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Diphenyl Sulfoxides as Selective Antagonists of the Muscarinic M_2 Receptor

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Abstract—Structure–activity studies on [4-(phenylsulfonyl)phenyl]methylpiperazine led to the discovery of 4-cyclohexyl-α-[4-[[4-methoxyphenyl](*S*)-sulfinyl]phenyl]-1-piperazineacetonitrile, **1**, an M_2 selective muscarinic antagonist. Affinity at the cloned human M_2 receptor was 2.7 nM; the M_1/M_2 selectivity is 40-fold.

Introduction

Considering that acetylcholine plays a key role in governing learning and memory processes,[1] and Alzheimer's disease has been associated with a reduction in cholinergic activity,[2] it is understandable that efforts to treat the disease have focused on enhancement of acetylcholine levels. Current approaches involve administration of acetylcholinesterase inhibitors,[3] or acetylcholine mimics such as post-synaptic M_1 agonists.[4] However, clinical trials with either have produced moderate or limited improvements in function.[4,5] An alternate approach is to develop drugs that enhance the release of acetylcholine.[2,6] In the CNS, data suggests that presynaptic M_2 receptors regulate acetylcholine release, and an M_2 antagonist will produce increased acetylcholine levels.[7,8] The M_1/M_2 selectivity is crucial since the postsynaptic receptor is of the M_1 subtype, and nonselective muscarinic antagonists such as scopolamine are known to produce cognitive deficits. Himbacine[9] and BIBN-99[10] are reported to be M_2 antagonists with 10- to 20-fold selectivity versus other muscarinic receptor subtypes, but in general the literature is devoid of compounds with high M_1/M_2 selectivity (Fig. 1). Based on this hypothesis we initiated a drug discovery program with the goal of identifying an M_2 selective muscarinic antagonist. Screening identified the lead compound, [4-(phenylsulfonyl)phenyl]methylpiperazine, with $K_i < 50$ nM at the M_2 receptor. This paper describes the discovery of **1**, a potent M_2 selective muscarinic antagonist.[11]

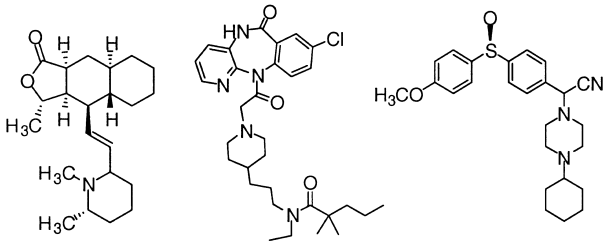

Figure 1.

Chemistry

The diphenylsulfones described in this paper were prepared as shown in Scheme 1. Reaction of the sodium salt of a benzenethiol and *p*-fluorobenzaldehyde, was followed by methyl Grignard addition. Oxidation of sulfur with *m*-chloroperoxybenzoic acid (MCPBA) gave the diphenylsulfone. The alcohol to chloride conversion was accomplished with thionyl chloride. Displacement of chloride with a substituted piperazine produced target compounds. Various benzenethiols are available commercially. *N*-Substituted piperazines were purchased or prepared from *N*-BOC piperazine via reductive amination with aldehydes, or alkylation with alkyl halides, followed by removal of the protecting group.

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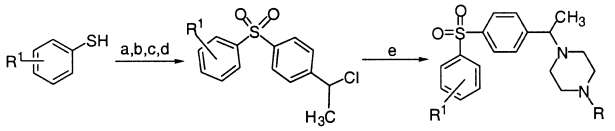
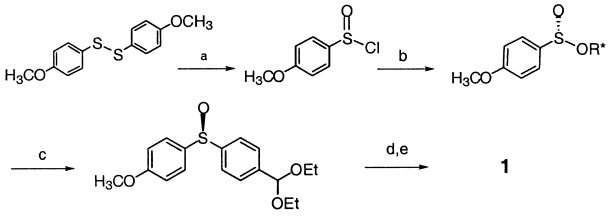


Scheme 1. (a) NaH, *p*-fluorobenzaldehyde; (b) methylmagnesium bromide; (c) MCPBA; (d) $SOCl_2$; (e) NaI, Et_3N, 4-(*R*)-piperazine.



Scheme 2. (a) $SOCl_2$; (b) *trans*-2-(*R*)-1-(*S*)-2-Ph-cyclohexanol, pyridine, THF; (c) *p*-MgBr-benzaldehyde diethylacetal; (d) *p*-TsOH; (e) cyclohexyl piperazine, Ti(*i*-OPr)$_4$, Et_2AlCN.

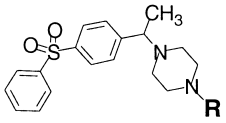
Compounds containing either the (*R*)- or (*S*)-sulfoxide were prepared from the reaction of a Grignard reagent and an optically active sulfinate ester[12,13] (Scheme 2). Typically one enantiomeric series is readily accessible, as one of the diastereomeric sulfinate esters tends to be crystalline.[14] However, since both the (*R*)- and (*S*)-sulfoxides were targets, we sought a method where both diastereomeric sulfinate esters were readily isolable. We found that the reaction of 4-methoxysulfinyl chloride with trans-2-(*R*)-1-(*S*)-2-phenylcyclohexanol,[14] gave a mixture of diastereomers (3:1 (*R*) to (*S*) sulfoxide) which were separable via flash chromatography. Menthol[13] was less effective as the chiral auxiliary because the diastereomeric sulfinate esters were not readily separable. Addition of the Grignard reagent derived from *p*-bromobenzaldehydedimethylacetal proceeded with inversion of configuration at sulfur and regeneration of the chiral alcohol. Following acetal hydrolysis, the Strecker amine was prepared by reaction of the aldehyde with cyclohexyl piperazine, titanium(IV) isopropoxide, and diethyl aluminum cyanide.

Separation of diastereomers at the benzylic center, with the (*R*)-sulfoxide, was accomplished on a Chiracel OD column (20% IPA/hexane). Diastereomers of the corresponding (*S*)-sulfoxide were separated by fractional crystallization (ethyl acetate/hexane).

Results

The binding data[15] in Table 1 show that variation of the *N*-(4)-*R*-group on the piperazine had a direct influence on affinity at the M_2 receptor. A lipophilic group, such as cyclohexyl, improved both the M_2 affinity and selectivity. However, M_2 affinity was decreased when the piperazine *N*-(4)-nitrogen was rendered nonbasic with an electron withdrawing substituent. This observation is consistent with reports of agonist binding to muscarinic receptors, where it is proposed that a basic nitrogen interacts with Asp 103 on the third transmembrane domain.[16,17]

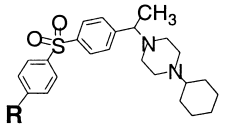
Table 1.



Compound	R	K_i (nM)		
		M_1	M_2	M_1/M_2
2	H	57	37	1.6
3	CH_3	96	34	3
4	CH_2CH_2OH	77	26	3
5	CO_2Et	200	135	1.5
6	Cyclohexyl	1.3	0.2	6
7	Ph	4770	4600	1

With the piperazine group fixed as cyclohexyl, Table 2 summarizes data for substitution on the phenylsulphonyl. M_1/M_2 selectivity was improved with 4-methoxy, 4-methyl, or 3-chloro. However, bulky groups such as *tert*-butyl or methylsulfonyl reduced affinity for the M_2 receptor.

Table 2.

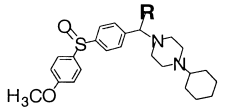


Compound	R	K_i (nM)		
		M_1	M_2	M_1/M_2
8	OCH_3	6	0.6	10
9	CH_3	11	0.8	14
10	Cl	8	0.6	13
11	*t*-Butyl	85	33	2.6
12	SO_2CH_3	106	36	3

As shown in Table 3, modification of the benzylic group, in combination with a sulfoxide bridging the phenyl rings led to compounds with affinity and selectivity comparable to the sulfone series. In addition, small groups were preferred at the benzylic position.

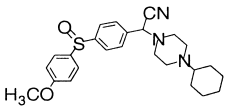
The influence of chirality at sulfur and the benzylic site on affinity and selectivity is summarized in Table 4. Configuration A was preferred at the benzylic site. The

Table 3.



Compound	R	K_i (nM)		
		M_1	M_2	M_1/M_2
13	CH_3	6	1	6
14	CN	117	8	14
15	Cyclohexyl	52	17	3
16	$CONH_2$	242	40	6
17	CF_3	23	5	5

Table 4.



Compound	SO	CN Isomer	K_i (nM) M_1	M_2	M_1/M_2
1	(*S*)	Mix	112	2.7	40
18	(*S*)	A	83	3.8	22
19	(*S*)	B	180	20	9
20	(*R*)	A	140	9	15
21	(*R*)	B	253	17	15

combination of the (*S*)-sulfoxide with benzylic configuration A gave the highest affinity and selectivity for M_2. However, we found that the benzylic cyanide undergoes facile racemization and were unable to isolate pure enantiomers. Therefore, the data in Table 4 reflect partial racemization of the benzylic center. Knowing that we could not isolate pure enantiomers at the benzylic site, we focused on **1**, which had the best profile overall. To provide the best estimation of the M_1/M_2 ratio, the K_i values for M_1 and M_2 were repeated with $n=9$ (all other determinations were performed in duplicate).

M_2 antagonism in a functional assay is reported for compound **1** in ref 11. The effect of **1** on acetylcholine release in rat brain was determined via a microdialysis paradigm. A dose-dependent response was observed following po administration with peak levels increasing over basal by almost three-fold. Compound **1** was also determined to improve memory in the young rat model of cognition (Fig. 2).[18]

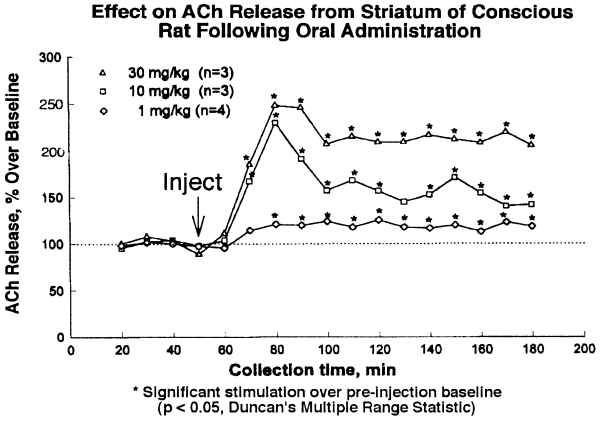


Figure 2.

Conclusion

Structure–activity relationship studies on [4-(phenylsulfonyl)phenyl]methylpiperazine led to discovery of a series of potent and M_2 selective ligands. Compound **1** displays an M_1/M_2 selectivity of 40-fold with an M_2 $K_i = 2.7$ nM. The compound has been characterized as an antagonist, was shown to increase acetylcholine levels in vivo and was effective in animal models of cognition.[18] These initial results are promising for the development of M_2 selective muscarinic antagonists for the treatment of Alzheimer's disease.

References and Notes

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