Brief Articles

Cyclohexylmethylpiperidinyltriphenylpropioamide: A Selective Muscarinic M_3 Antagonist Discriminating against the Other Receptor Subtypes

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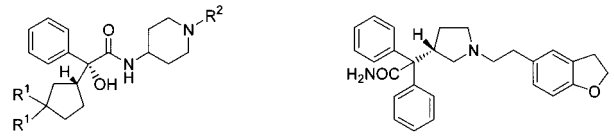
To discover a highly selective M_3 antagonist, a combinatorial library was prepared. The library was designed to identify a novel structural class of M_3 antagonists by exploring the spatial arrangement of the pharmacophores in known M_3 antagonists. After the evaluation of 1000 library members, a potent M_3 antagonist, **14a** (K_i = 0.31 nM), with novel structural features was identified. Compound **14a** showed high selectivity for M_3 receptors over the other muscarinic receptor subtypes (M_1/M_3 = 380-fold, M_2/M_3 = 98-fold, M_4/M_3 = 45-fold, M_5/M_3 = 120-fold).

Introduction

Acetylcholine (ACh) plays a number of pharmacological roles that are mediated by nicotinic and muscarinic receptors in central and peripheral nervous systems. Five muscarinic receptors (m1−m5) have been identified so far that mediate muscarinic functions.[1] These muscarinic receptors are homologous across receptor subtypes as well as across mammalian species[2] and are expressed predominantly in the parasympathetic nervous system. Although these receptors, with the exception of m5, have been proposed to participate in a number of physiologic functions, the roles of each receptor subtype in the specific muscarinic actions of ACh remain to be elucidated. For further characterization of the functions of each receptor, specific antagonists or agonists would be useful.

We have been interested in M_3 receptors from their pharmacological properties: the M_3 receptor subtype is homologous to other subtypes, M_1, M_2, M_4, and M_5,[3] and has been postulated to facilitate the parasympathetic stimulation of smooth muscle contraction and glandular secretion in the peripheral system.[4] It is widely expressed in the brain;[5] however, the physiologic roles remain unknown. A recent report demonstrated that M_3-deficient mice were hypophagic and lean, suggesting a role of central M_3 receptors in orexigenic activity.[6] To investigate the roles of peripheral and central M_3 receptors, selective and effective M_3 antagonists were explored.

Although extensive synthetic efforts have been devoted to discover selective M_3 antagonists, none of them have identified any compounds with sufficient selectivity toward M_3 receptors.[2,7] To identify a potent and selective M_3 receptor antagonist, we adopted a strategy using combinatorial chemistry that would effectively yield a series of structurally diverse antagonists. For the library design, we paid attention to spatial orientation and arrangement of the pharmacophores for M_3 antagonists. An increase in the flexibility of the spatial arrangement of the molecules may lead to more specific recognition of the binding site of M_3 receptors over those of the other receptor subtypes. Here, we report the design and synthesis using combinatorial chemistry and identify a new structure class of M_3 receptor antagonists. Subsequent derivation of this class provided a cyclohexylmethylpiperidinyltriphenylpropioamide (CPTP, **14a**), which showed potent binding affinity (K_i = 0.31 nM) to M_3 receptors and was 380-, 98-, 45-, and 120-fold more selective for M_3 over the M_1, M_2, M_4, and M_5 receptors, respectively.



1 R^1 = H, R^2 = 4-methyl-3-pentenyl

2 R^1 = F, R^2 = 6-aminopyridin-2-ylmethyl

Darifenacin

Figure 1. Known M_2-sparing M_3 antagonists.

Design and Synthesis

Analysis of the structural features of the known nonselective muscarinic antagonists and M_2-sparing M_3 antagonists such as **1**, **2**, and darifenacin[8] (Figure 1) suggested that a bulky aromatic cluster, a cationic nitrogen-containing part, and a hydrophobic part neighboring the nitrogen atom as the *N*-substituent are essential pharmacophores for M_3 potency. We postulated that new structure class of muscarinic antagonists with an increase in the molecular size and flexibility of the spatial arrangement of the known antagonists could lead to more specific recognition of the binding site of M_3 receptors over those of the other receptor subtypes. On the basis of this prediction, we designed muscarinic antagonists by incorporating a diverse spacer group between the aromatic cluster and the cationic site

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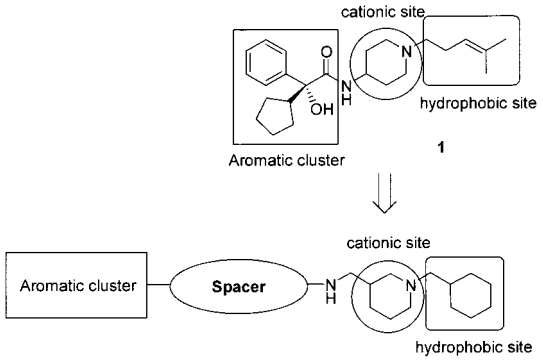
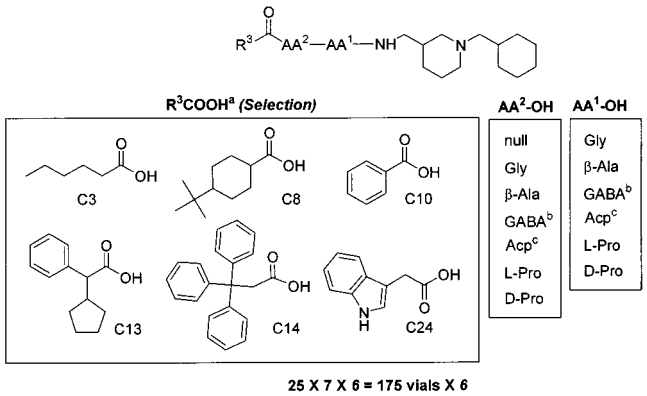


Figure 2. Design of a new class of muscarinic antagonists.

Scheme 1. Design of a Combinatorial Library[a]



[a] R^3COOH: structures of all 25 carboxylic acids are available in Supporting Information. [b]GABA: 4-aminobutyric acid. [c]Acp: 6-aminohexanoic acid.

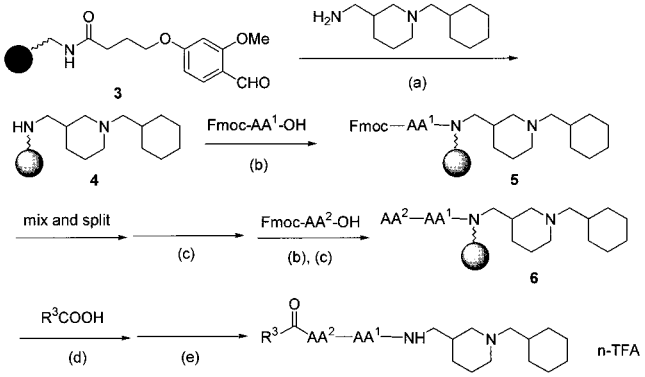
through a combinatorial chemistry approach. Simultaneously, optimization of the aromatic cluster was performed (Figure 2).

A library was constructed as follows (Scheme 1): Two variable sites were defined (AA^1 and AA^2) as the spacer moiety, and commercially available amino acids were incorporated into each site. Natural and unnatural amino acids with various sizes, including cyclic amino acids such as proline, were selected to increase the diverse display of conformations. A combination of these amino acids varied the size of the spacer widely, from three atoms (composed of null and glycine) to 14 atoms (composed of double 6-aminohexanoic acids).

The cationic tertiary amine core including a hydrophobic *N*-substituent was tentatively fixed to a racemic 1-cyclohexylmethyl-3-aminomethylpiperidine. With respect to the terminal acyl moiety, 25 commercially available carboxylic acids including aromatic, heteroaromatic, cycloalkyl, and acyclic carboxylic acids were selected.[9]

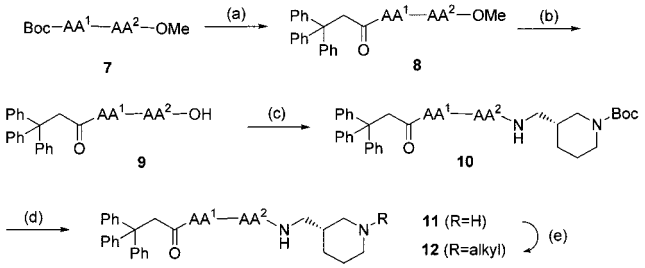
The construction of the library was performed using a (4-formyl-3-methoxyphenoxy)alkyl resin **3**,[10] which has been reported to give products with excellent purity[11] (Scheme 2). First, an aminomethyl piperidine core was introduced on the *o*-formyl resin by reductive alkylation,[12] and then spacer parts were condensed in order by the following two steps: The resultant amine **4** was acylated with the first spacer group (six Fmoc amino acid derivatives) by a standard PyBOP-DIEA method[13] to give six pools of **5**. After the six pools were mixed and split into seven groups, their Fmoc groups were removed by a standard protocol, and a second spacer group (Fmoc AA^2-OH; six amino acids and one deletion) was introduced by the same procedure. Finally each group of seven **6** was further split into 25 subgroups and coupled with 25 carboxylic acids using a WSC-HOBt method. The number of mixtures (six mixtures per vial) was chosen after several attempts for ease of product identification in ESI-MS spectra and for reducing the risk of producing false positive results in the binding assay. Finally, the products were cleaved from the resin by treatment with 80% TFA/DCM (containing 1% H_2O). In total, 175 vials and 1050 compounds (6 mixtures × 175 vials) were synthesized, and all samples were subjected to HPLC and mass spectral analysis. In most cases, the target compounds were produced in more than 85% purity, and all exhibited major peaks corresponding to the correct molecular ions in the ESI mass spectra.

Scheme 2. Library Synthesis[a]



[a] Reagents and conditions: (a) $NaBH(OAc)_3$, DCM; (b) PyBOP, DIEA, DMF; (c) 20% piperidine−DMF; (d) WSC, HOBt, DMF; (e) 1% H_2O−19% DCM−TFA.

Scheme 3. General Synthetic Method of the Selected Compounds[a]



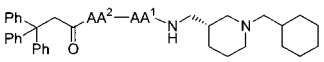
[a] Reagents: (a) (1) 10% HCl−MeOH, rt, (2) $Ph_3CCH_2CO_2H$, WSC, HOBt, NEt_3, $CHCl_3$, rt; (b) 4 N NaOH, MeOH, $CHCl_3$, rt; (c) (3*R*)-3-aminomethyl-1-*tert*-butoxycarbonylpiperidine, WSC, HOBt, $CHCl_3$, rt; (d) TFA, $CHCl_3$, rt; (e) aldehyde, $NaBCNH_3$−$ZnCl_2$, MeOH, rt.

The general synthetic method of the selected compounds (**13a**−**20a**) is highlighted in Scheme 3. An aminoester derived from **7** was condensed with 3,3,3-triphenylpropionic acid under the usual conditions to produce a diamide **8**. Hydrolysis of the ester moiety in **8** and subsequent coupling with an optically active 1-Boc-3-aminomethylpiperidine[14] yielded a triamide **10**. Finally, deprotection of the Boc group followed by reductive alkylation using an appropriate aldehyde and $NaBCNH_3$−$ZnCl_2$[15] produced a target product **12**.[16]

Results and Discussion

The primary screening of the library was performed by examining inhibition of [^{3}H]-NMS binding (1 μM) to

Table 1. Binding Affinities of the Selected Compounds to Human Muscarinic Receptor Subtypes



			binding affinity (K_i, nM)[a]					selectivity			
no.	AA^2	AA^1	m3	m1	m2	m4	m5	m1/ m3	m2/ m3	m4/ m3	m5/ m3
13a	null	Acp	1.0	110	72	30	210	110	72	30	200
14a	Gly	β-Ala	0.31	120	30	14	37	380	98	45	120
15a	Gly	GABA	3.3	300	270	74	1100	90	82	22	320
16a	β-Ala	β-Ala	0.76	36	44	31	30	48	58	41	40
17a	GABA	β-Ala	2.1	77	17	60	200	37	8.2	29	95
18a	L-Pro	Gly	2.2	150	13	43	2000	66	5.7	19	890
19a	L-Pro	β-Ala	0.27	150	12	11	58	550	45	42	210
20a	L-Pro	D-Pro	0.25	14	0.82	2.3	150	54	3.3	9.0	580
Atropine			0.50	0.25	1.5	0.34	0.54	0.96	2.9	1.3	3.3
Darifenacin			0.84	5.5	47	8.6	2.3	6.5	56	10	2.7

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

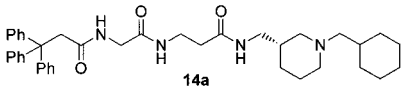
human m3 receptors expressed in CHO cells. Then the selected compounds were examined for their binding affinities (K_i values) to each of the five receptor subtypes (m1−m5).[7e] After evaluation of whole library members, only the vials derived from triphenylpropionic acid in R^3 position showed remarkable inhibition (>70% inhibition).[9] The hit vials were further examined by iterative deconvolution process including chiral resolution in the 3-aminomethylpiperidine moiety and proline spacers with respect to their affinity for m3 receptors and selectivity over the other receptor subtypes.[17]

As a result, (3*R*)-3-aminomethylpiperidine derivatives with particular combination of amino acid spacers were found to show highly potent M_3 binding affinities and moderate-to-high selectivities for m3 over m1 and m2 receptors (Table 1). Among them, compounds **13a**, **14a**, and **19a** showed greater than 100-fold m1/m3 selectivity, which was the highest m1/m3 selectivity reported to date. Especially, **14a** showed sub-nanomolar binding affinity for m3 receptors and excellent subtype selectivity over all the other receptor subtypes.

As shown in Table 1, the spacer structures of this class of compounds were found to significantly affect their selectivities for m3 over m1 and m2 receptors. For example, deletion of the amide moiety (**13a**) in the middle of the spacer group in **14a** resulted in a 3-fold decrease in both the m3 binding affinity and m1/m3 selectivity as compared to **14a**, while **13a** showed almost similar m2/m3 selectivity. Replacement of the glycyl moiety in **14a** with a β-alanyl group (**16a**) led to moderate m2/m3 selectivity, while replacement with a γ-aminobutyryl group (**17a**) resulted in almost complete loss of m2/m3 selectivity. Replacement of the β-alanyl group in **14a** with a γ-aminobutyryl group (**15a**) also resulted in a 10-fold reduction in the m3 binding affinity with a 4-fold decrease in m1/m3 selectivity, suggesting that the amide moiety in the middle of the spacer moiety in **14a** played an important role in enhancing m3 affinity and m1/m3 selectivity. In contrast, replacement of the glycyl moiety with a L-prolyl group (**19a**) produced enhanced m1/m3 selectivity (550-fold), despite decreased m2/m3 selectivity (45-fold), when compared with **14a**.

As the piperidine *N*-substituent was tentatively fixed to the cyclohexylmethyl moiety in the library, we examined the effect of the substituent on **14a** to find that cyclohexylmethyl gave the best result from the viewpoint of both m3 affinity and the selectivities.[18]

Table 2. In Vivo Selectivity of **14a** in Rats



14a

	ED_{50} (μg/kg, iv)				
	broncho-constriction M_3	bradycardia M_2	pressor response M_1	selectivity M_1/M_3	selectivity M_2/M_3
14a	15	1550	>3000	>200	100
Atropine	4.3	5.2	16	3.7	1.2

Although the detailed interaction of the compounds of this class and muscarinic receptors is not clarified, we speculate that the newly introduced spacer moieties may allow the acyl part, triphenylpropionamide, to interact with muscarinic receptors in a significantly different binding mode from those of the known M_3 antagonists.

Compound **14a** was subjected to evaluation of its functional selectivity using an in vitro system with rat tissues.[7e] In the isolated rat trachea, this compound effectively antagonized the acetylcholine (ACh)-induced responses, with a K_B value of 2.3 nM.[19] In the isolated rat atria, this compound showed less potent inhibition of carbachol-induced bradycardia, with a K_B value of 160 nM. These results indicated that the functional selectivity of this compound was 70-fold greater for tracheal M_3 receptors over cardiac M_2 receptors in rat, which was consistent with the selectivity observed in the binding assay.

The in vivo selectivity of **14a** was next examined in rat assay systems (Table 2). Intravenous administration of **14a** dose-dependently inhibited ACh-induced bronchoconstriction, with an ED_{50} value of 0.015 mg/kg in rats. In contrast, intravenous administration of **14a** up to 3 mg/kg did not affect the McN A-343-induced pressor response that is thought to be mediated by M_1 receptors.[20] Furthermore, **14a** inhibited ACh-induced bradycardia (M_2), with an ED_{50} value of 1.55 mg/kg after intravenous administration. These results may suggest that **14a** show greater than 200- and 100-fold selectivity for M_3 over M_1 and M_2 receptors in these assay systems, although its ADME properties were not elucidated.

Conclusion

We designed diverse classes of muscarinic antagonists using a combinatorial library approach based on the hypothesis that increases in the molecular size and conformational flexibility of the molecules produced more specific binding with m3 receptors than with the other receptor subtypes. We have found that 3,3,3-triphenylpropionamide derivatives with one or two amino acid residues between the triphenylpropionic acid moiety and the piperidinylmethylamine moiety showed potent binding affinities for m3 receptors with moderate-to-high subtype selectivities. Particularly, **14a** showed sub-nanomolar m3 affinity and high selectivity over the other receptor subtypes. Compound **14a** also showed high subtype selectivity (70-fold) for tracheal M_3 over cardiac M_2 receptors in the isolated rat tissues. Furthermore, **14a** showed greater than 100-fold in vivo

selectivity for M_3 over M_1 and M_2 receptors in the above rat assay systems. Therefore, **14a** would be useful as a pharmacological tool to clarify the roles of peripheral and central M_3 receptors. Elucidating the roles of central M_3 receptors that might be involved in food intake would be of particular interest.

Experimental Section

***N*-(2-[3-([(3*R*)-1-(Cyclohexylmethyl)-3-piperidinyl]methylamino)-3-oxopropyl]amino-2-oxoethyl)-3,3,3-triphenylpropioamide 14a.** To a solution of **14b**[18] (270 mg, 0.513 mmol) and cyclohexanecarbaldehyde (75 μL, 0.616 mmol) in MeOH (3 mL) was added $NaBH_3CN-ZnCl_2$ (0.3 mol/L, 3.1 mL),[15] and the mixture was stirred at room temperature for 40 min. The reaction was quenched by adding saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography ($CHCl_3$−MeOH, 20:2−20:3 elution) to give **14a** (261 mg, 0.419 mmol, 82%) as a white solid: mp 143.5−145.0 °C ($Et_2O-CHCl_3$); HPLC t_R = 23.7 min (system A), 17.2 min (system B); ^{1}H NMR ($CDCl_3$) δ 0.81−1.80 (m, 17H), 1.97−2.08 (m, 3H), 2.28−2.32 (m, 2H), 2.59−2.65 (m, 2H), 3.12−3.17 (m, 2H), 3.37−3.46 (m, 2H), 3.51−3.55 (m, 2H), 3.64 (s, 2H), 5.60−5.64 (m, 1H), 6.10−6.20 (m, 1H), 6.26−6.30 (m, 1H), 7.19−7.33(m, 15H). HRMS Calcd for $C_{39}H_{51}N_4O_3$ $(M + H)^+$: 623.3961. Found: 623.3967. Anal. Calcd for $C_{39}H_{50}N_4O_3$: C, 75.21; H, 8.09; N, 9.00. Found: C, 74.96; H, 8.26; N, 8.92.

Binding Assay. According to the reported method,[7e] the binding affinities were determined by inhibition of specific binding of [^{3}H]-NMS using membranes from CHO cells expressing cloned human m1−m5 receptors.

In Vivo Assay. According to the reported method,[7e] bronchoconstriction and bradycardia were evaluated. See Supporting Information for the method of pressor evaluation.

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Supporting Information Available: Detailed experimental procedures, characterization data for all new compounds, a list of all 25 carboxylic acids, a result of a primary screening of the library, and a table of the effect of piperidine substituents on **14a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) All new compounds were characterized by ^{1}H NMR, RP-HPLC, and mass spectra.

(17) The detailed discussion on the deconvolution process will be reported elsewhere.

(18) Linear chains (from ethyl to *n*-octyl), cyclooctylmethyl, cyclobutylmethyl, and no substituent were examined. (See Supporting Information.)

(19) The pA_2 value of **14a** in the rat trachea was 8.46.

(20) Evaluation using a selective M_1 agonist may be necessary, because McN A-343 is a partial agonist.

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