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RESEARCH ARTICLE

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Synthesis and antibacterial activities of 3H-benzooxazole-2, 2-dithiol and 3H-benzothiazole-2, 2-dithiol

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ABSTRACT

Benzo-heterocycles are very well known compounds that bind strongly and selectively to so many metal ions. They readily form chelates with all transition metal ions through its two donor sulphur atoms. In this study, two derivatives of benzo-heterocyclic compound (3H-Benzooxazole-2,2-dithiol and 3H-Benzothiazole-2,2-dithiol) which was synthesized by refluxing one mole of 2-amino-phenol with one mole of CS_2 in acetone, then one mole of 2-Amino-benzenethiol and one mole of CS_2 in acetone for two hours respectively, as indicated in our earlier report. These compounds were studied to determine their effectiveness in the treatment of diseases caused by the tested organisms. The antibacterial activities of these ligands were carried out using the disc diffusion method. Antibacterial activities were exhibited by 3H-Benzooxazole-2, 2-dithiol and 3H-Benzothiazole-2, 2-dithiol, against *Bacillus subtilis, Bacillus cereus, Pseudomonas aureginosa, Proteus mirabilis,* and *Salmonella typhi*. The minimum inhibitory concentration was 10mg/ml for both Ligands with a zone of inhibition range of 10.6mm-10.8mm for 3H-Benzooxazole-2, 2-dithiol and with the zone of inhibition range of 10.8mm-11.1mm for 3H-Benzothiazole-2, 2-dithiol. The ligands can compete favourably with gentamycin which served as the reference drug.

Keywords: Antibacterial activity, benzo-heterocycles, 3H-Benzooxazole-2,2-dithiol, 3H-Benzothiazole-2,2-dithiol

1. INTRODUCTION

Benzo-hectrocycles are versatile compounds with a wide range of chemistry, capable of forming complexes with most of the elements and able to stabilise transition metals in a variety of oxidation states [1]. This ability of stabilizing high oxidation states in metal complexes reflects strong o-bonding characteristic of these ligands [2]. A very large number of benzo-heterocyclic complexes with transition and non-transition metal ions have

been reported [3-5]. Some benzo-heterocyclic compounds with dithiocarbamate moiety have attracted attention because of their potential biological property [6]. The metal complexes of some of these compounds present striking structural features and have many applications, such as high pressure lubricants, fungicides, pesticides, and accelerators used in vulcanization [7].

Although the sulphur atoms of these ligands possess o-donor and n-back-donation characteristics of the same order of magnitude, these ligands have a special characteristics in that there is an additional n-electron flow from nitrogen to sulphur via a planar delocalized π-orbital system [2]. The effect of the delocalized π- orbital system results in a strong electron donation and hence a high electron density on the metal leading to its next higher oxidation state [8]. While a lot of hetero-cyclic complexes have been known for over the years, with many having been synthesized, the majority of these contain only simple alkyl substituents such as methyl and ethyl [2]. They are a class of metal-chelating, antioxidant compounds with various applications in medicine for the treatment of bacterial and fungal infections, and possible treatment of some other ailments like acquired immune deficiency syndrome [9].

A lot of work has been carried out on the antimicrobial activities of benzo-heterocyclic compounds. Most of the works reported on these activities are basically on their complexes. Mixed-ligand dithiocarbamate complexes of Nickel (II) have been reported to show good activity against *Escherichia coli, Pseudomonas aureginosa, Klebsiella oxytosa and Staphylococcus aureus* [10].

Jayaraju *et al.* (2012) reported the synthesis, characterization and antimicrobial activity of 2-Amino2-methyl 1-propanol Dithiocarbamate. Their report showed that the complexes have selective activity towards some microbes [11]. Ekennia *et al*., (2015) reported the synthesis, characterization, and antifungal activities of the pyridine adducts of N-methyl-N-phenyl dithiocarbamate complexes of Mn(II), Co(II), Ni(II), and Cu(II). They further reported, for the first time, the extractability of Ni (II) and Cu(II) ions by N-methyl-N-phenyl dithiocarbamate from aqueous phase into the organic phase. The compounds and the dithiocarbamate ligand were evaluated for antifungal properties against three important pathogens, namely, *Aspergillus flavus, Aspergillus niger,* and *Candida albicans*. Their report suggests that the products have potentials as lead compounds in broad spectrum research for antifungal agents, and that the ligand is a good reagent for pre-concentration and extraction of metal ions in different media [12].

Nabipour (2011) gave a report on cobalt complex, the *In vitro* antifungal activity and antibacterial activity. The antifungal activity was screened *in vitro* against *Candida albicans, Aspergillus flavus* and *Aspergillus niger* and the antibacterial activity screened *in vitro* against *Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus, Bacillus subtilis*. The report showed that the compounds exhibited significant antibacterial activity and the

bacterial strains with the zone of inhibition, 23 mm at minimum inhibitory concentration (MIC) of 30.0 µg/disc [2].

The purpose of this study is to find out if the two synthesized derivatives of benzo-hecterocyclic ligands (3H-Benzooxazole-2, 2-dithiol and 3H-Benzothiazole-2, 2-dithiol) can be effectively used as anti-bacterial agents.

2. METHOD AND MATERIAL

Syntheses of ligands

Syntheses of 3H-Benzooxazole-2,2-dithiol and 3H-Benzothiazole-2,2-dithiol have already been reported in earlier study and followed the same for further research [13].

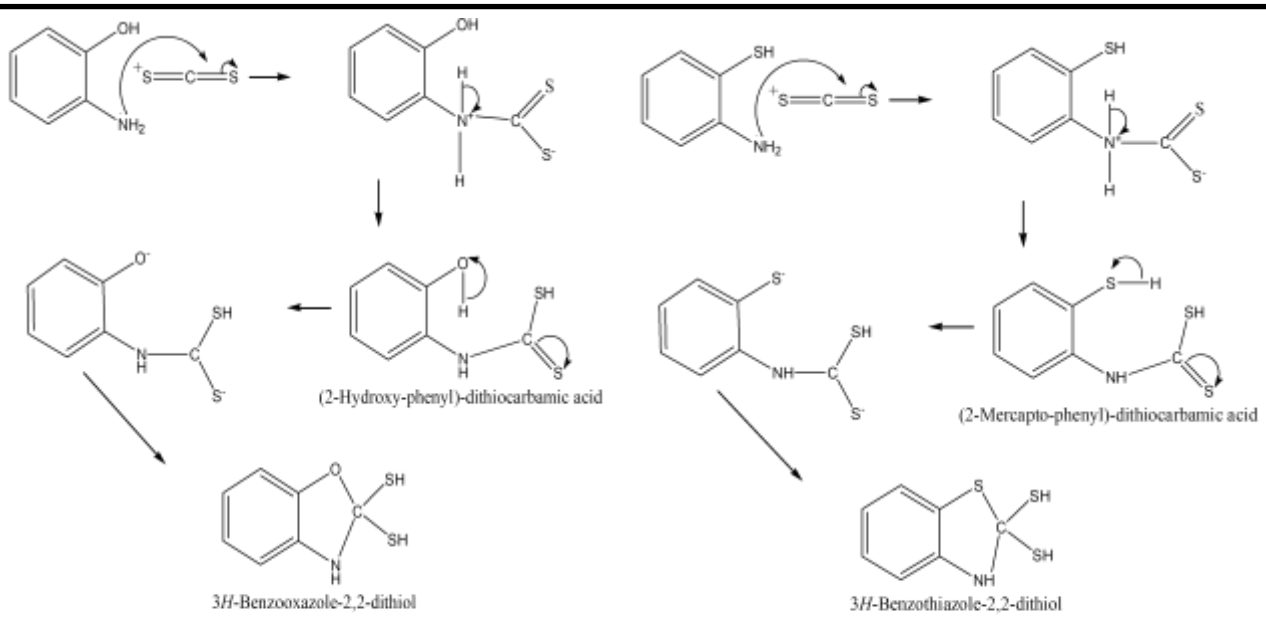
Antibacterial assay of the ligands

The ligands were tested against five standard strain of bacteria obtained from International Centre for Drug Research, Lucknow. The microbes were: *Bacillus subtilis* (ATCC 14579), *Bacillus cereus* (ATCC 33923), *Pseudomonas aureginosa* (ATCC 27856), *Proteus mirabilis* (ATCC 21784) and *Salmonella typhi* (ATCC 2785). Antibacterial activities of the ligands were investigated using the disc diffusion method [14].

Preparation of sample solution

A stock solution of each ligand was prepared by dissolving 200mg of the ligand in 2.0 ml of dimethylsulphoxide (DMSO), giving a concentration of 100mg/ml. From this, other standard solutions of 30, 25 20, 10, 15, 1.0 and 0.1 mg/ml were prepared for each of the ligand using DMSO as solvent. This was done to obtain the minimum inhibitory concentrations (MIC) of the ligands.

Twenty (20) sterilized paper discs were



A B

Figure A) Mechanism for the synthesis of 3H-Benzooxazole-2,2-dithiol B) Mechanism for the synthesis of 3H-Benzothiazole-2,2-dithiol

impregnated with 0.2 ml of the various prepared concentrations of the ligands. Also, 20 sterilized paper discs were impregnated with 0.2 ml each of DMSO and gentamycin (10mg/ml) in separate glass vials which served as the negative and positive controls respectively.

Inoculums preparation and disc placement

Inoculation was done using streaking method which involves streaking the inoculums around the peripheral region of the plate (Petri-dishes) using a sterilized cotton bud for each micro-organism. The Petri-dishes were divided into section with a marker indicating the various prepared concentrations of the ligands (100, 30, 25, 20, 15, 10, 1.0, 0.1 mg/ml), DMSO and gentamycin.

After the inoculation, a disc impregnated in the 0.2 ml of the various prepared concentrations of each of the ligand was picked with a syringe (each concentration with its own syringe) and dropped in the appropriate position as marked in the Petri-dish. This was done in triplicate for each bacterial to get an accurate reading. After the introduction of each of the disc in its appropriate position on the plates, the plates were carefully and properly packed into an incubator operated at 37 °C and allowed for about 24 h. Zones of inhibition were determined by measuring clear zones across the discs in mm. Mean values and standard deviations of triplicate readings were then determined and used in this research.

3. RESULTS AND DISCUSSION

Antibacterial activities of the ligands were determined using disc diffusion method. The activity was measured as zone of inhibition which is the diameter of the clear zone of inhibited bacterial growth. The ligands showed activity against the test organisms at concentrations of 10, 15, 20, 25, 30, and 100 mg/ml for both ligand (3H-Benzooxazole-2, 2-dithiol and 3H-Benzothiazole-2, 2-dithiol). The analysis was done in triplicate and the mean values of the results with their corresponding standard deviations (tables 1 and 2).

Chemicals or drugs are often administered to selectively eliminate or aid in the elimination of pathogenic organisms. Substances used for the treatment of bacterial diseases are referred to as chemotherapeutic substance or agent [15]. The synthesized ligands were studied to find out if they can be used as chemotherapeutic agents in the treatment of diseases caused by the five test organisms.

Both ligands were found to have antibacterial properties against all five tested organisms, with the same minimum inhibitory concentration (MIC) of 10mg/ml. The activity of 3H-Benzooxazole-2, 2-dithiol on *Salmonella typhi, Bacillus subtilis, Pseudomonas aureginosa, Bacillus cereus* and *Proteus mirabilis* as presented in table 1 showed zone of inhibition in the range of 20.4mm-20.7mm at the same concentration (100mg/ml). The minimum inhibitory concentration (MIC) of this ligand (10mg/ml), gave an activity in the range of 10.6mm-10.8mm (table1).

The increasing number and variety of drug resistance pathogens is a serious public health problem [16]. Recent studies show that *Bacillus subtilis* grow in anaerobic conditions and use

Table 1. Antibacterial activity of 3H-Benzooxazole-2, 2-dithiol

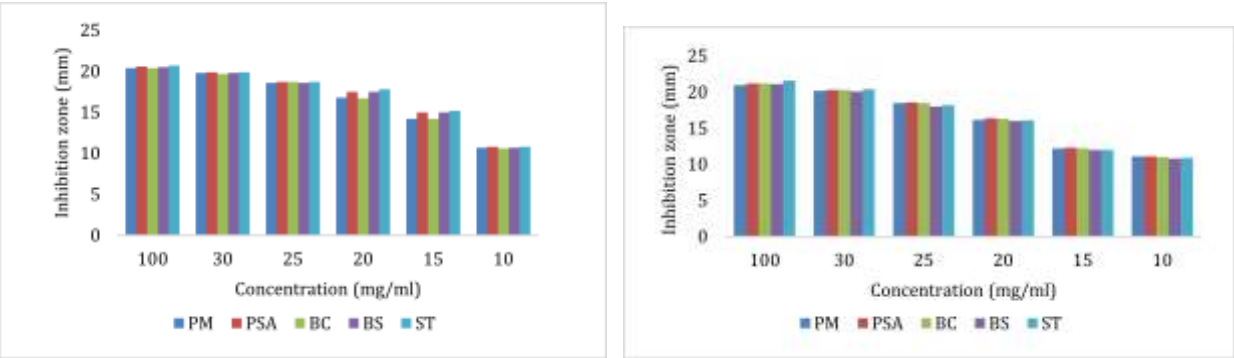
Bacteria	*Zone of inhibition (mm)									
	Ligand Concentration (mg/ml)								Gentamycin	
	100	30	25	20	15	10	1.0	0.1	(10mg/ml)	DMSO
PM	20.4±0.163	19.8±0.094	18.6±0.082	16.8±0.082	14.2±0.082	10.7±0.082	0	0	25.4±0.216	0
PSA	20.6±0.082	19.9±0.245	18.7±0.141	17.5±0.141	15.0±0.082	10.8±0.082	0	0	25.2±0.082	0
BC	20.4±0.141	19.7±0.082	18.7±0.141	16.7±0.082	14.2±0.082	10.6±0.163	0	0	24.8±0.082	0
BS	20.5±0.082	19.8±0.082	18.60±0.082	17.50±0.082	15.00±0.216	10.7±0.082	0	0	25.3±0.082	0
ST	20.7±0.141	19.9±0.082	18.7±0.163	17.8±0.082	15.2±0.082	10.8±0.082	0	0	24.6±0.082	0

*The values are average values of three readings
ST = *Salmonella typhi,* BS = *Bacillus subtilis,* BC = *Bacillus cereus,* PSA= *Pseudomonas aureginosa,* PM = *Proteus mirabilis*, Gentamycin = Positive Control, DMSO = Negative Control

Table 2. Antibacterial activity of 3H-Benzothiazole-2,2-dithiol

Bacteria	Zone of inhibition (mm)									
	Ligand Concentration (mg/ml)								Gentamycin	
	100	30	25	20	15	10	1.0	0.1	(10mg/ml)	DMSO
PM	21.0±0.00	20.2±0.082	18.5±0.082	16.2±0.082	12.2±0.082	11.1±0.082	0	0	25.5±0.082	0
PSA	21.2±0.082	20.3±0.082	18.6±0.082	16.4±0.082	12.3±0.082	11.1±0.249	0	0	25.0±0.o82	0
BC	21.2±0.082	20.3±0.082	18.5±0.082	16.3±0.082	12.2±0.082	11.0±0.082	0	0	24.7±0.082	0
BS	21.1±0.544	20.1±0.082	18.0±0.082	16.0±0.163	12.0±0.082	10.8±0.082	0	0	25.0±0.082	0
ST	21.6±0.141	20.4±0.082	18.2±0.082	16.1±0.082	12.0±0.163	10.9±0.082	0	0	25.1±0.082	0

*The values are average values of three readings
ST = *Salmonella typhi,* BS = *Bacillus subtilis,* BC = *Bacillus cereus,* PSA= *Pseudomonas aureginosa,* PM = *Proteus mirabilis*, Gentamycin = Positive Control, DMSO = Negative Control



A B

Figure A) Average zones of inhibition of the ligand 3H-Benzooxazole-2,2-dithiol B) Average zones of inhibition of the ligand 3H-Benzothiazole-2,2-dithiol

nitrite as terminal acceptor of electrons [17]. Its spores can survive the extreme heating that is often used to cook food, and is responsible for causing rapines [18].

Even through *Bacillus subtilis* which is a rod-shaped gram positive bacteria can gain protection more quickly against many stress situations such as acidic, alkaline, osmotic or oxidative conditions, heat and ethanol [19, 20], result of this Study (Table 1) has shown that 3H-Benzooxazole-2, 2-dithiol can be effective for the treatment of diseases caused by *Bacillus subtilis* at MIC of 10mg/ml concentration.

Gentamycin was found to inhibit the growth of the five tested organisms and the zone of inhibition was found to be in the range of 24.6-25.4mm, while the solvent (DMSO) showed no zone of inhibition against the tested organisms. This indicates that the antibacterial activities observed in the screening of 3H-Benzooxazole-2, 2-dithiol is as a result of the ligand and not due to the DMSO used in the preparation of different ligand concentrations.

MIC of the ligand (3H-Benzooxazole-2, 2-dithiol) was found to be 10mg/ml, with 10.8mm zone of inhibition for *Salmonella typhi* and *Pseudomonas aureginosa*, 10.7mm zone of inhibition for *Bacillus subtilis* and *Proteus mirabilis. Bacillus cereus* was inhibited by 3H-Benzooxazole-2, 2-dithiol with zone of inhibition of 10.6mm and MIC 10mg/ml; is an endemic, gram-positive rod shaped beta haemolytic bacteria that causes food borne illness [21], which get compounded when food is improperly refrigerated, allowing the spores to germinate, resulting in the production of enterotoxin. Its ingestion lead to illnesses like diarrohea and emetic syndrome [22]. Results show that 3H-Benzooxazole-2, 2-

dithiol can be used as a remedy for the treatment of any infection caused by *Bacillus cereus* and also for the treatment of infections caused by the other tested microbes.

The ligand 3H-Benzothiazole-2, 2-dithiol, was also found to have antibacterial property against the five organisms used, showing similar MIC of 10mg/ml as was observed in 3H-Benzooxazole-2,2-dithiol. Activity on *Salmonella typhi* showed the highest zone of inhibition (21.6mm) at concentration 100mg/ml, while other organisms showed zone of inhibition range 21.0 -21.2mm. Activity on *Bacillus cereus* and *Pseudomonas aureginosa* showed the same zone of inhibition (21.2mm) at concentration of 100mg/ml as shown in Table 2.

Pseudomonas aeruginosa grows in the absence of oxygen, if nitrate is available as respiratory election acceptor [23, 24]. Formation of bacteria capsule, slime layer and bio-film effectively protects cells from opsonisation, antibodies, complement deposition and phagocyte engulfment [25]. Properties of the bacterium undoubtedly contribute to its ecological success as an opportunistic pathogen [26]. The fatality rate in patients is near 50 percent and it is occasionally also a pathogen of plant [27-28]. The combination of gentamycin and carbencillin is frequently used for the treatment of severe *pseudomonas* infections [29]. But, with increased concentration, these ligands can also serve as

treatment for diseases caused by *Pseudomonas aeruginosa*.

This is indicated in the activity of the ligand 3H-Benzothiazole-2,2-dithiol which gave MIC of 10mg/ml with the zone of inhibition 11.1mm for *Pseudomonas aeruginosa* and *Proteus mirabilis*, 10.9mm and 10.8mm for *Salmonella typhi* and *Bacillus subtilis* respectively and 11.0mm for *Bacillus Cereus*. *Proteus mirabilis* which was inhibited by the ligand 3H-Benzothiazole-2,2-dithiol with 11.1mm zone of inhibition at MIC 10mg/m and 21.0mm zone of inhibition at 100mg/ml is a gram-negative, facultative anaerobic bacterium. They inhibit growth of unrelated strains resulting in a macroscopically visible line of reduced bacteria growth where two swarming strains intersect [30]. They have the ability to produce high level of urease which hydrolyses urea to ammonia and thus makes the urine more alkaline. If left untreated, the increased alkalinity can lead to the formation of crystals of struvite, calcium carbonate, and or apatite. The bacteria can be found throughout the stones, and these bacteria lurking in the stones can reinitiate after antibiotic treatment. Once the stone develop, overtime, they may grow large enough to cause obstruction and renal failures [31] with very distinct odour [32].

The results show that both ligands used in this study can compete favourably with gentamycin at an increased concentration. For an effective treatment of bacterial infections caused by any of these five tested organisms, concentration of not less than 10mg/ml is required for both ligands.

The present studies showed that the both ligands exhibited antibacterial activity for all test organisms at the same concentration (10mg/ml and above). However, the 3H-Benzothiazole-2,2-dithiol ligand gave a better activity at higher concentration compared to the 3H-Benzooxazole-2, 2-dithiol ligand which also gave a better activity at some concentration with respect to certain organisms tested. The enhanced activity of 3H-Benzothiazole-2, 2-dithiol ligand may be attributed to the presence of more sulphur compound compared to the other (3H-Benzooxazole-2, 2-dithiol). It may also be more stable with a high bonding ability [33].

4. CONCLUSION

Moderate antibacterial activities were exhibited by 3H-Benzothiazole-2,2-dithiol and 3H-Benzooxazole-2,2-dithiol ligands against all five tested standard isolates (*Bacillus subtilis, Bacillus cereus, Pseudomonas aureginosa, Proteus mirabilis* and *Salmonella typhi*). Results from this study shows that the minimum inhibitory concentration (MIC) is 10mg/ml for 3H-Benzooxazole-2,2-dithiol with zone of inhibition range 10.6mm-10.8mm and 10mg/ml also for 3H-Benzothiazole-2,2-dithiol with zone of inhibition range 10.8mm-11.1mm. It also indicated that with increased concentrations, the ligands can compete favourably with gentamycin which served as the reference drug. Commonly used antibiotics may have a lot of side effects, hence the need to explore other alternatives. Benzo-heteroclic ligands can act as perfect alternative or at least provide reasonable molecular scaffold on which future potent antibiotics could be based.

5. ACKNOWLEDGEMENT

NA

6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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No source of funding

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