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CONFORMATIONALLY RESTRICTED ANALOGUES OF NICOTINE AND ANABASINE

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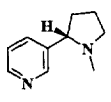
Abstract: A series of conformationally restricted analogues of nicotine has been synthesized and evaluated as agonists of neuronal acetylcholine receptors. Compound **2** (SIB-1663), which selectively activated human recombinant α2β4 and α4β4 nAChRs, was shown to be active in animal models of Parkinson's disease and pain.

Introduction

A combination of molecular biology, binding and electrophysiological studies have demonstrated the existence of multiple neuronal acetylcholine receptor (nAChR) subtypes in experimental animals and human brains.[1] In heterologous expression systems, nAChRs composed of pairwise combinations of α (α2, α3 or α4) and β (β2 or β4) subunits, three way combinations (e.g. α5 or α6 with αxβy) or α subunits alone (α7, α8 or α9) respond differently to well known nAChR agonists.[1] This implies that the design of subtype specific agonists is conceptually feasible. Furthermore, the therapeutic potential of nAChR agonists, such as nicotine, in Alzheimer's,[2] Parkinson's disease,[3] Tourette's syndrome,[4] and pain,[5] is being recognized. Thus, there is considerable interest in the search for subtype selective, centrally active nAChR agonists for many CNS disorders with the belief that these agents will be inherently more attractive as drugs than nicotine itself.[6] We have employed complementary approaches in the search for novel nAChR agonists - random screening and rational design. In each case, the primary assay evaluated the potential of the test compound to selectively activate recombinant human nAChRs (α2β4, α3β4, α4β4, α3β2, and α4β2) stably expressed in mammalian cell lines, to increase levels of intracellular free Ca^{++} ($[Ca^{++}]_i$). In this paper we present the results of one program which is based on the rational design of a family of conformationally restricted derivatives of nicotine **1**.



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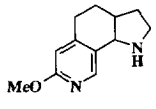

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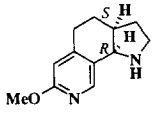

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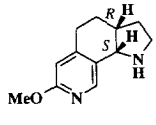

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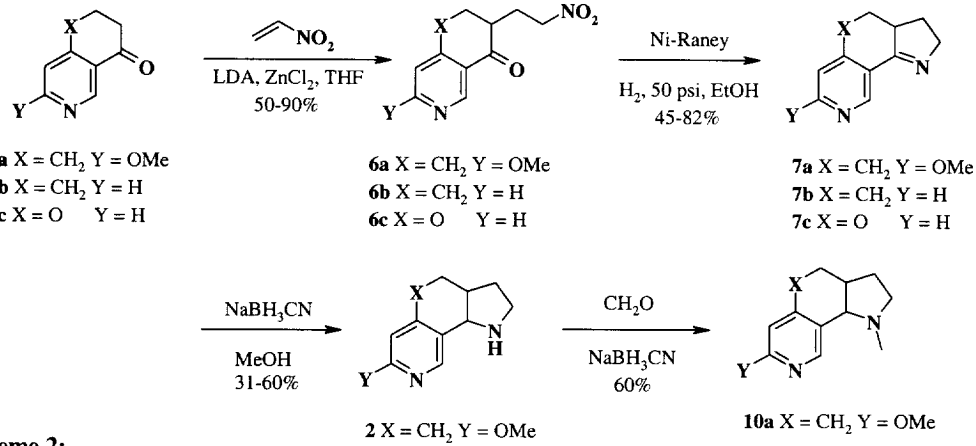
Compounds **2** (and enantiomers **8** and **9**), **3**[7], **4**, **10a** and **13** were selected for synthesis.

Synthesis

The synthesis of each of these molecules required the appropriate cyclic ketone, **5a**,[8] **5b**,[7] and **5c**,[9] as starting material (Scheme 1). Conversion of these quinolones into the corresponding fused 5-6-membered pyrrolidotetrahydroisoquinoline rings was achieved via the procedure described by Chavdarian and co-workers.[10] In all cases, the *cis* ring junction predominated (>95%) and could be fully purified by chromatography or recrystallization. Resolution of **2** into its enantiomers (**8** and **9**) was realized by fractional recrystallization using *D*- or *L*- di-*p*-toluoyltartaric acid, respectively; **8** was attributed the configuration (*R,S*) and **9** (*S,R*) by analogy with **3**.[7] *N*-Methylation of **2** to afford the conformationally restricted nicotine analogue **10a** was accomplished by a standard reductive methylation procedure with sodium cyanoborohydride and aqueous formaldehyde in acetonitrile.[10] The synthesis of the restricted anabasine analogue **13** (Scheme 2)

Scheme 1:



5a X = CH_2 Y = OMe
5b X = CH_2 Y = H
5c X = O Y = H

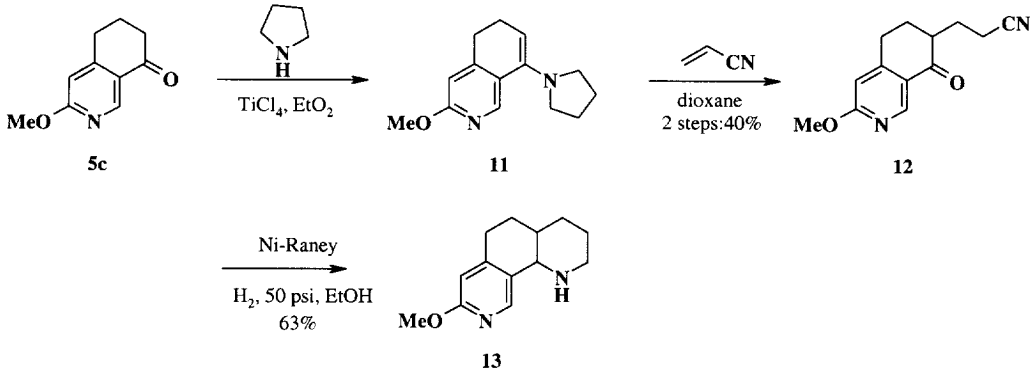
6a X = CH_2 Y = OMe
6b X = CH_2 Y = H
6c X = O Y = H

7a X = CH_2 Y = OMe
7b X = CH_2 Y = H
7c X = O Y = H

2 X = CH_2 Y = OMe
3 X = CH_2 Y = H
4 X = O Y = H

10a X = CH_2 Y = OMe

Scheme 2:



5c **11** **12** **13**

involve converting the cyclic ketone **5c** to the enamine[11] **11** for further elaboration. Conjugate addition of **11** with acrylonitrile in refluxing dioxane afforded **12**. Upon treatment with hydrogen (50 psi) in the presence of Raney Nickel, the nitrile group was reduced and the resulting amine underwent reductive cyclization to the restricted anabasine analogue **13**. In this case, **13** was obtained as an equiproportional mixture of the *cis* and *trans* fused ring junction.

Biological *in vitro* data

Compounds **2**, **(±)-3**, **4**, **8**, and **9** were tested for the ability to increase $[Ca^{++}]_i$ in human embryonic kidney (HEK293) cells stably transfected with cDNA encoding human nAChR subunits in pairwise combinations resulting in cell lines which express functional receptors comprised of α2β4, α3β4, α4β4, α3β2 or α4β2 subunits.[12] The functional efficacies at 100 μM, relative to a maximally effective concentration of **1**, are shown in Figure 1. The parent **(±)-3**[7] is a relatively non-selective nAChR agonist whereas incorporation of a methoxyl group **2** increased the selectivity and the efficacy for α2β4 and α4β4 cell lines. Evaluation of the functional efficacy of the enantiomers of **2** (**8** and **9**) revealed that although **8** is clearly the more efficacious agonist of α2β4 and α4β4, **9** is less efficacious but also extremely selective for α2β4 and α4β4 subtypes. This was anticipated because the three dimensional structure of **8** is very close to the low energy conformation of natural (*S*)-nicotine.[1b] Introduction of a heterocyclic oxygen atom afforded **4** which showed a similar profile to the parent **3**. Finally, the *N*-methyl derivative of **2** (**10a**) and the six-membered ring anabasine analogue **13** were only marginally active in this cell-based functional assay (data not shown).

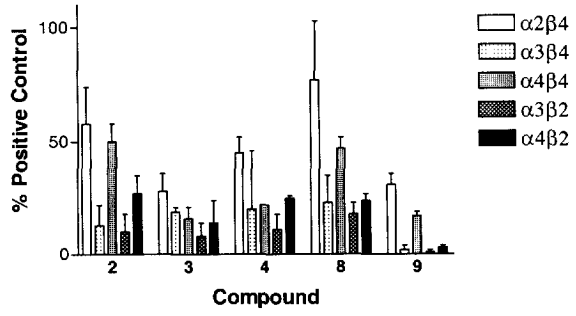


Figure 1. The ability of **2**, **3**, **4** and **8** to increase $[Ca^{++}]_i$ levels in HEK cells stably transfected with human nAChR subunits α2β4, α3β4, α4β4, α3β2 and α4β2, expressed as a percentage of the value for the maximally effective concentration of nicotine (**1**) in each cell line.

In order to study the activity of **2** in more detail at a molecular level, electrophysiological voltage-clamp measurements were also used to characterize its potency and efficacy on nAChR subtypes transiently expressed in *Xenopus* (Table I). In accordance with the cell-based Ca^{++} flux measurements, the results from this study

indicate a preference for the α2β4 and α4β4 receptor complexes. Only marginal activity was detected on oocytes expressing α7 (data not shown).

Agonist	α2β4	α3β4	α4β4	α4β2
EC_{50} (μM)[a]	87±7 (3)	81±51 (3)	32±13 (3)	3±1 (3)
Efficacy[b]	22%±4 (3)	5%±2 (3)	44%±7 (3)	8%±2 (3)

Table I. Potency and efficacy shown by **2** on various subtypes expressed in *Xenopus* oocytes under voltage-clamp conditions. [a]EC_{50} [μM; mean ± SEM (n)]; [b]efficacy [mean % ± SEM (n)] was normalized to the response produced by an EC_{100} concentration of acetylcholine or nicotine in the same cell[13].

In expanded dose response studies by electrophysiological (Figure 2) or Ca^{++} flux (Figure 3) methods, **2** at concentrations from 1 to about 50 μM, can be seen to selectively activate α2β4 and α4β4 receptors.

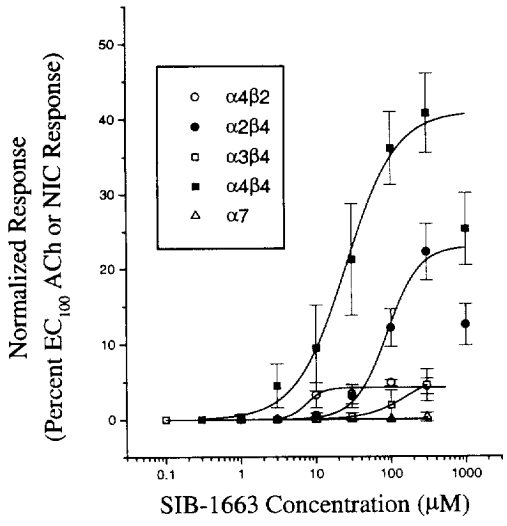


Figure 2: Efficacy and potency shown by **2** on various recombinant human nicotinic receptors expressed in *Xenopus Oocytes*. Responses were normalized to 100 μM of ACh or Nic. Each symbol represents the mean ± S.E.M. in the response in 3 oocytes.

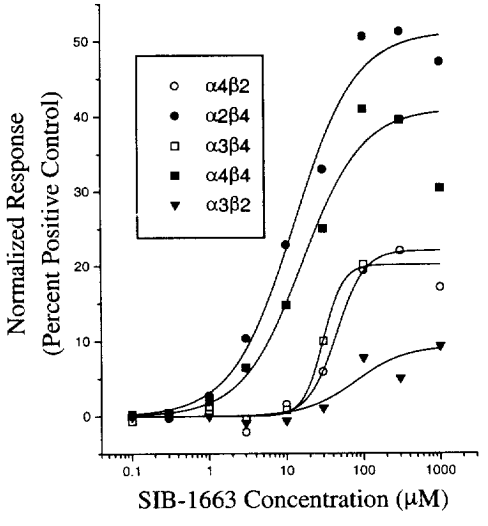


Figure 3: Efficacy and potency shown by **2** to increase $[Ca^{++}]_i$ levels in HEK cells transfected with various human nAChR subunits. Responses were normalized to the maximally effective concentration of nicotine in each cell line. Points represent the mean of 3-5 individual determinations done in duplicate.

Due to the subtype selectivity observed for **2**, this nAChR agonist was further profiled. The compound displaced tritiated nicotine from rat brain binding sites[14] with an IC_{50} value of 1.9 μM (c.f., IC_{50}(**1**) = 0.004 μM; IC_{50}(**3**) = 0.51 μM) and released dopamine from rat striatal slices[15] with an efficacy of 73 ± 20% (300 μM) compared to **1** (10 μM) (c.f., **3** = 56 ± 9%).

Biological *in vivo* data

The therapeutic potential of **2** was assessed in animal models of locomotor activity, nociception and cognition. The agonist **2** was as efficacious as nicotine in inducing locomotor activity in rats habituated to photocell activity cages[16] with a rapid onset of action (176 %, 30 mg/kg, s.c. compare to 187 %, 0.4 mg/kg, s.c. for nicotine; total activity over 120 min). Furthermore, **2** induced a dose-related ipsilateral rotation in rats (30 mg/kg, i.p.) with unilateral 6-hydroxydopamine lesions of the nigrostriatal dopamine pathway, suggesting an activation of the nigrostriatal dopamine system on the intact side[17]. This activity, which was similar to that observed with nicotine (0.35 mg/kg, i.p.), was attenuated by the nAChR antagonist mecamylamine, indicating that the effect of **2** was mediated through activation of central nAChRs. When assessed in the rat tail flick model of acute pain,[18] **2** (30 mg/kg, p.o.) showed 52% of the activity of morphine (2 mg/kg, s.c.). Interestingly, while the effect of morphine lasted 1 hour, **2** was still active at 3 hours. This antinociceptive activity was antagonized by mecamylamine, but not naloxone, suggesting that the antinociceptive activity is independent of opioid mechanisms. Finally, **2** was active in the rat passive avoidance model[19] of learning and memory. Compared to saline (100.60 ± 25.92 sec.), rats treated with **2** (15 mg/kg, s.c.) spent considerably longer time (221.16 ± 35.7 sec.) in the brightly lit compartment before leaving to enter the dark compartment where a mild electric shock was administered. Finally **2** (30 mg/kg, s.c.) did not induce any of the typical nicotine-like side effects (gastrointestinal and cardiovascular).

Discussion

The results presented here are in contrast with those presented by Glassco et al.[10] who reported that the closely related compound **(+)-3** possessed antinociceptive properties by a mecamylamine-insensitive mechanism. Furthermore **(+)-3** did not compete for rat brain membrane nicotine binding sites in contrast to the biologically inactive enantiomer **(-)-3**. In the present work, the desmethyl-methoxylated analogue **2** showed affinity for nicotine binding sites and released dopamine from rat brain striatal tissue which translated into activity in the unilateral 6-hydroxydopamine lesion model. As with most nAChR agonists that release dopamine, **2** also increased spontaneous locomotor activity. Like **(+)-3**, **2** was active in the rat tail flick model of antinociception. Compound **2** was also active in the inhibitory passive avoidance model which reflects an augmentation of memory performance. All *in vivo* activities for **2** could be attenuated by mecamylamine suggesting that central nAChR receptors were involved in the mechanism of action of this compound. The *in vivo* concentration of **2** in these experiments is probably in the range of 1 to 50 μM, assuming rapid absorption and minimal early metabolism. If these assumptions are correct, this biological activity could be attributed to the selective activation of α4β4- and /or α2β4-containing endogenous nAChRs.

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