Synthesis of a New Dithiocarbamate Compound and Study of Its Biological Properties

Hafezeh Nabipour,[a, b] Shahriar Ghammamy,[a,c,*] Shahriar Ashuri,[d] Zahra Shokri Aghbolagh[c, e]

[a]Department of Chemistry, Faculty of Science, Islamic Azad University, Takestan Branch, Takestan, Iran.

[b]Member of Young Researchers Club, Islamic Azad University, Takestan, Iran.

[c] Department of Chemistry, Faculty of Science, Imam Khomeini International University, Qazvin, Iran.

[d]Department of Chemistry, Faculty of Science, Urmia University, Urmia, Iran.

[e]Member of Young Researchers Club, Islamic Azad University, Qazvin Branch, Qazvin, Iran.

E-mail: *shghamami@yahoo.com, shghamami@ikiu.ac.ir*

Recevied date: September 20, 2010

Abstract

Dithiocarbamates (DTC) are well known compounds 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. Most of applications are based on complexation properties of DTC ligands with metal ions, especially with transition metal ions. DTC ligands readily form chelates with all transition metal ions through its two donor sulfur atoms. In this research a new dithiocarbamate compound was synthesized and characterized using elemental analyses, FT-IR, NMR. The antibacterial activities of synthesized compound 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***.**

Keywords: Antibacterial activity, Synthesis, Dithiocarbamate.

1. 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.[1] 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.[2] This property of stabilising high oxidation states in metal complexes reflects strong o-bonding characteristic of these ligands. Although the sulfur atoms of dithiocarbamate ligands possess o-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 π-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.[3] 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 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.[2] 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.[4]

Dithiocarbamate compound was synthesized and characterized using elemental analyses, FT-IR, NMR. The antibacterial activities of synthesized compound 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***.**

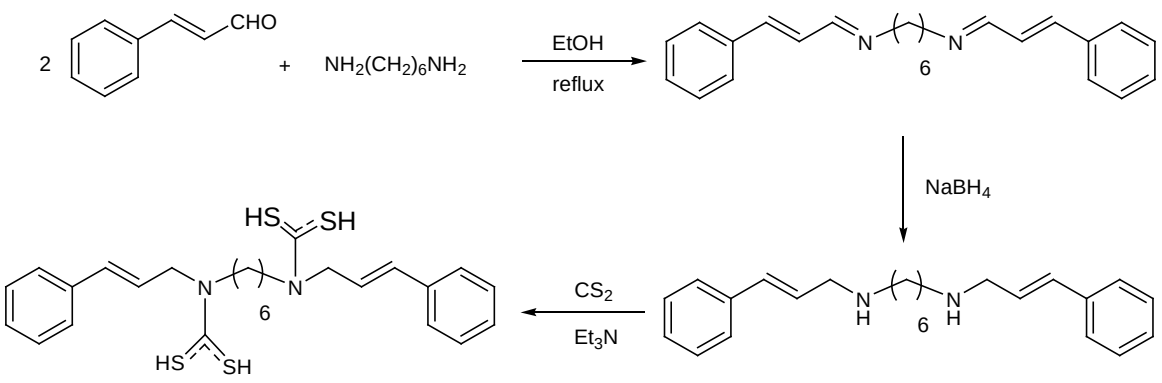
2. Results and Discussion

2.1. Synthesis of dithiocarbamate ligand

The secondary diamine, used as starting material for the preparation of the DTC compound, was obtained in 80% yield by condensation of cinnamaldehyde with 1,6-hexamethylenediamine in methanol followed by reduction of the Schiff base with $NaBH_4$ in methanol. One-pot synthesis from this amine, carbon disulfide, triethylamine gave 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 compound was carried out in two steps following procedures.

2.2. 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 cm^{-1} have wavenumbers that are intermediate when compared to those reported for C-N single bonds (1250-1360 cm^{-1}) and C=N double bonds (1640-1690 cm^{-1}), suggesting partial double bond character and, therefore, partial delocalization of electron density within the dithiocarbamate functions. For the CS_2 groups two bands were observed, $\nu(CS_2)_{as}$ and $\nu(CS_2)_s$ (1249, 988 cm^{-1} for **1**), which are characteristic for DTC compound.



Scheme 1. Preparation of the dithiocarbamate macrocycles.

A comparison of the ^{1}H-NMR spectra between the *N,N*-bis[(2E)-3-phenylprop-2-enyl]hexanediamine and the resulting product showed significant shift displacements to lower fields for the NCH_2benzyl and NCH_2 chain methylene hydrogen atoms (**1**: δ= 1. 21 and 1.53 ppm), thus indicating the formation of the dithiocarbamate. The NCS_2 carbon atoms gave signals at δ = 203.17 ppm, respectively. Interestingly, in the ^{1}H-NMR spectra all methylene hydrogen atoms gave rise to broad signals, indicating that the compounds are involved in at least one dynamic process.

2.3. In vitro antifungal study

In the current study (Table 1) the compound 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 80-230 µg/mL. The compound 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.

Table 1. In vitro antifungal studies of the DTC compound.

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

Where, MDA: Micro-dilution activity.

2.4. In vitro antibacterial study

In the antibacterial study (Table 2) of synthesized DTC compound 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 μg/disc.

Table 2. In vitro antibacterial studies of the DTC compound .

No	Sample	Zone of inhibitation (mm)			
		Escherichia coli	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Bacillus subtilis*
1	Dithiocarbamate (**1**)	8	8	9	10

3. Experimental

3.1. Materials and general methods

The reagents and solvents were of analytical grade. Aldehyde, amine, carbon disulfide were purchased from Merck Company. ^{1}H and ^{13}C NMR spectra of deuterated chloroform ($CDCl_3$) 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^{-1} 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*.

***N,N*-bis[(1*E*, 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^{-1}) ν(C=N), 1647; ν(C-N), 1213; ν(C-H), 3075; ν(C-H, out-of-plane), 733, ν(out-of-plane ring bend) 690; ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 8.0-8.1 (t, 2H, CH=N), 7.26-7.59 (m, 10H, C_6H_5), 6.92-6.94 (t, 4H, C_6H_5-CH=CH), 3.54-3.50 (t, 2H, C_6H_5-CH=CH), 1.67-1.72 (t, 8H, NCH_2CH_2), 1.39-1.42 (m, 4H, $NCH_2CH_2CH_2$); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ 27.34, 30.89, 51.79, 112.00, 124.17, 126.32, 129.11, 130.08, 135.54, 164.28.

***N,N*-bis[(2*E*)-3-phenylprop-2-enyl] hexanediamine.** *N,N* -bis[(1*E*,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 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 Na_2SO_4. After filtration the solvent was evaporated to obtain orange oil that was identified as the product (yield: 81 %). IR (KBr, cm^{-1}) ν(N-H) 3297; ν(C=C) 1652; ν(C-N), 1210, 1069; ν(C-H, out-of-plane), 742; ν(out-of-plane ring bend) 693; ^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 6.25-7.33 (m, 10H, C_6H_5), 4.30-4.32 (d, 2H, C_6H_5-CH=CH), 3.40-3.42 (d, 2H, C_6H_5-CH=CH), 2.63-2.67 (t, 2H, CH_2-N), 2. 28 (br, 2H, CH_2-NH), 1.53 (br, 8H, NCH_2CH_2), 1.21-1.26 (m, 4H, $NCH_2CH_2CH_2$); ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ 27.36, 32.14, 50.39, 124.6, 126.50, 128.11, 129.38, 135.74.

{(mercaptocarbonothioyl)[(2E)-3-phenylprop-2-enyl]amino}hexyl[(2E)-3-phenylprop-2-enyl]dithiocarbamate (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 yellow solution was purified with (1:1) n-hexane/ethyl acetate. (yield: 67 %), IR (KBr, cm^{-1}) ν(C-N), 1477; $\nu(CS_2)_{as}$, 1249; $\nu(CS_2)_s$, 988; ν(C-N), 1122; ν(C-H, out-of-plane), 750; ν(out-of-plane ring bend) 696; ^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ 26.31, 31.89, 49.22, 50.46, 54.19, 123.28, 126.10, 127.99, 132.18. 203.17. Anal. Calcd (%) for $C_{26}H_{30}N_2S_4$ (498.13 $gmol^{-1}$): C, 61.35; H, 6.10; N, 5.70; S, 25.66. Found: C, 62.00; H, 6.07; N, 5.62, S 25.67 %.

3.2. In vitro antifungal activity

The compound has been screened in vitro against *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*. Among several methods [5] available, the method [6,7] that is common in use in recent times has been adopted.

3.3. Microbroth dilution assay

The susceptibility of the fungi to various fractions of compounds 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 µL of medium was added to the wells of cell culture plates (Nunc Nunclon). The different concentrations in the range of 50-400 µg/mL of various fractions were prepared in duplicate wells and then the wells were incubated with 10 µL of conidial suspension containing 1×10^4 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.

3.4. 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 [8-11] consists in determining the antibacterial activity of the complex is to add it in known concentrations to the cultures of the test organisms.

3.5. Disc diffusion assay

Method of paper disc diffusion 0.05 mol/L aqueous solutions 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 5×10^5–5×10^6 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.

4. Conclusion

In summary, Dithiocarbamate compound was synthesized and characterized by using elemental analyses, FT-IR, NMR. The antibacterial activities of synthesized compound 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***.**

Acknowledgement

The authors are very much grateful to the Young Researchers Club, Islamic Azad University, Takestan and Ardabil branches, Iran, for giving all type of support in conducting this experiment.

References and Notes

1. Cookson, J.; Vickers, M. S.; Paul, R. L.; Cowley, A. R.; Beer, P. D. *Inorg. Chim. Acta*. **2008**, *361*, 1689.
2. Hogarth, G.; Rainford-Brent, E. J.; Kabir, S. E.; Richards, I.; Wilton-Ely, J. D. E. T.; Zhang, Q. *Inorg. Chim. Acta*. **2009**, *362*, 2020.
3. Pandeya, K. B.; Singh, R.; Mathur, P. K.; Singh. R. P. *Transition Met. Chem*. **1986**, *11*, 340.
4. Milacic, V.; Chen, D.; Giovagnini, L.; Diez, A.; Fregona, D.; Dou, Q. P. *Toxicol. Appl. Pharmacol*. **2008**, *231*, 24.
5. Raper, K. B.; Fennell, D. I. *The genus Aspergillus*. *The Williams and Wilkins Company, Baltimore*, **1965**, 686.
6. Sharma, R.; Sharma, G. L. *J. Ethnopharmacol.* **2002**, *80*, 193.
7. Dabur, R.; Singh, H.; Chhiller, A. K.; Ali, M.; Sharma, G. L. *Fitoterpia*..**2004** *75*, 389.
8. Blanc, D. S.; Wenger, A.; Bille, J. *J. Clin. Microb.* **2003** , *8*, 3499.
9. Saha, D.; Pal, J. *Lett. Appl. Microb*. **2002**, *34*, 311.
10. Tiwari, R. K.; Singh, D.; Singh, J.; Yadav, V.; Pathak, A. K.; Dabur, R.; Chhiller, A. K.; Singh, R.; Sharma, G. L.; Chandra, R.; Verma, A. K. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 413.
11. Eldeen, I. M. S.; Elgorashi, E. E.; Stadan, J. V. *J. Ethnopharmacol.* **2005**, *102*, 457.