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Design and synthesis of BAP-1 inhibitors

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Abstract: **Background**: Tumors pose a severe threat to human health, with malignant tumors requiring complex targeted therapies. BRCA1-binding protein 1 (BAP1), a key deubiquitinase, plays dual roles in tumorigenesis and progression, making it a promising therapeutic target. The second-generation BAP1 inhibitor IBAP-II exhibits good activity but suffers from poor solubility, limiting its clinical application. **Objectives**: To design and synthesize novel BAP1 inhibitors with improved properties based on IBAP-II, addressing its solubility issue and enhancing inhibitory potential. **Methods**: Using IBAP-II as the lead compound, five novel compounds (6-nitroquinoline, 5-nitroindole and 7-nitroquinoline derivatives) were synthesized via Michael addition and Suzuki-Miyaura reactions. Structural confirmation was performed by ^{1}H NMR spectroscopy. **Results**: All five compounds were successfully synthesized with yields ranging from 27.86% to 51.07%. NMR data validated their target structures and structural modifications (e.g., hydroxyl/nitro relocation, ring structure adjustment) were confirmed to potentially improve solubility and binding affinity. **Conclusion**: This study provides feasible synthetic routes for novel BAP1 inhibitors with optimized structures, laying a foundation for subsequent biological activity evaluations and offering new prospects for targeted tumor therapy, especially for small cell lung cancer.

Keywords: BAP1 inhibitor; Biological activity; Gene therapy; IBAP-II; Tumor

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INTRODUCTION

Tumor concept and research progress

A tumor is a new organism formed by local histiocytic proliferation or malignant transformation under the influence of various tumorigenic factors. According to the cell characteristics and the harm to the human body, tumors can be divided into two categories: benign tumors and malignant tumors. According to the location of the lesion, tumors can be divided into cancer and sarcoma. Cancer usually refers to the malignant tumor of epithelial tissue, sarcoma usually refers to the tumor of mesenchymal tissue, including fiber connective tissue, fat, muscle, coronary artery, bone and cartilage tissue (Woertler K., 2010).

Tumors pose a significant threat to the human body, necessitating thorough research into their pathogenesis and treatment modalities. Cancer stands as one of the deadliest diseases today and its eradication holds immense potential for extending human lifespan. However, due to the intricate heterogeneity of tumors and their propensity for recurrence and metastasis, treatment remains a formidable challenge in medicine. Presently, a diverse array of clinical treatments is employed, including surgical resection, radiotherapy, chemotherapy, photodynamic therapy (PDT) and immunotherapy, each tailored to specific patient types (Wang *et al*., 2019). Surgical resection is often effective for benign tumors, while chemotherapy and radiotherapy are preferred for malignant ones. Despite advances, current tumor treatments primarily operate at the cellular and tissue levels, necessitating further exploration of groundbreaking approaches like gene therapy and immunotherapy. The BAP1 inhibitor presented in this paper represents a novel treatment strategy, contributing significantly to the advancement of tumor therapy (Han A *et al.,* 2021).

Overview of BAP1

BAP1 and its role in tumorigenesis

The human BAP1 gene encodes a deubiquitinase enzyme composed of 729 amino acids (Song *et al*., 2019). Deubiquitinases (DUBs) are critical regulators in the ubiquitination modification pathway, modulating the stability and biological activity of target proteins through the specific cleavage or modification of ubiquitin chains. These enzymes are integral to various physiological and pathological processes in the body (Sahtoe *et al*., 2016). The DUB family comprises numerous members that can be categorized into six subfamilies: USP, UCH, OUT, MJD, JAMM and MCPIP, based on their functional and structural attributes. However, the intricate molecular mechanisms underlying their structure, function and action remain poorly understood (Heideker *et al*., 2015).

Recent research has illuminated the significance of DUBs in DNA damage repair, cellular development and growth (Murtaza *et al*., 2015), immune regulation (Ikeda *et al*., 2015) and tumor initiation and progression (Qian BZ and Pollard JW., 2010). Notably, BAP1, a member of the UCH subfamily of deubiquitinases, resides in the nucleus and plays a pivotal role in these processes. Extensive studies have demonstrated that BAP1 is frequently mutated or deleted in various tumor types, including renal clear cell carcinoma, uveal melanoma, pleural mesothelioma and cholangiocarcinoma. Its overexpression has been shown to impede tumor cell growth, thus positioning BAP1 as a tumor suppressor gene (Al-Shamsi *et al*., 2016). However,

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recent investigations have also revealed that BAP1 can promote breast cancer growth by regulating the deubiquitination of KLF5 (Qin *et al*., 2015), indicating that BAP1 may exhibit diverse functions and mechanisms of action in different tumor types (Sahtoe *et al*., 2016). Therefore, further research on BAP1 holds immense potential for advancing tumor therapy.

Physiological function of BAP1

The ubiquitin-proteasome pathway is a complex, efficient and rigorous protein degradation system, mainly responsible for the selective degradation of proteins in eukaryotic cells. This pathway plays an indispensable role in key physiological processes such as cell cycle, apoptosis, physiological health and disease occurrence and has always been a hot field of scientific research. Ubiquitin, a tiny protein ubiquitous in human eukaryotic cells, identifies and indicates proteins that need to be degraded through its unique labeling function. As an important part of this system, ubiquitin hydrolase affects protein degradation process by precisely regulating ubiquitination reaction, so as to maintain the homeostasis of intracellular environment（Hanpude P *et al.,* 2017).

BAP1 (BRCA1-related protein 1) is a member of this system and its function and action vary due to its unique structure and target. Studies have shown that BAP1 plays an important role in a variety of tumors. For example, in liver cancer, low expression of BAP1 has been shown to inhibit tumor growth (Yang *et al*., 2020). In malignant mesothelioma and invasive meningioma, the inactivation of BAP1 allele indicates that it is closely related to the occurrence and development of tumors (Li *et al*., 2019).

Research by the PLA Navy Medical University further revealed the immunomodulatory role of BAP1 in ovarian cancer and its high expression is closely related to multiple immune responses (Miao *et al*., 2022). In addition, loss of BAP1 expression can enhance the sensitivity of metastatic RCC to chemotherapy agents (Peña-Llopis *et al*., 2012) and the response of RCC to radiotherapy (Wei *et al*., 2018). Studies have revealed the role of BAP1 in oral mucosal melanoma, which is related to tumor cell differentiation and cell cycle regulation and affects the mRNA expression of histone deacetylase (HDAC) (Yang *et al.,* 2025). The study of the First Affiliated Hospital of Zhengzhou University found that the positive expression rate of BAP1 protein in meningioma tissues increased with the increase of tumor grade, indicating that BAP1 has a potential inhibitory effect on the development of meningioma (Song *et al*., 2016).

Although BAP1 has shown multiple roles in different tumor mechanisms, its specific mechanism of action is not fully understood. The current research mainly focuses on its correlation with tumorigenesis and development, but its detailed biological function still needs to be further explored. However, the regulation of the expression of BAP1 protein gene provides a new direction for the treatment of tumors and the development of BAP1 inhibitors is expected to provide a new auxiliary means for cancer treatment, which is of great significance for human health and medical development.

Overview of IBAP

In view of the differences in the role of BAP1 in different tumors, the types and targets of BAP1 inhibitors are also diverse, aiming to regulate the expression of BAP1 protein in different ways, that is, to regulate the translation expression of some fragments of BAP1 gene. For example, the ASXL1 gene codes for the core component of the BAP1 histone H2AK119Ub deubiquitination enzyme complex, which contains 1084 amino acids (Dey *et al*., 2012). Studies have shown that the mutation of ASXL1 gene is significantly associated with malignant myeloid diseases such as leukemia, but the specific regulatory mechanism is still unclear. The function of ASXL1 mutations and whether these mutations can be used as therapeutic targets remain to be further explored. Mutations within ASXL1 are known to have been described as loss-of-function mutations resulting in decreased PRC2 occupancy and Polycomb-mediated gene silencing (Abdel-Wahab *et al*., 2012), but their exact role in BAP1 protein function has not been determined.

However, studies have found that the catalytic activity of BAP1 is indeed involved in malignant tumors induced by ASXL1 mutations (Yang *et al*., 2018). In view of the importance of BAP1's catalytic activity in the malignant transformation of tumors, the researchers conducted an unbiased screening of small molecule inhibitors of BAP1's catalytic activity and identified a compound called IBAP (shown in Fig. 1). Treating ASXL1 mutated cells with IBAP was able to salvage abnormal oncogene expression patterns. This study provides a new perspective on the molecular mechanism by which ASXL1 mutations control BAP1 activity in cancer and provides a possible targeted therapy for ASXL1 mutated leukemia by inhibiting the catalytic activity of BAP1, thus advancing the field of leukemia treatment.

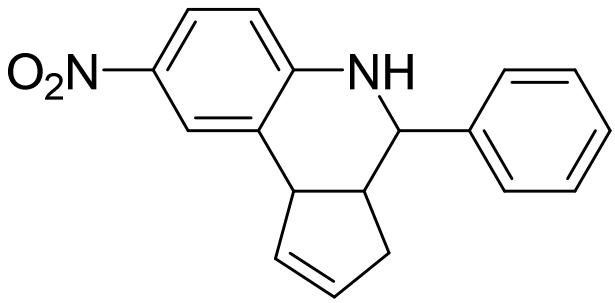

Fig. 1: Schematic diagram of IBAP structure

Subsequently, on the basis of IBAP, a new compound was synthesized by adding the modification of nitro and

hydroxyl group, which improved the inhibition effect of IBAP. In addition, the compound was found to have a higher affinity for BAP1 than other UCH family members and other ubiquitinating enzymes (Abdel-Wahab *et al*., 2012) and the compound was named IBAP-II (Fig. 2).

The primary signaling pathway through which IBAP-II affects SCLC cells is the ASCL1/MYCL/E2F pathway (refer to Fig. 3), a transcriptional signal reliant on BAP1.

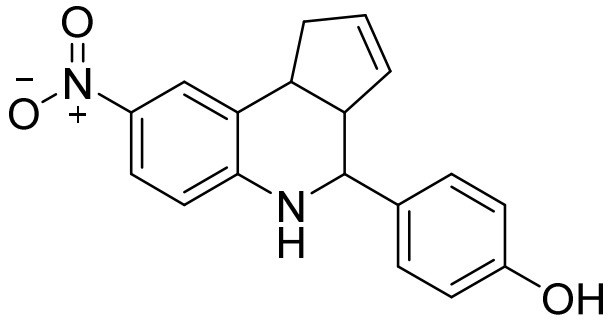

Fig. 2: Schematic diagram of IBAP-II classical structure

To verify the inhibitory effect of IBAP-II on small cell lung cancer, we administered DMSO, IBAP and IBAP-II to four distinct BAP1-WT SCLC cell lines: NCI-H1963, NCI-h748, NCI-h1882 and KP3 cells. Additionally, the BAP1-deficient SCLC cell line NCI-H226 was used as a negative control. Our findings revealed that both inhibitors effectively reduce cell viability in a laboratory setting. Notably, IBAP-II exhibited stronger inhibitory activity compared to IBAP across all four cell lines (Tsuboyama *et al*., 2022). This suggests that IBAP-II, as a successfully modified novel compound, enhances the inhibitory effect and improves activity beyond the original IBAP, thereby advancing the treatment of small cell lung cancer.

The above outlines the research and development trajectory of a BAP1 inhibitor. In other areas of tumor therapy, comprehending and identifying various gene codes, attempting to target different genes and designing novel BAP1 inhibitors to influence BAP1 protein expression and function, ultimately achieving the goal of intervening in tumor treatment, are pivotal projects for elevating the standard of tumor medical care.

MATERIALS AND METHODS

Based on the second generation of BAP1 inhibitor IBAP-II, five novel compounds were designed and synthesized. The choice of these two reactions was deliberate: Michael addition enables efficient incorporation of electron-donating and electron-withdrawing substituents, allowing fine-tuning of the electronic distribution within the scaffold (Ametsetor E., 2022), while Suzuki–Miyaura coupling is a well-established and versatile method for C–C bond formation, particularly suited for aromatic and heteroaromatic frameworks(Onnuch P *et al.,* 2024). These complementary strategies ensured both structural diversity and synthetic feasibility in the design of novel BAP1 inhibitors (XU *et al.,* 2022).

Design for pharmaceutical compounds

Given the increasing incidence of cancer, efforts to develop antitumor agents targeting BAP1 have intensified. Compared to the first-generation IBAP, IBAP-II exhibits superior selectivity and activity for BAP1, favors ubiquitination and shows a promising targeted therapeutic effect on small cell lung cancer. However, a key challenge lies in the poor solubility of IBAP-II, hindering its progress in drug formulation development.

BAP1 inhibitors

On the basis of this strategy, three types of compounds were designed and synthesized. The first type involved changing the saturation of the six-membered ring adjacent to the benzene ring and adjusting the position of the hydroxyl group, while maintaining the core structure of (IBAP-II) lacking the unsaturated five-membered ring. The retention of the amino group was pivotal as it can form hydrogen bonds with a specific amino acid in the human body, later identified as serine. This amino group linked to the benzene ring is thus a critical component of the drug's activity.

Based on this strategy, we designed and synthesized three distinct compounds. The first type involved modifying the saturation of the six-membered ring adjacent to the benzene ring, as well as adjusting the position of the hydroxyl group, while retaining the core structure of Analog#42 (IBAP-II) minus the unsaturated five-membered ring. The second type focused on rearranging the six-membered ring into an unsaturated five-membered ring and attaching different benzyl derivatives to the nitro para-amino group. Finally, the third type involved altering the relative positions of the amino and nitro groups, along with shifting the attachment site of the hydroxyl group on the benzene ring. These approaches explore diverse synthetic reactions to optimize the drug's properties.

Synthesis of BAP1 inhibitor

The target compounds were classified into three categories: 6-nitroquinoline derivatives, 5-nitroindole derivatives, and 7-nitroquinoline derivatives.

Synthesis of the first category (6-nitroquinoline derivatives)

In the process of synthesizing these compounds, to synthesize 6-nitroquinoline derivatives, 2-bromoquinoline was first nitrated to obtain 2-bromo-6-nitroquinoline and then Suzuki coupling was carried out on this basis to obtain 6-nitroquinoline derivatives with different substituents. In the process of synthesis, the intermediate product, 2-bromo-6-nitroquinoline, was coupled with 3-hydroxy-

phenylboric acid to obtain 3- (6-nitroquinolin-2-yl) phenol. Then the site of hydroxyl group was changed and 4- (6-nitroquinolin-2-yl) phenol was obtained by coupling with p-hydroxyphenylboric acid and intermediate products. The specific synthesis process is shown in the figure below.

Synthesis of 3- (6-nitroquinoline - 2-yl) phenol (1Aa)
Weigh 2-bromoquinoline (1eq, 2.1mmol) into 100mL nightshade bottle, add 3mL concentrated sulfuric acid under ice bath condition and then slowly drop concentrated nitric acid (0.6mL) into it. After 4 hours of reaction, the reaction was detected by thin layer chromatography (TLC) and the reaction was stopped. After quenching with water, the reaction was neutralized with saturated sodium bicarbonate solution first and then extracted with ethyl acetate. After three times of extraction (3×20mL), the organic phase was combined and an appropriate amount of anhydrous sodium sulfate was added for drying. Then silica gel column chromatography was used to separate and purify (the chromatography liquid polarity: petroleum ether: ethyl acetate = 25:1) to obtain 2-bromo-6-nitroquinoline. Then, 2-bromo-6-nitroquinoline (1eq, 1mmol) was weighed into a two-neck bottle and then 3-hydroxyphenylboric acid (1.1eq, 1.1mmol) and potassium carbonate (3eq, 3mmol) were added successively, dissolved with dioxane (5mL). Add pure water (1mL), then add (dppf) PdCl2 (0.03eq, 0.03mmol), stir and dissolve, replace nitrogen for about 5 times and react at 80 degrees Celsius. After 3 hours of reaction, the reaction was detected completely by thin layer chromatography, the reaction was stopped, the reaction was quenched with water, the pH was adjusted to acidity and then the organic phase was extracted with ethyl acetate. After four times of extraction (4×20mL), the organic phase was combined, an appropriate amount of anhydrous sodium sulfate was added to dry and the filtrate was extracted and then spin dried to obtain the crude product.

The product was separated and purified by silica gel column chromatography (the chromatography liquid polarity: dichloromethane: Ethyl acetate = 60:1).

3-(6-nitroquinolin-2-yl)phenol
Yellow powder,yield: 29.87%.1H NMR (300 MHz, DMSO-d6) δ 9.80 (s, 1H), 8.65 (d, J = 8.8 Hz, 1H), 8.39 – 8.19 (m, 3H), 7.82 – 7.58 (m, 3H), 7.39 (t, J = 8.0 Hz, 1H), 7.05 – 6.89 (m, 1H).

Synthesis of 4- (6-nitroquinoline - 2-yl) phenol (1Ab)
Weigh 2-bromoquinoline (1eq, 2.1mmol) into 100mL nightshade bottle, add 3mL concentrated sulfuric acid under ice bath condition and then slowly drop concentrated nitric acid (0.6mL) into it. After 4 hours of reaction, the reaction was detected completely by TLC analysis, the reaction was stopped, the reaction was quenched with water, neutralized with saturated sodium bicarbonate solution first and then extracted with ethyl acetate. After three times of extraction (3×20mL), the organic phase was combined, an appropriate amount of anhydrous sodium sulfate was added for drying and the filtrate was pumped and then spin dried to obtain the crude product. Then silica gel column chromatography was used to separate and purify (the chromatography liquid polarity: petroleum ether: ethyl acetate = 25:1) to obtain 2-bromo-6-nitroquinoline. After weighing 2-bromo-6-nitroquinoline (1eq, 1mmol) into a two-neck bottle, then adding 4-hydroxyphenylboric acid (1.1eq, 1.1mmol) and potassium carbonate (3eq, 3mmol) successively, dissolving with dioxane (5mL), Add pure water (1mL), then add (dppf) PdCl2(0.03eq, 0.03mmol), stir and dissolve, replace nitrogen for about 4 times and react at 80 degrees Celsius. After 3 hours of reaction, TLC analysis was used to detect the complete reaction, the reaction was stopped, the reaction was quenched with water, the pH was adjusted to acidity and then the organic phase was extracted with ethyl acetate. After four times of extraction (4×20mL), the organic phase was combined, an appropriate amount of anhydrous sodium sulfate was added for drying and the filtrate was extracted and then spin dried to obtain the crude product. Then the product was separated and purified by silica gel column chromatography (the chromatography liquid polarity: dichloromethane: ethyl acetate = 50:1).

4-(6-nitroquinolin-2-yl)phenol
Yellow powder, yield: 31.09%. 1H NMR (300 MHz, DMSO-d6) δ 10.07 (s, 1H), 8.57 (d, J = 8.8 Hz, 1H), 8.39 – 8.02 (m, 5H), 7.69 (t, J = 7.9 Hz, 1H), 7.09 – 6.86 (m, 2H).

The synthesis of the second category (5-nitroindole derivatives)
On the basis of the first category of compounds, the quinoline derivative is changed into indole derivative, that is, the unsaturated six-membered ring is designed as an unsaturated five-membered ring and the substituent group is directly connected to the amino group to form the second compound, that is, 5-nitroindole derivative. Therefore, 1-(4-methylbenzyl) -5-nitro-1H-indole was obtained by using 5-nitro-indole as substrate and reacting with 4-methylbenzyl chloride. In order to explore the effect of electron-withdrawing group on the activity of the compound, the electron-donating group was replaced by an electron-withdrawing group and 1- (4-fluorobenzyl) -5-nitro-1H-indole was obtained by reaction of 4-fluorobenzyl chloride with 5-nitro-indole. The specific synthesis process is shown in the figure below.

Synthesis of 1- (4-methylbenzyl) -5-nitro-1H-indole (1Ba)
Weigh 5-nitroindole (1eq, 0.7mmol) into 50mL nightshade bottle, then add 4-methylchlorbenzyl (1.2eq, 0.84mmol, 108μL), potassium carbonate (3eq, 2.1mmol), then add 5mLN, n-dimethylformamide and stir to dissolve. The reaction is carried out with nitrogen protection at 80 degrees Celsius.

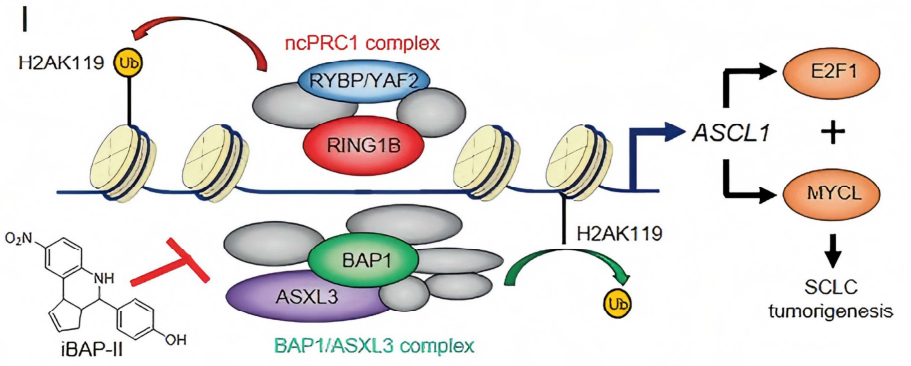

Fig. 3: Schematic diagram of the mechanism of IBAP-II in SCLC

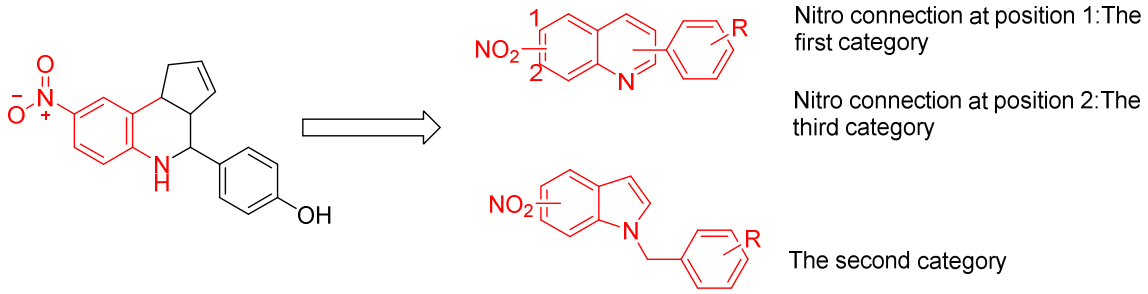

Fig. 4: Diagram of design for pharmaceutical compounds

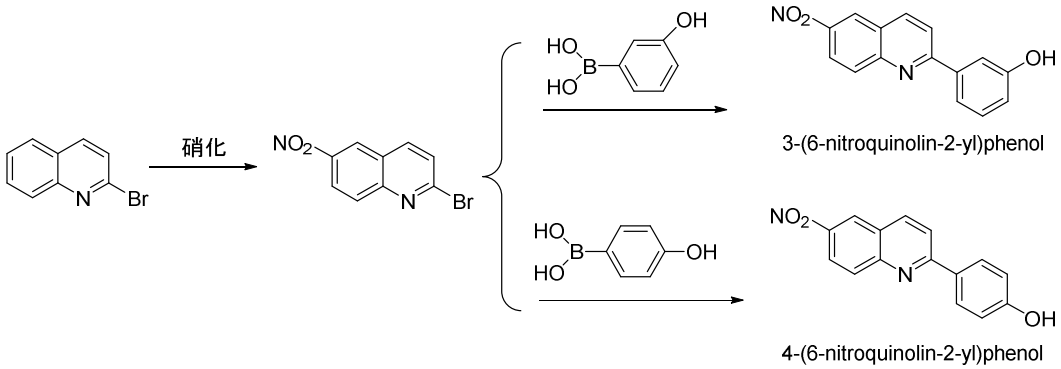

Fig. 5: Flow chart of synthesis of the first category of compounds

After reaction for 3 hours and 15 minutes, the reaction was detected by thin layer chromatography (TLC) and the reaction was stopped. After quenching with water, the reaction was extracted with ethyl acetate. After extraction for 3 times (3×20mL), the organic phase was combined and an appropriate amount of anhydrous sodium sulfate was added for drying. The product was separated and purified by silica gel column chromatography (the chromatographic liquid polarity: petroleum ether: ethyl acetate = 200:1)(Fig.4).

1-(4-methylbenzyl)-5-nitro-1H-indole

Light yellow solid,yield:48.86%.1H NMR (300 MHz, Chloroform-d) δ 8.66 – 8.52 (m, 1H), 8.13 – 7.96 (m, 1H), 7.38 – 7.20 (m, 2H), 7.14 (s, 2H), 7.01 (d, J = 7.3 Hz, 2H), 6.71 (d, J = 3.4 Hz, 1H), 5.32 (s, 2H), 2.32 (s, 3H).

Synthesis of 1- (4-fluorobenzyl) -5-nitro-1H-indole (1Bb)

Weigh 5-nitroindole (1eq, 0.8mmol) into 50mL nightshade bottle, then add 4-fluoro-chlorobenzyl (1.2eq, 0.96mmol, 115μL), potassium carbonate (3eq, 2.4mmol) and then add 5mLN, stir N-dimethylformamide to dissolve and react with nitrogen protection at 80°C. After reaction for 4 hours and 15 minutes, the reaction was detected by thin layer chromatography (TLC) and the reaction was stopped. After quenching with water, the reaction was extracted with ethyl acetate. After extraction for 4 times (4×20mL), the organic phase was combined and an appropriate amount of anhydrous sodium sulfate was added for drying. The product was separated and purified by silica gel column chromatography (the chromatographic liquid polarity: petroleum ether: ethyl acetate = 180:1).

1-(4-fluorobenzyl)-5-nitro-1H-indole

Light yellow solid, yield: 51.07%.1H NMR (300 MHz, Chloroform-d) δ 8.60 (d, J = 2.3 Hz, 1H), 8.08 (dd, J = 9.1, 2.3 Hz, 1H), 7.28 (d, J = 8.9 Hz, 2H), 7.17 – 6.93 (m, 4H), 6.73 (d, J = 3.3 Hz, 1H), 5.34 (s, 2H).

Synthetic of the third category (7-nitroquinoline derivative)

On the basis of the first category of compounds, the third category of compounds are considered to change the nitro site first, changing 6-nitroquinoline to 7-nitroquinoline and then switching the phenol junction site from site 2 to site 3. Therefore, 3-bromo-7-nitroquinoline was used as the reaction substrate and 4-hydroxyphenylborate Pinacol was Suzuki coupled with it to obtain 4- (7-nitroquinolin-3-yl) phenol. The specific synthesis route is shown in the figure below (Fig.5).

Weigh 3-bromo-7-nitroquinoline (1eq, 1mmol) into 50mL double-necked bottle, then add 4-hydroxyphenylborate pinalol ester (1.1eq, 1.1mmol) and potassium carbonate (3eq, 3mmol) successively, dissolve with 5mL Dioxane, then add 1mL pure water. Then add [1,1' -bis (diphenylphosphine) ferrocene] palladium dichloride (0.03eq, 0.03mmol), stir and dissolve and react with nitrogen protection at 80 degrees Celsius. After 3 hours of reaction, the reaction was detected completely by thin layer chromatography (TLC), the reaction was stopped, the reaction was quenched with water, the pH was adjusted to acidity and the organic phase was extracted with ethyl acetate, after 3 times of extraction (3×20mL), the organic phase was combined and an appropriate amount of anhydrous sodium sulfate was added for drying and the filtrate was extracted and then spin dried to obtain the crude product. The product was separated and purified by silica gel column chromatography (the chromatographic liquid polarity: petroleum ether: ethyl acetate = 5:1)(Fig.6).

4-(7-nitroquinolin-3-yl)phenol

Yellow powder, yield: 27.86%.1H NMR (300 MHz, DMSO-d6) δ 9.92 (s, 1H), 9.38 (d, J = 2.3 Hz, 1H), 8.91 (dd, J = 2.3, 0.9 Hz, 1H), 8.56 – 8.23 (m, 2H), 8.02 – 7.84 (m, 1H), 7.83 – 7.65 (m, 2H), 7.12 – 6.88 (m, 2H)(Fig.7).

RESULTS

Comprehensive analysis of data and results

In this study, five target compounds were successfully synthesized and their structures were confirmed by NMR spectroscopy, which was consistent with the expected results and all of them met the design requirements of BAP1 inhibitors. ^1H NMR spectra confirmed correct substitution patterns, absence of side-products and overall structural purity, thereby supporting the chemical stability of the compounds. Although NMR does not directly measure potency, it provides a reliable structural basis ensuring that subsequent biological tests reflect true activity. Characteristic signals—such as singlets at δ 9.8–10.0 ppm (hydroxyl protons) and downfield aromatic multiplets of nitro-substituted scaffolds—validated the intended modifications. Coupling constants further supported hydroxyl and nitro relocation. Yields ranging from 27.86% to 51.07% and reproducible spectra collectively demonstrate synthetic reliability despite the absence of formal reactivity indices. In the course of the experiment, the influence of different sites on the reaction activity was deeply observed.

Especially in the Suzuki coupling reaction, the first and third compounds showed significant differences in coupling site activity despite the same reaction conditions. Specifically, when the coupling groups are both 1Ca and 1Ab of 4-hydroxyphenol derivatives, the corresponding site of 1Ab shows higher coupling activity, which may be related to the location of the coupling group from the amino group. Therefore, in industrial scale-up synthesis or mass production, the first class compounds have more advantages due to their higher economy and efficiency.

Workup and yield limitations in Class I and Class III compound synthesis

In the synthesis of Class I and Class III compounds, we encountered similar challenges, namely the low extraction efficiency and the weak ability to collect organic phases in the extraction process. To solve this problem, we added a step to adjust pH after the reaction, which significantly improved the extraction efficiency and reaction yield. Nevertheless, the yields of certain products (e.g., 29.87% for 3-(6-nitroquinoline-2-yl)phenol) remain relatively low compared to expectations for multi-component reactions.

This limitation reflects the exploratory nature of the current study, which prioritized proof-of-concept design and synthesis. In future work, we plan to address this by optimizing catalyst loading, adjusting solvent polarity and refining purification strategies to further increase overall yields. We speculated that under alkaline conditions, the phenol derivatives would form salts and dissolve in the water phase due to the presence of hydroxyl groups. By adjusting the pH to acidity, these salts are broken down, allowing the product to be extracted more efficiently into the organic phase (Table1).

Reaction-site activity and substituent effects on yield and purification

It is worth noting that the second class of compounds synthesized with 5-nitroindole as reactants showed the highest reaction site activity and the reaction mechanism was simple and pure, which resulted in significantly higher yields than the first and third class of compounds. In addition, in the nucleophilic substitution reaction, although both 4-methyl chlorobenzene and 4-fluorobenzene participate as reactants, 4-fluorobenzene generates more by-products because one is an electron-withdrawing group and the other is an electron-giving group, which brings additional challenges to the subsequent purification process.

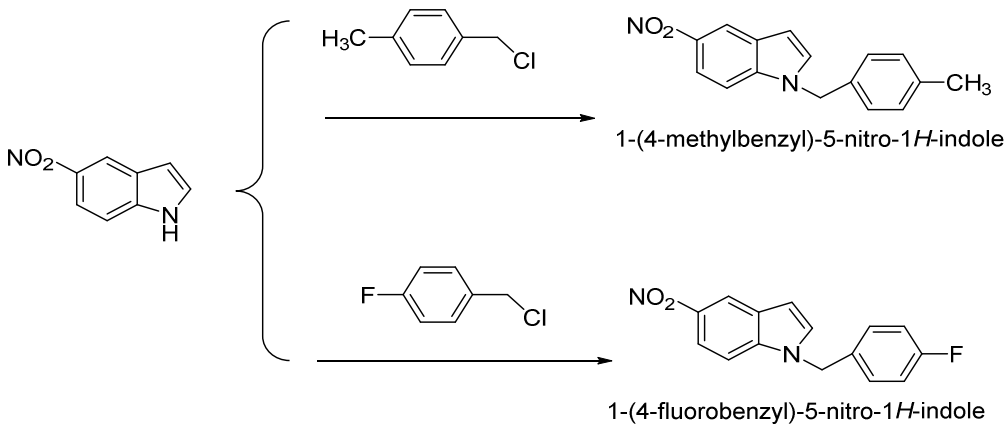

Fig. 6: Flow chart of synthesis of the second category of compounds

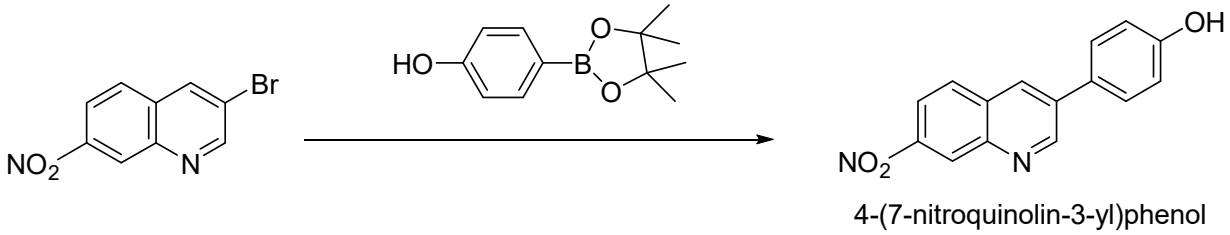

Fig. 7: Flowchart of synthesis of the third category of compounds

Table 1: Reagents and instruments used in the synthesis process

No.	Synthetic compound	Main reagents	Other reagents	Equipment
1.	3- (6-nitroquinoline - 2-yl) phenol (1Aa)	3-hydroxyphenylboric acid	2-bromoquinoline, concentrated sulfuric acid, concentrated nitric acid, [1,1' -bis (diphenylphosphine) ferrocene] Palladium dichloride [(dppf)PdCl2], dioxane,Potassium Carbonate, ethyl acetate (EA), Petroleum ether (PE), methylene chloride (DCM), purified water, silica gel, anhydrous sodium sulfate	85-1 magnetic stirrer, three ultraviolet analyzer, ZNHW- II electronic temperature control instrument, rotary evaporation instrument, SHB-3A circulating water multi-purpose vacuum pump, R201D constant temperature bath, beaker, liquid separation funnel, double neck bottle, nighthorn bottle, glass rod, magnetic stirrer, display cylinder, chromatographic column, balance, weighing paper
2.	4- (6-nitroquinoline - 2-yl) phenol (1Ab)	4-hydroxyphenylboric acid		
3.	1- (4-methylbenzyl) -5-nitro-1H-indole (1Ba)	5-nitroindole，4-methylbenzyl chloride，N, N-dimethylformamide (DMF)		
4.	1- (4-fluorobenzyl) - 5-nitro-1H-indole (1Bb)	5-nitroindole，4-fluorobenzyl chloride，N, N-dimethylformamide (DMF)		
5.	4-(7-nitroquinolin-3-yl)phenol	3-bromo-7-nitroquinoline, 4-hydroxyphenylborate pinalol ester，[1,1' -bis (diphenylphosphine) ferrocene] Palladium dichloride [(dppf)PdCl2], dioxane		

DISCUSSION

In this study, using IBAP-II as the lead scaffold, we designed and successfully synthesized five structurally simplified BAP1-inhibitor analogues spanning three chemotypes: (i) 6-nitroquinoline–phenol derivatives with hydroxyl positional isomerism, (ii) 5-nitroindole N-benzyl derivatives bearing electron-donating or electron-withdrawing substituents, and (iii) a 7-nitroquinoline–phenol derivative with altered coupling connectivity. All target structures were confirmed by ^1H NMR, and overall isolated yields ranged from 27.86% to 51.07%. Experimentally, we observed site-dependent differences in Suzuki–Miyaura coupling efficiency, and improving post-reaction workup by adjusting pH to acidic conditions enhanced extraction and yield for hydroxyl-containing products. In contrast, the N-alkylation route for the indole series proceeded with comparatively higher reaction-site activity and cleaner conversion, corresponding to higher yields overall. In summary, although these three types of compounds are simplified on the basis of the classical IBAP-II structure, they still retain the core characteristics of BAP1 inhibitors and exhibit different properties and reactivity due to their unique structure.

Compared with IBAP-II, the newly synthesized analogues feature improved synthetic accessibility and modifications intended to mitigate poor solubility, which is a known limitation of IBAP-II (Donati M *et al.,* 2023). While direct biological comparisons (e.g., IC_{50} values) are not yet available, these structural changes suggest potential advantages in drug-likeness and scalability. Future studies will conduct head-to-head inhibitory and pharmacological evaluations to confirm whether these modifications translate into superior therapeutic performance relative to IBAP-II and other reported BAP1 inhibitors. These findings not only provide valuable clues for us to further understand the mechanism of action of BAP1 inhibitors (Louie *et al.,* 2020), but also lay a solid foundation for future drug design and optimization. In particular, hydroxyl substitution was introduced to enable potential hydrogen-bonding interactions with the catalytic site of BAP1, thereby strengthening molecular binding, while relocation of the nitro group modifies the electron distribution of the aromatic ring, which may enhance affinity and selectivity. These mechanistic considerations explain why the structural modifications were expected to improve biological activity.

Strengths and Limitations

The innovation in this paper is that for classic Analog# 42 compounds, the parent nuclei are all six-membered rings connected. The six-membered rings connected with benzene ring are considered to be five-membered rings, the quinoline derivatives are changed into indole derivatives, the synthesis steps are reduced to improve the synthesis efficiency and the substituents are directly connected to heterocyclic N atoms to explore the effects of the substituent positions on drug activity.

In the current study, some limitations were faced. Due to time constraints, the synthesis steps were simplified during the synthesis, resulting in a relatively simple structure and a small number of substituents. While this simplification helps us to quickly verify the feasibility of the synthetic pathway, it also limits our in-depth exploration of the effects of different substituents on the inhibition function of BAP1 protein (Zhang *et al.,* 2018). Another important consideration is the impact of structural modifications on drug-likeness. The design strategy—particularly the removal of the unsaturated five-membered ring and relocation of hydroxyl and nitro groups—was intended to enhance aqueous solubility and potentially improve pharmacokinetic properties (Yan M *et al.,* 2020). Although binding affinity and ADME characteristics were not directly assessed in the present study, these modifications provide a rationale for improved drug-likeness, which will be systematically evaluated in future experimental work.

In the future, we plan to further expand the structural diversity of the compounds and study in detail the effects of different substituents on the inhibitory activity of BAP1 protein in order to find more potential BAP1 inhibitors. It should also be noted that the translation of BAP1 inhibitors from bench to bedside presents several important challenges (Masclef *et al.,* 2021). Key hurdles include limited aqueous solubility and drug delivery issues, potential off-target effects on other deubiquitinases and the emergence of resistance mechanisms with long-term use. Addressing these obstacles will require optimization of drug formulation, selectivity evaluation and integration with combination therapy strategies in future studies.

CONCLUSION

Based on the lead compound IBAP-II, five novel compounds were successfully designed and synthesized in this paper. In the face of the growing number of cancer patients worldwide and the urgent demand of patients for tumor treatment drugs or methods, we hope that the research on the BAP1 inhibitor can make a breakthrough as soon as possible to provide a solid theoretical basis and effective drug support for the treatment of cancer, especially small cell lung cancer. At the same time, we also hope to take this opportunity to improve the overall level of cancer treatment, to bring hope to those patients who are suffering from pain. There are still many things to be studied in the field of tumor therapy, such as in-depth understanding of the causes and regulation of gene mutations, which will greatly improve the level of medical treatment. Therefore, the further research and exploration of BAP1 inhibitor not only has important theoretical significance, but also provides new ideas and new methods for clinical tumor treatment. At present, no preclinical or clinical trial data are available; therefore, any therapeutic claims must be regarded as preliminary. Future work will

include enzymatic inhibition assays, cell-based activity tests, animal studies and pharmacokinetic evaluations to establish the efficacy and safety of these compounds before clinical translation. We look forward to further exploring the potential of BAP1 inhibitors in future studies to contribute more to the field of cancer treatment.

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Authors' contributions
X.Y and Z.Z: Conceptualization, methodology and validation; X.Y: Software, formal analysis, data curation and original draft preparation; Z.Z: Investigation, review and editing, visualization, supervision and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Data availability statement
The data presented in this study are available on request from the corresponding author. The data is not publicly available due to ethical, legal and privacy issues.

Ethical approval
Not applicable.

Conflicts of interest
The authors declare no conflict of interest. The founders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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