Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2247–2250

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design and Synthesis of Piperidinyl Piperidine Analogues as Potent and Selective M_2 Muscarinic Receptor Antagonists

Yuguang Wang,* Samuel Chackalamannil, Zhiyong Hu, John W. Clader, William Greenlee, William Billard, Herbert Binch, III, Gordon Crosby, Vilma Ruperto, Ruth A. Duffy, Robert McQuade and Jean E. Lachowicz

Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA

Received 5 June 2000; accepted 21 July 2000

Abstract—Identification of a number of highly potent M_2 receptor antagonists with >100-fold selectivity against the M_1 and M_3 receptor subtypes is described. In the rat microdialysis assay, this series of compounds showed pronounced enhancement of brain acetylcholine release after oral administration.

Alzheimer's disease (AD), the most common form of dementia, affects the independent living of the elderly population.[1] AD patients show a progressive loss of memory and cognitive function, which is due in part to the impairment of the cholinergic system.[2] Currently available cholinergic therapy for AD is based on increasing acetylcholine levels by inhibiting acetylcholinesterase, the enzyme that hydrolyzes acetylcholine.[2] Enhancement of acetylcholine levels could also be achieved by selectively inhibiting presynaptic M_2 muscarinic receptors, which regulate acetylcholine release by an inhibitory feedback mechanism.[2] It is essential that M_2 receptor antagonists are selective versus M_1 receptors because the post-synaptic M_1 receptors mediate the acetylcholine effect. It has been demonstrated that M_1 agonists improved cognition.[3] Additionally, selectivity versus M_3 receptors is also needed because inhibition of peripheral and central M_3 receptors can also cause side effects.[4]

The highly conserved amino acid sequences of muscarinic receptor subtypes renders design of a selective M_2 antagonist difficult.[5] In fact, despite several reports of potent M_2 antagonists,[3,6] there have not been any reports of a potent M_2 antagonist with >40-fold selectivity versus the M_1 and M_3 receptors. In an effort to discover selective M_2 antagonists, we selected the vinyl piperidine derivative **1** as our lead.[7] Compound **1** is a potent M_2 antagonist ($K_i = 0.17$ nM), but devoid of appreciable selectivity versus other receptor subtypes (Table 1). We wish to report here and in a subsequent communication that we have achieved this goal and identified a number of highly potent and highly selective M_2 antagonists.

Our design strategy was to generate additional sites of receptor interactions that could potentially serve to discriminate among various receptor subtypes. Toward this goal, an *N*-substituted piperidine surrogate that could provide steric and hydrogen-bonding interactions with receptor subtypes replaced the cyclohexyl ring of **1** (Fig. 1).

Compounds **6**, **7**, and **8** (Table 1), representing three structural subclasses, were synthesized as shown in Scheme 1. *N*-Boc protection of commercially available piperidine derivative **2** followed by displacement of fluorine atom with 4-methoxy thiophenol gave **3**. The ketone **3** was transformed to sulfone alkene **4** by sequential treatment with Tebbe reagent,[8] sulfide oxidation and deprotection.[9] Reductive amination of **4** followed by deprotection gave **5** which was converted to the final targets by treatment with different reagents such as sulfonyl chloride, acetyl chloride, and chlorofomate.[10]

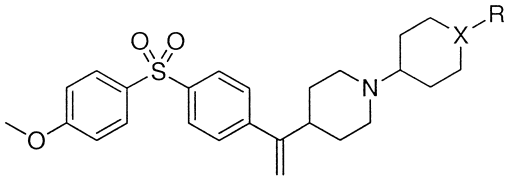
The binding affinity of the synthesized antagonists against cloned human muscarinic receptors were assayed according to the reported protocol.[11]

The results of varying *N*-substituent on the piperidine ring are presented in Table 1. The unsubstituted compound **5** showed reduced affinity toward the M_2 receptor, in

*Corresponding author. Tel.: +1-908-740-3508; fax: +1-908-740-4563; e-mail: yuguang.wang@spcorp.com

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00457-1

Table 1. Results of M_2 binding affinity and selectivity against M_1 and M_3



Compound	X	R	M_2 (K_i, nM)	M_1 (K_i, nM)	M_3 (K_i, nM)	M_1/M_2	M_3/M_2
1	C		0.17	2.8	0.48	16	3
5	N	H	1.14	9.1	3.4	8	3
6	N	COOEt	0.11	6.5	3.7	59	34
7	N	CO-*n*-C_3H_7	0.38	42.0	121.6	111	324
8	N	SO_2-*n*-C_3H_7	0.29	25.7	10.1	89	35

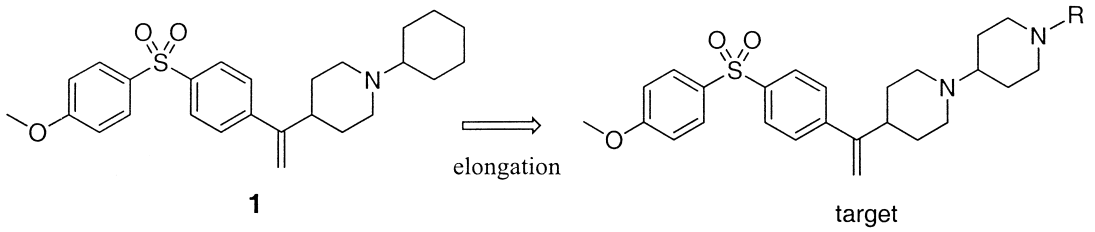
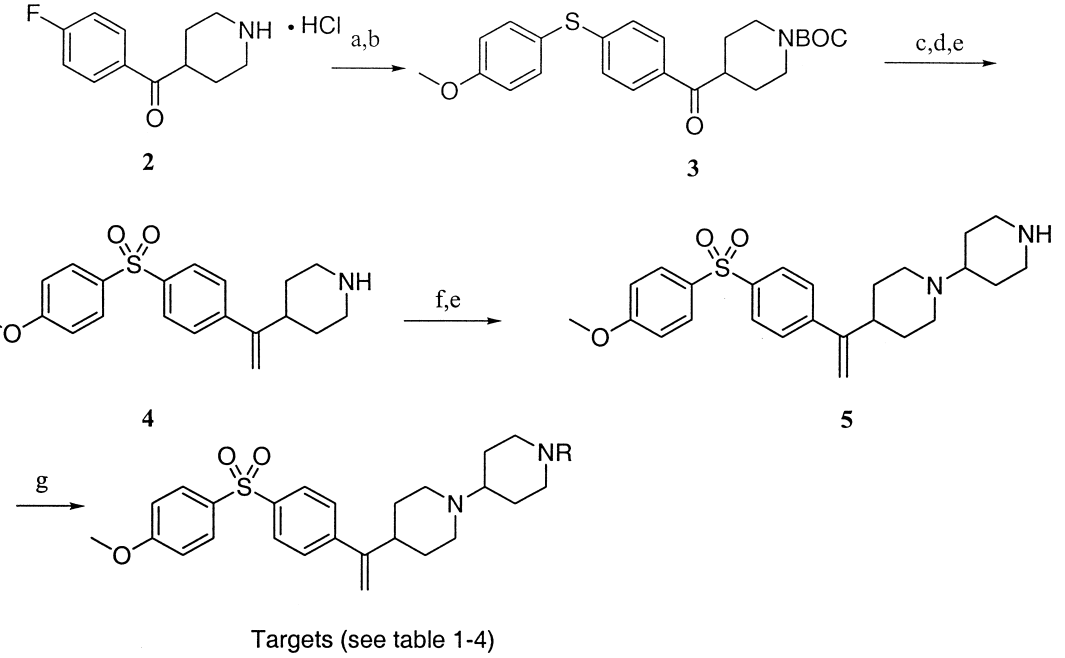


Figure 1. Elongation of **1**.

comparison to **1**, with little selectivity versus the M_1 and M_3 receptors. However, the carbamate derivative **6** and the propyl sulfonamide **8** showed improved selectivity versus M_1 and M_3 receptors, whereas the propyl amide **7** showed excellent selectivity. This enhanced selectivity is principally due to the decreased M_1 and M_3 receptor affinity, since the M_2 affinity remains unchanged.

In an effort to further optimize the binding profile of these compounds, each of the above subseries was further explored. The results of the amide subseries are in Table 2. As shown, the M_2 receptor affinity of these compounds is highly sensitive to the substitutes of the amide moiety. For example, amides **9**, **11**, and **12** lowered the M_2 receptor affinity considerably with concomitant loss of selectivity. On the other hand, amides **7** and **10** showed excellent M_2 affinity and M_1/M_2 and M_3/M_2 ratios.

The sulfonamide series afforded the most promising compounds in terms of both receptor affinity and subtype



Scheme 1. (a) $(BOC)_2O$, 10% NaOH/Et_2O, 89%; (b) NaH, DMF, 4-methoxythiophenol, 65 °C, 6 h, 89%; (c) Tebbe reagent, 90%; (d) $NaBO_3$, HOAc, 83%; (e) 30% TFA/CH_2Cl_2, 100%; (f) $NaBH(AcO)_3$, 1,2-DCE, 1-*t*-butoxycarbonyl-4-piperidone, 75%; (g) RCl, Et_3N, CH_2Cl_2.

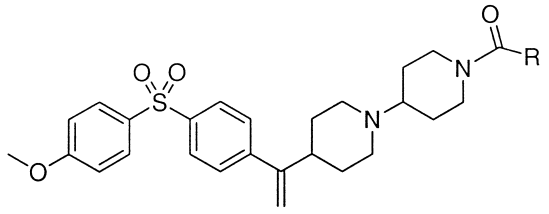
selectivity (Table 3). In this series, the M_2 receptor affinity was unaffected by the substitutes of the sulfonamides in most of the cases, whereas the M_1 affinity and M_3 affinity were quite sensitive to these substitutions. This property entailed a number of compounds with excellent M_2 potency and M_1 and M_3 selectivity. For example, sulfonamides **13**, **14**, and **15** have subnanomolar M_2 affinity and excellent selectivity toward M_1 and M_3 receptors.

The data for the carbamate derivatives are presented in Table 4. Although these derivatives showed uniformly high affinity in the M_2 binding assay, they were less selective toward M_1 and M_3 receptors than the corresponding amide and sulfonamide derivatives. The results of the three subseries demonstrated that the substitution changes in the amide and the sulfonamide series affected the M_1 and M_3 affinity much more than the carbamate series. As a result, high selectivity of M_2 versus M_1 and M_3 was observed in the amide series and the sulfonamide series, both of which have lowered M_1 and M_3 binding affinity.

The in vivo effect of the M_2 antagonist was measured using a microdialysis paradigm, in which the acetylcholine level in the rat striatum was monitored as a function of time through a dialysis membrane probe.[12] The acetylcholinesterase inhibitor neostigmine was perfused through the probe to produce an acetylcholine level high enough to activate the M_2 receptor inhibitory feedback mechanism and dampen the acetylcholine release. A stable baseline level of acetylcholine was routinely achieved under these conditions. When the M_2 receptor antagonist **6** was administered orally to rats, the level of acetylcholine was increased significantly over the baseline, as shown in Figure 2. This result is consistent with the blockade of the M_2 receptor by antagonist **6** and a consequent increased release of acetylcholine due to partial reversal of the M_2 inhibitory feedback mechanism. Additionally, the high level of the acetylcholine release also suggests that the carbamate **6** has good oral bioavailability and blood–brain barrier penetration.

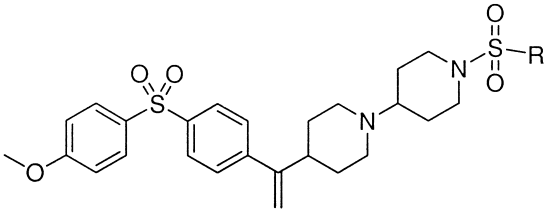
In summary, we have identified several potent and selective M_2 receptor antagonists. For example, compounds **10**, **13**, and **14** show subnanomolar K_i values for the M_2 receptor and greater than 150-fold selectivity against the M_1 and M_3 receptors. Additionally, the representative data presented for compound **6** demonstrated

Table 2. Results of M_2 affinity and selectivity of the amide series



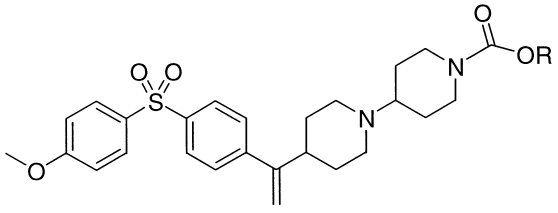
Compound	R	M_2 (K_i, nM)	M_1/M_2	M_3/M_2
9	Et	3.8	16	13
7	n-C_3H_7	0.38	111	324
10	Cyclopropyl	0.26	262	260
11	i-C_3H_7	2.2	53	40
12	CH_2Ph	2.99	32	48

Table 3. Results of M_2 affinity and selectivity of the sulfonamide series



Compound	R	M_2 (K_i, nM)	M_1/M_2	M_3/M_2
13	Et	0.46	162	382
8	n-C_3H_7	0.29	89	35
14	i-C_3H_7	0.16	198	440
15	n-C_4H_9	0.38	150	405
16	Ph	0.36	104	558
17	CH_2Ph	1.45	23	54

Table 4. Results of M_2 affinity and selectivity of the carbamate series



Compound	R	M_2 (K_i, nM)	M_1/M_2	M_3/M_2
6	Et	0.11	59	34
18	Me	0.99	61	55
19	n-C_3H_7	0.59	69	40
20	i-C_3H_7	0.41	36	n/a
21	n-C_4H_9	0.35	28	n/a
22	i-C_4H_9	0.30	35	n/a
23	CH_2Ph	0.45	24	n/a

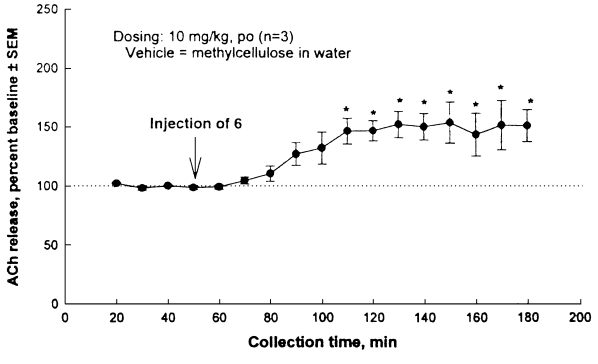


Figure 2. Effect of **6** on acetylcholine release in rat striatum following oral administration. Each data point represents a 10-min collection of microdialysate and constitutes the mean±SEM of three individual rats. Perfusion rate was 2 mL/min using Ringer's solution containing 1 mM neostigmine. Arrow indicates time of **6** administration (10 mg/kg, orally in water). *Significant stimulation over each of three pre-injection baseline points ($p < 0.05$, Duncan's mutiple range statistic).

that the M_2 antagonists upon oral administration, stimulate brain acetylcholine release in functional microdialysis assay. A detailed structure–activity relationship study of this class of compounds, as well as their in vivo efficacy in animal models of cognition, will be published in the future.

Acknowledgements

The authors would like to thank Mr. Yao H. Ing and Dr. P. Das for mass spectral data, and Dr. Murgolo for molecular modeling studies.

References and Notes

1. Brinton, R. D.; Yamazaki, R. S. *Pharm. Res.* **1998**, *15*, 386.
2. (a) Farlow, F. R.; Evans, R. M. *Neurology* 1998, *51*, S36. (b) Quirion, R.; Wilson, A.; Rowe, W.; Aubert, I.; Richard, J.; Doods, H. N.; Parent, A.; White, N.; Meaney, M. J. *J. Neurosci.* **1995**, *15*, 1455. (c) Doods, H. N. *Drugs Future* **1995**, *20*, 157.
3. (a) Avery, E. E.; Baker, L.; Asthana, S. *Drug Ther.* **1997**, *11*, 450. (b) Sauerberg, P.; Jeppessen, L.; Olesen, P. H.; Sheardown, M. J.; Finkjensen, A.; Rasmussen, T.; Rimvall, K.; Shannon, H. E.; Bymaster, F. P.; DeLapp, N. W.; Calligaro, D. O.; Ward, J. S.; Whitesitt, A. C.; Thomsen, C. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2897. (c) Xu, R.; Sim, M. K.; Go, M. L. *J. Med. Chem*, **1998**, *41*, 1404.
4. Andersson, K. E. *Drugs* **1988**, *35*, 477.
5. Brann, M. R.; Klimkowski, V. J.; Ellis, J. *Life Sci.* **1993**, *52*, 405.
6. (a) Lachowicz, J. E.; Lowe, D.; Duffy, R. A.; Ruperto, V.; Taylor, J. A.; Guzik, H.; Brown, J.; Berger, J. G.; Tice, M.; McQuade, R.; Kozlowski, J.; Clader, J.; Strader, C. D.; Murgolo, N. *Life Sci.* **1999**, *64*, 535. (b) Doller, D.; Chackalamannil, S.; Czarniecki, M.; McQuade, R.; Ruperto, V. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 901. (c) Engel, W. E.; Eberlein, W. G.; Mihm, G.; Hammer, R.; Trummlitz, G. *J. Med. Chem.* **1989**, *32*, 1718. (d) Malaska, M. J.; Fauq, A. H.; Kozikowski, A. P.; Aagaard, P. J.; Mckinney, M. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1247.
7. Billard, W.; Binch, H., III; Bratzler, K.; Chen, L. Y.; Crosby, G.; Duffy, R. A.; Dugar, S.; Lachowicz, J.; McQuade, R.; Pushpavanam, P.; Ruperto, V. B.; Taylor, L. A.; Clader, J. W. submitted together with this letter for publication.
8. Pine, S. H.; Shen, G. S.; Hoang, H. *Synthesis* **1991**, *165*.
9. Shimazaki, M.; Takahashi, M.; Komatsu, H.; Ohta, A.; Kajiii, K.; Kodama, Y. *Synthesis* **1992**, *555*.
10. All of the target compounds showed satisfactory result in the analyses of NMR, MS, LC/MS, and HRMS.
11. For radioligand binding analysis, each muscarinic receptor subtype was stably expressed in CHO-K1 cells. Clonal cell lines were selected which expressed receptors at levels between 1 and 9 pmol/mg protein. The K_d of QNB (l-quinuclidinyl benzilate) at each receptor subtype was determined by saturation binding using 5–2500 pM [^{3}H]QNB in 10 mM potassium phosphate buffer, pH 7.4. Protein concentrations were adjusted for each assay to achieve between 700 and 1500 cpm specific binding. Competition binding experiments were performed using 180 pM [^{3}H] QNB. All binding experiments were performed in the presence of 1% DMSO and 0.4% methylcellulose. Non-specific binding was defined by 0.5 mM atropine. After equilibrium was reached (120 min incubation at room temperature), bound and free radioactivity were separated by filtration using Whatman GF-C filters. Investigation of M_2 receptor antagonist activity was performed on related compounds by measuring the effects of the compounds adenylyl cyclase inhibition mediated by oxotremorine M (see **6a** for details).
12. Billard, W.; Binch, H., III; Crosby, G.; McQuade, R. *J. Pharmacol. Exp. Ther.* **1995**, *273*, 273.