Nicotinic Acetylcholine Receptor Efficacy And Pharmacological Properties Of 3-(Substituted Phenyl)-2Î²-Substituted Tropanes

F. Ivy Carroll
Bruce E. Blough
S. Wayne Mascarella
HernÃ¡n A. Navarro
J. Brek Eaton

See next page for additional authors

Follow this and additional works at: https://scholar.barrowneuro.org/neurobiology

Recommended Citation
Carroll, F. Ivy; Blough, Bruce E.; Mascarella, S. Wayne; Navarro, HernÃ¡n A.; Eaton, J. Brek; Lukas, Ronald J.; and Damaj, M. Imad, "Nicotinic Acetylcholine Receptor Efficacy And Pharmacological Properties Of 3-(Substituted Phenyl)-2Î²-Substituted Tropanes" (2010). Translational Neuroscience. 256.
https://scholar.barrowneuro.org/neurobiology/256

This Article is brought to you for free and open access by Barrow - St. Joseph's Scholarly Commons. It has been accepted for inclusion in Translational Neuroscience by an authorized administrator of Barrow - St. Joseph's Scholarly Commons. For more information, please contact suefue.espe@commonspirit.org.
Authors
F. Ivy Carroll, Bruce E. Blough, S. Wayne Mascarella, HernÃ¡n A. Navarro, J. Brek Eaton, Ronald J. Lukas, and M. Imad Damaj
Nicotinic Acetylcholine Receptor Efficacy and Pharmacological Properties of 3-(Substituted phenyl)-2β-substituted Tropanes

F. Ivy Carroll,* Bruce E. Blough,† S. Wayne Mascarella,‡ Hernán A. Navarro,‡ J. Brek Eaton,‡ Ronald, J. Lukas,§ and M. Imad Damaj§

†Center for Organic and Medicinal Chemistry, Research Triangle Institute, Research Triangle Park, North Carolina 27709-2194, United States, ‡Division of Neurobiology, Barrow Neurological Institute, 350 West Thomas Road, Phoenix, Arizona 85013, United States, and §Department of Pharmacology and Toxicology, Medical Campus, Virginia Commonwealth University, Richmond, Virginia 23219, United States

Received August 2, 2010

There is a need for different and better aids to tobacco product use cessation. Useful smoking cessation aids, bupropion (2) and varenicline (3), share some chemical features with 3-phenyltropanes (4), which have promise in cocaine dependence therapy. Here we report studies to generate and characterize pharmacodynamic features of 3-phenyltropane analogues. These studies extend our work on the multiple molecular target model for aids to smoking cessation. We identified several new 3-phenyltropane analogues that are superior to 2 in inhibition of dopamine, norepinephrine, and sometimes serotonin reuptake. All of these ligands also act as inhibitors of nicotinic acetylcholine receptor (nAChR) function with a selectivity profile that favors, like 2, inhibition of α3/4*β-nAChR. Many of these ligands also block acute effects of nicotine-induced antinociception, locomotor activity, and hypothermia. Importantly, all except one of the analogues tested have better potencies in inhibition of nicotine conditioned place preference than 2. We have identified new compounds that have utility as research tools and possible promise for treatment of nicotine dependence.

Introduction

Tobacco product use, principally through cigarette smoking, is the greatest preventable cause of premature mortality, contributing in the United States to over 435,000 deaths annually.1 Tobacco use cessation can halt and reverse the biological damage caused by smoking.2 It is now commonly accepted that smoking behavior is maintained by the reinforcing effects of nicotine (1) and aversive effects of nicotine withdrawal.3–6 Both nonpharmacological and pharmacological interventions have demonstrated efficacy in smoking cessation.7 At present, first-line pharmaceutical treatments include nicotine replacement therapy (NRT),8 bupropion (2) and varenicline (3).9,10 While these treatments are useful in helping 5–20% of smokers abstain over the long term, new pharmacotherapies are needed that are either more effective or can impact those individuals not helped by existing treatments.

The effectiveness of varenicline in smoking cessation is thought to be due to its action as a partial agonist at α4/β2-containing nicotinic acetylcholine receptors (nAChR).9,10 The mechanism for bupropion’s effectiveness as a smoking-cessation aid appears to be more multifaceted.11 Its behavioral and neurophysiological effects resemble those of psychomotor stimulants,12 and similar to other psychomotor stimulants, 2 inhibits the reuptake of dopamine (DA).13,14 It also inhibits the reuptake of norepinephrine (NE)13,14 and is a noncompetitive inhibitor of α3/4*-nAChR (where the * indicates that nAChR subunits are known or possible assembly partners in addition to those indicated) and α4/β2-nAChR.14 In animal behavioral pharmacology studies, 2 induced locomotor activity,15,16 generalized to cocaine and amphetamine in drug discrimination studies,17,18 produced conditioned place preference (CPP),19 and was self-administered by both rats20 and nonhuman primates.21

Over the past several years, we have synthesized a large number of 3-phenyltropane analogues and evaluated them for binding at monoamine transporters.22,23 Similar to 2, some analogues were better at inhibiting the dopamine (DA) transporter (DAT) and the norepinephrine (NE) transporter (NET) than the serotonin (5HT) transporter (SERT). Others were selective for DAT relative to NET and SERT, whereas others showed similar inhibition at all three transporters.22,23 In 1995, Lerner-Marmorosh et al. reported that a number of 3-phenyltropane analogues were effective in blocking nicotine-induced seizures in mice and that a good correlation was observed between pharmacological potencies and abilities to block [3H]mecamylamine binding to brain membranes.24 On the basis of these results, Lerner-Marmorosh et al. concluded that...
the 3-phenyltropane analogues are neuronal nicotinic antagonists acting on a similar site to that of mecamylamine, a noncompetitive nicotinic antagonist. In another study, we reported that several 3-phenyltropane analogues blocked nicotine-induced antinociception in the tail-flick test in mice with potencies greater than that of 2.25 These intriguing results suggested that an additional pharmacological study of the 3-phenyltropane class of monoamine uptake inhibitor might provide information about the mechanism of action of noncompetitive nicotinic antagonists like 2 and could provide lead compounds for development as aids to smoking cessation or as drugs for treatment of neurological or psychiatric disorders involving nicotinic mechanisms.

In this study, we report the synthesis and biological evaluation of 3-phenyltropane analogues 4a–l. All analogues show inhibitory potency at human DAT and NET and functional antagonism of human α3β4*-nAChR. Similar to 2, the compounds antagonize the antinociceptive, hypolocomotor, and/or hypothermic effects induced by an acute injection of nicotine in mice and blocked nicotine CPP after repeated injection.

Chemistry

The 3-phenyltropane analogues 4a–e, 4g, and 4l were synthesized as previously reported.25–29 Scheme 1 outlines the synthetic route used to prepare 3β-(4-chlorophenyl)-2β-(4’,5’-dimethylbenzimidazol-2’-yl)tropane (4f). 3β-(4-Chlorophenyl)tropane-2β-carboxylic acid (5)28 is converted to the acid chloride and then treated with 4,5-dimethyl-1,2-phenylenediamine to give amide 6. Treatment of 6 with phosphorus oxychloride gave the desired 4f. The nortropane analogue 4h was prepared from 4c as shown in Scheme 2. Treatment of 4c in 1,2-dichloroethane containing excess 1-chloroethyl chloroformate (ACE-Cl) followed by refluxing the intermediate urethane in methanol gave 4h.

The synthesis of the 3β-(4-chloro-3-methylphenyl)-2β-(3’-substituted isoxazol-5’-yl)tropanes (4i and 4j) is also outlined in Scheme 2. A solution of 4c was added to the dilithium salt of the appropriate ketone oxime in tetrahydrofuran (THF) at 0 °C under nitrogen, and the reaction mixture was allowed to warm to 25 °C. After a few hours, the reaction mixture was added to a THF solution containing sulfuric acid and refluxed for 1 h to give the desired products 4i and 4j.

The synthesis of the 3α,2β-tropane 4k is outlined in Scheme 3. Addition of a solution of (1R,5S)-2-(3’-methyl-1’,2’,4’-oxadiazol-5’-yl)-8-methyl-8-azabicyclo[3.2.1]oct-2-ene (7)30 in anhydrous THF at −78 °C to a solution of the 3-chloro-4-methylphenyllithium (prepared from the appropriate aryl bromide and butyllithium) followed by quenching with 1 N hydrochloric acid at −78 °C formed the 3α-(substituted phenyl)tropane-2α-(3’-methyl-1’,2’,4’-oxadiazol-5’-yl)-tropanes (8). In addition to the desired isomer, the 2α,3β-isomer was also formed, which was removed by flash chromatography or carried through and removed at the next reaction. Transformation of oxadiazole 8 to the desired methyl ester 4k was accomplished by reduction with nickel boride (generated in situ by reaction of sodium borohydride and nickel tetraacetate) and hydrochloric acid in refluxing methanol. Under such conditions, a complete epimerization of C-2 to form the 3α,2β-stereoisomer was observed.

In Vitro Assays. The 3-phenyltropane analogues 4a–l were evaluated for their ability to block reuptake of [3H]dopamine ([3H]DA), [3H]serotonin ([3H]SERT), and [3H]norepinephrine ([3H]NE) using human (h) DAT, hSERT, and hNET stably expressed in HEK293 cells using conditions similar to those previously reported.31,32 The results are given in Table 1.

The 3-phenyltropane analogues 4a–l were also evaluated for their ability to antagonize functional responses of α3β4*-nAChR and mechanisms involved for functional inhibition using previously reported methods.33 Results are given in Table 1 and in Figures 1 and 2.

In Vivo Assays. Acute Nicotine Testing. The 3-phenyltropane analogues 4a–l were also evaluated for their ability to antagonize behavioral responses to acute nicotine administration as previously described.31 Results are given in Table 2.

Nicotine Reward Using the CPP Test. Selected 3-phenyltropane analogues were also evaluated for their ability to antagonize the development of nicotine-induced CPP in mice using an unbiased paradigm.34 Results are given in Table 2.

Results

Effects on Monoamine Uptake. Compound 2 is a relatively weak monoamine uptake inhibitor. In contrast, all of the 3-phenyltropane analogues studied here have higher inhibitory potencies for DA uptake inhibition. 3β-(4-Methylphenyl)tropane-2β-carboxylic acid isopropyl ester (4a) and
β-(4-methylphenyl)-2β-(3,0-ethylisoxoazol-50-yl)tropane (4c), with IC50 values of 0.83 and 0.75 nM, respectively, were the most potent analogues as inhibitors of DA uptake. However, 3β-(4-chloro-3-methylphenyl)-2β-(3,0-methylisoxazol-50-yl)tropane-2β-carboxylic acid methyl ester (4g), and its nortropane analogue (4h), with IC50 values of 1.5, 2.0, and 2.3 nM, respectively, are almost as potent at inhibition of DA uptake as 4a and 4c. In addition, all of the 3-phenyltropane analogues were potent at NE uptake inhibitors. The most potent NE uptake inhibitors were the nortropane analogues 3R-(4-fluoro-3-methylphenyl)nortropane-2β-carboxylic acid methyl ester (4l) and 3β-(4-chloro-3-methylphenyl)-nortropane-2β-carboxylic acid methyl ester (4h), with IC50 values of 0.5 and 0.9 nM, respectively. 3α-(4-Chloro-3-methylphenyl)tropane-2β-carboxylic acid methyl ester (4g), 3β-(4-chloro-3-methylphenyl)-2β-(3,0-methylisoxazol-50-yl)tropane (4i), and 3α-(4-chloro-4-methylphenyl)tropane 2β-carboxylic acid methyl ester (4k), with IC50 values of 1.1, 6.3, and 6.5, respectively, also are very potent NE uptake inhibitors.

Most of the 3-phenyltropane analogues are inactive or have IC50 values greater than 100 nM for 5HT uptake inhibition. However, the 4-chloro-3-methylcarboxylic acid methyl ester 4g and its nortropane analogue 4h have IC50 values of 1 and 6.2 nM for 5HT uptake inhibition. Since these two analogues also have high potency for DA and NE uptake inhibition, they have high potency uptake inhibition at all three monoamine transporters.

Analogue 4g has a slight (~2-fold) preference for inhibition of 5HT and NE over DA uptake inhibition. Compound 4j has comparable inhibitory potencies for DA and NE uptake. Ligand 4h has slight (~2-fold) preference for NE over DA uptake inhibition. Compound 4i has 14-fold preference for...
inhibition of NE over DA uptake. Otherwise, all the other compounds have preference for inhibition of DA uptake over other monoamine transporters, as low as 2-fold for 4k and 4f to >60-fold for 4a.

Effects on nAChR Function. The effects of 3-phenyltropane analogues 4a–l on function of diverse human nAChR subtypes naturally or heterologously expressed by human cell lines were assessed using 86Rb+ efflux assays that are specific only for nAChR function in the cells used. None of the analogues has activity as agonists at α1*- or α3/β4*- or β4/β2-, or α4/β4-nAChR because 86Rb+ efflux in the presence of these ligands alone at concentrations from ~5 nM to 100 μM (data not shown here) was indistinguishable from responses in cells exposed only to efflux buffer.

86Rb+ efflux assays also were used to assess whether ligands had activity as antagonists at human nAChR. Representative concentration-response curves for selected ligands (Figure 1) illustrate nAChR in vitro inhibitory profiles (see also Table 1).

Compound 2 has IC50 values of 1.8, 12, 15, and 7.9 μM for functional antagonism of α3/β4*, α4/β2-, α4/β4-, and α1/β1*-nAChR, respectively. Analogues 4a, 4b, 4c, 4e, 4f, 4i, and 4j all have IC50 values equal to or lower than that for 2 at α3/β4*-nAChR. The most potent inhibitors of α3/β4*-nAChR function are 4f and 4i (IC50 = 0.57 and 0.73 μM, respectively). Similar to 2, all the 3-phenyltropane analogues show preference for functional inhibition of α3/β4*-nAChR over the other nAChR subtypes tested, with greatest overall preference for 4e (8-, >56-, and 11-fold over α4/β2-, α4/β4-, or α1*-nAChR) and 4i (11-, 8-, and 18-fold over α4/β2-, α4/β4-, or α1*-nAChRs). Only 4f had significantly better potency as a functional antagonist of α4/β2-nAChR than 2. Analogue 4j had the lowest overall preference for α3/β4*-nAChR (~2-fold), in part because it (and 4f) had the highest inhibitory potencies at α4/β4- and α1*-nAChR. Compounds 4f and 4g had the lowest preference (~3-fold), and compounds 4i and 4j had the highest preference (10- to 13-fold) for α3/β4*- over α4/β2-nAChR.

As was the case for 2, all of the 3-phenyltropane analogues tested inhibited nAChR function via an apparently noncompetitive mechanism, lowering apparent agonist efficacy without altering agonist EC50 values [representative data for compounds acting at α3/β4*-nAChR are shown (Figure 2)].

Comparing inhibitory potencies across classes of targets, 4g, 4h, and 4l have 3500- to 5700-fold selectivity for inhibition of NE uptake over α3/β4*-nAChR, and selectivity for inhibition of DA uptake over α3/β4*-nAChR ranges from 78- to 100-fold for 4f and 4j to >2000-fold for 4a and 4e.

In Vivo Effects. Compound 2 blocks nicotine-induced antinociception in the tail-flick and hot-plate tests with AD50 values of 1.2 and 15 mg/kg, respectively. Ten of the 3-phenyltropane analogues were more potent in blocking nicotine’s effects in the tail-flick assay than 2, having AD50 values between 0.002 and 0.28 mg/kg. The most potent analogue in the tail-flick test was 3α-(4-fluoro-3-methylphenyl)-2β-carboxylic acid methyl ester (4i),
The results from this study show that the 3-phenyltropane analogues have monoamine uptake inhibition and nAChR antagonist profiles similar to those of the 4- and 3β-substituted compounds. Some of these compounds show better potency than 2 as inhibitors of acute effects of nicotine and nicotine-induced CPP, which measures the acute rewarding effect of the drug.

Regardless of the type of substituent at the 2β- and 3β-position, all the 3-phenyltropane analogues (see structures 4a–1) had high potency in DA uptake inhibition. In addition, all the analogues except the 3β-cyclobutyl ester 4b and the 3β-4-chlorophenylisoxazole 4d also had high potency in NE uptake inhibition. From a structural perspective, extensive modifications of the 2β-group (see structures 4a–1) lowered analogue potency as 5HT uptake inhibitors more than they altered inhibitory potency as DA and NE uptake inhibitors.

Only analogues with smaller 2β-substituents (4g and 4h) retained high efficacy for 5HT uptake inhibition. On the basis of the limited comparisons, stereochemistry seems to influence activity for 5HT and NE uptake inhibition more than for DA uptake inhibition and 3β/4α-nAChR antagonist potency (compare 4g and 4k). With the exception of the 3β-4-chlorophenylisoxazole analogue 4d, all the analogues had similar relative potencies at 3β/4α- and 4α-β-nAChRs.

It is interesting to note that analogue 4c has 150 times more potency than 2 as an inhibitor of nicotine’s analgesic action in the tail-flick assay, 9-fold better activity in the hot-plate assay, 24-fold better activity in hypolocomotion studies, and a marginally more potent effect on body temperature, whereas it has ~8-fold better activity in the CPP assay. Analogue 4c does not differ much from 2 in its antagonistic potency at any of the nAChR subtypes studied, and it has 480-fold better activity as a DA uptake inhibitor, ~170-fold better activity as NE uptake inhibitor, and no less than 5-fold better activity as a 5HT uptake inhibitor than 2. Since analogue 4g (AD50 = 0.25 mg/kg) has activity comparable to that of 2 (AD50 = 0.35 mg/kg) in the CPP assay, is 6-fold more potent than 2 in the tail-flick assay, 40-, 44-, and 65-fold more potent than 2 in the hot-plate locomotor and hypothermia tests, and has about the same nAChR inhibition profile as 2 while having ~300 ~2000 > 10000 times more potency at DA/NE/5HT uptake inhibition, its CPP effects appear to be more related to antagonism of nAChR. A similar analysis of 4i and 4j shows that the 4- to 5-fold better CPP activity relative to 2 correlates better with their nAChR inhibition profile than with their monoamine transporter and acute nicotinic effects. Compound 4d with AD50 = 0.013 mg/kg has activity comparable to that of 2 (AD50 = 0.35 mg/kg) in the CPP assay, is 150 times more potent than 2 in the tail-flick assay, 40-, 44-, and 65-fold more potent than 2 in the hot-plate locomotor and hypothermia tests, and has about the same nAChR inhibition profile as 2 while having ~300 ~2000 > 10000 times more potency at DA/NE/5HT uptake inhibition, its CPP effects appear to be more related to antagonism of nAChR. A similar analysis of 4i and 4j shows that the 4- to 5-fold better CPP activity relative to 2 correlates better with their nAChR inhibition profile than with their monoamine transporter and acute nicotinic effects.

Regardless of the type of substituent at the 2β- and 3β-position, all the 3-phenyltropane analogues (see structures 4a–1) had high potency in DA uptake inhibition. In addition, all the analogues except the 3β-cyclobutyl ester 4b and the 3β-4-chlorophenylisoxazole 4d also had high potency in NE uptake inhibition. From a structural perspective, extensive modifications of the 2β-group (see structures 4a–1) lowered analogue potency as 5HT uptake inhibitors more than they altered inhibitory potency as DA and NE uptake inhibitors.

### Discussion

The results from this study show that the 3-phenyltropane analogues have monoamine uptake inhibition and nAChR antagonist profiles similar to those of 2. Some of the analogues also have slightly higher potency as antagonists of 3β/4α-nAChR, and all analogues retain preference across nAChR subtypes for blockade of 3β/4α-nAChR. Moreover, some of these compounds show better potency than 2 as inhibitors of acute effects of nicotine and nicotine-induced CPP, which measures the acute rewarding effect of the drug.

Regardless of the type of substituent at the 2β- and 3β-position, all the 3-phenyltropane analogues (see structures 4a–1) had high potency in DA uptake inhibition. In addition, all the analogues except the 3β-cyclobutyl ester 4b and the 3β-4-chlorophenylisoxazole 4d also had high potency in NE uptake inhibition. From a structural perspective, extensive modifications of the 2β-group (see structures 4a–1) lowered analogue potency as 5HT uptake inhibitors more than they altered inhibitory potency as DA and NE uptake inhibitors.
behavioral pharmacological properties. However, the reason for this lack of locomotor activity is still not fully understood. One possibility is that 4d is interacting with some as yet unidentified target, possibly a nAChR subtype other than those studied, that is responsible for its lack of locomotor activity and potent activity in the CPP test.

Although many analogues have much better potency in behavioral assays than 2, it is challenging to determine how those effects relate to ligand actions at molecular targets, in part because improvements over 2 for a given ligand can vary quite widely depending on the behavioral assay. Correlation plots comparing the in vitro and in vivo data did not provide any useful insights. Some of these challenges may be due to metabolism of analogues to more or less active forms. In addition, 2 in mice is converted in humans to hydroxy metabolites, one of which expresses much of the drug’s behavioral activity.

In summary, ligands have been developed that can serve as useful research tools, having different inhibitory potency profiles across nAChR and monoamine transporter targets. For example, 3β-(4-methylphenyl)-2β-(3'-ethyloxazol-5'-yl)-tropane (4c) has IC50 values of 0.75, 11, and 2600 nM for inhibition of DA, NE, and 5HT uptake and an IC50 of 1.7 nM for antagonism of α3β4*-nAChR. Like 2, 4c is active in all four acute tests of nicotine action in mice. Importantly, it is 150, 9, and 5 times more potent than the tail-flick, hot-plate, and locomotor tests, respectively, than 2. It also is 8 times more potent than 2 in blocking nicotine-CPP. Three other compounds (4g, 4h, 4i) have higher potencies than 4c as inhibitors in all four tests of acute nicotine effects, and these compounds as well as 4d, 4j, 4k, and 4l have potency as antagonists of nicotine-CPP significantly higher than that of 2. Compounds 4d and 4j are inactive or are less active than 2 in the acute test of nicotinic action. However, they retain high antagonist potency against α3β4*-nAChR and DA uptake inhibition. The current studies support the idea that nAChR antagonism, particularly inhibition of α3β4*-nAChR function, and inhibitory actions at monoamine transporters are pharmacodynamic features of 3-phenyltropanes. Perhaps most importantly, and certainly warranting further investigation, is the possible utility of these ligands as aids to smoking cessation, especially given their ability to inhibit acute effects of nicotine and a test of nicotine preference with better potencies than 2, which is a useful pharmacotherapy for nicotine dependence.

Experimental Section

Nuclear magnetic resonance (1H NMR and 13C NMR) spectra were recorded on a 300 MHz (Bruker AVANCE 300) spectrometer. Chemical shift data for the proton resonances were reported in parts per million (δ) relative to internal (CH3)2Si (δ 0.0). Elemental analyses were performed by Atlantic Microlab, Norcross, GA. Purity of compounds (> 95%) was established by elemental analysis. Analytical thin-layer chromatography (TLC) was carried out on plates precoated with silica gel GHLF (250 µm thickness). TLC visualization was accomplished with a UV lamp or in an iodine chamber. All moisture-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source. Anhydrous solvents were purchased from Aldrich Chemical Co. or VWR. CMA80 is a mixture of 80% chloroform, 18% methanol, and 2% concentrated ammonium hydroxide.

3β-(4-Chlorophenyl)-2β-(4′,5′-dimethylbenzimidazol-2′-yl)tropane (4f) Dihydrochloride. Compound 6 (3.8 g, 0.0995 mol) and POCl3 (50 mL) were mixed and stirred at reflux for 90 min. The reaction mixture was cooled and added to petroleum ether (2 L). Most of the solvent was decanted, and concentrated NH4OH—water (1:1) was added basic to litmus paper and then extracted with CH2Cl2. The organic layer was separated, dried (Na2SO4), and concentrated to yield 3.0 g of a tan amorphous solid. This solid was chromatographed on silica gel, eluting with EtOAc and then EtOAc—CMA80 (1:1) to afford 1.7 g (47%) of 4f.

The free base was dissolved in ether and treated with 1 M ethereal HCl to give 1.65 g of the dihydrochloride salt of 4f as a white solid: mp 224–227 °C; [α]D0 = −166.2 °C (c 0.69, MeOH). 1H NMR (CDCl3, free base) δ 1.73 (bd, 1H), 1.88 (q, J = 9.0 Hz, 2H), 2.22–2.35 (m, 4H), 2.31 (s, 1H), 2.33 (s, 1H), 2.35 (s, 1H), 3.20–3.48 (m, 4H), 6.67 (d, J = 9.0 Hz, 2H), 7.00 (d, J = 9.0 Hz, 2H), 7.26 (s, 2H). Anal. (C23H26ClN2O1·2H2O) C, H, N.

(−)-N-Nor-3β-(3-methyl-4-chlorophenyl)tropane-2β-carboxylic Acid Methyl Ester (4h) Tartrate. Compound 4e (0.85 g, 2.76 mmol) was dissolved in anhydrous CH2Cl2 (30 mL), and 1-chloroethyl chloroformate (ACE-Cl, 4.2 mL, 39 mmol) was added. The mixture was refluxed for 8 h. The reaction mixture was concentrated in MeOH (30 mL), and the solution refluxed overnight. The MeOH solution was concentrated and the residue partitioned between CH2Cl2 and 25 mL of NH4OH—H2O (1:1). The layers were separated and the aqueous layer extracted twice with CH2Cl2. The combined organic extracts were dried (Na2SO4), filtered, and concentrated to afford 0.85 g of yellow solid, which was chromatographed on 50 g of silica gel using 25% CMA80 in CH2Cl2 to obtain 0.54 g (67%) of 4h. This material was converted to the tartrate salt by dissolving 520 mg (1.77 mmol) of 4h in MeOH and adding a MeOH solution of β-tartaric acid 0.286 g (1.77 mmol). The salt was crystallized from MeOH—ether to give 0.7 g of 4h-tartrate: mp 156–158 °C; [α]D0 = −100.0 °C (c 1.00, MeOH). Anal. Calculated for C23H16ClNO5 C, 54.12; H, 5.90; N, 3.16; Cl, 7.99. Found: C, 53.88; H, 6.01; N, 3.13; Cl, 7.88. 1H NMR (CDCl3) δ 7.30 (d, 1H), 7.18 (s, 1H), 7.03 (d, 1H), 4.40 (s, 2H), 4.22 (m, 2H), 3.50 (m, 1H), 3.38 (s, 3H), 3.05 (d, 1H), 2.61 (t, 1H), 2.35 (s, 3H), 2.12–2.28 (m, 4H), 1.91 (d, 1H). Anal. (C27O2Cl13NO5) C, H, N.

(−)-3β-(3-Methyl-4-chlorophenyl)tropane-2β-(3′-methylisoxazol-5′-yl) hydrochloride. Acetone oxime (262 mg, 359 mmol) was dissolved under N2 in 7.5 mL of THF, and the solution was cooled to 0 °C. Butyllithium, 25 M in hexanes (2.9 mL, 7.18 mmol), was slowly added, and the solution was stirred in an ice bath for 2–3 h. Compound 4c (85 mg, 2.76 mmol) was dissolved in 4 mL of THF and slowly added to the cold reaction mixture. The mixture was stirred at room temperature for 3 h and then filtered from the complex mixture there for 1.6 h. A solution of 1.65 g of 36 NH4SO4, 2.1 mL of H2O2, and 7.65 mL of THF was slowly added, and the mixture was refluxed for 6 h before being stirred at room temperature for 16 h. The reaction mixture was concentrated and basified with NH4OH–H2O (1:1) to pH 10–11 and extracted with CH2Cl2 (3 × 10 mL). The combined organic extracts were dried (Na2SO4), filtered, and concentrated to give 0.8 g of product, which was purified by flash chromatography on silica gel using 10% Et2O–EtOH and 1% AcOH as solvents. The product was purified by recrystallization from MeOH–Et2O to afford 4h hydrochloride: mp 150 °C (dec); [α]D0 = −112.0 °C (c 1.00, MeOH). 1H NMR (CDCl3) δ 7.20 (d, 1H), 7.08 (s, 1H), 6.94 (d, 1H), 5.70, (s, 1H), 4.22 (m, 1H), 4.12 (m, 1H), 3.87 (m, 1H), 3.68 (m, 1H), 2.89 (s, 3H), 2.16–2.66 (m, 16H). Anal. (C29H22ClNO7) C, H, N.
of THF was slowly added, and the mixture was refluxed for 6 h before being stirred at room temperature 16 h. The reaction mixture was concentrated and basified with concentrated NH₄OH–H₂O (1:1) to pH 10–11 and extracted with CH₂Cl₂ (3 × 25 mL). The combined organic extracts were dried (Na₂SO₄), filtered, and concentrated to give 1.23 g of product, which was purified by flash chromatography on silica gel using 10% (Et₂O–Et₃N) (9:1) in 90% hexanes to give 0.39 g of 4c and 0.71 g (80% adjusted yield) of 4j. The 4j was crystallized from petroleum ether to give 0.59 g of fine white crystals: mp 139–141 °C. The product was converted to the HCl salt by adding 1 M HCl in Et₂O to an Et₂O solution of 4j. Recrystallization from MeOH–Et₂O gave 0.467 g of 4j·HCl: mp 278 °C (dec); [α]D₉₀⁻110.2 °C (c 1.00, MeOH). ¹H NMR (CD₂OD) δ 7.66 (m 2H), 7.43 (m 3H), 7.21 (d, 1H), 7.12 (s, 1H), 7.00 (d, 1H), 6.20 (s, 1H), 4.30 (m, 1H), 4.16 (m, 1H), 3.98 (m, 1H), 3.75 (m, 1H), 2.93 (s, 3H), 2.04–2.74 (m, 9H). Anal. (C₂H₅ClN₂O) C, H, N.

3-c-(4-Chloro-3-methylphenyl)tropane-2β-carboxylic Acid Methyln Ester (4k) Tosylate. To Ni(OAc)₂ (9.21 g, 0.037 mol) in MeOH (50 mL) was added Na₂H₂B₅ (1.4 g, 0.037 mol) in MeOH (20 mL). Compound 8 (2.20 g, 0.0074 mol) in MeOH (20 mL) was added followed by concentrated HCl (3.1 mL, 0.037 mol) in MeOH (5 mL). The black heterogeneous reaction mixture was stirred at reflux for 17 h. The mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was partitioned between Et₂O and concentrated NH₄OH–H₂O (1:1). The ether was separated, dried (Na₂SO₄), and concentrated in vacuo to give an orange oil. This oil was chromatographed on silica gel, eluting with ether–petroleum ether (1:1). The ether was separated, dried (Na₂SO₄), and concentrated in vacuo to give an orange oil. This oil was chromatographed on silica gel, eluting with ether–MeOH (1:1). The ether was separated, dried (Na₂SO₄), and concentrated in vacuo to give an orange oil. This oil was converted to the HCl salt by adding 8 M HCl in Et₂O to an Et₂O solution of the compound. 1H NMR (CDCl₃, free base) δ 1.32 (t, J = 5.8 Hz, 1H), 1.59 (m, 3H), 2.10 (m, 1H), 2.22 (s, 3H), 2.32 (s, 3H), 2.46 (m, 2H), 3.32 (m, 3H), 3.59 (s, 3H), 6.95 (d, J = 3.0 Hz, 1H), 6.98 (s, 1H), 7.25 (d, J = 5.5 Hz). Anal. (C₂H₅ClN₂O) C, H, N.

β-c-(4-Chlorophenyl)tropane-2β-N-(3-amino-4,5-dimethylphenyl Carboxamide) (6). To compound 5 (5.3 g, 0.0189 mol) in CH₂Cl₂ (100 mL) was added oxaly chloride (19.0 mL, 0.0378 mol, 2 M in CH₂Cl₂). The reaction mixture was stirred at room temperature for 2 h, then concentrated in vacuo. The resulting acid chloride was dissolved in CH₂Cl₂ (60 mL) and added to 4,5-dimethylimidazole (6.4 g, 0.0673 mol) in CH₂Cl₂ (50 mL). The reaction mixture was stirred, under nitrogen, for a period of 17 h. The solvent was decanted from a gummy residue, and 10% NaHCO₃ or NaOH as previously reported.

nAChR Functional Assays. Cells were harvested, seeded onto 24-well plates, and subjected to ⁸⁶Rb⁺ efflux assays as previously described. Specific ⁸⁶Rb⁺ efflux was assessed as the response to a fully efficacious concentration of carbamylcholine alone less that in the presence of efflux buffer alone. Any intrinsic agonist activity of test compounds was normalized, after subtraction of nonspecific efflux, to specific efflux. Antagonism of carbamylcholine-evoked ⁸⁶Rb⁺ efflux was assessed in samples containing the full agonist at a concentration where it stimulates 80–90% of maximal function. For studies of mechanism of antagonism, concentration–response curves were obtained using samples containing the full agonist, carbamylcholine, at the indicated concentrations alone or in the presence of 2 subunits), or transfected SH-EP1 epithelial cells heterologously expressing either human α4β2-nAChR, which is thought to be the most abundant, high affinity nicotine-binding nAChR in mammalian brain, or α4β4-nAChR. ⁸⁶Rb⁺ efflux was assessed in samples containing the full agonist at a concentration where it stimulates 80–90% of maximal function. For studies of mechanism of antagonism, concentration–response curves were obtained using samples containing the full agonist, carbamylcholine, at the indicated concentrations alone or in the presence of test antagonist, approached 100% of specific efflux as separately determined in sister samples exposed to fully efficacious concentrations of agonist. Note also that it has been repeatedly verified that functional parameters for nicotinic ligands and mechanisms of their action as determined using efflux assays are like those determined using whole-cell current recording techniques.

Behavior. All animal experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and Institutional Animal Care and Use Committee guidelines.

Animals. Male Institute of Cancer Research (ICR) mice (weighing 20–25 g) obtained from Harlan (Indianapolis, IN) were used throughout the study. Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care approved facility, were placed in groups of six, and had free access to food and water. Studies were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

Tail-Flick Test. Antinociception for pain mediated at the spinal level was assessed by the tail-flick method of D’Amour and Smith. In brief, mice were lightly restrained while a radiant heat source was shone onto the upper portion of the tail. To minimize tissue damage, a maximum latency of 10 s was
imposed. Latency to remove the tail from the heat source was recorded for each animal. A control response (2–4 s) was determined for each mouse before treatment, and a test latency was determined after drug administration (nicotine as an anagentic dose). Antagonism studies were carried in mice pretreated with either saline or 3-phenyltropane analogues 15 min after exposure to saline or nicotine. The difference in rectal temperature before and 30 min after subcutaneous injection of either saline or 3-phenyltropane analogue was determined as the percentage of maximum possible effect (%MPE), where %MPE = [(test latency in s − control latency in s)/(40 s − control latency in s)] × 100. Groups of 8–12 animals were used for each drug condition. Antagonism studies were carried in mice pretreated with either saline or 3-phenyltropane analogues 15 min before nicotine. The animals were then tested 5 min after administration of a subcutaneous dose of 2.5 mg/kg nicotine.

Locomotor Activity. Mice were placed into individual Omnitech photocell activity cages (28 cm × 16.5 cm; Omnitech Electronics, Columbus, OH) 5 min after subcutaneous administration of either 0.9% saline or nicotine (1.5 mg/kg). Interruptions of the photocell beams (two banks of eight cells each) were then recorded for the next 10 min. Data were expressed as the number of photocell interrumpptions. Antagonism studies were carried out by pretreating the mice with either saline or 3-phenyltropane analogues 15 min before nicotine. The animals were then tested 5 min after administration of a subcutaneous dose of 2.5 mg/kg nicotine.

Body Temperature. Rectal temperature was measured by a thermistor probe (inserted 24 mm) and digital thermometer (YSI Inc., Yellow Springs, OH). Readings were taken just before and 30 min after subcutaneous injection of either saline or 2.5 mg/kg nicotine. The difference in rectal temperature before and after treatment was calculated for each mouse. The ambient temperature of the laboratory varied from 21 to 24 °C from day to day. Antagonism studies were carried out by pretreating the mice with either saline or 3-phenyltropane analogues 15 min before nicotine. The animals were then tested 30 min after administration of a subcutaneous dose of 2.5 mg/kg nicotine.

Nicotine CPP Assessment. An unbiased CPP paradigm was utilized in this study as described in Kata et al. Briefly, place-conditioning chambers consisted of two distinct compartments separated by a smaller intermediate compartment with openings that allowed access to either side of the chamber. On day 1, adult male ICR mice were confined to the intermediate compartment for a 5 min habituation period and then allowed to move freely between compartments for 15 min. Time spent in each compartment was recorded. These data were used to separate the animals into groups of approximately equal bias. Days 2–4 were the conditioning days during which the saline group received saline in both compartments and drug groups received sc vehicle or 3-phenyltropane analogues 15 min before nicotine (0.5 mg/kg, sc) in one compartment and saline in the opposite compartment for 20 min. Drug-paired compartments were randomized among all groups. Day 5 was the drug free test day, and the procedure was the same as that of day 1. Activity counts and time spent on each side were recorded via photosensors using Med Associates interface and software. Separate groups of mice were conditioned with saline or 3-phenyltropane analogues alone to investigate if they induce CPP using the same procedure described above. Data were expressed as time spent on drug-paired side minus time spent on saline-paired side. A positive number indicated a preference for the drug-paired side, whereas a negative number indicated an aversion to the drug-paired side. A number at or near zero indicated no preference for either side.

Acknowledgment. This work was supported by National Institutes of Health National Cooperative Drug Discovery Group Grant U19 DA019377. Other effort was supported by grants (to R.L.L.) from the National Institutes of Health (Grant DA015389) and the Barrow Neurological Foundation. We thank Lawrence E. Biedundy for the synthesis of 4f and 4k and Fluvanna Josephson for the synthesis of 4h, 4i, and 4j.

Supporting Information Available: Elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

References


