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Bioorganic & Medicinal Chemistry Letters 15 (2005) 4385–4388
Bioorganic & Medicinal Chemistry Letters

Epibatidine analogues as selective ligands for the $\alpha_x\beta_2$-containing subtypes of nicotinic acetylcholine receptors

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Received 3 May 2005; revised 8 June 2005; accepted 9 June 2005
Available online 21 July 2005

Abstract—A series of epibatidine analogues was synthesized and characterized in vitro. These compounds are high affinity ligands for the nicotinic acetylcholine receptors (nAChR). They display binding selectivity for the $\alpha_x\beta_2$ subtypes of nAChRs over the $\alpha_x\beta_4$ subtypes, and especially for the $\alpha_4\beta_2$ and $\alpha_2\beta_2$ subtypes. Furthermore, most of these new nicotinic compounds display little, if any, agonist activities at $\alpha_3\beta_4$ nAChR. As a result they might become lead structures for the design and synthesis of highly selective ligands for nAChR subtypes containing the β_2 subunit.

The neuronal nicotinic acetylcholine receptors (nAChRs) belong to a family of ligand-gated ion channels that are present in the mammalian central nervous system (CNS) and peripheral nervous system (PNS). These receptors mediate neurotransmission at autonomic ganglia in the PNS and participate in the regulation of neurotransmission in the CNS.[1–4] In particular, nAChRs appear to play an important role in cognition and attention.[5,6] Previous studies have found alterations of nAChRs in a number of neurological and psychiatric disorders such as schizophrenia, autism, Alzheimer's disease, Parkinson's disease, and dementia with Lewy bodies.[7–13] As a result, nAChRs, in particular the $\alpha_4\beta_2$ and α_7 subtypes, have become the targets of drug development for the treatment of cognitive and attention impairment associated with these pathological states, as well as mood and anxiety disorders.[14–16] The availability of noninvasive in vivo imaging techniques such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT) will aid in the understanding of nAChRs in neurological and psychiatric disorders and in the development of therapeutic treatments for these diseases. Up to date most available PET and SPECT imaging agents have been nAChR agonists, which are toxic when administered in high doses.[17] More recently, Carroll et al. reported the synthesis of epibatidine-based nAChR antagonists that display much less toxicity.[18–20] Our interest in the development of PET imaging agents has prompted us to investigate the utility of nAChR antagonists as candidate PET ligands that might provide a wider safety margin in imaging studies. In this paper, we report the synthesis and in vitro characterization of epibatidine analogues as high affinity nAChR ligands.

Synthesis of epibatidine analogues is depicted in Scheme 1. Compound **1**, synthesized according to the procedures of Carroll et al.[18] underwent Suzuki coupling with a variety of substituted phenylboronic acids to provide compounds **2a**–**i** in 78–94% yield. Compounds **2a**–**c** were then converted to compounds **3a**–**c** in 36–43% yield by reaction with sodium nitrite and copper (I) chloride. Alternatively, reaction with sodium nitrite and tetrafluoroboronic acid afforded compounds **3d**–**k** in 31–67%. Reductive methylation of compound **3a** with formaldehyde and sodium cyanoborohydride in turn provided compound **4** in 69% yield.[21] The structures of compounds **3a**–**k** and **4** were confirmed by ^{1}H NMR and MS. Conversion of these compounds to their respective HCl salts was performed by addition of HCl in ether and isolation of the resulting solids. These salts were then used in pharmacological tests.

Candidate compounds were assayed for their affinities to the nAChRs in competitive binding experiments in vitro

Keywords: Synthesis; Antagonist; Nicotinic receptors.
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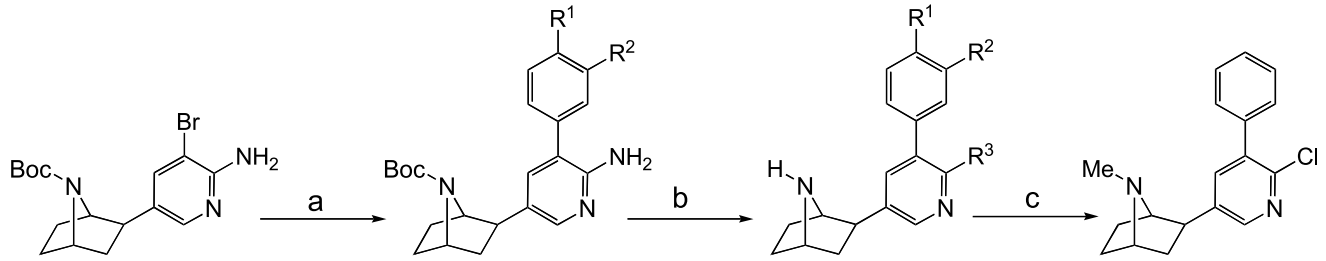
doi:10.1016/j.bmcl.2005.06.039



1

2a: R^1 = H, R^2 = H
2b: R^1 = Me, R^2 = H
2c: R^1 = OMe, R^2 = H
2d: R^1 = Cl, R^2 = H
2e: R^1 = F, R^2 = H
2f: R^1 = CN, R^2 = H
2g: R^1 = H, R^2 = Me
2h: R^1 = R^2 = Cl
2i: R^1 = F, R^2 = Me

3a: R^1 = R^2 = H, R^3 = Cl
3b: R^1 = Me, R^2 = H, R^3 = Cl
3c: R^1 = OMe, R^2 = H, R^3 = Cl
3d: R^1 = R^2 = H, R^3 = F
3e: R^1 = Me, R^2 = H, R^3 = F
3f: R^1 = H, R^2 = Me, R^3 = F
3g: R^1 = Cl, R^2 = H, R^3 = F
3h: R^1 = F, R^2 = H, R^3 = F
3i: R^1 = CN, R^2 = H, R^3 = F
3j: R^1 = R^2 = Cl, R^3 = F
3k: R^1 = F, R^2 = Me, R^3 = F

4

Scheme 1. Reagents and conditions: (a) arylboronic acid, $Pd(OAc)_2$, (*o*-tolyl)$_3$P, Na_2CO_3, DME; (b) 37% aq HCl, $NaNO_2$, Cu(I)Cl or 48% aq HBF_4, $NaNO_2$; (c) HCHO, $NaBH_3CN$, AcOH, MeCN.

using rat nAChRs ($\alpha_4\beta_2$, $\alpha_4\beta_4$, $\alpha_2\beta_2$, $\alpha_2\beta_4$, $\alpha_3\beta_2$, and $\alpha_3\beta_4$ subtypes) stably expressed on transfected cell lines and the radioligand [^{3}H]epibatidine.[22,23] In addition, compounds were tested for their agonist activities on $^{86}Rb^+$ efflux from KX$\alpha_3\beta_4$R2 cells that stably express functional rat $\alpha_3\beta_4$ nicotinic receptors.[22] Test results are listed in Table 1.

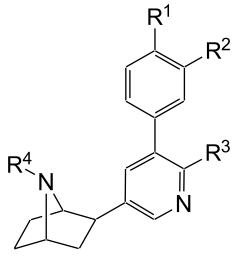
Inspection of the binding data reveals that most compounds display high affinities at the $\alpha_4\beta_2$, $\alpha_4\beta_4$, $\alpha_2\beta_2$, $\alpha_2\beta_4$, and $\alpha_3\beta_2$ subtypes of nAChR, with K_i values in the subnanomolar to nanomolar range, while binding affinities at the nAChR $\alpha_3\beta_4$ subtype are generally lower (K_i from 15.4 nM for compound **4** to 2940 nM for compound **3k**). Especially interesting is the binding selectivity these compounds display among the nAChR subtypes. In general, binding affinities for the $\alpha_x\beta_2$ subtypes are higher than those for the $\alpha_x\beta_4$ subtypes. For example, the binding affinities of all compounds for the $\alpha_4\beta_2$ subtype are one order of magnitude higher than those for the $\alpha_4\beta_4$ subtype, as well as for $\alpha_2\beta_2$ over $\alpha_2\beta_4$ subtype, while affinities of the compounds for the $\alpha_3\beta_2$ subtype are at least two orders of magnitude higher than those for the $\alpha_3\beta_4$ subtype. Among the $\alpha_x\beta_2$ combinations, all compounds have equal affinities for both the $\alpha_4\beta_2$ and $\alpha_2\beta_2$ subtypes, while affinities for the $\alpha_3\beta_2$ subtype are 2–10 times lower. The largest differences in binding affinities are between the $\alpha_4\beta_2/\alpha_2\beta_2$ and $\alpha_3\beta_4$ subtypes, with compound **3b** displaying the highest selectivity of 7397-fold for $\alpha_4\beta_2$ over $\alpha_3\beta_4$ and 9750-fold for $\alpha_2\beta_2$ over $\alpha_3\beta_4$. Taken together, it appears that these epibatidine analogues possess binding selectivity for the $\alpha_x\beta_2$ subtypes of nAChRs, and in particular for the $\alpha_4\beta_2$ and $\alpha_2\beta_2$ subtypes. This same subtype selectivity has been noted in nAChR agonists among the epibatidine analogues and pyridinyl ethers,[24–26] but not in the antagonists of Carroll et al.[18–20]

Results from functional assays indicate that all compounds, with the exception of compound **3i**, display negligible agonist activities at the nAChR $\alpha_3\beta_4$ subunit. Compound **3i**, on the other hand, appears to possess weak agonist activity (36.8% stimulation vs 100% for (−)-nicotine at 100 μM). In summary, data from functional assays indicate that compounds **3a**–**h**, **j**–**k**, and **4** are nicotinic antagonists at the $\alpha_3\beta_4$ subtype, while compound **3i** is a partial agonist.

Carroll et al.[18,19] have reported the synthesis, binding affinities at the αβ nAChR in rat cortical membrane, and antagonism of nicotine-elicited analgesia for compounds **3a**, **d**, **g**, and **h**. Binding affinities of these compounds at the αβ nAChR in rat cortical membrane are similar to the affinities for the rat $\alpha_4\beta_2$ and $\alpha_2\beta_2$ subtypes reported here. We have extended the observation of Carroll et al. by providing additional binding data of these compounds for other nAChR subtypes, and by demonstrating the binding selectivity of these compounds for nAChR subtypes containing the β_2 subunit. Further, we obtained data indicating the antagonist nature of these compounds at the $\alpha_3\beta_4$ nAChR subtype, in addition to their reported antagonism of nicotine-elicited analgesia that appears to be mediated by the $\alpha_4\beta_2$ subtype.[18,19] For these four compounds at least, it can be stated that they are antagonists at both the $\alpha_3\beta_4$ and $\alpha_4\beta_2$ nAChR subtypes. However, their functional activities at other nAChR subtypes remain to be elucidated.

Examination of structure–activity relationship offers several interesting observations. First, placement of a chlorine instead of a fluorine at the 2′-position of the pyridine ring increases the binding affinity across receptor subtypes. For example, the K_i's of compound **3a** (with a 2′-chlorine) are 17 (for $\alpha_2\beta_2$ subtype) to 40 times (for $\alpha_3\beta_4$ subtype) lower than those of **3d** (with a 2′-fluorine). Secondly, when chlorine is placed at the 2′-position of the pyridine ring, placement of a substituent at the 4-position of the 3′-phenyl ring maintains, but does not increase the binding affinities of the parent compound (compound **3a** vs **3b**–**c**). On the other hand, when fluorine is present at the 2′-position of the pyridine ring,

Table 1. Pharmacological properties of new epibatidine analogues: comparison with (−)-nicotine and (±)-epibatidine



Compound	R^1	R^2	R^3	R^4	K_i (nM)[a]						Agonist effect[b] % stimulation
					$\alpha_4\beta_2$	$\alpha_4\beta_4$	$\alpha_2\beta_2$	$\alpha_2\beta_4$	$\alpha_3\beta_2$	$\alpha_3\beta_4$	
(−)-Nicotine[c]					10	40	12	110	47	440	100
(±)-Epibatidine[c]					0.061	0.16	0.025	0.095	0.035	0.57	112
3a	H	H	Cl	H	0.039	1.15	0.037	0.470	0.122	24.0	6.1
4	H	H	Cl	CH_3	0.032	1.57	0.053	0.240	0.158	15.4	3.9
3b	CH_3	H	Cl	H	0.058	4.75	0.044	5.26	0.171	429	3.8
3c	OCH_3	H	Cl	H	0.034	1.57	0.040	0.862	0.100	54.5	3.1
3d	H	H	F	H	0.733	26.0	0.643	16.6	3.54	947	0.4
3e	CH_3	H	F	H	0.028	1.22	0.035	0.541	0.078	29.0	1.0
3f	H	CH_3	F	H	0.898	36.7	1.11	31.3	9.27	2400	0.1
3g	Cl	H	F	H	0.062	3.99	0.064	4.12	0.339	256	3.2
3h	F	H	F	H	0.078	6.42	0.079	6.12	0.504	405	3.6
3i	CN	H	F	H	0.039	3.60	0.066	3.67	0.351	289	36.8
3j	Cl	Cl	F	H	0.280	7.36	0.245	6.99	2.31	334	2.7
3k	F	CH_3	F	H	1.26	67.6	1.54	60.1	8.84	2940	−0.7

[a] Competitive binding experiments were conducted as described previously.[22] Ten concentrations of each compound were tested for their ability to compete with [^{3}H]epibatidine (0.5 nM) for binding sites in six stably transfected cell lines, each expressing one of the six defined receptor subtypes: $\alpha_2\beta_2$, $\alpha_2\beta_4$, $\alpha_3\beta_2$, $\alpha_3\beta_4$, $\alpha_4\beta_2$ and $\alpha_4\beta_4$. K_d values (nM) for [^{3}H]epibatidine used for calculating K_i values were 0.02 for $\alpha_2\beta_2$, 0.08 for $\alpha_2\beta_4$, 0.03 for $\alpha_3\beta_2$, 0.30 for $\alpha_3\beta_4$, 0.04 for $\alpha_4\beta_2$ and 0.09 for $\alpha_4\beta_4$.[23]

[b] Functional properties of compounds were determined by measuring $^{86}Rb^+$ efflux from $KX\alpha_3\beta_4R2$ cells as described previously.[22] The agonist activity of a compound was determined by measuring stimulated $^{86}Rb^+$ efflux in the presence of the compound (100 μM). Stimulated $^{86}Rb^+$ efflux by 100 μM of (−)-nicotine was defined as 100% of stimulation.

[c] Data for (−)-nicotine and (±)epibatidine were taken from the literature[23,28] and are shown here for comparison.

placement of an additional substituent at the 4-position of the 3′-phenyl ring increases binding affinities by at least one order of magnitude (compound **3d** vs **3e** and **3g–i**). However, substitution at the 3-position of the 3′-phenyl ring results in decreased affinities (compound **3d** vs **3f**). This later observation is in agreement with the results of Carroll et al. who demonstrated a 2–5 times difference in K_i's between the 3- and 4-substituted compounds for the rat cortical nAChR αβ subunits.[19] Disubstitution on the 3′-phenyl ring exerts a more complex effect on affinities. For example, 3,4-dichloro substitution increases the binding affinities (**3j**), while the 3-methyl-4-fluoro substitution decreases them (**3k**). Thirdly, the electronic nature of the substituent at the 4-position of the 3′-phenyl ring has minimal effect on the binding affinities (compare the K_i's of compounds **3e**, and **3g–i**). Finally, N-methylation of the 7-azabicyclo[2.2.1]heptane moiety induces no significant changes in binding affinities (**3a** vs **4**), as has been demonstrated elsewhere by other epibatidine analogues.[27]

In summary, a series of epibatidine analogues has been synthesized. Pharmacological studies indicate that the synthesized compounds bind with high affinities to all nicotinic receptor subtypes tested, except the $\alpha_3\beta_4$ subtype. In addition, these new compounds display binding selectivity for the $\alpha_x\beta_2$ subtypes of nicotinic acetylcholine receptor over the $\alpha_x\beta_4$ subtypes, and especially for the $\alpha_4\beta_2$ and $\alpha_2\beta_2$ subtypes. Carroll et al. have reported that similar compounds display low affinities for the α_7 subtype of nACh receptors.[19] Therefore, further structure–activity relationship studies of this class of compounds might offer the opportunity for the discovery of subtype selective nicotinic acetylcholine receptor ligands.

Acknowledgments

This research was supported by the National Institutes of Health (R21 MH66624-01), by the National Institute of Mental Health through the Psychoactive Drug Screening Program (Grant N01 MH32004), and by the Lieber Center for Schizophrenia Research at Columbia University.

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