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SYNTHESIS AND ACTIVITY OF ENANTIOPURE (*S*) (*m*-NITROBENZOYL) ALANINE, POTENT KYNURENINE-3-HYDROXYLASE INHIBITOR

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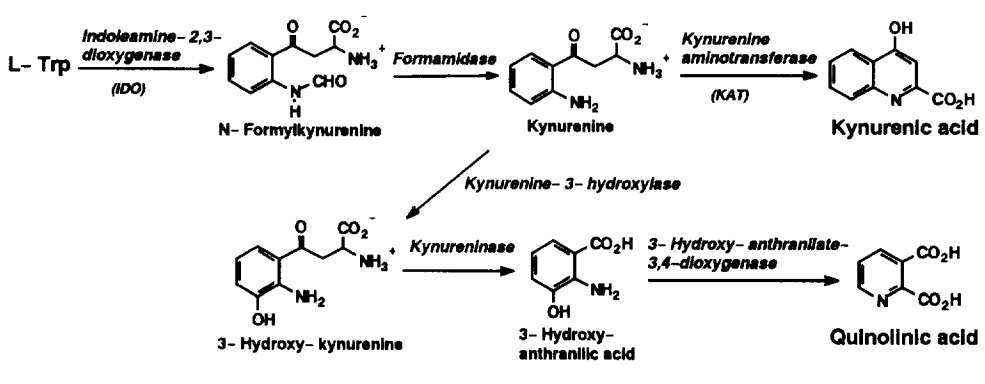
Abstract. The enantioselective synthesis of *S*(+) (*m*-nitrobenzoyl)alanine along with the inhibition of kynurenine-3-hydroxylase and kynureninase activity are reported.

Kynurenic acid (KYNA), an endogenous antagonist acting at the glycine recognition site present on the N-methyl-D-aspartate (NMDA) receptor ion-channel[1] is synthetized from kynurenine (KYN), a tryptophan metabolite, through the action of kynurenine transaminase (Scheme I).

Scheme I



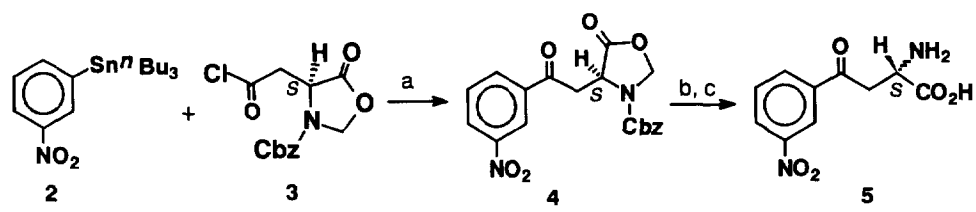
Since an overstimulation of NMDA receptors plays an important role in a variety of diseases including epilepsy, ischemia, neurodegenerative and inflammatory disorders of the central nervous system, an increase of KYNA concentration in the brain can have therapeutic utility. The production of KYNA

has, as a rate limiting step, the bioavailability of KYN which is also a substrate for kynurenine-3-hydroxylase and kynureninase, two enzymes of the metabolic pathway leading to the formation of the excitotoxic quinolinic acid and to NAD biosynthesis.[2] Over the past few years we have synthetized a number of KYN isosteres to be tested as inhibitors of the kynureninase and kynurenine-3-hydroxylase. Among them, (*m*-nitrobenzoyl)alanine (*m*-NBA, **1**), tested as a racemate, was shown to be a potent inhibitor, the first so far reported, of kynurenine-3-hydroxylase (IC_{50}= 1 μM).[3] When administered to rats having a microdialysis probe in their hippocampus, (±) *m*-NBA (**1**) significantly increased the concentration of KYNA in the dialysate (up to ten times over basal levels). This increased concentration of KYNA in brain extracellular spaces was correlated with sedation, increase of the convulsive threshold and mild analgesic effect in agreement with a reduced excitatory amino acid receptor function. The interesting pharmacological profile of (±) *m*-NBA (**1**) led us to undertake the stereoselective synthesis of its enantiomeric forms, that we report here.

Chemistry

The synthetic procedure for the preparation of **5** was based on the cross-coupling reaction of the stannane derivative[4] **2** with the protected aspartyl chloride[5,6] **3** catalyzed by Pd(II)[7] (Scheme II).

Scheme II



a) $(Ph_3P)_2PdCl_2$, PhMe, 70 °C, 10 h; b) 6N HCl, reflux, 2 h; c) $NaHCO_3$

In fact, when 3-nitrophenyl-tributyl-stannane (**2**) was reacted with (*S*) 3-Cbz-5-oxo-4-oxazolidinyl-acetyl chloride (**3**) in the presence of bis(triphenylphosphine)-palladium(II)chloride in anhydrous toluene (**2**:**3**:cat. =14:16:1) for 10 h at 70 °C, (*S*) 3-(3-Cbz-5-oxo-4-oxazolidinyl-acetyl)nitrobenzene (**4**) was obtained as an oil which was purified by flash chromatography (light petroleum containing 0-30% AcOEt, 25% yield). Acidic hydrolysis (6N HCl, reflux, 2h) of **4** afforded (*S*) (*m*-nitrobenzoyl)alanine hydrochloride (64% yield) which was then converted into the corresponding free base (**5**) by neutralization of an aqueous solution of the salt and following filtration of the white solid thus formed. The same procedure was applied for the preparation of the *R*-isomer.[8]

Experimental Procedure.

(S) 3-(3-Cbz-5-oxo-4-oxazolidinyl-acetyl)nitrobenzene (**4**).

Bis(triphenylphosphine)palladium(II)chloride (0.131 g, 0.187 mmol) was added to a solution of 3-(tributyl-stannyl)nitrobenzene (**2**, 1.10 g, 2.67 mmol) and 3-Cbz-5-oxo-4-oxazolidinyl-acetyl chloride (**3**, 0.874 g, 2.94 mmol) in anhydrous toluene (30 ml) and the resulting mixture was heated at

70 °C for 10 h. After cooling, the reaction mixture was diluted with ether (20 ml) and the catalyst was removed by filtration. The filtrate was washed with saturated $NaHCO_3$ (10 ml), water (3x10 ml), brine (10 ml) and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue (1.85 g) was submitted to flash chromatography: elution with light petroleum containing 10–30% of ethyl acetate yielded an oil which was further purified by extracting traces of the stannyl derivative with light petroleum thus obtaining pure **4** (0.257 g, 25%), mp 48–50 °C; ν_{max} ($CHCl_3$) 1800, 1710, 1533, 1497, 1353, 1316 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ3.70 (2H, m, CH_2CO); 4.50 (1H, s, 4–CH); 5.10 (2H, m, CH_2Ph); 5.50 (2H, d, J=18 Hz, 3–CH_2); 7.35 (5H, m, Ph); 7.65 (1H, t, J=8 Hz, 5'–CH); 8.20 (1H, s, 6'–CH); 8.40 (1H, d, J=8 Hz, 4'–CH); 8.70 (1H, s, 2'–CH); ^{13}C-NMR ($CDCl_3$) δ 38.93 ($COCH_2$); 50.84 (4–CH); 67.81 (CH_2Ph); 78.44 (3–CH_2); 122.92 (5'–CH); 127.95 (6'–CH); 128.11, 128.53, 135.21, 136.64 (Ph); 130.03 (4'–CH); 133.51 (2'–CH); 148.37 (1'–C); 152.52 (3'–C); 171.79 (2xCO_2); 194.41 (CO); GC–MS: 319 (9.39); 313.10 (100); 256.95 (43.30); 200.90 (48.78); 198.90 (74); 121.00 (30.27); 57.20 (30.61); $[\alpha]_D^{20}$ = +70.96 (c 1, $CHCl_3$).

S(+) (m–Nitrobenzoyl)alanine(**5**).

A suspension of **4** (0.153 g, 0.398 mmol) in 6N HCl (10 ml) was refluxed for 2 h. The reaction mixture was then extracted with ether (3x2 ml) and, after evaporation of the solvent, the solid residue was triturated with acetone (5 ml) thus affording **5** as a white solid hydrochloride salt (0.070 g, 64%), mp 196–7 °C; ν_{max}(KBr) 1700, 1530, 1480, 1360, 1310 cm^{-1}; ^{1}H–NMR (D_2O) δ 3.75 (2H, d, 3–CH2); 4.40 (1H, br s, 2–CH); 7.60 (1H, t, J=8 Hz, 5'–CH); 8.15 (1H, d, J=8 Hz, 6'–CH); 8.30 (1H, d, J=8 Hz, 4'–CH); 8.55 (1H, s, 2'–CH); ^{13}C–NMR ($CDCl_3$) δ39.42 (3–CH_2); 49.76 (2–CH); 123.84 (5'–CH); 129.46 (6'–CH); 131.41 (4'–CH); 135.27 (2'–CH); 137.07 (1'–C); 149.00 (3'–C); 172.17 (CO_2); 197.87 (CO); $[\alpha]_D^{22}$=+13.21 (c 1, H_2O). Following neutralization with $NaHCO_3$ of an aqueous solution of the hydrochloride salt followed by filtration afforded *S*(+) (*m*–nitrobenzoyl)alanine (**5**) as white solid free base.

Biological Results and Discussion.

The results of competitive inhibition studies are shown in Figure 1. The dose–response curves of the two compounds were obtained by using KYN as substrate (100 μM). When tested for the inhibition of kynurenine–3–hydroxylase activity the *R*(–)–isomer exhibited a 60% inhibition at 100 μM, while *S*(+) *m*–NBA (**5**), with an IC_{50} of 0.5 μM resulted to be the active constituent of the previously reported racemic mixture,[3] thus showing a concentration dependent stereoselective activity. A larger concentration of *S*(+) *m*–NBA also inhibits kynureninase, the second enzyme involved in the kynurenine pathway of tryptophan metabolism. In rat liver preparations, IC_{50} of *S*(+) *m*–NBA was 60 μM, while *R*(–) *m*–NBA (100 μM) was practically devoid of any inhibitory activity.

On the basis of the above results *S*(+) *m*–NBA (**5**) can now replace (±) *m*–NBA as an important biochemical and pharmacological tool for the characterization of the kynurenine pathway of tryptophan metabolism.

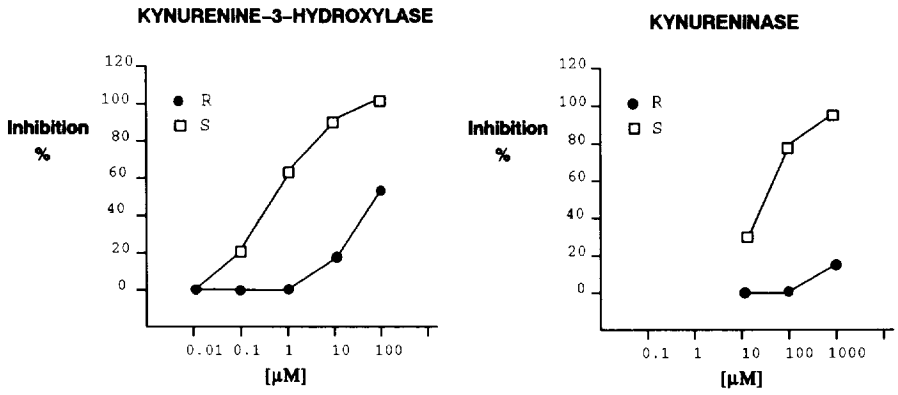


Fig. 1
Inhibition of kynurenine-3-hydroxylase and kynureninase by *R*(−) and *S*(+) *m*-NBA. Different concentrations of inhibitor were used while the substrate (KYN) was 100 μM. Each point represents the mean of at least four different experiments, each performed in duplicate. Standard errors were within 10%.[3]

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8. *R*(−) *m*-NBA, hydrochloride salt: $[\alpha]_D^{22}$=−14.7 (c 0.5, H_2O)

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