15 min reproducibly elicited the von Bezold-Jarisch reflex. Antagonists were injected iv 5 min prior serotonin, and their effect was expressed as percent inhibition of serotonin response. ED_{50} were calculated by linear regression analysis. Values shown are the means ± 95% CL.

Acknowledgment. Thanks are due to Dr. E. Dubini for assiduous analytical assistance. We are also indebted to M. Mondoni for skilled interpretation of the NMR spectra.

Registry No. **4a**·HCl, 123259-23-4; 4b·HCl, 123259-28-9; **4c**·HCl, 123259-24-5; **4d**·HCl, 127595-09-9; **4e**·HCl, 123259-26-7; **4f**·HCl, 123259-25-6; **4g**·HCl, 123259-27-8; **5a**·HCl, 123259-36-9; **5k**·HCl, 123259-43-8; **6a**, 123259-35-8; **6b**, 123259-52-9; **6c**, 123259-51-8; **6d**, 123279-50-5; **6e**, 123259-53-0; **6f**, 123259-54-1; **6g**, 127595-15-7; **6h**, 123259-49-4; **6i**, 123259-50-7; **6j**, 123259-55-2; **6k**, 123279-51-6; **6l**, 123259-56-3; **6m**, 123259-57-4; **6n**, 123259-58-5; **6o**, 123259-59-6; **6h**, 123259-60-9; **6q**, 123259-61-0; **7a**, 127595-10-2; **7b**, 127595-22-6; **7b**·HCl, 123258-88-8; **7c**, 127595-23-7; **7c**·HCl, 123258-87-7; **7d**, 127595-24-8; **7d**·HCl, 123258-89-9; **7e**, 127595-25-9; **7e**·HCl, 123258-90-2; **7f**, 127595-26-0; **7f**·HCl, 123258-91-3; **7g**, 123279-46-9; **7h**, 123279-43-6; **7i**, 123279-44-7; **7j**, 127595-27-1; **7j**·HCl, 123258-92-4; **7k**, 127595-28-2; **7k**·HCl, 123258-93-5; **7l**, 127595-29-3; **7l**·HCl, 123258-94-6; **7m**, 127595-30-6; **7m**·HCl, 123279-45-8; **7n**, 127595-31-7; **7n**·HCl, 123258-96-8; **7o**, 127595-32-8; **7o**·HCl, 123258-97-9; **7p**, 127595-33-9; **7p**·HCl, 123258-98-0; **7q**, 127595-34-0; **7q**·HCl, 123258-95-7; **8a**, 127595-35-1; **8a**·HCl, 123259-05-2; **8b**, 127595-36-2; **8b**·HCl, 127595-11-3; **9**, 615-16-7; **10**, 65657-53-6; **11a**, 123259-00-7; **11a**·HCl, 127595-12-4; **11b**, 127595-16-8; **11b**·$C_6H_8O_7$, 127595-17-9; **11c**, 127595-38-4; **11c**·HCl, 123259-02-9; **11d**, 123259-03-0; **11e**, 127642-62-0; **11e**·HCl, 127595-18-0; **11f**, 123288-60-8; **11f**·$C_6H_8O_7$, 123288-61-9; **11g**, 127595-39-5; **11g**·HCl, 127595-19-1; **12a**, 123258-84-4; **12a**·HCl, 127618-28-4; **12b**, 123258-83-3; **12b**·HCl, 123259-06-3; **12c**, 123259-07-4; **12d**, 123259-08-5; **12e**, 123259-09-6; **12f**, 123259-12-1; **12g**, 123259-13-2; **12h**, 127595-40-8; **12h**·HCl, 127595-20-4; **12i**, 127595-21-5; **13a**, 123259-10-9; **13b**, 127595-42-0; **13c**, 127595-41-9; **13c**·HCl, 127595-43-1; **14**, 2687-25-4; **15**, 127595-13-5; **16**, 127595-37-3; **16**·HCl, 127595-14-6; **17**, 123259-14-3; **18**, 123259-33-6; AA-OH·HCl, 2292-08-2; BB-OH, 135-97-7; JJ-OH, 26458-74-2; FF-OH, 1748-08-9; GG-OH, 18717-73-2; 2-$NO_2C_6H_4NH_2$, 88-74-4; HH-OH·HCl, 60205-58-5; AA-NH_2, 87571-88-8; II-OH, 6376-00-7; KK-OH, 99445-15-5; LL-OH, 16576-15-1; MM-OH, 37778-50-0; NN-OH, 5382-16-1; HH-NH_2, 76272-56-5; CC-NH_2, 6238-14-8; OO-NH_2, 127642-61-9; DD-NH_2, 41838-46-4; GG-NH_2, 76272-35-0; BB-NH_2, 81487-04-9; II-NH_2, 76272-41-8; 1-methyl-4-piperidinol, 106-52-5; 1,2,6-trimethyl-4-piperidinol, 90226-91-8; pseudopelletierine, 552-70-5.

Supplementary Material Available: The atomic coordinates of compounds 1 and **12a** in their active conformations (Figure 1) are available (2 pages). Ordering information is given on any current masthead page.

Synthesis and Biological Evaluation of New Antimuscarinic Compounds with Amidine Basic Centers. A Useful Bioisosteric Replacement of Classical Cationic Heads

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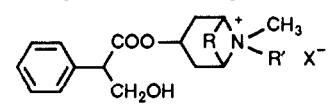
Amidines (guanidine, formamidine, and acetamidine) were introduced as substitutes for the cationic heads present in atropine, scopolamine, and corresponding quaternary derivatives. Amidine systems are intermediate in structure between tertiary amines and quaternary compounds, at least as regards ionization and electronic properties, but differ from the latter in shape (planar not tetrahedral). They have additional binding opportunities on account of their hydrogen-bond-forming capacity. The effect of the introduction of these cationic heads on the affinity for different muscarinic acetyl choline receptor (m-AcChR) subtypes was investigated in vitro, in binding displacement studies, and in functional tests on isolated organs. All new compounds (**3a**,b–**5a**,b) showed high affinity for the m-AcChR considered, comparable or slightly inferior to that of the parent drugs (**1a–e**). The new amidine derivatives proved effective as spasmolytic agents, with little tendency to cause central effects. However, no separation was achieved of spasmolytic and other untoward effects, like inhibition of salivation. Thus, amidine moieties are effective bioisosteric substitutes for conventional cationic heads present in antimuscarinic agents. Their unusual physical–chemical properties make them useful tools when modulation of pharmacokinetic or pharmacodynamic effects is required.

Muscarinic antagonists have long been employed in the treatment of several diseases in which it is useful to limit the effects of the natural transmitter acetylcholine. Their efficacy in the treatment of gastrointestinal disorders such as peptic ulcer and smooth-muscle spasms has led to the bothersome, sometimes troublesome side effects produced by their lack of selectivity in blocking muscarinic receptors (m-AcChR) in different organs.[1] Quaternization of the tertiary amino function is a commonplace chemical manipulation in this class of substances, widely utilized to control untoward effects especially in the central nervous system (CNS). Even though the inherent character of quaternary compounds negatively affects their absorption from the gastrointestinal tract, and hence their oral

Chart I. Classical Antimuscarinic Agents of Both Tertiary Amino and Quaternary Ammonium Type



1a: R = $-CH_2CH_2-$ R' = H (atropine)
b: R = $-CH(O)CH-$ R' = H (scopolamine)
c: R = $-CH_2CH_2-$ R' = CH_3 (atropine *N*-methyl bromide)
d: R = $-CH(O)CH-$ R' = CH_3 (scopolamine *N*-methyl bromide)
e: R = $-CH(O)CH-$ R' = $(CH_2)_3CH_3$ (scopolamine *N*-butyl bromide)

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Scheme I. Synthetic Pathways Followed for the Preparation of Compounds **3a,b–5a,b**

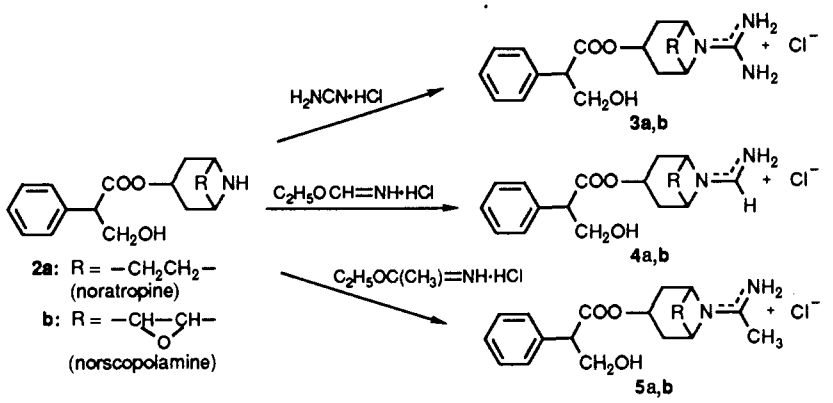
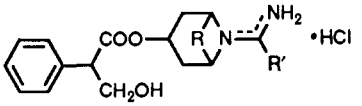


Table I. Physical Chemical Properties of Compounds **3a,b–5a,b**



compd	R	R′	recryst solv	mp, °C	% yield[a]	mol formula[b]	pK_a[c]
3a	$-CH_2CH_2-$	NH_2	diethyl ether	70–80 dec[d]	37	$C_{17}H_{24}ClN_3O_3$	11.6
3b	−CHOCH−	NH_2	acetone	160–161 dec	47	$C_{17}H_{22}ClN_3O_4$	10.84
4a	$-CH_2CH_2-$	H	diethyl ether	202–203 dec	41	$C_{17}H_{23}ClN_2O_3$	10.33
4b	−CHOCH−	H	ethanol	196–197 dec	56	$C_{17}H_{21}ClN_2O_4$	9.78
5a	$-CH_2CH_2-$	CH_3	diethyl ether	205–206 dec	46	$C_{18}H_{25}ClN_2O_3$	10.92
5b	−CHOCH−	CH_3	diethyl ether	182–185 dec	36	$C_{18}H_{23}ClN_2O_4$	10.74

[a] No attempt was made to optimize yields. [b] All compounds were analyzed for C, H, N, and Cl; the experimental data were within ±0.4% of the calculated values. [c] Atropine and scopolamine, used as reference compounds, showed the following pK_a values: 9.60 and 7.55, respectively (ref 28). Noratropine (**2a**) and norscopolamine (**2b**), used as starting materials, showed the following pK_a values: 10.00 and 7.09, respectively. [d] After lyophilization.

bioavailability, antimuscarinic substances of the onium type are still of therapeutic value as spasmolytic agents.

In our continuing effort to find new substances in which the desired spasmolytic activity could be separated from unwanted side effects, one line of research focused on chemical manipulation of the cationic portions commonly present in antimuscarinic drugs.

The onium head in antimuscarinic substances has an essential role in receptor recognition and interaction. For this reason only minor modifications have been made to this structural part, generally limited to substituent variation. Therefore, we introduced amidine moieties (guanidine, formamidine, acetamidine) as substitutes for the cationic heads present in atropine (**1a**), scopolamine (**1b**), or corresponding quaternary derivatives (**1c–e**) (Chart I).

Amidine function has been extensively evaluated in the histamine H_2-receptor field, and potent and selective antagonists have been developed with amidine groups as neutral polar moieties: cimetidine,[2] ranitidine,[3] nizatidine,[4] and famotidine[5] are now all marketed for the management of peptic ulcers. Compounds in which the amidine systems are present mainly as charged species have also been investigated: H_2-agonists like dimaprit[6] or ipromidine[7] and H_2-antagonists like mifentidine[8] are well-known examples. This paper describes the design, synthesis, and representative biological activity of the new derivatives **3a,b–5a,b**.

Chemistry

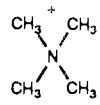
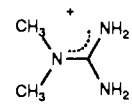
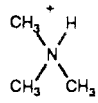
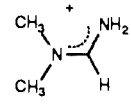
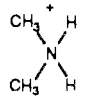
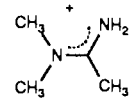
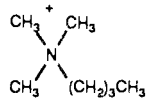
The synthetic pathways followed for the preparation of these amidine derivatives are outlined in Scheme I. Noratropine (**2a**) and norscopolamine (**2b**), easily obtained by Cl_3CCH_2OCOCl and $KMnO_4$ demethylation of their precursors (**1a,b**) according to known procedures,[9,10] were made to react with cyanamide, ethyl formimidate, or ethyl acetimidate. The respective guanidine (**3a,b**), formamidine (**4a,b**), and acetamidine derivatives (**5a,b**) were obtained in satisfactory yields (Table I).

Results and Discussion

Study Design. All evidence to date suggests that the muscarinic receptor requires a portion of high basic strength as its interacting counterpart so that at physiological pH a well-sized and -shaped cation is obtained capable of interacting with an Asp residue on the receptor protein by an electrostatic bond.[11,12] Moreover, van der

(1) Harvey, S. C. *Pharmaceutical Sciences*; Mack Publishing Co.: Easton, PA, 1980; Chapter 47, pp 850–860.
(2) Durant, G. J.; Emmet, J. C.; Ganellin, C. R.; Miles, P. D.; Parsons, M. E.; Prain, H. D.; White, G. R. *J. Med. Chem.* **1977**, *20*, 901.
(3) Bradshaw, J.; Brittain, R. T.; Cliterow, J. W.; Daly, M. J.; Jack, D.; Price, B. J.; Stubles, R. *Br. J. Pharmacol.* **1979**, *66*, 464.
(4) Lin, T. M.; Evans, D. C.; Warrick, M. W.; Pioch, R. P.; Ruffalo, R. R. *Gastroenterology* **1983**, *84*, 1231.
(5) Pendleton, R. G.; Torchiana, M. L.; Chung, C.; Look, P.; Wiese, S.; Clineschmidt, B. V. *Arch. Int. Pharmacodyn.* **1983**, *266*, 4.
(6) Parsons, M. E.; Owen, D. A. A.; Ganellin, C. R.; Durant, G. J. *Agent Actions* **1977**, *7*, 31.
(7) Durant, G. J.; Duncan, W. A. H.; Ganellin, C. R.; Parsons, M. E.; Blakemore, R. C.; Rasmussen, A. *Nature* **1978**, *276*, 403.
(8) Donetti, A.; Cereda, E.; Bellora, E.; Gallazzi, A.; Bazzano, C.; Vanoni, P. C.; Del Soldato, P.; Micheletti, R.; Pagani, F.; Giachetti, A. *J. Med. Chem.* **1984**, *27*, 380.
(9) Pfitzer, J. R. *J. Org. Chem.* **1978**, *43*, 4373.
(10) Banholtzer, R.; Pook, K. H. *Arzneim.-Forsch.* **1985**, *35*, 217.
(11) Holton, P.; Ing, H.; *Br. J. Pharmacol.* **1949**, *4*, 190.

Table II. van der Waals Volumes of Cation Heads

no.	ref molecule	vol of ref molecule, Å	no.	ref molecule	vol of ref molecule, Å
1c,d		90.6	3a,b		91.4
1a,b		75.1	4a,b		79.1
2a,b		59.2	5a,b		94.8
1e		142.0			

Waals contacts between the substitutents on the cation and nonpolar residues on the receptor are supposed to strengthen the ligand–receptor interaction.[13,14] The new cationic heads used as a primary pharmacophore were compared to the more traditional quaternary and tertiary ammonium heads, on the basis of several parameters (Figure 1). As far as basicity is concerned, both tertiary amines and quaternary compounds possess favorable features for a proper interaction with an anionic site on the receptor. Amidine systems, according to their pK_a values, which range from about 9 to 12,[15] can be considered intermediate structures between tertiary amines and quaternary onium compounds. The latter are fully ionized at any pH, and this has a detrimental effect on oral bioavailability. On the other hand, antimuscarinic compounds of the amine type exist as neutral species, at physiological pH, in a consistently high proportion. The pH-dependent amount of free base for an amidine system never exceeds 1% at pH 7.4 on account of the high pK_a, thus being present in a small but defined quantity. The unusual features of such systems led us to envisage an improved spasmolytic agent in which central side effects could be avoided without losing gastrointestinal absorption.

With regard to electronic properties, amidinium cations resemble quaternary ammonium compounds in their charge-delocalization pattern. The endocyclic N(1) and the exocyclic N(3) of an amidine base are hybridized sp^3 and sp^2, respectively. The protonation occurs first on the imino N(3), but by virtue of the charge delocalization through the central C atom, the N(1) also becomes charged and this last structure very probably is more important because of the better electron stabilization. Amidinium cations thus acquire the character of a quaternary salts.[16,17]

Concerning the steric requirements for a putative new type of cationic head, it was assumed that its bulk should be as close as possible to that of the classic muscarinic antagonists, such as atropine (**1a**) or *N*-methylatropine (**1c**), or the natural cholinergic neurotransmitter itself,

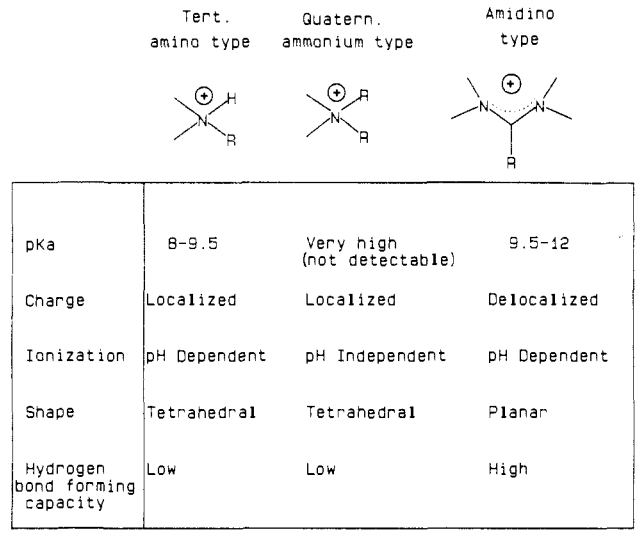


Figure 1. A comparison of different cationic heads on selected parameters.

acetylcholine. Model compounds were designed to represent traditional or unusual cationic heads. The van der Waals volume was the parameter of choice for assessing the size (bulk) of the charged species. The model compounds were built and manipulated and their volume was calculated with SYBYL[18] (Table II). The dimensions of the amidinium systems were intermediate between those of the tetra- and trimethylammonium model compounds, taken as representative of a quaternary (acetylcholine or *N*-methylatropine) and a tertiary onium head (atropine or scopolamine). The bulk of guanidinium and acetamidinium cations is in fact comparable to that of a quaternary system. As a consequence, a good fit with the complementary zone on the receptor should be assured, at least from the steric point of view.

As regards conformational properties, ionized basic moieties or the corresponding onium heads of conventional antimuscarinic agents have a tetrahedral spatial arrangement of the alkyl groups whereas amidinium cations prefer a planar structure.[19] These features would appear disadvantageous for receptor affinity if the theory of a muscarinic receptor shaped to interact through van der Waals contacts with four tetrahedrally disposed alkyl

(12) Foye, W. D. *Principles of Medicinal Chemistry*; Lea and Febiger: Philadelphia, 1989; pp 323–341.
(13) Belleau, B.; Puranen, J. *J. Med. Chem.* **1963**, *6*, 325.
(14) Burgen, A. S. W. *Trends Pharmacol. Sci.* **1989**, *10* (Suppl. Subtypes Muscarinic Receptor IV), 1.
(15) Patai, S. *The Chemistry of Amidine and Imidate*, Wiley Interscience: New York, 1975; pp 11–18.
(16) Vermouth, C. G.; Biziere, K. *Trends Pharmac. Sci.* **1986**, *7*, 421.
(17) Van der Brempt, C.; Evrard, G.; Durant, F. *Acta Crystallogr.* **1986**, *C42*, 1206.
(18) SYBYL program, Tripos Associates, St. Louis, MO 63117.
(19) Mazurek, A. P.; Topial, S.; Weinstein, H.; Osman, R. *Int. J. Quantum Chem.* **1983**, *24*, 293.

Table III. "In Vitro" Receptor Binding Studies (K_D[a] × 10^{-9} M)

compound	cerebral rat cortex[b]	rat heart[c]	submandibular rat gland[c]
3a	7 ± 1.5	11 ± 2.8	2.5 ± 0.31
3b	7.5 ± 1.9	23 ± 3.4	4.5 ± 0.78
4a	1.1 ± 0.12	1.6 ± 0.33	1.4 ± 0.15
4b	3.6 ± 0.51	10.0 ± 1.6	5 ± 0.95
5a	2.0 ± 0.27	10.0 ± 0.9	3.1 ± 0.70
5b	3.4 ± 0.44	6.7 ± 1.4	3.5 ± 0.68
atropine (**1a**)	1.3 ± 0.08	2.7 ± 0.6	0.63 ± 0.12
atropine methyl bromide (**1c**)	2.5 ± 0.17	2.7 ± 0.7	2.6 ± 0.24
scopolamine (**1b**)	0.75 ± 0.055	3 ± 0.45	0.65 ± 0.21
scopolamine methyl bromide (**1d**)	1.1 ± 0.2	0.87 ± 0.19	0.44 ± 0.09
scopolamine butyl bromide (**1e**)	300 ± 22	220 ± 19	220 ± 26

[a] See the Experimental Section for the method of determination and calculation. Standard deviations are given following the ± sign. Hill coefficients are not significantly different from 1. [b] Radioligand was [^{3}H]pirenzepine. [c] Radioligand was [^{3}H]NMS.

Table IV. "In Vitro" Functional Studies (K_B[a] × 10^{-9} M)

compound	guinea pig ileum[b]	guinea pig left atrium[b]
3a	1.92 ± 0.07	3.06 ± 0.34
3b	5.18 ± 0.77	16.4 ± 1.23
4a	0.72 ± 0.14	1.59 ± 0.25
4b	1.68 ± 0.13	6.55 ± 0.50
5a	1.61 ± 0.7	3.17 ± 0.20
5b	1.55 ± 0.44	5.02 ± 0.94
atropine (**1a**)	3.16 ± 0.39	0.66 ± 0.04
atropine methyl bromide (**1c**)	0.71 ± 0.10	1.29 ± 0.30
scopolamine (**1b**)	0.4 ± 0.09	0.17 ± 0.10
scopolamine methyl bromide (**1d**)	0.17 ± 0.03	0.38 ± 0.09
scopolamine butyl bromide (**1e**)	56.3 ± 3.86	58.4 ± 1.82

[a] See ref 23 for method of determination and calculation. Standard deviations are given following the ± sign. [b] Agonist bethanechol.

groups holds true.[13] Nevertheless, it was thought worthwhile to investigate amidinium systems when considering additional distinctive features. The driving force for the primary docking of amidinium cations with an anionic counterpart on the receptor is assumed to be an electrostatic interaction giving rise to the formation of a reinforced ionic bond,[20] but the possibilities of hydrogen bonding with surrounding areas of the receptor should not be overlooked. This could considerably influence the positioning of the interacting molecule in preferred orientations.[21] In conclusion, amidine systems offer more opportunities for interactions than traditional systems. It was reasonable to assume that these structural differences could affect affinity for the muscarine receptor subtypes and that the resulting selectivity profile might be therapeutically exploitable.

Biological Results. Binding affinity (K_D) of the new compounds (**3a,b–5a,b**) for m-AcChR subtypes was evaluated on different rat tissue homogenates (cerebral cortex, M_1; heart, M_2; submandibular gland, M_3)[22] (Table III). The compounds were also tested for their ability to antagonize a functional in vitro response (K_B) with guinea pig ileum and left atrium preparations[23] (Table IV). In the radioligand-binding assays, the formamidine (**4a,b**) and acetamidine (**5a,b**) derivatives displayed high affinity for the different muscarine receptor subtypes. Their affinity estimates were comparable to those shown by atropine, scopolamine, and their metho derivatives (**1a–d**). The guanidine derivatives showed somewhat weaker affinity (**3a–b**), particularly **3b**. The findings of the in vitro functional sstudies were consistent with the results from binding experiments. All the compounds behaved as competitive antagonists in inhibiting the contractile response of bethanechol on the guinea pig ileum, their activity being in the same range as those of the reference drugs. Antagonistic activity was lower in the guinea pig left atrium assay, in agreement with the lower binding affinity found in the heart preparation. On the whole, the in vitro results indicated that antagonistic activity was retained after amidine substitution.

Table V. "In Vivo" Activities (ED_{50}, μg/kg, iv, rat)

compound	oxotremorine-induced tremors	oxotremorine-induced salivation	carbachol-induced colonic spasms
3a	>3000	5.8	9.8
3b	>3000	18.8	18.6
4a	>10000	3.9	3.5
4b	1300	2.4	6.1
5a	>10000	6.2	3.3
5b	1700	4.1	3.9
atropine (**1a**)	453	8.6	4.5[a]
scopolamine (**1b**)	11	2.6	5.8
scopolamine butyl bromide (**1e**)	>3000	385	52.9

[a] See ref 24.

The in vitro m-AcChR antagonist activity of the compounds was reflected in the in vivo inhibition of carbachol-induced colonic spasms in the rat[24] (Table V). Compounds **4a**, **5a**, and **5b** showed the best spasmolytic activity within the class (ED_{50} = 3.5, 3.3, and 3.9 μg/kg, respectively). All the compounds were at least 3–10 times more potent than scopolamine *N*-butyl bromide, a clinically effective spasmolytic agent.[25] The low selectivity found among m-AcChR subtypes was reflected in the effectiveness of the compounds in inhibiting the salivation induced by oxotremorine in the rat[26] at the same dose. The compounds' ability to prevent the tremors induced by oxotremorine in the rat[26] was tested as an indication of their CNS-penetrating capacity. Compounds **3a,b**, **4a**, and **5a** up to a dose of 3000 μg/kg did not block oxotremorine's effects, displaying a profile closer to that of the quaternary compound scopolamine N-butyl bromide. Compounds **4b** and **5b** instead did antagonize the tremorigenic effect of oxotremorine, but their ED_{50} were always higher than those of atropine and scopolamine.

Discussion. Replacement of the conventional cationic heads of the tertiary amino or onium type by amidine moieties did not substantially affect receptor affinity in the class of the antimuscarinic substances investigated. The decrease in affinity for the cardiac preparation was very limited, meaning there was no tendency toward selectivity for ileal tissue. The large decrease of free energy following reinforced ionic bond formation (ΔG° = −10 kcal/mol)[27] at the receptor site probably outweighs the expected contribution of other, finer binding interactions,

(20) Walker, J. *J. Chem. Soc.* **1949**, 1996.
(21) Fastier, F. N. *Pharmacol. Rev.* **1962**, *14*, 37.
(22) Hammer, R.; Giraldo, E.; Schiavi, G. B.; Monferini, E.; Ladinsky, H. *Life Sci.* **1986**, *38*, 1653.
(23) Micheletti, R.; Montagna, E.; Giachetti, A. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 628.
(24) Schiavone, A.; Volontè, M.; Sagrada, A. *Arch. Int. Pharm. Ther.* **1989**, *301*, 7.
(25) Bauer, R.; Gross, E.; Scarselli, V.; Stockhaus, K.; Wick, M. *Arzneim.-Forsch.* **1968**, *18*, 1132.
(26) Del Soldato, P.; Pagani, F. *Pharmacol. Res. Commun.* **1982**, *14*, 279.
(27) Korolkovas, A. *Essential of Molecular Pharmacology*; Wiley Interscience: New York, 1970; Chapter 7, pp 137–186.

thus resulting in indiscriminate attachment to the different receptor subtypes. The high percentage of the ionized form present at physiological pH 7.4 accounts for the lack of CNS effects shown by the new amidine compounds. Atropine and scopolamine, depending on their pK_a values (9.60 and 7.55, respectively)[28] have about 1% and 40% of the uncharged form at pH 7.4 and are thus able to prevent oxotremorine-induced tremors in the rat at doses of 450 and 11 μg/kg, respectively. All the new compounds under evaluation were protonated, at least like atropine, with pK_a values ranging from 9.7 to 11.6, which accounts for less than 0.5% of the neutral molecule at pH 7.4. Atropine derivatives (compound **3a–5a**) behave as quaternary compounds in being unable to antagonize oxotremorine's effects up to 3000 μg/kg. Among the scopolamine derivatives, the guanidine derivative **3b** mimicked the quaternary structures, whereas the formamidine and acetamidine derivatives **4b** and **5b** lay between the tertiary amino compounds atropine (**1a**) and scopolamine (**1b**) and the quaternary derivative scopolamine *N*-butyl bromide (**1e**) in being able to block oxotremorine's effects at high but definite doses (1300 and 1700 μg/kg, respectively). It is likely that in this class of compounds the hydrogen-bond-forming capacity, beside the extent of protonation, plays a role in limiting access to the CNS. The observed rank order of potencies in inhibiting oxotremorine's CNS effects was tertiary amine > acetamidine > formamidine > guanidine ≥ onium compounds. Although the new compounds **3a,b–5a,b** had good spasmolytic activity, they blocked the salivation induced by oxotremorine in the same dose range. Therefore, separation of the desired spasmolytic activity from this untoward effect typical of conventional nonselective antimuscarinic agents was not achieved.

In conclusion, amidine moieties prove to be good bioisosteric substitutes for conventional basic centers of antimuscarinic substances. Physical–chemical properties associated with these moieties impart partition and ionic properties to the substances intermediate between those of tertiary amines and onium compounds. As a consequence, the present chemical manipulation may be of use in cases in which a modulation of bioavailability and pharmacokinetic profile of a substance is required.

Experimental Section

Chemistry. Noratropine[9] (**2a**), norscopolamine[10] (**2b**), ethyl formimidate hydrochloride,[29] and ethyl acetimidate hydrochloride[30] were prepared according to described procedures. Compounds **1a–d**, oxotremorine, carbachol, and bethanechol were purchased from Sigma Chemical Co., St. Louis, MO. Scopolamine *N*-butyl bromide and QNB were provided by Boehringer Ingelheim and K. Thomae Biberach, respectively. [^{3}H]NMS, 87 Ci/mmol and [^{3}H]PZ, 82.3 Ci/mmol were purchased from NEN, Boston, MA. Melting points were taken on a Büchi capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 337 spectrophotometer. ^{1}H NMR were recorded on a Varian CFT-20 spectrometer, operating at 80 MHz in the indicated solvent. Chemical shifts are reported as values relative to tetramethyl silane as internal standard. Mass spectra were recorded on a Finnigan 1020 spectrometer equipped with a CI source. TLC were performed with silica gel 60 GF 254 precoated plates (E. Merck, A.G. Darmstadt, F.R.G.) in the following systems: A, methylene dichloride–methanol–water 80:20:2; B, methylene dichloride–methanol–water 85:15:1.5; C, methylene dichloride–methanol–acetic acid 80:20:2. All the compounds were analyzed for C, H, N, and Cl. The analytical results were within ±0.4% of the theoretical values.

***N*-Amidinonoratropine Hydrochloride (3a).** A solution of noratropine hydrochloride (7.0 g, 22 mmol) and cyanamide (1.8 g, 42 mmol) in water was heated at 100 °C for 4 h. Water was then distilled off and the crude residue was purified by a flash chromatography technique on silica gel (system A, R_f 0.45) to give 2.9 g of the pure title compound as a hydrochloride salt: mp 78–80 °C dec (after lyophilization); ^{1}H NMR (Me_2SO-d_6–$CDCl_3$) δ 7.51 (br, 4 H), 7.32 (s, 5 H), 5.02 (br, 1 H), 4.43 (br, 2 H), 4.13 (br, 1 H), 3.58–4.12 (m, 3 H), 1.3–2.4 (br, 8 H); MS (CI) *m/e* 318 [M + H]. Anal. ($C_{17}H_{24}ClN_3O_3$) C, H, N, Cl.

(–)-*N*-Amidinonorscopolamine Hydrochloride (3b). This compound was similarly prepared starting from (–)-norscopolamine (8.2 g, 25.2 mmol), cyanamide (2.10 g, 50 mmol), and water (5 mL). From the crude residue after purification by a flash chromatography technique on silica gel (system B, R_f 0.25) 4.4 g of the pure title compound was obtained as a hydrochloride salt: mp 160–161 °C dec (from acetone); $[\alpha]_D$ = –26.09° (*C* 2% in water); ^{1}H NMR (Me_2SO-d_6–$CDCl_3$) δ 7.49 (br, 4 H), 7.27 (s, 5 H), 4.94 (br, 1 H), 4.44 (br, 2 H), 3.5–4.13 (m, 4 H), 3.25 (d, 1 H, *J* = 2 Hz), 1.5–2.4 (m, 4 H); MS (CI) *m/e* 332 [M + H]. Anal. ($C_{17}H_{22}ClN_3O_4$) C, H, N, Cl.

***N*-(Iminomethyl)noratropine Hydrochloride (4a).** A solution of freshly prepared ethyl formimidate hydrochloride (1.2 g, 10.8 mmol) and noratropine (3.0 g, 10.8 mmol) in absolute ethanol (15 mL) was stirred at room temperature for 48 h. The reaction mixture was evaporated to dryness, and from the crude residue after flash chromatography on silica gel (system C, R_f 0.35), 1.5 g of the pure title compound was obtained as a hydrochloride salt: mp 202–203 °C dec (from diethyl ether); ^{1}H NMR (D_2O) δ 7.87 (br, 1 H), 7.40 (s, 5 H), 5.08 (br, 1 H), 3.8–4.5 (m, 5 H), 1.5–2.4 (m, 8 H); MS (CI) *m/e* 303 [M + H]. Anal. ($C_{17}H_{23}ClN_2O_3$) C, H, N, Cl.

(–)-*N*-(Iminomethyl)norscopolamine Hydrochloride (4b). Freshly prepared ethyl formimidate hydrochloride (1.9 g, 17.4 mmol) was added portionwise to a well-stirred solution of (–)-norscopolamine (4.2 g, 14.5 mmol) in acetonitrile (85 mL) and methanol (30 mL). Stirring was continued for 7 h, then the reaction mixture was evaporated to dryness and 2.5 g of the pure title compound, as a hydrochloride salt, was obtained by recrystallization of the crude material: mp 196–197 °C dec (from ethanol); $[\alpha]_D$ = –25.4° (*C* 2% in water); ^{1}H NMR (Me_2SO-d_6–$CDCl_3$) δ 8–10 (br, 2 H), 8.17–8.19 (2 s, 1 H), 7–7.8 (s, 5 H), 5.03 (br, 2 H), 4.45 (br, 1 H), 4–6 (br, 1 H), 3.4–4.2 (m, 4 H), 3.2 (br d, 1 H), 1.6–2.6 (m, 4 H); MS (CI) *m/e* 317 [M + H]. Anal. ($C_{17}H_{21}ClN_2O_4$) C, H, N, Cl.

***N*-(Iminoethyl)noratropine Hydrochloride (5a).** A solution of ethyl acetimidate (1.35 g, 10.9 mmol) and noratropine (3.0 g, 10.8 mmol) in absolute ethanol (15 mL) was stirred overnight at room temperature. The reaction mixture was evaporated to dryness and the crude residue was purified by a flash chromatography technique on silica gel (system A, R_f 0.3) to give 1.8 g of the pure title compound as a hydrochloride salt: mp 205–206 °C dec (from diethyl ether); ^{1}H NMR (Me_2SO-d_6–$CDCl_3$) δ 7.34 (s, 5 H), 5.0 (br, 1 H), 4.75 (br, 1 H), 4.33 (br, 1 H), 4–6 (br, 3 H), 3.59–4.08 (m, 3 H), 2.32 (s, 3 H), 1.5–2.6 (m, 8 H); MS (CI) *m/e* 317 [M + H]. Anal. ($C_{18}H_{25}ClN_2O_3$) C, H, N, Cl.

(–)-*N*-(Iminoethyl)norscopolamine (5b). A solution of ethyl acetimidate (0.47 g, 5.4 mmol) and (–)-norscopolamine hydrochloride (1.6 g, 4.9 mmol) in acetonitrile (16 mL) and absolute ethanol (10 mL) was stirred at room temperature for 48 h. The reaction mixture was evaporated to dryness, and from the crude residue after purification by a flash chromatography technique (system A, R_f 0.23), 65 mg of the pure title compound was obtained as a hydrochloride salt: mp 186–185 °C dec (from diethyl ether); $[\alpha]_D$ = –25.3° (*C* 2% in water); ^{1}H NMR (Me_2SO-d_6–$CDCl_3$) δ 9.44 (br, 1 H), 9.03 (br, 1 H), 7.27 (s, 5 H), 4.94 (br t, 1 H), 4.77 (br, 1 H), 4.42 (br, 1 H), 4.16 (s, 1 H), 3.5–4.0 (m, 4 H), 3.34 (2 d, 1 H, *J* = 4.5 Hz), 2.24–2.27 (2 s, 3 H), 1.5–2.5 (m, 4 H); MS (CI) *m/e* 331 [M + H]. Anal. ($C_{18}H_{23}ClN_2O_4$) C, H, N, Cl.

Molecular Modeling. Molecular modeling studies were performed with an Evan and Sutherland PS 390 color raster graphics terminal coupled to a VAX 8250 computer. The model

(28) The pK_a values of atropine and norscopolamine have been reported: Albert, A.; Serjeant, E. P. *Ionisation Constants of Acid and bases*; Methuen and Co. Ltd.: London, 1981; pp 166–175 and 1–68.

(29) Ohme, R.; Schmitz, E. *Angew Chem. Int. Ed. Engl.* **1967**, *6*, 566.

(30) Reynand, P.; Moreau, R. C. *Bull. Soc. Chem. Fr.* **1964**, 2997.

compounds (Table II) were built, displayed, and manipulated by using the SYBYL program[18] (version 5.2.2). As the model compounds under investigation were charged species, the geometry optimization and the minimum energy conformations were calculated by using AMPAC[31] within SYBYL. The van der Waals volumes were calculated by using MVolume in SYBYL.

Determination of Macroscopic Ionization Constants. Macroscopic ionization constants were determined by potentiometric titration in water containing a few milliliters of methanol, according to the method of Albert and Serjeant,[28] by using a pHM Toptronic apparatus, equipped with a DG-111 Mettler electrode.

Biochemistry. Muscarinic Binding Assay.[22] Tissue Preparation. Rat tissues were removed, cleaned, homogenized (w/v: cerebral cortex, 1:80; whole heart, 1:130; submandibular salivary glands, 1:100) with an Ultra-Turrax at maximal speed for 30 s, followed by use of a Potter–Elvehjem homogenizer (30 strokes), in Na^+–Mg^{2+}-HEPES buffer, pH 7.4 (mM: NaCl, 100; $MgCl_2$, 10; HEPES, 20), and filtered through two layers of cheesecloth.

Binding Experiments. Binding curves for the different compounds were derived indirectly from competition experiments against 0.5 nM [^{3}H]-pirenzepine labeling the cerebral cortex m-AcChR and 0.3 nM [^{3}H]NMS for the m-AcChR of the heart and submandibular glands. A 1-mL portion of homogenate was incubated for 45 min at 30 °C in the presence of the marker ligand and different concentrations of the cold ligand. The incubation was terminated by centrifugation (12000 rpm for 3 min) at room temperature with an Eppendorf microcentrifuge. The resultant pellet was washed twice with 1.5 mL of saline to remove the free radioactivity and the final pellet was allowed to drain. The tips of the tubes containing the pellet were cut off and 200 μL of tissue solubilizer (Lumasolve, Lumac) was added and left to stand overnight. Radioactivity was then counted after addition of 4 mL of liquid scintillation solution (Lipolumo, Lumac). Assays were carried out in triplicate and the nonspecific binding was defined as the radioactivity bound or entrapped in the pellet when the incubation medium contained 1 μM 3-quinuclidinyl benzilate racemic mixture (QNB) in [^{3}H]NMS experiments and 1 μM atropine sulfate in [^{3}H]pirenzepine (PZ) experiments. Nonspecific binding averaged less than 10% and 3%, respectively. The corrected IC_{50} values or dissociation constants (K_D), were obtained by nonlinear regression analysis on the basis of a one or two binding site model with a Topfit pharmacokinetic for the radioligand occupancy shift with the equation of Cheng and Prusoff:[32] $K_D = IC_{50}/1 + {}^*C/{}^*K_D$, where $^*C$ represents the concentration and $^*K_D$ the dissociation constant of the radioligand used. Hill coefficients (nH) were calculated by linear-regression analysis and assessed for statistically significant deviation from unity (Student's t test).

Pharmacology. In Vitro Functional Tests. The antagonist affinities of the compounds on guinea pig ileal and left atrial muscarinic receptors were evaluated as described by Micheletti et al.[23]

Inhibition of Colon Spasms.[24] Male Sprague–Dawley rats (250–275 g, fasted overnight) were anesthetized with urethane (1.2 g/kg ip). After cannulation of the trachea and jugular vein, a small latex balloon was inserted through a midline abdominal incision into the colon 2–3 cm below the cecum. A polyethylene cannula (PE 50) was inserted into the abdominal aorta and pushed until its tip reached the superior mesenteric artery. The distal part of the aorta was occluded at the level of the common iliac arteries. Contractions of the colon were induced by intraaortic injection of carbachol (10 μg/kg) at 6-min intervals and recorded by means of a pressure transducer coupled to a chart recorder. Compounds were injected intravenously 2 min before each dose of spasmogen. The effect of the drugs was expressed as percent inhibition of pretreatment contractions and ED_{50} (dose causing 50% reduction of contractions), and 95% confidence limits were calculated by linear-regression analysis of the log dose–response curves.

Inhibition of Salivation and Tremors.[26] Tremors and salivation were induced in female albino rats (175–200 g, fasted for 24 h) by oxotremorine (100 μg/kg iv in the salivation test and 80 μg/kg in the tremors test). Antimuscarinic agents were administered (iv) immediately before oxotremorine. All were dissolved in 0.9% NaCl solution and the control rats were given the same amount of vehicle. After oxotremorine the animals were observed for 15 min by an investigator unaware of the treatment given. Results were expressed as percentages of animals without the symptoms of interest. ED_{50} (i.e. the dose protecting 50% of the animals from symptoms) was calculated according to the Litchfield and Wilcoxon procedure.[33]

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Registry No. **1a**, 51-55-8; 1b, 51-34-3; **1c**, 2870-71-5; **1d**, 155-41-9; **1e**, 149-64-4; **2a**, 16839-98-8; **2a**·HCl, 75559-01-2; **2b**, 4684-28-0; **3a**, 124064-71-7; **3a** (free base), 127571-29-3; **3b**, 124064-72-8; **3b** (free base), 127641-36-5; **4a**, 124065-23-2; **4a** (free base), 127571-30-6; **4b**, 124065-21-0; **4b** (free base), 127642-57-3; **5a**, 124065-42-5; **5a** (free base), 127571-31-7; **5b**, 127641-37-6; **5b**·HCl, 124065-43-6; H_2NCN, 420-04-2; $C_2H_5OCH{=}NH{\cdot}HCl$, 16694-46-5; $C_2H_5OC(CH_3){=}NH{\cdot}HCl$, 2208-07-3.

(31) AMPAC was developed by Dewar, M. J. S., University of Texas, Houston, TX, and Stewart, J. J. P., Seiler Research Labs, U.S. Air Force Academy, Colorado Spring, CO (AMPAC program is also available from the Quantum Chemistry Program Exchange).

(32) Cheng, Y. C.; Prusoff, N. H. *Biochem. Pharmacol.* **1973**, *22*, 3095.

(33) Litchfield, J. T.; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.