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A HYDROXYETHYL-HEPTADIENYL PHOSPHINE OXIDE (HEHPO) ETHANOL SOLUTIONS WITH ANTIBACTERIAL ACTIVITY

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ABSTRACT: *Background and Purpose: Antifungal effects of a HydroxyEthyl-Heptadieyl Phosphine Oxide (HEHPO) with unprotected hydroxy group (3-Diphenylphosphinoyl-5-methtlhepta-3,4-dien-2-ol) on pathogenic Gram-positives and Gram-negatives bacteria had been established. HEHPO (50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml) exerted different inhibitory effect on different bacterial cells in vitro. The effects of HEHPO on prokaryotic cells have not been studied. The present study was aimed to assess the antibacterial activity of HEHPO on pathogenic Gram-positive and Gram-negative bacteria. In vitro antimicrobial test: Escherichia coli 3398, Staphylococus aureus 745, Bacillus subtilis 6633, Salmonella Typhimurium 3591, Listeria monocytogen 863 and Enterobacter aerogenes 3691 were treated for 24 hours with HEHPO (50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml), Sefpotec (250 mg/ml). The antibacterial activity was assayed by the well diffusion method with digital caliper.Determination of minimum inhibitory concentrations (MICs): The MIC of HEHPO, that shows antimicrobial activity, were determined by methods as described by [17] and MICs were read in µg/ml after overnight incubation at 37°C. All experiments were made in replicate. Determination of Minimum bacteriocidal concentration (MBC): The MBC were carried out to check whether the test microbes were killed or only their growth was inhibited. Nutrient Agaragar was prepared and sterilized at 121°C for 15 minutes, the medium was poured into sterile petridishes and were allowed tocool and solidify. The contents of the MIC in the serial dilution were then subcultured onto the prepared medium, incubation was made at 37°C for24 h, after which each plate was observed forcolony growth. The lowest concentration of the HEHPO without a colony growth was recorded asthe MBC. HEHPO had higher antibacterial activity than tested antibiotic– Sefpotec. Key Results: The results revealed variability in the inhibitory concentrations of HEHPO for given bacteria. HEHPO at concentration 50 mg/ml for 24 hours notably inhibited growth of Gram-negative bacteria E. coli 3398 (28.77 mm mean zone of inhibition), E. aerogenes 3691 (20.87 mm mean zone of inhibition) and S. Typhimurium 745 (20.49 mm mean zone of inhibition). HEHPO did not inhibited Gram-positive bacteria S. aureus 745, B. subtilis 6633 and L. monocytogen 863. Conclusions and Implications: Based on the results obtained we can conclude that the examined HEAPO has bactericidal activity towards pathogenic bacteria, but in different concentrations. HEHPO possesses biological activity, which is not well studied. We know only from literary data that they are used for inhibiting the biosynthesis of sterol from the pathogen responsible for Pneumocystis-carinii pneumonia (PCP) -a disease similar to AIDS [2]. In our previous studies was shown that the Bifunctionalized Allene with protected hydroxy group (Dimethyl 3-methyl-1-[1-(tetrahydro-2H-pyran-2-yloxy)-ethyl]-hepta-1,2-dienephosphonate) (BA-1) exhibited antibacterial [8] and antifungal activity [9]. The results obtained show for the first time the existence of antifungal activity of HEHPO towards various pathogenic bacteria.*

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KEYWORDS: HydroxyEthyl-Heptadienyl Phosphine Oxide (HEHPO) (*3-Diphenylphosphinoyl-5-methtlhepta-3,4-dien-2-ol),* Antibacterial activity, Antibiotic.

INTRODUCTION

Antibiotics paved the way for unprecedented medical and societal developments, and are today indispensable in all health systems. Achievements in modern medicine, such as major surgery, organ transplantation, treatment of preterm babies, and cancer chemotherapy, which we today take for granted, would not be possible without access to effective treatment for bacterial infections. Within just a few years, we might be faced with dire setbacks, medically, socially, and economically, unless real and unprecedented global coordinated actions are immediately taken [14]. Health-care associated infections are also increasingly recognised in LMICs. Findings of a recent review [1] showed that pooled prevalence of health-care associated infections in resource-limited settings (15·5 per 100 patients) was twice the average prevalence in Europe (7·1 per 100 patients). Incidence of infections acquired in intensive care units in developing countries (pooled density 47·9 per 1000 patient-days) was three times the rate in the USA (13·6 per 1000 patient-days). Health-care associated infections in neonatal intensive care units in some countries (15·2–62·0 infections per 1000 patient-days) are up to nine times more common than in the USA (6·9 infections per 1000 patient-days). Both the need for antibiotics and the burden of resistance are likely to increase with the rate of health-care associated infections in LMICs. In low income and middle-income countries (LMICs), antibiotic use is increasing with rising incomes, high rates of hospitalisation, and high prevalence of hospital infections. Resistance arises as a consequence of mutations in microbes and selection pressure from antibiotic use that provides a competitive advantage for mutated strains. Suboptimum antibiotic doses help stepwise selection of resistance. Resistance genes are borne on chromosomal, and increasingly, on transmissible extrachromosomal elements. The resulting resistant clones—eg, meticilli nresistant

Staphylococcus aureus (MRSA) USA 300, *Escherichia coli* ST131, and *Klebsiella* ST258) are disseminated rapidly worldwide. This spread is facilitated by inter species gene transmission, poor sanitation and hygiene in communities and hospitals, and the increasing frequency of global, travel, trade, and disease transmission. These trends are globally consistent. Hospital data from developing countries suggest that resistance to the WHO recommended regimen of ampicillin and gentamicin in pathogens causing neonatal infections (in the fi rst 28 days of life) is common: 71% of isolates of *Klebsiella spp* and 50% of *E coli* are resistant to gentamicin [18]. Increasing rates of resistance to colistin and polymyxin B in Gram-negative organisms are being reported from countries around the world, including South Korea [11], Italy [6], Greece [3;12] and Saudi Arabia [4]. Moreover, there is some evidence of cross-resistance to colistin and host antimicrobial peptides that are part of the body's immune response [15].The decreasing effectiveness of antibiotics in treating common infections has quickened in recent years, and with the arrival of untreatable strains of carbapenem resistant *Enterobacteriaceae*, we are at the dawn of a postantibiotic era [7]. In high-income countries, continued high rates of antibiotic use in hospitals, the community, and agriculture have contributed to selection pressure that has sustained resistant strains [13], forcing a shift to more expensive and more broad-spectrum antibiotics. In this paper, the antibacterial activity of a HydroxyEthyl-Heptadienyl Phosphine Oxide (HEHPO) (*3-Diphenylphosphinoyl-5-methtlhepta-3,4-dien-2-ol*) has been studied as part of the exploration for new and novel bio-active compounds.

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MATERIALS AND METHODS

Test Organisms

Escherichia coli 3398, Staphylococus aureus 745, Bacillus subtilis 6633, Salmonella Typhimurium 3591, Listeria monocytogen 863 and Enterobacter aerogenes 3691 were obtained from the Collection of the Department of General and Applied Microbiology, Sofia University. All the isolates werechecked for purity and maintained in slants of Nutrient agar.

Media Used

Nutrient Agar (Biolife 272-20128, Milano, Italia) was the medium used as thegrowth medium for the microbes.

Compound Tested

HydroxyEthyl-Heptadienyl Phosphine Oxide (HEHPO) (*3-Diphenylphosphinoyl-5-methtlhepta-3,4-dien-2-ol)* was synthesised in the Laboratory of Toxicologycal Chemistry, Department of Organic Chemistry & Technology of the Konstantin Preslavsky University of Shumen, Bulgaria (figure 1) [10].

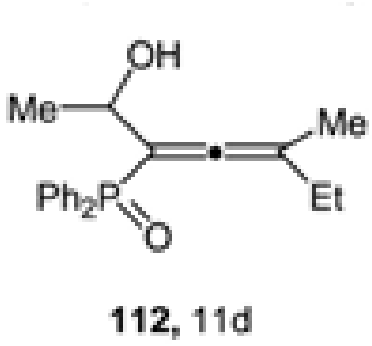

Figure 1.Structural formula of HEHPO

3-Diphenylphosphinoyl-5-methtlhepta-3,4-dien-2-ol (HydroxyEthyl-Heptadienyl Phosphine Oxide (HEHPO)). Light orange oil, yield: 87%. R_f 0.59; IR (neat, cm^{-1}): 1174 (P=O), 1441, 1490 (Ph), 1951 (C=C=C), 3369 (OH). ^{1}H-NMR (600.1 MHz): δ 0.86 (t, *J* = 7.4 Hz, 3H, Me-CH_2), 1.35 (dd, *J* = 6.2 Hz, *J* = 9.4 Hz, 3H, Me-CHO), 1.58 (d, *J* = 6.3 Hz, 3H, Me-C=), 1.78–1.90 (m, 2H, Me-CH_2), 2.70 (s, 1H, OH), 4.59– 4.63 (m, 1H, Me-CHO), 7.35– 7.90 (m, 10H, 2Ph). ^{13}C-NMR (150.9 MHz) δ = 12.4, 18.5 (*J* = 5.4 Hz), 22.4 (*J* = 7.6 Hz), 26.7, 64.2 (*J* = 7.4 Hz), 96.5 (*J* = 104.2 Hz), 105.1 (*J* = 13.4 Hz), 129.1– 132.4 (2Ph), 204.1 (*J* = 7.1 Hz). ^{31}P-NMR (242.9 MHz): δ 34.2. Anal. Calcd for $C_{20}H_{23}O_2P$ (326.37): C 73.60, H 7.10. Found: C 73.67, H 7.05.

Preparing the solution of HEHPO

The solutions of HEHPO (50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml) were freshly prepared in ethanol.

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Assay for Antibacterial Activity.

Antimicrobial assay was performed by the well diffusion method using soft 0,8% agar. Agar medium was added to sterile Petri dishes seeded with 100 µl of each test bacterial strains. Wells of equal distance were dug on the seeded plates. Each well was filled up with 100 µl of the HEHPO and antibiotics tested. After adjusting the pH at 6.5 by NaOH, the activity of the HEHPO was checked. The plates were incubated at 37°C for 48 hours. Th e antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well [5]. All experiments were performed in triplicate.

Determination of Minimum inhibitory concentrations (MICs)

The minimum inhibitory concentrations of HEHPO, that shows antimicrobial activity, were determined by 2-fold dilution methods as described by [17] and MICs were read in µg/ml after over night incubation at 37ºC. All experiments were made in replicate.

Determination of Minimum bacterial concentration (MBC)

The MBC were carried out to check whether the test microbes were killed or only their growth was inhibited. Nutrient Agaragar was prepared and sterilized at 121ºC for 15 minutes, the medium was poured into sterile petridishes and were allowed tocool and solidify. The contents of the MBC in the serial dilution were then subcultured onto the prepared medium, incubation was made at 37ºC for 24 h, after which each plate was observed for colony growth. The lowest concentration of the HEHPO without a colony growth was recorded asthe MBC.

RESULTS

In the present study the effects of HEHPO on six pathogenic Gram-positive and Gram-negative bacteria were evaluated. The effects were compared with widely used antibiotic Sefpotec. According to NCCLS, the antibiotic Sefpotec used is known to have broad spectrum antibacterial activity [16]. The effects of HEHPO on the microorganisms were summarized in Table 1.

HEHPO at concentration 50 mg/ml for 24 hours notably inhibited growth of Gram-negative bacteria *E. coli 3398* (28.77 mm mean zone of inhibition), E. *aerogenes 3691* (20.87 mm mean zone of inhibition) and *S. Typhimurium 745* (20.49 mm mean zone of inhibition). HEHPO did not inhibited Gram-positive bacteria *S. aureus 745*, *B. subtilis 6633* and *L. monocytogen 863* .

Table 1.Effect of HEHPO on test organisms.

Microorganisms	**Zone of inhibition (mm)[a]**
S. aureus 745 Gram- positive	-
E. *aerogenes 3691*Gram-negative	20.87±0.17
E. coli 3398 Gram-negative	28.77±0.05
*B. subtilis 6633*Gram- positive	-
L. monocytogen 863 Gram- positive	-
S. Typhimurium 745 Gram-negative	20.49±0.12
Ethanol (96%) (Negative control)	19.22±0.05
Sefpotec 250 µg/ml	13.78±0.05

[a]Data are presented as average values ± standard deviation in mm.

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Our assay for antibacterial activity of HEHPO was conducted by testing different concentrations of the compound on various pathogens to determine the MICs. We used five concentrations – 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml. The results are shown in Table 2.

Table 2. The MIC of HEHPO

Microorganisms		MIC (mg/ml)			
	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml
S. aureus 745	-	-	-	-	-
E. *aerogenes 3691*		+			
E. coli 3398			+		
B. subtilis 6633	-	-	-	-	-
L. monocytogen 863	-	-	-	-	-
S. Typhimurium 745		+			

[a]Results are mean ± SEM of three separate trails.

The results revealed variability in the inhibitory concentrations of HEHPO for given bacteria. MIC of HEHPO at concentration 25 mg/ml for 24 hours notably inhibited growth of E. *aerogenes 3691* and *Salmonella Typhimurium 745*. In contrast, MIC of HEHPO at concentration 12.5 mg/ml for 24 hours notably inhibited growth only of *E. coli 3398.*

Our next task was to determine the Minimum bacterial concentration (MBC) in regards with determining the bactericidal or bacteriostatic activity of the examined HEHPO. We used five concentrations – 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml. The results are shown in Table 3.

Table 3. The MBC of HEHPO

Microorganisms		MBC (mg/ml)[a]			
	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml
S. aureus 745	-	-	-	-	-
E. *aerogenes 3691*			+		
E. coli 3398			+		
B. subtilis 6633	-	-	-	-	-
L. monocytogen 863	-	-	-	-	-
Salmonella			+		
Typhimurium 745					

[a]Results are mean ± SEM of three separate trails.

MBC of HEHPO at concentration 25 mg/ml for 24 hours notably inhibited growth of *the three tested* Gram-negative bacteria *E. coli 3398*, E. *aerogenes 3691* and *S. Typhimurium 745*. For Gram-positive bacteria *S. aureus 745, B. subtilis 6633* and *L. monocytogen 863* MBC it was not reported.

Based on the results obtained we can conclude that the examined HEHPO has bactericidal activity towards both pathogenic bacteria, but in different concentrations.

HEHPO possesses biological activity, which is not well studied. We know only from literary

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data that they are used for inhibiting the biosynthesis of sterol from the pathogen responsible for *Pneumocystis-carinii* pneumonia (PCP) - a disease similar to AIDS [11]. In our previous studies was shown that the Bifunctionalized Allene with protected hydroxy group (*Dimethyl 3-methyl-1-[1-(tetrahydro-2H-pyran-2-yloxy)-ethyl]-hepta-1,2-dienephosphonate)* exhibited antibacterial [8] and antifungal activity [9]. The results obtained show for the first time the existence of antifungal activity of HEHPO towards various pathogenic bacteria.

Regulation, rational use, and infection control in human medicine Antibiotics are different from all other medicines in that the eff ects of their use extend way beyond individual patients. The societal eff ects of antibiotic use justifi es that they should belong to a special regulatory category. Antibiotic use should be strictly monitored and legislation to prevent over-the-counter sales without a prescription enforced, unless this would cause an unacceptable access problem (eg, in rural areas). Infection control interventions need to be reassessed and improved in an era with rapid transmission of multidrug-resistant bacteria and mobile antibiotic resistance genes. A fundamental obstacle in the management of antibiotic resistance in LMICs is the inadequate capacity and infrastructure to do basic microbiological laboratory analyses. These defi ciencies need to be addressed—eg, by mechanisms similar to the World Bank supported East Africa Public Health Laboratory Networking Project386 and the Danish supported Antibiotic Drug Use Monitoring and Evaluation.

The generation of reliable, relevant, and up-to-date information will be essential to respond to the negative effects of antibiotic resistance on public health. The poor understanding of the unique features and risks of antibiotic resistance is an important cause for the global complacency paving the way for the present crisis. Few studies have been done on the magnitude of the burden of antibiotic resistance and its contributions to excess mortality to convince policy makers of the need to react. Although antibiotic resistance is undermining the effective treatment of many important bacterial diseases with high mortality, especially in LMICs, it lacks the profile of HIV, tuberculosis, and malaria. Clear information on the health and economic burden of antibiotic resistance is urgently needed to make this complex problem tangible to policy makers [13].

Future studies should include testing Bifunctionalized Allenes and as a component of combined antibacterial therapy for invasive and refractory mould infections.

The occurrence of drug resistant strains with less susceptibility to antibiotics due to mutation challenges the researchers to invent newer drugs. At this scenario, evaluation of antimicrobial substances from various sources is considered to be a pivotal role. Nevertheless, further studies are required to explore the mechanism of biochemical active principle in the Bifunctionalized Allenes for the inhibitory action on various pathogens selected in the study.

CONCLUSION

HydroxyEthyl-Heptadienyl Phosphine Oxide (HEHPO) at 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml concentrations showed significant antibacterial activity on selected pathogens inclinical isolates.

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REFERENCES

Alexander J. Lepak and David R. Andes. (2015) Antifungal Pharmacokinetics and Pharmacodynamics. Cold Spring Harb Perspect Med May 5:a019653.

Allegranzi B, Bagheri Nejad S, et al. (2011) Burden of endemic health-careassociated infection in developing countries: systematic review and meta-analysis. Lancet 377 228–41.

Antoniadou A, Kontopidou F, Poulakou G, et al. (2007) Colistin-resistant isolates of *Klebsiella pneumoniae* emerging in intensive care unit patients: fi rst report of a multiclonal cluster. J Antimicrob Chemother 59 786–90.

Baadani AM, Thawadi SI, El-Khizzi NA, Omrani AS. (2013) Prevalence of colistin and tigecycline resistance in *Acinetobacter baumannii* clinical isolates from 2 hospitals in Riyadh Region over a 2-year period. Saudi Med J 34 248–53.

Bertrand-Harb C., Ivanova I., Dalgalarrondo M., Hartle T. (2003) Evolutionof -lactoglobulin and - lactalbumin content during yoghurt fermentation. Int. Dairy J., 13 39–45.

Capone A., Giannella M., Fortini D, et al, for the SEERBIO-GRAB network. (2013) High rate of colistin resistance among patients with carbapenem-resistant Klebsiella pneumoniae infection accounts for an excess of mortality. Clin Microbiol Infect 19 23–30.

Centers for Disease Control and Prevention (CDC). (2013) Vital signs: carbapenem-resistant Enterobacteriaceae. MMWR Morb Mortal Wkly Rep 62 165–70.

Ignatova-Ivanova, TS., Stefanova, I. ; Ismailov, I. E.; Ivanov, I. K.; Christov, V. Ch. (2015) *In Vitro* Studies of antifungal activity of a bifunctionalized allene ethanol extracts. Int. J. Res. Stud. BioSci. (IJRSB), 3 39–40.

Ignatova-Ivanova,TS., Stefanova, I.; Ismailov, I. E.; Ivanov, I. K.; Christov,V. Ch. (2015) *In Vitro* Studies of antibacterial activity of a bifunctionalized alleneethanol extracts. Int.J.Curr.Microbiol.App.Sci., 4589– 595.

Ismailov, I. E.; Ivanov, I. K.; Christov, V. Ch. (2014) Bifunctionalized allenes. Part XIII. A convenient and efficientmethod for regioselective synthesis of phosphorylatedα-hydroxyallenes with protected and unprotectedhydroxy group. Molecules, 19 6309–6329.

Ko KS, Suh JY, Kwon KT, et al. (2007) High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. J Antimicrob Chemother 60 1163–67.

Kontopidou F, Plachouras D, Papadomichelakis E, et al. (2011) Colonization and infection by colistin-resistant Gram-negative bacteria in a cohort of critically ill patients. Clin Microbiol Infect 17 9–11.

Laxminarayan R, Heymann DL. Challenges of drug resistance in the d eveloping world. *BMJ* 2012; 344**:** e1567.

Laxminarayan, R.,; Duse, A.; Wattal, C.;Zaidi, A.K.M.;Wertheim, H.F.L.;Sumpradit, N. (2013) Antibiotic resistance - the need for global solutions. Lancet. Infect. Dis., 13 1057–1098.

Napier BA, Burd EM, Satola SW, et al. (2013) Clinical use of colistin induces cross-resistance to host antimicrobials in Acinetobacter baumannii. MBio 4 e00021–13.

National Committee for Clinical Laboratory Standards (NCCLS). (1993) *Performance standards for antimicrobial disc suspectibility tests*. Approved standard NCCLS Publication, Villanova, PA, M2–A5, USA.

Omura, S. Pyl, D.V.D. Inokoshi, J. Takahashi Y., Takeshima H. (1993) Pepticinnamins, new farnesylproteintransferase inhibitors produced by an actinomycete. I. Producing strain, fermentation, isolation andbiological activity. J. Antibiotics, 46 222–228.

Zaidi AK, Huskins WC, Thaver D, Bhutta ZA, Abbas Z, Goldmann DA. (2005) Hospital-acquired neonatal infections in developing countries. Lancet 365 1175–88.

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