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Bioorganic & Medicinal Chemistry Letters
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Bioorganic & Medicinal Chemistry Letters 14 (2004) 1889–1896

Epibatidine structure–activity relationships

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Received 6 October 2003; revised 3 February 2004; accepted 3 February 2004

Abstract—Epibatidine is a potent but nonselective nAChR agonist. Its biological effects appear to be mediated largely by α4β2 nAChRs. Surprisingly, only a limited number of epibatidine analogues have been synthesized and evaluated in in vitro assays. Even fewer analogues have received in vivo pharmacological evaluation. In this paper, SAR studies directed toward epibatidine analogues will be reviewed.
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1. Introduction

Daly et al. reported the isolation, structural characterization, and potent analgesic activity of epibatidine (**1**) in 1992.[1] Subsequent studies showed that the analgesic activity resulted from interaction with acetylcholine nicotinic receptors (nAChRs).[2] The unique structure and biological activity of epibatidine generated considerable interest in this compound and precipitated the development of numerous routes for its synthesis from simple starting materials.[3–8] In addition, since the structural features of epibatidine were not consistent with previously proposed nicotinic pharmacophores, new pharmacophore models have been set forth to accommodate epibatidine (**1**).[9–12]

The synthetic methodology developed for the synthesis of epibatidine (**1**) provided the means for the synthesis of a number of epibatidine analogues, which have been evaluated for their biological activity. The structure–activity relationship (SAR) gained from these studies will be presented in this paper. The review will concentrate primarily on data from radioligand binding studies, however, some animal studies will also be presented. The in vitro SAR studies have involved the use of [^{3}H]nicotine, [^{3}H]cytisine, and [^{3}H]epibatidine as radioligands for α4β2 nAChRs and [^{125}I]α-bungarotoxin, [^{3}H]MLA, and [^{125}I]iodo-MLA as radioligands for α7 nAChRs. Most studies have used rat brain tissue as a source of nAChRs, however, in some cases mouse brain tissue or cloned receptors were used. The majority of the animal studies involved evaluating the compounds in the mouse tail-flick and rat or mouse hot-plate antinociception tests, but drug discrimination and spontaneous activity testing have also been used.

The SAR studies presented in this paper will be subdivided into the following sections. With few exceptions, the studies will be limited to analogues where only one modification to the epibatidine structure has been made:

1. Changes in stereochemistry;
2. Replacement of the N–H with other groups;
3. Changes in the 2′-chloropyridine ring;
4. Replacement of the 2′-chloropyridine ring with bioisosteric rings;
5. Changes in the 7-azabicyclo[2.2.1]heptane ring system; and
6. Conformationally-constrained analogues.

2. Changes in stereochemistry

Epibatidine (**1**) has a 7-azabicyclo[2.2.1]heptane structure to which is attached an *exo*-5-(2′-chloropyridinyl) substituent. Fletcher et al.[13] showed that the natural alkaloid possessed the (1*R*,2*R*,4*S*)-stereochemistry. Epibatidine (**1**) and its enantiomer possess negative and positive rotations of the plane of polarized light, respectively. However, salts showed opposite signs of rotation from the free bases.

Badio et al.[14] reported the inhibition of [^{3}H]nicotine binding to rat membranes by (−)- and (+)-epibatidine (**1**) and compared the results obtained to that of (−)- and (+)-nicotine (**2**) (Table 1). In contrast to nicotine where

Keywords: Epibatidine; Nicotine; SAR; Nicotinic receptors; nAChRs.
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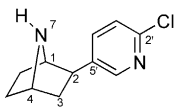
doi:10.1016/j.bmcl.2004.02.007

Table 1. Radioligand binding data and antinociception, spontaneous activity, and drug discrimination potencies for epibatidine stereoisomers

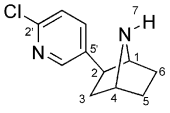
Compd	[^{3}H]Ligand K_i (nM)	Spontaneous activity ED_{50} (ug/kg)	Tail-Flick ED_{50} (ug/kg)	Drug discrimination ED_{50} (ug/kg)
(−)-Epibatidine (**1**)	0.058,[a] 0.055,[b] 0.026[c]	1.8[b]	9,[d] 6.1[b]	1.0[b]
(+)-Epibatidine (**1**)	0.045,[a] 0.055,[b] 0.018[c]	1.2[b]	7,[d] 6.6[b]	0.9[b]
(−)-Nicotine (**1**)	1.0[a]			
(+)-Nicotine (**1**)	38[a]			
endo-Epibatidine (**3**)	7.6[e,f]		>1000[d]	

[a] Taken from ref 14; [^{3}H]nicotine.
[b] Taken from ref 15; [^{3}H]nicotine.
[c] Taken from ref 16; [^{3}H]epibatidine.
[d] Taken from ref 17.
[e] Taken from ref 11; [^{3}H]cytisine.
[f] The material assayed was racemic.

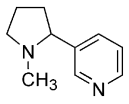
there is a 38-fold difference between the two isomers, (−)- and (+)-epibatidine possessed similar affinities. Damaj et al.[15] and Carroll et al.[16] have also reported that (−)- and (+)-epibatidine (**1**) have similar affinities for nAChRs (Table 1). Li et al.[17] and Damaj et al.[15] each evaluated (−)- and (+)-epibatidine (**1**) in the mouse tail-flick antinociception test and reported that the isomers possessed almost identical ED_{50} values (Table 1). Both isomers also showed potent antinociceptive activities in the mouse hot-plate test.[18] Damaj et al.[15] also reported that the ED_{50} values for spontaneous activity and drug-discrimination for the two isomers were very similar. *endo*-Epibatidine (**3**) which results from changing the 2-*exo*-(2′-chloropyridinyl) substituted in epibatidine (**1**) to the *endo*-position possesses relatively low affinity for the nAChR[11] and does not possess any antinociceptive activity in the tail-flick test at a dose of >1000 ug/kg.[17]



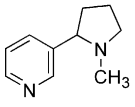
(*1R,2R,4S*)-epibatidine (**1**), Natural freebase, (-)-isomer salts, (+)-isomer



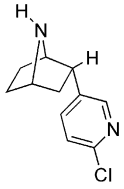
(*1S,2S,4R*)-epibatidine (**1**), freebase, (+)-isomer salts, (-)-isomer



(*S*)-(-)-nicotine (**2**)



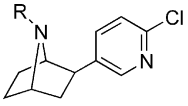
(*R*)-(+)-nicotine (**2**)



endo-epibatidine (**3**)

3. Replacement of the N–H with other groups

Replacement of the hydrogen on the N–H group of epibatidine with a methyl, ethyl, allyl, and an acetyl group provided the *N*-substituted analogues **4a–d**.[14,15,17] Replacement of the hydrogen of the NH group of (±)-, (−)-, and (+)-epibatidine with a methyl group to give (±)-, (−)-, and (+)-**4a** had very little effect on the nAChR binding affinity (Table 2).[14,17,19] The EC_{50} value of 9 ug/kg in the tail-flick antinociception test for (−)-**4a** is identical to that observed for (−)-epibatidine (**1**).[17] Replacement of the hydrogen of the N–H group of (±)-epibatidine with an ethyl group or (−)-epibatidine with an allyl group resulted in a large reduction of nAChR binding affinity (Table 2).[15,19] The binding affinity for the *N*-acetyl analogue **4d** has not been reported, however, it has very low activity (EC_{50} >1000 ug/kg) in the tail-flick antinociception test.[17]



4a, R = CH_3
b, R = C_2H_5
c, R = C_3H_7
d, R = CH_3CO

4. Changes in the 2′-chloropyridine ring

A number of 2′-substituted deschloro-epibatidine analogues **5a–i** have been synthesized and their nAChR binding properties were determined using various radioligands (Table 3).[16,18–20] It is interesting to note that epibatidine (**1**), the deschloro analogue **5a**, and the 2′-fluoro, 2′-bromo, and 2′-iodo-deschloro analogues, **5a–d**, respectively, all possess essentially the same K_i values. Thus, replacing the 2′-chloro substituent of epibatidine (**1**) with much larger iodo or bromo substituents or much smaller hydrogen and fluoro groups had very little effect on binding affinity. Contrarily, compounds **5e–g**, which have 2′-electron-donating groups, all have much

Table 2. Radioligand binding data and antinociception potency for *N*-substituted epibatidine analogues

Compd	[^{3}H]Ligand K_i (nM)	Tail-Flick ED_{50} (ug/kg)
(−)-**4a**	0.11[a]	9[b]
(+)-**4a**	0.26[a]	
(±)-**4a**	0.027[c]	
(±)-**4b**	13.6[c]	
(−)-**4c**	10.8[d]	
(−)-**4d**		>1000[b]

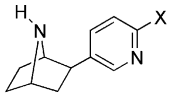
[a] Taken from ref 14; [^{3}H]nicotine.
[b] Taken from ref 17; [^{3}H]nicotine.
[c] Taken from ref 19; [^{3}H] (±)-epibatidine.
[d] Taken from ref 15; [^{3}H]nicotine.

Table 3. Radioligand binding data and antinociception potencies for 2′-substituted deschloroepibatidine analogues

Compd	[^{3}H]Ligand K_i (nM)	Tail-Flick, ED_{50} nmol/mouse[a]
(−)-Epibatidine (**1**)	0.026[a]	0.5
5a	0.020,[a] 0.030[b]	0.2
5b	0.027,[a] 0.037[c]	0.31
5c	0.023,[a] 0.045[c]	0.33
5d	0.070,[a] 0.09,[d] 0.48[b]	NT
5e	107[a]	52.4
5f	1.3[a]	27% @ 112
5g	26.4[a]	10% @ 32.5
5h	8.5[a]	19.2
5i	0.13[b]	NT

[a] Taken from ref 16; [^{3}H]epibatidine.
[b] Taken from ref 18; [^{3}H]nicotine.
[c] Taken from ref 19; [^{3}H]epibatidine.
[d] Taken from ref 20; This is K_d value of [^{125}I]analogue.

less binding affinity than epibatidine. The 2′-amino analogue **5f** with a K_i value of 1.3 nM has considerably greater affinity than the 2′-hydroxy analogue **5e**, which has a K_i value of 107 nM. Changing the 2′-amino compound to the 2′-*N*,*N*-dimethylamino analogue **5g** ($K_i = 26.4$) also results in a large loss of affinity. With the exception of **5f** and **g**, all of the compounds tested in the tail-flick test were nAChR agonists. There is a good correlation ($r^2 = 0.96$) between the K_i (nM) binding affinity values to rat brain [^{3}H]epibatidine sites and ED_{50} (nmol/mouse) values for antinociceptive potency.[16]



5a, X = H **5f**, X = NH_2
b, X = F **g**, X = $N(CH_3)_2$
c, X = Br **h**, X = CF_3SO_3
d, X = I **i**, X = CH_3
e, X = OH

Equilibrium binding affinities for epibatidine (**1**), the deschloro analogue **5a**, and the 2′-fluoro (**5b**), 2′-bromo (**5c**), 2′-hydroxy (**5e**), 2′-amino (**5f**), 2′-dimethylamino (**5g**), and 2′-trifluoromethanesulfonate (**5h**) analogues at α2β2, α2β4, α3β2, α3β4, α4β2, and α4β4 rat nAChRs expressed in Xenopus oocytes have been reported and are listed in Table 4.[21] In general, epibatidine (**1**) and the analogues showed higher affinities for the β2-containing receptors than the β4-containing receptors. For example, the deschloro analogue **5a** showed affinities 114- to 3500-fold greater at β2 than at β4-containing receptors. The largest difference in affinity was seen with α3β2 and α3β4 (a 3500-fold difference). The 2′-fluoro, 2′-bromo, and 2′-amino analogues **5b**, **c**, and **f** displayed affinities 52- to 895-fold, 4- to 55-fold, and 10- to 115-fold greater at β2 than at β4-containing receptors, respectively. The 2′-hydroxy (**5e**), 2′-dimethylamino (**5g**), and 2′-trifluoromethanesulfonate (**5h**) analogues possessed low affinity at all nAChRs studied.

The functional potency and efficacy for acetylcholine, epibatidine (**1**), deschloroepibatidine (**5a**) and the 2′-fluoro (**5b**), 2′-bromo (**5c**), and 2′-amino (**5f**) analogues for activation of α4β2, α3β4, and α4β4 neuronal nAChR subunit combinations have been reported and the data are listed in Table 5.[21] Epibatidine (**1**) showed similar potency and efficacy at α4β2 ($EC_{50} = 14$ and 58 Max%) and α4β4 ($EC_{50} = 17$ and 71 Max%) receptors. Its potency at the α3β4 receptor was 8–9-fold lower, with only slightly less efficacy. The deschloro analogue **5a** exhibited high efficacy at both α4β2 and α4β4 receptors (112 and 147%, respectively) with a significantly lower efficacy of 61% at α3β4 receptors. Deschloro epibatidine (**5a**) was less potent at α4β2 receptors ($EC_{50} = 3630$ nM) than at α3β4 and α4β4 ($EC_{50} = 702$ and 982 nM, respectively). The 2′-fluoro substituted analogue **5b** displayed the greatest improvement in subtype selectivity in terms of efficacy. Its efficacy of 131% at α4β4 was significantly higher than at α4β2 and α3β4 receptors (41 and 40%, respectively). However, its EC_{50} values were similar for all the receptor subtypes. 2′-Bromo analogue **5c** exhibited efficacies similar to acetylcholine at α4β2, α3β4, and α4β4 receptors (65, 82, and 102%). Its efficacy at α4β2 was significantly less than at α4β4. The compound was also significantly less potent at α4β2 ($EC_{50} = 94$ nM) and α3β4 ($EC_{50} = 189$ nM) receptors relative to α4β4 ($EC_{50} = 20$ nM). The 2′-amino analogue **5f** displayed an efficacy at the α3β4 receptor (82%) that was similar to acetylcholine, whereas, the efficacy at the α4β2 receptor was significantly lower (29%). The 2′-amino analogue **5f** was less potent at α4β2 ($EC_{50} = 7600$ nM) than at α3β4 and α4β4 ($EC_{50} = 3240$ and 1840 nM, respectively). Comparison of the data in Tables 4 and 5 for the α4β2 and α3β4 data shows that the affinity values for the 2′-substituted deschloro analogue (Table 4) were not predictive of the relative potencies determined in the functional assays (Table 5). For example,

Table 4. Equilibrium binding affinities for 2′-substituted deschloroepibatidine analogues on neuronal nAChRs[a]

Compd	[^{3}H]epibatidine, K_i (pM)					
	α2β2	α2β4	α3β2	α3β4	α4β2	α4β4
Epibatidine (**1**)	10	87	14	300	30	85
5a	28	3300	3.1	11,000	8.5	3200
5b	2.4	1200	3.8	2100	9.2	480
5c	13	55	4	220	10	44
5e	>100,000	N.D.	>100,000	N.D.	~100,000	N.D.
5f	62	3500	77	7200	350	4300
5g	>100,000	N.D.	>100,000	N.D.	>100,000	N.D.
5h	>10,000	N.D.	~100,000	N.D.	~100,000	N.D.

[a] Taken from ref 21.

Table 5. Functional potency and efficacy for epibatidine and 2′-substituted deschloroepibatidine analogues[a]

Compd	α4β2		α3β4		α4β4	
	Max%	EC_{50} (nM)	Max%	EC_{50} (nM)	Max%	EC_{50} (nM)
ACh	95	87,000	100	217,000	98	19,000
Epibatidine (**1**)	58	14	39	128	71	17
5a	112	3630	61	702	147	982
5b	41	254	40	319	131	239
5c	65	94	82	189	102	20
5f	29	7600	82	3240	41	1840

[a] Taken from ref 21.

Table 6. Radioligand binding data and antinociception properties for 3′-substituted epibatidine analogues[a]

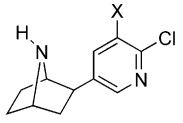
Compd	X	α4β2 [^{3}H]Epibatidine (K_i, nM)	α7 [^{125}H]iodo MLA (K_i, nM)	ED_{50} mg/kg Tail-Flick	ED_{50} mg/kg Hot-Plate	AD_{50} mg/kg	
						Tail-Flick	Hot-Plate
(−)-Epibatidine (**1**)	H	0.026	198	0.006	0.004		
(±)-Epibatidine (**1**)	H	0.035[b]	10.2[b]				
6a	Cl	0.014	1.12	0.05	0.03		
6b	F	0.025	3.43	0.008	0.009		
6c	Br	0.013	152	0.03	0.02		
6d	I	0.008	—	0.24	0.14	4% @ 0.01	0% @ 0.01
6e	NH_2	0.01	13.9	0.02	0.02	0.00003	0.0006
6f	C_6H_5	0.021[c]	—	0.7[c]	1.0[c]		
6g	N_3	0.027[b]	9.7[b]				

[a] Taken from ref 22.
[b] Taken from ref 24; [^{3}H]nicotine and [^{125}I]BTX was used for α4β2 and α7 nAChRs, respectively.
[c] Taken from ref 23.

the equilibrium binding of **5a** and **5b** for the α4β2 receptor was 1300- and 228-fold greater than the affinity for the α3β4 receptor. However, the functional potency of **5a** was five-fold greater at the α3β4 receptor than at the α4β2, and **5b** displayed equal functional potency at the α4β2 and α3β4 receptors. In contrast, there is a significant correlation ($r^2 = 0.96$) for affinity and EC_{50} values at the α4β4 receptor.

Several 3′-substituted epibatidine analogues **6a–g** have been synthesized and evaluated for inhibition of α4β2 nAChRs binding affinity using [^{3}H]epibatidine and α7 nAChR binding using [^{125}I]iodoMLA (Table 6).[22–24] The K_i values of (0.008 to 0.027 nM) of all the 3′-substituted epibatidine analogues (**6a–g**) were very similar to the K_i value of (−)-epibatidine ($K_i = 0.026$). The 3′-iodo (**6d**) and 3′-amino (**6e**) analogues with K_i values of 0.008 and 0.01 nM, respectively, possessed the highest affinity. The 3′-chloro (**6a**), 3′-fluoro (**6b**), 3′-amino (**6e**), and 3′-azido (**6g**) analogues all possessed significantly higher affinity at the α7 receptor relative to epibatidine (**1**). The 3′-bromo (**6c**) analogue and epibatidine possessed similar affinities at the α7 nAChR.

The antinociceptive properties for compounds **6a–f** using the tail-flick and hot-plate tests have been compared to those of epibatidine (**1**), and the results are summarized in Table 6.[22,25] All six analogues, **6a–f**, tested in the tail-flick and hot-plate tests were nAChR agonists with similar potencies in both antinociceptive assays. The 3′-fluoro analogue, **6b**, with ED_{50} values of 0.008 and 0.009 mg/kg in the tail-flick and hot-plate tests, respectively, as compared to 0.006 and 0.004 mg/kg for epibatidine (**1**) was the most potent analogue. The 3′-chloro and 3′-bromo analogues (**6a** and **c**, respectively) were 5–8-fold less potent than epibatidine. Since the 3′-iodo and 3′-amino analogues (**6d** and **e**, respectively) showed agonist activity below that expected from the K_i values for inhibition of [^{3}H]epibatidine binding, these two analogues were evaluated for nAChR antagonist activity in the tail-flick and hot-plate tests at lower doses.[22] The 3′-amino analogue **6e** (RTI-7527-33) was a potent nAChR antagonist in both tests with AD_{50} values of 30 and 600 ng/kg in the tail-flick and hot-plate tests, respectively. Compound **6d** lacked antagonist activity at a dose of 0.01 mg/kg in both tests.



6a, X = Cl **e**, X = NH_2
b, X = F **f**, X = C_6H_5
c, X = Br **g**, X = N_3
d, X = I

Table 7. Radioligand binding data and antinociceptive properties for 2′-fluoro-3-phenyl-deschloroepibatidine[a]

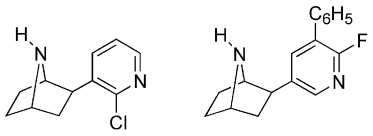
Compd	[^{3}H]epibatidine (K_i, nM)	ED_{50}[b]		AD_{50}[b]	
		Tail-Flick	Hot-Plate	Tail-Flick	Hot-Plate
Epibatine (**1**)	0.026	0.006	0.004		
(±)-**8**	0.24	3% @ 15	4% @ 15	0.5	1.2
(+)-**8**	0.24	7% @ 15	8% @ 15	1.0	2.4
(−)-**8**	0.26	5% @ 15	10% @ 15	0.08	0.7

[a] Taken from ref 23.
[b] Mg/kg or percent inhibition at 15 mg/kg.

Senokuchi et al. reported that the 6′-chloro analogue of epibatidine **7** showed an IC_{50} value of 3.7 nM compared to 0.5 nM for epibatidine using rat brain tissue and [^{3}H]methylcarboxylcholine as the radioligand.[26]

2′-Fluoro-3′-phenyldeschloroepibatidine (**8**) is a compound that involves two changes to the epibatidine structure (change of 2′-fluoro for 2′-chloro and the addition of a 3′-phenyl group). It is included in this review, since to our knowledge, it was the first epibatidine (**1**) analogue reported to show pure nAChR antagonists properties in vivo.[23] The results are listed in Table 7. The (±)-, (+)-, and (−)-**8** all have K_i values of about 0.24 nM for inhibition of [^{3}H]epibatidine binding, but show no nAChR agonist activity in the tail-flick or hot-plate tests at a dose of 15 mg/kg. Compounds (±)-, (+)-, and (−)-**8** blocked the antinociceptive effect of nicotine in the tail-flick test after s.c. administration with AD_{50} values of 0.5, 1.0, and 0.08 mg/kg, respectively. Thus, even though compound **8** did not show enantioselectivity in the binding assay (−)-**8** was 13-times more potent than (+)-**8** as an antagonist in the

tail-flick test. In the hot-plate test, (±)-, (+)-, and (−)-**8** blocked the antinociceptive effects of nicotine with AD_{50} values of 1.2, 2.4, and 0.7 mg/kg, respectively.



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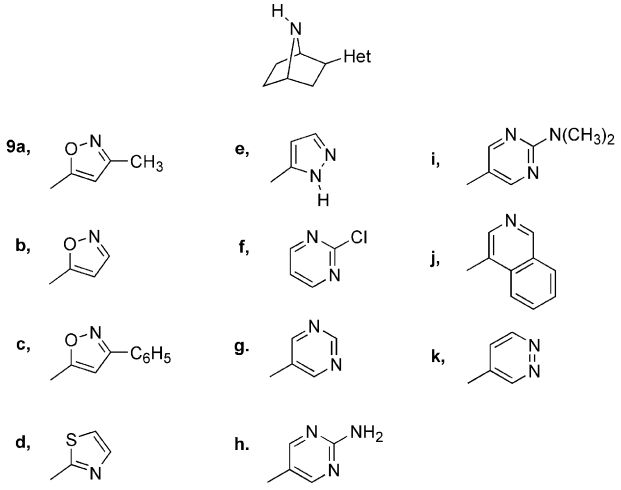
5. Replacement of the 2′-chloropyridine ring with bioisosteric rings

In order to gain information about the SAR for epibatidine class of nAChR and to possibly obtain compounds with a better pharmacological activity/toxicity ratios, several analogues were prepared where the 2′-chloropyridine ring was replaced by other heterocyclic groups. Badio et al. replaced the 2′-chloropyridine ring of epibatidine (**1**) with a 3′-methylisoxazolyl ring attached at the 5 position to give **9a**, an analogue that was named epiboxidine (Table 8).[27]



Compound **9a** was found to have 10-fold lower affinity in the [^{3}H]nicotine binding affinity assay and hot-plate antinociceptive test. Compound **9a** was reported to be 10-fold less toxic than epibatidine.[27] Singh et al. reported that **9a** had an IC_{50} value of 1.33 nM compared to 0.33 nM for epibatidine for inhibition of [^{3}H]cytisine binding.[28] They reported that **9a** was 9.2-fold less lethal than epibatidine. Singh et al. reported that the unsubstituted isoxazole analogue **9b** and the 3-phenylisoxazole analogue **9c** possessed IC_{50} values of 3.17 and 147 nM, respectively (Table 8). Seerden et al. reported that the 2-thiazolyl and 4-pyrazolyl analogues **9d** and **e**, respectively, possessed very low affinity for nAChRs.[29]

Table 8. Radioligand binding data and antinociception properties for 2′-chloropyridine ring replacement analogues

Compd	[^{3}H]Ligand (K_i, nM)	Hot-Plate [a]
Epibatidine (**1**)	0.058,[a] 0.33,[b] 0.16,[c] 0.18[d]	5 ug of Epibatidine gave same antinociceptive activity as 50 ug of **9a**.
9a	0.6,[a] 1.33[b]	
9b	3.17[b]	
9c	147[b]	
9d	5800[c]	
9e	18[c]	
9f	0.31[d]	
9g	0.81[c]	
9h	2.6[c]	
9i	29,500[c]	
9j	53[c]	

[a] Taken from ref 27; [^{3}H]nicotine.
[b] Taken from ref 28; IC_{50} values using [^{3}H]cytisine.
[c] Taken from ref 29; [^{3}H]cytisine.
[d] Taken from ref 30; [^{3}H]cytosine.

Four analogues, **9f–i**, have been reported where the 2′-chloropyridine ring of epibatidine (**1**) was replaced by pyrimidine and substituted pyrimidine rings (Table 8).[29,30] The most potent analogue is the 5-(2′-chloropyrimidinyl) compound **9f**, which has a K_i value of 0.31 nM compared to 0.18 nM for epibatidine. The 5-pyrimidinyl analogue **9g** with a K_i value of 0.81 nM is only slightly less potent. The 5-(2-aminopyrimidinyl) analogue **9h** is 8.6 times less potent than the 5-(2-chloropyrimidinyl) analogue **9f**. Changing **9h** to the 5-(2′-dimethylamino)pyrimidinyl analogue **9i** results in a large reduction in affinity for nAChRs (K_i value of 2.6 nM for **9h** compared to 29,500 nM for **9i**). The 4-isoquinolinoyl analogue **9j** also showed weak affinity for the nAChRs.

The 4-(pyridazinyl) analogue **9k** was assayed in vitro by whole-cell current recordings from Xenopus oocytes expressing nAChRs from rats.[31] Compound **9k** showed potencies relative to epibatidine of 0.25, 0.03, 0.01, and 0.20 at α4β2, α3β4, α7, and rat muscle receptors.[31]

6. Changes in the 7-azabicyclo[2.2.1]heptane ring

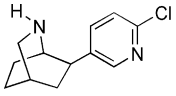
A number of epibatidine (**1**) analogues with changes in the 7-azabicyclo[2.2.1]heptane ring have been synthesized and evaluated. Krow et al. reported the nAChR

Table 9. Radioligand binding and antinociceptive potency of 7-azabicyclo[2.2.1]heptane ring modified analogues of Epibatidine

Compd	[^{3}H]Ligand (K_i, nM)	[^{125}I]α-BTX[b]	Tail-Flick
			EC_{50}, mg/g
Epibatidine (**1**)	0.090,[a] 0.02,[b] 0.04,[c] 0.26[d]		0.01[a]
10	0.47[a]		0.04[a]
11	0.34[a]		1.4[a]
12	0.056,[b] 6.6[c]	6.3	
13	>38,[b] 30[c]	3300	
14	0.045,[b] 0.032[c]	3.9	
15	>38[b]	1600	
(+)-**16**	0.13 [d]		
(−)-**16**	0.35[d]		
17	1.25[d]		
18	1.6[e]		
19	3.9[f]		
20	5.0[f]		

[a] Taken from ref 32; [^{3}H]cytisine.
[b] Taken from ref 25; [^{3}H]nicotine.
[c] Taken from ref 11; [^{3}H]cytisine.
[d] Taken from ref 33; [^{3}H] nicotine.
[e] Taken from ref 34; [^{3}H]epibatidine.
[f] Taken from ref 39; [^{3}H]cytisine.

binding data and tail-flick antinociceptive potency for 2′-(chloro-5′-pyridinyl)-2-azabicyclo[2.2.2]octanes **10** and **11** (Table 9).[32] These structures are nitrogen bridge homologsue of epibatidine where a methylene group has been inserted between the 7-aza group and the bridgehead positions. The vicinal (to NH group) analogue **10** and distal analogue **11** with K_i values of 0.47 and 0.34 nM are about 0.19- and 0.25-times less potent than epibatidine. The vicinal analogue **10** with an EC_{50} value of 0.04 mg/kg is about one-quarter as potent as epibatidine in the tail-flick test. The distal analogue **11** is much weaker in this test.



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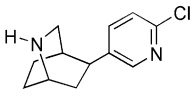
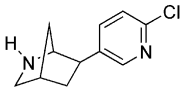


11

The 5- and 6-(2′-chloro-5′-pyridinyl)heptanes **12–15** can be viewed as epibatidine analogues where the 7-position nitrogen of epibatidine (**1**) has been moved to the 5- or 6-position of the 7-azabycyclo[2.2.1] ring. Cox et al. have synthesized all four analogues and evaluated their α4β2 and α7 nAChR potency for inhibition of [^{3}H]nicotine binding to rat cortical membranes and [^{125}I]BTX binding in rat hippocampal membranes, respectively.[25] These authors found that **12** and **14** with K_i values of 0.056 and 0.045 nM for α4β2 nAChRs were almost as potent as epibatidine (**1**), which showed a K_i value of 0.02 nM in their studies (Table 9). Compounds **12** and **14** showed K_i values of 6.3 and 3.9 nM for α7 nAChR (Table 9). Compounds **13** and **15** possessed low affinity for both α4β2 as well as α7 nAChRs. Dart et al. also synthesized analogues **12–14** and evaluated their α4β2 nAChR potency using whole rat brain tissue and [^{3}H]cytisine as the radioligand. These authors also found **14** to be the more potent compound and **15** to have low affinity. Under these conditions, compound **14** with a K_i value of 0.032 nM was equipotent to epibatidine with a K_i value of 0.04 nM. However, in contrast to Cox and Malpass et al.,[25] Dart et al. found analogue **12** to have relatively low affinity (K_i 6.6 nM compared to 0.056) for α4β2 nAChRs.



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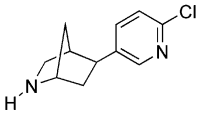

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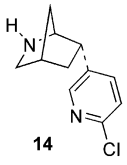

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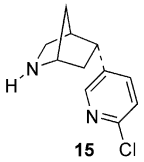

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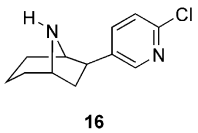

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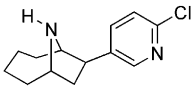

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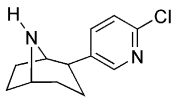
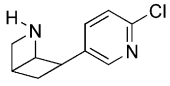


18

Malpass et al. reported the synthesis and evaluation of the homo- and dihomoepibatidine analogues **16** and **17** where one and two methylene groups have been inserted into the 5–6 bond of epibatidine (**1**).[33] Zhang et al. synthesized and evaluated the homoepibatidine analogue **18** where a methylene group has been inserted between the 2–3 bond of epibatidine.[34] The (+) and (−)-isomers of the 5–6 bond homo insertion analogue **16** have K_i values of 0.13 and 0.35 nM, respectively, for inhibition of [^{3}H]nicotine binding using rat brain tissue (Table 9). The (±)-isomer of **16** is reported to have about one-quarter the potency of epibatidine in the hot-plate test.[7] The addition of the second methylene to give the 5–6 bond insertion dihomo analogue **17** and one methylene to the 2–3 bond to give **18** results in significant loss of affinity (Table 9).

The highly rigidified 6-(2′-chloro-5-pyridinyl)hexanes structures **19** and **20** were synthesized and evaluated for inhibition of [^{3}H]cytisine binding to rat brain tissue.[35] Both compounds showed weak affinity at the α4β2 nAChRs relative to epibatidine. The amino vicinal isomer **19** with a K_i value of 3.9 nM was slightly more potent than the distal isomer **20** (Table 9).



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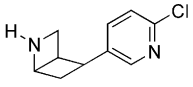

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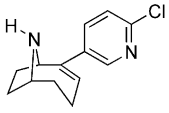

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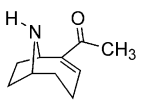


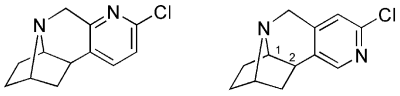
22 (*Anatoxin-a*)

The compound **UB-165** (**21**) was designed as an analogue of anatoxin-*a* (**22**). However, it can also be viewed as a 7-azabicyclo[2.2.1]heptane ring modified analogue of epibatidine (**1**). Sharples et al. reported that the K_i value for binding affinity of **UB-165** (**21**) at α4β2, α3β4, and α7 nAChRs were 0.27, 6.5, and 2760 nM.[36] Rat brain tissue and [^{3}H]nicotine and [^{3}H]MLA were used for the α4β2 and α7 assays, respectively. Cloned rat nAChR and [^{3}H]epibatidine were used for the α3β4 subtype. The K_i values obtained for epibatidine were 0.021, 0.077, and 101 nM for the α4β2, α3,β4, and α7 receptors, respectively. The **UB-165** (**21**) radioligand binding affinity for α4β2, α3β4, and α7 nAChRs were also reported by Gohlke et al.[37] The assays by these authors used rat brain tissue for α4β2 and α7 assay and pig adrenal glands for α3β4 assay. [^{3}H]epibatidine was used for the α4β2 and α3β4 assays and [^{3}H]MLA for the α7 assays. Under the conditions applied by these authors, **UB-165** show K_i values of 0.04, 1.3, and 12 nM at the α4β2, α3β4, and α7 nAChRs,[37] which are lower K_i values for **UB-165** at all three nAChRs than those reported by Sharples.[36] However, they also report lower values for epitabidine (K_i = 0.008, 0.022, and 4.0 nM for the α4β2, α3β4, and α7 subtypes).

7. Conformationally-constrained epibatidine analogues

There have been three attempts to explore the active conformation of epibatidine (**1**) by the synthesis and

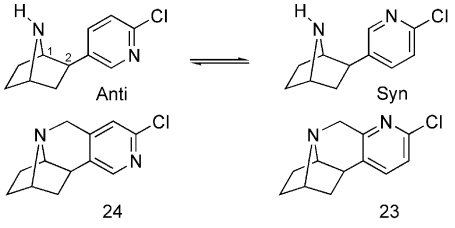
biological evaluation of conformationally-constrained epibatidine analogues. In one report,[38] compounds **23** and **24** were designed and synthesized as conformationally-locked analogues of epibatidine (**1**) comparable to the two principle low energy confirmations of the freely rotating pyridine ring in epibatidine.[9,11,39,40] In the calculated *syn* conformation of epibatidine, the N-C1-C2-N dihedral angle is ~42° and in compound **23**, the corresponding dihedral angle is ~43° (Table 10). In the '*anti*' conformation of epibatidine, the N–C1–C2-N dihedral angle is 133° and in **24**, the corresponding dihedral angle is ~138° (Table 10). The nitrogen-to-nitrogen distances are somewhat shorter in **23** and **24** than in the corresponding epibatidine (**1**) conformation, however, compound **24** with a nitrogen-to-nitrogen distance of 5.1 Å is well within the 4.5–5.5 Å range that has been proposed by several authors for the nicotinic pharmacophore (Table 10).[9,11,39,40] Even though the bridged analogues **23** and **24** possess several of the structural features in proposed pharmacophores for the nAChRs, neither compound showed high affinity for inhibition of [^{3}H]epibatidine binding using rat brain tissue. However, it is interesting to note that analogue **23** with the shorter N–N distance is more potent than analogue **24** (Table 11).



23 **24**

In another study, Wie et al. designed and synthesized the spirocyclic analogues **25** and **26** and the fused cyclic

Table 10. Comparison of the structural properties of **23** and **24** to those of epibatidine (**1**)



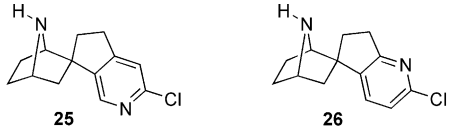
Compd	N–N Distance	N–C1–C2–N dihedral
23	3.8	43
24	5.1	138
syn-Epibatidine	4.6	42
anti-Epibatidine	5.6	133

Table 11. Radioligand binding data for bridged epibatidine compound **23** and **24**

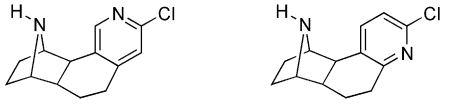
Compd	[^{3}H]Ligand (K_i, nM)
Epibatidine (**1**)	0.026[a]
23	1260[a]
24	3330[a]
29	136[b]
30	>1000[b]

[a] Taken from ref 38; [^{3}H]epibatidine.
[b] Taken from ref 42; [^{3}H]cytisine.

analogues **27** and **28** as conformationally-constrained analogues of epibatidine (1).[41] Similar to **23** and **24**, these four analogues did not show appreciable affinity at any of the six cloned nAChRs for which they were evaluated.[41] In a separate study, Abe et al.[42] synthesized the spirodihydrofuro-pyridine *syn* and *anti* analogues **29** and **30**. These authors also found that both analogues showed weak affinity for the nAChR using [^{3}H]cytisine as the radioligand. However, they did note that similar to the results with **23** and **24**, the *syn* analogue **29** was more potent than the *anti* analogue **30** (Table 11).

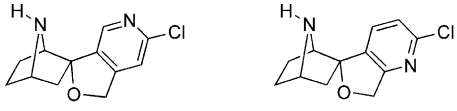


25 **26**

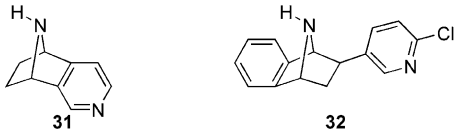


27 **28**

Lennox et al. reported the synthesis of the annulated epibatidine analogue **31**, but did not report any biological data.[43] We also synthesized this analogue (Carroll unpublished) and found that it possessed very low affinity for inhibition of [^{3}H]epibatidine. We also synthesized the analogue **32**, which is an epibatidine analogue with a benzene ring annulated to the 5,6-positions. This compound showed a K_i value of 90 nM for inhibition of [^{3}H]epibatidine binding (Carroll unpublished).



29 **30**



31 **32**

8. Summary

Unlike nicotine, (+)- and (−)-epibatidine showed similar radioligand binding affinities and activities in antinociceptive test. Conversion of epibatidine to its N–CH_3 analogue had only small effects on biological activity, whereas changes to larger groups or an acetyl group resulted in large losses of activity. Compounds with biological properties much like epibatidine can be obtained by replacement of the 2′-chloro group with other halogens or a hydrogen, while electron donating group in the 2′-position cause a large reduction in affinity. The addition of 3′-substituents to epibatidine can lead to compounds with high affinity for nAChR that are agonist or mixed agonist-antagonists. Most interestingly, 2′-fluoro-3′-phenyldeschloroepibatidine was found to be a potent nAChR antagonist in the tail-flick and hot-plate tests. With few exceptions, analogues resulting from replacement of the 2′-chloropyridine ring

of epibatidine with bioisosteric rings or changes in the 7-azabicyclo[2.2.1]heptane ring show reduced affinity for the nAChR relative to epibatidine. Nevertheless, several of these analogues are considerably more potent than nicotine. Thus far, all conformationally-constrained analogues of epibatidine have possessed very low affinity for the nAChRs.

Acknowledgements

This research was supported by the National Institute on Drug Abuse, Grant 12001.

References and notes

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