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Enhanced Antimicrobial Activity of a Supramolecular System Formed via Hydrogen Bonding Interaction of a 5, 10, 15, 20 *Meso tetrakis* Pentafluorophenyl 21H, 23H Porphine with Mesoporphyrin (ix) Dihydrochloride

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Antimicrobial activity of a supramolecular system resulting from hydrogen bonding interaction of mesoporphyrin(ix)dihydrochloride (3) with 5, 10, 15, 20 - meso tetrakis pentafluorophenyl 21H, 23H porphine (4) was investigated using the Agar diffusion and Poison plate methods. Compound (5) was shown to display enhanced antimicrobial activities against *S. aureus, C. albicans* and *E. coli* in comparison to that of either (3) or (4), synthetic ampicillin and nystatin.

Key words: Enhanced Antimicrobial activity, hydrogen bonding, supramolecular Chemistry, Agar well diffusion, Poison plate method.

An investigation of antimicrobial activity of synthesized compounds in recent years has been an area of intense research in the field of Pharmacy and medicines[11-13]. Also, recently, the search for novel antifungal and antibacterial compounds has received special attention as a result of enhanced microbial resistance to current pesticides[1]. Initially, plant extracts[2,3,4,5-10] and isolated pure natural products [2,3] have been used as antimicrobial agents. Subsequently, the isolation and structural elucidation of bioactive compounds from fractionated extracts have led chemists to mimic and synthesized similiar compounds as antimicrobial agents[11-12]. Via a modulation of the structure of those compounds (drugs), variation in the potency of their antimicrobial activity have been achieved[12-13]. In our continuing search for novel, potent and selective antimicrobial agents, we report here the antimicrobial activities of (5), a supramolecular system formed via hydrogen bonding interaction between Mesoporphyrin (ix) dihydrochloride (3) and 5, 10, 15, 20- *meso tetrakis* pentafluorophenyl 21H, 23H porphine (4) against *Candida albicans*, *S. aureus* and *E. coli* using Agar diffusion and Poison plate techniques [14, 20].

We have recently reported the use of Mesoporphyrin (ix) dihydrochloride and 5, 10, 15, 20 - *meso tetrakis* pentafluorophenyl 21H, 23H

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porphine as antimicrobial agents [21-22]. In this paper we found significant enhanced antimicrobial properties of compound (5) when an equal amount of both porphyrins (3) and (4) were mixed in solution and tested. Porphyrins and porphyrinoid compounds have been use as anion supermolecules in Supramolecular Chemistry [23-25] as antimicrobial agents [26-29] and as molecular wires [31-32].

An integral unit of a supramolecular system is the hydrogen bond. The latter may be regarded as a particular kind of dipole-dipole interaction in which a hydrogen atom attached to an electronegative atom (or electron withdrawing group) is attracted to a neighbouring dipole on an adjacent molecule or functional group. Because of its relatively strong and highly directional nature, hydrogen bonding has been described as the "masterkey" interaction in supramolecular Chemistry. An excellent example is the formation of carboxylic acid dimers and the base pairing in DNA by hydrogen bonding [30].

Microbial strains investigated were *Candida albicans, S.aureus and E. coli. Candida albicans* is a diploid fungus (i.e a form of yeast) and is a casual agent of opportunistic oral and genital infection in humans[17]. *S. aureus* induced furuncles (*i.e.* boils) and carbuncles[16]. *E. coli* can cause several intestinal and extra intestinal infections such as urinary tract infections, meningitis, peritonis, mastitis, septicemia and gram negative pneumonia[15].

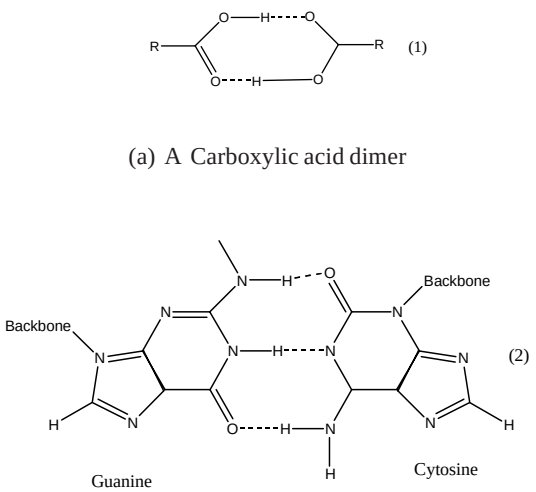

Fig. 1. Hydrogen bonding interactions in (a) Carboxylic acid dimer and (b) base pairing of Guanine and Cytosine

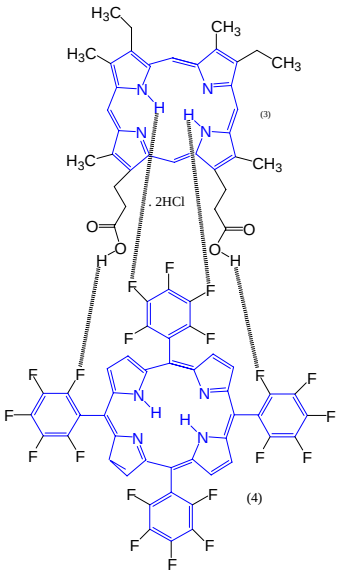

Fig. 2. Proposed hydrogen bonding interaction of mesoporphyrin (ix)dihydrochloride (3) and meso tetrakis pentafluorophenyl 21H, 23H porphin (4)

Procedure

Antimicrobial tests

Compound (5) was investigated for its antimicrobial activity using the Agar diffusion[14,20] and Poison Plate techniques[14] under aseptic conditions as reported for previously published work [21-22].

Reagents and materials

Mesoporphyrin (ix) dihydrochloride (1), *meso tetrakis* pentafluorophenyl 21H, 23H porphine and solvents were purchased from Aldrich. Antibiotics, Ampicillin, Nystatin, Mueller Hinton agar, agar plates and microbial discs were purchased locally. Bacterial and fungal culture were obtained at John's campus, Berbice, University of Guyana.

Preparation of Mesoporphyrin (ix) dihydrochloride (3)

Compound (3) was made up to the appropriate concentration of 10 mg in 10 ml (1mg

in 1 ml) of dichloromethane in a 25 ml round bottom flask and was stored under aseptic conditions.

Preparation of 5, 10, 15, 20- *meso tetrakis* pentafluorophenyl 21H, 23H porphine (4)

Compound (4) was made up to the appropriate concentration of 10 mg in 10ml (1mg in 1 ml) of dichloromethane in a 25 ml round bottom flask and was stored under aseptic conditions.

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

	Sample Number	Fungus, (*Candida albicans*) with Nystatin)	Bacteria, (*Staphylococcus aureus)* with Ampicillin	Bacteria, (*E.coli)* with Ampicillin
(Average of Triplicate)* ED_{50} (mm)	(1)	7.5	6.8	7.0
Area of inhibition (mm^2)*	(1)	176.63	145.19	153.86

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

	Sample Number	Fungus, (*Candida albicans*) with Nystatin)	Bacteria, (*Staphylococcus aureus)* with Ampicillin	Bacteria, (*E.coli)* with Ampicillin
(Average of Triplicate)* ED_{50} (mm)	-	<5	<5	<5
Area of inhibition (mm^2)*	-	<5	<5	<5

Table 3. Diffusion Plate (1 mg in 1 ml) for compound (5)

	Sample Number	Fungus, (*Candida albicans*) with Nystatin)	Bacteria, (*Staphylococcus aureus)* with Ampicillin	Bacteria, (*E.coli)* with Ampicillin
(Average of Triplicate)* ED_{50} (mm)	(5)	7.6	7.9	7.8
Area of inhibition (mm^2)*	(5)	181.37	195.96	191.04

Table 4. Poison Plate (1 mg in 1 ml) for compound (5)

	Sample Number	Fungus, (*Candida albicans*) with Nystatin)	Bacteria, (*Staphylococcus aureus)* with Ampicillin	Bacteria, (*E.coli)* with Ampicillin
(Average of Triplicate)* ED_{50} (mm)	(5)	7.9	7.5	7.7
Area of inhibition (mm^2)*	(5)	195.97	176.63	186.17

Table 5. Diffusion Plate (1 mg in 1 ml) for esoporphyrin (ix) *dihydrochloride* (3) 21

	Sample Number	Fungus, (*Candida albicans*) with Nystatin)	Bacteria, (*Staphylococcus aureus)* with Ampicillin	Bacteria, (*E.coli)* with Ampicillin
(Average of Triplicate)* ED_{50} (mm)	(3)	2.69	3.00	3.00
Area of inhibition (mm^2)*	(3)	22.38	28.26	28.62

Formation of Supramolecular system (5)

This was formed via the addition of 1 ml of (3) to 1 ml of (4) in dichloromethane

Source of microorganisms:

For the bacterial organisms, gram negative bacteria 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 Campus, Berbice.

Reference and Control

The references were antibiotic in nature. Ampicillin and Nystatin. Ampicillin was choosen 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].

Table 6. Poison Plate (1 mg in 1 ml) for mesoporphyrin (ix)dihydrochloride (3) [21]

	Sample Number	Fungus, (*Candida albicans*) with Nystatin)	Bacteria, (*Staphylococcus aureus)* with Ampicillin	Bacteria, (*E.coli)* with Ampicillin
(Average of Triplicate)* $ED_{50\ (mm)}$	(3)	2.90	2.90	2.67
Area of inhibition (mm^2)*	(3)	26.40	26.41	22.38

Table 7. Diffusion Plate (1 mg in 1 ml) for 5, 10, 15, 20-*meso tetrakis* pentafluorophenyl 21H, 23H porphine (4) [22]

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)*	(Average of Triplicate)* $ED_{50\ (mm)}$	Area of inhibition (mm^2)*
1	3.37	35.66	4.07	52.01	3.03	28.83

* Mean value

Table 8. Poison Plate (1 mg in 1 ml) for meso tetrakis pentafluorophenyl 21H, 23H porphine (4), [22]

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)*	(Average of Triplicate)* $ED_{50\ (mm)}$	Area of inhibition (mm^2)*
1	3.0	28.26	3.9	47.76	2.6	21.23

* Mean value

RESULTS

Results obtained in this experiment are as follows:

DISCUSSION

This paper focuses on the antimicrobial activity of supramolecular system (5) formed via hydrogen bonding interactions between Mesoporphyrin (ix) dihydrochloride (3) and 5, 10, 15, 20 *meso-tetrakis* pentafluorophenyl 21H, 23H porphine (4) against pathogenic microrganisms: *Candida albicans*, *E.coli* (gram negative bacteria) and *Staphylococcus aureus* (gram positive) using the Agar diffusion and Poison plate methods[14, 20]. For each microbial experiments, triplicates were done and the average value taken to calculate the area of inhibition.

The zone of inhibition (mm^2) 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 against antibiotics 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. For fungus *Candida albicans against* Nystatin and bacteria, *S. aureus* against ampicillin, the area of inhibition (mm^2) was 176.63 mm^2 (ED_{50} = 7.5 cm) and 145.19 mm^2 (ED_{50} = 6.8 cm) respectively. A controlled experiment was also investigated using the pure solvent, CH_2Cl_2 with the microorganism as the innoculant[20]. It was found that the pure solvent induced negligible zone of inhibition (< 5 mm) on the agar medium, Table 2. Thus, the zone of inhibition are indeed due to compound (3) rather than to the pure solvents.

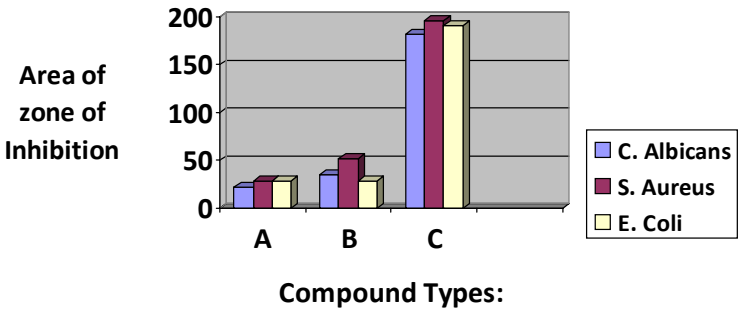

Fig. 1. Plot area of inhibition vs microbial strains for compds 1,2 & 3 using Diffusion Plate

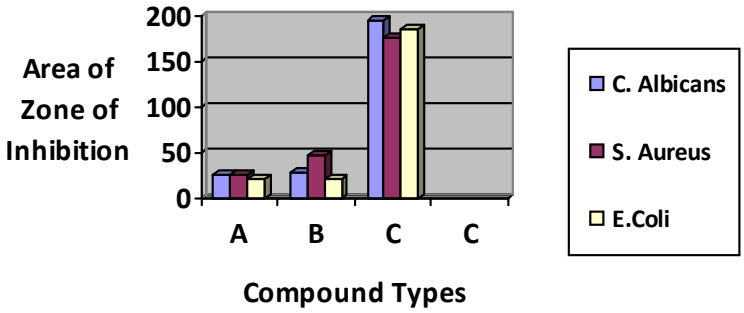

Fig. 2. Plot area of inhibition vs microbial strains for compds 1,2 & 3 using Poison Plate

A = Mesoporphyrin (ix) dihydrochloride = 3
B = 5,10,15,20-mesotetrakis pentafluorophenyl 21H, 23H, porphine = 4
C = Hydrogen bonded supermolecule = 5

Results indicate that for the Diffusion plate technique, the largest zone of inhibition was induced against bacterial strains *S.aureus* and the least against *Candida albicans* i.e the order of microbial resistance followed the sequence: *S.aureus* > *E. coli* > *C. albicans*. For example, zone of inhibition of 181.3 mm^2 (ED_{50} (mm) = 7.6 cm) against *Candida albicans* whereas the largest zone of inhibition of 195.96 mm^2 ($ED_{50(mm)}$ = 7.9cm) was induced by compound (3) against bacterial strains, *S. aureus*.

With the Poison plate technique, the largest zone of inhibition of 195.97mm^2 (ED_{50} (mm) = 7.9) was observed against Fungal strains, *Candida Albicans*. The smallest zone of inhibition was noted against bacterial strains, *S. aureus*, 176.63 mm^2 ($ED_{50\,(mm)}$ = 7.5 cm). The antimicrobial activity of compound (3) against microbial strain follow the sequence: *Candida albicans* > *S. aureus* > *E. coli*.

It is also evident that the area of inhibition induced by compound (5) against microbial strains was greater than those induced by reference compound Nystatin against *Candida albicans*, *Staphylococcus aureus* and *E. coli* against Ampicillin. This suggest that compound (5) is more potent as an antibiotic than synthetic Nystatin and Ampicillin against the above mentioned microbial strains.

Of greater significance, it is noteworthy that the area of inhibition induced by compound (5) is far greater than that induced by the individual component, *meso* porphyrin (ix) dihydrochloride, Tables 5 and Table 6 and 5, 10, 15, 20 - *meso tetrakis* pentafluorophenyl 21H, 23H porphine, Tables 7 and 8. For example, with Diffusion plate, an area of inhibition of 22.38 mm^2 (ED_{50} (mm) = 2.69) was obtained for Mesoporphyrin (ix) dichloride against *Candida albicans*. However, for compound (5), the corresponding zone of inhibition was 181.37 mm^2 ($ED_{50\,(mm)}$ = 7.6 mm^2).

In conclusion, compounds (3) and (4) form a hydrogen bonded supermolecule system (5) whose antimicrobial properties are more potent than either (3) and (4) or synthetic ampicillin and nystatin.

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