

**Original Research Article****Antibacterial and antifungal evaluation of some chalcogen bearing ligands, their transition and non-transition metal complexes**

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ABSTRACT

Eight chalcogen bearing compounds, 3-(4-fluorophenyl telluro) propylamine (1), 3-(phenyl telluro)propylammonium acetate salt (2), 3-(phenyl telluro)propylacetamide (3) and α -(phenylseleno) acetic acid (4) (1-4 are ligands), $[\text{Ph}_2\text{Sn}(\text{Cl})\cdot\text{I}](\text{NO}_3)$ (5), $[\text{Ph}_3\text{Sn}\cdot\text{I}](\text{BPh}_4)$ (6), $[\text{ZnCl}_2\cdot 2]$ (7) and $[\text{CdCl}_2\cdot 2]$ (8) (5-8 are complexes of 1 & 2) were synthesised and screened for antibacterial activity against Gram-positive bacterial strains of *Staphylococcus aureus*, *Bacillus anthracis* and the Gram-negative bacteria *Escherichia coli*. They were also tested for their antifungal activity against *Candida tropicalis*, *Trichophyton rubrum* and *Aspergillus niger*, by using the disk diffusion technique. Inhibition zones demonstrated that compounds 1-3 showed significant activity, due to the presence of N in the form of amine group however Compound 4 bearing an acidic group, shows higher activity against bacterial strains. Compounds 5-8 (having Sn, Zn and Cd in their framework) showed still higher activities, due to increase in the lipophilicity and easier penetration of the compounds into the outer cell wall of the microorganisms, which causes death due to cell membrane rupturing. Compounds 1-8 were most effective against *E. coli* (bacterial strains), as the cell wall of Gram-negative strains have thin outer lipid membrane, which is made up of lipopolysaccharides. These compounds showed slightly reduced antifungal activity, because the cell wall of fungi is made up of chitin, which is difficult to cross. It could be concluded, from the obtained results that the biological activity of compounds is essentially determined by the number and nature of the organic groups and central metal ion. The presence of NH_2 , COOH group as well as metal ion like Sn, Zn, Cd in the compounds leads to higher activity.

Introduction

The introduction of the antimicrobial agents into the general clinical use represents one of the landmark medical advances of modern medicine [1]. Tellurium found historical applications in the treatment of microbial infections prior to the discovery of antibiotics. Its oxyanion tellurite TeO_3^{2-} has been used in microbiology since 1930's, when Alexander Fleming reported its antibacterial properties [2]. In recent years, organotellurium compounds have attracted considerable attention, as they exhibit antioxidant, antitumor, antihelminthic and antibacterial activities. They work as protease inhibitors, as well as in photodynamic therapy (PDT) and have been developed as an alternative anticancer therapy [3].

Similarly, organoselenium compounds have also been known since the nineteenth century and comprise of wide variety of structures that display a remarkable range of properties. These compounds are used as antioxidants, enzyme modulators, antimicrobials, antihypertensives, cytokine inducers and have

also an antitumor activity [3]. Thus, the biochemistry and pharmacology of selenium based compounds is a subject of intense current interest, especially for public health [4]. The compounds containing 4-(dialkylamino)phenyl telluro group have been found to inhibit human primary cancer cell growth [5,6]. Antibacterial activity was also reported in some organotellurium and organoselenium compounds, derived from α -Bromo-4-methylacetophenone with tellurium analogues, showing higher activity than organoselenium compounds [7]. An organotellurium compound (RT-01), organotellurane, has been identified as a new antileishmanial agent [8]. Over the past decade, fungal infections have become an important complication and a major cause of morbidity and mortality in immunocompromised individuals [9].

In continuation of earlier work in the chemistry of chalcogen bearing compounds [10,11] the aim of the present work is to study the antibacterial and antifungal activity of hybrid organotellurium ligands and their complexes and eventually to exhibit through experimentation that the biological activity of compounds is essentially determined by the number and

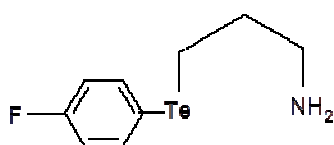
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nature of the organic groups, bound to the central metal atom, as well as by the nature of the central metal ion.

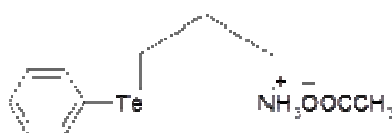
Materials and methods

Preparation of compounds 1–8

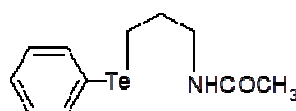
The compounds **1–4** (Figure 1) were synthesized by in situ borohydride reduction of Bis (4-Fluorophenyl)ditelluride, diphenyl ditelluride and diphenyl diselenide with different organic halides under dinitrogen atmosphere. Compounds **5–8** were synthesized by stirring of inorganic salts with **1** and **2** in dry ethanol in 1:1 molar ratio [12]. The used solvents were purified and dried by using a conventional method [13].



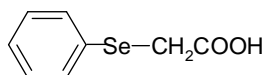
1. 3-(4-fluorophenyl telluro)propylamine



2. 3-(phenyl telluro)propylammonium acetate salt



3. 3-(phenyl telluro)propylacetamide



4. α -(phenylseleno) acetic acid

Figure 1: Studied ligands: 3-(4-fluorophenyl telluro)propylamine (1), 3-(phenyl telluro)propylammonium acetate salt (2), 3-(phenyl telluro)propylacetamide (3) and α -(phenylseleno) acetic acid (4)

Antibacterial and antifungal evaluation

Bacterial and fungal strains were procured from Department of Biochemistry, Birla institute of medical research and School of Studies in Biochemistry, Jiwaji University.

The antibacterial and antifungal evaluation of all the compounds was done against *S. aureus* (MTCC 1144), *B. anthracis* (MTCC 7303), *E. coli* (MTCC 433), *C. tropicalis* (MTCC 230), *T. rubrum* (MTCC 3272) and *A. niger* (MTCC 3272) by using the disk diffusion technique [14–16] on Luria-Bertani (LB) agar plates. Chloramphenicol and fluconazole were used as standard drugs.

The media, employed for the antimicrobial studies (Table 1), were prepared and autoclaved at 121°C for 20 min. The

agar/Sabouraud dextrose agar (SDA) Petri dishes (for bacterial and fungal study, respectively) were then dried for 30 min before inoculation, in order to prevent flow of inoculated material during incubation. Two to eight hour old microbial inoculum, containing 104–106 colony forming units (CFU)/mL, prepared in 2 mL–5 mL broth/ Sabouraud dextrose broth (SDB) (for bacterial and fungal study, respectively) were spread on the surface of the prepared Petri dishes with the help of a sterile cotton swab in air laminar flow. Sterile filter paper discs, impregnated with fixed doses of the compounds at concentrations of 20 μ g compound per mL of dimethylsulphoxide solvent and 40 μ g compound per mL of the same solvent, were placed on the pre-inoculated surface by flamed forceps. Discs, impregnated with dimethylsulphoxide solvent, were used as a control for the antibacterial and

antifungal activity, because of the free solubility of the test compounds. The standard drugs were tested at 40 µg/mL only.

with the fungal strains were incubated at 37 °C for 48 h. After this, the observations were noted. The results were expressed in terms of diameter of inhibition zone (mm).

The disc-bearing plates with the bacterial strains were incubated at 37 °C for 24 h, whereas the disc-bearing plates

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.

Results

The synthesis, characterization and antimicrobial screening of compounds 1–8, were carried out and the values of the inhibition zones (in mm) for the bacterial and fungal strains

are reported in **Table 2** and **Table 3**, respectively; no inhibition zone was shown by the control disc. The values, reported in the tables, are the average of three separate experiments.

Table 2: Antibacterial activity of compounds 1–8, applied in different concentrations

Compounds	Diameter of zone of inhibition (mm)					
	<i>S.aureus</i> (Gram positive) MTCC 1144		<i>B.anthraxis</i> (Gram positive) MTCC 7303		<i>E.coli</i> (Gram negative) MTCC 433	
	20µg/ml*	40µg/ml	20µg/ml	40µg/ml	20µg/ml	40µg/ml
1	20	22	18	22	29	32
2	20	25	20	24	33	36
3	18	16	18	22	30	33
4	22	26	33	38	29	32
5	28	30	25	28	38	40
6	32	35	28	32	40	42
7	24	22	22	25	30	33
8	22	22	24	22	35	38
Standard	-	31	-	31	-	30
Solvent	NA	NA	NA	NA	NA	NA

Note: * Compounds 1–8 were applied in concentrations 20 µg/mL and 40 µg/mL; Values, which represent the diameter (mm) of the 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 of compounds 1–8 applied in different concentrations

Compounds	Diameter of zone of inhibition (mm)					
	<i>C.tropicalis</i>		<i>T.rubrum</i>		<i>A.niger</i>	
	20µg/ml*	40µg/ml	20µg/ml	40µg/ml	20µg/ml	40µg/ml
1	18	22	23	26	16	17
2	21	24	22	24	17	18
3	21	22	21	22	17	18
4	21	23	22	26	12	14
5	28	30	26	32	18	20
6	30	34	28	35	20	22
7	20	22	24	30	20	25
8	22	28	24	28	22	28
Standard	-	30	-	25	-	30
Solvent	NA	NA	NA	NA	NA	NA

Note: *Compounds 1–8 were applied in concentrations 20 µg/mL and 40 µg/mL; Values, which represent the diameter (mm) of the inhibition zone, produced around each disc, are average of three separate experiments; DMSO was used as a control and fluconazole as standard drug.

Discussion

Interestingly, all of the compounds showed good activity against the bacterial strains. It is evident, anyway, that compounds 1–3 showed almost similar activity, whereas compounds 4–8 had an excellent inhibition activity of the bacterial growth (Table 2). Compounds 1–3 showed significant activity, due to the presence of N in the form of amine group however Compound 4 bearing an acidic group and Compounds 5–8 (having Sn, Zn and Cd in their framework) showed still higher activities, due to increase in the lipophilicity and easier penetration of the compounds into the outer cell wall of the microorganisms, which causes death due to cell membrane rupturing thereby boost the bioactivity of these compounds. The lipophilic characteristic is essential for determining the activity of the compounds.

Compounds 1–8 were most effective against *E. coli* (bacterial strains), as the cell wall of Gram-negative strains have thin outer lipid membrane, which is made up of lipopolysaccharides. The remaining two bacterial strains were Gram-positive (*S.aureus* & *B.anthraxis*) have thick outer membrane, made up of peptidoglycan which is difficult to penetrate. The remaining two bacterial strains were Gram-positive, which have thick outer membrane, made up of peptidoglycan.

It becomes clear from the tabulated values that the antibacterial and antifungal activities of the compounds were on the average, the same or slightly less than that of the standard drug.

Almost similar trend of activity of compounds was found against fungal strains, with slight deviation (Table 3). Here, compounds 1–3 also showed good activity, However, compound 4 showed minimum activity against *A. niger* which may be due to the presence of carboxylic groups, which are also components of the cell wall of this species. Therefore this

makes it difficult for compound 4, to easily penetrate into the cell wall of the strain. Compounds 5–8, showed significant activity same as that in case of bacterial strains. As these compounds are lipophilic, they disturb the cell wall, form pores and ultimately result in leaking out of the internal constituents, like cytoplasm and cause death to the cell by membrane rupturing. The results obtained from other studies, also points out that the bacterial and fungal growth inhibition activity of triorganotin compound is higher than that of diorganotin compounds [17].

Compound 8 contained Cd as a central metal, which is believed to disrupt the cell membrane by targeting ergosterol, either by binding to the sterol, forming pores and causing the membrane to become leaky, or by inhibiting the ergosterol biosynthesis, DNA transcription and cell division [18].

The compounds showed fungicidal activity against *C. tropicalis*, *T. rubrum* and *A. niger*. This may be due to the presence of organic group, amine (having nitrogen) and acidic groups, which form a tight bond with the heme iron of the fungal P450 enzyme and prevent substrate and oxygen binding.

It is evident from the data, obtained from antifungal studies that few fungal strains are slightly harder to treat, in comparison to bacteria, because of their cell wall, which is made up of chitin. Thus, the values of the zones of inhibition (Table 3) were found to be marginally lower than the values, obtained against bacterial strains, which are easier to penetrate. Compound 4 showed minimum activity against *A. niger*, which may be due to the carboxylic groups, which are also components of the cell wall of this species. Therefore this makes it difficult for compound 4, to easily penetrate into the cell wall of the strain [19].

A similar study, carried out by Chohan *et al.*, [20] 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 *in vitro* antibacterial activity against four Gram-negative (*E. coli*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and two Gram-positive (*B. subtilis* and *S. aureus*) bacterial strains and for *in vitro* antifungal activity against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glabrata*. The results of these studies showed 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 the 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. The images of the petri dish bearing Zone of inhibition for different compounds could be seen in supplementary file from Figure S2 to Figure S5. S1 could be seen here.

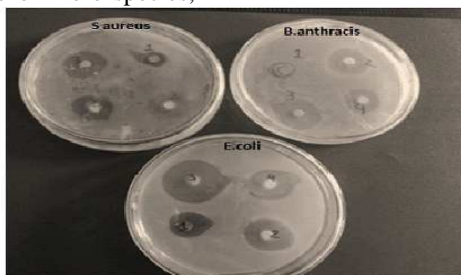


Figure S1. Zones of inhibition, surrounding the discs of compounds 1-4 at 20 µg/mL against *S. aureus*, *B. anthracis* and *E. coli*.

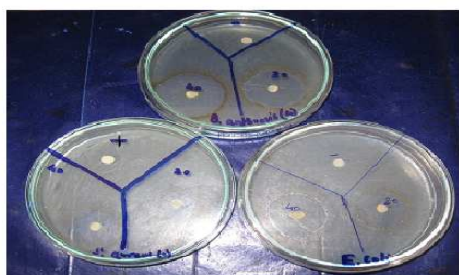


Figure S2. Zones of inhibition, surrounding the discs of compounds 5-7 at 20 µg/mL and 40 µg/mL against *E. coli*, *S. aureus* and *B. anthracis*.



Figure S3. Zone of inhibition, surrounding the disc of compound 3 at 20 µg/mL and 40 µg/mL against *Trubrum*.

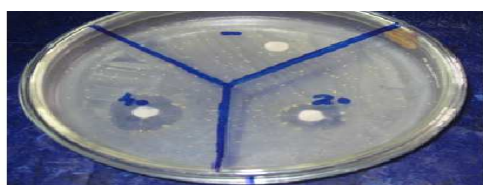


Figure S4. Zone of inhibition, surrounding the disc of compound 4 at 20 µg/mL and 40 µg/mL against *A. niger*.

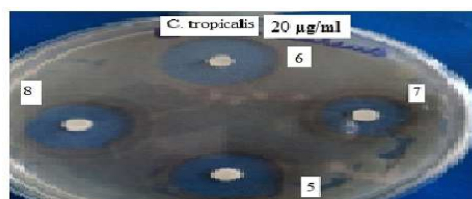


Figure S5. Zones of inhibition, surrounding the discs of compounds 5-8 at 20 µg/mL against *C. tropicalis*.

Conclusion

It could be concluded, from the obtained results that the biological activity is essentially determined by the number and nature of the organic groups, bound to the central metal atom, as well as by the nature of the central metal ion. The antibacterial activity of compounds 1-3, having chalcogen with N (as organic group, amine) in their frame work, showed good activity, though higher activity was found for compounds 4-8. Compound 4 had chalcogen along with acidic

group, whereas compounds 5-8 had Sn, Cd and Zn, as central metal ions in their framework. This increases their lipophilic characters and leads to rupturing of the cell wall. It is evident from the data that compounds 1-8 showed maximum activity against *E. coli*, due to the fact that the Gram-negative bacteria have thin outer lipid membrane, made up of lipopolysaccharides, which is easier to penetrate. The remaining two Gram-positive bacterial strains have thick outer membrane, made up of peptidoglycan. However slightly lower activity of all the compounds, in terms of inhibition zones,

was observed against the fungal strains as their cell wall made up of chitin are harder to treat, in comparison to bacteria. Further, a toxicity study needs to be done for utilizing those compounds as antimicrobial agents.

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Conflict of interest: We declare that we have no conflict of interest.

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