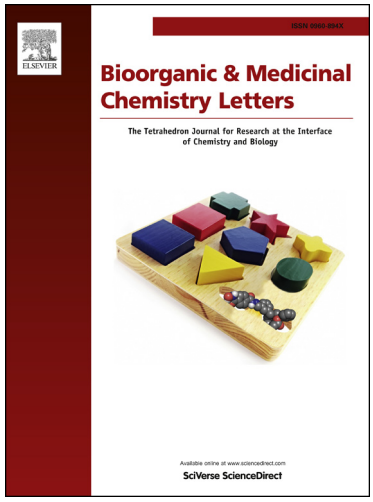

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PII: S0960-894X(13)01022-6
DOI: http://dx.doi.org/10.1016/j.bmcl.2013.08.082
Reference: BMCL 20820

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 13 June 2013
Revised Date: 14 August 2013
Accepted Date: 19 August 2013

Please cite this article as: Labadie, S., Barrett, K., Blair, W.S., Chang, C., Deshmukh, G., Eigenbrot, C., Gibbons, P., Johnson, A., Kenny, J.R., Kohli, P.B., Liimatta, M., Lupardus, P.J., Shia, S., Steffek, M., Ubhayakar, S., Abbema, A.v., Zak, M., Design and Evaluation of Novel 8-Oxo-pyridopyrimidine Jak1/2 Inhibitors, *Bioorganic & Medicinal Chemistry Letters* (2013), doi: http://dx.doi.org/10.1016/j.bmcl.2013.08.082

Design and Evaluation of Novel 8-Oxo-pyridopyrimidine Jak1/2 Inhibitors

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The Janus protein tyrosine kinases (Jak1, Jak2, Jak3, and Tyk2) mediate the intracellular signaling of numerous cytokines and thereby play critical roles in a variety of biological processes, including hematopoiesis and the regulation of immune and inflammatory responses.[1,2] Binding of a cytokine to its receptor leads to the activation of receptor associated Jak family kinases and subsequent recruitment and phosphorylation of STAT (Signal Transducer and Activator of Transcription) proteins.[3] The phosphorylated STATs form homo- or heterodimers that translocate to the nucleus and regulate transcription of target genes. Signaling by a large subfamily of cytokines, which includes IL-6, always involves Jak1 activation and is implicated in the pathogenesis of rheumatoid arthritis (RA).[1] A gain-of-function mutation (V617F) in the pseudo-kinase domain of the Jak2 isoform, which renders the kinase constitutively active, has been found in a significant portion of patients with myeloproliferative disorders (MPDs).[4] The deregulation of the Jak2 activity leads to the pathogenesis of MPDs.[5] Recent approval of tofacitinib **1**[6] (Xeljanz™, Figure 1) for RA and ruxolitinib **2**[7] (Jakafi™, Figure 1) for myelofibrosis (MF) has validated Jak1/2 targets in the treatment of these disorders. These molecules are inhibitors of both Jak1 and Jak2 isoforms and it is suggested that compounds with the ability to inhibit both Jak1/2 isoforms may have a broader therapeutic potential in neoplasia with disregulated Jak signaling.[8] Therefore, we were interested in the identification of novel Jak1/2 inhibitors which might complement our previously described Jak1 and Jak2 selective compounds.[9-11]

In our own efforts to discover novel potent Jak inhibitors, compounds bearing several distinct hinge-binding motifs were investigated. For example, a series of potent imidazopyrrolopyridine Jak1 selective inhibitors (**3**, Figure 2) and pyrazolopyrimidine

containing Jak2 selective inhibitors (**4**, Figure 2) were recently reported from our laboratories.[9-11] Numerous Jak1/2 inhibitors were also disclosed by several companies and some of these entities are in advanced clinical trials for the treatment of RA and MPDs.[12-14] We were intrigued by the excellent Jak2 activity of the tricyclic pyridone **5a** (Figure 3a) described by others in the literature.[15] Due to their rigid, planar structures, these compounds sometimes suffer from undesired physicochemical properties such as poor solubility.[15] We therefore envisioned modification of the pyridone-containing tricyclic template **5a** to the bicyclic structure **5b** while retaining the pyridone as the hinge binding moiety. Additional nitrogen atoms were incorporated in the new inhibitor design to facilitate analog synthesis and to obtain favorable direct or water mediated interactions with Jak1/2 proteins (**5c**, Figure 3a, b). Herein, we describe the synthesis of novel 8-oxo-pyridopyrimidine Jak1/2 inhibitors and their biochemical evaluation in related assays.

To test the viability of the modified template, compound **6** (Table 1) was synthesized and the Jak1/2 enzyme potencies were assessed. Encouragingly, compound **6** displayed activity for both Jak1 and Jak2 isoforms with high ligand-efficiency[16] (0.51 and 0.52 for Jak1 and Jak2, respectively). The inhibitor also displayed excellent aqueous kinetic solubility[17] (225 μM). To facilitate the medicinal chemistry efforts, a crystal structure of **6** bound to Jak1 was obtained (Figure 4). The pyridone oxygen makes an H-bond with the backbone NH of L959 while the pyridone NH interacts with the C=O of E957 in the Jak1 hinge region (4a). In addition, water mediated H-bond interaction between the C=O of L959 and N-2 of the pyrimidine ring is observed (4a). The cyclohexane moiety attached at C-4 of the core pyrimidine is

situated in close proximity to the Jak1 P-loop. The proximal N-H is situated within the H-bond distance from a water molecule that makes H-bond interactions with the C=O of G1020 and the carboxylic acid residue of D1021 (Figure 4b). The C-2 carbon of the pyrimidine ring is 5.4 Å away from a polar Jak1 side chain (E966) whose Jak2 counterpart is also polar in nature (D939).[18] These structural features suggested that modification of the cyclohexyl moiety at C-4 and functionalization of C-2 position were reasonable strategies to improve potency.

With the knowledge of the binding mode of the inhibitor **6**, a medicinal chemistry effort was initiated to further investigate the scope of this novel template. A systematic study of cyclohexylamine replacements at C-4 of the pyridopyrimidine ring was carried out to explore interactions with the P-loop region (**7**-**31**, Table 1). Among the un-substituted alkyl and cycloalkylamines tested, the molecule bearing a cyclopentylamine (**10**) moiety was approximately three-fold more potent than compound **6** for both the Jak1 and Jak2 isoforms. 3- and 4-Aminotetrahydropyranyl moieties at C-4 reduced the biochemical potency relative to **6** (**15** and **16**). Similarly, pyridopyrimidines with benzylamine and 4-chlorophenylanilino groups at C-4 gave undesired results (**17** and **18**). Molecules bearing tertiary amines (**19** and **20**) and a cyclopentyl ether moiety (**21**) exhibited loss of enzyme potencies for both Jak1 and Jak2 isoforms indicating that a hydrogen bond donor is required for the inhibitory activity. Compound **10** displayed excellent solubility (318 μM) and moderate permeability (MDCK P_{app} A:B = 2.5 x 10^{-6} cm/s). From these initial results, the cyclopentylamine at C-4 position appeared to be a favored substitution and we next explored functionalization of the cyclopentyl ring.

Accordingly, a number of substituted cyclopentylamine analogs were assessed as C-4 substituents in the inhibitor design. Inclusion of cyclopentylamines containing ester and hydroxyl groups resulted in a loss of enzyme potency relative to compound **10** (**22**-**25**). However, a nitrile group appended directly to the cyclopentane ring enhanced the biochemical potency (**26**-**31**). Among these derivatives, the *cis*-2-CN analog (**26**) was more potent than the *trans*- isomer (**27)**. These 2-substituted analogs were more potent than the 3-substituted analogs (**30** and **31**). The pure *cis*-enantiomer **28** exhibited low nanomolar enzyme inhibition for both Jak1 and Jak2 isoforms while the other enantiomer **29** was ten-fold less potent for these isoforms. Encouragingly, inhibitor **28** displayed good aqueous kinetic solubility (119 μM), human liver microsomal stability (CL = 3.3 mL/min/kg) and potency in both Jak1 (IL-6 pSAT3 EC_{50} = 214 nM) and Jak2 (EPO-pSTAT5 EC_{50} = 248 nM) driven measures of cell-based inhibition.[11] In addition, the molecule inhibited only one (MEKK2) non-Jak family kinases by >50% when tested against 40 such enzymes at a concentration of 1 μM (>100x Jak1/2 K_i).[19] Encouraged by these results, substitution at the C-2 of the pyrimidine ring of compound **26** (*cis*-racemate) was explored to further enhance enzyme and cell potencies.

Initially, a few H-bond donor/acceptor moieties were incorporated at the C-2 position of **26.** The N-Me analog **32** (Table 2) exhibited a moderate boost in the enzyme potency but a loss of cellular potency relative to **26** was observed. The loss of cell activity was believed to be due to higher tPSA[20] and an increase in the number of H-bond donors relative to **26** and therefore, tertiary amines at the C-2 position were explored. Piperidine and morpholine (**33** and **34**) moieties at C-2 deteriorated the enzyme potencies considerably. Similarly, incorporation of a hydroxyl moiety as the C-2

substituent did not provide improvement in enzyme inhibition (**35**). The analogs **32**-**35** displayed large shifts between the enzyme and cellular potencies. Again, this phenomenon is similar to the trend observed previously and is believed to be due to increase in the polarity of the molecules.[9] Therefore, a few analogs with C-linked substituents at the C-2 position of **26** were investigated (**36**-**38**). The *cis*-racemate **36** with the phenyl group at C-2 exhibited Jak2 potency comparable to **26** with a slight decrease in potency for Jak1. The 4-pyrazolyl group at C-2 (**37**) enhanced the biochemical potency for both Jak1/2. This analog, however, was inactive in the cellular assay, presumably due to poor permeability (MDCK P_{app} A:B = 0.2 $x10^{-6}$ cm/s). Incorporation of an ethyl group at C-2 provided compound **38** with excellent enzyme potencies for both Jak1 and Jak2. This molecule also displayed good cell potencies against Jak1 (IL-6 pSAT3 EC_{50} = 300 nM) and Jak2 (EPO-pSTAT5 EC_{50} = 115 nM).

The potency profile against the four Jak isoforms and in vitro DMPK properties of compound **38** are summarized in Table 3. The inhibitor was more selective for Jak1 and Jak2 isoforms relative to Jak3 and Tyk2. The compound had low human liver microsomal and human hepatocyte clearance and good aqueous kinetic solubility. The permeability of this molecule was lower than that observed for compound **10**. The compound did not inhibit Cytochrome-P450 (CYP) enzymes (1A2, 3A4, 2C9, 2C19, and 2D6; IC_{50} values = >10 μM) and only two out of 40 kinases (MEKK2, MEKK3) were inhibited greater than 50% when the compound was tested in non-Jak family kinases in a 40 membered panel tested at 1.0 μM.[19]

In order to understand the boost in potency obtained by incorporation of the nitrile on the cyclopentyl ring at C-4, co-crystallizations of nitrile containing inhibitors

with both Jak1 and Jak2 enzymes were attempted. These activities afforded a co-crystal of (*R*, S) enantiomer of **37** bound to Jak1 from the racemate (Figure 5). As expected, the pyridone moiety makes two H-bond interactions with the hinge region similar to that observed for compound **6.** The nitrile group points towards the G1020 region making multipolar interactions with the backbone carbonyl moieties. The backbone carbonyl oxygen of R1007 and the backbone carbonyl carbon of G1020 make polar interactions with the carbon atom and the nitrogen atom of the nitrile of the inhibitor **37**, respectively (Figure 5a). We surmise that these favorable polar interactions are responsible for the observed gain in potency relative to compound **10.**[21] As seen in the crystal structure of **6**, the N-H at C-4 makes H-bond interaction with the water molecule (Figure 5b). The pyrazolyl hetero-atom is situated within H-bond distance (2.8 Å) of the carboxylic acid moiety of E966 of the Jak1 protein. We hypothesize that similar interactions are also possible with the D939 of Jak2 protein and therefore similar potencies were observed for both Jak1 and Jak2.

The synthetic approach for compounds **5-31** is described in Scheme 1. The synthesis commenced with protection of 3-amino-2-chloropyridine **39** with the Boc group to provide **40**. Treatment of **40** with n-butyl lithium at -78 ^{o}C followed by quenching with carbon dioxide afforded acid **41**. Boc deprotection with TFA and subsequent heating in formamide at 140 ^{o}C overnight formed the pyrimidinone which, upon heating in $POCl_3$ at 100 ^{o}C for 2 h, afforded dichloride **42**. S_NAr displacement of the C-4 chloride in **42** with various amines in the presence of a base and subsequent hydrolysis of the C-8 chloride by heating with 6N HCl in THF provided the pyridones. When an acid labile group was present in the molecule, heating with NaOAc in acetic

acid was utilized for the hydrolysis of the 8-chloropyridylpyrimidines to the corresponding 8-oxo-pyridopyrimidines. The O-linked compound **21** was prepared by treating **42** with cyclopentanol in the presence of sodium hydride and subsequent hydrolysis of the chloride as described above.

The C-2 and C-4 disubstituted 8-oxo-pyridopyrimidines were obtained as depicted in Scheme 2. Acid **43** was prepared as described above starting from 2-methoxy-3-aminopyridine. Treatment of acid **43** with ammonia in the presence of 1,1-carbonyldiimidazole (CDI) provided amide **44** which was subsequently transformed to pyrimidinedione **45**. Reaction of **45** with $POCl_3$ formed trichloropyridylpyrimidine **46**, possibly via the *in-situ* demethylation of the methoxy group and subsequent chlorination by $POCl_3$. S_NAr displacement of the C-4 chloride in **46** with a mixture of *cis*- and *trans*-2-cyanocyclopentylamine[22] in the presence of a base provided **47.** The *cis*- and *trans*-isomers were separated by flash chromatography over silica gel. The displacement of the C-2 chloride of the *cis*-isomer with a variety of amines in the presence of a base and subsequent hydrolysis of the C-8 chloride in **48**-**51** generated compounds **32**-**35**. Suzuki coupling of **47** with phenyl or 4-pyrazoloboronic acid in the presence of a Pd(II) catalyst, followed by hydrolysis provided compounds **36** and **37**. Similarly, the Stille coupling of the intermediate **47** with vinyltributylstannane in the presence of a Pd(II) catalyst and subsequent hydrogenation followed by the hydrolysis of the C-8 chloride as described above provided the ethyl analog **38**.

In summary, we successfully designed a novel, potent and highly ligand efficient (LE = 0.51 and 0.52 for Jak1 and Jak2, respectively) bicyclic 8-oxo-pyridopyrimidine Jak1/Jak2 inhibitor (**6**). Structure-based design, based on the crystal structure of **6**

bound to Jak1 was then applied to further boost enzyme potency which eventually led to compound **38** with better ligand efficiency (LE = 0.56 and 0.59 for Jak1 and Jak2, respectively). During the optimization process, the polarity of the molecules was adjusted such that compounds not only exhibited excellent biochemical potency, but also improved cellular activity.

Acknowledgements

We thank the small molecule analytical and purification group for their support.

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Figure Captions

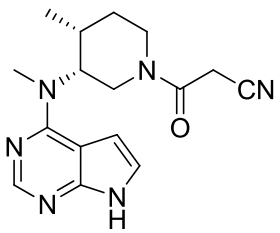
Figure 1. Marketed Jak1/2 inhibitors.

Figure 2. Previously described Jak1 and Jak2 selective inhibitors.

Figure 3. Design of 8-oxo-pyridopyrimidine template.

Figure 4: X-ray structure of **6** in complex with Jak1 (resolution = 2.4 Å, PDB code 4K77). Figures 4a and 4b provide two visual perspectives. 4b is rotated approximately 120 degrees relative to 4a. Compound **6** is depicted in yellow. Hydrogen bonds to the hinge region are shown as dashed red lines. Backbone hinge atoms contacting **6** are highlighted (E957 and L959). Notable crystallographic waters are denoted as red spheres. Hydrogen bonds to the water molecules are depicted as blue dashed lines. The double-headed black arrow shows the distance between the *C*-2 position of the ligand and the carboxylic acid moiety of the E966 in Jak1.

Figure 5: X-ray structure of **37** in complex with Jak1 (resolution = 2.73 Å, PDB code 4K6Z). Figures 5a and 5b provide two visual perspectives. 5b is rotated approximately 120 degrees relative to 5a. Compound **37** is depicted in yellow. Hydrogen bonds to the hinge region are shown as red dashed lines. Backbone hinge atoms contacting **37** are highlighted (E957 and L959). Dashed line in magenta shows interaction between the C-2 pyrazole and the carboxylic acid moiety of E966. Polar interactions between the CN and the backbone carbonyls of G1020 and R1007 are shown as cyan dashed lines. Hydrogen bonds to the water molecules are depicted as blue dashed lines.



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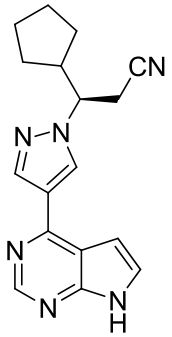

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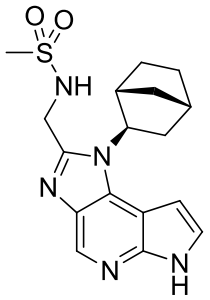

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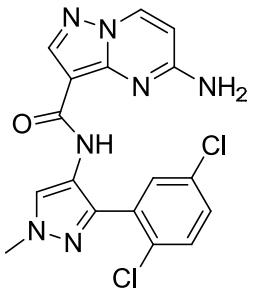
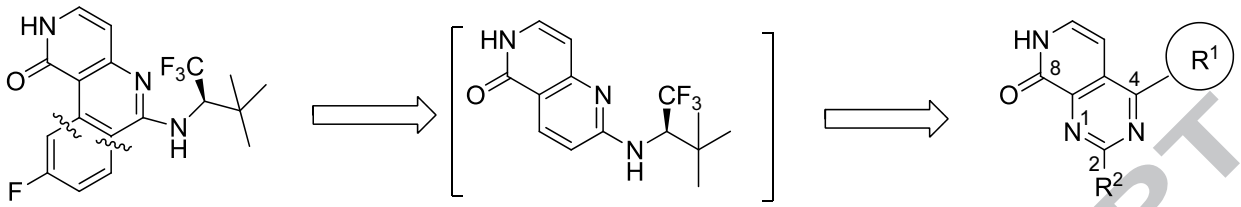


4

(a)

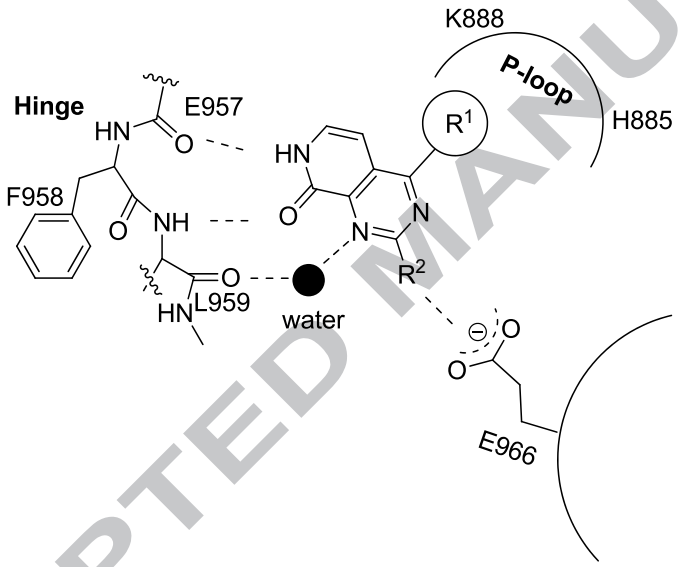


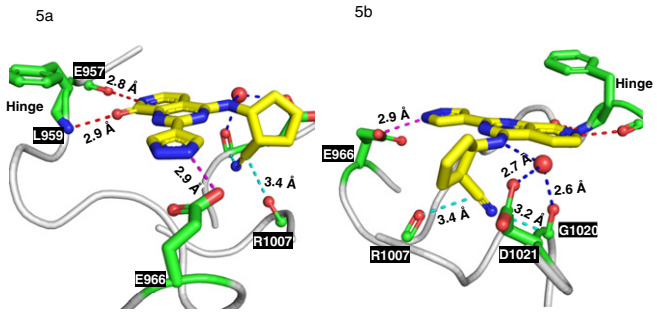
5a **5b** **5c**

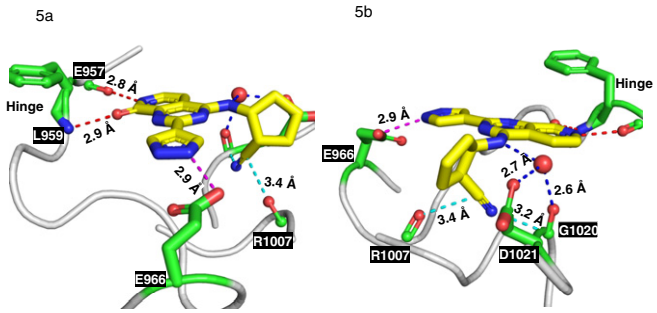
Jak2 K_i = 1 nM

Solubility = 3 μM

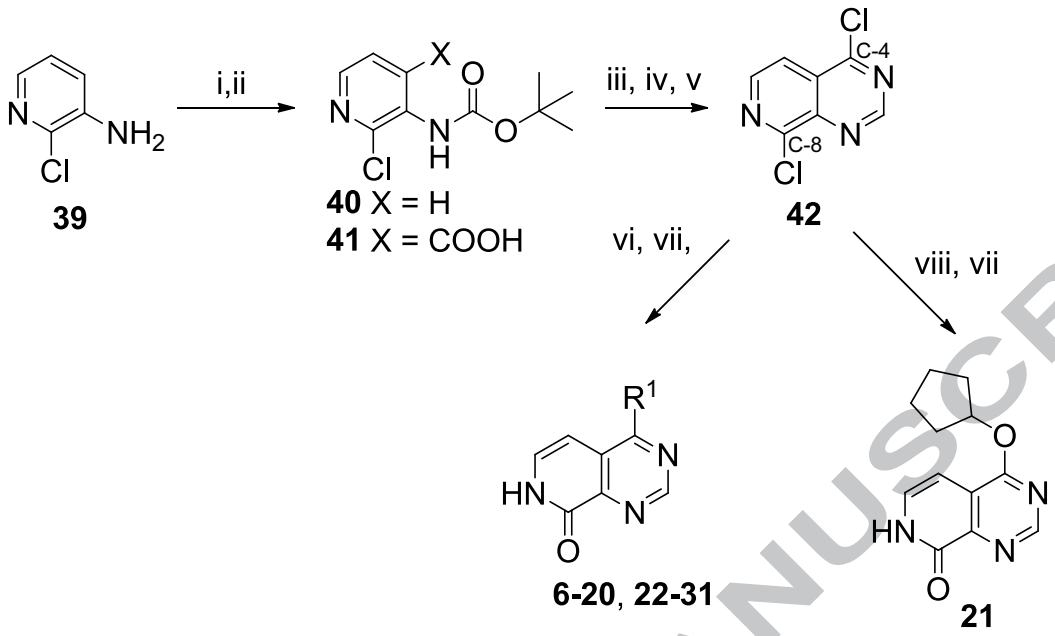
(b)





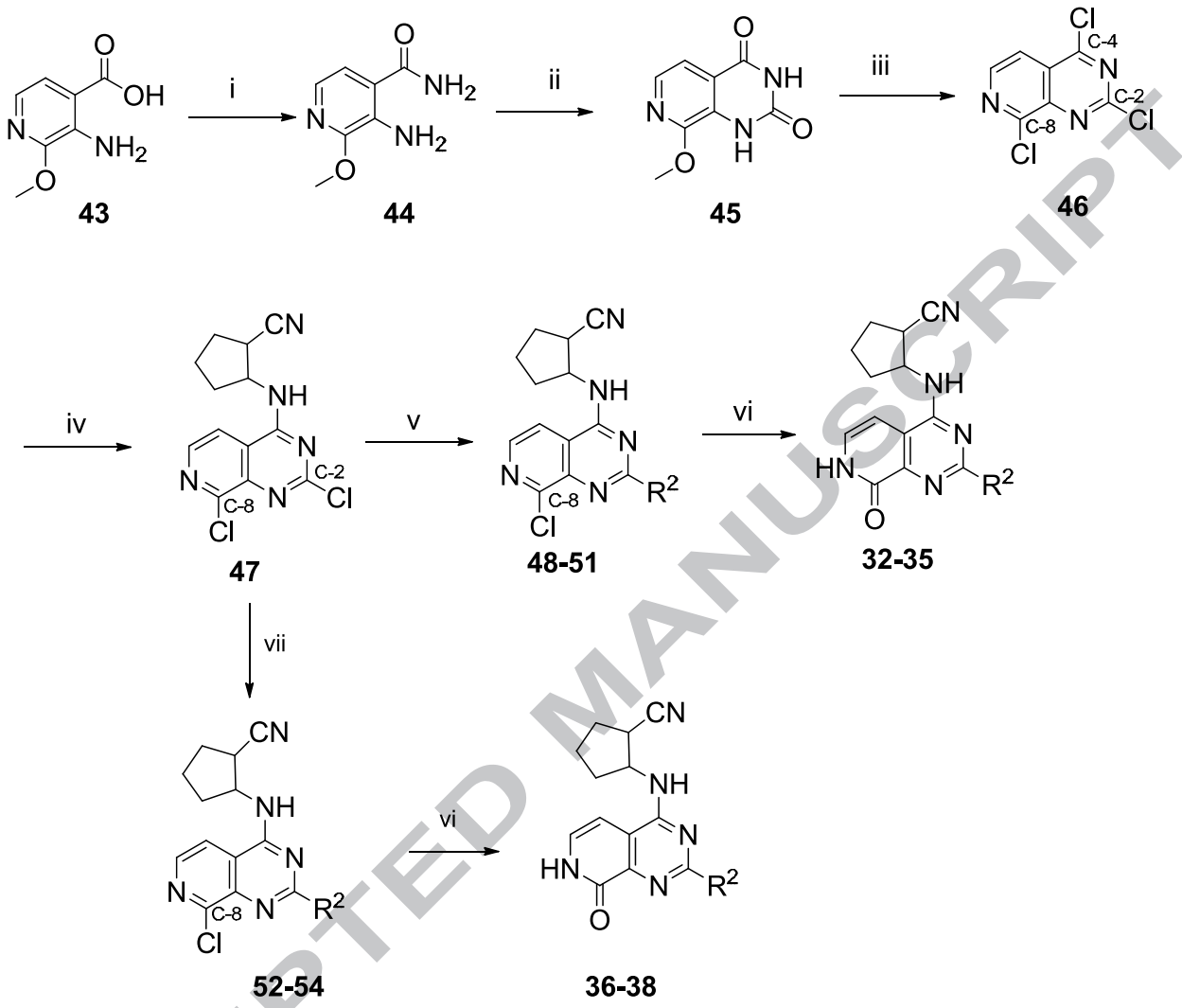


Scheme 1: Synthesis of compounds **6-31.**



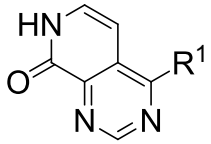
Reagents and reaction conditions: See Table 1 for R^1 substituents (i) 1M NaHMDS in THF, $(Boc)_2O$; (ii) 2.5 M n-BuLi in hexanes, TMEDA,-78 ^oC, CO_2; (iii) TFA, DCM; (iv) $HCONH_2$, 140 ^oC, 20 h; (v) $POCl_3$, 100 ^oC; 2 h (vi) primary/secondary amines, DIPEA, rt-60 ^oC, 20 h; (vii) 6N HCl, THF, reflux, 2h or NaOAc, AcOH, 80 ^oC, 20 h; (viii) cyclopentyl alcohol, NaH, DMF, 0.5 h.

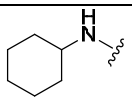
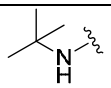
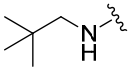
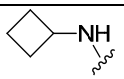
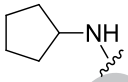
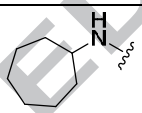
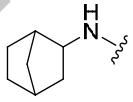
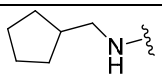
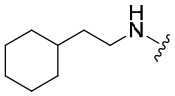
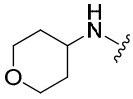
Scheme 2: Synthesis of compounds **32-38.**

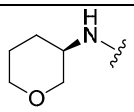
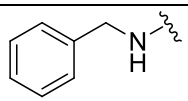
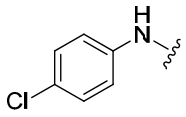
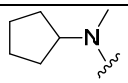
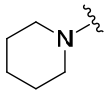
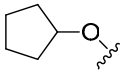
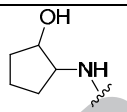
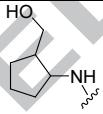
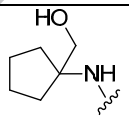
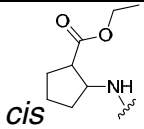
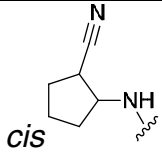


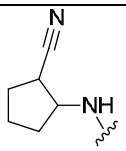
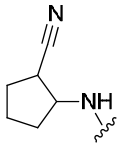
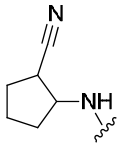
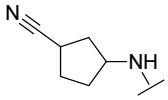
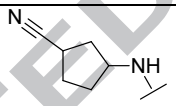
Reagents and reaction conditions: See Table 2 for R^2 substituents (i) CDI, ammonia, THF, 0 ^oC – rt; (ii) $(CCl_3CO)_2O$, 1,4-dioxane, 75 ^oC, 20 h; (iii) $POCl_3$, 100 ^oC, 20 h; (iv) 2-cyanocyclopentylamine(*cis-, trans*-mixture), DIPEA, 1,4-dioxane, 80 ^oC, 2 h; (v) primary/secondary amines, DIPEA, 60 ^oC, 20 h; (vi) NaOAc, AcOH, 60 ^oC, 20 h; (vii) $RB(OH)_2$, $PdCl_2(PPh_3)_2$, Na_2CO_3, 1,4-dioxane/water or tributylvinyltin, $PdCl_2(PPh_3)_2$, THF, 70 ^oC, 20 h and then H_2(balloon)/Pd-C/EtOAc.

Table 1: SAR of 8-oxo-pyridopyrimidine inhibitors **6-31.**



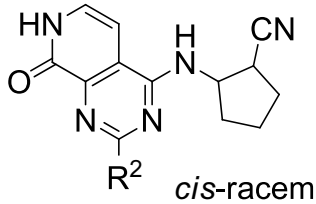
Compound No.	R^1	Jak1 Enzyme K_i (nM)[a,b]	Jak2 Enzyme K_i (nM)[a,b]
6		235	145
7		98	123
8		710	377
9		120	130
10		75	54
11		398	189
12[c]		313	158
13		644	550
14		151	201
15		893	766

16		661	574
17		720	690
18		610	380
19		3,500	3,200
20		>5,600	3,200
21		2,800	1,500
22[e]		453	567
23[d]		1,200	1,400
24[d]		408	575
25[d]		3,200	>5600
26[d,f]		13	9

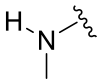
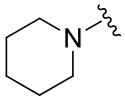
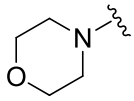
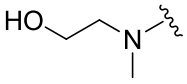
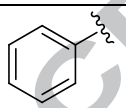
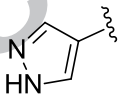
27[d]	 *trans*	65	63
28	*cis*-enantiomer 1 	7	4
29	*cis*-enantiomer 2 	68	40
30[d,g]	 *cis*	65	59
31[d,g]	 *trans*	151	150

[a]For compounds with enzyme potency of >100 nM, the enzyme data are from a single run. For those with enzyme potency of <100 nM, the data are the average of at least two separate runs. [b]For enzyme and cell based assay details see ref. 11. [c]*exo*-racemate. [d]racemate. [e]mixture of *cis*/*trans* diastereomers. [f]See ref. 22 for the synthesis of 2-cyanocyclopentyl amine. [g]See ref. 23 for the synthesis of 3-cyanocyclopentyl amine.

Table 2. SAR of 8-oxo-pyridopyrimidine inhibitors **32-38.**



cis-racemate

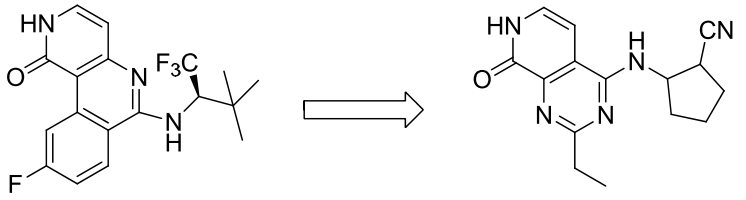
Compd No.	R^2	Jak1 Enzyme K_i (nM)[a,b]	Jak2 Enzyme K_i (nM)[a,b]	IL6-pSTAT3 EC_{50} (nM)[a,b]	EPO-pSTAT5 EC_{50} (nM)[a,b]	Solubility (μM)[c]	tPSA (Å)[d]
26	H	13	9	670	230	104	94
32		4.6	1.8	1000	350	74	106
33		440	21	10,000	4,100	8.6	97
34		380	13	>10,000	>10,000	51	106
35		280	23	>10,000	>10,000	19	117
36		18	3.0	800	426	11	94
37		2	0.6	7,700	6,300	24	123
38		3	1.0	300	115	140	94

[a]The data are the average of at least two separate runs. [b]For enzyme and cell based assay details see ref. 11. [c]Kinetic solubility measured at pH 7.4 in PBS buffer. [d]For tPSA see ref. 20.

Table 3. Enzyme potencies and in-vitro DMPK properties of **38**.

Enzyme K_i (nM) Jak1, Jak2, Jak3, Tyk2	MDCK P_{app} A:B ($x10^{-6}$ cm/s)	HLM CL-hep (mL/min/kg)	HH CL-hep (mL/min/kg)	CYP 450 IC_{50}(>10μM)
3.0, 1.0, 37, 11	0.6	0.3	2.8	1A2, 3A4. 2C9, 2C19, 2D6

Graphical abstract



Jak2 K_i = 1 nM
Solubility = 3 μM

cis-racemate

Jak1/2 K_i = 3/1 nM
Solubility = 140 μM