Original Research Article

Screening and Scoring of Microbial activity of -2-Pyridinealdazine

Abstract

The objective of this work was to screen the antibacterial and anti fungal activity of 2-Pyridinealdazine This azine is assessed for its antimicrobial activity against both gram positive and gram negative bacteria and fungal strains like and fungal strains *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium chrysogenum, Trichoderma viride*. 2-Pyridinealdazine is found to be effective against all pathogenic strains with the inhibition zone ranging from 10 to 30mm.

Key words; 2-Pyridinealdazine *Aspergillus flavus*, gram positive, gram negative bacteria

Introduction

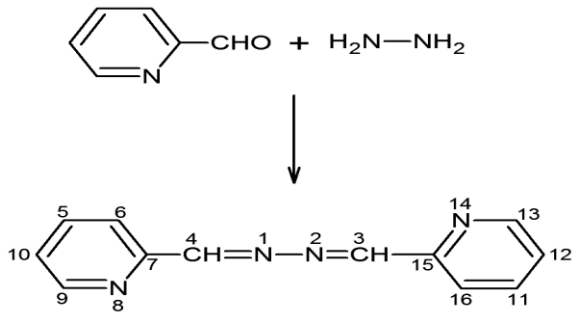
Aromatic azines represent a class of compounds that are known to be a good proton acceptors.The study of the intermolecular interactions of six –membered nitrogenated aromatic rings is of particular importance since they are known to constitute key building blocks of proteins,nucleotides,and many other important compounds[1-3].For example, the pyridine ring occurs in azines,the vitamins niacin and pyridoxal[4] and in the in vitro synthesis of DNA[5-6]. Azines have generated attention because of their ability to be used in a variety of chemical reactions, such as 1,3-dipolar cycloadditions with dieno-philes and 3 + 2 cycloadditions[7]in the construction of five-membered rings,which parallels the Diels –Alder reaction in construction of six-membered rings. Azines are receiving interest for their potential in bond formation reactions [8,9] bio-logical properties [10,11], the design of liquid crystals[12-19] and other materials applications. Symmetric azines having mesomorphism were reported by Deun et al. [20] in which the rare earth(e.g. lanthanum chloride) promotes decomposition of the hydrazide ligands.Recently we have a synthesised and determined the conformations of 2-pyridine carboxaldehyde by DFT studies.[21] Microorganisms are available naturally in the surrounding environment; therefore they can easily reach food during harvesting, slaughtering, processing, and packaging[22]. These microorganisms can survive under adverse conditions used in the food preservation such as low temperature, modified atmosphere packaging, vacuum packaging, as well as resist conventional pasteurization[23] .Thus, there is a considerable concern among consumers regarding the risk of using synthetic additives for human health, that led to decrease the use of these chemicals in food preservation [24] Therefore, new eco-friendly methodologies are required to reduce the growth of pathogenic bacteria and prolong the

self-life of food products, without using chemical preservatives. Hence the present investigation focussed biological deeds of symmetrical azine.

Experimental details

Preparation of symmetrical azine

2-Pyridinealdazine was first prepared by Lenart [22]. The2-pyridinecarboxaldehyde used in the present work was obtainedfrom the Aldrich chemical company and used without further purification. About 0.04 mol of 2-pyridinecarboxaldehyde and0.02 mol of hydrazine monohydrate was taken in a round bottomflask. The reaction mixture dissolved in ethanol and refluxed wellfor 1 h. The reaction mixture was kept at room temperature for an hour. The separated solid was filtered and purified by recrystal-lization from ethanol.



Scheme 1. Synthetic route of 2-pyridinealdazine.

Result and Discussions

Antibacterial activity

The following Gram–positive and Gram–negative strains have been used for the study.

1. *Escherichia coli* (Gram–negative)
2. *Salmonella typhi* (Gram–negative)
3. *Staphylococcus aureus* (Gram–positive)
4. *Bacillus subtilis* (Gram–positive)
5. *Streptococcus pyogenes* (Gram–positive)

Preparation of test inoculums

a) Sub-culture (preparation of seeded broth)

The strains of *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Salmonella typhi, Bacillus subtilis* and *Streptococcus pyogenes* were inoculated in conical flasks containing 100 mL of sterile nutrient broth. These conical flasks were incubated at 37±1°C for 24 h. This was used as seeded broth.

b) Standardization of seeded broth (viable count)

Dilutions

1 mL of seeded broth of each strain was diluted with 99 mL of steriled normal saline containing 0.05% tween 80 (8 drops of tween 80 in 1000 mL of normal saline). From that, 1 mL was further diluted to 10 mL with sterile normal saline. This was continued to until 10^{-10} mL of the dilution of seeded broth was obtained.

Incubation of nutrient agar petridishes

The dilutions were studied by inoculating 0.2 mL of each dilution on to the solidified nutrient agar medium by spread plate method after incubation at 37±1°C for 24 h. The number of well-formed colonie on the plates was counted. The seeded broth was then suitably diluted to have between 10^5–10^7 microorganisms per millimeter or cfu/mL. This was designated as the working stock and used for the antibacterial studies.

Preparation of solution of test compounds

The solution of test compounds were prepared by dissolving the same in dimethylsufoxide (DMSO) in specific gravity bottle and stored in refrigerator. The solution was removed from the refrigerator 1 h prior to its use and allowed to warm upto the room temperature. The test compounds were prepared at a concentration of 200 µg/mL. Similarly, the standard drug solutions of Amikacin and Amphotericin B were used respectively at a concentration of 200 µg/mL for finding the minimum inhibitory concentration solvent control of DMSO was also maintained throughout the experiments simultaneously.

c) Preparation of culture media

The following mediums were used for the bacterial growth:

i) Nutrient agar medium

ii) Nutrient broth medium

The media were sterilized by autoclaving at a pressure of 15 lb/sq at 121°C for 20 min.

i) Nutrient agar medium (Hi-Media)

The nutrient agar medium was prepared by dissolving 28 g of nutrient agar (procured from Hi-Media, Mumbai) in 1000 mL of distilled water.

Materials

Peptone	:	1%
Sodium chloride	:	0.5%
Beef extract	:	1%
Agar	:	2%
pH	:	7.4±0.2

ii) Nutrient broth medium (Hi-Media)

The nutrient broth medium was prepared by dissolving 13 g of nutrient broth (Hi-Media, Mumbai) in 1000 mL of distilled water.

Materials

Peptone	:	1%
Sodium chloride	:	0.5%
Beef extract	:	1%
pH	:	7.4±0.2

d) Determination of antibacterial activity by disc-diffusion method

Base plates were prepared by pouring 10 mL of autoclaved Muller-Hinton agar into sterile petridishes (9 cm) and allowing them to settle. Sterile blank discs (6 mm) were impregnated with 15 μL of known concentration of stock solution of tested complexes as to obtain discs containing 100 and 400 μg of each compound. Impregnated discs were air dried and cautiously placed on the surface of Mueller-Hinton agar plates freshly inoculated with microorganisms. After 10 min at room temperature the plated culture incubated for 24 h at 37°C. Experiments were conducted in quadruplicate (four discs with identical concentration of the same compound) and commercial antibiotic Amikacin (100 μg) impregnated discs used as positive controls. Susceptibility diameter zone was reported as the average value of replicates measurements.

Antifungal activity

The following fungal strains were used for the study.

1. *Aspergillus flavus*
2. *Aspergillus niger*
3. *Fusarium oxysporum*
4. *Penicillium chrysogenum*
5. *Trichoderma viride*

a) Preparation of culture media

Sabouraud's dextrose agar (SDA) medium was used for the growth of fungi and testing was done in Sabouraud's dextrose broth (SDB) medium.

b) Antifungal disc diffusion method

Mature conidia of fungal isolates were harvested from potato dextrose agar (PDA) plates and suspended in ringer solution and spore suspensions standardized with a haemocytometer (10^4 conidia mL^{-1}). Conidial suspension (1 mL) representing each fungal isolate was then spread on a 9 cm petridishes containing PDA (20 mL) with the excess of conidial suspension decanted and allow to dry. The compounds were dissolved in dimethyl sulphoxide (DMSO). Sterile 6 mm diameter test discs were impregnated with 15 μL of the solution of each test compound to certain 100 and 400 μg/disc in triplicates. Amphotericin B was used as a reference drug, for fungal inhibition. While DMSO was used as a negative control. Plates were incubating at room temperature

(22–25°C) for 3 days. The radius of the inhibition zone of fungal growth was measured after 3 days. Diameter zone was reported.

Preparation of culture media

(a)SDA medium

Formula

Dextrose	:	40g
Peptone	:	10g
Agar	:	15g
Distilled Water	:	1000g
pH	:	7.3±0.2

(b) SDA medium

Formula

Dextrose	:	40g
Peptone	:	10g
Distilled Water	:	1000 mL
pH	:	7.3±0.2

Antimicrobial studies

Determination of minimum inhibitory concentration (MIC) by two folds micro-broth dilution method against respective susceptible bacterial species. MIC (Minimum Inhibitory Concentration) is the lowest concentration of antibiotics or antimicrobials that can inhibit the growth of certain microbes. MIC values are specific for each combination of antibiotics and microbes. The MIC of an antibiotic against microbes is used to determine the sensitivity of

microbes to antibiotics. Based on the test results in various concentrations obtained a value of 10%, included in the intermediate category (CSLI category) [23]. Based on the clear zone produced and the MIC value it can be concluded that the azines compound, specifically 2-Pyridinealdazine has the potential to be developed as an antibacterial active ingredient to support the treatment of infectious diseases in the category of sensitive inhibition and is included in the broad spectrum because it can provide good resistance to gram bacteria positive or gram negative reinforced by MIC values included in the intermediate category.

The preliminary antimicrobial activity of azine is examined by disc diffusion method. The bacterial strains *viz.*, Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus, Streptococcus pyogenes*), Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) and fungal strains *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum, Penicillium chrysogenum, Trichoderma viride* were used for this study. The zone of inhibition (mm) of azine against both the tested bacterial and fungal strains are listed in Table 1. The 2-pyridinealdazine is shown to be good inhibitor of B. subtilis, E. coli and S. pyogenes but it shows minimum inhibitory activity towards *Staphylococcus aureus* and relatively poor inhibitor of ps*udomonas aeruginosa* compared with the reference compound.

Table-1 Diameter of zone of inhibition

Organisms	**Diameter of zone of inhibition (mm)**	**Reference**
	2-pyridinealdazine	
1. *Bacillus subtilis*	20	22
2. *Staphylococcus aureus*	16	18
3. *Streptococcus pyogenes*	26	23
4. *Escherichia coli*	22	16
5. *Pseudomonas aeruginosa*	10	31

Table-2 Diameter of zone of inhibition (cont.)

Organisms	Diameter of zone of inhibition (mm)	Reference
6. *Aspergillus flavus*	12	31
7. *Aspergillus niger*	13	28
8. *Fusarium oxysporum*	11	32
9. *Penicillium chrysogenum*	30	32
10. *Trichoderma viride*	16	31

From the antifungal activity data (Table 2), it could be observed that more or less equal activity isexhibited by azine towards *Aspergillus flavus and found to be good inhibitor of Penicillium chrysogenum.Comparetivly* 2-pyridinealdazine exhibit more or less good activity towards all the fungal strains given in the above table.

Conclusion

The 2-pyridinealdazine is effective in inhibiting the growth of Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus, Streptococcus pyogenes*), Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and fungal strains *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium chrysogenum, Trichoderma viride* were used for this study.

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