Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 22, pp. 2643-2646, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00483-0

Synthesis of Phosphinic Acid Transition State Analogues for the Reaction Catalysed by Kynureninase

Fiona C. Ross, Nigel P. Botting,*

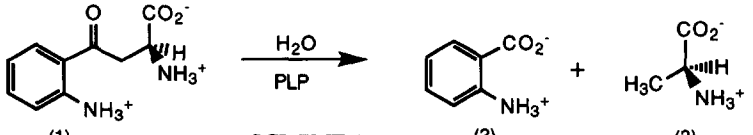
School of Chemistry, University of St. Andrews, St. Andrews, Fife, KY16 9ST.

and Paul D. Leeson.¶

Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex, CM20 2QR.

Abstract: The syntheses of phosphinic acid and methyl phosphinate analogues of kynurenine, designed as transition state mimics for the enzyme kynureninase are described, along with their inhibitory properties.

Kynureninase (EC 3.7.1.3) is a pyridoxal 5'-phosphate (PLP) dependent enzyme which catalyses the β,γ-cleavage of kynurenine (1) to give anthranilic acid (2) and L-alanine (3) (Scheme 1).[1] Kynureninase plays a key regulatory role on the neurologically important tryptophan metabolic pathway.[2] Quinolinic acid, which is a potent neurotoxin, is one of the biosynthetic products of the pathway. This metabolite has been implicated as an important etiological factor in various neurodegenerative disorders including epilepsy, Huntington's disease and AIDS-related dementia. Indeed, elevated endogenous levels of quinolinic acid have been observed in the brains of patients suffering from AIDS and very strong correlations have been found between these levels and the degree of neurological damage.[3] Inhibition of kynureninase offers a method of blocking the biosynthesis of quinolinic acid and therefore the enzyme is a potential therapeutic target for the treatment of these diseases.

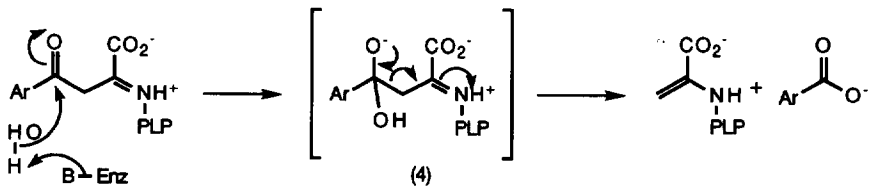


(1) (2) (3)

SCHEME 1

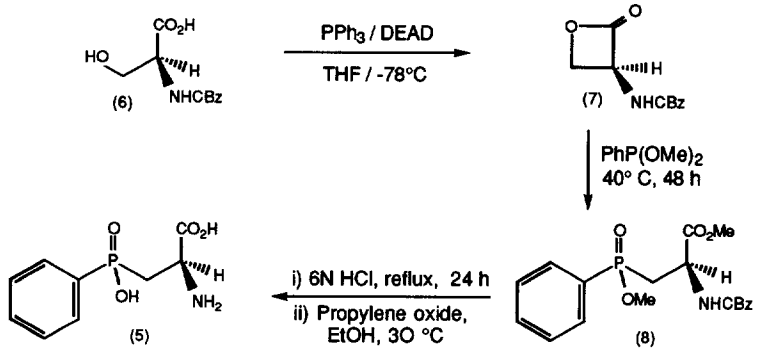
Until recently, few inhibitors had been reported for kynureninase[4] and these were non-selective inhibitors that would also inactivate other PLP dependent enzymes. We therefore sought to design more selective inhibitors, using the current knowledge concerning the chemical mechanism of the enzyme. Evidence to date, although not conclusive, points towards the reaction occurring via a pathway involving general base catalysed hydrolysis of the substrate/PLP ketimine (Scheme 2). Such a mechanism involves a tetrahedral intermediate (4), which can be used as a model for the transition state for the reaction. A stable analogue of kynurenine that mimics this structure should therefore bind very tightly to the enzyme.

* To whom correspondence should be addressed. FAX: 01334 463808, e-mail: npb@st-andrews.ac.uk



SCHEME 2

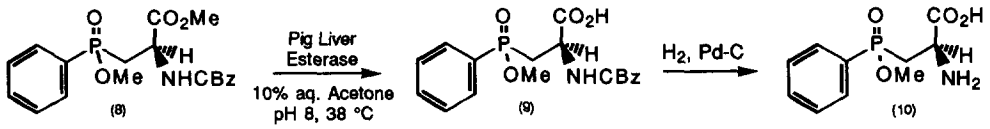
A phosphinic acid analogue (5) has been designed to serve as such a transition state mimic. In this first generation compound, the *ortho*-amino group has been omitted in order to simplify the synthesis. This will result in some loss of binding,[5] however removal of this group was reported to result in only a five fold increase in K_m. Compound (5) has been synthesised using the β-lactone methodology originally developed by Vederas.[6] This involves cyclisation of N-protected serine, in this case the N-carbobenzyloxy (N-CBz) derivative (6), to give the β-lactone (7). The lactonisation was carried out with triphenylphosphine and diethylazodicarboxylate (DEAD) and proceeded in 80% yield (Scheme 3). The strained β-lactone was then opened using a suitable nucleophile, in this case phenyl dimethyl phosphonite. Reaction at 40 °C, using the phosphonite as the solvent, proceeded smoothly to give the protected version of the inhibitor (8). (This is the first time a phosphonite nucleophile has been used to open a β-lactone.) The methyl ester is obtained due to migration of one of the methyl groups from the phosphonite onto the carboxylate produced on ring opening. Deprotection, without further purification, using 6N HCl, provides the target compound (5)[7] in 39% yield for the two steps (Scheme 3).



SCHEME 3

The inhibitory properties of the phosphinic acid were assessed using a preparation of kynureninase isolated from *Pseudomonas fluorescens*.[8] Kinetic studies showed that the compound was a competitive inhibitor relative to kynurenine (Figure 1). The binding constant, K_I, was found to be 4.28 ± 0.1 mM, while the K_m for the substrate was 25.6 ± 0.6 μM. This represents poor binding for an inhibitor that is supposed to be a mimic of the transition state for the reaction. One possible explanation is that at the pH of the assays, the phosphinic acid is negatively charged. Therefore there could be some destabilising interactions between the negative charge and other groups at the active site, which result in reduced binding. In order to test this theory the methyl phosphinate was also prepared.

The dimethyl ester (8) had been previously prepared *via* the β-lactone route, so attempts were made to selectively hydrolyse the carboxylate ester using lithium hydroxide. Although these proved to be unsuccessful, the desired transformation was achieved by an enzymic method. Thus incubation with pig liver esterase selectively removed the carboxylate ester giving the N-CBz compound in 44% yield. The final protecting group was then removed by hydrogenation to give the new inhibitor (10) in 67% yield.[9] This was obtained as a mixture of diastereomers, as there was no control over the stereochemistry at phosphorus, and used in this form.



SCHEME 4

Further kinetics showed that the methyl phosphinate was also a competitive inhibitor of kynureninase (Figure 1). The K_i was 0.88 ± 0.05 mM, implying improved binding relative to the free acid, by almost five fold. However, the inhibitor still bound more weakly than kynurenine itself. Therefore methylation does indeed improve binding, presumably by removing the negative charge and hence its interaction with the active site. Similar differences have been observed with phosphonamidate inhibitors of HIV-1 protease, although smaller improvements have been found in this case.[10]

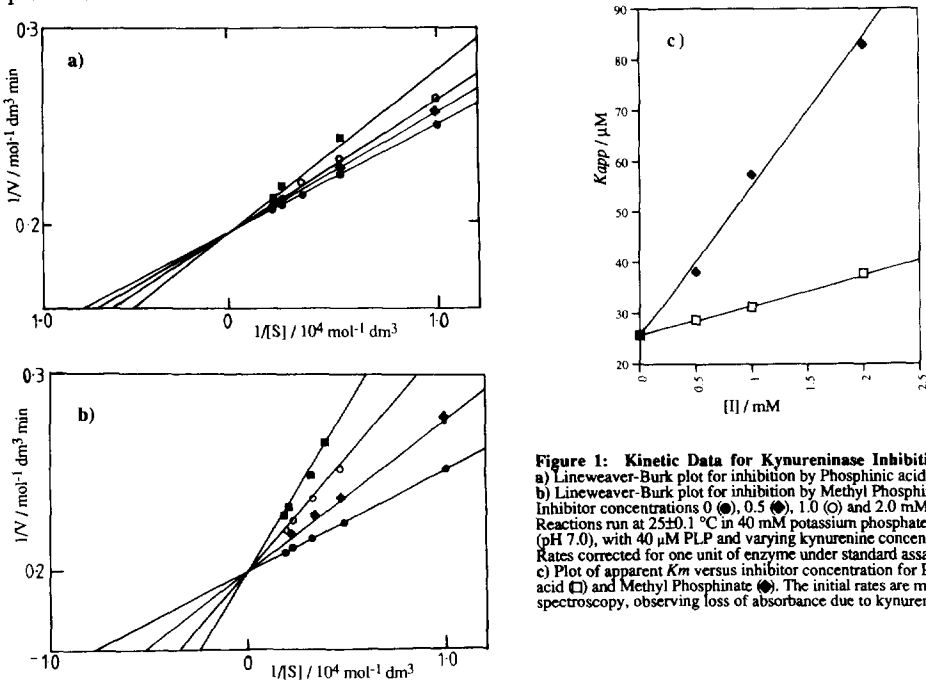


Figure 1: Kinetic Data for Kynureninase Inhibition
a) Lineweaver-Burk plot for inhibition by Phosphinic acid (5).
b) Lineweaver-Burk plot for inhibition by Methyl Phosphinate (10)
Inhibitor concentrations 0 (●), 0.5 (◆), 1.0 (○) and 2.0 mM (■).
Reactions run at 25±0.1 °C in 40 mM potassium phosphate buffer (pH 7.0), with 40 μM PLP and varying kynurenine concentration.
Rates corrected for one unit of enzyme under standard assay conditions.[11]
c) Plot of apparent *Km* versus inhibitor concentration for Phosphinic acid (□) and Methyl Phosphinate (◆). The initial rates are monitored by UV spectroscopy, observing loss of absorbance due to kynurenine at 360 nm.

Therefore it can be seen that both compounds are indeed inhibitors of kynureninase. Interestingly neither bind as well as the sulfone transition state analogue, *S*-(2-aminophenyl)-L-cysteine *S,S*-dioxide, prepared recently by

Phillips and Dua, which had a K_i of 70 nm.[12] The reduced binding may in part result from the absence of the *ortho*-amino group. The K_i for racemic desaminokynureine under our conditions was 23.4 ± 0.8 μM and the rate of turnover was *ca.* 100 fold less than that of kynurenine, implying that k_{cat} is reduced 100 fold. Thus it might be predicted that the *ortho*-amino substituted analogue of the methyl phosphinate would have a K_i of *ca.* 8.8 μM, still a hundred times greater than that of the sulfone. Interestingly, with the *S,S*-dioxides an *ortho*-amino group gave a 55-fold increase in the observed K_i value.[12] Whether the poor binding of the phosphinic acid transition state analogues has any implications for the proposed general base catalysed mechanism of kynureninase is as yet unclear. However, further mechanistic studies are underway in our laboratory to investigate the mechanism in more detail.

ACKNOWLEDGEMENTS. We wish to thank the Royal Society of Edinburgh/SOED for a Personal Research Fellowship (to NPB), MSD for a CASE studentship (to FCR), the EPSRC Mass Spectrometry Service at Swansea, and the University of St. Andrews for their generous support.

REFERENCES AND NOTES

¶ Current address; Wyeth-Ayerst, 865 Ridge Road, Monmouth Junction, NJ 08852, U.S.A.

1. Soda, K.; Tanizawa K.; *Adv. Enzymol.*, **1979**, *49*, 1-40.
2. Stone, T. W.; Connick, J. H.; *Neuroscience*, **1985**, *15*, 597-615.
3. Lipton, S. A.; *Trends in Neurosciences*, **1992**, *15*, 75-79.
4. Botting, N. P.; *Chem. Soc. Rev.*, **1995**, *24*, 401-412.
5. Tanizawa, K.; Soda, K.; *J. Biochem.*, **1979**, *86*, 1199-1209.
6. Arnold, L. D.; Kalantar, T.; Vederas, J. D.; *J. Am. Chem. Soc.*, **1985**, *107*, 7105-7109.
7. m.p. 210-212° C; (Found: C, 47.45; H, 5.45; N, 6.03; $[M + H]^+$, 230.0573. $C_9H_{13}NO_4P$ requires C, 47.17; H, 5.28; N, 6.11%; $[M + H]^+$, 230.0582); $[\alpha]_D^{20}$ +10.1° (c 0.4 in H_2O); υ_{max} (nujol)/cm^{-1} 3320 (OH acid), 1680 (CO acid), 1130, 1150 (P=O, R_2(OH)P=O); δ_H (200 MHz, ^2H_2O) 2.3 (2H, m, -PCH_2-), 4.0 (1H, m, α-H), 7.6 (5H, m, Ph); δ_C(74.76 MHz, ^2H_2O) 31.68 (d, C-3, J_{PC} 90 Hz), 50.07 (s, C-2), 129.50 (d, *ortho*-aromatic, J_{PC} 12 Hz) 131.63 (d, *meta*-aromatic, J_{PC} 10 Hz), 132.69 (s, *para*-aromatic), 135.17 (d, quat. aromatic, J_{PC} 130 Hz), 172.46 (s, CO2H); δ_P (121.49 MHz, ^2H_2O) 29.43; *m/z* (FAB) 230 ($[M+H]^+$, 12%).
8. Hayaishi, O.; Stanier, R. Y.; *J. Biol. Chem.*, 1952, **195**, 735-740.
9. m.p.188 °C (dec.); υ_{max} (nujol)/cm^{-1} 3320 (OH acid), 1690 (CO acid), 1225 (P=O, R_2(OH)P=O); δ_H (200MHz, ^2H_2O) 2.5 (2H, m, -PCH_2-), 3.6 (3H, d, $POCH_3$, J_{PH} 10Hz), 4.1 (1H, m, α-H), 7.35-7.80 (5H, m, Ph); δ_C(74.76 MHz, ^2H_2O) 29.63 (d, C-3, J_{PC} 100Hz), 49.45 (s, C-2), 52.57 (d, $POCH_3$, J_{PC} 5.6Hz), 128.74 (d, *ortho*-aromatic, J_{PC} 12Hz) 130.83 (d, *meta*-aromatic, J_{PC} 10Hz), 131.76 (s, *para*-aromatic), 133.06 (d, quat. aromatic, J_{PC} 169Hz), 172.27 (s, CO2H); δ_P (121.49 MHz, ^2H_2O) 47.94, 48.59; *m/z* (CI) 244 ($[M+H]^+$, 100%).
10. McLeod, D. A.; Brinkworth, R. I.; Ashley, J. A.; Janda, K. D.; Wirsching, P.; *Bioorg. Med. Chem. Lett.*, 1991, **1**, 653-658.
11. Phillips, R. S.; Dua, R. K.; *J. Am. Chem. Soc.*, **1991**, *113*, 7385-7397.
12. Dua, R. K.; Taylor, E. W.; Phillips, R. S.; *J. Am. Chem. Soc.*, **1993**, *115*, 1264-1270.

(*Received in Belgium* 16 *August* 1996; *accepted* 8 *October* 1996)