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Discovery of a novel series of quinolone α7 nicotinic acetylcholine receptor agonists

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ABSTRACT

High throughput screening led to the identification of a novel series of quinolone α7 nicotinic acetylcholine receptor (nAChR) agonists. Optimization of an HTS hit (**1**) led to 4-phenyl-1-(quinuclidin-3-ylmethyl)quinolin-2(1*H*)-one, which was found to be potent and selective. Poor brain penetrance in this series was attributed to transporter-mediated efflux, which was in turn due to high pK_a. A novel 4-fluoroquinuclidine significantly lowered the pK_a of the quinuclidine moiety, reducing efflux as measured by a Caco-2 assay.

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The α7 nicotinic acetylcholine receptor (nAChR) has been a target of significant and growing interest in neuroscience research for more than two decades as evidence has accumulated that it may be a viable target for treating the cognitive deficits and negative symptoms seen in schizophrenia.[1–3] Although current antipsychotic medications are able to manage the positive symptoms of schizophrenia (e.g., hallucinations, delusions), no treatments exist which alleviate the negative symptoms (e.g., reduced affect, anhedonia, social withdrawal) or cognitive deficits which are also core features of the disorder. Several α7 nAChR agonists have demonstrated improvement in preclinical models of memory and cognition, and a few examples have shown signs of alleviating both cognitive deficits and negative symptoms in clinical trials for schizophrenia.[2] α7 nAChR agonists have also become targets of interest for alleviating the cognitive deficits seen in Alzheimer's disease and as anti-inflammatory agents.[2,4]

Several known α7 nAChR agonists are shown in Figure 1.[5–7] Acetylcholine (ACh) is the natural ligand for the α7 nAChR, and most of the known α7 nAChR agonists can be considered ACh mimetics, containing a carbonyl or carbonyl isostere connected by a three-atom linker to a basic amine, expected to be highly charged at physiological pH.

A high-throughput screen of the Bristol–Myers Squibb compound collection for α7 nAChR agonists was performed using a Fluorometric Image Plate Reader (FLIPR) that measured calcium ion influx in HEK293 cells expressing the rat α7 nAChR.[8,9] Quinolone **1** (Fig. 2) was identified as a novel α7 nAChR agonist (α7 EC_{50} = 1.3 μM). Although the potency was only modest, we were

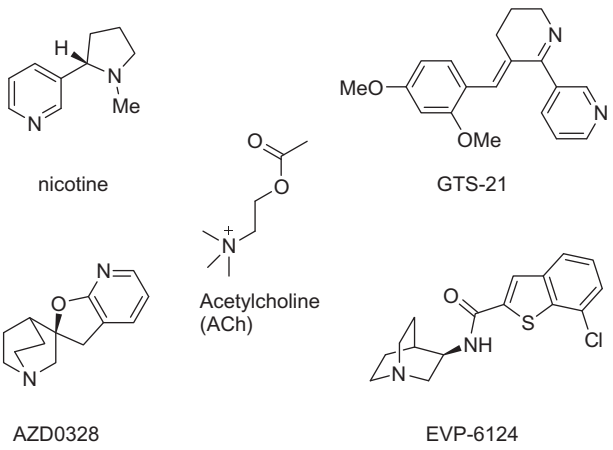

Figure 1. Examples of known α7 nAChR agonists.

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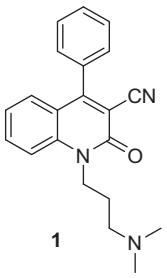
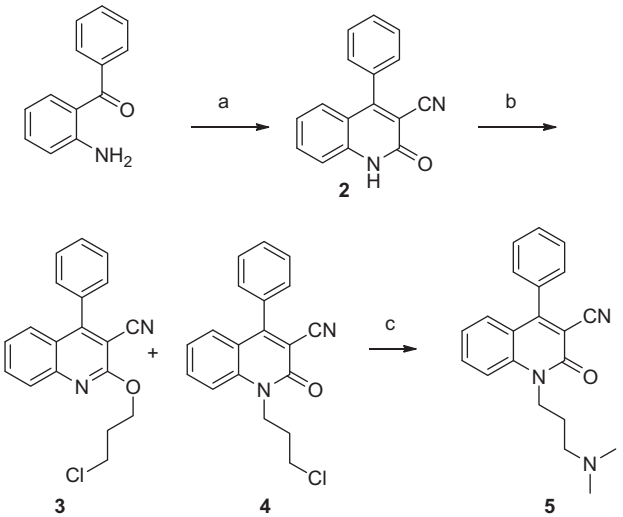
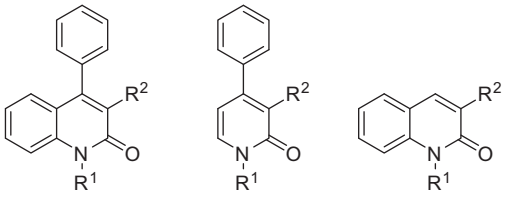


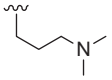
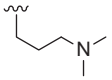
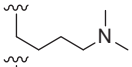
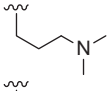
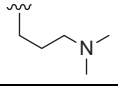
Figure 2. Lead compound from high-throughput screen.



Scheme 1. Reagents and conditions: (a) cyanoacetic acid, PCl_5,CH_2Cl_2, reflux 30 min, then NaOH (87%); (b) NaH, 3-chloro-1-iodopropane, DMF, rt, 3.5 h (3:21%, 4:38%); (c) dimethylamine, THF, NaI (cat) 70 °C, 4 h (60%).

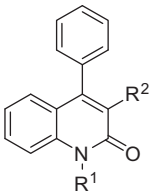
Table 1
α7 nAChR FLIPR data for selected compounds

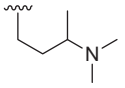
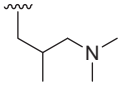
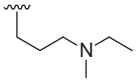
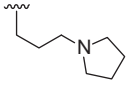
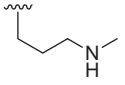
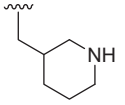
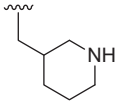
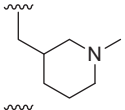
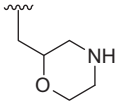
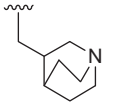


Compd	R^1	R^2	α7 nAChR EC_{50}[a] (μM)
1		CN	1.3
8		H	2.8
9		H	>10
10		H	>10
11		CN	>10
12		CN	>10

[a] All values are averages of at least two independent experiments.

Table 2
SAR of amine analogs

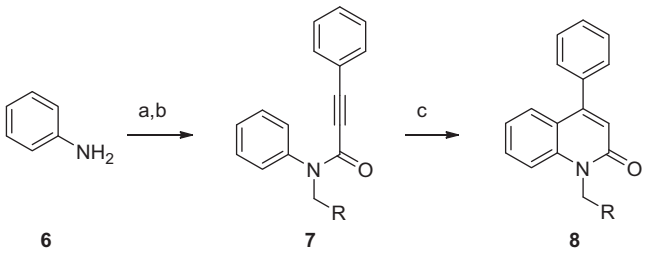


Compd	R^1	R^2	α7 nAChR EC_{50}[a] (μM)
13[b]		H	>10
14[b]		H	>10
15		CN	4.3
16		CN	9.3
17		CN	0.90
18[b]		CN	0.46
19		H	0.21
20[b]		CN	>10
21[c]		H	0.26
22[c]		H	0.15

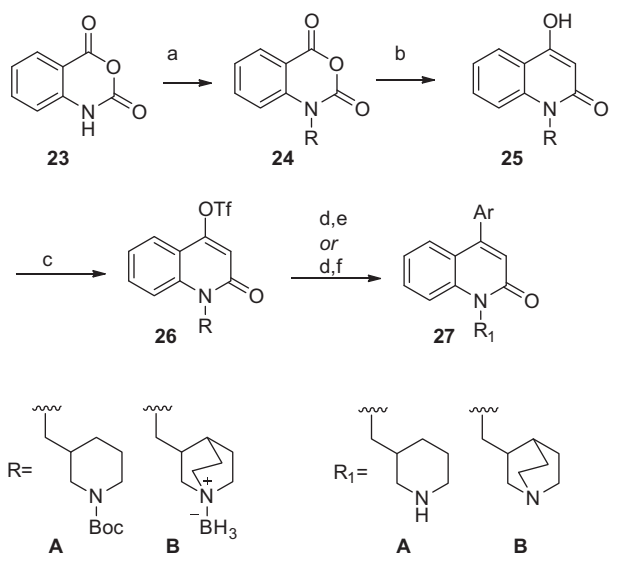
[a] All values are averages of at least two independent experiments.
[b] Racemic.
[c] Homochiral, final compounds separated by chiral supercritical fluid chromatography (SFC). Data reported for more potent enantiomer (absolute configuration not determined).

encouraged by good selectivity (EC_{50}s >100 μM at α3β4, α4β2 and α1β1δε; IC_{50} = 19 μM at the closely related $5HT_3$ receptor), relatively low molecular weight (331 g/mol) and novelty compared to other known α7 agonists. Another interesting aspect of quinolone **1** was that the 4 atom linker connecting the basic amine and carbonyl moieties was longer than those present in most known agonists (see Fig. 1 for comparison).[10]

The initial route to quinolone **1** and analogs varying at the amine or in linker length followed along the lines of the previously reported synthesis (Scheme 1).[11] Thus, acylation of *o*-aminobenzophenone with cyanoacetic acid followed by intramolecular Knoevenagel condensation led to cyanoquinolone **2**, which was treated with sodium hydride and iodochloropropane to afford a separable mixture of the O- and N-alkylated compounds (**3, 4**).



Scheme 2. Reagents and conditions: (a) RCHO, $NaHB(OAc)_3$, DCE, reflux (32–53%); (b) phenylpropiolic acid, $SOCl_2$, benzene (52–99%); (c) TfOH (neat), rt, 30 min (18–45%).



Scheme 3. Reagents and conditions: (a) R–OH, PPh_3, DIAD, THF, rt (58–85%); (b) EtOAc, LHMDS, THF, then warm in toluene (20–61%); (c) Tf_2O, Et_3N (55–76%); (d) $ArB(OH)_2$, $PdPPh_3$, K_2CO_3; (e) TFA, DCM; (f) 1:1, 3 N HCl/acetone (73–91%, 2 steps).

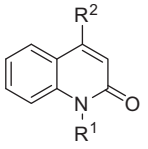
The desired N-alkylated compound **4** was then treated with a secondary amine and catalytic sodium iodide to afford the final compound (**5**). The syntheses of the de-annulated pyridone **11** and the des-phenyl quinolone **12**, were carried out in an analogous manner from the commercially available heterocyclic analogs of **2**.

To readily access analogs bearing more complex amine side-chains and avoid unproductive O-alkylation, an alternate synthesis was employed. Reductive alkylation of aniline with an aldehyde followed by acylation with phenylpropynoyl chloride led to amide **7** which was then rapidly cyclized in the presence of neat triflic acid to afford the des-cyano quinolone analogs **8**.[12]

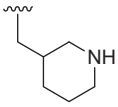
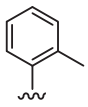
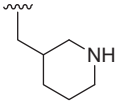
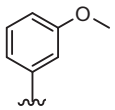
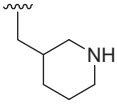
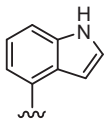
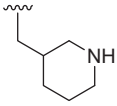
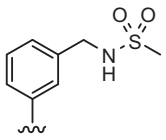
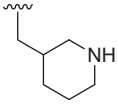
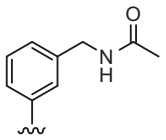
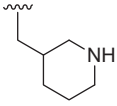
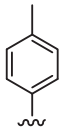
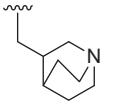
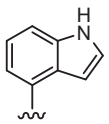
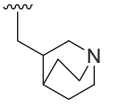
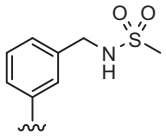
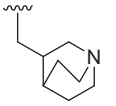
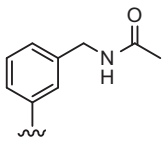
Results of core modification of quinolone **1** are shown in Table 1.[13] De-annulating the quinolone ring to the pyridone **11** or removing the pendant phenyl ring (**12**) led to a loss of α7 nAChR potency. However, the cyano group could be removed without substantially impacting potency (**8** vs **1**). Altering the chain length linking the quinolone core to the amine was not tolerated, with the 2-carbon (**9**) and 4-carbon (**10**) linkers showing a significant loss in activity.

Replacing the dimethylamino group with other tertiary amines also led to significant loss in potency, even with simple analogs such as ethylmethylamine **15** or pyrrolidine **16** (Table 2). Similarly, simple substitutions on the chain linking the amine to the quinolone core such as the α- and β-methyl compounds (**13, 14**) were not tolerated. Changes to the amine moiety that reduced terminal bulk or provided conformational restriction were more fruitful, with the secondary amine **17** showing a modest boost in potency and the conformationally restricted piperidines (**18**, **19**) and morpholine (**21**) showing a significant improvement in potency. While the cyano analog **1** was somewhat more potent than the corresponding des-cyano compound **8** in the dimethylaminopropyl series, for the piperidine analogs this was reversed with the des-cyano compound **19** showing improved potency. Interestingly, N-methylation of the piperidine was not tolerated (**20**), again highlighting the sensitivity of this moiety toward steric bulk. A

Table 3
α7 nAChR FLIPR data for various piperidine and quinuclidine substituted quinolones



28-36

Compd[a]	R^1	R^2	α7 nAChR EC_{50}[b] (μM)
28			1.5
29			1.3
30			0.11
31			0.077
32			0.10
33			>10
34[c]			0.023
35[c]			0.014
36[c]			0.032

[a] All compounds racemic.
[b] All values are averages of at least two independent experiments.
[c] At least 50-fold selective versus $5HT_3$, at least 100-fold selective vs. other nicotinic receptors (α3β4, α4β2 and α1β1δε).

Table 4
α7 Concentration response EP data for selected compounds

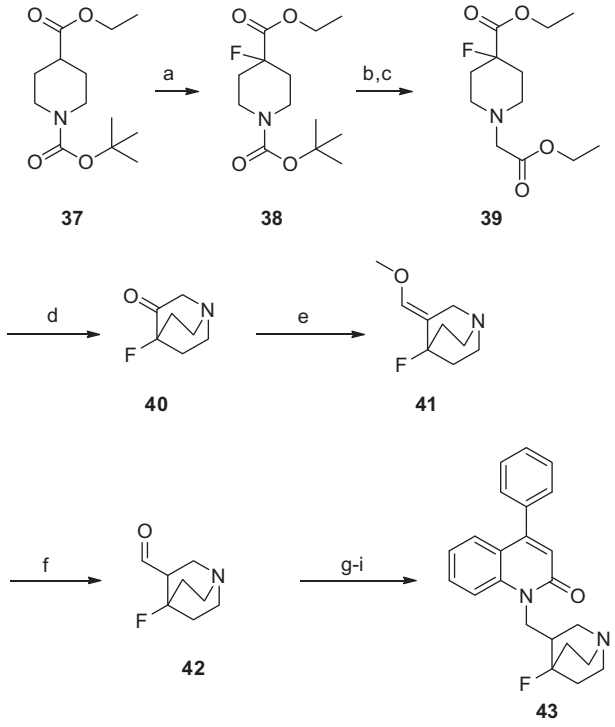
Compd	α7 EP EC_{50} (μM, area)	α7 EP Y_{max} (area) (%)	α7 EP $Y_{max,\ obs}$[a] (peak) (%)
1	8.2	44	15
19[b]	2.5	30	60
21[b]	1.2	41	34
22[b]	0.76	110	60
34[c]	0.54	80	62
35[c]	0.26	97[a]	21
36[c]	NF[d]	84[a]	21

[a] Observed Y_{max}.
[b] Homochiral, final compounds separated by chiral supercritical fluid chromatography (SFC). Data reported for more potent enantiomer (absolute configuration not determined).
[c] Racemic.
[d] Curve could not be fit to the data.

Table 5
α7 pK_a and penetrance data for selected compounds

Compound	PAMPA Pc (pH 7.4; nm/s)	pK_a	Caco-2 efflux ratio	Brain/plasma
AZD0328[a]	510	8.9	0.9	1.7–2.7[b]
21[a]	550	8.1	1.0	0.9[b]
22[a]	900	10.1	6.9	<0.02[c]
34[d]	400	ND[e]	>18	ND[e]
35[d]	0	ND[e]	ND[e]	ND[e]
36[d]	1	ND[e]	ND[e]	ND[e]
43[d]	380	7.6	0.6	ND[e]

[a] Homochiral.
[b] Rat, sc, 30 min.
[c] Rat, ip, 30 min.
[d] Racemic.
[e] Not determined.



Scheme 4. Reagents and conditions: (a) LHMDS, NFSI, THF (32%); (b) TFA/$CHCl_3$; (c) ethyl bromoacetate, Cs_2CO_3, THF (62%, 2 steps); (d) KOtBu, then HCl (42%); (e) dimethyl (1-diazo-2-oxopropyl)phosphonate, K_2CO_3, MeOH; (f) $HClO_4$, $CHCl_3$ (g) aniline, $NaHB(OAc)_3$, DCE, reflux (40%, 3 steps); (h) phenylpropiolic acid, $SOCl_2$, benzene (50%); (i) TfOH (neat), rt, 30 min (62%).

further modest boost in potency was observed with the more conformationally restricted quinuclidine **22**.

The two best amine groups (piperidine and quinuclidine) were chosen for further derivitization by changing the functionality and substitution of the pendant phenyl ring. In order to access such analogs efficiently an alternate sequence was devised, which allowed for late-stage incorporation of the aryl group. Thus, isatoic anhydride (**23**) and either *N*-Boc-3-hydroxymethyl piperidine or 3-hydroxymethyl quinuclidine-borane complex[14] were coupled under Mitsunobu conditions to afford heterocycles **24** (Scheme 3). Addition of the enolate of ethyl acetate provided the hydroxyquinolone **25**, which was then activated as the triflate to allow for Suzuki coupling with a variety of aryl and heteroaryl boronic acids. Final deprotection was carried out either with TFA for the Boc deprotection, or with a mixture of acetone and aqueous HCl for the borane-protected quinuclidines.

A broader SAR screen of aryl analogs was carried out on the more accessible piperidine compounds with the results guiding target selection in the quinuclidine series (Table 3). In the piperidine series, substitution at the 4-position of the phenyl ring was not tolerated (see example **33**) and substitution at the 2-position in most cases led to reduction in potency (see example **28**). Substitution at the 3-position was better tolerated, with polar functionality containing a hydrogen bond donor leading to a significant boost in potency (**30–32**). When applied to the quinuclidine series, the potency was even further improved for indole **34**, sulfonamide **35** and acetamide **36**.

Key compounds were further studied in a whole-cell patch-clamp electrophysiology paradigm using HEK cells stably expressing rat α7 along with human Ric3. In these experiments, both the peak intensity (peak) and area under the curve (area) were recorded, giving values for both potency and efficacy (Y_{max}; % response with respect to acetylcholine). It has been reported that area under the curve (also referred to as total charge) is a more accurate determination of the true electrophysiological response.[13,15] Data for selected compounds is shown in Table 4. As can be seen, the dimethylaminopropyl (**1**), piperidine (**19**) and morpholine (**21**) compounds were partial agonists while the quinuclidines (**22**, **34–36**) were either full agonists or high-intrinsic activity partial agonists as determined by area under the curve measures.

Unfortunately, the inclusion of polar functionality in the more potent compounds led to a loss of permeability, as shown by the low PAMPA values for the sulfonamide (**35**) and acetamide (**36**) analogs (Table 5). Although good permeability was observed for quinuclidine **22**, it was found to have very poor brain penetration (B/P <0.02). Follow-up experiments showed that all the quinuclidines tested had very high efflux ratios in a bidirectional Caco-2 assay, indicating that they may be substrates for transporter-mediated efflux. It has been previously demonstrated that PGP mediated efflux is often exacerbated by the presence of strongly basic amines,[16,17] and we hypothesized that this was the case here, since the quinuclidine–quinolones described herein are significantly more basic than other literature quinuclidine-based α7 nAChR ligands. This was attributed to the lack of a heteroatom substituent on C-3 of the quinuclidine ring for compounds such as **22** (see Fig. 1 for comparison), as a heteroatom substituent on the quinulidine would be expected to inductively reduce the pK_a of the amine In order to test whether efflux was driven by the presence of the strongly basic quinuclidine, we attempted to survey the basicity, efflux and brain penetration relationship of less basic analogs. Toward this end, a novel 4-fluoroquinuclidine was designed, with the goal of tempering the basicity of the quinuclidine nitrogen (Scheme 4).[17] Thus, α-fluorination of Boc-isonipecotate **37** followed by deprotection and coupling with ethyl bromoacetate led to piperidine **39**, which then underwent a

Dieckmann cyclization to give 4-fluoroquinuclidinone **40**. Two-step homologation to the aldehyde **42** was followed with conversion to quinolone **43** according to the prior method (see Scheme 2).

The pK_a of fluoroquinuclidine **43** was in fact substantially altered by the introduction of the fluorine, going from 10.1 for quinuclidine **22** to 7.6 for fluoroquinuclidine **43** (Table 5). Unfortunately, along with this drop in basicity, a very substantial loss in potency was observed in fluoroquinuclidine **43** (α7 nAChR FLIPR EC_{50} = 5.8 μM). The efflux that was seen in the more basic compounds was, however, no longer an issue, as evidenced by a Caco-2 efflux ratio near unity. This data, along with data for the morpholine compound **21**, showed that, at least for the limited number of compounds examined, high basicity contributed to poor brain-penetration, likely by increased transporter-mediated efflux.

In summary, optimization of a lead quinolone led to a series of potent quinuclidine/quinolone α7 nAChR agonists. Evidence supports the high pK_a of these compounds as reason for their lack of brain penetration. Less basic compounds in this series were found to lack transporter-mediated efflux and for morpholine **21**, this led to significantly improved brain penetration (Table 5).

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