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Metabolic Stabilization of Benzylidene Ketal M_2 Muscarinic Receptor Antagonists via Halonaphthoic Acid Substitution

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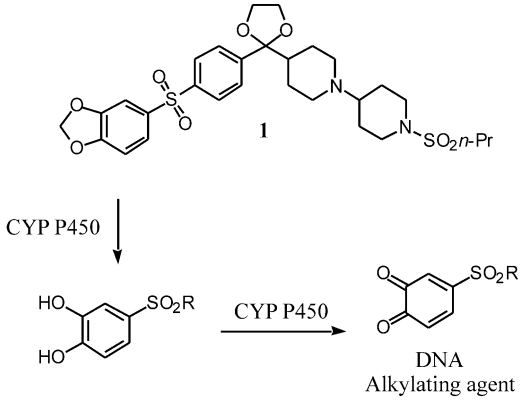
Abstract—The potential toxicological liabilities of the M_2 muscarinic antagonist **1** were addressed by replacing the methylenedioxyphenyl moiety with a *p*-methoxyphenyl group, resulting in M_2 selective compounds such as **3**. Several halogenated naphthamide derivatives of **3** were studied in order to improve the pharmacokinetic profile via blockage of oxidative metabolism. Compound **4** demonstrated excellent M_2 affinity and selectivity, human microsomal stability, and oral bioavailability in rodents and primates.

Alzheimer's disease (AD) is a neurodegenerative disease characterized by a steady decline in cognitive function and variations in effect. These changes lead to an incapacity for independent living and eventually to death. Several potential treatments for AD are currently under investigation, including activation of the cholinergic system and prevention of β-amyloid protein formation.[1] Currently, acetylcholinesterase inhibitors, which block the degradation of the neurotransmitter acetylcholine (ACh), are the marketed cholinergic therapy for AD.[1a,e] Muscarinic receptor agonists of the M_1 subtype could also increase ACh levels by stimulating postsynaptic cholinergic receptors.[1b,c,e] However, our focus has been on M_2 muscarinic receptor antagonists, which shut down the negative feedback mechanism of presynaptic receptors, thereby increasing ACh release in the CNS.[1b,c,e] Selectivity against M_1 and M_3 is necessary, as M_1 antagonism would be counterproductive, and M_3 receptors have been associated with adverse GI effects.[1d] Several compounds have been identified in our laboratories, but most of these compounds have M_2 selectivity and/or metabolic issues which could be improved upon.[2–4] We report here the identification of a series of selective M_2 antagonists and the use of substituted naphthoic acids to improve their metabolic stability.

Previously, we described the discovery of ketal **1**, a potent and selective M_2 muscarinic receptor antagonist.[3] The key feature of **1** was the stabilized benzylidene ketal moiety, which blocked the benzylic position of **1** from oxidative metabolism. In addition, **1** showed oral



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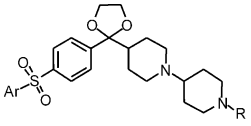
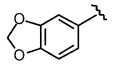
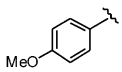
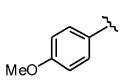
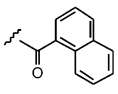
potency in rat models of cognitive activity. However, when **1** was orally dosed in cynomolgus monkeys, no parent compound was detected in the plasma. This observation was also made for other M_2 antagonists containing the methylenedioxydiarylsulfone moiety. Further metabolite ID studies concluded that in these compounds, the methylenedioxy group was cleaved in vivo.[5] Other reports on methylenedioxy groups indicate that cytochrome P450 (CYP P450) catalyzed oxidative cleavage of **1** could form a catechol intermediate, which upon further oxidation to an *ortho*-quinone, could increase toxicity by its potential as a DNA alkylating agent. In addition, drug–drug interaction could be a potential problem as methylenedioxy groups have been reported to inhibit and induce CYP P450.[6]

In light of the poor metabolic stability of the methylenedioxyphenyl group, we chose to replace this functionality with a *p*-methoxyphenyl group. Previous results demonstrated that M_2 potency could be achieved with the *p*-methoxy group in place.[3] Unfortunately, the direct analogue of **1**[7] had poor M_2 affinity and selectivity compared with the original lead **1** (Table 1).[8] However, replacement of the sulfonamide with a naphthamide moiety resulted in **3**, a compound with excellent M_2 binding affinity and selectivity.[9]

Although **3** had acceptable binding characteristics, it showed poor stability in human liver microsomes (Table 2), which has generally correlated with poor in vivo activity in our program.[10] In addition, the naphthamide moiety has been reported to be susceptible to metabolic oxidation to an arene oxide, which could promote toxicity via catechol formation or covalent binding to DNA.[6a,11] To block this undesired potential metabolism, we chose to study halogenated naphthamide analogues. These could improve metabolic stability both electronically and sterically. However, halogenated naphthoic acids were generally not commercially available for our synthesis. Therefore, we synthesized several new naphthoic acids[12] and allowed them to react with our piperidine core using EDC/DMAP conditions.[7]

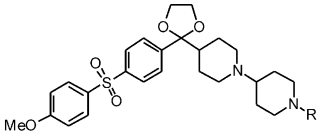
Table 1. Methylenedioxyphenyl versus *p*-methoxyphenyl derivatives

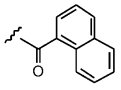
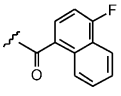
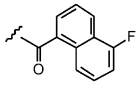
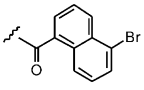
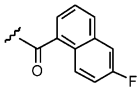
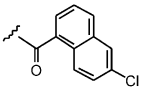
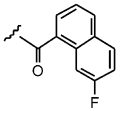
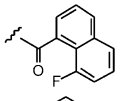
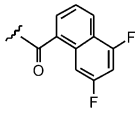
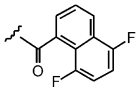


Compound	Ar	R	M_2 K_i (nM)	M_1/M_2	M_3/M_2
1			0.010	101.5	93.0
2			33.3	18.3	25.3
3			0.14	211.8	155.1

As shown in Table 2, all of the substituted naphthamides had good M_2 binding affinity, and most compounds also demonstrated selectivity of 100-fold versus M_1 and M_3 receptors. In order to differentiate these compounds by metabolic stability, incubation with human liver microsomes was performed, and the percentage of parent compound remaining after 20 min was measured.[13] Our desired stability was achieved by analogues with $>60\%$ parent compound remaining after incubation.[14] Using this standard, we discovered that the 4- and 5-positions of the naphthalene ring were optimal for substitution, as compounds **4**–**6** and **11**–**12** had microsomal stability greater than 2 times that of the parent naphthamide **3**. Conversely, compounds that only had substitution at the 6-, 7-, or 8-positions (**7**–**10**)

Table 2. Substituted naphthamide comparison



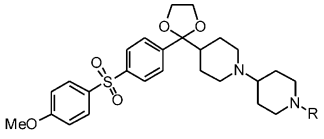
Compound	R	M_2 K_i (nM)	M_1/M_2	M_3/M_2	Micros[a]
3		0.14	211.8	155.1	31
4		0.51	124.9	102.7	76
5		0.20	234.4	164.6	85
6		0.51	312.7	313.5	72
7		0.81	83.8	104.8	11
8		1.4	194.5	199.6	41
9		1.2	74.6	44.0	5
10		0.99	84.1	143.1	19
11		0.23	335.4	233.3	68
12		0.29	435.8	216.2	84

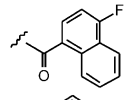
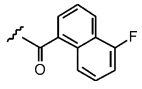
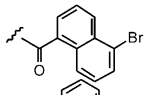
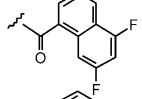
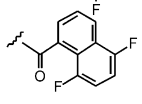
[a]% parent compound remaining after 20 min incubation with human liver microsomes.

had comparable or lower stability relative to **3**. These results suggested that oxidation was likely to occur in the lower region of the naphthalene ring (4- and 5-positions).

The 4- and 5-substituted naphthamide derivatives that satisfied in vitro binding and stability criteria were tested in vivo (Table 3). First, rats were dosed orally with these compounds (10 mg/kg, 20% HPβCD) to determine their plasma concentrations. AUC values were calculated from 0 to 6 h and used to rank order the compounds.[15] Second, microdialysis experiments were run in orally dosed rats to determine the extent of ACh release in the striatum, which measures blood–brain barrier penetration as well as in vivo potency.[16] 5-Halonaphthamides **5**, **11**, and **12** exhibited poor efficacy in microdialysis experiments, which correlated with their low plasma levels. However, both the 4-fluoronaphthamide **4** and the 5-bromonaphthamide **6** had good AUC values in orally dosed rats, but only **4** had positive microdialysis results. The physical properties of **4** versus **6** justified this discrepancy in oral potency. Both **4** and **6** had high molecular weights (659 and 720, respectively) and ClogP values[17] (4.3 and 5.0, respectively), but the higher values associated with compound **6** suggested that it would have poor absorption and blood–brain barrier penetration versus compound **4**.[18] Comparison of the ACh release during microdialysis (Fig. 1) suggested that **4** had a faster combination of absorption and CNS penetration than **6**.

Table 3. In vivo comparison of naphthamide derivatives



Compd	R	M_2 K_i (nM)	M_1/M_2	HM[a]	Rat AUC[b]	MD[c]	ClogP
4		0.51	124.9	76	603	180	4.3
5		0.20	234.4	85	130	140	4.3
6		0.51	312.7	72	702	125	5.0
11		0.23	335.4	68	367	133	4.4
12		0.29	435.8	84	NT	120	4.4

[a]% parent compound remaining after 20 min incubation with human liver microsomes.
[b]h·ng/mL, 0→6 h, 10 mg/kg, po, 20% HPβCD.
[c]Microdialysis: % ACh release above baseline in rat striatum, 0→2 h, 10 mg/kg, po.

Although **6** did not stimulate ACh release as **4** did, the slow onset of **6** suggested that it could be active at time points beyond the 2 h limit of the microdialysis experiment. In this regard it is worth noting that both **4** and **6** had similar rat plasma levels over 6 h, and the ACh release of both compounds appeared to be increasing at the final time points. To test the hypothesis that **6** could be active if tested over a longer period of time, a rodent cognition assay in which long pretreatment times are tolerated was employed. 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.[19] After a pretreatment time of 1 h, compound **4** was active at 0.1 and 0.3 mg/kg doses. However, when **6** was given to the rats 4 h before testing, cognitive improvement was shown at 0.3 and 1.0 mg/kg doses. These results supported the evidence that compound **6** had a slow onset of activity.

As both **4** and **6** demonstrated in vivo activity, their relative metabolic profiles were used to distinguish the two compounds. To this end, **4** and **6** were incubated with human microsomes for 60 min, and subjected to metabolite characterization by MS/MS analysis. Potential CYP P450 naphthalene oxidation products M + 16 (monohydroxylation), M + 32 (dihydroxylation), and M + 34 (epoxidation followed by H_2O attack to form diol) were searched for in the experiment. For compound **4**, only minor amounts of these metabolites were detected. However, for compound **6**, both M + 16 and M + 34 metabolites were found in significant quantities. These results suggested that the naphthalene A-ring was more susceptible to oxidative metabolism than the B-ring. Finally, we subjected **4** to c. monkey PK studies to see if any improvement was observed versus compound **1**. Indeed, compound **4** had an AUC (0→24 h) of 743 h·ng/mL when orally dosed in c. monkeys at 10 mg/kg in 20% HPβCD, as opposed to the AUC of 0 that was exhibited by **1** in a similar study.

In conclusion, by replacing the methylenedioxy moiety of **1** with a *p*-methoxy group, the terminal sulfonamide required replacement with a metabolically labile naphthamide to maintain acceptable M_2 receptor binding

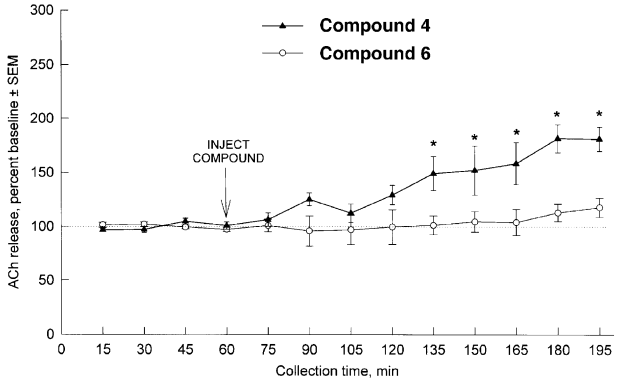


Figure 1. Microdialysis data. Compounds **4** and **6**, 10 mg/kg dose, po, 20% HPβCD. * = significant stimulation over pre-injection baseline ($p < 0.05$, Duncan's multiple range statistic).

affinity and selectivity. Several halogenated naphthamide analogues were synthesized from custom naphthoic acids in an attempt to improve metabolic stability. By screening not only against muscarinic receptors in vitro, but by also utilizing human liver microsomes and high-throughput rat PK screening, we were rapidly able to identify the selective M_2 muscarinic antagonist **4**, which demonstrated in vivo cholinergic activity and memory enhancement in rat microdialysis and PAR experiments. In addition, unlike the initial lead **1**, compound **4** exhibited good plasma levels when dosed in c. monkey, which was an essential parameter for the further development of the ketal series, as potential drug candidates must have acceptable plasma levels in multiple species for toxicological testing. Not only has the use of halogenated naphthamides proved useful for our muscarinic program, but these derivatives also have demonstrated potential to improve the metabolic profile of naphthalene analogues in general.

Acknowledgements

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4. For reviews of other published muscarinic agonists and antagonists, see refs 1d–g and: Baker, R.; Saunders, J. *Annu. Rep. Med. Chem.* **1989**, *24*, 31. Also, himbacine and its analogues have demonstrated modest M_2 affinity and selectivity. For further information, see the following articles and references cited therein: Doller, D.; Chackalamannil, S.; Czarniecki, M.; McQuade, R.; Ruperto, V. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 901. Kozikowski, A. P.; Fauq, A. H.; Miller, J. H.; McKinney, M. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 797.

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7. See ref 3 for synthesis of the ketal core. Sulfonylation or acylation of the piperidine intermediate afforded the compounds described in this paper.

8. See ref 2a 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.

9. See ref 2e for use of a naphthamide derivative to improve M_2 affinity and selectivity.

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

11. Silverman, R. B. *The Organic Chemistry of Drug Design and Drug Action*; Academic: San Diego, 1992; p 297.

12. Tagat, J. R.; McCombie, S. W.; Nazareno, D. V.; Kozlowski, J. A.; Boyle, C.; Josien, H.; Wang, Y.; Zhou, G. *Abstracts of Papers*, 36th National Organic Chemistry Symposium, Madison, WI, 1999; Abstract 259.

13. Procedure described in: Hecht, S.; Chen, C.; Hoffmann, D. *Cancer Res.* **1978**, *38*, 215.

14. Compounds which did not meet these criteria generally had poor plasma levels when orally dosed in rats.

15. 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.

16. For more details on microdialysis experiments, see ref 3 and: Billard, W.; Binch, H., III; Crosby, G.; McQuade, R. D. *J. Pharmacol. Exp. Ther.* **1995**, *273*, 273.

17. 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.

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19. For more details on PAR experiments, see ref 3 and: Smith, R. D.; Kistler, M. K.; Cohen-Williams, M.; Coffin, V. L. *Brain Res.* **1996**, *707*, 13.