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A RATIONALE FOR THE DESIGN AND SYNTHESIS OF m1 SELECTIVE MUSCARINIC AGONISTS.

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Abstract. Synthesis of potent and efficacious 1-azabicyclo[2.2.1]heptan-3-one oxime muscarinic agonists is described. These oximes have extended appendages designed to span the cavity defined by the seven helices of the muscarinic receptor.

Five muscarinic receptor subtypes (m1-m5) with unique protein structures have been described. These receptor subtypes belong to the G-protein coupled family of receptors.[1, 2] Receptors of this family are characterized by the proposed existence of seven helices[3,4] that define a transmembrane cavity. Within the cavity key amino acid residues appear to play a major role in positioning, orienting and binding an agonist to the receptor. Differences in amino acid sequence and internal topography among the subtypes is very limited.[1, 5] The design of subtype selective agonists, therefore, is difficult.

Because of the unique distribution of m1 receptors[1,6,7,8,9] in the peripheral and central nervous system, m1 agonists have the potential to enhance cognitive function without inducing unwanted cholinomimetic-mediated side-effects. Unfortunately, the most m1 selective muscarinic agonist yet described is only twofold selective for m1 over m2 receptor subtypes (McN-A-343[10]; Table I). This degree of selectivity is unlikely to translate to improved pharmacologic profiles. A new approach to the design and synthesis of muscarinic subtype selective agonists is required. Two general criteria must be met: 1) Compounds must be non-quaternary amines[11] to enhance brain penetration and must exhibit high efficacy at a selected muscarinic receptor subtype, having greater binding affinity for sites labelled by agonists than those labelled by antagonists. Efficacy in stimulating second messenger systems is required. 2) Compounds should be longer and larger than their non-selective counterparts to ensure maximum contact between the agonist and the internal surface of the binding cavity. Longer and larger compounds may bring the agonist into proximity with parts of the receptor unique to a particular subtype. Unique ligand receptor interactions may be required to achieve greater subtype selectivity.

The first criterion has been met by the recently described 1-azabicyclo[2.2.1]heptane oxadiazoles (1).[12, 13] The oxadiazole analogs and chemically related compounds[12, 14, 15, 16] are small molecules displaying little m1 subtype selectivity. Despite the lack of m1 selectivity in this

chemical series, we chose the 1-azabicyclo[2.2.1]heptane nucleus as a starting point in the design of the proposed compounds because of its demonstrated ability to yield high efficacy muscarinic agonists.

Fulfilling criterion 2 has been difficult. Muscarinic agonists tend to be small molecules. Introduction or extension of one carbon unit to an agonist leads to an antagonist or a partial agonist (Figure 1).[11, 12, 13] However, it may be possible to add bulkier groups in some cases and still maintain agonist activity. For example, agonist activity is retained when the methyl group in arecoline (4) is replaced with the bulkier O-propargyl group (8).[11] Additionally, retinal, a large/long molecule is an agonist for the G-protein linked receptor rhodopsin.[17] These observations led us to design and synthesize 1-azabicyclo[2.2.1]heptan-3-one oximes with a poly-ene/poly-yne appendage. The structures of the oximes, receptor binding data, subtype selectivity profiles and inositol phosphate stimulation data are given in Tables I and II.

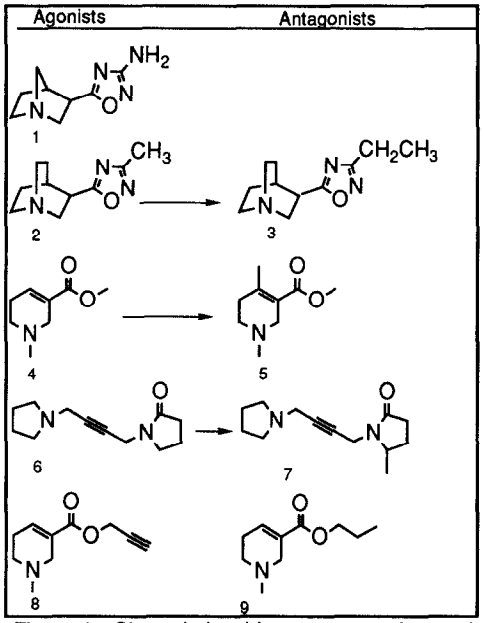


Figure 1. Size relationship among agonists and functional antagonists (partial agonists).

The target compounds were prepared by treating 1-azabicyclo[2.2.1]heptan-3-one (19)[18] with a hydroxylamine(13). When necessary, hydroxylamines were prepared from the appropriate alcohols (11) and N-hydroxyphthalimide (10) via the Mitsunobu[19] reaction to give the corresponding O-substituted phthalimides (12). Hydrolysis of the latter gave the desired hydroxylamines in good yields. Complex alcohols such as 2,5-hexadiyne-1-ol (16, R_1 = H) and terminally substituted 2,5-hexadiyne-1-ol (16, $R_1 \neq$ H), were prepared by Cu mediated coupling[20] of propargyl alcohol and propargyl bromides (15, terminally substituted/unsubstituted), Scheme I.

Biological activity was determined using muscarinic receptor binding followed by second messenger characterization. Muscarinic receptor binding assays were conducted using [^{3}H] quinuclidinyl benzilate to label antagonist sites (RQNB) and [^{3}H]-cis-methyldioxolane to label agonist sites (RCMD) in the rat neocortex.[21, 22] The ratio of RQNB/RCMD has been shown[23] to

predict agonist efficacy at muscarinic receptors. To confirm efficacy predictions from the binding assays, the ability of selected agonists to stimulate inositol phosphate accumulation[24] was determined (Table II). Selectivity for m1 over m2 muscarinic subtypes was determined by estimating agonist affinity for m1 and m2 receptor subtypes labelled by [^{3}H] QNB in CHO cells selectively expressing human m1 and m2 receptors.[25]

Our results indicate that synthesis of long/large, efficacious and potent 1-azabicyclo[2.2.1]heptan-3-one oxime muscarinic agonists is possible. The 2-propynyl (20h), 2,5 hexadiynyl (20p) and 2,5,8-nonatriynyl (20u) oximes are potent and efficacious muscarinic agonists (IC_{50} = 3.5 nM, 22 nM, 8.0 nM and ratios of RQNB/RCMD =1245, 402 and 119 respectively). These compounds meet our first criterion. Furthermore, these compounds are longer and larger than previously described muscarinic agonists. This may lead to greater subtype selectivity. As an early indication, compound 20v has greater affinity for m1 versus m2 receptor subtypes. Additional work to achieve greater agonist subtype selectivity is in progress .

Scheme 1

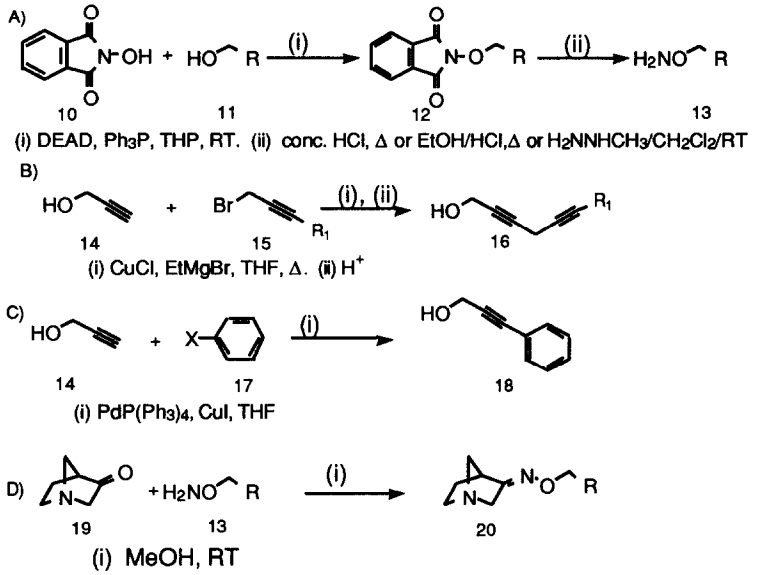
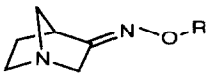


Table I. Muscarinic affinity and sub-type selectivity of alkyl and alkynyl 1-azabicyclo[2.2.1]heptan-3-one oximes

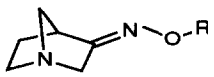


		IC_{50} nM*		RQNB	IC_{50} μm* RQNB		m2
NO	R	RCMD	RQNB	RCMD	m2	m1	m1
20a	H	>100	>1000	NA	NA	NA	NA
20b	CH_3	18.2	24558	1349	7.89	85.05	0.09
20c	CH_2CH3	12.5	3112	249	NA	NA	NA
20d	$CH_2CH_2CH_3$	120.0	3846	32	3.98	3.68	1.1
20e	$CH(CH_3)_2$	46.0	2030	44	3.15	2.61	1.2
20f	$C(CH_3)_3$	91.0	3540	39	4.46	3.84	1.2
20g	cBu	88.0	1156	13	1.63	1.11	1.5
20h+	$CH_2C{\equiv}CH$	3.5	4359	1245	1.04	9.51	0.1
20i	$CH_2C{\equiv}CCH_3$	4.0	4214	1054	1.70	5.81	0.3
20j	$CH_2C{\equiv}CCH_2CH_3$	3.6	5976	1660	8.60	4.90	1.8
20k+	$CH_2C{\equiv}CCH_2CH_2CH_3$	48.0	8689	181	20.6	9.64	2.1
20l+	$CH_2C{\equiv}CCH_2CH_2CH_2CH_3$	44.0	6045	137	9.65	7.89	1.2
20m	$CH(CH_3)C{\equiv}CH$	35.0	3984	114	5.28	3.69	1.4
20n	$CH_2CH_2C{\equiv}CH$	83.0	9806	118	15.59	14.45	1.1
20o+	$CH_2CH_2CH_2C{\equiv}CH$	111.0	3655	33	NA	NA	NA
20p	$CH_2C{\equiv}CCH_2C{\equiv}CH$	22.0	8834	402	2.84	10.01	0.3
20q+	$CH_2C{\equiv}CCH_2C{\equiv}CCH_3$	39.0	2914	75	5.08	5.68	0.9
20r+	$CH_2C{\equiv}CCH_2CH{=}CH_2$	32.0	7313	229	15.9	9.44	1.7
20s	$CH_2CH{=}CHC{\equiv}CH$	35.0	13850	396	30.2	12.6	2.4
20t	$CH_2C{\equiv}CCH_2OCH_3$	55.0	35434	644	59.3	42.4	1.4
20u+	$CH_2C{\equiv}CCH_2C{\equiv}CCH_2C{\equiv}CH$	8.0	958	120	2.18	2.03	1.1
20v+	$CH_2C{\equiv}CPh$	46.0	5012	109	25.8	5.42	4.8
	McN-A-343	25.0	5304	212	12.93	4.	2.6
	Carbachol	6.7	33000	4925	3.46	124.83	0.03
1	oxadiazole	0.3	88	293	0.02	0.4	0.05

*For IC_{50} determination, each drug was investigated in triplicate at 5-6 concentrations. NA = not active;

+Oximes isolated in pure Z form; the rest of the oximes were isolated as a mixture of E and Z isomers.

Table II. 1-Azabicyclo[2.2.1]heptan-3-one oxime Induced Inositol Phosphate Accumulation.



NO	R	RQNB RCMD	HMPA[a] %CCh[c]	HMPA[a] EC_{50}[d]	MIPA[b] %CCh[c]	MIPA[b] EC_{50}[d]
20b	CH_3	1349	NT		53	14.6
20c	$CH2CH_3$	249	NT		19	8.9
20d	$CH2CH_2CH_3$	32	44	27.00	3	ND
20h	$CH_2C{\equiv}CH$	1245	104	0.89	51	8.4
20i	$CH_2C{\equiv}CCH_3$	1054	63	1.90	49	8.8
20j	$CH_2C{\equiv}CCH_2CH_3$	1660	72	5.90	56	9.6
20p	$CH_2C{\equiv}CCH_2C{\equiv}CH$	402	79	4.70	14	16.4
20q	$CH_2C{\equiv}CCH_2C{\equiv}CCH_3$	75	81	5.05	2	ND
20u	$CH_2C{\equiv}CCH_2C{\equiv}CCH_2C{\equiv}CH$	120	50	5.10	0	ND
20v	$CH_2C{\equiv}CPh$	109	76	3.30	11	ND
	McN-A-343	212	57	3.50	3	ND
	Carbachol	4925	100	5.69	100	10.6
1	Oxadiazole	293	100	0.269	104	<1

[a]HMPA (Human Muscarinic Inositol Phosphate Accumulation) was measured in CHO cells expressing human m1 receptors. [b]MIPA (Muscarinic-stimulated Inositol Phosphate Accumulation) was measured in SK-N-SH neuroblastoma cells expressing human m3 receptors. [c]Compounds run at 100 μM; 100 μM of carbachol was used; results are means of two experiments done in triplicate at 5 concentrations. ND = not determined. NT = not tested. [d]EC_{50}s are in μM.

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