Analogues of Carbacholine: Synthesis and Relationship between Structure and Affinity for Muscarinic and Nicotinic Acetylcholine Receptors

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Summary

A series of acyclic and heterocyclic analogues of carbacholine (**1**) was synthesized using *N*-methylcarbacholine (MCC, **2**), *N,N*-dimethylcarbacholine (DMCC, **3**), and the corresponding tertiary amine (**4**) as leads. Whereas nicotinic acetylcholine receptor affinity was determined using [^{3}H]nicotine as the radioactive ligand, [^{3}H]oxotremorine-M ([^{3}H]Oxo-M) and [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB), in some cases supplemented with [^{3}H]pirenzepine ([^{3}H]PZ), were used as radioligands for muscarinic acetylcholine receptors on rat brain membranes. On the basis of receptor binding data, nicotinic/muscarinic (N/M) selectivity factors were determined, and muscarinic receptor efficacy (M agonist index) and M_1 selectivity (M_2/M_1 index) estimated. In most cases, quaternized analogues showed higher affinity than the corresponding tertiary amines for muscarinic and, in particular, nicotinic receptor sites. Among the new compounds, *N,N*-diethylcarbacholine (**9e**) (IC_{50} = 0.046 μM), (*S*)-1-methyl-2-(*N,N*-dimethylaminocarbonyloxymethyl)pyrrolidine (**17k**) (IC_{50} = 0.068 μM), and the corresponding quaternized analogue, **18k** (IC_{50} = 0.018 μM) showed the highest nicotinic receptor affinity. The tertiary amine, **17k** showed much higher nicotinic receptor affinity than the acyclic analogue, **4** (IC_{50} = 5.7 μM), and the N/M selectivity factor determined for **17k** (150) is an order of magnitude lower than that of nicotine (1400). The N/M selectivity factors for MCC (**2**) and DMCC (**3**), previously reported to be highly selective nicotinic receptor ligands, were shown to be 6.5 and 60, respectively, the latter value being comparable with that of **18k** (89).

Introduction

There is convincing evidence of major impairments of central acetylcholine (ACh) neurotransmission mechanisms in patients with the pathology characteristic of Alzheimer's disease (AD)[1–3]. This cholinergic deficit may be of particular relevance to disturbances in learning and memory capability of AD patients[4]. Postsynaptic M_1 muscarinic ACh receptors seem to survive the loss of cholinergic nerve terminals in cortical tissues, and the density of M_1 receptor sites actually is increased in the striatal region of brains from AD patients[3]. Provided that M_1 receptors survive in a functional state in AD, such receptors may be of primary interest as therapeutic targets[5–7].

The release of ACh from nerve terminals appears to be under dual cholinergic receptor control[8,9]. Whereas muscarinic M_2 receptors primarily function as autoreceptors and, thus, regulate ACh release in a negative feedback fashion[10], presynaptic nicotinic ACh receptors have been shown to modulate ACh release *via* a positive feedback mechanism[8,9].

The probability of successful cholinergic stimulation or replacement therapies in AD are assumed to be highest at the early stages of the disease[4]. At these stages, presynaptically located muscarinic M_2 and/or nicotinic ACh receptors may be relevant targets for symptomatic therapeutic interventions, making M_2 antagonists and nicotinic agonists pharmacologically interesting[11,12]. It seems likely that drugs with dual actions at ACh receptors, as for example mixed M_2 antagonists/M_1 agonists[13] or M_2 antagonists/nicotinic agonists, may have particular therapeutic interest in AD.

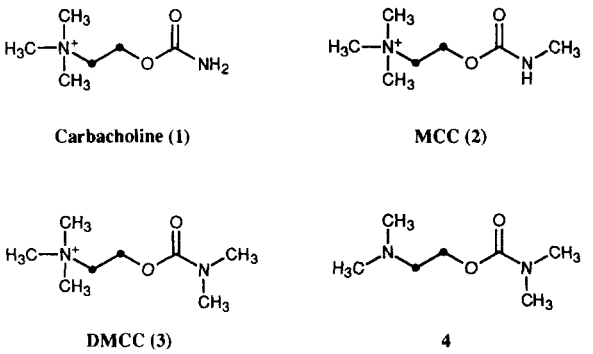

Chart 1. Structures of some carbamate ester acetylcholine receptor ligands.

A number of analogues of carbacholine (**1**), including *N*-methylcarbacholine (MCC, **2**)[14–16] and *N,N*-dimethylcarbacholine (DMCC, **3**)[17] (Chart 1), have recently been described as nicotinic ACh agonists, and [^{3}H]MCC has been introduced as a new radioligand for studies of nicotinic receptor sites [14,18]. The selectivity of these compounds as nicotinic ligands was claimed on the basis of their effects on the binding of [^{3}H]nicotine and the muscarinic antagonist, [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB)[18]. In pharmacological studies, MCC (**2**) was shown also to activate muscarinic receptors[16], although the binding of [^{3}H]MCC was not affected by muscarinic antagonists[14]. In order to gain further insight into the nicotinic/muscarinic (N/M) agonist selectivity of MCC (**2**) and DMCC (**3**), we have now studied these compounds as inhibitors of the nicotinic and muscarinic agonist ligands, [^{3}H]nicotine and [^{3}H]oxotremorine-M ([^{3}H]Oxo-M), respectively.

In this paper we also describe the synthesis of a series of conformationally restricted analogues of MCC (**2**) or DMCC

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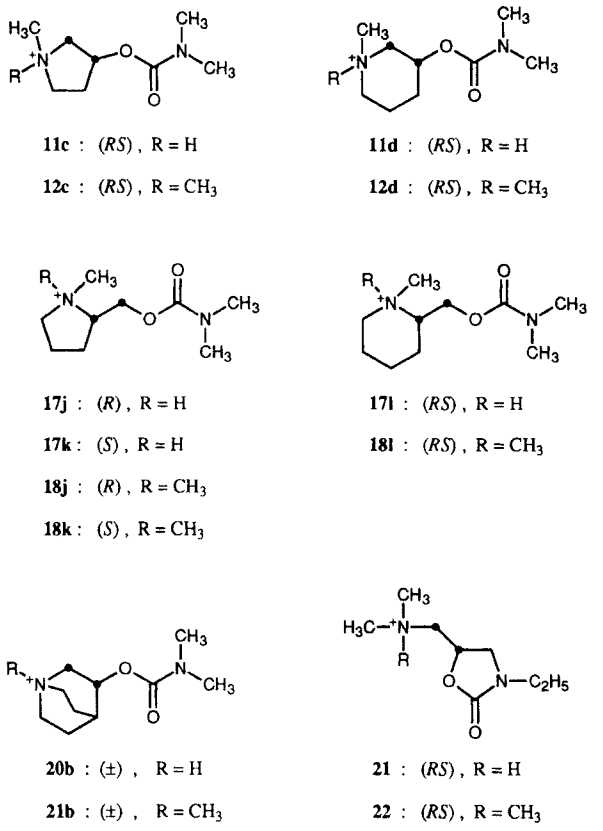


11c : (*RS*), R = H
12c : (*RS*), R = CH_3
11d : (*RS*), R = H
12d : (*RS*), R = CH_3
17j : (*R*), R = H
17k : (*S*), R = H
18j : (*R*), R = CH_3
18k : (*S*), R = CH_3
17l : (*RS*), R = H
18l : (*RS*), R = CH_3
20b : (±), R = H
21b : (±), R = CH_3
21 : (*RS*), R = H
22 : (*RS*), R = CH_3

Chart 2. Structures of some heterocyclic carbamate esters derived from carbacholine.

(**3**) (Chart 2) and studies of their effects on the binding of [^{3}H]nicotine, [^{3}H]Oxo-M, [^{3}H]QNB, and, in some cases, [^{3}H]pirenzepine ([^{3}H]PZ), a selective M_1 antagonist [19]. On the basis of receptor binding data for some of the muscarinic ligands, approximate values for agonist efficacy (M agonist index) and M_1 selectivity (M_2/M_1 index) were estimated following previously described procedures[20–22].

Methods and Results

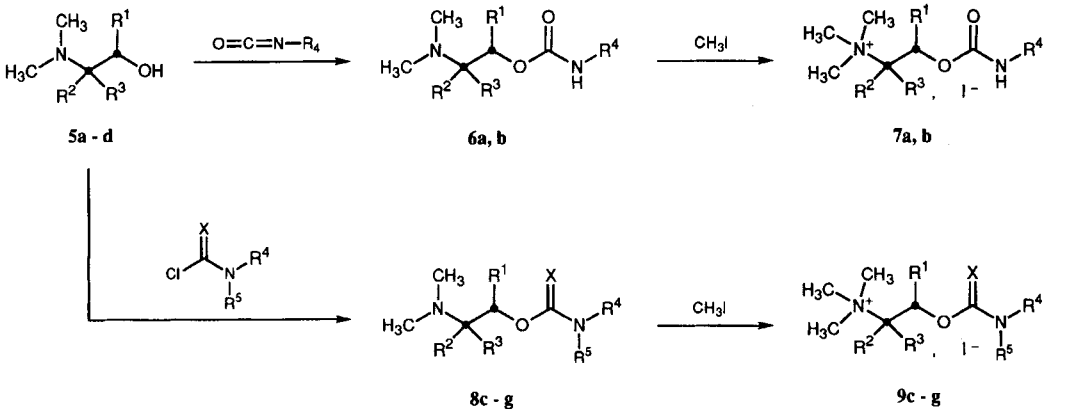
Chemistry

N-Mono- and *N,N*-dialkylated carbacholine analogues were synthesized by treatment of appropriate aminoalcohols with alkyl isocyanates or isothiocyanates and dialkylcarbamyl chlorides, respectively (Charts 3–6). In most cases, the reaction between cyclic aminoalcohols and alkyl isocyanates required high temperatures and prolonged reaction times. Attempts to facilitate these reactions by using CuCl as a catalyst[23] were unsuccessful. Under these reaction conditions, the tertiary aminoalcohols were partially converted into carbamate esters, in which the NH group had reacted with an additional molecule of alkyl isocyanate to produce urea derivatives, as shown by NMR spectroscopic analyses of the reaction products. A CuCl-catalyzed reaction was, however, successfully used in the syntheses of the tertiary amine, **25**, which was converted into the cyclic carbamate ester of choline, compound **26** (Chart 6). Thus, CuCl was used to catalyze the conversion of 1,3-dibromopropan-2-ol (**22**) into

Table 1. Physical and chemical properties of the synthesized compounds.

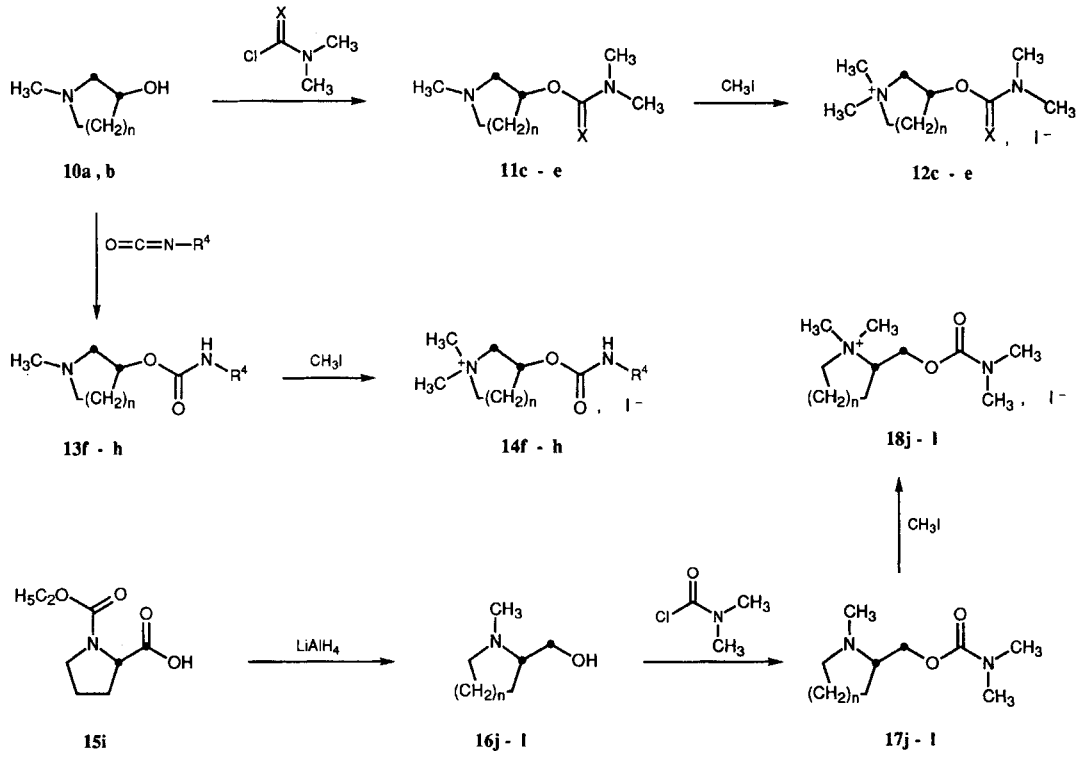
No.	Salt	Method[a]	Yield, %	Mp, °C	Recryst. solvent	Analyses
3[b]	iodide	E	90	203–206	MeOH/Et_2O	C,H, N, I
4[c]	chloride	C	61	136–137	MeCN/Et_2O	C, H, N, Cl
6a[c]	chloride	A	83	165–166	MeCN	C, H, N, Cl
6b	fumarate	A	78	71–73	*i*PrOH/Et_2O	C, H, N
7a[d]	iodide	E	85	120–123	EtOH/Et_2O	C, H, N, I
7b	iodide	E	60	133–136	EtOH/Et_2O	C, H, N, I
8c	fumarate	C	33	108–109	*i*PrOH	C,H,N
8d	fumarate	C	58	114–117	*i*PrOH/Et_2O	C, H, N
8e	fumarate	C	67	94–95	*i*PrOH/Et_2O	C, H, N
8f	fumarate	C	86	145–146	*i*PrOH/Et_2O	C, H, N
8g	fumarate	C	38	123–125	*i*PrOH/Et_2O	C, H, N
9c	iodide	E	66	205–207	EtOH/Et_2O	C, H, N, I
9d	iodide	E	69	135–137	EtOH/Et_2O	C, H, N, I
9e[e]	iodide	E	83	136–137	EtOH/Et_2O	C, H, N, I
9f	iodide	E	65	213–215	*i*PrOH/Et_2O	C, H, N, I
9g	iodide	E	54	161–165	MeOH/Et_2O	C, H, N, I
11c	fumarate	D	37	92–93	*i*PrOH/Et_2O	C, H, N
11d[f]	fumarate	D	54	134–135	*i*PrOH/Et_2O	C, H, N
11e	fumarate	C	45	146–147	*i*PrOH	C, H, N
12c	iodide	E	73	127–128	EtOH/Et_2O	C, H, N, I
12d[g]	iodide	E	66	203–204	EtOH/Et_2O	C, H, N, I
12e	iodide	E	34	166–167	EtOH	C, H, N, I
13f	fumarate	A	36	104–105	*i*PrOH/Et_2O	C,H,N
13g[h]	fumarate[i]	B	47	120–121	*i*PrOH/Et_2O	C, H, N
13h[h]	fumarate	B	51	133–134	*i*PrOH/Et_2O	C, H, N
14f	iodide	E	67	107–108	EtOH/Et_2O	C, H, N, I
14g[j]	iodide	E	69	118–120	EtOH/Et_2O	C, H, N, I
14h[k]	iodide	E	60	168–170	EtOH/Et_2O	C, H, N, I
17j[l]	bromide	C	34	130–132	*i*PrOH/Et_2O	C, H, N, Br
17k[m]	bromide	C	72	130–132	*i*PrOH/Et_2O	C, H, N, Br
17l	fumarate	C	71	123–124	*i*PrOH/Et_2O	C, H, N
18j[n]	iodide	E	51	134–135	*i*PrOH/Et_2O	C, H, N, I
18k[o]	iodide	E	86	134–136	*i*PrOH/Et_2O	C, H, N, I
18l	iodide	E	72	118–119	*i*PrOH/Et_2O	C, H, N, I
20a	fumarate[a]		41	163–164	*i*PrOH/Et_2O	C, H, N
20b	fumarate[a, p]		14	139–140	*i*PrOH/Et_2O	C, H, N
21a	iodide	F	91	118–120	EtOH/Et_2O	C, H, N, I
21b	iodide	F	68	161–162	EtOH	C, H, N, I
25	chloride[a]		25	193–194	EtOH/Et_2O	C, H, N, Cl
26	iodide	E	83	143–145	*i*PrOH/Et_2O	C, H, N, I

[a] For method of synthesis: see Experimental Section. [b] Previously reported compound[17,24,25]; mp of iodide: not given[17]; chloride: mp 185–187 °C[24] [c] Base previously reported[17]; bp not given. [d] Previously reported compound[17,24,25]; mp of iodide: not given[17]; chloride: mp 198–200 °C[24]. [e] Previously reported compound[24–26]; mp 142.5–143.5 °C[26]; mp 121–123 °C[24]; mp 105–108 °C[25]. [f] Previously reported compound[27]; chloride: mp 198–199 °C. [g] Previously reported compound[27]; bromide: mp 205–206 °C. [h] Base previously reported [28]. [i] Crystallized with 1.25 equivalent of fumaric acid. [j] Previously reported compound[28]; mp 265–267 °C. [k] Previously reported compound[28]; mp 172–174 °C. [l] $[\alpha]_D^{25}$ = –3.7° (*c* = 1.8 in MeOH). [m] $[\alpha]_D^{25}$ = + 3.7° (*c* = 1.7 in MeOH). [n] $[\alpha]_D^{25}$ = 1.2° (*c* = 1.0 in MeOH). [o] $[\alpha]_D^{25}$ = +1.3° (*c* = 1.0 in MeOH). [p] Crystallized with 1.5 equivalent of fumaric acid.



a:		$R^1 = H$	$R^2 = H$	$R^3 = H$	$R^4 = C_2CH_5$		
b:	(*RS*)	$R^1 = CH_3$	$R^2 = H$	$R^3 = H$	$R^4 = C_2CH_5$		
c:	(*RS*)	$R^1 = CH_3$	$R^2 = H$	$R^3 = H$	$R^4 = CH_3$	$R^5 = CH_3$	X = O
d:	(*RS*)	$R^1 = H$	$R^2 = CH_3$	$R^3 = H$	$R^4 = CH_3$	$R^5 = CH_3$	X = O
e:		$R^1 = H$	$R^2 = H$	$R^3 = H$	$R^4 = C_2CH_5$	$R^5 = C_2CH_5$	X = O
f:		$R^1 = H$	$R^2 = CH_3$	$R^3 = CH_3$	$R^4 = CH_3$	$R^5 = CH_3$	X = O
g:		$R^1 = H$	$R^2 = H$	$R^3 = H$	$R^4 = CH_3$	$R^5 = CH_3$	X = S

Chart 3. Synthesis of some acyclic carbacholine analogues.



a: (*RS*), n = 1	**d**: (*RS*), n = 2, X = O	**g**: (*RS*), n = 2, $R^4 = C_2H_5$	**j**: (*R*), n = 1
b: (*RS*), n = 1	**e**: (*RS*), n = 2, X = S	**h**: (*RS*), n = 2, $R^4 = CH_3$	**k**: (*S*), n = 1
c: (*RS*), n = 1, X = O	**f**: (*RS*), n = 1, $R^4 = C_2H_5$	**i**: (*R*)	**l**: (*RS*), n = 2

Chart 4. Synthesis of some pyrrolidinyl and piperidyl carbacholine analogues.

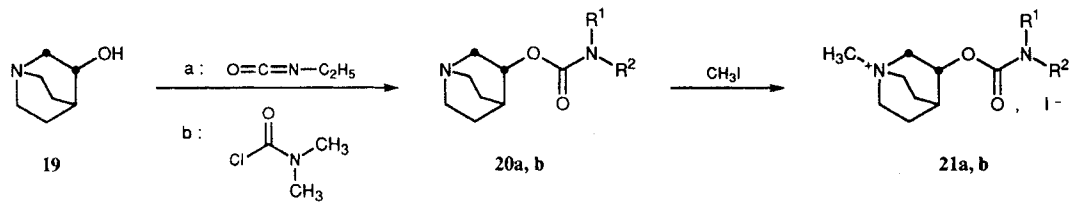


Chart 5. Synthesis of quinuclidinyl carbamate esters.

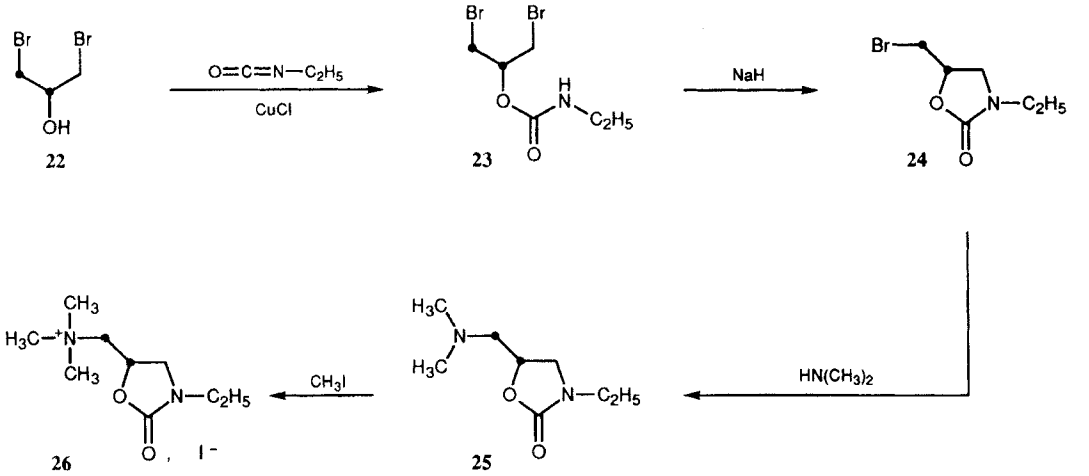


Chart 6. Synthesis of cyclic carbamate esters.

23, which, in turn, was cyclized under strongly basic conditions to give **24**, the precursor for **25**. The (*R*)- and (*S*)-forms of 1-methyl-2-(*N*,*N*-dimethylaminocarbonyloxymethyl)pyrrolidine, **17j** and **17k** (Chart 4), and the corresponding quaternized analogues, **18j** and **18k**, were synthesized using (*R*)- and (*S*)-proline, respectively, as starting materials. Some physical and chemical properties of all newly synthesized and some earlier described compounds are listed in Table 1.

Receptor Binding Studies and Estimation of Pharmacological Profile

Nicotinic ACh receptor binding studies were performed essentially as described using [^{3}H]nicotine as ligand[29]. In our unsuccessful attempts to use [^{3}H]MCC as a radioligand, we have used earlier described[18,30] experimental conditions and a variety of modifications of these procedures. Muscarinic ACh receptor binding studies were performed as described in detail elsewhere[22,31] using [^{3}H]Oxo-M, [^{3}H]QNB, and [^{3}H]PZ as ligands. The nicotinic/muscarinic (N/M) selectivity factors were calculated as the ratios between the IC_{50} values of the compounds as inhibitors of the receptor binding of [^{3}H]Oxo-M and [^{3}H]nicotine.

The *in vitro* pharmacological profiles of some selective muscarinic ligands were estimated on the basis of ligand receptor binding studies. PZ and QNB were used as relatively M_1-selective[13] and non-selective muscarinic antagonists, respectively. Oxo-M was used as a muscarinic agonist ligand in order to estimate the muscarinic agonist or partial agonist character of the compounds. The ratio between the K_i values of a compound determined in QNB (brain) and Oxo-M (brain) binding experiments was used as a muscarinic agonist index (M agonist index) of a compound. This method of estimating efficacy at muscarinic cholinergic receptors is analogous to that described earlier[21,22,31]. Agonist index values above 1500 reflect full agonism, whereas values of 20-200 and below predict partial agonism and antagonism, respectively, of muscarinic agents[21].

$$\text{M agonist index} = \frac{K_i\ (\text{QNB,brain})}{K_i\ (\text{Oxo-M,brain})} = \frac{IC_{50}\ (\text{QNB,brain})}{IC_{50}\ (\text{Oxo-M,brain})} \times\ 0.162$$

The ratio between K_i values of a compound determined in QNB (heart) and PZ (brain) binding experiments was used as an index of M_1-selectivity (M_2/M_1 index), higher values of this index indicating higher degrees of M_1-selectivity[20]. K_i values were calculated from IC_{50} values using the method of Cheng and Prusoff, and the factors of 0.162 and 0.125 comprise values of concentration and K_d of the ligands used in the respective binding experiments, as described previously in detail[20].

$$M_2/M_1 \text{ index} = \frac{K_i \text{ (QNB,heart)}}{K_i \text{ (PZ,brain)}} = \frac{IC_{50} \text{ (QNB,heart)}}{IC_{50} \text{ (PZ,brain)}} \times 0.125$$

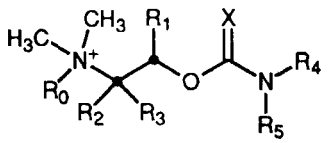
Structure-Activity Studies

Nicotine and carbacholine are highly selective ligands for nicotinic and muscarinic ACh receptor sites, showing N/M selectivity factors of 1400 and 0.007, respectively (Table 2). As described previously[17], DMCC is a selective nicotinic ligand, whereas MCC, which was introduced as a highly selective nicotinic ligand[14–16,18], was shown to bind to nicotinic and muscarinic receptor sites with comparable affinity, as indicated by a N/M selectivity factor of 6.5 (Table 2). In our hands, [^{3}H]MCC showed highly unusual binding curves under different experimental conditions including those described previously[18], and, as a consequence of this, affinity for nicotinic receptor sites was determined using [^{3}H]nicotine[29] as the only radioligand.

Structure-activity studies on a series of acyclic analogues of MCC or DMCC disclosed that quaternary compounds generally showed higher affinity for nicotinic as well as muscarinic ([^{3}H]Oxo-M binding data) receptor sites than the corresponding tertiary amines (Table 2). This difference is very pronounced for the nicotinic receptor affinity of the *N,N*-diethyl carbamate esters, **8e** and **9e**, the latter compound being a very potent nicotinic ligand. Whereas substitution of one methyl group for hydrogen at the C-atom α to the amino or quaternary ammonium groups had only minor effects on nicotinic receptor affinity, incorporation of two methyl groups into this position is only poorly tolerated by these receptor sites. Replacement of one hydrogen atom by a methyl group at the neighbouring C-atom completely abolished nicotinic receptor affinity, as shown for compounds **6b**, **7b**, **8c**, and **9c**. There is a marked difference between the potency of the test compounds as inhibitors of the binding of the agonist ligand, [^{3}H]Oxo-M, and the antagonist ligand, [^{3}H]QNB to muscarinic ACh receptor sites (Table 2).

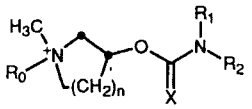
In Table 3, the relationship between structure and affinity for nicotinic or muscarinic receptor sites is illustrated. In the

Table 2. Effects of some acyclic carbacholine analogues on the binding of nicotinic and muscarinic ACh receptor ligands to rat brain membranes.

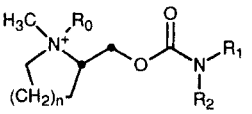


Compound	R0	R1	R2	R3	R4	R5	X	[^{3}H]Nicotine	[^{3}H]Oxo-M	[^{3}H]QNB	N/M selectivity factor
								IC_{50}, μM			
(*S*)-Nicotine								0.016	22	260	1400
Carbacholine (1)	CH_3	H	H	H	H	H	O	0.75	0.005	150	0.007
MCC (2)	CH_3	H	H	H	H	CH_3	O	0.023	0.15	270	6.5
4	H	H	H	H	CH_3	CH_3	O	5.7	18	620	3.2
DMCC (3)	CH_3	H	H	H	CH_3	CH_3	O	0.020	1.2	210	60
6a	H	H	H	H	H	C_2H_5	O	15	2.8	210	0.18
7a	CH_3	H	H	H	H	C_2H_5	O	0.16	0.72	81	4.5
6b	H	CH_3	H	H	H	C_2H_5	O	>100	38	410	<0.4
7b	CH_3	CH_3	H	H	H	C_2H_5	O	>100	3.6	61	<0.04
8c	H	CH_3	H	H	CH_3	CH_3	O	>100	81	790	<0.8
9c	CH_3	CH_3	H	H	CH_3	CH_3	O	>100	6.8	340	<0.07
8d	H	H	CH_3	H	CH_3	CH_3	O	2.3	11	240	4.8
9d	CH_3	H	CH_3	H	CH_3	CH_3	O	0.22	3.1	88	14
8e	H	H	H	H	C_2H_5	C_2H_5	O	5.6	5.2	68	0.93
9e	CH_3	H	H	H	C_2H_5	C_2H_5	O	0.046	0.57	29	12
8f	H	H	CH_3	CH_3	CH_3	CH_3	O	67	11	120	0.16
9f	CH_3	H	CH_3	CH_3	CH_3	CH_3	O	7.9	1.2	29	0.15
8g	H	H	H	H	CH_3	CH_3	S	47	12	280	0.26
9g	CH_3	H	H	H	CH_3	CH_3	S	1.9	2.5	95	1.3

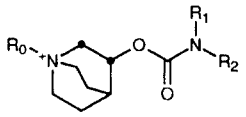
Table 3. Effects of some cyclic carbacholine analogues on the binding of nicotinic and muscarinic ACh receptor ligands to rat brain membranes.



Compound	R_0	R_1	R_2	X	*n*	[^{3}H]Nicotine IC_{50}, µM	[^{3}H]Oxo-M IC_{50}, µM	[^{3}H]QNB IC_{50}, µM	N/M selectivity factor
11c	H	CH_3	CH_3	0	1	>100	15	260	<0.15
12c	CH_3	CH_3	CH_3	0	1	15	3.6	110	0.24
13f	H	H	C_2H_5	0	1	>100	9.7	130	<0.1
14f	CH_3	H	C_2H_5	0	1	16	1.8	53	0.11
11d	H	CH_3	CH_3	0	2	>100	31	420	<0.3
12d	CH_3	CH_3	CH_3	0	2	9.4	7.6	250	0.81
11e	H	CH_3	CH_3	S	2	>100	39	180	<0.4
12e	CH_3	CH_3	CH_3	S	2	95	4.2	98	0.044
13g	H	H	C_2H_5	0	2	>100	2.2	38	<0.02
14g	CH_3	H	C_2H_5	0	2	91	3.4	100	0.037
13h	H	H	CH_3	0	2	>100	58	880	<0.6
14h	CH_3	H	CH_3	0	2	52	8.2	210	0.16



Compound	R_0	R_1	R_2	*n*	Stereo	IC_{50}, µM			
17j	H	CH_3	CH_3	1	*(R)*	0.52	3.6	64	6.9
18j	CH_3	CH_3	CH_3	1	*(R)*	0.19	1.3	46	6.8
17k	H	CH_3	CH_3	1	*(S)*	0.068	10	140	150
18k	CH_3	CH_3	CH_3	1	*(S)*	0.018	1.6	46	89
17l	H	CH_3	CH_3	2	*(RS)*	11	12	110	1.1
18l	CH_3	CH_3	CH_3	2	*(RS)*	0.76	2.2	55	2.9



Compound	R_0	R_1	R_2	IC_{50}, µM			
20a	H	H	C_2H_5	12	0.81	12	0.068
21a	CH_3	H	C_2H_5	32	2.1	26	0.066
20b	H	CH_3	CH_3	31	0.97	15	0.031
21b	CH_3	CH_3	CH_3	15	1.2	51	0.080
25				>100	>100	>1000	–
26				15	12	330	0.80

compounds shown in the upper and the lowest part of the table, the ethylene bridge of the molecules of MCC or DMCC has been incorporated into a pyrrolidine or quinuclidine ring, respectively. Interesting, all of these compounds, tertiary amines as well as the corresponding quaternary analogues, are very weak inhibitors of the binding of [^{3}H]nicotine. Furthermore, none of these compounds show impressive affinities for muscarinic receptor sites using [^{3}H]Oxo-M or [^{3}H]QNB as radioligands. In agreement with the data for compounds **6b**, **7b**, **8c**, and **9c** listed in Table 2, the latter series of receptor binding experiments generally provided very high IC_{50} values.

The compounds shown in the middle part of Table 3 are derived from MCC or DMCC by incorporation only of the C-atom neighbouring the ammonium groups into a pyrrolidine ring. Whereas the affinities of these compounds for muscarinic receptor sites generally are comparable with those of the other compounds listed in Table 3, they showed markedly

Table 4. Effects of some carbacholine analogues on the binding of muscarinic ACh receptor ligands to rat brain or heart membranes.

Compound	[^{3}H]QNB (brain)	[^{3}H]QNB (heart)	[^{3}H]PZ (brain)	[^{3}H]Oxo-M (brain)	M agonist index	M_2/M_1 index
	IC_{50},μM					
MCC (2)	270	12	21	0.15	290	0.071
DMCC (3)	210	51	15	1.2	28	0.43
7a	81	18	3.8	0.72	18	0.59
9e	29	11	1.7	0.57	8.2	0.81
9f	29	28	2.6	1.2	3.9	1.3
9g	95	35	12	2.5	6.1	0.36
12c	110	50	12	3.6	4.9	0.52
14f	53	26	4.1	1.8	4.8	0.79
21a	26	12	2.5	2.1	2.0	0.60
21b	51	19	4.7	1.2	6.9	0.51

higher affinity for nicotinic receptor sites. Thus, the high-affinity nicotinic receptor ligands, **17k** and **18k**, both having (*S*)-configuration, are very selective for this class of ACh receptor sites, the tertiary amine **17k** showing a N/M selectivity factor (150) an order of magnitude lower than that of nicotine (1400) (Table 2).

The compounds showing the highest affinity for muscarinic receptor sites, labelled by [^{3}H]Oxo-M, were subjected to more extensive receptor affinity testing in order to estimate their pharmacological profiles (Table 4). Whereas MCC (290) and DMCC (28) show M agonist index value predictive of partial agonism, the lower values for the rest of the compounds tested appear to reflect muscarinic antagonism, as described in an earlier section. Whereas the low M_2/M_1 index value for MCC (0.071) is predictive of a certain degree of M_2 selectivity, the relatively high value determined for compound **9f** (1.3) is indicative of a certain degree of M_1-selectivity of this compound. The M_2/M_1 index values for the rest of the analogues tested seem to indicate lack of significant M_1- or M_2-selectivity.

Discussion

There is a rapidly growing therapeutic interest in nicotinic ACh receptor ligands as novel agents for the treatment of AD, anxiety, and depression[32-35]. In AD, there may be a particular interest in nicotinic receptor ligands showing, in addition, a muscarinic M_2 antagonist profile.

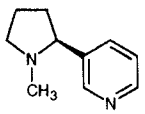
The work described in this paper represents the first step in our attempts to design therapeutically relevant nicotinic receptor ligands on a systematic and semirational basis. Whereas we have not been able to develop compounds combining high-affinity at nicotinic receptor sites and muscarinic M_2 antagonist profile, these structure-activity studies have shed important light on the structural requirement for effective binding to nicotinic receptor sites.

Studies on acyclic analogues of MCC or DMCC have disclosed that introduction of a methyl group at the C-atom neighbouring the oxygen atom of the choline moiety of the compounds results in complete loss of nicotinic receptor affinity, as exemplified by compounds **6b**, **7b**, **8c**, and **9c** (Table 2). Accordingly, the quinuclidine analogues **20a**, **21a**, **20b**, and **21b** and the cyclic carbamate esters **25** and **26**, all of which contain a ring residue in this position, and the compounds, in which the entire ethylene group is incorporated into a ring structure, are weak or inactive as inhibitors of [^{3}H]nicotine receptor binding (Table 3).

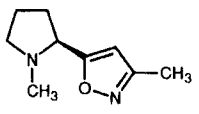
Introduction af a single methyl group into the methylene group neighbouring the ammonium groups is, on the other hand, not destructive for nicotinic receptor affinity, as exemplified by compounds **8d** and **9d** (Table 2). This observation prompted us to synthesize a number of pyrrolidine analogues of DMCC, in which only this particular C-atom was incorporated into the ring structure, and this approach led to compounds **17k** and **18k** as high-affinity and highly selective nicotinic receptor ligands (Table 3). From a pharmacological point of view, the tertiary amine structure of **17k**, the most selective nicotinic receptor ligand within this series of carbamate esters (Table 3), is particularly interesting.

In compounds **8g**, **9g** (Table 2), **11e**, and **12e** (Table 3), the carbonyl oxygen atoms of **4**, DMCC (**3**) (Table 2), **11d**, and **12d** (Table 3), respectively, have been replaced by sulphur. Whereas these C=O → C=S replacements do not affect muscarinic ACh receptor affinities significantly, such structural modifications quite markedly reduce affinities for nicotinic ACh receptor sites. Thus, whereas **11d** as well as **11e** are inactive as inhibitors of [^{3}H]nicotine binding, DMCC (**3**) is two orders of magnitude more potent than **9g**, and **4** and **12d** one order of magnitude more potent than their respective thioanalogues, **8g** and **12e**. These structure-activity relationships probably reflect that the carbonyl groups of nicotinic ACh agonists bind tightly to the recognition site(s) of nicotinic receptors[32].

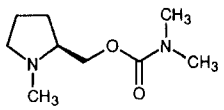
Nicotine and 3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole (ABT 418) (Chart 7) are, so far, the most extensively studied nicotinic agonists[32-35]. For both of these compounds, the nicotinic agonist effects reside in the (*S*)-enantiomers. The nicotinic receptor affinity of



(*S*)-Nicotine



(*S*)-ABT 418



17k

Chart 7. Structures of (*S*)-nicotine, (*S*)-ABT 418 and **17k**.

1-methyl-2-(*N,N*-dimethylaminocarbonyloxymethyl)pyrrolidine also resides in the (*S*)-enantiomer, **17k** (Chart 7), the (*R*)-enantiomer, **17j**, being an order of magnitude weaker as an inhibitor of [^{3}H]nicotine binding (Table 3). On the basis of these binding studies, it is not possible to conclude whether **17k** is an agonist, a partial agonist, or, perhaps, an antagonist at nicotinic receptors. Further studies are needed in order to establish the pharmacological profile of this compound. Studies along these lines and attempts to design and develop novel therapeutically interesting nicotinic receptor ligands on the basis of the present structure-activity studies are in progress.

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Experimental

Chemistry. General Procedures. Melting points were determined in capillary tubes and are uncorrected. Column chromatography (CC) was performed on silica gel 60 (70–230 mesh, ASTM, Merck).– ^{1}H NMR spectra were recorded for all compounds on a Bruker AC-200 F spectrometer and were consistent with the structures. Elemental analyses were within ± 0.4% of the theoretical values for C, H, and N, and when present, for I and S. The analyses were carried out by Mr. H. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Chemicals. The amino alcohols were commercially available except for **5d**[36] and **5f**[37], which were prepared by the literature methods.

Method A. 2-Dimethylaminoethyl N-Ethylcarbamate Hydrochloride (**6a**)

A solution of 2-dimethylaminoethanol (0.45 g, 5.0 mmol) and ethyl isocyanate (0.53 g, 7.5 mmol) in dry toluene (10 mL) was stirred at 50 °C for 24 h. After evaporation *in vacuo*, H_2O (15 mL) was added to the residue and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were dried and evaporated. The residue was dissolved in Et_2O (25 mL) and a 2.5 M solution of HCl in EtOAc was added to precipitate the hydrochloride salt. Recrystallization from MeCN gave 0.82 g (83%) of **6a**, mp 165–166 °C.

Method B. (RS)-1-Methyl-3-piperidyl N-Methylcarbamate fumarate (**13h**)

A solution of (*RS*)-1-methyl-3-hydroxypiperidine (0.34 g, 3.0 mmol) and methyl isocyanate (0.19 g, 3.2 mmol) in dry toluene (5 mL) was stirred for three days at room temp. After evaporation *in vacuo*, H_2O (15 mL) was added to the residue and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were dried and evaporated. CC of the residue (silica gel, triethylamine/EtOAc/light petroleum, 2:9:9) gave 0.34 g (66%) of the free base of **13h**. The fumarate salt was prepared by dissolving the base in Et_2O (15 mL) and adding a boiling solution of fumaric acid (0.23 g, 2.0 mmol) in *i*PrOH (4 mL). Recrystallization of the precipitate from *i*PrOH/Et_2O afforded 0.44 g (51%) of **13h**, mp 133–134 °C.

Method C. (RS)-2-Dimethylamino-1-propyl N,N-Dimethylcarbamate Fumarate (**8d**)

Sodium hydride (80% dispersion in mineral oil, 0.45 g, 15 mmol) was added portionwise to a solution of **5d**[36] (1.39 g, 13.5 mmol) in dry toluene (40 mL). After stirring for 4 h at room temp. *N,N*-dimethylcarbamoyl chloride (1.61 g, 15 mmol) was added and the mixture was stirred for 24 h at room temp. The reaction mixture was evaporated *in vacuo* and H_2O (40 mL) was added to the residue. The mixture was extracted with CH_2Cl_2 (3 × 50 mL) and the combined extracts were dried and evaporated. The residue was dissolved in Et_2O (30 mL) and a boiling solution of fumaric acid (1.56 g, 13.5 mmol) in *i*PrOH (15 mL) was added to precipitate the fumarate salt. Recrystallization from *i*PrOH/Et_2O gave 2.28 g (58%) of **8d**, mp 114–117 °C.

Method D. (RS)-1-Methyl-3-pyrrolidinyl N,N-Dimethylcarbamate Fumarate (**11c**)

The free base of the title compound was synthesized according to Method C from sodium hydride (80 % dispersion in mineral oil, 91 mg, 3.0 mmol), (*RS*)-1-methyl-3-hydroxypyrrolidine (276 mg, 2.73 mmol), *N,N*-dimethylcarbamoyl chloride (294 mg, 2.73 mmol) and toluene (10 mL). CC of the crude product (silica gel, triethylamine/EtOAc/light petroleum, 2:9:9) gave 245 mg (52%) of the free base of **11c**. The fumarate salt was prepared and recrystallized from *i*PrOH/Et_2O to give 291 mg (37%) of **11c**, mp 92–93 °C.

Method E. (RS)-2-(Dimethylaminocarbonyloxy)-1-N,N,N-trimethylpropanammonium Iodide (**9c**)

Sodium hydroxide (2 M, 5 mL) was cooled in ice and **8c** (0.50 g, 1.7 mmol) was added. The mixture was rapidly extracted with CH_2Cl_2 (3 × 10 mL). The combined extracts were dried and evaporated *in vacuo*. The residue was dissolved in EtOH (5 mL) and MeI (1.2 g, 8.5 mmol) was added. The mixture was stirred at 40 °C for 20 h and Et_2O (5 mL) was added to precipitate the title compound. Recrystallization from EtOH/Et_2O gave 0.35 g (66%) of **9c**, mp 205–207 °C.

(R)-1-Methyl-2-pyrrolidinemethanol (**16j**)

To a solution of (*R*)-pyrrolidine-2-carboxylic acid (1.5 g, 13 mmol) and K_2CO_3 (4.5 g, 32.6 mmol) in H_2O (7 mL) was added ethyl chloroformate (1.7 g, 15.6 mmol) dropwise during 20 min at 0 °C. The mixture was stirred at 0 °C for 1 h and at room temp. for 2 h. The mixture was acidified and extracted with CH_2Cl_2 (5 × 10 mL). The combined extracts were dried and evaporated to give 2.48 g (100%) of crude **15i** as an oil. ^{1}H NMR ($CDCl_3$): δ = 10.35 (br.s, 1H, COOH), 4.32 (m, 1H, NCH), 4.12 (m, 2H, OCH_2), 3.42 (m, 2H, NCH_2), 2.38–1.75 (m, 4H, 2 × CH_2), 1.21 (t, 3H, CH_3). A solution of crude **15i** (2.45 g, 13.4 mmol) in dry Et_2O (30 mL) was added dropwise during 20 min at room temp. to a suspension of $LiAlH_4$ (2.0 g, 53.9 mmol) in dry Et_2O (15 mL). The mixture was stirred at room temp. for 1 h and then refluxed for 15 h. Et_2O (100 mL) saturated with H_2O was added followed by a 5% aqueous solution of $NaHCO_3$ (45 mL). The mixture was stirred for 30 min and a 10% aqueous solution of Na_2CO_3 (5 mL) was added. The ethereal solution was decanted from the salt, which was further extracted with Et_2O (2 × 50 mL). The combined organic extracts were dried and evaporated to give 1.10 g of crude **16j**. Ball-tube distillation (2000 pa, 130 °C) gave 0.91 g (59%) of **16j** as an oil. $[\alpha]_D^{25}$ +47.3° (*c* = 1.4 in MeOH)[38]. ^{1}H NMR ($CDCl_3$): δ = 3.59 (dd, *J* = 11.0, 3.66 Hz, 1H, CH_2OH), 3.38 (dd, *J* = 11.0, 2.90 Hz, 1H, CH_2OH), 3.02 (m, 1H, NCH), 2.29 (s, 3H, CH_3), 2.25 (m, 2H, NCH_2), 1.90–1.58 (m, 4H, 2 × CH_2).

(±)-3-Quinuclidinyl N-Ethylcarbamate Fumarate (**20a**)

A mixture of (±)-3-hydroxyquinuclidine (0.50 g, 3.9 mmol), ethyl isocyanate (0.36 g, 5.0 mmol), triethylamine (0.05 mL) in dry DMF (20 mL) was

stirred at 50 °C for 24 h. After evaporation *in vacuo*, H_2O (15 mL) was added to the residue and the mixture was extracted with EtOAc (3 × 30 mL). The combined extracts were dried and evaporated. CC of the residue (silica gel,MeCN/AcOH/H_2O, 8:1:1) and extraction with EtOAc from aqueous K_2CO_3 gave 0.36 g (47%) of the free base **20a**. The fumarate salt was prepared by adding a boiling solution of the equivalent amount of fumaric acid to an ethereal solution of the free base. Recrystallization from *i*PrOH/Et_2O gave **20a**, 0.50 g (41%), mp 163–164 °C.

(±)-3-Quinuclidinyl N,N-Dimethylcarbamate Sesquifumarate **(20b)**

To a solution of (±)-3-hydroxyquinuclidine (0.50 g, 3.9 mmol) in dry DMF (20 mL) was added sodium hydride (80 % dispersion in mineral oil, 0.14 g, 4.7 mmol). The mixture was stirred at room temperature for 4 h and *N,N*-dimethylcarbamoyl chloride (0.50 g, 4.7 mmol) was added. After stirring for 24 h, the mixture was evaporated *in vacuo* and H_2O (30 mL) was added to the residue. Extraction with EtOAc (3 × 30 mL), drying and evaporation of the combined extracts gave an oil. CC as described above for compound **20a** gave 0.19 g (24%) of the free base of **20b**. Treatment of an ethereal solution of the free base with fumaric acid gave **20b**, 0.20 g (14%), mp 139–140 °C.

Method F. (±)-3-(Ethylaminocarbonyloxy)-1-methylquinuclidinium Iodide **(21a)**

MeI (0.85 g, 6.0 mmol) was added to a solution of the free base of **20a** (0.24 g, 1.2 mmol) in EtOH (3 mL). The mixture was stirred at 40 °C for 24 h. Et_2O (5 mL) was added and the precipitate was recrystallized from EtOH/Et_2O gave 0.37 g (91%) of **21a**, mp 118–120 °C.

1,3-Dibromo-2-propyl N-Ethylcarbamate **(23)**

To a mixture of 1,3-dibromopropan-2-ol (5.0 g, 23 mmol) and cuprous chloride (2.3 g, 23 mmol) in dry toluene (20 mL) was added dropwise during 10 min ethyl isocyanate (1.98 g, 25 mmol). After stirring for 2 h at room temp. the mixture was filtered and the filtrate evaporated *in vacuo* to give 6.52 g (98%) of **23** as an oil. ^{1}H NMR ($CDCl_3$): δ = 5.05 (quint, *J* = 5.1 Hz, 1H, CH), 4.89 (br. s, 1H, NH), 3.72–3.53 (m, 4H, 2 × CH_2Br), 3.23 (q, *J* = 5.0 Hz, 2H, NCH_2), 1.18 (t, *J* = 5.0 Hz, 3H, CH_3).

(RS)-5-Bromomethyl-3-ethyl-2-oxazolidinone **(24)**

To a suspension of sodium hydride (80 % dispersion in mineral oil, 1.38 g, 46 mmol) in dry THF (175 mL) was added **23** (6.5 g, 23 mmol). The mixture was stirred under nitrogen for 5 h and filtered. The filtrate was evaporated *in vacuo*. CC of the residue (silica gel, EtOAc/toluene, 1 : 20) gave 1.42 g (30%) of **24** as an oil. ^{1}H NMR ($CDCl_3$): δ = 4.68 (m, 1H, CH), 3.68 (t, *J* = 8.9 Hz, 1H, C4-H_2), 3.54 (dd, *J* = 10.1, 3.8 Hz, 1H, CH_2Br), 3.45 (dd, *J* = 10.1, 7.6 Hz, 1H, CH_2Br), 3.38 (dd, *J* = 8.9, 5.1 Hz, 1H, 4-H_2), 3.29 (q, *J* = 7.3 Hz, 2H, NCH_2), 1.14 (t, *J* = 7.3, Hz, 3H, CH_3).

(RS)-5-Dimethylaminomethyl-3-ethyl-2-oxazolidinone Hydrochloride **(25)**

A mixture of **24** (0.30 g, 1.4 mmol), sodium iodide (0.1 g), and dimethylamine (33% solution in EtOH, 4 mL, 22.8 mmol) was stirred at room temp. for 24 h. After evaporation, the residue was dissolved in 0.5 M HCl (10 mL) and the solution was washed with Et_2O (3 × 5 mL). Excess dilute sodium hydroxide was added to the aqueous phase and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined extracts were dried and evaporated *in vacuo*. The hydrochloride was prepared by dissolving the residue in a 2.5 M solution of HCl in EtOAc (3 mL) and adding Et_2O (10 mL). Recrystallization from EtOH/Et_2O gave 0.20 g (67%) of **25**, mp 193–194 °C.

Receptor Binding

Binding to muscarinic receptors was carried out essentially as described previously[31]. Briefly, rat brains were homogenized using a Teflon-glass homogenizer in 100 vol. (w/v) 10 mM sodium potassium phosphate buffer (pH 7.4) and diluted 1:10 with the same buffer. Aliquots (0.5 mg of tissue) were incubated with 0.12 nM [^{3}H]QNB (46 Ci/mmol, Amersham) alone or in the presence of test compound in a total volume of 5 mL for 30 min at 37 °C. Bound [^{3}H]QNB was separated from free ligand by rapid filtration through Whatman GF/B filters presoaked in 0.1% polyethyleneimine using a Brandel cell-harvester. The filters were rinsed twice with 3-mL aliquots of buffer and bound radioactivity estimated by liquid scintillation counting methods. On the basis of preliminary experiments, concentrations of test compounds showing 25–75% inhibition of radioligand binding were chosen for the determination of IC_{50} values. Each compound was tested in three different concentrations, and nonspecific binding estimated at 20 µM atropine. All estimations were made in triplicate, and each displacement experiment was repeated at least twice. IC_{50} values were determined using log-probit analyses.

Inhibition of [^{3}H]QNB binding to rat heart tissue was determined by the same procedure as described above with the exception that the tissue was homogenized in an Ultraturrax homogenizer and that 2 mg of tissue was used per assay.

Inhibition of [^{3}H]PZ binding to rat brain membranes was determined by a procedure analogous to that described above for [^{3}H]QNB binding: 3 mg of tissue were incubated with 1.0 nM [^{3}H]PZ (85 Ci/mmol, New England Nuclear) at 25 _C for 60 min in a total volume of 1.5 mL buffer. Nonspecific binding was estimated at 10 µM atropine.

The procedure for determination of inhibition of [^{3}H]Oxo-M binding to rat brain membranes was analogous to that described above for [^{3}H]QNB binding: 5 mg of tissue were incubated with 0.2 nM [^{3}H]Oxo-M (85 Ci/mmol, New England Nuclear) at 30 °C for 40 min in a total volume of 1.5 mL buffer. The reaction was stopped by adding 5 mL of ice-cold buffer, rapid filtration using a Brandel cell-harvester, and rinsing twice with 5-mL aliquots of buffer.

(*S*)-[^{3}H]Nicotine binding to cholinergic receptors in rat brain membranes was performed essentially as described previously[29]. Rat brains were homogenized (Ultraturrax) in 10 vol (w/v) buffer consisting of Na_2HPO_4, 8 mM; KH_2PO_4, 1.5 mM; KCl, 3 mM; NaCl, 120 mM; EDTA, 2 mM; HEPES, 20 mM; and iodoacetamide, 5mM (pH 7.4). The homogenate was centrifuged (50.000 × g; 20 min; 0 °C) and the pellet resuspended in 10 vol. cold standard assay buffer with the same composition as the buffer preparation described above, except for the addition of $MgCl_2$, 1mM and $CaCl_2$, 2 mM, and the elimination of EDTA and iodoacetamide. Aliquots (0.1 mg of tissue) were incubated with 5 nM (*S*)-[^{3}H]nicotine (78 Ci/mmol, Amersham) alone or in the presence of test compound in a total volume of 0.6 mL for 60 min at 0 °C. Incubation was terminated by adding 5 mL of ice-cold sodium potassium phosphate buffer (50 mM, pH 7.4) followed by rapid filtration through Whatman GF/B filters presoaked in 0.1% polyethyleneimine using a Brandel cell-harvester. Filters were washed with three 5-mL aliquots of cold sodium potassium phosphate buffer, and bound radioactivity estimated by liquid scintillation counting methods. Each compound was tested in three different concentrations, and nonspecific binding estimated at 0.5 mM nicotine-H-tartrate. All estimations were made in triplicate, and each displacement experiment was repeated at least three times.

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