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Substituted 2-(*R*)-Methyl Piperazines as Muscarinic M_2 Selective Ligands

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Abstract—A novel series of 2-(*R*)-methyl-substituted piperazines (e.g., **2**) is described. They are potent M_2 selective ligands that have >100-fold selectivity versus the M_1 receptor. In the rat microdialysis assay, compound **14** showed significantly enchanced levels of acetylcholine after oral administration.

People suffering from Alzheimer's Disease (AD) show a progressive loss of memory and cognitive function, which has been associated with impairment of the cholinergic system.[1] One method of treatment for AD is to increase acetylcholine levels through administration of acetylcholinesterase inhibitors.[2] An alternate approach is to develop drugs that enhance the release of acetylcholine.[1,3] The approach we have chosen is to enhance acetylcholine levels through antagonism of presynaptic M_2 muscarinic receptors.[4–6] The M_1/M_2 selectivity is crucial since antagonism of post-synaptic M_1 receptors, with agents such as scopolamine, is known to produce cognitive deficits.[7] Recently we reported on a series of M_2 selective antagonists **1**, which increased acetylcholine levels in a microdialysis paradigm in rats.[8] Although the chemical series described showed desirable activity in vitro and in vivo, we sought to find an alternative to the chiral sulfoxide, and α-cyano amine moieties. We wish to report our progress in this area as represented by structure **2**, wherein the sulfoxide and cyano moieties were replaced with sulfone, and (*S*)-methyl, respectively (Fig. 1).

Compounds containing a cyclohexylpiperazine, with R^2 hydrogen (Table 1), were prepared as shown in Scheme 1. Reaction of the sodium salt of benzenethiol and *p*-fluorobenzaldehyde in DMF, gave the coupled diphenylsulfide. Reaction with Ti(*i*-OPr)$_4$, 4-cyclohexyl-2-(*R*)-methylpiperazine, and either methylmagnesium bromide or diethylaluminum cyanide or sodium cyanoborohydride gave the product with R equal to methyl, cyano, or hydrogen, respectively. When R is methyl, the mixture of diastereomers was separated by flash chromatography. The cyclohexylpiperazine was prepared from the reaction of (*R*)-methyl piperazine with cyclohexanone, Ti(*i*-OPr)$_4$, and $NaCNBH_3$. Oxidation with *m*-chloroperoxybenzoic acid (*m*-CPBA) gave the sulfone. Stereochemical assignment of the benzylic methyl was done via NOE studies, following flash chromatographic separation of the diastereomers.

A non-racemic route to compounds containing an (*S*)-methyl group at the benzylic carbon is described in Scheme 2. Sodium hydrogensulfide was added to *p*-fluoroacetophenone, and BHT in hot DMF to

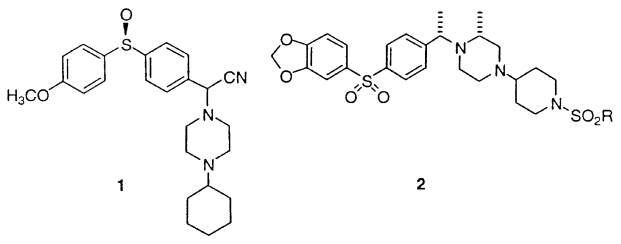

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Figure 1.

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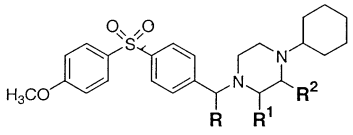
generate sodium-4-mercapto acetophenone, which was coupled in the same pot with 3,4-methylenedioxyiodobenzene under Suzuki's conditions.[9] After work up and chromatography, the ketone was isolated in ~80% yield. Oxidation of the diphenylsulfide to the sulfone was accomplished with *m*-CPBA. Corey's enantioselective reduction gave the chiral (*R*)-alcohol in >98% yield and 94% ee.[10] Mesylation and displacement with 4-*N*-Boc-2-(*R*)-methylpiperazine gave the (*S*)-benzylic methyl. Removal of the BOC with acid and reductive amination *N*-BOC-4-piperidinone proceeded in good yield.[11] Deprotection of the BOC gave the free piperidine, which was converted to amides, sulfonamides, and ureas with acid chlorides, sulfonyl chlorides, or isocyanates, respectively.

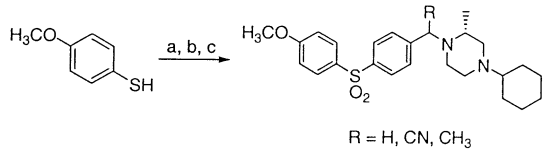
Compounds with R^2 equal to methyl (Table 1) were prepared from either (*R*)- or (*S*)-methyl lactate (Scheme 3). 2-Nitrophenylsulfonyl chloride was treated with aminoethanol, and the product was acylated with acetic anhydride in pyridine. This sulfonamide was treated with the triflate of either (*R*)- or (*S*)-methyl lactate, followed by reduction of the esters with borane-methyl sulfide complex. The piperazine was formed from reaction of the diol, activated with triflic anhydride, with the diphenylsulfonyl-α-methylbenzylamine, prepared as shown from α-methylbenzylamine. Removal of the sulfonamide protecting group, under Fukayama's conditions,[12] gave the free piperazine, which was converted to **9** or **10**.

The binding affinities for synthesized compounds described here were determined using cloned human muscarinic receptors, as previously described.[13] Our initial objective was to find a compound with affinity and selectivity comparable to or better than **1**, but without the chiral sulfoxide and α-cyano amine moieties. First we focused on switching the chiral sulfoxide of **1** to a sulfone, thereby simplifying the synthetic scheme and avoiding the possibility of metabolic racemization of the sulfoxide in vivo. The chirality lost in changing sulfoxide to sulfone, was introduced again by addition of a methyl to the piperazine ring (e.g., **3** and **4**, Table 1). A preference for the (*R*)-configuration for the R^1 methyl was apparent (e.g., **3**) with M_1/M_2 selectivity similar to **1** observed. In looking for alternates to the Strecker amine moiety, we focused on methyl as a non-racemizable replacement for the cyano group. Thus, substituting the cyano of compound **3** with methyl gave the diastereomeric compounds **6** and **7**. A slight preference for (*S*)-methyl at the benzylic site was observed, with **6** was apparent. The importance of the combination of the benzylic and piperazine methyl together was

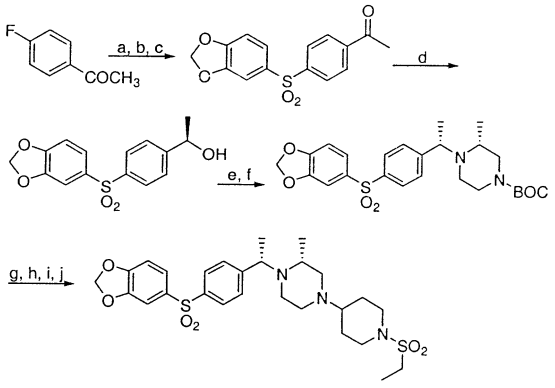
Table 1.



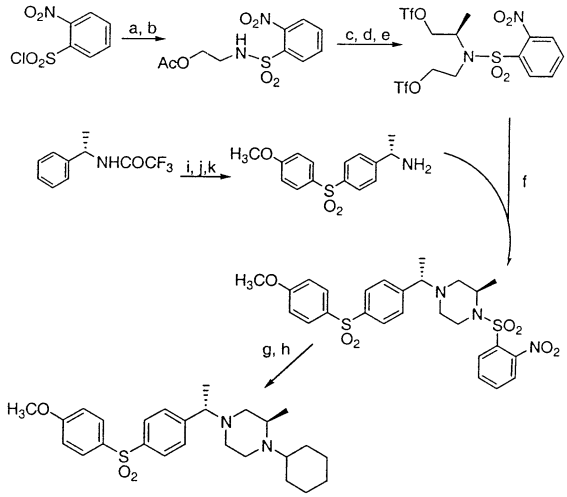
Compd	R	R^1	R^2	K_i (nM)	
				M_2	M_1/M_2
3	CN	(*R*)-CH_3	H	0.16	36
4	CN	(*S*)-CH_3	H	16	5
5	CH_3	H	H	0.6	10
6	(*S*)-CH_3	(*R*)-CH_3	H	0.3	44
7	(*R*)-CH_3	(*R*)-CH_3	H	0.15	30
8	H	(*R*)-CH_3	H	0.3	23
9	(*R*)-CH_3	H	(*S*)-CH_3	0.04	10
10	(*R*)-CH_3	H	(*R*)-CH_3	0.14	10



Scheme 1. (a) NaH, 0 °C, DMF, add *p*-fluorobenzaldehyde, warm to rt, 12 h; (b) 4-cyclohexyl-2-(*R*)-methylpiperazine, rt, Ti(*i*-OPr)$_4$, CH_3MgBr or Et_2AlCN or $NaCNBH_3$, for R = CH_3, CN, H, respectively, flash chromatography; (c) *m*-CPBA, CH_2Cl_2, rt.



Scheme 2. (a) NaHS, DMF, 95 °C, 2 h; (b) 3,4-methylenedioxyiodobenzene, CuI, K_2CO_3, DMF, 100 °C, 6 h; (c) *m*-CPBA, $NaHCO_3$, 1,2-dichloroethane, rt, 24 h; (d) 0.6 equiv $BH_3(CH_3)_2S$, THF, rt, 0.2 equiv Corey's oxoborolidine catalyst; (e) CH_3SO_2Cl, CH_2Cl_2, 0 °C, Et_3N; (f) tetramethylpiperidine, 2-(*R*)-methyl-4-BOC-piperazine, CH_3CN, reflux, 12 h; (g) 6 N HCl, reflux 7 h; (h) $NaHB(OAc)_3$, *N*-BOC-4-piperidinone; (i) 6 N HCl, rt 3 h; (j) $EtSO_2Cl$, Et_3N, rt.



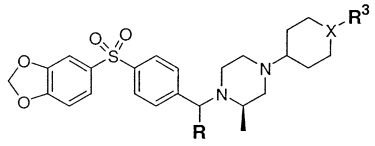
Scheme 3. (a) 2-Hydroxyethyl amine, rt, CH_2Cl_2; (b) acetic anhydride, pyridine, rt; (c) (*R*)-$CH_3CH(OSO_2CF_3)CO_2Et$; (d) $BH_3(CH_3)_2S$, THF; (e) Tf_2O, Collidine, CH_2Cl_2, 0 °C; (f) 2 equiv Na_2CO_3, CH_3CN, rt 24 h; (g) $HSCH_2CO_2H$, LiOH–H_2O; (h) Ti(*i*-OPr)$_4$, cyclohexanone, $NaCNBH_3$; (i) 1,3-dibromo-5,5-dimethylhydantoin, rt, $MeSO_3H$, CH_2Cl_2; (j) *n*-BuLi, THF, −70 °C, then $ArSO_2F$, warm to rt; (k) KOH, MeOH, rt.

demonstrated by removing each separately (e.g., **5** and **8**); a significant loss of selectivity resulted when either the benzylic or piperazine methyl was replaced with hydrogen.

The 3-methyl piperazines (R^2 = methyl and R^1 = hydrogen) indicate that the position of the methyl in the piperazine ring is important for optimizing M_2 selectivity, as these compounds showed good affinity but not selective.

To further optimize the binding profile of **6**, we chose to add more functionality to the structure by conversion of the cyclohexyl to a 4-piperidine, and replacing the 4-methoxyphenyl with 3,4-methylenedioxy phenyl. It was anticipated that additional steric and hydrogen-bonding sites could provide an opportunity for improved M_2 selectivity. Although this strategy was successful with piperidines,[14] it was untested with the methylated-piperazines described here. The results of these modifications are summarized in Table 2. When the 4-methoxyphenyl was exchanged with 3,4-methylenedioxy phenyl, an increase in M_2 affinity and selectivity was observed (e.g., **11** vs **6**). In general, piperidine derivatives such as sulfonamides and amides were more selective than cyclohexyl piperazine, but the free piperidine, **13**, had weaker affinity and lower selectivity. Aryl sulfonamides maintained M_2 affinity, but were less selective than the corresponding alkyl sulfonamides. Both amides and urea's lost affinity and selectivity relative to the equivalent alkyl sulfonamide.

Table 2.



Compd	R	X	R^3	K_i (nM)	
				M_2	M_1/M_2
11	(*S*)-CH_3	CH_2	—	0.03	61
12	(*R*)-CH_3	CH_2	—	0.03	30
13	(*S*)-CH_3	N	H	9	4
14	(*S*)-CH_3	N	SO_2–C_2H_5	0.7	109
15	(*S*)-CH_3	N	SO_2–*n*-C_3H_7	0.2	281
16	(*S*)-CH_3	N	SO_2–*n*-C_4H_9	0.2	198
17	(*S*)-CH_3	N	SO_2–Ph	0.8	40
18	(*S*)-CH_3	N	COPh	0.9	32
19	(*S*)-CH_3	N	CO–*n*-C_4H_9	1.3	15
20	(*S*)-CH_3	N	$CONHC_2H_5$	2.5	10

The in vivo effect of **14** was measured using a microdialysis paradigm,[5,15] in which the acetylcholine level was measured as a function of time through a dialysis probe inserted into the striatum of conscious rats. Compound **14** produced a sustained increase in acetylcholine levels (Fig. 2), which was not observed with the more selective **15** (data not shown). The affinity of **14** and **15** are similar, and since acetylcholine release is determined by M_2 potency, not M_1/M_2 selectivity, the differences observed in the microdialysis paradigm may reflect differences in the pharmacokinetic profiles of **14** and **15**.

We have demonstrated that both the chiral sulfoxide and Strecker amine moieties of **1** can be replaced with sulfone and methyl respectively, when a methyl group is added to the piperazine ring. The chirality of the benzylic and piperazine methyl groups is critical for maintaining good M_2 affinity and selectivity. The position of the piperazine methyl is important for M_2 selectivity. Further enhancements in M_2 affinity and selectivity were found through modification of the 4-methoxy phenyl and the cyclohexyl moieties. Alkyl

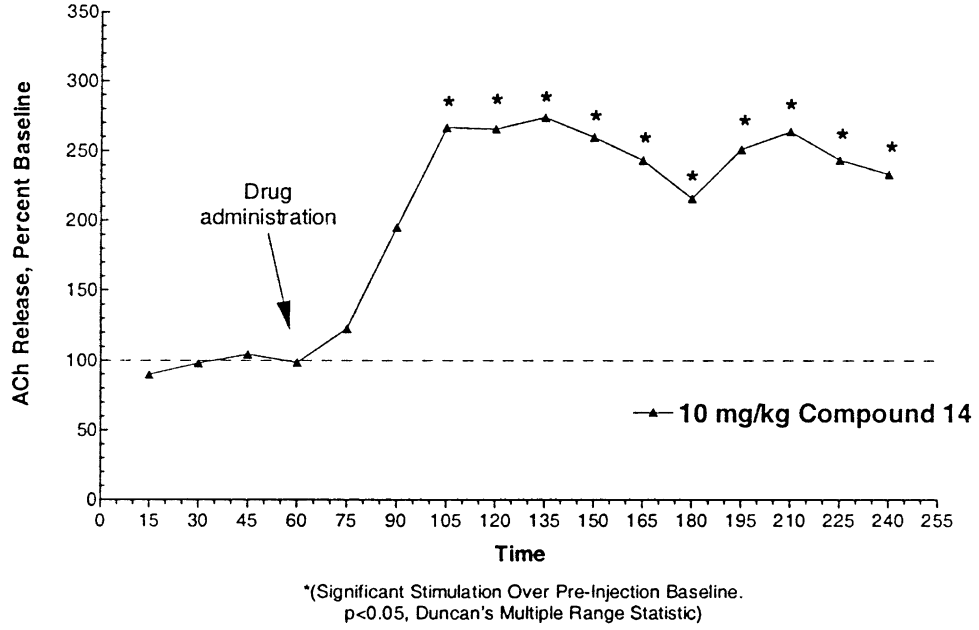


Figure 2. Acetylcholine release from striatum of conscious rat.

sulfonamides were the preferred capping groups for the piperidine nitrogen. Compound **14** displays a M_1/M_2 selectivity of 109-fold with a M_2 $K_i = 0.7$ nM. It produced a sustained increase in acetylcholine levels when administered orally in the microdialysis paradigm. These initial results are promising for the development of M_2 selective muscarinic antagonists for the treatment of Alzheimer's Disease.

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