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Research Article

Molecular Docking of FICZ (6-Formylindolo[3,2-b] carbazole) to Kynurenine Pathway Enzymes: Biological Basis of a Potential Drug

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Abstract

FICZ (6-formylindolo[3,2-b]carbazole), a photooxidation product and a metabolite of the essential amino acid L-tryptophan (Trp), is one of the strongest ligands of the aryl hydrocarbon receptor (AhR), binding to which activates the receptor to exert profound effects on body homeostasis, including immune function. FICZ is an effective antagonist of diseases in experimental models and thus a potential drug target. We hypothesise that it may act in inflammatory conditions, bacterial infections and the associated multi-drug resistance, and cancer by immunosuppression via proinflammatory kynurenine metabolites following induction of the extrahepatic Trp-degrading enzyme indoleamine 2,3-dioxygenase (IDO). We explored by molecular docking in silico the likely interaction of FICZ with relevant enzymes of the kynurenine pathway and of NAD+ synthesis and utilisation that may underpin its potential therapeutic actions. We demonstrate the ability of FICZ to dock in silico to IDO, other enzymes of the kynurenine pathway and enzymes of NAD+ synthesis and utilisation. We suggest that FICZ influences Trp metabolism both directly and indirectly via AhR activation and other effects on proinflammatory cytokines. We propose also for the first time that FICZ may be involved in the phototherapy of neonatal hyperbilirubinemia by counteracting bilirubin toxicity. In the light of our docking results, we outline potential future studies of the effects of FICZ on Trp metabolism in relation to the above and other conditions.

Keywords: Bacterial resistance; Cancer; Immune suppression; Inflammatory disease; Molecular docking; Neonatal jaundice; Tryptophan metabolism

Introduction

FICZ (6-formylindolo[3,2-b]carbazole (FICZ), is a compound produced by photooxidation of the essential amino acid *L*-tryptophan (Trp) in aqueous solution [1] and culture media [2]. It can also be produced metabolically by condensation of one molecule of the Trp metabolite indoleacetaldehyde with one molecule of its enol form [3]. FICZ exerts many effects on immune and other functions that render it a potential drug target, including adaptive responses to ultraviolet light, alterations in circadian rhythms, enhanced genomic instability, differentiation and growth of T cells, innate lymphoid cells and hematopoietic stem cells, protection against immune-related diseases and Natural Killer (NK) cell-mediated control of tumor growth (see the excellent reviews in [4-6]). Many of the experimental studies with FICZ could have implications for immune-related conditions, e.g., bacterial and viral infections and cancer. As immune function is influenced by metabolites of the essential amino acid *L*-tryptophan (Trp), the present molecular docking simulations were designed to explore the potential interaction of FICZ with enzymes of Trp metabolism that may pave the way for future biological and clinical studies (Figure 1).

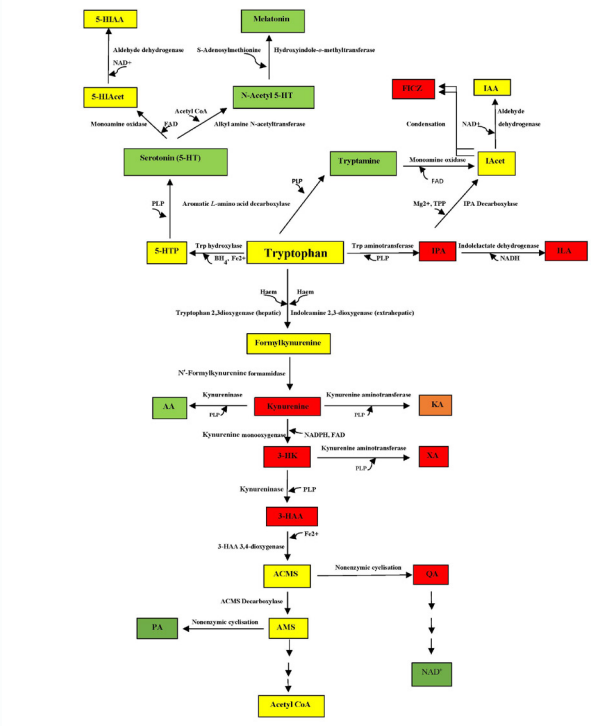

Figure 1: The tryptophan metabolic pathways.

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Trp is metabolised in mammals by 4 pathways, 3 of which are minor (each contributing ≤ 1%), whereas the fourth contributes 95% to dietary Trp degradation [7,8]. The 3 minor pathways are the transamination to Indol-3-Ylpyruvic Acid (IPA), decarboxylation to tryptamine and hydroxylation to serotonin (5-Hydroxytryptamine or 5-HT) and subsequent production of melatonin. The major pathway, the Kynurenine (Kyn) Pathway (KP), produces a range of metabolites. Gut microbiota also metabolise Trp, mainly to indoles, but many can also synthesise it. Many products of these pathways exert important physiological and biological effects that interact with many body systems and thus play important roles in health and disease [9]. Intermediates of the KP in particular exert important effects on immune function and have thus been implicated in the immune dysfunction associated with bacterial and viral infections and cancer. For example, tumors achieve their immune escape in part by inducing the key KP enzymes Trp 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) [10] to produce the Kyn metabolites 3-Hydroxykynurenine (3-HK), 3-Hydroxyanthranilic Acid (3-HAA) and Quinolinic Acid (QA) that undermine innate immunity [11,12]. Undermining immune defences in this way is also achieved by bacterial and viral infections [13]. Another key feature of activation of Trp metabolism in relation to immune function is production of Kynurenic Acid (KA) in the KP and of IPA in the transamination pathway. Both metabolites are relatively strong activating ligands of the Aryl Hydrocarbon Receptor (AhR), a transcription factor whose activation can result in protective as well as destructive effects on immune function [14]. While KA is the KP metabolite with the highest affinity for the AhR [15], IPA has a greater affinity [16]. FICZ is also a strong AhR agonist. Its affinity for the AhR is comparable to that of the most powerful exogenous ligand TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), with an IC_{50} of 1 nM each, but with FICZ being a stronger stimulant of AhR binding to DNA *in vitro*, (EC_{50} of 4.25 *vs* 7.5 nM) [17]. The AhR also regulates expression of the NAD^+-consuming enzyme poly (ADP-ribose) polymerase (PARP) [16,18]. As is the case with AhR activation, moderate PARP activation can be beneficial, whereas excessive activation can result in depletion of NAD^+ and hence of ATP, resulting in cell death [19,20]. PARP 1 and the other NAD^+-consuming enzyme SIRT 1 (silent mating type information regulation 2 homologue 1) are the largest NAD^+ consumers, with each consuming 33% of cellular NAD^+ [21]. In mammals, NAD^+ is synthesised mainly from Trp in the *de novo* biosynthetic pathway and to a lesser extent from nicotinic acid in the "Preiss-Handler", or from nicotinamide in the "Salvage", pathway (Figure 2).

As the changes in immune activity induced by FICZ can impact Trp metabolism along the KP, we considered it of interest to explore in the present molecular docking study if FICZ interacts directly with enzymes of the KP, including those leading to NAD^+ synthesis, and/or with enzymes whose activities can be influenced by KP metabolites or other related processes, with the aim of establishing or excluding a potential direct effect of FICZ on these enzymes in its mechanism(s) of action. Molecular docking was chosen as it tests direct interaction of ligands with target proteins in the absence of confounders existing in cell culture systems or *in vivo* that are likely to influence availability of the ligand, such as absorption, metabolism, enzyme reactions, protein binding and competition from other ligands.

Methods

Molecular docking of FICZ to enzymes of the KP, NAD^+ synthesis and other enzymes was performed using the Molegro virtual Docker (MVD) software as described previously [22,23]. Re-ranking was performed to improve accuracy. When re-ranked, 10 independent docking runs resulted in 10 solutions. The structure of FICZ (Supplementary Figure S1) was imported into the MVD work space in SDF format. All H atoms were added and important valence checked by using the utilities in MVD. The crystal structures of the enzymes studied were sourced from the protein data bank (http://www.rcsb.org/pdb) as detailed and referenced in Table 1 and imported into the MVD work region at reasonable resolution levels (≤ 2.8 Å). Enzymes were prepared by assigning the missing bonds, hydrogens and charges with the aid of the Swiss PDB viewer. The ligand was prepared in the same way using UCSF Chimera software. Scoring functions of the MVD and other docking details have been described [23] (Table 1 and 2).

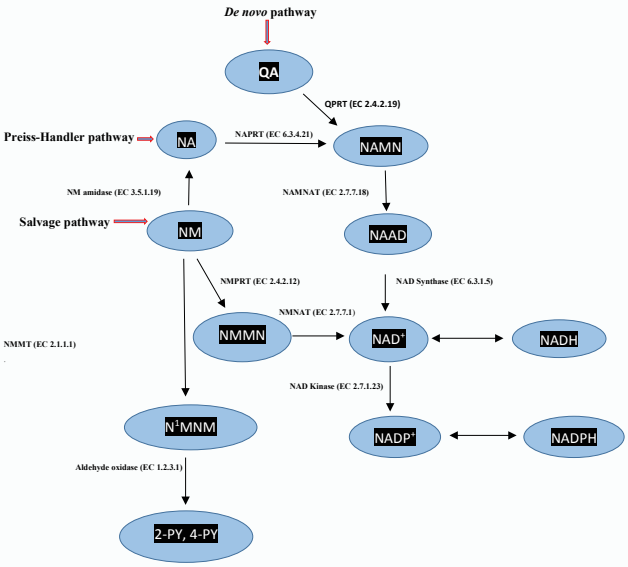

Figure 2: Pathways of NAD+ synthesis.

Results

Docking parameters are presented in Table 2, FICZ binding to enzymes is illustrated in supplementary Figure S2-S5, and amino acid residues at active sites and those binding FICZ are given in Supplementary Table S1 and Figure 3.

Docking of FICZ to enzymes of the kynurenine pathway

As shown in Table 2, Figure 3 and Figure S2 and S3, FICZ exhibited docking to 10 enzymes of the KP. The weakest docking was with TDO, whereas the strongest docking was with IDO, KAT I and KMO.

Docking of FICZ to enzymes of NAD^+ synthesis

FICZ also docked strongly to all 4 enzymes of NAD^+ synthesis (Table 2, Figure 3 and Figure S4).

Docking of FICZ to other enzymes related to tryptophan metabolism

As shown in Table 2, Figure 3 and Figure S5, FICZ also docked strongly to PARP1 and SIRT1, but less strongly to IL-4I1 (Phe oxidase).

Docking of FICZ to the AhR

Table 1: Sources of the crystal structures of the studied enzymes.

Enzyme	Source	Reference
TDO	Xanthomonas campestris in complex with ferrous heme and Trp (Northeast Structural Genomics Target XcR13: PDB ID: 2NW8)	Forouhar et al. [24] 2007
IDO	4-phenylimidazole bound form of human IDO	Sugimoto et al. [25] 2006
FAMID	Bacillus anthracis (PDB doi:10.2210/pdb4CO9/pdb)	Diaz-Saed et al. [26] 2014
KMO	Pseudomonas fluorescens KMO in complex with R061-8048 (PDB doi: 10.2210/pdb5X6Q/pdb)	Kim et al. [27] 2018
KAT I	human kynurenine aminotransferase-I bound to the PLP Cofactor (PDB doi:10.2210/pdb4WLH/pdb)	Nadvi et al. [28] 2017
KAT II	Human Kynurenine Aminotransferase II, PLP-bound Form (PDB doi: 10.2210/pdb5EUN/pdb)	Nematollahi et al. [29] 2016
KYNU	Homosapiens kynureninase (PDB doi: 10.2210/pdb2HZP/pdb)	Lima et al. [30], 2007
3-HAAO	3-Hydroxyanthranilate-3,4-dioxygenase I142P from Cupriavidus metallidurans in complex with 4-Cl-3-HAA (PDB doi: 10.2210/pdb5BTR/pdb)	Yang et al. [31], 2018
ACMSD	human dimeric ACMSD in complex with the inhibitor TES-1025 (PDB doi:10.2210/pdb7PWY/pdb)	Cianci et al. [32], 2022
QPRT	Human quinolinic acid phosphoribosyltransferase in Complex with its inhibitor phthalic acid (PDB doi: 10.2210/pdb4KWW/pdb)	Malik et al. [33], 2014
NAD synthetase	From Deinococcus radiodurans (PDB doi: 10.2210/pdb4Q16/pdb)	Lee et l. [34], 2014
NAMNAT	Human nicotinamide mononucleotide adenylyltransferase complexed with deamido-NAD	Zhou et al. [35], 2002
NMPRT	Human nicotinamide phosohoribosyltransferase phosphoribosyltransferase complexed with nicotinamide mononucleotide and pyrophosphate PDB DOI: 10.2210/pdb3DHD/pdb)	Burgos et al. [36], 2009
NPRT	Human Nicotinic acid phosphoribosyltransferase (PDB doi: 10.2210/pdb4YUB/pdb)	Marletta et al. [37], 2015
PARP1	The catalytic fraction of poly (ADP-ribose) polymerase complexed with the inhibitor isoindolinone (PDB doi: 10.2210/pdb6I8T/pdb)	Papeo et al. [38], 2019
SIRT1	Sirtuin1 in complex with resveratrol and an AMC- containing peptide (PDB DOI: 10.2210/pdb5BTR/pdb)	Cao et al. [39], 2015
Phe oxidase	Psuedomonas sp. P-501 (PDB doi: 10.2210/pdb2YR4/pdb)	Ida et al. [40], 2008

Abbreviations: TDO: tryptophan 2,3-dioxygenase; IDO: indoleamine 2,3-dioxygenase; FAMID, N´-formylkynurenine formamidase; KAT: kynurenine aminotransferase; KYNU: kynureninase; 3-HAAO: 3-hydroxyanthranilic acid 3,4-dioxygenase; ACMSD: 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase; QPRT: quinolinate phosphoribosyltransferase; NAMNAT: nicotinic acid mononucleotide adenylyl transferase; NMPRT NPRT: nicotinic acid phosphoribosyltransferase; PARP1: poly (ADP-ribose) polymerase 1; SIRT1: (silent mating type information regulation 2 homolog) 1; Phe oxidase also known as IL4-I1 (interleukin IL4-induced 1)

Table 2: Docking details of FICZ to kynurenine pathway and other enzymes.

Enzyme	Docking	Rerank	RMSD Score	Torsion	H bond energy
TDO	-1213.21	-10.94	0	1	-0.985
IDO	-135.44	-97.52	0	1	-2.403
FAMID	-18.92	-76.19	0	1	-4.697
KMO	-122.76	-92.23	0	1	-4.697
KAT I	-126.35	-95.49	0	1	0
KAT II	-118.92	-76.19	0	1	-0.449
KYNU	-108.19	-79.67	0	1	0
3-HAAO	-108.6	-67.12	0	1	0
ACMSD	-108.81	-62.42	0	1	0
QPRT	-107.24	-64.78	0	1	-3.896
NAD synthase	-118.72	-92.95	0	1	0
NAMNAT	-124.27	-94.82	0	1	-2.5
NMPRT	-132.32	-89.75	0	1	-0.843
NPRT	-115.9	-87.39	0	1	0
PARP 1	-116.833	-92.79	0	1	0
SIRT 1	-146.698	-91.68	0	1	0
Phe oxidase	-115.94	-43.52	0	1	-1.3185

RMSD (root mean square deviation). For other abbreviations, see Table 1.

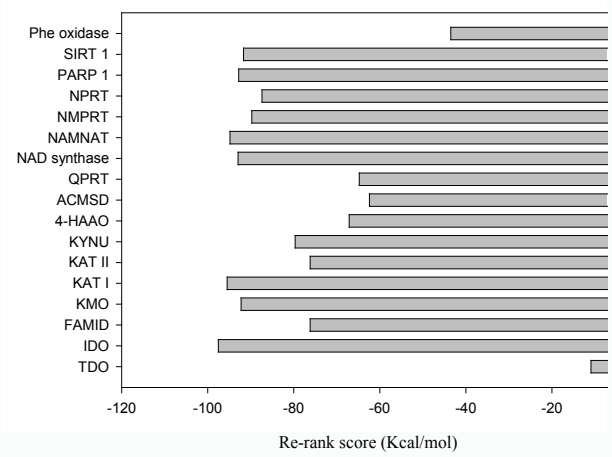

Figure 3: Molecular docking of FICZ to enzymes of tryptophan metabolism and related enzymes. For abbreviations, see the text.

Although this is a well-established property of FICZ, we have confirmed in the present study the strong docking of FICZ to the crystal structure of the AhR. With our Molegro Virtual Docker software, FICZ gave a MolDock score of -118.07 and a rerank score of -97.05 (Kcal/mol), in par with indirubin and a slightly stronger docking than the classical exogenous ligand TCDD (data not shown).

Discussion

The present molecular docking study with FICZ was designed to explore if this compound is likely to interact directly with enzymes of the major tryptophan degradative pathway, the KP, including those leading to production of the end product of the pathway, NAD^+, or its utilisation (by PARP1 and SIRT1). The results presented herein demonstrate the ability of FICZ to dock to all enzymes tested and strongly suggest that this metabolite and photooxidation product of Trp is likely to exert profound and direct effects on the metabolism of this amino acid. As far as we could ascertain, no studies with FICZ other than upregulation of IDO have been performed and so the present results open a new avenue of investigation of the potential effects of this molecule on Trp metabolism. Whereas molecular docking gives an indication of binding of ligands to target proteins, with a low (negative) binding energy reflecting strong affinity, the docking scores do not distinguish between agonists and antagonists. Accordingly, and with the limited published experimental evidence available, an attempt has been made here to deduce the type of effect FICZ is likely to exert on Trp-metabolising and related enzymes (Table 3).

Table 3: Potential indirect effects of FICZ on tryptophan metabolism.

Effect of FICZ	Ref	Mechanism	Outcome
↑ IL-1α, IL-1β, IL-6	[43]	IDO1 induction	↑ P-I Trp metabolites ® immunosuppression
AhR activation	[42]	↑ NK cells, ↑ IFN-g	↑ IDO1 ® ® immunosuppression
	[43,509]	↑ IL-6-STAT3 loop	↑ IDO1 ® ® immunosuppression
	[52]	↑ NEFA?	↑ Free Trp ® ↑ flux® ↑ P-I Trp
	[53]	↑ LAT-1	↑ Trp transport and flux
	[51,53]	↑ SLC7A5	↑ Trp transport and flux
	[16,18,19]	↑ PARPs	↑ NAD+, ↑ ATP, cell death

References: [16] (Sadek et al., 2020), [18] (Dere et al., 2006), [19] (Ha and Snyder, 1999), [42] (Tanaka et al., 2018), [43] (Vogel et al., 2008), [50] (Litzenberger et al., 2014), [51] (Kaiser et al., 2020), [52] (Boverhof et al., 2006), [53] (Tamblin et al., 2016).

Indirect effects of FICZ that may impact Trp metabolism

No direct effects of FICZ on activities of enzymes of the kynurenine pathway have been reported to-date. The only known effect of FICZ on KP enzymes is upregulation of IDO (see below). Indirect effects of FICZ on Trp metabolism observed *in vivo* can only be initiated by the actions of FICZ elsewhere. The most prominent, but not the only, effect of FICZ is its binding to and activation of the AhR [2]. Many of these AhR-dependent and -independent effects can impact Trp metabolism, as outlined and referenced in Table 3. By upregulating proinflammatory cytokines, such as IL-1α, IL-1β and IL-6 [41,42], FICZ is almost certain to cause induction of IDO1. IDO1 mRNA expression is enhanced by FICZ in human monocyte-derived U937 dendritic cells [43], mouse dendritic cells [44], human langerhans cells [45] and human monocytes [46]. Other studies however did not report upregulation of IDO1 expression [47,48]. Potential explanations of these opposite findings could be rapid degradation of FICZ by cytochrome CYP1A1 [49], cell type and duration of exposure. For example, AhR activation by FICZ in cultured keratinocytes reaches the highest level at 1h, but declines to a much lower level by 24h [42]. Also, AhR activation by FICZ, though strong, is reversible [41]. These latter authors also reported that FICZ increases Natural Killer (NK) cells and stimulates their production of IFN-γ, the most powerful IDO1 inducer. Thus, from the above discussion, it can reasonably be assumed that the strong docking of FICZ to IDO (Table 2) suggests that FICZ is an IDO agonist. Several other potential indirect mechanisms involve AhR activation. Thus, AhR activation induces IDO1 via an AhR-IL-6-STAT 3 autocrine loop [43,50] or a positive feedback loop [51]. AhR activation influences lipid metabolism in several ways (increased lipogenesis, enhanced mobilisation and transport) that could impact Trp metabolism. Thus, AhR activation by TCDD increases plasma Nonesterified Fatty Acids (NEFA) in rats and mice [52]; an effect that can cause the release of albumin-bound Trp and thus increase its flux down the KP to produce immunoactive metabolites [7]. A similar effect is likely to occur by FICZ activation of the AhR. Similarly, AhR activation by TCDD upregulates the amino acid transporters LAT-1 (SCL7A5) [51,53], secondarily to IDO induction, thus providing the positive feed-forward mechanism of action of AhR proposed by Kaiser et al. [51]. Furthermore, SLC7A5 is up-regulated by both IL-1β [54-56] and IL-2 [57]. These effects could therefore be exerted also by FICZ. AhR activation by TCDD [18] and by IL-4I1 (Phe oxidase), which is mediated by KA and/or IPA [16], upregulates poly (ADP-ribose) polymerase (PARP) gene expression. FICZ is therefore likely to activate PARPs. Activation of the AhR or PARPs is not intrinsically harmful, whereas over-activation could be. Harm can occur with the AhR inducing a state of heightened immune suppression [14], whereas with excessive PARP activation, depletion of NAD^+ and ATP can result in cell death [19]. Based on the above assumption, it is not clear if FICZ activates or inhibits PARP (see below). It is possible that the level of FICZ is the deciding factor. Accordingly, it is important to distinguish between the protective and harmful effects of AhR activation by FICZ. FICZ is protective at low concentrations, but toxic at high ones. The low physiological levels of FICZ (picomolar) and its rapid degradation by cytochrome *P*-450s ensure that high levels are not reached. The toxicity of FICZ is in general restricted to fish and birds [5]. It can therefore be assumed that FICZ is in the main protective, hence its usefulness in experimental disease models (see below).

Biological effects of FICZ: FICZ possesses a range of biological properties many of which are mediated by AhR activation [4-6]. Notable among these effects are self-renewal and differentiation of stem/progenitor cells, regulation of embryonic development, differentiation of immune cells and modulation of immune responses. Various effects of FICZ have been proposed in many preclinical studies as potential mechanisms of its therapeutic actions in relation to a range of clinical conditions, as summarised and referenced in Table 4.

Potential therapeutic effects of FICZ in clinical conditions: A number of clinical conditions have or can be been linked to a FICZ involvement. These are outlined with references in Table 4 and described below.

Inflammatory conditions: By far, most studies with FICZ are related to inflammation, with levels of various cytokines being modulated as stated in Table 3 (see also [5] and references cited therein). In skin inflammatory conditions, FICZ decreases skin inflammation in the imiquimod model [58], ameliorates skin inflammation in the above model by upregulating the chemokine (C-X-C motif) ligand 5 (Cxcl5) [59] and protects against mite-induced dermatitis [60]. In mouse models of other inflammatory conditions, such as inflammatory bowel disease, multiple sclerosis, peritonitis and rheumatoid arthritis, FICZ either lessens or increases the severity of the lesion (reviewed in [5]). In these experimental studies, decreased severity is associated with decreased levels of IL-17 and other cytokines, including IL-1β, IL-6, IFN-γ and TNF-α and increased levels of IL-10 and IL-22. By contrast, increased severity is associated with elevated levels of IL-17. A notable difference in these opposing responses is in the route of FICZ administration. Decreased severity occurs when FICZ is administered intraperitoneally in doses of 10 mg/kg once or 50 μg/kg once, 5 or 7 times. Decreased severity involving protection against bone loss was also demonstrated after tail-vein injection of a small (100 μg/kg) or a large (100 mg/kg) dose of FICZ in an osteoarthritis mouse model [65]. Intravenous pump [66] and oral [67] administration of FICZ to mice are not associated with harmful effects in studies of influenza A virus and peanut-induced allergy respectively.

Table 4: Experimental models of disease associated with a favourable effect of FICZ.

Condition	Model	Mechanism(s)	Reference
Inflammatory conditions:	Imiquimod model	↓ IL-17, ↓ IL-22	Di Meglio et al. [58], 2014
Skin inflammation	Imiquimod model	↑ Chemokine ligand 5 (Cxcl5) n	Smith et al. [59], 2017
Atopic dermatitis		↑Filaggrin, ↓ IL-22	Kiyomatsu-Oda et al. [60],
Bacterial infections	Listeria monocytogenes	Macrophage survival	Kimura et al. [61], 2013
	in mice	↑ ROS production	
	total bacteria and Enterobacteriaceae	↓ IL-6, KC, IL-18, apoptosis	Li et al. [62], 2020
Cancer	RMA-S Lymphoma	↑NK cells, ↑ IFN-g	Shin et al. [41], 2013
	B16 melanoma cells		
	(Lung metastatic tumors)		
	Mice in vivo		
	Breast cancer cells	↑ OVOL 1	Fan et al. [63], 2022
	Prostate cancer LNCaP cells	↓ Androgen-responsive	Arabnezhad et al. [64], 2020
		Target genes	
Neonatal hyperbilirubinemia		Antagonism of bilirubin toxicity	This study

Abbreviations: IFN: interferon; IL: interleukin; KC: keratinocyte-derived chemokine; NK: natural killer; OVOL 1: OVO-like transcriptional repressor 1; ROS: reactive oxygen species

By contrast, subcutaneous administration of a single 30 µg/kg body weight dose of FICZ is associated with increased severity. A possible explanation of these opposite responses may be related to differences in FICZ metabolism as a function of the administration route. The oral and 2 parenteral administration routes are likely to result in rapid metabolism of FICZ and a consequent availability of protective (low) levels, whereas more sustained (harmful) levels are likely to result from slower absorption of the lipophilic FICZ from subcutaneous tissues.

The relationships and interactions between the AhR, Cxcl5, IL-17 are complex and could also impact Trp metabolism. Thus, AhR activation by TCDD: a property also shared by FICZ, enhances the expression of the chemokine Cxcl5 [42], which recruits neutrophils, with the latter being a major player in innate immunity, as they are responsible for activation and recruitment of a range of immune cells to the site of infection, and for increased cytokine production [68]. Under these conditions, Trp metabolism is likely to be activated via IDO1 induction. IL-17 is considered a double-edged sword [69] and exhibits a reciprocal relationship with IDO1. For example, IFN-γ knock out mice show increased IL-17 -producing cells and exacerbation of collagen-induced arthritis [70]. By contrast, *IDO1* gene deletion in mice down-regulates IL-17 cells and protects against CCl_4-induced liver fibrosis [71].

OVO-like transcriptional repressor 1 (OVOL 1) can also influence IDO 1 activity via its inhibition of TGF-β signaling [63]. Thus, IDO1 is induced by TGF-β1 and both exhibit a positive correlation [72]. Thus, it appears likely that IDO 1 plays an important role in the potential clinical effects of FICZ in cancer, but in a manner opposite that in other inflammatory conditions, which may be explained by Fan et al. [63] having used a [FICZ] (5M) greatly higher than that (1 nM) capable of AhR activation. In the above described study of liver fibrosis [70], the authors reported a compensatory increase in *TDO2* gene expression in *IDO1* gene deleted mice. Some findings in *TDO* gene-deleted mice suggest that the reverse is also true [73.74].

Bacterial infections: Several studies have suggested that FICZ is effective in arresting bacterial growth and virulence. These include protection of mice against *Listeria monocytogenes* lethality by promoting macrophage survival and Reactive Oxygen Species (ROS) production [61] and protection of gut barrier against bacterial translocation, improving gut motility and decreasing bacterial numbers [62]. The efficacy of FICZ in reducing bacterial numbers and infections suggest that it could be a therapeutic target for combating bacterial infection and/or its resistance to other therapies. Regarding bacterial resistance, the Gram-negative *Acinetobacter baumannii* exhibits multi-drug resistance and using artificial intelligence followed by *in silico* prediction, Liu et al. [75] identified Abaucin, a compound with a narrow selective activity against the above bacterium that acts by inhibiting lipoprotein trafficking and was shown to control *A. baumannii* infection in a mouse wound model. Whether FICZ possesses antibacterial properties against *A baumannii* remains to be established. It is possible that the failure of *A. baumannii* to grow in a Trp-supplemented medium [76] may be due to FICZ formation and, if so, this would suggest that FICZ may be effective against this drug-resistant bacterium.

***Cancer*:** The study by Shin et al. [41] extends to cancer the theme of rapid metabolism of FICZ after its parenteral administration. Thus, the above authors reported that intraperitoneal administration of 3 doses of FICZ of 150 µg/kg each to mice infected with RMA-S lymphoma or B16 melanoma (breast cancer) reduced tumor growth and lung metastasis. In other breast cancer models, FICZ antagonises migration of cancer cells by activating the expression of the OVO-like transcriptional repressor 1 (OVOL1) [63]. As regards prostate cancer, FICZ inhibits the expression of androgen-responsive target genes and decreases levels of prostate-specific antigen and dihydrotestosterone [64]. While some of the above studies provided a strong link between FICZ and IDO1 in cancer, fewer studies of changes in KP enzymes beyond IDO have been performed. Thus, in renal cell carcinoma, QPRT is strongly down-regulated, resulting in increased QA levels, despite down-regulation of FAMID, KMO, and 3-HAAO [78]. KAT I and KAT II were also down regulated [77]. Down-regulation of KMO also occurs in many cancer types [78]. In this latter study of 28 cancer types, the numbers of significant upregulation and down-regulation of KP enzymes were as follows: TDO (13:2), IDO1 (10:4), IDO2 (6:11), FAMID (9:5), KMO (4:12), KAT (9:5), KYNU (5:6), 3-HAAO (1:18), ACMSD (3:9). With QPRT, an indirect estimate of 8:8 could be made. Thus, the most striking findings from these estimates are the preponderance of upregulation of TDO2, IDO1, FAMID and KAT and of down regulation of KMO and 3-HAAO. These relative changes in KP enzymes strongly suggest that the pathway will be diverted towards KA formation. This suggestion is consistent with the observed increase in [KA] in a range of cancer types [79]. FICZ

may therefore act as a KAT antagonist in cancer. As NAD^+ synthesis in the *de novo* pathway from Trp is inhibited in cancer, almost likely by QPRT down-regulation [77], FICZ is likely to act as agonist of this enzyme. By contrast, evidence exists for tumors upregulating enzymes of the salvage pathway of NAD^+ synthesis for their own requirements, from nicotinamide, namely NMPRT and NAMNAT [78]. The potential effects of FICZ docking to these two enzymes is therefore likely to be that of an antagonist (inhibitor), especially in view of the strong docking scores of FICZ with these two enzymes. Expression of NPRT, by contrast, appears to be decreased in many cancers [77,80], but whether FICZ activates this enzyme remains to be established. Both NAD^+-consuming enzymes PARP1 and SIRT1 are also activated in a range of cancers [77] and it may therefore be proposed on this basis that FICZ may inhibit both enzymes. A similar possibility applies to IL-4I1, as this immune check-point effector activity is enhanced in cancer [16] (Table 5). Based on our molecular docking findings and the above observations, a summary of known and potential effects of FICZ on KP and related enzymes based on the limited information available is given in Table 5. The predicted effects of FICZ are those likely to occur with moderate AhR activation by low concentrations of FICZ, but the ultimate proof must await experimental scrutiny.

Table 5: Predicted likely effects of FICZ on enzymes of the kynurenine pathway and of related processes.

Enzyme	FICZ as:	Enzyme	FICZ as:
TDO	?	QPRT	Agonist
IDO	Agonist	NAD synthase	?
FAMID	Agonist	NAMNAT	Antagonist
KMO	Agonist	NMPRT	Antagonist
KAT	Antagonist	NPRT	Agonist
KYNU	?	PARP 1	Antagonist
3-HAAO	Agonist	SIRT 1	Antagonist
ACMSD	Agonist	IL-4I1	?

Phototherapy of neonatal hyperbilirubinemia: Light therapy of Neonatal Hyperbilirubinemia (NNH) is likely to be achieved in part by photooxidation of Trp to FICZ. NNH can precipitate the condition of kernicterus that is associated with brain damage. Light therapy is aimed at photooxidation of bilirubin to biliverdin and other harmless or less toxic compounds. The main photooxidation product of bilirubin is lumirubin. Although bilirubin possesses both toxic and protective properties [81], the therapeutic efficacy of exposure to light suggests that the toxic effects outweigh the protective ones. The high bilirubin levels in kernicterus may be responsible for the neurological dysfunction in this condition. Bilirubin, however, induces cytochrome *P*-450 2A5. This and another *P*-450 (2A6) catalyse the oxidation of bilirubin to biliverdin [82]. How much biliverdin is recycled in this way and what is the balance between biliverdin and bilirubin levels in NNH remain to be established. Light therapy of NNH may not be limited to bilirubin photooxidation, but could extend to that of Trp, leading to the formation of FICZ. The low plasma albumin in the newborn infant results in increased free Trp availability. At birth, both free and total (free + albumin-bound) [Trp] in newborn plasma are double those in maternal circulation (for discussion and references, see [83]). Light therapy of NNH is almost certain to convert Trp into FICZ in infant tissues including the brain. With mild AhR activation at low FICZ levels, as would be expected during light therapy of NNH, an antiinflammatory state can arise, thereby countering bilirubin toxicity. Exploring FICZ formation and its actions could represent a novel approach in understanding the pathophysiology and therapy of NNH. The potential role of AhR activation in NNH could also be explored. In the accompanying paper, we demonstrate a strong docking of lumirubin to the crystal structure of the human AhR and to receptors influencing neuronal function and the failure of bilirubin and biliverdin to interact with these receptors (Figure 3).

Conclusions and Future Research Directions

The above accounts point to induction of IDO1 as a unifying hypothesis for the potential mechanism of action of FICZ in a range of clinical conditions. This brings into focus the kynurenine pathway of Trp metabolism as the major target of FICZ, which is capable of docking to all enzymes of the pathway tested. The potential effects of FICZ on Trp metabolism have not been explored and our molecular docking data suggest a range of studies in this respect. (1) The effects of FICZ on the kynurenine pathway require a systematic investigation involving measurement of gene expression and catalytic activities of the pathway enzymes and metabolite concentrations. (2) AhR activation by FICZ is likely to increase plasma [NEFA], which could result in elevation of plasma free [Trp] and hence enhancing the flux of Trp down the Kyn pathway. (3) The ability of FICZ to increase levels of the cytokines IL-1β and IFN-γ [42,43] suggests that expression of kynurenine monooxygenase (KMO) is likely to be enhanced [84-87], leading to increased production of immunomodulatory kynurenine metabolites. (4) AhR activation leads to increased expression of PARPs and a potential depletion of NAD^+. This is likely to occur with excessive and sustained AhR activation if FICZ is administered subcutaneously. (5) Pharmacokinetic, pharmacodynamic and safety studies should be performed with FICZ to distinguish between low and high doses, routes of administration, and safety or toxicity. (6) The effects on Trp metabolism and production of FICZ by exposure to light can yield supporting evidence for a potential role of FICZ in the phototherapy of neonatal hyperbilirubinemia.

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Supplementary Files

Supplementary Table 1: Amino acid residues at enzyme active sites and at FICZ docking.

Enzyme	Amino acid residues at active site	Ligand binding amino acids
TDO	Thr254, His55, Phe51, Leu120, Tyr120, Ser123, Tyr113, Gly121	Gly125, Tyr24, Ser123
IDO	Arg231, Gly262, 236, Ser263, Asn240	Gly261
FAMID	Pro125, Asn87, Thr111, Ser113, Asp72, Glu89, Leu88	Leu88, Asn87
KMO	Asp112, Ser52, Arg116, Asn110, Ile53, Arg49, Ala48, Thr46	Arg116, Asn110, Ser52
KAT I	Lys65, Thr64, 66, Tyr63, Phe278	Lys65, Thr66, Phe278, Trp18
KAT II	Ser77, Pro76, Gln289, Leu293, Tyr74	Ser77, Gln289
KYNU	Lys38, Leu39, Glu36, Asp37, Leu363	Asp37, Lys360
3-HAAO	Phe148, 149, Tyr118, Ile145	Tyr118, Phe148
ACMSD	His74, Trp191, 176, 194, Arg47, 243, Phe294	Phe294, Arg47
QPRT	Gly143, Glu25, Arg145, Leu146, Tyr162, Asp163	Leu146, Tyr162
NAD synthetase	Arg148, Leu88, Ile54, Ala49, Gly53	Arg148, Leu88
NAMNAT	Asn214, Arg207, Trp204, Gln234, Gly233, Val213	Gln234
NMPRT	Arg392, Arg196, Phe193, Gly383, Asp393	Arg392
NPRT	Arg171, Leu170, Gly169, Asn356, Ile358	Arg171, Gly169
PARP1	Tyr907, Gly863, Ser864, 904	Tyr907, Ser904
SIRT1	Thr209, Phe414, Pro447, Arg446	Lys444, Arg446
Phe xidase	Tyr896, 907, Lys903. Met890	Tyr896, Tyr907

Abbreviations: TDO, tryptophan 2,3-dioxygenase; IDO, indoleamine 2,3-dioxygenase; FAMID, N´-formylkynurenine formamidase; KAT, kynurenine aminotransferase; KYNU, kynureninase; 3-HAAO, 3-hydroxyanthranilic acid 3,4-dioxygenase; ACMSD, 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase; QPRT, quinolinate phosphoribosyltransferase; NAMNAT, nicotinic acid mononucleotide adenylyl transferase; NMPRT NPRT, nicotinic acid phosphoribosyltransferase; PARP1, poly (ADP-ribose) polymerase 1; SIRT1, (silent mating type information regulation 2 homolog) 1; Phe oxidase also known as IL4-I1 (interleukin IL4-induced 1).

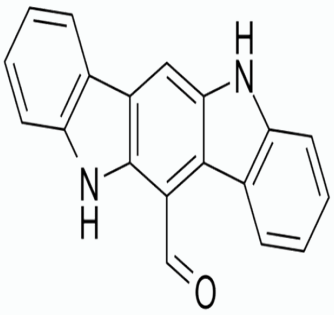


Figure S1: Structure of FICZ. Reproduced here courtesy of medchemexpress.com.

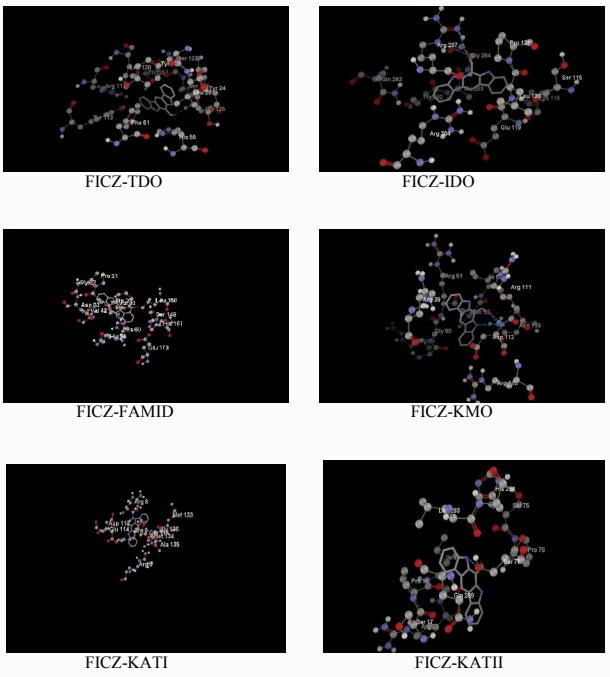
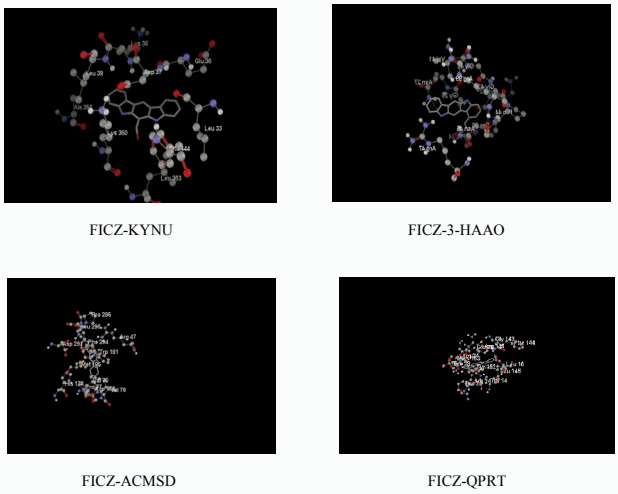


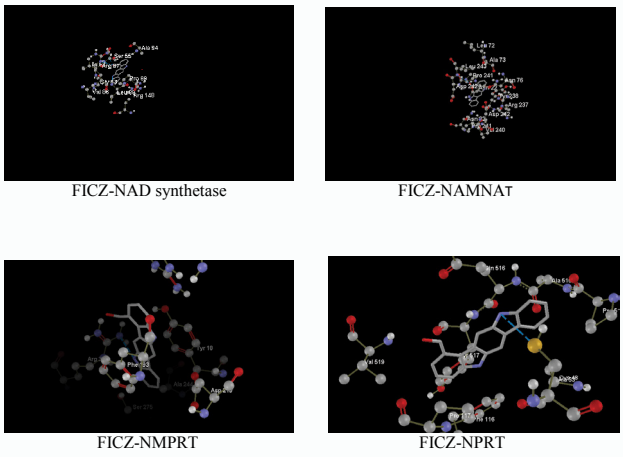
Figure S2: Docking of FICZ to TDO, IDO, FAMID, KMO, KAT I and KAT II. FICZ was docked as described in the Methods section to the crystal structures of the above enzymes of the kynurenine pathway: Abbreviations: TDO (Trp 2,3-dioxygenase), IDO (indoleamine 2,3-dioxygenase), FAMID (N´-formylkynurenine formamidase), KMO (kynurenine monooxygenase), KAT (kynurenine aminotransferase).



FICZ-KYNU FICZ-3-HAAO

FICZ-ACMSD FICZ-QPRT

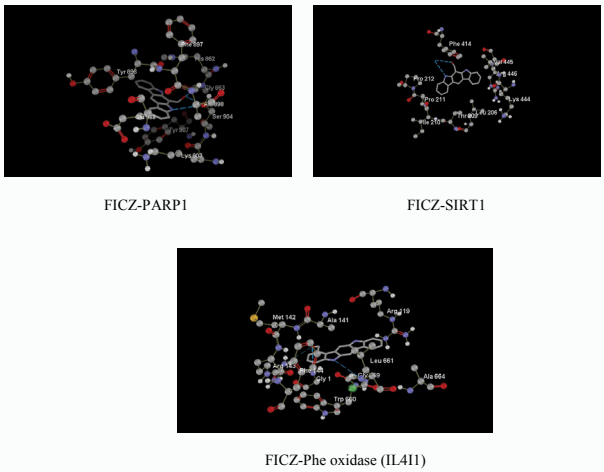
Figure S3: Docking of FICZ to 3-HAAO, KYNU, ACMSD and QPRT. FICZ was docked as described in the Methods section to the crystal structures of the above enzymes of the kynurenine pathway: Abbreviations: KYNU (kynureninase), 3-HAAO (3-hydroxyanthranilic acid 3,4-dioxygenase), ACMSD (2-amino-3-carboxymuconic acid-6-semialdehyde decarboxylase), QPRT (quinolinate phosphoribosyltransferase).



FICZ-NAD synthetase FICZ-NAMNAT

FICZ-NMPRT FICZ-NPRT

Figure S4: Docking of FICZ to enzymes of NAD+ synthesis. Docking was performed as described in the Methods section. Abbreviations: NAMNAT (nicotinamide mononucleotide adenylyltransferase), NMPRT (nicotinamide phosphoribosyltransferase), NPRT (nicotinate phosphoribosyltransferase).



FICZ-PARP1 FICZ-SIRT1

FICZ-Phe oxidase (IL4I1)

Figure S5: Molecular docking of FICZ to PARP1, SIRT1 and IL-4I1. Docking was performed as described in the Methods section. Abbreviations: PARP [poly (ADP-ribose) polymerase], SIRT (sirtuin: silent mating type information regulation), IL4I1(interleukin-4-induced-1 or L-phenylalanine oxidase).