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A novel chemotype of kinase inhibitors: Discovery of 3,4-ring fused 7-azaindoles and deazapurines as potent JAK2 inhibitors

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T.W. would like to dedicate this Letter to his PhD mentor, Professor Zhengming Li of Nankai University, Tianjin, China, in honor of his more than 50 years of teaching and research in organic chemistry

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ABSTRACT

Pictet–Spengler condensation of aldehydes or alpha-keto-esters with 4-(2-anilinophenyl)-7-azaindole (**11**) or deazapurine (**12**) gave high yields of the 3,4-fused cyclic compounds. SAR studies, by varying the substituted benzaldehyde components, lead to the discovery of a series of potent JAK2 kinase inhibitors.

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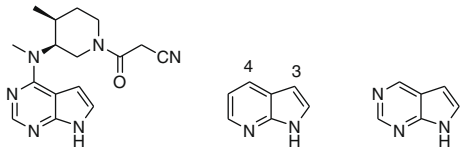
The Janus kinases (JAK) are a family of intracellular non-receptor tyrosine kinases that transduce cytokine-mediated signals via the JAK-STAT pathway. There are four known mammalian kinases in the JAK family: JAK1, JAK2, JAK3 and TYK2.[1] Among the JAK proteins, JAK3 has been a drug target for extensive study, cultimating in the discovery of a small molecule JAK3 inhibitor, CP-690550 (**1**) currently in advanced clinical trial for treatment of Rheumatoid Arthritis.[2]

In 2005 it was independently reported by several research teams that the occurrence of a single residue mutation (V617F) in JAK2 was commonly found in patients diagnosed with myeloproliferative disorders such as polycythemia vera, essential thrombocythemia and chronic idiopathic myelofibrosis.[3] The inhibition of cascade events mediated by JAK2 (V617F) therefore became an attractive approach for developing new targeted therapies against myeloproliferative disorders.[4] We became interested in the discovery of compounds that block the kinase activity of JAK2 by targeting the ATP binding site.

One class of JAK inhibitors contains an azaindole (**2a**) or deazapurine (**2b**) hinge-binding motif, as represented by CP-690550 (**1**). We started a program directed at the discovery of new JAK2 inhibitors where the 7-azaindole or deazapurine were of interest as starting points for design (Fig. 1).

Although mono- or poly-substituted **2a** and **2b** are documented in recent patent applications as novel kinase inhibitors, tricyclic, tetracyclic and polycyclic azaindoles and deazapurines have not been well represented.

Inspired by polycyclic *Ergot* indole alkaloid natural products, where a bridged ring exists between the 3 and 4 positions of indole,[5] we realized that an additional ring can be formed between 3 and 4 positions of 7-azaindole and deazapurine. This would re-



1 CP-690,550 **2a** **2b**

Figure 1.

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sult in a novel polycyclic chemotype, which should be rigid in nature and might have additional benefits of favorable physicochemical properties. Furthermore, screening of our corporate deck revealed that simple 3- and 4-substituted compounds such as **3** and **4** possessed moderate JAK2 activities. Overlaying the putative hinge binding orientations of **3** and **4** suggested that a hybrid structure such as **5**, with a bridge between positions 3 and 4 on the azaindole/deazapurine core, might provide a novel series of JAK2 inhibitors (Fig. 2).

With such a goal in mind, we investigated several possibilities to form the ring between the 3 and 4 positions on azaindole and deazapurine. After initial trial and error exploring several synthetic possibilities, we found a robust methodology based on Pictet–Spengler condensation to form such ring-fused systems. Our approach allowed rapid access to well over 150 examples of this chemotype and led to the discovery of a series of potent JAK2 inhibitors.

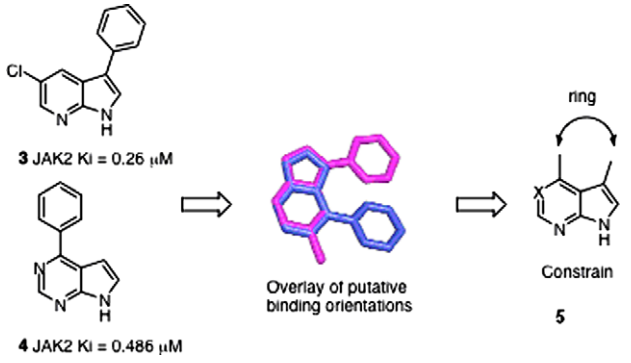
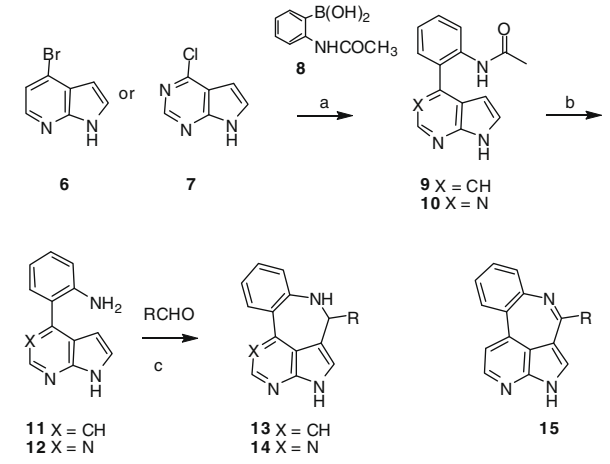


Figure 2.



Scheme 1. Reagents and conditions: (a) $Pd(PPh_3)_4$, K_2CO_3, DME, 128 °C, overnight; (b) concd HCl, reflux (99.9% for **11** and 84.1% for **12** each over two steps); (c) RCHO, MeOH, 4 N HCl-dioxane, 100 °C, 30 min, (>90%).

Suzuki coupling of 2-acetamidophenylboronic acid (**8**) with 4-bromo-7-azaindole (**6**) or 4-chlorodeazapurine (**7**) afforded intermediate 4-(2-acetamidophenyl)-azaindole (**9**) or deazapurine (**10**), which were treated in refluxing concentrated hydrochloric acid to produce high yields of **11** and **12** (Scheme 1). Next we examined the condensation between the anilines **11** and **12** with an aldehyde. To our delight, the Pictet–Spengler reaction proceeded well. Thus, heating either **11** or **12** with aldehydes in methanol and 4 N HCl-dioxane for 30 min, gave, after simple filtration and washing with ether, the resulting ring fused compounds **13** and **14**. Most workups were quite straightforward and consisted of partial evaporation of methanol, addition of 4–5 volumes of ethyl ether, filtration and washing with ether twice. For aldehydes containing basic nitrogen, one equivalent of the aldehyde could be used. For sterically demanding aldehydes such as *t*-pentaldehyde, longer reaction times and large excesses of the aldehydes were necessary. When the reaction was refluxed in methanol in a flask open to air for an extended period of time (2 days), the imino products **15** could be isolated in good yields. Obviously, **15** were the result of oxidation of products of **13**. This was further confirmed by refluxing of **13y** in methanol for two days to afford **15a** as a clean product.

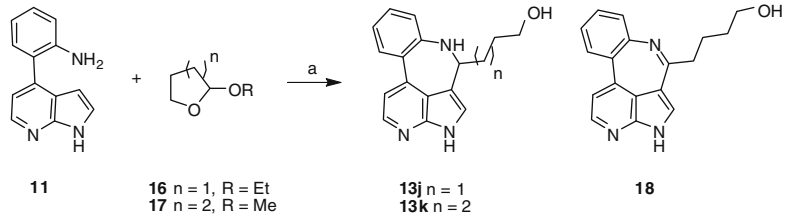
The cyclo-condensation could also be readily carried out with other aldehyde equivalents such as cyclized acetals **16** and **17**. Thus, heating **11** with either 2-ethoxytetrahydrofuran (**16**) or 2-methoxytetrahydro-2*H*-pyran (**17**) under microwave conditions (120 °C, 20 min; methanol, 4 N HCl-dioxane) afforded **13j** or **13k**, which released terminal primary alcohols. Likewise, extended heating of **17** with **11** (90 °C, overnight) yielded the imino product **18** (77%) (Scheme 2).

When esters of alpha-formal benzoate **19** were used in this reaction, the hexacyclic products of type **20** were obtained in almost quantitative yields (Scheme 3).

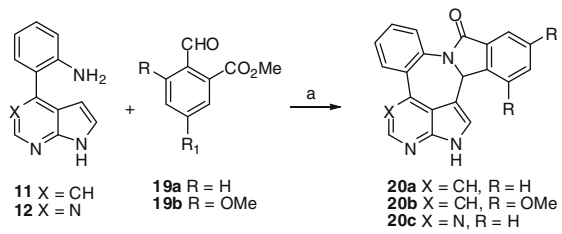
Activated ketones such as 2-oxo-2-phenylacetic esters **21** could also condense with **11** and **12** to form tetracycles **22** and **23** containing a quaternary center (Scheme 4).[6]

The methodology described in Scheme 1 was further expanded to include synthesis of an 8-membered 3,4-fused ring. Thus, Suzuki coupling of 4-bromoazaindole (**6**) with 2-cyanophenylboronic acid produced benzamide **24**, which condensed with aldehydes to afford lacams **25** and **26** (Scheme 5).

Table 1 summarizes JAK2 inhibition data for 3,4-ring-fused compounds **13**, **14**, **15**, **18, 20, 22**, **23**, **25** and **26**.[7] When R is aliphatic (**13a** to **13k**), only micromolar JAK2 activity was observed. In the deazapurine cases **14a** and **14b**, the observed K_i was greater than 5 μM. When R is aryl as in **13**, **14** and **15**, the enzyme inhibitory potency against JAK2 improved (**13l** to **13t**). Introduction of one or more fluorine atoms around the phenyl ring improved potency. The most potent fluoro analog was **13r** (2,3,6-trifluorophenyl) with a K_i of 56 nM. It was found that when R is a para-hydroxy phenyl group, very potent JAK2 activities were observed (**13u** to **13w**, **13y**, **13z**, **14e**, **14f** and **15a** in Table 1). This was also true in the case of eight-membered ring (**25**).



Scheme 2. Reagents and conditions: MeOH, 4 N HCl-dioxane, 120 °C, microwave, 20 min (30% for **13j** and 25% for **13k**).

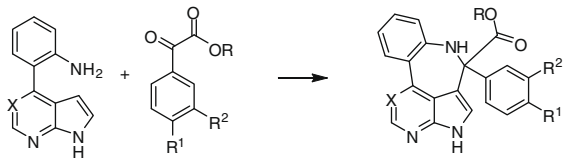


11 X = CH
12 X = N

19a R = H
19b R = OMe

20a X = CH, R = H
20b X = CH, R = OMe
20c X = N, R = H

Scheme 3. Reagents and conditions: (a) MeOH, 4 N HCl-dioxane, 100 °C, 30 min, >90%.

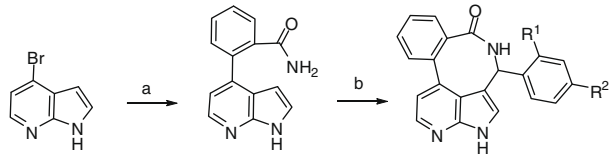


11 X = CH
12 X = N

21a R = Me, R^1 = NO_2, R^2 = H
21b R = Me, R^1 = OH, R^2 = F
21c R = Me, R^1 = OH, R^2 = H
21d R = Et, R^1 = OH, R^2 = H

22a X = CH, R = Me, R^1 = NO_2, R^2 = H
22b X = CH, R = Me, R^1 = OH, R^2 = F
22c X = CH, R = Me, R^1 = OH, R^2 = H
23 X = N, R = Et, R^1 = OH, R^2 = H

Scheme 4. Reagents and conditions: (a) MeOH, 4 N HCl-dioxane 100 °C, 30 min, >90%.



24

25 R^1 = F, R^2 = OH
26 R^1 = H, R^2 = OMe

Scheme 5. Reagents and conditions: (a) 2-Cyanophenylboronic acid, $Pd(PPh_4)_3$, Na_2CO_3, DME, 95 °C, 4 h (47% amide plus 48% nitrile); (b) aldehyde, MeOH, 4 N HCl-dioxane, 90 °C, 1 h (13% for **25** and **26**).

Several other polar substitutions at the *para*-phenyl position were studied (**13x**, **13aa** to **13ag**). The para-boronic acid analog **13x** also had potent activity. Methoxy analog **13aa** caused more than 20-folds loss in potency compared to the phenol analog **13u** while the hydroxymethyl group maintained activity (**13ab**). The 4-amino analog had a K_i of 71 nM (**13ac**) but the corresponding methylsulfonamide (**13ad**) caused a loss of potency (**13ae**). Carboxylic acid (**13ae**) and carboxamide (**13af**) groups did not help with the binding affinity. Finally, 4-pyridyl analog **13af** only had a K_i of 1.3 μM.

The role of the *para*-phenol functionality in high affinity binding to JAK2 was revealed by X-ray studies of a co-complex of **15a** with JAK2. As shown in Figure 3, compound **15a** forms traditional hinge hydrogen bonds to Leu 932 and Glu 930. In addition, its hydroxyl group donates a hydrogen bond to Glu 898 and accepts a hydrogen bond from the backbone NH of Phe 995. It is these latter two hydrogen bonds, buried deep in a hydrophobic pocket, that account for the extraordinary potency of the *para*-phenol containing analogues.

Since compounds **13** and **14** possess a chiral center, the effect of the chirality on activity was also examined. By a chiral column separation, we isolated two enantiomers of **13y**. The one enantiomer of **13y** had K_i of 2 nM while the other, 4 nM. When the chiral center in **13y** was eliminated by formation of imino **15a**, the potency was increased to a K_i of 0.8 nM.

Table 1
JAK2 K_i and cellular IC_{50} determinations

Compds	R	JAK2 K_i (μM)	TF1-GMCSF[8] IC_{50} (μM)
13a	Et	1.7	ND
13b	-*i*Bu	3.4	ND
13c	-*i*Pr	2	ND
13d	-*t*-Bu	2.1	ND
13e	CH_2CO_2Me	0.46	ND
13f	H	0.75	ND
13g	cyclohexyl	2.9	ND
13h	CH_2OCH_2Ph	1.8	ND
13i	CH_2Ph	1	ND
13j	$(CH_2)_4OH$	2.4	ND
13k	$(CH_2)_3OH$	2.4	ND
13l	Ph	0.37	ND
13m	2-F-Ph	0.22	ND
13n	3-F-Ph	0.2	ND
13o	4-F-Ph	0.49	ND
13p	2,6-F_2-Ph	0.13	ND
13q	2,4-F_2-Ph	0.21	ND
13r	2,3,6-F_3-Ph	0.056	ND
13s	2,3,4-F_3-Ph	0.28	ND
13t	2-OH-Ph	1.1	ND
13u	4-OH-Ph	0.0016	1.1
13v	3-F-4-OH-Ph	0.0005	0.86
13w	2-F-4-OH-Ph	0.0006	0.72
13x	4-$B(OH)_2$-Ph	0.013	>20
13y	2-Cl-4-OH-Ph	0.004	5.6
13z	3-Br-4-OH-Ph	0.002	5
13aa	4-MeO-Ph	0.042	ND
13ab	4-$HOCH_2$Ph	0.003	1.1
13ac	4-NH_2-Ph	0.071	ND
13ad	4-$MeSO_2NH$Ph	2.1	ND
13ae	4-CO_2H-Ph	3.1	ND
13af	4-MeNHCOPh	4.2	ND
13ag	4-Pyridyl	1.3	ND
14a	CF_3	>5	ND
14b	CH_2OCH_2Ph	>5	ND
14c	Ph	3.8	ND
14d	-3-F-Ph	1.5	ND
14e	2-F-4-OH-Ph	0.001	0.20
14f	3-F-4-OH-Ph	0.001	0.14
15a	2-Cl-4-OH-Ph	0.0008	0.16
18		0.4	ND
20a		4.5	ND
20b		>5	ND
20c		>5	ND
22a		0.21	ND
22b		0.029	ND
22c		0.21	ND
23		0.63	ND
25		0.034	ND
26		3.8	ND

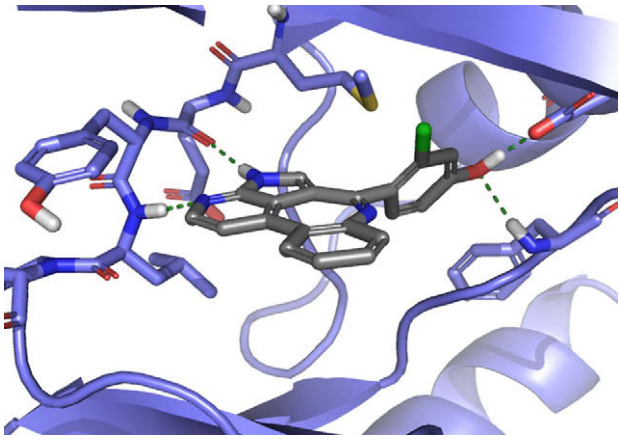


Figure 3. Crystal structure of compound **15a** bound to JAK2. Dashed lines represent hydrogen bonds. Crystallographic data for the structures has been deposited with the RCSB Protein Data Bank. Compound **15a**, PDB accession code: 3KCK.

The hexacyclic analogs **20** did not show JAK2 potency while the compounds **22** and **23**, with a quaternary center, still possessed reasonable potency against the JAK2 enzyme.

For potent JAK2 inhibitors **14e**, **14f**, **13v**, **13w** and **15a**, the JAK2 mediated cellular inhibitory activity was measured in a TF-1 cell line (an erythroleukemic cell line). In this assay, TF-1 cells were stimulated with GM-CSF and FACS was used to measure the intracellular concentration of pSTAT5.[8] As shown in Table 1, some of the most potent JAK2 inhibitors demonstrated sub-micromolar inhibitory IC_{50}.

In summary, we have developed synthetic methodology based on Pictet–Spengler like condensation that allows for quick synthesis of the 3,4-ring fused azaindoles and deazapurines. Assessment of these compounds for activity against the JAK2 enzyme identified a new class of potent JAK2 inhibitors, several of which also exhibited promising potencies in a GM-CSF stimulated TF-1 cell assay.

Acknowledgement

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7. JAK2 K_i Determination: ATP and polyE4Y were obtained from Sigma Chemical Co. (St. Louis, MO, USA). ^{33}P-γ-ATP, GF/B filter plates, and Ultima Gold™ scintillant were purchased from Perkin–Elmer Life Sciences (Boston, MA, USA). JAK2 used in Vertex assays were expressed and purified by the Gene Expression and Protein Biochemistry groups, respectively, at Vertex Pharmaceuticals Incorporated using standard recombinant methods.[a] *Methods*: The inhibitory activity of compounds in Table 1 against JAK2 was determined by following the residual kinase activity of JAK2 using a radiometric assay. The final concentration of the components in the assay were as follows: 100 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 1 mM DTT, 0.01% BSA, 0.6 nM JAK2, 0.5 mg/ml polyE4Y, and 12 μM ^{33}P-γ-ATP. A stock solution of inhibitor was made up in DMSO from which additional dilutions were made in DMSO; a 1.5 μL aliquot of DMSO or inhibitor in DMSO was added to each well. 50 μL of a 2× substrate mixture (100 mM HEPES, 10 mM $MgCl_2$, 1.0 mg/mL polyE4Y, and 24 μM ^{33}P-γ-ATP) was added and mixed with the inhibitor/DMSO. The reaction was initiated by the addition of 50 μL of a 2× enzyme mixture (100 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 2 mM DTT, 0.02% BSA, 1.0 nM JAK2). After 15 min, the reaction was quenched with 50 μL of 20%TCA. The quenched reaction was transferred to the GF/B filter plates and washed three times with 5% TCA. Following the addition of Ultimate Gold™ scintillant (50 μL), the samples were counted in a Packard TopCount. The radioactivity trapped is a measure of the residual JAK2 kinase activity. From the activity versus inhibitor concentration, the K_i value was determined by fitting the data to an equation for competitive tight binding inhibition kinetics[2] using Prism software, version 4.0, San Diego, CA, USA. (a) Fox, T.; Coll, J. T.; Ford, P. J.; Germann, U. A.; Porter, M. D.; Pazhanisamy, S.; Fleming, M. A.; Galullo, V.; Su, M.-S.; Wilson, K. P. *Protein Sci.* **1998**, 7, 2249; (b) Morrison, J. F.; Stone, S. R. *Comments Mol. Cell Biophys*. **1985**, *2*, 347.
8. *IC_{50} determinations*: TF-1 cells were obtained from American Type Culture Collection, Manassas, VA and cultured according to the provider's instructions in the presence of variable concentrations of compounds or DMSO. *Method*: JAK2-STAT5 signaling was stimulated with the addition of 2 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF, R&D systems, Minneapolis, MN) for fifteen minutes to TF-1 cells. Stimulated cells were fixed with the addition of 4% formaldehyde and permeabilized with 90% methanol. Phospho-STAT5 (pSTAT5) was quantified by flow cytometry in a Guava PCA-96 system (Guava Technologies, Hayward, CA) using an anti-STAT5 monoclonal antibody conjugated to phycoerythrin (BD Biosciences, San Jose, CA). IC_{50} values were calculated with Softmax Pro (Molecular Devices, Sunnyvale CA).