Discovery of 2-Aminothiazole-4-carboxamides, a Novel Class of Muscarinic M₃ Selective Antagonists, through Solution-Phase Parallel Synthesis

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Synthesis and structure–activity relationship of a new class of muscarinic M₃ selective antagonists were described. In the course of searching for a muscarinic M₃ 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 M₃ receptors in the human binding assays, we tried to improve its potency and selectivity for M₃ over M₁ and M₂ 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 cyclononylmethyl derivative (3f) as representative M₃ selective antagonists in this class.

Key words muscarine M₃ 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 (M₁, M₂, M₃, M₄) have been defined.^{6–8} Among these muscarinic receptor subtypes, M₃ receptors are localized in smooth muscle and mucosal glands and mediate contraction and mucus secretion. M₁ receptors, localized to the post-ganglionic cholinergic nerve terminals and glands, facilitate neurotransmission and gastric secretion. Neuronal M₂ 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 M₃ antagonists to complete the classification of the receptor subtypes and to provide more ideal therapeutic agents,^{11–13} however, few structure classes with sufficient M₃ selectivity have been discovered to date.¹⁴

As a part of our program for developing a muscarinic M₃ receptor antagonist for the treatment of pulmonary or urinary diseases, we pursued M₃ antagonists that are structurally distinct from a series of 2-(4,4-difluorocyclopentyl)-2-phenylacetamide derivatives such as Compound A¹³ and have selectivity for M₃ receptors two orders of magnitude greater than those for M₁ and M₂ 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. Avoid-

ing the structural complexity of 1 due to the five chiral centers, we first replaced the perhydonaphthylmethyl moiety with a naphthylmethyl 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 M₃ selective antagonists (3e, f) showing high potency ($K_i=ca.$ 1 nM) for M₃ receptors and greater than 100-fold selectivity for M₃ over M₁ and M₂ receptors.

In this paper, we describe the synthesis of aminothiazole derivatives, their binding affinities for M₁–M₃ receptors in the binding assay, and their selectivity for M₃ over M₁ and M₂ 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, CHCl₃, quant., (c); 1) HCl, MeOH, 2) 13, NaBH(OAc)₃, AcOH, THF, 76%, (d) Optical resolution, (e); 1) LAH, THF, 2) Boc₂O, THF, 3) MsCl, NEt₃, EtOAc, 4) NaN₃, DMF, 69%, (f) H₂, 10% Pd-C, MeOH, quant., (g) NH₂NHTs-*p*, HCl, MeOH, 80%, (h) *n*BuLi, THF-TMEDA, –78°C, then DMF, 71%.

Chart 1

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 μ M in the binding assay for the muscarinic M₃ receptor subtype in transfected CHO cells.¹⁷ Selected compounds showing greater than 50% inhibition at 1 μ M were subsequently purified or re-synthesized and tested in the binding assay for muscarinic receptor subtypes (M₁, M₂, M₃) to determine the K_i values¹⁸ and subtype selectivity (M₁/M₃, M₂/M₃).

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 (3*S*)-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 (R¹) on the aminothiazole ring, only an *N*-methylphenylamino group (**14a**) showed inhibitory activity comparable to **14**. The K_i values of **14a** for the M₁, M₂ and M₃ receptor subtypes were confirmed to be 1200, 54000 and 230 nM, respectively, and the selectivity for M₃ over the M₁ and M₂ 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 M₃ over M₂ receptors, while this moiety did not contribute to enhancement of the M₃ binding affinity. Thus, we selected an *N*-methyl-*N*-phenylamino group as the optimized R¹ segment.

Next, we tried to optimize the naphthylmethyl 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 M₃ receptors at 0.1 μ M. 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 M₃ receptors than **3a**. Also, **3b** had better selectivity for M₃ over M₂ receptors (M₂/M₃=74). Therefore, further optimization of the R² 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 M₃ Receptors and the Selectivity for M₃ over M₁ and M₂ Receptors

	R	M ₃		
		Selectivity		
		(K _i , nM)	M ₁ /M ₃	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 μ M to M₃ Receptors

R ¹	% inhibition at 1 μM
	52%
	60%

Table 4. The Binding Affinity of the Compounds (**3a–f**, **14a**) for M₃ Receptors and the Selectivity for M₃ over M₁ and M₂ Receptors

Compd.		M ₃		Selectivity		Compd.		M ₃		Selectivity	
R ²		(K _i , nM)		M ₁ /M ₃ M ₂ /M ₃		R ²		(K _i , nM)		M ₁ /M ₃ 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 M₃ Receptors and the Selectivity for M₃ over M₁ and M₂ Receptors

Compd.		M ₃		Selectivity		Compd.		M ₃		Selectivity	
R ³		(K _i , nM)		M ₁ /M ₃ M ₂ /M ₃		R ³		(K _i , nM)		M ₁ /M ₃ 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 M₃ over M₁ and M₂ receptors than the corresponding cyclooctylmethyl derivative (**3b**). Similarly, the cyclononylmethyl derivative (**3f**) was 10-fold more active and approximately 3-fold more selective for M₃ over M₂ receptors than the cyclononylmethyl derivative (**3c**).

Further derivatization of **3f** was performed for the optimization of the R³ segment (Table 5). These derivatizations indicated that the distance between the amide carbonyl and cationic amine parts, and a suitable structural rigidity as the R³ segment were important for the M₃ binding affinity. Based on these modifications, the (3*S*)-3-aminomethylpiperidine moiety was an optimal R³ segment. It is interesting to note that although both **3f** and Compound **B** (Fig. 1)¹⁴ have excellent M₃ potency and selectivity for M₃ over M₁ and M₂ 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 arrangement of the whole molecule plays a key role in the binding interaction between the M₃ receptors and the antagonists. On the other hand, Compound **A** (Fig. 1) with a conservative structure had a good potency for M₃ receptors, but was only selective for M₃ over M₂ receptors. Comparison of the structure of Compound **A** with those of the M₃ 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

enhanced selectivity for M₃ over M₁ receptors.

In conclusion, we have succeeded in identifying a new class of M₃ selective antagonists by derivatization of the lead compound (**1**) through the combinatorial approach. In this class, the cyclooctenylmethyl (**3e**) and cyclononylmethyl (**3f**) derivatives were found to show potent binding affinities for M₃ receptors, together with greater than 100-fold selectivity for M₃ over M₁ and M₂ receptors. These aminothiazole derivatives are the alternative examples showing greater than 100-fold selectivity for M₃ over M₁ and M₂ receptors,¹⁴ and these might be useful tools for complete characterization of the roles of M₃ receptor subtype and better understanding for the binding mode of the M₃ 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. ¹H-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 F₂₅₄ 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 NaHCO₃ solution and brine, dried over MgSO₄, 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 NH₄Cl solution, and the mixture was extracted with Et₂O. The organic phase was dried (MgSO₄), 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 Et₂O. The aqueous phase was acidified with 3*N* HCl, and extracted with CHCl₃. The organic phase was dried (MgSO₄), and evaporated to give **5** (1.7 g, 7.3 mmol, quant.) as a white solid: mp 159–162 °C; ¹H-NMR (400 MHz, CDCl₃) 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 (C₁₁H₁₀N₂O₂S – H)⁺.

***N*-(3*S*)-1-(Cyclononyl-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 CHCl₃ (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 NaHCO₃ solution, and the mixture was

extracted with Et₂O. The organic phase was washed with H₂O and brine, dried (MgSO₄), 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: ¹H-NMR (400 MHz, CDCl₃) 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 (C₂₂H₃₀N₄O₃S + 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 NaHCO₃ solution and extracted with CHCl₃. The organic phase was dried (MgSO₄) 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: ¹H-NMR (400 MHz, CDCl₃) 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 (C₁₇H₂₂N₄O₃S + 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 NaHCO₃ solution and extracted with CHCl₃. The organic phase was dried (Na₂SO₄), and evaporated. The residue was purified by preparative TLC (CHCl₃–MeOH=9:1) to give **3f** (27 mg, 0.058 mmol, 76%) as a colorless oil: ¹H-NMR (400 MHz, CDCl₃) 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 C₂₇H₃₉N₄SO (M+H)⁺: 467.2845, Found 467.2820; Anal. Calcd for C₂₇H₃₉N₄O·0.5H₂O·0.3CHCl₃: 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 [³H]-NMS using membranes from CHO cells expressing cloned human M₁–M₃ receptors. The K_i values of compounds were expressed the means of two or more independent assays.

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* (London), **323**, 411–416 (1986).
- 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.*, **209**, 367–372 (1986).
- 3) Peralta E. G., Ashkenazi A., Winslow J. W., Smith D. H., Ramachandran J., Capon D. J., *EMBO J.*, **6**, 3923–3929 (1986).
- 4) Bonner T. I., Buckley N. J., Young A. C., Brann M. R., *Science*, **237**, 527–532 (1987).
- 5) Bonner T. I., Young A. C., Brann M. R., Buckley N. J., *Neuron*, **1**, 403–410 (1988).
- 6) Hulme E. C., Birdsall N. J. M., Buckley N. J., *Ann. Rev. Pharmacol. Toxicol.*, **30**, 633–673 (1990).
- 7) Caulfield M. P., *Pharmacol. Ther.*, **58**, 319–379 (1993).
- 8) Barnes P. J., *Life Sci.*, **52**, 521–527 (1993).
- 9) Minette V. A., Barnes P. J., *J. Appl. Physiol.*, **64**, 2532–2537 (1988).
- 10) Fryer A. D., MacLagan J., *Eur. J. Pharmacol.*, **139**, 147–151 (1991).
- 11) Alabaster V. A., *Life Sci.*, **60**, 1053–1060 (1997).
- 12) Miyachi H., Kiyota H., Segawa M., *Biorg. Med. Chem. Lett.*, **8**, 2163–2168 (1998).
- 13) Mitsuya M., Kobayashi K., Kawakami K., Satoh A., Ogino Y., Kakikawa T., Ohtake N., Kimura T., Hirose H., Sato A., Numazawa T., Hasegawa T., Noguchi K., Mase T., *J. Med. Chem.*, **43**, 5017–5029 (2000).
- 14) Recently, we succeeded in identifying M₃ selective antagonists; Sagara Y., Sagara T., Mase T., Kimura T., Numazawa T., Fujikawa T., Noguchi K., Ohtake N., *J. Med. Chem.*, **45**, 984–987 (2002).
- 15) Hilpert K., Ackermann J., Banner D. W., Gast A., Gubernator K., Hadváry P., Labler L., Müller K., Schmid G., Tschopp T. B., Waterbeemd H., *J. Med. Chem.*, **37**, 3889–3901 (1994).
- 16) Naya A., Sagara Y., Ohwaki K., Saeki T., Ichikawa D., Iwasawa Y., Noguchi K., Ohtake N., *J. Med. Chem.*, **44**, 1429–1435 (2001).
- 17) Mitsuya M., Mase T., Tsuchiya Y., Kawakami K., Hattori H., Kobayashi K., Ogino Y., Fujikawa T., Satoh A., Kimura T., Noguchi K., Ohtake N., Tomimoto K., *Bioorg. Med. Chem.*, **7**, 2555–2567 (1999).
- 18) Cheng Y. C., Prusoff W. H., *Biochem. Pharmacol.*, **22**, 3099–3108 (1973).