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Diamino-1,2,4-triazole derivatives are selective inhibitors of TYK2 and JAK1 over JAK2 and JAK3

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ABSTRACT

Tyrosine kinase 2 (TYK2) is required for signaling of interleukin-23 (IL-23), which plays a key role in rheumatoid arthritis. Presented is the design and synthesis of 1,2,4-triazoles, and the evaluation of their inhibitory activity against the Janus associated kinases TYK2 and JAKs 1–3.

Blocking cytokine activity is an established strategy for the treatment of rheumatoid arthritis (RA) and other inflammatory diseases.[1–3] Biologic therapies such as etanercept (Enbrel) inhibiting tumor necrosis factor (TNF) have emerged as routine treatments for RA over the last decade. In addition to high costs and the requirement for clinical administration, these agents suffer from a high degree of non-responders. The small-molecule methotrexate (MTX), an antifolate agent, has been used for the treatment of RA for over two decades. MTX remains one of the front-line treatments for early RA, although many patients discontinue use due to lack of efficacy, toxicity or compliance issues. The current understanding of cytokine networks in vivo and the lack of response to current biologic therapies by certain patients indicate that new cytokines should be considered as therapeutic targets. Interleukin 23 (IL-23) has been shown to be a critical factor in the pathogenesis of animal models of RA.[4,5] IL-23 signaling requires activation of Janus associated kinase 2 (JAK2) and tyrosine kinase 2 (TYK2). Mutant mice with a naturally defective TYK2 gene do not develop arthritis.[6] Although either JAK2 or TYK2 could be targeted for blocking IL-23 activity, JAK2 is used more broadly in cytokine signaling. We therefore selected TYK2 as the target for our research, with an immediate goal of understanding the relationships between inhibitor structure and selectivity across the Janus associated kinases, JAKs 1–3 and TYK2. Here we present our initial studies toward selective TYK2 inhibitors.

Despite the recognition of TYK2 as a potential target in various inflammatory diseases, compounds that selectively inhibit TYK2 over JAKs 1–3 are nearly absent from the literature. The binding properties of a collection of drugs and drug candidates with a panel of kinases were recently reported.[7] Of these, a single compound, JNJ-7706621 (**1**),[8,9] is selective for TYK2 and JAK1 over JAK2 and JAK3. Previously, TG101348 (**2**),[10,11] INCB018424 (**3**),[12–15] and CP-690550 (**4**)[16] were designed as selective JAK inhibitors (Table 1). When we dock compounds **1**–**4** into the crystal structure of TYK2,[17] the binding interactions of these compounds with TYK2 define a common region extending from the hinge of the kinase domain to the phosphate binding region (Fig. 1). The hinge of the kinase domain consists of the stretch of peptide that connects the two lobes of the domain, providing an array of hydrogen bond donors and acceptors. These ligands also interact with the phosphate binding region of ATP, adjacent to the conserved DFG motif. This DFG sequence forms the start of the kinase activation loop and is within reach of the flexible G-rich loop that forms a flap over the top of ATP and substrates to shield water from the site of phosphate transfer. JNJ-7706621 and TG101348 project tail groups toward the other end of ATP binding site, where it opens toward solvent.

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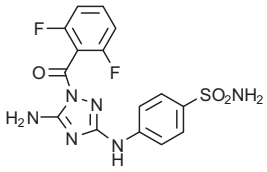
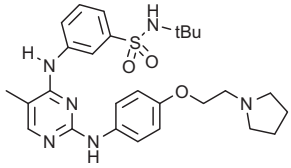
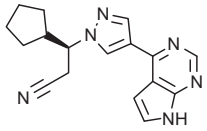
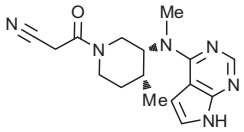
Table 1
Kinase inhibitors with activity against JAKs[a]

Compound		TYK2	JAK1	JAK2	JAK3
 **1:** JNJ-7706621	K_d (Ref. 7)	32 nM	21 nM	220 nM	180 nM
 **2:** TG101348	IC_{50} (Ref. 10)	150 nM	100 nM	3 nM	1 μM
 **3:** INCB018424	IC_{50} (Ref. 15)	19 nM	3 nM	3 nM	430 nM
 **4:** CP690550	K_d (Ref. 7)	620 nM	>10 μM	5 nM	2.2 nM

[a] Values listed are either dissociation constants (K_d) or enzyme inhibitory activities (IC_{50}) reported in the literature.

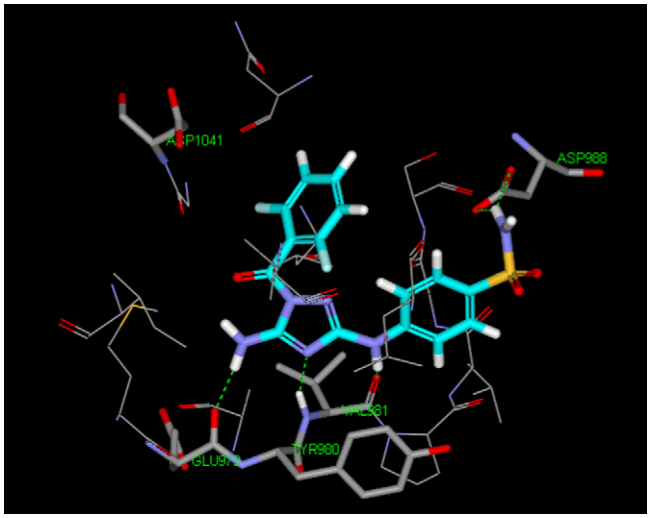


Figure 1. JNJ-7706621 docked into crystal structure of TYK2.

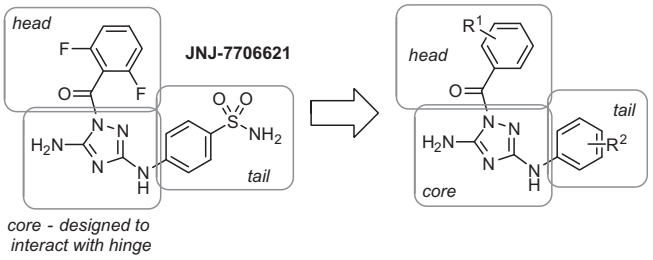
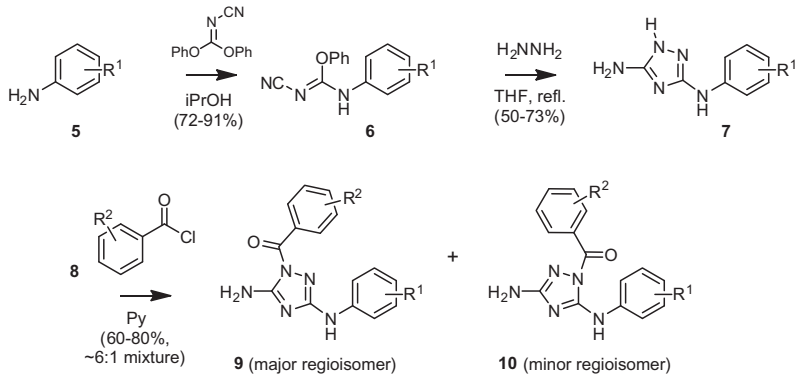


Figure 2. Deconstruction of JNJ-7706621.

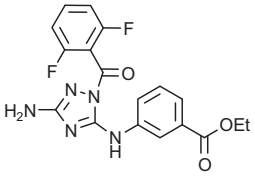
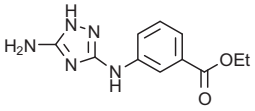
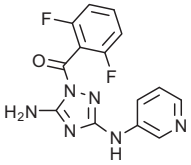
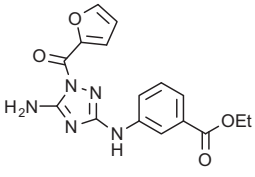
The near-perfect conservation of amino acids within the active site of the JAK family is well known. This conservation has the implication that the development of selective TYK2 inhibitors is not easily accomplished, but the examples of TG101348 and CP690550 prove that selectivity can be achieved within this kinase family. In our program, two observations were incorporated into the development of the compounds with the aim of achieving TYK2 selectivity. First, there is considerable sequence variability in the G-rich loop of JAK kinases and differences in its conformation might offer an opportunity for achieving differential response to TYK2. However, the inherent flexibility of this region makes it difficult to predict what chemical structures might offer selective inhibition across the JAKs. Therefore we turned our attention to a second observation. Residue Arg901 in Tyk2 is different in Jak2(Gln) and JAK3(Ser). Compounds that can form productive electrostatic interactions with this Arg could result in isoform selectivity. Much of the work on the compounds reported herein was executed prior to the release of the crystal structures of TYK2. In a homology model built from the JAK2 crystal structure, we considered Arg901 to be flexible, and thus available to be targeted by rationally designed inhibitors. This was called into question by TYK2 crystal structures that show Arg901 forming a hydrogen bond to Tyr980 from the hinge.[17] Of course, the crystal structure is a static representation and may not accurately describe the dynamic nature of the kinase.

Given the modest selectivity of JNJ-7706621, we used this compound as a starting point to develop SAR. The compound was



Scheme 1. General synthesis of TYK2 inhibitors.

Table 2
Inhibitory activity of 1,2,4-triazoles against TYK2 and JAKs 1–3

Compound	R^1	R^2	IC_{50} (nM)			
			TYK2	JAK1	JAK2	JAK3
1	4-SO_2NH_2	2,6-F_2	39	39	1700	950
11	3-SO_2NH_2	2,6-F_2	70	19	2500	1250
12	3-CO_2Et	2,6-F_2	1720	990	>10,000	>10,000
13	3-CO_2H	2,6-F_2	690	610	>10,000	>10,000
14	4-CO_2Me	2,6-F_2	760	500	>10,000	>10,000
15	4-CO_2H	2,6-F_2	42	45	2110	1470
16			>10,000	nt	nt	nt
17			>10,000	nt	nt	nt
18	H	2,6-F_2	360	230	⩾10,000	3250
19	3-NMe_2	2,6-F_2	70	39	5000	2000
20	4-NMe_2	2,6-F_2	70	80	2500	850
21			300	160	>10,000	5000
22	3-CO_2Et	H	>10,000	nt	nt	nt
23	3-CO_2Et	2-F	6040	⩾10,000	>10,000	>10,000
24	3-CO_2Et	4-CF_3	>10,000	nt	nt	nt
25	3-CO_2Et	4-NMe_2	>10,000	nt	nt	nt
26			>10,000	nt	nt	nt

deconstructed into three portions (Fig. 2): the core, which binds the hinge region; the head region, which occupies the phosphate binding region; and the tail, which is oriented toward Arg901 in our computational model. A series of compounds around the 1,2,4-triazole core of JNJ-7706621 were synthesized to probe the SAR of groups flanking the core according to the general scheme shown in Scheme 1, using methods developed by Webb.[18,19] Treatment of aniline **5** with diphenyl cyanocarbonimidate afforded the aniline adduct **6**, which was heated to reflux with hydrazine to give the diaminotriazole **7**. Reaction **of 7** with benzoyl chloride **8** gave a mixture of regioisomers **9** (major) and **10** (minor).

Kinase activity was determined by applying Invitrogen's LanthaScreen™ TR-FRET assays using the catalytic domain of recombinant human kinase. The inhibitory activity of compounds **1** and **11–26** is shown in Table 2. From these data, we are able to assess the potential of this scaffold to produce selective TYK2 inhibitors.

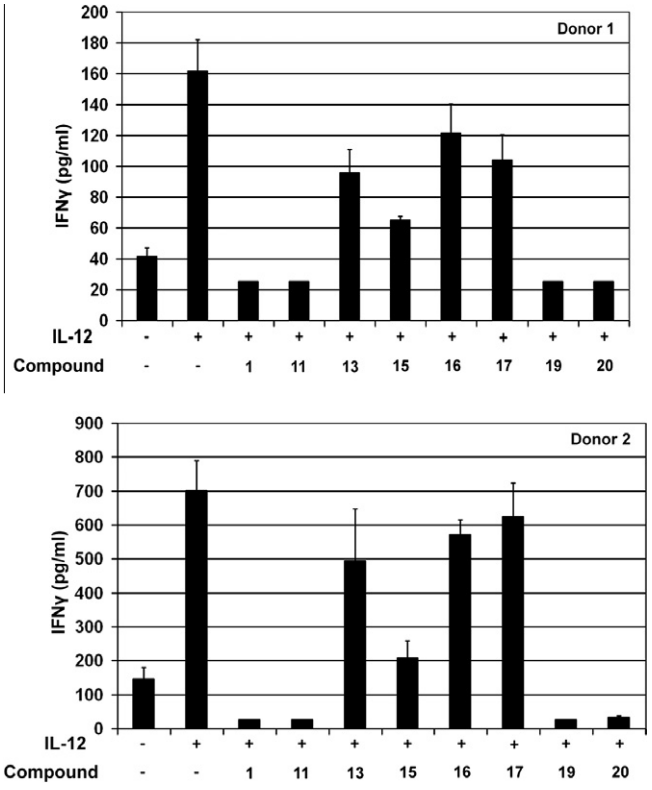


Figure 3. Inhibitory effect of compounds on IFNγ cytokine production. Human PHA blasts, from two different donors were derived from PBMCs cultured in PHA (0.1 μg/ml) and IL-2 (10 U/ml) for 7 days. PHA blasts were pre-incubated with inhibitors (10 μM) for 15 min and then stimulated with IL-12 (10 ng/ml). After 48 h, cell supernatants were collected and IFNγ production was measured by ELISA. Data are mean ± SEM of triplicate wells.

The activity and selectivity of JNJ-7706621 (**1**) was confirmed. TYK2 inhibitory activity was modulated by the tail group, where *para*-substituted compounds were more potent than their *meta*-congeners. This was most dramatic in carboxylates **15** versus **13**, with smaller effects evident in sulfonamides **1** versus **11** and esters **14** versus **12**. Stronger hydrogen bond acceptors also increased TYK2 inhibition; sulfonamides **1** and **11**, carboxylate **15** and dimethylamines **19** and **20** were better inhibitors than esters **12** and **14** and unsubstituted tail analog **18**. While this was consistent with the hypothesis that interacting with Arg901 would improve TYK2 activity, selectivity was relatively insensitive to these changes, except for a modest increase in JAK1 versus TYK2 selectivity between *para* and *meta* pairs (e.g. **1** vs**11**, **12** vs **14**, and **19** vs **20**). With respect to the head group, the 2,6-difluorobenzoyl head group was strongly preferred for TYK2 activity. Removal of the head group (**17**) or a 1,2-shift of the 2,6-difluorobenzoyl head group (**16**) gave inactive compounds. The 2,6-difluoro substitution appeared to be critical as the monofluoro compound **23** was significantly less active than **12** and non-halogenated **22** was inactive. The effect was not purely electronic, as trifluoromethyl analog **24** was inactive as well. Other head group variants showed no inhibition. While not a priority for the current research, these compounds likely represent novel CDK inhibitors given their structural similarity to JNJ-7706621 (**1**).

Next, we sought to determine whether these triazoles would also be capable of inhibiting TYK2 in a biologically relevant setting. We stimulated human PHA blasts with IL-12, a cytokine which requires TYK2 and JAK2 for signaling, in the absence or presence of inhibitors, and then examined the levels of IFNγ, a downstream product of IL-12 signaling. We found that compounds **11**, **19**, and **20** inhibited IFNγ production similar to JNJ-7706621 (**1**) whereas compounds **13** and **15** partially inhibited IFNγ (Fig. 3). Compounds **16** and **17** were included as negative controls since they showed no activity in the kinase assay. They showed minimal IFNγ inhibition.

In conclusion, we have identified four compounds, **11**, **15**, **19**, and **20**, with activity and selectivity similar to the most selective compound described in the literature JNJ-7706621 (**1**). Compounds **11**, **15**, and **20** maintain activity in a cellular context. Our future efforts will be directed toward developing compounds with higher selectivity for TYK2 over JAK1-3. Work will be guided by the new crystal structures, and we will seek new opportunities to achieve selectivity in this family of kinases.

Supplementary data

Supplementary data (synthetic procedures, NMR spectra of compounds **11**–**26**, and methods for enzymatic inhibition assays) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.026.

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