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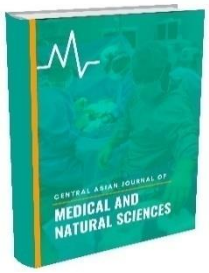

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# An Efficient Synthesis and Spectroscopic Characterizaton of Novel Thiosemicarbazone and Complexes

1. **Waseem Shoukat**
2. **Muhammad Nadeem Shoukat**
3. **Shujaat Hussain**
4. **Muhammad Masood**
5. **Rida Saeed**
6. **Muhammad Nouman Nazeer**

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[1,2,3] Institute of chemical Sciences, Bahauddin Zakariya University, Multan 60800, Punjab, Pakistan
merawaseem@gmail.com, nadeemshoukatk@gmail.com, shujaat416@gmail.com

[4] Sulaiman Bin Abdullah Aba Al-khail-Center for Interdisciplinary Research in Basic Sciences (SA-CIRBS), Faculty of Basic and Applied Sciences,International Islamic University, Islamabad, Pakistan
masood.shanu@gmail.com

[5] Department of Chemistry, Government college university, Lahore
ridasaeed23@gmail.com

[6] Department of Chemistry, Government College University, Faisalabad, Pakistan
noumannazeer72@gmail.com

**Abstract:** Thiosemicarbazones were obtained by reacting three different carbonyl moietes with thiosemicarbazide which were subjected to the metal salts give the required thiosemicarbazones and their metal complexes respectively. The prepared thisemicarbazones such as (2E) –2-[(2,4-dichlorophenyl) methylidene] hydrazine-1 carbothioamide and its complexes were characterized using different spectral techniques, such as UV–Vis., FT-IR, 1HNMR, 13CNMR and Elemental analysis. The results of the investigations support the formulation of each of the complexes as a metal ion surrounded by a planar, quadradentate ligand. Thus, the data provide strong support for the conclusion that the complexes were actually prepared. Finally, synthesized target compounds were screened for their antibacterial activity against Gram positive bacteria (Bacillus subtilis) and Gram negative bacteria (Escherichia coli).

**Key words:** Novel, Thiosemicarbazone.

## Introduction

Generally thiosemicarbazones coordinate as bidentate ligand via azomethine nitrogen and thione/thiolate sulfur but when additional coordination functionality is present in the proximity of donating centers, the ligands will coordinate in a tridentate manner. Due to their wide range of

biological application such as antibacterial, antifungal, antioxidants and anticancer agents1-6, heterocyclic thiosemicarbazones and their metal complexes still have considerable attention. The biological activities of thiosemicarbazones are based on the parent aldyehyde or ketone. In some cases the biological activities increased with metal complexation rather than the parent ligand. In this paper, we describethe synthesis and spectroscopic characterization of novel thiosemicarbazone ligands and their copper (II) complexes as part of our ongoing studies on the synthesis and properties of thiosemicarbazones derivatives.7-9.

Thiosemicarbazones are a class of small molecules that have been evaluated over the last 50 years as antivirals10-11 and as anticancer therapeutics 12 as well as for their parasiticidal action against Gram positive bacteria (*Bacillus subtilis*) and Gram negative bacteria (*Escherichia coli*) 13-14 which are the causative agents of malaria and Chagas's disease, respectively.Currently, a thiosemicarbazone, triapine, is being evaluated in human phase II trials as an antineoplastic therapeutic.15

In view of these SAR results, we synthesized a second generation series of thiosemicarbazones. These compounds were tested in vitro against Gram positive bacteria (*Bacillus subtilis*) and Gram negative bacteria (*Escherichia coli*). In addition, we screened all compounds, whether active against the target enzyme or not, in culture against the respective parasites, Gram positive bacteria (*Bacillus subtilis*) and Gram negative bacteria (*Escherichia coli*). All three parasites are major public health problems in tropical and subtropical countries, and for which new chemotherapies are desperately needed. Since thiosemicarbazones are known iron chelators and as such can destabilize or damage the non-heme iron stabilized tryosyl free radical and thus inhibit the catalytical function of RR. Mammalian ribonucleotide reductase is located in the cytoplasm and is regulated by the cell cycle. Ribonucleotide reductase is a multi submit enzyme responsible for the reduction of ribonucleotide to their corresponding deoxyribonucleotides, which are the building blocks for DNA replication and repair in all living cell. Thiosemicarbazones were prepared by simple process in which Nt-thiosemicarbazone moiety was replaced by aliphatic, arylic and cyclic amines. Literature survey revealed that the presence of certain bulky groups at Nt of the thiosemicarbazone moiety greatly enhances biological activity. Disease which is caused by microorganism (bacteria, fungi, viruses, protozoa and helminthes) and parasites after being transmitted from one host or reservoir to the other host is known as infection disease. These may be mild, severe or deadly to the host. Smallpox, distemper and measles are amongst the diseases known to have entered human populations at this time. Bacteria are grouped in a number of different ways. They usually have a size of 0.3-2.0 micrometer. Most bacteria are of one of three typical shapes, rod shaped (bacillus), round (coccus, e.g., streptococcus), and spiral (spirillum). The cytoplasm and plasma membrane of most bacterial cells are surounded by a cell wall, further classification of bacteria is based on cell wall characteristics. They can also be characterized by their patterns of growth, such as the chains formed by streptococci "Gram negative bacteria" are encased in a tripple layer. The outer most layers contain lipopolysaccharide (LPS) and are decolorized by the acetone /alcohol wash. The thiosemicarbazones can be used in screening for antibacterial activity against Gram positive bacteria (Staphylococcus aureus, Bacillus subtilis) and Gram negative bacteria (Escherichia coli, Vibrio cholera).Their antiparasitic activities in cell culture have not been determined.16-18

## 2. Experimental

### 2.1 Materials

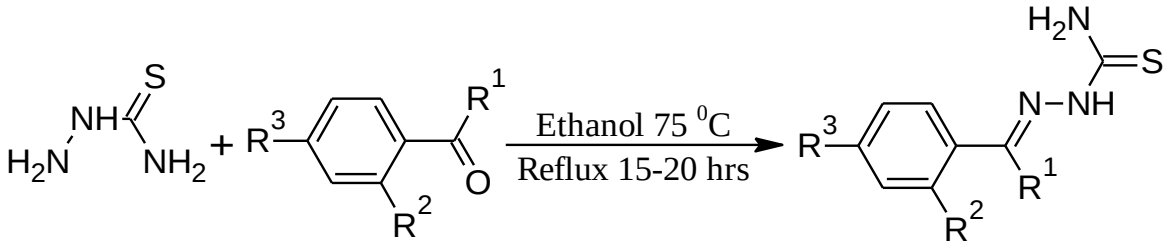
Thiosemicarbazide, 2,4-dichlorobenzaldehyde, Ethyl Acetate, Ethanol, Methanol, $CCl_4$, DCM, Dioxane, DMSO, DMF, Diethyl Ether, $SbCl_3$ , $CuCl_2$,$Zn(NO_3)_2$.

### 2.2 Methods

#### 2.2.1 Synthesis of thiosemicarbazones

**2.2.1.1 Synthesis of (2E) – 2 - [ (2,4-dichlorophenyl) methylidene] hydrazine-1-carbothioamide [C7H4Cl2O]**

20 mL ethanol along with 2.5 mmol of thiosemicarbazides in the round-bottom flask was stirred for 20 minutes with1 drop of conc. HCl. A solution of 2.5 mmol of 2,4-dichlorobenzaldehyde in 20 mL ethanol was added in above reaction mixture and stirred for 24 hours. On addition of 2,4-dichlorobenzaldehyde a colour change was observed. To check completion of reaction, TLC-technique was applied with short intervals using ethylacetate and petroleum ether as mobile phase (3:7).




$R^1$ =H, $R^2$=Cl, $R^3$=Cl

**Scheme 1.** General scheme for synthesis of thiosrmicarbazones

**M.P;** 268˚C **%age Yield;** 80 %. **IR (solid $cm^{-1}$); );** 1560 (-C=S), 1590 (-C=N-), 3407 (-NH-), 3239 (-$NH_2$), 669-880 (Aromatic Ring)**$^1$HNMR (DMSO, δppm);** 7.45-7.41 (m, 2H, Ar), 7.56-7.55 (d, 2H, Ar1H), 8.33 (S, 1H, $NH_2$), 8.38 (S, 1H, $NH_2$), 11.75 (S, 1H, -NH N=).**$^{13}$CNMR (DMSO, δppm);** 131.52, 134.48, 130.31, 129.74 (Ar), 138.13 (Ar, CH=N), 179.01 (C=S).

**2.2.2 Synthesis of thiosemicarbazones complexes**

**2.2.2.1 The synthesis for Zn-Complex with (2E)-2-[(2,4-dichlorophenyl)methylidene]hydrazine-1-carbothioamide($C_7H_4Cl_2S$)**

**In 20 mL of ethanol,** 2-mmol $C_7H_4Cl_2S$ was added in round-bottom flask and stirred till ligand become soluble. 1mmol $Zn(NO_3)_2$ was added when a clear solution was observed. A colour change of reaction mixture was observed. To check reaction completion TLC technique was applied with short intervals. Petroleum ether and ethylacetate was applied as solvent with 3:7 ratio. When reaction became complete, product was separated out through Whatmann filter paper and washed thoroughly with ethanol. Prepared complex was finally dried in oven. To check purity of product, TLC was done. For characterization IR, H-NMR, TGA, CHN, and EPR were done.

**M.P;** 320˚C **Yield; 80% IR (solid $cm^{-1}$);** 1585 (-C=S), 1605 (-C=N-), 3345 (-NH-), 3177 (-$NH_2$), 669-880 (Aromatic Ring)

**2.2.2.2 The synthesis for Sb-Complex with** (2E)-2-[(2,4-dichlorophenyl)methylidene]hydrazine-1-carbothioamide(**$C_7H_4Cl_2S$**)

**In 20 mL of ethanol,** 2-mmol $C_7H_4Cl_2S$ was added in round-bottom flask and stirred till ligand become soluble. 1mmol $SbCl_3$ was added when a clear solution was observed. A colour change of reaction mixture was observed. To check reaction completion TLC technique was applied with short intervals. Petroleum ether and ethylacetate was applied as solvent with 3:7 ratio. When reaction became complete, product was separated out through Whatmann filter paper and washed thoroughly with ethanol. Prepared complex was finally dried in oven. To check purity of product, TLC was done. For characterization IR, H-NMR, TGA, CHN, and EPR were done.

**M.P;** 336˚C **Yield; 75% IR (solid $cm^{-1}$);** 1588 (-C=S), 1605 (-C=N-), 3350 (-NH-), 3180 (-$NH_2$), 669-880 (Aromatic Ring)

**2.2.2.3 The synthesis for Cu-Complex with (2E)-2-[(2,4-dichlorophenyl)methylidene]hydrazine-1-carbothioamide($C_7H_4Cl_2S$)**

**In 20 mL of ethanol,** 2-mmol $C_7H_4Cl_2S$ was added in round-bottom flask and stirred till ligand become soluble. 1mmol $CuCl_2$was added when a clear solution was observed. A colour change of reaction mixture was observed. To check reaction completion TLC technique was applied with short intervals. Petroleum ether and ethylacetate was applied as solvent with 3:7 ratio. When reaction became complete, product was separated out through Whatmann filter paper and washed thoroughly with ethanol. Prepared complex was finally dried in oven. To check purity of product, TLC was done. For characterization IR, H-NMR, TGA, CHN, and EPR were done.

**M.P;** >232˚C **Yield; 70%IR (solid $cm^{-1}$);** 1586 (-C=S), 1604 (-C=N-), 3349 (-NH-), 3178 ($-NH_2$), 669-880 (Aromatic Ring)

**3. Results and Discussion**

The condensation reaction between *Thiosemicarbazide and 2,4-Dichlorobenzaldehyde gives (2E)-2-[(2,4-dichlorophenyl)methylidene]hydrazine-1-carbothioamide* in good yield. The synthesis of the target hybrid structure is outlined in Scheme 1.

**3.1 Elemental Analysis**

The elemental analysis for C, H, N and S revealed that the calculated and experimental data for the Schiff base are in good agreement suggesting the high percent purity of the compound which was further confirmed by mass spectrometry. The infrared absorption bands become very useful for determining the mode of coordination of the ligands to metal

**3.2 Characterization**

The structure of the thiosemicarbazone was established using **IR, $^1$HNMR,$^{13}$CNMR** spectroscopy. The infrared spectrum (Figure 1) was taken in 4000 - 400 $cm^{-1}$ region. Three bands at 3407, 3397 and 3350 $cm^{-1}$ representing stretching frequencies for the three (-NH) groups. Three bands between 690 and 760 $cm^{-1}$ indicating the presence of a substituted benzene ring. Other important bands were observed at 1605,1594,1590 $cm^{-1}$ (C=N), 1590,1580,1560 $cm^{-1}$ (C=S), 3180,3283,3239 $cm^{-1}$ ($-NH_2$).The infrared spectra of TSCs complexes showed a strong band at 1649-1595 $cm^{-1}$ attributed to C=N group. A negative shift of the order 31-42 cm-1 was observed for C=N stretching vibration on coordination due to the decrease of the bond order as a result of metal nitrogen bond formation which is in agreement with the work reported by. The next strong band at 1590-1303 $cm^{-1}$ is attributed to C=S group. a negative shift in the region of 864-717 and 854-719 cm-1 was observed in the complexes on coordination thereby indicating the involvement of thio sulfur in the coordination the metal ion. The bands in the range 3278 and 3350 $cm^{-1}$ are attributed to NH and $NH_2$ respectively. The negligible effect on these frequencies after complexation precludes the possibility of complexation at this group. On the other hand, the spectra of the complexes showed new bands around 550-478 $cm^{-1}$, 482-450 $cm^{-1}$ and 449-420 $cm^{-1}$ due to M-O,M-N and M-S respectively.

For **$^1$HNMR** prominent peaks were observed at 7.45 ppm corresponding to protons attached to aromatic ring, 8.33-.com8.38 ppm indicating nitrogen group and a broad singlet between 7.45 - 7.41 ppm suggesting that the aromatic ring.The $^{13}$CNMR spectrum gives data for the most deshielded peak appeared at 179.01 ppm and was attributed to C=S 131.52-129.74 ppm represents the aromatic ring.

The **$^{13}$CNMR** spectrum of TCSs was recorded. The most deshielded peak appeared at 179.01 ppm and was attributed to C=S, followed by a peak at 138.13 ppm which was assigned to C=N. Signals for the aromatic carbon atoms were observed in the range, 131.52-129.74 ppm.

### 3.3 Antibacterial Activity

The antibacterial activity of the (2*E*)-2-[(2,4-dichlorophenyl)methylidene]hydrazine-1-carbothioamide was determined using a modified Kirby-Bauer disc diffusion method. The antibacterial activity was done by using Gram positive bacteria (*Staphylococcus aureus*) and Gram negative bacteria (*Escherichia coli*). Ciprofloxacin was used as the standard. The percent activity index for the antibacterial was calculated as reported in literature.

**Table 1** For Anti-bacterial activity against *E.coli*(gram -ve) and *Staphylococcus aureus*(gram +ve)*respectively.*

| Sr. No | Sample (30. mg/mL) | *E.coli* (gram -ve) | *Staphylococcus aureus* (gram +ve) | AMP. (12.8 mg/mL) |
|---|---|---|---|---|
| 1. | L-1 | 13 | 20 | |
| 2. | Zn-$L_1$ | 15 | 15 | 40 |
| 3. | Sb-$L_1$ | 18 | 21 | |
| 4. | Cu-$L_1$ | 20 | 23 | |

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