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Discovery of DS28120313 as a potent orally active hepcidin production inhibitor: Design and optimization of novel 4,6-disubstituted indazole derivatives

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ABSTRACT

Hepcidin has emerged as the central regulatory molecule in systemic iron homeostasis, and its inhibition could be a favorable strategy for treating anemia of chronic disease (ACD). Here, we report the design, synthesis and structure–activity relationships (SAR) of a series of 4,6-disubstituted indazole compounds as hepcidin production inhibitors. The optimization study of multi-kinase inhibitor **1** led to the design of a potent and bioavailable hepcidin production inhibitor, **32** (DS28120313), which showed serum hepcidin-lowering effects in an interleukin-6-induced acute inflammatory mouse model.

The maintenance of serum iron levels is important since high and low iron concentrations induce oxidative organ damage and iron deficiency anemia, respectively.[1]

Anemia of chronic disease (ACD), is the second most prevalent form 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 basic disease such as rheumatoid arthritis.[2] Some patients with ACD are known to present with iron deficiency despite abundant body iron stores (termed *functional iron deficiency*).

Hepcidin was originally discovered as an antibacterial peptide,[3] and this hormone is inducible by inflammatory cytokines such as interleukin (IL)-6,[4] in addition to iron signaling. This peptide hormone is the 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. Therefore, controlling hepcidin levels would be a promising therapeutic strategy for treating hepcidin-induced functional iron deficiency. Indeed, a few biologics (e.g., NOX-H94, LY2928057, and LY2787106) are proceeding to clinical trials for the treatment of anemia. Here, we describe the design and optimization process aimed at lowering the multi-kinase inhibitory activity of **1** to discover methyl [6-(3-cyclopropyl-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indazol-4-yl]carbamate (DS79182026, **32**), a potent orally available hepcidin production inhibitor.

As previously reported,[7,8] we identified the indazole derivative **1** as a potently active lead compound with a half-maximal inhibitory concentration (IC_{50}) of 0.23 μM.[9]

Although compound **1** showed a hepcidin-lowering effect following oral administration, it inhibited numerous kinases at double the concentration of the IC_{50} value (Fig. 1).

Multi-kinase inhibitors are not considered suitable as medicines for chronic diseases and, therefore, we decided to carry out a derivatization to reduce the kinase inhibitory activity of our lead compound. Hydrogen bonds are among the most important specific interactions in biological recognition processes. In particular, for kinases, hydrogen bonds with hinge backbone residues are consid-

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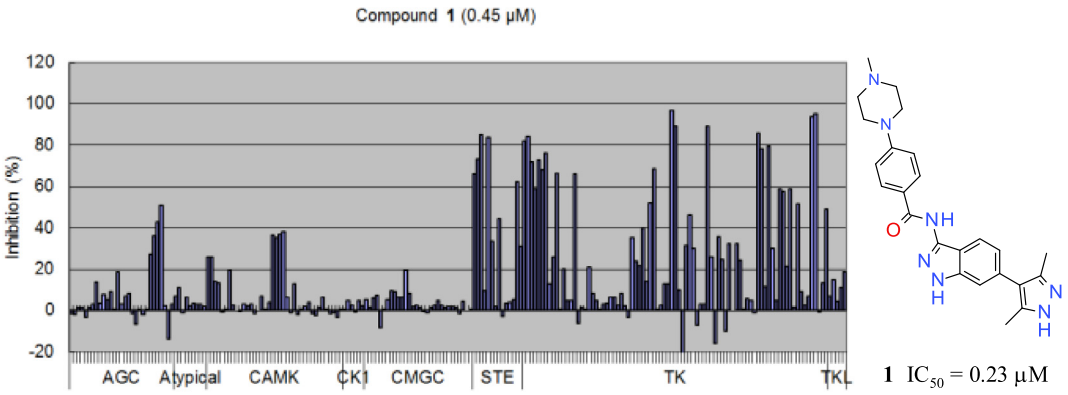
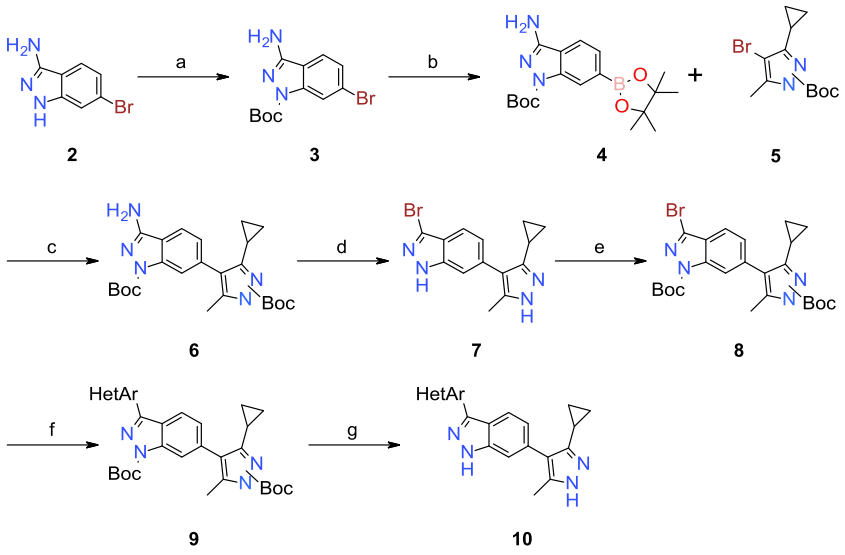


Fig. 1. IC_{50} values and kinase inhibitory profiles of compound **1**.



Scheme 1. Synthesis of 3-heteroaryl indazole derivatives. Reagents and conditions: (a) Boc_2O, Et_3N, DMAP, CH_3CN, 63%; (b) Bis(pinacolato)diboron, Pd(dppf)Cl_2-CH_2Cl_2, KOAc, 1,4-dioxane, 97%; (c) Pd(dppf)Cl2-CH2Cl2, K3PO4-nH2O, 1,2-dimethoxyethane/H2O, 91%; (d) CuBr, $NaNO_2$, HBr aq., AcOH/H_2O, 29%; (e) Boc_2O, Et_3N, DMAP, CH_3CN, 89%; (f) Heteroarylboronic acid, Pd(dppf)Cl_2-CH_2Cl_2, K_3PO_4-nH_2O, 1,2-dimethoxyethane/H_2O, 46–83%; (g) 4N-HCl/1,4-dioxane, 71–97%. (The Boc protected compounds were isolated as a single regioisomer, but the regio of Boc groups were not determined.)

ered the anchors of ligand binding, a generally indispensable interaction for potent enzyme inhibition.[10] We considered that the kinase inhibitory potency of **1** was probably due to its 3-aminoindazole moiety acting as a hinge binding site.[11] Therefore, we designed a scaffold, which transformed the amide moiety into a heteroaromatic ring. We thought that we could reduce the kinase inhibitory potency by transforming the NH group at 3-position of the indazole, which is thought to play the role of a hydrogen bond donor.

3-Heteroaryl substituted indazole derivatives were synthesized using the process illustrated in Scheme 1. The results of the analysis of the indazole derivatives with various functional groups on the 3-position are summarized in Table 1.

The transformation of amide substituents to simple heteroaromatic rings deteriorated the inhibitory activity (compounds **12**–**14**). However, the activity was effectively enhanced by introducing a cyclic amine moiety at the pyridine ring. Compounds **15** and **16** showed a significantly enhanced *in vitro* activity.

The 4-methylpiperazin-1-yl-pyridine derivative (**16**) showed good hepcidin inhibitory activity.

Although it improved slightly, compound **16** inhibited numerous kinases at a concentration that was 2-fold the IC_{50} value (Fig. 2).

We estimated that the hydrogen atom of the newly introduced pyridine ring functioned as a hydrogen bond donor. Therefore, we designed a new scaffold, which transferred the substituent from the 3- to 4-position of indazole. We hypothesized that the kinase inhibitory potency could be decreased by removing substituents that could function as hydrogen bond donors.

The 4-substituted indazole derivatives were synthesized using the process illustrated in Scheme 2.

The results of the analysis of the indazole derivatives with various functional groups on the 4-position are summarized in Table 2.

Compound **23** that lacked a substituent at the 3-position showed a considerably weaker activity than its parent compound, but compound **24** in which the amide moiety migrated to the 4-position showed moderate activity. The compounds with sterically smaller substituents on the amide group showed higher activity (**25** and **26**). The introduction of the 4-piperidynylaryl group,