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9*H*-Carbazole-1-carboxamides as potent and selective JAK2 inhibitors

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ABSTRACT

The discovery, synthesis, and characterization of 9*H*-carbazole-1-carboxamides as potent and selective ATP-competitive inhibitors of Janus kinase 2 (JAK2) are discussed. Optimization for JAK family selectivity led to compounds **14** and **21**, with greater than 45-fold selectivity for JAK2 over all other members of the JAK kinase family.

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JAK2 is a member of the Janus kinase family of non-receptor tyrosine kinases.[1] The other members in the family are JAK1, JAK3 and TYK2. Recently a gain-of-function point mutation in the pseudokinase domain (JH2) of JAK2 was discovered, where valine 617 is replaced with phenylalanine ($JAK2^{V617F}$) in myeloproliferative neoplasm (MPN) patients. This mutation was found in a majority of patients with polycythemia vera (PV) and approximately 50% of the patients suffering from essential thrombocythemia (ET) and myelofibrosis (MF).[2,3] The mechanism by which this and other JAK-STAT pathway mutations cause growth-factor independent activation is still under investigation. This discovery generated considerable interest in targeting JAK2 kinase as a potential treatment for MPNs. Prior to the introduction of the first JAK2 inhibitor, ruxolitinib, only palliative treatment options existed for MPNs. Ruxolitinib and the more recently reported momelotinib have shown modest activity in relieving constitutional symptoms and can temporarily improve the quality of life for patients with myelofibrosis. However, the side effects of these drugs result in high discontinuation rates.[4] JAK2 inhibitors also have the potential to treat other malignancies and inflammatory diseases that are caused by the aberrant activation of the JAK2/STAT signaling pathway.[5–8] A safe, orally bioavailable, small molecule, JAK2 inhibitor could significantly change the course of these diseases.[6] Since other members of the JAK family are involved in regulation of immune functions, their inhibition would likely cause undesired immunosuppression and would need to be avoided.[7] We present 9*H*-carbazole-1-carboxamides as novel, ATP-competitive JAK2 inhibitors with selectivity against other JAK family members.[9]

Screening of our internal compound collection resulted in the identification of **1** as a hit with good JAK2 potency but modest selectivity against other JAK family kinases and poor aqueous solubility.[10]

The X-ray crystal structure of **1** bound to the JAK2 kinase domain showed the inhibitor bound to the hinge region of the protein via a donor–acceptor–donor triad of hydrogen bonds, namely from the ring-NH to the carbonyl of Leu932, from the amide NH of Leu932 to the primary amide carbonyl, and from the amide NH of **1** to the carbonyl of Glu930 (Fig. 1).[11] The other NH of the primary amide is involved in a water-mediated hydrogen bond to the amide carbonyl of Gly993, an interaction that may contribute to the selectivity over JAK3 which has an Ala in this position. The Ala-Asp amide in JAK3 structures is flipped 180° compared to the

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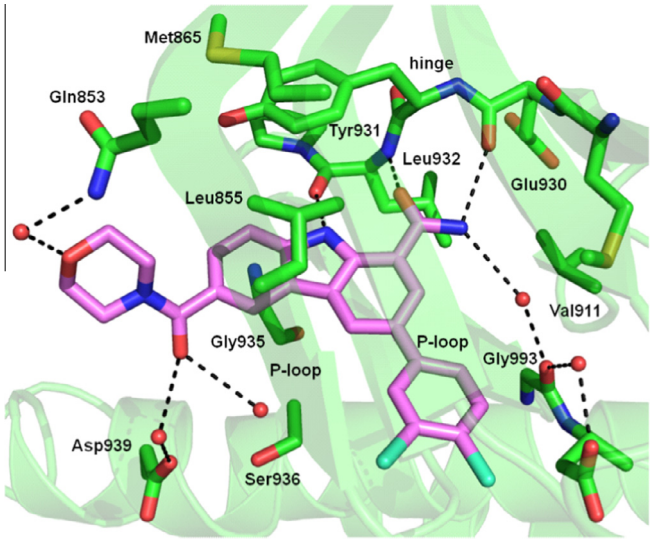

Figure 1. X-ray structure of compound **1** in the ATP binding site of the kinase domain of JAK2. Carbon atoms of **1** are colored in magenta. Hydrogen bonds shown as dotted lines.

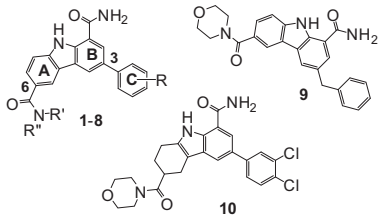

Figure 2. Structures of compounds **1**–**10**.

Gly993-Asp994 amide in JAK2, rendering it unable to form the above mentioned hydrogen bond.[12] The dichlorophenyl ring of **1** is tucked under the P-loop near the ribose-phosphate pocket at the start of the DFG motif of the activation loop.

The C-3-phenyl ring is slightly twisted relative to the carbazole core of the molecule, with a dihedral angle of ~30°. The-6-amido-morpholino group extends towards the solvent-exposed extended-hinge region, and participates in water-mediated hydrogen bonds with Gln853 and Asp939 (see Fig. 1 and Table 1).

The JAK family selectivity of **1** is likely due to nearby residue differences in the ribose pocket and solvent exposed extended hinge region (Fig. 1). In the ribose pocket these are Gly993 (Ala in JAK3) and Val911 (Ile in TYK2). The different amino acids in the solvent-exposed extended hinge region are Gln853 (Arg in JAK1 and TYK2 and Ser in JAK3), Met865 (Leu in the rest of the JAK family), Tyr931 (Phe in JAK1), Ser936 (Cys in JAK3), and Asp939 (Glu in JAK1). We first set out to study carbazole substitutions at the C-3 position that project towards the amino acid differences in the ribose binding pocket to improve JAK family selectivity (Fig. 2).

The ribose binding pocket in JAK2 is slightly larger than in TYK2 (Val911 v. Ile). Not unexpectedly, reducing the size of the substituents on the C-ring (e.g., des-chloro-compound **2**) resulted in the erosion of selectivity, but larger substituents (e.g., 3-propoxy compound **5**) reduced JAK2 potency. Ortho-substitution of the phenyl ring (e.g., **4**) leads to loss of JAK2 selectivity, likely due to further increase in the dihedral angle between the phenyl ring and the carbazole. Replacement of chloride substituents with methyl groups retained the activity (data not shown). Overall, one or two small substituents, in the meta or para position on the phenyl ring, are optimal for potency and selectivity.

We then turned our attention towards the C-6 and C-7 positions of the carbazole, to capitalize on the amino acid differences in the extended hinge region vs. other JAK family members, while at the same time attempting to improve ADME properties. We hypothesized that the introduction of a tertiary amine, protonated under physiological conditions, into the C-6 amide portion of the molecule (e.g., compounds **6**–**8**) would position a positive charge near Gln853 in JAK2 and less favorably near arginine in JAK1 and TYK2. Contrary to our expectations, these changes resulted in reduced selectivity for JAK2 versus JAK1 and TYK2.

Introducing a methylene spacer between the carbazole and the phenyl C-ring (compound **9**) reduced JAK2 potency, likely because of disturbed coplanarity between the 2 ring systems. Increasing the sp^3 content of the molecule by saturation of the A-ring (e.g., **10**) resulted in ~30-fold loss of JAK2 potency, suggesting the necessity of a flat aromatic ring in this position, to fit into the narrow gap between Leu855 and Gly935 (see Fig. 1).

Migrating the tertiary amide from C6 to C7 resulted in a small but consistent (~2–3-fold) loss of JAK2 potency (compounds **11**–**13**, Table 2). This is consistent with the fact that an amide carbonyl on C6, but not C7, can form the water-mediated H-bond with Asp939 that was observed in the X-ray crystal structure of compound **1** (Fig. 1). In contrast to the C6-amide series, the introduction of an additional basic nitrogen into the C-7 amide series (**14**) was tolerated and even beneficial for kinase selectivity, cell potency, and solubility. In particular, the

Table 1
Kinase and cell potency data for C-6 amides **1**–**10**

Compd	R′R″N	R	JAK2 (nM)	JAK1 (nM)	JAK3 (nM)	TYK2 (nM)	SET-2[a] (nM)	A549[b] (nM)
1		3,4-di-Cl	2.4	52	139	31	200	2140
2		H	2.6	18	48	16	120	2400
3		3-OMe	2.8	29	66	32	16	2160
4		2,4-di-Cl	6.8	31	119	40	216	3376
5		3-O-nC_3H_7	8.0	62	130	129	350	5820
6		4-Cl	1.2	5.7	35	2.8	71	1750
7		3,4-di-Cl	3.1	41	59	45	140	990
8		3-OMe	3.5	45	66	39	280	3730
9	See Figure 2		17	169	123	179	210	810
10	See Figure 2		86	1899	2021	608	n.d.	n.d.

[a] SET-2 cell proliferation IC_{50} (JAK2 dependent cell line).
[b] A549 cell proliferation IC_{50} (JAK2 independent cell line, used as counter-screen for non-selective cytotoxic compounds).

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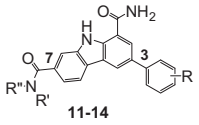
Table 2
Kinase and cell potency data for C-7 amides **11–14**

Compd	R′R″N	R	JAK2 (nM)	JAK1 (nM)	JAK3 (nM)	TYK2 (nM)	SET-2[a] (nM)	A549[b] (nM)
11		H	7.4	31	246	70	170	2640
12		3-OMe	5.7	49	193	171	210	3080
13		3-F-4-OMe	5.5	67	311	201	110	10,000
14		3-OMe	3.5	158	225	243	80	3840

[a] SET-2 cell proliferation IC_{50} (JAK2 dependent cell line).
[b] A549 cell proliferation IC_{50} (JAK2 independent cell line, used as counter-screen for non-selective cytotoxic compounds).



11-14

Figure 3. Structures of compounds **11–14**.

(*S*)-dimethylamino-pyrrolidine amide, **14**, demonstrated greater than 45-fold selectivity against other members of the JAK kinase family. The compound also displayed good cell potency (SET-2 IC_{50} = 80 nM) and cellular selectivity.[13] Compound **14** was further evaluated for its in-vitro ADME properties. The solubility of **14** is significantly higher than that of compound **1**. (>1.9 mg/mL at pH 1.0; 0.41 mg/mL at pH 6.5; compared to <0.001 mg/mL across pH range for **1**). The permeability of **14** is modest (PAMPA = 292 compared to >600 for e.g., **2**, **4**, **11**, **13**), as is metabolic stability (79% and 46% remaining compound after 10 min incubation with human and rat liver microsomes resp.) (Fig. 3).

We further explored 7-amino-carbazoles (Table 3). The 'reverse amide' **15** displayed poor JAK family selectivity. Several other amides of the 7-amino-carbazole (not shown) displayed a similar trend.

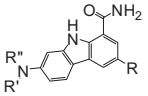
Tertiary amines (e.g., **16**) exhibited a significant improvement in the selectivity versus JAK3, however showed modest selectivity versus JAK1 and TYK2 and also reduced cellular potency. The introduction of a basic amine into the C7-substituent (e.g., **17–19**) further improved selectivity over JAK1 and TYK2. The effects on JAK family selectivity observed for various substituents at C7 are consistent with residue differences in its vicinity (Gln853 in JAK2 versus bulkier and more polar, positively charged Arg in JAK1 and TYK2).

Introduction of a pyridine ring as replacement for the phenyl C-ring gave compounds with low selectivity versus JAK1 (e.g., compound **20**). However, the N-methyl-indazole C-ring replacement was well tolerated when combined with the preferred C-7 substituent. Compound **21** has excellent kinase selectivity and good antiproliferative activity against JAK2-dependent SET-2 cells. Metabolic stability of **21** is comparable to **14**. (75% and 59% remaining compound after 10 min incubation with human and rat liver microsomes resp.). While the solubility of **21** at acidic pH was improved compared to the initial screening hit **1** (0.044 mg/ml at pH 1.0), its solubility at neutral pH and permeability were too low to achieve sufficient oral exposure and were inferior to **14** (<0.001 mg/ml at pH 6.5, PAMPA = 37 nm/s).

Compounds **14** and **21** were further tested in the AMBIT KinomeScan panel of 380 kinases to assess kinase selectivity. Compounds **14** and **21** bind to <6% and <3% of kinases in the panel, respectively.[14]

In summary, we herein report hit optimization of carbazole **1** leading to the identification of novel potent JAK2 inhibitors (**14** and **21**), with greater than 45-fold selectivity over other JAK family members (JAK1, JAK3, TYK2). These analogs also displayed excellent cellular potency against JAK2-dependent SET-2 cells (80 and 127 nM; respectively). In addition to having the desired kinase selectivity profile, compound **14** also provided improved pharmaceutics properties over **21**. Further studies towards improving the ADME properties of this series of JAK2 inhibitors will be published in due course (Fig. 4).

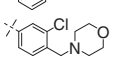
The syntheses of compounds **1–9** was carried out as per Scheme 1.[9] Para-amino-benzoic amides **22a** and **22b** undergo S_NAr reaction with 5-bromo-2-fluoro-benzonitrile **23** to give biphenylamines **24a**,**b**. Standard Suzuki conditions were used to install the ring C **25**. Cyclization to the carbazole and hydrolysis of the CN group to a primary amide was achieved upon heating **25** with palladium acetate in acetic acid. These harsh conditions also resulted in partial cleavage of the amide, leading to isolation of acid **26** as a byproduct, which was utilized to access amide **7**



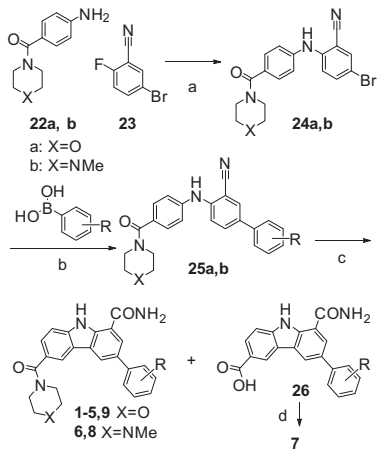
15-21

Figure 4. Structures of compounds **15–21**.

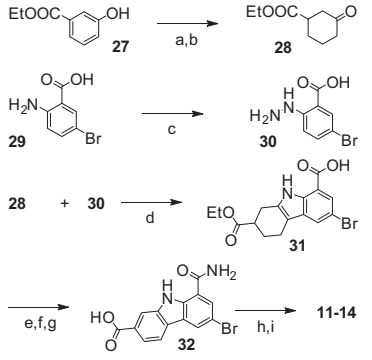
Table 3
Kinase and cell potency data for C-7 amines **15–21**

Compd	R′R″N	R	JAK2 (nM)	JAK1 (nM)	JAK3 (nM)	TYK2 (nM)	SET-2[a] (nM)	A549[b] (nM)
15			10.2	77	59	87	100	1310
16			7.4	218	990	158	500	10,000
17			2.1	141	264	71	370	3510
18			2.9	195	197	160	470	2730
19			5.2	432	242	197	260	930
20			6.1	100	442	117	120	2000
21			5.5	339	324	247	130	1410

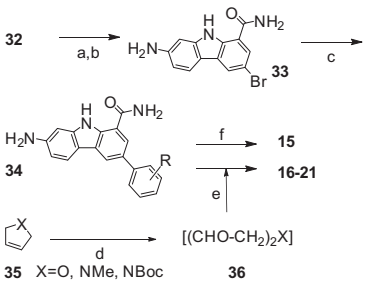
[a] SET-2 cell proliferation IC_{50} (JAK2 dependent cell line).
[b] A549 cell proliferation IC_{50} (JAK2 independent cell line, used as counter-screen for non-selective cytotoxic compounds).



Scheme 1. Synthesis of compounds **1**–**8**. Reagents and conditions: (a) KOtBu, DMSO, 1 h, 0 °C to rt, 70%.; (b) $Pd(PPh_3)_4$, Na_2CO_3, Toluene/MeOH 2:1, 105 °C, 40–80%; (c) 2.5 equiv. $Pd(OAc)_2$, HOAc, 2 h, 130 °C, 30–50%; (d) amine, HATU, DIPEA, DMAP, DMF 60%.



Scheme 2. Synthesis of compounds **11**–**14**. Reagents and conditions: (a) H_2 (4 bar), Rh-Al_2O_3, EtOH, 98%; (b) CrO_3, H_2SO_4, acetone, 95%; (c) $NaNO_2$, HCl, then $SnCl_2$ 89%; (d) AcOH, reflux, 70%; (e) EDC, HOBT, NH_4OH, THF + DCM, 94%; (f) DDQ, Tol, reflux, 93%; (g) NaOH, THF + MeOH + water, quant.; (h) EDC, HOBT, amine, 90%; (i) arylboronic acid, $Pd(PPh_3)_4$, aq Na_2CO_3, toluene, MeOH, 50–60%.



Scheme 3. Synthesis of compounds **15**–**21**. Reagents and conditions: (a) DPPA, Et_3N, *t*BuOH, 86%; (b) TFA, DCM, 96%; (c) arylboronic acid, $PdCl_2$(dppf), aq Na_2CO_3, DME, 50–70%; (d) Ozone, then PPh_3; (e) $NaBH_3CN$, $HC(OCH_3)_3$, HOAc, 20–50%; (f) tetrahydropyran-4-carboxylic acid, HOBT, EDC, DIPEA, DMF, 47%.

via standard amide coupling conditions. The synthesis of benzyl-analog **9** followed the same route, using 2-benzyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane instead of the substituted phenylboronic acids.

The C7-substituted amides **11**–**14** were prepared as shown in Scheme 2. Meta-hydroxy benzoic ester **27** was hydrogenated over rhodium on alumina, followed by Jones oxidation to give cyclohexanone **28**. Bromoanthranilic acid **29** was converted to the hydrazine **30** using standard conditions, then condensed with **28** to give **31**. Amide formation, followed by DDQ oxidation/dehydrogenation and ester hydrolysis gave **33**, which was converted to products **11**–**14** via amide formation followed by Suzuki coupling using standard methods.

The partially saturated analog 10 was made following the strategy shown in Scheme 2, using 4-oxocyclohexanecarboxylic acid instead of **28** and skipping the DDQ oxidation step. See Ref. 9a for details.

The C-7-anilines required for the synthesis of compounds **15**–**21** were prepared via Curtius rearrangement of **32** with diphenylphosphoryl azide in *t*-butanol, followed by BOC cleavage (Scheme 3). A Suzuki reaction installed the substituted aryl C ring. Dihydrofuran and N-substituted dihydopyrrole **35** were ozonized and the resulting di-aldehyde solutions used without isolation in reductive amination reactions to form the morpholine or piperidine rings of **16**–**21**.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.04.101.

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10. Aqueous solubility <0.001 mg/ml.
11. The atomic coordinates of compound **1** have been deposited in the RCSB Protein Data Bank (code 4ZIM). Experimental procedures for the JAK2 protein purification and crystallization are available in the Supplemental Material section.
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13. Cellular selectivity was defined as the ratio of IC_{50}'s for non-JAK2 dependent A549 cell line versus JAK2-dependent SET-2 cell line.
14. Less than 10% control activity at 1 micromolar concentration.