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Synthesis and antimicrobial activity of some substituted hydroxytriazenes

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ABSTRACT

In the present study, six substituted hydroxytriazenes were synthesized and confirmed by their elemental analysis. These synthesised hydroxytriazenes were screened for their antibacterial and antifungal activities. These compounds have shown very encouraging results showing inhibition zone from 15 mm to 45 mm against four bacterial and two fungal stains. Thus the study has brought about a novel application of this class of analytical reagents as an emerging class of bioactive chemicals.

INTRODUCTION

Hydroxytriazenes are well known chelating agents, having been widely used as both spectrophotometric and complexometric reagents for transition metal determinations. Their application as analytical reagents is quite established as shown by various reviews[1-3]. Recently a number of hydroxytriazenes have been screened for their insecticidal activity against Drosophila melanogaster Meig as well as for antimicrobial activities[4-7]. Very encouraging LC_{50} values, obtained for one of these compounds have further enhanced possibility of their use as bioactive compounds. In view of this in the present investigation six hydroxytriazenes were synthesized and screened for their antibacterial and antifungal activity against four bacterial and two fungal stains viz. *Escherichia coli; Salmonella typhi; Peudomonas aeruginosa; Bacillus subtilis; Aspergillus fumigatus, Candida albicans.*

EXPERIMENTAL SECTION

Synthesis of hydroxytriazenes

There are three methods for the synthesis of hydroxytriazenes reported in the literature. The first method involves reduction of nitrosobenzene or substituted nitrosobenzene with phenyl or substituted phenylhydrazenes to give hydroxytriazenes[8,9]. In the second method an alkyl or arylhydroxylamine is coupled with diazonium salt at 0-5^{o}C in 1:1 molar ratio to give the corresponding hydroxytriazenes[10-13]. The third method involves oxidation of diazoaminobenzene with peroxy benzoic acid under mild conditions[14].

The second method has advantages over other two methods in term of better yield and ease of preparation. Thus all the six hydroxytriazenes were synthesized using second method i.e. coupling an alkyl or aryl hydroxylamine with diazonium salt at 0-5^{o}C in 1:1 molar proportion, in acetate buffer medium of pH 5.0. The purity of each hydroxytriazenes was checked by physical characteristics and elemental analysis (Table 1).

Assay for antibacterial activity:

In vitro antibacterial activity was tested against four bacterial stains; *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Bacillus subtilis***.** These bacterial stains were obtained from Dept. of Microbiology, RNT Medical College, Udaipur.

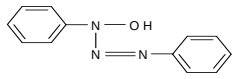
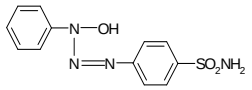
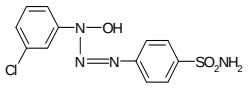
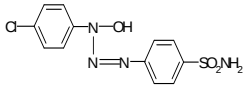
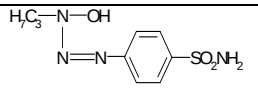
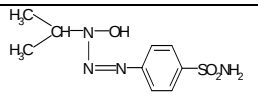
Antibacterial activity was tested by using agar well method[15] (cup plate method). The selected strains of bacteria were inoculated into 10 ml of sterile nutrient broth, and incubated at 37ºC for 16-18 hours. Using a sterile cotton swab, the nutrient broth cultures were swabbed on the surface of sterile nutrient agar plates. Eight wells were prepared with the help of sterilized cork borer with 8 mm diameter on inoculated agar plates. Using a micropipette, 100 µlitre solution of different hydroxytriazene compounds (HT-1, HT-2, HT-3, HT-4, HT-5 and HT-6) was added to different wells in the plate. Standard drug amikacin disk (30 µg) was placed in one well on the agar plate. The plates were incubated in an upright position at 37ºC for 24 hours. The diameter of zone of inhibition was measured in mm and the results were recorded. The inhibition zones with diameter less than 10mm were considered as having no antibacterial activity[16].

Assay for antifungal activity:

In vitro antifungal activity was tested against two fungi; *Aspergillus fumigatus* and *Candida albicans.* These fungal stains were obtained from Dept. of Microbiology, RNT Medical College, Udaipur.

Stock culture of fungal stains was maintained in sabouraud broth for 24 hrs. Sabouraud agar was used as the medium for antifungal assay by cup – plate method. Sabouraud agar plates were prepared and the proper concentration of fungal stain was spread on the plates. Wells were prepared with the help of sterilized cork borer with 8 mm diameter. In wells 100 µlitre of hydroxytriazene compounds solution (HT-1, HT-2, HT-3, HT-4, HT-5 and HT-6) and standard drug, Amphotracin B were poured by micropipette. The plates were incubated at 27 ^{o} – 28^{o} C for 48 hrs. The diameter of zone of inhibition was measured in mm and the results were recorded[17].

Table 1: Characterization data of the hydroxytriazenes

Compound	Structure	Colour and crystal shapes	Elemental analysis				M.P. (^{0}C)
				% C	% H	% N	
HT-1		Pale yellow niddle shaped crystals	Th Ex	67.60 67.60	5.16 5.18	19.72 18.70	119
HT-2		Light occur powdery mass	Th Ex	49.28 47.29	4.13 5.34	19.17 19.63	170
HT-3		Light yellow powder	Th Ex	44.10 42.88	3.36 3.00	17.15 16.36	161
HT-4		Light brown off white powder	Th Ex	44.10 44.98	3.36 3.30	17.15 16.30	150
HT-5		White shinning crystals	Th Ex	41.86 41.65	5.42 5.63	21.70 21.88	180
HT-6		White shinning microcrystals	Th Ex	41.86 42.28	5.42 5.49	21.70 21.53	190

Th: Theoretical; Ex: Experimental; C: Carbon; H: Hydrogen; N: Nitrogen

Table 2: Antimicrobial activity of hydroxytriazenes on bacterial and fungal stains

Stains	Zone of inhibition (mm)						
	HT-1	HT-2	HT-3	HT-4	HT-5	HT-6	Standard
Bacterial species							**Amikacin**
1. *E. coli*	16	17	25	18	15	21	28
2. *S. typhi*	18	17	22	17	19	17	24
3. *P. aeruginosa*	17	19	17	19	17	20	23
4. *B. subtilis*	15	16	16	19	18	20	27
Fungal species							**Amphotracin**
1. *A. fumigatus*	26	29	31	40	35	31	42
2. *C. albicans*	27	35	36	42	38	36	45

Abbreviations: E. Coli, Escherichia coli; S. typhi, Salmonella typhi; P. aeruginosa, Peudomonas aeruginosa; B. subtilis, Bacillus subtilis; A. fumigatus, Aspergillus fumigatus; C. albicans, Candida albicans. Standard; Amikacin: Bacterial species and Amphotracin: Fungal species

RESULTS AND DISCUSSION

The study showed that the hydroxytriazene compounds (HT-1, HT-2, HT-3, HT-4, HT-5 and HT-6) have antimicrobial activity against all four bacterial organisms and two fungal organisms at the concentration of 30 µg. In bacterial organisms, the zone of inhibition observed for hydroxytriazene compounds varied from 15 mm to 25 mm on various species of bacteria. The results indicate that the compounds have more antimicrobial activity against gram-negative organism than gram-positive. But these compounds show less antibacterial activity than the standard amikacin, which showed zone of inhibition 23 mm to 28 mm. In fungal organisms, hydroxytriazene compounds showed good antifungal activity, which was nearly equal to the

standard drug, amphotracin B. The zone of inhibition observed was from 26 mm to 45 mm. (Table 2).

The actual antimicrobial mechanism of hydroxytriazene compounds (HT-1, HT-2, HT-3, HT-4, HT-5 and HT-6) cannot be elucidated but a postulate can be drawn on the basis of the antimicrobial study conducted.
Hydroxytriazenes may act as chelating agents, due to the virtue of donor sites present in this chemical moiety i.e. oxygen and nitrogen. On the structural elucidation of different hydroxytriazene compounds, those hydroxytriazene compounds having sulphonamide group were found to be more active therefore it could be predicted that antimicrobial activity is due to the presence of sulphonamide group.

Antimicrobial agents normally act in one of the four ways to disrupt the microbial processes.
1. They hamper cell wall synthesis.
2. They inhibit microbial protein and nucleic acid synthesis.
3. They disrupt microbial membrane structure and function.
4. They block metabolic pathways through inhibition of key enzymes.

Most possible explanation for the mechanism seems to be on the basis of chelating property of hydroxytriazenes i.e. they form, chelate with the ingredients of the cell wall and hamper the wall synthesis. This explanation is further strengthened by the fact that basic difference between gram-positive and gram-negative bacteria is in their cell walls. The cell walls of gram-positive bacteria consists of 20-80 nm thick peptidoglycon layer where as gram-negative wall is 1-3 nm peptidoglycon layer. Thus the penetration of hydroxytriazenes to form chelate with cell wall ingredients is facilitated in both the type of bacteria, but more in gram-negative bacteria. The substituted hydroxytriazenes also contain the sulphanilamide moiety – a structural analogue of p-aminobenzoic acid (PABA), which is essential in the synthesis of folate. Hydroxytriazenes may compete with PABA for the enzyme involved in folate synthesis and thus may inhibit the metabolism in the bacteria.

All the hydroxytriazene compounds show a prominent antifungal activity compared to antibacterial activity. This may be due to difference in the chemical composition of cell wall of fungi and bacteria. The chemical composition of fungi allows penetration and formation of a chelate with the ingredients of the cell wall. Considering the above mentioned facts it could be concluded that hydroxytriazenes can be used as a potential antifungal agent and its mechanism of action is probably similar to that of amphotracin B, which acts by binding the cell membrane and interfere with permeability and transport functions**.**

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