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## Synthesis of dithiocarbamate ligand and study of their biological properties

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### ABSTRACT

Dithiocarbamates as ligands are well known to bind strongly and selectively to many metal ions, so in the past few years self-assembly directed by metal–dithiocarbamate coordination have emerged as a useful supramolecular methodology for the preparation of macrocycles, cages, catenanes, and nanoparticles. Dithiocarbamate (DTC) ligands are known for their anti-fungal, antibacterial and biocide activity. They form complexes with transition metals, leading to the stabilization of a wide range of oxidation states. These complexes have found applications in diverse areas such as materials science and medicine. The in situ generated dithiocarbamate formed by the reaction of an diamine and aldehyde and carbon disulfide in the presence of triethylamine. The antibacterial activity of dithiocarbamate tested against microorganism. © 2011 Trade Science Inc. - INDIA

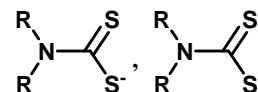
### KEYWORDS

Antibacterial activity;  
Synthesis;  
Dithiocarbamate.

### INTRODUCTION

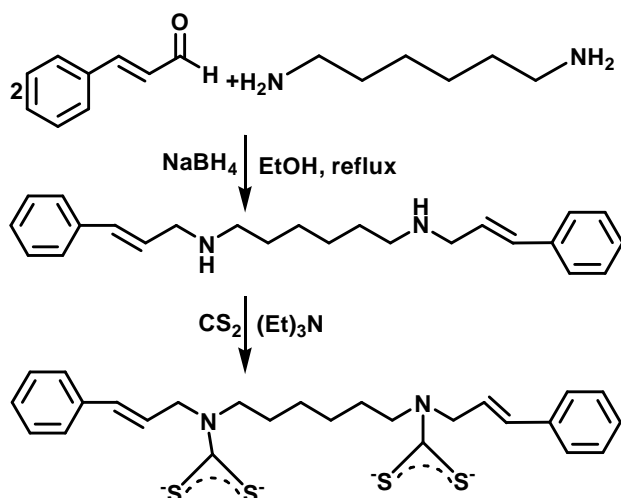
The continuing discovery of the many pivotal roles played by anions in chemical, biological and environmental processes has stimulated the construction of molecular host systems capable of complexing anionic guests<sup>[1]</sup>. Dithiocarbamates are versatile ligands capable of forming complexes with most of the elements and able to stabilise transition metals in a variety of oxidation states<sup>[2]</sup>. This property of stabilising high oxidation states in metal complexes reflects strong  $\sigma$ -bonding characteristic of these ligands. Although the sulfur atoms of dithiocarbamate ligands possess  $\sigma$ -donor and  $n$ -back-donation characteristics of the same order of magnitude, these ligands have a special feature in that there is an additional  $n$ -electron flow from nitrogen to

sulphur *via* a planar delocalised  $\pi$ - orbital system, as shown below:



This effect results in strong electron donation and hence a high electron density on the metal leading to its next higher oxidation state<sup>[3]</sup>. However, while dithiocarbamate complexes have been known for over a century, with many thousands having been prepared, the vast majority of these contain only simple alkyl substituents such as methyl and ethyl. A developing interest in the area of dithiocarbamate chemistry is the functionalization of the backbone such that new applications and interactions can be developed. This area is still in its early stages but already interesting potential

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Scheme 1 : Preparation of the dithiocarbamate ligand

applications have been noted including the functionalization of gold nanoparticles, the stepwise build-up of multimetallic arrays, the synthesis of dithiocarbamate-containing supramolecular systems which can be used for anion binding, the development of technetium radiopharmaceuticals<sup>[2]</sup>. Dithiocarbamates 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 AIDS<sup>[4]</sup>.

Dithiocarbamate ligand were synthesized and characterized by using elemental analyses, FT-IR, NMR. The antibacterial activities of synthesized compounds were studied against two Gram-negative species, *Escherichia coli*, *Klebsiella pneumoniae* and two Gram-positive species, *Staphylococcus aureus* and *Bacillus subtilis* and, for *in vitro* antifungal activity against, *Candida albicans*, *Aspergillus flavus*, *Aspergillus nigar*.

## EXPERIMENTAL

## Materials and general methods

The reagents and solvents were of analytical grade. Aldehyde, amine, carbon disulfide were purchased from Merck Company. <sup>1</sup>H and <sup>13</sup>C NMR spectra of deuterated chloroform (CDCl<sub>3</sub>) and solutions of the compounds were registered on a Bruker WM-300 spectrometer (300 MHz) using tetramethylsilane as internal standard. The infrared spectra of the compounds as KBr-disks were recorded in the range of 400–4000 cm<sup>-1</sup> with

a Mattson 1000 FT spectrometer. The microdilution broth method was used to determine the antibacterial activity of compounds against the bacteria: *S. aureus* ATCC 25923, *B. subtilis* ATCC 1023, and *K. pneumoniae* ATCC 10031, *E. coli* ATCC 8739, *C. albicans* ATCC 10231, *A. flavus* ATCC 9170, *A. nigar* ATCC 16404.

## Synthesis of dithiocarbamate ligand

The secondary diamine ligands, used as starting material for the preparation of the dithiocarbamate, were obtained in 78% yield by condensation of cinnamaldehyde with 1,6-hexamethylenediamine in methanol followed by reduction of the Schiff base with NaBH<sub>4</sub> in methanol. One-pot synthesis from this amine, carbon disulfide, triethylamine gave ligand dithiocarbamate (Scheme 1). These products are soluble in non polar solvents such as dichloromethane and chloroform, but insoluble in polar solvents such as acetone, ethanol, methanol, dimethyl sulfoxide, acetonitrile and water.

**The preparation of the ligand required was carried out in two steps following procedures reported in the literature**

(1) *N,N*-bis[(1*Z*,2*E*)-3-phenylprop-2-enylidene] hexanediamine

1,6-Hexamethylenediamine (3.37 g) and two equivalents of cinnamaldehyde (7.68 g) were dissolved in ethanol (25 mL) and the solution was refluxed for 2 h. After evaporation of the solvent the diimine was obtained in form of red oil (yield: 78 %). IR (KBr, cm<sup>-1</sup>)  $\nu$ (C=N), 1647;  $\nu$ (C-N), 1213;  $\nu$ (C-H), 3075;  $\nu$ (C-H, out-of-plane), 733,  $\nu$ (out-of-plane ring bend) 690; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>, ppm):  $\delta$  8.0-8.1 (d, 2H, CH=N), 7.26-7.59 (m, 10H, C<sub>6</sub>H<sub>5</sub>), 6.92-6.94 (d, 4H, C<sub>6</sub>H<sub>5</sub>-CH=CH), 3.54-3.50 (q, 2H, C<sub>6</sub>H<sub>5</sub>-CH=CH), 1.67-1.72 (br, 8H, NCH<sub>2</sub>CH<sub>2</sub>), 1.39-1.42 (m, 4H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>, ppm):  $\delta$  27.34, 31.89, 51.79, 112.00, 124.17, 126.32, 129.11, 130.08, 135.54, 164.28.

(2) *N,N'*-bis[(2*E*)-3-phenylprop-2-enyl] hexanediamine

*N,N'*-bis[(1*Z*,2*E*)-3-phenylprop-2-enylidene] hexanediamine (7.95 g) was dissolved in methanol and the solution was cooled to 0 °C. Sodium borohydride (3.18 g) was added under stirring and the mixture was

TABLE 1 : In vitro antifungal studies of the complexes

No.	Sample	<i>Candida albicans</i>	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>
		MDA (µg/ml)	MDA (µg/ml)	MDA (µg/ml)
1	Dithiocarbamate(1)	70	65	122

allowed to react over night. After removal of the solvent under vacuum, the resulting viscous liquid was washed with water and dichloromethane was added in order to extract the product. The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$ . After filtration the solvent was evaporated to obtain an orange oil that was identified as the product (yield: 81 %). IR (KBr,  $\text{cm}^{-1}$ )  $\nu(\text{N-H})$  3297;  $\nu(\text{C=C})$  1652;  $\nu(\text{C-N})$ , 1210, 1069;  $\nu(\text{C-H, out-of-plane})$ , 742;  $\nu(\text{out-of-plane ring bend})$  693;  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ , ppm):  $\delta$  6.25-7.33 (m, 10H,  $\text{C}_6\text{H}_5$ ), 5.40-5.42 (m, 2H,  $\text{C}_6\text{H}_5\text{-CH=CH}$ ), 4.30-4.32 (m, 2H,  $\text{C}_6\text{H}_5\text{-CH=CH}$ ), 3.63-3.67 (q, 4H,  $\text{CH}_2\text{-N}$ ), 2.28 (br, 2H,  $\text{CH}_2\text{-NH}$ ), 1.53 (br, 8H,  $\text{NCH}_2\text{CH}_2$ ), 1.21-1.26 (m, 4H,  $\text{NCH}_2\text{CH}_2\text{CH}_2$ );  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CDCl}_3$ , ppm):  $\delta$  27.36, 32.14, 50.39, 110.54, 126.38, 126.77, 128.08, 134.99, 135.94, 160.72.

### Dithiocarbamate ligand (1)

*N,N'*-bis[(2*E*)-3-phenylprop-2-enyl] hexanediamine (2.9 g), triethylamine (0.5 mL) and carbon disulfide (1.25 g) were dissolved in methanol (10 mL) and stirred for 2 h. The resulting solution yellow was purification with (1:1) n-hexane /ethyl acetate. (yield: 67 %), IR (KBr,  $\text{cm}^{-1}$ )  $\nu(\text{C-N})$ , 1477;  $\nu(\text{CS}_2)_{\text{as}}$ , 1249;  $\nu(\text{CS}_2)_{\text{s}}$ , 988;  $\nu(\text{C-N})$ , 1122;  $\nu(\text{C-H, out-of-plane})$ , 750;  $\nu(\text{out-of-plane ring bend})$  696;  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ , ppm):  $\delta$  6.25-7.33 (m, 10H,  $\text{C}_6\text{H}_5$ ), 5.21-5.19 (m, 2H,  $\text{C}_6\text{H}_5\text{-CH=CH}$ ), 4.33-4.30 (m, 2H,  $\text{C}_6\text{H}_5\text{-CH=CH}$ ), 3.52-3.50 (q, 4H,  $\text{CH}_2\text{-N}$ ), 2.00 (br, 2H,  $\text{CH}_2\text{-NH}$ ), 1.21 (br, 8H,  $\text{NCH}_2\text{CH}_2$ ), 1.28-1.21 (m, 4H,  $\text{NCH}_2\text{CH}_2\text{CH}_2$ );  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CDCl}_3$ , ppm)  $\delta$  26.31, 31.89, 55.46, 58.19, 123.28, 126.10, 127.00, 127.79, 132.18, 213.17. Anal. Calcd (%) for  $\text{C}_{26}\text{H}_{30}\text{N}_2\text{S}_4$  (498.13  $\text{g mol}^{-1}$ ): C, 62.60; H, 6.10; N, 5.60; S, 25.66. Found: C, 62.63; H, 6.07; N, 5.62, S 25.67 %.

### In vitro antifungal activity

The compound has been screened in vitro against *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*. Among several methods<sup>[5]</sup> available, the one

TABLE 2 : In vitro antibacterial studies of the complexes

No.	Sample	Zone of inhibition (mm)			
		<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
1	Dithiocarbamate (1)	8	8	9	10

method<sup>[6,7]</sup> that is common in use in recent times has been adopted.

### Microbroth dilution assay

The susceptibility of the fungi to various fractions of compound was assayed by microbroth dilution method. Sabouraud dextrose medium was dissolved in glass double distilled water and autoclaved at 10 psi for 15 min. A volume of 90  $\mu\text{L}$  of medium was added to the wells of cell culture plates (Nunc Nunclon). The different concentrations in the range of 50-400  $\mu\text{g/mL}$  of various fractions were prepared in duplicate wells and then the wells were incubated with 10  $\mu\text{L}$  of conidial suspension containing  $1 \times 10^4$  conidia. The plates were incubated at 37°C and examined macroscopically after 48 h for the growth of *Aspergillus* mycelia. All experiments were carried out in duplicate and the results were confirmed in three independent experiments.

### In vitro antibacterial activity

The compounds have been screened in vitro against *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*, *Bacillus subtilis*. Various methods are available for the evaluation of the antibacterial activity of different types of drugs. However, the most widely used method<sup>[8-11]</sup> consists in determining the antibacterial activity of the dithiocarbamate ligand is to add it in known concentrations to the cultures of the test organisms.

### Disc diffusion assay

Method of paper disc diffusion 0.05 mol/L aqueous solution of dithiocarbamate was prepared, and the antibacterial activity of the dithiocarbamate against *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* and *Bacillus subtilis* was studied. The bacterium suspension concentration was controlled as  $5 \times 10^5$ – $5 \times 10^6$  cfu/ml; the diameters of filter paper were 5 mm, and for the experiments, flat plates were incubated at 37°C (bacterium) for 16–18 h. Their inhibition diameter (including filter paper) was measured with a vernier caliper.

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### RESULTS AND DISCUSSIONS

#### Spectroscopic characterization

The IR spectra of 1 gave evidence for the formation of the dithiocarbamate functions. The bands resulting from the stretching vibrations of the C-N bonds at  $1477\text{ cm}^{-1}$  have wavenumbers that are intermediate when compared to those reported for C-N single bonds ( $1250\text{-}1360\text{ cm}^{-1}$ ) and C=N double bonds ( $1640\text{-}1690\text{ cm}^{-1}$ ), suggesting partial double bond character and, therefore, partial delocalization of  $\pi$ -electron density within the dithiocarbamate functions. For the  $\text{CS}_2$ -groups two bands were observed,  $\nu(\text{CS}_2)_{\text{as}}$  and  $\nu(\text{CS}_2)_{\text{s}}$  ( $1249, 988\text{ cm}^{-1}$  for 1). A comparison of the  $^1\text{H-NMR}$  spectra between the *N,N*-bis[(2*E*)-3-phenylprop-2-enyl]hexanediamine and the resulting products showed significant shift displacements to lower fields for the  $\text{NCH}_2$ benzyl and  $\text{NCH}_2$ chain methylene hydrogen atoms (1:  $\delta = 1.21$  and  $1.53$  ppm), thus indicating the formation of the dithiocarbamate. The  $\text{NCS}_2$  carbon atoms gave signals at  $\delta = 203.17$  ppm, respectively. Interestingly, in the  $^1\text{H-NMR}$  spectra all methylene hydrogen atoms gave rise to broad signals, indicating that the compounds are involved in at least one dynamic process.

#### *In vitro* antifungal study

In the current study (TABLE 1) of some synthesized dithiocarbamate ligand were tested against pathogenic fungal strains such as *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*. Ketoconazole was used as reference drug for fungi. The minimum inhibitory concentration (MICs) by microbroth dilution assays (MDA) is  $80\text{-}230\text{ }\mu\text{g/mL}$ . The ligand had highest *in vitro* antifungal activity against pathogenic fungal strains. The reason for the highest activity might be related to the presence of thio group in the dithiocarbamate.

#### *In vitro* antibacterial study

In the antibacterial study (TABLE 2) of some synthesized dithiocarbamate ligand was tested against pathogenic bacterial strains such as *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* and *Bacillus subtilis* using the disc diffusion method. Gentamycin was used as reference drug for bacteria.

In general, the compounds showed significant antibacterial activity and the bacterial strains with the zone of inhibition, 10 mm at minimum inhibitory concentration (MIC) of  $30.0\text{ }\mu\text{g/disc}$ .

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