Amides as antimicrobial agents

Raymond C.Jagessar [1]*, Davendra Rampersaud [2]

*[1]Lecturer, Department of Chemistry, University of Guyana, South America
[2] Microbiologist, St. Joseph Mercy hospital, Parade Street, Georgetown, Guyana, South America.

raymondjagessar@yahoo.com

Abstract: The antimicrobial activities of amides: p-bromoacetanilide, acetanilide, nitrobenzamide and benzamide were investigated using the Stokes Disc diffusion sensitivity technique and the pour plate method. All compounds with the exception of benzamide exhibited both antibacterial and antifungal activities. Furthermore, the presence of nitro and bromo substituent on the phenyl ring seem to augment the antimicrobial activity. [New York Science Journal. 2008;1(1):22-26]. (ISSN: 1554-0200).

Keywords: Amides, Antimicrobial activity, Stokes Disc sensitivity technique, Pour Plate method.

1.0 Introduction:

Amides, RCONHR' are known to play a pivitol role in molecular recognition, being important components in Supramolecular chemical anion sensors[1-3] technology. Furthermore, in nature, the selective binding for substrates such as anion is achieved via the positional alignment of the amide hydrogen bonds[3].While plant extracts[5] and isolated pure natural products[6,7] have been used for antimicrobial activities, there are few reports of amides as antimicrobial agents[8,9].

This paper focuses on the antimicrobial activity of commercially available Aldrich amides: acetanilide (1), p-bromoacetanilide (2), benzamide (3) and nitrobenzamide (4), Fig 1.0.

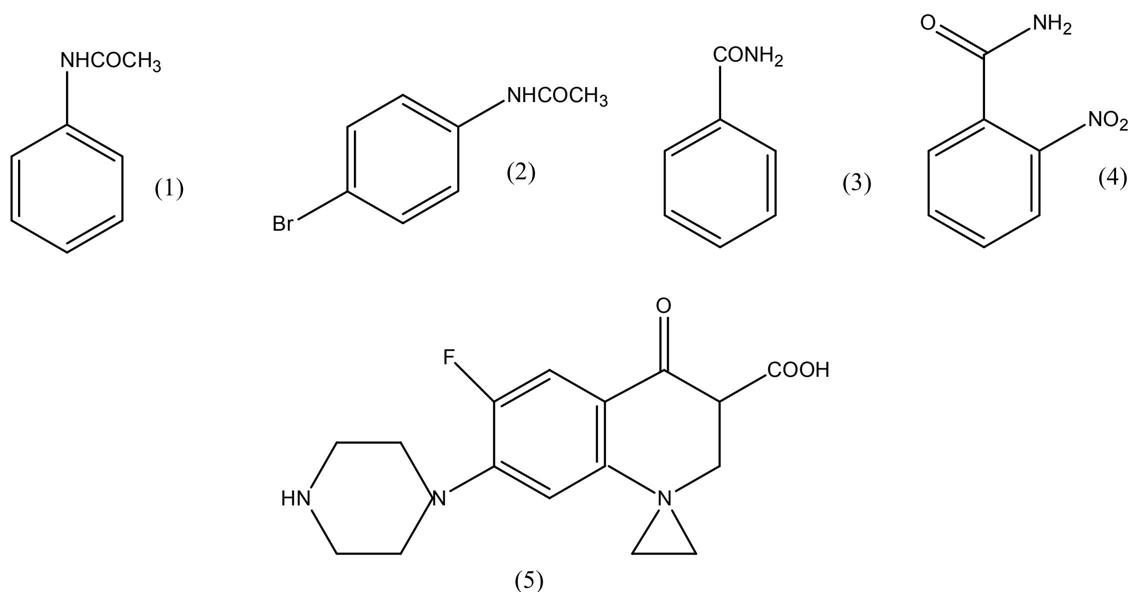

Fig. 1.0

2.0. Experiments:

2.1: Reagents and materials:

The amides, solvents and $BaCl_2.2H_2O$ were purchased from Aldrich. The antibiotic Ciprofloxacin and nystatin, Mueller Hinton agar, agar plates and microbial discs were purchased from the International Pharmacy Association in Guyana. Bacterial and fungal culture were obtained from Mercy hospital as necessary.

2.2. Preparation of Amide solution:

The amides were made to the appropriate concentration of 200 mg/ml in dichloromethane in a 25 ml round bottom flask and was sterilized by filtration through a 0.45 um membrane filter.

2.3 Preparation of Turbidity/opacity Standard (0.5 Mc Farland)

1% v/v solution of sulphuric acid was prepared by adding 1 ml of concentrated H_2SO_4 to 99 ml of distilled water and was mixed well.1.175% w/v solution of barium chloride was prepared ($BaCl_2.2H_2O$) by dissolving 2.35 g of dihydrate barium chloride ($BaCl_2.2H_2O$) in 200 ml of distilled water and was mixed well. To make the turbidity standard, 0.5 ml of the barium chloride solution was added to 99.5 ml of H_2SO_4 solution and was mixed thoroughly.The standard solution was dispensed into screw cap tubes as the same type as those for the preparation of the test and control inocula.The tubes were tightly sealed and stored in a dark room at room temperature (28-30ºC). The turbidity was vigorously agitated on a mechanical vortex mixer.

2.3. Source of microorganism:

For the bacterial organisms, both gram positive and gram negative bacteria were used. Gram positive and gram negative bacteria can be differentiated in the physical appearance of their cell envelopes. Gram positive and Gram negative bacteria used were *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) respectively. For the fungi, yeast of the *Candida albicans* (ATCC 1023) species were investigated. These microorganisms were stored in a refrigerator at the microbiology laboratory of the hospital.

2.4. Screening for Antimicrobial activities: Stokes Disc diffusion sensitivity technique.

Antimicrobial activities were investigated using Stokes Disc diffusion sensitivity and the pour plate method.Using Stokes Disc diffusion sensitivity testing technique[4] , an inoculum containing bacterial or yeast cells was applied on Muller-Hinton agar plates. On each plate, a reference or control antibiotic ciprofloxacin (CIPRO), 1–cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (5) was also applied.The reference antibiotic disc contained 10mg of antibiotic/disc. For fungal tests, nystatin was used. With the above testing technique, each disc was impregnated with the anticipated antimicrobial amide at appropriate concentration of 200 mg/ml using a microlitre syringe. This was then placed on a plate of sensitivity testing Mueller Hinton agar which was then incubated with the test organism:Bacteria/fungi. Incubation was done at 37ºC for 24 hr and 48 hr for the bacteria and *Candida albicans* species respectively. The antimicrobial compound diffuses from the disc into the medium. Following overnight incubation, the culture was examined for areas of no growth around the disc (zone of inhibition). The radius of the inhibition zone was measured from the edge of the disc to the edge of the zone.The end point of inhibition is where growth starts. Larger the inhibition zone diameter, greater is the antimicrobial activities. It is anticipated through the antimicrobial activity of amide, no area of growth will be induced around the disc.Bacteria or fungal strains sensitive to the antimicrobial are inhibited at a distance from the disc whereas resistant strains grow up to the edge of the disc.

2.5. Pour Plate Method.

With the pour plate method, 1 ml of microorganism (bacteria or fungi) was first tested against 1 ml of the amide solution i.e a 1:1 ratio of the microorganism to amide.This was followed with varying dilution of amide. This been 1:5, 1:10, 1:20 and 1:40 microorganism: amide concentration. The microorganism was placed in sterile water and was compared against the 1.0 Mc Farland standard so as to ensure that the turbidity exactly matches that of the standard. The density of the made up solution could be

adjusted either by (i) adding more colonies or (ii) adding more sterile water. For each ratio, the solution containing the microorganism and the amide solution were placed in vials and shaken vigorously so as to ensure a uniform mixture.

After the made up (adding water to the agar), nutrient agar was placed in the autoclave at 120°C for one and half hour, taken out and left to semi cool in a sterilized environment and then poured into 100 mm sterile glass plates with an even depth of 4mm on a level surface The mixture of microorganism and amide solution was vigourously shaken and then placed into the liquid, almost cool agar. A sterile glass rod was used to uniformly stir the mixture into the nutrient agar and left to harden or solidify in the glass plate. While this process is taking place, a 96% alcohol flame was used and the environment was sterilized with bleach and alcohol.

The inoculated plates were incubated for 48hrs at 37˚C in an inverted position (lid on bottom) to prevent collection of condensation on the agar surface. Unless the surface is dry, it will be difficult to obtain discrete surface colonies. The plates were examined for the appearance of individual colonies growing throughout the agar medium. The number of colonies were counted so as to determine how effective the amides were antibacterial and antifungal.

3.0. Results and Discussion: Interesting results were obtained as shown in **Table 1.0 – 5.0**.

Table 1.0 Antimicrobial activity of amides as shown by the inhibition zone diameter.

Gram-negative Bacteria, Area of inhibition.	**Gram-positive Bacteria, Area of inhibition.**	**Compounds (1)-(4)**	**Yeast (*candida* species) Area of inhibition.**	***Control disc***
17 mm	15 mm	Compound 1	12 mm	22 mm
21 mm	19 mm	Compound 2	11 mm	21 mm
No inhibition	No inhibition	Compound 3	No inhibition	19 mm
22 mm	17 mm	Compound 4	14 mm	22 mm

Compound (1), acetanilide and compound (2) induced an inhibition zone of 15 mm and 19 mm for gram positive bacteria, *Escherichia coli* respectively whereas inhibition zone of 17 mm and 21 mm were observed for compounds (1) and (2) against gram negative bacteria, *Staphylococcus aureus* respectively. For compound (4), nitrobenzamide, inhibition zone of 17 mm and 22 mm were obtained against gram positive and gram negative bacteria respectively. Compound (3), benzamide produced no inhibition zone on both gram positive and gram negative bacteria. Thus, compound (3) cannot be used as an antimicrobial compound. Both compounds (2) and (4) with electron withdrawing groups such as bromo and nitro exhibit a greater degree of inhibition against bacteria. For example, compound (2) and (4) induced zones of inhibition of 21mm and 22mm respectively against gram negative bacteria. Compound (1), (2) and (4) induced a smaller zone of inhibition against the fungal species, *candida albicans*. These been 12 mm, 11mm and 14 mm for compound (1), (2) and (4) respectively. It is interesting to note that compound (4) bearing a nitro group seem to produce the largest inhibition against gram negative bacteria and fungal strain. Again compound (3) produce no degree of inhibition on the fungal strain. These results are all summarized in Table 1.0. Pour plate results are summarized in Table 2.0-5.0.

Antimicrobial activity of amides as investigated using the pour plate method.

Table 2.0. compound 1.0

Bacteria	Fungi	Time(hrs)	Dilution(microorganism:amide)	Observations
S. aureus	*C.albicans*	48	1:1	No organism grown
S. aureus	*C.albicans*	48	1:5	No organism grown
S.aureus	*C.abicans*	48	1:10	No organism grown
S.aureus	*C.albicans*	48	1:20	Few colonies seen
S.aureus	*C.albicans*	48	1:40	Moderate amount of colonies seen

Table 3.0, **Compound 2**.

Bacteria	Fungi	Time (hrs)	Dilution (microorganism:amide)	Observations
S. aureus	*C.albicans*	48	1:1	No organism grown
S. aureus	*C.albicans*	48	1:5	No organism grown
S.aureus	*C.abicans*	48	1:10	No organism grown
S.aureus	*C.albicans*	48	1:20	Moderate colonies seen
S.aureus	*C.albicans*	48	1:40	Moderate colonies seen

Table 4.0. **Compound 3**.

Bacteria	Fungi	Time (hrs)	Dilution (microorganism:amide)	Observations
S. aureus	*C.albicans*	48	1:1	Few colonies seen
S. aureus	*C.albicans*	48	1:5	Few colonies seen
S.aureus	*C.abicans*	48	1:10	Moderate colonies seen
S.aureus	*C.albicans*	48	1:20	Large colonies seen
S.aureus	*C.albicans*	48	1:40	Large colonies seen

Table 5.0. **Compound 4.0**

Bacteria	Fungi	Time (hrs)	Dilution (microorganism:amide)	Observations
S. aureus	*C.albicans*	48	1:1	No organism grown
S. aureus	*C.albicans*	48	1:5	No organism grown
S.aureus	*C.abicans*	48	1:10	No organism grown
S.aureus	*C.albicans*	48	1:20	Few colonies seen
S.aureus	*C.albicans*	48	1:40	Moderate amount of colonies seen

The pour plate method indicates that as the concentration of the amide decrease (increasing dilutions) whilst the concentration of microorganism remaining constant, the number of colonies increase i.e the antimicrobial properties of the amide decrease with decreasing concentration of the amide.

All compounds show their strongest antimicrobial activities (i.e no organism growth or zero colonies) when the ratio of microorganism to amide is 1:1 and 1:5, Table, 2-5 Compound (1), (2) and (4) showed no organism growth at microorganism: amide ratio of 1:1, 1:5 and 1:10. With microorganism:amide ratio of 1:20 and 1:40, compound (1), (2) and (4) showed identical results. Thus, the results obtained via the pour plate method mirror that obtained via the disc diffusion method for compound (1), (2) and (4). Also, the pour plate method results for compound (3) also mirror those obtained via the disc diffusion method. For compound (3), colonies were seen at micro organism/amide ratio of 1:5, 1:10, 1:20, 1:40. These been few, moderate, large and large. With the exception of (3), no colonies were seen for

compound (1), (2) and (4) at microorganism:amide ratio of 1:1. These results strongly suggest that compound (3), benzamide, clearly is not an antimicrobial.

Conclusion:

In conclusion, the results obtained via the the disc diffusion and pour plate method support each other. Thus, based on the above, compounds (1), (2) and (4) can be used as antibacterial agents against gram positive bacteria and gram negative bacteria and as an antifungal agents against yeast or useful in the future design of drugs. It is anticipated that well designed macrocyclic amides will produce more potent results.

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Correspondence to:
Dr.Raymond C. Jagessar,
Department of Chemistry,
University of Guyana,
Turkeyen Campus,
Greater Georgetown,
South America
Email: raymondjagessar@yahoo.com

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