AN INVESTIGATION INTO THE MECHANISM OF ACTION OF CLAZAMYCIN AS AN ANTIBACTERIAL AGENT TOWARDS *PSEUDOMONAS AERUGINOSA*

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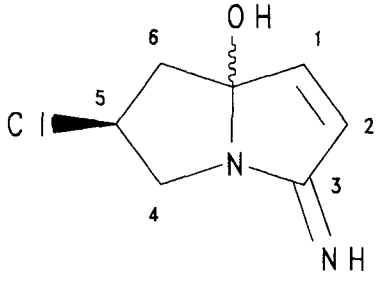
Clazamycin is a pyrrolizidine natural product produced by Streptomyces species that possesses antiviral, anti-pseudomonal and antitumour activity (Dolak et al 1980; Umezawa et al 1979).
It has been shown to react with nucleophiles at the C1-position via a novel Michael-type reaction (Thurston and Buechter, 1987). The objective of this work was to extend the studies with model nucleophiles to include amino acids, investigating their effect on the antibacterial activity of clazamycin towards P.aeruginosa. First, preliminary studies on the minimum inhibitory concentration (MIC) of clazamycin towards P. aeruginosa in the presence of selected amino acids was undertaken (Table 1).

Table 1

AMINO ACIDS	MIC(μg/ml)
control	20-24
alanine	28-32
methionine	28-32
lysine	24-30
serine	30-36
cysteine	>60
glutathione (tripeptide)	>60



Clazamycin

A concentration range of clazamycin was prepared by serial dilution in sterile tubes. A standard volume of culture medium (Davis and Mingioli medium containing sodium acetate) and the selected amino acids were added and the tubes inoculated with an overnight culture of P.aeruginosa (30μL/mL). The tubes were incubated and examined for turbidity to determine the MIC range.
The results indicate a significant rise in the MIC value for cysteine and glutathione, suggesting that clazamycin interacts with thiol functionalities, probably via Michael-type addition, to form inactive adducts.
Second, an investigation of the stoichiometry of the reaction between clazamycin and both cysteine (thiol nucleophile) and methionine (non-nucleophilic control) was carried out.
Solutions containing an appropriate concentration range of methionine or cysteine were used to establish the lowest concentration necessary to prevent the effect of an inhibitory concentration of clazamycin (32μg/ml). Values of 600-800 and 6-8μg/ml were found for methionine and cysteine respectively. From previous experiments, the MIC value for clazamycin in Davis and Mingioli medium (with no amino acids present) is 20-24 μg/ml. Therefore, the amino acid need only neutralise approximately 10 μg/ml (ie. 32-22 μg/ml) of clazamycin to abolish the inhibitory effect. In terms of molar equivalents, the experiment indicates a ratio of clazamycin:cysteine of approximately 1:1 and 1:104 for clazamycin:methionine. These results indicate that cysteine and clazamycin react in an approximate molar ratio of 1:1, which is consistent with the first experiment and the formation of a Michael-type adduct between clazamycin and cysteine.
Overall, these results support the notion that clazamycin exerts antimicrobial activity through alkylation of biological nucleophiles.

Dolak, L.A., DeBoer, C. (1980) J. Antibiot. 33: 83-84
Umezawa, H. et al (1979) J. Antibiot. 32: 762-764
Thurston, D.E., Buechter, D.D. (1987) J. Pharm. Pharmacol.(Suppl) 39: 111p