Discovery of 2-Aminothiazole-4-carboxamides, a Novel Class of Muscarinic M_3 Selective Antagonists, through Solution-Phase Parallel Synthesis

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Synthesis and structure–activity relationship of a new class of muscarinic M_3 selective antagonists were described. In the course of searching for a muscarinic M_3 antagonist with a structure distinct from those of the 2-(4,4-difluorocyclopentyl)-2-phenylacetamide derivatives, we identified a thiazole-4-carboxamide derivative (1) as a lead compound in our in-house chemical collection. Since this compound (1) showed relatively low binding affinity (K_i=140 nM) for M_3 receptors in the human binding assays, we tried to improve its potency and selectivity for M_3 over M_1 and M_2 receptors by derivatization of 1 through a combinatorial approach. A solution-phase parallel synthesis effectively contributed to the optimization of each segment of 1. Thus, we have identified a cyclooctenylmethyl derivative (3e) and a cyclononenylmethyl derivative (3f) as representative M_3 selective antagonists in this class.

Key words muscarine M_3 receptor; antagonist; 2-aminothiazole-4-carboxamide; parallel synthesis

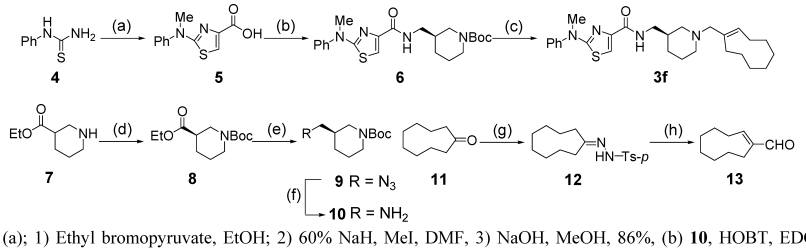
To date, five distinct but homologous gene sequences coding for muscarinic receptors (m1, m2, m3, m4, m5) have been identified and cloned.[1—5] Pharmacologically, four subtypes (M_1, M_2, M_3, M_4) have been defined.[6—8] Among these muscarinic receptor subtypes, M_3 receptors are localized in smooth muscle and mucosal glands and mediate contraction and mucus secretion. M_1 receptors, localized to the post-ganglionic cholinergic nerve terminals and glands, facilitate neurotransmission and gastric secretion. Neuronal M_2 receptors provide a functional negative feedback modulation of acetylcholine (ACh) release.[9,10] Extensive efforts have been directed to the identification of potent and subtype-selective M_3 antagonists to complete the classification of the receptor subtypes and to provide more ideal therapeutic agents,[11—13] however, few structure classes with sufficient M_3 selectivity have been discovered to date.[14]

As a part of our program for developing a muscarinic M_3 receptor antagonist for the treatment of pulmonary or urinary diseases, we pursued M_3 antagonists that are structurally distinct from a series of 2-(4,4-difluorocyclopentyl)-2-phenylacetamide derivatives such as Compound **A**[13] and have selectivity for M_3 receptors two orders of magnitude greater than those for M_1 and M_2 receptors. As a result of screening of our in-house chemical collection, a thiazole-4-carboxamide derivative (**1**) was identified as a new lead structure. Avoiding the structural complexity of **1** due to the five chiral centers, we first replaced the perhydronaphtylmethyl moiety with a naphtylmethyl group, without regard for the binding affinity of the compound. Optimization of the compound (**2**) by using a solution-phase parallel synthesis method led us to the identification of M_3 selective antagonists (**3e**, **f**) showing high potency (K_i=*ca.* 1 nM) for M_3 receptors and greater than 100-fold selectivity for M_3 over M_1 and M_2 receptors.

In this paper, we describe the synthesis of aminothiazole derivatives, their binding affinities for M_1—M_3 receptors in the binding assay, and their selectivity for M_3 over M_1 and M_2 receptors, and discuss their structure–activity and structure–selectivity relationships.

Chemistry

Preparation of **3f** was outlined in Chart 1 as a representative procedure for the series of 2-aminothiazole-4-carboxamide derivatives. The 2-aminothiazole-4-carboxylic acid (**5**) was derived from a thiourea (**4**) and ethyl 2-bromopyruvate by a conventional method in 86% yield. The (3*S*)-3-aminomethylpiperidine (**10**), a component of **3f**, was prepared from a racemic ethyl nipecotate (**7**).[15] Optical resolution of ethyl nipecotate was performed using a standard method using L-tartaric acid to give a (3*R*)-ethyl nipecotate (**8**). Following the reduction of **8** with LAH, the piperidine



Reagents: (a); 1) Ethyl bromopyruvate, EtOH; 2) 60% NaH, MeI, DMF, 3) NaOH, MeOH, 86%, (b) **10**, HOBT, EDCI, $CHCl_3$, quant., (c); 1) HCl, MeOH, 2) **13**, $NaBH(OAc)_3$, AcOH, THF, 76%, (d) Optical resolution, (e); 1) LAH, THF, 2) Boc_2O, THF, 3) MsCl, NEt_3, EtOAc, 4) NaN_3, DMF, 69%, (f) H_2, 10% Pd-C, MeOH, quant., (g) NH_2NHTs-*p*, HCl, MeOH, 80%, (h) *n*BuLi, THF-TMEDA, −78°C, then DMF, 71%.

Chart 1

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nitrogen was protected as a *tert*-butylcarbamate to afford the alcohol, which was converted to an azide (**9**) *via* the mesylate in 69% yield from **8**. **9** was hydrogenated to produce the amine (**10**) in quantitative yield. Coupling of the acid (**5**) with **10** was achieved using a standard protocol (EDCI and HOBT) to give an amide (**6**). Deprotection of the Boc group in **6** under acidic conditions, followed by reductive alkylation by treatment with an aldehyde (**13**)[16] in the presence of $NaBH(OAc)_3$ afforded **3f** in 76% yield.

Results and Discussion

Compounds prepared by a solution-phase parallel synthesis were tested in an initial screen to assess the percentage of inhibition at 1 μM in the binding assay for the muscarinic M_3 receptor subtype in transfected CHO cells.[17] Selected compounds showing greater than 50% inhibition at 1 μM were subsequently purified or re-synthesized and tested in the binding assay for muscarinic receptor subtypes (M_1, M_2, M_3) to determine the K_i values[18] and subtype selectivity (M_1/M_3, M_2/M_3).

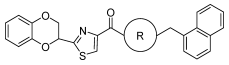
Before applying the solution-phase parallel synthesis for optimization of **2**, we prepared the three compounds (**14**—**16**) and tested their binding affinity to examine the necessity of the asymmetric carbon on the piperidine ring of **2**. Of them, compound (**14**) bearing a (*3S*)-piperidine moiety clearly showed the best binding and selectivity profiles (Table 1).

Thus, the (3*S*)-piperidine part being fixed, we tried to replace the 1,4-benzodioxane moiety of **14** with various functional groups using parallel synthesis (Table 2). Among 26 kinds of substituents introduced into the 2 position (R^1) on the aminothiazole ring, only an *N*-methylphenylamino group (**14a**) showed inhibitory activity comparable to **14**. The K_i values of **14a** for the M_1, M_2 and M_3 receptor subtypes were confirmed to be 1200, 54000 and 230 nM, respectively, and the selectivity for M_3 over the M_1 and M_2 receptors was 5- and 230-fold, respectively.

Comparison of these binding data with those of **14** indicated that an *N*-methylphenylamino group played an important role in improving the selectivity for M_3 over M_2 receptors, while this moiety did not contribute to enhancement of the M_3 binding affinity. Thus, we selected an *N*-methyl-*N*-phenylamino group as the optimized R^1 segment.

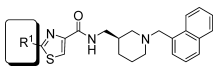
Next, we tried to optimize the naphtylmethyl moiety of **14a**, which was tentatively introduced into the piperidine nitrogen to avoid the complexity of the stereocenters of the perhydronaphthyl group in **1**, by substituting this moiety with various aromatic or cycloalkyl groups (Table 3). In this case, compounds were screened by the percentage of inhibition for M_3 receptors at 0.1 μM. Two substituents, a cyclohexylethyl and a cyclooctylmethyl group, seemed to be most effective in enhancing the binding affinity among 23 kinds of functional groups. Evaluation of the K_i values of the two compounds (**3a**, **b**) for the three receptor subtypes indicated that **3b** with a cyclooctylmethyl moiety displayed more potent activity (K_i=20 nM) for M_3 receptors than **3a**. Also, **3b** had better selectivity for M_3 over M_2 receptors (M_2/M_3=74). Therefore, further optimization of the R^2 segment in **3b** was conducted by replacing the cyclooctylmethyl moiety with larger ring-sized cycloalkylmethyl groups such as a cyclononyl- and cyclodecylmethyl groups (Table 4).

Table 1. The Binding Affinity of the Compounds (**14**—**16**) for M_3 Receptors and the Selectivity for M_3 over M_1 and M_2 Receptors



	R	M_3 (K_i, nM)	Selectivity M_1/M_3	Selectivity M_2/M_3
14		250	4.6	55
15		1100	1.3	25
16		1600	1.7	27

Table 2. Percent Inhibition of Compounds at 1 μM to M_3 Receptors



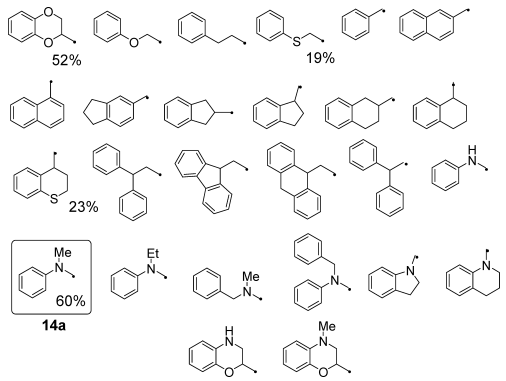
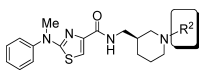
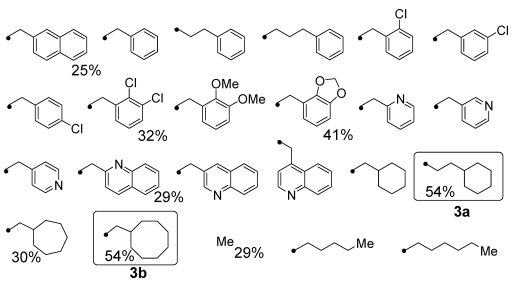
R^1	% inhibition at 1 μM
	52%, 19%, 23%, 60% (**14a**)

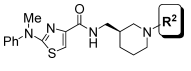
Table 3. Percent Inhibition of Compounds at 0.1 μM to M_3 Receptors



R^2	% inhibition at 0.1 μM
	25%, 32%, 41%, 29%, 54% (**3a**), 30%, 54% (**3b**), 29%

Replacement with a cyclononylmethyl group (**3c**) resulted in enhancement of the M_3 binding affinity to some extent, while the selectivity for M_3 over M_1 and M_2 receptors was maintained. Introduction of a cyclodecylmethyl group (**3d**) dramatically improved the binding affinity and selectivity for M_3 over M_2 receptors. In the process of identification of the 2-cyclopentyl-2-phenylacetamide derivatives,[17] we found that installment of a double bond into the piperidinyl side chain was effective in enhancing M_3 binding affinity. Therefore, we prepared cycloalkenylmethyl derivatives (**3e**, **f**)

Table 4. The Binding Affinity of the Compounds (**3a**—**f**, **14a**) for M_3 Receptors and the Selectivity for M_3 over M_1 and M_2 Receptors



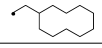
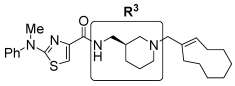
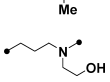
Compd.	R^2	M_3 (K_i, nM)	Selectivity M_1/M_3	Selectivity M_2/M_3	Compd.	R^2	M_3 (K_i, nM)	Selectivity M_1/M_3	Selectivity M_2/M_3
3a		52	33	32	**3e**		1.1	110	560
3b		20	34	74	**3f**		0.92	110	310
3c		10	42	93	**14a**		230	5.3	230
3d		1.0	64	200					

Table 5. The Binding Affinity of the Compounds (**3g**—**m**) for M_3 Receptors and the Selectivity for M_3 over M_1 and M_2 Receptors



Compd.	R^3	M_3 (K_i, nM)	Selectivity M_1/M_3	Selectivity M_2/M_3	Compd.	R^3	M_3 (K_i, nM)	Selectivity M_1/M_3	Selectivity M_2/M_3
3g		0.92	110	310	**3k**		1200	1.0	3.4
3h		28	33	8.6	**3l**		19	31	110
3i		420	2.6	9.5	**3m**		8.3	110	290
3j		210	5.0	11					

and evaluated their binding affinities. As expected, the cyclooctenylmethyl derivative (**3e**) displayed more potent binding activity and much better selectivity for M_3 over M_1 and M_2 receptors than the corresponding cyclooctylmethyl derivative (**3b**). Similarly, the cyclononenylmethyl derivative (**3f**) was 10-fold more active and approximately 3-fold more selective for M_3 over M_2 receptors than the cyclononylmethyl derivative (**3c**).

Further derivatization of **3f** was performed for the optimization of the R^3 segment (Table 5). These derivatizations indicated that the distance between the amide carbonyl and cationic amine parts, and a suitable structural rigidity as the R^3 segment were important for the M_3 binding affinity. Based on these modifications, the (3*S*)-3-aminomethylpiperidine moiety was an optimal R^3 segment. It is interesting to note that although both **3f** and Compound **B** (Fig. 1)[14] have excellent M_3 potency and selectivity for M_3 over M_1 and M_2 receptors, each of them has a different stereochemical structure in terms of the 3-aminomethylpiperidine portion. One of the reasons we assume is that the spatial arragangement of the whole molecule plays a key role in the binding interaction between the M_3 receptors and the antagonists. On the other hand, Compound **A** (Fig. 1) with a conservative structure had a good potency for M_3 recptors, but was only selective for M_3 over M_2 receptors. Comparison of the structure of Compound **A** with those of the M_3 selective antagonists (**3f** and Compound **B**) suggests that a larger size of the acid part or the cationic amine side chain would contribute to the

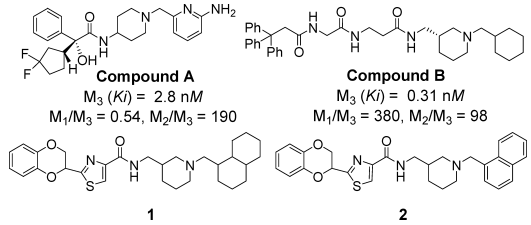


Fig. 1. Structures of Representative Compounds

enchnced selectivity for M_3 over M_1 receptors.

In conclusion, we have succeeded in identifying a new class of M_3 selective antagonists by derivatization of the lead compound (**1**) through the combinatorial approach. In this class, the cyclooctenylmethyl (**3e**) and cyclononenylmethyl (**3f**) derivatives were found to show potent binding affinities for M_3 receptors, together with greater than 100-fold selectivity for M_3 over M_1 and M_2 receptors. These aminothiazole derivatives are the alternative examples showing greater than 100-fold selectivity for M_3 over M_1 and M_2 receptors,[14] and these might be useful tools for complete characterization of the roles of M_3 receptor subtype and better understanding for the binding mode of the M_3 selective antagonist.

Experimental

Materials and Methods All reagents and solvents were of commercial quality and used without further purification unless otherwise noted. Melting points were determined with a Yanaco MP micromelting point apparatus and were not corrected. ^{1}H-NMR spectra were obtained on a JEOL AL400 with tetramethylsilane as an internal standard. Mass spectrometry was performed with a JEOL JMS-SX 102A. Elemental analysis was performed on an EA-1108 FISONS Instruments CHNOS analyzer. TLC was done using Merck Kieselgel F_{254} pre-coated plates. Silica gel column chromatography was carried out on Wako gel C-200.

2-(*N*-Methyl-*N*-phenylamino)thiazole-4-carboxylic Acid (5) 1) To a solution of **4** (3.0 g, 20 mmol) in EtOH (70 ml) was added ethyl bromopyruvate (3.0 ml, 1.24 mmol), and the mixture was heated at 90 °C for 3 h. After cooling to room temperature, the solvent was removed under reduced pressure, and the residue was diluted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, and evaporated. The suspension of the residue in toluene–hexane was heated, and the insoluble material was removed by filtration. The filtrate was concentrated under reduced pressure, and the resulting crystalline solid was collected by filtration, washed with toluene and dried to produce ethyl 2-(*N*-phenylamino)thiazole-4-carboxylate (3.4 g, 14 mmol, 70%).

2) To a solution of ethyl 2-(*N*-phenylamino)thiazole-4-carboxylate (2.5 g, 10 mmol) in DMF (25 ml) was added NaH (60% in mineral oil, 600 mg, 15 mmol) at 0 °C. After the mixture was stirred for 15 min at 0 °C, MeI (3.1 ml, 50 mmol) was added and the mixture was stirred for 2 h. The reaction was quenched by adding aqueous NH_4Cl solution, and the mixture was extracted with Et_2O. The organic phase was dried ($MgSO_4$), and evaporated. The residue was purified by silica gel column chromatography (hexane–EtOAc, 10 : 1 elution) to give ethyl 2-(*N*-methyl-*N*-phenylamino)thiazole-4-carboxylate (2.2 g, 2.4 mmol, 84%) as a colorless oil.

3) To a solution of ethyl 2-(*N*-methyl-*N*-phenylamino)thiazole-4-carboxylate (1.9 g, 7.2 mmol) in MeOH (40 ml) was added 3 N NaOH (15 ml), and the mixture was stirred for 15 h at room temperature. After completion of the reaction, the mixture was washed with Et_2O. The aqueous phase was acidified with 3 N HCl, and extracted with $CHCl_3$. The organic phase was dried ($MgSO_4$), and evaporated to give **5** (1.7 g, 7.3 mmol, quant.) as a white solid: mp 159—162 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.56 (3H, s), 7.34 (1H, t, J=7.8 Hz), 7.37 (2H, d, J=7.8 Hz), 7.44 (1H, s), 7.46 (2H, t, J=7.8 Hz); FAB-MS m/z 233 ($C_{11}H_{10}N_2O_2S$ $-H)^-$.

***N*-[(3*S*)-1-(Cyclononen-1-ylmethyl)piperidin-3-ylmethyl]-2-(*N*-methyl-*N*-phenylamino)thiazole-4-carboxamide (3f)** 1) To a mixture of **5** (551 mg, 2.4 mmol) and **10** (630 mg, 2.9 mmol) in $CHCl_3$ (15 ml) were added EDCI–HCl (500 mg, 2.6 mmol) and HOBt (540 mg, 3.5 mmol), and the mixture was stirred at room temperature for 16 h. The reaction was quenched by adding aqueous $NaHCO_3$ solution, and the mixture was

extracted with Et_2O. The organic phase was washed with H_2O and brine, dried ($MgSO_4$), and evaporated. The residue was purified by silica gel column chromatography (hexane–EtOAc, 1 : 1 elution) to give **6** (1.04 g, 2.4 mmol, quant.) as a white foam: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.20—1.33 (1H, m), 1.45 (9H, s), 1.61—1.92 (4H, m), 2.72 (1H, t, J=11.0 Hz), 2.90 (1H, ddd, J=13.0, 11.0, 2.9 Hz), 3.18—3.48 (2H, m), 3.54 (3H, s), 3.75—4.06 (2H, m), 7.26—7.33 (3H, m), 7.38 (2H, d, J=7.8 Hz), 7.45 (2H, t, J=7.8 Hz); FAB-MS m/z 431 ($C_{22}H_{30}N_4O_3S + H)^+$.

2) A mixture of **6** (1.04 g) in 10% HCl–MeOH (10 ml) was stirred at room temperature for 13 h. The reaction mixture was basified (pH 9) with aqueous $NaHCO_3$ solution and extracted with $CHCl_3$. The organic phase was dried ($MgSO_4$) and evaporated to give the crude product, *N*-[(3*S*)-piperidin-3-ylmethyl]-2-(*N*-methyl-*N*-phenylamino)thiazole-4-carboxamide (790 mg, 2.4 mmol, quant.) as a colorless oil: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.13—1.27 (1H, m), 1.40—1.53 (1H, m), 1.65—1.81 (3H, m), 1.83—1.91 (1H, m), 2.40 (1H, t, J=11.3 Hz), 2.58 (1H, td, J=11.3, 2.9 Hz), 3.00 (1H, d, J=12.2 Hz), 3.12 (1H, d, J=12.2 Hz), 3.22—3.39 (2H, m), 3.53 (3H, d, J=1.5 Hz), 7.26—7.34 (3H, m), 7.38 (2H, d, J=7.8 Hz), 7.44 (2H, t, J=7.8 Hz); FAB-MS m/z 331 ($C_{17}H_{22}N_4O_3S + H)^+$.

3) To a solution of *N*-[(3*S*)-piperidin-3-ylmethyl]-2-(*N*-methyl-*N*-phenylamino)thiazole-4-carboxamide (25 mg, 0.076 mmol) in THF (1 ml) was added **13** (30 mg, 0.20 mmol), AcOH (6 mg, 0.10 mmol), and $NaBH(OAc)_3$ (50 mg, 0.23 mmol), and the mixture was stirred at room temperature for 16 h. The reaction was quenched by adding saturated aqueous $NaHCO_3$ solution and extracted with $CHCl_3$. The organic phase was dried (Na_2SO_4), and evaporated. The residue was purified by preparative TLC ($CHCl_3$–MeOH=9 : 1) to give **3f** (27 mg, 0.058 mmol, 76%) as a colorless oil: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.01—2.01 (17H, m), 2.07—2.25 (4H, m), 2.69—2.93 (4H, m), 3.28—3.38 (2H, m), 3.54 (3H, s), 5.44 (1H, t, J=8.3 Hz), 7.25—7.35 (3H, m), 7.38 (2H, d, J=7.8 Hz), 7.44 (2H, t, J=7.8 Hz); HR-MS Calcd for $C_{27}H_{39}N_4SO$ $(M+H)^+$: 467.2845, Found 467.2820; *Anal.* Calcd for $C_{27}H_{39}N_4O \cdot 0.5H_2O \cdot 0.3CHCl_3$: C, 64.10; H, 7.74; N, 10.95. Found: C, 64.40; H, 7.93; N, 10.70.

Binding Assay According to the reported method,[17] the binding affinities were determined by inhibition of specific binding of [^{3}H]-NMS using membranes from CHO cells expressing cloned human M_1—M_3 receptors. The K_i values of compounds were expressed the means of two or more independent assays.

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