JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2010. Vol. 4(1), p. 41-46

Selective Antimicrobial Activity of 5, 10, 15, 20- *meso-tetrakis* Pentafluorophenyl 21H, 23H- porphine

S. Gomathinayagam[1] and R.C. Jagessar[2]*

[1]Department of Microbiology, Faculty of Agriculture, University of Guyana, Berbice Campus, John's Science Centre, Berbice, South America.
[2]Department of Chemistry, University of Guyana, Faculty of Natural Sciences, Turkeyen Campus, South America.

(Received: 31 August 2009; accepted: 22 October 2009)

Antimicrobial activity of 5, 10, 15, 20- *meso-tetrakis* pentafluorophenyl 21H, 23H-porphine (1) was investigated using the agar well diffusion and Poison plate methods. Compound (1) was shown to be both antibacterial and antifungal with a higher degree of selectivity for *S. aureus* and *C. albicans versus E. coli*

Key words: Antimicrobial activity, *meso tetrakis* porphyrin, agar well diffusion, poisons plate methods.

Over the years, plant extracts[1,4,5-9] and isolated pure natural products[2,3] have been used as initial antimicrobial agents. Following this, the isolation and structural elucidation of bioactive compounds from fractionated extracts have led chemists to mimic and synthesized similar compounds as antimicrobial agents[10-12]. Via a modulation of the structure of drugs, variation in the potency of their antimicrobial activity has been achieved[10-12]. Even though there are several reports of synthesized compounds such as porphyrins and other porphyinoid as antimicrobial agents[13-16], there is no documentation of 5,10,15,20- *meso tetrakis* pentafluorophenyl 21H, 23H- porphine as an antimicrobial agent. In our continuing search for novel, potent and selective antimicrobial agents, we report here an investigation of the antimicrobial activities of (1), 5, 10, 15, 20- *meso-tetrakis* pentafluorophenyl, 21H, 23H porphine via Agar well diffusion and Poison plate techniques[15-17]. Porphyrins are a versatile class of chemical compounds, having found applications in the field of supramolecular chemistry[21-23], catalysis [24-25], oxygen haemoglobin mimics[26-28] and in nanotechnology[29-30] etc.

* To whom all correspondence should be addressed.
E-mail: raymondjagessar@yahoo.com

Compound (1) was purchased from Aldrich chemical company.

MATERIAL AND METHODS

Reagents and materials

5,10,15,20- *meso-tetrakis* pentafluorophenyl 21H, 23H- porphine (1) and solvents were purchased from Aldrich. Antibiotic Ciprofloxacin and nystatin, Mueller Hinton agar, agar plates and microbial discs were purchased from the International Pharmacy Association in Guyana. Bacterial and fungal cultures were obtained at John's Science Centre, Berbice Campus, University of Guyana.

Preparation of 5,10,15,20- *meso-tetrakis* pentafluorophenyl 21H, 23H- porphine solution

Compound (1) was made up to the appropriate concentration of 10mg in 10ml (1mg in 1 ml) of dichloromethane in a 25 ml round bottom flask and was stored under aseptic conditions.

Source of microorganisms

For the bacterial organisms, a gram negative bacterium used was *Staphylococcus aureus* (ATCC 25923). For the fungi, yeast of the *Candida albicans* (ATCC 1023) species was investigated. These microorganisms were stored in a refrigerator at the microbiology laboratory at John's Science Centre, University of Guyana, Berbice Campus, Berbice.

Reference and Control

The references were antibiotic in nature. *Ampicillin* and *Nystatin*. *Ampicillin* was choose as the reference for all bacterial species used: *E.coli* and *S. aureus*. *Nystatin* was used as the reference for the fungus, *Candida.albicans.* The Control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion[20].

Antimicrobial tests

Compound (1) was investigated for their antimicrobial activity using the Agar diffusion[17-20] and Poison Plate techniques[17-20] under aseptic conditions.

Aseptic conditions

The aseptic chamber consists of a wooden box (1m x 1m x 0.5m) with a door which was cleaned with 70% ethanol and irradiated with short wave UV light for 1 hour.

Mother plates

These were made by culturing *C. albicans* on PDA (Potato Dextrose Agar). A sterilized 9 cm cork borer was used to cut agar discs in the plate.

Potato dextrose agar (PDA)

This is an agar medium on which the fungi was cultured. The potato was peeled and 200g weighed, finely chopped and boiled to a mash in distilled water. The dextrose was weighed (12.5g) and placed in a 1L measuring cylinder. Agar was weighed (12.5g) and added to the measuring cylinder (with the dextrose).The potato mash was stirred and strained into the cylinder. Distilled water was added to make up the solution to 500mL.The contents was continuously stirred until consistency was achieved and was then poured into a conical flask, plugged with cotton wool, over which aluminium foil was tightly wrapped. The flask was then autoclaved at 121 °C, 15psi for fifteen minutes.

Agar well diffusion Technique (Magalia *et al.*, 2004).

The spore suspension of pathogens was seeded into a molten PDA medium or poured into petri plates. When the medium solidified, a 9 cm well was made at the centre of the plate with the help of a sterile 9 cm cork borer. A solution of the test compound 5, 10, 15, 20- *meso tetrakis* pentafluorophenyl 21H, 23H- porphine (1), at a concentration of 1mg in 1ml was transferred into the well and incubated for three days. The zone of inhibition in mm^2 was measured for the test compound and recorded. From these values, the area of inhibition was calculated.

Poison Plate technique

Under aseptic conditions, the test compound was seeded into molten PDA medium and poured into Petri plates. The plates were covered and allowed to cool. As soon as the agar was solidified, a 9 cm sterile cork borer was used to make a disc on the pathogen plate (Mother plate). A pathogen disc was taken from pathogen plate (mother plate) and kept at the centre of test compound seeded plate with help of a sterile inoculum needle and was incubated for 3 to 4 days. The inoculum needle was sterilized with alcohol and flame before each application.

The zone of inhibition was measured for pathogenecity of test compound. The experiment was repeated thrice. Triplicate was maintained for each test compound at every 24 hours interval. The diameter of mycelium growth was measured and the average value taken.

RESULTS AND DISCUSSION

This paper focuses on the investigation of the antimicrobial activity of 5, 10, 15, 20- *meso tetrakis* pentafluorophenyl 21H, 23H porphine against pathogenic microrganisms: *Candida albicans*, *E.coli* (gram negative bacteria) and *Staphylococcus aureus* (gram positive) using the agar diffusion and poison plate methods[15-17]. For each microbial experiment, triplicates were done.

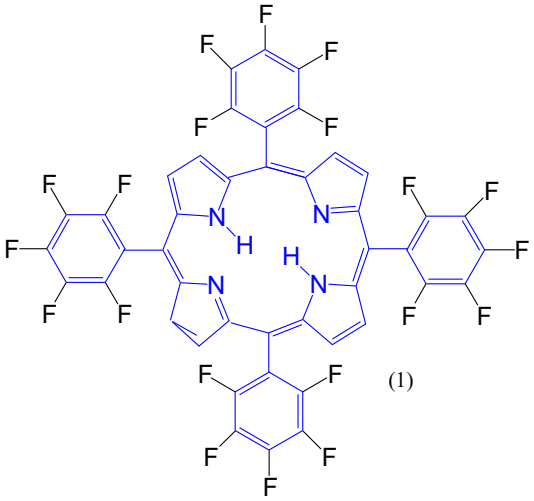
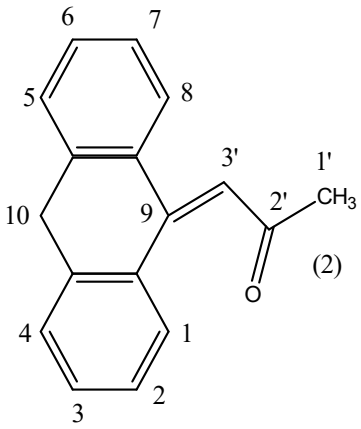


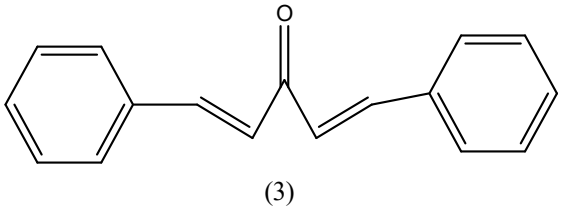
Fig. 1. 5, 10, 15, 20- *meso-tetrakis* pentafluorophenyl 21H, 23H porphine

The zone of inhibition (mm) is quoted at the ED_{50} value and as the area of inhibition (mm^2). ED_{50} is the effective dose concentration of the sample required to kill 50% of the pathogen growth. The zone of inhibition in mm at ED_{50} was calculated and converted into area of inhibition, mm^2. First, a random check of the compound antimicrobial activity was investigated using both methods, Table 1. These served as the reference experiments. Ampicillin was used as the reference for bacterial species and Nystatin as the reference for the fungal species. A controlled experiment was also investigated using the pure solvent with the microorganism as the innoculant[21]. It was found that the pure solvent induced negligible zone of inhibition on the agar medium, Table 0. Thus, the zone of inhibition are indeed due to compound (1) rather than to the pure solvents.

Results indicate that for both techniques, compound (1) induce the largest zone of inhibition against *Staphylococcus aureus* and the smallest zone of inhibition against *E. Coli,* Tables (1)- (4) and Graphs 1 (a) and (b). The order of zone of inhibition against microbial agents follow the sequence: *Staphylococcus. aureus* > *Candida. albicans* > *Eschericia. Coli*. For example, compound (1) induce zone of inhibition of 52.78 mm^2 (ED_{50} = 4.1 mm) against *S.aureus* compared with a zone of inhibition of 35.66 m^2 (ED_{50} = 3.37 mm) against *Candida albicans* and 28.83 mm^2



9-(propan-3-ene-1-methyl-2-one)anthracene



trans,*trans*-dibenzylideneacetone

Fig. 2. 9- (propan-3-ene-1-methyl-2-one)anthracene and *trans,trans*-dibenzylideneacetone

Table 1. Random check (1 mg in 1 ml) (Reference experiments)

Sample Name	*Candida albicans* (antibiotic Nyastatin)		*Staphylococcus aureus* (antibiotic Ampicillin)		*Eseterichia coli* (antibiotic Ampicillin)	
	(Average of Triplicate)* $ED_{50\ (mm)}$	Area of inhibition* (mm^2)	(Average of Triplicate)* $ED_{50\ (mm)}$	Area of inhibition (mm^2)* $ED_{50\ (mm)}$	(Average of Triplicate)*	Area of inhibition (mm^2)*
1	3.10	30.18	3.20	32.76	7.70	186.17

*Mean value

Table 2. Controlled experiment (1 mg in 1 ml)

Sample Name	*Candida albicans* (antibiotic Nyastatin)		*Staphylococcus aureus* (antibiotic Ampicillin)		*Eseterichia coli* (antibiotic Ampicillin)	
	(Average of Triplicate)* $ED_{50\ (mm)}$	Area of inhibition* (mm^2)	(Average of Triplicate)* $ED_{50\ (mm)}$	Area of inhibition (mm^2)* $ED_{50\ (mm)}$	(Average of Triplicate)*	Area of inhibition (mm^2)*
1	< 5	< 5	< 5	< 5	< 5	< 5

*Mean value

Table 3. Diffusion Plate (1 mg in 1 ml)

Sample Name	*Candida albicans* (antibiotic Nyastatin)		*Staphylococcus aureus* (antibiotic Ampicillin)		*Eseterichia coli* (antibiotic Ampicillin)	
	(Average of Triplicate)* $ED_{50\ (mm)}$	Area of inhibition* (mm^2)	(Average of Triplicate)* $ED_{50\ (mm)}$	Area of inhibition (mm^2)* $ED_{50\ (mm)}$	(Average of Triplicate)*	Area of inhibition (mm^2)*
1	3.37	35.66	4.07	52.01	3.03	28.83

*Mean value

Table 4. Poison Plate (1 mg in 1 ml)

Sample Name	*Candida albicans* (antibiotic Nyastatin)		*Staphylococcus aureus* (antibiotic Ampicillin)		*Eseterichia coli* (antibiotic Ampicillin)	
	(Average of Triplicate)* $ED_{50\ (mm)}$	Area of inhibition* (mm^2)	(Average of Triplicate)* $ED_{50\ (mm)}$	Area of inhibition (mm^2)* $ED_{50\ (mm)}$	(Average of Triplicate)*	Area of inhibition (mm^2)*
1	3.0	28.26	3.9	47.76	2.6	21.23

*Mean value

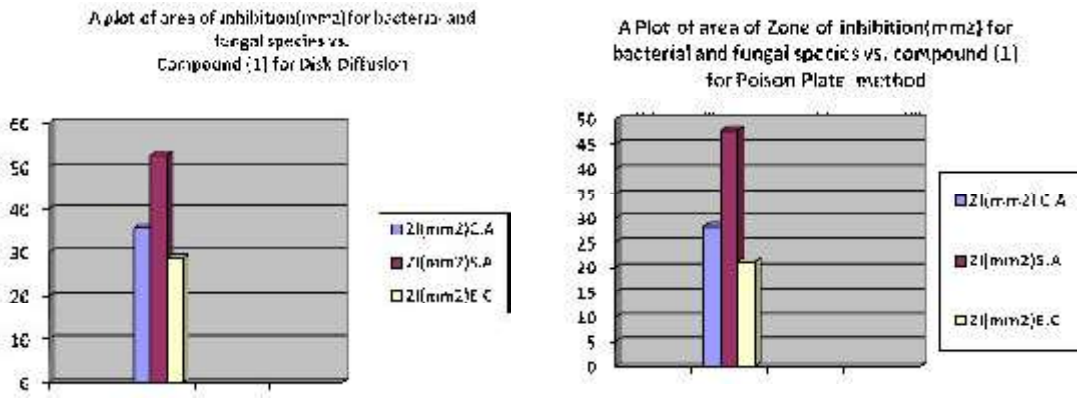


Fig. 3. A plot of zone of inhibition for bacterial and fungal species vs. compound (1) for diffusion and poison plate, methods

against *E. Coli* (ED_{50} =3.03 mm) . A similar microbial trend was noted for compound (1) using the poison plate technique. With this technique, zone of inhibition of 47.76 mm^2 (ED_{50} = 4.1 mm) was noted against *S. aureus* as compared with 28.26 mm^2(ED_{50} =3.37 mm) against *C. albicans*.

Thus, compound (1) is both an antibacterial and antifungal agent with the antimicrobial selectivity trend following the sequence: *S.aureus* > *C. albicans* > *E. Coli.* Both techniques conform this selectivity trend. This is in contrast to our recent finding where compound (2) [31] was found to be more antibacterial selective and compound (3) antifungal selective [32]. These results indicate that a variation in the structure of the compound can indeed induce variation in the antimicrobial selectivity. This structure activity relationship is further been investigated.

ACKNOWLEDGEMENTS

This research was financially supported by a small grant to Dr. R.C. Jagessar from the Royal Society of Chemistry, England and the University of Guyana. The use of the microbiology laboratory at John's Science Centre, University of Guyana, Berbice Campus, is acknowledged.

REFERENCES

1. Kandil, O., Redwan, N.M., Hassan, A.B.,Amer, A.M.M., El-Banna, H.A., "Extracts and fractions of *Thymus capitatus* exhibit antimicrobial activities". *Journal of Ethnopharmacology.,* 1994 **44**: 19-24
2. Barre, J.T., Bowden B.F., Coll J.C., Jesus. J., Fuente. V.E., Janairo, G.C., Ragasa, C.Y., "A bioactive triterpene from *Lantana camara"*, *Phytochemistry.,* 1997; **45**: 321-324.
3. Batista, O., Duarte. A., Nascimento. J., Simones, M.F., "Structure and antimicrobial activity of diterpenes from the roots of *Plectranthus heranthus"*, *J. Nat. Products*., 1994; **57**: 858-861.
4. Rojas, A., Hernandez. L., Pereda-Miranda. R., Mata. R, "Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants". *J. Ethnopharmacology*., **35**: 275-283.
5. Silva, O., Duarte. A., Cabrita. J., Pimentel. M., Diniz. A., Gomes. E., "Antimicrobial activity of Guinea-Bissau traditional remedies". *J.Ethnopharmacology*., 1996; 50: 55-59.
6. Jagessar. R.C., Mohamed. A., "Extractions and isolation of natural products from *Momordica Charantia*", Proceedings of the 15th Annual conference of the Caribbean Academy of Sciences, Guadeloupe, 2006; 40.
7. Jagessar R.C., Mohamed. N: Research abstracts, "Antimicrobial activities of selected plants", 1st International conference on the status of Biological Sciences in the Caribbean and Latin America Societies, Buddy's International hotel, Providence, Guyana, 2007; 17.
8. Jagessar R.C., Mohamed. A., Gomathinayagam, S: Research abstracts "Antimicrobial activities of selected tropical plants", 1st International conference on the status of Biological Sciences in the Caribbean and Latin America Societies,

Buddy's International hotel, Providence, Guyana, 2007; 39.

9. Jagessar R.C., Mohamed. N., Paper presentation "Antimicrobial activities of selected tropical plants", 1st International conference on the status of Biological Sciences in the Caribbean and Latin America Societies, Buddy's International hotel, Providence, Guyana, Reviewed and accepted for publication 2007.
10. Smith. C. M., Reynard. A.M.,"Textbook of Pharmacology", W.B.Saunders Company, 1992
11. Wood. A., "Topics in Drug design and discovery"., *Annual reports in medicinal Chemistry*., 2008; **41**: 353-409. Elsevier Inc.
12. Chemistry World, *Royal Society of Chemistry*, August., 2009; **6**(8): 16 .
13. Bozja. J., Yi. K., Shafer. W., Stojiljkovic. I., "Porphyrin-based compounds exert antibacterial action against the sexually transmitted pathogens", *International Journal of Antimicrobial Agents.*, **24(**6): 578-584,
14. Merchat, M. et al., "Studies on the Mechanism of Bacteria Photosensitization by *meso*-Substituted Cationic Porphyrins," *Journal of Photochemistry and Photobiology B: Biology* 1996; **35**: 149-157.
15. Merchat, M. *et* al., "*Meso*-Substituted Cationic Porphyrins as Efficient Photosensitizers of Gram- Positive and Gram-Negative Bacteria," *Journal of Photochemistry and Photobiology B: Biology.,* 1996; **32**: 153-157.
16. Nitzan, Y. et al., "Inactivation of Gram-Negative Bacteria by Photosensitized Porphyrins," *Photochemistry and Photobiology*., 1992; **55**(1): 89-96.
17. Huang, H.C, Hoes, J.A., "Penetration and infection of *Sclerotrinia sclerotiovum* by *covinilkuvum minitans"*, *Canada Journal. Botany*., 1976; **54**: 406-410.
18. Kondo. H., Oritani. T., Yamashita. K., *Agric.Biol.Chem.,* 1988; **52**(1): 129-133.
19. Mutak. S., Marsic.N., Dominics. M., Parlovic., D, *J.Med.Chem*., 2004; **47**(2): 411-431.
20. Murray,P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolke, R.H., "*Manual of Clinical Microbiology"*, 6th ed. Mosby Year Book, London, 1995.
21. Beer. P.D., Drew. M.G.B., Jagessar. R., "Selective anion recognition by novel 5,10,15,20 - *tetrakis* (o-ferrocenyl carbonylaminophenyl - substituted) zinc metalloporphyrin receptors"; *J. Chem. Soc., Dalton Trans.,* 1997; 881-886.
22. Jagessar. R.C.. Burns. D. H., "(Cis)-5,10,15,20 -Tetrakis (2-(arylurea) phenyl porphyrins": Novel neutral ligands for remarkablely selective and exceptionally strong chloride anion complexation in $(CD_3)_2SO$"; *J. Chem. Soc., Chem. Commun*., 1997; 1685-1686.
23. Cormode. D.P., Drew. M.G.B., Jagessar. R.C., Beer P.D., "Metalloporphyrin Anion sensors: The effect of the metal centre on the Anion binding properties of amido functionalized and *meso* tetraphenylporphyrins, (accepted for publications in *Dalton Transactions,* 2008.
24. Collman. J.P., Wagenknecht. P.S., Hutchison. J.E., "Molecular catalysts for multielectron redox reactions of small molecules", *Angnew.Chem. Int. Ed. Engl*., 1994; **20**: 1537-1554.
25. Benson. D.R., Valenzekovich. R., Diederich. F., "Catalytic cyclophanes: A porphyrin bridged cyclophane as a model for cytochrome P-450 enzymes"., *Angew. Chem. Int. Ed. Engl*., 1990; 29(2): 191-193.
26. Rose. E., Kossayl. A., Quelquajeu. M., Soleilhavoup. M., Duwarran. F., Burr. N., Lecas. A; "Synthesis of Biomimetic heme precursors: The double Picket Fence", 5, 10,15, 20-*tetrakis* (2', 6'-dinitro-4'-tert-butylphenyl) porphyrin., *J. Am. Chem. Soc*., 1996; 116(6).
27. Collman. J.P., Herrmann. P.C., lei. Fu, Eberspacher. T.A., Eubanks. M., Boitrel. B., P. Hayoz. P., Zhany. X., Brauman. J.I., Day. V.W., "Aza crown capped Porphyrin models of myoglobin: studies of the steric of gas binding. *J. Am.Chem. Soc*., 1991; **119**: 3481-3489.
28. Nelson. D. L., Cox. M. M., Lehninger, "Principles of Biochemistry", fourth edition., 2004; 157-189. W.H. Freeman and Company.
29. **"**Tour, J.M.; Rawlett, A.M.; Kozaki, M.; Yao, Y.; Jagessar, R.C.; Dirk, S.M.; Price, D.W.; Reed, M.A.; Zhou, C.-W.; Chen, J.;Wang, W.; Campbell, "Synthesis and Preliminary Testing of Molecular Wires and Devices," *Chem.Eur.J*., 2001; **7**: 5118-5134.
30. Jagessar. R.C., Tour. J.M.; "Synthesis of Porphyrins bearing *trans*-Thiols", ; *Organic letters.,* 2000; **2**, 111-113.
31. Jagessar. R.C., Gomathinayagam., Antifungal vs. antibacterial activity of a,b-unsaturated carbonyl compound, *Journal of Pure and Applied Microbiology*, 2010 (In press).