Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 2188–2193
Bioorganic & Medicinal Chemistry Letters

Preparation and affinity profile of novel nicotinic ligands

Yves Charton,[a] Claude Guillonneau,[a] Brian Lockhart,[b] Pierre Lestage[b] and Solo Goldstein[a,*]

[a]*SERVIER, Chemistry Research Division A, 11 rue des Moulineaux, 92150 Suresnes, France*
[b]*SERVIER, Division of Cerebral Pathology, 125 Chemin de Ronde, 78290 Croissy, France*

Received 17 October 2007; revised 20 December 2007; accepted 21 December 2007
Available online 26 January 2008

Abstract—Novel nicotinic ligands, characterized by the presence of an amino substituted cyclopropane ring connected to a pyridine nucleus, are described. Pharmacological investigation revealed that these compounds exhibit highest affinity for the rat α4β2 subtype of the nicotinic receptor with no affinity for the muscarinic receptor. No appreciable affinity for the muscular or for the ganglionic nicotinic receptor was observed at concentrations up to 10 μM. The increase in cortical ACh release as well as a positive effect on memory in a social recognition test in rat are exemplified.

Nicotine(*S*(−)nicotine) and nicotinic acetylcholine receptors (nAChR) have been known and studied for a long time, but it is only recently that renewed interest and research has focalized on their potential role in disease and therapy.[1]

Indeed, evidence coming from clinical investigations demonstrated that *S*(−)nicotine is beneficial for age-related memory impairments and in particular attention deficits.[2] However, until recently clinical use of nicotinic ligands has been thought to be severely limited by cardiovascular and neuromuscular side effects resulting from a non-selective activation of nAChR. Nevertheless, recent studies indicate that it is possible to develop selective nicotinic agents with marked effects on cognition but without some of the nicotine-related side effects.[2,3a]

Thus ispronicline (TC-1734) showed good affinities for the α4β2 nicotinic subtype and negligible affinities for other subtypes. Oral administration increases cortical ACh release (agonist effect) improving short and long time memory. Furthermore, encouraging clinical results have been reported recently.[3b]

Another example is the anabaseine analog GTS-21, a weak partial agonist at the α7 subtype[3c] and a more potent antagonist of receptors containing β2 and β4 subunits,[3d] possessing beneficial effects on neurodegenerescence and cognitive dysfunction.[3e]

Our own research effort was aimed at developing novel specific nicotinic ligands binding selectively to the α4β2 nAChR subtype.

Thus we have previously shown that ACh carbamate analogs (**I**) with reduced conformational flexibility induced by a cyclopropane ring are specific nicotinic ligand versus muscarinic receptors, binding selectively to the non-α7 subtype of nAChR. Investigation of related compounds possessing a pyridine ring as a nicotinic element (**II**) further demonstrated the strength of this approach for the design of new, powerful, and selective nicotinic ligands (Fig. 1).[4,5]

In this paper, we present synthetic details for compounds belonging to the general formula **II** as well as binding studies permitting the determination of the structural elements influencing potency and selectivity with respect to nicotinic receptors.

From the general formula **II** it is apparent that three structural elements can be modulated, namely the linker, the amino-head substitution, and the pyridine ring substitution.

We therefore set up a synthetic program aimed at the preparation of representative members of each class.

Keywords: Nicotinic; Subtypes; Ligands; Selectivity; α4β2; Substituted cyclopropanes; Pyridine ring.
* Corresponding author. Tel.: +33 1 55 72 25 99; fax: +33 1 55 72 24 30; e-mail: solo.goldstein@fr.netgrs.com

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.12.075

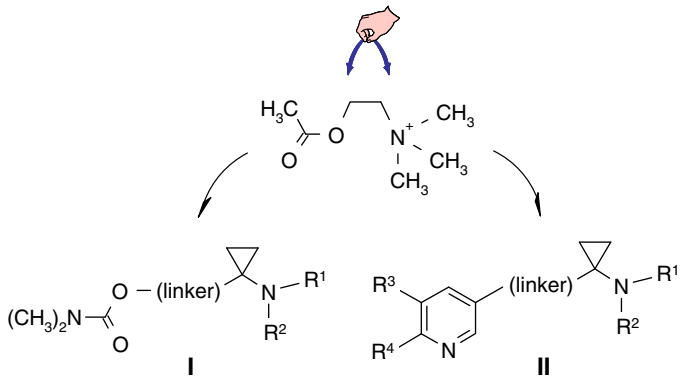


Figure 1. Nicotinic ligands with reduced conformational flexibility.

Most of the compounds have been obtained via a common alcohol intermediate, itself obtained from the commercially available diester of cyclopropane 1,1 dicarboxylic acid (Scheme 1).

Thus seven different linkers and 14 N-monomethylated analogs have been prepared from this key intermediate (Scheme 2). An Eischweiler–Clark reaction allowed the transformation of N-monomethylated compounds into N-dimethylated derivatives (Scheme 3) while N-unsubstituted analogs were obtained via an N-Boc carbamate already used for the synthesis of the common key alcohol (Scheme 4).

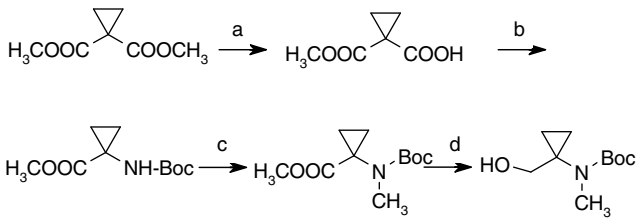
All compounds have been fully characterized by ^{1}H, ^{13}C NMR, and LCMS; final compounds possessed elemental analysis within accepted limits (±0.4%) from theory.[6]

Free bases were converted to their hydrochlorides or fumarate salts and purified by crystallization.

Binding experiments for α7, α4β2, and muscarinic receptors were carried out in a final volume of 250 μl in 96-well plates.[7–9]

The following tables illustrate the influence of each parameter mentioned above (linker, amino-head substitution, pyridine substitution) on the binding observed for the α4β2, α7, and muscarinic receptors.

Thus both heteroatoms containing and C-only linkers have been investigated and compared (Table 1).



Scheme 1. Synthesis of a key alcohol intermediate. Reagents and condition: (a) NaOH 1 N, CH_3OH (95%); (b) NEt_3, DPPA, toluene, *tert*-butanol (72%); (c) NaH, CH_3I, DMF (82%); (d) $LiBH_4$, THF (82%).

All synthesized compounds lack muscarinic activity; α4β2 nAChR specificity versus α7 nAChR is observed for all compounds with either type of linker.

The most powerful ligand in the series (K_i, 3 nM), is **1a** possessing an ether linker; carbon linkers (saturated or unsaturated) containing compounds are less powerful binders but are still selective for the α4β2 nAChR subtype.

The amino-head substitution has been examined (Table 2) on analogs of the most powerful ligand found, **1a**.

Specific binding for α4β2 nAChR subtype versus α7 nAChR and muscarinic receptors is observed.

The order of binding is $NHCH_3 > NH_2 > N(CH_3)_2$, compound **1a** still remaining the best in the series.

Increasing the steric hindrance on the amino-head decreases receptor binding (**1a** vs **15**, **1b** vs **16**).

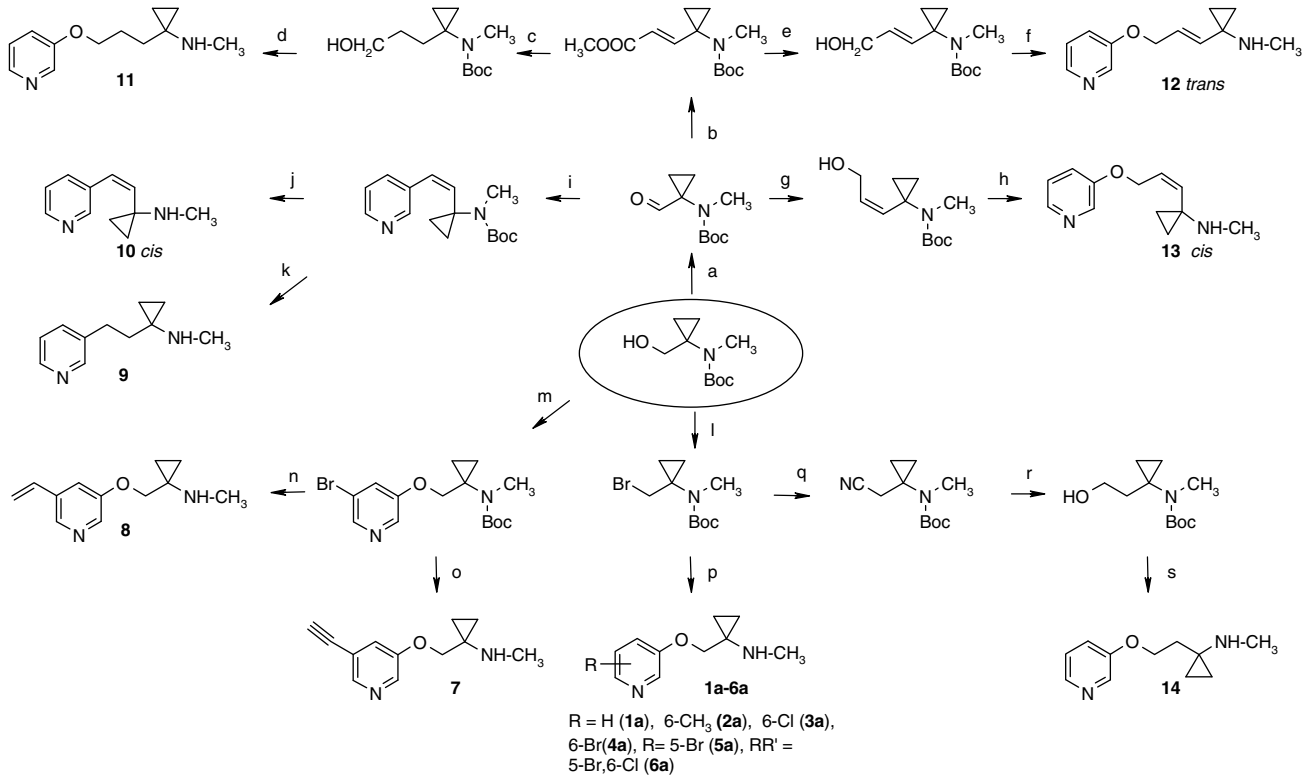
Next parameter studied was the pyridine ring substitution on positions 5 and 6 (monomethylated amino-head, Table 3).

For the first time a muscarinic affinity was observed for vinyl substituted pyridine **8**, the affinity for the α4β2 nAChR subtype being roughly 2-fold higher (no affinity for α7 subtype was observed).

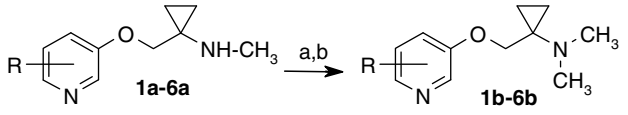
All other compounds are specific ligands for the α4β2 nAChR subtype. Substitution of the pyridine ring has a beneficial effect on binding leading to **5a** the most powerful compound obtained.

Although 5-bromo, 6-chloro di-substitution led to the very powerful and specific ligand **6a**, the order of magnitude was comparable with that obtained for mono-substituted, or non-substituted compounds **1a**, **7** (slightly less than **5a**).

Subtle effects on α4β2 binding depending on the nature of the substituents and their position was observed. Thus **5a** (Br in the 5 position) has the largest binding while a nearly 40-fold drop in binding is observed with

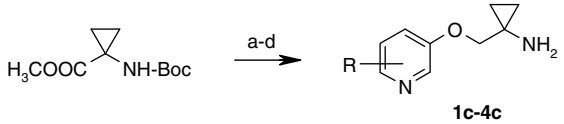


Scheme 2. Synthesis of N-monomethylated compounds. Reagents: (a) ClCOCOCl, DMSO, CH_2Cl_2 (87%); (b) $Ph_3P{=}CHCOOEt$, CH_2Cl_2 (*trans*/*cis* > 90/10) (95%); (c) H_2, Pd/C, EtOH (90%) then $LiBH_4$/THF (97%); (d) 3-hydroxypyridine, Ph_3P–PS resin, DIAD, THF (75%) then HCl/dioxan and fumaric acid, EtOH (60%); (e) dibal/hexane, CH_2Cl_2 (65%); (f) 3-hydroxypyridine, Ph_3P–PS resin, DIAD, THF (40%) then HCl/dioxan (50%); (g) $(PhO)_2POCH_2COOt$-Bu, Triton B, THF (75%) then dibal/hexane (45%); (h) 3-hydroxypyridine, Ph_3P–PS resin, DIAD, THF (45%) then HCl/dioxan (40%); (i) 3-(Ph_3P + CH_2)-pyridine, Cl^-, *t*-BuOK, DMSO (55%); (j) HCl/dioxan then fumaric acid, EtOH (50%); (k) H_2, Pd/C, MeOH (90%) then HCl/dioxan (95%); (l) CBr_4, PPh_3, ether (95%); (m) 3,5-diBr-pyridine, HNa, DMSO (81%); (n) $Pd(Ph_3P)_4$, $H_2C{=}CHSnBu_3$, toluene (80%) then HCl/dioxan (65%); (o) $Pd(PPh_3)_2Cl_2$, $(CH_3)_3SiCCH$, Et_3N, CuI (78%) then TBAF, THF and HCl/dioxan (80%); (p) 5 or 6-substituted 3-hydroxypyridine, Cs_2CO_3, butanone (70–90%) then HCl/EtOH (60–85%); (q) KCN, KI, DMSO (95%); (r) HCl/MeOH (73%) then Boc_2O, DMAP, CH_2Cl_2 (90%) and $LiBH_4$/THF (84%); (s) 3-hydroxypyridine, PH_3P, DIAD, THF (70%) then HCl/dioxan and fumaric acid, EtOH (80%).



R = H (**1a**,**1b**), 6-CH_3 (**2a**,**2b**), 6-Cl (**3a**,**3b**), 6-Br (**4a**,**4b**), R= 5-Br (**5a**,**5b**), RR' = 5-Br,6Cl (**6a**,**6b**)

Scheme 3. Synthesis of N-dimethylated compounds. Reagents: (a) HCHO, HCOOH (75–95%); (b) HCl, EtOH (73–96%).



R = H (**1c**), 6-CH_3 (**2c**), 6-Cl (**3c**), 6-Br(**4c**)

Scheme 4. Synthesis of N-unsubstituted compounds. Reagents: (a) $LiBH_4$, THF (95%); (b) CBr_4, PH_3P, ether (65%); (c) 6-substituted 3-hydroxypyridine, HNa, DMSO (45–65%); (d) HCl/dioxan (75–85%).

4a (Br in the 6 position); the same drop in binding is observed for the 6-methyl substituted pyridine **2a** while surprisingly a 6-chloro substitution induced a better binding.

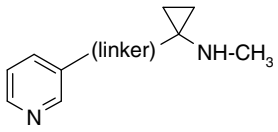
All these results are most probably due at least in part, to subtle steric and stereoelectronic effects influencing the interactions of the pyridine nitrogen and/or the pyridine ring as a whole with the α4β2 receptor site.

Table 4 below illustrates the results obtained when varying both the amino-head substitution (e.g., no substitution, methyl, dimethyl substitution) and the pyridine ring substitution (e.g., no substitution, methyl, chloro, bromo substitution).

Regardless of the substitution pattern on the pyridine ring, the binding ranking follows the order $NHCH_3 > NH_2 > N(CH_3)_2$, most potent compounds in the series being monomethylated **1a**, **5a** (5-bromo substitution on the pyridine ring), and **6a** (5-bromo, 6-chloro substitution on the pyridine ring).

The drop in affinity of $N(CH_3)_2$ versus $NHCH_3$ compounds can be anywhere between 10-fold and 300-fold (compare **5a**, **5b**); compared to $NHCH_3$ compounds non-substituted amino-head derivates

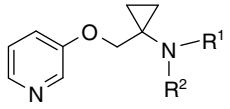
Table 1. Linker dependence of binding



Compound[a]	Linker	Nicotinic affinity		Muscarinic affinity (K_i, nM)
		α4β2 (K_i, nM)	α7 (K_i, nM)	
1a	O–CH_2	3	>10,000	>10,000
9	CH_2–CH_2	251	1910	>10,000
10	CH=CH	1160	3460	>10,000
11	O–CH_2–CH_2–CH_2	2460	>10,000	>10,000
12	O–CH_2–CH=CH*trans*	1100	>10,000	>10,000
13	O–CH_2–CH=CH*cis*	2280	>10,000	>10,000
14	O–CH_2–CH_2	109	>10,000	>10,000
ACh	—	39	3509	85
Nicotine	—	4	1500	>10,000

[a] Compounds **1**, **9**, **12**, and **13** were tested as HCl salts, compounds **10**, **11**, and **14** were tested as fumarates.

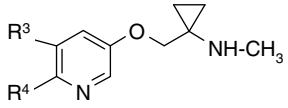
Table 2. Amino-head substitution dependence of binding



Compound[a]	N–R^1R^2	Nicotinic affinity		Muscarinic affinity
		α4β2 (K_i, nM)	α7 (K_i, nM)	
1a	$NHCH_3$	3	>10,000	>10,000
1b	$N(CH_3)_2$	95	>10,000	>10,000
1c	NH_2	34	>10,000	>10,000
15	C_2H_5–NH	1010	>10,000	>10,000
16	*N*-pyrrolidine	1610	>10,000	>10,000
ACh	—	39	3509	85
Nicotine	—	4	1500	>10,000

[a] Compounds **1**, **9**, **12**, and **13** were tested as HCl salts, compounds **10**, **11**, and **14** were tested as fumarates. Synthesis of compounds **15** and **16** is described in Ref. 5.

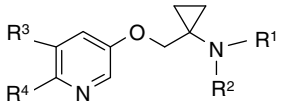
Table 3. Pyridine substitution dependence of binding



Compound[a]	Pyridine substitution	Nicotinic affinity		Muscarinic affinity (K_i, nM)
		α4β2 (K_i, nM)	α7 (K_i, nM)	
1a	—	3	>10,000	>10,000
2a	6-CH_3	40	>10,000	>10,000
3a	6-Cl	13	>10,000	>10,000
4a	6-Br	38.8	>10,000	>10,000
5a	5-Br	1.07	>10,000	>10,000
6a	5-Br,6-Cl	5.37	>10,000	>10,000
7	5-Ethynyl	6.56	>10,000	>10,000
8	5-Vinyl	70.1	>10,000	179
ACh	—	39	3509	85
Nicotine	—	4	1500	>10,000

[a] All compounds were tested as HCl salts.

Table 4. Binding dependence on cumulative pyridine and amino-head substitution



Compound[a]	Pyridine substitution	NR^1R^2	Nicotinic affinity α4β2 K_i (nM)
1a	—	$NHCH_3$	3
1b	—	$N(CH_3)_2$	95
1c	—	NH_2	34
2a	6-CH_3	$NHCH_3$	40
2b	6-CH_3	$N(CH_3)_2$	1090
2c	6-CH_3	NH2	266
3a	6-Cl	$NHCH_3$	13
3b	6-Cl	$N(CH_3)_2$	304
3c	6-Cl	NH2	98
4a	6-Br	$NHCH_3$	38.8
4b	6-Br	$N(CH_3)_2$	394
5a	5-Br	$NHCH_3$	1.07
5b	5-Br	$N(CH_3)_2$	273
6a	5-Br,6-Cl	$NHCH_3$	5.37
6b	5-Br,6-Cl	$N(CH_3)_2$	110

[a] All compounds were tested as HCl salts.

have somewhat lower but not negligible α4β2 binding affinities.

As far as pyridine substitution is concerned it is apparent from the data in Table 4 that Me or Br-substitution, and to a lesser extent Cl-substitution, on the 6 position of pyridine is detrimental for affinity. Br-substitution on the 5-position is beneficial only for the $NHCH_3$ but not for the $N(CH_3)_2$ amino-head.

Compared to mono-substitution on the pyridine ring, dihalogen, 5-Br,6-Cl, substitution leads to the only compounds (**6a**, **6b**) having affinity of the same level as unsubstituted pyridine compounds (**1a**, **1b**).

In summary novel potent and selective α4β2 nicotinic ligands have been obtained by combining structural elements of ACh and of nicotine while restraining the conformational mobility using a cyclopropane ring. For the vast majority of the synthesized compounds no muscarinic activity has been observed; most molecules have exhibited high specificity toward the α4β2 subtype versus the α7 subtype. The α4β2 nAChR affinity of these new ligands is modulated mainly by the nature and length of the linker between the pyridine moiety and the cyclopropane ring as well as by the degree of substitution of the amine. The best α4β2 affinities are obtained for the N-monomethylated compounds and an oxymethylene linker (ethers **1a**–**6a**, **7**). Good to excellent affinities for the α4β2 nAChR subtypes are obtained by the pyridine substitution on the 5 and/or 6 position. The affinity observed for the primary amines **1c**–**3c** is intermediate between that observed for secondary **1a**–**3a** amines or tertiary **1b**–**3b** amines. Despite the lesser steric hindrance this result might be explained by a lower interaction energy with the receptor site due to less fitting and/or loss of hydrophobic interaction coming from the lacking methyl group.

All investigated compounds tested at concentrations up to 10 μM did not demonstrate any appreciable affinity neither for muscular ($(\alpha 1)_2\beta\delta\gamma$) (binding assays using [^{125}I]α-bungarotoxin on *Torpedo marmorata* electroplax tissue) nor for the ganglionic nicotinic receptors (binding assays using [^{3}H]-epibatidine on IMR-32 cells).

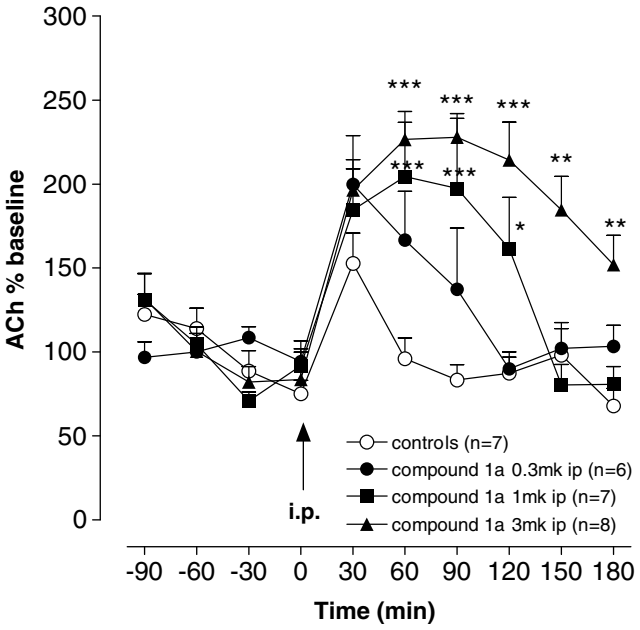


Figure 2. Effect of compound **1a** on acetylcholine release in prefrontal cortex of the freely moving rat. Values shown on percentage of control value. $^{*}p \leqslant 0.05$, $^{**}p \leqslant 0.01$, $^{***}p \leqslant 0.001$ versus control group.

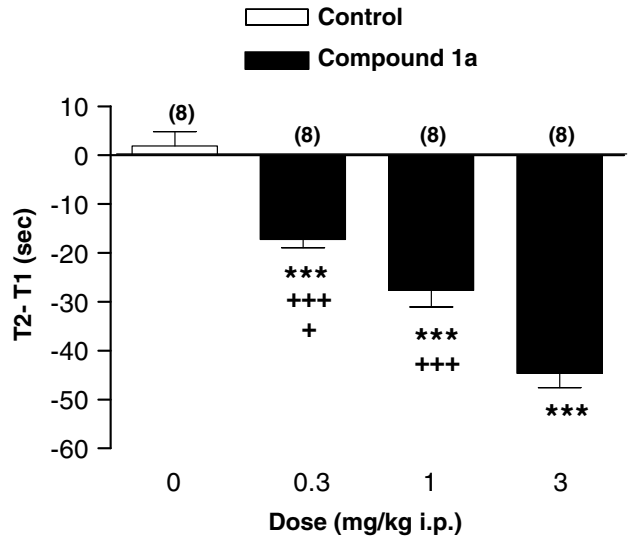


Figure 3. Effect of compound **1a** on the social Wistar rats. Values are means ± sem with the number of animals/group in parentheses. $^{***}p \leqslant 0.001$ versus control; $^{+}p \leqslant 0.05$ versus 1 mg/kg $^{+++}p \leqslant 0.001$ versus 3 mg/kg, ANOVA + Newman–Keuls.

Other pharmaceutical properties are exemplified for compound **1a**. Thus **1a** increased in a dose-dependant manner (microdialysis, freely moving rats), the release of cortical ACh (Fig. 2).[10] This effect is indicative of an agonist character. Moreover a positive effect on memory was observed in the social recognition test in Wistar rats (Fig. 3).[11]

Further pharmacological characterization of the above compounds is ongoing and will be reported in due time.

Acknowledgments

We gratefully acknowledge the Cerebral Pathology Division and the Chemistry Research Division A teams as well as Dr. J.P. Bouchet and Mrs. C. Schaeffer for analytical data and Mrs. D. Rostagni for the skillful typing of the manuscript.

References and notes

1. (a) Jensen, A. A.; Frølund, B.; Liljefors, T.; Krogsgaard-Larsen, P. *J. Med. Chem.* **2005**, *48*, 4705; (b) Romanelli, N.; Gratteri, P.; Guandalini, L.; Martini, E.; Bonaccini, C. *ChemMedChem* **2007**, *2*, 746.
2. (a) Dani, J. A. *Biol. Psychiatry* **2001**, *49*, 166; (b) Rezvani, A. H.; Levin, E. D. *Biol. Psychiatry* **2001**, *49*, 258.
3. (a) Levin, E. D.; Bradley, A.; Addy, N.; Sigurani, N. *Neuroscience* **2002**, *109*, 757; (b) Gatto, G. J.; Bohme, G. A.; Caldwell, W. S.; Letchworth, S. R.; Traina, V. M.; Obinu, M. C.; Laville, M.; Reibaud, M.; Pradier, L.; Dunbar, G.; Bencherif, M. *CNS Drug Rev.* **2004**, *10*, 147; (c) de Fiebre, C. M.; Meyer, E. M.; Henry, J. C.; Muraskin, S. I.; Kem, W. R.; Papke, R. L. *Mol. Pharmacol.* **1995**, *47*, 164; (d) Astles, P. C.; Baker, S. R.; Boot, J. R.; Broad, L. M.; Dell, C. P.; Keenan, M. *Curr. Drug Targets CNS Neurol. Disord.* **2002**, *1*, 337; (e) Kitagawa, H.; Takenouchi, T.; Azuma, R.; Wesnes, K. A.; Kramer, W. G.; Clody, D. E.; Burnett, A. L. *Neuropsychopharmacology* **2003**, *28*, 542.
4. Goldstein, S.; Guillonneau, C.; Charton, Y.; Lockhart, B.; Lestage, P. Eur. Patent 117081 A1, 2002; *Chem. Abstr.* **2002**, *136*, 86 625.
5. Lockhart, B.; Bonhomme, N.; Lebrun, C.; Roger, A.; Guillonneau, C.; Charton, Y.; Goldstein, S.; Lestage, P. *Pharmacologist* **2002**, *44*, A97.
6. *Selected data:* Compound **1a**: ^{1}H NMR (DMSO d_6-300 MHz) δ 9.85 (sb, 2H, NH_2^+), 8.65 (d, J = 2.0 Hz, 1H), 8.5 (d, J = 6.0 Hz, 1H), 8.05 (d, J = 7.6 Hz, 1H), 7.9 (m, 1H), 4.45 (s, 2H), 2.65 (s, 3H), 1.35–1.00 (m, 4H).
Compound **1b**: ^{1}H NMR (DMSO d_6-300 MHz) δ 11.4 (sb, 1H, NH^+), 8.65 (m, 1H), 8.5 (m, 1H), 8.00 (m, 1H), 7.85 (m, 1H), 4.50 (s, 2H), 2.85 (s, 6H), 1.5–1.10 (m, 4H).
Compound **1c**: ^{1}H NMR (DMSO d_6-300 MHz) δ 8.9 (sb, 3H, NH_3^+), 8.65 (d, J = 2.0 Hz, 1H), 8.5 (d, J = 6.0 Hz, 1H), 8.10 (dd, J = 7.6, 2 Hz, 1H), 7.9 (m, 1H), 4.35 (s, 2H), 1.15–0.95 (m, 4H).
Compound **5a**: ^{1}H NMR (DMSO d_6-300 MHz) δ 9.7 (sb, 2H, NH_2^+), 8.4 (m, 2H), 7.8 (m, 1H), 4.35 (s, 2H), 2.7 (s, 3H), 1.4–1.0 (m, 4H).
Compound **6a**: ^{1}H NMR (DMSO d_6, 300 MHz) δ 9.75 (sb, 2H, NH_2^+), 8.25 (d, J = 2 Hz, 1H), 8.00 (d, J = 2 Hz, 1H), 4.35 (s, 2H), 2.65 (s, 3H), 1.3–0.95 (m, 4H).
7. For labeling of α7 nicotinic subtype rat brain cell membranes (500 μg/ml) were incubated with [^{125}I]α-bungarotoxine (2 nM) for 5 h at 37 °C. Non-specific binding was determined by incubation of membrane preparations with 1 μM α-bungarotoxine Marks, M. J.; Stitzel, J. A.; Collins, A. C. *Pharmacol. Exp. Ther.* **1985**, *235*, 619.
8. For labeling of α4β2 receptors, rat brain cell membranes (250 μg/ml), were incubated with [^{3}H]cytisine for 2 h at room temperature. Non-specific binding was assessed by the incubation of membrane preparations with 10 μM *S*(−) nicotine Pabreza, L. A.; Dhawan, S.; Kellar, K. *Mol. Pharmacol.* **1990**, *39*, 9.
9. For labeling of muscarinic receptors (M_2/M_4) rat brain cell membranes (250 μg/ml) were incubated with [^{3}H]oxotremorine (2 nM) for 2 h at room temperature non-specific binding being determined by incubation of membrane preparations with 1 μM atropine Lockhart, B.; Closier, M.; Howard, K.; Stewart, C.; Lestage, P. *Naunyn Schmiedeberg Arch. Pharmacol.* **2001**, *363*, 429.
10. *ACh levels:* A guide-cannula was stereotaxically implanted in the prefrontal cortex (AP: +3.3, L: −0.6, V: −0.5 relative to bregma). After 5–7 days of recovery, the microdialysis probe (CMA11, 4 mm length, ∅ 0.23 mm) was lowered in the guide-cannula. The dialysis probe was continuously perfused at a flow rate of 1 μl/min with an artificial cerebrospinal fluid (composition in mM: NaCl 147, KCl 2.7, $MgCl_2$ 1, $CaCl_2$ 1.2, adjusted at pH 7.4 with 2 mM phosphate buffer). Two hours after probe implantation (stabilization period) in the rat prefrontal cortex, dialysates were collected every 30 min and analyzed by HPLC coupled to electrochemical detection. The first four dialysates were considered as baseline. Drug administration began at the end of the baseline. Data are expressed in percentage of the mean value of the baseline samples.
11. *Social recognition:* Adult male Wistar rats placed in their standard home cages were exposed twice to a juvenile rat (25–30 days old) in two 5 min sessions separated by a retention interval of 120 min. Manual chronometric evaluation of exploration times (s) of the juvenile rats was performed through a video-camera system. Decrease of the exploration time toward the juvenile rate on the 2nd session indicated that the adult rat recognized the juvenile (increase of memory retention).