measurements. Thanks are also extended to Drs. S. Saito, H. Nakajima, S. Harigaya, and S. Takeyama for their interest and encouragement.

Registry No. (±)-**2a**, 130605-15-1; (±)-**2a**·HCl, 130884-46-7; (+)-**2b**, 96125-53-0; (+)-**2b**·HCl, 96125-52-9; (+)-**2b**·maleate, 96128-92-6; (−)-**2b**, 110284-22-5; (−)-**2b**·HCl, 96125-59-6; (−)-**2b**·maleate, 130979-49-6; (±)-**2b**, 96451-06-8; (±)-**2b**·HCl, 96125-24-5; (+)-**2c**, 96125-41-6; (+)-**2b**·oxalate, 96125-42-7; (+)-**2d**, 96125-43-8; (+)-**2d**·oxalate, 96125-44-9; (+)-**2e**, 96125-45-0; (+)-**2e**·oxalate, 96125-46-1; (+)-**2f**, 96125-47-2; (+)-**2f**·oxalate, 96125-48-3; (±)-**2g**, 130884-75-2; (±)-**2g**·HCl, 130884-47-8; (±)-**2h**, 130884-48-9; (±)-**2h**·HCl, 121628-83-9; (±)-**2i**, 122666-34-6; (+)-**2i**, 122666-30-2; (+)-**2i**·oxalate, 122666-72-2; (+)-**2k**, 122682-52-4; (+)-**2k**·fumarate, 122682-53-5; (±)-**2l**, 120701-21-5; (±)-**2l**·oxalate, 120701-22-6; (+)-**2m**, 131099-95-1; (+)-**2m**·HCl, 104975-70-4; (+)-**2h**, 130884-49-0; (+)-**2h**·oxalate, 130979-50-9; (+)-**2o**, 96125-36-9; (+)-**2o**·L-tartrate, 96125-37-0; (±)-**2o**, 96142-59-5; (±)-**2o**·HCl, 96125-29-0; (+)-**2p**, 130884-76-3; (+)-**2p**·fumarate, 130981-21-4; (±)-**2q**, 96125-28-9; (±)-**2q**·oxalate, 96125-31-4; (+)-**2r**, 130884-50-3; (+)-**2r**·oxalate, 130979-51-0; (+)-**2s**, 130884-51-4; (+)-**2s**·oxalate, 130884-52-5; (+)-**2t**, 100893-29-6; (−)-**2t**, 100893-21-8; (−)-**2t**·oxalate, 131099-96-2; (±)-**2t**, 100893-31-0; (±)-**2t**·HCl, 100893-32-1; (+)-**2u**, 96125-27-8; (+)-**2u**·oxalate, 96125-40-5; (+)-**2v**, 130884-53-6; (+)-**2v**·fumarate, 130884-54-7; (−)-**2v**, 100893-02-5; (−)-**2v**·fumarate, 131099-97-3; (±)-**2w**, 130979-52-1; (±)-**2w**·HCl, 130979-53-2; (±)-**2w**·oxalate, 130979-54-3; (±)-**2x**, 130884-55-8; (±)-**2x**·HCl, 130884-56-9; (±)-**2x**, 130884-57-0; (±)-**2x**·HCl, 122666-47-1; (±)-**2z**, 130884-58-1; (±)-**2z**·HCl, 122666-59-5; (±)-**2aa**, 130884-59-2; (±)-**2aa**·HCl, 122666-64-2; (±)-**2bb**, 130884-60-5; (±)-**2bb**·HCl, 130884-61-6; (+)-**2cc**, 103921-09-1; (+)-**2cc**·HCl, 103920-99-6; (−)-**2cc**, 103921-10-4; (−)-**2cc**·HCl, 103921-02-4; (±)-**2cc**, 130695-87-3; (±)-**2cc**·HCl, 103920-96-3; (±)-**2dd**, 100601-02-3; (±)-**2dd**·HCl, 100601-01-2; (±)-**2ee**, 130884-77-4; (±)-**2ee**·HCl, 130903-47-8; (±)-**2ff**, 130884-78-5; (±)-**2ff**·HCl, 121664-35-5; (±)-**2gg**, 122666-41-5; (±)-**2gg**·$^1/_2$oxalate, 122666-42-6; (±)-**2hh**, 120701-27-1; (±)-**2hh**·$^1/_2$oxalate, 120701-28-2; (±)-**2ii**, 100601-03-4; (±)-**2ii**·HCl, 100601-04-5; (±)-**2jj**, 100600-75-7; (±)-**2jj**·HCl, 100600-76-8; (±)-**2kk**, 100600-77-9; (±)-**2kk**·HCl, 100600-78-0; (+)-**2ll**, 130790-20-4; (+)-**2ll**·HCl, 130979-55-4; (−)-**2ll**, 130790-24-8; (−)-**2ll**·HCl, 130979-56-5; **3a**, 40925-72-2; **3b**, 1004-00-8; **3c**, 23474-98-8; **3d**, 14482-33-8; **3e**, 33264-82-3; **3f**, 100493-32-1; **3** (X = 5,6-Cl_2), 6647-25-2; **3** (X = 4,5-Cl_2), 6647-24-1; (±)-**4a**, 96125-49-4; (±)-**4b**, 100493-13-8; (±)-**4c**, 130884-62-7; (±)-**5a**, 130884-63-8; (+)-**5b**, 96142-63-1; (−)-**5b**, 96125-56-3; (±)-**5b**, 96125-60-9; (±)-**5c**, 130884-64-9; (±)-**5d**, 122666-79-9; (±)-**5e**, 100902-58-7; (2*S*,3*S*)-**5e**, 100902-62-3; (2*R*,34R)-**5e**, 100902-60-1; (±)-**5f**, 100492-87-3; (±)-**5g**, 100492-85-1; (±)-**5h**, 100493-29-6; (±)-**5i**, 100601-38-5; (±)-**5j**, 100601-39-6; (+)-**5k**, 130979-57-6; (−)-**5k**, 130979-58-7; (±)-**6a**, 130884-65-0; (±)-**6b**, 96125-50-7; (±)-**6h**, 100601-58-9; (±)-**6j**, 100601-57-8; (±)-**7a**, 96142-62-0; (±)-**7b**, 122666-77-7; (±)-**7c**, 103921-06-8; (±)-**7d**, 100493-33-2; (±)-**7e**, 130884-66-1; (±)-**8a**, 96087-08-0; (±)-**8b**, 103921-05-7; (±)-**9a**, 130979-64-5; (+)-**10a**, 96054-27-2; (+)-**10a**·methyl L-(4-hydroxyphenyl)glycinate, 96054-28-3; (−)-**10a**, 96054-29-4; (−)-**10a**·methyl D-(4-hydroxyphenyl)glycinate, 96054-30-7; (±)-**10a**, 96125-51-8; (±)-**10b**, 122666-78-8; (−)-**10c**, 100902-61-2; (±)-**10c**, 103921-07-9; (±)-**11a**, 130884-67-2; (+)-**12a**, 96125-22-3; (+)-**12a**·L-lysine, 104966-84-9; (−)-**12a**, 96125-23-4; (−)-**12a**·L-lysine, 130884-68-3; (±)-**12a**, 96125-21-2; (±)-**13a**, 130979-65-6; **14a**, 611-07-4; **14b**, 603-86-1; (2*R*,3*R*)-**15a**, 100938-15-6; (2*S*,3*S*)-**15a**, 100902-59-8; (±)-**16a**, 130605-16-2; (±)-**16a**·HCl, 130884-69-4; (+)-**16b**, 96125-25-6; (+)-**16b**·oxalate, 96125-26-7; (−)-**16b**, 96125-57-4; (−)-**16b**·oxalate, 96125-58-5; (±)-**16b**, 105487-93-2; (±)-**16b**·HCl, 96125-61-0; (±)-**16c**, 130979-60-1; (+)-**16d**, 96125-34-7; (+)-**16d**·$HClO_4$, 96125-35-8; (±)-**16d**, 105487-94-3; (±)-**16d**·HCl, 96125-62-1; (±)-**16e**, 96087-06-8; (±)-**16e**·HBr, 96125-30-3; (+)-**16f**, 96125-38-1; (+)-**16f**·fumarate, 96125-39-2; (+)-**16g**, 131062-93-6; (+)-**16g**·HCl, 130979-61-2; (−)-**16g**, 100892-88-4; (−)-**16g**·HCl, 100892-89-5; (+)-**16h**, 100893-27-4; (+)-**16h**·$HClO_4$, 100893-28-5; (−)-**16h**, 100893-18-3; (−)-**16h**·$HClO_4$, 131099-98-4; (±)-**16h**, 100893-24-1; (±)-**16h**·oxalate, 130884-70-7; (+)-**16i**, 130884-71-8; (+)-**16i**·fumarate, 130979-62-3; (+)-**16g**, 130979-63-4; (+)-**16j**·HCl, 130884-72-9; (±)-**16k**, 130884-73-0; (±)-**16k**·HCl, 130884-74-1; (±)-**16l**, 122666-80-2; (+)-**16m**, 103920-97-4; (+)-**16m**·$HClO_4$, 103920-98-5; (−)-**16m**, 103921-00-2; (−)-**16m**·$HClO_4$, 103921-01-3; (±)-**16m**, 103920-95-2; (±)-**16m**·HCl, 103921-04-6; (±)-**16n**, 100601-05-6; (±)-**16n**·HCl, 100601-06-7; (±)-**16o**, 100601-07-8; (±)-**16o**·HCl, 100601-08-9; (±)-**16p**, 100600-97-3; (±)-**16p**·HCl, 100600-98-4; (±)-**16g**, 100600-99-5; (±)-**16q**·HCl, 100601-00-1; (+)-**16r**, 130790-21-5; (−)-**16r**, 130790-25-9; (+)-**17**, 110284-39-4; (*S*)-*N*-(2-naphthylsulfonyl)-2-pyrrolidinecarbonyl chloride, 91872-31-0.

Supplementary Material Available: Tables of structural data including Table XIII, giving final atomic coordinates and equivalent isotropic or isotopic thermal parameters with esd in parentheses, Table XIV, giving bond lengths with esd in parentheses, Table XV, giving bond angles with esd in parentheses, and Table XVI, giving results of Bijvoet pairs measurements and Figure 3, giving atomic nomenclature (5 pages); listing of structure factors (8 pages). Ordering information is given on any current masthead page.

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

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A series of 3-(3-alkyl-1,2,4-oxadiazol-5-yl)-1,2,5,6-tetrahydro-1-methylpyridines (**2a–q**) were synthesized and tested for central muscarinic cholinergic receptor binding affinity by using [^{3}H]oxotremorine-M and [^{3}H]QNB as ligands and in a functional assay using guinea pig ileum. The analogues with unbranched C_{1-8}-alkyl substituents (**2a–g**) were agonists, whereas the compounds with branched or cyclic substituents (**2h–m**) were antagonists. The alkyl ether analogues (**2o–q**) were also agonists but had lower receptor binding affinity than the corresponding alkyl analogues. The 3-(5-alkyl-1,2,4-oxadiazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine analogues had only very low affinity for the central muscarinic receptors and were weak antagonists in the ileum assay. A few 3-(3-butyl-1,2,4-oxadiazol-5-yl)-1,2,5,6-tetrahydro-1-methylpyridines substituted with methyl or hydrogen in the 1-, 5-, or 6-position were synthesized and tested. *N*-Desmethyl analogue **7** was a potent muscarinic agonist, whereas *N*-desmethyl-5-methyl analogue **11** and *N*-methyl-6-methyl analogue **13** both were antagonists with lower muscarinic receptor affinity. The 3-(3-butyl-1,2,4-oxadiazol-5-yl)quinuclidine (**17**) and tropane (**15**) analogues were both very potent antagonists with high affinity for central muscarinic receptors. The ratio [IC_{50}(QNB)/IC_{50}(Oxo-M)] × 0.162 proved to be a good indicator of the efficacy of the compounds in the guinea pig ileum assay.

The finding of a cholinergic deficit in the brain of patients with Alzheimer's disease has lead to the cholinergic hypothesis of Alzheimer's disease and to attempts at restoring cholinergic function by means of cholinomimetic

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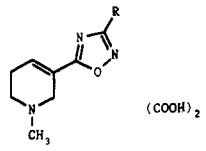
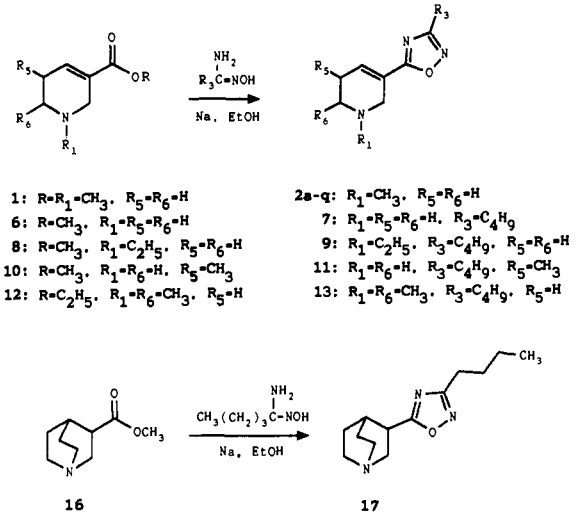
Table I. 3-(3-Alkyl-1,2,4-oxadiazol-5-yl)-1,2,5,6-tetrahydro-1-methylpyridine Oxalates



no.	R	% yield	mp, °C	recryst solvent	formula
2a	CH_3	44	159–160	EtOH/Et_2O	$C_{11}H_{15}N_3O_5$
2b	CH_2CH_3	25	168–169	EtOH/Et_2O	$C_{12}H_{17}N_3O_5$
2c	$CH_2CH_2CH_3$	32	153–154	EtOH/Et_2O	$C_{13}H_{19}N_3O_5$
2d	$CH_2(CH_2)_2CH_3$	36	121–123	EtOH/Et_2O	$C_{14}H_{21}N_3O_5$
2e	$CH_2(CH_2)_3CH_3$	40	149–150	EtOH/Et_2O	$C_{15}H_{23}N_3O_5$
2f	$CH_2(CH_2)_5CH_3$	33	94–95	EtOH/Et_2O	$C_{17}H_{27}N_3O_5$
2g	$CH_2(CH_2)_6CH_3$	19	122–123	EtOH/Et_2O	$C_{18}H_{29}N_3O_5$
2h	$CH(CH_3)_2$	37	133–134	EtOH/Et_2O	$C_{13}H_{19}N_3O_5$
2i	$C(CH_3)_3$	21	162–163	EtOH/Et_2O	$C_{14}H_{21}N_3O_5$
2j	c-C_3H_5	34	169–172	EtOH/Et_2O	$C_{13}H_{17}N_3O_5$
2k	c-C_4H_7	29	148–149	EtOH/Et_2O	$C_{14}H_{19}N_3O_5$
2l	c-C_5H_9	22	161–163	EtOH/Et_2O	$C_{15}H_{21}N_3O_5$
2m	c-C_7H_{13}	7	180–181	acetone	$C_{17}H_{25}N_3O_5$
2n	CH_2-c-C_3H_5	26	142–143	acetone	$C_{14}H_{19}N_3O_5$
2o	CH_2OCH_3	40	153–154	EtOH/Et_2O	$C_{12}H_{17}N_3O_5$
2p	$CH_2CH_2OCH_3$	29	140–141	EtOH/Et_2O	$C_{13}H_{19}N_3O_6$
2q	$CH_2CH_2OCH_2CH_3$	7	143–144	EtOH/Et_2O	$C_{14}H_{21}N_3O_6$

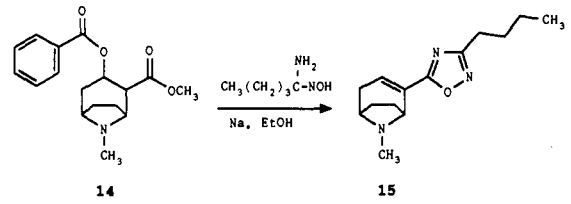
Scheme I



drugs.[1,2] Such agents include acetylcholine esterase inhibitors, precursors of acetylcholine and muscarinic agonists. It is on the latter approach, using muscarinic agonists, that we have focused our research.

The muscarinic agonist arecoline has previously been used as a lead structure to design conformationally restricted analogues[3] utilizing the 3-alkoxyisoxazole as an ester bioisoster. More recently, the replacement of the ester functionality in arecoline and in 3-acetoxyquinuclidine by 1,2,4-oxadiazole has produced very potent muscarinic agonists.[4] Emphasis in this later paper was

Scheme II



Scheme III

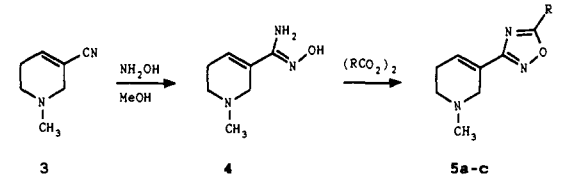
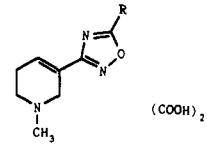


Table II. 3-(5-Alkyl-1,2,4-oxadiazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine Oxalates



no.	R	% yield	mp, °C	recryst solvent	formula
5a	CH_3	52	173–174	EtOH/Et_2O	$C_{11}H_{15}N_3O_5$
5b	CH_2CH_3	38	181–182	EtOH/Et_2O	$C_{12}H_{17}N_3O_5$
5c	$CH_2CH_2CH_3$	65	170–171	EtOH/Et_2O	$C_{13}H_{19}N_3O_5$

the structure–activity relationship (SAR) of the quinuclidine and of the 1-azanorbornane ring systems with 3-methyl- or 3-amino-1,2,4-oxadiazol-5-yl as the ester isostere. In these two azabicyclic ring systems the substituent at the 1,2,4-oxadiazole should be small and hydrophilic in order to retain muscarinic agonist activity.

In this paper we report on the SAR of 1,2,4-oxadiazole arecoline analogues with more lipophilic substituents. The affinity of the compounds to central muscarinic receptors and their effects on peripheral ileal muscarinic receptors in vitro are described.

Chemistry

3-Alkyl-1,2,4-oxadiazole compounds **2a–q** (Table I) were synthesized by refluxing arecoline (1) and the appropriate amide oxime in dry ethanol with 1 equiv of sodium and molecular sieves (Scheme I). Under identical conditions compounds **7**, **9**, **11**, **13**, and **17** were made from the corresponding esters (**6** (norarecoline), 8,[5] 10,[6] 12[7] and 16[8]). Tropane analogue **15** was obtained under similar conditions from cocaine (**14**) (Scheme II). 5-Alkyl-1,2,4-oxadiazole compounds **5a–c** (Table II) were synthesized with 3-cyano-1,2,5,6-tetrahydro-1-methylpyridine[9] as starting material (Scheme III). The cyano compound (**3**) was treated with hydroxylamine to give amide oxime **4**, which upon heating with the appropriate acid anhydride gave the analogues **5a–c**. The structures of all of the new com-

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Table III. In Vitro Muscarinic Effects of 3-(3-Alkyl-1,2,4-oxadiazol-5-yl)-1,2,5,6-tetrahydro-1-methylpyridine Oxalate

no.	binding to rat brain membranes: IC_{50} ± SEM		muscarinic agonist index[a]	effects on guinea pig ileum		
				agonism		antagonism:
	[^{3}H]Oxo-M, nM	[^{3}H]QNB, μM		EC_{50} ± SEM, μM	% max[b]	K_B, μM
2a	12.3 ± 0.5	41.0 ± 3.7	540	0.032 ± 0.003	100	
2b	11.0 ± 1.0	35.3 ± 2.5	520	0.20 ± 0.01	100	
2c	6.3 ± 1.0	17.4 ± 0.3	447	0.10 ± 0.01	100	
2d	14.1 ± 2.5	15.8 ± 0.3	181	0.15 ± 0.07	100	
2e	36.9 ± 1.8	6.7 ± 0.6	29	0.62 ± 0.02	40	
2f	28.3 ± 1.0	9.1 ± 0.2	52	0.13 ± 0.03	100	
2g	59.9 ± 0.9	11.0 ± 0.4	30	1.46 ± 0.01	100	
2h	182 ± 9	15.8 ± 0.3	14	>30	0	0.2
2i	286 ± 32	9.0 ± 0.1	5	>30	0	0.2
2j	78 ± 17	6.8 ± 0.7	14	>30	0	0.15
2k	191 ± 6.8	12.9 ± 1.0	11	>30	0	0.65
2l	102 ± 21	10.8 ± 0.6	15	>30	0	1.55
2m	>850	16.0 ± 0.7	<3	>30	0	0.16
2n	7.8 ± 0.4	11.3 ± 0.3	235	1.00 ± 0.03	100	
2o	43.5 ± 5.0	66.9 ± 6.7	249	0.42 ± 0.04	100	
2p	144 ± 5.8	41.5 ± 3.8	47	0.72 ± 0.06	100	
2q	107 ± 8.0	42.8 ± 6.1	65	1.28 ± 0.01	60	
arecoline (1)	77 ± 17	106 ± 13	223	0.073 ± 0.003	100	

[a] $[IC_{50}(QNB)/IC_{50}(Oxo\text{-}M)] \times 0.162$. [b] Maximal contraction compared to acetylcholine.

Table IV. In Vitro Muscarinic Effects of 3-(5-Alkyl-1,2,4-oxadiazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine Oxalate

no.	binding to rat brain membranes: IC_{50} ± SEM		muscarinic agonist index[a]	effects on guinea pig ileum		
				agonism		antagonism:
	[^{3}H]Oxo-M, nM	[^{3}H]QNB, μM		EC_{50} ± SEM, μM	% max[b]	K_B, μM
5a	955 ± 123	401 ± 41	68	>30	0	8.00
5b	954 ± 15	156 ± 3.5	26	>30	0	5.75
5c	681 ± 81	88 ± 8.4	80	>30	0	3.38

[a] $[IC_{50}(QNB)/IC_{50}(Oxo\text{-}M)] \times 0.162$. [b] Maximal contraction compared to acetylcholine.

pounds were established by ^{1}H NMR (**2a–q** and **5a–c** in the supplementary material) and supported by elemental analyses.

Evaluation of Biological Effects

In vitro receptor binding studies were used to determine the affinity of the compounds for muscarinic receptor sites in rat brain. The ability of the compounds to displace tritiated oxotremorine M (Oxo-M) was interpreted as the affinity for the "agonist conformational state", whereas the ability to displace quinuclidinyl benzilate (QNB) was the affinity for the "antagonist conformational state". The ratio $[IC_{50}(QNB)/IC_{50}(Oxo\text{-}M)] \times 0.162$[3,10] was used as a muscarinic agonist index to predict agonist efficacy. This method for prediction of efficacy at muscarinic receptors is analogous to that described earlier.[11] Freedman et al.[11] correlated the ability of the ligands to induce inositol phosphate turnover in rat cerebral cortex to the muscarinic agonist index. According to this agonist index scale, values of the index above 4000 indicate full agonism, whereas values of 100–300 and below 15 predict partial agonism and antagonism, respectively. The isolated guinea pig ileum was used as a functional test for evaluation of the pharmacological profile of the compounds at muscarinic receptors.

Structure–Activity Relationships

The observation that replacement of the ester group in benzodiazepine esters[12] and in β-carboline esters[13] by 1,2,4-oxadiazoles produced compounds with increased affinity and/or efficacy prompted us to design 1,2,4-oxadiazole arecoline analogues (**2** and **5**). In the benzodiazepine and β-carboline series both the 3-alkyl-1,2,4-oxadiazol-5-yl and the 5-alkyl-1,2,4-oxadiazol-3-yl groups were ester bioisosteres producing compounds with agonist character. It soon became clear that the muscarinic receptors only recognized the 3-alkyl-1,2,4-oxadiazol-5-yl moiety as an isostere to the arecoline ester functionality (Table III). 3-(5-Alkyl-1,2,4-oxadiazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridines **5a–c** (Table IV) had only very low affinity to central muscarinic receptors and acted as weak antagonists in the ileum assay. In the 3-(3-alkyl-1,2,4-oxadiazol-5-yl)-1,2,5,6-tetrahydro-1-methylpyridine series, all tested compounds with unbranched C_{1-8} alkyl substituents (**2a–g**) had good affinity to central muscarinic receptors and were full muscarinic agonists on the guinea pig ileum, except **2e** (pentyl), which was partial. All these agonists (**2a–g**) were partial according to the muscarinic agonist index values. With longer chain length the index value declined accordingly.

Substitution of a carbon with an oxygen in the alkyl substituent as in **2o–q** afforded a decrease in the receptor affinity and in the ileum agonist potency. The methoxymethyl (**2o**) and the methoxyethyl (**2p**) analogues were full agonists on the ileum, whereas the ethoxyethyl (**2q**) analogue was a partial agonist.

Isopropyl, *tert*-butyl (**2h–i**), and cyclic alkyl (**2j–m**) side chains produced compounds with low affinity for the Oxo-M binding site and low muscarinic agonist index

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(11) Freedman, S. B.; Harley, E. A.; Iversen, L. L. *Br. J. Pharmacol.* 1988, *93*, 437.
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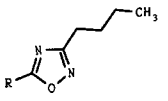
(13) Bondo Hansen, J.; Engelstoft, M.; Honoré, T.; Wätjen, F.; Schmiechen, R., 3-(1,2,4-oxadiazolyl)-β-carboline derivative as benzodiazepine receptor ligands. Data presented at the IXth International Symposium on Medicinal Chemistry, Berlin, Germany, Sept. 14–18, 1986.

Table V. In Vitro Muscarinic Effects of 3-(3-Butyl-1,2,4-oxadiazol-5-yl) Derivatives



no.	R	binding to rat brain membranes: IC_{50} ± SEM		muscarinic agonist index[a]	effects on guinea pig ileum		
					agonism		antagonism:
		[^{3}H]Oxo-M, nM	[^{3}H]QNB, μM		EC_{50} ± SEM, μM	% max[b]	K_B, μM
7		12.1 ± 3.4	25.3 ± 0.7	339	0.32 ± 0.07	100	
2d		14.1 ± 2.5	15.8 ± 0.3	181	0.15 ± 0.07	100	
9		86.2 ± 3.1	20.6 ± 2.5	39	>30	0	0.7
11		685 ± 183	64.3 ± 8.0	15	>30	0	NT
13		145 ± 3.7	10.2 ± 1.1	11	>30	0	0.13
15		181 ± 3.0	8.9 ± 1.2	8	>30	0	0.22
17		58.5 ± 14	1.5 ± 0.09	4	>30	0	0.07

[a] [IC_{50}(QNB)/IC_{50}(Oxo-M)] × 0.162. NT = not tested. [b] Maximal contraction compared to acetylcholine.

values. In the functional assay they were all antagonists. An exception was the cyclopropylmethyl (**2n**) analogue, which had good affinity to central muscarinic receptors and acted as a full agonist on the ileum.

According to the literature[4] the substituent at the 1,2,4-oxadiazole should be small and hydrophilic in order to retain muscarinic agonist activity. This conclusion was based on biochemical evaluation of 3-(3-substituted-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.2]octanes and 3-(3-substituted-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptanes. Substitution with methyl in either the 5- or the 6-position of 3-(3-methyl-1,2,4-oxadiazol-5-yl)-1,2,5,6-tetrahydro-1-methylpyridine (**2a**) was reported[14] to produce compounds with reduced muscarinic receptor affinity and efficacy. Comparing the reported SAR to our own results in Table III suggested that the unsubstituted tetrahydropyridine ring was better accepted by the muscarinic receptor, allowing the 3-alkyl-1,2,4-oxadiazole substituents to be more lipophilic without losing the muscarinic agonist activity.

In an attempt to demonstrate that the unsubstituted tetrahydropyridine had the highest affinity to the agonist conformational state when the 1,2,4-oxadiazole substituent was more lipophilic, a series of 3-butyl-1,2,4-oxadiazoles was prepared (Table V). *N*-Desmethyl analogue **7** had equal affinity for the Oxo-M binding site but a higher agonist index value than arecoline analogue **2d** (Table V). This was in accordance with results published on **2a** and the *N*-desmethyl analogue.[14] Compound **7** was also a full agonist on the guinea pig ileum. However, substitution of the *N*-methyl for a *N*-ethyl (**9**) or substitution of a hydrogen with a methyl group in the 5 (**11**) or 6 (**13**) position produced in all cases compounds with lower affinity for the Oxo-M binding site and lower agonist index values. The compounds were all antagonists on the ileum. Two azabicyclic ring systems, tropane (**15**) and quinuclidine (**17**), were also tested. Both compounds were antagonists on the ileum and displaced QNB at lower concentrations than Oxo-M, resulting in very low muscarinic agonist index values.

Discussion

Arecoline has shown in clinical trials with patients with presenile dementia of the Alzheimer type a significant enhancement of performance in recognition tests.[15] The ester functionality in benzodiazepine esters and in β-carboline esters had been replaced by 1,2,4-oxadiazoles, producing compounds with improved affinity and efficacy.[12,13] On the basis of these facts we designed and synthesized a series of 3-alkyl-1,2,4-oxadiazol-5-ylarecoline derivatives.

A screening procedure, including [^{3}H]Oxo-M and [^{3}H]QNB receptor binding assays and guinea pig ileum as functional test, has identified unbranched 3-(3-alkyl-1,2,4-oxadiazol-5-yl)-1,2,5,6-tetrahydro-1-methylpyridines **2a–g,n–q** (Table III) as muscarinic agonists. The receptor binding data was generated on rat brain membranes, where the receptor population is different from the receptor population in the ileum. It was therefore not surprising that there was poor correlation between the receptor binding data and the ileum data. The muscarinic agonist index was correlated to the inositol phosphate turnover in rate cerebral cortex,[11] but our data (Table III and V) demonstrate that this index also is an indicator of the agonist activity on the guinea pig ileum. However, since the index scale was based on central muscarinic affinity and efficacy, the scale value should only be taken as an indicator of the efficacy on the muscarinic receptors in the ileum. The ileum has a large muscarinic receptor reserve and, consequently, most agonists show up as full agonists. Only two of the agonists were partial on the ileum, pentyl analogue **2e** and its oxygen analogue **2q**. Arecoline analogues with unbranched alkyl substituents on the "reverse" 1,2,4-oxadiazole (5-alkyl-1,2,4-oxadiazol-3-yl) **5a–c** (Table

(14) Showell, G. A.; Merchant, K.; Baker, R.; Freedman, S. B.; Saunders, J. *Trends Pharmacol. Sci., Suppl. Subtypes Muscarinic Recept. IV* **1989**, 112.

(15) Christie, J. E.; Shering, A.; Ferguson, J.; Clen, A. I. M. *Br. J. Psychiatry* **1989**, *138*, 46.

II) had only very low affinity for muscarinic receptors (Table IV). Thus, the muscarinic agonist index values are probably very unreliable. An explanation of the dramatic difference in the receptor affinity between the 3-alkyl and the 5-alkyl-1,2,4-oxadiazole tetrahydropyridines could be the differences in electron distribution in the two oxadiazoles. It is not possible to superimpose both the two double bonds in the oxadiazoles and the substituents at the same time.

3-(3-Alkyl-1,2,4-oxadiazol-5-yl)-1,2,5,6-tetrahydro-1-methylpyridine analogues with branched (**2h–i**) or cyclic (**2j–m**) alkyl side chains close to the oxadiazole ring had lower affinity for the Oxo-M binding site and were antagonists in the ileum preparation. This indicates that space-filling substituents close to the oxadiazole ring are not accepted by the muscarinic receptors. One example, cyclopropylmethyl analogue **2n** suggests that the muscarinic receptor might be activated by analogues with the branched alkyl further away from the oxadiazole ring.

3-Butyl-1,2,4-oxadiazole was chosen as the esterisostere because it had a substituent of intermediate length and lipophilicity. Removal of the *N*-methyl group, which gave a compound **7** with lower ring inversion barrier and therefore greater flexibility than **2d**, increased the muscarinic agonist index (Table V), indicating a more full agonist. Decreasing the ring flexibility and adding steric factors by *N*-ethyl substitution (**9**) or by methylation on the 5 (**11**) or 6 (**13**) positions of the tetrahydropyridine ring decreased the muscarinic receptor affinity and the muscarinic agonist index value (Table V). Replacement of the tetrahydropyridine ring with the more space filling azabicyclic ring systems tropane (**15**) and quinuclidine (**17**) resulted in compounds with high affinity to the QNB binding site and very low agonist index values (Table V). All of the 3-butyl-1,2,4-oxadiazole compounds with either ring substituents (**9**, **11**, **13**) or azabicyclic ring systems (**15**, **17**) were antagonists on the ileum preparation. Probably both steric factors and the flexibility of the cyclic amine have impact on the affinity and the efficacy of the ligands to the muscarinic receptors.

These findings could indicate that the very flexible unsubstituted tetrahydropyridine ring fits extremely well into the anionic cavity of the muscarinic receptors in the agonist conformational state. The receptor recognizes even compounds with long unbranched alkyl substituents on the 1,2,4-oxadiazole ring as agonists (**2a–g**). Changing the conformation or the flexibility of the tetrahydropyridine ring as in **7**, **9**, **11**, **13**, **15**, and **17** leads to compounds where the substituent must be small in order to activate the receptor. These more hindered azacycles and azabicycles probably impose some changes on the receptor, which reduce the space available at the receptor site.

Further work is ongoing to determine the muscarinic receptor subtype selectivity of the compounds.

Experimental Section

Chemistry. Melting points were determined automatically in a Mettler FP 51 connected to a Mettler FP5. ^{1}H NMR spectra were recorded at 60 MHz on a Hitachi Perkin-Elmer R-248 spectrometer in either $CDCl_3$ or D_2O with Me_4Si or sodium 3-(trimethylsilyl)propanesulfonate, respectively, as internal standards. Elemental analyses were performed by Novo Microanalytical Laboratory, Denmark, and were within ±0.4% of calculated values.

General Procedure for the Synthesis of 3-(3-Alkyl-1,2,4-oxadiazol-5-yl)-1,2,5,6-tetrahydro-1-methylpyridine Oxalates (2) (Table I). To a solution of sodium (180 mg, 7.8 mg-atom) in dry ethanol (20 mL) and molecular sieves (type 4A, 5 g) was added alkylcarboxamide oxime (8 mmol). The mixture was stirred at room temperature for 10 min whereafter arecoline hydrobromide (1, 1.0 g, 4.23 mmol) was added. The mixture was heated at 80 °C for 12 h, filtered, and evaporated in vacuo. To the residue was added water (10 mL) and the mixture was extracted with ether (3 × 25 mL). The combined extracts were dried ($MgSO_4$) and evaporated in vacuo to give the free base of the wanted product. The residue was dissolved in ethanol (10 mL) and a solution of oxalic acid in ethanol (10 mL) was added. The title compound crystallized after addition of ether. Recrystallization from ethanol–ether gave an analytically pure product.

General Procedure for the Synthesis of 3-(5-Alkyl-1,2,4-oxadiazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine Oxalates (5) (Table II). A solution of sodium (575 mg, 25 mg-atom) and hydroxylamine hydrochloride (1.74 g, 25 mmol) in dry methanol (30 mL) was stirred at room temperature for 30 min and filtered. A solution of 3-cyano-1,2,5,6-tetrahydro-1-methylpyridine[9] (**3**, 1.65 g, 13.5 mmol) in methanol (20 mL) was added to the filtrate. The reaction mixture was stirred at room temperature for 20 h and evaporated in vacuo. The residue was extracted with ethanol (50 mL), filtered, and evaporated to give the intermediate amide oxime (**4**) as a solid which was used without further purification.

A solution of crude amide oxime **4** (200 mg, 1.29 mmol) in the appropriate acid anhydride (5 mL) was heated at 80 °C for 24 h. After evaporation in vacuo the residue was dissolved in 4 N sodium hydroxide (5 mL) and extracted with ether (3 × 25 mL). The ether phases were dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was dissolved in ethanol (5 mL) and added to a solution of oxalic acid in ethanol (5 mL). Addition of ether afforded the title compound as a crystalline solid. Recrystallization gave an analytically pure product.

3-(3-Butyl-1,2,4-oxadiazol-5-yl)-1,2,5,6-tetrahydropyridine Hemioxalate (7). The compound was synthesized as described above under the general procedure for synthesizing compounds **2a–q** with norarecoline hydrochloride (**6**) instead of arecoline hydrobromide (**1**). Crystallization and recrystallization from ethanol/ether gave the wanted oxadiazole **7** in 20% yield: mp 207–208 °C; ^{1}H NMR (D_2O) δ 7.3 (1 H, m), 4.15 (2 H, m), 3.45 (2 H, t, J = 6 Hz), 3.0–2.6 (4, H, m), 2.0–1.0 (4 H, m), 0.91 (3 H, t, J = 6 Hz). Anal. ($C_{12}H_{18}N_3O_3$) C, H, N.

3-(3-Butyl-1,2,4-oxadiazol-5-yl)-1-ethyl-1,2,5,6-tetrahydropyridine Oxalate (9). The compound was synthesized as described above under the general procedure for the synthesis of compounds **2a–q** using 1-ethyl-1,2,5,6-tetrahydro-3-(methoxycarbonyl)pyridinium chloride (**8**) instead of arecoline hydrobromide (**1**). Crystallization and recrystallization from ethanol/ether gave the title compound (**9**) in 25% yield: mp 102–104 °C; ^{1}H NMR (D_2O) δ 7.3 (1 H, m), 4.4–4.0 (2 H, m), 3.9–3.2 (4 H, m), 3.0–2.6 (4 H, m), 1.9–1.1 (4 H, m), 1.40 (3 H, t, J = 6 Hz), 0.90 (3 H, t, J = 6 Hz). Anal. ($C_{15}H_{23}N_3O_5$) C, H, N.

(*RS*)-3-(3-Butyl-1,2,4-oxadiazol-5-yl)-1,2,5,6-tetrahydro-5-methylpyridine Oxalate (11). The compound was synthesized as described under the general procedure for the synthesis of the compounds **2a–q** using (*RS*)-1,2,5,6-tetrahydro-3-(methoxycarbonyl)-5-methylpyridine[6] oxalate (**10**) instead of arecoline hydrobromide (**1**). Crystallization and recrystallization from ethanol/ether gave the wanted product (**11**) in 12% yield: mp 190–191 °C; ^{1}H NMR (D_2O) δ 7.2 (1 H, m), 4.1–3.9 (2 H, m), 3.7–3.2 (1 H, m), 3.45 (3 H, t, J = 6 Hz), 3.0–2.5 (2 H, m), 2.0–1.0 (4 H, m), 1.2 (3 H, t, J = 7 Hz), 0.90 (3 H, t, J = 6 Hz). Anal. ($C_{14}H_{21}N_3O_5$) C, H, N.

(*RS*)-1,6-Dimethyl-3-(3-butyl-1,2,4-oxadiazol-5-yl)-1,2,5,6-tetrahydropyridine Oxalate (13). The compound was synthesized as described above for the synthesis of compounds **2a–q** using (*RS*)-1,6-dimethyl-3-(ethoxycarbonyl)-1,2,5,6-tetrahydropyridine[7] oxalate (**12**) instead of arecoline hydrobromide (**1**). Crystallization and recrystallization from ethanol/ether gave the desired oxadiazole (**13**) in 16% yield: mp 141–142 °C; ^{1}H NMR (D_2O) δ 7.2 (1 H, m), 4.3–4.0 (2 H, m), 4.0–3.3 (2 H, m), 3.05 (3 H, s), 3.0–2.6 (3 H, m), 2.9–1.1 (7 H, m), 0.85 (3 H, t, J = 6 Hz). Anal. ($C_{15}H_{23}N_3O_5$) C, H, N.

2-(3-Butyl-1,2,4-oxadiazol-5-yl)-8-methyl-8-azabicyclo-[3.2.1]oct-2-ene Oxalate (15). Sodium (101 mg, 4.4 mg-atom) was dissolved in dry ethanol (20 mL). Molecular sieves (Type 4A, 2.3 g) and pentanamide oxime (474 mg, 5.9 mmol) were added, and the resulting mixture was vigorously stirred for 15 min before addition of cocaine hydrochloride (669 mg, 2.2 mmol). The reaction mixture was heated at 80 °C for 20 h. The solution was

then filtered from the molecular sieves and the solvent was evaporated in vacuo. The residue was dissolved in water (40 mL) and extracted with ether (3 × 150 mL). The dried ($MgSO_4$) ether phases were evaporated, and the residue was purified by flash chromatography [eluent: EtOAc/MeOH (2:1)]. The free base was dissolved in ethanol (10 mL) and a solution of oxalic acid in ethanol (2 mL) was added. The title compound crystallized after addition of ether in 37% yield: mp 174–175 °C; ^{1}H NMR (D_2O) 7.1 (1 H, m), 4.2 (1 H, m), 2.95 (3 H, s), 3.0–2.3 (7 H, m), 2.0–1.1 (4 H, m), 0.90 (3 H, t, J = 6 Hz). Anal. ($C_{16}H_{23}N_3O_5$) C, H, N.

3-(3-Butyl-1,2,4-oxadiazol-5-yl)quinuclidine Oxalate (17). The compound was synthesized as described under the general procedure for the synthesis of the compounds **2a–q** using 3-(methoxycarbonyl)quinuclidine[8] hydrochloride (**16**) instead of arecoline hydrobromide (**1**). Crystallization and recrystallization from ethanol/ether gave the title compound **17** in 46% yield: mp 122–124 °C; ^{1}H NMR (D_2O) δ 3.85 (2 H, s), 3.6–3.2 (3 H, m), 2.75 (2 H, t, J = 6 Hz), 2.6 (1 H, m), 2.5–1.1 (10 H, m), 0.90 (3 H, t, J = 6 Hz). Anal. ($C_{15}H_{23}N_3O_5$) C, H, N.

Muscarinic Cholinergic Agonism and Antagonism in Guinea Pig Ileum. Male guinea pigs were killed, and the terminal part of the ileum was removed and mounted in an organ bath containing tyrode solution of the following composition (mM): NaCl (136.9), KCl (2.68), $CaCl_2$ (0.9), $MgCl_2$ (1.05), $NaHCO_3$ (11.9), NaH_2PO_4 (0.42), glucose (5.55) maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. Dose–response curves to muscarinic agonists were constructed sequentially using 30-s contact time. Antagonists were added to the tyrode solution 5 min before the retesting of the agonist responses. Results are expressed as EC_{50} values for agonists and K_B values for antagonists. The K_B values were calculated by regression analysis with a minimum of four points and an n = 4–8 for each point. The efficacy of the agonists were compared to the full agonist acetylcholine, and the effects are expressed as percent of maximal contraction.

Receptor Binding to Rat Brain Homogenates. All preparations are performed at 0–4 °C unless otherwise indicated.

Displacement of [^{3}H]QNB. Fresh whole forebrain from male Wistar rats (200–250 g) was homogenized by an Ultra-Turrax homogenizer (5–10 s) in volumes of 0.32 M sucrose. The homogenate was centrifuged at 4300g for 5 min. The pellet was discarded and the supernatant centrifuged at 40000g for 15 min. The final pellet was rehomogenized in 50 mM KH_2PO_4, pH 7.1 (1000 mL/per g of original tissue), and the membrane homogenate was used for binding assays. To 2.5 mL of tissue suspension was added 25 μL of test substance and 25 μL [^{3}H]QNB (1 nM final concentration). Samples were thoroughly mixed and incubated at 37 °C for 20 min. After incubation, samples were poured directly onto Whatman GF/C glass-fiber filters under suction and immediately washed two times with 10 mL of buffer at 0 °C. Nonspecific binding was determined in duplicate with atropine (3 μM, final concentration) as the test substance. The amounts of radioactivity on the filters were determined by conventional liquid scintillation counting. Specific binding was total binding minus nonspecific binding.

Displacement of [^{3}H]Oxotremorine-M. Fresh cortex (0.1–1 g) from male Wistar rats (150–250 g) was homogenized for 5–10 s in 10 mL of 20 mM Hepes, pH 7.4, with an Ultra-Turrax homogenizer. The homogenizer was rinsed with 10 mL of buffer and the combined suspension centrifuged for 15 min at 40000g. The pellet was washed three times with buffer. In each step the pellet was homogenized as before in 2 × 10 mL of buffer and centrifuged for 10 min at 40000g. The final pellet was homogenized in 20 mM Hepes, pH 7.4 (100 mL per g of original tissue), and used for binding assay. Aliquots of 0.5 mL were added to 25 μL of test solution and 25 μL of [^{3}H]oxotremorine-M (1.0 nM, final concentration), and the solution was mixed and incubated for 30 min at 25 °C. Nonspecific binding was determined in triplicate with arecoline (3 μM, final concentration) as the test substance. After incubation samples were added to 5 mL of ice-cold buffer and poured directly onto Whatman GF/C glass-fiber filters under suction and immediately washed two times with 5 mL of ice-cold buffer. The amount of radioactivity on the filters was determined by conventional liquid scintillation counting. Specific binding is total binding minus nonspecific binding.

IC_{50} values were calculated from the inhibitory effects of at least four different concentrations of test compounds by using the Hill equation.

Supplementary Material Available: ^{1}H NMR spectral data and microanalysis for 3-(3-alkyl-1,2,4-oxadiazol-5-yl)-1,2,5,6-tetrahydro-1-methylpyridine oxalates (**2a–q**) and for 3-(5-alkyl-1,2,4-oxadiazol-3-yl)-1,2,5,6-tetrahydro-1-methylpyridine oxalates (**5a–c**) (3 pages). Ordering information is given on any current masthead page.