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Optimization of a novel kinase inhibitor scaffold for the dual inhibition of JAK2 and FAK kinases

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ABSTRACT

The elaboration of a novel scaffold for the inhibition of JAK2 and FAK kinases was targeted in order to provide a dual inhibitor that could target divergent pathways for tumor cell progression.

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Janus kinase 2 (JAK2) has received a great deal of attention upon the identification of the JAKV617F mutation in patients with myeloproliferative neoplasms (MPNs).[1] In subsequent years following this discovery, the dysregulation of the JAK/signal transducers and activators of transcription (STAT) signaling pathway has been implicated in hematopoietic malignancies and solid tumors. Constitutive activation of the JAK/STAT pathway has been detected in a wide spectrum of human cancers and correlated with a particularly malignant and metastatic phenotype.[2,3] The JAK/STAT signaling pathway is involved in mechanisms of tumorigenesis including cell proliferation, survival, angiogenesis, and immune evasion and surveillance. The JAK family of kinases is comprised of four members: JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2). Several potent JAK inhibitors have been identified including CEP-701,[4] AZD1480[5] and INCB-18424[6] (Ruxolitinib) which is likely to be approved for myelofibrosis in the US later in 2011.[7]

One strategy for the development of improved treatments in oncology is the development of kinase inhibitors targeting multiple pathways of tumorigenesis. Coupling JAK2 inhibition with inhibitory activity toward another kinase such as focal adhesion kinase (FAK), which plays a role in tumor invasion and metastasis, would provide a powerful treatment for a wide spectrum of malignancies. Activated FAK promotes tumor cell motility, invasiveness/metastasis, and angiogenesis while activated JAK2 plays a critical role in tumor cell proliferation, anti-apoptotic signaling, chemoresistance to cytotoxic therapies, and immune evasion. Therefore, dual FAK–JAK2 inhibition offers the potential for simultaneous targeting of all major mechanisms of tumor pathogenesis and progression. The research objective for the team was to optimize a chemotype for both potent FAK and JAK2 inhibition in order to provide a more beneficial and effective chemotherapeutic agent.

Cephalon has recently disclosed the synthesis[8] and utility of a novel series of 2-anilino-7-aryl-pyrrolo[2,1-*f*][1,2,4]triazines as ALK inhibitors (Fig. 1).[9,10] Screening of **1** showed a subnanomolar IC_{50} in the JAK2 enzyme assay (IC_{50} = 0.2 nM) with good potency in the FAK assay (IC_{50} = 17 nM) (Table 1). The bioavailable ALK inhibitor **2** showed equipotent FAK enzymatic activity, with modest inhibition of JAK2. These results compared well with the known Novartis inhibitor **4**, featuring a closely related heterocyclic system, previously disclosed to be active in enzymatic assays for both FAK (IC_{50} = 200 nM)[11] and JAK2 (IC_{50} = 5.9 nM).[12]

Knowing that the *o*-methoxy group in the aniline at C2 of **2** provides pan-kinase selectivity[13,14] and may be detrimental to the JAK2 potency, we embarked on an SAR campaign wherein the aniline present in **2** was profiled as its desmethoxy congener paired with a variety of R-groups at C7 (Fig. 1,3). The desmethoxy C2 aniline was prepared from 4-phenylpiperidine in three steps

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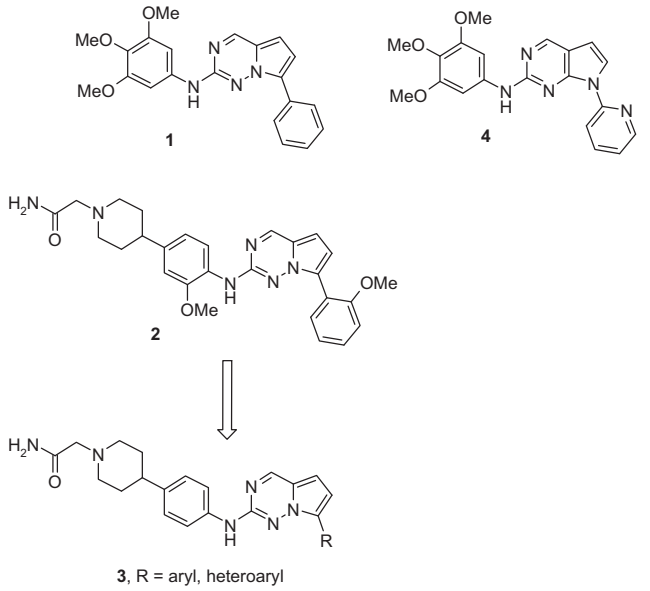
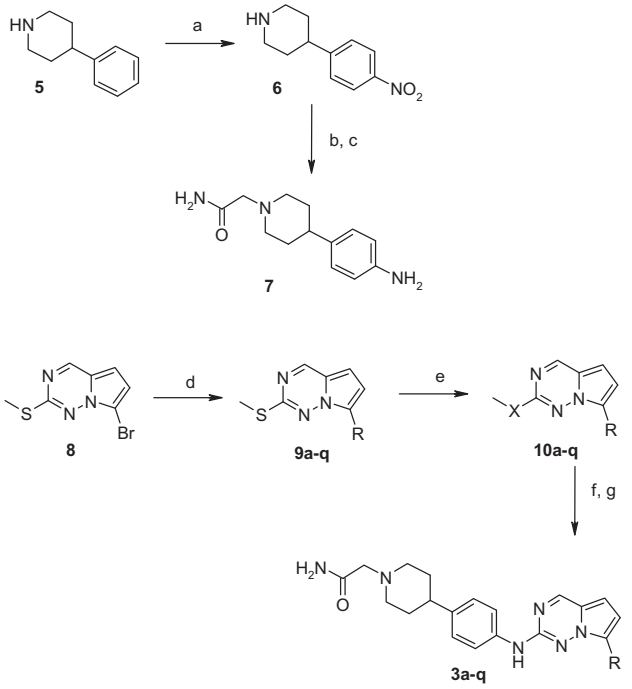


Figure 1. Cephalon pyrrolotriazine analogs and Novarti's related pyrrolopyrimidine.



Scheme 1. Reagenta and conditions: (a) HNO_3, H_2SO_4, AcOH, 40%; (b) iodoacetamide, Cs_2CO_3, MeCN, 95%; (c) H_2, Pd/C, MeOH, 85%; (d) boronic acid/ester, $Pd(PPh_3)_4$; (e) *m*-CPBA (X = SO) or H_2O_2, Na_2WO_4 (X = SO_2); (f) NaOH; (g) $PhN(Tf)_2$ then **7**.

Table 1
Screening of initial pyrrolo[2,1-*f*][1,2,4]triazine leads versus Novartis' pyrrolopyrimidine

Compd	IC_{50}[a,b] (nM)	
	JAK2 enzyme	FAK enzyme
1	0.20	17
2	250	21
4	5.9	200

[a] IC_{50} values are reported as the average of at least two separate determinations.
[b] Values for **4** are literature values; see Refs. 11 (FAK) and 12 (JAK2).

(Scheme 1). Nitration gave a mixture of *ortho* and *para* nitration, but the desired *para* derivative was selectively crystallized from cyclohexane. Capping of the piperidine nitrogen with iodoacetamide and hydrogenation of the nitro group furnished the desired aniline **7**.

Heterocyclic bromide **8**[8] readily undergoes arylation at C7 when coupled with a variety of boronic acids or esters to furnish **9a–q**. Oxidation of **9a–q** with either *m*-CPBA or a hydrogen peroxide/sodium tungstate system proceeded efficiently to activate C2 for hydrolysis with aqueous sodium hydroxide from either the sulfoxide or sulfone. As previously described,[8] in situ formation of the triflate followed by displacement with aniline **7** gave the targeted analogs **3a–q**.

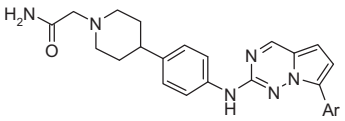
The enzymatic activity of analogs **3a–q** showed a wide range of aromatic and heteroaromatic substitution that was tolerated for inhibition of JAK2 (Table 2). In the cellular assay, analog **3b** showed the highest potency (IC_{50} = 15 nM) with most of the remaining analogs meeting program criteria of IC_{50} <100 nM.[15] In the FAK assays, analog **3c**, featuring a *m*-*tert*-butylsulfonamide, showed the greatest inhibition (IC_{50} = 5.7 nM) with analog **3b** again showing good potency (IC_{50} = 14 nM). Derivatives bearing a basic heterocyle at C7 (**3k–m**, **3q**) or a basic alkylamine (**3o**) showed the least activity, as did fused ring systems (**3o**, **3p**). Inhibition of FAK in the cellular assay was not as robust in this series as it was for JAK2. Cellular IC_{50} values ranged from 610 nM to >10 μM, with analogs **3a**, **3b**, **3e** and **3j** showing IC_{50} values below 1 μM.

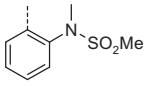
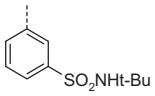
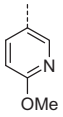
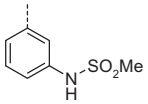
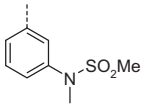
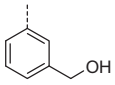
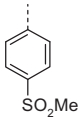
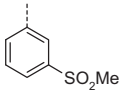
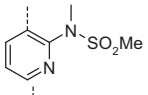
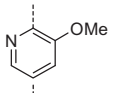
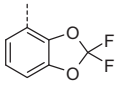
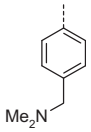
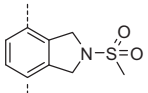
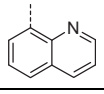
The electron rich C5 of the pyrrolo[2,1-*f*][1,2,4]triazine system was previously shown[10] to be a metabolic liability and was investigated in this series using the 2-(*N*-methyl)methanesulfonamidophenyl substituent at C7. Utilizing the regiomeric bromide **11**,[8] the C5 bromide was converted (Scheme 2) into either the methyl analog (via Suzuki coupling) or the chloride (using $NiCl_2$).[16] Subsequent halogenation at C7 and Suzuki coupling afforded intermediates **12a–b**. Following methylation of the sulfonamide, the methyl sulfide at C2 was oxidized and displaced to furnish the hydroxyl analogs, which were activated for displacement and finally reacted with aniline **7** to furnish **14a–b**.

While C5 methyl analog **14a** lost activity in the cellular assays (Table 3), **14b** exhibited slight improvement in both FAK and JAK2 cellular assays. As expected, the stability of **14b** in liver microsomes improved in all four species as compared to **3b** (**14b**: Mouse: 26 min, Rat: 21 min, Dog: 17 min, Human: 11 min vs **3b**: Mouse: 15 min, Rat: 6 min, Dog: 11 min, Human: <5 min). Analog **14b** was profiled in Sprague–Dawley rats (Table 4) and exhibited a desirable profile, with an iv half-life of 1.0 h, moderate distribution (Vd = 1.3 ± 0.3 L/kg) and measurable oral exposure, resulting in a bioavailability of 12 ± 2%. Internal selectivity screening showed **14b** to be equipotent against JAK3 (enzyme IC_{50} = 2.9 nM) and selective against the insulin receptor (enzyme IC_{50} = 380 nM). Further profiling was conducted at Invitrogen; **14b** displayed >90% inhibition against 19 of 53 oncology-related kinases (36%) when screened at 1 μM.[17]

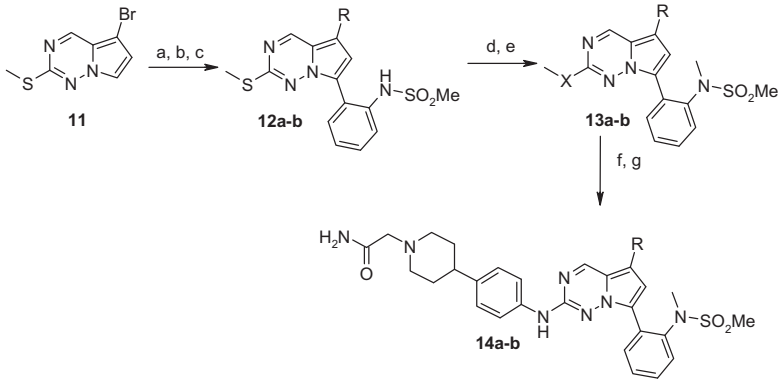
A PK/PD experiment was conducted, dosing **14b** as a single oral dose (55 mg/kg) and monitoring pSTAT3 and pFAK phosphorylation in CWR22 tumor xenografts (Fig. 2). pSTAT3 signaling was inhibited >75% out to 12 h, with return of signal at 24 h, indicative of robust inhibition of JAK2. The high plasma protein binding of **14b** (99.4% in mouse) is likely contributing to strong inhibition of pSTAT3 at the 12 h timepoint. The inhibition of pFAK at 2 h was >75%, but decreased at both the 6—and 12-h timepoints, before returning to that of vehicle at 24 h. This short inhibition of FAK

Table 2
Biological data for compounds **3a**–**k**



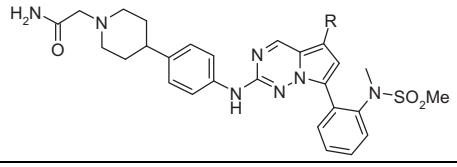
Compd	Ar	IC_{50}[a] (nM)			
		JAK2 enzyme	JAK2 cell	FAK enzyme	FAK cell
3a		0.60	41	55	690
3b		2.0	15	14	610
3c		0.64	90	5.7	1340
3d		0.77	62	39	1190
3e		0.22	60	21	660
3f		0.09	45	48	>10,000
3g		0.27	27	33	1390
3h		0.22	29	114	>10,000
3i		0.52	58	25	1010
3j		2.1	38	14	750
3k		16	1140	2040	>10,000
3l		85	NT	>10,000	NT
3m		8.9	282	551	NT
3n		13	>1000	122	NT
3o		2900	NT	>1000	NT
3p		16	NT	205	NT
3q		6.3	170	167	NT

[a] IC_{50} values are reported as the average of at least two separate determinations.



Scheme 2. Reagents and conditions: (a) R = Me: trimethylboroxine, $Pd(PPh_3)_4$; R = Cl: $NiCl_2$; (b) R = Me: NBS; R = Cl: NIS; (c) boronic acid, $Pd(PPh_3)_4$; (d) methyl sulfate; (e) R = Me: *m*-CPBA (X = SO); R = Cl: H_2O_2, Na_2WO_4 (X = SO_2); (f) NaOH; (g) $PhN(Tf)_2$ then **7**.

Table 3
Biological data for compounds **3b**, **14a–b**



Compd	R	IC_{50}[a] (nM)			
		JAK2 enzyme	JAK2 cell	FAK enzyme	FAK cell
3b	H	2.0	15	14	610
14a	Me	3.6	39	11	1230
14b	Cl	2.5	12	5.0	420

[a] IC_{50} values are reported as the average of at least two separate determinations.

Table 4
Pharmacokinetic parameters of **14b** in Sprague–Dawley rats[a]

	PK parameters	
iv	Dose (mg/kg)	1
	$t_{1/2}$ (h)	1.0 ± 0
	$AUC_{0\text{-}t}$ (ng h/mL)	1320 ± 299
	Vd (L/kg)	1.3 ± 0.3
	CL (mL/min/kg)	14 ± 4
po	Dose (mg/kg)	5
	C_{max} (ng/mL)	185 ± 24
	t_{max} (h)	2.2 ± 1
	$AUC_{0\text{-}\infty}$ (ng h/mL)	832 ± 121
	F%	12 ± 2

[a] Values are the average ± SEM from three animals.

in vivo is consistent with the cellular potency of **14b** (IC_{50} = 420 nM) and observed levels in tumor and plasma (Fig. 3).

In summary, progress toward a dual inhibitor of both FAK and JAK2 was achieved, providing a bioavailable analog that demonstrated knockdown of pSTAT3 in vivo over 12 h and to a lesser extent, reduction in FAK phosphorylation. Further refinement of this or another chemical series is necessary to provide a more potent dual inhibitor that would be capable of validating the FAK–JAK2 dual inhibition hypothesis.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.049.

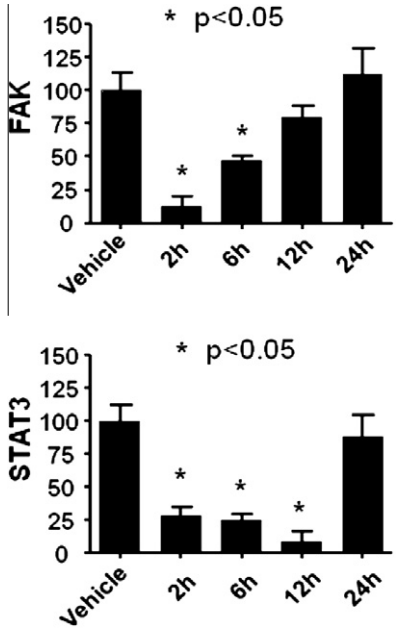


Figure 2. Inhibition of pFAK and pSTAT3 phosphorylation in CWR22 tumor xenografts in mice by **14b** with a single oral dose (55 mg/kg).

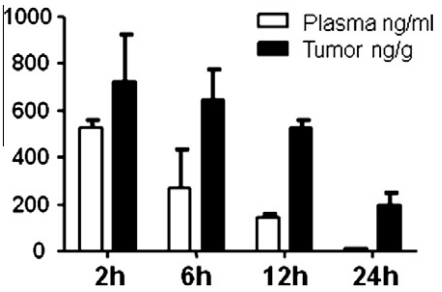


Figure 3. PK data for **14b** from PK/PD study in mice with a single oral dose (55 mg/kg).

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14. The *o*-methoxy group in **2** is accommodated by the gatekeeper+2 residue in FAK (leucine) but not in JAK2 (tyrosine). For a similar binding analysis, see: Galkin, A. V.; Melnick, J. S.; Kim, S.; Hood, T. L.; Li, N.; Li, L.; Xia, G.; Steensma, R.; Chopiuk, G.; Jiang, J.; Wan, Y.; Ding, P.; Liu, Y.; Sun, F.; Schultz, P. G.; Gray, N. S.; Warmuth, M. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 270.
15. For this chemical series, robust JAK2 enzyme inhibition was consistently observed but translation into cell varied widely. The enzyme assay is a partial sequence construct for JAK2, whereas for FAK it is the full sequence. Translation to cell for FAK was more consistent for this series. For SAR purposes, the JAK2 cell was the gating result, followed by FAK cell inhibition. For a full description of the assays, see the Supplementary data.
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