Research on Key Processes in the Synthesis of Highly Potent Small Molecule Inhibitors

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Abstract [**Objectives**] To explore the synthetic process of PD-L1 small molecule inhibitors, focusing on optimizing key reaction conditions and synthetic routes. [**Methods**] By analyzing the pharmacophore design of PD-L1 small molecule inhibitors and combining the optimization of synthetic methods and the improvement of reaction conditions, an efficient synthetic process was developed. [**Results**] Through optimization of reaction conditions, not only were the purity and yield of the products improved, but the inhibitory activity of the compounds was also significantly enhanced. Some compounds demonstrated strong anti-tumor effects in both *in vitro* and *in vivo* models. [**Conclusions**] This study aims to provide theoretical support and technical guidance for the efficient synthesis of small molecule inhibitors, offering new ideas and practical foundations for drug development in tumor immunotherapy.

Key words Small molecule inhibitor, PD-L1, Immune checkpoint, Synthetic process

1 Introduction

With the continuous development of tumor immunotherapy, immune checkpoint inhibitors have become an important component of anti-tumor therapy. Inhibition of the PD-1/PD-L1 pathway provides a new therapeutic strategy for tumor immunotherapy, and related antibody drugs have achieved significant clinical efficacy[1]. Small molecule inhibitors, due to their high affinity, good selectivity, and cost advantages in drug development, are gradually becoming a research hotspot. However, despite the advantages demonstrated by monoclonal antibodies in immunotherapy, the high cost and limitations in treatment response rates drive small molecule inhibitors to be a research focus. Small molecule inhibitors, owing to their cost advantages in drug development, convenience of oral administration, and broader targeting capabilities, are gradually emerging as a new solution for treating tumors. Through the precise design of inhibitors with high affinity and good selectivity, it is possible to effectively block the PD-1/PD-L1 pathway, enhance the anti-tumor effects of the immune system, thereby providing new therapeutic options for the clinic. This study aims to investigate the synthetic process of PD-L1 small molecule inhibitors, focusing on optimizing key reaction conditions and developing efficient synthetic routes. This will be achieved through systematic analysis of the pharmacophore design, synthetic methods, and key processes of small molecule inhibitors.

2 Pharmacophore and design principles of small molecule inhibitors

2.1 Overview of pharmacophore theory Pharmacophore theory is an important concept in molecular drug design. It analyzes the structural features within a molecule that influence pharmacological activity and proposes pharmacophore models to guide the optimization and design of drug molecules. A pharmacophore typically refers to the structural units within a molecule possessing specific chemical and spatial characteristics that can interact with the active site of a biological target to exert a biological effect. By constructing pharmacophore models, researchers can understand the impact of different chemical groups on drug activity and, based on this, perform rational structural modifications to optimize the activity and selectivity of drugs. Pharmacophore theory is widely applied in the design of small molecule drugs, particularly in areas such as anti-tumor, antibacterial, and antiviral fields[2]. Successful pharmacophore design relies on a deep understanding of drug-target interactions. Utilizing tools such as computational chemistry methods and molecular docking techniques enables the efficient screening of potential drug molecules and the systematic optimization of pharmacophores to improve their pharmaceutical properties.

2.2 Pharmacophore analysis of PD-L1 small molecule inhibitors The design of PD-L1 small molecule inhibitors relies on the application of pharmacophore theory, particularly for the targeted intervention of the PD-1/PD-L1 immune checkpoint pathway. The pharmacophore of PD-L1 small molecule inhibitors typically includes specific structural units capable of generating strong interactions with the binding site of the PD-L1 protein (Fig. 1). In the design of PD-L1 small molecule inhibitors, the selection of the pharmacophore directly determines the binding affinity and specificity of the molecule for PD-L1. Taking BMS-8 as an example, structural analysis indicates that the core part of its pharmacophore is an aromatic ring system that engages in $\pi - \pi$ stacking interactions with the immune checkpoint region of PD-L1, thereby enhancing inhibitory activity. Furthermore, other auxiliary pharmacophores, such as fluorine atoms or amino groups, further enhance the selectivity of the molecule for the target by forming interactions like hydrogen bonds and salt bridges. Different pharmacophores influence the binding strength and stability of the molecule with PD-L1 by altering its electronic properties, spatial configuration, hydrophobicity, *etc.*, thereby affecting the inhibitory effect[3].

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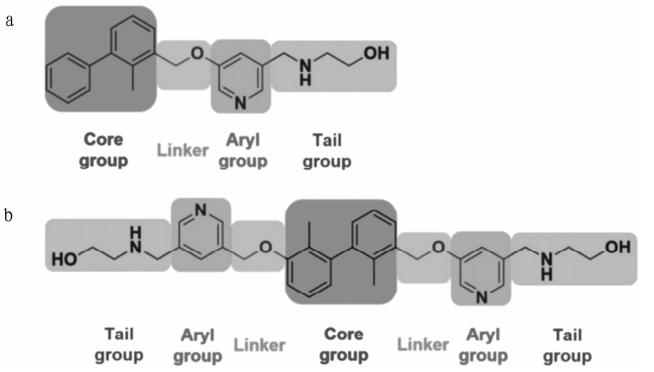

Fig. 1 Pharmacophore of PD-L1 small molecule inhibitors

2.3 Key factors in pharmacophore design Key factors in pharmacophore design include structural units such as molecular linkers, aryl groups, and tail groups, which play important roles in the biological activity and selectivity of drugs. Firstly, the molecular linker serves as a bridge connecting different pharmacophores. Its design not only affects the spatial configuration of the pharmacophore, but also influences the overall rigidity and flexibility of the molecule, thereby impacting the binding mode of the molecule to the target. Secondly, aryl groups, as important components of the pharmacophore, determine the affinity of the molecule for the target through their electronic properties and spatial arrangement. Modifications to the aromatic ring system can modulate the hydrophobicity of the drug and its interaction forces with the target. Furthermore, the role of the tail group in the pharmacophore should not be overlooked. The tail group typically binds to the target by forming hydrogen bonds or ionic bonds, optimizing the selectivity and stability of the drug. During the pharmacophore design process, the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of the drug must also be considered to ensure its feasibility for clinical application.

3 Synthetic methods and key processes for small molecule inhibitors

3.1 Synthetic routes The synthetic routes for small molecule PD-L1 inhibitors primarily aim to enhance the activity and selectivity of the drug through the optimized design of different linking groups and core structures. Researchers from Southern Medical University proposed in their patent a synthetic route based on 1,3-dihydroxybenzene derivatives, which involved the introduction of chlorine atoms and different tail chains, adding a tail group at the para position of the ether chain to optimize the affinity of the compound for PD-1/PD-L1 interaction (Fig. 2). For example, compound 1 exhibited a half maximal inhibitory concentration (IC_{50}) of 9.1 nM measured by homogeneous time-resolved fluorescence (HTRF) binding assay, and demonstrated good water solubility (17.61 mg/mL). Researchers from the Shanghai Institute of Materia Medica employed different types of solubilizing groups, such as sulfonic acid and polyols, to optimize the synthetic route. Compound 2 showed a strong inhibitory effect with an IC_{50} of 0.88 nM. By selecting appropriate tail groups and linker types, it is possible to improve the pharmacokinetic properties while ensuring drug efficacy, thereby enhancing its potential for clinical application.

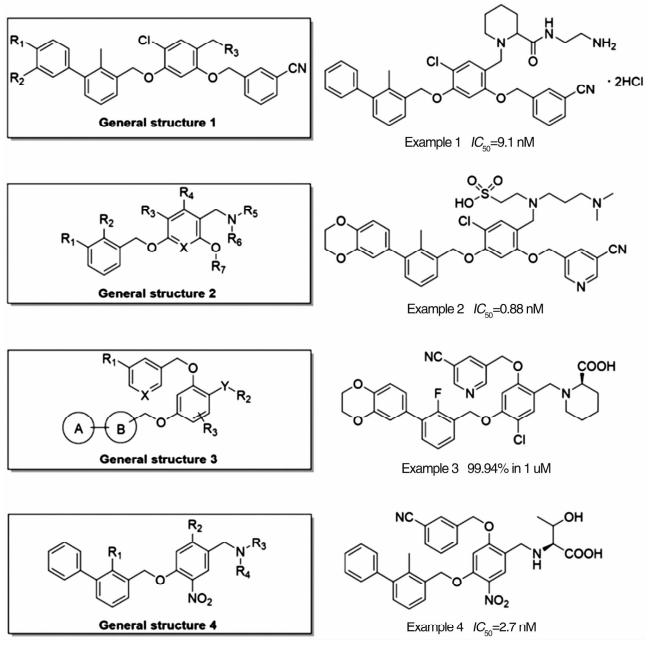

Fig. 2 Structures of patented compounds from Southern Medical University and China Pharmaceutical University

3.2 Optimization of key reaction conditions In the synthesis of small molecule PD-L1 inhibitors, the optimization of key reaction conditions is crucial for improving yield and selectivity. Taking compound 2 as an example, the Shanghai Institute of Materia Medica optimized the reaction conditions by adjusting reaction temperature, solvent, and reaction time, achieving an IC_{50} of 0.88 nM for this compound. Studies have shown that the choice of temperature and solvent significantly impacts the reaction rate and the purity of the product. Compound 2 significantly inhibited tumor growth in a melanoma model in efficacy experiments at 40 mg/kg. This result also underscores the importance of optimizing reaction conditions for enhancing the biological activity of the compound. Similarly, compound 4 showed good inhibitory efficacy with an IC_{50} of 2.7 nM in the HTRF binding assay and exhibited no cytotoxicity in cellular experiments. These data indicate that selecting appropriate reaction conditions not only improves the purity of the product but can also influence the biological activity and pharmacokinetic properties of the compound to some extent.

3.3 Exploration and application of efficient synthetic processes For the efficient synthesis of small molecule PD-L1 inhibitors, researchers have explored more efficient and greener synthetic pathways based on optimizing reaction conditions. For example, compounds utilizing cyclic ether linkers and alkene-based linkers demonstrated high inhibitory activity and low cytotoxicity. Researchers from Maxinovel Pharma proposed a new synthetic process for alkene-based linkers. Compounds 18 and 19 from this process exhibited IC_{50} values of 2.3 and 2.9 nM, respectively, in *in vitro* PD-1/PD-L1 binding assays, indicating their good efficacy in inhibiting PD-1/PD-L1 interaction (Fig. 3). Furthermore, compound MAX-10181 has entered Phase I clinical trials, demonstrating that the efficiency and efficacy of this synthetic process

have also been validated in clinical research. By adopting more environmentally friendly reaction routes and reducing reaction steps, not only is the synthetic efficiency improved, but also the production cost is reduced to a certain extent.

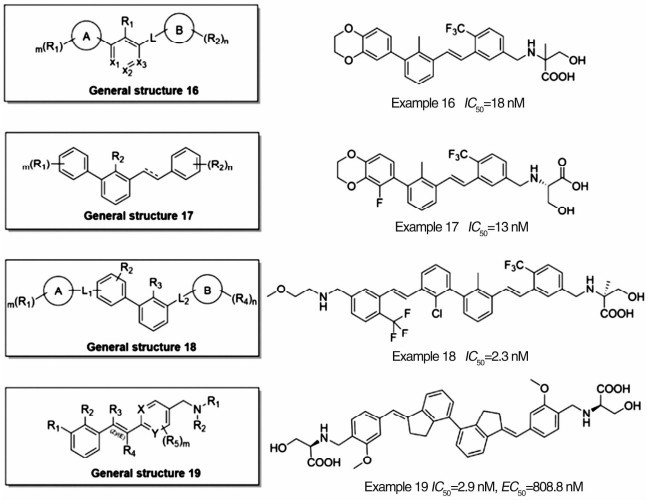

Fig. 3 Compounds patented by Maxinovel Pharma

4 Efficacy evaluation and preclinical studies of small molecule inhibitors

4.1 *In vitro* activity assessment The PD-1/PD-L1 binding inhibition assay is an important method for evaluating the inhibitory effect of immune checkpoint inhibitors *in vitro*. To accurately detect the inhibitory capacity of the drug on PD-1/PD-L1 binding, this study employed the HTRF method for fluorescence signal measurement. Experimental data showed a positive correlation between drug concentration and fluorescence signal change. As the drug concentration increased, the fluorescence signal significantly intensified, indicating that the drug inhibits PD-1/PD-L1 binding (Table 1). By calculating the IC_{50} value, we can further evaluate the inhibitory efficiency of the drug. The experimental results showed an IC_{50} value of 2.5 μM, meaning this drug possesses strong inhibitory capability. In cellular experiments, cells were treated with different concentrations of the drug, and cell survival rates were monitored to analyze its impact on immune cell function. The IC_{50} value analysis provides crucial reference data for further *in vivo* experiments and preclinical studies of the drug.

Table 1 Analysis of drug concentration, fluorescence signal change, and IC_{50} values

Drug concentration // μM	Fluorescence signal change // %	IC_{50} value // μM
0.01	12.5	–
0.1	35.6	–
1.0	58.2	–
10.0	83.9	–
100.0	95.2	2.5

4.2 Animal experiments and efficacy evaluation To further evaluate the *in vivo* efficacy of the small molecule inhibitor, this study utilized a mouse tumor model to test the inhibitory effect of different drug doses on tumor growth. The experimental results showed that as the drug dose increased, the tumor volume significantly decreased, indicating that the antitumor inhibitory effect of this small molecule inhibitor is dose-dependent. For the high-dose group (50 mg/kg), tumor volume decreased by approximately 70%, while for the low-dose group (10 mg/kg), it was only 20% (Table 2). In terms of safety, the experiment showed no significant adverse reactions in the medium-dose group (25 mg/kg), while the high-dose group exhibited mild weight loss and weakness symptoms, which were not fatal. These results demonstrate that this small molecule inhibitor has significant antitumor efficacy in the mouse tumor model and possesses good safety.

Table 2 Therapeutic efficacy and body weight changes in the mouse tumor model

Group	Drug dose mg/kg	Treatment duration // d	Tumor volume change // %	Body weight change // %
Control	0	14	+35	+2
Low-dose	10	14	−20	−1
Medium-dose	25	14	−50	−3
High-dose	50	14	−70	−5

4.3 Pharmacokinetic, pharmacodynamic, and toxicological studies In terms of pharmacokinetics, this study further analyzed the absorption, distribution, metabolism, and excretion processes of the small molecule inhibitor in mice. Experiments showed that the drug reached peak plasma concentration 30 min after administration and began to rapidly distribute to organs such as the liver and kidneys, indicating favorable *in vivo* distribution characteristics of the drug. Specific data indicated that the drug concentration in plasma decreased to about 60% of the initial value within 4 h, while concentrations in the liver and kidneys were maintained for a longer duration (Table 3).

Table 3 Pharmacokinetic data of the small molecule inhibitor

Time point // h	Plasma concentration // ng/mL	Liver concentration // ng/g	Kidney concentration // ng/g
0.5	500	120	150
1.0	450	100	140
2.0	350	80	120
4.0	200	50	80
8.0	120	30	60
12.0	80	15	40

In terms of pharmacodynamics, the antitumor activity of the drug lasted for a relatively long duration, and no significant drug resistance phenomenon was observed. Toxicological studies showed that the drug did not exhibit significant toxic reactions at low and medium doses, but weight loss and some mild discomfort reactions occurred in the high-dose group (Table 4). Based on this, it is recommended to further optimize the dosing regimen in preclinical studies to ensure the maximum safety and efficacy of the drug.

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Table 4 Toxicological study of the small molecule inhibitor

Dose//mg/kg	Toxicity manifestation	Main observations	Assessment
0	None	No abnormalities	Non-toxic
10	Mild	Decreased appetite, mild weight loss	Safe
25	Moderate	Significantly decreased appetite, marked weight loss	Safe
50	Severe	Extreme weakness, rapid weight loss, shortness of breath	Not recommended

5 Conclusion

This study conducted an in-depth exploration of the synthetic process for PD-L1 small molecule inhibitors, focusing on optimizing reaction conditions and synthetic routes, and exploring efficient and green synthetic pathways, thereby providing a potential new solution for tumor immunotherapy. Through the optimization of pharmacophore design, the affinity and selectivity of the molecules for PD-L1 were successfully improved, significantly enhancing inhibitory activity. Preclinical studies demonstrated that the synthesized inhibitors exhibit good antitumor efficacy and safety both *in vitro* and in animal models, supporting their potential for application in future clinical trials. Looking ahead, with the continuous advancement of synthetic technologies and the application of precision design methods, PD-L1 small molecule inhibitors are expected to become an important weapon in the field of tumor immunotherapy.

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