Communications to the Editor

4-[[2-(1-Methyl-2-pyrrolidinyl)ethyl]thio]-phenol Hydrochloride (SIB-1553A): A Novel Cognitive Enhancer with Selectivity for Neuronal Nicotinic Acetylcholine Receptors

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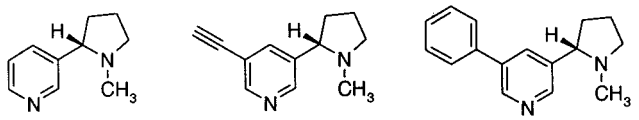
Recent studies have revealed the existence of a family of presynaptic, neuronal acetylcholine ion channel receptors (nAChRs) in the brain which function to modulate the release of neurotransmitters,[1] such as acetylcholine (ACh), dopamine (DA), and other monoamines implicated in learning and memory processes.[2] There is convincing evidence to implicate a deficit of nAChRs in the symptomatology of Alzheimer's disease (AD). For example, studies have demonstrated a substantial loss of nAChRs from cortical[3] and hippocampal[4] brain regions of AD patients and that this loss is dependent upon the particular nAChR subtype composition.[5] It is likely that efficacious nAChR agonists will stimulate the activity of the remaining intact nAChRs to compensate for this loss. Furthermore, the neurotoxin β-amyloid has been shown to attenuate nicotine-induced release of ACh and DA.[6] Finally, the prototypical nAChR agonist nicotine (**1**) has been shown to ameliorate some of the symptoms of AD[7,8] and, in a number of animal models, to have neuroprotective effects.[9] Thus, subtype-selective nAChR agonists have the potential to treat a number of central nervous system (CNS) disorders such as Alzheimer's disease.

As a part of a program to identify compounds that selectively activate nAChRs which stimulate the release of certain neurotransmitters, we have reported that SIB-1508Y (**2**) is being developed to treat the symptoms of Parkinson's disease which can be biochemically defined as a deficiency of dopamine.[10] To develop a useful therapeutic agent for Alzheimer's disease, we sought selective nAChR agonists which stimulate the release of acetylcholine from the hippocampus and cortex, without concomitant activation of peripheral nAChRs.[11]

In this Communication, we wish to report the synthesis and pharmacological evaluation of 4-[[2-(1-methyl-2-pyrrolidinyl)ethyl]thio]phenol hydrochloride (**5**, SIB-1553A), a novel selective agonist of human neuronal nAChRs. In contrast with other nicotinic agonists reported to be active in animal models of memory and learning, such as ABT-418,[12] ABT-089,[13] and GTS-21,[14] **5** markedly increases acetylcholine levels from the hippocampus and cortex in addition to other neurotransmitters relevant for cognitive processes. The overall pharmacological profile strongly suggests that **5** will be useful in treating the cognitive deficits of Alzheimer's disease.



1 **2** **3**

It was observed that replacement of the ethyne moiety of **2** with a phenyl group (i.e., **3**) shifted the subtype selectivity in the calcium flux assay using human recombinant nAChRs from a tendency to activate β2-containing recombinant nAChRs to β4-containing receptors,[15] although both compounds were highly potent at displacing [^{3}H]nicotine from rat cortical membranes (IC_{50} = 4 and 37 nM, respectively). Aiming to capitalize on this observation, we designed compound **4**, incorporating the phenyl and pyrrolidine rings of **3** but lacking the pyridine ring of **1**−**3**. Although **4** did selectively activate β4-containing nAChR cell lines, it lacked potency and was relatively inefficient in stimulating the release of neurotransmitters from brain slices from different regions of rat brain. However, extensive structure−activity relationship studies led to the observation that incorporation of a hydroxyl group in the *para*-position of the phenyl ring afforded a very potent and efficacious nAChR agonist (**5**).

Condensation of thiophenols **6** and **7** with 2-(2-chloroethyl)-1-methylpyrrolidine **8** led to the synthesis of the hydrochloride salts of racemic **4** and **5**, respectively, in high yield. To prepare (*S*)-**5** and (*R*)-**5**, it was first necessary to resolve the chloro derivative **8** with di-*p*-toluoyl-D(or-L)-tartaric acid to afford (*S*)-**8** and (*R*)-**8**[16] (Scheme 1). The enantiomerically enriched compounds (*S*)-**5** (ee ≥ 98%) and (*R*)-**5** (ee ≥ 98%) were synthesized, and the enantiomeric excess was determined by chiral HPLC.[17]

Evaluation of **5** acting on human embryonic kidney (HEK) cells, stably expressing human nAChR subunit combinations $\alpha2\beta4$, $\alpha3\beta4$, $\alpha4\beta4$, $\alpha3\beta2$, and $\alpha4\beta2$, to increase intracellular calcium ion concentration [$(Ca^{2+})_i$] (Figure 1),[18] confirmed the selectivity for β4-containing nAChR subtypes. Tested under voltage clamp conditions and measuring inward currents elicited by agonists in *Xenopus* oocytes expressing the human nAChR subunit combinations $\alpha2\beta2$, $\alpha3\beta2$, $\alpha4\beta2$, $\alpha2\beta4$, $\alpha3\beta4$, $\alpha4\beta4$, and $\alpha7$,[19] **5** was most efficacious on $\alpha4\beta4$ and had weak activity on $\alpha3\beta4$ and $\alpha7$ receptors (Figure 2).[21]

When the ability to displace [^{3}H]nicotine from rat cortical membranes was determined, **5** was found to be significantly less potent than nicotine. This is an indica-

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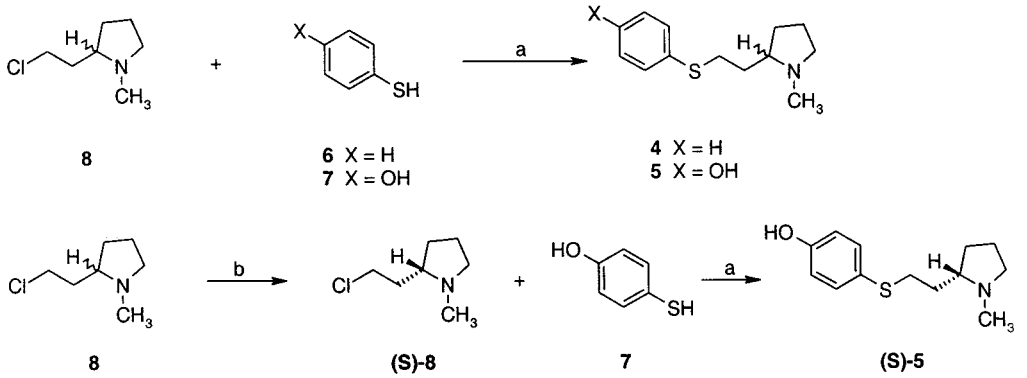
Scheme 1[a]



[a] (a) K_2CO_3, DMF, 25 °C (85% yield); (b) di-*p*-toluoyl-D-tartaric acid, MeOH.

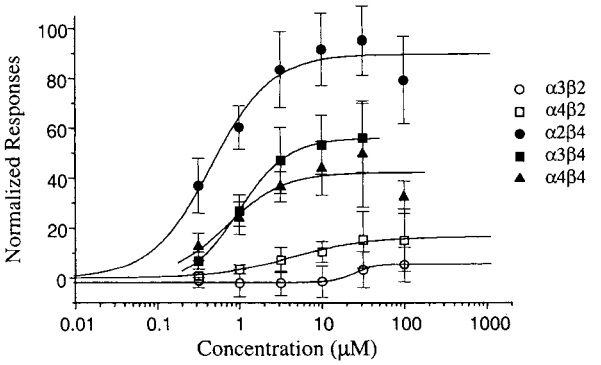


Figure 1. Relative efficacy and potency shown by **5** to increase intracellular calcium ion levels in HEK cells transfected with various human nAChR subunits. Responses were normalized to the maximally effective dose of nicotine in each cell line (except for α3*β*2 where DMPP was used). Points represent the mean of five individual determinations run in duplicate.

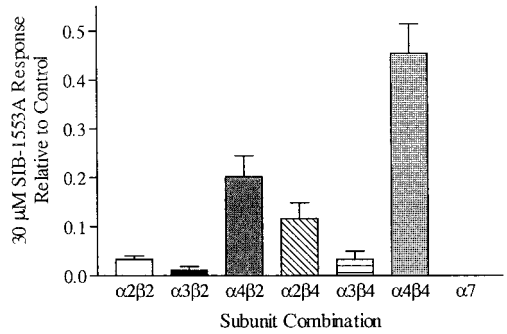


Figure 2. Current evoked by **5** (30 μM) under voltage clamp recording conditions[20] on *Xenopus* oocytes expressing the designated recombinant human nAChR subtypes. The ordinate shows the fractional response relative to a saturating dose of nicotine (for α2*β*4, α3*β*4, α4*β*2, and α4*β*4), acetylcholine (α2*β*2 and α7), or DMPP (α3*β*2). No current was detectable on α7 under these conditions.

tion that **5** binds preferentially to a less abundant endogenous nAChR subtype than to the most abundant subtype which is believed to be composed of α4 and *β*2 subunits.[22] Compound **5** released neurotransmitters extremely efficiently in vitro and/or in vivo (Table 1). In particular, **5** (40 mg/kg), as well as both enantiomers ((*S*)-**5** or (*R*)-**5**), led to a 10−12-fold increase of acetylcholine from the hippocampi of freely moving rats, and this effect could be attenuated by the nAChR antagonists mecamylamine and dihydro-*β*-erythroidine.

Table 1. Ligand Binding and Neurotransmitter Release

compd	[^{3}H]Nic IC_{50} (nM)[a]	[^{3}H]DA striatum[b]	[^{3}H]NE hippocampus[b]	[^{3}H]NE prefrontal cortex[b]	rats: dose (mg/kg, sc)	in vivo ACh (*n*)[c]
1	2.4 ± 0.3	1	1	1	0.4[d]	1 ± 0.5 (6)
4	ND	1.5 ± 0.4	ND	ND	40	0.6 ± 0.4 (2)
5	110 ± 61	6.5 ± 1.5	0.2 ± 0.05	0.6 ± 0.03	40	12 ± 1.5 (6)
(*S*)-**5**	ND	4.3 ± 0.9	0.3 ± 0.1	0.35 ± 0.1	40	13 ± 1.2 (2)
(*R*)-**5**	ND	4.1 ± 0.8	0.2 ± 0.05	0.3 ± 0.05	40	10 ± 6 (2)

[a] The data represent the mean of two independent experiments each performed in triplicate. The binding affinities at nAChRs represent the ability of the compound to displace the binding of [^{3}H]nicotine from rat cortical membrane preparations.[3a] [b] The efficacy of the test compound (300 μM) to stimulate [^{3}H]DA and [^{3}H]NE from brain slice preparation.[23] The data represent the mean ± SD of 3−6 experiments and are normalized to the response for the maximally efficacious concentration of nicotine for each brain region/neurotransmitter. [c] Acetylcholine release data in Sprague−Dawley rats.[24] Compounds were administered subcutaneously. Data represent the mean ± SEM of 2−6 independent experiments. *p < 0.05 versus saline control using two-way repeated measures ANOVA with Student−Newman−Keuls post-hoc test. The effect of nicotine is defined as 1. [d] Most efficacious dose of nicotine.

Since **5** stimulated the release of ACh, DA, and NE in brain regions known to play an important role in learning and memory processes, it was evaluated for potential cognitive enhancement effects in an animal model. In a sequential spontaneous alternation task, **5** was found to reverse the working memory deficits of aged C57BL/6 mice. In this paradigm, **5** was more efficacious and better tolerated than **1**. At a dose of 2.28 μmol/kg, **5** increased alternation over controls by 53.3% with no side effects.[25] Furthermore, **5** exhibited a broad spectrum of activity in other animal models of working memory.[26] Interestingly, neither (*S*)-**5** nor (*R*)-**5** alone was as active as the racemate **5** in any of these behavioral experiments. In conclusion, these data suggest that **5** is a novel nAChR agonist which may have therapeutic potential for the symptomatic treatment of Alzheimer's disease and other cognitive disorders.

Supporting Information Available: Experimental details. This information is available free of charge via the Internet at http://pubs.acs.org.

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