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Benzylidene Ketal Derivatives as M_2 Muscarinic Receptor Antagonists

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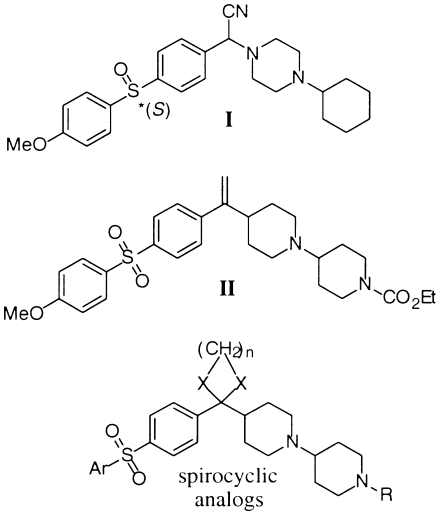
Abstract—Benzylidene ketal derivatives were investigated as selective M_2 receptor antagonists for the treatment of Alzheimer's disease. Compound **10** was discovered to have subnanomolar M_2 receptor affinity and 100-fold selectivity against other muscarinic receptors. Also, **10** demonstrated in vivo efficacy in rodent models of muscarinic activity and cognition.

Alzheimer's disease (AD) is characterized initially by a decline in memory function that hinders normal daily living, followed by a more severe loss of cognitive function and eventual morbidity and mortality. Cholinergic marker loss and selective degeneration of cholinergic neurons in the basal forebrains of AD patients have implicated cholinergic system enhancement as a potential AD treatment.[1] The current available cholinergic pharmacotherapy for AD is in the form of acetylcholinesterase inhibitors, which hinder the degradation of acetylcholine (ACh) in the synapse.[1a] Elevation of synaptic ACh levels could also be achieved by selectively inhibiting presynaptic muscarinic receptors of the M_2 subtype, agonist-induced stimulation of which shuts off ACh release.[1b,c] Since inhibition of postsynaptic M_1 receptors could offset the beneficial effects of presynaptic M_2 receptor inhibition, and M_3 receptors are associated with GI side effects, it is essential that such an agent be selective for the M_2 receptor.[1d]

Compounds **I**[2] and **II**[3] are reported M_2 receptor antagonists with a modest level of selectivity against the M_1 and M_3 receptors.[4] Although these compounds have M_2 selectivity, structural changes could confer increased selectivity over M_1 and M_3 receptors. In addition, presence of the metabolically labile styrene moiety in **II** and a chemically labile benzylic cyano group in **I** preclude the development of these compounds as clinical candidates. We wish to report here the identification of new compounds which have improved upon these earlier leads by incorporating metabolically stable spirocycles at the benzylic position as well as raising M_2 selectivity to 100-fold over other muscarinic receptors. Additionally, these new compounds demonstrate promising oral activity in rodents.



The syntheses of the spirocyclic targets are outlined in Scheme 1. A diarylsulfide was formed via an arylthiol addition/elimination reaction on the *N*-BOC protected

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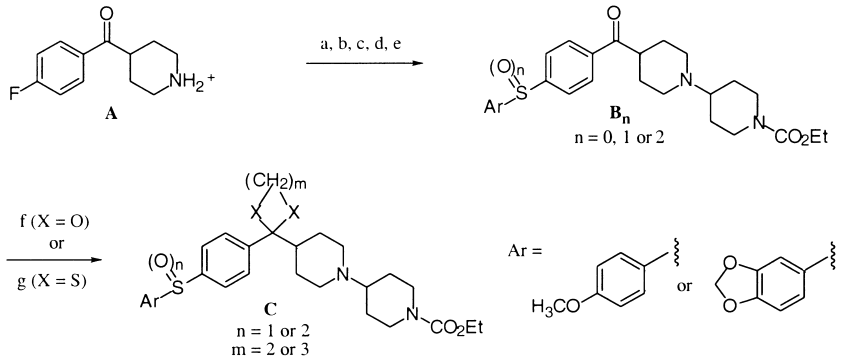
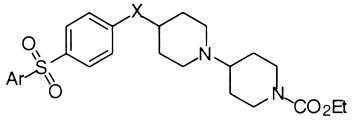


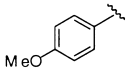
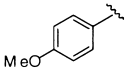
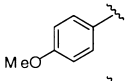
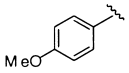
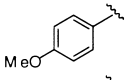
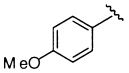
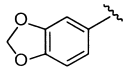
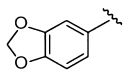
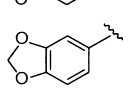
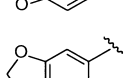
Scheme 1. General synthesis of ketal analogues. Reagents and conditions: (a) $(BOC)_2O$, 10% NaOH, Et_2O; (b) ArSH, NaH, DMF; (c) TFA, CH_2Cl_2; (d) Ti(O-*i*-Pr)$_4$, *N*-carbethoxy-4-piperidone, CH_2Cl_2, then $NaBH_3CN$, MeOH; (e) $NaBO_3 \cdot 4H_2O$ (1 or 2 equiv), AcOH; (f) $HO(CH_2)_mOH$, *p*-TsOH, $HC(OEt)_3$, $PhCH_3$, Δ; (g) $HS(CH_2)_mSH$, $BF_3 \cdot OEt_2$, CH_2Cl_2.

analogue of **A**. Subsequent deprotection followed by reductive amination afforded the bis-piperidine $\mathbf{B_0}$. Sulfide oxidation provided the desired sulfoxide and sulfone intermediates $\mathbf{B_1}$ and $\mathbf{B_2}$. From $\mathbf{B_1}$ and $\mathbf{B_2}$, simple ketalization provided the benzylidene ketals **C**.[5]

Table 1 summarizes initial studies with analogues of **II**.[6] Replacement of the methylene unit of **II** with oxygen decreased M_2 affinity by 40-fold and selectivity over the other muscarinic receptors by 2-fold. However, the five-membered spirocyclic ketals **2** and **4** had M_2 affinity comparable to that of **II**. This phenomenon could be due to the ketone of **1** deactivating the aromatic ring to a greater degree than the ketal or the olefin. Also, the ketal heteroatoms could be in a more favorable position for M_2 receptor binding than the ketone oxygen. Note that only a limited amount of steric bulk was tolerated as the dioxane and dithiane compounds had decreased M_2 affinity over their five-membered ring counterparts.

Table 1. Initial analogues of **II**



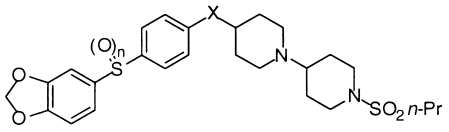
Compound	Ar	X	M_2 K_i (nM)	M_1/M_2	M_3/M_2
II			0.07	68.3	21.9
1			2.91	26.8	10.2
2			0.16	28.3	7.1
3			0.72	21.0	14.4
4			0.11	22.5	3.0
5			14.3	4.4	2.9
6			0.13	47.6	7.5
7			0.073	4.1	1.3
8			0.030	21.7	46.0
9			1.82	10.2	6.1

Having shown promise, this ketal series was synthesized with a methylenedioxyphenyl replacement for the *p*-methoxyphenyl moiety in order to avoid the metabolic demethylation often associated with methoxyphenyl groups.[7] The ketone intermediate **6** had good M_2 affinity and selectivity. As with the *p*-methoxyphenyl derivatives, the ketals generally improved M_2 affinity over the ketone, but suffered from a lack of M_2 selectivity. However, compound **8** demonstrated that modifications to the ketal structure could enhance M_2 selectivity without adversely affecting the M_2 K_i value. Only specific positioning of steric bulk could be tolerated, as there was a 60-fold difference in M_2 affinity between **8** and its enantiomer **9**.

After displaying the potential of the ketal to positively effect M_2 potency without hurting selectivity, we replaced the ethyl carbamate with an *n*-propylsulfonamide group in order to avoid the decarboxylation that could occur with **II**. To this end, ketal derivatives of compound **7** were treated with KOH in refluxing ethylene glycol to remove the ethyl carbamate. The resulting intermediate was exposed to *n*-propylsulfonyl chloride to provide the final ketal compounds **10**–**19**. Both the ketal moiety and the oxidation state of the biaryl sulfur linkage were modified.

In the sulfone series (Table 2: even numbers), the non-substituted dioxolane **10** exhibited the most desirable M_2 affinity/selectivity profile. The tetrahydrofuran derivative **16**[8] demonstrated that both heteroatoms of the ketal were required for M_2 potency, as **16** had reduced M_2 affinity of 500- and 160-fold versus the dioxolane **10** and dithiolane **14**, respectively. Also, the extra heteroatom present in **10** and **14** imparted significant selectivity

Table 2. *n*-Propylsulfonamide series



Compound	X	M_2 K_i (nM)	M_1/M_2	M_3/M_2
10 (n = 2)		0.010	101.5	93.0
11 (n = 1)		0.823	10.8	10.0
12 (n = 2)		1.22	8.7	46.1
13 (n = 1)		9.09	7.5	12.9
14 (n = 2)		0.031	62.6	57.4
15 (n = 1)		0.82	10.7	12.8
16 (n = 2)		5.07	0.22	0.24
17 (n = 1)		12.1	0.49	0.50
18 (n = 2)		1.02	42.6	93.4
19 (n = 1)		34.8	4.6	11.8

over the other muscarinic receptors, as the tetrahydrofuran **16** displayed a lower K_i value for the *other* muscarinic receptors when compared to M_2. In comparison to the sulfone series, the sulfoxide series (Table 2: odd numbers) was disappointing. The compounds were all less potent and less selective than their sulfone analogues.

Before undergoing in vivo studies, both the chemical and metabolic stability of **10** were examined. The hydrochloride salt of **10** was treated with 0.1 N HCl (pH 1), and after several days there was no evidence of ketal hydrolysis. This ketal stability to acid was likely due to the electron withdrawing *p*-sulfone substituent. In addition, **10** showed stability in rat and human microsomes.[9]

In vivo, **10** (po dosing) was studied in microdialysis and passive-avoidance response (PAR) assays. In the microdialysis experiment, ACh levels in the rat striatum were sampled via a microdialysis probe and measured by HPLC/ECD.[10] A detectable level of ACh was achieved by addition of neostigmine, an acetylcholinesterase inhibitor. After attaining a stable baseline, **10** was dosed orally, and ACh levels were monitored over a period of approximately 2 h. As shown in Figure 1, compound **10** promoted a prolonged release of ACh in the rat brain, demonstrating blood–brain barrier penetration and M_2 potency. The ACh levels of **10** surpass that of **II** in a similar study,[3] which implies that the ketal derivative has improved metabolic stability, CNS levels, and/or receptor binding kinetics over the original lead.

For the PAR experiment, **10** was dosed orally to a young rat before testing in a reference memory paradigm (very young rats exhibit memory deficits when tested in such an assay).[11] The rat was given a training session in a dark/light box, in which the rat had a natural aversion to the light chamber. Entry to the darkened area was followed by a mild electrical shock to the feet. In order to avoid the electrical shock, the rat

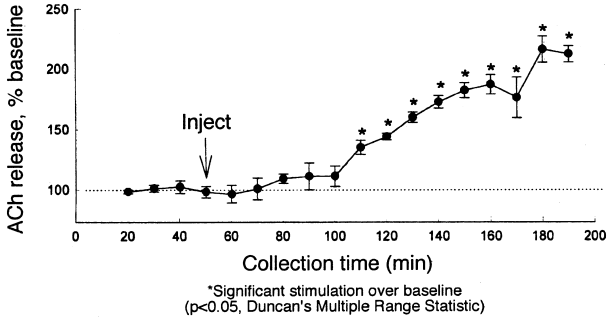


Figure 1. Microdialysis data. Compound **10**, 10 mpk dose, po, methyl cellulose in water.

needed to simply stay in the light chamber and not enter the darkened area, hence the name passive-avoidance response. In a test session given 24 h later, the latency time (length of time the rats avoided the dark chamber) was measured, with longer latency denoting better reference memory. Previous work has indicated that cholinergic enhancing drugs improve learning as measured in this PAR experiment, and that these conditions served as a useful model of impaired memory caused by cholinergic hypofunction. When **10** was dosed to young rats 2 h before training followed by testing 24 h later, a significant increase in latency time was observed with doses of 0.1 mg/kg or greater (Fig. 2). This enhancement of reference memory in vivo demonstrated the utility of **10** to improve cognition via cholinergic system stimulation.

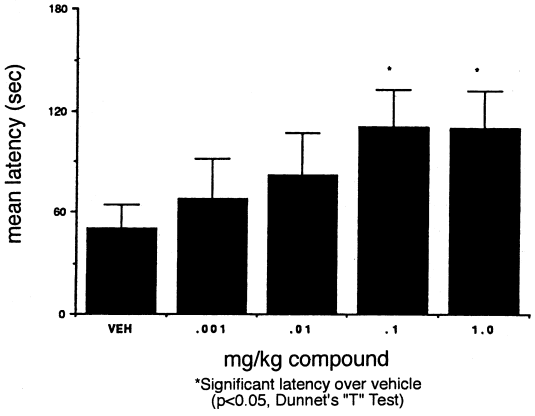


Figure 2. PAR data. Compound **10**, po, methyl cellulose in water.

In conclusion, **10** improved upon leads such as **I** and **II** not only by improving M_2 selectivity versus other muscarinic receptors, but also by replacing metabolically unstable benzylic moieties with the chemically and metabolically stable ketal. Furthermore, **10** was shown to improve cholinergic system activity and memory in orally dosed in vivo microdialysis and PAR studies. Ongoing studies in this promising ketal series for the treatment of AD will be described in future publications.

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