Bioorganic & Medicinal Chemistry Letters xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of DS79182026: A potent orally active hepcidin production inhibitor

Takeshi Fukuda [a,*], Riki Goto [b], Toshihiro Kiho [c], Kenjiro Ueda [d], Sumie Muramatsu [d], Masami Hashimoto [d], Anri Aki [b], Kengo Watanabe [e], Naoki Tanaka [a]

[a] Rare Disease & LCM Laboratories, Daiichi Sankyo Co, Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[b] Biologics & Immuno-Oncology Laboratories, Daiichi Sankyo Co, Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[c] Modality Research Laboratories, Daiichi Sankyo Co, Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[d] Pain & Neuroscience Laboratories, Daiichi Sankyo Co, Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[e] Drug Metabolism & Pharmacokinetics Research Laboratories, Daiichi Sankyo Co, Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan

ARTICLE INFO

Article history:
Received 19 April 2017
Revised 27 June 2017
Accepted 1 July 2017
Available online xxxx

Keywords:
Hepcidin
Anemia of chronic disease
Benzisoxazole
Pyrazole
Kinase

ABSTRACT

Hepcidin has emerged as the central regulatory molecule of systemic iron homeostasis. Inhibition of hepcidin could be a strategy favorable to treating anemia of chronic disease (ACD). We report herein the synthesis and structure-activity relationships (SARs) of a series of benzisoxazole compounds as orally active hepcidin production inhibitors. The optimization study of multi kinase inhibitor **1** led to a potent and bioavailable hepcidin production inhibitor **38** (DS79182026), which showed serum hepcidin lowering effects in a mouse IL-6 induced acute inflammatory model.

Hepcidin is a peptide hormone, and is known as the master regulator for systemic iron mobilization.[1] 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.[2] As hepcidin was originally discovered as an antibacterial peptide,[3] this hormone is inducible by inflammatory cytokines such as IL-6,[4] in addition to iron signaling.

Anemia of chronic disease (ACD), which includes anemia of inflammation, is a heterogenic anemic condition due to chronic inflammation from a basic disease, such as rheumatoid arthritis.[5] Some ACD patients are known to present iron deficiency despite abundant body iron store (termed *functional iron deficiency*). Recently, high hepcidin induction based on inflammatory status was recognized as the cause of functional iron deficiency. Hepcidin expression deficiency is a common phenotype of hereditary hemochromatosis. The controlling of hepcidin level would be a promising therapeutic strategy for treating hepcidin caused functional iron deficiency. Indeed, a few such biologics (e.g. NOX-H94, LY2928057 and LY2787106) are entering clinical trials for treatment of anemia. Herein we describe the derivatization aimed at the enhancement of bioavailability and lowering of the multi kinase inhibitory activity of **1** to discover methyl {6-[5-methyl-3-(pyridin-2-yl)-1*H*-pyrazol-4-yl]-1,2-benzisoxazol-3-yl}carbamate (DS79182026, **38**), a potent orally available hepcidin production inhibitor.

As previously reported,[6] we identified the indazole derivative **1** as a potent active lead compound (IC_{50} = 0.13 μM).[7] Although compound **1** showed hepcidin lowering effect in mice by the intraperitoneal administration, pharmacokinetic (PK) profiles supported a lack of exposure to blood in the oral administration (Table 1).

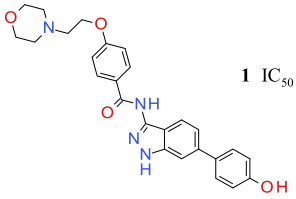
The indispensable *para*-hydroxyphenyl group also might be responsible for this low exposure because of the *O*-glucuronidation via UDP-glucuronosyltransferase (UGT). As an entirely fresh start, we started to explore the alternatives of the *para*-hydroxyphenyl group.

First, we examined the bicyclic phenolic bioisosteres. The bicyclic mimetics were designed to address the phenolic H-bond donor based on the localization afforded by the complementary fused heterocyclic rings.[8] However, the benzimidazole **3**, pyrolopyridine **4** and oxindole **5** were found to be surprisingly poor mimetics. Then, we investigated the monocyclic heteroaromatics. The transformations to pyrrole **6** drastically lost and 4-pyridyl group **7** deteriorated hepcidin inhibitory activity. However, the dimethylisoxazole **8** showed moderate inhibitory activity.

* Corresponding author.
E-mail address: fukuda.takeshi.zv@daiichisankyo.co.jp (T. Fukuda).

http://dx.doi.org/10.1016/j.bmcl.2017.07.004
0960-894X/

Table 1
IC_{50} values and physicochemical properties and PK parameters of **1**.



1 IC_{50} = 0.13 μM

LogD	MS[a] (%)	UGT[a] (%)	Cmax (μg/mL)	Tmax (h)	AUC (h*μg/mL)
3.4	89	76	2.64[b] 0.32[c]	1.33[b] 1.50[c]	6.93[b] 0.89[c]

[a] Remaining (%) of the tested compound after 0.5 h incubation with mouse liver microsomes (0.5 mg/mL).
[b] Average of two values dosed at 30 mg/kg i.p. with C57BL/6J mice (0.5% Methylcellulose, suspension).
[c] Average of two values dosed at 30 mg/kg p.o. with C57BL/6J mice (0.5% Methylcellulose, suspension).

Surprisingly, the dimethylpyrazole **9** showed inhibitory activity comparable to compound **2** (Table 2).

Subsequently, we investigated the combinations of the alternative pyrazole and potent benzamide moieties.[6]

The compounds **10** and **11** retained potent *in vitro* activity (Table 3).

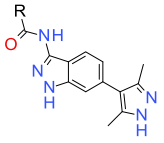
PK parameters of compound **11** are summarized in Table 4.

Compound **11** showed high stability against UGT. PK profiles of **11** were evaluated and found to be improved compared to compound **1**, presumably due to the interruption of the glucuronidation.

Indeed, **11** showed higher plasma exposure than **1** and was considered to be a suitable profile as an oral agent.

Next, the hepcidin lowering effect of compound **11** was evaluated by a mouse interleukin-6 (IL-6) induced acute inflammatory model.

Table 3
SAR of 6-dimethylpyrazole derivatives.



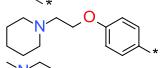
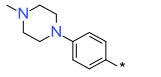
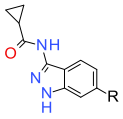
Compound	R	IC_{50} (μM)
9		0.33
10		0.26
11		0.23

Table 2
Alternatives of *para*-hydroxyphenyl group.



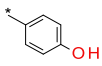
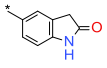
Compound	R	IC_{50} (μM)	Compound	R	IC_{50} (μM)
2		0.40	**6**		>30
3		>3	**7**		7.2
4		11	**8**		1.0
5		>30	**9**		0.33

Table 4
Physicochemical properties and PK parameters of **11**.

LogD	MS[a] (%)	UGT[a] (%)	Cmax[b] (μg/mL)	Tmax[b] (h)	AUC[b] (h*μg/mL)
2.4	80	94	0.92	3.25	2.55

[a] Remaining (%) of the tested compound after 0.5 h incubation with mouse liver microsomes (0.5 mg/mL).
[b] Average of two values dosed at 30 mg/kg p.o. with C57BL/6J mice (0.5% Methylcellulose, suspension).

As hepcidin is known as an acute-phase protein responding to chronic inflammatory conditions, we evaluated by intravenous injection for the acute-phase induction of hepcidin in serum levels in response to mouse IL-6. Beforehand, we conducted a time course study in which serum hepcidin levels began increasing as early as 1 h after injection of IL-6, reached plateau at 4 h, and stayed constant until 6 h after injection (data not shown). Compound was administered orally to 9-week-old male C57BL/6J mice 30 min before IL-6 administration. Blood was collected at 4 h after the IL-6 injection and the hepcidin concentration was determined.

Oral administration of 30 mg/kg doses of compound **11** inhibited hepcidin production triggered by IL-6 (Fig. 1).

Although compound **11** showed a hepcidin lowering effect in oral administration, it inhibited many kinases at double the concentration of IC_{50} value (Fig. 2).

As multi kinase inhibitors are not considered to be suitable as medicines for chronic diseases, we decided to carry out the derivatization with the intention of reducing the kinase inhibitory activity. Generally, hydrogen bonds are among the most important specific interactions in biological recognition processes. In particular for kinases, hydrogen bonds with hinge backbone residues are considered the anchors of ligand binding, a generally indispensable interaction for potent enzyme inhibition.[9] We considered that the kinase inhibitory potency of **11** was probably due to its 3-aminoindazole moiety acting as a hinge binding site.[10]

Therefore, we designed a benzisoxazole scaffold, which replaced oxygen of the indazole ring with nitrogen. The benzisoxazole derivative **13** showed moderate hepcidin inhibitory activity (Table 5).

As expected, the kinase inhibitory potency of compound **13** was significantly reduced at double the concentration of IC_{50} value (Fig. 3).

Since we were able to find that the benzisoxazole scaffold had a lower kinase inhibitory activity, we then examined the substituent at the 3-position of benzisoxazole.

3,6-Disubstituted benzisoxazole derivatives were synthesized as illustrated in Scheme 1. Starting from a bromopyrazole **14**, *ortho*-fluorobenzonitrile **16** was obtained by Boc-protection and Suzuki-coupling reaction. Cyclization with acetohydroxamic acid yielded 3-amino-6-substituted benzisoxazole intermediate **17** accompanying Boc-deprotection. After reprotection of the pyrazole, amidation with acid chloride yielded 3-amide benzisoxazole derivative **19**.

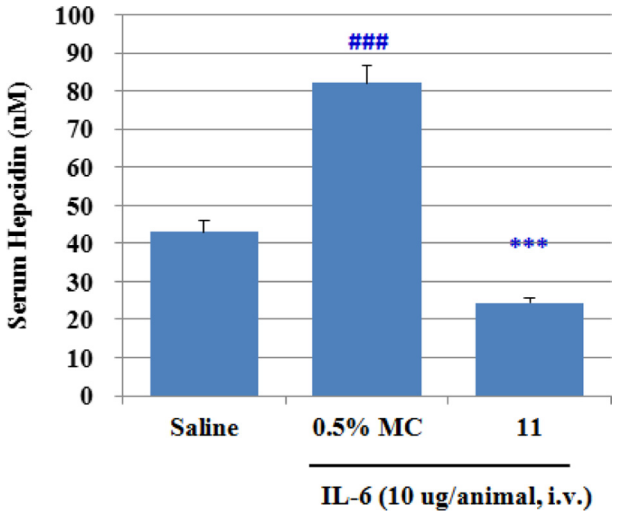
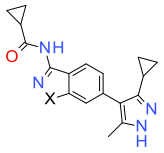


Fig. 1. Effect of compound **11**. The compound was administered to mice at dose of 30 mg/kg (p.o., 0.5% Methylcellulose, suspension, n = 4) before IL-6 treatment. ###, $p < 0.001$ vs Saline treated group (*t*-test), ***, $p < 0.001$ vs 0.5% MC treated group (*t*-test).

Table 5
In vitro activity of indazole and benzisoxazole scaffolds.



Compound	X	IC_{50} (μM)
12	NH	0.082
13	O	0.29

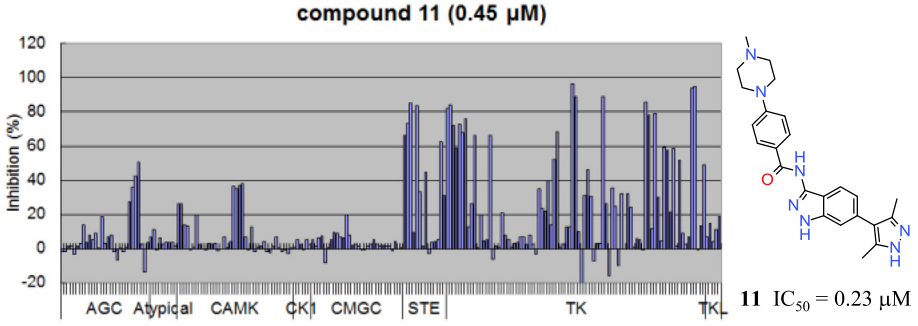


Fig. 2. IC_{50} values and kinase inhibitory profiles of compound **11**.

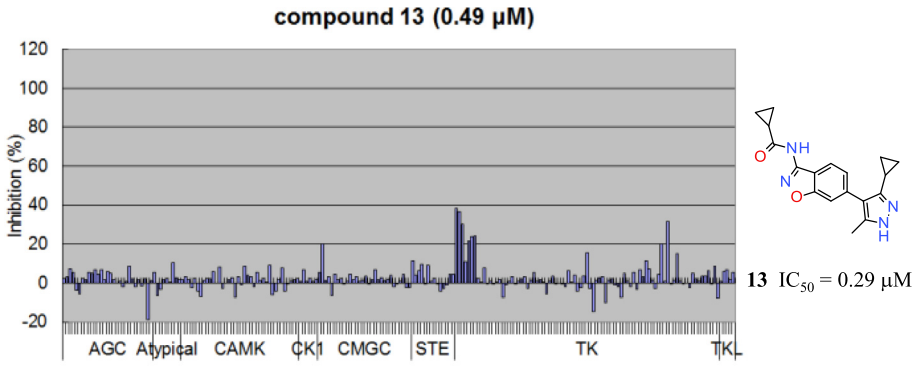
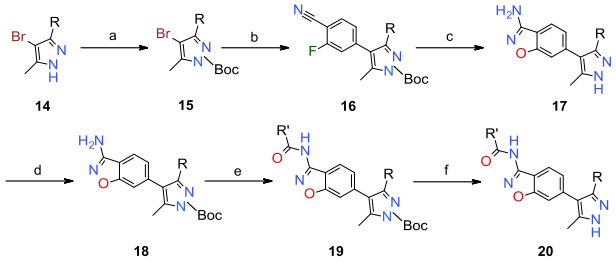
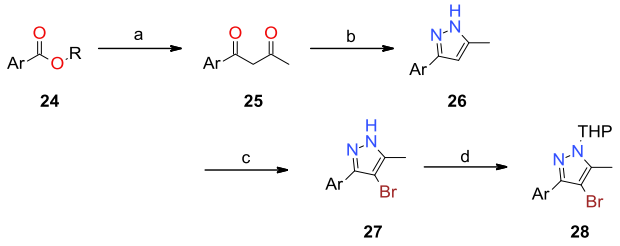


Fig. 3. IC_{50} values and kinase inhibitory profiles of compound **13**.



Scheme 1. Synthesis of 6-substituted benzisoxazole derivatives. Reagents and conditions: (a) Boc_2O, Et_3N, DMAP, THF, 63–99%; (b) 4-Cyano-3-fluorophenylboronic acid, Pd(dppf)Cl_2-CH_2Cl_2, K_3PO_4-nH_2O, 1,2-dimethoxyethane/H_2O, 7–58%; (c) Acetohydroxamic acid, potassium *tert*-butoxide, DMF, 58–100%; (d) Boc2O, Et_3N, DMAP, THF, 24–95%; (e) acyl chloride, pyridine, CH2Cl2 or alkyl chloroformate, pyridine, CH_2Cl_2 or Ethyl isocyanate, Et_3N, THF, 20–95%; (f) 4N-HCl/dioxane, 71–97%. (The Boc protected compounds were single regioisomer, but the regio of Boc groups were not determined.)



Scheme 2. Synthesis of 2-Aryl-5-methyl pyrazoles. Reagents and conditions: (a) Acetone, Sodium methoxide, THF, quant.; (b) Hydrazine monohydrate, EtOH, 69–94%; (c) *N*-bromosuccinimide, CH_2Cl_2/CCl_4, 55–100%; (d) 3,4-Dihydro-2H-pyran, p-Toluenesulfonic acid monohydrate, THF, 69–94%.

Using isocyanate and alkyl chloroformate in place of acyl chloride gave urea and carbamate derivatives. Finally, deprotection of the Boc group with an acidic condition gave the objective compounds.

The results of benzisoxazole derivatives variously functionalized on the 3-position are summarized in Table 6.

A primary amine derivative **21** showed a weaker activity. On the other hand, it was found that the carbamate derivative **22** showed a similar activity to compound **13**. Moreover, urea **23** maintained a moderate activity.

Next, various substituents on the pyrazole were examined. Regarding the preparation of various bromopyrazole **28**, the synthetic routes are summarized in Scheme 2. Arylmethylpyrazole **26** was prepared from aryl ester **24** by Claisen condensation and following cyclization with hydrazine monohydrate. The THP-protected bromopyrazole **28** was obtained from **26** by bromination and treatment of 2,3-dihydropyran with p-Toluenesulfonic acid.[11] The objective derivants were provided by the similar method illustrated in Scheme 1 with the use of bromopyrazole **28**.

The results of methylpyrazole derivatives variously substituted are summarized in Table 7.

As a consequence of tolerability of alkyl substituents, the bulkiness negatively affected the *in vitro* activity (compound **31**, **32**). Moreover, various polar groups such as ether, alcohol, ester, and amide deteriorated the activity (compounds **33**–**36**).

Presuming by the results of the effective cyclopropyl group, the sp^2-carbon character of the substituent was important for enhancement of the activity. As expected, the installation of the phenyl group resulted in a moderate activity. So it was found that the aromatic ring enhanced inhibitory activity, and we next focused on various heteroaromatic ring transformations. Results of the nitrogen-containing heteroaromatic ring, 2-pyridyl derivative **38**, provided a significant leap to *in vitro* activity.

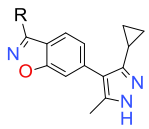
We next turned our attention to the introduction of a substituent to the versatile 2-pyridyl ring (Table 8).

Installation of a methyl group at the 3- and 4-positions of pyridine decreased activity (compounds **42**, **43**). In contrast, the 6-methylpyridine derivative **44** retained potent activity, but a bulky substituent at the 6-position weakened the activity (compound **46**). These results encouraged us to further optimize compound **38**.

Next, the substituent of carbamate at the 3-position was examined (Table 9).

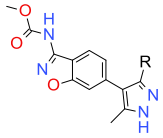
Linear substituents such as methyl carbamate **38** and ethyl carbamate **47** were acceptable, but the branched isopropyl carbamate **48** was slightly less active. Meanwhile, fluoroethyl carbamate **49** and methoxyethyl carbamate **50** showed preservation of the favorable hepcidin inhibitory activity.

Table 6
SAR of 3-substituted benzisoxazole derivatives.



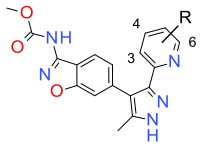
Compound	R	IC_{50} (μM)
13		0.29
21		1.4
22		0.21
23		0.72

Table 7
SAR of 6-substituted benzisoxazole derivatives.



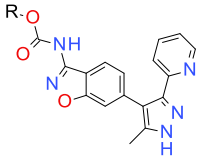
Compound	R	IC_{50} (μM)	Compound	R	IC_{50} (μM)
22	c-Pr	0.21	**35**	CO_2Me	2.9
29	Me	0.58	**36**	CONHMe	4.4
30	Et	0.46	**37**	Ph	0.54
31	i-Pr	0.91	**38**		0.039
32	t-Bu	>10	**39**		0.35
33	CH_2OMe	1.1	**40**		0.21
34	CH(OH)Me	1.2	**41**		2.9

Table 8
SAR of 2-pyridyl pyrazole derivatives.



Compound	R	IC_{50} (μM)	Compound	R	IC_{50} (μM)
38	H	0.039	**44**	6-Me	0.042
42	3-Me	4.8	**45**	6-OMe	0.40
43	4-Me	2.6	**46**	6-i-Pr	2.6

Table 9
SAR of substituted carbamate derivatives.



Compound	R	IC_{50} (μM)	Compound	R	IC_{50} (μM)
38	Me	0.039	**49**	FCH_2CH_2-	0.047
47	Et	0.040	**50**	$MeOCH_2CH_2$-	0.069
48	i-Pr	0.16			

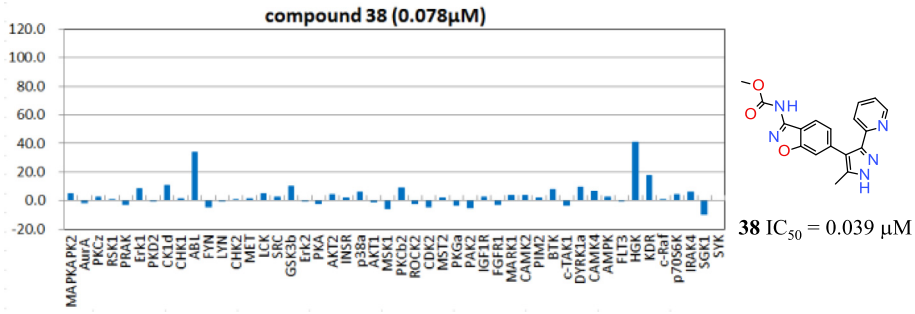


Fig. 4. IC_{50} values and kinase inhibitory profiles of compound **38**.

Table 10
Physicochemical properties and PK parameters of DS79182026.

LogD	MS[a] (%)	PB (free%)	Cmax[b] (μg/mL)	Tmax[b] (h)
2.5	87	17	9.17	1.0
AUC[b] (h*μg/mL)	Vd[c] (L/kg)	Cl[c] (mL/min/kg)	BA[b] (%)	
36.2	0.91	14.5	100	

[a] Remaining (%) of the tested compound after 0.5 h incubation with mouse liver microsomes (0.5 mg/mL).
[b] Average of two values dosed at 30 mg/kg p.o. with C57BL/6J mice (0.5% Methylcellulose, suspension).
[c] Average of two values dosed at 30 mg/kg i.v. with C57BL/6J mice (92 mM SBE7-β-CyD, solution).

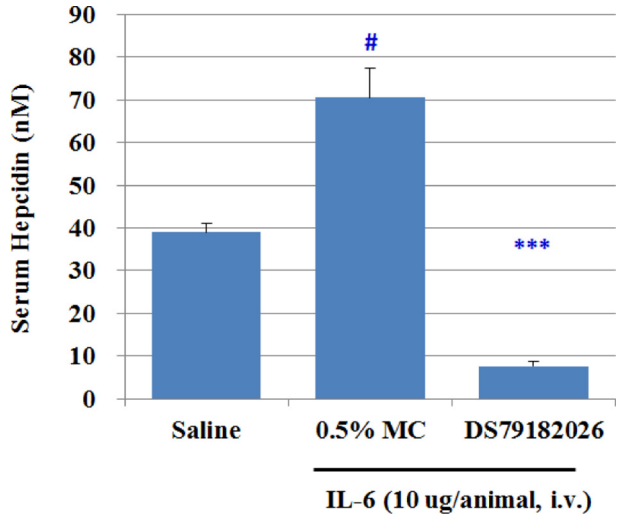


Fig. 5. Effect of DS79182026. The compound was administered to mice at dose of 30 mg/kg (p.o., 0.5% Methylcellulose, suspension, $n = 4$) before IL-6 treatment. #, $p < 0.05$ vs Saline treated group (*t*-test), ***, $p < 0.001$ vs 0.5% MC treated group (*t*-test).

In addition, the highly potent compound **38** retained its low kinase inhibitory activity through the derivatization at the pyrazole ring (Fig. 4).

Thus, orally active hepcidin production inhibitor **38**, named DS79182026, was selected for further investigations.

Pharmacokinetic (PK) parameters of DS79182026 were evaluated (Table 10).

Appropriate lipophilic compound DS79182026 possessed good metabolic stability and low plasma protein binding (*fu*,*p* = 17%). DS79182026 showed high plasma exposure in mice and was considered to have a suitable profile as an oral agent.

Next, the hepcidin lowering effect of DS79182026 was evaluated by mouse acute inflammatory model (Fig. 5).

DS79182026 significantly reduced blood hepcidin level at a dose of 30 mg/kg by the oral administration.

In conclusion, we discovered a series of benzisoxazole derivatives as potent and orally bioavailable hepcidin production inhibitors. Starting from the multi kinase inhibitor **1**, we acquired the benzisoxazole as a scaffold with a lower kinase inhibition by transformation of the hinge binding site.

With the optimization of substituents at the 3- and 6-positions, DS79182026 was found to possess potent *in vitro* activity. DS79182026 inhibited hepcidin production in HepG2 cells and lowered serum hepcidin levels in an IL-6 induced mouse acute inflammatory model.

The target molecule of the mechanism of action is not known exactly, but DS79182026 was found to be a promising compound as an oral agent showing *in vivo* efficacy.

The target identification to understand the mechanism of action of these compounds is ongoing and will be reported in due course.

Acknowledgments

We would like to thank Dr. Nakayama, Dr. Machinaga for their technical assistance and helpful discussions.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.07.004.

References

1. Ganz T. Hepcidin a regulator of intestinal iron absorption and iron recycling by macrophages. *Best Pract Res Clin Haematol.* 2005;18:171–182.
2. De Domenico I et al. Regulation of iron acquisition and storage: consequences for iron-linked disorders. *Nat Rev Mol Cell Biol.* 2008;9:72–81.
3. Krause A et al. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. *FEBS Lett.* 2000;480:147–150.
4. Nemeth E et al. IL–6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest.* 2004;113:1271–1276.

5. Weiss G et al. Medical progress: anemia of chronic disease. *N Engl J Med*. 2005;352:1011–1023.
6. Fukuda T et al. Synthesis and SAR studies of 3,6-disubstituted indazole derivatives as potent hepcidin production inhibitors. *Bioorg Med Chem Lett*. 2017;27:2148–2152. http://dx.doi.org/10.1016/j.bmcl.2017.03.056.
7. Hepcidin inhibitory activity of each compound was tested in HepG2 cell line, stimulated with 50 ng/mL of BMP6 for Hepcidin mRNA expression induction. Data fit for IC50 was determined using GraphPad Prism's nonlinear regression equation.
8. Meanwell Nicholas A. Synopsis of some recent tactical application of bioisosteres in drug design. *J Med Chem*. 2011;54:2529–2591.
9. Dy Grace K et al. Understanding, recognizing, and managing toxicities of targeted anticancer therapies. *CA Cancer J Clin*. 2013;63:249–279.
10. Xing Li et al. Kinase hinge binding scaffolds and their hydrogen bond patterns. *Bioorg Med Chem*. 2015;23:6520–6527.
11. McLaughlin M. The THP-protected pyrazoles were isolated the single regioisomer. The regiochemistry were determined by comparing the chemical shift of 2-position of tetrahydropyran with reported 1H NMR data. *J Org Chem*. 2008;73:4309–4312.

Please cite this article in press as: Fukuda T., et al. Bioorg. Med. Chem. Lett. (2017), http://dx.doi.org/10.1016/j.bmcl.2017.07.004