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S-ARYL CYSTEINE *S,S*-DIOXIDES AS INHIBITORS OF MAMMALIAN KYNURENINASE

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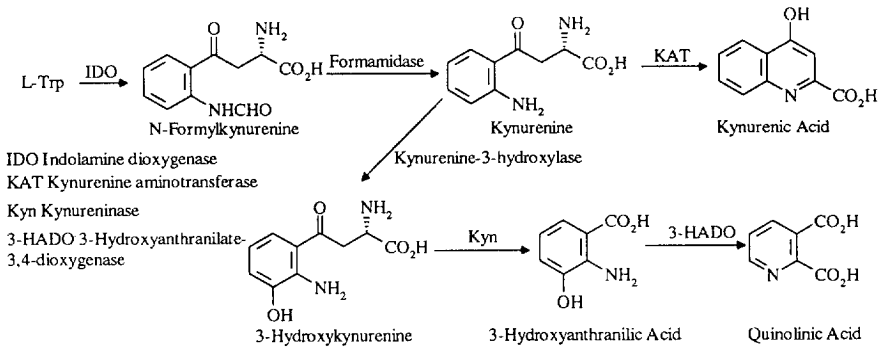
Abstract: A series of 2-amino-S-aryl cysteine *S,S*-dioxides have been synthesised and shown to inhibit kynureninase an important enzyme in the biosynthesis of the known excitotoxic moiety quinolinic acid. The most potent of these, 2-amino-5-methyl-S-phenyl cysteine *S,S*-dioxide **6d**, inhibits interferon-γ induced synthesis of quinolinic acid in human macrophages.

Quinolinic acid (QUIN) is a metabolite of tryptophan (Trp) (Figure 1) which behaves as an NMDA receptor agonist and has been shown to be neurotoxic [1, 2]. In normal brains,

FIGURE 1

QUIN metabolism pathway from L-Trp



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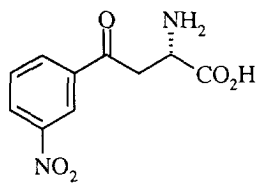
concentrations of QUIN are low [3, 4], reflecting the very low levels of the enzymes indolamine dioxygenase (IDO), kynureninase and kynurenine-3-hydroxylase in the CNS. However, in certain inflammatory disease states where the CNS has been compromised, significant increases in QUIN concentrations have been observed [5], consistent with induction of IDO within CNS infiltrating macrophage [6, 7].. Strikingly a significant correlation between QUIN concentrations in AIDS patients and the clinically assessed severity of neuropsychological deficits was reported [8]. Also progressive slowing of reaction times has been correlated with increasing QUIN levels in HIV-infected individuals [9].

Recently Natalini *et al* [10] reported on S-(*m*-nitrobenzoyl)alanine **1** as an inhibitor of



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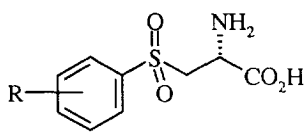


2 a R = 2-NO_2; **b** R = 2-NH_2;
c R = 4-NO_2; **d** R = 4-NH_2.

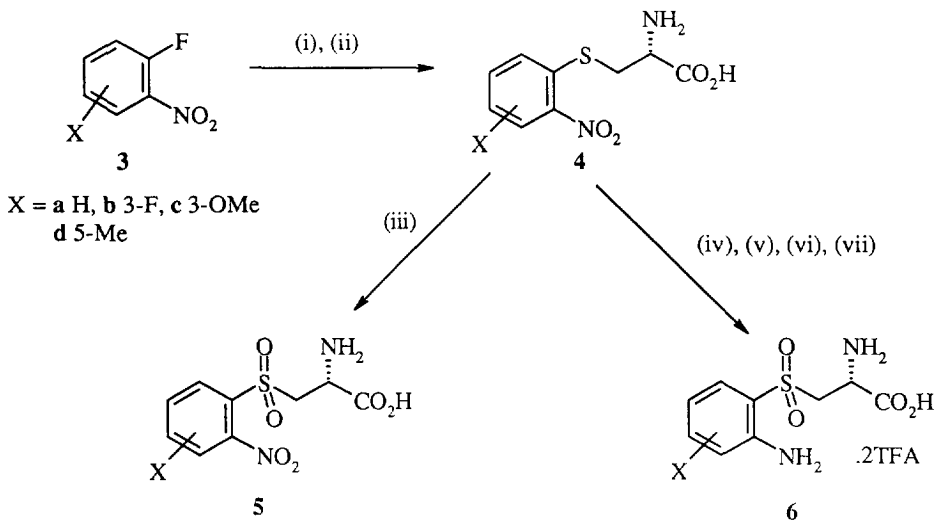
mammalian kyurenine-3-hydroxylase and Dua *et al* [11] described some S-aryl-L-cysteine *S,S*-dioxides **2** as inhibitors of bacterial kynureninase.

This report describes the activities of novel inhibitors of kynureninase on mammalian kynureninase activity *in vitro* as well as the effect on QUIN synthesis in macrophage cultures.

Chemistry

Reaction of the appropriately substituted 2-fluoronitrobenzene derivatives **3** with N-acetyl-L-cysteine followed by deprotection[12] gave the S-aryl-L-cysteine derivatives **4** (Scheme 1). Oxidation with hydrogen peroxide and 98% formic acid[11] gave the 2-nitro-S-aryl-L-cysteine S, S-dioxides **5**. Elaboration of **4** to **6** involved protection of the α-amino group as the *t*-Boc derivative under standard conditions and reduction of the aromatic nitro-compounds to the aniline using zinc and ammonium chloride in methanol. Formation of the benzhydryl ester using diphenyldiazomethane was followed by oxidation of sulfide to sulfone with *m*-CPBA and final deprotection with TFA/anisole yielded the amino acids **6** as the ditrifluoroacetates

(Scheme 1).



Reagents:

(i) N-Acetyl-L-cysteine, $NaHCO_3$, H_2O, EtOH; (ii)c.H_2SO_4, H_2O; (iii) 98% formic acid, 30% H_2O_2; (iv) Boc_2O, $NaHCO_3$, H_2O, dioxane; (v) Zn, NH_4Cl, MeOH; (vi) $Ph_2C{=}NNH_2$, NiB_4, EtOAc; (vii)*m*-CPBA, CH_2Cl_2; (viii) TFA, anisole.

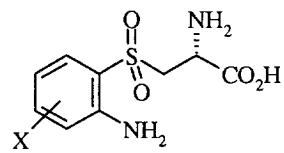
SCHEME 1

Discussion

Whilst none of the 2-nitro-S-aryl cysteine S,S-dioxides **5** showed any inhibition against kynureninase, the 2-amino derivative **6a** gave an IC_{50} = 36μM (Table 1). At the substrate concentration used, a K_i for **6a** can be estimated at 18μM. Though this is considerably lower than the K_i of **6a** against bacterial kynureninase (0.07μM)described previously[11], this is consistant with the different substrate preference of the mammalian enzyme[13] compared to bacterial kynureninase. Introduction of substituents at the 3-position such as **6b** (3-F, IC_{50} = 20μM) and **6c** (3-OMe, IC_{50} = 29μM) gave some improvement but the best compound was

the 5-Me derivative **6d** with an IC_{50} = 11μM.

TABLE 1



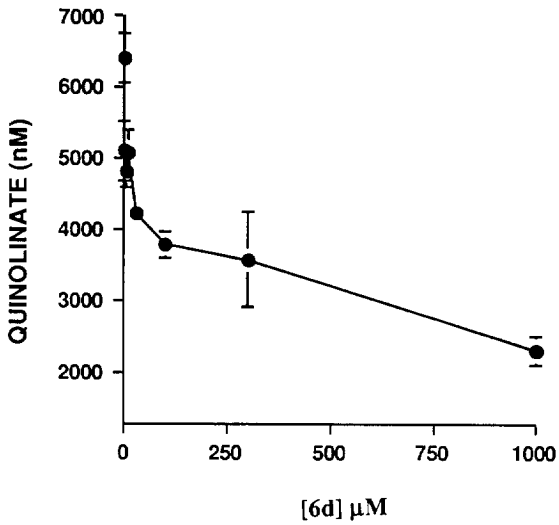
Compound	X	Inhib. of mammalian Kyn (IC_{50}; μM)[a]
6a	H	36(33-39)
6b	3-F	20(16-22)
6c	3-OMe	29(22-37)
6d	5-Me	11(8-14)

a) Rat liver kynureninase was used as described previously[14], assayed at Km concentrations of the kynurenine substrate. The values given are the mean and range from at least 3 separate experiments.

Additionally, using primary cultures of cultured human peripheral blood monocyte-derived macrophages the effect of **6d** was tested on the interferon-γ induced synthesis of QUIN. The data demonstrate inhibition of QUIN formation, though, at doses higher than for the isolated enzyme. Such a difference could be attributed to either poor cellular entry, high levels of the competing substrate or both. Cell viability, assessed by the trypan blue dye exclusion test, was not impaired over the time course of the experiment. Effects of such a compound could have utility under conditions where QUIN is elevated and may be pathogenic. One such example is spinal cord injury in the guinea pig, where partial inhibition of QUIN significantly improved disease outcome [15]. Thus, inhibitors such as those described herein could have utility in spinal cord injury.

FIGURE 2

Inhibition of QUIN formation in Human Macrophages. Quinolinate was measured in the cellular media 48 hours after stimulation with human interferon-γ as described previously [6].



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