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Research paper

Design of novel 3,6-diazabicyclo[3.1.1]heptane derivatives with potent and selective affinities for $\alpha_4\beta_2$ neuronal nicotinic acetylcholine receptors

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ABSTRACT

New analogues (**3a**–**l**) of the previously described $\alpha_4\beta_2$ selective ligand 3-(6-halopyridin-3-yl)-3,6-diazabicyclo[3.1.1]heptanes (**2a**,**b**) have been synthesized and their binding activity for neuronal acetylcholine receptor subtypes $\alpha_4\beta_2$ and α_7 were assayed.

Six of these compounds (**3a**,**b**,**c**,**j**,**k** and **l**) showed high affinity and selectivity for $\alpha_4\beta_2$ receptors. The phenylpyridyl-diazabicycloheptane **3c** displayed K_i value of 11.17 pM for $\alpha_4\beta_2$, in line with that of the halogenated homologues **3a**,**b**, although it was characterized by an improved selectivity ($Ki = 17$ μM for α_7 receptors). The influence of substitutions on the phenylpyridyl moiety on binding at both $\alpha_4\beta_2$ and α_7 receptors has been examined through the Topliss decision tree analysis. Substitution with electron-donating groups (as CH_3 and OCH_3) resulted in a good affinity for $\alpha_4\beta_2$ receptors and substantially no affinity for α_7. Amongst all the tested phenyl-substituted compounds, the *p*-NO_2-phenyl substituted analogue **3j** exhibited the highest $\alpha_4\beta_2$ affinity, with *Ki* value comparable to that of **3c**.

Intrinsic $\alpha_4\beta_2$ receptor mediated activity in [^{3}H]-DA release assay was showed by compound **3a** as well as by the reference analogue **2a**, whereas phenyl substituted derivative **3c** exhibited $\alpha_4\beta_2$ antagonist activity.

1. Introduction

Severe pain states, such as physiological, inflammatory, and neuropathic, often accompain inflammatory disease and neuropathic insults and can be treated with commonly prescribed analgesic agents [1]. Unfortunately, the therapeutic potential of non-steroidal anti-inflammatory drugs (NSAIDs) and opioids has been limited by safety concerns, as well as by unpleasant side effects with consequent poor patient compliance [2–5].

Thus, the treatment of various form of pain is a hugely unmet medical need, and the search for novel and efficacious analgesics with improved therapeutic index receives considerable attention [6].

Among a number of novel approaches to pain relief currently under investigation, nicotinic acetylcholine receptors (nAChRs) hold considerable potential as therapeutic targets for the development of analgesic drugs [7]. The neuronal nicotinic acethylcholine receptors are prototypic ligand-gated ion channel receptors that are widely expressed through the central and peripheral nervous system and mediate fast synaptic transmission [8,9]. Neuronal nAChRs are pentameric proteins comprising either combinations of two different types of subunit (α and β) or five copies of the same α subunit symmetrically arranged around a central ion pore. The multiple nAChR subunits form a plethora of different receptor subtypes among which the most abundant in the CNS are the $\alpha_4\beta_2$*

Abbreviations: CNS, central nervous system; nAChRs, nicotinic acetylcholine receptors; ACh, acetylcholine; GABA, gamma amino butyric acid; Trp-149, Tryptophan-149; Leu-119, Leucine-119; Asn-107, Asparagine-107; [^{3}H]-DA, [^{3}H]-dopamine; BINAP, (2,2′-*bis*(diphenylphosphino)-1,1′-binaphthyl; DCPD, 3,6-dichloropiridazine; IPr·HCl, 1,3-*bis*(2,6-diisopropylphenyl)imidazolium chloride; Boc, *tert*-Butyloxycarbonyl; FC, flash chromatography.

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and the α_7* receptors (the asterisk indicates the potential presence of other subunits), whereas the $\alpha_3\beta_4$ receptor is the predominant subtype at ganglionic synapses [10,11].

The neuronal nAChRs are involved in a wide range of physiological and pathophysiological processes, and they have been proposed as potential therapeutic targets in a number of neurodegenerative disorders such as Alzheimer's disease [12] and Parkinson's disease [13], neurological disorders such as Tourette's syndrome [14], psychic disorders such as schizophrenia [15], anxiety and depression [16], epilepsy [17], in various form of pain [7], and nicotine addiction [18]. Recently, was also suggested their potential use in cancer therapy [19].

The $\alpha_4\beta_2$ receptor subtype has generated significant interest among academic and industrial researchers as a result of its role in pain reflexes [20–22]. Thus, the generation of potent $\alpha_4\beta_2$ nAChR subtype selective ligands has been a goal for the advancement of novel nAChR-based analgesics with a minimum of side effects [23].

Interest in neuronal nAChRs as targets for the development of novel analgesic compounds was greatly increased by the discovery of epibatidine (**1**, Fig. 1), a toxin extracted from the skin of an ecuadorian frog (Epipedobates tricolor) [24]. The natural compound evidenced remarkable $\alpha_4\beta_2$ affinity (K_i = 0.045 nM) and significant analgesic activity. Epibatidine was in fact 200 times more effective than morphine in pain treatment in a pain hotplate test [25]. The absence of typical side effects of opioids, such as tolerance and dependence, suggested the use of epibatidine as potential alternative therapeutic strategy for severe pain states. However, its lack of subtype selectivity led to a very low therapeutic index (it has negative effects on the CNS and the respiratory, gastrointestinal and cardiovascular systems) and epibatidine development was discontinued, nevertheless synthetic modifications of its structure have been performed in order to improve potency and selectivity while reducing the toxicity.

These endeavours, based upon examination of the two key structural elements of **1**, that are the (i) azabicyclo amine moiety and the (ii) pyridine heterocycle, led to a number of promising substances, many of them closely related but also not related to epibatidine.

In this context, we previously reported a series of compounds with a 3,6-diazabicyclo[3.1.1]heptane structure typified by derivatives **2a**,**b** (Fig. 1) exhibiting potent and selective affinities for the $\alpha_4\beta_2$ subtype receptor [26].

Among the new derivatives, compound **2a**, bearing a 2-chloropyridine ring on the 3,6-diazabicyclo[3.1.1]heptane system, showed 3.6-fold higher binding affinity than epibatidine. This interesting result suggest that derivatives **2a**,**b** could be assumed as lead compounds for the design, synthesis and biological evaluation of novel selective ligands for nAChRs.

In this work different 3,6-diazabicyclo[3.1.1]heptane derivatives **3** (Table 1) have been synthesized by the modulation of the substituents on the pyridine ring in order to evaluate the effect of such modifications on the affinity, selectivity and functionality towards nAChR subtypes.

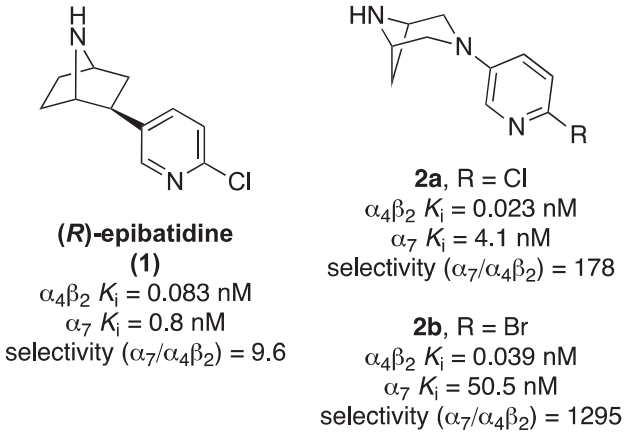

Fig. 1. Epibatidine and 3,6-diazabicyclo[3.1.1]heptanes.

2. Results and discussion

2.1. Chemistry

6-*tert*-Butyloxycarbonyl-3,6-diazabicyclo[3.1.1]heptane (**4**) was synthesized according an improved protocol previously reported [27]. *N*-Boc-protected bridged piperazine **4** was readily *N3*-arylated by Buckwald–Hartwig cross-coupling reaction with the appropriate 3,5-dihalopyridine to furnish derivatives **5** and **6** which serve both as starting compounds for next synthetic steps and for the preparation of **3a** and **3b** (Scheme 1).

The syntheses of derivatives **3c-l** was performed by a Suzuki coupling reaction starting from a suitable aryl boronic acid (**7-16**) and **6** followed by *N*-Boc deprotection of aryl-heteroaryl derivatives **17-26** (Scheme 2).

2.2. Pharmacology

The goal of this study was to prepare a series of 3-(5′-substituted-pyridin-3′-yl)-3,6-diazabicyclo[3.3.1]heptane analogues of the $\alpha_4\beta_2$ ligands **2a** and **2b** [26], and to measure their affinity towards $\alpha_4\beta_2$ and α_7 receptors. The binding affinities values are reported in Table 1.

Compounds **3a** and **3b** have a chlorine or a bromine atom at the 5′-position of the pyridyl ring as compared to **2a** and **2b**, respectively. Both maintained high $\alpha_4\beta_2$ receptor affinities and relatively low α_7 receptor affinities. The 5′-bromo-pyridyl derivative **3b** exhibited higher $\alpha_4\beta_2$ to α_7 selectivity (30769-fold) than did both the **2a** (16475-fold) and the **2b** (9307-fold).

Compound **3c** has one phenyl group at C5′ of the pyridyl ring. The derivative showed significant $\alpha_4\beta_2$ affinity, with K_i value which was approximately 2-fold higher relative to those of compounds **3a**,**b**. However, this compound possessed a remarkable $\alpha_4\beta_2$ vs α_7 selectivity ratio (K_i α_7/K_i $\alpha_4\beta_2$ = 1,521,934). Phenylpyridyldiazabicycloheptane **3c** serves as a convenient benchmark for all of the others in term of presenting structure-binding affinity relationships for the substitutions on the phenyl ring.

Initial attempts to improve the $\alpha_4\beta_2$ receptor binding affinity of **3c** employed the Topliss method, a practical technique guideline in choice of substituents to identify the most potent agents. The Topliss method takes into account the electronic, lipophilic, and steric parameters for substitution on an unfused benzene ring using basic Hansch principle in a nonmathematical, nonstatistical, and non computerized manner. So, the optimization at the R site of **3c** *via* the Topliss tree was undertaken choosing substituents from the Craig plot. Compounds **3d**,**e**,**f** have a halogen atom (Cl, F, Br, respectively) at *para* position of the unfused benzene ring and their K_i values indicated that they had lower affinities for $\alpha_4\beta_2$ receptors as compared to **3c** and no significant affinity for α_7 receptors (K_i > 1000 nM). Compounds **3g**,**h**,**i** contained a 2,4-Cl_2, 3,4-Cl_2 and 2,5-Cl_2-substituted phenyl group, respectively. Their $\alpha_4\beta_2$ receptor affinities were decreased when compared to **3c**. The 3,4-Cl_2-substituted analogue **3h** showed the lowest affinity among the three compounds.

The compounds containing an electron-donating substituent **3k** (CH_3) and **3l** (OCH_3) maintained a $\alpha_4\beta_2$ receptor affinity comparable to that of **3c**.