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Novel JAK1-Selective Benzimidazole Inhibitors with Enhanced Membrane Permeability

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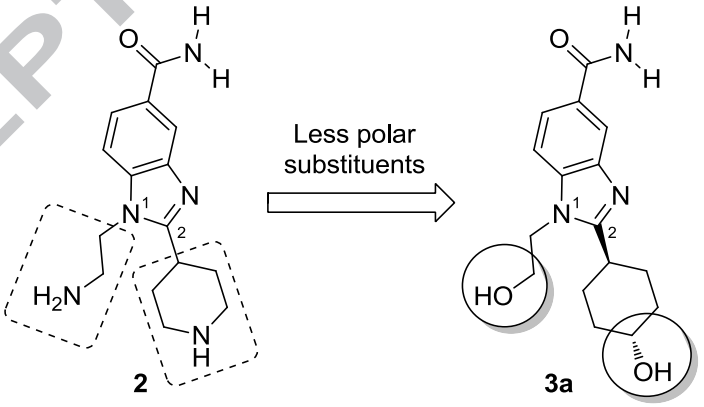
Graphical Abstract



	JAK Inhibition (IC_{50}, μM)				Property	
	JAK1	JAK2	JAK3	Tyk2	LogP	LogP_e
2	0.05	3.2	1.3	3.7	-0.37	-5.12
3a	0.08	3.2	1.8	4.3	0.69	-4.12

Keywords: Janus kinase, Rheumatoid arthritis, Benzimidazole, Selective inhibition

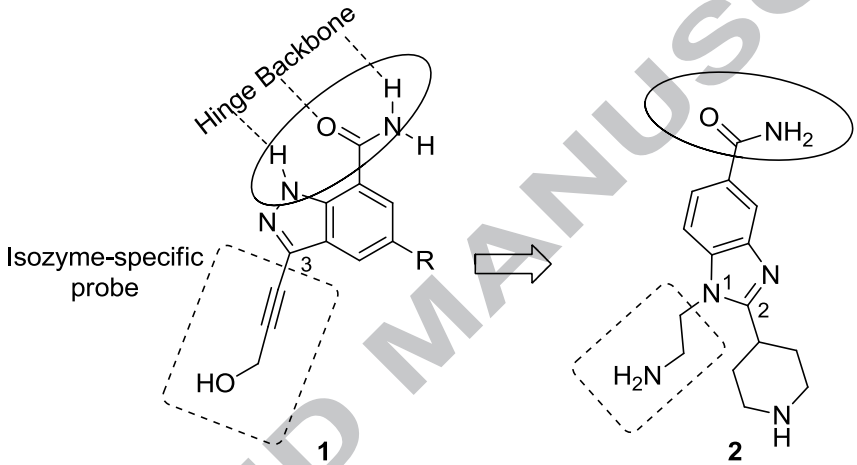
ABSTRACT

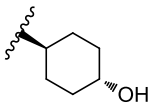
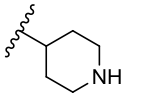
The previously identified Janus kinase 1 (JAK1)-selective inhibitor, 1-(2-aminoethyl)-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole-5-carboxamide (**2**), suffered from low cell permeability, which resulted in poor pharmacokinetic properties. In this study, by introducing less polar hydrogen bond donors at N^1 (a hydroxyalkyl or a methylaminoalkyl group) and C2 (a cyclohexanol group) positions, a series of novel benzimidazole derivatives were prepared, which exhibited selective JAK1 inhibitory activity (IC_{50} against JAK1 = 0.08 ~ 0.15 μM; JAK1-selectivity = 26 ~ 40 fold *vs* JAK2, 12 ~ 23 fold *vs* JAK3, and 38 ~ 54 fold *vs* Tyk2) along with significantly increased lipophilicity (3.3 ~ 15.8 times) as well as membrane permeability (6.3 ~ 12 times).

Cytokine receptors are specifically coupled to various Janus protein tyrosine kinases (JAK1, JAK2, JAK3 and Tyk2). Upon binding to cytokines, the cytokine receptors transmit information related to immune function,[1] inflammation,[2] or hematopoiesis[3-5] by way of JAK-STAT (signal transducer and activator of transcription) pathway. In particular, cytokines such as interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21 bind to the cytokine receptors with γ_c chain which is exclusively associated with JAK3 and JAK1.[2] Therefore, both JAK3 and JAK1 have been targeted for therapeutic intervention of immunologic disorders such as rheumatoid arthritis (RA).[2] Xeljanz (Tofacitinib, CP-690,550) was the first-in-class JAK inhibitor approved for the treatment of RA.[6-7] However, because Xeljanz was revealed as a potent pan-JAK inhibitor with low isozyme-selectivity,[8] debate continues about whether highly isozyme-specific JAK inhibitors are necessarily the best approach for the control of RA with an optimized risk/benefit ratio. Another unresolved point is whether the signal transduction through γ_c-containing cytokine receptors is governed by JAK3 or JAK1.[9-11] Thus, in order to put an end to this controversy and to facilitate JAK inhibitor-based anti-RA drug discovery, identification of novel JAK3-selective[12-13] or JAK1-selective[14-15] inhibitors is urgently required.

Over the past few years, we have been in active pursuit of the JAK1-selective inhibitors based on the initial observation that a linear tether (dashed box, **1**, Fig. 1) attached to a hinge-binding motif (circle, **1**, Fig. 1) of an ATP-competitive kinase inhibitor 3-alkynolyl-indazole-7-carboxamide derivative (**1**, Fig. 1) serves as an isozyme-specific probe group to provide selective inhibition of JAK1 over JAK2.[16] Various scaffolds with a linear tether have been prepared, and recently we identified 1-(2-aminoethyl)-2-(piperidin-4-yl)-1*H*-benzo[*d*]imidazole-5-carboxamide (**2**, Fig. 1) as a potent JAK1-selective inhibitor (IC_{50} against JAK1 = 0.05 μM; 63-fold *vs* JAK2, 25-fold *vs* JAK3, and 74-fold *vs* Tyk2).[17]

However, due to the lack of cell permeability conferred by the polar hydrogen bond donors (aminoethyl and piperidinyl groups) at N^1 and C2 positions, compound **2** showed poor pharmacokinetic properties.[17] In this study, by introducing less polar hydrogen bond donors at N^1 (a hydroxyalkyl or a methylaminoalkyl group) and C2 (a cyclohexanol group) positions of the benzimidazole core (**3a** ~ **3g**, Fig. 1), we attempted to discover novel membrane-permeable benzimidazole derivatives with potent and selective JAK1 inhibitory activity.



	R^1	R^2
3a	$(CH_2)_2OH$	
3b	$(CH_2)_3OH$	
3c	(*R*)-$CH_2CHOHCH_3$	
3d	$(CH_2)_2NHCH_3$	
3e	$(CH_2)_3NH_2$	
3f	$(CH_2)_2NHCH_3$	
3g	$(CH_2)_3NH_2$	

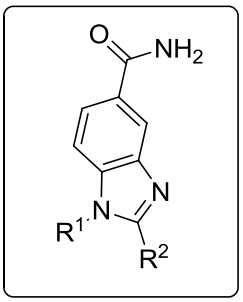
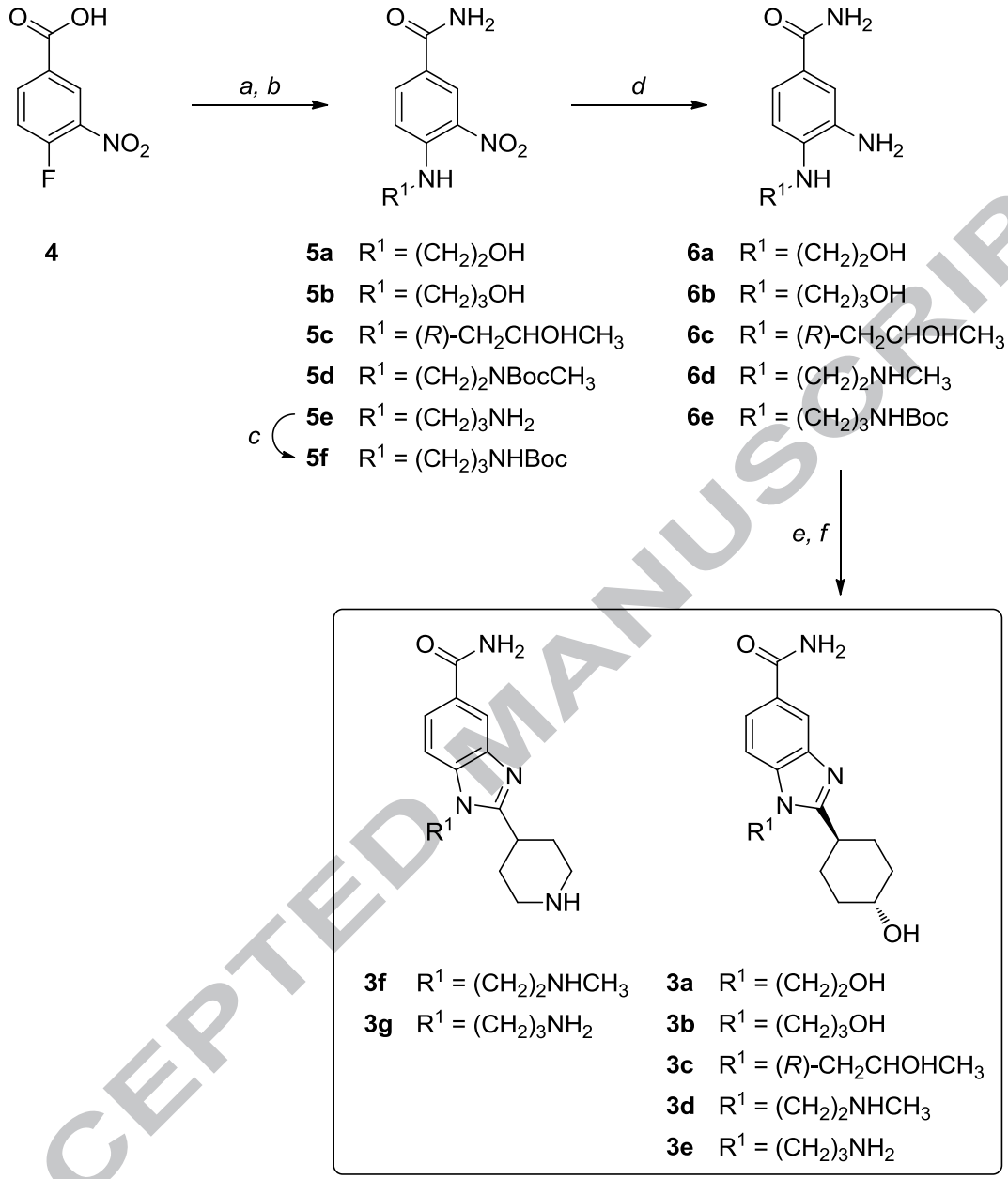


Figure 1. Design of novel benzimidazole derivatives (**3a** ~ **3g**) based on the previously identified JAK1-selective inhibitors (**1** and **2**)

The title compounds (**3a** ~ **3g**) were synthesized from commercially available 4-fluoro-3-nitrobenzoic acid (**4**) in 5 steps (Scheme 1).[17] EDC [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide] coupling of **4** with 1-hydroxy-1*H*-benzotriazole (HOBt)-

ammonium salt (NH_3·HOBt) in a 1:4 mixture of *N*,*N*-dimethylformamide (DMF) and acetonitrile (CH_3CN) provided 4-fluoro-3-nitrobenzamide (97% yield), which underwent nucleophilic aromatic substitution with various amines to give 4-amino-3-nitrobenzamides **5a** ~ **5e** in 74% ~ 96% yields. After reduction, **5a** ~ **5e** were converted to the corresponding 3,4-diaminobenzamides **6a** ~ **6e** in 52% ~ 61% yields. Formation of the benzimidazole core structure was accomplished by condensation of **6a** ~ **6e** with *trans*-4-((*tert*-butyldimethylsilyl)oxy)cyclohexanecarbaldehyde or *tert*-butyl 4-formylpiperidine-1-carboxylate in the presence of sodium metabisulfite ($Na_2S_2O_5$)[18]. The protecting groups in the resulting benzimidazole derivatives were then removed by treatment with HCl or TFA to provide a series of the benzimidazole derivatives with a C2-cyclohexanol substituent (**3a** ~ **3e**) or with a C2-piperidine substituent (**3f** ~ **3g**) in 16% ~ 30% yields.



Reagents & Conditions : (a) EDC, NH_3-HOBt, DMF/CH_3CN; (b) R^1NH_2, DIPEA, *i*-PrOH, 80 °C; (c) $(Boc)_2O$, K_2CO_3, acetone-H_2O (1:1); (d) H_2, Pd/C, MeOH; (e) (1*R*,4*R*)-4-((*tert*-butyldimethyl silyl)oxy)cyclohexanecarbaldehyde or *tert*-butyl 4-formylpiperidine-1-carboxylate, $Na_2S_2O_5$, DMF, 80 °C; (f) TFA (or HCl), MeOH

Scheme 1. Synthesis of the benzimidazole derivatives (**3a ~ 3g**)

In vitro inhibitory activity of the benzimidazole derivatives (**3a ~ 3g)** on the JAK isozymes was determined using Z′-LYTE™ Kinase Assay Kit-Tyr 6 Peptide (JAK1, JAK2 and JAK3)

and Tyr 3 Peptide (Tyk2) (Invitrogen). Pyridone-6,[19] a pan-JAK inhibitor which is used for quality control of the assay kit, was used as a positive control. Assays were performed at the ATP K_m, and the IC_{50} values of **3a** ~ **3g** against each JAK isozyme as well as their JAK1-selectivities over other JAK isozymes are summarized in Table 1.

Table 1. Inhibitory activity (IC_{50}) of the benzimidazole derivatives against JAK isozymes

	IC_{50} (μM)[a]				JAK1 Selectivity[b]		
	JAK1	JAK2	JAK3	Tyk2	*vs* JAK2	*vs* JAK3	*vs* Tyk2
3a	0.08±0.01	3.2±0.2	1.8±0.1	4.3±0.5	40	23	54
3b	7.0±0.2	29.7±0.9	20.6±1.2	42.2±2.8	4	3	6
3c	2.0±0.1	12.1±0.3	8.5±0.2	25.7±1.5	6	4	13
3d	0.15±0.08	3.9±0.2	1.9±0.1	5.6±0.2	26	12	38
3e	5.3±0.3	18.5±0.9	12.4±0.5	30.7±1.4	4	2	6
3f	0.09±0.02	3.1±0.3	1.5±0.1	4.1±0.4	34	16	46
3g	5.4±0.9	27.3±3.4	14.3±1.2	31.0±3.8	5	3	6
2[c]	0.05±0.01	3.2±0.2	1.3±0.1	3.7±0.3	63	25	74

[a]Each experiment was repeated at least three times

[b]Selectivity = (IC_{50} against JAK2 or JAK3) / (IC_{50} against JAK1)

[c]Reference 19

The benzimidazole derivatives synthesized in this study (**3a** ~ **3g**) showed modest to potent inhibition against a series of JAK isozymes. In particular, **3a**, **3d** and **3f** showed potent (IC_{50} against JAK1 = 0.08 ~ 0.15 μM) and selective (26 ~ 40 fold *vs* JAK2, 12 ~ 23 fold *vs* JAK3, and 38 ~ 54 fold *vs* Tyk2) inhibitory activity against JAK1 (Table 1). As demonstrated in our previous work[17], structural comparison of these compounds with others revealed that **3a**, **3d** and **3f** share common structural elements for selective inhibition of JAK1; hydrogen bond donors are substituted at the N^1 position via an ethylene linker. In contrast, one-carbon

homologues with a N^1-propyl side chain (**3b**, **3e** and **3g**) showed only moderate inhibitory activity (IC_{50} against JAK1 = 5.3 ~ 7.0 μM) with low JAK1 selectivity (4 ~ 5 fold *vs* JAK2, 2 ~ 3 fold *vs* JAK3, and 6 fold *vs* Tyk2). Furthermore, introduction of a methyl group to the N^1-ethylene linker was detrimental for JAK1 inhibition, which resulted in significant decrease in inhibitory activity as well as JAK1 selectivity of **3c** (IC_{50} against JAK1 = 2.0 μM; 6-fold *vs* JAK2, 4-fold *vs* JAK3, and 13-fold *vs* Tyk2). Unlike substitution at the N^1-ethylene moiety, methylation at the terminal amino group did not affect the inhibitory activity of the benzimidazole derivatives, and **3f** with a N^1-2-(methylamino)ethyl substituent showed similar inhibitory activity as well as JAK1 selectivity (IC_{50} against JAK1 = 0.09 μM; 34-fold *vs* JAK2, 16-fold *vs* JAK3, and 46-fold *vs* Tyk2) compared with **3a** (IC_{50} against JAK1 = 0.08 μM; 40-fold *vs* JAK2, 23-fold *vs* JAK3, and 54-fold *vs* Tyk2). On the other hand, 4-hydroxycyclohexyl (**3a** ~ **3e**) or 4-piperidinyl (**3f** and **3g**) group substituted at the C2 position did not affect the inhibitory activity of the resulting benzimidazole derivatives; **3d** (IC_{50} against JAK1 = 0.15 μM; 26-fold *vs* JAK2, 12-fold *vs* JAK3, and 38-fold *vs* Tyk2) and **3f** (IC_{50} against JAK1 = 0.09 μM; 34-fold *vs* JAK2, 16-fold *vs* JAK3, and 46-fold *vs* Tyk2) showed similar inhibitory activity and JAK1 selectivity.

The logP value of a compound, the logarithm of its partition coefficient between n-octanol and water, is a well-established measure of the compound's lipophilicity. The logP values of the benzimidazole derivatives with potent and selective inhibitory activity against JAK1 (**3a**, **3d** and **3f**) were thus calculated (Table 2) and, as anticipated, they showed significantly increased partition coefficient (P) values (3.3 ~ 15.8 times) compared with **2** (Fig. 1).

The novel JAK1-selective inhibitors **3a**, **3d** and **3f** were also evaluated for their ability to pass through an artificial membrane. The parallel artificial membrane permeability assay (PAMPA), a gold standard *in vitro* method for determining drug permeability, was

performed[20] by using the pre-coated plate which includes polystyrene filter plate with polyvinylidene fluoride membrane. The PAMPA data summarized in Table 2 show that, in terms of passive permeability (effective permeability coefficients, P_e), the benzimidazole derivatives **3a**, **3d** and **3f** were more permeable than **2** by 10.0, 12.0 and 6.30 times, respectively.

Table 2. Lipophilicity (LogP)[a] and permeability across artificial membranes[b] of JAK1-selective inhibitors (**2**) and benzimidazole derivatives (**3a**~**3g**)

Compd.	**3a**	**3d**	**3f**	**2**
LogP	0.69	0.83	0.15	-0.37
Log P_e (cm/s)	-4.12±0.02	-4.04±0.04	-4.32±0.06	-5.12±0.06

[a]LogP was calculated by ChemDraw Ultra 12.0

[b]Assay was performed in triplicate.

In summary, current attempt to improve membrane permeability of the JAK1-selective inhibitor **2** includes introduction of less polar hydrogen bond donors at N^1 and C2 positions of the benzimidazole core. In particular, changing the polar amino groups at N^1 and C2 positions of **2** by methylation and/or substitution with a 4-hydroxycyclohexyl group resulted in significant increase in lipophilicity (3.3 ~ 15.8 times) as well as membrane permeability (6.3 ~ 12 times) of the resulting benzimidazole derivatives (**3a**, **3d** and **3f**) without substantial loss of inhibitory activity against JAK1 (IC_{50} against JAK1 = 0.08 ~ 0.15 μM; JAK1-selectivity = 26 ~ 40 fold *vs* JAK2, 12 ~ 23 fold *vs* JAK3, and 38 ~ 54 fold *vs* Tyk2). A comprehensive *in vitro* and *in vivo* assessment of the biological activity of **3a**, **3d** and **3f** are underway to confirm their therapeutic potential against RA.

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