Please download and read the Referee Guidelines

Inhibitors of the kynurenine pathway as neurotherapeutics:
a patent review (2012-2015)

SCHOLARONE™
Manuscripts

Abstract

Introduction: The proven pathological alterations in the kynurenine pathway of tryptophan

metabolism either in preclinical models of neurological and psychiatric disorders or in human

samples themselves elicited numerous attempts to restore the altered balance via

pharmaceutical manipulation of the pathway.

Areas covered: The aim of the authors was to conduct a review of relevant scientific data on

enzyme inhibitors of the kynurenine pathway, with a special attention to pipeline drug

development strategies based on relevant patent literature, covering the period of 2012-2015.

Considering the magnitude of the topic, only the most prominent examples of lead

compounds and substances necessary to enlight structure activity relationships were reported.

Expert opinion: Although the clinical and preclinical data are reassuring, there is a lack of

applicable drugs in daily clinical practice. However, the recent determination of enzyme

structures considerably promoted the development of potent inhibitors, most of them have

been designed as a structural analog of the natural enzyme substrate. Especially, the inhibition

of indolamine 2,3-dioxygenase in central nervous system tumors, the inhibition of kynurenine

aminotransferase in cognitive dysfunction and the inhibition of kynurenine 3-monooxygenase

in neurodegenerative disorders, such as Huntington's disease, alike provide a great promise.

Keywords: inhibitors, kynurenine pathway, neurotherapeutics, tryptophan metabolism

Article highlights

- The kynurenine pathway of tryptophan metabolism serves as an interesting drug target in certain neurological and psychiatric conditions.

- Amongst therapeutic options, the development of enzyme inhibitors would be one of the leading options for~~in~~ pharmaceutical manipulation of pathological alterations.

- In light of preclinical data, especially the inhibitors of indolamine 2,3-dioxygenase, kynurenine aminotransferase and kynurenine 3-monooxygenase could exert beneficial effects in future clinical studies.

- Probably the most extensively studied field is that of the kynurenine 3-monooxygenase inhibitors, highlighting the lead compounds of *trans*-2-substituted-cyclopropane-1-carboxylic acids, phenylthiazole, phenylthiadiazole and pyrimidine benzenesulfonamides, and aryl pyrimidines.

- The reassuring preclinical data warrant future well-designed clinical studies to ~~be~~enable ~~to determine which~~ the identification of compounds that may provide relief in some devastating neurological and psychiatric illnesses.

Abbreviations

3-HAO – 3-hydroxyanthranilate 3,4-dioxygenase

3-OH-ANA – 3-hydroxyanthranilic acid

3-OH-L-KYN – 3-hydroxy-L-kynurenine

4-chloro-L-KYN – 4-Cl-L-KYN

AD – Alzheimer's disease

AMPAR – α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors

BBB – blood-brain barrier

CNS – central nervous system

FAD – flavin adenine dinucleotide

GPR35 – G protein-coupled receptor 35

HD – Huntington's disease

IC_{50} – half maximal inhibitory concentration

IDO – indolamine 2,3-dioxygenase

IFN – interferon

KAT – kynurenine aminotransferase

KMO – kynurenine 3-monooxygenase

KYN – kynurenine

KYNA – kynurenic acid

mitAAT – mitochondrial aspartate aminotransferase

NAD^+ – nicotinamide adenine dinucleotide

$NADP^+$ – nicotinamide adenine dinucleotide phosphate

NMDAR – N-methyl-D-aspartate receptor

PLP – pyridoxal-5'-phosphate

QUIN – quinolinic acid

SAR – structure activity relationship

TDO – tryptophan 2,3-dioxygenase

TRP – tryptophan

1. The kynurenine pathway of tryptophan metabolism

The kynurenine (KYN) pathway is the main route of tryptophan (TRP) metabolism (Fig. 1.; [1]), more than 95% of TRP is metabolized through kynurenines[2]. This pathway consists of several neuroactive metabolites and ~~ensures~~ provides the major source of nicotinate [3] for the production of nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate $(NADP^+)$ coenzymes. The neuroactive kynurenines include kynurenic acid (KYNA), 3-hydroxy-L-KYN (3-OH-L-KYN), and quinolinic acid (QUIN), and alterationsin their levels have proven pathogenic roles in several neurological and psychiatric conditions [4]. KYNA is a wide-spectrum endogenous antagonist of ionotropic excitatory amino acid receptors[5]. In micromolar concentrations, in addition to exerting a weak antagonistic effect on α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) and kainate receptors [6]it displays competitive antagonism at the strychnine-insensitive glycine-binding site of the N-methyl-D-aspartate receptor (NMDAR; [7]). In addition to its inhibitory profile, in nanomolar concentrations it is capable of facilitating AMPAR responses, raising the possibility of partial agonism[8]. Previously it was believed that ~~D~~despite the above-described broad action at glutamatergic receptors, the main mechanism of action of KYNA on glutamatergic neurotransmission at physiologically relevant nanomolar concentrations would be mediated via the non-competitive blockade of α7-nicotinic acetylcholine receptors at glutamatergic terminals[9], the activation of which is involved in the regulation of glutamate release[10]. However, the results of the study presented by Hilmas et al. [9]could not be repeated by experiments of four different, independent laboratories, indicating that KYNA may not influence glutamate release via the blockade of α7-nicotinic acetylcholine receptors[11-14]. These contradictory findings may be attributable to a non-specific effect produced by dimethyl sulfoxide applied as a solvent for

KYNA.Nevertheless other possible reasons such as regional or species differences, or the age of the preparation could not be accounted for the observed contradictory results [14].

Although it has been reported as well that KYNA can elicit inositol trisphosphate production and Ca^{2+} mobilization through the activation of G protein-coupled receptor GPR35 (GPR35) [15], the role of this phenomenon is questionable as regards the central nervous system (CNS) due to the limited expression of GPR35 in the brain. In addition to acting on cell surface receptors, it is suggested that KYNA can also exert potent free radical scavenging and antioxidant effects[16]. With regard to 3-OH-L-KYN, its deleterious effects are proposed to be mediated by free radicals and not by glutamate receptors[17]. Some of the toxic actions of 3-OH-L-KYN may be due attributable to its metabolite, 3-hydroxyanthranilic acid (3-OH-ANA), which readily undergoes auto-oxidation with the production of O_2^-[18], resulting in neurotoxic properties [19]. QUIN is a weak, but specific competitive agonist with low receptor affinity at the NMDAR subgroup containing GLUN2A and GLUN2B subunits [20], the latter having a special role in mediating glutamate-induced excitotoxicity [21]. In addition to the direct activation of NMDARs, QUIN can evoke the release and inhibit the uptake of glutamate as well [22]. Furthermore, the production of reactive oxygen intermediates and the induction of lipid peroxidation also contribute to the neurotoxic effects of QUIN [23]. Several neurological conditions can be characterized by the absolute or relative decrease in KYNA concentrations and the elevation of 3-OH-L-KYN and QUIN [4,24-26]. However, the excessive elevation of KYNA can also have detrimental effects, such as the impairment of cognitive functioning in schizophrenia [27,28]and Alzheimer's disease (AD) [29-31]. In light of the above-mentioned data, there are 3 main ways of achieving neuroprotection targeting the kynurenine pathway: the prodrug, the KYNA analog and the metabolic shift concepts [4]. With regard to the metabolic shift concept, many enzyme inhibitors have been developed with the aim of restoratversing pathological alterations at the level of neuroactive

kynurenines.Although the question may arise whether the inhibition of TRP metabolism results in pathological decrease of de novo NAD^+ biosynthesis [32], the existence of the salvage pathway most probably provides a sufficient compensation through an appropriate exogenous intake of niacin, especially in light of the fact that niacin deficiency could result in pellagra, a disease linked to chronic systemic depletion of NAD^+.

2. Structure and function of certain enzymes of the kynurenine pathway and their inhibitors as neurotherapeutics

2.1 Indolamine 2,3-dioxygenase 1 and 2, tryptophan 2,3-dioxygenase

The conversion of L-TRP by indolamine 2,3-dioxygenase (IDO, also known as IDO1, EC: 1.13.11.52) and tryptophan 2,3-dioxygenase (TDO, also known as TDO2, EC: 1.13.11.11) enzymes to N-formyl-KYN is the rate-limiting step of the synthesis of L-KYN and the KYN pathway itself. Although IDO1 shares very limited homology with TDO at amino acid level, both enzymes are *b*-type heme-dependent cytosolic dioxygenases and their mechanism of action includes the incorporation of two atoms of molecular oxygen into the indole ring of their substrate [33]. Enzyme-structure studies, yielding three dimensional models as well, demonstrated that IDO is a monomeric, two-domain enzyme comprising one molecule of heme b [34], whereas TDO is a tetrameric protein comprising four molecules of heme b[35,36]. A third, more recently identified tryptophan catabolic enzyme is IDO2 (also known as INDOL1, EC: 1.13.11.-), which is structurally similar to IDO and is encoded adjacently [37,38]. It is, however, unclear if IDO2 is functionally active in humans [39,40], and its relevance in conditions affecting the human neural system has not yet been demonstrated. IDO is widely expressed in the body including the CNS; however, it is more abundantly expressed in immune cells (especially antigen-presenting cells such as macrophages and dendritic cells) and the placenta. In contrast, TDO is preferentially expressed in the liver.

Unlike TDO, IDO is readily inducible by various inflammatory stimuli, including interferon-α

(IFNα), IFNβ and IFNγ, interleukin-1 and -2, tumor necrosis factor, cytotoxic T lymphocyte

antigen 4, transforming growth factor β, and lipopolysaccharide [41]. The activation of the

KYN pathway has in turn immunosuppressive properties, mediated in part via TRP depletion,

and the elevated levels of KYN, 3-OH-KYN, 3-OH-ANA, and QUIN, which lead to the

suppressed proliferation and increased apoptosis of activated T helper 1 lymphocytes, as well

as the increase in the number of regulatory T cells [4]. The immunosuppressive effect of

placental IDO activation and downstream kynurenines is thought to be responsible for

maternofetal immune tolerance, and its fingerprint in terms of elevated KYN/TRP ratio has

recently been evidenced from human umbilical cord vein serum samples [42]. Increased IDO

activation has been linked to various neurological and psychiatric disorders where

neuroinflammation is implicated at some point of the pathogenesis, including Parkinson's

disease, Huntington's disease (HD), AD, multiple sclerosis, amyotrophic lateral sclerosis,

cerebral ischemia, CNS infections and major depression (widely reviewed in [4]). On the

other hand, elevated TDO expression, increased KYNA levels, and elevated kynurenine 3-

monooxygenase (KMO) activity have been observed in regions preferentially involved in

schizophrenic patients, with increased KYNA and decreased QUIN levels measured in the

cerebrospinal fluid, alterations suggested to essentially contribute to the cognitive deficits

observed in the disease [4,43,44]. The development of cognitive dysfunction via increased

KYNA levels maybe attributableto its excessive anti-glutamatergic effect, as detailed later.

The high expression of both IDO and TDO has been linked to a variety of tumors (including

that formed in the CNS,[45,46]), and they are thought to be in part responsible for mediating

tumor camouflage via providing an immunosuppressive milieu. The role of IDO2 in tumor

survival has also recently gained an intensive research interest. By far the most extensively

examined inhibitor of IDO is the substrate analogue 1-methyl-TRP, more specifically theits D

stereoisomer **1** (1-methyl-D-TRP,also known as indoximod, NLG8189). Interestingly, while

1-methyl-L-TRP is far more effective in inhibiting IDO in vitro in cell-free and some cell

culture assays (Ki = 19 μM), the D stereoisomer is reported to be highly superior among in

vivo conditions,:it shows anti-tumor effect [47], and it has already been involved in clinical

trials as standalone or add-on therapy to boost anti-cancer immunity [45].In particular, **1** is

used together with temozolomide in a trial enrolling patients with primary brain malignancies

(NCT02052648). In addition to the lead molecule, a hydroxamidine derivative **2**

(INCB024360; (4ε)-4-[(3-chloro-4-fluoroanilino)-nitrosomethylidene]-1,2,5-oxadiazol-3-

amine) with most advanced pharmacological profile (IC_{50} = 7-15 nM in cell culture assays, 72

nM in cell-free assay; [48]), and **3** (NGL919; (1-cyclohexyl-2-(5H-imidazo[5,1-*a*]isoindol-5-

yl)ethanol) with similar pharmacological properties (IC_{50} = 7 nM[49]) and with an in vivo

effect on murine glioblastoma comparable to that achieved with **1**[50]have also been

introduced as candidate biological therapies in clinical trials of malignancies, though not yet

of CNS tumors. A comprehensive compilation of natural and synthetic IDO inhibitors with

different pharmacological properties is provided in a prior publication [4].

The lead TDO inhibitor molecule is **4** (680C91; 6-Fluoro-3-[(1ε)-2-(3-pyridinyl)ethenyl]-1H-

indole; Ki = 51 nM[51]), whereas and the development of orally available indole derivatives

have also been reported [52], including compound **5** (LM10; 6-fluoro-3-[(E)-2-(1H-tetrazol-5-

yl)vinyl]-1H-indole) with the most promising profile (Ki = 5.5 μM). LM10 appears to be

effective in preclinical use [53].

The role of IDO/TDO inhibition in neurodegenerative disorders where alterations have been

published is not as deeply explored as in case of malignancy. Compound **6** (coptisine

chloride; 6,7-dihydro-bis(1,3)benzodioxolo[5,6-*a*:4',5'-*g*]quinolizinium chloride; IC_{50} = 6.3

μM), a component of a traditional Chinese medication Oren-gedoku-to hasve been reported to

be beneficial in a murine model of AD [54]. On the other hand, genetic inhibition of TDO has

been shown to ameliorate the phenotype of a transgenic Drosophila model of HD [55]and

similarly, genetic inhibition of IDO was protective against QUIN-induced striatal

neurotoxicity in mice [56]. Furthermore, inhibition of IDO with **1** abrogated systemic

inflammation-induced depressive behavior in mice [57,58]. The genetic ablation or **1**-induced

inhibition of IDO in mice, however, did not influence infarct size and overall outcome in an

acute stroke model of mice [59]. Further research is needed to establish the therapeutic

potential of IDO/TDO inhibition in disorders associated with neurodegeneration and/or

neuronal dysfunction. The structure of the above-mentioned inhibitors of IDO and TDO are

demonstrated in Fig. 2.

2.2 Kynurenine aminotransferases

There are four subtypes of kynurenine aminotransferases (KATs, EC: 2.6.1.x; reviewed in

[60]: KAT-I-II [61], KAT-III [62]and mitochondrial aspartate aminotransferase (mitAAT, also

called KAT-IV) [63]). KATs belong to the α-family of pyridoxal-5‘-phosphate (PLP)-

dependent enzymes and they are responsible for the irreversible transamination of L-KYN

resulting in the production of KYNA. The KATs are abundantly expressed in astrocytes [64],

and the expression of KAT-II is confined entirely to these cell types [65],with only weak

granular staining visible in neurons [66]. With regard to the expression level of different KAT

subtypes, a difference between species can be observed: KAT-II has been demonstrated to be

the main KYNA-producing enzyme in the rat and human brains, while in the mouse brain

mitAAT has the highest activity [63]. However, following the discovery that the neglected

role of KAT-I under physiological conditions ~~would~~ can be attributed to methodological

reasons, it can be assumed that KAT-I may also contribute to the in vivo formation of KYNA

[60]. In addition to the assessment of the pathobiochemistry of the KYN pathway, in light of

the fact that genetic elimination of KAT-II can improve cognitive functioning [31], the

development of KAT inhibitors may have therapeutic potential, as well [4,67]. The rationale ofa potential therapeutic improvement of cognitive functionsin conditions characterized by elevated brain KYNA levels, such as schizophrenia or AD, would be that normalizing KYNA levels would result in the decrease of pathological inhibition of glutamatergic neurotransmission and thereby long term potentiation, phenomena necessary for memory formation.Studies on the structure function relationship of human KAT-II revealed that within each of the 2 active sites in the functional dimer the PLP-binding pocket harbors the cofactor molecule in Schiff-base linkage to the ε-amino group of the catalytic residues Lys-263 of the corresponding subunit [68]. These observations in three dimensional models are essential for drug design targeting the active site of the enzyme. With regard to KAT-II inhibition, non-selective (e.g. **7**; dichlorovinylcysteine; IC_{50} = 30-100 μM [69]) and selective inhibitors also exist (Fig. 3.). The spectrum of selective KAT-II inhibitors consists of less potent natural (e.g. **8**; quisqualic acid; ((2S)-2-amino-3-(3,5-dioxo-1,2,4-oxadiazolidin-2-yl)propanoic acid); IC_{50} = 200-520 μM [69,70]), endogenous natural (e.g. **9**; S-adenosylhomocysteine; IC_{50} = 30-100 μM [71]), and synthetic (e.g. **10**; 4-carboxy-3-hydroxyphenylglycine; 49% inhibition at 200 μM [70]), as well as more potent endogenous natural (e.g. **11**; L-cysteinsulphinic acid; IC_{50} = 2 μM [72]) and synthetic (e.g. **12**; (S)-(4)-(ethylsulfonyl)benzoylalanine; IC_{50} = 6 μM [73]) substances. Compounds **11** and **12** were demonstrated to be not only pure competitive inhibitors, but also to be substrate analogs[74]. Accordingly, whichthe latter one could significantly improve the performance in the Morris water maze cognitive test following intracerebroventricular administration [75]. FurthermoreHowever, in addition to the development of second-generation drugs with better inhibitory potency (e.g. **13**; BFF-122; (S)-10-(4-aminopiperazin-1-yl)-9-fluoro-3-methyl-7-oxo-3,7-dihydro-2H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid; 43% inhibition at 1 μM [76]), there are 2 other problems emerging: the penetrationacrossthrough the blood-brain barrier (BBB) and the observation

that sequence variants in human and rat KAT-II would cause significant cross-species enzyme

potency shifts for **12**[77,78], i.e. this substance was disappointingly almost inactive on

humans. Compound **14** (BFF-816; (5Z)-5-[(3-{11-oxo-1-azatricyclo[6.3.1.0{4,12}]dodeca-

4(12),5,7-trien-7-yl}phenyl)methylidene]-1,3-thiazolidine-2,4-dione) is capable of reducing

extracellular levels of KYNA in the striatum, hippocampus and prefrontal cortex following

oral administration at the dose of 30 mg/kg in rats and could improve cognitive performance

in the Morris water maze test [79]. Compound **15** (PF-04859989; (3S)-3-amino-1-hydroxy-

3,4-dihydroquinolin-2(1H)-one) is selective inhibitor of human KAT-II (IC_{50} = 23 nM) over

human KAT-I, KAT-III and KAT-IV (IC_{50} values of 22, 11, and > 50 μM, respectively [80]).

Furthermore, the potency shift between human and rat KAT-II (IC_{50} = 263 nM) is considered

to be modest, which allows the application of this compound in preclinical rodent models.

Similarly to **13**, **15** covalently binds to the enzyme cofactor PLP in the catalytic pocket,

creating an irreversible adduct. Additionally, the functional triad of the 3-aminohydroxamic

acid moiety of **15** consists of the H-bond formations of carbonyl oxygen with the side chain

of Asn-202 and that of the other oxygen atom in the hydroxamic acid functionality with Arg-

399. The structure-activity relationship (SAR) studies with regard to assessing the role of 3-

aminohydroxamic acid moiety in inhibitory activity revealed that the (S)-enantiomer is

approximately 10-fold more potent compared to the (R)-enantiomer, and the methylation of

hydroxamic acid group or the introduction of a methyl group at the C-3 position likewise

resulted in a complete loss of activity. Furthermore, the fused phenyl moiety of the molecule

contributes to the van der Waals interactions with the hydrophobic pocket of KAT-II formed

by Leu-40, Tyr-74 and Leu-293. Preliminary SAR data suggest that substitutions at C-6 and

C-7 positions would be used for further optimization of properties. The administration of **15**

successfully ameliorated ketamine-induced cognitive dysfunction in rats [81].

2.3 Kynurenine 3-monooxygenase

3-OH-L-KYN is formed by the action of KMO (EC: 1.14.13.9 [82]). KMO is a flavin adenine

dinucleotide (FAD)-containing enzyme localized in the outer mitochondrial membrane [83].

The QUIN-producing branch of the kynurenine pathway, including KMO, is mainly localized

within microglia and macrophages [84], which associate the neurodegenerative effects of

neurotoxic metabolites with neuroinflammation. Although the expression level of KMO is

relatively low in the brain ~~and higher~~ as compared with that in the liver and kidney [85-87],

the transport of 3-OH-L-KYN through the BBB [88]enables the exertion of its neurotoxic

effects in the CNS following a peripheral activation. After L-KYN binding, NADPH reduces

FAD before oxygen binding and the formation of L-KYN-FAD-hydroperoxide intermediate

[89]. The next step is the oxidation of L-KYN,yielding 3-OH-L-KYN and water. The

demonstration of the three dimensional enzyme structure [90]would further facilitate the

design of KMO inhibitors.~~with the aim of decreasing the concentration of toxic KYN~~

~~pathway metabolites or shifting the pathway toward KYNA production would be one of the~~

~~leading therapeutic strategies~~The rationale in the development of these inhibitors would be the

restoration of the physiological balance in the metabolite levels, i.e. decreasing the production

of neurotoxic compounds (such as 3-OH-L-KYN and QUIN) and shifting the pathway into

the production of the anti-excitotoxicKYNA, metabolites present in many neurodegenerative

diseases in a relative excess and deficit, respectively[83]. Indeed, as the majority of 3-OH-L-

KYN is produced in the periphery, the penetration of KMO inhibitors through the BBB is not

obligatory. With regard to KMO inhibition, the early attempts resulted in the synthesis of

~~un~~on-selective KMO inhibitors with low potency (e.g. **16**; nicotinylalanine; IC_{50} = 900 μM

[91]) with inhibitory effect on kynureninase as well. However, the addition of **16** to L-KYN

and probenecid increased the potency to protect against QUIN-mediated neurotoxicity in the

striatum [92]and in the nigrostriatal dopaminergic system [93]. Furthermore, **16**has

anticonvulsant effects, as well [91,94,95]. The development of 4-aryl-4-oxobutanoic acids,

such as **17** (mNBA; (m-nitrobenzoyl)alanine) resulted in higher potency (IC_{50} = 900 nM[96])

with still ~~un~~non-selective activity. The lead compound **17** exerted protective effects both in

vitro (organotypic hippocampal slice cultures) and in vivo (transient bilateral carotid

occlusion in gerbils, permanent unilateral middle cerebral artery occlusion in rats) models of

cerebral ischemia via the reduction of 3-OH-KYN and the increase in KYNA concentrations

[97-99]. Furthermore, **17**could alleviate kainate-induced neurotoxicity [100], and has

anticonvulsant activity [91], as well. Compound **17** also reduced QUIN formation in immune-

activated mice [101]. Compound **18** (PNU 156561, formerly known as FCE 28833A; (R,S)-

3,4-dichlorobenzoylalanine) is also capable of exerting anti-ischemic effects, probably via

increasing brain KYNA levels [102,103]. With regard to SAR studies in benzoylalanines, it

can be concluded that the S-enantiomers ~~would be~~ are more potent (IC_{50} = 500 nM[104]), the

carboxyl group is obligatory for inhibition, whereas the amine group is not [105]. Therefore

the amine can be replaced with a methylene bridge, resulting in compound **19** (UPF 648;

(1S,2S)-2-(3,4-dichlorobenzoyl)cyclopropanecarboxylic acid) with much higher potency (IC_{50}

= 40 nM) and selectivity [76,106]. In neuron-depleted striata (7 days following QUIN

injection) **19** not only decreased 3-OH-KYN and QUIN production (by 77% and 66%,

respectively), but moderately increased KYNA synthesis (by 27%)~~,~~ as well [76]~~.~~and was

proved protective in a QUIN model of HD [107]. Furthermore, **19**was protective in a

Drosophila model of HD~~, too~~ as well[50]. However, the formation of hydrogen peroxide

probably via the decay of the intermediate hydroperoxyflavin, would limit the application of

these L-KYN-like molecules as KMO inhibitors such as, **17** or **19**[90]. Sulfonamides are

structural analogs of L-KYN~~,~~ as well, containing an FAD-binding benzene ring, a five- or six-

membered aromatic ring as linker, a sulfonamide as amino-acid bioisostere and an extendable

benzene ring [83]. The inhibitory potential is a function of the size of the linking ring, ranging

from a minimal inhibition in case of six-membered rings ($\leq 25\%$ at 10 µM [108]) to a

significantly more potent inhibition in case of five-membered rings, such as thiazoles[109].

Small substituents, such as methoxy groups, are required on both benzene rings for optimal in

vitro binding. Accordingly, the lead compound of phenylthiazolebenzenesulfonamides is **20**

(Ro 61-8048; 3,4-dimethoxy-N-[4-(3-nitrophenyl)thiazol-2-yl]-benzenesulfonamide; IC_{50} =

37 nM). Although drugs belonging to the sulfonamide group of KMO inhibitors have poor

penetration through the BBB, this would not necessarily be a problem in light of the above-

mentioned fact that the majority of 3-OH-L-KYN is produced in the periphery. Accordingly,

20increased the serum levels of KYN and KYNA and decreased the incidence of L-DOPA-

induced dyskinesias in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated cynomolgus

monkeys, but did not affect the antiparkinsonian effect of L-DOPA [110,111]. Compound **20**

successfully improved dystonia in a genetic model of paroxysmal dyskinesias[112,113]. With

regard to HD, **20**was proved protective in yeast and Drosophila models of the disease

[55,114]. Furthermore, similarly to **17**, **20** reduced QUIN formation in immune-activated mice

[101]and also exerted protective effects in both in vitro and in vivo models of cerebral

ischemia via the reduction of 3-OH-KYN and the increase in KYNA concentrations [97-

99].Similarly to **16**, **20**proved to have anticonvulsant effects [91]. Furthermore, compound **20**

exerted substantial anti-inflammatory effects in animal models of cerebral malaria and

trypanosomiasis [115,116]. To overcome the problem that **20** is a metabolically unstable

compound, a potential slow-release prodrug, **21** (JM6; 2-(3,4-dimethoxybenzene-

sulfonylamino)-4-(3-nitrophenyl)-5-(piperidin-1-yl)-methylthiazole) has been were

developed. Although it successfully ameliorated the neurodegenerative processes in the R6/2

transgenic mouse model of HD and a transgenic mouse model of AD overexpressing human

amyloid precursor protein [117], later the validity of these results has been criticized in light

of pharmacokinetic studies that **21** is neither a prodrug for **20** nor a potent KMO inhibitor

[118]. Aryl pyrimidines would be promising candidates as KMO inhibitors, as well [119](SAR

to be detailed later). 4-Aryl-oxobutanoates and their esters (e.g. **22**;[(Z)-4-(3-fluorophenyl)-2-

hydroxy-4-oxo-but-2-enyl] acetate; IC_{50} = 1.6 μM [120,121]), phenylcinnamic acids (e.g.

23;2,4-dichloro-1-[(E)-2-(2-chloro-4-methoxy-phenyl)-1-methyl-vinyl]benzene; IC_{50} = 0.5

μM [115]), phenyloxadiazoles, pyrroloquinolones and 3-oxopropanenitrile molecules havealso

been reported as well;however the available experimental data are limited [83].The structures

of the above-mentioned inhibitors of KMO are demonstrated in Fig.4.

2.4 Kynureninase

Kynureninase (EC: 3.7.1.3), similarly to KATs, is a PLP-dependent enzyme, as well. It is

responsible for the β,γ-hydrolytic cleavage of 3-OH-L-KYN (human and other eukaryotic

constitutive kynureninases) and L-KYN (many prokaryotic inducible kynureninases),

resulting in the formation of 3-OH-ANA or anthranilic acid, respectively, and L-alanine [122].

With regard to the structure of human recombinant kynureninase, the enzyme possesses two

substrate binding sites: a regulatory non-catalytic site and a catalytic site [123]. The

explanation for the possibility of two substrates despite the highly conserved active sites

would be a slight difference in enzyme structure due to conserved point mutations, as

demonstrated by three dimensional models[124]. In prokaryotes, the Trp69 side chain phenyl

group γ-carbon of Thr282 form a hydrophobic patch which is unfavorable for hydrogen

binding with the 3-hydroxyl group of 3-OH-L-KYN. Conversely, in eukaryotes, the side

chain of His102 and the δ nitrogen of Asn333 would promote that interaction. With regard to

the expression pattern of human kynureninase in extrahepatic cells, it is mainly expressed in

macrophages and microglia, similarly to KMO[84]. In addition to the above-mentioned unon-

selective inhibitors, such as **16** (IC_{50} = 800 μM [91]) and **17** (IC_{50} = 100 μM [96]), some more

potent and selective compoundsexist (Fig. 5.). With regard to SAR studies in a structural

analog of L-KYN, **24**(2-amino-4-(3-hydroxyphenyl)-4-hydroxybutanoic acid; $K(i) = 40$ nM and 100 nM for recombinant human or rat hepatic kynureninase, respectively [125,126]), revealed that the 2-amino group is crucial for activity, whereasile the 3-hydroxyl group playsed an important role in binding at the active site of kynureninase, presumably via hydrogen bonding. 2-Amino-S-aryl-cysteine-S,S-dioxides (the most potent is **25**; 2-amino-5-methyl-S-phenyl cysteine S,S-dioxide; $IC_{50} = 11$ µM) would present another option for the selective inhibition of kynureninase[127]. The 2-amino moiety appears to be essential for enzyme inhibition because neither of the 2-nitro-substituted compounds exerted inhibitory activity. With regard to the inhibition of kynureninase from Pseudomonas fluorescens, brominated compounds, such as **26** ((4R)-5-bromodihydro-L-kynurenine; $K(i) = 55$ nM) with slow substrate activity, as well, hasve been synthesized as well[128]. The (4S)-epimer is also a potent inhibitor ($K(i) = 170$ nM) withoutno demonstrated substrate activity.

2.5 3-Hydroxyanthranilate 3,4-dioxygenase

3-Hydroxyanthranilate 3,4-dioxygenase (3-HAO, EC: 1.13.11.6) is responsible for the formation of QUIN from 3-OH-ANA [129]. 3-HAO, as demonstrated by three dimensional models as well, is a cytosolic homodimer protein, with each subunit containing two non-heme ferrous irons, and it catalyzes the cleavage of the benzene ring of 3-OH-ANA [130,131]. The direct product of this reaction is an intermediate, α-amino-β-carboxymuconic acid ε-semialdehyde. This unstable compound can either spontaneously arrange to QUIN or can be converted to picolinic acid after decarboxylation. 3-HAO is mainly localized in the liver and kidney, but it is also present in the CNS in low amounts [129,132]. With regard to CNS, the main source of 3-HAO, being the part of the QUIN-producing branch of the KYN pathway, are microglia [84];however, it is also expressed by astrocytes [64]. Following the early exploration of weak inhibitors of 3-HAO, such as **27** (m-hydroxybenzoic acid; $IC_{50} = 480$ µM

[133]), more potent compounds have been developedlater (Fig. 6.). The systemic

administration of 4-chloro-L-KYN (4-Cl-L-KYN), the BBB-penetrable pro-drug of 7-Cl-

KYNA, ~~which~~a molecule exert~~s~~ing enhanced antagonism at the glycine/NMDA receptors,

resulted in the prevention of QUIN- and malonate-induced neurotoxicity in the rat striatum

[134]. 4-Cl-L-KYN is metabolized to **28** (4-chloro-3-hydroxyanthranilic acid), an inhibitor of

QUIN synthesis ($IC_{50} = 32$ μM [135-137]), which may further broaden the possibility for

neuroprotection. Compound **28** successfully improved functional recovery and preserved

white matter after spinal cord injury in adult Hartley guinea pigs [138]. 4,5-, 4,6-Disubstituted

and 4,5,6-trisubstituted 3-OH-ANA derivatives were also synthesized and demonstrated to

have inhibitory action on 3-HAO [139]. The positioning of electron withdrawing groups in the

4- and 5-positions increased the potency of enzyme inhibition. NCR-631 is also a 3-

hydroxyanthranilate analog (structure is not available) which exerted protective effects

against anoxia and neuroinflammation-related damage [140]and demonstrated anticonvulsant

effects, as well [141]. 2-Aminonicotinic acid 1-oxides, e.g. **29** (6-methyl-2-aminonicotinic

acid 1-oxide; $IC_{50} = 1.1$ μM), represent another promising group with inhibitory activity on 3-

HAO [142]. Although the inhibitors of both kynureninase and 3-HAO would yield interesting

compounds for future drug development, they are currently not in the pipeline with regard to

the modulation of TRP metabolism inneurotherapeutic aspect.

3. Kynurenine pathway inhibitors: advances in the recent patent literature (2012-2015)

Thomson Innovation Patent Export was utilized to search for patents with the

demonstration~~lineation~~ of inhibitors of the kynurenine pathway as neurotherapeutics applying

the assessment period of 2012-2015. Twenty patents ~~have been~~ were found, out of which

EP2664615 [143]was excluded because the invention related to novel compounds for the inhibition of glycogen synthase kinase-3β and/or modulation of NMDA channel activity, not for the inhibition of enzymes of the kynurenine pathway. There were several overlaps amongst the remaining 19 patents, due to the publication of the same inventions under the egis of World Intellectual Property Organization, United States Patent and Trademark Office and European Patent Office. Therefore, finally, 11 inventions could be identified out of which one deals with the inhibitors of IDO1, 2 and TDO, and the remaining deal with the inhibitors of KMO.

3.1 Indolamine 2,3-dioxygenase 1 and 2, tryptophan 2,3-dioxygenase

3.1.1 2,3-diamino-furo[2,3-*c*]pyridine and 2,3-diamino-benzo[*b*]thiophene derivatives

The patent document WO2014186035 [144](EP2970173) reports the synthesis and experimental data on 2,3-diamino-furo[2,3-*c*]pyridine and 2,3-diamino-benzo[*b*]thiophene derivatives as inhibitors of IDO1,2 and TDO (Fig. 7.). The IC_{50} values were determined applying human in vitro IDO1, IDO2 and TDO biochemical and cellular enzyme assays. The lead compounds of the two derivative groups are **30** (N^3-(3-chloro-4-fluorophenyl)furo[2,3-*c*]pyridine-2,3-diamine; IC_{50} values are < 200 nM for IDO1, < 1000 nM for IDO2 and 500-2000 nM for TDO) and **31** (N^3-(3-chloro-4-fluorophenyl)benzo[*b*]thiophene-2,3-diamine; IC_{50} values are < 200 nM for IDO1 and < 500 nM for TDO). With regard to SAR, the modifications of substitutions on the phenyl ring would have significant impact on inhibitory potency. Although the application of different substituents ~~on~~ at different positions have diverse effect on the inhibition of IDO1 and TDO, it can be highlighted that when the halogenation was applied in both the C-2 and C-4 positions (e.g. **32**; N^3-(2-chloro-4-fluorophenyl)furo[2,3-*c*]pyridine-2,3-diamine) or trisubstitution with halogens was performed (e.g. **33**; N^3-(3,5-dichloro-4-fluorophenyl)furo[2,3-*c*]pyridine-2,3-diamine) the inhibitory

potency on IDO1 became considerably decreased without affecting ~~the~~ inhibitory potency on

TDO (IC_{50} values are > 1000 nM for IDO1, 500-2000 nM for TDO and > 1000 nM for IDO1,

500-2000 nM for TDO, respectively). On the other hand, the lack of substitution (**34**; N^3-

phenylfuro[2,3-*c*]pyridine-2,3-diamine) resulted in a slight decrease ~~i~~on IDO1 inhibition (IC_{50}

= 200-1000 nM) and a slight increase ~~i~~on TDO inhibition (IC_{50}< 500 nM). With regard to the

application of substituents ~~on~~at the furo[2,3-*c*]pyridine moiety, it can be concluded that the

application of substituents ~~in~~atthe C-7 position (e.g. **35**; N^3-(3-chloro-4-fluorophenyl)-7-

(pyridine-4-yl)furo[2,3-*c*]pyridine-2,3-diamine; IC_{50}< 200 nM for IDO1 and < 500 nM for

TDO; **36**; N^3-(3,4-difluorophenyl)-7-(pyridine-4-yl)furo[2,3-*c*]pyridine-2,3-diamine; IC_{50}<

200 nM for IDO1, < 1000 nM for IDO2 and < 500 nM for TDO; **37**; 7-chloro-N^3-(3-chloro-4-

fluorophenyl)furo[2,3-*c*]pyridine-2,3-diamine; IC_{50}< 200 nM for IDO1 and < 500 nM for

TDO) d~~id~~oes not considerably influence the activity of the molecule on IDO1 and IDO2, but

may alter the activity of molecule on TDO depending on the applied substituents (e.g. **38**; N^3-

(3-chloro-4-fluorophenyl)-7-propylfuro[2,3-*c*]pyridine-2,3-diamine; IC_{50}< 200 nM for IDO1

and > 2000 nM for TDO). The amidation of amine moiety either ~~in~~atthe C-2 position (e.g. **39**;

ethyl(3-((3-chloro-4-fluorophenyl)amino)furo[2,3-*c*]pyridin-2-yl)carbamic acid) or at both

positions (e.g. **40**; methyl-N-(3-chloro-4-fluoro-phenyl)-N-[2-

(methoxycarbonylamino)furo[2,3-*c*]pyridin-3-yl]carbamate) led to ~~the~~a decrease ~~of~~in

inhibitory activity (IC_{50} values are > 1000 nM for IDO1 and > 2000 nM for TDO in both

cases). The SAR in 2,3-diamino-benzo[*b*]thiophene derivatives is quite similar to that of 2,3-

diamino-furo[2,3-*c*]pyridine derivatives. The in vivo effects of some of the above-mentioned

compounds have been tested in different preclinical models. Amongst others, the drugs **30**,**31**,

35, **36** and **37** were applied orally following intraperitoneal lipopolysaccharide injection into

mice to model immune activation and to study IDO1 expression and activity. Plasma KYN

levels ~~have been~~were measured as the biochemical indicator of decreased IDO activity. The

use of compounds **30**, **35**, **36** and **37** resulted in ~~more than 50%~~ a reduction more than 50% in KYN level, wh~~ile~~ereas**31** resulted in a reduction of between 25-50%. Compounds **30**, **35**, **36** and **37** significantly decreased tumor growth following oral administration when CT-26 mouse colorectal tumor forming cells were injected subcutaneously in the right flank of Balb/c mice. With regard to the effect on KYN levels in plasma and tumor tissue, **30** induced a81% and a 62% decrease, respectively, wh~~ile~~ereas**35** induced a 81% and a 67% decrease, respectively. Compounds **30** and **35**~~were~~ proved capable of penetrating ~~across~~the BBB following intravenous injection, indicating that these compounds can be useful for IDO or TDO-associated brain diseases as well.

3.2 Kynurenine 3-monooxygenase

3.2.1 Aryl pyrimidines

As mentioned above, aryl pyrimidines would be promising candidates of KMO inhibition (Fig. 8.)[113]. The patent documents US20130029988[145](WO2011091153, EP252844), US20130331370[146](WO2010017179, EP2331095), WO2013016488[147](US20140329816, EP2736337), WO2013033068[148](US20140329795, EP2751086). and WO2013033085[149](EP2750677) also deal with inventions in the field of aryl pyrimidines as KMO inhibitors. The aryl pyrimidine skeleton was proposed on the basis that the pyrimidine N-3 nitrogen would mimic the carbonyl oxygen of L-KYN and some of its analog~~ue~~s (**17**, **19**), wh~~ile~~ereas the N-1 nitrogen would mimic the amino group of the above-mentioned compounds [119]. The above-mentioned patent documents and the comprehensive work of Toledo-Sherman et al. [119]delineate the effects of modifications of the aryl ring at the C-4 position, ~~the effects of~~ substituents at the pyrimidine core,~~and the effects of~~ carboxylic acid modifications (mainly

via esterification or amidation), the application of acid bioisosteres and the non-acidic

replacement at the C-6 position on inhibitory potency of the molecule. The inhibitory potency

was tested via the application of biochemical assays using mitochondrial fractions derived

from mice, rats and human liver tissues and via the application of cellular assays using CHO

cell lines expressing human or rodent KMO enzymes and the physiologically more relevant

primary cells with endogenous levels of KMO. The level of inhibition was expressed either as

the percentage of inhibition at 10 μM concentration of the tested compounds or as IC_{50}

values. With regard to the modifications of the aryl ring at the C-4 position, **41** (4-(3,4-

dichlorophenyl)-pyrimidine-6-carboxylic acid; 105.5%, IC_{50} = 0.6 nM) and **42** (4-(3-

chlorophenyl)-pyrimidine-6-carboxylic acid; 105.6%, IC_{50} = 0.5 nM) would serve as lead

compounds with excellent inhibitory potency. The removal of chlorines (**43**; 4-phenyl-

pyrimidine-6-carboxylic acid; IC_{50} = 38 nM), the saturation of the phenyl ring (**44**; 4-

cyclohexyl-pyrimidine-6-carboxylic acid; IC_{50} = 340 nM) the replacement of the phenyl ring

with a heteroaryl ring (e.g. **45**; 4-pyridine-3-yl-pyrimidine-6-carboxylic acid; IC_{50} = 990 nM)

and the introduction of large substituents (e.g. **46**; 4-biphenyl-3-yl-pyrimidine-6-carboxylic

acid; 70%, IC_{50} = 2.13 μM) similarly resulted in substantial decrease in inhibitory potency.

The application of methyl group in at the C-2 position in **41**or **47** (2-[6-(3,4-

dichlorophenyl)pyrimidin-4-yl]-4-methyl-oxazole; 100.65%) considerably reduced the

potency of inhibition (**48**; 4-(3,4-dichlorophenyl)-2-methyl-pyrimidine-6-carboxylic acid;

IC_{50} = 12 nM; **49**;2-[6-(3,4-dichlorophenyl)-2-methyl-pyrimidin-4-yl]-4-methyl-oxazole;

46.84%) which would can be attributed to steric properties interfering with the hydrogen

bonding interaction of KMO with the N-1 nitrogen. The application of a methyl group in at

the C-5 position in **41**also considerably decreased the potency of inhibition (**50**; 4-(3,4-

dichlorophenyl)-5-methyl-pyrimidine-6-carboxylic acid; IC_{50} = 10 nM), probably due to

disturbing the co-planarity of the biaryl ring system via posing substantial strain on ring

torsions. Although the penetration of the BBB is not obligatory in case of KMO

inhibitors, it would broaden the mechanism of action by local CNS effects. The presence of

carboxylic acid moiety is critical to form an ionic salt bridge with a guanidinium

moiety of an arginine of KMO; however it considerably decreases the penetration

through the BBB. It seems that esterification (e.g. **51**; isopropyl-6-(3,4-

dichlorophenyl)pyrimidine-4-carboxylate; 100%) or amidation (e.g. **52**; 6-(3,4-

dichlorophenyl)-N-methyl-pyrimidine-4-carboxamide; 100.31%) retains the inhibitory

potential of the majority of molecules; however it depends on the side-chain with some

exceptions (e.g. **53**; N-(2-amino-1-methyl-2-oxo-ethyl)-6-(3,4-dichlorophenyl)pyrimidine-4-

carboxamide; 47.73%). With regard to the application of acid bio-isosteres, such as 1H-

tetrazole (**54**; 4-(3,4-dichlorophenyl)-6-(1H-tetrazol-5-yl)pyrimidine; 102.87%, IC_{50} = 2 nM),

oxadiazolone (**55**; 3-[4-(3,4-dichlorophenyl)pyrimidin-6-yl]-4H-1,2,4-oxadiazol-5-one;

101.78%, IC_{50} = 12 nM) or acidic acyl sulfonamide (e.g. **56**; N-[4-(3,4-

dichlorophenyl)pyrimidine-6-carbonyl]-benzenesulfonamide; 100.73%, IC_{50} = 25 nM) at the

C-6 position in 3,4-dichlorophenyl-substituted molecules at the C-4 position resulted in

retained inhibition of KMO. However, non-acidic replacement with moieties such as

carbonitrile (**57**; 4-(3,4-dichlorophenyl)pyrimidine-6-carbonitrile; 68.36%, IC_{50} = 4 μM),

1,2,4-oxadiazole (**58**; 4-(3,4-dichlorophenyl)-6-(1,2,4-oxadizol-3-yl)pyrimidine; 68.52%, IC_{50}

= 5.06 μM), thiazole (**59**; 4-(3,4-dichlorophenyl)-6-thiazol-2-yl)pyrimidine; 48%), or 1H-

pyrazole (**60**; 4-(3,4-dichlorophenyl)-6-(1H-pyrazol-3-yl)pyrimidine; 19%) provided

decreased potency of inhibition. As substitution of the pyrimidine moiety at the C-6 position

is restricted, further optimization of BBB penetration with retained KMO inhibition has been

proposed via the substitutions on the phenyl ring. Accordingly, **61** (4-(3-chloro-4-

cyclopropoxy-phenyl)-pyrimidine-6-carboxylic acid; 100%, IC_{50} = 0.5 nM) could be detected

in brain tissues following intravenous or per os administration as well, and exerted profound

elevation in extracellular KYN, anthranilic acidAA and KYNA levels.

3.2.2 Pyrimidine benzenesulfonamides

In addition to the delineation of pyrimidine carboxylic acids, in the patent documentWO2013033085 [149](EP2750677), the patent document also reports the percentage of inhibition at 10 μM of some 4-(3-chloro-4-cyclopropoxyphenyl)-substituted pyrimidine benzenesulfonamides (Fig. 9.). Although the application of the pyrimidine benzenesulfonamide moiety itself did not result in notable KMO inhibition (**62**; N-[4-(3-chloro-4-cyclopropoxyphenyl)pyrimidine-6-yl]-benzenesulfonamide; 73%), the fluorination of the phenyl ring either in para (**63**; N-[4-(3-chloro-4-cyclopropoxyphenyl)pyrimidine-6-yl]-4-fluorobenzene-1-sulfonamide; 98%) or in ortho position (**64**; N-[4-(3-chloro-4-cyclopropoxyphenyl)pyrimidine-6-yl]-2-fluorobenzene-1-sulfonamide; 96%) could considerably increase the potency of inhibition.

3.2.3 Phenylthiazolebenzenesulfonamides

US8710237[150](priority publications: WO2008022281, US20120022052, EP2054397) reports experimental data on phenylthiazolebenzenesulfonamides (Fig. 10.), including **20** and **21**. Although the validity of results demonstrating the beneficial effects of **21** has been criticized in light of pharmacokinetic studies that **21** is neither a prodrug for **20** nor a potent KMO inhibitor [118], the invention reports several other compounds with promising inhibitory effects. The percentage of inhibition at 10 μM concentration of the tested compounds applying rat liver homogenates has been investigated in some molecules. With regard to SAR, the effects of substitution at the nitrogen of sulfonamide moiety and at the C-5 position of thiazole moiety were assessed. The application of ethylcarboxylate group (**65**; 3,4-dimethoxy-N-[4-(3-nitrophenyl)thiazole-2-yl]-N-(ethylcarboxylate)benzenesulfonamide)

resulted in higher inhibitory potency (84%) compared to that of a simple methyl group (**66**; 3,4-dimethoxy-N-[4-(3-nitrophenyl)thiazole-2-yl]-N-(methyl)benzenesulfonamide; 52%). Compound **65** significantly increased the brain concentrations of KYN and significantly decreased that of 3-OH-KYN in a dose-dependent manner 5 h following intraperitoneal administration, whereasile**66** only decreased 3-OH-KYN concentrations. Nevertheless these drugs did not affect the levels of either brain KYNA and QUIN, or plasma KYN, KYNA, 3-OH-KYN and QUIN levels. The application of a six-membered N-containing ring (**67**; 3,4-dimethoxy-N-[4-(3-nitrophenyl)-5-(piperidin-1-ylmethyl)-1,3-thiazol-2-yl]benzenesulfonamide), especially that of comprising oxygen atin para position (**68**; 3,4-dimethoxy-N-[4-(3-nitrophenyl)-5-morpholinomethyl-1,3-thiazol-2-yl]benzenesulfonamide) resulted in higher inhibitory potency (24% and 64%, respectively) compared to that of a five-membered N-containing ring (**69**; 3,4-dimethoxy-N-[4-(3-nitrophenyl)-5-(pyrrolidin-1-ylmethyl)-1,3-thiazole-2-yl]benzenesulfonamide; 9%). Compound **67** significantly decreased the brain concentrations of 3-OH-KYN and QUIN levels in a dose-dependent manner 5 h following intraperitoneal administration, while Compound **68** and **69** only decreased 3-OH-KYN concentrations. Nevertheless these drugs did not affect the levels of either brain or plasma KYN and KYNA. In light of its inhibitory potency, the above-mentioned effect of **69** is surprising and it would be attributed to prodrug properties.

3.2.4 Phenylthiadiazolebenzenesulfonamides

The patent document US20120046324[151](EP2420494, WO2008022286) reports some scientific data about on the assessment of phenylthiadiazolebenzenesulfonamides (Fig. 11.). In light of the percentage of inhibition at 10 µM, it can be concluded that the application of 1,2,4-thiadiazole moiety (e.g. **70**; 3,4-dimethoxy-N-[3-(phenyl)-1,2,4-thiadiazol-5-yl]benzenesulfonamide; 73.61%) resulted in higher inhibitory potency compared to the 1,3,4-

thiadiazole moiety (e.g. **71**; 3,4-dimethoxy-N-[5-(phenyl)-1,3,4-thiadiazol-2-

yl]benzenesulfonamide; 60.03%).

3.2.5 *trans*-2-Substituted-cyclopropane-1-carboxylic acids

The further development of *trans*-2-substituted-cyclopropane-1-carboxylic acids is based on

the promising preclinical data obtained from the experiments with **19**. The patent documents

WO2013151707[152](EP2833879, US20150057238) and WO2015047978[153]report

molecules ~~of~~developed either as a result of modification of the benzoyl ring or further

substitution of the cyclopropane moiety, or the modification of carboxylic acid moiety via

amidation or application of acid bioisosteres (Fig. 12.). In light of the percentage of inhibition

at 30 µM, it can be concluded that modification of the benzoyl ring did not considerably

influence the inhibitory potential (e.g. **72**; (1S,2S)-2-(7-chloro-2,3-dihydro-1-benzofuran-5-

carbonyl)cyclopropane-1-carboxylic acid; 100.3%). The methyl-substitution of cyclopropane

moiety was assessed, as well. The application of a methyl group ~~in~~ at the C-1 position did not

considerably affect the inhibitory potency (**73**; (1S,2S)-2-(3,4-dichlorobenzoyl)-1-

methylcyclopropane-1-carboxylic acid; 98.6%).However, when this methyl group was

introduced~~in~~at the C-3 position, its effects depended on steric conditions (**74**; (1S,2S,3S)-2-

(3,4-dichlorobenzoyl)-3-methylcyclopropane-1-carboxylic acid; 52.5%; **75** (enantiomer of

74); (1R,2R,3R)-2-(3,4-dichlorobenzoyl)-3-methylcyclopropane-1-carboxylic acid; 100.6%;

76 (C-3 epimer of **74**); (1S,2S,3R)-2-(3,4-dichlorobenzoyl)-3-methylcyclopropane-1-

carboxylic acid; 96.7%). However, the disubstitution of methyl groups ~~in~~at the same position

considerably decreased the inhibitory potency (**77**; (1R,3R)-3-(3,4-dichlorobenzoyl)-2,2-

dimethylcyclopropanecarboxylic acid; 54%; **78** (enantiomer of **77**); (1S,3S)-3-(3,4-

dichlorobenzoyl)-2,2-dimethylcyclopropanecarboxylic acid; 15.4%). ~~With regard to t~~The

application of acid bioisosteres, such as 1H-tetrazole (**79**; (3,4-dichlorophenyl)-[(1S,2S)-2-

(1H-tetrazol-5-yl)cyclopropyl]methanone; 101%) or oxadiazolone (**80**; 3-[(1S,2S)-2-(3,4-

dichlorobenzoyl)cyclopropyl]-4H-1,2,4-oxadiazol-5-one; 101%), resulted in retained

inhibition of KMO. However, amidation of carboxylic acid moiety considerably decreased the

potency of inhibition (**81**; (1S,2S)-2-[(3-chloro-4-

cyclopropoxyphenyl)carbonyl]cyclopropane-1-carboxamide; 42%).

3.2.6 Isoxazoles and isoxazolines

The patent document US20120329812[154](WO2010017132A1) delineates several 3,4- or

3,5-disubstituted isoxazoles and isoxazolines as KMO inhibitors (Fig. 13.). With regard to

SAR, the effects of the partial saturation of the isoxazole moiety and the effect ofor the

application of substituents at the C-4 or C-5 position in molecules substituted with the 3,4-

dichlorophenyl substituted molecules moiety at the C-3 position could bewere assessed via

the percentage of inhibition at 10 µM. The application of carboxylic moiety itself either at the

C-4 (**82**; [3-(3,4-dichlorophenyl)-isoxazol-4-yl]-acetic acid; 74.7%) or at the C-5 position (**83**;

[3-(3,4-dichlorophenyl)-isoxazol-5-yl]-acetic acid; 74.53%) alikewise resulted in reduced

potency of inhibition. However, the partial saturation of the isoxazole moiety in the former

molecule considerably increased the inhibitory potency (**84**; [3-(3,4-dichlorophenyl)-4,5-

dihydro-isoxazol-4-yl]-acetic acid; 101.21%). Although esterification did not influence the

activity (e.g. **85**; [3-(3,4-dichlorophenyl)-4,5-dihydro-isoxazol-4-yl]-acetic acid methyl ester;

102.45%), amidation (e.g. **86**; 2-[3-(3,4-dichlorophenyl)-4,5-dihydro-isoxazol-4-yl]-

acetamide; 60.4%) or the introduction of a methyl group at the C-5 position (e.g. **87**; [3-(3,4-

dichlorophenyl)-5-methyl-4,5-dihydro-isoxazol-4-yl]-acetic acid; 84.5%) alikeboth

considerably decreased KMO inhibition. These results would be attributed to steric effects.

4. Conclusions

Certain enzymes of the KYN pathway of TRP metabolism, especially those having a central initiating role in the pathway (such as IDO and TDO) or that are responsible for the formation of neuroactive kynurenines (such as KATs, KMO, kynureninase, and 3-HAO) serve as interesting targets for pharmaceutical drug design, in light of the involvement of the alterations of the pathway in numerous disorders, including neurological conditions. There has been a considerable development in this field in recent decades with emerging preclinical data supporting the beneficial effects of these compounds. Although there is a constant need for further development of lead compounds to achieve drugs with appropriate effect/side-effect profile, some of the available substances are already worth testing in clinical trials.

5. Expert opinion

In recent years, numerous research has been done to explore the involvement of TRP metabolism in neurological and psychiatric disorders. Although clinical and preclinical data are reassuring and yield several possibilities for therapeutic intervention, there is a paucity of applicable drugs in daily clinical practice. With regard to KYNA derivativesanalogs, most of them, such as GV150526 in stroke [155], were unable to exert significant improvement in clinical outcome. However, there are some other promising candidates, including halogenated compounds and KYNA amides, which could be utilized in future clinical studies [4,67,156,157]. 4-Cl-KYN (also known as AV-101), the BBB-penetrable prodrug of 7-Cl-KYNA,a moleculewhichdemonstratinges enhanced antagonism at glycine/NMDA receptors, was successfully assessed in a Phase I trial (ClinicalTrials.gov identifier: NCT01483846) and an investigational new drug application for neuropathic pain has been filed with the United States Food and Drug Administration. Furthermore, there is anongoing recruiting periodment for a trial the assessingmentthe therapeutic potential of this drug in major depressive disorder (ClinicalTrials.gov identifier: NCT02484456). With regard to the metabolic shift concept

with the aim of restoration ofversing pathological alterations at the level of neuroactive

compounds of the KYN pathway of TRP metabolism, several enzyme inhibitors have been

developed. The determination of enzyme structures considerably promoted the development

of potent inhibitors,; most of them were designed as a structural analog of the natural enzyme

substrate. As IDO/TDO activation would serve as a double-edged sword in the CNS, i.e.

protective immunosuppressive response vs. considerable elevation in the levels of cytotoxic

TRP metabolites, the manipulation of these enzymes should be implemented very cautiously.

Nevertheless,**1** and **2** seems to be beneficial in preclinical studies on their antitumor effect.

The enrollment of patients in a Phase I/II study assessing the efficacy of **1** in combination

with temozolomidein primary malignant brain tumors is ongoing (ClinicalTrials.gov

identifier: NCT02052648). In light of recent preclinical data with 2,3-diamino-furo[2,3-

c]pyridine and 2,3-diamino-benzo[*b*]thiophene derivatives, these compounds deserve

attention for future clinical studies as well. Although most neurological disorders can be

characterized by the relative deficiency of the neuroprotectantKYNA, the excessive elevation

of this compound would may result in deteriorated cognitive functions. Therefore, the

application of KAT inhibitors in these conditions, such as schizophrenia, would have

rationalejustification. Although **13**, **14** and **15** all have promising preclinical properties, there

are noclinical studies ongoing with KAT inhibitors. Probably the hottest topic in the field of

inhibitors of KYN pathway as neurotherapeutics is the development of KMO inhibitors with

the aim of reducing the levels of toxic compounds and shifting the pathway toward the

production of neuroprotective KYNA to restore the balance of altered metabolite levels.

Although **19** and substances achieved from the further development of *trans*-2-substituted-

cyclopropane-1-carboxylic acids provided reassuring preclinical data, there are currently no

clinical trials ongoing with any of these compounds. Furthermore, the situation is the same

with the more potent phenylthiazolebenzenesulfonamides, including **20**,

phenylthiadiazolebenzenesulfonamides, isoxazolesand isoxazolines. The advanced

development of aryl pyrimidines and pyrimidine benzenesulfonamides based on well-

designed SAR studies in recent years holds the promise of ~~the achievement~~ development of

potent and selective compounds. Hopefully, some of these molecules would be appropriate

for future clinical testing if sufficient reassuring preclinical data were gathered. Although

promising kynureninase and 3-HAO inhibitors also exist, currently the design of KMO

inhibitors seems to be the pipeline of drug development with the aim of the reduction of the

levels of neurotoxic compounds of the KYN pathway. With regard to future research in this

field, in addition to the development of drugs with much higher selectivity, enhanced potency

and better side-effect profile, a special attention should also be paid to the better

characterization of neurochemical alterations and the exploration of possible genetic

background ~~as well~~ to enablethe establishment of~~setting up~~ the best individualized therapy.

Declaration of interest

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest

(••) to readers

1.   Zadori D, Klivenyi P, Plangar I, et al. Endogenous neuroprotection in chronic

neurodegenerative disorders: with particular regard to the kynurenines. J Cell Mol Med

2011;15:701-17

• **Interesting review on the treatment options in neurodegenerative disorders via natural endogenous targets.**

2.   Wolf H. The effect of hormones and vitamin B6 on urinary excretion of metabolites of the kynurenine pathway. Scand J Clin Lab Invest Suppl 1974;136:1-186

3.   Beadle GW, Mitchell HK, Nyc JF. Kynurenine as an intermediate in the formation of nicotinic acid from tryptophane by Neurospora. Proc Natl Acad Sci U S A 1947;33:155-8

4.   Vecsei L, Szalardy L, Fulop F, Toldi J. Kynurenines in the CNS: recent advances and new questions. Nat Rev Drug Discov 2013;12:64-82

•• **Comprehensive review describing the role of alterations in the KYN**kynurenine **pathway of TRP**tryptophan **metabolism in certain neurological conditions.**

5.   Perkins MN, Stone TW. An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. Brain Res 1982;247:184-7

6.   Birch PJ, Grossman CJ, Hayes AG. Kynurenate and FG9041 have both competitive and non-competitive antagonist actions at excitatory amino acid receptors. Eur J Pharmacol 1988;151:313-5

7.   Kessler M, Terramani T, Lynch G, Baudry M. A glycine site associated with N-methyl-D-aspartic acid receptors: characterization and identification of a new class of antagonists. J Neurochem 1989;52:1319-28

8.   Rozsa E, Robotka H, Vecsei L, Toldi J. The Janus-face kynurenic acid. J Neural Transm 2008;115:1087-91

9.   Hilmas C, Pereira EF, Alkondon M, et al. The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications. J Neurosci 2001;21:7463-73

10.   Marchi M, Risso F, Viola C, et al. Direct evidence that release-stimulating alpha7* nicotinic cholinergic receptors are localized on human and rat brain glutamatergic axon terminals. J Neurochem 2002;80:1071-8

11.   Stone TW. Kynurenic acid blocks nicotinic synaptic transmission to hippocampal interneurons in young rats. Eur J Neurosci 2007;25:2656-65

12.   Arnaiz-Cot JJ, Gonzalez JC, Sobrado M, et al. Allosteric modulation of alpha 7 nicotinic receptors selectively depolarizes hippocampal interneurons, enhancing spontaneous GABAergic transmission. Eur J Neurosci 2008;27:1097-110

13.   Mok MH, Fricker AC, Weil A, Kew JN. Electrophysiological characterisation of the actions of kynurenic acid at ligand-gated ion channels. Neuropharmacology 2009;57:242-9

14.   Dobelis P, Staley KJ, Cooper DC. Lack of modulation of nicotinic acetylcholine alpha-7 receptor currents by kynurenic acid in adult hippocampal interneurons. PLoS One 2012;7:e41108

15.   Wang J, Simonavicius N, Wu X, et al. Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. J Biol Chem 2006;281:22021-8

16.   Lugo-Huitron R, Blanco-Ayala T, Ugalde-Muniz P, et al. On the antioxidant properties of kynurenic acid: free radical scavenging activity and inhibition of oxidative stress. Neurotoxicol Teratol 2011;33:538-47

17.   Eastman CL, Guilarte TR. The role of hydrogen peroxide in the in vitro cytotoxicity of 3-hydroxykynurenine. Neurochem Res 1990;15:1101-7

18.   Dykens JA, Sullivan SG, Stern A. Oxidative reactivity of the tryptophan metabolites 3-hydroxyanthranilate, cinnabarinate, quinolinate and picolinate. Biochem Pharmacol 1987;36:211-7

19.   Jhamandas K, Boegman RJ, Beninger RJ, Bialik M. Quinolinate-induced cortical cholinergic damage: modulation by tryptophan metabolites. Brain Res 1990;529:185-91

20.   de Carvalho LP, Bochet P, Rossier J. The endogenous agonist quinolinic acid and the non endogenous homoquinolinic acid discriminate between NMDAR2 receptor subunits. Neurochem Int 1996;28:445-52

21.   Liu Y, Wong TP, Aarts M, et al. NMDA receptor subunits have differential roles in mediating excitotoxic neuronal death both in vitro and in vivo. J Neurosci 2007;27:2846-57

22.   Connick JH, Stone TW. Quinolinic acid effects on amino acid release from the rat cerebral cortex in vitro and in vivo. Br J Pharmacol 1988;93:868-76

23.   Rios C, Santamaria A. Quinolinic acid is a potent lipid peroxidant in rat brain homogenates. Neurochem Res 1991;16:1139-43

24.   Zadori D, Klivenyi P, Szalardy L, et al. Mitochondrial disturbances, excitotoxicity, neuroinflammation and kynurenines: novel therapeutic strategies for neurodegenerative disorders. J Neurol Sci 2012;322:187-91

25.   Schwarcz R, Guidetti P, Sathyasaikumar KV, Muchowski PJ. Of mice, rats and men: Revisiting the quinolinic acid hypothesis of Huntington's disease. Prog Neurobiol 2010;90:230-45

• **An interesting review about the role of neurotoxic KYN~~kynurenine~~ pathway metabolites in Huntington's disease.**

26.   Stone TW, Mackay GM, Forrest CM, et al. Tryptophan metabolites and brain disorders. Clin Chem Lab Med 2003;41:852-9

• **An interesting review describing how the absolute or relative concentrations of neuroactive kynurenines are implicated in certain neurological conditions, such as aquired immune deficiency syndrome~~AIDS~~-dementia complex or HD~~Huntington's disease~~.**

27.   Schwarcz R, Rassoulpour A, Wu HQ, et al. Increased cortical kynurenate content in schizophrenia. Biol Psychiatry 2001;50:521-30

28.   Chess AC, Simoni MK, Alling TE, Bucci DJ. Elevations of endogenous kynurenic

acid produce spatial working memory deficits. Schizophr Bull 2007;33:797-804

29.   Zadori D, Veres G, Szalardy L, et al. Glutamatergic dysfunctioning in Alzheimer's

disease and related therapeutic targets. J Alzheimers Dis 2014;42 Suppl 3:S177-87

30.   Baran H, Jellinger K, Deecke L. Kynurenine metabolism in Alzheimer's disease. J

Neural Transm (Vienna) 1999;106:165-81

31.   Potter MC, Elmer GI, Bergeron R, et al. Reduction of endogenous kynurenic acid

formation enhances extracellular glutamate, hippocampal plasticity, and cognitive behavior.

Neuropsychopharmacology 2010;35:1734-42

32.   Braidy N, Guillemin GJ, Grant R. Effects of kynurenine pathway inhibition on NAD

metabolism and cell viability in human primary astrocytes and neurons. Int J Tryptophan Res

2011;4:29-37

33.   De Laurentis W, Khim L, Anderson JL, et al. The second enzyme in pyrrolnitrin

biosynthetic pathway is related to the heme-dependent dioxygenase superfamily.

Biochemistry 2007;46:12393-404

34.   Sugimoto H, Oda S, Otsuki T, et al. Crystal structure of human indoleamine 2,3-

dioxygenase: catalytic mechanism of O2 incorporation by a heme-containing dioxygenase.

Proc Natl Acad Sci U S A 2006;103:2611-6

• This paper reports the three dimensional structure of IDO.

35.   Forouhar F, Anderson JL, Mowat CG, et al. Molecular insights into substrate

recognition and catalysis by tryptophan 2,3-dioxygenase. Proc Natl Acad Sci U S A

2007;104:473-8

36.   Zhang Y, Kang SA, Mukherjee T, et al. Crystal structure and mechanism of

tryptophan 2,3-dioxygenase, a heme enzyme involved in tryptophan catabolism and in

quinolinate biosynthesis. Biochemistry 2007;46:145-55

• **This paper reports the three dimensional structure of TDO.**

37.   Ball HJ, Sanchez-Perez A, Weiser S, et al. Characterization of an indoleamine 2,3-dioxygenase-like protein found in humans and mice. Gene 2007;396:203-13

38.   Metz R, Duhadaway JB, Kamasani U, et al. Novel tryptophan catabolic enzyme IDO2 is the preferred biochemical target of the antitumor indoleamine 2,3-dioxygenase inhibitory compound D-1-methyl-tryptophan. Cancer Res 2007;67:7082-7

39.   Lob S, Konigsrainer A, Schafer R, et al. Levo- but not dextro-1-methyl tryptophan abrogates the IDO activity of human dendritic cells. Blood 2008;111:2152-4

40.   Lob S, Konigsrainer A, Zieker D, et al. IDO1 and IDO2 are expressed in human tumors: levo- but not dextro-1-methyl tryptophan inhibits tryptophan catabolism. Cancer Immunol Immunother 2009;58:153-7

41.   Mandi Y, Vecsei L. The kynurenine system and immunoregulation. J Neural Transm (Vienna) 2012;119:197-209

42.   Grozdics E, Berta L, Bajnok A, et al. B7 costimulation and intracellular indoleamine-2,3-dioxygenase (IDO) expression in peripheral blood of healthy pregnant and non-pregnant women. BMC Pregnancy Childbirth 2014;14:306

43.   Kegel ME, Bhat M, Skogh E, et al. Imbalanced kynurenine pathway in schizophrenia. Int J Tryptophan Res 2014;7:15-22

44.   Sathyasaikumar KV, Stachowski EK, Wonodi I, et al. Impaired kynurenine pathway metabolism in the prefrontal cortex of individuals with schizophrenia. Schizophr Bull 2011;37:1147-56

• **This paper describes the importance of elevated prefrontal KYNA~~kynurenic acid~~ level in the pathogenesis of cognitive dysfunctions in schizophrenia.**

45.   Vacchelli E, Aranda F, Eggermont A, et al. Trial watch: IDO inhibitors in cancer therapy. Oncoimmunology 2014;3:e957994

46.   Opitz CA, Litzenburger UM, Opitz U, et al. The indoleamine-2,3-dioxygenase (IDO) inhibitor 1-methyl-D-tryptophan upregulates IDO1 in human cancer cells. PLoS One 2011;6:e19823

47.   Hou DY, Muller AJ, Sharma MD, et al. Inhibition of indoleamine 2,3-dioxygenase in dendritic cells by stereoisomers of 1-methyl-tryptophan correlates with antitumor responses. Cancer Res 2007;67:792-801

48.   Liu X, Shin N, Koblish HK, et al. Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity. Blood 2010;115:3520-30

49.   Mautino MR, Jaipuri FA, Waldo J, et al. NLG919, a novel indoleamine-2,3-dioxygenase (IDO)-pathway inhibitor drug candidate for cancer therapy [abstract]. In: Proceedings of the 104th Annual Meeting of the American Association for Cancer Research; 2013 Apr 6-10; Washington, DC Philadelphia (PA). Cancer Res 2013;73:Abstract nr 491

50.   Li M, Bolduc AR, Hoda MN, et al. The indoleamine 2,3-dioxygenase pathway controls complement-dependent enhancement of chemo-radiation therapy against murine glioblastoma. J Immunother Cancer 2014;2:21

51.   Salter M, Hazelwood R, Pogson CI, et al. The effects of a novel and selective inhibitor of tryptophan 2,3-dioxygenase on tryptophan and serotonin metabolism in the rat. Biochem Pharmacol 1995;49:1435-42

52.   Dolusic E, Larrieu P, Moineaux L, et al. Tryptophan 2,3-dioxygenase (TDO) inhibitors. 3-(2-(pyridyl)ethenyl)indoles as potential anticancer immunomodulators. J Med Chem 2011;54:5320-34

53.   Pilotte L, Larrieu P, Stroobant V, et al. Reversal of tumoral immune resistance by inhibition of tryptophan 2,3-dioxygenase. Proc Natl Acad Sci U S A 2012;109:2497-502

54.   Yu D, Tao BB, Yang YY, et al. The IDO inhibitor coptisine ameliorates cognitive impairment in a mouse model of Alzheimer's disease. J Alzheimers Dis 2015;43:291-302

55.   Campesan S, Green EW, Breda C, et al. The kynurenine pathway modulates

neurodegeneration in a Drosophila model of Huntington's disease. Curr Biol 2011;21:961-6

56.   Mazarei G, Budac DP, Lu G, et al. The absence of indoleamine 2,3-dioxygenase

expression protects against NMDA receptor-mediated excitotoxicity in mouse brain. Exp

Neurol 2013;249:144-8

57.   O'Connor JC, Lawson MA, Andre C, et al. Induction of IDO by bacille Calmette-

Guerin is responsible for development of murine depressive-like behavior. J Immunol

2009;182:3202-12

58.   O'Connor JC, Lawson MA, Andre C, et al. Lipopolysaccharide-induced depressive-

like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. Mol Psychiatry

2009;14:511-22

59.   Jackman KA, Brait VH, Wang Y, et al. Vascular expression, activity and function of

indoleamine 2,3-dioxygenase-1 following cerebral ischaemia-reperfusion in mice. Naunyn

Schmiedebergs Arch Pharmacol 2011;383:471-81

60.   Han Q, Cai T, Tagle DA, Li J. Structure, expression, and function of kynurenine

aminotransferases in human and rodent brains. Cell Mol Life Sci 2010;67:353-68

61.   Okuno E, Nakamura M, Schwarcz R. Two kynurenine aminotransferases in human

brain. Brain Res 1991;542:307-12

62.   Yu P, Li Z, Zhang L, et al. Characterization of kynurenine aminotransferase III, a

novel member of a phylogenetically conserved KAT family. Gene 2006;365:111-8

63.   Guidetti P, Amori L, Sapko MT, et al. Mitochondrial aspartate aminotransferase: a

third kynurenate-producing enzyme in the mammalian brain. J Neurochem 2007;102:103-11

64.   Guillemin GJ, Kerr SJ, Smythe GA, et al. Kynurenine pathway metabolism in human

astrocytes: a paradox for neuronal protection. J Neurochem 2001;78:842-53

•• **This paper describes the kynurenine pathway in astrocytes with a special attention to**

KYNAkynurenic acid production.

65.   Guidetti P, Hoffman GE, Melendez-Ferro M, et al. Astrocytic localization of

kynurenine aminotransferase II in the rat brain visualized by immunocytochemistry. Glia

2007;55:78-92

66.   Roberts RC, Du F, McCarthy KE, et al. Immunocytochemical localization of

kynurenine aminotransferase in the rat striatum: a light and electron microscopic study. J

Comp Neurol 1992;326:82-90

67.   Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain:

when physiology meets pathology. Nat Rev Neurosci 2012;13:465-77

• **This interesting review delineates the role of neuroactive kynurenines in brain**

functioning and dysfunctioning.

68.   Rossi F, Garavaglia S, Montalbano V, et al. Crystal structure of human kynurenine

aminotransferase II, a drug target for the treatment of schizophrenia. J Biol Chem

2008;283:3559-66

• **This paper reports the three dimensional structure of KAT-II.**

69.   Guidetti P, Okuno E, Schwarcz R. Characterization of rat brain kynurenine

aminotransferases I and II. J Neurosci Res 1997;50:457-65

70.   Battaglia G, Rassoulpour A, Wu HQ, et al. Some metabotropic glutamate receptor

ligands reduce kynurenate synthesis in rats by intracellular inhibition of kynurenine

aminotransferase II. J Neurochem 2000;75:2051-60

71.   Luchowska E, Luchowski P, Paczek R, et al. Dual effect of DL-homocysteine and S-

adenosylhomocysteine on brain synthesis of the glutamate receptor antagonist, kynurenic

acid. J Neurosci Res 2005;79:375-82

72.   Kocki T, Luchowski P, Luchowska E, et al. L-cysteine sulphinate, endogenous

sulphur-containing amino acid, inhibits rat brain kynurenic acid production via selective

interference with kynurenine aminotransferase II. Neurosci Lett 2003;346:97-100

73.   Pellicciari R, Rizzo RC, Costantino G, et al. Modulators of the kynurenine pathway of tryptophan metabolism: synthesis and preliminary biological evaluation of (S)-4-(ethylsulfonyl)benzoylalanine, a potent and selective kynurenine aminotransferase II (KAT II) inhibitor. ChemMedChem 2006;1:528-31

74.   Passera E, Campanini B, Rossi F, et al. Human kynurenine aminotransferase II--reactivity with substrates and inhibitors. FEBS J 2011;278:1882-900

75.   Pocivavsek A, Wu HQ, Potter MC, et al. Fluctuations in endogenous kynurenic acid control hippocampal glutamate and memory. Neuropsychopharmacology 2011;36:2357-67

76.   Amori L, Guidetti P, Pellicciari R, et al. On the relationship between the two branches of the kynurenine pathway in the rat brain in vivo. J Neurochem 2009;109:316-25

77.   Pellicciari R, Venturoni F, Bellocchi D, et al. Sequence variants in kynurenine aminotransferase II (KAT II) orthologs determine different potencies of the inhibitor S-ESBA. ChemMedChem 2008;3:1199-202

78.   Casazza V, Rossi F, Rizzi M. Biochemical and structural investigations on kynurenine aminotransferase II: an example of conformation-driven species-specific inhibition? Curr Top Med Chem 2011;11:148-57

79.   Wu HQ, Okuyama M, Kajii Y, et al. Targeting kynurenine aminotransferase II in psychiatric diseases: promising effects of an orally active enzyme inhibitor. Schizophr Bull 2014;40 Suppl 2:S152-8

80.   Dounay AB, Anderson M, Bechle BM, et al. Discovery of brain-penetrant, irreversible kynurenine aminotransferase II inhibitors for schizophrenia. ACS Med Chem Lett 2012;3:187-92

81.   Kozak R, Campbell BM, Strick CA, et al. Reduction of brain kynurenic acid improves cognitive function. J Neurosci 2014;34:10592-602

82.   Battie C, Verity MA. Presence of kynurenine hydroxylase in developing rat brain. J

Neurochem 1981;36:1308-10

83.   Smith JR, Jamie JF, Guillemin GJ. Kynurenine-3-monooxygenase: a review of

structure, mechanism, and inhibitors. Drug Discov Today 2016;21:315-24

•• **A comprehensive review on KMOkynurenine 3-monooxygenase inhibitors.**

84.   Espey MG, Chernyshev ON, Reinhard JF, Jr., et al. Activated human microglia

produce the excitotoxin quinolinic acid. Neuroreport 1997;8:431-4

•• **This paper describes the production of neurotoxic KYNkynurenine pathway**

metabolites in microglia.

85.   Erickson JB, Flanagan EM, Russo S, Reinhard JF, Jr. A radiometric assay for

kynurenine 3-hydroxylase based on the release of 3H2O during hydroxylation of L-[3,5-

3H]kynurenine. Anal Biochem 1992;205:257-62

86.   Guillemin GJ, Smith DG, Smythe GA, et al. Expression of the kynurenine pathway

enzymes in human microglia and macrophages. Adv Exp Med Biol 2003;527:105-12

•• **This paper describes the production of neurotoxic KYNkynurenine pathway**

metabolites in microglia.

87.   Heyes MP, Chen CY, Major EO, Saito K. Different kynurenine pathway enzymes

limit quinolinic acid formation by various human cell types. Biochem J 1997;326 (Pt 2):351-

6

88.   Fukui S, Schwarcz R, Rapoport SI, et al. Blood-brain barrier transport of kynurenines:

implications for brain synthesis and metabolism. J Neurochem 1991;56:2007-17

• **This paper describes the mechanism of the transport of kynurenines via the blood-**

brain barrier.

89.   Crozier-Reabe KR, Phillips RS, Moran GR. Kynurenine 3-monooxygenase from

Pseudomonas fluorescens: substrate-like inhibitors both stimulate flavin reduction and

stabilize the flavin-peroxo intermediate yet result in the production of hydrogen peroxide. Biochemistry 2008;47:12420-33

90.   Amaral M, Levy C, Heyes DJ, et al. Structural basis of kynurenine 3-monooxygenase inhibition. Nature 2013;496:382-5

• This paper reports the three dimensional structure of KMO and the mechanism of its inhibition.

91.   Carpenedo R, Chiarugi A, Russi P, et al. Inhibitors of kynurenine hydroxylase and kynureninase increase cerebral formation of kynurenate and have sedative and anticonvulsant activities. Neuroscience 1994;61:237-43

92.   Harris CA, Miranda AF, Tanguay JJ, et al. Modulation of striatal quinolinate neurotoxicity by elevation of endogenous brain kynurenic acid. Br J Pharmacol 1998;124:391-9

93.   Miranda AF, Boegman RJ, Beninger RJ, Jhamandas K. Protection against quinolinic acid-mediated excitotoxicity in nigrostriatal dopaminergic neurons by endogenous kynurenic acid. Neuroscience 1997;78:967-75

94.   Connick JH, Heywood GC, Sills GJ, et al. Nicotinylalanine increases cerebral kynurenic acid content and has anticonvulsant activity. Gen Pharmacol 1992;23:235-9

95.   Russi P, Alesiani M, Lombardi G, et al. Nicotinylalanine increases the formation of kynurenic acid in the brain and antagonizes convulsions. J Neurochem 1992;59:2076-80

96.   Pellicciari R, Natalini B, Costantino G, et al. Modulation of the kynurenine pathway in search for new neuroprotective agents. Synthesis and preliminary evaluation of (m-nitrobenzoyl)alanine, a potent inhibitor of kynurenine-3-hydroxylase. J Med Chem 1994;37:647-55

97.   Moroni F, Cozzi A, Peruginelli F, et al. Neuroprotective effects of kynurenine-3-hydroxylase inhibitors in models of brain ischemia. Adv Exp Med Biol 1999;467:199-206

98.   Moroni F, Carpenedo R, Cozzi A, et al. Studies on the neuroprotective action of

kynurenine mono-oxygenase inhibitors in post-ischemic brain damage. Adv Exp Med Biol

2003;527:127-36

99.   Cozzi A, Carpenedo R, Moroni F. Kynurenine hydroxylase inhibitors reduce ischemic

brain damage: studies with (m-nitrobenzoyl)-alanine (mNBA) and 3,4-dimethoxy-[-N-4-

(nitrophenyl)thiazol-2yl]-benzenesulfonamide (Ro 61-8048) in models of focal or global

brain ischemia. J Cereb Blood Flow Metab 1999;19:771-7

100.   Behan WM, Stone TW. Role of kynurenines in the neurotoxic actions of kainic acid.

Br J Pharmacol 2000;129:1764-70

101.   Chiarugi A, Moroni F. Quinolinic acid formation in immune-activated mice: studies

with (m-nitrobenzoyl)-alanine (mNBA) and 3,4-dimethoxy-[-N-4-(-3-nitrophenyl)thiazol-

2yl]-benzenesul fonamide (Ro 61-8048), two potent and selective inhibitors of kynurenine

hydroxylase. Neuropharmacology 1999;38:1225-33

102.   Speciale C, Wu HQ, Cini M, et al. (R,S)-3,4-dichlorobenzoylalanine (FCE 28833A)

causes a large and persistent increase in brain kynurenic acid levels in rats. Eur J Pharmacol

1996;315:263-7

103.   Speciale C, Cini M, Wu HQ, et al. Kynurenic acid-enhancing and anti-ischemic

effects of the potent kynurenine 3-hydroxylase inhibitor FCE 28833 in rodents. Adv Exp Med

Biol 1996;398:221-7

104.   Natalini B, Mattoli L, Pellicciari R, et al. Synthesis and activity of enantiopure (S) (m-

nitrobenzoyl) alanine, potent kynurenine-3-hydroxylase inhibitor. Bioorg Med Chem Lett

1995;5:1451-1454

105.   Giordani A, Pevarello P, Cini M, et al. 4-Phenyl-4-oxo-butanoic acid derivatives

inhibitors of kynurenine 3-hydroxylase. Bioorg Med Chem Lett 1998;8:2907-2912

106.   Pellicciari R, Amori L, Costantino G, et al. Modulation of the kynurine pathway of

tryptophan metabolism in search for neuroprotective agents. Focus on kynurenine-3-

hydroxylase. Adv Exp Med Biol 2003;527:621-8

107.   Sapko MT, Guidetti P, Yu P, et al. Endogenous kynurenate controls the vulnerability

of striatal neurons to quinolinate: Implications for Huntington's disease. Exp Neurol

2006;197:31-40

108.   The J. David Gladstone Institutes, a testamentary trust established under the will of J.

David Gladstone; University of Maryland; Muchowski, PJ; Muchowski, JM; Schwarcz, R;

Guidetti, P. Small molecule inhibitors of kynurenine-3-monooxygenase. WO2008022286

(2008)

109.   Rover S, Cesura AM, Huguenin P, et al. Synthesis and biochemical evaluation of N-

(4-phenylthiazol-2-yl)benzenesulfonamides as high-affinity inhibitors of kynurenine 3-

hydroxylase. J Med Chem 1997;40:4378-85

110.   Gregoire L, Rassoulpour A, Guidetti P, et al. Prolonged kynurenine 3-hydroxylase

inhibition reduces development of levodopa-induced dyskinesias in parkinsonian monkeys.

Behav Brain Res 2008;186:161-7

111.   Ouattara B, Belkhir S, Morissette M, et al. Implication of NMDA receptors in the

antidyskinetic activity of cabergoline, CI-1041, and Ro 61-8048 in MPTP monkeys with

levodopa-induced dyskinesias. J Mol Neurosci 2009;38:128-42

112.   Richter A, Hamann M. The kynurenine 3-hydroxylase inhibitor Ro 61-8048 improves

dystonia in a genetic model of paroxysmal dyskinesia. Eur J Pharmacol 2003;478:47-52

113.   Hamann M, Sander SE, Richter A. Effects of the kynurenine 3-hydroxylase inhibitor

Ro 61-8048 after intrastriatal injections on the severity of dystonia in the dt sz mutant. Eur J

Pharmacol 2008;586:156-9

114.   Giorgini F, Guidetti P, Nguyen Q, et al. A genomic screen in yeast implicates

kynurenine 3-monooxygenase as a therapeutic target for Huntington disease. Nat Genet

2005;37:526-31

115. Clark CJ, Mackay GM, Smythe GA, et al. Prolonged survival of a murine model of cerebral malaria by kynurenine pathway inhibition. Infect Immun 2005;73:5249-51

116. Rodgers J, Stone TW, Barrett MP, et al. Kynurenine pathway inhibition reduces central nervous system inflammation in a model of human African trypanosomiasis. Brain 2009;132:1259-67

117. Zwilling D, Huang SY, Sathyasaikumar KV, et al. Kynurenine 3-monooxygenase inhibition in blood ameliorates neurodegeneration. Cell 2011;145:863-74

118. Beconi MG, Yates D, Lyons K, et al. Metabolism and pharmacokinetics of JM6 in mice: JM6 is not a prodrug for Ro-61-8048. Drug Metab Dispos 2012;40:2297-306

119. Toledo-Sherman LM, Prime ME, Mrzljak L, et al. Development of a series of aryl pyrimidine kynurenine monooxygenase inhibitors as potential therapeutic agents for the treatment of Huntington's disease. J Med Chem 2015;58:1159-83

•• **A comprehensive review on aryl pyrimidines as KMOkynurenine 3-monooxygenase inhibitors.**

120. Guidetti P, Eastman CL, Schwarcz R. Metabolism of [5-3H]kynurenine in the rat brain in vivo: evidence for the existence of a functional kynurenine pathway. J Neurochem 1995;65:2621-32

121. Reinhard JF, Jr. Pharmacological manipulation of brain kynurenine metabolism. Ann N Y Acad Sci 2004;1035:335-49

122. Botting NP. Chemistry and neurochemistry of the kynurenine pathway of tryptophan metabolism. Chemical Society Reviews 1995;24:401

123. Walsh HA, Botting NP. Purification and biochemical characterization of some of the properties of recombinant human kynureninase. Eur J Biochem 2002;269:2069-74

124. Lima S, Khristoforov R, Momany C, Phillips RS. Crystal structure of Homo sapiens

kynureninase. Biochemistry 2007;46:2735-44

125.   Walsh HA, Leslie PL, O'Shea KC, Botting NP. 2-Amino-4-[3'-hydroxyphenyl]-4-

hydroxybutanoic acid; a potent inhibitor of rat and recombinant human kynureninase. Bioorg

Med Chem Lett 2002;12:361-3

• **This paper reports the three dimensional structure of kynureninase.**

126.   Walsh HA, O'Shea KC, Botting NP. Comparative inhibition by substrate analogues 3-

methoxy- and 3-hydroxydesaminokynurenine and an improved 3 step purification of

recombinant human kynureninase. BMC Biochem 2003;4:13

127.   Drysdale MJ, Reinhard JF. S-aryl cysteine S,S-dioxides as inhibitors of mammalian

kynureninase. Bioorg Med Chem Lett 1998;8:133-8

128.   Heiss C, Anderson J, Phillips RS. Differential effects of bromination on substrates and

inhibitors of kynureninase from Pseudomonas fluorescens. Org Biomol Chem 2003;1:288-95

129.   Foster AC, White RJ, Schwarcz R. Synthesis of quinolinic acid by 3-

hydroxyanthranilic acid oxygenase in rat brain tissue in vitro. J Neurochem 1986;47:23-30

130.   Malherbe P, Kohler C, Da Prada M, et al. Molecular cloning and functional expression

of human 3-hydroxyanthranilic-acid dioxygenase. J Biol Chem 1994;269:13792-7

131.   Zhang Y, Colabroy KL, Begley TP, Ealick SE. Structural studies on 3-

hydroxyanthranilate-3,4-dioxygenase: the catalytic mechanism of a complex oxidation

involved in NAD biosynthesis. Biochemistry 2005;44:7632-43

• **This paper reports the three dimensional structure of 3-HAO.**

132.   Kohler C, Okuno E, Flood PR, Schwarcz R. Quinolinic acid

phosphoribosyltransferase: preferential glial localization in the rat brain visualized by

immunocytochemistry. Proc Natl Acad Sci U S A 1987;84:3491-5

133.   Vescia A, Di Prisco G. Studies on purified 3-hydroxyanthranilic acid oxidase. J Biol

Chem 1962;237:2318-24

134.   Guidetti P, Wu HQ, Schwarcz R. In situ produced 7-chlorokynurenate provides

protection against quinolinate- and malonate-induced neurotoxicity in the rat striatum. Exp

Neurol 2000;163:123-30

135.   Parli CJ, Krieter P, Schmidt B. Metabolism of 6-chlorotryptophan to 4-chloro-3-

hydroxyanthranilic acid: a potent inhibitor of 3-hydroxyanthranilic acid oxidase. Arch

Biochem Biophys 1980;203:161-6

136.   Walsh JL, Todd WP, Carpenter BK, Schwarcz R. 4-Halo-3-hydroxyanthranilates are

potent inhibitors of 3-hydroxyanthranilate oxygenase in the rat brain in vitro and in vivo. Adv

Exp Med Biol 1991;294:579-82

137.   Walsh JL, Wu HQ, Ungerstedt U, Schwarcz R. 4-Chloro-3-hydroxyanthranilate

inhibits quinolinate production in the rat hippocampus in vivo. Brain Res Bull 1994;33:513-6

138.   Yates JR, Heyes MP, Blight AR. 4-chloro-3-hydroxyanthranilate reduces local

quinolinic acid synthesis, improves functional recovery, and preserves white matter after

spinal cord injury. J Neurotrauma 2006;23:866-81

139.   Linderberg M. Synthesis and QSAR of substituted 3-hydroxyanthranilic acid

derivatives as inhibitors of 3-hydroxyanthranilic acid dioxygenase (3-HAO). European

Journal of Medicinal Chemistry 1999;34:729-744

140.   Luthman J, Radesater AC, Oberg C. Effects of the 3-hydroxyanthranilic acid analogue

NCR-631 on anoxia-, IL-1 beta- and LPS-induced hippocampal pyramidal cell loss in vitro.

Amino Acids 1998;14:263-9

141.   Luthman J. Anticonvulsant effects of the 3-hydroxyanthranilic acid dioxygenase

inhibitor NCR-631. Amino Acids 2000;19:325-34

142.   Vallerini GP, Amori L, Beato C, et al. 2-Aminonicotinic acid 1-oxides are chemically

stable inhibitors of quinolinic acid synthesis in the mammalian brain: a step toward new

antiexcitotoxic agents. J Med Chem 2013;56:9482-95

143.   Neurim Pharmaceuticals (1991) Limited. Substituted aryl-indole compounds and their

kynurenine/kynuramine-like metabolites as therapeutic agents. EP2664615 (2013)

144.   Curadev Pharma Private Ltd. Inhibitors of the kynurenine pathway. WO2014186035

(2014)

145.   Dominguez, C; Toledo-Sherman, LM; Winkler, D; Brookfield, F; De Aguiar Pena,

PC. Certain kynurenine-3-monooxygenase inhibitors, pharmaceutical compositions, and

methods of use thereof. US20130029988 (2013)

146.   Wityak, J; Toledo-Sherman, LM; Dominguez, C; Courtney, SM; Yarnold, CJ; De

Aguiar Pena, PC; Scheel, A; Winkler, D. Certain kynurenine-3-monooxygenase inhibitors,

pharmaceutical compositions, and methods of use thereof. US20130331370 (2013)

147.   Dominguez, C; Toledo-Sherman, LM; Courtney, SM; Prime, M; Mitchell, W; Brown,

CJ; De Aguiar Pena, PC; Johnson, P. Certain kynurenine-3-monooxygenase inhibitors,

pharmaceutical compositions, and methods of use thereof. WO2013016488 (2013)

148.   Courtney, SM; Mitchell, W; Brown, CJ; De Aguiar Pena, PC; Johnson, P; Dominguez,

C; Toledo-Sherman, LM; Munoz, I. Certain kynurenine-3-monooxygenase inhibitors,

pharmaceutical compositions, and methods of use thereof. WO2013033068 (2013)

149.   Courtney, SM; Prime, M; Mitchell, W; Brown, CJ; De Aguiar Pena, PC; Johnson, P;

Dominguez, C; Toledo-Sherman, LM; Munoz, I. Certain kynurenine-3-monooxygenase

inhibitors, pharmaceutical compositions, and methods of use thereof. WO2013033085 (2013)

150.   The J. David Gladstone Institute; University of Maryland. Small molecule inhibitors

of kynurenine-3-monooxygenase. US8710237 (2014)

151.   The J. David Gladstone Institutes, a testamentary trust established under the will of J.

David Gladstone; University of Maryland. Small molecule inhibitors of kynurenine-3-

monooxygenase. US20120046324 (2012)

152.   CHDI Foundation, Inc. Kynurenine-3-monooxygenase inhibitors, pharmaceutical

compositions, and methods of use thereof. WO2013151707 (2013)

153.   CHDI Foundation, Inc. Kynurenine-3-monooxygenase inhibitors, pharmaceutical

compositions, and methods of use thereof. WO2015047978 (2015)

154.   Wityak, J; Toledo-Sherman, LM; Dominguez, C; Courtney, SM; Yarnold, CJ; De

Aguiar Pena, PC; Scheel, A; Winkler, D. Certain kynurenine-3-monooxygenase inhibitors,

pharmaceutical compositions, and methods of use thereof. US20120329812 (2012)

155.   Sacco RL, DeRosa JT, Haley EC, Jr., et al. Glycine antagonist in neuroprotection for

patients with acute stroke: GAIN Americas: a randomized controlled trial. JAMA

2001;285:1719-28

156.   Stone TW. Development and therapeutic potential of kynurenic acid and kynurenine

derivatives for neuroprotection. Trends Pharmacol Sci 2000;21:149-54

157.   Fulop F, Szatmari I, Vamos E, et al. Syntheses, transformations and pharmaceutical

applications of kynurenic acid derivatives. Curr Med Chem 2009;16:4828-42

• **A comprehensive review on KYNAkynurenic acid derivatives as therapeutic**

substances in neurological disorders.

Figure captions

Figure 1. The schematic depiction of the kynurenine pathway of tryptophan metabolism.

Figure 2.Reference inhibitors of indolamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase
reported in the literature.

Figure 3.Reference inhibitors of kynurenine aminotransferase reported in the literature.

Figure 4.Reference inhibitors of kynurenine 3-monooxygenase reported in the literature.

Figure 5.Reference inhibitors of kynureninase reported in the literature. It should be noted that **16**and **17**in Fig. 4. can also exert inhibitory effect on kynureninase.

Figure 6.Reference inhibitors of 3-hydroxyanthranilate 3,4-dioxygenase reported in the literature.

Figure 7. Some 2,3-diamino-furo[2,3-*c*]pyridine and 2,3-diamino-benzo[*b*]thiophene derivatives as inhibitors of indolamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase.

Figure 8. Certain aryl pyrimidines as inhibitors of kynurenine 3-monooxygenase.

Figure 9. Some pyrimidine benzenesulfonamides as inhibitors of kynurenine 3-monooxygenase.

Figure 10. Some phenylthiazolebenzenesulfonamides as inhibitors of kynurenine 3-monooxygenase. It should be noted that **20** and **21** in Fig. 4. also belong to this chemical group.

Figure 11. Two examples of phenylthiadiazolebenzenesulfonamides as inhibitors of kynurenine 3-monooxygenase.

Figure 12. Certain *trans*-2-substituted-cyclopropane-1-carboxylic acids as inhibitors of kynurenine 3-monooxygenase. It should be noted that **19** in Fig. 4. also belongs to this

chemical group.

Figure 13. Some isoxazoles (**82** and **83**) and isoxazolines(**84-87**) as inhibitors of kynurenine

3-monooxygenase.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
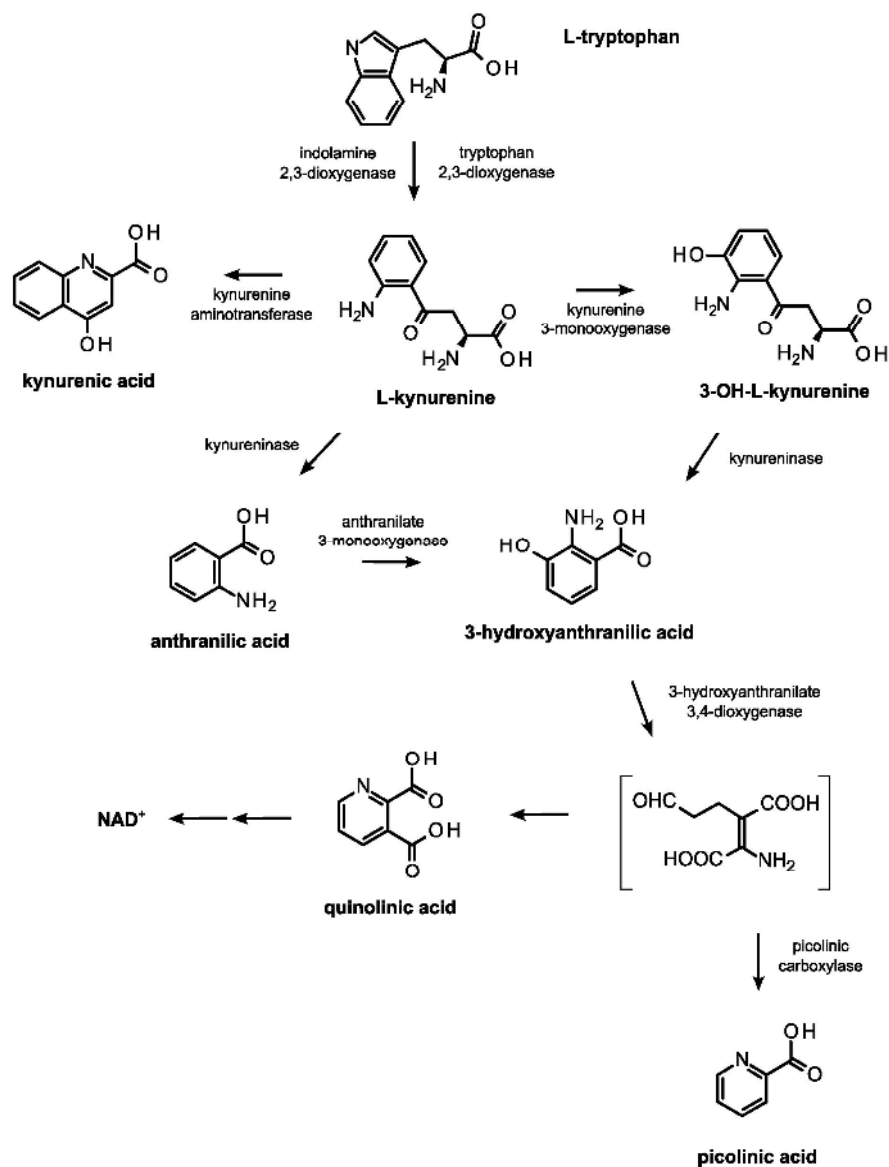
502x671mm (96 x 96 DPI)
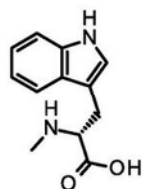
1
indoximod
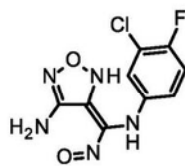
2
INCB024360
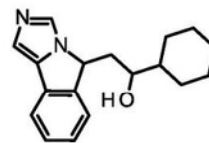
3
NGL919
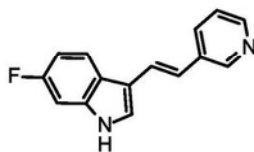
4
680C91
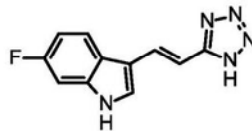
5
LM10
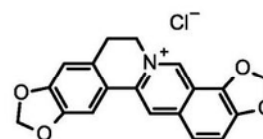
6
coptisine

361x207mm (72 x 72 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
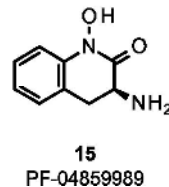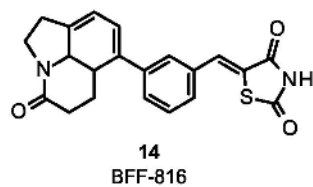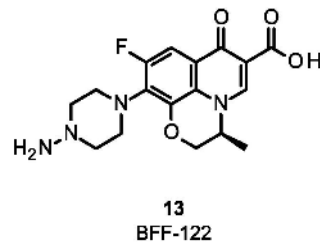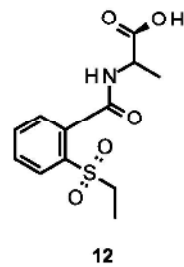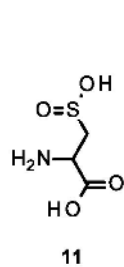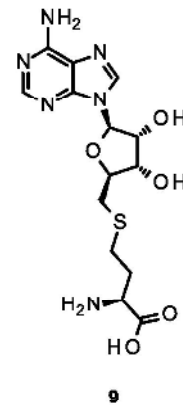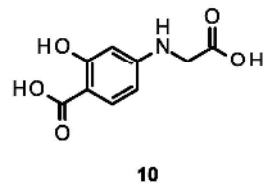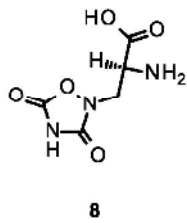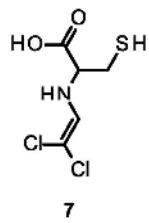
543x437mm (96 x 96 DPI)
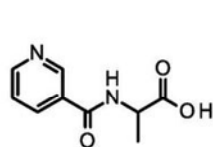
16
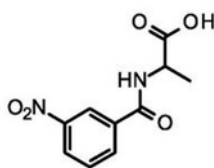
17
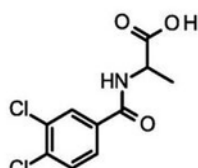
18
PNU 156561
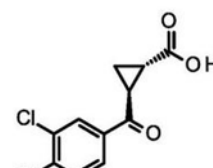
19
UPF 648
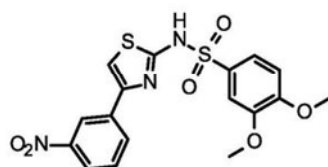
20
Ro 61-8048
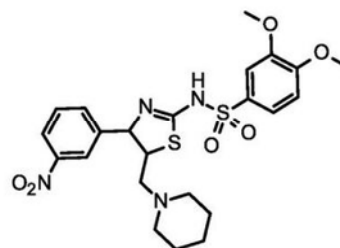
21
JM6
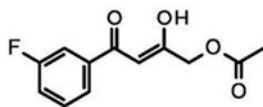
22
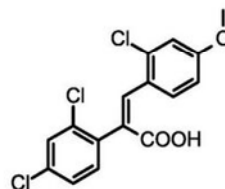
23

313x270mm (72 x 72 DPI)
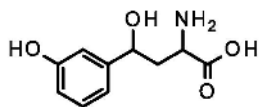
24
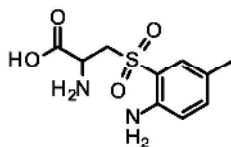
25
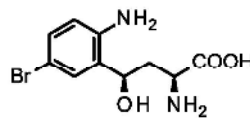
26

543x124mm (96 x 96 DPI)
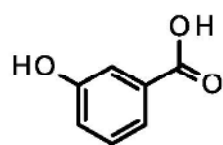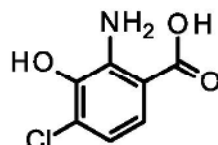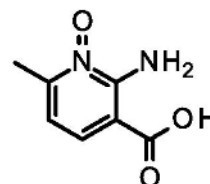
403x124mm (96 x 96 DPI)
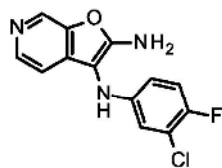
30
WO2014186035
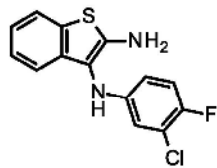
31
WO2014186035
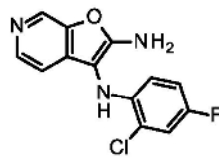
32
WO2014186035
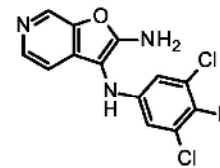
33
WO2014186035
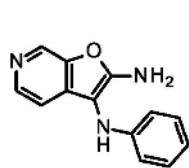
34
WO2014186035
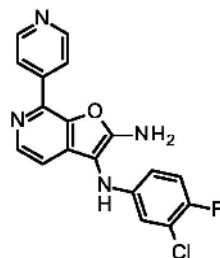
35
WO2014186035
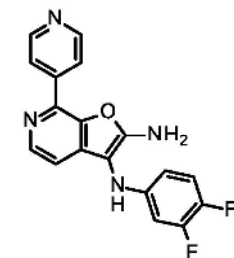
36
WO2014186035
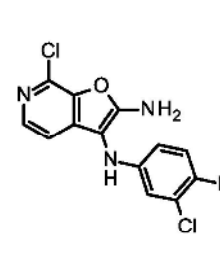
37
WO2014186035
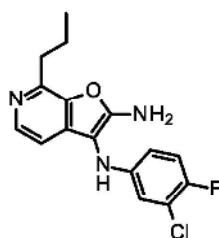
38
WO2014186035
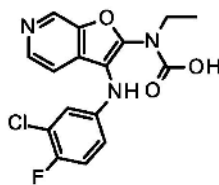
39
WO2014186035
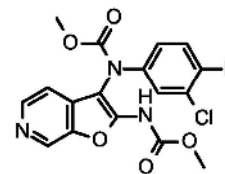
40
WO2014186035

543x468mm (96 x 96 DPI)
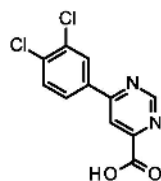
41
WO2013033068
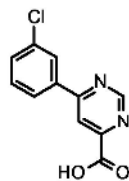
42
WO2013033068
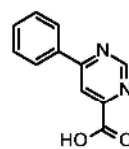
43
WO2013033068
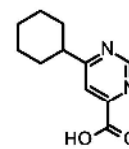
44
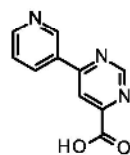
45
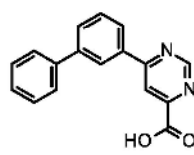
46
WO2013033068
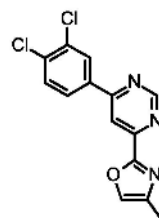
47
WO2013016488
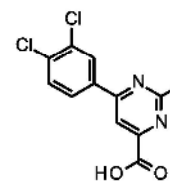
48
WO2013033068
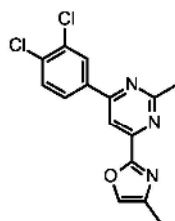
49
WO2013016488
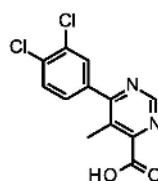
50
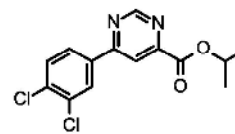
51
WO2013033068
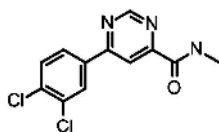
52
WO2013033068
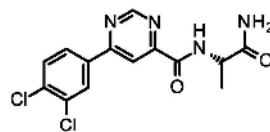
53
WO2013033068
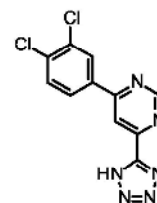
54
US20130029988

543x731mm (96 x 96 DPI)

55
US20130029988

56
WO2013033068

57
US20130029988

58
US20130029988

59
US20130029988

60
US20130029988

61
WO2013033085
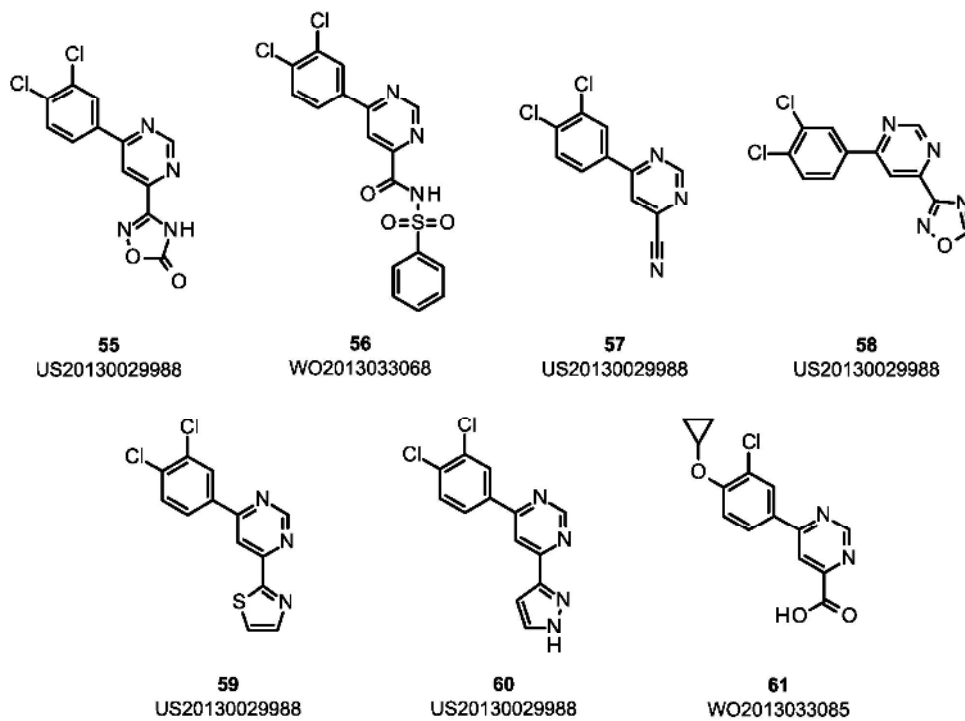
543x406mm (96 x 96 DPI)
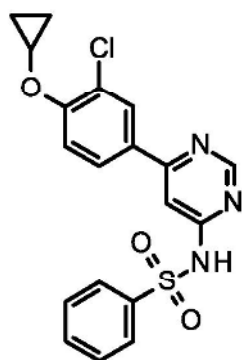
62
WO2013033085
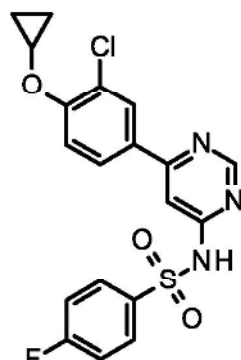
63
WO2013033085
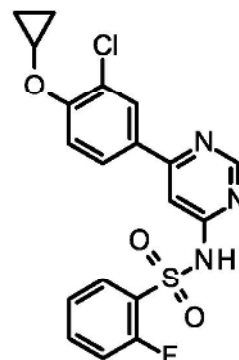
64
WO2013033085

403x187mm (96 x 96 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
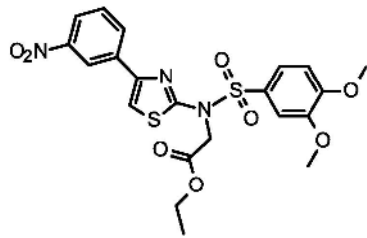
65
US8710237
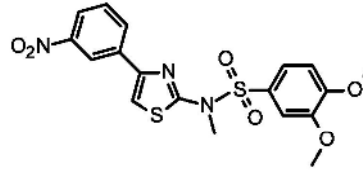
66
US8710237
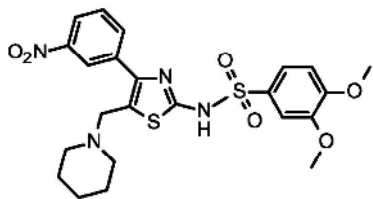
67
US8710237
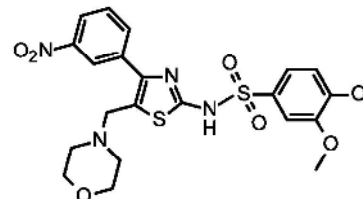
68
US8710237
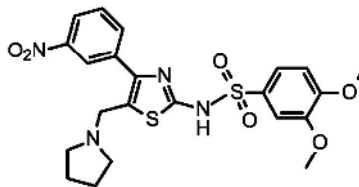
69
US8710237

543x500mm (96 x 96 DPI)
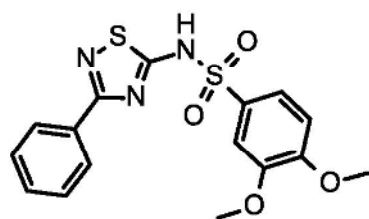
70
US201220046324
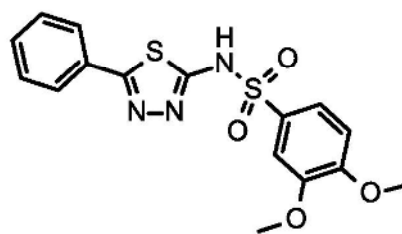
71
US201220046324

403x134mm (96 x 96 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

72
WO2015047978

73
WO2013151707

enantiomers

74
WO2013151707

75
WO2013151707

76
WO2013151707

enantiomers

77
WO2013151707

78
WO2013151707

79
WO2013151707

80
WO2013151707
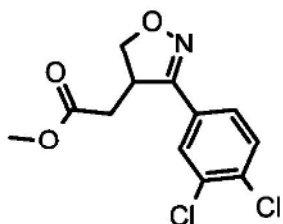
81
WO2013151707
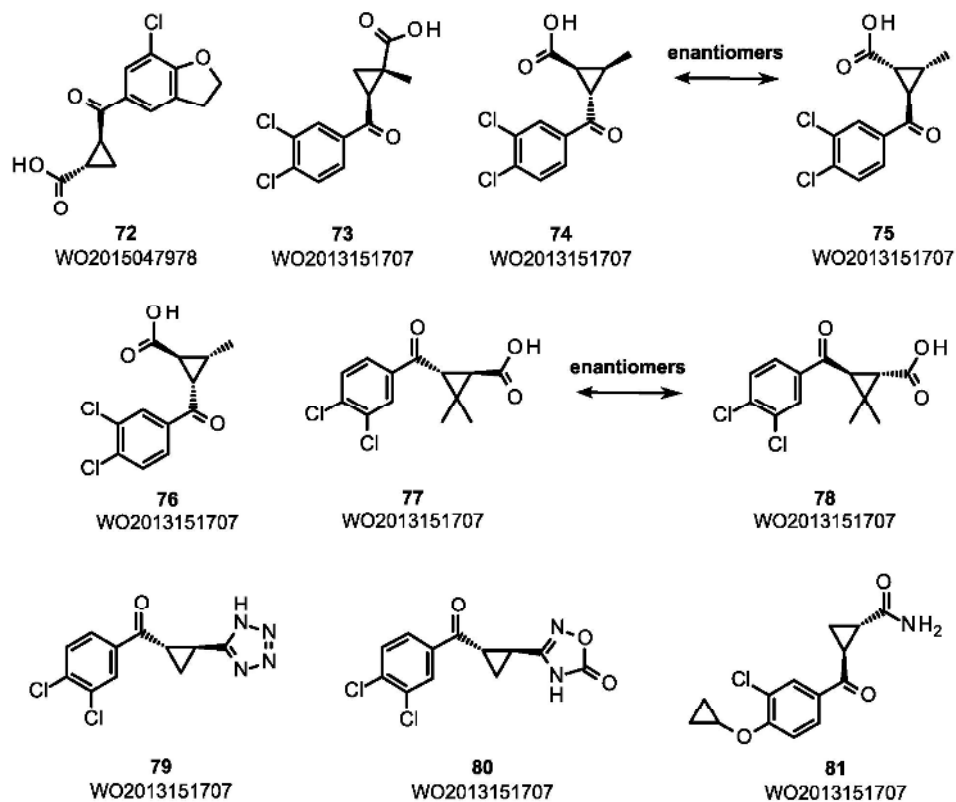
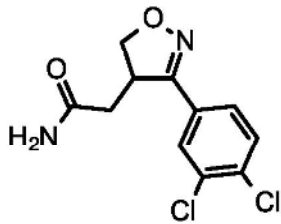543x437mm (96 x 96 DPI)
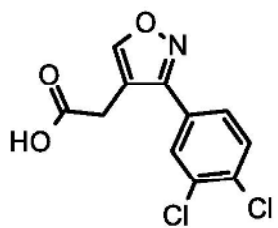
82
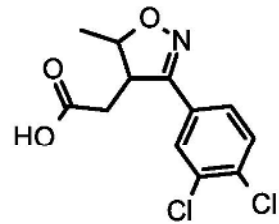US20120329812
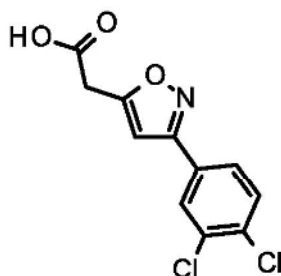
83
US20120329812
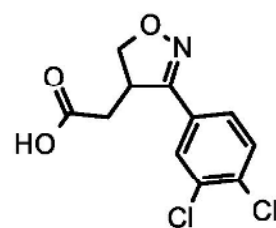
84
US20120329812

85
US20120329812

86
US20120329812

87
US20120329812

403x303mm (96 x 96 DPI)