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Design, Synthesis and Activity of a Series of Arylpyrid-3-ylmethanones as Type I Positive Allosteric Modulators of α7 Nicotinic Acetylcholine Receptors

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Abstract

A series of novel arylpyrid-3-ylmethanones (**7a-aa**) were designed as modulators of α7 nicotinic acetylcholine receptors (nAChRs). The methanones were found to be Type I positive allosteric modulators (PAMs) of human α7 nAChRs expressed in *Xenopus* ooctyes. Structure activity relationship (SAR) studies resulted in the identification of compound **7v** as a potent and efficacious Type I PAM with maximum modulation of a nicotine EC_5 response of 1200% and $EC_{50} = 0.18$ μM. Compound **7z** was active in reversing the effect of scopolamine in the novel object recognition (NOR) paradigm with a minimum effective i.p. dose of 1.0 mg/kg (2.7 μmol/kg). This effect was blocked by the selective α7 nAChR antagonist methyllycaconitine (MLA). These compounds are potent Type I positive allosteric modulators of α7 nAChRs that may have therapeutic value in restoring impaired sensory gating and cognitive deficits in schizophrenia and Alzheimer's disease.

Introduction

The α7-subtype of nicotinic acetylcholine receptors (nAChR) has emerged as an important target for the development of central nervous system (CNS) agents for the treatment of a variety of disorders involving cognitive deficits and neurodegeneration, including schizophrenia, attention deficit hyperactivity disorder (ADHD) and Alzheimer's disease.[1] The α7 nAChR is part of the Cys-loop family that includes γ-aminobutyric acid$_A$ (GABA$_A$), serotonin 3 (5-HT$_3$) and glycine receptors. The α7 receptor is a pentamer that forms an ion channel that binds acetylcholine (choline) as the endogenous ligand and enhances Ca^{++} conductance when open.[2] Selective agonists[3] and partial agonists[4] have been described for the α7 nAChR as well as positive allosteric modulators (PAMs).[5] PAMs may have advantages over compounds acting at the orthosteric ligand binding site because they maintain the normal temporal and spatial pattern of neurotransmission due to their lack of

activity in the absence of an agonist. Clinical studies with agonists have shown that targeting the α7 nAChR can be an effective method for improving cognitive deficits in schizophrenia.[6,7] The fact that 80% of schizophrenics smoke also points to the importance of nAChRs in the disease.[8] PAMs at nicotinic receptors have been further characterized as Type I and II, depending on their interactions with the receptor.[5] Type I modulators leave the fast native kinetics of the channel and its desensitization characteristics unperturbed, while Type II compounds significantly retard channel kinetics and may reverse desensitization. Structures of representative Type I and II PAMs are given in Figure 1. Compounds have also been characterized with properties in between the two extremes[5]. Because the α7 nAChR is highly Ca^{++} permeable, Type II modulators that reverse desensitization may have the potential to be cytotoxic, although this has not been observed *in vivo*.[9] With some type II modulators, the currents elicited from desensitized channels were drastically reduced at physiological temperature compared to rt.[10a] Despite these reduced currents, these Type II modulators have been found to be excitotoxic under more physiological conditions (e.g., 37°C plus serum factors).[10b]

We have described the activity of a series of enaminone esters and amides as potent allosteric modulators of $GABA_A$Rs.[11] A small library of these compounds were screened for activity as PAMs of human α7 nAChRs expressed in *Xenopus* oocytes. Compounds were identified with activity at both receptors and modifications to these hits resulted in the identification of novel compounds with selectivity for α7 nAChRs.[9] The enaminone esters and amides, exemplified by compounds **1a, b** and **c** (Figure 2) emerged from these efforts, but were not optimized as potential drugs. For example, *in vitro*, the compounds were not selective for α7 nAChRs. Compound **1a** was equally active at α7 nACh and $GABA_A$Rs, showing essentially equal efficacy at the nicotinic receptor and $α_1β_2γ_{2L}$ $GABA_A$Rs. The 4-fluoro analog **1b** was more efficacious at α7 receptors but still showed $GABA_A$R activity.[9] In addition to this lack of selectivity, the ^{1}H NMR spectra of both esters indicate that they are mixtures of *Z-* and *E-*isomers.[11] Based on the chemical shift for the aniline NH in the *Z-*isomers of **1a** and **b** (**Z-1**), a hydrogen-bond (H-bond) with the ester carbonyl oxygen atom is implicated (see Figure 2). The major isomer, at least under NMR conditions in CDCl$_3$ or DMSO-d$_6$, is **Z-1**, presumably due to the stronger H-bond with the ester in **Z-1** compared to the ketone in **E-1**. Unlike the corresponding esters, enaminone amides like **1c** that form two internal H-bonds (one between the amide NH and the ketone and a second between the amide oxygen atom and the aniline NH) are known to be single isomers.[11] *In vitro*, **1c** was similar in activity at α7 nAChRs to **1a** with I/I$_{max}$ = 1.5 and also exhibited potent activity at $GABA_A$Rs based on inhibition of the binding of [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]TBPS, IC$_{50}$ = 0.1 μM).[12] Furthermore the enaminone amides are alkylated anilines with the potential for the formation of toxic metabolites.

Assuming that the enaminone esters bind to the α7 nAChR as the major *Z-*isomer, and the amides bind similarly, it was hoped that the H-bonded array in **1c** could be replaced with a pyridine ring as in compound **2** (Figure 3). This tactic has been employed in a number of other chemical series, where groups of H-bonded atoms have been replaced with six-membered rings and vice-versa.[13] In many cases, the biological activity of the H-bonded series has been maintained or improved. Several criteria were felt to be important for the compounds to meet: (1) They should be selective for α7 nAChRs over heteromeric nAChRs and $GABA_A$Rs. (2) The compounds should be Type I modulators of α7 nAChRs that preserve the native kinetics of the receptor and do not reverse desensitization. (3) The efficacy of the compounds at α7 nAChRs should be maximized without decreasing their potency. This paper describes the synthesis and biological activity of **2** as PAMs of α7 nAChRs.

Chemistry

The pyridines **2** were prepared by using one of three methods depending on the substituents on the aryl group of the benzoyl moiety. Starting with arylacetic acids (Method A; Scheme 1), reaction with $POCl_3$/DMF (Vilsmeier reagent) afforded the aryl(dimethylamino)propenal **3**,[14] which was condensed with cyanoacetamide in the presence of sodium methoxide to give the pyridone **4**.[14,15] Reaction of **4** with $POCl_3$ followed by addition of an amine afforded the 2-amino-5-aryl-3-cyanopyridine **6**. Addition of an arylmagnesium halide and hydrolysis to the ketone then gave the products **7a-k**.

The intermediate aminocyanopyridines **6** were also prepared as shown in Scheme 2 (Method B), starting with 2-chloronicotinonitrile. Reaction with propylamine gave **8**, followed by bromination with *N*-bromosuccinimide (NBS) afforded 5-bromo-2-propylamino-3-pyridinecarbonitrile **9**. Reaction with arylboronic acids was carried out under Suzuki coupling conditions. Conversion of **6** to the final product still necessitated the use of an arylmagnesium halide. This final step was found to be low yielding and limited by the availability and stability of the Grignard reagents. In order to avoid these issues and to allow for the preparation of a wider variety of arylketones, a better method for introducing the benzoyl group was needed.

As shown in Scheme 3, use of 2-fluoropyridine as starting material (Method C) improved the versatility of the synthetic method, allowing for a more modular construction of the desired product. With the wide variety of benzaldehydes available a larger variety of substitutions to the benzoyl group were easily accessible.

Ortholithiation of 2-fluoropyridine with lithium diisopropylamide[16] (LDA) at −78 °C followed by addition of a substituted benzaldehyde and oxidation with pyridinium chlorochromate (PCC) gave the 3-benzoyl-2-fluoropyridines **11**. Addition of an amine R1NH2 in DMSO or EtOH at rt gave the intermediate 2-alkylamino-3-benzoylpyridines **12**. Bromination with NBS followed by Suzuki coupling then gave the desired compounds **7p-z** and **7aa**. This method generally worked well except in the case of 3-(3,4-difluorobenzoyl)-2-fluoropyridine (**14**, Scheme 4). Addition of amines to **14** resulted in the formation of the desired compound **15** along with significant displacement of the 4-fluorine on the benzoyl group to give **16** (identified by mass spectroscopy). The two products were separated by using flash chromatography. This side-reaction was not observed with 4-chloro- or 4-fluorobenzoyl groups.

In vitro Pharmacology

Compounds were tested for *in vitro* activity at human α7 nAChRs expressed in frog oocytes at rt using previously published protocols.[9] Compounds were inactive in the absence of direct agonists, generally nicotine at an EC_5 concentration (concentration that evokes 5% of the maximum nicotine response (I_{max})), confirming that the compounds are allosteric modulators. Acetylcholine (ACh) and choline responses were also enhanced in the presence of the modulators. Concentration-response curves were generated for the compounds in Tables 1, 2, 3 and 4 and the maximum modulation (generally at 10 μM or the limit of solubility) and EC_{50} (concentration at which half the maximum modulation is observed) values determined. Table 1 examines the effect of changes to the 2-alkylamino group on the activity of the arylpryidin-3-ylmethanones. The unsubstituted 2-amino compound **7p** was active as were compounds incorporating methyl, ethyl or propyl groups as in **7b, q, r** and **7a**. Increasing the size of the 2-alkylamino group generally led to increases in potency with the ethyl and propylamines showing 15- and 18-fold improvements over the unsubstituted amino group (**7p**) in EC_{50}, respectively. Attempted substitution with larger 2-alkylamines,

including 3-pentyl (**7c**), 2,5-difluorobenzyl- (**7d**) and phenethylamine (**7e**) gave inactive compounds. The tertiary amine **7i** was also found to be inactive.

Modifications to the substitution on the benzoyl group were made within the 5-(4-ethoxyphenyl)-2-propylaminopyridine series using a Topliss tree approach[17]. *In vitro* data are given in Table 2. Moving the 4-chlorine in **7a** to the 2-position gave compound **7s** which was poorly active as a modulator of $\alpha 7$ nAChRs with maximum modulation at 10 μM of only 35%. The 2-methyl compound (**7f**) was found to be inactive. Similarly, 2,4-dichloro substitution as in **7t** gave poor maximum modulation of 40%. The isomeric 2-, 3- and 4-fluorobenzoyl compounds were synthesized and tested for activity. While the 3- and 4-fluoro groups (**7h** and **7g**) were active and exhibited similar potencies and maximum modulations, moving the fluorine atom to the 2-position dramatically reduced activity (**7u**). Adding a 3-fluorine to **7g** gave the 3,4-difluoride **7v** which exhibited excellent activity with maximum modulation of 1200% and $EC_{50} = 0.18$ μM. The isomeric 2,5-difluoride, **7w**, was poorly active with maximum modulation of only 200% and an $EC_{50} = 3$ μM. Substitutions with fluorine at the 3- and 5-positions gave compound **7x** with poor maximum modulation of 130%. With no substitution on the benzoyl group as in **7y**, potent modulation was observed ($EC_{50} = 0.21$ μM) with modest maximum modulation (300%).

Next, modifications to the 4-ethoxy group were made in order to explore the role of this group in the activity of the PAMs (Table 3). Replacement of the 4-ethoxy in **7a** with a 4-chloride (**7j**) resulted in essentially complete loss of activity. Within the 4-fluorobenzoyl series, activity was the best for 4-ethoxy group as in **7g** while replacement of the 4-ethoxy group with trifluoromethoxy, propyl, phenoxy or ethylthio groups abolished activity (**7l, m, n** and **o**, respectively). It was found, however, that the ethoxy group could be replaced with a 4-fluorine as in **7k**, resulting in good potency ($EC_{50} = 0.22$ μM) but with a 2-fold loss in maximum efficacy compared to **7a**.

Based on the compounds presented in Tables 1, 2 and 3, **7v** emerged as a compound with both potent activity as a modulator of $\alpha 7$ nAChRs ($EC_{50} = 0.18$ μM) and excellent maximum modulation (1200%). Close analogs were prepared to examine the effect of changes in the 2-alkylamino group on the activity of this compound. *In vitro* data are given in Table 4. The methyl and ethyl analogs, **7z** and **7aa**, showed large losses in potency compared to **7v** and were less efficacious with maximum modulations of 770 and 470%, respectively.

Unfortunately, initial testing in oocytes indicated that **7z** was more potent as a modulator of $\alpha 7$ nAChRs than **7v** leading to the fuller characterization of **7z** *in vitro* before additional oocyte data gave the relative EC_{50}'s reported in Table 4. Compound **7z** at its EC50 concentration of 1 μM induced a leftward shift in the concentration-response curve for ACh as shown in Figure 4. The EC50 value for ACh was reduced from 206 μM in the absence of **7z** to 123 μM in the presence of the modulator, although the change was not significant. At all concentrations of ACh tested, **7z** significantly increased modulation of control currents. At 10 μM, **7z** did not significantly inhibit the binding of the $\alpha 7$ nAChR antagonist α-bungarotoxin (percent inhibition 5 ± 5, n = 2), consistent with its action as a PAM of $\alpha 7$ nAChRs. Compound **7z** was tested for selectivity among nAChRs and for activity at $\alpha_1\beta_2\gamma_{2L}$ GABA$_A$Rs (Table 5). The compound had little activity at $\alpha_4\beta_2$ and $\alpha_3\beta_4$ nAChRs and unlike the enaminone amides and esters from which it was derived, poor activity was observed with $\alpha_1\beta_2\gamma_{2L}$ GABA$_A$Rs.

The pyridines were found to be Type I modulators that do not alter the rapid desensitization kinetics of the native channel. (see Figure 5 for a representative current trace). Compound **7z** did not block the desensitization of the channel initiated by 100 μM nicotine (see Figure 6).

This behavior is in stark contrast to Type II modulators such as PNU-120596 (*N*-(5-chloro-2,4-dimethoxyphenyl)-*N* -(5-methyl-3-isoxazolyl)urea)[18] that can reopen the desensitized channel.[9]

in vivo Pharmacology

As mentioned previously, initial *in vitro* studies had over-estimated the potency of **7z** leading to its testing *in vitro* and *in vivo*. A pharmacokinetic (PK) study with compound **7z** was undertaken to establish that the compound was able to cross the blood-brain barrier (BBB) after intraperitoneal (i.p.) administration. When given to CD1 mice, a 3 mg/kg (8.1 μmol/kg) i.p. dose of **7z** in 80% PEG 400/20% saline gave brain levels at 30, 60 and 120 min of 0.64 (± 0.03) μM, 0.26 (± 0.04) μM and 0.012 (± 0.007) μM, respectively. The corresponding plasma levels were found to be 1.75 (± 0.14) μM, 0.34 (± 0.07) μM and 0.06 (± 0.02) μM. With confirmation of exposure to the CNS, compound **7z** was tested for activity in the novel object recognition (NOR) paradigm.[19] CD1 mice were dosed with 0.7 mg/kg (2.1 μmol/kg) subcutaneous (s.c.) scopolamine hydrochloride to disrupt their ability to distinguish between a novel and a familiar object. A dose response of **7z** (formulated in 80% PEG 400/20% saline) is shown in Figure 7 where doses greater than 0.3 mg/kg (0.81 μmol/kg) reversed the effect of scopolamine. The lack of a change in activity for the active doses can be explained by the nature of the NOR paradigm where activity shows a threshold phenomenon. The effect of 3 mg/kg (8.1 μmol/kg) **7z** was reversed by 3 mg/kg (3.4 μmol/kg) MLA.

Discussion

Conformational restriction of the presumed active isomer of the enaminone amide **1c** resulted in a series of arylpyrid-3-ylmethanones that maintained activity as Type I PAMs of human α7 nAChRs. Unlike **1c**, however, the methanones were poorly active at $\alpha_1\beta_2\gamma_{2L}$ GABA$_A$Rs. In addition, they were inactive at $\alpha_4\beta_2$ and $\alpha_{,3}\beta_4$ nAChRs at 10 μM. Three areas of the pyridine structure were varied as part of SAR studies: (1) changes to the 2-amino group (2) changes in substitutions to the 3-benzoyl group and (3) changes in substitutions to the 5-aryl group. The potency of the pyridines as PAMs generally increased as the 2-amino substitutient (R1) increased in size from H (**7p**, EC$_{50}$ = 2.5 μM) to Me (**7q**, EC$_{50}$ = 0.38 μM), Et (**7r**, EC$_{50}$ = 0.16 μM) and Pr (**7a**, EC$_{50}$ = 0.14 μ,M). This trend was also observed within the series with R$_2$ = 4-F and 3,4-diF where activity improved from R$_1$ = Me (**7b** and **7z**, EC$_{50}$ = 1.9 μM and 1.2 μM, respectively) to R$_1$ = Pr (**7g** and **7v**, EC$_{50}$ = 0.13 μM and 0.18 μM, respectively). With Et substitution as in **7aa**, a 4-fold loss in potency was observed compared to Me substitution (**7z**). Improvements in the maximum modulation measured were striking for **7q** (1200%) versus compounds with small alkyl group substitution that generally showed maximum modulations of 500–700% (Table 1). A limit on the size of R$_1$ was noted in the loss of activity for R$_1$ groups larger than propyl, including 3-pentyl, 2,5-difluorobenzyl and phenethyl (**7c**, **7d** and **7e**). The tertiary amine **7i** was also devoid of activity, indicating either that **7i** is unable to act as a H-bond donor that is required for activity or the *N*-methylpropylamino group forces the benzoyl group further out of the plane of the pyridine ring resulting in poor activity.

Changes to the 3-benzoyl group indicated that ortho substitution (R$_2$ = 2-F, 2-Me, 2-Cl and 2,4-diCl as in **7u**, **7f**, **7s** and **7t**) is not accommodated by the α7 nAChR. In the case where the ortho substituent is Me or Cl, this is likely a steric effect that twists the phenyl ring of the benzoyl group further out of the plane of the pyridine ring. With 2-fluorine substitution, the strong C-F dipole can orient to reduce the overall dipole of the molecule by at least partially opposing the dipole of the carbonyl group. This change in conformation would be less important in the 3- and 4-fluorobenzoyl groups (**7h** and **7g**). This effect of fluorine position

on conformational preference has been noted in the isomeric fluoroacetophenones where the 2′-isomer has a large barrier for rotation about the phenyl-carbonyl bond and the molecule exists almost completely as the s-trans conformer (dipoles of the C-F and carbonyl bond opposed to each other). With the 3′-and 4′-fluoroacetophenones free rotation about the aryl-carbonyl bond is observed and the molecules exist in both the s-cis and s-trans conformers[20]. In order to better understand the effect of the 2-fluorobenzoyl group on the α7 nAChR activity of **7u**, a comparison with the unsubstituted benzoyl compound **7y** and the 3,4-difluoro analog **7z** was made using conformational analysis. Rotation about the bond between the phenyl group and carbonyl carbon of the benzoyl group (ψ in Figure 8) confirmed that the barrier to rotation is much larger for **7u** than for **7y** or **7z** (see supporting material). The preferred conformation for activity at the α7 nAChR is likely one that is somewhat removed from the minima found in the conformational analysis. **7y** and **7z** can more easily access this active conformation because its energy is closer to the energy of the lowest energy conformer, while **7u** cannot.

In the isomeric difluoro compounds, 3,4-substitution as in **7v** resulted in the most potent and efficacious compound tested (EC_{50} = 0.18 μM, maximum modulation = 1200%) while the 2,5- and 3,5-isomers were less active (**7w** and **7x**). The improvement in activity in **7v** compared to the 3- and 4-monofluoro analogs may involve a specific H-bonding interaction[21]. The poor activity for the 2,5-isomer may involve similar dipole interactions as noted with mono fluorination at the 2-position.

Modifications to the 5-aryl group showed that 4-ethoxy substitution gave the most efficacious compounds. Replacing the ethoxy group with other groups including trifluoromethoxy, propyl, phenoxy and ethylthio abolished activity. The only other compound that showed similar potency to the 4-ethoxy group (**7g**) was the 4-fluoro (**7k**) but exhibited a 2-fold loss in efficacy. It is clear from these data that the size of the ethoxy group is not the deciding factor in its activity because the propyl analog (**7m**) is inactive. It is likely that the ether oxygen is acting as a H-bond acceptor. This may also explain the activity of the 4-fluoro substituted analog (**7k**) that may also benefit from a H-bonding interaction with the receptor[21].

Based on these SAR studies and PK studies that showed that compound **7z** crossed the BBB, **7z** was tested for activity in the NOR paradigm after i.p. dosing. Enhancement of NOR performance was observed with a minimum effective dose i.p. of 1.0 mg/kg (2.7 μmol/kg). This effect was reversed by the selective α7 nAChR antagonist MLA, which is consistent with activity mediated by α7 nAChRs.

Conclusions

A series of substituted arylpyrid-3-ylmethanones (**7**) were designed from enaminone esters and amides (**1a-c**) that had been described as allosteric modulators of both GABA$_A$ and α7 nAChRs.[9] The methanones were found to be Type I PAMs at α7 nAChRs that maintain the native kinetics of the receptor and unlike Type II PAMs do not reverse receptor desensitization. The compounds were poorly active at $\alpha_1\beta_2\gamma_{2L}$ GABA$_A$Rs and inactive at $\alpha_4\beta_2$ and $\alpha_3\beta_4$ nAChRs. SAR studies resulted in the synthesis of compound **7v** that exhibited potent and efficacious modulation of nicotine EC5 responses (EC50 = 0.18 μM, max. mod. = 1200%). Compound **7z** was found to penetrate the CNS after i.p. dosing in mice and was active in the NOR paradigm with a minimum effective dose i.p. of 1.0 mg/kg (2.7 μmol/kg). Additional studies are underway to more fully characterize the activity of these compounds in other animal models relevant to the treatment of neuropsychiatric disorders including schizophrenia, ADHD and Alzheimer's disease.

Experimental Section

Chemistry

^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova spectrometer at 400 and 100 MHz, respectively, or with a Bruker 400 or 500 MHz NMR in CDCl3 referenced to CHCl$_3$ (7.26 ppm) or to tetramethylsilane (0.00 ppm) and to CDCl$_3$ at 77.00 ppm or DMSO-d6 referenced to DMSO-d$_5$ (2.50 ppm). Melting points were determined on an Electrothermal MEL-TEMP 3.0 apparatus (Barnstead International, Dubuque, IA) and are not corrected. Compounds were determined to have purity >95% using elemental analysis performed by Robertson Microlit Laboratories, Madison, NJ and are within 0.4% of theoretical values unless otherwise noted (see supporting information). Flash chromatography was carried out by using the method of Still[22] *et al*, on 230–400 mesh silica gel (silica gel 60, Geduran) obtained from EMD. Solvents were HPLC grade and were obtained from EMD. DMSO, nicotine, GABA, scopolamine hydrochloride (Scop) and anhydrous solvents were obtained from Aldrich Chemical Co. (Milwaukee, WI). Methyllycaconitine citrate (MLA) was obtained from Tocris Biosciences (Bristol, UK). Reactions were carried out under an atmosphere of dry nitrogen gas. Inhibition of a-bungarotoxin binding by **7z** was carried out by CEREP (Redmond, WA).

Method A: Synthesis of (4-chlorophenyl)[5-(4-ethoxyphenyl)-2-propylamino-3-pyridinyl]methanone (7a) starting from 4-ethoxyphenylacetic acid

α-[(Dimethylamino)methylene]-4-ethoxybenzeneacetaldehyde (3a)—Phosphorus oxychloride (6.4 mL) was slowly added to DMF (8 mL) at 0°C. To the mixture was added 4-ethoxyphenylacetic acid (Acros; 4.53 g; 25.1 mmol) and the reaction was heated at 75 °C for 6 h. After cooling to rt the reaction was added to ice-water and brought to pH 11 with 13.0 g of solid NaOH. After heating at 100°C for 1h, the mixture was allowed to cool to rt. The resulting solid was collected by filtration, washed with cold water and air dried. The crude product was treated with 100 mL of CH$_2$Cl$_2$, filtered to remove insoluble material, dried (MgSO$_4$), filtered and conc. to dryness. The residue was dissolved with heating in 100% EtOAc and subjected to flash chromatography (elution with 100% EtOAc) to give 3.57 g (65% yield) of the title compound as a light yellow solid, mp 101–102°C. TOF MS ES$^+$ m/z 220 (M + H$^+$). ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.08 (s, 1H), 7.08 (d, 2H, *J* = 8.5 Hz), 6.87 (d, 2H, *J* = 8.5 Hz), 6.76 (br s, 1H), 4.03 (q, 2H, *J* = 6.8 Hz), 2.83 (br s, 6H), 1.41 (t, 3H, *J* = 7.0 Hz).

1,2-Dihydro-5-(4-ethoxyphenyl)-2-oxo-3-pyridinecarbonitrile (4a)—Sodium metal (0.70 g, 30 mmol) was added to 50 mL of MeOH and the resulting solution was treated with cyanoacetamide (1.26 g, 15 mmol). The mixture was stirred at rt for 1 h, and solid α-[(dimethylamino)methylene]-4-ethoxybenzeneacetaldehyde (2.50 g, 11.0 mmol) was added. The mixture was heated for 4 h at reflux and allowed to cool to rt. The resulting yellow precipitate was collected and washed with cold MeOH. The solid was then dissolved in hot water (150 mL), filtered, and the filtrate was acidified with a 1N aqueous HCl solution. The resulting white solid was collected, washed with cold water, and dried, affording 1.90 g (70% yield) of **4a**. TOF MS ES$^+$ m/z 241, 263. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 12.40 (s, 1H), 8.47 (s, 1H), 7.99 (s, 1H), 7.49 (d, 2H, *J* = 8.5 Hz), 6.91 (d, 2H, *J* = 8.5 Hz), 4.00 (q, 2H, *J* = 7.0 Hz), 1.28 (t, 3H, *J* = 7.0 Hz). ^{1}H NMR matches lit data[23].

2-Chloro-5-(4-ethoxyphenyl)-3-pyridinecarbonitrile (5a)—**4a** (515 mg, 2.14 mmol) was heated in phosphorus oxychloride (3 mL) at 120–130°C overnight. The resulting solution was evaporated to dryness and the residue was treated with toluene and conc. in vacuo. This material was partitioned between a sat. aq. NaHCO$_3$ solution and CH$_2$Cl$_2$. The aqueous layer was washed twice with CH$_2$Cl$_2$ and the pooled organic layers were extracted

with a sat. aq. NaHCO$_3$ solution and brine. The emulsion that formed was filtered through Celite. The CH$_2$Cl$_2$ layer was then dried (MgSO$_4$), filtered and conc. to dryness. The solid that formed was dissolved in CH$_2$Cl$_2$ and subjected to flash chromatography. Elution with CH$_2$Cl$_2$ gave 340 mg (61% yield) of **5a** as a white solid, mp 162–162.5°C. TOF MS ES$^+$ m/z 259, 261. ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.75 (d, 1H, $J = 2.5$ Hz), 8.11 (d, 1H, $J = 2.5$ Hz), 7.47 (d, 2H, $J = 9.0$ Hz), 7.02 (d, 2H, $J = 8.5$ Hz), 4.10 (q, 2H, $J = 7.0$ Hz), 1.46 (t, 3H, $J = 7.0$ Hz).

5-(4-Ethoxyphenyl)-2-propylamino-3-pyridinecarbonitrile (6a)—

A solution of 2-chloro-5-(4-ethoxyphenyl)-3-pyridinecarbonitrile (0.30 g 1.16 mmol) in 3 mL of DMSO was treated with neat propylamine (0.47 mL, 5.8 mmol). The mixture was heated at 60–70°C overnight and then poured into ice-water. The resulting precipitate was collected, washed with cold water, and dried to give 0.28 g (90% yield) of **6a** as a light yellow solid with mp 103.6–104.6°C. TOF MS ES$^+$ m/z 282. ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.48 (d, 1H, $J = 2.5$ Hz), 7.80 (d, 1H, $J = 2.5$ Hz), 7.34 (d, 2H, $J = 8.5$ Hz), 6.96 (d, 2H, $J = 8.5$ Hz), 5.18 (br s, 1H), 4.07 (q, 2H, $J = 7.0$ Hz), 3.50 (q, 2H, $J = 6.7$ Hz), 1.44 (t, 3H, $J = 7.0$ Hz), 1.02 (t, 3H, $J = 7.2$ Hz).

(4-Chlorophenyl)[5-(4-ethoxyphenyl)-2-propylamino-3-pyridinyl]methanone (7a)—

A solution of **6a** (56 mg, 0.20 mmol) in THF was treated with a solution of 4-chlorophenylmagnesium bromide (1 mL of 1M solution in ether; 1.0 mmol) giving a yellow precipitate. After heating for 20 h at 70°C, the resulting solid was allowed to cool and then treated with 3 mL of an aqueous 6N HCl solution. After heating at reflux for 30 min, the reaction was diluted with water, neutralized with solid K$_2$CO$_3$ (0.5 g) and extracted with EtOAc (3 × 5 mL). The organic layers were pooled, dried (Na$_2$SO$_4$), and concentrated. Purification by flash chromatography eluting with CH$_2$Cl$_2$, followed by crystallization from MeOH gave **7a** (10 mg, 12% yield) as a yellow solid. TOF MS ES$^+$ m/z 395, 397. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.70 (t, $J = 5.1$ Hz, 1H) 8.50 (s, 1H), 7.77 (s, 1H), 7.52 (d, $J = 8.7$ Hz, 2H), 7.40 (d, $J = 8.7$ Hz, 2H), 7.24 (d, $J = 8.9$ Hz, 2H), 6.86 (d, $J = 8.8$ Hz, 2H), 3.98 (q, $J = 7.0$ Hz 2H), 3.52 (q, $J = 7.0$ Hz, 2H), 1.68 (sxt, $J = 7.2$ Hz, 2H), 1.35 (t, $J = 7.0$ Hz, 3H), 0.99 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 196.82 (1C), 158.32 (1C), 157.98 (1C), 152.71 (1C), 140.68 (1C), 137.49 (1C), 137.12 (1C), 130.49 (2C), 129.87 (1C), 128.11 (2C), 127.11 (2C), 123.40 (1C), 115.09 (2C), 111.58 (1C), 63.61 (1C), 42.99 (1C), 22.80 (1C), 14.89 (1C), 11.76 (1C) ppm. Anal. (C$_{23}$H$_{23}$ClN$_2$O$_2$) C, H, N.

Compounds 7b-k were prepared by using Method A and the appropriate starting arylacetic acids, alkylamines and arylmagnesium halides

[5-(4-Ethoxyphenyl)-2-methylamino-3-pyridinyl](4-fluorophenyl)methanone (7b)—

The title compound was prepared by using the procedure described for the synthesis of **7a** except that 4-chlorophenylmagnesium bromide was replaced with 4-fluorophenylmagnesium bromide and propylamine was replaced with methylamine. TOF MS ES$^+$ m/z 351, 352. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.60 (m, 2H), 7.86 (s, 1H), 7.66 (m, 2H), 7.32 (d, $J = 8.8$ Hz 2H), 7.16 (t, $J = 8.6$ Hz, 2H), 6.92 (d, $J = 8.7$ Hz, 2H), 4.03 (q, $J = 7.0$ Hz, 2H), 3.16 (d, $J = 4.9$ Hz 3H), 1.42 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 196.68 (1C), 165.70 (1C), 163.69 (1C), 158.36 (1C), 152.37 (1C), 140.63 (1C), 135.61 (1C), 131.60 (1C), 131.53 (1C), 129.96 (1C), 127.15 (2C), 123.47 (1C), 115.69 (1C), 115.52 (1C), 115.09 (2C), 112.19 (1C), 63.61 (1C), 28.09 (1C), 14.89 (1C) ppm. Anal. (C$_{21}$H$_{19}$FN$_2$O$_2$) C, H, N.

(4-Chlorophenyl)[5-(4-ethoxyphenyl)-2-[(1-ethylpropyl)amino]-3-pyridinyl]methanone (7c)—

7c was prepared as described above for the synthesis of **7a** except that propylamine was replaced with 1-ethylpropylamine. ^{1}H NMR (CDCl$_3$, 500

MHz) δ 8.64 (t, *J* = 8.2 Hz, 1H) 8.47 (s, 1H), 7.76 (s, 1H), 7.52 (d, *J* = 8.7 Hz, 2H), 7.40 (d, *J* = 8.6 Hz, 2H), 7.24 (d, *J* = 8.8 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 4.23 (sxt, *J* = 6.1 Hz 1H), 3.97 (q, *J* = 7.1 Hz, 2H), 1.64 (p, *J* = 7.1 Hz, 2H), 1.55 (p, *J* = 7.1 Hz, 2H), 1.35 (t, *J* = 7.0 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 6H). ^{13}C NMR (CDCl$_3$, 125 MHz) 197.40 (1C), 158.20 (1C), 152.91 (1C), 140.79 (1C), 137.97 (1C), 137.63 (1C), 130.51 (2C), 130.44 (1C), 130.25 (1C), 130.05 (1C), 128.72 (2C), 127.06 (2C), 124.26 (1C), 123.09 (1C), 115.07 (2C), 63.60 (1C), 52.82 (1C), 27.05 (1C), 17.68 (1C), 14.89 (1C), 10.25 (1C) ppm. Anal. (C$_{25}$H$_{27}$ClN$_2$O$_2$) C, H, N.

(4-Chlorophenyl)[2-[(2,5-difluorobenzyl)amino]-5-(4-ethoxyphenyl)-3-pyridinyl]methanone (7d)—7d was prepared as described above for the synthesis of **7a** except that propylamine was replaced with 2,5-difluorobenzylamine. The compound was isolated as a yellow solid with mp 140.5–143°C. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 9.08 (t, *J* = 5.9 Hz, 1H), 8.63 (s, 1H), 8.41 (s, 1H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.52 (d, *J* = 8.5 Hz, 2H), 7.39 (d, *J* = 8.7 Hz, 2H), 7.20 (ddd, *J* = 3.1, 5.3, 8.8 Hz, 1H), 7.08 (sxt, *J* = 4.4 Hz, 1H), 7.00 (d, *J* = 8.7 Hz, 2H), 6.97 (m, 1H), 4.96 (d, *J* = 6.0 Hz, 2H), 4.11 (q, *J* = 7.0 Hz, 2H), 1.33 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 196.88 (1C), 159.87 (1C), 158.48 (1C), 157.52 (1C), 156.33 (1C), 155.21 (1C), 152.43 (1C), 140.61 (1C), 137.99 (1C), 137.54 (1C), 130.59 (2C), 129.67 (1C), 128.81 (2C), 127.71 (1C), 127.24 (2C), 124.59 (1C), 116.42 (1C), 115.12 (2C), 114.75 (1C), 112.16 (1C), 63.61 (1C), 38.51 (1C), 14.89 (1C) ppm. Anal. (C$_{27}$H$_{21}$F$_2$N$_2$O$_2$) C, H, N.

(4-Chlorophenyl)[5-(4-ethoxyphenyl)-2-(phenethylamino)-3-pyridinyl]methanone (7e)—7e was prepared as described above for the synthesis of **7a** except that propylamine was replaced with phenethylamine. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.70 (t, *J* = 5.0 Hz, 1H) 8.57 (s, 1H), 7.85 (s, 1H), 7.65 (m, 2H), 7.32 (m, 5H), 7.22 (m, 2H), 7.16 (t, *J* = 8.6 Hz, 2H), 6.94 (d, *J* = 8.6 Hz, 2H), 4.05 (q, *J* = 7.0 Hz 2H), 3.88 (q, *J* = 7.0 Hz, 2H), 3.02 (t, *J* = 7.2 Hz, 2H), 1.42 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 196.55 (1C), 165.62 (1C), 163.61 (1C), 158.29 (1C), 157.71 (1C), 152.31 (1C), 140.54 (1C), 139.46 (1C), 135.55 (1C), 131.52 (1C), 129.91 (1C), 128.84 (2C), 128.52 (2C), 127.09 (2C), 126.34 (1C), 123.59 (1C), 115.61 (1C), 115.44 (1C), 115.04 (2C), 111.96 (1C), 63.56 (1C), 42.69 (1C), 35.87 (1C), 14.83 (1C) ppm. Anal. (C$_{28}$H$_{25}$FN$_2$O$_2$) C, H, N.

[5-(4-Ethoxyphenyl)-2-propylamino-3-pyridinyl](2-methylphenyl)methanone (7f)—7f was prepared as described above for the synthesis of **7a** except that 2-chlorophenylmagnesium chloride was replaced with 2-tolylmagnesium chloride. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 9.14 (t, *J* = 4.9 Hz, 1H), 8.54 (s, 1H), 7.63 (s, 1H), 7.36 (t, *J* = 7.0 Hz, 1H), 7.28 (d, *J* = 7.6 Hz 2H), 7.25 (m, 3H), 6.88 (d, *J* = 8.8 Hz, 2H), 4.02 (q, *J* = 7.0 Hz, 2H), 3.62 (q, *J* = 7.0 Hz 2H), 2.29 (s, 3H), 1.77 (sxt, *J* = 7.3 Hz, 2H), 1.40 (t, *J* = 7.0 Hz, 3H), 1.07 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 200.36 (1C), 158.20 (1C), 157.84 (1C), 152.94 (1C), 141.24 (1C), 139.51 (1C), 135.30 (1C), 130.82 (1C), 129.92 (1C), 129.59 (1C), 127.29 (1C), 127.10 (2C), 125.42 (1C), 123.58 (1C), 114.96 (2C), 112.49 (1C), 63.53 (1C), 42.91 (1C), 22.77 (1C), 19.59 (1C), 14.82 (1C), 11.73 (1C) ppm. Anal. (C$_{24}$H$_{26}$N$_2$O$_2$) C, H, N.

[5-(4-Ethoxyphenyl)-2-propylamino-3-pyridinyl](4-fluorophenyl)methanone (7g)—7g was prepared by using the procedure described for the synthesis of **7a** except that 4-chlorophenylmagnesium bromide was replaced with 4-fluorophenylmagnesium bromide. The compound was isolated as a yellow solid with mp 104.2–104.7°C. TOF MS ES$^+$ 379. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.72 (t, *J* = 5.0 Hz, 1H) 8.56 (s, 1H), 7.86 (s, 1H), 7.66 (m, 2H), 7.33 (d, *J* = 8.8 Hz, 2H), 7.17 (d, *J* = 8.7 Hz, 2H), 6.93 (d, *J* = 8.7 Hz, 2H), 4.04 (q, *J* = 7.0 Hz 2H), 3.59 (q, *J* = 7.1 Hz, 2H), 1.74 (t, *J* = 7.3 Hz, 2H), 1.42 (t, *J* = 7.0 Hz, 3H),

1.06 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 196.64 (1C), 165.62 (1C), 163.61 (1C), 158.25 (1C), 157.43 (1C), 152.43 (1C), 140.63 (1C), 135.63 (1C), 131.50 (1C), 129.96 (1C), 127.05 (2C), 123.29 (1C), 115.62 (1C), 115.45 (1C), 115.03 (2C), 111.71 (1C), 63.55 (1C), 42.94 (1C), 22.75 (1C), 14.83 (1C), 11.71 (1C) ppm. Anal. (C$_{23}$H$_{23}$FN$_2$O$_2$) C, H, N.

[5-(4-Ethoxyphenyl)-2-propylamino-3-pyridinyl](3-fluorophenyl)methanone (7h)—**7h** was prepared by using the procedure described for the synthesis of **7a** except that 4-chlorophenylmagnesium bromide was replaced with 3-fluorophenylmagnesium bromide. TOF MS ES$^+$ 379. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.72 (t, J = 5.0 Hz, 1H) 8.56 (s, 1H), 7.86 (s, 1H), 7.65 (dd, J = 8.7, 5.5 Hz, 2H), 7.32 (d, J = 8.8 Hz, 2H), 7.17 (d, J = 8.7 Hz, 2H), 6.93 (d, J = 8.7 Hz, 2H), 4.05 (q, J = 7.0 Hz 2H), 3.59 (q, J = 7.1 Hz, 2H), 1.75 (t, J = 7.3 Hz, 2H), 1.42 (t, J = 7.0 Hz, 3H), 1.06 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 196.73 (1C), 165.69 (1C), 163.68 (1C), 158.32 (1C), 157.99 (1C), 152.50 (1C), 140.71 (1C), 135.68 (1C), 131.57 (1C), 130.03 (1C), 127.12 (2C), 123.36 (1C), 115.69 (2C), 115.10 (2C), 111.78 (1C), 63.61 (1C), 43.00 (1C), 22.80 (1C), 14.89 (1C), 11.77 (1C) ppm. Anal. (C$_{23}$H$_{23}$FN$_2$O$_2$-½MeOH) C, H, N.

[5-(4-Ethoxyphenyl)-2-(N-methylpropylamino)-3-pyridinyl](4-fluorophenyl)methanone (7i)—**7i** was prepared as described above for the synthesis of **7a** except that 4- chlorophenylmagnesium bromide was replaced with 4-fluorophenylmagnesium bromide and propylamine was replaced with *N*-methylpropylamine. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.56 (s, 1H), 7.98 (m, 2H), 7.79 (s, 1H), 7.46 (d, J = 8.8 Hz, 2H), 7.21 (t, J = 8.6 Hz, 2H), 7.00 (d, J = 8.9 Hz, 2H), 4.11 (q, J = 7.0 Hz, 2H), 3.57 (q, J = 7.0 Hz 2H), 2.83 (s, 3H), 1.49 (t, J = 7.0 Hz, 3H), 1.17 (t, J = 7.0 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 194.58 (1C), 166.83 (1C), 164.80 (1C), 158.35 (1C), 157.19 (1C), 147.74 (1C), 137.61 (1C), 133.22 (1C), 132.61 (1C), 129.90 (1C), 127.33 (2C), 125.00 (1C), 118.85 (1C), 115.84 (2C), 115.05 (2C), 63.59 (1C), 46.71 (1C), 38.46 (1C), 14.90 (1C), 12.15 (1C) ppm. Anal. (C$_{23}$H$_{23}$FN$_2$O$_2$) C, H, N.

(4-Chlorophenyl)[5-(4-chlorophenyl)-2-propylamino-3-pyridinyl]methanone (7j)—**7j** was prepared by using the procedure described for the synthesis of **7a** except that 4-ethoxyphenylacetic acid was replaced with 4-chlorophenylacetic acid. **7j** was isolated as a yellow solid with mp 130–131°C. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.83 (t, J = 5.0 Hz, 1H) 8.57 (s, 1H), 7.86 (s, 1H), 7.58 (d, J = 8.5 Hz, 2H), 7.48 (d, J = 6.5 Hz, 2H), 7.37 (d, J = 8.5 Hz, 2H), 7.33 (d, J = 6.5 Hz, 2H), 3.60 (q, J = 7.0 Hz, 2H), 1.75 (sxt, J = 7.5 Hz, 2H), 1.06 (t, J = 7.5 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 196.71 (1C), 158.35 (1C), 152.75 (1C), 140.89 (1C), 137.97 (1C), 137.65 (1C), 136.04 (1C), 133.07 (1C), 130.48 (2C), 129.24 (2C), 128.82 (2C), 127.20 (2C), 122.31 (1C), 111.59 (1C), 43.01 (1C), 22.76 (1C), 11.74 (1C) ppm. Anal. (C$_{21}$H$_{18}$Cl$_2$N$_2$O) C, H, N.

(4-Fluorophenyl)[5-(4-fluorophenyl)-2-propylamino-3-pyridinyl]methanone (7k)—**7k** was prepared by using the procedure described for the synthesis of **7a** except that 4-ethoxyphenylacetic acid was replaced with 4-fluorophenylacetic acid and 4-chlorophenylmagnesium bromide was replaced with 4-fluorophenylmagnesium bromide. **7k** was isolated as a yellow solid with mp 98.8–99.8°C. TOF MS ES$^+$ m/z 353. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.82 (t, J = 5.9 Hz, 1H), 8.61 (s, 1H), 7.92 (s, 1H), 7.73 (m, 2H), 7.42 (m, 2H), 7.24 (t, J = 8.6 Hz, 2H), 7.15 (t, J = 8.6 Hz, 2H), 3.66 (t, J = 7.0 Hz, 2H), 1.81 (sxt, J = 7.2 Hz, 2H), 1.12 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 196.62 (1C), 163.18 (1C), 161.22 (1C), 158.58 (1C), 152.58 (1C), 140.95 (1C), 135.53 (1C), 133.77 (1C), 131.50 (2C), 127.58 (2C), 122.61 (1C), 115.91 (2C), 115.58 (2C), 111.75 (1C), 43.00 (1C), 22.77 (1C), 11.75 (1C) ppm. Anal. (C$_{21}$H$_{18}$F$_2$N$_2$O) C, H, N.

Method B: Use of 2-Chloronicotinonitrile as Starting Material. Synthesis of (4-fluorophenyl) [2-propylamino-5-[(4-trifluoromethoxy)phenyl]-3-pyridinyl]methanone (7l)

2-Propylamino-3-pyridinecarbonitrile (8)—2-Chloronicotinonitrile (2-chloro-3-cyanopyridine; 1.46 g, 10.5 mmol) was dissolved in 5 mL of DMSO and neat propylamine (4.0 mL, 2.88 g, 48.7 mmol) was added. After stirring at rt for 2 days, the reaction was treated with ice and water. The resulting precipitate was collected, washed with water and dried, affording 1.44 g (85% yield) of the title compound as a white solid, mp 31–33°C. Recrystallization from hexane (reflux to 10°C) gave **8** as a solid, mp 34–35.3°C. Lit[24a] mp 33–35°C, 38°C[24b]. ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.28 (dd, 1H, J = 5.0, 1.5 Hz), 7.63 (d, 1H, J = 7.5 Hz), 6.57 (dd, 1H, J = 7.5, 5.0 Hz), 5.17 (br s, 1H), 3.46 (q, 2H, J = 6.8 Hz), 1.66 (sxt, 2H, J = 7.0 Hz), 1.00 (t, 3H, J = 7.5 Hz).

5-Bromo-2-propylamino-3-pyridinecarbonitrile (9)—A solution of **8** (1.02 g, 6.30 mmol) in 5 mL of dry DMF at 0 °C was treated dropwise with a solution of *N*-bromosuccinimide (1.25 g, 7.00 mmol) in 5 mL of DMF. After stirring overnight at room temperature, the reaction mixture was poured into ice-water (20 mL), and the pH was brought to 12 with an aqueous 10% KOH solution. The resulting precipitate was collected, washed with cold water and air dried, affording 1.46 g (96% yield) of the title compound as a light yellow solid. This solid was recrystallized from 9:1 hexanes/EtOAc to give **9** as colorless needles, mp 106.4–106.9°C. TOF MS ES$^+$ m/z 240, 242. ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.29 (d, 1H, J = 2.0 Hz), 7.71 (d, 1H, J = 2.5 Hz), 5.21 (br s, 1H), 3.44 (q, 2H, J = 6.8 Hz), 1.65 (sxt, 2H, J = 7.0 Hz), 0.99 (t, 3H, J = 7.5 Hz).

2-Propylamino-[5-(4-trifluoromethoxy)phenyl]-3-pyridinecarbonitrile (6l)—A solution **9** (450 mg, 1.87 mmol) and 4-(trifluoromethoxy)phenylboronic acid (397 mg, 1.94 mmol) in a mixture containing toluene (15 mL), a solution of Na$_2$CO$_3$ (259 mg) in water (4 mL) and EtOH (4 mL) was treated with 113 mg of Pd(PPh$_3$)$_4$ and heated at reflux for 16 h. Once at rt, the reaction was diluted with 25 mL each of toluene and water. The aqueous layer was washed with toluene (2 × 15 mL). The pooled organic layer was washed with water and brine, dried (MgSO$_4$), filtered and conc. The residue was dissolved in CH$_2$Cl$_2$ purified by flash chromatography (elution with 100% CH$_2$Cl$_2$) affording 490 mg (81% yield) of **6l** as an off-white solid, mp 117.5–118°C. ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.50 (d, 1H, J = 2.0 Hz), 7.82 (d, 1H, J = 2.5 Hz), 7.47 (d, 2H, J = 9.0 Hz), 7.29 (d, 2H, J = 8.0 Hz), 5.27 (br s, 1H), 3.52 (q, 2H, J = 7.0 Hz), 1.70 (sxt, 2H, J = 7.2 Hz), 1.02 (t, 3H, J = 7.5 Hz).

(4-Fluorophenyl)[2-propylamino-5-[(4-trifluoromethoxy)phenyl]-3-pyridinyl]methanone (7l)—A solution of **6l** (160 mg, 0.50 mmol) in THF (2 mL) was treated with a 1M solution of 4-fluorophenylmagnesium bromide in THF (5 mL, 5 mmol). The solution was heated at 65°C overnight and then allowed to cool. At rt, the reaction was treated with 5 mL of a 6N HCl solution and refluxed for 30 min. The reaction was then partitioned between EtOAc and water. The two phase mixture was treated with solid K$_2$CO$_3$ until neutralized. The organic layer was dried (Na$_2$SO$_4$), filtered and conc. in vacuo. Flash chromatography (4:1 hexanes/EtOAc) gave the crude product which was recrystallized from MeOH affording 40 mg (20% yield) of **7l** as a yellow solid, mp 91.3–92°C. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.79 (t, J = 4.9 Hz, 1H) 8.57 (s, 1H), 7.88 (s, 1H), 7.66 (m, 2H), 7.41 (d, J = 8.6 Hz, 2H), 7.25 (d, J = 8.4 Hz, 2H), 7.18 (t, J = 8.5 Hz, 2H), 3.60 (q, J = 6.9 Hz 2H), 1.74 (sxt, J = 7.2 Hz, 2H), 1.06 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 196.58 (1C), 165.78 (1C), 163.77 (1C), 158.40 (1C), 152.62 (1C), 148.35 (1C), 141.03 (1C), 135.48 (1C), 134.32 (1C), 131.59 (1C), 131.52 (1C), 127.30 (2C), 122.12 (1C), 121.66 (1C), 119.51 (1C), 115.61 (2C), 111.79 (1C), 43.01 (1C), 22.76 (1C), 11.73 (1C) ppm. Anal. (C$_{22}$H$_{18}$F$_4$N$_2$O$_2$) C, H, N.

Compounds 7m, 7n and 7o were prepared using Method B

(4-Fluorophenyl)[2-propylamino-5-(4-propylphenyl)-3-pyridinyl]methanone
(7m)—7m was prepared by using the procedure described for the synthesis of **7l** except that
(4-trifluoromethoxy)phenylboronic acid was replaced with 4-propylphenylboronic acid.
Purification by flash chromatography (10:1 hexanes/EtOAc) followed by recrystallization
from MeOH gave a 46 % yield of the product as a yellow solid. ^{1}H NMR (CDCl$_3$, 500
MHz) δ 8.75 (t, J = 4.7 Hz, 1H), 8.60 (s, 1H), 7.90 (s, 1H), 7.66 (dd, J = 5.5, 8.7, 2H), 7.32
(d, J = 8.1 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 7.17 (t, J = 8.7 Hz, 2H), 3.59 (q, J = 6.6 Hz,
2H), 2.60 (t, J = 7.7 Hz 2H), 1.75 (sxt, J = 7.3 Hz, 2H), 1.64 (sxt, J = 7.5 Hz, 2H), 1.06 (t, J
= 7.5 Hz, 3H), 0.95 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 196.74 (1C), 165.70
(1C), 163.69 (1C), 158.19 (1C), 152.73 (1C), 141.71 (1C), 140.99 (1C), 135.67 (1C), 135.02
(1C), 131.54 (1C), 129.22 (2C), 125.90 (2C), 123.54 (1C), 115.53 (2C), 111.81 (1C), 43.03
(1C), 37.70 (1C), 24.64 (1C), 22.82 (1C), 13.93 (1C), 11.79 (1C) ppm. Anal. (C$_{24}$H$_{25}$FN$_2$O)
C, H, N.

(4-Fluorophenyl)[5-(4-phenoxyphenyl)-2-propylamino-3-pyridinyl]methanone
(7n)—7n was prepared by using the procedure described for the synthesis of **7l** except that
(4-trifluoromethoxy)phenylboronic acid was replaced with 4-phenoxyphenylboronic acid.
The crude product was purified by flash chromatography (10:1 hexanes/EtOAc) and then
recrystallized from MeOH affording a 50% yield of the product. ^{1}H NMR (CDCl$_3$, 500
MHz) δ 8.75 (t, J = 5.0 Hz, 1H), 8.57 (s, 1H), 7.88 (s, 1H), 7.67 (m, 2H), 7.37 (d, J = 7.3 Hz,
2H), 7.31 (d, J = 7.4 Hz, 2H), 7.17 (t, J = 8.7 Hz, 2H), 7.09 (t, J = 7.4 Hz, 2H), 7.02 (m,
3H), 3.60 (q, J = 7.1 Hz, 2H), 1.74 (sxt, J = 7.3 Hz, 2H), 1.04 (t, J = 7.4 Hz, 3H). ^{13}C NMR
(CDCl$_3$, 125 MHz) 196.58 (1C), 165.65 (1C), 163.64 (1C), 158.10 (1C), 156.92 (1C),
156.65 (1C), 152.50 (1C), 140.77 (1C), 135.58 (1C), 131.52 (1C), 131.45 (1C), 129.80 (2C),
127.28 (2C), 123.48 (1C), 122.90 (1C), 119.26 (2C), 119.02 (2C), 115.47 (2C), 111.73 (1C),
42.95 (1C), 22.74 (1C), 11.69 (1C) ppm. Anal. (C$_{27}$H$_{23}$FN$_2$O$_2$) C, H, N.

[5-[(4-Ethylthio)phenyl]-2-propylamino-3-pyridinyl](4-fluorophenyl)methanone
(7o)—7o was prepared by using the procedure described for the synthesis of **7l** except that
4-(trifluoromethoxy)phenylboronic acid was replaced with 4-(ethylthio)phenylboronic acid.
The product was isolated in 30% yield after flash chromatography (4:1 hexanes/EtOAc) and
recrystallization form MeOH. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.77 (t, J = 5.2 Hz, 1H), 8.59
(s, 1H), 7.90 (s, 1H), 7.67 (dd, J = 8.5, 5.4 Hz, 2H), 7.34 (dd, J = 12.1, 8.6 Hz, 4H), 7.18 (t,
J = 8.4 Hz, 2H), 3.59 (q, J = 7.0 Hz, 2H), 2.94 (q, J = 7.4 Hz, 2H), 1.74 (sxt, J = 7.3 Hz,
2H), 1.32 (t, J = 7.3 Hz, 3H), 1.06 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 196.57
(1C), 165.67 (1C), 163.66 (1C), 158.15 (1C), 140.78 (1C), 135.48 (1C), 135.05 (1C), 131.42
(2C), 129.57 (2C), 126.26 (2C), 122.77 (1C), 115.68 (2C), 115.50 (1C), 111.77 (1C), 42.97
(1C), 27.69 (1C), 22.72 (1C), 14.39 (1C), 11.69 (1C) ppm. Anal. (C$_{23}$H$_{23}$FN$_2$OS) C, H, N.

Method C: Use of 2-fluoropyridine as starting material, synthesis of (3,4-difluorophenyl)[5-(4-ethoxyphenyl)-2-methylamino-3-pyridinyl]methanone (7z)

α-(3,4-Difluorophenyl)-2-fluoro-3-pyridinemethanol (10z)—A solution of 2-
fluoropyridine (1.49 g, 15.3 mmol) in 4 mL of dry THF was cooled in a dry-ice/acetone bath
for 10 min and a 1.5M solution of LDA-THF in cyclohexane (Aldrich; 10.2 mL, 15.3 mmol)
was added dropwise via syringe over 36 min. After stirring cold for 2 hrs, neat 3,4-
difluorobenzaldehyde (1.7 mL, 2.2 g, 15.4 mmol) was added dropwise via syringe over 20
min. The reaction was allowed to warm to rt overnight. The reaction was cooled in an ice-
water bath and quenched with water and ice. The mixture was then extracted with EtOAc (3
× 25 mL). The EtOAc layers were washed with brine, dried (MgSO$_4$), filtered and conc to
dryness affording 3.96 g of crude product. The residue was an orange oil that solidified on
standing. Recrystallization from 40 mL of 3:1 hexanes/EtOAc gave 1.75 g of the product as

a light yellow solid, mp 108.6–111°C. The mother liquor was purified by flash chromatography affording an additional 902 mg of the product. Total yield 2.65 g (72%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.12 (d, 1H, J = 3.6 Hz), 7.97 (t, 1H, J = 6.0 Hz), 7.27–7.22 (m, 2H), 7.16–7.12 (m, 2H), 6.03 (s, 1H), 2.78 (s, 1H).

(3,4-Difluorophenyl)(2-fluoro-3-pyridinyl)methanone (11z)—A solution of **10z** (2.42 g, 10.1 mmol) in CH$_2$Cl$_2$ (70 mL) was treated with NaOAc (2.5 g) and Celite (4.1 g). Solid PCC (3.16 g) was added in portions. An additional 2.37 g of PCC was added over 2 h. The resulting dark mixture stirred at rt for an additional 1 h and then added to a flash column. Elution with 100% CH$_2$Cl$_2$ gave 2.30 g (96%) of0025;) of **11z** as a colorless oil that solidified on standing, mp 47.6–48.3°C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.45 (d, 1H, J = 3.5 Hz), 8.05 (dt, 1H, J = 8.0, 1.8 Hz), 7.71 (m, 1H), 7.57 (m, 1H), 7.40 (m, 1H), 7.29 (dd, 1H, J = 17.0, 8.5 Hz).

(3,4-Difluorophenyl)(2-methylamino-3-pyridinyl)methanone (12z)—A solution of **11z** (596 mg, 2.51 mmol) in EtOH (3 mL) was treated with a solution of methylamine in EtOH (Aldrich, 33 weight %; 0.77 mL, 257 mg, 8.26 mmol). The light yellow solution was stirred at rt overnight and the resulting yellow ppt that formed was isolated by filtration and washed with EtOH (3 mL). The mother liquor showed recovered starting material by TLC. The solid and mother liquor were comb. and conc. to dryness. The residue was dissolved in EtOH and treated with 0.5 mL of methylamine solution. After stirring overnight, a ppt had formed. The mixture was filtered and the solid was washed with EtOH (3 mL) affording 294 mg (47 %) of the product, mp 114–115.5°C. The mother liquor was conc. in vacuo. and adsorbed onto silica gel. Flash chromatography (4:1 hexanes/EtOAc) gave an additional 234 mg of the product. Total yield 528 mg (85%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.64 (br s, 1H), 8.37 (d, 1H, J = 5.0 Hz), 7.70 (d, 1H, J = 7.5 Hz), 7.45 (t, 1H, J = 9.5 Hz), 7.34 (m, 1H), 7.29 (m, 1H), 6.52 (m, 1H), 3.13 (d, 3H, J = 5.0 Hz).

(5-Bromo-2-methylamino-3-pyridinyl)(3,4-difluorophenyl)methanone (13z)—A solution of **12z** (489 mg, 1.97 mmol) in 8 mL of DMF at 0°C was treated with solid NBS (Aldrich, MW 177.98; 357 mg, 2.01 mmol). The reaction was allowed to warm to rt and stirred for 4 h. The reaction was diluted with ice-water and extracted with EtOAc. The aqueous layer was washed with EtOAc (2 × 15 mL). The pooled EtOAc layers were washed with water (2 ×15 mL), a 10% aqueous thiosulfate solution (40 mL), water and brine. After drying (MgSO$_4$), the mixture was filtered and conc. to dryness, affording 613 mg of the product, mp 150.4–151.7°C. ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.58 (br s, 1H), 8.37 (s, 1H), 7.75 (s, 1H), 7.46 (t, 1H, J = 8.0 Hz), 7.34 (m, 1H), 7.30 (m, 1H), 3.10 (d, 3H, J = 5.0 Hz).

(3,4-Difluorophenyl)[5-(4-ethoxyphenyl)-2-methylamino-3-pyridinyl]methanone (7z)—A mixture of **13z** (453 mg, 1.38 mmol) and 4-ethoxyphenylboronic acid (Aldrich; 250 mg, 1.51 mmol) was dissolved in toluene (12 mL) and EtOH (3 mL). A soln of Na$_2$CO$_3$ (213 mg 2.01 mmol) in 3 mL of HPLC grade water was added. An N$_2$ stream was bubbled through the mixture for 15 min. Solid Pd(PPh$_3$)$_4$ (77 mg) was added and the mixture was placed in an oil bath at 90°C for 3.5 h. Once at rt, the reaction was partitioned between toluene and water (25 mL of each). The aqueous layer was extracted with toluene (2 × 20 mL) and the pooled toluene layers were washed with water and brine, dried (MgSO$_4$), filtered and conc. The crude product was dissolved in CH$_2$Cl$_2$, and purified by flash chromatography. Elution with 10% EtOAc/CH$_2$Cl$_2$ gave 456 mg (90%) of the product as a yellow solid with mp 142.5–144°C. TOF MS ES[+] m/z 369. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.60 (s, 1H), 8.58 (m, 1H), 7.83 (s, 1H), 7.50 (m, 1H), 7.38 (ddd, J = 8.2, 4.4, 2.1 Hz, 1H), 7.32 (d, J = 8.7 Hz, 2H), 7.26 (t, J = 9.0 Hz, 1H), 6.93 (d, J = 7.7 Hz, 2H), 4.05 (q, J = 7.0 Hz, 2H), 3.16 (d, J = 5.0 Hz, 3H), 1.42 (t, J = 7.0 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz)

195.23 (1C), 158.46 (1C), 153.53 (1C), 153.43 (1C), 151.41 (1C), 149.32 (1C), 140.43 (1C), 136.31 (1C), 129.81 (1C), 127.20 (2C), 125.87 (1C), 123.64 (1C), 118.60 (1C), 117.49 (1C), 115.16 (2C), 111.66 (1C), 63.64 (1C), 28.13 (1C), 14.90 (1C) ppm. Anal. ($C_{21}H_{18}F_2N_2O_2$-MeOH) C, H, N.

The synthesis of 7p–7y and 7aa used Method C as described above for the synthesis of 7z

[2-Amino-5-(4-ethoxyphenyl)-3-pyridinyl](4-chlorophenyl)methanone (7p)—7p
was prepared in 41% yield as described above for the synthesis of **7z** except that 3,4-difluorobenzaldehyde was replaced with 4-chlorobenzaldehyde and methylamine was replaced with ammonia. TOF MS ES$^+$ m/z 337, 339. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.84 (t, J = 5.1 Hz, 1H), 8.48 (s, 1H), 7.86 (s, 1H), 7.62 (d, J = 8.4 Hz, 2H), 7.47 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.6 Hz, 2H), 6.93 (d, J = 8.6 Hz, 2H), 6.82 (s, 2H), 4.04 (q, J = 7.0 Hz, 2H), 1.42 (t, J = 7.0 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 196.48 (1C), 158.57 (1C), 158.53 (1C), 152.22 (1C), 140.18 (1C), 138.15 (1C), 137.40 (1C), 130.63 (2C), 129.58 (1C), 128.85 (2C), 127.32 (2C), 125.62 (1C), 115.14 (2C), 112.35 (1C), 63.65 (1C), 14.90 (1C) ppm. Anal. ($C_{20}H_{17}ClN_2O_2$) C, H, N.

(4-Chlorophenyl)[5-(4-ethoxyphenyl)-2-methylamino-3-pyridinyl]methanone
(7q)—7q was prepared as described above for the synthesis of **7z** except that 3,4-difluorobenzaldehyde was replaced with 4-chlorobenzaldehyde. **7q** was isolated as a yellow solid with mp 135.5–136°C. TOF MS ES$^+$ m/z 367. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.55 (t, J = 3.5 Hz, 1H), 8.51 (s, 1H), 7.76 (s, 1H), 7.49 (d, J = 8.3 Hz, 2H), 7.38 (d, J = 8.3 Hz, 2H), 7.23 (d, J = 8.5 Hz, 2H), 6.84 (d, J = 8.6 Hz, 2H), 3.97 (q, J = 7.0 Hz, 2H), 3.09 (d, J = 4.9 Hz, 3H), 1.32 (t, J = 7.0 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 196.80 (1C), 158.45 (1C), 158.36 (1C), 152.58 (1C), 140.61 (1C), 137.97 (1C), 137.75 (1C), 130.49 (2C), 129.88 (1C), 128.75 (2C), 127.15 (2C), 123.51 (1C), 115.10 (2C), 111.99 (1C), 63.61 (1C), 28.09 (1C), 14.89 (1C) ppm. Anal. ($C_{21}H_{19}ClN_2O_2$) C, H, N.

(4-Chlorophenyl)[5-(4-ethoxyphenyl)-2-ethylamino-3-pyridinyl]methanone (7r)
—7r was prepared as described above for the synthesis of **7z** except that 3,4-difluorobenzaldehyde was replaced with 4-chlorobenzaldehyde and methylamine was replaced with ethylamine. TOF MS ES$^+$ m/z 381. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.74 (t, J = 5.1 Hz, 1H), 8.63 (s, 1H), 7.90 (s, 1H), 7.64 (d, J = 8.5 Hz, 2H), 7.53 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 8.7 Hz, 2H), 6.99 (d, J = 8.7 Hz, 2H), 4.11 (q, J = 7.0 Hz, 2H), 3.72 (q, J = 7.0 Hz, 2H), 1.49 (t, J = 7.0 Hz, 3H), 1.41 (t, J = 7.3 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 196.84 (1C), 158.34 (1C), 157.88 (1C), 152.70 (1C), 140.67 (1C), 137.86 (1C), 137.74 (1C), 130.50 (2C), 129.98 (1C), 128.76 (2C), 127.13 (2C), 123.46 (1C), 115.10 (2C), 111.63 (1C), 63.61 (1C), 35.99 (1C), 14.95 (1C), 14.89 (1C) ppm. Anal. ($C_{22}H_{21}ClN_2O_2$) C, H, N.

(2-Chlorophenyl)[5-(4-ethoxyphenyl)-2-propylamino-3-pyridinyl]methanone
(7s)—7s was prepared using the procedure for **7z** except that 3,4-difluorobenzaldehyde was replaced with 2- chlorobenzaldehyde and methylamine was replaced with propylamine. TOF MS ES$^+$ m/z 395, 397. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 9.08 (t, J = 5.1 Hz, 1H), 8.55 (s, 1H), 7.57 (s, 1H), 7.48 (d, J = 7.9 Hz, 1H), 7.42 (t, J = 7.3 Hz, 1H), 7.37 (t, J = 7.3 Hz, 1H), 7.32 (d, J = 7.6 Hz, 1H), 7.25 (d, 2H), 6.90 (d, J = 8.8 Hz, 2H), 4.02 (q, J = 7.0 Hz, 2H), 3.63 (q, J = 7.0 Hz, 2H), 1.77 (sxt, J = 7.3 Hz, 2H), 1.40 (t, J = 7.0 Hz, 3H), 1.07 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 196.41 (1C), 158.35 (1C), 157.82 (1C), 153.62 (1C), 141.29 (1C), 138.96 (1C), 130.85 (1C), 130.82 (1C), 130.10 (1C), 130.05 (1C), 128.69 (1C), 127.27 (2C), 126.90 (1C), 123.93 (1C), 115.02 (2C), 111.75 (1C), 63.59 (1C), 43.00 (1C), 22.81 (1C), 14.88 (1C) 11.77 (1C) ppm. Anal. ($C_{23}H_{23}ClN_2O_2$) C, H, N.

(2,4-Dichorophenyl)[5-(4-ethoxyphenyl)-2-propylamino-3-pyridinyl]methanone (7t)—**7t** was prepared as described above for the synthesis of **7z** except that 3,4-difluorobenzaldehyde was replaced with 2,4-dichlorobenzaldehyde and methylamine was replaced with propylamine. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 9.04 (t, J = 5.1 Hz, 1H), 8.55 (s, 1H), 7.54 (s, 1H), 7.50 (s, 2H), 7.45 (d, J = 8.7 Hz, 2H), 7.35 (d, J = 8.2 Hz, 2H), 7.26 (d, J = 8.4 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 4.02 (q, J = 7.0 Hz, 2H), 3.63 (q, J = 6.6 Hz, 2H), 1.77 (sxt, J = 7.3 Hz, 2H), 1.39 (t, J = 7.0 Hz, 3H), 1.06 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 196.40 (1C), 158.30 (1C), 157.81 (1C), 153.61 (1C), 141.27 (1C), 138.95 (1C), 130.85 (1C), 130.82 (1C), 130.09 (1C), 130.03 (1C), 128.69 (1C), 127.26 (2C), 126.89 (1C), 123.93 (1C), 115.02 (2C), 111.75 (1C), 63.58 (1C), 43.00 (1C), 22.82 (1C), 14.88 (1C) 11.78 (1C) ppm. Anal. (C$_{23}$H$_{22}$Cl$_2$N$_2$O$_2$) C, H, N.

[5-(4-Ethoxyphenyl)-2-propylamino-3-pyridinyl](2-fluorophenyl)methanone (7u)—**7u** was prepared as described above for the synthesis of **7z** except that 3,4-difluorobenzaldehyde was replaced with 2-fluorobenzaldehyde and methylamine was replaced with propylamine. TOF MS ES[+] m/z 379. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 9.06 (t, J = 5.0 Hz, 1H), 8.55 (s, 1H), 7.74 (s, 1H), 7.49 (m, 1H), 7.44 (t, J = 8.1 Hz, 1H), 7.28 (d, J = 8.3 Hz, 2H), 7.25 (d, J = 7.4 Hz, 1H), 7.18 (t, J = 9.2 Hz, 1H), 6.90 (d, J = 8.6 Hz, 2H), 4.02 (q, J = 7.0 Hz, 2H), 3.61 (q, J = 7.0 Hz, 2H), 1.75 (sxt, J = 7.4 Hz, 2H), 1.41 (t, J = 7.0 Hz, 3H), 1.06 (t, J = 7.4 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 194.37 (1C), 160.03 (1C), 158.31 (1C), 158.04 (1C), 157.70 (1C), 153.45 (1C), 141.20 (1C), 132.28 (1C), 129.03 (1C), 127.77 (1C), 127.26 (2C), 124.47 (1C), 123.01 (1C), 116.18 (1C), 115.04 (2C), 112.30 (1C), 63.59 (1C), 42.98 (1C), 22.81 (1C), 14.88 (1C), 11.78 (1C) ppm. Anal. (C$_{23}$H$_{23}$FN$_2$O$_2$) C, H, N.

(3,4-Difluorophenyl)[5-(4-ethoxyphenyl)-2-propylamino-3-pyridinyl]methanone (7v)—**7v** was prepared in 40% yield as described above for the synthesis of **7z** except that methylamine was replaced with propylamine. **7v** was isolated as a yellow solid with mp 84–85.5°C. TOF MS ES[+] m/z 397. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.70 (t, J = 5.0 Hz, 1H), 8.57 (s, 1H), 7.83 (s, 1H), 7.50 (m, 2H), 7.38 (ddd, J = 6.1, 4.2, 2.3 Hz, 2H), 7.30 (d, J = 8.4 Hz, 2H), 7.27 (q, J = 8.7 Hz, 2H), 6.93 (d, J = 8.6 Hz, 2H), 4.04 (q, J = 7 Hz, 2H), 3.59 (q, J = 6.9 Hz, 2H), 1.74 (sxt, J = 8.3 Hz, 3H), 1.41 (t, J = 7.0 Hz, 3H), 1.04 (t, J = 7.5 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 195.22 (1C), 158.40 (1C), 157.99 (1C), 152.97 (1C), 151.65 (1C), 150.32 (1C), 140.45 (1C), 136.39 (1C), 129.86 (1C), 127.25 (2C), 125.85 (1C), 123.50 (1C), 118.60 (1C), 117.32 (1C), 115.13 (2C), 111.21 (1C), 63.61 (1C), 43.00 (1C), 22.79 (1C), 14.88 (1C), 11.74 (1C) ppm. Anal. (C$_{23}$H$_{22}$F$_2$N$_2$O$_2$) C, H, N.

(2,5-Difluorophenyl)[5-(4-ethoxyphenyl)-2-propylamino-3-pyridinyl]methanone (7w)—**7w** was prepared as described above for the synthesis of **7z** except that 3,4-difluorobenzaldehyde was replaced with 2,5-difluorobenzaldehyde and methylamine was replaced with propylamine. ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.01 (t, J = 4.9 Hz, 1H), 8.56 (s, 1H), 7.70 (s, 1H), 7.29 (d, J = 8.7 Hz, 2H), 7.16 (m, 2H), 6.92 (d, J = 8.7 Hz, 2H), 4.04 (q, J = 7.0 Hz, 2H), 3.62 (q, J = 7.0 Hz, 2H), 1.76 (sxt, J = 7.3 Hz, 3H), 1.42 (t, J = 7.0 Hz, 3H), 1.07 (t, J = 7.5 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 192.58 (1C), 159.55 (1C), 158.40 (1C), 157.70 (1C), 155.85 (1C), 153.93 (1C), 140.94 (1C), 129.97 (1C), 128.96 (1C), 127.31 (2C), 124.10 (1C), 118.84 (1C), 117.74 (1C), 116.43 (1C), 115.09 (2C), 111.78 (1C), 63.62 (1C), 43.00 (1C), 22.82 (1C), 14.90 (1C), 11.77 (1C) ppm. Anal. (C$_{23}$H$_{22}$F$_2$N$_2$O$_2$) C, H, N.

(3,5-Difluorophenyl)[5-(4-ethoxyphenyl)-2-propylamino-3-pyridinyl]methanone (7x)—**7x** was prepared as described above for the synthesis of **7z** except that 3,4-difluorobenzaldehyde was replaced with 3,5-difluorobenzaldehyde and methylamine was replaced with propylamine. TOF MS ES[+] m/z 397. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.79 (t, J

= 4.3 Hz, 1H), 8.58 (s, 1H), 7.81 (s, 1H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.14 (d, *J* = 5.6 Hz, 2H), 7.00 (tt, *J* = 8.5, 2.3 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 2H), 4.04 (q, *J* = 7.0 Hz, 2H), 3.61 (t, *J* = 6.6 Hz, 2H), 1.75 (sextet, *J* = 7.2 Hz, 2H), 1.43 (t, *J* = 7.0 Hz, 3H), 1.06 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 195.11 (1C), 163.70 (1C), 161.70 (1C), 158.43 (1C), 158.00 (1C), 153.45 (1C), 142.56 (1C), 140.53 (1C), 129.81 (1C), 127.22 (2C), 123.67 (1C), 115.13 (2C), 112.05 (1C), 111.83 (1C), 110.86 (1C), 106.60 (1C), 63.62 (1C), 43.00 (1C), 22.78 (1C), 14.88 (1C), 11.73 (1C) ppm. Anal. (C$_{23}$H$_{22}$F$_2$N$_2$O$_2$) C, H, N.

[5-(4-Ethoxyphenyl)-2-propylamino-3-pyridinyl]phenylmethanone (7y)—7y was prepared as described above for the synthesis of **7z** except that 3,4-difluorobenzaldehyde was replaced with benzaldehyde and methylamine was replaced with propylamine. **7y** was isolated as a yellow solid with mp 88–88.5°C. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.81 (t, *J* = 5.0 Hz, 1H), 8.56 (s, 1H), 7.91 (s, 1H), 7.63 (d, *J* = 7.0 Hz, 2H), 7.56 (t, *J* = 7.3 Hz, 1H), 7.49 (t, *J* = 7.4 Hz, 2H), 7.32 (d, *J* = 8.8 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 2H), 4.04 (q, *J* = 7.0 Hz, 2H), 3.60 (q, *J* = 6.5 Hz, 2H), 1.75 (sxt, *J* = 7.2 Hz, 2H), 1.42 (t, *J* = 7.0 Hz, 3H), 1.06 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 198.26 (1C), 158.26 (1C), 158.06 (1C), 152.41 (1C), 141.08 (1C), 139.58 (1C), 131.42 (1C), 130.16 (1C), 129.09 (2C), 128.43 (2C), 127.16 (2C), 123.32 (1C), 115.05 (2C), 111.92 (1C), 63.60 (1C), 43.00 (1C), 22.82 (1C), 14.89 (1C), 11.78 (1C) ppm. Anal. (C$_{23}$H$_{24}$N$_2$O$_2$) C, H, N.

(3,4-Difluorophenyl)[5-(4-ethoxyphenyl)-2-ethylamino-3-pyridinyl]methanone (7aa)—7aa was prepared as described above for the synthesis of **7z** except that methylamine was replaced with ethylamine. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.61 (t, *J* = 4.3 Hz, 1H), 8.58 (s, 1H), 7.83 (s, 1H), 7.51 (m, 1H), 7.38 (m, 1H), 7.32 (d, *J* = 8.7 Hz, 2H), 7.26 (t, *J* = 7.3 Hz, 1H), 6.93 (d, *J* = 8.7 Hz, 2H), 4.05 (q, *J* = 7.0 Hz, 2H), 3.66 (m, 2H), 1.62 (d, *J* = 7.0 Hz, 3H), 1.34 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (CDCl$_3$, 125 MHz) 195.24 (1C), 158.40 (1C), 157.79 (1C), 152.94 (1C), 151.31 (1C), 149.30 (1C), 140.46 (1C), 136.36 (1C), 129.84 (1C), 125.92 (2C), 125.84 (1C), 123.57 (1C), 118.44 (1C), 117.48 (1C), 115.13 (2C), 111.28 (1C), 63.62 (1C), 36.01 (1C), 14.93 (1C), 14.88 (1C) ppm. Anal. (C$_{22}$H$_{20}$F$_2$N$_2$O$_2$) C calcd 69.10, found 68.58, H, N.

Electrophysiology—Individual compounds were tested for modulation of submaximal nicotine-evoked currents in oocytes expressing human α7 nAChRs. For each oocyte, the maximal nicotine-evoked currents were determined in response to 3 mM nicotine. All other currents were scaled to this value. The concentration of nicotine was adjusted to evoke a fractional current of approximately 0.05 (5% of max, or "EC$_5$"), and this concentration of nicotine was used to generate EC$_5$ control currents. Increasing concentrations of test compounds were applied to oocytes alone (pretreatment) and then in combination with the EC$_5$ concentration of nicotine (co-application). This protocol allowed measurement of both direct effects of test compounds on α7 nAChRs, and modulatory effects of compounds on nicotine-evoked responses. mRNA was prepared and stored using conventional techniques from cDNA clones encoding the human nAChR subunits. Preparation, micro-injection and maintenance of oocytes were performed as reported in detail previously . Individual oocytes were injected with 5 – 50 ng of each subunit mRNA. Following injections, oocytes were maintained at 16–17°C in Barth's medium. Two-electrode voltage clamp recordings were made 3–14 days following mRNA injections at a holding voltage of −70 mV unless specified. The nicotinic recordings were done in Ca^{++}-free Ringer solution (mM: NaCl, 115; KCl, 2; BaCl$_2$, 1.8; HEPES, 5; pH 7.4) to limit Ca^{++}-activated chloride and muscarinic currents. Drug and wash solutions were applied using a microcapillary "linear array" in order to allow rapid application of agonists. Currents were recorded on a chart recorder and/ or PC-based computer for subsequent analysis. Test compounds were made up in DMSO over a concentration range of 0.001 – 10 mM and diluted 1000–3000-fold into the

appropriate saline just prior to testing (final [DMSO] < 0.1%). The concentration-dependence of modulation was analyzed using GraphPad "Prism" curve-fitting software. For $GABA_A$Rs, individual oocytes were microinjected with 1–5 ng of cRNA encoding human α1, β2, and γ2L $GABA_A$ receptor subunits. Membrane current responses were measured at a holding potential of −70 mV using conventional two-electrode voltage clamp techniques, 3–11 days following injection. Modulatory effects of compounds were assayed on control responses that were 10% of the maximum GABA response (EC_{10}) in an individual oocyte.

Novel Object Recognition (NOR)

Adult CD1 mice (Charles River Laboratories, Wilmington, MA) were used in the NOR paradigm. Experiments were performed in accordance with the Animals Scientific Procedures Act (1986) and were approved by the Institutional Animal Care and Use Committee (IACUC). The animals were allowed to explore the chamber in the absence of any objects for two 15 min sessions on day 1 to ensure habituation to the empty apparatus and testing room. On day 2 animals were subject to acquisition for one 10 min period with two identical objects. The familiar and novel objects were counter-balanced to ensure there was no object preference. A tall glass test tube (A) and a 15 mL plastic test tube (B) were used as familiar/novel objects. Immediately after acquisition the mice were dosed with 0.7 mg/kg (2.1 μmol/kg) scopolamine hydrochloride subcutaneously (s.c.). Half the animals were then dosed with either compound **7z** dissolved in 80% PEG/20% saline or an equivalent dose of a vehicle (80% PEG 400/20% saline solution). 30 min after injection the animals were video recorded exploring for one 5 min session with one familiar object from acquisition and one novel object. A single-blinded experimenter watching the recorded video scored the animal's exploration of novel and familiar objects and analyzed the mice for their retention of the familiar object. Object exploration is defined as an interaction with the object, which includes sniffing, licking, touching, or closely staring at the objects. The objects were chosen so that the animals could not sit or stand on them. The percent time spent with the novel object was defined as the time spent with novel divided by the total time spent with both novel and familiar objects. Results were analyzed as a mean ± S.E.M. Analysis of the data was accomplished using a one-way ANOVA followed by a post hoc Dunnet's t-test.

Pharmacokinetic Studies

Tissue Extraction and Sample Preparation—Blood was removed at thirty min after drug administration via cardiac puncture under halothane anesthesia and centrifuged at 1000X's *g* for 6 min to separate the plasma. After euthanization, brains were perfused with saline and removed. Brain and plasma samples were stored at −20°C until processed for extraction and liquid chromatography/mass spectrometry (LC/MS) analysis. Approximately 0.5 g (brain) was combined with 1 mL phosphate-buffered saline (pH 7.4) and homogenized with a Tissue-Tearor (Biospec Products, Bartlesville, OK). Homogenized brain samples were combined with 2 mL EtOAc and vortexed for 30 s. Plasma samples (250 μL) were combined with 2 mL acetonitrile (ACN) and vortexed for 30 s. Brain and plasma extract/solvent mixtures were spun 500X's *g* for 5 min. The entire plasma extraction supernatant was decanted into new tubes and evaporated to dryness under an air stream. A 1 mL aliquot of the brain extraction supernatant was transferred to new tubes and evaporated to dryness under an air stream. Each evaporated sample received 250 μL of ACN and was sonicated for 5 min. Samples were vortexed and spun 500X's *g* for 5 min. The sample supernatant was pipetted into sample tubes containing 1 μL of internal standard.

Sample Analysis—Plasma and brain samples were acquired through a Shimadzu SIL-HTc autosampler and run through a Shimadzu LC-10ADvp HPLC with a 90% ACN/10% H_2O mobile phase using a C18 column (Poroshell 120 EC-C18 4.6 × 50 mm, 2.7 μm;

Agilent Technologies, Santa Clara CA). Detection of analyte was performed via MRM monitoring on a Quattro Premier (Waters, Milford, MA) using MassLynx v4.0 software. The parent/daughter transition mass (369.3 > 140.5) was detected at 1.07 min with a 0.3 mL/min rate of infusion using a cone voltage of 55 V, collision energy of 30 eV, dwell time of 0.1 s, 3.89 kV capillary voltage, 8V extractor, source temperature of 100°C, desolvation temperature 400°C and collision gas flow of 0.6 mL/min. Compound 522-054 [26] was used as an internal standard.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations Used

ADHD	attention deficit hyperactivity disorder
MLA	methyllycaconitine citrate
Mod	modulation
NOR	novel object recognition paradigm
PAM	positive allosteric modulator
Scop	scopolamine hydrochloride
TBPS	*tert*-butylbicyclophosphorothionate

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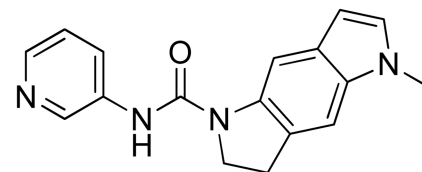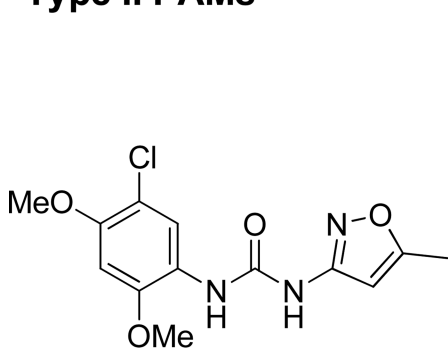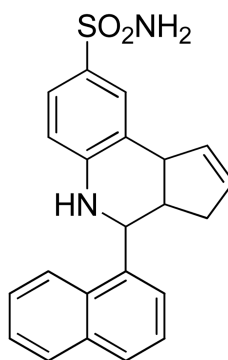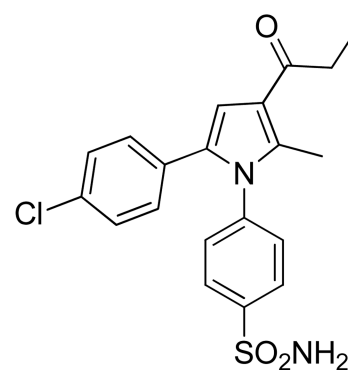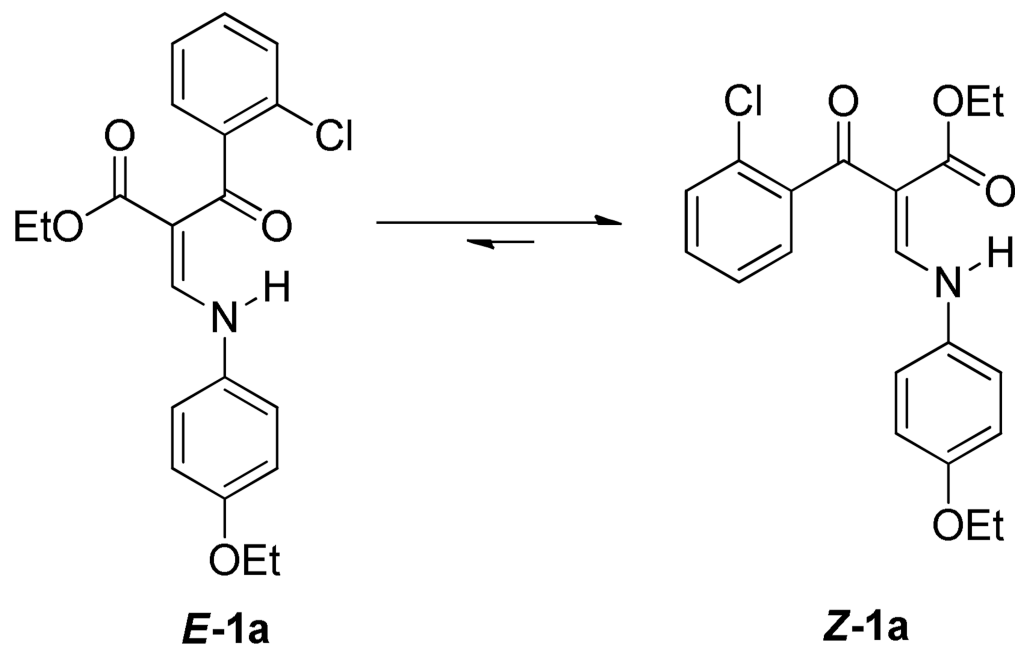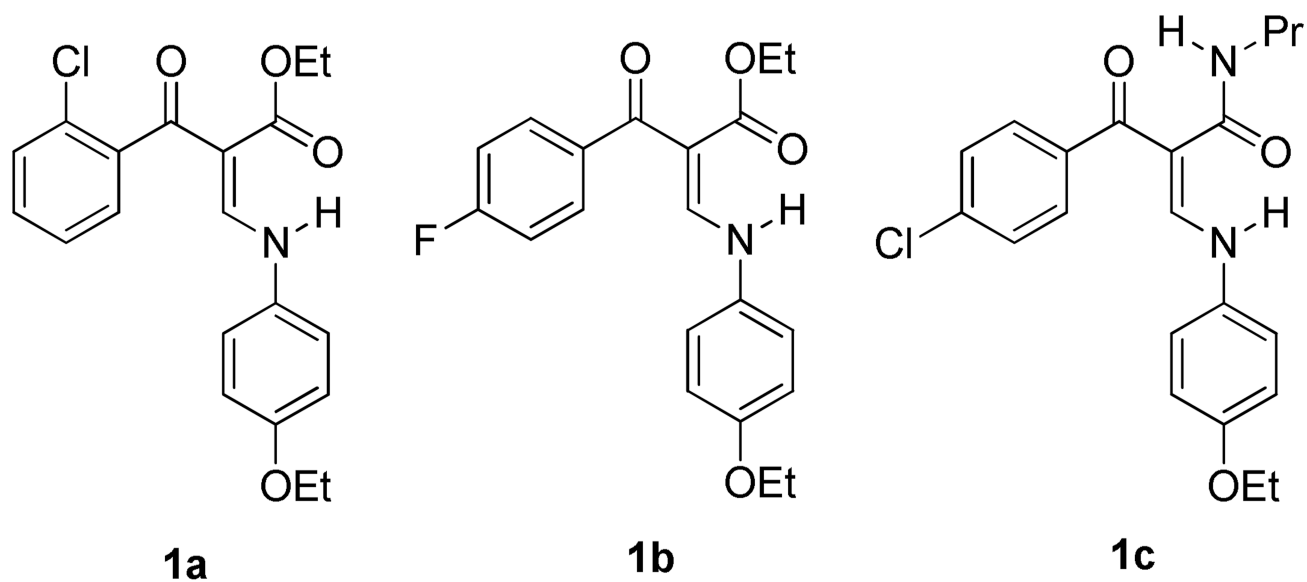
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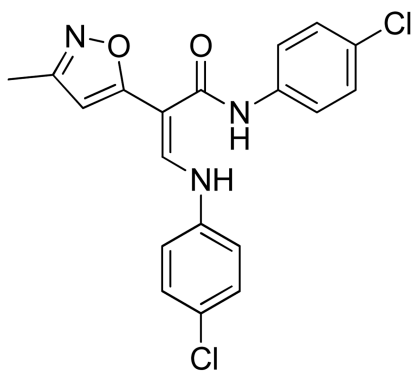
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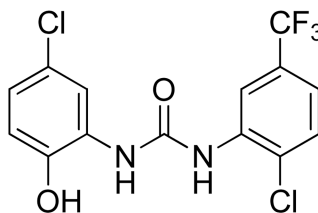
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SB-206553

Type II PAMs

PNU-120596

THQ

A-867744

Figure 1.
Structures of Type I and Type II PAMs[5]

Figure 2.
Structures of enaminone esters (**1a** and **1b**), amide **1c** and the *E*- and *Z*-isomers of **1a**.
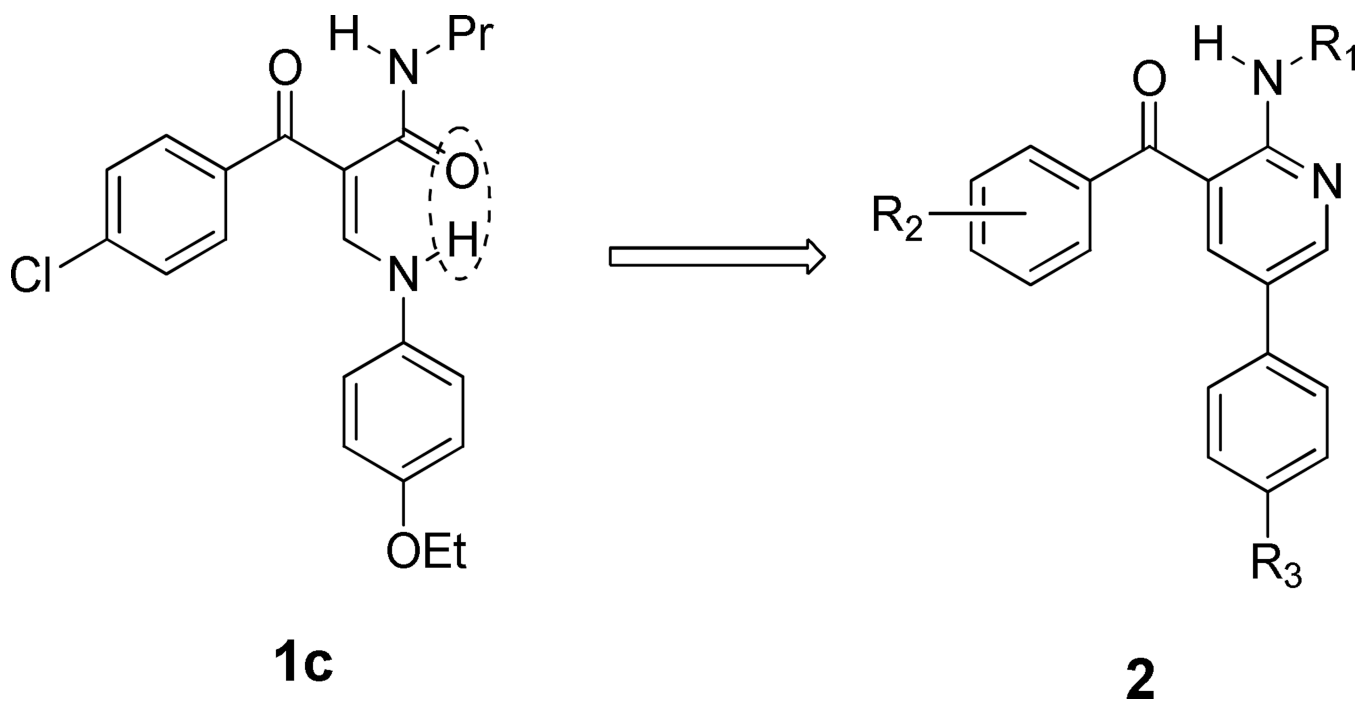
Figure 3.
Design of (2-alkylamino-5-arylpyridine-3-yl)arylmethanones **2** from enaminone amide **1c**.
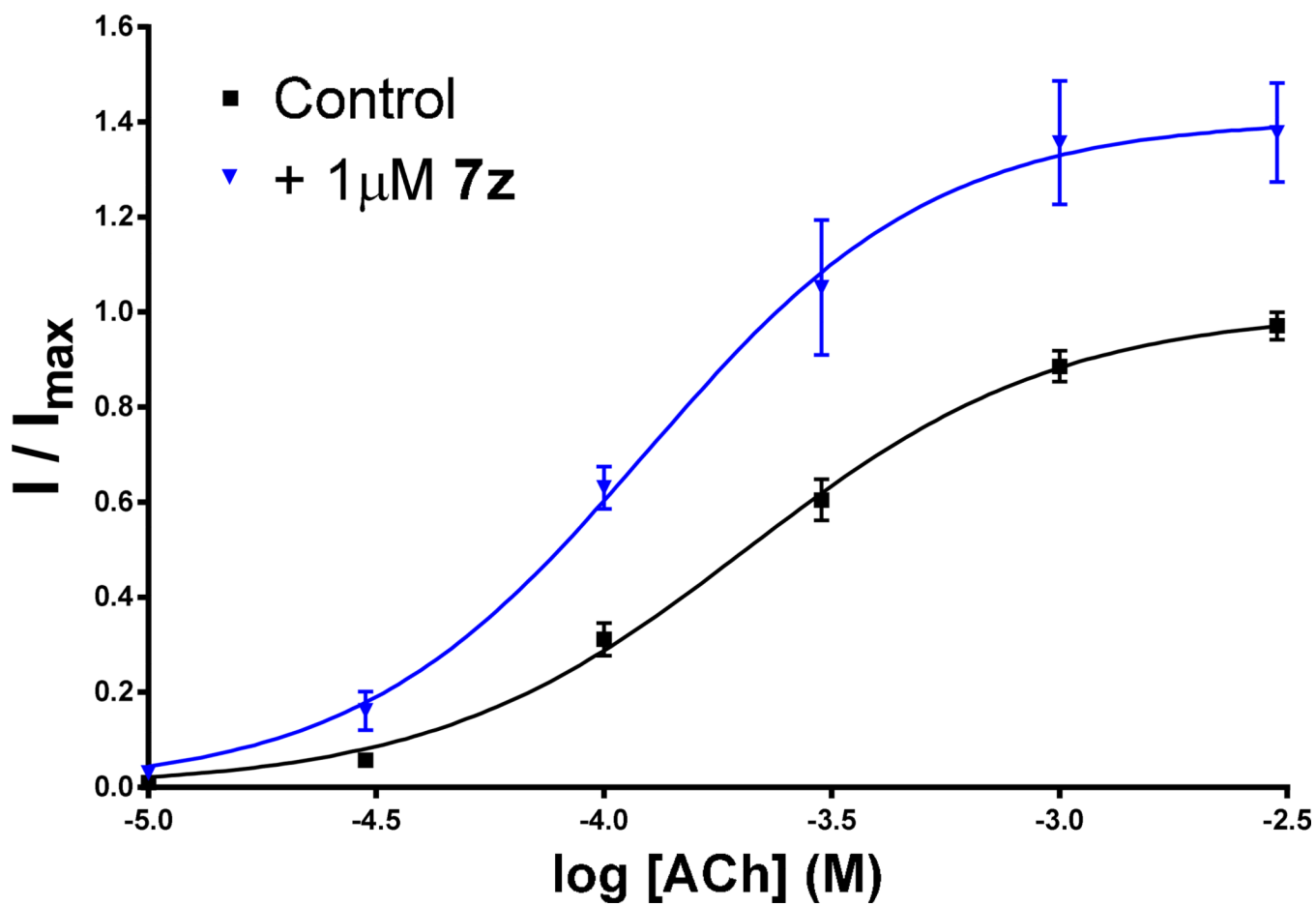
Figure 4.
Acetylcholine (ACh) concentration-response curves in the presence and absence of an EC_{50} concentration of compound **7z**.
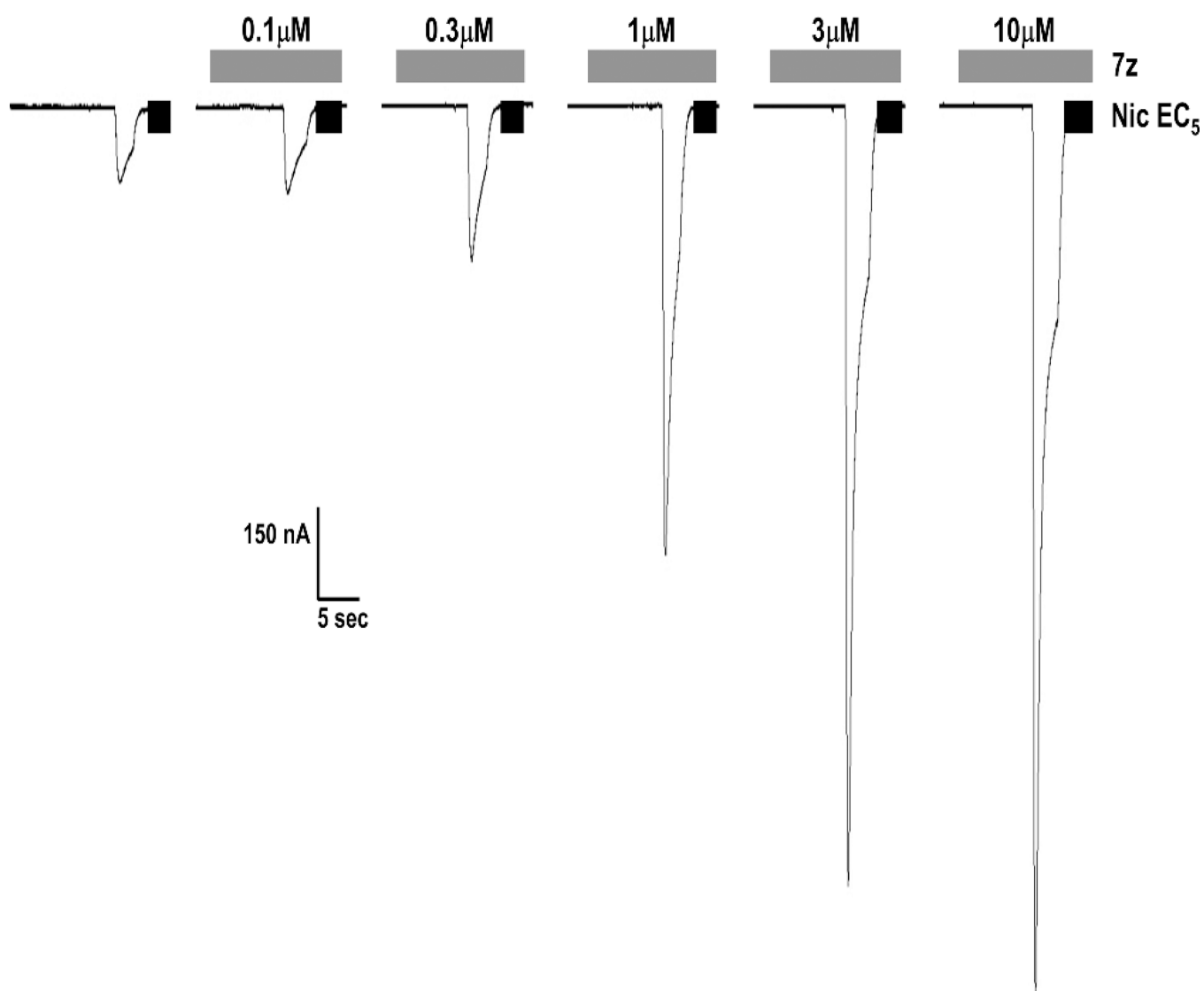
Figure 5.
Modulation of nicotine EC_5 evoked currents in *Xenopus* oocytes expressing human $\alpha 7$ nAChRs with increasing concentrations of **7z**.
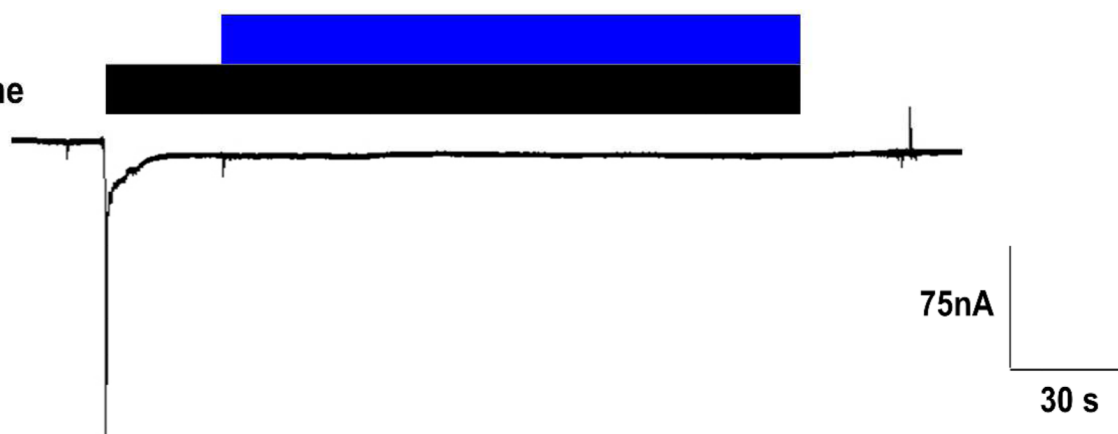
Figure 6.
Current trace of 1μM **7z** in the presence of 100 μM nicotine showing no reversal of desensitization
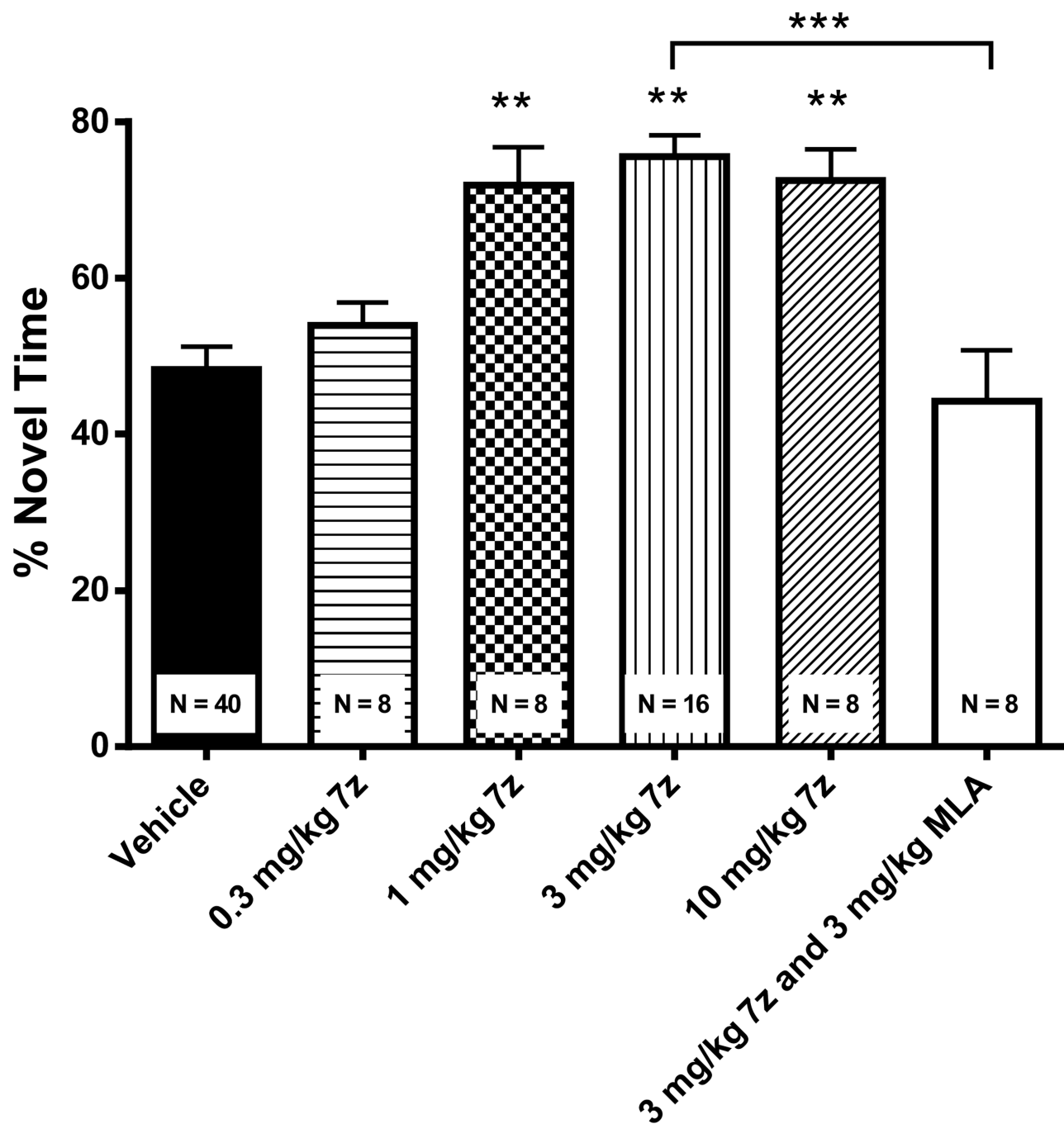
Figure 7.
Effect of **7z** (0.3 to 10 mg/kg, 0.81 to 27 μmol/kg) i.p. in 80% PEG 400/20% saline on the % time spent with a novel object (% novel time) in the mouse (CD-1) NOR paradigm in the presence of 0.7 mg/kg (2.1 μmol/kg) s.c. scopolamine hydrochloride (Scop) versus those treated with drug, vehicle and Scop alone. All animal groups received Scop. Significant One-way ANOVA P<0.0001. Post Hoc Dunnet's Multiple Comparison Test **P<0.01. The effect of 3 mg/kg (8.1 μmol/kg) **7z** is reversed by 3 mg/kg (3.4 μmol/kg) MLA (far right bar). Post Hoc Bonferroni's Multiple Comparison Test ***P<0.001.
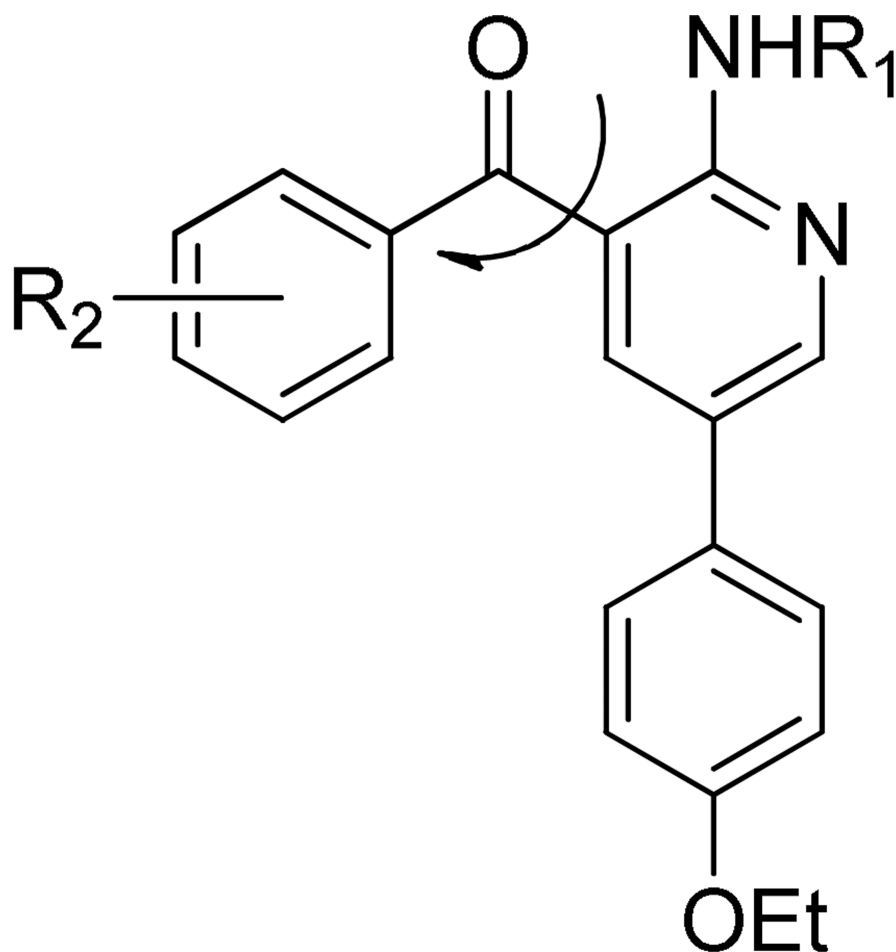
7u: R₁ = Pr, R₂ = 2-F
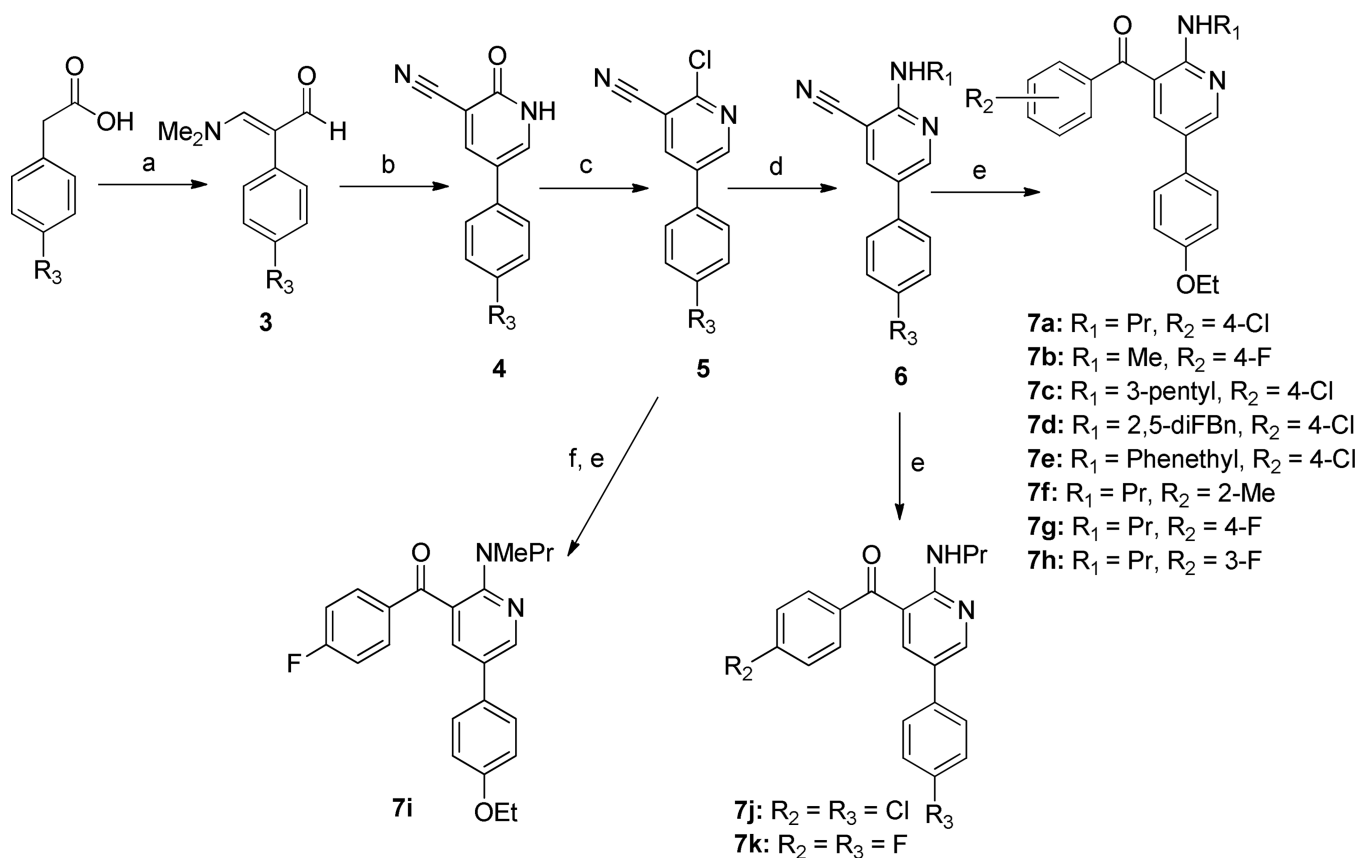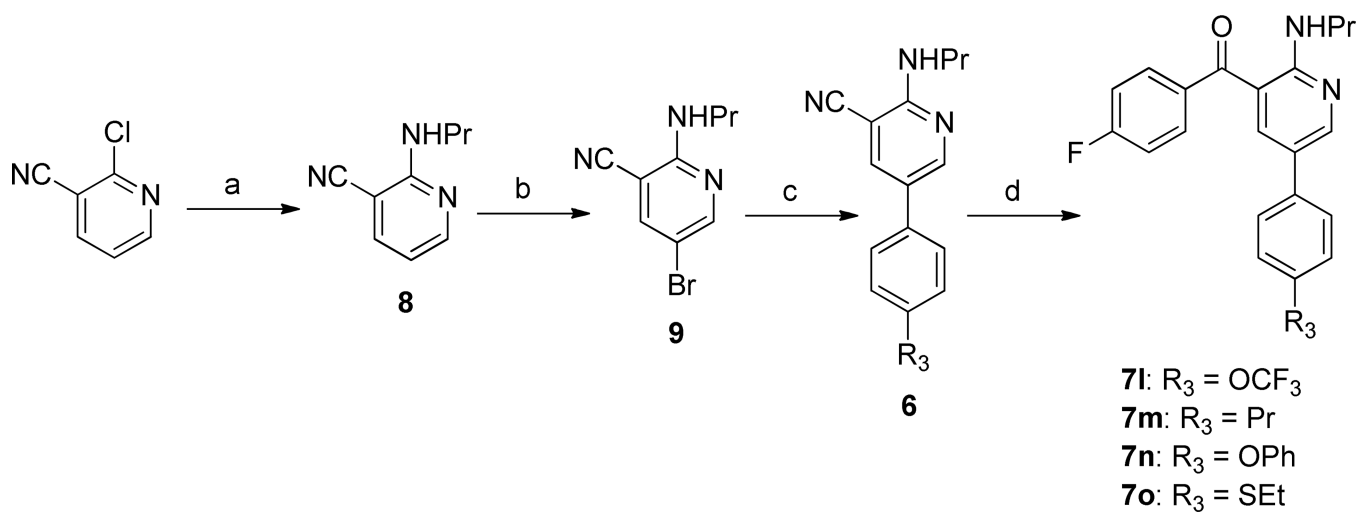**7y**: R₁ = Pr, R₂ = H
7z: R₁ = Me, R₂ = 3,4-diF

Figure 8.
Dihedral angle (ψ) varied in conformational analysis

Scheme 1.
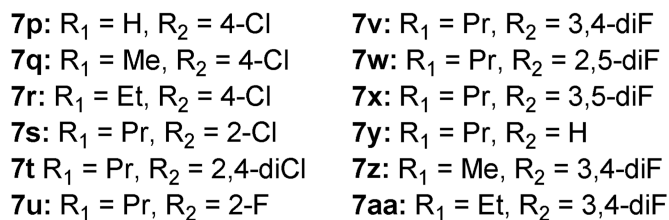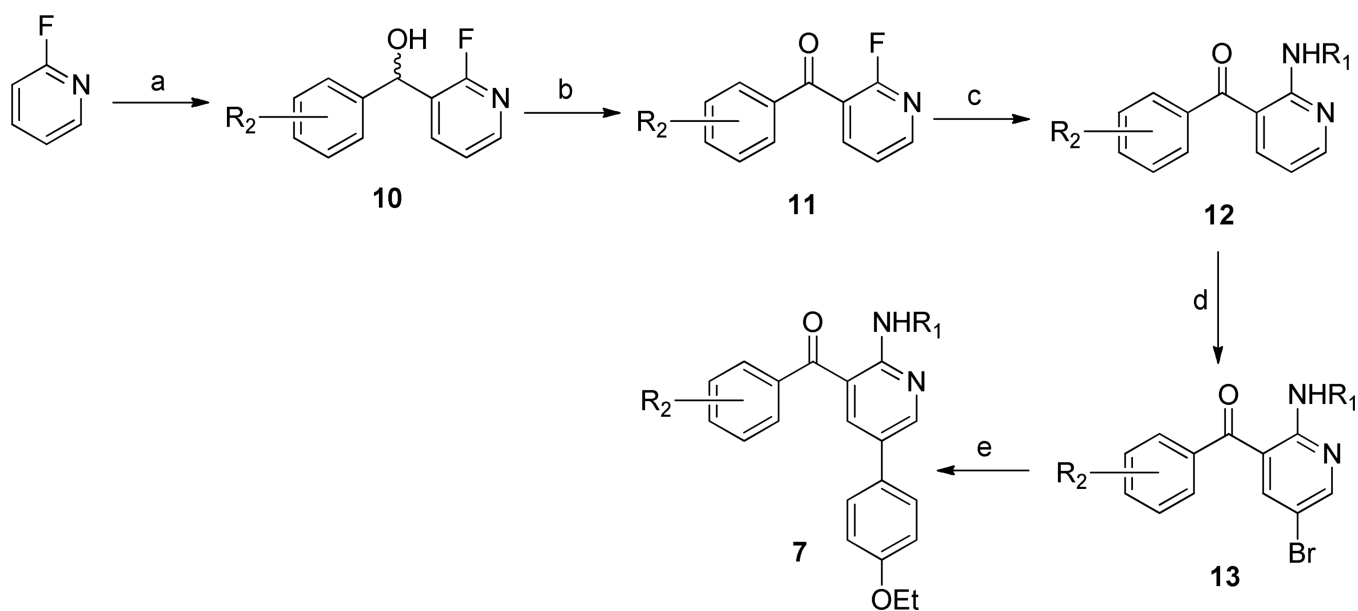Method A: Synthesis of Arylpyridylmethanones Starting with Arylacetic Acids.
Reagents/Solvents: a) POCl$_3$/DMF/70 °C, then aq. NaOH/100 °C b) cyanoacetamide/
NaOMe/MeOH c) POCl$_3$, reflux d) R$_1$NH$_2$/DMSO e) R$_2$PhMgX/THF reflux then aq. HCl,
reflux f) MeNHPr/DMSO.

Scheme 2.

Method B: Conversion of 2-chloronicotinonitrile to **7l, m, n** and **o**.

Reagents/Solvents: a) Propylamine/DMSO b) NBS/DMF c) ArylB(OH)$_2$, toluene/EtOH/aq. Na$_2$CO$_3$/Pd(PPh$_3$)$_4$ d) 4-Fluorophenylmagnesium bromide/THF, reflux then aq. HCl, reflux

7p: R$_1$ = H, R$_2$ = 4-Cl   **7v:** R$_1$ = Pr, R$_2$ = 3,4-diF
7q: R$_1$ = Me, R$_2$ = 4-Cl   **7w:** R$_1$ = Pr, R$_2$ = 2,5-diF
7r: R$_1$ = Et, R$_2$ = 4-Cl   **7x:** R$_1$ = Pr, R$_2$ = 3,5-diF
7s: R$_1$ = Pr, R$_2$ = 2-Cl   **7y:** R$_1$ = Pr, R$_2$ = H
7t R$_1$ = Pr, R$_2$ = 2,4-diCl   **7z:** R$_1$ = Me, R$_2$ = 3,4-diF
7u: R$_1$ = Pr, R$_2$ = 2-F   **7aa:** R$_1$ = Et, R$_2$ = 3,4-diF
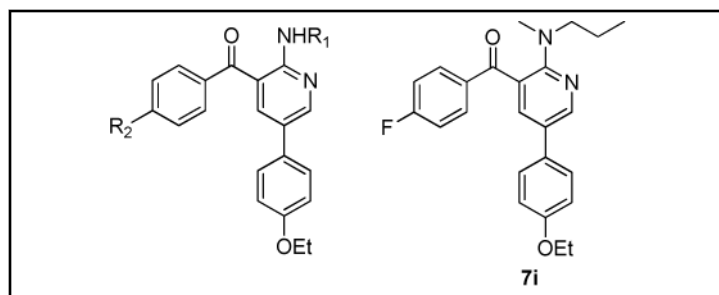
Scheme 3.
Method C: Use of 2-fluoropyridine as starting material
Reagents/Solvents: a) LDA/THF/R$_2$PhCHO, −78 °C b) PCC/CH$_2$Cl$_2$ c) R$_1$NH$_2$/DMSO or EtOH, rt d) NBS/DMF e)4-EtOPhB(OH)$_2$, toluene/EtOH/aq. Na$_2$CO$_3$/Pd(PPh$_3$)$_4$.
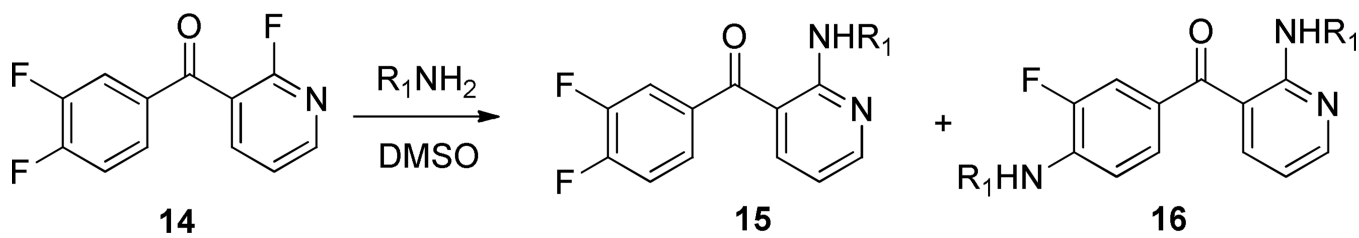
Scheme 4.
Reaction of 3-(3,4-difluorobenzoyl)-2-fluoropyridine (**14**) with amines.

Table 1

In vitro activity of [2-alkylamino-5-(4-ethoxyphenyl)pyridin-3-yl]arylmethanones in oocytes expressing human α7 nAChRs.[a]
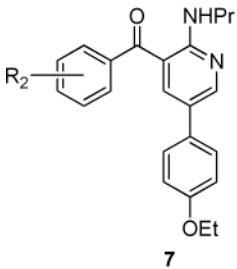

Compound	R_1	R_2	EC_{50} (μM)	Max. mod. (% at 10 μM)
7p	H	Cl	2.5	500
7b	Me	F	1.9	600
7q	Me	Cl	0.38	1200
7r	Et	Cl	0.16	700
7a	Pr	Cl	0.14	600
7c	3-pentyl	Cl	ND	55
7d	2,5-difluorobenzyl	Cl	ND	IA
7e	phenethyl	Cl	ND	IA
7i	-	-	ND	40

[a]Data are from 2–5 individual oocytes treated with a nicotine EC_5 (concentration that evokes 5% of the maximum nicotine response). The EC_{50} is the drug concentration that gives half of the maximum modulation (max mod). 95% Confidence intervals are given in supplementary information, ND = Not determined, IA = inactive
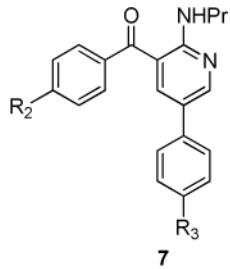
Table 2

In vitro activity of aryl[5-(4-ethoxyphenyl)-2-(propylamino)pyridin-3-yl]methanones **7** in oocytes expressing human α7 nAChR.[a]

7

Compound	R$_2$	EC$_{50}$ (μM)	Max. mod. (%, 10 μM)
7a	4-Cl	0.14	600
7s	2-Cl	ND	35
7f	2-Me	ND	IA
7t	2,4-diCl	ND	40
7g	4-F	0.13	600
7h	3-F	0.45	700
7u	2-F	ND	75
7v	3,4-diF	0.18	1200
7w	2,5-diF	3.0	200
7x	3,5-diF	ND	130
7y	H	0.21	300

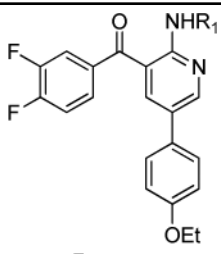[a]See footnote to Table 1. ND = Not determined, IA = inactive

Table 3

In vitro activity of aryl[5-aryl-2-(propylamino)pyridin-3-yl]methanones **7** in oocytes expressing human α7 nAChRs.[a]

Compound	R_2	R_3	EC_{50} (μM)	Max. mod. (%, 10 μM)
7a	Cl	OEt	0.14	600
7j	Cl	Cl	ND	40
7g	F	OEt	0.13	600
7l	F	OCF_3	ND	55
7m	F	Pr	ND	30
7n	F	OPh	0.56	30
7o	F	SEt	ND	40
7k	F	F	0.22	300

[a]See footnote to Table 1, ND = not determined.

Table 4

In vitro activity of [2-alkylamino-5-(4-ethoxyphenyl)pyridin-3-yl](3,4-difluorophenyl)-methanones (**7v, z** and **aa**) in *Xenopus* oocytes expressing human α7 nAChRs.[a]

7v, z, aa

Compound	R_1	EC$_{50}$ (µM)	Max. mod. (%, 10 µM)
7z	Me	1.2	770
7aa	Et	4.9	470
7v	Pr	0.18	1200

[a] See footnote to Table 1.

Table 5

Activity of **7z** at human $\alpha_4\beta_2$ nAChRs, rat $\alpha_3\beta_4$ nAChRs and human $\alpha_1\beta_2\gamma_{2L}$ GABA$_A$Rs expressed in *Xenopus* oocyctes.

Receptor	Max. mod. (% at 10 µM)
$\alpha_4\beta_2$ nACh	-2 ± 5
$\alpha_3\beta_4$ nACh	-11 ± 3
$\alpha_1\beta_2\gamma_{2L}$ GABA$_A$	71 ± 12