Syntheses, Transformations and Pharmaceutical Applications of Kynurenic Acid Derivatives

Ferenc Fülöp,[a]* István Szatmári,[a] Enikő Vámos,[b] Dénes Zádori,[b] József Toldi,[c] László Vécsei[b]

[a]*Institute of Pharmaceutical Chemistry and Research Group for Stereochemistry, Hungarian Academy of Sciences, University of Szeged, H-6720 Szeged, Eötvös u. 6, Hungary*

[b] *Department of Neurology, University of Szeged, H-6725 Szeged, Semmelweis u. 6, Hungary*

[c] *Department of Physiology, Anatomy and Neuroscience, University of Szeged, H-6726 Szeged, Hungary*

KEYWORDS: Kynurenic acid, Kynurenic acid derivatives, Neurological disorders, Schizophrenia, Retinal damage, Diabetes mellitus, Hypertension

Abstract

The syntheses and transformations of 4-hydroxyquinoline-2-carboxylic acid, kynurenic acid, are reviewed, and special attention is paid to the pharmacological activities and pharmaceutical applications of its derivatives.

1. Introduction

4-Hydroxyquinoline-2-carboxyxlic acid, also known as kynurenic acid (KYNA, Fig. 1), is an endogenous product of the tryptophan (TRP) metabolism, a pathway (Fig. 2) known to be responsible for the production of nicotinamide adenine dinucleotide (NAD^+) and NAD phosphate ($NADP^+$) [1,2]. In this pathway, TRP is converted into various compounds, including L-kynurenine (L-KYN), which can be metabolized in two separate ways, one branch furnishing KYNA, and the other 3-hydroxykynurenine (3-OH-KYN) and quinolinic acid (QUIN), the precursors of NAD [3,4].

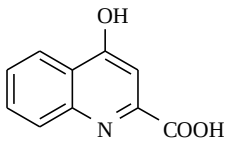


Fig. 1

Among the important features of KYNA is the fact that it is one of the few known endogenous excitatory amino acid receptor blockers with a broad spectrum of antagonistic properties in supraphysiological concentrations. One of its recently confirmed sites of action is the - 7-nicotinic acetylcholine (- 7-nACh) receptor and, interestingly, a more recently identified one is a higher-affinity positive modulatory binding site at the α amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor [5].

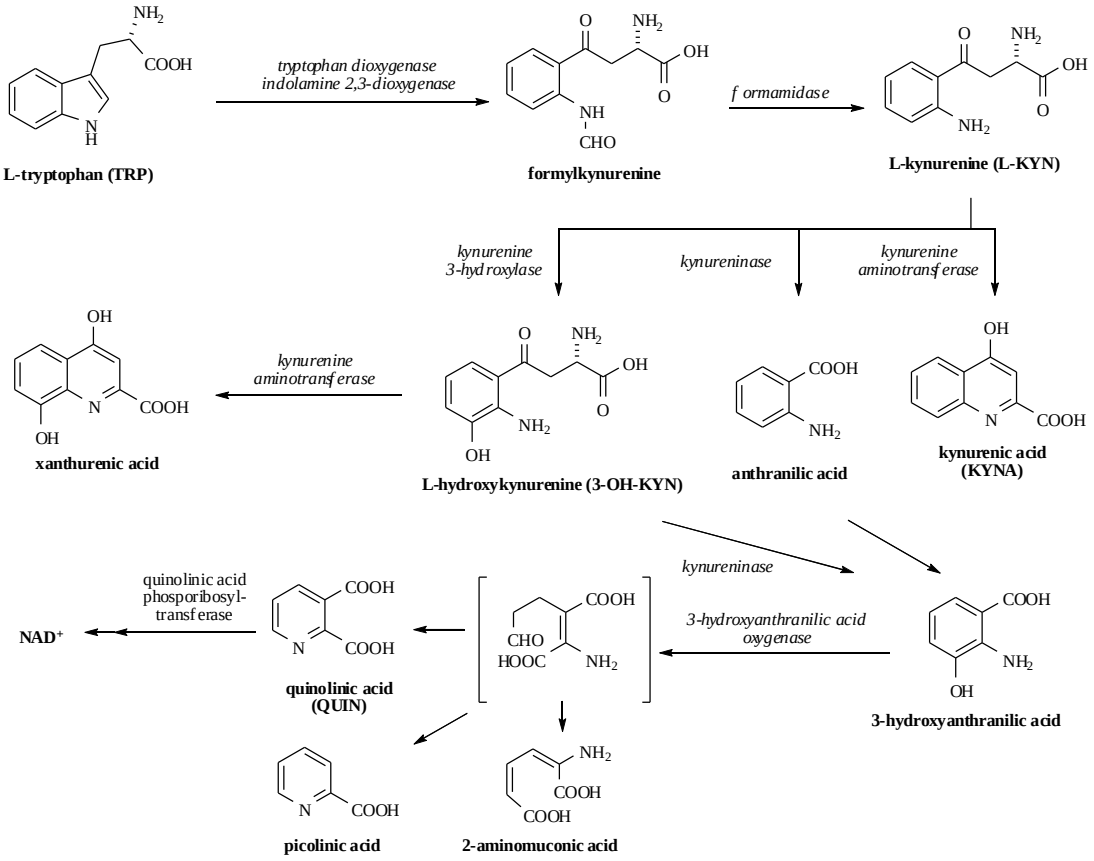


Fig. 2

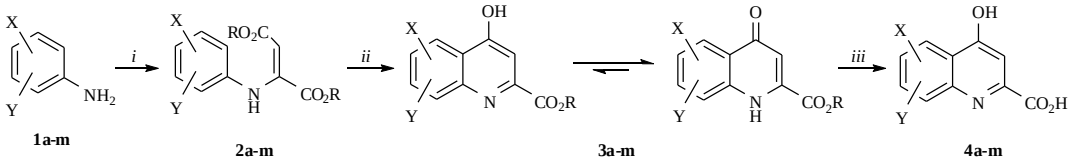
Since KYNA is a TRP metabolite produced by astrocytes and neurons via the KYN pathway in both humans and rodents [6-9], and since it is an endogenous neuroprotective agent able to prevent neuronal loss following excitotoxic, ischemia-induced and infectious neuronal injuries [10,11], there has recently been increasing interest in the synthesis and pharmacological studies of KYNA derivatives.

The primary aim of the present review is to survey the methods available for the synthesis and transformations of KYNA derivatives. Additionally, the pharmacological activities of these derivatives will be reviewed.

2. Syntheses and transformations of KYNA derivatives

2.1. Syntheses of KYNA derivatives

The general procedure for the synthesis of 4-hydroxyquinolinic acid involves a modified Conrad-Limpach method, starting from the commercially available substituted arylamines **1**. The first step comprises enamine bond formation through the use of dimethyl acetylenedicarboxylate (DMAD) or diethyl acetylenedicarboxylate (DEAD), resulting in **2a-m** (Scheme 1).



Scheme 1. *Reagents and conditions: i*), *ii*) see Table 1; *iii*) NaOH, MeOH, H_2O, rt; then HCl (aq)

The intermediate fumarates **2a-m** are then cyclized by using diphenyl ether or Downtherm at high temperature, yielding methyl 4-hydroxy-2-carboxylates (**3a-e** and **3i-m**) or ethyl 4-hydroxy-2-carboxylates (**3f-h**), respectively. The reaction conditions for the preparation of different substituted 4-hydroxyquinolinic acid derivatives are listed in Table 1. For **3a-m**, the enol-oxo tautomerism is possible. This equilibrium is shifted in favor of the oxo form when C-2 contains an ester function (**3a-m**), while the presence of a carboxylic group at C-2 (**4a-m**) favors the enol form (Scheme 1).

For the transformations of the 4-hydroxy-quinoline derivatives, the free acids (KYNA derivatives) are needed. Hydrolysis of the esters is generally performed in methanolic alkaline medium followed by acidification with HCl (Scheme 1).

Table 1. Reagents and conditions for the synthesis of KYNA derivatives **3a-m**

Compounds	X	Y	R	Reaction conditions *i* **1 → 2**	Reaction conditions *ii* **2 → 3**	References
3a	H	H	Me	DMAD, MeOH, 55 °C	diphenyl ether, reflux or Downtherm, 240 °C	[12,13]
3b	6-F	H	Me	DMAD, MeOH, 55 °C	diphenyl ether, reflux	[14-16]
3c	7-OMe	H	Me	DMAD, MeOH, 55 °C	diphenyl ether, reflux	[14]
3d	6,7 -OCH_2O		Me	DMAD, MeOH, 55 °C	diphenyl ether, reflux	[14]
3e	5,7–di-Cl		Me	DMAD, MeOH, 55 °C	diphenyl ether, reflux	[17]
3f	5,7–di-Cl		Et	DEAD, MeOH, 55 °C	diphenyl ether, reflux	[18]
3g	5,7–di-Br		Et	DEAD, MeOH, 55 °C	diphenyl ether, reflux	[18]
3h	5,7–di-I		Et	DEAD, MeOH, 55 °C	diphenyl ether, reflux	[18]
3i	7-Cl	H	Me	DMAD, MeOH, 55 °C	diphenyl ether, reflux	[19]
3j	7-OBn	H	Me	DMAD, MeOH, reflux	Downtherm, 240 °C	[12,13]
3k	6-OBn	H	Me	DMAD, MeOH, reflux	Downtherm, 240 °C	[12,13]
3l	6-NO_2	H	Me	DMAD, MeOH, reflux	Downtherm, 240 °C	[12,13]

3m	6,7 -NH-COO-	Me	DMAD, MeOH, reflux	Downtherm, 240 °C	[12,13]

2.2. Transformations of KYNA derivatives

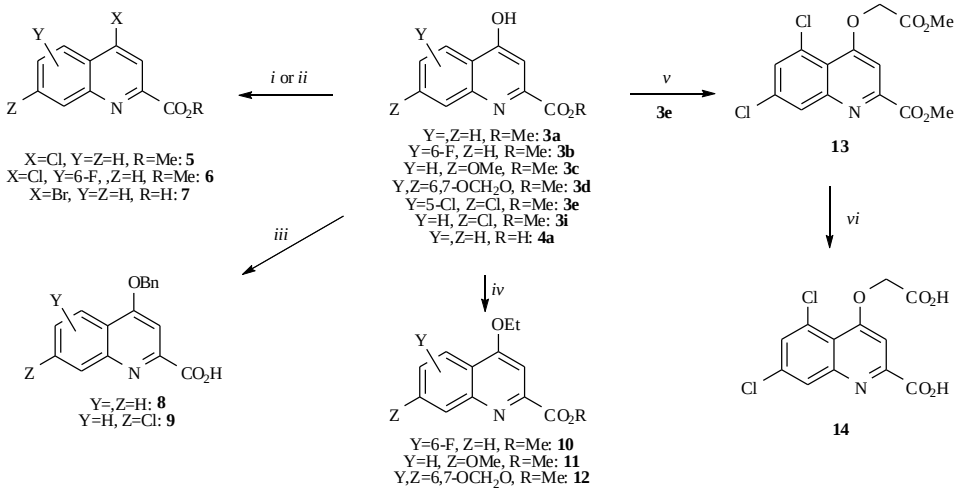
The transformations of KYNA derivatives can be achieved in different ways: transformation of the synthetically active 4-OH group, and conversion of the 2-carboxylic function to the pharmacologically interesting ester or amide derivatives of KYNA.

2.2.1. Transformations of the hydroxy group at position 4

The 4-OH group can be chlorinated by refluxing the starting materials **3a** and **3b** in $POCl_3$ for some hours [14], resulting in **5** and **6**. A new approach in this area is the use of P_2O_5/Bu_4NBr as brominating agent, furnishing **7** (Scheme 2) [20].

The reactions of **3a** and **3i** with benzyl bromide (BnBr) in basic medium (K_2CO_3), followed by hydrolysis of the ester moiety with NaOH, led to *O*-protected acid derivatives **8** [21,22] and **9** [19], respectively. When ethyl bromide was applied under similar reaction conditions, the OH group in **3b-3d** was transformed to an OEt group (**10-12**, Scheme 2) [14].

On the use of methyl bromoacetate in the presence of NaH in DMF, an ether moiety containing an additional ester function was incorporated at C-4 to yield **13**, hydrolysis of which led to dicarboxylic acid **14** (Scheme 2) [17].

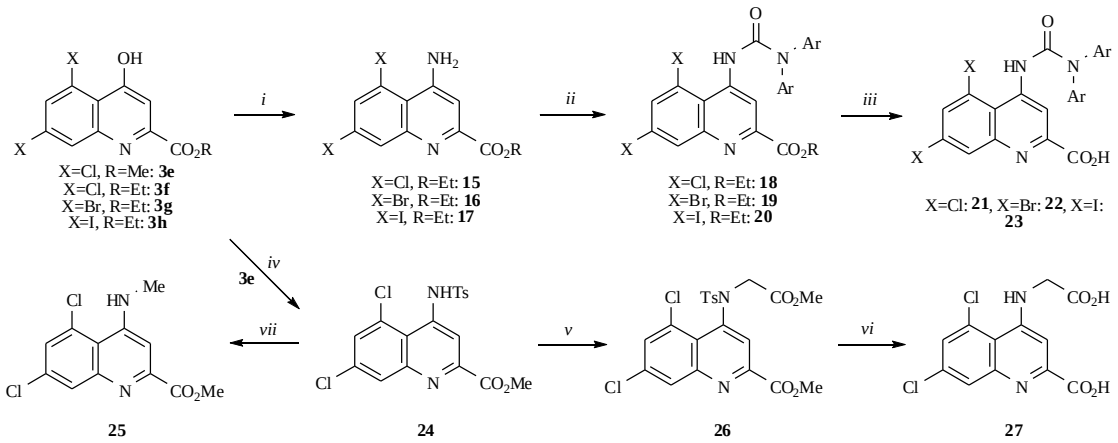


Scheme 2. *Reagents and conditions: i*) $POCl_3$, reflux, 4 h; *ii*) P_2O_5/Bu_4NBr, 100 °C, toluene; *iii*) BnBr, K_2CO_3, DMF then NaOH, H_2O, MeOH; *iv*) K_2CO_3, EtBr, DMF; *v*) methyl bromoacetate, NaH, DMF; *vi*) 1.0 M LiOH, MeOH

A series of KYNA derivatives bearing an amide moiety at C-4 (**21-23**) have been prepared through transformation of the OH group to NH_2 by refluxing **3f-h** with chlorosulfonyl isocyanate, followed by the coupling of **15-17** with diphenylcarbamoyl chloride in the presence of NaH. The hydrolysis of **18-20** 10% NaOH and then HCl (aq) led to the desired substituted KYNA derivatives **21-23** (Scheme 2) [18].

27, the nitrogen-bridged analog of **14**, was prepared via the reaction of **3e** with *p*-tolylsulfonyl isocyanate, which resulted in **24**. Substitution of **24** with methyl bromoacetate and subsequent hydrolysis of the dimethyl ester gave the desired dicarboxylic acid **27** [17,23,24].

The heating of **24** with MeI in the presence of Na_2CO_3 yielded methyl 4-methylamino-5,7-dichloroquinoline-2-carboxylate (**25**, Scheme 3) [23,25].

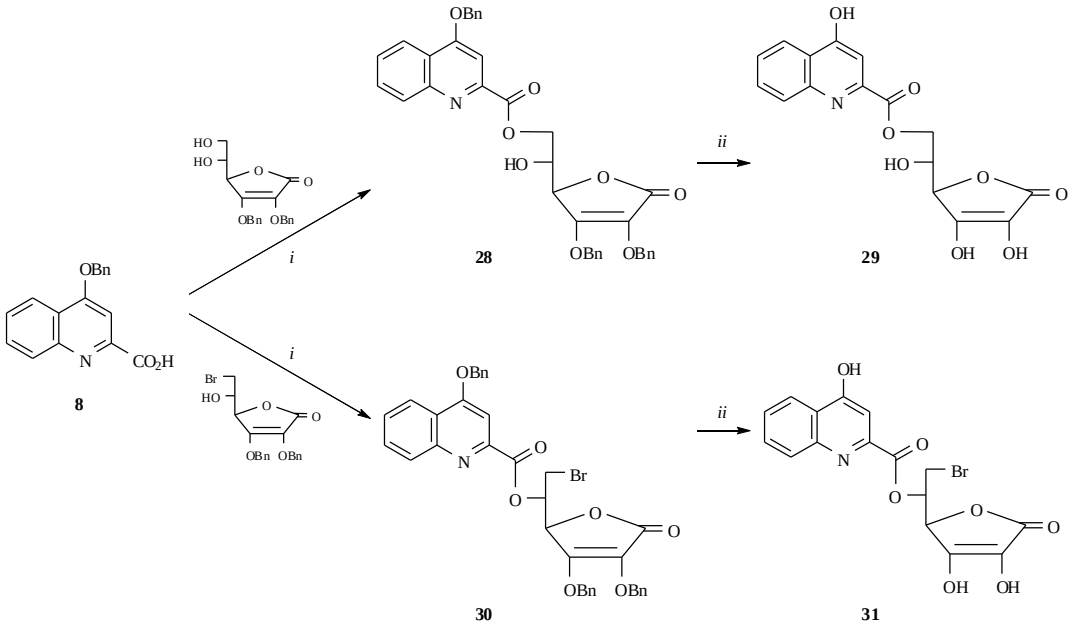


Scheme 3. *Reagents and conditions: i*) chlorosulfonyl isocyanate, , MeCN; *ii*) diphenylcarbamoyl chloride, NaH, 0 °C, DMF; *iii*) 10% NaOH (aq) then HCl (aq); *iv*) *p*-tolylsulfonyl isocyanate, , MeCN; *v*) methyl bromoacetate, NaH, DMF; *vi*) 90% H_2SO_4, 0 °C; *vii*) MeI, Na_2CO_3, reflux, MeCN

2.2.2. Transformations of the ester/carboxylic function at position 2

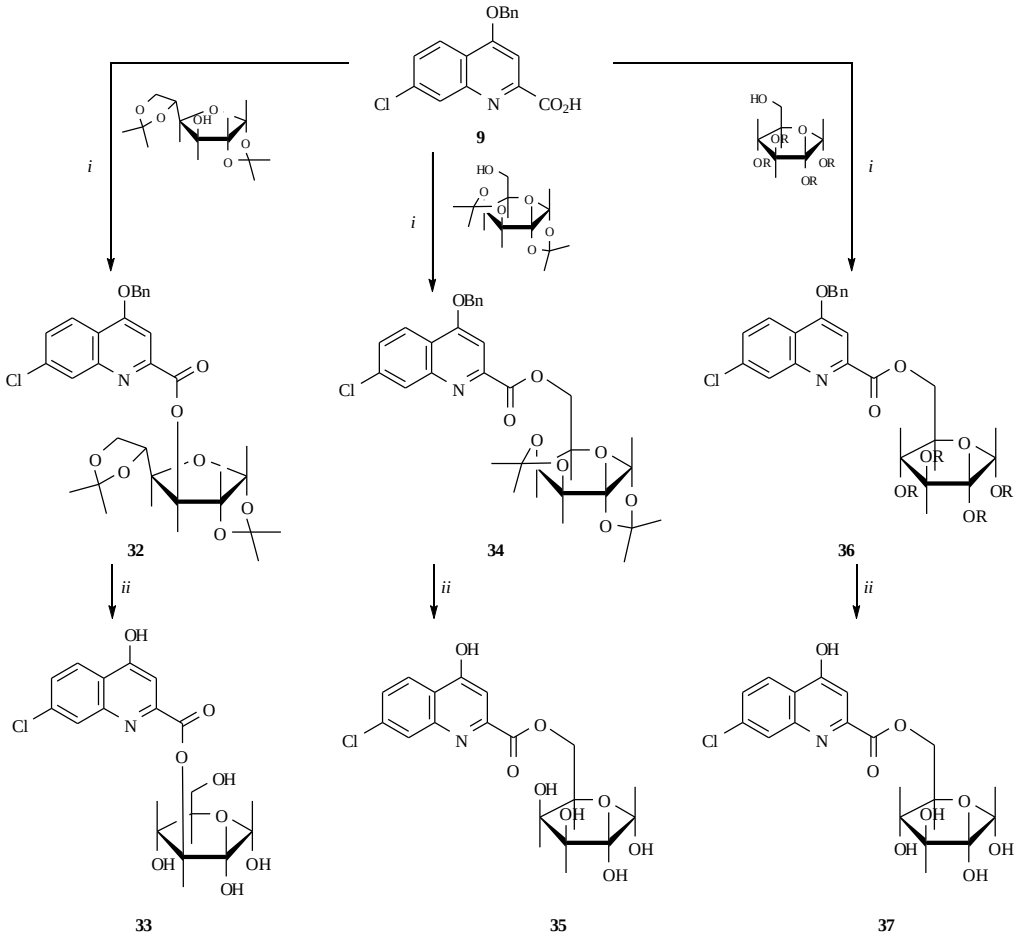
KYNA derivatives are potent NMDA receptor antagonists, but they exert only weak activity after systemic administration. In order to overcome the blood-brain barrier (BBB), new esters that are actively transported across the BBB have been prepared by chemical conjugation with essential nutrients such as ascorbic acid (AA), glucose and galactose.

Esterification with AA is carried out by a general coupling procedure involving a protected precursor, the 2,3-diBn derivative of AA, in the presence of *N*,*N*'-dicyclohexylcarbodiimide (DCC), leading to **28** (Scheme 4). Since BrAA is endowed with better SVCT2 affinity than that of AA, BrAA derivatives of KYNA were prepared as second-generation compounds. The coupling between BrAA and **8** in the presence of DCC resulted in **30** (Scheme 4). The 6-*O*-ascorbates **28** and **30** were then deprotected by hydrogenolysis, affording the desired KYNA esters **29** and **31** (Scheme 4) [21,22].



Scheme 4. *Reagents and conditions: i*) DMAP, DCC, CH_2Cl_2 or CH_2Cl_2/DMF; *ii*) TFA, CH_2Cl_2, then H_2, C/Pd 10%, MeOH

Esterification of **9** with 1,2:4,5-di-*O*-isopropylidene- - D-glucofuranose, 1,2:3,4-di-*O*-isopropylidene- - D-galactopyranose and 1,2, 3,4-*O*-tetramethylcarbonate-D-glucopyranose, in the presence of 1,1'-carbonyldiimidazole (CDI) furnished **32**, **34** and **36** (Scheme 5). The Bn group was cleaved off by hydrogenolysis and the protecting groups of the resultant esters were removed with trifluoroacetic acid (TFA) in CH_2Cl_2 to yield **33**, **35** and **37** [19].



Scheme 5. *Reagents and conditions: i*) CDI, CH_2Cl_2, DMF; *ii*) TFA, CH_2Cl_2, then H_2, C/Pd 10%, MeOH

The free carboxylic function at C-2 in KYNA derivatives provides a new synthetic possibility: transformation to amide derivatives by using the corresponding amines. The strategy of choosing amines follows that for ester formation, *e.g.* by using different "natural-like" amines, the BBB can be overcome.

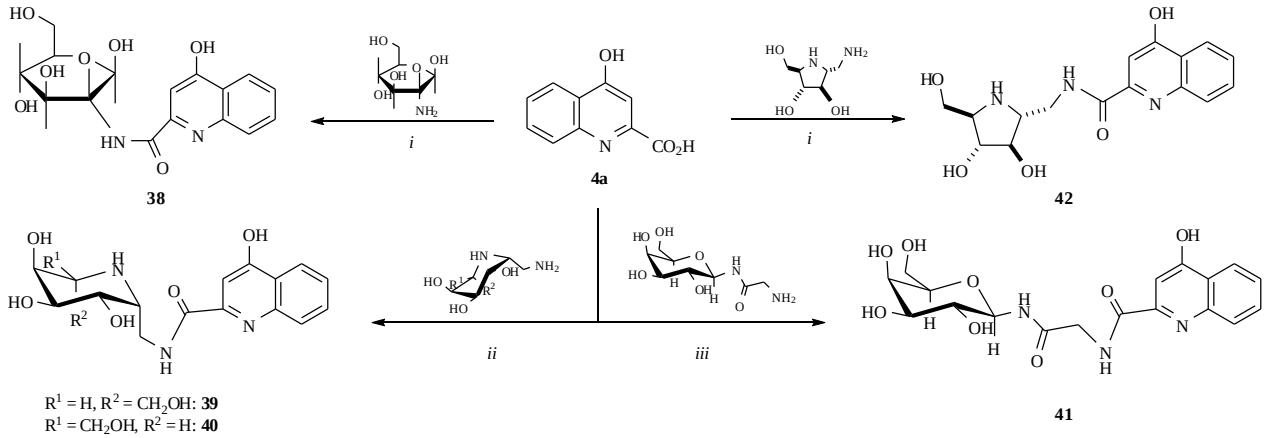
The amidation of KYNA (**4a**) with glucosamine (GLUC) in the presence of 1-hydroxybenzotriazole (1-HOBT) and DCC led to the formation of **38** (Scheme 6) [26].

As iminosugars are known to be inhibitors of many carbohydrate-processing enzymes and to exhibit various biological activities, a series of

iminosugar core structures were constructed and (without any purification) were screened directly against tumor cell lines. Among these compounds, **39** and **40**, were synthetized and tested as new KYNA derivatives (Scheme 6) [27].

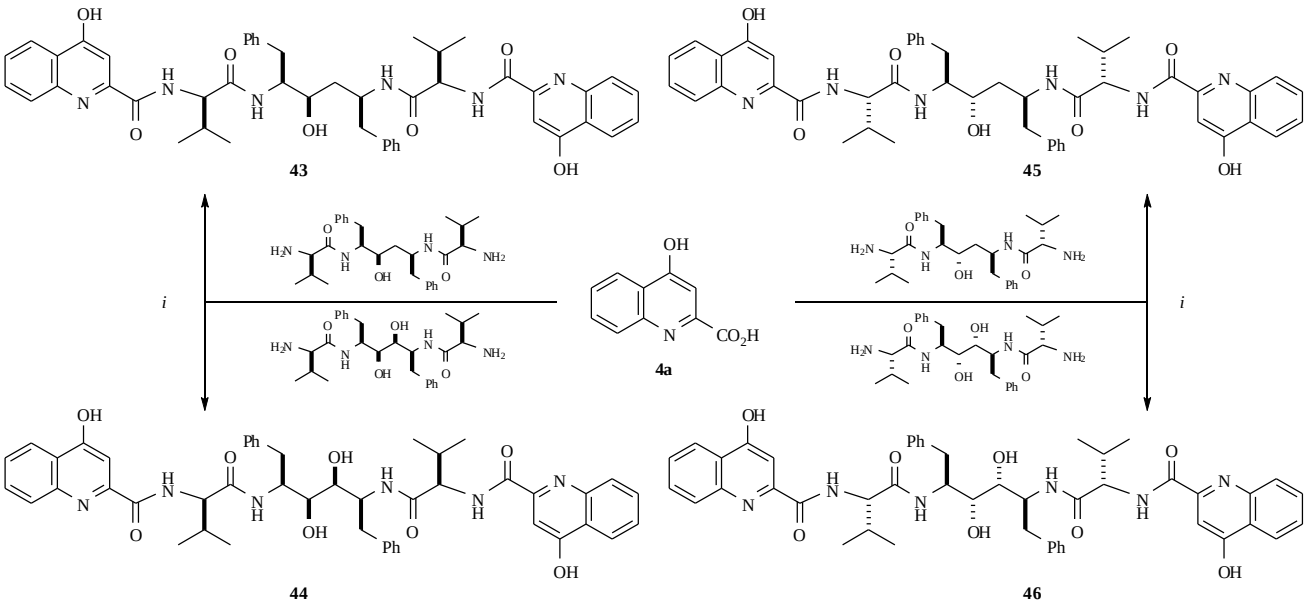
When *N*-glycyl- - glycopyranosylamine was reacted with **4a** in the presence of DCC and *N*-hydroxysuccinimide in DMSO, **41** (Scheme 6) was isolated [28].

A further extension of this type of amidation of KYNA involves the use of iminocyclitol as amine component. The coupling reaction is performed with DCC in the presence of 1-HOBT, furnishing **42** (Scheme 6) [29].



Scheme 6. *Reagents and conditions: i*) DCC, 1-HOBT, DMF; *ii*) HBTU, DIEA, DMF; *iii*) DCC, *N*-hydroxysuccinimide, DMSO

On the other hand, **4a** provided a new strategy via which to synthetize new asymmetric monohydroxyethylene (**43** and **45**) or symmetric (**44** and **46**) peptidomimetics as potential HIV-1 [30] and *C. albicans* aspartic [31] protease inhibitors (Scheme 7).

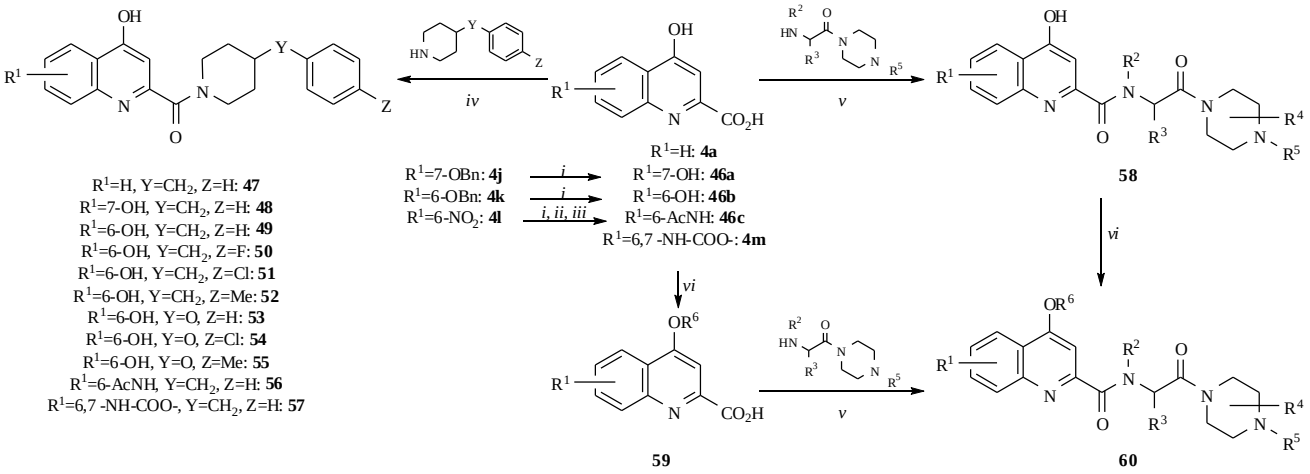


Scheme 7. *Reagents and conditions: i*) DCC, 1-HOBT, DMF

The syntheses of some new amides of KYNA were recently reported. The intensified interest in these types of compounds is demonstrated by the number of patents that have appeared in this field. New KYNA amides containing a substituted piperidine amide moiety at C-2 (**47-57**) have been prepared through coupling of the corresponding KYNA derivatives with the amines, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) serving as coupling agent [12,13].

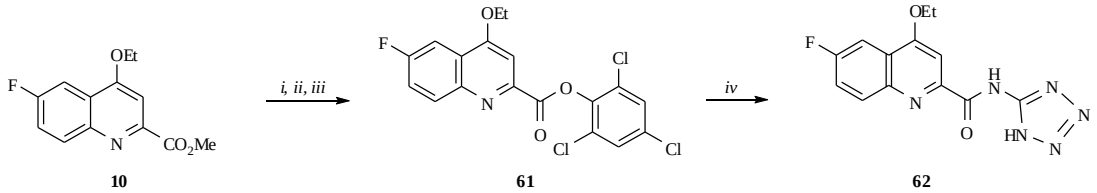
A new series of KYNA amides bearing a substituted piperazine moiety following the double amide function (**58**, Scheme 8) were succesfully synthetized in DMF in the presence of 1-HOBT and 3-dimethylaminopropyl-3-ethylcarbodiimide (EDCI) [32]. **60**, a 4-substituted analog of **58**, was obtained in two different pathways: transformation of the OH group of KYNA derivatives to OR^6 by the standard Williamson synthesis, followed by coupling with the corresponding amines, or application of the Williamson synthesis to amide **58**, leading to **60** (Scheme 8) [32].

Another subgroup of KYNA amides bearing a water-soluble side-chain in the amide moiety have been synthetized and studied by the present authors [33,34].



Scheme 8. *Reagents and conditions: i*) H_2, 10% Pd/C, MeOH, rt; *ii*) Ac_2O, reflux; *iii*) NaOH, MeOH, H_2O, rt, then HCl; *iv*) 1-HOBT, EDCI; DMF; *v*) HBTU, Et_3N, DMF; *vi*) R^6X, Cs_2CO_3, DMF

In order to obtain the carbonylaminotetrazole derivative **62**, an activated 2,4,6-trichlorophenyl ester (**61**) was first isolated, which was then reacted with 5-aminotetrazole to yield **62** (Scheme 9) [14].

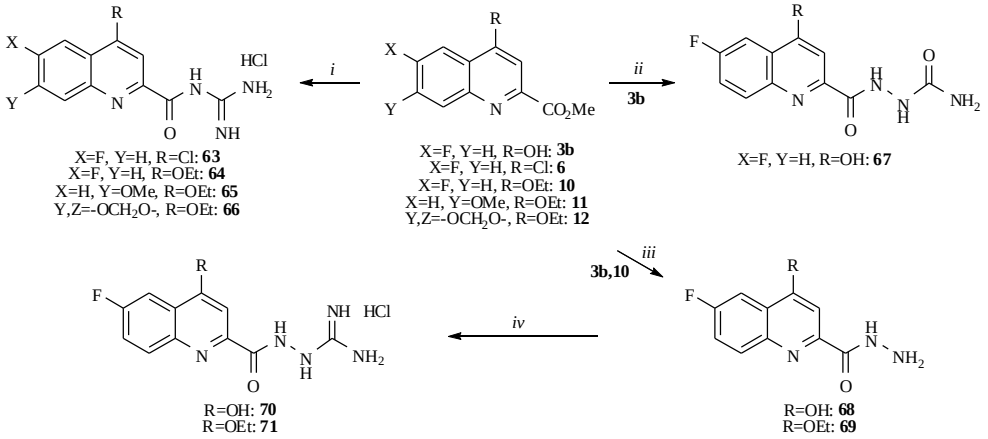


Scheme 9. *Reagents and conditions: i*) NaOH/MeOH, reflux, 2h; *ii*) $SOCl_2$, rt, overnight; *iii*) 2,4,6-trichlorophenol, pyridine, toluene, 3h; *iv*) aminotetrazole monohydrate, Et_3N, acetone, reflux

The preparation and transformation of a hydrazide-type moiety in the side-chain led to new KYNA derivatives. When KYNA methyl esters **3b**, **6**, **10-12** were treated with guanidine in DMF, **63-66** were formed (Scheme 10) [14].

Following the reaction of 6-F-KYNA methyl ester (**3b**) with semicarbazide, **67** (Scheme 10) was isolated [14].

Hydrazide derivatives **68** and **69** were prepared from **3b** and **10** by treatment with hydrazine hydrate, and were subsequently guanylated with 1*H*-pyrazole-1-carboxamide to give **70** and **71** (Scheme 10) [14,15].



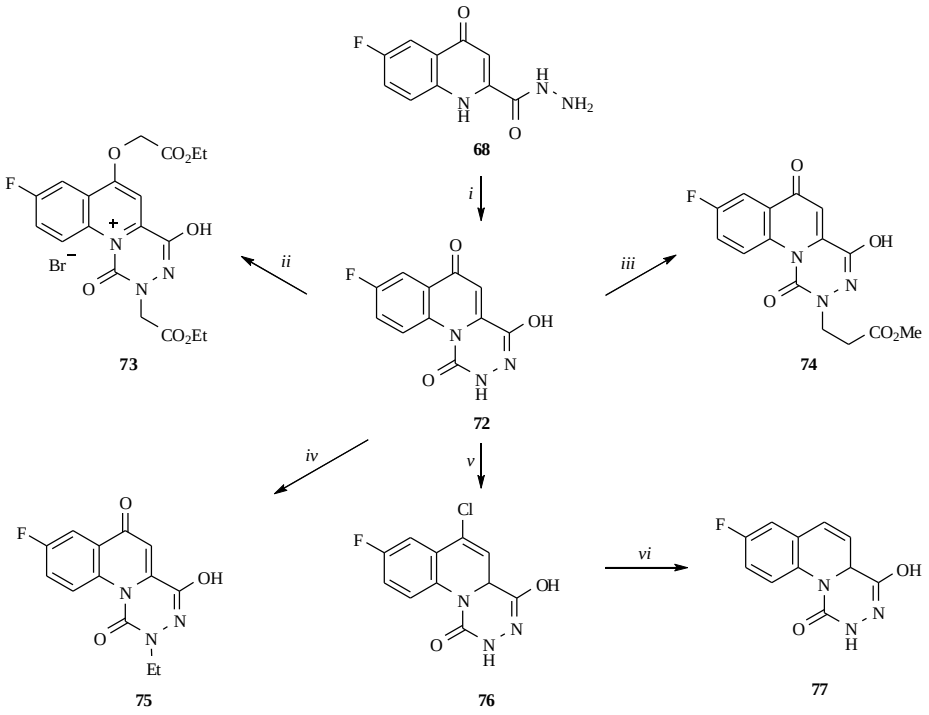
Scheme 10. *Reagents and conditions: i*) guanidine, DMF, rt, then EtOH/HCl or HCl aq. (10%); *ii*) semicarbazide, DMF/dioxane; *iii*) NH_2NH_2, 1.5 H_2O, reflux, 2 h; *iv*) 1*H*-pyrazole-1-carboxamide hydrochloride, DMF, 100 °C, 3 h

The ring closure of **68** with triphosgene led to 8-fluoro-4-hydroxy-1*H*-[1,2,4]triazino[4,5-*a*]quinoline-1,6(2H)-dione (**72**) [15]. Alkylation of **72** with 2 equivalents of K_2CO_3 and ethyl bromoacetate resulted in double alkylation (Scheme 11, **73**) without any trace of monoalkylated compound.

In contrast, a Michael addition with methyl acrylate in the presence of Et_3N led to the selective introduction of a methyl propanoate group onto N-2 (Scheme 11, **74**) [15].

Regioselective alkylation on N-2 occurred upon reaction with EtI in the presence of NaH as base (Scheme 11, **75**) [15].

As a further transformation, [1,2,4]triazino compound **72**, was chlorinated with $POCl_3$, yielding **76**. In order to ascertain the position of the Cl substituent, **76** was subsequently reduced by catalytic hydrogenation under pressure, leading to **77** (Scheme 11) [15].



Scheme 11. *Reagents and conditions: i*) triphosgene, Py, CH_2Cl_2; *ii*) ethyl bromoacetate, K_2CO_3, DMF, 50 °C; *iii*) methyl acrylate, Et_3N, DMF, 100 °C; *iv*) EtI, NaH, DMF; *v*) $POCl_3$, reflux; *vi*) Pd-C (10%), H_2, DMF

3. Pharmacological activities of KYNA derivatives

It is well known that KYNA has neuroactive properties (reviewed in [3,5,9]). It is synthesized from L-KYN in reactions mediated by mitochondrial aspartate aminotransferase [35] and kynurenine aminotransferases (KATs): KAT-I, KAT-II [36-38] and KAT-III [39] in astrocytes, glial cells [40] and neurons [41]. The largest amount of KYNA is produced by KAT II in the rat [36]. Less information is available concerning the role of KYNA in the periphery than in the central nervous system (CNS). Recent data indicate that major amounts of serum KYNA are produced and liberated from the vascular endothelium [42,43].

KYNA displays a broad spectrum of antagonistic properties. In the human cerebrospinal fluid (CSF), the concentration of KYNA is in the nanomolar range [44]. It can act competitively, primarily at the strychnine-insensitive glycine-binding site of the NMDA receptors [45-47]. At higher concentration, it can competitively inhibit the AMPA and kainate receptors, too [45,46,48,49]. Interestingly, Prescott et al. [50] reported that KYNA in nanomolar to micromolar concentrations is able to facilitate the AMPA receptor responses. However, the concentration ranges are controversial, because it was recently demonstrated that KYNA in micromolar concentrations still has a neuroinhibitory effect, whereas in nanomolar concentrations it behaves as a facilitator [1]. Moreover, KYNA non-competitively blocks the - 7-nACh receptors [51], activation of which at the presynaptic site takes part in the regulation of glutamate release [52], and hence in both glutamatergic and cholinergic neurotransmission. Furthermore, in nanomolar concentrations, acting on - 7-nACh receptors, it can reduce the extracellular level of dopamine in the rat

striatum. This fact suggests that even modest increases in the brain KYNA level can contribute to modulation of the dopaminergic transmission [53].

GPR35, which is a G-protein-coupled receptor in immune and gastrointestinal tissues, was also found to be selectively activated by KYNA, but not by other kynurenines [54].

In view of its pharmacological properties, an increase of the level of KYNA in the CNS seems to be a potential therapeutic strategy. However, KYNA can cross the BBB only poorly [55], whereas L-KYN is transported across much more easily by a neutral amino acid carrier [55]. This is often utilized in animal models, where L-KYN is administered in monotherapy or in combination with probenecid to elevate the KYNA concentration in the CNS.

However, an excessive elevation of the KYNA level could have adverse effects. The intracerebroventricular injection of KYNA to rats resulted in reduced exploratory activity, ataxia, stereotypy, sleeping and respiratory depression (mainly in higher doses), while there was only a slight effect on the learning ability [56]. The precursor L-KYN did not exert any marked effect when applied in equimolar doses. KYNA has been shown to be an anxiolytic compound [57], while L-KYN is anxiogenic [58].

In consequence of its elevated CNS level, KYNA behaves as an endogenous neuroprotective agent and it is able to prevent neuronal loss following excitotoxic, ischemia-induced, and infectious neuronal injuries [10,11,59]. The changed level of KYNA in the CNS correlates with several disorders (summarized in Table 2).

KYNA and its derivatives under pathological conditions

Many reviews have been published on the relationships between oxidative stress, free radicals, neurological diseases and the metabolites of the kynurenine pathway [60,61]. Stone [23] has also surveyed the therapeutic potential of the neuroactive kynurenines and their analogs. Thus, the aim of this chapter is to give a concise overview of the pharmacological activity of the base molecule KYNA, concentrating mainly on the CNS, and to consider the possible therapeutic significance of the novel KYNA derivatives in the past decade.

Table 2. KYNA concentrations under pathological conditions

Disorders	Possible changes in level of KYNA	References
Ischemia	No alteration in KYNA level or KAT activities	[11]
Alzheimer's disease	A slightly elevated KYNA concentration in the putamen, caudate nucleus and hippocampus in post-mortem brains	[62]
Parkinson's disease	Lowered KYNA concentrations in the frontal cortex, putamen and substantia nigra pars compacta	[63]
Huntington's disease	A KYNA level deficiency and decreased activities of KAT-I and KAT-II in the striatum	[64,65]
	An imbalance between the levels of neuroactive kynurenines in the striatum in different stages of this disease	[66]
Multiple sclerosis	A lower KYNA level in the CSF in patients with stable relapsing-remitting multiple sclerosis	[67]
	An increased KYNA content during relapse of the disease	[68]
Epilepsy	A decreased KYNA level in the CSF of subjects with West syndrome	[69]

	No correlation between the development of complex partial seizures and the CSF KYNA concentration	[70]
Schizophrenia	An elevated KYNA level in the prefrontal cortex	[71]
	An increased concentration of KYNA in the CSF	[72]
Diabetes mellitus	Elevated KYNA levels in senile nuclear human cataracts	[73]

Ischemia

The excitotoxicity caused by overexcitation of the glutamate receptors is known to play an important role in brain ischemia [74]. It was recently demonstrated that KYN pretreatment preserves the hippocampal pyramidal cells in the CA1 area after global ischemia in gerbils and reduces the focal infarction in mice [10]. In very high doses (400-1800 mg/kg b.w.), KYNA can cross the BBB and induce an increase in the KYNA level of the CNS. This increased concentration exerts a neuroprotective effect after bilateral carotid occlusion in transient forebrain ischemia in gerbils [75]. Peripherally administered L-KYN, which causes an increased KYNA level in the brain, increases the normal and the unilateral carotid artery occlusion-induced ischemic corticocerebral blood flow in conscious rabbits [76]. It has been reported that L-KYN administration can mitigate the delayed cortical neuronal damage in an animal model of transient global ischemia [77]. In the four-vessel occlusion model in the rat, combined treatment with L-KYN and probenecid proved able to rescue the Schaffer collateral-CA1 synapses from impaired long term potentiation induction after transient global ischemia [78]. Taken together, these findings suggest that an

increased concentration of KYNA has an important modulating role in the neuronal cell damage after ischemic conditions.

Neurodegenerative disorders *(Alzheimer's disease, Parkinson's disease, Huntington's disease and multiple sclerosis)*

The ratio TRP/L-KYN in the plasma is elevated in Alzheimer's disease [79], as an indication of the importance of the kynurenine pathway in this disorder. An increased KYNA level has been demonstrated in the putamen and caudate nucleus of Alzheimer's disease patients, accompanied by significant increases in KAT I activity in both nuclei [62].

The ratio TRP/L-KYN is decreased in both the serum and the CSF of patients with Parkinson's disease as compared with normal controls [80]. Lowered KYNA concentrations have been measured in the frontal cortex, putamen and substantia nigra pars compacta of such patients [63]. The bilateral injection of 5,7-di-Cl-KYNA (**4f**) into the rostral striatum inhibited the haloperidol-induced muscle rigidity in the model of parkinsonian-like muscle rigidity in the rat [81].

A decreased KYNA level and a reduced activity of KAT have been found in the striatum of Huntington's disease patients [64,65]; these alterations are suggested to be related to the development of the condition. An imbalance of this disease has also been demonstrated between the levels of neuroactive kynurenines in the striatum in the different stages [66], with a slight striatal KYNA elevation in the early stages and a decreased or normal concentration in the advanced stages. The ratio TRP/L-KYN is lower in subjects with Huntington's disease than in healthy controls. The higher levels of L-KYN in such patients do not result in any differences in KYNA levels in an advanced stage, providing no

evidence of endogenous neuroprotective compensation by KYNA [82]. Intrastriatal injections of QUIN cause significantly larger lesions in mice with a targeted deletion of KAT II than in wild-type animals, which indicates that KYNA controls the vulnerability of the striatal neurons to QUIN [83]. These data demonstrate abnormalities in the kynurenine pathway which may play a role in the pathomechanism of Huntington's disease.

Lower KYNA levels have been found in the CSF in patients with stable relapsing-remitting multiple sclerosis as compared with healthy controls [67]. Recent findings indicate an increased KYNA content during the relapse of the disease [68], when immune cells are presumed to be abundant in the CNS, catabolizing TRP to produce L-KYN metabolites. In the periphery, the erythrocytes of multiple sclerosis patients display increased levels of KAT I and II [84], suggesting the presence of general activation mechanisms in the disease affecting the CNS. However, stable multiple sclerosis patients did not exhibit significant changes in the plasma KYNA concentration during 24 h of monitoring [85].

Epilepsy

Combined L-KYN and probenecid administration, which elevates the CNS KYNA level, attenuates pentylenetetrazole-induced seizures in the rat brain [86,87]. In WAG/Rij rats, which are a genetic model of absence epilepsy, the KYNA levels are significantly lower in the frontal lobe [88], suggesting its involvement in the higher excitability. Scharfman et al. [89] showed that, following the bath-application of L-KYN, *de novo* synthetized KYNA is much more effective than normally-synthetized KYNA against low extracellular magnesium-induced spontaneous epileptiform activity. *In vitro* studies have

indicated that many antiepileptic drugs enhance KYNA production in rat cortical slices, pointing to an alternative target of such medication [90].

Magnesium-induced seizures were successfully attenuated by 7-Cl-KYNA (**4i**) applied *in vitro* to hippocampal and entorhinal cortical slices [91]. In pilocarpine-induced epilepsy, an enhanced 7-Cl-KYNA concentration and a higher extracellular KYNA level were detected by an *in vivo* microdialysis technique. Furthermore, *in vitro* studies (tissue slices) have revealed an enhanced synthesis of both KYNA and 7-Cl-KYNA (**4i)** in the same area. This may suggest that the brain can synthesize the NMDA receptor antagonist under pathological conditions [92]. Battaglia et al. [93] reported that D-glucose or D-galactose esters of 7-Cl-KYNA (**33**,**35** and **37**) readily cross the BBB and are converted to 7-Cl-KYNA or KYNA by astrocytes and neurons in the brain. These data indicate that the glucose-conjugate may have a role in transporting drugs across the BBB. At the same dose, glucose-conjugated esters exhibited neuroprotective effects against NMDA toxicity and epileptic seizures.

In an *in vitro* study, mice were pretreated with 7-Cl-KYNA (**4i)** or one of its esters (**33**,**35** and **37**) before NMDA injection. The NMDA-induced seizures were not mitigated in the animals injected with 7-Cl-KYNA (**4i**) and ester **35**. It was hypothesized that **35** could not penetrate successfully through the BBB. Esters **33** and **37**, in the same dose, did attenuate NMDA-induced seizures in mice [19]. Both *in vivo* and *in vitro* experiments suggest that **37** may be of potential in the therapy of epilepsy.

Systemic administration of a newly synthetized compound, GLUC–KYNA (**38**), which readily penetrates the BBB, leads to an increased KYNA concentration in the CNS [94]. Intracerebroventricularly administered GLUC–KYNA (**38**) induces the same behavioral effects in rats as those of KYNA [26]. In

electrophysiological experiments, intravenously injected GLUC–KYNA (**38**) decreased the amplitudes of the barrel field potentials evoked by contralateral whisker pad stimulation. GLUC–KYNA (**38**) in a lower dose significantly decreased the amplitude of the cortical responses relative to those of KYNA (given intravenously in the same dose;) [26]. In *in vivo* experiments, systemically administered GLUC–KYNA (**38**), but not pure KYNA, mitigated the evoked activity in the hippocampal CA1 area [94].

Administration of 5,7-di-Cl-KYNA (**4f**) into the hilus of the gyrus dentate prevented both the behavioral and the electrographic manifestations of status epilepticus [95]. In animal experiments 5,7-di-Cl-KYNA (**4f**) suppressed both glutamate- and glycine-induced seizures [96]. When the neuroprotective effects of 4-*trans*-2-carboxy-5,7-dichloro-4-phenylaminocarbonylamino-1,2,3,4-tetrahydroquinoline (L-689,560), 5,7-di-Cl-KYNA (**4f**) and 7-Cl-KYNA (**4i**) were tested on a primary culture of rat cortical neurons, all afforded protection against glutamate-induced excitotoxicity [97].

Recent findings demonstrated that 9 of 12 4-urea-5,7-di-Cl-KYNA derivatives tested exerted anticonvulsant activity in mice in maximal electroshock, subcutaneous pentylenetetrazole and threshold tonic extension tests [25]. The methyl ester of diphenylureido-di-Cl-KYNA appeared to be protective against audiogenic seizures in DBA/2 mice [18].

While epilepsy seems to be the most widely examined pathological condition in relation to kynurenine pathway, several derivatives of KYNA have been tested in experimental models in the last decade, too, giving the possibility of studying the structure-activity relationship (Table 3).

Table 3. The pharmacological activity of the systemically administered KYNA derivatives in the experimental models of epilepsy

Derivatives	Pharmacological activity	References
4i **35**	The NMDA-induced seizures were not mitigated.	
33 **37**	The NMDA-induced seizures were attenuated (probably due to better BBB penetration).	[19]
38	A greater decrease in the amplitude of the cortical responses relative to KYNA.	[26]
38	Mitigated the evoked activity in the hippocampal CA1 area (but not KYNA).	[94]

Migraine

Migraine has a complex pathophysiology in which both central and peripheral components of the trigeminal pain pathway probably play significant parts. The role of glutamate is strongly indicated at different sites of migraine pathogenesis [98]. In one animal model of trigeminovascular activation after electrical stimulation of the Gasserian ganglion, decreased KAT expression was observed among the dural Schwann cells [99]. In combination with probenecid, L-KYN is able to reduce *c-fos* activation in the caudal trigeminal nucleus in the above model [100]. The periaqueductal grey cell activity involved in nociception can also be altered by the administration of KYNA [101]. These findings suggest that kynurenines can influence the brain stem structures involved in the pathogenesis of migraine.

In one migraine model, SZR72, is a novel synthetic KYNA analog, significantly attenuated the nitroglycerine-induced increase in the number of *c-fos*-immunoreactive neurons in the caudal trigeminal nucleus in the rat [33].

Such findings suggest the influence of the kynurenine pathway on a wide range of neurons involved in the process.

Pain

It has been reported that NMDA receptors play an important part in spinal nociceptive transmission [102-104]. The peripheral role of the metabolites of the kynurenine pathway in pain processing was postulated by Simone et al. [105]. Iontophoric administration of KYNA to the spinal cord reduces the nociceptor response of the spinal cord neurons after cutaneous and muscular noxious stimulation [106].

Microinjections of KYNA or 7-Cl-KYNA (**4i**) significantly inhibit the acquisition of formalin-induced conditioned place avoidance (CPA) without reducing electric foot-shock CPA and acute formalin-induced nociceptive behavior in rats [107,108]. *In vitro* electrophysiological examinations demonstrated that NMDA-evoked currents, but not formalin-induced nociceptive behavior, were successfully inhibited by 7-Cl-KYNA (**4i**) in rostral anterior cingulate cortex slices [108].

Animal investigations suggest that 5,7-di-Cl-KYNA (**4f**) can inhibit intraplantar formalin-induced nocifensive behavior and tissue injury in a dose-dependent manner without any side-effects [109]. At a dose of 46.4 mg/kg bodyweight (i.p.), it reduced the pain score by approximately 88% as compared with the control group. It also inhibited tactile allodynia in a neuropathic pain model in the rat.

Diphenylureido-di-Cl-KYNA (**21**) was synthetized with a view to achieving a combination of the effects of carbamazepine and KYNA. Diphenylureido-di-Cl-

KYNA (**21**) and its derivatives (**22** and **23**), which easily cross the BBB, inhibit both NMDA channels at the strychnine-insensitive glycine binding site and sodium channels. Diphenylureido-di-Cl-KYNA (**21**) and its methyl ester diminished glutamate-induced excitotoxic cell death in primary cultures of cerebellar granule cells. The ethyl ester of diphenylureido-di-Cl-KYNA (**21**) attenuated tactile and thermal hyperalgesia in animals following spinal nerve ligation, and in C57BL/6 mice exerted an anxiolytic effect in the elevated-plus maze test [18]. Taken together, these observations point to the involvement of metabolites of the kynurenine pathway at various sites of nociception.

Schizophrenia

An elevated KYNA level has been detected in the prefrontal cortex of schizophrenic patients [71], and the concentration of KYNA has also been found to be increased in the CSF of such patients [72]. The enhanced brain KYNA level gives rise to electrophysiological effects [110-112], similarly as observed after NMDA receptor administration [113]. These findings are in good accordance with the glutamatergic hypofunction seen in schizophrenia [114], which can manifest in an elevated firing activity of midbrain dopaminergic neurons via inhibition of the tonic GABAergic input, which normally dampens the neuronal activity [111].

The neurobiological background of the sensory gating deficits in schizophrenia is not fully understood. An elevated KYNA level in rats has been reported to be associated with an altered responsiveness to auditory stimuli, caused by an interaction with the - 7-nACh receptor [115].

Retinal damage

KYNA and KAT activities have been observed in mammalian eyes [116]. In the retina of DBA/2J mice, the impairment of KYNA biosynthesis that is demonstrated may be one of the mechanisms of retinal degeneration related to ocular hypertension [117]. A dysfunction of the glutamatergic system has been described in various pathological conditions in the retina, e.g. retinal damage such as glaucoma [118] and optic neuropathy [119]. Interestingly, KYNA level changes have been observed in both the rat and the chicken retina during ontogeny [120].

In vivo experimental data have revealed that 5,7-di-Cl-KYNA (**4f**) inhibits NMDA-induced cell loss in the retinal ganglion cells, thinning of the inner plexiform layer, and the occurrence of DNA fragmentation revealed by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL). These findings suggest that glycine site stimulation by endogenous ligands is obligatory for NMDA-induced retinal damage [121].

Diabetes mellitus

Changed levels of kynurenines may play an important role in diabetic cataract [122]. Kynurenines and their glycoside derivatives in the ocular lens absorb ultraviolet radiation and thereby possibly help protect the retina from ultraviolet light. Elevated KYNA levels have been reported in senile nuclear human cataracts and in cataractous lenses of rats with experimentally induced diabetes [73].

In rats with diabetes mellitus of type II, the glucose concentrations have been measured in the plasma. Variation of the different groups at position 4 (**5**,

6, **10-12**), did not cause an increase in the hypoglycemic activity [14]. A carbonylguanidine group at position 2 (**66**) enhanced hypoglycemic activity as compared with one of position 3 [14].

Hypertension

The rostral ventrolateral medulla plays an important role in regulation of the arterial blood pressure. Injection of KYNA into this organ in spontaneously hypertensive rats decreased the mean arterial pressure, whereas this effect was not observed in Wistar-Kyoto rats [123].

3. Conclusions and outlook

The evidence indicates that KYNA and its derivatives play crucial roles in maintaining the normal brain function.

Animal studies have demonstrated that elevated levels of KYNA or KYNA derivatives in the brain may reduce the overactivation of excitatory amino acid receptors and thereby modify or arrest the progression of various disorders. In view of structure-activity relationship, there are only few available data reflecting the advances in systematic drug design in the past decade. While the earlier attempts mainly focused on enhancing the potency (e.g. substitution with halogen atoms), the recent activities particularly deal with the conversion of the 2-carboxylic function to the pharmacologically interesting derivatives of KYNA. This can offer a novel therapeutic opportunity, and the development of these powerful new compounds promises to be the key to brain neuroprotection.

Acknowledgments

This work was supported by grants ETT-215/2006, NKTH 08/2004 and OTKA K75628.

References

1. Rozsa, E.; Robotka, H.; Vecsei, L.; Toldi, J. The Janus-face kynurenic acid. *J Neural Transm.,* **2008** , 115, 1087-1091.

2. Stone, T.W. Kynurenic acid antagonists and kynurenine pathway inhibitors. *Expert. Opin. Investig. Drugs,* **2001** , 10, 633-645.

3. Nemeth, H.; Toldi, J.; Vecsei, L. Role of kynurenines in the central and peripheral nervous systems. *Curr. Neurovasc. Res.,* **2005** , 2, 249-260.

4. Nemeth, H.; Toldi, J.; Vecsei, L. Kynurenines, Parkinson's disease and other neurodegenerative disorders: preclinical and clinical studies. *J Neural Transm. Suppl,* **2006** , 285-304.

5. Sas, K.; Robotka, H.; Toldi, J.; Vecsei, L. Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. *J Neurol. Sci.,* **2007** , 257, 221-239.

6. Rajda, C.; Bergquist, J.; Vecsei, L. Kynurenines, redox disturbances and neurodegeneration in multiple sclerosis. *J Neural Transm. Suppl,* **2007** , 323-329.

7. Giles, G.I.; Collins, C.A.; Stone, T.W.; Jacob, C. Electrochemical and in vitro evaluation of the redox-properties of kynurenine species. *Biochem. Biophys. Res. Commun.,* **2003** , 300, 719-724.

8. Nemeth, H.; Robotka, H.; Toldi, J.; Vecsei, L. Kynurenins in the central nervous system: recent developments. *Central Nervous System Agents in Medicinal Chemistry,* **2007** , 7, 45-46.

9. Robotka, H.; Toldi, J.; Vecsei, L. L-kynurenine: metabolism and mechanism of neuroprotection. *Future Neurol,* **2008** , 3, 169-188.

10. Gigler, G.; Szenasi, G.; Simo, A.; Levay, G.; Harsing, L.G., Jr.; Sas, K.; Vecsei, L.; Toldi, J. Neuroprotective effect of L-kynurenine sulfate administered before focal cerebral ischemia in mice and global cerebral ischemia in gerbils. *Eur. J Pharmacol.,* **2007** , 564, 116-122.

11. Luchowska, E.; Luchowski, P.; Sarnowska, A.; Wielosz, M.; Turski, W.A.; Urbanska, E.M. Endogenous level of kynurenic acid and activities of kynurenine aminotransferases following transient global ischemia in the gerbil hippocampus. *Pol. J Pharmacol.,* **2003** , 55, 443-447.

12. Borza, I.; Horvath, C.; Farkas, S.; Nagy, J.; Kolok, S. Kynurenic acid amide derivatives as NR2B receptor antagonists. *WO2006010967,* **2006** ,

13. Borza, I.; Kolok, S.; Galgoczy, K.; Gere, A.; Horvath, C.; Farkas, S.; Greiner, I.; Domany, G. Kynurenic acid amides as novel NR2B selective NMDA receptor antagonists. *Bioorg. Med. Chem. Lett.,* **2007** , 17, 406-409.

14. Edmont, D.; Rocher, R.; Plisson, C.; Chenault, J. Synthesis and evaluation of quinoline carboxyguanidines as antidiabetic agents. *Bioorg. Med. Chem. Lett.*, **2000**, 10, 1831-1834.

15. Edmont, D. Chenault, J. 8-Fluoro-4-hydroxy-1*H*-[1,2,4]triazino[4,5-*a*]quinoline-1,6(2*H*)-dione: synthesis and reactivity. *J. Heterocyclic Chem.*, **2003**, 40, 789-793.

16. Muirhead, K.M. Botting, N.P. The synthesis of 5-fluorokynurenine and 6-fluorokynurenic acid as metabolic probes. *Arkivoc*, **2002**, iii, 37-45.

17. Harrison, B.L.; Baron, B.M.; Cousino, D.M.; McDonald, I.A. 4-[(Carboxymethyl)oxy]- and 4-[(carboxymethyl)amino]-5,7-dichloroquinoline-2-carboxylic acid: new antagonists of the strychnine-insensitive glycine binding site on the N-methyl-D-aspartate receptor complex. *J Med. Chem.*, **1990**, 33, 3130-3132.

18. Tabakoff, B. Method for treating chronic pain. *WO2007044682*, **2007**

19. Bonina, F.P.; Arenare, L.; Ippolito, R.; Boatto, G.; Battaglia, G.; Bruno, V.; de, C.P. Synthesis, pharmacokinetics and anticonvulsant activity of 7-chlorokynurenic acid prodrugs. *Int. J Pharm.*, **2000**, 202, 79-88.

20. Kato, Y.; Okada, S.; Tomimoto, K.; Mase, T. A facile bromination of hydroxyheteroarenes. *Tetrahedron Lett.*, **2001**, 42, 4849-4851.

21. Manfredini, S.; Pavan, B.; Vertuani, S.; Scaglianti, M.; Compagnone, D.; Biondi, C.; Scatturin, A.; Tanganelli, S.; Ferraro, L.; Prasad, P.; Dalpiaz, A. Design, synthesis and activity of ascorbic acid prodrugs of nipecotic, kynurenic and diclophenamic acids, liable to increase neurotropic activity. *J Med. Chem.*, **2002**, 45, 559-562.

22. Manfredini, S.; Vertuani, S.; Pavan, B.; Vitali, F.; Scaglianti, M.; Bortolotti, F.; Biondi, C.; Scatturin, A.; Prasad, P.; Dalpiaz, A. Design, synthesis and in vitro evaluation on HRPE cells of ascorbic and 6-bromoascorbic acid conjugates with neuroactive molecules. *Bioorg. Med. Chem.*, **2004**, 12, 5453-5463.

23. Stone, T.W. Development and therapeutic potential of kynurenic acid and kynurenine derivatives for neuroprotection. *Trends Pharmacol. Sci.*, **2000**, 21, 149-154.

24. Stone, T.W. Inhibitors of the kynurenine pathway. *Eur. J Med. Chem.*, **2000**, 35, 179-186.

25. Nichols, A.C. Yielding, K.L. Anticonvulsant activity of 4-urea-5,7-dichlorokynurenic acid derivatives that are antagonists at the NMDA-associated glycine binding site. *Mol. Chem. Neuropathol.*, **1998**, 35, 1-12.

26. Fuvesi, J.; Somlai, C.; Nemeth, H.; Varga, H.; Kis, Z.; Farkas, T.; Karoly, N.; Dobszay, M.; Penke, Z.; Penke, B.; Vecsei, L.; Toldi, J. Comparative study on the effects of kynurenic acid and glucosamine-kynurenic acid. *Pharmacol. Biochem. Behav.*, **2004**, 77, 95-102.

27. Zhang, L.; Sun, F.; Li, Y.; Sun, X.; Liu, X.; Huang, Y.; Zhang, L.H.; Ye, X.S.; Xiao, J. Rapid synthesis of iminosugar derivatives for cell-based in situ screening: discovery of "hit" compounds with anticancer activity. *ChemMedChem.*, **2007** , 2, 1594-1597.

28. Likhosherstov, L.M.; Novikova, O.S.; Zheltova, A.O.; Shibaev, V.N. *N*-Glycyl-b-glycopyranosyl-amines, derivatives of mono- and disaxxharides, and their use for the preparation of carboxylic acid glycoconjugates. *Russ. Chem. Bull. , Int. Ed.*, **2000** , 49, 1454-1459.

29. Liu, J.; Numa, M.M.; Liu, H.; Huang, S.J.; Sears, P.; Shikhman, A.R.; Wong, C.H. Synthesis and high-throughput screening of N-acetyl-beta-hexosaminidase inhibitor libraries targeting osteoarthritis. *J. Org. Chem.*, **2004** , 69, 6273-6283.

30. Brik, A.; Lin, Y.C.; Elder, J.; Wong, C.H. A quick diversity-oriented amide-forming reaction to optimize P-subsite residues of HIV protease inhibitors. *Chem. Biol.*, **2002** , 9, 891-896.

31. Tossi, A.; Benedetti, F.; Norbedo, S.; Skrbec, D.; Berti, F.; Romeo, D. Small hydroxyethylene-based peptidomimetics inhibiting both HIV-1 and C. albicans aspartic proteases. *Bioorg. Med. Chem.*, **2003** , 11, 4719-4727.

32. Bryant, J.; Buckman, B.; Islam, I.; Mohan, R.; Morrissey, M.; Wei, G.P.; Xu, W. 2-Aminocarbonyl-quinoline compounds as platelet adenosine diphosphate receptor antagonists. *WO2004052366*, **2004** ,

33. Knyihar-Csillik, E.; Mihaly, A.; Krisztin-Peva, B.; Robotka, H.; Szatmari, I.; Fulop, F.; Toldi, J.; Csillik, B.; Vecsei, L. The kynurenate analog SZR-72 prevents the nitroglycerol-induced increase of c-fos immunoreactivity in the rat caudal trigeminal nucleus: comparative studies of the effects of SZR-72 and kynurenic acid. *Neurosci. Res.*, **2008** , 61, 429-432.

34. Vecsei, L.; Boros, M.; Kaszaki, J.; Fulop, F.; Toldi, J.; Szatmari, I. Use of kynurenic acid and derivatives thereof in the treatment of conditions of the gastrointestinal tract accompanied by hypermotility and inflammation or gout or multiple sclerosis. *WO2008087461*, **2008** ,

35. Guidetti, P.; Amori, L.; Sapko, M.T.; Okuno, E.; Schwarcz, R. Mitochondrial aspartate aminotransferase: a third kynurenate-producing enzyme in the mammalian brain. *J. Neurochem.*, **2007** , 102, 103-111.

36. Guidetti, P.; Okuno, E.; Schwarcz, R. Characterization of rat brain kynurenine aminotransferases I and II. *J. Neurosci. Res.*, **1997** , 50, 457-465.

37. Okuno, E.; Nakamura, M.; Schwarcz, R. Two kynurenine aminotransferases in human brain. *Brain Res.*, **1991** , 542, 307-312.

38. Schmidt, W.; Guidetti, P.; Okuno, E.; Schwarcz, R. Characterization of human brain kynurenine aminotransferases using [3H]kynurenine as a substrate. *Neuroscience*, **1993** , 55, 177-184.

39. Yu, P.; Li, Z.; Zhang, L.; Tagle, D.A.; Cai, T. Characterization of kynurenine aminotransferase III, a novel member of a phylogenetically conserved KAT family. *Gene*, **2006** , 365, 111-118.

40. Ceresoli-Borroni, G.; Guidetti, P.; Schwarcz, R. Acute and chronic changes in kynurenate formation following an intrastriatal quinolinate injection in rats. *J. Neural Transm.*, **1999** , 106, 229-242.

41. Guillemin, G.J.; Cullen, K.M.; Lim, C.K.; Smythe, G.A.; Garner, B.; Kapoor, V.; Takikawa, O.; Brew, B.J. Characterization of the kynurenine pathway in human neurons. *J. Neurosci.*, **2007** , 27, 12884-12892.

42. Stazka, J.; Luchowski, P.; Wielosz, M.; Kleinrok, Z.; Urbanska, E.M. Endothelium-dependent production and liberation of kynurenic acid by rat aortic rings exposed to L-kynurenine. *Eur. J. Pharmacol.*, **2002** , 448, 133-137.

43. Stazka, J.; Luchowski, P.; Urbanska, E.M. Homocysteine, a risk factor for atherosclerosis, biphasically changes the endothelial production of kynurenic acid. *Eur. J. Pharmacol.*, **2005** , 517, 217-223.

44. Erhardt, S.; Schwieler, L.; Emanuelsson, C.; Geyer, M. Endogenous kynurenic acid disrupts prepulse inhibition. *Biol. Psychiatry*, **2004** , 56, 255-260.

45. Birch, P.J.; Grossman, C.J.; Hayes, A.G. Kynurenic acid antagonises responses to NMDA via an action at the strychnine-insensitive glycine receptor. *Eur. J. Pharmacol.*, **1988** , 154, 85-87.

46. 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, 1319-1328.

47. Parsons, C.G.; Danysz, W.; Quack, G.; Hartmann, S.; Lorenz, B.; Wollenburg, C.; Baran, L.; Przegalinski, E.; Kostowski, W.; Krzascik, P.; Chizh, B.; Headley, P.M. Novel systemically active antagonists of the glycine site of the N-methyl-D-aspartate receptor: electrophysiological, biochemical and behavioral characterization. *J. Pharmacol. Exp. Ther.*, **1997** , 283, 1264-1275.

48. Bertolino, M.; Vicini, S.; Costa, E. Kynurenic acid inhibits the activation of kainic and N-methyl-D-aspartic acid-sensitive ionotropic receptors by a different mechanism. *Neuropharmacology*, **1989** , 28, 453-457.

49. Stone, T.W. Connick, J.H. Quinolinic acid and other kynurenines in the central nervous system. *Neuroscience*, **1985** , 15, 597-617.

50. Prescott, C.; Weeks, A.M.; Staley, K.J.; Partin, K.M. Kynurenic acid has a dual action on AMPA receptor responses. *Neurosci. Lett.*, **2006** , 402, 108-112.

51. Hilmas, C.; Pereira, E.F.; Alkondon, M.; Rassoulpour, A.; Schwarcz, R.; Albuquerque, E.X. The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications. *J. Neurosci.*, **2001** , 21, 7463-7473.

52. Marchi, M.; Risso, F.; Viola, C.; Cavazzani, P.; Raiteri, M. Direct evidence that release-stimulating alpha7 nicotinic cholinergic receptors are localized on human and rat brain glutamatergic axon terminals. *J. Neurochem.*, **2002** , 80, 1071-1078.

53. Rassoulpour, A.; Wu, H.Q.; Ferre, S.; Schwarcz, R. Nanomolar concentrations of kynurenic acid reduce extracellular dopamine levels in the striatum. *J. Neurochem.*, **2005** , 93, 762-765.

54. Wang, J.; Simonavicius, N.; Wu, X.; Swaminath, G.; Reagan, J.; Tian, H.; Ling, L. Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. *J. Biol. Chem.*, **2006** , 281, 22021-22028.

55. Fukui, S.; Schwarcz, R.; Rapoport, S.I.; Takada, Y.; Smith, Q.R. Blood-brain barrier transport of kynurenines: implications for brain synthesis and metabolism. *J. Neurochem.*, **1991** , 56, 2007-2017.

56. Vecsei, L. Beal, M.F. Comparative behavioral and pharmacological studies with centrally administered kynurenine and kynurenic acid in rats. *Eur. J. Pharmacol.*, **1991** , 196, 239-246.

57. Schmitt, M.L.; Graeff, F.G.; Carobrez, A.P. Anxiolytic effect of kynurenic acid microinjected into the dorsal periaqueductal gray matter of rats placed in the elevated plus-maze test. *Braz. J. Med. Biol. Res.*, **1990** , 23, 677-679.

58. Vecsei, L. Beal, M.F. Intracerebroventricular injection of kynurenic acid, but not kynurenine, induces ataxia and stereotyped behavior in rats. *Brain Res. Bull.*, **1990** , 25, 623-627.

59. Smith, D.H.; Okiyama, K.; Thomas, M.J.; McIntosh, T.K. Effects of the excitatory amino acid receptor antagonists kynurenate and indole-2-carboxylic acid on behavioral and neurochemical outcome following experimental brain injury. *J. Neurosci.*, **1993** , 13, 5383-5392.

60. Lewen, A.; Matz, P.; Chan, P.H. Free radical pathways in CNS injury. *J. Neurotrauma*, **2000** , 17, 871-890.

61. Guillemin, G.J.; Meininger, V.; Brew, B.J. Implications for the kynurenine pathway and quinolinic acid in amyotrophic lateral sclerosis. *Neurodegener. Dis.*, **2005** , 2, 166-176.

62. Baran, H.; Jellinger, K.; Deecke, L. Kynurenine metabolism in Alzheimer's disease. *J. Neural Transm.*, **1999** , 106, 165-181.

63. Ogawa, T.; Matson, W.R.; Beal, M.F.; Myers, R.H.; Bird, E.D.; Milbury, P.; Saso, S. Kynurenine pathway abnormalities in Parkinson's disease. *Neurology,* **1992** , 42, 1702-1706.

64. Beal, M.F.; Matson, W.R.; Swartz, K.J.; Gamache, P.H.; Bird, E.D. Kynurenine pathway measurements in Huntington's disease striatum: evidence for reduced formation of kynurenic acid. *J. Neurochem.,* **1990** , 55, 1327-1339.

65. Jauch, D.; Urbanska, E.M.; Guidetti, P.; Bird, E.D.; Vonsattel, J.P.; Whetsell, W.O., Jr.; Schwarcz, R. Dysfunction of brain kynurenic acid metabolism in Huntington's disease: focus on kynurenine aminotransferases. *J. Neurol. Sci.,* **1995** , 130, 39-47.

66. Guidetti, P.; Luthi-Carter, R.E.; Augood, S.J.; Schwarcz, R. Neostriatal and cortical quinolinate levels are increased in early grade Huntington's disease. *Neurobiol. Dis.,* **2004** , 17, 455-461.

67. Rejdak, K.; Bartosik-Psujek, H.; Dobosz, B.; Kocki, T.; Grieb, P.; Giovannoni, G.; Turski, W.A.; Stelmasiak, Z. Decreased level of kynurenic acid in cerebrospinal fluid of relapsing-onset multiple sclerosis patients. *Neurosci. Lett.,* **2002** , 331, 63-65.

68. Rejdak, K.; Petzold, A.; Kocki, T.; Kurzepa, J.; Grieb, P.; Turski, W.A.; Stelmasiak, Z. Astrocytic activation in relation to inflammatory markers during clinical exacerbation of relapsing-remitting multiple sclerosis. *J. Neural Transm.,* **2007** , 114, 1011-1015.

69. Yamamoto, H.; Murakami, H.; Horiguchi, K.; Egawa, B. Studies on cerebrospinal fluid kynurenic acid concentrations in epileptic children. *Brain Dev.,* **1995** , 17, 327-329.

70. Heyes, M.P.; Saito, K.; Devinsky, O.; Nadi, N.S. Kynurenine pathway metabolites in cerebrospinal fluid and serum in complex partial seizures. *Epilepsia,* **1994** , 35, 251-257.

71. Schwarcz, R.; Rassoulpour, A.; Wu, H.Q.; Medoff, D.; Tamminga, C.A.; Roberts, R.C. Increased cortical kynurenate content in schizophrenia. *Biol. Psychiatry,* **2001** , 50, 521-530.

72. Erhardt, S.; Schwieler, L.; Nilsson, L.; Linderholm, K.; Engberg, G. The kynurenic acid hypothesis of schizophrenia. *Physiol Behav.,* **2007** , 92, 203-209.

73. Zarnowski, T.; Rejdak, R.; Zielinska-Rzecka, E.; Zrenner, E.; Grieb, P.; Zagorski, Z.; Junemann, A.; Turski, W.A. Elevated concentrations of kynurenic acid, a tryptophan derivative, in dense nuclear cataracts. *Curr. Eye Res.,* **2007** , 32, 27-32.

74. Dohmen, C.; Kumura, E.; Rosner, G.; Heiss, W.D.; Graf, R. Extracellular correlates of glutamate toxicity in short-term cerebral ischemia and reperfusion: a direct in vivo comparison between white and gray matter. *Brain Res.,* **2005** , 1037, 43-51.

75. Salvati, P.; Ukmar, G.; Dho, L.; Rosa, B.; Cini, M.; Marconi, M.; Molinari, A.; Post, C. Brain concentrations of kynurenic acid after a systemic neuroprotective dose in the gerbil model of global ischemia. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **1999** , 23, 741-752.

76. Sas, K.; Csete, K.; Vecsei, L.; Papp, J.G. Effect of systemic administration of L-kynurenine on corticocerebral blood flow under normal and ischemic conditions of the brain in conscious rabbits. *J Cardiovasc. Pharmacol.,* **2003** , 42, 403-409.

77. Robotka, H.; Sas, K.; Agoston, M.; Rozsa, E.; Szenasi, G.; Gigler, G.; Vecsei, L.; Toldi, J. Neuroprotection achieved in the ischaemic rat cortex with L-kynurenine sulphate. *Life Sci.,* **2008** , 82, 915-919.

78. Sas, K.; Robotka, H.; Rozsa, E.; Agoston, M.; Szenasi, G.; Gigler, G.; Marosi, M.; Kis, Z.; Farkas, T.; Vecsei, L.; Toldi, J. Kynurenine diminishes the ischemia-induced histological and electrophysiological deficits in the rat hippocampus. *Neurobiol. Dis.,* **2008** , 32, 302-308.

79. Widner, B.; Leblhuber, F.; Walli, J.; Tilz, G.P.; Demel, U.; Fuchs, D. Tryptophan degradation and immune activation in Alzheimer's disease. *J. Neural Transm.,* **2000** , 107, 343-353.

80. Widner, B.; Leblhuber, F.; Fuchs, D. Increased neopterin production and tryptophan degradation in advanced Parkinson's disease. *J. Neural Transm.,* **2002** , 109, 181-189.

81. Ossowska, K.; Lorenc-Koci, E.; Konieczny, J.; Wolfarth, S. The role of striatal glutamate receptors in models of Parkinson's disease. *Amino. Acids,* **1998** , 14, 11-15.

82. Stoy, N.; Mackay, G.M.; Forrest, C.M.; Christofides, J.; Egerton, M.; Stone, T.W.; Darlington, L.G. Tryptophan metabolism and oxidative stress in patients with Huntington's disease. *J. Neurochem.,* **2005** , 93, 611-623.

83. Sapko, M.T.; Guidetti, P.; Yu, P.; Tagle, D.A.; Pellicciari, R.; Schwarcz, R. Endogenous kynurenate controls the vulnerability of striatal neurons to quinolinate: Implications for Huntington's disease. *Exp. Neurol.,* **2006** , 197, 31-40.

84. Hartai, Z.; Klivenyi, P.; Janaky, T.; Penke, B.; Dux, L.; Vecsei, L. Kynurenine metabolism in multiple sclerosis. *Acta Neurol. Scand.,* **2005** , 112, 93-96.

85. Amirkhani, A.; Rajda, C.; Arvidsson, B.; Bencsik, K.; Boda, K.; Seres, E.; Markides, K.E.; Vecsei, L.; Bergquist, J. Interferon-beta affects the tryptophan metabolism in multiple sclerosis patients. *Eur. J. Neurol.,* **2005** , 12, 625-631.

86. Nemeth, H.; Robotka, H.; Kis, Z.; Rozsa, E.; Janaky, T.; Somlai, C.; Marosi, M.; Farkas, T.; Toldi, J.; Vecsei, L. Kynurenine administered together with probenecid markedly inhibits pentylenetetrazol-induced seizures. An

electrophysiological and behavioural study. *Neuropharmacology,* **2004** , 47, 916-925.

87. Vecsei, L.; Miller, J.; MacGarvey, U.; Beal, M.F. Kynurenine and probenecid inhibit pentylenetetrazol- and NMDLA-induced seizures and increase kynurenic acid concentrations in the brain. *Brain Res. Bull.,* **1992** , 28, 233-238.

88. Kaminski, R.M.; Zielinska, E.; Dekundy, A.; van, L.G.; Turski, W. Deficit of endogenous kynurenic acid in the frontal cortex of rats with a genetic form of absence epilepsy. *Pol. J. Pharmacol.,* **2003** , 55, 741-746.

89. Scharfman, H.E.; Hodgkins, P.S.; Lee, S.C.; Schwarcz, R. Quantitative differences in the effects of de novo produced and exogenous kynurenic acid in rat brain slices. *Neurosci. Lett.,* **1999** , 274, 111-114.

90. Kocki, T.; Wielosz, M.; Turski, W.A.; Urbanska, E.M. Enhancement of brain kynurenic acid production by anticonvulsants--novel mechanism of antiepileptic activity? *Eur. J. Pharmacol.,* **2006** , 541, 147-151.

91. Wu, H.Q.; Lee, S.C.; Scharfman, H.E.; Schwarcz, R. L-4-chlorokynurenine attenuates kainate-induced seizures and lesions in the rat. *Exp. Neurol.,* **2002** , 177, 222-232.

92. Wu, H.Q.; Rassoulpour, A.; Goodman, J.H.; Scharfman, H.E.; Bertram, E.H.; Schwarcz, R. Kynurenate and 7-chlorokynurenate formation in chronically epileptic rats. *Epilepsia,* **2005** , 46, 1010-1016.

93. Battaglia, G.; La, R.M.; Bruno, V.; Arenare, L.; Ippolito, R.; Copani, A.; Bonina, F.; Nicoletti, F. Systemically administered D-glucose conjugates of 7-chlorokynurenic acid are centrally available and exert anticonvulsant activity in rodents. *Brain Res.,* **2000** , 860, 149-156.

94. Robotka, H.; Nemeth, H.; Somlai, C.; Vecsei, L.; Toldi, J. Systemically administered glucosamine-kynurenic acid, but not pure kynurenic acid, is effective in decreasing the evoked activity in area CA1 of the rat hippocampus. *Eur. J. Pharmacol.,* **2005** , 513, 75-80.

95. Mazarati, A.M. Wasterlain, C.G. N-methyl-D-asparate receptor antagonists abolish the maintenance phase of self-sustaining status epilepticus in rat. *Neurosci. Lett.,* **1999** , 265, 187-190.

96. Sierra-Paredes, G.; Senra-Vidal, A.; Sierra-Marcuno, G. Effect of extracellular long-time microperfusion of high concentrations of glutamate and glycine on picrotoxin seizure thresholds in the hippocampus of freely moving rats. *Brain Res.,* **2001** , 888, 19-25.

97. Ohtani, K.; Tanaka, H.; Yoneda, Y.; Yasuda, H.; Ito, A.; Nagata, R.; Nakamura, M. In vitro and in vivo antagonistic activities of SM-31900 for the NMDA receptor glycine-binding site. *Brain Res.,* **2002** , 944, 165-173.

98. Vikelis, M. Mitsikostas, D.D. The role of glutamate and its receptors in migraine. *CNS. Neurol. Disord. Drug Targets.,* **2007** , 6, 251-257.

99. Knyihar-Csillik, E.; Chadaide, Z.; Okuno, E.; Krisztin-Peva, B.; Toldi, J.; Varga, C.; Molnar, A.; Csillik, B.; Vecsei, L. Kynurenine aminotransferase in the supratentorial dura mater of the rat: effect of stimulation of the trigeminal ganglion. *Exp. Neurol.,* **2004** , 186, 242-247.

100. Knyihar-Csillik, E.; Toldi, J.; Mihaly, A.; Krisztin-Peva, B.; Chadaide, Z.; Nemeth, H.; Fenyo, R.; Vecsei, L. Kynurenine in combination with probenecid mitigates the stimulation-induced increase of c-fos immunoreactivity of the rat caudal trigeminal nucleus in an experimental migraine model. *J. Neural Transm.,* **2007** , 114, 417-421.

101. Jiang, M. Behbehani, M.M. Physiological characteristics of the projection pathway from the medial preoptic to the nucleus raphe magnus of the rat and its modulation by the periaqueductal gray. *Pain,* **2001** , 94, 139-147.

102. Hu, J.Y. Zhao, Z.Q. Differential contributions of NMDA and non-NMDA receptors to spinal Fos expression evoked by superficial tissue and muscle inflammation in the rat. *Neuroscience,* **2001** , 106, 823-831.

103. Alvarez-Vega, M.; Baamonde, A.; Gutierrez, M.; Hidalgo, A.; Menendez, L. Intrathecal N-methyl-D-aspartate (NMDA) induces paradoxical analgesia in the tail-flick test in rats. *Pharmacol. Biochem. Behav.,* **2000** , 65, 621-625.

104. Zhang, Y.Q.; Ji, G.C.; Wu, G.C.; Zhao, Z.Q. Excitatory amino acid receptor antagonists and electroacupuncture synergetically inhibit carrageenan-induced behavioral hyperalgesia and spinal fos expression in rats. *Pain,* **2002** , 99, 525-535.

105. Simone, D.A.; Nolano, M.; Johnson, T.; Wendelschafer-Crabb, G.; Kennedy, W.R. Intradermal injection of capsaicin in humans produces degeneration and subsequent reinnervation of epidermal nerve fibers: correlation with sensory function. *J. Neurosci.,* **1998** , 18, 8947-8959.

106. Song, X.J. Zhao, Z.Q. Differential effects of NMDA and non-NMDA receptor antagonists on spinal cutaneous vs muscular nociception in the cat. *Neuroreport,* **1993** , 4, 17-20.

107. Johansen, J.P. Fields, H.L. Glutamatergic activation of anterior cingulate cortex produces an aversive teaching signal. *Nat. Neurosci.,* **2004** , 7, 398-403.

108. Ren, K.; Williams, G.M.; Hylden, J.L.; Ruda, M.A.; Dubner, R. The intrathecal administration of excitatory amino acid receptor antagonists selectively attenuated carrageenan-induced behavioral hyperalgesia in rats. *Eur. J. Pharmacol.,* **1992** , 219, 235-243.

109. Christoph, T.; Reissmuller, E.; Schiene, K.; Englberger, W.; Chizh, B.A. Antiallodynic effects of NMDA glycine(B) antagonists in neuropathic pain: possible peripheral mechanisms. *Brain Res.,* **2005** , 1048, 218-227.

110. Erhardt, S.; Blennow, K.; Nordin, C.; Skogh, E.; Lindstrom, L.H.; Engberg, G. Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. *Neurosci. Lett.,* **2001** , 313, 96-98.

111. Erhardt, S. Engberg, G. Increased phasic activity of dopaminergic neurones in the rat ventral tegmental area following pharmacologically elevated levels of endogenous kynurenic acid. *Acta Physiol Scand.*, **2002** , 175, 45-53.

112. Erhardt, S.; Schwieler, L.; Engberg, G. Kynurenic acid and schizophrenia. *Adv. Exp. Med. Biol.*, **2003** , 527, 155-165.

113. French, E.D. Phencyclidine and the midbrain dopamine system: electrophysiology and behavior. *Neurotoxicol. Teratol.*, **1994** , 16, 355-362.

114. Stone, T.W.; Forrest, C.M.; Mackay, G.M.; Stoy, N.; Darlington, L.G. Tryptophan, adenosine, neurodegeneration and neuroprotection. *Metab Brain Dis.*, **2007** , 22, 337-352.

115. Shepard, P.D.; Joy, B.; Clerkin, L.; Schwarcz, R. Micromolar brain levels of kynurenic acid are associated with a disruption of auditory sensory gating in the rat. *Neuropsychopharmacology*, **2003** , 28, 1454-1462.

116. Zarnowski, T.; Rejdak, R.; Zagorski, Z.; Juenemann, A.G.; Zrenner, E.; Kocki, T.; Urbanska, E.M.; Turski, W.A. Content of kynurenic acid and activity of kynurenine aminotransferases in mammalian eyes. *Ophthalmic Res.*, **2004** , 36, 124-128.

117. Rejdak, R.; Kohler, K.; Kocki, T.; Shenk, Y.; Turski, W.A.; Okuno, E.; Lehaci, C.; Zagorski, Z.; Zrenner, E.; Schuettauf, F. Age-dependent decrease of retinal kynurenate and kynurenine aminotransferases in DBA/2J mice, a model of ocular hypertension. *Vision Res.*, **2004** , 44, 655-660.

118. Guo, L.; Salt, T.E.; Maass, A.; Luong, V.; Moss, S.E.; Fitzke, F.W.; Cordeiro, M.F. Assessment of neuroprotective effects of glutamate modulation on glaucoma-related retinal ganglion cell apoptosis in vivo. *Invest Ophthalmol. Vis. Sci.*, **2006** , 47, 626-633.

119. Levkovitch-Verbin, H.; Quigley, H.A.; Martin, K.R.; Zack, D.J.; Pease, M.E.; Valenta, D.F. A model to study differences between primary and secondary degeneration of retinal ganglion cells in rats by partial optic nerve transection. *Invest Ophthalmol. Vis. Sci.*, **2003** , 44, 3388-3393.

120. Rejdak, R.; Zarnowski, T.; Turski, W.A.; Kocki, T.; Zagorski, Z.; Guenther, E.; Kohler, K.; Zrenner, E. Changes of kynurenic acid content in the rat and chicken retina during ontogeny. *Graefes Arch. Clin. Exp. Ophthalmol.*, **2002** , 240, 687-691.

121. Hama, Y.; Katsuki, H.; Tochikawa, Y.; Suminaka, C.; Kume, T.; Akaike, A. Contribution of endogenous glycine site NMDA agonists to excitotoxic retinal damage in vivo. *Neurosci. Res.*, **2006** , 56, 279-285.

122. Andrzejewska-Buczko, J.; Pawlak, D.; Tankiewicz, A.; Matys, T.; Buczko, W. Possible involvement of kynurenamines in the pathogenesis of cataract in diabetic patients. *Med. Sci. Monit.*, **2001** , 7, 742-745.

123. Ito, S.; Komatsu, K.; Tsukamoto, K.; Sved, A.F. Excitatory amino acids in the rostral ventrolateral medulla support blood pressure in spontaneously hypertensive rats. *Hypertension,* **2000** , 35, 413-417.