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Discovery and SAR of potent, orally available 2,8-diaryl-quinoxalines as a new class of JAK2 inhibitors

Carole Pissot-Soldermann *, Marc Gerspacher, Pascal Furet, Christoph Gaul, Philipp Holzer, Clive McCarthy, Thomas Radimerski, Catherine H. Regnier, Fabienne Baffert, Peter Drueckes, Gisele A. Tavares, Eric Vangrevelinghe, Francesca Blasco, Giorgio Ottaviani, Flavio Ossola, Julien Scesa, Janitha Reetz

Novartis Institutes for Biomedical Research, Novartis Pharma AG, WKL-136.4.96, CH-4002 Basel, Switzerland

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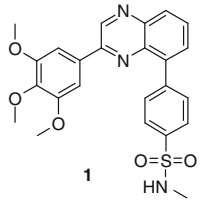
ABSTRACT

We have designed and synthesized a novel series of 2,8-diaryl-quinoxalines as Janus kinase 2 inhibitors. Many of the inhibitors show low nanomolar activity against JAK2 and potently suppress proliferation of SET-2 cells in vitro. In addition, compounds from this series have favorable rat pharmacokinetic properties suitable for in vivo efficacy evaluation.

The Janus kinase (JAK) family comprises of four non-receptor protein tyrosine kinases, JAK1, JAK2, JAK3 and TYK2, which play an important role in cell survival, proliferation and differentiation.[1] The discovery of somatic mutations in JAK2, particularly that of $JAK2^{V617F}$, in patients with chronic myeloproliferative neoplasms (MPNs) marked an important milestone in our understanding of the pathogenesis of these disorders.[2,3] The $JAK2^{V617F}$ mutation is present in nearly every patient with polycythemia vera and in almost 50% of patients with essential thrombocythemia or primary myelofibrosis.[4] Thus, JAK2 represents a promising target for the treatment of MPNs and considerable efforts are ongoing to discover and develop small molecule inhibitors of its kinase activity. Herein, we describe the discovery of 2,8-diaryl-quinoxalines as potent JAK2 inhibitors, and report on their JAK family selectivity, anti-proliferative effects in $JAK2^{V617F}$ bearing SET-2 cells and pharmacokinetic properties in rats.

Screening an internal proprietary kinase compound collection resulted in the identification of a lead compound, which through iterative scaffold morphing exercises[5,6] and optimization afforded a class of 2,8-diaryl-quinoxalines, represented by the prototype compound (**1**; Fig. 1). Compound **1** is a potent inhibitor of JAK2 (IC_{50} = 42 nM), with a 30-fold JAK2/JAK3 selectivity in enzymatic assays and an overall favorable kinase selectivity profile. The hypothetical binding mode for this compound was confirmed by the crystal structure of the active JAK2 protein kinase domain in complex with **1** (Fig. 2)[7] and revealed that the quinoxaline N1 atom is involved in a critical interaction with the hinge part of the kinase through a hydrogen-bond with the backbone amide of Leu932. In addition, this quinoxaline ring makes hydrophobic contacts with side chains of residues Ala880, Met929 and Leu983. Favorable hydrophobic contacts are also observed between the aromatic ring of the trimethoxyphenyl substituent of **1** and the side chain of Leu855 and backbone of Gly935. The methylsulfonamide group participates in multiple hydrogen-bond interactions with water molecules located in the phosphate region of the ATP binding site. The phenyl ring that links the quinoxaline core to the methylsulfonamide moiety, occupies a hydrophobic part of the cavity, surrounded by the side chains Val863, Leu983 and backbone of Gly993.



1

Figure 1. Structure of prototype 2,8-diaryl-quinoxaline (**1**).

* Corresponding author. Tel.: +41 61 696 18 90; fax: +41 61 696 62 46.
E-mail address: carole.pissot@novartis.com (C. Pissot-Soldermann).

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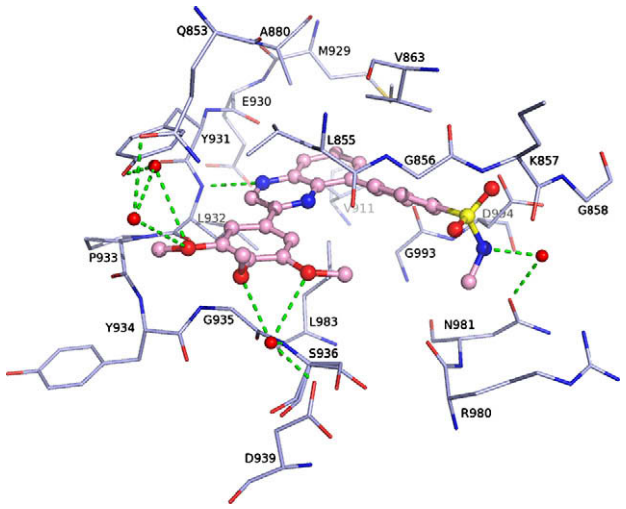
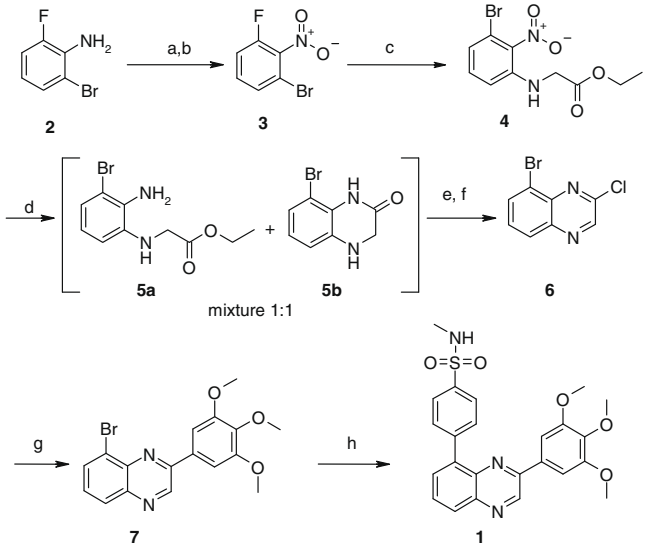


Figure 2. Crystal structure of **1** in complex with the JAK2 kinase domain solved at 2.00 Å resolution. Polar contacts between the inhibitor, solvent and the protein are indicated with dotted lines.

The pharmacokinetic profile of **1** was evaluated in conscious rats. After intravenous bolus administration, **1** shows a rather high total clearance (87 mL min^{-1} kg^{-1}) with a large volume of distribution (6.75 L kg^{-1}) and a moderate terminal half-life (1.69 h) and, after oral administration (3 mg/kg as a suspension in 0.5% carboxymethyl cellulose in bidistillated water) the AUC was 4 μg min mL^{-1}, corresponding to 34% oral bioavailability.

Based upon the in vitro/in vivo profile of **1**, we considered this chemotype as an attractive starting point and embarked on an extensive medicinal chemistry program to optimize the molecule.

The compounds were readily synthesized via the sequence outlined in Scheme 1, starting from 2-bromo-6-fluoro-aniline (**2**). Oxidation of the aniline in two steps yielded the nitro compound (**3**) and fluoride displacement with ethyl glycinate afforded (**4**). Reduction of the nitro group via hydrogenation in the presence of Raney–Nickel led to a mixture of the corresponding aniline derivative **5a** and the cyclized intermediate **5b**. Treatment of this mixture with KOBu-*t* in the presence of air (oxygen) yielded 8-bromo-1*H*-quinoxaline-2-one which was chlorinated with $POCl_3$ to provide 8-bromo-2-chloro quinoxaline (**6**). Intermediate (**6**) was a used to introduce structural diversity in the two final steps via consecutive selective palladium-catalyzed Suzuki coupling reactions to introduce two aryl moieties.



Scheme 1. Reagents and conditions: (a) *m*CPBA, CH_2Cl_2, reflux, 30 min 93%; (b) H_2O_2 30% AcOH, fuming HNO_3, rt then 90 °C, 30 min 66% (crude); (c) ethylglycinate·HCl, DMA, DIEA, 80 °C, 18 h, 62%; (d) H_2, RaNi, EtOH, THF 1:1, quant. (crude); (e) KOBu-*t*, THF, MeOH, air, rt, 16 h, 66%; (f) $POCl_3$, 55 °C, 3 h, 88%; (g) 3,4,5-trimethoxyphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DMF, 105 °C, 68%; (h) S-Phos, $Pd_2(dba)_3$, K_3PO_4, 1,2-DME, 4-*N*-methyl (aminosulfonylphenyl) boronic acid, 110 °C, 15 h, 71%.

The effects of the 2,8-diaryl-quinoxalines on JAK family members were assessed in biochemical and JAK2-dependent cellular assays (Table 2).[8,9] The initial SAR studies focused on variation of substituent R^2. Replacement of the methyl sulfonamide by 4-phenylacetic morpholine amide (**8**) or 4-phenylacetic thiomorpholine dioxide amide (**9**) gave a 3-8 fold gain in JAK2 potency, which also translated into cell growth inhibition (GI_{50}) of 228 nM and 119 nM, respectively, in the SET-2 $JAK2^{V617F}$ proliferation assay. Reducing the planar morpholine amide moiety to the more flexible and slightly basic benzyl morpholine was tolerated (compare **13** with **14** and **15** with **16**). Exploration of the effect of additional small substituents (F or Me) on the phenyl ring in R^2 showed that introduction in the *meta*-position resulted in a fourfold increase in potency (compare entry **13** with **16** and **17**), whereas the same residues in the *ortho*-position yielded a drastic loss of potency (compare entries **18** and **19** with **13**). In comparison to **22**, introduction of a 3,5-difluoro substituent further improved JAK2 inhibitory activity providing compound **23** with a very good inhibition of SET-2 $JAK2^{V617F}$ cell proliferation (GI_{50} = 135 nM). This SAR is fully supported by the crystal structure of JAK2 in complex with **1**. This structure suggests that *meta*-substituents can provide additional favorable hydrophobic contacts with residues Val863 and Gly856 of the P-loop on the one hand and the carbon atoms of the side chain of Asp994 on the other hand. In contrast, *ortho*-substituents, by increasing the twist between the quinoxaline core and the phenyl ring R^2, lead to a steric clash of the substituent with either Val863 or Leu983. As already mentioned, the phenyl ring in R^2 lay in proximity to the backbone of Gly993, which corresponds to Ala966 in JAK3. This difference, coupled with the observation that in a structure of JAK3 recently disclosed[10], Ala966 adopts a ψ backbone conformation of opposite direction compared to that of Gly993 in JAK2 provides a rationale to explain the selectivity for JAK2 against JAK3 obtained with our analogs. The R^2 phenyl ring and its substituents appear to be better accommodated by the less sterically demanding Gly993 residue of JAK2. Introduction of small R^1 groups (F or Me) resulted in significant loss of potency (entry **11** and **12**). As expected from the structure, this R^1 position is too close to the carbonyl group of Glu930 to tolerate any substituent.

Exploration of the R^3 substituent was pursued to optimize the physicochemical properties of the compounds as well as to improve JAK2 affinity (compare **10** with **20** and **15** with **21**). The R^3 substituent is located in the hydrophobic channel formed by residues Gly935 and Leu855 at the entrance of the cavity. Compounds **20** to **23**, in which the trimethoxyphenyl moiety is replaced by carboxamide-substituted phenyl residues, show potent cellular

Table 1
Comparison of measured molecular properties of compounds[11,12]

Compounds	Log $1/S_0$ (M)	Log *P*	ΔSL	MP	pK_a
20	4.80	2.80	2.00	204.03	3.9/6.5
24	4.60	4.00	0.60	168.41	4.5/7.2
25	5.06	3.80	1.26	227.28	3.6/6.9
26	3.98	3.20	0.78	137.30	5.5/9.1

S_0, intrinsic aqueous solubility, Log *P* logarithm of the partition coefficient *P*, based on experimental Log *P*, ΔSL = Log $1/S_0$ − Log *P*, MP: melting point, pK_a: (minus) logarithm of the ionization constant K_a, measured by potentiometry.