Synthesis of 2-(3-Substituted-1,2,4-oxadiazol-5-yl)-8-methyl-8-azabicyclo[3.2.1]octanes and 2α-(3-Substituted-1,2,4-oxadiazol-5-yl)-8-methyl-8-azabicyclo[3.2.1]oct-2-enes as Potential Muscarinic Agonists

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Radioligand binding affinities of seven muscarinic receptor ligands which possess an oxadiazole ring side chain have been determined in rat heart, rat brain, and m_1- or m_3-transfected CHO cell membrane preparations to determine the selectivity for subtypes of muscarinic receptor. The ratios of binding constants in brain membranes were measured as an indicator of potential agonist activity against [^{3}H]QNB and [^{3}H]Oxo-M. These muscarinic ligands did not discriminate the subtypes of muscarinic receptors. Six muscarinic ligands which have a 3-amino- or 3-methyl-1,2,4-oxadiazol-5-yl groups attached to the 8-methyl-8-azabicyclo[3.2.1]oct-2-ene or 8-methyl-8-azabicyclo[3.2.1]octane head group show binding constants between 2.04×10^{-6} and 1.79×10^{-5} *M* in rat heart, rat brain, and m_1- or m_3-transfected CHO cell membrane preparations. 1-Methyl-2-[3-amino-1,2,4-oxadiazol-5-yl]piperidine shows low binding constants of approximately 10^{-4} *M* in rat heart and rat brain. (1*R*,5*S*)-2-[3-Amino-1,2,4-oxadiazol-5-yl]-8-methyl-8-azabicyclo-[3.2.1]oct-2-ene [(1*R*,5*S*)-17] was the most active compound.

KEY WORDS: muscarinic agonist; oxadiazole; radioligand binding; azabicyclo[3.2.1]octanes; organic synthesis.

INTRODUCTION

Among some transmitter alterations in Alzheimer's disease, the cholinergic deficit in cerebral cortex and hippocampus is a consistent alteration correlating with the severity of dementia (1,2). This deficit has been linked to cognition dysfunction, especially memory and learning ability loss. Accordingly, cholinergic agents such as acetylcholine (ACh) esterase inhibitors, acetylcholine precursor, and muscarinic agonists have been employed in clinical efforts to improve cholinergic function.

Recently, five muscarinic genes, m_1 to m_5, have been cloned, where m_1, m_2, and m_3 appear to correspond to the pharmacologic subtypes M_1, M_2, and M_3, respectively (3). In cerebral cortex and hippocampus, the M_1 muscarinic receptor subtype, which is coupled to phosphoinositide (PI) turnover, is assumed to be responsible for cholinergic function and the M_2 subtype seems to function as a presynaptic autoreceptor which regulates ACh release. Muscarinic agonists such as arecoline (1), bethanechol (2), RS-86 (3), oxotremorine (4), and pilocarpine (5) have been employed for Alzheimer's disease (4–6); however, because of their low efficacy and selectivity, employment of these drugs for Alzheimer's disease was not successful. M_1-selective, high-efficacy muscarinic agonists that penetrate the blood–brain barrier may have better therapeutic properties for the treatment of some cognition dysfunctions in Alzheimer's disease.

Some 1,2,4-oxadiazole-based tertiary amines produce a high muscarinic efficacy (see Fig. 1). For example, oxadiazole derivatives **6–9** of the *N*-methyltetrahydropyridine (7), quinuclidine (8), azabicyclo[2.2.1]heptane (9), and 2-azabicyclo[2.2.2]octane (9) ring system, respectively, have been reported to give potent muscarinic agonists (10). In addition, a patent application claimed structures of general formula **10** as agents for the treatment of Alzheimer's disease (11). However, Sauerberg and co-workers (12) recently reported that (1*R*,5*S*)-2-(3-butyl-1,2,4-oxadiazol-5-yl)-8-methyl-8-azabicyclo[3.2.1]oct-2-ene (**11**) was a weak muscarinic agonist.

We prepared seven muscarinic ligands, which possess an oxadiazole ring at the 2-position of 8-methyl-8-azabicyclo[3.2.1]oct-2-ene or 8-methyl-8-azabicyclo[3.2.1]octane or the 2-position of 1-methylpiperidine head groups, and observed the affinities in rat heart, rat brain, and m_1- and m_3-transfected CHO cell membrane preparations. The relative affinities of muscarinic agonists in displacing agonist and antagonist radioligands, the [^{3}H]NMS:[^{3}H]Oxo-M ratio, was introduced by Freedman *et al.* as an index of efficacy at cerebral cortical muscarinic receptors (8,13). A relationship between this ratio and functional efficacy was indicated since high and low ratios corresponded to high and low phosphatidylinositol turnover, respectively, in cerebral cortex preparations (8,13,14). We previously compared [^{3}H]NMS:[^{3}H]Oxo-M and [^{3}H]QNB:[^{3}H]Oxo-M ratios and found them not to be significantly different and to approximate literature data (15).

MATERIALS AND METHODS

Synthesis

Melting points were determined on a Thomas Hoover capillary tube apparatus. All optical rotations were determined at the sodium D line using a Rudolph Research Autopol III polarimeter (1-dm cell). NMR spectra were recorded on a Bruker WM-250 spectrometer using tetramethylsilane as an internal standard. High-resolution mass spectra were obtained on a VG Analytical ZAB E spectrometer. Thin-layer chromatography was carried out on Whatman silica gel 60 TLC plates using $CHCl_3$–MeOH–conc. NH_4OH (40:9:1) unless otherwise noted. Visualization was accomplished under UV or in an iodine chamber. For column chromatography, a 230- to 400-mesh silica gel and $CHCl_3$–MeOH–conc. NH_4OH (40:9:1) mixture was used as

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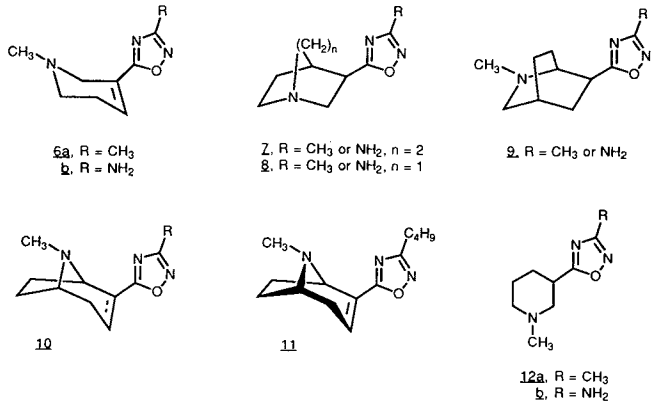

Fig. 1. Selected muscarinic agonist reported in the literature.

eluent. Microanalyses were carried out by Atlantic Microlab, Inc.

General Procedure for the Synthesis of 3-Methyl-1,2,4-oxadiazoles. Acetamide oxime (500 mg, 6.75 mmol) suspended in THF (50 ml) under nitrogen was heated at 60°C with NaH (132 mg, 5.5 mmol in oil dispersion) for 1 hr. The appropriate ester (2.75 mmol) and 4-Å molecular sieves (2 g) were added and the reaction mixture was heated under reflux for 3 hr. After cooling, the reaction was filtered and solvent removed on a rotary evaporator. The residue was chromatographed on a silica gel column eluting with $CHCl_3$–CH_3OH (95:5) to give the free base of the 3-methyl-1,2,4-oxadiazole analogue. Compounds were characterized as their hydrochloride salts (see Table I).

General Procedure for the Synthesis of 3-Amino-1,2,4-oxadiazoles. To a solution of sodium metal (0.72 g, 0.031 g-at) in dry ethanol (40 ml) and 4-Å molecular sieves (5 g) under argon was added hydroxyguanidine hemisulfate hemihydrate (1.56 g, 0.0058 mol). After stirring for 1 hr, the appropriate ester (2.76 mmol) in ethanol (2 ml) was added and the reaction mixture heated under reflux for 4 hr. After cooling, the reaction was filtered and the filtrate concentrated on a rotary evaporator. The residue was chromatographed on silica gel using $CHCl_3$–CH_3OH–conc. NH_4OH (40:9:1) as the eluent. Except for compound (1*S*,2*S*,5*S*)-**18**, which was characterized as its tartrate salt, all compounds were characterized as their hydrochloride salts (see Table I).

(1R,2R,5R)-2α-Carbomethoxy-8-methyl-8-azabicyclo[3.2.1]octane [(1R,2R,5R)-14] Tartrate. A solution of (1*R*,5*S*)-**13** (16) (1.25 g, 0.0069 mol) in CH_3OH (10 ml) was stirred under an atmosphere of H_2 with 10% Pd/C (800 mg) for 3 hr. The catalyst was removed by filtration and was washed with CH_3OH. The filtrate and washings were concentrated on a rotary evaporator. The residue was chromatographed on a silical gel column. Eluting with a solvent mixture of $CHCl_3$–CH_3OH–NH_4OH (40:9:1) gave 900 mg (71%) of **14** as an oil and a small amount of a second isomer, presumably the β-(1*R*,2*S*,5*R*)-isomer, which was isomerized to the title compound on treatment with sodium methoxide in methanol (17).[4] ^1H NMR ($CDCL_3$, 150 MHz) δ 1.39–2.01 (m, 8), 2.35 (s, 3, NCH_3), 2.87 (m, 1, H-2), 3.18 (m, 1, H-5), 3.46 (m, 1, H-1), 3.66 (s, 3, OCH_3).

An analytical sample was prepared as the tartrate salt and recrystallized from a mixture of CH_3OH and ether; mp 132°C; $[\alpha]_D^{20}$ − 12.50° (c, 0.24, CH_3OH). *Anal.* Calcd for $C_{14}H_{23}NO_8$ (C, H, N).

(1S,2S,5S)-2α-Carbomethoxy-8-methyl-8-azabicyclo[3.2.1]octane [(1S,2S,5S)-14]. The title compound was prepared in an 88% yield by a procedure analogous to that described for (1*R*,2*R*,5*R*)-**14**. The TLC and ^1H NMR spectra were identical to (1*R*,2*R*,5*R*)-**14**. This compound was used without further characterization for the preparation of (1*S*,2*S*,5*S*)-**18**.

Biological

Cell Cultures. Chinese hamster ovary cell (CHO) lines specifically expressing transfected rat m_1 or m_3 muscarinic receptor subtypes were employed (18). Cells were cultured in monolayers containing nutrient mixtures of 90% F-12 (Ham's), 10% fetal bovine serum, 2 m*M* L-glutamine, 50 μU/ml penicillin, 50 μg/ml streptomycin in a humidified atmo-

[4] Zirkle *et al.* (17) reported that catalytic reduction of **12** using Raney nickle catalyst gave mainly the 2β-isomer of **14**, which could be isomerized to **14** by treatment with sodium methoxide in methanol.

Table I. Physical Properties of Compounds **17–21**[a]

Compound	m.p. (°C)[b]	$[\alpha]_D^{23}$ (c) MeOH	^1H NMR ($CDCl_3$) δ of free base						
			NCH_3	C-CH_3	H-3	H-2	H-1	H-5	Formula[c]
(1*R*,5*S*)-**16**	208–209	−33.3° (0.51)	2.40 (s)	2.41 (s)	6.88 (t)		3.32 (m)	3.94 (d)	$C_{11}H_{16}ClN_3O$[d]
(1*S*,5*R*)-**16**	211–212	+33.92° (0.625)	2.40 (s)	2.41 (s)	6.88 (t)		3.32 (m)	3.95 (m)	$C_{11}H_{16}ClN_3O$[d]
(1*R*,5*S*)-**17**	208	−38.05° (0.51)	2.40	—	6.81 (t)		3.91 (m)	3.31 (m)	$C_{10}H_{15}ClN_4O$
(1*R*,2*R*,5*R*)-**18**	224–226	−2.86° (0.84)	2.36	2.38	—	3.26	3.49 (m)	3.19 (m)	$C_{11}H_{18}ClN_3O$
(1*S*,2*S*,5*S*)-**18**	141–142	−7.5° (0.17)	2.30	2.35	—	3.20 (m)	3.45 (m)	3.20 (m)	$C_{15}H_{23}N_3O_7$
(1*R*,2*R*,5*R*)-**19**	207 (dec)	−2.9° (0.17)	2.34	—		3.21 (m)	3.45 (m)	3.26 (m)	$C_{10}H_{17}ClN_4O$
20	173	—	2.20			3.34			$C_8H_{15}ClN_4O$[d]

[a] General procedures for the synthesis of compounds **17–21** are given under Materials and Methods.

[b] All salts were hydrochlorides except (1*S*,2*S*,5*S*)-**18**, which was characterized as the tartrate salt. The salts were recrystallized from a methanol and ethyl ether mixture.

[c] All compounds except (1*S*,2*S*,5*S*)-**18** were analyzed for C, H, N, and Cl. The results agreed to ±0.4% with theoretical values.

[d] This salt was hydrated with 0.25 mol of water.

sphere of 95% O_2:5% CO_2. Geneticin, 50 μg/ml, was added to the medium. Cells were subcultured from 1:5 to 1:10 and used when it became confluent, by day 2 or 3.

Preparation of Tissues and Cell Cultures for Radioligand Binding. Microsomal fractions of rat heart and rat brain were prepared as described previously in our laboratory (15,19). In brief, male Sprague–Dawley rats (weight, 250–300 g) were killed by decapitation. Rat heart and brain minus brain stem and cerebellum were removed. Rat brain portions were minced with scissors in 15 vol/g wet weight in ice-cold Tris (pH 7.2, 25°C) buffer and homogenized with 10 passes of a motor-driven (TRI-R-Stirrer) glass–Teflon homogenizer at setting 7. Rat heart homogenates were prepared in a similar fashion, save that they were first homogenized in a Brinkman Polytron at setting 7 for 5 sec. The m_1 or m_3 receptor-expressing CHO cell lines were suspended in ice-cold 50 m*M* Tris buffer and homogenized with a motor-driven glass–Teflon homogenizer at setting 5 with 10 passes.

The homogenates were centrifuged at 1100*g* for 20 min, and the supernatants were centrifuged at 45,000*g* for 45 min at 4°C. The resultant pellets were homogenized in 50 m*M* ice-cold Tris buffer at a concentration of 20–30, 120–150, 5–8, and 10–15 μg/5-ml binding assay volume for brain, heart, and m_1- and m_3-expressing cells, respectively, for [^{3}H]QNB binding assay. For [^{3}H]Oxo-M binding, rat brain membrane preparations were prepared in the same way in 20 m*M* HEPES buffer (pH 7.4), and the pellets were washed once and centrifuged at 45,000*g* for 45 min. The resultant pellet was homogenized at a concentration of 30–40 μg/1 ml binding assay volume. Protein concentrations were measured by the method of Bradford (20) with bovine serum albumin as standard.

[^{3}H]QNB, [^{3}H]Oxo-M Radioligand Binding. Membrane fractions were incubated with 6.08×10^{-11} *M* [^{3}H]QNB and various concentrations of muscarinic agents in a 5-ml binding assay volume for 60, 90, and 120 min for tissue preparations and m_1- and m_3-expressing cell membrane preparations, respectively. For [^{3}H]Oxo-M binding assay, 1.14×10^{-9} *M* [^{3}H]Oxo-M was incubated with muscarinic agonists for 60 min in a 1-ml binding assay volume. After incubation samples were filtered through Whatman GF/B filters and washed twice with 5 ml of ice-cold Tris buffer using a cell harvester (Model M-24R, Brandel Instrument, Gaithersberg, MD). For [^{3}H]Oxo-M assay, samples were filtered through GF/C filters presoaked in 0.05% polyethyleneimine. The radioactivity of filters in 5 ml of scintillation fluid was counted using a scintillation counter at an efficiency of approximately 45%. Nonspecific binding was measured in the presence of 10^{-5} *M* atropine.

Materials

[^{3}H]QNB (L-Benzilic-4,4′-[^{3}H]quinuclidinyl benzilate; sp act, 32.9 Ci/mmol) and [^{3}H]Oxo-M ([*methyl*-^{3}H]-oxotremorine acetate; sp act, 87.5 Ci/mmol) were purchased from DuPont–New England Nuclear (Boston, MA). Tissue culture media and supplements were obtained from GIBCO Laboratories (Grand Island, NY).

Data Analysis

Radioligand binding data were analyzed using a nonlinear curve-fitting program (BDATA, CDATA, EMF Software, Knoxville, TN). The IC_{50} values for ligand competition with [^{3}H]QNB were converted to K_i values with the Cheng and Prusoff equation (21) and the known values of K_D for [^{3}H]QNB determined separately in these membrane preparations (19).

RESULTS

Chemistry

(1*R*,5*S*)- and (1*S*,5*R*)-Anhydroecgonine methyl esters (**13**) prepared as reported by Clarke and co-workers (16) were used to prepare the target compounds. The reaction schemes are shown in Fig. 2. Catalytic reduction of (1*R*,5*S*)- and (1*S*,5*R*)-**13** using 10% palladium on carbon catalyst in methanol gave (1*R*,2*R*,5*R*)- and (1*S*,2*S*,5*S*)-2α-carbomethoxy-8-methyl-8-azabicyclo[3.2.1]octanes (**14**), respectively. Condensation of the esters **13** and **14** with acetamide oxime and sodium hydride in refluxing tetrahydrofuran provided the 3-methyloxadiazole analogues (1*R*,5*S*)- and (1*S*,5*R*)-**16** and (1*R*,2*R*,5*R*)- and (1*S*, 2*S*, 5*S*)-**18**. Similarly, treatment of esters (1*R*, 5*S*)-**13** and (1*R*,2*R*,5*R*)-**14** and com-

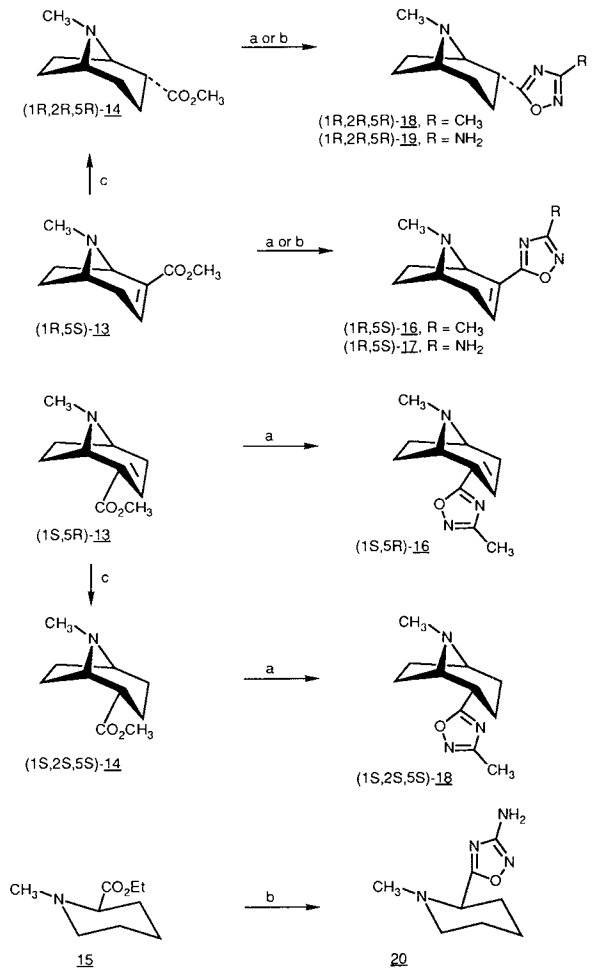

Fig. 2. Reagents: (a) NaH, THF, $CH_3C(=NOH)NH_2$, reflux; (b) Na, C_2H_5OH, powdered molecular sieve (type 4Å), $NH_2C(=NOH)NH_2 \cdot H_2SO_4 \cdot H_2O$, reflux; (c) H_2, Pd/C (10%), CH_3OH.

mercially available *N*-methyl-2-carboethoxypiperidine (**15**) with hydroxyguanidine sulfate and sodium ethoxide in ethanol at reflux gave the 3-aminooxadiazoles (1*R*,5*S*)-**17**, (1*R*,2*R*,5*R*)-**19**, and **20**. Structural assignments for compounds **16–20** were based on elemental and ^{1}H NMR analysis.

Receptor Binding

Competititon Against [^{3}H]QNB. Table II represents the affinities of muscarinic receptor ligands for the inhibition of binding of [^{3}H]QNB in rat heart, rat brain, and m_1- or m_3-transfected CHO cell line membrane preparations. Seven muscarinic ligands did not distinguish the subtypes of muscarinic receptors. (1*R*,5*S*)-**17** and (1*R*,2*R*,5*R*)-**19**, which possess a 2-(3-amino-1,2,4-oxadiazo-5-yl) substituent on the 8-methyl-8-azabicyclo[3.2.1]oct-2-ene and 8-methyl-8-azabicyclo[3.2.1]octane, respectively, show similar affinities between 4.69×10^{-6} and 1.79×10^{-5} *M* in rat heart, rat brain, and m_1- or m_3-transfected CHO cell membrane preparations. The affinities of (1*R*,5*S*)- and (1*S*,5*R*)-enantiomers of 2-[3-methyl-1,2,4-oxadiazol-5-yl]-8-methyl-8-azabicyclo[3.2.1]oct-2-ene (**16**) were similar in all four systems and are two to three times higher than that of (1*R*,5*S*)-**17**. The (1*S*,2*S*,5*S*)-enantiomer of 2α-[3-methyl-1,2,4-oxadiazol-5-yl]-8-methyl-8-azabicyclo[3.2.1]octane (**18**) show four to seven times increased affinity over (1*R*,2*R*,5*R*)-**19**. However, the (1*R*,2*R*,5*R*)-enantiomer of **18** shows similar affinities to (1*R*,2*R*,5*R*)-**19** in rat heart, rat brain, and m_1-or m_3-transfected CHO cell membrane preparations. Compound **20** shows low affinities, approximately 10^{-4} *M*, in rat heart and rat brain.

Competition Against [^{3}H]QNB and [^{3}H]Oxo-M. Table III summarizes the binding affinities of seven ligands against [^{3}H]QNB and [^{3}H]Oxo-M in rat brain membrane preparations. Compound (1*R*,5*S*)-**17** shows a [^{3}H]QNB/[^{3}H]Oxo-M ratio of 71. (1*R*,5*S*)-**16** and (1*R*,2*R*,5*R*)-**19** show ratios of 16 and 18, respectively. The other agonists show ratios of below 10.

DISCUSSION

The "cholinergic hypothesis" for Alzheimer's disease, involving synaptic cholinergic neurons in the hippocampus and cerebral cortex, has generated interest in agents acting as agonists at postsynaptic muscarinic receptors in the cortex (22–24). For example, arecoline (**1**) has been clinically evaluated for cognitive improvement in Alzheimer's patients (4). Possibly because of the compound's short duration of action and low efficacy, only marginal cognitive improvement in Alzheimer's patients was observed. The replacement of the ester function in arecoline (**1**) by the metabolically and hydrolytically more stable ester isostere, 3-methyl- or 3-amino-1,2,4-oxadiazol-5-yl group, led to compounds **6a** (7,12) and **6b** (7), which showed increased an affinity for the agonist state of the receptor and a high efficacy. Compounds (1*R*,5*S*)-**16** and -**17** and (1*S*,5*R*)-**16** are analogues of **6a** and **6b** with an ethano bridge between carbon-2 and carbon-6 of the tetrahydropyridine ring. In addition to adding steric bulk at the 2- and 6-positions of the heterocyclic ring, these changes provide analogues of **6a** and **6b** with much less conformational freedom. The low affinity of **11** for the agonist state of the muscarinic receptor may be due to the fact that the (1*R*,5*S*) configuration places the 1,2,4-oxadiazol-5-yl group in a region where it cannot act as a good hydrogen bond acceptor or that the 3-butyl group on the oxadiazole exceeds the steric volume in the receptor area normally occupied by the acetyl methyl group of acetylcholine. The weak binding for the agonist state observed for both (1*R*,5*S*)- and (1*S*,5*R*)-enantiomers of **16**, which also possess a methyl group in the 3-position of the 1,2,4-oxadiazol-5-yl group, suggests that the low potency in these more rigid analogues of **6a** is due to factors other than the absolute stereochemistry of the ring system or to the size of the substituent on the oxadiazole ring.

Street and co-workers (9) reported that the 3-amino analogue **6b** was two times as potent as **6a** in binding to the agonist state of the receptor. This increase in binding potency was attributed to the electron-donating amino group enhancing the hydrogen-bonding acceptor properties of the oxadiazole ring nitrogens. Since the 3-amino analogue (1*R*,5*S*)-**17** is 1.5 times more potent than the 3-methyl compound, (1*R*,5*S*)-**16**, the oxadiazole ring of these two analogues may be interacting with the receptor so that they can accommodate the two hydrogen bond donors of the muscarinic agonist receptor site.

Compounds (1*R*,2*R*,5*R*)- and (1*S*,2*S*,5*S*)-**18** and (1*R*,2*R*,5*R*)-**19** showed a weak affinity for the agonist state of the receptor. This was not surprising since the piperidine

Table II. Inhibition of [^{3}H]QNB Binding by Muscarinic Agonists in Rat Heart, Rat Brain, m_1 CHO, and m_3 CHO Cell Membrane Preparations

	Heart			Brain			m_1 CHO			m_3 CHO		
Drug	K_i	n_H	*n*	K_i	n_H	*n*	K_i	n_H	*n*	K_i	n_H	*n*
(1*R*,5*S*)-**17**	$4.69 \pm 0.51 \times 10^{-6}$	0.80	4	$6.36 \pm 0.86 \times 10^{-6}$	0.87	5	$9.54 \pm 1.96 \times 10^{-6}$	0.98	4	$1.14 \pm 0.27 \times 10^{-5}$	0.88	4
(1*R*,5*S*)-**16**	$2.09 \pm 0.21 \times 10^{-6}$	0.96	4	$2.15 \pm 0.12 \times 10^{-6}$	0.92	4	$2.68 \pm 0.59 \times 10^{-6}$	0.98	5	$5.87 \pm 0.77 \times 10^{-6}$	0.97	4
(1*S*,5*R*)-**16**	$2.16 \pm 0.15 \times 10^{-6}$	1.06	4	$2.04 \pm 0.34 \times 10^{-6}$	0.98	4	$2.61 \pm 0.15 \times 10^{-6}$	0.97	5	$6.87 \pm 1.33 \times 10^{-6}$	0.87	4
(1*R*,2*R*, 5*R*)-**19**	$9.10 \pm 1.28 \times 10^{-6}$	0.82	4	$5.45 \pm 0.53 \times 10^{-6}$	0.88	4	$9.54 \pm 1.39 \times 10^{-6}$	0.83	4	$1.79 \pm 0.55 \times 10^{-5}$	0.99	4
(1*R*,2*R*, 5*R*)-**18**	$6.96 \pm 1.07 \times 10^{-6}$	1.06	4	$3.91 \pm 0.57 \times 10^{-6}$	1.00	4	$7.57 \pm 2.08 \times 10^{-6}$	0.87	4	$1.24 \pm 0.18 \times 10^{-5}$	0.87	4
(1*S*,2*S*, 5*S*)-**18**	$2.73 \pm 0.17 \times 10^{-6}$	0.91	4	$1.02 \pm 0.18 \times 10^{-6}$	0.94	4	$1.41 \pm 0.08 \times 10^{-6}$	0.97	4	$3.20 \pm 0.30 \times 10^{-6}$	0.89	4
20	$1.42 \pm 0.09 \times 10^{-4}$	0.82	3	$1.48 \pm 0.13 \times 10^{-4}$	0.93	3	19.13% (10^{-4} *M*)			14.45% (10^{-4} *M*)		

Table III. Inhibition of [^{3}H]Oxo-M or [^{3}H]QNB Binding by Muscarinic Agonists in Rat Brain Membrane Preparations

Drug	[^{3}H]Oxo-M			[^{3}H]QNB			QNB/Oxo-M
	K_i	n_H	n	K_i	n_H	n	
Atropine	$4.59 \pm 1.65 \times 10^{-10}$	0.92	3	$3.37 \pm 0.48 \times 10^{-10}$	1.00	4	0.73
Carbachol	$1.31 \pm 0.16 \times 10^{-8}$	1.11	4	$1.88 \pm 0.32 \times 10^{-5}$	0.56	4	1435.11
Pilocarpine	$8.29 \pm 3.35 \times 10^{-8}$	0.95	4	$1.01 \pm 0.16 \times 10^{-6}$	0.95	5	12.18
(1*R*,5*S*)-**17**	$8.96 \pm 0.22 \times 10^{-8}$	0.97	5	$6.36 \pm 0.86 \times 10^{-6}$	0.87	5	71
(1*R*,5*S*)-**16**	$1.37 \pm 0.23 \times 10^{-7}$	0.97	4	$2.15 \pm 0.12 \times 10^{-6}$	0.92	4	16
(1*S*,5*R*)-**16**	$2.83 \pm 0.13 \times 10^{-7}$	1.17	4	$2.04 \pm 0.34 \times 10^{-6}$	0.98	4	7
(1*R*,2*R*,5*R*)-**19**	$2.96 \pm 0.28 \times 10^{-7}$	1.05	4	$5.45 \pm 0.53 \times 10^{-6}$	0.88	4	18
(1*R*,2*R*,5*R*)-**18**	$4.73 \pm 0.76 \times 10^{-7}$	1.16	5	$3.91 \pm 0.57 \times 10^{-6}$	1.00	4	8
(1*S*,2*S*,5*S*)-**18**	$5.71 \pm 0.83 \times 10^{-7}$	1.00	5	$1.02 \pm 0.18 \times 10^{-6}$	0.94	4	2
20	$2.47 \pm 0.30 \times 10^{-5}$	0.84	4	$1.48 \pm 0.13 \times 10^{-4}$	0.93	3	6

compounds **12a** and **12b** were much less potent than the arecoline analogues **6a** and **6b** (9). Compound **20**, which has the 3-amino-1,2,4-oxadiazol-5-yl group on the carbon adjacent to the piperidine nitrogen, showed the weakest affinity for the receptor.

CONCLUSIONS

Muscarinic ligands which have a 3-amino- or 3-methyl-1,2,4-oxadiazol-5-yl group attached to 8-methyl-8-azabicyclo[3.2.1]oct-2-ene or 8-methyl-8-azabicyclo[3.2.1]octane at the 2-position of the azabicyclic ring show a weak affinity for the muscarinic agonist state and do not discriminate the subtypes of muscarinic receptors. The observation that both (1*R*,5*S*)- and (1*S*,5*R*)-**16** show a low affinity for the agonist state relative to **6a** suggests that the receptor cannot accommodate the steric bulk imposed by the 6,7-ethano bridge in structure **16**.

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APPENDIX

1. (1*R*,2*R*,5*R*)-2α-[3-Methyl-1,2,4-oxadiazol-5-yl]-8-methyl-8-azabicyclo[3.2.1]octane Hydrochloride [(1*R*,2*R*,5*R*)-**18** · HCl]. *Anal.* Calcd for $C_{11}H_{18}ClN_3O$: C, 54.20; H, 7.44; Cl, 14.54; N, 17.24. Found: C, 54.22; H, 7.46; Cl, 14.50; N, 17.23.

2. (1*S*,2*S*,5*S*)-2α-[3-Methyl-1,2,4-oxadiazol-5-yl]-8-methyl-8-azabicyclo[3.2.1]octane [(1*S*,2*S*,5*S*)-**18**] Tartrate. *Anal.* Calcd for $C_{15}H_{23}N_3O_7$: C, 50.39; H, 6.48; N, 11.75. Found: C, 50.34; H, 6.51; N, 11.69.

3. (1*R*,2*R*,5*R*)-2α-[3-Amino-1,2,4-oxadiazol-5-yl]-8-methyl-8-azabicyclo[3.2.1]octane Hydrochloride [(1*R*,2*R*,5*R*)-**19** · HCl]. *Anal.* Calcd for $C_{10}H_{17}ClN_4O$: C, 49.08; H, 7.00; Cl, 14.49; N, 22.89. Found: C, 49.12; H, 7.04; Cl, 14.58; N, 22.81.

4. (1*R*,5*S*)-2α-[3-Methyl-1,2,4-oxadiazol-5-yl]-8-methyl-8-azabicyclo[3.2.1]oct-2-ene Hydrochloride [(1*R*,2*R*,5*R*)-**16** · HCl]. *Anal.* Calcd for $C_{11}H_{16}ClN_3O \cdot 0.25\ H_2O$: C, 53.65; H, 6.75; Cl, 14.40; N, 17.07. Found: C, 53.80; H, 6.78; Cl, 14.52; N, 16.98.

5. (1*S*,5*R*)-2α-[3-Methyl-1,2,4-oxadiazol-5-yl]-8-methyl-8-azabicyclo[3.2.1]oct-2-ene Hydrochloride [(1*S*,2*S*,5*S*)-**16** · HCl]. *Anal.* Calcd for $C_{11}H_{16}ClN_3O \cdot 0.25\ H_2O$: C, 53.65; H, 6.75; Cl, 14.40; N, 17.07. Found: C, 53.98; H, 6.72; Cl, 14.61; N, 17.06.

6. (1*R*,5*S*)-2α-[3-Methyl-1,2,4-oxadiazol-5-yl]-8-methyl-8-azabicyclo[3.2.1]oct-2-ene Hydrochloride [(1*R*,2*R*,5*R*)-**17** · HCl]. *Anal.* Calcd for $C_{10}H_{15}ClN_4O$: C, 49.48; H, 6.22; Cl, 14.60; N, 23.08. Found: C, 49.44; H, 6.22; Cl, 14.84; N, 22.88.

7. 1-Methyl-2-[3-amino-1,2,4-oxadiazol-5-yl]piperidine Hydrochloride (**20** · HCl). *Anal.* Calcd for $C_8H_{15}ClN_4O \cdot 0.25\ H_2O$: C, 43.05; H, 7.00; Cl, 15.88, N, 25.11. Found: C, 43.08; H, 7.01; Cl, 15.98; N, 25.12.

REFERENCES

1. E. K. Perry. The cholinergic hypothesis ten years on. *Br. Med. Bull.* 42(1):63–69 (1986).
2. E. Giacobini and R. Becker (eds.). *Current Research in Alzheimer Therapy,* Taylor and Francis, New York, 1988.
3. R. R. Levine and N. J. M. Birdsall (eds.). Subtypes of Muscarinic Receptors IV. *Trends Pharmacol. Sci. Suppl.* (1989).
4. P. N. Tariot, R. M. Cohan, J. A. Walkowitz, T. Sundarland, P. A. Nawhouse, D. L. Murphy, and H. A. Weigartner. Multiple-dose arecoline infusions in Alzheimer's disease. *Gen. Psychiat.* 45:901–909 (1988).
5. S. L. Read, J. Frazee, J. Shapira, C. Smith, J. L. Cummings, and U. Tomiyasu. Intracerebroventricular bethanechol for Alzheimer's disease. Variable dose-related responses. *Arch. Neurol.* 47:1025–1031 (1990).
6. E. Hollander, M. Davidson, R. C. Mohs, T. B. Horvath, B. M. Davis, Z. Zemishlany, and K. L. Davis. RS 86 in the treatment of Alzheimer's disease: Cognitive and biological effects. *Biol. Psychiat.* 22:1067–1078 (1987).
7. G. A. Showell, T. L. Gibbons, C. O. Kneen, A. M. MacLeod, K. Merchant, J. Saunders, S. B. Freedman, S. Patel, and R. Baker. Tetrahydropyridyloxadiazoles: Semirigid muscarinic ligands. *J. Med. Chem.* 34:1086–1094 (1991).
8. J. Saunders, M. Cassidy, S. B. Freedman, E. A. Harley, H. L. Iversen, C. Kneen, A. M. MacLeod, K. J. Merchant, R. J. Snow, and R. Baker. Novel quinuclidine-based ligands for the muscarinic cholinergic receptor. *J. Med. Chem.* 33:1128–1138 (1990).
9. L. J. Street, R. Baker, T. Book, C. O. Kneen, A. M. MacLeod, K. J. Merchant, G. A. Showell, J. Saunders, R. H. Herbert, S. B. Freedman, and E. A. Harley. Synthesis and biological

activity of 1,2,4-oxadiazole derivatives: Highly potent and efficacious agonists for cortical muscarinic receptors. *J. Med. Chem.* 33:2690–2697 (1990).

10. S. B. Freedman, E. A. Harley, S. Patel, N. R. Newberry, M. J. Gilbert, A. T. McKnight, J. K. Tang, J. J. Maguire, N. T. Mudunkotuwa, R. Baker, L. J. Street, A. M. MacLeod, J. Saunders, and L. L. Iversen. A novel series of non-quaternary oxadiazoles acting as full agonists at muscarinic receptors. *Br. J. Pharmacol.* **101**:575–580 (1990).
11. L. Nielsen, F. Watjen, J. W. Kindtler, P. H. Olesen, and P. Sauerberg. European Patent Application 88118593.8 (1988).
12. P. Sauerberg, J. W. Kindtler, L. Nielsen, M. J. Sheardown, and T. Honore. Muscarinic cholinergic agonists and antagonists of the 3-(3-alkyl-1,2,4-oxadiazol-5-yl)-1,2,5,6-tetrahydropyridine type. Synthesis and structure-activity relationships. *J. Med. Chem.* 34:687–692 (1991).
13. S. B. Freedman, E. A. Harley, and L. L. Iversen. Relative affinities of drugs acting at cholinoceptors in displacing agonist and antagonist radioligands: The NMS/Oxo-M ratio as an index of efficacy at cortical muscarinic receptors. *Br. J. Pharmacol.* 93:437–445 (1988).
14. B. S. Orlek, F. E. Blaney, F. Brown, M. S. G. Clark, M. S. Hadley, J. Hatcher, G. J. Riley, H. E. Rosenberg, H. J. Wadsworth, and P. Wyman. Comparison of azabicyclic esters and oxadiazoles as ligands for the muscarinic receptor. *J. Med. Chem.* 34:2726–2735 (1991).
15. D. J. Triggle, Y. W. Kwon, P. Abraham, J. B. Pitner, S. W. Mascarella, and F. I. Carroll. Synthesis, molecular modeling studies and muscarinic agonist/antagonist activity of azaprophen analogs. *J. Med. Chem.* (in press).
16. R. L. Clarke, S. J. Daum, A. J. Gambino, M. D. Aceto, J. Pearl, M. Levitt, W. R. Cumiskey, and E. F. Bogado. Compounds affecting the central nervous system. 4. 3β-Phenyltropane-2-carboxylic esters and analogs. *J. Med. Chem.* 16:1260–1267 (1973).
17. C. L. Zirkle, T. A. Geissman, M. Bloom, P. N. Craig, F. R. Gerns, Z. K. Indik, and A. M. Pavloff. 3-Substituted tropane derivatives. I. The synthesis and stereochemistry of the tropane-3-carboxylic acids and their esters. A comparison of positional isomers in the tropane series. *J. Org. Chem.* 27:1269–1279 (1962).
18. C. M. Fraser, C.-D. Wang, D. A. Robinson, J. D. Gocayne, and J. C. Venter. Site-directed mutagenesis of m_1 muscarinic acetylcholine receptors: Conserved aspartic acids play important roles in receptor function. *Mol. Pharmacol.* 36:840–847 (1989).
19. K. Jim, G. T. Bolger, D. J. Triggle, and G. Lambrecht. Muscarinic receptors in guinea pig ileum. A study by agonist-^{3}H-labelled antagonist competition. *Can. J. Physiol. Pharmacol.* 60:1707–1714 (1982).
20. M. M. Bradford. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254 (1976).
21. Y.-C. Cheng and W. H. Prusoff. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzyme reaction. *Biochem. Pharmacol.* 22:3099 (1973).
22. W. H. Moos, R. E. Davis, R. D. Schwarz, and E. R. Gamzu. Cognition activators. *Med. Res. Rev.* 8(3):353–369 (1988).
23. R. E. Becker and E. Giacobini. Mechanisms of cholinesterase inhibition in senile dementia of the Alzheimer type: Clinical, pharmacological, and therapeutic aspects. *Drug Dev. Res.* 12(3–4):163–195 (1988).
24. A. Wettstein and R. Speigel. Clinical trials with the cholinergic drug R586—Alzheimer's disease (AD) and senile dementia of the Alzheimer type (SDAT). *Psychopharmacology* 84:572–573 (1984).