3-(2-Benzofuranyl)quinuclidin-2-ene Derivatives: Novel Muscarinic Antagonists†

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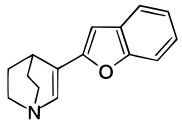
A series of 26 derivatives of the novel muscarinic antagonist 3-(2-benzofuranyl)quinuclidin-2-ene (**1**) has been synthesized and evaluated for muscarinic and antimuscarinic properties. The affinity of the compounds was determined by competition experiments in homogenates of cerebral cortex, heart, parotid gland, and urinary bladder from guinea pigs using (−)-[^{3}H]-3-quinuclidinyl benzilate as the radioligand, and the antimuscarinic potency was determined in a functional assay on isolated guinea pig urinary bladder using carbachol as the agonist. The 5-fluorobenzofuranyl derivative was slightly more potent than **1**. The 7-bromo-substituted **8** displayed a 14-fold tissue selectivity ratio for muscarinic receptors in the cortex versus the parotid gland. Comparative molecular field analysis and quantitative structure−activity relationship models were developed for this series of substituted benzofuranyl derivatives.

Introduction

Considerable research efforts have been devoted to the development of centrally active muscarinic agonists as a potential remedy for Alzheimer's disease.[1] Attempts have also been made to develop novel subtype-selective muscarinic antagonists; M_2 receptor-selective drugs might be beneficial in the treatment of heart disorders[2] and possibly also in the therapy of Alzheimer's disease by acting as antagonists at autoreceptors in the central nervous system (CNS).[3] Furthermore, the use of antagonists with selectivity for M_1 and M_3 receptors in the trachea has been suggested in the treatment of lung disorders,[4] and antagonists with selectivity for muscarinic receptors in the bladder would be useful in the treatment of incontinence.[5] However, fairly few receptor subtype-selective muscarinic antagonists have been reported, and, in addition, their selectivity is moderate.[6] Therefore, novel muscarinic antagonists with increased subtype selectivity are of interest. We have previously reported[7] a number of achiral muscarinic antagonists with structures related to previously published chiral quinuclidine-derived agonists.[8] The most potent compound was 3-(2-benzofuranyl)quinuclidin-2-ene (**1**).



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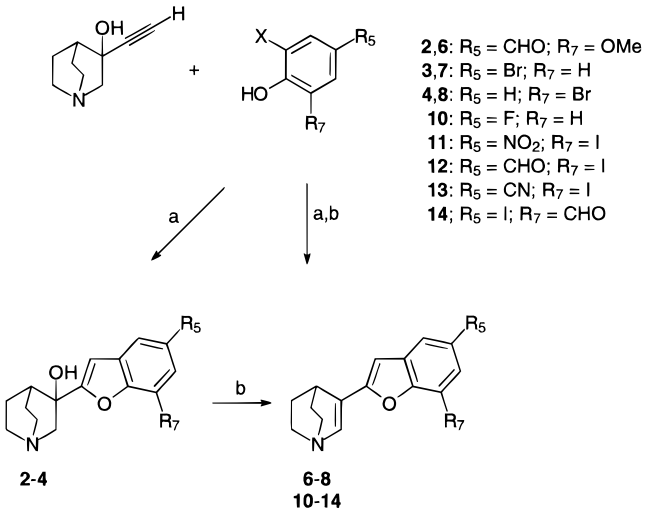
We now present a series of 3-, 5-, and 7-substituted derivatives of **1**. The compounds were investigated for their ability to displace (−)-[^{3}H]-3-quinuclidinyl benzilate [(−)-[^{3}H]QNB] from muscarinic receptors in cerebral cortex, heart, parotid gland, and urinary bladder from guinea pigs. In addition, the antimuscarinic potencies were evaluated in a functional assay on the isolated guinea pig urinary bladder.

Scheme 1[a]



[a] Reagents: (a) Cu_2O, pyridine; (b) HCOOH, 100 °C.

Chemistry

Synthesis. The syntheses of the derivatives of **1** are outlined in Schemes 1−5. Physical data of intermediates and test compounds are presented in Table 1. 3-(2-Benzofuranyl)quinuclidin-3-ol derivatives **2**−**5** (Schemes 1 and 2) were synthesized by using the Stephens−Castro reaction (or Castro cyclization).[9−11] This procedure provides an efficient method for substituted benzofuran derivatives from readily available starting materials. Thus, 3-ethynylquinuclidin-3-ol[12] and the appropriate ortho-halogenated phenol were heated with Cu_2O in pyridine (method I).[13] Dehydration of **2**−**4** to the corresponding quinuclidin-2-ene derivatives **6**−**8** was accomplished by heating in concentrated formic acid (method II).[14] However, furo[3,2-*b*]pyridinyl derivative **5**, which was prepared from 3-hydroxy-2-iodopyridine, could not be dehydrated under these conditions and was instead heated in concentrated methanesulfonic acid at 200 °C to produce **9** (Scheme 2).[7,15] Compounds **10**−**14** were prepared without isolation of the intermediate alcohol (method III).

The 3-bromobenzofuran derivative **16** was prepared by a conceptually different route (Scheme 3) since it was inaccessible by the Stephens−Castro procedure. 3-Bro-

† Dedicated to Professor Ernst Mutschler on the occasion of his 65th birthday.
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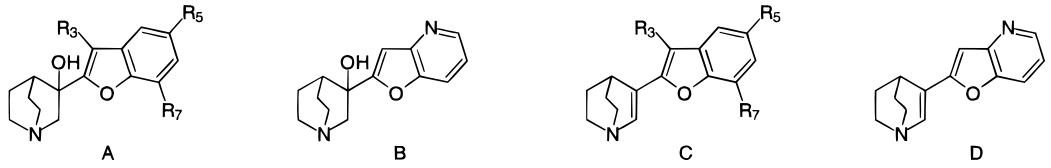
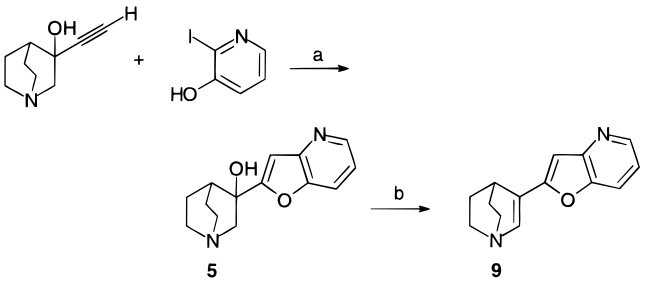
Table 1. Yields and Physical Data of Intermediates and Compounds Tested



compd	general structure	R_3	R_5	R_7	prepn method[a]	yield, %	mp, °C	recrystn solvents[b]	formula
2	A	H	CHO	OMe	I	84	227−228	A	$C_{17}H_{19}NO_4$
3	A	H	Br	H	I	63	244−245	B	$C_{15}H_{16}BrNO_2$
4	A	H	H	Br	I	54	210−211	C	$C_{15}H_{16}BrNO_2$
5	B	H	H	H	I	53	203−204	A	$C_{14}H_{16}N_2O_2$
6	C	H	CHO	OMe	II	86	220 dec	D	$C_{17}H_{17}NO_3 \cdot (COOH)_2$
7	C	H	Br	H	II	96	168−169	D	$C_{15}H_{14}BrNO \cdot (COOH)_2 \cdot 0.25H_2O$
8	C	H	H	Br	II	93	237−239	D	$C_{15}H_{14}BrNO \cdot HCl$
9	D	H	H	H	*a*	58	212 dec	D	$C_{14}H_{14}N_2O \cdot (HCl)_2$
10	C	H	F	H	III	6	238−240	D	$C_{15}H_{14}FNO \cdot HCl$
11	C	H	NO_2	I	III	47	255 dec	D	$C_{15}H_{15}IN_2O_3 \cdot HCl$
12	C	H	CHO	I	III	64	270 dec	E	$C_{16}H_{14}INO_2 \cdot HCl$
13	C	H	CN	I	III	18	255 dec	D	$C_{16}H_{13}IN_2O \cdot HCl \cdot 0.67H_2O$
14	C	H	I	CHO	III	54	210 dec	F	$C_{16}H_{14}INO_2 \cdot (COOH)_2$
15	A	Br	H	H	*a*	91	157−159	G	$C_{15}H_{16}BrNO_2$
16	C	Br	H	H	II	82	dec	F	$C_{15}H_{14}BrNO \cdot HCl$
17	C	H	NO_2	H	IV	65	237 dec	F	$C_{15}H_{14}N_2O_3 \cdot HCl$
18	C	H	CHO	H	IV	87	270 dec	F	$C_{16}H_{15}NO_2 \cdot HCl$
19	C	H	CN	H	IV	75	255 dec	F	$C_{16}H_{14}N_2O \cdot HCl$
20	C	H	H	CHO	IV	79	255 dec	F	$C_{16}H_{15}NO_2 \cdot HCl \cdot 0.33H_2O$
21	C	H	CH_2OH	OMe	V	99	203−204	D	$C_{17}H_{19}NO_3 \cdot (COOH)_2$
22	C	H	CH_2OH	I	V	99	245 dec	D	$C_{16}H_{16}INO_2 \cdot HCl$
23	C	H	I	CH_2OH	V	82	233−235	D	$C_{16}H_{16}INO_2 \cdot 0.5(COOH)_2$
24	C	H	CH_2OH	H	V	98	230 dec	F	$C_{16}H_{17}NO_2 \cdot HCl$
25	C	H	H	CH_2OH	V	91	235 dec	F	$C_{16}H_{17}NO_2 \cdot HCl$
26	C	CH_3	H	H	VI	75	246 dec	F	$C_{16}H_{17}NO \cdot HCl$
27	C	H	CH_3	H	*a*	53	241−243	F	$C_{16}H_{17}NO \cdot HCl$
28	C	H	H	CH_3	VI	83	209−210	H	$C_{16}H_{17}NO \cdot HCl$
29	C	Ph	H	H	VII	65	241−243	F	$C_{21}H_{19}NO \cdot HCl$
30	C	H	Ph	H	VII	99	242−244	F	$C_{21}H_{19}NO \cdot HCl$
31	C	H	H	Ph	VII	99	240−242	F	$C_{21}H_{19}NO \cdot HCl$
32	C	H	NH_2	H	*a*	73	280 dec	I	$C_{15}H_{16}N_2O \cdot (HCl)_2 \cdot 0.33H_2O$

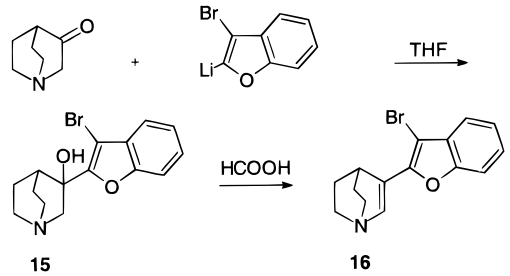
[a] See the Experimental Section. [b] A, ethyl acetate; B, chloroform; C, ethyl acetate−MeOH; D, MeOH−ether; E, acetonitrile−MeOH−ether; F, acetonitrile; G, ethyl acetate−light petroleum; H, acetonitrile−ether; I, acetonitrile−MeOH.

Scheme 2[a]



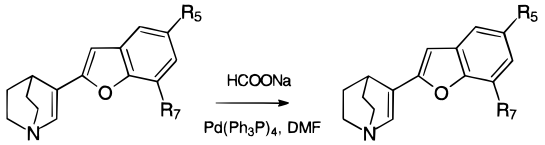
[a] Reagents: (a) Cu_2O, pyridine; (b) methanesulfonic acid, 200 °C.

Scheme 3



mo-2-lithiobenzofuran,[16] formed by treatment of 3-bromobenzofuran with lithium diisopropylamide, was quenched with 3-quinuclidinone. The resulting alcohol **15** was dehydrated in concentrated formic acid (method II) to afford **16**.

Scheme 4

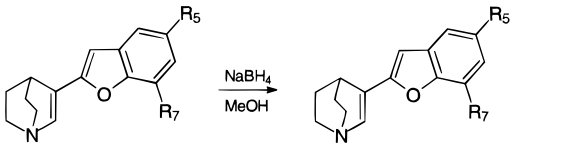


11: $R_5 = NO_2$; $R_7 = I$
12: R_5 = CHO; $R_7 = I$
13: R_5 = CN; $R_7 = I$
14: R_5 = I; R_7 = CHO

17: $R_5 = NO_2$; $R_7 = H$
18: R_5 = CHO; $R_7 = H$
19: R_5 = CN; $R_7 = H$
20: R_5 = H; R_7 = CHO

The substituted 3-(2-benzofuranyl)quinuclidin-2-ene derivatives were converted into additional compounds by use of a number of functional group transformations. Dehalogenation of the aromatic iodides **11**−**14** to the corresponding protium analogues **17**−**20** was accomplished by palladium-catalyzed reduction using sodium formate as proton donor (method IV; Scheme 4).[17] The formyl-substituted compounds **6**, **12**, **14**, **18**, and **20** were reduced by treatment with $NaBH_4$ in MeOH to give **21**−**25**, respectively (method V; Scheme 5). Methyl and phenyl substituents were introduced in the 3-, 5-, and 7-positions of the benzofuran ring by submitting the bromo-substituted derivatives **16**, **7**, and **8** to palladium-catalyzed Stille[18] and Suzuki[19] type coupling reactions, with tetramethyltin and phenylboronic acid, respectively (methods VI and VII; Scheme 6). This procedure produced the methyl-substituted derivatives **26** and **28** and phenyl-substituted derivatives **29**−**31**. The 5-methyl-substituted benzofuran derivative **27** was prepared

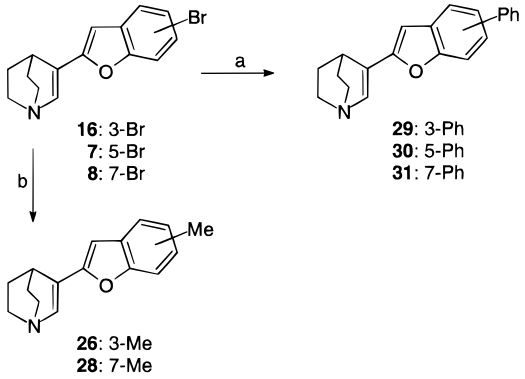
Scheme 5



6: R_5 = CHO; R_7 = OCH_3
12: R_5 = CHO; R_7 = I
14: R_5 = I; R_7 = CHO
18: R_5 = CHO; R_7 = H
20: R_5 = H; R_7 = CHO

21: R_5 = CH_2OH; R_7 = OCH_3
22: R_5 = CH_2OH; R_7 = I
23: R_5 = I; R_7 = CH_2OH
24: R_5 = CH_2OH; R_7 = H
25: R_5 = H; R_7 = CH_2OH

Scheme 6[a]



16: 3-Br
7: 5-Br
8: 7-Br

29: 3-Ph
30: 5-Ph
31: 7-Ph

26: 3-Me
28: 7-Me

[a] Reagents: (a) phenylboronic acid, $Pd(Ph_3P)_4$, Na_2CO_3, DME; (b) $(CH_3)_4Sn$, $Pd(OAc)_2$, tri-*o*-tolylphosphine, DMF.

from the corresponding 5-hydroxymethyl derivative **24** by treatment with trifluoroacetic acid and triethylsilane.[20,21] The 5-nitro substituent of **17** was reduced to an amino group, producing **32**, using $SnCl_2$/HCl as reducing agent.

Pharmacological Results and Discussion

Receptor binding affinities (K_i; Table 2) of the 3-(2-benzofuranyl)quinuclidin-2-ene[22] derivatives for muscarinic receptors in the cerebral cortex, heart, parotid gland, and urinary bladder from guinea pigs were indirectly determined by competition experiments with the radioligand (−)-[^{3}H]QNB (3-quinuclidinyl [*phenyl*-4-^{3}H]benzilate).[23−26] Antimuscarinic potencies (K_B; Table 2) were evaluated by functional *in vitro* studies on isolated guinea pig bladder, using carbachol as the agonist. In the presence of antagonist, the concentration−response curves to carbachol were shifted in parallel toward higher concentrations, but the maximal responses remained unaffected. Thus, the inhibition seemed to be competitive since it always could be overcome by an increase in the carbachol concentration. None of the compounds exhibited any muscarinic agonist activity in the isolated urinary bladder when tested in concentrations of 10−1000 μM.

The following effects on the receptor affinity for muscarinic receptors in cortex of the various substitutions in **1** were apparent: Substituents in the 3-position had a limited effect on the affinity, lowering it 2−4-fold. Introduction of a fluoro substituent in the 5-position of the benzofuran ring (**10**) increased the affinity slightly. All other 5-substituted compounds displayed lower affinity than the unsubstituted **1**. The 5-hydroxymethyl-substituted **24** had 140-fold lower affinity than **1**.

Introduction of substitutents in the 7-position generally caused less reduction in affinity. A 7-methyl or bromo substituent lowered the affinity for muscarinic receptors about 2−3-fold, and other substituents produced a more significant reduction in affinity. Changing the heteroaromatic ring system of **1** to furo[3,2-*b*]-pyridine (**9**) reduced the affinity 2-fold. Since this does not affect the size of the ring system, the affinity may be reduced due to the increased hydrophilicity and/or different electronic properties.

All of the 5,7-disubstituted compounds exhibited moderate to low affinity for muscarinic receptors. Interestingly, when introducing a 7-iodo substituent in the

Table 2. Affinities (K_i) for Muscarinic Receptors, Determined by Competition Experiments with (−)-[^{3}H]QNB, and Functional *in Vitro* Data (K_B), Determined on Isolated Urinary Bladder Strips from Guinea Pig vs Carbachol[a]

	K_i, nM				K_B, nM
compd	cerebral cortex	heart	parotid gland	urinary bladder	urinary bladder
1[b]	9.6 ± 0.1	31 ± 4	59 ± 4	67 ± 15	33 ± 5
6	1170 ± 10	2040 ± 30	7600 ± 340	3900 ± 800	nd[c]
7	39 ± 3	86 ± 1	109 ± 24	156 ± 1	178 ± 50
8	15 ± 2	44 ± 5	216 ± 55	97 ± 2	123 ± 18
9	21 ± 1.7	61 ± 2	110 ± 26	83 ± 22	nd
10	6.3 ± 0.4	34 ± 9	30 ± 8	37 ± 5	nd
11	>150	≈1900	>1000	≈1600	nd
12	221 ± 11	517 ± 29	1300 ± 260	1000 ± 300	nd
13	440 ± 150	nd	nd	nd	nd
14	358 ± 22	314 ± 17	2300 ± 270	600 ± 130	nd
16	40 ± 0.2	106 ± 21	161 ± 38	116 ± 8	nd
17	139 ± 0.4	460 ± 41	515 ± 12	nd	nd
18	364 ± 38	920 ± 154	1571 ± 14	1300 ± 270	nd
19	60 ± 0.6	160 ± 11	236 ± 0.8	nd	nd
20	648 ± 0.3	1612 ± 53	3023 ± 84	1700 ± 150	nd
21	3665 ± 52	5400 ± 150	≈20000	9400 ± 200	≈10000
22	197 ± 2	462 ± 52	970 ± 200	802 ± 2	nd
23	480 ± 60	1100 ± 200	2700 ± 300	2460 ± 40	nd
24	1400 ± 130	1840 ± 70	10700 ± 1600	2900 ± 600	13400 ± 450
25	330 ± 6	1360 ± 60	1024 ± 6	2000 ± 30	nd
26	25.3 ± 0.8	77.8 ± 0.1	204 ± 52	nd	nd
27	116 ± 1	238 ± 8	295 ± 12	364 ± 39	nd
28	25 ± 2	76 ± 15	76 ± 6	nd	nd
29	20 ± 2	72 ± 7	56 ± 4	107 ± 8	148 ± 8
30	289 ± 4	376 ± 24	410 ± 61	332 ± 7	nd
31	135 ± 0.7	260 ± 27	410 ± 80	330 ± 60	nd
32	212 ± 22	837 ± 27	683 ± 8	nd	nd

[a] Values are means ± SEM of two to three experiments performed in triplicate. [b] Data are taken from ref 7. [c] nd = not determined.

5-hydroxymethyl derivative **24**, the affinity increased 7-fold (**22**), whereas when introduced in the 5-cyano derivative **19**, the affinity was reduced 7-fold (**13**). The other iodo-substituted derivatives showed only marginal change in affinity compared to the corresponding desiodo derivative.

It is noteworthy that the 7-bromo-substituted **8** displayed 14-fold selectivity for muscarinic receptors in cortex versus parotid gland. This selectivity ratio is similar to that previously found for pirenzepine (8-fold).[27] None of the other benzofuran derivatives displayed significant tissue selectivity. Since all compounds except **8** showed limited selectivity, we used only receptor affinities for cortex in the structure−activity relationship (SAR) analysis.

Structure−Activity Relationships. This set of substituted benzofuran derivatives showed moderate affinities for muscarinic receptors. In order to be able to understand the molecular properties that affect the affinity, we chose to use two different methods: traditional quantitative structure−activity relationship (QSAR) and comparative molecular field analysis (CoMFA).[28] QSAR is attractive in this case since a large number of descriptors are available for aromatic substituents and because it is straightforward to calculate additional descriptors. The CoMFA method appealed to us because the compounds are conformationally restricted, and this makes the alignment less ambiguous. CoMFA has the advantage of being able to visualize regions in 3D-space where substituents would increase or decrease the affinity.

The QSAR analysis was performed on 22 of the substituted benzofuran derivatives. Only the 5- and 7-substituted compounds and the unsubstituted **1** were included in the analysis. The 3-substituted derivatives were omitted due to their limited numbers. In addition, **9** was omitted because it contains a different heterocycle, and **11** was excluded because of lack of exact biological data. The properties of the substituents were decribed by using π,[29] σ-meta, σ-para, molar refractivity (MR),[29] and verloops steric descriptors (B1, B5, and L).[30] In addition 3-21 G* *ab initio* calculations were performed on these compounds using SPARTAN 3.1.[31] The keyword QSAR was used in the calculations to generate a number of molecular descriptors. These included HOMO and LUMO energies, electronegativity, hardness,[32] molecular volume, surface area, ovality,[33] lipophilicity, and dipole moment. Also included were the atom charges of the aromatic atoms in the benzofuran ring which were based on fits to the electrostatic potential. The matrix, containing in total 32 descriptors, was evaluated in SIMCA-S using PLS.[34] The cross-validated r^2 (r^2_{cv}) was used to evaluate the models. The derived model gave a r^2 of 0.56 but a r^2_{cv} of only 0.27 (one principal component). To improve the model, the number of variables was reduced by selecting only the variables with a VIP number[35] larger than 1.0. In addition π-5, π-7, and the SPARTAN-calculated log *P* were selected since the it has been suggested that lipophilicity is important for muscarinic antagonism.[36] This procedure reduced the number of variables to 15 (π-5,[37] MR-5, B1-5, B5-5, L-5, π-7, B5-7, L-7, ovality, surface area, molecular volume, log *P*, electrostatic charge at C7, LUMO energy, and electronegativity). The resulting model gave the same r^2 but showed an increase in r^2_{cv} to 0.40 using one principal component.

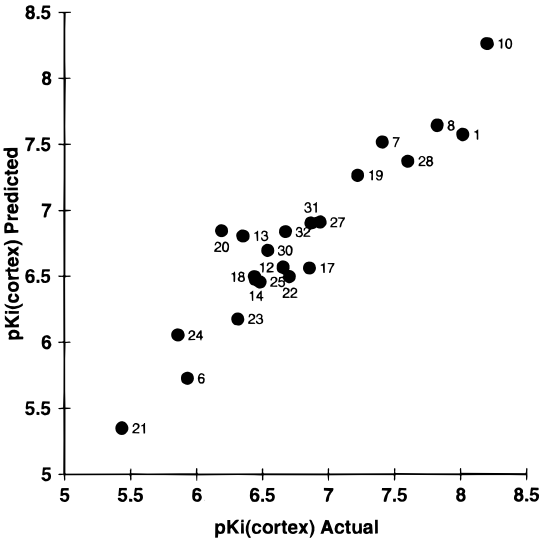


Figure 1. Plot of actual versus predicted affinities derived from the QSAR model.

By including the square of the ovality descriptor, the model improved considerably ($r^2 = 0.87$ and $r^2_{cv} = 0.67$, two principal components). An interaction between the substituents in the 5- and 7-positions is not unlikely. Therefore we also included the cross-term between π-5 and L-7 which gave an additional slight improvement to yield a model with $r^2 = 0.88$ and $r^2_{cv} = 0.71$ using two principal components (Figure 1).[38] In addition, a PLS model for only the mono-5-substituted benzofuran derivatives using π, MR, σ-meta, σ-para, L, B1, and B5 as descriptors produced a good model ($r^2 = 0.92$ and $r^2_{cv} = 0.82$).[39]

The QSAR analysis indicates that the receptor affinity for cortex of the benzofuran analogues is positively influenced by the lipophilicity of the substituent in both the 5- and 7-positions but negatively influenced by the size of the substituents in these positions as decribed by a number of size descriptors. The electronic properties of the aromatic ring appear to be of less importance, but the models suggest that they have some influence on the affinity as judged from the negative influence of LUMO, electronegativity, and charge at C7. The QSAR model suggests that for optimum cortical muscarinic affinity, the benzofuran ring should be substituted with small, lipophilic substituents in the 5- and 7-positions.

The CoMFA was performed on the same set of compounds. Also in this study **11** was omitted, but the furopyridine derivative **9** was included in the analysis. The compounds were aligned by fitting all heavy atoms in the quinuclidin-2-ene ring using **1** as a template. They were superimposed in the same energetically favored rotameric form, the benzofuran ring being coplanar with the double bond in the quinuclidin-2-ene ring and the benzofuran oxygen located distant from the quinuclidin-2-ene nitrogen.[7] Semiempirical PM3 charges and geometries were used in the study. Conformational preferences of the substituents were taken from dihedral drives using the PM3 Hamiltonian and from the literature.[40] Throughout the superpositions, the same conformation of a substituent was used for all compounds. The compounds were included in a box, and the steric and electrostatic interactions were evaluated at grid points in the box. The CoMFA was performed with a grid size of 2 Å[41] using a positively charged carbon as the probe atom. The variables were scaled

Table 3. Summary of CoMFA Results for Models 1−4 (See Figure 3 for a graphical representation of model 2)

model	regressors	r^2_{cv}	principal components	r^2	standard error of estimate	*F*-value
1	CoMFA	0.52	2	0.72	0.40	25.8
2	CoMFA + π	0.60	3	0.84	0.31	33.4
3	CoMFA[a]	0.61	5	0.78	0.36	34.5
4	CoMFA + π[a]	0.71	4	0.92	0.23	47.9
5	CoMFA + π[b]	0.26	3	0.80	0.30	19.2

[a] Compound **22** was excluded. [b] Compounds **10**, **13**, **18**, **21**, and **28** were excluded.

Table 4. Relative Contributions of the Steric and Electrostatic Fields and the π Values for CoMFA Models 1−4

CoMFA model	relative contribution		
	steric	electrostatic	π
1	0.73	0.27	
2	0.65	0.17	0.18
3	0.75	0.25	
4	0.68	0.14	0.18

using CoMFA standard scaling which gives identical weight to CoMFA fields and additional variables (block scaling). No column filtering was used in the analysis. The steric and electrostatic cutoff values were set to 30 kcal/mol. The electrostatic interaction was dropped for each compound within the steric cutoff values. The analysis was made with cross-validation using the "leave one out" procedure. The r^2_{cv}, which is a measure of the predictive power of a model, was used to evaluate the models. This value is always lower than the conventional r^2, and a $r^2_{cv} > 0.5$ is considered to indicate a good predictive ability. We also included the π-substituent values, summed between the 5- and 7-substituents, in the CoMFA since it has been suggested that lipophilic properties increase the affinity of antagonists.[36] This gave a slightly higher r^2_{cv} (see Table 3). The derived CoMFA model, which included the π-values, gave $r^2_{cv} = 0.60$ (model 2, Table 3). The steric factors made the major contribution to the CoMFA model (Table 4). Compound **22** was the most poorly predicted by the model. The r^2_{cv} increased when **22** was excluded (models 4 and 5, Table 3). This compound was the only iodo-substituted derivative that showed a considerably higher affinity than the corresponding monosubstituted analogue, which may help to rationalize why this compound is less well predicted. However, we still chose to include it in the final model (model 2, Figure 2). To further test the CoMFA correlation, we excluded five compounds (**10**, **13**, **18**, **21**, and **28**) from the set and generated a CoMFA model of the remaining 16 compounds. This lowered the r^2_{cv} to 0.26 ($r^2 = 0.80$). This model was, however, still able to predict the excluded compounds. A correlation coefficient (r^2) of 0.96 was found for a plot of actual versus predicted affinity (Figure 3).

The CoMFA contour values were chosen from a steric field distribution histogram to find contour values with sufficient data points with a location in 3D-space where the influence of field properties on affinity is the greatest. Figure 4 shows that increased steric bulk in the 5- and 7-positions of the benzofuran ring leads to decreased affinity (yellow contours). Any positive influence of steric bulk could not be seen as indicated by the abscence of green contours even at the 0.0001 level. The CoMFA results also suggest that an increased negative potential in the benzofuran ring would lead to an

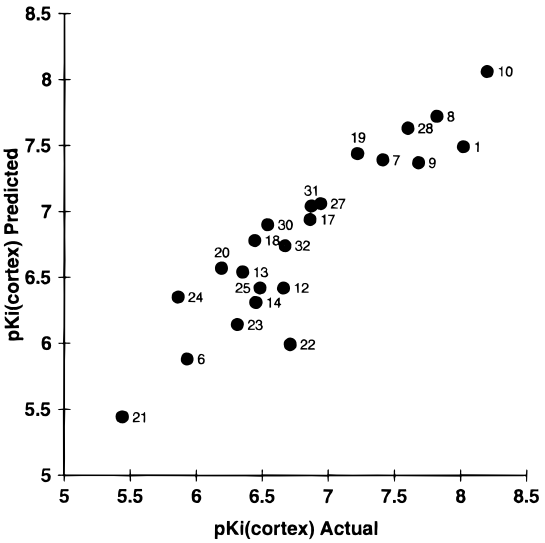


Figure 2. Plot of actual versus predicted affinities derived from CoMFA model 2.

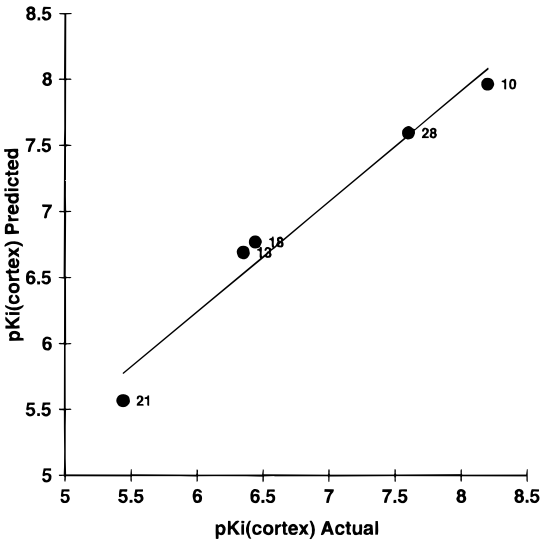


Figure 3. Plot of actual versus predicted affinities of the test set (**10**, **13**, **18**, **21**, and **28**) when using a CoMFA model including π but generated without these compounds.

increased affinity (red contours). In addition, the two blue contours indicate regions were addition of groups providing positive electrostatic potential may increase affinity.

In conclusion, we have evaluated the structure−activity relationships of a series of rigid muscarinic antagonists using two different methods, QSAR and CoMFA. Both methods produce acceptable models, as judged by r^2_{cv}. They predict that steric bulk in the 5- and 7-positions of the benzofuran ring is detrimental for affinity to muscarinic receptors in the cortex and that increased lipophilicity increases the affinity. The models also provide information on the influence of electronic properties of the benzofuran ring on affinity, and they suggest that higher affinity may be obtained by making the ring system more electron rich. Our data further suggest that the studied molecules bind to the muscarinic receptors in a region which is lipophilic and sterically constrained allowing little room to add more steric bulk in the investigated regions of the molecules.

Experimental Section

Chemistry. General Comments. Melting points (uncorrected) were determined in open glass capillaries on a Thomas-

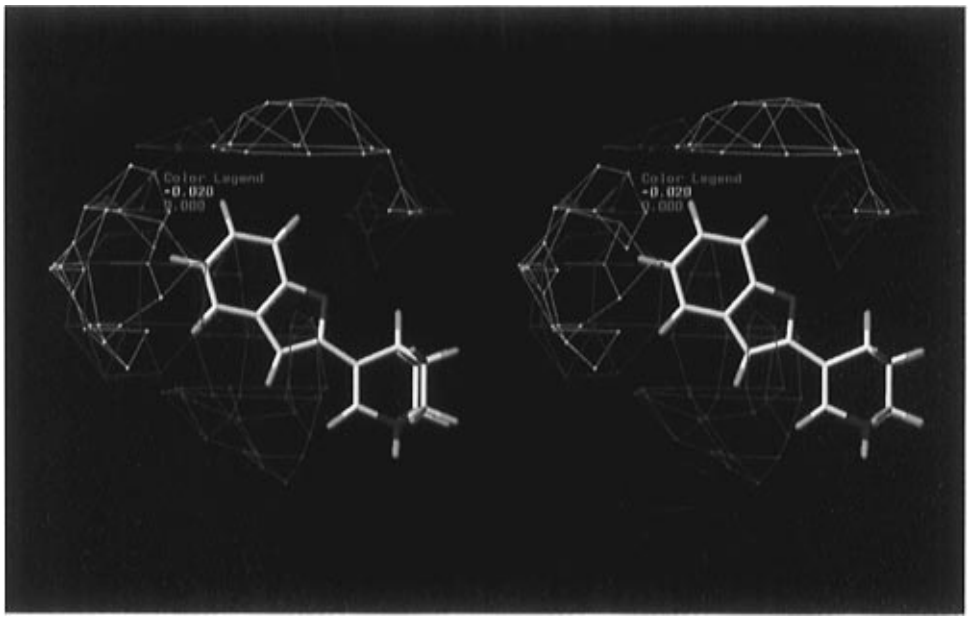


Figure 4. Stereoscopic representation of CoMFA model 2. Included within the CoMFA contours is compound **1**. The contour levels are made using actual STDEV*COEFF values. The red electrostatic contours (−0.002) indicate areas where negative groups are beneficial for activity, i.e., where they lower the K_i value. Blue contours (0.0002) indicate areas where positive groups increase the affinity. The absence of green contours (0.0001) indicates that there are no areas where an increase in steric bulk would increase the activity. Yellow contours (−0.02) indicate areas where steric bulk is detrimental to the biological activity.

Hoover apparatus. IR spectra were recorded on a Perkin Elmer 298 infrared spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL FX 90Q spectrometer at 89.55 and 22.5 MHz, respectively, or on a JEOL JNM-EX 270 spectrometer at 270.2 and 67.9 MHz, respectively, and referenced to internal tetramethylsilane. All spectra were in accordance with the assigned structures. Capillary GLC analyses were performed on a Carlo Erba 6000 Vega instrument equipped with a FID-40 flame ionization detector and a LDC Milton Roy CI-10B integrator; GLC column: DB-5 fused silica gel (30 m, i.d. = 0.32 mm); carrier helium (50−80 kPa). Thin-layer chromatography was carried out on aluminum sheets precoated with silica gel 60 F_{254} (0.2 mm) or aluminum oxide 60 F_{254} neutral (type E) (E. Merck). Column chromatography was performed on silica gel using Kiselgel 60 (230−400 mesh), E. Merck, or on aluminum oxide using aluminum oxide 90, E. Merck. Chromatographic spots were visualized by UV and/or I_2 vapor. The elemental analyses were performed by Mikro Kemi AB, Uppsala, Sweden, and were within ±0.4% of the calculated values.

Synthesis. Below are given representative examples of the reactions presented in Table 1.

3-(5-Formyl-7-methoxy-2-benzofuranyl)quinuclidin-3-ol (2). Method I. 3-Ethynylquinuclidin-3-ol[12] (3 g, 19.8 mmol) was added to a suspension of 5-iodovanillin (5.52 g, 19.8 mmol) and Cu_2O (1.7 g, 11.9 mmol) in dry pyridine (50 mL). The mixture was refluxed under N_2 over night. The pyridine was evaporated under reduced pressure, and the residue was purified by chromatography on Al_2O_3 using gradient elution ($CHCl_3$−$CHCl_3$ + 5% MeOH) to yield 5.0 g (84%) of **2**: R_f (base) 0.49 (Al_2O_3, $CHCl_3$ + 5% MeOH); mp 227−228 °C; ^{1}H NMR (DMSO-d_6) δ 10.01 (s, 1H, CHO), 7.84 (s, 1H), 7.37 (s, 1H), 7.08 (s, 1H), 5.62 (s, 1H, OH), 4.01 (s, 3H, CH_3O), 3.45−3.35 (1H, partly obscured), 2.90−2.60 (m, 5H), 2.25−2.17 (m, 1H), 2.15-2.00 (m, 1H), 1.55−1.15 (m, 3H); ^{13}C NMR δ 192.29, 164.20, 146.63, 145.30, 132.94, 129.63, 118.51, 104.60, 103.25, 68.84, 60.65, 55.78, 46.45, 45.64, 30.96, 23.09, 20.40. Anal. ($C_{17}H_{19}NO_4$) C, H, N.

3-(5-Formyl-7-methoxy-2-benzofuranyl)quinuclidin-2-ene Oxalate (6). Method II. Compound **2** (4.18 g, 13.9 mmol) was dissolved in concentrated formic acid (60 mL). The solution was stirred under reflux for 12 h. The mixture was made basic with 5 M NaOH and extracted with $CHCl_3$ (3 × 150 mL). The combined organic layers were dried (K_2CO_3) and concentrated. The residue was purified by chromatography on Al_2O_3 using $CHCl_3$ as eluent to give 3.37 g (86%) of pure **6**. A small sample was converted into the oxalate and recrystallized in MeOH−ether: R_f (base) 0.65 (Al_2O_3, $CHCl_3$); mp 220 °C dec; ^{1}H NMR (DMSO-d_6) δ 10.03 (s, 1H, CHO), 7.92 (s, 1H), 7.59 (s, 1H), 7.46 (s, 1H), 7.23 (s, 1H), 3.65−3.50 (m, 3H), 3.15−3.00 (m, 2H), 2.15−2.00 (m, 2H), 1.80−1.65 (m, 2H); ^{13}C NMR δ 192.18, 151.08, 146.97, 145.35, 135.83, 133.53, 129.52, 126.68, 118.74, 107.28, 106.30, 56.01, 49.34 (C6 and C7), 26.76 (C4), 23.43 (C5 and C8). Anal. ($C_{17}H_{17}NO_3$·$(COOH)_2$) C, H, N.

3-(5-Formyl-7-iodo-2-benzofuranyl)quinuclidin-2-ene Hydrochloride (12). Method III. 3-Ethynylquinuclidin-3-ol (3 g, 19.8 mmol)[12] was added to a suspension of 3,5-diiodo-4-hydroxybenzaldehyde (5.52 g, 19.8 mmol) and Cu_2O (1.7 g, 11.9 mmol) in dry pyridine (50 mL). The mixture was refluxed under N_2 for 2 h. The pyridine was evaporated under reduced pressure, and the residue was purified by chromatography on Al_2O_3 using gradient elution ($CHCl_3$−$CHCl_3$ + 5% MeOH). The product was triturated with ether to yield 6.0 g of 3-(5-formyl-7-iodo-2-benzofuranyl)quinuclidin-3-ol. Part of this product (3.0 g, 7.55 mmol) was taken out and dissolved in concentrated formic acid (25 mL). The solution was refluxed for 3 days, alkalinized with 5 M NaOH, extracted with $CHCl_3$ (3 × 150 mL), dried (K_2CO_3), and concentrated under reduced pressure to yield 2.42 g (64% overall yield) of pure **12**. The product was converted into the hydrochloride and recrystallized from MeCN−MeOH-ether: R_f (base) 0.69 (Al_2O_3, $CHCl_3$); mp 270 °C dec; ^{1}H NMR (DMSO-d_6) δ 10.01 (s, 1H, CHO), 8.29 (s, 2H), 7.79 (s, 1H), 7.18 (s, 1H, C2-H), 3.70−3.55 (m, 3H, C4-H, C6-H, C7-H), 3.20−3.05 (m, 2H, C6-H, C7-H), 2.15−2.00 (m, 2H, C5-H, C8-H), 1.85−1.65 (m, 2H, C5-H, C8-H); ^{13}C NMR δ 191.32 (C=O), 157.90, 150.85, 135.47, 135.18, 134.07, 127.94, 125.55, 124.33, 108.48, 76.89 (benzofuran C7), 49.70 (C6 and C7), 26.58 (C4), 22.93 (C5 and C8). Anal. ($C_{16}H_{14}INO_2$·HCl) C, H, N.

3-(5-Formyl-2-benzofuranyl)quinuclidin-2-ene Hydrochloride (18). Method IV. A mixture of **12** (0.96 g, 2.55 mmol), sodium formate (0.87 g, 12.73 mmol), and $Pd(Ph_3P)_4$ (0.088 g, 0.076 mmol, 3%) in DMF (20 mL) was heated at 120 °C for 10 min. The mixture was concentrated under reduced pressure, and the residue was purified by chromatography on SiO_2 using $CHCl_3$ + 15% MeOH as eluent to give 0.56 g (87%) of pure **18**. A sample was converted into the hydrochloride and recrystallized from MeCN: R_f (base) 0.42 (Al_2O_3, $CHCl_3$); mp 270 °C dec; ^{1}H NMR (DMSO-d_6) δ 10.08 (s, 1H, CHO), 8.31 (d, J = 1.65 Hz, 1H), 7.96 (dd, J = 1.65, 8.58 Hz, 1H), 7.82 (d, J = 8.58 Hz, 1H), 7.66 (s, 1H), 7.20 (s, 1H), 3.70−3.55 (m, 3H),

3.20−3.00 (m, 2H), 2.15−2.00 (m, 2H), 1.85−1.65 (m, 2H); ^{13}C NMR δ 192.29 (CHO), 157.66, 150.87, 135.88, 132.52, 128.32, 126.95, 125.16, 124.85, 112.02, 107.47, 49.70 (C6 and C7), 26.61 (C4), 22.96 (C5 and C8). Anal. ($C_{16}H_{15}NO_2$·HCl) C, H, N.

3-[7-(Hydroxymethyl)-5-iodo-2-benzofuranyl]quinuclidin-2-ene Oxalate (23). Method V. $NaBH_4$ (0.015 g, 0.40 mmol) was added to a solution of **14** (0.30 g, 0.79 mmol) in MeOH (15 mL). The mixture was stirred for 2 h at room temperture, aqueous HCl (3 mL, 1 M) was added, and the MeOH was evaporated under reduced pressure. The residue was alkalinized with 5 M aqueous NaOH and extracted with $CHCl_3$ (5 × 40 mL). The combined organic layers were dried (K_2CO_3) and concentrated under reduced pressure. The residue was purified by chromatography on Al_2O_3 using $CHCl_3$ + 5% MeOH as eluent. The product was converted into the oxalate and recrystallized from MeOH−ether to provide 0.28 g (82%) of **23**: R_f (base) 0.56 (Al_2O_3, $CHCl_3$ + 5% MeOH); mp 233−235 °C; ^{1}H NMR (DMSO-d_6) δ 7.90 (s, 1H), 7.62 (s, 1H), 7.19 (s, 1H), 7.14 (s, 1H), 4.76 (s, 1H, OH), 3.40−3.20 (m, 3H), 2.87−2.70 (m, 2H), 2.00−1.85 (m, 2H), 1.70−1.50 (m, 2H); ^{13}C NMR δ 151.35, 151.03, 136.49, 132.99, 131.48, 130.51, 128.19, 127.99, 103.63, 87.24, 56.85, 49.15 (C6 and C7), 26.92 (C4), 25.41 (C5 and C8). Anal. ($C_{16}H_{16}INO_2$·0.5(COOH)$_2$) C, H, N.

3-(7-Methyl-2-benzofuranyl)quinuclidin-2-ene Hydrochloride (28). Method VI. A mixture of **8** (0.20 g, 0.66 mmol), tetramethyltin (1.18 g, 6.6 mmol), $Pd(OAc)_2$ (0.003 g, 0.013 mmol, 2%) and tri-*o*-tolylphosphine (0.016 g, 0.053 mmol, 8%) in DMF (4 mL) was heated in a small, tightly sealed round bottom flask (5 mL) at 120 °C for 18 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by chromatography on SiO_2 using $CHCl_3$ + 10% MeOH as eluent to provide 0.13 g (83%) of **28**. The product was converted into the hydrochloride and recrystallized from MeCN−ether: R_f (base) 0.38 (SiO_2, $CHCl_3$ + 10% MeOH); mp 209−210 °C; ^{1}H NMR (DMSO-d_6) δ 7.55−7.40 (m, 2H), 7.20−7.15 (m, 2H), 7.13 (s, 1H, C2-H), 3.7−3.5 (m, 3H), 3.15-3.00 (m, 2H), 2.49 (s, 3H), 2.15−2.00 (m, 2H), 1.80−1.65 (m, 2H); ^{13}C NMR δ 153.49, 148.66, 136.40, 127.18, 126.81, 123.77, 123.48, 120.82, 119.15, 107.46, 49.74 (C6 and C7), 26.59 (C4), 23.00 (C5 and C8), 14.46 (CH_3). Anal. ($C_{16}H_{17}NO$·HCl) C, H, N.

3-(7-Phenyl-2-benzofuranyl)quinuclidin-2-ene Hydrochloride (31). Method VII. $Pd(Ph_3P)_4$ (0.086 g, 0.074 mmol, 3%) was added to a solution of **8** (0.75 g, 2.5 mmol) in dimethoxyethane (8 mL). The mixture was stirred for 10 min, and phenylboronic acid (0.33 g, 2.73 mmol) and Na_2CO_3 (1 M, 6 mL) were added. The reaction mixture was refluxed for 2.5 h, concentrated under reduced pressure, and extracted with $CHCl_3$ (4 × 30 mL). The combined organic layers were dried (K_2CO_3) and concentrated under reduced pressure. The residue was purified by chromatography on first Al_2O_3 using ether + 10% MeOH as eluent and then on SiO_2 using $CHCl_3$ + 10% $CHCl_3$ as eluent to afford 0.74 g (99%) of pure **31**. The product was converted into the hydrochloride and recrystallized from MeCN: R_f (base) 0.41 (SiO_2, $CHCl_3$ + 10% MeOH); mp 240−242 °C; ^{1}H NMR (DMSO-d_6) δ 7.92−7.86 (m, 2H), 7.73−7.52 (m, 5H), 7.49−7.37 (m, 2H), 7.07 (s, 1H, C2-H), 3.70−3.55 (m, 3H, C4-H, C6-H, C7-H), 3.20−3.00 (m, 2H, C6-H, C7-H), 2.15−2.00 (m, 2H, C5-H, C8-H), 1.80−1.65 (m, 2H, C5-H, C8-H); ^{13}C NMR δ 151.55, 149.20, 136.08, 135.25, 128.78, 128.62, 128.10, 127.90, 125.21, 124.45, 124.17, 124.08, 121.08, 107.33, 49.77 (C6 and C7), 26.65 (C4), 23.00 (C5 and C8). Anal. ($C_{21}H_{19}NO$·HCl) C, H, N.

3-(5-Methyl-2-benzofuranyl)quinuclidin-2-ene Hydrochloride (27). Triethylsilane (2.73 mL, 17.1 mmol) and trifluoroacetic acid (2.42 mL, 31.4 mmol) were added to a solution of **24** (0.69 g, 2.7 mmol) in $CHCl_3$ (2 mL). The mixture was stirred for 1 h at room temperature, an additional 2 mL of trifluoroacetic acid was added, and the temperature was raised to 60 °C. After 5 h the reaction was quenched by addition of 5 M aqueous NaOH. The mixture was extracted with $CHCl_3$, and the combined organic layers were dried (K_2CO_3) and condensed under reduced pressure. The residue was purified by chromatography on SiO_2 using $CHCl_3$ + 10% MeOH as eluent to provide 0.34 g (53%) of **27**. The product was converted into the hydrochloride and recrystallized from MeCN: R_f (base) 0.62 (Al_2O_3, $CHCl_3$); mp 241−243 °C; ^{1}H NMR (DMSO-d_6) δ 7.52−7.46 (m, 2H), 7.41 (s, 1H), 7.24−7.18 (m, 1H), 7.08 (m, s, 1H), 3.7−3.5 (m, 3H), 3.15−3.00 (m, 2H), 2.40 (s, 3H, CH_3), 2.15−1.95 (m, 2H), 1.80−1.60 (m, 2H); ^{13}C NMR δ 153.41, 149.45, 136.82, 132.90, 128.18, 127.75, 123.83, 121.71, 111.07, 107.41, 49.99 (C6 and C7), 27.03 (C4), 23.35 (C5 and C8), 21.16 (CH_3). Anal. ($C_{16}H_{17}NO$·HCl) C, H, N.

3-(5-Amino-2-benzofuranyl)quinuclidin-2-ene Hydrochloride (32). A solution of $SnCl_2$·$(H_2O)_2$ (2.50 g, 11.1 mmol) in concentrated HCl (37%, 10 mL) was added to a solution of **17** (1.0 g, 3.7 mmol) in concentrated acetic acid (10 mL). The mixture was stirred at room temperature for 14 h, made basic with 5 M NaOH, and extracted with $CHCl_3$ (4 × 150 mL). The combined organic layers were dried (K_2CO_3) and concentrated under reduced pressure. The residue was purified by chromatography on SiO_2 using $CHCl_3$ + 10% MeOH as eluent to afford 0.65 g (73%) of pure **32**. The product was converted into the hydrochloride and recrystallized from MeCN−MeOH: R_f (base) 0.27 (Al_2O_3, $CHCl_3$); mp 280 °C dec; ^{1}H NMR (DMSO-d_6) δ 7.78−7.71 (m, 2H), 7.58 (s, 1H), 7.45−7.38 (m, 1H), 7.16 (s, 1H), 3.70−3.50 (m, 3H), 3.20−3.00 (m, 2H), 2.20−2.00 (m, 2H), 1.80−1.60 (m, 2H); ^{13}C NMR δ 153.35, 150.69, 136.10, 128.50, 128.05, 124.64, 121.22, 116.11, 112.28, 107.20, 49.72 (C6 and C7), 26.72 (C4), 22.99 (C5 and C8). Anal. ($C_{15}H_{16}N_2O$·$(HCl)_2$·0.33H_2O) C, H, N.

3-(3-Bromo-2-benzofuranyl)quinuclidin-3-ol (15). A solution of LDA (11.7 mL, 2 M, 23.4 mmol) in THF/*n*-heptane was added dropwise to a stirred solution of 3-bromobenzofuran (4.23 g, 21.5 mmol) in dry THF (100 mL) at −78 °C. After stirring for 2 h a solution of 3-quinuclidinone (2.44 g, 19.5 mmol) in dry THF (10 mL) was added. The mixture was allowed to slowly reach room temperature over 10 h. The reaction was quenched by addition of water (5 mL), and the mixture was concentrated under reduced pressure. The residue was purified by chromatography on Al_2O_3 columns, initially with gradient elution (EtOAc → EtOAc + 10% MeOH) and then with EtOAc + 5% MeOH as eluent to yield 5.74 g (91%) of **15**: R_f (base) 0.72 (Al_2O_3, $CHCl_3$ + 5% MeOH); mp 157−159 °C; ^{1}H NMR ($CDCl_3$) δ 8.55 (s, 1H, OH), 7.58−7.25 (m, 4H), 4.01 (d, J = 14.4 Hz, 1H, C2-H), 3.17 (d, J = 14.4 Hz, 1H, C2-H), 3.10−2.95 (m, 1H), 2.90−2.60 (m, 4H), 2.40−2.25 (m, 1H), 1.70−1.35 (m, 3H); ^{13}C NMR δ 156.24, 152.49, 128.95, 125.34, 123.45, 119.66, 111.37, 93.89, 71.34, 59.87, 46.67, 45.77, 31.27, 23.00, 20.61. Anal. ($C_{15}H_{16}BrNO_2$) C, H, N.

3-(2-Furo[3,2-*b*]pyridinyl)quinuclidin-2-ene Hydrochloride (9). Compound **5** (0.30 g, 1.23 mmol) was dissolved in concentrated methanesulfonic acid (20 mL). The solution was heated at 200 °C for 4 h. The mixture was made basic with NaOH and extracted with $CHCl_3$ (3 × 150 mL). The combined organic layers were dried (K_2CO_3) and concentrated under reduced pressure. The residue was purified by column chromatography, first on Al_2O_3 using $CHCl_3$ as eluent and then on SiO_2 using $CHCl_3$ + 5% MeOH as eluent to give 0.16 g (58%) of pure **9**. The product was converted into the hydrochloride and recrystallized from MeOH−ether: R_f (base) 0.38 (Al_2O_3, $CHCl_3$); mp 212 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.63 (d, J = 4.78 Hz, 1H), 8.20 (d, J = 8.41 Hz, 1H), 7.76 (s, 1H), 7.52 (dd, J = 4.78, 8.41 Hz, 1H), 7.30 (s, 1H, C2-H), 3.75−3.55 (m, 3H, C4-H, C6-H, C7-H), 3.20−3.00 (m, 2H, C6-H, C7-H), 2.20−2.00 (m, 2H, C5-H, C8-H), 1.85−1.70 (m, 2H, C5-H, C8-H); ^{13}C NMR δ 153.31, 148.28, 145.78, 145.14, 135.99, 126.07, 120.90, 120.03, 106.59, 49.58 (C6 and C7), 26.50 (C4), 22.86 (C5 and C8). Anal. ($C_{14}H_{14}N_2O$·$(HCl)_2$) C, H, N.

Muscarinic Receptor Binding Studies. The tissue preparations and general methods used have been described in detail elsewhere for the parotid gland,[23] urinary bladder,[24] heart,[25] and cerebral cortex,[25] respectively. Cerebral cortex in rat expresses a mixture of muscarinic receptor subtypes (M_1 40%, M_2 30%, M_3 5%, and M_4 20%) as found with immunoprecipitation.[42,43] The heart expresses a homogeneous population of the M_2 subtype, in rat and rabbit.[44,45] Parotid gland contains 93% of M_3, determined with specific antibodies for M_1−M_5, in rat.[46] In the urinary bladder the predominant subtype is M_2, and a smaller population of M_3 is present, in rat, rabbit, guinea pig, and human bladder.[47]

Male guinea pigs (250−400 g of body weight) were killed by a blow on the neck and exsanguinated. The brain was placed on ice for dissection of the cerebral cortex (gray matter only). Urinary bladders, hearts, and parotid glands were dissected in a Krebs−Henseleit buffer (pH 7.4) containing 1 mM phenylmethanesulfonyl fluoride (PMSF; a protease inhibitor). Dissected tissues were homogenized in an ice-cold sodium−potassium phosphate buffer (50 mM, pH 7.4) containing 1 mM PMSF, using a Polytron PT-10 instrument (bladder, heart, parotid) and a Potter-Elvehjem Teflon homogenizer (cortex). All homogenates were diluted with ice-cold phosphate/PMSF buffer to a final protein concentration of ≤0.3 mg/mL and immediately used in the receptor binding assays. Protein was determined by the method of Lowry et al.,[48] using bovine serum albumin as the standard.

The muscarinic receptor affinities of the unlabeled compounds were derived from competition experiments in which the ability to inhibit the receptor specific binding of (−)-[^{3}H]-QNB (32.9 Ci/mmol) was monitored as previously described.[25,26] Each sample contained 10 μL of (−)-[^{3}H]QNB solution (final concentration 2 nM), 10 μL of a solution of test compound, and 1.0 mL of tissue homogenate. Triplicate samples were incubated under conditions of equilibrium, i.e., at 25 °C for 60 (urinary bladder), 80 (heart and cerebral cortex), or 210 (parotid gland) min. Nonspecific binding was determined in the presence of 10 μM unlabeled atropine. Incubations were terminated by centrifugations,[24] and the radioactivity in the pellets was determined by liquid scintillation spectrometry.[24]

IC_{50} values (concentration of unlabeled compound producing 50% inhibition of the receptor specific (−)-[^{3}H]QNB binding) were graphically determined from the experimental concentration−inhibition curves. Affinities, expressed as the inhibition constants (K_i values), were calculated by correcting the IC_{50} for the radioligand-induced parallel shift and differences in the receptor concentration, using the method of Jacobs et al.[49] The binding parameters for (−)-[^{3}H]QNB (K_D and receptor densities) used in these calculations have been determined in separate series of experiments.[23−25]

Functional *in Vitro* Studies. Male guinea pigs, weighing about 300 g, were killed by a blow on the neck and exsanguinated. Smooth muscle strips of the urinary bladder were dissected in a Krebs−Henseleit solution (pH 7.4). The strip preparations were vertically mounted between two hooks in the thermostatically controlled (37 °C) organ baths (5 mL). One of the hooks was adjustable and connected to a force transducer (FT 03, Grass Instrument). The Krebs−Henseleit solution was continuously bubbled with carbogen gas (93.5% O_2/6.5% CO_2) to maintain the pH at 7.4. Isometric tension was recorded by a Grass polygraph (Model 79D). A resting tension of approximately 5 mN was initially applied on each muscle strip, and the preparations were washed several times during the stabilization. The urinary bladder strips were used for evaluation of antimuscarinic and muscarinic activity (see Pharmacological Results and Discussion). Carbachol (carbamoylcholine chloride) was used as the agonist. Concentration−response curves to carbachol were generated by cumulative dose−response technique.

In studies of antagonism, a control concentration−response curve to carbachol was generated by cumulative addition of carbachol to the bladder strip (i.e., a stepwise increase of the agonist concentration until the maximal contractile response was reached) followed by washing out and a resting period of at least 15 min prior to addition of a fixed concentration of the test compound (antagonist) to the organ bath. After 60 min of incubation with antagonist, a second cumulative concentration−response curve to carbachol was generated. Responses were expressed as percent of the maximal response to carbachol. EC_{50} values for carbachol in the absence (control) and presence of antagonist were graphically derived, and dose ratios (r) were calculated. Dissociation constants, K_B, for the antagonists were then calculated by $K_B = [A]/r - 1$, where [A] is the concentration of the test compound.[50]

Computational Methods. The PLS analyses were performed in Simca-S for Windows version 5.1.[51] PM3 and *ab initio* calculations were performed in SPARTAN 3.1.[31]

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Supporting Information Available: Assigned ^{13}C NMR shifts (DMSO-d_6) of the quinuclidin-2-ene (Q2−Q8) and benzofuran (C2−C7a) carbons of the 5- and 7-substituted quinuclidin-2-ene derivatives. The ^{13}C NMR spectra were assigned by using standard shift theory, comparisons with selected benzofuran derivatives, and ^{1}H−^{13}C heteronuclear NMR correlation experiments (2 pages).[52] Ordering information is given on any current masthead page.

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(37) This indicates the π value for the substituent in the 5-position of the benzofuran ring. Similarly, a suffix of −7 indicates the 7-position.

(38) The PLS equation for the model generated is pK_i(cortex) = 9.59 + 0.20*π−5 − 0.07*MR-5 − 0.11*L-5 − 0.10*B1-5 − 0.19*B5-5 + 0.16*π-7 − 0.07*L-7 − 0.16*B5-7 − 0.15*LUMO + 0.02*Elneg − 0.11*mol vol − 0.13*surface area − 0.16*ovality + 0.17*log *P* − 0.14*(charge at C7) + 0.29*(ovality)^2 − 0.13*π-5*L-7.

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