvements in cognitive performance in animal models and in schizophrenic patients, suggesting a beneficial role for a selective α7 nAChR agonist for the treatment of schizophrenia.[5] The diversity of nicotinic ligands has expanded greatly over the past decade, with the focus on selective agents of particular interest.[6]

Chart 1



Compound **1** (Chart 1) was identified via high-throughput screening as a weak agonist of a chimera of the α7 (α7-5HT_3)[7] receptor (Figure 1). It had been shown that **1** is a 5-HT_3 antagonist with a K_i of 1 nM and is typical of the quinuclidine amide template, a fertile source of potent 5-HT_3 inhibitors.[8] More generally, this compound is a member of the class of 1,2-diamine-monoamides such as sulpiride, which have found utility as modulators of a variety of CNS receptors. We therefore chose to explore the potential of this diamine-amide class of molecules as agonists of the α7 nAChR. At the time **1** was discovered, there were only a few α7 selective agonists in the literature, including GTS-21[9] and AR-R17779.[10] Structurally related carbamates had also been disclosed in the literature,[11] while more recently, the 5-HT_3 antagonist tropisetron was shown to have α7 agonist activity.[12]

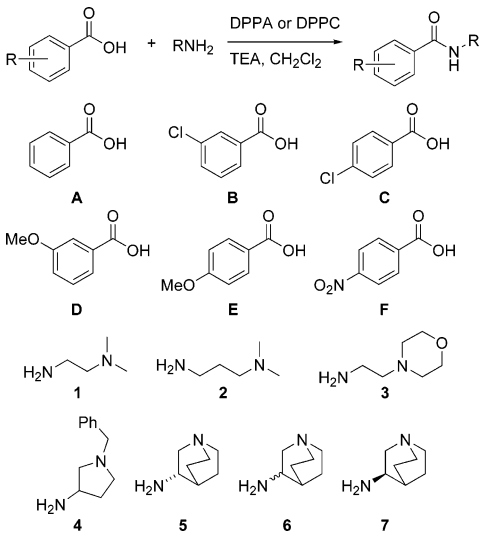


Figure 1. Makeup of the benzamide library. PNU-282987 is compound combination **C7**.

To follow up the discovery of this hit and rapidly assess whether α7-5HT_3 selectivity could be attained, we chose a strategy of preparing and testing small focused libraries of amides. These compounds could be

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rapidly prepared on the benchtop without specialized equipment to attain this goal. By following up on the resulting hits with iterative small libraries, one or two chemists were able to get a quick overview of the SAR in a few weeks by making tens of compounds rather than hundreds or thousands. The wide array of available amide bond forming procedures allowed us to screen a variety of reactions, evaluating them on the basis of product purity.[13] We screened carbodiimides such as DCC and DIC, phosphonium reagents such as BOP and PyBOP, mixed anhydrides from pivaloyl chloride or BOPCl, and diphenylphosphorylazide (DPPA). Since all of the products were amines, we used a capture/release technique to purify the products. The crude reaction mixtures were simply poured onto a plug of acidic resin and washed thoroughly. The products were then eluted with methanolic triethylamine to give the desired amides. While all of the reactions gave the desired amides in good yield, they varied widely in the purity of the final products. Both the carbodiimide and the phosphonium methods worked poorly by this criterion. The byproducts of these reactions, ureas and phosphoroamidates, were strongly retained on the resin and required prohibitively large wash volumes to elute. This is probably due to the weak basicity of such compounds ($pK_a \approx 0$ for simple ureas), causing them to be partially retained by the acidic ion-exchange resin.

The mixed anhydride procedure gave better results; however, pivaloyl chloride often reacted slowly, while reactions with BOPCl gave products contaminated with impurities arising from decomposition of the reagent. DPPA was found to give the purest products in good yields. Despite its advantages in terms of product purity, a significant problem was encountered when using DPPA with 2-substituted benzoic acids. In these cases, a competitive Curtius rearrangement occurred, yielding the corresponding urea in addition to the desired amide. For this reason, we excluded this group of acids from the initial library. With the methodology set, we synthesized a small library using six aryl acids and seven amine-starting materials (Figure 1).

The resulting library of 42 compounds was tested in a FLIPR assay using SHEP cells expressing the α7-5HT$_3$ chimera (Figure 2). All of the analogues with significant activity contained 3-aminoquinuclidine as the amine half of the amide (amines **5**−**7**). Within the quinuclidine series, the *R* enantiomer (amine **7**) was preferred over the *S* (amine **5**), with the racemic compounds falling in between (amine **6**). This is in contrast to the 5-HT$_3$ SAR in which the *S* enantiomer is the more potent. Finally, there was a positive effect of having a substituent in the para position of the benzamide over having the same substituent in the meta position or having no substituent at all (the phenyl benzamide **A**). The best compound from this library was the 4-chlorobenzamide (**C7**), PNU-282987, which had an EC_{50} at the chimera of 154 nM. This was followed by the 4-methoxybenzamide (**E7**), which had an EC_{50} of 360 nM, and the 4-nitrobenzamide (**F7**), with an EC_{50} of 1 μM.

The SAR of the quinuclidine amides was investigated in more detail with the construction of a more focused library (Table 1). In this experiment, (*R*)-3-aminoquinuclidine was the only amine used and the benzoic acid

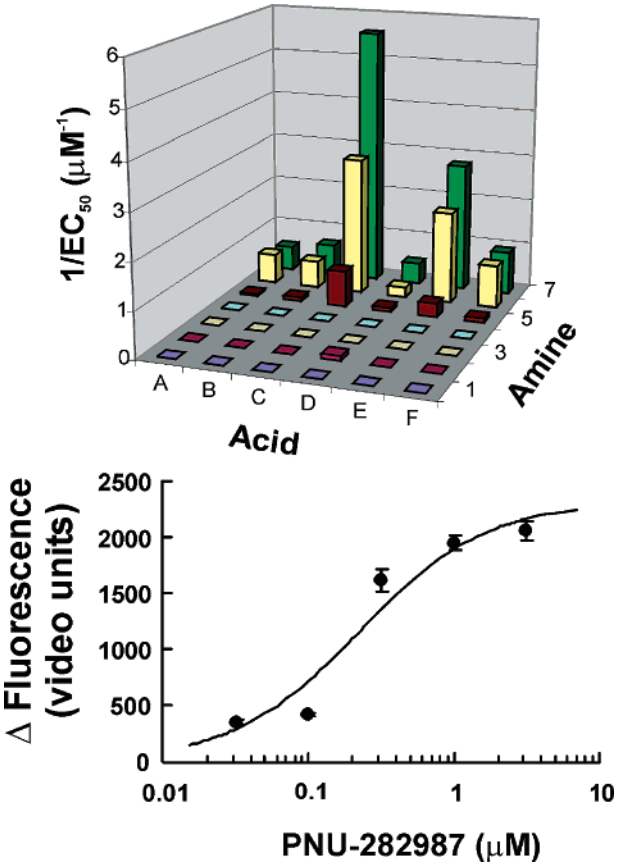


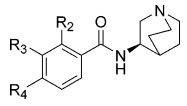
Figure 2. Functional activity of the benzamide library toward the α7-5HT$_3$ chimera (top) and an example of the concentration−response for PNU-282987 (bottom). See Figure 1 for the labeling of acid and amine structures.

was varied more widely. To include 2-substituted benzoic acids, an alternative experimental procedure was required to avoid the complicating Curtius rearrangement. We found that diphenylphosphoryl chloride (DPPC) worked as well as DPPA as a reagent for the construction of the amide bond, and because there was no azide present, the Curtius rearrangement was precluded.

The resulting compounds showed a wide range of activity, but the SAR was very clear. Ortho-substituted benzamides were very poor α7-5HT$_3$ chimera agonists in contrast to the 5-HT$_3$ SAR where an *o*-alkoxy group is required for potent binding. The 2-fluorobenzamide (**11**) had an EC_{50} of over 16 μM, while the 2-methoxy analogue (**14**) was essentially inactive. For the meta-substituted compounds, the methoxy (**15**) and fluoro (**12**) substituents showed intermediate activity while the para-substituted analogues were the most active, thus establishing the relationship para > meta ≫ ortho.

The remainder of this quinuclidine amide library was focused on the SAR of the critical 4-position of the benzamide. The most potent analogues contained small substituents in the para position. Compounds with chloro, fluoro, methyl, or methoxy groups had similar activity; however, increasing in size from methyl (**26**) to ethyl (**27**) led to an order of magnitude increase in EC_{50}, and the *n*-propyl (**28**) and *i*-propyl (**29**) analogues were inactive. In fact, all compounds in this study with a 4-substituent that contained more than two non-hydrogen atoms showed poor activity ($EC_{50} \geq 30$ μM) with the exception of the 4-nitro analogue (**24**), demon-

Table 1. Activity of the Focused Quinuclidine Benzamide Library toward the α7-5HT$_3$ Chimera



compd	R_2	R_3	R_4	EC_{50}(α7-5HT$_3$) (nM)
10	H	H	H	1900 (829[a])
11	F	H	H	16700
12	H	F	H	3300
13	H	H	F	506 (570[a])
14	OMe	H	H	>100000
15	H	OMe	H	2200
16	H	H	OMe	360 (300[a])
17	H	H	SMe	164 (241[a])
18	H	Cl	H	1500
19	H	H	Cl	128 (154[a])
20	H	H	Br	206 (126[a])
21	H	H	I	494 (349[a])
22	H	H	OBn	>100000
23	H	H	SBn	100000
24	H	H	NO_2	1000
25	H	H	Ac	30000
26	H	H	Me	324
27	H	H	Et	3760
28	H	H	*n*Pr	>100000
29	H	H	*i*Pr	>100000
30	H	H	SO_2Me	>100000
31	H	H	NMe_2	30000
32	H	H	CF_3	>100000
33	H	H	NHAc	>100000

[a] Activity in parentheses obtained from individually resynthesized compounds.

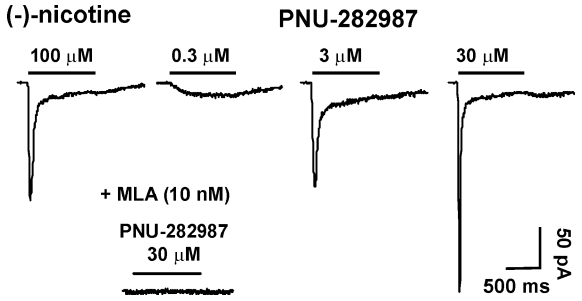


Figure 3. Activity of PNU-282987 on cultured rat hippocampal neurons.

strating a critical dependence of the activity on the steric bulk of this substituent.

Several assays were used to validate that the chimera assay could be used to identify agonists of native receptors. In a binding assay, PNU-282987 displaced the α7 selective antagonist methyllycaconitine (MLA) from rat brain homogenates with a K_i of 27 nM. Also, when applied to cultured rat hippocampal neurons, PNU-282987 evoked a rapidly desensitizing inward whole-cell current that was concentration-dependent and blockable by MLA, consistent with opening of the α7 receptor (Figure 3).[14]

The selectivity of PNU-282987 over related receptors was also evaluated. In particular we were concerned with agonism of the neuromuscular junction form of the receptor ($\alpha1\beta1\gamma\delta$) and the predominant ganglionic nAChR ($\alpha3\beta4$). Activation of these receptors was shown to cause many of the undesirable effects of nonspecific agonists such as epibatidine and nicotine.[15] PNU-282987 showed no detectable agonist activity up to 100 μM and negligible antagonist activity ($IC_{50} \geq 60$ μM) at both receptor subtypes. Further, PNU-282987 did not

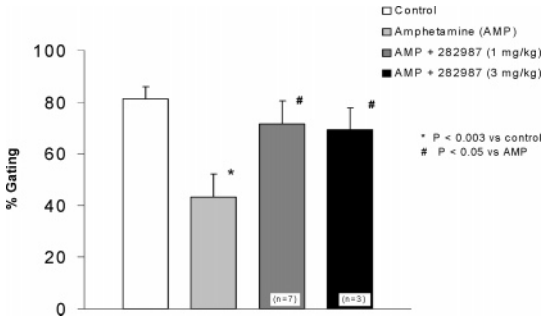


Figure 4. Effect of PNU-282987 on the auditory gating deficit in amphetamine treated rats.

significantly displace tritiated cytisine from rat brain homogenates at 1 μM (14% inhibition), suggesting a high selectivity over the α4β2 subtype.[16] With respect to the 5-HT$_3$ receptor, PNU-282987 displaced tritiated GR-65630 with a K_i of 1662 nM,[17] translating into a selectivity of about 62-fold for α7 compared to the high selectivity of **1** for the 5-HT$_3$R (over 500-fold). In a cell-based FLIPR assay, PNU-282987 was found to be a functional antagonist of the 5-HT$_3$ receptor ($IC_{50} = 4541$ nM). Broader selectivity of PNU-282987 was evaluated in a screen of 32 receptors, ion channels, and enzymes at Cerep (Rueil-Malmaison, France). At a test concentration of 1 μM, PNU-282987 produced <30% inhibition of specific binding or enzyme activity at all targets except the 5-HT$_3$ receptor.

We also tested this compound in a rat model of the impaired sensory gating, which had been validated with the known α7 partial agonist GTS-21.[18] Systemic administration of D-amphetamine (0.3 or 1 mg/kg, iv) significantly disrupted auditory gating in anesthetized rats because of a combination of simultaneous decreases of conditioning responses with corresponding increases in test responses. Subsequent administration of the α7 nAChR agonist PNU-282987 (iv, 1 or 3 mg/kg, $n = 10$) significantly reversed amphetamine-induced gating deficit (Figure 4). In contrast, application of vehicle in control rats did not normalize the amphetamine-induced gating deficit ($n = 9$). Furthermore, PNU-282987 (1 mg/kg) had no significant effect on normal gating ($n = 4$) in anesthetized rats.

In conclusion, using parallel synthesis, we have explored the SAR of tertiary-amine-containing arylamides as α7 nAChR agonists. Of the five structurally different amines tested, only 3-aminoquinuclidine yielded active analogues. The *R* enantiomers were more active than the corresponding *S* enantiomers. On the benzamide portion, small substituents in the para position gave the most active analogues. The most potent analogue in this series was the 4-chlorobenzamide, PNU-282987. Several experiments confirm that the α7-5HT$_3$ chimera assay is predictive of native α7 nAChR activity. PNU-282987 displaced MLA from rat brain homogenates with a K_i of 27 nM, and it evoked currents in rat hippocampal neurons in a concentration-dependent and MLA blockable manner. We also tested this compound in a rat model of impaired sensory gating. Treatment of gating impaired rats with PNU-282987 led to a reversal of the gating deficit. These results demonstrate that (*R*)-3-aminoquinuclidine arylamides such as PNU-282897 are a template for finding α7

nAChR agonists that may be useful for treating the cognitive and attentional deficits of schizophrenia.

Supporting Information Available: List of abbreviations, experimental description, and analytical data for library compounds, details of the biological assays, and results from broad selectivity profiling performed at Cerep. This material is available free of charge via the Internet at http://pubs.acs.org.

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