Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 57–60

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Identification of Novel Muscarinic M_3 Selective Antagonists with a Conformationally Restricted Hyp-Pro Spacer

Yufu Sagara,* Toshifumi Kimura, Toru Fujikawa, Kazuhito Noguchi and Norikazu Ohtake

Banyu Tsukuba Research Institute in collaboration with Merck Research Laboratories, Okubo-3, Tsukuba 300-2611, Ibaraki, Japan

Received 3 September 2002; accepted 3 October 2002

Abstract—The identification of potent and selective muscarinic M_3 antagonists that are based on the recently discovered triphenylpropioamide derivative, **1**, and have a unique amino acid spacer group is described. The introduction of a hydroxyproline-proline group to the spacer site and the use of a propyl or cyclopropylmethyl group as the piperidine *N*-substituent led to the discovery of the novel M_3 selective antagonists [**8c**, **8g**; $K_i < 2$ nM (M_3), $M_1/M_3 > 700$-fold, $M_2/M_3 > 180$-fold], which have a more rigid structure than **1**.

There are five muscarinic acetylcholine receptor subtypes (M_1–M_5) known to date.[1–5] These receptor subtypes play a number of pharmacological roles both centrally and peripherally.[6,7] For example, the M_1 receptor is located at the postganglionic cholinergic nerve terminals and glands, which facilitate neurotransmission and gastric secretion, respectively. The neuronal M_2 receptor provides functional negative feedback modulation of acetylcholine (ACh) release in addition to its role as a cardiac M_2 receptor, which regulates heart rate. The M_3 receptor is located in smooth muscle and mucosal glands, which mediate contraction and mucus secretion, respectively.[8]

Orally active muscarinic antagonists such as oxybutynin have been used for the treatment of urinary tract disorders, including urinary incontinence (UI), via blockade of the M_3 receptor. However, their subtype non-selective profiles may cause adverse effects such as a dry mouth, blurred vision, constipation, and tachycardia, which limit their clinical utility.[9] M_3 selective antagonists may reduce these adverse effects, but their advantages over the non-selective antagonists remains to be elucidated because of lack of M_3 selective antagonists. Therefore, pharmaceutical research into therapeutic agents that are selective for muscarinic receptor subtypes has focused on the exploration of M_3 selective antagonists.

As a part of our research in developing a muscarinic M_3 receptor antagonist, we discovered the novel M_3 selective antagonist, **1**, using a rationally designed combinatorial library.[10] Compound **1** possesses a novel structure that is distinct from the existing muscarinic antagonists, and it exhibits excellent M_3 binding affinity and selectivity profile as shown in Table 1. A structure activity relationship (SAR) analysis of analogues of **1** revealed that amino acid moiety plays a major role in the M_3 selectivity toward the other receptor subtypes and that particular combinations of amino acids show a high M_1/M_3 selectivity (37–550-fold), which would not be attainable with known M_3 antagonists.

To develop a better pharmacological tool for clarifying the role of the M_3 receptor, further improvements in the subtype selectivity of this novel class of M_3 antagonist were planned. However, the highly flexible structural feature of **1** might interrupt the understanding of the SAR and the binding mode in this class of compounds. Among the novel M_3 antagonists identified from the library, **2a** possess a conformationally restricted spacer (L-Pro-D-Pro) while compound **1** has a flexible spacer structure. The number of possible conformers of **2a** was predicted to be less than 0.07% of that of **1**.[11] However, **2a** had low subtype selectivity toward the other receptor subtypes, especially toward the M_2 receptor (Table 1).

*Corresponding author. Tel.: +81-298-77-2000; fax: +81-298-77-2029; e-mail: oowadayf@banyu.co.jp

0960-894X/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00843-0

Based on the above observations, we assumed that **2a** might be an alternative starting point for further chemical modifications to identify more subtype-selective compounds, if we could find the strategy to improve its subtype selectivity. Therefore, we attempted to identify compounds that are analogues of **2a** but have comparable subtype selectivity with **1**.

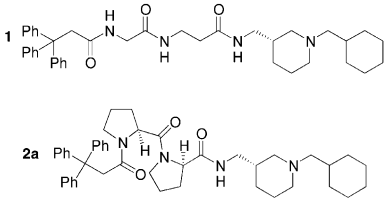
Here, we report the synthesis and SARs of the analogues of **2a** and the identification of the hydroxyproline derivatives, **8c** and **8g**, which have significantly improved M_1/M_3, M_2/M_3, and M_5/M_3 selectivity compared with **1**.

The synthesis of the representative compounds, **8**, is shown in Scheme 1. Methyl esterification of commercially available D-benzyloxycarbonylproline **3** was followed by deprotection and subsequent amidation with *N*-α-benzyloxycarbonyl-*O*-*tert*-butyl-L-4-*trans*-hydroxyproline to yield **4**. This monoamide **4** was transformed to diamide **5** by deprotection and condensation with 3,3,3-triphenylpropionic acid. Hydrolysis of **5** and subsequent coupling with (3*R*)-3-aminomethyl-1-*tert*-butoxycarbonylpiperidine afforded triamide **6**. Deprotection of both *tert*-butoxycarbonyl and *tert*-butyl of **6** by treatment with TFA afforded **7**. Finally, either reductive amination or alkylation of **7** provided the target compounds **8**.

The binding affinities of the synthesized compounds were evaluated using cloned human M_1–M_5 receptors according to a method[10] described previously, and the selectivity for M_3 toward the other receptor subtypes was examined.

In the previously reported library,[10] only a set of L-Pro and D-Pro were tested with a racemic 3-aminomethyl-1-cyclohexylmethyl piperidine core. Because additional interactions between the hydroxy group(s) on the pyrrolidine ring in **2a** and the M_3 receptor were expected, a set of hydroxyprolines were combined with the same racemic piperidine template as in the first step of the SAR study around **2a**. L-Pro and D-Pro in **2a** were initially replaced with commercially available *trans*-L-4-hydroxyproline and *trans*-D-4-hydroxyproline, respectively (Table 2).

Table 1. M_3 antagonists from the former library[10]

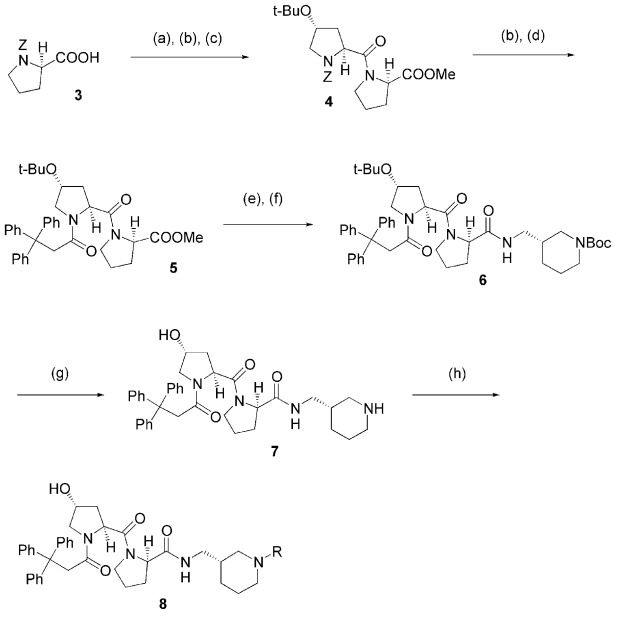


Compd	Binding affinity (K_i, nM)[a]					Selectivity			
	M_3	M_1	M_2	M_4	M_5	M_1/M_3	M_2/M_3	M_4/M_3	M_5/M_3
1	0.31	120	30	14	37	390	97	45	120
2a	0.25	14	0.82	2.3	150	56	3.3	9.2	600
Atropine	0.50	0.25	1.5	0.34	0.54	0.50	3.0	0.68	1.1

[a]Values are the mean of two or more independent assays.

The substitution of L-Pro with L-hydroxyproline (**9**) maintained the M_3 binding affinity. Interestingly, **9** showed significantly improved selectivity for the M_3 receptor toward the M_1, M_2, and M_5 receptors in comparison with **2a**, while the M_3 selectivity toward the M_4 receptor was comparable. Replacement of D-Pro with D-hydroxyproline (**10**) resulted in a 14-fold reduced affinity to the M_3 receptor and lowered selectivity toward the M_1, M_2, and M_5 receptor subtypes compared with those of **9**. The combination of L-hydroxyproline and D-hydroxyproline (**11**) resulted in a 4-fold decrease in M_3 receptor affinity, while **11** was more selective toward the M_1 and M_2, and M_5 receptors, than was **9**. These results suggest that the hydroxy group on the pyrrolidine ring of L-Pro played an important role in M_1/M_3, M_2/M_3, M_5/M_3 selectivity, but that the hydroxy group did not affect the M_4/M_3 selectivity.

Considering their M_3 affinity and selectivity, **9** was subjected to further modifications. In order to examine the role of the hydroxy group on the pyrrolidine, the (*S*)-hydroxy- (**8a**), (*R*)-hydroxy- (**12**), (*S*)-amino- (**13**), and (*R*)-amino (**14**) analogues were prepared and assayed. In these analogues, an optically active 3-(3*R*)-aminomethyl-1-cyclohexylmethyl piperidine core was used in place of the racemic one. The (*S*)-hydroxy analogue, **8a**, was 2- to 3-fold more M_3 selective than the (*R*)-hydroxy analogue **12** toward the M_1, M_2, M_5 receptors. The (*S*)-



Scheme 1. General synthesis of hydroxyproline derivative **8**. Reagents and conditions: (a) MeOH, DMAP, WSC, $CHCl_3$; (b) H_2, $Pd(OH)_2$, MeOH; (c) *N*-α-carbobenzoxy-*O*-*tert*-butyl-L-4-hydroxyproline, WSC, HOBt, $CHCl_3$; (d) 3,3,3-triphenylpropionic acid, WSC, HOBt, $CHCl_3$; (e) aq NaOH, MeOH; (f) (3*R*)-3-aminomethyl-1-*tert*-butoxycarbonylpiperidine, WSC, HOBt, $CHCl_3$; (g) TFA (neat); (h) aldehyde, $NaBCNH_3$–$ZnCl_2$, MeOH, rt; or RX, K_2CO_3, CH_3CN, heat.

amino analogue (**13**), and (*R*)-amino analogue (**14**) showed potent M_3 affinity comparable to that of **8a**. **13** had higher M_1/M_3, M_2/M_3, and M_5/M_3 selectivity than did **14**. A similar trend was observed for the hydroxy derivatives, **8a** and **12**.

These results indicate that the regio- and stereo chemistry of the hydroxy or amine group were important for the M_3 affinity and selectivity toward the M_1, M_2, and M_5 receptors and suggest that an additional hydrogen bonding interaction between these analogues and the M_3 receptor resulted in their improved binding affinity and selectivity (Table 3).

Since the M_1/M_3 selectivity of **13** was a half that of **8a**, the *trans*-L-4-hydroxyproline analogue, **8a**, was selected for further modification.

The effects of the piperidine substituents in both **2a** (spacer: L-Pro-D-Pro) and **8a** (spacer: L-hydroxyproline-D-Pro) on the M_3 affinity and selectivity were also explored, because our previous SAR study on the analogues of **1** revealed that a substituent on the piperidine nitrogen substantially affected the M_3 affinity and selectivity.[10]

Replacement of the cyclohexylmethyl group in **2a** with ethyl (**2b**), *n*-propyl (**2c**) and *n*-butyl (**2d**) improved the M_1/M_3, M_2/M_3, and M_4/M_3 selectivity but resulted in a greater than 10-fold reduction in the M_3 affinity (Table 4). A similar trend was observed for the analogues of **8a**. After the introduction of ethyl, *n*-propyl, *n*-butyl, and *n*-hexyl onto the nitrogen of piperidine, subtype selectivity greatly improved (M_1/M_3; 300–870-fold, M_2/M_3; 35–410-fold, M_4/M_3; 13–38-fold); and M_3 affinity ranged from 0.12 to 8.3 nM. Among the linear alkyl derivatives, **8c**, which has an *n*-propyl group, was the most balanced compound [K_i (M_3); 1.5 nM, M_1/M_3; 870-fold, M_2/M_3; 180-fold, M_4/M_3; 38-fold, M_5/M_3; 2300-fold].

Trends in selectivity were similar between the linear alkyl derivatives and the cycloalkylmethyl derivatives. The cyclopropylmethyl analogue, **8g**, showed the best selectivity (M_1/M_3; 700-fold, M_2/M_3; 190-fold, M_4/M_3; 36-fold, M_2/M_3; 2000-fold) among the three compounds; however, its M_3 affinity was moderate.

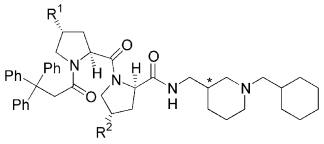
The SAR in Table 4 suggests that selective binding of these ligands to the M_3 receptor was affected by both the size of piperidine *N*-substituents and the hydroxy group at L-Pro. When the piperidine *N*-substituent was small, the resulting ligand possibly did not fit into the pockets of the receptor subtypes other than the M_3 receptor. Independently, the results of the increased selectivity when the hydroxy group was introduced to L-Pro in **2a** indicate that there was a counterpart within the M_3 receptor pocket that interacted with the hydroxy group presumably by forming a hydrogen bond.

The representative compounds, **8c** and **8g**, were subjected to evaluation of their functional antagonism using an in vitro system with rat tissues.[12] In the isolated rat trachea, **8c** and **8g** antagonized the acetylcholine (ACh)-induced muscle contractile responses effectively, with a K_B value of 1.6 and 2.7 nM, respectively. Therefore, both compound **8c** and **8g** showed an antagonistic activity comparable to their binding affinity to the M_3 receptor.

In conclusion, the SAR studies of the new triphenylpropioamide class of M_3 antagonists that were conducted to identify more subtype-selective and conformationally restricted compounds compared with **1** led to the identification of **8c** and **8g**, which have a spacer composed of L-hydroxyproline and D-Pro.

In particular, **8c** showed a K_i value of 1.5 nM for the M_3 receptor with excellent M_3 selectivity (M_1/M_3; 870-fold, M_2/M_3; 180-fold, M_4/M_3; 38-fold, and M_5/M_3; 2300-fold), and **8c** was 2-fold more selective than **1** in terms of the M_1/M_3 and M_2/M_3 selectivity. Furthermore, since **8c** possesses a decreased conformational flexibility as mentioned above, this compound may be a more useful template for understanding of the binding mode of this triphenylpropioamide class of compounds. Further SAR studies around the unique hydroxyproline-proline analogues are in progress.

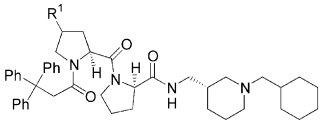
Table 2. Effects of the introduction of a hydroxy group to the prolines[a]



No.	R^1	R^2	Binding affinity (K_i, nM)[b]					Selectivity			
			M_3	M_1	M_2	M_4	M_5	M_1/M_3	M_2/M_3	M_4/M_3	M_5/M_3
9	OH	H	0.17	48	2.7	1.4	220	280	16	8.2	1300
10	H	OH	2.4	170	11	29	1600	71	4.6	12	670
11	OH	OH	0.75	260	21	9.6	1700	350	28	13	2300

[a]Compounds **9**–**11** are racemic at 3-aminomethyl-1-cyclohexylmethyl piperidine.
[b]Values are the mean of two or more independent assays.

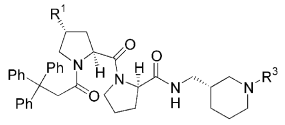
Table 3. Stereochemistry of hydroxy and amine groups at L-proline



No.	R^1	Binding affinity (K_i, nM)[a]					Selectivity			
		M_3	M_1	M_2	M_4	M_5	M_1/M_3	M_2/M_3	M_4/M_3	M_5/M_3
8a	HO	0.13	18	2.1	0.81	130	140	16	6.2	1000
12	HO	0.34	27	1.5	3.6	230	79	4.4	11	680
13	H_2N	0.13	9.5	1.9	1.3	130	73	15	10	1000
14	H_2N	0.17	9.0	0.50	1.9	82	53	2.9	11	480

[a]Values are the mean of two or more independent assays.

Table 4. Effects of piperidine *N*-substituent on receptor binding and selectivity



No.	R^1	R^3	Binding affinity (K_i, nM)[a]					Selectivity			
			M_3	M_1	M_2	M_4	M_5	M_1/M_3	M_2/M_3	M_4/M_3	M_5/M_3
15	H	H	99	2500	3100	1600	5000	25	31	16	51
2b	H	Ethyl	37	>2500	2600	970	>5100	>68	70	26	>140
2c	H	*n*-Propyl	6.9	800	150	220	4500	120	22	32	650
2d	H	*n*-Butyl	2.6	200	26	61	1500	77	10	23	580
2a	H	Cyclohexylmethyl	0.25	14	0.82	2.3	150	56	3.3	9.2	600
7	OH	H	23	>2500	6300	740	>5000	>110	270	32	>220
8b	OH	Ethyl	8.3	>2500	3400	270	>5000	>300	410	33	>600
8c	OH	*n*-Propyl	1.5	1300	270	57	3500	870	180	38	2300
8d	OH	*n*-Butyl	0.60	290	45	13	1000	480	75	22	1700
8e	OH	*n*-Hexyl	0.12	44	4.2	1.5	150	370	35	13	1300
8f	OH	*n*-Octyl	0.34	27	1.5	3.6	230	79	4.4	11	680
8g	OH	Cyclopropylmethyl	2.0	1400	380	72	4000	700	190	36	2000
8a	OH	Cyclohexylmethyl	0.13	18	2.1	0.81	130	140	16	6.2	1000
8h	OH	Cyclooctylmethyl	0.13	7.9	0.82	0.30	37	61	6.3	2.3	280

[a]Values are the mean of two or more independent assays.

Acknowledgements

We are grateful to Ms. Nami Sakaizumi, Ms. Sachie Arai-Otsuki, and Ms. Minaho Uchiyama for technical support and Ms. Jocelyn Winward, Merck & Co., Inc., for her assistance in preparation of the manuscript.

References and Notes

1. Kubo, T.; Fukuda, K.; Mikami, A.; Maeda, A.; Takahashi, H.; Mishina, M.; Haga, T.; Haga, K.; Ichiyama, A.; Kanagawa, K.; Kojima, M.; Matsuo, H.; Hirose, T.; Numa, S. *Nature* **1986**, *323*, 411.
2. Kubo, T.; Maeda, A.; Sugimoto, K.; Akiba, I.; Mikami, A.; Takahashi, H.; Haga, T.; Haga, K.; Ichiyama, A.; Kanagawa, K.; Matsuo, H.; Hirose, T.; Numa, S. *FEBS Lett*. **1986**, *209*, 367.
3. Peralta, E. G.; Ashkenazi, A.; Winslow, J. W.; Smith, D. H.; Ramachandran, J.; Capon, D. J. *EMBO J*. **1987**, *6*, 3923.
4. Bonner, T. I.; Buckley, N. J.; Young, A. C.; Brann, M. R. *Science* **1987**, *237*, 527.
5. Bonner, T. I.; Young, A. C.; Brann, M. R.; Buckley, N. J. *Neuron* **1988**, *1*, 403.
6. Hulme, E. C.; Birdsall, N. J. M.; Buckley, N. J. *Ann. Rev. Pharmacol. Toxicol*. **1990**, *30*, 633.
7. Caulfield, M. P. *Pharmacol. Ther*. **1993**, *58*, 319.
8. The precise physiological roles of the M_4 and M_5 receptors remain to be elucidated. See: (a) Gomeza, J.; Zhang, L.; Kostenis, E.; Felder, C.; Bymaster, F.; Brodkin, J.; Shannon, H.; Xia, B.; Deng, C.; Wess, J. *Proc. Natl. Acad. Sci. U.S.A*. **1999**, *96*, 10483. (b) Gomeza, J.; Zhang, L.; Kostenis, E.; Felder, C. C.; Bymaster, F. P.; Brodkin, J.; Shannon, H.; Xia, B.; Duttaroy, A.; Deng, C.; Wess, J. *Life Sci*. **2001**, *68*, 2457. (c) Bymaster, F. P.; Carter, P. A.; Zhang, L.; Falcone, J. F.; Stengel, P. W.; Cohen, M. L.; Shannon, H. E.; Gomeza, J.; Wess, J.; Felder, C. C. *Life Sci*. **2001**, *68*, 2473. (d) Eglen, R. M.; Nahorski, S. R. *Br. J. Pharmacol*. **2000**, *130*, 13.
9. Eglen, R. M.; Choppin, A.; Dillon, M. P.; Hegde, S. *Curr. Opin. Chem. Biol*. **1999**, *3*, 426.
10. Sagara, Y.; Sagara, T.; Mase, T.; Kimura, T.; Numazawa, T.; Fujikawa, T.; Noguchi, K.; Ohtake, N. *J. Med. Chem*. **2002**, *45*, 984.
11. A systematic search was performed on the amino acid spacer site of **1** and **2a** employing the conformational search functionality of SYBYL. (SYBYL Molecular Modeling Package, version 6.8; Tripos, Inc., St. Louis, MO, USA). Increments of bond rotation were set to 30, 10 and 180° for all rotatable single bonds, ring bonds and an amide bond, respectively, and the van der Waals bump coefficient was set at 0.95 to eliminate unfeasible conformations suffered from van der Waals repulsion. The number of predicted conformers were 97,410 and 64 for **1** and **2a**, respectively.
12. Hirose, H.; Aoki, I.; Kimura, T.; Fujikawa, T.; Numazawa, T.; Sasaki, K.; Sato, A.; Hasegawa, T.; Nishikibe, M.; Mitsuya, M.; Ohtake, N.; Mase, T.; Noguchi, K. *J. Pharmacol. Exp. Ther*. **2001**, *297*, 790.