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Original article

Synthesis and binding studies of epibatidine analogues as ligands for the nicotinic acetylcholine receptors

Linjing Mu, Konstantin Drandarov, William H. Bisson, Anita Schibig, Christa Wirz, P. August Schubiger, Gerrit Westera *

Center for Radiopharmaceutical Sciences, Swiss Federal Institute of Technology Zurich, Paul Scherrer Institute, Villigen and University Hospital of Zürich, Rämistrasse 100, CH-8091 Zürich, Switzerland

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Abstract

Neuronal nicotinic acetylcholine receptors (nAChRs) are transmembrane ligand-gated ion channels. Recent research demonstrated that selective nAChR ligands may have therapeutic potential in a number of CNS diseases and disorders. The alkaloid epibatidine is a highly potent non-opioid analgesic and nAChR agonist, but too toxic to be a useful ligand. To develop ligands selective for distinct nAChR subtypes and with reduced toxicity, a series of epibatidine and homoepibatidine analogues were synthesized. (±)-8-Methyl-3-(pyridin-3-yl)-8-azabicyclo[3,2,1]oct-2-ene, showed high affinity towards α4β2 (K_i = 2 nM), subtype selectivity (α4β2/α7 affinity ratio > 100) and relatively low toxicity in mice and can be labeled with ^{11}C and ^{18}F as positron emission tomography (PET) tracers for imaging of nAChRs.

Keywords: Nicotinic acetylcholine receptors (nAChRs); Epibatidine analogues; Affinity

1. Introduction

The neuronal nicotinic acetylcholine receptors (nAChRs) are excitatory ligand-gated cation channels, localized in the central and peripheral nervous systems and neuromuscular junctions. They mediate cholinergic neurotransmission and in CNS are involved in cognitive processes like learning and memory [1,2]. Until now 17 different nAChR subunits (α1–α10, β1–β4, γ, δ, and ε) have been identified in vertebrate species, which can co-assemble to generate a wide variety of heterologous or homologous pentamers, thus enabling a variety of nAChR subtypes [3]. The major population in mammalian brain consists of α4β2 nAChRs. Since the neurodegenerative Alzheimer and Parkinson diseases, Tourette syndrome, anxiety, pain and depression might be connected with selective changes in the density of the population of α4β2 nicotine receptors in certain brain regions, this subtype appears an important target for developing of new ligands as potential therapeutics [1] or radiotracers [1,4–10] for in vivo diagnostic purposes.

The alkaloid epibatidine (**1**, Fig. 1), isolated from the skin of the Ecuadorian poison frog *Epipedobates tricolor* by Spande et al. [11] is a very specific agonist for the nAChRs [1,2,12]. It exhibits subnanomolar affinity towards the neuronal heteromeric α4β2 nAChRs. Unfortunately, the use of epibatidine as a therapeutic agent is precluded by its very high toxicity [1]. Nevertheless, the binding and agonistic potential of epibatidine (**1**) had tremendous stimulating impact on the nACh receptors research.

A number of ligands with high in vitro affinity for the α4β2 subtype have been radiolabeled, and tested for in vivo imaging of the brain nAChRs [4–10]. Remarkable high affinities to the nAChRs have been registered in simple azetidinyl-pyridyl and pyrrolidinyl-pyridyl ethers (A-85380 and A-84543, Fig. 1) [8]. However, in many cases additional, pharmacokinetic, toxicological or selectivity factors are preventing the clinical use of them. Therefore, the in vivo imaging requires development of new radioligands with high binding affinity and at the same time with high subtype selectivity, low non-specific binding,

* Corresponding author. Tel.: +41 44 255 3560; fax: +41 44 255 4428.
E-mail address: gerrit.westera@dmr.usz.ch (G. Westera).

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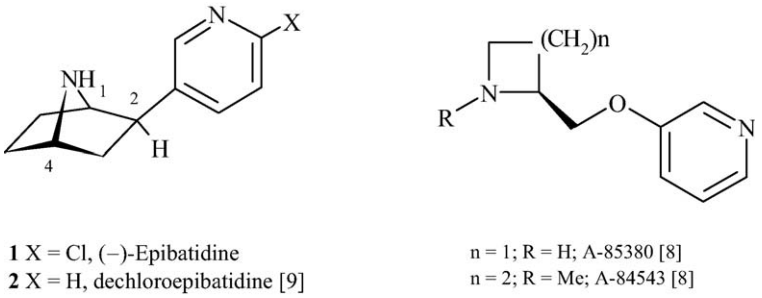


1 X = Cl, (−)-Epibatidine
2 X = H, dechloroepibatidine [9]

n = 1; R = H; A-85380 [8]
n = 2; R = Me; A-84543 [8]

Fig. 1. Structures of epibatidine, dechloroepibatidine, A-85380, and A-84543.

appropriate lipophilicity for brain penetration, appropriate metabolism and low toxicity.

In the present work the synthesis and the in vitro binding affinities for α4β2 and α7 nAChRs of a series of epibatidine and homoepibatidine analogues as potential lead compounds for the development of new brain nAChRs radioligands are reported. Structure–binding affinity relationships are discussed.

2. Chemistry

The compounds included in the present paper were designed with respect to the wide accepted key structural features of the Sheridan's "three point" model for the nAChR pharmacophore: (a) a basic aliphatic nitrogen atom positively charged; (b) an aromatic ring with a nitrogen lone pair electron as hydrogen bond acceptor with a definite distance from (a); and (c) a central hydrophobic area [1,2,13–15]. In order to examine derivatives with different distances between the sp^3 (a) and the sp^2 (b) hybridized N atoms, epibatidine analogues 7-aza-bicyclo [2.2.1]heptane derivatives (compounds **9** and **12**) and homoepibatidine analogues 8-methyl-8-aza-bicyclo[3.2.1]octane derivatives (compounds **14**–**19**) were synthesized. For compounds **15**–**19**, the azabicycle and the pyridine moieties are connected directly, while for compounds **9**, **12**, and **14** the two moieties are connected through an oxygen bridge. Compound **16** contains an electron withdrawing (fluoro) substituent and compound **15** contains a tertiary alcoholic group (a H-bond donor/acceptor) both situated in the spacer part of the molecule.

*2.1. Synthesis of 7-aza-bicyclo[2.2.1]heptyloxy pyridines **9** and **12***

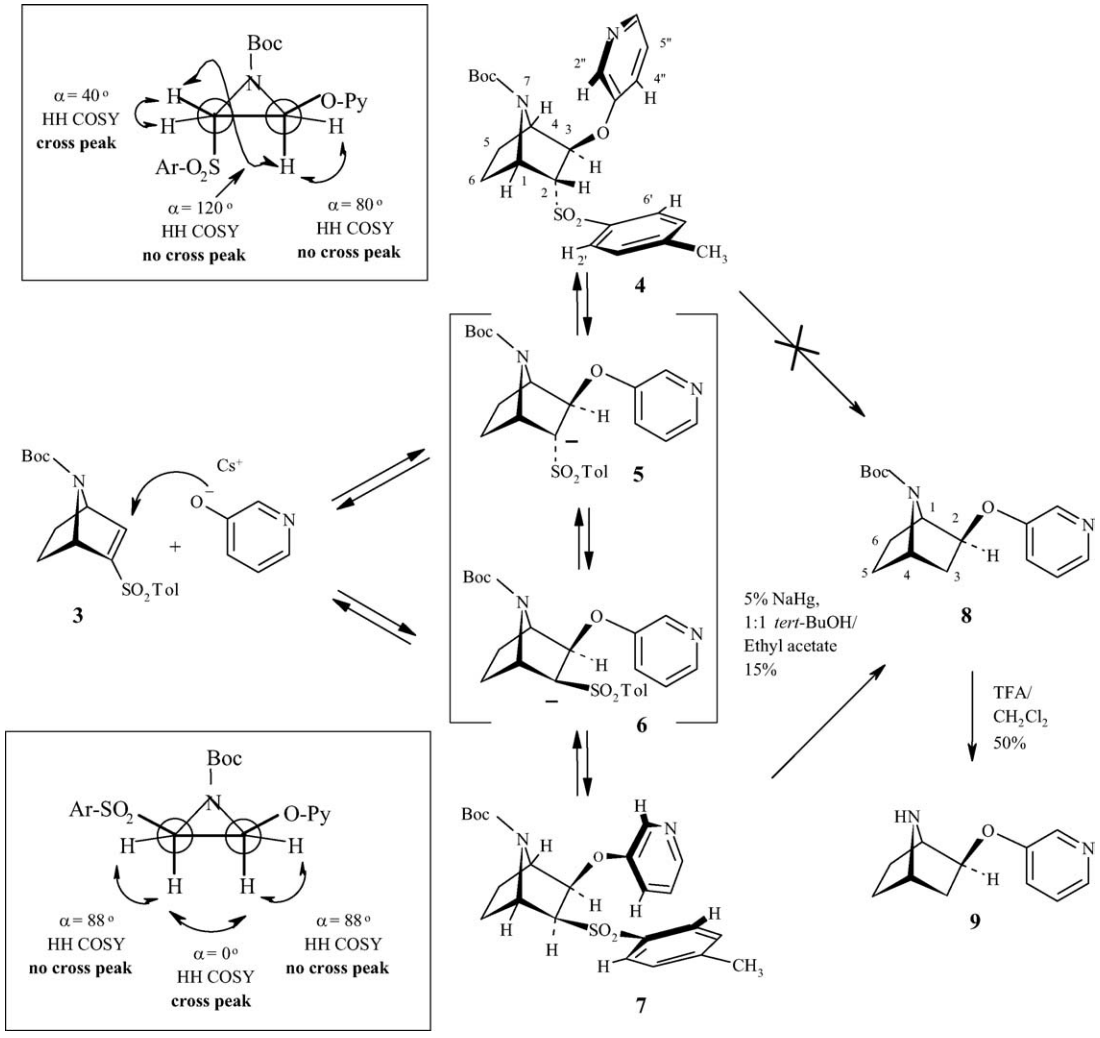
Compound (±)-**9** was synthesized according to Scheme 1. The Boc-protected and activated olefin (±)-**3** was prepared using described procedures [7]. Michael type addition of 3-hydroxypyridine to compound (±)-**3** in the presence of cesium carbonate as a base afforded a mixture of two diastereoisomeric adducts, which could be separated by column chromatography in 1:2 ratio of compound (±)-**4** to (±)-**7**. These two isomers behave differently in the next desulfonation reaction. Compound (±)-**4** was rapidly destroyed by β-elimination to the starting olefin **3** and 3-hydroxypyridine with sodium amalgam (Na/Hg) in the 1:1 mixture of ethyl acetate and *tert*-butyl alcohol [7], whereas compound (±)-**7** gave the desired desulfonated product (±)-**8** in 15% yield. Since the known alternative desulfonation methods such as electrolysis [18] or samarium (II) iodide reduction [19] were ineffective in this case, the approach shown here was the sole described method for the preparation of compound (±)-**8** although with a low yield. Finally, the removal of the Boc-protecting group of compound (±)-**8** with trifluoroacetic acid (TFA) leads to the target compound (±)-**9** in 50% yield.

The synthesis of compound (±)-**12** is illustrated in Scheme 2. Compound (±)-**11** was obtained in 75% yield via coupling of the *exo*-alcohol (±)-**10**, which was prepared according to published procedures [20–22], with 2-bromopyridine in the presence of NaH and tris(dibenzylideneacetone)dipalladium chloroform complex ($Pd_2(dba)_3$) with (S)-(−)-2,2′-bis(di-*p*-tolylphosphino-1,1′binaphthyl (S-Tol-BINAP) as the catalyst [23]. The desired (±)-**12** was obtained in 64% yield after removal of the *N*-Boc protecting group from (±)-**11** with TFA. Unfortunately, a similar approach starting from 3-bromopyridine failed to give the above discussed 3-pyridyloxy analogue (±)-**9**.

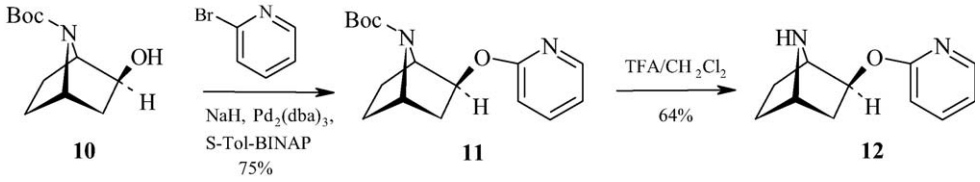
The stereochemistry of compounds (±)-**4** and (±)-**7**, illustrated in Scheme 1, was elucidated from their 1D and 2D ^1H NMR spectra. Their HH COSY cross correlations between the ^1H NMR signals of the vicinal protons C(1)H–C(2)H, C(2)H–C(3)H, and C(3)H–C(4)H were clear.

As the rate of spin–spin interaction ^3J depends on the dihedral angle between the interacting vicinal protons, the HH COSY cross-peaks observed only between C(1)H and C(2)H, but not between C(2)H and C(3)H neither between C(3)H and C(4)H evidently confirmed that the 2,3-*trans*-configuration of compound (±)-**4** with 2-*endo*-3-*exo*-attachment of the substituents. Only such an arrangement, with H–C(1)–C(2)–H_{exo} (dihedral angle of 40°, higher magnitude of ^3J), H_{exo}–C(2)–C(3)–H_{endo} (dihedral angle of about 120°, lower magnitude of ^3J), and H_{endo}–C(3)–C(4)–H (dihedral angle of about 80°, lower magnitude of ^3J) would correspond to the obtained HH COSY cross-correlation. Additionally, the ROESY spectrum of compound (±)-**4** shows a well pronounced NOE cross-peak between C(3)H_{endo} and C(5)H_{endo}, which supports the structure depicted in Scheme 1.

Similarly, the HH COSY spectrum of compound (±)-**7** shows spin–spin correlation between C(2)H and C(3)H but no correlation between C(1)H and C(2)H neither between C(3)H and C(4)H. This corresponds to *cis*-orientation of the substituents at C(2)–C(3) in compound (±)-**7** with 2-*exo*-4-tolylsulfonyl and 3-*exo*-3-pyridyloxy moieties as depicted in Scheme 1.



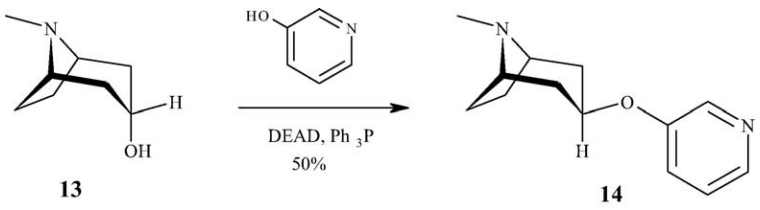
Scheme 1. Preparation of (±)-2-(pyridin-3-yl)oxy-7-aza-bicyclo[2.2.1]heptane (**9**). The Michael adducts **4** and **7** are shown in their most populated conformers according to the 2D ^{1}H NMR (NOESY) data. The Newman projections from the C(2)–C(3) faces of compounds **4** and **7** are given for better illustration of the obtained HH COSY cross-correlations (see the text).



Scheme 2. Synthesis of compound **12**.

This arrangement of the substituents at C(2)–C(3) is in agreement with the higher magnitude of ^{3}J H_{endo}–C(2)–C(3)–H_{endo} (dihedral angle of about 0°) and lower magnitudes of ^{3}J for H–C(1)–C(2)–H_{endo} and H_{endo}–C(3)–C(4)–H vicinal pairs of protons (both dihedral angles of about 88°). The ROESY experiment of compound (±)-**7** shows NOEs between C(2)H_{exo} and C(3)H and C(5)H_{endo} and (6)H_{endo}, which confirms the structure depicted in Scheme 1.

For both compounds (±)-**4** and (±)-**7**, the 3-pyridyloxy substitution is attached to the 7-azabicyclo[2.2.1]heptane nucleus in *exo*-orientation, which suggests that the 3-pyridinolate anion attacks the olefin **3** exclusively from its *exo*-side to form the *trans*-adduct/carbanion **5** or the *cis*-adduct/carbanion **6**, which after further protonation gives the 2-*endo*-3-*exo*-compound (±)-**4** and the 2-*exo*-3-*exo*-compound (±)-**7**, respectively. An isomerization of the *trans*-adduct/carbanion intermediate **5** to the thermodynamically more favored (all *exo*) *cis*-carbanion intermediate **6** is expectable. Such *endo* to *exo* isomerization of closely related compounds in the presence of base has been studied earlier [16]. Both compounds (±)-**4** and (±)-**7** display pronounced sensitivity towards strong bases and undergo β-elimination to the starting olefin **3** and 3-hydroxypyridine.



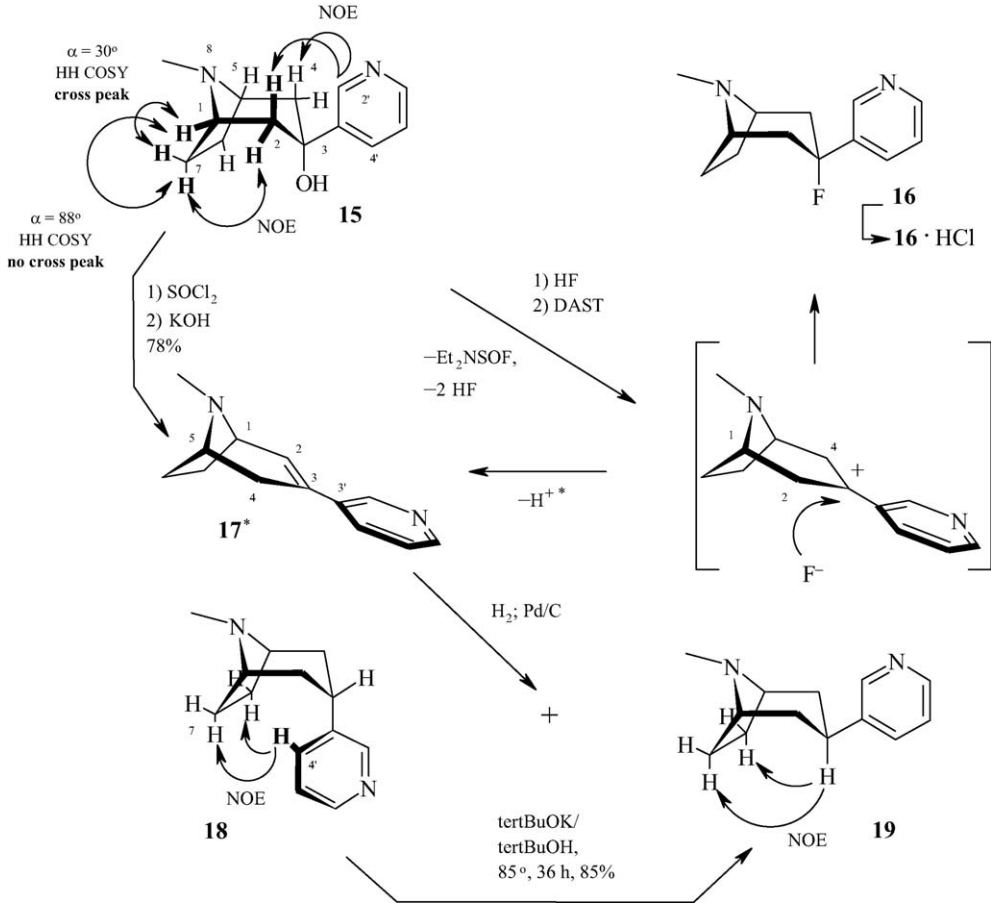
Scheme 3. Synthesis of compound **14**.

Compound (±)-**4** shows somewhat stronger sensitivity which is obviously due to the appropriate geometry of the H–C(2)–C(3)–O bonds (dihedral angle of about 0°) just ideal for a concerted *cis*-elimination (E2). This reaction has been studied earlier on similar (norbornyl) systems, which display reactivity analogous to those of compounds (±)-**4** and (±)-**7** [17]. Therefore, a mutual conversion between compounds (±)-**4** and (±)-**7** (via the intermediates **5**, **6**, and the starting compound **3** and 3-hydroxypyridine), leading to an equilibrium mixture of these compounds during the Michael addition reaction is reasonable (Scheme 1).

The HH-COSY spectra of both compounds (±)-**8** and (±)-**9** do not show spin–spin interaction between C(1)H and C(2)–H_{exo}, which again confirms the *exo*-attachment of the 3-pyridyloxy functionality in these compounds (Scheme 1).

2.2. The 8-methyl-8-aza-bicyclo[3.2.1]octane derivatives (homoepibatidine analogues) *14–19*

The 8-methyl-8-aza-bicyclo[3.2.1]octyloxy pyridine **14** was prepared (Scheme 3) from commercially available *endo*-tropine (**13**) and 3-hydroxypyridine *via* Mitsunobu reaction. Its *exo*-configuration was confirmed by 2D ^{1}H NMR (ROESY) where pronounced NOEs between the C(3)H and the C(6)H_{endo}, C(7)H_{endo} were observed.



Scheme 4. Synthesis of compounds **16**–**19**.

* The deprotonation of the intermediate carbocation (in brackets) can proceed with equal probability from both C(2) or C(4) yielding the both possible enanthiomers of the 2,3-unsaturated derivative **17**. The product of the deprotonation from C(4)H (1*R*)(5*S*)-enantiomer of **17** is shown. The free rotation of the pyridine ring around C(3)–C(3′), due to conjugation is partially restricted. It is unclear whether one of both possible rotamers with synperiplanar (shown) and antiperiplanar orientation of the pyridine N-lone pair towards the double bond is energetically more favored.

The tertiary *exo*-alcohol **15** was prepared similarly to published procedures [24,27] by addition of 3-lithiated-pyridine to the commercially available tropinone. Its *exo*-configuration was confirmed by HH COSY and ROESY experiments as shown in Scheme 4. The signal corresponding to C(1)H + C(5)H shows HH COSY cross peak with $C(6)H_{exo}$ + $C(7)H_{exo}$, but does not show cross peak with the $C(6)H_{endo}$ + $C(7)H_{endo}$. Further, a ROESY cross peak was observed between $C(6)H_{endo}$ + $C(7)H_{endo}$ and $C(2)H_{endo}$ + $C(4)H_{endo}$. Finally, the signals corresponding to $C(2)H_{exo}$ and $C(4)H_{exo}$ show ROESY cross interaction with the C(2′)H and C(4′)H suggesting a free rotation of the pyridine ring. The lack of cross-NOEs between the pyridine ring and $C(6)H_{endo}$ + $C(7)H_{endo}$ confirms the 3-*exo*-configuration of compound **15**.

The fluorinated derivative **16** (isolated as hydrochloride in 17% yield) was prepared together with the elimination product **17** (31%) by treatment of the dihydrofluoride of the *exo*-alcohol **15** with diethylaminosulfur trifluoride (DAST). The introduction of the free base of compound **15** in this reaction led to a lower yield with a mixture of by-products. Although during the fluorination reaction with DAST hydrogen fluoride is released, the use of preliminary prepared dihydrofluoride of compound **15** suppressed the rate of side reactions and improved the yield of **16**. The use of the hydrofluoride of compound **15** as starting material in this reaction was necessary also in order to avoid the introduction of alternative nucleophiles in the reaction mixture, which could compete with F^- to give the corresponding by-products. The NOESY experiment of compound **16**·HCl did not display any cross-NOE between the pyridine part and the C(6)H and C(7)H. Therefore, we should consider that the fluorination of the starting alcohol **15** with DAST proceeds by S_N1 mechanism with retention of the configuration via a resonance stabilized pyridinyl carbocation intermediate, which is further attacked by the fluoride anion from the *endo*-face to form the thermodynamically more stabile isomer **16** with *exo*-oriented bulky 3-pyridyl substituent at C(3) (Scheme 4). Alternatively, the deprotonation of the intermediate carbocation yields the olefin (±)-**17**.

Compound (±)-**17** was also prepared as a sole product by the consecutive chlorination of the alcohol **15** with thionyl chloride followed by dehydrohalogenation with aqueous potassium hydroxide [24,25,27].

The catalytic hydrogenation of olefin **17** gave a mixture of *endo*- and *exo*-compounds **18** and **19** in a 9:1 ratio. The *endo*-tropane derivative **18** was quantitatively epimerized to its thermodynamically more favored 3-*exo*-isomer **19** by heating with *tert*-BuONa in *tert*-butyl alcohol at 85 °C [20,26].

3. Pharmacology

3.1. In vitro binding studies

The inhibition constants (K_i) of the ligands towards the α4β2 and α7 neuronal nicotinic receptors were estimated using the inhibition competition binding assay method with [^{3}H]-cytisine [29] and [^{125}I]-α-bungarotoxin [28], respectively. The results are summarized in Table 1. Data for both enantiomers of epibatidine (**1**) and dechloroepibatidine [9] (**2**) are included for comparison.

The N–N distances of the lowest energy conformers of epibatidine (**1**) are frequently used for superimposition with the optimized conformers of new potential nAChRs ligands [30]. Although this distance is not the only important factor for the binding affinity, it is the easiest way to estimate if an energetically optimized structure complies with the main features of the nAChRs pharmacophore. Therefore, the N–N distances calculated for both possible terminal rotamers of the pyridine ring in epibatidine (**1**), dechloroepibatidine (**2**) and the calculated optimized structures of the studied compounds are listed in Table 1 as well.

3.2. Toxicity studies of compound (±)-17

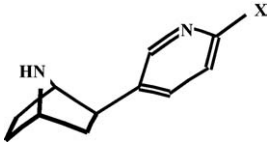
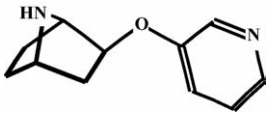
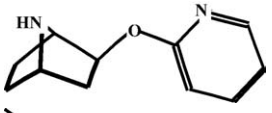
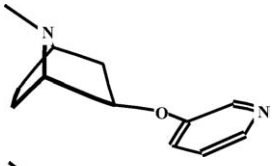
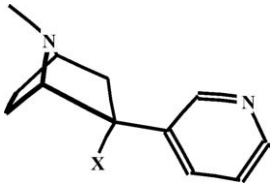
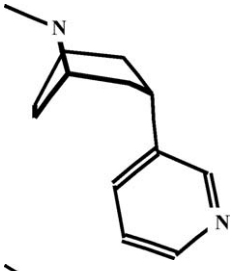
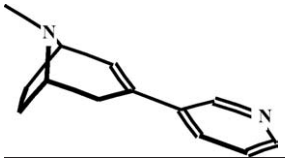
Mice were treated with an intravenous dose of 10 μg per mouse (20–30 g) and with the exception of one drug-independent mouse death, all animals survived. The median lethal dose (LD_{50}) of compound (±)-**17** after a single intravenous injection in mice of both sexes, observed over a period of 14 days, was thus established to be greater than 2.5 μmol/kg body weight. Thus, compound (±)-**17** shows more than 50 times lower toxicity compared to epibatidine (**1**). The intravenous dose of 10 μg per mouse was chosen by multiplicating the minimal dose needed for a human PET investigation by 10^4 times. Thus a dose with a wide range of safety should be obtained. This methodology avoids the unnecessary sacrifice of many mice and gives toxicology results for single applications, which are reliable and with a satisfactory safety profile.

4. Results and discussions

All compounds exhibit low affinity towards the α7, but higher for the α4β2 neuronal nicotinic receptors. Their affinities towards the α4β2 neuronal nicotinic receptor vary by orders of magnitude depending on the structure.

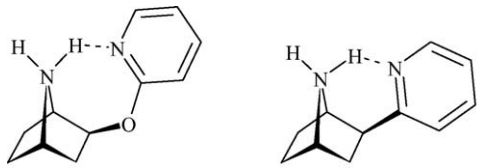
The insertion of an oxygen bridge between the 7-azanorbornyl moiety and the pyridine ring in compound **9** decreases dramatically the affinity of this compound for both α4β2 and α7 neuronal nicotinic receptors, compared to those of epibatidine (**1**) and dechloroepibatidine (**2**). All three pyridyl ethers (**9**, **12**, and **14**) possess high conformational flexibility and the N–N distances in different local conformers vary over a wide range. The lower energy conformers of these compounds with one of the largest possible N–N distances were arbitrarily chosen to be shown in Table 1. The much lower affinity of the pyridyl ether **9** (K_i = 17.4) suggests that this compound can not assume an energetically unfavorable conformation which fits better to the α4β2 ACh receptor site, as it has been assumed for the high affinity 3-pyridyl ethers (A-85380 and A-84543, Fig. 1) [8,31]. This inability for conformational adaptation to the receptor steric requirements is even more pronounced in the tropane derivative **14**, which displays much lower affinity (K_i = 955 nM).

Table 1
Binding affinities towards rat neuronal α4β2 and α7 nAChRs

Optimized structures[a]	Calculated N–N distances/Å[a] (after 180° rotation about the C-pyridyl bond)	Compounds	X	K_i ± S.E.[b] (nM)	
				[^{3}H]-cytisine	[^{125}I]-α-bungarotoxin
	4.8 (5.8)	(+)-**1**	Cl	0.053 ± 0.009	16 ± 3
		(−)-**1**	Cl	0.073 ± 0.01	17 ± 3
		(+)-**2**	H	0.064 ± 0.004[c]	
		(−)-**2**	H	0.015 ± 0.003[c]	
	6.4 (6.4)[d]	(±)-**9**		17.4 ± 3.2	1290 ± 47
	5.2 (5.2)[d]	(±)-**12**		620 ± 272	2460 ± 369
	7.2 (6.8)[d]	**14**		955 ± 86	17800 ± 2910
	6.0 (6.7)	**15**	OH	> 20000	> 20000
		16	F	38.8 ± 6.4	486 ± 184
		19	H	14.5 ± 2.8	
	6.6 (5.8)[e]	**18**		1100 ± 500	
	6.4 (6.4)	(±)-**17**		2.07 ± 0.61	327 ± 144

[a] The structures of the studied compounds were optimized using SYBYL 6.8 provided by Tripos Inc. (St. Louis, USA). The Chem3D Pro replicas of the minimized structures are included here for guidance only. In the cases of chiral compounds only one of the possible enantiomers is shown.
[b] See Section 6. Data are presented as mean ± standard deviation.
[c] IC_{50}.
[d] The pyridyl ethers **9**, **12,** and **14** possess high conformational flexibility. The lower energy conformers shown were arbitrarily chosen to be included in Table.
[e] The 2D ^{1}H NMR spectra (ROESY) (NOE between the C(4') and the C(5)H_{endo} and C(6)H_{endo}) show that in $CDCl_3$ solution compound **18** exists mainly in the conformation presented here.

The 36-fold lower affinity of the 2-(pyridin-2-yl)oxy derivative **12** (K_i = 620) than that of its (2-(pyridin-3-yl)oxy) isomer **9** (K_i = 17.4) lies in the entirely different orientation of its pyridine N-lone electron pair. A similar effect of strong reduction (100-fold) of the affinity when the pyridine ring is attached to the 7-azanorbornane at C(2′) (instead at C(3′) as in epibatidine, **1**) to form *iso*-dechloroepibatidine (2PABH, Fig. 2) has been noted earlier [9]. In both compounds **12** and 2PABH [9] a



12 *iso*-dechloroepibatidine (**2PABH**) [9]

Fig. 2. Structures of compound **12** and **2PABH**.

strong intramolecular hydrogen bond between the protonated (at physiological pH) N-atom of the azabicycle and the pyridine N-atom, may be expected due to the appropriate molecular geometry. Such H-bonding presumably would compete with the specific receptor/ligand H-bond interactions in the receptor site leading to low affinity.

Compared to compound **9**, the azabicyclo and heteroaromatic moieties of the tropane-derived isostereomers **15**–**19** are connected directly. The fact that the affinity of compound **19** ($K_i = 14.5$) is commensurate with that of compound **9** ($K_i = 17.4$) indicates that the presence of the ether bridge in compound **9** does not contribute significantly to its binding via an electronic effect as additional H-bond acceptor.

The introduction of an electron withdrawing fluoro-substituent at position 3 of compound **19** ($K_i = 14.5$), yielding its 3-fluoro isostereomer **16** ($K_i = 38.8$), has no remarkable influence on the binding affinity, whereas the introduction of H-bonding donor/acceptor OH-functionality at the same position results in a complete lack of affinity of the isosteric 3-OH-derivative **15**. Presumably, this is due to the tertiary alcohol group, which leads to higher polarity of this compound, compared to its isostereomers **16** and **19**.

The 3-*endo*-compound **18** ($K_i = 1100$) shows 76-fold less affinity than that of its 3-*exo*-epimer **19**. This is similar to *endo*-epibatidine, which possesses 130 times less affinity than the natural *exo*-isomer [32]. The 2D ^{1}H NMR spectra (ROESY) show that in solution ($CHCl_3$ an H-donor) compound **18** exists mainly in chair conformation of the piperidine part of the azabicycle with the lone electron pair of the pyridine N-atom out of the azabicycle (NOE between the C(4′) and the C(5)H_{endo} and C(6)H_{endo}) as presented in Table 1.

From the group of ligands studied, compound (±)-**17** displays the highest affinity (K_i ~ 2 nM) towards the α4β2 nAChRs. This is the conformationally most rigid member of the series studied. Compared to epibatidine (**1**), which in fact is also a quite rigid molecule, the presence of a double bond conjugated with the pyridine nucleus in compound (±)-**17** restricts even the free rotation of the pyridine ring. On the other hand, the internitrogen distance of compound (±)-**17** (N–N 6.4 Å) is much longer than that of epibatidine. These facts strongly suggest that an alternative accommodation mode of compound (±)-**17** exists in the ACh receptor active pocket than that of epibatidine (**1**), but probably sharing a common onium site in the receptor. The presence of a basic sp^3 hybridized N-atom and the conjugated π-system of compound (±)-**17**, which forms a flat, lipophillic, π-basic area seems to fulfill exactly the requirements of the Barlow "point plus flat area" model for the AChR chromophore [13,31,32]. If so, the enantiomers of compound **17** (footnote in Scheme 4) may not show significantly different binding affinities toward nAChRs. Although the ACh agonistic activity of (±)-**17** and similar compounds has been noticed [24,25,27,33], their molecular features as selective α4β2 nAChRs ligands have not been recognized and they have not been studied for nAChR-imaging.

The significant subtype selectivity (α4β2/α7 affinity ratio > 100) and relatively high affinity (K_i ~ 2 nM) of (±)-**17** motivated its further toxicological studies. The LD_{50} > 2.5 μmol/kg body weight (see Section 6) shows that compound (±)-**17** is more than 50 times less toxic than epibatidine (**1**) and confirms its potential, when labeled with ^{11}C or ^{18}F, for clinical use as a positron emission tomography (PET) tracer for nAChR imaging.

Recently the crystal structure of the molluscan ACh-binding protein was resolved [34] and the binding of nicotine and carbamoylcholine to the ACh-binding protein was described in [35]. As it was proposed before [36,37] a cation–π interaction between the protonated sp^3 N-atom of the ligand and a tryptophan residue from the active site of the receptor seems to be involved. The results of our molecular docking experiments of a number of nAChRs ligands (including compound (±)-**17**) to modeled binding sites of α4β2 and α3β4 can be found elsewhere [38].

5. Conclusion

In order to find new ligands for α4β2 subtypes of nAChRs with high affinity and, compared to the lead compounds, better subtype selectivity and lower toxicity, some epibatidine and homoepibatidine analogues have been synthesized and evaluated by binding studies. The compounds **9**, **12**, **14**–**19**[1] show reasonably high affinity towards α4β2 nAChRs. From this series of compounds (±)-**17** displays the highest affinity for the α4β2 nAChRs subtype (K_i ~ 2 nM) and relatively low toxicity in mice. Thus, the N(8)–$^{11}CH_3$-analogue of compound (±)-**17** is expected to be useful as a PET tracer for imaging the brain nAChRs.

6. Experimental

6.1. Chemistry

All commercially available materials were used without further purification. Solvents for the measurement of spectra were spectrograde. Preparative separations were performed with flash column chromatography (Merck silica gel 60, particle size 40–60 μm) and analytical TLC was performed on precoated plates (Merck silica gel 60 F_{254}); detection by UV absorption or exposure to I_2 vapors. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-300 (300 MHz for ^{1}H and 75.6 MHz for ^{13}C) spectrometer. Chloroform-*d* was used as the solvent unless otherwise stated; Me_4Si (δ 0.00) was used as an internal standard. All NMR chemical shifts are reported as δ values and coupling constants (J) are given in hertz (Hz). The splitting pattern abbreviations are as follows; singlet (s); doublet (d); triplet (t); quartet (q); multiplet (m); and broad (br). The electrospray ionization (ESI) mass spectra were carried out using a Finnigan Mat Instrument TSQ 7000. Four-

[1] Interestingly, the structures of compounds **9**, **14**, and **19** have been discussed theoretically as probable ACh agonists, prior to their synthesis and biological evaluation presented here [39].

ier transform mass spectrometry (FTMS) on IonSpec HiResESI instrument.

*6.1.1. tert-Butyl (±)-2-endo-3-exo-2-(4-methylphenylsulfonyl)-3-(pyridin-3-yl)oxy-7-azabicyclo[2.2.1] heptane-7-carboxylate (**4**) and tert-butyl (±)-2-exo-3-exo-2-(4-methylphenylsulfonyl)-3-(pyridin-3-yl)oxy-7-azabicyclo[2.2.1] heptane-7-carboxylate (7)*

A mixture of **3** (560 mg, 1.6 mmol) 3-hydroxypyridine (300 mg, 3.2 mmol) and Cs_2CO_3 (1 g, 3.2 mmol) in 6 ml acetonitrile was stirred at 50 °C for 5 days. Water was added and the mixture was extracted with chloroform. The organic extract was washed with 20% aqueous K_2CO_3 solution and evaporated. The residual yellow oil was purified by flash chromatography (silica gel, consecutive elution with hexane/ethyl acetate 7:3 then 1:1) to afford (±)-**4** (130 mg, 18%) and (±)-**7** (270 mg, 38%) both as colorless glasslike solids (overall yield 56%).

Data for compound (±)-**4**: TLC: hexane/THF (8:5) $R_f = 0.6$. ^{1}H NMR: δ 8.25 (d, $J = 4$ Hz, 1H, C(6″)H), 8.10 (br. s, 1H, C(2″)H), 7.73 (dd, $J = 6.58$, 1.78 Hz, 2H, C(2′)H + C(6′)H), 7.28 (d, $J = 7.7$ Hz, 2H, C(3′)H + C(5′)H), 7.18 (m, 1H, C(5″)H, 7.07 (m, 1H, C(4″)H, 4.74 (br. 1H, C(3)H), 4.58 (t, $J = 4.28$ Hz, 1H, C(1)H), 4.40 (d, $J = 5.56$ Hz, 1H, C(4)H). 3.75 (br. s, 1H, C(2)H), 2.55–2.25 (m, 4H, C(6)H_{endo} + NCH_3), 2.05–1.85 (m, 1H, C(6)H_{exo}), 1.85–1.70 (m, 1H, C(5)H_{exo}), 1.70–1.55 (m, 1H, C(5)H_{endo}), 1.40–1.10 (br. s, 9H, Boc). High resolution ESI-MS: calculated for $[M + H]^+$ $C_{23}H_{29}N_2O_5S$: 445, 1792, found: 445.1786.

Data for compound (±)-**7**: TLC: hexane/THF (8:5) R_f 0.5. ^{1}H NMR ($CDCl_3$): δ 8.26 (d, $J = 4$ Hz, 1H, C(6″)H), 8.19 (br. s, 1H, C(2″)H), 7.75 (m, $J = 7.6$, 2H, C(2′)H + C(6′)H), 7.40–7.15 (m, 4H, C(3′), C(5′)H, C(4″)H, C(5″)H), 4.91 (br. s, 1H, C(3)H), 4.61 (d, $J = 4.8$ Hz, 1H, C(1)H), 4.52 (br, 1H, C(4)H), 3.19 (d, $J = 4$ Hz, 1H, C(2)H), 2.39 (s, 3H, NCH_3), 2.1–1.8 (m, 2H, C(5)H_{exo} + C(6)H_{exo}), 1.78–1.50 (m, 2H, C(5)H_{endo} + C(6)H_{endo}), 1.45 (s, 9H, Boc). High resolution ESI-MS: calculated for $[M + H]^+$ $C_{23}H_{29}N_2O_5S$: 445, 1792, found: 445.1787.

*6.1.2. tert-Butyl (±)-exo-2-(pyridin-3-yl)oxy-7-azabicyclo [2.2.1]heptane-7-carboxylate (**8**)*

To a stirred mixture of Na_2HPO_4 (255 mg, 1.8 mmol) and NaH_2PO_4 (216 mg, 1.8 mmol) in 5 ml of EtOAc and *tert*-butyl alcohol (1:1), 5% Na/Hg (3.3 g, 7.2 mmol) was added at 0 °C under argon, (±)-**7** (160 mg, 0.36 mmol) in 2 ml EtOAc and *tert*-butyl alcohol (1:1) was added dropwise to the reaction mixture. After 1 h stirring at 0 °C and 3 h at room temperature, the solid was removed by filtration and washed with CH_2Cl_2 and EtOAc. The organic solvents were removed under vacuum. The residue was partitioned between H_2O and CH_2Cl_2. The aqueous phase was extracted twice with CH_2Cl_2, the organic extract was dried over Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography on silica gel (EtOAc/MeOH/hexane, 3:1:6) to give 16 mg of **8** as colorless oil in 15% yield. TLC: hexane/EtOAc (7:3) R_f 0.2. ^{1}H NMR: δ 8.27 (br. s, 1H, C(2′)H), 8.23 (m, 1H, C(6′)H), 7.40–7.20 (m, 2H, C(4′)H + C(5′)H), 4.70 (m, C(2)H), 4.45 (br. s, C(1)H), 4.24 (br. t, C(4)H), 2.45–2.30 (m, 1H), 2.30–2.10 (br. m, 1H), 1.90–1.20 (m, 13H). High resolution ESI-MS: calculated for $[M + H]^+$ $C_{16}H_{22}N_2O_3$: 291.1703, found: 291.1699.

*6.1.3. (±)-exo-2-(Pyridin-3-yl)oxy-7-azabicyclo[2.2.1]heptane (**9**)*

To a solution of compound (±)-**8** (15 mg) in CH_2Cl_2 (0.5 ml) was added TFA (0.5 ml). The resulting mixture was stirred at room temperature for 1 h then evaporated to dryness. The trace of remaining TFA was removed by repetitive addition and evaporation of CH_2Cl_2 and MeOH. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH/Et_3N, 10:1:0.1) to give compound (±)-**9** as yellowish oil (5 mg, 50%). TLC: $CHCl_3$/MeOH/25% aqueous ammonia (9:1:0.1) R_f 0.4. ^{1}H NMR: δ 8.24 (br. s, C(2′)H), 8.16 (br. s, C(6′)H), 7.22–708 (m, C(4′)H + C(5′)H), 4.61 (m, C(2)H), 3.86 (m, C1)H), 3.37 (m, C(4)H), 2.22–2.05 (m, 2H), 1.75–1.10 (m, 2H). High resolution ESI-MS: calculated for $[M + H]^+$ $C_{11}H_{14}N_2O$: 191.1179, found: 191.1176.

*6.1.4. tert-Butyl (±)-exo-2-(pyridin-2-yl)oxy-7-azabicyclo [2.2.1]heptane-7-carboxylate (**11**)*

To a solution of (±)-**10** (97 mg, 0.45 mmol) in xylene (9 ml), sodium hydride (95% NaH, 23 mg, 0.91 mmol) was added under argon. The mixture was stirred at 70 °C for 25 min. After cooling down to room temperature 2-bromopyridine (72 mg, 0.45 mmol), tris(dibenzylideneacetone)dipalladium chloroform complex ($Pd_2(dba)_3$, 15 mg, 6% Pd), and (S)-(–)-2,2′-bis(di-*p*-tolylphosphino)-1,1′-binaphthyl (Tol-BINAP, 23 mg, 0.03 mmol) were added and the mixture was stirred at 90 °C for 20 h. After removal of the solvents under vacuum the residue was purified by column chromatography (silica gel, hexane/EtOAc, 4:1) to give the title compound (±)-**11** (100 mg, 75%) as colorless oil. TLC: hexane/EtOAc (7:3) R_f 0.6. ^{1}H NMR: δ 8.12 (d, $J = 3.6$ Hz, 1H), 7.54 (t, $J = 7.2$ Hz, 1H), 6.84 (t, $J = 5.9$ Hz, 1H), 6.69 (d, $J = 8.3$ Hz, 1H), 4.98 (dd, $J = 6.7$, 2.6 Hz, 1H), 4.41 (d, $J = 4.9$ Hz, 1H), 4.31 (br. s, 1H), 2.1–1.6 (m, 4H), 1.5–1.2 (m, 11H). ESI-MS: 313 $[M + Na]^+$.

*6.1.5. (±)-exo-2-(Pyridin-2-yl)oxy-7-azabicyclo[2.2.1]heptane (**12**)*

To a solution of the compound (±)-**11** (95 mg, 0.32 mmol) in CH_2Cl_2 (2.5 ml) was added TFA (1 ml). The reaction mixture was stirred at room temperature for 1 h. After evaporation to dryness at reduced pressure the trace of remaining TFA was removed by repetitive addition and evaporation of CH_2Cl_2 and MeOH. The residue was purified by column chromatography (silica gel, EtOAc/hexane/Et_3N, 1:1:0.02) to yield compound (±)-**12** as colorless oil (40 mg, 64%). TLC: $CHCl_3$/MeOH/25 % aqueous ammonia (9:1:0.1) R_f 0.56. ^{1}H NMR: δ 8.13 (d, $J = 3.8$ Hz, 1H), 7.55 (t, $J = 8.2$ Hz, 1H), 6.84 (t, $J = 5.8$ Hz, 1H), 6.69 (d, $J = 8.3$ Hz, 1H), 4.99 (m, 1H), 3.71 (m, 2H), 2.00 (m, 1H), 1.7–1.5 (m, 3H), 1.45–1.20 (m, 3H). High resolution

ESI-MS: calculated for $[M + H]^+$ $C_{11}H_{14}N_2O$: 191.1179, found: 191.1176.

6.1.6. exo-8-Methyl-3-(pyridin-3-yl)oxy-8-azabicyclo [3,2,1] octane (**14**)

Tropine hydrate (**13**, 0.7 g, 4.96 mmol) was dissolved in 15 ml of dry THF, then triphenylphosphine (1.56 g, 5.95 mmol) and 3-hydroxypyridine (0.57 g, 5.95 mmol) were added. The solution was cooled down to 0 °C and diethylazodicarboxylate (DEAD, 1.04 g in 5 ml dry THF, 5.95 mmol) was added dropwise. After 10 min, the ice bath was removed and the reaction mixture was stirred at room temperature for 20 h. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2 and washed with 1 N HCl. The aqueous layer was alkalinized with aqueous Na_2CO_3 and extracted with CH_2Cl_2. The extract was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 9:1) to give compound **14** as white solid in 50% yield. M.p. 160 °C. TLC: $CHCl_3$/MeOH/25% aqueous ammonia (9:1:0.1) R_f 0.48. ^{1}H NMR: δ 8.27 (s, 1H, C(2′)H); 8.17 (m, 1H, C(6′)H), 7.17 (m, 2H, C(4′)H + C(5′)H), 4.51 (m, 1H, C(3)H), 3.26 (br. m, 2H C(1)H + C(5)H), 2.36 (s, 3H, NCH_3), 2.10 (m, 2H, $C(6)H_{exo}$ + $C(7)H_{exo}$), 1.99 (m, 2H, $C(2)H_{endo}$ + $C(4)H_{endo}$), 1.94 (m, 2H, $C(2)H_{exo}$ + $C(4)H_{exo}$), 1.62 (m, 2H, $C(6)H_{endo}$ + $C(7)H_{endo}$). High resolution ESI-MS: calculated for $[M + H]^+$ $C_{13}H_{19}N_2O$: 219.1492, found: 219.1489.

6.1.7. exo-8-Methyl-3-(pyridin-3-yl)-8-azabicyclo[3,2,1]octan-3-ol (**15**)

Compound **15** was prepared according to Ref. [24] in 64% yield as a white solid. TLC: THF/MeOH/Et_3N (9:1:0.3) R_f = 0.1; ^{1}H NMR: δ 8.77 (d, J = 2.60 Hz, 1H, C(2′)H), 8.45 (dd, J = 4.6, 1.6 Hz, 1H, C(6′)H), 7.80 (m, 1H, C(4′)H), 7.21 (m, 1H, C(5′)H), 3.26 (br. m, 2H, C(1)H + C(5)H), 2.41–2.32 (m, 5H, incl. $C(2)H_{exo}$ + $C(4)H_{exo}$ and NCH_3 at 2.35), 2.21 (m, 2H, $C(6)_{endo}$ + $C(7)H_{endo}$), 2.05 (m, 3H, $C(6)_{exo}$ + $C(7)H_{exo}$)), 1.90–1.75 (m, 2H, $C(2)H_{endo}$ + $C(4)H_{endo}$). ESI-MS: 219 $[M + H]^+$.

6.1.8. exo-3-Fluoro-8-methyl-3-(pyridin-3-yl)-8-azabicyclo [3,2,1]octane (**16**) and (±)-8-methyl-3-(pyridin-3-yl)-8-azabicyclo[3,2,1]oct-2-ene (**17**)

A solution of 500 mg (8.6 mmol) of KF in 5 ml of water was passed through a short column containing 6 g of Amberlite CG-120 (strong cation exchanger in H^+ form). The strongly acidic eluate, containing HF was added to 270 mg (1.2 mmol) of the free base of compound **15**. The water was removed by evaporation to dryness. The excess of HF in the residue was removed by repetitive addition and evaporation of ethanol. The trace of ethanol was then removed by repetitive addition and evaporation of toluene. Dry CH_2Cl_2 (25 ml) was added and the suspension was cooled to –70 °C. DAST (250 μl, 307 mg, 1.9 mmol) was added and the mixture was left to reach r.t. during approximately 1 h, then a new portion of 250 μl DAST was introduced and the reaction mixture was stirred additional 14 h at r.t. The orange liquid phase was decanted. The solid residue was washed once again with CH_2Cl_2 and washing solvent was added to the decanted liquor. Water was added to the solid residue. The suspension was filtered to give 50 mg unidentified polymeric material. The filtrate was alkalinized with $NaHCO_3$ and extracted with CH_2Cl_2. The organic extract was washed with water, acidified with few drops of ethanolic HCl and evaporated to dryness without heating. The residue was dissolved in minimal amount of mixture—ethyl acetate/methanol/5% aqueous solution of ammonium chloride (5:4:1) and introduced into a silica gel column, which was eluted first with ethyl acetate and then with ethyl acetate/methanol/5% aqueous solution of ammonium chloride (5:4:1). The eluate containing **16**·HCl was evaporated. The residual crystalline ammonium chloride was washed with chloroform. The **16**·HCl, which is soluble in chloroform, passes in solution whereas the ammonium chloride remains as precipitate, which was removed by filtration and the chloroform solution of the **16**·HCl was evaporated to give 55 mg (17%) of **16**·HCl as colorless glass like solid. From the washing CH_2Cl_2 after evaporation and flash chromatography ($CHCl_3$/MeOH/25% aqueous NH_3 9:1:0.1) compound **17** (78 mg, 31%) was isolated as yellowish oil.

Data for compound **16** (free base): TLC: THF/MeOH/Et_3N (9:1:0.3) R_f = 0.4; ^{1}H NMR ($CDCl_3$): 8.89 (s, 1H, C(2′)H), 8.6 (br. s, 1H, C(6′)H), 7.9 (m, 1H, C(4′)H), 7.28 (m, 1H, C(5′)H), 3.32 (br. s, 2H, C(1)H + C(5)H), 2.64–2.59 (m, 2H, $C(2)H_{exo}$ + $C(4)H_{exo}$), 2.49-2.45 (m, 2H, $C(2)H_{endo}$ + $C(4)H_{endo}$), 2.42 (s, 3H, NCH_3), 1.93–1.90 (m, 2H, $C(6)H_{exo}$ + $C(7)H_{exo}$), 1.30–1.28 (m, 2H, $C(6)H_{endo}$ + $C(7)H_{endo}$).

Data for compound **16**·HCl: (CD_3OD): δ 9.5 (br. s, 1H, C(2′)H), 8.72 (br. s, 1H, C(6′)H), 8.28 (br. d, J = 7.8 Hz, 1H, C(4′)H), 7.64 (br. m, 1H, C(5′)H), 4.10 (br. s, 2H, C(1)H + C(5)H), 3.3–2.95 (m, 2H, $C(2)H_{exo}$ + $C(4)H_{exo}$), 2.95–2.8 (m, 5H, $C(2)H_{endo}$ + $C(4)H_{endo}$ + NCH_3 at 2.86), 2.4–2.2 (br. m, 2H, C(6)H_{exo} + $C(7)H_{exo}$), 1.95–1.65 (m, 2H, $C(6)H_{endo}$ + $C(7)H_{endo}$). High resolution ESI-MS: calculated for $[M + H]^+$ $C_{13}H_2N_2F$: 221.1449, found: 221.1446.

Data for compound **17**: TLC: THF/MeOH/Et_3N (9:1:0.3) R_f = 0.3; ^{1}H NMR ($CDCl_3$): δ 8.59 (d, J = 1.6 Hz, 1H), 8.42 (dd, J = 4.7, 1.5 Hz, 1H), 7.60 (m, 1H), 7.19 (m, 1H), 6.29 (d, J = 5.5 Hz, 1H), 3.47 (m, 2H), 2.86 (dd, J = 17, 3.7 Hz, 1H), 2.8–1.5 (m, 5H). ESI-MS 201 $[M + H]^+$.

6.1.9. endo-8-Methyl-3-(pyridin-3-yl)-8-azabicyclo [3,2,1] octane (**18**) and exo-8-methyl-3-(pyridin-3-yl) -8-azabicyclo [3,2,1]octane (**19**)

Method A. A mixture of 184 mg (0.92 mmol) and 200 mg 10% Pd/C in 20 ml ethanol was stirred at r.t. in H_2 atmosphere for 22 h. After filtration the filtrate was concentrated under reduced pressure. The residual oil was purified by flash chromatography on silica gel (chloroform/methanol/25% aqueous ammonia 9:1:0.1) to afford **18** (67 mg, 36%) and **19** (6 mg, 3%) as colorless oils.

Method B. A mixture of 20 mg **18** (0.1 mmol), 1 g *tert*-butyl alcohol and 100 mg potassium tert-butylate was heated at 85 °C for 24 h. Water was added to the mixture. The solution

was saturated with K_2CO_3 and extracted three times with chloroform. The organic extract was washed with water, dried with Na_2SO_4 and evaporated to afford **19** (17 mg, 85%) as colorless oil. Data for compound **18**: TLC: chloroform/methanol/25% aqueous ammonia (9:1:0.1) R_f 0.38. ^{1}H NMR: δ 8.57 (s, C(2′)H), 8.42 (s, C(6′)H), 7.58 (d, 1H, $J = 8$ Hz, C(4′)H), 7.21–7.18 (m, 1H, C(5′)H), 3.25 (br. m, 2H, C(1)H + C(5)H), 3.09 (m, 1H, C(3)H), 2.55–2.40 (m, 2H, $C(2)H_{exo}$ + $C(4)H_{exo}$), 2.27 (s, 3H, NCH_3), 2.16–2.01 (m, 2H, $C(6)H_{exo}$ + $C(7)H_{exo}$), 1.66–1.58 (m, 2H, $C(2)H_{endo}$ + $C(4)H_{endo}$), 1.43 (m, 2H, $C(6)H_{endo}$ + $C(7)H_{endo}$). High resolution ESI-MS: calculated for $[M + H]^+$ $C_{13}H_{19}N_2$: 203.1543, found: 203.1539.

Data for compound **19**: TLC: chloroform/methanol/25% aqueous ammonia (9:1:0) R_f 0.33. ^{1}H NMR: δ 8.47 (br. s, 2H, C(2′)H and C(6′)H), 7.66 (d, 1H, $J = 7.7$ Hz, C(4′)H), 7.23 (br. s. 1H, C(5′)H), 3.37 (s, 2H, C(1)H + C(5)H), 2.90 (m, 1H, C(3)H), 2.42 (s, 3H, NCH_3), 2.19–2.13 (m, 2H, $C(6)H_{exo}$ + $C(7)H_{exo}$), 2.08 (m, 2H, $C(2)H_{exo}$ + $C(4)H_{exo}$), 1.80 (m, 2H, $C(6)H_{endo}$ + $C(7)H_{endo}$), 1.72–1.67 (m, 2H, $C(2)H_{endo}$ + $C(4)H_{endo}$). High resolution ESI-MS: calculated for $[M + H]^+$ $C_{13}H_{19}N_2$: 203.1543, found: 203.1540.

6.2. In vitro binding studies

6.2.1. Materials

(−)-[^{3}H]-cytisine (32.4 Ci/mmol) and [^{125}I]-α-bungarotoxin (146 Ci/mmol) were obtained from PerkinElmer Life Sciences (Boston, USA). (−)-Nicotine and α-bungarotoxin were obtained from Sigma-Aldrich (Steinheim, Germany) and all buffer chemicals from Fluka (Buchs, Switzerland). Frozen Sprague–Dawley rat brains were purchased from RCC Ltd. (Füllinsdorf, Switzerland) and Charles River Labs (Sulzfeld, Germany).

6.2.2. Competition binding assay

Binding assays of all the compounds synthesized for the α4β2 and α7 neuronal nicotinic receptor subtypes were performed at the final volume of 0.2 ml containing 100 μg of protein (membrane preparations of frozen Sprague–Dawley rat brains) in a BSS buffer at pH 7.4. The concentration of [^{3}H]-cytisine (72,000–59,600 dpm/pmol) was 2 nM. After 1 h at 0 °C, the samples were vacuum-filtered on glass fiber filters (Whatman GF/C) which were then washed with ice-cold buffer (2 × 4 ml). Non-specific binding was estimated in the presence of 0.1 mM (−)-nicotine. Packard Ultima Gold was the cocktail used for liquid scintillation counting with a Tri-Carb 2200CA analyzer. The concentration of [^{125}I]-alpha-bungarotoxin (213,839–102,091 dpm/pmol) was 0.5 nM. After 2 h at 37 °C, the samples were vacuum-filtered on glass fiber filters (Whatman GF/B), which were then washed with ice-cold buffer (3 × 4 ml). Non-specific binding was estimated in the presence of 0.01 mM alpha-bungarotoxin. The radioactivity was counted with a Packard Cobra II autogamma.

6.2.3. Data analysis

The binding results from the counter were analyzed using the Kell Programme from Biosoft using, respectively, $K_d = 1$ nM for (−)-[^{3}H] cytisine (α4β2 subtype) and $K_d = 0.7$ nM for [^{125}I]-α-Bungarotoxin (α7 subtype).

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