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Design and Synthesis of Ether Analogues as Potent and Selective M_2 Muscarinic Receptor Antagonists

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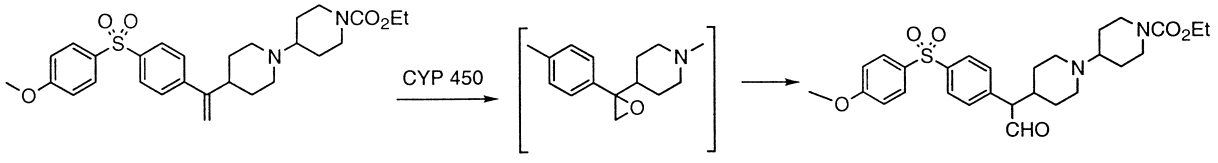
Abstract—Novel, selective M_2 muscarinic antagonists, which replace the metabolically labile styrenyl moiety of the prototypical M_2 antagonist **1** with an ether linkage, were synthesized. A detailed SAR study in this class of compounds has yielded highly active compounds that showed M_2 K_i values of <1.0 nM and >100-fold selectivity against M_1, M_3, and M_5 receptors.

Acetylcholine is an important neurotransmitter that plays a key role in learning and memory.[1] The cognitive effects of acetylcholine are mediated by agonist-induced stimulation of post-synaptic nicotinic and muscarinic M_1 receptors.[2] Under normal physiological conditions, synaptic acetylcholine levels are modulated by a feedback shut off mechanism initiated by acetylcholine-induced stimulation of presynaptic M_2 receptors. The senile dementia associated with Alzheimer's disease is directly correlated with depleted cholinergic activity in the cortical and hippocampal areas of brain and the current form of therapy addresses this issue by inhibiting cholinesterase, which breaks down acetylcholine. There have also been considerable efforts to develop an M_1 agonist for the treatment of Alzheimer's disease. Several M_1 agonists have been demonstrated to improve cognitive functions in clinical trials.[3] Alternatively, selective inhibition of presynaptic M_2 muscarinic receptors has been shown to enhance acetylcholine levels in vitro as well as in vivo.[4] The success of this approach would greatly depend on selectively achieving M_2 receptor inhibition, since inhibition of post-synaptic M_1 receptors and peripheral M_1 and M_3 receptors carries the potential liabilities of serious side effects.

In a previous communication,[5] we have described the design and synthesis of highly potent and selective M_2 antagonists represented by structure **1** (Fig. 1). In vitro metabolism studies on these compounds using rat liver microsomes gave the aldehyde **3**[6] as the principal metabolite which presumably arises through the rearrangement of the putative intermediate epoxide **2**. Since the epoxide **2** as well as the aldehyde **3** has potential toxic liabilities, we redirected our efforts toward identifying an ethylidine replacement. Herein we wish to report the successful outcome of our efforts.

An ether linkage was explored as a surrogate for the ethylidine unit (Fig. 2). The C−O−C bond angle of ethers ranges from 124° (diphenyl ether) to 111.5°



1 2 3

Figure 1. Metabolism of **1**.

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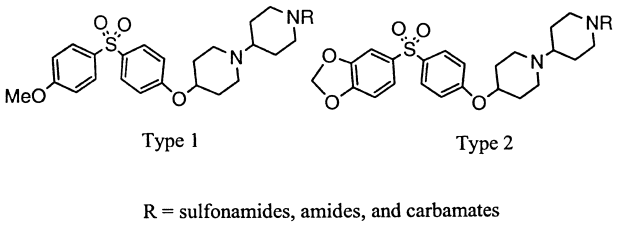


Figure 2. Designed targets.

(dimethyl ether),[7] roughly corresponding to an sp^2 carbon and the lone pair of electrons somewhat emulating the potential role, if any, of the π electrons of the ethylidine unit. Additionally, we decided to replace the *p*-methoxy substitute of the phenyl ring by a metabolically less labile methylenedioxy group.[8] Based on this rationale, the following two types of targets were explored.

The synthesis of these compounds from commercially available starting materials is described in Scheme 1. Reduction of ketone **4** was followed by coupling with 4-iodophenol to produce ether **5**. Displacement of the iodo group of **5** with suitably substituted benzenethiol gave intermediate **6**,[9] deprotection of which with trifluoroacetic acid followed by reductive amination afforded **8**. Oxidation of **8** with *m*CPBA followed by deprotection gave sulfone **10**, which was transformed to the final targets by functionalization of the piperidine nitrogen as shown in Scheme 1.[10]

The binding affinity of the newly synthesized targets against cloned human muscarinic receptors, assayed according to the reported protocol,[11] is presented in Table 1.

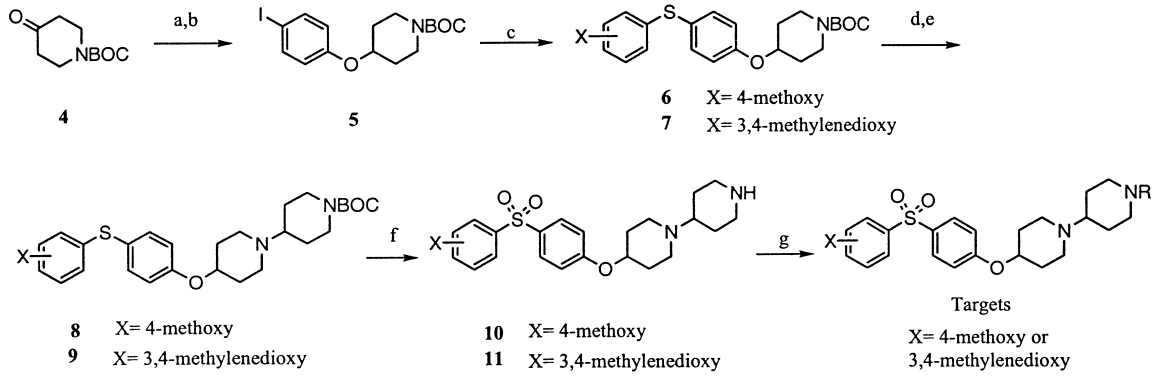
As shown in Table 1, the direct replacement of the ethylidine moiety of **12** with an oxygen atom to generate compound **13** resulted in reduced M_2 binding affinity by about 1000-fold. However, the lost binding affinity of **13** could be restored to a reasonable level by replacement of the 4-methoxy group with a 3,4-methylenedioxy group (**14**). These results indicated that the decreased binding affinity of ether derivatives could be compensated for by appropriate structural modification in other parts of the molecule. Due to the excellent M_2 binding affinity of compound **14**, we selected the type 2 targets containing the methelenedioxyphenyl moiety for further SAR studies by varying the substituents on the piperidine nitrogen.

A representative SAR of the sulfonamide series is presented in Table 2. It is evident that the binding affinity is affected by the size of the alkyl sulfonyl group. Compounds with smaller and unbranched sulfonyl alkyl groups (**14** and **15**) demonstrated higher M_2 binding affinity. However, their selectivity versus M_1, M_3, and M_5 are low. Aromatic 1-naphthyl sulfonamide **19** showed low M_2 binding affinity and selectivity.

The data for carbamate derivatives are presented in Table 3. Since most of the carbamates reported in the previous communication had high M_2 binding affinity with low selectivity,[5] a few carbamates were prepared in the ether series also. The results in Table 3 are consistent with the results previously reported. In spite of the excellent M_2 binding affinity, we have so far been unable to achieve acceptable selectivity in the carbamate series.

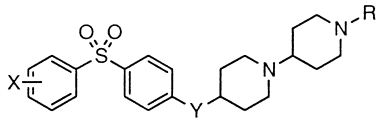
The most promising results were obtained from the amide series (Table 4). The selection of the R groups for the amide series greatly affects M_2 binding affinity and selectivity. Substituted alkyl amides **23** and **24** are less potent and selective. Aromatic amide **25** improved M_2 binding affinity by 10-fold, but it has low M_2 selectivity versus other receptor subtypes. Unlike the sulfonamide series (**19**), introduction of a 1-naphthyl group (**26**) in the amide series dramatically increased both the M_2 binding affinity and selectivity. This result provided a new direction for SAR modification.

Since the amide series offered superior M_2 binding and selectivity profile compared with the sulfonamide series, we focused further modifications on the amide series. A series of amides with 1-naphthyl bioisosteres was prepared. The results are shown in Table 5. Among the quinoline analogues, the position of the nitrogen atom plays an important role for M_2 binding and selectivity. For example, 4-quinoline analogue **27** is highly potent with 100-fold selectivity versus M_1, M_3, and M_5 while the 8-quinoline analogue (**29**) showed low M_2 potency and selectivity. Introduction of 2,3-dimethyl phenyl



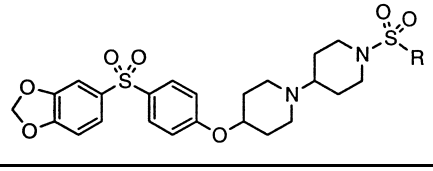
Scheme 1. (a) $NaBH_4$, EtOH, 100%; (b) Ph_3P, DEAD, THF, 4-iodophenol, 64%; (c) 4-methoxybenzenethiol or 3,4-methylenedioxybenzenethiol, DMPU, CuI, K_2CO_3, 50–70%; (d) 30% TFA/CH_2Cl_2, 100%; (e) $NaBH(AcO)_3$, 1,2-dichloroethane, **4**, 70–85%; (f) $MeSO_3H$/*m*CPBA, 25%TFA/CH_2Cl_2, 50–70%; (g) sulfonyl chloride, chloroformate, or acyl chloride, Et_3N, CH_2Cl_2, 85–98%.

Table 1. Comparison of M_2 binding affinity among different series



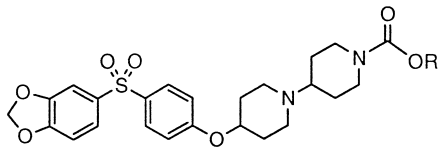
Compound	X	Y	R	M_2 (K_i, nM)
1	4-Methoxy	$CH{=}CH_2$	CO_2Et	0.11
12	4-Methoxy	$CH{=}CH_2$	SO_2-*n*-C_3H_7	0.29
13	4-Methoxy	O	SO_2-*n*-C_3H_7	138.8
14	3,4-Methylenedioxy	O	SO_2-*n*-C_3H_7	1.6

Table 2. Results of M_2 binding affinity and selectivity of the sulfonamide series



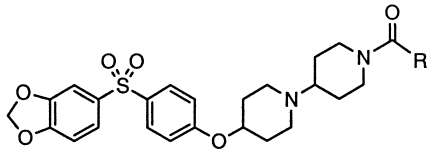
Compound	R	M_2, K_i (nM)	M_1/M_2	M_3/M_2	M_4/M_2	M_5/M_2
15	Et	2.0	49	122	12	46
14	*n*-C_3H_7	1.6	58	46	25	39
16	*i*-C_3H_7	14.0	10	2	2	16
17	*n*-C_4H_9	6.9	35	50	4	35
18	CH_2Ph	15.0	19	12	4	1
19	1-Naphthyl	34.7	2	8	1	4

Table 3. Results of M_2 binding affinity and selectivity of the carbamate series



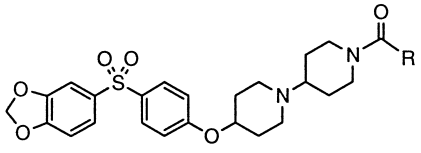
Compound	R	M_2, K_i (nM)	M_1/M_2	M_3/M_2	M_4/M_2	M_5/M_2
20	Et	6.0	4	2	1	14
21	*i*-C_3H_7	3.2	59	19	7	10
22	Ph	1.1	48	61	11	69

Table 4. Results of M_2 binding affinity and selectivity of the amide series



Compound	R	M_2, K_i (nM)	M_1/M_2	M_3/M_2	M_4/M_2	M_5/M_2
23	$CH_2OC_2H_3$	17.9	6	5	2	13
24	CH_2OPh	59.5	8	5	3	13
25	Ph	1.6	18	6	6	15
26	1-Naphthyl	0.5	357	216	28	50

Table 5. Results of M_2 binding affinity and selectivity of 1-naphthyl bioisosteres



Compound	R	M_2, K_i (nM)	M_1/M_2	M_3/M_2	M_4/M_2	M_5/M_2
26	1-Naphthyl	0.5	357	216	28	50
27	4-Quinoline	1.0	140	117	18	1485
28	5-Quinoline	0.5	233	124	21	64
29	8-Quinoline	12	9	10	2	4
30	2,3-DiMePh	0.4	155	132	31	56
31	2-(3-Methylthiophenyl)	0.2	234	109	23	196

group (**30**) also resulted in excellent M_2 binding affinity and selectivity. Compounds **27** and **31** have the best overall M_2 binding and selectivity profile among the ether analogues.

In summary, the metabolically labile alkene group has been successfully replaced with an ether linker. The results of SAR studies showed that the 1-naphthyl group and its bioisosteres contribute to high M_2 binding affinity and selectivity in the amide series. Complete results of SAR studies, as well as the in vivo efficacy studies for the amide series, will be reported in due course of time.

Acknowledgements

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6. Metabolite **3** was confirmed by analysis with an authentic sample.
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9. 3,4-Methylenedioxybenzenethiol was prepared according to the procedure in ref 8.
10. All of the target compounds showed satisfactory results in the analyses of NMR, MS, LC/MS, and HRMS.
11. For radioligand binding analysis, each muscarinic receptor subtype was stably expressed in CHO-K1 cells. Clonal cell lines were selected which expressed receptors at levels between 1 and 9 pmol/mg protein. The K_d of QNB (l-quinuclidinyl benzilate) at each receptor subtype was determined by saturation binding using 5–2500 pM [^{3}H]QNB in 10 mM potassium phosphate buffer, pH 7.4. Protein concentrations were adjusted for each assay to achieve between 700 and 1500 cpm specific binding. Competition binding experiments were performed using 180 pM [^{3}H] QNB. All binding experiments were performed in the presence of 1% DMSO and 0.4% methylcellulose. Non-specific binding was defined by 0.5 mM atropine. After equilibrium was reached (120 min incubation at rt), bound and free radioactivity were separated by filtration using Whatman GF-C filters. Investigation of M_2 receptor antagonist activity was performed on related compounds by measuring the effects of the compound's adenylyl cyclase inhibition mediated by oxotremorine M. K_i was expressed as mean of duplicate value (SEM < 15%). All determinations were performed at least twice.