Synthesis of novel epibatidine-related derivatives through 1,3-dipolar cycloaddition of pyridinenitrile oxides

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Abstract

The Δ^2-isoxazoline derivatives **3a-c** and **4a-c**, structurally related to epibatidine, and the simplified analogues **5a-c** were synthesized by means of a 1,3-dipolar cycloaddition of regioisomeric pyridinenitrile oxides to suitable dipolarophiles. Target compounds were assayed at α4β2 and α7 neuronal acetylcholine receptor (nAChR) subtypes. Competition binding experiments at α4β2 nAChRs showed an overall significant reduction in affinity for the compounds under study in comparison to the reference radioligand [³H]-epibatidine. On the other hand, compounds **3b**, **3c**, and **4b** exhibited a noticeable affinity for the α7 receptors and **3c** showed also a slight degree of α7 over α4β2 selectivity.

Keywords: Synthesis, 1,3-dipolar cycloaddition, nitrile oxides, epibatidine analogues, neuronal nicotinic receptors, binding affinity

Introduction

The nicotinic acetylcholine receptors (nAChRs) are a superfamily of ligand-gated ion channels, which also includes GABA$_{A,C}$, glycine, and 5-HT$_3$ receptors.[1,2] Neuronal nAChRs are widely distributed in the human brain, where they are frequently associated with modulatory events and, to a lesser extent, mediate synaptic transmission.[3] Their molecular structure is characterized by pentameric combinations of homologous, genetically distinct α and β subunits (α2-α10 and β2-β4), whose differential association confers specific structural and functional properties to the resulting receptor subtypes.[4-7] The different neuronal nAChRs, which are stimulated by (*S*)-nicotine **1** (Figure 1) or by synthetic compounds,[8,9] are involved in a number of functional processes such as cognition, learning and memory, cerebral blood flow and metabolism, as well as in an array of pathological conditions such as Alzheimer's and Parkinson's diseases, schizophrenia, epilepsy, Tourette's syndrome, anxiety, depression and nicotine addiction.[6,10-12] The role played by neuronal nAChRs in a variety of pathological states stimulated the

development of novel and potentially useful therapeutic agents promoted by the identification of suitable pharmacophore models.[13-17] Research efforts have mainly concerned ligands selectively acting at the α4β2 and α7 receptor subtypes,[18] the two major populations of nAChRs found in the brain.

The discovery of epibatidine **2** (Figure 1), an alkaloidal toxin isolated from the skin of the Ecuadorian poisonous frog *Epipedobates tricolor*,[19] has renewed the interest in targeting nAChRs for analgesia.[20-22] Epibatidine has potent nAChR mediated analgesic activity since it agonizes neuronal α4β2 subtypes with a binding affinity that is 30 times higher than that of nicotine.[23] The main obstacle to the clinical use of epibatidine as an analgesic drug is its very narrow therapeutic index, which has been attributed to the lack of any marked selectivity for a single nAChR subtype.[24-26] Nevertheless, epibatidine is a reference compound for all investigations on nAChRs and a model structure for the design of novel, high affinity and subtype-selective nAChR ligands. Epibatidine-related derivatives have been synthesized by varying the heteroaryl moiety,[27-29] or functionalizing and expanding the alicyclic skeleton,[30-33] or modifying the substituent and/or the position of the epimino nitrogen atom.[34,35]

In the light of the above discussed evidences 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 distanced by the insertion of a Δ^2-isoxazoline moiety, either spiro-condensed (derivatives **3a-c**) or fused (derivatives **4a-c**) with the azanorbornane core (Figure 1). In addition, we synthesized and tested compounds **5a-c** (Figure 1), in which the ethylene bridge of the bicyclic system of **4a-c** was removed. The aim of this study was to investigate the effect of the variation of both the epimino-*N*/pyridine-*N* distance and the conformational profile on the affinity/selectivity for the nAChR subtypes. Since the chlorine atom located on the pyridine ring makes a minor contribution to the affinity of epibatidine,[36] we decided to synthesize the unsubstituted pyridinyl regioisomers only. Moreover, the novel chiral derivatives **3a-c** and **4a-c** were prepared as racemates by taking into account the similar receptor binding efficiencies of the epibatidine enantiomers.[28,37,38] This paper reports the synthesis of compounds **3-5** and the evaluation of their binding affinity at α4β2 and α7 nAChR subtypes. The present results represent a further application of the cycloaddition strategy to the synthesis of biologically active heterocycles, which has characterized our research in the recent years with a focus on chiral Δ^2-isoxazoline derivatives selectively acting at the different glutamic acid receptors and transporters.[39-42]

1   **2**

3a: X = N, Y = CH, Z = CH
3b: X = CH, Y = N, Z = CH
3c: X = CH, Y = CH, Z = N

4a: X = N, Y = CH, Z = CH
4b: X = CH, Y = N, Z = CH
4c: X = CH, Y = CH, Z = N

5a: X = N, Y = CH, Z = CH
5b: X = CH, Y = N, Z = CH
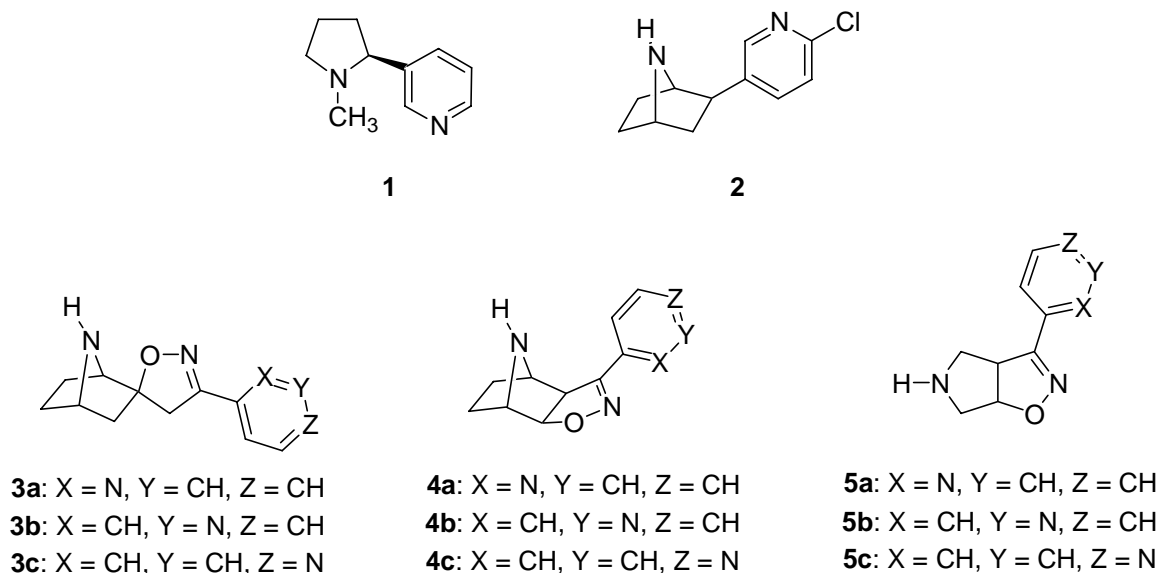**5c**: X = CH, Y = CH, Z = N

Figure 1. Structure of model and target ligands for neuronal nAChR subtypes.

Results and Discussion

The synthetic approach to the desired final compounds is outlined in Scheme 1. Intermediate Δ^2-isoxazolines **9a-c**, **11a-c**, and **13a-c** were prepared by a 1,3-dipolar cycloaddition involving the appropriate pyridinenitrile oxides, generated in situ upon treatment of the corresponding hydroximoyl chlorides **6a-c**[43] with triethylamine, and alkenes **8**, **10**, and **12**, respectively. A Wittig reaction performed on the known 7-*tert*-butoxycarbonyl-7-aza-byciclo[2.2.1]heptan-2-one **7**[44] afforded dipolarophile **8**, whereas 7-*tert*-butoxycarbonyl-7-aza-byciclo[2.2.1]hept-2-ene **10**[28] and 1-*tert*-butoxycarbonyl-Δ^3-pyrroline **12**[45] were prepared following published procedures.

As expected, pyridinenitrile oxides attack the less hindered face of bicyclic olefin **10** yielding exclusively the *exo*-cycloadducts **11a-c**. According to the literature,[46-48] the observed *syn* facial selectivity is dictated by the *anti* pyramidalization of the olefinic hydrogens in the dipolarophile. The structural assignment to adducts **11a-c** was unequivocally established by ^{1}H-NMR spectroscopy, since the absence of vicinal coupling constants between protons H-1 and H-2 as well as between protons H-3 and H-4 (Scheme 1) is a clear indication of their *exo* configuration.[49,50] By taking into account the outcome of reactions carried out on olefins structurally related to **8**,[51,52] we assigned the *exo* configuration even to spirocyclic intermediates **9a-c**, which were the only isomers isolated in each cycloaddition step. Finally, the pericyclic reaction of pyridinenitrile oxides with olefin **12** yielded bicyclic cycloadducts **13a-c**. Treatment of cycloadducts **9a-c**, **11a-c**, and **13a-c** with trifluoroacetic acid provided the desired epibatidine analogues as the free bases **3a-c**, **4a-c**, and **5a-c**, respectively. The latter compounds were converted into the corresponding fumarates by treatment with fumaric acid in methanol and the salts obtained were submitted to biological testing.
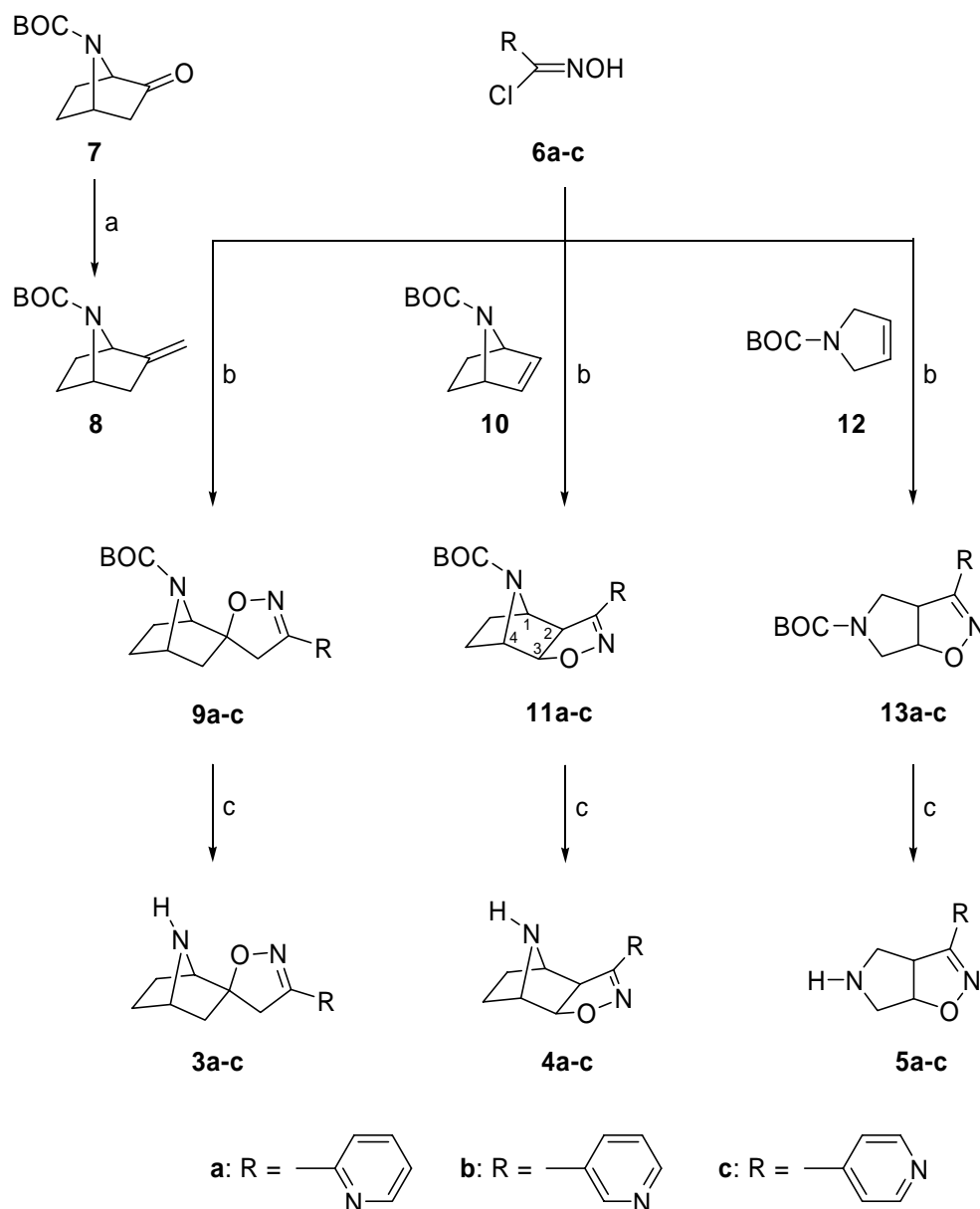
Scheme 1. a: Ph₃P=CH₂, Et₂O; b: (C₂H₅)₃N, CH₃CN, 60 °C; c: CF₃COOH, CH₂Cl₂.

The fumarates of the target compounds **3a-c**, **4a-c**, and **5a-c** were assessed for their binding affinity to α4β2 and α7 rat nicotinic receptor subtypes using [³H]-epibatidine and [¹²⁵I]-α-bungarotoxin as radioligands, respectively. The K_i values were calculated from the competition curves by means of the LIGAND program.[53] On the whole, the insertion of the Δ^2-isoxazoline moiety between the azanorbornane system and the pyridine ring greatly reduced or abolished the binding affinity for the α4β2 nAChR subtype, since the K_i value of the reference radioligand falls within the 20-40 pM concentration range. Among the studied compounds, only derivative **5b** (K_i = 72 μM) and the fused analogues **4b** (K_i = 86 μM) and **4c** (K_i = 68 μM), which are characterized by the pyridine nitrogen in position 3' and 4' respectively, retained a residual and comparable

affinity for the α4β2 nAChRs. Conversely, the two spirocyclic analogues **3b** (K_i = 41 μM) and **3c** (K_i = 28 μM), in which the pyridine nitrogen atoms are located in position 3' and 4' respectively, showed the highest affinity for the α7 nAChR subtype, thus behaving quite similarly to the fused analogue **4b** (Ki = 32 μM). In addition, derivative **3c** evidenced a slight degree of selectivity for α7 over α4β2 receptor subtypes (K_i values of 28 vs 400 μM, respectively).

The above discussed results on the group of novel epibatidine-related derivatives will be examined by taking into account both the ligand-based pharmacophore models reported in the literature and a recently proposed molecular model of the α4β2 receptor subtype.[54]

In conclusion, homologation of the structure of epibatidine, a prototype α4β2 selective agonist of nAChRs, led to the appearance of an unexpected, non negligible affinity for the α7 receptors. Moreover, among the compounds under study, the spirocyclic derivative **3c** showed a degree of α7 vs α4β2 selectivity, a result which could be exploited in designing novel selective agonists of this nAChR subtype.

Acknowledgments

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