2-Fluoro-4-pyridinylmethyl Analogues of Linopirdine as Orally Active Acetylcholine Release-Enhancing Agents with Good Efficacy and Duration of Action

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In an effort to improve the pharmacokinetic and pharmacodynamic properties of the cognition-enhancer linopirdine (DuP 996), a number of core structure analogues were prepared in which the 4-pyridyl pendant group was systematically replaced with 2-fluoro-4-pyridyl. This strategy resulted in the discovery of several compounds with improved activity in acetylcholine (ACh) release-enhancing assays, in vitro and in vivo. The most effective compound resulting from these studies, 10,10-bis[(2-fluoro-4-pyridinyl)methyl]-9(10*H*)-anthracenone (**9**), is between 10 and 20 times more potent than linopirdine in increasing extracellular hippocampal ACh levels in the rat with a minimum effective dose of 1 mg/kg. In addition to superior potency, **9** possesses an improved pharmacokinetic profile compared to that of linopirdine. The half-life of **9** (2 h) in rats is 4-fold greater than that of linopirdine (0.5 h), and it showed a 6-fold improvement in brain−plasma distribution over linopirdine. On the basis of its pharmacologic, pharmacokinetic, absorption, and distribution properties, **9** (DMP543) has been advanced for clinical evaluation as a potential palliative therapeutic for treatment of Alzheimer's disease.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that is accompanied in its later stages by a variety of deficiencies that include deterioration of memory, loss of cognitive function, and privation of personality.[1] This condition is reflective of the widespread pathology found in the brain of AD patients at autopsy. Both familial and sporadic forms of the disease have been identified, and causative roles for abnormal amyloid deposition,[2,3] phosphorylated microtubule proteins,[4] and neuroinflammatory mechanisms[5−7] have been put forth.

In addition to the presence of plaques and tangles, neurochemical alterations are also included as hallmarks of the disease. In particular, decreases in the markers of cortical cholinergic innervation are one of the earliest and most consistently present correlates of AD. These observations have led to the hypothesis that associates this type of dementia with cholinergic losses.[8]

Neurotransmitter receptor sites are also altered in AD. Both muscarinic M2[9] and nicotinic[10,11] receptors decrease with the progression of the disease, whereas the number of muscarinic M1 receptors does not change.[12] Patients with AD show reduced activity of acetylcholine esterase (AChE), high-affinity choline uptake (HACU), and choline acetyltransferase (ChAT) reflective of a damaged cholinergic system and directly related to their cognitive impairments.[8,13−17] Moreover various animal models support the role of ACh in cognition and learning.[18−22] The slow nature of the degenerative process as well as the redundancy of the connectivity of the brain area provides a window of opportunity for palliative therapy early in the disease. The positive effects of the cholinesterase inhibitors tacrine[23] and donepezil[24,25] observed in a subpopulation of AD patients support this concept and the basis that compounds that do indeed bolster the cholinergic system should provide theraputic benefit.

Over the past several years, we have identified a series of compounds that possess the unique ability to enhance the potassium-evoked release of acetylcholine (ACh).[26,27] Since this neurotransmitter is known to be deficient in AD,[16,17] these compounds are potential therapeutics for the treatment of this disease. Unlike direct receptor agonists or cholinesterase inhibitors, we anticipated these agents would not cause receptor down-regulation due to sustained receptor interaction.[28] Moreover, since these agents only affect evoked release, they should not cause neurotransmitter depletion or overload toxicity, a problem with agents affecting basal release.[13,29,30] The first member of this series to be tested in clinical trials was linopirdine (AVIVA, DuP 996), a compound whose development was largely supported by behavioral pharmacology.[31,32] The results from these studies, however, were mixed and unconvincing.[33] More recent animal pharmacokinetic studies have suggested that the absence of a more definitive clinical effect with linopirdine may have been due to its short and variable half-life and poor blood−brain barrier (BBB) penetration.[34,35] Therefore, the neurotransmitter release en-

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Scheme 1

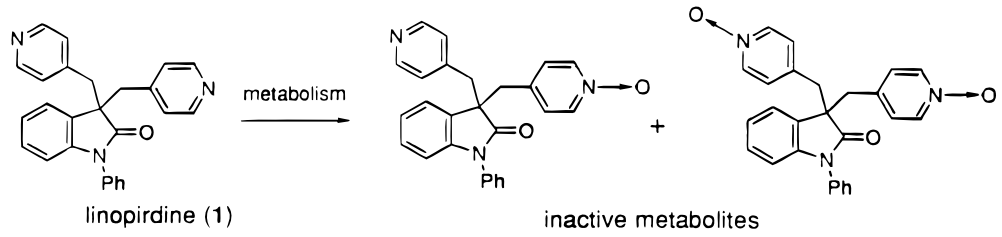
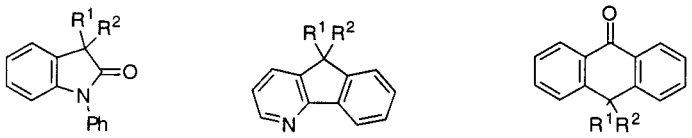
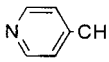
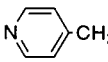
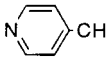
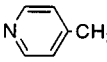
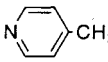
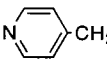
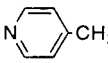
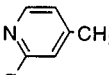
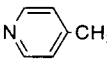
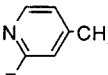
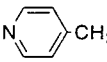
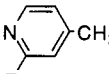
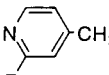
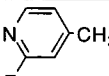
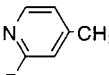
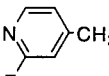
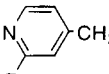
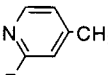


Table 1. Physicochemical Properties of Linopirdine Analogues



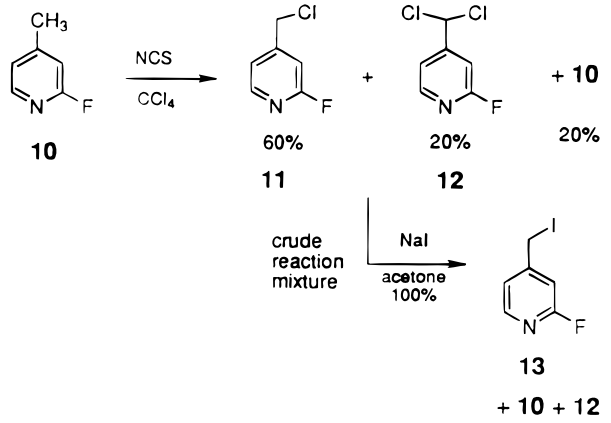
A B C

compd no.	compd type	R^1	R^2	mp °C	% yield	Anal.
1, linopirdine (DuP 996)	A			185-7[45]	90	C,H,N
2	B			169-171	81	C,H,N
3	C			231-232	33	C,H,N
4	A			174-6	33	C,H,N
5	B			164-5	49	C,H,N
6	C			199-201	12	C,H,N
7	A			143-5	42	C,H,N
8	B			137-141	57	C,H,N
9	C			159-161	58	C,H,N,F

hancement approach to AD may not have been adequately tested with this drug.

Our requirements, then, for a second-generation neurotransmitter release-enhancing compound were greater brain penetration and longer duration of action than that of linopirdine. To achieve these goals, two important observations were taken into consideration. First, other core structures, such as 4-azafluorene and anthrone, when dialkylated with the 4-pyridinylmethyl group, were not only more potent than linopirdine but also more lipophilic, a property that can facilitate BBB penetration.[36] Second, it had been shown that the pyridinylmethyl pendant groups of linopiridne were subject to N-oxidation causing this compound to have a short plasma half-life.[34] Metabolic N-oxidation of aromatic nitrogens, such as pyridine, is known to be a cytochrome P-450-dependent process[37] and may result in rapid metabolism or clearance of active substance. Indeed, the major oxidative metabolites of linopirdine (DuP 996) were the bis- and mono-*N*-oxides in rats, dogs, and humans[34] (Scheme 1). It was expected that substituting a fluorine atom for the hydrogen ortho to the pyridine nitrogens would reduce the basicity of the heterocycles as well as their ability to undergo N-

Scheme 2



oxidation.[38] There is no evidence in the literature to suggest that 2-fluoro-substituted pyridines undergo metabolic N-oxidation.[39]

To assess the effects of fluorine substitution into the 2-position of the pyridinyl pendant groups, we systematically replaced 4-pyridinylmethyl with 2-fluoro-4-pyridinylmethyl in three core structure types: oxindole (**1**, **4**, **7**), 4-azafluorene (**2**, **5**, **8**), and anthrone (**3**, **6**, **9**) (Table 1). These compounds were then evaluated for their ability to enhance release of acetylcholine in a potassium-evoked slice assay and in vivo using microdialysis in conscious animals.

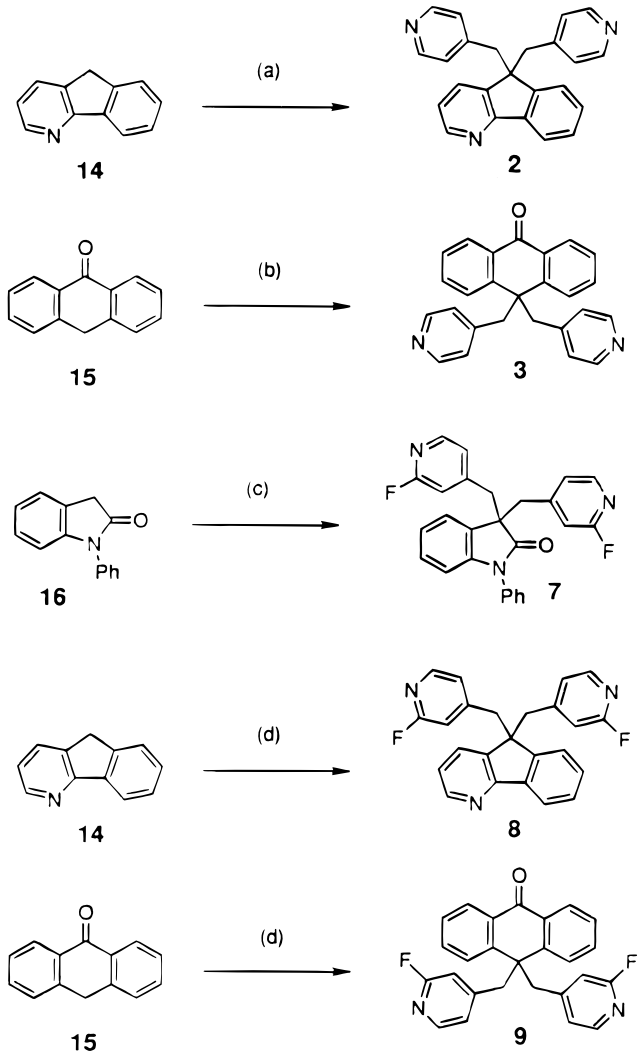
Chemistry

Chlorination of commercially available 2-fluoro-4-methylpyridine (**10**) with *N*-chlorosuccinimide under free radical conditions provided a mixture of 2-fluoro-4-(chloromethyl)pyridine (**11**), 2-fluoro-4-(α,α-dichloromethyl)pyridine (**12**), and unreacted starting material (3:1:1) (Scheme 2). Subsequent treatment of this mixture under Finkelstein conditions gave only one iodinated compound, 2-fluoro-4-(iodomethyl)pyridine (**13**). The byproducts from the chlorination reaction are not reactive and are readily removed by chromatography after the subsequent alkylation reaction.

The symmetrically dialkylated compounds **2**, **7**, **8**, and **9** were prepared according to Scheme 3, utilizing *N*-phenyloxindole,[40] 4-azafluorene,[41,42] and anthrone and either 4-picolyl chloride, 2-fluoro-4-(chloromethyl)pyridine, or 2-fluoro-4-(iodomethyl)pyridine in the presence of base (such as sodium hydride). The anthrone product **3** was best prepared from 4-picolyl chloride hydrochloride and anthrone under phase-transfer catalyzed conditions.[43] In the case of **9**, the yield using 2-fluoro-4-(iodomethyl)pyridine as an alkylating agent was usually greater than 55%, whereas from the chloromethyl compound it was typically less than 25%.

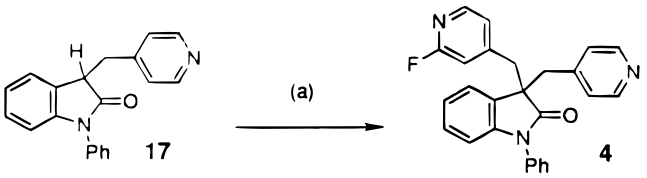
Unsymmetrically dialkylated compounds ("mixed pendant group" compounds) **4**, **5**, and **6** were also prepared to determine if one fluorinated pyridine would be sufficient to improve pharmacodynamics while maintaining or increasing potency. Oxindole **4** was prepared from the well-known 1,3-dihydro-1-phenyl-3-(4-pyridinylmethyl)-2*H*-indol-2-one[44,45] and 2-fluoro-4-(chloromethyl)pyridine in the presence of sodium hydride (Scheme 4). The 4-azafluorene analogue **5** was synthesized via the procedure in Scheme 5, starting from

Scheme 3[a]



[a] (a) Picolyl chloride (2 equiv), NaH, THF, room temperature; (b) picolyl chloride hydrochloride (2 equiv), benzyltriethylammonium chloride, toluene, NaOH, 50 °C; (c) 2-fluoro-4-(chloromethyl)pyridine (2 equiv), NaH, THF, room temperature; (d) 2-fluoro-4-(iodomethyl)pyridine (2 equiv), NaH, THF, room temperature.

Scheme 4[a]



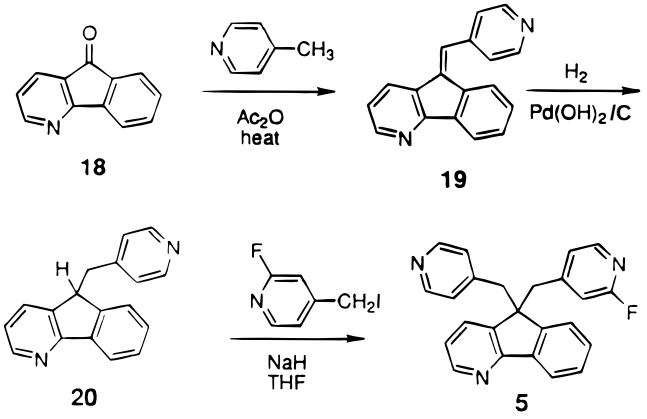
[a] (a) 2-Fluoro-4-(iodomethyl)pyridine (2 equiv), NaH, THF, room temperature.

4-azafluorenone.[46] Anthrone **6** was produced according to Scheme 6, by the presence of equivalent amounts of both alkylating agents. The desired unsymmetrically appended product **6** was purified from a mixture with the symmetrically appended compounds **3** and **9**. Attempts to prepare a monoalkylated anthrone and convert it to **6** were not successful.

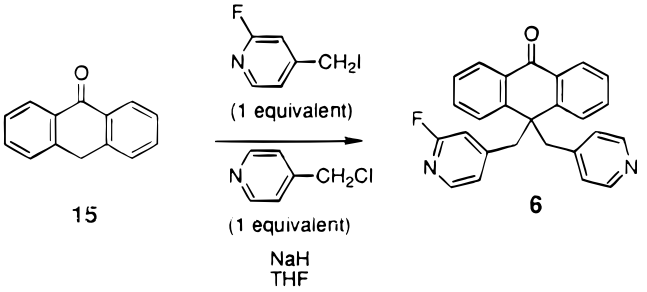
Biological Activity

Compounds were initially assessed for their ability to enhance the K^+-stimulated release of [^{3}H]acetylcholine from rat hippocampal slices preloaded with [^{3}H]-

Scheme 5



Scheme 6



choline.[47] This assay is a standard slice superfusion procedure in which release is stimulated for 4 min at two points, denoted S1 and S2, by increasing the concentration of potassium in the superfusion medium to 25 mM. Test compound is introduced after the control stimulation period (S1) and remains in the superfusion medium until the end of the second stimulation period (S2). The ratio of S2/S1 is used to measure the degree of drug-induced release enhancement.

Figure 1 shows dose−response curves for ACh release enhancement for linopirdine and **9**. From these curves, the EC_{50} value, which is the concentration that causes an enhancement that is 50% of the maximal response of a given compound, was calculated. For **9** and linopirdine, these values are 0.83 and 4.5 μM, respectively, with the maximum response of **9** higher than that observed for linopirdine. The EC_{50}'s for the other compounds are shown in Table 2. With the exception of the oxindoles **4** and **7**, *within* a core series, release activity follows nonfluoro ≈ monofluoro > difluoro > linopirdine.

The exact mechanism of ACh release enhancement by these compounds has yet to be unequivocally proven. However, the association of the ability of linopirdine and related compounds to block certain K^+ channels at concentrations comparable to those causing neurotransmitter release enhancement has been reported.[48] In particular, blockade of M-type potassium channels[49] currently serves as a possible molecular target responsible for the ability of these agents to cause ACh release enhancement. Further work is currently underway to support this proposal.

To determine neurotransmitter release-enhancing activity in vivo, compounds were evaluated in microdialysis assays that measure extracellular brain concentrations of ACh in freely moving rats.[50−52] In this

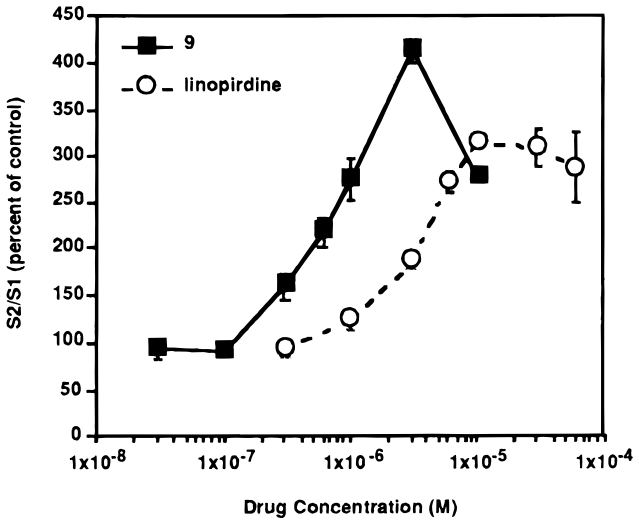


Figure 1. In vitro ACh release-enhancing effects of **9** and linopirdine: dose response. Assays were performed as described previously.[47] Each point represents data from at least three individual determinations. EC_{50} values calculated using peak efficacy as 100% activity were found to be 0.83 μM for **9** and 4.5 μM for linopirdine.

experimental procedure, an animal is surgically implanted with a guide-cannula into the hippocampus 2−4 days prior to testing. On the day of testing, a microdialysis probe is placed through the guide and is perfused with artificial CSF. Physostigmine, a cholinesterase inhibitor, is also perfused in the system to prevent the degradation of released ACh, which diffuses into the probe and is collected. The dialysate is injected every 20 min onto an HPLC system which measures ACh. Test compound or vehicle is administered orally to animals after the fourth 20-min collection period. Experiments are usually carried out for 14 collection periods. Results are expressed as percent increase of ACh over baseline for each collection period. Linopirdine showed a rapid onset of action, a short duration, and inactivity below 10 mg/kg, po (Table 2, Figure 2). The monofluorinated and bisfluorinated oxindoles **4** and **7** are likewise inactive at doses lower than 10 mg/kg. The azafluorenes and anthrones show an increased ability to enhance ACh release. Generally, upon oral administration, the nonfluorinated compounds **2** and **3** and the monofluorinated compounds **5** and **6** showed a similar fast onset and short duration of action like linopirdine. The bisfluorinated compounds **8** and **9**, however, show a slower onset of action, along with a very extended duration (Figure 2). Compounds **8** and **9** are less water-soluble and more lipophilic than linopirdine by >1 log unit. These properties can influence absorption and distribution of agents in vivo and most likely are contributing to the in vivo pharmacological profile.

The fact that these compounds showed good activity upon oral administration implies that they have a higher bioavailability, greater brain penetration, longer half-life (compared to linopirdine), or a combination of effects. Indeed, the most effective compound resulting from these studies is **9**, being between 10 and 20 times more potent than linopirdine in increasing extracellular hippocampal ACh levels in the rat with a minimum effective dose of 1 mg/kg (Table 2). In addition to

Table 2. Acetylcholine Release Enhancement Data

compd	in vitro ACh release EC_{50} (μM)	in vitro ACh release max efficacy[a]	in vivo microdialysis ACh release (po)		
			dose, vehicle[b]	peak[c]	duration[d]
1 (linopirdine)	4.5 ± 1.24	337% at 10 μM	20, methocel	117 ± 36	140
			10, methocel	65 ± 13	80
			5, water	50 ± 16	20
2	4.12 ± 2.28	725% at 10 μM	10, methocel	IA	
3	0.45 ± 0.12	600% at 10 μM	10, water	127 ± 31	100
			5, water	18 ± 15	NSP
4	10.0 ± 10.1	433% at 10 μM	10, methocel	27 ± 38	NSP
5	1.08 ± 0.2	317% at 10 μM	10, water	52 ± 10	>40
			5, water	47 ± 32	NSP
6	0.41 ± 0.23	452% at 1 μM	5, methocel	75 ± 12	100
7	15.2 ± 6.67	326% at 10 μM	10, methocel	44 ± 32	NSP
8	1.60 ± 0.95	440% at 3 μM	5, methocel	201 ± 68	>80
			1, methocel	74 ± 29	120
9	0.83 ± 0.28	413% at 3 μM	1, methocel	115 ± 27	>120
			0.5, methocel	40 ± 21	NSP

[a] Percent control, control = 100%, at the dose specified; determined as described previously.[47] [b] Doses in mg/kg; methocel, compound suspended in 25% methocel in water and bead-milled overnight. [c] Percent increase over control, control = 0; IA, inactive. [d] Number of consecutive minutes at significance; NSP, no significant points.

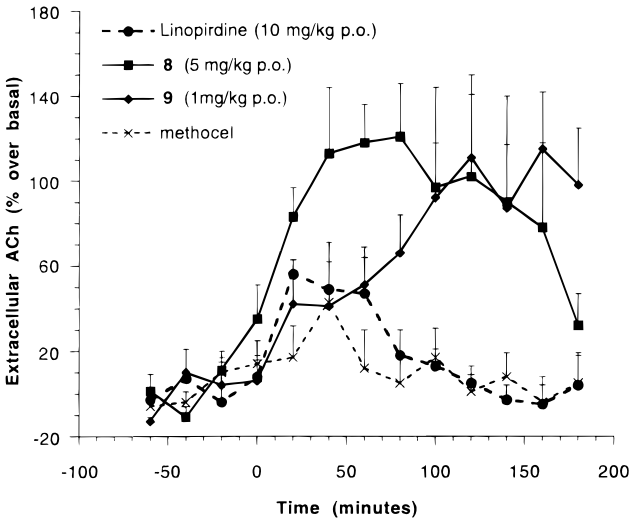


Figure 2. In vivo microdialysis comparison of test compounds **8** and **9** to linopirdine. Values are expressed as percent control. Drug was administered at time zero.

superior potency, **9** possesses an improved pharmacokinetic profile compared to that of linopirdine. The half-life of **9** (2 h) in rats is 4-fold greater than that of linopirdine (0.5 h). The oral bioavailability of both **9** and linopirdine is approximately 30%. The levels of linopirdine were previously evaluated in plasma and brain upon oral dosing. The brain-to-plasma ratio of linopirdine was approximately 1:6.[53,55] The brain-to-plasma ratio of **9** observed in parallel studies was approximately 1:1.[56] Thus, this improvement in brain−plasma distribution of **9** and the in vivo effects of this compound suggest that it may also possess the better brain penetration, as compared to linopirdine, that we sought.

In summary, we have discovered that substitution of 2-fluoro-4-pyridinylmethyl pendant groups for 4-pyridinylmethyl groups, in conjuction with a more potent tricyclic systems, has led to a compound possessing superior pharmacolgical and pharmacodynamic properties relative to linopirdine. On the basis of this pharmacological and pharmacokinetic profile, **9** (DMP 543) is undergoing clinical development.[54]

Experimental Section

Chemistry. Melting points were determined on a Electrothermal capillary apparatus and are uncorrected. Proton NMR spectra were obtained using Varian Unity 300 spectrometers (Varian Instruments, Palo Alto, CA); chemical shifts were recorded in ppm (δ) from an internal TMS standard in deuteriochloroform or deuteriodimethyl sulfoxide as specified. Coupling constants were measured in hertz (Hz). Mass spectra were measured with a Hewlett-Packard 5988A or a Finnigan MAT 8230 mass spectrometer with a particle beam interface using NH_3 or CH_4 for chemical ionization. High-resolution mass spectra (HRMS) were obtained on a VG 70-VSE instrument with NH_3 as a carrier gas for chemical ionization. Elemental analyses were performed by Quantitative Technologies, Inc., Whitehouse, NJ, or Micro-Analysis, Inc., Wilmington, DE. Solvents and reagents were obtained from commercial vendors and used without further purification unless otherwise indicated.

2-Fluoro-4-(chloromethyl)pyridine (11). To a solution of 2-fluoro-4-picoline (**10**; 13.33 g, 120 mmol) and *N*-chlorosuccinimide (23.98 g, 180 mmol) in 500 mL of carbon tetrachloride was added 1.5 g of benzoyl peroxide, and the mixture was heated at reflux. After 4 h, the mixture was briefly cooled to room temperature, an additional 0.5 g of benzoyl peroxide was added, and reflux was continued overnight. After cooling to room temperature, the reaction mixture was filtered through Celite, washed with saturated sodium thiosulfate, saturated sodium bicarbonate, water, and brine, and dried over magnesium sulfate. The solvent was removed in vacuo to give a yellow liquid, 17.845 g, which contained 60 mol % **11**, 24 mol % **12**, and 16 mol % starting material **(10)** by ^{1}H NMR (300 MHz, $CDCl_3$) integration. This product was used directly in alkylation reactions or converted to the iodide **13**.

2-Fluoro-4-(iodomethyl)pyridine (13). To a solution of sodium iodide (19.79 g, 0.132 mol) in 500 mL of acetone was added **11** (17.46 g, 0.120 mol; 29.6 g of the chlorination mixture), and the solution was heated at reflux for 2 h. The precipitated sodium chloride was filtered off through Celite, and the solvent was removed in vacuo. The residue was redissolved in ether, washed with saturated sodium thiosulfate, water, and brine, and dried over magnesium sulfate. The solvent was evaporated to give a yellow oil, 33.29 g. ^{1}H NMR (300 MHz, $CDCl_3$) showed that the methylene singlet of the chloride at 4.57 ppm had shifted to 4.34 ppm and the 6-H pyridine doublet had shifted from 8.23 to 8.16 ppm. The spectrum showed 100% conversion of **11** to **13** and the other unchanged side products of the chlorination reaction (**12** and **10**). This product was used directly in alkylation reactions.

5,5-Bis(4-pyridinylmethyl)-5*H*-indeno[1,2-*b*]pyridine, Hemihydrate (2). To a suspension of sodium hydride (60% in oil, 3.34 g, 83.5 mmol) in 100 mL of THF at room

temperature were added a solution of 4-azafluorene (**14**)[41,42] (4.16 g, 25 mmol) and 1 drop of ethanol. A solution of 4-picolyl chloride in benzene (prepared from 9.79 g, 59.7 mmol, of the hydrochloride salt, free-based with sodium carbonate, extracted into benzene, and dried over sodium sulfate) was subsequently added to the reaction mixture via addition funnel. After the mixture stirred at room temperature for 3 h, saturated ammonium chloride was added, and the mixture was extracted with ethyl acetate. The aqueous layer was washed with more ethyl acetate, and the combined organic solution was washed with water and brine and dried over magnesium sulfate. The product was purified via column chromatography (silica gel, 5% methanol in methylene chloride) and recrystallized from ethyl acetate/hexane to give **2** as white crystals, 7.09 g, 81% yield: mp 169−171 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.40 (s, 4H), 6.51 (d, J = 6.2 Hz, 4H), 7.18 (dd, J = 4.8, 7.7 Hz, 1H), 7.37 (dt, J = 0.7, 7.3 Hz, 1H), 7.47 (dt, J = 0.7, 7.3 Hz, 1H), 7.59 (d, J = 7.7 Hz, 1H), 7.67 (dd, J = 1.5, 7.7 Hz, 1H), 7.74 (d, J = 7.3 Hz, 1H), 8.15 (dd, J = 1.5, 4.8 Hz, 4H), 8.45 (dd, J = 1.5, 5.1 Hz, 1H); MS (CI/NH_3) *m/e* 350 (M + H). Anal. ($C_{24}H_{19}N_3 \cdot 0.5H_2O$) C, H, N.

10,10-Bis(4-pyridinylmethyl)-9(10*H*)-anthracenone (3). To a mechanically stirred slurry of 50 g (0.257 mol) of anthrone, 92.9 g (0.566 mol) of 4-picolyl chloride hydrochloride, and 5.14 g (0.0226 mol) of benzyltriethylammonium chloride in 1000 mL of toluene was added 129 mL of 50% NaOH via addition funnel over the period of 15 min. After addition, the mixture was slowly heated to 50 °C and maintained at that temperature for 18 h. The mixture was then diluted with 50 mL of water and cooled to room temperature. The toluene layer was separated, washed with water and brine, dried over magnesium sulfate, and evaporated in vacuo to give a dark-brown solid. The product was dissolved in methylene chloride, chromatographed on silica gel with 10% ethyl acetate/hexane, and recrystallized from methylene chloride/hexane to give **3** as white crystals, 32 g, 33% yield: mp 231−2 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.73 (s, 4H), 6.19 (dd, J = 1.5, 4.8 Hz, 4H), 7.54 (dt, J = 1.1, 7.5 Hz, 2H), 7.79 (dt, J = 1.5, 7.5 Hz, 2H), 7.98 (d, J = 8.1 Hz, 2H), 8.02 (dd, J = 1.5, 4.8 Hz, 4H), 8.12 (dd, J = 1.5, 7.7 Hz, 2H); IR (KBr) 1656, 1600, 1328, 1316, 694 cm^{-1}; MS (CI/NH_3) *m/e* 377 (M + H). Anal. ($C_{26}H_{20}N_2O$) C, H, N.

3-[(2-Fluoro-4-pyridinyl)methyl]-3-(4-pyridinylmethyl)-1,3-dihydro-1-phenyl-2*H*-indol-2-one (4). This compound was prepared from **17**[44,45] (710 mg, 2.36 mmol) and **11** (2.69 mmol from the crude chlorinated mixture) using sodium hydride (60% in oil, 3.37 mmol, 135 mg) in THF in the same manner described for **2**. Purification via column chromatography (2:1 hexane/ethyl acetate) and recrystallization from methylene chloride/hexane provided **4** as white crystals, 318 mg, 33% yield: mp 174−6 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.22 (d, J = 12 Hz, 1H), 3.26 (d, J = 12 Hz, 1H), 3.46 (d, J = 13 Hz, 1H), 3.52 (d, J = 13 Hz, 1H), 6.29 (d, J = 8 Hz, 1H), 6.52 (s, 1H), 6.63 (d, J = 10 Hz, 2H), 6.80 (d, J = 5 Hz, 1H), 6.85 (d, J = 6 Hz, 2H), 7.10 (m, 2H), 7.34 (m, 2H), 7.43 (d, J = 8 Hz, 2H), 7.93 (d, J = 5 Hz, 1H), 8.30 (d, J = 6 Hz, 2H); IR (KBr) 3394, 3070, 2928, 1708, 1612, 1564, 1502, 1466, 1412, 1382, 1282, 1270, 1238, 1220, 768 cm^{-1}; MS (CI/NH_3) *m/e* 410 (M + H). Anal. ($C_{26}H_{20}FN_3O$) C, H, N.

5-(4-Pyridinylmethylene)-5*H*-indeno[1,2-*b*]pyridine (19). A mixture of 4-azafluorenone **(18)** (15.21 g, 84 mmol), 4-picoline (88 mmol, 8.21 g, 8.6 mL), and acetic acid (8.4 mL) in 100 mL of acetic anhydride was heated at 130 °C for 24 h. Excess acetic anhydride was removed in vacuo, and the residue was partitioned between methylene chloride and saturated sodium carbonate. The organic layer was washed with water and brine, and dried over magnesium sulfate. The solvent was removed under reduced pressure, and the residue was purified via column chromatography (silica gel, 10% THF in methylene chloride) and recrystallized from methylene chloride/hexane to give **19** as pink plates, 6.0 g, 28% yield: mp 194−6 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.18 (dd, J = 4.8, 8.1 Hz, 1H), 7.54 (m, 2H), 7.60 (d, J = 5.5 Hz, 2H), 7.69 (dd, J = 1.5, 7.7 Hz, 1H), 7.94 (m, 1H), 8.04 (s, 1H), 8.12 (m, 1H), 8.53 (dd, J = 1.5, 4.8 Hz, 1H), 8.73 (dd, J = 1.5, 4.4 Hz, 2H); MS (CI/NH_3) *m/e* 257 (M + H). Anal. ($C_{18}H_{12}N_2$) C, H, N.

5-(4-Pyridinylmethyl)-5*H*-indeno[1,2-*b*]pyridine (20). To a solution of **19** (0.724 g, 2.8 mmol) in ethanol was added 20% $Pd(OH)_2$/C (150 mg), and the mixture was shaken under 50 psi of hydrogen (Parr bottle) for 1 h. The catalyst was filtered off, the solvent was removed in vacuo, and the residue was triturated with ether/petroleum ether to give **20** as a white solid, 0.355 g, 49% yield: mp 126−8 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.03 (dd, J = 8.0, 13.9 Hz, 1H), 3.27 (dd, J = 6.6, 13.9 Hz, 1H), 4.26 (t, J = 7.3 Hz, 1H), 7.09 (d, J = 5.5 Hz, 2H), 7.11 (d, J = 5.1 Hz, 1H), 7.37 (m, 2H), 7.47 (dt, J = 1.5, 7.3 Hz, 1H), 8.05 (d, J = 7.3 Hz, 1H), 8.51 (dd, J = 1.5, 4.4 Hz, 2H), 8.57 (1.1, J = 5.1 Hz, 1H); MS (CI/NH_3) *m/e* 259 (M + H). Anal. ($C_{18}H_{14}N_2 \cdot 0.2H_2O$) C, H, N.

5-[(2-Fluoro-4-pyridinyl)methyl]-5-(4-pyridinylmethyl)-5*H*-indeno[1,2-*b*]pyridine (5). This compound was prepared from **20** (180 mg, 0.697 mmol) and **11** (0.794 mmol from the crude chlorinated mixture) using sodium hydride (60% in oil, 0.997 mmol, 40 mg) in THF in the same manner described for **2**. Purification via column chromatography (ethyl acetate) and recrystallization from methylene chloride/hexane provided **5** as white crystals, 125 mg, 49% yield: mp 164−5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.40 (s, 2H), 3.46 (d, J = 4 Hz, 2H), 6.13 (s, 1H), 6.34 (dt, J = 1.5, 5.1 Hz, 1H), 6.53 (d, J = 6 Hz, 2H), 7.20 (dd, J = 4.8, 7.7 Hz, 1H), 7.41 (dt, J = 1.1, 7.3 Hz, 1H), 7.51 (dt, J = 1.7, 7.3 Hz, 1H), 7.60 (d, J = 6.8 Hz, 1H), 7.70 (dd, J = 1.8, 7.7 Hz, 1H), 7.73 (d, J = 5.1 Hz, 1H), 7.76 (d, J = 7.3 Hz, 1H), 8.17 (d, J = 6 Hz, 2H), 8.47 (dd, J = 1.5, 5.1 Hz, 1H); IR (KBr) 1610, 1568, 1558, 1452, 1412, 824, 750 cm^{-1}; MS (CI/NH_3) *m/e* 368 (M + H). Anal. ($C_{24}H_{18}FN_3 \cdot 0.25H_2O$) C, H, N.

10-[(2-Fluoro-4-pyridinyl)methyl]-10-(4-pyridinylmethyl)-9(10*H*)-anthracenone (6). To a mechanically stirred slurry of 1.94 g (10 mmol) of anthrone, 1.64 g (10 mmol) of 4-picolyl chloride hydrochloride, 1.46 g (10 mmol) of 2-fluoro-4-(chloromethyl)pyridine, and 0.2 g (0.87 mmol) of benzyltriethylammonium chloride in 50 mL of toluene was added 5 mL of 50% NaOH via addition funnel over a period of 15 min. After addition, the mixture was stirred at room temperature for 3 days. The mixture was then diluted with 50 mL of water and diluted with ethyl acetate. The organic layer was washed with water and brine, dried over magnesium sulfate, and evaporated in vacuo to give a dark-brown oil. The crude product was dissolved in methylene chloride, chromatographed on silica gel with 1:1 ethyl acetate/hexane, and recrystallized from methylene chloride/hexane to give **6** as beige crystals, 468 mg, 12% yield: mp 199−201 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.76 (s, 2H), 3.81 (s, 2H), 5.89 (s, 1H), 6.11 (d, J = 5 Hz, 1H), 6.22 (d, J = 5 Hz, 2H), 7.48 (t, J = 7 Hz, 2H), 7.64 (d, J = 5 Hz, 1H), 7.85 (m, 2H), 8.05 (s, 3H), 8.07 (s, 1H), 8.17 (d, J = 9 Hz, 2H); IR (KBr) 3030, 2950, 1654, 1600, 1558, 1460, 1412, 1322, 1152, 816 cm^{-1}; MS (CI/NH_3) *m/e* 395 (M + H). Anal. ($C_{26}H_{19}FN_2O$) C, H, N.

3,3-Bis[(2-fluoro-4-pyridinyl)methyl]-1,3-dihydro-1-phenyl-2*H*-indol-2-one (7). This compound was prepared from **16**[40] (2.09 g, 10 mmol) and **11** (22 mmol from the crude chlorinated mixture) using sodium hydride (60% in oil, 40 mmol, 960 mg) in THF in the same manner described for **2.** Purification via column chromatography (2% methanol in chloroform) and recrystallization from ethyl acetate/hexane provided **7** as pale-yellow crystals, 1.77 g, 42% yield: mp 143−5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.25 (d, J = 12.8 Hz, 2H), 3.53 (d, J = 12.8 Hz, 2H), 6.34 (d, J = 7.3 Hz, 1H), 6.50 (s, 2H), 6.65 (m, 2H), 6.80 (d, J = 5.1 Hz, 2H), 7.17 (m, 2H), 7.37 (m, 4H), 7.95 (d, J = 5.1 Hz, 2H); MS (CI/NH_3) *m/e* 428 (M + H). Anal. ($C_{26}H_{19}F_2N_3O$) C, H, N.

5,5-Bis[(2-fluoro-4-pyridinyl)methyl]-5*H*-indeno[1,2-*b*]-pyridine (8). This compound was prepared from **14**[41,42] (1.22 g, 7.3 mmol) and **13** (16 mmol from the iodination mixture) using sodium hydride (60% in oil, 25 mmol, 1.0 g) in THF in the same manner described for **2**. Purification via column chromatography (1.5% methanol/chloroform) and recrystallization from ether/petroleum ether provided **8** as white crystals,

1.60 g, 57% yield: mp 137−41 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.43 (d, J = 13 Hz, 2H), 3.49 (d, J = 13 Hz, 2H), 6.14 (s, 2H), 6.35 (dt, J = 1.5, 3.3 Hz, 2H), 7.22 (dd, J = 5.1, 7.7 Hz, 2H), 7.43 (dt, J = 1.1, 7.3 Hz, 1H), 7.53 (dt, J = 1.1, 7.3 Hz, 1H), 7.60 (d, J = 7.3 Hz, 1H), 7.75 (m, 4H), 8.48 (dd, J = 1.5 Hz, 4.8H); MS (CI/NH_3) *m/e* 386 (M + H). Anal. ($C_{24}H_{17}N_3F_2$) C, H, N.

10,10-Bis[(2-fluoro-4-pyridinyl)methyl]-9(10*H*)-anthracenone (9). The title compound was prepared from anthrone (5.0 g, 26 mmol) and **13** (57 mmol from the iodination mixture) using sodium hydride (60% in oil, 57 mmol, 2.28 g) in THF in the same manner described for **2**. Purification via column chromatography (1:1 ethyl acetate/hexane) and recrystallization from ether/methylene chloride provided **9** as white crystals, 6.17 g, 58% yield: mp 159−61 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.76 (s, 4H), 5.85 (s, 2H), 6.04 (t, J = 3 Hz, 2H), 7.50 (t, J = 8 Hz, 2H), 7.63 (d, J = 5 Hz, 2H), 7.83 (t, J = 8 Hz, 2H), 7.97 (d, J = 8 Hz, 2H), 8.16 (d, J = 8 Hz, 2H); IR (KBr) 3068, 3032, 2940, 1660, 1604, 1558, 1460, 1410, 1326, 1272, 936, 702 cm^{-1}; MS (CI/NH_3) *m/e* 413 (M + H). Anal. ($C_{26}H_{18}F_2N_2O$) C, H, N.

Acetylcholine Release Assay. These experiments were performed as described previously.[47]

In Vivo Microdialysis Assay. Compounds were evaluated for their ability to enhance the release of acetylcholine in vivo as previously described.[55]

Pharmacokinetic Studies. Pharmacokinetic parameters were determined in male CD rats that received a 20-h or 7-day dosing of **9** at 1 or 4 mg/kg/day orally in 0.25% methylcellulose suspension or 4 mg/kg/day iv. For the iv study, **9** was dissolved in propylene glycol and administered through ALZET pumps implanted in a jugular cannula at an infusion rate of 8 or 10 μL/h. At 0.5, 1, 2, 4, 8, and 16 h after dosing, rats were sacrificed and blood samples were collected into tubes containing EDTA. Blood and brain tissue were collected at the end of the infusion. Plasma was separated by centrifugation and stored frozen until analysis. Compounds were extracted from plasma by simple liquid−liquid extraction. LC/MS/MS analysis was performed on a Sciex (Thornhill, Ontario) model APIIII triple quadrapole mass spectrometer interfaced with a turbo ion spray ionization source. The liquid chromatography consisted of a Perkin-Elmer series 200 solvent delivery system (Norwalk, CT), a Perkin-Elmer ISS 200 autoinjector, and a Waters Symmetry octyl minibore column (2.1 × 50 mm).

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