DUAL INHIBITORS OF INDOLEAMINE-2,3-DIOXYGENASE (IDO) AND TRYPTOPHAN-2,3-DIOXYGENASE (TDO) AS ANTI-TUMOR IMMUNE MODULATORS

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ABSTRACT

Indoleamine-2,3-dioxygenase (IDO) and tryptophan-2,3-dioxygenase (TDO) are enzymes that catalyze the rate-limiting step of the kynurenine pathway. Recent literature reports IDO and TDO upregulation in tumor cells leading to L-tryptophan depletion and accumulation of tryptophan metabolites. This process represents an essential mechanism for tumor-induced immunosuppression. Following the failure of Epacadostat, an IDO inhibitor, in phase 3 clinical trials, numerous studies have shifted to a dual inhibition scheme to overcome the compensation mechanism linked to TDO. Therefore, the dual inhibition of IDO and TDO using a single molecule emerges as a highly promising therapeutic approach. This comprehensive review aims to discuss the successful scaffolds of reported dual inhibitors and their inhibitory values against both enzymes. The reported active compounds have the potential to form a novel chemical class of anti-tumor immune modulator drugs.

Keywords: Indoleamine-2,3-dioxygenase, tryptophan-2,3-dioxygenase, dual inhibitors, anti-tumor immune modulators.

1. Introduction:

1.1. Cancer disease

According to statistics from the World Health Organization (WHO), Cancer is the second leading cause of death globally with 9.6 million deaths in 2020. Approximately 18% of deaths result from lung cancer, 10% from colon and rectum cancer, 8% from liver cancer, 8% from stomach cancer, and 7% from breast cancer. The underlying causes of cancer development are not completely understood, however, there are risk factors that increase the incidence of cancer development. Adjustable risk factors, such as tobacco use, alcohol consumption, excessive body weight, poor diet, and lack of physical activity, can be controlled. Conversely, inherited genetic mutations represent an unalterable risk factor. Cancer by definition is a group of diseases that can affect all parts of the body. It is characterized by uncontrolled growth of abnormal cells. These cells, undergoing mutations disrupting normal division, lead to tumor formation. Cancer cells acquire eight hallmarks, as outlined in Hanahan and Weinberg's research, collectively defining their aggressiveness and resilience, posing challenges in treatment (Hanahan et al. 2011). Various pathways, including surgery, chemotherapy, radiation therapy, targeted therapy, immune-modulators, hormonal therapy, and precision medicine, are employed for cancer management (Debela et al. 2021).

1.2. Anti-tumor Immune modulators

Anti-tumor immune modulators have emerged in the last decade as effective and possible treatments for cancer. They are agents that target specific immune pathways to promote the immune response against tumors and enhance their rejection. Currently, diverse types of anti-tumor immune modulators are being developed (National Cancer Institute 2019). Examples include (I) cytokines; proteins produced by white blood cells integral to the body's normal immune response (Nicholas et al. 2011). (II) BCG (Bacillus Calmette-Guérin); a weakened form of the tuberculosis-causing bacteria, triggers an immune reaction against cancer (Han et al. 2020). (III) Thalidomide and its derivatives hinder angiogenesis and immune evasion (Xia et al. 2021). Our research group contributed much to this field and got promising results (El-Zahabi et al. 2020, Abdallah et al. 2021, Kotb et al. 2022, El-Zahabi et al. 2023, Elkady et al. 2023, Mabrouk et al. 2023). (IV) IDO/TDO inhibitors, target both enzymes involved in suppressing the immune response against cancer (Chen et al. 2021). Dual IDO and TDO inhibitors are of particular interest in this review.

2. Indoleamine-2,3-dioxygenase

2.1. Physiological role:

Indoleamine-2,3-dioxygenase is an oxidoreductase heme-containing enzyme. It is widely distributed across various tissues. Initially discovered in the liver, it has since been found in other organs like the placenta, and peripheral and central nervous systems (Booth et al. 2015). Encoded by the IDO gene located in chromosome 8p12 of the human genome (Soliman et al. 2010). This gene expresses two isoforms: Indoleamine-2,3-dioxygenase 1 (IDO1) and a less pervasive and effective form, Indoleamine-2,3-dioxygenase 2 (IDO2) (Ball et al. 2009). IDO controls L-tryptophan levels and acts as an immune suppressive in our human body. Through the kynurenine pathway (KP), Its

role extends to safeguarding immune-privileged areas such as the eyes, testes, and placenta, protecting the foetus from maternal immune rejection (Routy et al. 2016). It starts its biological function by metabolizing the L-Trp into the Kynurenine in the KP (Dolivo et al. 2018). It catalyzes the first rate-limiting step via oxidative cleavage of the pyrrole ring of L-tryptophan converting it to *N*-formyl-kynurenine. Subsequently, the formamidase enzyme transforms it into Kynurenine, as depicted in **Figure 1**.

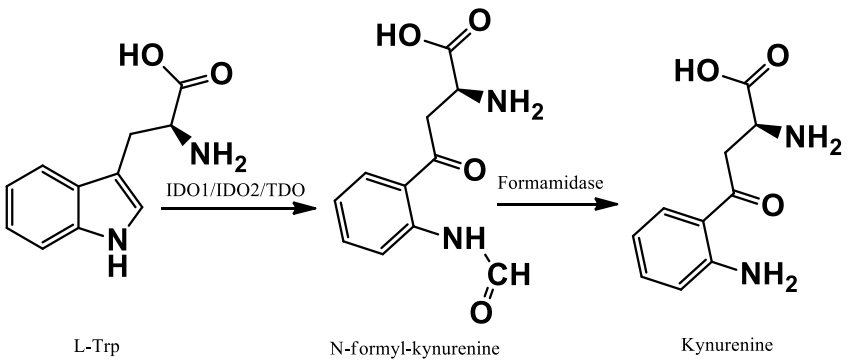


Figure 1: Pathway of Kynurenine formation

2.2. Roles in tumor immunity

Many findings report IDO1 expression in many tumours by cancer cells (Uyttenhove et al. 2003), plasmacytoid dendritic cells in draining lymph nodes (Munn et al. 2004), and human monocyte–derived macrophages (Munn et al. 1999). This upregulation leads to L-tryptophan depletion and the accumulation of tryptophan metabolites, which cause activation of the human aryl hydrocarbon receptor (AhR) leading to immunosuppression and tumour growth promotion. This happens as follows (i) it suppresses T cells and natural killer cells (NK) proliferation (Fallarino et al. 2002, Frumento et al. 2002, Terness et al. 2002, Della Chiesa et al. 2006), (ii) stimulates differentiation and maturation of T regulatory cells inducing systemic tolerance against the presenting antigen (Fallarino et al. 2003), and (iii) supports the activity of myeloid-derived suppressor cells (MDSC) (Smith et al. 2012), **Figure 2** (Austin et al. 2015). Moreover, the role of IDO1 expression extends to tumour inflammation (Muller et al. 2008, Muller et al. 2010), angiogenesis, and metastasis (Smith et al. 2012). Furthermore, recent literature reports that pharmacological inhibition of IDO leads to immune rejection of tumors in preclinical mice (Uyttenhove et al. 2003). Consequently, IDO1 is an important validated drug target for cancer treatment, which is being intensively investigated (Matsuno et al. 2010, Dolusic et al. 2011, Dolusic et al. 2013, Fung et al. 2013).

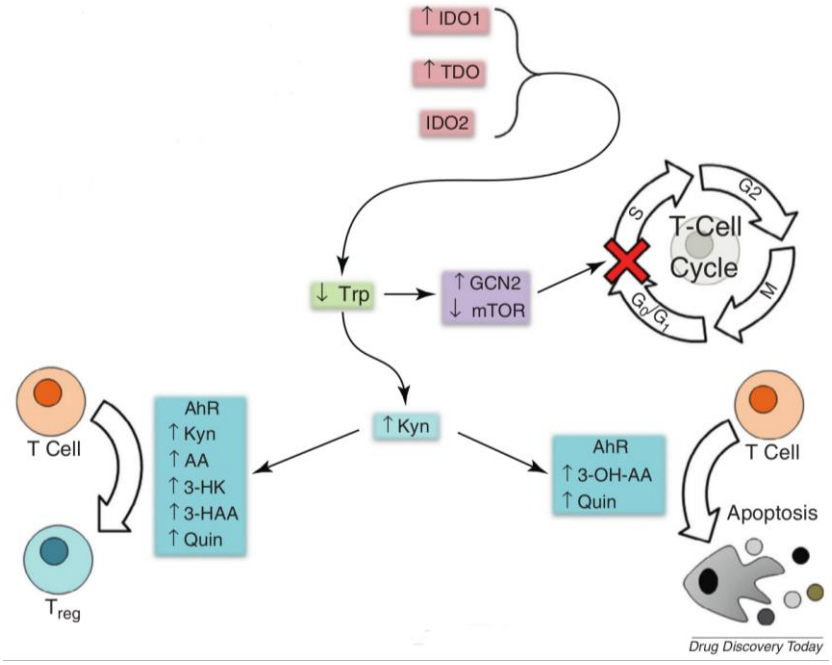


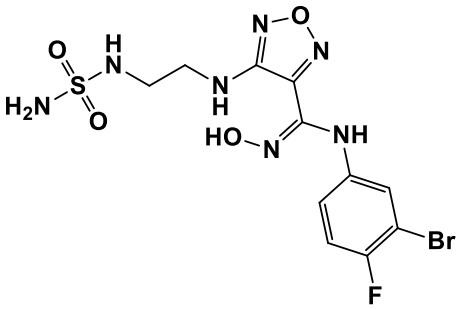
Figure 2: Role of IDO and TDO in tumor induced immunosuppression

2.3. IDO reported inhibitors

Since the role of IDO in tumor immunity was revealed, many inhibitors have been developed to counteract the immune inhibition and promote tumor rejection by the immune system. Diverse structures and chemical scaffolds find their way to enter clinical trials for approval. Seven clinical candidates showed encouraging results either alone or in combination therapy, depicted in **Table 1** and **Figure 3,** (Tang et al. 2021). Unfortunately, most of them put on hold due to limited benefits (Garber 2018). The most promising candidate Epacadostat **(1)** combined with targeted immune checkpoint therapy PD-1 unfortunately failed in phase 3 in 2018 (Long et al. 2019). The underlying causes were not obvious but the researchers suggest that the failure may be mainly due to the compensation mechanism happened by TDO (Muller et al. 2019). Other reasons were speculated as insufficient drug dose, incompatible drug combination, and insufficient time to complete the inhibition of tryptophan degradation by IDO inhibitor (Muller et al. 2019).

Table 1: Some reported IDO inhibitors:

Name	Reference
Epacadostat **(1)**	(Kelly et al. 2018)
LY3381916 (**2**)	(Kotecki et al. 2021)
NLG802 **(3)**	(Kumar et al. 2020)
BMS-986205 **(4)**	(Huynh et al. 2023)
PF-0684003 **(5)**	(Tumang et al. 2016)
Indoximod **(6)**	(Soliman et al. 2016)
Navoximod **(7)**	(Jung et al. 2019)



Epacadostat
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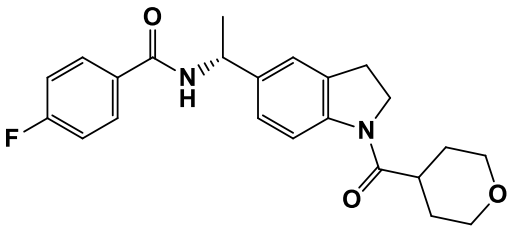

LY3381916
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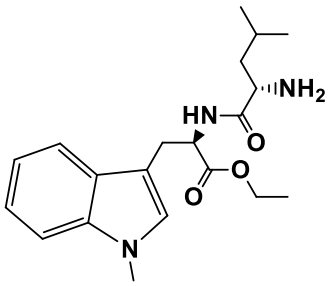

NLG802
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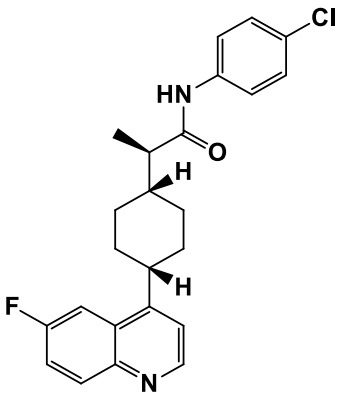

BMS-986205
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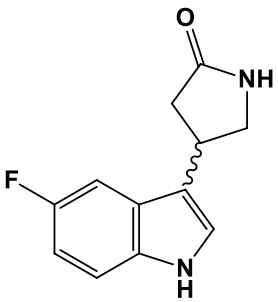

PF-0684003
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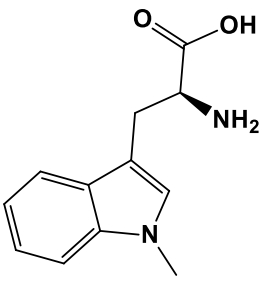

Indoximod
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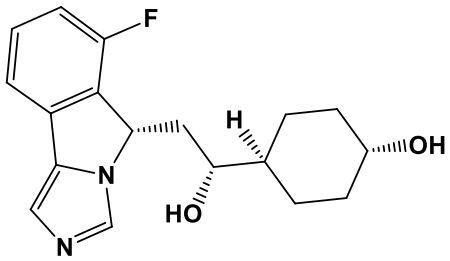


Navoximod
(7)

Figure 3: Some reported IDO inhibitors

3. Tryptophan-2,3-dioxygenase

3.1. Physiological role:

Tryptophan-2,3-dioxygenase is an oxidoreductase heme-containing enzyme expressed mainly in liver and to lesser extent in the brain (Knox et al. 1950, Zhang et al. 2007). It controls systemic tryptophan levels by catalysing l-Trp in the kynurenine pathway. It regulates the same first rate-limiting step as IDO, figure 1 (Fallarino et al. 2002). Through the KP, it acts as immune suppressive enzyme leading to immune privilege (Routy et al. 2016).

3.2. Roles in tumor immunity

In 2012, Pilotte's study reported TDO upregulation in many tumors (Pilotte et al. 2012). TDO expression in cancer cells causing the activation of human aryl hydrocarbon receptor (AhR) by the kynurenine. This leads to immune cells inhibition and tumor growth promotion in the same way as of IDO, **Figure 2** (Opitz et al. 2011, Pilotte et al. 2012). Furthermore, pharmacological inhibition of TDO leads to immune rejection of tumors in preclinical mice (Pilotte et al. 2012). Therefore, TDO is increasingly investigated as a validated target in cancer immune modulation (Dolusic et al. 2011, Pantouris et al. 2014).

3.3. Reported TDO inhibitors

Since the role of TDO discovered, several researchers and companies start their development for TDO inhibitors to block l-Trp degradation and stimulate the immune response (Kozlova et al. 2019). Many inhibitors have been synthesized and showed high range of activity against TDO as depicted in **Figure 4** (CAUWENBERGHS 2015, stefano. 2015, Kozlova and Frédérick 2019). Although it is a highly active area of research, only one series of inhibitors has been succeeded to be selective TDO inhibitor but did not find its way for clinical trial (stefano. 2015). For this reason, no studies currently focus on selective TDO inhibitors.

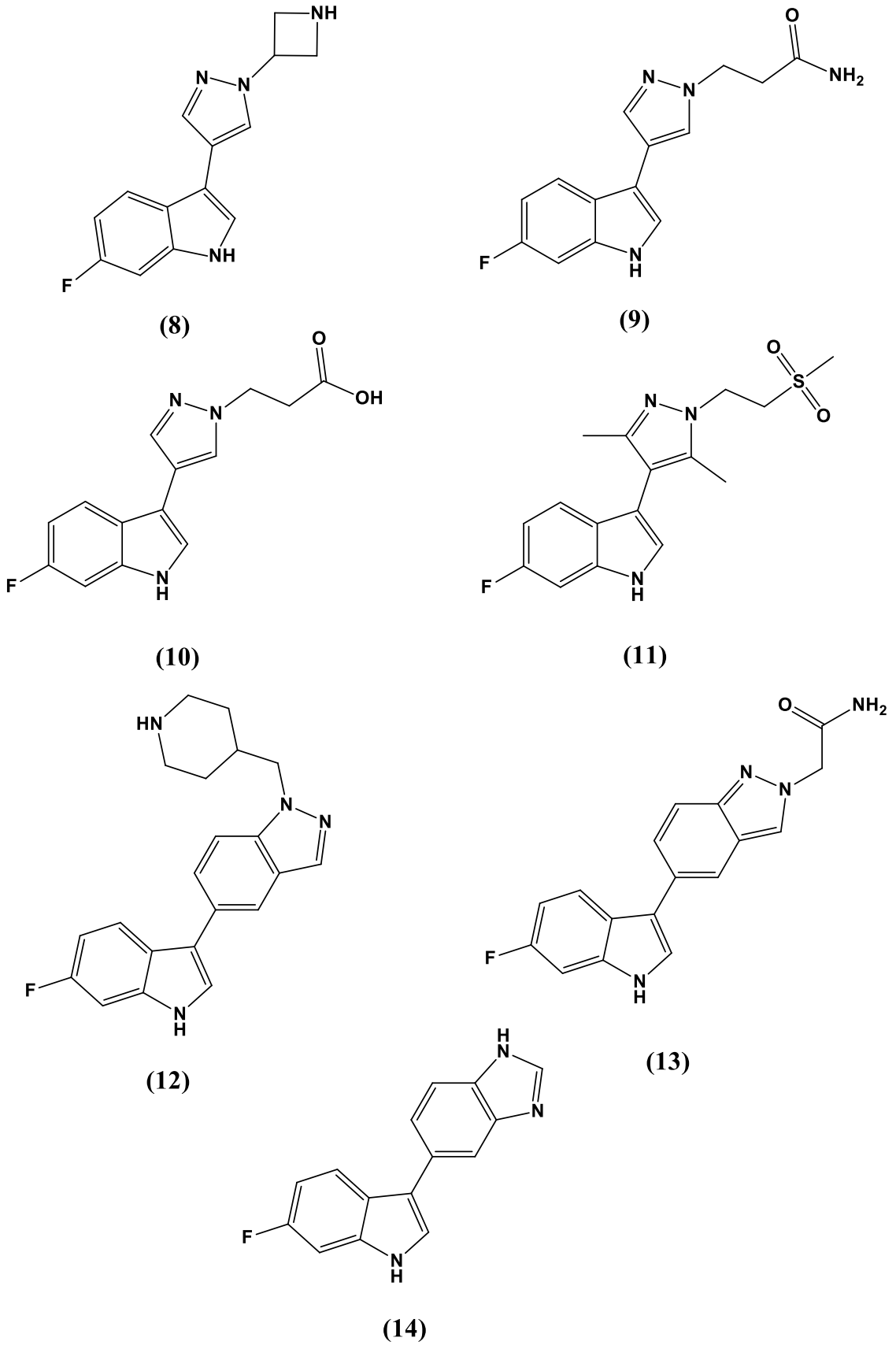


Figure 4: Some reported TDO inhibitors

4. Significance of dual inhibition

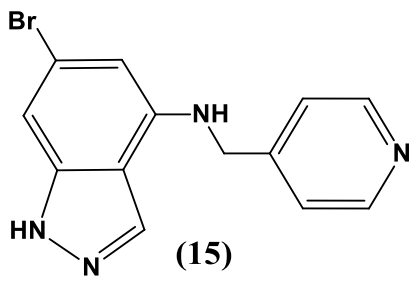
The failure of Epacadostat in phase 3 clinical trials has shifted attention to the compensation mechanism linked to L-Trp depletion through TDO and IDO2. IDO2 isn't as a significant target as IDO1 and TDO in human tumors due to its lower expression and reduced capacity for L-Trp degradation (van Baren et al. 2015). Therefore, complete inhibition of Trp degradation through dual IDO and TDO inhibition emerges as a promising strategy, offering higher efficacy and better anti-tumor activity compared to a single IDO inhibitor strategy (Feng et al. 2019). Dual IDO/TDO inhibition brings multiple advantages. Firstly, since significant proportions of tumor cells express both IDO and TDO, dual inhibition allows targeting a wider range of malignancy types, reaching up to 51% of the human tumour set, compared to only 31% by IDO inhibitors or 35% by TDO inhibitors alone (Pilotte et al. 2012). Secondly, compared to using a combination of two drugs, it provides fewer side effects and avoids issues such as drug interactions, variances in biological distribution, and metabolism discrepancies. Third, targeting multiple proteins with weak inhibition may be more effective than targeting one with selective high affinity inhibitors in networked systems (Agoston et al. 2005). Last, reaching a better control of simultaneous IDO and TDO activity holds promise for managing other conditions related to chronic infection (Potula et al. 2005, Favre et al. 2010), depression (Plangar et al. 2012), and schizophrenia (Linderholm et al. 2012).

5. Reported IDO/TDO dual inhibitors

In this review we have complied reported dual inhibitors from 2018 to 2023

5.1. 4,6-Substituted-1*H*-indazole derivatives

In 2019, Yang and his team developed 38 derivatives of 4-amino-6-bromo-1*H*-indazole (Yang et al. 2019). The objective was to find potent dual inhibitors and evaluate their inhibition against both IDO and TDO. They introduced different substituents to the 4-amino of the indazole core. Notably, compounds **15** and **16** exhibited moderate inhibition percentage against IDO and TDO at concentration of 10 µM. However, compound **17** showed the most significant inhibitory activity against IDO1 and TDO in both enzymatic and cell-based assay. Compound **17** featured a methyl benzoxadiazole-at the 4-amino of the 6-Bromo-1*H*-indazole nucleus. It displays IDO1 IC_{50} value of 0.74 µM and TDO IC_{50} value of 2.93 µM in enzymatic assay.



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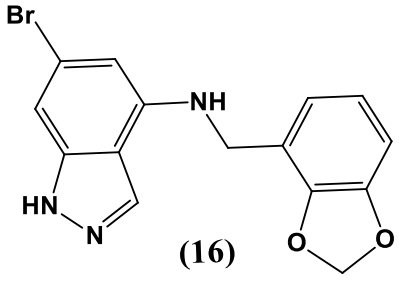

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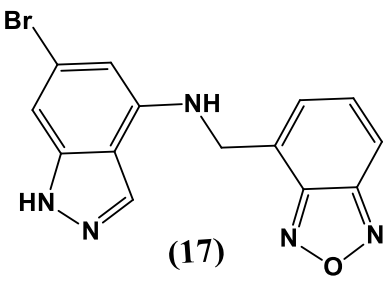

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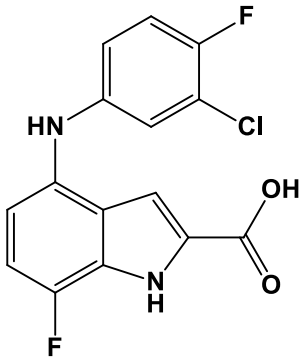
5.2. Indole-2-carboxylic acid derivatives

In 2020, Cui and his team synthesized diverse analogues for screening dual IDO/TDO hits: compound **18** and **19** (Cui et al. 2020). Their aim was to find potent lead structures and study the structure activity relationship (SAR). They initially synthesized various 6- substituted derivatives of hits, eventually reaching compound **19**, which contain acetamide at position 6 of the indole-2-carboxylic core. Compound **19** shows an IDO IC_{50} value of 8.40 µM and a TDO IC_{50} value of 8.48 µM. Following this, they varied the aryl part of the 4 amino of indole-2-carboxylic acid nucleus with different substituents. As a result, they identified five more potent compounds, labeled from **20** to **25.** Among these, compound **24** displayed the highest activity, with IDO IC_{50} value of 1.17 µM and a TDO IC_{50} value of 1.55 µM.



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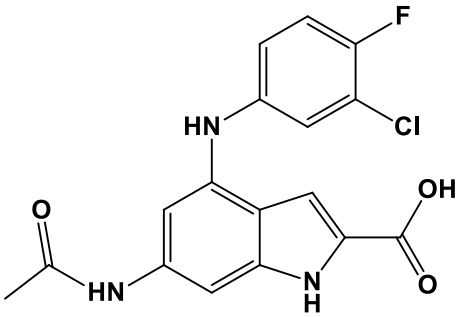

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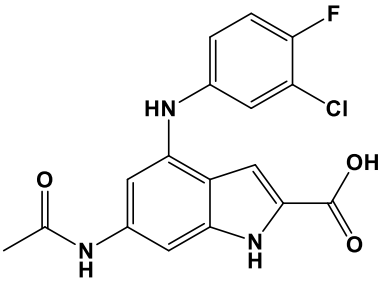

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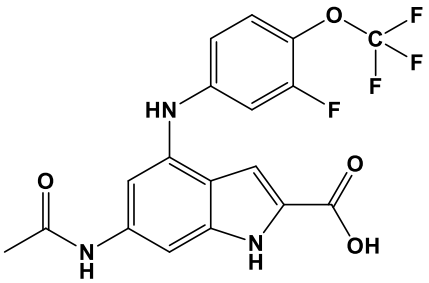

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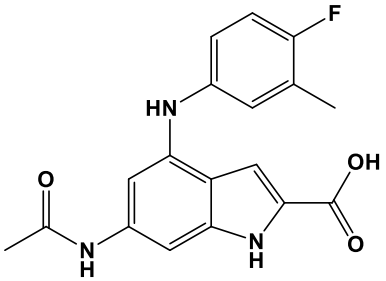

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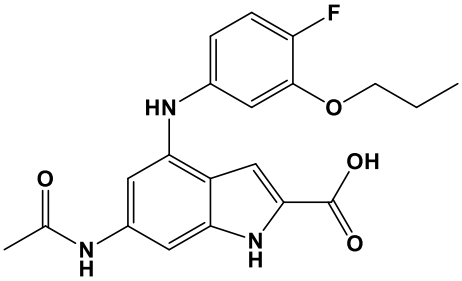

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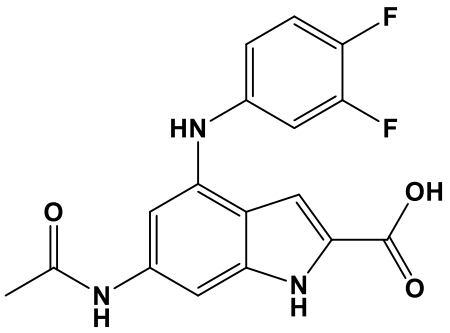

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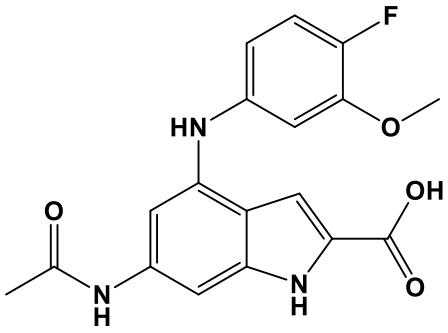

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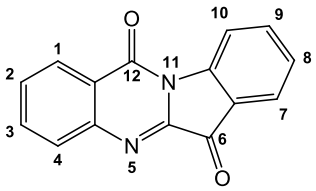
5.3. N-benzyl/aryl substituted tryptanthrin derivatives

In 2019, Yang and his team synthesized derivatives of their previously reported IDO inhibitor compound **26**, known as tryptanthrin (Yang et al. 2019). Their aim was to discover potent dual IDO/TDO inhibitors and determine their inhibition type and magnitude. Introducing various *N*-benzyl and aryl substituents to position 2 of the tryptanthrin nucleus resulted in compounds **27**, **28**, **29** and **30**, displaying similar high activity levels. Among these, Compound **29** emerged as the most promising candidate, exhibiting an IDO IC_{50} of 0.11µM and a TDO IC_{50} of 0.41 µM. Additionally, they tested compound **27** in mice and observed increasing of T cells proliferation and tumor growth suppression.



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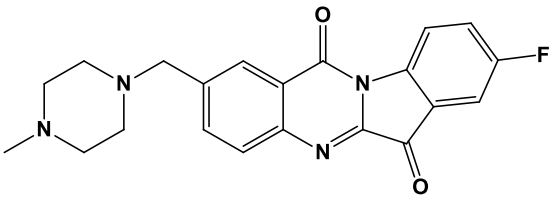

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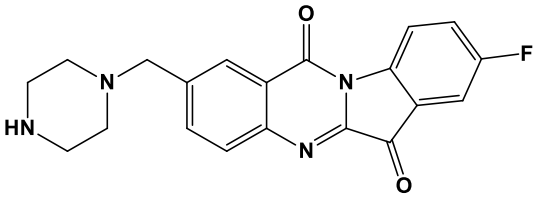

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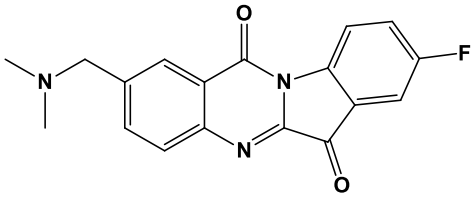

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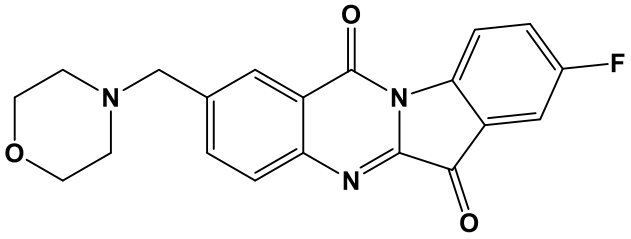

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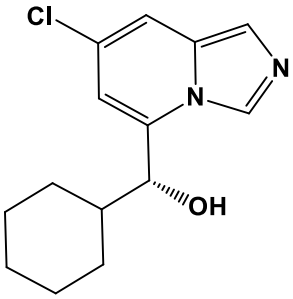
5.4. Substituted imidazole [1,5-*a*] pyridines derivatives

In 2020, Wang and his team synthesized a series of substituted imidazole [1,5-a] pyridine (WANG et al. 2020). The purpose was to invent patent scaffold for dual IDO/TDO inhibitors. They developed different derivatives at position 5,6,7,8 of the imidazole [1,5-a] pyridine nucleus. They found up that substitution of hydroxyl group at the α chiral carbon attached to position 5 or 8 significantly increases the activity. Moreover, they noted that different substitutes at othro and/or meta positions concerning the α hydroxyl at positon 5 or 8 bear more activity. Ultimately, they identified 3 active dual inhibiters: **31, 32** and **33.** Among them **31** demonstrated the highest activity with IDO IC_{50} of 9.6 nM and TDO IC_{50} of 29 nM.



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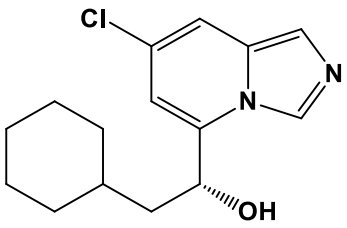

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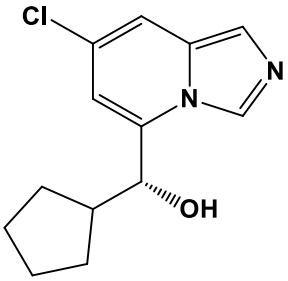

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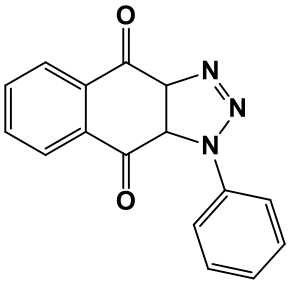
5.5. 1-Phenyl-1H-naphtho[2,3-*d*][1,2,3]triazole-4,9-dione

In 2020, Pan and his team developed and tested a series composed of 41 compounds (Pan et al. 2020) with the purpose of finding potent dual inhibitors and performing SAR analysis. They initially screened an in house library composed of 5000 compounds and identified compound **34** as a promising hit, demonstrating an IDO IC_{50} value of 0.100 μM and a TDO IC_{50} value of 0.07 μM. Subsequently, they introduced various substituted aryl groups in the 1-NH position of the triazole ring until they found the most active derivative. Further modifications were made to this derivative at positions 6 and 7 with different functional groups. They discovered three active compounds **35, 36** and **37** where compound **36** displayed the highest activity, exhibiting an IC_{50} value of 5 nM for IDO and 4 nM for TDO. The less active and inactive compounds in this series provided valuable insights into factors contributing in decreasing the activity.



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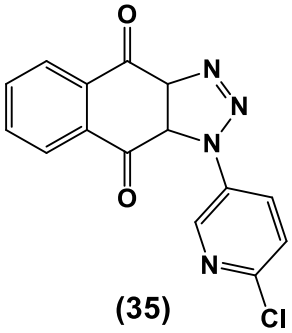

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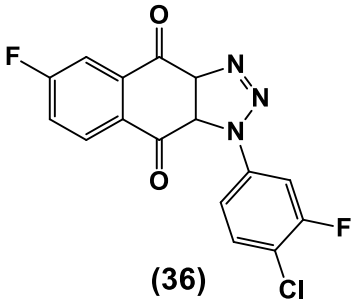

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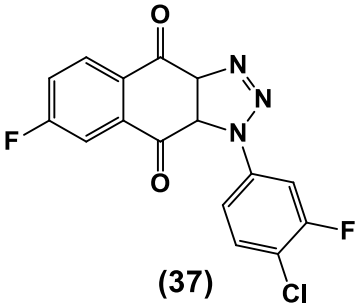

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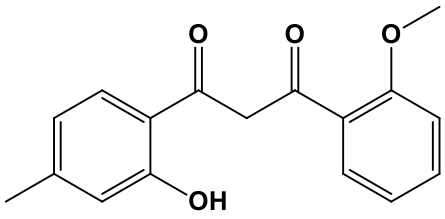
5.6. Propanedione derivatives

In 2019, Sari and his teams performed a virtual screening for 64000 compounds to identify scaffolds capable of inhibiting both IDO and TDO (Sari et al. 2019). They utilized 3D similarity shape and 3D pharmacophore models as screening filters. They identified three hits **38, 39** and **40**. Among them compound **40** emerged as the most active compound with IDO and TDO IC_{50} value of 3.42 µM.



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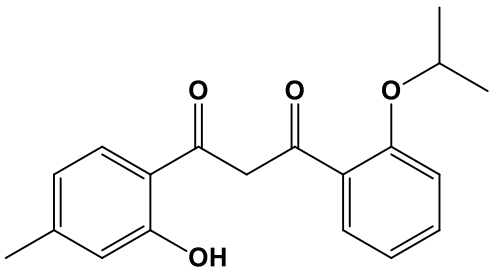

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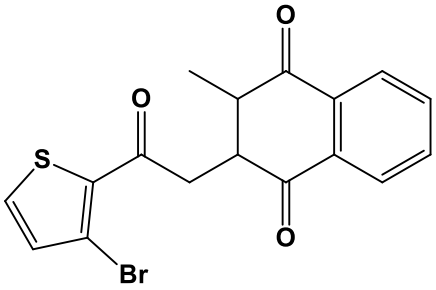

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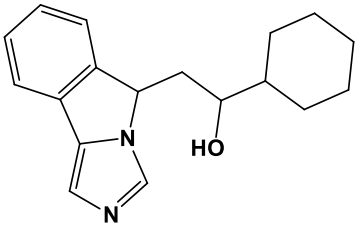
5.7. Imidazoisoindole derivatives

In 2019, Tu and his team synthesized 26 imidazoisoindole derivatives aimed at discovering potent dual inhibitors while eliminating cytochrome p450 (CYP) activity (Tu et al. 2019). Starting from the clinical candidate selective IDO inhibitor compound **41** (NLG-919), they performed multiple rounds of SAR studies, exploring various substituents to develop potent dual inhibitors. Eventually they developed three active derivatives: compound **42, 43** and **44**. Compound **44** emerged as the most active with IDO IC_{50} of 9.7 nM and TDO IC_{50} of 47 nM. This compound represents a promising lead scaffold for dual inhibitor also exhibiting a favorable pharmacokinetic profile.



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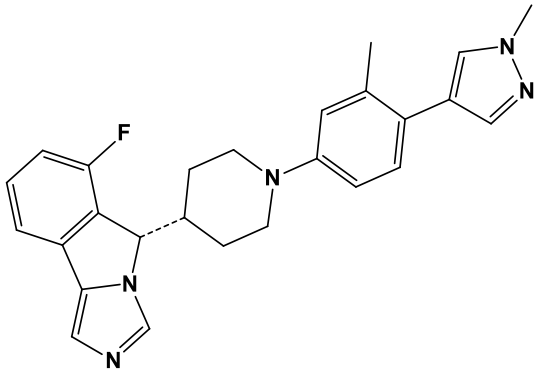

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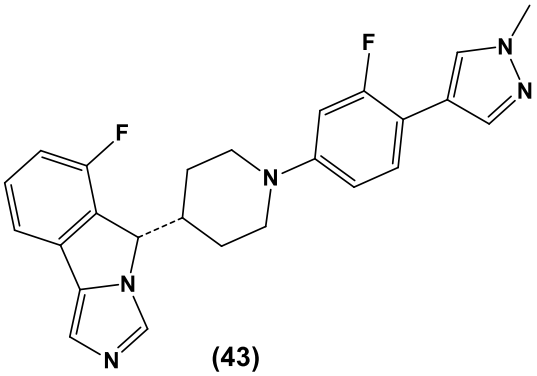

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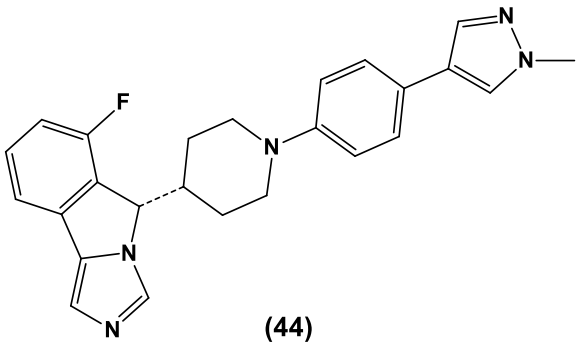

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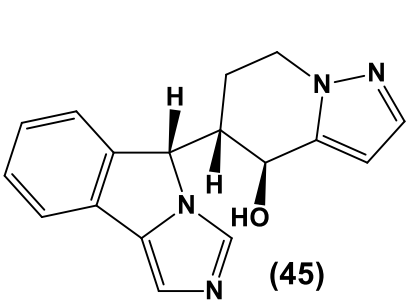
In 2020, Parr and the research team continued their exploration of imidazoisoindole nucleus and synthesized as series of 31 compounds for tryptophan-mediated enzyme inhibition (Parr et al. 2020). Their objective was to discover selective dual IDO/TDO inhibitor over CYP. Using a structure-based drug design technique, they optimized a group of imidazoisoindole inhibitors to identify potent dual inhibitors. Among the three active inhibitors **45, 46**, and **47**, compound **47** demonstrated the highest activity, displaying an IDO IC_{50} of 0.69 μM and a TDO IC_{50} of 0.06 μM. it is worth mentioning, their work introduced a valuable parameter to check the selectivity over cytochrome P450 (CYP) for the developed inhibitors.



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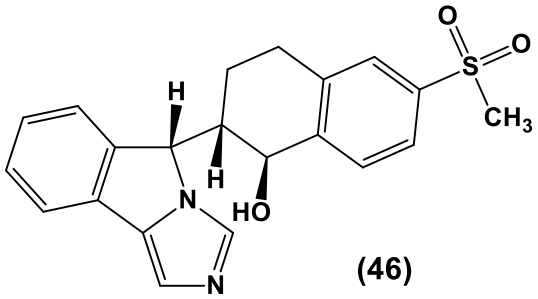

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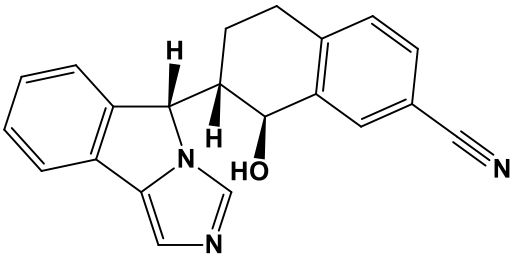

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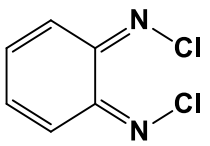
5.8. NCI hits

In 2021, Capochiani de Iudicibus and his research group conducted a screening of 5,682 compounds from the National Cancer Institute (NCI) library and their in house library, aiming to find dual IDO/TDO inhibitors and selective TDO inhibitors (Capochiani de Iudicibus et al. 2021). They developed an effective and accurate assay that can measure the inhibition percentage of compounds concurrently against IDO and TDO. This assay allowed them to rapidly screen this high numbers of compounds. They found ten active dual inhibitors, among them three compounds **48**, **49** and **50** that have relatively low IC_{50}. Compound **48** is the most active one with IC_{50} of 8.43 µM and 9.17 µM for IDO and TDO respectively.



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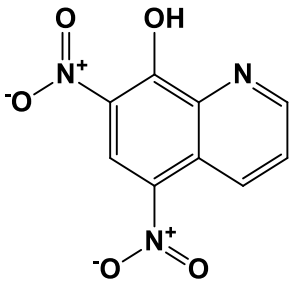

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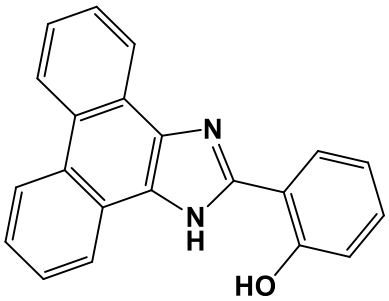

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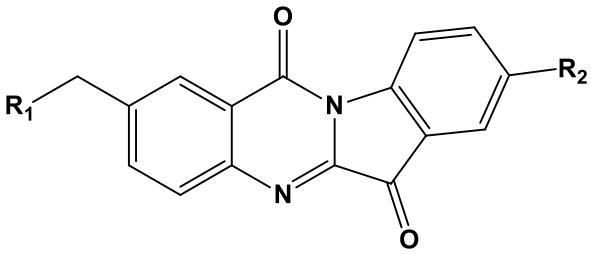
5.9. 2,8- Substituted indole quinazoline-6,12-dione

In 2021, Liang and his team continued their investigation on 2,8 substituted indole quinazoline-6,12-dione nucleus (Liang et al. 2021). They developed compound

51 in their lab as a dual IDO-TDO inhibitor for treatment of pancreatic cancer. The specific substituents on the nucleus remain undisclosed. It shows a high significant inhibition in both cell and enzymatic assays, displaying an IC_{50} value of 0.025 µM for both IDO and TDO in cell based assay. Furthermore, they tested it against tumor mouse model to evaluate its preclinical efficacy. It inhibits the tumor growth, promotes its apoptosis and stops the metastasis. This outcome highlights the potential of the dual inhibition strategy.



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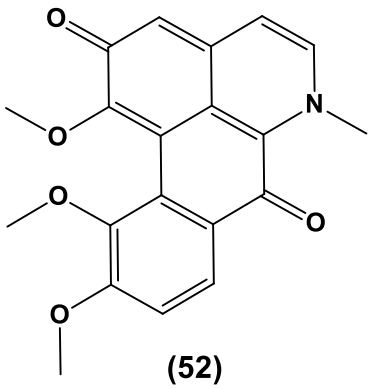
5.10. Natural alkaloids

In 2023, Bao and his team extract natural alkaloids form famous medicinal plant in China, known as Dactylicapnos scandes (Bao et al. 2023). Their aim was to explain the anti-tumor activity associated to this plant. They conducted root extraction using bioassay guidance followed by semi-synthesis, yielding 37 compounds. Among these compounds, compound **52** exhibited dual inhibition against IDO and TDO with a Ki value of 1.9 µM and 3.1 µM, respectively.



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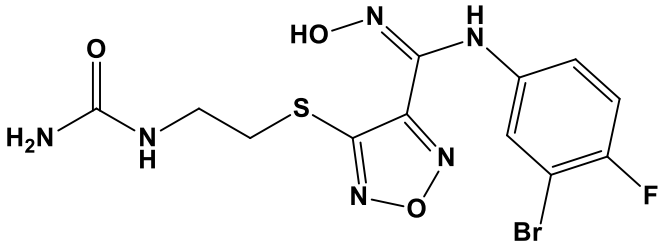
5.11. Clinical candidates

Until now, three clinical candidate have entered clinical trials as dual IDO/TDO inhibitors: LPM-3480226 (Nanjing Kanghai Phospholipid Biological Technology Co. 2019), DN-1406131 (Shanghai De Novo Pharmatech Co. 2018) and SHR9146 (Jiangsu Hengrui Pharmaceuticals Co. 2018). They are currently undergoing the phase 1 evaluation.



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LPM3480226

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المثبطات المزدوجه لانزيمات ال IDO و TDO كمعدلات مناعيه مضاده للسرطان

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الملخص :

انزيم IDO و انزيم TDO هما عبارة عن انزيمات تحفز مسار الكينورينين. تشير الأدبيات الحديثة إلى زيادة وجود IDO و TDO في الخلايا السرطانية مما يؤدي إلى استنزاف L-tryptophan وتراكم المستقلبات. تمثل هذه العملية آلية أساسية لتثبيط المناعة في حال السرطان . بعد فشل Epacadostat، وهو مثبط IDO، في المرحلة الثالثة من التجارب السريرية، تحولت العديد من الدراسات إلى نظام تثبيط مزدوج للتغلب على آلية التعويض المرتبطة بـ TDO . ولذلك، فإن التثبيط المزدوج لـ IDO وTDO باستخدام جزيء واحد يظهر كنهج علاجي واعد للغاية. تهدف هذه المراجعة الشاملة إلى مناقشة المثبطات المزدوجة الناجحه المنشوره في الدوريات العلميه وقيمها المثبطة ضد كلا الإنزيمين. المركبات النشطة المنشوره لديها القدرة على تشكيل فئة كيميائية جديدة من الأدوية المعدلة للمناعة المضادة للورم.

الكلمات المفتاحية: IDO، TDO، مثبطات مزدوجة ، معدّلات مناعية مضادة للورم