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Published in:
Expert Opinion on Therapeutic Patents

DOI:
10.1080/13543776.2022.2045276

Publication date:
2022

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Expert Opinion on Therapeutic Patents

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ietp20

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To cite this article: Jingjing Deng, Zhengqi Cheng, Juyang Long, Alexander Dömling, Micky Tortorella & Yuanze Wang (2022) Small Molecule Inhibitors of Programmed Cell Death Ligand 1 (PD-L1): A Patent Review (2019–2021), Expert Opinion on Therapeutic Patents, 32:5, 575-589, DOI: 10.1080/13543776.2022.2045276

To link to this article: https://doi.org/10.1080/13543776.2022.2045276

Published online: 10 Mar 2022.

EXPERT OPINION ON THERAPEUTIC PATENTS
2022, VOL. 32, NO. 5, 575–589
https://doi.org/10.1080/13543776.2022.2045276

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REVIEW

Small Molecule Inhibitors of Programmed Cell Death Ligand 1 (PD-L1): A Patent Review (2019–2021)

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ABSTRACT

Introduction: The blockade of immune checkpoints, especially the PD-1/PD-L1 pathway with therapeutic antibodies, has shown success in treating cancers in recent years. Seven monoclonal antibodies (mAbs) targeting PD-1 or PD-L1 have been approved by FDA. However, mAbs exhibit several disadvantages as compared to small molecules such as poor permeation, high manufacturing costs, immunogenicity as well as lacking oral bioavailability. Recently, small-molecule inhibitors targeting PD-L1 have been disclosed with the ability to modulate the PD-1/PD-L1 pathway.

Areas covered: The authors reviewed small molecules targeting PD-L1 that block the PD-1/PD-L1 protein–protein interaction for the treatment of various diseases.

Expert opinion: Compared with mAbs, PD-1/PD-L1 small-molecule inhibitors show several advantages such as improved tissue penetration, low immunogenicity, well-understood formulation and lower manufacturing costs. They can serve as complementary or synergistically with mAbs for immune therapy. However, at this time most of the reported inhibitors are still inferior to therapeutic antibodies in their inhibitory activities due to smaller molecular weight. Therefore, better small molecules need to be developed to improve their potencies. Moreover, although several PD-L1 small-molecule inhibitors have shown excellent preclinical results, their safety and efficacy in the clinic still awaits further validation.

ARTICLE HISTORY
Received 23 November 2021
Accepted 18 February 2022

KEYWORDS
Immune therapy; immune checkpoints; PD-1; PD-L1; small molecule inhibitors; cancers

1. Introduction

The T-cell-based immune system plays an important role in recognizing and destroying aberrant cells through binding of their T-cell receptor(s) (TCR) to peptide-major histocompatibility complexes (MHC) on target cells, thereby controlling and eradicating diseases such as cancer. This process largely is regulated by a series of co-stimulatory and co-inhibitory receptors expressed on immune cells and their ligands (also known as immune checkpoint molecules). By taking advantage of this mechanism, aberrant cells such as tumor cells and pathogen-infected cells often develop strategies to interfere with these immune checkpoint molecules suppressing the immune system, thus escaping surveillance and clearance. Therefore, development of agents blocking immune checkpoints have recently emerged as a promising approach for cancer immunotherapy. Among all immune checkpoints, the programmed cell death-1 (PD-1) and its ligand PD-L1 as therapeutic targets have stood out due to their remarkable clinical efficacy in a large number of malignancies with durable responses and low toxicity. To date, seven anti-PD-1/PD-L1 monoclonal antibodies (mAbs) have been approved by the FDA for the treatment of various cancers [1–7].

PD-1, also known as CD279, is a cell surface receptor and often expressed by activated B cells, T cells, regulatory T cells (Tregs), dendritic cells (DCs), natural killer cells (NKs), and macrophages that reside in the tumor microenvironment. It functions as an intrinsic negative regulator of immune responses and plays an important role in the suppression of antigen-specific T cell response in diseases like viral infection and cancer [8–10]. PD-L1, also named B7 homolog1 (B7-H1) or CD274, has been identified as the major ligand of the co-inhibitory receptor PD-1. Under physiologic conditions, PD-L1 is constitutively expressed in different tissues of activated immune cells, including bone marrow (BM)-derived mast cells, dendritic cells (DCs), mesenchymal stem cells (MSCs), monocytes, T and B lymphocytes, and various immune privileged organs. However, it is also commonly upregulated in tumor cells and its expression is further increased after induction of IFN-γ. The overexpression of PD-L1 allows the tumor cells to protect themselves in multiple ways. It can inactivate the tumor infiltrating lymphocytes (TIL) through binding to its PD-1 receptor resulting in an increase of apoptosis in tumor-specific T cells. Moreover, it can also promote the expression of IL-10 by T cells to reduce the overall immune response. Since PD-L1 is seldom expressed in normal human tissues, it can be used as a selective target to treat tumors [11].

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Article highlights

- Blocking immune checkpoints is a highly promising therapeutic method to fight various diseases, especially cancers.
- Despite PD-1/PD-L1 antibodies have gained great medical and commercial success in treating cancers, they also have various disadvantages.
- The pharmacophores of PD-L1 small-molecule inhibitors for cancer immunotherapy are summarized.
- The PD-L1 inhibitors disclosed in patents of recent 3 years are classified by different linkers they used.

This box summarizes key points contained in the article.

The progress in the development of small molecules that interfere with the PD-1/PD-L1 pathway is lagging behind that of antibodies. It is well known that development of small-molecule inhibitors that block the PD-1/PD-L1 protein–protein interaction has been a difficult task as its interface is large and flat and lacking a deep pocket for binding. However, this problem was recently solved by researchers at BMS who published a patent on PD-L1 small-molecule inhibitors along with the first co-crystal structure (BMS-8)/PD-L1 [12,13]. Since then, dozens of PD-L1-based patents have been published. Encouragingly, five PD-L1 small-molecule inhibitors have entered clinical trials like drug INCB086550 from Incyte Corporation that has just reached phase II trials in September of 2021 (Table 1). A patent review summarizing PD-L1 inhibitors discovered and disclosed from 2015 to 2018 was published [14] and in this review we summarize new small-molecule PD-L1 inhibitors developed and revealed from 2019 to 2021.

2. Patents on PD-L1 small molecule inhibitors

2.1. Binding mode of the PD-L1 inhibitors and pharmacophores

Based on the pharmacophore model deduced from our first cocrystal structure of a small molecule in PD-L1, thousands of PD-L1 inhibitors have been published in patents and papers over the last few years with 10 compounds being co-crystallized with the PD-L1 protein and deposited in the RCSB protein date bank (5J8O, 5J89, 5NIU, 5N2F, 5N2D, 6RPG, 6R3K, 7NLD, 7BEA, 6VQN) [15–20]. These co-crystals validated that PD-L1 inhibitors exert their effect on blocking the PD-1/PD-L1 interface by stabilizing the PD-L1 dimer. The crystal structure of BMS-202/PD-L1 complex illustrated in Figure 1 showed that BMS-202 binds into a cylindrical, hydrophobic pocket created at the interface of the PD-L1 dimer. The distal biphenyl core of BMS-202 is stabilized by π-alkyl interactions with nearby amino acids and forms a π-stacking interaction with the side chain of ATyr56. The major interaction of the methoxy-pyridine moiety is π-π stacking with $_B$Tyr56. The extended *N*-(2-aminoethyl)acetamide moiety extends toward the solvent area and creates a number of electrostatic interactions.

By analyzing the data collectively, we have summarized the SAR of disclosed compounds by classifying them into two pharmacophores. The first pharmacophore consists of a core group, linker, aryl moiety and tail group (Figure 2A). The core is generally a biphenyl moiety that is located in a hydrophobic pocket composed by the amino acids Tyr56, Met115, and Ala121. The aryl group is normally a five- or six-membered aromatic ring or fused ring that is connected with the core group by a linker. The terminus tail is oriented toward the solvent area and could form interactions with nearby residues via hydrogen binding. The second pharmacophore has the same composition as the first, but it has an extra linker, aryl and tail group on the other side of the core moiety (Figure 2B). Compounds with this pharmacophore normally have higher binding affinity with the PD-L1 protein because their

Table 1. Ongoing clinical trials of PD-L1 small-molecule inhibitors.

Drug	Company	Phase	Status	NCT Number
INCB086550	Incyte Corporation	Phase 2	Recruiting	NCT04629339
GS-4224	Gilead Sciences	Phase 1b/2	Terminated	NCT04049617
MAX-10181	Maxinovel Pharma	Phase 1	Recruiting	NCT04122339
IMMH-010	Tianjin Chasesun Pharma	Phase 1	Not yet recruiting	NCT04343859
AN4005	Adlai Nortye Biopharma	Phase 1	Recruiting	NCT04999384

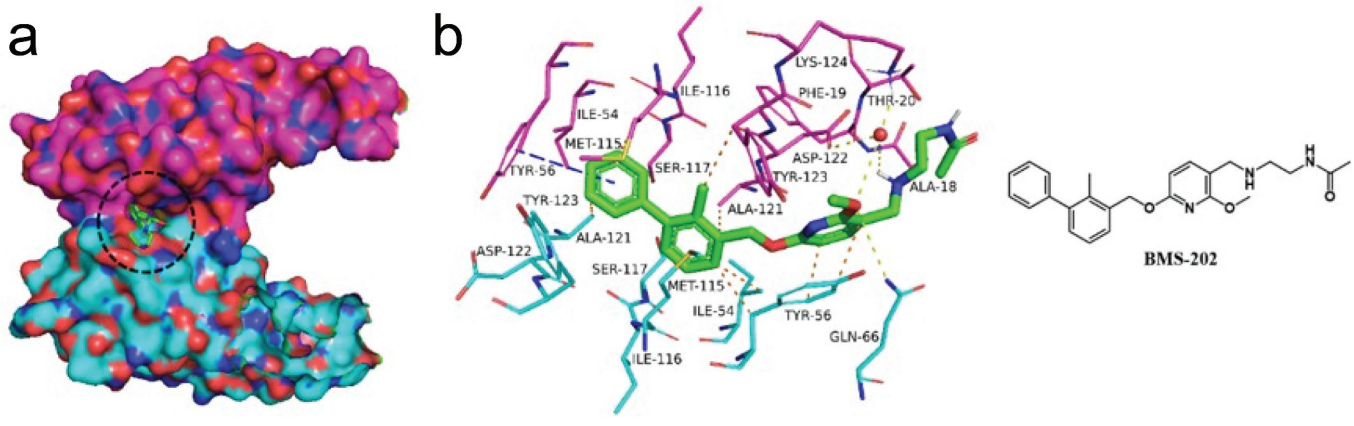


Figure 1. Binding mode of PD-L1 small molecule inhibitors (PDB: 5J89).

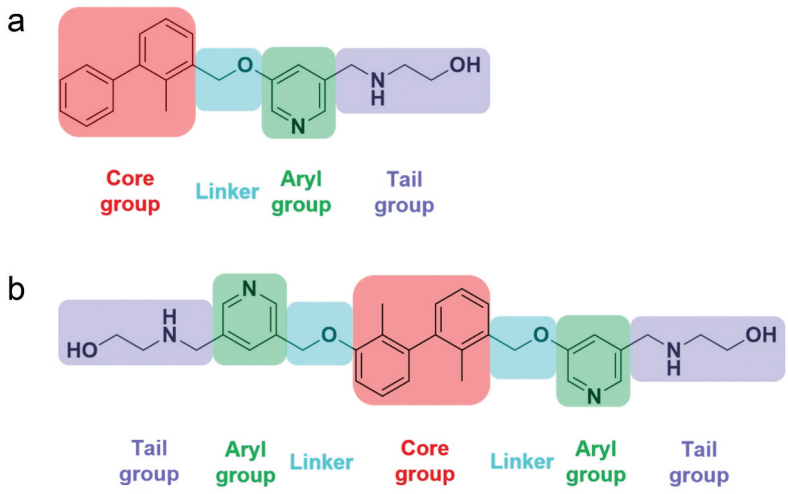


Figure 2. Pharmacophores of PD-L1 small molecule inhibitors.

molecular weight is much larger. The molecules can be symmetrical, pseudo-symmetrical or unsymmetrical depending on the linkers, aryl and tail groups used. In this review, we will discuss new PD-L1 inhibitors disclosed in patents in recent years using different linker types.

2.2. PD-L1 inhibitors with ether linker

2.2.1. Linear ether linker

Researchers from Southern Medical University published two patents describing 1,3-dihydroxy phenyl derivatives as PD-1/PD-L1 inhibitors (Figure 3) [21,22]. The compounds are derivatives based on the BMS compounds (US 2015/0291549 Al) and belong to the first pharmacophore. As shown in structure **1**, a chloro group was introduced in the aryl moiety as well as a different tail group chain in the para-position of the ether chain. One of the compounds, **1** later published in a manuscript showed in vitro inhibition of the PD-1/PD-L1 interaction with an IC_{50} value of 9.1 nM as measured in a homogeneous time-resolved fluorescence binding assay (HTRF) [23]. In addition, **1** was able to promote HepG2 cell death in a HepG2/PD-L1 co-culture cell model in a concentration-dependent manner. Compound **1** showed good water solubility (17.61 mg/mL), moderate pharmacokinetics ($t_{1/2}$ = 20 h, F = 12%) and minimal cytotoxicity in cancer cells (IC50 > 5 μM). Moreover, **1** showed efficacy in humanized PD-1 C57BL/6 J-Pdcd$^{em1(hPDCD1)/Smoc}$ mice implanted with B16-F10 melanoma.

Later, chemists from Shanghai Institute of Materia Medica (Chinese Academy of Sciences) described 156 1,3-dihydroxy phenyl derivatives. (Figure 3) [24] In this series of compounds different types of soluble groups were used, for example, sulfonic acids and polyalcohols were introduced for the tail group. One of the most potent molecules, **2** exhibited an IC_{50} of 0.88 nM in the in vitro PD-1/PD-L1 binding assay. Meanwhile, in an efficacy experiment at a concentration of 40 mg/kg, **2** was able to significantly inhibit tumor growth in a melanoma model. In another patent, the Shanghai Institute of Organic Chemistry (Chinese Academy of Sciences) disclosed 106 compounds with similar structures [25]. In an HTRF binding assay, at a dose of 1 μM, compound **3** showed 99.94% inhibition of PD-1/PD-L1 interaction. China Pharmaceutical University has filed a patent with 49 molecules that use nitrobenzene as the aryl moiety [26]. **4** displayed an IC_{50} of 2.7 nM in blocking the binding of the PD-1/PD-L1 interaction in vitro without any cytotoxicity against Lewis lung carcinoma (LLC) cells. In addition, **4** was able to dose-dependently elevate interferon-γ production and counteract PD-1/PD-L1 induced immunosuppression.

Chemists from Sunshine Lake Pharma disclosed multiple small molecules that inhibit the PD-1/PD-L1 interaction, which also belong to the first pharmacophore, but an extra-aliphatic ether chain with different terminal spiro or fused rings were introduced to the core group. (Figure 4) [27] The in vitro inhibition of the PD-1/PD-L1 interaction as measured in the Cisbio assay revealed that the most potent compound was **5** with IC_{50} of 1.1 nM. China Pharmaceutical University has filed another patent which also adopted an aliphatic ether chain connected with a biphenyl core group with different primary or second amines used as the terminal group in most cases [28]. In the aryl group, the benzooxadiazole or benzothiadiazole moiety is substituted in the meta position of the ether linker. Molecule **6** was reported to inhibit PD-1/PD-L1 binding in vitro and showed efficacy in a cell-based assay with an IC_{50} of 2.33 nM and EC_{50} of 0.21 μM respectively. In addition, the pharmacokinetics, chemical stability, microsome stability, acute toxicity, hERG inhibition and in vivo efficacy in a B16-F10 melanoma tumor model was also reported using several different compounds. Shenzhen Chipscreen Biopharma has also described a patent with 26 biaryl derivatives that can bind to the PD-L1 protein [29]. As shown in the general structure **7**, an aliphatic chain is introduced to the terminal benzene ring by an alkene instead of an ether linker. Compound **7** showed an IC_{50} of 1 nM in the HTRF binding assay.

China Pharmaceutical University has filed another patent describing 46 PD-L1 small-molecule inhibitors [30]. As shown by the general structure of **8** (Figure 5), a benzo-[c]oxadiazole core is used as the aryl component. Among

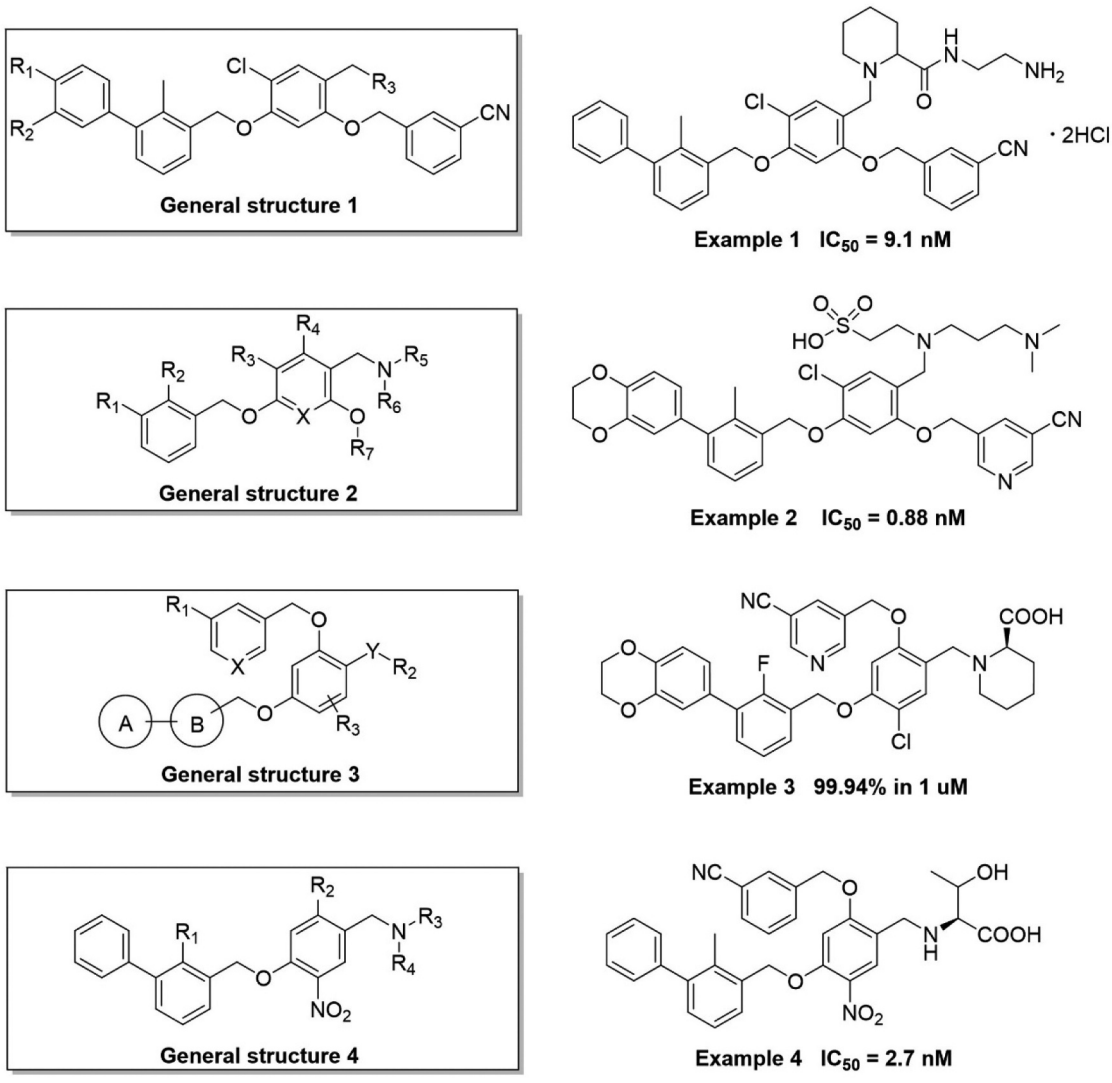


Figure 3. The general structures and examples of compounds patented by Southern Medical University and China Pharmaceutical University.

the reported examples, **8** is the most potent molecule with IC_{50} of 1.8 nM as measured in the HTRF binding assay. Chemists from the Jubilant Biosys Limited have also published two patents of 1,3-dihydroxy phenyl derivatives with general structures exemplified by molecules **9** and **10** [31,32]. Both of them belong to the first pharmacophore and have a similar core group. However, structure **9** uses pyrimidine as its aryl group while a dihydroindene or 1,2,3,4-tetrahydronaphthalene fused ring is introduced in the structure of **10**. Similarly, both of their tail groups lie in the para position of the ether linker. The example compounds from these two patents **9** and **10** were evaluated as PD-L1 inhibitors in the HTRF binding assay and showed IC_{50} values of less than 100 nM.

2.2.2. Cyclized ether linker

ChemoCentryx Inc. filed three patents describing four series of compounds with general structures highlighted by molecules **11, 12, 13** and **14**. (Figure 6) [33–36] All the compounds in these patents used a cyclized ether chain connected between the core and the aryl group. In addition, they share similar aryl and tail groups. For compounds with the general structure of **11**, 329 compounds were synthesized and 141 of them had IC_{50} values of less than 100 nM as measured in HTRF assay. **12** has an extra linear ether chain connected with the core group and shows an IC_{50} of less than 5 nM. All the reported molecules with the general structure of **13** are symmetrical and their ability to block the PD-1/PD-L1 interaction was measured by an enzyme-linked immunosorbent assay (Elisa). The structure of **14** has an uncommon substituted phenol group attached to its core group and molecule **14** showed an IC_{50} of less than 100 nM. Researchers from Bristol-Myers Squibb (BMS) also reported small-molecule PD-L1 inhibitors with a cyclized ether chain as shown by the general structure of **15** [37]. As a representative example **15** could inhibit the PD-1/PD-L1 interaction in vitro with an IC_{50} value of 1 nM.

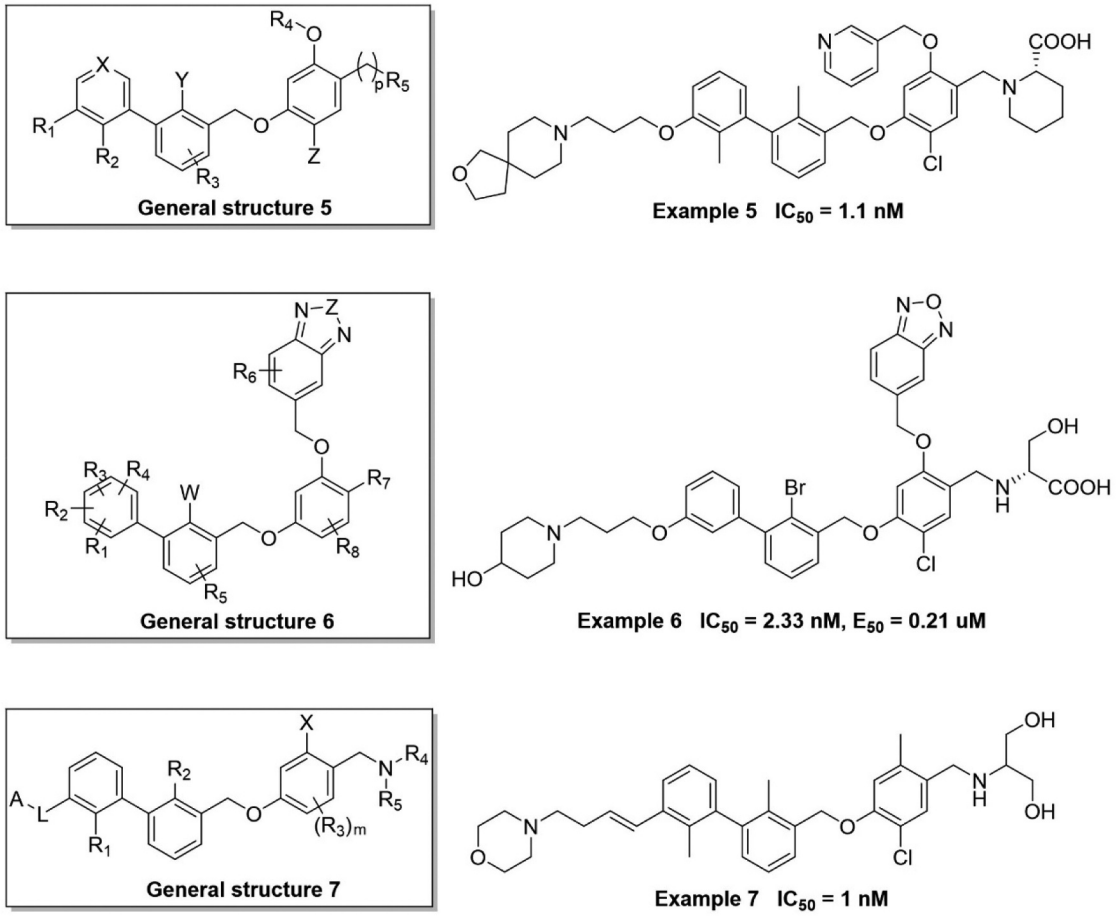


Figure 4. The general structures and examples of compounds patented by Sunshine Lake Pharma, China Pharmaceutical University and Shenzhen Chipscreen Biopharma.

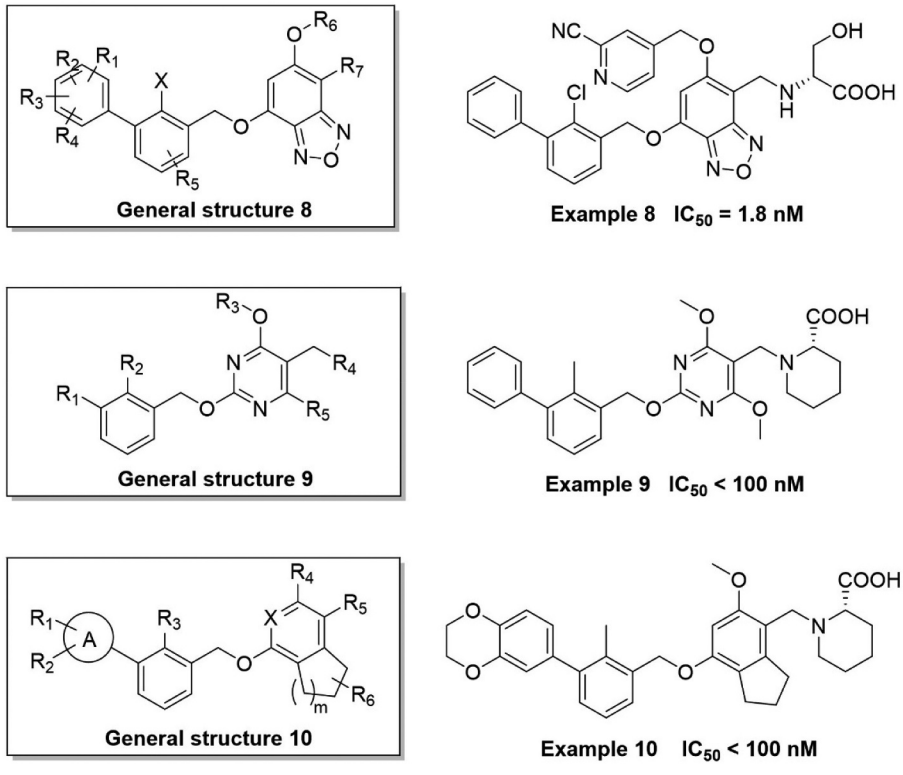


Figure 5. The general structures and examples of compounds patented by China Pharmaceutical University and Jubilant Biosys Limited.

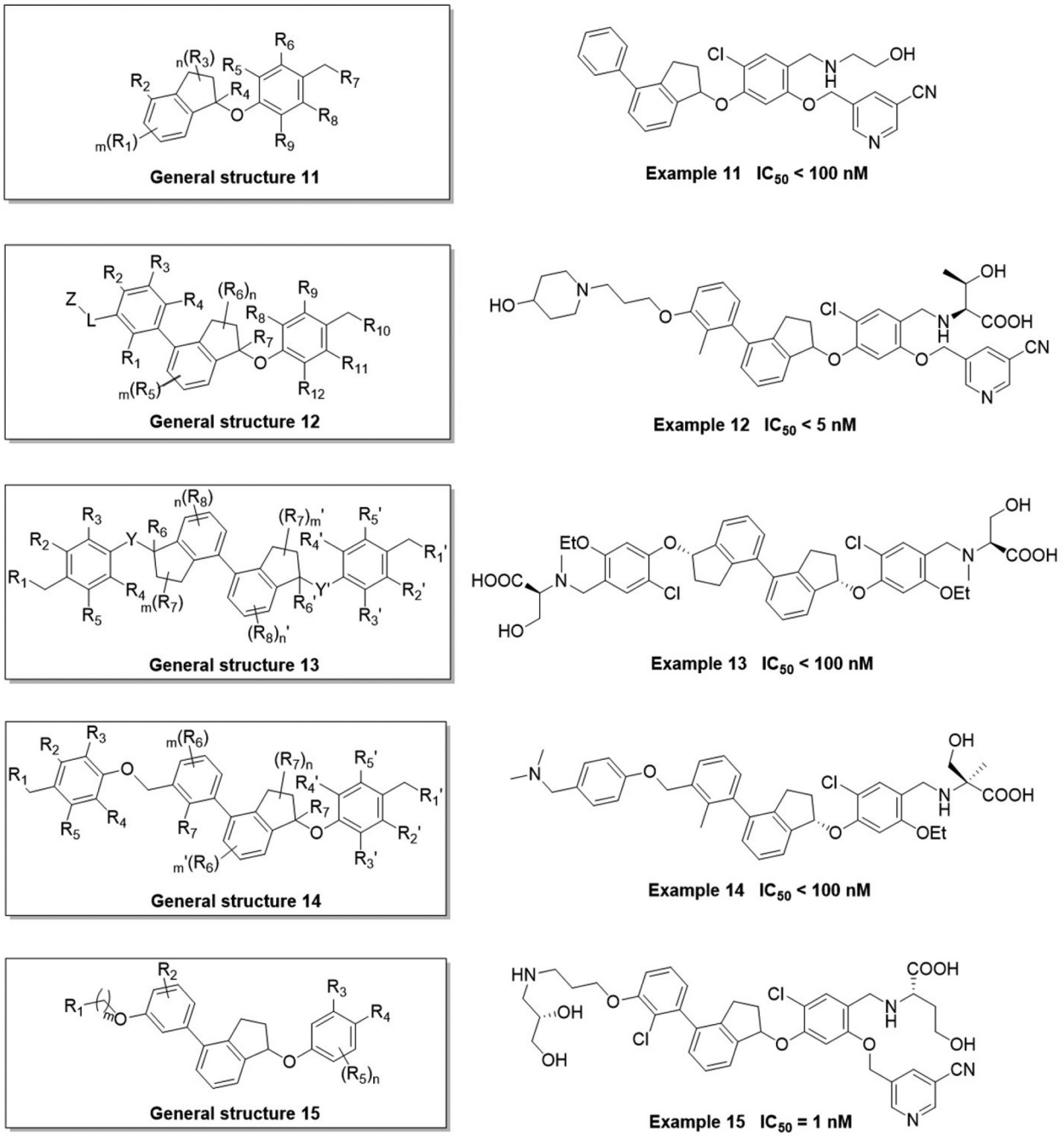


Figure 6. The general structures and examples of compounds patented by ChemoCentryx Inc. and BMS.

2.3. PD-L1 inhibitors with alkene linker

To obtain good affinity toward the PD-L1 dimer it is well known that the biphenol core is important, but the linker also play an important role by connecting and allowing the aryl and tail groups sitting in the proper position where they could form stronger interactions with nearby amino acid residues. Therefore, some other linkers have been explored by different companies. Maxinovel Pharma has filed several patents that adopt the alkene as the linker instead of ether. (Figure 7) [38–41] From the compounds they disclosed, **18** and **19** showed an IC_{50} of 2.3 and 2.9 nM in the in vitro PD-1/PD-L1 binding assay, respectively. In addition, compound **19** also inhibited cell-based PD-1/PD-L1 binding with an EC_{50} of 808.8 nM. More importantly, one of the compounds (MAX-10181) has entered phase I clinical trials although the exact structure has not yet been disclosed. (NCT04122339)

2.4. PD-L1 inhibitors with amide linker

In addition to the ether and alkene linker, the amide has also been proven to be another privileged alternative linker that can be used considering its drug-like properties. The amide linker was first disclosed by Incyte and later on adopted by

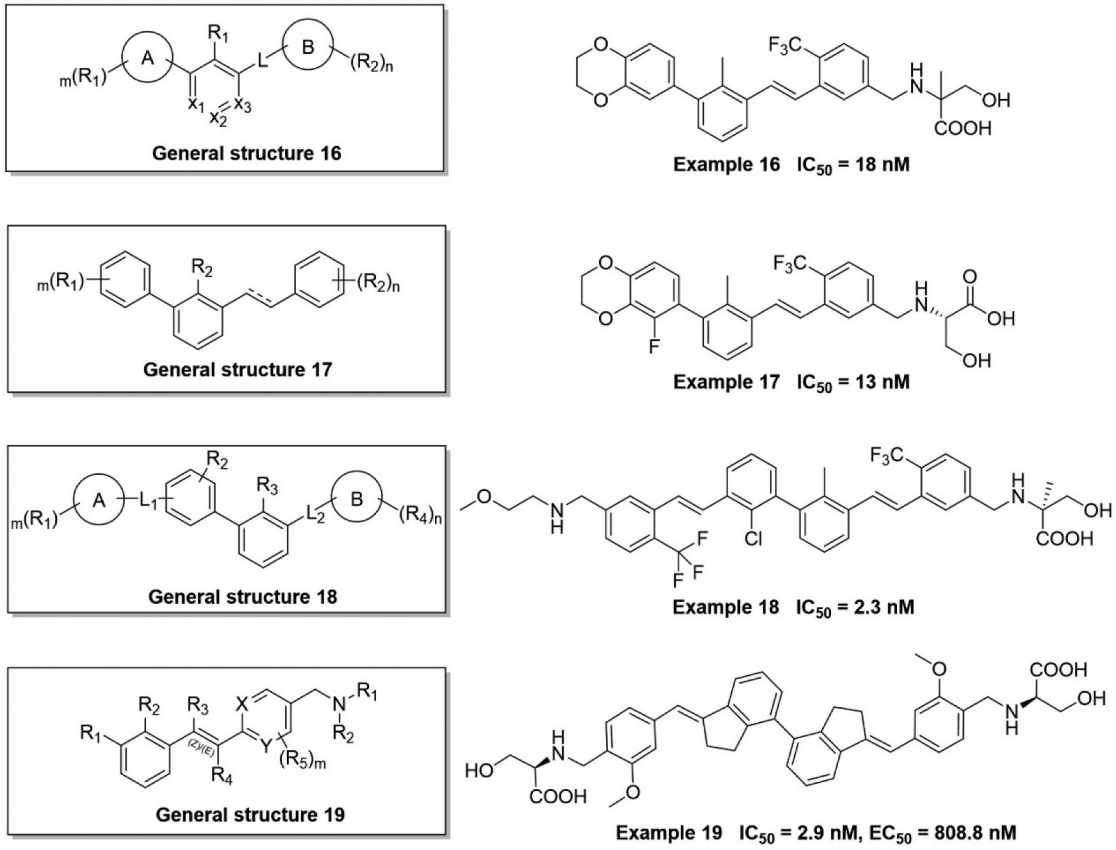


Figure 7. The general structures and examples of compounds patented by the Maxinovel Pharma.

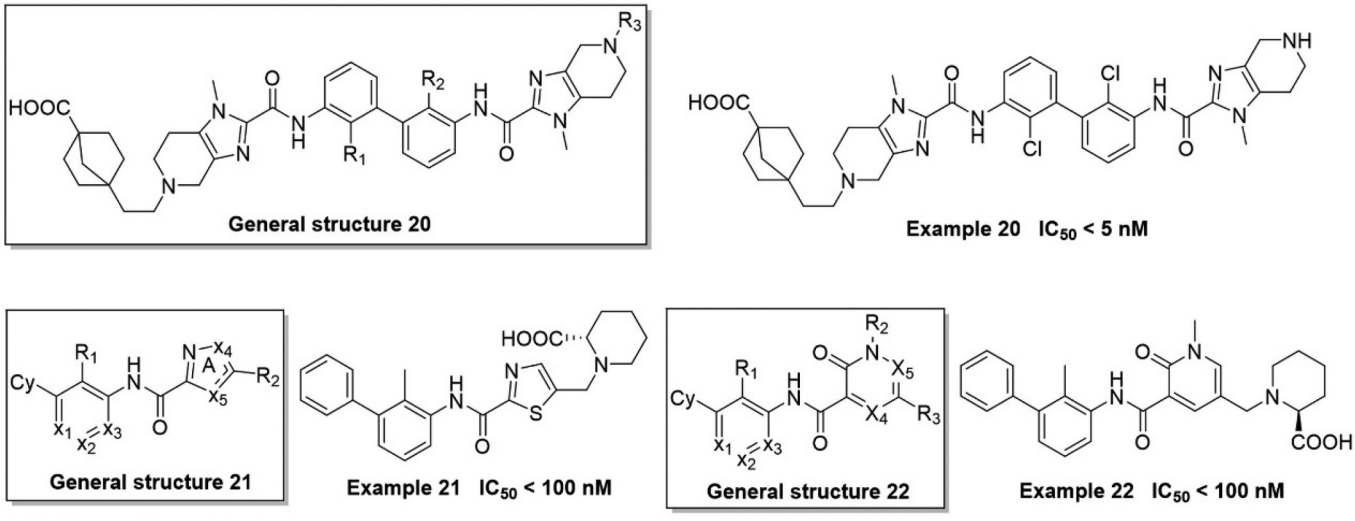


Figure 8. The general structures and examples of compounds patented by Incyte.

several other companies. Continuing their efforts in developing PD-L1 inhibitors, Incyte published 3 patents with structures that highlight the amide linker starting from 2019. (Figure 8) [42–44] The general structure highlighted by **20**, 1-methyl-4,5,6,7-tetrahydro-1 *H*-imidazo[4,5-c]pyridine is used as the aryl group and all the compounds disclosed in this patent belong to the second pharmacophore. The biphenol core, amide linker and the aryl group of these compounds is symmetric but a different tail group was introduced. A bridged cyclic ring with a terminal carboxylic acid is connected with the biphenol group by an ethyl linker on the left side, while various substitutions are used on the other side. For patent US2020/0172533, five-membered heterocycles are used as the aryl group. A thiazole group is used in example **21** and the tail

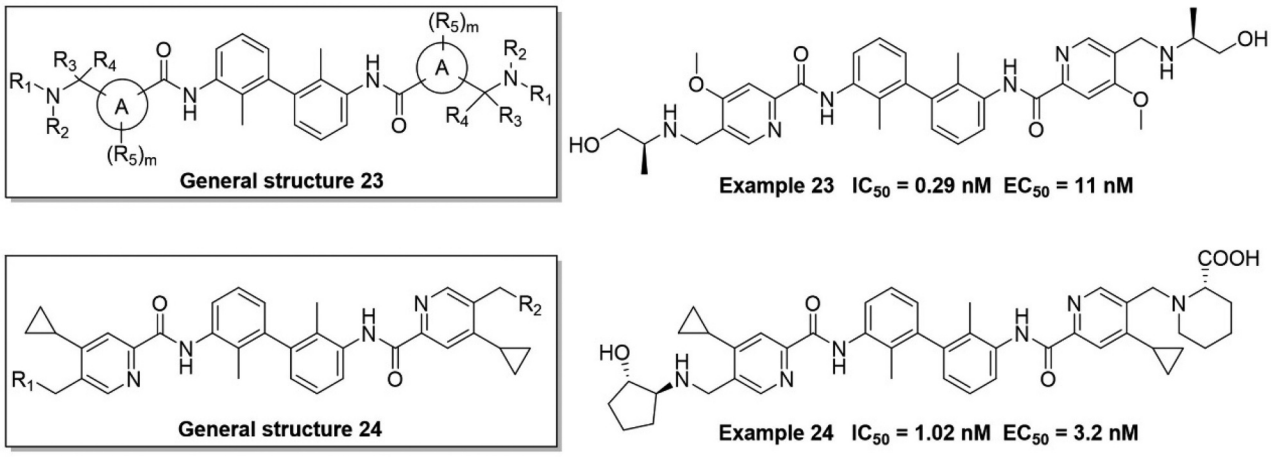


Figure 9. The general structures and examples of compounds patented by Abbisko Therapeutics.

is substituted with a L-pipecolinic acid. Similarly, six-membered heterocycles are used as the aryl group in patent US2020/0283423. A 1-methylpyridin-2(1 H)-one group is used in example **22** and the same L-pipecolinic acid is used as the tail. Both **21** and **22** showed an IC_{50} less than 100 nM in in vitro PD-1/PD-L1 binding assay.

Scientists from Abbisko Therapeutics disclosed a series of symmetrical compounds as new PD-1/PD-L1 antagonists for the treatment of cancer, infectious diseases and immune related diseases. (Figure 9) [45] The PD-1/PD-L1 HTRF binding assay and Jurkat reporter gene cellular assay were used to investigate the in vitro activity of these compounds. In addition, the pharmacokinetic behavior of several selected examples was tested in ICR mice. The results showed that one of their best compounds **23** displayed an IC_{50} of 0.29 nM in HTRF assay and EC_{50} of 11 nM in the cell assay. However, it showed poor pharmacokinetic characteristics with a half-life of 2.4 h and an exposure AUC_{last} of less than 500 hr*ng/mL. In another patent (WO2021/008491 A1), pyridine is used as the aryl moiety and a cyclopropyl group is substituted to the meta position of the amide linker as illustrated by compound **24** and showed an IC_{50} of 1.02 nM in HTRF binding assay with a comparable EC_{50} (3.2 nM) to **23** in the cell-based assay.

Researchers from Shenyang Pharmaceutical University disclosed a patent with 39 compounds that use a [1,2,4]triazolo [4,3-a]pyridine scaffold as the aryl group. (Figure 10) [46] One of their best compounds, **25** (later on published in a separate paper) showed an IC_{50} of 92.3 nM in the HTRF binding assay [47]. The docking study showed that both the triazole and pyridine moiety of **25** formed a face-to-face π-π interaction with Asp122. This demonstrated that a fused ring for the aryl group could be a valuable strategy for rational design of new PD-L1 small-molecule antagonists. In addition, **25** significantly promoted the T-cell response. In a co-culture model of Hep3B/OS-8/hPD-L1 and CD3 T cells, **25** induced elevations of interferon-γ production in a dose-dependent manner. In the same year, this group published another series of compounds with a cyclized amide linker as illustrated by the structure of

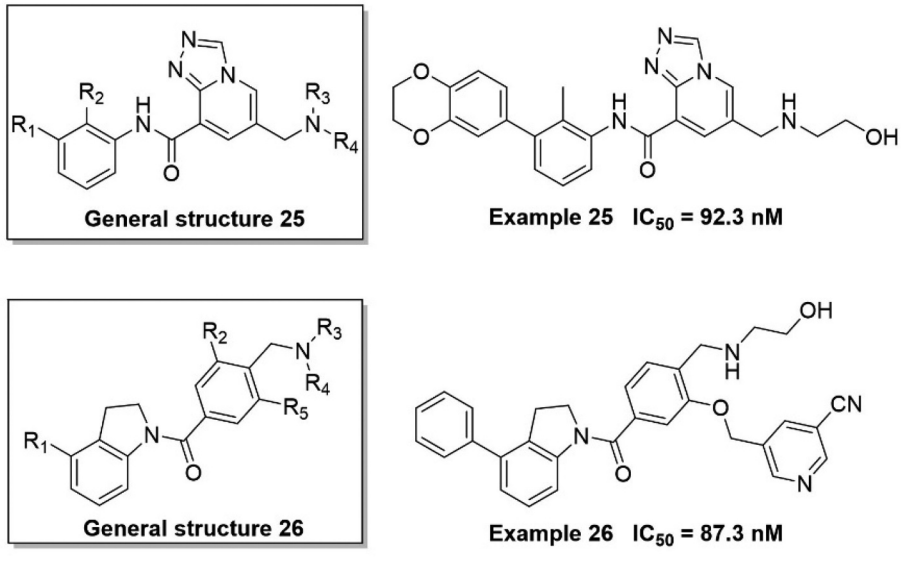


Figure 10. The general structures and examples of compounds patented by Shenyang Pharmaceutical University.

compound **28** [48]. Sixty-three analogs are included in this patent, but most of them showed only moderate PD-1/PD-L1 inhibition activity in vitro.

Chemists from Betta Pharma has disclosed two patents with an amide as the linker. (Figure 11) [49] Their compounds belong to the second pharmacophore and most of them have symmetrical or semi-symmetrical structures. The inventors investigated the ability of their compounds to inhibit PD-1/PD-L1 interaction using the HTRF binding assay. In general structure **27**, Q and Q′ are each independently selected from absent, C(O) or $C(R_2)_2$; R3 and R3′ are each independently selected from H, or $C_{1\text{-}8}$alkyl; R_2 and R_2′ are each independently selected from H, halogen, CN, or $C_{1\text{-}8}$alkyl; R_2′ and R3′ together with the atoms to which they are attached form a five- to six- membered heterocyclic ring; R1 and R5 are each independently selected from heterocyclic ring or heteroaryl ring optionally comprising 1, 2 or 3 hetero atoms independently selected from N, S, or O, wherein the heterocyclic ring or heteroaryl ring is monocyclic or bicyclic, optionally substituted with $C_{1\text{-}8}$alkyl, -(CH_2)p-COOH, -(CH2)p-CONR, -(CH2)pOH, -(CH2)p–heterocyclyl, -C3-7cycloalkyl, -C3-7heterocyclyl, or -(CH2)p-NR; s and p are each independently selected from O, 1, 2 or 3. The symmetrical compound **27** showed an IC_{50} of 0.37 nM in blocking PD-1/PD-L1 binding in vitro. For the general structure of 28, the linker is limited to a cyclized amide, m is 0, 1, 2 or 3 [50]. Ring A is a five- and six-membered heterocyclic ring; wherein the heterocyclic ring is optionally comprising 1, 2 or 3 hetero atoms independently selected from N, S or O. 58 examples of compounds are included in this patent and most of them showed IC_{50} values of less than 25 nM in the HTRF binding assay.

2.5. PD-L1 inhibitors without linker

Researchers from Gilead disclosed two patents describing molecules where the core group is directly connected to

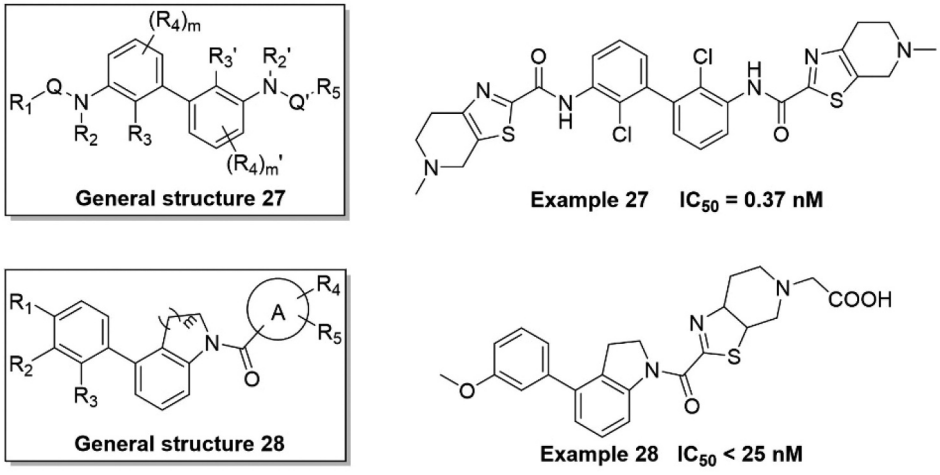


Figure 11. The general structures and examples of compounds patented by Betta Pharma.

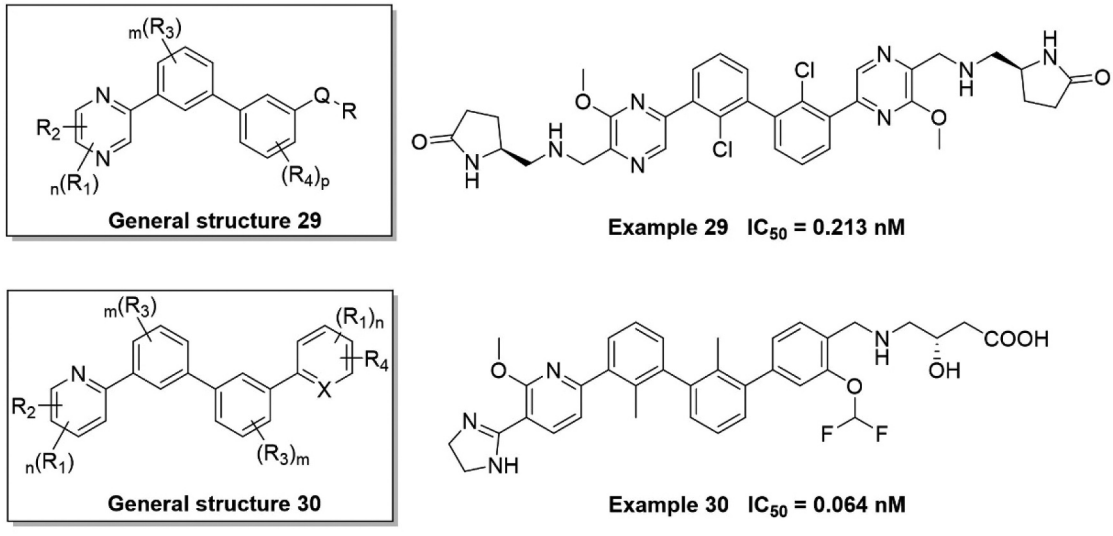


Figure 12. The general structures and examples of compounds patented by Gilead.

the aryl group without any linker. (Figure 12) [51–53] As demonstrated by the general structure of **29**, the hydrophobic core is a biphenyl group with various substituted groups such as alkyls, halogens and haloalkyl moieties. The pyrazine is used as the aryl group and a wide variety of groups were selected as the hydrophilic moieties. The published data showed that the compounds in this patent were generally potent (IC_{50} < 0.5 nM) at blocking the PD-1/PD-L1 interaction. The selected example of **29** showed an IC_{50} of 0.213 nM in a bead-based amplified luminescent proximity homogeneous assay and an EC_{50} of 119 nM in a PD-1/PD-L1 NFAT reporter assay. In addition, **29** was also evaluated for its effects on activation of HBV-specific T cells from CHB patient PBMCs. Results showed **29** enhances the antiviral/effector functions of HBV-specific $CD8^+$ and $CD4^+$ T cells from CHB patients in vitro to a degree comparable to those of the anti-PD-L1 monoclonal antibody durvalumab. Moreover, **29** showed greater than 90% target occupancy (TO) on tumors for a duration of at least 24 hours post dosing following intraperitoneal administration of 10 (QD), 25 (BID), 50 mg/kg (QD) in a human PD-L1-expressing MC38 mouse colorectal tumor model. In patent US10710986, the pharmaceutical composition of **29** and its effect on the treatment of colorectal cancer is further described.

Chemists from Betta pharma also disclosed two patents in which benzo[d]oxazole is used as the aryl group directly attached to the biphenol core. (Figure 13) [54,55] In the aryl and the hydrophilic part of the structure of compound **31**, Y is selected from O, S, -NR_9-; R_6 is selected from H, halogen, CN, -C_{1-8}alkyl, -C_{1-4}haloalkyl, -C_{2-8}alkenyl, sulfonyl, sulfinyl, provided that if Y is O, then R_6 is not -C_{1-8}alkyl; R_7 and R_8 are each independently selected from H, -C_{1-8}alkyl, -C_{1-6}alkyl-COOH, -C_{5-6} aryl, wherein -C_{1-6}alkyl-COOH, and -C_{5-6} aryl optionally substituted with -C_{1-8}alkyl, -C_{0-4}alkyl-COOH, -C_{0-4} alkyl-OH; or R_7 and R_8 together with the atoms to which they are attached form a three- to seven- membered heterocyclic ring; wherein the heterocyclic ring is optionally comprising 1, 2 or 3 hetero atoms independently selected from N, S, or O; the heterocyclic ring optionally substituted with oxo, -C_{1-8} alkyl, -C_{0-4}alkyl-COOH, -C_{0-4}alkyl-OH; As an example, molecule **31** showed an IC_{50} of 1.8 nM in the PD-1/PD-L1 homogenous HTRF binding assay. For the general structure of **32** from patent WO2020015717, an extra chain is substituted to the terminal benzene ring. For **32**, a morpholine group is attached by an aliphatic ether linker to the ortho position of the methyl group of the biphenol core.

Additionally, some PD-1/PD-L1 inhibitors that also do not contain any linker between the biphenol core and the aryl group were published by Sunshine Lake pharma (CN111793077), Incyte (US20200172541), ChemoCentryx Inc. (WO2020257549 and US20210002229), Shanghai Ennovabio pharma (WO2021121282). (Figure 14) [56–60] Different five- and six-membered aromatic rings or five- and six-membered fused heterocycles are used as the aryl group.

2.6. PD-L1 inhibitors with two different linkers in combination

Except for the compounds discussed above, there are still some PD-L1 inhibitors that have two different linkers attached on both sides of the core group. This class of compounds generally belongs to the second pharmacophore and have unsymmetrical structures. These compounds can modulate the PD-1/PD-L1 protein-protein interaction and thus may be useful for therapeutic administration to enhance immunity against diseases and disorders such as cancer and infectious diseases. PD-1/PD-L1 HTRF binding assays with recombinant human PD-L1 protein were used to validate the activity of these compounds. Structures **37**–**49** are summarized in Figure 15 [61–77]. Among them, compounds **38** and **39**

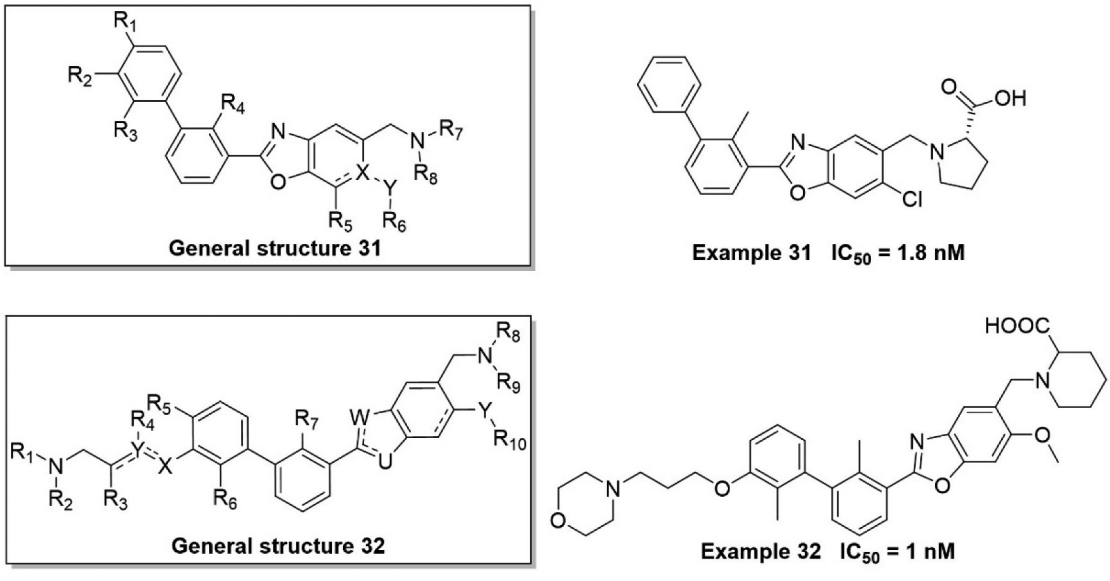


Figure 13. The general structures and examples of compounds patented by Betta Pharma.

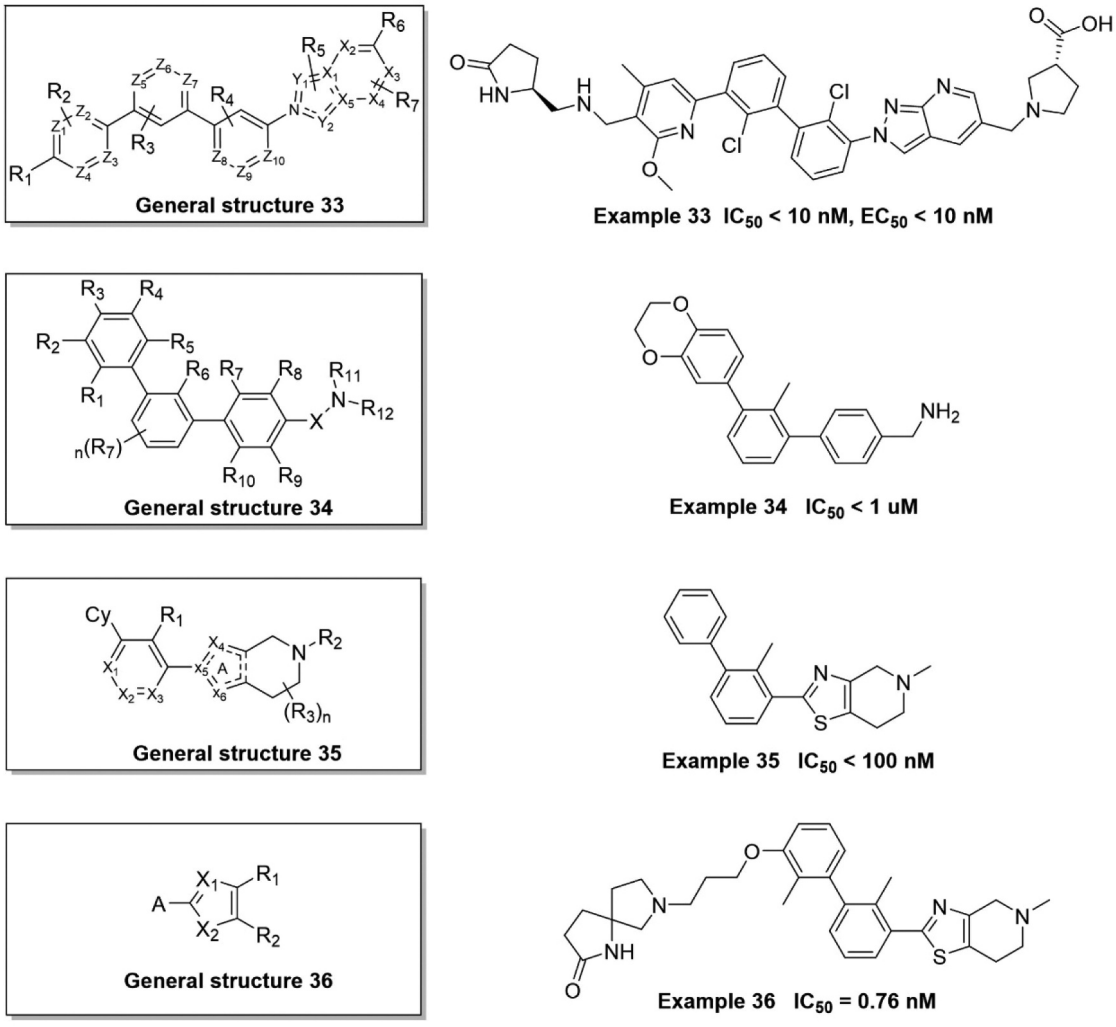
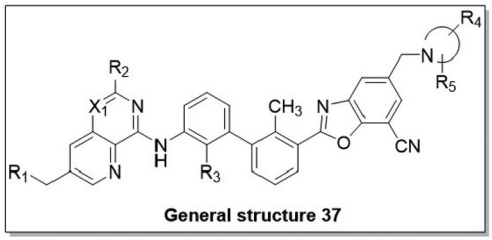


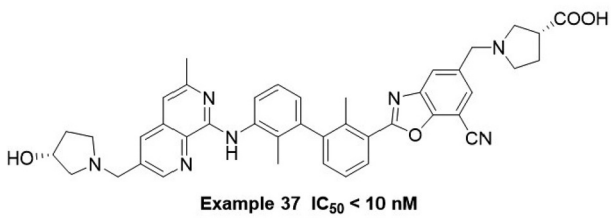
Figure 14. The general structures and examples of compounds patented by Sunshine Lake pharma, Incyte, ChemoCentryx Inc. and Shanghai Ennovabio pharma.

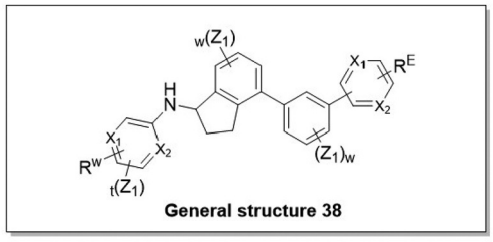
disclosed by Gilead showed extraordinary in vitro activity with an IC_{50} of less than 0.064 nM in ALPHA assay.

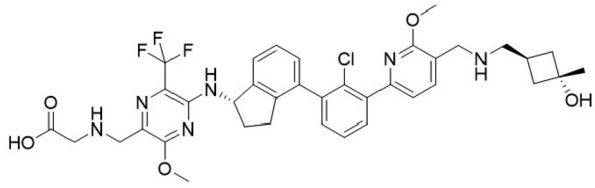
3. Expert opinion

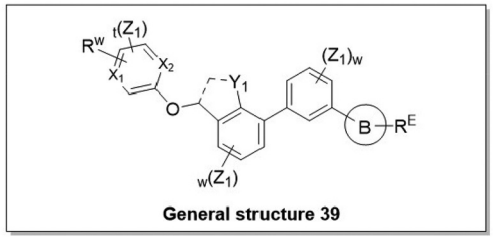
Immune checkpoint blockade therapy, especially PD-1/PD-L1 mAbs, has become a new therapeutic modality and garnered great success in treating cancers. To date, this approach has shown benefit in various cancer types, such as melanoma, cutaneous squamous cell carcinoma (CSCC), recurrent or advanced endometrial cancer, non-small cell lung cancer, metastatic merkel-cell carcinoma and so on. However, despite the medical and commercial success, PD-1/PD-L1 mAbs also have several limitations. First, because of poor diffusion and permeation profiles, their impressive clinical efficacy has been limited to a small fraction of patients. Second, the unfavorable pharmacokinetic characteristics of mAbs are responsible for immunogenicity and toxicities, leading to severe immune-related adverse events (irAEs). Moreover, the manufacturing cost of the mAbs is high, which will increase the economic burden on patients. As a result, using small molecule inhibitors to block PD-1/PD-L1 interactions may be an alternative approach to activate the innate immune system to fight against cancers. Small-molecule inhibitors have several advantages such as oral bioavailability, increased tumor penetration, accessible to intracellular targets and lower production costs, which might be advantages over antibody-based therapeutics. Small molecule inhibitors could be used as a monotherapy or combined with other drugs including antibodies and may provide an important complementary or potentially synergistic benefit in immune therapy. Since the first PD-L1 small molecule patent published by BMS, over 100 patents have been filled in the World Intellectual Property Organization. In addition, different bioassays have been developed to explore the in vitro and in vivo activities of these PD-L1 inhibitors, for example, the PD-1/PD-L1 assay kit from Cisbio and the cell-based PD-1/PD-L1 kit from Promega. Moreover, ten small molecules with PD-L1 protein have been co-crystallized and their structures have been published, which paves the way for better rational design of more potent PD-L1 inhibitors. One important hurdle in rational drug discovery of small molecule antagonists is to discover the pivotal pharmacophores that can be built upon. In this review, we summarized two pharmacophores based on the structure and activity of known PD-L1 inhibitors disclosed from

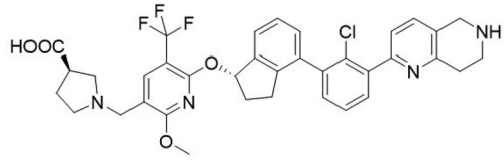


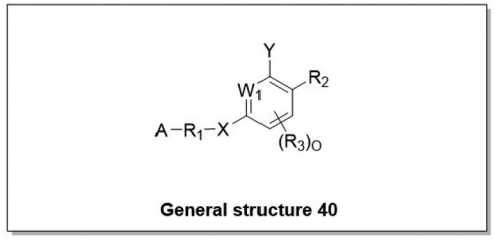


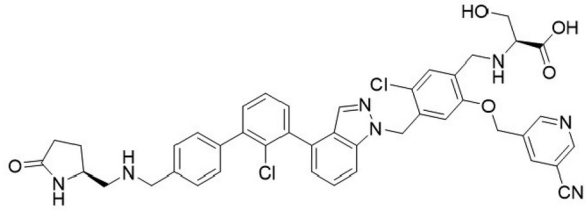


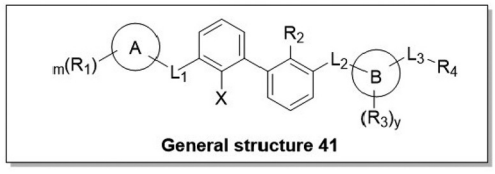


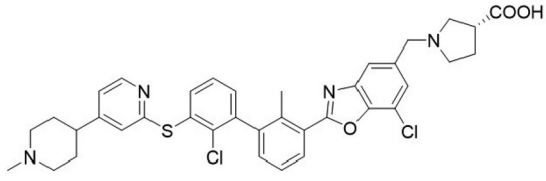


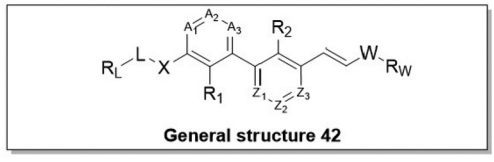


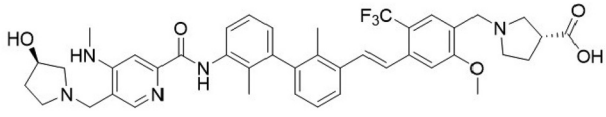


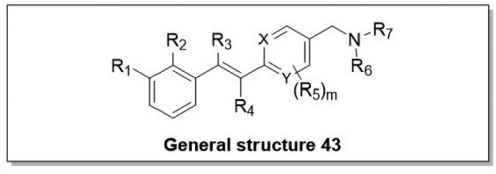


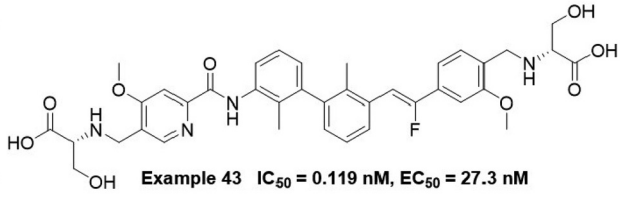


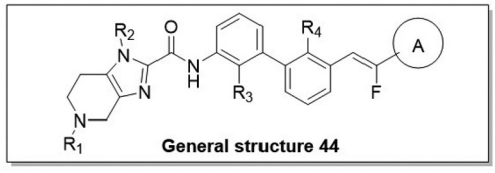












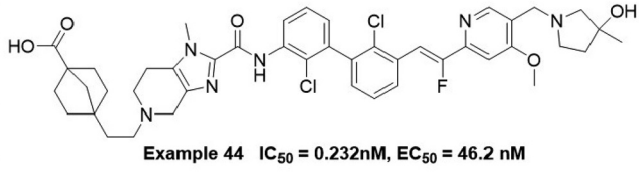


Figure 15. PD-L1 inhibitors with two different linkers in combination.

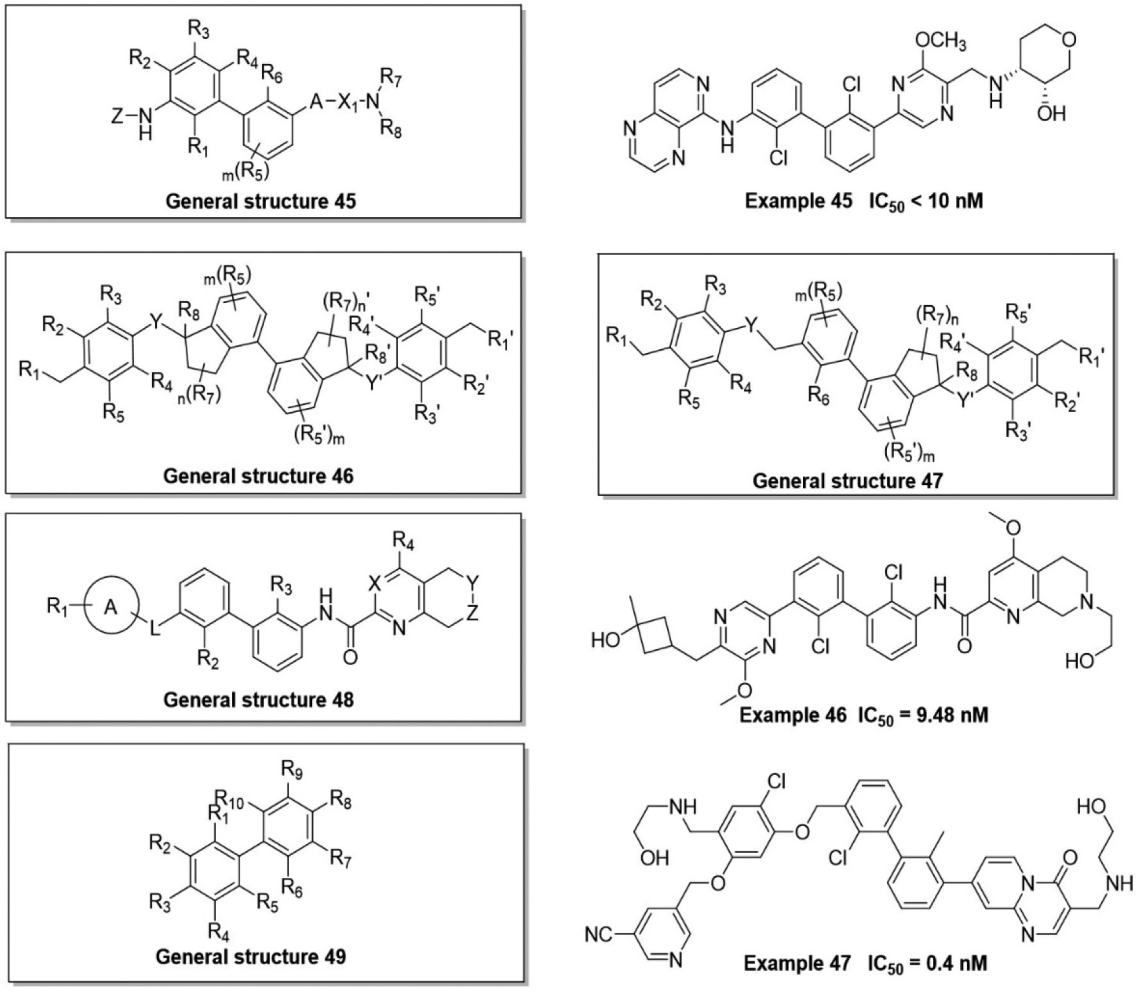


Figure 15. (Continued).

Table 2. IC_{50} values of the example compounds and their cancer types addressed in the patents.

Example	IC_{50}(nM)	Cancer types	Example	IC_{50}	Cancer types
1	9.1	Hepatoma, Melanoma	25	92.3	NM
2	0.88	Melanoma	26	87.3	NM
3	-	Lung cancer	27	0.37	NM
4	2.7	Lung cancer, Melanoma, carcinoma pancreatic cancer	28	<25	NM
5	1.1	NM	29	0.213	Colorectal cancer
6	2.33	Melanoma, carcinoma	30	0.064	NM
7	1	Lung cancer	31	1.8	NM
8	1.8	Breast cancer	32	1	NM
9	<100	Renal cancer	33	<10	NM
10	<100	NM	34	<1000	NM
11	<100	NM	35	<100	NM
12	<5	Melanoma	36	0.76	NM
13	<100	NM	37	<10	NM
14	<100	NM	38	<0.064	NM
15	1	NM	39	<0.064	NM
16	18	NM	40	-	NM
17	13	NM	41	20	NM
18	2.3	NM	42	<1	NM
19	2.9	NM	43	0.119	NM
20	<5	NM	44	0.232	NM
21	<100	NM	45	<10	NM
22	<100	NM	46	9.48	NM
23	0.29	NM	47	0.4	NM
24	1.02	NM			

NM: not mentioned in the patent

recent patents and papers. In addition, IC_{50} values of each of the compounds described in the manuscript, as well as in the various types of cancer addressed in the patents is summarized in Table 2. Encouragingly, some PD-L1 small molecule inhibitors exhibited comparable activity to those of anti-PD-L1 antibodies. In addition, five compounds have shown excellent preclinical results and entered clinical trials. However, the development of PD-L1 small molecules is still in its infancy compared with the development PD-1/PD-L1 antibodies. Whether these compounds can exhibit a clinical performance as good as antibodies remains unknown. In addition, compared with the huge number of small molecules that disclosed by patents, the number of molecules have progressed to clinical trials is relatively small. It seems that the discovery of inhibitors relying only on protein-based assays (e.g. HTRF) present certain limitations. Many compounds are indeed lacking a clear translation to the biological activity in a more complex cellular environment. The molecules which exhibit promising potency in both protein-based assays and cellular assays (for example, ARB-272572) probably have greater opportunities to succeed [20]. Moreover, some inhibitors can bind to the mouse PD-L1 significantly more weakly than to the human PD-L1. This highlights the requirement for analyzing the binding of the identified inhibitors not only to the human but also to the mouse PD-L1 so as to minimize the risk of unspecific and off-target effects during preclinical investigations in mouse models. Therefore, more efforts are still needed to design better PD-L1 small molecule inhibitors and investigate their safety and efficacy clinically to accelerate their approval.

Funding

This work was supported by GuangDong Basic and Applied Basic Research Foundation (No. 2020A1515110430); The authors also thank the Bioland Laboratory for financial support.

Declaration of interests

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

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