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Gamma-lactams—A novel scaffold for highly potent and selective α7 nicotinic acetylcholine receptor agonists

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ABSTRACT

A novel class of α7 nicotinic acetylcholine receptor (nAChR) agonists has been discovered through high-throughput screening. The *cis* γ-lactam scaffold has been optimized to reveal highly potent and selective α7 nAChR agonists with in vitro activity and selectivity and with good brain penetration in mice.

Nicotinic acetylcholine receptors (nAChRs) belong to a superfamily of ligand gated ion channels that include the receptors for GABA, glycine and serotonin (5-HT_3). For centrally expressed nAChRs several genes have been cloned to date including nine α subunits (α2–α10) and three β subunits (β2–β4). Among the various polymeric constellations that these subunits may adopt, the α7 nAChR can form functional homopentamers that bind α-bungarotoxin with high affinity. Recent findings indicate that the α7 subunit may also assemble with other subunits.[1]

A substantial body of evidence suggests that the α7 nAChR are involved in the pathophysiology of several human diseases. Centrally, agonism of the α7 nAChR has been discussed as a therapeutic option for a number of neurological disorders such as; age associated memory impairment (AAMI),[2] Alzheimer's disease and schizophrenia.[3] Additionally, peripherally α7 nAChRs are expressed in macrophages and stimulation of the α7 nAChR inhibits the release of inflammatory cytokines (e.g. TNF-α, IL-1) as well as HMGB-1.[4] Thus, the clinical use of agonists of the α7 nAChR could represent a viable strategy against a multitude of human disorders.[1]

Recently several α7 nAChR agonists have been described, some of which have progressed to the clinic. The common pharmacophore of nicotine and α7 nAChR agonists usually consists of a strong base (e.g. pyrolidine in nicotine **1**, piperidine or tetrahydropyridine in anabasein-derived scaffolds **2** or a bicyclic amine in **3**–**5**, most prominently quinuclidine as in **3** and **4**) connected to a hydrophobic element by a linker (e.g. carbamate, ether or amide) (Fig. 1).[5–7]

In view of the populated chemical space around these general scaffolds we sought to find alternative templates. Herein we describe the discovery of a series of γ-lactams as α7 nAChR agonists

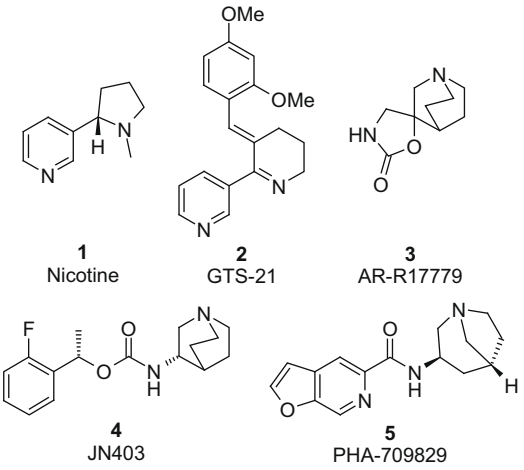

Figure 1. Representative α7 nAChR agonists.

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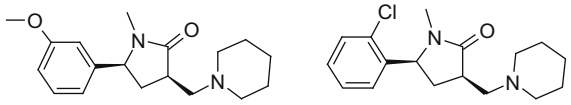
that display high potency, selectivity and interesting in vivo pharmacokinetic properties.

In a recent screening campaign a small cluster of *cis* gamma-lactams emerged as a structurally distinct hit class represented by **6** and **7** (Fig. 2).[8]

These hits were found to be α7 nAChR agonists with pEC_{50} values of 5.9 (**6**) and 6.1 (**7**) in a Ca-influx assay.[9] Both compounds also showed good relative efficacy in comparison to the known agonists nicotine (**1**), GTS-21 (**2**) and AR-R17779 (**3**) as well as (+)-epibatidine which is a full agonist at the α7 nAChR.[9] Intrigued by the discovery of a novel α7 agonist scaffold of good potency, hit-to-lead optimization was initiated. We started with the exploration of the piperidine moiety which presumably mimics the basic nitrogen of nicotine (Table 1).

Replacing the piperidine in **6** by a smaller pyrolidine led to loss of potency (e.g. **8**, Table 1). Expanding the ring in **7** to a seven-membered ring (**9**) also led to lower potency.

Further efforts focused on improving potency by modifying the aromatic portion of the scaffold. A positional scan of the *meta*-methoxy or *ortho*-chloro substituent in the original hits (e.g. **6**, **10**, **11**, and **7**, **12**, **13**, Table 2) had little effect on potency. However replacing the *para* position with a bromide (**14**) or methyl (**15**) led to a small but significant improvement. Introduction of a more lipophilic group, for example a cyclohexyl (**16**) or cyclopentyl (**17**) moiety, led to improved potency albeit at a detriment to efficacy. Continuing in this vein the central aromatic ring was substituted with a phenyl (**18**) which proved inferior. However the potency could be resurrected and even further improved by placing an *ortho*-fluoro (**19**) or *ortho*-chloro substituent (**20**) providing the most potent agonist of this series. Presumably the *ortho* substituent provides the ideal biaryl conformation to fill the lipophilic pocket.

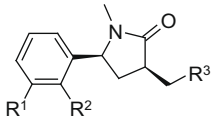


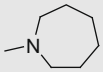
6
pEC_{50} = 5.9
Emax = 128

7
pEC_{50} = 6.1
Emax = 89

Figure 2. *Cis* γ-lactam HTS hits **6** and **7**.

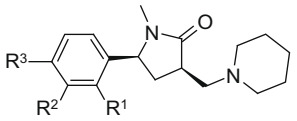
Table 1
Initial hit exploration of γ-lactam hits **6** and **7**



Compound[a]	R^1	R^2	R^3	pEC_{50}[b]	E_{max}
1	—	—	—	5.8	100
2	—	—	—	5.7	71
3	—	—	—	6.4	104
(+)-Epibatidine	—	—	—	7.4	100
6	MeO	H		5.9	128
8	MeO	H		5.1	80
7	H	Cl		6.1	89
9	H	Cl		5.7	82

[a] Compounds tested as diastereomerically pure *cis* racemates.
[b] Agonist-activity in GH3 cells recombinantly expressing human α7 nAChR. Compounds measured in duplicate. EC_{50} values generated from individual 8-point concentration response curves.[9]

Table 2
Exploration of hydrophobic moiety



Compound[a]	R1	R^2	R^3	pEC_{50}[b]	E_{max} (%)
10	OMe	H	H	5.9	107
6	H	OMe	H	5.9	128
11	H	H	OMe	5.9	124
7	Cl	H	H	6.1	89
12	H	Cl	H	6.0	102
13	H	H	Cl	6.0	110
14	H	H	Br	6.2	89
15	H	H	Me	6.2	114
16	H	H	Cy	6.5	60
17	H	H		6.5	28
18	H	H	Ph	5.9	61
19	H	H	2-F-Ph	6.5	47
20	H	H	2-Cl-Ph	6.9	62

[a] Compounds tested as diastereomerically pure *cis* racemates.
[b] Agonist-activity in GH3 cells recombinantly expressing human α7 nAChR. Compounds measured in duplicate. EC_{50} values generated from individual 8-point concentration response curves.[9]

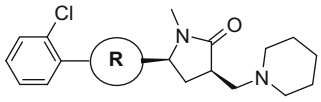
We hypothesized that the central phenyl served as a hydrophobic linker positioning the lactam and the distal hydrophobic moiety in optimal distance for potency. Thus we sought to explore by modifying the linker in order to investigate the effect it would have on potency. Having attained good potency with **20**, we also started to monitor selectivity against the α3β4 nAChR sub-type. The replacement of the phenyl linker by a thiazole led to a significant improvement in potency and efficacy and an improvement in selectivity (**21**, Table 3). Other heterocyclic linkers such as 'naked' or substituted thiophene (**22** and **23**) or imidazoles (**24** and **25**) were less potent. The breakthrough came by removing the cyclic element altogether and linking the γ-lactam and the hydrophobic moiety with an alkyne which led to a potent, efficacious and selective lead (**26**).

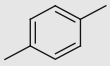
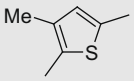
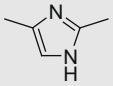
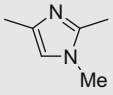
The desirable profile of the alkyne led us to further examine this linker. It was found that the distal *ortho*-chloro phenyl moiety, which in **20** had proven decisive to obtain potency, could be exchanged with an *ortho*-methyl (**27**, Table 4) or even an unsubstituted phenyl (**28**) to yield potent and efficacious compounds. Potency could be improved by replacing the phenyl with a *N*-methyl indole (**29**), albeit without improving selectivity. To our satisfaction the selectivity could be further improved by replacing the phenyl with benzofuran or dihydrobenzofuran (**30** and **31**) yielding highly potent and selective α7 agonists.

The alkynes **28**–**31** were profiled further in our ligand gated ion channel panel, which included the α3β4, α4β2, and α1β1γδ nicotinic sub-types, as well as the 5-hydroxytryptamine 3 ($5\text{-}HT_3$) receptors (Table 5).

Overall the alkynes displayed excellent selectivity against the nAChR sub-types as well as the $5\text{-}HT_3$ receptor (all > 100-fold). Especially noteworthy is the selectivity against the $5\text{-}HT_3$ receptor

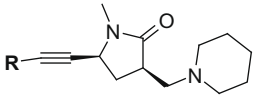
Table 3
Exploration of linker

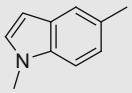
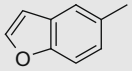
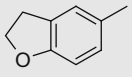


Compound[a]	R	pEC_{50}[b]	E_{max} (%)	Selectivity α7/α3β4[c]
20		6.9	62	3
21		7.6	89	14
22		7.5	79	26
23		6.2	81	nd
24		5.9	61	nd
25		6.2	47	nd
26		7.6	89	52

[a] Compounds tested as diastereomerically pure *cis* racemates.
[b] Agonist-activity in GH3 cells recombinantly expressing human α7 nAChR. Compounds measured in duplicate. EC_{50} values generated from individual 8-point concentration response curves.[9]
[c] Antagonistic activity in cells expressing the different nAChR[10] and 5-HT_3[5] receptors.

Table 4
Optimization of alkyne linked α7 agonists



Compound[a]	R	pEC_{50}[b]	E_{max} (%)	Selectivity α7/α3β4[c]
26	2-Cl-Ph	7.6	82	52
27	2-Me-Ph	7.8	83	104
28	Ph	8.0	104	126
29		8.1	100	111
30		8.1	90	123
31		8.1	80	154

[a] Compounds tested as pure enantiomers.
[b] Agonist-activity in GH3 cells recombinantly expressing human α7 nAChR. Compounds measured in duplicate. EC_{50} values generated from individual 8-point concentration response curves.[9]
[c] Antagonistic activity in cells expressing the different nAChR[10] and 5-HT_3[5] receptors.

Table 5
Selectivity profile of alkynes **28**–**31** compared to JN403 (**4**)

Compound[a]	α7 pEC_{50}[b]	α3β4 pIC_{50} (ratio)[c]	α4β2 pIC_{50} (ratio)[c]	α1β1γδ pIC_{50} (ratio)[c]	5-HT_3 pIC_{50} (ratio)[c]
4	7.0	4.7 (200)	4.6 (251)	3.9 (1259)	4.7 (200)
28	8.0	5.9 (126)	5.1 (820)	4.9 (1299)	3.6 (26368)
29	8.1	6.0 (111)	5.7 (232)	5.4 (431)	4.6 (3221)
30	8.1	6.1 (123)	5.3 (660)	4.0 (16982)	4.0 (16982)
31	8.1	6.0 (154)	5.0 (1263)	5.2 (773)	4.3 (5389)

[a] Compounds tested as pure enantiomers.
[b] Agonist-activity in GH3 cells recombinantly expressing human α7 nAChR. Compounds measured in duplicate. EC_{50} values generated from individual 8-point concentration response curves.[9]
[c] Antagonistic activity in cells expressing the different nAChR[10] and 5-HT_3[5] receptors.

when compared to the quinuclidene derivative **4**. When tested against human recombinant muscarinic receptors the compounds also displayed IC_{50} > 10 μM on all sub-types (M1–M5).

In vitro cardiovascular safety profiling was also undertaken, with the compounds submitted for patch-clamp hERG K+ channel assay. In general the compounds display reasonable cardiosafety, for example, alkyne **28**, having an IC_{50} of 6.85 μM. Patch clamp experiments at the cardiac sodium channel were also performed, and alkyne **28** was found to display a 33% inhibition at a concentration of 30 μM. Finally to investigate the safety window in an ex vivo whole heart assay, we submitted alkyne **28** to the "Langendorff" isolated rabbit heart assay, and found to have no QT-interval prolongation with doses up to 10 μM.

The ADME and pharmacokinetic data were also assessed in vitro and in vivo (Table 6).

The phenyl-substituted alkyne **28** showed rapid clearance and short half-lives in the mouse liver microsome preparations (ER = 90%, $t_{1/2}$ = 6.6). The benzofuran **30** showed improved in vitro metabolic stability, displaying an ER and $t_{1/2}$ of 67% and 12.5 in MLM and 68% and 25.8 in HLM, respectively. The rapid in vitro clearance of **28** was reflected in the low plasma levels when administered orally to mice. In spite of the low plasma levels the compound showed excellent brain exposure and no retention. The improved microsomal stability of the benzofuran **30**, translated into improved in vivo pharmacokinetic properties. High brain and plasma levels were observed at one and four hours following administration. As was the case with phenyl-substituted alkyne **28** the brain/plasma ratios at both time points indicated good brain penetration.

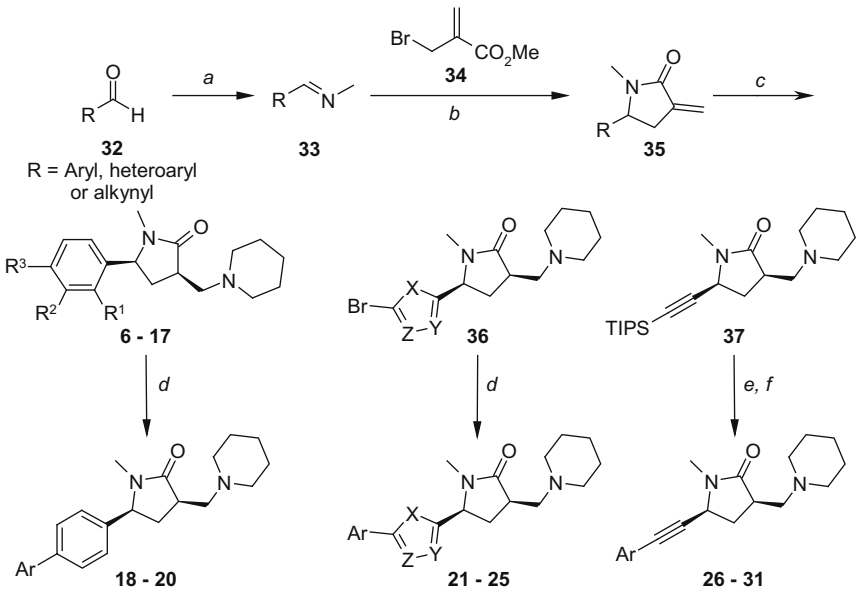
Compounds were prepared following two general procedures outlined below (Schemes 1 and 2). The diastereomerically pure racemates were prepared as summarized in Scheme 1. Various aldehydes (e.g. **32**, R = aryl, heteroaryl or alkyne) were converted to methyl-imines (e.g. **33**). From which the corresponding lactams **35** were generated by treating the imine with an organozinc reagent derived in situ from bromide **34**. The resulting α,β-unsaturated lactams were treated with piperidine and the major *cis* isomers (**6–17**, **36**, and **37**) were isolated (typical *cis*/*trans* ratio 3/1) in good overall yields. The aryl linked agonists **18–20**, could be obtained by Suzuki[12] coupling of **14** with the corresponding aryl boronic acids. The heteroaryl linked agonists **21–25** were obtained in a similar fashion from the corresponding brominated heterocycle **36**. Finally, the alkyne linked derivatives were obtained by desilylation of **37** followed by Sonogashira[13] coupling. In the examples **26–31**, pure enantiomers could be isolated after chromatography on chiral support.[14]

Additionally we developed an asymmetric route to access this scaffold. This sequence is exemplified for compound **20** below in Scheme 2. The route was based on the work of Villiéras[15] and Yamamoto.[16] Aldehyde **38** was condensed with (*S*)-phenyl glycidol to afford imine **39**. The diastereoselective addition of an organozinc reagent derived from bromide **34** in situ onto the corresponding

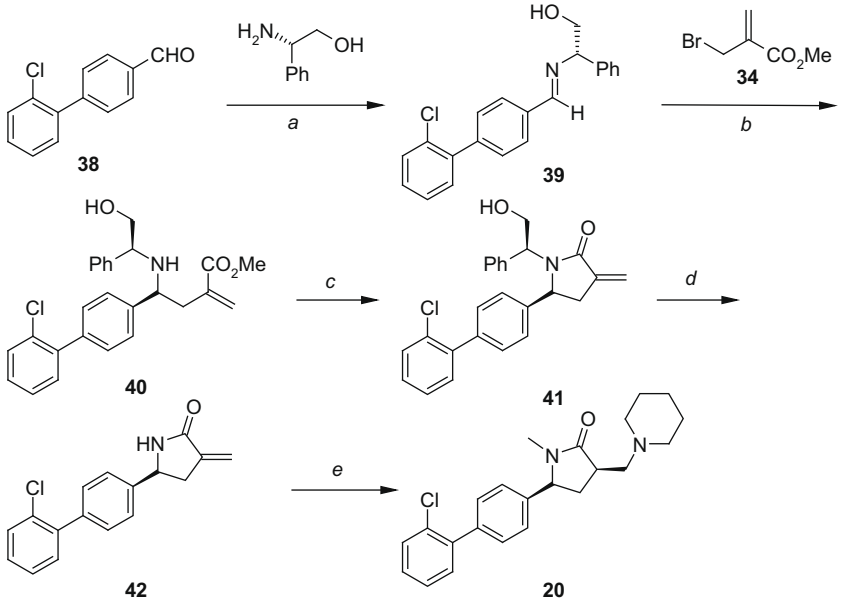
Table 6
ADME and pharmacokinetic data

Compound	Log*P* (o/w)	Plasma protein binding (human/rat)[a]	MLM ER/$t_{1/2}$[b]	HLM ER/$t_{1/2}$[b]	Mouse in vivo pharmokokinetic data[c]	
					Brain/plasma levels 1 h (pmol/g)	Brain/plasma levels 4 h (pmol/g)
28	3.7	17%/31%	90%/6.6	nd	239/18	64/4
30	3.9	nd	67%/12.5	68%/25.8	6533/1001	3676/544

[a] Plasma protein binding expressed as free fraction.
[b] MLM and HLM, mouse liver microsomes or human liver microsomes, respectively, in vitro hepatic extraction ratios (ER) are reported as% compound depleted at completion of experiment.
[c] Mouse in vivo pharmacology: Mice (n = 6) were treated with 30 μmol/kg po of compound. Plasma and brain concentrations were assessed at the time points indicated.[11] For full protocols see Ref. 11.



Scheme 1. General synthetic route for the preparation of γ-lactams. Reagents and conditions: (a) $MeNH_2$ (33% in EtOH), 25 °C, 4 h (85–95%); (b) **34**, Zn in THF, 25 °C, 20 min, followed by addition of **33**, then stirred for 3.5 h at 25 °C (70–90% over two steps); (c) piperidine, 2 h, 85 °C, (50–60%); (d) arylboronic acid, $Pd(OAc)_2$, Na_2CO_3, PhMe, 12 h, 110 °C (40–65%); (e) TBAF, THF, 2 h, 25 °C (90%); (f) ArI, $(PPh_3)_2PdCl_2$, CuI, THF/Et_3 N, 2 h, 25 °C (40–60%).



Scheme 2. General synthetic route for the preparation of enantiomerically pure γ-lactams. Reagents and conditions: (a) (*S*)-phenyl glycidol, CH_2Cl_2, 4 Å molecular sieves, reflux, 3 h (95%); (b) **34**, Zn in THF, 25 °C, 20 min, followed by addition of **37** then stirred for 3.5 h, 25 °C; (c) i—NaOH (1 M), THF, 24 h, 25 °C; ii—mesitylenesulfonyl chloride, diethyl isopropyl amine, NBu_4HSO_4, 4.5 h, 25 °C (37% from **37**); (d) i—$SOCl_2$,THF, 45 min, reflux; ii—DBU, CH_2Cl_2, 24 h, 25 °C (79%); (e) i—KOtBu, MeI, THF, 45 min, 25 °C; ii—piperidine, 3.5 h, 90 °C (60%).

chiral imine afforded the methyl acrylate **40** as a single diastereomer. This was directly transformed into the γ-lactam **41** after hydrolysis of the ester and cyclization of the mixed anhydride generated in situ. The chiral auxiliary in **41** was removed by conversion of the hydroxyl to the chloride followed by DBU assisted elimination to the enamine and subsequent hydrolysis. Finally, alkylation with MeI of **42** and conjugate addition of piperidine afforded **20** in a separable *cis*/*trans* mixture (3:1). The exemplified compound **20** was shown to have an ee > 99% by chiral chromatography.[13]

In summary, we have discovered and optimized a series of *cis* γ-lactams as α7 nAChR agonists that display excellent potency, subtype selectivity and favorable in vitro and in vivo pharmacokinetic properties. Additionally, the scaffold displays good cardiosafety profile. The scaffold was discovered through high-throughput screening, and is devoid of the classical bicyclic amine, thus providing a valuable lead series for further development.

Acknowledgment

We wish to thank Dr. Eric Francotte for chiral separation support.

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8. The *cis* diastereomer was 40- to 60-fold more potent to the corresponding *trans* diastereomer (data not shown). Moreover the eutomer/distomer relationship for **7** was ca. 3-fold with pEC_{50} values 6.34 versus 6.01, respectively. The depicted (*S*,*S*)-configuration was later confirmed to be the eutomer by enantioselective synthesis of **20** (vide infra Scheme 2). All stereochemistry is therefore given as the (*S*,*S*)-configuration.
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