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2-Amino-4-[3′-hydroxyphenyl]-4-hydroxybutanoic Acid; A Potent Inhibitor of Rat and Recombinant Human Kynureninase

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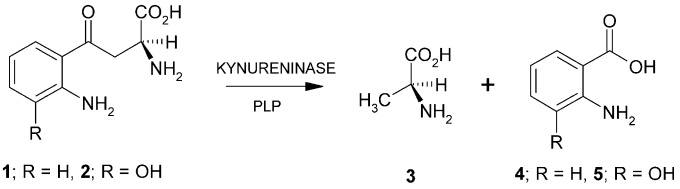
Abstract—A novel structural analogue of kynurenine, 2-amino-4-[3′-hydroxyphenyl]-4-hydroxybutanoic acid **6**, was synthesised as an inhibitor of kynureninase. The compound had a significant inhibitory effect on kynureninase from both rat and human, giving a K_i of 100 nM. It was thus found that removal of the aryl amino group coupled with a reduction of the carbonyl group at position 7 of the alanine side chain greatly enhanced potency of the inhibitor.

The excitotoxin quinolinic acid is implicated as an etiological factor in a number of neurodegenerative diseases such as AIDS related dementia, Alzheimer's disease and Parkinson's disease as a result of its agonist activity at the NMDA receptor.[1] One method of regulating the effects of this metabolite would involve the use of synthetic inhibitors of the enzymes on the kynurenine pathway of tryptophan metabolism, which is the biosynthetic source of quinolinic acid.[2] Kynureninase (L-kynurenine hydrolase, E.C. 3.7.1.3), which catalyses the β,γ-hydrolytic cleavage of kynurenine **1** or 3-hydroxykynurenine **2** (Scheme 1), is one potential therapeutic target.

To date there have been relatively few reported inhibitors for kynureninase and many lack specificity.[3,4] A number of inhibitors have been developed that mimic the transition state for the kynureninase catalysed reaction, including (4*S*)- and (4*R*)-dihydro-L-kynurenine,[5] a series of *S*-aryl-L-cysteine *S,S*-dioxides[6] and a phosphinic acid analogue of kynurenine,[7] which are all competitive inhibitors of bacterial kynureninase with varying potency. Drysdale and Reinhard[8] have also examined *S*-aryl-L-cysteine *S,S*-dioxides as inhibitors of mammalian kynureninase from rat liver and observed good inhibition. Recently this laboratory synthesised a series of novel bicyclic kynurenine analogue inhibitors of which a naphthyl analogue proved to be the most potent against both the bacterial and recombinant human enzymes.[9] It is known that the constitutive rat and human enzymes share an 85% amino acid sequence homology[10] and display a similar substrate-selectivity and therefore it is possible that the potency of inhibitory compounds in this instance could be similar.

As part of our ongoing studies on kynureninase, 2-amino-4-[3′-hydroxyphenyl]-4-hydroxybutanoic acid **6** was synthesised as a potential inhibitor for the enzyme. The compound was designed to include a hydroxyl group at the 7-position as a mimic of the putative tetrahedral



1; R = H, **2**; R = OH — **3** — **4**; R = H, **5**; R = OH

Scheme 1.

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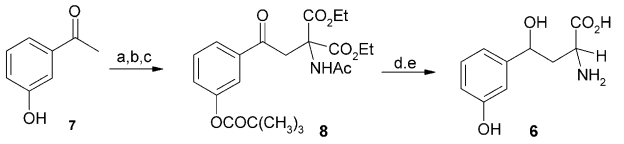
transition state, plus a hydroxyl group at C-3 to give it greater specificity for the mammalian enzymes. The amino group at C-2′ was not included, as previously, in order to simplify the synthesis. The compound was synthesised using a modification of our previous methods (Scheme 2),[9] including a step to protect the hydroxyl group at the 3′-position as a pivaloyl derivative. The carbonyl group was reduced by catalytic hydrogenation and all the protecting groups removed in one step, using acid hydrolysis. Following final recrystallisation, the spectral data[11] indicated the presence of a single diastereomer of the final product as a racemic mixture. Comparison with previous literature data[5] indicated that the two enantiomers were likely to be of the (4*S*,2*S*) and (4*R*,2*R*) configuration.

The inhibitory activity of the racemic 2-amino-4-[3′-hydroxyphenyl]-4-hydroxybutanoic acid **6** was then assessed with rat liver kynureninase,[9] recombinant human kynureninase[9,12] and bacterial kynureninase



Scheme 2. (a) $(CH_3)_3CCOCl$, pyridine (79%); (b) $CuBr_2$, EtOAc/$CHCl_3$ (63%); (c) $CH(CO_2Et)_2NHAc$, NaH, DMF (57%); (d) H_2, 5% Pd/C, EtOAc (66%); (e) 6 N HCl (98%).

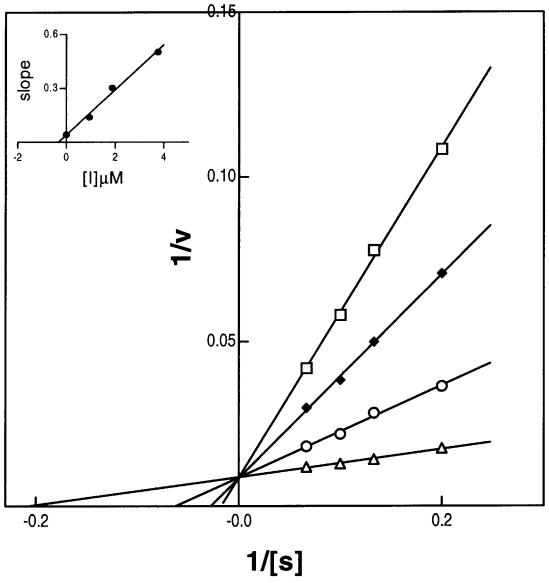


Figure 1. Kinetic data of inhibition of rat hepatic kynureninase 2-amino-4-[3′-hydroxyphenyl]-4-hydroxybutanoic acid. The inhibition of rat kynureninase is depicted with respect to 3-hydroxykynurenine as substrate. Data are presented as a Lineweaver–Burk plot for the following inhibitor concentrations: 0 μM (Δ), 0.95 μM (○), 1.90 μM (◆), 3.80 μM (□). Initial rates of reaction (v = nmol/min/mg protein) were assayed as described in ref 9. The concentration of substrate was varied between 0 and 20 μM. An enzyme concentration of 3.4 mg/mL was employed. The graph shown is the mean of three triplicate experiments. Measurements were performed spectrofluorimetrically. The inset is a secondary plot of slope against the concentration of inhibitor, which was used to calculate K_i.

using a spectrofluorimetric assay.[13] The compound was found to inhibit all three enzymes in a dose dependent and reversible manner with equimolar affinity, giving $K_i = 130$ nM for the rat enzyme (Fig. 1) and $K_i = 100$ nM for the human enzyme (Fig. 2). However, the inhibitor was approximately 100-fold less potent with the bacterial enzyme, giving a K_i of 10 μM. The inhibition was distinctly mixed for the recombinant human enzyme (Fig. 2). At low substrate concentrations the inhibition was competitive whilst at higher levels the inhibition became non-competitive. This is consistent with some recent observations in our laboratory, which have indicated the presence of a second regulatory binding site on the human kynureninase.[14] The degree of inhibition with 5 μM 3′-hydroxykynurenine as substrate was 55% for the recombinant human enzyme (data not shown).

Our experimental findings reveal that 2-amino-4-[3′-hydroxyphenyl]-4-hydroxybutanoic acid **6** is one of the most potent inhibitors of human kynureninase yet designed and synthesised. The importance of the 3′-hydroxy group as a recognition element for the mammalian enzyme is highlighted by the poorer inhibition observed with the bacterial enzyme. Indeed, recent results in our laboratory have shown that purified homogeneous recombinant human kynureninase only hydrolyses 3′-hydroxykynurenine and shows no activity at all with kynurenine.[14] It is possible that this compound could provide a good lead for the development of structurally related drugs with a greater potency for inhibiting the activity of human kynureninase, which may ultimately be used to regulate the availability of quinolinate concentrations at the level of the receptor.

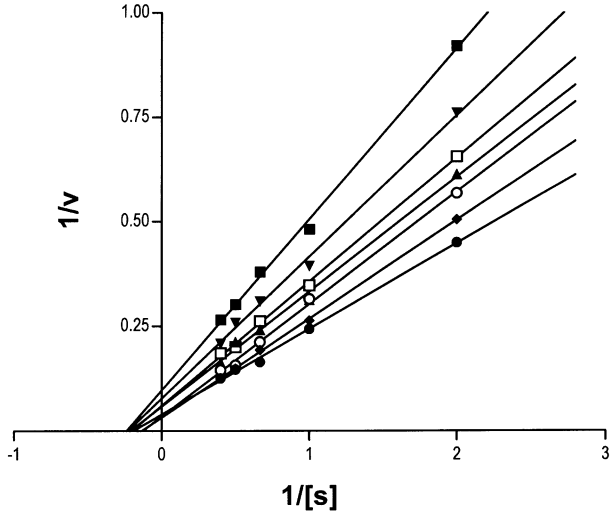


Figure 2. Kinetic data of inhibition of recombinant human kynureninase 2-amino-4-[3′-hydroxyphenyl]-4-hydroxybutanoic acid. The inhibition of human recombinant kynureninase is depicted with respect to 3-hydroxykynurenine as substrate. Data are presented as a Lineweaver–Burk plot for the following inhibitor concentrations: 0 nM (●), 160 nM (◆), 320 nM (○), 640 nM (▲), 1280 nM (□), 2.56 μM (▼) and 5 μM (■). Experiments were performed as described for Figure 1 and detailed in ref 9. An enzyme concentration of 1.8 mg/mL was employed.

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