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

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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 optionsfor~~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 ~~GPR~~35 (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 $O_2^{\cdot-}$[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 function~~ing~~ 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 re~~storat~~versing 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 analog~~ue~~ 1-methyl-TRP, more specifically ~~the~~its 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 has~~ve~~ 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, ~~which~~the latter one could significantly improve the performance in the Morris water maze cognitive test following intracerebroventricular administration [75]. Furthermore~~However~~, 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 penetration~~across~~through 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 ~~u~~non-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 ~~u~~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 (≤ 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, **20**increased 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 ~~u~~non-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, whereas~~ile~~ the 3-hydroxyl group plays~~ed~~ 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,~~ has~~ve~~ been synthesized as well[128]. The (4S)-epimer is also a potent inhibitor (K(i) = 170 nM) with~~out~~no 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, ~~whicha~~ 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 de~~monstration~~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 ion IDO1 inhibition (IC_{50} = 200-1000 nM) and a slight increase ion TDO inhibition (IC_{50}< 500 nM). With regard to the application of substituents onat the furo[2,3-*c*]pyridine moiety, it can be concluded that the application of substituents inatthe 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) didoes 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 inatthe 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 thea decrease ofin 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~~across~~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 ~~would be~~ is critical to form an ionic salt bridge with a guanidinium moiety of an arginine of KMO;~~, but~~ however it considerably decreases the penetration ~~across~~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~~,~~;~~but~~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%)~~, and~~ 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 i~~o~~n 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 acid~~AA~~ 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 of~~for 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 be~~were 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%) ~~a~~likewise 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%) ~~alike~~both 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 derivatives~~analogs~~, 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 molecule~~which~~demonstrating~~es~~ 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 a nongoing recruit~~ing period~~ment for a trial ~~the~~ assessing~~ment~~the therapeutic potential of this drug in major depressive disorder (ClinicalTrials.gov identifier: NCT02484456). With regard to the metabolic shift concept

with the aim of re~~storation of~~versing 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 ~~rationale~~justification. 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

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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.

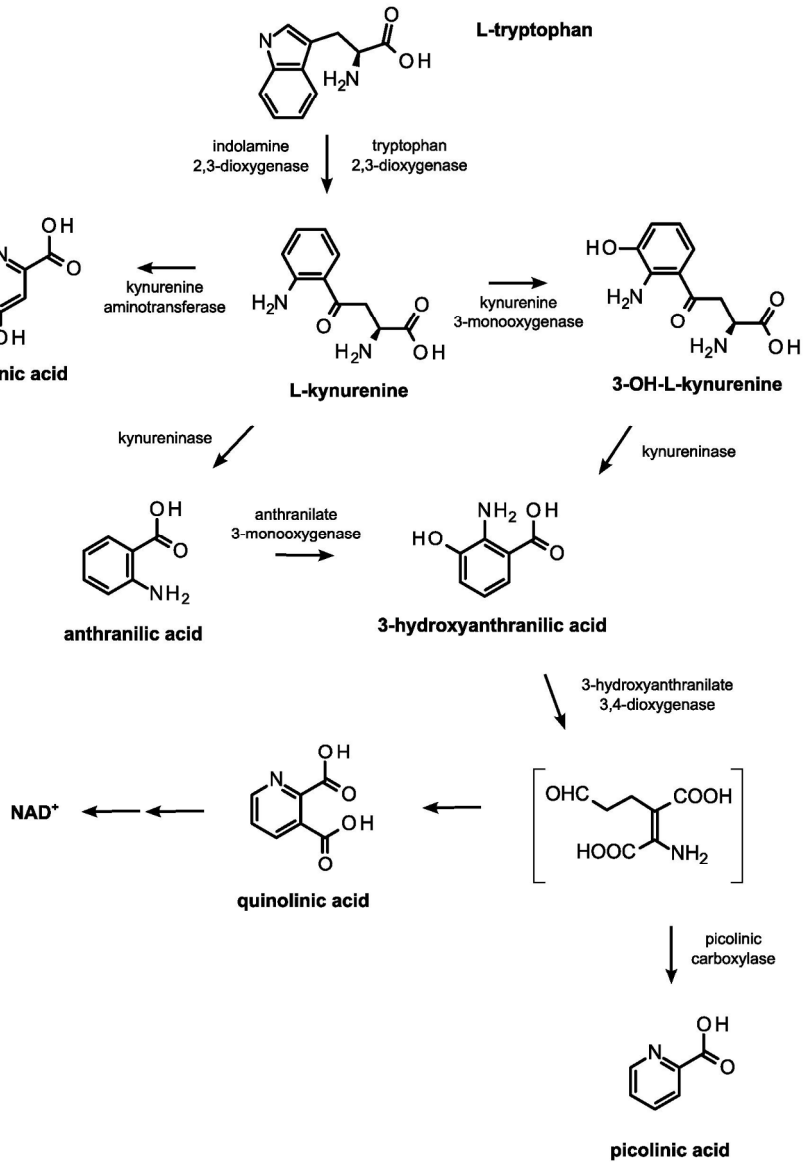
Figure 10. Some phenylthiazolebenzenesulfonamides as inhibitors of kynurenine 3-monooxygenase. It should be noted that **20** and **21** in Fig. 4. also belong to this chemical group.

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.



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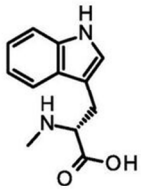

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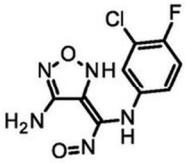

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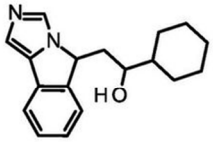

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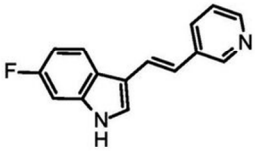

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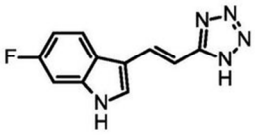

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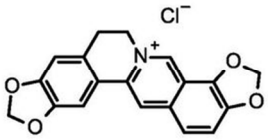

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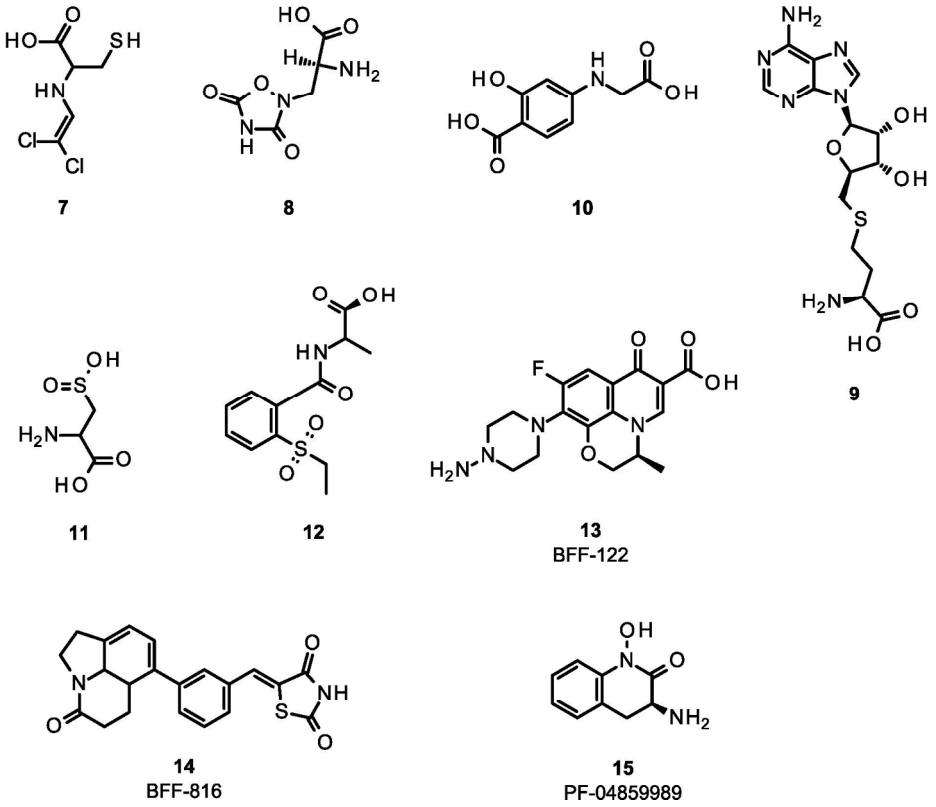

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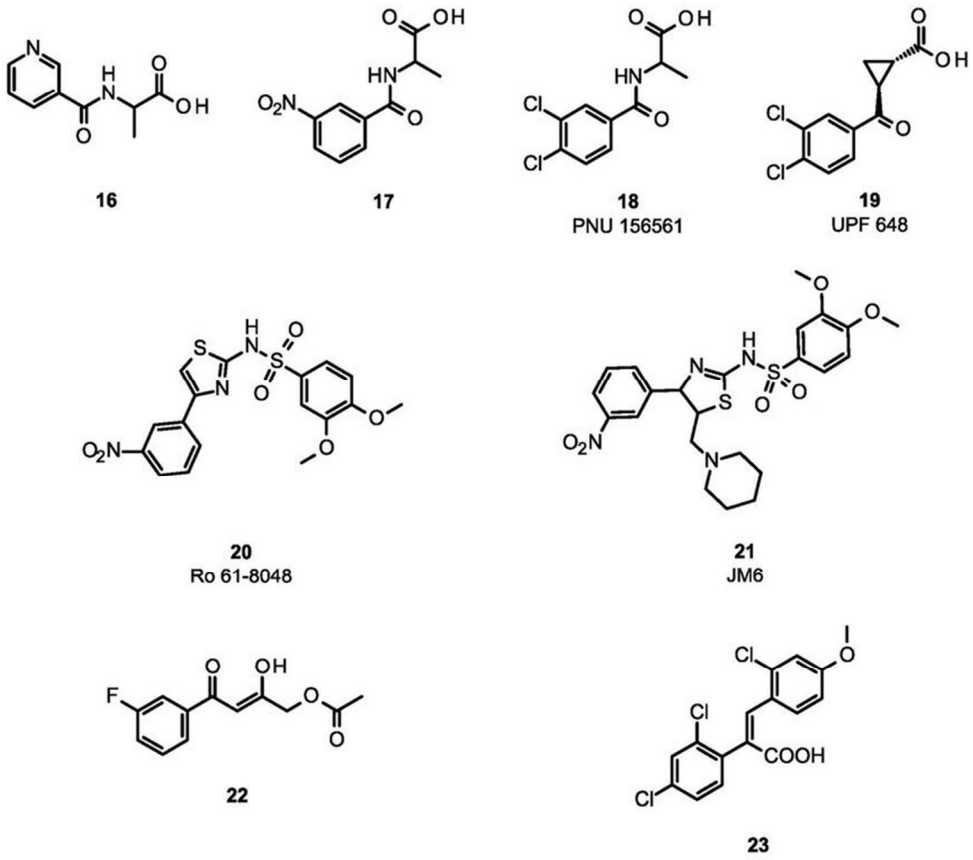

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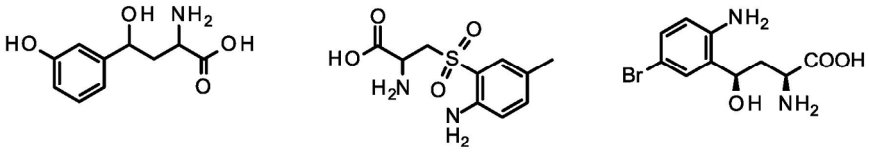

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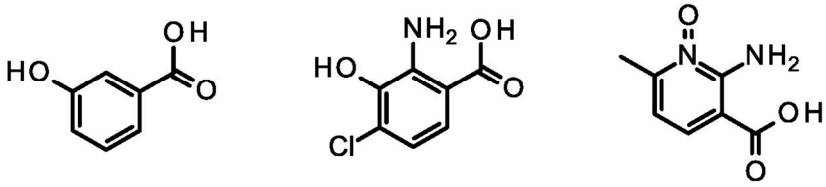

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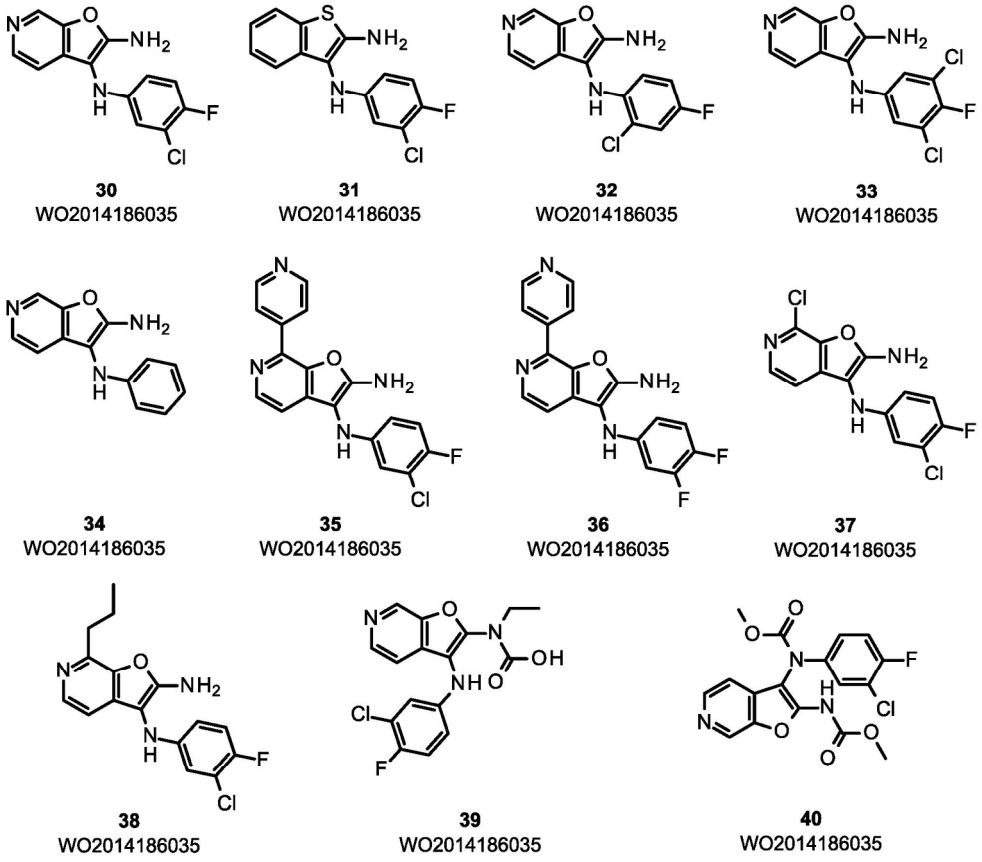

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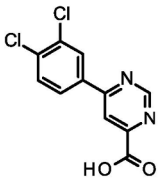

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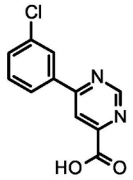

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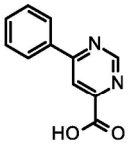

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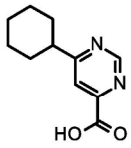

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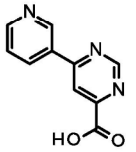

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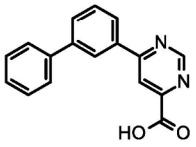

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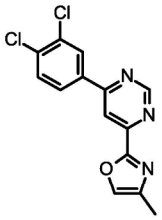

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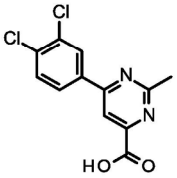

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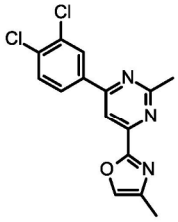

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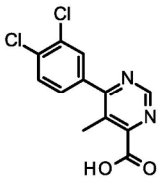

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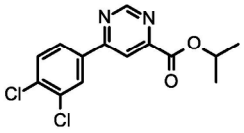

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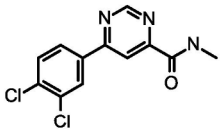

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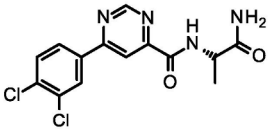

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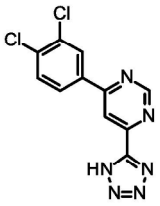

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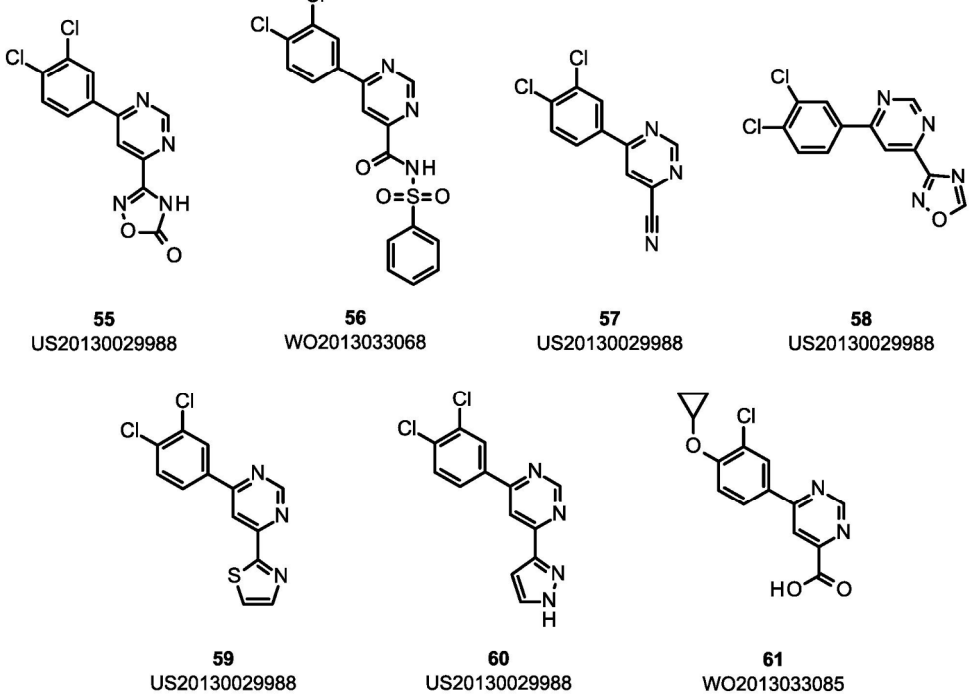

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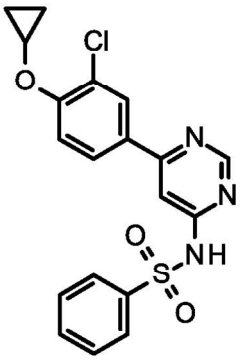

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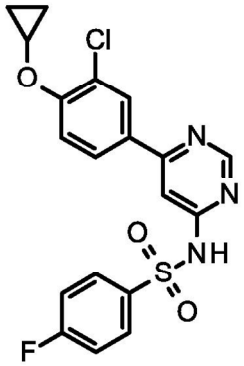

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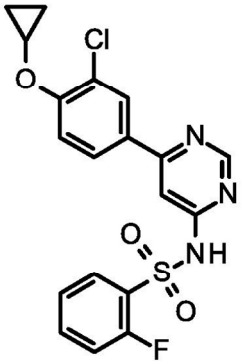

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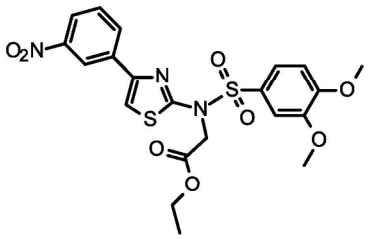

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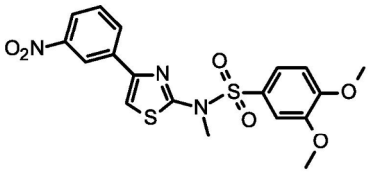

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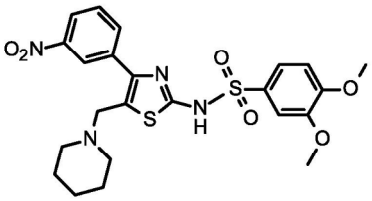

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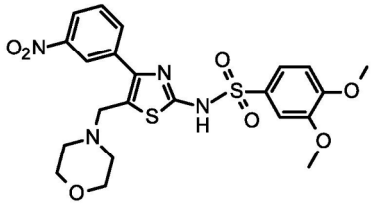

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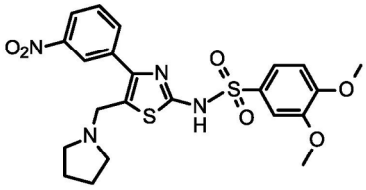

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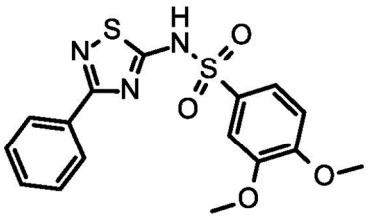

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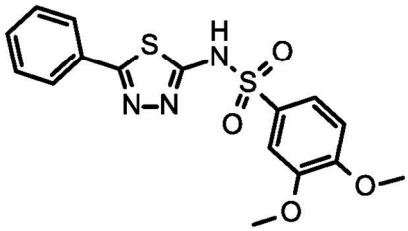

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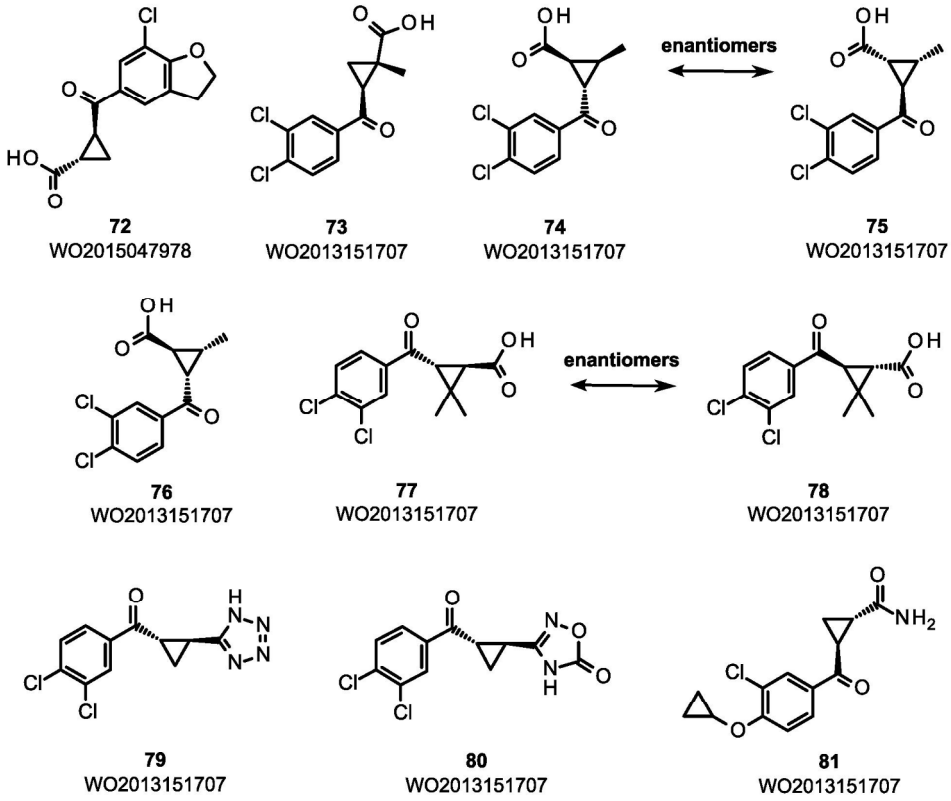

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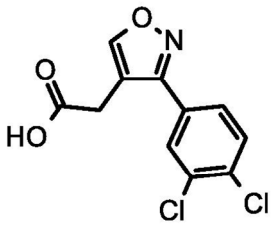

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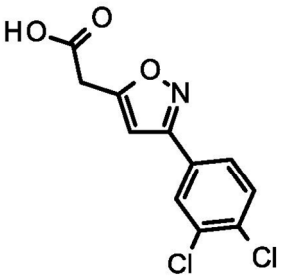

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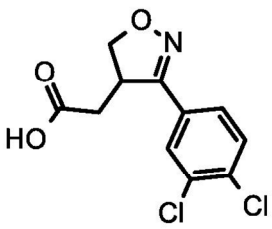

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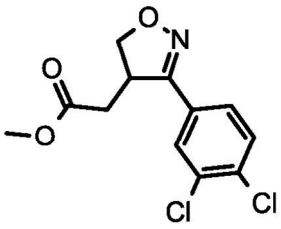

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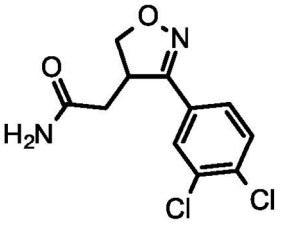

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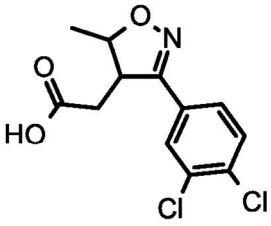

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