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6-(2-Phenylethyl)nicotine: A novel nicotinic cholinergic receptor ligand

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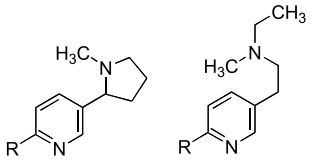
Abstract—6-(2-Phenylethyl)nicotine (**1b**; K_i = 15 nM) was unexpectedly found to bind at α4β2 nicotinic cholinergic (nACh) receptors. Although this compound failed to produce nicotine-like agonist action in several functional assays, **1b** antagonized the antinociceptive effects of nicotine (mouse tail-flick assay) in a dose-dependent fashion when administered via an intrathecal route.

There has been recent interest in nicotinic cholinergic (nACh) receptors because of their possible role in the treatment of pain.[1–4] Several years ago, on examining a series of 6-substituted derivatives of nicotine (**1a**; **1** where R = –H) in a QSAR study, we derived a relating equation suggesting that α4β2 nACh receptor affinity was related to the lipophilicity of the 6-position substituent. The relating equation further indicated that as the size of the substituent increased, affinity decreased.[5] That is, affinity appeared to be dictated by the lipophilicity of the 6-position substituent but was further tempered by substituent size.



1 **2**

Consistent with these results is that nicotine analogs bearing hydrophilic substituents at the 6-position (e.g., **1**; R = COOH, K_i > 10,000 nM) lacked significant affinity, as did nicotine analogs bearing bulky substituents (e.g., **1**; R = Ph, K_i = 9440 nM).[5] Subsequent QSAR studies by other laboratories resulted in similar conclusions.[6,7] However, as might be expected, QSAR studies are only as reliable as the compounds that are included in the investigation. Previous QSAR studies were limited to nicotine analogs (or other related nicotinic agents) bearing relatively small 6-position substituents. Recently, we identified a novel nicotine analog that challenges our earlier findings and we now wish to report that 6-(2-phenylethyl)nicotine (6-PEN; **1b**; **1** where R = $–CH_2CH_2Ph$) binds at nACh receptors.

As the size/lipophilicity of the nicotine 6-position substituent was incrementally increased from –H to –*n*Pr (K_i = 1.3, 1.8, 5.6, and 17 nM for **1**; R = –H, $–CH_3$, $–C_2H_5$, and $–nC_3H_7$, respectively) affinity decreased.[5,8] However, we found that the (±)- and *S*(−)*n*-propyl analogs (i.e., **1**; R = $–CH_2CH_2CH_3$), unlike other members of this homologous series, behaved not as nicotinic agonists, but as antagonists.[8] This prompted us to examine additional 6-substituted nicotine analogs including the bulkier **1b**. Furthermore, because aminoethylpyridines **2** (e.g., **2a** where R = –H; K_i = 18 nM) have been demonstrated to bind at nACh receptors,[9] we also prepared the 6-(2-phenylethyl) analog **2b** (i.e., **2** where R = $–CH_2CH_2Ph$).

Compound **1b** was prepared from 6-methylnicotine (**3**) as shown in Scheme 1. Reaction of **3** with *n*BuLi formed the anion, which was allowed to react with

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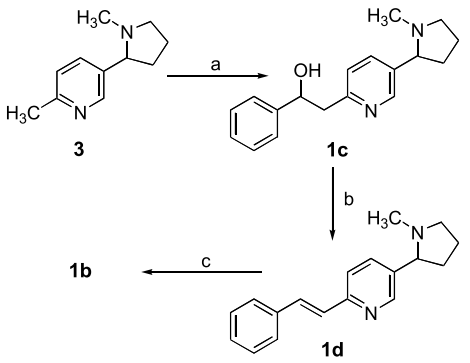


Scheme 1. Reagents and conditions: (a) (i) *n*BuLi/THF, −78 °C, (ii) PhCHO; (b) H_3PO_4 (85%), Δ; and (c) 10% Pt/C, H_2, EtOH.

benzaldehyde to afford the diastereomeric alcohol **1c**. Dehydration of the alcohol to alkene **1d** followed by catalytic reduction provided **1b**.

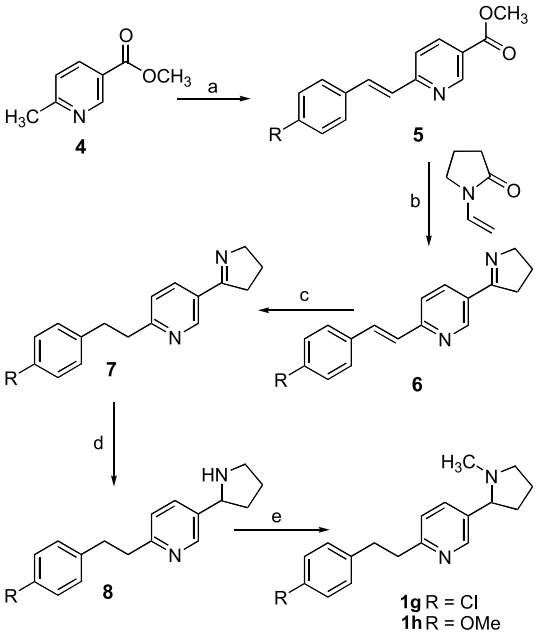
Compounds **1e** and **1f** were prepared by alkylation of the individual optical isomers of 6-methylnicotine using *n*BuLi and (2-bromoethyl)benzene. The 4′-substituted compounds **1g** and **1h** were prepared as shown in Scheme 2. Condensation of methyl 6-methylnicotinate (**4**) with the appropriate benzaldehyde followed by coupling with 1-vinyl-2-pyrrolidinone afforded styryl derivative **6**. Subsequent reduction of the olefins using Ra–Ni followed by reduction of the imine with sodium cyanoborohydride provided the nornicotine analogs **8**, which were reductively methylated to **1g** and **1h**.

Methyl 6-methylnicotinate (**4**) was converted to the known[10] **9** and allowed to react with (*N*-ethyl-*N*-methyl)aminoacetonitrile (**10**) in the presence of sodium bis(trimethylsilyl)amide to afford β-keto amide **11** (Scheme 3). The amide and ketone functions were reduced in a two-step process to provide **2b**.

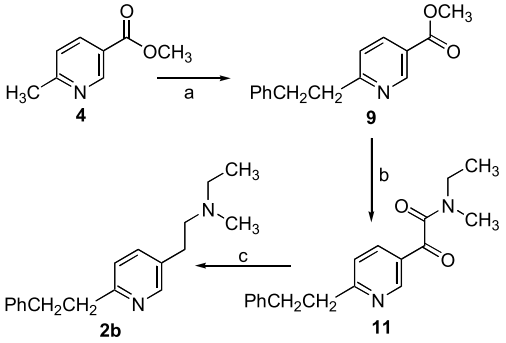
Compound **1b** (K_i = 15 nM) was found to bind[11] at α4β2 nACh receptors with about 7-fold lower affinity than nicotine (**1a**, Table 1), but with an affinity comparable to (−)6-*n*-propylnicotine (**1**, R = $CH_2CH_2CH_3$; K_i = 17 nM). The hydroxy (i.e., **1c**, K_i = 275 nM) and styryl (**1d**, K_i = 350 nM) synthetic intermediates showed reduced affinity, as did the chain-lengthened compounds **1e** and **1f** (K_i = 72 and >10,000 nM, respectively), and the ethylaminopyridine counterpart of **1b** (**2b**, K_i > 10,000 nM) (Table 1). Introduction of an electron withdrawing chloro group (**1g**, K_i = 40 nM) or electron donating methoxy group (**1h**, K_i = 10 nM) at the phenyl ring 4-position of **1b** had little effect on affinity (Table 1).

Compound **1b** was examined for possible nicotine-like effects in several assays indicative of such action: a mouse spontaneous activity assay, mouse hypothermia assay, mouse tail-flick assay, and in rats trained to discriminate (−)nicotine from saline vehicle.[13] In the spontaneous activity and hypothermia assays, **1b** failed to produce nicotine-like actions up to a subcutaneous dose of 15 mg/kg (data not shown). Compound **1b** also failed to produce an antinociceptive effect, producing only 2% of the maximal possible effect (MPE) at the highest dose tested (i.e., 15 mg/kg) (data not shown). In tests of stimulus generalization employing rats trained to discriminate 0.6 mg/kg of (−)nicotine from vehicle (ED_{50} = 0.1 mg/kg), **1b** produced a maximum of 6% nicotine-appropriately responding at doses of up to 1 mg/kg (i.e., 10 times the ED_{50} dose of nicotine) and disrupted the animals' behavior at higher doses (data not shown). Clearly, **1b** failed to produce nicotine-like actions in any of the assays.

Given that **1b** binds at nACh receptors, it was additionally examined as a possible nicotine antagonist in each of the assays. In the tail-flick assay, **1b** at a dose of 15 mg/kg administered in combination with 2.5 mg/kg of (−)nicotine displayed no significant antagonism of the response. Likewise, **1b** failed to significantly attenuate the spontaneous activity, hypothermic action, or

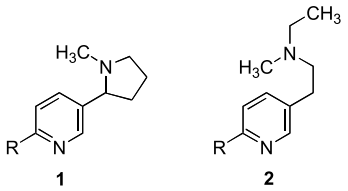


Scheme 2. Reagents and conditions: (a) Ac_2O, (4-Cl)PhCHO, Δ, or AcOH, piperidine, (4-OMe)PhCHO, Δ; (b) NaH, THF, Δ; (c) H_2. Ra–Ni, rt; (d) $NaCNBH_3$, MeOH, AcOH; and (e) $NaHB(OAc)_3$, H_2CO.



Scheme 3. Reagents and conditions: (a) (i) PhCHO, (ii) H_2, 10% Pd/C; (b) **10**, sodium bis(trimethylsilyl)amide; and (c) (i) $LiAlH_4$, (ii) H_2, 10% Pd/C.

Table 1. Physicochemical properties and α4β2 nACh receptor binding affinities



	R	Isomer	Recryst. solvent	Melting point (°C)	Empirical formula[a]	K_i, nM (±SEM)[b]
1a	–H	(±)	—	—	—	2 (±1)
1b	$-CH_2CH_2-Ph$	(±)	MeOH/Et_2O	192–195	$C_{18}H_{22}N_2 \cdot 2HCl$[c]	15 (±4)
1c	$-CH_2-CH(OH)-Ph$	(±)	2-PrOH/Et_2O	203–206	$C_{18}H_{22}N_2O \cdot 2HCl$[d]	275 (±20)
1d	–CH=CH–Ph	(±)	2-PrOH/Et_2O	225–228	$C_{18}H_{20}N_2 \cdot 2HCl$[c]	350 (±25)
1e	$-CH_2CH_2CH_2-Ph$	(−)	2-PrOH/Et_2O	160–162	$C_{19}H_{24}N_2 \cdot 2HCl$[e]	72 (±5)
1f	$-CH_2CH_2CH_2-Ph$	(+)	2-PrOH/Et_2O	159–161	$C_{19}H_{24}N_2 \cdot 2HCl$[f]	>10,000
1g	$-CH_2CH_2-Ph$(4-Cl)	(±)	EtOH/Et_2O	112–113	$C_{18}H_{21}ClN_2 \cdot 1.25C_2H_2O_4$	40 (±2)
1h	$-CH_2CH_2-Ph$(4-OMe)	(±)	EtOH/Et_2O	114–118	$C_{19}H_{23}N_2O \cdot 1.5C_2H_2O_4$[c]	10 (±2)
2a	–H	—	—	—	—	18[g]
2b	$-CH_2CH_2-Ph$	—	EtOH/Et_2O	133–136	$C_{18}H_{24}N_2 \cdot 2.5C_2H_2O_4$	>10,000

[a] All compounds analyzed within 0.4% of theory for C, H, and N; $C_2H_2O_4$ = oxalate salt. Assigned structures are consistent with ^1H NMR spectral data.
[b] Previously published procedures were used for the binding assays.[11]
[c] Crystallized with 0.25 mol H_2O.
[d] Crystallized with 0.5 mol H_2O.
[e] Crystallized with 1.25 mol H_2O.
[f] Crystallized with 0.6 mol H_2O.
[g] Synthesis and K_i value reported earlier.[9]

stimulus effects of (−)nicotine. However, administered via an intrathecal (i.t.) route, **1b** fully antagonized the antinociceptive actions of nicotine in a dose-dependent fashion, whereas by itself it produced no (5% MPE) antinociceptive effect (Fig. 1). That is, a combination of 20 μg/mouse of (−)nicotine plus 70 μg/mouse of **1b** produced saline-like effects.

To obtain a preliminary indication of selectivity for α4β2 versus other nACh receptors, **1b** was submitted to the NIMH/PDSP.[15] Apparently, **1b** binds with somewhat reduced affinity at cloned rat α2β2 (K_i = 490 nM), α2β4 (1200 nM), α3β2 (2000 nM), α3β4 (4600 nM), and α4β4 (400 nM) binding sites, and binds at cloned rat α4β2 receptors (K_i = 200 nM) with about 6-fold lower affinity than nicotine (K_i = 35 nM) when [^{3}H]epibatidine is used as radioligand.

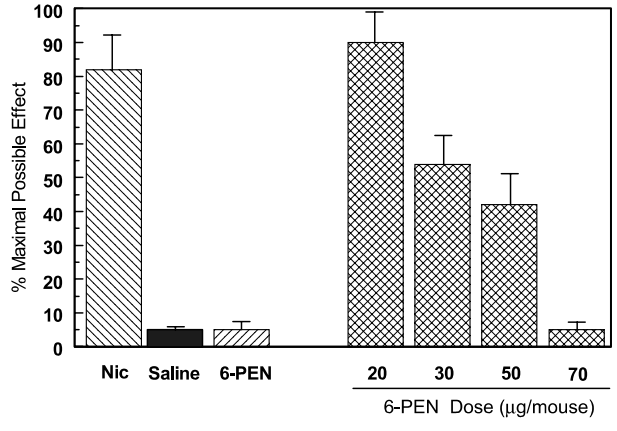


Figure 1. 6-PEN (**1b**) dose-dependently antagonized the antinociceptive actions of (−)nicotine when co-administered via the i.t. route 5 min prior to nicotine (20 μg/mouse). By themselves, (−)nicotine (NIC, 20 μg/mouse) produced >80% MPE whereas **1b** (70 μg/mouse) produced only 5% MPE.

6-(2-Phenylethyl)nicotine (**1b**) was unexpectedly found to bind with high affinity at α4β2 nACh receptors. Although its chloro- and methoxy analogs **1g** and **1h** retained affinity, alkyl chain extension (**1e**/**1f**) led to reduced affinity, as did incorporation of a 2-phenylethyl substituent at the 6-position of an aminoethylpyridine (i.e., **2b**). Administered via the sc route, compound **1b** failed to either produce or antagonize nicotine-like actions in several functional assays including the mouse spontaneous activity, hypothermia, tail-flick, and rat drug discrimination assays. Interestingly, **1b** was able to antagonize the antinociceptive actions of (−)nicotine in the tail-flick assay when administered via the intrathecal route. One explanation for these findings is that the receptor population that mediates this effect might be inaccessible to **1b** when administered via the sc route for pharmacokinetic or pharmacodynamic reasons. Although both the racemate and *S*(−)-isomer of 6-*n*-propylnicotine (**1** where R = –*n*Pr) bind with similar affinity to α4β2 receptors and behave as antagonists, future studies will examine the individual optical isomers of **1b** to determine if they act in a manner similar to the racemate, or whether one of the isomers behaves differently.

Acknowledgment

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13. The functional assays were conducted as previously described.[12] Each is briefly described below. Male ICR mice (20–25 g; Harlan Labs, Indianapolis, IN), housed in an AALAC approved facility in groups of six, had free access to food and water. Male Sprague–Dawley rats (Charles River Laboratories; Wilmington, MA), initially weighing 350–400 g, were used in the drug discrimination studies. All studies were approved by the Institutional Animal Care and Use Committee of VCU. *Tail-flick Assay*. Antinociceptive response was calculated as % maximum possible effect (%MPE), where %MPE = [(test − control)/(10-control)] × 100. Mice (n = 6–12) were tested 5 min after either sc or i.t. injections. Antagonism studies were performed by pretreating mice with saline or **1b** 5 min prior to administration of (−)nicotine. Intrathecal injections (volume = 5 μL) were performed free-hand between the L5 and L6 lumbar space in unanesthetized mice according to the method of Hylden and Wilcox[14] using a 30-gauge needle attached to a glass microsyringe. *Spontaneous activity*. Mice (minimum of six/dose) were placed into individual Omnitech photocell activity cages (28 × 16.5 cm) 5 min after sc administration of 0.9% saline or **1b**. Interruptions of the photocell beams were recorded for the next 10 min. *Body temperature*. Rectal temperature was measured by a thermistor probe (inserted 24 mm) and digital thermometer (Yellow Springs Instrument Co., Yellow Springs, OH). Readings were taken just before and at 30 min after the sc injection of either saline or **1b**. Each dose of each agent was examined in a minimum of six animals. *Drug Discrimination*. Rats (n = 6) were trained (standard two-lever operant chambers, Model E10-10, Coulbourn Instruments, Lehigh Valley, PA) to discriminate 0.6 mg/kg of (−)nicotine hydrogen tartrate from vehicle (sterile 0.9% saline) under a variable-interval 15-s schedule of sweetened milk reinforcement using a 15-min training session. Once the animals had been trained to discriminate nicotine from saline vehicle, generalization and antagonism tests were conducted as described.[12]
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