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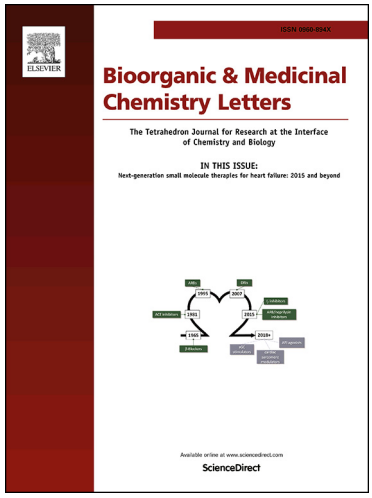

Discovery of DS42450411 as a potent orally active hepcidin production inhibitor: Design and optimization of novel 4-aminopyrimidine derivatives

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Discovery of DS42450411 as a potent orally active hepcidin production inhibitor: Design and optimization of novel 4-aminopyrimidine derivatives

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ABSTRACT

Hepcidin has emerged as the central regulatory molecule in systemic iron homeostasis. The inhibition of hepcidin may be a favorable strategy for the treatment of anemia of chronic disease. Here, we have reported the design, synthesis, and structure-activity relationships (SAR) of a series of 4-aminopyrimidine compounds as inhibitors of hepcidin production. The optimization study of **1** led to the design of a potent and bioavailable inhibitor of hepcidin production, **34** (DS42450411), which showed serum hepcidin-lowering effects in a mouse model of interleukin-6-induced acute inflammation.

The maintenance of serum iron level is important since a high iron concentration induces oxidative organ damage, and a low iron concentration results in iron deficiency anemia.[1]

Anemia of chronic disease (ACD) is the second most prevalent form of anemia after that caused by iron deficiency and occurs in patients with acute or chronic immune activation. ACD, which includes inflammation-associated anemia, is a heterogenic anemic condition caused by chronic inflammation from a common disease such as infections, cancer and rheumatoid arthritis.[2] Some patients with ACD are known to present with iron deficiency, despite abundant body iron stores (termed *functional iron deficiency*).

Hepcidin, originally discovered as an antibacterial peptide,[3] is induced by inflammatory cytokines such as interleukin (IL)-6,[4] in addition to its iron signaling effects. This peptide hormone is a homeostatic regulator of intestinal iron absorption, iron recycling by macrophages, and iron mobilization from hepatic stores.[5]

Recently, high hepcidin induction based on inflammatory status was recognized as the cause of functional iron deficiency.[6] Hepcidin expression deficiency is a common phenotype of hereditary hemochromatosis. Thus, control of the hepcidin level would be a promising therapeutic strategy for treating ACD. Indeed, a few biologics (e.g., NOX-H94, LY2928057, and LY2787106) have proceeded to clinical trials for the treatment of ACD. We thought an orally administrable hepcidin

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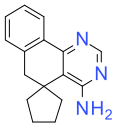
production inhibitor could contribute to patient's QoL (Quality of Life) improvement and started research.

Herein, we have described the lead optimization of **1** to produce 8-{1-[(1*R*,4*R*)-4-aminocyclohexyl]ethenyl}-5,5-dimethyl-5,6-dihydrobenzo[*h*]quinazolin-4-amine (DS42450411, **34**), an orally available potent inhibitor of hepcidin production.

Through the screening of our compound library, we identified a 4-aminopyrimidine compound **1** as an inhibitor of hepcidin production (IC_{50} = 4.2 μM).[7]



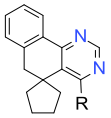
1 IC_{50} = 4.2 μM

Figure 1. HTS-hit of hepcidin production inhibitor **1** with IC_{50} values.

To begin optimization, we first investigated the derivatization of the 4-aminopyrimidine moiety.

The results are summarized in Table 1; only the primary amine showed inhibitory activity.

Table 1. SAR of 4-position of pyrimidine derivatives



compound	R	IC_{50} (μM)
1	NH_2	4.2
2	H	> 30
3	OH	> 30
4	NHMe	> 30
5	NMe_2	> 30

Next, the spiro-structure at 5-position was modified to simplify the derivatization (Table 2).

Table 2. SAR of 5-substituted pyrimidine derivatives

compound	R, R'	IC_{50} (μM)
1	cyclopentyl	4.2
6	H, H	> 30
7	Me, Me	4.5

Although compound **6**, without a substituent at the 5-position, showed a dramatic loss of activity, we found that conversion to a simple dimethyl group maintained the same activity as that of spiro-cyclopentane.

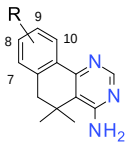
Next, various substituents on the left-hand benzene ring were examined.

The results for the four methyl-substituted derivatives revealed that a substituent at the 8-position was favored (compounds **8**–**11**, Table 3).

After it was determined that the substitution at the 8-position enhanced inhibitory activity, we then investigated the derivatization of the various substituents at the 8-position.

The aryl derivative **12** exhibited slightly weak activity and the ester derivative **13** showed decreased hepcidin inhibitory activity. In contrast, the methoxy derivative **14** showed slightly improved activity.

Table 3. SAR of Substituted 4-aminopyrimidine derivatives

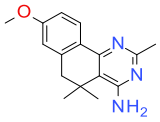


compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
7	H	4.5	**11**	10-Me	4.7
8	7-Me	5.0	**12**	8-Ph	1.5
9	8-Me	0.60	**13**	8-CO_2Me	11
10	9-Me	2.8	**14**	8-OMe	0.53

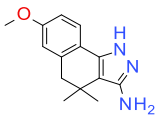
Next, we attempted the conversion of the aminopyrimidine moiety. Only the adoption of the methyl group at the 2-position resulted in a loss of activity. The pyrazole and isoxazole scaffolds also exhibited a loss of activity (Figure 2).

The above results revealed that the 4-aminopyrimidine scaffold was essential for hepcidin inhibitory activity.

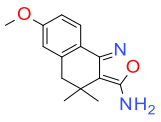
Figure 2. In vitro activity of other scaffolds



15 IC_{50} > 5.0 μM



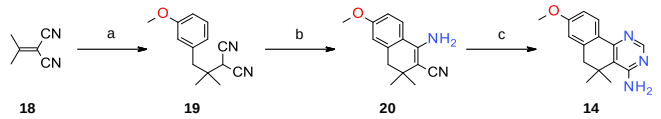
16 IC_{50} > 30 μM



17 IC_{50} > 30 μM

The synthetic route of **14** is illustrated in Scheme 1. Starting from isopropylidenemalononitrile **18**, propanedinitrile **19** was obtained by Michael addition of 3-methoxybenzyl magnesium chloride.

Subsequently, cyclization using trifluoromethanesulfonic acid yielded the 1-amino-3,4-dihydro-6-methoxy-2-naphthalenecarbonitrile intermediate **20** as single isomer. Finally, cyclization with formamide yielded compound **14**.



Scheme 1. Synthesis of compound **14**. Reagents and conditions: (a) 3-Methoxybenzylmagnesium chloride, THF, 40%; (b) Trifluoromethanesulfonic acid, CH_2Cl_2, quantative; (c) Formamide, 72%.

We conducted a kinase profiling assay using **14**, which had moderate *in vitro* activity. The results demonstrated that **14** had strong inhibitory effects on CLK2, DYRK1a, and DYRK1b, belonging to the CMGC kinase family (Figure 3).

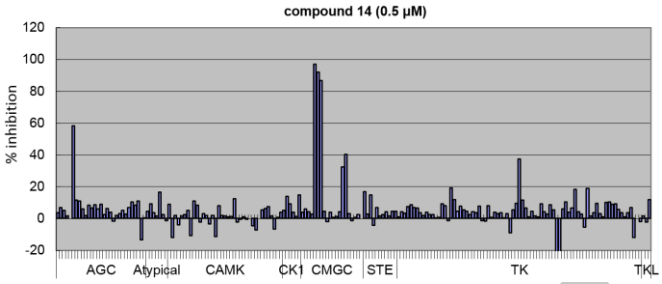


Figure 3. Kinase inhibitory profiles of compound **14** (192 kinases)

We hypothesized that the mechanism of inhibition of hepcidin production of this series of compounds is due to inhibition of DYRK1a or kinases belonging to CMGC family.

Therefore, we carried out the X-ray co-crystal structure analysis of **14** and DYRK1a, one of CMGC family proteins.[8]

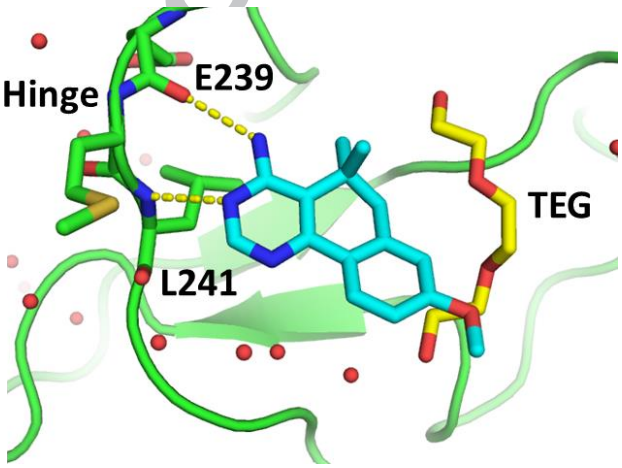


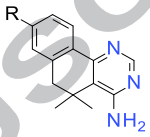
Figure 4. Co-crystal structure of DYRK1a and compound **14** determined at 1.5 Å resolution (PDB: 6A1F).
Triethylene glycol (TEG) was placed to the position of unidentified density observed within the ATP-binding pocket of DYRK1a.

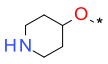
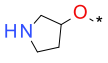
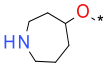
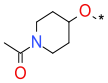
In the crystal structure, compound **14** bound to the ATP-binding pocket of DYRK1a with its aminopyrimidine moiety interacting with the hinge region. It was also suggested from the crystal structure that there is a space tolerance in the ATP-binding pocket beyond the 8-methoxy groups.

Next, various alkoxy substituents at the 8-position were examined (Table 4).

As the isopropoxy derivative **22** showed equivalent activity as **14**, the space tolerance was confirmed. Next, the feasibility of the functional groups was investigated.

Table 4. SAR of 4-amino-8-substituted pyrimidine derivatives



compound	R	IC_{50} (μM)	compound	R	IC_{50} (μM)
14	MeO	0.53	**25**		0.23
21	EtO	0.42	**26**		0.18
22	i-PrO	0.49	**27**		0.24
23	$HOCH_2CH_2O$	1.1	**28**		0.76
24	HO_2CCH_2O	11	**29**		0.81

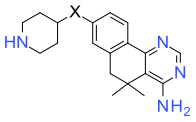
The hydroxyethyl derivative **23** exhibited slightly weak activity. In addition, the carboxyl group (**24**) was not acceptable. In contrast, incorporation of a cyclic amino group effectively enhanced the inhibitory activity (compounds **25–27**, Table 4).

As the *in vitro* activity was diminished by the conversion of an NH group to an acetamide of the piperidine ring (**28**), basicity was essential for the enhancement of activity. Further, as the direct attachment of piperidine derivative **29** resulted in no enhancement of the activity, the role of the linker was suggested to be important.

Therefore, we studied various linkers as a replacement for the ether in order to enhance activity (Table 5).

The conversion of ether oxygen to ketone did not result in an enhancement of activity (**30**), but the *exo*-olefin linker (**32**) significantly increased the *in vitro* activity.

Table 5. SAR of various linkers at 8-position



Compound	X	IC50 (μM)
25	O	0.23
30	C=O	0.30
31	*rac*-CHMe	0.073
32	C=CH_2	0.021

We carried out the X-ray co-crystal structure analysis of the highly active compound **32** and DYRKla to understand the structural basis of the significant leap in *in vitro* activity (Figure 5).

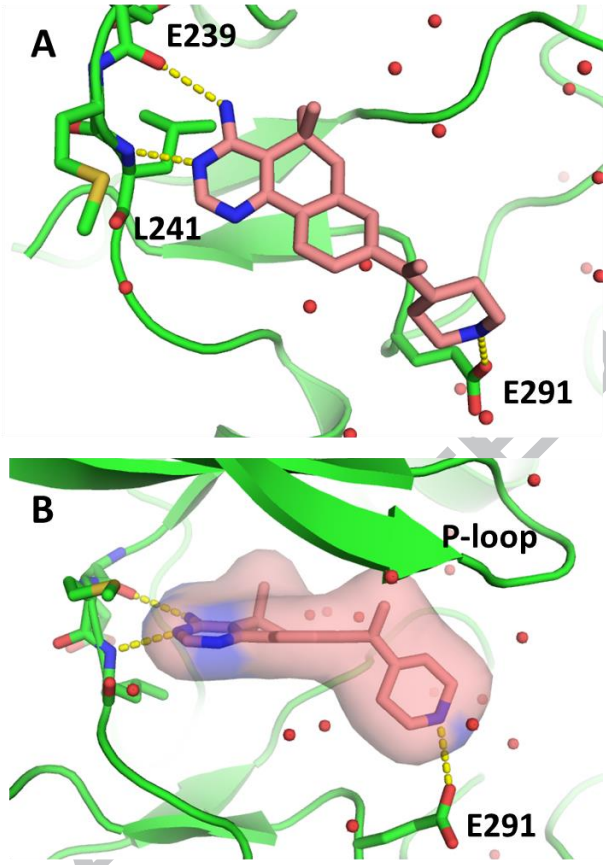


Figure 5. Co-crystal structure of DYRK1a and compound **32** determined at 2.15 Å resolution (PDB: 6A1G).

While compound **32** maintained the interaction with hinge region similar to compound **14**, the NH group of newly introduced piperidine at 8-position formed a hydrogen bond with the glutamic acid 291. In addition to this, *exo*-olefin linker was considered to improve the spatial complementarity with the *P*-loop. These interactions could explain the robust *in vitro* activity of compound **32**.

As shown in Table 6, the inhibitory activity of

Table 6. SAR of inhibitory activity between hepcidin production and DYRK1a

compound	Hepcidin IC50 (μM)	DYRK1a IC50 (μM)
14	0.53	0.33
6	> 30	57
15	>5.0	2.7
25	0.23	0.56
32	0.021	0.054

DYRK1a of compound **32** was greater than that of compound **14**.

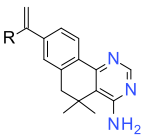
In addition, ALK2 and ALK3 is recognized as important kinase regulating Hepcidin production via BMP ligand / BMP receptor kinase / SMAD pathway. We have checked for ALK2, and DS42450411 didn't inhibit ALK2 at higher concentration (around 30 μM) compared to Hepcidin production inhibition concentration.

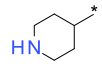
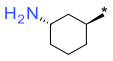
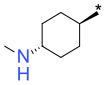
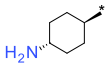
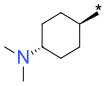
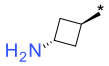
The mechanism of the inhibition of hepcidin production of this series of compounds was suggested to be through the inhibition of DYRK1a or a kinase belonging to CMGC family.

Then, we conducted optimization studies of the substituent at the 8-*exo*-olefin site.

The results of the different functionalized *exo*-olefin derivatives on the 8-position are summarized in Table 7.

Table 7. SAR of 8-*exo*-olefin derivatives



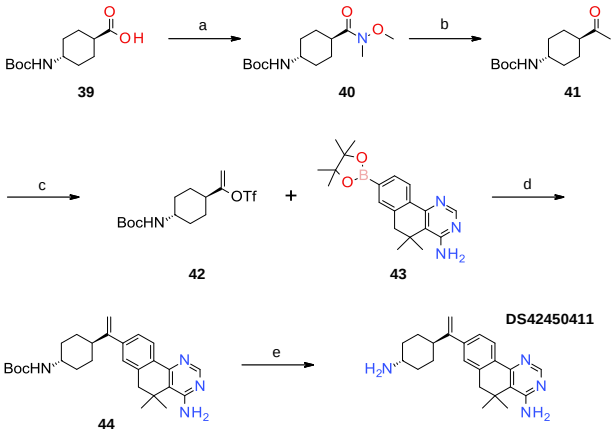
compound	R	IC50 (μM)	compound	R	IC50 (μM)
32		0.021	**36**		0.12
33		0.042	**37**		0.045
34		0.032	**38**		0.11
35		0.031			

Thus, the potently active hepcidin production inhibitor **34**, named DS42450411, was selected for further investigation.

DS42450411 was synthesized using the process illustrated in Scheme 2.

Starting from carboxylic acid **39**, the vinyl triflate intermediate **42** was synthesized via Weinreb amide

40 and methyl ketone **41**. Subsequently, a Suzuki-coupling reaction with the boronic acid pinacol ester **43** yielded the *exo*-olefin skeleton. Finally, deprotection of the Boc group in acidic conditions produced DS42450411.



Scheme 2. Synthesis of DS42450411. Reagents and conditions: (a) N,O-Dimethylhydroxyamine, EDC-HCl, HOBt, Diisopropylethylamine, CH_2Cl_2, 99%; (b) Methylmagnesium bromide, THF, 28%; (c) $NaN(TMS)_2$, $PhNTf_2$, THF, 63%; (d) XPhosG2-Pd, K_3PO_4-nH_2O, 1,4-dioxane/H_2O, 69%; (e) trifluoroacetic acid, CH_2Cl_2, 75%.

To evaluate the *in vivo* efficacy, we assessed the pharmacokinetic (PK) parameters of DS42450411 (Table 8).

Table 8. Physicochemical properties and pharmacokinetic parameters of DS42450411

LogD	PB (free %)	Cmax[a] (μg/mL)	Tmax[a] (h)	$T_{1/2}$[a] (h)	CL[a] (mL/min/kg)	AUC[a] (h*μg/mL)
1.6	6.5	0.61	1.17	4.59	191	2.58

[a] Average of two values dosed at 30 mg/kg orally (p.o.) in C57BL/6J mice (0.5% methylcellulose suspension).

DS42450411 was appropriately lipophilic and possessed moderate plasma protein binding (*fu,p* = 6.5%). Further, it showed high plasma exposure in mice and was considered to have a suitable profile as an oral agent.

Next, the hepcidin-lowering effect of DS42450411 was evaluated in an *in vivo* mouse model.

Hepcidin is known as an acute-phase protein produced in response to chronic inflammatory conditions. Prior to this evaluation, we conducted a time-course study, in which serum hepcidin levels were found to increase as early as 1 h after the injection of IL-6, plateaued at 4 h, and then remained constant until 6 h after injection (data not shown). Therefore, we evaluated the effect of DS42450411 against the acute-phase induction of hepcidin in serum in response to the intravenous injection of mouse IL-6.

DS42450411 was administered orally to 9-week-old male C57BL/6J mice 30 min before IL-6 administration. Blood samples were collected 4 h after the IL-6 injection, and the hepcidin concentration was determined.

As shown in Figure 6, DS42450411 significantly reduced the blood hepcidin concentration when administered orally at 30 mg/kg.

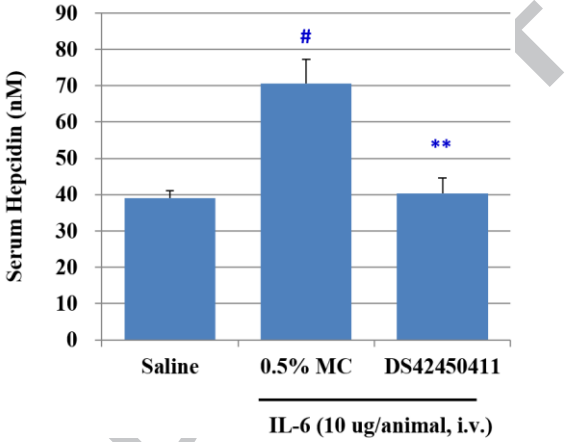


Figure 6. Effect of DS42450411. The compound was administered to mice at dose of 30 mg/kg (p.o., 0.5% Methylcellulose, suspension, $n = 4$) before IL-6 treatment. #, $p < 0.05$ vs Saline treated group (t-test), **, $p < 0.01$ vs 0.5% MC treated group (t-test).

In conclusion, we reported the discovery of a series of 4-aminopyrimidine derivatives as potent and orally bioavailable inhibitors for hepcidin production. Starting from compound **1**, the introduction of the amine moiety at the 8-position and the transformation from ether linker to *exo*-olefin significantly increased the activity.

The optimization of the amine moiety at 8-position yielded DS42450411, which possessed potent *in vitro* activity. Furthermore, DS42450411 was confirmed as a promising compound for oral administration and lowered the serum hepcidin levels in a mouse model of IL-6-induced acute inflammation.

The mechanism of the inhibition of hepcidin production of this series of compounds was suggested to be through the inhibition of DYRK1a or a kinase belonging to CMGC family. Up to the present, there isn't any known information linking DYRK1a kinase with Hepcidin production. DS42450411 didn't inhibit ALK2 at higher concentration compared to Hepcidin production inhibition concentration. As most of ALK2 inhibitor has inhibitory activity on ALK3, we supposed that our compound do not inhibit Hepcidin production by this classical BMP/BMPR/SMAD pathway.

Further investigation will be needed by discovering more potent and specific inhibitor for true target identification.

We are currently performing multifaceted validation, including whether DYRK1a is a true pharmacological target, which will be reported in due course.

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References and notes

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Graphical Abstract

Discovery of DS42450411 as a potent orally active hepcidin production inhibitor: Design and optimization of novel 4-aminopyrimidine derivatives

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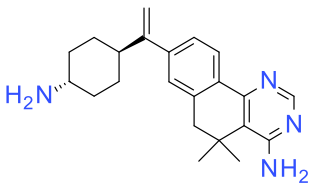

34 (DS42450411)

Hepcidin mRNA expression
IC_{50} = 0.032μM

Highlights

- Hepcidin, a peptide hormone, is a master regulator of systemic iron mobilizations (83 characters)
- Hepcidin inhibition could be a strategy for treating anemia of chronic disease (80 characters)
- 4-Aminopyrimidine compound was founded as a hit of Hepcidin production inhibitor (82 characters)
- Optimization study led to a potent and bioavailable Hepcidin production inhibitor (83 characters)
- DS42450411 showed serum Hepcidin-lowering effects in an acute inflammation model (82 characters)