INTERACTION WITH 4-NITROANISOLE DEMETHYLASE BY SOME PYRROLIDINE-2,5- AND IMIDAZOLIDINE-2,4-DIONES

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In a previous report, we demonstrated that close structural analogues of aminoglutethimide (1), namely 3-(4-aminophenyl)-3-ethylpyrrolidine-2,5-dione (2) and 5-(4-aminophenyl)-5-ethylimidazolidine-2,4-dione (3) differed markedly in their inhibition of human placental aromatase (Daly et al, 1984). We have further studied the inhibitory activity of mixtures of enantiomers of these and structurally related agents on another cytochrome P-450 isozyme, namely rat hepatic microsomal 4-nitroanisole demethylase. The structures of the compounds studied are shown in figure 1. The demethylase system used was that described by Netter and Seidel (1964), in which 4-nitroanisole is converted to 4-nitrophenol via a hepatic cytochrome P-450-dependent oxidative metabolic pathway. Liver microsomes, prepared from male rats (250-300g), were added to assay tubes containing 4-nitroanisole (1 μM, Km 0.27μM), co-factors (NADP 0.5μM; glucose-6-phosphate 10μM; Mg^{2+} 12.5μM in 50mM phosphate buffer, pH 7.4) containing test compounds (added in ethanol). The reaction was terminated after 20 min with 20% trichloroacetic acid, the mixture centrifuged and the supernatant made alkaline with 4N NaOH. The absorbance of the solution was measured at 405nm, and enzyme kinetics analysed by Eadie-Hofstee plots using least squares linear regression. The results obtained are shown in table 1.

FIGURE 1

COMPOUND	R	X
1	AMINOGLUTETHIMIDE	
2	H	CH_2
3	H	NH
4	CH_3	CH_2
5	CH_3	NH

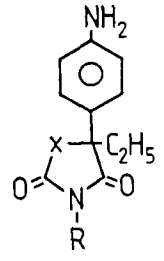

TABLE 1

COMPOUND	KINETICS	% INHIBITION
1	NON-COMPETITIVE	57
2	NON-COMPETITIVE	47
3	NON-COMPETITIVE	36
4	VARIABLE	24
5	****	10

(% inhibition - Refers to percent inhibition at an inhibitor concentration of 25μM after 20min. **** - Kinetics not determined)

Each compound inhibited the nitroanisole reaction, with the exception of 5. Compound 4 displayed variable kinetics, acting as an uncompetitive inhibitor at low, and as a non-competitive inhibitor at high concentration, due to N-demethylation *in vitro* to 2. Therapy with 1 has been demonstrated to induce the metabolism of many co-administered drugs, reflective of affinity of binding to cytochrome P-450, with potentially serious clinical results (Lonning et al, 1984). Our findings indicate that the new compound 4 would be a weaker inducing agent than 1, which would undergo N-demethylation *in vivo* to the active aromatase inhibitor 2. The reduced affinity of 4 for binding to cytochrome P-450 indicates that this agent may possess therapeutic advantages over 1.

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