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Comparison of Synthetic Routes of Ruxolitinib And Its Application

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Abstract. Ruxolitinib is known as a selective kinase inhibitor that acts by inhibiting the activator of the transcription pathway. JAK-STAT signaling pathway plays an important role in the signal transduction of many growth factors and cytokines related to cell proliferation, growth and hematopoiesis. Myelofibrosis is a JAK1/JAK2 signaling-specific gene expression upregulation, resulting in an overall sustained disruption of the JAK-STAT signaling pathway. Ruxolitinib effectively and selectively inhibits JAK2V617F mediated signal transduction and proliferation, significantly increases apoptosis in a dose-dependent manner, and leads to the doubling of depolarized mitochondria at 64 nm in Ba/F3 cells. Ruxolitinib has been confirmed to be more effective than other treatments for the same period in the treatment of some diseases such as polycythemia vera, so a safe, efficient, and cost-effective synthesis of ruxolitinib is warranted. The advantages of route 1 are high economy, safety, and atomic utilization, while the disadvantage is a large number of reaction steps and low overall yield. The advantage of route 2 is the small number of reaction steps and high convenience, while the disadvantage is the expensive raw materials, complex experimental conditions, and low atomic utilization efficiency. The advantage of route 3 is the high overall yield and also more convenient, while the disadvantage is the expensive raw materials and complex experimental conditions. This is the result of the route comparison, and this paper can find that each route has its advantages and disadvantages. On balance, we recommend synthetic route one. It is economical and safe, although the yield is low, it is still acceptable.

Keywords: Ruxolitinib, Synthesis, JAK inhibitor, Application

1. Introduction

Ruxolitinib is the first oral drug that can be used to treat bone marrow fibrosis. It belongs to a tyrosine kinase inhibitor, namely a small molecule inhibitor of protein kinase JAK1 and JAK2 [1], which exhibits great selectivity for JAK3 and other kinases just like CHK2 or MET. Ruxolitinib is more suitable for the treatment of high-risk bone marrow fibrosis, and also suitable for the treatment of polycythemia vera. In recent years, ruxolitinib has also been proved to play an important role in the treatment of graft versus host disease. In addition, ruxolitinib has even been proved to be able to treat

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different types of skin diseases, such as atopic dermatitis. Ruxolitinib is one kind of selective kinase inhibitor that acts by inhibiting the activator of the transcription pathway. JAK-STAT signaling pathway has been proved to exist in many growth factors and cytokine signal transduction systems related to cell proliferation, growth and hematopoiesis. MF is a JAK1/JAK2 signaling-specific gene expression upregulation that leads to an overall sustained disruption of the JAK-STAT signaling pathway. Ruxolitinib selectively and efficiently inhibited JAK2V617F-mediated signaling and proliferation, significantly increased apoptosis in a dose-dependent manner, and resulted in a doubling of depolarized mitochondria at 64 nM in Ba/F3 cells. In addition, ruxolitinib was significantly effective against erythroid colony formation (IC50=67nM), it also has the potency to inhibit the proliferation of red lineage progenitor cells in normal donors (IC50=407nM) and patients with polycythemia vera (IC50=223nM) [2].

In terms of pharmacokinetics, Shilling et al. studied the way ruxolitinib is metabolized in healthy adults using a carbon 14 labeling assay [3]. Its presence in plasma was mainly in the form of the parent drug. Ruxolitinib is metabolized in the body primarily by the oxidative pathway and its hydroxyl and oxo metabolites are eventually excreted in the urine. In addition, this study found no significant difference between the concentrations of parent drug and metabolites on Day 1 and Day 10 in healthy subjects taking continuous daily doses of ruxolitinib, indicating no significant accumulation of either parent drug or metabolites. In recent years, there have been many studies demonstrating that ruxolitinib has shown better results than other treatment options in the treatment of various diseases. Vannucchi et al. compared the outcomes of patients with polycythemia vera receiving ruxolitinib or standard therapy have shown that patients receiving ruxolitinib achieved hematocrit control or complete hematologic remission at a much higher rate than patients receiving standard therapy, and some patients had a significant reduction in spleen volume [4]. Notably, after 32 weeks of treatment, in addition to symptom reduction, many patients treated with ruxolitinib reported a reduction in their individual symptoms and improvements in quality of life and health status, in contrast to patients receiving standard treatment. Moreover, patients in the ruxolitinib group had a significantly lower rate of arterial thrombosis than the standard therapy group, so ruxolitinib should be considered for inclusion in the standard therapy for erythrocytosis.

Ruxolitinib also demonstrates many advantages when used for the treatment of myelofibrosis. A pooled analysis by Oritani et al. of Japanese patients' outcomes in a clinical study of ruxolitinib for myelofibrosis showed that after 24 weeks of ruxolitinib treatment [5], more than two-thirds of the patients had a reduction in total symptom score (TSS) of more than 50% from baseline, along with substantial improvement in their quality of life. The analysis also showed that the dose of ruxolitinib was associated with efficacy, with higher doses resulting in better outcomes. The use of ruxolitinib for myelofibrosis significantly reduced the size of the spleen and improved individual symptoms due to splenomegaly, thereby improving quality of life and survival outcomes.

As for the efficacy of treatment regimens using ruxolitinib for steroid-refractory graft-versus-host disease, the ORR within one month of ruxolitinib treatment was higher compared to most second-line treatments and that ruxolitinib treatment was safe and effective in pediatric patients. And the outcomes of Chinese patients with steroid-refractory graft-versus-host disease treated with ruxolitinib provides additional evidence of the safety and efficacy of ruxolitinib in the treatment of steroid-refractory graft-versus-host disease.

The excellent results of ruxolitinib in the treatment of these diseases indicate that it is an excellent drug and, therefore, the synthesis of ruxolitinib is also very important. This paper aims to present three commonly used synthetic routes for ruxolitinib and compare their advantages and disadvantages. Through the summary of the literature review, we found that all three routes start from common chemical raw materials and have their advantages in terms of economy, safety and yield. Through the study of this thesis, different routes can be selected in the synthesis to achieve the desired goal according to the actual situation and our conditions.

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2. Synthetic routes I

The synthesis route I for ruxolinib [6] is a synthetic route with a high degree of self-selectivity, which means that many high-valent catalysts for asymmetric catalysis will be avoided, as shown in Fig. 1. Cyclopentyl formaldehyde and malonic acid were added to 1 L of pyridine to react to give 3-cyclopentylacrylic acid. The hydrazine hydrate was cooled down to 0~5 ℃, and 3-cyclopentylacrylic acid was added with stirring, and the reaction was increased to 70~75 ℃ for 0.5 hours after addition. After the reaction, it was concentrated into oil under reduced pressure, added purified H_2O, stirred to dissolve, cooled to 0~5 ℃, and stirred to precipitate overnight. The filter cake was washed with isopropyl ether and dried at 45 ℃ to obtain 5-cyclopentylpyrazolidin-3-one. Add the racemic 5-cyclopentylpyrazolidin-3-one to acetone, stir and dissolve, add D-tartaric acid, stir for 30 minutes, then cool down to 0~5 ℃ and precipitate, filter, and the filter cake is washed with acetone. The filter cake was dried at 45 ℃ to obtain (R)-5-cyclopentylpyrazolidin-3-one D-tartrate, where the ee value is 99.4%. Sodium hydroxide solution was added slowly dropwise to (R)-5-cyclopentylpyrazolidin-3-one D-tartrate, cooled to 0~5 °C in an ice bath and concentrated with stirring by slowly adding concentrated hydrochloric acid dropwise until the material solution was turbid, then adjusting the pH of the solution to neutral with hydrochloric acid, adding dichloromethane in three extractions, combining the organic layers, drying anhydrous magnesium sulfate, filtering, collecting the filtrate, and concentrating under reduced pressure to obtain compound 4. The next compounds were substituted with benzyl and the next compounds were substituted with benzyloxycarbonyl to protect the in situ amino groups. Compound 6 was obtained by adding 4-chloro-7H-pyrrolo[2,3-d]pyrimidine and DPPF palladium dichloride catalyst to tetrahydrofuran in a cross-coupling reaction. The 4-methyl-7H-pyrrolo[2,3-d]pyrimidine was added to a mixture of DMF and dioxane, cooled to below 0 °C, and trichloroxaphos was added dropwise with stirring, controlling the temperature of the material below 20 ℃ and then warmed to 80 ℃ and stirred for 3 hours. This can continue to concentrate dioxane and DMF under reduced pressure, add tetrahydrofuran to the residue, adjust the pH to 10~12 with 25% sodium hydroxide aqueous solution, raise the temperature to 60 °C, and stir for 2 hours. Then compounds 4 and 7 were heated to reflux in anhydrous ethanol and reacted to obtain compound 8. Next, compound 8 was added to a mixture of dichloromethane and NMP, and oxalyl chloride was added dropwise to control the temperature not exceeding 5 °C to obtain compound 9. Then compound 9 was obtained by adding Then, ammonia was added to replace chlorine with amine to obtain compound 10. Then, it was reacted with trichloroxaphos in dichloromethane to obtain compound 11. Finally, benzyloxycarbonyl was removed by reaction with trifluoroacetic acid under acidic conditions to obtain the final product ruxolitinib.

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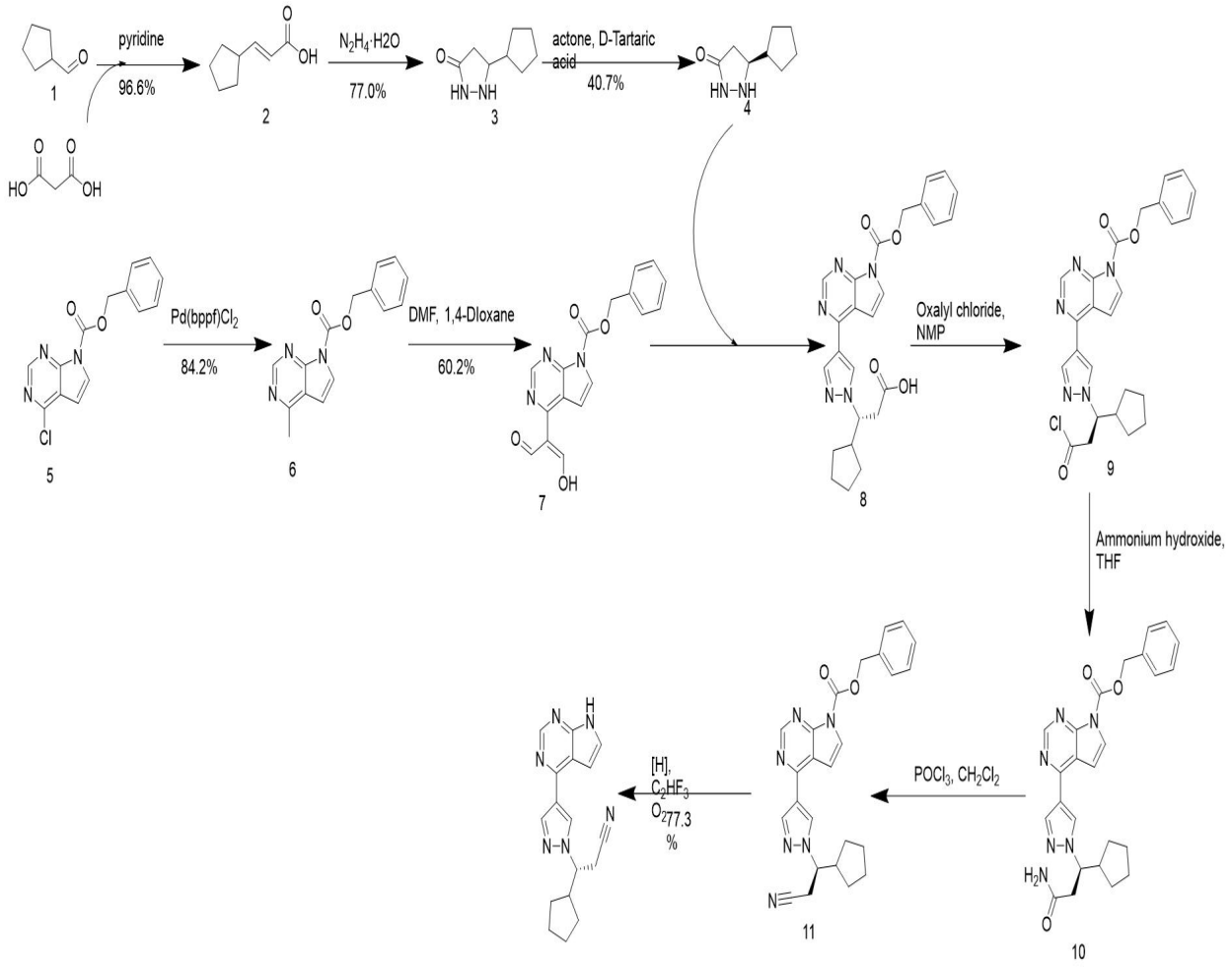

Figure 1. Synthesis of ruxolitinib (route 1) [6].

3. Synthetic routes II

Haydl et al. reported the regioselective and enantioselective synthesis of N-substituted pyrazole, which can be used for the synthesis of ruxolitinib [7]. They believed that this new method could be further applied to the efficient synthesis of roxolitinib. This methodology is fundamental to the scheme in question as it increases the yield rate and ee rate significantly. And more interestingly, there is an existing flaw in this methodology. The preparation of chiral pyrazole still needs many steps. Haydl's research team has developed a new alternative method, which is used for the preparation of metal catalyzed allyl substitution and oxidation to form dendritic allyl products, which to some extent solves the defects of traditional synthesis methods.

As shown in Fig. 2, this scheme uses compound 1 to react with compound 2 with the presence of CuBr and LiBr in THF to produce compound 3. The yield rate of this step is rather low and has a considerable amount of unreacted compound which is a limitation to its industrial application. The yield rate for this rate is merely 30%. Compound 3 is then reacted with compound 4 to produce compound 5. The yield rate for this step is 95%. This step also has high enantioselectivity (90% ee). This is the step where the result of the research applied, and this proves the practicability of this method in chiral centers synthesis. The application of the method renders the scheme possible. Compound 5 undergoes brown hydroboration by reacting with 9-BBN, which is followed by reacting with H_2O_2 and producing compound 6. The total yield for the mentioned two steps is 99%. This step gives Compound 6 corresponding alcoholic group. Compound 6 then undergoes Swern oxidation

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which converts the alcoholic group into the aldehyde group and becomes compound 7. The accompanying yield rate is 97%. This step gives Compound 7 aldehyde group that possesses the ability to react with NH_4OH in the process of I_2. Compound 7 reacts with NH_4OH in the presence of I_2 in a THF-H_2O system and generates compound 8. This step gives compound 8 the required nitrile which is able to react with B2pin2. Compound 8 is then reacted with B2pin2 in the presence of AcOK with the catalysis of [$PdCl_2$(dppf)] in DMSO to give compound 9. The yield rate for this step is 98% and it takes 24 h with a temperature of 90 °C. The compound 9 is then reacted with compound 10 and gives ruxolitinib via Suzuki coupling reaction.

There are two steps in this scheme that require Rh or Pd. The corresponding catalysts are quite expensive and hard to be removed or reused which renders this scheme unlikely to be applied in the commercial application. This first step that synthesis Compound 3 also set a limitation for the application of this method in mass production. It causes the total yield to decline significantly. The step 1 is considered as a major barrier of the utility of this method. If there are better way to produce Compound 3, this method would be much better.

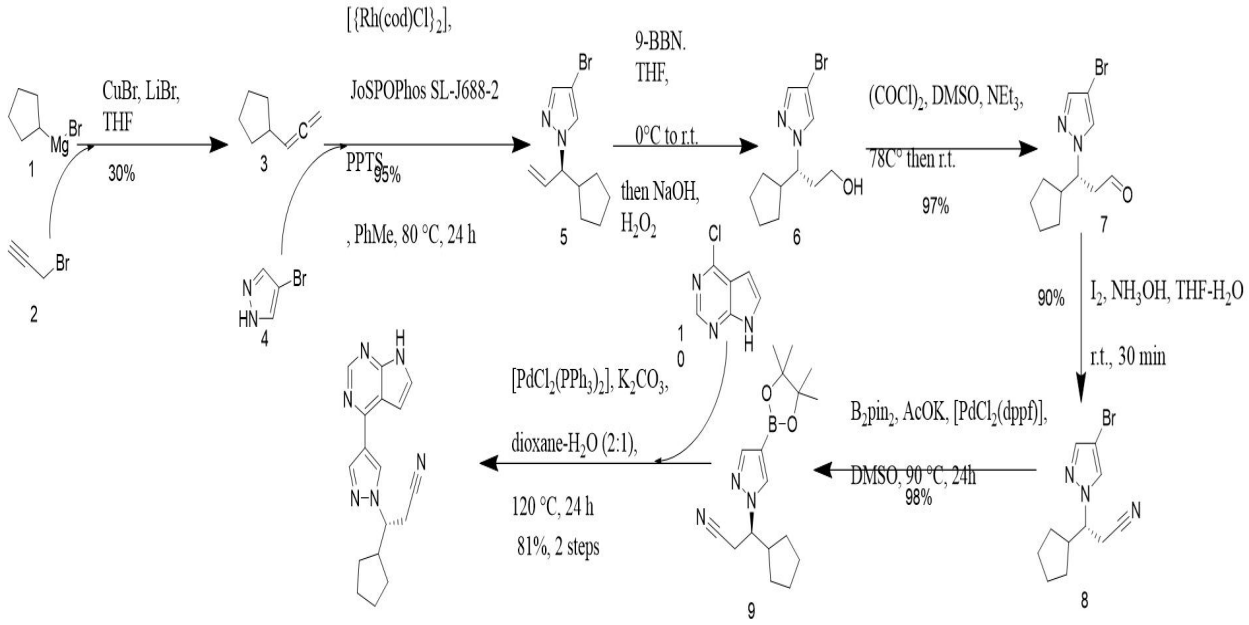

Figure 2. Synthesis of ruxolitinib (route 2) [7].

4. Synthetic routes III

As shown in Fig. 3, the synthesis route III for ruxolitinib is a validated route for the asymmetric synthesis of ruxolitinib [8]. Compound 1 was first treated with NaH and SEM-Cl in DMAC as solvent at 0-5°C to give compound 2 with SEM-protected amino group in 89% yield. After compound 3 was reacted with compound 4 under acidic toluene solvent conditions to give amino protected compound 5, then medium with organoborane compound 6 with format reagent iPrMgCl in the next Suzuki coupling reaction was carried out with the previously obtained compound 2 with compound 7 using $Pd(PPh_3)_4$ as a catalyst to give compound 8, which was subsequently hydrolyzed to give compound 9, in 82% yield for both steps. Thereafter, compound 10 was additionally refluxed with Ph_3P=CHCHO in benzene to give compound 11. however, the resulting compound 11 was contaminated with about 14% of compound 11a. Compound 11a could be removed by preparative HPLC, but its presence was shown to have no significant effect on the subsequent Michael addition, so it did not need to be removed. The subsequent Michael addition reaction of compound 9 with five times the amount of compound 11 using benzene as a solvent with p-nitrobenzoic acid as an acidic additive was performed at room temperature for 24 h to produce compound 12 in 84% yield with 89% ee. This reaction was

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enantioselective at low temperatures, but the catalyst used was slow to react at zero degrees, so the reaction was chosen to be carried out at room temperature. The use of nitro-substituted benzoic acid as an acidic additive speed up the reaction (in contrast to benzoic acid), but a further increase in acidity leads to a decrease in enantioselectivity. Compound 12 was subsequently treated with ammonia and then oxidized with iodine to give compound 13 in 82% yield, and finally, the SEM group was removed using lithium tetrafluoroborate and ammonia to give ruxolitinib in 84% yield (compound 14).

Incyte Corporation optimized this reaction route by using commercially available 3-Cyclopentylacrylonitrile for direct Michael addition to compound 9, also eliminating the subsequent iodine oxidation step, improving the atomic utilization of the reaction and saving time. Moreover, acetonitrile with DBU as a reaction condition can avoid the use of the expensive catalyst in the original route, which needs to be synthesized from a purchased catalyst and avoiding the use of this catalyst can save much cost and reduce the price of the drug.

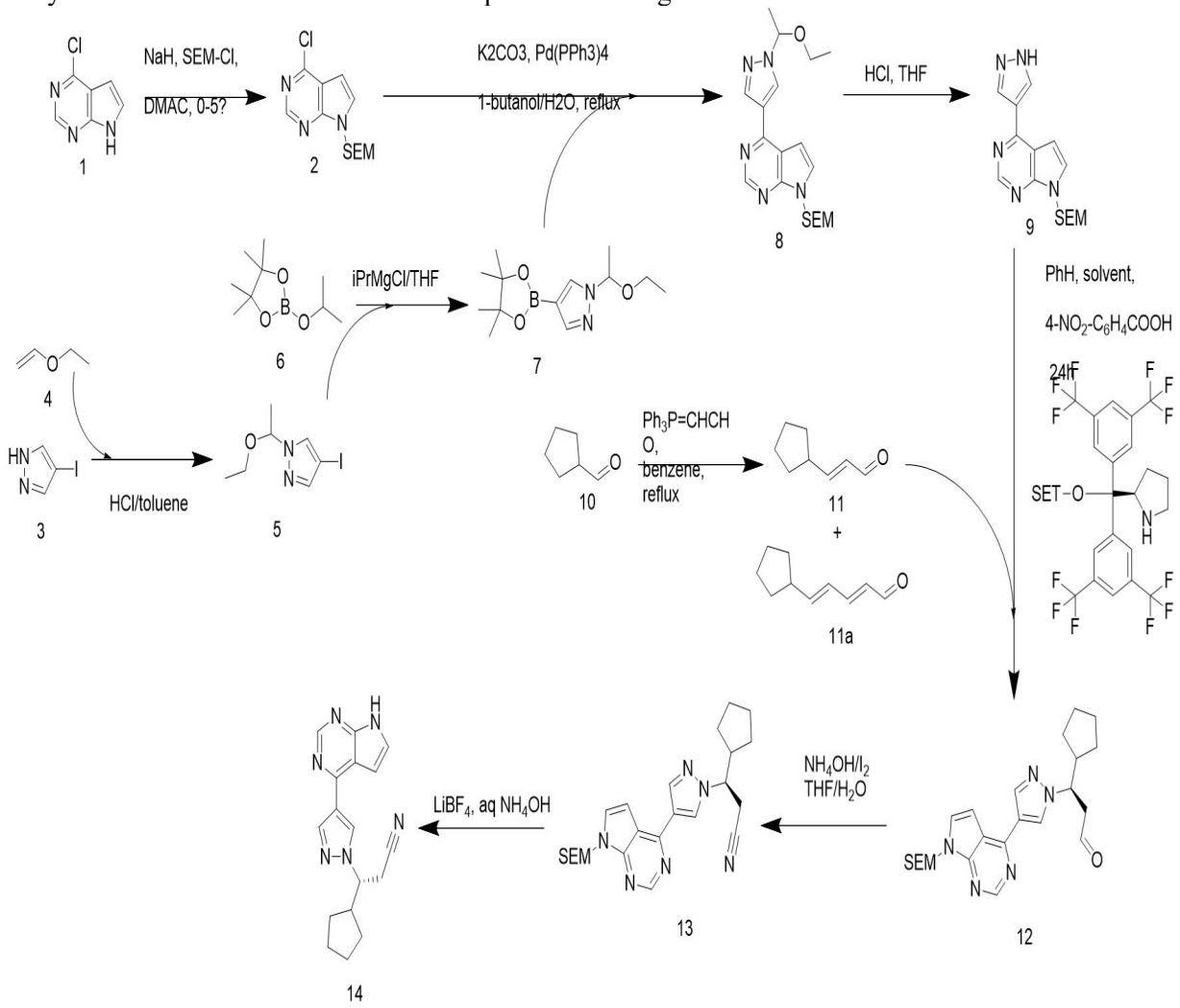

Figure 3. Synthesis of ruxolitinib (route 3) [8].

5. Comparative analysis

This article will make a comparative analysis of the above three synthetic routes in six aspects: safety, convenience, economy, advancement, yield, and atomic utilization efficiency, as shown in Table 1. Here we take the form of a table to present the nature of each route so that you can see the results of the analysis for each route.

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The advantage of route one is high economy, good safety, and high atomic utilization, while the disadvantage is a large number of reaction steps and low overall yield. The advantage of route two is the small number of reaction steps and high convenience, while the disadvantage is the expensive raw materials, complex experimental conditions, and low atomic utilization efficiency. The advantage of route three is the high overall yield and also more convenient, while the disadvantage is the expensive raw materials and complex experimental conditions. This is the result of the route comparison, and we can find that each route has its advantages and disadvantages. On balance, we recommend synthetic route one. It is economical and safe, although the yield is low, it is still acceptable.

Table 1. Comparative analysis of three synthetic routes

Route	Reaction conditions	Reaction Method	Reaction steps	Total yield	Atomic utilization efficiency (without considering by-product recovery)	Economy
Route 1	Mild conditions, safe raw materials, no need for high temperature and pressure	Condensation reaction, Chloroformation reaction, Suzuki coupling reaction	11	14.1%	79.55%	No high-priced catalyst demand
Route 2	There is a strictly anhydrous environment, mainly alkaline organic solvent environment	The accumulated diolefins were first prepared using Grignard's reagent, and the reaction was completed by Suzuki coupling	8	19.8%	23.77%	Expensive raw materials and catalysts for Grignard reagent and Suzuki coupling
Route 3	Mainly alkaline organic solvent environment	Mainly Suzuki coupling with Michael addition reaction	9	42.0%	25.64%	Suzuki coupling reaction with Michael addition reaction catalyst is expensive

6. Drug application

The U.S. FDA officially approved ruxolitinib (trade name Jakafi) in 2011 as the first drug for myelofibrosis (MF) treatment and granted it rare disease drug status. It is applicable to the treatment of moderate or high-risk bone marrow fibrosis, such as primary bone marrow fibrosis, bone marrow fibrosis after polycythemia vera and bone marrow fibrosis after primary thrombocytosis.

Ruxolitinib can also be used to treat a fatal childhood immune disease, HLH. Following an outbreak of pneumonia caused by the COVID-19 virus, Chinese researchers tested the efficacy of ruxolitinib for COVID-19 and showed that the drug was well tolerated, had low toxicity, no new safety signals, and, compared to controls, patients in the experimental group using ruxolitinib had significantly reduced levels of seven cytokines levels were significantly reduced, significantly reversing respiratory and multisystem inflammation in patients with severe COVID-19.

Ruxolitinib acts mainly by blocking STAT factor phosphorylation through inhibition of JAK kinase, which causes excessive cellular inflammation. And clinical evidence suggests that this inhibitor of JAK1 and JAK2 may reduce the inflammatory response in the lungs and may avoid the use of intensive care. Some literature reports that the use of ruxolitinib shortens the recovery time from lymphopenia and reduces the COVID-19 inflammation score as well as IL-6 and CRP levels. In addition, patients treated with oral tyrosine kinase inhibitors had a shorter time to clinical improvement of respiratory symptoms than controls and did not progress from noninvasive to invasive assisted ventilation [9].

In addition, ruxolitinib has been confirmed to be useful for atopic dermatitis (AD) treatment and its cream formulation results in a rapid reduction in pruritus index in patients within 36 hours [10]. There are also ongoing studies exploring the safety and efficacy of ruxolitinib in adolescent and adult regimens, as well as the feasibility of its use in pediatric AD patients.

7. Conclusion

The yield of our chosen reaction route one was greatly compromised due to the synthetic chirality. After searching for information, we believe that there are three ways to change this situation. We can choose better reactions and catalysts. By changing the catalyst (e.g. using selective catalytic enzymes or asymmetric large-site platinum-based metal complexes) the selectivity of the reaction can be substantially improved. Natural chiral compounds can be used. Compounds with chiral activity can be found by screening various natural compounds. And among these compounds can be found compounds that can be used as reaction materials. These compounds can help us to eliminate the step of preparing chiral centers, and the production process can be optimized. By optimizing the production process (e.g. by using continuous flow technology) by-products (e.g. corresponding spin isomers) can be reused. This not only increases the overall yield but also improves atomic utilization.

References

[1] Silvennoinen O, Ihle JN, Schlessinger J, Levy DE. Interferon-induced nuclear signalling by Jak protein tyrosine kinases. Nature. 1993, 366: 583-585.

[2] Quintás-Cardama A, Vaddi K, Liu P, et al. Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms. Blood. 2010, 115(15): 3109-3117.

[3] Shilling AD, Nedza FM, Emm T, et al. Metabolism, excretion, and pharmacokinetics of [14C]INCB018424, a selective Janus tyrosine kinase 1/2 inhibitor, in humans. Drug Metab Dispos. 2010, 38(11): 2023-2031.

[4] Vannucchi AM, Kiladjian JJ, Griesshammer M, et al. Ruxolitinib versus standard therapy for the treatment of polycythemia vera. N Engl J Med. 2015, 372(5): 426-435.

[5] Oritani K, Ohishi K, Okamoto S, et al. Effect of ruxolitinib therapy on the quality-of-life of Japanese patients with myelofibrosis. Curr Med Res Opin. 2018, 34(3): 531-537.

[6] Zhang XQ, Zhang, AM, Zhou, Z, et al. Synthesis process of ruxolitinib. WO2017114461A1. 2016. Dec.29.

[7] Haydl AM, Xu K, Breit B. Regio- and enantioselective synthesis of N-substituted pyrazoles by rhodium-catalyzed asymmetric addition to allenes. Angew Chem Int Ed Engl. 2015, 54(24): 7149-7153.

[8] Lin Q, Meloni D, Pan Y, et al. Enantioselective synthesis of Janus kinase inhibitor INCB018424 via an organocatalytic aza-Michael reaction. Org Lett. 2009, 11(9): 1999-2002.

DOI: 10.54254/2753-8818/4/20220552

[9] Marcuzzi A, Rimondi E, Melloni E, et al. New Applications of JAK/STAT Inhibitors in Pediatrics: Current Use of Ruxolitinib. Pharmaceuticals. 2022; 15(3): 374.
[10] Zhou S, Qi F, Gong Y, et al. Biological Therapies for Atopic Dermatitis: A Systematic Review. Dermatology. 2021, 237(4): 542-552.