Synthesis of Some Dibenzodiazepinone Derivatives as Potent and m2-Selective Antimuscarinic Compounds

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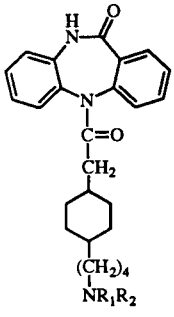
Received August 5, 1993
Revised January 20, 1994

Two series of 5-[[4-[4-(dialkylamino)butyl]-1-cyclohexyl]acetyl], and 5-[(dialkylamino)acyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-ones were synthesized as potential m2-selective ligands **1**,**2**. Their affinity and selectivity for the muscarinic cholinergic receptor *m*-AChR subtypes were determined. Replacing a nitrogen with CH in the piperidine ring of 5-[[4-[4-(dialkylamino)butyl]-1-piperidinyl]acetyl]-10,11-dihydro-5*H*-dibenzo-[*b*,*e*][1,4]diazepin-11-ones **3** significantly altered the affinity and selectivity to the muscarinic receptor subtypes.

J. Heterocyclic Chem., **31**, 787 (1994).

Recent molecular biological studies have demonstrated five distinct subtypes of *m*-AChR, each of which has been found to be localized to discrete areas of the brain or peripheral tissues [1-3]. These five subtypes are distinct proteins, in some cases having no more than 50% homology in their amino acid sequence [1,3]. Furthermore, the mRNA for the five subtypes are differentially distributed in the brain, as shown by *in situ* hybridization studies [1] and have distinct pharmacological [4] and biochemical [5] profiles. Since 3-quinuclidinyl 4-iodobenzilate (4-IQNB) binds to each of these receptor subtypes with a similar affinity [6] we are continuing to develop a new generation of subtype-selective radioligands that can specifically be used for the tomographic imaging of each of these receptor subtypes. In our current work, we have focused on developing an m2-selective radioligand.

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 [7]. 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 **3**, (R = ethyl) is the most potent compound in the series but it does not sufficiently cross the blood-brain barrier [7].



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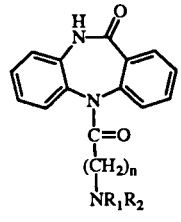

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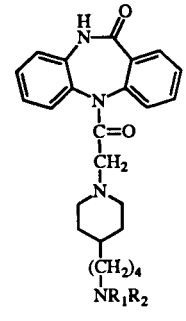


3

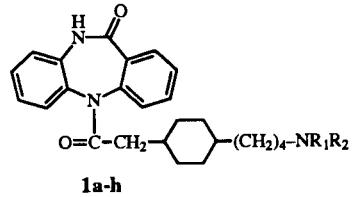
We therefore focused our effort on increasing the lipophilicity further. This was accomplished by formulating a bioisoster through replacing the nitrogen of the piperidine ring with carbon **1** or by replacing the piperidine ring with a straight hydrocarbon chain **2**.

Chemistry.

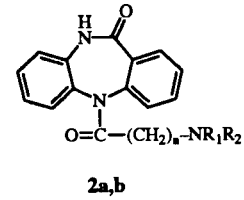
Reaction of 4-[4-(chloro)butyl]cyclohexylacetic acid (**4**), 10-chlorodecanoic acid (**5a**), and 11-bromoundecanoic

Table 1

Data on 5-[[4-[4-(Dialkylamino)butyl]-1-cyclohexyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-ones **1a-h** and 5-[(Dialkylamino)-acyl]-10,11-dihydro5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-ones **2a,b**



1a-h

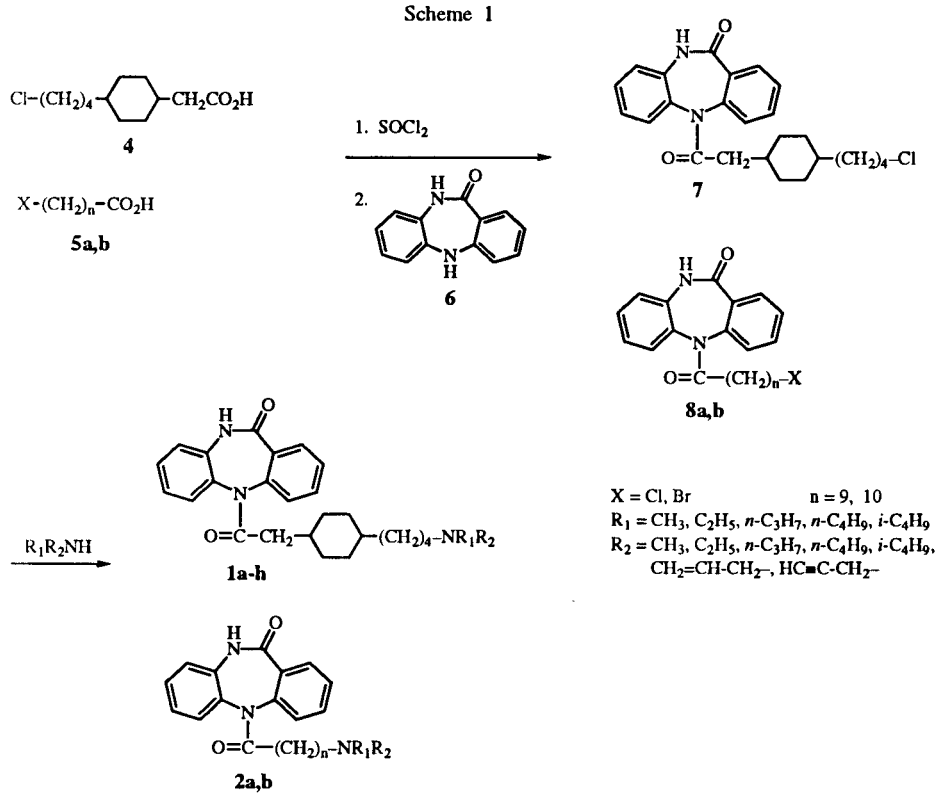


2a,b

Product	R_1	R_2	n	yield (%)	mp °C	R_f [a]
1a	CH_3	CH_3		49	73-76	0.51
1b	C_2H_5	C_2H_5		67	53-55	0.56
1c	n-C_3H_7	n-C_3H_7		32	46-49	0.50 [b]
1d	n-C_4H_9	n-$C4H_9$		49	29-33	0.55 [c]
1e	i-C_4H_9	i-C_4H_9		37	52-55	0.79 [d]
1f	CH_3	n-C_3H_7		70	48-52	0.21 [e]
1g	CH_3	CH_2=CH-CH_2-		51	38-41	0.47 [f]
1h	CH_3	HC≡C-CH_2-		59	62-66	0.45 [g]
2a	C_2H_5	C_2H_5	9	82	24-27	0.57
2b	C_2H_5	C_2H_5	10	68	45-48	0.57

[a] Methanol/ammonium hydroxide (98:2). [b] Ethyl acetate/methanol (4:1). [c] Ethyl acetate/methanol 10:1). [d] Ethyl acetate/hexane (1:1). [e] Ethyl acetate/ methanol (1:1). [f] Ethyl acetate/methanol (3:1). [g] Ethyl acetate.

Scheme 1



acid (**5b**) with thionyl chloride, followed by condensation with 11-oxo-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepine (**6**), provided 5-[[4-[4-(chloro)butyl]-1-cyclohexyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one (**7**), 5-(10-chlorodecanoyl)-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one (**8a**), and 5-(11-bromoundecanoyl)-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one (**8b**). Subsequent reaction of **7**, **8a**, and **8b** with secondary amines yielded compounds **1a-h** and **2a,b** (Scheme I and Table 1 and 2).

Results and Discussion.

We have recently reported the synthesis and evaluation of 5-[[[(dialkylamino)alkyl]-1-piperidinyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-ones [7]. Among these, compound **3**, (R = ethyl) is the most potent m2-selective antagonist known to date, but it did not cross the blood-brain barrier sufficiently to function as a potential muscarinic receptor radiotracer [7]. To increase the lipophilicity of this compound, we replaced the nitrogen in the piperidine ring with carbon. However, this change led to a significant reduction in selectivity (Table 3). Compounds **1a-h, 2a,b** and three reference compounds (Table 3) were tested for their apparent affinities for the m1 and m2 subtypes. The IC_{50}'s for the muscarinic ligands were determined by competitive ligand binding assay against [^{3}H]QNB. These data demonstrate that several of the compounds in the series are equally potent and m2-selective.

The data (Table 4) reveal that neither preinjection nor coinjection of the m2-selective compound **1b** at a dose of 500 nmoles or 2000 nmoles per animal affects the regional (*R,R*)-[^{125}I]IQNB accumulation in the rat brain. The use of *N,N*-dimethylformamide instead of ethanol as the solvent for dissolving the compound did not affect the results (Table 4).

Efforts to synthesize related compounds with increased lipophilicity and/or reduced size are underway, and will be reported in subsequent articles.

EXPERIMENTAL

The melting points were obtained on a Fisher-John apparatus. The ^{1}H nmr spectra were recorded on a Bruker AC-300 instrument and are expressed as parts per million (δ) from internal tetramethylsilane. Diethylamine, dibutylamine, diisobutylamine, dipropylamine, and *N,N*dimethylformamide were obtained from Aldrich.

11-Oxo-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepine (**6**).

It was prepared by method reported by Giani *et al.* [8], and recrystallized from *n*-butanol.

Table 2

NMR, and Elemental Analytical Data on 5-[[4-[4-(Dialkylamino)butyl]-1-cyclohexyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-ones **1a-h**, and 5-[(Dialkylamino)acyl]-10, 11-dihydro-5*H*-dibenzo[b,e][1,4]diazepin-11-ones 2a,b

Compound	^{1}H NMR: δ [a]	Formula	Elemental Analysis Calcd/Found		
			C	H	N
1a	1.17 (m, 7H), 1.39 (m, 8H), 2.02 (m, 1H), 2.21 (s, 6H), 2.24 (m, 4H), 7.22 (m, 2H), 7.35 (m, 2H), 7.44 (m, 2H), 7.60 (m, 1H), 7.98 (m, 1H), 9.04 (br, 0.5H), 9.50 (br, 0.5H)	$C_{27}H_{35}N_3O_2$	74.79 74.36	8.14 8.20	9.69 9.45
1b	1.01 (t, J = 7.1 Hz, 6H), 1.17 (m, 6H), 1.37 (m, 8H), 1.69 (m, 1H), 2.09 (m, 1H), 2.27 (m, 2H), 2.38 (m, 2H), 2.51 (q, J = 7.1 Hz, 4H), 7.24 (m, 2H), 7.33 (m, 2H), 7.46 (m, 2H), 7.59 (m, 1H), 7.99 (m, 1H), 10.21 (br, 1H)	$C_{29}H_{39}N_3O_2$	75.45 74.98	8.51 8.80	9.10 9.11
1c	0.86 (t, J = 7.1 Hz, 6H), 1.17 (m, 6H), 1.43 (m, 12H), 1.72 (m, 1H), 2.05 (m, 1H), 2.32 (m, 8H), 7.24 (m, 2H), 7.38 (m, 4H), 7.59 (m, 1H), 8.00 (m, 1H), 9.70 (br, 0.5H), 10.15 (br, 0.5H)	$C_{31}H_{43}N_3O_2$	76.04 75.75	8.85 9.00	8.58 8.39
1d	0.90 (t, J = 7.1 Hz, 6H), 1.17 (m, 7H), 1.26 (m, 6H), 1.36 (m, 9H), 1.71 (m, 1H), 2.04 (m, 1H), 2.29 (m, 2H), 2.39 (m, 6H), 7.27 (m, 2H), 7.33 (m, 2H), 7.46 (m, 2H), 7.59 (m, 1H), 8.00 (m, 1H), 10.01 (br, 1H)	$C_{33}H_{47}N_3O_2$	76.55 76.15	9.15 9.22	8.12 7.93
1e	0.85 (d, J = 6.5 Hz, 12H), 1.15 (m, 7H), 1.65 (m, 2H), 1.70 (m, 1H), 2.02 (d, J = 7.2 Hz, 4H), 2.05 (m, 1H), 2.25 (m, 4H), 7.23 (m, 2H), 7.42 (m, 4H), 7.59 (m, 1H), 8.00 (m, 1H), 9.14 (br, 0.5H), 9.57 (br, 0.5H)	$C_{33}H_{47}N_3O_2$	76.55 76.32	9.15 9.27	8.12 8.02
1f	0.88 (t, J = 7.3 Hz, 3H), 1.21 (m, 7H), 1.46 (m, 9H), 1.70 (m, 1H), 2.08 (m, 1H), 2.20 (s, 3H), 2.25 (m, 6H), 7.23 (m, 2H), 7.35 (m, 2H), 7.46 (m, 2H), 7.58 (m, 1H), 8.00 (m, 1H), 10.45 (br, 1H)	$C_{29}H_{39}N_3O_2$	75.45 75.08	8.51 8.63	9.10 8.96
1g	1.16 (m, 8H), 1.33 (m, 6H), 1.70 (m, 1H), 2.09 (m, 1H), 2.19 (s, 3H), 2.25 (m, 4H), 2.98 (m, 2H), 5.15 (m, 2H), 5.86 (m, 1H), 7.22 (m, 2H), 7.31 (m,2H), 7.43 (m, 2H), 7.58 (m, 1H), 7.98 (m, 1H), 8.67 (br, 0.5 H), 9.08 (br, 0.5H)	$C_{29}H_{37}N_3O_2$	75.78 75.47	8.11 8.10	9.14 8.98
1h	1.22 (m, 7H), 1.38 (m, 7H), 1.69 (m, 1H), 2.01 (m, 1H), 2.20 (s, 3H), 2.34 (m, 3H), 3.33 (d, J = 2.4 Hz, 2H), 7.25 (m, 2H), 7.34 (m, 2H), 7.44 (m, 2H), 7.59 (m, 1H), 8.00 (m, 1H), 9.08 (br, 0.5H), 9.51 (br, 0.5H)	$C_{29}H_{35}N_3O_2$	76.12 75.80	7.71 7.78	9.18 9.13
2a	1.03 (t, J = 7.2 Hz, 6H), 1.21 (br, 10H), 1.41 (m, 2H), 1.59 (m, 2H), 2.18 (m, 1H), 2.41 (m, 3H), 2.55 (q, J = 7.2 Hz, 4H), 7.23 (m, 2H), 7.40 (m, 4H), 7.60 (t, J = 7.3 Hz, 1H), 7.98 (m, 1H), 9.10 (br, 1H)	$C_{27}H_{37}N_3O_2$	74.44 74.23	8.56 8.60	9.64 9.52
2b	1.01 (t, J = 7.2 Hz, 6H), 1.20 (br, 12H), 1.40 (m, 2H), 1.60 (m, 2H), 2.19 (m, 1H), 2.40 (m, 3H), 2.52 (q, J = 7.2 Hz, 4H), 7.24 (m, 2H), 7.41 (m, 4H), 7.60 (t, J = 7.4 Hz, 1H), 7.98 (m, 1H), 9.72 (br, 1H)	$C_{28}H_{39}N_3O_2$	74.79 74.57	8.74 8.75	9.34 9.26

[a] The ^{1}H nmr spectra were obtained in deuteriochloroform solution.

5-[[4-[4-(Chloro)butyl]-1-cyclohexyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-one (**7**).

To a solution of 4-[4-(chloro)butyl]cyclohexylacetic acid [9] (**4**, 8.5 g, 0.038 mole), in chloroform (100 ml), was added 15 ml of thionyl chloride. The mixture heated at reflux for 3 hours. The solvent and the excess of 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**, 7.73 g, 0.037 mole), *N,N*-dimethylaniline (2.7 ml), tetrahydrofuran (150 ml), and the mixture was refluxed for 5 hours. The reaction mixture was evaporated to dryness, potassium bicarbonate solution was added and stirring was continued for 10 minutes. The solid was filtered, and washed with water and petroleum ether. The residue was purified by flash column chromatography (elution with hexane/ethyl acetate first 10:1 and then 1:1 and finally with 100% ethyl acetate or methanol). Yield 9.6 g (62%), mp 65-67°; tlc [silica gel, chloroform/methanol (20:1)] Rf 0.77; ^{1}H nmr (deuteriochloroform): 0.86 (m, 1H), 1.16 (m, 4H), 1.40 (m, 6H), 1.71 (m, 4H), 2.04 (m, 1H), 2.25 (m, 2H), 3.49 (t, J = 6.7 Hz, 2H), 7.21 (m, 3H), 7.40 (m, 3H), 7.40 (m, 3H), 7.60 (m, 1H), 7.97 (m, 1H), 8.73 (br, 0.5H), 9.20 (br, 0.5H).

Anal. Calcd. for $C_{25}H_{29}ClN_2O_2$: C, 70.66; H, 6.88; N, 6.59; Cl, 8.34. Found: C, 70.66; H, 7.02; N, 6.53; Cl, 8.08.

Table 3

IC_{50} Values of 5-[[4-[4-(Dialkylamino)butyl]-1-cyclohexyl]-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-ones **1**, 5-[(Dialkylamino)acyl]-10,11-dihydro-5*H*-dibenzo[*b,e*] [1,4]diazepin-11-ones 2,5-[[4-[4-(Diethylamino)butyl]-1-piperidinyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]-diazepin-11-one (**3** , R = ethyl), 11-[[4-[4-(Dethylamino)butyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (AQ-RA 741), and 3-Quinuclidinyl Benzilate (QNB)

Compound	IC_{50}ml (nM)	IC_{50}m2 (nM)	Selectivity IC_{50}ml/IC_{50}m2
1a	75.0	15.00	5.00
1b	29.3	7.33	3.99
1c	26.5	7.75	3.42
1d	45.0	8.00	5.62
1e	31.5	10.00	3.15
1f	27.5	6.50	4.23
1g	27.5	7.25	3.79
1h	30.0	7.00	4.29
2a	40.0	13.00	3.08
2b	40.0	18.00	2.22
3 (R = ethyl)	30.0	3.25	9.23
AQ-RA 741	32.5	7.69	4.22
QNB	0.663	0.754	0.878

Table 4

In Vivo (*R,R*)-[^{125}I]IQNB Brain Accumulation as a Function of the Injection of the m2-Selective Compound **1b**

Brain Region	Dose (nmole) 500[a] m2	500[a] control	2000[a] m2	2000[a] control	2000[b] m2	2000[b] control
left corpus striatum	0.3345	0.3176	0.2408	0.2283	0.2842	0.2682
	0.0330	0.0474	0.0043	0.0187	0.0185	0.0108
right corpus striatum	0.3290	0.3061	0.2609	0.2354	0.2931	0.2755
	0.0258	0.0382	0.0013	0.0143	0.0291	0.0201
hippocampus	0.2569	0.2451	0.2045	0.1876	0.2386	0.2402
	0.0200	0.0237	0.0113	0.0098	0.0245	0.0090
right cerebral cortex	0.3220	0.3066	0.2553	0.2376	0.2684	0.2747
	0.0211	0.0370	0.0088	0.0117	0.0124	0.0053
left cerebral cortex	0.3380	0.3206	0.2488	0.2376	0.2728	0.2759
	0.0312	0.0351	0.0100	0.0148	0.0162	0.0059
superior colliculus	0.2008	0.1819	0.1791	0.1562	0.1184	0.1271
	0.0020	0.0149	0.0042	0.0118	0.0100	0.0084
left thalamus	0.2316	0.2098	0.1882	0.1649	0.1525	0.1714
	0.0161	0.0213	0.0079	0.0129	0.0067	0.0101
right thalamus	0.2352	0.1953	0.1911	0.1739	0.1548	0.1621
	0.0056	0.0149	0.0103	0.0110	0.0072	0.0077
inferior colliculus	0.1900	0.1919	0.1945	0.1620	0.1129	0.1431
	0.0084	0.0098	0.0036	0.0136	0.0027	0.0253
cerebellum	0.0906	0.0747	0.1013	0.0883	0.0657	0.0754
	0.0027	0.0065	0.0005	0.0051	0.0014	0.0028
pons	0.1937	0.1937	0.1709	0.1558	0.1219	0.1262
	0.0063	0.0102	0.0078	0.0138	0.0067	0.0068
medulla	0.1542	0.1455	0.1500	0.1364	0.0973	0.1058
	0.0074	0.0077	0.0012	0.0121	0.0050	0.0056

[a] Used ethanol as solvent. [b] Used 10% dimethylformamide and 10% emulphor in distilled water as solvent: values are means ± SEM and are expressed as % dose/gram.

General Procedure for the Preparation of 5-[[4-[4-(Dialkylamino)-butyl]-1-cyclohexyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-ones (Tables 1 and 2, **1a-h**). 5-[[4-[4-(Diethylamino)butyl]-1-cyclohexyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-one (**1b**).

To a solution of 5-[[4-[4-(chloro)butyl]-1-cyclohexyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-one (**7**, 1.4 g, 3.2 mmoles), in acetonitrile (20 ml), diethylamine (10 ml), and sodium carbonate (1 g), were added and the resulting mixture was stirred at reflux for 72 hours. The solvent was removed under reduced pressure and the residue was partitioned between chloroform and water. The organic layer was washed with water, dried over sodium sulfate, and evaporated to dryness. The residue was purified by flash column chromatography (elution with methanol/ammonium hydroxide, 98:2).

5-(10-Chlorodecanoyl)-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-one (**8a**).

To the chlorodecanoic acid (**5a**, 4.13 g, 0.02 mole), thionyl chloride (10 ml) was added. The mixture was heated at reflux for 4 hours. The excess thionyl chloride was then distilled. To the residue were added 11-oxo-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepine (**6**, 4.2 g, 0.02 mole), *N,N*-dimethylaniline (2 g), and tetrahydrofuran (150 ml), and the mixture was stirred at room temperature for 72 hours. The mixture was basified with sodium bicarbonate, and the organic layer was washed with water and dried over magnesium sulfate. The solvent was then removed under reduced pressure and the residue was purified by flash column chromatography on silica gel, using ethyl acetate/hexane (1:1) as eluent (yield 5.2 g, 65%), mp 54-57°; tlc [silica gel, ethyl acetate/hexane (1:1)] Rf 0.66; ^{1}H nmr (deuteriochloroform): δ 1.21 (br, 8H), 1.34 (m, 2H), 1.65 (m, 2H), 1.77 (m, 2H), 2.18 (m, 1H), 2.33 (m, 1H), 3.48 (t, J = 6.8 Hz, 2H), 7.23 (m, 1H), 7.37 (m, 5H), 7.61 (t, J = 7.4 Hz, 1H), 8.00 (m, 1H), 9.35 (br, 0.5H), 9.78 (br, 0.5H).

Anal. Calcd. for $C_{23}H_{27}ClN_2O_2$: C, 69.24; H, 6.82; N, 7.02; Cl, 8.88. Found: C, 68.92; H, 7.02; N, 7.33; Cl, 8.64.

5-(11-Bromoundecanoyl)-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-one (**8b**).

This compound was prepared by following the procedure for the synthesis of **8a**. It was recrystallized from hexane, mp 91-92°, yield 37%, tlc [silica gel, ethyl acetate/hexane (1:1)] Rf 0.67; ^{1}H nmr (deuteriochloroform): δ 1.20 (br, 10H), 1.35 (m, 2H), 1.66 (m, 2H), 1.80 (m, 2H), 2.17 (m, 1H), 2.36 (m, 1H), 3.36 (t, J = 6.9 Hz, 2H), 7.23 (m, 1H), 7.38 (m, 5H), 7.61 (t, J = 7.4 Hz, 1H), 8.00 (m, 1H), 9.55 (br, 0.5H), 9.99 (br, 0.5H).

Anal. Calcd. for $C_{24}H_{29}BrN_2O_2$: C, 63.01; H, 6.39; N, 6.12; Br, 17.46. Found: C, 63.06; H, 6.58; N, 6.32; Br, 17.63.

General Procedure for the Preparation of 5-(Dialkylaminoacyl)-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-ones (Tables 1 and 2, **2a,b**). 5-[10-(Diethylamino)decanoyl]-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-one (**2a**).

To a solution of 5-(10-chlorodecanoyl-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-one (**8a**, 3.98 g, 0.01 mole) in acetonitrile (75 ml), were added diethylamine (10 ml), and potassium carbonate (3 g). The mixture was stirred at room temperature for 72 hours and then evaporated to dryness under reduced pressure. The residue partitioned between ethyl acetate and water. The

organic layer was separated, washed with water, and dried over magnesium sulfate. After removal of the solvent, a solid remained which was crystallized from petroleum ether to yield 3.6 g (68%) of product.

Biological Assays.

Tissue Preparation.

Membranes containing m1 receptors were isolated from CHO cells that had been transfected with the gene coding for the m1 subtype. These transfected cell lines were obtained from Dr. Mark Brann (NIH) and were grown as previously described [10]. Rat heart was used as the source of the m2 receptor. Cell membranes were prepared by lysing heart in 10 m*M* Tri-HCl, *p*H 7.4, containing 2 m*M* EDTA, as described by Bonner *et al.* [2]. Membranes were resuspended in the Tri/EDTA buffer at a protein concentration of 3 mg/ml and stored at −70° until used.

Determination of IC_{50}'s.

The IC_{50}'s for the muscarinic ligands were determined by competitive ligand binding assay against [^{3}H]QNB [11]. The compound was dissolved in 100% ethanol and added to 4 ml of Tris-buffered (10 m*M*, *p*H 7.4) 0.9% saline containing 2.5 x 10^{-10} *M* [^{3}H]QNB at a final concentration of 0.5% ethanol. Competition curves were generated with 12 concentrations of unlabled compounds in the range of 1 x 10^{-12} *M* to 1 x 10^{-5} *M*. Aliquots (0.1 ml) of the tissue preparation were added, and the mixture was vortexed and incubated at 30 for 2 hours. The incubation mixture was rapidly filtered on a GF/C filter paper, washed with 15 ml of ice-cold saline, air dried, placed in Cytoscint (ICN Biomedicals, Inc.) scintillation cocktail, and counted for 2 minutes each. Data were analysed with the LIGAND program of Munson and Rodbard [12]. The IC_{50}'s were obtained from pooled data of at least 2 determinations in duplicate on separate days.

In Vivo Studies.

Radiopharmaceuticals.

(*R,R*) [^{125}I]4-IQNB was prepared and purified according to the method of Cohen *et al* [13].

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 i.p.) 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, animals were sacrificed by decapitation and 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 minutes in an autogamma counter (GammaTrac 1193, Tm Analytic; Elk Grove Village, II) 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* accumulaion of (*R,R*)-[^{125}I]IQNB between the right *versus* left cerebral hemispheres, the left and right cerebral cortex, corpus striatum, and thalamus were dissected and studied as separate entities.

Experimental Design.

Three different experimental protocolls were performed: a) animals were preinjected with a single bolus of the m2-selective compound (**1b**, 500 nmoles in a final volume of 0.1 ml normal saline containing up to 50% ethanol) or normal saline into the exposed jugular vein. After an hour, (*R,R*)-[^{125}I]IQNB (10μCi in a final volume of 0.1 ml normal saline containing up to 30% ethanol) was injected i.v. Animals were sacrificed 1 hour after the injection of (*R,R*)-[^{125}I]IQNB. b) Animals were coinjected with a single bolus of the m2-selective compound (**1b**, 2000 nmoles) or normal saline mixed with (*R,R*)-[^{125}I]IQNB (10 μCi in a final volume of 0.18 ml of normal saline containing up to 60% ethanol) into the exposed jugular vein. After an hour the animals were sacrificed. c) Animals were injected with a single bolus of the m2-selective compound (**1b**, 2000 nmoles in a final volume of 0.1 ml of distilled water containing 10% *N,N*-dimethylformamide and 10% Emulphor EL-620 or the solvent alone into the exposed jugular vein. Immediately after the injection of the m2-selective compound **1b**, animals received a bolus of (*R,R*)-[^{125}I]IQNB (10 μCi in a final volume of 0.10 ml normal saline containing up to 30% ethanol). After an hour the animals were sacrificed. The brains were rapidly removed, and tissue samples were dissected and treated as described above.

Data Analysis.

In all studies, a minimum of 3 animals were used per reported result unless otherwise stated. The results for (*R,R*)-[^{125}I]IQNB accumulation (which includes both specific and nonspecific binding) are expressed as the percent of (*R,R*)-[^{125}I]IQNB injected dose per gram (% dose/gram) of tissue (wet weitht) and as percent change in (*R,R*)-[^{125}I]IQNB accumulation after blockade of the *m*-AChR by the m2-selective compound. All % dose/gram values are presented as the mean ± SEM.

Acknowledgment.

This work was supported in part by Grant NS22215 from the National Institute of Health, DHEW, and by contrat DE FG05 88ER60649 from the Department of Energy.

REFERENCES AND NOTES

[1] T. I. Bonner, N. J. Buckley, A. C. Young and M. R. Brann, *Science*, **237**, 527 (1987).

[2] T. I. Bonner, A. C. Young, M. R. Brann and N. J. Buckley, *Neuron*, **1**, 403 (1988).

[3] E. T. Peralta, A. Ashkenazi, J. W. Winslow, D. H. Smith, J. Ramachandran and D. J. Capon, *EMBO J.*, **6**, 3923 (1987).

[4] N. J. Buckley, T. I. Bonner, C. M. Buckley and M. R. Brann, *Mol. Pharmacol.*, **35**, 469 (1989).

[5] E. G. Peralta, A. Ashkenazi, J. W. Winslow, J. Ramachandran and D. J. Capon, *Nature*, **334**, 434 (1988).

[6] B. A. Zeeberg, M. S. Gitler, J. Baumgold, R. A. DeLaCruz and R. C. Reba, *Biochem. Biophys. Res. Commun.*, **179**, 768 (1991).

[7] V. I. Cohen, J. Baumgold, B. Jin, R. DeLaCruz, W. J. Rzeszotarski and R. C. Reba, *J. Med. Chem.*, **36**, 162 (1993).

[8] R. P. Giani, M. Borsa, E. Parini and G. C. Tonon, *Synthesis*, 550 (1985).

[9] V. I. Cohen and B. Jin, unpublished results.

[10] J. Baumgold and T. White, *Biochem. Pharmacol.*, **38**, 1605 (1989).

[11] W. J. Rzeszotarski, R. E. Gibson, W. C. Eckelman, D. A. Simms, E. M. Jagoda and R. C. Reba, *J. Med. Chem.*, **25**, 1103 (1982).

[12] P. J. Munson and D. Rodbard, *Anal. Biochem.*, **107**, 220 (1980).

[13] V. I. Cohen, W. J. Rzeszotarski, R. E. Gibson, L. H. Fan and R. C. Reba, *J. Pharm. Sci.*, **78**, 833 (1989).