Eur J Med Chem (1995) 30, 61–69

Novel potent and m_2-selective antimuscarinic compounds which penetrate the blood–brain barrier

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(Received 18 January 1994; final version received and accepted 29 September 1994)

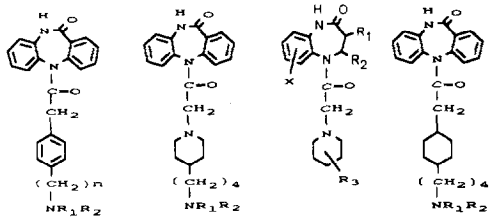
Summary — A series of 5-[[[(dialkylamino)alkyl]-1-phenyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-ones **1** were prepared as potential m_2-selective ligands. The binding affinities and selectivities of these compounds for the muscarinic cholinergic receptor subtypes were determined. The best m_2-selective antimuscarinic agent studied was 5-[[4-[4-(diisobutylamino)butyl]-1-phenyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one **1h** (DIBD), which caused a significant reduction in (R,R)-3-quinuclidinyl-[^{125}I]-4-iodobenzilate ((R,R)-[^{125}I]-4IQNB) binding in brain regions known to contain a high percentage of m_2-receptors. Thus DIBD penetrates the blood–brain barrier and exhibits *in vivo* selectivity for the m_2 subtype. In contrast, neither DIBA, AF-DX 116, nor AQ-RA 741 caused a significant m_2-selective reduction in (R,R)-[^{125}I]-4IQNB binding in the brain regions studied.

m_2-selective antimuscarinic agent / blood–brain barrier

Introduction

In a continuation of our effort to develop potent m_2-selective ligands, we have synthesized a number of derivatives of 5-[[[(dialkylamino)alkyl]-1-piperidinyl]-acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-ones **2** [1]. The data demonstrate that 5-[[4-[4-(diethylamino)butyl]-1-piperidinyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one **2**, (R = R' = ethyl; DIBA) is the most potent compound in the series. This compound is almost nine times as selective for m_2-receptors than for m_1-receptors. These results also demonstrate that DIBA (IC_{50} = 3.25 nM, table I; K_i = 0.3 nM [2]) is more potent at m_2 receptors than other reported compounds, such as AQ-RA 741 (IC_{50} = 7.69 nM; table I), AF-DX 116 (K_i = 73.4 nM) [2], and BIBN 99 (K_i = 30 nM) [3]. However, DIBA cannot cross the blood–brain barrier (BBB) sufficiently to function as a potential *in vivo* m_2 radiotracer [1].

These results suggest that we must increase the lipophilicity further or reduce the size of the molecule. Consequently, we decided to reduce the size of the



1 2 3 4

tricycle and prepare substituted 1,5-benzodiazepines **3** [4]. However, this reduction in size resulted in a loss of affinity. An increase in lipophilicity resulting from replacement of the piperidine ring in **2** with cyclohexane **4** does not impart BBB permeability [5]. Most recently, on the basis of existing structure–activity relationships, we have designed and synthesized a series of m_2-selective antagonists **1** (by replacing the piperidine ring in **2** with benzene) capable of crossing the BBB. The best m_2-selective antimuscarinic agent in this series is 5-[[4-[4-(diisobutylamino)butyl]-1-phenyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]-diazepin-11-one **1h** (DIBD).

Table I. IC_{50} of 5-[[4-[4-(dialkylamino)butyl]-1-phenyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-ones **1a–n**, QNB, DIBA and AQ-RA 741.

Compound	R_1	R_2	*n*	IC_{50} m_1 *(nM)*	IC_{50} m_2 *(nM)*	*Selectivity* IC_{50} m_1/IC_{50} m_2
1a	C_2H_5	C_2H_5	2	78.73	44.95	1.75
1b	*i*-C_4H_9	*i*-C_4H_9	3	1491.00	326.10	4.57
1c	CH_3	CH_3	4	65.00	20.00	3.25
1d	C_2H_5	C_2H_5	4	30.00	6.00	5.00
1e	*n*-C_3H_7	*n*-C_3H_7	4	35.00	6.50	5.38
1f	*i*-C_3H_7	*i*-C_3H_7	4	23.00	3.00	7.66
1g	*n*-C_4H_9	*n*-C_4H_9	4	25.00	10.00	2.50
1h	*i*-C_4H_9	*i*-C_4H_9	4	23.00	3.50	6.57
1i	CH_3	*n*-C_3H_7	4	23.50	5.00	4.70
1j	CH_3	CH_2=CH-CH_2-	4	29.00	7.00	4.14
1k	CH_3	CH≡C-CH_2-	4	13.00	6.25	2.08
1l	*i*-C_5H_{11}	*i*-C_5H_{11}	4	45.00	19.00	2.36
1m	C_2H_5	C_2H_5	5	14.82	12.66	1.71
1n	*i*-C_4H_9	*i*-C_4H_9	5	563.40	112.36	5.01
QNB				0.663	0.754	0.878
DIBA				30.00	3.25	9.23
AQ-RA 741				32.50	7.69	4.22

Chemistry

Compounds **1a–n** (tables II and III) were prepared according to scheme 1. Reaction of 4-(bromoalkyl)-phenylacetic acid **5a–d** with thionyl chloride, followed by condensation with 11-oxo-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepine **6**, yielded the 5-[[4-(bromoalkyl)-1-phenyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-ones **7a–d** (tables IV and V). The reaction of **7a–d** with secondary aliphatic amines provided **1a–n**.

Pharmacological results

Compounds **1a–n** and three reference compounds were tested for their apparent affinities at m_1 and m_2 subtypes (table I). The IC_{50} values were determined in displacement studies of [^{3}H]-3-quinuclidinyl benzilate ([^{3}H]QNB) binding to membranes from m_1-transfected cells or from heart tissue (for m_2). These data demonstrate that **1h** (DIBD) is one of the most potent compounds in the series (table I). This compound is selective for m_2 receptors over m_1 receptors by almost 7-fold.

Table II. Data on 5-[[4-[4-(dialkylamino)alkyl]-1-phenyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-ones **1a–n**.

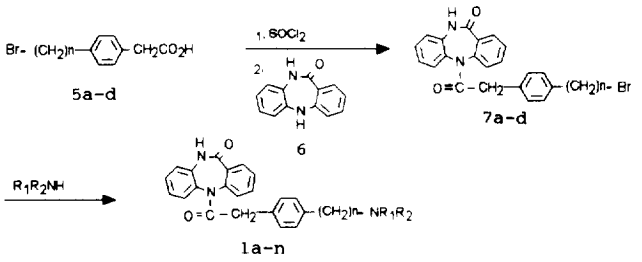
Compound	R_1	R_2	*n*	*Yield (%)*	*Mp (°C)*	R_f^a	*Formula*
1a	C_2H_5	C_2H_5	2	62	53–57	0.60	$C_{27}H_{29}N_3O_2$
1b	*i*-C_4H_9	*i*-C_4H_9	3	55	55–59	0.36[b]	$C_{32}H_{39}N_3O_2$[g]
1c	CH_3	CH_3	4	57	50–53	0.45	$C_{27}H_{29}N_3O_2$
1d	C_2H_5	C_2H_5	4	57	45–48	0.47	$C_{29}H_{33}N_3O_2$[h]
1e	*n*-C_3H_7	*n*-C_3H_7	4	52	35–38	0.48[c]	$C_{31}H_{37}N_3O_2$
1f	*i*-C_3H_7	*i*-C_3H_7	4	38	54–56	0.50	$C_{31}H_{37}N_3O_2$[i]
1g	*n*-C_4H_9	*n*-C_4H_9	4	57	30–32	0.60[d]	$C_{33}H_{41}N_3O_2$
1h	*i*-C_4H_9	*i*-C_4H_9	4	17	50–54	0.40[b]	$C_{33}H_{41}N_3O_2$[j]
1i	CH_3	*n*-C_3H_7	4	55	45–50	0.32[e]	$C_{29}H_{33}N_3O_2$[k]
1j	CH_3	CH_2=CH-CH_2-	4	51	32–35	0.54[d]	$C_{29}H_{31}N_3O_2$
1k	CH_3	HC≡C-CH_2-	4	56	48–53	0.53[f]	$C_{29}H_{29}N_3O_2$
1l	*i*-C_5H_{11}	*i*-C_5H_{11}	4	31	35–39	0.38[f]	$C_{35}H_{45}N_3O_2$
1m	C_2H_5	C_2H_5	5	56	42–44	0.43	$C_{30}H_{31}N_3O_2$[l]
1n	*i*-C_4H_9	*i*-C_4H_9	5	59	40–43	0.45[b]	$C_{34}H_{43}N_3O_2$

[a]MeOH/NH_4OH (98:2); [b]$CHCl_3$/MeOH (20:1); [c]EtOAc/MeOH (1:1); [d]EtOAc/MeOH (2:1); [e]$CHCl_3$/MeOH (10:1); [f]EtOAc; [g]C: calc, 77.23, found, 76.78; [h]C: calc, 76.45, found, 75.67; [i]C: calc, 76.99, found, 76.24; [j]C: calc, 77.46, found, 76.89; [k]C: calc, 76.45, found, 75.77; [l]C: calc, 76.73, found, 76.03.

Table III. ^{1}H-NMR data on 5-[[4-[4-(dialkylamino)butyl]-1-phenyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-ones **1a–n**.

Compound	*^{1}H-NMR (δ)*[a]
1a	8.94 (1H, br), 7.91 (1H, d, J = 7.7 Hz), 7.55 (1H, t, J = 7.0 Hz), 7.38 (4H, m), 7.22 (1H, m), 6.99 (4H, m), 6.83 (1H, t, J = 7.2 Hz), 3.65 (2H, s), 2.68 (4H, q, J = 7.1 Hz), 2.65 (4H, m), 1.03 (6H, t, J = 7.1 Hz)
1b	8.31 (1H, br), 7.96 (1H, d, J = 7.6 Hz), 7.59 (1H, t, J = 7.5 Hz), 7.37 (4H, m), 7.23 (1H, m), 7.02 (4H, m), 6.91 (1H, d, J = 7.1 Hz), 3.62 (2H, s), 2.59 (2H, m), 2.33 (2H, t, J = 6.9 Hz), 2.04 (4H, d, J = 7.2 Hz), 1.66 (4H, m), 0.87 (12H, d, J = 6.6 Hz)
1c	9.04 (1H, br), 7.93 (1H, d, J = 7.7 Hz), 7.55 (1H, t, J = 7.6 Hz), 7.38 (4H, m), 7.18 (1H, m), 7.02 (4H, m), 6.88 (1H, d, J = 7.5 Hz), 3.62 (2H, s), 2.47 (2H, t, J = 7.0 Hz), 2.18 (2H, t, J = 7.2 Hz), 2.16 (6H, s), 1.52 (2H, m), 1.40 (2H, m)
1d	8.89 (1H, br), 7.93 (1H, d, J = 7.6 Hz), 7.54 (1H, t, J = 7.7 Hz), 7.36 (4H, m), 7.21 (1H, m), 7.02 (4H, m), 6.87 (1H, d, J = 7.5 Hz), 3.6 (2H, s), 2.48 (4H, q, J = 7.2 Hz), 2.47 (2H, t, J = 7.2 Hz), 2.37 (2H, t, J = 7.3 Hz), 1.53 (2H, m), 1.43 (2H, m), 0.98 (5H, t, J = 7.2 Hz)
1e	8.98 (1H, br), 7.94 (1H, d, J = 7.6 Hz), 7.57 (1H, t, J = 7.5 Hz), 7.38 (4H, m), 7.20 (1H, m), 7.01 (4H, m), 6.89 (1H, d, J = 7.5 Hz), 3.61 (2H, s), 2.50 (2H, t, J = 6.8 Hz), 2.32 (4H, t, J = 7.3 Hz), 2.32 (2H, m), 1.51 (2H, m), 1.41 (4H, sextet, J = 7.3 Hz), 1.39 (2H, m), 0.83 (6H, t, J = 7.3 Hz)
1f	9.33 (1H, br), 7.96 (1H, d, J = 7.7 Hz), 7.59 (1H, t, J = 7.6 Hz), 7.43 (2H, m), 7.32 (2H, m), 7.22 (1H, m), 7.08 (4H, m), 6.93 (1H, d, J = 7.9 Hz), 3.63 (2H, m), 2.97 (2H, sept, J = 6.5 Hz), 2.48 (2H, t, J = 7.5 Hz), 2.33 (2H, t, J = 7.3 Hz), 1.5 (2H, m), 1.40 (2H, m), 0.97 (12H, d, J = 6.5 Hz)
1g	8.49 (1H, br), 7.93 (1H, d, J = 7.6 Hz), 7.56 (1H, t, J = 7.4 Hz), 7.35 (4H, m), 7.20 (1H, m), 7.03 (4H, m), 6.86 (1H, d, J = 7.4 Hz), 3.61 (2H, s), 2.51 (1H, t, J = 6.8 Hz), 2.37 (4H, t, J = 7.0 Hz), 2.37 (2H, m), 1.53 (2H, m), 1.38 (6H, m), 1.26 (4H, sextet, J = 7.2 Hz), 0.88 (6H, t, J = 7.2 Hz)
1h	8.33 (1H, br), 7.94 (1H, d, J = 7.4 Hz), 7.59 (1H, t, J = 7.7 Hz), 7.38 (4H, m), 7.23 (1H, m), 7.01 (4H, m), 6.88 (1H, d, J = 6.9 Hz), 3.63 (2H, s), 2.53 (2H, t, J = 7.2 Hz), 2.30 (2H, t, J = 7.0 Hz), 2.02 (4H, d, J = 7.2 Hz), 1.63 (4H, m), 1.42 (2H, m), 0.85 (12H, d, J = 6.6 Hz)
1i	8.66 (1H, br), 7.89 (1H, d, J = 7.2 Hz), 7.56 (1H, t, J = 7.4 Hz), 7.38 (4H, m), 7.20 (1H, m), 7.03 (4H, m), 6.82 (1H, d, J = 7.2 Hz), 3.62 (2H, s), 2.52 (2H, t, J = 7.2 Hz), 2.34 (4H, m), 2.22 (3H, s), 1.53 (2H, m), 1.46 (4H, m), 0.86 (3H, t, J = 7.3 Hz)
1j	8.67 (1H, br), 7.93 (1H, d, J = 7.6 Hz), 7.56 (1H, t, J = 7.3 Hz), 7.35 (4H, m), 7.20 (1H, m), 7.04 (4H, m), 6.85 (1H, d, J = 7.2 Hz), 5.83 (1H, m), 5.13 (2H, m), 3.63 (2H, s), 2.96 (2H, d, J = 6.6 Hz), 2.52 (2H, t, J = 7.2 Hz), 2.30 (2H, t, J = 7.3 Hz), 2.16 (3H, s), 1.55 (2H, m), 1.45 (2H, m)
1k	8.75 (1H, br), 7.91 (1H, d, J = 7.2 Hz), 7.57 (1H, t, J = 7.5 Hz), 7.36 (4H, m), 7.20 (1H, m), 7.02 (4H, m), 6.83 (1H, d, J = 7.2 Hz), 3.63 (2H, s), 3.31 (2H, d, J = 2.2 Hz), 2.52 (2H, t, J = 7.2 Hz), 2.38 (2H, t, J = 7.5 Hz), 2.27 (3H, s), 2.19 (1H, t, J = 2.2 Hz), 1.55 (2H, m), 1.40 (2H, m)
1l	8.26 (1H, br), 7.93 (1H, d, J = 7.4 Hz), 7.56 (1H, t, J = 6.7 Hz), 7.35 (4H, m), 7.20 (1H, m), 6.99 (4H, m), 6.85 (1H, d, J = 6.9 Hz), 3.61 (2H, s), 2.53 (2H, t, J = 7.2 Hz), 2.42 (6H, m), 1.50 (6H, m), 1.30 (4H, m), 0.86 (12H, d, J = 6.5 Hz)
1m	8.52 (1H, br), 7.96 (1H, d, J = 7.6 Hz), 7.59 (1H, t, J = 7.5 Hz), 7.39 (4H, m), 7.22 (1H, m), 7.03 (4H, m), 6.91 (1H, d, J = 7.2 Hz), 3.62 (2H, s), 2.59 (2H, t, J = 7.2 Hz), 2.51 (4H, q, J = 7.1 Hz), 2.39 (2H, t, J = 7.7 Hz), 1.58 (2H, m), 1.45 (2H, m), 1.27 (2H, m), 1.01 (6H, t, J = 7.1 Hz)
1n	8.30 (1H, br), 7.95 (1H, d, J = 7.6 Hz), 7.59 (1H, t, J = 7.4 Hz), 7.36 (4H, m), 7.23 (1H, m), 7.01 (4H, m), 6.90 (1H, d, J = 7.2 Hz), 3.62 (2H, s), 2.52 (2H, m), 2.27 (2H, t, J = 7.0 Hz), 2.02 (4H, d, J = 7.1 Hz), 1.65 (4H, m), 1.36 (4H, m), 0.85 (12H, d, J = 6.6 Hz)

*The ^{1}H-NMR spectra were obtained in deuterochloroform solution.



Scheme 1.

Compound **1h** (DIBD) was studied *in vivo*. The percentage change in (R,R)-3-quinuclidinyl-[^{125}I]-4-iodobenzilate ((R,R)-[^{125}I]-4IQNB) in specific brain regions induced by unlabeled **1h** is depicted in table VI. At a dose of 1000 nmol, **1h** caused a statistically significant reduction of (R,R)-[^{125}I]-4IQNB (table VI). This is seen in regions containing a high percentage of m_2 receptors [6–9] (table VII). The effect was especially clear in the superior and inferior colliculi, pons, and medulla. At a higher dose (3000 nmol) the effect of **1h** was enhanced (table VIII). Comparison of these results with the ineffectiveness of compound **4** indicates that a planar ring in the cationic head may be an important factor.

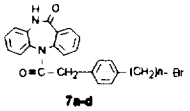
The above results were obtained when dimethylformamide (DMF) and emulphor were contained in the solvent used to dissolve **1h**. DMF and emulphor (table VI), in comparison with normal saline and ethanol (table IX), exhibit a slight elevation in the % dose/g in the controls. In contrast to the results with DMF and emulphor, 1000 nmol of unlabeled **1h** in normal saline containing 65% ethanol caused a uniform decrease in (R,R)-[^{125}I]-4IQNB binding in all brain regions, indicating either a statistical fluctuation in the % dose/g between animals or a possible nonspecific effect upon delivery of (R,R)-[^{125}I]-4IQNB. There is no evidence for *in vivo* m_2 selectivity (table IX).

Table IV. Data on 5-[4-[(bromoalkyl)-1-phenyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-ones **7a–d**.

Compound	*n*	*Yield (%)*	*Mp (°C)*	R_f^a	*Formula*
7a	2	50	69–73	0.71[b]	$C_{23}H_{19}BrN_2O_2$[c]
7b	3	54	70–72	0.56	$C_{24}H_{21}BrN_2O_2$[d]
7c	4	66	70–73	0.52	$C_{25}H_{23}BrN_2O_2$
7d	5	48	66–68	0.60	$C_{26}H_{25}BrN_2O_2$[e]

[a]Hexane/ethyl acetate (1:1); [b]chloroform/methanol (10:1); [c]Br: calc 18.36, found 19.01; [d]Br: calc 17.78, found 18.35; [e]Br: calc 16.74, found 17.28.

Table V. ^{1}H-NMR data on 5-[4-[(bromoalkyl)-1-phenyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-ones **7a–d**.



Compound	*n*	*^{1}H-NMR (δ)*[a]
7a	2	8.87 (1H, br), 7.94 (1H, d, J = 7.7 Hz), 7.60 (1H, t, J = 7.3 Hz), 7.38 (4H, m), 7.23 (1H, m), 7.03 (4H, m), 6.89 (1H, d, J = 7.5 Hz), 3.68 (1H, m), 3.67 (2H, s), 3.53 (1H, m), 2.99 (2H, m)
7b	3	8.56 (1H, br), 7.95 (1H, d, J = 7.8 Hz), 7.61 (1H, t, J = 7.8 Hz), 7.42 (4H, m), 7.23 (1H, m), 7.04 (4H, m), 6.96 (1H, m), 3.65 (2H, s), 3.48 (1H, t, J = 6.3 Hz), 3.36 (1H, m), 2.68 (2H, m), 2.10 (2H, m)
7c	4	8.81 (1H, br), 7.95 (1H, d, J = 8.1 Hz), 7.61 (1H, t, J = 7.6 Hz), 7.40 (4H, m), 7.23 (1H, m), 7.07 (4H, m), 6.94 (1H, m), 3.64 (2H, s), 3.37 (2H, t, J = 6.7 Hz), 2.53 (2H, m), 1.75 (4H, m)
7d	5	8.20 (1H, br), 7.93 (1H, d, J = 7.7 Hz), 7.60 (1H, t, J = 7.8 Hz), 7.39 (4H, m), 7.22 (1H, m), 7.01 (4H, m), 6.89 (1H, m), 3.63 (2H, s), 3.40 (2H, t, J = 6.8 Hz), 2.54 (2H, m), 1.86 (2H, m), 1.61 (2H, m), 1.45 (2H, m)

[a]The ^{1}H-NMR spectra were obtained in deuterochloroform solution.

Table VI. Percentage change in (R,R)-[^{125}I]-4IQNB in specific brain regions by unlabeled 5-[[4-[4-(diisobutylamino)butyl]-1-phenyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one **1h** (DIBD).

Brain region	*% Dose/G*		p^c	*% Change*	
	Control[a]	*1000 nmol DIBD*[b]		*Total*[d]	*Specific*[e]
csr	0.29 ± 0.025	0.28 ± 0.005	0.3528	–4	–6
csl	0.29 ± 0.025	0.29 ± 0.005	0.8453	–1	–1
ccr	0.30 ± 0.025	0.29 ± 0.006	0.5592	–2	–3
ccl	0.30 ± 0.025	0.29 ± 0.007	0.7492	–2	–2
thr	0.20 ± 0.017	0.19 ± 0.005	0.2129	–7	–11
thl	0.20 ± 0.018	0.19 ± 0.005	0.2476	–6	–9
cb	0.09 ± 0.007	0.07 ± 0.002	0.0001	–23	NA
sc	0.19 ± 0.016	0.15 ± 0.004	0.0001	–21	–31
ic	0.19 ± 0.016	0.14 ± 0.004	0.0001	–26	–40
hipp	0.23 ± 0.019	0.23 ± 0.005	0.6743	2	3
pons	0.17 ± 0.014	0.14 ± 0.004	0.0001	–19	–31
med	0.14 ± 0.011	0.11 ± 0.003	0.0001	–18	–35

[a]Control value ± standard error of the mean; [b]DIBD was dissolved in distilled water containing 10–13% DMF and 10–13% emulphor; [c]$p \leq 0.05$ was considered a significant difference; [d]computed as (DIBD region – control region)/control region x 100%; [e]computed as [(DIBD region – DIBD cb) – (control region – DIBD cb)]/(control region – DIBD cb) x 100%. Abbreviations: left corpus striatum (csl), right corpus striatum (csr), hippocampus (hipp), left cerebral cortex (ccl), right cerebral cortex (ccr), left thalamus (thl), right thalamus (thr), medulla (med), pons (pons), cerebellum (cb), inferior colliculus (ic), and superior colliculus (sc).

Table VII. Subtype concentrations (nM) in brain regions*.

	csl/csr	*hipp*	*ccl/ccr*	*thl/thr*	*med*	*pons*	*cb*
m_1	72.79	76.14	70.38	13.76	2.60	2.60	0.42
m_2	30.12	27.54	39.33	36.12	36.40	36.40	15.75
m_3	16.32	16.20	20.70	5.16	2.86	2.86	1.05
m_4	112.95	30.78	49.68	17.20	2.60	2.60	0.53

*Computed from the data reported in refs [6–9]. Superior and inferior colliculi were not determined by Wolfe but we assume that their composition is similar to that of pons and medulla. For abbreviations see table VI.

Table VIII. Percentage change in (R,R)-[^{125}I]-4IQNB in specific brain regions by unlabeled 5-[[4-[4-(diisobutylamino)butyl]-1-phenyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one **1h** (DIBD).

Brain region	*% Dose/G*		p^c	*% Change*	
	Control[a]	*3000 nmol DIBD*[b]		*Total*[d]	*Specific*[e]
csr	0.30 ± 0.007	0.26 ± 0.004	0.0059	–12	–15
csl	0.31 ± 0.012	0.25 ± 0.005	0.0056	–18	–23
ccr	0.29 ± 0.010	0.26 ± 0.007	0.0644	–10	–14
ccl	0.29 ± 0.010	0.26 ± 0.008	0.0910	–9	–12
thr	0.21 ± 0.008	0.16 ± 0.007	0.0035	–22	–34
thl	0.20 ± 0.012	0.16 ± 0.005	0.0135	–21	–32
cb	0.08 ± 0.004	0.07 ± 0.003	0.0391	–17	NA
sc	0.19 ± 0.006	0.11 ± 0.007	0.0001	–41	–64
ic	0.21 ± 0.014	0.13 ± 0.005	0.0008	–40	–60
hipp	0.24 ± 0.011	0.22 ± 0.009	0.2911	–7	–10
pons	0.18 ± 0.007	0.11 ± 0.004	0.0001	–36	–60
med	0.14 ± 0.007	0.10 ± 0.003	0.0048	–30	–62

[a]Control ± standard error of the mean; [b]DIBD was dissolved in distilled water containing 10–13% DMF and 10–13% emulphor; [c]$p \leq 0.05$ was considered a significant difference; [d]computed as (DIBD region – control region)/control region x 100%; [e]computed as [(DIBD region – DIBD cb) – (control region – DIBD cb)]/(control region – DIBD cb) x 100%. For abbreviations see table VI.

Table IX. Percentage change in (R,R)-[^{125}I]-4IQNB in specific brain regions by unlabeled 5-[[4-[4-(diisobutylamino)butyl]-1-phenyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-one **1h** (DIBD).

Brain region	*% Dose/G*		*p*[c]	*% Change*	
	Control[a]	*1000 nmol DIBD*[b]		*Total*[d]	*Specific*[e]
csr	0.35 ± 0.010	0.29 ± 0.015	0.0135	–17	–22
csl	0.36 ± 0.010	0.31 ± 0.013	0.0320	–13	–17
ccr	0.34 ± 0.007	0.29 ± 0.008	0.0061	–13	–17
ccl	0.35 ± 0.008	0.30 ± 0.007	0.0028	–14	–19
thr	0.25 ± 0.008	0.22 ± 0.009	0.0341	–13	–19
thl	0.25 ± 0.005	0.22 ± 0.009	0.0163	–13	–20
cb	0.10 ± 0.005	0.08 ± 0.002	0.0079	–20	NA
sc	0.22 ± 0.006	0.19 ± 0.006	0.0037	–15	–24
ic	0.23 ± 0.007	0.18 ± 0.005	0.0008	–19	–30
hipp	0.27 ± 0.010	0.25 ± 0.015	0.2593	–9	–13
pons	0.22 ± 0.005	0.19 ± 0.005	0.0021	–14	–22
med	0.18 ± 0.006	0.16 ± 0.004	0.0202	–12	–23

[a]Control ± standard error of the means; [b]DIBD was dissolved in distilled water containing 67.5% ethanol; [c]$p \leq 0.05$ was considered a significant difference; [d]computed as (DIBD region – control region)/control region x 100%; [e]computed as [(DIBD region – DIBD cb) – (control region – DIBD cb)]/(control region – DIBD cb) x 100%. For abbreviations see table VI.

DMSO (table X), used in the studies with AF-DX 116, in comparison with normal saline and ethanol (table XI), exhibits a slight decrease in the % dose/g in the controls, indicating either a statistical fluctuation in the % dose/g between animals or a possible nonspecific effect upon delivery of (R,R)-[^{125}I]-4IQNB. DIBA, AF-DX 116, and AQ-RA 741 (1000 nmol each) were studied *in vivo*. With DIBA, there was no significant change in (R,R)-[^{125}I]-4IQNB binding (table XI). This result confirmed an earlier finding using a different animal model and experimental procedure [1]. There was little or no decrease in (R,R)-[^{125}I]-4IQNB binding with AF-DX 116 (table X) or AQ-RA 741 (table XII). The change was no greater than 17% and no m_2 selectivity was seen.

Conclusion

In conclusion, two potential approaches for increasing BBB penetration have previously failed. Our findings show that a reduction in size and molecular weight (**3**) resulted in a loss of affinity. An increase in lipophilicity (by replacing the piperidine ring in **2** with cyclohexane (**4**)) does not enhance *in vivo* binding [5]. However, a new approach described here, replacing the nonplanar cyclohexane ring (> 99% chair-shaped) in the cationic head in **4** with a benzene ring, resulted in a series of compounds (**1**) in which the ring of the cationic head has a planar shape. The best m_2-selective antimuscarinic agent studied was 5-[[4-[4-(diisobutylamino)butyl]-1-phenyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-one **1h** (DIBD), which caused a significant reduction in (R,R)-[^{125}I]-4IQNB binding in brain regions known to contain a high percentage of m_2 receptors. Thus DIBD penetrates the blood–brain barrier and exhibits *in vivo* selectivity for the m_2 subtype.

Experimental protocols

Chemistry

The melting points were obtained on a Fisher-John apparatus. ^{1}H-NMR spectra were recorded on a Bruker AC-300 instrument and are expressed as parts per million (δ) from internal tetramethylsilane. DIBA **2** (R = R' = ethyl) was prepared as reported [1]. AF-DX 116 [10], AQ-RA 741 [1, 10] were synthesized using literature procedures.

*General procedure for the preparation of 5-[4-[(Bromoalkyl)-1-phenyl]acetyl]-10,11-dihydro-5H-dibenzo[b,e][1,4]diazepin-11-ones **7a–d**. 5-[[4-[2-(Bromo)ethyl]-1-phenyl]acetyl]-10,11-dihydro-5H-dibenzo[b,e][1,4]diazepin-11-one **7a***

To the 4-[2-(bromo)ethyl]phenylacetic acid **5a** (12.15 g, 0.05 mol) in 100 ml chloroform, was added thionyl chloride (30 ml), and the mixture was heated at reflux for 3 h. The solvent and the excess thionyl chloride were evaporated under reduced pressure. To the residue was added 11-oxo-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepine **6** (10.5 g, 0.05 mol), *N,N*-dimethylaniline (3 ml) and THF (100 ml), and the mixture was refluxed for 5 h. The mixture was evaporated to dryness under reduced pressure. A potassium bicarbonate solution was added and the mixture was extracted with chloroform. The chloroform extract was washed with water, dried over sodium sulfate, and evaporated to dryness under reduced pressure. Purification by flash chromatography on silica gel, using ethyl acetate/hexane 1:1 as eluent, provided the product.

Table X. Percentage change in (R,R)-[^{125}I]-4IQNB in specific brain regions by unlabeled AF-DX 116.

Brain region	*% Dose/G*		*p*[c]	*% Change*	
	Control[a]	*1000 nmol AF-DX 116*[b]		*Total*[d]	*Specific*[e]
csr	0.34 ± 0.022	0.30 ± 0.009	0.1812	–12	–13
csl	0.33 ± 0.022	0.30 ± 0.006	0.2693	–10	–10
ccr	0.33 ± 0.022	0.30 ± 0.004	0.4234	–7	–7
ccl	0.32 ± 0.022	0.30 ± 0.007	0.5841	–5	–5
thr	0.19 ± 0.010	0.17 ± 0.004	0.2471	–8	–9
thl	0.19 ± 0.012	0.17 ± 0.004	0.2221	–10	–11
cb	0.02 ± 0.003	0.02 ± 0.001	0.3875	–13	NA
sc	0.14 ± 0.008	0.12 ± 0.003	0.1571	–11	–13
ic	0.13 ± 0.008	0.12 ± 0.001	0.3077	–8	–10
hipp	0.27 ± 0.017	0.25 ± 0.002	0.3016	–8	–9
pons	0.13 ± 0.008	0.13 ± 0.003	0.4910	–5	–6
med	0.08 ± 0.005	0.08 ± 0.002	0.5897	–4	–5

[a]Control ± standard error of the mean; [b]AF-DX 116 was dissolved in 2.5X PBS with 10% DMSO and 4% 1 N HCl; [c]$p \leq 0.05$ was considered a significant difference; [d]computed as (AF-DX region – control region)/control region x 100%; [e]computed as [(AF-DX region – AF-DX cb) – (control region – AF-DX cb)]/(control region – AF-DX cb) x 100%. For abbreviations see table VI.

Table XI. Percentage change in (R,R)-[^{125}I]-4IQNB in specific brain regions by unlabeled DIBA.

Brain region	*% Dose/G*		*p*[c]	*% Change*	
	Control[a]	*1000 nmol DIBA*[b]		*Total*[d]	*Specific*[e]
csr	0.45 ± 0.025	0.42 ± 0.027	0.5460	–6	–7
csl	0.43 ± 0.030	0.42 ± 0.026	0.7449	–4	–4
ccr	0.43 ± 0.022	0.43 ± 0.022	0.8910	–1	–1
ccl	0.44 ± 0.018	0.42 ± 0.025	0.7833	–3	–3
thr	0.24 ± 0.006	0.23 ± 0.011	0.7547	–2	–3
thl	0.24 ± 0.002	0.23 ± 0.011	0.5778	–4	–5
cb	0.03 ± 0.000	0.03 ± 0.002	0.4705	–8	NA
sc	0.17 ± 0.002	0.17 ± 0.010	0.9876	0	0
ic	0.16 ± 0.002	0.17 ± 0.011	0.5427	7	8
hipp	0.34 ± 0.019	0.35 ± 0.023	0.9519	1	1
pons	0.16 ± 0.003	0.16 ± 0.007	0.6420	–3	–4
med	0.11 ± 0.002	0.10 ± 0.005	0.3328	–8	–10

[a]Control ± standard error of the mean; [b]DIBA was dissolved in 60% ethanol, 40% normal saline; [c]$p \leq 0.05$ was considered a significant difference; [d]computed as (DIBA region – control region)/control region x 100%; [e]computed as [(DIBA region – DIBA cb) – (control region – DIBA cb)]/(control region – DIBA cb) x 100%. For abbreviations see table VI.

General procedure for the preparation of 5-[[4[(dialkylamino) alkyl]-1-phenyl]acetyl]-10,11-dihydro-5H-dibenzo[b,e][1,4]-diazepin-11-ones ***1a–n****. 5-[[4-[4(Diisobutylamino)butyl-1-phenyl]-acetyl]-10,11-dihydro-5H-dibenzo[b,e][1,4]diazepin-11-one* ***1h*** *(DIBD)*

5-[[4-[4-(Bromo)butyl]-1-phenyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-one **7c** (2.32 g, 5 mmol), diisobutylamine (3 ml) and sodium carbonate (3 g), in acetonitrile (40 ml), were refluxed for 5 h. The solvent was removed under reduced pressure and the residue was partitioned between chloroform/water. The organic layer was separated, washed with water, and dried over sodium sulfate. The solvent was removed under reduced pressure and the residue purified by preparative TLC using chloroform/methanol, 20:1 (for the first purification), and then 40:1 (for the second purification) as eluent.

Biological evaluation

In vitro studies

Tissue preparation. Membranes containing m_1 receptors were isolated from CHO cells that had been transfected with these genes. These transfected cell lines were obtained from M Brann (NIH) and were grown as previously described [11]. The CHO cells expressing m_2 receptors expressed relatively low densities of this receptor, and were therefore not used. Instead, rat heart was used as the source of m_2 receptor.

Cell membranes were prepared by lysing cells or heart in 10 mM Tris-HCl, pH 7.2, 2 mM EDTA. Membranes were resuspended in the Tris/EDTA buffer at a protein concentration of 3 mg/ml and stored at –70°C until used.

Table XII. Percentage change in (R,R)-[^{125}I]-4IQNB in specific brain regions by unlabeled AQ-RA 741.

Brain region	*% Dose/G*		*p*[c]	*% Change*	
	Control[a]	*1000 nmol AQ-RA 741*[b]		*Total*[d]	*Specific*[e]
csr	0.29 ± 0.013	0.32 ± 0.019	0.3565	11	14
csl	0.30 ± 0.012	0.32 ± 0.016	0.4729	8	11
ccr	0.30 ± 0.012	0.30 ± 0.014	0.7944	–1	–0.97
ccl	0.30 ± 0.012	0.30 ± 0.012	0.9332	0	0.52
thr	0.20 ± 0.007	0.21 ± 0.007	0.7371	3	4
thl	0.20 ± 0.009	0.21 ± 0.007	0.8309	3	4
cb	0.09 ± 0.006	0.07 ± 0.001	0.2133	–17	NA
sc	0.19 ± 0.007	0.19 ± 0.005	0.5974	–3	–5
ic	0.19 ± 0.007	0.18 ± 0.007	0.3462	–6	–10
hipp	0.23 ± 0.010	0.27 ± 0.009	0.1561	17	24
pons	0.18 ± 0.008	0.17 ± 0.007	0.5067	–5	–8
med	0.14 ± 0.007	0.13 ± 0.005	0.4254	–7	–14

[a]Control ± standard error of the mean; [b]AQ-RA was dissolved in deionized water containing 10% DMF and 10% emulphor; [c]$p \leq 0.05$ was considered a significant difference; [d]computed as (AQ-RA region – control region)/control region x 100%; [e]computed as [(AQ-RA region – AQ-RA cb) – (control region – AQ-RA cb)]/(control region – AQ-RA cb) x 100%. For abbreviations see table VI.

Determination of IC_{50} values. The IC_{50} values for the muscarinic ligands were determined by competitive ligand binding assay with [^{3}H]QNB as the radiotracer. Competition curves were generated with 12 concentrations of unlabeled compounds: 10^{-12} to 10^{-6} M. The compounds were dissolved in 100% EtOH and added to 4 ml tris(hydroxymethyl)aminomethane-buffered (10 mM, pH 7.4) and 0.9% saline containing 2.5 x 10^{-10} M [^{3}H]QNB at a final concentration of 0.5% EtOH. Aliquots (0.1 ml) of cell membrane were added, and the mixture was vortexed and incubated at 30°C for 2 h. The incubation mixture was rapidly filtered on a GF/C filter paper. The filter was washed with 15 ml ice-cold saline, air dried, placed in Cytoscint (ICN Biomedicals, Inc) scintillation cocktail, and counted for 2 min each. Data were analyzed with the LIGAND program of Munson and Rodbard [12]. The IC_{50} values were obtained from pooled data of at least 2 determinations in duplicate on separate days (table I).

In vivo studies

Radiopharmaceuticals. (R,R)-[^{125}I]-4IQNB was prepared by two different methods, as follows.

1. An exchange procedure using a kit formulated by Nordion International Inc, Canada. Sodium [^{125}I]iodide was supplied with the kit. Purification of the labeled product was carried out using a C-18 Sep-pack cartridge. Radiochemical purity was determined by thin-layer chromatography. A labeling yield of 25% was obtained. The specific activity of (R,R)-[^{125}I]-4IQNB was approximately 450 Ci/mmol. It was determined by comparing the radioactivity specifically bound to a caudate nucleus homogenate to that of [^{3}H]QNB of known specific activity [13].
2. The triazene method of Cohen *et al* [13] was used to determine the specific activity, which was approximately 900 Ci/mmol. It was previously [14] incorrectly stated that the (R,R)-[^{125}I]-4IQNB used in the DIBD experiments was prepared using the triazene [13] method.

General experimental procedures. Male Sprague–Dawley rats weighing 200–250 g were used in the experiments. Animals were anesthetized with Ketamine/xylazine (100:10 mg/kg either ip or im) and the right jugular vein was exposed for intravenous injection of all compounds. Animals were maintained under anesthesia until time of sacrifice. At the end of each study, the animals were sacrificed by decapitation, the brains were rapidly removed, blotted free of excess blood and placed on ice. Tissue samples (20–70 mg) of specific brain regions were counted for 2 min in an autogamma counter (GammaTrac 1193, Tm Analytic; Elk Grove Village, IL, USA) with a counting efficiency of 78% for ^{125}I.

The brain regions of interest included the cerebral cortex, corpus striatum, thalamus, hippocampus, superior colliculus, inferior colliculus, pons, medulla, and cerebellum. In order to determine if there were any differences in the *in vivo* accumulation of (R,R)-[^{125}I]-4IQNB between the right and left cerebral hemispheres, the left and right cerebral cortex, corpus striatum, and thalamus were dissected and studied as separate entities.

The (R,R)-[^{125}I]-4IQNB injected dose per gram (% dose/g) of tissue (wet weight) was calculated and the mean ± the SEM of each group was obtained. The mean value of the experimental and control groups was used to determine the percentage change in (R,R)-[^{125}I]-4IQNB binding in specific rat brain regions by the unlabeled compounds.

Experimental design

DIBD. Two different experimental protocols were performed. In *Protocol 1* animals were injected with a single bolus of 1000 or 3000 nmol of **1h** (DIBD) in a final volume of 0.1 ml distilled water containing 10% *N,N*-dimethylformamide (DMF) (Aldrich Chemical Co Inc, Milwaukee, WI, USA) and 10% emulphor EL-620 (Rhone-Poulenc, Cranbury, NJ, USA) or the solvent alone. Immediately after the injection of **1h**, animals received a bolus injection of (R,R)-[^{125}I]-4IQNB (prepared by the exchange method; 6 μCi in a final volume of 0.10 ml normal saline containing up to 55% ethanol). Animals were sacrificed after 1 h.

In *Protocol 2* animals were coinjected with a single bolus of the m_2-selective compound (5-[[4-[4-(diisobutylamino)butyl]-1-phenyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-one **1h**, DIBD; 1000 nmol) or normal saline mixed with

(R,R)-[^{125}I]-4IQNB (prepared by the exchange method; 8 μCi in a final volume of 0.25 ml normal saline containing up to 65% ethanol) into the exposed jugular vein. Animals were sacrificed after 1 h.

Four to 12 rats were used per reported result unless otherwise stated (tables VI, VIII and IX).

DIBA. Animals were injected with a single bolus of DIBA (1000 nmol) in a final volume of 0.10 ml distilled water containing 60% ethanol or the solvent alone. One hour after the injection of DIBA, the animals received a bolus injection of (R,R)-[^{125}I]-4IQNB (prepared by the triazene method; 10 μCi in a final volume of 0.10 ml normal saline containing up to 10% ethanol). The animals were sacrificed after 3 h. Three control animals and 5 animals receiving DIBA were used (table XI).

AF-DX 116. Animals were injected with a single bolus of AF-DX 116 (1000 nmol) in a solvent suggested by J Baumgold (personal communication). This solvent consisted of a final volume of 0.10 ml 2.5X PBS (Biofluids Inc, Rockville, MD, USA) containing 10% dimethylsulfoxide (DMSO) (Fisher Scientific Company, Pittsburgh, PA) and 4% 1 N HCl (Fisher Scientific Company, Fairlawn, NJ, USA). Control rats received the solvent alone. One hour after the injection of AF-DX 116, the animals received a bolus injection of (R,R)-[^{125}I]-4IQNB (prepared by the triazene method; 10 μCi in a final volume of 0.10 ml normal saline containing up to 10% ethanol). The animals were sacrificed after 3 h. Four control animals and 4 animals receiving AF-DX 116 were used (table X).

AQ-RA 741. Animals were injected with a single bolus of AQ-RA 741 (1000 nmol) in a final volume of 0.10 ml distilled water containing 10% *N,N*-dimethylformamide (DMF) (Aldrich Chemical Co, Inc, Milwaukee, WI, USA) and 10% emulphor EL-620 (Rhone-Poulenc, Cranbury, NJ, USA) or the solvent alone. Immediately after the injection of AQ-RA, the animals received a bolus injection of (R,R)-[^{125}I]-4IQNB (prepared by the triazene method; 20 μCi in a final volume of 0.09 ml normal saline containing up 25% ethanol). The animals were sacrificed after 1 h. Four animals received AQ-RA (table XII).

The unpaired Student *t*-test was used to compare differences between the control and the experimental animals. A value of $p < 0.05$ was considered a significant difference.

Acknowledgments

This work was supported in part by grant NS22215 from the National Institutes of Health, and by contract DEFG05 88ER60649 from the Department of Energy.

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