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ANTIMICROBIAL AND ANTIOXIDANT SCREENING OF SOME CHALCOGEN BEARING LIGANDS AND THEIR TRANSITION METAL COMPLEXES

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ABSTRACT

Eight tellurium bearing compounds, bis[2-(1,3-dioxan-2-yl)ethyl] telluride (1), N-[(4-phenyl telluro) butyl] phthalimide (2), bis[4-(N-phthalimido) butyl] telluride (3) and N-[2-(phenyl telluro) ethyl] piperidine (4), [AgSCN.(1)] (5), [$CdCl_2$.(2)] (6), [CdI_2.(3)] (7) and [$ZnCl_2$.(4)] (8) are tested for their antibacterial activity against gram positive bacteria Streptococcus aureus, Enterobacter aerogens and gram negative bacteria Escherechia coli. They were also screened against fungal strains viz Aspergillus niger, Mucor species by disc diffusion method. In antibacterial screening, inhibition zones demonstrated that compounds 4 and 8 found most active against Streptococcus aureus and Enterobacter aerogens, which may be due to presence of N-CH_2 group. In antifungal screening, inhibition zones demonstrated that compounds 6 and 7 shows good fungicidal activity against Aspergillus niger and Mucor species. The antioxidant activity reveals that the compounds 1, 3, 5-7 shows moderate activity, but compounds 4 and 8 shows higher activity than others due to the presence of Nitrogen donor and its specific interaction with metal i.e. Zn.

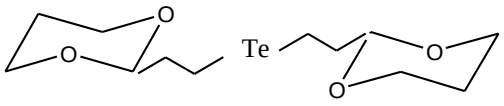
KEY WORDS

bis[2-(1,3-dioxan-2-yl) ethyl] telluride, N-[(4-phenyl telluro) butyl] phthalimide, bis[4-(N-phthalimido)butyl] telluride, N-[2-(phenyl telluro) ethyl] piperidine, S. aureus, E. aerogens, E. coli, A. niger and Mucor sp.

INTRODUCTION:

In the recent years, organotellurium compounds have attracted considerable attention as antioxidants, antitumoral activity, antihelmenthic, antibacterial activity and protease inhibitors. Where Photodynamic therapy (PDT) has been developed as an alternative cancer therapy.[1] Tellurium dichalcogenides are more efficient antioxidants than selenium counterparts. Organotellurium compounds are readily oxidized from the divalent to tetravalent state. Consequently, this property makes tellurides attractive scavangers of reactive oxidizing agents such as hydrogen peroxide, hypo chloride and proxy radicals.

Over the past decade, fungal infections have become an important complication and a major cause of morbidity and mortality in immune compromised individuals[2]. In comparison to organoselenium compounds,[3] the fungal activity of tellurium bearing compounds are scarce. The antifungal agents currently in use are limited either by their ineffectiveness or toxicity.[4,5] In continuation of our earlier work on the chemistry of chalcogen bearing compounds.[6,7] We communicate the results of antibacterial, antifungal and antioxidant activity of hybrid organotellurium compound compounds **1-4** and their complexes [AgSCN**.(1)**] (**5**), [$CdCl_2$**.(2)**] (**6**), [CdI_2**.(3)**] (**7**) and [$ZnCl_2$**.(4)**] (**8**).



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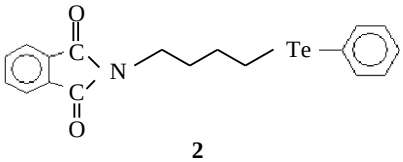

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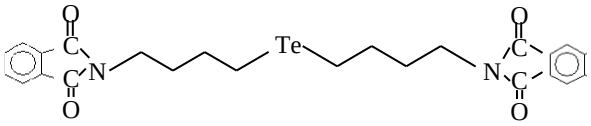

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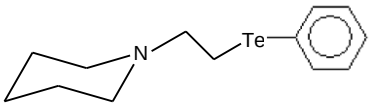

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MATERIALS AND METHODS:

The solvents ($CHCl_3$ and DMSO) were purified and dried by conventional methods.[8] The preparation and characterization of compounds **1-8** have been reported elsewhere.[9]

Preparation of 1-8

The compounds **1-4** were synthesized by *insitu* borohydride reduction of tellurium and diphenyl ditelluride with different organic halides under dinitrogen atmosphere, whereas compounds **5-8** were synthesized by stirring of inorganic salts with **1-4** in dry ethanol in 1:1 molar ratio.[9]

Antimicrobial activity

Synthesized compounds **1-8** were screened *in vitro* for the antimicrobial activities against three bacterial strains namely *Enterobacter aerogens, Escherechia Coli, Streptococcus aureus* and two fungal strains namely *Aspergillus niger* and *Mucor sp.* by disc diffusion method[10-12]. The tested compounds were dissolved in chloroform or DMSO at two different concentrations 20 µg/ml and 40 µg/ml. The bacterial and fungal cultures were maintained on nutrient agar (Table 1). The agar media were incubated in petriplates. The filter paper (Whatman No.1) sterile discs of 5 mm diameter impregnated with the tested compounds were placed on these petriplates with different bacterial and fungal strains, along with standard. After 24 hours of incubation at 37°C for bacteria and 48 hours of incubation, for fungi the diameter of the inhibition zone (mm) (**Table 2** and **3**) was measured. Chloramphenicol and Fluconazole were used as standard drugs respectively.

Table 1: List of the used media for the bacterial and fungal studies

S.No.	Media used*		Constituent/l		pH	
	For bacteria	For fungi	For bacteria	For fungi	For bacteria	For fungi
1	NB	SDB	Peptone-10g Beefextract-5g NaCl-5g	Peptone-5g Dextrose-20g	7.2 ± 0.2	5.6 ± 0.2
2	NA	SDA	Peptone-10g Beefextract-5g NaCl-5g Bacteriological agar-16g	Peptone-5g Dextrose-20g Agar-7.5g	7.2 ± 0.2	5.6 ± 0.2

NA- Nutrient agar, NB- Nutrient broth, SDB- Sabouraud dextrose broth, SDA- Sabouraud dextrose agar.

Antioxidant activity

Synthesized compounds **1-8** were screened *in vitro* for antioxidant activity. DPPH radical (Dipicryl diphenyl hydrazine) scavenging activity was determined by standard method of Singh *et al.*[13]. 5 ml of methanol solution of DPPH (0.1 mM) was added to 1ml of the sample solutions at different concentrations (1000-25µml) and vortexed. The mixtures were incubated at room temperature (25-30°C) for 20 min. Changes in the absorbance were measured at 517 nm and were compared with the Control i.e ascorbic acid (2 mg/ml).

Determination of anti-oxidant activity (DPPH radical scavenging activity)

4.3 mg of DPPH (1, 1-Diphenyl-2-picrylhydrazyl) was dissolved in 3.3 ml methanol, it was protected from light by covering the test tubes with aluminum foil. 150 µl

DPPH solution was added to 3 ml methanol and absorbance was taken immediately at 517 nm for control reading. 50 µl of various concentrations of synthesized compounds as well as standard compound (Ascorbic acid) were taken and the volume was made uniformly to 150 µl using methanol. Each of the samples was then further diluted with methanol up to 3 ml and to each 150 µl DPPH was added. Absorbance was taken after 15 minutes at 517 nm using methanol as blank on UV-visible spectrometer Shimadzu, UV-1601, Japan. The IC_{50} values for each drug compounds as well as standard preparation were calculated. The DPPH free radical scavenging activity was calculated using the following formula:

$$\% \text{ scavenging} = \frac{[\text{Absorbance of control} - \text{Absorbance of test sample}] \times 100}{\text{Absorbance of control}}$$

RESULTS

The antibacterial and antifungal evaluation of chalcogen bearing ligands and their complexes (compounds **1-8**) in terms of zone of inhibition is reported in **Table 2** and **3** respectively while antioxidant activity is reported in Table 4. The lipophilic characteristic is essential for deciding the activity of the compound. The increased activity of the compounds having Cd and Zn metal may increase the lipophilic characters of the central metal ion which has pronounced effect on the normal cell process thereby having higher activity against bacteria. These compounds show lipophilicity so it disturbs the cell wall and causes pores which ultimately results in leaking out of the internal constituents like cytoplasm and causes death to the cell membrane rupturing.

In antifungal studies, the fungal strains are hard to treat in comparison to bacteria because of the cell wall is made up of chitin. However, the compounds showed good fungicidal activity, may be due to the presence of organic group phthalimide having nitrogen, which forms a tight bond with heame iron of fungal P450 enzyme preventing substrate and oxygen binding. The images of the petri dish bearing zone of inhibition for different compounds could be seen in **Figures 1-3**

Table 2: Antibacterial activity of compounds 1-8

Compounds	Diameter of zone of inhibition (mm)					
	S.aureus		*E.coli*		*E.aerogens*	
	20µg/ml	40µg/ml	20µg/ml	40µg/ml	20µg/ml	40µg/ml
1	14	18	11	14	12	13
2	15	19	13	15	14	15
3	15	16	15	17	13	15
4	22	25	16	17	15	18
5	12	15	12	15	14	17
6	18	20	19	23	19	22
7	15	20	15	19	18	22
8	19	24	20	24	21	29
STD	-	31	-	30	-	31
Solvent	NA	NA	NA	NA	NA	NA

[Values represent the diameter (mm) of inhibition zone produced around each disc are average of three separate experiments, DMSO was used as a control and chloramphenicol as standard drug]

Table 3: Antifungal activity data of compounds 1-8

Compounds	Diameter of zone of inhibition (mm)			
	A.niger		*Mucor*	
	20µg/ml	40µg/ml	20µg/ml	40µg/ml
1	15	16	11	16
2	20	25	14	23
3	19	20	15	17
4	28	34	22	24
5	17	19	11	13
6	32	34	23	27
7	33	35	25	29
8	22	26	18	21
STD	-	30	-	31
Solvent	NA	NA	NA	NA

[Values represent the diameter (mm) of inhibition zone produced around each disc are average of 3 separate experiments, DMSO was used as a control and fluconazole as standard drug]

Antioxidant activity data i.e. absorbance at different concentration i.e.2, 4, 8, 20 and 40 reported in **Table 4**. Reveals that the all compounds **1-8** shows moderate activity, but **4** and **8** shows higher activity than others. The tellurium, which is presents in its structure coordinating zinc, exhibits a strong secondary interaction with nitrogen, play an important role in the catalytic antioxidant activity of these compounds.[14-17] The bar graph represents the relative activity of reported compounds in **Figure 4.**

Table 4: Antioxidant data of samples 1-8 showing absorbance at different concentration

Compounds	Concentration of compounds (µl)				
	2	4	8	20	40
STD	2.77	5.52	24.31	57.79	00.00
1	17.03	18.35	18.88	35.56	35.31
2	11.76	10.02	14.98	23.80	35.70
3	10.30	13.40	14.14	23.80	36.30
4	10.60	13.30	14.30	24.80	40.48
5	13.10	13.80	14.90	23.00	37.23
6	11.10	13.40	14.30	22.50	37.46
7	11.70	14.00	15.30	22.70	38.63
8	10.80	14.50	14.80	23.00	40.20

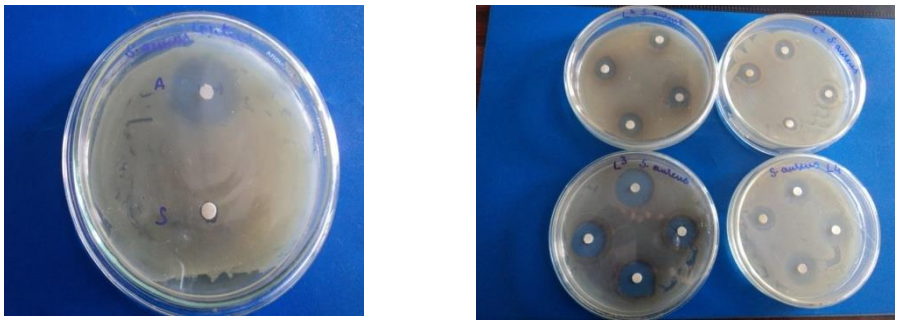

Figure 1: Zone of inhibition surrounding the disc of standard antibiotic chloramphenicol and compound 1-4 against *E. coli and S. aureus*

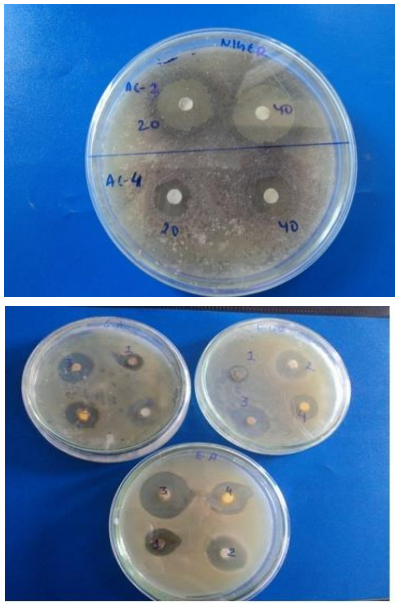

Figure 2: Zone of inhibition surrounding the disc of compounds 4-8 against *E. coli, E. aerogen and S. aureus.* and zone of inhibition surrounding the disc of compound 3 and 4 against *A. niger*

Figure 3: Zone of inhibition surrounding the disc of compounds 5-8 against *A. niger and Mucor sp.*

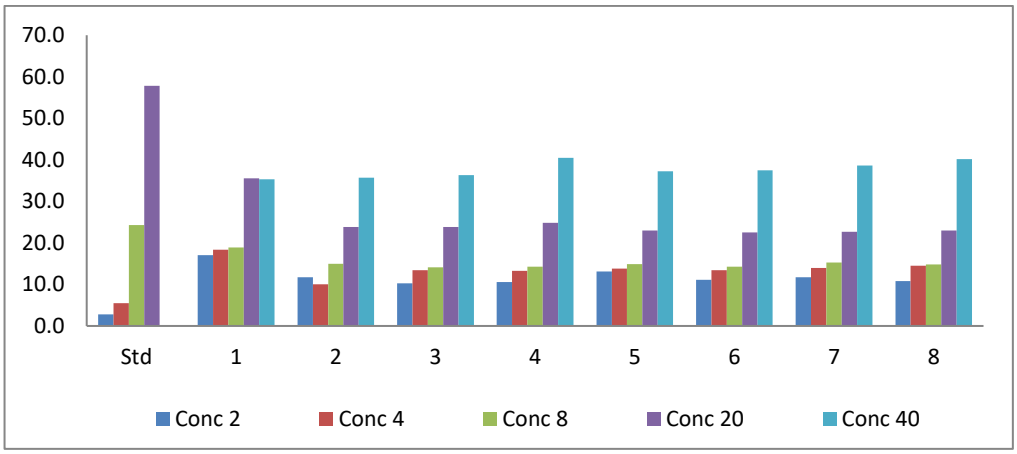

Figure 4: Antioxidant activity graph of compounds 1-8

DISCUSSION

Among all the tested compounds the Antibacterial activity of compounds **4** and **8** found highest against *S. aureus* and *E.aerogens,* which may be due to presence of N-CH_2 group which increases the lipophilicity and easier penetration of compound in outer cell wall of the bacteria which boost the bioactivity of these compounds.[18] Compounds **1** and **5** showed least activity which may be due to absence of nitrogen whereas 2, 3, 6 and 7 showed moderate activity against all the bacterial strains which may be due to presence of O=C-N group in the formula. It is evident from the data that

on an average compound **6-8** (metal complexes) are more effective in comparison to **1-4** (ligands)[19].

The increased activity of the compounds having Cd and Zn metal may increase the lipophilic characters of the central metal ion which have pronounced effect on the normal cell process thereby having higher activity against bacteria. As the compounds **6-8** are liphophilic so it disturbs the cell wall and causes pores and ultimately results in leaking out of the internal constituents like cytoplasm and causes death to the cell membrane rupturing.

It is also evident from the reported data that as cell wall of gram (-) bacterial strains have more antigenic properties due to the presence of thin outer lipid membrane made up of lipopolysaccharides, therefore compounds show higher activity against them while reduced activity is seen against gram (+) strains having thick outer membrane made up of peptidoglycon.

In antifungal studies, the fungul strains are hard to treat in comparison to bacteria because of the cell wall is made up of chitin. Compounds **6** and **7** showed fungicidal activity against *A. niger and Mucor* may be due to the presence of organic group phthalimide having nitrogen, which forms a tight bond with heame iron of fungal P450 enzyme preventing substrate and oxygen binding. They also possess Cd as a central metal which is believed to disrupt the cell membrane do so by targeting ergosterol, either by binding to the sterol, forming pores and causing the membrane to become leaky or inhibiting ergosterol biosynthesis and inhibition of cell division or DNA transcription.[20]

A similar study, carried out by Chohan *et al*.[18] showed similar results, where cobalt (II), copper (II), nickel (II) and zinc (II) metal complexes of amino acid-derived compounds have been synthesized and screened for their antibacterial activity against four Gram-negative and Gram-positive bacterial strains and for *in vitro* antifungal activity. The results of these studies showed that the metal (II) complexes are more active against one or more species, when compared to the ligands. It has also been suggested that the ligands with nitrogen and oxygen donor systems might inhibit the enzyme production, since enzymes, which require these groups for their activity, appear to be especially more susceptible to deactivation by the metal ions upon chelation. This supports the results obtained by us.

Antioxidant activity reveals that the all compounds **1-8** shows moderate activity, however comparison suggests that compound **4** and **8** shows higher activity than others. The tellurium presents in the structure of compound **4** on coordinating with zinc, exhibits a strong secondary interaction with nitrogen, plays an important role in the catalytic antioxidant activity of these compounds.[14-17]

CONCLUSION

In vitro, antibacterial and antifungal activity of tellurium bearing compounds **1**-**8** were studied by disc diffusion method against various microorganisms at two different concentrations 20 µg/mL & 40 µg/mL. As the compounds were insoluble in water, their DMSO solutions were used for test. After incubation, inhibition zone surrounding the disc was measured. All the compounds have Te in their framework however compound **1** has Oxygen while **2-4** have Nitrogen along with Tellurium, which facilitates the breakage of the outer membrane of bacteria and thus showed good activity. The outer membrane in these bacteria consists of characteristic lipopolysaccharide-a lipoprotein complex and is inaccessible to the most of the antibiotics including penicillin. The organotellurium compounds used in the present study might have crossed the outer membranes of these strains resulting in possible activity. However, compounds **4-8** showed higher activity than **1-4**, which suggest that the metal (II) complexes are more active against one or more species when compared to the uncomplexed ligands. It has also been suggested[21,22] that the ligands with nitrogen and oxygen donor systems might inhibit the enzyme production, since enzymes, which require these groups for their activity, appear to be especially more susceptible to deactivation by the metal ions upon chelation. This supports the results obtained by us. All the compounds were found to be more active at higher concentration (40µg/mL)

While compound **4** and **8** showed higher activities than others due to strong interactions. Antioxidant activity is all about the reduction or removal of free radicals and nascent oxygen from an environment. On the other hand, many bacteria could also depend on this nascent oxygen for survival in any given environment. Thus, or removal of free radicals or oxidants could relate to antimicrobial activity.

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