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Enhancement of Pharmacokinetic Properties and In Vivo Efficacy of Benzylidene Ketal M_2 Muscarinic Receptor Antagonists Via Benzamide Modification

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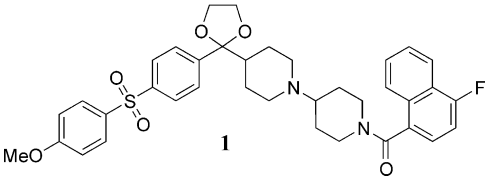
Abstract—We previously reported the initial discovery of a novel class of stabilized benzylidene ketal M_2 receptor antagonists. This paper discusses new analogues consisting of benzamide modifications which not only improved M_2 receptor affinity and selectivity, but also enhanced the pharmacokinetic properties of the series. These changes led to the discovery of a highly potent and selective M_2 antagonist, which demonstrated in vivo efficacy and had good bioavailability in multiple species.

Alzheimer's disease (AD) is a neurodegenerative disease characterized by a steady decline in cognitive function and variations in affect. Potential therapies for AD include activation of the cholinergic system[1,2] and prevention of β-amyloid protein formation.[2] Acetylcholinesterase inhibitors, which block the degradation of the neurotransmitter acetylcholine (ACh), are the current cholinergic therapy for AD.[1] M_1 muscarinic receptor agonists also increase cholinergic activity.[1,3] Our research has involved M_2 muscarinic receptor antagonists, which shut down the negative feedback mechanism of presynaptic receptors, thereby increasing ACh release in the CNS.[1,3] Selectivity against M_1 and M_3 receptors is necessary to avoid peripheral side effects and also diminished efficacy in the case of M_1 antagonism.[1,2] Here we report benzamide modifications to a ketal series of M_2 antagonists, which led to the discovery of a compound with excellent in vivo efficacy and pharmacokinetic properties in multiple species.

Recently, we reported the discovery of the fluoronaphthamide derivative **1**.[4] Compound **1** has good M_2 receptor affinity and selectivity, and demonstrated in vivo efficacy in rat models of cognition. However, the potential for arene oxide formation and low cynomolgus monkey bioavailability of compound **1** led us to study lower molecular weight substituted benzamides.



1

In a structurally related series from our muscarinic program, it was discovered that *o*-toluoyl amides have good M_2 receptor affinity and selectivity.[5] We incorporated the toluamide into the ketal series and studied the effects of varying the left hand aryl linked moiety and the ketal functionality (Table 1).[6,7] The methylenedioxyphenyl compound **2** had an order of magnitude improved M_2 binding compared with the *p*-methoxyphenyl derivatives **3**–**7**, but the selectivity over M_1 and M_3 was poor. Comparison of sulfones **3** and **4** with sulfoxides **6** and **7** demonstrated that a sulfone linker was preferred over the sulfoxide by the M_2 receptor by a 3- to 5-fold difference in affinity. In the ketal ring, bulk

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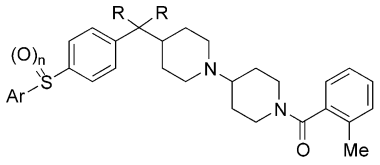
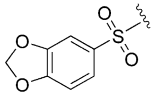
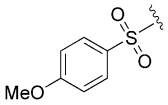
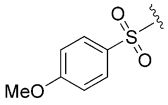
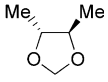
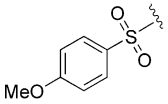
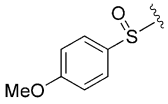
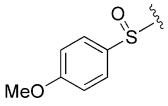
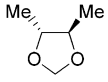
Table 1. *o*-Toluamide derivatives



Compd	$ArS(O)_n$		M_2 K_i (nM)[a]	M_1/M_2	M_3/M_2
2			0.065	28	8
3			0.54	158	33
4			18.0	32	—
5			0.86	89	15
6			2.5	146	35
7			51.0	14	—

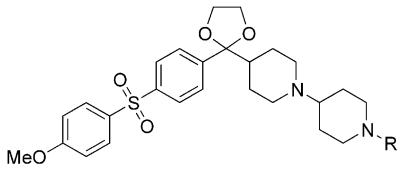
[a]Mean of duplicate values (SEM <15%). All determinations were performed at least twice.

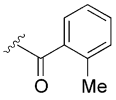
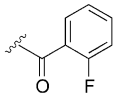
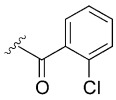
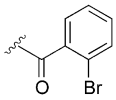
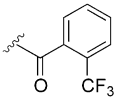
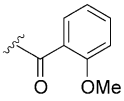
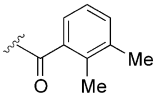
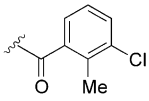
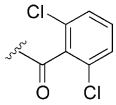
was not tolerated, as the dimethyldioxolanes **4** and **7** had much worse affinity for the M_2 receptor than their non-substituted dioxolane and dithiolane counterparts. Finally, the dioxolane **3** had better M_2 affinity and selectivity than the dithiolane **5**.

Based on the results from Table 1, we chose the template represented by ketal **3** to continue with our benzamide modification studies. By changing the *o*-substituents or adding a second substituent, we first attempted to improve the selectivity of the ketal **3** to 100-fold or greater against the M_1 and M_3 receptors. Secondly, we targeted substituents with varying size and electronic properties in order to affect metabolic stability, which we measured via human liver microsomal incubation.[8,9] Replacement of the methyl group with a halogen or trifluoromethyl group did not improve the M_2 selectivity or microsomal stability (Table 2, compounds **8**–**11**). The electron donating methoxy substituent did improve the M_2 selectivity, but compound **12** had very poor stability in human liver microsomes. Extra substituents also did not help the M_2 selectivity, as disubstituted derivatives **13**–**15** only had 40- to 60-fold selectivity over M_1 and M_3.

Since our goal was not only to improve M_2 selectivity, but also to improve the PK of the naphthamide **1**, we

Table 2. Substituted benzamides



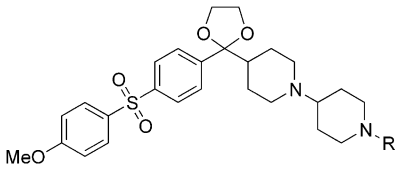
Compd	R	M_2 K_i (nM)[a]	M_1/M_2	M_3/M_2	Human micros[b]
3		0.54	158	33	57
8		4.8	49	8	—
9		1.6	96	24	41
10		1.8	102	21	34
11		1.0	43	50	49
12		1.2	137	107	18
13		1.4	66	40	—
14		0.72	42	—	—
15		2.3	54	57	53

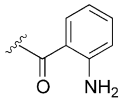
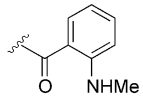
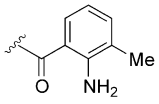
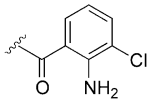
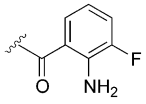
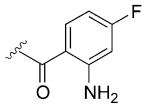
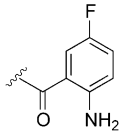
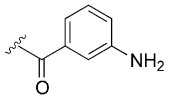
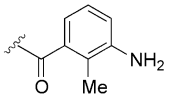
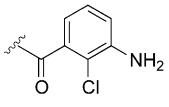
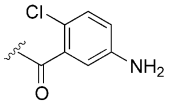
[a]Mean of duplicate values (SEM <15%). All determinations were performed at least twice.
[b]% parent compound remaining after 20 min incubation with human liver microsomes.[8]

synthesized 2-amino derivatives (anthranilamides) and 3-amino derivatives (mesalamine analogues). These compounds served both criteria. The amine as an electron donating group could aid selectivity similarly to the methoxy derivative **12**. Also, the amino group lowers overall logP values, which could result in improved PK over the lipophilic naphthamide **1**.

The anthranilamide **16** (Table 3) did not improve upon the binding properties of previous benzamide derivatives. Substituting the amine with a methyl group (**17**) improved selectivity, but likely because of demethylation,

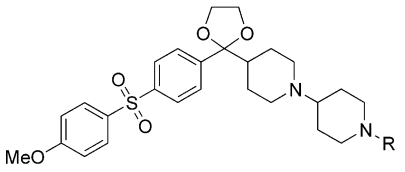
Table 3. Amino substituted benzamides

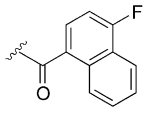
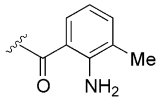
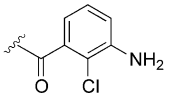


Compd	R	M_2 K_i (nM)[a]	M_1/M_2	M_3/M_2	Human micros[b]
16		1.5	142	54	80
17		0.90	238	126	20
18		0.39	623	250	77
19		1.1	153	44	42
20		0.51	283	110	33
21		0.44	464	213	50
22		0.68	209	211	42
23		0.78	209	119	72
24		0.40	240	203	79
25		0.53	235	360	73
26		0.53	323	360	38

[a]Mean of duplicate values (SEM <15%). All determinations were performed at least twice.
[b]% parent compound remaining after 20 min incubation with human liver microsomes.[8]

Table 4. In vivo comparison of lead ketals



Compd	R	Human micros[a]	Rat AUC[b]	Micro-dialysis[c]
1		76	603	180
18		77	1572	194
25		73	—	135

[a]% parent compound remaining after 20 min incubation with human liver microsomes.[8]
[b]Area Under the Curve: h ng/mL, 0 → 6 h, 10 mg/kg, po, 20% hydroxypropyl-β-cyclodextrin (HPβCD).[11]
[c]% ACh release compared with baseline (baseline = 100%).[10]

the microsomal stability was decreased. Even though adding an extra methyl group at the 3-position did not help with the binding data in the toluamide series (**3** vs **13**), the anthranilamide **18** had excellent M_2 affinity and selectivity over M_1 and M_3. Halogen substitution around the aromatic ring produced several compounds with good M_2 binding and selectivity data (**19**–**22**), but none had the same microsomal stability as the methyl compound **18**. The mesalamine analogues **23**–**26** all had excellent affinity and selectivity, and most had good microsomal stability as well.

Representatives from the anthranilamide and mesalamine series were tested further in vivo (Table 4). Efficacy was tested in a rat microdialysis assay, in which ACh levels were measured from perfusate collected from striatum.[10] The anthranilamide **18** had improved activity over the original naphthamide **1**, but the mesalamine **25** showed minimal response in the microdialysis assay. This may be due to the higher basicity of the 3-aminobenzamide **25** over the vinylogous amide **18** or the naphthamide **1**. The more basic **25** may have been unable to cross the blood–brain barrier, or be more susceptible to metabolic oxidation. In addition to its effects on ACh release, the plasma levels of anthranilamide **18** following oral administration were assessed.[11] Plasma levels of **18** were 2.5 times greater than those of the naphthamide **1**, which followed the trend of the compounds' relative efficacy in vivo. It was hoped that the increase in rat plasma concentration of the anthranilamide **18** would result in higher bioavailability in other species, particularly if this observation was due to

greater absorption. As the ClogP of **18** was 3.3 compared to 5.0 for the lipophilic naphthamide **1**, increased absorption may indeed explain the increased plasma exposure.[12]

The improved binding profile and in vivo activity of anthranilamide **18** prompted further in vivo testing in a rat model of cognition. In the rat passive avoidance response (PAR) experiment, longer latency times to enter a darkened chamber in which a foot shock was previously delivered have been shown to be indicators of improved reference memory.[13] After a pretreatment time of 1 h, **18** was active at oral doses ranging from 0.001 to 0.1 mg/kg. Since M_2 receptors are also present in the heart,[1] it was necessary to test the effects of compound **18** on heart rate. At oral doses of 3 and 10 mg/kg, **18** produced an increase in heart rate in rats, but at 1 mg/kg the heart rate was not affected.[14] This result shows that a significant multiple exists between the in vivo effective dose of **18** and the dose that precipitates undesirable cardiovascular effects. In addition, **18** improved upon the PK of naphthamide **1** by demonstrating high oral bioavailability in rats and acceptable bioavailability in cynomolgus monkeys.[15] These results are essential for the further advancement of **18**, as in vivo efficacy in cognition models has been demonstrated, and because toxicological studies require bioavailability in multiple species.

Acknowledgements

The authors wish to thank Professor Ronald Breslow, Dr. John Clader, Dr. William Greenlee, and Dr. Catherine Strader for helpful discussions. We also thank Dr. Pradip Das for obtaining analytical data, Dr. James Kaminski for logP calculations, and Dr. Robert Watkins for obtaining heart rate data.

References and Notes

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7. See ref 6 for the methodology used to obtain muscarinic receptor binding data. The data presented is the mean of duplicate values (SEM $<15\%$). All determinations were performed at least twice.

8. Procedure described in: Hecht, S.; Chen, C.; Hoffmann, D. *Cancer Res.* **1978**, *38*, 215. Compounds (10 μg/mL concentration) were incubated in human microsomes and their metabolic stability was determined by the percent of the parent compound remaining after 20 min. The stability was compared to that observed for SCH 72788 (Lachowicz, J. E.; Duffy, R. A.; Ruperto, V.; Kozlowski, J.; Zhou, G.; Clader, J.; Billard, W.; Binch, H., III; Crosby, G.; Cohen-Williams, M.; Strader, C. D.; Coffin, V. *Life Sci.* **2001**, *68*, 2585), which was used as a reference incubated at the same time under the same conditions. A difference of 30% was considered significant and compounds that showed stabilities 30% more than SCH 72788 were considered further. SCH 72788 typically had 15–30% parent remaining after 20 min.

9. We have generally found in our M_2 program that compounds which had poor microsomal stability in vitro also had poor in vivo stability, and the resulting metabolites did not demonstrate M_2 activity: Cox, K. Unpublished results.

10. For more details on microdialysis experiments, see ref 6 and: Billard, W.; Binch, H., III; Crosby, G.; McQuade, R. D. *J. Pharmacol. Exp. Ther.* **1995**, *273*, 273. Reported values represent significant stimulation over baseline ACh levels ($p<0.05$, Duncan's Multiple Range Statistic, $n=3$).

11. Procedure described in: Cox, K. A.; Dunn-Meynell, K.; Korfmacher, W. A.; Broske, L.; Nomeir, A. A.; Lin, C. C.; Cayen, M. N.; Barr, M. N. *Drug Discov. Today* **1999**, *4*, 232. %CV values typically range from 10 to 30 in this assay ($n=2$).

12. ClogP values were calculated using SYBYL version 6.6 accessed via the ClogP column type and various expression generators with a special license (Biobyte) available from Tripos, Inc.

13. For more details on PAR experiments, see ref 6 and: Smith, R. D.; Kistler, M. K.; Cohen-Williams, M.; Coffin, V. L. *Brain Res.* **1996**, *707*, 13.

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15. Bioavailability of naphthamide **1**: rat = 44%, c. monkey = 9%. Bioavailability of anthranilamide **18**: rat = 80%, c. monkey = 15%.