Synthesis of tert-butyl 3-(2-(4-amino-3-(2-aminobenzo[d]oxazole-5-yl)-1H-pyrazole[3,4-d] pyrimidine-1-yl) ethoxy) propionate

Qi Zhang[a], Yao Huang[b], Heran Cao[c], Fangyi Gong[d], Qingye Gan[e] and Beibei Mao*

College of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan 250355, China

[a]1115309640@qq.com, [b]3137335047@qq.com, [c]145705187@qq.com, [d]1120057187@qq.com

[e]1348030692@qq.com, *Corresponding author e-mail: maobeibei89@163.com

Abstract. Tert-butyl 3-(2-(4-amino-3-(2-aminobenzo[d]oxazol-5-yl)-1H-pyrazole[3,4-d] pyrimidine-1-yl) ethoxy) propionate plays an important role in the whole synthesis route as an intermediate of target mTOR targeted PROTAC molecule PRO1. In this experiment, palladium catalyzed Suzuki reaction was used, the target compound tert-butyl 3-(2-(4-amino-3-(2-aminobenzo[d]oxazol-5-yl)-1H-pyrazole[3,4-d]pyrimidine-1-yl)ethoxy)propionate (compound 3) was synthesized from 5-(4,4,5,5-tetramethyl-1,3,2-dioxobenzaldehyde-2-yl) benzo[d]oxazole-2-amine (compound 1) and 3-(2-{4-amino-3-iodine-1H-pyrazole[3,4-d]pyrimidine -1-ethoxy}propionic acid) tert-butyl ester (compound 2). The structure of the product was confirmed by 1H NMR, 13C NMR and HRMS. When the reaction time is 6 hours, the ratio of reactants n (compound 1): n (compound 2) = 1:2, the reaction solvent is DME: H2O (2:1, volume ratio), the reaction temperature is 90°C, the catalyst is Pd (PPh3)4, the base condition is Na2CO3, the best reaction condition can be obtained, and the yield is 96.7%.

Keywords: mTOR; Synthetic; Condition optimization; PROTAC; Suzuki-Miyaura coupling reaction.

1. Introduction

Suzuki-Miyaura coupling reaction, as one of the widely used synthesis reactions at present, takes organic borides and halogenated compounds as raw materials, and under the catalysis of palladium catalysts in alkaline conditions, can efficiently construct new C-C bonds, which has obvious advantages such as low toxicity, high efficiency, high synthesis yield and wide substrate universality [1]. This reaction, as a closed catalytic cycle reaction, roughly includes three steps: (1) zero-valent palladium is inserted into the carbon halide bond, and the valence is increased by oxidation to form divalent palladium; (2) the organic boride undergoes a metallation reaction to transfer nucleophilic groups to palladium; and (3) the reduction and elimination reaction generates coupling products and zero-valent palladium.

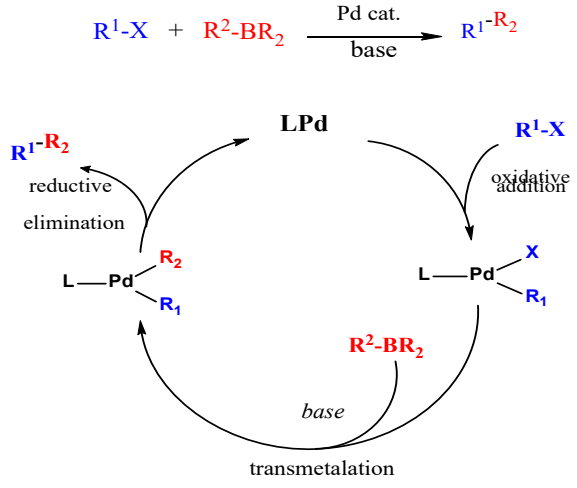

Fig. 1 Suzuki-Miyaura coupling reaction mechanism

At present, people often use aryl pinacol borate instead of traditional aryl boric acid as organic boride in Suzuki reaction [2-4], and have achieved very good results [5]. In this experiment, 5-(4,4,5,5-tetramethyl-1,3,2-dioxbenzaldehyde-2-yl) benzo[d]oxazole-2-amine, a pinacol borate as an organic boride and Pd (PPh3)4 as a catalyst were used, and finally a good reaction yield was obtained.

Mammalian rapamycin target protein (mTOR), as a serine/threonine protein kinase, is used to regulate cell growth, apoptosis, reproduction and autophagy [6]. At present, the abnormal expression of mTOR signaling pathway has been confirmed to be related to the occurrence of many diseases, such as breast cancer, lung cancer, multiple sclerosis, diabetic nephropathy, systemic lupus erythematosus, etc[6-9]. Therefore, mTOR signaling pathway inhibitors have become the research focus of targeted therapy. The pyrazole[3,4-d] pyrimidine skeleton derivatives explored in this experiment, as important mTOR inhibitors and anti-tumor active substances [10-11], are widely used in clinical targeted therapy.

Protein degradation targeting chimera (PROTAC) is a bifunctional molecule: its one end is a target protein ligand, and the other end is a structure that can recruit a protein degradation system. The two parts are connected together by a suitable linker [12]. It recruits E3 ubiquitin ligase, specifically ubiquitinate the target protein and degrade the target protein through proteasome system in a targeted way, thus achieving the efficacy of targeted therapy [13]. According to this technology, we designed a PROTAC molecule for mTOR signaling pathway inhibitor. The target product tert-butyl 3-(2-(4-amino-3-(2-aminobenzo[d]oxazol-5-yl)-1H-pyrazole[3,4-d] pyrimidine-1-yl) ethoxy) propionate is an important intermediate plays an important role in the whole synthetic route.

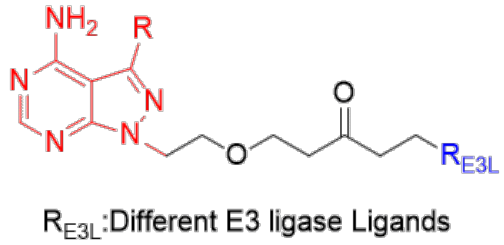

Fig. 2 Structure of PRO1

In this experiment, we used Suzuki reaction principle, 5-(4,4,5,5-tetramethyl-1,3,2-dioxbenzaldehyde-2-yl)benzo[d]oxazole-2-amine (compound 1) and tert-butyl 3-(2-(4-amino -3-iodine-1H-pyrazole[3,4-d]pyrimidine-1-yl)ethoxy)propionate (compound 2) were used as raw material, Pd(PPh3)4 was used as catalyst to synthesize the target compound tert-butyl 3-(2-(4- amino-3-(2-aminobenzo[d]oxazol-5-yl)-1H-pyrazole[3,4-d]pyrimidine-1-yl)ethoxy)propionate (compound 3). The final product was confirmed by 1H NMR, 13C NMR and HRMS, and at the same time, the effects of reaction time, reaction material ratio, reaction temperature, base, reaction solvent and nitrogen protection factors on the product yield were explored, and the optimum reaction conditions were determined [14]. This method provided ideas for improving the product yield of Suzuki-Miyaura reaction.

2. Materials and Methods

2.1 Raw materials and instruments

Reagent: 5-(4,4,5,5-tetramethyl-1,3,2-dioxbenzaldehyde-2-yl)benzo[d]oxazole-2-amine from Bide Pharmatech Ltd.; Dimethyl ether (DME) comes from Shanghai Macklin Biochemical Co., Ltd; The silica gel plate (50×100 mm) is from Qingdao Ocean Chemical Co., Ltd.; Column silica gel is from Qingdao Ocean Chemical Co., Ltd.; Potassium carbonate, sodium carbonate, anhydrous magnesium sulfate, ethyl acetate (EA), petroleum ether (PE) and other reagents are commercially available Analytical Reagent, AR.

Instrument: Rotary evaporator N-1300D-WB is from EYELA Tokyo Physical and Chemical Instrument Co., Ltd.; ADVANCEIII HD-600MHz nuclear magnetic resonance instrument is from Bruker Company, Germany; Time-of-flight mass spectrometer Agilent 1260 HPLC-1290 UPLC/6230 TOF is from Agilent Technologies, USA.

2.2 Experimental method

2.2.1 Synthetic route

According to the method of reference [13], 3-(2-{4-amino-3-iodine-1H-pyrazolo[3,4-d] pyrimidine-1-ethoxy} propionic acid) tert-butyl ester and 5-(4,4,5,5-tetramethyl-1,3,2-dioxbenzaldehyde-2-yl) benzo[d]oxazole-2-amine were used as the starting materials. After extraction, drying and column chromatography, the final product tert-butyl 3-(2-(4-amino-3-(2-aminobenzo[d]oxazole-5-yl) -1H-pyrazole[3,4-d] pyrimidine-1-yl) ethoxy) propionate was obtained. The synthetic route is shown in Figure 3.

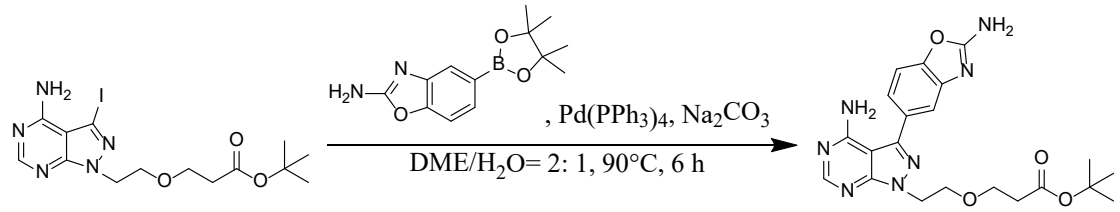

Fig. 3 Synthetic route of *tert*-butyl 3-(2-(4-amino-3-(2-aminobenzo[*d*]oxazol-5-yl)-1*H*-pyrazole [3,4-*d*] pyrimidine-1-yl) ethoxy) propionat

2.2.2 Experimental procedure

Synthesis: Add 3-(2-{4-amino-3-iodine-^{1}H-pyrazole[3,4-d]pyrimidine-1-ethoxy}propionic acid) tert-butyl ester 50.00 mg (0.120 mmol) and 5-(4,4,5,5-tetramethyl-1,3,2-dioxbenzaldehyde-2-yl) benzo[d]oxazole-2-amine 62.42 mg (0.240 mmol) into a round bottom flask, add 6 mL DME: H2O (2: 1, volume ratio), stir to completely dissolve the raw materials, add 63.59 mg (0.600 mmol) of sodium carbonate and 13.87 mg (0.0120 mmol) of Pd(PPh3)4, stir them to fully mix, connect a nitrogen protection device, and heat the system to 110℃ to react and reflux for 6 hours. During the reaction, TLC was used for tracking and monitoring (the developing agent was the mixture of dichloromethane and methanol with the volume ratio of 15: 1) until the compond 1 was completely disappeared.

Post-treatment of the reaction: after the reaction, add 30 mL of ethyl acetate and 30 mL of water into the reaction solution, extract and separate, collect the ethyl acetate layer, continuously extract the water layer for three times, and combine the extractive solutions. Adding anhydrous magnesium sulfate into the extract, and drying overnight. Suction filtration and spin drying.

Purification by silica gel column chromatography: using the mixture of dichloromethane and methanol with the volume ratio of 20: 1 as eluent, 49.10 mg of white solid was obtained after purification, and the yield was 96.7%. ^{1}H NMR (600 MHz, DMSO-d6) δ 8.24 (s, ^{1}H), 7.54 (s, 2H), 7.47 (d, J = 8.1 Hz, ^{1}H), 7.40 (d, J = 1.5 Hz, ^{1}H), 7.23 (dd, J = 8.1, 1.7 Hz, ^{1}H), 4.46 (t, J = 5.6 Hz, 2H), 3.87 (t, J = 5.6 Hz, 2H), 3.58 (t, J = 6.1 Hz, 2H), 2.32 (t, J = 6.^{1} Hz, 2H), 1.28 (s, 9H). 13C NMR (151 MHz, DMSO-d6) δ 170.71, 163.89, 158.32, 154.83, 148.80, 144.80, 128.94, 120.90, 115.45, 109.35, 97.78, 80.09, 68.43, 66.45, 46.45, 36.18, 28.08. HRMS (ESI) calcd for C21H26N7O4 [M+H] + m/z 440.2046, found 440.2004.

3. Results and discussion

3.1 Effect of material ratio on product yield

At 90℃, Pd (PPh3)4 was used as palladium catalyst, DME: H2O (2: 1, volume ratio) was used as solvent, sodium carbonate was used as alkali, and the reaction was carried out under the protection

of nitrogen for 6 hours. The effect of the ratio of reactants on the yield of the product was investigated. The final results are shown in Table 1.

As can be seen from Table 1, the reaction yield is 80.1% when the ratio of n(compound 1): n(compound 2)=1: 1.0, and the reaction yield is 90.2% when the ratio of n(compound 1): n(compound 2)=1: 2.0, and when n(compound 1): n(compound 2)=1: 3.0, the yield increased slightly to 90.4%. To sum up, it can be found that the reaction material ratio has little influence on the final yield. Considering the cost and yield comprehensively, it can be considered that n(compound 1): n(compound 2)=1:2.0 is the best feed ratio.

Table 1. Effect of material ratio on product yield

Number	Material ratio	Yield, %
1	1: 1.0	80.1
2	1: 1.5	85.5
3	1: 2.0	90.2
4	1: 3.0	90.4

3.2 Effect of reaction temperature on product yield

It was found that the final yield of the product was significantly different at different reaction temperatures when other conditions were the same and were the best.

Table 2 shows that, when the reaction temperature is 120℃, the reaction yield is only 55.7%. After the reaction, TLC detection shows that there are many and dense side reaction products at 120℃, and the polarity difference is small. However, when the reaction temperature is 90℃, the reaction yield suddenly rises to 96.7%. At this time, there is a negative correlation between the reaction temperature and the yield result, which may be due to the dehalogenation and protonation of halogenated hydrocarbon in compond 1 when the temperature is too high. The steric hindrance of reactant 2 is large, the thermal stability is general, and it is prone to deboron protonation and boric acid self-coupling reaction. When the reaction temperature is 80℃, the product yield decreases to 86.4%. Therefore, the optimum reaction temperature is 90℃.

Table 2. The effect of temperature on the yield of product

Number	Reaction temperature/℃	Yield, %
1	120	55.7
2	110	76.5
3	100	86.5
4	90	96.7
5	80	86.4

3.3 The influence of alkali types on product yield

As one of the important factors affecting Suzuki reaction, alkali often plays a decisive role in the reaction yield. The strength of alkali, base groups, etc. affect the selectivity of the reaction. At present, some studies have shown that strong base and organic base will inhibit Suzuki reaction process to a certain extent and increase the generation probability of cyclization by-products [15-16]. Based on this conclusion, we discussed the effects of NaOH, Na_2CO_3, K_2CO_3 and ethylenediamine on the reaction yield when the molar ratio of them to compound 1 is 5: 1. The results are shown in Table 3.

Table 3. Effect of the type of base on the product yield

Number	Base	Yield, %
1	NaOH	32.7
2	Na_2CO_3	96.7
3	K_2CO_3	90.0
4	ethylenediamine	56.6

As can be seen from Table 3, when the base is NaOH and ethylenediamine, the reaction yield is both low, only 32.7% and 56.6%, while when the base is sodium carbonate, the reaction yield greatly increases to 96.7%, which is higher than that of potassium carbonate. Therefore, we can determine that the reaction yield is the highest when the alkali used is sodium carbonate.

3.4 Effect of solvent on reaction yield

As a common and necessary solvent for Suzuki reaction, aqueous solvent has been widely used in synthesis. The effects of different water-soluble solvents on the reaction yield were discussed in this experiment.

It can be seen from Table 4 that the reaction yield is not optimistic when water or dimethyl ether is used as the reaction solvent alone: on the one hand, when water or organic solvents are used alone, the polarity of solvents and reactants does not match, and the solubility of reactants is poor; On the other hand, in pure water or organic solvents, the catalytic efficiency of palladium catalyst is low, which may also be related to solubility. However, if the two are mixed, the final yield will be greatly improved. The yield is as follows. Considering the yield and the concept of green chemistry, reducing the proportion of organic solvent as much as possible, the best reaction solvent can finally be determined as DME: H_2O (2: 1, volume ratio).

Table 4. Effect of solvent on product yield

Number	Solvent	Yield, %
1	DME	39.7
2	Water	35.0
3	DME: H_2O (2: 1, volume ratio)	96.7
4	DME: H_2O (3: 2, volume ratio)	97.0

3.5 Effect of nitrogen protection on reaction yield

Inert gas protection also plays an important role in Suzuki reaction. Its main purpose is to isolate the oxygen in the air and prevent the oxidation of palladium catalyst, so as to improve the product yield. Under the same other conditions, two groups of control experiments with and without nitrogen protection were set. The yields of these two groups are shown in Table 5.

As can be seen from Table 5, the yield of the group with nitrogen protection is obviously higher than that of the group without nitrogen protection. During the experiment, we also found that the palladium black is obvious and the oxidation degree is high in the group without nitrogen protection after 5 hours of reaction, while the group with nitrogen protection hardly produces palladium black, which shows that nitrogen protection is very important for this reaction.

Table 5. Effect of nitrogen protection on product yield

Number	With or withoutnitrogen protection	Yield, %
1	Yes	96.7
2	No	72.0

3.6 NMR analysis of the target compound

^{1}H NMR analysis of the target compound shows that, δ 8.24 has a single peak, the integral is one hydrogen, which is the hydrogen on the pyrazolopyrimidine skeleton, δ 7.54 has a single peak, the integral is two hydrogens, which is the hydrogen on the amino group; δ 7.47 double peaks, integrating one hydrogen δ 7.23 has double double peaks, integrating one hydrogen δ 7.23 has double peaks, integrating one hydrogen, three hydrogen in totle, which are the hydrogens on the benzoxazole skeleton; δ 4.46 has triple peak, integrating two hydrogens, δ 3.87 triple peak, integrating two hydrogens, δ 3.58 triple peak, integrating two hydrogens, δ 2.32 triple peak, integrating two hydrogens, totally eight hydrogens, which are hydrogen on four methylene groups; δ 1.28 has a single peak with an integral of 9 hydrogens, which are the hydrogens on tert-butyl.

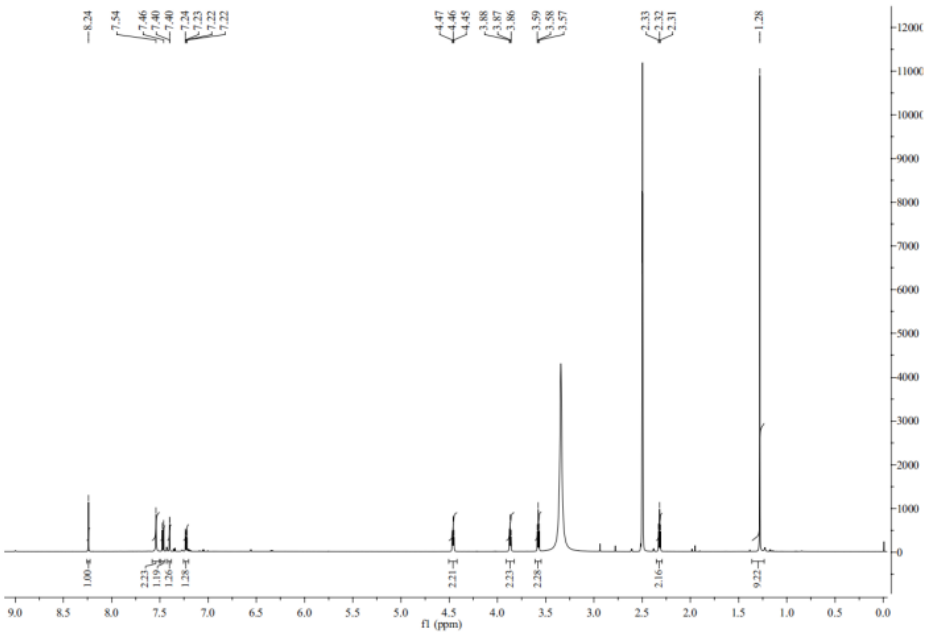

Fig. 4 The ^{1}H NMR of target compound

13C NMR analysis of the target compound showed that δ 170.71, 163.89, 158.32, 154.83 and 148.80 were the carbon of pyrazolopyrimidine skeleton. δ 144.80, 128.94, 120.90, 115.45, 109.35 and 97.78 are the carbon on the skeleton of benzoxazole; δ 80.09, 68.43, 66.45 and 46.45 are carbon on methylene; δ 36.18 is tert-butyl carbon; δ 28.08 is the carbon on the methyl group.

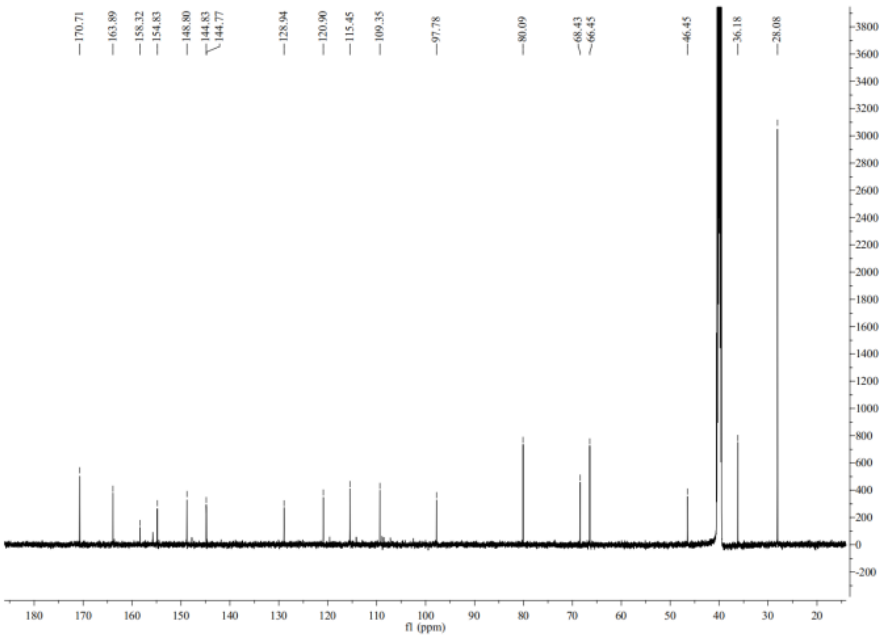

Fig. 5 The ^{13}C NMR of target compound

4. Summary

(4,4,5,5-tetramethyl-1,3,2-dioxbenzaldehyde-2-yl)benzo[d]oxazole-2-amine (compound 1) and tert-butyl 3-(2-(4-amino-3-iodine-1H-pyrazole[3,4-d]pyrimidine-1-yl)ethoxy)propionate (compound 2) were used as raw material, Pd(PPh3)4 was used as catalyst to synthesize the target compound tert-butyl 3-(2-(4-amino-3-(2-aminobenzo[d]oxazol-5-yl)-1H-pyrazole[3,4-d]pyrimidine -1-yl)ethoxy) propionate (compound 3), The final product was confirmed by 1H NMR, 13C NMR and HRMS,and at the same time, the effects of reaction time, reaction material ratio, reaction temperature, base,

reaction solvent and nitrogen protection factors on the product yield were explored, and the optimum reaction conditions were determined. It was determined that when the reaction time was 6 hours, the reaction ratio of n (compound 1): n (compound 2) =1:2, the reaction solvent was DME: H2O (2:1, volume ratio), the reaction temperature was 90℃, and the catalyst was Pd (PPh3)4. This method provides an idea for Suzuki reaction in aqueous solvent.

Acknowledgement

This work was supported by the Natural Science Foundation of Shandong Province (ZR2019BB031).

References

[1] Zhang lei, Yang Chen, Guo Xuefeng, et al. Research progress of Suzuki-Miyaura coupling reaction mechanism [J]. Organic Chemistry,2021,41(09):3492-3510.

[2] Xu Yuliang, Fang Hao. Research progress in synthesis of arylboronic acid compounds[J]. Organic Chemistry,2018,38(04):738-751.

[3] Lou Xin, Zhang Zhenqi. Application of pinacol borate B_2pin_2 in coupling reaction [J]. Chemical Reagents,2015,37(06):511-514.

[4] Li Yingqi, Peng Yuchun. Research progress in synthesis of aryl boric acid and its esters [J]. Chemical Reagents, 2006(03):149-152.

[5] Zhang Jie. Synthesis of substituted aryl borate in aqueous system [D]. Nanjing University of Science and Technology,2012.

[6] Song Xiaohong, Liu Mingming, Tong Honggang. Research progress of mTOR signaling pathway and related diseases [J]. Chinese Journal of Microcirculation,2018,28(03):64-70.

[7] Xu Gongjun, Shen Zhen, Lan Yanqin, et al. Research progress of mTOR signaling pathway in skin malignant tumors [J]. Medical Innovation of China, 2021,18(19):180-184.

[8] Hu Xueju, Wang Ruiying, Jiang Fei, et al. Interaction mechanism of PI3K/AKT/mTOR and MAPK/ERK signaling pathways in gastric cancer cells [J]. Chinese Health,2021,37(07):1125-1128.

[9] Cheng Xingyi, Chi WU, Tian Yuan Liang Zi, et al. Research progress of inhibitors in mTOR and IIS signaling pathways and senile diseases [J]. Chemistry of Life,2019,39(04):692-70

[10] Hu Xueju, Wang Ruiying, Jiang Fei, et al. Interaction mechanism of PI3K/AKT/mTOR and MAPK/ERK signaling pathways in gastric cancer cells [J]. Chinese Health, 2021,37(07):1125-1128.

[11] ZHANG M M, WEI W, PENG C J, et al. Discovery of novel pyrazolopyrimidine derivatives as potent mTOR/HDAC bi-functi-onal inhibitors via pharmacophore-merging strategy [J]. Bioorganic & Medicinal Chemistry Letters, 2021 Oct 1;49:128286.

[12] Chen Ran, Wang Baoshi, Liu Mingming, et al. Antitumor screening of pyrazolo [4,3-d] pyrimidine derivatives and its mechanism [J]. Acta Universitatis Medicinalis Anhui, 2019,54(03): 452-457.

[13] VANESSA S, RODRIK O, MASANORI O, et al. Abstract 2147:O-vercoming mTOR resistance mutations with a new generation mTOR inhibitor [J]. Cancer Research, 2016,76(14_Supplement):2147-2147.

[14] Wang Yongqi, Tong Hongjuan. Synthesis of 3- benzoyl imine -1- tert-butoxycarbonyl piperidine -4- one [J]. Contemporary Chemical Industry, 2020,49(04): 560-563.

[15] Liu Ning, Liu Chun, Jinlin. Suzuki reaction catalyzed by aqueous palladium [J]. Organic Chemistry,2012,32(05):860-876.

[16] Li Xinmin. Suzuki reaction of aqueous aryl borides catalyzed by palladium [D]. Dalian University of Technology,2016.