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Discovery of triazolo [1,5-a] pyridine derivatives as novel JAK1/2 inhibitors

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Abstract

Small molecule JAK inhibitors have been demonstrated efficacy in rheumatoid arthritis, inflammatory bowel disease, and psoriasis with the approval of several drugs. Aiming to develop potent JAK1/2 inhibitors, two series of triazolo [1,5-a] pyridine derivatives were designed and synthesized by various strategies. The pharmacological results identified the optimized compounds **J-4** and **J-6**, which exerted high potency against JAK1/2, and selectivity over JAK3 in enzyme assays. Furthermore, **J-4** and **J-6** effectively suppressed proliferation of JAK1/2 high-expression BaF3 cells accompanied with acceptable metabolic stability in liver microsomes. Therefore, **J-4** and **J-6** might serve as promising JAK1/2 inhibitors for further investigation.

Keywords: JAK1/2 inhibitors; Anti-inflammatory; Triazolo [1,5-a] pyridine derivatives; Drug design

Autoimmune diseases are a pathophysiological state wherein immune responses are directed against and damage the body's own tissues or organs including rheumatoid arthritis (RA), inflammatory bowel disease etc. Dysregulation of cytokine signal pathway plays a critical role in the occurrence and development of these diseases. It is well documented that janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway mediates the signal of numerous cytokines and growth factors involved in the regulation of immunity, inflammation, and hematopoiesis [1]. JAKs are a family of cytoplasmic protein tyrosine kinases including JAK1, JAK2, JAK3, and TYK2. Upon binding to the cytokine, its receptor units dimerize or polymerize which leads to the phosphorylation and activation of JAKs. Subsequently, the corresponding

receptor is phosphorylated and activated JAKs recruit and phosphorylate STAT proteins which translocate to the nucleus and modulate gene transcription [2]. The overexpression and mutation of JAKs in this pathway will initiate severe symptoms including autoimmune diseases and hematological disorders [3]. To date, diverse JAK inhibitors have been developed for the treatment of immune-related disorders (**Figure 1**). Tofacitinib **1** is the first approved JAK inhibitor for treating rheumatoid arthritis. However, tofacitinib is a pan-JAK inhibitor, systemic administration of tofacitinib may cause serious adverse events such as infection and thrombocytopenia [4]. Based on significant therapeutic benefit, ruxolitinib **2** and baricitinib **3**, potent JAK1/2 inhibitors have been approved for myelofibrosis and rheumatoid arthritis respectively with low therapeutic dose and slight side effects [5]. Recently, selective JAK1 inhibitor upadacitinib **4** has been approved by FDA for the treatment of medium or severe RA. Notably, filgotinib **5**, selective JAK1 inhibitor, is reported to be successful in Phase III studies for RA and atopic dermatitis respectively. In addition, in the Phase II clinical treatment of crohn's disease, filgotinib showed significant efficacy and acceptable safety profile [6].

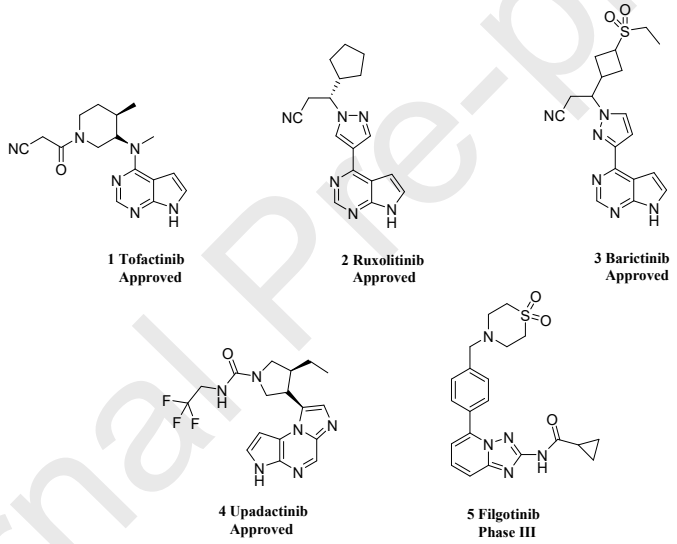


Figure 1. Chemical structures of representative JAK inhibitors

However, adverse effects were reported in filgotinib's toxicological tests in rat and dog models [7]. Due to the moderate activity of filgotinib in vivo, it is necessary to increase the drug dose (100-200 mg) to achieve the expected potency [6]. By analysing the SAR of filgotinib, it was found that its triazolopyridine and exocyclic NH were crucial for maintain the activity. The substitution of cyclopropanecarbonyl group with other acyl or alkyl group led to the decline of the activities. Besides, the phenyl moiety has little effect on the activity, while the property and size of the terminal polar groups have significant effect on the activity and selectivity. As show in **Figure 2**, the terminal thiomorpholine dioxide group was modified by diverse polar motifs with CN or F atom (**series I**) to further increase the potency and selectivity of the compounds. On the other hand, **series II** were designed by replacing the phenyl moiety of filgotinib with unsaturated piperidine to investigate the effect of flexibility on the activities and selectivity.

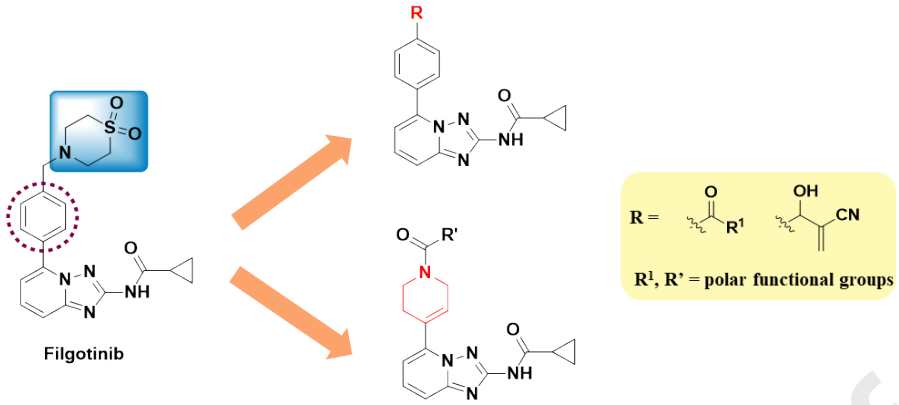
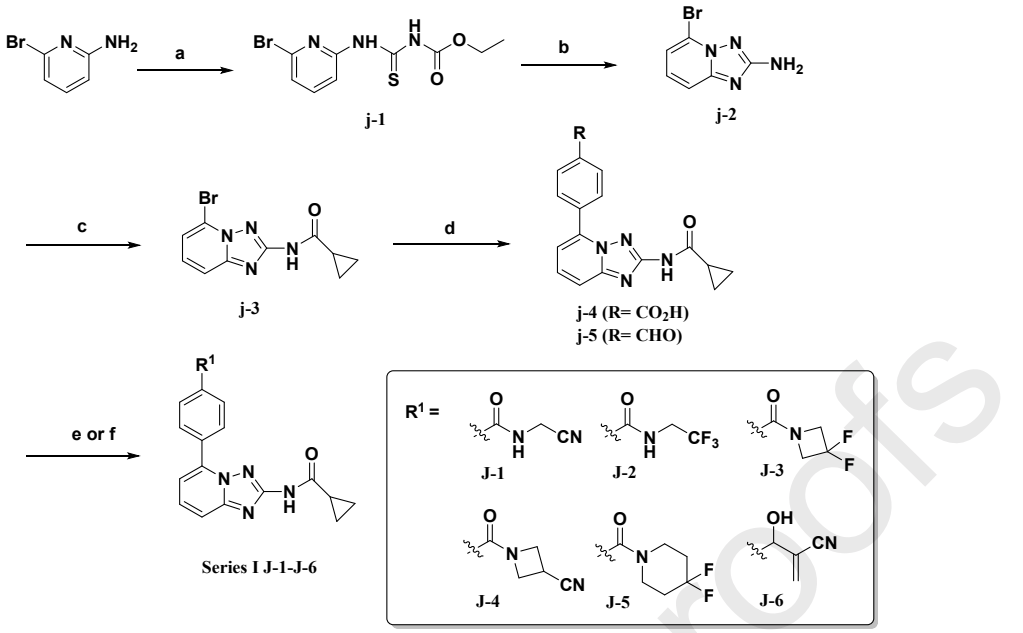


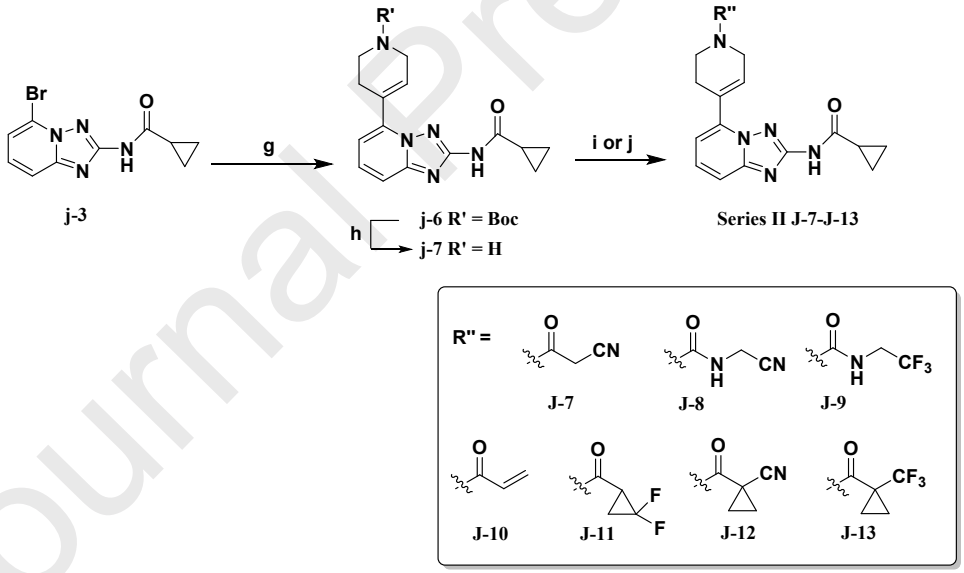
Figure 2. Design strategy for the target compounds

Herein, we describe the identification and characterization of a novel triazolopyridine series as JAK1/2 inhibitors. Synthetic method and detailed enzymatic and cell-based studies of the target compounds were presented and discussed. The results showed that compounds **J-4** and **J-6** exhibited high potency against JAK1/2 in the enzymatic and cell-based assay accompanied with acceptable metabolic stability.

The synthetic procedure for the target compounds is described in **Scheme 1** and **Scheme 2**. Commercially available 2-amino-hydroxylaino-6-bromo-pyridine was condensed with ethoxycarbonyl isothiocyanate and then reacted with hydroxylamine to afford **j-2**. Next, intermediate **j-2** was acylated with cyclopropanecarboxyl chloride to yield **j-3**, followed by a Suzuki reaction to yield **j-4** and **j-5** [8]. The target compounds **J-1**-**J-5** were yielded by condensation reaction between **j-4** and corresponding amines. **J-6** was synthesised by Baylis-Hillman reaction between **j-5** and acrylonitrile at room temperature. Furthermore, *N*-Boc-1,2,5,6-tetrahydropyridine-4-boronic acid pinacol ester was coupled via a Suzuki reaction with **j-3** to provide **j-6** followed by removing the Boc protecting group to yield **j-7** (**Scheme 2**). Compounds **J-7** and **J-10**-**J-13** were accessible using different functionalization of carboxylic acids by condensation reaction with **j-7**. Using preformed imidazole-1-carboxamides under weak alkaline conditions with **j-7** provided compounds **J-8** and **J-9** [9].



Scheme 1. Reagents and conditions: (a) ethoxycarbonyl isothiocyanate, DCM, rt; (b) hydroxylamine hydrochloride, DIPEA, EtOH/MeOH, reflux; (c) Cyclopropanecarbonyl chloride, Et_3N, MeCN; (d) Ar-BPin, Pd(dppf)Cl_2, K_2CO_3, 1,4-dioxane/water, 90°C; (e) HNR^2R^3, HATU, DIPEA, DMF, rt; (f) Acrylonitrile, DABCO, MeCN/H_2O, rt.

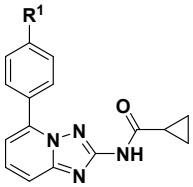


Scheme 2. Reagents and conditions: (g) *N*-Boc-1,2,5,6-tetrahydropyridine-4-boronic acid pinacol ester, Pd(dppf)Cl_2, K_2CO_3, 1,4-dioxane/water, 100°C; (h) 1,4-dioxane/HCl, rt; (i) R^4CO_2H, HATU, DIPEA, DMF, rt ; (j) *N*-substituted-1H-imidazole-1-carboxamide, TEA, EtOH, 60°C for **J-8/9**.

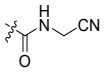
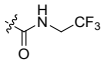
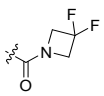
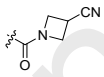
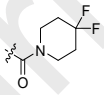
The enzymatic potency assay was conducted using a Caliper assay with the ATP concentration at the Km for each of the JAK targets. As shown in **Table 1**, the steric effect and property of R^1 groups had an impact on activities and selectivity of **series I** against JAKs. Compared to filgotinib, **J-1** and **J-2** showed similar selectivity with relatively low potency against JAK-1/2. Through further modification, **J-3-J-5** were

obtained by the incorporation of cyclic amine with CN or di-F group. Interestingly, the potency of the compounds with substituted azetidine (**J-3**, **J-4**) were improved 2-5 folds (**J-3**, **J-4** vs **J-1**, **J-2**). **J-4** exhibited high potency against JAK1/2 (IC_{50} = 61 nM and 79nM) and showed good selectivity over JAK3 (JAK 3/1 ratio = 10.8). However, **J-5** with 4,4-difluoropiperidine moiety was inactive (JAK1 IC_{50} = 393 nM) which may be due to its steric effect. To further increase the inhibitory activity of the compound, **J-6** with α-hydroxy acrylonitrile moiety was designed to increase polarity interaction with the P-loop of JAKs. As we expected, **J-6** had increased potency for all JAK isoforms. **J-6** effectively inhibited the activities of JAK1 and JAK2 with the value of IC_{50} 95 nM and 55 nM respectively and 3-5 folds selectivity against JAK3.

Table 1. Enzymatic Potencies for series I compounds against JAK1, JAK2, and JAK3



Series I

compound	R^1	JAK1 IC_{50} (nM)[a]	JAK2 IC_{50} (nM)[a]	JAK3 IC_{50} (nM)[a]
J-1		286	299	1120
J-2		230	214	1015
J-3		124	139	941
J-4		61	79	659
J-5		393	401	923
J-6		95	55	285
Filgotinib[b]		115	74	623

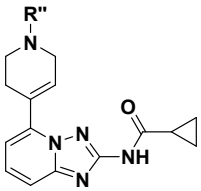
a Experiments were performed in duplicate

b Used as positive control

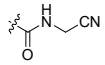
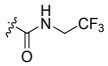
Then, we investigated the conversion of phenyl moiety of filgotinib to tetrahydropyridine and the effect of R'' group on the activities of the compounds. As shown in **Table 2**, Compared with **J-1** and **J-2**, compounds **J-7** and **J-8** with similar terminal cyano group displayed similar potency against JAK1/2. Subsequently, the introduction of CF_3 or difluorocyclopropyl at R'' group showed a decreased potency against all the three isoforms (**J-9**, **J-10** vs **J-7**, **J-8**). The substitution of R'' group by acryloyl group led to a significant decline of activities against JAKs (JAK1, JAK2 and JAK3 IC_{50} = 877, 424 and 2767 nM, respectively). Besides, compounds with CN or

CF_3 substituted cyclopropyl group obtained low potency (**J-12** and **J-13**). The results indicated that the introduction of tetrahydropyridine could maintain the activities of the compounds (**J-7**, **J-8** vs **J-1**, **J-9** vs **J-2**) and suitable polar functional group was crucial for high potency and selectivity.

Table 2. Enzymatic Potencies for series II compounds against JAK1, JAK2, and JAK3



Series II

compound	R''	JAK1 IC_{50} (nM)[a]	JAK2 IC_{50} (nM)[a]	JAK3 IC_{50} (nM)[a]
J-7		193	157	1640
J-8		170	175	983
J-9		234	347	1740
J-10		283	192	2409
J-11		877	424	2767
J-12		1123	441	1547
J-13		861	317	5649
Filgotinib[b]		115	74	623

a Experiments were performed in duplicate

b Used as positive control

Based on the enzymatic assays, compounds **J-4** and **J-6** were selected for further study the anti-proliferation activities on BaF3-JAKs cells. As shown in **Table 3**, similarly to filgotinib, **J-4** exhibited high potency against BaF3-JAK1 cells (IC_{50} = 2.59 μM) and selectivity over BaF3-JAK2 cells (JAK 2/1 ratio = 2.9) which was inactive against JAK3. Interestingly, **J-6** effectively inhibited the proliferation of BaF3-JAK1 cells and BaF3-JAK2 cells with 7 folds selectivity against BaF3-JAK3 cells.

Table 3. Antiproliferation Activities of compounds against BaF3-JAKs cells

compound	BaF3-JAK1 IC_{50}[a] (μM)	BaF3-JAK2 IC_{50}[a] (μM)	BaF3-JAK3 IC_{50}[a] (μM)
J-4	2.59	7.43	＞30
J-6	1.18	1.03	7.05
Filgotinib[b]	1.43	4.45	13.26

a Experiments were performed in duplicate

b Used as positive control

Moreover, we performed the study of **J-4** and **J-6** in incubation with human and rat liver microsomes in vitro (**Table 4**). The studies revealed that **J-4** and **J-6** possessed high $T_{1/2}$ and low CL in both HLM and RLM. It demonstrated that **J-4** and **J-6** were stable in human and rat liver microsomes.

Table 4. In Vitro Liver Microsome Stability

compound	HLM[a] $T_{1/2}$ (min)	HLM[a] CL (μL/min/mg)	RLM[b] $T_{1/2}$ (min)	RLM[b] CL (μL/min/mg)
J-4	＞1000	＜2	550	6.3
J-6	381	9.1	129	26.9

a human liver microsomes

b rat liver microsomes

To further elucidate the binding mode, molecular docking analysis of compound **J-4** and **J-6** was performed using Molegro Virtual Docker 2010 (v4.1) (Molegro ApS, Aarhus, Denmark). As shown in **Figure 3A** and **3B**, **J-4** and **J-6** showed similar binding mode with the ATP binding pocket of JAK1. Exocyclic NH and the triazolo nitrogen N3 of the triazolo[1,5-a]pyridine formed two critical hydrogen bonds with Leu959 residue and phenyl ring was located in the hydrophobic pocket. azetidine-3-carbonitrile (**J-4**) and hydroxymethyl-acrylonitrile (**J-6**) are oriented to the glycine rich loop respectively. **Figure 3C** and **3D** expounded the binding mode of **J-4** and **J-6** with JAK2. triazolo[1,5-a]pyridine skeleton of both compounds formed two hydrogen bonds interactions with Leu932. The hydrophobic interactions were formed between 11e and Ala880, Leu983, Val863 and Val911. The docking analysis indicated that **J-4** and **J-6** possess high potency against JAK1/2 in activity assays.

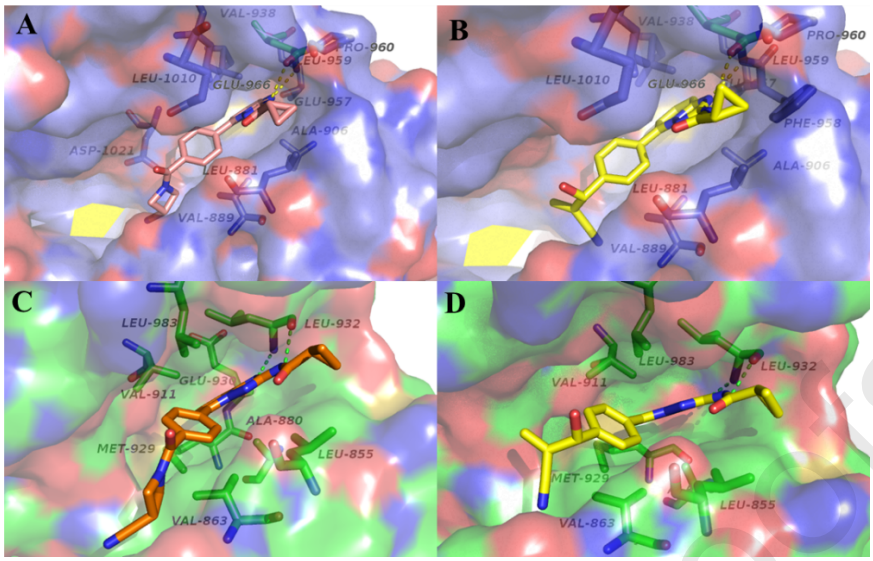


Figure 3. Docking mode of compound **J-4** and **J-6** with JAK1 (PDB code 4P7E) and JAK2 (PDB code 6DBN). (A) binding conformation for **J-4** in the binding site of JAK1, (B) binding conformation for **J-6** in the binding site of JAK1. (C) binding conformation for **J-4** in the binding site of JAK2, (D) binding conformation for **J-6** in the binding site of JAK2, The H-bond (yellow or green) are displayed as dotted arrows.

In summary, two series of triazolo[1,5-a]pyridine derivatives were designed, synthesized as JAK1/2 inhibitors. Most compounds exhibited moderate to high potency against JAK1/2. In particular, compounds **J-4** and **J-6** showed high potency against JAK1/2 and selectivity over JAK3. Anti-proliferation assays showed that **J-4** and **J-6** could effectively inhibit the proliferation of JAK1 and JAK2 high-expression BaF3 cells. Furthermore, **J-4** and **J-6** exhibited preferable liver microsome stability. Overall, **J-4** and **J-6** were identified as promising lead for JAK inhibitors for the treatment of inflammatory diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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References

[1] (a) Shuai K, Liu B. Regulation of JAK-STAT signaling in the immune system. *Nat. Rev. Immunol*. 2003, 3, 900−910. (b) Clark J D, Flanagan M E, Telliez J B. Discovery and development of Janus kinase (JAK) inhibitors for inflammatory diseases. *J Med Chem*, 2014, 57(12), 5023-5038.

[2] (a) Cai B, Cai J P, Luo Y L, Chen C, Zhang S. The specifc roles of JAK/STAT signalling pathway in sepsis. *Inflammation*, 2015, 38(4), 1599-1608. (b) Kisseleva T, Bhattacharya S, Braunstein J, Schindler C W. Signalling Through the JAK/STAT Pathway, Recent Advances and Future Challenges. *Gene*, 2002, 285, 1-24.

[3] (a) Babon J J, Lucet I S, Murphy J M, Nicola N A, Varghese L N. The molecular regulation of Janus kinase (JAK) activation. *Biochemical Journal*, 2014, 462(1), 1-13. (b) Senkevitch E, Durum S. The promise of Janus kinase inhibitors in thetreatment of hematological malignancies. *Cytokine*, 2017, 98, 33-41.

[4] (a) Fleischmann R, Cutolo M, Genovese M C, Lee E B, Kanik K S, Sadis S, Connell C A, Gruben D, Krishnaswami S, Wallenstein G, Wilkinson B E, Zwillich S H. Phase 2B DoseRanging Study of the Oral JAK Inhibitor Tofacitinib (CP-690,550) or Adalimumab Monotherapy Versus Placebo in Patients with Active Rheumatoid Arthritis with an Inadequate Response to DMARDs. *Arthritis Rheum*. 2012, 64, 617−629. (b) Jatiani S S, Baker S J, Silverman L R, Reddy E P. JAK/STAT Pathways in Cytokine Signaling and Myeloproliferative Disorders: Approaches for Targeted Therapies. *Genes Cancer* 2010, 1, 979−993.

[5] (a) Mesa, R. A.; Yasothan, U.; Kirkpatrick, P. Ruxolitinib. *Nat. Rev.Drug Discovery* 2012, 11, 103−104. (b) Genovese MC, Kremer J, Zamani O, et al. Baricitinib in patients with refractory rheumatoid arthritis. *N Engl J Med*. 2016, 13,1243–1252.

[6] Vermeire S, Schreiber S, Petryka R, Kuehbacher T, Hebuterne X, Roblin X, Klopocka M, Goldis A, Wisniewska-Jarosinska M, Baranovsky A, Sike R, Stoyanova K, Tasset C, Vander A A, Harrison P. Clinical remission in patients with moderate-to-severe Crohn's disease treated with filgotinib (the FITZROY study): results from a phase 2, double-blind, randomised, placebo-controlled trial. *Lancet* 2017, 389 (10066), 266-275.

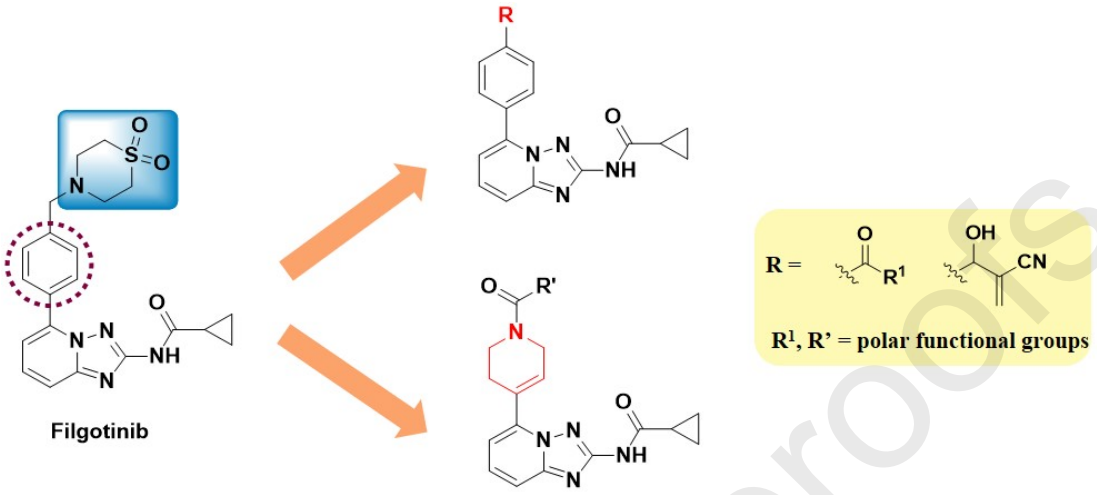
[7] Galapagos NV. Risks related to product development, regulatory approval and commercialization http://reports.glpg.com/annual-report-2016/en/riskfactors/ product -development-regulatory-approval-and-commercialization.html; 2016 Accessed 17.08.30

[8] Christel J M, Stephen R F, Guy V L, Raphael G, Javier B, Koen S, Nolwenn J,Ellen V A, Philippe C, Liên L, René G, Béatrice V, Luc N, Thierry C, Reginald B, Luc V R, Fabrice C, Murielle U, and Pierre D. Triazolopyridines as selective JAK1 inhibitors: from hit identification to GLPG0634. *J. Med. Chem*, 2014, 57,9323-9342.

[9] Andrew F, Catherine M. A, Eric A, Mary E. B, Matthew F. B, Jill C, James D. C, Martin E. D, Ivan V. E, Andrew F, Brian S. G, Ariamala G, Matthew M. H,Martin H, Brett D. H, Jason J, John D. K, David C. L, David L,Tsung H. L,Betsy S. P, Eddine S, Raman S, Peter T. S,Jean-Baptiste T, John I. T, Felix F. V, Fabien V, Zhao K W, Li X, Xiaojing Y, Xin Yang, and Liying Z. Dual Inhibition of TYK2 and JAK1 for the Treatment of Autoimmune Diseases: Discovery of ((S)- 2,2-Difluorocyclopropyl)((1R,5S)- 3-(2-((1-methyl- 1H- pyrazol-4-yl)amino) pyrimidin-4-yl)-3,8- diazabicyclo[3.2.1]octan-8-yl)methanone (PF-06700841). *J. Med. Chem*, 2018, 61, 8597-8612.

Conflict of interest

The authors declared that they have no conflicts of interest to this work. Neither the entire paper nor any part of its content has been published or has been accepted elsewhere.



Compd.	Enzymatic potencies against JAKs (IC_{50}/nM)			Antiproliferation activities against BaF3-JAKs cells (IC_{50}/μM)		
	JAK1	JAK2	JAK3	BaF3-JAK1	BaF3-JAK2	BaF3-JAK3
J-4	61	79	659	2.59	7.43	>30
J-6	95	55	285	1.18	1.03	7.05
Filgotinib	115	74	623	1.43	4.45	13.26

- A novel series of triazolo [1,5-a] pyridine derivatives were designed, synthesized and evaluated as JAK1/2 inhibitors.
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- Compounds **J-4** and **J-6** effectively suppressed proliferation of BaF3-JAK1/2 cells with selectivity over BaF3-JAK3 cells.
- Compounds **J-4** and **J-6** possessed acceptable metabolic stability in liver microsomes.