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Brief Article

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# Benzimidazole Derivatives as Potent JAK1-Selective Inhibitors

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*KEYWORDS. Benzimidazole, Aminoalkyl, Piperidin-4-yl, Janus kinase 1, Selectivity.*

**ABSTRACT:** The Janus kinase (JAK) family comprises four members (JAK1, JAK2, JAK3, and Tyk2) that play a key role in mediating cytokine receptor signaling. JAK inhibition thus modulates cytokine-mediated effects. In particular, selective inhibition of JAK1 or JAK3 may provide an efficient therapeutic agent for the treatment of inflammatory diseases, with minimized side effects. In this study, as part of our continued efforts to develop a selective JAK1 inhibitor, a series of 1,2-disubstituted benzimidazole-5-carboxamide derivatives was prepared and their inhibitory activities against all four JAK isozymes were evaluated. A clear structure-activity relationship was observed with respect to JAK1 selectivity; this highlighted the importance of hydrogen bond donors at both $N^1$ and $R_2$ positions located within a specific distance from the benzimidazole core. One of the synthesized compounds, 1-(2-aminoethyl)-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole-5-carboxamide (**5c**), showed remarkable JAK1 selectivity (63-fold *vs* JAK2, 25-fold *vs* JAK3, and 74-fold *vs* Tyk2). Molecular docking revealed that the 2-aminoethyl and piperidin-4-yl substituents of **5c** function as probes to differentiate the ATP-binding site of JAK1 from that of JAK2, resulting in preferential JAK1 binding. A kinase panel assay confirmed the JAK1 selectivity of **5c**, which showed no appreciable inhibitory activity against 26 other protein kinases at 10 μM.

## INTRODUCTION

The cytoplasmic domains of cytokine receptors that mediate the intracellular signaling of type I/II cytokines are associated with specific Janus protein tyrosine kinases (JAK1, JAK2, JAK3, and Tyk2). For example, interleukins (IL) responsible for adaptive immune functions (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) specifically bind to cytokine receptors that contain a common gamma chain ($\gamma_c$, CD132) and are associated with intracellular JAK3 and JAK1.[1] JAK3 is characterized by its exclusive association with $\gamma_c$, and loss-of-function mutation of JAK3 results in complete abrogation of signaling by $\gamma_c$-cytokines; this causes severe combined immunodeficiency[2]. In contrast, JAK1 is widely expressed and, by pairing with JAK2 and/or Tyk2, is involved in a broad range of cytokine signaling to affect several proinflammatory cytokines associated with the innate immune response ($\gamma_c$, gp130, type I/II interferon, IL-6, IL-10 subfamily)[3]. JAK2 is exclusively associated with the receptors for various cytokines and growth factors (IL-3, IL-5, granulocyte macrophage colony-stimulating factor, erythropoietin, and thrombopoietin) and functions as a homodimer to play an essential role in red blood cell formation[4]. As a result, JAK2-knockout mice develop anemia[5], while a unique gain-of-function JAK2 point mutation (V617F) results in myeloproliferative disorders (MPD) in humans[6]. Taken together, studies of cytokine-mediated signaling have revealed pathologic roles of specific cytokines in inflammatory diseases and MPD, which can be abrogated by inhibition of the associated JAK isozymes[7]. Consequently, enormous efforts have been devoted to the development of JAK inhibitors, which culminated in the recent approval of two JAK inhibitors, ruxolitinib[8] and tofacitinib[9], for the treatment of MPD and rheumatoid arthritis (RA), respectively (Chart 1); ruxolitinib is a JAK1/JAK2-selective inhibitor with reduced potency against JAK3[8], while tofacitinib is a pan-JAK inhibitor that inhibits JAK1, JAK2, and JAK3 to an approximately equal extent[9]. However, it is still unclear whether

**Chart 1. Structures of the JAK inhibitors**

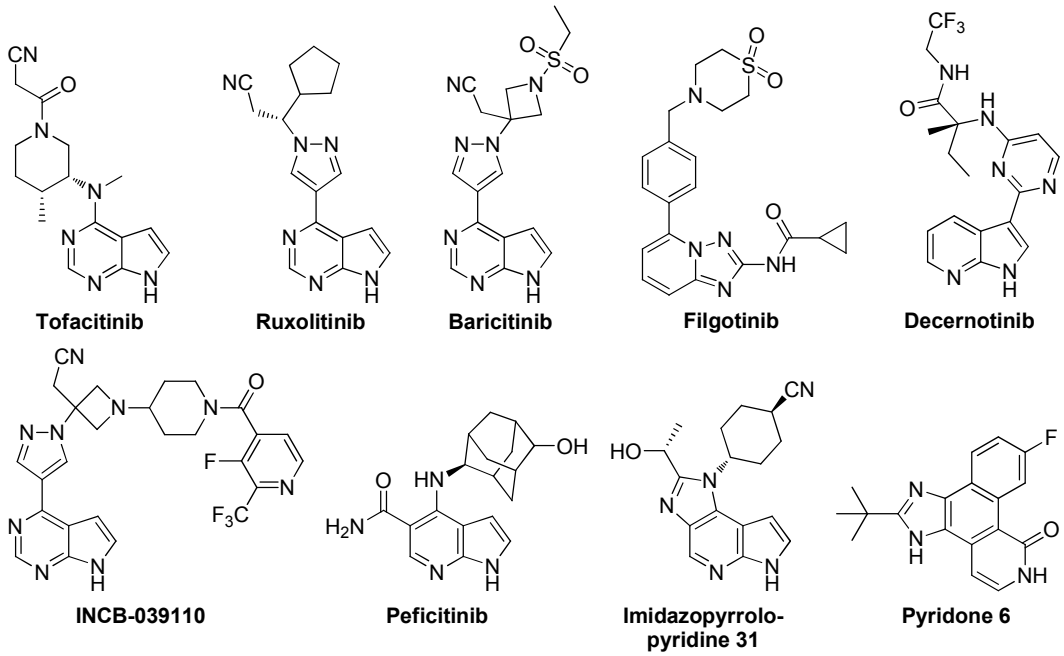



**Table 1. JAK1-selectivity of known inhibitors in enzyme assay**

| Compds | $IC_{50}$ (nM) | | | | Selectivity[a] | | |
|---|---|---|---|---|---|---|---|
| | JAK1 | JAK2 | JAK3 | Tyk2 | JAK1/JAK2 | JAK1/JAK3 | JAK1/Tyk2 |
| Baricitinib | 6 | 6 | >400 | 53 | 1 | >67 | 9 |
| Decernotinib | 11 | 13 | 2.5[b] | 11 | 1 | 0.2 | 1 |
| Filgotinib | 10 | 25 | 810 | 116 | 3 | 81 | 12 |
| INCB-039110[c] | - | - | - | - | >20 | >200 | - |
| Peficitinib | 3.9 | 5.0 | 0.71 | 4.8 | 1 | 0.2 | 1 |
| ABT-494 | - | - | - | - | 74[d] | - | - |
| Imidazopyrrolopyridine-**31** | 1.9[e] | - | 280[e] | 12[e] | 36 | 147 | 6 |

[a]Selectivity in enzyme assay = ($IC_{50}$ against JAK2, JAK3, or Tyk2) / ($IC_{50}$ against JAK1). [b]$K_i$ value. [c]Under development for oncology indications. [d]Selectivity in cellular assays. [e]Inhibition constants ($K_i$'s)

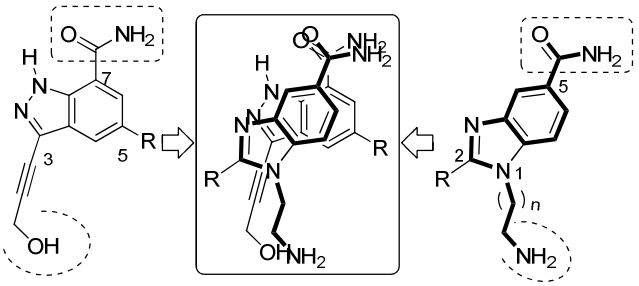
inhibition of multiple JAK isozymes is a prerequisite for the clinical efficacy of JAK inhibitors, or if an isozyme-selective inhibitor would show improved efficacy and safety profiles. In this context, it is worth noting that, even though the clinical efficacy of tofacitinib in patients with RA has been attributed to the suppression of JAK3 activity[8,10], a dominant role of JAK1 over JAK3 in RA has been strongly proposed[11]. On the other hand, a recent report by Pfizer stated that inhibition of either JAK1 or JAK3 was enough to abrogate cytokine signaling[12]. Accordingly, there is a compelling need for the development of selective JAK1 or JAK3 inhibitors. Moreover, from a therapeutic point of view, inhibition of JAK2 should be avoided because the induction of anemia, a dose-limiting side effect of tofacitinib, has been ascribed to the concomitant inhibition of JAK2[13].

Currently, several JAK inhibitors with different isoform specificity profiles[14-20] are in the clinical or preclinical stage for the treatment of autoimmune diseases including RA (Table 1). Among those, filgotinib[16], INCB-039110[17], ABT-494[19] and imidazopyrrolopyridine-**31** [20] are reported to show JAK1-selectivity while decernotinib[15] is the best characterized selective JAK3 inhibitor. In particular, ABT-494, a highly selective JAK1 inhibitor (74-fold vs. JAK2), turned out to be effective in RA patients without unfavorable effects on erythropoietin signaling and peripheral NK cell counts.[19] Thus, it is anticipated that the clinical studies with JAK1 or JAK3 selective inhibitors will confirm a relationship between the isoform selectivity and side-effect profiles. In this context, further investigation to identify novel scaffolds of JAK1-selective inhibitors is required.

Recently, our research group reported that 3-alkynolylindazole-7-carboxamide derivatives with a linear tether, 3-alkynol (dashed circle, Chart 2), which serves as an isozyme-specific probe group, showed selective inhibition of JAK1 over other JAK isozymes[21]. Based on this observation, the present study developed compounds with a novel

benzimidazole-5-carboxamide scaffold and an aminoalkyl functionality (dashed circle, Chart 2). In addition, to optimize selectivity and potency for the target enzyme, the benzimidazole core structure was further substituted with an alkyl substituent at the 2-position. Here, we describe the synthesis and biological evaluation of a series of these 1-aminoalkyl-2-alkyl-1*H*-benzo[*d*]imidazole-5-carboxamide derivatives, resulting in the identification of a potent JAK1 inhibitor with remarkable JAK1 selectivity.

**Chart 2. Design of the title compound**




## RESULTS AND DISCUSSION

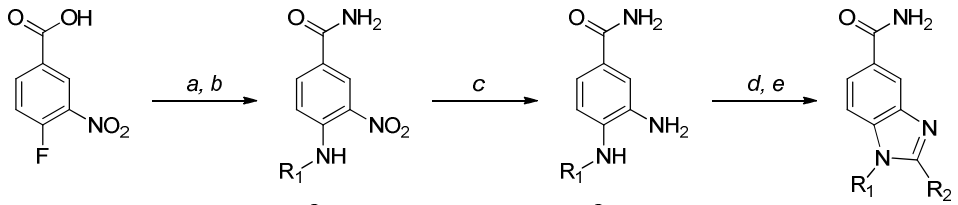
The 1-aminoalkyl-2-alkyl-1*H*-benzo[*d*]imidazole-5-carboxamides (**4** and **5**), were prepared from commercially available 4-fluoro-3-nitrobenzoic acid (**1**) in 5 steps (Scheme 1). First, EDC [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide] coupling of **1** with 1-hydroxy-1*H*-benzotriazole (HOBT)-ammonium salt ($NH_3$·HOBT) in a 4:1 mixture of $CH_3CN$ and *N*,*N*-dimethylformamide (DMF) yielded the corresponding benzamide, which underwent nucleophilic aromatic substitution by 1,3-diaminopropane or ethylenediamine followed by *N*-Boc protection, to give the corresponding 4-alkylamino-3-nitrobenzamide (**2a** or **2b**), with 75% or 79% yield. The nitro functionality of **2a** or **2b** was then reduced to give the key intermediate, 4-alkylamino-3-aminobenzamide (**3a** or **3b**), with 67% or 91% yield. These diaminobenzamides were then cyclized to the corresponding benzimidazole-5-carboxamides by treatment with the appropriate aldehydes in the presence of sodium hydrogen sulfite ($NaHSO_3$)[22] with 10–67% yields. Finally, deprotection of the Boc groups provided the desired compounds (**4** and **5**).

Z′-LYTE™ Kinase Assay Kits (Invitrogen) were used to determine the *in vitro* inhibitory activity of the benzimidazole derivatives against the JAK isozymes; the Tyr 6 peptide was used to analyze JAK1 and JAK3 effects and the Tyr 3 peptide was used for Tyk2. Pyridone-6 (Chart 1)[23], a pan-JAK inhibitor which is used for quality control of the assay kits, was used as a positive control. Assays were performed at the ATP $K_m$ and the $IC_{50}$ values of the synthesized compounds against each JAK isozyme, as well as their JAK1-selectivities, are summarized in Table 2. The benzimidazole derivatives with a 3-aminopropyl group substituted at the $N^1$ position (**4a** and **4b**) showed only moderate inhibitory activity against JAK1 ($IC_{50}$ = 6.1 and 11.3 μM, respectively) and JAK3 ($IC_{50}$ = 1.5 and 1.8 μM, respectively). Surprisingly, however, shortening the 3-aminopropyl chain of **4a** by one carbon potentiated the JAK1-inhibitory activity of the resulting benzimidazole derivative **5a** by more than 60-fold ($IC_{50}$ = 0.1 μM) without altering inhibition of other JAK isozymes. As a result, the 2-aminoethyl-substituted benzimidazole derivative **5a** showed high JAK1 selectivity over other JAK isozymes (29-fold *vs* JAK2, 15-fold *vs* JAK3, and 31-fold *vs* Tyk2). Based on this observation, the $N^1$ substituent was fixed as a 2-aminoethyl group and a series of benzimidazole derivatives with various $R_2$ substituents was prepared (**5b–5o**). Within the series, the $R_2$-alkyl derivatives (**5a–5d**) showed a clear structure-activity relationship whereby an amino group located within a specific distance from the benzimidazole core played a key role in maintaining potent JAK1-inhibitory activity. Thus, compounds with 2-aminoethyl (**5a**) or piperidin-4-yl (**5c**) functionalities at $R_2$ showed potent inhibitory activity against JAK1, with $IC_{50}$ values of 0.1 and 0.05 μM, respectively, while derivatives with a longer $R_2$-alkylamino group [**5b**, $R_2$ = $(CH_2)_3NH_2$] or with an $R_2$-hydrocarbon substituent [**5d**, $R_2$ = cyclohexyl] were significantly less potent. On the other hand, the $R_2$-alkyl substituents did not affect the inhibitory activities of the corresponding benzimidazole derivatives (**5a–5d**) against other JAK isozymes (JAK2, JAK3, and Tyk2). Taken together, the $R_2$-dependence of the inhibitory activities of these benzimidazole derivatives was specific to JAK1, and compound **5c**, with a piperidin-4-yl group at $R_2$, showed remarkable JAK1 selectivity (63-fold *vs* JAK2, 25-fold *vs* JAK3, and 74-fold *vs* Tyk2). On the other hand, among the benzimidazole derivatives with an aromatic substituent at $R_2$ (**5e–5o**), only **5k** ($R_2$ = 4-OH-Ph) with a hydroxyl group at the 4-position of the aromatic group showed JAK1-selective inhibitory activity (11-fold *vs* JAK2, 20-fold *vs* JAK3, and 26-fold *vs* Tyk2). Based on these observations, common structural features within the set of JAK1-selective inhibitors (**5a**, **5c**, and **5k**) could be identified; an alkyl chain bearing a hydrogen bond donor (-NH or -OH) is substituted at the 1-position of the benzimidazole core.

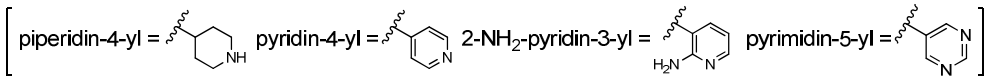
In a separate experiment, the inhibitory activities of **5c** against the JAK isozymes were measured in the presence of 1 mM ATP (Table 3), instead of at the $K_m$[12,24], in order to mimic the physiological level of ATP. As expected[12,24], the observed $IC_{50}$ values for **5c** were 114–205-fold higher in the presence of 1 mM ATP than those obtained at the $K_m$ values (Table 2); these data shifts were smallest for JAK1, which has the highest $K_m$ for ATP. As a result, the JAK1 selectivity of **5c** became more prominent under these physiologically relevant assay conditions.

The JAK1 selectivity of this benzimidazole derivative was then investigated using a molecular docking study. The most potent JAK1-selective inhibitor, **5c**, was docked into the ATP-binding site of JAK1 (PDB ID = 4EHZ)[20] or JAK2 (PDB ID = 4Fo9)[20] using the flexible ligand docking software, Glide, incorporated into the Schrödinger molecular modeling software suite; the best docking poses are shown in Figure 1. The ATP-binding sites of JAK1 and JAK2 are differentiated by two amino acids, one in the

**Scheme 1.** Synthesis of the benzimidazole derivatives (**4** and **5**).




| | $R_1$ | $R_2$ | | $R_1$ | $R_2$ | | $R_1$ | $R_2$ |
|---|---|---|---|---|---|---|---|---|
| **4a** | $(CH_2)_3NH_2$ | $(CH_2)_2NH_2$ | **5e** | $(CH_2)_2NH_2$ | Ph | **5j** | $(CH_2)_2NH_2$ | 4-$CF_3$-Ph |
| **4b** | | Ph | **5f** | | $CH_2$Ph | **5k** | | 4-OH-Ph |
| **5a** | $(CH_2)_2NH_2$ | $(CH_2)_2NH_2$ | **5g** | | 4-CN-Ph | **5l** | | 2,4-di-OH-Ph |
| **5b** | | $(CH_2)_3NH_2$ | **5h** | | 4-$CH_3$-Ph | **5m** | | pyridin-4-yl |
| **5c** | | piperidin-4-yl | **5i** | | 4-*i*Pr-Ph | **5n** | | 2-$NH_2$-pyridin-3-yl |
| **5d** | | cyclohexyl | | | | **5o** | | pyrimidin-5-yl |




*Reagent & Conditions* : (a) EDC, $NH_3$-HOBT, DMF/$CH_3CN$; (b) $R_1NH_2$, DIPEA, i-PrOH, 80 °C; (c) $H_2$, Pd/C, MeOH; (d) $R_2CHO$, $NaHSO_3$, MeOH, reflux (e) HCl, MeOH

**Table 2. Inhibitory activities of the benzimidazole derivatives for the indicated enzymes**

| Compds | $IC_{50}$ (μM)[a] | | | | Selectivity[b] | | |
|---|---|---|---|---|---|---|---|
| | JAK1 | JAK2 | JAK3 | Tyk2 | JAK1/JAK2 | JAK1/JAK3 | JAK1/Tyk2 |
| **4a** | 6.1±0.8 | 2.2±0.3 | 1.5±0.1 | 6.4±0.9 | 0.4 | 0.2 | 1 |
| **4b** | 11.3±0.8 | 20.5±1.2 | 1.8±0.3 | 2.7±0.1 | 2 | 0.2 | 0.2 |
| **5a** | 0.1±0.01 | 2.9±1.3 | 1.5±0.3 | 3.1±0.2 | 29 | 15 | 31 |
| **5b** | 2.2±0.3 | 1.6±0.2 | 1.1±0.1 | 2.5±0.2 | 0.7 | 0.5 | 1 |
| **5c** | 0.05±0.01 | 3.15±0.2 | 1.26±0.1 | 3.7±0.3 | 63 | 25 | 74 |
| **5d** | 4.3±0.3 | 4.1±0.2 | 2.5±0.1 | 3.3±0.2 | 1 | 0.6 | 0.8 |
| **5e** | 11.6±1.2 | 2.8±0.3 | 14.1±0.6 | 4.2±0.2 | 0.2 | 1 | 0.4 |
| **5f** | 18.2±0.7 | 2.7±0.3 | 2.1±0.3 | 7.5±0.2 | 0.1 | 0.1 | 0.4 |
| **5g** | 2.9±0.2 | 2.6±0.3 | 1.9±0.3 | 2.5±0.1 | 0.9 | 0.7 | 0.9 |
| **5h** | 3.7±0.2 | 2.6±0.1 | 2.3±0.1 | 3.5±0.3 | 0.7 | 0.6 | 0.9 |
| **5i** | 3.1±0.1 | 5.9±0.5 | 1.9±0.2 | 2.7±0.2 | 2 | 0.6 | 0.9 |
| **5j** | 9.3±0.5 | 4.8±0.2 | 2.1±0.2 | 3.2±0.3 | 0.5 | 0.2 | 0.3 |
| **5k** | 0.1±0.0 | 1.3±0.2 | 2.4±0.1 | 3.2±0.1 | 11 | 20 | 26 |
| **5l** | 3.1±0.3 | 2.5±0.2 | 2.9±0.3 | 3.7±0.1 | 0.8 | 0.9 | 1 |
| **5m** | 32.4±1.4 | 13.5±0.9 | 10.5±0.4 | 5.8±0.2 | 0.4 | 0.3 | 0.2 |
| **5n** | 8.1±0.4 | 4.7±0.6 | 1.9±0.2 | 4.3±0.5 | 0.6 | 0.2 | 0.5 |
| **5o** | 3.4±0.1 | 1.6±0.2 | 2.5±0.1 | 2.8±0.3 | 0.5 | 0.7 | 0.8 |
| **Pyridone-6**[c] | 0.004 | 0.001 | 0.01 | 0.005 | 2 | 0.2 | 0.2 |

The ATP concentration ($K_m$) was 87 μM for JAK1, 35 μM for JAK2, 16 μM for JAK3, and 25 μM for Tyk2. [a]The means ± SD of three independent experiments are shown. [b]Selectivity = ($IC_{50}$ against JAK2, JAK3, or Tyk2) / ($IC_{50}$ against JAK1). [c]Pan-JAK inhibitor[23] used as a positive control in this study.

αD-helix (Glu966 in JAK1, Asp939 in JAK2) and the other in the glycine-rich loop (His885 in JAK1, Asn859 in JAK2) (Figure 1). Comparison of these two pairs of amino acid residues (Glu966 vs Asp939, His885 vs Asn859) revealed

**Table 3. Inhibitory activity of 5c against JAK isozymes in the presence of 1 mM ATP.**

| Compds | $IC_{50}$ (μM)[a] | | | | Selectivity[b] | | |
|---|---|---|---|---|---|---|---|
| | JAK1 | JAK2 | JAK3 | Tyk2 | JAK1/JAK2 | JAK1/JAK3 | JAK1/Tyk2 |
| **5c** | 5.7±0.8 | 437.3±3.1 | 232.5±7.5 | 759.5±5.6 | 76 | 41 | 133 |
| **Pyridone-6** | 0.6±0.1 | 1.4±0.3 | 0.9±0.2 | 1.7±0.3 | 2 | 1 | 3 |

[a]The means ± SD of three independent experiments. [b]Selectivity = ($IC_{50}$ against JAK2, JAK3, or Tyk2) / ($IC_{50}$ against JAK1).

that they had different side chain lengths. Thus, amino acids with longer side chains, Glu966 (JAK1) and Asn859 (JAK2), protrude into the ATP-binding site, which results in constriction of ligand binding in the "upper" portion of JAK1 (Glu966–Leu881, 5.2 Å, Figure 1a) and the "lower" portion of JAK2 (Arg980–Asn859, 4.1 Å, Figure 1b).

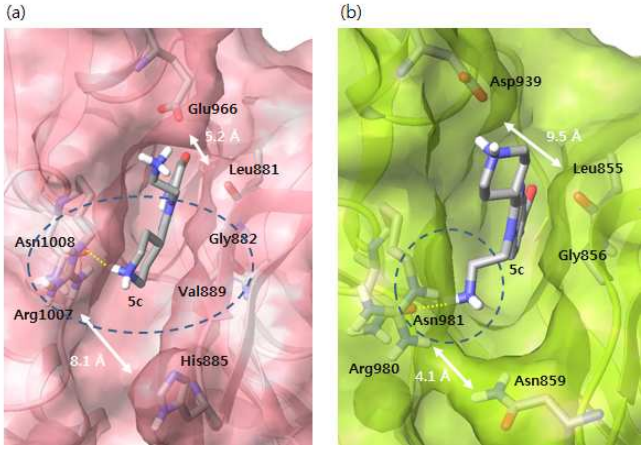



**Figure 1.** Docked structures of **5c** to (a) JAK1 (PDB ID: 4EHZ) and (b) JAK2 (PDB ID: 4Fo9). Dotted lines show hydrogen bonds. Hydrophobic contacts between **5c** and the ATP-binding sites are shown as dashed circles. Block arrows indicate the widths of the ATP-binding sites.

Interestingly, the best docking poses of **5c** to JAK1 and JAK2 showed that the more sterically demanding piperidin-4-yl moiety of **5c** was positioned away from the constricted area. The binding mode of **5c** to JAK1 is characterized by positioning the piperidin-4-yl group at the "lower" open side of the ATP-binding pocket (Figure 1a), forming a hydrogen bond with Asn1008 and multiple favorable contacts with Arg1007, Gly882, Val889, and Asp1021. In addition, the smaller and charged 2-aminoethyl group is placed near the polar side chain of Glu966. On the other hand, upon binding to JAK2, the benzimidazole moiety of **5c** rotates about the aryl-carbonyl bond (~190.4°) to locate the 2-aminoethyl and piperidin-4-yl substituents at the opposite side of the ATP-binding pocket (Figure 1b); the piperidin-4-yl group, at the upper side of the ATP-binding pocket, is located between Asp939 and Leu855, while the 2-aminoethyl functionality forms a hydrogen bond to Asn981 at the bottom of the ATP-binding site. Overall, the binding mode of **5c** to JAK2 is less favorable because of the lack of hydrophobic contacts around the 2-aminoethyl substituent, as well as the positioning of the nonpolar hydrocarbons in the piperidin-4-yl ring between the polar side chain of Asp939 and the backbone carbonyl group of Leu855. The preference of **5c** for JAK1 was reflected by the docking scores (G-score; -8.95 for JAK1 and -7.75 for JAK2). Taken together, this molecular docking information clearly showed that the 2-aminoethyl and piperidin-4-yl moieties (attached to the benzimidazole core of **5c**) differentiate the ATP-binding sites of JAK1 and JAK2; **5c** preferentially binds to the ATP-binding site of JAK1 by positioning the bulkier piperidin-4-yl substituent in the open space and forming multiple favorable contacts with the enzyme.The commercial KinaseProfiler™ Service (Eurofins Scientific, Inc.) was utilized to evaluate the selectivity of compound **5c** against a panel of 27 kinases (Figure 2). KinaseProfiler™ assay protocols measure the percent inhibition of phosphorylation of a peptide substrate in the presence of fixed concentrations of ATP that were close to the $K_m$ for each kinase (Figure 2). Except JAK1, no appreciable inhibition of these protein kinases was observed in the presence of 10 μM **5c**, with most maintaining >65% of their control activity.

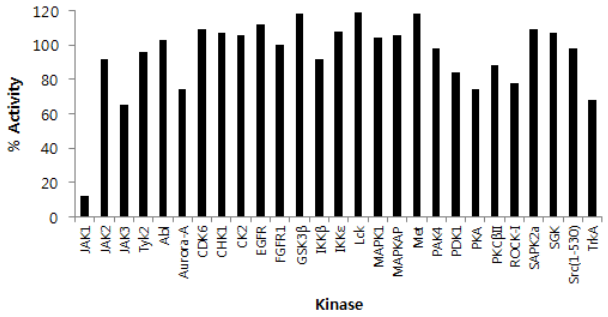



**Figure 2.** Kinase panel assays were performed in the presence of **5c** (10 μM) and ATP ($K_m$ concentration) using Eurofins KinaseProfiler™ service. The y-axis represents percentage of remaining kinase activity (% Activity).

However, due to high polarity, the JAK1-selective inhibitor **5c** showed low PAMPA permeability ($P_e$ = 0.1 x $10^{-6}$ cm/s), lack of activity in cell-based assay[25] (Supporting Information) and poor oral bioavailability (0.7%) in rat. On the other hand, **5c** demonstrated generally favorable pharmacokinetic properties: following intravenous (i.v.) administration to rats, **5c** exhibited moderate volumes of distribution ($V_{ss}$, 0.5 l/kg) and low systemic plasma clearance (CL, 0.4 l/hr/kg) resulting in plasma half-life ($t_{1/2}$) of 9.1 h (Table S1, Supporting Information). In light of the unsatisfactory pharmacokinetic properties, the JAK1-selective 1,2-disubstituted benzimidazole-5-carboxamide scaffold needs further optimization.

## CONCLUSIONS

Since each JAK isozyme is employed in various cytokine pathways regulating immune function, specific inhibition of a single JAK isozyme can provide a beneficial therapeutic option in patients with immunologic disorders such as RA. Pfizer's pan-JAK inhibitor, tofacitinib, was recently approved for the treatment of RA, and its clinical efficacy has been attributed to the suppression of JAK3 activity. However, since both JAK1 and JAK3 are associated with $\gamma_c$ cytokine receptors, it is unclear whether the clinical efficacy of tofacitinib results from its concomitant inhibition of JAK3 and JAK1, or selective inhibition of one JAK isozyme. The development of selective JAK1 or JAK3 inhibitors, and investigation of their effects, is therefore required in order to address this issue. For this purpose, we set out to develop JAK1-selective inhibitors, based on our previous observations that a linear alkyl tether can function as a probe to differentiate the ATP-binding site of JAK1 from those of the other JAK isozymes. We synthesized a series of 1,2-disubstituted benzimidazole derivatives and evaluated their inhibitory activities against all four JAK isozymes. These benzimidazole derivatives showed clear structure-activity relationships that confirmed the importance of hydrogen bond donors at both $N^1$ and $R_2$ positions for JAK1 selectivity; a 2-aminoethyl group and an alkyl chain bearing a hydrogen bond donor were favored at $N^1$ and $R_2$, respectively. Compound **5c**, with a piperidin-4-yl group at $R_2$, showed unprecedented JAK1 selectivity (63-fold *vs* JAK2, 25-fold *vs* JAK3, and 74-fold *vs* Tyk2). A molecular docking study revealed that **5c** preferentially bound to the ATP-binding site of JAK1 by positioning the bulkier piperidin-4-yl substituent at the open space and by forming multiple favorable contacts with the enzyme. A kinase panel assay also showed that compound **5c** (10 μM) did not inhibit a panel of 26 other protein kinases. The JAK1-selective benzimidazole derivatives identified in this study thus have the potential to elucidate the biological consequences of specific JAK1 inhibition, specifically in terms of therapeutic intervention for RA.

## EXPERIMENTAL SECTION

**General procedure for the preparation of 3-amino-4-alkylaminobenzamide (3a and 3b).** Palladium on charcoal (10% w/w) was added to a solution of **2** (1.4 mmol) in MeOH (15 ml) and charged with hydrogen (balloon, 1 atm). After stirring for 8 h at room temperature, solvent was removed under reduced pressure to give a crude product which was purified by column chromatography ($SiO_2$, $CH_2Cl_2$:MeOH:$NH_4OH$:water = 60:35:5:5) to give **3**.

**General procedure for the preparation of benzimidazole derivatives 4 and 5.** Preparation of 2-(2-aminoethyl)-1-(3-aminopropyl)-1*H*-benzo[*d*]imidazole-5-carboxamide (**4a**) is provided as a representative example. A solution of **3a** (22 mg, 0.07 mmol) in MeOH (3 ml) was added to *tert*-butyl (3-oxopropyl)carbamate (14 mg, 0.08 mmol) and sodium bisulfate (13 mg, 0.1 mmol). After stirring for 1 h at 60°C, inorganic material was collected by filtration and rinsing with MeOH. The filtrate was concentrated under reduced pressure to give a crude product, which was purified by column chromatography ($SiO_2$, $CH_2Cl_2$:MeOH:$NH_4OH$:water = 80:20:1:1) to give the benzoimidazole-derivative as a dark yellow powder. This benzoimidazole-derivative was then dissolved in MeOH (3 ml) and treated with 6 N HCl (0.1 ml). The reaction mixture was stirred at room temperature for 8 h and then concentrated under reduced pressure. The crude product was purified by column chromatography ($SiO_2$, $CH_2Cl_2$:MeOH:$NH_4OH$:water = 60:35:5:5) to give **4a** (11 mg, 60% yield) as a yellow powder.

## ASSOCIATED CONTENT

### Supporting Information

General experimental procedures, $^1H$ and $^{13}C$ nuclear magnetic resonance (NMR) spectra for new compounds, and methods for the molecular docking study. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Author Contributions

All authors have given approval to the final version of the manuscript.

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## ABBREVIATIONS

JAK, Janus kinase; IL, interleukin; MPD, myeloproliferative disorders; RA, rheumatoid arthritis; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBT, 1-hydroxy-1*H*-benzotriazole; DMF, *N*,*N*-dimethylformamide; Boc, *tert*-butoxycarbonyl; EtOAc, ethyl acetate; MeOH, methanol; PAMPA, parallel artificial membrane permeability assay.

## REFERENCES

(1) Rochman, Y.; Spolski, R.; Leonard, W. J. New insights into the regulation of T cells by $\gamma_c$ family cytokines. *Nat. Rev. Immunol.* **2009**, *9*, 480-490.

(2) Noguchi, M.; Yi, H.; Rosenblatt, H. M.; Filipovich, A. H.; Adelstein, S.; Modi, W. S.; McBride, O. W.; Leonard, W. J. Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. *Cell* **1993**, *73*, 147-157.

(3) Schindler, C.; Levy, D. E.; Decker, T. JAK-STAT signaling: from interferons to cytokines. *J. Biol. Chem.* **2007**, *282*, 20059-20063.

(4) Wu, H.; Liu, X.; Jaenisch, R.; Lodish, H. F. Generation of committed erythroid BFU-E and CFU-E progenitors does not require erythropoietin or the erythropoietin receptor. *Cell* **1995**, *83*, 59-67.

(5) Levy, D. E.; Darnell, J. E., Jr. STATS: Transcriptional control and biological impact. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 651-662.

(6) Quintás-Cardama, A.; Kantarjian, H.; Cortes, J.; Verstovsek, S. Janus kinase inhibitors for the treatment of myeloproliferative neoplasias and beyond. *Nat. Rev. Drug Discovery* **2011**, *10*, 127-140.

(7) Tam, C. S.; Verstovsek, S. Investigational Janus kinase inhibitors. *Expert Opin. Investig. Drugs* **2013**, *22*, 687-699.

(8) Quintás-Cardama, A.; Vaddi, K.; Liu, P.; Manshouri, T.; Li, J.; Scherle, P. A.; Caulder, E.; Wen, X.; Li, Y.; Waeltz, P.; Rupar, M.; Burn, T.; Lo, Y.; Kelley, J.; Covington, M.; Shepard, S.; Rodgers, J. D.; Haley, P.; Kantarjian, H.; Fridman, J. S.; Verstovsek, S. Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms. *Blood* **2010**, *115*, 3109-3117.

(9) Flanagan, M. E.; Blumenkopf, T. A.; Brissette, W. H.; Brown, M. F.; Casavant, J. M.; Shang-Poa, C.; Doty, J. L.; Elliott, E. A.; Fisher, M. B.; Hines, M.; Kent, C.; Kudlacz, E. M.; Lillie, B. M.; Magnuson, K. S.; McCurdy, S. P.; Munchhof, M. J.; Perry, B. D.; Sawyer, P. S.; Strelevitz, T. J.; Subramanyam, C.; Sun, J.; Whipple, D. A.; Changelian, P. S. Discovery of CP-690,550: a potent and selective Janus kinase (JAK) inhibitor for the treatment of autoimmune diseases and organ transplant rejection. *J. Med. Chem.* **2010**, *53*, 8468-8484.

(10) Kremer, J M.; Cohen, S.; Wilkinson, B. E.; Connell, C. A.; French, J. L.; Gomez-Reino, J.; Gruben, D.; Kanik, K. S.; Krishnaswami, S.; Pascual-Ramos, V.; Wallenstein, G.; Zwillich, S. H. A phase IIb dose-ranging study of the oral JAK inhibitor tofacitinib (CP-690,550) versus placebo in combination with background methotrexate in patients with active rheumatoid arthritis and an inadequate response to methotrexate alone. *Arthritis Rheum.* **2012**, *64*, 970-981.

(11) Haan, C.; Rolvering, C.; RAulf, F.; Kapp, M.; Druckes, P.; Thoma, G.; Behrmann, I.; Zerwes, H. G. Jak1 has a dominant role over Jak3 in signal transduction through γc-containing cytokine receptors. *Chem. Biol.* **2011**, *18*, 314-323.

(12) Thorarensen, A.; Banker, M. E.; Fensome, A.; Telliez, J. –B.; Juba, B.; Vincent, F.; Czerwinski, R. M.; Casimiro-Garcia, A. ATP-mediated kinome selectivity: the missing link in understanding the contribution of individual JAK kinase isoforms to cellular signaling. *ACS Chem. Biol.* **2014**, *9*, 1552-1558.

(13) Verstovsek, S.; Kantarjian, H.; Mesa, R. A.; Pardanani, A. D.; Cortes-Franco, J.; Thomas, D. A.; Estrov, Z.; Fridman, J. –S.; Bradley, E. C.; Erickson-Viitanen, S.; Vaddi, K.; Levy, R.; Tefferi, A. Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. *N. Engl. J. Med.* **2010**, *363*, 1117-1127.

(14) Fridman, J. S.; Scherle, P. A.; Collins, R.; Burn, T. C.; Li, Y.; Li, J.; Covington, M. B.; Thomas, B.; Collier, P.; Favata, M. F.; Wen, X.; Shi, J.; McGee, R.; Haley, P. J.; Shepard, S.; Rodgers, J. D.; Yeleswaram, S.; Hollis, G.; Newton, R. C.; Metcalf, B.; Friedman, S. M.; Vaddi, K. Selective Inhibition of JAK1 and JAK2 Is Efficacious in Rodent Models of Arthritis: Preclinical Characterization of INCB028050. *J. Immunol.* **2010**, *184*, 5298-5307.

(15) Mahajan, S.; Hogan, J. K.; Shlyakhter, D.; Oh, L.; Salituro, F. G.; Farmer, L.; Hoock, T. C. VX-509 (decernotinib) is a potent and selective janus kinase 3 inhibitor that attenuates inflammation in animal models of autoimmune disease. *J. Pharmacol. Exp. Ther.* **2015**, *353*, 405-414.

(16) Van Rompaey, L.; Galien, R.; van der Aar, E. M.; Clement-Lacroix, P.; Nelles, L.; Smets, B.; Lepescheux, L.; Christophe, T.; Conrath, K.; Vandeghinste, N.; Vayssiere, B.; De Vos, S.; Fletcher, S.; Brys, R.; van 't Klooster, G.; Feyen, J. H.; Menet, C. Preclinical characterization of GLPG0634, a selective inhibitor of JAK1, for the treatment of inflammatory diseases. *J. Immunol.* **2013**, *191*, 3568-3577.

(17) Luchi, M.; Fidelus-Gort, R. K.; Douglas, D.; Zhang, H.; Flores, R.; Newton, R.; Scherle, P.; Yeleswaram, S.; Chen, X.; Sandor, V. A randomized, dose-ranging, placebo-controlled, 84-day study of INCB039110, a selective Janus kinase-1 inhibitor, in patients with active rheumatoid arthritis. *Arthritis Rheum.* **2013**, *65(Suppl 10)*, 1797.

(18) Yamazaki, S.; Inami, M.; Ito, M.; Fujii, Y.; Hanaoka, K.; Yamagami, K.; Okuma, K. ASP015K: a novel JAK inhibitor demonstrated potent efficacy in adjuvant-induced arthritis model in rats. *Arthritis Rheum.* **2012**, *64 (Suppl 10)*, 2084.

(19) Voss, J.; Graff, C.; Schwartz, A.; Hyland, D.; Argiriadi, M.; Camp, H.; Dowdling, L.; Friedman, M.; Frank, K.; George, J.; Goedken, E.; Lo Schiavo, G.; Morytko, M.; O'Brien, R.; Padley, R.; Rozema, M.; Rosebraugh, M.; Stewart, K.; Wallace, G.; Wishart, N.; Murtaza, A.; Olson, L. THU0127 Pharmacodynamics of a novel JAK1 selective inhibitor in rat arthritis and anemia models and in healthy human subjects. *Ann. Rheum. Dis.* **2014**, *73 (Suppl 2)*, 222.

(20) Zak, M.; Hurley, C. A.; Ward, S. I.; Bergeron, P.; Barrett, K.; Balazs, M.; Blair, W. S.; Bull, R.; Chakravarty, P.; Chang, C.; Crackett, P.; Deshmukh, G.; DeVoss, J.; Dragovich, P. S.; Eigenbrot, C.; Ellwood, C.; Gaines, S.; Ghilardi, N.; Gibbons, P.; Gradl, S.; Gribling, P.; Hamman, C.; Harstad, E.; Hewitt, P.; Johnson, A.; Johnson, T.; Kenny, J. R.; Koehler, M. F. T.; Bir Kohli, P.; Labadie, S.; Lee, W. P.; Liao, J.; Liimatta, M.; Mendonca, R.; Narukulla, R.; Pulk, R.; Reeve, A.; Savage, S.; Shia, S.; Steffek, M.; Ubhayakar, S.; van Abbema, A.; Aliagas, I.; Avitabile-Woo, B.; Xiao, Y.; Yang, J.; Kulagowski, J. J. Identification of C-2 hydroxyethyl imidazopyrrolopyridines as potent JAK1 inhibitors with favorable physicochemical properties and high selectivity over JAK2. *J. Med. Chem.* **2013**, *56*, 4764-4785.

(21) Kim, M. K.; Shin, H.; Cho, S. Y.; Chong, Y. Linear propargylic alcohol functionality attached to the indazole-7-carboxamide as a JAK1-specific linear probe group. *Bioorg. Med. Chem.* **2014**, *22*, 1156-1162.

(22) Yamashita, T.; Yamada, S.; Yamazaki, Y.; Tanaka, H. New procedure for the synthesis of 2-alkylbenzimidazoles. *Synth. Comm.* **2009**, *39*, 2982-2988.

(23) Thompson, J. E.; Cubbon, R. M.; Cummings, R. T.; Wicker, L. S.; Frankshun, R.; Cunningham, B. R.; Cameron, P. M.; Meinke, P. T.; Liverton, N.; Weng, Y.; DeMartino, J. A. Photochemical preparation of a pyridone containing tetracycle: a Jak protein kinase inhibitor. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1219-1223.

(24) Clark, J. D.; Flanagan, M. E.; Telliez, J. –B. Discovery and development of Janus kinase (JAK) inhibitors for inflammatory diseases. *J. Med. Chem.* **2014**, *57*, 5023-5038.

(25) Nakano, M.; Fujii, T.; Hashimoto, M.; Yukawa, N.; Yoshifuji, H.; Ohmura, K.; Nakaizumi, A.; Mimori, T. Type I interferon induces CX3CL1 (fractalkine) and CCL5 (RANTES) production in human pulmonary vascular endothelial cells. *Clin. Exp. Immunol.* **2012**, *170*, 94-100.

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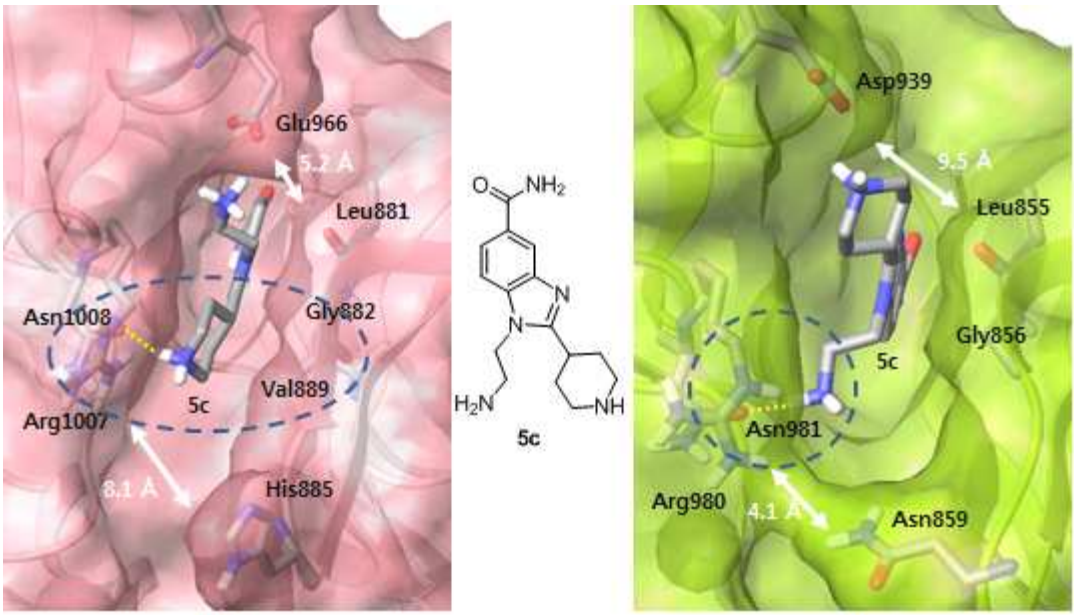


| [ATP] | $IC_{50}$ (μM) | | | | Selectivity | | |
|---|---|---|---|---|---|---|---|
| | JAK1 | JAK2 | JAK3 | Tyk2 | JAK2 | JAK3 | Tyk2 |
| *Km* | 0.05±0.01 | 3.2±0.2 | 1.3±0.1 | 3.7±0.3 | 63 | 25 | 74 |
| 1 mM | 5.7±0.8 | 437.3±3.1 | 232.5±7.5 | 759.5±5.6 | 76 | 41 | 133 |