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REVIEW

IDO as a drug target for cancer immunotherapy: recent developments in IDO inhibitors discovery

Shan Qian,*a Man Zhang,∗a Quanlong Chen,∗a Yanying He,a Wei Wanga and Zhouyu Wangab

Received 00th January 20xx, Accepted 00th January 20xx
DOI: 10.1039/x0xx00000x

www.rsc.org/advances

1. Immunotherapy is an exciting strategy of cancer therapy

The interactions between immune system and the developments of tumors are complex and dynamic. On one hand, inflammation suppresses the immune response to actually promote tumors progression; on the other hand, tumor cells are also able to evade or subvert the immune response on the pressure of immune surveillance. It will be effective and critical to breach immune suppressive mechanisms established by tumor cells in the treatment of cancer patients. In 2010, the first therapeutic vaccine Sipuleucel-T was approved for the treatment of metastatic prostate cancer by the Food and Drug Administration (FDA). Since then, immunotherapeutic approaches are unleashed and rapidly expanding worldwide in the field of cancer research. In 2011, the first cytotoxic T lymphocyte antigen-4 (CTLA-4) antibody Ipilimumab was approved for the treatment of metastatic melanoma (MM). Programmed cell death 1 and its ligand (PD-1 and PD-L1) antibodies as new generations of ‘immune checkpoint blockade’ are now under intense clinical investigation in MM and non-small-cell lung cancer (NSCLC). PD-1/PD-L1 antibodies Nivolumab and Pembrolizumab are evaluated as the blockbuster drugs in the near future. Immunotherapeutics technologies of chimeric T cell antigen receptors (CAR-T) have achieved remarkable success in the treatment of leukemia and solid tumor (pancreatic carcinoma). Cancer immunotherapy has been nominated for ‘Year Breakthrough of Cancer Research 2013’ by Science magazine. More than 50 phase III trials are currently on-going in cancer immunotherapy. Because of distinguished effectiveness and novelty, cancer immunotherapies promise to be an innovation in the treatment of cancer following operative treatment, chemotherapy, radiotherapy and targeted therapy.

2. IDO is a key mediator of immune escape and pathogenic inflammation in cancer

Indoleamine 2, 3-dioxygenase (IDO), an extrahepatic tryptophan catabolising enzyme encoded by the IDO1 gene, degrades the essential amino acid tryptophan (TRP) into kynurenine (KYN), known as the initial and rate-limiting step of kynurenine pathway. Expression of the IDO1 gene by tumor cells or host APCs can inhibit tumor-specific effector CD8+ T cells and enhance the suppressor activity of T regulatory cells (Tregs). IDO-mediated catabolism of TRP are identified as an important immune effector pathway in the tumor cells to escape a potentially effective immune response, which is induced by innate and adaptive immune responses. IDO affects differentiation and proliferation of T cells, triggering downstream signaling through GCN2, mTOR and AhR (Fig. 1). Firstly, TRP deprivation in the tissue microenvironment that leads to the accumulation of TRP-tRNA in uncharged conformation. General control non-derepressible 2 (GCN2) is a stress-response kinase containing an allosteric regulatory site that responds to uncharged tRNA. The activation of GCN2 kinase leads to phosphorylation and attenuation of its downstream target, eukaryotic translation initiation factor-2α (eIF-2α) kinase. Phosphorylated eIF-2α prevents readout of most RNA transcripts and limits protein translation in response to this condition. Secondly, another signaling molecule inhibited due to the absence of TRP is master metabolic regulator mTOR (mTORC1), which is regulatory target of the master amino acid-sensing kinase 1 (GLK1). Local TRP degradation can block

*Department of Pharmaceutical Engineering, Xihua University, Chengdu 610039, P.R. China.
a Department of Chemistry, Xihua University, Chengdu 610039, P.R. China.
E-mail: qians33@163.com; zhousywang77@gmail.com; Fax: +86-28-87720552.
Fig. 1 IDO signaling effector pathways.

IDO mediators such as interferon-gamma (IFN-γ) when host responses against the tumor and produces inflammatory environment. The deregulation of IDO in tumor cells is due to cancer suppressive gene called bridging integrator 1 (Bin1). Bin1 is a c-myc interacting protein with the features of tumor suppressor that interacts with the N-terminal of c-Myc protein to neutralize its transformation and transactivation effect. Clinical observations have suggested that frequent loss or attenuation of Bin1 and high IDO expression are seen in various cancers, containing advanced breast cancer, prostate cancer, melanoma, astrocytoma, neuroblastoma, lymphocytic leukemia and colon cancer, and are associated with worse clinical prognosis in patients with a variety of malignancies. Bin1 can restrict tumor outgrowth and limit tumor immune tolerance through its effect on down regulation of IDO expression. On the contrary, mouse knockout studies indicated that deletion of the Bin1 results in the STAT1 and NF-kB dependent super-expression of IDO following IFN-γ stimulation. Bin1 knockout mice are expected to result in large tumor growth when are transduced with c-myc and mutant ras oncogenes. IDO inhibition can prevent the tumor growth in these mice, suggesting the direct link between Bin1 and IDO activity.

3. Therapeutic strategies and challenges of IDO inhibitors in cancer immunotherapy

Targeting the IDO pathway via inhibition of the IDO enzyme or blocking its downstream signaling effects is a prime target for small-molecule immunomodulatory drugs in cancer. It has also been widely recognized that IDO inhibitors might be useful in combination with traditional chemotherapeutic drugs or therapeutic cancer vaccines. Damaged or dying tumor cells exposed to chemotherapeutic regimens can express many antigens to activate immune responses against the tumor. However, this almost never triggers a curative immune response against established human tumor, because tumor can rapidly reestablish tolerance following each cycle of chemotherapy by upregulating immunological checkpoints, including IDO.

In addition, chemotherapy induces the transient lymphopenia and cytokine-rich environment that may promote T cells to break tolerance to homeostatic recovery. Finally, Chemotherapy can also deplete and blunt regulatory T cells which can generate a short-lived but beneficial anti-tumor immune response. Thus, inhibiting IDO in the period of post-chemotherapy could promote T cells to more receptively break tolerance and potentially enhance the effectiveness of chemotherapeutics by active immunotherapy.

Multiple immune inhibitory mechanisms are present concurrently in the tumor microenvironment, so single agent treatments targeting one immunological checkpoint are difficult to achieve optimal efficiency. Allison et al. indicated that IDO is a critical resistance mechanism in antitumor T cell immunotherapy targeting CTLA-4. IDO blockade strongly synergizes with CTLA-4 antibody ipilimumab, which achieved a striking delay in B16 melanoma tumor growth and increased overall survival. Spranger et al. also reported that multiple inhibitions of IDO, CTLA-4 and PD-1/PD-L1 showed greater effect in activating the immune system and inhibiting tumor growth than either treatment alone.
showed more pronounced activation (proliferation+cytokine production) of intratumoral CD8^+ T cells. These data provided a strong incentive to explore combination clinical therapies using IDO inhibitors regardless with IDO expression by the tumor cells.

Recently, the development of IDO inhibitors are ongoing intensively in academia and pharmaceutical companies, containing with Pfizer Pharmaceuticals Ltd., Roche, Bristol-Myers Squibb, and three small-molecule compounds (Indoximod, Epacadostat and GDC-0919) have entered clinical trials. As a kynurenine pathway inhibitor, Indoximod (NLG8189) improves the efficacy of multiple chemotherapeutics (Paclitaxel, Docetaxel, Temozolomide and Gemcitabine), and some immunological checkpoints mediators (Ipilimumab and anti-CD28 monoclonal antibody) in Phase I/II clinical studies for metastatic breast cancer, metastatic melanoma, non-small cell lung cancer, primary malignant brain tumors, metastatic pancreatic cancer, as well as metastatic prostate cancer (source http://www.clinicaltrials.gov). Epacadostat (INC8024360) was obtained following a high throughput screening (HTS) of Incyte’s corporate collection (IC_{50} = 67nM). It promotes T and natural killer (NK)-cell growth, increases IFN-γ production, and reduces conversion to Treg-like cells. Epacadostat suppresses KYN generation and tumor growth in immunocompetent, but not immunodeficient mice. In mice bearing CT26 colon carcinoma, Epacadostat also inhibits the growth of IDO-expressing tumors. Epacadostat is evaluated at present mainly in Phase I/II clinical combination drug trials in gynecological and peritoneal cancers, melanoma, malignant solid tumor, lymphoma, breast, lung, as well as renal cell cancers with CTLA-4 and PD-1/PD-L1 antibodies, respectively. GDC-0919 (NLG919) was obtained by rational structural design based on the X-ray crystal structure for IDO complexed with 4-phenyl-imidazole (PIM). It is a strong competitive IDO inhibitor and potently inhibits IDO pathway in vitro and in cellular assays (K_i=7nM, EC_{50}=75nM). Treatment with GDC-0919, upon vaccination of B16F10 tumor-bearing mice, resulted in an increase in the T effector cell response, leading to improved anti-tumor efficacy (~95% reduction in tumor volume). Combined treatment with GDC-0919 and chemo-radiation therapy enhanced survival in mice bearing intracranial glioblastoma tumors. Chemotherapy treatment alone resulted in collections of perivascular leukocytes within the tumor microenvironment, but no complement degradation. Adding IDO-blockade led to upregulation of vascular cell adhesion molecule-1 (VCAM-1) on vascular endothelium within tumors, and further led to widespread complement deposition at sites of tumor growth. In Phase I clinical trial for the treatment of advanced-stage solid tumors, GDC-0919 demonstrated the high dose tolerance (well tolerated up to 800 mg BID on a 21/28 day cycle), but revealed that single-agent therapy with an IDO inhibitor failed to cause tumor eradication and to prevent disease progression (best response was limited to stable disease in 7 out of 17 patients). GDC-0919 is now evaluated in phase Ib in combination with PD-L1 inhibitor atezolizumab.

4. Structure-based IDO inhibitors design

The crystal structure of IDO bound to the ligand PIM published by Sugimoto et al. in 2006 [Protein Data Bank (PDB) file "2D0T"] opened the door for structure-based in silico design of IDO inhibitors. IDO is a monomeric heme-containing enzyme. In the PIM-bound X-ray structure, the ligand is bound in a deep binding site with one imidazole nitrogen coordinated to the Fe iron at the distance of 2.1Å and its phenyl ring inside a hydrophobic pocket (pocket A). The PIM binding sites consist of residues Tyr126, Cys129, Val130, Phe163, Phe164, Ser167, Leu234, Gly262, Ser263, Ala264, and the heme ring. Possible hydrogen bonding sites are the Cys129-SH, Ser167-OH, Gly262-CO, Ala264-NH and the heme 7-propionate group. In 2014, two crystal structures of IDO bound to the larger ligands Amg-1 (PDB file "4PK5") and imidazothiazole compound (PDB file "4PK6") were published (Fig. 3). Another hydrophobic pocket (pocket B) at the entrance of pocket A is provided by multiple residues and the heme ring. In addition to occupy pocket A, the larger ligands can also interact with residues located at pocket B, containing Phe226, Arg231, Ser235, Phe291, Ile354, and Leu384. Moreover, the additional hydrogen bond with the side chain of Arg231 is possible. The ligands occupied both pocket A and pocket B may obtain better inhibitory activities.

Röhrig et al. investigated the binding modes of all known IDO inhibitors via docking algorithm EA Dock and developed a pharmacophore model to devise new compounds to be tested for IDO inhibition. Fragment-based approach was also used to design and to optimize small organic molecule inhibitors. Both approaches yielded several novel small-molecular inhibitor scaffolds and the most active compound 1 showed nanomolar inhibitory activity. On the basis of the binding modes between IDO and these ligands, they concluded that an ideal IDO inhibitor should contain the following features: (i) an aromatic ring with at least two cycles, to fill into pocket A. Almost all known IDO inhibitors comply with this rule; (ii) an atom with a free electron pair (such as oxygen, sulfur, or nitrogen) that can coordinate to the Fe iron. The ligands not obeyed this rule generally show low inhibitory activities; (iii) groups in larger ligands that can form van der Waals interactions with the binding sites in the pocket B; (iv) groups in larger ligands that can form hydrogen bond to Ser167, Gly262, Ala264, and Arg231 or to the heme 7-propionate. At the same year, a series of hypoxia-targeting novel IDO hybrid inhibitors have been designed and synthesized by Nakashima et al. Among these compounds, L-Trp-tirapazamine hybrid 2 was able to bind the enzyme-substrate complex and showed the potent IDO inhibitory activity with an IC_{50} value of 15.3μM. Smith et al. have developed three pharmacophores to search novel IDO inhibitors, coupled with refinement of hits through Kier flexibility scoring and “What-if” docking analysis. Eighteen compounds were tested in vitro, yielding compound 3 (IC_{50}: 20μM) with an ester imidamide linker between two aromatic regions, which has much greater interactions with the active site compared to many of the simple IDO inhibitors reported to date. Fragment screening has the capacity to identify novel IDO inhibitors, including those can’t identified by enzymatic screening. 2-hydrazinobenzothiazole was identified as a strong IDO inhibitor.
The different series of 1,2,3-triazoles were thus synthesized for ring by a triazole ring, which has widely applied in pharmaceuticals. The triazole scaffold shares structural similarity with PIM and it replaces the imidazole pharmacophore of potent IDO inhibitors. The triazole scaffold coordinated bond with the heme iron to stable the complex through screening of the Zenobia fragment library. It forms a co-ordinated bond with the heme iron to stable the complex through hydrazine rather than through the sulphur in the benzothiazole ring. Phenylhydrazine was 32-fold more potent (IC<sub>50</sub>: 0.25μM) than 2-hydrazinobenzothiazole (4) (IC<sub>50</sub>: 8.0μM). The enzyme assay indicated that the hydrazine interaction is sufficient for IDO inhibitory, and the benzothiazole group was less favorable for inhibition of IDO, because it may restrict the hydrazine-heme interaction.

Based on the co-crystal structure of IDO with PIM, Huang et al. discovered that 1H-1,2,3-triazole might be a new key pharmacophore of potent IDO inhibitors. The triazole scaffold shares structural similarity with PIM and it replaces the imidazole ring by a triazole ring, which has widely applied in pharmaceuticals. The different series of 1,2,3-triazoles were thus synthesized for screening high efficacy IDO inhibitors, and compound 5 showed potent inhibitory activity with an IC<sub>50</sub> value of 86 μM. Molecular docking studies indicated that substituent group on 4-aryl should not be too large and an electron-withdrawing group near the NH group of the triazoles was prerequisite of inhibitory activities.

5. IDO inhibitors discovered by HTS

HTS strategy is extensively carried out on library screening by measuring kynurenine formation in enzymatic assay with purified recombinant human IDO protein. The benzisothioura derivative 6 was identified as noncompetitive IDO inhibitors via screening campaign. Subsequent optimization of 6 lead to the identification of nanomolar inhibitor 7, which suppressed kynurenine production in A431 cells. Structure-activity relationships (SARs) analysis revealed that the distance from the isothioura moiety and the halogen atom at the C-4 of phenyl ring were necessary for inhibitory activities. The keto-indole derivative 8 was identified as a potent IDO inhibitor via virtual screening strategy combining various filters including HTS docking. Then, a novel series of IDO inhibitors are synthesized based on this indol-2-yl ethanone scaffold, leading to the compound 9 with an IC<sub>50</sub> value of 13μM. Preliminary SARs analysis revealed an iron coordinating group on the linker is essential to retain inhibitory activity. Methylthiohydantoin-tryptophan (MTH-Trp, 10) was discovered by library screening in 2005. A series of novel tryptoline derivatives were synthesized based on its structure and the most active compound 11 showed potent inhibitory activity with an IC<sub>50</sub> value of 46.1μM.

Matsuno et al. have discovered the anti-hypertensive drug candesartan cilexetil as an IDO inhibitor with an IC<sub>50</sub> of 12μM by screening. This agent is an angiotensin II receptor blocker (ARB) with wide utilities in clinics. A series of candesartan derivatives have subsequently been synthesized and the most active compound 12 showed potent inhibitory activity with an IC<sub>50</sub> value of 1.0μM. SARs analysis and docking results indicated that candesartan derivatives may uniquely bind to the entrance of the active site of IDO, not to the heme ferrous iron. Recently, a series of phenyl benzenzesulfonylhydrazides were synthesized in order to improve cellular inhibitory activities of 2-phenyl benzeneethanesulfonyl-hydrazide 13, which was identified as a potent IDO inhibitor via HTS. The compound 14 showed good inhibitory activity with an IC<sub>50</sub> value of 61nM in vitro and an EC<sub>50</sub> value of 172nM in the HeLa cell. SARs analysis revealed that the interactions between 14 and IDO conclude the coordination of sulfone and heme iron, the hydrogen bonding and hydrophobic interactions.

6. Natural-source IDO inhibitors

It is well established that various benzoquinone and naphthoquinones display low micromolar activities as IDO inhibitors, e.g. exiguamine A (15) and annulin B (16), suggesting these moieties to be key pharmacophores and likely indolemimetic. Bridewell et al. have tested a series of pyranonaphthoquinone natural products, leading triazole 17 with potent IDO inhibitory activity (IC<sub>50</sub>: 6.0μM) and cellular activity against IDO.
Benzoferanquinones 18 containing a CH₂OR group at C-3 was an analogue of the marine metabolite annulin A 38. It was a potent IDO inhibitor with an IC₅₀ value of 0.24 μM, and this quinone didn't generate significant oxidative stress at its IDO inhibitory concentration. Pasceri et al. 39 have prepared a range of 2-aminothiazol-3-ones by oxidative cyclocondensation of 2-aminophenols. The cinnamonic acid 19 containing additional electron-withdrawing carboxylate groups on the phenyl rings showed potent inhibitory activity with an IC₅₀ value of 0.46μM.

Meroterpenoid 20 was isolated from the marine sponge Xestospongia vansoesti and showed potent IDO inhibitory activity with an IC₅₀ value of 0.11μM 40. SARs analysis revealed that the orientation of the dioxydihydrothiazine ring fusion to C-14/C-15 is important and the hydroxyl at C-3 is significantly detrimental to inhibitory activity. These results described above have extended the quinone series with IDO inhibitory activities based on natural products.

Many natural alkaloids have also been discovered to be novel IDO inhibitors. Tryptanthrin (indolo[2,1-b]quinazolin-6,12-dione, 21), a potent IDO inhibitor, was isolated from Chinese medicinal plants Polygonum tinctorum and Isatis tinctoria 41. Three series of tryptanthrin derivatives were synthesized and the compound 22 showed potent inhibitory activity with an IC₅₀ value of 0.16μM. SARs analysis revealed that an electron-withdrawing group at the C-8 contributed to IDO inhibition.

In the screening course of the extracts of fungus for IDO inhibitors, a new benzodiazepine alkaloid named benzomalvin E (23) and a new benzofuran alkaloid named thielavin Q (24) were isolated from soil fungus 42, 43. They exhibited potent IDO inhibitory activity with IC₅₀ values of 21.4μM and 26.5μM, respectively. Williams et al. 44 have reported new meroterpenoid named Halicloic acid A (25), was isolated from the marine sponge Haliclona (Haliclona) sp. collected in the Philippines. This compound exhibited potent IDO inhibitory activity with an IC₅₀ value of 11μM. SARs analysis indicated that the hexaprenoid moiety is critical to retain IDO inhibitory activity.

7. Conclusions and perspectives
The structure of IDO consists of a highly conserved sequence AGGSGAG (residues 260–265), which provides conformational flexibility in several proteins. Thus, the main chain of IDO exhibits backbone flexibility and a large conformational change, leading to different active site conformations characterized by larger pocket A and different shapes and sizes of pocket B. Therefore, it is important in the approach of IDO docking studies that protein flexibility and heme interactions could be taken into account.

IDO is an intercellular monomeric enzyme constituted by a heme Fe iron center as a site for transferring oxygen atoms into pyrrole ring. IDO is active when the Fe iron is in its reduced ferrous state, so the continuous presence of reductants is necessary to maintain enzyme activity, since IDO is prone to autooxidation. Cytochrome reductases and cytochrome b₅ are putative intracellular reducing agents, and these reductants are substituted by methylene blue and ascorbic acid in vitro assay, to keep the Fe iron in its reduced form. A large number of natural IDO inhibitors are redox-cycling compounds. Some experts have presumed these compounds contain problematic functional groups such as quinines, which will not only affect the kynurenine pathway of TRP degradation but also interfere with many other cellular pathways. Therefore, it should be avoided to use these compounds as IDO inhibitors.

IDO is an attractive target for anticancer therapy and the discovery of IDO inhibitors has been intensely ongoing in both academic research laboratories and pharmaceutical organizations. Over the recent five years, several new IDO inhibitor scaffolds have been discovered by structure-based design, HTS, and natural product screening. We hope the information provided herein will be helpful for the development of novel IDO pharmacological inhibitors in the treatment of cancer.

Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>AhR</td>
<td>aryl hydrocarbon receptor</td>
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<tr>
<td>ARB</td>
<td>angiotensin II receptor blocker</td>
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<td>Bin1</td>
<td>bridging integrator 1</td>
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<tr>
<td>CAR-T</td>
<td>chimeric T cell antigen receptors</td>
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<tr>
<td>CTLA-4</td>
<td>cytotoxic T lymphocyte antigen-4</td>
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<tr>
<td>DMBA</td>
<td>dimethylbenzanthracene</td>
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<tr>
<td>eIF-2α</td>
<td>eukaryotic translation initiation factor-2α</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>GCN2</td>
<td>general control non-derepressible 2</td>
</tr>
<tr>
<td>GLK1</td>
<td>master amino acid-sensing kinase 1</td>
</tr>
<tr>
<td>HTS</td>
<td>high throughput screening</td>
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<tr>
<td>IDO</td>
<td>indoleamine 2, 3-dioxygenase</td>
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<tr>
<td>IFN-γ</td>
<td>interferon-gamma</td>
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<tr>
<td>KYN</td>
<td>kynurenine</td>
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<tr>
<td>MM</td>
<td>metastatic melanoma</td>
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<tr>
<td>MTH-Trp</td>
<td>methylthiohydantoin-tryptophan</td>
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<tr>
<td>mTOR</td>
<td>mammalian target of rapamycin</td>
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<tr>
<td>mTORC1</td>
<td>master metabolic regulator mTOR</td>
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<tr>
<td>NF-xB</td>
<td>nuclear factor-xB</td>
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<tr>
<td>NK</td>
<td>natural killer</td>
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<tr>
<td>NSCLC</td>
<td>non-small-cell lung cancer</td>
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Acknowledgements

The work was supported by the National Natural Science Foundation of China (81302647), the Scientific Research Fund of Sichuan Provincial Education Department (15ZA0132), the Open Research Subject of Key Laboratory of Advanced Scientific Computation and Simulation (SZJJ2014-083) and innovation fund of post graduate from Xihua University (ycjj2015139).

References


IDO as a drug target for cancer immunotherapy: recent developments in IDO inhibitors discovery

Shan Qian a, *, Man Zhang a, Quanlong Chen a, Yanying He a, Wei Wang a and Zhouyu Wang b, *

a Department of Pharmaceutical Engineering, Xihua University, Chengdu 610039, P.R. China.
b Department of Chemistry, Xihua University, Chengdu 610039, P.R. China.
*Address correspondence to these authors at Xihua University, Chengdu 610039, P.R. China; E-mail: qians33@163.com; zhouyuwang77@gmail.com; Fax: +86-28-87720552.

This review highlights recent advances (2010 -- 2015) in the research related to the role of IDO in immune escape and pathogenic inflammation in cancer, and novel small-molecule IDO inhibitors with an emphasis on their chemical structures and modes of action.