Tryptophan Metabolism as a Pharmacological Target

Morgane Modoux, Nathalie Rolhion, Sridhar Mani, Harry Sokol

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Review

Tryptophan Metabolism as a Pharmacological Target

Morgane Modoux,[1,2] Nathalie Rolhion,[1,2] Sridhar Mani,[3] and Harry Sokol [1,2,4,*]

L-Tryptophan is an essential amino acid required for protein synthesis. It undergoes an extensive and complex metabolism along several pathways, resulting in many bioactive molecules acting in various organs through different action mechanisms. Enzymes involved in its metabolism, metabolites themselves, or their receptors, represent potential therapeutic targets, which are the subject of dynamic research. Disruptions in L-tryptophan metabolism are reported in several neurological, metabolic, psychiatric, and intestinal disorders, paving the way to develop drugs to target it. This review will briefly describe L-tryptophan metabolism and present and discuss the most recent pharmacological developments targeting it.

Highlights

L-Tryptophan (L-Trp) is metabolized via three pathways: the indole pathway in bacteria and the kynurenine and serotonin pathways in mammalian cells.

Disruptions in L-Trp metabolism are reported in several diseases making L-Trp metabolism a promising therapeutic target.

Manipulating L-Trp metabolism is an attractive therapeutic strategy.

Key enzymes of L-Trp metabolism are targets of inhibitors currently undergoing clinical trials in cancerology, dermatology, and gastroenterology.

Serotonin and aryl hydrocarbon receptor (AhR) receptors are targeted in the treatment of gastrointestinal diseases, inflammation, and many cancers.

Next-generation probiotics producing indoles are being developed for their ability to activate AhR in the gut.

Introduction

L-Tryptophan (L-Trp) is an essential amino acid required for protein biosynthesis. It is also a biochemical precursor of metabolites that significantly affect mammalian physiology, including gastrointestinal functions, immunity, metabolism, and the nervous system. In the gastrointestinal tract L-Trp metabolism can follow three significant pathways, all of which are influenced by the gut microbiota: (i) the kynurenine pathway (KP) in both immune and epithelial cells, (ii) the **serotonin** (5-hydroxytryptamine, **5-HT**; see Glossary) production pathway in enterochromaffin cells (ECCs), a specialized subtype of intestinal epithelial cell, and (iii) direct transformation by the gut microbiota of L-Trp into several molecules, including ligands of the **aryl hydrocarbon receptor (AhR)** (Figure 1). Enzymes involved in these pathways, metabolites themselves, or their receptors represent therapeutic targets. Alterations in L-Trp metabolism have been reported recently in several neurological, metabolic, psychiatric, and intestinal diseases, paving the way for developing drugs to target it. Here, we will discuss in particular: (i) inhibitors of enzymes of the KP and analogs of neuroprotective metabolites, (ii) antagonists of 5-HT peripheral receptors and inhibitors of 5-HT synthesis, (iii) different strategies to target AhR via agonists or antagonists, (iv) direct administration of L-Trp metabolites, and (v) the use of live biotherapeutic products for the potential exploitation of their enzymatic machinery in modulating L-Trp metabolism.

Tryptophan Metabolism

Host Tryptophan Metabolism

In mammalian cells, most L-Trp is metabolized via the KP, while the remainder is utilized in the synthesis of 5-HT and melatonin (MT). In KP, L-Trp is catabolized into the unstable derivative N-formyl-L-kynurenine (NFK) by rate-limiting enzymes **tryptophan 2,3-dioxygenase (TDO)** and **indoleamine 2,3-dioxygenases (IDO1/IDO2)** (Figure 1). Globally, the enzymes of KP are expressed in a tissue-specific manner. *TDO* is expressed in the liver, whereas *IDO1* is expressed in many cell types and tissues and is inducible by cytokines [1]. NFK is rapidly metabolized by kynurenine formamidase (expressed in liver, kidney, and brain) to form L-kynurenine (L-kyn). L-kyn is a crucial metabolite with potent immunoregulatory functions through its binding to AhR [1–3]. L-kyn is mainly metabolized by **kynurenine monooxygenase** (KMO) to form 3-hydroxykynurenine (3-HK). 3-HK is then degraded to 3-hydroxyanthranilic acid (3-HAA) by **kynureninase (KYNU)**. KYNU is subsequently metabolized to 2-amino-3-carboxymuconic 6-semialdehyde (ACMS) by 3-

[1]Sorbonne Université, INSERM, Centre de Recherche Saint-Antoine, CRSA, AP-HP, Saint Antoine Hospital, Gastroenterology Department, F-75012 Paris, France
[2]Paris Centre for Microbiome Medicine FHU, Paris, France
[3]Molecular Pharmacology, Genetics and Medicine, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[4]INRA, UMR1319 Micalis & AgroParisTech, Jouy en Josas, France

*Correspondence:
harry.sokol@aphp.fr (H. Sokol).

hydroxyanthranilic acid 3,4-dioxygenase (3-HAO). The former is expressed in the liver, kidney, central nervous system (CNS), and placenta, while the latter has a broad tissue distribution [3]. ACMS can be cyclized to quinolinic acid (QUIN) or metabolized by the enzyme 2-amino-3-carboxymuconate-semialdehyde decarboxylase (ACMSD), found mainly in the kidney and to a lesser extent in the liver, and responsible for the synthesis of 2-aminomuconic-6-semialdehyde (AMS). AMS is either metabolized by 2-aminomuconic semialdehyde dehydrogenase (AMSD) to result in acetyl-CoA or cyclized nonenzymatically to form picolinic acid (PICA). In the CNS, QUIN is mainly produced by microglia. It acts as a neurotoxic agent on astrocytes mainly by its selective agonist effect on ionotropic glutamate glutamatergic N-methyl-D-aspartate (NMDA) receptors (Figure 2). However, mechanisms determining the engagement of KP in its synthesis remain undetermined [4,5]. QUIN is also a precursor for the *de novo* synthesis pathway for NAD via the enzyme **quinolinate phosphoribosyltransferase (QPRT)** expressed mainly in the liver and kidney. NAD is a cofactor for numerous enzymes involved in cellular energy metabolism, adaptive responses of cells to bioenergetic and oxidative stress, and genome stability. Its deficiency affects tissues that need high cellular energy, such as the brain, gut, and skin, causing pellagra [6]. Finally, PICA is a neuroprotective molecule whose concentration is reduced in the serum of patients with autism, and plasma and cerebrospinal fluid (CSF) of subjects who have attempted suicide [7,8].

Hepatic and cerebral **kynurenine aminotransferase (KAT)** synthesizes kynurenic (KYNA) and xanthurenic acid (XANA) from L-kyn and 3-HK, respectively [3]. Four KATs have been identified in the mammalian brain (KAT I–IV), but KAT II activity accounts for the highest proportion (60%) of the total KAT activity in the mammalian brain [9]. KYNA is a neuroprotective metabolite that acts as an AhR ligand and has an antagonist effect on NMDA and α-amino-3-hydroxy-5-methylisoazol-4-propionate (AMPA) receptors [3]. It also participates in tissue homeostasis and inflammation regulation via its binding to the orphan receptor GPR35 [10,11]. KYNA has been reported to antagonize the α-7 acetylcholine receptor, but this effect remains controversial [12]. Its structural analog XANA acts on AhR and the metabotropic glutamate receptors mGlu2 and mGlu3 [13].

The KP is organized with several intersections, and the flux through various routes with the KP is not equal. This means that the production of different end-products of KP, such as KYNA, XANA, PICA, or QUIN, is not identical, and the balance between them can change according to the situation.

The remaining L-Trp not metabolized in KP leads to intestinal and cerebral 5-HT production through the **Trp hydroxylase (TPH)** enzymes. More than 90% of 5-HT is produced in the gut, particularly in ECCs through TPH1, the activity of which is modulated by intestinal microbiota [14–16]. Synthesis of 5-HT at the central level is carried out via the enzyme TPH2. 5-HT is an important gastrointestinal signaling molecule that conveys signals from the gut to intrinsic or extrinsic neurons and influences intestinal peristalsis, motility, secretion, vasodilatation, and nutrient absorption. Thus, it represents an attractive target for the treatment of several intestinal disorders. 5-HT can be metabolized secondarily to MT, a circadian hormone promoting sleep, but also has anti-inflammatory properties [17].

Microbial Tryptophan Metabolism

Intestinal microorganisms metabolize unabsorbed L-Trp into several molecules, such as indole derivatives [indole-3-aldehyde (IAld), indole-3-acetic acid (IAA), indole-3-propionic acid (IPA), indole-3-acetaldehyde (IAAld), indole-3-lactic acid (ILA), and indole acrylic acid] but also tryptamine and skatole. These metabolites are involved in intestinal permeability, regulation of inflammation, and host immunity [18–24]. Several of these metabolites are ligands for AhR

Glossary

Aryl hydrocarbon receptor (AhR): the AhR transcription factor belongs to the basic helix–loop–helix (bHLH) – Per-Arnt-Sim (PAS) family involved in environment sensing. Cytoplasmic in the basal state, it translocates in the nucleus after fixation of a ligand to induce the transcription of target genes including the cytochromes (cyp) 1a1, 1a2 and 1b1. Pharmacological duality according to the immune or cancerous nature of the pathology to be treated.

Indoleamine 2,3-dioxygenase (IDO) 1: ubiquitous and nonspecific enzyme that catabolizes L-Trp into NFK. IDO1 is inducible by proinflammatory stimuli. Pharmacological target in cancers.

Kynureninase (KYNU): enzyme expressed mainly in the liver and kidneys and responsible for the metabolism of 3-HK to 3-HAA.

Kynurenine aminotransferase (KAT): enzyme which metabolizes L-kyn and 3-HK to KYNA and XANA, respectively. Unlike other KP enzymes, KAT expression decreases in response to inflammatory stimuli favoring the shift of KP toward the production of neurotoxic QUIN. Promising target in schizophrenia where the increase in KYNA leads to the hypofunction of glutamatergic transmission.

Kynurenine monooxygenase (KMO): catalyzes the hydroxylation of L-kyn to form 3-HK. Absent from astrocytes but predominant in microglia where it actively participates in the synthesis of precursors of the neurotoxic metabolite QUIN. Promising target in neurodegenerative diseases to stop the synthesis of deleterious metabolites (3-HK and QUIN).

Probiotics: microorganisms which, when administered live and in adequate amounts to the host, bring beneficial health effects [World Health Organization (WHO)].

Quinolinate phosphoribosyltransferase (QPRT): enzyme responsible for the conversion of the neurotoxic QUIN to coenzyme NAD involved in numerous reactions of energy and tissue homeostasis. The administration of recombinant QPRT could overcome the rapid saturation of the endogenous enzyme and remove the excess of QUIN.

Serotonin (5-HT): endogenous monoamine involved in a wide range of physiological processes such as behavior, vascular function, hemostasis,

[24,25]. Of note, it was recently shown that some of these molecules are not only synthesized by microbiota, but also by tumor cells through the effect of an L-amino acid oxidase, IL-4-induced-1 (IL-4I1), metabolizing L-Trp into indole-3-pyruvic acid and subsequently into IAA, IAld, and ILA, thus allowing escape from the immune system, survival, and tumor motility in an AhR-dependent manner [25]. AhR signaling is considered a vital component of the immune response at barrier sites. Thus, it is crucial for intestinal homeostasis by acting on epithelial renewal, barrier integrity, and many immune cell types, such as intra-epithelial lymphocytes, T helper (Th)17 cells, innate lymphoid cells, macrophage dendritic cells, and neutrophils [26]. Dietary molecules and xenobiotics directly activate AhR. Also, many AhR ligands are processed and inactivated by cytochrome p450 family proteins, such as cyp1a1, which is a direct AhR transcriptional target constituting a feedback loop for AhR signaling [27]. However, some metabolites such as 5-HT act indirectly on AhR through a CYP inhibiting mechanism, thereby expanding receptor ligands [28]. Interestingly, 5-hydroxy indole acetic acid (5-HIAA) is, unlike 5-HT, an AhR agonist [29].

Pharmacological Targeting of L-Trp Metabolism

L-Trp metabolism leads to the production of several essential molecules for host physiology. It is perturbed in many diseases, notably neurological, psychiatric, metabolic, infectious, intestinal diseases, and cancer cells, making it an ideal pharmacological target [5,12–18].

Enzymatic Modulation

Dioxygenases Inhibitors

While present in healthy tissues, an increase and constitutive IDO expression has been described in multiple cancers, contributing to immune suppression and neovascularization [30]. IDO1 inhibitors allow the restoration of immune cell function [1]. These inhibitors are currently being evaluated in Phase I or II clinical trials (indoximod, epacadostat, navoximod, EOS200271, and BMS-986205; Table 1). They are well-tolerated, but their effects as a monotherapy are insufficient. They are now evaluated in combination with immune checkpoint inhibitors. However, the results obtained are mixed [31,32]. A possible explanation for the failure of this combination may result from the induction of IL-4I1 by IDO1, allowing the immune escape of the tumor clone [25]. Under these circumstances, a tritherapy comprising an inhibitor of IDO1, a checkpoint inhibitor, and an inhibitor of IL-4I1 could be an interesting avenue. Research for new IDO1 inhibitors continues actively [25,33]. Beyond cancer, targeting of IDO1 could also concern patients with metabolic disorders since obesity is associated with an increase of IDO1 activity in the gut, which hijacks L-Trp to the KP at the production expense of indoles by the microbiota. Genetic or pharmacological inhibition of IDO1 improves insulin sensitivity, decreases endotoxemia, and regulates lipid metabolism [34]. Initially, TDO was thought to be only constitutively expressed in the liver. However, *TDO* was also shown to be expressed in various cancer cells, including breast cancer, ovarian carcinoma, and gliomas. It is involved in progression and immune suppression through the TDO-L-kyn-AhR pathway, and inhibition of TDO contributes to reverse immune escape [1,35,36]. Currently, TDO inhibitors are mainly in preclinical stages. However, research is moving toward dual *IDO1* and *TDO* inhibitors since both are involved in the pathophysiology of cancer through the synthesis of the immunomodulatory metabolite L-kyn. Inhibitors targeting both enzymes include HTI-1090, DN14066131, RG70099, and EPL-1410. The oral inhibitor HTI-1090 (SHR9146) is being evaluated in a Phase I clinical trial alone or in combination with a programmed cell death protein 1 (PD-1) or vascular endothelial growth factor receptor (VEGFR) inhibitor for the treatment of solid tumors (NCT03208959 and NCT03491631; https://www.clinicaltrials.gov/).

KAT Inhibitors

The impaired glutamatergic activity of the NMDA receptors is involved in schizophrenia, with a potential role for the NMDA antagonist KYNA. A meta-analysis of 13 studies showed an elevation

hepatic regeneration, intestinal motility, insulin secretion, erythropoiesis, adipocytes differentiation, immune responses, and the fibrosis process.

Tryptophan 2,3-dioxygenase (TDO): specific enzyme that catabolizes the same reaction as IDO at physiological level. Constitutively expressed on the liver, it is also involved in physiopathology of many cancers where it plays a role in immune escape. Pharmacological target in cancers.

Tryptophan hydroxylase (TPH): 5-HT synthesis enzyme from L-Trp. Tph1 and Tph2 isoforms are responsible, respectively, for the synthesis of 5-HT in ECCs of the intestine, pancreatic β cells, fat cells, lung, pineal gland, CNS, and enteric nervous system neurons. Interesting pharmacological target for inhibiting pathophysiological mechanisms such as inflammation, angiogenesis, fibrosis, and cell proliferation. Pharmacological target in intestinal diseases associated with transit disorders.

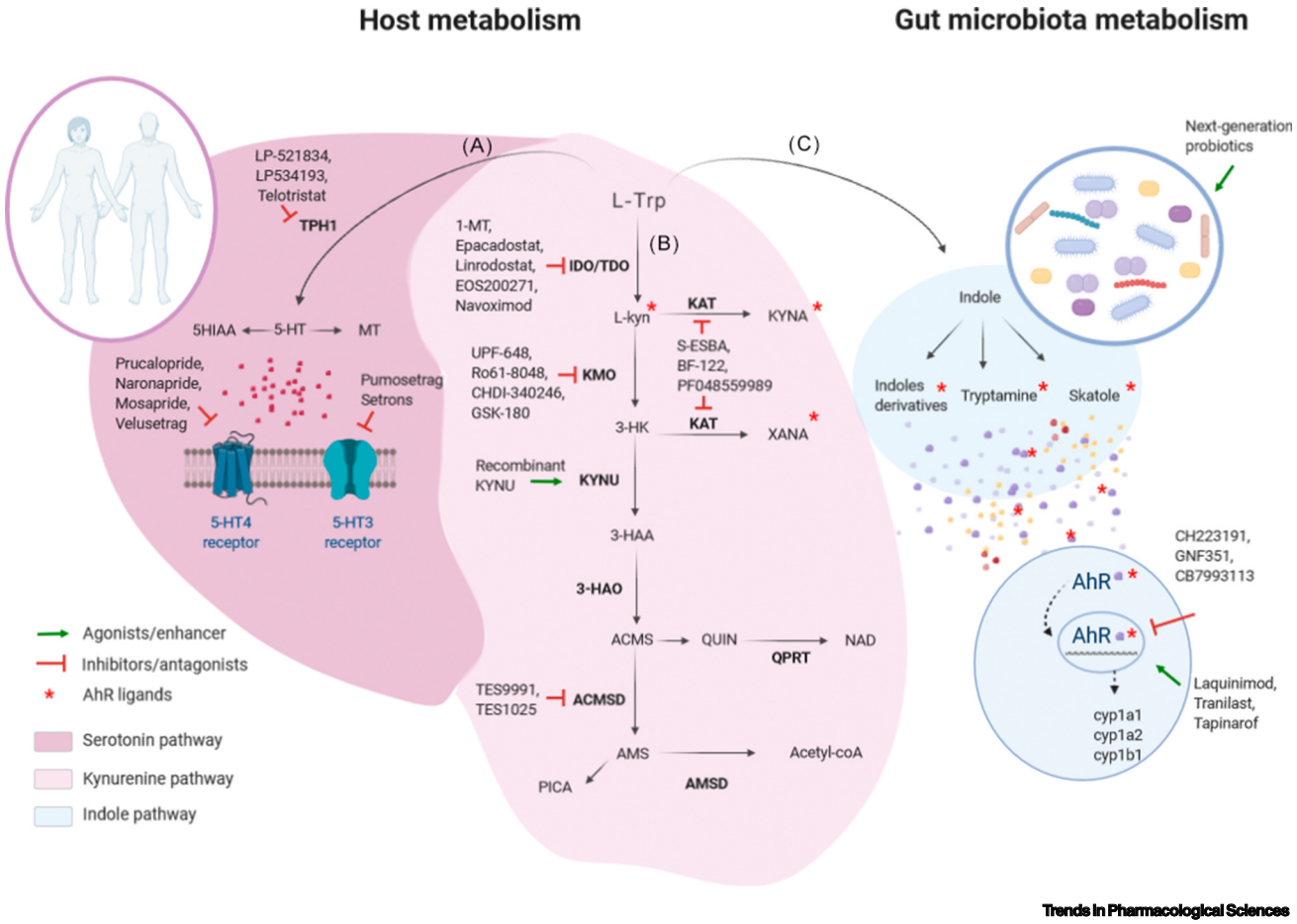


Figure 1. L-Trp Metabolism and Pharmacological Targets. Host L-Trp metabolism follows the 5-HT and the kynurenine pathways in which several pharmacological targets are currently under development. (A) L-Trp is metabolized to 5-HT which can either be degraded to 5-HIAA or metabolized to MT. Pharmacological targets are the rate-limiting enzyme in 5-HT synthesis TPH and the peripheral 5-HT receptors. (B) The L-kyn pathway involves a series of enzymatic reactions which constitute potential therapeutic targets. (C) Unabsorbed L-Trp is metabolized by intestinal microorganisms into several molecules, such as indole derivatives, tryptamine, and skatole, some of which are AhR ligands. After binding of its ligand in the cytosol, AhR translocates in the nucleus and binds to specific DNA sequences resulting in the transcription of target genes including those of the cytochrome p450 family. AhR could be targeted by antagonist or agonist molecules. Administration of next-generation probiotics could also modulate L-Trp metabolism. Abbreviations: 1-MT, 1-methyltryptophan; 3-HAA, 3-hydroxyanthranilic acid; 3-HAO, 3-hydroxyanthranilic acid 3,4-dioxygenase; 3-HK, 3-hydroxykynurenine; 5HIAA, 5-hydroxyindole acetic acid; 5-HT, serotonin; ACMS, 2-amino-3-carboxymuconic acid-6-semialdehyde; ACMSD, 2-amino-3-carboxymuconate-semialdehyde decarboxylase; AhR, aryl hydrocarbon receptor; AMSD, 2-aminomuconic semialdehyde dehydrogenase; IDO, indoleamine 2,3-dioxygenase; KAT, kynurenine aminotransferase; KMO, kynurenine monooxygenase; KYNA, kynurenic acid; KYNU, kynureninase; L-kyn, L-kynurenine; L-Trp, L-tryptophan; MT, melatonin; NAD, nicotinamide; PICA, picolinic acid; QPRT, quinolinate phosphoribosyltransferase; QUIN, quinolinic acid; TDO, tryptophan 2,3-dioxygenase; TPH1, tryptophan hydroxylase 1; XANA, xanthurenic acid.

of KYNA in the CSF and CNS of patients with schizophrenia. In rats, the cerebral level of KYNA has been positively correlated with cognitive deficits similar to the ones observed in schizophrenia [37–39] (Figure 2). In this context, KAT inhibitors showed promising effects on cognitive functions, likely through decreased production of KYNA [40]. Several KAT inhibitors, such as PF-04859989, BFF-122, and S-ESBA (Table 2), have been identified. PF-04859989 and BFF-122 act as KAT II inhibitors by irreversibly binding to the pyridoxal phosphate (PLP) cofactor [41,42]. This vitamin B6-derived coenzyme plays a role in a wide variety of enzymatic reactions. However, because over 300 PLP-dependent enzymes and proteins have been identified, irreversible binding to PLP may cause side effects. Such is the case with carbidopa that irreversibly binds to free PLP and PLP-dependent enzymes [43].

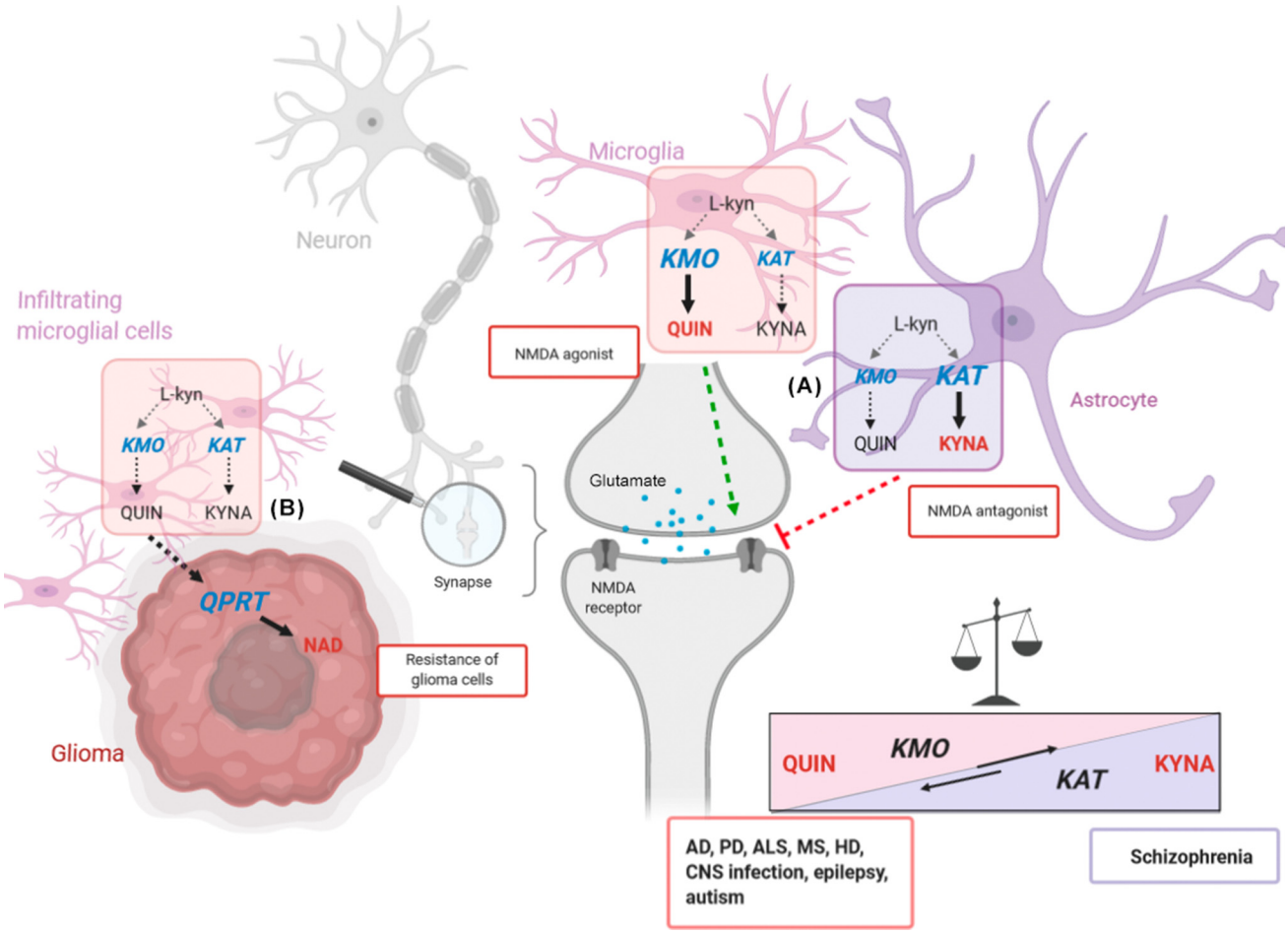
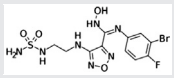
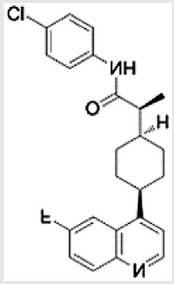
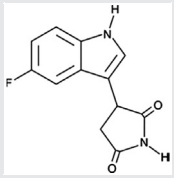
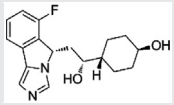


Figure 2. Pathological Deregulation of L-Trp Metabolism in the CNS. (A) In the CNS, KYNA is mainly produced by astrocytes while QUIN is produced in microglia by KAT and KMO, respectively. Any imbalanced ratio of these two pathways leads to pathological repercussions. (B) As glioma cells are unable to produce QUIN, microglial production of QUIN and its transformation into NAD by glioma cells allows their proliferation and survival. Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; CNS, central nervous system; HD, Huntington's disease; KAT, kynurenine aminotransferase; KMO, kynurenine monooxygenase; KYNA, kynurenic acid; L-kyn, L-kynurenine; L-Trp, L-Tryptophan; MS, multiple sclerosis; NMDA, N-methyl-D-aspartate; PD, Parkinson's disease; QUIN, quinolinic acid.

Moreover, recently a central function for vitamin B6 and PLP in the homeostatic host–microbiota crosstalk through L-Trp metabolism has been shown [44]. It may be hazardous to exacerbate an imbalance in L-Trp metabolism in patients since dysregulation of host–microbiota homeostasis has shown its involvement in many diseases. S-ESBA has shown interesting effects in rats, but its inhibitory activity on the human enzyme is too weak to consider its clinical use [45]. Given potential toxicity or lack of efficacy, these molecules have not reached the threshold for clinical trials, and research has moved toward the development of inhibitors targeting the active site of the enzyme [46].

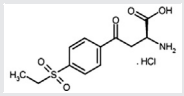
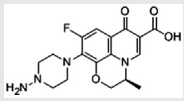
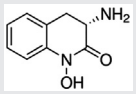
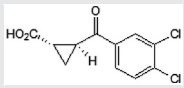
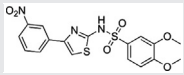
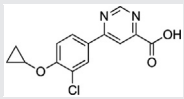
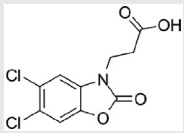
Finally, KAT inhibition by itself might be detrimental. In inflammatory bowel disease (IBD), the increased KAT activity is thought to be a compensatory mechanism for modulating inflammation and reducing cytotoxicity [47]. However, it should be noted that the elevation of KYNA observed in schizophrenia could be caused by an increase of peripheral L-kyn synthesis, making it more available for its passage through the blood–brain barrier (BBB) [38]. Also, the elevation of KYNA in patients with

Table 1. List of Currently Investigated IDO1 Inhibitors[a]

Molecule	Structure and properties	Investigations	Published studies	Active or recruiting studies[b]
1-MT-L-Trp	Analog of L-Trp Nonspecific competitive inhibitor of IDO1 Increases the effectiveness of anticancer drugs and increases KYNA *in vivo* and *ex vivo* regardless of IDO	Fundamental research [83]	Advanced malignancies: well tolerated (monotherapy) [67]	Phase I/II: breast (NCT01042535, NCT01792050), pancreatic (NCT02077881), prostate (NCT01560923), non-small cell lung cancer (NCT02460367), solid (NCT00567931, NCT01191216), brain tumors (NCT04049669, NCT02052648, NCT02502708), leukemia (NCT02835729), and melanoma (NCT03301636, NCT02073123)
1-MT-D-Trp (indoximod)	Low *in vitro* activity but effective *in vivo*, preferentially inhibit IDO2 May promote tumor growth by off-target effect Prodrug: NLG802	Cancers (alone or in combination) [84,85]		
Epacadostat INCB024360 	Selective reversible competitive inhibitor of IDO1 Antitumoral (decreases Tregs, increases synthesis of IFNγ by T cells) but lack of activity as a monotherapy Metabolized by the intestinal microbiota and the enzyme UGT1A9 (AhR target)	Cancers (only in combination) [86,87]	Ovarian cancer: no benefit [88] Tumors: well tolerated and had encouraging antitumor activity [89] Metastatic melanoma: no benefit [31]	Phase I/II: thymic carcinoma (NCT02364076), naso-pharyngeal (NCT04231864), gastric (NCT03196232), gastrointestinal (NCT03291054), pancreatic (NCT03006302), urothelial bladder (NCT03832673), non-small cell lung (NCT03322566, NCT03322540), and rectal (NCT03516708) cancers, melanoma (NCT01961115), sarcoma (NCT03414229), metastatic solid tumors (NCT03347123) Phase III: urothelial (NCT03361865, NCT03374488) and renal carcinoma (NCT03260894), head and neck carcinoma (NCT03358472)
Linrodostat BMS-986205 	Potent, selective, and irreversible IDO1 inhibitor, restores T-cell proliferation and reduces intratumoral L-kyn up to 90%	Cancers [90–92]	Tumors: well tolerated (± nivolumab), need further investigations for efficacy [91]	Phase I/II: pharmacokinetics (NCT03378310, NCT03312426) and safety (NCT03192943), Endometrial (NCT04106414), liver (NCT03695250), gastric (NCT02935634) head and neck (NCT03854032) and bladder (NCT03519256) cancers, solid tumors (NCT03792750, NCT03459222, NCT02658890) glioblastoma (NCT04047706) Phase III: bladder cancer (NCT03661320, NCT03661320), melanoma (NCT03329846)
EOS200271 	IDO1 specific non-competitive inhibitor Oral use Brain permeable	Glioma Association with PD-L1 inhibitors [93,94]	Malignant glioma: well tolerated [94]	
Navoximod, GDC-0919, or NLG-919 	Moderately selective noncompetitive reversible inhibitor Dose-dependent activation and proliferation of effector T cells, Regression of large established tumors Synergy with indoximod Increases survival (± chemotherapy) currently optimized by prodrug formulation	Cancers [96]	Recurrent advances solid tumors: well tolerated and reduced plasmatic L-kyn [97]	Phase I/II: solid tumors (NCT02471846, NCT02048709)

[a]Abbreviations: IFN, interferon; Treg, regulatory T cell.
[b]Clinical trials can be accessed at https://www.clinicaltrials.gov/.

Table 2. List of Currently Investigated KAT and KMO Inhibitors

Molecule	Structure and properties	Investigations
KAT inhibitors		
S-ESBA 	L-kyn analog Selective competitive inhibitor of KAT II Decreases KYNA and increases dopamine in rat brain but has a very low activity against human KAT II Synergizes with QUIN in the induction of striatum lesions	Discontinued (weak human KAT inhibitor) [49,98]
BF-122 	Levofloxacine analog Potent human KAT II inhibitor Decreases KYNA without affecting QUIN and increases dopamine	Discontinued (pyridoxal phosphate binding) [49,98]
PF-04859989 	Selective and irreversible inhibitor Brain-penetrable Decreases KYNA in the prefrontal cortex (50%) Restores nicotine-induced glutamatergic activity Low oral bioavailability and rapid metabolism Lead compound for the synthesis of numerous derivatives	Discontinued (pyridoxal phosphate binding) [49,98]
KMO inhibitors		
UPF648 	L-kyn derivative Selective inhibitor Increases KYNA (dose-dependent), decreases QUIN and 3-HK synthesis Protects against QUIN-mediated toxicity	[49]
Ro61-8048 	L-kyn derivative Inhibits QUIN synthesis Increases KYNA Analgesic effect	Parkinson's, Alzheimer's, Huntington's Addiction treatment Multiple sclerosis, CNS infection [49,99]
CHDI-340246 	Chlorine derivative Low BBB pass Increases striatal and plasma L-kyn, KYNA Decreases plasma 3-HK	Huntington's [49,51,100]
GSK-180 	Oxazolidinone derivative Potent and specific but low cell penetration	Acute pancreatitis [101]

neurodegenerative disease remains controversial. Recently, an increase in KYNA has been shown in the CSF of patients with Alzheimer's disease [48]. This underlines the need to deepen our knowledge of the regulatory mechanisms of KP to provide more targeted therapeutic interventions. A more detailed review of the development of the KAT inhibitors is provided by Jacobs *et al.* [49].

KMO Inhibitors

KMO is located at a critical branching point in KP, leading to the synthesis of downstream metabolites 3-HK and QUIN. Given the deleterious effects of these two metabolites as observed in neurodegenerative diseases, epilepsy, autism, CNS infection (Figure 2), acute pancreatitis, pain, and certain cancers, KMO represents a key target in the treatment of these conditions. *KMO* is mainly expressed in microglia, where its inhibition induces a decrease of 3-HK and QUIN and shifts L-kyn metabolism toward the production of neuroprotective KYNA [50,51]. KMO inhibitors include halogenated L-kyn derivatives (UPF648, Ro61-8048), chlorine derivative (CHDI-340246), and

chlorinated benzisoxazole (GSK-180). However, all are still at the preclinical phase of development (Table 2). Peripheral KMO inhibition is sufficient to increase cerebral levels of KYNA via increasing L-kyn transport to the brain. However, KMO inhibitors that cross the BBB would be more attractive for treating neuropsychiatric conditions and are currently being developed [52]. Finally, diclofenac, a potent non-steroidal anti-inflammatory drug, was recently shown to exhibit a KMO inhibition effect, which can account for some of its efficacy [53–55].

KYNU Inhibitors

While cancers are characterized by upregulation of *IDO1*, dermatological diseases such as psoriasis or atopic dermatitis are associated with an increased expression of *KYNU*, which correlates with the severity of the disease and tends to decrease with anti-inflammatory treatments [56]. To date, no efficient KYNU inhibitors have been developed. The use of this type of molecule may be limited by the inhibitory role of KYNU on cancer cell proliferation [57].

QPRT Inhibitors

QPRT is involved in the *de novo* synthesis of NAD, an essential cofactor for many cellular functions, including oxidative phosphorylation, macrophage physiology, and global immune system homeostasis. This cofactor is crucial for the survival of glioma cells. The neoplastic transformation of normal astrocytes into glioma cells is associated with a QPRT-mediated switch in NAD metabolism. It exploits microglia-derived QUIN as an alternative source to replenish intracellular NAD pools [58]. Blocking *QPRT* seems attractive for glioma treatment, but it causes an accumulation of neurotoxic QUIN, which is linked to many neurological disorders such as Parkinson's, autism, epilepsy, Huntington disease, multiple sclerosis, Alzheimer's disease, and depression [7,8,59–61]. To date, the only known inhibitor of QPRT is phthalic acid, a QUIN analog acting as a moderately potent competitive inhibitor leading to increased urinary excretion of QUIN [62]. Moreover, as NAD is essential for global metabolic and immune homeostasis, QPRT inhibitors for cancer therapy could target malignant cells selectively.

ACMSD Inhibitors

By blocking the pathway leading to PICA and acetyl-CoA, ACMSD inhibitors such as phthalate esters shift the KP toward QUIN synthesis and downstream metabolite NAD. However, phthalate esters being endocrine disruptors are excluded from any therapeutic use. Thus, other molecules, such as TES-991 and TES-1025, have been developed [63,64]. The first proved to reduce hepatic steatosis, inflammation, and hepatic lipid accumulation in a murine model of non-alcoholic fatty liver disease. Simultaneously, the second had a protective effect in a mouse model of acute kidney injury [63]. It is important to emphasize that the synthesis of NAD requires the metabolism of QUIN by QPRT. This enzyme being easily saturable, the efflux of QUIN secondary to the inhibition of ACMSD could lead to the deleterious accumulation of this neurotoxic metabolite [8]. There is currently no attempt to inhibit ACMSD in humans pharmacologically and, NAD supplementation, which is much safer, is preferred.

Recombinant KYNUs

Beyond *IDO/TDO* inhibition, L-kyn depletion can rely on the use of recombinant KYNU. Because the human enzyme preferentially degrades 3-HK, research is directed toward bacterial KYNUs, which have a significant catalytic activity toward L-kyn. Bacterial polyethylene glycol (PEG)ylated KYNUs can deplete L-kyn produced by human cancer cells expressing *IDO1* and *TDO*. A single subcutaneous dose of KYNU can deplete L-kyn in both plasma and tumors and increase the intratumoral effector T-cells in mice [65]. KYNU combined with anti-PD-1 showed greater efficacy than epacadostat/anti-PD-1 combination in tumor-bearing mice and resulted in complete tumor eradication in 60% of the animals [66].

TPH Inhibitors

Besides its physiological functions, 5-HT is involved in cancer (including carcinoid syndrome), gastrointestinal disorders, thrombosis, inflammation, diabetes, obesity, pulmonary hypertension, and fibrosis. Inhibiting its production by targeting TPH is thus a strategy explored in these indications. Several TPH inhibitors have been developed (LP-521834, LP-534193, and telotristat), but only telotristat is currently used. This molecule is a non-brain-permeable TPH inhibitor recently approved by the FDA to treat diarrhea in carcinoid syndrome combined with a somatostatin analog [67]. It also reduces the severity of dextran sodium sulfate (DSS)-induced colitis in mice and increases the number of goblet cells that produce protective mucus in the colon [68].

Receptor Modulators

5-HT Receptors

5-HT3 and 5-HT4 receptors expressed in the intestine are the subjects of clinical investigations for the treatment of gastrointestinal disorders, notably irritable bowel syndrome (IBS). The 5-HT3 receptor is expressed on excitatory cells, including afferent and efferent nerve fibers. It is the target for antagonists belonging to the class of 'setrons' useful for treating nausea and vomiting during chemotherapy. As one of their side effects is constipation, they have also been investigated in the treatment of predominantly diarrheal IBS. Conversely, the 5-HT3 receptor could also be targeted with partial agonists such as pumosetrag for predominantly diarrheal IBS treatment to decrease receptor activation when the 5-HT concentration in the cellular environment is too high. The 5-HT4 receptor is expressed on ECCs and enteric neurons. It facilitates the release of acetylcholine to relax colonic smooth muscles. This receptor is targeted by prokinetic agents used in the treatment of constipation associated or not with IBS, but the lack of selectivity of the first molecules, such as tegaserod or cisapride, has led to the development of more selective 5-HT4 receptor agonists including prucalopride, naronapride, mosapride, and velusetrag, which have been marketed (except naronapride).

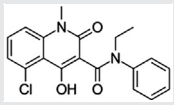
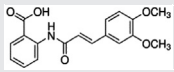
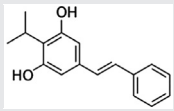
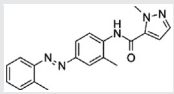
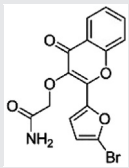
AhR

AhR is an attractive therapeutic target in autoimmune, cancerous, neurodegenerative, or intestinal disorders owing to its involvement in a wide variety of physiological and pathological processes. In oncology, AhR is involved in an immunotolerance loop that allows tumor escape and receptor antagonists, such as CH223191, CB7993113, and GNF351 (Table 3) have been used as antitumor agents. However, because of the pleiotropic effect of AhR, its inhibition could lead to deleterious consequences.

StemRegenin 1 (SR1) is a compound originally isolated from donor blood for its ability to increase the number of CD34+ cells for hematopoietic stem cell transplantation (HSC) [69,70]. Its *ex vivo* use makes it possible to overcome the pleiotropic effects of AhR antagonism, and the molecule is currently under clinical investigation for malignant hemopathies and neutropenia treatment. SR-1 is also named HSC-835 or MGTA-456.

Research on AhR mainly focuses on its anti-inflammatory potential via the use of agonist molecules such as tranilast, laquinimod, and tapinarof (Table 3). The former is already marketed for the treatment of bronchial asthma. It is currently undergoing Phase II (rheumatoid arthritis) and Phase III (hyperuricemia, pterygium) clinical trials. Beyond AhR activation, it has many other functions widely reviewed in [71]. Laquinimod is a KYNA-like molecule with a quinoline structure, currently under development for the treatment of multiple sclerosis, active lupus arthritis (NCT01085084), lupus nephritis (NCT01085097), and Huntington's disease (NCT02215616). It has shown positive results in a Phase III randomized controlled trial (RCT) in Crohn's disease [72], but the effects were mixed in multiple sclerosis. Tapinarof is in Phase III clinical trials for psoriasis treatment. Beyond conventional

Table 3. List of Currently Investigated AhR Agonists and Antagonists

Molecule	Structure and properties	Investigations	Published studies	Active or recruiting studies[c]
AhR agonists				
Laquinimod 	Quinoline 3-carboxamide structural similar to KYNA AhR-dependent effects on encephalomyelitis Mixed results (Phase II and III clinical trials – multiple sclerosis) Allows remyelination	Huntington's Multiple sclerosis Crohn's disease [102,103]	Multiple sclerosis: well tolerated, significant reduction in brain atrophy [104,105] Crohn's disease: well tolerated, promising effects [72]	Phase I/II: efficacy and safety in relapsing multiple sclerosis (NCT01047319), Huntington's disease (NCT02215616), lupus arthritis (NCT01085084),lupus nephritis (NCT01085097), Crohn's disease (NCT00737932), relapsing multiple sclerosis (NCT01975298)
Tranilast 	Synthetic analog of ANA	Asthma (marketed) Rheumatoid arthritis Multiple sclerosis Hyperuricemia Cancer [71]	Prostate cancer: benefit on prognosis [71]	Phase I/II: mucinoses (NCT03490708) scleredema diabeticorum (NCT03512873) sarcoidosis (NCT03528070), cryopyrin-associated periodic syndrome (NCT03923140), pterygium (NCT01003613), hyperuricemia (NCT00995618, NCT01052987), gout (NCT01109121), rheumatoid arthritis (NCT00882024)
Tapinarof (benvitimod) 	Bacterial stilbene Free radical scavenger Dermal application	Psoriasis atopic dermatitis [106]	Psoriasis and atopic dermatitis: well tolerated [107,108]	Phase I/II: safety, tolerability, and pharmacokinetics of tapinarof cream, 1% (extensive plaque psoriasis) (NCT04042103) Phase III: efficacy and safety of topical tapinarof cream, 1% (plaque psoriasis) (NCT03956355)
AhR antagonists				
CH223191 	Competitive selective antagonist No antagonistic activity with non-HAH ligands[a]	Fundamental research but may be a promising effect in pancreatic cancer [109]	No active clinical trials	
CB7993113 	Good oral bioavailability Blocks tumor cell migration and reduces the invasive phenotype of ER–/PR–/HER2–[b] breast cancer cells *in vitro*	[70,95]		
StemRegenin-1	*Ex vivo* application Expand CD34+ cells	Stem cell transplantation Neutropenia Thrombocytopenia	CD34+ cell expansion [69,70]	Malignant hemopathies (NCT01474681 and NCT01930162) Neutropenia and thrombocytopenia (NCT03406962)

[a]Non-HAH ligands (halogenated aromatic hydrocarbons) include polycyclic aromatic hydrocarbons (PAHs) as well as endogenous L-Trp ligands. HAHs are distinguished from PAHS and endogenous ligands by very slow metabolism and a prolonged effect on the AhR receptor.
[b]Abbreviations: ER, estrogen receptor; HER, human epidermal growth factor receptor 2; PR, progesterone receptor.
[c]Clinical trials can be accessed at https://www.clinicaltrials.gov/.

agonists and antagonists, the new concept of selective AhR modulators (SAhRMs) designates any AhR ligand that lacks agonist activity but can repress the expression of acute inflammatory phase genes. These molecules are reviewed in [73].

Administration of KYNA and its Derivatives

To date, neuroprotective KYNA is the only KP metabolite directly used for therapeutic purposes. It is well-tolerated by the dermal route and could be used in the prevention of scars. It does not cross the skin barrier, thus avoiding systemic effects [74]. Owing to its short half-life and reduced

brain permeability, chlorinated analogs crossing the BBB have been synthesized, such as 4-chloro-kynurenine. This KYNA prodrug has an anti-epileptogenic effect by reducing seizure duration, hippocampal lesions, and has an antidepressant effect [75]. KAT metabolizes it to 7-chloro-kynurenic acid, an NMDA receptor inhibitor. Despite encouraging results *in vivo* and good tolerance in humans, a Phase II RCT (NCT02484456) did not affect the treatment of depression [76–78]. The AhR agonist laquinimod, mentioned earlier, is also a derivative of KYNA. At present, no other metabolite of KP has been investigated.

Beyond KP, several molecules derived from the bacterial metabolism of L-Trp have shown promising effects *in vivo* on colitis and metabolic syndrome through their agonist effect on AhR [21,24,79]. Thus, the administration of live microbes able to metabolize L-Trp into therapeutic indole derivatives could also be envisaged.

Next-Generation Probiotics

With the rapid improvement of knowledge on intestinal microbiota, a significant effort to identify and characterize new microbial strains with therapeutic potential isolated from the intestine (live biotherapeutic products) has been carried out to target mechanisms of action and diseases [80]. For example, the administration of *Lactobacillus*, which naturally produces AhR agonists, improves colitis severity in mice and dietary-induced metabolic impairments, suggesting therapeutic interventions for IBD [24] and metabolic disorders [79]. Next-generation **probiotics** have been mostly identified based on comparative analysis of microbiota compositions between healthy and unhealthy individuals [81]. They also include recombinant microorganisms overexpressing genes of interest and could represent an excellent alternative approach to modulate host physiology. Genes of interest could be human genes with therapeutic potential or bacterial genes involved in the synthesis of indole derivatives. The main obstacles to developing next-generation probiotics are the lack of a clearly defined regulatory pathway and the manufacturing, as many of these microorganisms require highly demanding conditions to grow [82]. However, probiotics may allow local delivery of desired metabolites and modulate other beneficial signaling pathways in the host.

Concluding Remarks

While the immunomodulatory role of metabolites such as L-kyn and KYNA seems protective, a deleterious function is attributed to them in cancer and schizophrenia, respectively. In addition, QUIN and 3-HK are involved in neurodegenerative diseases. The former is also the precursor of the essential cofactor NAD. This same duality is found for AhR since the therapeutic strategy differs according to the disease reserving the agonists for inflammatory diseases and the antagonists for targeting cancer. Targeting 5-HT receptors can also have dual effects, depending on the context. The metabolism of L-Trp is thus a promising therapeutic target. However, it requires in-depth knowledge of the regulatory mechanisms and their interconnections to define the appropriate intervention for each supposed indication, and to have the ability to precisely act on the targeted metabolite or enzyme (see Outstanding Questions).

Besides the multiple molecules currently under clinical investigation, next-generation probiotics carrying the appropriate enzymatic machinery for the synthesis of indoles are also in development. They offer numerous possibilities for modulation of the pleiotropic receptor AhR.

Outstanding Questions

What are the consequences of blocking one L-Trp metabolism pathway on the others (undesirable effects, disease development)?

How do you selectively target a specific enzyme or metabolite in cells of interest?

What are the effects of indole derivatives produced by bacteria on KP and on the 5-HT production pathway?

What are the interconnections between the three major pathways of L-Trp metabolism?

What are the precise regulatory mechanisms of KP?

What would be the implications of development of kynureninase inhibitors?

How do you leverage gut microbiota to modulate L-Trp metabolism in a therapeutic perspective?

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