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Identification of 4-(2-furanyl)pyrimidin-2-amines as Janus kinase 2 inhibitors

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ABSTRACT

Janus kinases inhibitor is considered to have therapeutic potential for the treatment of oncology and immune-inflammatory diseases. Two series of 4-(2-benzofuranyl)pyrimidin-2-amine and 4-(4,5,6,7-tetrahydrofuro[3,2-*c*]pyridin-2-yl)pyrimidin-2-amine derivatives have been designed and synthesized. Primary SAR studies resulted in the discovery of a novel class of 4,5,6,7-tetrahydrofuro[3,2-*c*]pyridine based JAK2 inhibitors with higher potency (IC_{50} of 0.7 nM) and selectivity (>30 fold) to JAK3 kinase than tofacitinib.

1. Introduction

Janus kinases (JAKs) are a family of non-receptor tyrosine kinase. They play an important role in hematopoiesis and immune response. Upon stimulation of cytokine signaling, JAKs function as subsequent activation of the downstream signal transducer and activator of transcription (STAT).[1] There are four isoforms identified in human: JAK1, JAK2, JAK3, and TYK2. Among these, JAK3 is expressed mostly in lymphoid cells and binds exclusively to the common γ-chain of the IL-2 family of receptors.[2] A mutation or loss of JAK3 results in severe combined immune deficiency.[3] In contrast, JAK2 is expressed on multiple cell transmitting signals of hormones and growth factors, which is critical for controlling the generation of blood cells from hematopoietic stem cells. JAK2 mutations lead to impaired erythropoiesis.[4] Besides, gene silencing studies showed that loss of JAK1 or TYK2 leads to defective lymphopoiesis.[5] Therefore, JAKs have been regarded as potentially effective targets for the treatment of oncology and immune-inflammatory diseases.

Indeed, several small-molecule JAK inhibitors have been developed for treatment of rheumatoid arthritis (RA), myelofibrosis, psoriasis, leukemia, lymphoma, etc (Fig. 1). Ruxolitinib was the first approved JAK inhibitor by FDA in late 2011 for use in myelofibrosis treatment.[6] Tofacitinib, a pan-JAK inhibitor, was launched for RA one year later.[7] Following that, impressive progress in this field has been made recently (Fig. 1).[8] According to the inhibition of primary isoform, they will be examplified by JAK3 inhibitor (VX-509[9]), JAK2 inhibitor (CEP-701,[10] SB1518,[11] LY2784544,[12] BMS-911543[13]), and JAK1 inhibitor (GLPG0634[14]). In contrast to the current JAK-targeting biological agents, they are featured by the action mechanism and oral availability.

The molecular structure of the JAK inhibitors is divided into four parts marked as A, B, C and D (Fig. 2). The structural cores of part A varied from pyrrolopyrimidines, pyrrolopyridines, 2-aminopyrimidines, to triazolopyridines. The tail C emerged mainly as amide, sulfonamide, and cyano group. By means of modification in part C, better selectivity could be achieved. For example, replacement of the N-(cyanomethyl)benzamide moiety (CYT387[15]) by 2-pyrrolidine carboxamide (XL019[16]) leads to more than 30-fold JAK2 selectivity enhancement within JAK family. Finally, the linker B could be the key to discover new lead structures. Pyrazole, pyrimidine, and benzene were successfully adopted to replace the tertiary amine linker of tofacitinib. Based on the knowledge of structural and molecular biology, inhibition of JAK2 may be an effective approach for the treatment of myeloproliferative neoplasms (MPNs).[17] During which, primary myelofibrosis (MF) represents a significant unmet clinical need. Structure modification of

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0968-0896/

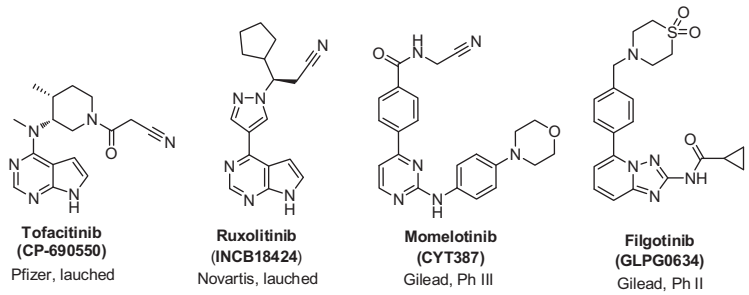

Figure 1. Representative JAK inhibitors.

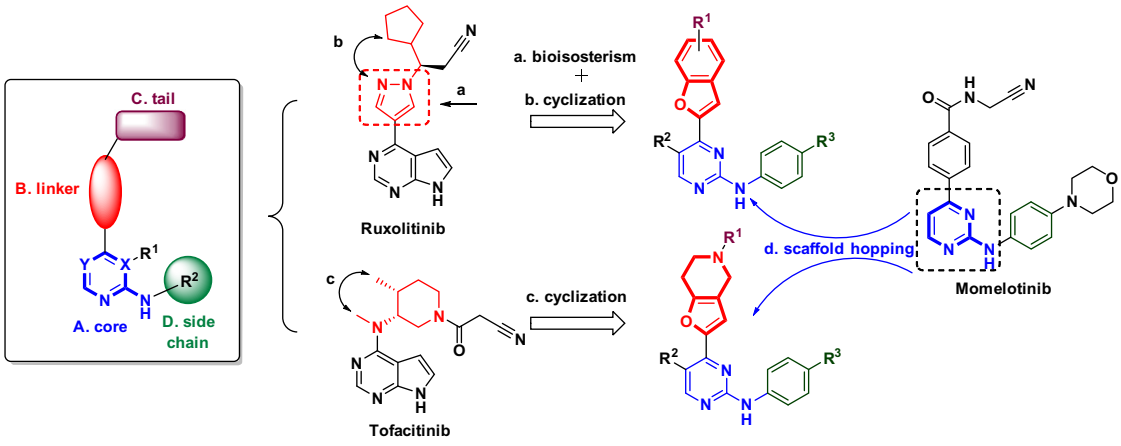

Figure 2. Design of the target compounds.

part B and C offers an opportunity to discover potentially selective JAK2 inhibitors.

The fused-furan was widely existed in the world, such as nature products, agrochemicals, pharmaceutical products, dyes and spices. The two kinds of fused-furan framework, benzofuran and 4,5,6,7-tetra-hydrofuro[3,2-*c*]pyridine moieties, came forth as privileged structures thereof commonly used in medicinal chemistry.

Based on the above considerations, pyrimidine, the core structure of momelotinib, was firstly choosed as our leading core. Based on the linker structure of Ruxolitinib, 4-(2-benzofuranyl)-pyrimidin-2-amine derivatives was designed with the strategy of bioisosterism (a, pyzole to furan) and cyclization (b). With the strategy of cyclization (c), 4-(4,5,6,7-tetrahydrofuro-[3,2-*c*]pyridin-2-yl)pyrimidin-2-amine derivatives was designed from the linker structure of tofacitinib. Herein we describe the synthesis, biochemical and cellular evaluation of the 4-(2-furanyl)pyrimidin-2-amine derivatives as JAK2 inhibitors.

2. Results and discussion

2.1. Chemistry

The novel 4-(2-benzofuranyl)pyrimidin-2-amine (**5a**–**5k**) and 4-(4,5,6,7-tetrahydrofuro[3,2-*c*]pyridin-2-yl)pyrimidin-2-amine derivatives (**16a**–**16h**) were prepared as illustrated in Schemes 1–4.

As indicated in Schemes 1 and 2, our key steps in the synthesis of 4-(2-benzofuranyl)pyrimidin-2-amine derivatives (**5a**–**5h**) involved two palladium-catalyzed cross-coupling reactions. Based on the reactivity difference between two chloride atoms of pyrimidine core, Suzuki coupling of 2,4-dichloro-5-methyl pyrimidine and 2-benzofuranyl boronic acid **3** gave compounds **4a**–**4d**, then Buchwald-Hartwig coupling with various anilines afforded compounds **5a**–**5f** (Scheme 1). When the substitute at the 5-position of pyrimidine was trifluoromethyl, the aniline moieties **6a**–**6b** were first installed selectively to 2-position of 2,4-dichloro-5-trifluoromethyl pyrimidine to generate intermediates **7a**–**7b** mediated by Lewis acid.[18] Then compound **7** was subjected to Suzuki coupling with 2-benzofuranyl boronic acids **3** to produce target compounds **5g**–**5h** (Scheme 2). Compound **5b** was hydrolyzed to generate free acid **5i**. Hydrolysis followed by condensation of ester **5d** produced α,β-unsaturated hydroximic acid **5j**. Removal Boc of **5g** gave free piperazine **5k** (Scheme 3).

As shown in Scheme 4, a similar method was used to construct the 4-(4,5,6,7-tetrahydrofuro-[3,2-*c*]pyridin-2-yl)pyrimidin-2-amine derivatives **16a**–**16h**. The key building block **12** used in this study was prepared from feedstock furfural for the first time, which avoids the need of more expensive 3-furaldehyde.[19] For this route, condensation of nitromethane with furfural, followed by reduction with $LiAlH_4$ in refluxing *tert*-butyl methyl ether to afford amine **10**. Then protection with benzyl and Pictet-Spengler reaction with aqueous formaldehyde gave 5-benzyl-4,5,6,7-tetrahydrofuro[3,2-*c*]pyridine hydrochloride **12**. Lithiation of **12** followed by quench with chlorotributyltin resulted compound **13**. Then Stille coupling of the tin regent **13** and 2,4-dichloro pyrimidine produced **14a**–**14c** with 71–89% yield. At this point, attempt to perform Suzuki coupling from the corresponding boronic acid failed.