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Full Length Research Paper

Antibacterial and antifungal activities of novel hydroxytriazenes

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In a search for new leads potent antimicrobial agents, an array of novel hydroxytriazenes i-xi were synthesized and characterized through their melting point, crystal shape, colour and elemental analysis. *In vitro* microbiological evaluations were carried out for all the synthesized compounds against both bacterial and fungi using the turbidimetric method. The reagent number x and xi showed significant antibacterial activities against two gram positive [*Streptococcus feacalis, Staphylococcus aureus*, penicillin resistance (2500 units)]. All synthesized hydroxytriazenes except reagent number viii showed antifungal activities against five fungi [*Candida albicans, Cryptococcus neoformans, Sporotrichum schenckii, Trichophyton mantagrophytes, Aspergillus fumigatus*}. The minimum inhibitory concentration (mic) values against these bacteria and fungi ranged from <12.5 to 50 µg/ml. Some hydroxytriazenes (x, xi) showed an unusual combined antibacterial and antifungal action, which suggest that hydroxytriazenes revealed a wide range of antimicrobial activity.

Key words: Antibacterial, antifungal, antimicrobial, hydroxytriazenes, mic, turbidimetric.

INTRODUCTION

Over the past few years, health related quality of human life benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses, not only because many of them produce toxic reactions but also due to emergence of drug resistant microbes (Agwara et al., 2010; Atebda, 2003; Edewor and Usman, 2011; Gopalakrishnan et al., 2011). Hydroxytriazenes constitute an important new class of biologically active compound (Goswami, 2002; Hura et al., 2003).

Recently, a number of hydroxytriazenes were screened for their insecticidal activity against *Drosophila melanogaster* Meig (Ombaka and Gichumbi, 2011). Very encouraging LC50 values were obtained from two of these compounds and this further enhanced the possibility of their use as bioactive compounds. Hence, the present study was undertaken to determine the antibacterial and antifungal activities of eleven hydroxytriazenes.

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METHODOLOGY

Synthesis of hydroxytriazenes

The synthesis of hydroxytriazenes involves coupling of aryl diazonium salt with aryl or alkylhydroxylamine. A general scheme of the synthesis involves four steps as follows.

Preparation of aryl or alkylhydroxylamine

In the preparation of arylhydroxylamine, 0.25 moles of nitroaryl compound, 15 g of ammonium chloride, 400 ml of water, 100 ml of rectified spirit were mixed and stirred mechanically at 40°C and then 40 g of zinc dust was added in the small lots such that, the temperature of the reaction remained between 58 and 62°C. The reaction mixture was filtered under suction and the residue was washed with warm water (40°C). The filtrate was kept in refrigerator at about 0°C (Chauhan et al., 2010).

In the preparation of alkylhydroxylamine 0.3 moles of nitroalkane, 30 9 of ammonium chloride 250 ml of water were taken against 0.2 moles of aromatic primary amine. Furthermore, the temperature of reacting mixture was maintained between 0 and 15°C and the residue was washed with ice cold water (Jain et al., 2011).

Preparation of aryl diazonium salt

0.2 moles of aromatic primary amine was dissolved in a warm mixture of 50 ml concentration of HCl and 50 ml water and then stirred. 13.9 g of sodium nitrite was dissolved using 40 ml water in another beaker. Both solutions were kept in a freezer for 3 h. Thereafter, a beaker containing aromatic primary amine hydrochloride solution was transferred in an ice bath in order to keep its temperature between 0 and 5°C. To this solution, sodium nitrite solution was added drop by drop with stirring (Naulakha et al., 2009).

Coupling and crystallization

Aryl or alkyl hydroxylamine was transferred in an ice bath, and then it was mechanically stirred. To this, aryldiazonium salt was slowly added in small portions. The pH was maintained between 5 and 6 by addition of small portions of sodium acetate. The temperature of the mixture was filtered under sunction. The residue was washed under sunction. The residue was washed with ice cold water. The filtrate obtained was subjected to charcoal treatment and crystallization using appropriate solvent (Prabhat et al., 2011; Poja et al., 2011).

Microbiological assays

A number of methods for microbiological assays are available which include: (1) cylinder plate or cup-plate method (2) Turbidimetric method (3) filter paper disc method (4) agar cup method (Gangadevi et al., 2008; Hassan et al., 2009; Ilias et al., 2011; Mann et al., 2008).

Throughout these investigations, turbidimetric method was used. The method depends upon the growth of a microbial culture in a solution of antibiotic in a fluid medium that is favorable to its rapid growth in the absence of antibiotic compounds (Mahesh and Satish, 2008). This method has an advantage of a shorter incubation period for the growth of the test organism which is usually three to four hours. However, the presence of the solvent residue, affects this assay more than the cylinder plate assay (Orak et al., 2011); hence, care was taken to ensure freedom from such substance in the test solution. In the present investigations, the maximum concentration tested was 100 µg/ml in dimethylsulfoxide (DMSO) and method of serial dilution was used.

RESULTS AND DISCUSSION

Physical characteristic and elemental analysis

Table 1 shows the physical characteristic and elemental analysis of hydroxytriazenes. These compounds were either light yellow needles, light yellow shining needles, bright yellow shining needles, orange yellow needles or light yellow powder. The melting point of these compounds ranged from 62 to 180°C. Ethanol or acetone was used to crystallize these compounds. The result of C, H and N indicates that the theoretical values and experimental values were identical.

Antibacterial, antifungal activities of hydroxytriazenes

Table 2 shows the antibacterial and antifungal activity of hydroxytriazenes measured by the turbidimetric method against selected pathogenic bacteria and fungi. Reagent number x and xi showed good inhibitory effects towards *S. aureus* (mic value 25 µg/ml) as compared to other hydroxytriazenes and the results are better compared to those obtained by (Hasan et al., 2009). All hydroxytriazenes synthesized except reagent number (vii), revealed antifungal activity. Reagent number (i) showed good inhibitory effects towards *Trichophton mentagrophytes, Aspergillus fumigatus* with mic value < 12.5 µg/ml.

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.

The probable explanation for the mechanism seems to be on the basis of chelating property of hydroxytriazenes, that is, they form chelate with the ingredients of the cell wall and hamper the cell wall synthesis. This explanation is further strengthened by the fact that basic difference between gram positive and gram negative bacterial is in their cell walls. Hence, the penetration of hydroxytriazenes to form chelate with the wall's ingredient is not facilitated in case of gram negative bacteria, and thus, no activity was against them, while the absence of outer membrane in case of gram positive bacteria allows their penetration and chelate formation. Therefore, reasonable activities have been shown. Further antifungal activities were shown by all the compounds except reagent number (viii) which did not shown any activity. This is possible because fungi and bacteria have essential difference in the chemical composition of their cell walls. The chemical composition of fungi allows penetration and formation of a chelate with the ingredients in the cell wall.

Conclusion

Hydroxytriazenes are biologically active compounds that can be exploited for possibility of replacing antibiotics in the case of emergence of drug resistant microbes.

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Table 1. Physical characteristics and elemental analysis of hydroxytriazenes.

Reagent number	synthesized Hydroxytriazene	Physical characteristic			Elemental analyses						Molecular formula
		Colour and shape of crystals	Crystallized from	M.P	%C		%H		%N		
					Th	Exp	Th	Exp	Th	Exp	
i	3-Hydroxy-3-p-tolyl-1-(2,4,6-tribromophenyl)-triazene	Light yellow needles	Ethanol	126	33.63	33.41	2.17	1.58	9.05	9.51	$C_{13}H_{10}N_3OBr$
ii	3-Hydroxy-3-m-tolyl-1-(2,4,6-tribromophenyl)-triazene	light yellow needles	Ethanol	85	33.63	32.89	2.17	2.99	9.05	9.76	$C_{13}H_{10}N_3OBr$
iii	3-Hydroxy-3-m-tolyl-1-(3,4-dichlorophenyl)-triazene.	Light yellow shining needles	Acetone	136	52.70	50.14	3.74	4.42	14.19	14.47	$C_{13}H_{11}N_3OCl$
Cl	3-Hydroxy-3-o-tolyl-1-p--chloro phenyltriazene	Light yellow shining needles	Acetone	106	59.64	57.67	4.62	5.65	16.06	16.70	$C_{13}H_{12}N_3OCl$
v	3-Hydroxy-3-o-tolyl-1-o-chlorophenyltriazene	Bright yellow needles	Acetone	62	59.64	58.73	4.62	4.81	16.06	16.63	$C_{13}H_{12}N_3OCl$
vi	3-Hydroxy-3-n-propyl-1-m-chlorophenyltriazene	Light yellow shining needles	Ethanol	80	50.56	50.24	5.66	5.37	19.67	20.28	$C_9H_{12}N_3OCl$
vii	3-Hydroxy-3-n-propyl-1-m-chlorophenyltriazene	Light yellow shining needles	Ethanol	60	50.56	49.76	5.66	4.90	19.67	19.24	$C_9H_{12}N_3OCl$
viii	3-Hydroxy-3-p-tolyl-1-m-hydroxyphenyltriazene	Orange- yellow needles	Ethanol	124	64.16	63.40	5.38	5.03	17.28	17.89	$C_{13}H_{13}N_2O_2$
ix	3-Hydroxy-3-m-tolyl-1-o-nitrophenyltriazene	Light yellow powder	Acetone	180	57.32	57.74	4.44	4.26	20.58	18.78	$C_{13}H_{13}N_4O_3$
x	3-Hydroxy-3-p-tolyl-1-o-carboxyphenyltriazene	Yellow shining needles	Ethanol	179	61.96	60.96	4.83	4.66	15.49	14.67	$C_{14}H_{13}N_3O_3$
xi	3-Hydroxy-3-o-tolyl-1-o-carboxyphenyltriazene	Light yellow shining needles	Ethanol	138	61.96	60.48	4.83	4.63	15.49	14.69	$C_{14}H_{13}N_3O_3$

Th, values obtained on theoretical data; Exp, values obtained based on experimental data.

Table 2. Antibacterial and antifungal activities of hydroxytriazenes in DMSO.

Reagent number	Synthesized hydroxytriazene	Minimum inhibitory concentration (mic) in µg/ml against									
		Bacteria						Fungi			
		(i)	(ii)	(iii)	(iv)	(v)	(vi)	(vii)	(viii)	(ix)	(x)
(i)	3-Hydroxy-3-p-tolyl-1-(2,4,6-tribromophenyl)-triazene.	-	-	-	-	-	-	50	50	<12.5	<12.5
(ii)	3-Hydroxy-3-m-tolyl-1-(2,4,6-tribromophenyl)-triazene.	-	-	-	-	-	-	-	50	50	-
(iii)	3-Hydroxy-3-m-tolyl-1-(3,4-dichlorophenyl)-triazene.	-	-	-	-	-	-	-	-	50	-
(iv)	3-Hydroxy-3-o-tolyl-1-p--chloro phenyltriazene	-	-	-	-	-	-	-	-	50	-
(v)	3-Hydroxy-3-o-tolyl-1-o-chlorophenyltriazene	-	-	-	-	-	-	-	-	50	-
(vi)	3-Hydroxy-3-n-propyl-1-m-chlorophenyltriazene	-	-	-	-	-	-	-	-	25	50
(vii)	3-Hydroxy-3-n-propyl-1-m-chlorophenyltriazene	-	-	-	-	-	-	-	-	25	50
(viii)	3-Hydroxy-3-p-tolyl-1-m-hydroxyphenyltriazene	-	-	-	-	-	-	-	-	-	-
(ix)	3-Hydroxy-3-m-tolyl-1-o-nitrophenyltriazene	-	-	-	-	-	-	-	50	50	-
(x)	3-Hydroxy-3-p-tolyl-1-o-carboxyphenyltriazene	25	-	-	-	<12.5	-	-	-	50	-
(xi)	3-Hydroxy-3-o-tolyl-1-o-carboxyphenyltriazene	25	-	-	-	<12.5	50	-	-	25	50

(i) *S. feacalis* (ii) *Klebsiella pneumoniae* (iii) *Escherichia coli* (iv) *Pseudomonas aeruginosa* (v) *S. aerus* penicillin resistance (2500 units) (vi) *C. albicans* (vii) *C. neoformans* (viii) *S. schenckii* (ix) *T. mentagrophytes* (x) *A. fumigatus.*

Note: The control disc used for solvent had no zone of inhibition, so the data was omitted from the listed data.

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