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2-Benzimidazolyl-9-(chroman-4-yl)-purinone derivatives as JAK3 inhibitors

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ABSTRACT

A novel class of Janus tyrosine kinase 3 (JAK3) inhibitors based on a 2-benzimidazoylpurinone core structure is described. Through substitution of the benzimidazoyl moiety and optimization of the *N*-9 substituent of the purinone, compound **24** was identified incorporating a chroman-based functional group. Compound **24** shows excellent kinase activity, good oral bioavailability and demonstrates efficacy in an acute mechanistic mouse model through inhibition of interleukin-2 (IL-2) induced interferon-γ (INF-γ) production.

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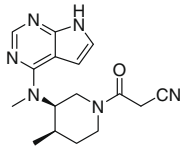
The Janus kinase (JAK) family of cytoplasmic protein tyrosine kinases consists of four members (JAK1, JAK2, JAK3 and TYK2) that help regulate cellular functions in the lympho-hematopoietic system that are critical for cell proliferation, survival and differentiation. JAK3 has limited tissue distribution, specifically expressed in T cells and other hematopoietic lineages, whereas the other JAK's are ubiquitously expressed in both hematopoietic and non-hematopoietic lineages. JAK3 interacts with cytokine receptors that contain the gamma common chain (γc), the receptor subunit for interleukins (IL)-2, -4, -7, -9, -15 and -21.[1] Interleukin binding to the γc cytokine receptor initiates heterodimerization of JAK3 and JAK1 resulting in JAK activation, which in turn, phosphorylates and activates signal transducers and activators of transcription (STAT). STAT dimerization leads to migration to the nucleus where they regulate gene expression. Dysfunction of the γc-JAK3 signaling results in severe combined immunodeficiency (SCID) phenotype.[2,3] Current immunosuppressive drugs have narrow therapeutic indices as a consequence of toxicity related to the ubiquitous distribution of their targets. For example, the molecular target of the immunosuppressants Tacrolimus and Cyclosporin is calcineurin which is widely expressed in all tissues. Thus, the development of a selective JAK3 inhibitor may provide a novel immunosuppressive agent that gives an optimal therapeutic window for the treatment of immune cell-mediated diseases.[4] JAK2 is highly homologous to JAK3. JAK2 plays a critical role in signaling via many cytokines, such as erythropoietin, granulocyte-macrophage colony-stimulating factor and thrombopoietin. Inhibition of JAK2 may result in anemia, granulocytopenia and thrombocytopenia. A number of JAK3 inhibitors have been reported,[5] including CP-690,550 (Fig. 1), which is currently in phase III clinical trials.[6] The aim of this research was to identify novel and potent JAK3 inhibitors with selectivity over JAK2 to avoid potential side effects.

Compound **5a** (Table 1) was identified as an early JAK3 inhibitor, exhibiting an IC_{50} value of 115 nM in a JAK3 kinase assay and approximately 28-fold selectivity versus JAK2.[7] Preparation of **5a** was achieved through regioselective arylation of 4-aminotetrahydropyran with 2,4-dichloro-5-nitropyrimidine[8] (**1**) to give **2** (Scheme 1). Displacement of the 2-position chlorine of **2** with benzimidazole provided **4** (R = H) which was then followed by reduction of the nitro group[9] and generation of the purinone via cyclization with 1,1′-carbonyldiimidazole.[10]



CP-690,550

Figure 1. JAK3 Inhibitor CP-690,550.

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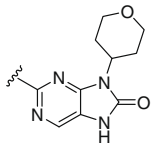
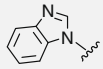
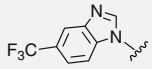
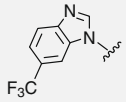
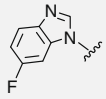
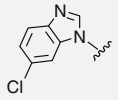
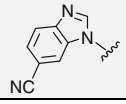
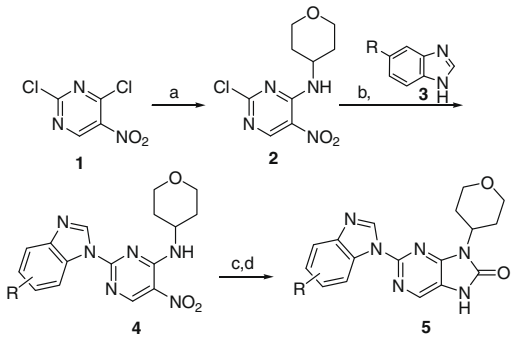
Table 1



Compound	R	JAK3 IC_{50} (nM)	JAK2 IC_{50} (nM)
5a		115	3250
5b		245	1800
5c		68	560
5d		38	3400
5e		14	393
5f		7	470

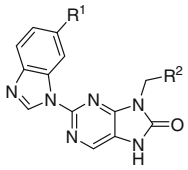
Reaction of a 5-monosubstituted benzimidazole **3** with **2** provided a mixture of the 5- and 6-position regioisomers **4**, which were separated by flash chromatography. A two to 15-fold increase in kinase activity was observed on incorporation of electron withdrawing substituents in the 6-position of the benzimidazole (**5c–f**), with the 6-cyano analog **5e** exhibiting an IC_{50} value of 7 nM in the kinase assay. The 5-trifluoromethyl-substituted benzimidazole derivative **5b** was approximately fourfold weaker than the corresponding 6-position isomer **5c**. Of the initial analogs synthesized, **5d** and **5f** demonstrated the highest level of selectivity versus JAK2 (89- and 67-fold, respectively).

Concurrent studies incorporating a substituted benzyl group at *N*-9 of the purinone core using a similar procedure to that outlined in Scheme 1 demonstrated that halogenated benzylic *N*-9 substituents also provided compounds with good JAK3 inhibitory activity. Compounds were either tested as a mixture of 5- and 6-substituted benzimidazoles, or where possible, separated to provide the 6-substituted analogs (Table 2). Monofluorinated benzylic substituents at the *N*-9 position (**6d–f**) provided increased potency in comparison to the corresponding monochloro analogs (**6a–c**).



Scheme 1. Reagents and conditions: (a) 4-aminotetrahydropyran, *i*Pr_2NEt, THF, −78 °C; (b) *i*Pr_2NEt, THF, 25 °C; (c) $Na_2S_2O_4$, $NaHCO_3$, THF, H_2O, MeOH, 25 °C; (d) 1,1′-carbonyldiimidazole, CH_2Cl_2, 25 °C.

Table 2



Compound	R^1	R^2	JAK3 IC_{50} (nM)	JAK2 IC_{50} (nM)
6a	CN	2-Chlorophenyl	76[a]	930[a]
6b	CN	3-Chlorophenyl	245[a]	4000[a]
6c	CN	4-Chlorophenyl	1450[a]	3200[a]
6d	CN	2-Fluorophenyl	25[a]	260[a]
6e	CN	3-Fluorophenyl	38[a]	1000[a]
6f	CN	4-Fluorophenyl	70[a]	230[a]
6g	F	2,3-Difluorophenyl	108	1000
6h	CN	2,3-Difluorophenyl	17	270
6i	F	2,6-Difluorophenyl	70	320
6j	CN	2,6-Difluorophenyl	6	28
6k	CN	2,6-Dichlorophenyl	220	1550

[a] Tested as a mixture of 5- and 6-substituted benzimidazoles.

Difluorinated analogs (**6g–6j**) provided a further increase in potency, with the 2,6-difluorobenzyl analog **6j** displaying an IC_{50} value of 6 nM.

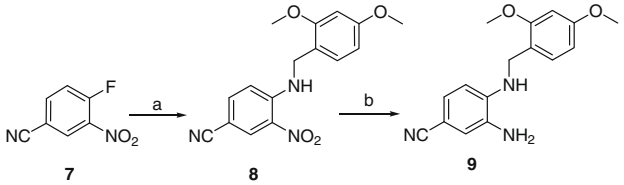
Based on the inhibitory activity of the 6-substituted benzimidazole analogs, a regiospecific process whereby the benzimidazole was constructed via arylation of a suitably protected substituted dianiline was investigated (Schemes 2–4). The devised synthesis would ideally allow late incorporation of the *N*-9 substituent to allow further exploration of the SAR related to this position.

For generation of the 6-cyano-substituted benzimidazole, intermediate **9** was synthesized in two steps (Scheme 2) via arylation of 2,4-dimethoxybenzylamine with 4-fluoro-3-nitrobenzonitrile (**7**) followed by nitro group reduction.

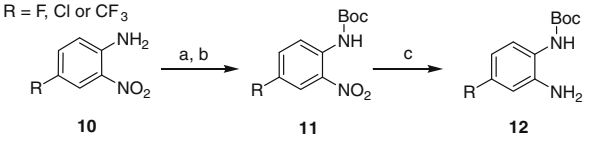
For generation of the 6-fluoro, 6-chloro and 6-trifluoromethyl-substituted benzimidazoles, a similar monoprotected dianiline intermediate (**12**) was synthesized from the corresponding *ortho*-aminonitrobenzene **10** (Scheme 3).

Boc protection of **10** using dimethylaminopyridine (DMAP) resulted in the generation of a bis-boc intermediate, which was partially deprotected with stoichiometric trifluoroacetic acid to provide **11**. Subsequent nitro group reduction of **11** provided **12**.

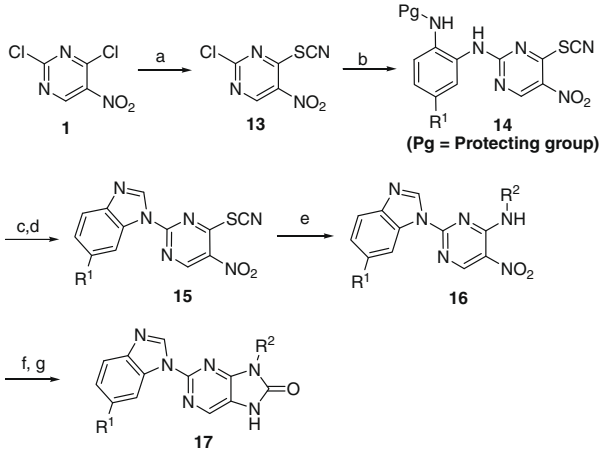
Construction of the benzimidazole moiety at the 2-position of the purinone core required manipulation of pyrimidine precursor **1** to suppress reactivity at the 4-position. Consequently, reaction of **1** with sodium thiocyanate provided **13**, which reacts preferen-



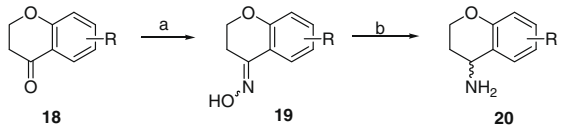
Scheme 2. Reagents and conditions: (a) 2,4-dimethoxybenzylamine, *i*Pr_2NEt, THF, 25 °C; (b) $Na_2S_2O_4$, $NaHCO_3$, THF, H_2O, MeOH, 25 °C.



Scheme 3. Reagents and conditions: (a) di*tert*butyldicarbonate, DMAP, CH_2Cl_2, 25 °C; (b) TFA, CH_2Cl_2, 25 °C; (c) $Na_2S_2O_4$, $NaHCO_3$, THF, H_2O, MeOH, 25 °6C.



Scheme 4. Reagents and conditions: (a) sodium thiocyanate, EtOH, 0 °C; (b) **9** or **12**, K_2CO_3, CH_3CN, 25 °C; (c) TFA, Et_3SiH, CH_2Cl_2, 25 °C (Pg = dimethoxybenzyl) or TFA, CH_2Cl_2, 25 °C (Pg = Boc); (d) trimethylorthoformate, MeOH, 25 °C; (e) R^2–NH_2, THF, iPr_2NEt, 25 °C; (f) $Na_2S_2O_4$, $NaHCO_3$, THF, H_2O, MeOH, 25 °C; (g) 1,1′-carbonyldiimidazole, THF, 25 °C.



Scheme 5. Reagents and conditions: (a) hydroxylamine hydrochloride, NaOAc, EtOH, 25 °C; (b) H_2, Raney Ni, EtOH, 25 °C.

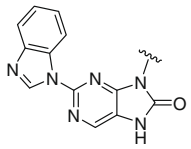
tially at the 2-position with the protected dianiline (**9** or **12**) to give **14** (Scheme 4). Protecting group removal under acidic conditions followed by reaction with trimethylorthoformate provided **15**, effectively controlling the regiochemistry of the 6-substituted benzimidazole.

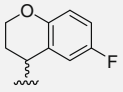
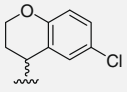
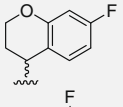
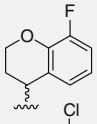
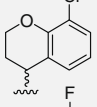
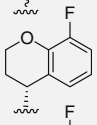
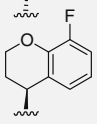
Incorporation of the *N*-9 substituent can subsequently be achieved by thiocyanate displacement of **15** with a primary amine (R^2–NH_2) to provide **16**, which is converted to the purinone analogs **17** via nitro reduction and ring formation with 1,1'-carbonyldiimidazole. Generation of analogs incorporating the *N*-9 substituent as tetrahydropyran confirmed the regiochemical assignment of **5c–f** as the 6-substituted benzimidazole.

A study was initiated whereby the structural features of the THP functionalized analogs **5** and the substituted benzyl analogs **6** were combined, resulting in the incorporation of a series of substituted chromans at *N*-9 of the purinone. 4-Aminochroman synthesis was achieved *via* catalytic hydrogenation of an oxime **19** generated from the corresponding chromanone **18** to provide racemic **20** (Scheme 5).

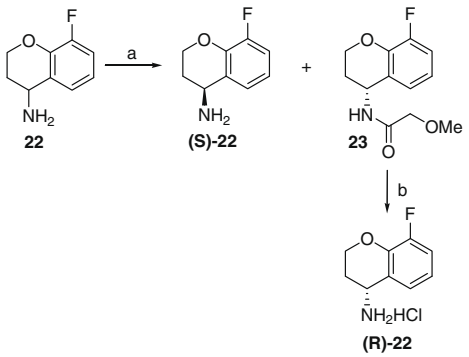
The unsubstituted chroman analog **21a**, incorporating benzimidazole at the 2-position, showed moderate JAK3 potency (Table 3), comparable to that of the corresponding THP derivative **5a**. JAK2 selectivity, however, was significantly reduced in comparison to **5a**. Fluorination at the 5- (**21b**), 6- (**21c**) and 8-positions (**21f**) of the chroman showed a slight increase in inhibitory activity, whereas the 7-fluorochroman analog **21e** was approximately threefold weaker. As with the substituted benzylic analogs **6**, chlorine substitution (**21d** and **21g**) was found to be inferior to both fluorine and hydrogen. Of the substituted chroman-based analogs, **21f** provided the best balance between JAK3 activity and JAK2 selectivity (~10-fold), and subsequent resolution of the stereoisomers of **21f** demonstrated a preference for the (*R*)-enantiomer (**(*R*)-21f**) of the *N*-9 chromanyl purinone.

Table 3
Chroman-based JAK3 inhibitors



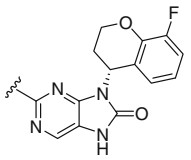
Compound	R	JAK3 IC_{50} (nM)	JAK2 IC_{50} (nM)
21a		95	170
21b		48	97
21c		28	83
21d		365	500
21e		260	95
21f		30	285
21g		170	840
(*R*)-**21f**		28	333
(*S*)-**21f**		540	7000

The stereochemical assignment of the preferred (*R*)-enantiomer was made following resolution of 8-fluoro-4-aminochroman using Lipase B (Scheme 6).[11] Enzymatic acylation of the (*R*)-enantiomer of **22** allowed separation of the components to give **(*S*)-22** and subsequent acid mediated hydrolysis of **23** provided **(*R*)-22**. Incorpo-



Scheme 6. Reagents and conditions: (a) novozyme 435 (*Candida antarctica*), *t*BuOMe, methoxymethylacetate, 25 °C; (b) HCl, EtOH, 25 °C.

Table 4
(*R*)-8-fluorochroman-based JAK3 inhibitors



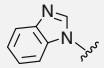
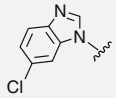
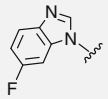
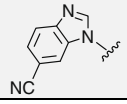
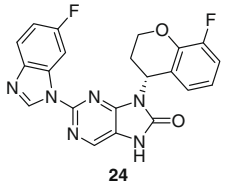
Compound	R	JAK3 IC_{50} (nM)	JAK2 IC_{50} (nM)
(*R*)-**21f**		28	333
23		15	56
24		3	26
25		1	5

Table 5
Pharmacokinetic parameters for **24** in female Balb/c mice at 10 mpk po and 2 mpk iv



24

$AUC_{po\ 0-\infty}$	13,224 ng h/mL
F_{po} (%)	100
$C_{max,\ po}$ (ng/mL)	5472
CL_{iv} (mL/min/kg)	14.6
$T_{1/2,\ iv}$ (h)	1.41

ration of the resolved enantiomers of **22** into the purinone analogs allowed the stereochemical assignment.

Following isolation of (*R*)-**22**, the (*R*)-8-fluoro-4-aminochroman substituent was subsequently combined with 6-substituted benzimidazole components at C-2 of the purinone following the synthesis outlined in Scheme 4 (Table 4).

A significant increase in potency was observed for the chloro-, fluoro- and cyano-substituted benzimidazoles, in comparison to (*R*)-**21f**, with **24** and **25** showing a 10- and 30-fold increase in JAK3 kinase activity, respectively. Moderate JAK2 selectivity was observed for **24** and **25** (nine and fivefold, respectively).

The 6-fluorobenzimidazole analog **24** demonstrated good JAK3 kinase activity and approximately ninefold selectivity over JAK2 in the kinase assay, and was selected for further characterization. Oral dosing in mice showed that **24** was orally bioavailable and had an excellent PK profile (Table 5).

The favorable PK profile exhibited by **24** supported further in vivo characterization of the compound in an acute mechanistic mouse model whereby IL-2-induction of IFN-γ production in mice is determined. Compound **24** was administered orally to female Balb/c mice and after 1 h, IL-2 (100,000 IU/mouse) or its carrier PBS was administered ip. After an additional 3 h, the mice were sacrificed and the blood was collected by cardiac puncture. The amount of INF-γ in serum was determined by ELISA. Compound **24** was efficacious in reducing the IFN-γ response to IL-2. A dose-dependent reduction in IFN-γ was observed resulting in an ED_{50} of 5 mpk ($n = 2$) (Fig. 2).

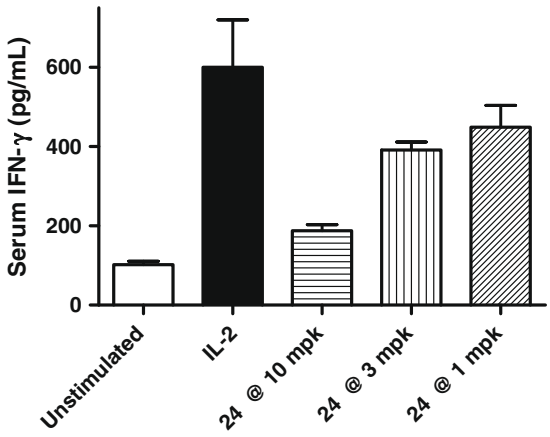


Figure 2. Efficacy of **24** in an acute mechanistic IL-2 mouse model.

In summary, key structural features associated with 2-benzimidazolyl-9-(chroman-4-yl)-purinone derivatives as potent JAK3 inhibitors have been identified. The development of a regioselective synthesis has allowed the substitution pattern of the benzimidazole moiety to be explored. In addition, combination of SAR related to *N*-9 substitution has led to extremely potent JAK3 inhibitors with excellent pharmacokinetic properties, allowing the demonstration of in vivo efficacy in an acute mechanistic mouse IL-2 model. Further optimization of this series of inhibitors with respect to JAK3 potency, JAK2 selectivity and in vivo characterization will be reported elsewhere.

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