Asian Journal of Chemical Sciences

10(4): 108-112, 2021; Article no.AJOCS.81912
ISSN: 2456-7795

Evaluation of Anti Microbial Activity of -2-Pyridinealdazine

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJOCS/2021/v10i419110
Editor(s):
(1) Dr. Sung Cheal Moon, Korea Institute of Materials Science, Korea.
Reviewers:
(1) Md. Tarek Hossain, American International University, Bangladesh.
(2) El Khattabi El Hassan, University Hassan II Casablanca, Morocco.
Complete Peer review History, details of the editor(s), Reviewers and additional Reviewers are available here: https://www.sdiarticle5.com/review-history/81912

Original Research Article

Received 20 October 2021
Accepted 22 December 2021
Published 23 December 2021

ABSTRACT

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

Keywords: 2-Pyridinealdazine Aspergillus flavus; gram positive; gram negative bacteria.

1. INTRODUCTION

Azines are compounds possesing -C=N-N=C- moiety, representing a class of compounds that are known to be a good proton acceptors. Azines have generated attention because of their ability to be used ina variety of chemical reactions, such as 1,3-dipolar cycloadditions with dieno-philes and 3 + 2 cycloadditions [1] in the construction of five-membered rings, which parallels the Diels – Alder reaction in construction of six-memberedrings. The study of the intermolecular interactions of six –membered nitrogenated aromatic rings is of particular importance since

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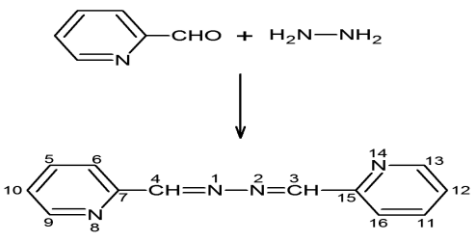
they are known to constitute key building blocks of proteins, nucleotides and many other important compounds [1-3].For example, the pyridine ring occurs in azines, the vitamins niacin and pyridoxal [4] and in the in vitro synthesis of DNA [5-6]. Azines are receiving interest for their potential in bond formation reactions [7-9] biological 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 ofthe hydrazide ligands. Recently synthesis and determination of the conformations of 2-pyridine carboxaldehyde by DFT studies was reported but only few reports on the literature on the biological aspects of azines [21-25]. Hence the present investigation focussed on biological deeds of symmetrical azine**.**

2. METHODS

2.1. Preparation of Symmetrical Azine

The starting material used for the preparation of 2-pyridinealdazine is 2-pyridinecarboxaldehyde and it was purchased from the Aldrich chemical company so used as such without further purification. Ethanolic solution of 0.04 mol of 2-pyridinecarboxaldehyde and 0.02 mol of hydrazine monohydrate was taken in a round bottomflask. and refluxed well for 1 h. The reaction mixture was kept at room temperature for an hour. The separated solid was filtered and purified by recrystallization from ethanol.



Scheme 1. Synthetic route of 2-pyridinealdazine

3. RESULTS AND DISCUSSIONS

3.1 Antibacterial Activity

The following bacterial strains have been used for the study. Among them two (*Escherichia coli& Salmonella typhi)* are Gram–negative all the remaining are Gram–positive

1. *Escherichia coli*
2. *Salmonella typhi*
3. *Staphylococcus aureus*
4. *Bacillus subtilis*
5. *Streptococcus pyogenes*

3.2 Preparation of Test Inoculums

3.2.1 Sub-culture (preparation of seeded broth)

In conical flasks containing 100 mL of sterile nutrient broth the strains of *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Salmonella typhi, Bacillus subtilis*and *Streptococcus pyogenes* were inoculated. Incubation of the conical flsks were done at 37±1°C for 24 h. This broth is called as seeded broth.

3.2.2 Standardization of seeded broth (viable count)

Dilutions: About 99 mL of steriled normal saline containing 0.05% tween 80 is used to dilute 1 mL of seeded broth of each strain (8 drops of tween 80 in 1000 mL of normal saline). One mL from that was further diluted to 10 mL with sterile normal saline and it was continued til 10^{-10} mL of the dilution of seeded broth was obtained.

3.2.3 Incubation of nutrient agar Petri dishes

Dilutions were monitored 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. Total number of well-formed colonies on the plates were taken in to account. Suitably diluted seeded broth having 10^5to10^7 microorganisms per millimeter or cfu/mL was designated as the working stock and used for the antibacterial studies.

3.2.4 Preparation of solution of test compounds

The 2-pyridinealdazine was dissolved in the dimethyl lsufoxide (DMSO) at a concentration of 200 µg/mL. in specific gravity bottle and stored in refrigerator. 1 h prior to its use the solution was removed from the refrigerator and allowed to attain the room temperature.The standard drug solutions of Amikacin and Amphotericin B were prepared similarly, at a concentration of 200 µg/mL for finding the minimum inhibitory concentration solvent control of DMSO was also maintained throughout the experiments simultaneously.

3.2.5 Preparation of culture media

Nutrient agar medium and Nutrient broth medium were used for the bacterial growth:

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.

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.

3.2.6 Determination of antibacterial activity by disc-diffusion method

By pouring 10 mL of autoclaved Muller-Hinton agar into sterile petridishes (9 cm) base plates were prepared and were allowed to settle. Impregnation of sterile blank discs (6 mm) was done with 15 μL of known concentration of stock solution of tested complexes as to obtain discs containing 100 and 400 μg of each compound. All the impregnated discs were air dried and cautiously placed on the surface of Mueller-Hinton agar plates freshly inoculated with microorganisms. The plated culture incubated for 24 h at 37°C and experiments were conducted in quadruplicate and commercial antibiotic Amikacin (100 μg) impregnated discs used as positive controls. Susceptibility diameter zone was reported as the average value of replicates measurements.

3.2.7 Antifungal activity

The fungal strains used for the study were given below

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

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.

3.2.8 Antifungal disc diffusion method

Potato dextrose agar (PDA) plates used to harvest mature conidia of fungal isolates and then it was suspended in ringer solution and spore suspensions .Further it was standardized with a haemocytometer (10^4conidia mL^{-1}). Conidial suspension (1 mL) representing each fungal isolate was then spread on a 9 cm Petri dishes containing PDA (20 mL) and the excess of conidial suspension was decanted and allowed to dry. 2-pyridinealdazine was 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. For fungal inhibition Amphotericin B was used as a reference drug,. While DMSO was used as a negative control. Incubation of plates were done at room temperature (22–25°C) for 3 days. After 3 days the radius of the inhibition zone of fungal growth was measured and diameter of the zone was reported.

3.2.9 Preparation of culture media

(a)SDA medium:

Formula

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

(b) SDA medium

Formula

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

3.3 Antimicrobial Studies

The lowest concentration of antibiotics or antimicrobials is known as MIC (Minimum Inhibitory Concentration) that can inhibit the growth of certain microbes. for each For every antibiotics and microbes the MIC values are specific. 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

Table 1. Diameter of zone of inhibition

Organisms	Diameter of zone of inhibition (mm)	
	2-pyridinealdazine	Reference
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

10%, included in the intermediate category (CSLI category) [23]. From 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 positive or gram negative bacteria 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-pyridinealdazineis 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*eudomonas aeruginosa* compared with the reference compound.

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.Comparatively*2-pyridinealdazine exhibit more or less good activity towards all the fungal strains given in the above table.

4. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ebrahimi A, Habibi M, Masoodi HR. chem. phys. 2007;340:85.
2. Abadleh M, Peifer C, Kinkel K, Sccholimeyer D, Laufer S. Acta Crystallogr. 2007;63:1423.
3. Ghosh K, Masanta G. Supramol. chem. 2005;17:331.
4. Tarr JB. J. Arditti, plantphysiol. 1982;69:553.
5. Mac Gregor JA, Hamilton CM, Kubicek JE, Mirsalis JC. J. Appl. Toxicol. 2000;20:389.
6. Gao EJ, et al. Eur J Med chem. 2010;45:4531.
7. Schweizer EE, Cao Z, Rheingold AL, Brunch M. Reactions of azines, 16:Preparation of 6-(phenylmethylene)-

5H-pyrazolo[1,5-d] [2,4] benzoxazepines, 5H,7H-pyrazolo [1,5-d] [2] benzazepin-6-one, and indeno [2,3-c] pyridine-3,9-diones.J. Org. Chem. 1993;58:4339–4345.
8. Schweizer EE, Cao Z, Rheingold AL, Bruch M. J.Org. Chem. 1993;58:4339–4345.
9. Marek R, Sta´ Stna´-Sedla´ Cková I, Jousek J, Marek, J, Potacek M. Bull. Soc. Chim. Belg. 1997;106:645-649.
10. Demus D, Goodby J, Gray GW, Spiess HW, Vill V. Handbook of Liquid Crystals; Wiley VCH, Weinheim; 1998.
11. Khodair AI, Bertrand P. Tetrahedron. 1998;54:4859–4872.
12. Yiang Z, Zheng J, Yang CJ, Lanz. Univ., J. SichuanUnion Univ., Eng. Sci. Ed. 2004;40:51–54.
13. Hoelzemann G. Expert Opin. Ther. Pat. 2006;16:1175–1178.
14. Screda SV, Antipin MY, Timofeeva TV, Struchkov YT, Sov YT. Phys. Crystallogr. 1988;33:66–70.
15. Astheimer H, Watz L, Haase W, Loub J, Muller HJ, Gallardo M. Mol. Cryst. Liq. Cryst. 1985; 131:343–351.
16. Centore R, Panunzi B, Tuzi AZ. Kristallog. 1996;211:31–38.
17. Centore R, Garzillo CJ. Chem. Soc. Perkin. 1997;2:79–84.
18. Espinet P, Etxebarria J, Remon A, Serrano JL. Angew Chem. Int. Ed. Engl. 1989;28:1065–1066
19. Melendez E, Serrano JL. Mol. Cryst. Liq. Cryst.1983;91:173–185.
20. Deun RV, Parac-Vogt TN, Hecke KV, Meervelt LV, Binnemans K, Guillon D, Donnio B. J. Mater. Chem. 2003;13:1639–1645.
21. Arulmani R, Balachander R, Vijaya P, Sankaran KR. spectrochim. Acta A. 2015;138:660-666.
22. Hatab et al. J. Food Sci. 2016;81:1987–1995.
DOI: 10.1111/1750-3841.13382\
23. Joux F, et al. Microbes Infect. 2000;2: 1523–1535.
DOI: 10.1016/S1286-4579(00)01307-1
24. Lenart GH. Liebigs Ann. Chem. 1915;410: 101–103.
25. Lim CK, Tho LY, Lim LM, Shah SAA, Weber JFF. Lett. Org. Chem. 2012;9(8): 549-555.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/81912