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Sulfide Analogues as Potent and Selective M_2 Muscarinic Receptor Antagonists

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Abstract—We have discovered highly potent, selective sulfide M_2 receptor antagonists with low molecular weight and different structural features compared with our phase I clinical candidate Sch 211803. Analogue **30** showed superior M_2 receptor selectivity profile over Sch 211803. More importantly, this study provided new leads for the discovery of M_2 receptor antagonists as potential drug candidates.

The successful characterization of five muscarinic receptor subtypes (M_1–M_5) has greatly contributed to the understanding of the physiological relationships between the neurotransmitter acetylcholine (ACh) and the muscarinic receptors.[1] Among the five muscarinic receptor subtypes, the post-synaptic M_1 receptors interact with ACh to promote cognition, memory and learning, while the pre-synaptic muscarinic M_2 receptors regulate the synaptic levels of ACh via a feedback inhibition of ACh release. The current treatment of cognitive disorders such as Alzheimer's Disease (AD) is focused on the means to increase ACh levels.[2] Suppression of the M_2 receptor induced-feedback inhibition of ACh release is an alternative mechanism for the continuous release of ACh to the synaptic cleft. Indeed, M_2 receptor antagonists have been reported to enhance the ACh release in animal models.[3] As part of our research program for the treatment of AD, we have been involved in the development of M_2 receptor antagonists. One of these compounds, Sch 211803, has entered phase 1 clinical trials.[4] Our next goal was to identify a backup candidate for Sch 211803. We would like the backup candidate to have different structural features with lower molecular weight, while maintaining high M_2 receptor binding affinity (K_i <5 nM) and selectivity versus M_1 and M_3 receptors (>100-fold).

Our strategy for the discovery of a backup candidate was to simplify the left-hand portion of the structure of Sch 211803 to reduce its molecular weight. We first explored the feasibility of replacing the chlorophenylsulfonyl group with an isopropylsulfonyl group (see **1** and **2** in Fig. 1). The isopropyl group was expected to partially mimic the steric environment of the aromatic ring. The selection of 1-naphthyl amide found in **2** was based on our previous study.[5]

The targets were prepared as outlined in Scheme 1.

Reductive amination of commercially available piperidine derivative **3** afforded piperidinyl piperidine **4**. Displacement of the fluorine atom of **4** with isopropylthiol yielded thiol ketone **5**. The benzylic carbonyl group of **5** was reduced to a benzylic methylene group (**6**) via treatment with $NaBH_4$ followed by Et_3SiH/TFA treatment. The amine **6** was transformed to the aryl amides **7** and **8** by coupling with corresponding aryl acids. The final products **1** and **2** were obtained after oxidation of sulfur to sulfone.[6]

The sulfone targets **1** and **2** as well as their sulfide intermediates **7** and **8** were assayed with cloned human muscarinic receptors according to the reported protocol (Table 1).[7]

The M_2 receptor binding affinities of sulfones **1** and **2** were disappointing in comparison to that of Sch 211803. However, the sulfide precursors **7** and **8** demonstrated stronger M_2 receptor binding affinity than their sulfone derivatives. Especially, sulfide **8** showed excellent M_2 receptor binding affinity and selectivity.

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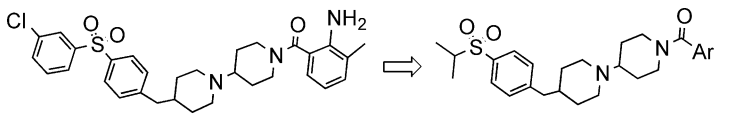
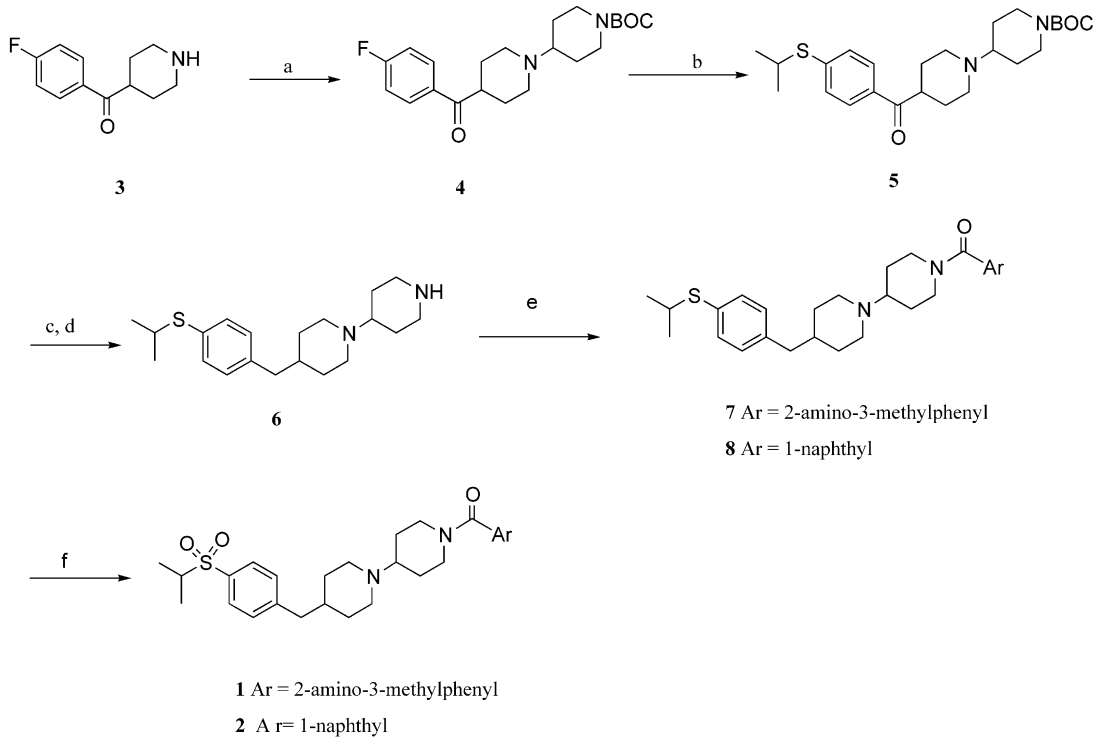


Sch 211803

1 Ar = 2-amino-3-methyl phenyl
2 Ar = 1-naphthyl

Figure 1. Modification of Sch 211803.



3 **4** **5**

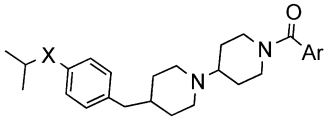
6

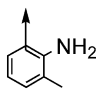
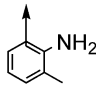
7 Ar = 2-amino-3-methylphenyl
8 Ar = 1-naphthyl

1 Ar = 2-amino-3-methylphenyl
2 A r= 1-naphthyl

Scheme 1. (a) $NaBH(AcO)_3$, 1,2-DCE, 1-*t*-butoxycarbonyl-4-piperidone, 75–84%; (b) NaH, DMF, isopropylthiol, 65 °C, 6 h, 70–80%; (c) $NaBH_4$, MeOH, 90–98%; (d) TFA, Et_3SiH, 70–85%; (e) ArCOOH, EDCI, DMAP, CH_2Cl_2, 85–96%; (f) *m*CPBA $MeSO_3H$, CH_2Cl_2, 60–75%.

Table 1. The results of M_2 binding and selectively of prepared analogues



Compd	X	Ar	M_2, K_i (nM)	M_1/M_2	M_3/M_2	M_4/M_2	M_5/M_2
Sch 211803			0.89	734	787	69	95
1	SO_2		104	11	na	na	na
2	SO_2		17	21	na	na	na
7	S		20	37	na	na	na
8	S		0.9	205	454	12	241

na, not available.

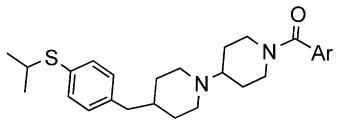
Therefore, we turned our attention to the optimization of SAR of sulfide analogue **8**. First, the 1-naphthyl ring was replaced with other types of aromatic rings. The M_2 K_i values of several selected analogues are shown in Table 2. Replacement of 1-naphthyl ring with 2-naphthyl ring (**9**) resulted in loss of activity by more than 200-fold. Modification of phenyl substitution (**10**–**16**) as well as incorporation of heteroaryl moieties (**17**–**19**) similarly produced compounds that are much less active. These results suggest that the 1-naphthyl group is essential for the high M_2 receptor binding affinity.

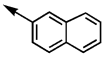
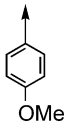
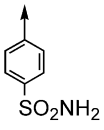
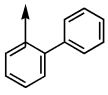
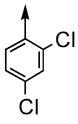
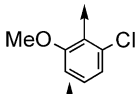
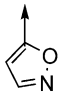
Next, a series of substituted 1-naphthyl groups and isosteric bicyclic heteroaryl derivatives was examined. The results are shown in Table 3.

Generally, substitution of 1-naphthyl moiety drastically reduced M_2 receptor binding affinity compared to compound **8** except compound **28**. Compound **20**, with M_2 K_i of 6.1 nM, showed excellent selectivity versus other receptor subtypes. The M_2 receptor binding affinities of fluoronaphthyl derivatives suggested a modest advantage for 7-fluoro substitution (**23**). Among the quinoline derivatives, 6-isoquinoline derivative **28** showed excellent

M_2 receptor binding affinity albeit poor selectivity versus other receptor subtypes, whereas the isomeric quinoline derivatives (**25**–**27**) as well as the cinnoline derivative **29** were much less active.

Table 2. The results of M_2 binding and selectivity of selected analogues from modification of **8**

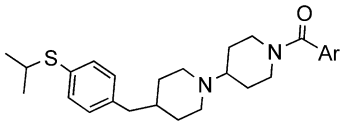


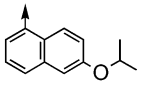
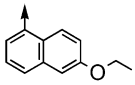
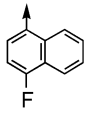
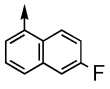
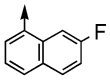
Compd	Ar	M_2, K_i (nM)	M_1/M_2	M_3/M_2	M_4/M_2	M_5/M_2
8		0.9	205	454	12	241
9		241	na	na	na	na
10		260	na	na	na	na
11		192	na	na	na	na
12		108	na	na	na	na
13		129	na	na	na	na
14		250	na	na	na	na
15		134	na	na	na	na
16		237	na	na	na	na
17		254	na	na	na	na
18		134	na	na	na	na
19		923	na	na	na	na

na, not available.

Finally, in Table 4, groups capable of hydrogen bonding were chosen to replace the isopropyl group of **8** based on previous modeling of M_2 receptor antagonists which suggested there might be a hydrogen bonding interaction at the left-hand side of the antagonist with M_2 receptor.[8] A series of analogues (**30**–**32**) was prepared by following the synthetic route of Scheme 1 where methyl thioglycolate was used instead of isopropyl thiol. Hydrolysis of methyl ester **30** afforded carboxylic acid **35** while reduction of **30** provided alcohol **36**. Other esters (**33** and **34**) and amides (**37** and **38**) were synthesized by coupling of acid **35** with appropriate alcohol or amine.

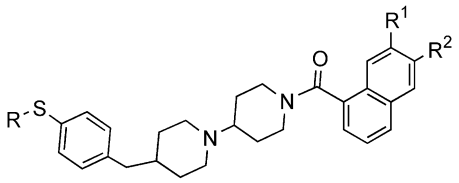
Table 3. The results of M_2 binding and selectivity of selected 1-naphthyl bioisosteres

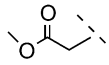
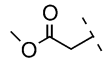
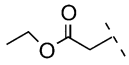
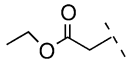
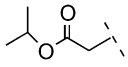
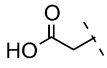
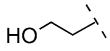
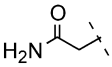
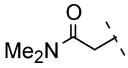


Compd	Ar	M_2, K_i (nM)	M_1/M_2	M_3/M_2	M_4/M_2	M_5/M_2
8		0.9	205	454	12	241
20		6.1	163	190	65	219
21		14	68	na	na	na
22		22	37	na	na	na
23		22	37	na	na	na
24		8.6	98	na	na	na
25		20	39	na	na	na
26		11	79	na	na	na
27		256	3	na	na	na
28		1.0	58	na	na	na
29		256	3	na	na	na

na, not available.

Table 4. The results of M_2 binding and selectivity of selected analogues of sulfide modification



Compd	R	R^1	R^2	M_2, K_i (nM)	M_1/M_2	M_3/M_2	M_4/M_2	M_5/M_2
8		H	H	0.9	205	454	12	241
30		H	H	0.7	1395	1532	57	409
31		F	H	4.3	246	301	12	218
32		H	OEt	2.7	380	467	32	538
33		H	H	40	na	na	na	na
34		H	H	48	na	na	na	na
35		H	H	1500	na	na	na	na
36		H	H	26	na	na	na	na
37		H	H	18	52	na	na	na
38		H	H	12	73	na	na	na

na, not available.

Methyl esters **30**–**32** showed excellent M_2 receptor binding affinity and selectivity. Among them, ester **30** showed the best selectivity versus other receptor subtypes. Interestingly, there was no obvious hydrogen bonding effect based on the comparison of the M_2 receptor binding affinity of **8** with **30**, and **33**–**38**. On the other hand, methyl glycolates **31** and **32** had a modest increase of M_2 receptor binding affinity versus their isopropyl analogues **23** and **21** (Table 3). Esters **33** and **34** had lower M_2 receptor binding affinities perhaps due to steric hindrance. Likewise, amides **37** and **38** demonstrated 10- to 20-fold reduction in M_2 receptor binding affinity compared to methyl ester **30**. The carboxylic acid **35** and alcohol **36** were also less active. These results showed that steric bulk and acidity are not tolerated for the left-hand side modifications.

In summary, we have discovered highly potent, selective sulfide M_2 receptor antagonists **8**, **30**–**32**. Sulfide **8** has low molecular weight ($M_r = 486$) and different structural features compared with Sch 211803 ($M_r = 566$). Analogue **30** ($M_r = 516$, $K_i = 0.7$ nM) showed superior M_2 receptor selectivity profile over Sch 211803. The oral efficacy of the sulfide analogues was determined in the microdialysis assay which measures the increase of Ach release due to the blockade of the M_2 inhibitory feedback mechanism.[3] Analogue **8** showed much lower oral activity than Sch 211803 perhaps due to its low metabolism stability and high lipophilicity.[9] Since the sulfide atom can be metabolically oxidized and the naphthyl group is lipophilic, our next SAR modification will focus on the replacement of the sulfide atom with metabolically stable bioisosteres and the naphthyl group with less lipophilic groups. These SAR studies led to a new generation of M_2 receptor antagonists with low molecular weight, different structural features, and improved oral bioavailability and efficacy. The results of these studies will be reported in the future.

Acknowledgements

The authors would like to thank Mr. Yao H. Ing and Dr. P. Das for mass spectral data, Dr. N. J. Murgolo for molecular modeling studies, and Dr. J. W. Clader for helpful discussion.

References and Notes

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6. All of the target compounds showed satisfactory result in the analyses of NMR, MS, LC/MS, and HRMS.
7. 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. Nonspecific binding was defined by 0.5 mM atropine. After equilibrium was reached (120-min incubation at room temperature), 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 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.

8. Part of the molecular modeling work for the sulfoxide series has been reported. See: Lachowicz, J. E.; Lowe, D.; Duffy, R. A.; Ruperto, V.; Taylor, L. A.; Guzick, H.; Brown, J.; Berger, J. G.; Tice, M.; McQuade, R.; Kozlowski, J.; Clader, J. W.; Strader, C. D.; Murgolo, N. *Life Sci*. **1999**, 64.

9. At 10 mpk oral dosing, the AUC (0–2.5 h) of analogue **8** is 1009 while the AUC (0–2.5 h) of Sch 211803 is 7800 (details will be reported by W. Billard). During the human microsomal incubation, there was 26% of analogue **8** left compared with 90% of Sch 211803 (details will be reported by K. A. Cox). The Clog P of analogue **8** is 6.3 (details will be reported by J. Kaminski).