Research objective 2 – Blockade of specific branches of the kynurenine metabolic pathway

Activity 1 - Inhibition of production of kynurenic acid as a new antipsychotic mechanism of the effect of pharmaceuticals

The aim of this activity is the design, synthesis and biological evaluation of new inhibitors of kynurenine-aminotransferase, reducing the level of kynurenic acid in the brain. The design of the substances shall take place by in silico methods of molecular modelling (extraction of pharmacophore, molecular docking etc.). The individual targets of this study are as follows:

- Research into active structures, extraction of pharmacophore and design of new substances with the aid of molecular modelling. The design of the structures shall focus in particular on inhibitors of kynurenine-aminotransferase li (KAT li), which is the main isoform of this enzyme occurring in the brain.
- Preparation of proposed substances by methods of organic synthesis and analysis of their inhibition activity in vitro. The most promising substances will be further structurally optimised in order to attain the best possible candidates.
- Subsequent in vivo testing of the most promising candidates. Not only acute toxicity and bioavailability, but also pharmacokinetics will be observed.
- Evaluation of the relationships between biological activity and structure (SAR) and feedback for the design of new KAT li inhibitors.

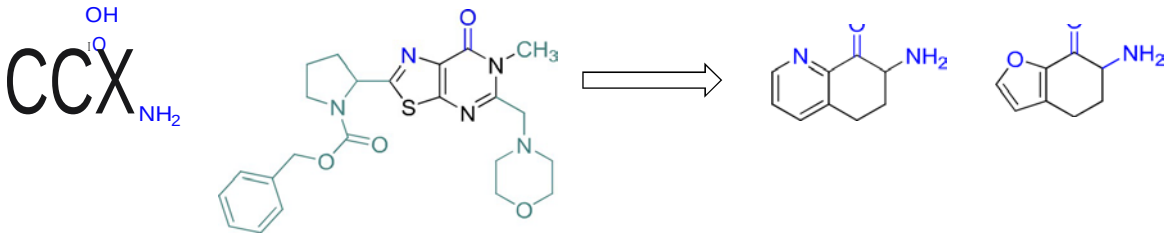
The kynurenine metabolic pathway is the main metabolism of tryptophan. The products of this pathway are neuroactive metabolites, referred to in summary as kynurenines. In the first step of the kynurenine pathway, tryptophan is converted into N-formylkynurenine with the aid of one of a triad of enzymes, indolamine-2.3-dioxygenase 1 (1D01), indolamine-2.3-dioxygenase 2 (1D02) or tryptophan-2.3-dioxygenase (TDO). This conversion is the main regulating step of the entire kynurenine pathway. The subsequent hydrolysis of N-formylkynurenine by the enzyme formamidase generates the key metabolite kynurenine (KYN), which may be further metabolised by a number of enzymes representing different branches of the kynurenine pathway. These main enzymes are kynurenine-aminotransferase (KAT) and kynurenine-3-monooxygenase (KMO). The first of these enzymes (KAT) converts kynurenine into kynurenic acid (KYNA), which is a dead end metabolite. KMO converts kynurenine into 3-hydroxykynurenine, which is subsequently further metabolised by the enzyme kynureninase (KYNU) and 3-hydroxyanthranilate-3.4-dioxygenase (3-HAO) into quinolinic acid (QUIN). KYNA is a highly potent antagonist of the glutamatergic *N*-methyl-O-aspartate receptors (NMDAR), and its effect is considered neuroprotective, whilst QUIN is a highly patent agonist of the NMDA receptors, and through this mechanism has a pronounced neurotoxic eff ect. Dysregulation of these two main branches of the kynurenine pathway, and thus dysregulation of levels of individua! metabolites, has been observed in connection with the main psychiatrie disorders such as depression, bipolar disorder and schizophrenia, as well as in the pathogenesis of neurodegenerative disorders such as Huntington's or Alzheimer's disease (Fujigaki et al., 2017; Dounay et al., 2015).

KYNA is produced in astrocytes from KYN with the aid of KAT (Guillemin et al., 1999). There are a total of four described isoforms of KAT (KAT I, 11, Ill and IV), with KAT li responsible for the majority of production of KYNA in the brain (Kozak et al., 2014; Schwarcz et al., 2012). In addition to its antagonist effect on the NMDAR, KYNA is also an antagonist of the a-7-nicotinic acetylcholine receptor (a7nAChR) (Hilmas et al., 2001). Studies have demonstrated raised levels of KYNA in the CSF and brain tissue in patients suffering from schizophrenia (Erhardt et al., 2001; Linderholm et al., 2012; Nilsson et al., 2005; Schwarcz et al., 2001). These raised levels of KNYA may lead to hypofunction of the NMDAR (Shepard et al.,

2003) and to a reduction of glutamatergic and cholinergic (and indirectly also dopaminergic) neurotransmission, and may cause symptoms connected with schizophrenia (Amori et al., 2009). Inhibition of KAT li activity may represent a potential target in the treatment of schizophrenia.

Preclinical study of the KAT li inhibitor PF-04859989 showed that systemic administration of the KAT li inhibitor reversed the KYN-induced elevation of brain KYNA concentrations and restored glutamate release events evoked by pressure ejections of nicotine into the prefrontal cortex of rats with elevated brain KYNA concentrations (Koshy Cherian et al., 2014). More recent studies have also indicated that systemic administration of the same KAT li inhibitor penetrates BBB, efficiently reduces brain levels of KYNA and decreases firing activity of midbrain dopamine neurons (Linderholm et al., 2015). This study indicates that a reduction in levels of KYNA has potential in the treatment of hyperdopaminergic states in connection with schizophrenia.

Within the framework of the presented activity, so far 2 potential KAT li inhibitors have been proposed, based on a structure of published inhibitors, specifically the aforementioned PF-04859989 (1) (Dounay et al., 2012) and compounds containing 1.3-thiazolo[5.4-d]pyrimidine-7(6H)-on 2 (). In the case of the proposed substances, inhibition of KAT li activity is expected, and in the case of its demonstration the substances shall be further optimised. *(Fig . 1).*



PF-04859989 (1) inhibitor 2 new proposed potential inhibitors KAT li

Fig. 1: Selected published active inhibitors and new proposed potential inhibitors.

Involved subjects

- NUDZ - Professor Jiří Horáček, PhD.; Dr. Karel Valeš, PhD; Dr. Tomáš Páleníček, PhD.; Martin Kuchař, PhD.

Activity 2 - Antidepressant and neuroprotective influence of inhibition of quinolinic acid production

The targets of this activity are based on the previous activity. Within the framework of the project new kynurenine-3-monooxygenase {KMO) inhibitors and 3-hydroxyanthranilate-dioxygenase {3HAO) inhibitors will be designed, synthesised and biologically evaluated, influencing the level of quinolinic acid in the bran. As in the case of the previous activity, the design of the structures shall take place by in silico methods of molecular modelling (extraction of pharmacophore, molecular docking etc.). The individua! targets of this study are as follows:

- Research into active structures, extraction of pharmacophore and design of new substances with the aid of molecular modelling. The design of the structures will be focused on inhibitors of kynurenine-3-monooxygenase {KMO) and 3-hydroxyanthranilate-dioxygenase {3HAO), which are the main enzymes in the branch of the kynurenine pathway responsible for the production of quinolinic acid.
- Preparation of proposed substances by methods of organic synthesis and analysis of their inhibition

activities in vitro. The most promising substances will be further structurally optimised in order to attain the best possible candidates.

- Subsequent in vivo testing of the most promising candidates. Not only acute toxicity and bioavailability, but also pharmacokinetics will be observed.
- Evaluation of the relationships between biological activity and structure (SAR) and feedback for the design of new KMO and 3HAO inhibitors.

The kynurenine metabolic pathway is the main catabolic pathway of the tryptophan metabolism. The products of this pathway are neuroactive metabolites, referred to in summary as kynurenine s. ln the first step of the kynurenine pathway, tryptophan is converted into N-formylkynurenine with the aid of one of a triad of enzymes, indolamine-2 .3-dioxygenase 1 {IDOl), indolamine-2.3-dioxygenase 2 {IDO2) or tryptoph an-2.3-dioxygenase (TDO). This conversion is the main regulating step of the entire kynurenine pathway. The subsequent hydrolysis of N-formylkynurenine by the enzyme formamidase generates the key metabolite kynurenine (KYN), which may be further metabolised by a number of enzymes representing different branches of the kynurenine pathway. These main enzymes are kynurenine-aminotransferase (KAT) and kynurenine-3-monooxygenase {KMO). The first of these enzymes (KAT) converts kynurenine into kynurenic acid (KYNA), which is a dead end metabolite. KMO converts kynurenine into 3- hydroxykynurenine, which is subsequently further metabolised by the enzyme kynureninase (KYNU) and 3- hydroxyanthranilate-3.4-dioxygenase {3-HAO) into quinolinic acid (QUIN). KYNA is a highly patent antagonist of the glutamatergic N-methyl-O-aspartate receptors {NMDAR), and its effect is considered neuroprotective, whilst QUIN is a highly patent agonist of the NMDA receptors, and through this mechanism has a pronounced neurotoxic effect. Dysregulation of these two main branches of the kynurenine pathway, and thus dysregulation of levels of individual metabolites has been observed in connection with the main psychiatrie disorders such as depression, bipolar disorder and schizophrenia, as well as in the pathogenesis of neurodegenerative disorders such as Huntington's or Alzheimer's disease
{Fujigaki et al., 2017; Dounay et al., 2015).

Kynurenine-3-monooxygenase (KMO) is another of the enzymes that metabolise kynurenine. lt is the main enzyme in the branch of the kynurenine pathway leading to QUIN, and under normal physiological conditions this branch is the main pathway of the kynurenine metabolism (Bender and McCreanor, 1982). KMO is produced especially in microglia, monocytes and macrophages, and is localised on the outer membrane of the mitochondria (Heyes et al., 1992; Alberati-Giani et al., 1996). The product of kynurenine conversion with the aid of KMO is 3-hydroxykynurenine (3-HK), which is considered to be a toxic metabolite, especially due to the effect of oxidative cell damage. Studies indicate that neuronal damage generated by 3-HK is caused by the action of free radicals and not by interaction with the glutamate receptors (Eastman and Guilarte, 1989). ln further steps 3-HK is metabolised by KYNU into 3- hydroxyanthranilate (3-HA), which is further metabolised with the aid of 3-HAO into QUIN, the dead end metabolite of the KMO branch of the kynurenine pathway. Although 3-HAO is a direct producer of QUIN, it remains little known with regard to its physiological role in the CNS.

As mentioned above, QUIN is a neurotoxic metabolite of the kynurenine pathway, acting as an NMDAR agonist, thereby increasing neuronal activity and also raising the intracellular level of calcium ions. (Stone and Perkins, 1981). QUIN can also elicit cytotoxicity by increasing neuronal glutamate release, inhibiting its uptake by astrocytes, and inhibiting astroglial glutamine synthetase, leading to excessive microenvironmental glutamate concentrations and neurotoxicity (Baverel et al., 1990; Ting et al., 2009).

From a medical-chemical perspective, KMO is a very good target for the treatment of psychiatrie and neurodegenerative disorders in connection with increased QUIN production, such as depression,

bipolar disorders, Huntington's disease (HD) and Alzheimer's disease (AD). Microglia is a validated local source of neurotoxic QUIN production in HD because microglia expresses KMO, following induction by proinflammatory stimuli. The KP metabolism in the CNS of patients with HD may shift to the KMO branch, resulting in the accumulation of QUIN. An interesting recent study has demonstrated that JM6, a smallmolecule prodrug KMO inhibitor, extended life span, prevented synaptic loss, and decreased microglial activation in a mouse model of HD (Zwilling et al., 2011). ln a number of studies raised levels of 3- HK and QUIN have been demonstrated, together with a reduced level of KYNA, in patients suffering from AD (Gulaj et al., 2010; Hartai et al., 2007). lt has been suggested that there are positive correlations between cognitive function tests and plasma KYNA levels, whereas inverse correlations exist between these tests and QUIN levels in dementia. As the accumulation of amyloid-b plaques and tangles result in microglial activation and neuroinflammation, the KP metabolism in patients with AD may be accelerated through the KMO branch, causing the accumulation of 3-HK and QUIN in the CNS (Gulaj et al., 2010).

Dysregulation of the kynurenine pathway is documented also in depressive patients however contrary to schizophrenia QUIN levels are increased. Patients suffering severe depression had a significantly increased density of QUIN-positive cells in the subregions of anterior cingulate cortex compared to controls (Steiner et al., 2011). QUIN levels in CSF of suicide attempters are around 300% of the levels in healthy controls (Erhardt et al., 2013). CSF kynurenine/tryptophan ratio was also increased in suicidal attempters indicating an activation of the kynurenine pathway (Brundin et al., 2016). The elevated QUIN level in CSF of suicidal patients persisted over 18-month period after suicidal attempt (Bay-Richter et al, 2015). Serum and CSF QUIN elevations were accompanied by decreases of KYNA concentrations (Bay-Richter et al, 2015; Savitz et al., 2015). lt was suggested that the generation of QUIN is induced by an inflammatory process (Bryleva and Brundin, 2017).

The immune system is activated in both diseases thus upregulation of kynurenine pathway enzymes might be detected in schizophrenia as well as depre ssion. Divergent effect of immune activation on the kynurenine metabolism depends on type-specific cytokine activation associated either with schizophrenia or depression. lncreased proinflammatory type-1 cytokines were found in major depression while schizophrenia is characterized by type-1/type-2 cytokine imbalance (Schwarz et al., 2001). Th2 dominant immune response (Th2 shift) found in the subpopulation of schizophrenic patients (Chiang et al., 2004; Avgustin et al., 2005) is associated with overexpression of TDO and the activation of astrocytes leading to accumulation of KYNA. On the other hand Thl dominant immune response is more frequently found in major depression where IDO and KMO are induced by the type-1 cytokines. Similarly; in those schizophrenics with Thl dominant immune response the kynurenine pathway changes would be more similar to those found in depression (Muller et al., 2009) .

Involved subjects

- NUDZ - Professor Jiří Horáček, PhD.; Dr. Karel Valeš, PhD; Dr. Tomáš Páleníček, PhD.; Martin Kuchař, PhD.

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