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ACYLHYDRAZONES AS M1/M3 SELECTIVE MUSCARINIC AGONISTS

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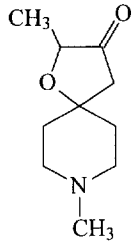
Abstract: To improve receptor binding affinity and to investigate functional selectivity of 2,8-dimethyl-1-oxa-8- azaspiro[4.5]decan-3-one acetylhydrazone **2** at muscarinic receptor subtypes, a series of acylhydrazones **A** was synthesized. The SAR indicates that the binding affinity in the pirenzepine assay (M1) correlates well with lipophilicity. Intrinsic activity (30% of carbachol response) of agonists at M1 remains unchanged. Compounds with n = 0 and 6, where X = $NHCO(CH_2)_nMe$, did not inhibit cAMP formation in rat heart membrane (M2). Most of the compounds are more efficacious at the M3 receptor than at M1. The results suggest that the M1 and M3 receptors can better tolerate bulky and long chained substituents than the M2 receptor.

Development of centrally acting cholinergic M1-selective agonists has become a current trend in the design and synthesis of muscarinic agonists for Alzheimer's disease (AD). M1 receptor activation is thought to be important not only in reversing cognitive deficits associated with AD but also in reducing β-amyloid deposition, an effect that may alter disease progression.[1] Traditional muscarinic agonists have little or no receptor subtype selectivity, limiting their clinical usefulness due to side effects associated with stimulation of the M2 and M3 muscarinic receptors.[2] Elaboration of the highly efficacious albeit nonselective muscarinic compound 2,8-dimethyl-1-oxa-8-azaspiro[4.5]decan-3-one[3] (**1**) was seen as a basis for developing subtype selective compounds. During the structural modification of **1**, compound **2** was found to exhibit no M2 functional activity, but a robust effect in improving short term memory of aged rats in the T-maze paradigm.[4] However, the affinity of this compound for M1, assessed by a [H^3]pirenzepine (PZ) binding assay, was quite low.[3a] The molecular modeling studies suggested that a cavity or pocket exists in the trans-membrane helical bundle at both the binding and efficacy sites of muscarinic receptors, which could accommodate a bulky agonist ligand. Exploiting the presumed subtle differences in ligand size tolerance of the various subtypes was elected as the means of creating discriminative ligand-receptor interactions as well as improving M1 affinity.[5] The ease of synthesis and opportunity for structural modification made acylhydrazones **A** an attractive choice for the exploration of the steric and electronic requirements of the M1, M2, and M3 receptors with an eye toward finding auxiliary binding sites unique to each subtype.



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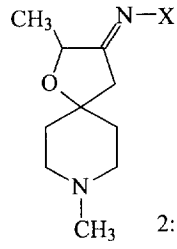


A

X = NR'COR
2: X = NHCOMe

Chemistry

Synthesis The acylhydrazones were prepared by the reaction of the spirofuranone **1** citrate with the appropriate hydrazides in methanol at room temperature. NOE information indicates an antirelationship between the methyl and acetylhydrazone groups of compound **2**.[3a]

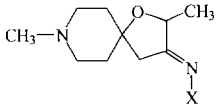
Molecular Modeling Models of the 7 trans-membrane domains of the M1, M2, and M3 muscarinic receptor subtypes were constructed and optimized by a combination of molecular dynamics and mechanics using the Sybyl suite of programs.[6a] The individual helicies were arranged into the appropriately organized bundles based upon alignment to the electron cryo-microscopic structure of bacteriorhodopsin.[6b,c] Ligands were docked into either a capture or binding site of the M1 receptor centered around Asp105, at the extracellular margin of the helical bundle, or into the address or efficacy site around Asp79, buried within the trans-membrane cavity (see Fig. 2).

Results and Discussion

The steric tolerance of the M1 receptor was systematically examined by synthesizing an extensive series of straight chained, branched, cyclic alkyl and aromatic acylhydrazones, and testing for affinity (PZ displacement) as well as efficacy (stimulation of phosphotidyl inositol, PI, hydrolysis as compared to that of carbachol).[7] The results are shown in Table 1. In the homologous series of alkyl acylhydrazones a linear correlation[8] was found between affinity and cLog P[9] (Fig. 1) as the length of the chain is increased from C1-C9. The affinity peaked at ca 10 uM when R is $\geq C_7$. Replacing NH with NMe, as seen in compounds **3** and **7**, enhanced the binding. The relationship between affinity and cLog P is also demonstrated by the cyclohexyl (**18-21**) and the arylalkyl (**22-26**) analogs. Compound **25**, a phenylcyclohexyl analog, displayed the best affinity (4.8 uM) in Table 1. Limited studies which measured potency at M1 receptors showed a good agreement with affinity and is consistent with earlier unpublished studies which have compared Ki and EC_{50} values for partial agonists at M1 receptors. For example, compounds **1**, **2**, and **27** had EC_{50} values for stimulation of PI

hydrolysis of 3.5, 42, and 46 μM respectively, which closely parallel measured Ki values (Table1). When R is NH_2 (semicarbazone **27**) or OMe (carbomethoxyhydrazone **28**), the intrinsic activity was slightly reduced as compared to **2**. The wide range of affinity values was not reflected in M1 efficacy which remained at ~ 30% of maximum. The size limit for agonism was C7 in the simple alkyl acylhydrazone series beyond which the

Table 1 Biological Activities[7] and cLog P of Acylhydrazone Derivatives

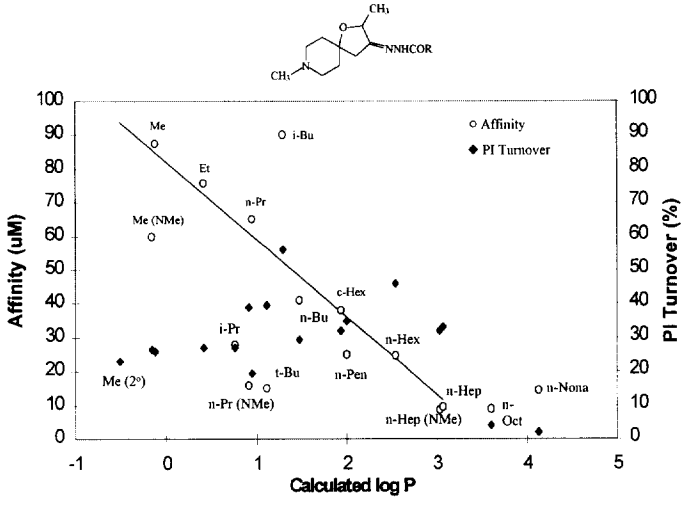


#	X	PZ (M1) Ki (μM)	PI % max[b]	cLog P[9]
1		3.8	64	
2	NHCOMe	100	26	-0.12
3	NMeCOMe	60	26	-0.16
4[a]	NHCOMe	180	23	-0.51
5	NHCOEt	76	27	0.41
6	NHCO-n-Pr	65	20	0.95
7	NMeCO-n-Pr	16	39	0.91
8	NHCO-i-Pr	28	27	0.76
9	NHCO-n-Bu	41	30	1.47
10	NHCO-i-Bu	90	43	1.29
11	NHCO-t-Bu	15.2	40	1.11
12	NHCO-n-Pen	25	35	2.00
13	NHCO-n-Hex	25	46	2.54
14	NHCO-n-Hep	9.5	33	3.07
15	NMeCO-n-Hep	8.8	32	3.03
16	NHCO-n-Oct	9.0	4	3.60
17	NHCO-n-Nona	14.4	2	4.13
18	NHCO-c-Hex	38	32	1.94
19	$NHCOCH_2$-c-Hex	30	39	2.47
20	$NHCO(CH_2)_3$-c-Hex	13.6	23	3.53
21	$NHCO(CH_2)_4$-c-Hex	8.0	5	4.06
22	NHCOPh	78	33	1.63
23	$NHCO\text{-}CH_2Ph$	30	11	1.66
24	$NHCO(CH_2)_3Ph$	34	32	2.50
25	NHCOPh-4-c-Hex	4.8	38	4.15
26	$NHCOCH_2$-2-Naph	10	20	2.89
27	$NHCONH_2$	100	26	-0.63
28	$NHCO_2CH_3$	25	19	0.33

[a]8-desmethyl derivative. [b]Intrinsic activity as percent of carbachol response.

compounds become antagonists. Compounds with M1 intrinsic activity greater than 20 % and with M1 affinity ranging from 4.8 to 180 uM were further examined for M2 and M3 activity. Compounds **2**, **14**, **25**, and **26** showed marked functional selectivity for the M1 and M3 receptor subtypes over M2 (Table 2). These compounds showed no M2 activity as compared to **4** (a full M2 agonist), **8**, and **12**. The semicarbazone **27** exhibited good M2 activity. All compounds tested in the isolated guinea pig trachea assay showed a high level of M3 intrinsic activity.

Fig. 1 cLog P vs Affinity and M1 Efficacy[a]

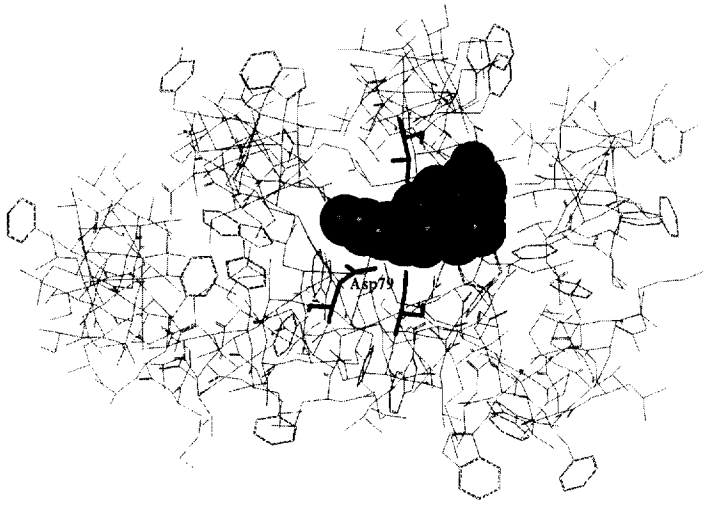


[a] For clarity, labels are only placed on affinity (open circle) for each compound.

Table 2 Intrinsic Activitities of Selected Acyl Hydrazones at the Three Muscarinic Receptor Subtypes

#	X	Intrinsic Activity (%) Hip (M1)	Heart(M2)	Tr (M3)
1		64	100	83
2	NHCOMe	26	0	91
4	NHCOMe	23	100	
8	NHCO-i-Pr	27	40	
12	NHCO-n-Pen	35	34	
14	NHCO-n-Hep	33	0	81
25	NHCOPh-4-c-Hex	38	0	78
26	$NHCOCH_2$-2-Naph	20	0	
27	$NHCONH_2$	26	51	

Fig. 2 Interaction of 14 with the Efficacy Site of the M1 Receptor



In the molecular modeling study, compound **14**, X = NHCO-n-Hep, was docked within the efficacy site of the trans-membrane domain of the M1 receptor (Fig. 2). The side-chain terminal methyl moiety is closely enveloped by two tyrosine residues that form part of the binding site. Longer side-chains in the acylhydrazone series will be sterically excluded from the efficacy site and thus be functionally inactive. This study indicates that the M1 and M3 receptors can better accommodate bulky and long chained substituents than the M2 receptor.

These results suggest that an agonist ligand with a long side-chain or bulky group in this series can still fit into both the receptor recognition site (binding site) and the activation site (efficacy site) of the M1 and M3 receptor subtypes and that they may be excluded from the M2 efficacy site by either steric occlusion or detrimental interaction. Furthermore, the affinity for the M1 receptor, obtained from the pirenzepine assay, was found to vary widely with little change in intrinsic activity within the series, consistent with the view that the recognition site for agonists and antagonists differs in muscarinic receptor subtypes and is distinct from the efficacy site.[10]

In summary, acylhydrazone derivatives of 2,8-dimethyl-1-oxa-8-azaspiro[4.5]decan-3-one (**1**) are efficacious muscarinic agonists, exhibiting hippocampal PI hydrolysis in the 20-50% range of cabachol. The M1 and M3 receptors can accommodate bulky and long chained substituents thereby distinguishing them from the M2 subtype. However, preferential selectivity for M1 over M3 receptor subtype remains to be addressed.

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7. M1 Receptors: Affinity for M1 receptors was measured by the ability of the test compound to displace [^{3}H] pirenzepine in rat hippocampal membranes.[11] Intrinsic activity at M1 receptors was determined by measuring stimulation of phosphotidyl inositol (PI) hydrolysis in rat hippocampal tissue with a concentration range of test compound which included at least 10 times the measured affinity (0.1-1 mM). In some instances a full concentration range was used to calculate EC_{50} values.[12,13] Results are expressed as the percentage of the PI hydrolysis rate produced by 5 mmol concentration of carbachol, a full agonist.
   M2 Receptors: M2 coupled inhibition of adenylate cyclase (AC) was measured in rat heart membranes using 100 and 300 μM of test compound and intrinsic activity was compared as above to that of carbachol[14] and intrinsic activity is compared as above to that of carbachol.
   M3 Receptors: Agonist parameters at M3 were determined by measuring isometric contractions of the isolated guinea pig trachea using a 10^{-8} to 10^{-4} M concentration range of test compound and compared to the response elicited by full concentration response curves to carbachol within the same tissue.[15,16]
8. Affinity constants at the muscarinic receptor have been correlated with lipophilicity: Banerjee, S.; Lien, E. *Pharm. Res.* **1990**, *7*, 746.
9. The cLog P values for this series were calculated using the MedChem Software by Daylight. The validity of these calculations was verified by the experimental determination of the log P of **2**. This compound was partitioned between octanol/buffer at pH 12.08 and the log P was obtained from the assay of both phases. This pH was selected to suppress the ionized form of the compound. The pKa for this series has been determined to fall in the 8 to 9 range. The experimental log P for **2** was found to be -0.24 which is in good agreement with the MedChem value of -0.12 (see Fig. 1).
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