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Synthesis of Epibatidine-Related Δ^2-Isoxazoline Derivatives and Evaluation of Their Binding Affinity at Neuronal Nicotinic Acetylcholine Receptors

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The group of Δ^2-isoxazoline derivatives **5a–c** and **6a–c**, structurally related to epibatidine, and the simplified analogues **7a–c** were synthesized by means of a 1,3-dipolar cycloaddition-based strategy and tested at α4β2 and α7 neuronal acetylcholine receptor (nAChR) subtypes. Competition binding experiments at α4β2 nAChR subtypes showed an overall significant reduction in affinity for the compounds under study in comparison to the reference radioligand [^{3}H]-epibatidine. These outcomes have been rationalized by taking into account the ligand-based pharmacophore models reported in the literature and the recently proposed molecular model of the α4β2 receptor subtype. Conversely, compounds **5b**, **5c**, and **6b** exhibited a noticeable affinity for the α7 receptors and, in the case of **5c**, also some subtype selectivity.

Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs) make up a family of ligand-gated ion channels that are widely distributed in the human brain, where they are frequently associated with modulatory events and, to a lesser extent, mediate synaptic transmission.[1] Their pentameric molecular structure is characterized by homomeric (α7) or heteromeric (α2-α6) combinations of homologous, genetically distinct subunits, whose differential association confers specific structural and functional properties to the resulting subtypes.[2] These properties include a high degree of intrinsic Ca^{2+} permeability: heteromeric neuronal nAChRs containing α and β subunits have a fractional Ca^{2+} current of 2–5%, whereas that of homomeric α7 receptors ranges from 6% to 12%.[3] The complex Ca^{2+}-mediated responses following activation of neuronal nAChRs amplify the information beyond the initial receptive neuron, facilitating the interface with intracellular processes. These mechanisms underlie the varied neuronal activities of (*S*)-nicotine (**1**) (Scheme 1) in the brain and account for the involvement of neuronal nAChRs in a number of functional processes such as cognition, learning and memory, cerebral blood flow and metabolism, as well as in a series of pathological conditions such as Alzheimer's disease, Parkinson's disease, schizophrenia, epilepsy, Tourette's syndrome, anxiety, depression and nicotine addiction.[2c,4] Given the involvement of nAChRs in a variety of pathological states, the most recent investigations have focused on the development of new and potentially useful therapeutic agents guided by suitable pharmacophore models.[5] Research efforts have mainly concerned ligands acting on the α4β2 and α7 receptor subtypes,[6] which represent the two major nAChR subtypes in the human brain.

Over the last decade, the discovery of epibatidine (**2**) (Scheme 1), an alkaloidal toxin isolated from the skin of the poisonous Ecuadorian frog *Epipedobates tricolor*[7] provided with an analgesic potency, roughly one hundred times higher than morphine, has renewed the interest in targeting nAChRs for analgesia.[8] As a matter of fact, the potent analgesic activity of epibatidine is due to its high affinity for the α4β2 nAChR subtype which is 30 times higher than that of nicotine.[9] The main obstacle to the clinical use of epibatidine as an analgesic drug is its very narrow therapeutic index, which is due to the lack of subtype selectivity.[10] Nevertheless, epibatidine has become a reference compound for studying nAChRs structure and a lead compound for the design of novel, high-affinity and subtype-selective nAChR ligands. Epibatidine-related derivatives have been synthesized by varying the heteroaryl moiety,[11] or functionalizing and expanding the alicyclic skeleton,[12] or modifying the substituent and/or the position of the nitrogen atom in the bicyclic compound.[13] Moreover, bivalent and conformationally constrained epibatidine analogues have been synthesized and tested.[14] The spirocyclic compounds **3a–b** and fused cyclic derivatives **4a–b** (Scheme 1) were assayed at six different nicotinic nAChR subtypes. Interestingly, the fused compound **4a** had higher

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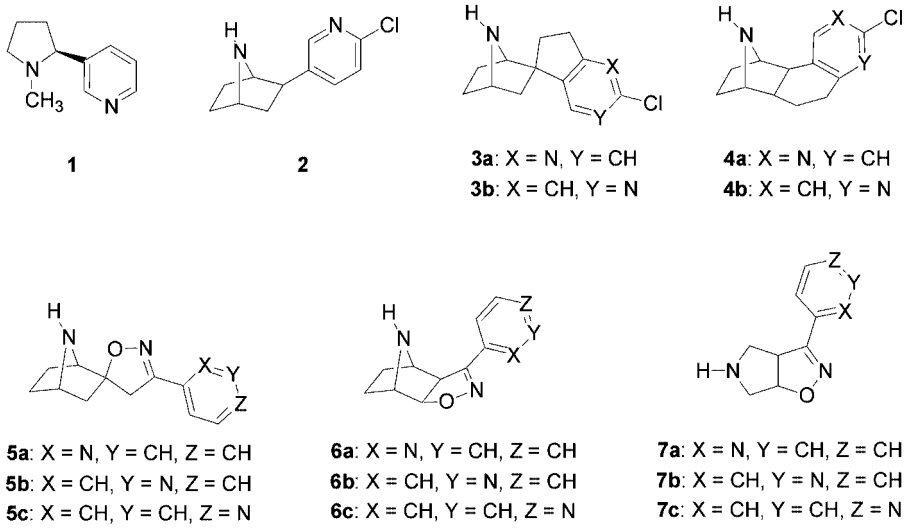


Scheme 1. Reference and target structures of the ligands for nAChRs.

affinity for the nAChRs containing the β2 rather than the β4 subunit, whereas its regioisomer **4b** showed a reversed selectivity.[14b] These outcomes have been rationalized by means of conformational analysis and molecular modeling studies,[14b,15] which revealed an overall correspondence of the pharmacophore elements of epibatidine with those of ligands **4a**–**b**, although enough differences exist to account for the inversion of their affinity profile.

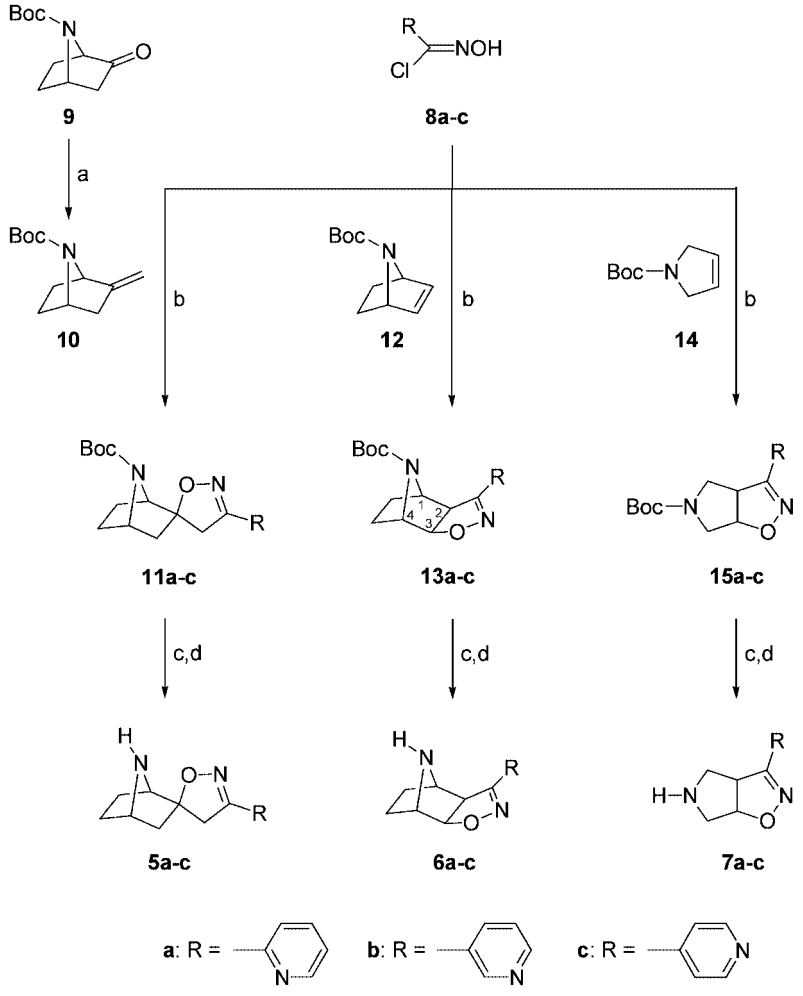
In the light of the above-commented results we designed a group of novel compounds in which the two structural elements featuring epibatidine (i.e. the 7-azabicyclo[2.2.1]-heptane system and the pyridine ring) were kept away by the insertion of a Δ^2-isoxazoline moiety, either spiro-condensed (derivatives **5a**–**c**) or fused (derivatives **6a**–**c**) with the azanorbornane core (Scheme 1). In addition, we synthesized and tested compounds **7a**–**c** (Scheme 1), in which the ethylene bridge of the bicyclic system of **6a**–**c** was removed. The aim of this study was to investigate the effect of the variation of both the N[aza(bi)cycle]–N(pyridine) distance and the conformational profile on the affinity/selectivity for the nAChR subtypes. Because the chlorine atom located on the pyridine ring makes a minor contribution to the affinity of epibatidine,[16] we chose to synthesize the three unsubstituted pyridinyl regioisomers. Moreover, the novel chiral derivatives **5a**–**c**, and **6a**–**c** were prepared as racemates based on the negligible stereoselectivity observed for the epibatidine enantiomers.[11b,17] This paper describes the synthesis of compounds **5**–**7**, the evaluation of their binding affinity at α4β2 and α7 nAChR subtypes, and an investigation of their affinity profile by direct and indirect molecular modeling approaches.

Results and Discussion

The synthetic routes to the desired final compounds are outlined in Scheme 2. Intermediates **11a**–**c**, **13a**–**c**, and **15a**–**c** were prepared by a 1,3-dipolar cycloaddition involving the appropriate pyridinenitrile oxides, generated in situ upon treatment of the corresponding hydroximoyl chlorides **8a**–**c**[18] with triethylamine, and the dipolarophiles **10**, **12**, and **14**, respectively. The alkene **10** was prepared by a Wittig methylenation reaction carried out on known *tert*-butyl 2-oxo-7-azabicyclo[2.2.1]heptane-7-carboxylate (**9**),[19] whereas *tert*-butyl 7-azabicyclo[2.2.1]hept-2-ene-7-carboxylate (**12**)[11b] and *tert*-butyl Δ^3-pyrroline-1-carboxylate (**14**)[20] were obtained following published procedures.

As expected, pyridinenitrile oxides attack the less hindered face of the bicyclic olefin **12** yielding exclusively the *exo* cycloadducts **13a**–**c**. According to the literature,[21] the observed *syn*-facial selectivity is dictated by the *anti* pyramidalization of the olefinic hydrogen atoms in the dipolarophile. The structural assignment to the adducts **13a**–**c** was established by analyzing their ^{1}H NMR spectra and those of the corresponding bases **6a**–**c**. The absence of a coupling constant between the protons 1-H and 2-H as well as between 3-H and 4-H (Scheme 2) is a clear indication of their *exo* configuration.[22] By considering the outcome of reactions carried out on olefins structurally related to **10**,[23] we assigned the *exo* configuration even to the spirocyclic intermediates **11a**–**c**, which were the only isomers isolated from the cycloaddition step. Treatment of **11a**–**c**, **13a**–**c**, and **15a**–**c** with trifluoroacetic acid provided the desired epibatidine analogues as the free bases **5a**–**c**, **6a**–**c**, and **7a**–**c**, which were then converted into the corresponding fumarates.

The binding affinities of the fumarates of target compounds **5a**–**c**, **6a**–**c**, and **7a**–**c** for the rat α4β2 and α7 nAChR subtypes were assessed using [^{3}H]-epibatidine and [^{125}I]-α-bungarotoxin as radioligands. The K_i values were calculated from the competition curves by means of the LIGAND program.[24] Inspection of the data reported in Table 1 clearly shows that the insertion of the Δ^2-isoxazoline moiety between the azanorbornane system and the pyridine ring greatly reduces or abolishes the binding affinity for the α4β2 and the α7 nAChR subtypes, because the K_i values of the reference radioligands fall within the 20–40 pM and the 0.8–1 nM concentration ranges, respectively. When



Scheme 2. a: $Ph_3P{=}CH_2$, Et_2O; b: $(C_2H_5)_3N$, CH_3CN, 60 °C; c: CF_3COOH, CH_2Cl_2; d: $C_4H_4O_4$, MeOH.

tested at final concentrations up to 200 μM, compounds **7a** and **7c** did not inhibit radioligand binding to either subtype, and derivatives **6a**, **6c**, and **7b** did not inhibit binding to the α7 subtype. Therefore, the K_i values of the above-cited compounds have been indicated as higher than 100 μM.

Derivative **7b** (K_i = 72 μM) and the fused analogues **6b** (K_i = 86 μM) and **6c** (K_i = 68 μM), which have the pyridine nitrogen in position 3′ and 4′, respectively, retained a residual and comparable affinity for the α4β2 nAChRs. Conversely, the two spirocyclic analogues **5b** (K_i = 41 μM) and **5c** (K_i = 22 μM), which are characterized by 3′ and 4′ pyridine nitrogen atoms, respectively, had the highest affinity for the α7 nAChR subtype, thus they behave quite similarly to the fused analogue **6b**. In addition, derivative **5c** was slightly more selective for the α7 than the α4β2 receptor subtypes (K_i = 22 and 400 μM, respectively).

The above-discussed results on the group of novel epibatidine-related derivatives were examined by molecular modeling investigations. Among the proposed ligand-based models for the interaction with nicotinic receptors, initially Beers and Reich[25] suggested a distance of 5.9 Å between the center (A) of a positive charge (N^+) and a hydrogen-bond acceptor moiety (B). Later on Sheridan,[26] in addition to the above-cited A and B pharmacophoric elements, introduced a dummy atom (C) defining the line along which the hydrogen bond may take place. In this model, the optimal distances were 4.8 ± 0.3 Å for A–B, 4.0 ± 0.3 Å for A–C,

Table 1. Binding affinities (K_i, μM) of **5a**–**c**, **6a**–**c**, and **7a**–**c** to α4β2 and α7 nAChR subtypes.

Compound	α4β2; [^{3}H]-Epibatidine; (K_i, μM)	α7; [^{125}I]α-BgTx; (K_i, μM)
5a	222	124
5b	104	41
5c	400	22
6a	102	>100
6b	86	32
6c	68	>100
7a	>100	>100
7b	72	>100
7c	>100	>100
3a	29[a]	n.d.[b]
3b	7[a]	n.d.[b]
4a	0.07[a]	n.d.[b]
4b	0.3[a]	n.d.[b]
Epibatidine	32 pM	0.8 nM
α-BgTx.	n.d.[b]	0.9 nM

[a] Ref.[14b]. [b] Not determined.

and 1.2 ± 0.3 Å for B–C. Subsequently, Glennon proposed a longer A–B distance (5.5 Å) based on the minimum-energy conformation of epibatidine,[27] and Abreo et al. suggested a higher value (6.1 Å)[28] for α4β2 selective nicotinic agonists by considering the conformational profile of nicotine-related derivatives which are equipotent with epibatidine. Moreover, an overview of the most recent patents on selective nicotinic ligands[29] reveals the tendency to design ligands whose A–B distance is longer than that of the compounds included in the above-cited pharmacophore models. In this respect, our compounds reproduce a variety of A–B distances (Table 2), ranging from 4.7 Å (**6a**) to 8.8 Å (**5c**). Worth noting, the novel derivatives **6b**, **6c**, and **7b** with the highest residual affinity for the α4β2 receptors display values of the A–B distance falling in the range 6–7 Å (Table 2).

Table 2. Pharmacophoric distances *d* (N^+–N_{pyr}) for compounds **3**–**7**.

Compound	*d* [Å]
3a	5.9
3b	5.9
4a1	4.9
4a2	5.0
4b1	4.4
4b2	5.1
5a	6.8–6.9
5b	8.1–8.2
5c	8.8
6a	4.7–5.2
6b	5.9–6.3
6c	6.7
7a	4.8–5.4
7b	6.0–6.5
7c	6.8

On the other hand, compounds **6b**, **5b**, and **5c**, characterized by a comparable high μM affinity for the α7 receptors, show quite dissimilar values of the A–B distance (5.9–6.3 Å, 8.1–8.2 Å, and 8.8 Å, respectively, Table 2). Thus, within the set of compounds under study, such a pharmacophoric feature plays a less predictable role in addressing the ligands to the α7 receptor binding sites. In any case, the appearance of a non-negligible affinity for the α7 nAChRs deserves an additional in-depth investigation to define the pharmacological profile of the most interesting compounds.

In a parallel study, we utilized the model for the extracellular domain of the neuronal α4β2 rat nAChR subtype, recently optimized by Westera et al.[30] At first we considered the active conformation of (–)-epibatidine docked into the model in accordance with the reported[30] first cluster of binding orientations (Figure 1, A), then we inserted the energetically favoured conformation of the reference compound **4a**,[14b] which was superimposed to (–)-epibatidine by a rigid overlapping of the common structural moieties, i.e. the azabicycloheptane system and the pyridine ring (Figure 1, B). The relevant interactions for the activation of the α4β2 receptor are substantially retained in the rigidified epibatidine analogue **4a**, because the presence of the fused cyclohexene ring does not preclude the binding of the N_{pyr} to the lateral chain of the Lys77 (chain A) residue. The decrease in affinity on passing from epibatidine to **4a** can be

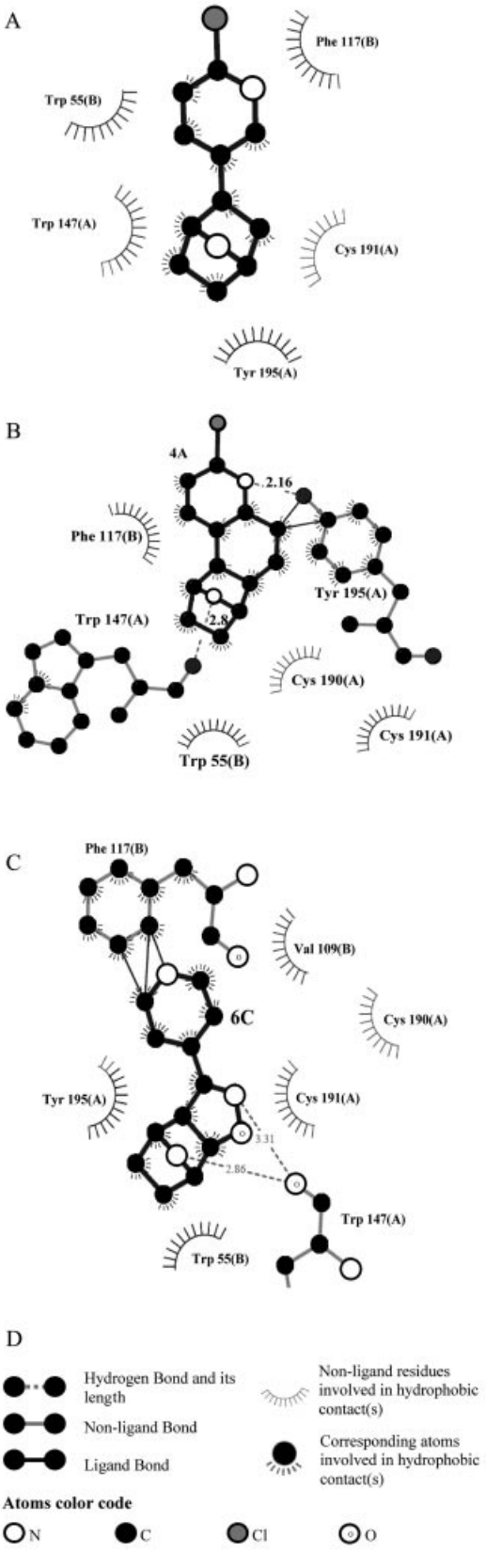


Figure 1. (–)-Epibatidine (**A**), compounds **4a** (**B**), and **6c** (**C**) docked into the α4β2 model described in ref.[30]. Cation–π and hydrogen-bond interactions have been omitted for simplicity. Illustrations were obtained by LIGPLOT v.4.4.2 (ref.[31]).

tentatively attributed to the steric clash between the two extra methylene groups of the rigidifying cyclohexene ring of **4a** and the lateral chain of Tyr 195 (chain A). We hypothesize that derivatives **6** may adopt a binding topology to the α4β2 receptor subtype similar to that of (–)-epibatidine. Superimposition by the common azabicycloheptane moiety as reported for **6c** (Figure 1, C), the compound showing the highest residual affinity for the α4β2 receptor, puts in evidence a significant steric clash involving the pyridine ring of **6c** and the lateral chain of Phe177 (chain B).

Therefore, in the light of this investigation on the rat α4β2 receptor model, we propose an explanation for the inadequacy of our homologation/rigidification approach applied to epibatidine in the search for potent and selective agonists of the α4β2 subtype.

Conclusions

Δ^2-Isoxazoline derivatives **5a**–**c**, **6a**–**c**, and **7a**–**c**, structurally related to epibatidine, were prepared and tested to gain further insights into the structural requirements necessary to impart affinity and selectivity at the neuronal nicotinic α4β2 and α7 receptors. Competition binding data demonstrated that some of the new ligands retained residual affinity for the α4β2 subtype in the high-μM concentration range. Surprisingly, a meaningful affinity for the α7 subtype emerged for other compounds of the series and, among them, the spirocyclic derivative **5c** showed a degree of α7 vs. α4β2 selectivity. In conclusion, homologation of the structure of a prototype α4β2 selective agonist led to the appearance of an unexpected affinity for the α7 receptors, a result which could be exploited in designing novel selective agonists of this nAChR subtype.

Experimental Section

Chemistry

Materials and Methods: Hydroxyimoyl chlorides **8a**–**c**,[18] *tert*-butyl 2-oxo-7-azabyciclo[2.2.1]heptane-7-carboxylate (**9**),[19] *tert*-butyl 7-azabicyclo[2.2.1]hept-2-ene-7-carboxylate (**12**),[10b] and Δ^3-pyrroline[20a] were prepared according to literature procedures. The transformation of the secondary amine of Δ^3-pyrroline into the corresponding *N*-Boc derivative **14** was accomplished along a standard methodology. ^{1}H NMR spectra were recorded with a Varian Mercury 300 (300 MHz) spectrometer in $CDCl_3$ solutions (unless otherwise indicated) at 20 °C. Chemical shifts (δ) are expressed in ppm and coupling constants (J) in Hz. TLC analyses were performed on commercial silica gel 60 F_{254} aluminum sheets; spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution. Melting points were determined with a model B 540 Büchi apparatus and are uncorrected. Microanalyses (C,H,N) of new compounds agreed with the theoretical value within ±0.4%.

***tert*-Butyl 2-Methylene-7-azabyciclo[2.2.1]heptane-7-carboxylate (10):** To an ice-cooled stirred suspension of potassium *tert*-butoxide (700 mg, 6.23 mmol) in anhydrous diethyl ether (20 mL) was added portionwise methyltriphenylphosphonium bromide (2.41 g, 6.75 mmol). After heating at reflux for 1 h, the suspension was cooled to room temp. A solution of **9**[19] (940 mg, 4.45 mmol) in anhydrous ethyl ether (10 mL) was then added dropwise. The mixture was stirred at room temperature for about 1 h, until disappearance of the starting material; the progress of the reaction was monitored by TLC (10% ethyl acetate/petroleum ether). Acetone (5 mL) and water (25 mL) were then added, the organic phase was separated and the aqueous phase was extracted with diethyl ether (3 × 25 mL). The combined organic extracts were dried with anhydrous sodium sulfate, and the solvent was removed under reduced pressure. A silica gel column chromatography of the residue (10% ethyl acetate/petroleum ether) afforded 759 mg (82% yield) of the expected methylene derivative. Colorless oil, b.p. 85–90 °C/1.9 mbar; R_f = 0.69 (10% ethyl acetate/petroleum ether). ^{1}H NMR: δ = 1.43 (s, 9 H), 1.49 (m, 2 H), 1.86 (m, 2 H), 2.05 (d, J = 15.7 Hz, 1 H), 2.43 (dd, J = 4.5 and 15.7 Hz, 1 H), 4.26 (dd, J = 4.5 and 4.5 Hz, 1 H), 4.42 (d, J = 4.5 Hz, 1 H), 4.72 (s, 1 H), 4.92 (s, 1 H) ppm. $C_{12}H_{19}NO_2$ (209.28): calcd. C 68.87, H 9.15, N 6.69; found C 68.51, H 9.37, N 7.01.

General Procedure for the Synthesis of Cycloadducts 11a–c, 13a–c, and 15a–c: Reaction of hydroxyimoyl chloride **8a** with alkene **10** is described as a typical example. To a suspension of **10** (200 mg, 0.96 mmol) and 2-pyridinecarbohydroxyimoyl chloride (**8a**) (186 mg, 0.96 mmol) in toluene (12 mL) was added dropwise a solution of triethylamine (0.27 mL, 1.91 mmol) in toluene (2 mL). While heating at reflux for 5 days, further amounts of **8a** (371 g, 1.91 mmol) and triethylamine (0.53 mL, 3.83 mmol) were added portionwise to the reaction mixture. The progress of the reaction was monitored by TLC (ethyl acetate). Water (10 mL) was added to the reaction mixture, the phases were separated and the aqueous layer was extracted with ethyl acetate (6 × 15 mL). After the usual work-up, the crude residue was purified by silica gel column chromatography (50% petroleum ether/ethyl acetate), which afforded 164 mg (52% yield) of cycloadduct **11a**.

***tert*-Butyl 3′-(Pyridin-2-yl)spiro[7-azabicyclo[2.2.1]heptane-2,5′-(4′*H*)-isoxazole]-7-carboxylate (11a):** Colorless prisms (from ethyl acetate), m.p. 167–170 °C (dec); R_f = 0.42 (ethyl acetate). ^{1}H NMR: δ = 1.48 (s, 9 H), 1.54–1.72 (m, 5 H), 2.42 (dd, J = 4.2 and 10.9 Hz, 1 H), 3.39 (d, J = 19.0 Hz, 1 H), 3.61 (d, J = 19.0 Hz, 1 H), 4.25 (br. s, 1 H), 4.35 (br. s, 1 H), 7.29 (dd, J = 4.8 and 8.2 Hz, 1 H), 7.72 (dd, J = 6.4 and 8.2 Hz, 1 H), 8.01 (d, J = 6.4 Hz, 1 H), 8.60 (d, J = 4.8 Hz, 1 H) ppm. $C_{18}H_{23}N_3O_3$ (329.39): calcd. C 65.63, H 7.04, N 12.76; found C 65.80, H 6.78, N 12.92.

***tert*-Butyl 3′-(Pyridin-3-yl)spiro[7-azabicyclo[2.2.1]heptane-2,5′-(4′*H*)-isoxazole]-7-carboxylate (11b):** Yield: 40%, 125 mg; colorless prisms (from ethyl acetate), m.p. 157–162 °C; R_f = 0.33 (ethyl acetate). ^{1}H NMR: δ = 1.48 (s, 9 H), 1.46–1.92 (m, 5 H), 2.44 (dd, J = 5.2 and 13.4 Hz, 1 H), 3.22 (d, J = 16.4 Hz, 1 H), 3.42 (d, J = 16.4 Hz, 1 H), 4.24 (br. s, 1 H), 4.38 (br. s, 1 H), 7.38 (m, 1 H), 8.07 (d, J = 8.1 Hz, 1 H), 8.63 (br. s, 1 H), 8.78 (br. s, 1 H) ppm. $C_{18}H_{23}N_3O_3$ (329.39): calcd. C 65.63, H 7.04, N 12.76; found C 65.34, H 7.07, N 12.51.

***tert*-Butyl 3′-(Pyridin-4-yl)spiro[7-azabicyclo[2.2.1]heptane-2,5′-(4′*H*)-isoxazole]-7-carboxylate (11c):** Yield 26%, 188 mg; colorless prisms (from ethyl acetate), m.p. 190–197 °C (dec); R_f = 0.27 (ethyl acetate). ^{1}H NMR: δ = 1.48 (s, 9 H), 1.54–1.91 (m, 5 H), 2.46 (dd, J = 4.0 and 13.9 Hz, 1 H), 3.22 (d, J = 16.8 Hz, 1 H), 3.39 (d, J = 16.8 Hz, 1 H), 4.23 (br. s, 1 H), 4.36 (br. s, 1 H), 7.51 (d, J = 5.1 Hz, 2 H), 8.66 (d, J = 5.1 Hz, 2 H) ppm. $C_{18}H_{23}N_3O_3$ (329.39): calcd. C 65.63, H 7.04, N 12.76; found C 65.76, H 7.06, N 12.47.

Cycloadditions of hydroxyimoyl chlorides **8a**–**c** (1.5 mmol) to the dipolarophile **12** (1.5 mmol) were carried out in acetonitrile at room temp. for 8 h using a twofold excess of triethylamine.

***tert*-Butyl 5-(Pyridin-2-yl)-3-oxa-4,10-diazatricyclo[5.2.1.0^{2,6}]dec-4-ene-10-carboxylate (13a):** Yield 37%, 188 mg; colorless prisms (from acetone), m.p. 155–156 °C; R_f = 0.50 (40% ethyl acetate/petroleum ether). ^{1}H NMR: δ = 1.12 (m, 2 H), 1.39 (s, 9 H), 1.82 (m, 2 H), 3.95 (m, 1 H), 4.69 (m, 1 H), 4.83 (m, 1 H), 7.26 (m, 1 H), 7.70 (m, 1 H), 7.93 (m, 1 H), 8.60 (m, 1 H) ppm. $C_{17}H_{21}N_3O_3$ (315.37): calcd. C 64.74, H 6.71, N 13.32; found C 64.52, H 6.52, N 13.60.

***tert*-Butyl 5-(Pyridin-3-yl)-3-oxa-4,10-diazatricyclo[5.2.1.0^{2,6}]dec-4-ene-10-carboxylate (13b):** Yield 74%, 242 mg; colorless prisms (from ethyl acetate), m.p. 157–158 °C; R_f = 0.36 (ethyl acetate). ^{1}H NMR: δ = 1.10–1.60 (m, 11 H), 1.86 (m, 2 H), 3.77 (d, $J_{2,3}$ = 8.4 Hz, 1 H, 2-H), 4.53 (br. s, 1 H), 4.65 (br. s, 1 H), 4.89 (d, $J_{3,2}$ = 8.4 Hz, 1 H, 3-H), 7.37 (m, 1 H), 8.09 (m, 1 H), 8.64 (m, 1 H), 8.85 (m, 1 H) ppm. $C_{17}H_{21}N_3O_3$ (315.37): calcd. C 64.74, H 6.71, N 13.32; found C 64.87, H 6.85, N 13.10.

***tert*-Butyl 5-(Pyridin-4-yl)-3-oxa-4,10-diaza-tricyclo[5.2.1.0^{2,6}]dec-4-ene-10-carboxylate (13c):** Yield 77%, 187 mg; colorless prisms (from ethyl acetate), m.p. 152–153 °C; R_f = 0.53 (ethyl acetate). ^{1}H NMR: δ = 1.05–1.65 (m, 11 H), 1.89 (m, 2 H), 3.75 (d, $J_{2,3}$ = 8.0 Hz, 1 H, 2-H), 4.50 (br. s, 1 H), 4.60 (br. s, 1 H), 4.99 (d, $J_{3,2}$ = 8.0 Hz, 1 H, 3-H), 7.78 (m, 2 H), 8.73 (m, 2 H) ppm. $C_{17}H_{21}N_3O_3$ (315.37): calcd. C 64.74, H 6.71, N 13.32; found C 64.51, H 6.74, N 13.09.

Compounds **15a**–**c** were prepared by reacting hydroxyimoyl chlorides **8a**–**c** (6 mmol), dipolarophile **14** (3 mmol) and triethylamine (12 mmol) in refluxing acetonitrile for 5 days.

***tert*-Butyl 3-(Pyridin-2-yl)-3a,4,6,6a-tetrahydropyrrolo[3,4-*d*]isoxazole-5-carboxylate (15a):** Yield 49%, 432 mg; viscous pale yellow oil; R_f = 0.54 (5% methanol/dichloromethane). ^{1}H NMR: δ = 1.39 (s, 9 H), 3.64 (m, 2 H), 4.00 (m, 2 H), 4.24 (m, 1 H), 5.27 (m, 1 H), 7.28 (dd, J = 4.2 and 5.8 Hz, 1 H), 7.72 (dd, J = 5.8 and 7.1 Hz, 1 H), 8.02 (d, J = 7.1 Hz, 1 H), 8.62 (d, J = 4.2 Hz, 1 H) ppm. $C_{15}H_{19}N_3O_3$ (289.33): calcd. C 62.27, H 6.62, N 14.52; found C 61.95, H 6.84, N 14.70.

***tert*-Butyl 3-(Pyridin-3-yl)-3a,4,6,6a-tetrahydropyrrolo[3,4-*d*]isoxazole-5-carboxylate (15b):** Yield 31%, 266 mg; viscous pale yellow oil; R_f = 0.47 (5% methanol/dichloromethane). ^{1}H NMR: δ = 1.42 (s, 9 H), 3.55–3.90 (m, 3 H), 4.01 (m, 1 H), 4.26 (m, 1 H), 5.36 (m, 1 H), 7.38 (dd, J = 4.3 and 7.9 Hz, 1 H), 8.06 (d, J = 7.9 Hz, 1 H), 8.66 (d, J = 4.3 Hz, 1 H), 8.79 (s, 1 H) ppm. $C_{15}H_{19}N_3O_3$ (289.33): calcd. C 62.27, H 6.62, N 14.52; found C 62.48, H 6.60, N 14.27.

***tert*-Butyl 3-(Pyridin-4-yl)-3a,4,6,6a-tetrahydropyrrolo[3,4-*d*]isoxazole-5-carboxylate (15c):** Yield 26%, 183 mg; viscous pale yellow oil; R_f = 0.33 (ethyl acetate). ^{1}H NMR: δ = 1.39 (s, 9 H), 3.50–3.80 (m, 3 H), 4.00 (m, 1 H) 4.18 (m, 1 H), 5.37 (dd, J = 5.7 and 9.5 Hz, 1 H), 7.50 (d, J = 5.4 Hz, 2 H), 8.68 (d, J = 5.4 Hz, 2 H) ppm. $C_{15}H_{19}N_3O_3$ (289.33): calcd. C 62.27, H 6.62, N 14.52; found C 62.30, H 6.47, N 14.37.

General Procedure for the Synthesis of Derivatives 5a–c, 6a–c, 7a–c and Their Fumarates: Reaction of compound **11a** is described as a typical example. To a stirred solution of **11a** (146 mg, 0.45 mmol) in dichloromethane (0.81 mL) was added dropwise trifluoroacetic acid (0.35 mL, 4.5 mmol), and the mixture was stirred at room temp. for 12 h. The progress of the reaction was monitored by TLC (10% methanol/dichloromethane). After concentration at reduced pressure, the residue was dissolved in water (2 mL) and treated with diethyl ether (3 × 3 mL). The residual aqueous phase was made alkaline by portionwise addition of solid Na_2CO_3 and extracted with ethyl acetate (6 × 3 mL). The combined organic extracts were dried with anhydrous sodium sulfate, and the solvent was evaporated under vacuum to afford 92 mg (89% yield) of the desired free base **5a**.

3′-(Pyridin-2-yl)spiro[7-azabicyclo[2.2.1]heptane-2,5′(4′*H*)-isoxazole] (5a): Yellow oil; R_f = 0.45 (10% methanol/dichloromethane). ^{1}H NMR: δ = 1.31 (m, 2 H), 1.48 (m, 1 H), 1.69 (m, 2 H), 2.22 (dd, J = 5.5 and 14.1 Hz, 1 H), 3.44 (d, J = 17.9 Hz, 1 H), 3.57 (br. s, 1 H), 3.59 (d, J = 17.9 Hz, 1 H), 3.73 (br. s, 1 H), 7.29 (dd, J = 4.8 and 8.2 Hz, 1 H), 7.72 (dd, J = 6.4 and 8.2 Hz, 1 H), 8.01 (d, J = 6.4 Hz, 1 H), 8.60 (d, J = 4.8 Hz, 1 H) ppm.

To a solution of **5a** (59 mg, 0.26 mmol) in methanol (1.5 mL) was added a solution of fumaric acid (33 mg, 0.29 mmol) in methanol (1.5 mL). After stirring at room temp. for 12 h, the reaction mixture was concentrated under reduced pressure affording quantitatively the corresponding fumarate, which was crystallized from methanol.

5a·$C_4H_4O_4$: Colorless prisms, m.p. 141–144 °C; R_f = 0.48 (20% methanol/dichloromethane). ^{1}H NMR (CD_3OD): δ = 1.82 (m, 1 H), 2.00 (m, 3 H), 2.23 (d, J = 14.3 Hz, 1 H), 2.49 (dd, J = 4.3 and 14.3 Hz, 1 H), 3.61 (d, J = 18.0 Hz, 1 H), 3.83 (d, J = 18.0 Hz, 1 H), 4.18 (br. s, 1 H), 4.27 (br. s, 1 H), 6.68 (s, 2 H), 7.44 (dd, J = 5.0 and 8.0 Hz, 1 H), 7.86 (dd, J = 7.6 and 8.0 Hz, 1 H), 7.96 (d, J = 7.6 Hz, 1 H), 8.61 (d, J = 5.0 Hz, 1 H) ppm. $C_{17}H_{19}N_3O_5$ (345.35): calcd. C 59.12, H 5.55, N 12.17; found C 58.96, H 5.78, N 12.08.

3′-(Pyridin-3-yl)spiro[7-azabicyclo[2.2.1]heptane-2,5′(4′*H*)-isoxazole] (5b): Yield: 86%, 90 mg; pale yellow oil; R_f = 0.39 (10% methanol/dichloromethane). ^{1}H NMR: δ = 1.32–1.78 (m, 5 H), 2.18 (dd, J = 4.3 and 14.7 Hz, 1 H), 3.65 (d, J = 16.1 Hz, 1 H), 3.57 (br. s, 1 H), 3.95 (d, J = 16.1 Hz, 1 H), 3.73 (br. s, 1 H), 7.34 (dd, J = 4.3 and 6.4 Hz, 1 H), 8.02 (d, J = 6.4 Hz, 1 H), 8.61 (d, J = 4.3 Hz, 1 H), 8.78 (s, 1 H) ppm.

5b·$C_4H_4O_4$: Colorless powder, m.p. 170–182 °C (dec); R_f = 0.30 (20% methanol/dichloromethane). ^{1}H NMR (CD_3OD): δ = 1.81 (m, 1 H), 2.00 (m, 3 H), 2.21 (d, J = 14.3 Hz, 1 H), 2.47 (dd, J = 4.0 and 14.3 Hz, 1 H), 3.59 (d, J = 17.9 Hz, 1 H), 3.81 (d, J = 17.9 Hz, 1 H), 4.18 (br. s, 1 H), 4.28 (br. s, 1 H), 6.68 (s, 2 H), 7.53 (dd, J = 4.4 and 7.0 Hz, 1 H), 8.16 (d, J = 7.0 Hz, 1 H), 8.61 (d, J = 4.4 Hz, 1 H), 8.84 (s, 1 H) ppm. $C_{17}H_{19}N_3O_5$ (345.35): calcd. C 59.12, H 5.55, N 12.17; found C 59.33, H 5.38, N 11.85.

3′-(Pyridin-4-yl)spiro[7-azabicyclo[2.2.1]heptane-2,5′(4′*H*)-isoxazole] (5c): Yield: 91%, 89 mg; yellow oil; R_f = 0.40 (15% methanol/dichloromethane). ^{1}H NMR: δ = 1.20–1.50 (m, 3 H), 1.60–1.78 (m, 2 H), 2.19 (dd, J = 5.4 and 13.9 Hz, 1 H), 3.24 (d, J = 15.0 Hz, 1 H), 3.38 (d, J = 15.0 Hz, 1 H), 3.54 (m, 1 H), 3.73 (m, 1 H), 7.47 (d, J = 5.4 Hz, 2 H), 8.63 (d, J = 5.4 Hz, 2 H) ppm.

5c·3/2$C_4H_4O_4$: Colorless powder, m.p. 176–182 °C; R_f = 0.30 (25% methanol/dichloromethane). ^{1}H NMR (CD_3OD): δ = 1.84 (m, 1 H), 2.01 (m, 3 H), 2.23 (d, J = 14.3 Hz, 1 H), 2.49 (dd, J = 5.1 and 14.3 Hz, 1 H), 3.58 (d, J = 17.9 Hz, 1 H), 3.79 (d, J = 17.9 Hz, 1 H), 4.21 (br. s, 1 H), 4.30 (br. s, 1 H), 6.71 (s, 3 H), 7.69 (d, J = 6.2 Hz, 2 H), 8.63 (d, J = 6.2 Hz, 2 H) ppm. $C_{13}H_{15}N_3O\cdot 3/2C_4H_4O_4$ (403.39): calcd. C 56.57, H 5.25, N 10.42; found C 56.88, H 5.02, N 10.69.

5-(Pyridin-2-yl)-3-oxa-4,10-diazatricyclo[5.2.1.0^{2,6}]dec-4-ene (6a): Yield: 89%, 83 mg; yellow oil; R_f = 0.47 (10% methanol/dichloromethane). ^{1}H NMR: δ = 1.27 (m, 2 H), 1.53 (m, 1 H), 1.71 (m, 1 H), 3.87 (m, 3 H), 4.83 (d, J = 7.7 Hz, 1 H), 7.29 (dd, J = 4.8 and 5.5 Hz, 1 H), 7.70 (dd, J = 5.5 and 8.1 Hz, 1 H), 7.97 (d, J = 8.1 Hz, 1 H), 8.61 (d, J = 4.8 Hz, 1 H) ppm.

6a·$C_4H_4O_4$: Colorless prisms, m.p. 182–183 °C; R_f = 0.44 (20% methanol/dichloromethane). ^{1}H NMR (CD_3OD): δ = 1.70 (m, 1

H), 1.90 (m, 3 H), 4.21 (d, $J_{2,3}$ = 8.5 Hz, 1 H, 2-H), 4.34 (br. s, 1 H), 4.45 (br. s, 1 H), 5.04 (d, $J_{3,2}$ = 8.5 Hz, 1 H, 3-H), 6.69 (s, 2 H), 7.44 (dd, *J* = 4.5 and 5.2 Hz, 1 H), 7.86 (dd, *J* = 5.2 and 7.7 Hz, 1 H), 8.01 (d, *J* = 7.7 Hz, 1 H), 8.64 (d, *J* = 4.5 Hz, 1 H). $C_{16}H_{17}N_3O_5$ (331.32): calcd. C 58.00, H 5.17, N 12.68; found C 58.29, H 4.86, N 12.88.

5-(Pyridin-3-yl)-3-oxa-4,10-diazatricyclo[5.2.1.0^{2,6}]dec-4-ene (6b): Yield: 92%, 77 mg; colorless oil; R_f = 0.31 (10% methanol/dichloromethane). ^{1}H NMR: δ = 1.29 (m, 2 H), 1.48 (m, 1 H), 1.73 (m, 1 H), 3.70 (d, $J_{2,3}$ = 8.0 Hz, 1 H, 2-H), 3.79 (m, 1 H), 3.91 (m, 1 H), 4.89 (d, $J_{3,2}$ = 8.0 Hz, 1 H, 3-H), 7.35 (dd, *J* = 3.4 and 7.3 Hz, 1 H), 8.08 (d, *J* = 7.3 Hz, 1 H), 8.64 (d, *J* = 3.4 Hz, 1 H), 8.87 (s, 1 H) ppm.

6b·3/2C$_4$H$_4$O$_4$: Colorless prisms, m.p. 192–194 °C; R_f 0.49 (20% methanol/dichloromethane). ^{1}H NMR (CD_3OD): δ = 1.70 (m, 1 H), 1.91 (m, 3 H), 4.25 (d, $J_{2,3}$ = 8.4 Hz, 1 H, 2-H), 4.30 (m, 2 H), 5.07 (d, $J_{3,2}$ = 8.4 Hz, 1 H, 3-H), 6.69 (s, 3 H), 7.54 (dd, *J* = 4.4 and 7.7 Hz, 1 H), 8.22 (d, *J* = 7.7 Hz, 1 H), 8.63 (d, *J* = 4.4 Hz, 1 H), 8.94 (s, 1 H) ppm. $C_{12}H_{13}N_3O·3/2C_4H_4O_4$ (389.36): calcd. C 55.52, H 4.92, N 10.79; found C 55.31, H 4.95, N 11.07.

5-(Pyridin-4-yl)-3-oxa-4,10-diazatricyclo[5.2.1.0^{2,6}]dec-4-ene (6c): Yield: 90%, 84 mg; pale yellow leaflets (from 40% ethyl acetate/hexane), m.p. 129–130 °C; R_f = 0.25 (10% methanol/dichloromethane). ^{1}H NMR: δ = 1.29 (m, 2 H), 1.50 (m, 1 H), 1.74 (m, 1 H), 3.69 (d, $J_{2,3}$ = 7.7 Hz, 1 H, 2-H), 3.75 (m, 1 H), 3.90 (m, 1 H), 4.92 (d, $J_{3,2}$ = 7.7 Hz, 1 H, 3-H), 7.56 (d, *J* = 5.8 Hz, 2 H), 8.68 (d, *J* = 5.8 Hz, 2 H) ppm.

6c·3/2C$_4$H$_4$O$_4$: Colorless prisms, m.p. 200–211 °C (dec); R_f = 0.28 (15% methanol/dichloromethane). ^{1}H NMR (CD_3OD): δ = 1.68 (m, 1 H), 1.91 (m, 3 H), 4.22 (d, $J_{2,3}$ = 8.5 Hz, 1 H, 2-H), 4.34 (br. s, 1 H), 4.38 (br. s, 1 H), 5.12 (d, $J_{3,2}$ = 8.5 Hz, 1 H, 1 H, 3-H), 6.69 (s, 3 H), 7.71 (d, *J* = 5.7 Hz, 2 H), 8.61 (d, *J* = 5.7 Hz, 1 H) ppm. $C_{12}H_{13}N_3O·3/2C_4H_4O_4$ (389.36): calcd. C 55.52, H 4.92, N 10.79; found C 55.80, H 4.71, N 10.55.

3-(Pyridin-2-yl)-4,5,6,6a-tetrahydro-3a*H*-pyrrolo[3,4-*d*]isoxazole (7a): Yield: 62%, 173 mg; yellow oil; R_f = 0.56 (20% methanol/dichloromethane). ^{1}H NMR: δ = 2.91 (dd, *J* = 3.1 and 14.6 Hz, 1 H), 3.11 (dd, *J* = 7.3 and 14.6 Hz, 1 H), 3.48 (m, 2 H), 4.35 (m, 1 H), 5.36 (dd, *J* = 3.1 and 7.3 Hz, 1 H), 7.28 (dd, *J* = 4.5 and 6.3 Hz, 1 H), 7.69 (dd, *J* = 6.3 and 7.3 Hz, 1 H), 8.02 (d, *J* = 7.3 Hz, 1 H), 8.62 (d, *J* = 4.5 Hz, 1 H) ppm.

7a·3/4C$_4$H$_4$O$_4$: Colorless powder, m.p. 158–162 °C (dec); R_f = 0.21 (20% methanol/dichloromethane). ^{1}H NMR (CD_3OD): δ = 3.27 (dd, *J* = 4.4 and 12.7 Hz), 3.38 (dd, *J* = 9.2 and 12.7 Hz, 1 H), 3.62 (m, 2 H), 4.57 (m, 1 H), 5.45 (dd, *J* = 4.4 and 9.2 Hz, 1 H), 6.67 (s, 1.5 H), 7.42 (dd, *J* = 4.8 and 6.1 Hz, 1 H), 7.85 (dd, *J* = 6.1 and 8.1 Hz, 1 H), 8.01 (d, *J* = 8.1 Hz, 1 H), 8.61 (d, *J* = 4.8 Hz, 1 H) ppm. $C_{10}H_{11}N_3O·3/4C_4H_4O_4$ (276.27): calcd. C 56.52, H 5.11, N 15.21; found C 56.73, H 4.82, N 15.50.

3-(Pyridin-3-yl)-4,5,6,6a-tetrahydro-3a*H*-pyrrolo[3,4-*d*]isoxazole (7b): Yield: 86%, 132 mg; yellow oil; R_f = 0.48 (20% methanol/dichloromethane). ^{1}H NMR: δ = 2.93 (dd, *J* = 3.7 and 13.6 Hz, 1 H), 3.10 (dd, *J* = 7.7 and 12.5 Hz, 1 H), 3.30 (d, *J* = 12.5 Hz, 1 H), 3.52 (d, *J* = 13.6 Hz, 1 H), 4.17 (dd, *J* = 7.7 and 8.4 Hz, 1 H), 5.39 (dd, *J* = 3.7 and 8.4 Hz, 1 H), 7.36 (dd, *J* = 4.8 and 8.1 Hz, 1 H), 8.07 (d, *J* = 8.1 Hz, 1 H), 8.64 (d, *J* = 4.8 Hz, 1 H), 8.84 (s, 1 H) ppm.

7b·3/2C$_4$H$_4$O$_4$: Colorless prisms, m.p. 182–185 °C (dec); R_f = 0.23 (20% methanol/dichloromethane). ^{1}H NMR (CD_3OD): δ = 3.45–3.55 (m, 3 H), 3.76 (d, *J* = 13.2 Hz, 1 H), 4.70 (m, 1 H), 5.52 (dd, *J* = 4.4 and 9.2 Hz, 1 H), 6.71 (s, 3 H), 7.54 (dd, *J* = 4.8 and 8.1 Hz, 1 H), 8.19 (d, *J* = 8.1 Hz, 1 H), 8.62 (d, *J* = 4.8 Hz, 1 H), 8.89 (s, 1 H) ppm. $C_{10}H_{11}N_3O·3/2C_4H_4O_4$ (363.33): calcd. C 52.89, H 4.72, N 11.56; found C 53.11, H 4.95, N 11.39.

3-(Pyridin-4-yl)-4,5,6,6a-tetrahydro-3a*H*-pyrrolo[3,4-*d*]isoxazole (7c): Yield: 78%, 125 mg; pale yellow oil; R_f = 0.56 (20% methanol/dichloromethane). ^{1}H NMR: δ = 2.92 (dd, *J* = 3.7 and 13.6 Hz, 1 H), 3.08 (dd, *J* = 7.7 and 12.5 Hz, 1 H), 3.26 (d, *J* = 12.5 Hz, 1 H), 3.52 (d, *J* = 13.6 Hz, 1 H), 4.12 (dd, *J* = 7.7 and 8.4 Hz, 1 H), 5.41 (dd, *J* = 3.7 and 8.4 Hz, 1 H), 7.53 (d, *J* = 5.3 Hz, 2 H), 8.67 (d, *J* = 5.3 Hz, 2 H) ppm.

7c·3/2C$_4$H$_4$O$_4$: Colorless prisms, m.p. 200–202 °C (dec); R_f = 0.41 (20% methanol/dichloromethane). ^{1}H NMR (CD_3OD): δ = 3.41 (dd, *J* = 4.7 and 13.2 Hz, 1 H), 3.50 (m, 2 H), 3.76 (d, *J* = 13.2 Hz, 1 H), 4.63 (m, 1 H), 5.57 (dd, *J* = 4.7 and 9.7 Hz, 1 H), 6.71 (s, 3 H), 7.74 (d, *J* = 5.8 Hz, 2 H), 8.65 (d, *J* = 5.8 Hz, 2 H) ppm. $C_{10}H_{11}N_3O·3/2C_4H_4O_4$ (363.33): calcd. C 52.89, H 4.72, N 11.56; found C 53.15, H 4.61, N 11.39.

Receptor Binding Assay

Membranes Binding of [^{3}H]-Epibatidine and [^{125}I]-α-Bungarotoxin: The cortex tissues were dissected, immediately frozen on dry ice and stored at –80 °C for later use. In each experiment, the cortex tissues from two rats were homogenized in 10 mL of a buffer solution (50 mM Na_3PO_4, 1 M NaCl, 2 mM EDTA, 2 mM EGTA and 2 mM PMSF, pH 7.4) using a potter homogenizer; the homogenates were then diluted and centrifuged at 60000 g for 1.5 h. The total membrane homogenization, dilution and centrifugation procedures were performed twice, then the pellets were collected, rapidly rinsed with a buffer solution (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$ and 2 mM PMSF, pH 7), and resuspended in the same buffer containing a mixture of 20 μg/mL of each of the following protease inhibitors: leupeptin, bestatin, pepstatin A, and aprotinin.

[^{3}H]-Epibatidine Binding: (±)-[^{3}H]-Epibatidine with a specific activity of 56–60 Ci/mmol was purchased from Perkin–Elmer (Boston MA); the nonradioactive α-bungarotoxin, nicotine, and epibatidine were purchased from Sigma. It has been previously reported that [^{3}H]-epibatidine also binds to α-bungarotoxin binding receptors with nM affinity.[10b] In order to prevent the binding of [^{3}H]-epibatidine to the α-bungarotoxin binding receptors, the membrane homogenates were pre-incubated with 2 μM α-bungarotoxin and then with [^{3}H]-epibatidine. The saturation experiments were performed by incubating aliquots of cortex membrane homogenates with 0.01–2.5 nM concentrations of (±)-[^{3}H]-epibatidine overnight at 4 °C. Nonspecific binding was determined in parallel by means of incubation in the presence of 100 nM unlabelled epibatidine. At the end of the incubation, the samples were filtered through a GFC filter soaked in 0.5% polyethylenimine and washed with 15 mL of a buffer solution (10 mM Na_3PO_4, 50 mM NaCl, pH 7.4) and the filters were counted in a β counter.

[^{125}I]-α-Bungarotoxin Binding: The saturation binding experiments were performed using aliquots of cortex membrane homogenates incubated overnight with 0.1–10 nM concentrations of [^{125}I]-α-bungarotoxin (specific activity 200–213 Ci/mmol, Amersham) at room temp. Non-specific binding was determined in parallel by means of incubation in the presence of 1 μM unlabelled α-bungarotoxin. After incubation, the samples were filtered as described above and the bound radioactivity was directly counted in a γ counter.

nACh Receptor Affinity of Derivatives 5a–c, 6a–c, and 7a–c: The inhibition of radioligand binding by epibatidine, nicotine and the test compounds was measured by pre-incubating cortex homoge-

nates with increasing doses (10 pM – 10 mM) of the reference nicotinic agonists, epibatidine or nicotine, and the drug to be tested for 30 min at room temp., followed by overnight incubation with a final concentration of 0.075 nM [^{3}H]-epibatidine or 1 nM [^{125}I]-α-bungarotoxin at the same temperatures as those used for the saturation experiments. These ligand concentrations were used for the competition binding experiments because they are within the range of the K_D values of the ligands for the two different classes of nAChRs. For each compound, the experimental data obtained from the three saturation and three competition binding experiments were analyzed by means of a non-linear least square procedure, using the LIGAND program as described by Munson and Rodbard.[24] The binding parameters were calculated by simultaneously fitting three independent saturation experiments and the K_i values were determined by fitting the data of three independent competition experiments. The errors in the K_D and K_i values of the simultaneous fits were calculated using the LIGAND software, and were expressed as percentage coefficients of variation (% CV). When final compound concentrations up to 200 μM did not inhibit radioligand binding, the K_i value was defined as being > 100 μM based on the Cheng and Prusoff's equation.[32]

Theoretical Calculations

Structures, built by GaussView03[33] and preliminarily minimized at semiempirical-PM3 level, were submitted to a geometry optimization at B3LYP/6-31G* level by the Gaussian03 package.[33] To avoid strong intramolecular electrostatic interactions, that would lead to unrealistic geometries during optimizations in vacuo, the azanorbonane nitrogen was considered not charged; such a method provides geometries that compare satisfactorily with those of the corresponding solvated ionized species.[34] We fully optimized all the starting geometries deriving from the pseudorotational path of the five-membered carbocyclic ring and from rotation around the exocyclic single bonds. The energies of the conformers were recalculated in a polarizable conductor-like continuum solvation model (C-PCM)[35] to obtain values conceivable for water solutions. Compounds **4a** and **6c** were manually overlapped by simple superimposition of the azanorbornane ring shared with (–)-epibatidine, oriented as in the cluster 1 of the binding orientation proposed by Westera et al.[30] The obtained dockings were evaluated and refined by SYBYL 7.1 provided by Tripos Inc (St. Louis, USA).

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[1] F. Dajas-Bailador, S. Wonnacott, *Trends Pharmacol. Sci.* **2004**, *25*, 317–324.
[2] a) C. P. Fenster, M. F. Rains, B. Noerager, M. W. Quick, R. A. J. Lester, *J. Neurosci.* **1997**, *17*, 5747–5759; b) Q. Nai, J. M. McIntosh, J. F. Margiotta, *Mol. Pharmacol.* **2003**, *63*, 311–324; c) C. Gotti, F. Clementi, *Progr. Neurobiol.* **2004**, *74*, 363–396; d) A. A. Jensen, B. Frølund, T. Liljefors, P. Krogsgaard-Larsen, *J. Med. Chem.* **2005**, *48*, 4705–4745.
[3] S. Fucile, *Cell Calcium* **2004**, *35*, 1–8.
[4] a) J. Lindström, *Mol. Neurobiol.* **1997**, *15*, 193–222; b) D. Paterson, A. Nordberg, *Progr. Neurobiol.* **2000**, *61*, 75–111; c) G. K. Lloyd, M. Williams, *J. Pharmacol Exp. Ther.* **2000**, *292*, 461–467.
[5] a) M. W. Holladay, M. J. Dart, J. K. Lynch, *J. Med. Chem.* **1997**, *40*, 4169–4194; b) R. A. Glennon, M. Dukat, *Pharm. Acta Helv.* **2000**, *74*, 103–114; c) M. W. Holladay, N. D. P. Cosford, I. A. McDonald, in: *Neuronal Nicotinic Receptors: Pharmacology and Therapeutic Opportunities* (Eds.: S. P. Arneric, J. D. Brioni), Wiley-Liss, New York, **1999**, pp. 253–270; d) J. E. Tønder, P. H. Olesen, *Curr. Med. Chem.* **2001**, *8*, 651–674; e) R. A. Glennon, M. Dukat, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1841–1844.
[6] J. D. Schmitt, *Curr. Med. Chem.* **2000**, *7*, 749–800.
[7] T. F. Spande, H. M. Garraffo, M. W. Edwards, H. J. C. Yeh, L. Pannell, J. W. Daly, *J. Am. Chem. Soc.* **1992**, *114*, 3475–3478.
[8] a) M. D. Meyer, M. W. Decker, L. E. Rueter, D. J. Anderson, M. J. Dart, K. H. Kim, J. P. Sullivan, M. Williams, *Eur. J. Pharmacol.* **2000**, *393*, 171–177; b) J. W. Daly, H. M. Garraffo, T. F. Spande, M. W. Decker, J. P. Sullivan, M. Williams, *Nat. Prod. Rep.* **2000**, *17*, 131–135; c) J. W. Daly, *J. Med. Chem.* **2003**, *46*, 445–452.
[9] T. C. Li, C. G. Qian, J. Eckman, D. F. Huang, T. Y. Shen, *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2759–2764.
[10] a) J. P. Sullivan, A. W. Bannon, *CNS Drug Rev.* **1996**, *2*, 21–39; b) V. Gerzanich, X. Peng, F. Wang, G. Wells, R. Anand, S. Fletcher, J. Lindstrom, *Mol. Pharmacol.* **1995**, *48*, 774–782; c) J. P. Sullivan, M. W. Decker, J. D. Brioni, D. L. Donnelly-Roberts, D. A. Anderson, A. W. Bannon, C. H. Kang, P. Adams, M. Piattoni-Kaplan, M. J. Buckley, M. Gopalakrishnan, M. Williams, S. P. Arneric, *J. Pharmacol. Exp. Ther.* **1994**, *271*, 624–631.
[11] a) F. I. Carroll, J. R. Lee, H. A. Navarro, L. E. Brieaddy, P. Abraham, M. I. Damaj, B. R. Martin, *J. Med. Chem.* **2001**, *44*, 4039–4041; b) F. I. Carroll, F. Liang, H. A. Navarro, L. E. Brieaddy, P. Abraham, M. I. Damaj, B. R. Martin, *J. Med. Chem.* **2001**, *44*, 2229–2237; c) F. I. Carroll, J. R. Lee, H. A. Navarro, W. Ma, L. E. Brieaddy, P. Abraham, M. I. Damaj, B. R. Martin, *J. Med. Chem.* **2002**, *45*, 4755–4761.
[12] a) Z.-L. Wei, Y. Xiao, C. George, K. J. Kellar, A. P. Kozikowski, *Org. Biomol. Chem.* **2003**, *1*, 3878–3881; b) C. G. V. Sharples, G. Karig, G. L. Simpson, J. A. Spencer, E. Wright, N. S. Millar, S. Wonnacott, T. Gallagher, *J. Med. Chem.* **2002**, *45*, 3235–3245; c) H. Gohlke, D. Gündisch, S. Schwarz, G. Seitz, M. C. Tilotta, T. Wegge, *J. Med. Chem.* **2002**, *45*, 1064–1072; d) H. Gohlke, S. Schwarz, D. Gündisch, M. C. Tilotta, A. Weber, T. Wegge, G. Seitz, *J. Med. Chem.* **2003**, *46*, 2031–2048.
[13] a) A. G. Horti, U. Scheffel, A. S. Kimes, J. L. Musachio, H. T. Ravert, W. B. Mathews, Y. Zhan, P. A. Finley, E. D. London, R. F. Dannals, *J. Med. Chem.* **1998**, *41*, 4199–4206; b) C. D. Cox, J. R. Malpass, J. Gordon, A. Rosen, *J. Chem. Soc., Perkin Trans. 1* **2001**, 2372–2379.
[14] a) Z. L. Wei, Y. Xiao, K. J. Kellar, A. P. Kozikowski, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1855–1858; b) Z.-L. Wei, P. A. Petukhov, Y. Xiao, W. Tückmantel, C. George, K. J. Kellar, A. P. Kozikowski, *J. Med. Chem.* **2003**, *46*, 921–924; c) H. Abe, Y. Arai, S. Aoyagi, C. Kibayashi, *Tetrahedron Lett.* **2003**, *44*, 2971–2973; d) L. E. Brieaddy, S. W. Mascarella, H. A. Navarro, R. N. Atkinson, M. I. Damaj, B. R. Martin, F. I. Carroll, *Tetrahedron Lett.* **2001**, *42*, 3795–3797.
[15] J. E. Tønder, P. H. Olesen, J. B. Hansen, M. Begtrup, I. Pettersson, *J. Comput.-Aided Mol. Des.* **2001**, *15*, 247–258.
[16] M. N. Romanelli, F. Gualtieri, *Med. Res. Rev.* **2003**, *23*, 393–426.
[17] a) B. Badio, D. Shi, M. Garraffo, J. W. Daly, *Drug Dev. Res.* **1995**, *36*, 46–59; b) M. I. Damaj, W. Glassco, M. Dukat, E. L. May, R. A. Glennon, B. R. Martin, *Drug Dev. Res.* **1996**, *38*, 177–187.
[18] S. Kanemasa, H. Matsuda, A. Kamimura, T. Kakinami, *Tetrahedron* **2000**, *56*, 1057–1064.
[19] C. Zhang, M. L. Trudell, *J. Org. Chem.* **1996**, *61*, 7189–7191.
[20] a) S. Brandänge, B. Rodriguez, *Synthesis* **1988**, 347–348; b) P. Conti, C. Dallanoce, M. De Amici, C. De Micheli, R. Fruttero, *Tetrahedron* **1999**, *55*, 5623–5634.

[21] a) C. De Micheli, R. Gandolfi, R. Oberti, *J. Org. Chem.* **1980**, *45*, 1209–1213; b) P. Caramella, P. Grünanger, in: *1,3-Dipolar Cycloaddition Chemistry*, vol. 3 (Ed.: A. Padwa), John Wiley & Sons, Inc., **1984**, pp. 291–392; c) R. Gandolfi, G. Tonoletti, A. Rastelli, M. Bagatti, *J. Org. Chem.* **1993**, *58*, 6038–6048.
[22] a) R. J. Baker, S. Chiu, C. Klein, J. W. Timberlake, L. M. Trefonas, R. Majesté, *J. Org. Chem.* **1980**, *45*, 482–485; b) L. Dal Bo, M. De Amici, C. De Micheli, R. Gandolfi, K. N. Houk, *Tetrahedron Lett.* **1989**, *30*, 807–810.
[23] a) D. P. Curran, *J. Am. Chem. Soc.* **1983**, *105*, 5826–5833; b) H. C. Brown, W. J. Hammar, J. H. Kawakami, I. Rothberg, D. L. Vander Jagt, *J. Am. Chem. Soc.* **1967**, *89*, 6381–6382.
[24] P. J. Munson, D. Rodbard, *Anal. Biochem.* **1980**, *107*, 220–239.
[25] W. H. Beers, E. Reich, *Nature* **1970**, *228*, 917–922.
[26] R. P. Sheridan, R. Nilakantana, J. S. Dixon, R. Venkataraghavan, *J. Med. Chem.* **1986**, *29*, 899–906.
[27] R. A. Glennon, J. L. Herndon, M. Dukar, *Med. Chem. Res.* **1994**, *4*, 461–473.
[28] M. A. Abreo, N.-H. Lin, D. S. Garvey, D. E. Dunn, A.-M. Hettinger, J. T. Wasicak, P. A. Pavlik, M. Y. C. Artin, D. L. Donnelly-Roberts, D. J. Anderson, J. P. Sullivan, M. Williams, S. P. Arneric, M. W. Holladay, *J. Med. Chem.* **1996**, *39*, 817–825.
[29] L. Toma, D. Barlocco, A. Gelain, *Expert Opin. Ther. Pat.* **2004**, *14*, 1029–1040.
[30] W. H. Bisson, L. Scapozza, G. Westera, L. Mu, P. A. Schubiger, *J. Med. Chem.* **2005**, *48*, 5123–5130.
[31] A. C. Wallace, R. A. Laskowski, T. J. M. Hornton, *Protein Eng.* **1995**, *8*, 127–134.
[32] Y.-C. Cheng, W. H. Prusoff, *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
[33] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery Jr, T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, *Gaussian 03*, Revision B.05, Gaussian, Inc., Wallingford, CT, **2004**.
[34] P. A. Nielsen, T. Liljefors, *J. Comput. Aided Mol. Des.* **2001**, *15*, 753–763.
[35] V. Barone, M. Cossi, *J. Phys. Chem. A* **1998**, *102*, 1995–2001.

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