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Der Pharmacia Sinica, 2013, 4(2):171-175

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Synthesis and antimycobacterial activity of Cu (II) complexes containing thiosemicarbazones ligand

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ABSTRACT

Copper (II) thiosemicarbazone complexes derived from simple thiosemicarbazone. The compounds were characterised using various spectroscopic and analytical techniques, including NMR spectroscopy, mass spectrometry, infrared spectroscopy and elemental analysis. The copper (II) complexes were screened for in vitro antitubercular activity. Incorporation of the copper (II) centre into thiosemicarbazone scaffolds enhanced their efficacy against the Mycobacterium tuberculosis while this trend was not observed for the selected simple thiosemicarbazones against the Mycobacterium tuberculosis virulent strain NCSMTB.

Keywords: PEG-400; Thiosemicarbazones; Cu (II) complexes; antimycobacterial.

INTRODUCTION

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), is responsible for the death of almost two million people every year [1-3]. Indeed, no new drugs have been developed against mycobacterium since, the 1960s and there is an urgent need to develop new anti-TB therapeutics [4]. Therefore taking into account what is reported above there is a pressing need to develop new and more effective anti-tubercular agents.

Thiosemicarbazones are currently well established as an important class of sulphur donor ligands particularly for transition metal ions [5–7]. Their metal complexing ability attracted considerable attraction due to remarkable biological activities. This compound presents a great variety of biological activity ranging from antitumor, fungicide, bactereocide, anti-inflammatory and antiviral activities [8–11]. It is well known from the literature that semicarbazide compounds containing the amide moiety have a strong ability to form metal complexes. Schiff base ligands based on thiosemicarbazones and their complexes have received considerable attention since, because of their pharmacological properties, they have enormous applications, such as antibacterial and anticancer agents [12–14].

The Cu (II) ion forms a series of co-ordination compounds with well defined structures. They can yield mono or poly nuclear complexes, some of which are biologically relevant [15– 18]. It plays a vital role in the numerous biological processes that involve electron transfer reactions or the activation of some antitumor substances [19]. Copper is an essential micronutrient for feeding and a co-factor of several enzymes involved in oxidative metabolism. Apart from its numerous functions in metabolic processes, copper is also recognized as a part in the immune function [20].

Inspired from earlier works suggest that complexes offer an excellent opportunity for the study of the effects of ligands system on the formation of complex and also the importance of the compounds containing thiosemicarbazide

moiety in medicinal uses. Here, we reported the synthesis of Cu (II) complexes of thiosemicarbazones derivative. The synthesized ligands and the copper complexes evaluated for their antimycobacterial activity against the *Mycobacterium tuberculosis*.

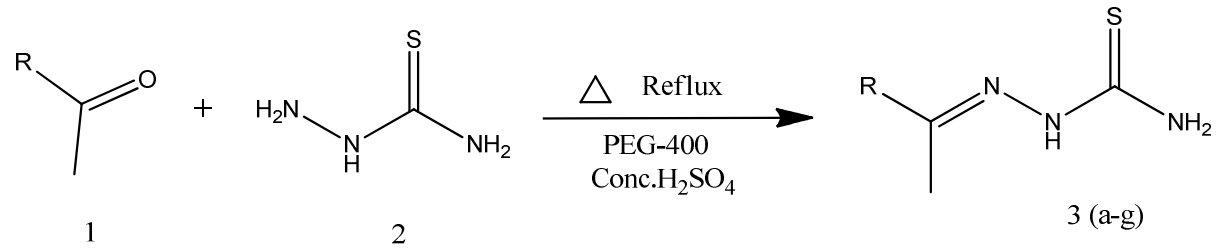
MATERIALS AND METHODS

Chemistry

All chemicals and solvents used were laboratory grade and purified prior to use. Melting points were determined by open capillary method and are uncorrected. ^{1}H NMR spectra were recorded (in DMSO-d_6 δ ppm) on AVANCE-300 MHz spectrometer using TMS as an internal standard (s = singlet, d = doublet, t = triplet, m = multiplates and br = brod). IR spectra were recorded (in KBr pallets) on SCHIMADZU spectrophotometer. The mass spectra were recorded on GC-MS SHIMDZU (Q2010 PLUS) in EI mode spectrometer. Elemental analyses were performed on a Perkins-Elmer C, H, N, elemental analyzer. All reactions were monitored by using thin layer chromatography (TLC) using 0.2 mm silica gel plates 60 F_{254} (MERCK). Reaction components were visualized in UV (255 and 365 nm) and iodine chamber.

Synthesis of thiosemicarbazone:

Thiosemicarbazones were synthesized by condensation of different acetophenone and thiosemicarbazide, reaction is carried out in green solvent PEG-400 in presence of few drops of conc. sulphuric acid. Reaction completion was monitored on TLC. Generally thermodyanamic product in reaction forms in 2 to 3 hrs.



Scheme 1: Green way synthesis of thiosemicarbazone

***Where R**= Phenyl, 4-nitrophenyl, 4-hydroxyphenyl, 4-chlorophenyl, 3-nitrophenyl, 4-methylphenyl, 4-bromophenyl.*

2-(1-phenylethylidene)hydrazinecarbothioamide: IR data (KBr, cm^{-1}): 3373.3, 3151.5 (s, N-H, NH2), 3249.8 (s, N-H, NH); 2900.7, 2858.3, 2815.9 (m, C-H, CH3, CH); 1605.5 (s, C=N); 1595.0, 1504.4 (s, C-C, phenyl); 1182.3 (s, C=S). Elemental Anal.(%) Calc.: C, 55.93; H, 5.74; N, 21.74; S, 16.59 Found: C, 56.10; H, 5.84; N, 22.15; S, 15.91.

2-(1-(4-nitrophenyl) ethylidene) hydrazinecarbothioamide: IR data (KBr, cm^{-1}): 3376.3, 3154.5 (s, N-H, NH2), 3240.6 (s, N-H, NH); 2905, 2868, 2825.6 (m, C-H, CH3, CH); 1610 (s, C=N); 1597, 1508.6 (s, C-C, phenyl); ; 1546.0, 1340.0 (s, N-O, NO2); 1188.0 (s, C=S). Elemental Anal. (%) Calc.: C, 45.37; H, 4.23; N, 23.51; O, 13.43; S, 13.46 Found: C, 45.85; H, 4.40; N, 23.12; O, 13.42; S, 13.21.

2-(1-(4-hydroxyphenyl) ethylidene) hydrazinecarbothioamide: IR data (KBr, cm^{-1}): 3540 (s, O-H, OH); 3368.3, 3152.5 (s, N-H, NH2), 3235.0 (s, N-H, NH); 2915.7, 2850.0, 2824.9 (m, C-H, CH3, CH); 1615.0 (s, C=N); 1575.0, 1510.4 (s, C-C, phenyl); 1172.3 (s, C=S). Elemental Anal.(%) Calc.: C, 51.65; H, 5.30; N, 20.08; O, 7.65; S, 15.32 Found: C, 51.60; H, 5.40; N, 20.16; O, 7.35; S, 15.49.

2-(1-(4-chlorophenyl) ethylidene) hydrazinecarbothioamide: IR data (KBr, cm^{-1}): 3358.3, 3145.0 (s, N-H, NH2), 3247.1 (s, N-H, NH); 2889.1, 2857.2, 2812.4 (m, C-H, CH3, CH); 1595.5 (s, C=N); 1575.0, 1504.4 (s, C-C, phenyl); 1176.3 (s, C=S); 740 (s, C-Cl, Ar-Cl). Elemental Anal.(%) Calc.: C, 47.47; H, 4.43; Cl, 15.57; N, 18.45; S, 14.08 Found: C, 47.87; H, 4.67; Cl, 15.43; N, 18.65; S, 13.38.

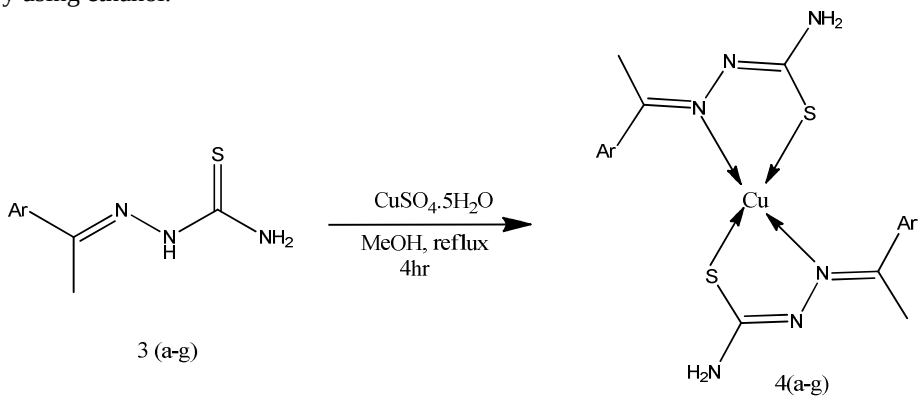
2-(1-(3-nitrophenyl) ethylidene) hydrazinecarbothioamide: IR data (KBr, cm^{-1}): 3372.0, 3148.5 (s, N-H, NH2), 3253.0 (s, N-H, NH); 2915.7, 2865.3, 2810.9 (m, C-H, CH3, CH); 1618.5 (s, C=N); 1570.0, 1525.4 (s, C-C, phenyl); ; 1535.6, 1355.0 (s, N-O, NO2); 1162.3 (s, C=S). Elemental Anal. (%) Calc.: C, 45.37; H, 4.23; N, 23.51; O, 13.43; S, 13.46 Found: C, 45.57; H, 4.37; N, 23.31; O, 13.73; S, 13.02.

2-(1-(p-tolyl) ethylidene) hydrazinecarbothioamide: IR data (KBr, cm^{-1}): 3378.3, 3165.5 (s, N-H, NH2), 3240.8 (s, N-H, NH); 2895.2, 2868.0, 2807.0 (m, C-H, CH3, CH); 1625.5 (s, C=N); 1595.0, 1504.4 (s, C-C, phenyl); 1182.3 (s, C=S). Elemental Anal.(%) Calc.: C, 57.94; H, 6.32; N, 20.27; S, 15.47 Found: C, 58.14; H, 6.40; N, 20.45; S, 15.01.

2-(1-(4-bromophenyl) ethylidene) hydrazinecarbothioamide: IR data (KBr, cm^{-1}): 3381.0, 3160.0 (s, N-H, NH2), 3264.8 (s, N-H, NH); 2910.0, 2855.4, 2820.3 (m, C-H, CH3, CH); 1614.8 (s, C=N); 1598.0, 1516.4 (s, C-C, phenyl); 1178.3 (s, C=S); 610 (s, C-Br, Ar-Br). Elemental Anal.(%) Calc.: C, 39.72; H, 3.70; Br, 29.36; N, 15.44; S, 11.78 Found: C, 39.42; H, 3.80; Br, 29.76; N, 15.74; S, 11.28.

Synthesis and characterization of copper (II) thiosemicarbazone complexes:

The complexes were synthesized by reaction of corresponding thiosemicarbazones with $CuSO_4.5H_2O$ in methanol at 40-60^{0}C for the time period of 3-4 hrs. The ratio of thiosemicarbazones with $CuSO_4.5H_2O$ was taken as 2:1. The reaction completion was monitored by TLC. If the precipitate was not formed during workup then add alcoholic ammonia solution drop wise till solution became alkaline and then again reflux it for 2hrs. Filter the product and recrystalise it by using ethanol.



Scheme 2: Synthesis of Copper (II) thiosemicarbazone complexes

Copper (II) complexes of 2-(1-phenylethylidene)hydrazinecarbothioamide: IR data (KBr, cm^{-1}): 3373.3, 3151.5 (s, N-H, NH2), 3249.8 (s, N-H, NH); 2900.7, 2858.3, 2815.9 (m, C-H, CH3, CH); 1605.5 (s, C=N); 1595.0, 1504.4 (s, C-C, phenyl); 789 (s, C-S). ^{1}H NMR (DMSO-d6, δ ppm): 2.24 (s, 3H); 6.7 (s, 1H); 7.78 (d, 2H); 7.52(d, 3H); 8.42 (s, 2H). Elemental Anal. (%) Calc.: C, 48.03; H, 4.93; Cu, 14.12; N, 18.67; S, 14.25 Found: C, 48.03; H, 4.93; Cu, 14.12; N, 18.67; S, 14.25.

Copper (II) complexes of 2-(1-(4-nitrophenyl)ethylidene)hydrazinecarbothioamide: IR data (KBr, cm^{-1}): 3376.3, 3154.5 (s, N-H, NH2), 3240.6 (s, N-H, NH); 2905, 2868, 2825.6 (m, C-H, CH3, CH); 1610 (s, C=N); 1597, 1508.6 (s, C-C, phenyl); ; 1546.0, 1340.0 (s, N-O, NO2); 792 (s, C-S). ^{1}H NMR (DMSO-d6, δ ppm): 2.24 (s, 3H); 6.8 (s, 1H); 7.90 (d, 2H); 8.10 (d, 3H); 8.46 (s, 2H). Elemental Anal. (%) Calc.: C, 40.03; H, 3.73; Cu, 11.77; N, 20.75; O, 11.85; S, 11.87 Found: C, 40.03; H, 3.73; Cu, 11.77; N, 20.75; O, 11.85; S, 11.87.

Copper (II) complexes of 2-(1-(4-hydroxyphenyl) ethylidene) hydrazinecarbothioamide: IR data (KBr, cm^{-1}): 3540 (s, O-H, OH); 3368.3, 3152.5 (s, N-H, NH2), 3235.0 (s, N-H, NH); 2915.7, 2850.0, 2824.9 (m, C-H, CH3, CH); 1615.0 (s, C=N); 1575.0, 1510.4 (s, C-C, phenyl); 785.3 (s, C-S). ^{1}H NMR (DMSO-d6, δ ppm): 2.28 (s, 3H); 6.85 (d, 2H); 7.70 (d, 2H); 7.0 (s, 1H); 8.36 (s, 2H); 8.62 (s, 1H). Elemental Anal.(%) Calc.: C, 44.85; H, 4.60; Cu, 13.18; N, 17.43; O, 6.64; S, 13.30 Found: C, 44.85; H, 4.60; Cu, 13.18; N, 17.43; O, 6.64; S, 13.30.

Copper (II) complexes of 2-(1-(4-chlorophenyl) ethylidene) hydrazinecarbothioamide: IR data (KBr, cm^{-1}): 3358.3, 3145.0 (s, N-H, NH2), 3247.1 (s, N-H, NH); 2889.1, 2857.2, 2812.4 (m, C-H, CH3, CH); 1595.5 (s, C=N); 1575.0, 1504.4 (s, C-C, phenyl); 792.1 (s, C-S); 740 (s, C-Cl, Ar-Cl). ^{1}H NMR (DMSO-d6, δ ppm): 2.26 (s, 3H); 6.7 (s, 1H); 7.32 (d, 2H); 7.68 (d, 2H); 8.26 (s, 2H). Elemental Anal.(%) Calc.: C, 41.66; H, 3.88; Cl, 13.66; Cu, 12.24; N, 16.19; S, 12.36 Found: C, 41.66; H, 3.88; Cl, 13.66; Cu, 12.24; N, 16.19; S, 12.36.

Copper (II) complexes of 2-(1-(3-nitrophenyl)ethylidene)hydrazinecarbothioamide: IR data (KBr, cm^{-1}): 3372.0, 3148.5 (s, N-H, NH2), 3253.0 (s, N-H, NH); 2915.7, 2865.3, 2810.9 (m, C-H, CH3, CH); 1618.5 (s, C=N); 1570.0, 1525.4 (s, C-C, phenyl); ; 1535.6, 1355.0 (s, N-O, NO2); 788.6 (s, C-S). ^{1}H NMR (DMSO-d6, δ ppm): 2.22 (s, 3H); 6.7 (s, 1H); 7.38 (t, 1H); 7.54 (d, 1H); 7.68 (s, 1H), 8.22 (s, 2H). Elemental Anal. (%) Calc.: C, 40.03; H, 3.73; Cu, 11.77; N, 20.75; O, 11.85; S, 11.87 Found: C, 40.03; H, 3.73; Cu, 11.77; N, 20.75; O, 11.85; S, 11.87.

Copper (II) complexes of 2-(1-(p-tolyl)ethylidene)hydrazinecarbothioamide: IR data (KBr, cm^{-1}): 3378.3, 3165.5 (s, N-H, NH2), 3240.8 (s, N-H, NH); 2895.2, 2868.0, 2807.0 (m, C-H, CH3, CH); 1625.5 (s, C=N); 1595.0, 1504.4 (s, C-C, phenyl); 782.9 (s, C-S). ^{1}H NMR (DMSO-d6,δ ppm): 2.26 (s, 3H); 2.32 (s, 3H), 6.8 (s, 1H); 7.28 (d, 2H); 7.68 (d, 2H); 8.26 (s, 2H). Elemental Anal.(%) Calc.: C, 50.24; H, 5.48; Cu, 13.29; N, 17.58; S, 13.41 Found: C, 50.24; H, 5.48; Cu, 13.29; N, 17.58; S, 13.41.

Copper (II) complexes of 2-(1-(4-bromophenyl) ethylidene) hydrazinecarbothioamide: IR data (KBr, cm^{-1}): 3381.0, 3160.0 (s, N-H, NH2), 3264.8 (s, N-H, NH); 2910.0, 2855.4, 2820.3 (m, C-H, CH3, CH); 1614.8 (s, C=N); 1598.0, 1516.4 (s, C-C, phenyl); 798.0 (s, C-S); 610 (s, C-Br, Ar-Br). ^{1}H NMR (DMSO-d6, δ ppm): 2.32 (s, 3H); 6.7 (s, 1H); 7.38 (d, 2H); 7.68 (d, 2H); 8.10 (s, 2H). Elemental Anal.(%) Calc.: C, 35.57; H, 3.32; Br, 26.29; Cu, 10.45; N, 13.83; S, 10.55 Found: C, 35.57; H, 3.32; Br, 26.29; Cu, 10.45; N, 13.83; S, 10.55.

Determination of Antimycobacterial Activity:

The sensitivity of test *Mycobacterium* strains towards the synthesized compounds **4(a-g)** was demonstrated by agar diffusion method[22]. Stock solutions (0.1, 0.5 and 1.0 mg/mL) of the individual compounds were prepared in dimethyl sulfoxide (DMSO). A sterile cork borer of 7 mm diameter was used to bore holes into the inoculum seeded solidified nutrient agar. A 30 µl volume of individual compounds was loaded into the labelled well in the prepared media plate using sterile pipette. The test was performed in two parallel experiments. The plates were kept in refrigerator for prediffusion of the sample and incubated at 37ºC for 48 h. Growth of test organisms was observed after the incubation of 48 h and the diameter of inhibition zone was measured. The antimycobacterial activity of Rifampicin (a standard antituberculoid drug) was also demonstrated simultaneously.

The MIC of the synthesized compounds was determined using the broth micro-dilution assay against the test *Mycobacterium* strains [21]. Tests were performed in sterile 96-well microplates by dispensing into each well a total volume of 200 µl, comprising 100 µl of standardized suspension of either *Mycobacterium* strains ($1x10^6$ cells/mL) and 100 µl of different concentrations of the synthesized compounds and incubated up to 48 h at 37°C. Microbial growth was determined by absorbance measurement at 620 nm using Thermo make Automatic Ex-Microplate Reader (M 51118170). The MIC of Rifampicin was also calculated for comparison purpose. The MIC was defined as the lowest concentration of the sample that inhibited the growth of test microorganisms.

RESULTS AND DISCUSSION

Chemistry

The synthetic pathway of the compounds is outlined in the **scheme 1** and preparation of complexes is outlined in the **scheme 2** under the frame of 'green chemistry'. Thus the formation of hydrazones is confirmed by NMR spectral analysis. A peak observed at δ 2.31 ppm is corresponding to methyl group (CH3). The aromatic proton ortho to imine bond is observed at δ 7.71ppm as a multiplet, where as the remaining aromatic protons resonated at δ 7.41 ppm as a multiplet. Similarly the NH_2 protons of hydrazide part is observed at δ 8.91 ppm as a broad singlet, whereas the NH proton is observed at δ 6.92 ppm. The complexes are soluble in DMSO and sparingly soluble in common organic solvents. The formations of complexes were confirmed by means of FTIR spectroscopy. Absorption bands associated with the (C=S) stretch appear at lower wave numbers. This suggests participation of the sulphur donor atom in copper (II) coordination. The absorption bands due to the imine moiety ν(C=N) appear in the range 1580-1700 cm^{-1} and are consistent with coordination of copper to the imine nitrogen and the formation of new C=N bond due to the removal of hydrogen atom in -NH upon copper (II) complexation.

Biology

Antimycobacterial activity: The results of the antimycobacterial activity of the synthesized compounds are summarized in **Table1,** which clearly shows the differential sensitivity of *Mycobacterial* strains towards the test samples. The insertion of copper (II) in the compounds to form the complexes with thiosemicarbazones may be attributed with effective growth inhibition of *Mycobacterium tuberculosis* as these compounds shared same MIC values. The strain *Mycobacterium tuberculosis* was observed to be resistant towards the synthesized compounds **3(a-g)**, while the compounds with copper (II) complexes shown to be active. The few compounds like **4d** (MIC, 100 µg /mL)**, 4g** (MIC, 50µg/mL) and **4b (**MIC, 60 µg /mL) were found to be effective growth inhibitors of this strain. The presence of 4-chloro phenyl thiosemicarbazone with copper (II), 4-bromo phenyl thiosemicarbazone with copper (II) and 4-Nitro phenyl thiosemicarbazone with copper (II) moiety in these compounds might have relevance in imparting the growth inhibition activity against *Mycobacterium tuberculosis.* However, the compounds **4a, 4c, 4f and 4e** (MIC, 1 mg/mL) showed moderate activity against *Mycobacterium tuberculosis.*

The results of the above studies signifies the importance of copper(II) complexes of thiosemicarbazone derivatives as novel scaffold and the compounds especially **4d, 4g** and **4b** as lead molecules in the design and development of novel and effective antimycobacterial agents. The result also holds importance on the eve of MDR crisis, where searching novel, effective and safe antimycobacterial agents has remained a major thrust area in the mainstream of anti- TB research.

Table 1- Antimycobacterial screenig of synthesized complex of Cu (II)

Sr. No.	Compound Code	Zone of Inhibition *Mycobacterium tuberculosis*			
		0.1 mg/ml	0.5 mg/ml	1.0 mg/ml	MIC mg/ml
1	4 a	NR	NR	1.8	1
2	4 b	2.4	3.6	5.8	0.06
3	4c	NR	NR	1.5	1
4	4d	2	2.5	3.4	0.1
5	4e	NR	NR	2	1
6	4f	NR	NR	1.7	1
7	4g	2.4	3.6	5.8	0.06
8	Rifampicin	ND	ND	ND	0.48

The results shown are the mean values of two parallel experiments. [B] The concentrations (0.1, 0.5 and 1.0 mg/ml) mentioned above are the stock concentrations of the individual samples, however the actual individual stock sample volume (30 µl) used for the agar diffusion assay corresponds to 3, 15, 30 µg.

CONCLUSION

The synthesis and biological evaluation of thiosemicarbazones and copper (II) thiosemicarbazone derived complexes against *M. tuberculosis* is reported. Modification of thiosemicarbazones into thiosemicarbazone based copper (II) complexes significantly improved their antitubercular activity. Thiosemicarbazone based copper (II) octahedral complexes synthesised by 2:1 ratio of thiosemicarbazone with copper sulphate are more potent than simple thiosemicarbazone.

Acknowledgements
SDP and RDK are thankful to CSIR, New Delhi for JRF. APA is thankful to UGC for teacher fellowship under FDP scheme. Authors gratefully acknowledge to Director, School of Chemical Sciences, S.R.T.M. University, Nanded and Vishnu chemicals, Hyderabad for spectral analysis.

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