2-(2-Piperidyl)- and 2-(2-Pyrrolidyl)chromans as Nicotine Agonists: Synthesis and Preliminary Pharmacological Characterization

Simon M. N. Efange,*,† Zhude Tu,† Krystyna von Hohenberg,† Lynn Francesconi,‡ Robertha C. Howell,‡ Marilyn V. Rampersad,‡ Louis J. Todaro,‡ Roger L. Papke,§ and Mei-Ping Kung||

Departments of Radiology, Medicinal Chemistry, and Neurosurgery, and Graduate Program in Neuroscience, University of Minnesota, Minneapolis, Minnesota 55455, Department of Chemistry, Hunter College, City University of New York, New York, New York 10021, Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, Florida 32610, and Department of Radiology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

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As part of an effort to develop a new class of subtype selective nicotine agonists, we have synthesized and tested a group of 12 hydroxylated 2-(2-piperidyl)- and 2-(2-pyrrolidyl)chromans. In rat brain membranes, all 12 compounds displayed poor affinity for [^{125}I]-α-bunagarotoxin binding sites. In contrast, three compounds, **17c**, **24**, and **26**, displayed moderate to high affinity for [^{3}H]cytisine binding sites, while three (**17b**, **18b**,**c**) and six (**17a**,**d**,**e** and **18a**,**d**,**e**) compounds showed weak and poor affinity, respectively, for these same sites. In subsequent studies, compounds **17a** and **17c** were found to stimulate the efflux of $^{86}Rb^{+}$ from rat cortical synaptosomes, an indication of agonist activity. Further, both **17c** and **26** displayed high intrinsic activity in stimulating the release of [^{3}H]dopamine from striatal synaptosomes; however, only **17c** was effective at stimulating the release of [^{3}H]acetylcholine from cortical synaptosomes, suggesting differential selectivity. In cloned human nicotinic acetylcholine receptors (nAChR) expressed in *Xenopus* oocytes, both **17c** and **26** activated α7 and α3β2 receptor subtypes in a dose-dependent manner, but **26** was clearly the more potent agonist. Last, neither compound displayed dose-dependent activation of α4β2 nAChRs. We conclude that 2-(2-azacyclic)chromans appear to be a promising new class of nicotine agonists.

Introduction

The actions of the neurotransmitter acetylcholine (ACh) are mediated by two major classes of neuroreceptors, muscarinic and nicotinic. The classification is based on the effects of the prototypical cholinergic agonists nicotine (**1**) and muscarine (**2**) (reviewed in ref 1). Stimulation of muscarinic receptors results in G-protein-mediated signal transduction, while nicotinic receptor stimulation is linked to activation of ion channels. Nicotine, the prototypical nicotinic acetylcholine receptor (nAChR) ligand, elicits a wide range of cellular and pharmacological effects including (1) stimulation of neurotransmitter release, (2) modulation of cardiovascular, endocrine, and metabolic function, and (3) cognition-enhancing, neuroprotective, anxiolytic, and analgesic activities.[2,3] Although many of its pharmacological properties are beneficial to human health, the clinical utility of nicotine is limited by its cardiovascular (such as hypertension, tachycardia, and peripheral vasoconstriction), gastrointestinal (nausea, abdominal pain), and neuromuscular side effects and addictive liability. Consequently, there is a need to develop nicotine-like compounds that exhibit the beneficial properties of **1** but are devoid of its undesirable effects.

Recent advances in molecular biology suggest that the multiple actions of **1** can be attributed to the existence of multiple nicotinic receptor subtypes.[4−6] The discovery of specific agonist or antagonist therapies that are based on selective modulation of nAChR subtypes may therefore result in new and potentially useful therapeutic agents. Mammalian nicotinic receptors belong to a class of pentameric ligand-gated ion channels. In the rat brain, at least eight α (α_2−α_9) and three β (β_2−β_4) subunits have been cloned. Chick and human nAChR subunit genes have also been cloned.[4−6] Corresponding rat, human, and chick nAChR genes exhibit a high degree of sequence homology. This diversity of subunits provides for a multitude of potential combinations, and thus a large pool of nAChR subtypes. Although the subunit composition of native nAChR is poorly understood, at least eight nAChR subtypes have been identified in heterologous expression systems.[6] Moreover, many of these display pharmacological and physiological properties that are similar to the native receptors found in the CNS. Both α and β subunits are widely distributed in the mammalian brain. However, each nAChR subtype exhibits a distinct anatomical distribution in the brain.

The large number of potential nAChR subtypes has fueled interest in the development of subtype-selective nicotine agonists and antagonists. Compounds developed as part of this effort include the nicotine agonists **3**,[7] **4**,[8] **5**,[9] **6**,[10] **7**,[11] **8**,[12] and **9**[13] (Chart 1). Herein we describe yet another class of nicotine agonists.

Two of the most interesting compounds reported above, **6** and **7**, are flexible pyridyl alkyl ethers, and the

* To whom correspondence should be addressed. Address: Department of Radiology (MMC 292), University of Minnesota, Academic Health Center, 420 Delaware Street, S.E., Minneapolis, MN 55455. Phone: (612) 626-6646. Fax: (612) 626-1951. E-mail: efang001@tc.umn.edu.
† University of Minnesota.
‡ Hunter College, City University of New York.
§ University of Florida College of Medicine.
|| University of Pennsylvania.

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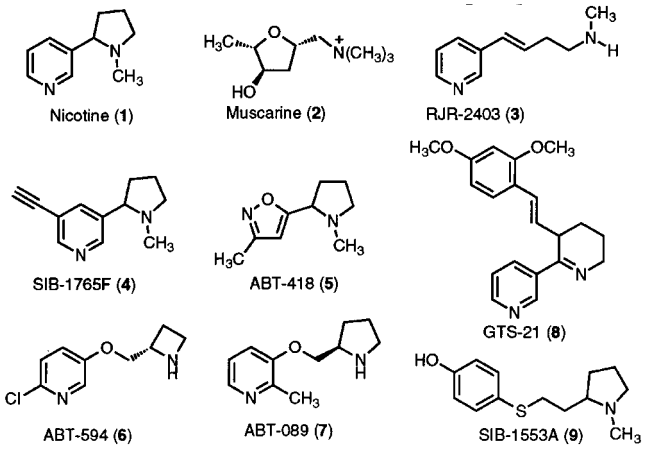
Chart 1. Naturally Occurring and Synthetic Cholinergic Agonists



third, **9**, is a phenol carrying a flexible side chain. Compound **6** is a potent inhibitor of [^{3}H]nicotine binding to the neuronal $\alpha_4\beta_2$ nAChR subtype in rat brain (K_i = 37 pM) and in cells expressing the human receptor (K_i = 55 pM).[14] The compound displays 28 000- and 180 000-fold lower affinity for the human α_7 nAChR and the neuromuscular subtype labeled by [^{125}I]-α-bungarotoxin, respectively. In functional assays, **6** stimulated $^{86}Rb^+$ efflux in kidneys cells transfected with human $\alpha_4\beta_2$ receptors and in cells expressing the sympathetic ganglion-like nAChR subtype.[14] However, the compound was more potent at $\alpha_4\beta_2$ receptors than at ganglionic nAChRs, suggesting a potential for reduced side-effect liability relative to **1**. Compared to **6**, compound **7** is about 300-fold less potent as an inhibitor of [^{3}H]nicotine binding to the $\alpha_4\beta_2$ nAChR subtype.[15] However, **7** displays equally poor affinity for the α_7 neuronal subtype and ganglionic nAChRs. Therefore, both **6** and **7** display a high degree of subtype selectivity despite their obvious flexibility. In our search for subtype-selective nAChR ligands, these compounds provided the inspiration for the design of a class of nicotine agonists, which is the subject of this manuscript.

Results and Discussion

Among nicotine agonists and antagonists, the pyridyl alkyl ethers, represented herein by **6** and **7**, are distinguished by their structural simplicity and the presence of a flexible oxymethylene bridge that separates the pyridyl and aminocycloalkyl fragments. In an attempt to develop a class of nicotine agonists that incorporate the latter structural attribute, we chose to constrain the oxymethylene bridge within a bicyclic structure (see structure **A** in Chart 2). Because compounds of type **A** are conformationally restrained, it was expected that their subtype selectivity profiles would be different from those of **6** and **7**. In an effort to expand the investigation into new chemotypes, we also chose to replace the pyrido fragment in **A** with a benzo fragment, thus resulting in compounds of the type **B** (Chart 2). Precedent for the latter substitution was provided by the work of Elliott et al.[16] on phenylpyrrolidines as simplified analogues of the nicotine antagonist erysodine (**10**) (Chart 2) and by the relatively high subtype selectivity displayed by **9**.[13] In the present manuscript, we describe the results of our initial investigation of 2-(2-piperidyl)- and 2-(2-pyrrolidyl)chromans, derived from structure **B** (Chart 2), as nicotine agonists.

Chemistry

The synthesis of the target 2-(2-piperidinyl)chromans was elaborated as shown in Scheme 1. The reaction of a substituted anisaldehyde with 2-acetylpyridine provided the chalcones **12a**−**e** in yields of 73−94%. Reduction of the latter was accomplished in two steps to yield the substituted propyl alcohols **14a**−**e**, which were smoothly demethylated with BBr_3 (91−98%) to provide the corresponding phenols **15a**−**e**. Intramolecular cyclization via the Mitsunobu reaction produced the hydroxylated 2-(2-pyridyl)chromans **16a**−**e**. N-methylation of the latter was followed by stepwise reduction to provide the diastereomeric mixtures of **17a**−**e** and **18**−**e**. Overall yields for the sequence ranged between 12% and 34%. Yields have not been optimized. With one exception, **17b**/**18b**, the more abundant member of each diastereomeric pair was the erythro isomer. The ratio of erythro to threo ranged from 2:1 to 5:1.

The synthesis of the 2-(2-pyrrolidyl)chromans **24** and **26** (Scheme 2) began with the previously reported **19**.[17] Condensation of the latter with 2,4-dimethoxyanisaldehyde provided **20** in 69% yield. Treatment of the latter with $LiAlH_4$ resulted in complete reduction of the acryloyl moiety, thus yielding a mixture of two diastereomers, **21** and **22**, in approximately equal amounts. Chromatographic separation of the diastereomers followed by intramolecular cyclization via the Mitsunobu reaction provided the target compounds **24** and **26** in yields of 31% and 60%, respectively. An alternative route (Scheme 3) was provided to give an equimolar mixture of **21** and **22** in a combined yield of 39% from 2-acetylpyrrole.

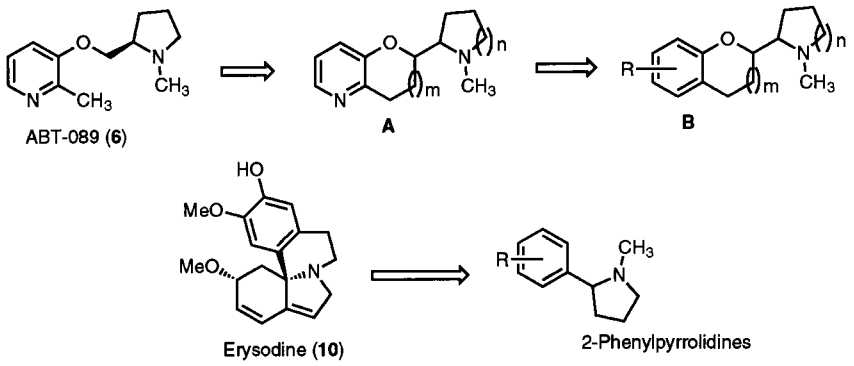
X-ray Crystallography

Unequivocal determination of the structures of **17c** and **24** was accomplished by X-ray diffraction studies. Compound **17c** was found to be the threo isomer of the **17c**/**18c** pair, while **24** was shown to be the erythro isomer of the **24**/**26** pair. Since **24** is erythro, compound **26** is then assigned the threo configuration, and the relative stereochemistry of C2−C2′ positions in the precursors **21** and **22** can, in turn, be inferred from the mechanism of the Mitsunobu reaction.[18]

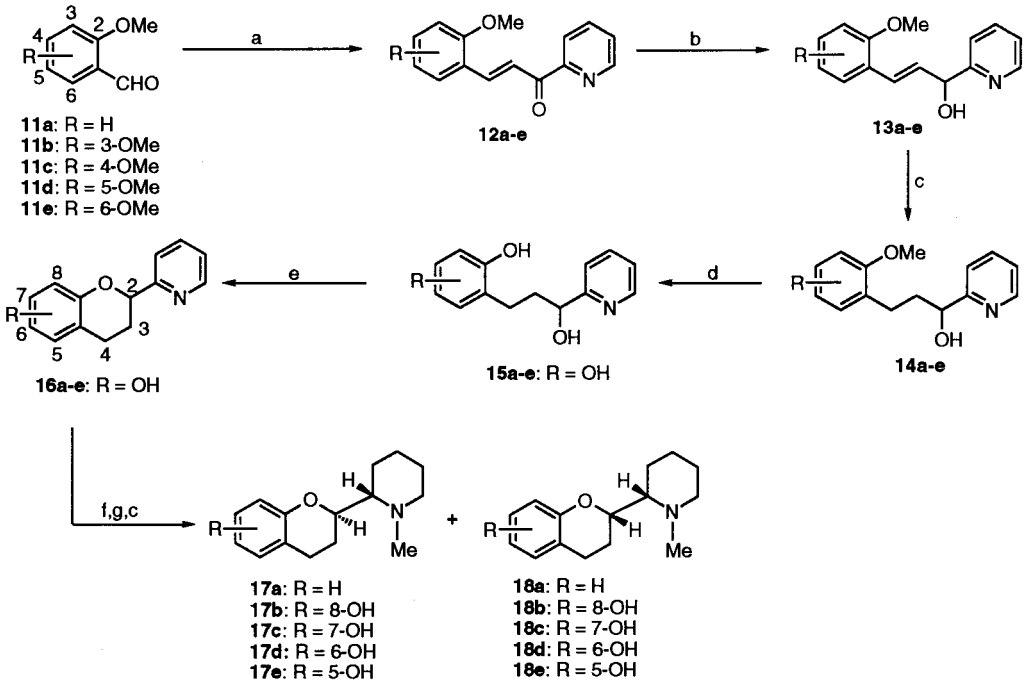
Pharmacology

1. In Vitro Binding Studies. 1.1. Nicotinic Receptors. In vitro binding studies were performed with [^{3}H]cytisine and [^{125}I]-α-bungarotoxin according to published procedures.[19] These two ligands bind preferentially to α4β2 and α7 nAChR subtypes, respectively. The relative affinities of the test compounds for the two binding sites are provided in Table 1. The unsubstituted threo isomer **17a** displayed only moderate affinity for the [^{3}H]cytisine binding site. In contrast, the erythro isomer **18a** was found to exhibit poor affinity for this binding site. The introduction of a hydroxyl group at the C8 position of the chroman skeleton led to a 4-fold increase in affinity (compare **17a** vs **17b**), while a C7

Chart 2. Design of Conformationally Restricted Cholinergic Agonists



Scheme 1. Synthesis of 2-(2-Piperidyl)chromans



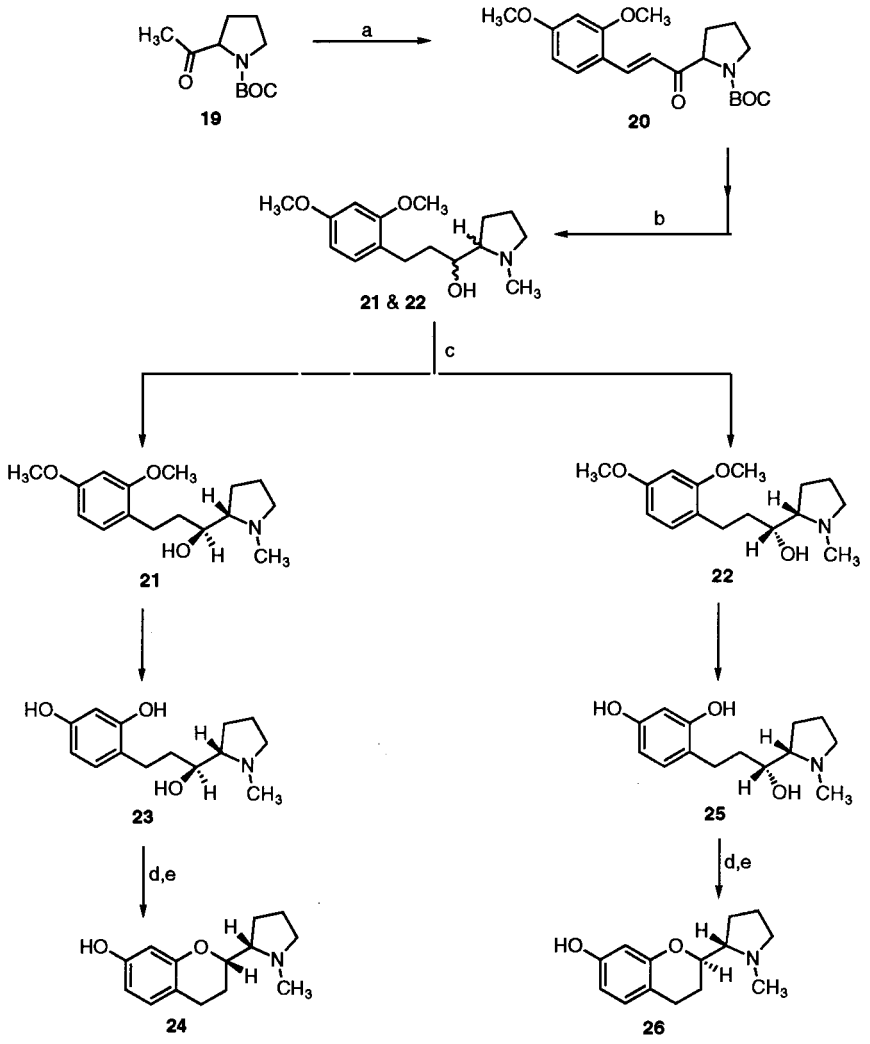
a: 2-Acetylpyridine, KOH, EtOH; b: $NaBH_4$, MeOH; c: H_2/Pd-C; d: BBr_3, CH_2Cl_2; e: DEAD, Ph_3P, THF; f: MeI, acetone; g: $NaBH_4$, MeOH; h: ACE-Cl, dichloroethane, reflux.

hydroxyl group increased affinity for the [^{3}H]cytisine binding site almost 70-fold (**17a** vs **17c**). On the other hand, moving the hydroxyl group to C5 (**17e**) or C6 (**17d**) proved to be unfavorable because the resulting compounds displayed poor affinity for the [^{3}H]nicotine binding site. Compound **17c** thus emerged as the most potent piperidine analogue tested. When this substitution pattern (C7-hydroxy) was extended to the 2-(2-pyrrolidyl)chromans, the affinity increased 4- to 9-fold over the corresponding piperidines (compare **17c** vs **26** and **18c** vs **24**). Abood et al.[20] report that nicotine displays a 10-fold higher affinity for nAChRs than anabasine. Therefore, we conclude that with respect to the aminocycloalkyl fragment the effects of ring size are similar for both the aminocycloalkyl-substituted pyridines (of which nicotine and anabasine are members) and the corresponding chromans.

In a previous study of 2-phenylpyrrolidines as simplified analogues of the naturally occurring nicotinic antagonist erysodine, Elliott et al.[16] found that 1-methyl-2-phenylpyrrolidine itself displayed only moderate to weak affinity for nicotinic receptors. However, the presence of certain substituents on the phenyl group (such as 3-bromo, 4-chloro, 4-fluoro, 3,4-dichloro, and 3,4-methylenedioxy) significantly increased affinity for nicotinic receptors, leading the authors to suggest that these substituted phenyl groups are bioisosteres of the 3-pyridyl fragment in nicotine. In the present study, we have extended these earlier observations by showing that a non-nitrogenous bicyclic fragment can also replace the 3-pyridyl fragment of nicotine.

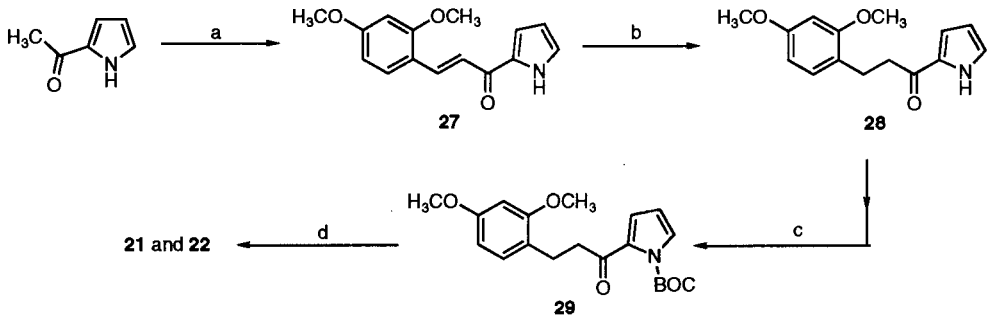
In two out of five diastereomeric pairs within the piperidine series, the threo isomer displayed 10- to 17-fold higher affinity for the [^{3}H]cytisine binding site than the corresponding erythro isomer (**17a** vs **18a** and **17c** vs **18c**). However, with a hydroxyl group at C8, both isomers displayed comparable affinity (**17b** vs **18b**). Because the disparity between threo and erythro seems to be greatest with the unsubstituted compounds (**17a** and **18a**) and the C7-hydroxy analogues (**17c** and **18c**), we suggest that substituents at the latter position may be involved in a critical interaction, possibly involving H-bonding, with protein residues at the ligand binding site. The presence of a H-bond acceptor is consistent

Scheme 2. Synthesis of *threo*- (**24**) and *erythro*-7-Hydroxy-2-(2-pyrrolidyl)chroman (**26**)



a: 2,4-dimethoxybenzaldehyde, 10% aq. NaOH, EtOH; b: $LiAlH_4$, THF, reflux;
c: chromatography (silica gel); d: i. BBr_3, CH_2Cl_2, -40 C, ii. EtONa, EtOH;
e: PPh_3, DEAD, dioxane-DMF.

Scheme 3. Alternative Synthesis of **24** and **26**



a: 2,4-dimethoxybenzaldehyde, 10% aq. NaOH, EtOH; b: 10% Pt-C, H2, MeOH;
c: Di-tert-butyldicarbonate, t-BuOK, THF, reflux; d: $LiAlH_4$, THF, reflux.

with the pharmacophore model originally proposed by Beers and Reich[21] and subsequently extended by Sheridan et al.[22] With respect to the discrimination between threo and erythro isomers, we have observed from molecular models of **17a** and **18a** (data not shown) and from X-ray crystallographic studies that the threo isomer is an extended structure while the erythro isomer is folded. Since the threo isomers generally display higher affinity, we therefore conclude that the extended structure is optimum for binding.

In the nicotine series, substitution at the C5 position has been found to result in analogues with altered subtype selectivity.[13] Moreover, when the pyridyl group of **7** is replaced with a phenyl moiety, the presence of electron-withdrawing groups at the meta position (of the phenyl group) increases affinity for [^{3}H]nicotine binding

Table 1. Relative Affinities of Piperidyl- and Pyrrolidylchromans for [^{3}H]Cytisine and [^{125}I]-α-Bungarotoxin Binding Sites

compound	K_i, [^{3}H]cytisine (nM)	K_i, [^{125}I]-α-BT (nM)
(±)-**17a**	6200 ± 450	>10000
(±)-**17b**	1620 ± 1100	*c*
(±)-**17c**	93.4 ± 30	
(±)-**17d**	>10000	>10000
(±)-**17e**	>5000	*c*
(±)-**18a**	61800 ± 5010	>10000
(±)-**18b**	1660 ± 1450	*c*
(±)-**18c**	1560 ± 250	>10000
(±)-**18d**	>10000	>10000
(±)-**18e**	>5000	*c*
(±)-**24**	170 ± 36	*c*
(±)-**26**	21.3 ± 9.4	*c*
cytisine	2.78 ± 0.58 (0.9 ± 0.1)[a]	*c*
α-bungarotoxin	NT	14
S-(−)-nicotine	1.0 ± 0.02[b]	4000 ± 890[b]
(*S*)-**7**	17 ± 2[b]	>10000[b]
(*R*)-**7**	39 ± 4[b]	>10000[b]

[a] Assay performed at 4 °C (ref 19). [b] Reference 15. [c] Not tested.

sites.[23] The C7 position of the 2-piperidylchromans may therefore provide fertile ground for additional structure modification.

None of the compounds tested were found to display even moderate affinity for the [^{125}I]-α-bungarotoxin binding site.

1.2. Dopamine Receptors and Monoamine Transporters. The 2-(2-piperidyl)chromans were screened for binding to other receptors because structurally related compounds such as the tetralins 2-dipropylamino-7-hydroxy- and 2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene (7-OH-DPAT and 8-OH-DPAT)[24,25] and 2-(aminomethyl)chromans[26] display high affinity for dopamine and serotonin receptors. Six compounds, **17a**, **18a**, **17c**, **18c**, **24**, and **26**, were screened for binding to dopamine D2 receptors and to dopamine and serotonin transporters, using published procedures.[27−30] The first four compounds displayed weak affinity (K_i > 5 μM) for these targets (data not shown). In addition, **24** and **26** failed to inhibit radioligand binding even at concentrations as high as 3 μM. Therefore, these piperidyl and pyrrolidylchromans do not appear to bind to dopamine receptors or monoamine transporters.

2. In Vitro Functional Assays. 2.1. Stimulation of Rubidium-86 Release in Rat Cortex. Drug-induced release of $^{86}Rb^+$ from rat cortical synaptosomes was determined by published procedures.[31] This assay has been used to assess the ability nicotine agonists to modulate ion fluxes across the plasma membrane. *S*-(−)-Nicotine and compound **17c** were studied at four concentrations (1, 5, 10, and 100 μM), while compound **17a** was studied at 10 and 100 μM because of its lower affinity for [^{3}H]cytisine binding sites (vide supra). At the concentrations studied, *S*-(−)-**1** and racemic **17c** displayed comparable potency in their ability to stimulate the release of $^{86}Rb^+$ from rat cortical synaptosomes (Figure 1). In addition, the relative efficacy of racemic **17c** was comparable to that of *S*-(−)-**1**. Consistent with its reduced affinity for [^{3}H]cytisine binding sites, **17a** displayed lower potency in this assay. However, **17a** could match the peak response of *S*-(−)-**1** when tested at 100 μM. We conclude that **17c** and the congeneric 2-(2-piperidyl)chromans are effective activators of ion channels.

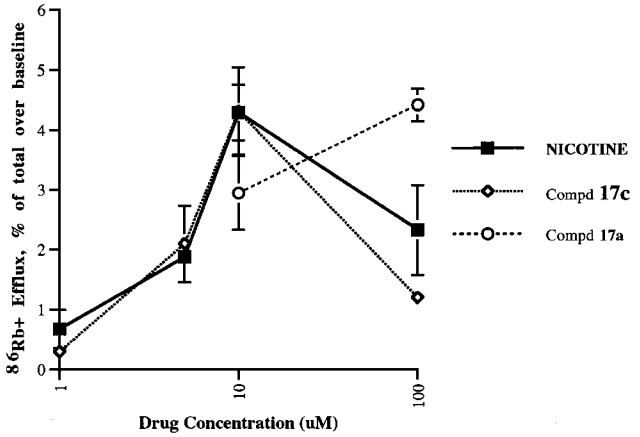


Figure 1. Drug-induced release of $^{86}Rb^+$ from rat cortical synaptosomes.

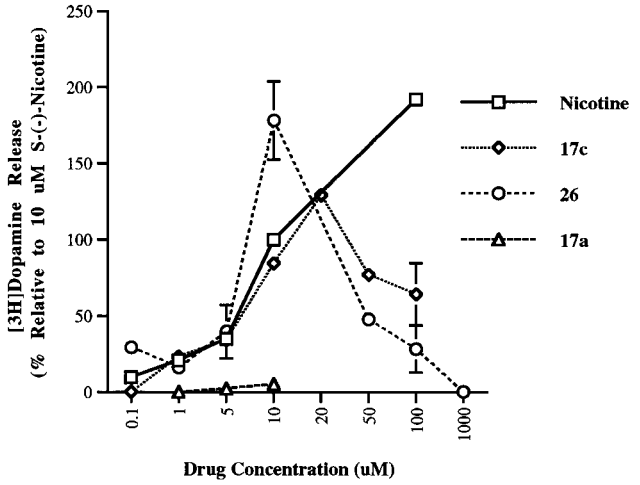


Figure 2. Agonist-induced release of [^{3}H]dopamine from rat striatal synaptosomes.

2.2. Stimulation of Dopamine Release in Rat Striatum. The ability of **17a**, **17c**, and **26** to evoke the release of [^{3}H]DA from striatal synaptosomes was assayed according to published procedures.[15] The viability of the synaptosomal preparation was confirmed by measuring K^+-evoked release of [^{3}H]DA. This was followed by measurement of nicotine-induced neurotransmitter efflux. The test compounds were evaluated at the end. Consistent with previous reports, nicotine-induced release of [^{3}H]DA was dose-dependent. Compound **17a** failed to stimulate the release of [^{3}H]DA when tested at doses up to 10 μM (Figure 2). On the other hand, **17c** displayed dose-dependent stimulation of [^{3}H]DA release. The compound was equipotent with *S*-(−)-**1** at 1, 5, and 10 μM. Higher doses of **17c** were less effective, suggesting that peak effects are obtained at or near 10 μM. Indeed, at doses greater than 20 μM, **17c** suppresses K^+-evoked release of [^{3}H]DA. Compound **26** also stimulates the release of [^{3}H]DA. Moreover, the compound appears to be more potent and more efficacious than both nicotine and **17a**. We conclude that both **17c** and **26** behave as nicotine agonists in this assay.

2.3. Stimulation of [^{3}H]Acetylcholine Release in Rat Cortex. Stimulation of [^{3}H]ACh release from rat cortical synaptosomes was assayed according to published methods.[15] These studies showed that **17c** stimulates release of [^{3}H]ACh (Figure 3) and that the compound may be as potent as nicotine in this

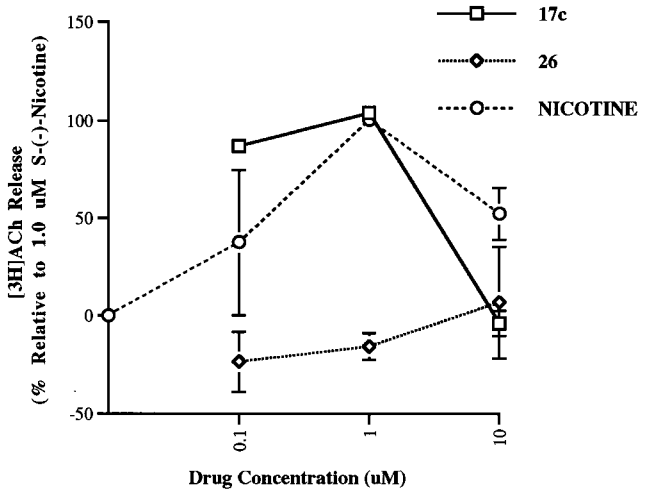


Figure 3. Drug-induced release of [^{3}H]acetylcholine from rat cortical synaptosomes.

assay. In contrast, **26** displays negligible activity in this assay. Therefore, compound **17c** appears to be an effective agonist while **26** may well be an antagonist in this assay.

2.4. Stimulation/Inhibition of Cloned Human Nicotinic Acetylcholine Receptors. To further characterize this class of compounds, the pharmacological properties of **17c** and **26** were evaluated on three human nAChRs (hnAChRs), α4β2, α3β2, and α7, expressed in *Xenopus* oocytes. The results are summarized in Table 2. Membrane currents were measured as described previously.[32] Responses evoked by test compounds are normalized to those evoked by the following ACh control doses in the same cells: 30 μM for α4β2 and α3β2; 300 μM for α7. These values correspond to EC_{30}, EC_{15}, and EC_{50}, respectively, for ACh on these receptors. To probe for residual inhibitory effects, both activation and recovery data were collected. Activation effects were assessed by measuring the response stimulated by the application of the test compound. Recovery effects were assayed by washing for 5 min, applying ACh, and measuring the response this compound. Both **17c** and **26** displayed dose-dependent activation of α3β2 and α7 nAChRs; however, in each case **26** was more potent than **17c**. While neither compound showed inhibitory aftereffects at α3β2 receptors, **26** was clearly more effective at causing residual inhibition at the α7 subtype. Indeed, following the application of 100 μM **26** to α7 nAChRs, profound inhibition of subsequent ACh responses was observed. Neither compound displayed dose-dependent activation of α4β2 nAChRs, although **26** evoked a stronger response than **17c**. Therefore, the latter is a less effective activator at all subtypes but with proportionately fewer inhibitory aftereffects.

3. Comparative Pharmacological Profiles of *S*-(−)-Nicotine, Compound 7, and Chroman-Based Agonists 17 and 26. Table 3 compares the pharmacological profiles of *S*-(−)-**1**, (*S*)-**7**, (±)-**17c**, and (±)-**26**. All four compounds display poor affinity for the [^{125}I]-α-bungarotoxin binding site but moderate to high affinity for the [^{3}H]cytisine site. Racemic **26** is particularly noteworthy because it displays affinity comparable with that of (*S*)-**7**. Optical resolution of the former might even increase the affinity beyond that of (*S*)-**7**, suggesting that conformationally restrained benzenoid structures of this kind are recognized by nAChRs. The results of three functional assays also suggest significant qualitative differences. While both **17c** and **26** markedly enhance the release of [^{3}H]dopamine from rat striatal synaptosomes, (*S*)-**7** was notably lacking in potency in this assay.[15] On the other hand, both (*S*)-**7** and **17c** stimulate the release of [^{3}H]ACh from cortical synaptosomes while **26** displays low efficacy and potency. In this respect, the latter also differs significantly from *S*-(−)-nicotine.

Nicotinic ACh receptor-mediated release of [^{3}H]ACh, [^{3}H]dopamine, and $^{86}Rb^+$ from rodent brain preparations is presumed to reflect activation of various nAChR subtypes acting alone or in combination.[31,33−36] Consequently, disparities in pharmacological profiles such as those observed here can be reasonably attributed to differential activation of nAChR subtypes. Our radioligand binding data, which are only for two subtypes, therefore provide insufficient information regarding the subtype selectivity of these compounds. However, the functional assays provide a window into the pharmacological profiles of **17c** (chromaperidine) and **26** (chromaproline).

Nicotine-stimulated striatal dopamine release is presumed to be mediated by at least two nAChR subtypes, α3β2 and α4β2*.[33−36] On the other hand, nicotine-evoked ACh release is attributed to the activation of α4β2 nAChRs.[31] Since **17c** displays comparable efficacy in both the [^{3}H]ACh and [^{3}H]dopamine release assays, it is reasonable to conclude that this compound activates both α4β2 and α3β2 nAChRs. On the other hand, **26**, despite its higher affinity for α4β2 nAChRs (relative to **17c**), fails to evoke cortical [^{3}H]ACh release but produces robust stimulation of striatal [^{3}H]dopamine release. This would suggest that **26** selectively activates α3β2 nAChRs. Support for this view is provided by the observation that **26** activates human α3β2 receptors expressed in *Xenopus* oocytes but appears to inhibit α4β2 receptors expressed in the same cells. The combination of conformational restriction and isosteric replacement embodied in this class of substituted chromans is, therefore, accompanied by changes in subtype selectivity that distinguish the chromans from the flexible pyridyl alkyl ethers that inspired their design. Given the pharmacological profiles describe herein, we suggest that the 2-aminocycloalkyl-substituted chromans represent a new class of nAChR agonists that deserve further investigation.

Experimental Section

1. Materials. Synthetic intermediates were purchased from Aldrich Chemical Co. (Milwaukee, WI) and were used as received. Tetrahydrofuran (THF) was distilled from sodium hydride immediately prior to use. Dimethylacetamide and toluene were distilled from sodium shortly before use. *p*-Dioxane was dried over potassium hydroxide. All other reagents and solvents were purchased as reagent grade and used without further purification.

2. General. All air-sensitive reactions were carried out under nitrogen. Standard handling techniques for air-sensitive materials were employed throughout this study. Yields were not optimized. Melting points were determined on a Haake-Buchler melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a 300 MHz GE spectrometer. NMR spectra are referenced to the deuterium lock frequency of the spectrometer. With this condition, the chemical shifts (in ppm) of residual solvents are observed at 7.26 ($CHCl_3$), 4.78 (CD_3OH). Spectral peaks are described by the following abbrevia-

Table 2. Activation and Recovery of Cloned Human nAChRs after Treatment with Hydroxylated Chromans

compound concentration (μM)	h$\alpha4\beta2$		h$\alpha3\beta2$		h$\alpha7$	
	activation[a]	recovery[a]	activation[a]	recovery[a]	activation[a]	recovery[a]
17c						
10	0.054 ± 0.016	0.931 ± 0.031	0.134 ± 0.028	0.944 ± 0.038	0.199 ± 0.018	1.192 ±0.214
100	0.062 ± 0.005	0.925 ± 0.036	0.885 ± 0.040	1.003 ± 0.020	0.410 ± 0.012	0.851 ± 0.115
26						
10	0.290 ± 0.052[b]	0.647 ± 0.050[b]	0.548 ± 0.029	1.002 ± 0.030	0.596 ± 0.035	0.600 ± 0.154
100	0.220 ± 0.043[b]	0.668 ± 0.178[b]	2.317 ± 0.158	1.075 ± 0.048	0.962 ± 0.067	0.197 ± 0.088

[a] Activation and recovery data are expressed relative to ACh control responses in the same cells. The control ACh concentrations are 30 μM for $\alpha4\beta2$, 30 μM for $\alpha3\beta2$, and 300 μM for $\alpha7$. These values correspond to the EC_{30}, EC_{15}, and EC_{50}, respectively, for ACh at these receptors. Receptor activation was measured after application of the compound. Following a 5 min wash, ACh was reapplied and the responses were measured to determine the extent of recovery or inhibition. Data are presented as mean ± standard deviation of three experiments. [b] Average of two determinations.

Table 3. Comparative Pharmacologic Profiles of *S*-(−)-Nicotine, ABT-089, and Chroman-Based Nicotinic Agonists

compound	K_i, [^{3}H]cytisine (nM)	K_i, α-BT[a] (nM)	$^{86}Rb^+$ [b]	[^{3}H]DA[b]	[^{3}H]ACh[b]
S-(−)-**1**	1.0 ± 0.1	6000 ± 876	1.0 (100%)	1.0 (100%)	1.0 (100%)
(*S*)-**7**	17 ± 2	>10000	0.2 (34%)	0.04 (70%)	0.3 (100%)
(±)-**17c**	93 ± 30	>10000	∼1 (100%)[c]	>1.0 (100%)[c]	>1.0 (80%)[c]
(±)-**26**	21 ± 9	>10000	*d*	>1.0 (200%)[c]	<0.01 (<5%)[c]

[a] α-BT = α-bungarotoxin. [b] Data presented as relative potency (relative efficacy). [c] Estimated from incomplete dose−response curves. Data for *S*-(−)-**1** and (*S*)-**7** are taken from ref 15. [d] Not available.

tions wherever appropriate: b = broad, d = doublet, t = triplet, q = quartet, m = multiplet. Preparative chromatography was performed on a Harrison Research Chromatotron using Merck 60 PF254 silica gel or a preparative HPLC system (Rainin Instrument Co.) using a 41.1 mm i.d. Dynamax silica gel column (delivering solvent at 60−80 mL/min). Analytical TLC was carried out on Analtech GHLF silica gel glass plates, and visualization was aided by UV and/or methanolic iodine.

3. Procedure A: General Method for the Synthesis of Chalcones 12a−d. 3.1. *trans*-3-(2-Methoxyphenyl)-1-(2-Pyridyl)propen-1-one (12a). A solution of 2-acetylpyridine (6.06 g, 50 mmol) in EtOH (10 mL) was added dropwise over 30 min to a cold (ice bath), stirring solution of *o*-anisaldehyde (6.81 g, 50 mmol) in EtOH (100 mL) and 10% aqueous NaOH (50 mL). After the addition, stirring and cooling were continued for 3 h. The reaction mixture was then allowed to come to room temperature and stirred overnight. After 15 h at room temperature, the resulting mixture was filtered to give a yellow precipitate that was washed with 50% aqueous EtOH and dried under reduced pressure at 50 °C to provide 9.9 g (83%) of the chromatographically homogeneous product. ^{1}H NMR ($CDCl_3$): δ 3.90 (s, 3H, methoxyl), 6.90−6.98 (m, 2H), 7.3−7.45 (m, 2H), 7.77−7.80 (m, 2H), 8.10−8.40 (m, 3H), 8.70 (d, 1H).

3.2. *trans*-3-(2,3-Dimethoxyphenyl)-1-(2-pyridyl)propen-1-one (12b). Yield {from 2,3-dimethoxybenzaldehyde (10.0 g, 60.2 mmol) and 2-acetylpyridine (7.5 g, 61.9 mmol)}, 73%. ^{1}H NMR ($CDCl_3$): δ 3.80−4.00 (s, 6H, methoxyl), 6.90−7.00 (d, 1H, −CH=*CH*−CO), 6.90−7.20 (t, 1H,-**CH**=CH−CO), 7.20−8.80 (m, 7H, aryl).

3.3. *trans*-3-(2,4-Dimethoxyphenyl)-1-(2-pyridyl)propen-1-one (12c). Yield {from 2,4-dimethoxybenzaldehyde (5.0 g, 30.0 mmol) and 2-acetylpyridine (3.8 g, 31 mmol)}, 90%. ^{1}H NMR ($CDCl_3$): δ 3.70−4.00 (s, 6H, methoxyl), 6.40−6.60 (m, 2H, vinyl), 7.20−8.80 (m, 7H, aryl).

3.4. *trans*-3-(2,5-Dimethoxyphenyl)-1-(2-pyridyl)propen-1-one (12d). Yield {from 2,5-dimethoxybenzaldehyde (15.0 g, 90.3 mmol) and 2-acetylpyridine (11.0 g, 90.8 mmol)}, 77%. ^{1}H NMR (DMSO-d_6): δ 3.60−4.00 (s, 6H, methoxyl), 6.90−7.10 (m, 2H, vinyl), 7.20−8.80 (m, 7H, aryl).

3.5. *trans*-3-(2,6-Dimethoxyphenyl)-1-(2-pyridyl)propen-1-one (12e). Yield {from 2,6-dimethoxybenzaldehyde (5.70 g, 34.3 mmol) and 2-acetylpyridine (4.28 g, 35.3 mmol)}, 94%. ^{1}H NMR ($CDCl_3$): δ 3.90−4.00 (s, 6H, methoxyl), 6.50−6.60 (m, 2H, vinyl), 7.20−8.80 (m, 7H, aryl).

4. Procedure B: General Method for the Reduction of Chalcones 12a−e. 4.1. *trans*-3-(2-Methoxyphenyl)-1-(2-pyridyl)propen-1-ol (13a). Sodium borohydride (9.54 g, 0.25 mol) was added portionwise over 30 min to a cooled (ice bath) stirring suspension of chalcone **12a** (14.73 g, 61.6 mmol) in anhydrous MeOH (200 mL). Complete dissolution was obtained at the end of the addition. The ice bath was removed, and stirring was continued for 5 h. Monitoring by TLC (20% acetone−hexane on silica gel) confirmed that the reaction had gone to completion. The reaction mixture was concentrated to a residue that was diluted with H_2O and extracted with CH_2Cl_2 (3 × 100 mL). The organic extracts were combined, dried over anhydrous Na_2SO_4, and concentrated to a yellow syrup (quantitative). ^{1}H NMR ($CDCl_3$): δ 3.75 (s, 3H, methoxyl), 5.38 (d, 1H, methine), 6.20−6.40 (q, 1H, vinyl), 6.80 (m, 2H), 7.15 (m, 2H), 7.30−7.40 (dd, 2H), 7.65 (m, 1H), 8.6 (d, 1H).

4.2. *trans*-3-(2,3-Dimethoxyphenyl)-1-(2-pyridyl)propen-1-ol (13b). Yield, 95%. ^{1}H NMR ($CDCl_3$): δ 3.80−4.00 (s, 6H, methoxyl), 4,90−5,10 (s, 1H, hydroxyl), 5.30−5.50 (m, 1H, methine−H), 6.20−6.40 (q, 1H, −CH=C*H*(OH)−), 6.80−6.90 (d, 1H, −C*H*=CH(OH)−), 6.90−8.80 (m, 7H, aryl).

4.3. *trans*-3-(2,4-Dimethoxyphenyl)-1-(2-pyridyl)propen-1-ol (13c). Yield, 83%. ^{1}H NMR ($CDCl_3$): δ 3.60−4.00 (s, 6H, methoxyl), 4.70−5.00 (s, 1H, hydroxyl), 5.20−5.40 (s, 1H, methine−H), 6.20−6.60 (m, 2H, vinyl), 6.90−8.80 (m, 7H, aryl).

4.4. *trans*-3-(2,5-Dimethoxyphenyl)-1-(2-pyridyl)propen-1-ol (13d). Yield, 89%. ^{1}H NMR ($CDCl_3$): δ 3.60−3.90 (s, 6H, methoxyl), 4.90−5.10 (s, 1H, hydroxyl), 5.30−5.40 (d, 1H, methine−H), 6.20−6.40 (m, 2H, vinyl), 6.70−8.80 (m, 7H, aryl).

4.5. *trans*-3-(2,6-Dimethoxyphenyl)-1-(2-pyridyl)propen-1-ol (13e). Yield, 99%. ^{1}H NMR ($CDCl_3$): δ 3.70−3.90 (s, 6H, methoxyl), 4.40−5.00 (s, 1H, hydroxyl), 5.30−5.40 (d, 1H, methine), 6.50−6.60 (d, 2H, vinyl), 6.70−8.60 (m, 7H, aryl).

5. Procedure C. Synthesis of Propyl Alcohols 14a−d. 5.1. 3-(2-Methoxyphenyl)-1-(2-pyridyl)propan-1-ol (14a). A solution of **3a** (10.4 g, 43.3 mmol) in methanol (40 mL) was treated with 10% Pd−C (220 mg) and hydrogenated at 50 psi in a Parr hydrogenator for 24 h. The mixture was filtered to remove the catalyst, and the filtrate was passed through a short column of silica gel (50% acetone−hexane). Concentration of the eluent yielded the product as a syrup (quantitative). ^{1}H NMR ($CDCl_3$): δ 1.70−2.30 (m, 2H, methylene), 2.50−3.00 (m, 2H, methylene), 3.60−3.95 (s, 3H, methoxyl), 4.60−4.90 (m, 1H, methine), 6.60- -8.70 (m, 8, aryl).

5.2. 3-(2,3-Dimethoxyphenyl)-1-(2-pyridyl)propan-1-ol (14b). Yield, 95%. ^{1}H NMR ($CDCl_3$): δ 1.80−2.90 (m, 4H, methylene), 3.60−3.90 (s, 6H, methoxyl), 4.20−4.70 (s, 1H, hydroxyl), 4.70−4.80 (m, 1H, methine), 6.70−8.60 (m, 7H, aryl).

5.3. 3-(2,4-Dimethoxyphenyl)-1-(2-pyridyl)propan-1-ol (14c). Yield, 96%. ^{1}H NMR ($CDCl_3$): δ 1.80−2.90 (m, 4H,

methylene), 3.60−4.00 (s, 6H, methoxyl), 4.00−4.60 (s, 1H, hydroxyl), 4.60−4.90 (m, 1H, methine), 6.30−8.70 (m, 7H, aryl).

5.4. 3-(2,5-Dimethoxyphenyl)-1-(2-pyridyl)propan-1-ol (14d). Yield, 96%. ^{1}H NMR ($CDCl_3$): δ 1.80−2.90 (m, 4H, methylene), 3.60−3.80 (s, 6H, methoxyl), 4.00−4.50 (s, 1H, hydroxyl), 4.70−4.80 (m, 1H, methine), 6.60−8.60 (m, 7H, aryl).

5.5. 3-(2,6-Dimethoxyphenyl)-1-(2-pyridyl)propan-1-ol (14e). Yield, 98.8%. ^{1}H NMR ($CDCl_3$): δ 1.80−2.90 (m, 4H, methylene), 3.70−3.90 (s, 6H, methoxyl), 4.00−4.60 (s, 1H, hydroxyl), 4.60−4.80 (m, 1H, methine), 6.50−8.80 (m, 7H, aryl).

6. Procedure D. General Method for Synthesis of Phenols 15a−d. 6.1. 3-(2-Hydroxyphenyl)-1-(2-pyridyl)-propan-1-ol (15a). Boron tribromide (7.79 g, 31.1 mmol) in methylene chloride (15 mL) was added dropwise over 30 min to a cooled (dry ice−acetone) stirring solution of **14a** in dry methylene chloride (40 mL). The dry ice−acetone bath was maintained for an additional 30 min and subsequently replaced with a regular ice bath. Stirring and cooling were continued for 2.5 h, at which time the reaction was shown to be complete (TLC, silica gel, 50% acetone−hexane). The reaction mixture was then returned to a dry ice−acetone bath and carefully quenched with methanol (40 mL). The resulting solution was concentrated under reduced pressure, and the residue was treated with a saturated solution of sodium bicarbonate (100 mL, aqueous). Extraction of the mixture with methylene chloride (3 × 70 mL) followed by drying over anhydrous sodium sulfate and concentration provided the product as an orange syrup (3.4 g, quantitative). ^{1}H NMR ($CDCl_3$): δ 1.80−2.30 (m, 2H, methylene), 2.50−3.00 (m, 2H, methylene), 4.50−4.80 (m, 1H, methine), 5.00−6.40 (s, 2H, hydroxyl), 6.60−8.70 (m, 8H, aryl).

6.2. 3-(2,3-Dihydroxyphenyl)-1-(2-pyridyl)propan-1-ol (15b). Yield, 93%. ^{1}H NMR ($CDCl_3$): δ 1.60−2.70 (m, 4H, methylene), 4.40−4.60 (m, 1H, methine), 5.20−5.60 (m, 1H, hydroxyl), 6.40−8.40 (m, 7H, aryl), 8.90−9.30 (s, 2H, phenolic).

6.3. 3-(2,4-Dihydroxyphenyl)-1-(2-pyridyl)propan-1-ol (15c). Yield, 91%. ^{1}H NMR ($CDCl_3$): δ 0.80−1.80 (m, 4H, methylene), 3.60−3.80 (m, 1H, hydroxyl), 4.30−4.50 (m, 1H, methine), 5.10−7.70 (m, 7H, aryl), 7.80−8.20 (s, 1H, phenolic).

6.4. 3-(2,5-Dihydroxyphenyl)-1-(2-pyridyl)propan-1-ol (15d). Yield, quantitative. ^{1}H NMR ($CDCl_3$): δ 1.60−2.80 (m, 4H, methylene), 4.40−4.60 (m,1H, methine), 5.30−5.40 (m, 1H, hydroxyl), 6.20−8.40 (m, 7H, aryl), 8.30−8.50 (s, 2H, phenolic).

6.5. 3-(2,6-Dihydroxyphenyl)-1-(2-pyridyl)propan-1-ol (15e). Yield, 89%. ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1.80−2.75 (m, 4H, methylene), 4.30−4.40 (m, 1H, methine), 4.50−5.50 (m, 1H, hydroxyl), 6.10−9.50 (m, 9H, aryl and phenolic).

7. Procedure E. General Method for the Synthesis of 2-(2-Pyridyl)chromans 16a−d. 7.1. 2-(2-Pyridyl)chroman (16a). Triphenylphosphine (12.0 g, 45.8 mmol) and **15a** (9.6 g, 39.5 mmol) were dissolved in dry THF (100 mL), and the solution was cooled in an ice bath. Diethyl azodicarboxylate (DEAD, 8.0 g, 46.0 mmol) was added dropwise under nitrogen, and the reaction mixture was allowed to come to room temperature. Stirring was continued at room temperature, and the progress of the reaction was monitored by TLC (10% isopropyl alcohol−hexane, silica gel). The reaction was complete after 1 week. Solvent was removed under reduced pressure, and the residue was passed through a short column of silica gel (50% acetone−hexane). The desired fractions were concentrated to a residue that solidified on cooling. The latter was triturated with diethyl ether, cooled, filtered, and dried to provide the product as a white solid (6.5 g, 78%). ^{1}H NMR ($CDCl_3$): δ 2.00−2.60 (m, 2H, methylene), 2.60−3.20 (m, 2H, methylene), 5.10−5.40 (m, 1H, methine), 6.60−8.70 (m, 8H, aryl). Conversion to the hydrochloride was accomplished by treating the free base with methanolic HCl. The hydrochloride was recrystallized from isopropyl alcohol; mp 164−166 °C. Anal. ($C_{14}H_{13}NO\cdot HCl$) C, H, N.

7.2. 8-Hydroxy-2-(2-pyridyl)chroman (16b). Yield, 60%. ^{1}H NMR ($CDCl_3$): δ 2.10−3.10 (m, 4H, methylene), 5.10−5.20 (q, 1H, methine), 6.00−6.20 (s, 1H, phenol), 6.80−8.80 (m, 7, aryl). Anal. ($C_{14}H_{13}NO_2\cdot HCl$) C, H, N.

7.3. 7-Hydroxy-2-(2-pyridinyl)chroman (16c). Yield, 84%. ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1.80−2.80 (m, 4H, methylene), 4.90−5.10 (m, 1H, methine), 6.20−8.60 (m, 7H, aryl), 8.90−9.00 (s, 1H, phenol). The free base was converted to the corresponding hydrochloride by dissolution in cold methanolic HCl, concentration of the solution, and recrystallization from ethyl alcohol. Anal. ($C_{14}H_{13}NO_2\cdot HCl$) C, H, N.

7.4. 6-Hydroxy-2-(2-pyridyl)chroman (16d). Yield, 60%. ^{1}H NMR ($CDCl_3$): δ 1.90−3.00 (m, 4H, methylene), 5.10−5.20 (m. 1H, methine), 6.50−7.80 (m, 7, aryl), 8.50−8.70 (s, 1H, phenol). Anal. ($C_{14}H_{13}NO_2\cdot HCl$) H, N, C: calcd, 63.86; found, 63.31.

7.5. 5-Hydroxy-2-(2-pyridyl)chroman (16e). Yield, 56%. ^{1}H NMR ($CDCl_3$): δ 1.90−2.90 (m, 4H), 5.10−5.30 (m, 1H), 6.40−8.80 (m, 8H). The free base was converted to the hydrochloride with cold methanolic HCl and recrystallized from absolute ethanol; mp 246−248 °C. Anal. ($C_{14}H_{13}NO\cdot HCl$) H, N, C: calcd, 63.86; found, 66.74.

8. Procedure F. Synthesis of 2-(2-Piperidyl)chromans 17a−d and 18a−d. 8.1. *threo*-2-(1-Methylpiperidin-2-yl)-chroman (17a) and *erythro*-2-(1-Methylpiperidin-2-yl)-chroman (18a). A solution of **16a** (6.5 g, 30.8 mmol) and iodomethane (26.2 g, 184.8 mmol) in anhydrous acetone was stirred at room temperature over 2 days. The resulting yellow precipitate was collected by filtration, washed with ether, and dried to give 8.5 g (78%) of the pyridinium methiodide. {In certain instances, heating was required to bring the reaction to completion.} The pyridinium methiodide was used without further purification. Sodium borohydride (3.75 g, 99.1 mmol) was added portionwise over 30 min to a cooled (ice bath) stirring solution of the methiodide (7.0 g, 19.83 mmol) in methanol (100 mL). The reaction mixture was allowed to slowly come to room temperature, and stirring was continued overnight. After 15 h, the resulting solution was concentrated under reduced pressure to produce a colorless residue. The latter was partitioned between ethyl acetate (80 mL) and water (50 mL). After separation of the organic phase, the aqueous layer was re-extracted with ethyl acetate (2 × 80 mL). The organic extracts were combined, dried over anhydrous sodium sulfate, and concentrated to provide 4.5 g (quantitative) of the tetrahydropyridine. A solution of the latter in methanol (40 mL) was treated with 10% Pd−C (150 mg) and hydrogenated on a Parr hydrogenator at 60 psi for 24 h. The reaction mixture was filtered to remove the catalyst and other insoluble material, and the filtrate was concentrated to yield a mixture of two compounds **17a** and **18a**. Chromatographic separation by radial flow chromatography on silica gel (5% isopropyl alcohol−hexane plus 1% Et_3N) provided the diastereomers **17a** and **18a**. In this system, the threo isomer **17a** was found to elute before the erythro isomer **18a**. Compound **17a**: yield, 21%; mp 164−166 °C. ^{1}H NMR ($CDCl_3$): δ 1.00−3.10 (m, 16H, alkyl), 4.27−4.34 (m, 1H, C2−methine), 6.70−7.20 (m, 4H, phenyl). Anal. ($C_{15}H_{21}NO\cdot HCl$) C, H, N. Compound **18a**: yield, 55%; mp 202−204 °C. ^{1}H NMR ($CDCl_3$): δ 1.20−3.10 (m, 16H, alkyl), 4.20−4.26 (m, 1H, C2−methine), 6.70−7.20 (m, 4H, phenyl). Anal. ($C_{15}H_{21}NO\cdot HCl$) C, H, N.

8.2. *threo*-8-Hydroxy-2-(1-methylpiperidin-2-yl)chroman (17b) and *erythro*-8-Hydroxy-2-(1-methylpiperidin-2-yl)chroman (18b). Compound **17b**: yield, 35%; mp 315−317 °C. ^{1}H NMR (DMSO-d_6): δ 1.10−2.90 (m, 16H, alkyl), 3.90−4.20 (m, 1H, C2−methine), 6.40−6.70 (m, 3H, aryl), 8.80−9.20 (s, 1H, phenolic). Anal. ($C_{15}H_{21}NO_2\cdot HCl$) C, H, N. Compound **18b**: yield, 15%; mp 291−293 °C. ^{1}H NMR (DMSO-d_6): δ 1.20−3.10 (m, 16H, alkyl), 4.00−4.20 (m, 1H, C2−methine), 6.40−6.60 (m, 3H, aryl), 7.00−8.20 (s, 1H, phenolic). Anal. ($C_{15}H_{21}NO_2\cdot HCl$) H, N, C: calcd, 63.57; found, 62.94.

8.3. *threo*-7-Hydroxy-2-(1-methylpiperidin-2-yl)chroman (17c) and *erythro*-7-Hydroxy-2-(1-methylpiperidin-2-yl)chroman (18c). Compound **17c**: yield, 13%; mp (hydrochloride) 251−253 °C. ^{1}H NMR (DMSO-d_6): δ 1.00−3.60 (m, 16H, alkyl), 4.15−4.19 (m, 1H, C2−methine), 6.00−6.80 (m, 3H, phenyl), 9.00−9.10 (s, 1H, phenolic). Anal. ($C_{15}H_{21}NO_2\cdot$

HCl) C, H, N. Compound **18c**: yield, 66%; mp (hydrochloride) 265−267 °C. ^{1}H NMR ($CDCl_3$): δ (free base) 1.00−3.60 (m, 16H, alkyl), 3.98−4.10 (m, 1H, C2−methine), 6.00−6.80 (m, 3H, aryl), 9.00−9.10 (s, 1H, phenolic). Anal. ($C_{15}H_{21}NO_2$·HCl) C, H, N.

8.4. *threo*-6-Hydroxy-2-(1-methylpiperidin-2-yl)chroman (17d) and *erythro*-6-Hydroxy-2-(1-methylpiperidin-2-yl)chroman (18d). Compound **17d**: yield, 10%; mp 289−291 °C. ^{1}H NMR (DMSO-d_6) δ 1.50−4.00 (m, 16H, alkyl), 4.30−4.40 (m, 1H, C2−methine), 6.40−6.80 (m, 3H, aryl), 8.80−9.00 (s, 1H, phenol), 9.80−10.80 (br s, 1H, acidic). Anal. ($C_{15}H_{21}NO_2$·HCl) C, H, N. Compound **18d**: yield, 60%; mp 286−288 °C. ^{1}H NMR (DMSO-d_6): δ 1.30−3.60 (m, 16H, alkyl), 3.40−3.60 (s, 3H), 4.30−4.40 (m, 1H, C2−methine), 6.40−6.80 (m, 3H, aryl), 8.80−9.00 (s, 1H, phenol), 9.80−10.80 (br s, 1H, acidic). Anal. ($C_{15}H_{21}NO_2$·HCl) C, H, N.

8.5. *threo*-5-Hydroxy-2-(1-methylpiperidin-2-yl)chroman (17e) and *erythro*-5-Hydroxy-2-(1-methylpiperidin-2-yl)chroman (18e). Compound **17e**: yield, 11%; mp 287−289 °C. ^{1}H NMR (DMSO-d_6): δ 1.00−3.10 (m, 16H), 4.20−4.30 (m, 1H), 6.25−7.00 (m, 4H). Anal. ($C_{15}H_{21}NO_2$·HCl) C, H, N. Compound **18e**: yield, 47%; mp 252−256 °C. ^{1}H NMR (DMSO-d_6): δ 1.20−3.10 (m, 16H), 4.10−4.30 (m, 1H), 5.00−6.20 (s, 1H), 6.25−7.00 (m, 4H). Anal. ($C_{15}H_{21}NO_2$·HCl) C, H, N.

9. Procedure G. Method for the Preparation of Chalcones 20 and 27. 9.1. *trans*-3-(2,4-Dimethoxyphenyl)-1-(1-butoxycarbonylpyrrolidin-2-yl)propen-2-one (20). A cold solution of 10% aqueous NaOH (100 mL) was added dropwise to a cold stirring solution of 2,4-dimethoxybenzaldehyde (10.0 g, 60 mmol) and **19**[17] (14.0 g, 66 mmol) in ethanol (100 mL). Upon completion of the addition, the reaction mixture was stirred for 20 h and monitored by TLC (30% ethyl acetate−hexane, silica gel). After 20 h, the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (4 × 60 mL). The combined organic extracts were washed consecutively with 0.6 N HCl (50 mL) and saturated aqueous sodium bicarbonate (100 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to yield the crude product. The latter was purified by column chromatography on silica gel (10% ethyl acetate) to provide 15 g (69%) of **20**. ^{1}H NMR ($CDCl_3$): δ 1.20−1.50 (d, 9H, *tert*-butyl), 1.50−3.70 (m, 7H, pyrrolidyl), 3.80−3.90 (d, 6H, methoxyl), 6.40−8.00 (m, 5H, aryl and vinyl). EIMS (FAB) calcd for $C_{20}H_{27}NO_5$ *m/z*, 361.1889; found, 362.1998 $(M + H)^+$, 32%.

9.2. *threo*-3-(2,4-Dimethoxyphenyl)-1-(1-methylpyrrolidin-2-yl)propan-1-ol (21) and *erythro*-3-(2,4-Dimethoxyphenyl)-1-(1-methylpyrrolidin-2-yl)propan-1-ol (22). A solution of **20** (15.0 g, 41.6 mmol) in 10 mL of freshly distilled THF was added under nitrogen to a cold (ice bath) stirring suspension of $LiAlH_4$ (20.0 g, 527 mmol). The mixture was heated slowly to reflux and maintained in this condition overnight. The reaction mixture was then cooled in an ice bath, diluted with THF (200 mL), and quenched by careful dropwise addition of water (20 mL) and 15% NaOH (60 mL), consecutively. The resulting mixture was stirred for 1 h and filtered to remove a white precipitate. The latter was washed with EtOAc (200 mL) and set aside. The filtrate was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to provide a crude mixture of **21** and **22**. Compounds **21** and **22** were separated by silica gel chromatogaphy (5% i-PrOH−hexane). Under these conditions, compound **21** eluted first. Compound **21**: yield, 4.6 g (79%). ^{1}H NMR ($CDCl_3$): δ 1.50−3.30 (m, 15H), 3.70−3.80 (m, 1H), 3.80−3.90 (s, 6H), 6.40−7.20 (m, 3H). Compound **22**: yield, 3.9 g (69%). ^{1}H NMR ($CDCl_3$): δ 1.45−3.30 (m, 16H), 3.70−3.80 (s, 6H), 6.40−7.20 (m, 3H). EIMS (FAB) calcd for $C_{16}H_{25}NO_3$ *m/z*, 279.1834; found, 280.1938 $(M + H)^+$, 100%.

9.3. *threo*-3-(2,4-Dihydroxyphenyl)-1-(1-methylpyrrolidin-2-yl)propan-1-ol (23). Compound **21** (6.0 g, 21.5 mmol) was dissolved in 250 mL of methylene chloride, and the resulting solution was cooled in a dry ice−acetone bath and stirred under nitrogen. A solution of boron tribromide (6.0 mL, 63.9 mmol) in 20 mL of dry methylene chloride was then added dropwise over 30 min. The dry ice−acetone bath was replaced with an ice bath, and stirring was continued overnight while the reaction temperature was allowed to rise slowly to room temperature. After 20 h, the reaction mixture was cooled in a dry ice−acetone bath and the reaction was quenched by careful addition of methanol (10 mL). The pH of the solution was adjusted to 9−11 with aqueous NaOH, and the layers were separated. The aqueous layer was further extracted with EtOAc (4 × 100 mL) and set aside. The organic extracts were combined, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to yield 2.6 g (48%) of **23**. ^{1}H NMR (acetone-d_6 + DMSO-d_6): δ 1.40−3.30 (m, 14H), 3.60−3.80 (m, 1H), 5.90−6.70 (m, 3H).

9.4. *erythro*-7-Hydroxy-2-(1-methylpyrrolidin-2-yl)chroman (24). Compound **23** (2.6 g, 10.4 mmol) and triphenylphosphine (2.9 g, 11.1 mmol) were added to a dried flask containing 150 mL of freshly distilled 1,4-dioxane and 30 mL of dry DMF, and the resulting solution was cooled in an ice bath. Diethyl azodicarboxylate (2.0 g, 11.5 mmol) was added dropwise under nitrogen. Following the addition, the reaction temperature was slowly raised to room temperature over 2 h. Stirring was then continued overnight. After 16 h, the reaction was found to be incomplete. The mixture was refluxed for 20 h, cooled, and concentrated to remove excess solvent. The residue was redissolved in ethyl acetate (20 mL) and chilled to precipitate triphenylphosphine oxide. The latter was removed by filtration, and the filtrate was concentrated to a residue that was chromatographed on a silica gel column (30% acetone−hexane) to give the crude product. Further purification of the latter was obtained by radial flow chromatography on silica gel (30% THF−hexane) to yield 0.7 g (31%) of **24** as the free base. ^{1}H NMR [free base] ($CDCl_3$): δ 1.60−3.80 (m, 13H), 3.10−3.30 (m, 1H), 4.00−4.10 (m, 1H), 6.30−6.90 (m, 3H). EIMS (FAB) calcd for $C_{14}H_{19}NO_2$ *m/z*, 233.1416; found, 234.1481 $(M + H)^+$, 100%. The corresponding hydrochloride was obtained by dissolving the free base in cold methanolic HCl. After solvent removal, the hydrochloride was crystallized from absolute ethanol as a white solid; mp (hydrochloride) 241−243 °C. Anal. ($C_{14}H_{19}NO_2$·HCl) C, H, N.

9.5. *erythro*-3-(2,4-Dihydroxyphenyl)-1-(1-methylpyrrolidin-2-yl)propan-1-ol (25). Compound **22** (5.6 g, 20.1 mmol) was reacted with boron tribromide (6.0 mL, 63.9 mmol) under the conditions described above for **21**. After the reaction was quenched with methanol (10 mL), the mixture was concentrated to a residue. The residue was redissolved in absolute ethanol (20 mL), and the solution was added to a solution of sodium (0.55 g, 25.9 mmol) in 30 mL of absolute ethanol. After being stirred for 30 min, the mixture was filtered to remove precipitated NaBr. Concentration of the filtrate provided a residue that was passed through a short column of silica gel (with ethyl acetate as mobile phase) to yield 5.0 g (quantitative) of the phenol **25**. ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1.20−3.30 (m, 14H), 3.30−3.50 (m, 1H), 4.00−4.6.00 (s, 3H), δ 5.90−6.70 (m, 3). EIMS (FAB) calcd for $C_{14}H_{21}NO_3$ *m/z*, 251.1521; found, 252.1603 $(M + H)^+$, 100%.

9.6. *threo*-7-Hydroxy-2-(1-methylpyrrolidin-2-yl)chroman (26). Compound **24** (5.0 g, 19.9 mmol) was reacted with triphenylphosphine (6.0 g, 22.9 mmol) and diethyl azodicarboxylate (4.0 g, 22.9 mmol) in the same manner as described above for **23**. Workup and chromatographic purification yielded 2.8 g (60%) of **26** as the free base. ^{1}H NMR ($CDCl_3$): δ 1.25−2.80 (m, 10H), 2.80−2.90 (s, 3H), 3.20−3.30 (m, 1H), 3.80−4.00 (t, 1H), 6.30−6.90 (m, 3H), 10.0−12.0 (s, 1H). EIMS (FAB) calcd for $C_{14}H_{19}NO_2$ *m/z*, 233.1416; found, 234.1497 $(M + H)^+$, 100%. Compound **26** was converted to the hydrochloride and recrystallized from absolute ethanol as described above; mp (hydrochloride) 254−256 °C. Anal. ($C_{14}H_{19}NO_2$·HCl) H, N, C: calcd, 62.43; found, 61.44.

9.7. *trans*-3-(2,4-Dimethoxyphenyl)-1-(2-pyrrolyl)propen-1-one (27). The reaction of 2,4-dimethoxybenzaldehyde (10.66 g, 60 mmol) with 2-acetylpyrrole (7.0 g, 66.0 mmol) yielded 12.3 g (74%) of the chalcone. ^{1}H NMR ($CDCl_3$): δ 3.80−4.00 (s, 6H), 6.30−8.20 (m, 8H).

9.8. 3-(2,4-Dimethoxyphenyl)-1-(2-pyrrolyl)propan-1-one (28). A solution of **27** in methanol (20 mL) was treated

under nitrogen with 10% Pt−C (170 mg) and hydrogenated in a Parr hydrogenator at 70 psi for 8.5 h. The catalyst was removed by filtration over Celite, and the filtrate was concentrated to yield 2.0 g (quantitative) of the ketone. ^{1}H NMR ($CDCl_3$): δ 2.90−3.10 (m, 4H), 3.70−3.90 (s, 6H), 6.20−7.20 (m, 6H), 7.70−8.30 (s, 1H).

9.9. 3-(2,4-Dimethoxyphenyl)-1-(1-*tert*-butoxycarbonylpyrrol-2-yl)propan-1-one (29). Di-*tert*-butyldicarbonate (8.1 g, 37.1 mmol) was added to a mixture of **28** (8.0 g, 30.9 mmol) and t-BuOK (0.35 g, 3.1 mmol) in 10 mL of freshly distilled THF, and the mixture was refluxed overnight under nitrogen. After 20 h, the reaction mixture was cooled, diluted with 100 mL of ethyl acetate, and washed with a saturated solution of $NaHCO_3$ (3 × 60 mL). The organic extract was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to yield the crude product. The latter was purified by column chromatography on silica gel (eluting with 10% ethyl acetate−hexane) to provide 8.0 g (73%) of the desired compound. ^{1}H NMR ($CDCl_3$): δ 1.50−1.65 (m, 4H), 2.90−3.10 (m, 4H), 3.70−3.90 (s, 6H), 6.10−7.40 (m, 6H). Reduction of this intermediate with $LiAlH_4$, as described for compound **20**, yielded a mixture of **21** and **22** in the same ratio as obtained from **20**.

10. X-ray Crystallography. Suitable crystals for diffraction were obtained by slow diffusion of hexane into a solution of the hydrochloride in acetonitrile. The structures of **17c** and **24** were determined by single-crystal X-ray analyses. For both compounds, the unit cell was found to contain two independent molecules, that is, two molecules not related by crystallographic symmetry. The intensity data were measured on an Enraf-Nonius CAD4 diffractometer (graphite-monochromated Mo Kα radiation, $\omega-2\theta$ scans). The dimensions of the crystals used for data collection were approximately 0.16 mm × 0.60 mm × 0.96 mm and 0.12 mm × 0.20 mm × 0.92 mm for **17c** and **24**, respectively. The data were not corrected for absorption. For **17c**, 1429 of 2625 independent reflections were considered observed [$I > 3.0\sigma(I)$] for $\theta < 25°$. For **24**, 1622 of 5014 independent reflections were considered observed [$I > 3.0\sigma(I)$] for $\theta < 25°$. The structures were solved by a multiple-solution procedure[37] and were refined by full-matrix least squares. In the final refinement, the nonhydrogen atoms were refined anisotropically. The hydrogen atoms were included in the structure factor calculations, but their parameters were not refined. The final discrepancy indices are $R = 0.044$ and $R_w = 0.052$ for the 1429 observed reflections, and $R = 0.053$ and $R_w = 0.054$ for the 1622 observed reflections. The final difference maps have no peaks greater than ±0.27 e Å^{-3}. The X-ray data are provided as Supporting Information.

11. Radioligand Binding Studies. 11.1. Membrane Preparation. Membranes were prepared according to Pabreza et al.[19] with slight modification. Rats (250−350 g) were decapitated under diethyl ether anesthesia. Brains were rapidly removed, and the cerebral cortex was dissected. With a Teflon−glass homogenizer, the tissue was homogenized in 25 volumes (per weight of tissue) of 50 mM Tris-HCl buffer (pH 7.45 at room temperature) containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 2.5 mM $CaCl_2$. The crude homogenate was centrifuged for 15 min at 18 000*g* in a refrigerated Sorval RC-5B centrifuge using an SS-34 rotor. The supernatant was discarded, and the pellet was suspended in the above buffer and centrifuged for 15 min at 18 000*g*. The final membrane pellet was resuspended in Tris-HCl buffer containing 0.2 mM PMSF and 1.0 mM EDTA at 18 mg of protein/mL and stored at −80 °C.

11.2. Binding to Brain Nicotinic ACh Receptors: [^{3}H]-Cytisine and [^{125}I]-α-Bungarotoxin Binding Sites. All binding assays were carried out in 50 mM Tris-HCI buffer pH 7.45 containing 120 mM NaCl, 5mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. Aliquots of homogenate equivalent to 2.5 mg of tissue (250 μg of protein) in 400 μL of buffer were added to test tubes containing 50 μL of a competing ligand of appropriate concentration (range 0.0001−10 μM). A total of 30 min of incubation time was allowed for competing ligands to reach the receptor. A total of 50 μL of [^{3}H]-(−)-cytisine yielding a final concentration of 1.5 nM was then added, and the mixture was incubated for 90 min at room temperature (22−25 °C). Nonspecific binding was determined from membranes incubated in parallel with 10 μM *S*-(−)-nicotine bitartrate before the addition of radioligand.

Binding of [^{125}I]-α-bungarotoxin was assayed in the above buffer supplemented with 4.0 mg/mL of bovine serum albumin. Competition experiments were conducted at 1.5−2.0 nM [^{125}I]-α-bungarotoxin and 0.001−10 μM competing ligands at 37 °C for 4.5 h. Nonspecific binding was determined in the presence of 1.0 μM cold α-bungarotoxin.

Scintillation counting was performed with a Packard Instruments counter, and data were analyzed with EBDA/LIGAND.

11.3. Binding to Dopamine D2 Receptors, Dopamine, and Serotonin Reuptake Sites. Binding to dopamine receptors was assayed in rat striatal homogenates as previously described.[27] Binding of candidate molecules to dopamine and serotonin transporters was tested with rat striatal and cortical homogenates, respectively, using previously described methods.[28,29] Alternatively, monoamine transporter binding was tested in LLC cells.[30]

12. In Vitro Functional Assays. 12.1. Preparation of Synaptosomal Fraction (P_2). Rats were sacrificed by decapitation under diethyl ether anesthesia. The brains were removed quickly and dissected on an ice-cold platform to remove either striatal tissue or cerebral cortex. The tissue was placed in 10 volumes (w/v) of ice-cold 0.32 M sucrose buffered to pH 7.5 with 5 mM HEPES and was homogenized by hand with the aid of a Teflon−glass homogenizer. The homogenate was diluted to 25 volumes with ice-cold 0.32 M sucrose and centrifuged for 10 min at 1000*g* at 4 °C. The supernatant was transferred to a separate tube, and the pellet was resuspended in 10 volumes of homogenization medium, homogenized again, and centrifuged for 10 min at 1000*g*. The resulting supernatant was combined with the original supernatant and centrifuged for 20 min at 18 000*g* to yield the P_2 pellet. The cortical (P_2) synaptosomal pellet was used to study $^{86}Rb^+$ and [^{3}H]ACh efflux, while the striatal pellet was used to measure [^{3}H]-dopamine efflux.

12.2. $^{86}Rb^+$ Efflux Assay. Determination of $^{86}Rb^+$ efflux was performed as described by Marks et al.[31] with some modifications introduced by Gattu et al.[38] Synaptosomes (250 mg of protein/mL) were incubated for 30 min at 22 °C in 35 μL/sample of perfusion buffer containing 4 μCi of $^{86}Rb^+$ (1Ci/g) and 120.0 mM NaCl, 1.5 mM KCl, 2.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, 50 mM HEPES, pH 7.5, and 20 mM D-glucose. Tetrodotoxin (50 nM) and $CsCl_2$ (5 mM) were added to the perfusion buffer to block (non-nicotinic) Na^+ channels and to reduce the basal efflux rate, respectively. The tissue was harvested and separated from the incubation medium by passing the mixture through 6 mm diameter glass fiber filters (type GC 50, Adventec MFS, Inc., Pleasanton, CA) under gentle vacuum, followed by three washes with 0.6 mL of incubation buffer at room temperature. The filters containing the $^{86}Rb^+$-loaded synaptosomes were placed on 13 mm glass fiber filters (type GC 50, Adventec MFS, Inc., Pleasanton, CA) and perfused continuously at 22 °C. After a wash period of 8 min, fractions were collected every 30 s for 2 min before exposure of the tissue to the nicotine agonist or antagonist. Interaction of candidate molecules with nicotinic receptors occurs 3 min into the 10 min collection period. *S*-(−)-Nicotine (10 μM) was included in each experiment as a control to account for variations between experiments. Radioactivity in the samples was measured by liquid scintillation counting.

The magnitude of $^{86}Rb^+$ efflux was calculated by determining the increase in radioactivity above the baseline after stimulation of the tissue. The average baseline underlying the peak was calculated by averaging the radioactivity present in the tubes immediately before and after the peak. Peak size was determined by subtracting the average baseline value from each fraction in the peak EC_{50} values, and the maximum response obtained for stimulation of $^{86}Rb^+$ efflux is calculated by use of Inplot (Graphpad, San Diego, CA). Data are analyzed

by a two-way ANOVA with use of StatView II. The criterion for statistical significance was $P < 0.05$.

12.3. Measurement of Striatal [^{3}H]Dopamine Release. Measurement of [^{3}H]dopamine uptake and efflux from striatal synaptosomes was performed as described by Rowell et al.[39] with some modifications described by Teng et al.[40] Striatal synaptosomes (160 mg of protein/mL) were resuspended in 15 mM HEPES buffer, pH 7.5 (containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 10 mM glucose, 10 μM pargyline, and 10 μM ascorbic acid), preincubated for 10 min at 34 °C, and loaded with 100 nM [^{3}H]-dopamine for 15 min. The entire assay was performed in an atmosphere of 95%/5% O_2/CO_2. The tissue was harvested and separated from the incubation medium by filtration onto 1.2 mm diameter glass fiber filters (type GC 50, Adventec MFS, Inc., Pleasanton, CA) under gentle vacuum, followed by two washes with 1.0 mL of incubation buffer at room temperature.

The filters containing the [^{3}H]dopamine-loaded synaptosomes were placed on 2.1 mm glass fiber filters (type GC 50, Adventec MFS, Inc., Pleasanton, CA) in a superfusion chamber and washed for 45 min at 0.8 mL/min with incubation buffer supplemented with 10 nM nomifensine, a dopamine reuptake inhibitor. After the wash period, fractions were collected every 60 s for 10 min before exposure of the tissue to *S*-(−)-nicotine and the study compounds. *S*-(−)-Nicotine and other study compounds were introduced for 5 min into a 30 min collection period. Radioactivity was measured by liquid scintillation counting. The magnitude of the [^{3}H]dopamine efflux was calculated by determining the increase in radioactivity above baseline following stimulation of the tissue. The average baseline underlying the peak was calculated by averaging the radioactivity present in the tubes before and after the peak. Peak size was determined by subtracting the average baseline value from each fraction in the peak. Data were then normalized to the response elicited by a fixed concentration of *S*-(−)-nicotine.

13. Measurement of [^{3}H]ACh Release. Drug-induced release of [^{3}H]ACh from hippocampal synaptosomes was measured as described by Sullivan et al.[15] The F4 synaptosomal fraction was washed with Kreb's buffer (containing 118.5 mM NaCl, 25 mM $NaHCO_3$, 1.2 mM KCl, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 2.5 mM $MgCl_2$, and 10 mM glucose gassed with 95%/5% O_2/CO_2 to pH 7.4) and resuspended at 1 mg of protein/mL. The synaptosomes were loaded with [^{3}H]choline by incubation with 0.8 μM [^{3}H]choline (2 Ci/mmol). Aliquots of the incubation mixture were then loaded into the perfusion chamber of a Brandell harvester and perfused with Kreb's buffer (37 °C) at a flow rate of 0.25 mL/min. Three-minute fractions were collected and measured for radioactivity. Drugs for study were administered as 20 s pulses. The [^{3}H]-ACh release evoked by study compounds was normalized to (−)-nicotine-evoked release and to the total radioactivity accumulated by synaptosomes. Evoked release of [^{3}H]ACh from rat hippocampal synaptosomes was measured as the area under the peak above basal release.

14. Measurement of Drug-Induced Receptor Activation and Inhibition in Cloned Human nAChRs. These studies were performed as previously reported.[32]

14.1. Preparation of RNA and Expression in *Xenopus* Oocytes. RNA transcripts were prepared in vitro using the appropriate message machine kit from Ambion, Inc. (Austin, TX) after linearization and purification of cloned cDNAs. Two to three ovarian lobes were surgically removed from a *Xenopus laevis* female (Nasco, Ft. Atkinson, WI). The ovarian tissue was then cut open to expose the oocytes and treated with collagenase from Worthington Biochemical Corporation (Freehold, NJ) for 2 h at room temperature in calcium-free Barth's solution (88 mM NaCl, 10 mM HEPES, pH 7.6, 0.33 mM $MgS0_4$, 0.1 mg/mL gentamicin sulfate). Subsequently, stage 5 oocytes were isolated and injected with 50 nL each of a mixture of the appropriate subunit cRNAs following harvest. Recordings were made 1−7 days after injection depending on the cRNAs being tested.

Electrophysiology oocyte recordings were made with a Warner Instruments (Hamden, CT) OC-725C oocyte amplifier and RC-8 recording chamber interfaced to a Macintosh personal computer. Data were acquired using Labview software (National Instruments) and filtered at a rate of 6 Hz. Oocytes were placed in a Warner recording chamber with a total volume of about 0.6 mL and perfused at room temperature by frog Ringer's solution (115 mM NaCl, 2.5 mM KCl, 10 mM HEPES, pH 7.3, 1.8 mM $CaCl_2$) containing 1 mM atropine to block potential muscarinic responses. A Mariotte flask filled with Ringer's solution was used to maintain a constant hydrostatic pressure for drug deliveries and washes. Drugs were diluted in perfusion solution and loaded into a 2 mL loop at the terminus of the perfusion line. A bypass of the drug-loading loop allowed bath solution to flow continuously while the drug loop was loaded, and then drug application was synchronized with data acquisition by using a two-way electronic valve. The rate of bath solution exchange and all drug applications was 6 mL/min. Current electrodes were filled with a solution containing 250 mM CsCl, 250 mM CsF, and 100 mM EGTA and had resistances of 0.5−2 MΩ. Voltage electrodes were filled with 3 M KCl and had resistances of 1−3 MΩ. Oocytes with resting membrane potentials more positive than −30 mV were not used.

14.2. Experimental Protocols and Data Analysis. Current responses to drug application were studied under a two-electrode voltage clamp at a holding potential of −50 mV. Holding currents immediately prior to agonist application were subtracted from measurements of the peak response to agonist. All drug applications were separated by a wash period of 5 min unless otherwise noted. At the start of recording, all oocytes received a control application of ACh. Subsequent drug applications were normalized to the second ACh application in order to control for the level of channel expression in each oocyte. The second application of control ACh was used to minimize the affects of rundown that occasionally occurred after the initial ACh-evoked response. To measure residual inhibitory effects, an experimental application of ACh with inhibitor or of inhibitor alone was followed by a second application of ACh alone and compared to the preapplication control ACh response. Means and standard errors (SEM) were calculated from the normalized responses of at least three oocytes for each experimental concentration unless otherwise noted.

For each of the receptor subtypes tested, a control ACh concentration was selected that was sufficient to stimulate the receptors to a level representing a reasonably high value of popen at the peak of the response while minimizing rundown with successive ACh applications.

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Supporting Information Available: Two tables of X-ray diffraction parameters for **17c** and **24** at 295 K and two tables of atomic coordinates for **17c** and **24**. This material is available free of charge via the Internet at http://pubs.acs.org.

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