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CHOLINERGIC AGENTS: 2-OXAZOLIDINONE ANALOGUES OF THE ACETYLCHOLINE-RECEPTOR MUSCARINIC AGONIST PILOCARPINE

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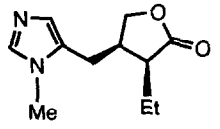
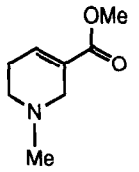
Abstract: An oxazolidinone analogue of pilocarpine demonstrates muscarinic agonist-like properties *in vitro* at the acetylcholine-receptor. Replacement of the carbon alpha to the carbonyl in pilocarpine with a nitrogen represents the most active 2-oxazolidinone, comparable to pilocarpine. Further modifications abolished much of the activity, demonstrating the strict structure activity relationships intrinsic to this series.

Cholinomimetics continue to hold promise as potential therapeutic agents in the symptomatic treatment of senile cognitive decline (SCD)[4]. Unfortunately, the clinical usefulness of classical cholinomimetics is limited by peripheral parasympathetic effects, which are often observed at doses below those necessary to elicit desired central nervous system effects. Consequently, there is considerable interest in muscarinic acetylcholine-receptor (mAcChR) subtype-selective agents, which may provide cholinomimetics with reduced liability for side effects. Pilocarpine, arecoline, and oxotremorine have the same principal site of action as muscarine and acetylcholine and are therefore classified as having muscarinic action.



pilocarpine

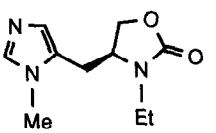


arecoline

oxotremorine

Pilocarpine has been reported to exhibit selectivity for the genetically defined m1 muscarinic receptor subtype[5]. Comparatively little structure-activity relationship (SAR) work in this area has appeared[6], despite the availability of facile synthetic routes to analogues[6-8].

Knowing that a strict SAR pertains to muscarinic agonism[4], we decided to study a series of isomers and derivatives which differ from pilocarpine by replacement of the carbon alpha to the carbonyl in pilocarpine with a nitrogen. These 2-oxazolidinones are more stable than the pilocarpine lactone ring while maintaining muscarinic activity. We have discovered that the most active oxazolidinone, PD 133315[6, 8] possesses muscarinic cholinergic properties *in vitro* and also exhibits some selectivity for central muscarinic sites[9, 10].

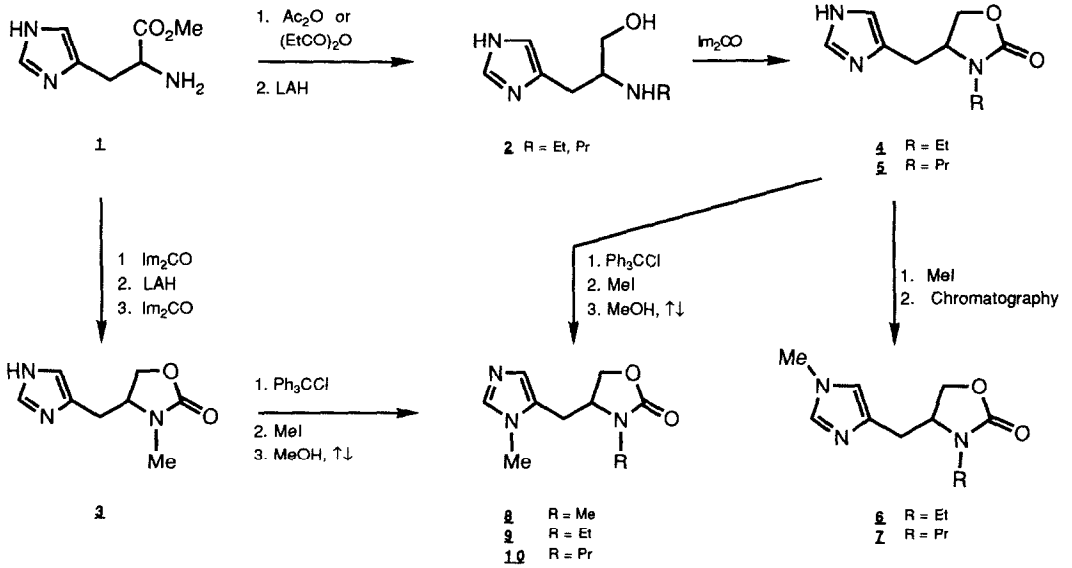


PD 133315 (9a)

The series of substituted imidazolylmethyl-2-oxazolidinones was synthesized as shown in Scheme 1. The synthetic route utilized was similar to the routes of Rapoport[6] and Gonzalez[8]. All approaches used (D or L)-histidine as the starting material. However, in the present case, the order of synthetic manipulations and the selection of reagents differ to provide a convergent route which allows for the generation of a variety of substitution patterns.

Alkylation of histidine methyl ester, 1, with 1,1'-carbonyldiimidazole (Im_2CO, for R=Me) or the appropriate anhydride (for R=Et, Pr) was followed by LAH reduction to afford an amino alcohol (2). Acylation with Im_2CO yielded 3-alkyl-4-imidazolylmethyl-2-oxazolidinones (3-5). Methylation of 4 and 5 to afford predominantly 3-alkyl-4-[(1-methyl-1H-imidazol-4-yl)methyl]-2-oxazolidinones (6 and 7) was achieved with iodomethane and chromatographic purification. The 3-alkyl-4-[(1-methyl-1H-imidazol-5-yl)methyl]-2-oxazolidinones, namely 8, 9, and 10, were obtained regiospecifically by the reaction of 4 or 5 with triphenylmethyl chloride followed by quaternization with iodomethane and then deprotection in refluxing methanol.

SCHEME 1. Synthesis of 3-alkyl-imidazolylmethyl-2-oxazolidinones

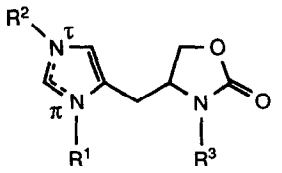


The activity of 2-oxazolidinone analogues is sensitive to changes in substitution patterns as demonstrated in Table 1. The [^{3}H]quinuclidinyl benzilate receptor binding assay (RQNB)[11] assesses the ability of the test compound to compete with the muscarinic antagonist, QNB. Similarly, the [^{3}H]cis-methyldioxolane receptor binding assay (RCMD)[12] assesses the ability of the test compound to compete with the muscarinic agonist, CMD. Data are reported as the percent inhibition at 0.1 μM of test compound for CMD or 1.0 μM of test compound for QNB. The concentration which inhibits binding by 50% (IC_{50}) is determined when the percent inhibition in RCMD or RQNB is greater than 20%.

Simple replacement of the carbon alpha to the carbonyl in pilocarpine with a nitrogen (PD 133315, 9a) gave the most active 2-oxazolidinone, comparable to pilocarpine. Further manipulations, however, abolished much of the activity. Several modifications to PD 133315 which resulted in loss of biological activity are: methylation at the τ nitrogen of the imidazole ring (6 and 7), removal of the methyl group on the imidazole altogether (3-5), and replacement of the ethyl group at R^3 with a methyl group (8).

The biological activity is sensitive to stereochemistry as demonstrated by a comparison of PD 133315 (9a) and 9b which differ only in their chirality. The S isomer, PD 133315 (9a), is the most potent of the oxazolidinones, while the R isomer, 9b, has little appreciable muscarinic activity at the concentrations tested.

TABLE 1. SAR of 3-alkyl-imidazolylmethyl-2-oxazolidinones



Compound	R^1	R^2	R^3	Stereo-chemistry	RCMD IC_{50} or % inhibition @ 0.1 μM	RQNB IC_{50} or % inhibition @ 1.0 μM
3	H	-	Me	S	0%	0%
4a	H	-	Et	S	4%	4%
4b	H	-	Et	R	0%	2%
5	H	-	Pr	S	2%	4%
6	-	Me	Et	R	0%	1%
7	-	Me	Pr	S	2%	10%
8	Me	-	Me	S	11%	2%
PD 133315 (9a)	Me	-	Et	S	43 nM	8900 nM
9b	Me	-	Et	R	6%	0%
10	Me	-	Pr	S	73 nM	3300 nM

Receptor binding assays, RCMD and RQNB, assess the ability of the test compounds to displace a muscarinic agonist or antagonist, [^{3}H]quinuclidinyl benzilate or [^{3}H]cis-methyldioxolane respectively, in rat cortical tissue. Data are reported as the mean of at least three determinations. The standard errors of measurement are within 10% of the reported values.

The ability of the test compound to stimulate [^{3}H]inositol phosphate accumulation[13] was assessed through activation of endogenous muscarinic receptors in human SK-N-SH neuroblastoma cells (Table 2). Data are expressed as the maximum stimulation of phosphoinositide (PI) turnover by the test compound relative to stimulation by 100 μM of carbachol (CCh). The ratio of IC_{50} values obtained for muscarinic antagonist/agonist binding (RQNB/RCMD) is predictive of the intrinsic efficacy of muscarinic agonists[14] for stimulation of PI turnover *in vitro* .

Muscarinic agonists generally exhibit a RQNB/RCMD ratio of ≥100, antagonists have a ratio of ≤1, while partial agonists usually have ratios between 1 and 100. Table 2 shows that PD 133315 (9a) exhibits a muscarinic agonist ratio of 214 and stimulates PI turnover. This profile of activity is indicative of a muscarinic agonist and is comparable to oxotremorine and pilocarpine. Although replacement of the ethyl group at R^3 with a propyl group (10) retained binding activity, the ratio of RQNB/RCMD of 45 is indicative of a partial agonist.

The subtype selectivity of muscarinic agonists was determined by comparing displacement of the muscarinic antagonist QNB in a genetically transformed rat cell line (m1C2) transfected with cloned m1 receptors[5, 15] ($m1\text{-}QNB_{m1c2}$) and rat heart homogenate containing predominantly the pharmacologic M2 receptor[5] ($M2\text{-}QNB_{heart}$) respectively. Data are expressed as the concentration of the test compound that inhibits binding of QNB by 50% (IC_{50}). Though less potent than pilocarpine or oxotremorine, PD 133315 exhibits a comparable degree of specificity for m1 versus M2 muscarinic receptors.

TABLE 2. Relative Efficacy and Selectivity of 2-Oxazolidinones and Reference Agonists

Compound	RQNB/RCMD (Efficacy Ratio)	PI Turnover (% Carbachol)	M2-QNB$_{heart}$ IC_{50} (nM)	m1-QNB$_{m1c2}$ IC_{50} (nM)	M2/m1 (Selectivity Ratio)
PD 133315 (9a)	214	16	30910	12690	2.4
10	45				
pilocarpine	142	33	8800	4430	2.0
oxotremorine	308	33	860	360	2.4
arecoline	604	69	9150	11920	0.8
carbachol	2202	100	18900	44370	0.4

The efficacy ratio (RQNB/RCMD) is predictive of muscarinic agonist activity. Phosphoinositide (PI) turnover assesses the ability of the test compound to stimulate [^{3}H]inositol phosphate accumulation in human SK-N-SH neuroblastoma cells relative to carbachol. Subtype selectivity assesses displacement of the muscarinic antagonist QNB in a genetically transformed rat cell line (m1C2) transfected with cloned m1 receptors (m1-QNB$_{m1c2}$) and rat heart homogenate containing the pharmacologic M2 receptor (M2-QNB$_{heart}$) respectively. Data are reported as the mean of at least three determinations. The standard errors of measurement are within 20% of the reported values

While limited synthetic and biological studies on some of these compounds (4a, 6, and 9a) were reported during the course of our work[6, 8], we have developed an additional, versatile synthetic route, extended the SAR via new analogues, and are the first to access muscarinic sub-type selectivity comparable to pilocarpine.

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