Substituted Pentacyclic Carbazolones as Novel Muscarinic Allosteric Agents: Synthesis and Structure−Affinity and Cooperativity Relationships

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Two series of pentacyclic carbazolones, **22** and **23**, have been synthesized utilizing a facile intramolecular Diels−Alder reaction and are allosteric modulators at muscarinic acetylcholine receptors. Their affinities and cooperativities with acetylcholine and the antagonist *N*-methylscopolamine (NMS) at M_1−M_4 receptors have been analyzed and compared. All of the synthesized compounds are negatively cooperative with acetylcholine. In contrast, the majority of the compounds exhibit positive cooperativity with NMS, particularly at M_2 and M_4 receptors. The subtype selectivity, in terms of affinity, was in general $M_2 > M_1 > M_4 > M_3$. The largest increases in affinity produced by a single substitution of the core structure were given by the 1-OMe (**22b**) and 1-Cl (**22d**) derivatives. The position of the N in the ring did not appear to be important for binding affinity or cooperativity. Two compounds **22y** and **23i**, both trisubstituted analogues, were the most potent compounds synthesized, with dissociation constants of 30−100 nM for the M_2 NMS-liganded and unliganded receptor, respectively. The results indicate that the allosteric site, like the primary binding site, is capable of high-affinity interactions with molecules of relatively low molecular weight.

Introduction

Until recently, it has proven difficult to discover competitive antagonists that exhibit a substantial selectivity for any one of the five muscarinic receptor subtypes (M_1−M_5) over all the other subtypes.[1−4] In fact, no M_5 selective antagonist and no agonists of substantial subtype selectivity, at least based on "affinity", have been described. This has been disappointing in view of the importance of muscarinic receptors as therapeutic targets in a variety of diseases, including asthma, disorders of intestinal motility, urinary bladder dysfunction, and Alzheimer's disease, and in the control of pain.[2]

The difficulty in designing subtype selective competitive muscarinic agents is probably because of the strong conservation of sequence in regions considered to bind agonists.[5,6] Muscarinic receptors contain a second, allosteric, binding site in addition to the "primary" site, which binds acetylcholine (ACh), as well as competitive antagonists such as *N*-methylscopolamine and atropine (for recent reviews, see refs 7−12). It is conceivable that there could be greater sequence differences between the amino acid residues contributing to the allosteric sites on different muscarinic receptor subtypes, hence providing a prospect of developing agents with greater subtype selectivity, based on affinity. Moreover, ligands binding to the allosteric site modulate the binding and actions of ligands, including ACh, at the primary binding site on muscarinic receptors. Intrinsic to allosteric agents is a form of selectivity that is based on differences in cooperativity between subtypes. This is not available to antagonists and agonists where affinity and efficacy (in the case of agonists) are the determinants of relative receptor subtype selectivity.

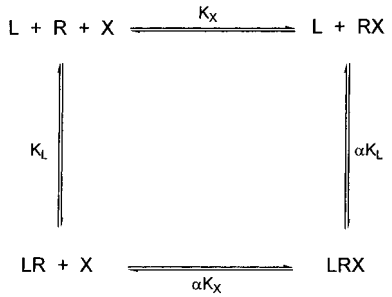

Figure 1. Ternary complex model of allosteric action of a ligand L with an allosteric agent X at a receptor R.

The simplest mechanism depicting the simultaneous interactions of two ligands with a receptor is the allosteric ternary complex model[13] (Figure 1) in which the affinity constant K_X of the binding of the ligand X to the primary site on the receptor R is modified by a factor α, the cooperativity factor, by the binding of the allosteric ligand L to the second site on the receptor. In a complementary manner, the affinity of L, K_L, is changed by the same factor α by the binding of X to the receptor. The cooperativity of the interaction of the two ligands with the receptor is defined by the factor α: if $\alpha > 1$, there is positive cooperativity; if $\alpha < 1$, there is negative cooperativity. For the special case where $\alpha = 1$, there is neutral cooperativity and the binding of L to R does not affect the binding of X. The binding, actions, and subtype selectivity of the allosteric ligand are therefore defined by two parameters: its affinity K_L,

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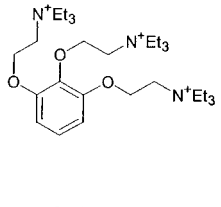
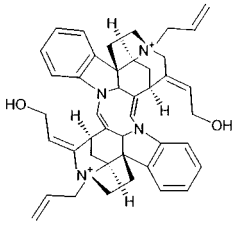
which is just dependent on the nature of R, and α, which depends on *both* R *and* X. The subtype selectivity and the pharmacological activity of L for modifying the action of X at different subtypes are therefore manifest by differences in *either or both* K_L and α.

Design of Novel Muscarinic Allosteric Agents

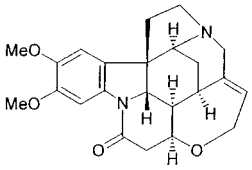
The first ligand shown to interact with the allosteric site on muscarinic receptors was gallamine **1**,[13,14] which



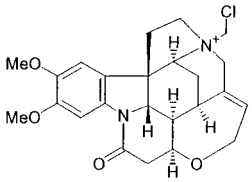
Gallamine (**1**)



Alcuronium (**2**)



Brucine (**3**)



N-Chloromethylbrucine (**4**)

exhibits an M_2 selectivity as regards K_L. In equilibrium and kinetic studies gallamine obeyed the predictions of the allosteric ternary complex model.[13] The α values were also found to be different (but all much less than 1) for the interaction between gallamine, and a variety of agonists and antagonists at a given subtype also differed for a given competitive ligand at different subtypes.[13] A similar M_2 selectivity to gallamine (as regards K_L) is exhibited by alcuronium **2**, and it also shows a subtype selectivity in α, with positive cooperativity with the antagonist *N*-methylscopolamine (NMS) being found at M_2 receptors but not at other subtypes.[15]

Ligands based around the structure of alcuronium or with flexible spacers between the two quaternary nitrogens have been investigated intensively.[16−24] A number of these molecules are more potent than gallamine and alcuronium, especially under low ionic strength conditions. One such ligand, [^{3}H]-dimethyl W-84, has been used to label an allosteric site on the M_2 receptor.[25]

The quantitative analyses of the allosteric interactions in these studies have involved primarily the determination of the potency of the ligands in slowing down the dissociation rate of [^{3}H]-NMS from M_2 receptors, i.e., the parameter αK_L where X = NMS. However, because our aim is to develop allosteric molecules for eventual therapeutic use, it is important to estimate the two parameters that define their actions on the different receptor subtypes in vivo, namely, K_L and α, where X is the endogenous ligand ACh. The bis-onium ligands, where investigated, are strongly negatively cooperative with ACh at all subtypes.

The first ligands reported to exhibit positive cooperativity with ACh in binding and functional studies at one or more subtypes were brucine **3** and its quaternized derivatives.[26−30] For this interesting series of compounds, subtype selectivity was primarily based on α (with ACh) and not K_L; for example, brucine is an

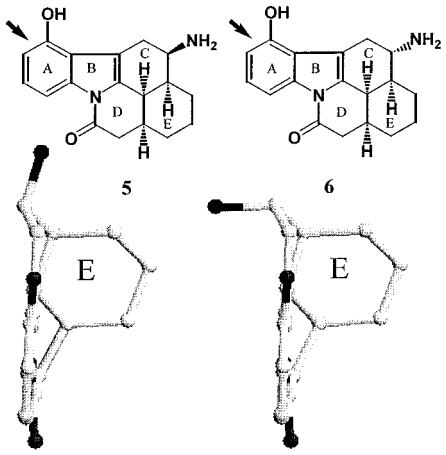


Figure 2. Side view (from the direction of the arrow) of the solution NMR structure of diasterioisomers **5** and **6**.

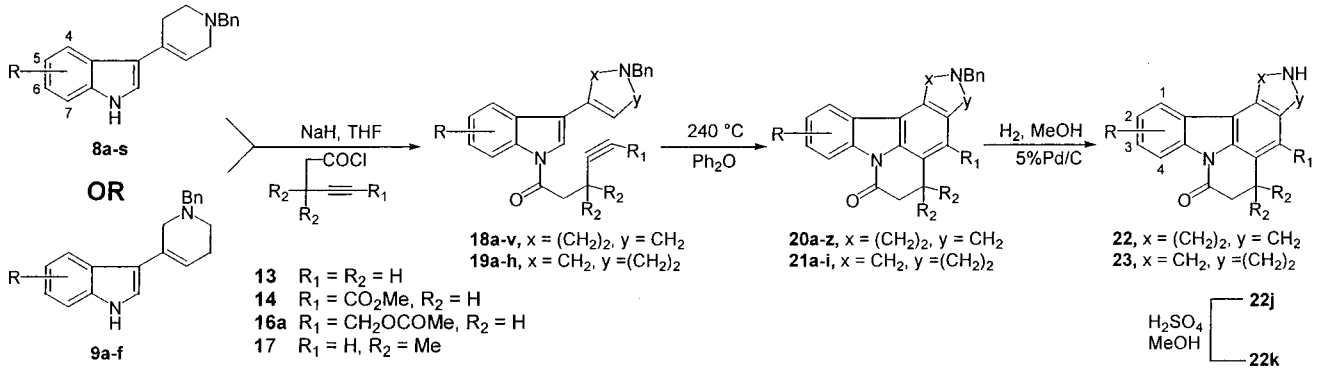
allosteric enhancer at only M_1 receptors, and *N*-chloromethylbrucine **4** is an enhancer at only M_3 receptors.[26,28,29]

Our studies on the brucine analogues and also on a series of strychnine derivatives pointed to the fact that small changes in structure can produce large changes in cooperativity and subtype selectivity.[28,30] However, the complex structures of strychnine and brucine were viewed as a major limitation in their use as a template for the development of allosteric muscarinic drugs. Subsequent screening of compounds with fused indole ring systems, structurally related to brucine, led to the discovery of other multicyclic agents with allosteric properties. Among these, the aminotetrahydroisoquinolinocarbazole **5** was our most interesting hit.[31] This agent is more potent than brucine at all muscarinic receptor subtypes. In addition, **5** displays neutral or positive cooperativity with NMS at all subtypes and low negative cooperativity with ACh at M_1, M_3, and M_4 receptors, but most interestingly, it has a 3-fold positive cooperativity with ACh at M_2 receptors.[31] This is the only well-characterized allosteric enhancer of ACh at M_2 receptors identified to date. In contrast, its diastereoisomer **6** exhibits a 8−20-fold higher affinity at M_1, M_3, and M_4 receptors but also has a high negative cooperativity with both NMS and ACh at all receptor subtypes.[31]

These stereoisomers have structures simpler than that of brucine and were regarded as suitable lead agents for the synthesis of more potent muscarinic allosteric agents. The reported solution NMR structures of **5** and **6** (Figure 2) have rings ABCD forming a rigid and an almost planar structure, with ring E perpendicular to this plane and in a chair conformation.[31] The only difference between the two isomers is the spatial orientation of the exocyclic amino group relative to the pentacyclic ring system; the amino group is equatorial in **5** and exists in the plane of the ABCD ring structure, whereas it is axial in **6** and approximately perpendicular to the ring system. One conclusion is that the spatial position of the amino group relative to the ABCD ring plays a major role in the binding at unliganded and liganded receptors and consequently in their cooperativity with primary ligands.

On the basis of these observations, we designed an analogous pentacyclic ring system (**7**) that incorporates

Scheme 1. Intramolecular Cyclization of 3-THPIs with Subsequent Debenzylation

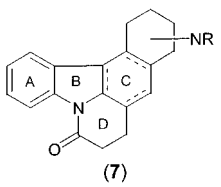


Cmpd.	R
8a	H
8b	4-OMe
8c	4-Cl
8d	4-COOMe
8e	5-Me
8f	5-OMe
8g	5-OBn
8h	5-Cl
8i	5-COOBn
8j	5-CON$(CH_2CH_2)_2O$
8k	5-$CH_2N(CH_2CH_2)_2O$
8l	6-OMe
8m	6-Cl
8n	6-COOMe
8o	6-CN
8p	7-OMe
8q	5,6-$(OMe)_2$
8r	5,6-OCH_2O
8s	4,5,6-$(OMe)_3$
9a	H
9b	5-OMe
9c	5-OBn
9d	5-COOMe
9e	6-OMe
9f	5,6-$(OMe)_2$

Cmpd.	x	y	R	R_1	R_2
18a	1	0	H	H	H
18b	1	0	4-OMe	H	H
18c	1	0	4-Cl	H	H
18d	1	0	4-COOMe	H	H
18e	1	0	5-Me	H	H
18f	1	0	5-OMe	H	H
18g	1	0	5-OBn	H	H
18h	1	0	5-Cl	H	H
18i	1	0	5-COOBn	H	H
18j	1	0	5-CON$(CH_2CH_2)_2O$	H	H
18k	1	0	5-$CH_2N(CH_2CH_2)_2O$	H	H
18l	1	0	6-OMe	H	H
18m	1	0	6-Cl	H	H
18n	1	0	6-COOMe	H	H
18o	1	0	6-CN	H	H
18p	1	0	7-OMe	H	H
18q	1	0	5,6-$(OMe)_2$	H	H
18r	1	0	5,6-OCH_2O	H	H
18s	1	0	4,5,6-$(OMe)_3$	H	H
18t	1	0	5,6-$(OMe)_2$	COOMe	H
18u	1	0	5,6-$(OMe)_2$	CH_2OCOMe	H
18v	1	0	4-OMe	H	Me
19a	0	1	H	H	H
19b	0	1	5-OMe	H	H
19c	0	1	5-OBn	H	H
19d	0	1	5-COOMe	H	H
19e	0	1	6-OMe	H	H
19f	0	1	5,6-$(OMe)_2$	H	H
19g	0	1	H	H	Me
19h	0	1	5,6-$(OMe)_2$	H	Me

Cmpd.	x	y	R	R_1	R_2
20a	1	0	H	H	H
20b	1	0	1-OMe	H	H
20c	1	0	1-OH	H	H
20d	1	0	1-Cl	H	H
20e	1	0	1-COOMe	H	H
20f	1	0	2-Me	H	H
20g	1	0	2-OMe	H	H
20h	1	0	2-OBn	H	H
20i	1	0	2-Cl	H	H
20j	1	0	2-COOBn	H	H
20k	1	0	2-CON$(CH_2CH_2)_2O$	H	H
20l	1	0	2-$CH_2N(CH_2CH_2)_2O$	H	H
20m	1	0	3-OMe	H	H
20n	1	0	3-OH	H	H
20o	1	0	3-Cl	H	H
20p	1	0	3-COOMe	H	H
20q	1	0	3-CN	H	H
20r	1	0	4-OMe	H	H
20s	1	0	4-OH	H	H
20t	1	0	2,3-$(OMe)_2$	H	H
20u	1	0	2,3-OCH_2O	H	H
20v	1	0	1,2,3-$(OMe)_3$	H	H
20w	1	0	2,3-$(OMe)_2$	COOMe	H
20x	1	0	2,3-$(OMe)_2$	CH_2OCOMe	H
20y	1	0	1-OMe	H	Me
20z	1	0	1-OH	H	Me
21a	0	1	H	H	H
21b	0	1	2-OMe	H	H
21c	0	1	2-OBn	H	H
21d	0	1	2-COOMe	H	H
21e	0	1	3-OMe	H	H
21f	0	1	3-OH	H	H
21g	0	1	2,3-$(OMe)_2$	H	H
21h	0	1	H	H	Me
21i	0	1	2,3-$(OMe)_2$	H	Me

the ABCD ring structure of brucine and **5** but, in addition, offers the potential for locating the basic amino group in a variety of endo- and exocyclic positions. Ring E of **5** was eliminated (for ease of synthesis), and the compounds described in this paper do not contain stereocenters. This enabled both the nature and the spatial position of the amino group to be readily investigated in the anticipation that both affinity and cooperativity might be optimized. We synthesized several novel ring scaffolds of type **7** (in which ring C was



(**7**)

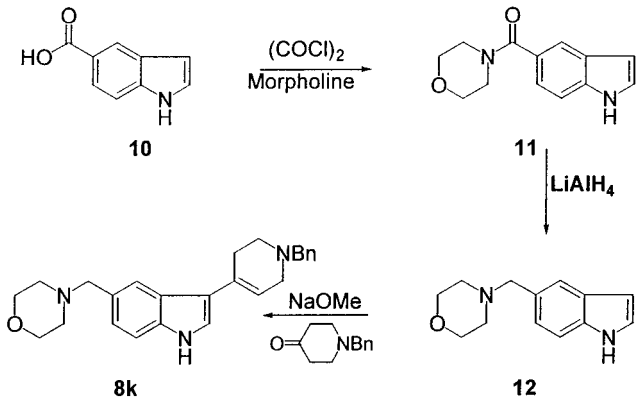
both saturated and unsaturated), readily accessible by an intramolecular [4 + 2]π cycloaddition procedure of 1-substituted-3-(tetrahydropyridinyl)indoles previously reported.[32] Taking into consideration the affinity and cooperativity of these ring systems and their relative ease of synthesis, the pentacyclic carbazolones **22a** and **23a** were chosen as templates for the development of more potent allosteric agents. In this paper we present our data on the effect of ring substitution in **22a** and **23a** where the aim was to increase affinity while maintaining $\alpha > 1$ or at least close to unity for the antagonist NMS and for ACh. A preliminary report of some of the findings has been presented.[33]

Chemistry

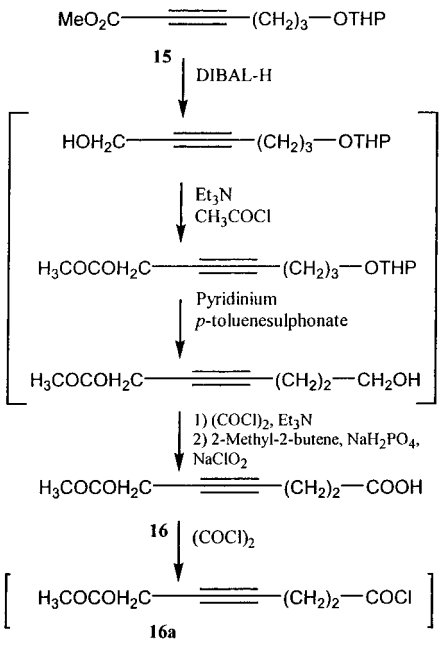
We previously described an efficient four-step synthetic route to a variety of pentacyclic carbazolones via the intramolecular [4 + 2]π cycloaddition of 3-(tetrahydropyridinyl)indoles (THPIs).[32] Following this procedure, we were able to prepare two series of pentacyclic carbazolones **22a**−**aa and 23a**−**i**, illustrated by Schemes 1 and 4 and incorporating a variety of substituents.

The prerequisite 3-THPIs **8a**−**s** and **9a**−**f** were prepared from the one-step condensation of appropriately

Scheme 2



Scheme 3



substituted indole and *N*-benzylpiperidone under either acidic or basic conditions according to our previously published procedures.[34] In the case of **8k**, the starting 5-morpholinomethylindole **12** was obtained from the $LiAlH_4$ reduction of the corresponding amide **11**, which in turn was prepared from the commercially available indole-5-carboxylic acid **10** according to Scheme 2.

Depending on the nature of R_1 and R_2 in the target molecules, the 3-THPIs were acylated with the appropriate acid chlorides (**13, 14, 16a**, and **17)** under anhydrous conditions to generate the Diels−Alder precursors **18a**−**v** and **19a**−**h**, using NaH as base. The synthesis of the hexynoyl chloride **16a**, not previously described, was achieved starting from **15**[35] followed by subsequent reduction, acetylation, deprotection, and oxidation to yield **16**, which was converted to **16a** upon treatment with oxalyl chloride in CH_2Cl_2 as illustrated in Scheme 3.

The thermal cyclization of the Diels−Alder precursors and the subsequent aromatization by loss of H_2 could only be affected in diphenyl ether at elevated temperatures (∼240 °C). This procedure furnished the cycloadducts **20a**−**z** and **21a**−**i** in good yields and usually free from impurities. Cyclization appeared to be unaffected by the position or the nature of the substituents on the indole ring (diene) or on the acetylene moiety (dieneophile).

Removal of the benzyl group was achieved by catalytic hydrogenation of the Diels−Alder adducts, using 5% Pd/C in MeOH at about 40 °C. This procedure worked well for most of the benzylated analogues (**20** and **21**), and good yields of the target ligands (**22** and **23**) were obtained. However, debenzylation of the 3-chloro-substituted analogue **20o** by catalytic hydrogenation also resulted in extensive dechlorination of the molecule, and only poor yields of **22p** were obtained. This problem was overcome for the other chloro-substituted analogues (e.g., **20d** and **20i**) by simply exchanging the protecting groups. Thus, reaction of **20d** and **20i** with vinyl chloroformate in THF followed by in situ acid hydrolysis of the corresponding carbamate intermediate furnished the debenzylated chloro-substituted analogues **22d** and **22i** in high yields as illustrated in Scheme 4.

The analogues incorporating a hydroxyl substituent (**22c, 22o, 22t, 22aa**, and **23f**) were obtained by the demethylation of the corresponding methoxy compounds (e.g., **20c, 20n, 20s, 20z**, and **21f**, respectively) using BBr_3 in CH_2Cl_2 followed by hydrogenolysis to remove the benzyl group (Scheme 4). The methyl ester **22k** was obtained by acid-catalyzed esterification of the corresponding carboxylic acid **22j** in MeOH (Scheme 4).

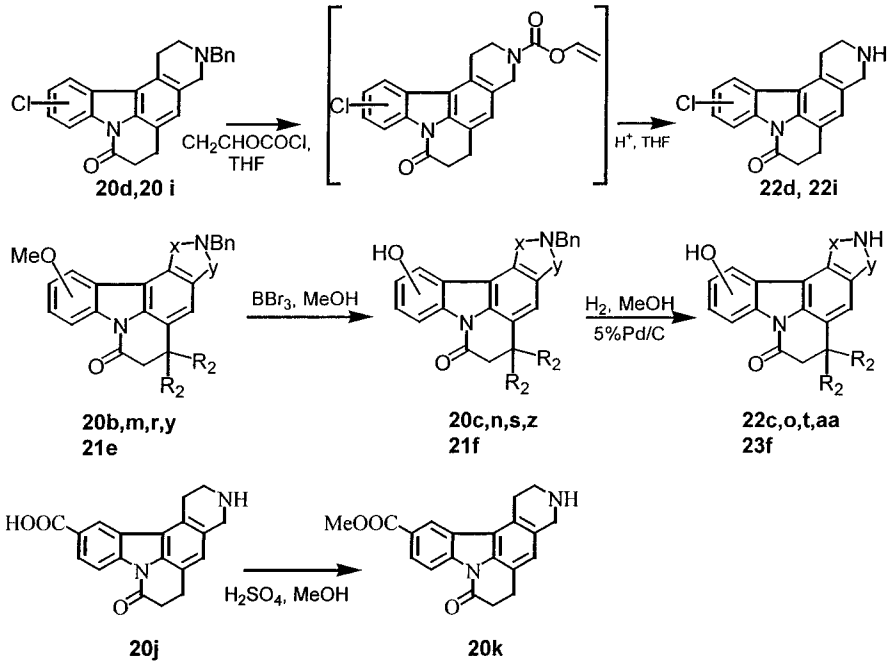
Pharmacology. Radioligand Binding Assays and Data Analysis

The binding assays and analyses have been described previously.[28,36,37] These provide estimates of the affinities of the allosteric compounds for the unliganded and NMS-liganded M_1−M_4 receptors (Table 1) together with the cooperativities of the ligands with NMS (shown symbolically in Table 2). Two types of experimental design were used: equilibrium assays to measure the effects of allosteric agents on the binding of ^{3}H−NMS and ACh, and "off-rate" assays to measure the effects of the allosteric ligands on ^{3}H−NMS dissociation. The experimental data were analyzed by nonlinear least-squares analysis using equations derived from the allosteric ternary complex model (Figure 1). The compounds in both series in general exhibited Hill coefficients greater than 1 for their binding to both unliganded and NMS-liganded receptors. Possible explanations for this are discussed later under "Apparent Positive Cooperativity". Detailed descriptions of the methodology and the data analysis are provided in the Experimental Section and in Supporting Information. It should be noted that the binding measurements are made in a Na/Mg/Hepes buffer and not in a low ionic strength buffer where affinities of certain allosteric agents are considerably higher.[18,38]

Results and Discussion

The binding affinities of 36 substituted pentacyclic carbazolones (**22a**−**aa** and **23a**−**i**) at the free and NMS-liganded human M_1−M_4 muscarinic receptors were measured using our "semiquantitative equilibrium" and "off-rate" assays and are listed in Table 1. The affinities of the ligands for the NMS-occupied receptor, estimated from the equilibrium and kinetic experiments, were in excellent agreement with each other; in only 3 out of

Scheme 4



140 possible comparisons were the differences greater than 3-fold. Such a concordance is in agreement with the predictions of the allosteric ternary complex model if the equilibrium and kinetic measurements reflect the same binding processes. Because of their higher precision, the estimates of the affinity at the NMS-occupied receptor in Table 1 are those from the off-rate experiments.

As discussed earlier, the pentacyclic carbazolones **22a** and **23a** were regarded as suitable allosteric templates for the development of more potent muscarinic allosteric agents. For our initial attempt, we chose to introduce a variety of substituents into all available positions of the indole ring (i.e., positions 1, 2, 3, and 4). The choice of the substituents was largely dependent on the commercial availability of the starting indoles and included an array of hydrophilic, hydrophobic, and hydrogen bond donors and acceptors.

Effect of Substituents on Affinity at Free and NMS-Liganded M_1−M_4 Receptors. The unsubstituted pentacyclic carbazolone **22a** binds with the logarithm of the affinity of 4.6−5.5 at free and NMS-liganded muscarinic receptor subtypes. In general, the results from the substitution study reveal that addition of substituents to positions 1−4 of the carbazolone ring system gives rise to an increase in affinity at both free and NMS-liganded receptors for all subtypes. The only exception is the 2-morpholinoamido derivative (**22l**) where a small but consistent reduction of affinity at all subtypes is apparent.

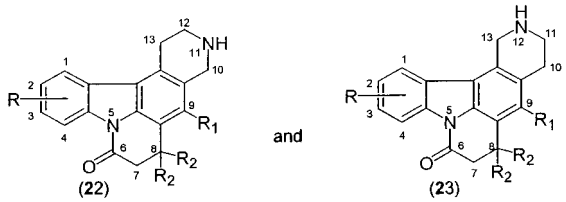
At the unliganded M_1 receptor the maximum increase in affinity for a single substituent is about 10-fold and is found for the 1-OMe substituent (**22b**). A similar gain in affinity is observed at free and NMS-liganded M_2−M_4 subtypes. Relocation of this group to positions 2, 3, and 4 (i.e., **22g**, **22n**, and **22s**, respectively) has little effect or reduces affinity at all free and NMS-liganded subtypes with the 4-position being the least tolerated. Further additions of methoxy groups (i.e., **22u** and **22w**) appear to have little further effect on affinity. The SAR observed for the 1-OMe substituent is also generally true for the 1-OH substitution (**22c**), although this agent binds with slightly lower affinity at most free and liganded M_2−M_4 subtypes.

Substitution with a Cl atom in the 1-position (**22d**) results in the highest gain in affinity achieved for a single substituent at free and liganded M_1−M_4 subtypes (with the exception of free M_1 receptors). For example, the logarithm of the affinity at NMS-liganded M_2 receptors is 7.1, a gain of over 40-fold in affinity over **22a**. Relocation of the Cl atom to the 2- or 3-position (**22i** and **22p**) slightly lowers the affinity particularly at the free and NMS-liganded M_3 and M_4 subtypes. Introduction of an electron-withdrawing group such as a COOMe into the 1-position (**22e**) makes little difference to the overall binding of the molecule. However, relocation of this group to either the 2- or 3-position (**22k** and **22q**) increases affinity at all subtypes particularly at the unliganded receptors.

Other substituents such as a 2-Me (**22f**) or 2,3-methylenedioxy (**22v**) slightly enhance affinity, whereas 2-COOH (**22j**), 2-morpholinomethyl (**22m**), and 3-CN (**22r**) do not have significant effects on binding. The 2,3-dimethoxy analogue **22u** has good aqueous solubility and binds with moderate affinities. This agent was further functionalized with a methyl ester at the 8-position (i.e., **22x**), but no improvement in affinity was observed. However, functionalization with an acetoxymethyl group (**22y**) at that position results in an increase in affinity (up to 5-fold) at all free and NMS-liganded subtypes. This agent **22y**, with a logarithm of the affinity of 7.2 at NMS-liganded M_2 receptors, is the most potent positive allosteric ligand in this series. Analogues **22b** and **22c** were both functionalized at the 7-position with methyl groups (i.e., **22z** and **22aa**, respectively), but no gain in affinity was observed.

Compound **23a** has an allosteric binding profile similar to that of its isomer **22a**. The only difference in the two ring systems is the position of the basic endocyclic nitrogen atom, and that results in a slight

Table 1. Estimated Logarithm of the Affinities (log M^{-1}) of the Pentacyclic Carbazolones at the Free and NMS-Liganded Muscarinic (M_1−M_4) Receptors



				M_1		M_2		M_3		M_4	
compd	R	R_1	R_2	free	NMS	free	NMS	free	NMS	free	NMS
22a	H	H	H	5.49 ± 0.03 (2)	5.49 ± 0.03 (2)	5.31 ± 0.10 (2)	5.49 ± 0.01 (2)	4.88 ± 0.03 (2)	4.64 ± 0.01 (2)	4.99 ± 0.08 (2)	5.37 ± 0.09 (2)
22b	1-OMe	H	H	6.50 ± 0.15 (2)	5.95 ± 0.09 (2)	6.43 ± 0.01 (2)	6.70 ± 0.04 (2)	5.58 ± 0.04 (2)	5.52 ± 0.07 (2)	6.25 ± 0.04 (2)	6.26 ± 0.08 (2)
22c	1-OH	H	H	5.84 ± 0.25 (2)	6.11 ± 0.14 (3)	5.99 ± 0.22 (2)	6.28 ± 0.19 (3)	5.43 ± 0.18 (2)	5.27 ± 0.13 (3)	5.60 ± 0.24 (2)	6.13 ± 0.18 (3)
22d	1-Cl	H	H	6.24 ± 0.05 (2)	6.58 ± 0.01 (2)	6.60 ± 0.03 (2)	7.12 ± 0.02 (2)	5.92 ± 0.08 (2)	6.05 ± 0.06 (2)	6.26 ± 0.03 (2)	6.66 ± 0.01 (2)
22e	1-COOMe	H	H	5.47 ± 0.03 (2)	5.34 ± 0.07 (2)	5.50 ± 0.05 (2)	5.82 ± 0.00 (2)	4.98 ± 0.05 (2)	4.78 ± 0.02 (2)	5.60 ± 0.24 (2)	5.58 ± 0.00 (2)
22f	2-Me	H	H	5.90 ± 0.24 (3)	5.69 ± 0.24 (3)	6.09 ± 0.25 (3)	6.47 ± 0.25 (3)	5.71 ± 0.02 (3)	5.08 ± 0.31 (3)	6.26 ± 0.03 (2)	5.97 ± 0.26 (3)
22g	2-OMe	H	H	5.78 ± 0.26 (2)	5.23 ± 0.08 (2)	5.96 ± 0.15 (2)	6.15 ± 0.21 (2)	5.04 ± 0.20 (2)	4.54 ± 0.07 (2)	5.60 ± 0.14 (2)	5.57 ± 0.21 (2)
22h	2-OH	H	H	5.79 ± 0.23 (3)	6.07 ± 0.04 (3)	5.61 ± 0.21 (3)	6.25 ± 0.09 (3)	4.89 ± 0.23 (3)	5.47 ± 0.05 (3)	5.65 ± 0.30 (3)	6.05 ± 0.04 (3)
22i	2-Cl	H	H	5.66 ± 0.03 (2)	5.58 ± 0.03 (2)	5.93 ± 0.01 (2)	6.54 ± 0.08 (2)	5.16 ± 0.01 (2)	5.23 ± 0.05 (2)	5.61 ± 0.10 (2)	6.14 ± 0.12 (2)
22j	2-COOH	H	H	5.43 ± 0.24 (2)	4.57 ± 0.17 (3)	5.31 ± 0.08 (4)	5.60 ± 0.10 (3)	4.51[a]	3.94 ± 0.19 (3)	5.39 ± 0.20 (3)	5.32 ± 0.17 (3)
22k	2-COOMe	H	H	6.32 ± 0.02 (2)	5.62 ± 0.09 (2)	6.44 ± 0.02 (2)	6.43 ± 0.12 (2)	5.64 ± 0.13 (2)	5.09 ± 0.02 (2)	5.55 ± 0.17 (2)	6.00 ± 0.05 (2)
22l	2-CON$(CH_2CH_2)_2$O	H	H	5.10 ± 0.12 (2)	4.70 ± 0.02 (2)	4.71 ± 0.09 (3)	5.21 ± 0.01 (2)	4.45[a]	3.57 ± 0.16 (2)	5.14 ± 0.04 (4)	4.54 ± 0.08 (2)
22m	2-CH_2N$(CH_2CH_2)_2$O	H	H	5.47 ± 0.06 (2)	5.35 ± 0.07 (2)	5.53 ± 0.16 (2)	5.79 ± 0.00 (2)	4.56 ± 0.04 (3)	3.93 ± 0.10 (2)	6.12 ± 0.18 (2)	5.35 ± 0.06 (2)
22n	3-OMe	H	H	5.84 ± 0.16 (2)	6.07 ± 0.12 (2)	5.90 ± 0.11 (2)	6.35 ± 0.07 (2)	5.33 ± 0.04 (2)	5.20 ± 0.04 (2)	4.64[a]	6.14 ± 0.04 (2)
22o	3-OH	H	H	6.01 ± 0.12 (2)	5.62 ± 0.05 (2)	5.24 ± 0.02 (2)	5.34 ± 0.07 (2)	5.23 ± 0.05 (2)	5.29 ± 0.09 (2)	5.38 ± 0.09 (2)	5.36 ± 0.01 (2)
22p	3-Cl	H	H	6.06 ± 0.06 (2)	6.51 ± 0.09 (2)	6.46 ± 0.02 (2)	6.81 ± 0.16 (2)	5.39 ± 0.00 (2)	5.48 ± 0.04 (2)	5.61 ± 0.11 (2)	6.47 ± 0.02 (2)
22q	3-COOMe	H	H	6.14 ± 0.01 (2)	5.72 ± 0.00 (2)	6.39 ± 0.08 (2)	6.35 ± 0.10 (2)	5.43 ± 0.07 (2)	4.83 ± 0.07 (2)	5.04 ± 0.09 (2)	6.01 ± 0.13 (2)
22r	3-CN	H	H	5.87 ± 0.13 (2)	5.46 ± 0.00 (2)	5.79 ± 0.08 (2)	5.58 ± 0.14 (2)	5.19 ± 0.02 (2)	4.68 ± 0.09 (2)	5.85 ± 0.01 (2)	5.36 ± 0.02 (2)
22s	4-OMe	H	H	5.40 ± 0.07 (2)	5.07 ± 0.04 (2)	5.19 ± 0.09 (2)	5.55 ± 0.09 (2)	5.06 ± 0.05 (2)	4.75 ± 0.05 (2)	5.97 ± 0.14 (2)	5.11 ± 0.09 (2)
22t	4-OH	H	H	5.49 ± 0.15 (2)	5.38 ± 0.10 (2)	5.36 ± 0.10 (2)	5.67 ± 0.11 (2)	5.06 ± 0.02 (2)	4.37 ± 0.09 (2)	5.43 ± 0.01 (2)	5.25 ± 0.11 (2)
22u	2,3-$(OMe)_2$	H	H	6.05 ± 0.11 (3)	5.81 ± 0.02 (2)	6.15 ± 0.05 (2)	6.47 ± 0.09 (2)	5.20 ± 0.18 (2)	5.15 ± 0.04 (2)	4.80 ± 0.04 (2)	6.05 ± 0.09 (2)
22v	2,3-OCH_2O	H	H	6.06 ± 0.08 (2)	5.99 ± 0.06 (2)	6.18 ± 0.04 (2)	6.31 ± 0.04 (2)	5.61 ± 0.03 (2)	5.06 ± 0.05 (2)	5.29 ± 0.16 (2)	5.73 ± 0.10 (2)
22w	1,2,3-$(OMe)_3$	H	H	6.74 ± 0.09 (2)	6.52 ± 0.16 (3)	6.69 ± 0.06 (3)	6.67 ± 0.16 (3)	5.63 ± 0.10 (3)	5.33 ± 0.05 (3)	5.97 ± 0.04 (3)	6.21 ± 0.07 (3)
22x	2,3-$(OMe)_2$	COOMe	H	5.97 ± 0.07 (2)	6.05 ± 0.00 (2)	6.22 ± 0.14 (2)	6.37 ± 0.04 (2)	5.11 ± 0.08 (2)	4.90 ± 0.04 (2)	5.86 ± 0.02 (2)	5.96 ± 0.07 (2)
22y	2,3-$(OMe)_2$	CH_2OCOMe	H	6.09 ± 0.07 (2)	6.16 ± 0.07 (3)	6.63 ± 0.01 (2)	7.21 ± 0.18 (3)	5.69 ± 0.09 (2)	5.85 ± 0.06 (3)	5.91 ± 0.03 (2)	6.55 ± 0.06 (3)
22z	1-$(OMe)_2$	H	Me	6.65 ± 0.05 (2)	5.54 ± 0.13 (3)	6.21 ± 0.23 (2)	6.16 ± 0.03 (2)	4.92[a]	4.83 ± 0.12 (2)	5.94 ± 0.13 (2)	5.79 ± 0.16 (3)
22aa	1-OH	H	Me	5.80 ± 0.02 (2)	5.84 ± 0.07 (2)	5.56 ± 0.05 (2)	5.88 ± 0.12 (2)	5.15 ± 0.24 (2)	4.84 ± 0.05 (2)	5.75 ± 0.00 (2)	5.97 ± 0.16 (2)
23a	H	H	H	5.85 ± 0.05 (3)	5.70 ± 0.05 (2)	5.51 ± 0.07 (2)	5.57 ± 0.05 (2)	5.78 ± 0.04 (2)	4.69 ± 0.00 (2)	5.31 ± 0.04 (2)	5.48 ± 0.03 (2)
23b	2-OMe	H	H	6.10 ± 0.05 (2)	5.91 ± 0.18 (2)	5.87 ± 0.07 (2)	6.05 ± 0.03 (2)	5.21 ± 0.06 (2)	4.76 ± 0.03 (2)	5.71 ± 0.03 (2)	5.74 ± 0.03 (2)
23c	2-OH	H	H	6.04 ± 0.02 (2)	6.07 ± 0.06 (2)	5.66 ± 0.05 (2)	5.81 ± 0.04 (2)	5.13 ± 0.06 (2)	5.12 ± 0.01 (2)	5.56 ± 0.01 (2)	5.90 ± 0.06 (2)
23d	2-COOMe	H	H	6.19 ± 0.02 (2)	5.85 ± 0.01 (2)	6.14 ± 0.05 (2)	6.03 ± 0.09 (2)	5.55 ± 0.17 (2)	4.88 ± 0.01 (2)	5.84 ± 0.01 (2)	5.91 ± 0.10 (2)
23e	3-OMe	H	H	6.13 ± 0.03 (3)	6.17 ± 0.07 (2)	5.92 ± 0.02 (2)	6.18 ± 0.19 (3)	5.14 ± 0.05 (2)	4.86 ± 0.12 (2)	5.68 ± 0.01 (2)	6.00 ± 0.11 (2)
23f	3-OH	H	H	6.59 ± 0.02 (2)	6.13 ± 0.04 (2)	5.60 ± 0.02 (2)	5.64 ± 0.05 (2)	5.60 ± 0.02 (2)	5.23 ± 0.10 (2)	5.52 ± 0.08 (2)	5.65 ± 0.05 (2)
23g	2,3-$(OMe)_2$	H	H	6.19 ± 0.15 (3)	6.44 ± 0.06 (3)	6.28 ± 0.13 (3)	6.97 ± 0.20 (3)	5.19 ± 0.02 (2)	5.53 ± 0.04 (3)	5.90 ± 0.01 (2)	6.57 ± 0.09 (3)
23h	H	H	Me	5.60 ± 0.10 (2)	5.69 ± 0.16 (2)	5.69 ± 0.03 (3)	5.87 ± 0.21 (2)	4.86 ± 0.11 (3)	4.56 ± 0.06 (2)	5.42 ± 0.06 (3)	5.80 ± 0.10 (2)
23i	2,3-$(OMe)_2$	H	Me	6.88 ± 0.03 (3)	6.29 ± 0.13 (2)	7.08 ± 0.11 (2)	6.70 ± 0.16 (2)	6.03 ± 0.09 (2)	5.47 ± 0.07 (2)	6.67 ± 0.04 (2)	6.37 ± 0.16 (2)

[a] Data were limited by the small amounts of the material.

Table 2. Cooperativity of **22a**−**aa** and **23a**−**i** with NMS at M_1−M_4 Receptors[a]

compd	M_1	M_2	M_3	M_4
22a	+	+	−	+
22b	−	+	0	0
22c	++	++	0	++
22d	+	++	0	+
22e	−	+	−	−
22f	−	+	−	+
22g	−	+	−	0
22h	++	++	++	++
22i	0	++	0	++
22j	−	++	−	++
22k	− −	0	−	−
22l	− −	++	− −	−
22m	−	+	−	0
22n	+	+	−	+
22o	−	+	0	+
22p	+	+	0	++
22q	−	−	−	−
22r	−	−	−	0
22s	−	+	−	+
22t	−	+	−	−
22u	−	+	0	+
22v	−	+	−	−
22w	−	0	−	+
22x	−	+	−	+
22y	0	++	0	++
22z	−	+	−	0
22aa	0	+	−	+
23a	−	+	−	+
23b	−	+	−	0
23c	0	+	0	+
23d	−	−	−	0
23e	0	+	−	+
23f	−	0	−	0
23g	+	++	+	++
23h	+	+	−	+
23i	−	−	−	−

[a] Cooperativity estimates are from the primary screens and the data in Table 1. Cooperativity values are as follows: (− −) <0.2; (−) 0.2−0.8; (0) 0.81−1.19; (+) 1.2−3; (++) >3.

increase in affinity, particularly at unliganded M_3 receptors. The effect of a small number of substituents in **23a** was also examined. As with the first series, the majority of substituents incorporated led to an increase in the overall affinity of the molecule. Analogues incorporating the following substituents 2-MeO (**23b**), 2-OH (**23c**), 2-COOMe (**23d**), 3-MeO (**23e**), and 3-OH (**23f**) exhibit binding affinities similar to those of their corresponding isomers (i.e., **22g**, **22h**, **22k**, **22n**, and **22o** respectively). This provides a strong indication that the position of the basic nitrogen atom does not play a significant role in the binding of these molecules. Similarly, the 2,3-dimethoxy analogue **23g** binds with almost equal affinities at the unliganded receptor subtypes compared to its isomer **22u**, although at NMS-liganded receptors, this agents displays higher affinities than **22u** (up to 4-fold). In contrast, functionalization of **23g** with methyl groups at the 7-position (i.e., **23i)** increases affinity at the unliganded receptors with virtually no effect at NMS-liganded subtypes. Interestingly, addition of methyl groups at the 7-position of **23a** (i.e., **23h**) has little or no effect on muscarinic binding.

Subtype Selectivity. Affinity. In general, the pattern of affinity found for both series of compounds is $M_2 > M_1 > M_4 > M_3$. However, none of the analogues discussed here exhibit significant selectivity for one subtype versus all other subtypes. The only marginal exceptions are the 3-OH substituted analogues **22o** and **23f**. These isomers display about a 10-fold selectivity at M_1 over the M_2−M_4 subtypes, although the observed selectivity is only apparent at the unliganded receptors.

Subtype Selectivity. Cooperativity with NMS. The pattern of occurrence of positive cooperativity is $M_2 > M_4 > M_1 > M_3$, which is somewhat different from that observed for the subtype selectivity pattern of affinity. Most of the substituents examined give rise to positive cooperativity with NMS at M_2 and M_4 receptors. However, only a small number of substituents support positive cooperativity at the M_1 and M_3 subtypes (Table 2). Two compounds were positively cooperative with NMS at all subtypes, and two were negatively cooperative at all subtypes. Within this series of compounds there is an organization in the cooperativity pattern between subtypes. Compounds that are positive at M_4 are positive at M_2. All compounds that are positive at M_1 are positive at both M_2 and M_4, and all compounds that are positive at M_3 are positive at the other three subtypes. The only exception is the trisubstituted **22w**, which is positive at M_4 and neutral at M_2.

The magnitude and nature of the observed cooperativity also appear to be highly dependent on the ring position and electronic character of the substituents as well as on the receptor subtype. For example, electron-donating substituents such as a hydroxyl group in ring positions 1 and 2 (i.e., **22c** and **22h**, respectively) exhibit strong positive cooperativities at M_1, M_2, and M_4 receptors, whereas the same substituent in the 3- or 4-position (i.e., **22o** and **22t**, respectively) manifest weak positive, neutral, or low negative cooperativities depending on the receptor subtype. Furthermore, electron-withdrawing substituents such as a methyl ester (**22e**, **22k**, and **22q**) or a nitrile (**22r**) tend to mostly support negative cooperativities.

Subtype Selectivity. Cooperativity with ACh. All of the synthesized compounds inhibited the binding of ACh at all muscarinic receptor subtypes as assessed from our "semiquantitative equilibrium" binding assay.

The cooperativity of (**23g)** with ACh at M_1 receptors was estimated by a simultaneous analysis of ACh/^{3}H−NMS competition curves measured in the absence and presence of three concentrations of **23g.** The estimated value of the cooperativity with ACh (0.10 ± 0.01, $n = 2$) indicated that **23g** is not strongly negatively cooperative with ACh at this subtype. A comparable experiment with **22b** at M_1 receptors gave an α value versus ACh of 0.13.

Apparent Positive Cooperativity. One interesting and distinguishing feature of this series of compounds is that in both the equilibrium and kinetic measurements the slope factors required to describe the interactions of the compounds with the receptors were greater than 1. A possible artifact that could give rise to such a phenomenon is the loss of the ligand by adsorption to the plastic tubes during dilution or in the assay or by depletion of the free ligand concentration by its partitioning into the membranes. Control experiments involving multiple transfers of dilutions between tubes and the measurement of the optical density of supernatants from binding assays, as well as the slope factors not being the same for different receptor subtypes,

excluded this possibility. At M_1, M_2, M_3, and M_4 receptors, the slope factors in equilibrium studies were 1.70 ± 0.31, 1.72 ± 0.22, 1.44 ± 0.29, and 1.76 ± 0.23 (mean ± SD, $n = 36$), respectively, with an overall range of 0.9−2.2. The equivalent slope factors estimated from the "off-rate" assays were 1.78 ± 0.26, 1.76 ± 0.26, 1.66 ± 0.31, and 1.77 ± 0.22 with an overall range of 1.0−2.4. The data with slope factors greater than 2 could also be fitted satisfactorily if the slope factors were constrained to 2. The slope factors at M_3 receptors appeared to be lower than that at the other subtypes. An analogous result has been observed for the weaker ligand tacrine.[39]

Because the slope factors are greater than 1 for the ligands binding to the unoccupied and NMS-occupied receptors, it cannot be argued that the apparent positive cooperativity results from the ligands binding to both the allosteric site and the competitive site. One explanation is that the ligands are binding in a positively cooperative fashion to two different allosteric sites on muscarinic receptors. Indeed, it has recently been demonstrated that there are two allosteric sites on these receptors,[12,37] and it could be possible, using the methods described, to test whether these ligands bind to both sites.

An alternative explanation is that muscarinic receptors can exist as dimers (or higher oligomers),[40,41] and the binding of these allosteric ligands to its allosteric site on the receptor either modulates the extent of receptor dimerization or generates a positive homotropic interaction transmitted via the receptor−receptor interface. If this were to be true, then these molecules would have an action additional to their allosteric action at the competitive site. Such actions could have important repercussions on receptor function if the precise dimerization state of the receptor is crucial for receptor activation. Because agonist action and binding affinity are related to the induction or selection of a specific receptor conformation, one may speculate that the observed negative cooperativity between acetylcholine and the ligands described in this paper may be a consequence of their apparent (or real) positive homotropic cooperativity.

Summary and Conclusions

The existence of allosteric sites on muscarinic receptors raises the possibility of designing highly subtype selective ligands. This selectivity arises from two parameters: the *binding affinity* for the allosteric site and the *cooperativity* of interaction with the endogenous agonist. This latter parameter is not available to directly acting agonists and antagonists, where affinity oreffi-cacy is the only determinant of relative receptor subtype selectivity. Subtype selective cooperativity with ACh has already been demonstrated for brucine and **5** in both binding and function.[26,28,29,31] However, their low affinities for muscarinic receptors limit their usefulness as research tools. In an effort to develop high-affinity allosteric modulators of ACh, key pharmacophoric features in the lead molecules were identified and incorporated into simpler ring systems. We then probed different aromatic positions of these novel ring systems with a variety of substituents to delineate the receptor requirement for both affinity and cooperativity with competitive agents.

In this paper we have been able to demonstrate, through substitution effects, that both affinity and cooperativity with NMS can be altered substantially in a subtype-dependent manner. Notable compounds, in terms of affinity, are **22d**, **22i**, **22w** (M_1−M_4), and **22y** (M_2), and these suggest that high-affinity interactions at the allosteric site should be possible with molecules of relatively low molecular weight. In terms of receptor selectivity, **23f** binds with a 10-fold higher affinity at unliganded M_1 versus M_2−M_4 receptors, although this agent lacks a comparable selectivity at the NMS-liganded receptors.

All of the agents are negatively cooperative with the neurotransmitter molecule acetylcholine, though the degree of negative cooperativity appears to be low. We have demonstrated that, through structural manipulation of an allosteric pharmacophore, negative cooperativity with NMS can be converted to positive cooperativity and vice versa. In principle, this should apply to any ligand, including ACh. Therefore, continued synthetic work with this series and other analogous multicyclic ring systems should enable the identification of agents with positive cooperativity with acetylcholine.

Experimental Section

General Methods. Starting materials were prepared according to literature procedures as indicated, and if no reference is quoted, they are available commercially. Anhydrous THF and MeOH were obtained according to the procedures described by Perrin and Armarego.[42] In the text, petrol refers to petroleum ether (80−100 °C) and ammonia refers to concentrated ammonia ($d = 0.88$). All reactions were carried out under a slow stream of argon unless otherwise stated. Melting points (mp) were carried out in open capillaries on an Electrothermal digital melting point apparatus (model IA9100) and are uncorrected. The ^{1}H NMR spectra were recorded on Bruker AM 400 WB spectrometer using the facilities at the MRC Biomedical NMR Centre, National Institute for Medical Research, Mill Hill. Chemical shifts are reported in ppm downfield of internal tetramethylsilane (TMS) or 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt (TSP) as indicated. Mass spectra were run by fast-atom bombardment on a VG Analytical ZAB-SE double-focusing magnetic sector mass spectrometer. Elemental analyses were performed by the microanalytical section of the Department of Chemistry, University College London, and were within ±0.4% of the calculated values. Column chromatography was carried out on Merck silica gel 60 (70−230 mesh ASTM).

5-Morpholinomethyl-3-(1-benzyl-1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole (8k). A solution of indole-5-carboxylic acid **10** (7.2 g, 44.7 mmol) in THF (250 mL) containing oxalyl chloride (11.3 g, 89.3 mmol) was stirred at room temperature for 10 h. The reaction mixture was taken to dryness in vacuo, and the residue was redissolved in anhydrous THF (100 mL) and cooled to 5 °C. A solution of morpholine (7.8 g, 89.3 mmol) in THF (50 mL) was added, and the mixture was stirred at room temperature for 1 h. The mixture was filtered and the filtrate taken to dryness. The residue was partitioned between EtOAc and aqueous K_2CO_3. The organic layer was dried over Na_2SO_4 and taken to dryness in vacuo to yield **11** as a white solid (3.0 g, 28%, yield not maximized). ^{1}H NMR (DMSO-d_6) δ 3.52 (m, 4H), 3.60 (m, 4H), 6.49 (s, 1H), 7.14 (dd, $J = 1.6$ and 8.4 Hz, 1H), 7.39−7.44 (m, 2H), 7.62 (s, 1H), 11.25 (br s, 1H).

A suspension of $LiAlH_4$ (0.93 g, 24.5 mmol) in THF (25 mL) was added to a solution of **11** (1.87 g, 8.1 mmol) in THF (125 mL) and refluxed for 3 h. The mixture was cooled to 0 °C, and wet EtOAc (10 mL) was added. The mixture was filtered and then taken to dryness. The resulting residue was stirred in dilute HCl. The aqueous layer was filtered, made alkaline with dilute K_2CO_3, and extracted with EtOAc. The organic layer

was dried over Na_2SO_4 and evaporated in vacuo to give **12** as a white solid (1.1 g, 63%). ^{1}H NMR ($CDCl_3$) δ 2.47 (t, J = 4.5 Hz, 4H), 3.59 (s, 2H), 3.71 (t, J = 4.5 Hz, 4H), 6.51 (m, 1H), 7.18 (m, 2H), 7.32 (d, J = 8.4 Hz, 1H), 7.56 (s, 1H), 8.20 (br s, 1H).

A solution of **12** (1.08 g, 5 mmol), 1-benzyl-4-piperidone (2.84 g, 15 mmol), and sodium methoxide (1.62 g, 30 mmol) in anhydrous methanol (75 mL) was refluxed under argon for 48 h. The reaction mixture partioned between H_2O and EtOAc. The organic layer was separated and taken to dryness. The resulting residue was warmed in 10% MeOH in EtOAc (50 mL). Ether was added until precipitation occurred. The product **8k** was collected by filtration and dried in vacuo (0.80 g, 41%, yield not maximized):

Preparation of Acid Chloride 16a. A 1.0 M solution of diisobutylaluminum hydride in hexane (20 mL) was added dropwise to a solution of **15** (2.26 g, 10 mmol) in CH_2Cl_2 (23 mL) at −70 °C, and the mixture was stirred for 30 min. The mixture was partitioned between EtOAc and H_2O. The organic layer was washed with aqueous NaCl, dried over Na_2SO_4, and removed under reduced pressure. The resulting alcohol was dissolved in THF (20 mL) and cooled to 0 °C, and triethylamine (1.2 g, 12 mmol) and acetyl chloride (0.86 g, 11 mmol) were added successively. The solution was stirred for 30 min, diluted with H_2O, and extracted with EtOAc. The organic layer was washed with aqueous NaCl, dried over Na_2SO_4, and removed under reduced pressure. The product was dissolved in EtOH (200 mL) containing pyridinium *p*-toluenesulfonate (0.3 g, 1.2 mmol), and the mixture was stirred at 45 °C for 3 h. The solution was concentrated under reduced pressure and chromatographed on silica, eluting with a mixture of EtOAc/petrol (1:1). The free alcohol, obtained as a colorless oil, was dissolved in CH_2Cl_2 (50 mL). A solution of DMSO (1.47 g, 19 mmol) in CH_2Cl_2 (1 mL) was added to a 2 M solution of oxalyl chloride in CH_2Cl_2 (7.2 mL) at −75 °C, and the mixture was stirred for 10 min. This was then added to the previous solution, and the mixture was stirred at −75 °C for 30 min and then at −45 °C for 40 min and then cooled back to −75 °C before Et_3N (7.5 mL, 53 mmol) was added. The mixture was then allowed to reach room temperature over a period of 1 h and then was diluted with a mixture of EtOAc and H_2O. The organic layer was washed successively with dilute HCl, H_2O, and aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The resulting aldehyde was dissolved in tBuOH (20 mL) containing 2-methyl-2-butene (2.5 g, 35.5 mmol) and was added to a solution of $NaH_2PO_4 \cdot H_2O$ (1.11 g, 7.1 mmol) in H_2O (10 mL). This was cooled to ∼10 °C, and $NaClO_2$ (0.83 g, 9.23 mmol) was added in portions over a period of 10 min. The mixture was stirred at room temperature for 30 min, acidified with dilute HCl, and extracted with EtOAc. The organic layer was taken to dryness, and the residue was chromatographed on silica, eluting with EtOAc/petrol (1:1) to give the acid **16** as a colorless oil that crystallized on cooling in the fridge (1.18 g, 69%). ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.09 (s, 3H), 2.58 (s, 4H), 4.65 (s, 2H).

To a cold solution of **16** (1.15 g, 6.75 mmol) in CH_2Cl_2 (20 mL) was added a 2 M solution of oxalyl chloride (4.05 mL, 8.1 mmol) in CH_2Cl_2. The solution was stirred at room temperature for 18 h and refluxed for a further hour. The solvent was removed, and the crude residue was distilled, collecting **16a** as the fraction boiling at 124−125 °C and 5 mmHg (0.65 g, 51%).

General Procedure for the Acylation of 3-(1-Benzyl-1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles. A suspension of sodium hydride (60% w/v dispersion in mineral oil, 32 mmol) in THF (30 mL) was added to a cold solution of 3-(tetrahydropyridinyl)indole (30 mmol) in THF (300 mL). The mixture was stirred at room temperature for 1 h and then cooled to 0−5 °C. A solution of the appropriate acid chloride (32 mmol) in THF (70 mL) was then added dropwise, and the resulting mixture was allowed to stir at room temperature for 2 h. The mixture was concentrated to about a half of its original volume and then acidified with 2 N hydrochloric acid. Unless otherwise stated, the resulting white precipitate was collected by filtration, washed successively with THF, H_2O, and ether, and if necessary recrystallized as stated. Compounds **18a**−**v** and **19a**−**h** were synthesized following the above procedure for acylation.

1-(4-Pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole Hydrochloride (18a). 18a was prepared from the reaction of **8a** and **13** according to the general acylation procedure. Yield 76%; mp 263−265 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.61 (td, J = 6.8 and 2.4 Hz, 2H), 2.72 (t, J = 2.4 Hz, 1H), 2.87 (br s, 2H), 3.29 (t, J = 6.8 Hz, 2H), 3.45 (br s, 2H), 3.84 (s, 2H), 4.39 (s, 2H), 6.35 (br s, 1H), 7.37 (t, J = 8 Hz, 1H), 7.43 (t, J = 8 Hz, 1H), 7.51−7.57 (m, 5H), 7.91 (d, J = 8 Hz, 1H), 7.96 (s, 1H), 8.44 (d, J = 8 Hz, 1H).

4-Methoxy-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole Hydrochloride (18b). 18b was prepared from the reaction of **8b** and **13** according to the general acylation procedure. Yield 73%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 338 K) δ 2.60 (m, 1H), 2.65 (m, 2H), 2.85 (t, J = 5.8 Hz, 2H), 3.22 (m, 4H), 3.66 (m, 2H), 3.78 (s, 2H), 3.88 (s, 3H), 5.88 (m, 1H), 6.89 (d, J = 8.2 Hz, 1H), 7.33 (t, J = 8.2 Hz, 1H), 7.40 (m, 5H), 7.48 (s, 1H), 7.99 (d, J = 8.2 Hz, 1H).

4-Chloro-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole Hydrochloride (18c). 18c was prepared from the reaction of **8c** and **13** according to the general acylation procedure. Yield 83%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 343 K) δ 2.60 (t, J = 2.6 Hz, 1H), 2.67 (td, J = 2.6 and 6.7 Hz, 2H), 2.79 (br s, 2H), 3.24 (t, J = 6.7 Hz, 2H), 3.48 (t, J = 6.0 Hz, 2H), 3.84 (br s, 2H), 4.40 (s, 2H), 5.83 (m, 1H), 7.38 (m, 2H), 7.55 (m, 5H), 7.75 (s, 1H), 8.37 (d, J = 7.6 Hz, 1H).

Methyl 1-(4-Pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole-4-carboxylate Hydrochloride (18d). 18d was prepared from the reaction of **8d** and **13** according to the general acylation procedure. Yield 58%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 333 K) δ 2.58 (m, 1H), 2.63 (m, 2H), 3.28 (t, J = 6.4 Hz, 2H), 3.44 (br s, 2H), 3.78 (br s, 2H), 3.88 (s, 3H), 3.92 (s, 2H), 4.44 (s, 2H), 5.64 (br s, 1H), 7.49−7.63 (m, 6H), 7.63 (dd, J = 1 and 7.6 Hz, 2H), 7.91 (s, 1H), 8.66 (dd, J = 1 and 7.6 Hz, 1H).

5-Methyl-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole Hydrochloride (18e). 18e was prepared from the reaction of **8e** and **13** according to the general acylation procedure. Recrystallized from MeOH; yield 75%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 333 K) δ 2.54−2.64 (m, 3H), 2.85 (br s, 2H), 3.24 (t, J = 6.7 Hz, 2H), 3.42 (br s, 2H), 3.82 (br s, 2H), 3.91 (s, 3H), 4.35 (s, 2H), 6.31 (br s, 1H), 7.25 (d, J = 8.6 Hz, 1H), 7.49−7.61 (m, 5H), 7.69 (s, 1H), 7.84 (s, 1H), 8.29 (d, J = 8.6 Hz, 1H).

5-Methoxy-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole Hydrochloride (18f). 18f was prepared from the reaction of **8f** and **13** according to the general acylation procedure. Yield 89%; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.61 (td, J = 2.4 and 6.7 Hz, 2H), 2.68 (t J = 2.4 Hz, 1H), 2.86 (br s, 2H), 3.25 (t, J = 6.7 Hz, 2H), 3.47 (br s, 2H), 3.82 (s, 3H), 3.84 (s, 2H), 4.39 (s, 2H), 6.31 (br s 1H), 7.04 (dd, J = 2.3 and 9.0 Hz, 1H), 7.32 (d, J = 2.3 Hz, 1H), 7.52−7.57 (m, 5H), 7.91 (s, 1H), 8.34 (d, J = 9 Hz, 1H).

5-Benzyloxy-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole Hydrochloride (18g). 18g was prepared from the reaction of **8g** and **13** according to the general acylation procedure. Yield 76%; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.61 (td, J = 2.4 and 6.8 Hz, 2H), 2.68 (t J = 2.4 Hz, 1H), 2.83 (br s, 2H), 3.24 (t, J = 6.8 Hz, 2H), 3.44 (br s, 2H), 3.81 (s, 2H), 4.36 (s, 2H), 5.17 (s, 2H), 6.25 (br s, 1H), 7.11 (dd, J = 2.2 and 9.1 Hz, 1H), 7.34−7.58 (m, 11H), 7.90 (s, 1H), 8.33 (d, J = 9.1 Hz, 1H).

5-Chloro-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole Hydrochloride (18h). 18h was prepared from the reaction of **8h** and **13** according to the general acylation procedure. Yield 87%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 333 K) δ 2.57 (m, 1H), 2.64 (m, 2H), 2.87 (br s, 2H), 3.28 (t, J = 6.7 Hz, 2H), 3.48 (br s, 2H), 3.86 (br s, 2H), 4.41 (s, 2H), 6.27 (br s, 1H), 7.44 (dd, J = 1.8 and 8.9 Hz, 1H),

7.51−7.62 (m, 5H), 7.90 (d, J = 1.8 Hz, 1H), 7.97 (s, 1H), 8.43 (d, J = 8.9 Hz, 1H).

Benzyl 1-(4-Pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole-5-carboxylate Hydrochloride (18i). 18i was prepared from the reaction of **8i** and **13** according to the general acylation procedure. Yield 75%; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.61−2.65 (m, 3H), 2.87 (br s, 2H), 3.29 (t, J = 6.3 Hz, 2H), 3.48 (br s, 2H), 3.86 (s, 2H), 5.40 (s, 2H), 6.31 (br s, 1H), 7.33−7.58 (m, 10H), 8.02 (s, 1H), 8.05 (d, J = 8.8 Hz, 1H), 8.48 (s, 1H), 8.53 (d, J = 8.8 Hz, 1H).

5-Morpholinocarbonyl-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole (18j). 18j was prepared from the reaction of **8j** and **13** according to the general acylation procedure. The reaction mixture was made alkaline with aqueous K_2CO_3 and extracted with EtOAc. The organic layer was taken to dryness to give **18j** as a light-brown gum in quantitative yield: ^{1}H NMR ($CDCl_3$) δ 2.05 (t, J = 2.4 Hz, 1H), 2.58 (br s, 2H), 2.73 (m, 4H), 3.18 (m, 2H), 3.22 (m, 2H), 3.65 (s, 2H), 3.4−3.8 (br s, 8H), 6.28 (m, 1H), 7.25−7.42 (m, 7H), 7.91 (s, 1H), 8.51 (d, J = 8.0 Hz, 1H).

5-Morpholinomethyl-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole Dihydrochloride (18k). 18k was prepared from the reaction of **8k** and **13** according to the general acylation procedure. Yield 76%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 333 K) δ 2.59 (m, 3H), 2.91 (br s, 2H), 3.09 (br s, 4H), 3.31 (t, J = 7.4 Hz, 2H), 3.51 (br s, 2H), 3.81 (br s, 4H), 3.88 (br s, 2H), 4.29 (s, 2H), 4.42 (s, 2H), 6.36 (br s, 1H), 7.49−7.63 (m, 6H), 7.97 (s, 1H), 7.99 (s, 1H), 8.48 (d, J = 8.9 Hz, 1H).

6-Methoxy-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole (18l). 18l was prepared from the reaction of **8l** and **13** according to the general acylation procedure. Yield 82%; ^{1}H NMR ($CDCl_3$) δ 2.04 (t, J = 2.6 Hz, 1H), 2.56 (m, 2H), 2.70−276 (m, 4H), 3.15 (t, J = 7.3 Hz, 2H), 3.23 (m, 2H), 3.67 (s, 2H), 3.88 (s, 3H), 6.26 (m, 1H), 6.91 (dd, J = 2.5 and 8.8 Hz, 1H), 7.21 (s, 1H), 7.26−7.41 (m, 5H), 7.66 (d, J = 8.8 Hz, 1H), 8.12 (d, J = 2.5 Hz, 1H).

6-Chloro-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole Hydrochloride (18m). 18m was prepared from the reaction of **8m** and **13** according to the general acylation procedure. Yield 83%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 333 K) δ 2.60 (t, J = 5.9 Hz, 1H), 2.66 (td, J = 2.6 and 6.6 Hz, 2H), 2.87 (br s, 2H), 3.26 (t, J = 6.6 Hz, 2H), 3.48 (t, J = 5.9 Hz, 2H), 3.86 (s, 2H), 4.38 (s, 2H), 6.27 (br s, 1H), 7.41 (dd, J = 2.0 and 8.6 Hz, 1H), 7.55 (m, 5H), 7.86 (m, 2H), 8.44 (d, J = 2.0 Hz, 1H).

Methyl 1-(4-Pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole-6-carboxylate Hydrochloride (18n). 18n was prepared from the reaction of **8n** and **13** according to the general acylation procedure. Yield 83%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 333 K) δ 2.57 (m, 1H), 2.66 (m, 2H), 2.91 (br s, 2H), 3.29 (t, J = 6.7 Hz, 2H), 3.90 (br s, 2H), 3.92 (s, 2H), 3.93 (s, 3H), 4.43 (s, 2H), 6.34 (br s, 1H), 7.52−7.63 (m, 5H), 7.95−8.04 (m, 2H), 8.09 (s, 1H), 9.05 (s, 1H).

6-Cyano-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole Hydrochloride (18o)?? 18o was prepared from the reaction of **8o** and **13** according to the general acylation procedure. Yield 72%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 343 K) δ 2.59 (m, 1H), 2.67 (td, J = 2.5 and 6.7 Hz, 2H), 2.77 (t, J = 6.8 Hz, 2H), 3.21 (t, J = 2.5 Hz, 2H), 3.30 (t, J = 6.8 Hz, 2H), 3.60 (s, 2H), 3.68 (s, 2H), 6.29 (m, 1H), 7.39 (m, 5H), 7.68 (d, J = 8.0 Hz, 1H), 7.97 (s, 1H), 8.03 (d, J = 8.0 Hz, 1H), 8.74 (s, 1H).

7-Methoxy-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole Hydrochloride (18p). 18p was prepared from the reaction of **8p** and **13** according to the general acylation procedure. The product was recrystallized from a mixture of MeOH/Et_2O. Yield 48% (not maximized); ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 333 K) δ 2.56−2.62 (m, 3H), 2.83 (br s, 2H), 3.23 (m, 2H), 3.43 (br s, 2H), 3.83 (br s, 2H), 3.92 (s, 3H), 4.36 (s, 2H), 6.24 (br s, 1H), 7.05 (d, J = 8.0 Hz, 1H), 7.34 (t, J = 8.0 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.50−7.60 (m, 5H), 7.78 (s, 1H).

5,6-Dimethoxy-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole Hydrochloride (18q). 18q was prepared from the reaction of **8q** and **13** according to the general acylation procedure. Yield 80%; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.61 (td, J = 2.5 and 6.7 Hz, 2H), 2.68 (t J = 2.5 Hz, 1H), 2.84 (m, 2H), 3.25 (t, J = 6.7 Hz, 2H), 3.44 (m, 2H), 3.63 (t, J = 6.3 Hz, 2H), 3.83 (s, 6H), 4.37 (s, 2H), 6.32 (m 1H), 7.30 (s, 1H), 7.51−7.59 (m, 5H), 7.79 (s, 1H), 8.07 (s, 1H).

5,6-Methylenedioxy-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole (18r). 18r was prepared from the reaction of **8r** and **13** according to the general acylation procedure and that described for **18j**. Yield 80%; ^{1}H NMR ($CDCl_3$) δ 2.03 (t, J = 2.6 Hz, 1H), 2.53 (m, 2H), 2.68−2.76 (m, 4H), 3.13 (t, J = 7.4 Hz, 2H), 3.22 (m, 2H), 3.66 (s, 2H), 6.00 (m, 2H), 6.15 (m, 1H), 7.17 (s, 1H), 7.21 (s, 1H), 7.26−7.41 (m, 5H), 8.06 (s, 1H).

5,6,7-Trimethoxy-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole (18s). 18s was prepared from the reaction of **8s** and **13** according to the general acylation procedure and that described for **18j**. Yield 89%; ^{1}H NMR (HCl salt, DMSO-d_6/D_2O, TSP-salt, 338 K) δ 2.50 (t, J = 2.5 Hz, 1H), 2.65 (m, 2H), 2.73 (m, 2H), 3.20 (t, J = 6.8 Hz, 2H), 3.27 (t, J = 5.8 Hz, 2H), 3.63 (m, 2H), 3.83 (s, 3H), 3.84 (s, 3H), 3.89 (s, 3H), 4.20 (s, 2H), 5.99 (m, 1H), 7.49−7.52 (m, 6H), 7.90 (s, 1H).

5,6-Dimethoxy-1-(5-methoxycarbonyl-4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole (18t). 18t was prepared from the reaction of **8q** and **14** according to the general acylation procedure. The reaction mixture was partitioned between EtOAc and aqueous $NaHCO_3$. The organic layer was taken to dryness, and the resulting residue was chromatographed on silica, eluting with EtOAc to give the pure product as a white powder in 18% yield (not maximized). ^{1}H NMR ($CDCl_3$) δ 2.56 (m, 2H), 2.77 (t, J = 5.7 Hz, 2H), 2.86 (t, J = 7.8 Hz, 2H), 3.19−3.25 (m, 4H), 3.68 (s, 2H), 3.77 (s, 3H), 3.92 (s, 3H), 3.96 (s, 3H), 6.21 (m, 1H), 7.16 (s, 1H), 7.20 (s, 1H), 7.27−7.41 (m, 5H), 8.11 (s, 1H).

5,6-Dimethoxy-1-(6-acetoxy-4-hexynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole (18u). 18u was prepared from the reaction of **8q** and **16a** according to the general acylation procedure. The reaction mixture was partitioned between EtOAc and aqueous $NaHCO_3$. The organic layer was concentrated, and the resulting white precipitate was collected by filtration, Yield 17% (not maximized); ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3H), 2.5−2.9 (br, 2H), 2.76 (m, 2H), 3.14 (t, J = 7.3 Hz, 2H), 3.2−3.8 (br, 4H), 3.93 (s, 3H), 3.96 (s, 3H), 4.1−4.5 (m, 2H), 4.66 (t, J = 2.0 Hz, 2H), 6.10 (m, 1H), 7.11 (s, 1H), 7.29 (s, 1H), 7.46−7.71 (m, 5H), 8.12 (s, 1H).

4-Methoxy-1-(3,3-dimethyl-4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-4-yl)indole (18v). 18v was prepared from the reaction of **8b** and **17** according to the general acylation procedure. Yield 46%; ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 6H), 2.14 (s, 1H), 2.56 (m, 2H), 2.73 (t, J = 5.6 Hz, 2H), 3.00 (s, 2H), 3.17 (m, 2H), 3.67 (s, 2H), 3.89 (s, 3H), 5.80 (m, 1H), 6.72 (d, J = 8.0 Hz, 1H), 7.22−7.41 (m, 7H), 8.17 (d, J = 8.0 Hz, 1H).

1-(4-Pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-3-yl)indole Hydrochloride (19a). 19a was prepared from the reaction of **9a** and **13** according to the general acylation procedure. Yield 76%; mp 226−227 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.60−2.65 (m, 4H), 2.69 (t, J = 2.5 Hz, 1H), 3.26 (t, J = 6.7 Hz, 2H), 3.30 (m, 2H), 4.15 (s, 2H), 4.42 (s, 2H), 6.53 (m, 1H), 7.39 (t, J = 8 Hz, 1H), 7.45 (t, J = 8 Hz, 1H), 7.50−7.57 (m, 5H), 7.87 (d, J = 8 Hz, 1H), 7.88 (s, 1H), 8.43 (d, J = 8 Hz, 1H).

5-Methoxy-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-3-yl)indole Hydrochloride (19b). 19b was prepared from the reaction of **9b** and **13** according to the general acylation procedure. Yield 78%; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.57 (t, J = 2.4 Hz, 1H), 2.60−2.66 (m, 4H), 3.22 (t, J = 6.4 Hz, 2H), 3.34−3.7 (br, 2H), 3.82 (s, 3H), 4.08 (s, 2H), 4.48 (s, 2H), 6.49 (m, 1H), 7.06 (dd, t, J = 2.2 and 9.1 Hz, 1H), 7.24 (d, J = 2.2 Hz, 1H), 7.36−7.60 (m, 5H), 7.77 (s, 1H), 8.32 (d, J = 9.1 Hz, 1H).

5-Benzyloxy-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-3-yl)indole Hydrochloride (19c). 19c was prepared from the reaction of **9c** and **13** according to the general acylation procedure. Yield 65%; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.59−2.65 (m, 5H), 3.20 (t, J = 6.5 Hz, 2H), 3.40 (br s, 2H), 4.05 (s, 2H), 4.45 (s, 2H), 5.16 (s, 2H), 6.40 (m, 1H), 7.11 (dd, t, J = 2.2 and 9.0 Hz, 1H), 7.28 (d, J = 2.2 Hz, 1H), 7.36−7.58 (m, 10H), 7.77 (s, 1H), 8.32 (d, J = 9.0 Hz, 1H).

Methyl 1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-3-yl)indole Carboxylate (19d). 19d was prepared from the reaction of **9d** and **13** according to the general acylation procedure and as described for **18j**. Recrystallized from a mixture of EtOAc/*n*-hexane; yield 49%; ^{1}H NMR ($CDCl_3$) δ 2.03 (t, J = 2.5 Hz, 1H), 2.44 (m, 2H), 2.67−2.75 (m, 4H), 3.16 (t, J = 7.3 Hz, 2H), 3.36 (m, 2H), 3.73 (s, 2H), 3.95 (s, 3H), 6.38 (m, 1H), 7.27−7.42 (m, 6H), 8.05 (dd, J = 1.5 and 8.8 Hz, 1H), 8.49 (d, J = 1.5 Hz, 1H), 8.52 (d, J = 8.8 Hz, 1H).

6-Methoxy-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-3-yl)indole (19e). 19e was prepared from the reaction of **9e** and **13** according to the general acylation procedure and that described for **18j**. Yield 68%; ^{1}H NMR ($CDCl_3$) δ 2.03 (t, J = 2.6 Hz, 1H), 2.40 (m, 2H), 2.67 (t, J = 5.8 Hz, 2H), 2.72 (td, J = 2.6 and 7.3 Hz, 2H), 3.14 (t, J = 7.3 Hz, 2H), 3.34 (m, 2H), 3.71 (s, 2H), 3.87 (s, 3H), 6.31 (m, 1H), 6.92 (dd, J = 2.4 and 8.7 Hz, 1H), 7.15 (s, 1H), 7.26−7.42 (m, 5H), 7.63 (d, J = 8.7 Hz, 1H), 8.11 (d, J = 2.4 Hz, 1H).

5,6-Dimethoxy-1-(4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-3-yl)indole Hydrochloride (19f). 19f was prepared from the reaction of **9f** and **13** according to the general acylation procedure. Yield 73%; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.6−2.7 (m, 5H), 3.23 (t, J = 5.7 Hz, 2H), 3.31 (br s, 2H), 3.82 (s, 3H), 3.85 (s, 3H), 3.97 (br s, 2H), 4.42 (s, 2H), 6.48 (br s, 1H), 7.22 (s, 1H), 7.5−7.6 (m, 5H), 7.67 (s, 1H), 8.06 (s, 1H).

1-(3,3-Dimethyl-4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-3-yl)indole Hydrochloride (19g). 19g was prepared from the reaction of **9a** and **17** according to the general acylation procedure. Yield 86%; ^{1}H NMR (DMSO-d_6/D_2O) δ 1.40 (s, 6H), 2.63 (s, 1H), 2.64 (br s, 2H), 3.14 (s, 2H), 3.38 (br s, 2H), 4.01 (s, 2H), 4.42 (s, 2H), 6.54 (br s, 1H), 7.39−7.48 (m, 2H), 7.52−7.58 (m, 5H), 7.81 (s, 1H), 7.86 (d, J = 8 Hz, 1H), 8.45 (d, J = 8 Hz, 1H).

5,6-Dimethoxy-1-(3,3-dimethyl-4-pentynoyl)-3-(1-benzyl-1,2,5,6-tetrahydropyridin-3-yl)indole Hydrochloride (19h). 19h was prepared from the reaction of **9f** and **17** according to the general acylation procedure. Yield 57%; ^{1}H NMR (DMSO-d_6/D_2O) δ 1.40 (s, 6H), 2.62 (m, 2H), 2.68 (s, 1H), 3.13 (s, 2H), 3.32 (br s, 2H), 3.82 (s, 3H), 3.85 (s, 3H), 3.95 (br s, 2H), 4.40 (s, 2H), 6.47 (br s, 1H), 7.5−7.6 (m, 5H), 7.69 (s, 1H), 7.81 (s, 1H), 8.08 (s, 1H).

General Procedure for Intramolecular Diels−Alder Reactions. The Diels−Alder precursor (0.01 mol) was suspended in diphenyl ether (400 mL) at 230−240 °C for about 2 h or until the starting material was fully consumed under a slow stream of argon gas. The solution was cooled to room temperature, and unless otherwise stated, ether containing hydrogen chloride was added until precipitation occurred. The resulting precipitate was collected by filtration, washed exhaustively with ether, and dried in vacuo. The following Diels−Alder adducts **20a**−**z** and **21a**−**i** were synthesized using the above procedure for cyclization.

11-Benzyl-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one (20a). Diphenyl ether was removed in vacuo, and the resulting residue was chromatographed on silica, eluting with EtOAc/toluene (1:1) to give **20a** as a white crystalline solid in 56% yield. Mp 126−127 °C; ^{1}H NMR ($CDCl_3$) δ 2.93 (t, J = 5.9 Hz, 2H), 3.01 (t, J = 7.5 Hz, 2H), 3.20 (t, J = 7.5 Hz, 2H), 3.36 (t, J = 5.9 Hz, 2H), 3.76 (s, 4H), 6.93 (s, 1H), 7.25−7.45 (m, 6H), 7.50 (t, J = 8 Hz, 1H), 7.98 (d, J = 8 Hz, 1H), 8.54 (d, J = 8 Hz, 1H).

11-Benzyl-1-methoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (20b). Yield ∼50% (not maximized); ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 343 K) δ 3.00 (t, J = 7.5 Hz, 2H), 3.20 (t, J = 7.5 Hz, 2H), 3.61 (br s, 2H), 3.72 (m, 2H), 3.85 (m, 4H), 3.99 (s, 3H), 7.00 (d, J = 7.7 Hz, 1H), 7.14 (s, 1H), 7.40 (t, J = 7.7 Hz, 1H), 7.52−7.61 (m, 5H), 8.12 (d, J = 7.7 Hz, 1H).

11-Benzyl-1-hydroxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrobromide (20c). A solution of 1 N BBr_3 in CH_2Cl_2 (16 mL) was added with stirring to a cold solution of **20b** (0.8 g, 2 mmol) in CH_2Cl_2 (50 mL) under a slow stream of Ar. The reaction mixture was allowed to stir at room temperature for 8 h and then cooled to 0 °C and carefully diluted with MeOH. The mixture was heated at 50 °C for 15 min, cooled, filtered, and concentrated, and if necessary, ether was added until precipitation occurred. The resulting light-brown precipitate was collected by filtration and dried in vacuo (0.48 g, 52%).

11-Benzyl-1-chloro-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (20d). Recrystallized from MeOH; yield 83%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 333 K) δ 3.03 (t, J = 7.3 Hz, 2H), 3.26 (t, J = 7.3 Hz, 2H), 3.56 (br s, 2H), 3.95 (t, J = 6.1 Hz, 2H), 4.47 (s, 2H), 4.48 (s, 2H), 7.27 (s, 1H), 7.52−7.64 (m, 7H), 8.60 (d, J = 8.0 Hz, 1H).

Methyl 11-Benzyl-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one-1-carboxylate Hydrochloride (20e). Yield 80%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 313 K) δ 3.05 (t, J = 7.5 Hz, 2H), 3.21 (br s, 2H), 3.28 (t, J = 7.5 Hz, 2H), 3.56 (br s, 2H), 3.98 (s, 3H), 4.50 (s, 2H), 4.51 (s, 2H), 7.27 (s, 1H), 7.56−7.63 (m, 6H), 7.69 (t, J = 8 Hz, 1H), 8.69 (d, J = 8 Hz, 1H).

11-Benzyl-2-methyl-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (20f). Yield 63%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 333 K) δ 3.01 (t, J = 8.0 Hz, 2H), 3.24 (t, J = 8.0 Hz, 2H), 3.59 (t, J = 8.0 Hz, 2H), 3.67 (t, J = 8.0 Hz, 2H), 3.91 (s, 3H), 4.43 (s, 2H), 4.48 (s, 2H), 7.15 (s, 1H), 7.42 (d, J = 8.6 Hz, 1H), 7.56−7.63 (m, 5H), 7.89 (s, 1H), 8.30 (d, J = 8.6 Hz, 1H).

11-Benzyl-2-methoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (20g). Yield >95%; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.99 (t, J = 7.5 Hz, 2H), 3.22 (t, J = 7.5 Hz, 2H), 3.58 (br s, 2H), 3.64 (br s, 2H), 3.89 (s, 3H), 4.42 (s, 2H), 4.47 (s, 2H), 7.17 (s, 1H), 7.21 (dd, J = 2.2 and 9.0 Hz, 1H), 7.52 (d, J = 2.2 Hz, 1H), 7.54−7.60 (m, 5H), 8.33 (d, J = 9.0 Hz, 1H).

11-Benzyl-2-benzyloxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one hydrochloride (20h). Yield 92%; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.98 (t, J = 7.5 Hz, 2H), 3.22 (t, J = 7.5 Hz, 2H), 3.55 (br s, 2H), 3.63 (br s, 2H), 4.42 (s, 2H), 4.47 (s, 2H), 5.24 (s, 2H), 7.17 (s, 1H), 7.28 (dd, J = 1.8 and 8.9 Hz, 1H), 7.36−7.61 (m, 11H), 8.32 (d, J = 8.9 Hz, 1H).

11-Benzyl-2-cholro-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (20i). Yield 60% (not maximized); ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 333 K) δ 3.04 (t, J = 7.5 Hz, 2H), 3.27 (t, J = 7.5 Hz, 2H), 3.59 (br s, 2H), 3.65 (br s, 2H), 4.42 (s, 2H), 4.46 (s, 2H), 7.22 (s, 1H), 7.51−7.62 (m, 5H), 7.63 (dd, J = 2.0 and 8.9 Hz, 1H), 8.09 (d, J = 2.0 Hz, 1H), 8.90 (d, J = 8.9 Hz, 1H).

Benzyl 11-Benzyl-2−7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one-2-carboxylate Hydrochloride (20j). Yield 94%; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.04 (t, J = 7.5 Hz, 2H), 3.25 (t, J = 7.5 Hz, 2H), 3.55−3.90 (br s, 2H), 3.63 (br s, 2H), 4.48 (s, 2H), 4.53 (s, 2H), 5.43 (s, 2H), 7.00 (d, J = 8.5 Hz, 2H), 7.18 (d, J = 7.8 Hz, 1H), 7.22 (s, 1H), 7.39−7.62 (m, 7H), 8.21 (d, J = 8.6 Hz, 1H), 8.50 (d, J = 8.6 Hz, 1H), 8.61 (s, 1H).

11-Benzyl-2-morpholinocarbonyl-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (20k). Yield >95%; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.06 (t, J = 7.2 Hz, 2H), 3.28 (t, J = 7.2 Hz, 2H), 3.49 (m, 2H), 3.64 (m, 6H), 3.79 (m, 4H), 4.49 (s, 2H), 4.53 (s, 2H), 7.22 (s, 1H), 7.48−7.62 (m, 5H), 7.65 (d, J = 9 Hz, 1H), 8.10 (s, 1H), 8.51 (d, J = 9 Hz, 1H).

11-Benzyl-2-morpholinomethyl-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Dihydrochloride (20l). Yield 85%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt,

333 K) δ 3.05 (m, 4H), 3.28 (t, J = 7.3 Hz, 2H), 3.60 (br s, 2H), 3.81 (br s, 4H), 3.92 (br s, 4H), 4.31 (br s, 2H), 4.40 (br s, 2H), 4.42 (br s, 2H), 7.22 (s, 1H), 7.48−7.61 (m, 5H), 7.69 (d, J = 8.2 Hz, 1H), 8.22 (s, 1H), 8.49 (d, J = 8.2 Hz, 1H).

11-Benzyl-3-methoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (20m). Recrystallized from MeOH; yield 62%; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.00 (t, J = 7.4 Hz, 2H), 3.01 (t, J = 7.4 Hz, 2H), 3.52 (br s, 2H), 3.63 (br s, 2H), 3.88 (s, 3H), 4.42 (s, 2H), 4.48 (s, 2H), 7.11 (s, 1H), 7.11 (dd, J = 2.5 and 8.6 Hz, 1H), 7.53−7.62 (m, 5H), 7.97 (d, J = 2.5 Hz, 1H), 7.99 (d, J = 8.6 Hz, 1H).

11-Benzyl-3-hydroxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrobromide (20n). **20n** was prepared as described for **20c**, starting with **20m**. Recrystallized from MeOH; yield 83%; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.99 (t, J = 7.4 Hz, 2H), 3.21 (t, J = 7.4 Hz, 2H), 3.51 (br s, 2H), 3.65 (br s, 2H), 4.44 (s, 2H), 4.50 (s, 2H), 6.95 (dd, J = 0.9 and 8.7 Hz, 1H), 7.08 (s, 1H), 7.53−7.61 (m, 5H), 7.89 (d, J = 0.9 Hz, 1H), 7.90 (d, J = 8.7 Hz, 1H).

11-Benzyl-3-chloro-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (20o). Recrystallized from MeOH; yield 81%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 323 K) δ 3.04 (t, J = 7.4 Hz, 2H), 3.26 (t, J = 7.4 Hz, 2H), 3.56 (t, J = 6.0 Hz, 2H), 3.67 (t, J = 6.0 Hz, 2H), 4.44 (s, 2H), 4.48 (s, 2H), 7.20 (s, 1H), 7.50−7.62 (m, 6H), 8.08 (d, J = 8.5 Hz, 1H), 8.40 (d, J = 1.8 Hz, 1H).

Methyl 11-Benzyl-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one-3-carboxylate Hydrochloride (20p). Yield 80%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 333 K) δ 2.92 (t, J = 5.6 Hz, 2H), 3.05 (t, J = 7.5 Hz, 2H), 3.26 (t, J = 7.5 Hz, 2H), 3.34 (t, J = 5.6 Hz, 2H), 3.74 (s, 2H), 3.76 (s, 2H), 3.96 (s, 3H), 7.14 (s, 1H), 7.30−7.46 (m, 5H), 8.08 (dd, J = 1.5 and 8.2 Hz, 1H), 8.24 (d, J = 8.2 Hz, 1H), 9.05 (d, J = 1.5 Hz, 1H).

11-Benzyl-3-cyano-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (20q). Recrystallized from MeOH; yield 75%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 323 K) δ 3.06 (t, J = 7.4 Hz, 2H), 3.29 (t, J = 7.4 Hz, 2H), 3.60 (t, J = 5.4 Hz, 2H), 3.67 (t, J = 5.4 Hz, 2H), 4.46 (s, 2H), 4.48 (s, 2H), 7.29 (s, 1H), 7.55−7.62 (m, 5H), 7.83 (d, J = 8.2 Hz, 1H), 8.24 (d, J = 8.2 Hz, 1H), 8.66 (s, 1H).

11-Benzyl-4-methoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (20r). Yield 73%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 343 K) δ 3.03 (t, J = 7.4 Hz, 2H), 3.24 (t, J = 7.4 Hz, 2H), 3.58(br s, 2H), 3.64 (br s, 2H), 3.97 (s, 3H), 4.40 (s, 2H), 4.44 (s, 2H), 7.16 (s, 1H), 7.26 (d, J = 8 Hz, 1H), 7.43−7.60 (m, 6H), 7.71 (d, J = 8.0 Hz, 1H).

11-Benzyl-4-hydroxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrobromide (20s). **20s** was prepared as described for **20c**, starting with **20r**. Yield 75%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 323 K) δ 3.11 (t, J = 7.4 Hz, 2H), 3.23 (t, J = 7.4 Hz, 2H), 3.56 (br s, 2H), 3.80 (br s, 2H), 4.48 (s, 2H), 4.52 (s, 2H), 7.04 (d, J = 8 Hz, 1H), 7.21 (s, 1H), 7.42 (t, J = 8 Hz, 1H), 7.56−7.61 (m, 6H).

11-Benzyl-2,3-dimethoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (20t). Yield 91%; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 343 K) δ 3.00 (t, J = 7.4 Hz, 2H), 3.22 (t, J = 7.4 Hz, 2H), 3.57(br s, 2H), 3.62 (br s, 2H), 3.87 (s, 3H), 3.90 (s, 3H), 4.40 (s, 2H), 4.49 (s, 2H), 7.09 (s, 1H), 7.50 (s, 1H), 7.53−7.62 (m, 5H), 7.99 (s, 1H).

11-Benzyl-2,3-methylenedioxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (20u). Yield 81%; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.99 (t, J = 7.4 Hz, 2H), 3.20 (t, J = 7.4 Hz, 2H), 3.50 (br s, 2H), 3.62 (br s, 2H), 4.42 (s, 2H), 4.48 (s, 2H), 6.14 (s, 2H), 7.09 (s, 1H), 7.53−7.61 (m, 5H), 7.57 (s, 1H), 7.93 (s, 1H).

11-Benzyl-1,2,3-trimethoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (20v). Yield 36% (not maximized); ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 318 K) δ 3.00 (t, J = 7.5 Hz, 2H), 3.21 (t, J = 7.5 Hz, 2H), 3.60−4.05 (m, 4H), 3.86 (s, 3H), 3.93 (s, 3H), 4.00 (s, 3H), 4.47 (s, 2H), 4.51 (s, 2H), 7.11 (s, 1H), 7.50−7.62 (m, 5H), 7.97 (s, 1H).

Methyl 11-Benzyl-2,3-dimethoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one-9-carboxylate (20w). Yield 41% (not maximized); ^{1}H NMR ($CDCl_3$) δ 2.88 (t, J = 6.0 Hz, 2H), 3.00 (t, J = 7.5 Hz, 2H), 3.33 (t, J = 7.5 Hz, 2H), 3.34 (t, J = 6.0 Hz, 2H), 3.75 (s, 2H), 3.86 (s, 2H), 3.89 (s, 3H), 3.98 (s, 3H), 4.03 (s, 3H), 7.26−7.42 (m, 5H), 7.45 (s, 1H), 8.17 (s, 1H).

11-Benzyl-9-acetoxymethyl-2,3-dimethoxy-7,8,10,11,-12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one hydrochloride (20x). Yield 66%; ^{1}H NMR (DMSO-d_6/D_2O) δ 1.94 (s, 3H), 3.02 (t, J = 7.4 Hz, 2H), 3.33 (t, J = 7.4 Hz, 2H), 3.61 (s, 4H), 3.89 (s, 3H), 3.91 (s, 3H), 4.45 (s, 2H), 4.49 (s, 2H), 5.13 (s, 2H), 7.54−7.8 (m, 6H), 8.05 (s, 1H).

11-Benzyl-8,8-dimethyl-1-methoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (20y). Yield 85%; ^{1}H NMR (DMSO-d_6/D_2O) δ 1.36 (s, 6H), 2.91 (s, 2H), 3.52 (br s, 2H), 3.81 (br s, 2H), 3.97 (s, 3H), 4.42 (s, 4H), 7.06 (d, J = 8 Hz, 1H), 7.27 (s, 1H), 7.53−7.60 (m, 6H), 8.12 (d, J = 8 Hz, 1H).

11-Benzyl-8,8-dimethyl-1-hydroxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrobromide (20z). 20z was prepared as described for **20c**, starting with **20y**. Recrystallized from MeOH; yield 73%; ^{1}H NMR (DMSO-d_6/D_2O) δ 1.36 (s, 6H), 2.90 (s, 2H), 3.50 (br s, 2H), 3.90 (br s, 2H), 4.38 (s, 2H), 4.41 (s, 2H), 6.90 (d, J = 8 Hz, 1H), 7.22 (s, 1H), 7.40 (t, J = 8 Hz, 1H), 7.52−7.61 (m, 5H), 8.00 (d, J = 8 Hz, 1H).

12-Benzyl-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[4,3-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (21a). Yield 94%; mp 291−292 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.03 (t, J = 7.4 Hz, 2H), 3.20−3.30 (m, 4H), 3.53 (br s, 2H), 4.56 (s, 2H), 4.81 (s, 2H), 7.28 (s, 1H), 7.51 (t, J = 8 Hz, 1H), 7.56−7.65 (m, 6H), 7.84 (d, J = 8 Hz, 1H), 8.45 (d, J = 8 Hz, 1H).

12-Benzyl-2-methoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[4,3-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (21b). Yield 96%; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.98 (t, J = 7.5 Hz, 2H), δ 3.23 (t, J = 7.5 Hz, 2H), 3.26 (br s, 2H), 3.65 (br s, 2H), 3.85 (s, 3H), 4.63 (s, 2H), 4.76 (s, 2H), 7.10 (d, J = 1.8 Hz, 1H), 7.19 (dd, J = 1.8 and 8.9 Hz, 1H), 7.27 (s, 1H), 7.56−7.71 (m, 5H), 8.31 (d, J = 8.9 Hz, 1H).

12-Benzyl-2-benzyloxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[4,3-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (21c). Yield 83%; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.98 (t, J = 7.5 Hz, 2H), δ 3.23 (t, J = 7.5 Hz, 2H), 3.26 (br s, 2H), 3.66 (br s, 2H), 4.64 (s, 2H), 4.76 (s, 2H), 5.13 (s, 2H), 7.20 (d, J = 2.3 Hz, 1H), 7.26 (dd, J = 2.3 and 8.9 Hz, 1H), 7.28 (s, 1H), 7.39−7.72 (m, 10H), 8.31 (d, J = 8.9 Hz, 1H).

Methyl 12-Benzyl-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[4,3-*c*:1′,2′,3′-lm]carbazol-6-one-2-carboxylate Hydrochloride (21d). Yield 78%; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.06 (t, J = 7.4 Hz, 2H), δ 3.28 (m, 4H), 3.62 (br s, 2H), 3.98 (s, 3H), 4.64 (s, 2H), 4.82 (s, 2H), 7.35 (s, 1H), 7.59−7.73 (m, 5H), 8.20 (dd, J = 1.6 and 8.6 Hz, 1H), 8.28 (d, J = 1.6 Hz, 1H), 8.52 (d, J = 8.6 Hz, 1H).

12-Benzyl-3-methoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[4,3-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (21e). Yield 78%; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.02 (t, J = 7.4 Hz, 2H), δ 3.19−3.26 (m, 4H), 3.52 (br s, 2H), 3.88 (s, 3H), 4.59 (s, 2H), 4.81 (s, 2H), 7.10 (d, J = 8.6 Hz, 1H), 7.18 (s, 1H), 7.55−7.67 (m, 5H), 7.76 (d, J = 8.6 Hz, 1H), 7.99 (s, 1H).

12-Benzyl-3-hydroxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[4,3-*c*:1′,2′,3′-lm]carbazol-6-one Hydrobromide (21f). 21f was prepared as described for **20c**, starting with **21e**. Recrystallized from MeOH; yield 70%; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.00 (t, J = 7.5 Hz, 2H), δ 3.18−3.24 (m, 4H), 3.45 (br s, 2H), 4.62 (br s, 2H), 4.82 (s, 2H), 6.94 (dd, J = 2.3 and 8 Hz, 1H), 7.16 (s, 1H), 7.56−7.68 (m, 6H), 7.91 (d, J = 2.3 Hz, 1H).

12-Benzyl-2,3-dimethoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[4,3-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (21g). Yield 88%; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.00 (t, J = 7.4 Hz, 2H), δ 3.19 (br s, 2H), 3.24 (t, J = 7.4 Hz, 2H), 3.53 (br

s, 2H), 3.85 (s, 3H), 3.87 (s, 3H), 4.64 (s, 2H), 4.72 (s, 2H), 7.05 (s, 1H), 7.19 (s, 1H), 7.5−7.8 (m, 5H), 8.00 (s, 1H).

12-Benzyl-8,8-dimethyl-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[4,3-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (21h). Yield 88%; ^{1}H NMR (DMSO-d_6/D_2O) δ 1.40 (s, 6H), 2.96 (s, 2H), δ 3.26 (br s, 2H), 3.57 (br s, 2H), 4.63 (s, 2H), 4.90 (s, 2H), 7.41 (s, 1H), 7.5−7.7 (m, 7H), 7.88 (d, J = 8 Hz, 1H), 8.44 (d, J = 8 Hz, 1H).

12-Benzyl-8,8-dimethyl-2,3-dimethoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[4,3-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (21i). Yield 91%; ^{1}H NMR (DMSO-d_6/D_2O) δ 1.38 (s, 6H), 2.92 (s, 2H), 3.28 (br s, 2H), 3.63 (br s, 2H), 3.85 (s, 3H), 3.88 (s, 3H), 4.62 (s, 2H), 4.72 (s, 2H), 7.08 (s, 1H), 7.30 (s, 1H), 7.55−7.72 (m, 5H), 8.00 (s, 1H).

General Procedure for Debenzylation Reactions. A suspension of the Diels−Alder adduct (1.0 g) and 5% Pd/C (0.5 g) in MeOH (75 mL) was stirred initially under argon (10 min) and then under hydrogen at atmospheric pressure at 40 °C for 2 h or until the reaction was complete. The reaction mixture was filtered, and unless otherwise stated, the filtrate was concentrated under reduce pressure and the resulting precipitate was collected by filtration. If necessary, further purification was carried out as described. Unless otherwise stated, the following compounds were obtained by the above debenzylation procedure.

7,8,10,11,12,13-Hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]-carbazol-6-one Hydrochloride (22a). Recrystallized from small quantities of MeOH as colorless needles; yield 64%; mp 287−290 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.99 (t, J = 7.5 Hz, 2H), 3.22 (t, J = 7.5 Hz, 2H), 3.47 (t, J = 6.1 Hz, 2H), 3.61 (t, J = 6.1 Hz, 2H), 4.44 (s, 2H), 7.21 (s, 1H), 7.49 (t, J = 8 Hz, 1H), 7.57 (t, J = 8 Hz, 1H), 8.03 (d, J = 8 Hz, 1H), 8.34 (d, J = 8 Hz, 1H); MS *m*/*e* 277 (MH^+, 100). Anal. ($C_{18}H_{16}N_2O$·HCl) C, H, N.

1-Methoxy-7,8,10,11,12,13-Hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (22b). Recrystallized from small quantities of MeOH; yield 63%; mp 284−286 °C; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt) δ 2.99 (t, J = 7.5 Hz, 2H), 3.21 (t, J = 7.5 Hz, 2H), 3.60 (t, J = 6.0 Hz, 2H), 3.75 (t, J = 6.0 Hz, 2H), 4.02 (s, 3H), 4.51 (s, 2H), 7.04 (d, J = 8.2 Hz, 1H), 7.20 (s, 1H), 7.55 (t, J = 8.2 Hz, 1H), 8.03 (d, J = 8.2 Hz, 1H); MS *m*/*e* 307 (MH^+, 100). Anal. ($C_{19}H_{18}N_2O_2$.HCl·0.1H_2O) C, H, N.

1-Hydroxy-7,8,10,11,12,13-Hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrobromide (22c). The product was obtained from **20c** by the general procedure for debenzylation and then recrystallized from small quantities of MeOH. Yield 62%; mp > 300 °C; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 298 K) δ 2.97 (t, J = 7.6 Hz, 2H), 3.18 (t, J = 7.6 Hz, 2H), 3.53 (t, J = 6.2 Hz, 2H), 3.82 (t, J = 6.2 Hz, 2H), 4.43 (s, 2H), 6.87 (d, J = 8 Hz, 1H), 7.12 (s, 1H), 7.37 (t, J = 8 Hz, 1H), 7.92 (d, J = 8 Hz, 1H); MS *m*/*e* 293 (MH^+, 100). Anal. ($C_{18}H_{16}N_2O_2$.HBr·0.5H_2O) C, H, N.

1-Chloro-7,8,10,11,12,13-Hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (22d). To a suspension of **20d** (60 mg, 0.14 mmol) in anhydrous THF (25 mL) and K_2CO_3 (39 mg, 0.28 mmol) was added with stirring vinyl chloroformate (250 mg, 2.35 mmol). The mixture was stirred at room temperature for 8 h and then filtered. The filtrate was taken to dryness, and the residue was redissolved in MeOH (25 mL) containing trifluoroacetic acid (80 mg, 0.7 mmol). The solution was refluxed for 10 h and cooled to room temperature, ether containing HCl (10 mL) and charcoal (100 mg) was added, and the mixture was stirred for 1 h. The mixture was filtered and the filtrate taken to dryness. The resulting residue was recrystallized from MeOH to give **22d** as a white powder (35 mg, 73% yield). Mp 281−284 °C; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 338 K) δ 3.06 (t, J = 7.5 Hz, 2H), 3.29 (t, J = 7.5 Hz, 2H), 3.50 (t, J = 6.3 Hz, 2H), 3.91 (t, J = 6.3 Hz, 2H), 4.47 (s, 2H), 7.31 (s, 1H), 7.56 (m, 2H), 8.57 (d, J = 8 Hz, 1H); MS *m*/*e* 313 and 311 (MH^+, 33 and 100, respectively). Anal. ($C_{18}H_{15}N_2OCl$·HCl) C, H, N.

Methyl 7,8,10,11,12,13-Hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one-1-carboxylate Hydrochloride (22e). Recrystallized twice from a mixture of MeOH/H_2O; yield 73%; mp >300 °C; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 313 K) δ 3.06 (t, J = 7.5 Hz, 2H), 3.11 (t, J = 6.3 Hz, 2H), 3.29 (t, J = 7.5 Hz, 2H), 3.43 (t, J = 6.3 Hz, 2H), 4.02 (s, 3H), 4.45 (s, 2H), 7.32 (s, 1H), 7.61 (d, J = 8 Hz, 1H), 7.71 (t, J = 8 Hz, 1H), 8.72 (d, J = 8 Hz, 1H); MS *m*/*e* 335 (MH^+, 100). Anal. ($C_{20}H_{18}N_2O_3$·HCl) C, H, N.

2-Methyl-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (22f). Yield 63%; mp 265−268 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.49 (s, 3H), 2.97 (t, J = 7.5 Hz, 2H), 3.21 (t, J = 7.5 Hz, 2H), 3.48 (t, J = 6 Hz, 2H), 3.63 (t, J = 6 Hz, 2H), 4.45 (s, 2H), 7.18 (s, 1H), 7.39 (d, J = 8 Hz, 1H), 7.44 (s, 1H), 8.22 (d, J = 8 Hz, 1H); MS *m*/*e* 335 (MH^+, 100). Anal. ($C_{19}H_{18}N_2O$·HCl) C, H, N.

2-Methoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (22g). Recrystallized from a mixture of MeOH/H_2O as colorless needles; yield 83%; mp 285−290 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.99 (t, J = 7.4 Hz, 2H), 3.22 (t, J = 7.4 Hz, 2H), 3.47 (t, J = 5.4 Hz, 2H), 3.61 (t, J = 5.4 Hz, 2H), 3.90 (s, 3H), 4.40 (s, 2H), 7.20 (d, J = 8.9 Hz, 1H), 7.22 (s, 1H), 7.52 (d, J = 1.8 Hz, 1H), 8.32 (d, J = 8.9 Hz, 1H); MS *m*/*e* 307 (MH^+, 50). Anal. ($C_{19}H_{18}N_2O_2$·HCl) C, H, N.

2-Hydroxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (22h). Recrystallized from a mixture of MeOH/H_2O as colorless needles; yield 56%; mp >300 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.99 (t, J = 7.5 Hz, 2H), 3.23 (t, J = 7.5 Hz, 2H), 3.46 (t, J = 6.2 Hz, 2H), 3.57 (t, J = 6.2 Hz, 2H), 4.40 (s, 2H), 7.05 (dd, J = 2.2 and 8.8 Hz, 1H), 7.20 (s, 1H), 7.44 (d, J = 2.2 Hz, 1H), 8.24 (d, J = 8.8 Hz, 1H); MS *m*/*e* 293 (MH^+, 100). Anal. ($C_{18}H_{16}N_2O_2$·HCl·0.6H_2O) C, H, N.

2-Chloro-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (22i). 22i was prepared as described for **22d**, starting with **20i**. Recrystallized from MeOH; yield 55%; mp 289−292 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.82 (t, J = 7.5 Hz, 2H), 3.24 (t, J = 7.5 Hz, 2H), 3.42 (t, J = 5.9 Hz, 2H), 3.49 (t, J = 5.9 Hz, 2H), 4.32 (s, 2H), 7.21 (s, 1H), 7.42 (d, J = 8 Hz, 1H), 7.90 (s, 1H), 8.21 (d, J = 8 Hz, 1H); HRMS ($C_{18}H_{16}ClN_2O$) found *m*/*z* 311.0970, calcd 311.0958.

7,8,10,11,12,13-Hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]-carbazol-6-one-2-carboxylic Acid Hydrochloride (22j). Recrystallized from MeOH as colorless needles; yield 76%; mp > 330 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.03 (t, J = 7.4 Hz, 2H), 3.25 (t, J = 7.4 Hz, 2H), 3.48 (t, J = 6.2 Hz, 2H), 3.64 (t, J = 6.2 Hz, 2H), 4.46 (s, 2H), 7.27 (s, 1H), 8.05 (d, J = 8.6 Hz, 1H), 8.36 (d, J = 8.6 Hz, 1H), 8.44 (s, 1H); MS *m*/*e* 321 (MH^+, 100). Anal. ($C_{19}H_{16}N_2O_3$·HCl·1.2H_2O) C, H, N.

Methyl 7,8,10,11,12,13-Hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one-2-carboxylate Hydrochloride (22k). A solution of **22j** (0.32 g) in MeOH (50 mL) containing catalytic amounts of 98% H_2SO_4 was refluxed overnight. The mixture was cooled, and the white precipitate was collected by filtration and recrystallized from a mixture of MeOH/H_2O as colorless needles. Yield 76%; mp >300 °C; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt) δ 3.00 (t, J = 7.5 Hz, 2H), 3.23 (t, J = 7.5 Hz, 2H), 3.41 (t, J = 6 Hz, 2H), 3.63 (t, J = 6 Hz, 2H), 3.96 (s, 3H), 4.44 (s, 2H), 7.28 (s, 1H), 8.05 (d, J = 8 Hz, 1H), 8.32 (d, J = 8 Hz, 1H), 8.33 (s, 1H); MS *m*/*e* 335 (MH^+, 100). Anal. ($C_{20}H_{18}N_2O_3$·0.5H_2SO_4·1H_2O) C, H, N.

2-Morpholinocarbonyl-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (22l). The residue obtained from the reaction was chromatographed on silica, eluting with 4% concentrated ammonia in MeOH. The product was redissolved in MeOH, and Et_2O containing HCl was added. The white precipitate was collected by filtration and dried in vacuo. Yield 88%; mp 250 °C (dec); ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt) δ 3.09 (t, J = 7.5 Hz, 2H), 3.31 (t, J = 7.5 Hz, 2H), 3.57 (m, 4H), 3.66 (m, 4H), 3.83 (m, 4H), 4.45 (s, 2H), 7.31 (s, 1H), 7.69 (d, J = 8 Hz, 1H), 8.15 (s, 1H), 8.53 (d, J = 8 Hz, 1H); MS *m*/*e* 390 (MH^+, 100). Anal. ($C_{23}H_{23}N_3O_3$·HCl·H_2O) C, H, N.

2-Morpholinomethyl-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Dihydrochloride (22m).

Yield 73%; mp 230 °C (dec); ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 313 K) δ 3.08 (t, J = 7.5 Hz, 2H), 3.25 (t, J = 7.5 Hz, 2H), 3.39 (br s, 4H), 3.65 (t, J = 6 Hz, 2H), 3.78 (t, J = 6 Hz, 2H), 3.93 (br s, 4H), 4.52 (s, 2H), 4.58 (s, 2H), 7.26 (s, 1H), 7.67 (d, J = 8 Hz, 1H), 8.05 (s, 1H), 8.43 (d, J = 8 Hz, 1H); MS *m/e* 376 (MH^+, 100). Anal. ($C_{23}H_{25}N_3O_2$·2HCl·1H_2O) C, H, N.

3-Methoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (22n). Recrystallized from MeOH as colorless needles; yield 77%; mp >300 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.02 (t, J = 7.4 Hz, 2H), 3.24 (t, J = 7.4 Hz, 2H), 3.43 (t, J = 6.0 Hz, 2H), 3.54 (t, J = 6.0 Hz, 2H), 3.88 (s, 3H), 4.38 (s, 2H), 7.11 (dd, J = 2.4 and 8.7 Hz, 1H), 7.15 (s, 1H), 7.99 (d, J = 2.4 Hz, 1H), 8.01 (d, J = 8.7 Hz, 1H); MS *m/e* 307 (MH^+, 100). Anal. ($C_{19}H_{16}N_2O_2$·HCl·0.5H_2O) C, H, N.

3-Hydroxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrobromide (22o). The product was obtained from **20n** following the general procedure for debenzylation. Recrystallized from MeOH as colorless needles; yield 56%; mp >300 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.00 (t, J = 7.4 Hz, 2H), 3.22 (t, J = 7.4 Hz, 2H), 3.42 (t, J = 6.0 Hz, 2H), 3.52 (t, J = 6.0 Hz, 2H), 4.38 (s, 2H), 6.94 (dd, J = 2.1 and 8.7 Hz, 1H), 7.11 (s, 1H), 7.90 (d, J = 2.1 Hz, 1H), 7.91 (d, J = 8.7 Hz, 1H); MS *m/e* 293 (MH^+, 100). Anal. ($C_{18}H_{16}N_2O_2$·HBr·0.5H_2O) C, H, N.

3-Chloro-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (22p). Recrystallized twice from MeOH as colorless needles; yield 19%; mp 295−298 °C; ^{1}H NMR (DMSO-d_6/D_2O,TSP-salt, 298 K) δ 3.06 (t, J = 7.5 Hz, 2H), 3.26 (t, J = 7.5 Hz, 2H), 3.44 (t, J = 6 Hz, 2H), 3.51 (t, J = 6 Hz, 2H), 4.35 (s, 2H), 7.26 (s, 1H), 7.57 (d, J = 8.5 Hz, 1H), 8.14 d, J = 8.5 Hz, 1H), 8.44 (s, 1H); HRMS ($C_{18}H_{16}ClN_2O$) found *m/e* 311.0980, calcd 311.0955.

Methyl 7,8,10,11,12,13-Hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride-3-carboxylate Hydrochloride (22q). The reaction mixture was filtered, and ether containing HCl was added. The resulting precipitate was recrystallized twice from MeOH/H_2O. Yield 64%; mp >300 °C; ^{1}H NMR (DMSO-d_6/D_2O, 323 K) δ 3.04 (t, J = 7.5 Hz, 2H), 3.26 (t, J = 7.5 Hz, 2H), 3.42 (br s, 2H), 3.63 (t, J = 6 Hz, 2H), 3.96 (s, 3H), 4.43 (s, 2H), 7.23 (s, 1H), 7.93−8.02 (m, 2H), 8.71 (s, 1H); MS *m/e* 335 (MH^+, 100). Anal. ($C_{20}H_{18}N_2O_3$·HCl) C, H, N.

3-Cyano-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (22r). Recrystallized from a mixture of MeOH/H_2O; yield 64%; mp 282−285 °C; ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 298 K) δ 2.86 (t, J = 7.3 Hz, 2H), 3.25 (t, J = 7.3 Hz, 2H), 3.61 (s, 2H), 3.63 (s, 2H), 4.43 (s, 2H), 7.23 (s, 1H), 7.63 (d, J = 8 Hz, 1H), 8.07 (s, 1H), 8.33 (d, J = 8 Hz, 1H); MS *m/e* 302 (MH^+, 100). Anal. ($C_{19}H_{15}N_3O$·HCl) C, H, N.

4-Methoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (22s). Recrystallized from MeOH/H_2O; yield 67%; mp 288−290 °C; ^{1}H NMR (DMSO-d_6/D_2O, 323 K) δ 3.03 (t, J = 7.3 Hz, 2H), 3.25 (t, J = 7.3 Hz, 2H), 3.53 (br s, 2H), 3.61 (br s, 2H), 3.96 (s, 3H), 4.44 (s, 2H), 7.23 (s, 1H), 7.29 (d, J = 8 Hz, 1H), 7.53 (t, J = 8 Hz, 1H), 7.75 (d, J = 8 Hz, 1H); MS *m/e* 307 (MH^+, 100). Anal. ($C_{19}H_{18}N_2O_2$·HCl) C, H, N.

4-Hydroxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrobromide (22t). The product was obtained from **20s** following the general procedure for debenzylation and then recrystallized from MeOH/H_2O. Yield 90%; mp >300 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.11 (t, J = 7.5 Hz, 2H), 3.24 (t, J = 7.5 Hz, 2H), 3.50 (t, J = 6 Hz, 2H), 3.65 (t, J = 6 Hz, 2H), 4.47 (s, 2H), (d, J = 8 Hz, 1H), 7.23 (s, 1H), 7.41 (t, J = 8 Hz, 1H), 7.56 (d, J = 8 Hz, 1H); MS *m/e* 293 (MH^+, 100). Anal. ($C_{18}H_{16}N_2O_2$·HBr) C, H, N.

2,3-Dimethoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido-[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (22u). Recrystallized from MeOH as colorless needles; yield 60%; mp 288−290 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.00 (t, J = 7.4 Hz, 2H), 3.23 (t, J = 7.4 Hz, 2H), 3.49−3.55 (m, 4H), 3.88 (s, 3H), 3.92 (s, 3H), 4.39 (s, 2H), 7.13 (s, 1H), 7.53 (s, 1H), 8.02 (s, 1H); MS *m/e* 377 (MH^+, 100). Anal. ($C_{20}H_{20}N_2O_3$·HCl·1.5H_2O) C, H, N.

2,3-Methylenedioxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (22v). Recrystallized from MeOH as colorless needles; yield 69%; mp 294−298 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.00 (t, J = 7.4 Hz, 2H), 3.22 (t, J = 7.4 Hz, 2H), 3.41 (t, J = 6.0 Hz, 2H), 3.52 (t, J = 6.0 Hz, 2H), 4.36 (s, 2H), 6.15 (s, 2H), 7.12 (s, 1H), 7.60 (s, 1H), 7.95 (s, 1H); MS *m/e* 321 (MH^+, 100). Anal. ($C_{19}H_{16}N_2O_3$·HCl·0.2H_2O) C, H, N.

1,2,3-Trimethoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (22w). Recrystallized from MeOH; yield 55%; mp 210 °C (dec); ^{1}H NMR (DMSO-d_6/D_2O, TSP-salt, 298 K) δ 2.98 (t, J = 7.5 Hz, 2H), 3.21 (t, J = 7.5 Hz, 2H), 3.56 (t, J = 6 Hz, 2H), 3.64 (t, J = 6 Hz, 2H), 3.89 (s, 3H), 3.92 (s, 3H), 4.03 (s, 3H), 4.46 (s, 2H), 7.18 (s, 1H), 7.86 (s, 1H); MS *m/e* 367 (MH^+, 100). Anal. ($C_{21}H_{22}N_2O_4$·HCl) C, H, N.

Methyl 2,3-Dimethoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one-9-carboxylate Hydrochloride (22x). Yield 65%; mp 270 °C (dec); ^{1}H NMR (DMSO-d_6/D_2O) δ 3.00 (t, J = 7.4 Hz, 2H), 3.32 (t, J = 7.4 Hz, 2H), 3.54 (s, 4H), 3.89 (s, 3H), 3.92 (s, 3H), 3.94 (s, 3H), 4.43 (s, 2H), 7.53 (s, 1H), 8.01 (s, 1H); MS *m/e* 395 (MH^+, 100). Anal. ($C_{22}H_{22}N_2O_5$·HCl·0.6H_2O) C, H, N.

9-Acetoxymethyl-2,3-dimethoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (22y). Yield 75%; mp 290 °C (dec); ^{1}H NMR (DMSO-d_6/D_2O) δ 2.06 (s, 3H), 3.02 (t, J = 7.4 Hz, 2H), 3.34 (t, J = 7.4 Hz, 2H), 3.53 (s, 4H), 3.88 (s, 3H), 3.92 (s, 3H), 4.49 (s, 2H), 5.22 (s, 2H), 7.51 (s, 1H), 8.01 (s, 1H); MS *m/e* 409 (MH^+, 100). Anal. ($C_{23}H_{24}N_2O_5$·HCl·1.1H_2O) C, H, N.

8,8-Dimethyl-1-methoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (22z). Recrystallized from MeOH as colorless needles; yield 50%; mp 275−278 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 1.39 (s, 6H), 2.90 (s, 2H), 3.44 (t, J = 6.4 Hz, 2H), 3.85 (t, J = 6.4 Hz, 2H), 3.98 (s, 3H), 4.39 (s, 2H), 6.91 (d, J = 8 Hz, 1H), 7.29 (s, 1H), 7.40 (t, J = 8 Hz 1H), 8.0 (d, J = 8 Hz, 1H); MS *m/e* 335 (MH^+, 100); HRMS ($C_{21}H_{22}N_2O_2$) found *m/z* 335.1790, calcd 335.1772.

8,8-Dimethyl-1-hydroxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[3,4-*c*:1′,2′,3′-lm]carbazol-6-one Hydrobromide (22aa). The product was obtained from **20z** following the general procedure for debenzylation. Recrystallized from MeOH as colorless needles; yield 50%; mp >330 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 1.38 (s, 6H), 2.91 (s, 2H), 3.44 (t, J = 6.4 Hz, 2H), 3.83 (t, J = 6.4 Hz, 2H), 4.40 (s, 2H), 6.91 (d, J = 8 Hz, 1H), 7.30 (s, 1H), 7.40 (t, J = 8 Hz 1H), 8.0 (d, J = 8 Hz, 1H); MS *m/e* 321 (MH^+, 100). Anal. ($C_{20}H_{20}N_2O_2$·HBr·0.2H_2O) C, H, N.

7,8,10,11,12,13-Hexahydro-6*H*-dipyrido[4,3-*c*:1′,2′,3′-lm]-carbazol-6-one Hydrochloride (23a). Recrystallized from MeOH/H_2O as colorless needles (97% yield before recrystallization); mp 295−298 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.02 (t, J = 7.4 Hz, 2H), 3.21 (t, J = 6.0 Hz, 2H), 3.26 (t, J = 7.4 Hz, 2H), 3.53 (t, J = 6.0 Hz, 2H), 4.77 (s, 2H), 7.28 (s, 1H), 7.52 (t, J = 8 Hz, 1H), 7.61 (t, J = 8 Hz, 1H), 7.93 (t, J = 8 Hz, 1H), 8.40 (t, J = 8 Hz, 1H); MS *m/e* 277 (MH^+, 100). Anal. ($C_{18}H_{16}N_2O$·HCl·0.6H_2O) C, H, N.

2-Methoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[4,3-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (23b). Recrystallized from MeOH/H_2O as colorless needles (91% yield before recrystallization); mp 288−295 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.98 (t, J = 7.4 Hz, 2H), 3.18 (t, J = 5.9 Hz, 2H), 3.23 (t, J = 7.4 Hz, 2H), 3.49 (t, J = 5.9 Hz, 2H), 3.90 (s, 3H), 4.75 (s, 2H), 7.19 (dd, J = 2.4 and 8.9 Hz, 1H), 7.26 (s, 1H), 7.35 (d, J = 2.4 Hz, 1H), 8.31 (d, J = 8.9 Hz, 1H), 8.40 (t, J = 8 Hz, 1H); MS *m/e* 307 (MH^+, 100). Anal. ($C_{19}H_{18}N_2O_2$·HCl) C, H, N.

2-Hydroxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[4,3-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (23c). Recrystallized from MeOH/H_2O as colorless needles (83% yield before recrystallization); mp >300 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 2.96 (t, J = 7.5 Hz, 2H), 3.19 (t, J = 7.5 Hz, 2H), 3.21 (t, J = 6.0

Hz, 2H), 3.52 (t, J = 6.0 Hz, 2H), 4.69 (s, 2H), 7.00 (dd, J = 1.9 and 8.8 Hz, 1H), 7.21 (d, J = 1.9 Hz, 1H), 7.24 (s, 1H), 8.16 (d, J = 8.8 Hz, 1H); MS *m/e* 293 (MH^+, 100). Anal. ($C_{18}H_{16}N_2O_2 \cdot HCl \cdot H_2O$) C, H, N.

Methyl 7,8,10,11,12,13-Hexahydro-6*H*-dipyrido[4,3-*c*:1′,2′,3′-lm]carbazol-6-one-2-carboxylate Hydrochloride (23d). Recrystallized from MeOH as colorless needles; yield 83%; mp >300 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.06 (t, J = 7.3 Hz, 2H), 3.18 (t, J = 6.0 Hz, 2H), 3.28 (t, J = 7.3 Hz, 2H), 3.48 (t, J = 6.0 Hz, 2H), 3.94 (s, 3H), 4.84 (s, 2H), 7.33 (s, 1H), 8.20 (dd, J = 1.5 and 8.6 Hz, 1H), 8.47 (d, J = 1.5 Hz, 1H), 8.52 (d, J = 8.6 Hz, 1H); MS *m/e* 335 (MH^+, 100). Anal. ($C_{20}H_{18}N_2O_3 \cdot HCl \cdot 0.2H_2O$) C, H, N.

3-Methoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[4,3-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (23e). Recrystallized from MeOH as colorless needles; yield 65%; mp >300 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.02 (t, J = 7.4 Hz, 2H), 3.14 (t, J = 5.8 Hz, 2H), 3.24 (t, J = 7.4 Hz, 2H), 3.45 (t, J = 5.8 Hz, 2H), 3.89 (s, 3H), 4.72 (s, 2H), 7.11 (dd, J = 2.3 and 8.6 Hz, 1H), 7.18 (s, 1H), 7.88 (d, J = 8.6 Hz, 1H), 7.99 (d, J = 2.3 Hz, 1H); MS *m/e* 307 (MH^+, 100); HRMS ($C_{19}H_{19}N_2O_2$) found *m/z* 307.1470, calcd 307.1460.

3-Hydroxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[4,3-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (23f). The product was obtained from **21f** following the general procedure for debenzylation and then recrystallized from MeOH/H_2O as colorless needles; yield 75%; mp >300 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.00 (t, J = 7.5 Hz, 2H), 3.13 (t, J = 5.7 Hz, 2H), 3.22 (t, J = 7.5 Hz, 2H), 3.45 (t, J = 5.7 Hz, 2H), 4.69 (s, 2H), 6.95 (dd, J = 2.3 and 8.6 Hz, 1H), 7.14 (s, 1H), 7.78 (d, J = 8.6 Hz, 1H), 7.90 (d, J = 2.3 Hz, 1H); MS *m/e* 293 (MH^+, 100). Anal. ($C_{18}H_{16}N_2O_2 \cdot HBr \cdot 0.2H_2O$) C, H, N.

2,3-Dimethoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido-[4,3-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (23g). Recrystallized from MeOH as colorless needles; yield 29%; mp 285−287 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 3.00 (t, J = 7.4 Hz, 2H), 3.15 (t, J = 5.8 Hz, 2H), 3.24 (t, J = 7.4 Hz, 2H), 3.47 (t, J = 5.8 Hz, 2H), 3.86 (s, 3H), 3.93 (s, 3H), 4.80 (s, 2H), 7.16 (s, 1H), 7.40 (s, 1H), 8.02 (s, 1H); MS *m/e* 337 (MH^+, 100). Anal. ($C_{20}H_{20}N_2O_3 \cdot HCl \cdot H_2O$) C, H, N.

8,8-Dimethyl-7,8,10,11,12,13-hexahydro-6*H*-dipyrido-[4,3-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (23h). Recrystallized from MeOH as colorless needles; yield 89%; mp 292−296 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 1.41 (s, 6H), 2.96 (s, 2H), 3.20 (t, J = 5.9 Hz, 2H), 3.50 (t, J = 5.9 Hz, 2H), 4.81 (s, 2H), 7.39 (s, 1H), 7.52 (t, J = 8 Hz, 1H), 7.63 (t, J = 8 Hz, 1H), 8.01 (d, J = 8 Hz, 1H), 8.44 (d, J = 8 Hz, 1H); MS *m/e* 305 (MH^+, 100). Anal. ($C_{20}H_{20}N_2O \cdot HCl \cdot 0.5H_2O$) C, H, N.

8,8-Dimethyl-2,3-dimethoxy-7,8,10,11,12,13-hexahydro-6*H*-dipyrido[4,3-*c*:1′,2′,3′-lm]carbazol-6-one Hydrochloride (23i). Recrystallized from MeOH as colorless needles; yield 77%; mp 285−288 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 1.39 (s, 6H), 2.93 (s, 2H), 3.18 (t, J = 5.7 Hz, 2H), 3.48 (t, J = 5.7 Hz, 2H), 3.89 (s, 3H), 3.93 (s, 3H), 4.82 (s, 2H), 7.28 (s, 1H), 7.42 (s, 1H), 8.02 (s, 1H); MS *m/e* 365 (MH^+, 100). Anal. ($C_{22}H_{24}N_2O_3 \cdot HCl \cdot 0.2H_2O$) C, H, N.

Radioligand Binding. CHO cells stably expressing cDNAs encoding human muscarinic M_1−M_4 receptors were grown to confluence in α-MEM medium (GIBCO) containing 10% (v/v) newborn calf serum, 50 U/mL penicillin, 50 mg/mL streptomycin, and 2 mM glutamine at 37 °C under 5% CO_2 and harvested by scraping in a hypotonic medium (20 mM Hepes + 10 mM EDTA, pH 7.4). Membranes were prepared at 0 °C by homogenization with a Polytron followed by centrifugation (40 000*g*, 15 min), were washed once in 20 mM Hepes + 0.1 mM EDTA, pH 7.4, and were stored at −70 °C in the same buffer at protein concentrations of 2−5 mg/mL.

The binding assays and analyses have been described previously.[28,36,37] In general, binding of ^{3}H−NMS was conducted in a buffer containing 20 mM Hepes + 100 mM NaCl + 10 mM $MgCl_2$ (pH 7.4) at 30 °C in a volume of 1 mL. Membranes were collected by filtration over glass fiber filters (Whatman GF/B) presoaked in 0.1% polyethylenimine, using a Brandel cell harvester (Semat, Herts, U.K.), extracted overnight in scintillation fluid (ReadySafe, Beckman), and counted for radioactivity in Beckman LS6000 scintillation counters. Membrane protein concentrations (5−50 μg/mL) were adjusted so that not more than about 15% of added radioligand was bound. Nonspecific binding was measured in the presence of 10^{-6} M QNB (an antagonist with picomolar potency) and accounted for 1−5% of total binding. Test compounds were dissolved in DMSO that, at the highest final concentration of 1%, had no effect on binding.

The equilibrium assays utilized an incubation time of at least 2 h in the presence of GTP (2×10^{-4} M) and ^{3}H−NMS concentrations of 0.1−0.7 nM, depending on receptor subtype. In most assays binding of ^{3}H−NMS in the absence and presence of an IC_{50} concentration of ACh was measured alone and in the presence of three or more concentrations of test agent. Selected compounds were studied further with an equilibrium assay that measured inhibition curves with ACh alone and in the presence of three concentrations of test agent and/or with a nonequilibrium assay in which the receptors in half the tubes were equilibrated with ^{3}H−NMS before addition of ACh and test agent, while the receptors in the remaining tubes were exposed to ACh and test agent before exposure to ^{3}H−NMS. In all cases the data were fitted to the allosteric model using nonlinear regression analysis (SigmaPlot, SPSS, Erkrath, Germany) to provide quantitative estimates of the affinity of the agent at the unliganded receptor and its cooperativity with ^{3}H−NMS and ACh. When the analysis required that kinetic effects on ^{3}H−NMS binding be taken into account, values of 0.075, 0.361, 0.064, and 0.066 min^{-1} were used for the dissociation rate constants of ^{3}H−NMS at M_1, M_2, M_3, and M_4 receptors, respectively.[28]

In the off-rate assay, a high concentration of membranes (2−4 mg protein/mL) was incubated with a high concentration of ^{3}H−NMS (5 nM) for about 15 min. Then 10 μL aliquots were distributed into tubes that were empty or contained 1 mL of 10^{-6} M QNB alone and in the presence of at least four concentrations of allosteric agent. Nonspecific binding was measured in separately prepared tubes containing 10 μL of membrane and 2 mL of ^{3}H−NMS + QNB. Sometime later, about 2.5 dissociation half-lives, the samples were filtered. The data were transformed to dissociation rate constants, expressed as the percentage inhibition of the "true" ^{3}H−NMS dissociation rate constant measured in the absence of allosteric agent, and fitted to a logistic function using nonlinear regression analysis. This curve corresponds theoretically to the occupancy curve of the allosteric agent at the ^{3}H−NMS-occupied site, and the regression analysis provides a quantitative estimate of the affinity of the agent for the ^{3}H−NMS-occupied receptor.

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Supporting Information Available: Additional information on the methodology for performing and analyzing the equilibrium and nonequilibrium binding assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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