Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(1):648-652

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

MIC for determination of antibacterial activity of Di-2-ethylaniline phosphate

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ABSTRACT

To determine the novelty and potency of the compound with antibacterial activity minimum inhibitory concentration (MIC) was studied against four different Gram negative bacteria using the broth dilution assay. For this purpose test compound Di-2-ethylaniline phosphate was prepared in the range from 50-10,000 µg/ml in dimethyl sulfoxide. Highest percentage growth inhibition in terms of minimum inhibitory concentration was found when 2 ml of 600 µg/ml concentration was used against Gram negative bacteria. Decreasing optical density was reported with increasing concentration of test compound. So it has been concluded that the minute concentration of Di-2-ethylaniline phosphate have the antibacterial activity with high potency.

Keywords: Di-2-ethylaniline phosphate, Minimum inhibitory concentration, Antibacterial activity, Optical density.
Abbreviations: *MIC- Minimum inhibitory concentration, OD- Optical density, mm-millimetre, NAM- Nutrient agar media, DMSO - Dimethyl sulfoxide.*

INTRODUCTION

The phosphodiester linkage is highly stable toward solvolytic cleavage and is widely utilized throughout nature as the linker joining the backbone components of DNA and RNA polymers [1]. The phosphonoformate trianion ("foscarnet"), (PFA), is active against herpes simplex virus and AIDS-related human cytomegalovirus [2]. Organophosphorus (OP) pesticides are widely used for protection of agricultural crops from variety of insects where, Azodrin a 3-Hydroxy-N-methyl-cis-crotonamid Dimethyl Phosphate has been reported effective against some insects that attack sweet corn and cotton [3,4].

Phosphoryl and thiophosphoryl transfer reactions are of prime importance in biological systems [5]. The transfer of a phosphoryl group between ATP and ADP is the fundamental mechanism for energy transfer that allows the processes of synthesis, active transport, muscle action and nerve function to occur [6]. Nucleoside phosphates and their phosphonate analogues have proven to be exceedingly important agents for anticancer and antiviral therapy, [7,8] and phosphonate-containing drugs are increasingly being explored in other therapeutic areas [9,10].

Many of the currently available classes of antibacterial were developed between the 1940s and 1960s [11-13]. Global antibacterial resistance is becoming an increasing public health problem [14]. A variety of reasons, including inappropriate and excessive use of antibiotics, has led to the emergence of pathogenic bacterial strains that are highly resistant to most or all current antibiotics [15-18]. Thus there is a significant need for discovery of new types of antimicrobials to treat infections and disease caused by resistant organisms. Still much work has not been reported on antibacterial properties of phosphate ester, so present investigation concerned antibacterial activity of Di-2-ethylaniline phosphate against four different Gram negative bacteria, which are common pathogens in human disease.

EXPERIMENTAL SECTION

The classical method involves diffusion assays in which the antibiotic is placed on the surface of an agar plate that has been inoculated with test bacteria. During the incubation the antibiotic diffuses, creating a concentration gradient that produces a zone of bacterial growth inhibition [19, 20]. In the early 1970s, automated systems were developed for assay of bacterial antibiotic susceptibility. These systems were an automated version of the classical procedures in which the antibiotic is added to a liquid bacterium to measure the bacterial growth [21].

To test the antibacterial activity of Di-2-ethylaniline phosphate Bacterial samples were taken from School of Studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India and named as bacteria A, B, C and D. The pure cultures of these bacteria were maintained on NAM (Hi media, Laboratories Ltd. Bombay, India) at 37°C. Characterizations of all the selected bacteria were done by Gram's and acid fast staining techniques [22]. Bacterial cultures maintained on nutrient agar slants were aseptically inoculated into 10 ml of sterile broth these were shacked thoroughly and incubated at 37^{o}C for 24 hours; this was designated as the working stock that was used for antibacterial studies. On the other hand 10 ml of nutrient broth medium were taken in different test tubes were autoclaved.

Solutions of test compound (Di-2-ethylaniline phosphate) were prepared in dimethyl sulphoxide (DMSO) and designed a set of concentrations (50-10,000 µg/ml) in nutrient broth medium by diluting the stock solution 20,000 µg/ml and these was used to test antibacterial activity of Di-2-ethylaniline phosphate employing broth dilution assay [23]. For primilary test 1 ml of each concentration (50-10,000 µg/ml) was added into test tubes containing nutrient broth medium and simultaneously another set of test tubes were added by 2 ml as final volume of same concentrations. Each tube was inoculated with 100 µl of bacterial suspension and incubated at 37^{o}C for 24 hours. The growth of all selected four bacteria were detected by optical density (OD) (Spectrophotometer Elico, SL27) at 600 nm and percentage bacterial growth inhibition was calculated as formula given below.

$$\textbf{Percentage growth inhibition} = \frac{\text{OD of control}}{\text{OD of control - OD of test}} \times 100$$

RESULTS AND DISCUSSION

In the present investigation antibacterial activity of Di-2-ethylaniline phosphate was studied by minimum inhibitory concentration against four selected Gram negative bacteria. Growth of these bacteria was measured at 600 nm after 24 hours by spectrophotometer in terms of optical density. Control measurements were carried out without addition of Di-2-ethylaniline phosphate. Interestingly primary test results of control for all Gram negative bacteria, optical density was noted as 0.037, 0.038, 0.118 and 0.033 respectively for bacteria A, B, C and D which were found to be decreased with addition of 1 ml of 50 µg/ml concentration of test compound and appeared 0.032, 0.028, 0.100 and 0.030 optical density for above same bacteria. Similarly in another set of test, it was regularly 0.033, 0.031, 0.113 and 0.032 for all selected Gram negative bacteria A, B, C and D. However with inoculation of 2 ml of 50µg/ml of test compound bacterial growth was inhibited by reducing turbidity. 1 ml of each concentration (50-10,000 µg/ml) reduced the bacterial growth accordingly with increasing concentration, while 2 ml of same concentrations decreased the growth up to 50% as compared to 1 ml.

Percentage growth inhibition was, calculated with only the finding of 2 ml of each concentration, which indicated that the values of optical density were further decreased by reducing visual growth or turbidity of bacterial strength, shown in **Figure 1** and inhibition increases with concentration. **Figure 2** showed that percentage growth inhibition, test results of all Gram negative bacteria, where bacteria D aligned with maximum percentage growth inhibition. Highest percentage growth inhibition (74.3%) was obtained for bacteria C whereas it was 48.5%, 19.5% and 62.5% for bacteria A, B and D at minimum 50 µg/ml concentration of test compound. Bacteria D showed almost 93.7% growth inhibition at 2 ml of 600 µg/ml while bacteria A, B and C showed growth inhibition below 90% at higher concentration 10,000 µg/ml of same volume. Some of the optical density was found to be similar and constant at different concentrations for all the bacteria.

Table 1 Optical density and percent growth inhibition of Di-2-ethylaniline phosphate determined by spectrophotometer at 600 nm

µg/ml	Gram negative bacteria											
	A (OD)			B (OD)			C (OD)			D (OD)		
	1 ml	2 ml	% Growth inhibition of 2 ml	1 ml	2 ml	% Growth inhibition of 2 ml	1 ml	2 ml	% Growth inhibition of 2 ml	1 ml	2 ml	% Growth inhibition of 2ml
Control	0.037	0.033	---	0.038	0.031	---	0.118	0.113	---	0.033	0.032	---
50	0.032	0.017	48.5	0.028	0.025	19.5	0.100	0.029	74.3	0.030	0.012	62.5
100	0.031	0.016	51.5	0.026	0.023	25.8	0.085	0.022	80.5	0.030	0.008	75
200	0.030	0.015	54.5	0.025	0.020	35.5	0.075	0.021	81.4	0.029	0.006	81.3
300	0.029	0.015	54.5	0.023	0.015	51.6	0.069	0.020	82.3	0.029	0.003	90.6
400	0.029	0.014	57.6	0.023	0.014	54.8	0.067	0.019	83.2	0.028	0.003	90.6
500	0.029	0.013	60.6	0.019	0.013	58.1	0.066	0.019	83.2	0.027	0.002	93.7
600	0.025	0.013	60.6	0.018	0.013	58.1	0.064	0.018	84.1	0.026	0.002	93.7
700	0.025	0.012	63.6	0.017	0.012	61.3	0.063	0.018	84.1	0.024	---	---
800	0.025	0.012	63.6	0.015	0.012	61.3	0.061	0.018	84.1	0.022	---	---
900.	0.024	0.012	63.6	0.015	0.012	61.3	0.058	0.017	84.9	0.021	---	---
1000	0.024	0.011	66.7	0.014	0.011	64.5	0.055	0.017	84.9	0.021	---	---
2000	0.023	0.011	66.7	0.013	0.011	64.5	0.051	0.016	85.8	0.020	---	---
3000	0.023	0.011	66.7	0.012	0.010	67.7	0.047	0.016	85.8	0.019	---	---
4000	0.021	0.010	69.7	0.012	0.010	67.7	0.042	0.015	86.7	0.017	---	---
5000	0.020	0.010	69.7	0.011	0.009	70.9	0.040	0.015	86.7	0.014	---	---
8000	0.020	0.010	69.7	0.011	0.008	74.2	0.032	0.014	87.6	0.014	---	---
10000	0.018	0.009	72.7	0.010	0.007	77.4	0.026	0.012	89.4	0.012	---	---

OD- Optical Density, µg/mL -parts per million, "--"- nil

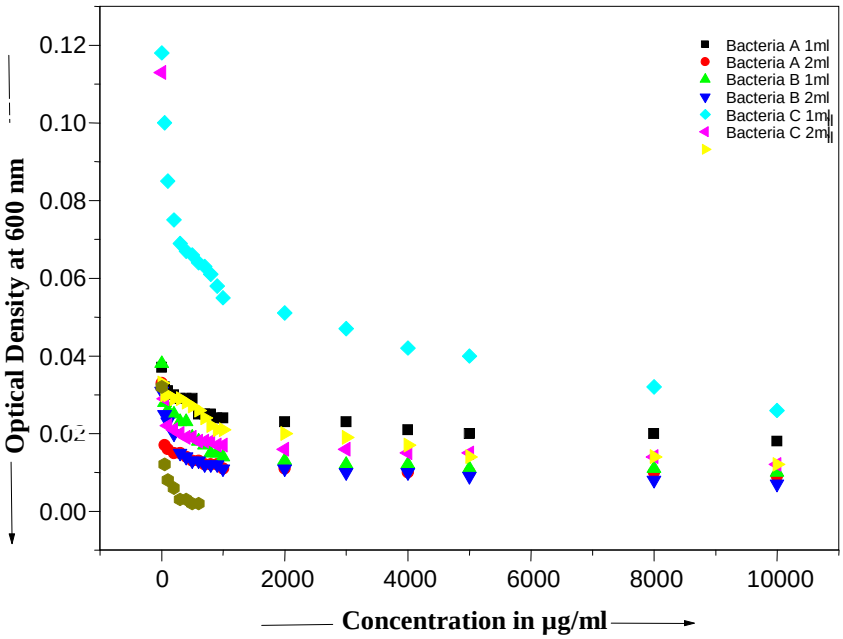

Figure 1 Minimum inhibitory concentration (MIC) of Di-2-ethylaniline phosphate by broth dilution method

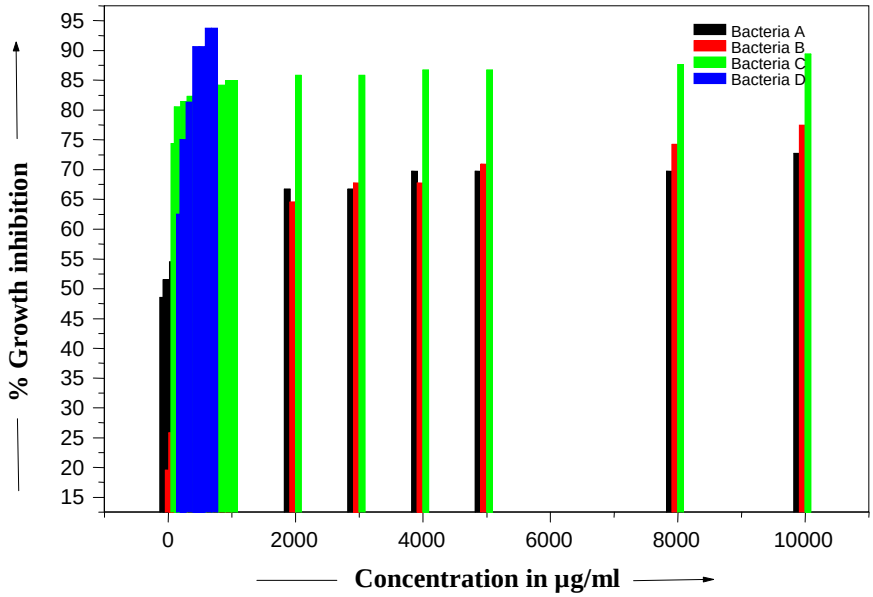

Figure 2 Percent growth inhibition of Di-2-ethylaniline phosphate against all Gram negative bacteria

The MIC is the lowest concentration of antibiotic in which there was no visible growth, or, in the case of bacteriostatic antibiotics, the lowest concentration in which there was no turbidity greater than the faint turbidity present in all tubes reported by [24]. In this way 1 ml of 100, 800, 800 and 500 µg/ml, respectively for bacteria A, B, C and D was the lowest concentrations that produced no visible bacterial growth (no turbidity) when compared with control tubes. The observed optical density values for Di-2-ethylaniline phosphate and percentage bacterial growth inhibition summarized in **Table 1**. In the present investigation, highest percentage growth inhibition in terms of minimum inhibitory concentration was obtained in 2 ml of 600 µg/ml concentrations against bacteria D whereas constantly higher growth inhibition exhibited against bacteria C while moderate antibacterial activity were examined for bacteria A and B.

CONCLUSION

Di-2-ethylaniline phosphate has been reported as active antibacterial compound with high potency and novelty, which may be further useful in Pharmaceutical Chemistry as well as in drug development.

Acknowledgement
The authors are thankful to Dr. K. S. Patel, Prof. and Head, School of Studies in Chemistry and Dr. K. L. Tiwari, former Prof. and Head, School of Studies in Biotechnology Pt. Ravishankar Shukla University Raipur, C.G. India for providing research facilities. First author is also thankful to the UGC - New Delhi for providing financial assistance.

REFERENCES

[1] Schroeder GK, Lad C, Wyman P, Williams NH, Wolfenden, *Proc. Natl. Acad. Sci.* U.S.A. **2006**, 103: 4052.
[2] Physician Desk Reference, *Pharmacol. Ther.* **2000**, 40: 213.
[3] Young JR, *Ofjc. Agr. Chemists*, **1965**, 48: 938.
[4] Shell CC, Agriculture Chemical Division New York, N.Y.,"Summary of Basic Data for Technical Bidrin Insecticide," and "Summary of Basic Data for Azodrin Insecticide, **1963**.
[5] Han X, Balakrishnan VK, Buncel E, *Langmuir,* **2007**, 23: 6519.
[6] Um IH, Shin YH, Lee SE, Yang K, Buncel E, *J. Org. Chem.* **2008**, 73: 923.
[7] Narule MN, *J. Chem. Pharm. Res.* **2011**, 3 (3): 38-47.
[8] Shivananda MK, Shet PM *J. Chem. Pharm. Res.* **2011**, 3 (5): 61-66.
[9] Hecker SJ, Erion DM, *J. Med. Chem.* **2008**, 51 (8): 2328.
[10] Shivananda M K, Shet Prakash M, *J. Chem. Pharm. Res*. **2011**, 3 (2): 303-307.
[11] Labischinski H, *Int. J. Med. Microbiol*. **2001**, 291 (5): 317.
[12] McDevitt D, Rosenberg M, *Trends. Microbiol*. **2001**, 9: 611.
[13] Spellberg B, Powers JH, Brass EP, Miller LG, Edwards JE, *Clin. Infect. Dis*. **2004**, 38: 1279.
[14] Bax R, Mullan N, Verhoef J., *Int. J. Antimicrob. Agents*. **2000**, 16: 51-59.
[15] Silver LL, Bostian KA, *Antimicrob. Agents Chemother.* **1993**, 37: 377.
[16] Alanis AJ, *Arch. Med. Res.* **2005**, **36**: 697-705.
[17] Norrby SR, Nord CE, Finch R, *Lancet. Infect. Dis.* **2005**, **5**: 115.
[18] Mittal M, Sarode SM, Shingare RM, Vidyasagar G, Shrivastava B, *J. Chem. Pharm. Res*, **2011**, 3 (3): 766-774
[19] Bauer AW, Kirby WM, Sherris JC, Turck M., *Amrican. J. Clin. Pathol.* **1966**, 45: 493.
[20] Kahan JS, Kahan, FM, Goegelman R, Currie SA, Jackson M, Stapley EO, *J. Antibiot.* **1979**, 32: 1.
[21] Fuente RDL, Sonawane ND, Arumainayagam D, Verkman AS, *British. J. Pharmacol.* **2006**, 149: 551.
[22] Hawker LE, Linton AH, The bacterial spore London: Academic press, **1979**.
[23] Stalons DR, Thornsberry C, *Antimicrob. Agents Chemother*. **1975**, **7** (1): 15.
[24] Wilkins TD, Holdman LV, Abramson IJ, Moore WEC, *Antimicro. Agents and Chemoth.* **1972**, 1 (6): 451.