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The identification of novel structural compound classes exhibiting high affinity for neuronal nicotinic acetylcholine receptors and analgesic efficacy in preclinical models of pain

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Abstract

Neuronal nicotinic acetylcholine receptors represent a new and potentially useful target for the development of novel non-opioid, non-NSAID (nonsteroidal antiinflammatory drug) analgesic agents. A variety of nicotinic acetylcholine receptor agonists such as nicotine, epibatidine and the azetidinyl ether, (*R*)-5-(2-azetidinylmethoxy-2-chloropyridine (ABT-594) possesses significant efficacy in preclinical models of pain. A preponderance of evidence suggests that nicotinic acetylcholine receptor agonists produce their analgesic effects predominantly via activation of descending inhibitory pain pathways originating in the key brainstem regions of the nucleus raphe magnus, dorsal raphe, and locus coeruleus, and that $\alpha 4$-containing nicotinic acetylcholine receptor subunits mediate these effects. Although these studies may provide a pharmacological target for the development of nicotinic acetylcholine receptor analgesics, the rational design of selective ligands based on the protein structure of the binding site is hampered by insufficient structural information. Using an approach based upon homology to known high-affinity ligands for the $\alpha 4\beta 2$ binding site, a four-point model is proposed which defines distance and directionality parameters common to this set of nicotinic acetylcholine receptor ligands.

Keywords: Nicotinic acetylcholine receptor; Molecular model; Epibatidine; ABT-594; A-85380; Analgesia; Antinociception

It has been recognized for many years that nicotine possesses antinociceptive properties; however, relatively high doses are required to produce antinociception and the effect is relatively modest and of short duration (Davis et al., 1932). The much more recent observation that epibatidine (**1**) (Fig. 1), a compound originally isolated from the skin of an Ecuadorian tree frog *Epipedobates tricolor*, produces profound antinociceptive effects in rodents with efficacy comparable to morphine and potency as much as 200 times greater than morphine has stimulated the recent resurgence in interest in nicotinically-mediated analgesia (Badio and Daly, 1994; Badio et al., 1995; Sullivan et al., 1994).

Several compounds from the azetidinyl ether class have recently been described to exhibit potent antinociceptive activity across a range of preclinical models of acute, persistent and neuropathic pain, including (*R*)-5-(2-azetidinylmethoxy-2-chloropyridine (ABT-594) (**2**) (Fig. 1) and (*S*)-5-(2-azetidinylmethoxypyridine (A-85380) (**3**) (Fig. 1; Bannon et al., 1998a,b; Curzon et al., 1998). Several approaches have been used to evaluate the mechanism(s) underlying the antinociceptive effects of these compounds. A central nervous system (CNS) site of action is implicated from studies which demonstrate that intracerebroventricular (i.c.v.) administration of chlorisondamine, a nicotinic acetylcholine receptor antagonist with a very long duration of action in the CNS, produces complete or near-complete blockade of the antinociceptive effects of ABT-594 in rat models of acute thermal pain (hot-box) and persistent chemical pain (formalin test) (Bannon et al., 1998a). These results strongly suggest that

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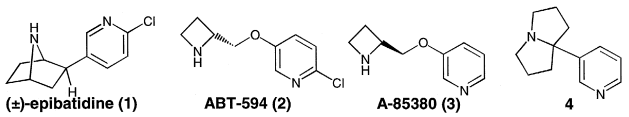

Fig. 1. High affinity ligands for the α4β2 nicotinic acetylcholine receptor.

activation of central (brain or spinal cord) nicotinic acetylcholine receptors plays a critical role in the antinociceptive effects of cholinergic channel modulators. Using induction of the immediate-early gene c-*fos* as a marker of neuronal activation, it has been demonstrated that systemic administration of antinociceptive doses of ABT-594 and A-85380 activates a number of brainstem nuclei believed to be important in pain modulation, such as the locus coeruleus and the nucleus raphe magnus (Bitner et al., 1998b; Nikkel et al., 1998). Neurons containing norepinephrine in the locus coeruleus and serotonin (5-HT) in the nucleus raphe magnus provide descending inhibition of spinal pain processing, and activation of these pathways may be important mediators of nicotinic acetylcholine receptor-induced antinociception. It has also been demonstrated that antinociception can be produced by intra-nucleus raphe magnus administration of A-85380 and ABT 594 (Bannon et al., 1998b; Curzon et al., 1998).

Nicotine-induced antinociception can be attenuated by intrathecal administration of 5-HT receptor and α-adrenoceptor antagonists, supporting the view that descending projections of brainstem norepinephrine and 5-HT neurons mediate the antinociceptive effects of this agent (Iwamoto and Marion, 1993). The antinociceptive effects of systemically administered A-85380 in the hot-box test were evaluated after intrathecal administration of a variety of neurotransmitter antagonists. Idazoxan (a selective α2-adrenoceptor antagonist), methysergide ($5\text{-HT}_1/5\text{-HT}_2$ receptor antagonist), scopolamine (muscarinic receptor antagonist) and tropine 3,5-dichloro-benzoate (MDL 72222) (5-HT_3 receptor antagonist) significantly attenuated the effects of A-85380, whereas prazosin (α1-adrenoceptor antagonist) and mecamylamine (nicotinic acetylcholine receptor antagonist) did not. Moreover, intrathecal A-85380 did not have antinociceptive effects in the hot-box test, but rather produced dose-dependent hyperalgesia and irritation at higher doses, further suggesting that the spinal cord is not the primary site of antinociceptive action in the rat (Rueter et al., 1999).

A role for activation of brainstem serotonergic and noradrenergic pathways in the antinociceptive effects of nicotinic acetylcholine receptor agonists is further suggested by the finding that depletion of norepinephrine and 5-HT by chemical lesioning fully attenuated the antinociceptive effects of systemically-administered A-85380 in the hot-box model of acute thermal pain (Nikkel et al., 1998). Additional studies have demonstrated that selective destruction of 5-HT neurons (5,7-dihydroxytryptamine (5,7-DHT) lesioning) partially attenuated the antinociceptive effects of A-85380 in models of acute thermal pain (Decker et al., 1998). The generality of these results to other pain models has not been established.

Emerging are data that support a role for α4-containing nicotinic acetylcholine receptor subtypes in the mediation of antinociceptive activity. Double labeling studies have confirmed the co-localization of the α4 subunit on serotonergic nuclei within the nucleus raphe magnus (Bitner et al., 1998b). The functional importance of the α4 subunit has also been assessed using an antisense approach. An oligonucleotide targeting the region of the start codon for the α4 subunit was designed. Infusion of the antisense oligo, but not a missense oligo, decreased both α4 staining in the brainstem and the antinociceptive effects of A-85380 in the hot-box model. Moreover, animals recovered the antinociceptive response to A-85380 5 days after the antisense infusions were discontinued, indicating that the effects of the antisense were not the result of permanent and non-specific damage to this brain region (Bitner et al., 1998a). Consistent with the antisense results, Changeux has demonstrated that α4-knockout and β2-knockout mice do not show antinociceptive responses to nicotine in the hot-plate test, a model of supraspinal nociception (Marubio et al., 1999). By contrast, in the α4 and β2-knockout mice, nicotine retained analgesic properties (albeit at higher doses) in the tail flick assay, a model believed to represent a spinal reflex response. These results are consistent with nicotinic acetylcholine receptor-mediated activation of descending inhibitory pathways in which α4 subunit-containing receptors are principally responsible for the initiation of this response.

Although α4-containing nicotinic acetylcholine receptor subtypes may represent a viable molecular target for a novel analgesic, the design of selective ligands is not a straightforward process. In principle, ligand design can be based on knowledge of the protein binding site structure or it can be based on homology to known high affinity nicotinic acetylcholine receptor ligand molecules. Although the level of structural information available for the nicotinic acetylcholine receptor continues to expand, the precision required to design ligands based on that information is still insufficient. The work of Unwin using electron microscopy techniques applied to the *Torpedo* receptor provides a level of detail never before observed (Miyazawa et al., 1999). However, assignment of backbone amino acid structure has not yet been achieved. Site-directed mutagenesis and affinity labeling studies (for a comprehensive review, see Hucho et al., 1996) have defined a highly conserved agonist binding domain rich in aromatic amino acid residues, and numerous studies have supported a π–cation interaction as being critical to stabilization of agonist binding (Dougherty, 1996; Sugiyama et al., 1996; Williamson et al., 1998; Zhong et al., 1998; Schmitt et al., 1999). Translation of this information into a three-dimensional model with a predictive value remains an elusive

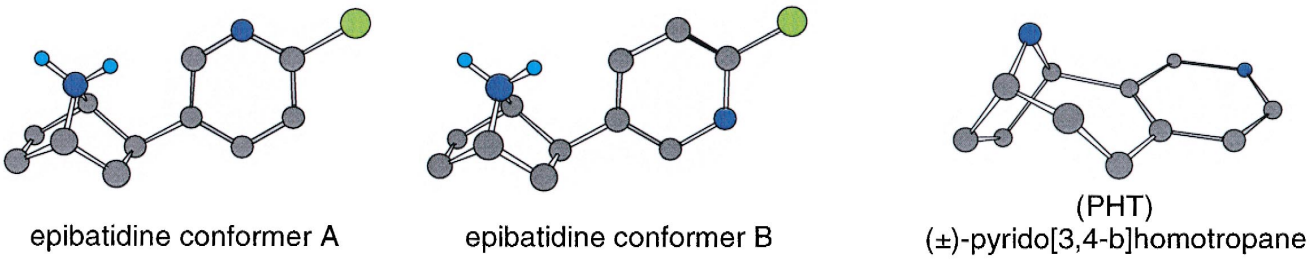

Fig. 2. Approximated structures of families of low energy epibatidine conformers and the structure of PHT.

goal. Consequently, we have instead developed a ligand-based model to predict affinity for the α4β2 binding site based on common structural elements observed across a range of high affinity ligands for this site. A four-point model is proposed.

The four-point model described differs from existing models of the nicotinic acetylcholine receptor receptor in that it places greater emphasis on the directionality of interaction of the positively charged basic amine substructure and its protein counterpart and on the hydrogen bond donating electron rich substructure and its protein counterpart, and relatively lesser emphasis on the importance of N–N distance between the charged amine and the pyridine nitrogen (Beers and Reich, 1970; Sheridan et al., 1986; Dukat et al., 1994; Glennon et al., 1994; Abreo et al., 1996; Barlocco et al., 1998; Koren et al., 1998). Two key ligands used in the design of this model were epibatidine (Fig. 1, **1**) and the pyrrolizidine (**4**) (Fig. 1).

(−)-Epibatidine (Fig. 1, **1**) was utilized as the reference compound in developing a pharmacophore model for binding to the α4β2 receptor. The positively charged sp^3 nitrogen, its electron rich complementary protein site, the pyridine nitrogen, and its complementary hydrogen bond donor site point were chosen as pharmacophoric elements for superposition in this four-point model (Abreo et al., 1996). Epibatidine possesses a rigid 7-azabicyclo[2.2.1]-heptane skeleton with a rotatable chloropyridyl substituent. This rotation accommodates a large range of internitrogen distances (Bencherif et al., 1998), a parameter that has been emphasized in various nicotinic pharmacophore models. Calculations typically generate two families of low energy epibatidine conformers (approximated by conformers A and B, Fig. 2) that differ by roughly 180° in the rotation of the pyridyl ring. Although the internitrogen distances vary greatly (from ~4.5 to 5.5 Å), these conformers have quite similar energy and are separated by a very low barrier (Bencherif et al., 1998; Campillo et al., 1998). Therefore, at the outset it is not clear which low energy epibatidine conformer family should be utilized as the reference template when attempting to superimpose the putative pharmacophoric elements of various ligands. A preliminary analysis indicated that a better superposition is obtained between epibatidine conformer A and the conformationally restricted nicotine analog pyrido[3,4-*b*]homotropane (PHT), which possesses α4β2 receptor binding affinity approximately equivalent to nicotine (Kanne and Abood, 1988). Therefore, we chose initially to examine conformer A in greater detail as the template for superposition, orienting the pyridine ring nitrogen proximal to the sp^3 nitrogen (N–N distance of 4.79 Å), although a suitable

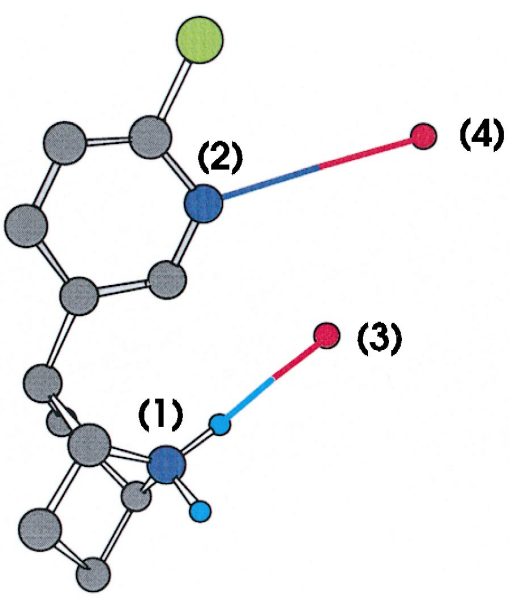

Fig. 3. Pharmacophoric element selection for (−)-epibatidine (**1**). The nitrogen atoms (dark blue) are pharmacophoric elements (1) and (2); elements (3) and (4) are points on the receptor (red) with which elements (1) and (2) interact.

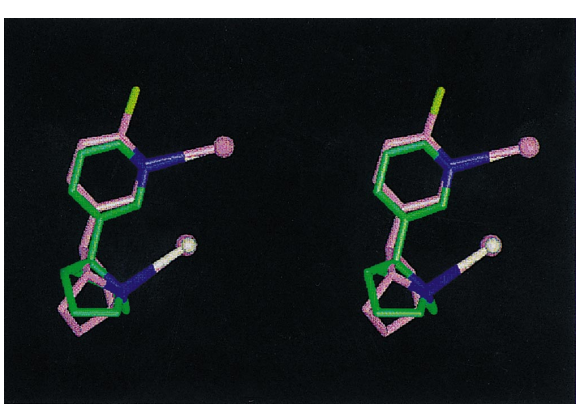

Fig. 4. Superposition of the putative pharmacophoric elements of (−)-epibatidine (**1**) (pink) and pyrrolizidine **4** (green).

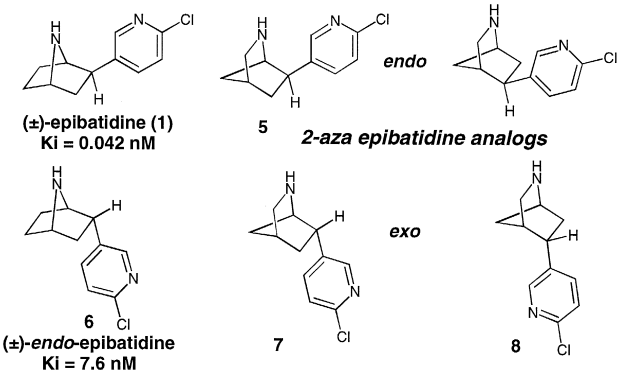

Fig. 5. Structures of epibatidine, endo-epibatidine, and 2-aza epibatidine analogs.

overlap of conformer B with the pharmacophoric elements of various ligands may also be possible.

A second key decision point required the assignment of directionality to the interaction of the charged basic amine residue for the ligand to the complementary protein site. Whether the protonated site is viewed as interacting with a π cloud of aromatic residues or as a hydrogen bond to a negatively charged amino acid, we believe it is appropriate to address the directionality of that interaction. The high affinity $\alpha4\beta2$ ligand ($K_i = 0.32$ nM) 7*a*-(3-pyridinyl)-hexahydro-1*H*-pyrrolizine (Fig. 1, **4**) was a useful tool compound for assigning directionality. The structure of **4** can be conceptualized as a hybrid between the individual enantiomers of nicotine. Fig. 3 illustrates the four points selected as pharmocophoric elements for **1** (Fig. 1), and Fig. 4 shows the superposition of the low energy conformation of **1** with **4**.

The model was next evaluated using three series of compounds exhibiting varying degrees of affinity for the

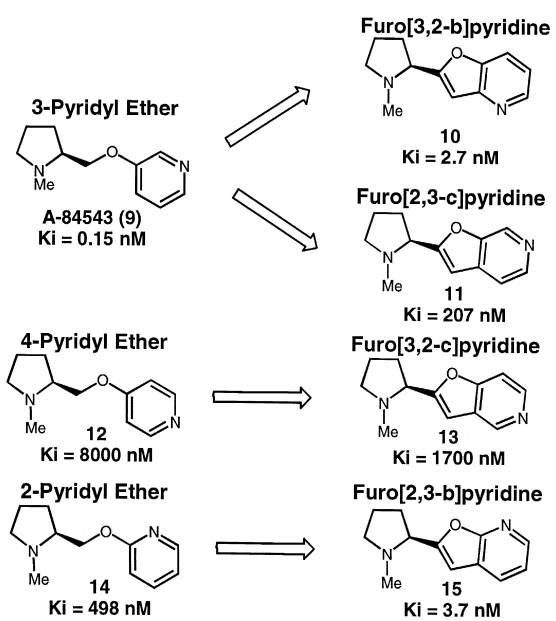

Fig. 6. [^{3}H]Cytisine binding affinities of various pyridyl ethers and their constrained furopyridine analogs.

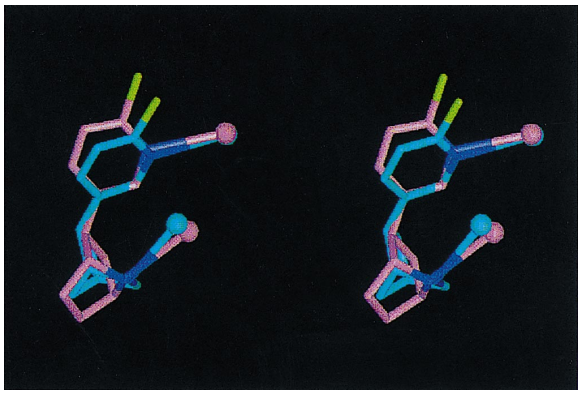

Fig. 7. Superposition of the putative pharmacophoric elements of (−)-epibatidine **1** (pink) and 2-azabicycloheptane **5** (blue).

$\alpha4\beta2$ binding site. A series of epibatidine analogs (Fig. 5), a series of pyridyl ether analogs, and a series of four ''ring constrained'' furopyridine analogs derived by conformational restriction of the pyridyl ethers (Fig. 6) were examined. Within each structural series, the model correctly predicted the relative affinity for the $\alpha4\beta2$ binding site.

The first series evaluated included the endo epimer of epibatidine (Fig. 5, **6**), and three analogs (Fig 5, **5**, **7**, **8**) possessing the 2-aza[2.2.1]bicycloheptane ring system (Fig. 5). In this new series, the azabicyclic ring nitrogen of epibatidine has been translocated from the 7- to the 2-position of the rigid bicyclo[2.2.1]heptane framework (Hodgson et al., 1998; Kasyan et al., 1998; Malpass and Cox, 1999). The 6-endo 2-aza epibatidine analog **5** exhibits very high binding affinity for $\alpha4\beta2$ receptors ($K_i = 0.032$ nM) in comparison to epibatidine. As expected, a unified superposition may be obtained between the pharmacophoric elements of **1** (Fig. 1) and compound **5** (Fig. 7), and this may account for their similar $\alpha4\beta2$ binding profiles.

(±)-Endo-epibatidine (**6**) (Fig. 5) exhibits > 150-fold weaker binding affinity than the natural exo-isomer **1** (Wypij and Shen, 1994), and a similar trend is observed for the exo-2-azabicyclo[2.2.1]heptane epibatidine analogs **7** and **8** ($K_i = 6.6$ nM and 30 nM, respectively). Molecular

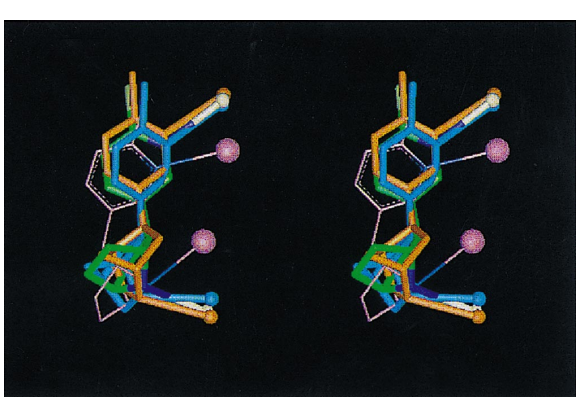

Fig. 8. Superposition of the putative pharmacophoric elements of (−)-epibatidine **1** (thin pink), endo-epibatidine **6** (green), 6-exo analog **7** (blue), and 5-exo analog **8** (orange).

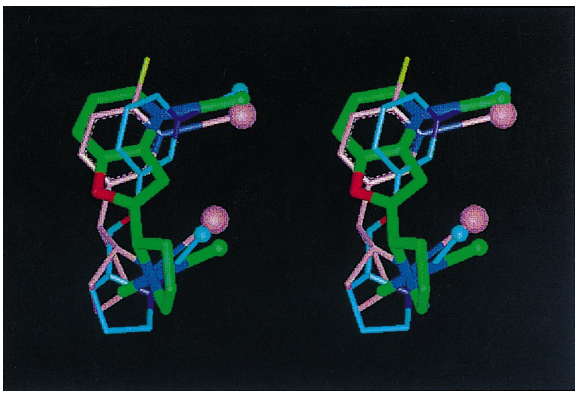

Fig. 9. Superposition of putative pharmacophoric elements of (−)-epibatidine (pink), furo[3,2-*b*]pyridine **10**, and A-84543 (**9**) (faint blue).

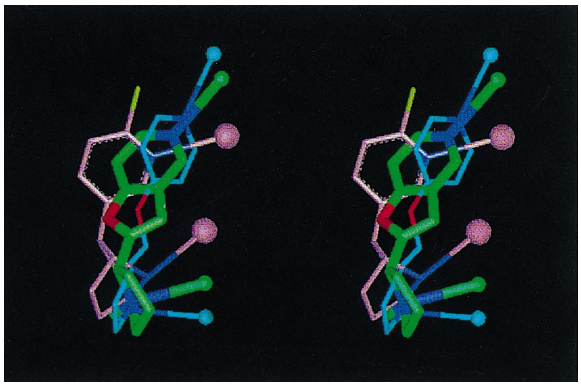

Fig. 11. Superposition of putative pharmacophoric elements of (−)-epibatidine (pink), furo[3,2-*c*]pyridine **13**, and 4-pyridyl ether **12** (blue).

modeling indicates that a very close superposition of the pharmacophoric elements of endo-epibatidine (Fig. 5, **6**) and the two exo-substituted 2-azabicycloheptane compounds **7** and **8** can be obtained; however, the overlap with epibatidine is not good (Fig. 8). The distances between putative protein site points and internitrogen distances of **6**, **7**, and **8** are much longer than the optimum distances for **1** or compound **5**, and this may account for the reduced $\alpha 4\beta 2$ receptor binding affinity of these analogs.

The three geometric isomers of the pyridyl ether structure and the four resulting ring constrained furopyridine analogs (Elliott et al., 1997) represent a useful group of compounds exhibiting widely divergent affinities for the $\alpha 4\beta 2$ nicotinic acetylcholine receptor binding site (Fig. 6). The 3-pyridyl ether (*S*)-5-(2-pyrrolidinylmethoxypyridine (A-84543) (**9**) (Fig. 6) may be constrained by tethering the oxymethyl group through an sp^2 carbon to the 2-pyridyl position to yield the furo[3,2-*b*]pyridine compound **10**, which exhibits low nanomolar binding affinity ($K_i = 2.7$ nM) for the $\alpha 4\beta 2$ nicotinic acetylcholine receptor. Conversely, the derivative resulting from tethering **9** to the 4-pyridyl position, the furo[2,3-*c*]pyridyl pyrrolidine **11**, is significantly less potent ($K_i = 207$ nM). The constrained furo[3,2-*c*]pyridine analog **13** of the 4-pyridyl ether **12** displayed only micromolar binding affinity. Whereas the 2-pyridyl ether **14** showed modest binding affinity ($K_i =$ 498 nM), the furo[2,3-*b*]pyridine analog **15** exhibited significantly improved binding affinity ($K_i = 3.7$ nM) for the $\alpha 4\beta 2$ receptor (Fig. 6).

Figs. 9–12 illustrate the superpositions of (−)-epibatidine with the pyridyl ethers and their corresponding furopyridine analogs. The best overlap of the flexible 3-pyridyl ether molecule **9** with (−)-epibatidine (Figs. 9 and 10) was obtained with a comparatively high energy conformation ($\Delta E = 2.2$ kcal/mol), which agrees with previous reports (Abreo et al., 1996). However, higher energy conformers of either the 2- or 4-pyridyl ether compounds **14** and **12** did not result in a significantly improved overlap. Both the furo[3,2-*b*]pyridine **10** (Fig. 9) and the furo[2,3-b]pyridine **15** (Fig. 12) achieve a reasonable superposition with (−)-epibatidine. In contrast, the furo[2,3-*c*]pyridine **11** (Fig. 10), furo[3,2-*c*]pyridine **13**, and 4-pyridyl ether **12** (Fig. 11) compounds contain much longer distances between nitrogens and putative protein site points compared to (−)-epibatidine. The pyridine ring of the 2-pyridyl ether **14** does not overlap well with the aromatic ring of either (−)-epibatidine or **15** (Fig. 10), and

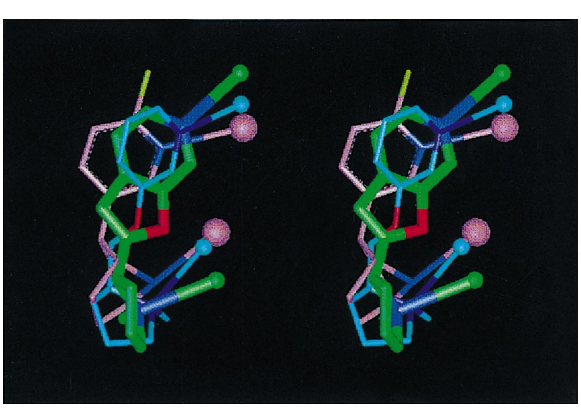

Fig. 10. Superposition of putative pharmacophoric elements of (−)-epibatidine (pink), furo[2,3-*c*]pyridine **11**, and A-84543 (**9**) (faint blue).

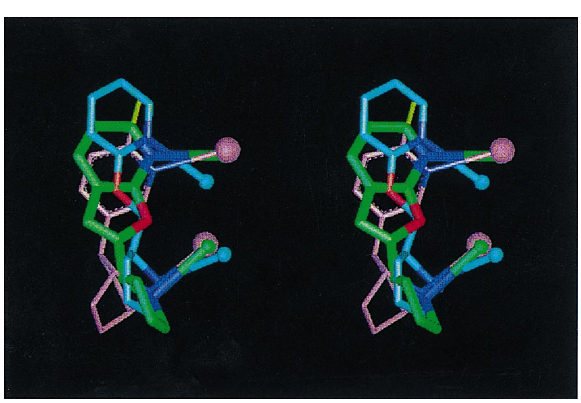

Fig. 12. Superposition of putative pharmacophoric elements of (−)-epibatidine (pink), furo[2,3-*b*]pyridine **15** (green), and 2-pyridyl ether **14** (blue).

may occupy space that the receptor does not tolerate. In general, the compounds having low energy conformations that achieve the best fit superposition with the putative pharamcophoric elements of epibatidine also exhibit the highest binding affinity for the $\alpha 4\beta 2$ nicotinic acetylcholine receptor.

The analgesic potential of nicotinic acetylcholine receptor ligands has been demonstrated with structurally diverse molecules such as epibatidine and ABT-594. Mechanistic studies strongly implicate $\alpha 4$-containing nicotinic acetylcholine receptor subtypes, and likely the $\alpha 4\beta 2$ subtype as being critically important to the analgesic activity of this class. Using the pyrrolizidine nucleus of **4** and azabicyclo[2.2.1]heptane skeleton of epibatidine, a ligand-based model with predictive value for assessing affinity for the $\alpha 4\beta 2$ binding site has been proposed. A diverse set of ligands, including various isomeric azabicycloheptanes, pyridyl ethers, and furopyridines has been used to validate the model. The relationship between affinity for the $\alpha 4\beta 2$ subtype of the nicotinic acetylcholine receptor and analgesic efficacy is less clear. Although affinity for this site may be a prerequisite for a nicotinic acetylcholine receptor-mediated analgesic effect, affinity alone does not guarantee efficacy. Selectivity for this site over other nicotinic acetylcholine receptor subtypes, which may function to counteract the analgesic activity of a given compound, must be considered. The ability to design novel structural classes exhibiting affinity for the $\alpha 4\beta 2$ nicotinic acetylcholine receptor subtype will aid in our understanding of the therapeutic potential of this class of drugs in the treatment of pain.

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