Synthesis and Biological Evaluation of Bupropion Analogues as Potential Pharmacotherapies for Smoking Cessation

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Bupropion (2a) analogues were synthesized and tested for their ability to inhibit monoamine uptake and to antagonize the effects of human α3β4*, α4β2, α4β4, and α1* nAChRs. The analogues were evaluated for their ability to block nicotine-induced effects in four tests in mice. Nine analogues showed increased monoamine uptake inhibition. Similar to 2a, all but one analogue show inhibition of nAChR function selective for human α3β4*-nAChR. Nine analogues have higher affinity than α3β4*-nAChRs than 2a. Four analogues also had higher affinity for α4β2 nAChR. Analogues 2r, 2m, and 2n with AD50 values of 0.014, 0.015, and 0.028 mg/kg were 87, 81, and 43 times more potent than 2a in blocking nicotine-induced antinociception in the tail-flick test. Analogue 2x with IC50 values of 31 and 180 nM for DA and NE, respectively, and with IC50 of 0.62 and 9.8 μM for antagonism of α3β4 and α4β2 nAChRs had the best overall in vitro profile relative to 2a.

Introduction

Tobacco-related diseases remain as the predominant cause of premature mortality. An estimated one billion individuals worldwide are smokers.1 Despite recent declines, it is estimated that 21% of the adult population in the U.S. are active smokers.2 Since less than 10% of smokers are capable of quitting unwanted, improvements in the clinical management of smoking are needed.3

Nicotine (1, Chart 1) is the main active ingredient in tobacco smoke that causes and maintains tobacco addiction. Nicotine produces a myriad of profound behavioral and physiological effects and is able to initiate and support drug-seeking behavior in humans and in laboratory animals.4 The pharmacological and behavioral effects result from the activation of different nicotinic acetylcholine receptor (nAChR) subtypes, which are members of an ionotropic neurotransmitter receptor superfamily.5 nAChRs containing α4 and β2 or α7 subunits (α4β2- and α7-nAChR, respectively) are the two major subtypes found in the brain, although appreciable amounts of α3β4* and α6β2*-nAChRs also are present in brain regions implicated in reward and drug dependence such as the substantia nigra, the ventral tegmental area (VTA), and the medial habenula system.6,7,8

Although nAChRs are the initial sites of action of nicotine in the brain, downstream events involving dopaminergic reward pathways may be critical in reinforcing smoking behavior. Nicotine, similar to other abused substances, is thought to be reinforcing because of the stimulation of mesolimbic dopamine reinforcement pathways.9 Cigarette smoking acutely increases dopamine (DA) concentration in the ventral striatum/nucleus accumbens, key brain regions in the reward pathway.10

The first line medications on the market today to treat nicotine addiction are various nicotine replacement (NRT) formulations (nicotine gum, transdermal nicotine patches, vapor inhaler, nicotab, nasal spray, lozenges), presumed to mimic effects of tobacco-derived nicotine, the antidepressant bupropion (2a), and varenicline (3).11 Both varenicline and bupropion SR (a sustained-release formulation) reduce symptoms of withdrawal, cigarette craving, and smoking reinforcement and produce smoking cessation with efficacy equal to or better than nicotine replacement.12 However, none of these therapies has proven to be ideal, as smoking relapse still occurs at alarmingly high rates even after successful short-term therapies.

The use of 2a for treating nicotine addiction resulted from serendipitous observations that patients taking 2a as an antidepressant were more successful in smoking cessation attempts. It was known that 2a inhibited DA and norepinephrine (NE) uptake activity, but the discovery that it also preferentially antagonized α3β4*-nAChR13,14 suggested that more than one of these targets might be involved in its smoking cessation efficacy. It is possible that bupropion SR is achieving its effects by increasing dopamine levels through DA uptake inhibition and shielding against nicotine induction of nAChR-mediated dopamine elevation. Although it is not clear what part if any is played by the ability of 2a to inhibit NE uptake in its smoking cessation activity, it is likely to contribute to nicotine withdrawal amelioration. Surprisingly, very little effort has been devoted toward the development of 2a analogues with improved smoking cessation properties.15,16 analogues with im-I-19

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†Abbreviations: DA, dopamine; 5HT, serotonin; NE, norepinephrine; SR, sustained release; HEP, human embryonic kidney; DAT, dopamine transporter; SERT, serotonin transporter; NET, norepinephrine transporter; nAChR, nicotinic acetylcholine receptor; VTA, ventral tegmental area; NRT, nicotine replacement therapy; CTDP, Cocaine Treatment Discovery Program; NIDA, National Institute on Drug Abuse; MPE, maximum possible effect.

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Compound 2a inhibits DA reuptake, increasing the synaptic levels of DA. Compound 2a also inhibits nicotine-induced DA and NE overflow from superfused striatal and hippocampal slices, respectively. Thus, 2a might be functioning as an indirect DA agonist by reward modulation of this downstream action of nicotine. The ability of 2a to alleviate withdrawal symptoms is consistent with an indirect dopamine agonist mechanism. Compound 2a noncompetitively inhibits carbamylcholine-induced 86Rb+ efflux from human neuroblastoma cells expressing α3β4*-nAChR.15 and function of α3β2-, α4β2-, and α7-nAChR heterologously expressed in Xenopus oocytes.16 Other studies suggest that α3β4*-nAChR plays a major role in nicotine-evoked NE release from hippocampus.23,24

We recently reported the synthesis of a number of 2a analogues that were evaluated for their abilities to inhibit neurotransmitter uptake by the dopamine, norepinephrine, or serotonin (5HT) transporters, DAT, NET, or SERT, respectively, under the National Institute on Drug Abuse (NIDA) Cocaine Treatment Discovery Program (CTDP).25 Those studies sought to identify entities with better potency and selectivity toward DAT than 2a and whose activity as an indirect dopamine agonist might reveal a novel pharmacology for treating cocaine addiction. In the current study, we designed and developed 2a analogues as smoking cessation aids, seeking entities that possess increased inhibitory activity for DA and NE uptake inhibition and/or nAChR antagonism while retaining the druglike properties of the lead compound. In this study we report the synthesis and biological evaluation of 2a analogues 2b–ff, 4, and 5. Some of the analogues have higher inhibitory potencies than 2a at DAT and NET as well as at α3β4*-nAChR. In addition, some of the compounds antagonize the antinociceptive, hyperlocomotor, and hypothermic effects of acutely administered nicotine in mice with potencies greater than that of 2a. 2-(N-tert-Butylamino)-3,4-dichloropentanophenone (2x), which was 41- and 7.5-fold more potent in inhibition of DA and NE uptake and 3-fold more potent as an α3β4*-nAChR antagonist than 2a, is one of the more interesting compounds. Compound 2x is also 9 times more potent than 2a as an antagonist of nicotine-induced antinociception in the mouse tail-flick test.

**Chemistry**

The 2a analogues 2b–d, 2g–p, 2r, 2w–z, 2ff, 4, and 5 were synthesized as previously reported.25 The new 2a analogues 2e, 2f, 2q, 2s–v, and 2aa–ee were prepared using procedures exactly analogous to those used to synthesize the reported analogues. Thus, bromination of the ketones 6a–e with bromine in acetic acid afforded the bromoketones 7a–e (Scheme 1). Treatment of 7a–e with tert-butylamine, cyclopentylamine, and piperidine yielded the desired 2a analogues 2e, 2f, 2q, 2s–v, 2aa–ee, 4, and 5.

**In Vitro Assays.** The 2a analogues 2b–ff, 4, and 5 were evaluated for their ability to block reuptake of [3H]dopamine ([3H]DA), [3H]serotonin ([3H]5HT), and [3H]norepinephrine ([3H]NE) using (h)DAT, (h)SERT, and (h)NET stably expressed in HEK293 cells using conditions similar to those previously reported.26,27 The results are given in Table 1. [3H]DA, [3H]5HT, and [3H]NE uptake values for 2a and analogues 2b–d, 2g–p, 2r, and 2w–z were obtained as a part of the NIDA CTDP and previously reported.25 For the most part, the relative potency in both evaluations was the same. However, in general, the efficacy in this study tended to be higher (lower IC50 values) for all analogues than the efficacies obtained in the CTDP program.25 There were some exceptions. For example, in the case of 2o, we obtained IC50 values of 209, 607, and 16,000 nM for the inhibition of [3H]DA, [3H]SERT, and [3H]NE uptake compared to 31 and 969 nM and inactive in the CTDP program.

**In Vivo Assays.** Compound 2a and analogues 2b–ff, 4, and 5 were also evaluated for their ability to antagonize functional responses of α3β4*, α4β2, α4β4, and α1*-nAChR using previously reported methods modified as described in Experimental Section. Results are given in Table 1 and in Figures 1 and 2.27

**Results**

Compound 2a and its analogues were evaluated for their ability to inhibit DA, NE, and 5-HT uptake inhibition using
### Table 1. Analogue Inhibition of Monoamine Uptake and Nicotinic Acetylcholine Receptor (nAChR) Function

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<th>R</th>
<th>R₁</th>
<th>R₂</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>[3H]DA IC₅₀ (nM)</th>
<th>[3H]NE IC₅₀ (nM)</th>
<th>[3H]SERT IC₅₀ (nM)</th>
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<td>6500 ± 270</td>
<td>IA</td>
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<td>2520 ± 900</td>
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Values for mean ± standard error of three independent experiments, each conducted with triplicate determination. μM with an SEM range of 1.8 to 1.15 μM or 1.6–2.1 μM. IA: IC₅₀ > 100 μM.
human DAT, NET, and SERT homologously expressed by HEK293 cells. Compound 2a has IC$_{50}$ values of 658 and 1850 nM for inhibition of DA and NE uptake, respectively, and is inactive at SERT (Table 1). Very few 2a analogues had appreciably better IC$_{50}$ values for DA uptake inhibition than 2a. Compounds 2o and 2p, where the α-methyl group in 2a was replaced by ethyl and propyl groups, respectively, have IC$_{50}$ values for inhibition of DA uptake of 209 and 56 nM, respectively, indicating that replacement of the α-methyl group with larger alkyl chains produced ligands with better efficacy, a finding that also is borne out by the improvement in inhibitory potency at DAT for 2w (IC$_{50}$ = 118 nM) and 2x (IC$_{50}$ = 31 nM) relative to 2k (IC$_{50}$ = 463 nM) in the 3,4-dichlorophenyl analogue group. In each case, the 3,4-dichlorophenyl analogue has higher inhibitory potency for DA uptake inhibition than the otherwise equivalent monochlorophenyl analogue. Each 3,4-dichlorophenyl compound (2k, 2w, 2x) also has slightly better inhibitory potency for NE uptake (1670, 389, and 180 nM IC$_{50}$ values, respectively) greater than the equivalent monochlorophenyl compounds (2a, 2o, 2p; 1850, 607, 370 nM IC$_{50}$ values, respectively). The propyl 2a analogue (2p) and 3,4-dichlorophenyl propyl analogue 2x have 7- and 6-fold selectivity for DA over NE uptake inhibition.

Compound 2aa has 4- and 14-fold elevated inhibitory potency relative to 2a for DA uptake inhibition (IC$_{50}$ = 156 nM) and NE uptake inhibition (IC$_{50}$ = 135 nM). Compound 2y is the only compound tested that has higher selectivity...
nor any of the analogues tested possess intrinsic activity as agonists at α1*-nAChR, α3/4*-nAChR, or α4/β4-nAChR. 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 2a or its analogues had activity as antagonists at human nAChR. With few exceptions (noted in Table 1), 2a and each of its analogues exhibited concentration-dependent inhibition of ion flux responses elicited by EC90 concentrations of carbamylcholine for α1*-nAChR, α3/4*-nAChR, and α4/β4-nAChR. Representative concentration response curves for 2a itself, for compounds having substituted phenyl groups (2c, 2h, 2l; Figure 1), or for compounds having amine or alkyl charges (2y, 2o, 2x, 2w; Figure 2) illustrate some of the features of these ligands (see also Table 1).

Compounds 2a has IC₅₀ values of 1.8, 12, 15, and 7.9 μM for α3/4*-nAChR, α4/β4-nAChR, and α1*-nAChR, respectively, and thus is 4- to 8-fold selective for the α3/4* relative to the other nAChRs. All of the analogues except 2x (approximately equipotent at α3/4*- and α4/β4-nAChR) block function of α3/4*-nAChR at concentrations lower than those needed to inhibit function of the other nAChR subtypes. That is, all of the analogues are selective in their antagonism for α3/4*-nAChR. Compounds 2l, 2o, 2p, and 2x have slightly higher potencies than 2a, the α4/β2 nAChR (IC₅₀ = 7.7–9.8 μM).
The 4-fold selectivity of 2a for the α3β4*-nAChR over other subtypes is increased to >7-fold for the 3-bromo, 3-methyl, and 3-methoxyphenyl analogues 2c, 2d, and 2e, respectively, and to >10-fold for the 3,4-difluorophenyl and N-methyl analogues 2f and 2g, respectively.

In addition to the improvements in selectivity for α3β4*-nAChR over other subtypes, the 3-chloro, 4-methyl substituted phenyl analogue 2i (IC50 = 0.65 μM) has ~3-fold higher inhibitory potency at α3β4*-nAChR relative to 2a. Both mono- and dichloro-substituted phenyl compounds 2o, 2p, 2q, and 2r (IC50 = 0.51–0.70 μM), having extended alkyl side chains, have ~3-fold higher potency at α3β4*-nAChR relative to 2a with the 3,4-dichlorophenyl ethyl and propyl compounds 2s and 2t, respectively, being >10-fold more potent than the unextended 3,4-dichlorophenyl analogue (2k; IC50 = 6.8 μM). These compounds also are remarkable because they also had higher inhibitory potency and at selectivity for DA uptake inhibition and higher potency for NE uptake inhibition than 2a, so they all represent analogues with higher affinity than 2a for three of its molecular targets.

Compound 2u blocks nicotine-induced antinociception in the tail-flick and hot-plate tests with AD50 values of 1.2 and 15 mg/kg, respectively (Table 2). It also blocks nicotine-induced locomotor activity and hypothermia with AD50 values of 4.9 and 9.2 mg/kg, respectively. Seventeen of the 33 analogues had AD50 values of 0.014–0.5 mg/kg in the tail-flick test, showing higher inhibitory potency than 2a. Analogues 2u, 2v, and 2w with AD50 values of 0.014, 0.015, and 0.028 mg/kg were 86, 80, and 43 times more potent than 2a in blocking nicotine-induced antinociception in the tail-flick test. Surprisingly, none of these analogues has higher potency than 2a for DA, NE, and SERT uptake inhibition or any nAChR subtype. Analogues 2u, 2v, 2x, and 2y with AD50 values of 0.05 mg/kg were the next most potent in the tail-flick assay, being 24-fold more potent than 2a. Analogues 2v, 2w, 2y, and 2x (AD50 = 0.09–0.15 mg/kg) have ~10-fold better inhibitory potency than 2a against nicotine-mediated analgesia in the tail-flick assay. Thus, 2x is an analogue that has increased potency relative to 2a as an inhibitor of DA and NE uptake, and at α3β4*-nAChR, analogues 2v and 2x have potencies comparable to those of 2a across those targets. The 3,4-dichlorophenyl analogues 2k, 2m, and 2n are ~4-, ~10-, and ~77-fold more potent than their 3-chlorophenyl substituted equivalents 2a, 2o, and 2p, respectively. However, the tail-flick assay results are not particularly illuminating about the molecular targets contributing to analogue effects, perhaps because effects at higher levels might override the presumed spinal level of nicotine-mediated antinociception.

In the hot-plate assay, 2d and 2l (potent and selective at α3β4*-nAChR) and 2y (selective for NE uptake inhibition and α3β4*-nAChR) have about 2-fold more potency than 2a, and 2n (inactive at transporters and less potent than 2a at α3β4*-nAChR) has about 3-fold more potency. These findings suggest that several mechanisms might impact supraspinal mechanisms of nicotine-mediated antinociception.

Of all the analogues, only 2d has potency like 2a in blocking nicotine’s effects on locomotion, and only 2d and 2l rival the ability of 2a to inhibit nicotine’s effects on body temperature. Similar to 2a, substituted phenyl analogues 2c, 2d, and 2l (roughly sharing the in vitro fingerprint of 2a) had potency in all four behavioral tests as nicotine antagonists. Compound 2x, a potent DA and NE uptake inhibitor and α3β4*-nAChR antagonist, had potency in three of four of the in vivo tests. Compounds 2p, 2w, and 2x, having the highest potencies as DA uptake inhibitors, differ nearly 200-fold in potency in the tail-flick assay, and only 2x shows activity in the hot-plate or locomotor tests. These analogues also are among the most potent at NE uptake inhibition.

Discussion

Several 2a analogues were synthesized and tested for their ability to inhibit monoamine uptake and to antagonize function of four different nAChR subtypes. The analogues were also evaluated for their ability to block nicotine-induced antinociception, locomotor activity, and hypothermia.

We succeeded in creating and characterizing analogues with significantly lower IC50 values relative to 2a for inhibition of both DA or NE uptake inhibition (2o, 2p, 2w, 2x, and 2aa). The current efforts also succeeded in generating nine agents with higher (2l, 2o, 2p, 2w, 2x) or slightly higher (2c, 2d, 2h, 2y) antagonist potency relative to 2a at α3β4*-nAChR. Of these, 2c, 2d, 2l, and especially 2y also have improved selectivity for α3β4*-nAChR over other nAChR subtypes relative to 2a. In addition, analogues 2e and 2j show improved selectivity for α3β4*-nAChR although without having lower IC50 values than 2a.

Compounds were also developed that had altered target selectivity between nAChR and transporters. For example, 2c had improved selectivity for α3β4*-nAChR over DA and NE uptake inhibition, 2y improved selectivity for α3β4*-nAChR over DA uptake inhibition, and 2p, 2w, and 2x improved selectivity for DA and NE uptake inhibition over α3β4*-nAChR antagonist relative to 2a. Thus, several new compounds have been developed that have higher potency and/or selectivity at specific neurotransmitter transporters or α3β4*-nAChR than 2a, thereby providing leads for further target-directed drug development. These compounds also afford, in principle, opportunities to dissect roles of specific molecular targets in nicotine-mediated behavioral effects. Structure-activity relationships are complex and difficult to generalize because phenyl substitutions and amine/alkyl changes seem to interact in terms of altering potencies at and selectivities for specific targets in vitro. However, compounds with alkyl chain extensions in mono-chlorophenyl (like 2a) or dichlorophenyl configurations (2o, 2p, 2w, and 2x) had higher affinity than 2a for all three targets, DAT, NET, and α3β4*-nAChR, implicated in 2a action. The selectivity increase for 2a analogues in blocking α3β4*- nAChRs subtypes is very relevant, since these subtypes are highly expressed in the medial habenula and its primary target, the interpeduncular nucleus. Recent data suggest an important role for the habenulo-interpeduncular system and the nicotinic receptor subunits expressed therein in nicotine withdrawal.28

Seventeen of the analogues had higher potency than 2a as antagonists of nicotine-induced antinociception in the tail-flick test, having AD50 values that ranged from ~2- to ~86-fold lower than that for 2a. However, five analogues (2d, 2i, 2o, 2n, and 2y) were only slightly (~2-fold) better than 2a as antagonists of nicotine-induced antinociception in the hot plate assay, just one analogue (2d) rivaled or bettered 2a as an antagonist of nicotine’s effects on locomotion, and only 2d, 2i, 2l, and 2aa rivaled or bettered the antagonistic potency of 2a toward nicotine-induced hypothermia. Analogues 2m, 2n, and 2r have the lowest AD50 values in the tail-flick assay, and yet only 2n in the hot-plate assay shows any other form of nicotine
behavioral antagonism. Every ligand except 2p that had higher potency than 2a at α3β4*-nAChR also showed an improvement over 2a in antagonistic potency in the tail-flick assay as did three of the ligands (2o, 2w, and 2x but not 2aa and 2p) that had higher potency than 2a as antagonists for DA and NE uptake inhibition. Thus, increased potency at α3β4*-nAChR correlates with but is not necessary for improvement in ligand antagonist potency in the tail-flick assay. Moreover, when improvement in activity for DA and NE uptake inhibition is dissociated from improvement in activity at α3β4*-nAChR (i.e., for 2aa), there is no improvement in behavioral antagonism in the tail-flick assay. Perhaps compounds like 2g, 2i, 2k, 2j, 2f, 3, 4, or even 2m, 2n, and 2r that are not as active as 2a in vitro are metabolized in vivo to forms that are active behaviorally and might also have higher affinity for DA and NE uptake inhibition and/or α3β4*-nAChR antagonism, but drug exposure times in vivo are short.

In summary, several 2a analogues were synthesized and tested for their ability to inhibit monoamine uptake and to antagonize the effects of α3β4*, α4/2β, α4β/4, and α1β1 nAChRs. The analogues were also evaluated for their ability to block nicotine-induced antinociception, locomotor activity, and hypothermia. Analogue 2o, 2p, 2r, and 2x had significantly better IC50 values for DA uptake inhibition relative to 2a. Analogue 2x also had a significantly better IC50 value for NE uptake inhibition relative to 2a. Analogue 2l, 2o, 2p, 2s, 2w, and 2x had IC50 values of 2.6 to 3.6 times better than 2a for the antagonism of the α3β4*-nAChR. Seventeen of the 2a analogues had better AD50 values for blocking nicotine-induced antinociception in the tail-flick test, with analogues 2m, 2n, and 2r having the lowest AD50 values. Analogue 2x with IC50 values of 31 and 180 nM for DA and NE uptake inhibition compared to 658 and 1850 nM for 2a and an IC50 of 0.62 and 9.8 μM for antagonism of the α3β4*- and α4/2β-nAChRs, respectively, compared to 1.8 and 12 μM for 2a had the best overall in vitro profile. This compound also had an AD50 of 0.13 mg/kg in the tail-flick test compared to an AD50 of 1.2 mg/kg for 2a.

Overall, the findings support the idea that multiple molecular targets can play roles in mediating nicotine’s behavioral effects and that these new 2a analogues have potential not only as pharmacological tools to study targets and mechanisms involved but also as new pharmacotherapies with potentially higher efficacy as aids to smoking cessation.

**Experimental Section**

**Chemistry.** 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). Optical rotations were measured on an AutoPol III polarimeter, purchased from Rudolf Research. 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.

2-(N-tert-Butylamino)-3-methoxypropio phenone (2f) Fumarate. To a stirred solution of 2-bromo-3'-nitropropio phenone (7d) (400 mg, 1.5 mmol) in 3 mL of CH3CN was added tert-butylamine. The mixture was stirred for 10 h at room temperature. The reaction solution was diluted with EtOAc, washed with aqueous NaHCO3, water, and brine, and then dried over Na2SO4. The solvent was evaporated, and the residue was purified by column chromatography on silica gel using MeOH–CH2Cl2 (1:1) as the eluent to afford 138 mg (39%) of 2f. The 2f was immediately dissolved in Et2O, 1.0 equiv of fumaric acid (dissolved in minimal amount of MeOH) was added dropwise, and the mixture was stirred overnight. The solid was collected by filtration, washed with Et2O, and vacuum-dried to yield 150 mg of 2f fumarate as a white solid: mp 152–153 °C. 1H NMR (CD3OD) δ 7.58 (s, 2H), 5.37 (q, J = 7.1 Hz, 1H), 4.88 (s, 3H), 1.66 (d, J = 4.4 Hz, 3H), 1.55 (d, J = 5.7 Hz, 2H), 1.35 (s, 9H), 1.12 (s, 6H). 13C NMR (CD3OD) δ 193.6, 170.7, 161.5, 135.7, 134.5, 131.2, 122.0, 114.2, 59.0, 55.7, 54.3, 26.2, 18.5. LCMS (ESI) m/z: 236.5 (M + 1)+. Anal. (C8H12HNO4C) C, H, N.

2-(N-tert-Butylamino)-3-nitropropio phenone (2f) Fumarate. To a stirred solution of 2-bromo-3'-nitropropio phenone 7e (300 mg, 1.16 mmol) in 3 mL of CH3CN was added tert-butylamine (0.170 g, 3.0 mmol). The mixture was stirred for 6 h at 40 °C. The solution was filtered to remove the white precipitate. The solution was diluted with EtOAc, washed with aqueous NaHCO3, water, and brine, and then dried over Na2SO4. The solvent was evaporated, and the residue was purified by column chromatography on silica gel using MeOH–CH2Cl2 (1:1) as the eluent to afford 211 mg (73%) of 2f. The 2f was immediately dissolved in Et2O, 1.0 equiv of fumaric acid (dissolved in minimal amount of MeOH) was added dropwise, and the mixture was stirred overnight. The solid was collected by filtration, washed with Et2O, and vacuum-dried to give 182 mg (37%) of 2f fumarate as a white solid: mp 166–167 °C. 1H NMR (CD3OD) δ 8.94 (s, 1H), 8.59 (dd, 2H), 7.90 (t, 1H), 6.68 (s, 2H), 5.30 (q, 1H), 1.61 (d, 3H), 1.37 (s, 9H). 13C NMR (CD3OD) δ 198.5, 172.8, 152.1, 137.9, 137.5, 133.9, 131.9, 126.5, 60.9, 56.8, 29.5, 28.7, 20.6. LCMS (ESI) m/z: 252.3 (M + 1)+. Anal. (C12H16N2O4C) C, H, N.

2-(N-Cyclopentylamino)-3-fluoropropio phenone (2g) Fumarate. To a CH3CN solution (3 mL) of 2-bromo-3'-fluoropropiophene 7a (290 mg, 1.26 mmol) was added cyclopentylamine (125 μL, 1.26 mmol), and the mixture was allowed to react for 3 h at room temperature. The precipitate was filtered off. The filtrate was concentrated, and the residue was purified by column chromatography on silica gel using cyclohexane–EtOAc (2:1) with 1% NH4OH to give 50 mg (64%) of 2g fumarate, as a white solid: mp 166–167 °C. 1H NMR (CD3OD) δ 7.95 (s, 1H), 7.85 (d, 1H), 7.64 (m, 1H), 7.51 (m, 1H), 5.16 (m, 1H), 3.64 (m, 1H), 2.15 (m, 2H), 1.61–1.85 (m, 6H), 1.60 (d, 3H). 13C NMR (CD3OD) δ 196.4, 165.1, 163.2, 136.8, 136.7, 133.0, 126.6, 123.5, 123.2, 117.0, 116.7, 59.2, 58.9, 31.2, 25.2, 17.0. LCMS (ESI) m/z: 236.4 (M + 1)+. Anal. (C9H12F2NO4C) C, H, N.

2-(N-Cyclopentylamino)-3'-bromopropio phenone (2s) Fumarate. To a CH3CN solution (3 mL) of 2-bromo-3'-bromopropio phenone 7b (480 mg, 1.65 mmol) was added cyclopentylamine (330 μL, 3.31 mmol), and the mixture was allowed to react for 6 h at 40 °C. After the mixture was cooled to room temperature, the precipitate was removed by filtration. The filtrate was concentrated, and the residue was purified by column chromatography on silica gel using cyclohexane–EtOAc (2:1 to 1.0 with 1% NH4OH) as the eluent to give 182 mg (37%) of 2s as light-yellow oil. The 2s was dissolved in 50 mL of Et2O, a MeOH solution (1 mL) of fumaric acid (71 mg) was added dropwise, and the mixture was stirred overnight. The precipitate was collected, washed with Et2O, and dried under vacuum to give 240 mg (35%) of 2s fumarate as a white solid: mp 152–153 °C. 1H NMR (CD3OD) δ 8.25 (s, 1H), 8.07 (d, 1H), 7.89 (d, 1H), 7.54 (t, 1H), 6.68 (s, 2H), 5.13 (q, 1H),
added dropwise, and the mixture was stirred overnight. The precipitate was collected, washed with EtOAc, and dried under vacuum to give 180 mg (54%) of 2aa-HCl as a white solid: mp 184–185 °C. 1H NMR (CD3OD) δ 7.81 (d, 1H), 7.64 (m, 1H), 7.52 (m, 1H), 5.32 (m, 1H), 3.75 (m, 2H), 3.02 (m, 2H), 2.09–2.16 (m, 4H), 1.63 (d, 3H). 13C NMR (CD3OD) δ 196.3, 166.2, 162.9, 132.7, 132.6, 126.2, 123.3, 121.3, 116.7, 116.4, 66.9, 55.8, 53.4, 24.4, 16.8. LCMS (ESI) m/z 222.6 (M + H)+. Anal. (C13H17ClFNO3) C, H, N.

2-(N-Pyridinylidinyl)-3'-methylpropionophenone (2bb) Fumarate. To a CH3CN/H2O solution (4 mL/2 mL) of 2-bromo-3'-methylpropionophenone 7b (700 mg, 2.4 mmol) was added pyrrolidine (200 μL, 2.4 mmol), and the mixture was allowed to react for 4 h at room temperature. The reaction solution was diluted with EtOAc, washed with aqueous NaHCO3, water, and brine, and then dried over Na2SO4. The solvent was evaporated, and the residue was purified by column chromatography on silica gel using cyclohexane–EtOAc (2:1 to 1.0 with 1% NH4OH) as the eluent to give 252 mg (67%) of 2bb as a light-yellow oil. The 2bb was dissolved in 50 mL of EtOAc, a MeOH solution (1 mL) of fumaric acid (1 equiv) was added dropwise, and the mixture was stirred overnight. The precipitate was collected, washed with EtOAc, and dried under vacuum to give 2bb fumarate as a white solid: mp 134–135 °C. 1H NMR (CDCl3) δ 8.25 (s, 1H), 8.05 (d, 1H), 7.67 (d, 1H), 7.33 (t, 1H), 3.91 (q, 1H), 2.61 (m, 4H), 1.80 (m, 4H), 1.37 (d, 9H). 13C NMR (CDCl3) δ 199.7, 137.8, 135.8, 131.8, 130.1, 127.3, 122.8, 65.0, 51.1, 23.6, 15.8. LCMS (ESI) m/z 284.7 (M + H)+. Anal. (C13H17BrNO3) C, H, N.

2-(N-Pyridinylidinyl)-3'-methylpropionophenone (2cc) Fumarate. To a CH3CN–H2O solution (4 mL/2 mL) of 2-bromo-3'-methylpropionophenone 7c (400 mg, 1.76 mmol) was added pyrrolidine (150 μL, 1.76 mmol), and the mixture was allowed to react for 4 h at room temperature. The reaction solution was diluted with EtOAc, washed with aqueous NaHCO3, water, and brine, and then dried over Na2SO4. The solvent was evaporated, and the residue was purified by column chromatography on silica gel using cyclohexane–EtOAc (2:1 to 1.0 with 1% NH4OH) as the eluent to give 2cc as a light-yellow oil. The 2cc was dissolved in EtOAc, a MeOH solution (1 mL) of fumaric acid (1 equiv) was added dropwise, and the mixture was stirred overnight. The precipitate was collected, washed with EtOAc, and dried under vacuum to give 320 mg (55%) of 2cc fumarate as a white solid: mp 131–132 °C. 1H NMR (CD3OD) δ 7.86 (m, 2H), 7.57 (d, J = 7.6 Hz, 1H), 7.48 (t, J = 7.6 Hz, 1H), 6.69 (s, 2H), 5.23 (q, J = 7.1 Hz, 1H), 3.55–3.33 (m, 4H), 2.45 (s, 3H), 2.12 (m, 1H), 1.60 (d, J = 7.1 Hz, 3H). 13C NMR (CD3OD) δ 201.2, 175.0, 144.4, 140.6, 139.9, 138.2, 130.0, 70.3, 58.0, 28.0, 25.0, 20.6. LCMS (ESI) m/z 218.1 (M + H)+. Anal. (C13H15NO3) C, H, N.

2-(N-Pyridinylidinyl)-3'-methylpropionophenone (2dd) Fumarate. To a CH3CN–H2O solution (4 mL/2 mL) of 2-bromo-3'-methylpropionophenone 7d (600 mg, 2.47 mmol) was added pyrrolidine (200 μL, 2.4 mmol), and the mixture was allowed to react for 4 h at room temperature. The reaction solution was diluted with EtOAc, washed with aqueous NaHCO3, water, and brine, and then dried over Na2SO4. The solvent was evaporated, and the residue was purified by column chromatography on silica gel using cyclohexane–EtOAc (2:1 to 1.0 with 1% NH4OH) as the eluent to give 2dd as a light-yellow oil. The 2dd was dissolved in 50 mL of EtOAc, a MeOH solution (1 mL) of fumaric acid (1 equiv) was added dropwise, and the mixture was stirred overnight. The precipitate was collected, washed with EtOAc, and dried under vacuum to give 340 mg (30%) of 2dd fumarate as a white solid: mp 121–122 °C. 1H NMR (CD3OD) δ 7.67 (d, J = 7.7 Hz, 1H), 7.56 (s, 1H), 7.47 (t, J = 8.1 Hz, 1H), 7.24 (dd, J = 8.2, 2.6 Hz, 1H), 6.58 (s, 1H), 4.46 (m, 1H), 3.82 (s, 3H), 2.76 (s, 4H), 1.74 (s, 4H), 1.29 (d, J = 6.8 Hz, 3H). 13C NMR (CD3OD) δ 197.7, 165.0, 157.8, 135.0, 132.7, 128.3, 119.4, 117.8, 111.6, 61.2, 53.7, 48.6, 21.6, 12.8. LCMS (ESI) m/z 234.3 (M + H)+. Anal. (C15H15NO3) C, H, N.
2-(N-Pyridylidene)-3-nitropropiophenone (2ce) Hydrochloride. To a CH$_2$CN–H$_2$O solution (4 mL/2 mL) of 7e (310 mg, 1.2 mmol) was added pyridoline (100 µL, 1.2 mmol), and the mixture was allowed to react for 2 h at room temperature. The reaction solution was diluted with EtOAc, washed with aqueous NaHCO$_3$, water, and brine, and then dried over Na$_2$SO$_4$. The solvent was evaporated, and the residue was purified by column chromatography on silica gel using cyclohexane–EtOAc (2:1 to 1:0 with 1% NH$_3$OH) as the eluent to give 120 mg (40%) of 2ce as a light-yellow oil. The 2ce was dissolved in 50 mL of Et$_2$O, a solution of hydrochloric acid in ether was added dropwise, and the mixture was stirred overnight. The precipitate was collected, washed with Et$_2$O, and dried under vacuo to give 60 mg (18%) of 2ce-HCl as an off-white solid: mp 35–36°C. $^1$H NMR (CD$_3$OD) δ 8.86 (s, 1H), 8.59 (s, 1H), 8.50 (s, 1H), 7.91 (s, 1H), 5.50 (s, 1H), 3.96 (s, 2H), 3.40 (m, 2H), 2.14 (m, 4H), 1.70 (s, 3H). $^{13}$C NMR (CD$_3$OD) δ 196.2, 150.7, 136.8, 136.1, 132.9, 130.6, 125.3, 67.9, 56.8, 54.6, 25.3, 25.2, 17.7. LCMS (ESI) m/z: 249.2 (M + 1)$^+$. Anal. (C$_{13}$H$_{17}$ClN$_2$O$_3$·H$_2$O) C, H, N.

**General Procedure for 2-Bromo-3-Substituted Propiophenones 7a–e.** To a solution of the appropriate propiophenone 6a–e (25 mmol) in acetic acid (45 mL), bromine (25 mmol) was added dropwise. After the mixture was stirred overnight, the acetic acid was removed under vacuum, and the resulting residue was dissolved in EtOAc, washed with saturated NaHCO$_3$ solution, brine, dried (Na$_2$SO$_4$), and concentrated to give the bromoketones 7a–e. The un purified 7a–e were used to prepare the 2a analogues without further purification.

**Cell Lines and Culture.** HEK-293 cells stably expressing human DAT, NET, or SERT were maintained as previously described. Use was made of several human cell lines that naturally or heterologously express specific, functional, human nAChR subtypes. Cells of the TE671/RD line naturally expresses muscle-type nAChR (αβ1β2δ) and α1*·nAChR, and SH-SY5Y neuroblastoma cells naturally expresses autonomic α3β4*·nAChRs (containing α3, β4, probably α5, and sometimes β2 subunits). Different clones of SH-EP1 epithelial cell line have been engineered to heterologously express either α4β2·nAChR, which is thought to be the most abundant, high affinity nicotine-binding nAChR in mammalian brain, or α4β4·nAChR, another possible brain nAChR subtype (SH-EP1-hα4β2 or α4β4 cells, respectively). These cells were maintained as low passage number (1–26 from our frozen stocks) cultures to ensure stable expression of native or heterologously expressed nAChR as previously described. Cells were passaged once weekly by splitting just-confluent cultures 1:300 (TE671/RD), 1/5 (SH-SY5Y), or 1/20 (transfected SH-EP1) in serum-supplemented medium to maintain log-phase growth.

**Transporter Assays.** The abilities of 2a and its analogues to inhibit uptake of [H]dopamine ([H]DA), [H]serotonin ([H]5-HT), or [H]norepinephrine ([H]NE) by the respective human transporters were evaluated using the appropriate HEK-293 cell line as previously reported. nAChR Functional Assays. Cells were harvested at confluence from 100 mm plates by mild trypsinization (Irvine Scientific, Santa Ana, CA) and triturated or (for SH-SY5Y cells) by trituration alone before being suspended in complete medium and evenly seeded at a density of 1.25–2 confluent 100 mm plates per 24-well plate (Falcon; ~100–125 µg of total cell protein per well in a 500 µL volume). After cells had adhered (generally overnight but no sooner than 4 h later), the medium was removed and replaced with 250 µL per well of complete medium supplemented with ~350000 cpm of $^{86}$Rb$^+$ (PerkinElmer Life and Analytical Sciences, Boston, MA) and counted at 40% efficiency using Cerenkov counting (TriCarb 1900 liquid scintillation analyzer, 59% efficiency; PerkinElmer Life Sciences). After at least 4 h and typically overnight, $^{86}$Rb$^+$ efflux was measured using the “flip-plate” technique. Briefly, after aspiration of the bulk of $^{86}$Rb$^+$ loading medium from each well of the “cell plate”, each well containing cells was rinsed 3× with 2 mL of fresh $^{86}$Rb$^+$ efflux buffer (130 mM NaCl, 5.4 mM KCl, 2 mM CaCl$_2$, 5 mM glucose, 50 mM HEPES, pH 7.4) to remove extracellular $^{86}$Rb$^+$. Following removal of residual rinse buffer by aspiration, the flip-plate technique was used again to simultaneously introduce 1.5 mL of fresh efflux buffer containing drugs of choice at indicated final concentrations from a 24-well “efflux/drug plate” into the wells of the cell plate. After a 5 min incubation, the solution was “flipped” back into the efflux/drug plate, and any remaining buffer in the cell plate was removed by aspiration. Cells remaining in the cell plate were lysed and suspended by addition of 1.5 mL of 0.1 M NaOH, 0.1% sodium dodecyl sulfate to each well. Suspensions in each well were then subjected to Cerenkov counting (Wallac Micobeta Trilux 1450; 25% efficiency) after placement of inserts (Wallac 1450–109) into each well to minimize cross-talk between wells.

For quality control and normalization purposes, the sum of $^{86}$Rb$^+$ in cell plates and efflux/drug plates was defined to confirm material balance (i.e., that the sum of $^{86}$Rb$^+$ released into the efflux/drug plates and $^{86}$Rb$^+$ remaining in the cell plate was the same for each well). This ensured that $^{86}$Rb$^+$ efflux was the same whether measured in absolute terms or as a percentage of loaded $^{86}$Rb$^+$. Similarly, the sum of $^{36}$Rb$^+$ in cell plates and efflux/drug plates also determined the efficiency of $^{86}$Rb$^+$ loading (the percentage of applied $^{86}$Rb$^+$ actually loaded into cells).

Control, total $^{86}$Rb$^+$ efflux was assessed in the presence of only a fully efficacious concentration of carbamylcholine (1 mM for SH-EP1-hα4β2, SH-EP1-hα4β4/3 cells or TE671/RD cells; 3 mM for SH-SY5Y cells). Control, nonspecific $^{86}$Rb$^+$ efflux was measured either in the presence of the full efficacious concentration of carbamylcholine plus 10 µM mecamylamine, which gave full block of agonist-induced and spontaneous nAChR-mediated ion flux, or in the presence of efflux buffer alone. Either determination of nonspecific efflux was equivalent. Specific efflux was then taken as the difference in control samples between total and nonspecific $^{86}$Rb$^+$ efflux. Any intrinsic agonist activity of test drugs was ascertained using samples containing test drug only at different concentrations and was normalized, after subtraction of nonspecific efflux, to specific efflux in test drug-free, control samples.

Antagonism of carbamylcholine-evoked $^{86}$Rb$^+$ efflux was assessed in samples containing the full agonist at a concentration where it stimulates 80–90% of maximal function (i.e., its EC$_{80}$–EC$_{50}$ value) when exposed alone to a given nAChR subtype (i.e., 460 µM for TE671/RD cells, 200 µM for SH-SY5Y cells, 200 µM for SH-EP1-hα4β2 or α4β4 cells and test drugs, and 200 µM for SH-SY5Y cells). antagonists were assayed using samples containing test drug only at different concentrations and was normalized, after subtraction of nonspecific efflux, to specific efflux in control samples. 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 a concentration of the test ligand close to its IC$_{50}$ value for inhibition of nAChR function. In other studies, cells were pre-exposed to analogues for 1 h (over the last hour of $^{86}$Rb$^+$ loading) or 1 day (with $^{86}$Rb$^+$ loading occurring during the final 4 h of drug pretreatment) before effects on nAChR function were assessed and was normalized (during extracellular $^{86}$Rb$^+$ removal) or in the continued presence of drug.

Ion flux assay results were fitted using Prism (GraphPad) to the Hill equation, $F = F_{	ext{max}}/(1 + (X/Z)^{n})$, where $F$ is the test sample specific ion flux as a percentage of control, $F_{\text{max}}$ is specific ion flux in the absence of test drug (i.e., for control samples), $X$ is the test ligand concentration, $Z$ is the EC$_{50}$ (n > 0 for agonists) or IC$_{50}$ (n < 0 for antagonists), and $n$ is the Hill coefficient. All concentration–ion flux response curves were simple and fit well, allowing maximum and minimum ion flux values to be determined by curve fitting, but in cases where antagonists had weak functional potency, minimum ion flux was set at 0% of control. Note that because agonist concentrations used for test ligand antagonism assessments were EC$_{50}$–EC$_{90}$ values, not all of the data, even at the lowest concentrations of test antagonist, approached 100% of specific efflux, as separated determined in...
sister samples exposed to fully efficacious concentrations of agonist.

**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 analgesic 5 min after subcutaneous administration at 2.5 mg/kg; nicotine administration 15 min after exposure to saline of 2a analogue to assess the latter drug’s ability to block nicotine-mediated antinociception). Antinociceptive response was calculated as the percentage of maximum possible effect (%MPE), where %MPE = [(test control)/(10 control)] × 100.

**Hot-Plate Test.** Mice were placed into a 10 cm wide glass cylinder on a hot plate (Thermojust Apparatus) maintained at 55 °C for assessment of pain responses mediated at supraspinal levels. To minimize tissue damage, a maximum exposure to the hot plate of 40 s was imposed. Measures of control latencies (time until the animal jumped or licked its paws; typically 8–12 s) were done twice for stimuli applied at least 10 min apart for each mouse. Antinociceptive responses after test drug administrations were determined and calculated as the %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 2a metabolite 15 min before nicotine. The animals were then tested 5 min after administration of a subcutaneous dose of 2.5 mg/kg nicotine. The difference in tail withdrawal latency 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 2a metabolite 15 min before nicotine. The animals were then tested 30 min after administration of a subcutaneous dose of 2.5 mg/kg nicotine.

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**Supporting Information Available:** Results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

**References**


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