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Discovery and SAR of a novel series of Natriuretic Peptide Receptor-A (NPR-A) agonists

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ABSTRACT

Novel thienopyrimidine compounds **2** and **3** were discovered from high-throughput screening as Natriuretic Peptide Receptor A (NPR-A) agonists. Scaffold hopping of a thienopyrimidine ring to a quinazoline ring, introduction of the basic functional group and optimization of the substituent on the 6-position of the benzene ring of quinazoline led to improved agonistic activity. We discovered compound **48**, which showed potent agonistic activity for NPR-A with an EC_{50} value of 0.073 μM, indicating 350-fold potency compared to the hit compound **3**.

Atrial natriuretic peptide (ANP) is a cardiovascular hormone that consists of 28 amino acids. ANP is mainly secreted from the cardiac atria and binds to the Natriuretic Peptide Receptor-A (NPR-A), which exerts diuresis vasodilation.[1,2] Human ANP (HANP®) is already being used clinically for the treatment of acute heart failure in Japan.[3,4] Recent studies have shown that ANP has a potent inhibitory effect on cardiac fibrosis and hypertrophy, and exhibits anti-inflammatory effects.[4,5] These results suggest that the non-peptidic NPR-A agonists that are suitable for chronic setting might also be useful for the treatment of cardiocirculatory diseases such as chronic heart failure, chronic kidney failure and arteriosclerosis obliterans. The clinical utility of ANP is limited to the acute setting because of its rapid clearance from the body, as is often the case with other peptide drugs.[5]

It has been reported that ANP binds to the dimeric NPR-A and causes a twist motion of the two extracellular domains, which lead to the conformational changes in the intracellular guanylyl cyclase domain and the activation of enzymatic activity.[6] It is a big challenge to develop small molecules which mimic the action of ANP. We have previously reported non-peptidic small molecules with a novel triazine scaffold that showed NPR-A agonistic effects like ANP. We succeeded in the discovery of the potent NPR-A agonist **1** via a dimerization approach (Fig. 1).[7] Compound **1** has a high molecular weight (~700) and 20 nitrogen atoms in its structure, and these attributes make it an unsuitable lead compound for clinical candidate. Based on the above consideration, we re-conducted a high-throughput screening campaign of the Daiichi Sankyo compound library to discover new small scaffolds using human NPR-A expressing CHO cells.[5] As a result, we identified the thienopyrimidine derivatives **2** and **3** which enhanced the production of cyclic guanosine monophosphate (cGMP) in the assay. These compounds did not act on NRR-B, another Natriuretic Peptide Receptor subtype, expressing and mock CHO cells in a counter assay at 20 μg/mL, and we considered compounds **2** and **3** to selectively activate NPR-A (Fig. 1). Since these compounds showed only weak agonistic activity but had lower molecular weights and a fewer number of nitrogen atoms than those of the triazine compound **1**, we decided to optimize their structures to improve their agonistic activity.

In order to elucidate the structure-activity relationship, compounds that could be modified at the 2- and 4-positions of the thienopyrimidine core were prepared, as shown in Scheme 1. The urea **5** was prepared by reacting commercially available methyl 3-amino-5-phenylthiophene-2-carboxylate **4** with trichloroacetyl isocyanate. After deprotection of the trichloroacetyl group with ammonia, cyclization under alkaline conditions afforded the bicyclic core **6**, which was then chlorinated with phosphorus oxychloride to give the intermediate **7**. Aminomethylpyridines and aminobenzylamines were introduced to the 4-position of **7**, and subsequent introduction of alkylamines to the 2-position assisted

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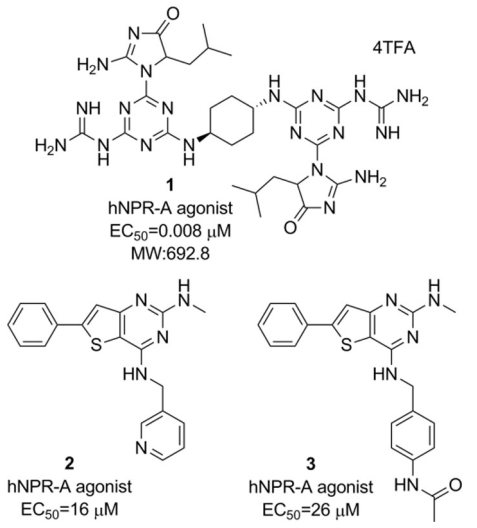


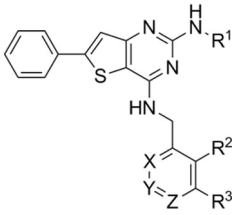
Fig. 1. Triazine compound **1** and hit compounds from high throughput screening.

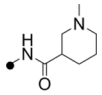
microwave irradiation yielded compounds **13b**–**17b**. Acetylation of the compounds **16b** and **17b** afforded **18** and **19**, respectively, and the condensation of **17b** with 1-methyl-3-piperidinecarboxylic acid[8] led to the formation of compound **20**.

We evaluated the NPR-A agonistic activity[9] of compounds **13b**–**15b** and **18**–**20** using human NPR-A expressing CHO cells (Table 1). Our initial efforts focused on varying the size of the alkylamino group at the 2-position of compound **2**. It was found that the agonistic activity was slightly improved as the size of the alkylamino group increased (**14a** and **14b**), although compound **14c** with the larger isobutylamino group showed decreased agonistic activity. Therefore, the substituent at the 2-position was fixed as isopropylamino group for further study.

Compounds **13b** and **15b** with different positions of the nitrogen atom on the pyridine ring did not improve the agonistic activity. However, changing the position of the acetylamino group of compound **3** decreased NPR-A agonistic activity (**18** and **19**). Compound **20** was designed with the basic functional group 1-methyl-3-piperidine on the acetylamino group of compound **19** because the negatively charged amino acids typically cluster at the ANP binding site of NPR-A receptor.[10] In fact, compound **20** showed

Table 1
EC_{50} values of thienopyrimidine NPR-A agonists. The concentration of each compound required for half-maximum accumulation of cGMP (EC_{50}) was determined from an analysis of the plots of cGMP contents versus log concentration of the compound in human NPR-A expressing CHO cells. Maximum cGMP concentration induced by human ANP was regarded as 100% (EC_{50} = 0.1 nM).

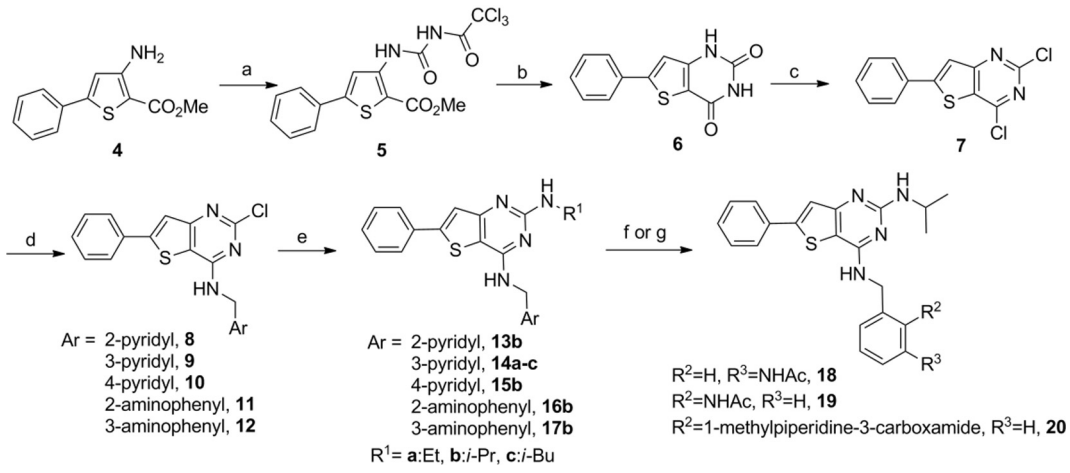


Compd.	R^1	R^2	R^3	X	Y	Z	hNPR-A agonist EC_{50} (μM)
14a	Et	H	H	CH	N	CH	10
14b	*i*-Pr	H	H	CH	N	CH	7.9
14c	*i*-Bu	H	H	CH	N	CH	22
13b	*i*-Pr	H	H	N	CH	CH	13
15b	*i*-Pr	H	H	CH	CH	N	13
18	*i*-Pr	H		CH	CH	CH	45
19	*i*-Pr		H	CH	CH	CH	30
20[a]	*i*-Pr		H	CH	CH	CH	5.0

[a] Racemate.

improved agonistic activity with an EC_{50} value of 5.0 μM. Therefore, it seems that the introduction of a basic functional group at this position is effective in improving agonistic activity.

Subsequently, we designed quinazoline derivatives based on the reports that a benzene ring could be treated as an equivalent of a thiophene ring.[11] The synthesis of the quinazoline compounds shown in Tables 2 and 3 is described in Scheme 2. Intermediates **27** and **28** were prepared from commercially available **21** and **22** via a synthetic method similar to that used to obtain **7**. Compounds **29**–**33** were prepared by the introduction of aminobenzylamines and *trans*-1,4-cyclohexanediamine at the 4-position of intermediates **27** and **28**. The introduction of the isopropylamino group at the



Scheme 1. Reagents and conditions: (a) trichloroacetyl isocyanate, THF, r.t., 96%; (b) 7 N NH_3/MeOH, r.t.; then 4 N NaOH, 100 °C, 80%; (c) phosphorus oxychloride, *N*,*N*-diethylaniline, 110 °C, 93%; (d) Ar-CH_2NH_2, DIPEA, THF, r.t., 60–93%; (e) R_1-NH_2, H_2O, microwave, 160 °C, 69–99%; (f) AcCl, DIPEA, THF r.t., 91–94%; (g) 1-methyl-3-piperidinecarboxylic acid, HATU, DIPEA, CH_2Cl_2, r.t., 72%.