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THE SYNTHESIS AND STRUCTURE ACTIVITY RELATIONSHIPS OF ENANTIOMERICALLY PURE HYDROXYLATED OXOTREMORINE DERIVATIVES

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Abstract: The synthesis and radioligand binding data of optically active hydroxylated oxotremorine derivatives are described. There are significant pharmacological differences between the enantiomers, most notably at the 3- and 4-position of the pyrrolidinone ring. In addition, there appears to be one side of the pyrrolidinone ring that accommodates substitutents better than the other (facial selectivity).

Senile Dementia of the Alzheimer's Type (SDAT) is a neurodegenerative disease that results in progressive memory impairment and dementia. Autopsied brain tissue from SDAT patients has shown a consistent and significant decrease in cholinergic markers, such as choline acetyltransferase and acetylcholine esterase, most notably in the nucleus basalis of Meynert, cerebral cortex and hippocampus.[1] It has been suggested that the degeneration occurs presynaptically while the postsynaptic muscarinic receptors remain intact.[2] These observations in part form the basis for the cholinergic hypothesis of age related memory loss.[3] A series of drug discovery projects[4] have been initiated in our geriatric program to find cholinergic agents to ameliorate the cognitive symptoms of this degenerative disease.

To achieve the therapeutic goal, target structures can be screened for pharmacological as well as muscarinic receptor subtype selectivity to find agents that will enhance central cholinergic function with minimal peripheral cholinergic effects. Therefore, we have focused our attention on the search for a partial muscarinic agonist. Using conventional means of administration, a partial agonist will be accessible to most brain regions but, depending upon the level of receptor reserve,[5] may facilitate cholinergic transmission only in the presence of low levels of acetylcholine.

The introduction of a substitutent into a neurotransmitter mimic may decrease the affinity of the compound for its receptor by introducing steric hindrance between it and the receptor binding domain. Rather than a disadvantage, this effect may be useful as a possible method to construct a molecule with the optimal degree of partial agonism and possibly with receptor subtype selectivity. The addition of a

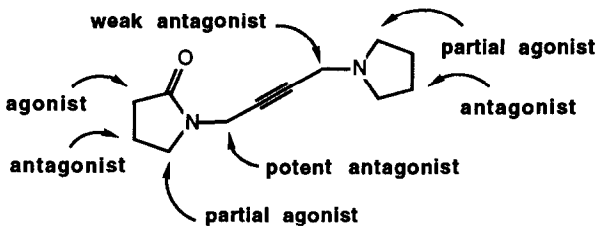


Figure 1. Structure activity relationships for methylated oxotremorine derivatives

methyl group to a series of cholinergic agonists has been shown to dramatically decrease *in vivo* and *in vitro* cholinergic agonist activity. One such investigation has been the synthesis and pharmacological studies of oxotremorine (Figure 1).[6] The systematic addition of a methyl group to oxotremorine produced compounds with pronounced pharmacological differences. These results provide evidence for the amount of steric tolerance in oxotremorine for lipophilic substitutents within the agonist binding domain.

Similar in size to a methyl group, amino, hydroxyl and thiol functionalities may not only probe the steric environment in the receptor binding domain but also may test for the presence of auxiliary polar functionalities. These modifications may result in subtype selectivity as well as pharmacological activity. In addition, these functional groups provide synthetic handles to enable the preparation of prodrug forms of the oxotremorine derivatives. The prodrug forms provide a second approach to search for, and/or improve the pharmacological selectivity of the target compounds.

To test these hypotheses, a chemical synthesis program was initiated to enantioselectively prepare hydroxyl, thiol and amino analogs of the cholinergic muscarinic agonist oxotremorine. This preliminary report will describe the synthesis and biochemical data of hydroxylated derivatives of oxotremorine (Figure 2).

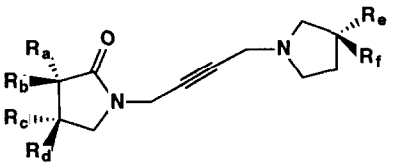


Figure 2. Targeted structures. One of the substitutents R_a, R_b R_c, R_d, R_e or R_f is OH.

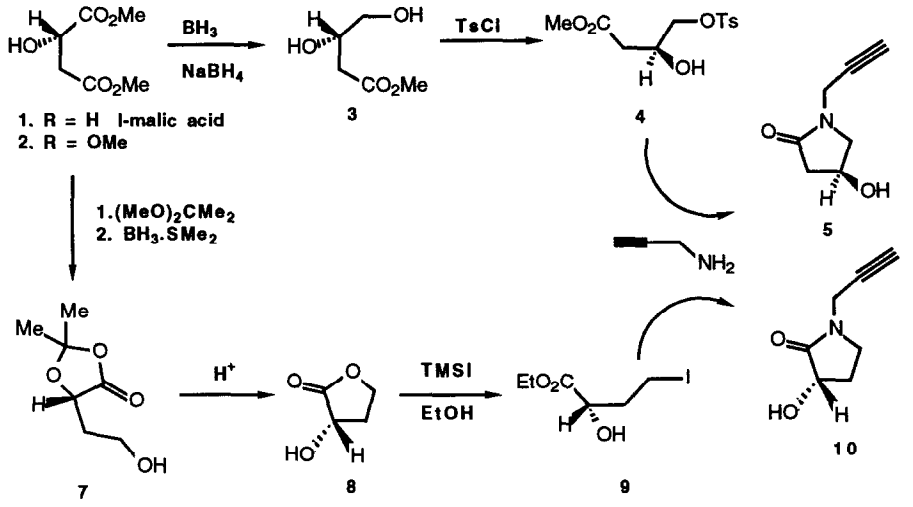
Chemistry.

The target structures were prepared as outlined in **Schemes I-III**. Esterification of *l*-malic acid **1** with methanolic hydrogen chloride provided the diester **2**. Reaction of **2** with one equivalent of borane and, when the hydrogen evolution ceased, addition of 5 mole percent of sodium borohydride produced an exothermic reaction and diol **3**.[7] The differentiation of the two esters in the reduction was presumably the result of intramolecular alkoxyborane activation of the desired ester via a five membered transition state rather than via a six membered transition state for the undesired ester. Tosylation of the primary alcohol in **3** with tosyl chloride and pyridine gave the crystalline tosylate **4**. Displacement of the tosylate group in **4** with propargylamine followed by concomitant cyclization led to the propargylic pyrrolidinone **5** in a 25% overall yield from **1**.

Alternatively, treatment of *l*-malic acid **1** with dimethoxypropane afforded the crystalline acetonide acid **6** which was reduced with borane dimethylsulfide complex to alcohol **7** Reaction of **7** with trifluoroacetic acid produced lactone **8** as a distillable liquid [8] Ring opening of lactone **8** and esterification of the resulting acid using trimethylsilyl iodide in ethanol produced the iodoester **9**.[9] Treatment of **9** with propargylamine resulted in the formation of pyrrolidinone **10** in a 45% overall yield from **1**.

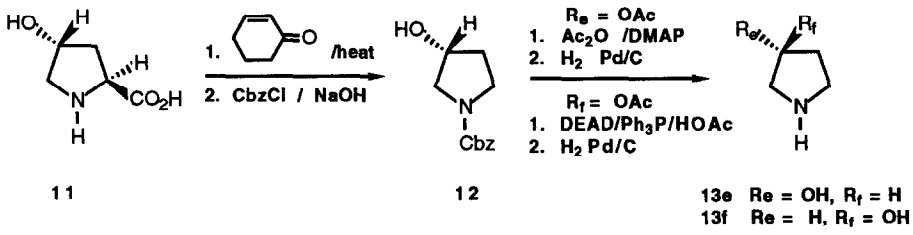
In a similar manner, the enantiomers of **5** and **10** were prepared starting from d-malic acid to provide the four enantiomerically pure hydroxylated pyrrolidinone derivatives **15a-d**.

Scheme I



The 3-acetoxypyrrolidines **13e,f** were readily prepared from trans- 4-hydroxy-1-proline (**11**). Decarboxylation of **11** using cyclohexenone in refluxing cyclohexanol[10] followed by protection of the secondary amine with benzyl chloroformate afforded **12**. Acetylation of the alcohol **12** with acetic anhydride followed by hydrogenolysis of the carbobenzyloxy group gave **13e**. Alternatively, reaction of **12** with diethyl azodicarboxylate, triphenylphosphine and acetic acid yielded the inverted acetate which upon removal of the carbobenzyloxy group by catalytic hydrogenation gave **13f**.

Scheme II

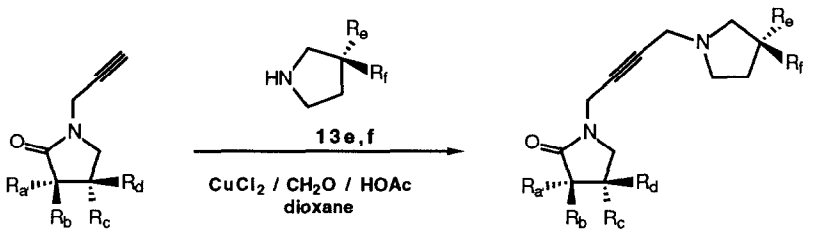


11 12

13e Re = OH, Rf = H
13f Re = H, Rf = OH

The preparation of the oxotremorine derivatives was achieved via a Mannich reaction. Protection of the hydroxyl groups in **14a-d** (R_a, R_b, R_c or R_d = OH) as acetates using acetic anhydride gave the propargylpyrrolidinones of general structure **15a-d**. Reaction of the enantiomerically pure pyrrolidinones **15a-d** or propargyl pyrrolidinone **15** (R_a, R_b, R_c, R_d = H) with pyrrolidine or 3-acetoxypyrrolidine **13e,f**, paraformaldehyde and a catalytic amount of cuprous chloride in dioxane and acetic acid yielded the acetoxyoxotremorine derivatives **16a-f**. Saponification of the acetates with sodium carbonate in methanol produced the target hydroxylated oxotremorine derivatives **17a-f** The optical purity of the derivatives was determined by the NMR spectra of the corresponding Mosher's ester derivatives[11] and, in each case, was found to be greater than 95% optically pure

Scheme III



14a-d R_a, R_b, R_c or R_d = OH
15a-d R_a, R_b, R_c or R_d = OAc

16a-f R_a, R_b, R_c, R_d R_e or R_f = OAc
17a-f R_a, R_b, R_c, R_d R_e or R_f = OH

Table 1. Summary of chemical and biochemical data.

Compd	Substitutent	Optical Rotation[b]	^{3}H-QNB Ki, μM[c] (rat)	S.D.[d] (+/-)	^{3}H-CD Ki, μM[c] (rat)	S.D.[d] (+/-)	Ki Ratio ^{3}H-QNB/ ^{3}H-CD
Oxotremorine			0.06	0.01	0.0005	0.0001	120
17a	R_a-OH	-63	1.4	0 5	0.009	0.0023	150
17b	R_b-OH	+60	2.1	0.8	0.12	0.006	17
17c	Rc-OH	+8	3	0.8	0.078	0 028	38
17d	R_d-OH	-7	7.5	1.3	8.56	2.9	0.9
17e	R_e-OH	-4	1.8	0.7	2.38	0.87	0.7
17f	R_f-OH	+4	1.2	0.6	0.61	0.19	2

[a] each compound gave a satisfactory elemental analysis; [b] optical rotations were determined in methanol and are reported as $[\alpha]_D^{26^0}$; [c] each value is the mean of three to five determinations using rat cortical tissue; [d] standard deviation from the mean

Pharmacology.

Displacement of ^{3}H-quinuclidinyl benzilate (^{3}H-QNB) from muscarinic acetylcholine receptors in binding assays has been used extensively as a rapid and efficient screen to compounds that interact at cholinergic receptors.[12] Little information regarding the potential intrinsic activity or pharmacological selectivity of the compound for muscarinic receptor subtypes is obtained in this assay. Meaningful estimates of intrinsic activity and pharmacological selectivity can nevertheless be determined from the apparent equilibrium dissociation constants (K_i) for test compounds. This information can be obtained using a series of assays that employ tritiated agonists as well as antagonists to label receptor populations in several different tissues.

A method for using the relative affinities (K_i values) of test compounds to displace ^{3}H-N-methylscopolamine (^{3}H-NMS, antagonist) and ^{3}H-oxotremorine-M (^{3}H-Oxo-M, agonist) as an index of their efficacy at cortical muscarinic receptors (NMS/Oxo-M ratio) has been described.[13] Alternatively, ^{3}H-quinuclidinyl benzilate (^{3}H-QNB, antagonist) can be used to label both the high and low affinity states[14] and ^{3}H-*cis*-methyltrimethylammoniummethyl-1,3-dioxolane (^{3}H-CD, agonist) to label the high affinity agonist state of the muscarinic receptor in competition assays.[15] The experimental conditions were chosen to maximize the difference between agonists and antagonists, and the ratio of K_i values for the two assays provides a useful index of agonist efficacy. Muscarinic antagonists display similar affinity in both binding assays (K_i ratio is ~1.0). Full muscarinic agonists displace ^{3}H-CD from the muscarinic receptor with high affinity relative to their displacement of ^{3}H-QNB. Thus, full agonists such as carbachol exhibit large ratios (K_i ratios ~ 500 to 2000). Compounds that display partial muscarinic agonist activity such as oxotremorine in functional assays have intermediate ratios (K_i ratio ~10-500). The ratio of K_i values determined in the two assays correlates well with the ability of agonists to stimulate cortical phosphatidylinositol turnover.[16]

Results.

All of the hydroxylated oxotremorine target structures **17a-f** have weaker affinities for the muscarinic receptor than the parent compound oxotremorine. Target **17a** and oxotremorine have similar ^{3}H-QNB/^{3}H-CD ratios suggesting that **17a** has comparable agonist-like properties to oxotremorine but with a lower affinity for the receptor. Amongst the enantiomers **17a-d** there are differences in affinity and agonist-like effects. Compounds **17a** and **17b** have similar affinities in the ^{3}H-QNB binding assay but **17a** has a tenfold greater affinity than **17b** in the ^{3}H-CD binding assay. Taken together, **17a** has a greater agonist-like profile than **17b**. Similarly, **17c** and **17d** have similar affinities in the ^{3}H-QNB binding assay but there is approximately a one hundred-fold difference in affinity in the ^{3}H-CD binding assay. Unlike **17c** which retains some partial agonist like effects, **17d** is best described as an antagonist and is similar to the 4-methyl substituted compound described by Ringdahl.[6] When these data are combined, there appears to be one side of the pyrrolidine ring which better accommodates hydroxyl substitutents (**17a,c**) than the other side (**17b,d**). Compounds **17e** and **17f** have weak affinity for the muscarinic

receptor and are clearly antagonists.

The present study describes the synthesis and biochemical data of compounds having hydroxyl substitutents on the muscarinic agonist oxotremorine. The data are consistent with the pharmacological effects observed by Ringdahl for the methylated derivatives with the exception of **17c** which has been characterized as a partial agonist. In addition, the biochemical data for the optical isomers at the 3- and 4-positions of the pyrrolidinone ring (**17a-d**) provide the first evidence of the effects of the steric environment of the oxotremorine molecule within the agonist binding domain. In summary, the initial biochemical profile of a series of hydroxylated oxotremorine derivatives as partial muscarinic acetylcholine agonists has been presented as potential therapeutic agents for the treatment of SDAT.

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