Synthesis and Structure–Activity Relationship of Some 5-[[[(Dialkylamino)alkyl]-1-piperidinyl]acetyl]-10,11-dihydro-5*H*-benzo[*b*,*e*][1,4]diazepin-11-ones as M_2-Selective Antimuscarinics

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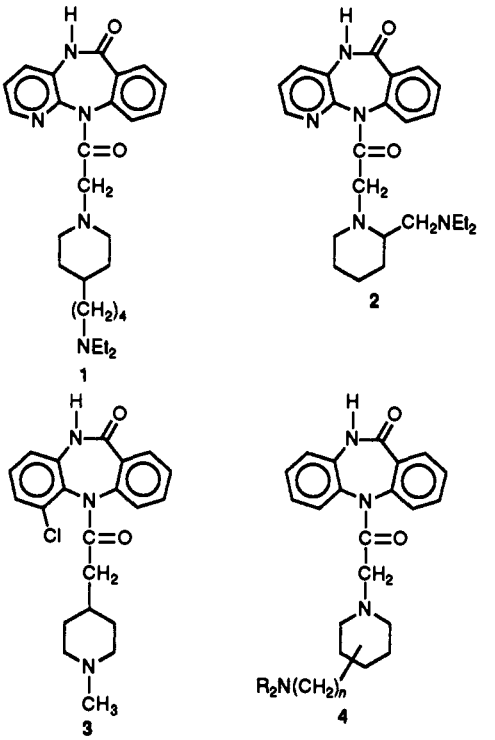
A series of 5-[[[(dialkylamino)alkyl]-1-piperidinyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]-diazepin-11-ones were prepared as potential M_2-selective ligands. The compounds were evaluated for their affinity and selectivity for the muscarinic cholinergic receptor. The best M_2-selective antimuscarinic agent studied is 5-[[4-[4-(diethylamino)butyl]-1-piperidinyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one, which is approximately 10 times more potent at M_2 receptors than previously known compounds such as 11-[[4-[4-(diethylamino)butyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (AQ-RA 741).

Our group has developed 1-azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-(4-iodophenyl)-α-phenylacetate (4IQNB), a potent m-AChR antagonist with high affinity but non-selectivity for subtypes.[1-4] In order to generate m-AChR radioligands with improved pharmacokinetics, we have developed a group of M_1-selective AChR antagonists possessing lipophilicity lower than that of IQNB.[5,6] Since 4IQNB binds to each of receptor subtype with a similar affinity, and since only the M_2 subtype is lost in Alzheimer's disease, we have focused on developing an M_2-selective radioligand.

The importance of developing subtype ligands is illustrated by the following findings. When assayed with nonselective radioligands such as [^{3}H]QNB, the density of m-AChR in brain from Alzheimer patients has been reported to be unchanged compared to age-matched controls.[7] However, when these studies were repeated using radioligands which are selective for the M_2 subtype such as 11-[[2-[(diethylamino)methyl-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one-([^{3}H]AF-DX 116), it became clear that M_2 receptors are selectively lost by as much as 61%.[8-10] Since brain contains a high density of M_1 receptors and a relatively low density of M_2 receptors, even a 61% loss of M_2 receptors was not detected using nonselective ligands. Therefore, in the present work a significant effort was devoted to developing M_2-selective ligands suitable for PET and SPECT studies of the central nervous system (CNS).

Although a large number of compounds have been reported to possess a high selectivity toward the M_1 muscarinic receptor subtype,[11] the structure–activity relationship (SAR) of other subtype-selective compounds has barely begun to emerge, and so far has produced only a few compounds that are 10-fold selective for either M_2 or M_3 muscarinic receptors.[12]

According to Eberlein et al.,[12] the selectivity of tricyclic antagonists for a particular muscarinic receptor subtype is vested primarily in the side chain carrying the amino group, with only slight contribution by the tricyclic ring system. Therefore, in developing M_2-selective ligands we used the cationic heads from the M_2-selective compounds like 11-[[4-[4-(diethylamino)butyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (AQ-RA 741) (1) and 11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]-



benzodiazepin-6-one (AF-DX 116) (2). We then combined them with the lipophilic dibenzodiazepinone bulk group from the M_1-selective 6-chloro-5-[(1-methyl-4-piperidinyl)acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one (UH-AH 37) (3) to obtain the compounds with general formula 4.

Chemistry

The required 11-oxo-10,11-dihydro-5*H*-dibenzo[*b*,*e*]-[1,4]diazepin (9) was prepared from the reaction between 2-chlorobenzoic acid and *o*-phenylenediamine. The reaction between 2- and 4-vinylpyridines (**5a**,**b**) and dialkylamines **6a–e** provided 2-(dialkylamino)ethylpyridines **7a–g** (Table I). 4-Picolyllithium (**5c**) reacted with 3-(dimethyl- or 3-(diethylamino)propyl chloride (**6f**,**g**) to provide 4-[4-dimethyl- or (diethylamino)butyl]pyridines (**7i**,**j**). [(Dialkylamino)alkyl]pyridines **7a–j** were converted into the respective piperidines **8a–j** by catalytic reduction (platinum oxide). Condensation of **9** with chloroacetyl chloride

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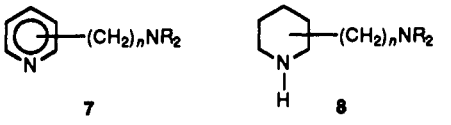
Table I. Data on [(*N,N*-Dialkylamino)alkyl]pyridines 7a–h and -piperidines 8a–h



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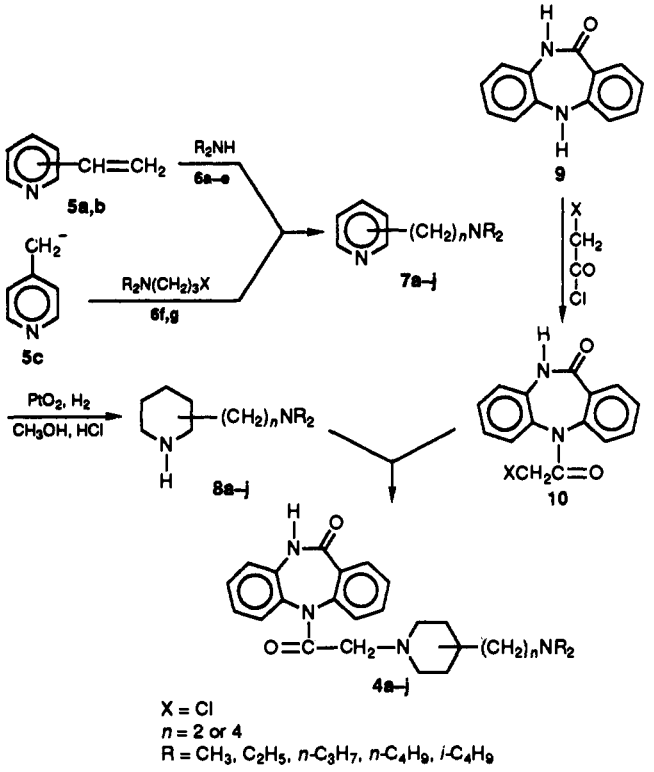
product	R	*n*	position	% yield	bp, °C (pressure, mm)
7a	*n*-C_3H_7	2	2	17	75–76 (0.2)
7b	*n*-C_4H_9	2	2	41	93 (0.1)
7c	*i*-C_4H_9	2	2	38	70–79 (0.1)
7d	CH_3	2	4	41	40 (0.1)
7e	C_2H_5	2	4	36	72 (0.3)
7f	*n*-C_3H_7	2	4	42	76–77 (0.08)
7g	*n*-C_4H_9	2	4	16	92 (0.1)
7h	*i*-C_4H_9	2	4	37	80–90 (0.1)
8a	*n*-C_3H_7	2	2	86	96–98 (0.95)
8b	*n*-C_4H_9	2	2	90	91–94 (0.15)
8c	*i*-C_4H_9	2	2	70	75 (0.1)
8d	CH_3	2	4	91	60 (0.65)
8e	C_2H_5	2	4	89	70 (0.4)
8f	*n*-C_3H_7	2	4	92	66 (0.05)
8g	*n*-C_4H_9	2	4	89	109–110 (0.4)
8h	*i*-C_4H_9	2	4	85	81–82 (0.08)

Scheme I



provided chloroacetyl compounds **10**. Subsequent reaction of [(dialkylamino)alkyl]piperidines **8a–j** with **10** yielded compounds **4a–j** (Scheme I and Table II).

Results and Discussion

Compounds **4a–g,i,j** and two reference compounds (Table III) were tested for their apparent affinities at M_1 and M_2 subtypes. The K_i values (means ± SD) are from displacement binding studies of [^{3}H]NMS binding to membranes from brain (mostly M_1 receptors), from M_1-transfected cells, or from heart tissue (for M_2). These data demonstrate that **4j** is the most potent compound in the series. This compound is selective for M_2 receptors over M_1 receptors (the most abundant in brain) by almost 8-fold. These results also demonstrate that **4j** is approximately 10 times more potent at M_2 receptors than previously known compounds such as AQ-RA 741.

Using Rekker's tables of fragmental partition coefficients we have calculated that **4j** should be approximately 15 times more lipophilic than AQ-RA 741. Furthermore, we determined the CNS penetrability of this compound. For these studies, mice were injected (ip) with this compound, and after 3 h, their brains were homogenized and the number of unoccupied receptors was determined by [^{3}H]NMS binding. We found that 3 h following injection of 0.05 mg/mouse of this compound, 0.042% of the dose penetrated the brain. In contrast, upon injection of the same doses, no detectable amount of AQ-RA 741 penetrated the brain. This result indicates that **4j** crosses the blood–brain barrier better than AQ-RA 741 but not enough for evaluation as a potential muscarinic receptor radiotracer.

Experimental Section

The melting points were obtained on a Fisher-John apparatus. The IR spectra of the compounds were obtained on a Perkin-Elmer 1710 infrared Fourier transform spectrometer. ^{1}H NMR spectra were recorded on a Bruker AC-300 instrument and expressed as parts per million (δ). HPLC was performed on an Altex Model 110A. 2-Chlorobenzoic acid, *o*-phenylenediamine, 2- and 4-vinylpyridines, 4-picoline, dialkylamines, chloroacetyl chloride, and platinum oxide were obtained from Aldrich.

11-Oxo-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepine (9) was prepared by condensation of *o*-phenylenediamine with 2-chlorobenzoic acid in chlorobenzene by method reported by Giani et al.[13] It was recrystallized from *n*-butanol.

5-(Chloroacetyl)-10,11-dihydrodibenzo[*b,e*][1,4]diazepin-11-one (10). A solution of **9** (16.4 g, 0.078 mol), *N,N*-dimethylaniline (5.5 g), and chloroacetyl chloride (15.6 mL) in THF (350 mL) was refluxed with stirring for 5 h.[14] The mixture was washed with 5% potassium bicarbonate and water. The solvent was removed under reduced pressure. The crude product was recrystallized from *n*-butanol (91%). Mp: 241–242 °C. TLC [silica gel, EtOAc/hexane (1:1)]: R_f 0.50; IR (KBr): 1685, 1661 cm^{-1}. Anal. ($C_{15}H_{11}N_2O_2Cl$): C, H, Cl, N.

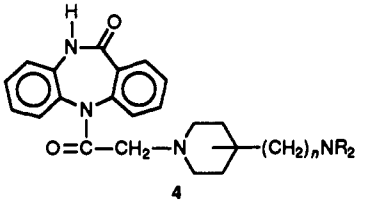
Typical Procedure for the Preparation of 2- and 4-[2-(Dialkylamino)ethyl]pyridines (Table I, 7a–h). 2-[2-(Dipropylamino)ethyl]pyridine (7a). The procedure described by Reich et al.[15] was followed. A suspension of 2-vinylpyridine (**5a**, 21 g, 0.2 mol), dipropylamine (**6c**, 20.25 g, 0.2 mol), and acetic acid (10 mL) was refluxed with stirring overnight. The mixture was made strongly basic with 25% sodium hydroxide solution, saturated with anhydrous potassium carbonate, and extracted with ether. The combined extracts were dried over magnesium sulfate, and the solvent was removed under reduced pressure. The residue distilled in vacuum to give 7.2 g of **7a** (Bp: 75–76 °C/0.2 mm).

4-[4-(Dimethyl- and (Diethylamino)butyl]pyridines (Scheme I, 7i,j) and -piperidines (Scheme I, 8i,j). To a solution of 4-picolyllithium (**5c**) in ether was added 3-dimethyl- or 3-diethylpropyl chloride (**6f,g**) at 0 °C. The resulting solution was then stirred at 0 °C for 30 min. The mixture was poured into water and extracted with ether. The ethereal extract was washed with water, dried, and distilled to give 7i (Bp: 50 °C/0.03 mm) and 7j (Bp: 66–68 °C/0.03 mm).

General Procedure for the Preparation of [(Dialkylamino)alkyl]piperidines (Table I, 8a–j). A mixture of 0.1 mol of [(dialkylamino)alkyl]pyridine (**7**), 100 mL of methanol, 15 mL of concentrated hydrochloric acid, and 0.3 g of platinum dioxide was hydrogenated to obtain product **8**.

General Procedure for the Preparation of 5-[[[(Dialkylamino)alkyl]-1-piperidinyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-ones (Table II, 4a–j). The chloroacetyl derivative **10** (2.87 g, 0.01 mol), [(dialkylamino)alkyl]piperidine (**8**; 0.01 mol), and potassium carbonate (1 g) in 30 mL of acetonitrile were refluxed for 5 h. The solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate and water. The organic layer was separated, washed

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



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compound	R	*n*	position	% yield	mp, °C	R_f[a]	relative lipophilicity,[c] t_R, min	formula
4a	*n*-C_3H_7	2	2	34	71–72	0.67	11.4	$C_{28}H_{38}N_4O_2$[d]
4b	*n*-C_4H_9	2	2	28	67–68	0.75	16.4	$C_{30}H_{42}N_4O_2$[e]
4c	*i*-C_4H_9	2	2	31	82–83	0.39[b]	13.6	$C_{30}H_{42}N_4O_2$[f]
4d	CH_3	2	4	27	101–105	0.40	8.8	$C_{24}H_{30}N_4O_2$[g]
4e	C_2H_5	2	4	42	82–85	0.46	8.8	$C_{26}H_{34}N_4O_2$
4f	*n*-C_3H_7	2	4	21	73–76	0.73	9.8	$C_{28}H_{38}N_4O_2$
4g	*n*-C_4H_9	2	4	41	58–61	0.76[b]	12.8	$C_{30}H_{42}N_4O_2$[h]
4h	*i*-C_4H_9	2	4	45	86–89	0.69	11.5	$C_{30}H_{42}N_4O_2$[i]
4i	CH_3	4	4	59	157–158	0.35	8.5	$C_{28}H_{34}N_4O_2$
4j	C_2H_5	4	4	71	70–72	0.35	9.0	$C_{28}H_{38}N_4O_2$[j]

[a] MeOH/NH_4OH (98:2). [b] $CHCl_3$/MeOH (10:1). [c] Compounds were chromatographed on a Waters 8MB C_{18} 10 Radial Pak column eluted at 2 mL/min with methanol/water/acetonitrile (50:40:10) containing 1 g/L of sodium 1-octanesulfonate and 1.2 mL/L of formic acid. The compounds were detected by absorption at 280 nm. [d] C: calcd, 72.69; found, 72.16. [e] C: calcd, 73.43; found, 72.87. [f] C: calcd, 73.43; found, 72.74. [g] C: calcd, 70.91; found, 70.48. [h] C: calcd, 73.43, found, 72.93. [i] C: calcd, 73.43, found, 72.90. [j] C: calcd, 72.69; found, 71.97.

Table III. Affinity Constants of 5-[[[(Dialkylamino)alkyl]-1-piperidinyl]acetyl]-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepin-11-ones (4), AQ-RA 741 (1), and AF-DX 116 (2)[a]

compound	R	*n*	position	brain	heart
4a	*n*-C_3H_7	2	2	56.6 ± 6.2	34.3 ± 5.2
4b	*n*-C_4H_9	2	2	177.0	88.0
4c	*i*-C_4H_9	2	2	99.0	74.0
4d	CH_3	2	4	51.0 ± 6	19.5 ± 5.7
4e	C_2H_5	2	4	35.0 ± 7	16.0 ± 3
4f	*n*-C_3H_7	2	4	97.0 ± 19	47.0 ± 2
4g	*n*-C_4H_9	2	4	100.0 ± 35	47.0 ± 2
4i	CH_3	4	4	11.0*	3.7
4j	C_2H_5	4	4	4.0 ± 1*	0.3 ± 0.1
1 (AQ-RA 741)				34.0 ± 5*	3.7 ± 0.4
2 (AF-DX 116)				740.0 ± 87*	73.0 ± 2

[a] K_i values for displacement of [^{3}H]NMS binding to brain (mostly M_1) or to heart (M_2) muscarinic receptors. Varying concentration of compounds were incubated with 0.5 nM [^{3}H]NMS and membranes from either rat brain, rat heart, or (*) A9 L cells transfected with m_1 receptors for 60 min at 37 °C. IC_{50} values were converted to K_i using the Cheng–Prusoff equation. K_d values for [^{3}H]NMS binidng to brain, heart, and M_1-transfected cell membranes were 350, 420, and 235 pM, respectively. Unless otherwise indicated, data are means ± SD of triplicate determinations.

with water, and dried over magnesium sulfate. The ethyl acetate was removed under reduced pressure and the residue purified by flash chromatography on silica gel using methanol/NH_4OH (100:2) as eluent.

Biological Methods. Membranes from M_1-transfected A9 L cells, from rat heart and from rat brain were obtained as previously described.[16] Displacement studies were performed by incubating (37 °C for 60 min) varying concentrations of compound, 0.5 nM [^{3}H]NMS, and 100–300 g of membrane in a total volume of 1 mL of phosphate-buffered saline (pH 7.2). The reaction was terminated by rapid filtration over GF/B filters, and the filters were counted in a scintillation counter following equilibration with scintillation fluid. IC_{50} values were obtained by nonlinear regression analysis of the resulting data, as performed by the Graphpad computer program (San Diego, CA), and were converted to K_i's using the Cheng–Prusoff equation.[17]

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Supplementary Material Available: Tables listing ^{1}H NMR data of compounds 4a–j, 7a–h, and 8a–h (2 pages). Ordering information is given on any current masthead page.

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