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 (Kᵢ = 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 cyclononenylmethyl derivative (3f) as representative M₃ selective antagonists in this class.

Key words muscarinic 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. Avoiding the structural complexity of 1 due to the five chiral centers, we first replaced the perhydronaphthylmethyl 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 M₃ selective antagonists (3e, f) showing high potency (Kᵢ = 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 (3S)-3-aminoethylpiperidine (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 D-tartaric acid to give a (3R)-ethyl nipecotate (8). Following the reduction of 8 with LAH, the piperidine

Reagents: (a) 1) Ethyl bromopyruvate, EtOH; 2) 60% NaOH, MeOH, DMF, 3) NaOH, MeOH, 86%. (b) 10, HOBT, EDCl, CHCl₃, quant., (c) 1) HCl, MeOH, 2) 13, NaBH₄(OAc)₂, AcOH, THF, 76%, (d) Optical resolution, (e) 1) LAH, THF, 2) Boc-O, THF, 3) MsCl, NEt₃, EtOAc, 4) NaCN, DMF, 69%, (f) H₂, 10% Pd-C, MeOH, quant., (g) NH₂,NH₂,HCl, MeOH, 80%, (h) nBuLi, THF, TMEDA, ~78°C, then DMF, 71%.

Chart 1

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nitrogen was protected as a tert-butylicarbamate 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) 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 M₃ receptor subtype in transfected CHO cells. 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 (M₁, M₂, M₃) to determine the Kᵢ values and subtype selectivity (M₂/M₃, M₂/M₄).

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 (3S)-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 (R²) on the aminothiazole ring, only an N-methylphenylamino group (14a) showed inhibitory activity comparable to 14. The Kᵢ values 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 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 M₁ 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 Kᵢ values of the two compounds (3a, b) for the three receptor subtypes indicated that 3b with a cyclooctylmethyl moiety displayed more potent activity (Kᵢ=20 nM) for M₃ receptors than 3a. Also, 3b had better selectivity for M₃ over M₁ 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 cyclocdecymethyl groups (Table 4).

Replacement with a cyclononylmethyl group (3c) resulted in enhancement of the M₃ binding affinity to some extent, while the selectivity for M₃ over M₁ and M₂ receptors was maintained. Introduction of a cyclocdecymethyl group (3d) dramatically improved the binding affinity and selectivity for M₃ over M₂ receptors. In the process of identification of the 2-cyclopentyl-2-phenylacetamide derivatives, we found that installment of a double bond into the piperidinyl side chain was effective in enhancing M₃ binding affinity. Therefore, we prepared cycloalkenylmethyl derivatives (3e, f)
on these modifications, the R3 segment were important for the M3 binding affinity. Based binding activity and much better selectivity for M3 over M1 tors and the Selectivity for M3 over M1 and M2 Receptors. On the whole molecule plays a key role in the binding interaction in terms of the 3-aminomethylpiperidine portion. One of receptors, each of them has a different stereochemical struc-
ture had a good potency for M3 receptors, but was only selective for M3 over M2 receptors. These aminothiazole derivatives are the alternative examples showing greater than 100-fold selectivity for M3 over M1 and M2 receptors, 13 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.

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 (3S)-3-aminomethylpiperidine moiety was an optimal R3 segment. It is interesting to note that although both 3f and Compound B (Fig. 1) 13 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 M1 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 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 cyclononenylmethyl (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, 13 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 FISON 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 3f (3.0 g, 20 mmol) in EtOH (70 ml) was added ethyl bromopru-
vate (3.0 ml, 52 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.

2) To a solution of 2-(N-methyl-N-phenylamino)thiazole-4-carboxylic acid (1.9 g, 7.2 mmol) in MeOH (40 ml) was added 3 N HCl, and the mixture was stirred for 2 h. The reaction mixture was quenched by adding aqueous NaHCO3 solution, and the mixture was extracted with EtOAc. The organic phase was dried (MgSO4), and evaporated. The residue was purified by silica gel column chromatography (hexane–EtOAc, 10:1 elution) to give 2-(N-methyl-N-phenylamino)thiazole-4-carboxylate (2.2 g, 2.4 mmol, 84%) as a colorless oil.

3) To a solution of 2-(N-methyl-N-phenylamino)thiazole-4-carboxylate (1.9 g, 7.2 mmol) in THF (50 ml) was added a solution of LiAlH4 (3.0 mmol) in THF (50 ml) at 0 °C. After the mixture was stirred for 1 h, the reaction mixture was quenched by adding aqueous NaHCO3 solution, and the mixture was extracted with EtOAc. The organic phase was dried (MgSO4), and evaporated. The residue was purified by silica gel column chromatography (hexane–EtOAc, 10:1 elution) to give 2-(N-methyl-N-phenylamino)thiazole-4-carboxamide (1.9 g, 7.2 mmol, 84%) as a colorless oil.

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

<table>
<thead>
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<th>Compd. R2</th>
<th>M1 (Kᵢ, nM)</th>
<th>Selectivity</th>
<th>Compd. R2</th>
<th>M1 (Kᵢ, nM)</th>
<th>Selectivity</th>
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<tr>
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<td>34</td>
<td>3h</td>
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<tr>
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<td>3d</td>
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<td>64</td>
<td>3j</td>
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</table>

### Table 5. The Binding Affinity of the Compounds (3g—m) for M3 Receptors and the Selectivity for M3 over M1 and M4 Receptors

<table>
<thead>
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<th>Compd. R1</th>
<th>M1 (Kᵢ, nM)</th>
<th>Selectivity</th>
<th>Compd. R1</th>
<th>M1 (Kᵢ, nM)</th>
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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, dd, J=13.0, 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 (C₁₇H₂₂N₄O₃S + 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 (Na₂SO₄), and evaporated. The residue was purified by preparative TLC (CHCl₃–MeOH 9:1) to give 13 (0.5 mg, 0.02 mmol), AcOH (6 mg, 0.10 mmol), and NaBH₄(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 (MgSO₄) and evaporated to give the crude product, N-[3(S)-piperidin-3-ylmethyl]-2-(3-phenylamino)thiazole-4-carboxamide (790 mg, 2.4 mmol, quant.) as a colorless oil: 1H-NMR (400 MHz, CDCl₃) δ: 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), 3.32—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-[(3S)-piperidin-3-ylmethyl]-2-(3-phenylamino)thiazole-4-carboxamide (25 mg, 0.076 mmol) in THF (1 ml) was added 1 (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-[(3-phenylamino)thiazole-4-carboxamide (790 mg, 2.4 mmol, quant.) as a colorless oil: 1H-NMR (400 MHz, CDCl₃) δ: 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), 3.32—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)⁺.

References and Notes


