



Original Research

Molecular Insights into the Interaction of Tryptophan Metabolites with the Human Aryl Hydrocarbon Receptor *in Silico*: Tryptophan as Antagonist and no Direct Involvement of Kynurenine

Abdulla A-B Badawy^{1,*} , Shazia Dawood² 

¹Formerly School of Health Sciences, Cardiff Metropolitan University, CF5 2YB Wales, UK

²Pharmacy and Allied Health Sciences, Iqra University, 7580 Karachi, Pakistan

*Correspondence: badawyabdulla@yahoo.com (Abdulla A-B Badawy)

Academic Editor: Catarina M. Paquete

Submitted: 30 June 2024 Revised: 8 August 2024 Accepted: 21 August 2024 Published: 24 September 2024

Abstract

Background: A direct link between the tryptophan (Trp) metabolite kynurenine (Kyn) and the aryl hydrocarbon receptor (AhR) is not supported by metabolic considerations and by studies demonstrating the failure of Kyn concentrations of up to 100 μ M to activate the receptor in cell culture systems using the proxy system of cytochrome *P*-450-dependent metabolism. The Kyn metabolite kynurenic acid (KA) activates the AhR and may mediate the Kyn link. Recent studies demonstrated down regulation and antagonism of activation of the AhR by Trp. We have addressed the link between Kyn and the AhR by looking at their direct molecular interaction *in silico*. **Methods:** Molecular docking of Kyn, KA, Trp and a range of Trp metabolites to the crystal structure of the human AhR was performed under appropriate docking conditions. **Results:** Trp and 30 of its metabolites docked to the AhR to various degrees, whereas Kyn and 3-hydroxykynurenine did not. The strongest docking was observed with the Trp metabolite and photooxidation product 6-Formylindolo[3,2-*b*]carbazole (FICZ), cinnabarinic acid, 5-hydroxytryptophan, N-acetyl serotonin and indol-3-ylactic acid. Strong docking was also observed with other 5-hydroxyindoles. **Conclusions:** We propose that the Kyn-AhR link is mediated by KA. The strong docking of Trp and its recently reported down regulation of the receptor suggest that Trp is an AhR antagonist and may thus play important roles in body homeostasis beyond known properties or simply being the precursor of biologically active metabolites. Differences in AhR activation reported in the literature are discussed.

Keywords: biological factors; cytochrome *P*-450-catalysed reactions; gene deletion; ligand binding; molecular docking; tryptophan metabolism

1. Introduction

The present study illustrates the value of metabolism in revealing important principles and resolving controversial issues in biomedical research, namely the link between the tryptophan (Trp) metabolite kynurenine (Kyn) and the aryl hydrocarbon receptor (AhR). The AhR is a major player in body homeostasis. In addition to control of xenobiotic metabolism, it is involved in many cellular processes, including cellular differentiation, stem cell maintenance and immune function [1–3]. Its involvement in immune function has been the subject of intense investigation ever since it was recognised that its activation by the industrial pollutant dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin or TCDD) induces immunosuppression in humans and animals [4]. Activation of the AhR can be either beneficial or harmful, hence its description as “friend or foe?” [4]. It is activated not only by industrial toxins such as TCDD, polycyclic hydrocarbons and many other chemicals, but also by endogenous ligands. Some endogenous ligands, such as indole, indirubin and the tryptophan (Trp) photooxidation product and microbial metabolite 6-formylindolo[3,2-*b*]carbazole (FICZ) are potent activators (see [5] and references cited therein). It is also activated by various Trp

metabolites (Fig. 1, Ref. [6]) and by those produced by gut microbiota (Fig. 2, Ref. [6]) [7]. The physiological implications of AhR activation by Trp metabolites are numerous and are determined by extent of activation, type of immune cells targeted and biological transformations of metabolites catalysed in part by the AhR-linked cytochrome *P*-450 system [4]. Surprisingly, the parent compound Trp itself has not been investigated for interaction with the AhR until only recently [8,9] and by us in the present work (see the Discussion below). Kyn interaction with the AhR received much attention and is believed to underpin the Kyn effects on immune function, e.g., promotion of T-regulatory (Treg) cell development, suppression of T helper type 17 (Th17) differentiation and reduction of Interleukin 13 (IL-13) and IL-10 and Interferon gamma (IFN- γ) production [10,11]. Kynurenic acid (KA) possesses both pro- and anti-inflammatory properties [12] and while its protect against inflammation in certain circumstances, it can be associated with pathological states in others, e.g., cancer. Interaction of other Kyn metabolites with the AhR has received little attention. By contrast, Trp metabolites formed by intestinal microbiota (Fig. 2) have been the focus of AhR interaction. Indole and its derivatives retaining the indole structure are



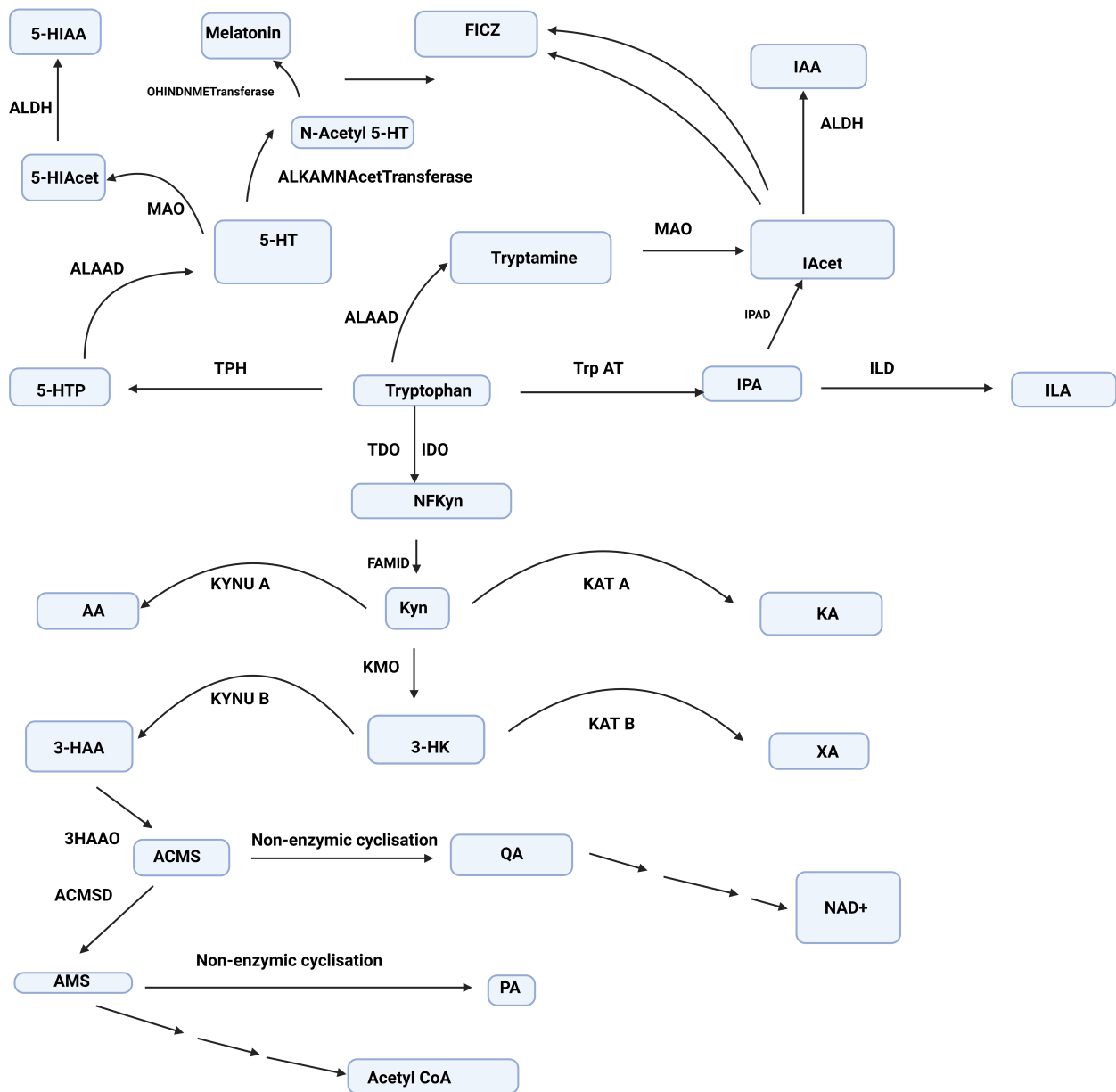


Fig. 1. The tryptophan metabolic pathways. This Figure is based on Fig. 1 in [6] by Badawy AA-B. Tryptophan metabolism and disposition in cancer biology and immunotherapy. *Biosci Rep* 2022; 42: BSR20221682. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9653095/>. Abbreviations used: Acet, acetaldehyde; ACMS, 2-Amino-3-carboxymuconic acid-6-semialdehyde: also known as acrolein aminofumarate; ACMSD, 2-Amino-3-carboxymuconic acid-6-semialdehyde decarboxylase; ALAAD, aromatic *L*-amino acid decarboxylase; AlcAm, alkyl amino; ALDH, aldehyde dehydrogenase; AMS, 2-Aminomuconic acid -6-semialdehyde; AA, anthranilic acid; FAMID, N-formylkynurenine formamidase; FICZ, 6-formylindolo[3,2-b]carbazole; 3-HAA, 3-hydroxyanthranilic acid; 5-HIAcet, 5-hydroxyindoleacetaldehyde; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HIAcet, 5-hydroxyindoleacetaldehyde; 3-HK, 3-hydroxykynurenine; 5-HT, 5-hydroxytryptamine or serotonin; 5-HTP, 5-hydroxytryptophan; IDO, indoleamine 2,3-dioxygenase; IAcet, indole acetaldehyde; IAA, indol-3-ylacetic acid; ILA, indol-3-ylactic acid; ILD, indole lactate decarboxylase; IPAD, indolepyruvic acid decarboxylase; IPA, indol-3-ylpyruvic acid; KA, kynurenic acid; KYNU, kynureninase; KAT, kynurenine aminotransferase; NFKyn, N-formylkynurenine; MAO, monoamine oxidase; NAD⁺, oxidised nicotinamide-adenine dinucleotide; OHIndole, hydroxyindole; OHINDMETransferase, hydroxyindole methyl transferase; PA, picolinic acid; QA, quinolinic acid; TDO, tryptophan 2,3-dioxygenase; Trp AT, tryptophan aminotransferase; TPH, tryptophan hydroxylase; XA, xanthurenic acid; kyn, kynurenine; KMO, Kynurenine-3-monooxygenase; 3HAAO, 3-hydroxyanthranilate 3,4 dioxygenase.

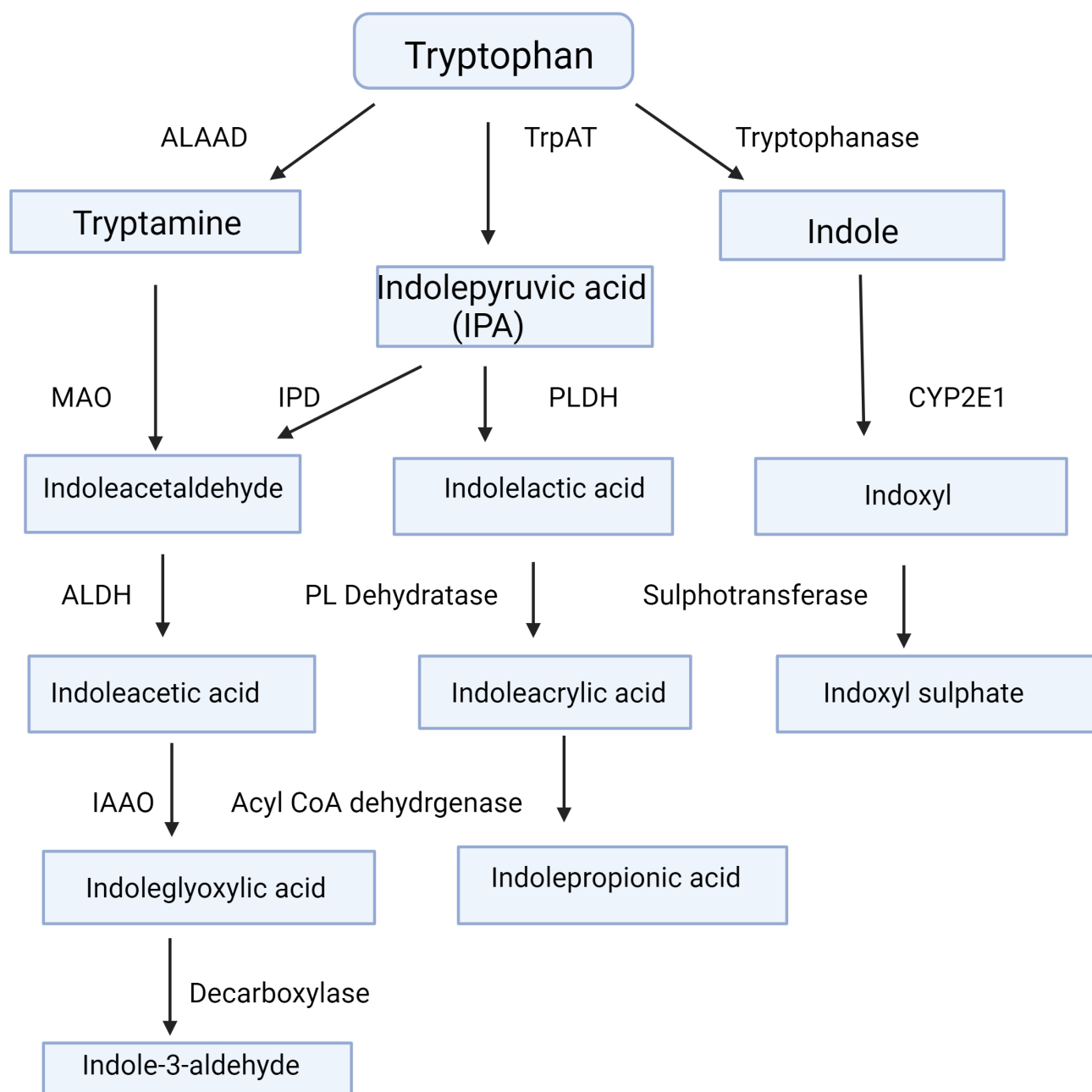


Fig. 2. Tryptophan metabolism by gut microbiota. This Figure is based on Fig. 2 in [6] by Badawy AA-B. Tryptophan metabolism and disposition in cancer biology and immunotherapy. *Biosci. Rep* 2022; 42: BSR20221682. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9653095/>. Abbreviations: ALAAD, aromatic *L*-amino acid decarboxylase; ALDH, aldehyde dehydrogenase; CYP2E1, cytochrome *P*-450 2E1; IAAO, indoleacetic acid oxidase; IPD, indolepyruvate decarboxylase; MAO, monoamine oxidase; PL Dehydratase, phenyllactate dehydratase; PLDH, phenyllactate dehydrogenase; TrpAT, tryptophan aminotransferase.

all AhR ligands, with varying binding capacities. Their interaction with the AhR enables them to exhibit antiinflammatory properties and exert protective effects supporting intestinal homeostasis [13–16].

The majority of previous studies however used the ability of potential AhR ligands to activate cytochrome *P*-450-dependent metabolism, particularly that catalysed by Cytochrome P450 1A1 (CYP1A1), which is upregulated following ligand binding to and activation of the receptor

[9]. This proxy approach is not a sufficient proof of ligand affinity. The latter requires a more direct approach, that of binding assessment or ligand binding assay [5] and/or gene deletion experiments or molecular docking. An almost universally held view by immunologists and others is that Kyn is a direct AhR agonist and that its AhR activation is at the root of many immune diseases involving stimulation of the Kyn pathway (KP) of Trp degradation (Fig. 1) [17].

Most studies involving the Kyn-AhR link have assessed it by using relatively high concentrations of Kyn, e.g., 30–70 μM [18], 50 μM [19] or 100 μM [20] in cultured cell systems. Under these experimental conditions, Kyn could have undergone metabolic transformation to either kynurenic acid (KA) or anthranilic acid (AA) by the catalytic actions of Kyn aminotransferase (KAT) or kynureninase (KYNU) respectively (Fig. 1), and/or to trace derivatives with potent AhR binding properties [21]. Using the HepG2 40/6 reporter cell line and *P*-450-dependent drug-metabolising activity, DiNatale *et al.* [22] reported that 10 μM KA, but not Kyn, activates the AhR, with xanthurenic acid being much less active. A 10 μM [Kyn] is at least 4-fold higher than its normal plasma values [23]. Also, because plasma [Kyn] is much higher than [KA] (μM vs. nM), a moderate (47%) increase in [Kyn] induced by a small oral Trp dose (1.15 g: ~ 16 mg/kg) consumed by healthy volunteers results in a 209% increase in [KA] [23]. A recent study [9] showed that *AhR* expression was not enhanced in wild-type HEK293-E cells incubated for 72 h with 80 μM Kyn. These latter authors reported that Kyn enhances *AhR* expression in the absence of Trp, with Trp depletion being a key determinant, but, nevertheless, this enhancement could not be attributed to Kyn itself. Taken together, the above studies show that Kyn may not activate the AhR directly, but can do so via a metabolite(s). Based purely on metabolic grounds, KA is the most likely mediator, given the very low affinity of KAT for Kyn [24]. Assessment of AhR ligand affinity by receptor activation in cell cultures or *in vivo* using *P*-450-related metabolism is not a direct measure of binding or affinity. Molecular docking to the crystal structure of the AhR can provide a more objective indication of binding of potential ligands in the absence of metabolic and other biological modulators. Using this technique, we now report the failure of Kyn and 3-hydroxykynurenine (3-HK) to dock to the AhR and suggest that Kyn activates this receptor via KA, but possibly also other metabolites depending on the complement of Kyn pathway enzymes present in the experimental cell system or *in vivo*. Our strong docking of Trp and its reported [8,9] down-regulation and inactivation of the AhR suggest that Trp is an AhR antagonist with potential implications for body homeostasis. We further suggest that differences in AhR activation by Trp metabolites reported in the literature are due to the prevailing metabolic and other biological conditions.

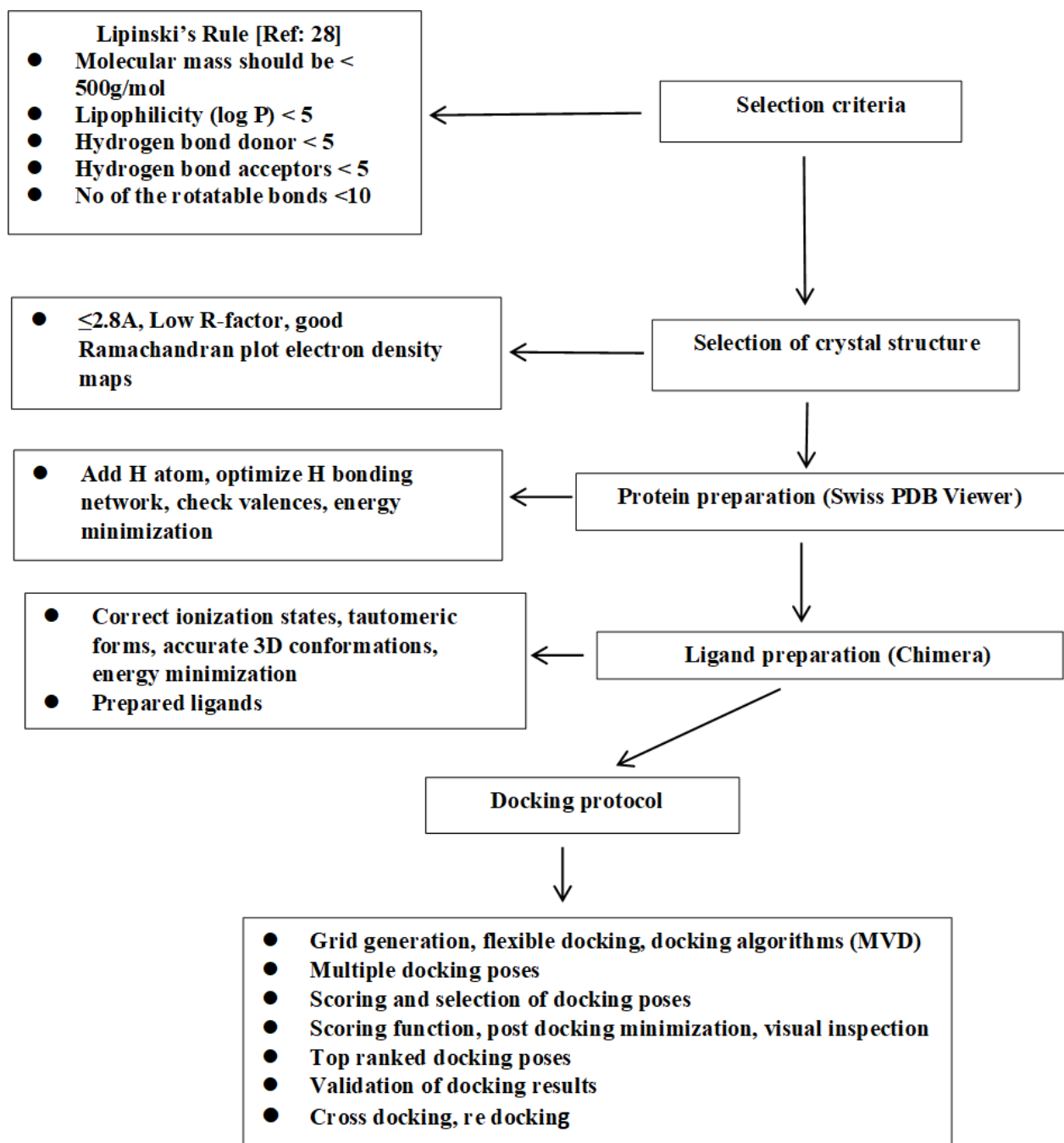
2. Methods

Molegro virtual Docker (MVD) software was utilised for the molecular docking of Trp and its metabolites as described previously [25,26]. Re-ranking was performed to improve accuracy. When re-ranked, 10 independent docking runs resulted in 10 solutions. The structures of Trp and 30 of its metabolites were imported into the MVD workspace in Structure Data File (SDF) format. All H atoms were added and important valence checked. The crystal

structure of the human AhR was obtained from the protein data bank (<https://www.rcsb.org/>) (PDB ID: 5Y7Y [27]) and imported into the MVD work region at reasonable resolution levels ($\leq 2.8\text{\AA}$).

Scheme 1 (Ref. [28]) provides a flow chart of the docking procedure. Some docking details have already been described [26]. Additionally, the following details may be relevant in the light of the results obtained. The MolDock score is a negative score where lower scores indicate better binding affinities. The score combines various interaction terms to give an overall indication of the binding strength between the ligand and the protein. Positive docking scores generally indicate poor binding or unfavourable interactions within the binding site. This can occur due to significant steric hindrances between the ligand and the protein, unfavourable electrostatics, with repulsive electrostatic interactions outweighing attractive forces, and poor conformational fit, wherein the ligand cannot adopt a favourable conformation within the binding site. The docking results of ligands giving positive docking scores, despite being biologically active compounds, suggest that their specific conformations or functional groups are not well-suited for binding to the active site of the target protein in the modelled conditions.

Only crystal structures with a resolution of 2.8 \AA or better were selected for docking studies. High-resolution structures provide more accurate atomic positions and reliable geometry of the binding sites, which are critical for precise docking simulations. Preparation of the protein structure for docking was performed using the SWISS PDB VIEWER tool (<https://spdbv.unil.ch/>). Addition of hydrogen atoms to the protein, which are often missing in crystal structures, was performed using the software of the Protein Preparation SWISS PDB Viewer. This step is essential for accurately modeling hydrogen bonding interactions and ensuring proper protonation states. The protonation states of ionizable residues (e.g., Asp, Glu, His, Lys, and Arg) were assigned based on physiological pH (7.4). This ensures that the protein environment is correctly modelled for docking. Orientation of hydrogen atoms in hydroxyl, thiol, and amino groups was optimized to form the most favourable hydrogen bonds. This involves reorienting groups to maximize hydrogen bonding interactions within the protein and with potential ligands. Valences of all atoms, especially those in the binding site, were checked to ensure there are no unrealistic bond lengths or angles. This step helps in avoiding errors in the docking simulation due to incorrect valence states. If the binding site includes metal ions or cofactors, their correct coordination states and geometry were verified and, if necessary, adjusted. Finally, the prepared protein structures were subjected to a restrained energy minimization to relieve any steric clashes or unfavourable interactions introduced during the preparation steps. Tools such as AMBER (<https://ambermd.org/>), CHARMM (<https://academiccharmm.org/>), or GRO-



Scheme 1. Flow chart and criteria of the docking procedure.

MACS (<https://www.gromacs.org/>) were used for this purpose, applying a force field appropriate for the protein system. All other details are in the Mole Gro software website (<http://molexus.io/molegro-virtual-docker/>).

3. Results

A summary of docking to the AhR of Trp, 30 of its metabolites and three established agonists of the receptor is illustrated in Fig. 3, with docking details given in Table 1 and amino acid residues at the AhR active site and those binding the ligands listed in Supplementary Table 1.

3.1 Docking of the AhR Own Ligand and Established Agonists

As shown in Table 1 and illustrated in **Supplementary Fig. 1**, the powerful AhR agonists indirubin and the Trp photooxidation product and metabolite FICZ showed equal binding potencies that were slightly greater than that by TCDD and are therefore included here as reference agonists and for comparison with Trp metabolites.

Table 1. Docking of tryptophan metabolites and other compounds to the human aryl hydrocarbon receptor.

Compound	Docking score	Re-ranked score	Log P	RMSD	Torsion number	HB	M.Wt.
FICZ and others							
Own ligand	-48.91	-44.47	-1.8	0	2	-10.279	92.09
Indirubin	-144.08	-97.16	2.7	0	1	-3.794	262.26
FICZ	-118.07	-97.05	4.3	0	1	-1.938	282.29
TCDD	-119.79	-93.66	6.3	0	0	0	321.97
Kynurenine pathway							
Trp	-107.21	-83.16	-1.1	0	3	-6.780	204.23
NFKyn	-100.97	-25.09	-2.3	0	5	-4.066	237.23
Kyn	2002.36	65.71	-2.2	0	4	-6.574	208.20
3-HK	2994.42	31.92	-2.5	0	4	-8.947	224.21
KA	-98.9427	-82.66	1.3	0	0	-5.571	189.17
AA	-79.9362	-66.75	1.2	0	1	-5.987	137.13
XA	-97.26	-77.59	0.9	0	0	-5.925	205.17
3-HAA	-89.31	-71.64	0.9	0	1	-5.163	153.13
CBA	-118.07	-97.50	0.6	0	2	-12.544	302.24
QA	-94.87	-78.20	0.2	0	2	-8.546	167.12
PA	-74.82	-60.65	0.7	0	1	-4.942	123.11
Serotonin pathway							
Trp	-107.21	-83.16	-1.1	0	3	-6.780	204.23
5-HTP	-118.23	-96.49	-1.7	0	3	-6.265	220.23
5-HT	-108.20	-89.97	-1.7	0	2	-4.595	176.22
5-HIAA	-112.93	-86.26	1.1	0	2	-7.39	191.18
N-Acetyl 5-HT	-129.26	-95.23	0.5	0	3	-4.70	218.25
Melatonin	-110.81	-76.61	0.8	0	4	-0.809	232.28
Decarboxylation and microbiota pathways							
Tryptamine	-105.60	-88.50	1.6	0	2	0.128	161.22
Skatole	-87.04	-70.51	2.6	0	0	-2.367	131.17
Indole	-76.33	-63.95	2.1	0	0	-0.593	117.15
I-3-A	-76.33	-63.95	2.1	0	0	-0.593	145.16
IAcetald	-102.91	-84.17	1.4	0	2	-2.244	159.18
IAA	-99.78	-78.88	1.4	0	2	-2.500	175.19
IAcetamide	-89.46	-52.19	0.8	0	2	-4.766	174.20
ILA	-114.52	-91.14	1.5	0	3	-5.035	205.21
IAcrylA	-103.36	-83.63	2.2	0	1	-2.787	187.19
IEth	-99.58	-76.57	1.8	0	2	-2.447	229.20
IPA	-110.06	-88.81	1.3	0	2	-2.154	203.19
IPropA	-111.94	-84.72	1.8	0	3	-4.258	189.21
Indoxyl	1950.3	-39.34	1.2	0	0	-4.257	133.15
Indoxyl SO ₄	2972.35	-11.98	1.3	0	2	-3.535	213.21

Docking scores are in Kcal/mol. Only the more accurate re-ranked scores are in bold. Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; HB, hydrogen bond energy (kcal/mol); M.Wt., molecular mass; P, partition coefficient; RMSD, root mean square deviation; CBA, cinnabarinic acid; I-3-A, Indole-3-aldehyde; IAcrylA, indoleacrylic acid.

3.2 Docking of Tryptophan and Metabolites of the Kynurenine Pathway

The data illustrated in Fig. 3, Table 1 and **Supplementary Figs. 2,3** show that, of Trp and 10 metabolites of the KP, only Kyn and 3-HK failed to dock to the human AhR crystal structure. By contrast, Trp and other kynurenines docked strongly, with cinnabarinic acid (CBA) exhibiting the strongest binding, followed by Trp = KA > QA > XA

> 3HAA > AA > PA > NFKyn. Docking of CBA was comparable with that of the 3 reference compounds and that by Trp and KA followed closely.

3.3 Docking of Metabolites of the Serotonin Pathway

The results in Fig. 3, Table 1 and **Supplementary Fig. 4** show that all three 5-hydroxyindoles (5-HTP, 5-HT and 5-HIAA) docked strongly to the human AhR, with that of 5-

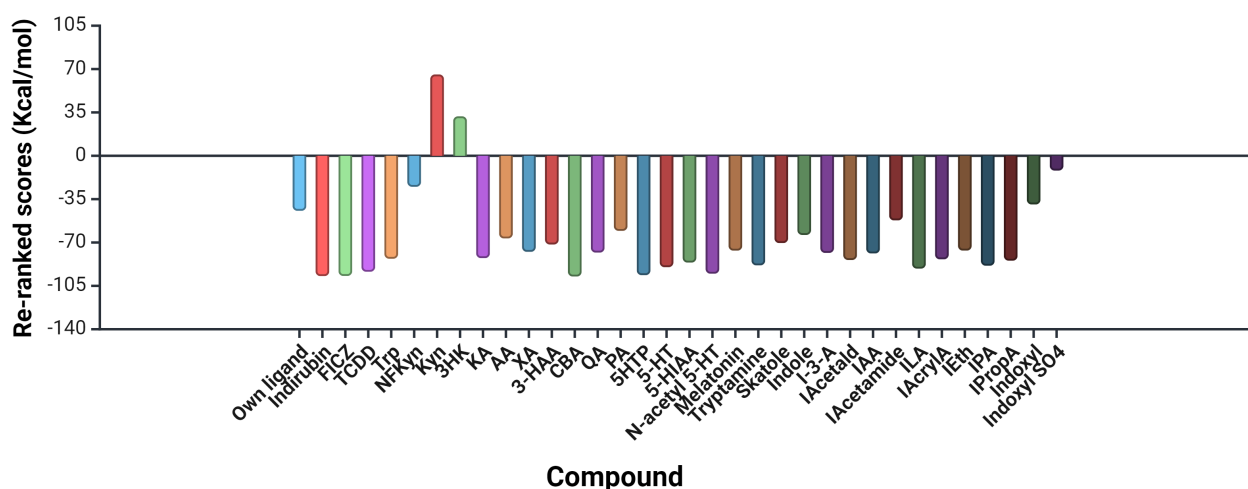


Fig. 3. Molecular docking of tryptophan and 30 of its metabolites to the human aryl hydrocarbon receptor in comparison with known ligands. FICZ, 6-formylindolo[3,2-b]carbazole; TCDD, 2,3,7,8- tetrachlorodibenzo-p-dioxin; NFKyn, Nformylkynurenine; Kyn, kynurenine; 3HK, 3-hydroxykynurenine; KA, kynurenic acid; AA, anthranilic acid; XA, xanthurenic acid; 3-HAA, 3-hydroxyanthranilic acid; CBA, cinnabaric acid; QA, quinolinic acid; PA, picolinic acid; 5HTP, 5-Hydroxytryptophan, ; 5-HIAA, 5-hydroxyindoleacetic acid; IEth, indole-3-ethanol, also known as tryptophol; ILA, indolelactic acid.

HTP being close to that of the reference compounds. Docking of N-acetyl serotonin was comparable to that of 5-HTP and stronger than that of the melatonin metabolite.

3.4 Docking of Metabolites of other Pathways and those Produced by Microbiota

Docking of metabolites of the decarboxylation (tryptamine) and transamination (indole pyruvic acid) pathways and of related metabolites produced by gut microbiota is also detailed in Table 1, summarised in Fig. 3 and illustrated in **Supplementary Figs. 5,6**. All 13 metabolites docked to the AhR to varying degrees. The strongest docking was observed with indolelactic acid (ILA), indolepyruvic acid (IPA) and tryptamine and the weakest was with indoxyl SO₄. To test if this weak docking is limited to the human AhR, docking of indoxyl SO₄ to the AhR of *drosophila melanogaster* and the mouse was performed. Indoxyl SO₄ docked even more weakly to the mouse AhR and failed to dock to the fly AhR (Table 2, **Supplementary Table 2, Supplementary Figs. 7,8**).

To establish if the failure of Kyn to interact with the AhR is limited to the human receptor, docking of Kyn to AhR structures of 2 other species was also performed: those of *Drosophila melanogaster* (PDB 7vni) [29] and mouse (PDB 4m4x) [30]. As shown in Table 2, detailed in **Supplementary Table 2** and illustrated in **Supplementary Figs. 7,8**, Kyn failed to dock to the AhR of these 2 species, as well as to that of human. By contrast, KA docked to all three structures as did TCDD. Only FICZ (and indoxyl SO₄) failed to dock to the *Drosophila* AhR structure. Binding of KA, TCDD and FICZ appears to be strongest with the human AhR.

Table 2. Lack of docking of kynurenine to the aryl hydrocarbon receptor of several species.

Compound	Re-ranked AhR docking scores (Kcal/mol)		
	Human	Drosophila	Mouse
Kyn	65.71	72.03	32.72
KA	-82.66	-65.17	-39.08
TCDD	-93.66	-52.97	-70.05
FICZ	-97.05	37.16	-94.85
Indoxyl SO ₄	-11.98	14.87	-7.58

Abbreviations: AhR, aryl hydrocarbon receptor; FICZ, 6-formylindolo[3,2-b]carbazole; KA, kynurenic acid; Kyn, kynurenine; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

3.5 Correlation of Docking Scores and Receptor Activation of Trp Metabolites

In order to correlate the AhR docking scores of Trp metabolites with published binding parameters obtained by other methods, we identified the median effective concentration (EC₅₀) values of 12 metabolites from the review by Kumar *et al.* [31]. As shown in Fig. 4 (Ref. [31]), there appears to be no significant correlation between the docking score and the EC₅₀ (Pearson Product Moment correlation gave a coefficient *r* of 0.172 and a *p* value of 0.5933).

4. Discussion

Our molecular docking results confirm the absence of a direct interaction between kynurenine and the human aryl hydrocarbon receptor (Fig. 3, Table 1 and **Supplementary Table 1**). Whereas species differences in AhR ligand binding are well documented [5,32,33], species differences do not provide an explanation of the absence of a Kyn inter-

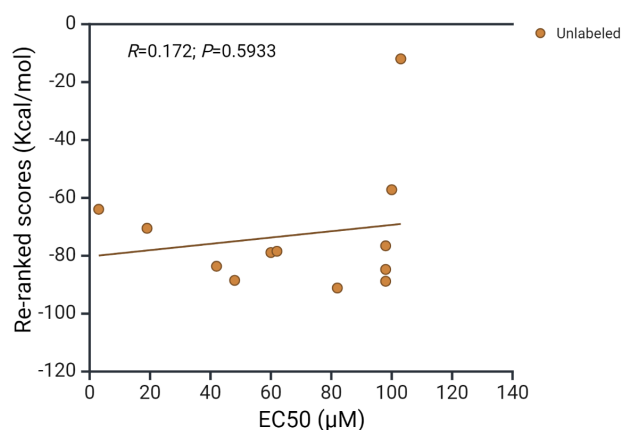


Fig. 4. Correlation of docking scores with AhR receptor activation by tryptophan metabolites. The EC₅₀ values of the 12 compounds listed have been compiled from the review by Kumar *et al.* [31]. The 12 metabolites are: tryptamine, skatole, indoxyl SO₄, indole acetamide, picolinic acid, indole ethanol, indole acrylic acid, indole propionic acid, indole lactic acid, indole-3-aldehyde, indole acetic acid and indole.

action with the human AhR, as no docking was observed between Kyn and the AhR of two other species: the mouse and *Drosophila melanogaster* (Fig. 3, Table 2 and **Supplementary Table 2**). The failure of FICZ and indoxyl SO₄ to dock to the fly AhR (Table 2) is however a further example of species differences.

There are several factors involved in the failure of Kyn and 3-HK to bind (dock) to the AhR. These are molecular shape and size, hydrogen bonding and electrostatic interactions, steric hindrance, binding pocket conformation, energy landscape and binding affinity, and solvent effects. Thus, Kyn is a linear molecule with several functional groups, including an amino group and a carboxyl group (Fig. 5). The linear structure might not fit well into the binding pocket of the AhR if the pocket requires a more compact or differently shaped molecule. 3-HK has an additional hydroxyl group compared to Kyn, which can increase its polarity and potentially introduce steric hindrance if the hydroxyl group does not fit well within the binding pocket. The amino and carboxyl groups in Kyn can form hydrogen bonds, but if the binding site of the AhR does not have suitable hydrogen bond acceptors or donors positioned correctly, interactions may not occur. Whereas the hydroxyl group in 3-HK can participate in hydrogen bonding, it may also increase the overall polarity of the molecule, potentially leading to unfavourable electrostatic interactions if the binding site of AhR has regions of similar polarity or charge. Both Kyn and 3-HK might experience steric clashes with the amino acid residues lining the binding pocket of AhR. If the binding pocket is narrow or has a specific shape, larger or differently shaped molecules might not fit properly. The conformation of AhR's binding pocket might not be compatible with the linear structure of Kyn or the addi-

tional hydroxyl group in 3-HK. Proteins often have binding pockets tailored to specific ligand shapes, and even small deviations can prevent proper binding. The binding energy landscape might not favour the interaction between AhR and Kyn or 3-HK. If the energy required for the molecules to adopt a binding-competent conformation is too high, or if the resulting binding energy is not sufficiently negative, stable docking will not occur. Finally, solvation can play a significant role in docking. If Kyn or 3-HK forms strong interactions with solvent molecules (e.g., water), this could compete with the binding interactions within the AhR binding pocket, leading to failed docking attempts. The structure of kynurenic acid (Fig. 5), by contrast, does not suffer from these restrictive influences, enabling it to bind to the AhR. Further structural studies are likely to clarify the roles of these potential determinants of the failure of Kyn and 3-HK to bind to the AhR.

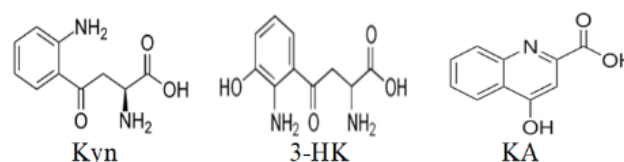


Fig. 5. Structures of kynurenine (Kyn), 3-hydroxykynurenine (3-HK) and kynurenic acid (KA).

The absence of a direct link between Kyn and the AhR is not surprising, as studies have demonstrated the failure of Kyn to activate the AhR or induce its mRNA expression at ligand concentrations of 10–100 μM [9,22]. Indirect AhR activation by Kyn can be visualised on biochemical grounds, with kynurenic acid being the primary mediator. Thus, as KAT exhibits a low affinity for Kyn, the impetus for KA formation is the substrate concentration [24]. Almost all studies of Kyn-AhR interaction have been conducted with larger than physiologically relevant concentrations of Kyn in cell cultures or *in vivo*, with no control over potential metabolic transformation of Kyn. Our results are the first to demonstrate the absence of a direct interaction of Kyn with the AhR. Previous studies suggesting a direct Kyn-AhR link have used the proxy system of cytochrome P-450-dependent drug metabolism or induction of AhR target genes. This is no substitute for a pharmacological or a biochemical approach, such as competitive ligand binding assays, gene deletion models [5,34] or molecular docking *in silico*. Future studies using these techniques should confirm the absence or otherwise of a direct Kyn-AhR link, if experimental conditions are well controlled to block Kyn metabolism. In the meantime, we propose that diagrams of this link should read: Kyn → KA → AhR.

In the recent study by Solvay *et al.* [9], the authors reported that Trp depletion by culturing HEK-293-E cells (expressing KAT, but not Kynurenine-3-monooxygenase

(KMO) or KYNU) in a Trp-free medium supplemented with foetal bovine serum (FBS: providing ~26 μ M Trp) for 72 h, but not for 24 h, allows Kyn and various KP metabolites to enhance expression of AhR target genes. The 72 h culture ensures depletion of the Trp present in the FBS. Although Solvay *et al.* [9] proposed that Kyn may act via KA through KAT upregulation, an additional possibility is that the Trp provided by FBS can also be transaminated by KAT (or by Trp aminotransferase, if expressed) to IPA that is known to be a more powerful AhR agonist [35]. Solvay *et al.* [9] also reported that Trp down-regulates the AhR and proposed that its absence allows the above “weak” AhR agonists to exert a stronger activation. The Trp interaction with the AhR in the present study coupled with this AhR down regulation strongly suggest that Trp is an AhR antagonist and therefore functions in the opposite direction to neutralise AhR activation by agonists: a notion supported by the Trp dose-response data of the above authors [9] and also by the earlier reported [8] ability of Trp to block AhR activation by agonists. Given that the indole structure is amenable to AhR binding and that a whole range of indole metabolites of Trp are known AhR agonists, it is surprising that Trp itself has not been included in previous studies. The concept of Trp as an AhR antagonist may prove to have important biological and clinical consequences (see below) and should be the subject of future studies.

Our docking data in Fig. 3 and Table 1 show that most Trp metabolites exhibited docking scores of -50 Kcal/mol or lower, with only one exception, that of indoxyl SO_4 giving a much less negative score. A similarly moderate docking of indoxyl SO_4 to the mouse AhR and no docking to the Fly AhR were observed (Table 2). This moderate docking of indoxyl SO_4 together with its relatively high EC_{50} value (~ 100 μ M) (Fig. 4) suggest that this metabolite exerts effects largely and/or additionally unrelated to direct AhR activation. Indoxyl SO_4 is formed by sulphuration of indoxyl by aryl (phenol) sulphotransferase (EC 2.8.2.1) in the reversible reaction: Indoxyl + 3'-phosphoadenylylsulphate \leftrightarrow indoxyl sulphate + adenosine 3',5'-diphosphate [36]. It is possible that, when often tested at relatively high concentrations (200 μ M) in cell systems [37] or high doses (600–800 mg/kg) *in vivo* [38,39], indoxyl SO_4 may undergo de-sulphuration to indoxyl, which is a stronger AhR ligand (Fig. 3). Whereas indolelactic acid (ILA) is often portrayed as a weak AhR ligand, our docking data (Fig. 3) show that it is little different from the strong docking by indolepyruvic (IPA), indoleacrylic (IAcryLA) or indolepropionic (IPropA) acids. Any differences *in vivo* may be due to metabolic and other factors, e.g., substrate concentrations, enzyme reaction, redox state, competition from other ligands and protein binding. This is borne out by the absence of a significant correlation between docking scores and the EC_{50} for 12 Trp metabolites (Fig. 4).

The little known Trp metabolite, cinnabaric acid (CBA) exhibited the strongest interaction with the AhR

among Kyn and other metabolites (Table 1, Fig. 3). CBA is formed by auto-oxidation of 3-hydroxyanthranilic acid (3-HAA) with O_2 first via the anthranilyl radical and $\text{O}_2^{\cdot-}$, followed by dismutation of superoxide to H_2O_2 and O_2 and subsequent oxidation of the anthranilyl radical to CBA [40]. CBA exerts important effects, including generation of reactive oxygen species, apoptosis of thymocytes [41] and activation of NMDA (N-methyl-D-aspartate) receptors [42]. Its AhR activation is likely to play a part in the former effects. Utilisation of these properties of CBA may yield important indicators for therapeutic targeting.

Whereas kynurenine metabolites and indole derivatives produced by intestinal microbiota have been the focus of AhR research, little attention has been given to the potential links between 5-hydroxyindoles and the AhR. Our docking results show that 5-HTP, 5-HT and 5-HIAA are strong AhR ligands, despite earlier doubts regarding 5-HT [43]. 5-HTP [44], 5-HT [43] and 5-HIAA [45] have previously been suggested to activate the AhR, with 5-HT having been proposed to modify AhR activation by interfering with CYP1A1 clearance of AhR ligands [43]. The awaited demonstration of direct interaction of melatonin with the AhR [46] has recently been demonstrated [47] and is also reported in the present study. Our finding that the melatonin precursor N-acetyl serotonin docks to the AhR more strongly than melatonin is consistent with this precursor being a stronger antioxidant [48]. An intriguing observation is that of N-acetyl 5-HT being a positive allosteric regulator of Indoleamine 2,3 dioxygenase 1 (IDO1) resulting in restraining neuroinflammation [49]. Melatonin however is a competitive inhibitor of Trp 2,3-dioxygenase with an half maximal inhibitory concentration (IC_{50}) of 2.7 μ M [50]. These are clear examples of the cross talk between the serotonin and kynurenine pathways. Whether many of the beneficial effects of serotonin and melatonin [51,52] involve their interaction with the AhR remains to be assessed. Table 3 (Ref. [1,7–9,12,22,43,44,46,47,53–75]) lists single examples of effects of Trp metabolites related to their interaction with the AhR. A detailed account of AhR-related effects is clearly outside the scope of the present discussion. However, effects of FICZ, KA and Trp merit further discussion.

The Trp photooxidation product and bacterial metabolite FICZ is one of the most powerful AhR ligands, as illustrated in the present study. It exerts a range of biological effects that render it a potential drug in the making (reviewed in [75]). It exerts antiinflammatory [76–78], antibacterial [79,80] and anticancer [81–83] effects in experimental models via a range of mechanisms including, among others, decreased IL-17 and IL-22, increased chemokine ligand 5 and filaggrin, increased macrophage survival and ROS production, decreased IL-6, keratinocyte-derived chemokine and apoptosis, and increased NK cells, IL-18 and IFN- γ . As stated in Table 3, the route of FICZ administration determines the type of effect. Protective effects are associated with oral and parenteral administra-

Table 3. Examples of AhR-dependent effects of tryptophan metabolites.

Metabolite	Reranked AhR docking score	Example of properties/effects associated with AhR binding [References]
Trp	−83.16 Antagonist	Production of AhR agonists [1], Blockade of AhR activation [8], Down-regulation of the AhR [9]
NFKyn	−25.09	Precursor of Kyn and subsequent metabolites
Kyn	65.71 (−)	Indirectly activates the AhR via KA and AA
3-HK	31.92 (−)	Indirectly activates the AhR via XA and 3-HAA
KA	−82.66	Physiological Kyn pathway AhR agonist [9,22] Pro- and anti-inflammatory [12,53]
AA	−66.75	Pro- or anti-inflammatory, acting via 3-HAA [53]
XA	−77.59	Promotes tumor cell migration and parasite development [46]
3-HAA	−71.64	Enhances AhR activation by nuclear coactivator 7 [54], apoptotic [55,56]
CBA	−97.50	Protects against oxidative stress [57], enhances production of IL-22 [58]
QA	−78.20	Skin anti-inflammatory: inhibits NLRP3 inflammasome activation [59]
PA	−60.65	Anti-inflammatory [60,61], but docking suggests AhR agonism
5-HTP	−96.49	AhR activator: mediates IL-2-induced T cell exhaustion in cancer [44]
5-HT	−89.97	Indirect AhR activator [43], but may also be an AhR antagonist
5-HIAA	−86.26	Reverses LPS production of proinflammatory Trp metabolites [62]
N-Acetyl 5-HT	−95.23	Docking suggests AhR agonism, which may explain some of its protective properties
Melatonin	−76.61	AhR agonism [47] may explain some of its protective properties
Tryptamine	−88.50	Docking suggests AhR agonism, also a prolignand acting via FICZ [63]
Skatole	−70.51	Regulates intestinal epithelial function [64]
Indole	−63.95	AhR agonist: potentiates IL-1b-mediated IL-6 mRNA induction [65]
I3A	−63.95	Epithelial protection and antifungal resistance [7]
IAcetald	−84.17	Agonist [66], hepatoprotective [67]
IAA	−78.88	Maintains intestinal epithelial homeostasis via mucin sulfation [68]
IAcetamide	−52.19	Alleviates alcoholic liver injury [69]
ILA	−91.14	Anti-inflammatory [70]
IAcrylA	−83.63	Anti-inflammatory [71]
IEth	−76.57	Regulates gut integrity by correcting permeability [72]
IPA	−88.81	Enhances barrier integrity [72]
IPropA	−84.72	Improves gut-blood barrier function [73]
Indoxyl	−39.34	Untested: likely to mediate the indoxyl SO ₄ effects
Indoxyl SO ₄	−11.98	Induces oxidative stress [74]
FICZ	−97.05	Route-dependent metabolism determines type of effect [75]

tion, whereas harmful effects are observed after subcutaneous administration. Metabolism of FICZ may explain this dichotomy, with rapid disposal of FICZ after oral or parenteral administration of even large doses resulting in a lower bioavailability, and slow absorption following subcutaneous administration ensuring persistent and prolonged availability [75]. FICZ also interacts with (docks to) most enzymes of the kynurenine pathway of Trp degradation [75] and is thus likely to influence Trp metabolism significantly. These and other considerations should be the subject of pharmaco-therapeutic developmental studies with FICZ. As a Trp photooxidation product, FICZ may be formed during phototherapy of neonatal hyperbilirubinemia. Its molecular interactions with glutamate and γ -aminobutyrate (GABA) receptors (AA-B Badawy & S Dawood, in press) suggest that it may counteract the neuronal dysfunction that characterizes kernicterus in the newborn.

As our data and those of others [8,9] suggest that Kyn activates the AhR indirectly via KA, the question arises as to whether some AhR-related, among the many, effects attributed to Kyn [84,85] may in fact be exerted by KA. The answer awaits future studies. The association of KA with the AhR however is an important issue that cannot be over-emphasised. Whereas the “Janus-faced” KA is both pro- and anti-inflammatory [12,53], it exerts a long-term influence on immune function through this association. With IDO1 induction by the AhR-IL-6-STAT3 autocrine loop [86,87], activation of the kynurenine pathway can be maintained, with continued production of KA. This can lead to increased IL-6 production and with inflammation, increased IL-6 can induce IDO1 to produce more KA and activate the AhR [22]. A vicious circle is thus established and it is notable that KA levels are increased in tumors, sera, bone marrow and intestinal mucosal material of patients with a range of cancer types [88].

Tryptophan is an AhR antagonist [8,9] and this study is a landmark statement in bioscience. It elevates Trp from being the simple precursor of biologically active metabolites to an orchestrator of defensive mechanisms against excessive AhR-related harm. Trp assumes the role of a protective parent of metabolites. While Trp supplementation increases the production of AhR activators in an *in vitro* simulator of intestinal microbial ecosystem, it was reported [8] to antagonise AhR signaling. Recently, Trp was reported to inhibit the gene expression and ligand activation of the AhR [9] and the authors suggested that Trp depletion enables weak ligands to exert a strong AhR activation. However, the Trp concentration-response data [9] suggest that Trp acts in the opposite direction, namely antagonising AhR activation by ligands. AhR antagonism by Trp now sheds more light on the earlier controversy of the role of Trp depletion versus kynurenine metabolite formation in bacterial and viral infections [89,90]. While current opinion emphasises the importance of both phenomena, Trp depletion acquires a new meaning, synonymous with loss of protection against excessive AhR activation. Trp supplementation may be a valid option in appropriate circumstances. Although one of us [91] and others [92] have warned against the use of Trp in therapy of SARs-CoV-2 (COVID-19) infection to avoid production of the AhR-activating and T-cell damaging effects of Kyn metabolites, AhR antagonism by Trp suggests that Trp could be effective provided its use is combined with inhibitors of tryptophan-2,3-dioxygenase (TDO), indoleamine-2,3-dioxygenase (IDO) and aminotransferases. This should ensure blockade of production of the AhR agonists KA and IPA.

5. General Conclusions, Limitations and Comments

The present molecular docking findings strongly suggest that kynurenine activates the aryl hydrocarbon receptor indirectly via kynurenic acid. Investigators should therefore revise the Kyn-AhR link accordingly and insert KA as an intermediate. As this is the first stage in elucidating the nature of the Kyn-AhR link, a limitation of this study is the absence of further experimental scrutiny of this link. The proposed Kyn → KA → AhR link should therefore be the subject of future experimental studies with Trp and Kyn in various cell systems expressing or lacking key enzymes of the Kyn pathway, in particular, KAT, Trp aminotransferase, Kyn monooxygenase and kynureninase. AhR gene expression, protein content and target gene activation using Kyn should be performed in the absence of Trp and presence of strong inhibition of KAT and also potentially of KMO and KYNU. If KAT is expressed in cells, the effect of Kyn should be studied in the absence and presence of a strong KAT inhibitor. Cell models involving Knock-out of KAT, KYNU and KMO can also be studied. Under these conditions, no AhR activation is a likely outcome. A KAT null mutant yeast can also be tested with Kyn and KA. With

Trp, AhR activation by TCDD or FICZ can be examined in the presence of various concentrations of Trp in cells expressing or lacking TDO/IDO to confirm antagonism. Similar experiments with *KAT* and *Trp AT* gene deletion can be informative. The demonstration in the present study of the strong interaction of Trp with the human AhR and its reported [8,9] antagonism by Trp in cell systems open an important new avenue of investigation of the physiological functions of this essential amino acid beyond known properties or simply being the precursor of many biologically active metabolites. In this regard, the notion suggested by Solvay *et al.* [9] that Trp depletion enables Trp metabolites to overcome their weak activation of the AhR suggests that Trp acts in the opposite direction by virtue of being an antagonist. This property could be utilised in clinical situations requiring blockade of AhR activation, e.g., in cancer, bacterial and viral infections. In conclusion, the present study has illustrated the importance of metabolic considerations and molecular docking in clarifying controversies in our understanding of biological processes, proposing solutions and opening new avenues of investigation.

Availability of Data and Materials

All data in this submission have been included. The source of the protein structures tested was the protein data bank (<http://www.rcsb.org/>).

Author Contributions

SD and AABB designed the study. SD directed, performed and curated the experimental data. AABB analysed the data and wrote the first draft. AABB and SD edited and approved the final version. Both authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/j.fbl2909333>.

References

- [1] Gasiewicz TA, Singh KP, Bennett JA. The Ah receptor in stem cell cycling, regulation, and quiescence. *Annals of the New York Academy of Sciences*. 2014; 1310: 44–50.
- [2] Avilla MN, Malecki KMC, Hahn ME, Wilson RH, Bradfield CA. The Ah Receptor: Adaptive Metabolism, Ligand Diversity, and the Xenokine Model. *Chemical Research in Toxicology*. 2020; 33: 860–879.
- [3] Gutiérrez-Vázquez C, Quintana FJ. Regulation of the Immune Response by the Aryl Hydrocarbon Receptor. *Immunity*. 2018; 48: 19–33.
- [4] Julliard W, Fechner JH, Mezrich JD. The aryl hydrocarbon receptor meets immunology: friend or foe? A little of both. *Frontiers in Immunology*. 2014; 5: 458.
- [5] Hubbard TD, Murray IA, Perdew GH. Indole and Tryptophan Metabolism: Endogenous and Dietary Routes to Ah Receptor Activation. *Drug Metabolism and Disposition: the Biological Fate of Chemicals*. 2015; 43: 1522–1535.
- [6] Badawy AAB. Tryptophan metabolism and disposition in cancer biology and immunotherapy. *Bioscience Reports*. 2022; 42: BSR20221682.
- [7] Puccetti M, Paolicelli G, Oikonomou V, De Luca A, Renga G, Borghi M, *et al.* Towards Targeting the Aryl Hydrocarbon Receptor in Cystic Fibrosis. *Mediators of Inflammation*. 2018; 2018: 1601486.
- [8] Koper JE, Troise AD, Loonen LM, Vitaglione P, Capuano E, Fogliano V, *et al.* Tryptophan Supplementation Increases the Production of Microbial-Derived AhR Agonists in an *In Vitro* Simulator of Intestinal Microbial Ecosystem. *Journal of Agricultural and Food Chemistry*. 2022; 70: 3958–3968.
- [9] Solvay M, Holfelder P, Klaessens S, Pilote L, Stroobant V, Lamy J, *et al.* Tryptophan depletion sensitizes the AHR pathway by increasing AHR expression and GCN2/LAT1-mediated kynurenine uptake, and potentiates induction of regulatory T lymphocytes. *Journal for Immunotherapy of Cancer*. 2023; 11: e006728.
- [10] Sorgdrager FJH, Naudé PJW, Kema IP, Nollen EA, Deyn PPD. Tryptophan Metabolism in Inflammaging: From Biomarker to Therapeutic Target. *Frontiers in Immunology*. 2019; 10: 2565.
- [11] Stone TW, Clanchy FIL, Huang YS, Chiang NY, Darlington LG, Williams RO. An integrated cytokine and kynurenine network as the basis of neuroimmune communication. *Frontiers in Neuroscience*. 2022; 16: 1002004.
- [12] Wirthgen E, Hoefflich A, Rebl A, Günther J. Kynurenic Acid: The Janus-Faced Role of an Immunomodulatory Tryptophan Metabolite and Its Link to Pathological Conditions. *Frontiers in Immunology*. 2018; 8: 1957.
- [13] Zelante T, Iannitti RG, Cunha C, De Luca A, Giovannini G, Pieraccini G, *et al.* Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity*. 2013; 39: 372–385.
- [14] Alexeev EE, Lanis JM, Kao DJ, Campbell EL, Kelly CJ, Battista KD, *et al.* Microbiota-Derived Indole Metabolites Promote Human and Murine Intestinal Homeostasis through Regulation of Interleukin-10 Receptor. *The American Journal of Pathology*. 2018; 188: 1183–1194.
- [15] Krishnan S, Ding Y, Saedi N, Choi M, Sridharan GV, Sherr DH, *et al.* Gut Microbiota-Derived Tryptophan Metabolites Modulate Inflammatory Response in Hepatocytes and Macrophages. *Cell Reports*. 2018; 23: 1099–1111.
- [16] Konopelski P, Konop M, Gawrys-Kopczynska M, Podsadni P, Szczepanska A, Ufnal M. Indole-3-Propionic Acid, a Tryptophan-Derived Bacterial Metabolite, Reduces Weight Gain in Rats. *Nutrients*. 2019; 11: 591.
- [17] Nebert DW, Dalton TP. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. *Nature Reviews. Cancer*. 2006; 6: 947–960.
- [18] Opitz CA, Litzenburger UM, Sahn F, Ott M, Tritschler I, Trump S, *et al.* An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature*. 2011; 478: 197–203.
- [19] Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, Bradfield CA. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *Journal of Immunology (Baltimore, Md.: 1950)*. 2010; 185: 3190–3198.
- [20] Bessede A, Gargaro M, Pallotta MT, Matino D, Servillo G, Brunacci C, *et al.* Aryl hydrocarbon receptor control of a disease tolerance defence pathway. *Nature*. 2014; 511: 184–190.
- [21] Seok SH, Ma ZX, Feltenberger JB, Chen H, Chen H, Scarlett C, *et al.* Trace derivatives of kynurenine potently activate the aryl hydrocarbon receptor (AHR). *The Journal of Biological Chemistry*. 2018; 293: 1994–2005.
- [22] DiNatale BC, Murray IA, Schroeder JC, Flaveny CA, Lahoti TS, Laurenzana EM, *et al.* Kynurenic acid is a potent endogenous aryl hydrocarbon receptor ligand that synergistically induces interleukin-6 in the presence of inflammatory signaling. *Toxicological Sciences: an Official Journal of the Society of Toxicology*. 2010; 115: 89–97.
- [23] Badawy AAB, Dougherty DM. Assessment of the Human Kynurenine Pathway: Comparisons and Clinical Implications of Ethnic and Gender Differences in Plasma Tryptophan, Kynurenine Metabolites, and Enzyme Expressions at Baseline and After Acute Tryptophan Loading and Depletion. *International Journal of Tryptophan Research: IJTR*. 2016; 9: 31–49.
- [24] Badawy AAB. Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects. *International Journal of Tryptophan Research: IJTR*. 2017; 10: 1178646917691938.
- [25] Dawood S, Zarina S, Bano S. Docking studies of antidepressants against single crystal structure of tryptophan 2, 3-dioxygenase using Molegro Virtual Docker software. *Pakistan Journal of Pharmaceutical Sciences*. 2014; 27: 1529–1539.
- [26] Dawood S, Bano S, Badawy AAB. Inflammation and serotonin deficiency in major depressive disorder: molecular docking of antidepressant and anti-inflammatory drugs to tryptophan and indoleamine 2,3-dioxygenases. *Bioscience Reports*. 2022; 42: BSR20220426.
- [27] Sakurai S, Shimizu T, Ohto U. The crystal structure of the AhRR-ARNT heterodimer reveals the structural basis of the repression of AhR-mediated transcription. *The Journal of Biological Chemistry*. 2017; 292: 17609–17616.
- [28] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*. 1997; 23: 3–25.
- [29] Dai S, Qu L, Li J, Zhang Y, Jiang L, Wei H, *et al.* Structural insight into the ligand binding mechanism of aryl hydrocarbon receptor. *Nature Communications*. 2022; 13: 6234.
- [30] Wu D, Potluri N, Kim Y, Rastinejad F. Structure and dimerization properties of the aryl hydrocarbon receptor PAS-A domain. *Molecular and Cellular Biology*. 2013; 33: 4346–4356.
- [31] Kumar P, Lee JH, Lee J. Diverse roles of microbial indole compounds in eukaryotic systems. *Biological Reviews of the Cambridge Philosophical Society*. 2021; 96: 2522–2545.
- [32] Faber SC, Soshilov AA, Giani Tagliabue S, Bonati L, Denison MS. Comparative In Vitro and In Silico Analysis of the Selectivity of Indirubin as a Human Ah Receptor Agonist. *International Journal of Molecular Sciences*. 2018; 19: 2692.
- [33] Xu X, Zhang X, Yuan Y, Zhao Y, Fares HM, Yang M, *et al.* Species-Specific Differences in Aryl Hydrocarbon Receptor Responses: How and Why? *International Journal of Molecular Sciences*. 2021; 22: 13293.
- [34] Creeden JF, Gordon DM, Stec DE, Hinds TD, Jr. Bilirubin as a metabolic hormone: the physiological relevance of low

- levels. *American Journal of Physiology. Endocrinology and Metabolism*. 2021; 320: E191–E207.
- [35] Sadik A, Somarrivas Patterson LF, Öztürk S, Mohapatra SR, Panitz V, Secker PF, *et al*. IL4I1 Is a Metabolic Immune Checkpoint that Activates the AHR and Promotes Tumor Progression. *Cell*. 2020; 182: 1252–1270.e34.
- [36] Duffel MW, Jakoby WB. On the mechanism of aryl sulfotransferase. *The Journal of Biological Chemistry*. 1981; 256: 11123–11127.
- [37] Lano G, Laforêt M, Von Kotze C, Perrin J, Addi T, Brunet P, *et al*. Aryl Hydrocarbon Receptor Activation and Tissue Factor Induction by Fluid Shear Stress and Indoxyl Sulfate in Endothelial Cells. *International Journal of Molecular Sciences*. 2020; 21: 2392.
- [38] Ichii O, Otsuka-Kanazawa S, Nakamura T, Ueno M, Kon Y, Chen W, *et al*. Podocyte injury caused by indoxyl sulfate, a uremic toxin and aryl-hydrocarbon receptor ligand. *PloS One*. 2014; 9: e108448.
- [39] Walker JA, Richards S, Belghasem ME, Arinze N, Yoo SB, Tashjian JY, *et al*. Temporal and tissue-specific activation of aryl hydrocarbon receptor in discrete mouse models of kidney disease. *Kidney International*. 2020; 97: 538–550.
- [40] Iwahashi H, Ishii T, Sugata R, Kido R. Superoxide dismutase enhances the formation of hydroxyl radicals in the reaction of 3-hydroxyanthranilic acid with molecular oxygen. *The Biochemical Journal*. 1988; 251: 893–899.
- [41] Hiramatsu R, Hara T, Akimoto H, Takikawa O, Kawabe T, Isobe KI, *et al*. Cinnabarinic acid generated from 3-hydroxyanthranilic acid strongly induces apoptosis in thymocytes through the generation of reactive oxygen species and the induction of caspase. *Journal of Cellular Biochemistry*. 2008; 103: 42–53.
- [42] Fazio F, Lionetto L, Molinaro G, Bertrand HO, Acher F, Ngomba RT, *et al*. Cinnabarinic acid, an endogenous metabolite of the kynurenine pathway, activates type 4 metabotropic glutamate receptors. *Molecular Pharmacology*. 2012; 81: 643–656.
- [43] Manzella CR, Ackerman M, Singhal M, Ticho AL, Ceh J, Alrefai WA, *et al*. Serotonin Modulates AhR Activation by Interfering with CYP1A1-Mediated Clearance of AhR Ligands. *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology*. 2020; 54: 126–141.
- [44] Liu Y, Zhou N, Zhou L, Wang J, Zhou Y, Zhang T, *et al*. IL-2 regulates tumor-reactive CD8⁺ T cell exhaustion by activating the aryl hydrocarbon receptor. *Nature Immunology*. 2021; 22: 358–369.
- [45] Zhao L, Xiao HT, Mu HX, Huang T, Lin ZS, Zhong LLD, *et al*. Magnolol, a Natural Polyphenol, Attenuates Dextran Sulfate Sodium-Induced Colitis in Mice. *Molecules (Basel, Switzerland)*. 2017; 22: 1218.
- [46] Ma N, He T, Johnston LJ, Ma X. Host-microbiome interactions: the aryl hydrocarbon receptor as a critical node in tryptophan metabolites to brain signaling. *Gut Microbes*. 2020; 11: 1203–1219.
- [47] Slominski AT, Kim T-K, Slominski RM, Song Y, Qayyum S, Placha W, *et al*. Melatonin and its metabolites can serve as agonists on the aryl hydrocarbon receptor and peroxisome proliferator-activated receptor gamma. *International Journal of Molecular Sciences*. 2023; 24: 15496.
- [48] Wölfler A, Abuja PM, Schauenstein K, Liebmman PM. N-acetylserotonin is a better extra- and intracellular antioxidant than melatonin. *FEBS Letters*. 1999; 449: 206–210.
- [49] Mondanelli G, Coletti A, Greco FA, Pallotta MT, Orabona C, Iacono A, *et al*. Positive allosteric modulation of indoleamine 2,3-dioxygenase 1 restrains neuroinflammation. *Proceedings of the National Academy of Sciences of the United States of America*. 2020; 117: 3848–3857.
- [50] Walsh HA, Daya S, Whiteley CG. Mode of inhibition of rat liver tryptophan 2,3-dioxygenase by melatonin. *Journal of Pineal Research*. 1994; 16: 188–192.
- [51] Herr N, Bode C, Duerschmied D. The Effects of Serotonin in Immune Cells. *Frontiers in Cardiovascular Medicine*. 2017; 4: 48.
- [52] Mondanelli G, Volpi C. The double life of serotonin metabolites: in the mood for joining neuronal and immune systems. *Current Opinion in Immunology*. 2021; 70: 1–6.
- [53] Badawy AAB. Hypothesis kynurenine and quinolinic acids: The main players of the kynurenine pathway and opponents in inflammatory disease. *Medical Hypotheses*. 2018; 118: 129–138.
- [54] Gargaro M, Vacca C, Massari S, Scalisi G, Manni G, Mondanelli G, *et al*. Engagement of Nuclear Coactivator 7 by 3-Hydroxyanthranilic Acid Enhances Activation of Aryl Hydrocarbon Receptor in Immunoregulatory Dendritic Cells. *Frontiers in Immunology*. 2019; 10: 1973.
- [55] Terness P, Bauer TM, Röse L, Dufter C, Watzlik A, Simon H, *et al*. Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. *The Journal of Experimental Medicine*. 2002; 196: 447–457.
- [56] Fallarino F, Grohmann U, Vacca C, Bianchi R, Orabona C, Spreca A, *et al*. T cell apoptosis by tryptophan catabolism. *Cell Death and Differentiation*. 2002; 9: 1069–1077.
- [57] Joshi AD, Carter DE, Harper TA, Jr, Elferink CJ. Aryl hydrocarbon receptor-dependent stanniocalcin 2 induction by cinnabarinic acid provides cytoprotection against endoplasmic reticulum and oxidative stress. *The Journal of Pharmacology and Experimental Therapeutics*. 2015; 353: 201–212.
- [58] Lowe MM, Mold JE, Kanwar B, Huang Y, Louie A, Pollastri MP, *et al*. Identification of cinnabarinic acid as a novel endogenous aryl hydrocarbon receptor ligand that drives IL-22 production. *PloS One*. 2014; 9: e87877.
- [59] Qiao P, Zhang C, Yu J, Shao S, Zhang J, Fang H, *et al*. Quinolinic Acid, a Tryptophan Metabolite of the Skin Microbiota, Negatively Regulates NLRP3 Inflammasome through AhR in Psoriasis. *The Journal of Investigative Dermatology*. 2022; 142: 2184–2193.e6.
- [60] Proding J, Loacker LJ, Schmidt RLJ, Ratzinger F, Greiner G, Witzeneder N, *et al*. The tryptophan metabolite picolinic acid suppresses proliferation and metabolic activity of CD4⁺ T cells and inhibits c-Myc activation. *Journal of Leukocyte Biology*. 2016; 99: 583–594.
- [61] Narayan R, Sharma M, Yadav R, Biji A, Khatun O, Kaur S, *et al*. Picolinic acid is a broad-spectrum inhibitor of enveloped virus entry that restricts SARS-CoV-2 and influenza A virus in vivo. *Cell Reports. Medicine*. 2023; 4: 101127.
- [62] Han Q, Liu R, Wang H, Zhang R, Liu H, Li J, *et al*. Gut Microbiota-Derived 5-Hydroxyindoleacetic Acid Alleviates Diarrhea in Piglets via the Aryl Hydrocarbon Receptor Pathway. *Journal of Agricultural and Food Chemistry*. 2023; 71: 15132–15144.
- [63] Vikström Bergander L, Cai W, Klocke B, Seifert M, Pongratz I. Tryptamine serves as a prolignand of the AhR transcriptional pathway whose activation is dependent of monoamine oxidases. *Molecular Endocrinology (Baltimore, Md.)*. 2012; 26: 1542–1551.
- [64] Kurata K, Kawahara H, Nishimura K, Jisaka M, Yokota K, Shimizu H. Skatole regulates intestinal epithelial cellular functions through activating aryl hydrocarbon receptors and p38. *Biochemical and Biophysical Research Communications*. 2019; 510: 649–655.
- [65] Hubbard TD, Murray IA, Bisson WH, Lahoti TS, Gowda K, Amin SG, *et al*. Adaptation of the human aryl hydrocarbon receptor to sense microbiota-derived indoles. *Scientific Reports*.

2015; 5: 12689.

- [66] Agus A, Planchais J, Sokol H. Gut Microbiota Regulation of Tryptophan Metabolism in Health and Disease. *Cell Host & Microbe*. 2018; 23: 716–724.
- [67] Shang H, Huang C, Xiao Z, Yang P, Zhang S, Hou X, *et al.* Gut microbiota-derived tryptophan metabolites alleviate liver injury via AhR/Nrf2 activation in pyrrolizidine alkaloids-induced sinusoidal obstruction syndrome. *Cell & Bioscience*. 2023; 13: 127.
- [68] Li M, Ding Y, Wei J, Dong Y, Wang J, Dai X, *et al.* Gut microbiota metabolite indole-3-acetic acid maintains intestinal epithelial homeostasis through mucin sulfation. *Gut Microbes*. 2024; 16: 2377576.
- [69] Wang M, Feng X, Zhao Y, Lan Y, Xu H. Indole-3-acetamide from gut microbiota activated hepatic AhR and mediated the remission effect of *Lactiplantibacillus plantarum* P101 on alcoholic liver injury in mice. *Food & Function*. 2023; 14: 10535–10548.
- [70] Meng D, Sommella E, Salvati E, Campiglia P, Ganguli K, Djebali K, *et al.* Indole-3-lactic acid, a metabolite of tryptophan, secreted by *Bifidobacterium longum* subspecies *infantis* is anti-inflammatory in the immature intestine. *Pediatric Research*. 2020; 88: 209–217.
- [71] Wlodarska M, Luo C, Kolde R, d’Hennezel E, Annand JW, Heim CE, *et al.* Indoleacrylic Acid Produced by Commensal *Peptostreptococcus* Species Suppresses Inflammation. *Cell Host & Microbe*. 2017; 22: 25–37.e6.
- [72] Scott SA, Fu J, Chang PV. Microbial tryptophan metabolites regulate gut barrier function via the aryl hydrocarbon receptor. *Proceedings of the National Academy of Sciences of the United States of America*. 2020; 117: 19376–19387.
- [73] Konopelski P, Mogilnicka I. Biological Effects of Indole-3-Propionic Acid, a Gut Microbiota-Derived Metabolite, and Its Precursor Tryptophan in Mammals’ Health and Disease. *International Journal of Molecular Sciences*. 2022; 23: 1222.
- [74] Lu CL, Zheng CM, Lu KC, Liao MT, Wu KL, Ma MC. Indoxyl-Sulfate-Induced Redox Imbalance in Chronic Kidney Disease. *Antioxidants* (Basel, Switzerland). 2021; 10: 936.
- [75] Dawood S, Badawy AA-B. Molecular docking of FICZ (6-formylindolo[3,2-b]carbazole) to kynurenine pathway enzymes: biological basis of a potential drug. *Journal of Pharmacology and Pharmaceutics*. 2024; 1: 1004.
- [76] Di Meglio P, Duarte JH, Ahlforth H, Owens NDL, Li Y, Villanova F, *et al.* Activation of the aryl hydrocarbon receptor dampens the severity of inflammatory skin conditions. *Immunity*. 2014; 40: 989–1001.
- [77] Smith KJ, Boyer JA, Muku GE, Murray IA, Gowda K, Desai D, *et al.* Editor’s Highlight: Ah Receptor Activation Potentiates Neutrophil Chemoattractant (C-X-C Motif) Ligand 5 Expression in Keratinocytes and Skin. *Toxicological Sciences: an Official Journal of the Society of Toxicology*. 2017; 160: 83–94.
- [78] Kiyomatsu-Oda M, Uchi H, Morino-Koga S, Furue M. Protective role of 6-formylindolo[3,2-b]carbazole (FICZ), an endogenous ligand for aryl hydrocarbon receptor, in chronic mite-induced dermatitis. *Journal of Dermatological Science*. 2018; 90: 284–294.
- [79] Kimura A, Abe H, Tsuruta S, Chiba S, Fujii-Kuriyama Y, Sekiya T, *et al.* Aryl hydrocarbon receptor protects against bacterial infection by promoting macrophage survival and reactive oxygen species production. *International Immunology*. 2014; 26: 209–220.
- [80] Li X, Luck ME, Hammer AM, Cannon AR, Choudhry MA. 6-Formylindolo (3, 2-b) Carbazole (FICZ)-mediated protection of gut barrier is dependent on T cells in a mouse model of alcohol combined with burn injury. *Biochimica et Biophysica Acta. Molecular Basis of Disease*. 2020; 1866: 165901.
- [81] Shin JH, Zhang L, Murillo-Sauca O, Kim J, Kohrt HEK, Bui JD, *et al.* Modulation of natural killer cell antitumor activity by the aryl hydrocarbon receptor. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110: 12391–12396.
- [82] Fan C, Wang Q, van der Zon G, Ren J, Agaser C, Sliker RC, *et al.* OVOL1 inhibits breast cancer cell invasion by enhancing the degradation of TGF- β type I receptor. *Signal Transduction and Targeted Therapy*. 2022; 7: 126.
- [83] Arabnezhad MR, Montazeri-Najafabady N, Chatraborty N, Ghafarian Bahreman A, Mohammadi-Bardbori A. Anti-androgenic effect of 6-formylindolo[3,2-b]carbazole (FICZ) in LNCaP cells is mediated by the aryl hydrocarbon-androgen receptors cross-talk. *Steroids*. 2020; 153: 108508.
- [84] Kolodziej LR, Paleolog EM, Williams RO. Kynurenine metabolism in health and disease. *Amino Acids*. 2011; 41: 1173–1183.
- [85] Marszalek-Grabska M, Walczak K, Gawel K, Wicha-Komsta K, Wnorowska S, Wnorowski A, *et al.* Kynurenine emerges from the shadows - Current knowledge on its fate and function. *Pharmacology & Therapeutics*. 2021; 225: 107845.
- [86] Vogel CFA, Goth SR, Dong B, Pessah IN, Matsumura F. Aryl hydrocarbon receptor signaling mediates expression of indoleamine 2,3-dioxygenase. *Biochemical and Biophysical Research Communications*. 2008; 375: 331–335.
- [87] Litzenburger UM, Opitz CA, Sahm F, Rauschenbach KJ, Trump S, Winter M, *et al.* Constitutive IDO expression in human cancer is sustained by an autocrine signaling loop involving IL-6, STAT3 and the AHR. *Oncotarget*. 2014; 5: 1038–1051.
- [88] Walczak K, Wnorowski A, Turski WA, Plech T. Kynurenine acid and cancer: facts and controversies. *Cellular and Molecular Life Sciences: CMLS*. 2020; 77: 1531–1550.
- [89] Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, *et al.* Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science (New York, N.Y.)*. 1998; 281: 1191–1193.
- [90] Badawy AAB, Namboodiri AMA, Moffett JR. The end of the road for the tryptophan depletion concept in pregnancy and infection. *Clinical Science (London, England: 1979)*. 2016; 130: 1327–1333.
- [91] Badawy AAB. Immunotherapy of COVID-19 with poly (ADP-ribose) polymerase inhibitors: starting with nicotinamide. *Bio-science Reports*. 2020; 40: BSR20202856.
- [92] Chilosi M, Doglioni C, Ravaglia C, Martignoni G, Salvagno GL, Pizzolo G, *et al.* Unbalanced IDO1/IDO2 Endothelial Expression and Skewed kynurenine Pathway in the Pathogenesis of COVID-19 and Post-COVID-19 Pneumonia. *Biomedicines*. 2022; 10: 1332.