Accepted Manuscript

Design, synthesis and structure-activity relationship study of aminopyridine derivatives as novel inhibitors of Janus Kinase 2

Wanqi Wang, Yanyan Diao, Wenjie Li, Yating Luo, Tingyuan Yang, Yuyu Zhao, TianTian Qi, Fangling Xu, Xiangyu Ma, Huan Ge, Yingfan Liang, Zhenjiang Zhao, Xin Liang, RuiWang, Lili Zhu, Honglin Li, Yufang Xu

PII: S0960-894X(19)30220-3
DOI: https://doi.org/10.1016/j.bmcl.2019.04.011
Reference: BMCL 26378

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 18 March 2019
Revised Date: 30 March 2019
Accepted Date: 6 April 2019

Please cite this article as: Wang, W., Diao, Y., Li, W., Luo, Y., Yang, T., Zhao, Y., Qi, T., Xu, F., Ma, X., Ge, H., Liang, Y., Zhao, Z., Liang, X., RuiWang, Zhu, L., Li, H., Xu, Y., Design, synthesis and structure-activity relationship study of aminopyridine derivatives as novel inhibitors of Janus Kinase 2, *Bioorganic & Medicinal Chemistry Letters* (2019), doi: https://doi.org/10.1016/j.bmcl.2019.04.011

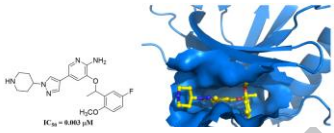
Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Design, synthesis and structure-activity relationship of aminopyridine derivatives as novel inhibitors of Janus Kinase 2

Compounds **12l** potently and selectivity inhibited the enzymatic activity of JAK2

WanqiWang,[#,a]YanyanDiao,[#,a]Wenjie Li,[#,a]YatingLuo,[a]Tingyuan Yang,[a] YuyuZhao,[a]TianTian Qi,[a] FanglingXu,[a]XiangyuMa,[a]HuanGe,[a]YingfanLiang,[a] Zhenjiang Zhao,[a] Xin Liang,[a]RuiWang,[a] LiliZhu,*[,a] Honglin Li *[,a] and Yufang Xu *[,a]



Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Design, synthesis and structure-activity relationship study of aminopyridine derivatives as novel inhibitors of Janus Kinase 2

Wanqi Wang,[#,a] Yanyan Diao,[#,a] Wenjie Li,[#,a] Yating Luo,[a] Tingyuan Yang,[a] Yuyu Zhao,[a] TianTian Qi,[a] Fangling Xu,[a] Xiangyu Ma,[a] Huan Ge,[a] Yingfan Liang,[a] Zhenjiang Zhao,[a] Xin Liang,[a] RuiWang,[a] Lili Zhu,[*,a] Honglin Li [*,a] and Yufang Xu [*,a]

[a]*Shanghai Key Laboratory of New Drug Design, State Key Laboratory of Bioreactor Engineering, School of Pharmacy, East China University of Science & Technology, Shanghai 200237, China*
[#]*Authors contributed equally to this work.*

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
JAK2
Rheumatoid arthritis
Crizotinib
Molecular docking

ABSTRACT

Janus Kinase 2 (JAK2) is a kind of intracellular non-receptor protein tyrosine kinase and has been certified as an important target for the treatment of myeloproliferative neoplasms and rheumatoid arthritis. However, the low selectivity and potential safety issues restrict the clinical applications of JAK2 inhibitors. Here we found that crizotinib showed good inhibitory activity against JAK2 by enzymatic assays (IC_{50} = 27 nM). Then we carried out structure-based drug design and synthesized a series of compounds with an aminopyridine scaffold. Finally, compound 12k and 12l were identified as the promising inhibitors of JAK2, which exhibited high inhibitory activity (IC_{50} =6 nM and 3 nM, respectively) and selectivity for JAK2 over JAK1 and JAK3, and showed potent antiproliferative activities toward HEL human erythroleukemia cells. Moreover, 12k suppressed symptoms of the collagen-induced arthritis (CIA) model in rats.

Janus kinase is a family of soluble non-receptor protein tyrosine kinases with four members, JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2).[1-3] JAK1, JAK2 and TYK2 are widely present in cells and tissues, while JAK3 is mainly expressed in myeloid and lymphoid cells. Each of the four members of the JAK family plays an individual role in the oncogenesis of the immune system.[4, 5] Cytokines stimulate cells through cytokines receptors on the cell membrane, thereby activating downstream signals and affecting the biological functions of cells.[6] This process is called cell signal transduction. Janus kinase, signal transducers and activators of transcription constitute the JAK-STAT pathway, which is a hot signal pathway in the current cytokine research and also plays important roles in many biological reactions in the body.[7] It regulates cell proliferation, differentiation and apoptosis, and also participates in the physiological processes such as immunity and inflammatory reactions of the body.[8] The main process of JAK-STAT signalling is described as follows: firstly, cytokines bind to their receptors outside the cell and activate receptors, which dimerize and phosphorylate JAKs on the receptor, and generate docking sites for STATs; after that, the STAT is phosphorylated and dimerizes; ultimately, STATs enter the nucleus in the form of homo- or hetero-dimerization and multimerization, and then bind to DNA and participate in the regulation of gene transcription and expression.[9-11]

Abnormalities in the JAK-STAT pathway have been linked to both inflammatory diseases and cancer, such as rheumatoid arthritis (RA), psoriasis and myeloproliferative neoplasms (MPNs).[12-17] Rheumatoid arthritis is a chronic systemic inflammatory disease that cocurs in 0.5-1% of adults worldwide. Although it has a variety of treatment options, for instance, biotic and abiotic disease-modifying anti-rheumatic disease drugs (DMARDs), there is still a significant unmet medical need. Therefore, new treatment methods are required to address the needs of patients with RA.[18] Studies have shown that the JAKs and STATs are significantly activated by cytokines in synovial cells and synovial tissues in RA patients, so inhibiting the JAK-STAT pathway can reduce the activation and proliferation of inflammatory cells, thereby relieving rheumatoid arthritis. Therefore, the development of drugs acting on the JAK-STAT pathway has become an important research direction for the treatment of RA.[19] For example, tofacitinib is a JAK1/3 inhibitor from Pfizer and has been approved for the treatment of RA by the

* Corresponding author. Tel.: +86-21-64250213; fax: +86-21-64250213; email: zhulfl@ecust.edu.cn, hlli@ecust.edu.cn; yfxu@ecust.edu.cn

US Food and Drug Administration (FDA) in 2012.[20,21] After that, several JAK kinase inhibitors were developed for RA, such as baricitinib and peficitinib. These JAK inhibitors have potential application for treating RA.[22, 23]

MPNs are malignant hematological diseases that originate from bone marrow hematopoietic stem cells. Polycythemiavera, essential thrombocythemia and primary myelofibrosis are the three most common myeloproliferative cancers.[24] Studies show that JAK2 V617F mutation are found in 80% of cancer patients with MPNs. This mutation causes the excessive activation of JAK2, which leads to the excessive proliferation of tumour cells.[25-27] The discovery of JAK2 V617F mutation in MPNs has prompted the development of molecularly targeted therapy.

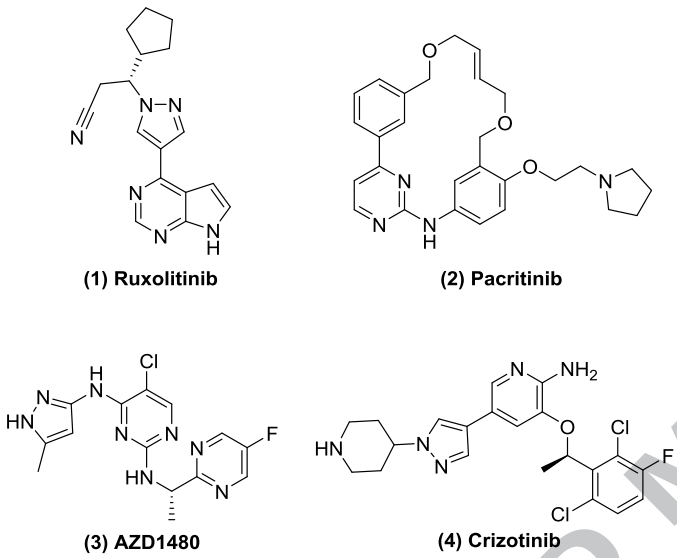


Figure1. Structures of (1) Ruxolitinib, (2) Pacritinib, (3) AZD1480, and (4) Crizotinib.

At present, some JAK2 inhibitors have entered clinical research, such as ruxolitinib, pacritinib and AZD1480 (Figure 1).[28-30] Ruxolitinib is the first JAK inhibitor approved by FDA, targeting JAK2 kinase (IC_{50} = 2.8 nM) for treating primary myelofibrosis.[31] These JAK2 inhibitors have shown good efficacy in a number of disease areas, but have known on-target toxicities, such as increased risk of infection, anemia (platelets, red and white blood cells), hypercholesterolemia, increased transglutaminases and creatinine levels, which have led to clinical safety issues.[32-33] Therefore, the discovery of selective JAK2 inhibitors is an area of high interest. In addition, due to high homology amongst JAK family members, particularly in the catalytic domain, the identification of selective JAK2 inhibitors has represented a significant challenge. Herein, we screened our compound library containing 672 listed drugs and tried to find candidate inhibitors targeting JAK2.

Through surface plasmon resonance assays (Figure S1), protein thermal shift assays (Table S1), and Z'-LYTE™ fluorescence assays, we finally obtained the lead compound, crizotinib, has a good inhibitory effect on JAK2. Crizotinib was reported to be a ALK/c-Met/ROS1 multitarget tyrosine kinase inhibitor, and it was approved by FDA for the treatment of ALK-positive locally advanced or metastatic non-small cell lung cancer (NSCLC). [34]

In order to improve the activity and selectivity, a series of crizotinib derivatives with an aminopyridine scaffold were designed and synthesized.

Firstly, we used the Sf9 eukaryotic expression system to express the kinase domain of JAK2 and obtained the purified recombinant protein by separation and purification technologies.[35] In order to identify new compounds binding to JAK2, we performed SPR-based screening from our in-house compound library containing 672 listed drugs. Ruxolitinib, which is a reported compound targeting JAK2, was chosen as the positive control for the binding assays. Initially, the compounds from our library were screened at 50 μM to evaluate whether they can bind to JAK2. After the initial screening, the dose-response assays were performed to calculated the K_D values. Finally, we identified that the most potent compound crizotinib bound to JAK2 with a K_D value of 10.99 nM (Figure S1). This result showed that crizotinib had a strong binding affinity with JAK2 protein. To further confirm their interaction, we performed fluorescence-based thermal shift assay and found the melting temperature of JAK2 was increased in the presence of crizotinib, with an increase of 8℃(Table S1). Subsequently, we conducted the Z'-LYTE™ Kinase Assay to measure the kinase level of inhibitory activity of crizotinib (IC_{50} = 27 nM for JAK2) and obtained the selectivity for JAK2 over JAK1 and JAK3 (20- and 50-fold), respectively. In addition, the result of the Mobility shift assay showed that crizotinib has a higher selectivity for JAK2 over TYK2, about 47-fold (Table 2).

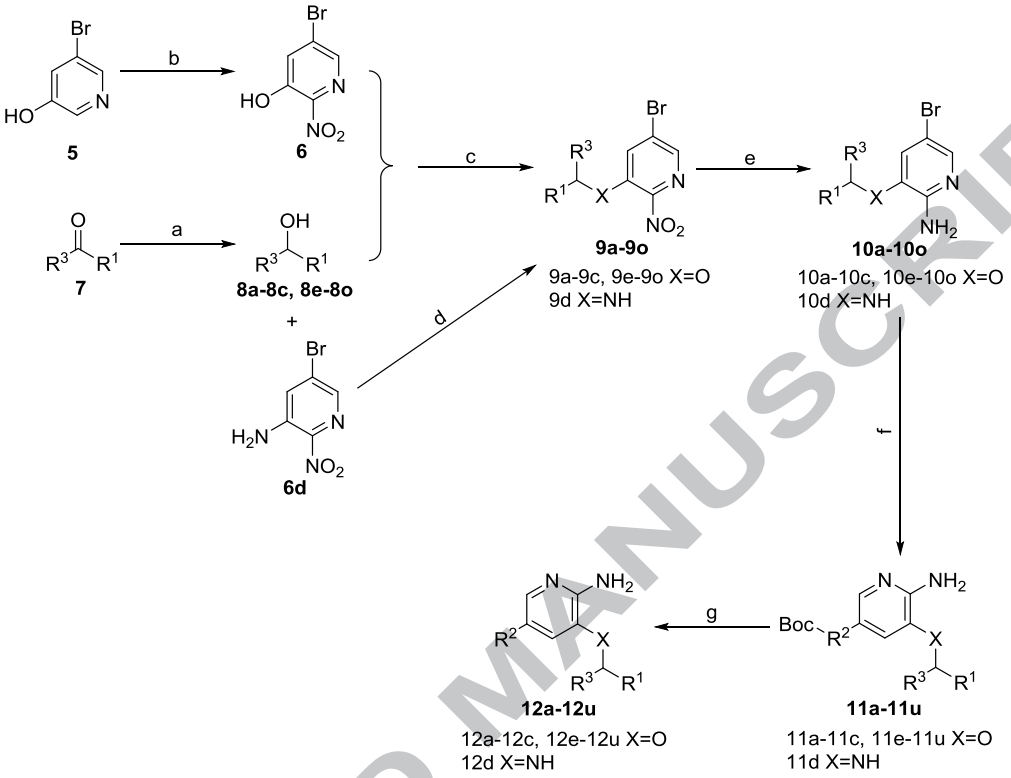
In order to further elucidate the mechanism of crizotinib at the cellular level, we carried out cell proliferation assay and investigated the effects of crizotinib on the JAK2-STAT signaling pathway. We found that crizotinib had an inhibition effect on HEL cells proliferation (IC_{50} = 4.725 μM) (Table 3). Moreover, crizotinib had effects on inducing concentration dependent inhibition of phosphorylation on STAT3 and STAT5, and increasing JAK2 autophosphorylation level at the Y1007/8 in HEL cells (Figure S2), which are general effects of JAK2 inhibitors[36]. Based on the above, we confirmed that crizotinib is a new lead compound targeting JAK2.

The strategies for the synthesis of compounds adopted in this research are shown in Schemes 1.[37] 3-Hydroxy-5-bromopyridine was used as a raw material to obtain compound **6** by nitration. Mitsunobu reaction was then performed with the alcohol substrate **8a-8c**, **8e-8o** to obtain intermediates of **9a-9c, 9e-9o**. The **9d** was obtained by an electrophilic substitution reaction. After being reduced with iron powder, a Suzuki reaction occurred with R^2-Boc to obtain intermediates of **11a-11u**. Finally Boc is removed under acidic conditions to give final product of **12a-12u** which were racemic compounds.

Crizotinib is a previously reported c-MET/ALK inhibitor and the crystal structures of crizotinib binding to the kinase domains of these two kinases, respectively, have been resolved.[38] In view of the highly conserved nature of the ATP-binding pocket within the kinase domain, we envisioned that crizotinib may adopt a similar conformation when interacting with JAK2. After super imposing the crystal structure of ALK in complex with crizotinib (PDB code 2XP2) onto JAK2 in complex with NVP-BSK805 (PDB code 3KRR), it was found that the shared piperidinyl-pyrazole moieties between the two crystallographic ligands overlaid well with each other.[39] To investigate the proposed binding mode of crizotinib with JAK2 and explore significant clues for further structural optimization, crizotinib was extracted and merged into the ATP-binding pocket of JAK2 (Figure 2A).

The 2-aminopyridine core of crizotinib putatively participates in hydrogen bond interactions with the backbone atoms of residues Gly930 and Leu932 in the hinge region (Figure 2B). The 3-benzyloxy moiety inserts into the hydrophobic pocket toward the activation loop, however, it does not seem to fit the pocket very well. On the one hand, the methyl group protrudes to the N-terminal lobe, leading to steric clashes with the side chain of

residue Val863. On the other hand, an unfavourable conflict could happen between the 2-chlorine of the phenyl ring and the oxygen on the backbone of residue Gly993, as the distance between these two atoms is as short as 1.6 Å. Owing to the formation of van der Waals effects between the 6-chlorine and residues Leu855 and Gly856, enhanced inhibitory activity could be pursued by introduction of larger groups to fulfill the hydrophobic subpocket. At the other end of crizotinib, the piperidinyl-pyrazole moiety extends to the hinge region near the entrance of the ATP-binding pocket, where the nonconserved residues may be critical for the design of compounds capable of selectively discriminating within the JAK family.[40]



Scheme 1. Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C to room temperature, 6 h; (b) HNO_3, H_2SO_4, 0 °C to room temperature, 6 h; (c) DIAD, PPh_3, THF, 0 °C to room temperature, 4 h; (d) TBAB, KI, Cs_2CO_3, CH_3CN, 80 °C, 10 h; (e) Fe, AcOH/EtOH, reflux, 3 h; (f) BPin-R^2-Boc, Pd(dppf)Cl_2, Cs_2CO_3, Toluene, 80 °C, 12 h; (g) Trifluoroacetic acid, CH_2Cl_2, 0 °C to room temperature, 12 h.

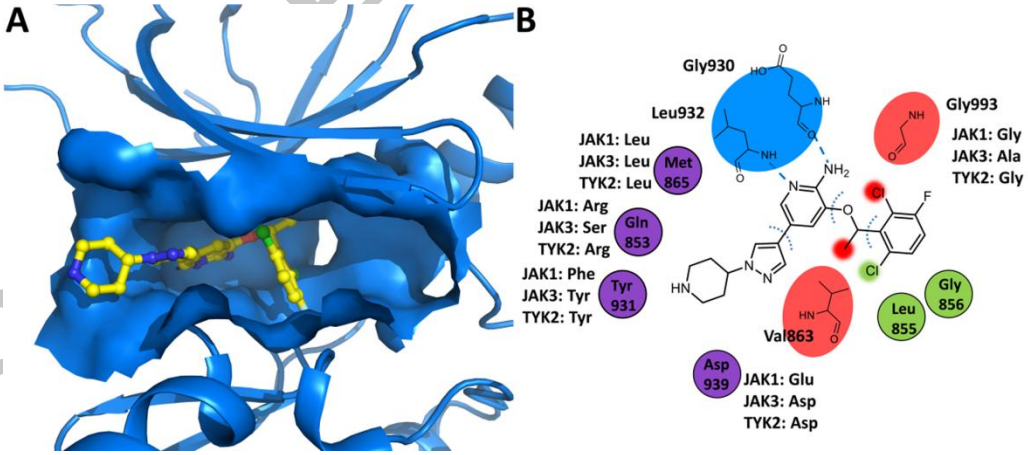


Figure 2. (A) Overview of crizotinib in the ATP-binding site of JAK2 kinase domain (PDB code 3KRR). The receptor is shown in marine cartoon, and the ligand is presented as a ball and stick model. (B) Detailed binding interactions of crizotinib against JAK2 with only key residues displayed for clarity. The hydrogen bonds are indicated as blue dashed lines. The residues and atoms of the ligand involved in steric clashes and hydrophobic effects are highlighted in red and green, respectively. Key residues which differ in the JAK family are also labeled.

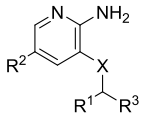
On the basis of the putative binding mode, the essential 2-aminopyridine core was fixed, and our initial efforts were performed on the optimization of the linker between the pyridine ring and the terminal 2,6-dichloro-3-fluorophenyl group. Inconsistent with our model, the removal of the methyl group yielded compound **12b**, with 6-fold activity loss against JAK2 compared to the racemic crizotinib **12a** (IC_{50} = 0.572 vs 0.092 μM). While the introduction of a larger ethyl group based on **12b** led to 5-fold enhanced potency of **12c**, the IC_{50} value of which is comparable to that of **12a**. These unexpected results might be ascribed to the plasticities of the ligand and the residues in the N-terminal lobe, which may adjust their conformations to tolerate each other. In addition, the replacement of -O- by -NH- did not make obvious improvements in terms of inhibitory effect against JAK2 (compound **12d**, IC_{50}= 0.126 μM).

Subsequently, an alternative strategy was carried out by modification of the R^2 group, which may significantly influence the potency and selectivity against JAK2. Compounds **12e-12i**, bearing substituents with different sizes and electrostatic effects in the R^2 position, were therefore developed. Compound **12e**,

containing a tetrahydropyran group, displayed slightly improved potency against JAK2, but its IC_{50} value was still in the same range as that of **12a** (0.065 vs 0.092 μM). When the terminal piperazinyl group was moved from 5- to 6-position of the pyridin-3-yl moiety, up to 6-fold discrepancy with respect to potency against JAK2 was found between compounds **12f** and **12g** (IC_{50}= 0.119 vs 0.804 μM), implying that the region at the entrance of the cavity is relatively sensitive to the substituents harbored in it. The removal of the piperazinyl ring resulted in a substantial drop in potency as observed for compound **12h** with a single-digit micromolar IC_{50} value. Furthermore, compound **12i**, which had an electronically neutral phenyl ring attached to the 2-aminopyridine core, almost suffered a complete loss of activity, suggesting that polar basic groups are probably preferential at the R^2 position for better inhibitory potency against JAK2.

Table 1

Structure and activity profiles of compounds 12a-12u against JAK2 kinase



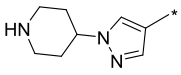
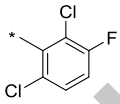
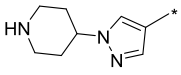
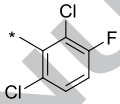
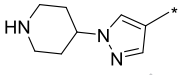
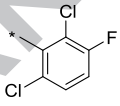
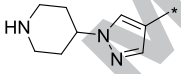
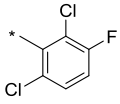
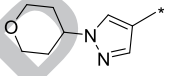
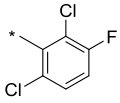
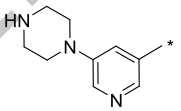
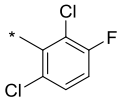
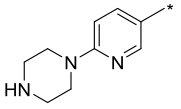
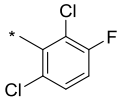
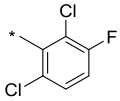
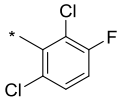
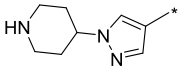
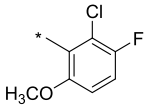
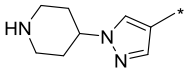
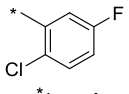
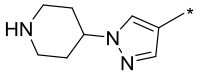
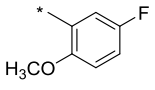
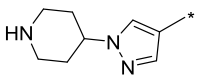
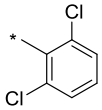
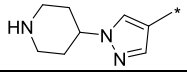
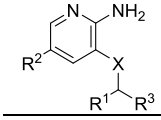
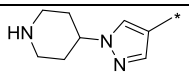
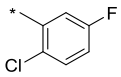
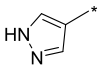
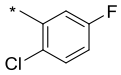
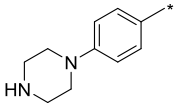
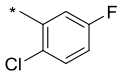
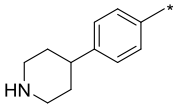
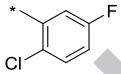
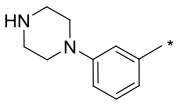
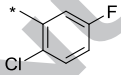
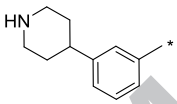
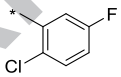
Compd	R^1	X	R^2	R^3	IC_{50} (μM)
12a	CH_3	O			0.092±0.007
12b	H	O			0.572±0.020
12c	C_2H_5	O			0.100±0.002
12d	CH_3	NH			0.126±0.005
12e	CH_3	O			0.065±0.001
12f	CH_3	O			0.119±0.008
12g	CH_3	O			0.804±0.009
12h	CH_3	O			3.049±0.204
12i	CH_3	O			7.152% (inhibition rate at1μM)
12j	CH_3	O			0.026±0.002
12k	CH_3	O			0.006±0.001
12l	CH_3	O			0.003±0.001
12m	CH_3	O			0.442±0.003
12n	CH_3	O			0.215±0.012

Table 1 *(continues)*



Compd	R^1	X	R^2	R^3	IC_{50} (μM)
12o	CH_3	O			3.400±0.431
12p	CH_3	O			0.461±0.002
12q	CH_3	O			0.016±0.001
12r	CH_3	O			0.037±0.003
12s	CH_3	O			0.048±0.008
12t	CH_3	O			0.027±0.002
12u	CH_3	O			0.035±0.001

In accordance with our previous hypothesis, compared to compound **12a**, the introduction of a larger methoxy group at the 6-position of the phenyl ring led to about 3-fold potency increase of compound **12j** (IC_{50}= 0.092 vs 0.026 μM). Meanwhile, the removal of the 2-chlorine atom yielded a more potent compound **12k** (IC_{50}= 0.006 μM), which may be attributed to avoiding the steric conflicts with Gly993. Although the structure of **12k** has been reported as a mutant ALK inhibtor, we have found its potential to be a novel selective JAK2 inhibitor.[35] On the basis of compound **12k**, the replacement of chlorine with methoxy group generated the most potent JAK2 inhibitor (compound **12l**, IC_{50}= 0.003 μM) of this chemical series. To thoroughly delineate the underlying SAR, compound **12l** was docked to the ATP-binding pocket of JAK2 (PDB code 3KRR) using Glide SP mode (Maestro v10.1, Schrödinger Inc.). In the predicted docking pose, as shown in Figure S2, **12l** displayed a very similar conformation to that of crizotinib binding to ALK (PDB code 2XP2). The alkyl linker tilts slightly toward the inside of the pocket, leaving enough space for the methyl group to be away from the N-terminal lobe to avoid the steric conflicts with Val863. Moreover, the methoxy group on the phenyl ring putatively participates in favorable hydrophobic interactions with around residues such as Leu855 and Gly856. The 3-fluorine atom appears to be essential to obtain potent inhibitory effect against JAK2, and its absence gave rise to 4-fold diminished potency of compound **12m** in relative to **12a**. Likewise, the smaller phenyl (**12n**) or cyclohexyl (**12o**) group in the R^3 position also did not make any improvements for the inhibitory activity against JAK2.

Table 2

Evaluation of the inhibitory activities of selected compounds against JAK1, JAK3 and TKY2.

Compd	Enzyme inhibitory activity (IC_{50}/μM)				Enzyme Selectivity		
	JAK1	JAK2	JAK3	TYK2	JAK1/JAK2 ratio	JAK3/JAK2 ratio	TYK2/JAK2 ratio
12j	1.223±0.013	0.026±0.002	1.759±0.063	0.774±0.041	46	66	30
12k	0.660±0.005	0.006±0.001	0.513±0.023	1.298±0.318	110	86	216
12l	1.796±0.041	0.003±0.001	1.584±0.044	0.794±0.203	598	528	264
12q	0.082±0.003	0.016±0.001	0.965±0.006	1.453±0.162	5	60	91
12t	0.120±0.001	0.027±0.002	2.788±0.051	2.530±0.437	4	107	94
Crizotinib	0.563±0.011	0.027±0.002	1.360±0.074	1.269±0.150	20	50	47
Ruxolitinib	0.004±0.001	0.0006±0.0001	0.051±0.002	0.011±0.002	6	85	18

Based on the previously established proper 2-chloro-5-fluorophenyl moiety, compounds **12p**-**12u** possessing various R^2 groups were synthesized, most of which exhibited IC_{50} values at two-digit nanomolar level. It is noteworthy that compound **12p** retrieved the inhibitory ability against JAK2 in comparison with **12i** (0.461 vs >10 μM). The addition of the piperidinyl or

piperazinyl increased the inhibitory potency, moreover, the para-substituent was more favoured than the meta-substituent (**12r** vs **12t**, **12s** vs **12u**). As illustrated in Figure S2, the -NH- group on the piperazinyl moiety of compound **12t** could form an extra hydrogen bond with residue Asp939, which would contribute to the enhanced potency against JAK2.

Five compounds with high inhibitory activities against JAK2 were selected from the obtained compounds, and selective experiments of the JAK family were performed (Table2). The results showed that compounds **12k** and **12l** dispalyed very good selectivity for JAK2 over other JAKs, and **12l** was 598-, 528-, and 264-fold selective over JAK1, JAK3, and TYK2, respectively. We speculated that the superior selectivity of compound **12l**, relative to the hit compound crizotinib (**12a**), was partially ascribed to the removal of the chloride atom from the phenyl ring at the 2-position. This group extended to the activation loop which has a glycine residue in JAK2, JAK1 and TYK2 but an alanine residue in JAK3. In addition, the methoxy group appeared to significantly influence the selectivity of compound **12l** within the JAK family, as its introduction resulted in reversed potency changes against JAK1 and JAK3. As displayed in Figure S2, the methoxy group is located towards the flexible glycine-rich loop. Although the non-conserved residues in this region did not directly interact with the inhibitor, they may contribute to the conformational plasticity and affect the selectivities of inhibitors.

On the basis of enzymatic assays, we performed the antiproliferative activity assays on the HEL human erythroleukemia cell line, which is known to contain the JAK2 V617F mutation and proliferate activity is sensitive to the JAK2-STAT signaling pathway. The results showed that crizotinib and some of its derivatives showed potent inhibition on the proliferation of HEL cells (Table 3 and Table S3). Among them, crizotinib, **12k** and **12l** inhibited HEL cells proliferation with IC_{50} equal to 4.725 μM, 15.024 μM and 4.194 μM, respectively.

Table 3

The antiproliferative activities on HEL cells of **12k** and **12l**

Compd	Cellular Antiproliferative Activity (IC_{50}/μM)
12k	15.024±0.032
12l	4.194±0.056
Crizotinib	4.725±0.049
Ruxolitinib	7.639±0.363

Based on the molecular level, **12k** and **12l** have comparable activities (6 nM and 3nM). And compared with **12l**, **12k** is easier to synthesize to obtain the larger amounts of material, so we chose **12k** for *in vivo* experiments. In order to evaluate the effect of **12k** in the collagen-induced arthritis (CIA) model in rats, all rats were weighted every third day and their clinical scores were recorded every day. Compared to the control group, the growth of the weight in the RA group increased slowly after the onset of disease due to serious illness, especially from day 10 post immunization to the end of the experiment. The rats in methotrexate group showed a significant increase in body weight compared with RA group and had no disease phenotype. Although treatment with **12k** had no obvious effect on the growth of body weight (Figure 4A), **12k** markedly decreased the arthritis swelling score **(**at dose of 50 mg/kg) and alleviated foot swelling in a dose dependent manner (Figure 4B and 4C). The incidences were lower in three **12k**-treated groups than models (data were not shown). All those data indicated that compounds **12k** could suppressed symptoms of the collagen-induced arthritis (CIA) model in rats.

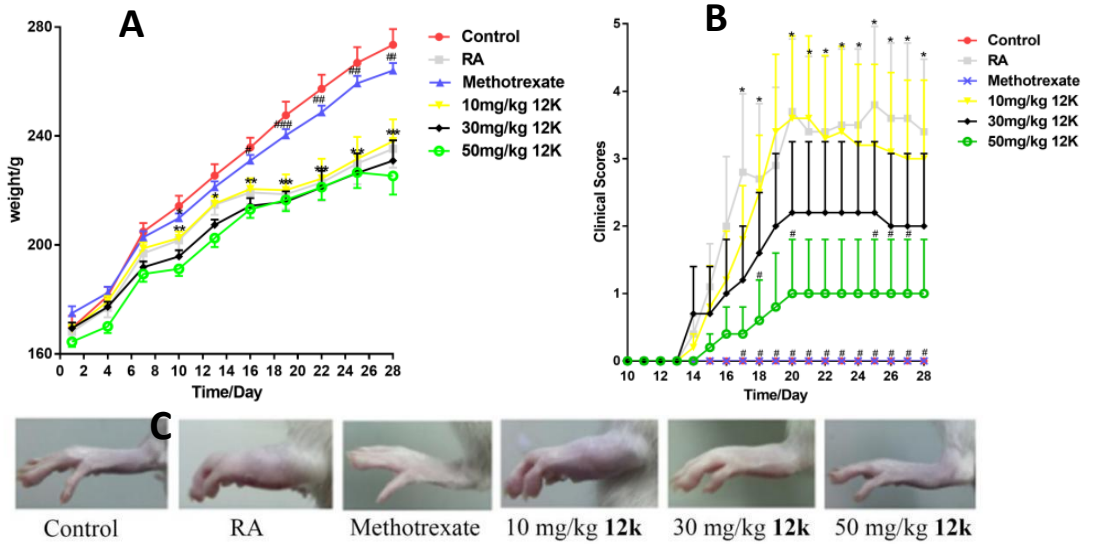


Figure 4. The effects of compound **12k** on CIA rats *in vivo*. Arthritis was induced in Wistar rats by twice immunization with type II collagen on day 0 and day 8. Compound **12k** was orally administered at 10 mg/kg, 30 mg/kg and 50 mg/kg until the end of experiment. Methotrexate, as the positive control, was administered at dose of 0.3 mg/kg. (A) Measurement of body weight was taken every three days. (B) Arthritis scores were evaluated every day. (C) The hind paws representative photos of different treatment groups. All quantitative data were expressed as mean ± SEM. n=10/group. Statistic difference was indicated as * P < 0.05, ** P < 0.01, ***P <0.001 vs control; $^{\#}$ P < 0.05 $^{\#\#}$ P < 0.01, $^{\#\#\#}$ P <0.001 vs RA. RA, Rheumatoid Arthritis.

In summary, we successfully identified crizotinib displaying good inhibitory activity against JAK2 and synthesized a series of derivatives with an aminopyridine scaffold as JAK2 inhibitors. Among them, compound **12k** and **12l** had high inhibitory effects

(IC_{50} = 6 nM and 3 nM, respectively) and selectivity for JAK2 over JAK1, JAK3 and TYK2. In conjunction with molecular docking, we had extensively explored the structure-activity relationships of this series of compounds. The pharmacodynamic results in vivo showed that **12k** had a certain preventive effect on rheumatoid arthritis. In conclusion, our study indicated that a new class of JAK2 inhibitors with an aminopyridine scaffold had the potential for further development and application in the field of rheumatoid arthritis. And drug metabolism and pharmacokinetic properties of these compounds will be studied in the future.

Acknowledgments

The research is supported in part by the National Key Research and Development Program (Grant 2016YFA0502304), the National Natural Science Foundation of China (grant 81825020), the National Science & Technology Major Project "Key New Drug Creation and Manufacturing Program", China (No. 2018ZX09711002), the Special Program for Applied Research on Super Computation of the NSFC-Guangdong Joint Fund (the second phase) under Grant No. U1501501. Honglin Li is also sponsored by National Program for Special Supports of Eminent Professionals and National Program for Support of Top-Notch Young Professionals.

References and notes

1. Shuai, K.; Liu, B. *Nat. Rev. Immunol.* **2003**, *3*, 900.
2. Williams, N. K.; Bamert, R. S.; Patel, O.; Wang, C.; Walden, P. M.; Wilks, A. F.; Fantino, E.; Rossjohn, J.; Lucet, I. S. *J. Mol. Biol.* **2009**, *387*, 219.
3. Hitoshi, Y.; Lin, N.; Payan, D. G.; Markovtsov, V. *Int. J. Hematol.* **2010**, *91*, 189.
4. Kawamura, M.; Mcvicar, D. W.; Johnston, J. A..; Blake, T. B.; Chen, Y. Q.; Lal, B. K.; Lloyd, A. R.; Kelvin, D. J.; Staples, J. E.; Ortaldo, J. R.; O'SHEA, J. J. *Proc. Natl. Acad. Sci. USA.* **1994**, *91*, 6374.
5. Haan, C.; Behrmann, I.; Haan, S. *J. Cell. Mol. Med.* **2010**, 14, 504.
6. Vainchenker, W.; Dusa, A.; Constantinescu, S. N. *Semin. Cell. Dev. Biol.* **2008**, *19*, 385.
7. Bromberg, J.; Jr, J. E. D. *Oncogene*. **2000**, *19*, 2468.
8. G. R. Stark, G. R.; Jr, J. E. D. *Immunity*. **2012**, *36*, 503.
9. Schwartz, D. M.; Kanno, Y. ; Villarino, A.; Ward, M.; Gadina, M.; O'Shea, J. J. *Nat. Rev. Drug Discov.* **2017**, *17*, 843.
10. O'Shea, J. J.; Schwartz, D. M.; Villarino, A. V.; Gadina, M.; McInnes, I. B.; Laurence. A. *Annu. Rev. Med.* **2015**, *66*, 311.
11. Furqan, M.; Mukhi, N.; Lee, B.; Liu, D. L. *Biomark. Res.* **2013**, *1*, 1.
12. Van Vollenhoven, R. F.; Fleischmann, R.; Cohen, S.; Lee, E. B.; Meijide, J. A. G.; Wagner, S.; Forejtova, S.; Zwillich, S. H.; Gruben, D.; Koncz, T.; Wallenstein, G, V.; Krishnaswami, S.; Bradley, J. D.; Wilkinson, B. *N. Engl. J. Med.* **2012**, *367*, 508.
13. Yamaoka, K. *Curr. Opin. Chem. Biol.* **2016**, *32*, 29.
14. Schleicher, S. M. *Clin. Podiatr. Med. Surg.* **2016**, *33*, 355.
15. Roskoski, R. J. *Pharmacol. Res.* **2016**, *111*, 784.
16. Malemud, C. J. *Ther. Adv. Musculoskel. Dis.* **2018**, *10*, 117.
17. Passamonti, F.; Maffioli, M. *Blood*. **2018**, *131*, 2426.
18. Klünder, B.; Mohamed, M. F.; Othman, A. A.; *Clin. Pharmacokinet*. **2018**, *57*, 977.
19. Ivashkiv, L. B.; Hu. X. *Arthritis Rheum.* **2003**, *48*, 2092.
20. Flanagan, M. E.; Blumenkopf, T. A.; Brissette, W. H.; Brown, M. F.; Casavant, J. M.; Shang-Poa, C.; Doty, J. L.; Elliott, E. A.; Fisher, M. B.; Hines, M.; Kent, C.; Kudlacz, E. M.; Lillie, B. M.;; Magnuson, K. S.; McCurdy, S. P.; Munchhof, M. J.; Perry, B. D.; Sawyer, P. S.; Strelevitz, T. J.; Subramanyam, C.; Sun, J.; Whipple, D. A.; Changelian, P. S. *J. Med. Chem.* **2010**, *53*, 8468.
21. Mori, S.; Yoshitama, T. Ueki, Y. *Intern. Med.* **2018**, *57*, 663.
22. Genovese, M. C.; Kremer, J.; Zamani, O.; Ludivico, C.; Krogulec, M.; Xie, L.; Beattie, S. D.; Koch, A. E.; Cardillo, T. E.; Rooney, T. P.; Macias, W. L.; de Bono, S.; Schlichting, D. E.; Smolen, J. S. *N. Engl. J. Med.* **2016**, *374*, 1243.
23. Kivitz, A. J.; Gutierrez-Ureña, S. R.; Poiley, J.; Genovese, M. C.; Kristy, R.; Shay, K.; Wang, X.; Garg, J. P.; Zubrzycka-Sienkiewicz, A. *Arthritis Rheumatol.* **2017**, *69*, 709
24. Saridomichelakis, M. N.; Olivry, T. *Vet. J.* **2016**, *207*, 29.
25. Quintás-Cardama, A.; Kantarjian, H.; Cortes, J.; Verstovsek, S. *Nat. Rev. Drug Discov.* **2011**, *10*, 127.
26. Kota, J.; Caceres, N.; Constantinescu, S. N. *Leukemia*. **2008**, *22*, 1828.
27. Levine, R. L.; Pardanani, A.; Tefferi, A.; Gilliland, D. G. *Nat. Rev. Cancer*. **2007**, *7*, 673.
28. Harrison, C.; Kiladjian, J. J.; Al-Ali, H. K.; Gisslinger, H.; Waltzman, R.; Stalbovskaya, V.; McQuitty, M.; Hunter, D. S.; Levy, R.; Knoops, L.; Cervantes, F.; Vannucchi, A. M.; Barbui, T.; Barosi, G. *N. Engl. J. Med*. **2012**, *366*, 787.
29. Pardanani, A.; Gotlib, G. R.; Jamieson, C.; Cortes, J. E.; Talpaz, M.; Stone, R. M.; Silverman, M. H.; Gilliland, D. G.; Shorr, J.; Tefferi, A. *J. Clin. Onco.* **2011**, *29*, 789.
30. Plimack, E. R.; Lorusso, P. M.; McCoon, P.; Tang, W.; Krebs, G. Curt, A. D.; Eckhardt, S. G. *Oncologist*. **2013**, *18*, 819.
31. Quintás-Cardama, A.; Vaddi, K.; Liu, P.; Manshouri, T.; Li, J.; Scherle, P. A.; Caulder, E.; Wen, X.; Li, Y.; Waeltz, P.; Rupar, M.; Burn, T.; Lo, Y.; Kelley, J.; Covington, M.; Shepard, S.; Rodgers, J. D.; Haley, P.; Kantarjian, H.; Fridman, J. S.; Verstovsek, S. *Blood*. **2010**, *115*, 3109.
32. Grimster, N. P.; Anderson, E.; Alimzhanov, M.; Bebernitz, G.; Bell, K.; Chuaqui, C.; Deegan, T.; Ferguson, A. D.; Gero, T.; Harsch, A.; Huszar, D.; Kawatkar, A.; Kettle, J. G.; Lyne, P.; Read, J. A.; Costa, C. R.; Ruston, L.; Schroeder, P.; Shi, J.; Su, Q.; Throner, S.; Toader, D.; Vasbinder, M.; Woessner, R.; Wang, H.; Wu, A.; Ye, M.; Zheng, W.; Zinda, M. *J. Med. Chem.*, **2018**, *61*, 5235.
33. Bose, P.; Verstovsek, S. *Blood*. **2017**, *130*, 115.
34. You, L. K.; Shou, J. W.; Deng, D. C.; Jiang, L. M.; Jing, Z.; Yao, J. L.; Li, H.; Xie, J. S.; Wang, Z. G.; Pan, Q.; Pan, H. M.; Huang, W. D.; Han, W. D. *Oncotarget*, **2015**, *6*, 40268.
35. Lucet, I. S.; Fantino, E.; Styles, M.; Bamert, R.; Patel, O.; Broughton, S. E.; Walter, M.; Burns, C. J.; Treutlein, H.; Wilks, A. F.; Rossjohn, J. *Blood*, **2006**, *107*, 176.
36. Hart, S.; Goh, K. C.; Novotny-Diermayr, V.; Hu, C. Y.; Hentze, H.; Tan, Y. C.; Madan, B.; Amalini, C.; Loh, Y. K.; Ong, L. C.; William, A. D.; Lee, A.; Poulsen, A.; Jayaraman, R.; Ong, K. H.; Ethirajulu, K.; Dymock, B. W.; Wood, J. W. *Leukemia*. **2011**, *25*, 1751.
37. Huang, Q.; T. W. Johnson, T. W.; Bailey, S.; Brooun, A.; Bunker, K. D.; Burke, B. J.; Collins, M. R.; Cook, A. S.; Cui, J. J.; Dack, K. N.; Deal, J. G.; Deng, Y.; Dinh, D.; Engstrom, L. D.; He, M.; Hoffman, J.; Hoffman, R. L.; Johnson, P. S.; Kania, R. S.; Lam, H.; Lam, J. L.; Le, P. T.; Li, Q.; Lingardo, L.; Liu, W.; Lu, M. W.; McTigue, M.; Palmer, C. L.; Richardson, P. F.; Sach, N. W.; Shen, H.; Smeal, T.; Smith, G. L.; Stewart, A. E.; Timofeevski, S.; Tsaparikos, K.; Wang, H.; Zhu, H.; Zhu, J.; Zou, H. Y.; Edwards, M. P. *J. Med. Chem*. **2014**, *57*, 1170.
38. Cui, J. J.; Tran-Dubé, M.; Shen, H.; Nambu, M.; Kung, P.; Pairish, M.; Jia, L.; Meng, J.; Funk, L.; Botrous, I.; McTigue, M.; Grodsky, N.; Ryan, K.; Padrique, E.; Alton, G.; Timofeevski, S.; Yamazaki, S.; Li, Q.; Zou, H.; Christensen, J.; Mroczkowski, B.; Bender, S.; Kania, R. S.; Edwards, M. P. *J. Med. Chem.* **2011**, *54*, 6342.
39. Baffert, F.; Régnier, C. H.; Pover, A. D.; Pissot-Soldermann, C.; Tavares, G. A.; Blasco, F.; Brueggen, J.; Chène, P.; Drueckes, P.; Erdmann, D.; Furet, P.; Gerspacher, M.; Lang, M.; Ledieu, D.; Nolan, L.; Ruetz, S.; Trappe, J.; Vangrevelinghe, E.; Wartmann, M.; Wyder, L.; Hofmann, F.; Radimerski, T. *Mol. Cancer Ther.* **2010**, *9*, 1945.
40. Chrencik, J. E.; Patny, A.; Leung, I. K.; Korniski, B.; Emmons, T. L.; Hall, T.; Weinberg, R. A.; Gormley, J. A.; Williams, J. M.; Day, J. E.; Hirsch, J. L.; Kiefer, J. R.; Leone, J. W.; Fischer, H. D.; Sommers, C. D.; Huang, H.; Jacobsen, E. J.; Tenbrink, R. E.; Tomasselli, A. G.; Benson, T. E. *J. Mol. Biol*. **2010**, *400*, 413.