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CHOLINERGIC AGENTS: ALDEHYDE, KETONE, AND OXIME ANALOGUES OF THE MUSCARINIC AGONIST UH5

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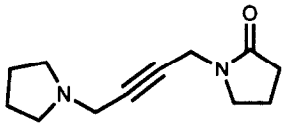
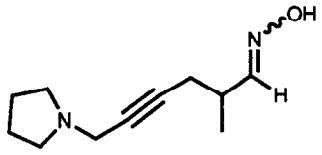
Abstract: There is considerable interest in muscarinic acetylcholine receptor (mAChR) subtype selective agents as cholinomimetics for the treatment of senile dementia of the Alzheimer's type (SDAT). A series of substituted analogs similar to the muscarinic agonists UH5 and UH28 (analogs of the muscarinic agonist oxotremorine) were synthesized and evaluated pharmacologically. Several oxime analogues of UH5 demonstrate agonist-like properties *in vitro* at muscarinic receptors. One oxime in this series (Compound **18**) was found to be almost five fold more m_1 selective than UH5.

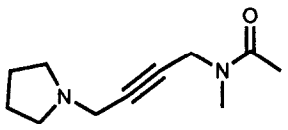
Although the involvement of several neurotransmitter systems has been implicated in Alzheimer's disease, the loss of forebrain cholinergic function is a consistent neurobiological abnormality.[4] Thus, cholinomimetics may hold promise as potential therapies for SDAT. Classical cholinomimetics are clinically ineffective due to peripheral parasympathetic effects, which are often observed at very low doses.



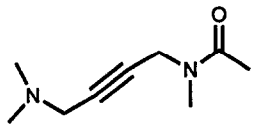
Oxotremorine



18



UH5

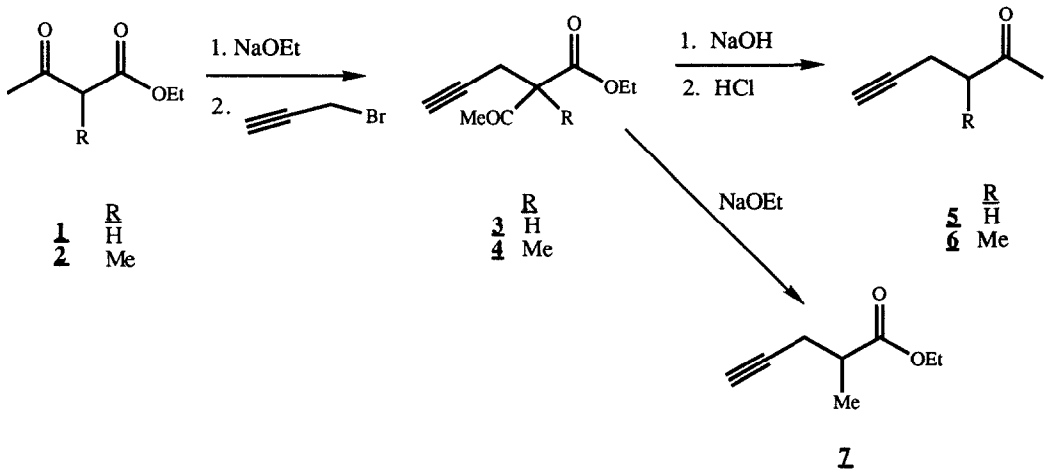


UH28

Five sequence-unique muscarinic cholinergic receptor subtypes (mAChRs), m_1 through m_5, have recently been identified.[6] These subtypes have been so classified by the cloning and expression of five receptors containing unique amino acid sequences. These receptors mediate some of the actions of acetylcholine, mainly in tissues localized in the brain (m_1, m_3, and m_5), and in the heart and gastrointestinal system (m_2 and m_4). The m_1, m_2 and m_3 receptors correlate pharmacologically to the M_1 (brain), M_2 (heart) and M_3 (glandular M_2) receptors, respectively.[7,8] While selective antagonists have been developed for most of the subtypes, selective agonists have not been readily identified. Recently, UH5 and UH28 (analogs of the classical muscarinic agonist, oxotremorine) have been reported to be somewhat subtype selective muscarinic agonists.[5] Efforts reported here are focused on the identification of muscarinic agonist analogs of UH5 and UH28 which may provide cholinomimetics with both a reduced liability for side effects (through subtype selectivity) and increased hydrolytic stability.

Previous work in the muscarinic agonist area suggested that modification of the lactam in oxotremorine may protect the compound from enzymatic oxidation and maintain agonist activity.[9,10] We anticipated that opening the lactam ring and replacing the amide nitrogen with a carbon atom might also enhance *in vivo* stability. Bradbury[11] optimized the tertiary amine region of these molecules. Combining these functional optimizations, we synthesized a series of ketone and oxime analogs of UH5 and UH28.

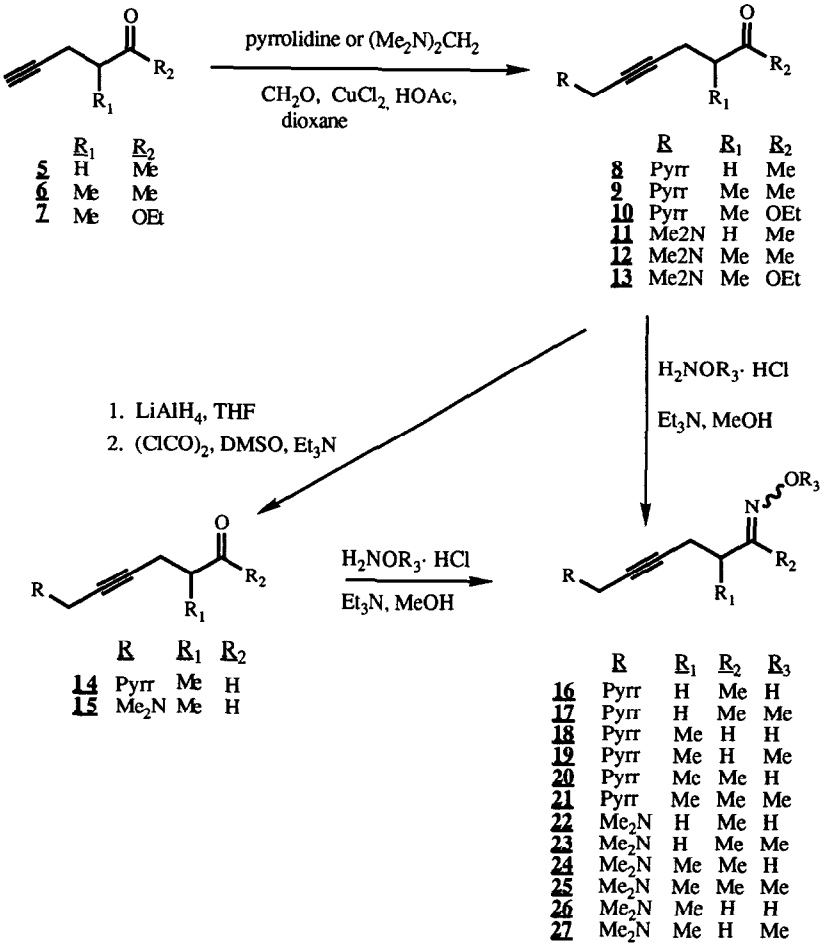
Scheme 1. Synthesis of Ketone and Ester Intermediates



Intermediates **3**, **4** and **7** were synthesized from ethyl acetoacetate or ethyl 2-methylacetoacetate following the methods of Ritter[12] and Barbot.[13] The route used to obtain the ketones **5** and **6** was similar to that of Resul[14] and Bradbury[11] (Scheme 1). Mannich condensations were used to couple the terminal acetylenes with pyrrolidine or N,N-dimethylamine (generated *in situ* from bis (N,N-dimethylamino)methane), yielding compounds **8-13**. Esters **10** and **13** were reduced with $LiAlH_4$ to the corresponding alcohols and oxidized to the aldehydes **14** and **15** under standard Swern conditions.

Aldehydes **14** and **15**, and ketones **8**, **9**, **11** and **12** were converted to their corresponding oximes, **16-27**, by reaction with the appropriate alkoxyamine hydrochlorides in the presence of triethylamine (Scheme 2). All compounds gave satisfactory analytical and spectroscopic results.[15]

Scheme 2. Synthesis of Target Compounds



	R_1	R_2
5	H	Me
6	Me	Me
7	Me	OEt

	R	R_1	R_2
8	Pyrr	H	Me
9	Pyrr	Me	Me
10	Pyrr	Me	OEt
11	Me2N	H	Me
12	Me2N	Me	Me
13	Me2N	Me	OEt

	R	R_1	R_2
14	Pyrr	Me	H
15	Me_2N	Me	H

	R	R_1	R_2	R_3
16	Pyrr	H	Me	H
17	Pyrr	H	Me	Me
18	Pyrr	Me	H	H
19	Pyrr	Me	H	Me
20	Pyrr	Me	Me	H
21	Pyrr	Me	Me	Me
22	Me_2N	H	Me	H
23	Me_2N	H	Me	Me
24	Me_2N	Me	Me	H
25	Me_2N	Me	Me	Me
26	Me_2N	Me	H	H
27	Me_2N	Me	H	Me

Biological Methods

Receptor binding assays were conducted in rat neocortex. The [^{3}H]-quinuclidinyl benzilate receptor binding assay (RQNB) assesses the ability of the test compound to compete with the muscarinic antagonist QNB. Similarly, the [^{3}H]-cis-methyldioxolane receptor binding assay (RCMD) assesses the ability of the test compound to compete with the muscarinic agonist CMD.[18] Muscarinic subtype selectivity is assessed through a comparison of displacement of the muscarinic antagonist QNB in a genetically transformed mouse cell line (m1C2) transfected with cloned m_1 receptors (m_1-QNB_{m1C2}) and rat heart homogenate containing the pharmacologic M_2 receptor (M_2-QNB_{heart}) respectively.[19,20]

Results

The structure-activity relationships of these analogs are summarized in Table 1. The efficacy ratio (RQNB/RCMD, as defined in Table 1) is predictive of muscarinic agonist efficacy. Muscarinic agonists generally exhibit a ratio greater than 100, antagonists have a ratio of approximately 1, while partial agonists have ratios between 1 and 100.[16,17] In our assays UH5, a full muscarinic agonist, has an efficacy ratio of 547; while compound **18** has an efficacy ratio of 96, classifying it as a partial agonist.

Table 1. Receptor Binding and Efficacy Ratios

Compound	RCMD IC_{50}(nM) or % inhib @0.1μM	RQNB IC_{50}(nM) or % inhib.@ 1.0μM	RQNB/RCMD (Efficacy Ratio)
Ketones			
8	214	7280	34
9	59	5696	97
11	68	65600	965
12	101	55400	549
Aldehydes			
14	1%	9%	-
15	0%	0%	-
Oximes			
16	373	1254	3
17	5%	17%	-
18	21	1928	92
19	27%	15%	-
20	21%	15%	-
21	21%	26%	-
22	139	36769	265
23	5%	1%	-
24	12%	9%	-
25	5%	3%	-
26	30	13230	441
27	6%	4%	-
Reference Compounds			
oxotremorine	1.5	407	308
UH5	4.5	2447	544
UH28	21.6	87876	4068
McNeil-A-343	64	5388	84

Data are expressed as the concentration of test compound that inhibits binding of 0.1 nM [^{3}H]-CMD or 0.03nM [^{3}H]-QNB by 50% (IC_{50}). The IC_{50} values were determined from 5-7 concentrations tested in triplicate.

The target compounds display a wide range of muscarinic activity (Table 1). Within this small series the selectivity ranges from m_1 selective partial agonists, such as compound **18**, to slightly M_2 selective compounds (Table 2). Small changes in structure had significant, although unpredictable, effects on affinity and selectivity.

Table 2. Relative Efficacy Values and Selectivity Ratios

Compound	RQNB/RCMD Efficacy Ratio	[^{3}H]-QNB IC_{50}(nM) Heart M_2	[^{3}H]-QNB IC_{50}(nM) m1C2(m_1)	M_2/m_1 Selectivity Ratio
18	92	5500	920	4.8
9	97	6500	2800	2.3
26	441	34200	25900	1.3
11	965	18900	51800	0.4
UH5	544	2010	2450	0.9
UH28	4068	4460	87880	0.2
oxotremorine	308	858	364	2.4
McNeil-A-343	84	34116	7240	4.7

Data are expressed as the concentration of test compound that inhibits binding by 50% (IC_{50}). Data are reported as the mean of at least three determinations.

Substitution of pyrrolidine with N,N-dimethylamine consistently reduced muscarinic **antagonist** affinity by a factor of ten in the ketone and oxime cases (e.g. **8** vs **11** and **20** vs **26**). This may reflect lipophilic and/or steric differences of the N,N-dimethylamine group versus pyrrolidine and its resultant ability to bind at the muscarinic antagonist binding site.

Several of the ketone analogs display satisfactory affinity and efficacy at the muscarinic receptor when R_1 is methyl or hydrogen (**9**, **11** and **12**). Three of four oximes display affinity and efficacy when R_1 or R_2 is hydrogen (**18**, **22** and **26**). Incorporation of methyl groups in both R_1 and R_2 positions (**20**, **24** and **25**) eliminates most affinity for both the agonist and antagonist binding sites. O-Methyl oxime substitution in all cases resulted in a total loss of affinity. The steric bulk of the methyloximes may hinder binding at the muscarinic receptor and the oxime hydrogen may also be involved in some necessary hydrogen bonding at this location.

In our assays for subtype selectivity (Table 2), UH5 shows no selectivity. Oxime **18** displays a five fold increase in selectivity for the m_1 receptor subtype in comparison to UH5. The substitution of N,N-dimethylamine for pyrrolidine, in oxime **26**, produces a loss of selectivity for the m_1 subtype vs the M_2 subtype. This selectivity decrease is accompanied by a ten fold decrease in antagonist affinity. A similar phenomenon can be observed in the ketone series between compounds **9** and **11**. In this case, the replacement of R_1 with a hydrogen in addition to the change from N,N-dimethylamino to pyrrolidine causes a ten fold decrease in antagonist affinity. This suggests that if the efficacy ratio increases the selectivity decreases. In this series of UH5 analogs we have discovered a range of muscarinic agonist affinities and selectivities for the M_2 or m_1 muscarinic agonist receptors. In general, the oxime and ketone analogs were found to have reasonable affinity for the muscarinic receptor, while the methyloximes and aldehydes showed low/no activity. Oxime **18** is the

most potent and m_1 selective partial muscarinic agonist in this series. It exhibits a receptor binding profile consistent with muscarinic partial agonist activity and has five fold better selectivity for the m_1 agonist receptor than UH5. Oxime **18** also has the desirable quality of being a lipid-soluble free amine. While it remains unclear whether this selectivity ratio will be meaningful *in vivo*, its *in vitro* selectivity is comparable to the quaternary salt McNeil-A-343, the most selective muscarinic agonist in the literature.

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