THE IDENTIFICATION OF NOVEL ANTILEPROTIC DRUGS FROM A STUDY OF TYROSINASE INHIBITORS USING A RAPID IN-VITRO SCREENING TEST

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Tyrosinase occurs widely in plants and animals, and of particular interest to us is the identification of the presence of a tyrosinase enzyme or other 3,4-dihydrophenylalanine (dopa) oxidising system in M. leprae (Prabhakaran, 1977). This 'enzyme' provides a rational target for the development of novel anti-leprotic drugs. (Hooper, 1985). However since M. leprae cannot be cultivated in vitro this source of the 'enzyme' is impracticable. We therefore chose to use mushroom tyrosinase as the basis of an inexpensive in vitro screen suitable for the rapid evaluation of large numbers of compounds for possible inhibitory effect or ability to form potentially toxic metabolites with this enzyme.

Tyrosinase inhibition was estimated spectrophotometrically using mushroom tyrosinase (Sigma) in phosphate buffer (0.5M, pH 6.5) with dopa as substrate in slight excess with respect to tyrosinase, and a molar ratio of 2:1 test compound to dopa was used. Enzymic activity was measured by monitoring the formation of oxidation products at 470nm or 300nm. Inhibition was determined by a comparison of the oxidation rates in the presence and absence of the test compound. A similar method was used to check for metabolism of test compounds, excluding dopa from the system. The antileprotic drugs dapsone, rifampcin, thiambutosine and the experimental drug deoxyfructo-serotonin all showed inhibitory activity in this screen. 145 compounds, obtained by synthesis or available commercially were screened and 30 compounds showed more than 50% inhibition whilst 16 were significantly metabolised.

In particular we have identified an extensive range of structure-activity relationships in a variety of carboxylic acids. For significant inhibitory activity it is necessary to have, i) a planar carboxylate group (the tetrahedral phosphonate and sulphonate groups greatly reduce activity), ii) the carboxylate group must be linked directly to a co-planar phenyl ring (benzoic acid derivatives) or through an intervening planar trans ethene group (cinnamic acids) or linear ethynyl group (phenylpropiolic acids). Activity is greatly reduced if a tetrahedral methylene group is used as a link (phenylacetic acids); iii) the single most important factor enhancing inhbitory activity is the lipophilicity of the substituents in the 4-position of the phenyl ring. This is true whether the substituent is electron releasing (CH_3) or electron withdrawing (CF_3); iv) steric factors are important, ortho substitution in the phenyl ring reduces activity (salicylic acids) and naphthalene-1-carboxylic acid is a much weaker inhibitor than the 2-carboxylic acid; v) reducing the size and lipophilicity of the aromatic ring leads to reduced inhibitory activity, the order being thiophene > pyrrole > furan which is the same order as ring resonance energies and lipophilicities. Enzyme kinetic studies show that some of these compounds act as competitive inhibitors,others as non-competive inhibitors, and others as mixed inhibitors.

6 of the more potent inhibitors have been submitted for evaluation against M.leprae in a tissue culture system (Mahadevan, et al, 1984). To date, 3 compounds show appreciable antimicrobial activity.

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