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COMPARATIVE *in vitro* ANTIBACTERIAL ACTIVITY OF HETRO VINYL OXAZINO COUMARIN DERIVATIVE

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ABSTRACT

The *in vitro* activity of hetero vinyl oxazino coumarin derivative has been compared with those of seven antibiotics (β-Lactam and non β-lactam) against 40 multiple resistant becteria from clinical samples. These included strain of *Escherichia coli, Staphylococcus aureus, Klebsiella spp, Pseudomonas aeruginosa, Proteus vulgaria and P. mirabilis*. The test compound was the most active agent against all these bacteria, while the other antibiotics employed could not exhibit promising activity. The LD_{50} value of the compound was also determined.

INTRODUCTION

The continuing problem of the multiple resistance of clinically important bacteria of β-Lactam antibiotics as well as other non-β Lactam antibiotics has encouraged the global medicinal chemists and microbiologist to search for more effective inhibitors of these organisms. Over the last five decades enormous work has been done to discover noval β-lactum and non β-lactam antibiotics against multiple resistant bacteria[1,2,4-9,13,14]. The useful biological properties of coumarins have been outlined by some earlier workers[7,10]. Also reports have been published in the been few years on the antibacterial, antimycobacterial and antiprotozoal activities of various coumarin derivatives[16,17]. However the reports on antimicrobial activity of coumarins against multiple drug resistant bacteria are too little and fragmentary. In the present study we have screened the newly synthesized coumarin dertivative[17], 9-hydroxy-ethyl-4-[2-(5'-nitro-2'-fury) Vinyl] - 8,10 - dihydro-2H-pyrano [2,3-f] [1,3] benzoxazin - 2- ones, against various clinically resistant bacteria.

MATERIAL AND METHODS

Organisms

Clinical isolates of *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella spp, Proteus vulgaris and P. mirabilis* resistant to variety of β-lactam and non β-lactam antibiotics were chosen of study. All these strains were collected from Osmania General Hospital and Osmania Medical College, Hyderabad, India. They were identified according to conventional criteria[12] and tested for β-lactamse activity following iodometric method described by Catlin[3]. All the organisms were maintained in stock by periodical subculturing onTrypticase Soy Agar (TSA).

Antibiotics

The test compound, coumarin derivative was synthesized by the fundamental chemistry section of our IDPL Research Center, Hyderabad. The following standard antibiotics were employed for comparative studies : sodium methicillin (Bristol Laboratories, Syracuse, NY), ampicillin anhydrous (Larks S.P.S. Millan Italy), chloramphenicol (Park - Davis, India), doxycycline (IDPL, Hyderabad, India). Cephalexin (IDPL, Hyderabad, India), sodium penicillin (IDPL, Rishikesh, India), streptomycin (IDPL, Reshikesh, India). These were supplied by the makers in powder form with known potencies, stored in dry atmosphere, and reconstituted according to the manufacturer's recommendation with adjustments for potency.

Minimum Inhibitory Concentration (MIC)

The drugs were dissolve in appropriate

solvents (20 mg/2ml), further diluted in sterile distilled water and finally incorporated into Trypticase Soy Broth (TSE) and distributed into tubes. The control tubes received the same amount of the solvent present in those with the highest concentration of the testing agent. Inocula were prepared from 6 hr. old broth cultures diluted such that the such final inoculum size was approximately 10^5 colony forming units (CFUs) per ml. The inoculated tubes were incubated at 37°±1°C for 24 hrs. The minimum inhibitory concentration (MIC) was defined as the lowest amount of drug completely inhibiting the growth of bacteria which was evaluated by visual inspection.

Determination of LD_{50}

Swiss albino mice (male 18-22 g) were used. They were housed in air-conditioned room (22±0.5°C) with food in pelletes and tap water. The test compound was administered by oral route and intraperitoneally in 2% *Gum acacia* suspension in varying doses. Groups of 10 animals were used for each does and after an observation of 24 hrs the number of survivors was recorded and LD_{50} of the compound was determined[11]. The animals were anaesthetized and necropsy was done to see the presence of the compound in stomach.

RESULTS AND DISCUSSION

The coumarin derivative showed the greatest degree of in vitro activity, inhibiting all the strains of *Pseudomonas, Proteus, Escherichia, Staphylococcus* and *Klebsiella*. All these strains were found to be highly resistant to sodium penicillin, ampicillin, methicillin, chloramphenicol, streptomycin, doxycycline and cephalexin as shown in table 1.

The test compound was most active against the clinically important *S. aureus* cultures inhibiting at a concentration ranging from 0.025 to 10 μg./ml. All the β-lactam antibiotics used were least effective against the gram-positive bacterium with MIC values 100-1000 μg./ ml. Cephalexin, which is of third generation of cephalosporins did not show any activity even at 1000μg/ml. Chloramphenicol and doxycyclins showed moderate activity with MIC values 100 and 500 μg./ml respectively where all the ten strains were inhibited.

Against enteric Gram - negative cultures the test compound showed significant activity as compared to the seven antibiotics studied. It was quite against all the strains of *E.coli, Klebsiella, P. vulgaris* and *P. mirabilis*. There is relatively less difference in their respectively MIC values against these organisms with a range of 0.25-20 μg./ml. In case of *E.coli* the trend of activity reflects more or less the same as evidenced in case of *S.aureus.* Cephalexin and methicillin did not posses any inhibitory effect upto 1000 μg/ml against all the six strains tested. Penicillin and ampicillin exhibited weak activity with MIC values ranging from 100 to 1000 μg/ml., followed by chloramphenicol and streptomycin with the value in the range of 50 - 1000 and 50-500 μg./ml) respectively. Of all the antibiotics, doxycyline only appears to be somewhat effective against. *E.coli* (50-100 μg/ml.), while and test compound was at least 20 fold more active than doxycyline.

The coumarine compound was also found active to the three strains of *Klebsiella spp.* inhibiting in the range of 0.25 - 5 μg/ml chloramphenicol and strephtomycin were found to be moderatively active MIC value ranging from 50-100 μg/ml. All other β-lactam antibiotics least active to this bacterium with MIC values ranging from 50 1000 μg/ml.

The test compound showed greater activity (MIC 5- 10 μg/ml) against five strains of *Proteus* though the MIC values was not so low as it was in the case of *S. aureus, E.coli* and *Klebsiella spp*. Most of the cultures were highly resistant to cephalexin, ampicillin and methiclin as they could not be inhibited even upto 100 μg./ml of the drug concentrations. Rest of the drugs of the showed moderate activity with doxycyline having an edge over others.

The lest compound was the most active against *Pseudomonas aeruginosa* which is normally observed to be highly resistant to majority of the antibiotics. All the sixteen strains were inhibited by the test compounds in the range of 0.1 - 30 μg./ml, while they were found to be resistant to cephalexin, penicillin, ampicillin, methicillin, chloramphenicol and streptomycin even at 1000 μg/ml concentration. Only doxycycline showed slightly better activity over the others with its MIC values between 50 - 100 μg/ml.

The LD_{50} of the test compound was 58.4 μg/kg body weight when administered intraperitoneally . Threre was no mortality in animals when a dose of 1000 μg/kg body weight of the compound was given per oral, but the

Table - 1: Comparative efficacy of antibiotics with hetero/vinyl oxazino coumarin (HOC 13) derovatove against resistant bacteria

Organism	Number of Strains	Agent	MIC Range µg/ml
Escherischia coli	(6)	Sodium Penicillin	100-1000
		Ampicillin	100-1001
		Sod. Methicillin	1000
		Chloramphenicol	50-1000
		Streptomycin	50-500
		Doxycycline	50-100
		Cephalexin	1000
		HOC - 13	25-20
Staphylococcus aureus	(10)	Sodium Penicillin	100-1000
		Ampicillin	50-1000
		Sod. Methicillin	50-1000
		Chloramphenicol	50-100
		Streptomycin	50-1000
		Doxycycline	50-500
		Cephalexin	1000
		HOC - 13	0.025-10
Klebsiella	(3)	Sodium Penicillin	100-1000
		Ampicillin	50-1000
		Sod. Methicillin	50-1000
		Chloramphenicol	50-100
		Streptomycin	50-100
		Doxycycline	25-50
		Cephalexin	500
		HOC - 13	0.25-5
Pseudomonas aeruginosa	(16)	Sodium Penicillin	100-1000
		Ampicillin	100-1000
		Sod. Methicillin	1000
		Chloramphenicol	100-1000
		Streptomycin	500-1000
		Doxycycline	50-500
		Cephalexin	1000
		HOC - 13	0.1-30
Proteus vulgaris	(3)	Sodium Penicillin	500-1000
		Ampicillin	1000
		Sod. Methicillin	1000
		Chloramphenicol	100-1000
		Streptomycin	50-1000
		Doxycycline	50-500
		Cephalexin	1000
		HOC - 13	5-10
P. mirabilis	(2)	Sodium Penicillin	500-1000
		Ampicillin	50-1000
		Sod. Methicillin	50-1000
		Chloramphenicol	50-1000
		Streptomycin	50-100
		Doxycycline	50
		Cephalexin	1000
		HOC - 13	5-10

Each datum in table in average of four independent determinations

absorption of the compound was found to be incomplete at this dose.

In conclusion, the interesting *in vitro* activity of hetero vinyl oxazino coumarin derivative against a wide range of organisms and the low incidence of resistance suggest the possibility of its becoming a useful chemotherapetuic agent, even in the treatment of infection with multi - resistant organisms.

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REFERENCES

1. Abraham, E.P. and Newton, G.G.F. A comparison of the action of Pennicillinase on benzylpenicillin and cephalosporin N and the competitive inhibition of penicillinase by cephalosporin C. *Biochem*. J., **63**:628 - 634 (1956)
2. Bucourt, R. Synthetic or hemisynthetic pathways. An open question for the routes to cephalosporins of the new generation. In Recent advance in chemistry of β- lactam antibiotics; 1-25 (the Royal society of chemistry, London 1980)
3. Catline B.W. Idometirc detection of Haemophilus influenzae β-lactamase Rapid presumptive test for ampicillin resistence. *Antimicrobial agent and chemotherapy*, **7**(**3**):265-270(1975)
4. Cole, M. Inhibition of β-lactam. In β-lactomases (ed. J.M.T. Himilton miller & J.T. Simith_Ch 9 : 205-289 (New York/ London : Academic Press 1979)
5. Cole, M. Inhibitors of antibiotics - inactivating enzymes. Antibiotic interactions (Ed. J.D. Williams), 99-135 (London ; Academic Press 1979 b)
6. Cole, M Elson S. and Fullbrook, P.D. Inhibition of the beta - lactamases of *Escherichia coli* and *Klebsiella aerogens* by semisynthetic pencillins, *Biochem. J.,* **127**; 295-308 (1972)
7. Feur,G. The mechanism and biological action of coumarins. In Ellis, G.P. west, G.B. (Eds.) *Progress in Medicinal Chemistry*. **10** : 85-158 (1974)
8. Ganguly, A.k. Girizavallabhan, V.M., Micombie, S., Pinto, P. Rizvi, Jeferry, P.D. and Lin, S. Synthesis of Sch 29482 - a noval penem antibiotic. *Journal of Antimicrobial Chemotherphy,* **9** : Suppl. C., 1 -5 (1982)
9. Hamashima, Y. Matsumara, H., Matsuura, S., Nagata,W. Narasida, M and Hoshida, T. Synthesis and Structure Activity Relationship of 1 - oxacephem derivatives. In Recent advance in the chemistry of β- lactam antibiotics, 57-79. (The Royal society of chemistry. London. 1980)
10. Kulkarni M.V. Vemanna, D.P., Vasant N.B. and siddaiah Nanjappa. Synthesis and biological properties of some 3 - heterocyclic substituted coumarins. *Arch. Pharm*. **314**(**5**): 435 - 439 (1981)
11. Litchefield J.T. Jr. and wilcoxon, F.A. simplified methods of evaluating dose response experiments. *Journal of pharmacology and experimental therapy,* **96** : 99-113 (1940)
12. Mac Faddin, J.F. Biochemical tests for identification of medical bacteria. Williams a Wilkins, Baltimore/London (1980)
13. Muggleton, P.W. why ceftazidime? *Journal of Antimicrobial Chemotherapy,* **8** : B, 1-3 (1981)
14. Piller, N.B. The resolution of thermal oedema at various temperatures under coumarin treatment. *British Journal of Experimental Pathology,* **56** : 83 -91 (1975)
15. Ralinson G.N. Stevens, S., Batchelor, F.R. Cameron - wood, J.and chain, E.B., *Bacteriological studies on a new pencillin - BRL 1241* (Celbenin). Lancet ii : 564 - 567, (1960)
16. Shridhar, D.R. Lal B., Vaidya, N.K., Bhopale, K.K. and Tripathi, H.N. Synthesis and biological activity of some 3 - [2-(Heteroaryl) - vinyl] - 2 H-I, 4 - benzoxazin -2-ones. *Indian Journal of chemistry*. **18B**. 251-253 (1979)
17. Shridhar, D.R. Reddy Sastry, C,V, Lal, B., Reddi G.S. Bhopale, K.K., Khokar, R.S. and Tripahti, K. Synthesis and biological activity of 9-substituted 4-[2-(5'-Nitro-2'-furyl-&5' nitro - 2' thinyl)vinyl]-8,10-dihydro-2/H-pyrano [2,3,-f] [1,3] - benzoxazin -2- ones. *Indian Journal of Chemistry*, **198** : 1065 - 1067 (1980)