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Muscarinic Antagonist Activity of 3-(5-alkoxy-oxazol-2-yl)-1,2,5,6-tetrahydropyridines.

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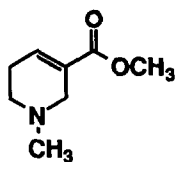
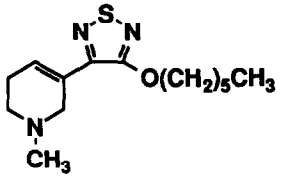
Abstract: A series of 3-(5-alkoxy-oxazol-2-yl)-1,2,5,6-tetrahydropyridines (OXTP) were found to have high affinity for muscarinic receptors and to be potent muscarinic antagonists as measured by blockade of acetylcholine stimulated PI hydrolysis in rat cortex or by blockade of oxotremorine induced tremors in mice.

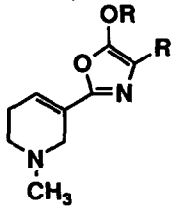
We wish to report potent muscarinic antagonist activity for a series of 1-methyl-3-(5-alkoxy-4-alkyloxazol-2-yl)-1,2,5,6-tetrahydropyridines (OXTP) **1**. The tetrahydropyridine moiety of arecoline has been the basis for many structure-activity studies directed at the discovery of new ligands for muscarinic receptors.[1] Arecoline itself has undergone clinical evaluation as a cholinomimetic agent for neurotransmitter replacement therapy in Alzheimer's disease.[2] Due to rapid ester hydrolysis, the relatively short half-life of arecoline *in vivo* has limited the clinical usefulness of this drug. Bioisosteric replacement of the ester functionality with different heterocycles has been investigated by several different laboratories.[3] Some examples of previously reported heterocyclic replacements include 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, 1,2,5-oxadiazoles, and 1,2,5-thiadiazoles.[3] These efforts have generally focused on analogs with varying degrees of muscarinic agonist activity, such as the 1,2,5-thiadiazole found in Xanomeline, now undergoing clinical trials for Alzheimer's disease.[4] In contrast, we have found that replacement with a substituted oxazole group results in compounds with potent muscarinic antagonist activity. Muscarinic antagonists are potentially valuable therapeutic entities for the treatment of Parkinson's disease, motion sickness, gastrointestinal and genitourinary disorders.[5]



Arecoline (2)



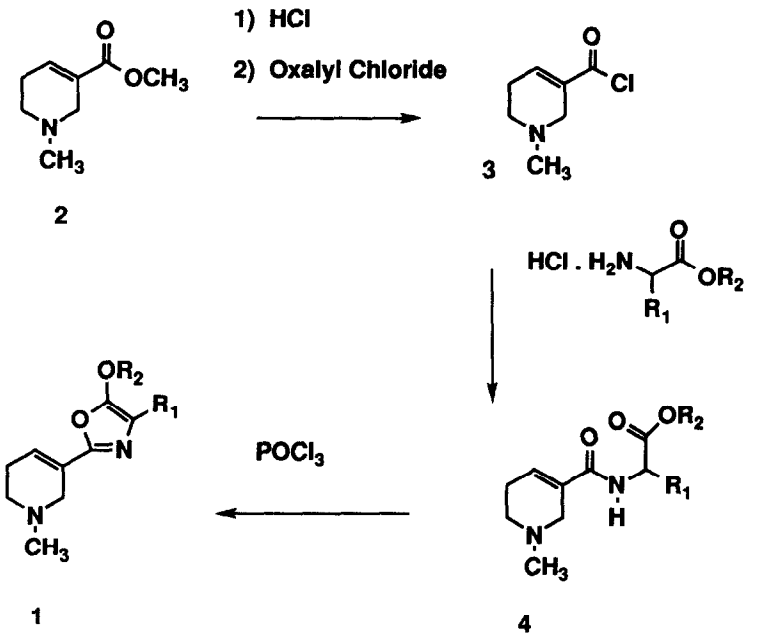
Xanomeline



OXTP (1)

Chemistry

The alkoxy-oxazoles were synthesized from arecoline and amino acid precursors. While arecaidine is commercially available, it was more economical to prepare it by acid hydrolysis of arecoline (**2**).[6] Arecaidine was converted to its corresponding acid chloride (**3**) by treatment with oxalyl chloride in CH_2Cl_2 along with catalytic DMF.[7] This acid chloride was then used to acylate a number of different α-amino acid esters. α-Amino acid esters not commercially available were prepared by reaction of the amino acid with thionyl chloride in the presence of the desired alcohol.[8] The α-amino acid esters, as hydrochloride salts, were

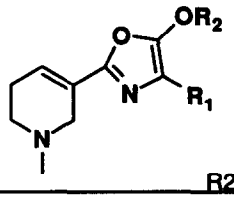


then reacted with the acid chloride in refluxing CH_2Cl_2 for 24-48 hours, without any additional base present.[9] The resulting amido-esters **4**, were then cyclized with $POCl_3$ in refluxing $CHCl_3$ to give the desired alkoxy-oxazole-tetrahydropyridine **1**.[10]

Pharmacology

Affinity for muscarinic receptors was measured based on inhibition of [^{3}H]-pirenzepine binding rat cortical homogenates.[11,12] Muscarinic antagonist activity was measured *in vitro*, using blockade of acetylcholine induced phosphitidyl inositol hydrolysis in a rat cerebral cortex slice preparation.[13] Antagonist activity *in vivo* was measured by blockade of the tremors induced by oxotremorine in mice (1mg/kg, i.p.).[12,14] Compounds also were examined alone in the above systems for agonist activity. None of the compounds in the series were found to have agonist activity on their own either *in vitro* or *in vivo*.

Increasing the length and bulk of the $\mathbf{R_1}$ and $\mathbf{R_2}$ substituents influenced the muscarinic antagonist potency in this series of tetrahydropyridyl-oxazoles. Simply increasing the size of the $\mathbf{R_1}$ substituent from hydrogen to methyl to ethyl to butyl results in a corresponding increase in the potency of the compounds in the *in vitro* binding and functional assays. Branching with a sec-butyl group further enhanced the potency both *in vitro* and *in vivo*. In some cases, such as **8**, **10** and **12**, the *in vivo* potency is substantially less than that expected from the *in vitro* potency. This difference is most pronounced for compound **8**, being inactive at blocking oxotremorine-induced tremors at the highest doses tested (30 mg/kg), while retaining substantial potency (216nM) for blockingACh-induced PI stimulation in rat cortex. The penetration into brain of **8** is demonstrated by its activity for disrupting memory in a spatial alternation paradigm in rats, with a minimum effective dose of 1 mg/kg (s.c.). The difference between *in vitro* and *in vivo* activity for **8**, in comparison



Cmpd	R1	R2	In Vitro: Receptor Binding ^{3}H-Pz K_i[a] nM	In Vitro: Inhibition ACh Stimul. PI Hydrol. IC_{50}[a] nM	In Vivo: Inhibition Oxotrem. Induced Tremors AD_{50}[b] mg.kg. ip
5	H	CH_3	>1000		>30
6	CH_3	CH_3	90		>30
7	CH_2CH_3	CH_3	30	646	10
8	$(CH_2)_3CH_3$	CH_3	11	284	>30
9	$CH(CH_3)_2$	CH_3	10	216	3.5
10	$CH_2CH(CH_3)_2$	CH_3	25	752	>30
11	$CH(CH_3)CH_2CH_3$	CH_3	2	102	2.5
12	CH_2PH	CH_3	16	758	>30
13	CH_3	CH_2CH_3	33	629	10
14	$CH(CH_3)CH_2CH_3$	CH_2CH_3	0.8	24	0.77
15	$CH(CH_3)CH_2CH_3$	$(CH_2)_2CH_3$	0.8	32	0.97
16	$CH(CH_3)CH_2CH_3$	$(CH_2)_3CH_3$	1.5	40	1.6
17	$CH(CH_3)CH_2CH_3$	$(CH_2)_5CH_3$	22		
scopolamine			0.08	8.9	0.1

[a] Determined in triplicate, standard error of the mean <25%. [b] Dose required for 50% reduction in the tremor response to oxotremorine (1 mg/kg, i.p.), five mice per dose, standard error of the mean <10%.

with other compounds in the series, may be a result of selectivity for subtypes of muscarinic receptors. Further characterization of the receptor selectivity and pharmacology of **8** will be published elsewhere.[14]

The compounds with a sec-butyl substituent for $\mathbf{R_1}$ are the most potent in the series. For example, compound **11** is the most potent of the compounds with $\mathbf{R_2}$ equal to methyl. Elongation of the $\mathbf{R_2}$ substituent further enhanced muscarinic antagoist potency. Compounds **14**, **15** with ethyl and propyl groups respectively at $\mathbf{R_2}$, were the most potent antagonists in this series. These latter compounds are within approximately one order of magnitude of the potency of scopolamine in these same assays. Further increase in the $\mathbf{R_2}$ group to the hexyl chain of **17** resulted in a decrease in affinity for pirenzepine binding.

All compounds examined in this series of alkoxy-oxazolyl-tetrahydropyridines were found to be devoid of muscarinic agonist activity in the test systems described above. Many examples in the series are muscarinic antagonists, as determined by blocking acetylcholine stimulated PI hydrolysis in rat cortex *in vitro* or by blockade of oxotremorine induced tremors *in vivo*. Evidence was found for compound **8** being relatively M1

selective, based on its relatively high potency for inhibiting acetylcholine stimulated PI turnover *in vitro* and for memory disruption in rats *in vivo* in comparison with its lack of activity for blocking oxotremorine induced tremors *in vivo*. Compound **14** was found to be the most potent antagonist in the series, having affinity for pirenzepine binding of 0.8 nM and and IC_{50} of 24 nM for blocking acetylcholine-stimulated PI hydrolysis in rat cortex and an AD_{50} of 0.77 mg/kg for blocking oxotremorine-induced tremors in rats. In the spatial alternation model, **14** disrupted memory in rats with a minimum effective dose of 0.3 mg/kg (s.c.).[14]

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References

1. Moos, W. H.; Bergmeier, S. C.; Coughenour, L. L.; Davis, R. E.; Hershenson, F. M.; Kester, J. A.; McKee, J. S.; Marriott, J. G.; Schwarz, R. D.; Tecle, H.; Thomas, A. J. *J. Pharm. Sci.*, **1992**, *81*, 1015-1019.

2. Soncrant, T. T.; Raffaele, K. C.; Asthana, S.; Berardi, A.; Morris, P. P.; Haxby, J. V. *Psychopharmacology* **1993**, *112*, 421-427.

3. a) Sauerberg, P.; Olesen, P. H., Suzdak, P. D.; Sheardown, M. J.; Mitch, C. H.; Quimby, S. J.; Ward, J. S.; Bymaster, F. P.; Sawyer, B. D.; Shannon, H. E.; *Bioorg. Med. Chem. Lett.* **1992**, *2*, 809-814. b) Showell, G. A.; Gibbons, T. L.; Kneen, C. O.; MacLeod, A. M.; Merchant, K.; Saunders, J.; Freedman, S. B.; Patel, S.; Baker, R. *J. Med. Chem.* **1991**, *34*, 1086-1094. c) Sauerberg, P.; Larsen, J. J.; Falch, E.; Krogsgaard-Larsen, P *J. Med. Chem.* **1986**, *29*, 1004-1009. d) Wadsworth, H. J.; Jenkins, S. M.; Orlek, B. S.; Cassidy, F.; Clark, M. S. G.; Hawkins, J.; Naylor, C. B. *J. Med. Chem.* **1992**, *35*, 1280-1290.

4. Sauerberg, P.; Olesen, P. H.; Nielsen, S.; Treppendahl, S.; Sheardown, M. J.; Honore, T.; Mitch, C. H.; Ward, J. S.; Pike, A. J.; Bymaster, F. P.; Sawyer, B. D.; Shannon, H. E. *J. Med. Chem.* **1992**, *35*, 2274-2283.

5. Weiner, N. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, seventh edition; Gilman, A. G.; Goodman, L. S.; Rall, T. W.; Murad, F., Ed.; Macmillan Publishing, New York, 1985, pp. 140-144.

6. Boyland, E.; Nery, R. *Biochem. J.* **1969**, *113*, 123-130.

7. Burgstahler, A. W.; Weigel, L. O.; Shaefer, C. G. *Synthesis* **1976**, 767-768.

8. Brenner, M.; Huber, W. *Helv. Chim. Acta* **1953**, *36*, 1109-1115.

9. Prelog, V.; Wieland, P *Helv. Chim. Acta* **1946**, *29*, 1128-1132.

10. Lakhan, R.; Ternai, B. *Advances in Heterocyclic Chemistry* ; Katritzky, A. R.; Boulton, A. J., Ed.; Academic Press: New York, 1974; Vol. 17, pp. 99-211.

11. Potter, L. T.; Ferrendelli, C. A.; Hanchett, H. E. *Cell Molec. Neurobiol.* **1988**, *8*, 181-191.

12. Shannon, H. E.; Bymaster, F. P.; Calligaro, D. O.; Greenwood, B.; Mitch, C. H.; Sawyer, B. D.; Ward, J. S.; Olesen, P. H.; Sheardown, M. J.; Swedberg, M. D. B.; Suzdak, P. D.; Sauerberg *J. Pharmacol. Exp. Ther.* **1994**, *269*, 271-281.

13. Schoepp, D. D.; Johnson, B. G.; Smith, E. C. R.; McQuaid, L. A. *Molecular Pharmacology*, **1990**, *38* 222-228.

14. Shannon, H. E.; Bymaster, F. P.; Hendrix, J. P.; Quimby, S. J.; Mitch, C. H. *Psychopharmacology* in press.

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