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

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

Antimicrobial activity of 5, 10, 15, 20- meso-tetrasik 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 \textit{S. aureus} and \textit{C. albicans} versus \textit{E. coli}. 

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

Over the years, plant extracts\textsuperscript{1,4,5-9} and isolated pure natural products\textsuperscript{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\textsuperscript{10-12}. Via a modulation of the structure of drugs, variation in the potency of their antimicrobial activity has been achieved\textsuperscript{10-12}. Even though there are several reports of synthesized compounds such as porphyrins and other porphyrinoid as antimicrobial agents\textsuperscript{13-16}, there is no documentation of 5,10,15,20- meso tetrasik 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-tetrasik pentafluorophenyl, 21H, 23H porphine via Agar well diffusion and Poison plate techniques\textsuperscript{15-17}. Porphyrins are a versatile class of chemical compounds, having found applications in the field of supramolecular chemistry\textsuperscript{21-27}, catalysis\textsuperscript{24-25}, oxygen haemoglobin mimics\textsuperscript{26-28} and in nanotechnology\textsuperscript{29-30} etc.

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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. The Control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion.

**Antimicrobial tests**

Compound (1) was investigated for their antimicrobial activity using the Agar diffusion technique and Poison Plate techniques 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 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 pathogenicity 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-\textit{meso} tetrakis pentafluorophenyl 21H, 23H porphine against pathogenic microorganisms: \textit{Candida albicans}, \textit{E.coli} (gram negative bacteria) and \textit{Staphylococcus aureus} (gram positive) using the agar diffusion and poison plate methods\textsuperscript{15-17}. For each microbial experiment, triplicates were done.

The zone of inhibition (mm) is quoted at the ED\textsubscript{50} value and as the area of inhibition (mm\textsuperscript{2}). ED\textsubscript{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\textsubscript{50} was calculated and converted into area of inhibition, mm\textsuperscript{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 inoculant\textsuperscript{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 \textit{Staphylococcus aureus} and the smallest zone of inhibition against \textit{E. Coli}, Tables (1)- (4) and Graphs 1 (a) and (b). The order of zone of inhibition against microbial agents follow the sequence: \textit{Staphylococcus. aureus} > \textit{Candida. albicans} > \textit{Eschericia. Coli}. For example, compound (1) induce zone of inhibition of 52.78 mm\textsuperscript{2} (ED\textsubscript{50} = 4.1 mm) against \textit{S. aureus} compared with a zone of inhibition of 35.66 m\textsuperscript{2} (ED\textsubscript{50} = 3.37 mm) against \textit{Candida albicans} and 28.83 mm\textsuperscript{2}
Table 1. Random check (1 mg in 1 ml) (Reference experiments)

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Candida albicans (antibiotic Nyastatin)</th>
<th>Staphylococcus aureus (antibiotic Ampicillin)</th>
<th>Escherichia coli (antibiotic Ampicillin)</th>
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<tr>
<td></td>
<td>(Average of Triplicate)* ED&lt;sub&gt;50&lt;/sub&gt; (mm)</td>
<td>Area of inhibition* (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>Area of inhibition* (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
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*Mean value

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

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*Mean value

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

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<th>Staphylococcus aureus (antibiotic Ampicillin)</th>
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<td>Area of inhibition* (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
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*Mean value

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

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<th>Sample Name</th>
<th>Candida albicans (antibiotic Nyastatin)</th>
<th>Staphylococcus aureus (antibiotic Ampicillin)</th>
<th>Escherichia coli (antibiotic Ampicillin)</th>
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<td>(Average of Triplicate)* ED&lt;sub&gt;50&lt;/sub&gt; (mm)</td>
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*Mean value

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) was found to be more antibacterial selective and compound (3) antifungal selective. 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.

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