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Modulators of the Kynurenine Pathway of Tryptophan Metabolism: Synthesis and Preliminary Biological Evaluation of (*S*)-4-(Ethylsulfonyl)benzoylalanine, a Potent and Selective Kynurenine Aminotransferase II (KAT II) Inhibitor

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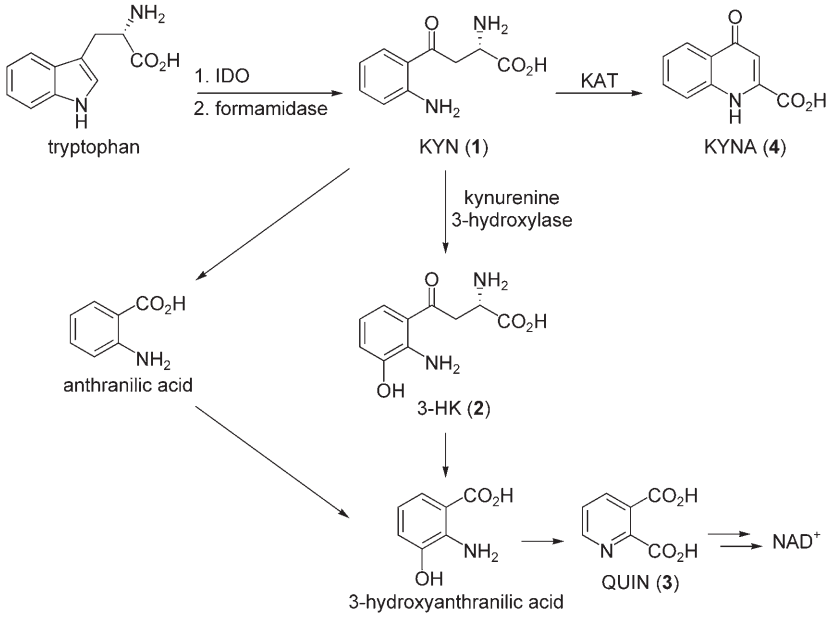
In mammals, most non-proteinogenic L-tryptophan is metabolized by a complex enzymatic cascade known as the kynurenine pathway (KP, Scheme 1), which ultimately leads to the production of NAD^+. The KP has received considerable interest over the last decade since several of its components are endowed with neuroactive properties.[1]

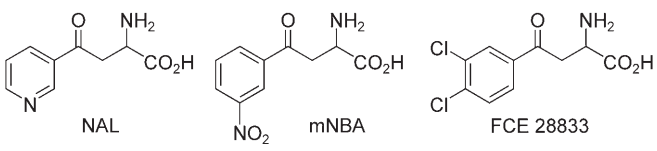
Thus, in a branch of the pathway initiated by the rate-limiting enzyme kynurenine 3-hydroxylase, the central metabolite kynurenine (KYN, **1**) is transformed into the pro-excitotoxic free-radical generator 3-hydroxykynurenine (3-HK, **2**) and then further into the excitotoxin quinolinic acid (QUIN, **3**).[2] A second branch of the KP leads from KYN to kynurenic acid (KYNA, **4**), a competitive antagonist of the Gly_B site of the NMDA receptor complex[3] and a noncompetitive antagonist of the α7* nicotinic acetylcholine receptor.[4] The transamination of KYN to KYNA is irreversible and catalyzed by kynurenine aminotransferases (KATs). At least two isoforms (KAT I and KAT II) are present in the mammalian brain and have been characterized by biochemical and genetic methods.[5–7]

The KP provides several targets for drugs that could be of use in the treatment of CNS diseases and disorders.[8] For example, the inhibition of kynurenine 3-hydroxylase decreases 3-HK and QUIN production and causes an increase in KYNA formation, leading to protection against excitotoxic insults. This concept has been verified using in vitro and in vivo models of brain ischemia,[9] although it has not yet been successfully translated into clinically deliverable drugs. In contrast, the inhibition of KAT, and especially of KAT II, results in decreased KYNA production.[10] In the brain, this causes diminished inhibition of α7* nicotinic acetylcholine receptor function and enhanced glutamate release.[11,12] KAT inhibitors could therefore be useful in disorders related to glutamatergic and cholinergic hypofunction, such as learning and memory deficits. So far, however, only a few potent KAT inhibitors have been described.[13] During the past years, we and others have shown that the benzoylalanine nucleus is particularly amenable to decoration, resulting in potent and selective kynurenine 3-hydroxylase inhibitors such as NAL, mNBA, and FCE 28833.[14,15]



Scheme 1. The kynurenine pathway (KP) of tryptophan metabolism. IDO: indoleamine 2,3-dioxygenase.

In the frame of this project, we recently became interested in the synthesis of sulfonylalkyl-substituted benzoylalanine derivatives, with the aim of taking advantage of the versatility of this moiety for its size, hydrophobic/hydrophilic balance, and



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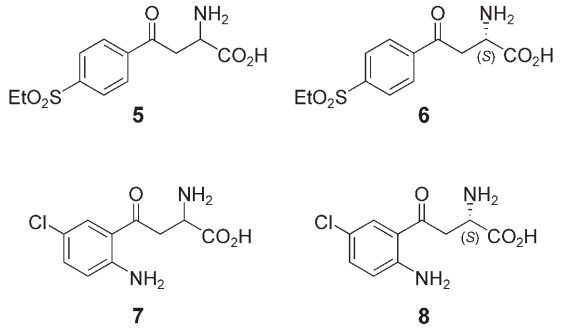
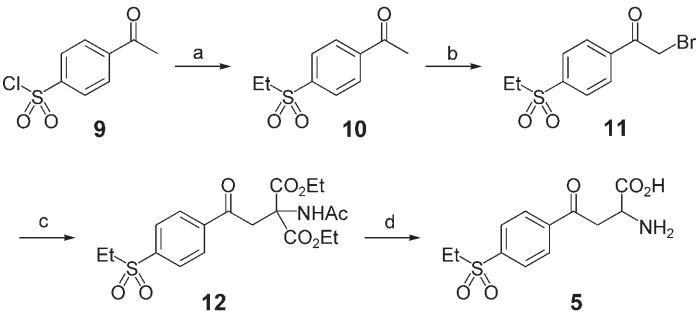
capacity for accepting hydrogen bonds. In the course of these studies, we noticed that D,L-4-(ethylsulfonyl)benzoylalanine (**5**) showed the unprecedented profile of a KAT inhibitor without effect on kynurenine 3-hydroxylase and kynureninase. Of particular interest is the notion that **5** cannot be transaminated by KAT owing to the lack of the kynurenine-like 2-amino group. Thus, unlike endogenous competing substrates such as α-aminoadipate or the more recently reported L-cysteine sulfinate[16] or S-adenosylhomocysteine,[17] **5** is expected to act as a pure inhibitor, thus being endowed with enhanced potential as a pharmacological tool for the characterization of the physiopathological role of KATs. Herein, we report the synthesis of D,L-4-(ethylsulfonyl)benzoylalanine (**5**), and of its active enantiomer (*S*)-4-(ethylsulfonyl)benzoylalanine (**6**), along with preliminary pharmacological evaluations in vitro and in vivo. We also compare **6** with D,L-5-Cl-KYN (**7**) and L-5-Cl-KYN (**8**), which are two known KAT inhibitors.[13] Finally, we show that the new benzoylalanine derivative, but not L-5-Cl-KYN (**8**), effectively decreases brain KYNA concentrations in vivo. This difference between the two compounds is explained in terms of their properties as KP enzyme inhibitors.

The synthetic protocol for the preparation of the racemic benzoylalanine derivative **5** (Scheme 2) involved classical acetamidomalonate addition/decarboxylative hydrolysis as the key step for the generation of the amino acidic moiety starting from 4-ethylsulfonyl-α-bromoacetophenone (**11**),[13] prepared in two steps from commercially available 4-acetyl-benzenesulfonyl chloride (**9**). Thus, **9** treated with sodium sulfite in basic aqueous conditions yielded the corresponding sodium sulfinate, which, by subsequent addition of 1-bromoethane, furnished the corresponding 4-ethylsulfonylacetophenone derivative **10** in 40% yield. Treatment of **10** with bromine in glacial acetic acid resulted in the corresponding α-bromo derivative **11** in 54% yield, which was then treated with diethyl acetamidomalonate sodium salt in DMF to give the desired aminomalonic acid derivative **12** (55% yield). Decarboxylative acidic hydrolysis (6 N HCl, 95 °C) of the intermediate **12** gave the desired D,L-(4-ethylsulfonyl)benzoylalanine (**5**) in 72% yield.

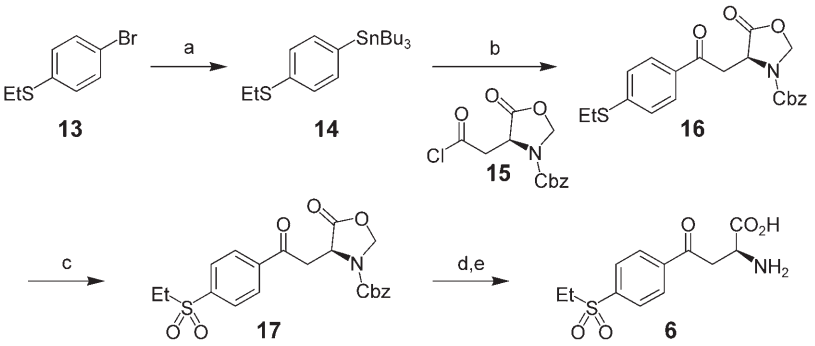




Scheme 2. Reagents and conditions: a) Na_2SO_3, $NaHCO_3$, H_2O, 75 °C, then EtBr, 105 °C, 40%; b) Br_2, AcOH, CH_2Cl_2, room temperature, 54%. c) diethyl-acetoamidomalonate, NaH, DMF, room temperature, 55%; d) 6 N HCl, 95 °C, 72%. DMF = *N*,*N*-dimethylformamide.

The enantioselective synthesis of (*S*)-(4)-(ethylsulfonyl)benzoylalanine (**6**) was then carried out as depicted in Scheme 3. Thus, according to the procedure reported by Salituro and McDonald[18] and applied previously by us to the enantioselective preparation of (*S*)-(*m*-nitrobenzoyl)alanine (mNBA),[19] 4-ethylthio-1-bromobenzene (**13**) was converted by lithium–halogen exchange (*t*BuLi, THF, −78 °C) followed by quenching with tributylstannyl chloride into the corresponding arylstannane **14**. Palladium(0)-catalyzed coupling of **14** with (*S*)-3-(benzyloxycarbonyl)-5-oxo-4-oxazolidineacetyl chloride (**15**)[20] ([Pd_2(dba)$_3$]·$CHCl_3$, toluene, 70 °C) gave the protected 4-substituted benzoylalanine derivative **16** in 67% yield after purification by flash chromatography. The 4-ethylthio-substituted derivative **16** was then oxidized, by using *m*CPBA (2.5 equiv) in chloroform solution, into the corresponding ethylsulfonyl derivative **17** (95%). This was finally deprotected in 6 N hydrochloric acid to obtain, after purification by ion-exchange chromatography, the desired (*S*)-4-(ethylsulfonyl)benzoylalanine (**6**) in 96% yield.

Compounds **5** and **6** were initially tested against KAT, kynurenine 3-hydroxylase, and kynureninase in rat liver tissue homogenate (Table 1). The two compounds inhibited KAT with IC_{50} values of 100 and 71 μM, respectively, but were essentially ineff-



Scheme 3. Reagents and conditions: a) 1) *t*BuLi, THF, −78 °C, 2) Bu_3SnCl, −78 °C→room temperature; b) [Pd_2(dba)$_3$]·$CHCl_3$, toluene, 70 °C, 67%; c) mCPBA, $CHCl_3$, room temperature, 95%; d) 6 N HCl, 95 °C, e) Dowex 50WX2-200, 10% pyridine, 96%. dba = *trans*,*trans*-dibenzylideneacetone, mCPBA = *meta*-chloroperoxybenzoic acid.

Table 1. Inhibition of KAT, kynurenine 3-hydroxylase, and kynureninase from rat liver.[a]

Compound	KAT	Kynurenine 3-hydroxylase	Kynureninase
D,L-5-Cl-KYN (**7**)	16 ± 1	5 ± 1	233 ± 46
L-5-Cl-KYN (**8**)	14 ± 1	3 ± 0.4	86 ± 14
5	100 ± 10	> 1000	> 1000
6	71 ± 5	> 1000	> 1000

[a] Assays were performed with crude tissue homogenate. Data are expressed as IC_{50} values (μM) and are the mean ± SEM of three experiments.

fective as inhibitors of kynurenine 3-hydroxylase and kynureninase. D,L-5-Cl-KYN (**7**) and L-5-Cl-KYN (**8**), used as reference compounds, caused more pronounced KAT inhibition than **5** and **6**, but also showed substantial inhibition of kynureninase and especially kynurenine 3-hydroxylase (three determinations for each compound and each enzyme). To further characterize the properties of the ethylsulfonyl-substituted benzoylalanine, the *S* enantiomer was tested against KAT I and KAT II using partially purified preparations of rat liver as the enzyme source.[7] In line with the effect of the racemic form (see above), **6** was a potent KAT II inhibitor (IC_{50} = 6.1 μM), but it showed no KAT I inhibition at 1 mM. Moreover, the reference compound **8** inhibited KAT II potently and preferentially, but also showed significant KAT I inhibition at 1 mM (Figure 1).

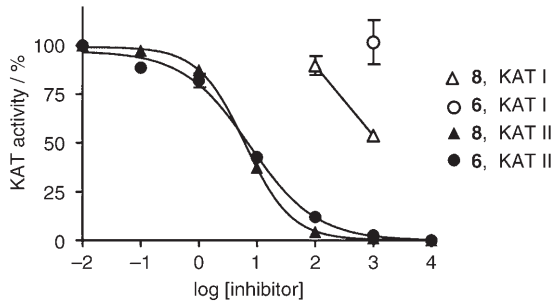


Figure 1. Inhibition of partially purified rat liver KAT I and KAT II by L-5-Cl-KYN (**8**) and **6**. Data are expressed as percent of control and are the mean ± SEM of three experiments.

The effects of **6** and **8** were evaluated and compared in vivo. To this end, each compound (5 mM) was infused by reverse dialysis into the hippocampus of unanaesthetized rats, and the effects on extracellular KYNA levels were recorded over time.[21] As illustrated in Figure 2, the KYNA content in the microdialysate decreased during the application of **6** but rose during tissue perfusion with **8**. In both groups, extracellular KYNA levels returned to baseline values soon after the drug treatment was discontinued. Additional kynurenine 3-hydroxylase inhibition likely explains the failure of **8** to decrease extracellular KYNA levels in vivo. The increase in KYNA after perfusion of **8** was, in fact, similar to that caused by specific kynurenine 3-hydroxylase inhibitors.[16,17]

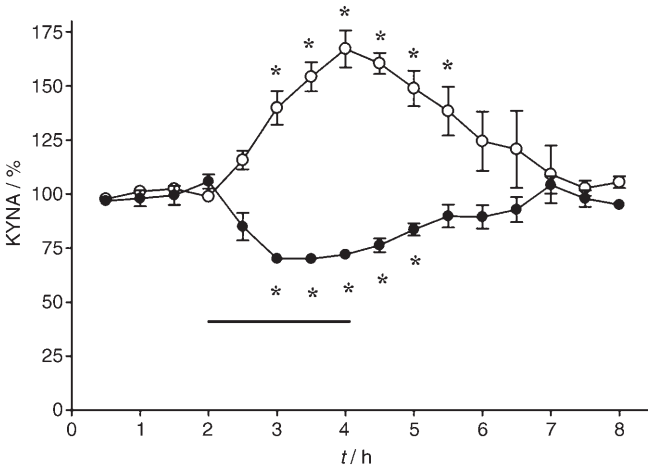


Figure 2. Effect of L-5-Cl-KYN (**8**, ○) and **6** (●) on extracellular KYNA concentrations (expressed as percent of basal levels) in the rat hippocampus. Data are the mean ± SEM of four rats per group. Drugs were infused at 5 mM for 2 h (bar). *$p < 0.05$ compared with basal values (two-way repeated measures ANOVA with the Bonferroni post-hoc test).

In conclusion, compound **6** is the first selective, synthetic KAT II inhibitor described so far and should prove to be a useful tool for elucidating pivotal aspects of KYNA neurobiology, including the role of KYNA as an endogenous modulator of glutamatergic and cholinergic neurotransmission. After appropriate modification, **6** or a congener may also be capable of transiently lowering KYNA levels in the human brain. The expected result, that is, increased activity of NMDA and α7* nicotinic receptors, may be beneficial toward cognitive processes in normal individuals and is a desirable goal in the treatment of catastrophic brain diseases such as Alzheimer's disease and schizophrenia.

Keywords: enzymes · kynurenic acid · kynurenine aminotransferase II · kynurenine pathway · medicinal chemistry

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