4-Amino-pyrrolopyridine-5-carboxamide: A Novel Scaffold for JAK1-Selective Inhibitors

Heerim Shin,[#] Mi Kyoung Kim,[#] and Youhoon Chong*

Department of Bioscience and Biotechnology, Bio/Molecular Informatics Center, Konkuk University; Hwayang-dong, Gwangjin-gu, Seoul 143–701, Korea.

Received October 14, 2013; accepted December 24, 2013

Despite a high level of interest in selective Janus kinase 1 (JAK1) inhibitors and their potential for the treatment of inflammatory diseases such as rheumatoid arthritis (RA), only a few such inhibitors have been reported to date. In this study, a novel 4-amino-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxamide scaffold was designed through structural modification of the potent JAK1-selective inhibitor, C2-methyl imidazopyrrolopyridine. Among the series studied, the 4-(2-aminoethyl)amino-pyrrolopyridine derivative, 2j, exhibited a significant 24.7-fold JAK1/JAK2 selectivity along with reasonable inhibitory activity against JAK1 ($IC_{50}=2.2\ \mu M$). The noticeable JAK1-selectivity of 2j was then tackled through a molecular docking study, which showed that the aminoethyl functionality of 2j is well positioned to discriminate the subtle but significant difference in the size of the ligand binding sites between JAK1 and JAK2.

Key words Janus kinase 1 (JAK1); pyrrolopyridine; rheumatoid arthritis

The Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway, a major signaling cascade in response to inflammatory and proliferative signals, consists of the Janus protein tyrosine kinase family (JAK3, JAK2, JAK1, and TYK2) and STAT family of transcription factors. Upon ligand-receptor binding, the JAKs get activated, resulting in the phosphorylation, dimerization, and nuclear translocation of the downstream STAT proteins, which regulate the transcription of STAT-dependent genes. Depending on the specific cytokines coupled with JAKs, ligand-receptor binding culminates in an intracellular response such as an immune function,¹⁾ inflammation,²⁾ or hematopoiesis.^{3,4)} For instance, the gamma common (γ_c) family of cytokines, *i.e.*, interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21, is exclusively associated with JAK1 and JAK3,⁵⁾ and a loss-of-function mutation in the γ_c chain or JAK3 results in severe combined immunodeficiency (SCID). As a result, abrogation of signaling by γ_c -cytokines through the inhibition of γ_c -linked JAK3 has long been regarded as an attractive target for the treatment of immunologic disorders such as rheumatoid arthritis (RA).^{2,6)} Pfizer's Xeljanz (Tofacitinib, CP-690550), the first-in-class JAK inhibitor approved for the treatment of RA,⁷⁾ was initially reported to be a selective JAK3 inhibitor,⁶⁾ spurring many pharmaceutical companies to launch JAK3 medicinal chemistry programs.

However, it has become clear that tofacitinib is not as spe-

cific for JAK3 as initially thought, but it is rather a potent pan-JAK inhibitor,⁸⁾ suggesting that the clinical efficacy of the compound against RA is unlikely to be driven by the selective inhibition of JAK3. In addition, accumulated evidence has revealed that in signal transduction through γ_c -containing cytokine receptors, JAK1 plays a dominant role, while JAK3 exhibits only a secondary functional activity that merely enhances the effect of JAK1.^{9–13)} Accordingly, JAK1 inhibition has become a relevant treatment option for RA. For successful treatment of RA, JAK1 inhibitor has also been anticipated to have a high JAK1-over-JAK2 selectivity because the therapy-related anemia and thrombocytopenia observed with JAK2 inhibitors are particularly dangerous for patients receiving immunomodulating therapy.¹⁴⁾

Despite a high level of interest in selective JAK inhibitors and their potential for immune-modulating therapies, only a few JAK1 inhibitors have been reported to date.^{15,16)} Among them, C2-methyl imidazopyrrolopyridine (**1**, Fig. 1) shows potent and selective inhibitory activity against JAK1, *i.e.*, K_i of 10 nM, and JAK1 selectivity of 20,¹⁶⁾ which guided us to design a novel JAK1-selective inhibitor based on a pyrrolopyridine scaffold. 4-Amino-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxamide (**2**, Fig. 1), thus designed, has amino and carboxamide functionalities at the 4- and 5-positions of the pyrrolopyridine scaffold, respectively, which are anticipated to mimic the imidazole moiety of **1** through the formation of an intramolecular hydrogen bond. A short and modular synthesis of the structural variants for an extensive study on the structure–activity relationship is an additional advantage of exploring 4-amino-pyrrolopyridine-5-carboxamides as a scaffold for the design of a novel JAK1-selective inhibitor.

The synthesis of 4-amino-pyrrolopyridine-5-carboxamide derivatives **2a** through **k** was accomplished starting from the commercially available 7-azaindole (Chart 1). In a three-step sequence, 7-azaindole was selectively chlorinated at the 4-position to give **3** in 84% yield,¹⁷⁾ which was then treated with triisopropylsilyl chloride (TIPSCI) to provide N1-protected 4-chloropyrrolopyridine **4** in 76% yield.¹⁸⁾ The *ortho*-lithiation of **4**, followed by trapping with methyl chloroformate, furnished the key intermediate **5** in 65% yield.¹⁹⁾ Nucleophilic aromatic substitution of **5** with various amines,²⁰⁾ followed

Fig. 1. Structure of C-2 Methyl Imidazopyrrolopyridine (**1**) and 4-Amino-1*H*-pyrrolo[2,3-*b*]pyridine-5-carboxamide (**2**)

The authors declare no conflict of interest.

[#]These authors contributed equally to this work.

*To whom correspondence should be addressed. e-mail: chongy@konkuk.ac.kr

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Reagent and conditions: (a) mCPBA, DME/heptane; (b) POCl₃, 85°C; (c) NaOH, H₂O, pH 10; (d) NaH, TIPSCl, THF, 80°C; (e) *sec*-BuLi, THF, 30 min, -78°C; (f) methyl chloroformate, THF; (g) RNH₂, DIPEA, *i*-PrOH; (h) NaOH, EtOH; (i) EDC, NH₄Cl, HOBt, DIPEA, CH₃CN/DMF.

Chart 1. Syntheses of the Title Compounds, **2a–k**

by hydrolysis and amidation,²¹⁾ gave the desired products **2a** through **k** in combined yields of 23 to 61%.

The *in vitro* inhibitory activity of 4-amino-pyrrolopyridine-5-carboxamide derivatives **2a** through **k** on the JAK isozymes was determined using Z'-LYTE™ Kinase Assay Kit-Tyr 6 Peptide (JAK1 through JAK3) and Tyr 3 Peptide (Tyk2) (Invitrogen) according to the manufacturer's instruction. The pan-JAK inhibitor, "pyridine 6," was used as a positive control,²²⁾ and the percentage of inhibition of each JAK isozyme obtained using 10 μM of the title compounds is summarized in Table 1.

Depending on the amino functionality attached at the 4-position of the pyrrolopyridine scaffold, the title compounds can be divided into three groups: arylalkyl (**2a–d**), alkyl (**2e–h**), and hydroxyalkyl/aminoalkyl amine (**2i–k**) derivatives. Among these series, the alkylamine derivatives, **2e–k**, inhibited JAK1 kinase activity more efficiently than did the arylalkylamine-substituted derivatives, **2a–d**. Interestingly, the JAK1 inhibitory activity was significantly decreased with increased steric bulk to give compounds with unsubstituted and short amino functionality, **2a** and **e**, which were the most active in each series. In particular, pyrrolopyridine derivatives with short alkylamine substituents such as **2e** and **f** showed the most potent inhibitory activity against JAK1 with 65.4% and 58.2% inhibition at 10 μM, respectively (Table 1).

Based on the inhibitory activity at 10 μM concentration,

three compounds with promising JAK isozyme selectivity profiles were selected (**2i–k**) and their IC₅₀ values were evaluated (Table 2).

As anticipated, the 4-hydroxyalkylamino- (**2i**) and the 4-aminoalkylamino- (**2j,k**) pyrrolopyridine derivatives exhibited significant JAK1/JAK2 selectivity of 20.5–24.7, along with reasonable inhibitory activity against JAK1 (IC₅₀=2.2–2.6 μM). Also noteworthy is the JAK1/Tyk2 selectivity of 9.8–11.3 observed with this class of compound. In addition, the high membrane permeability of the most promising compound **2j** measured in a parallel artificial membrane permeability assay (PAMPA, log *P*_e=−3.21, Table 2)^{23,24)} warrants further development of the 4-aminoalkylamino-pyrrolopyridine derivatives as potential JAK1-selective inhibitors.

The noticeable JAK1 selectivity of this particular series of pyrrolopyridine derivatives was then tackled through a molecular docking study. The compound **2j** was therefore docked into the ATP-binding site of JAK1 (PDB ID=4EHZ)¹⁶⁾ and JAK2 (PDB ID=4F09)¹⁶⁾ by using the flexible ligand docking software Glide incorporated into the Schrödinger molecular modeling software suite. Although the ligand-binding sites of JAK1 and JAK2 share almost the same features, the best docking poses of **2j** for the two enzymes showed subtle, but significant, differences (Fig. 2).

A comparison of the ligand-binding sites of JAK1 and JAK2 revealed that the distance between the hinge motif,

i.e., Leu959/Glu957 for JAK1 and Leu932/Glu930 for JAK2, and the bottom of the ligand-binding site, *i.e.*, Arg1007/Asn1008 for JAK1 and Arg980/Asn981 for JAK2, is about 0.3–0.6 Å longer for JAK2 (the block arrow shown in Fig. 2). The docking pose of **2j** to JAK1 showed that the aminoethyl functionality of **2j** is well positioned for discriminating the different sizes of the ligand-binding sites of JAK1 and JAK2. Specifically, compound **2j** fits snugly in the ligand-binding site of JAK1 to form two hydrogen bonds with backbone and side-chain carbonyl groups of Arg1007 and Asn1008, respectively (Fig. 2). In contrast, owing to the larger binding site of JAK2, compound **2j** binds at a slightly higher region of the ligand-binding site (double-headed arrow shown in Fig. 2), resulting in the formation of only one hydrogen bond with the backbone carbonyl group of Arg980 (Fig. 2), and lowering the docking score (G-score=−8.22) compared to that of JAK1 (G-score=−8.45).

Overall, the title compounds showed significantly decreased activity compared with the C-2 methyl imidazopyrrolopyridine¹⁶⁾ (**1**, Fig. 1). Presumably, this loss of activity might have resulted from the competition for hinge motif binding between the 4-amino-5-carboxamide functionalities and the pyrrolopyridine scaffold. Nevertheless, this proof-of-concept study revealed that

Table 1. % Inhibition of the JAK Isozymes by 10 μM of the Title Compounds

Compound	% Inhibition ^{a)}			
	JAK1	JAK2	JAK3	Tyk2
2a	50.3±3.1	15.7±1.8	43.3±2.1	32.8±1.6
2b	41.4±2.6	15.9±1.2	46.8±3.4	13.5±0.8
2c	37.6±2.4	21.1±0.8	41.9±2.0	13.9±1.2
2d	27.1±1.9	21.2±0.8	48.3±1.5	35.7±1.7
2e	65.4±3.7	28.8±2.1	54.5±1.9	18.4±1.3
2f	58.2±2.1	30.7±2.3	42.6±2.0	21.5±1.5
2g	45.2±1.8	24.4±1.8	38.5±1.2	20.1±1.0
2h	40.7±2.2	29.4±2.3	45.5±2.1	31.6±1.9
2i	49.4±2.4	12.6±1.1	30.5±1.3	11.5±0.9
2j	52.6±3.2	10.4±0.8	32.5±1.7	15.7±1.3
2k	49.8±3.6	15.2±1.6	42.6±1.4	10.8±1.0
Pyridine 6 ^{b)} (nm)	4.3 (3.3) ^{c)}	1.3 (2.5) ^{c)}	12.6 (10.9) ^{c)}	5.1 (3.6) ^{c)}

a) Each experiment was repeated at least three times. b) IC₅₀ values (nm) of the pan-JAK inhibitor, 'pyridine 6'.²²⁾ c) IC₅₀ values reported in ref. 22.

Table 2. JAK Isozyme Selectivity and PAMPA Permeability of Three Selected Compounds (**2i–k**)

Compound	IC ₅₀ (μM)				Selectivity ^{a)}		log P _e (PAMPA) (cm/s) ^{b)}
	JAK1	JAK2	JAK3	Tyk2	JAK1/JAK2	JAK1/Tyk2	
2i	2.4	49.2	1.9	27.1	20.5	11.3	ND ^{c)}
2j	2.2	54.4	1.7	24.1	24.7	11.0	−3.21±0.02
2k	2.6	59.4	2.0	25.6	22.8	9.8	ND ^{c)}

a) Selectivity=IC₅₀ against JAK2 (or Tyk2)/IC₅₀ against JAK1. b) P_e (effective permeability) was determined at 25 μM concentration of the test compound after incubation for 1 h. c) Not determined.

the hydroxyalkyl and aminoalkyl substituents endow a 4-amino-pyrrolopyridine-5-carboxamide scaffold with JAK1 selectivity over JAK2, an observation supported through a molecular docking study. Based on the results, an extensive study on the structure–activity relationship of pyrrolopyridine derivatives as JAK1-selective inhibitors is ongoing, and the results will be reported elsewhere.

Acknowledgments This research was supported by a Grant from the Korean Health Technology R&D project, Ministry of Health & Welfare, Republic of Korea (A111698), by the Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093824), and by a Grant from Next Generation Bio-Green 21 PJ007982 (Korea Rural Development Administration).

Fig. 2. Superposition of the Docking Modes of **2j** to JAK1 (Atoms in Atom-Type Color, Ribbons in Pink) and JAK2 (Atoms and Ribbons in Green)

Hydrogen bonds are shown as dotted lines, and the size of the ligand binding site is denoted as a block arrow. The double-headed arrow indicates an upward shift of **2j** upon binding to JAK2. (Color images were converted into gray scale.)

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