Novel *N*-aryl nicotinamide derivatives: taking stock on 3,6-diazabicyclo[3.1.1]heptanes as ligands for neuronal acetylcholine receptors.

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ABSTRACT

We designed the synthesis of a small library of 3-substituted-3,6-diazabicyclo[3.1.1]heptanes whose affinity on neuronal nicotinic receptors (nAChRs) was evaluated. Among the synthesized compounds, the 5-(3,6-diazabicyclo[3.1.1]heptane-3-yl)-*N*-(2-fluorophenyl)nicotinamide **43** proved to be the most interesting compound with $\alpha_4\beta_2$ *Ki* value of 10 pM and a very high $\alpha_7/\alpha_4\beta_2$ selectivity. Furthermore, compounds **35**, **39** and **43** elicited a selective partial agonist activity for $\alpha_4\beta_2$ nAChR subtype.

Finally, in this paper we also report the conclusions on the 3,6-diazabicyclo[3.1.1]heptanes as ligands for nAChRs, resulting from our consolidated structure activity relationship (SAR) studies on this template.

Keywords: 3,6-diazabicyclo[3.1.1]heptanes, synthesis, nAChRs, partial agonists, $\alpha_4\beta_2$ selectivity, tobacco addiction, molecular docking.

1. Introduction

The activation of ionotropic acetylcholine nicotinic receptors (nAChRs) is responsible for several physiological processes; therefore, their modulation could be useful in the treatment of different pathological conditions [1]. Due to their potential therapeutic significance, in particular in the frame of the fight against tobacco addiction, nAChRs have been the subject of extensive researches that led to individuation of bridged piperazines (**Figure 1**) [2] derived from chemical modification of epibatidine, as unusual pharmacophore for nicotinic ligands [3].

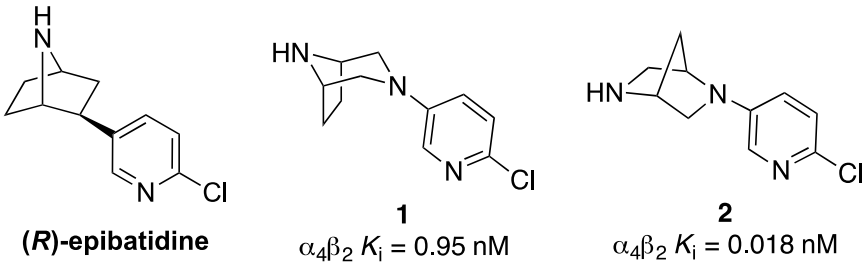


Figure 1. Bridged piperazines as nAChRs ligands.

The 3,8-diazabicyclo[3.2.1]octane system of **1** (**Fig. 1**) showed high $\alpha_4\beta_2$ affinity and a potent central analgesic activity in mouse hot plate paradigm, in analogy with that of epibatidine. Through theoretical calculations and high-field proton magnetic resonance (^{1}H-NMR) spectroscopy resulted that the tricyclic system of **1** has one conformation similar to that of epibatidine, responsible of the analogy in their pharmacological profile [4].

In 2007 were reported the analgesic properties of a novel series of compounds, related to epibatidine, among which compound **2** (**Figure 1**), typified by a 2,5-diazabicyclo[2.2.1]heptane structure, represents the best compound of the whole series [5]. Unfortunately, although endowed with analgesic activities in several animal models of neuropathic pain, its clinical evaluation was precluded, due to its unfavourable pharmacokinetic profile.

However, this new class of compounds served to hypothesize the common steric and electronic features useful to ensure the optimal interactions with nAChR $\alpha_4\beta_2$ and α_7, leading to identifying in

bridged piperazine bearing a pyridine ring a new pharmacophore model for potential nAChR ligands [5]. Starting from the new pharmacophore model of the 2,5-diazabicyclo[2.2.1]heptane core, we designed an intriguing structural modification and synthesized a new series of compounds typified by a 3,6-diazabicyclo[3.1.1]heptane (3,6-DBH) structure as derivatives **3-8** reported in **Figure 2**, some of which are endowed with an interesting $\alpha_4\beta_2$ subtype receptor profile showing high affinity and selectivity [6,7].

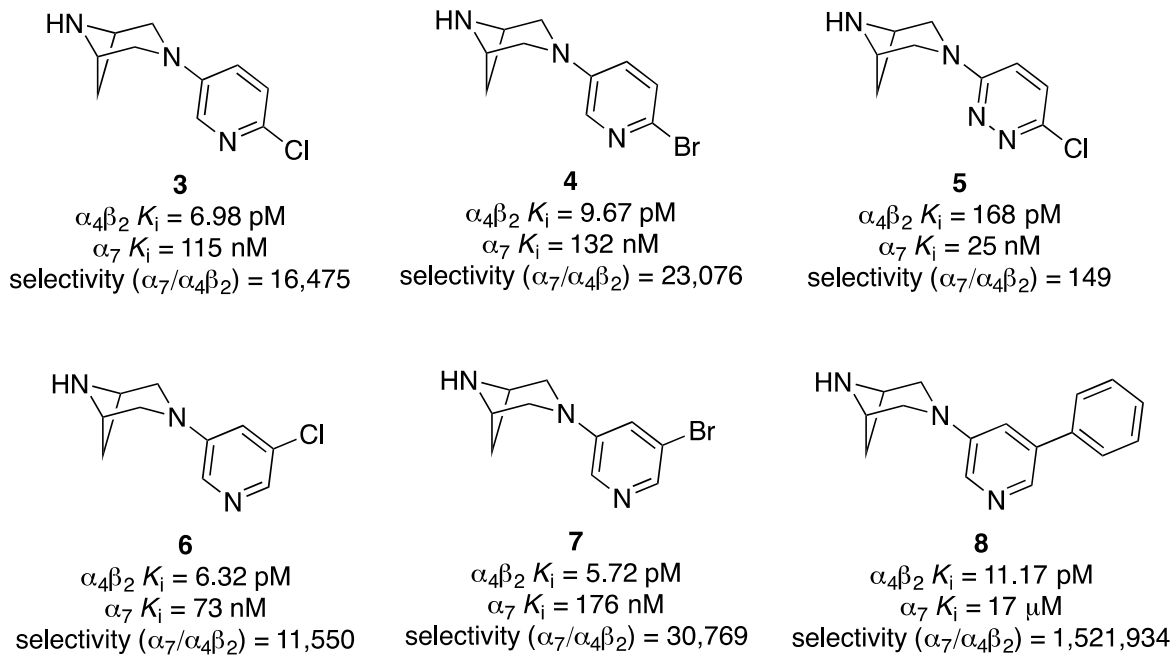


Figure 2. 3,6-Diazabicyclo[3.3.1]heptanes.

Among the new synthesized compounds, those with a pyridine bearing a halogen or an aryl ring, displayed a spectrum of $\alpha_4\beta_2$ binding affinities comparable to that of epibatidine and the lead compound **2**. The substitution of an halogen atom in α or β position of the pyridine ring (**3**, **4**, **6** and **7**) seems not to be preferential for the affinity of these compounds; at the same time, the introduction of electron-donating and/or electron-withdrawing groups on the aryl ring (**8**) had minimal effect on binding indicating a sensitivity of $\alpha_4\beta_2$ subtype to positive π values together with negative or positive

σ values. Moreover, by induced inhibition of [^{3}H]-DA release assay on aryl derivatives, compound **8** and its analogues proved to be antagonists towards nAChRs [7].

Therefore, to further evaluate the structure-activity relationship (SAR) studies on the 3,6-DBH structure, we planned the synthesis of new derivatives and the evaluation of their biological profile on nAChRs, designing the introduction of different substituted anilines both in the 3- and 2- position of the heteroaromatic ring of the pyridinyl-3,6-DBH system, and on its pyridazine analogue, leading to compounds **9-11** (**Figure 3**).

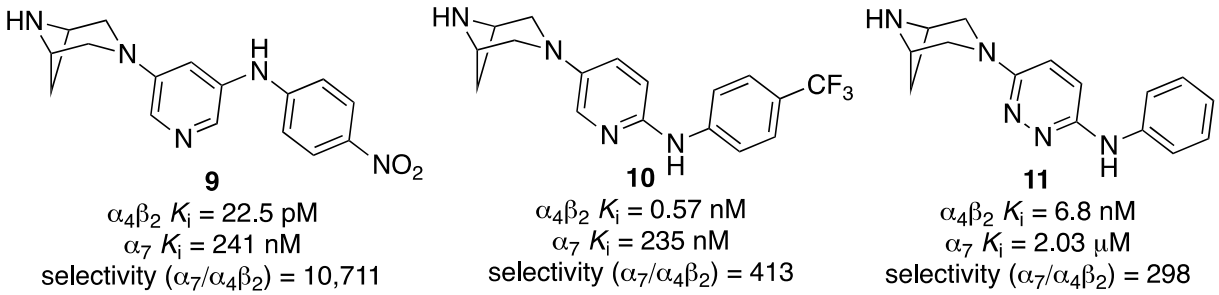


Figure 3. Heteroaromatic 3,6-diazabicyclo[3.3.1]heptanes functionalizated.

Within these series, the pharmacomodulation on the heteroaromatic ring linked to 3,6-DBH system with different anilines, is responsible for high $\alpha_4\beta_2$ receptor affinity, with K_i ranging from pM to μM values, and $\alpha_7/\alpha_4\beta_2$ selectivity. Indeed, the substitution with different anilines is well tolerated in term of receptor affinity, mostly for $\alpha_4\beta_2$ subtype and in minor extent for $\alpha_3\beta_4$, whereas seems harmful for α_7 receptor subtype, particularly for derivatives of the series typified by **9** [1].

To complete the picture on 3,6-DBH ligands, therein we report the synthesis and binding data of the new series of compounds, reported in **Figure 4** and **Table 1**, in which we evaluated the effects on affinity and selectivity towards different nAChRs, of the introduction of a carboxamidic group between the pyridine, on the DBH skeleton, and a second aromatic ring.

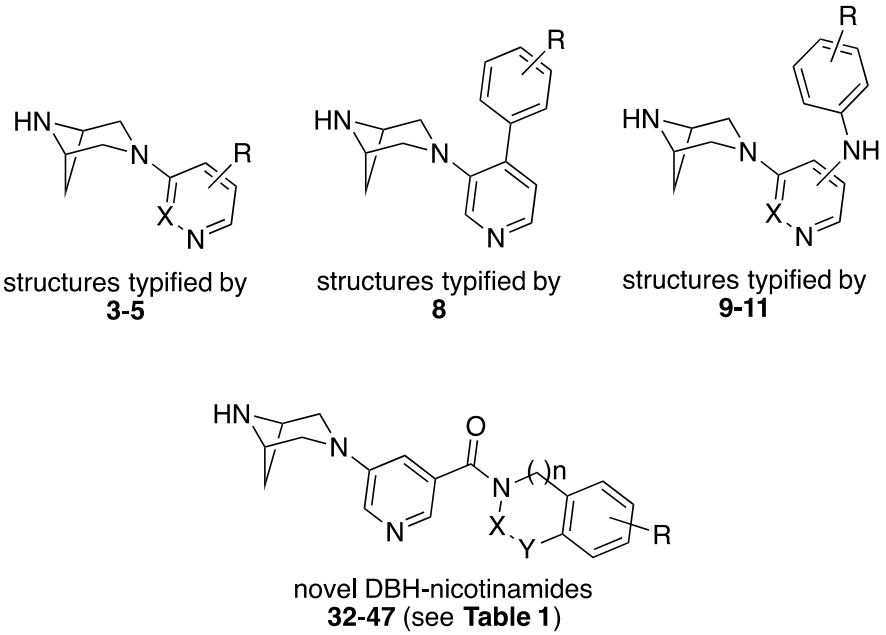


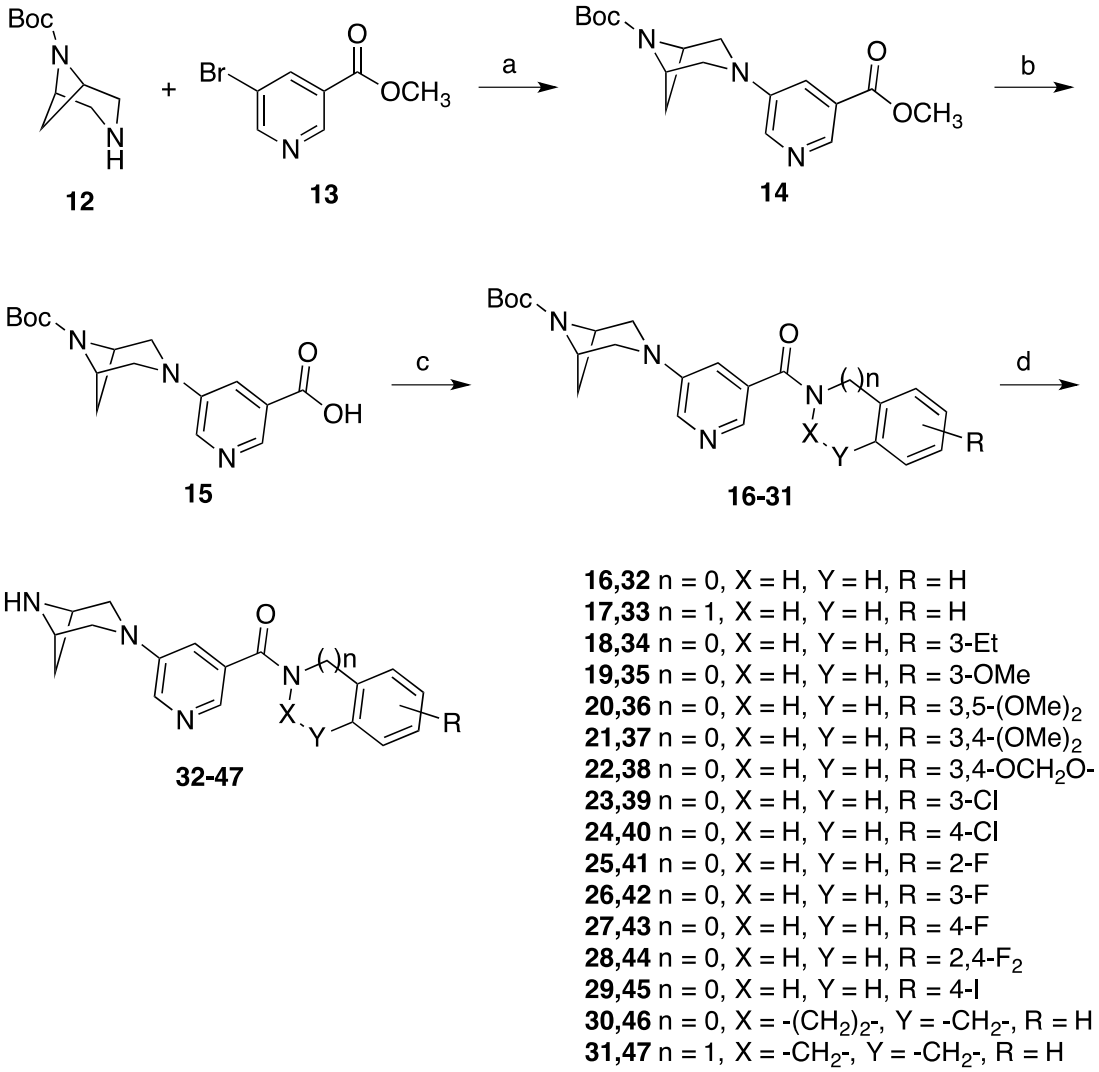
Figure 4. Related 3,6-diazabicyclo[3.3.1]heptanes.

The resulting structures were also docked to the binding sites of nAChR α_7 and $\alpha_4\beta_2$ to evaluate possible binding modes and compare them to epibatidine.

2. Results and discussion

2.1. Chemistry

The previously reported [7] *tert*-butyl 3,6-diazabicyclo[3.1.1]heptane-6-carboxylate, derivative **12**, serves as starting compound for the synthesis of derivatives **32-47**. The coupling reaction under microwave conditions, between key intermediate **12** and methyl 5-bromonicotinate **13,** led to compound **14**, whose hydrolysis (**15**) followed by acylation with the appropriate anilines or amines gave Boc-protected derivatives **16-31**. The final *N*-Boc deprotection yielded compounds **32-47** (**Scheme 1**).



16,32 n = 0, X = H, Y = H, R = H
17,33 n = 1, X = H, Y = H, R = H
18,34 n = 0, X = H, Y = H, R = 3-Et
19,35 n = 0, X = H, Y = H, R = 3-OMe
20,36 n = 0, X = H, Y = H, R = 3,5-$(OMe)_2$
21,37 n = 0, X = H, Y = H, R = 3,4-$(OMe)_2$
22,38 n = 0, X = H, Y = H, R = 3,4-OCH_2O-
23,39 n = 0, X = H, Y = H, R = 3-Cl
24,40 n = 0, X = H, Y = H, R = 4-Cl
25,41 n = 0, X = H, Y = H, R = 2-F
26,42 n = 0, X = H, Y = H, R = 3-F
27,43 n = 0, X = H, Y = H, R = 4-F
28,44 n = 0, X = H, Y = H, R = 2,4-F_2
29,45 n = 0, X = H, Y = H, R = 4-I
30,46 n = 0, X = -$(CH_2)_2$-, Y = -CH_2-, R = H
31,47 n = 1, X = -CH_2-, Y = -CH_2-, R = H

Scheme 1. Reagents and conditions: a) Cs_2CO_3, $Pd_2(dba)_3$, Xantphos, dioxane, MW (normal abs) 130 °C, 1-3 h; b) NaOH, H_2O, CH_3OH, 5 h, 60 °C; c) amine, EDC, HOBt, DCM, rt, 12 h,; d) HCOOH, rt, 12 h.

2.2. nAChR binding affinities

We synthesized a small library of 5-(3,6-diazabicyclo[3.1.1]heptan-3-yl)-*N*-arylnicotinamides (**32-47**) with the view to test the effect of the introducing a carboxamide group in our 3,6-DBH template. **Table 1** shows the affinities (K_i) of the newly synthesized compounds towards the $\alpha_4\beta_2$ and α_7 nAChR subtypes determined using [^{3}H]-Epibatidine as a ligand for the $\alpha_4\beta_2$ subtype and [^{125}I]α-Bungarotoxin for the α_7 subtype. The *K*i values reported in the table are obtained from five independent experiments.

Most derivatives displayed α_7 nAChRs low affinity, ranging in K_i from 1.07 μM (**33**) to > 50 μM (**34**, **35**, **44** and **46**).

First of all, we wanted to evaluate the effect of the anilide group with respect to the carboxamide one on the $\alpha_4\beta_2$ affinity and selectivity of this new series of compounds. Both the introduction of aniline ring (**32**) and benzylamine group (**33**) in the carboxylic moiety provided good $\alpha_4\beta_2$ affinity with K_i values of 1.1 nM and 1.8 nM, respectively. Due to its better $\alpha_7/\alpha_4\beta_2$ selectivity (2182), compound **32** was chosen as lead compound of the SARs on DBH-nicotinamide series.

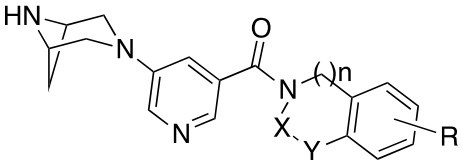
Then, we planned the modulation of the aromatic aniline ring, by inserting a 3-Et (**34**) and a 3-OCH_3 group (**35**), whereas the ethyl group maintained the same $\alpha_4\beta_2$ affinity (K_i = 1.3 nM) with respect to **32**, the introduction of the methoxy group in meta position of the aniline ring (**35**) led to a slight increase of $\alpha_4\beta_2$ receptors affinity (K_i = 560 pM), resulting both derivatives highly $\alpha_4\beta_2$ selective (K_i $\alpha_7/\alpha_4\beta_2$ > 38461 and > 89286, respectively). Therefore, was evaluated the effect both of a double methoxy group substitution (**36**, **37**) and of the introduction of a 3,4-methylenedioxy functional group (**38**). The latter compound showed a significant increase both in $\alpha_4\beta_2$ affinity (K_i = 106 pM) and selectivity (K_i $\alpha_7/\alpha_4\beta_2$ = 79245).

The most interesting compounds resulted those of the halo-aniline series (**39-45**). Particularly, derivative **43**, bearing a 4-fluorine substituted aniline ring, showed the best $\alpha_4\beta_2$ receptor profile with a K_i value of 10 pM and a very high selectivity (K_i $\alpha_7/\alpha_4\beta_2$ = 3320000). The shift of the fluorine atom in *meta*-position (**42**), resulted in 4.4-fold loss in $\alpha_4\beta_2$ affinity (K_i = 44 pM) with respect to **43**, whereas the *ortho*-monoflourine (**41**) showed a 31.5-fold loss in $\alpha_4\beta_2$ affinity (K_i = 315 pM). The double substitution, to the 2,4-difluorine derivative (**44**) led to a compound with $\alpha_4\beta_2$ receptor affinity and selectivity similar to **42**.

The introduction of an alogen atom, as chlorine (**40**) or iodine (**45**), gave compounds with comparable affinity (K_i $\alpha_4\beta_2$ = 404 pM and K_i $\alpha_4\beta_2$ = 366 pM, respectively), whereas the 3-chlorine derivative **39** showed $\alpha_4\beta_2$ affinity (K_i = 42 pM) comparable with 3-fluorine analogue.

Finally, we incorporated the *N*-atom of the carboxamide moiety in a 1,2,3,4-tetrahydroquinoline- (**46**) or 1,2,3,4-tetrahydroisoquinoline-bicyclic system (**47**). This modification led to $\alpha_4\beta_2$ affinity values of 196 pM and 31 pM, respectively.

Table 1. $\alpha\beta$ and α_7 nAChR binding affinity for compounds 32-47.

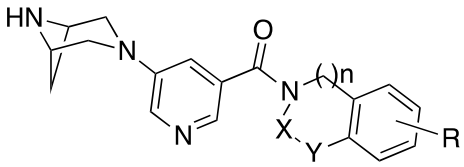


Compound	n	X	Y	R	$\alpha\beta$ (pM)	CV (%)[a]	α (μM)	CV (%)	Selectivity $\alpha_7/\alpha_4\beta_2$
32	0	H	H	H	1100	55	2.4	40	2182
33	1	H	H	H	1800	68	1.07	35	594
34	0	H	H	3-Et	1300	60	> 50	-	> 38461
35	0	H	H	3-OMe	560	56	> 50	-	> 89286
36	0	H	H	3,5-$(OMe)_2$	985	62	43.1	60	43756
37	0	H	H	3,4-$(OMe)_2$	828	63	2.3	20	2778
38	0	H	H	3,4-OCH_2O-	106	49	8.4	32	79245
39	0	H	H	3-Cl	42	49	22.9	40	545238
40	0	H	H	4-Cl	404	56	13	35	32178
41	0	H	H	2-F	315	65	3.4	30	10794
42	0	H	H	3-F	44	60	27.9	29	634091
43	0	H	H	4-F	10	91	33.2	42	3320000
44	0	H	H	2,4-F_2	51	65	> 50	-	> 980392
45	0	H	H	4-I	366	71	23.4	35	63934
46	0	-$(CH_2)_2$-	-CH_2-	H	196	81	> 50	-	255102
47	1	-CH_2-	-CH_2-	H	31	61	1	29	32258
[^{3}H]-Epi[b]					0.050	12	N.D.[c]		
[^{125}I]α-BgTx[d]					N.D.		1.1		

[a]Coefficient of variation on three independent experiments. [b][^{3}H]-Epibatidine. [c]Not Determined. [d][^{125}I]α-Bungarotoxin.

The $\alpha_3\beta_4$ nAChRs affinity for selected compounds (**Table 2**) found that, overall, derivatives with high $\alpha_4\beta_2$ affinity displayed $\alpha_3\beta_4$ K_i values in the nM range, as **39** (K_i $\alpha_3\beta_4$ = 59 nM, K_i $\alpha_4\beta_2$ = 42 pM), **42** (K_i $\alpha_3\beta_4$ = 28 nM, K_i $\alpha_4\beta_2$ = 44 pM), **43** (K_i $\alpha_3\beta_4$ = 81 nM, K_i $\alpha_4\beta_2$ = 10 pM), **44** (K_i $\alpha_3\beta_4$ = 50 nM, K_i $\alpha_4\beta_2$ = 51 pM) and **47** (K_i $\alpha_3\beta_4$ = 21 nM, K_i $\alpha_4\beta_2$ = 31 pM). Among these compounds, the 4-fluorine derivative, **43**, resulted the most $\alpha_4\beta_2$ selective compound (K_i $\alpha_3\beta_4$/ K_i $\alpha_4\beta_2$ = 8100).

Table 2. $\alpha_3\beta_4$ nAChR binding affinity for compounds 32-47.



Compound	n	X	Y	R	$\alpha_3\beta_4$ (nM)	CV (%)[a]	Selectivity $\alpha_3\beta_4/\alpha_4\beta_2$
32	0	H	H	H	192	28	174
33	1	H	H	H	16	26	889
34	0	H	H	3-Et	306	20	235
35	0	H	H	3-OMe	265	28	473
36	0	H	H	3,5-$(OMe)_2$	2	40	2030
37	0	H	H	3,4-$(OMe)_2$	516	27	623
38	0	H	H	3,4-OCH_2O-	113	45	1066
39	0	H	H	3-Cl	59	26	1405
40	0	H	H	4-Cl	123	20	304
41	0	H	H	2-F	102	26	324
42	0	H	H	3-F	28	30	636
43	0	H	H	4-F	81	27	8100
44	0	H	H	2,4-F_2	50	65	980
45	0	H	H	4-I	103	37	281
46	0	$-(CH_2)_2-$	$-CH_2-$	H	62	41	316
47	1	$-CH_2-$	$-CH_2-$	H	21	43	677
[^{3}H]-Epi[b]					0.150	30	

[a]Coefficient of variation on three independent experiments. [b][^{3}H]-Epibatidine.

2.3. Functional analysis on the nAChRs

Based on the binding data, we selected compounds **35**, **39** and **43** for a functional analysis of their agonism on human $\alpha_4\beta_2$ and $\alpha_3\beta_4$ heteromeric nAChRs (**Figure 5**). All tested compounds acted as partial agonists, being able to elicit inward whole-cell currents in GH4C1 cells transfected with human α_4 and β_2 subunits, with similar EC_{50} values: 0.6±0.2 μM, 1.0±0.2 μM and 0.52±0.04 μM for compounds **35**, **39** and **43**, respectively. Even the maximal evoked currents were very similar, being 38±11 %, 37±7 % and 41±1 % of those elicited by 1 mM ACh. All dose-response curves exhibited $n_H<1$ (**Figure 5**). The selectivity of these compounds was confirmed at the functional level, given that their agonist effects on human $\alpha_3\beta_4$ nAChRs were visible only at high concentrations (>1 μM), with EC50 values >10 μM (**Figure 5**). In particular, compound **39** was never able to elicit currents in GH4C1 cells expressing human $\alpha_3\beta_4$ nAChRs.

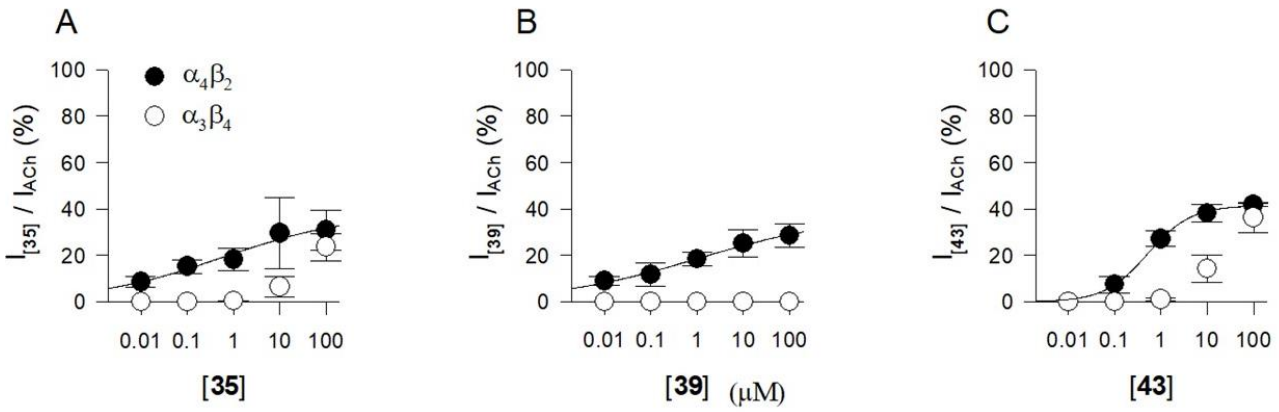


Figure 5. Partial agonism of compounds **35, 39** and **43** on $\alpha_4\beta_2$ nAChR. Dose-response relations were obtained by applying compounds **35** (A), **39** (B) or **43** (C) to GH4C1 cells transiently transfected with cDNA coding for human α_4 and β_2 subunits (●) or human α_3 and β_4 subunits (○). The elicited whole-cell currents were normalized to those evoked by 1 mM ACh. Symbols represent mean±SEM. Solid lines represent the best fit to a single Hill equation. The corresponding parameters are: PANEL A, $I_{[\mathbf{35}]max}$=38±11 %, n_H=0.3, EC_{50}=0.6±0.2 μM (n=6 cells); PANEL B, $I_{[\mathbf{39}]max}$=37±7 %, n_H=0.3, EC_{50}=1.0±0.2 μM (n=5); PANEL C, $I_{[\mathbf{43}]max}$=41±1 %, n_H=0.9, EC_{50}=0.52±0.04 μM (n=10).

2.4. Molecular docking

To gain an insight into the possible binding modes for the compounds they were docked to the binding sites of both $\alpha_4\beta_2$ and α_7. The simulation showed a clear difference in the ligand positioning between the two subtypes. In the crystal structure of nAChR α_7 epibatidine is shown to interact with multiple residues in the binding site, among them Leu106, Gln114, Tyr91 and most prominently an ionic interaction between the nitrogen of the azabicycloheptane moiety and Trp145 and Tyr191 (**Figure 6A**). Similar interactions occur in the structure of the $\alpha_4\beta_2$ subtype between the pyrrolidine nitrogen and Trp156 and Tyr204 (**Figure 6B**). Compared to epibatidine the novel compounds (exemplary shown with compound **43**) are turned around in in the binding pocket of the α_7 subtype, since the bindig site is closed off on the side of the pyridyl and does not allow for larger

substitutions. This leads to a loss of the ionic interaction (Figure **7A**). On the other hand, in the $\alpha_4\beta_2$ subtype the compounds align with epibatidine and retain the ionic interaction. This could explain the high selectivity of the larger structures.

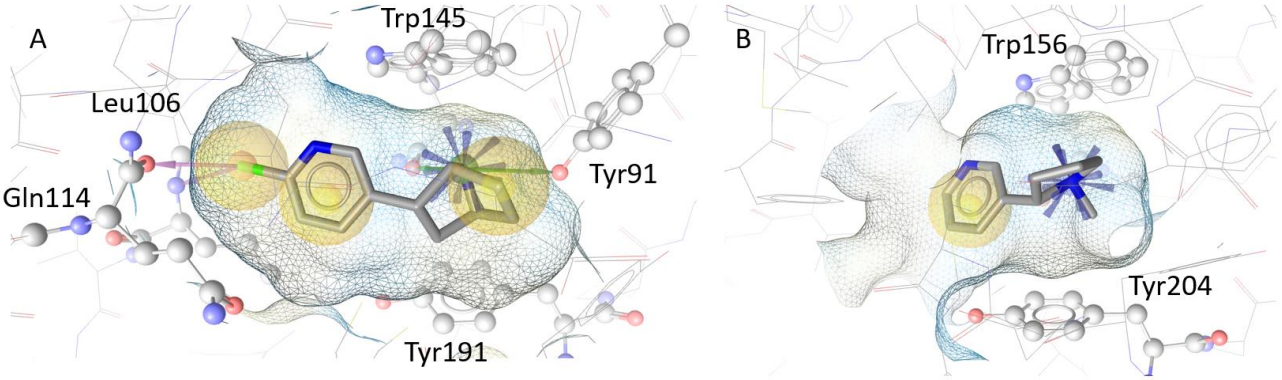


Figure 6. (**A**) Epibatidine in the binding site of nAChR α_7. It forms polar interactions with Leu106 and Gln114 (pink arrows), an hydrogen bond with Tyr91 (green arrow) and an ionic interaction with Trp145 and Tyr191(blue star). (**B**) Nicotine in the binding site of nAChR $\alpha_4\beta_2$ forming ionic interactions with Trp156 and Tyr204.

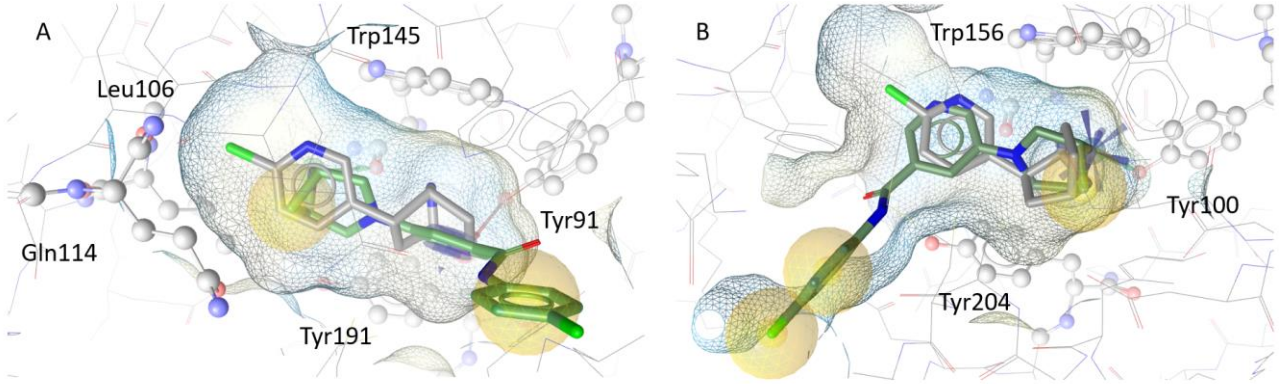


Figure 7. (**A**) Compound **43** in the binding site of nAChR α_7. The molecule forms hydrogen bonds with Trp145 and Tyr91, whereas the core structure is turned around and does not form the ionic interaction. (**B**) Compound **43** in the binding site of nAChR $\alpha_4\beta_2$. The key ionic interaction Trp156 and Tyr204 is retained and the core scaffold aligns with epibatidine.

3. Conclusions

A small library of 5-(3,6-diazabicyclo[3.1.1]heptan-3-yl)-*N*-arylnicotinamide derivatives have been designed. The 12 compounds synthesized and tested in this study, acting as partial agonists, allowed us to deepen the SARs on the 3,6-DBH core as template for the synthesis of potent nicotinic $\alpha_4\beta_2$ receptor ligands, with K_i binding values better than epibatine for all series. The main structural features of whole DBH compounds, synthesized by us to today, are below summarized.

The initial synthesis of derivatives combining the 3,6-DBH system and various halo-pyridines (**3**, **4**, **6** and **7**) and a chloropiridazine (**5**) furnished compounds endowed with high $\alpha_4\beta_2$ affinity and selectivity. This evidence let us to assume the halo-pyridine structure as lead compound and the 5'-position of the pyridine ring as preferred for the modulation of this system, due to the interesting $\alpha_4\beta_2$ binding values of agonists **6** and **7**, (K_i $\alpha_4\beta_2$ = 6.32 pM and 5.72 pM, respectively).

The modulation of C-5' aniline position with various substituted aryls, compounds typified by structure **11** (K_i $\alpha_4\beta_2$ = 11.17 pM), displayed high $\alpha_4\beta_2$ receptors affinity in accordance with that of halogenated homologues and very high selectivity values, the latter influenced by mono-phenyl substitution. Interestingly, the introduction of aryl rings on C-5' of pyridine nucleus exhibited $\alpha_4\beta_2$ antagonist activity.

The introduction of an amine group between the heteroaromatic ring of the DBH system and the aryl ring gave three different classes of compounds, typified by compounds **9-11**, resulting compounds of the 3-(anilino)pyridine series those with the high $\alpha_4\beta_2$ K_i values ranging in the pM field (**9**, K_i $\alpha_4\beta_2$ = 11.17 pM) and an affinity and selectivity slightly decreased with respect to the lead compound.

Finally, the introduction of the *N*-aryl nicotinamide function, allowed to identify the halo-aryl nicotinamides as the best derivatives of this series, resulting the compound **43** having a K_i $\alpha_4\beta_2$ = 10 pM, similar to that of the lead compound of the whole DBH series.

In conclusion, tacking stock on 3,6-diazabicyclo[3.1.1]heptane ligands, we can conclude that the DBH-pyridine template represents a good pharmacophore for the design of nAChR ligands endowed with very high $\alpha_4\beta_2$ affinity and selectivity, influenced by the nature of substituent on pyridine ring.

Indeed, even if all tested compound of different DBH series synthesized by us maintain high $\alpha_4\beta_2$ affinity and selectivity, from our SARs emerged that the α or β, both chlorine and bromine, substitution on the pyridine ring led to the best compounds of the DBH series with a very high affinity and selectivity for $\alpha_4\beta_2$ nAChR subtype, better than epibatidine.

4. Experimental section

4.1. General procedures

The reactions involving air or moisture-sensitive compounds were performed under argon atmosphere. Unless otherwise specified, all materials, solvents, reagents and precursors were obtained from commercial suppliers and were used without further purification. The standard configuration of the Biotage® Microwave Initiator Eight 2.5 was used for microwave experiments, which were carried out in sealed microwave process vials under normal absorption. A 4560 Parr Apparatus and an H_2PEM-100 Parker Balston Hydrogen Generator were used for hydrogenation reactions. Purification by flash column chromatography (FC) was performed on Flash-master (Biotage®) with pre-packed Biotage® SNAP silica gel cartridges or manually on silica gel (Kieselgel 60, 0.040−0.063 mm, Merck®). The thin layer chromatography (TLC) was used to monitor reactions and was performed with Polygram SIL N-HR/HV_{254} pre-coated plastic sheets (0.2 mm) on aluminum sheets (Kieselgel 60 F254, Merck®). Melting points were determined on a Köfler melting point apparatus and are uncorrected. IR spectra were measured as KBr pallets with a Jasco FT/IR 460 plus spectrophotometer and are expressed in ν (cm^{-1}). NMR experiments were recorded on a Varian Unity 200 spectrometer (200.07 MHz for ^1H, and 50.31 MHz for ^{13}C, respectively) and the spectra were acquired using deuterated chloroform (chloroform-d) as solvent. Chemical shifts (δ) for ^1H- and ^{13}C-NMR spectra are reported in parts per million (ppm) using the residual non-deuterated solvent resonance as the internal standard (for chloroform-d: 7.26 ppm, ^1H and 77.16 ppm, ^{13}C; for DMSO-d_6: 2.50 ppm, ^1H, 39.52 ppm, ^{13}C). Data are reported as follows: chemical shift, multiplicity (s for

singlet, bs for broad singlet, d for doublet, t for triplet, q for quadruplet, qu for quintuplet, m for multiplet), integration and coupling constants (J) in Hertz (Hz). All final compounds displayed ≥ 95% purity as determined by elemental analysis on a Perkin-Elmer 240-B analyser, for C, H, and N.

*4.1.1. Methyl 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotinate (**14**)*

To a solution of the Boc-bridged-piperazine **12** (200 mg, 1.00 mmol) [7] in dioxane (5 mL), nicotinic bromo-ester **13** (283 mg, 1.31 mmol), $Pd_2(dba)_3$ (28 mg, 0.03 mmol), Xantophos (58 mg, 0.10 mmol) and Cs_2CO_3 (657 mg, 2.01 mmol) were added. The mixture was reacted under MW irradiation (12 h, 110 °C, low abs) and the suspension was filtered on celite®. After in vacuum concentration of the filtrate, the resulting residue was dissolved in EtOAc, washed (H_2O), dried (Na_2SO_4) and concentrated in vacuum. The title compound **14** was isolated by FC (petroleum ether/EtOAc 5:5) as an orange oil. Yield 86% (292 mg, 0,87 mmol). ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.62 (s, 1H), 8.29 (s, 1H), 7.57 (s, 1H), 4.33 (d, 2H, J = 5.4 Hz), 3.80-4.05 (m, 5H), 3.35 (d, 2H, J = 10.0 Hz), 2.60-2.80 (m, 1H), 1.52 (d, 1H, J = 8.8 Hz), 1.35 (s, 9H).

*4.1.2. 5-[6-(tert-Butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotinic acid (**15**)*

To a solution of the ester **14** (100 mg, 0.30 mmol) in methanol (1.35 mL) a solution of NaOH (394 mg) in H_2O (0.7 mL) was added. The mixture was stirred at room temperature for 2 h, then diluted with EtOAc. The aqueous phase was acidified to pH 4 and extracted with CH_2Cl_2, which was dried (Na_2SO_4), filtered and concentrated in vacuum. Title compound **15** was isolated as a yellow solid. Yield 99% (94 mg, 0.30 mmol). Mp = 225-226 °C, ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.75 (s, 1H), 8.36 (s, 1H), 7.73 (s, 1H), 4.35 (d, 2H, J = 5.8 Hz), 3.86-4.15 (m, 2H), 3.38 (d, 2H, J = 10.2 Hz), 2.62 (bs, 2H, OH exch. with D_2O), 1.54 (d, 1H, J = 8.8 Hz), 1.37 (s, 9H).

*4.1.3. General procedure for the synthesis of amides **16-31***

To a solution of nicotinic acid derived **15** (0.75 mg, 0.235 mmol) in CH_2Cl_2 (4 ml) EDC (56 mg, 0.329 mmol), hydroxy-benzotriazole (38 mg, 0.282 mmol) and DMAP (9 mg,0.08 mmol) were added. The solution was stirred at room temperature for 2 h, then the required amine (0.282 mmol)

was added and the whole stirred at the same temperature for 4-8 h. The organic solution was washed (H_2O), dried (Na_2SO_4), filtered and concentrated in vacuum. The analytically pure product was isolated by FC as indicated below.

*4.1.4. N-Phenyl 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotine carboxamide (**16**)*

Reaction between **15** and aniline gave a crude product whose purification by FC (chloroform/methanol 9:1) afforded **16**. Pale oil; yield 94% (87 mg); mp 179-182 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.44 (bs, 1H, NH exch. with D_2O), 8.20-8.30 (m, 1H), 7.50-7.73 (m, 2H), 7.30-7.45 (m, 3H), 7.10-7.18 (m, 1H), 6.62-6.82 (m, 1H), 4.26-4.39 (m, 2H), 3.83-3.96 (m, 2H), 3.35 (d, 2H, J = 10.8 Hz), 2.60-2.75 (m, 1H), 1.51 (d, 1H, J = 8.6 Hz), 1.35 (s, 9H).

*4.1.5. N-Benzyl 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotine carboxamide (**17**)*

Reaction between **15** and benzylamine gave a crude product whose purification by plug chromatography (EtOAc) afforded **17**. White solid; yield 94% (90 mg); mp 65-67 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.29 (s, 1H), 8.20-8.25 (m, 1H), 7.48 (s, 1H), 7.27-7.42 (m, 5H), 6.49 (bs, 1H, NH exch. with D_2O), 4.67 (d, 2H, J = 5.4 Hz), 4.31 (d, 2H, J = 6.0 Hz), 3.80-4.10 (m, 2H), 3.35 (d, 2H, J = 11.0 Hz), 2.60-2.80 (m, 1H), 1.51 (d, 1H, J = 8.8 Hz), 1.38 (s, 9H).

*4.1.6. N-(3-Ethylphenyl) 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotine carboxamide (**18**)*

Reaction between **15** and 3-ethylaniline gave a crude product whose purification by FC (petroleum ether/EtOAc 4:6) afforded **18**. White solid; yield 94% (93 mg); mp 91-92 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.40-8.50 (m, 1H), 8.27 (d, 1H, J = 3.0 Hz), 7.94 (bs, 1H, NH exch. with D_2O), 7.38-7.58 (m, 3H), 7.32 (d, 1H, J = 7.6 Hz), 7.02 (d, 1H, J = 8.2 Hz), 4.32 (d, 2H, J = 6.4 Hz), 3.85-4.15 (m, 2H), 3.36 (d, 2H, J = 11.0 Hz), 2.60-2.80 (m, 3H), 1.52 (d, 1H, J = 8.4 Hz), 1.35 (s, 9H), 1.26 (t, 3H, J = 7.4 Hz).

*4.1.7. N-(3-Methoxyphenyl) 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotine carboxamide (**19**)*

Reaction between **15** and 3-methoxyaniline gave a crude product whose purification by FC (chloroform/methanol 98:2) afforded **19**. White solid; yield 97% (97 mg); mp 77-80 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.25-7.35 (m, 1H), 7.38-7.50 (m, 1H), 8.04 (bs, 1H, NH exch. with D_2O), 7.40-7.56 (m, 2H), 7.20-7.35 (m, 1H), 7.12 (d, 1H, J = 7.2 Hz), 6.73 (dd, 1H, J_o = 2.8 Hz, J_m = 8.4 Hz), 4.32 (d, 2H, J = 6.0 Hz), 3.86-4.18 (m, 2H), 3.84 (s, 3H), 3.35 (d, 2H, J = 10.8 Hz), 2.60-2.80 (m, 1H), 1.52 (d, 1H, J = 8.6 Hz), 1.35 (s, 9H).

*4.1.8. N-(3,5-diMethoxyphenyl) 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotine carboxamide (**20**)*

Reaction between **15** and 3,5-dimethoxyaniline gave a crude product whose purification by FC (petroleum ether/EtOAc 4:6) afforded **20**. White solid; yield 90% (96 mg); mp 189-191 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.43 (s, 1H), 8.18-8.30 (m, 2H), 7.56 (bs, 1H, NH exch. with D_2O), 6.90-7.00 (m, 2H), 6.25-6.32 (m, 1H), 4.32 (d, 2H, J = 5.8 Hz), 3.85-4.10 (m, 2H), 3.80 (s, 6H), 3.35 (d, 2H, J = 10.2 Hz), 2.60-2.80 (m, 1H), 1.52 (d, 1H, J = 8.8 Hz), 1.35 (s, 9H).

*4.1.9. N-(3,4-diMethoxyphenyl) 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotine carboxamide (**21**)*

Reaction between **15** and 3,4-dimethoxyaniline gave a crude product whose purification by FC (petroleum ether/EtOAc 3:7) afforded **21**. White solid; yield 90% (96 mg); mp 83-84 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.43 (s, 1H), 8.20-8.35 (m, 1H), 7.91 (bs, 1H, NH exch. with D_2O), 7.51 (s, 2H), 7.00-7.10 (m, 1H), 6.87 (d, 1H, J = 8.6 Hz), 4.33 (d, 2H, J = 5.8 Hz), 3.95-4.20 (m, 2H), 3.93 (s, 3H), 3.90 (s, 3H), 3.37 (d, 2H, J = 10.8 Hz), 2.60-2.80 (m, 1H), 1.53 (d, 1H, J = 9.0 Hz), 1.36 (s, 9H).

*4.1.10. N-(3,4-Methylendioxyphenyl) 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotine carboxamide (**22**)*

Reaction between **15** and 3,4-methylendioxyaniline gave a crude product whose purification by FC (petroleum ether/EtOAc 3:7) afforded **22**. White solid; yield 93% (96 mg); mp 78-80 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.18-8.28 (m, 1H), 8.11 (bs, 1H, NH exch. with D_2O), 7.52 (s, 1H), 7.37 (s, 1H), 6.90-7.00 (m, 1H), 6.79 (d, 1H, J = 8.6 Hz), 5.99 (s, 2H), 4.32 (d, 2H, J = 5.6 Hz), 3.85-4.10 (m, 2H), 3.35 (d, 2H, J = 10.6 Hz), 2.60-2.80 (m, 1H), 1.51 (d, 1H, J = 8.8 Hz), 1.35 (s, 9H).

*4.1.11. N-(3-Chlorophenyl) 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotine carboxamide (**23**)*

Reaction between **15** and 3-chloroaniline gave a crude product whose purification by FC (chloroform/methanol 98:2) afforded **23**. White solid; yield 85% (85 mg); mp 114 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.38-8.50 (m, 1H), 8.25-8.35 (m, 1H), 7.93 (bs, 1H, NH exch. with D_2O), 7.75-7.88 (m, 1H), 7.45-7.60 (m, 2H), 7.33 (d, 1H, J = 8.4 Hz), 7.16 (d, 1H, J = 7.8 Hz), 4.27-4.48 (m, 2H), 3.85-4.18 (m, 2H), 3.30-3.50 (m, 2H), 2.60-2.80 (m, 1H), 1.53 (d, 1H, J = 9.0 Hz), 1.38 (s, 9H).

*4.1.12. N-(4-Chlorophenyl) 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotine carboxamide (**24**)*

Reaction between **15** and 4-chloroaniline gave a crude product whose purification by FC (chloroform/methanol 98:2) afford **24**. White solid; yield 89% (89 mg); mp 130-132 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.35-8.48 (m, 1H), 8.20-8.33 (m, 1H), 7.96 (bs, 1H, NH exch. with D_2O), 7.63 (d, 2H, J = 8.8 Hz), 7.40-7.56 (m, 1H), 7.36 (d, 2H, J = 8.8 Hz), 4.33 (d, 2H, J = 6.4 Hz), 3.85-4.18 (m, 2H), 3.36 (d, 2H, J = 10.4 Hz), 2.65-2.80 (m, 1H), 1.52 (d, 1H, J = 8.8 Hz), 1.35 (s, 9H).

*4.1.13. N-(2-Fluorophenyl) 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotine carboxamide (**25**)*

Reaction between **15** and 2-fluoroaniline gave a crude product whose purification by FC (petroleum ether/EtOAc 4:6) afforded **25**. Yellowish solid; yield 93% (90 mg); mp 111-113 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.38-8.50 (m, 2H), 8.20-8.35 (m, 1H), 8.14 (bs, 1H, NH exch. with D_2O),

7.48-7.58 (m, 1H), 7.05-7.28 (m, 3H), 4.33 (d, 2H, J = 5.6 Hz), 3.90-4.10 (m, 2H), 3.37 (d, 2H, J = 10.6 Hz), 2.60-2.80 (m, 1H), 1.53 (d, 1H, J = 8.8 Hz), 1.36 (s, 9H).

*4.1.14. N-(3-Fluorophenyl) 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotine carboxamide (**26**)*

Reaction between **15** and 3-fluoroaniline gave a crude product whose purification by FC (petroleum ether/EtOAc 3:7) afforded **26**. Yellowish solid; yield 94% (91 mg); mp 188-189 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.43 (s, 1H), 8.27 (s, 1H), 8.19 (bs, 1H, NH exch. with D_2O), 7.60-7.75 (m, 1H), 7.42-7.55 (m, 1H), 7.20-7.40 (m, 2H), 6.80-7.00 (m, 1H), 4.32 (d, 2H, J = 6.4 Hz), 3.85-4.10 (m, 2H), 3.34 (d, 2H, J = 10.8 Hz), 2.60-2.80 (m, 1H), 1.52 (d, 1H, J = 8.6 Hz), 1.35 (s, 9H).

*4.1.15. N-(4-Fluorophenyl) 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotine carboxamide (**27**)*

Reaction between **15** and 4-fluoroaniline gave a crude product whose purification by FC (petroleum ether/EtOAc 3:7) afforded **27**. Yellowish solid; yield 96% (93 mg); mp 193-195 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.43 (s, 1H), 8.20-8.35 (m, 1H), 8.11 (bs, 1H, NH exch. with D_2O), 7.57-7.70 (m, 2H), 7.45-7.55 (m, 1H), 7.08 (t, 2H, J = 8.8 Hz), 4.33 (d, 2H, J = 6.4 Hz), 3.85-4.15 (m, 2H), 3.34 (d, 2H, J = 10.4 Hz), 2.60-2.80 (m, 1H), 1.52 (d, 1H, J = 9.0 Hz), 1.35 (s, 9H).

*4.1.16. N-(2,4-diFluorophenyl) 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotine carboxamide (**28**)*

Reaction between **15** and 2,4-difluoroaniline gave a crude product whose purification by FC (petroleum ether/EtOAc 3:7) afforded **28**. Yellowish solid; yield 95% (92 mg); mp 157-158 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.25-8.45 (m, 3H), 8.00 (bs, 1H, NH exch. with D_2O), 7.52 (s, 1H), 6.85-7.10 (m, 2H), 4.34 (d, 2H, J = 6.6 Hz), 3.90-4.15 (m, 2H), 3.37 (d, 2H, J = 10.6 Hz), 2.60-2.80 (m, 1H), 1.52 (d, 1H, J = 9.0 Hz), 1.36 (s, 9H).

*4.1.17. N-(4-Iodophenyl) 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotine carboxamide (**29**)*

Reaction between **15** and 4-iodoaniline gave a crude product whose purification by FC (petroleum ether/EtOAc 3:7) afforded **29**. Beige solid; yield 89% (109 mg); mp 218-219 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.44 (s, 2H), 8.22 (d, 1H, J = 3.2 Hz), 7.68 (d, 2H, J = 8.8 Hz), 7.53 (bs, 1H, NH exch. with D_2O), 7.47 (d, 2H, J = 8.8 Hz), 4.32 (d, 2H, J = 6.0 Hz), 3.85-4.10 (m, 2H), 3.34 (d, 2H, J = 10.6 Hz), 2.60-2.80 (m, 1H), 1.50 (d, 1H, J = 8.8 Hz), 1.34 (s, 9H).

*4.1.18. N-(1,2,3,4-Tetrahydroisoquinoline) 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotine carboxamide (**30**)*

Reaction between **15** and 1,2,3,4-tetrahydroisoquinoline gave a crude product whose purification by FC (petroleum ether/EtOAc 3:7) afforded **30**. Beige solid; yield 89% (109 mg); mp 73-74 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.18-8.25 (m, 1H), 8.09 (s, 1H), 7.15-7.32 (m, 4H), 7.00-7.10 (m, 1H), 4.80-5.00 (m, 1H), 4.55-4.68 (m, 1H), 4.31 (d, 2H, J = 3.8 Hz), 3.90-4.10 (m, 3H), 3.60-3.80 (m, 1H), 3.33 (d, 2H, J = 10.6 Hz), 2.81-3.00 (m, 2H), 2.60-2.80 (m, 1H), 1.35-1.55 (m, 10H).

*4.1.19. N-(1,2,3,4-Tetrahydroquinoline) 5-[6-(tert-butoxycarbonyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl]nicotine carboxamide (**31**)*

Reaction between **15** and 1,2,3,4-tetrahydroquinoline gave a crude product whose purification by FC (petroleum ether/EtOAc 3:7) afforded **31**. Beige solid; yield 55% (56 mg); mp 85-87 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.02 (d, 1H, J = 3.2 Hz), 7.73 (s, 1H), 6.80-7.45 (m, 5H), 4.28 (d, 2H, J = 5.8 Hz), 3.80-4.00 (m, 3H), 3.40-3.50 (m, 1H), 3.20 (d, 2H, J = 10.6 Hz), 2.83 (t, 2H, J = 6.6 Hz), 2.60-2.80 (m, 1H), 2.06 (q, 2H, J = 6.6 Hz), 1.48 (d, 1H, J = 8.8 Hz), 1.35 (s, 9H).

*4.1.20. General procedure for the preparation of final compounds **32-47**.*

The appropriate *tert*-butoxycarbonyl derivative (**16 – 31**) (0.323 mmol) was dissolved in HCOOH (2 mL) and the solution was stirred at room temperature for 20 h. Then H_2O was added and the acid solution was extracted with $CHCl_3$. The aqueous layer was basified with 10% K_2CO_3 aqueous solution and extracted with $CHCl_3$. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure to afford the analytically pure product.

*4.1.21. 5-(3,6-Diazabicyclo[3.1.1]heptane-3-yl)-N-(phenyl)nicotinamide (**32**)*

Title compound was synthesized starting from **16**. Yellowish solid; yield 75% (71 mg); mp 126-128 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.38-8.50 (m, 1H), 8.20-8.30 (m, 1H), 8.04 (bs, 1H, NH exch. with D_2O), 7.66 (d, 2H, J = 8.6 Hz), 7.50-7.55 (m, 1H), 7.30-7.47 (m, 2H), 7.12-7.22 (m, 1H), 3.93 (d, 2H, J = 6.0 Hz), 3.55-3.70 (m, 4H), 2.70-2.90 (m, 1H), 1.58-1.80 (m, 2H, NH exch. with D_2O). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 164.58 (C=O), 137.93 (C), 134.90 (CH), 134.89 (CH), 129.04 (2 x CH), 124.70 (CH), 120.45 (2 x CH), 116.06 (C), 115.79 (C), 55.78 (2 x CH_2), 50.99 (2 x CH), 31.23 (CH_2). IR (nujol) ν: 3502 (2 x NH), 1651 (C=O). Elemental analysis calculated (%) for $C_{17}H_{18}N_4O$: C 69.37, H 6.16, N 19.03. Found: C 69.46, H 6.20, N 19.05.

*4.1.22. 5-(3,6-Diazabicyclo[3.1.1]heptane-3-yl)-N-(benzyl)nicotinamide (**33**)*

Title compound was synthesized starting from **17**. Yellowish solid; yield 90% (89 mg); mp 104-105 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.28 (s, 1H), 8.21 (s, 1H), 7.49 (s, 1H), 7.36 (s, 5H), 6.61 (bs, 1H, NH exch. with D_2O), 4.67 (d, 2H, J = 5.4 Hz), 3.93 (d, 2H, J = 5.4 Hz), 3.50-3.70 (m, 4H), 2.70-2.90 (m, 1H), 1.72 (bs, 1H, NH exch. with D_2O), 1. 61 (d, 1H, J = 8.6 Hz). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 166.31 (C=O), 144.66 (C), 137.99 (C), 134.91 (CH), 130.24 (C), 128.70 (2 x CH), 127.85 (2 x CH), 127.55 (CH), 118.01 (CH), 115.79 (CH), 55.60 (2 x CH_2), 51.06 (2 x CH), 44.05 (CH_2), 31.20 (CH_2). IR (nujol) *ν*: 3489 (2 x NH), 1654 (C=O). Elemental analysis calculated (%) for $C_{18}H_{20}N_4O$: C 70.11, H 6.57, N 18.17. Found: C 70.20, H 6.52, N 18.20.

*4.1.23. 5-(3,6-Diazabicyclo[3.1.1]heptane-3-yl)-N-(3-ethylphenyl)nicotinamide (**34**)*

Title compound was synthesized starting from **18**. White solid; yield 92% (96 mg); mp 186-187 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.42 (s, 1H), 8.23-8.36 (m, 1H), 7.99 (bs, 1H, NH exch. with D_2O), 7.47-7.65 (m, 2H), 7.45 (s, 1H), 7.32 (d, 1H, J = 7.6 Hz), 7.02 (d, 1H, J = 7.2 Hz), 3.93 (d, 2H, J = 6.0 Hz), 3.55-3.78 (m, 4H), 2.70-2.88 (m, 1H), 2.68 (q, 2H, J = 7.3 Hz), 1.63 (d, 1H, J = 9.0 Hz), 1.55 (bs, 1H, NH exch. with D_2O), 1.26 (t, 3H, J = 7.6 Hz). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 163.99 (C=O), 143.43 (CH), 137.68 (C), 135.29 (CH), 133.66 (CH), 129.75 (C), 127.37 (C), 122.53 (CH),

119.14 (CH), 117.05 (CH), 114.50 (CH), 112.11 (C), 54.36 (2 x CH_2), 49.93 (2 x CH), 30.17 (CH_2), 27.69 (CH_3), 14.54 (CH_2). IR (nujol) ν: 3502 (2 x NH), 1651 (C=O). Elemental analysis calculated (%) for $C_{19}H_{22}N_4O$: C 70.78, H 6.88, N 17.38. Found: C 70.86, H 6.90, N 17.35.

*4.1.24. 5-(3,6-Diazabicyclo[3.1.1]heptane-3-yl)-N-(3-methoxyphenyl)nicotinamide (**35**)*

Title compound was synthesized starting from **19**. White solid; yield 87% (91 mg); mp 153-155 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.38-8.48 (m, 1H), 8.22-8.35 (m, 1H), 7.92 (bs, 1H, NH exch. with D_2O), 7.48-7.56 (m, 1H), 7.40-7.47 (m, 1H), 7.20-7.38 (m, 1H), 7.12 (d, 1H, J=7.8 Hz), 6.73 (dd, 1H, J_o=1.4 Hz, J_m=7.8 Hz), 3.95 (d, 2H, J=5.4 Hz), 3.85 (s, 3H), 3.60-3.80 (m, 4H), 2.70-2.90 (m, 1H), 1.63 (d, 1H, J=8.8 Hz), 1.57 (bs, 1H, NH exch. with D_2O). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 164.52 (C=O), 159.95 (CH), 144.83 (C), 139.35 (CH), 134.61 (2 x CH), 130.63 (C), 129.63 (CH), 116.23 (C), 112.65 (CH), 110.26 (C), 106.25 (CH), 55.48 (2 x CH_2), 55.24 (OCH_3), 50.83 (2 x CH), 31.21 (CH_2). IR (nujol) ν: 3505 (2 x NH), 1721 (C=O). Elemental analysis calculated (%) for $C_{18}H_{20}N_4O_2$: C 66.65, H 6.21, N 17.27. Found: C 66.77, H 6.27, N 17.30.

*4.1.25. 5-(3,6-Diazabicyclo[3.1.1]heptane-3-yl)-N-(3,5-dimethoxyphenyl)nicotinamide (**36**)*

Title compound was synthesized starting from **20**. White solid; yield 93% (110 mg); mp 216-219 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.40 (s, 1H), 8.27 (s, 1H), 7.98 (bs, 1H, NH exch. with D_2O), 7.45-7.55 (m, 1H), 6.91 (s, 2H), 6.30 (s, 1H), 3.94 (d, 2H, J = 7.0 Hz), 3.85 (s, 6H), 3.55-3.75 (m, 4H), 2.70-2.90 (m, 1H), 1.65 (bs, 2H, NH exch. with D_2O).

^{13}C-NMR (50 MHz, $CDCl_3$) δ 164.66 (C=O), 160.06 (CH), 143.89 (C), 139.88 (CH), 135.74 (C), 134.27 (CH), 130.28 (C), 117.58 (CH), 115.16 (C), 98.23 (2 x CH), 95.93 (C), 54.98 (2 x CH_2), 54.65 (2 x OCH_3), 50.39 (2 x CH), 30.64 (CH_2). IR (nujol) ν: 3495 (2 x NH), 1721 (C=O). Elemental analysis calculated (%) for $C_{19}H_{22}N_4O_3$: C 64.39, H 6.26, N 15.81. Found: C 64.45, H 6.29, N 15.83.

*4.1.26. 5-(3,6-Diazabicyclo[3.1.1]heptane-3-yl)-N-(3,4-dimethoxyphenyl)nicotinamide (**37**)*

Title compound was synthesized starting from **21**. Beige solid; yield 84% (96 mg); mp 204-205 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.42 (s, 1H), 8.20-8.30 (m, 1H), 8.08 (bs, 1H, NH exch. with

D_2O), 7.51 (s, 2H), 6.95-7.15 (m, 1H), 6.86 (d, 1H, J = 8.4 Hz), 3.80-4.10 (m, 8H), 3.50-3.70 (m, 4H), 2.70-2.90 (m, 1H), 1.63 (d, 1H, J = 9.6 Hz), 1.55 (bs, 1H, NH exch. with D_2O).

^{13}C-NMR (50 MHz, $CDCl_3$) δ 164.27 (C=O), 148.84 (C), 145.91 (C), 144.83 (C), 134.51 (CH, C), 131.64 (CH), 117.97 (C), 116.10 (CH), 112.37 (CH), 111.11 (CH), 105.20 (CH), 55.98 (2 x OCH_3), 55.49 (2 x CH_2), 50.93 (2 x CH), 31.22 (CH_2). IR (nujol) ν: 3495 (2 x NH), 1721 (C=O). Elemental analysis calculated (%) for $C_{19}H_{22}N_4O_3$: C 64.39, H 6.26, N 15.81. Found: C 64.45, H 6.30, N 15.86.

*4.1.27. 5-(3,6-Diazabicyclo[3.1.1]heptane-3-yl)-N-(3,4-methylendioxyphenyl)nicotinamide (**38**)*

Title compound was synthesized starting from **22**. Beige solid; yield 88% (96 mg); mp 201-202 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.30-8.50 (m, 1H), 8.26 (d, 1H, 3.0 Hz), 7.98 (bs, 1H, NH exch. with D_2O), 7.45-7.58 (m, 1H), 7.30-7.45 (m, 1H), 6.95 (dd, 1H, J_o = 2.2 Hz, J_m = 8.6 Hz), 6.80 (d, 1H, J = 8.4 Hz), 5.99 (s, 2H), 3.94 (d, 2H, J = 6.4 Hz), 3.55-3.75 (m, 4H), 2.70-2.90 (m, 1H), 1.58-1.80 (m, 2H, NH exch. with D_2O). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 164.49 (C=O), 147.71 (CH), 144.82 (C), 144.42 (C), 135.73 (2 x CH), 132.25 (C), 130.76 (C), 116.09 (C), 113.76 (CH), 108.05 (CH), 103.20 (CH), 101.28 (CH_2), 55.95 (2 x CH_2), 50.91 (2 x CH), 31.22 (CH_2). IR (nujol) ν: 3500 (2 x NH), 1723 (C=O). Elemental analysis calculated (%) for $C_{18}H_{18}N_4O_3$: C 63.89, H 5.36, N 16.56. Found: C 64.01, H 5.40, N 16.60.

*4.1.28. 5-(3,6-Diazabicyclo[3.1.1]heptane-3-yl)-N-(3-chlorophenyl)nicotinamide (**39**)*

Title compound was synthesized starting from **23**. White solid; yield 97% (103 mg); mp 147-149 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.35-8.48 (m, 1H), 8.20-8.30 (m, 1H), 8.11 (bs, 1H, NH exch. with D_2O), 7.81 (s, 1H), 7.38-7.58 (m, 2H), 7.33 (d, 1H, J = 7.8 Hz), 7.15 (d, 1H, J = 7.8 Hz), 3.87-4.10 (m, 2H), 3.50-3.80 (m, 4H), 2.70-2.90 (m, 1H), 1.60-1.90 (m, 2H, NH exch. with D_2O). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 164.79 (C=O), 143.90 (C), 139.49 (CH), 135.75 (C), 134.38 (C), 133.32 (CH), 130.02 (C), 129.13 (CH), 123.28 (CH), 119.95 (CH), 118.10 (CH), 115.23 (CH), 54.97 (2 x CH_2), 50.33 (2 x CH), 30.58 (CH_2). IR (nujol) ν: 3503 (2 x NH), 1654 (C=O). Elemental analysis calculated (%) for $C_{17}H_{17}ClN_4O$: C 62.10, H 5.21, N 17.04. Found: C 62.23, H 5.29, N 17.11.

*4.1.29. 5-(3,6-Diazabicyclo[3.1.1]heptane-3-yl)-N-(4-chlorophenyl)nicotinamide (**40**)*

Title compound was synthesized starting from **24**. White solid; yield 86% (91 mg); mp 188-190 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.36-8.48 (m, 1H), 8.20-8.32 (m, 1H), 7.93 (bs, 1H, NH exch. with D_2O), 7.63 (d, 2H, J = 8.8 Hz), 7.45-7.55 (m, 1H), 7.36 (d, 2H, J = 8.8 Hz), 3.90-4.10 (m, 2H), 3.62-3.68 (m, 4H), 2.78-2.90 (m, 1H), 1.60-1.85 (m, 2H, NH exch. with D_2O). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 164.29 (C=O), 143.56 (C), 141.23 (C), 136.74 (CH), 135.48 (CH), 134.06 (C), 129.97 (C), 127.52 (3 x CH), 121.02 (2 x CH), 54.68 (2 x CH_2), 49.93 (2 x CH), 30.23 (CH_2). IR (nujol) ν: 3503 (2 x NH), 1654 (C=O). Elemental analysis calculated (%) for $C_{17}H_{17}ClN_4O$: C 62.10, H 5.21, N 17.04. Found: C 62.23, H 5.24, N 17.12.

*4.1.30. 5-(3,6-Diazabicyclo[3.1.1]heptane-3-yl)-N-(2-fluorophenyl)nicotinamide (**41**)*

Title compound was synthesized starting from **25**. White solid; yield 85% (85 mg); mp 86-87 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.38-8.55 (m, 2H), 8.25-8.35 (m, 1H), 8.09 (bs, 1H, NH exch. with D_2O), 7.10-7.35 (m, 3H), 3.96 (d, 2H, J = 5.6 Hz), 3.60-3.80 (m, 4H), 2.70-2.90 (m, 1H), 1.55-1.78 (m, 2H, NH exch. with D_2O). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 165.01 (C=O), 143.90 (CH), 137.23 (CH), 136.23 (C), 135.57 (CH), 135.22 (C), 130.22 (C), 125.26 (CH), 125.03 (C), 124.31 (CH), 116.38 (CH), 115.61 (CH), 56.95 (2 x CH_2), 49.27 (2 x CH), 30.15 (CH_2). IR (nujol) ν: 3506 (2 x NH), 1684 (C=O). Elemental analysis calculated (%) for $C_{17}H_{17}FN_4O$: C 65.37, H 5.49, N 17.94. Found: C 65.43, H 5.51, N 17.90.

*4.1.31. 5-(3,6-Diazabicyclo[3.1.1]heptane-3-yl)-N-(2-fluorophenyl)nicotinamide (**42**)*

Title compound was synthesized starting from **26**. Yellowish solid; yield 87% (87 mg); mp 208-210 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.25-8.33 (m, 1H), 8.03 (bs, 1H, NH exch. with D_2O), 7.60-7.75 (m, 1H), 7.45-7.56 (m, 1H), 7.25-7.38 (m, 2H), 6.85-6.98 (m, 1H), 3.95 (d, 2H, J = 5.6 Hz), 3.55-3.70 (m, 4H), 2.70-2.90 (m, 1H), 1.55-1.80 (m, 2H, NH exch. with D_2O). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 164.20 (C=O), 143.36 (C), 139.60 (C), 135.18 (CH), 133.83 (CH), 129.43 (CH), 128.49 (C), 114.86 (CH), 114.47 (CH), 112.06 (CH), 106.74 (CH), 106.23 (C), 54.33 (2 x CH_2),

49.79 (2 x CH), 30.09 (CH_2). IR (nujol) ν: 3505 (2 x NH), 1684 (C=O). Elemental analysis calculated (%) for $C_{17}H_{17}FN_4O$: C 65.37, H 5.49, N 17.94. Found: C 65.45, H 5.54, N 17.89.

*4.1.32. 5-(3,6-Diazabicyclo[3.1.1]heptane-3-yl)-N-(4-fluorophenyl)nicotinamide (**43**)*

Title compound was synthesized starting from **27**. White solid; yield 87% (87 mg); mp 250-252 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.29 (s, 1H), 7.86 (bs, 1H, NH exch. with D_2O), 7.58-7.70 (m, 2H), 7.50-7.56 (m, 1H), 7.09 (d, 2H, J = 8.4 Hz), 3.90-4.00 (m, 2H), 3.62-3.79 (m, 4H), 2.70-2.90 (m, 1H), 1.50-1.80 (m, 2H, NH exch. with D_2O). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 164.68 (C=O), 144.50 (C), 136.18 (C), 135.12 (2 x C), 135.08 (CH), 130.33 (CH), 122.46 (CH), 122.44 (CH), 115.55 (CH), 115.15 (2 x CH), 54.97 (2 x CH_2), 50.22 (2 x CH), 30.64 (CH_2). IR (nujol) ν: 3505 (2 x NH), 1684 (C=O). Elemental analysis calculated (%) for $C_{17}H_{17}FN_4O$: C 65.37, H 5.49, N 17.94. Found: C 65.49, H 5.52, N 17.89.

*4.1.33. 5-(3,6-Diazabicyclo[3.1.1]heptane-3-yl)-N-(2,4-difluorophenyl)nicotinamide (**44**)*

Title compound was synthesized starting from **28**. Beige solid; yield 86% (92 mg); mp 193-194 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.25-8.45 (m, 3H), 7.99 (bs, 1H, NH exch. with D_2O), 7.40-7.60 (m, 1H), 6.80-7.05 (m, 2H), 3.95 (d, 2H, J = 6.0 Hz), 3.60-3.80 (m, 4H), 2.70-2.90 (m, 1H), 1.64 (d, 1H, J = 9.0 Hz), 1.50 (bs, 1H, NH exch. with D_2O). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 164.62 (C=O), 160.12 (C), 157.67 (C), 154.19 (C), 151.73 (C), 144.90 (CH), 135.90 (CH), 134.85 (CH), 130.30 (CH), 123.29 (CH), 122.43 (C), 115.53 (CH), 55.72 (2 x CH_2), 51.24 (2 x CH), 31.35 (CH_2).
IR (nujol) ν: 3502 (2 x NH), 1662 (C=O). Elemental analysis calculated (%) for $C_{17}H_{16}F_2N_4O$: C 61.81, H 4.88, N 16.96. Found: C 61.95, H 4.91, N 16.90.

*4.1.34. 5-(3,6-Diazabicyclo[3.1.1]heptane-3-yl)-N-(4-iodophenyl)nicotinamide (**45**)*

Title compound was synthesized starting from **29**. Yellowish solid; yield 86% (117 mg); mp 238-240 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.40 (s, 1H), 8.27 (d, 1H, J = 3.0 Hz), 8.10 (bs, 1H, NH exch. with D_2O), 7.69 (d, 2H, J = 8.8 Hz), 7.46 (d, 2H, J = 8.8 Hz), 3.93 (d, 2H, J = 6.0 Hz), 3.50-3.70 (m, 4H), 2.70-2.90 (m, 1H), 1.55-1.80 (m, 2H, NH exch. with D_2O). ^{13}C-NMR (50 MHz, $CDCl_3$)

δ164.48 (C=O), 143.70 (C), 137.97 (C), 136.56 (2 x CH), 134.15 (C), 134.14 (CH), 121.82 (2 x CH), 117.44 (CH), 114.89 (CH), 86.41 (C), 54.72 (2 x CH_2), 50.23 (2 x CH), 32.28 (CH_2). IR (nujol) ν: 3507 (2 x NH), 1693 (C=O). Elemental analysis calculated (%) for $C_{17}H_{17}IN_4O$: C 48.59, H 4.08, N 13.33. Found: C 48.69, H 4.15, N 13.38.

*4.1.35. 5-(3,6-Diazabicyclo[3.1.1]heptane-3-yl)-N-(1,2,3,4-tetrahydroisoquinoline)nicotinamide (**46**)*

Title compound was synthesized starting from **30**. Yellowish solid; yield 73% (79 mg); mp 157-160 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ8.22 (d, 1H, J = 3.2 Hz), 8.10 (s, 1H), 7.10-7.45 (m, 4H), 7.07 (s, 1H), 4.91 (s, 1H), 4.60-4.75 (m, 1H), 3.85-4.15 (m, 3H), 3.45-3.80 (m, 5H), 2.70-3.10 (m, 3H), 1.40-1.70 (bs, 2H, NH exch. with D_2O). ^{13}C-NMR (50 MHz, $CDCl_3$) δ168.79 (C=O), 143.91 (CH), 143.68 (CH), 134.49 (C), 133.92 (CH), 132.61 (CH), 132.47 (CH), 132.07 (C), 127.06 (C), 126.73 (2 x CH), 115.41 (C), 58.16 (2 x CH_2), 57.54 (2 x CH), 48.01 (CH_2), 45.43 (CH_2), 44.82 (CH_2), 29.68 (CH_2). IR (nujol) ν: 3421 (NH), 1719 (C=O). Elemental analysis calculated (%) for $C_{20}H_{22}N_4O$: C 71.83, H 6.63, N 16.75. Found: C 71.92, H 6.70, N 16.80.

*4.1.36. 5-(3,6-Diazabicyclo[3.1.1]heptane-3-yl)-N-(1,2,3,4-tetrahydroquinoline)nicotinamide (**47**)*

Title compound was synthesized starting from **31**. White solid; yield 81% (87 mg); mp 57-58 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ8.08 (s, 1H), 7.79 (s, 1H), 6.87-7.20 (m, 4H), 6.70-6.80 (m, 1H), 3.85-3.96 (m, 4H), 3.45-3.55 (m, 4H), 2.86 (d, 2H, J = 6.4 Hz), 2.74-2.82 (m, 1H), 2.07 (qu, 2H, J = 6.4 Hz), 1.58-1.78 (bs, 1H, NH exch. with D_2O), 1.61 (d, 1H, J = 8.8 Hz). ^{13}C-NMR (50 MHz, $CDCl_3$) δ168.50 (C=O), 146.61 (CH), 145.68 (CH), 137.49 (C), 134.02 (CH), 133.69 (CH), 133.47 (CH), 131.87 (C), 127.06 (C), 125.73 (2 x CH), 115.41 (C), 57.16 (2 x CH_2), 55.57 (2 x CH), 49.15 (CH_2), 46.43 (CH_2), 44.86 (CH_2), 24.68 (CH_2). IR (nujol) ν: 3421 (NH), 1719 (C=O). Elemental analysis calculated (%) for $C_{20}H_{22}N_4O$: C 71.83, H 6.63, N 16.75. Found: C 71.90, H 6.71, N 16.83.

4.2. Binding studies

The affinities (K_i) of the synthetized compounds for the $\alpha_4\beta_2$, $\alpha\beta$ and α_7 receptors were determined as previously described [1] using [^{3}H]-Epibatidine labeled rat cerebral cortex membranes ($\alpha_4\beta_2$), or membranes of HEK 243 cells transiently trasfected with human $\alpha\beta$ nAChR, [^{125}I] α-Bungarotoxin labelled rat hippocampus membranes (α_7).

The *K*i (% of coefficient of variation) values shown were calculated by using the LIGAND program to fit the data obtained from at least three independent saturation and competition binding experiments for each compound using the $\alpha_4\beta_2$, $\alpha_3\beta_4$, and α_7 subtypes.

4.3. Functional activity.

The electrophysiological measurements were made as previously described [8].

4.4. Molecular docking.

The docking simulations were performed with GOLD version 5.2 (Cambridge Crystallographic Data Centre, Cambridge, UK). This program uses a genetic algorithm to calculate up to ten docking poses per input-ligand. GoldScore Fitness was selected as a scoring function, which takes hydrogen bonding, ligand internal strains, and steric aspects of the receptor-ligand complex into account.

Docking simulations were conducted on nAChR $\alpha_4\beta_2$ and α_7 subtypes. For the α_7 subtype a crystal structure co-crystallized with epibatidine was available (PDB entry 3SQ6) [9] and used for docking. A redocking was conducted to optimize the docking parameters and led to an RMSD of 1.18 with the final settings. In the course of the protein preparation, hydrogens were added. The binding site was defined in a 8 Å radius around the co-crystallized ligand of chain A.

For the α_7 subtype a crystal structure co-crystallized with epibatidine was available (PDB entry 3SQ6) and used for docking. A redocking was conducted to optimize the docking parameters and led to an RMSD of 1.18 with the final settings. In the course of the protein preparation, hydrogens were added. The binding site was defined in a 8 Å radius around the co-crystallized ligand of chain A.

For the $\alpha_4\beta_2$ subtype a crystal structure co-crystallized with nicotine was used (PDB entry 5KXI) [10], since the substrate is similar in size to epibatidine. A redocking of nicotine resulted in an RMSD of 1.22 with the final settings. In the course of the protein preparation, hydrogens were added. The binding site was also defined in a 8 Å radius around the co-crystallized ligand of chain A to keep the settings similar to the settings used in the docking on the α_7 subtype.

Appendix A. Supplementary data

^{1}H-NMR and ^{13}C-NMR spectra of of representative compounds **35**, **39**, and **43** are available.

Conflict of interest

None of the authors have conflict of interest to declare

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