Bioorganic & Medicinal Chemistry 18 (2010) 4498–4508

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Novel tricyclic Δ^2-isoxazoline and 3-oxo-2-methyl-isoxazolidine derivatives: Synthesis and binding affinity at neuronal nicotinic acetylcholine receptor subtypes

Clelia Dallanoce [a,*], Fabio Frigerio [a], Giuliana Martelli [a], Giovanni Grazioso [a], Carlo Matera [a], Diego Yuri Pomè [a], Luca Pucci [b], Francesco Clementi [b], Cecilia Gotti [b], Marco De Amici [a]

[a] Dipartimento di Scienze Farmaceutiche 'Pietro Pratesi', Università degli Studi di Milano, Via Mangiagalli 25, 20133 Milano, Italy
[b] CNR, Istituto di Neuroscienze, Farmacologia Cellulare e Molecolare e Dipartimento Farmacologia, Chemioterapia e Tossicologia Medica, Università degli Studi di Milano, Via Vanvitelli 32, 20129 Milano, Italy

ARTICLE INFO

Article history:
Received 2 December 2009
Revised 20 April 2010
Accepted 21 April 2010
Available online 27 April 2010

Keywords:
Δ^2-Isoxazoline and 3-oxo-isoxazolidine derivatives
Neuronal nicotinic acetylcholine receptors
Cytisine and ferruginine analogues
Binding affinity
Molecular modeling

ABSTRACT

A group of novel tricyclic Δ^2-isoxazolines (**4b**, **5b**, **7a–b**, and **8a–b**) and 3-oxo-isoxazolidines (**6a–b** and **9a–b**), structurally related to cytisine or norferruginine, was prepared through 1,3-dipolar cycloadditions involving suitable olefins and bromonitrile oxide. The target compounds were assayed at α4β2 and α7 neuronal acetylcholine receptors (nAChRs). The results of competition binding experiments indicated for the new derivatives a reduction of the affinity at the α4β2 subtype in comparison with the reference molecules, coupled with an overall negligible affinity at the α7 subtype. The binding mode of the bromo-Δ^2-isoxazolines **4b** and **7b**, which were the highest affinity ligands in the series (K_i = 0.92 and 0.75 μM, respectively), was analyzed by applying a recently developed model of the α4β2 nAChRs.

1. Introduction

Acetylcholine receptors have been historically subdivided into muscarinic and nicotinic receptors based on selective activation by two natural products, muscarine and nicotine, respectively.[1] Over the last two decades, research efforts in this field have been mainly addressed to natural as well as synthetic nicotinic ligands, and took advantage of the characterization of nicotinic acetylcholine receptors (nAChRs) as major modulators of neuronal excitability throughout the central nervous system (CNS).[2–4] These receptors belong to the superfamily of ligand-gated ion channels and are formed by pentameric combinations of nine α (α2–α10) and three β (β2–β4) subunits, whose association brings about differences in structural, pharmacological, and functional properties.[5–7] Indeed, ligands selective for specific nAChR subtypes in the CNS have been suggested to hold promise for therapeutic intervention in a range of pathologies, among them Alzheimer's and Parkinson's diseases, epilepsy, schizophrenia, Tourette's syndrome, attention deficit/hyperactivity disorder, and nicotine addiction.[5–10]

As far as the structure of naturally occurring nAChR ligands is taken into account, several azabicyclic derivatives were reported to activate nicotinic receptor populations.[11] (−)-Cytisine **1** (Fig. 1), a plant alkaloid originally isolated from the golden chain tree (*Laburnum anagyroides*),[12,13] and (+)-ferruginine **2** (Fig. 1), a compound extracted from the arboreal species *Darlingia ferruginea*,[14] have been among the studied nicotinic ligands. Worth mentioning, (+)-**2** had a negligible affinity for the most abundant heteropentameric α4β2 neuronal nAChR, whereas its synthetic antipode (−)-ferruginine **2** showed about 60-fold higher affinity for the same receptor subtype.[11,15,16] When the 'unnatural' *nor*-analogue (−)-**3** (Fig. 1) was prepared and tested, its affinity at α4β2 receptors matched that showed by (−)-**2**.[11,15,16]

A charged nitrogen atom and a hydrogen bond acceptor-π (HBA-π) moiety,[2] that is an aromatic or conjugated carbonyl function, in an appropriate spatial arrangement are the essential structural motifs characterizing the most populated class of nAChR ligands, including compounds (−)-**1** and (−)-**3**. However, the development of derivatives with a pronounced selectivity for a specific receptor subtype has been hampered by the relatively high number of nAChRs and the slight differences in their structures. In addition, coexpression of multiple nAChR subtypes in most neurons could have complex effects on neurotransmitter release probability.[4,8]

Among the various natural compounds tested as potential nicotinic drugs, cytisine (−)-**1** has been exploited as a template for the

* Corresponding author. Tel.: +39 02 50319327; fax: +39 02 50319326.
E-mail address: clelia.dallanoce@unimi.it (C. Dallanoce).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.04.065

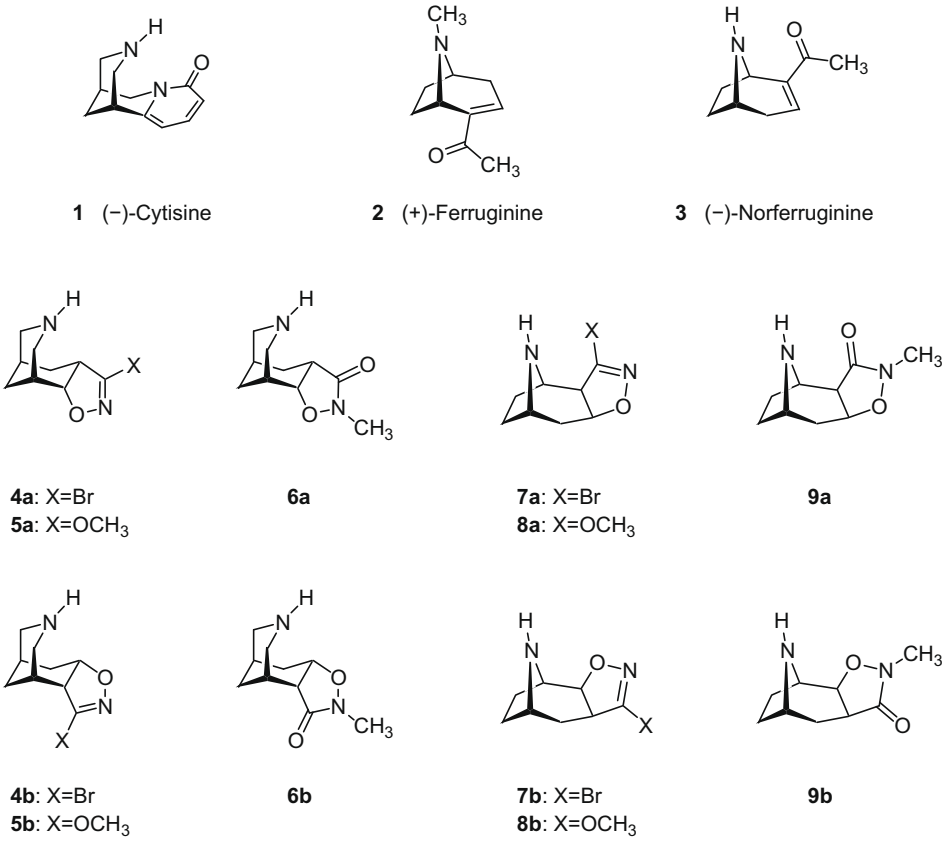


Figure 1. Structures of (−)-cytisine, (+)-ferruginine, (−)-norferruginine, and the compounds investigated in this study.

synthesis of structural analogues.[17] In terms of affinity, (−)-**1** selectively binds α4β2 nAChRs, behaving as a partial agonist at β2-containing channels when its functional responses are compared with those of acetylcholine and nicotine.[18,19] The main structural variations to the cytisine skeleton aimed at creating novel analogues with improved subtype selectivity and/or pharmacokinetic properties. These modifications involved primarily the introduction of different substituents on the aromatic 2-pyridone ring[17,20–23] and on the basic alicyclic nitrogen.[11,24,25] On the other hand, a group of structural analogues of norferruginine (−)-**3** was synthesized an tested, in which the methylketone function was replaced by a few heteroaromatic moieties.[16]

As an extension of our ongoing investigations on heterocyclic ligands of neuronal nAChRs,[26–29] we designed novel Δ^2-isoxazolines and 3-oxo-isoxazolidines incorporating the 3-azabicyclo-[3.3.1]nonane skeleton of cytisine, that is, compounds **4a–6a** and **4b–6b**, and the 8-azabicyclo[3.2.1]octane nucleus of norferruginine, that is, compounds **7a–9a** and **7b–9b**. Thus, we aimed at analyzing the effect produced by changes in conformation between the two essential pharmacophoric elements, that is, the hydrogen bond acceptor moiety and the protonatable center, on the affinity profile of the new derivatives at neuronal nAChR subtypes.

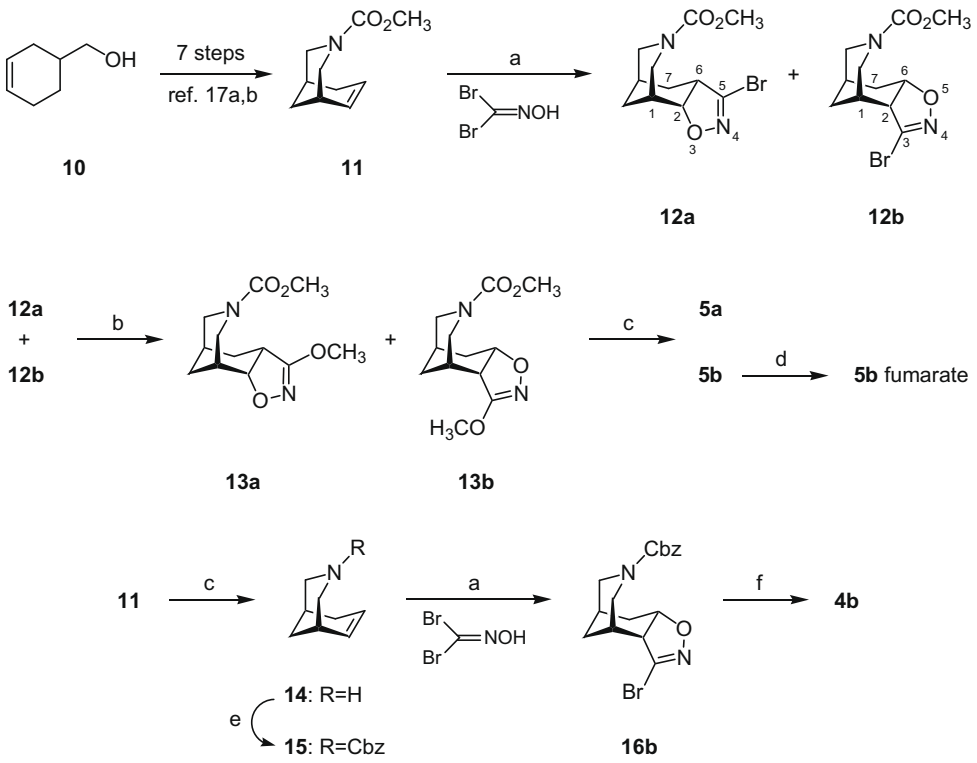
This paper describes the synthetic approach to the racemic forms of compounds **4a–9a** and **4b–9b**, the assessment of their binding at the α4β2 and α7 nAChR subtypes, and the estimation of their affinity in the light of molecular modeling studies.

2. Results and discussion

The desired final compounds were all synthesized through the initial 1,3-dipolar cycloaddition of bromonitrile oxide,[30] generated in situ by treatment of dibromoformaldoxime with solid $NaHCO_3$, to an appropriate unsaturated partner. At first, methyl 3-azabicyclo[3.3.1]non-6-ene 3-carboxylate **11** was prepared following a known reaction sequence,[31,32] which started from the commercially available 3-cyclohexene-1-methanol **10** (Scheme 1). Bromonitrile oxide added to the less hindered face of bicyclic olefin **11**, yielding the two regioisomeric *exo*-cyloadducts **12a** and **12b** exclusively (for details on the *exo*/*endo* notation see Refs. 33 and 34). This outcome was confirmed by DFT/b3lyp/6-31g* calculations with the GAUSSIAN03 program package,[35] since the energy of the *exo*-cycloadducts was about 2.6 kcal/mol lower than that of the *endo*-cycloadducts. Similarly, the transition state (TS) leading to the *exo*-cycloadducts was characterized by a 3.7 kcal/mol lower energy content than the TS of the corresponding *endo*-cycloadducts (Fig. S1 as Supplementary data).

Unfortunately, we were unable to separate the two *exo*-regioisomers, whose relative amount was inferred by identifying meaningful signals in the ^{1}H NMR spectrum of the column chromatographed mixture of the two cycloadducts. In the major regioisomer **12b**, the H-2 proton (Scheme 1) resonated as a doublet (J = 9.6 Hz) at 3.37 ppm, whereas the corresponding H-2 proton of the minor regioisomer **12a** (d, J = 8.3 Hz) resonated at 4.46 ppm. As a consequence, the pericyclic reaction produced **12a** and **12b** in about a 1:5 ratio.

The mixture of bromo-Δ^2-isoxazolines **12a** and **12b** was treated with a suspension of K_2CO_3 in methanol under reflux to afford a mixture of the corresponding methoxy derivatives **13a** and **13b**, which, once again, could not be separated by preparative chromatography (Scheme 1). Cleavage of the methoxycarbonyl protective group by reaction with hydrazine hydrate and KOH in refluxing ethanol led to the free bases **5a** and **5b**. Only the major regioisomer **5b** was isolated as a pure compound, which was converted into the corresponding fumarate.



Scheme 1. Reagents and conditions: (a) $NaHCO_3$, AcOEt; (b) K_2CO_3, MeOH, reflux; (c) NH_2NH_2/KOH, EtOH; (d) $C_4H_4O_4$, MeOH; (e) $ClCO_2CH_2C_6H_5$/TEA-DMAP, THF; (f) $(Me)_3SiI$, CH_3CN.

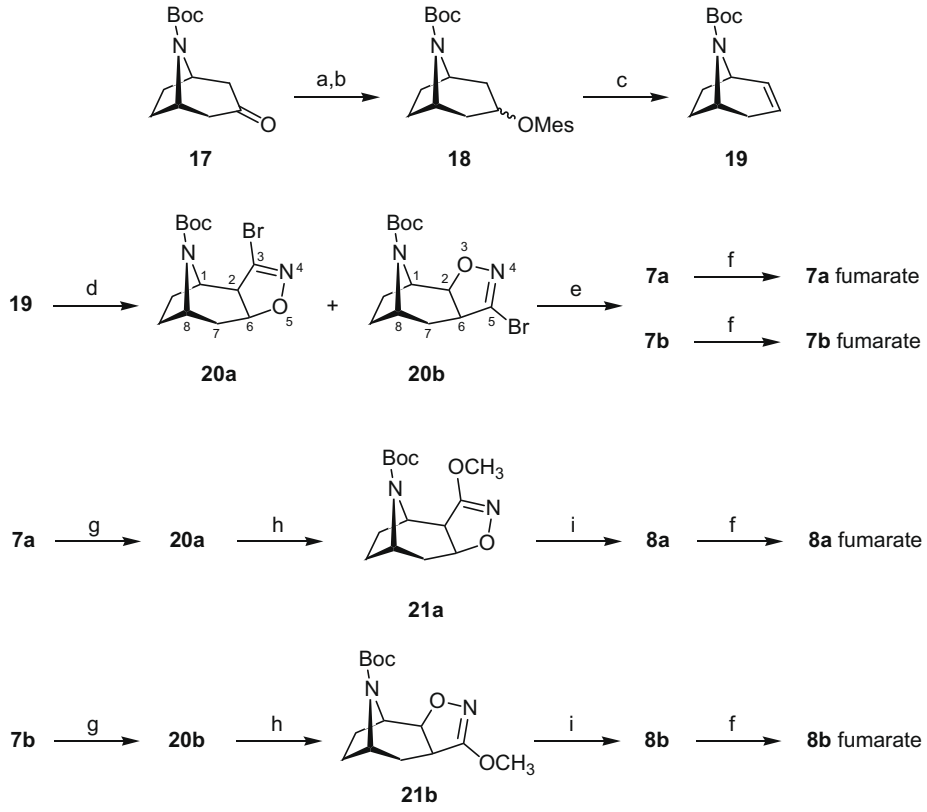
We synthesized also dipolarophile **15**, whose reaction with bromonitrile oxide produced the *N*-Cbz protected *exo* cycloadduct **16b** as the sole regioisomer (Scheme 1). Hence, we could isolate the corresponding fused bromo-Δ^2-isoxazoline **4b** as a crystalline derivative upon treatment of **16b** with trimethylsilyl iodide in acetonitrile.

With a parallel reaction sequence, we synthesized *N*-Boc-nortropene **19**[36,37] by standard conversion of known 3-oxo-8-azabicyclo[3.2.1]octane-8-carboxylic acid *tert*-butyl ester **17**[38] into mesylate **18** (*exo*/*endo* isomeric mixture),[39] followed by reaction with DBU (Scheme 2). Consistent with the behavior of similar dipolarophiles,[26] the pericyclic reaction involving **19** brought about the *exo* addition of bromonitrile oxide, with formation of regioisomers **20a** and **20b** in a 3:1 relative ratio (Scheme 2), as estimated from the ^{1}H NMR spectrum of the purified mixture of cycloadducts (see Section 4). The two isomers were separated by flash chromatography only after acidic treatment, and the resulting free bases **7a** and **7b** were converted into the corresponding fumarates or retransformed into the individual *N*-Boc derivatives **20a** and **20b**. The latter compounds produced the two regiomeric methoxy-Δ^2-isoxazolines **21a** and **21b**, which afforded the free bases **8a** and **8b**, and their corresponding crystalline fumarates (Scheme 2).

Finally, we prepared the two couples of 2-methyl-isoxazolidin-3-ones **6a–6b** and **9a–9b** as summarized in Scheme 3. In both cases, we made use of either the mixture of bromo-Δ^2-isoxazolines **12a/12b** (Scheme 1) or their related analogues **20a** and **20b** (Scheme 2), which were reacted with a NaOH suspension in acetone under microwave heating. This treatment allowed chromatographic separation of the intermediate hydroxyl-substituted regioisomers **22a** and **22b** bearing the 3-azabicyclo[3.3.1]nonane nucleus and, in addition to the desired substitution reaction, caused the *N*-Boc cleavage of **20a** and **20b** having the 8-azabicyclo[3.2.1]octane skeleton. Hence, the same protective group was promptly reinserted to give rise to regioisomers **24a** and **24b**. The two couples of 3-oxo-2-methyl-isoxazolidines (**23a** and **23b**, **25a** and **25b**) were then obtained by reaction of their precursors (**22a**, **22b**, and **24b**) with methyl iodide in the presence of K_2CO_3 or, in the case of **24a**, by treatment with lithium diisopropylamide.[40,41] The desired crystalline fumarates of **6a**, **6b**, **9a**, and **9b** were then prepared and tested.

The target compounds **4b**, **5b**, **6a–9a**, and **6b–9b** were assessed for their binding affinity to α4β2 and α7 rat nAChR subtypes using [^{3}H]epibatidine and [^{125}I]α-bungarotoxin as radioligands, respectively. The K_i values were calculated from the competition curves by means of the LIGAND program.[42] Inspection of the data reported in Table 1 shows that fusion of the Δ^2-isoxazoline and isoxazolidin-3-one moieties with the azabicyclic skeletons of both cytisine and norferruginine is detrimental for the affinity at the α4β2 nAChRs. Indeed, the K_i value of (−)-cytisine at this subtype (0.40 nM) is more than three orders of magnitude lower than the K_i values (from 0.92 to 79 μM) determined for its related ligands **4b**, **5b**, and **6a**–**b**. Conversely **7b**, the best α4β2 ligand among the norferruginine-related compounds **7a**–**b**, **8a–b**, **9a–b**, displayed a K_i value (0.75 μM), which is about one order of magnitude higher than that known (0.094 μM)[11] for (−)-norferruginine. In addition, the novel studied compounds had K_i values at the homomeric α7 subtype which were generally higher but comparable with those observed at the heteromeric α4β2 receptors. When tested at final concentrations up to 200 μM, derivatives **6b**, **9a**, and **9b** did not inhibit radioligand binding to the α7 subtype. Hence, their K_i values have been indicated as higher than 100 μM.

Furthermore, the nature of the substituent on the Δ^2-isoxazoline nucleus, that is bromine (**4b**, **7a**, and **7b**) or methoxy (**5b**, **8a**, and **8b**), and the variation of the fused ring, that is insertion of the isoxazolidin-2-methyl-3-one moiety (**6a–b** and **9a–b**), marginally affected the molecular recognition process at both investigated receptor subtypes. Thus, the relatively high K_i values of the new



Scheme 2. Reagents and conditions: (a) $NaBH_4$, MeOH; (b) CH_3SO_2Cl/TEA, CH_2Cl_2; (c) DBU, toluene; (d) $Br_2C{=}NOH/NaHCO_3$, AcOEt; (e) 4 N HCl/dioxane; (f) $C_4H_4O_4$, MeOH; (g) $(Boc)_2O$/TEA, CH_2Cl_2; (h) K_2CO_3, MeOH, reflux; (i) CF_3CO_2H, CH_2Cl_2.

derivatives, in particular those observed at the α4β2 subtype, should be the result of a poor conformational fitting within the receptor binding cleft.

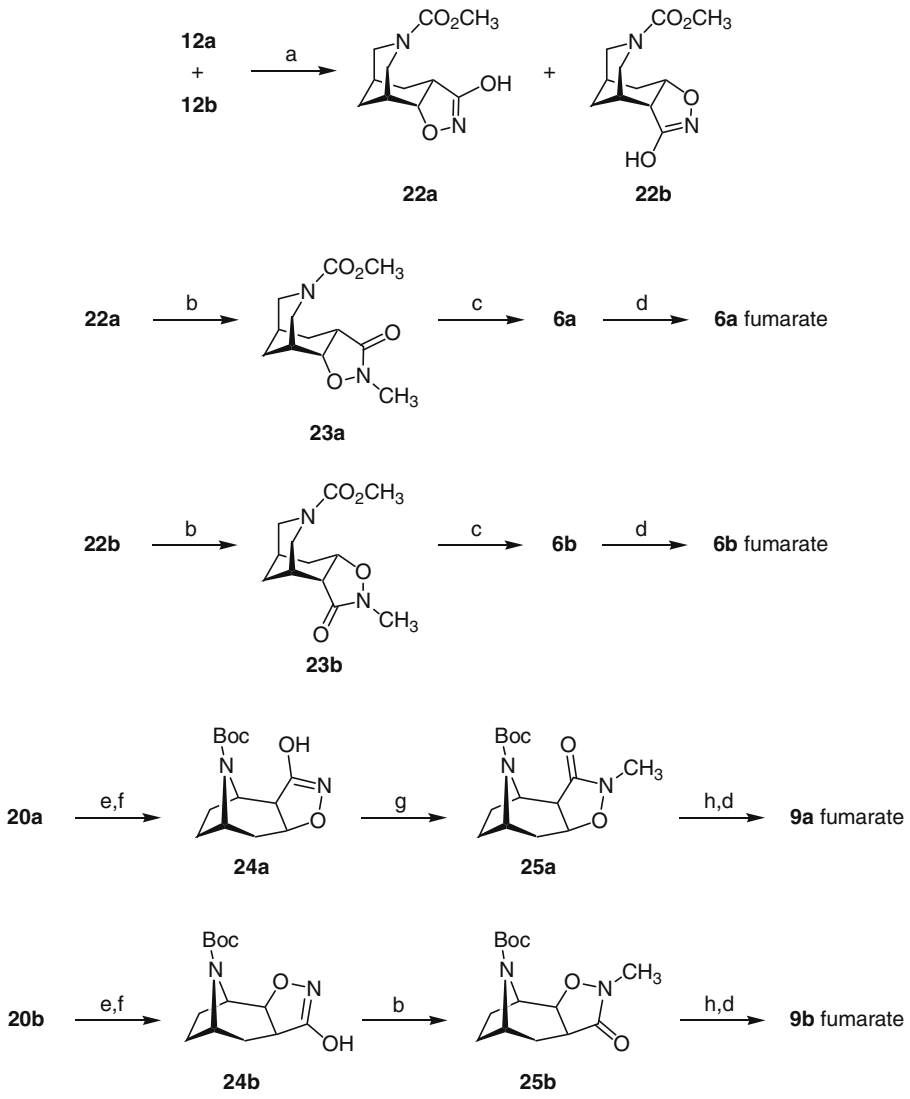
The binding modes of the two bromo-Δ^2-isoxazolines **4b** and **7b**, that is, the compounds with the highest affinity for the α4β2 nAChRs (K_i equal to 0.92 and 0.75 μM, respectively), were compared with those of their corresponding reference derivatives. To this end, we made use of a recently published molecular model for this receptor subtype, which allowed the theoretical evaluation of the binding free energies for a set of α4β2 selective nicotinic ligands.[43] The binding mode of (−)-cytisine **1** (Fig. 2A, cyan) was found to be similar to that of reference agonists such as epibatidine and nicotine in locating the cationic head close to the indole ring of α4-Trp147.[43] The ligand–receptor interaction was further strengthened by formation of H-bonds between the same protonated nitrogen and (a) the α4-Trp147 carbonyl group, and (b) the side chain of α4-Tyr91. An additional stabilization in the receptor binding pocket involved the H-bond between the cytisine carbonyl group and the side chain of α4-Tyr195. We investigated the three-dimensional structure of compounds **4b–6b** (as an example the geometry of **4b** is reported in Fig. S2 as Supplementary data). If we hypothesize a binding mode similar to that of (−)-cytisine, the Δ^2-isoxazoline oxygen, which is the putative H-bond acceptor site of regioisomers **4b**, **5b**, and **6b**, is 3.9 Å far from the side chain of α4-Tyr195. In addition, the rigidity of the molecular skeleton hinders the adjustment of these ligands in the binding cleft selected by (−)-cytisine.

For the best docked enantiomer of **4b** (Fig. 2A, green), we suggest that the same H-bond network of the (−)-cytisine basic moiety should be operative. Such an orientation in the binding cleft of the α4β2 receptor favors a correct insertion of the bromine on the Δ^2-isoxazoline nucleus in a hydrophobic pocket formed at the interface of β2-Phe117, β2-Leu119 and α4-Cys190. This additional interaction might in part account for the highest affinity of **4b** when compared with **5b** and **6b**. The lowest affinity shown by **6b** may be attributed to the presence of the *N*-Me group leading to a steric clash with the β2-Phe117 side chain.

At variance with the results on the cytisine-related derivatives, the docking calculations on the two enantiomers of **7b** showed that they both could adopt a binding mode similar to that predicted for their reference compound (−)-norferruginine, that is, a cation-π and a H-bond interaction between the charged nitrogen of the ligand and the indole ring and the carbonyl group of α4-Trp147, respectively (Fig. 2B). The insertion of (−)-norferruginine in the receptor binding cleft is further stabilized by a H-bond between the ligand carbonyl function and the side chain of α4-Tyr91. This supplementary H-bond stabilization is precluded to compounds **7b–9b** due to a higher distance (3.7 Å) between the oxygen of the Δ^2-isoxazoline ring and the phenol of α4-Tyr91. However, the overall analogy in the binding mode with the reference molecule coupled with the fruitful projection of the bromine of **7b** in the hydrophobic area near β2-Trp55 (located at the α4–β2 interface) might explain its partially conserved affinity at the α4β2 subtype, with a K_i value about one order of magnitude higher than that reported for (−)-norferruginine. Replacement of bromine with a methoxy group, that is, Δ^2-isoxazoline **8b**, causes some steric clash in the direction of the side chain of β2-Trp55. This hindrance turns out to be critical when the methyl group is directly linked to the ring nitrogen, as in the isoxazolidin-3-one derivative **9b**.

3. Conclusions

New Δ^2-isoxazoline and isoxazolidin-3-one derivatives, designed taking cytisine and norferruginine as model compounds, were synthesized by applying a 1,3-dipolar cycloaddition-based



Scheme 3. Reagents and conditions: (a) 1.5 N NaOH, acetone (MW, 100 W, 80 °C, 5 min); (b) K_2CO_3/CH_3I, acetone; (c) $(Me)_3SiI$, $CHCl_3$; (d) $C_4H_4O_4$, MeOH; (e) 1.5 N NaOH, acetone (MW, 120 W, 120 °C, 20 min); (f) $(Boc)_2O$, 1.5 N NaOH; (g) LDA/CH_3I, THF; h: CF_3CO_2H, CH_2Cl_2.

Table 1
Affinity of derivatives **4b**, **5b**, **6a–b**, **7a–b**, **8a–b**, and **9a–b** for native α4β2 and α7 nAChR subtypes present in rat cortical membranes, labeled by [^{3}H]epibatidine and [^{125}I]α-bungarotoxin

Compd	α4β2 [^{3}H]Epibatidine (K_i, μM)	α7 [^{125}I]α-BgTx (K_i, μM)
4b	0.92 (39)	28.5 (42)
5b·**1/2C$_4$H$_4$O$_4$**	31 (75)	170 (67)
6a·**3/4C$_4$H$_4$O$_4$**	1.8 (30)	9.5 (56)
6b·**3/4C$_4$H$_4$O$_4$**	79 (68)	>100
7a·**3/4C$_4$H$_4$O$_4$**	260 (57)	109 (33)
7b·**C$_4$H$_4$O$_4$**	0.75 (46)	20 (65)
8a·**1/2C$_4$H$_4$O$_4$**	53 (80)	68 (74)
8b·**C$_4$H$_4$O$_4$**	4.5 (50)	28.2 (29)
9a·**1/2C$_4$H$_4$O$_4$**	50 (36)	>100
9b·**3/4C$_4$H$_4$O$_4$**	100 (44)	>100
(−)-Cytisine **1**	0.40 nM (25)[a]	0.30 (28)[a]
(−)-Norferruginine **3**	0.094[b]	>10[b]
(±)-Epibatidine	0.035 nM[c]	10.2 nM[c]
α-BgTx	n.d.[d]	0.9 nM[a]

Numbers in brackets represent %coefficients of variation.
[a] This paper.
[b] Ref. 11.
[c] Ref. 49.
[d] Not determined.

approach. Competition binding data at neuronal nicotinic acetylcholine receptors showed that the novel tricyclic compounds, particularly those related to cytisine, showed a meaningful reduction of the affinity at the α4β2 subtype when compared to their reference molecules. We studied the geometrical features of the 3-bromo-Δ^2-isoxazolines **4b** and **7b**, that is, the derivatives in the series endowed with the highest affinity (K_i values in the low micromolar range) at the α4β2 receptors, and the two compounds were then docked into a molecular model for this subtype. This analysis put in evidence that, for **4b**, the drop in affinity has to be attributed to a different molecular recognition of this ligand and cytisine by the receptor protein. Conversely, we found that the binding mode of **7b** is similar to that adopted by its reference compound norferruginine, which could account for the less severe reduction of affinity.

4. Experimental section

4.1. Chemistry

4.1.1. Materials and methods

Dibromoformaldoxime,[30] methyl 3-azabyciclo[3.3.1]non-6-ene-3-carboxylate **11**,[32] 3-azabyciclo[3.3.1]non-6-ene **14**,[32] and

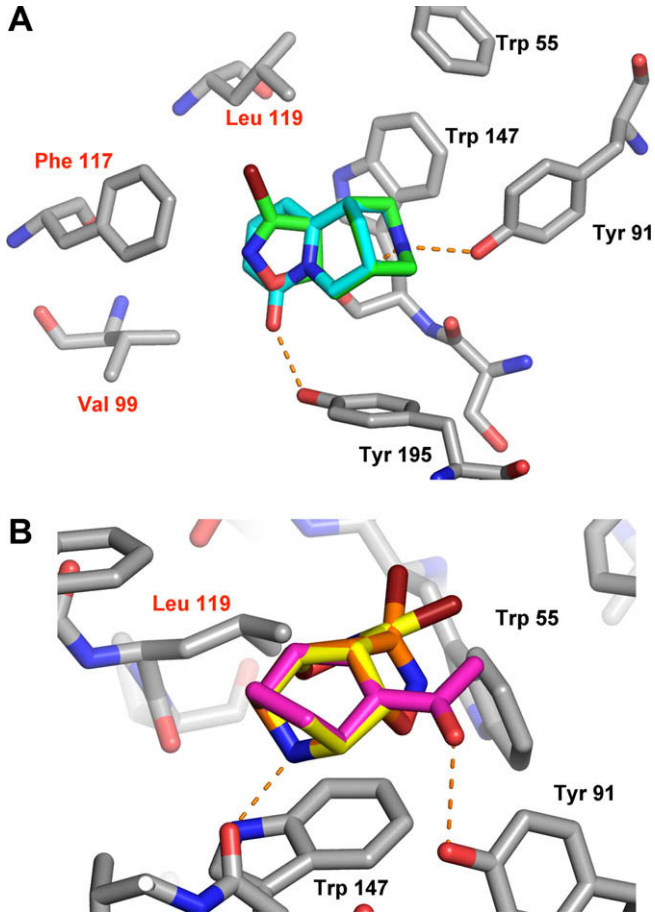


Figure 2. Binding of compounds **4b** (A) and **7b** (B) in the active site of heteromeric α4β2 nAChR. Residues from α4 and β2 subunits are labeled in black and red, respectively. Receptor model residues are depicted as stick model, and carbon atoms are colored in gray. Ligands are colored according to the following atom color code: carbon atoms are in green for the best docked enantiomer of **4b** and cyan for (−)-cytisine (A), yellow and orange for both enantiomers of **7b**, and magenta for (−)-norferruginine (B). Some residues have been omitted for sake of clarity.

tert-butyl 3-oxo-8-azabicyclo[3.2.1]octane-8-carboxylate **17**[38] were prepared according to literature procedures. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Mercury 300 (^{1}H, 300.063; ^{13}C, 75.451 MHz) spectrometer in $CDCl_3$ solutions (unless otherwise indicated) at 20 °C. ESI Mass spectra were obtained on a Varian 320 LC–MS–MS instrument (Supplementary data). Data are reported as mass-to-charge ratio (*m/z*). TLC analyses were performed on commercial Silica Gel 60 F_{254} aluminum sheets, and spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution. Microwave oven reactions were performed using a CEM Discovery apparatus. Melting points were determined on a model B 540 Büchi apparatus and are uncorrected. Microanalyses (C, H, N) of new compounds agreed with the theoretical value within ±0.4%.

4.1.2. Methyl 5-bromo-3-oxa-4,10-diazatricyclo[6.3.1.0^{2,6}]dodec-4-ene-10-carboxylate (12a) and Methyl 3-bromo-5-oxa-4,10-diazatricyclo[6.3.1.0^{2,6}]dodec-3-ene-10-carboxylate (12b)

A suspension of alkene **11**[31,32] (1.82 g, 10 mmol), $NaHCO_3$ (10.92 g, 0.130 mol), and dibromoformaldoxime (2.23 g, 5.50 mmol) in ethyl acetate (50 mL) was stirred at room temperature for 2 days. The progress of the reaction was monitored by TLC (20% ethyl acetate/petroleum ether). The solid was filtered off, the solvent was removed under reduced pressure and the crude residue was purified by silica gel column chromatography (10% ethyl acetate/petroleum ether), which afforded 2.70 g (89% yield) of a mixture of cycloadducts **12a** and **12b** (R_f = 0.40, 20% ethyl acetate/*n*-hexane) as a yellow viscous oil.

4.1.3. Methyl 5-methoxy-3-oxa-4,10-diazatricyclo[6.3.1.0^{2,6}]dodec-4-ene-10-carboxylate (13a) and Methyl 3-methoxy-5-oxa-4,10-diazatricyclo[6.3.1.0^{2,6}]dodec-3-ene-10-carboxylate (13b)

A stirred suspension of **12a** and **12b** (852 mg, 2.81 mmol) and K_2CO_3 (3.88 g, 28.10 mmol) in methanol (30 mL) was stirred at reflux for 4 h. The progress of the reaction was monitored by TLC (40% ethyl acetate/petroleum ether). The solid was filtered off, the solvent was removed under reduced pressure and the crude residue was purified by silica gel column chromatography (20% ethyl acetate/petroleum ether), which afforded 679 mg (95% yield) of a mixture of methoxy derivatives **13a** and **13b** as a pale yellow oil (R_f = 0.47, 40% ethyl acetate/petroleum ether).

4.1.4. 5-Methoxy-3-oxa-4,10-diazatricyclo[6.3.1.0^{2,6}]dodec-4-ene (5a) and 3-Methoxy-5-oxa-4,10-diazatricyclo[6.3.1.0^{2,6}]-dodec-3-ene (5b)

To a stirred solution of the mixture **13a** and **13b** (630 mg, 2.48 mmol) in ethanol (20 mL) were added powdered KOH (3.47 g, 61.84 mmol) and hydrazine monohydrate (600 μL, 12.37 mmol).[32] The reaction mixture was heated at reflux for 4 h, then concentrated and poured into water (4 mL). The aqueous phase was repeatedly extracted with dichloromethane, the combined organic layers were dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The crude residue was purified by column chromatography (5% dichloromethane/methanol) affording two fractions: 165 mg (**5a** + **5b**) and 185 mg (pure **5b**), in 72% overall yield.

4.1.4.1. Compound 5b. Colorless oil; R_f = 0.51 (10% dichloromethane/methanol). ^{1}H NMR δ: 1.56 (m, 2H), 1.68–1.85 (m, 3H), 2.05 (m, 1H), 2.15 (br s, 1H), 2.82 (m, 4H), 3.27 (d, *J* = 9.4 Hz, 1H), 3.86 (s, 3H), 5.27 (ddd, *J* = 7.8, 7.8 and 9.4 Hz, 1H) ppm. ^{13}C NMR δ: 28.9, 29.3, 29.7, 29.9, 35.2, 49.1, 52.4, 57.4, 80.2, 169.6 ppm. MS (ESI) *m/z* 197.1 $[M+H]^+$, calcd for $C_{10}H_{16}N_2O_2$ 196.3.

To a solution of **5b** (100 mg, 0.51 mmol) in methanol (2 mL) was added a solution of fumaric acid (59 mg, 0.51 mmol) in methanol (3 mL). After stirring at room temperature for 18 h, the reaction mixture was concentrated under reduced pressure to quantitatively provide the corresponding salt, which was crystallized from methanol/diethyl ether 1:1.

4.1.4.2. Compound 5b·1/2$C_4H_4O_4$. Colorless prisms, mp 184.5–185 °C. ^{1}H NMR (CD_3OD) δ: 1.76 (br s, 1H), 1.97 (m, 1H), 2.06 (m, 1H), 2.19 (m, 1H), 2.41 (br s, 1H), 3.19 (m, 1H), 3.25 (m, 4H), 3.41 (d, *J* = 9.1 Hz, 1H), 3.89 (s, 3H), 5.09 (ddd, *J* = 7.3, 7.3 and 9.1 Hz, 1H), 6.68 (s, 1H) ppm. ^{13}C NMR (CD_3OD) δ: 24.8, 25.2, 26.3, 29.6, 31.3, 47.0, 48.7, 57.1, 77.2, 134.4, 168.1, 168.8 ppm. $C_{12}H_{18}N_2O_4$ (254.28): calcd C, 56.68; H, 7.13; N, 11.02. Found: C, 56.32; H, 7.34; N, 11.23.

4.1.5. 3-Bromo-5-oxa-4,10-diazatricyclo[6.3.1.0^{2,6}]dodec-3-ene (4b)

3-Azabicyclo[3.3.1]non-6-ene **14** was prepared by cleavage of the carbamate function of **11** according to the procedure reported in Ref. 32. To a stirred solution of **14** (320 mg, 2.60 mmol), triethylamine (1.5 mL, 10.40 mmol) and 4-(dimethylamino)pyridine (0.06 g, 0.52 mmol) in THF (30 mL) at 0 °C was added dropwise benzyl chloroformate (1.54 mL, 10.8 mmol). After stirring at room temperature for 12 h, the reaction mixture was concentrated at reduced pressure. The crude residue was purified by silica gel column chromatography (5% ethyl acetate/petroleum ether) which gave the desired *N*-Cbz protected alkene (388 mg, 58% yield).

4.1.5.1. Benzyl 3-azabicyclo[3.3.1]non-6-ene-3-carboxylate (15). Pale yellow oil, R_f = 0.74 (20% ethyl acetate/petroleum ether). ^{1}H NMR δ: 1.62–1.82 (m, 2H), 1.87–2.18 (m, 2H), 2.22–2.38 (m, 2H), 2.82–3.06 (m, 2H), 3.86–4.04 (m, 1H), 4.09–4.32 (m, 1H), 5.12 (m, 2H), 5.70 (m, 2H), 7.34 (m, 5H) ppm.

Dipolarophile **15** (350 mg, 1.36 mmol) was reacted following the protocol above reported for olefin **11**. The resulting cycloadduct was purified by column chromatography, which provided 351 mg (68% yield) of **16b**.

4.1.5.2. Benzyl 3-bromo-5-oxa-4,10-diazatricyclo[6.3.1.0^{2,6}]dodec-3-ene-10-carboxylate (16b). Yellow oil; R_f = 0.49 (20% ethyl acetate/petroleum ether). ^{1}H NMR δ: 1.92–2.08 (m, 3H), 2.39 (br s, 1H), 2.95–3.10 (m, 3H), 3.32 (m, 1H), 4.05–4.25 (m, 3H), 4.76 (m, 1H), 5.13 (br s, 2H), 7.33 (m, 5H) ppm.

To a stirred solution of bromo-Δ^2-isoxazoline **16b** (540 mg, 1.42 mmol) in CH_3CN (10 mL), maintained at −5 °C, was added dropwise iodotrimethylsilane (808 μL, 5.68 mmol) in three portions every 30 min. The reaction mixture was then stirred at room temperature for 2 days and was monitored by TLC (20% ethyl acetate/petroleum ether). After treatment with 2 N HCl (5 mL), the water layer was extracted with diethyl ether (3 × 5 mL), then was made alkaline by portionwise addition of a solution of NaOH (pH 12), and extracted with ethyl acetate (10 × 5 mL). The pooled organic extracts were dried over anhydrous sodium sulfate and the crude residue was purified by silica gel column chromatography (5% methanol/dichloromethane) to provide the desired free base **4b** (108 mg, 31% yield).

4.1.5.3. Compound 4b. Colorless powder (from methanol), mp 91–92 °C; R_f = 0.36 (15% methanol/dichloromethane). ^{1}H NMR (CD_3OD) δ: 1.74–1.88 (m, 2H), 2.04 (d, J = 14.0 Hz, 1H), 2.13 (m, 1H), 2.33 (br s, 1H), 2.55 (br s, 1H), 3.22–3.48 (m, 4H), 3.54 (d, J = 7.7 Hz, 1H), 4.36 (m, 1H), 4.87 (br s, 1H) ppm. ^{13}C NMR (CD_3OD): δ= 25.4, 26.5, 30.4, 35.2, 40.0, 45.1, 46.9, 62.4, 118.8 ppm. $C_9H_{13}BrN_2O$ (245.12): calcd C, 44.10; H, 5.35; N, 11.43. Found: C, 44.42; H, 5.09; N, 11.68.

4.1.6. *tert*-Butyl 3-bromo-5-oxa-4,11-diazatricyclo[6.2.1.0^{2,6}]undec-3-ene-11-carboxylate (20a) and *tert*-butyl 5-bromo-3-oxa-4,11-diazatricyclo[6.2.1.0^{2,6}]undec-4-ene-11-carboxylate (20b)

Reduction of *tert*-butyl 3-oxo-8-azabicyclo[3.2.1]octane-8-carboxylate **17**[38] (6.50 g, 28.85 mmol) with excess sodium borohydride in methanol afforded the crude *exo*/*endo* secondary alcohols[44] which were transformed into the corresponding *exo*/*endo* mesylates **18** with a known procedure.[39] The reaction residue was purified by silica gel column chromatography (20% ethyl acetate/petroleum ether), which provided mesylates **18** (6.70 g, 76% overall yield) as a pale yellow oil, R_f = 0.49 (30% ethyl acetate/petroleum ether).

To a stirred solution of the chromatographed *exo*/*endo* mixture **18** (5.80 g, 19.0 mmol) in toluene (20 mL) was added dropwise 1,8 diazabicyclo[5.4.0]undecen-7-ene (DBU, 8.5 mL, 57 mmol). The solution was stirred at reflux for 24 h and the progress of the reaction was monitored by TLC (30% ethyl acetate/petroleum ether). After concentration under reduced pressure, the residue was purified by silica gel column chromatography (15% ethyl acetate/petroleum ether), which provided the wanted *tert*-butyl 8-azabicyclo[3.2.1]oct-2-ene-8-carboxylate **19** as a pale yellow oil (3.38 g, 85% yield), R_f = 0.69 (20% ethyl acetate/petroleum ether). The spectroscopic features of **19** matched those previously described for (+)-**19**.[36]

The pericyclic reaction was performed on dipolarophile **19** (2.80 g, 13.38 mmol) following the protocol above reported for olefin **11**. The crude reaction was purified by column chromatography (10% ethyl acetate/petroleum ether), which afforded 4.12 g (93% yield) of a mixture of cycloadducts **20a** and **20b** (R_f = 0.28, 15% ethyl acetate/petroleum ether) as a beige viscous oil. ^{1}H NMR analysis allowed to evaluate their 3:1 ratio, by considering the two multiplets centered at 4.94 and 3.22 δ, which have been attributed to the H-6 proton (see Scheme 2) of the major (**20a**) and minor (**20b**) regioisomer, respectively.

4.1.7. 3-Bromo-5-oxa-4,11-diazatricyclo[6.2.1.0^{2,6}]undec-3-ene (7a) and 5-bromo-3-oxa-4,11-diazatricyclo[6.2.1.0^{2,6}]undec-4-ene (7b)

To a stirred solution of the mixture of regioisomers **20a** and **20b** (3.05 g, 9.21 mmol) in dioxane (10 mL), at 0 °C, was added dropwise a solution of 4 N HCl in dioxane (3.75 mL, 15 mmol). The reaction mixture was stirred at room temperature for about 1 h, and was monitored by TLC (5% methanol/dichloromethane). The solvent was removed in vacuo and a saturated $NaHCO_3$ solution was added (pH 8). The aqueous layer was extracted with diethyl ether (5 × 5 mL) and, after the usual work-up, isomers **7a** and **7b** were isolated by silica gel column chromatography (2% methanol/dichloromethane) in 65% overall yield.

4.1.7.1. Compound 7a. Yield 50%, 1.062 g; colorless leaflets (from *n*-hexane), mp 69–70 °C; R_f = 0.45 (5% methanol/dichloromethane + 0.1% concd NH_3). ^{1}H NMR δ: 1.58–1.77 (m, 4H), 1.83–2.08 (m, 3H), 3.27 (d, J = 9.4 Hz, 1H), 3.52 (br s, 1H), 3.83 (d, J = 6.1 Hz, 1H), 4.87 (ddd, J = 7.9, 7.9, and 9.4 Hz, 1H) ppm. ^{13}C NMR δ: 28.5, 30.6, 38.2, 52.6, 53.6, 57.6, 76.4, 142.8 ppm. MS (ESI) *m/z* 231.0 [M]$^+$, calcd for $C_8H_{11}BrN_2O$ 231.1.

4.1.7.2. Compound 7b. Yield 15%, 320 mg; pale yellow oil, R_f = 0.35 (5% methanol/dichloromethane + 0.1% concd NH_3). ^{1}H NMR δ: 1.24 (m, 3H), 1.39 (m, 1H), 1.58 (m, 2H), 1.86 (dd, J = 9.1 and 13.0 Hz, 1H), 3.15 (ddd, J = 9.1, 9.1, and 8.3 Hz, 1H), 3.59 (br s, 1 H), 3.77 (d, J = 5.0 Hz, 1H), 4.19 (d, J = 8.3 Hz, 1H) ppm. ^{13}C NMR δ: 26.7, 29.9, 34.3, 45.2, 51.0, 53.1, 83.1, 144.8 ppm. MS (ESI) *m/z* 231.0 [M]$^+$, calcd for $C_8H_{11}BrN_2O$ 231.1.

According to the procedure previously described for derivative **5b**, the free bases **7a** and **7b** were treated with fumaric acid to produce the corresponding fumarates, which were crystallized from methanol/diethyl ether 1:1.

4.1.7.3. Compound 7a·3/4$C_4H_4O_4$. Colorless prisms, mp 169.5–170 °C. ^{1}H NMR (CD_3OD) δ: 1.79 (m, 1H), 1.98 (m, 1H), 2.13 (m, 2H), 2.27 (m, 2H), 3.79 (d, J = 9.9 Hz, 1H), 3.91 (br s, 1H), 4.25 (br s, 1H), 5.13 (ddd, J = 8.0, 8.0, and 9.8 Hz, 1H), 6.69 (s, 1.5H) ppm. ^{13}C NMR (CD_3OD) δ: 24.2, 26.3, 27.2, 34.0, 53.4, 56.0, 73.6, 134.5, 139.5, 168.3 ppm. $C_{11}H_{14}BrN_2O_4$ (318.14): calcd C, 41.53; H, 4.44; N, 8.81. Found: C, 41.37; H, 4.70; N, 8.51.

4.1.7.4. Compound 7b·$C_4H_4O_4$. Colorless prisms, mp 186–187 °C. ^{1}H NMR (CD_3OD) δ: 1.74 (m, 1H), 2.03 (m, 2H), 2.08–2.32 (m, 3H), 3.57 (ddd, J = 8.8, 8.8, and 8.5 Hz, 1H), 4.02 (br s, 1H), 4.28 (br s, 1H), 4.58 (d, J = 8.5 Hz, 1H), 6.69 (s, 2H) ppm. ^{13}C NMR (CD_3OD) δ: 23.8, 24.0, 30.7, 43.3, 52.6, 53.5, 81.9, 134.4, 144.4, 168.1 ppm. $C_{12}H_{15}BrN_2O_5$ (347.16): calcd C, 41.52; H, 4.36; N, 8.07. Found: C, 41.38; H, 4.55; N, 8.29.

4.1.8. 3-Methoxy-5-oxa-4,11-diazatricyclo[6.2.1.0^{2,6}]undec-3-ene (8a)

To a stirred and ice-cooled solution of **7a** (850 mg, 3.68 mmol) and triethylamine (1.14 mL, 8.13 mmol) in dichloromethane (25 mL) di-*tert*-butyl dicarbonate (1.12 g, 5.12 mmol) was added portionwise. The reaction mixture was stirred at room temperature for 12 h and the disappearance of the starting material was controlled by TLC (5% methanol/dichloromethane). After addition of 1 N HCl (25 mL), the aqueous layer was extracted with diethyl

ether (5 × 20 mL). The combined organic phases were dried, concentrated under reduced pressure, and the crude residue was purified by silica gel column chromatography (15% ethyl acetate/petroleum ether), which afforded the *N*-Boc protected derivative **20a** (1.048 g, 86% yield).

4.1.8.1. *tert*-Butyl 3-bromo-5-oxa-4,11-diazatricyclo[6.2.1.0^{2,6}]-undec-3-ene-11-carboxylate (20a). Colorless leaflets (from *n*-hexane), mp 78–78.5 °C. ^{1}H NMR δ: 1.45 (s, 9H), 1.58 (m, 1H), 1.70 (m, 1H), 1.87 (m, 1H), 1.95–2.15 (m, 3H), 3.31 (d, *J* = 9.1 Hz, 1H), 4.37 (br s, 1H), 4.67 (br s, 1H), 4.94 (ddd, *J* = 7.9, 7.9, and 9.1 Hz, 1H) ppm. ^{13}C NMR δ: 27.6, 28.5, 30.2, 36.9, 50.7, 52.2, 58.4, 76.1, 80.5, 141.3, 153.1 ppm.

Compound **20a** (810 mg, 2.44 mmol) was reacted following the protocol previously described to prepare the mixture of methoxy-Δ^2-isoxazolines **13a** and **13b**. Intermediate **21a** was purified by column chromatography (497 mg, 72% yield).

4.1.8.2. *tert*-Butyl 3-methoxy-5-oxa-4,11-diazatricyclo[6.2.1.0^{2,6}]-undec-3-ene-11-carboxylate (21a). Colorless leaflets (from *n*-hexane), mp 84–84.5 °C; R_f = 0.63 (40% ethyl acetate/petroleum ether). ^{1}H NMR δ: 1.45 (s, 9H), 1.53–1.62 (m, 1H), 1.62–1.73 (m, 1H), 1.85–2.10 (m, 4H), 3.26 (d, *J* = 9.0 Hz, 1H), 3.86 (s, 3H), 4.32 (br s, 1H), 4.59 (br s, 1H), 4.90 (ddd, *J* = 8.2, 8.2, and 9.0 Hz, 1H) ppm. ^{13}C NMR δ: 27.0, 28.5, 29.5, 30.3, 36.8, 50.7, 51.4, 57.4, 76.1, 79.6, 152.7, 170.9 ppm.

To a stirred solution of **21a** (426 mg, 1.51 mmol) in dichloromethane (10 mL) at 0 °C was added dropwise trifluoroacetic acid (1.12 mL, 15.1 mmol) and the mixture was stirred at room temperature for 12 h. The progress of the reaction was monitored by TLC (eluant: 5% methanol/dichloromethane), then it was concentrated at reduced pressure and the residue was dissolved in water (5 mL) and treated with diethyl ether (3 × 5 mL). The residual aqueous phase was made alkaline by portionwise addition of solid K_2CO_3 (pH 10) and extracted with dichloromethane (3 × 5 mL). The title compound **8a** (220 mg, 80% yield) was obtained after the usual work-up and concentration of the pooled organic phases.

4.1.8.3. Compound 8a. Pale yellow viscous oil; R_f = 0.69 (5% methanol/dichloromethane). ^{1}H NMR δ: 1.57–1.80 (m, 4H), 1.83–2.04 (m, 2H), 3.20 (d, 8.5 Hz, 1H), 3.53 (m, 1H), 3.75 (m, 1H), 3.92 (s, 3H), 4.67 (br s, 1H), 4.81 (ddd, *J* = 8.0, 8.0, and 8.5 Hz, 1H) ppm. ^{13}C NMR δ: 28.1, 30.8, 38.2, 51.4, 52.6, 53.0, 57.7, 76.6, 168.7 ppm. MS (ESI) *m/z* 183.1 [M+H]$^+$, calcd for $C_9H_{14}N_2O_2$ 182.2.

4.1.8.4. Compound 8a·1/2$C_4H_4O_4$. Colorless prisms (from methanol/diethyl ether 1:1), mp 170.5–171.5 °C. ^{1}H NMR (CD_3OD) δ: 1.82–1.94 (m, 2H), 1.98–2.10 (m, 2H), 2.10–2.23 (m, 2H), 3.62 (d, *J* = 9.4 Hz, 1H), 3.83 (br s, 1H), 3.92 (m, 3 H), 4.06 (br s, 1H), 4.98 (ddd, *J* = 7.8, 7.8, and 9.4 Hz, 1H), 6.65 (m, 1H). ^{13}C NMR (CD_3OD) δ: 24.1, 27.2, 33.7, 49.4, 52.3, 53.8, 57.5, 73.9, 134.5, 166.2, 168.4 ppm. $C_{11}H_{16}N_2O_4$ (240.26): calcd C, 54.99; H, 6.71; N, 11.66. Found: C, 54.65; H, 6.98; N, 11.84.

4.1.9. 5-Methoxy-3-oxa-4,11-diazatricyclo[6.2.1.0^{2,6}]undec-4-ene (8b)

The procedure above described for **7a** was applied to **7b** (232 mg, 1 mmol), and afforded the corresponding *N*-Boc protected derivative **20b** (252 mg, 76% yield).

4.1.9.1. *tert*-Butyl 5-bromo-3-oxa-4,11-diazatricyclo[6.2.1.0^{2,6}]-undec-4-ene-11-carboxylate (20b). Pale yellow oil. ^{1}H NMR δ: 1.46 (m, 9H), 1.55–1.67 (m, 2H), 1.83 (m, 2H), 2.09 (m, 2H), 3.22 (ddd, *J* = 8.4, 8.4, and 9.9 Hz, 1H), 4.27 (br s, 1H), 4.33 (d, *J* = 9.9 Hz, 1H) ppm. ^{13}C NMR δ: 26.4, 26.9, 28.5, 32.0, 45.4, 50.4, 52.8, 80.1, 83.8, 143.7, 153.9 ppm.

Compound **20b** (240 mg, 0.725 mmol) was reacted following the protocol previously described for methoxy-Δ^2-isoxazolines **13a** and **13b**. Intermediate **21b** was purified by column chromatography (168 mg, 82% yield).

4.1.9.2. *tert*-Butyl 5-methoxy-3-oxa-4,11-diazatricyclo[6.2.1.0^{2,6}]-undec-4-ene-11-carboxylate (21b). Colorless prisms (from methanol/diethyl ether 1:1), mp 285–295 °C dec; R_f = 0.47 (40% ethyl acetate/petroleum ether). ^{1}H NMR δ: 1.48 (m, 9H), 1.53–1.67 (m, 3H), 1.82 (m, 1H), 1.95–2.08 (m, 2H), 2.94 (ddd, *J* = 9.2, 9.2, and 7.4 Hz, 1H), 3.82 (s, 3H), 4.25 (d, *J* = 7.4 Hz, 1H), 4.31 (br s, 1H), 4.46 (br s, 1H) ppm. ^{13}C NMR δ: 26.6, 28.5, 31.6, 37.8, 50.6, 53.4, 57.3, 60.5, 79.7, 83.6, 154.1, 171.8 ppm.

Derivative **21b** (141 mg, 0.50 mmol) was converted into the corresponding free base following the procedure described for **8a**. The title compound **8b** (75 mg, 82% yield) was obtained after the usual work-up and concentration of the pooled organic phases.

4.1.9.3. Compound 8b. Pale yellow viscous oil; R_f = 0.46 (5% methanol/dichloromethane). ^{1}H NMR δ: 1.52–1.66 (m, 2H), 1.84 (ddd, *J* = 13.2, 9.1, and 1.4 Hz, 1H), 1.88–2.03 (m, 2H), 2.50 (br s, 1H), 2.88 (ddd, *J* = 9.1, 9.1, and 7.7 Hz, 1H), 3.58 (br s, 1H), 3.67 (m, 1H), 3.84 (s, 3H), 4.17 (dd, *J* = 7.7 and 1.4 Hz, 1H) ppm. ^{13}C NMR δ: 26.6, 29.9, 34.1, 37.5, 51.3, 53.7, 57.4, 83.0, 172.9 ppm. MS (ESI) *m/z* 183.1 [M+H]$^+$, calcd for $C_9H_{14}N_2O_2$ 182.2.

4.1.9.4. Compound 8b·$C_4H_4O_4$. Light beige powder (from methanol/diethyl ether 1:1), mp 158–158.5 °C. ^{1}H NMR (CD_3OD) δ: 1.87–2.08 (m, 3H), 2.14–2.22 (m, 3H), 3.25 (ddd, *J* = 9.2, 9.2, and 7.7 Hz, 1H), 3.85 (s, 3H), 4.05 (br s, 1H), 4.17 (m, 1H), 4.54 (dd, *J* = 7.7 and 2.5 Hz, 1H), 6.70 (s, 2H) ppm. ^{13}C NMR (CD_3OD) δ: 23.9, 24.0, 30.4, 35.8, 53.0, 54.1, 57.0, 81.7, 134.6, 168.7, 172.6 ppm. $C_{13}H_{18}N_2O_6$ (298.29): calcd C, 52.34; H, 6.08; N, 9.39. Found: C, 52.49; H, 6.33; N, 9.11.

4.1.10. Methyl 5-hydroxy-3-oxa-4,10-diazatricyclo[6.3.1.0^{2,6}]dodec-4-ene-10-carboxylate (22a) and Methyl 3-hydroxy-5-oxa-4,10-diazatricyclo[6.3.1.0^{2,6}]dodec-3-ene-10-carboxylate (22b)

To an acetone solution (2 mL) of the mixture of bromo-Δ^2-isoxazolines **12a/12b** (500 mg, 1.65 mmol) in a MW tube was added a solution of 1.5 N NaOH (6 mL). The mixture was heated (power: 100 W; *T*: 80 °C; *p*: 240 psi) for 5 min and monitored by TLC (10% methanol/dichloromethane). The crude reaction was extracted with diethyl ether (3 × 5 mL), the residual aqueous phase was made acid (pH 2) by addition of 2 N HCl and extracted with dichloromethane (3 × 5 mL). The pooled organic extracts were dried over anhydrous sodium sulfate and the residue was purified by silica gel column chromatography (5% methanol/dichloromethane), affording hydroxy-Δ^2-isoxazolines **22a** and **22b**.

4.1.10.1. Compound 22a. Yield 11%, 44 mg; yellow viscous oil; R_f = 0.47 (ethyl acetate). ^{1}H NMR (DMSO-d_6, 60 °C) δ: 1.52–1.68 (m, 2H), 1.77 (m, 1H), 1.90 (m, 2H), 2.04 (m, 1H), 2.34 (m, 1H), 2.90–3.05 (m, 2H), 3.60 (s, 3H), 3.96 (br s, 1H), 4.00 (br s, 1H), 4.35 (d, *J* = 6.6 Hz, 1H) ppm. ^{13}C NMR δ: 26.5, 27.0, 29.4, 30.1, 40.2, 47.1, 49.1, 53.2, 82.9, 156.5, 177.4 ppm.

4.1.10.2. Compound 22b. Yield 45%, 178 mg; yellow viscous oil; R_f = 0.57 (ethyl acetate). ^{1}H NMR (DMSO-d_6, 60 °C) δ: 1.42–1.58 (m, 2H), 1.74 (m, 1H), 1.86–1.98 (m, 2H), 2.25 (br s, 1H), 2.87 (d, *J* = 8.8 Hz, 1H), 2.90–3.02 (m, 2H), 3.61 (s, 3H), 3.85–3.98 (m, 2H), 4.65 (ddd, *J* = 8.2, 8.2, and 8.8 Hz, 1H) ppm. ^{13}C NMR δ: 28.9, 29.0, 29.2, 32.9, 47.3, 49.1, 49.9, 53.1, 78.1, 156.6, 173.8 ppm.

4.1.11. 4-Methyl-3-oxa-4,10-diazatricyclo[6.3.1.0^{2,6}]dodecan-5-one (6a)

A suspension of **22a** (140 mg, 0.58 mmol) and potassium carbonate (0.33 g, 2.37 mmol) in acetone (5 mL) was stirred at room temperature for 5 min, then iodomethane (360 μL, 5.80 mmol) was added. After stirring for 3 h at room temperature, the solvent was removed in vacuo and the residue was purified by silica gel column chromatography (30% ethyl acetate/petroleum ether) to give 83 mg (56% yield) of the desired isoxazolidin-3-one **23a**.

4.1.11.1. Methyl 4-methyl-5-oxo-3-oxa-4,10-diazatricyclo[6.3.-1.0^{2,6}]dodecane-10-carboxylate (23a). Pale yellow oil; R_f = 0.49 (ethyl acetate). ^{1}H NMR (CD_3OD) δ: 1.64 (m, 2H), 1.92–2.16 (m, 4H), 2.60 (m, 1H), 3.11 (m, 2H), 3.17 (s, 3H), 3.70 (s, 3H), 4.11 (br s, 1H), 4.15 (br s, 1H), 4.49 (d, J = 6.3 Hz, 1H) ppm. ^{13}C NMR δ: 26.5, 27.1, 29.3, 30.0, 31.7, 40.5, 47.1, 49.1, 53.0, 80.1, 156.0, 172.5 ppm.

To a stirred solution of **23a** (80 mg, 0.315 mmol) in chloroform (3 mL) at 0 °C was added dropwise iodotrimethylsilane (448 μL, 3.15 mmol). After stirring at room temperature for 8 h, the title compound **6a** (53 mg, 86% yield) was obtained with the procedure described above for the preparation of **4b**.

4.1.11.2. Compound 6a. Pale yellow thick oil; R_f = 0.84 (5% methanol/dichloromethane). ^{1}H NMR δ: 1.50–1.62 (m, 1H), 1.62–1.75 (m, 2H), 1.78–1.88 (m, 2H), 1.98 (br s, 1H), 2.09 (m, 1H), 2.84–3.00 (m, 4H), 3.14 (s, 3H), 3.28 (ddd, J = 9.4, 9.4, and 6.6 Hz, 1H), 4.44 (d, J = 6.6 Hz, 1H) ppm. ^{13}C NMR δ: 27.4, 28.1, 29.9, 30.9, 31.6, 42.5, 50.0, 52.2, 81.7, 173.3 ppm. MS (ESI) m/z 197.1 [M+H]$^+$, calcd for $C_{10}H_{16}N_2O_2$ 196.3.

4.1.11.3. Compound 6a·3/4$C_4H_4O_4$. Colorless prisms (from methanol/diethyl ether 1:1), mp 182.5–184 °C. ^{1}H NMR (D_2O) δ: 1.68 (d, J = 14.0 Hz, 1H), 1.76 (m, 1H), 1.86 (d, J = 14.0 Hz, 1H), 2.03 (m, 1H), 2.15 (br s, 1H), 2.32 (br s, 1H), 3.08 (s, 3H), 3.18 (m, 1H), 3.20–3.37 (m, 4H), 4.65 (m, 1H), 6.54 (s, 1.5H) ppm. ^{13}C NMR (D_2O) δ: 23.6, 23.8, 27.0, 30.7, 31.3, 39.1, 45.2, 47.6, 79.2, 134.9, 171.7, 171.9 ppm. $C_{13}H_{19}N_2O_5$ (283.30): calcd C, 55.11; H, 6.76; N, 9.89. Found: C, 54.80; H, 7.08; N, 9.71.

4.1.12. 4-Methyl-5-oxa-4,10-diazatricyclo[6.3.1.0^{2,6}]dodecan-3-one (6b)

Hydroxy-Δ^2-isoxazoline **22b** (240 mg, 1.0 mmol) was converted into the corresponding *N*-methylated derivative **23b** (183 mg, 72% yield) following the procedure described for **23a**.

4.1.12.1. Methyl 4-methyl-3-oxo-5-oxa-4,10-diazatricyclo[6.3.-1.0^{2,6}]dodecane-10-carboxylate (23b). Pale yellow oil; R_f = 0.46 (60% ethyl acetate/petroleum ether). ^{1}H NMR (DMSO-d_6, 60 °C) δ: 1.42–1.57 (m, 2H), 1.68 (m, 1H), 1.92–2.03 (m, 2H), 2.27 (br s, 1H), 2.85–3.02 (m, 3H), 3.05 (s, 3H), 3.61 (s, 3H), 3.91 (d, J = 14.7 Hz, 1H), 3.94 (d, J = 14.7 Hz, 1H), 4.61 (m, 1H) ppm. ^{13}C NMR δ: 29.0, 29.7, 29.9, 32.0, 33.6, 47.7, 49.2, 50.0, 53.0, 75.8, 156.6, 168.9 ppm.

Intermediate **23b** (150 mg, 0.59 mmol) was converted into the title compound **6b** (90 mg, 78% yield) by applying the reaction conditions described for **6a**.

4.1.12.2. Compound 6b. Pale yellow thick oil; R_f = 0.82 (15% methanol/dichloromethane). ^{1}H NMR δ: 1.25 (br s, 1H), 1.45–1.63 (m, 3H), 1.76 (m, 1H), 1.90 (br s, 1H), 2.07 (m, 1H), 2.37 (br s, 1H), 2.82–2.90 (m, 3H), 2.96 (d, J = 8.8 Hz, 1H), 3.17 (s, 3H), 5.29 (ddd, J = 8.2, 8.2, and 8.8 Hz, 1H) ppm. ^{13}C NMR δ: 29.8, 29.9, 30.4, 31.9, 34.4, 48.6, 52.0, 52.6, 76.9, 170.0 ppm. MS (ESI) m/z 197.1 [M+H]$^+$, calcd for $C_{10}H_{16}N_2O_2$ 196.3.

4.1.12.3. Compound 6b·3/4$C_4H_4O_4$. Colorless prisms (from methanol/diethyl ether 1:1), mp 163.5–165 °C. ^{1}H NMR (D_2O) δ: 1.55 (d, J = 13.9 Hz, 1H), 1.66 (d, J = 13.9 Hz, 1H), 1.90 (m, 1H), 2.12 (m, 1H), 2.19 (br s, 1H), 2.45 (br s, 1H), 3.09 (s, 3H), 3.13–3.35 (m, 5H), 5.11 (ddd, J = 8.0, 8.0, and 8.6 Hz, 1H), 6.55 (s, 1.5H) ppm. ^{13}C NMR (D_2O) δ: 24.8, 25.1, 26.3, 30.6, 31.1, 45.8, 47.2, 47.4, 74.7, 134.4, 167.8, 171.4 ppm. $C_{13}H_{19}N_2O_5$ (283.30): calcd C, 55.11; H, 6.76; N, 9.89. Found: C, 55.37; H, 6.98; N, 10.12.

4.1.13. 4-Methyl-5-oxa-4,11-diazatricyclo[6.2.1.0^{2,6}]undecan-3-one (9a)

To a solution of bromo-Δ^2-isoxazoline **20a** (662 mg, 2.0 mmol) in acetone (2 mL) in a microwave tube was added a solution of 1.5 N NaOH (6 mL). The reaction mixture was heated at the MW apparatus (power: 120 W; T: 120 °C; p: 240 psi) for 20 min, and the disappearance of the starting material was monitored by TLC (15% ethyl acetate/petroleum ether). After treatment with diethyl ether (3 × 5 mL), to the residual aqueous phase was added di-*tert*-butyl dicarbonate (625 mg, 2.87 mmol) and the mixture was vigorously stirred overnight. Diluted HCl was then added (pH 3) and the aqueous phase was extracted with diethyl ether (5 × 5 mL). After the usual work-up, the residue of the pooled organic was column chromatographed (5% methanol/dichloromethane) to provide 349 mg (65% overall yield) of intermediate **24a**.

4.1.13.1. *tert*-Butyl 3-hydroxy-5-oxa-4,11-diazatricyclo[6.2.1.0^{2,6}]-undec-3-ene-11-carboxylate (24a). Colorless leaflets (from *n*-hexane), mp 186–186.5 °C; R_f = 0.39 (5% methanol/dichloromethane). ^{1}H NMR δ: 1.45 (s, 9H), 1.56–1.73 (m, 2H), 1.88–1.97 (m, 1H), 2.02–2.12 (m, 2H), 2.15–2.22 (m, 1H), 2.45 (br s, 1H), 3.17 (dd, J = 8.5 and 1.7 Hz, 1H), 4.40 (br s, 1H), 4.83 (br s, 1H), 4.98 (ddd, J = 8.0, 8.0, and 8.5 Hz, 1H) ppm. ^{13}C NMR δ: 26.4, 28.5, 28.7, 29.8, 35.0, 49.9, 52.0, 75.8, 80.2, 153.2, 173.1 ppm.

To a stirred solution of **24a** (268 mg, 1.0 mmol) in anhydrous THF (20 mL), kept under a nitrogen atmosphere at of −78 °C, was added dropwise a 2 M solution of LDA in THF/heptane/ethylbenzene (0.5 mL, 1.0 mmol). The reaction mixture was gradually let to warm at −50 °C, then, on cooling again at −78 °C, iodomethane (62 μL, 1.0 mmol) was added. The reaction mixture was stirred for additional 12 h at room temperature, then a saturated solution of $NaHCO_3$ (20 mL) was added and the aqueous layer was extracted with ethyl acetate (3 × 20 mL). The pooled organic extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure. Purification of the crude residue was by silica gel column chromatography (40% ethyl acetate/petroleum ether) afforded compound **25a** (209 mg, 74% yield).

4.1.13.2. *tert*-Butyl 4-methyl-3-oxo-5-oxa-4,11-diazatricyclo-[6.2.1.0^{2,6}]undecane-11-carboxylate (25a). Yellow oil; R_f = 0.44 (60% ethyl acetate/petroleum ether). ^{1}H NMR δ: 1.46 (s, 9H), 1.48–1.72 (m, 2H), 1.92–2.09 (m, 4H), 2.89 (d, J = 9.1 Hz, 1H), 3.10 (s, 3H), 4.37 (br s, 1H), 4.76 (m, 2H) ppm. ^{13}C NMR δ: 27.0, 28.4, 29.8, 32.0, 35.4, 50.3, 50.9, 52.3, 73.0, 80.2, 127.9, 153.4 ppm.

The *N*-Boc protected derivative **25a** (187 mg, 0.66 mmol) was treated with trifluoroacetic acid, as described for its analogue **21a**, producing the free base **9a** (104 mg, yield 86%).

4.1.13.3. Compound 9a. Pale yellow thick oil; R_f = 0.37 (10% methanol/dichloromethane). ^{1}H NMR δ: 1.52–1.70 (m, 2H), 1.70–1.88 (m, 3H), 1.90–2.07 (m, 2H), 2.85 (d, J = 8.3 Hz, 1H), 3.13 (s, 3H), 3.54 (br s, 1H), 3.91 (br s, 1H), 4.70 (dd, J = 8.3 and 8.3 Hz, 1H) ppm. ^{13}C NMR δ: 27.9, 29.9, 32.1, 37.2, 50.2, 53.1, 53.5, 74.0, 168.6 ppm.

4.1.13.4. Compound 9a·1/2$C_4H_4O_4$. Colorless prisms (from ethanol/diethyl ether 4:1), mp 184.5–185.5 °C. ^{1}H NMR (CD_3OD) δ:

1.83–1.98 (m, 3H), 2.02–2.15 (m, 2H), 2.22 (m, 1H), 3.15 (s, 3H), 3.29 (br s, 1H), 3.84 (br s, 1H), 4.11 (d, J = 4.7 Hz, 1H), 4.97 (m, 1H), 6.67 (s, 1H) ppm. ^{13}C NMR (CD_3OD) δ: 23.4, 24.0, 26.4, 31.1, 32.3, 53.5, 54.1, 71.4, 134.3, 165.5, 167.9 ppm. $C_{11}H_{16}N_2O_4$ (240.26): calcd C, 54.99; H, 6.71; N, 11.66. Found: C, 54.71; H, 6.48; N, 11.34.

4.1.14. 4-Methyl-3-oxa-4,11-diazatricyclo[6.2.1.0^{2,6}]undecan-5-one (9b)

Bromo-Δ^2-isoxazoline **20b** (331 mg, 1.0 mmol) was converted into the corresponding hydroxy-Δ^2-isoxazoline **24b** (190 mg, 71% overall yield) as described for **24a**.

4.1.14.1. *tert*-Butyl 5-hydroxy-3-oxa-4,11-diazatricyclo[6.2.1.0^{2,6}]-undec-4-ene-11-carboxylate (24b). Light orange oil; R_f = 0.44 (5% methanol/dichloromethane). ^{1}H NMR (DMSO-d_6, 60 °C) δ: 1.37 (m, 9H), 1.64 (m, 2H), 1.76 (m, 2H), 1.96 (m, 2H), 2.08 (br s, 1H), 2.76 (m, 1H), 3.13 (m, 1H), 4.13 (m, 1H), 4.23 (m, 2H) ppm. ^{13}C NMR δ: 26.5, 28.5, 29.6, 30.6, 37.2, 51.5, 53.3, 80.0, 81.7, 154.1, 175.8 ppm.

Derivative **24b** (210 mg, 0.78 mmol) was converted into isoxazolidin-3-one **25b** (197 mg, 89% yield) following the protocol reported for the synthesis of **23a**.

4.1.14.2. *tert*-Butyl 4-methyl-5-oxo-3-oxa-4,11-diazatricyclo-[6.2.1.0^{2,6}]undecane-11-carboxylate (25b). Yellow oil; R_f = 0.45 (80% ethyl acetate/petroleum ether). ^{1}H NMR (DMSO-d_6, 60 °C) δ: 1.40 (s, 9H), 1.64 (m, 2H), 1.73 (m, 2H), 1.88 (m, 2H), 2.89 (m, 1H), 3.00 (s, 3H), 4.11 (br s, 1H), 4.25 (m, 2H) ppm. ^{13}C NMR (CD_3OD) δ: 25.6, 27.6, 29.4, 30.9, 37.2, 51.3, 52.1, 53.1, 79.4, 79.9, 154.3, 171.8 ppm.

The *N*-Boc protected derivative **25b** (170 mg, 0.60 mmol) was treated with trifluoroacetic acid as described for its analogue **21a**, affording 68 mg (62% yield) of the title free base.

4.1.14.3. Compound 9b. Colorless thick oil; R_f = 0.44 (5% methanol/dichloromethane). ^{1}H NMR δ: 1.61 (m, 2H), 1.63–1.72 (m, 1H), 1.75 (br s, 1H), 1.88–2.02 (m, 3H), 2.71 (m, 1H), 3.19 (s, 3H), 3.59 (m, 2H), 4.11 (m, 1H) ppm. ^{13}C NMR δ: 26.5, 31.8, 31.9, 32.3, 37.2, 51.8, 53.4, 78.6, 171.7 ppm. MS (ESI) m/z 183.0 $[M+H]^+$, calcd for $C_9H_{14}N_2O_2$ 182.2.

4.1.14.4. Compound 9b·3/4$C_4H_4O_4$. Colorless prisms (from 2-propanol/diethyl ether 1:1), mp 170–172 °C. ^{1}H NMR (D_2O) δ: 1.82–1.96 (m, 3H), 2.02–2.18 (m, 3H), 3.05 (m, 1H), 3.10 (s, 3H), 4.09 (br s, 1H), 4.15 (br s, 1H), 4.61 (m, 1H), 6.56 (s, 1.5H) ppm. ^{13}C NMR (D_2O) δ: 22.7, 23.2, 27.8, 31.1, 34.8, 52.9, 53.1, 77.3, 134.4, 170.0, 171.3 ppm. $C_{12}H_{17}N_2O_5$ (269.27): calcd C, 53.52; H, 6.36; N, 10.40. Found: C, 53.80; H, 6.13; N, 10.07.

4.2. Receptor binding assays

4.2.1. 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 60,000g 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.

4.2.2. [^{3}H]Epibatidine binding

(±)-[^{3}H]Epibatidine with a specific activity of 56–60 Ci/mmol was purchased from Perkin–Elmer (Boston MA); the non radioactive α-bungarotoxin, epibatidine, and (−)-cytisine **1** were purchased from Sigma. It has been previously reported that [^{3}H]epibatidine also binds to α-bungarotoxin binding receptors with nM affinity.[45] In order to prevent the binding of [^{3}H]epibatidine to the α-bungarotoxin binding receptors, the membrane homogenates were pre-incubated with 1 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 on 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.

4.2.3. [^{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, GE Healthcare) at room temperature. Nonspecific 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.

4.2.4. nAChR affinity of derivatives 4b, 5b, 6a–b, 7a–b, 8a–b, and 9a–b

The inhibition of radioligand binding by epibatidine, (−)-cytisine, and the test compounds, dissolved in DMSO and diluted 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), was measured by pre-incubating cortex homogenates with increasing doses (10 pM–10 mM) of the reference nicotinic agonists, epibatidine or (−)-cytisine, and the drug to be tested for 30 min at room temperature, followed by overnight incubation with a final concentration of 0.050 nM [^{3}H]epibatidine or 1 nM [^{125}I]α-bungarotoxin at the same temperatures as those of 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.[42] 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. 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.[46]

4.3. Theoretical calculations

The DFT/b3lyp/6-31g* calculations were performed with GAUSSIAN03 package.[35] The structures of the *exo*- and the *endo*-cycloadducts were built with GAUSSVIEW3.0 and, after a preliminary

optimization of the geometry at the b3lyp/6-31g* level, we optimized also the TS using suitable g03 keywords.

The ligands docked into the receptor binding clefts were built by SYBYL 8.0 (Tripos Inc., 1699 South Hanley Rd., St. Louis, MO 63144) and preliminarily minimized at the DFT/b3lyp/6-31g* level as implemented in GAUSSIAN03.[35] The amino groups were considered in the ionized form to better simulate the physiological conditions. In this theoretical study we made use of a recently published model of α4β2 nAChRs, which was elaborated in collaboration with the University of Florence.[43] Docking experiments of the tested ligands were performed by means of the program GOLD 3.2;[47] the goldscore fitness function and the distribution of torsion angles were chosen as indicators of the quality of the docking results. Van der Waals and hydrogen bonding radii were set at 4.0 and 3.0 Å, respectively while genetic algorithm parameters were kept at the default value. The resulting complexes were then optimized by means of a molecular mechanics method implemented in the Sybyl 8.0 software. Figs. 2, S1, and S2 were acquired by the PyMOL software.[48]

Acknowledgments

G.G. is indebted to Dr. Laura Legnani for the helpful discussion on the thermodynamic/kinetic control of the pericyclic reactions. The authors are also grateful to Giacomo Luca Visconti for recording LC–MS/MS spectra. This research project was financially supported by the Italian research grants: FIRB # RBNE03FH5Y and PRIN # 20072BTSR2 (to M.D.A.), and by the EC grant # 202088 Neurocypres (to C.G.).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.04.065.

References and notes

1. Dale, H. H. *Pharmacol. Rev.* **1954**, *6*, 7.
2. Schmitt, J. D. *Curr. Med. Chem.* **2000**, *7*, 749.
3. Dajas-Bailador, F.; Wonnacott, S. *Trends Pharmacol. Sci.* **2004**, *25*, 317.
4. Exley, R.; Cragg, S. J. *Br. J. Pharmacol.* **2008**, *153*, S283.
5. Jensen, A. A.; Frølund, B.; Liljefors, T.; Krogsgaard-Larsen, P. *J. Med. Chem.* **2005**, *48*, 4705.
6. Romanelli, M. N.; Gratteri, P.; Guandalini, L.; Martini, E.; Bonaccini, C.; Gualtieri, F. *ChemMedChem* **2007**, *2*, 746.
7. Kalamida, D.; Poulas, K.; Avramopoulou, V.; Fostieri, E.; Lagoumintzis, G.; Lazaridis, K.; Sideri, A.; Zouridakis, M.; Tzartos, S. J. *FEBS J.* **2007**, *274*, 3799.
8. Gotti, C.; Clementi, F. *Prog. Neurobiol.* **2004**, *74*, 363.
9. Gotti, C.; Zoli, M.; Clementi, F. *Trends Pharmacol. Sci.* **2006**, *27*, 482.
10. Cincotta, S. L.; Yorek, M. S.; Moschak, T. M.; Lewis, S. R.; Rodefer, J. S. *Curr. Opin. Invest. Drugs* **2008**, *9*, 47.
11. Daly, D. W. *Cell. Mol. Neurobiol.* **2005**, *25*, 513. and references cited therein.
12. Partheil, A. *Ber.* **1890**, *23*, 3201.
13. Ing, H. R. *J. Chem. Soc.* **1931**, 2195.
14. Bick, R. C.; Gillard, J. W.; Leow, H. M. *Austr. J. Chem.* **1979**, *32*, 2537.
15. Gohlke, H.; Gündish, D.; Schwartz, S.; Seitz, G.; Tilotta, M. C.; Wegge, T. *J. Med. Chem.* **2002**, *45*, 1064.
16. Gohlke, H.; Schwartz, S.; Gündish, D.; Tilotta, M. C.; Weber, A.; Wegge, T.; Seitz, G. *J. Med. Chem.* **2003**, *46*, 2031. and references cited therein.
17. Cassels, B. K.; Bermúdez, I.; Dajas, F.; Abin-Carriquiri, J. A.; Wonnacott, S. *Drug Disc. Today* **2005**, *10*, 1657.
18. Meyer, E. L.; Xiao, Y.; Kellar, K. J. *Mol. Pharmacol.* **2001**, *60*, 568.
19. Eaton, J. B.; Peng, J. H.; Schroeder, K. M.; George, A. A.; Fryer, J. D.; Krishnan, C.; Buhlman, L.; Kuo, Y. P.; Steinlein, O.; Lukas, R. J. *Mol. Pharmacol.* **2003**, *64*, 1283.
20. Slater, Y. E.; Houlihan, L. M.; Maskell, P. D.; Exley, R.; Bermúdez, I.; Lukas, R. J.; Valdivia, A. C.; Cassels, B. K. *Neuropharmacology* **2003**, *44*, 503.
21. Imming, P.; Klaperski, P.; Stubbs, M. T.; Seitz, G.; Gündisch, D. *Eur. J. Med. Chem.* **2001**, *36*, 375.
22. Houlihan, L. M.; Slater, Y. E.; Guerra, D. L.; Peng, J. H.; Kuo, Y. P.; Lukas, R. J.; Cassels, B. K.; Bermúdez, I. *J. Neurochem.* **2001**, *78*, 1029.
23. Kozikowski, A. P.; Chellappan, S. K.; Xiao, Y.; Bajjuri, K. M.; Yuan, H.; Kellar, K. J.; Petukhov, P. A. *ChemMedChem* **2007**, *2*, 1157.
24. Carbonnelle, E.; Sparatore, F.; Canu-Boido, C.; Salvagno, C.; Baldani-Guerra, B.; Terstappen, G.; Zwart, R.; Vijverberg, H.; Clementi, F.; Gotti, C. *Eur. J. Pharmacol.* **2003**, *471*, 85.
25. Tasso, B.; Canu-Boido, C.; Terranova, E.; Gotti, C.; Riganti, L.; Clementi, F.; Artali, R.; Bombieri, G.; Meneghetti, F.; Sparatore, F. *J. Med. Chem.* **2009**, *52*, 4345.
26. Dallanoce, C.; Bazza, P.; Grazioso, G.; De Amici, M.; Gotti, C.; Riganti, L.; Clementi, F.; De Micheli, C. *Eur. J. Org. Chem.* **2006**, 3746. and references cited therein.
27. De Micheli, C.; De Amici, M.; Dallanoce, C.; Clementi, F.; Gotti, C. PCT Int. Appl. WO 2008000469, 2008.
28. Rizzi, L.; Dallanoce, C.; Matera, C.; Magrone, P.; Pucci, L.; Gotti, C.; Clementi, F.; De Amici, M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4651.
29. Grazioso, G.; Pomè, D. Y.; Matera, C.; Frigerio, F.; Pucci, L.; Gotti, C.; Dallanoce, C.; De Amici, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6353.
30. Vyas, D. M.; Chiang, Y.; Doyle, T. W. *Tetrahedron Lett.* **1984**, *25*, 487.
31. Esch, P. M.; Hiemstra, H.; de Boer, R. F.; Speckamp, W. N. *Tetrahedron* **1992**, *48*, 4659.
32. Udding, J. H.; Papin, N.; Hiemstra, H.; Speckamp, W. N. *Tetrahedron* **1994**, *50*, 8853.
33. *Stereochemistry of Organic Compounds*; Eliel, E. L., Wilen, S. H., Eds.; John Wiley & Sons: New York, 1994; pp 665–834. Chapter 11.
34. In Basic Terminology of Stereochemistry (IUPAC Recommendations 1996), available at the site http://www.chem.qmul.ac.uk/iupac/stereo/DE.html.
35. *GAUSSIAN03, Revision B.04*, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery Jr., J. A.; Vreven, T.; Kudin, K. N. J.; Burant, C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, S.; Clifford, J.; Cioslowski, B. B.; Stefanov, G.; Liu, A.; Liashenko, P.; Piskorz, I.; Komaromi, A. G.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian: Wallingford CT, 2004.
36. Lin, R.; Castells, J.; Rapoport, H. *J. Org. Chem.* **1998**, *63*, 4069.
37. Aggarwal, V. K.; Astle, C. J.; Rogers-Evans, M. *Org. Lett.* **2004**, *6*, 1469.
38. Berdini, V.; Cesta, M. C.; Curti, R.; D'Anniballe, G.; Di Bello, N.; Nano, G.; Nicolini, L.; Topai, A.; Allegretti, M. *Tetrahedron* **2002**, *58*, 5569.
39. Napier, S. E.; Bingham, M. J.; Dunbar, N. A. PCT Int. Appl. WO 2007/063071, 2007.
40. Baldwin, J. E.; Ng, S. C.; Pratt, A. J. *Tetrahedron Lett.* **1987**, *28*, 4319.
41. Hanamoto, T.; Egashira, M.; Ishizuka, K.; Furuno, H.; Inanaga, J. *Tetrahedron* **2006**, *62*, 6332.
42. Munson, P. J.; Rodbard, D. *Anal. Biochem.* **1980**, *107*, 220.
43. Sgrignani, J.; Bonaccini, C.; Grazioso, G.; Chioccioli, M.; Cavalli, A.; Gratteri, P. *J. Comp. Chem.* **2009**, *30*, 2443.
44. Pedersen, H.; Sinning, S.; Bülow, A.; Wiborg, O.; Falborg, L.; Bols, M. *Org. Biomol. Chem.* **2004**, *2*, 2861.
45. Gerzanich, V.; Peng, X.; Wang, F.; Wells, G.; Anand, R.; Fletcher, S.; Lindstrom, J. *Mol. Pharmacol.* **1995**, *48*, 774.
46. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
47. GOLD v. 3.2, Cambridge Crystallographic Data Centre: Cambridge, UK.
48. DeLano, W. L. The PyMOL Molecular Graphics System, 2002, DeLano Scientific: Palo Alto, CA, USA. http://www.pymol.org.
49. Zhang, N.; Tomizawa, M.; Casida, J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 525.