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Synthesis, characterization and antimicrobial study of newly thiosemicarbazone

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ABSTRACT

A newly synthesis of 1-arylidenethiosemicarbazide by condensation of substituted aldehyde with thiosemicarbazide is carried out. The compounds were characterized on the basis of elemental analysis, Mass, IR, ^{1}H NMR and ^{13}C NMR spectral data. The structures were investigated for their antibacterial and antifungal activity.

Keywords: Thiosemicarbazone, Spectral characterization and Biological activity.

INTRODUCTION

Infections caused by microorganisms pose a serious challenge to the medical community and there is a need for a reliably effective therapy for such infectious diseases that had previously caused extensive mortality, morbidity and fear. The resurgence of infections in industrialized countries as well as the appearance of multidrug resistant (MDR) strains of bacterial and fungal pathogens has prompted the quest for new drugs acting both as antibacterial and antifungal agents, without cross- resistance with known antimicrobials. Development of MDR**[1,2]** to existing drugs is a constant growing phenomenon that has concerned researchers world-wide, and now has reached an alarming level for certain infectious diseases lacking ready treatment regimens**[3–5]**.

There are two basic approaches to develop a new drug for microbial infections: (i) Synthesis of analogues, modifications or derivatives of existing compounds for improving microbial treatment, (ii) Searching for novel structures, that the concerned organisms has never encountered, for the treatment of MDR microbial infections, which may act either by the same or a new mechanism**[6]**. To pursue this goal, our research efforts are directed to finding new chemical classes of antimicrobial agents. The methods of investigation enabled us to find some new pharmacophores of the above mentioned activities. Many studies have been conducted on compounds bearing –N=N-, -N-C=S, -CH=N- and electron withdrawing moieties as a pharmacophore**[7–10]**. Various substituted aromatic rings were utilised as a basis to constitute a large series of N- and S heteroatomic compounds. Special attention was paid to correlate their biological activity with their structure which proved that, activity is enhanced by electron withdrawing groups attached to aryl ring**[11–16]**. Furthermore, interest in the chemistry, synthesis and biology of these pharmacophores continues to be fuelled by their wide range of biological properties viz. antifungal, anticancer, antibacterial, antiviral, antiamoebic, antiproliferative, antitubercular, antitumor, anticonvulsant, antimalarial and trypanocidal activities**[17–37]**.

MATERIALS AND METHODS

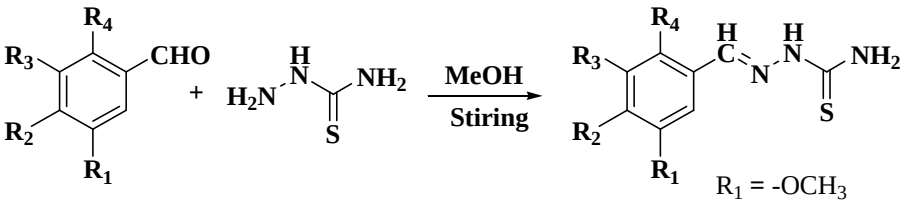
All the chemicals and solvents used for the synthesis of thiosemicarbazones were purchased from Merck and all chemicals and solvents were of laboratory reagent. Melting point of ligand was taken in open capillary and was incorrect, Thin-layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F254 (Merck). Visualization was made with UV light (254 and 365nm) or with an iodine vapor.

The elemental analysis of all compounds were carried out at EURO EA Elemental Analyzer, EA-3000, RS-232, The mass spectra of thiosemicarbazones were taken on SHIMADZU-QP2010 spectrometer, The IR spectra of thiosemicarbazones were scanned on SHIMADZU-FTIR 8400 spectrophotometer as KBr discs over the frequency range 4000-400 cm^{-1}, The NMR spectra were scanned on BRUKER AVANCE II 400 MHz NMR spectrometer by using DMSO as a solvent.

Synthesis

Preparation of 1-substituted aryl thiosemicarbazide

The thiosemicarbazide (0.01M) was dissolved in 10 ml of methanol in a 100 ml round bottom flask, a solution of 0.01M substituted aromatic aldehyde in methanol was added drop wise over a 10 min. period with continues stirring, after addition the reaction mixture was stirred for 3 hours at room temperature, completion the reaction solvent was evaporated and residue was washed with cold methanol and dried at room temperature.



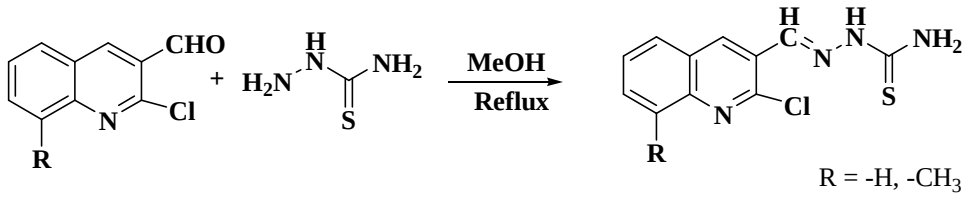
R_1 = -OCH_3
R_2 = -OH, -OCH_3
R_3 = -Br, -H
R_4 = -H, -NO_2

1-(3-bromo-4-hydroxy-5-methoxybenzylidene)thiosemicarbazide(RMT-1) is white **colour** powder; **yield** 86.0%; **M. P.** 200°C; **R_f:** 0.31 (8:2 benzene:acetone); **Anal. Calcd**. For $C_9H_{10}BrN_3O_2S$ (304.16 g/mol): C, 35.54%; H, 3.31%; N, 13.81%; S, 10.54%. **Found:** C, 35.41%; H, 3.26%; N, 13.68%; S, 10.37%; **MS** (m/z): 305 (M+1); **IR (KBr, cm^{-1}):** *ν*(OH) 3471; *ν*(NH) 3354; *ν*(NH_2) 3252; *ν*(C=N) 1678; *ν*(N-N) 1047; *ν*(C=S) 1284; δ(C=S) 835; *ν*(Ar–C-H) 3136-3010; *ν*(Ar–C=C) 1498; *ν*(C-Br) 607; **^{1}H-NMR (DMSO-d_6):** δ 3.87 (s, 3H, OMe); 7.41, (d, 1H, *J* = 1.6 Hz, Ar-H); 7.47, (d, 1H, *J* = 1.6 Hz, Ar-H); 8.05, 8.12 (d, 2H, NH_2); 8.43 (s, 1H, HC=N); 9.98 (s, br, 1H, OH); 11.32 (s, 1H, NH); **^{13}C-NMR (DMSO-d_6):** *δ* 56.40 (O-CH_3); 109.0, 109.29, 124.26, 126.49, 145.44, 148.52 (Ph); 141.25 (HC=N); 177.64 (C=S).

1-(4,5-dimethoxy-2-nitrobenzylidene)thiosemicarbazide(RMT-2) is Yellow **colour** powder; **yield** 82.0%; **M. P.** 288°C; **R_f:** 0.34 (8:2 benzene:acetone); **Anal. Calcd**. For $C_{10}H_{12}N_4O_4S$ (284.29 g/mol): C, 42.25%; H, 4.25%; N, 19.71%; S, 11.28%. **Found:** C, 42.14%; H, 4.20%; N, 19.62%; S, 11.19%; **MS** (m/z): 284 (M); **IR (KBr, cm^{-1}):** *ν*(NH) 3375; *ν*(NH_2) 3292; *ν*(C=N) 1628; *ν*(N-N) 1060; *ν*(C=S) 1286; δ(C=S) 833; *ν*(Ar–C-H) 3171-3005; *ν*(Ar–C=C) 1527; *ν*(C-NO_2) 1323; **^{1}H-NMR (DMSO-d_6):** δ 3.87, (s, 3H, OMe); 3.95, (s, 3H, OMe); 7.56, (s, 1H, Ar-H); 7.69, (s, 1H, Ar-H); 8.14, 8.34 (d, 2H, NH_2); 8.55 (s, 1H, HC=N); 11.33 (s, 1H, NH); **^{13}C-NMR (DMSO-d_6):** *δ* 56.14, 56.54 (O-CH_3); 107.52, 108.68, 123.06, 138.04, 149.46, 152.77 (Ph); 141.37 (HC=N); 178.19 (C=S).

Preparation of 1-substituted quinolinyl thiosemicarbazide

A mixture of a thiosemicarbazide (0.01M) and substituted quinoline aldehyde (0.01 M) was dissolved in 70-80 ml of methanol and add 1 ml of 20% NaOH solution, the resulting reaction mixture was reflux for 10 hours on boiling water bath. After completion of reaction, the reacthio mixture was poured in ice-cold water, it was filtered and washed repeatedly with cold methanol and dried at room temperature.



R = -H, -CH_3

1-((2-chloroquinolin-3-yl)methylene)thiosemicarbazide(RMT-3) is Greenish **colour** powder; **yield** 79.0%; **M. P.** 232°C; **R_f:** 0.50 (8:2 benzene:acetone); **Anal. Calcd**. For $C_{11}H_9ClN_4S$ (264.73 g/mol): C, 49.91%; H, 3.43%; N, 21.16%; S, 12.11%. **Found:** C, 49.86%; H, 3.35%; N, 21.03%; S, 12.08%; **MS** (m/z): 264 (M); **IR (KBr, cm^{-1}):** *ν*(NH) 3310; *ν*(NH_2) 3234; *ν*(C=N) 1591; *ν*(N-N) 1082; *ν*(C=S) 1292; δ(C=S) 862; *ν*(Ar–C-H) 3144-2966; *ν*(Ar–C=C) 1475; *ν*(C-Cl) 765; **^{1}H-NMR (DMSO-d_6):** δ 7.67, (tri, 1H, Ar-H); 7.80, (tri, 1H, Ar-H); 7.93, (d, 1H, *J* = 8.4

Hz, Ar-H); 7.97, (d, 1H, *J* = 8.0 Hz, Ar-H); 8.25, (s, 1H, Ar-H); 8.45, 8.49 (d, 2H, NH_2); 9.28 (s, 1H, HC=N); 11.79 (s, 1H, NH); **^{13}C-NMR (DMSO-d_6):** *δ* 126.15, 126.96, 127.70, 127.82, 128.42, 131.49, 135.98, 146.90, 148.40 (Ph); 136.74 (HC=N); 178.40 (C=S).

1-((2-chloro-8-methylquinolin-3-yl)methylene)thiosemicarbazide(RMT-4) is Yellow **colour** powder; **yield** 81.0%; **M. P.** 244°C; **R_f:** 0.53 (8:2 benzene:acetone); **Anal. Calcd**. For $C_{12}H_{11}ClN_4S$ (278.76 g/mol): C, 51.70%; H, 3.98%; N, 20.10%; S, 11.50%. **Found:** C, 51.63%; H, 3.88%; N, 20.04%; S, 11.42%; **MS** (m/z): 278 (M); **IR (KBr, cm^{-1}):** *ν*(NH) 3352; *ν*(NH_2) 3271; *ν*(C=N) 1599; *ν*(N-N) 1107; *ν*(C=S) 1280; δ(C=S) 850; *ν*(Ar–C-H) 3140-3001; *ν*(Ar–C=C) 1491; *ν*(C-Cl) 759; **^{1}H-NMR (DMSO-d_6):** δ 2.66, (s, 3H, CH_3); 7.58, (tri, 1H, Ar-H); 7.69, (d, 1H, *J* = 7.2 Hz, Ar-H); 7.832, (d, 1H, *J* = 8.0 Hz, Ar-H); 8.27, (s, 1H, Ar-H); 8.46, 8.51 (d, 2H, NH_2); 9.28 (s, 1H, HC=N); 11.80 (s, 1H, NH); **^{13}C-NMR (DMSO-d_6):** *δ* 17.25 (Ar-CH_3); 125.91, 126.33, 127.04, 127.57, 131.43, 135.55, 136.32, 146.03, 147.52 (Ph); 136.84 (HC=N); 178.40 (C=S).

Antimicrobial evaluation

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using microdilution broth method according to NCCLS standards**[38]**. Serial dilutions of the test compounds and reference drugs were prepared in Muellere-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Muellere-Hinton agar were performed to obtain the required concentrations. In primary screening 1000 µg mL^{-1}, 500 µg mL^{-1} and 250 µg mL^{-1} concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution at 200 µg mL^{-1}, 100 µg mL^{-1}, 50 µg mL^{-1}, 25 µg mL^{-1}, 12.5 µg mL^{-1}, and 6.25 µg mL^{-1} concentration against all microorganisms. The tubes were inoculated with 108 cfu mL^{-1} (colony forming unit/mL) and incubated at 37 ºC for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied.

RESULTS AND DISCUSSION

The structure of the new compounds synthesized were elucidated by spectral data. In the IR spectra, some significant stretching bands due to –N-H, -N-N- and –C=S were observed at 3460-3215 cm^{-1}, 1120-1020 cm^{-1} and 1310-1240 cm^{-1} respectively. The specific band for thiosemicarbazone (-CH=N-) was observed at 1670-1590 cm^{-1}.

In the ^{1}H NMR spectra, the signal due to –CH=N protons, present in all compounds, appeared at 8.5-9.2 ppm as a singlet. The, -N-NH and NH_2 protons were observed at 11.30-11.55 and 8.10-8.30 ppm as a singlet, respectively. All the aromatic protons were observed in the expected regions.

In the ^{13}C NMR spectra the signal due to (-CH=N-) carbon, appeared at 136.74-141.37 ppm and -C=S carbon, appeared at 177.64-178.40 ppm and all the aromatic carbons were observed in the expected regions. The ^{13}C NMR spectrum provides direct information about the carbon skeleton of the synthesized compound.

Mass spectra of compounds showed a (M + 1) and (M + 2) peaks, in agreement with their molecular formula.

ANTIFUNGAL ACTIVITY OF SYNTHESISED THIOSEMICARBAZONES

		MINIMAL FUNGICIDAL CONCENTRATION (µG/ML)			
Sr. No.	**CODE NO.**	**C. ALBICANS MTCC 227**	**A. NIGER MTCC 282**	**S. CEREVISIAE MTCC 170**	**E. FLOCCOSUM MTCC 7880**
1	RMT - 1	500	200	500	>1000
2	RMT – 2	500	500	500	500
3	RMT – 3	250	500	250	500
4	RMT – 4	250	500	250	>1000
5	Nystatin	100	100	100	100
6	Greseofulvin	500	100	500	500

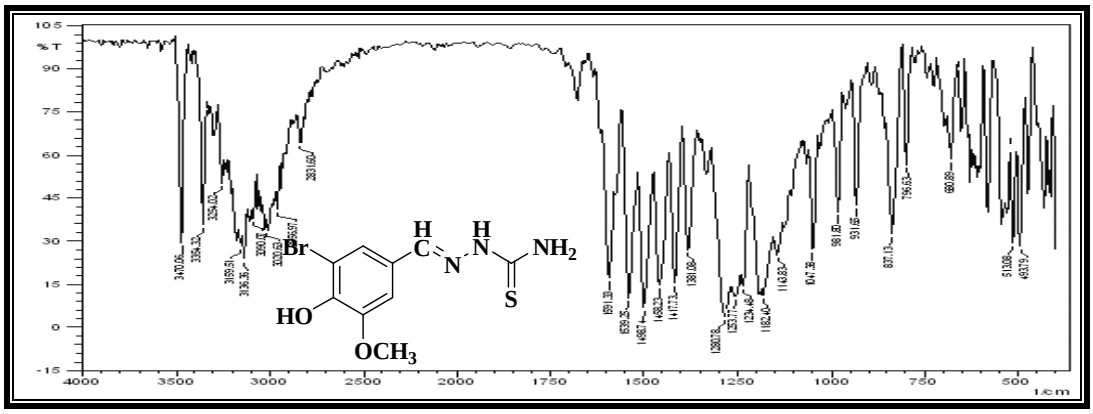
All the synthesized compounds were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method**[38,39]** with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus epidermidis* MTCC 442, one Gram-negative bacteria *Escherichia coli* MTCC 443 and four fungal strains *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282, *S. cerevisiae* MTCC 170, *E. floccosum* MTCC 7880 taking gentamycin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and greseofulvin as standard drugs.

The results obtained from antimicrobial susceptibility testing are depicted in Table.

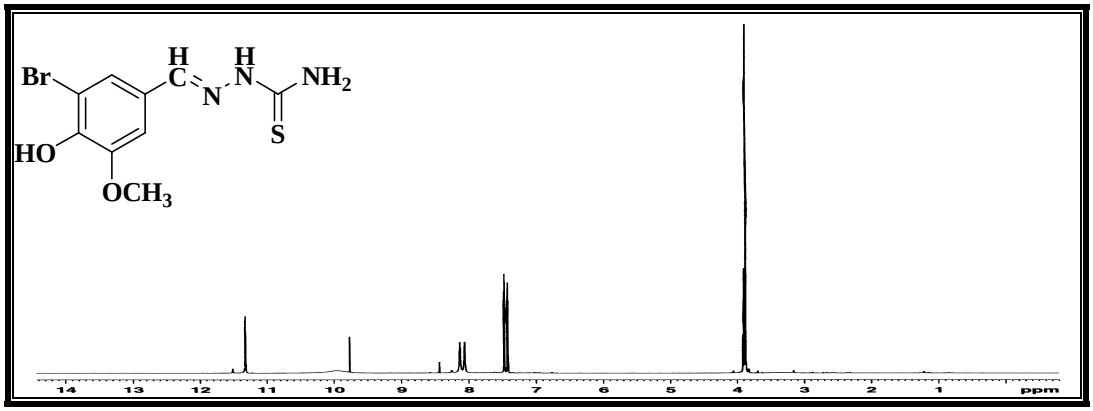
ANTIBACTERIAL ACTIVITY OF SYNTHESISED THIOSEMICARBAZONES

MINIMUM INHIBITION CONCENTRATION (µG/ML)				
Sr. NO.	**CODE no.**	**E. COLI MTCC 443**	**S. EPIDERMIDIS MTCC 442**	**S. AUREUS MTCC 96**
1	RMT - 1	200	100	62.5
2	RMT – 2	500	250	150
3	RMT – 3	250	250	200
4	RMT – 4	125	500	250
5	Gentamycin	0.05	0.25	0.25
6	Ampicillin	100	200	250
7	Chloramphenicol	50	25	50
8	Ciprofloxacin	25	50	50
9	Norfloxacin	10	15	10

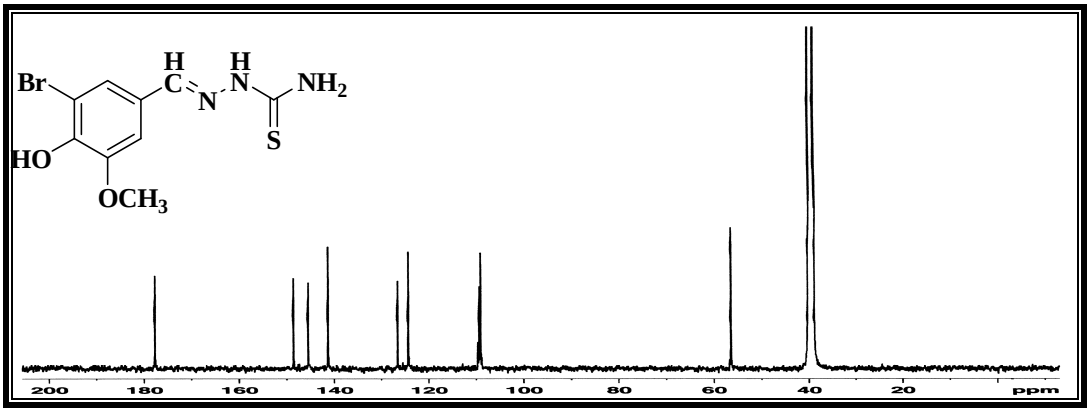
IR spectrum of RMT-1



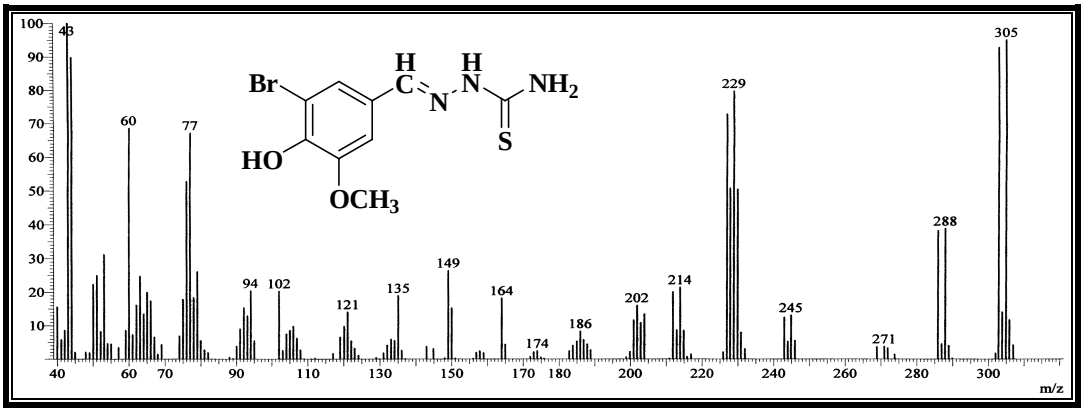
^{1}H NMR spectrum of RMT-1



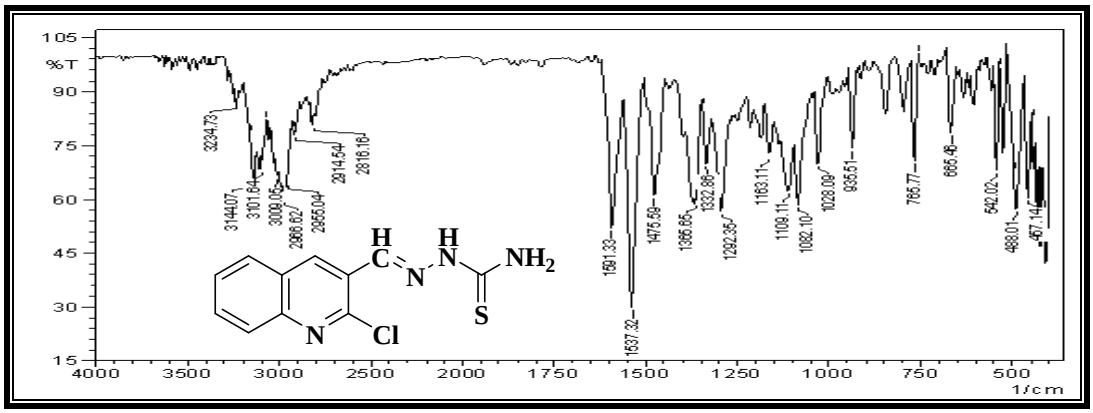
^{13}C NMR spectrum of RMT-1



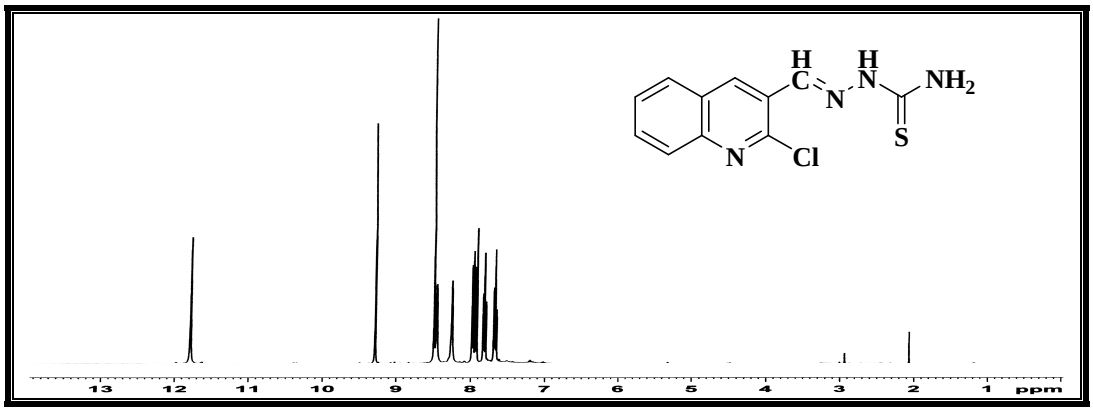
Mass spectrum of RMT-1



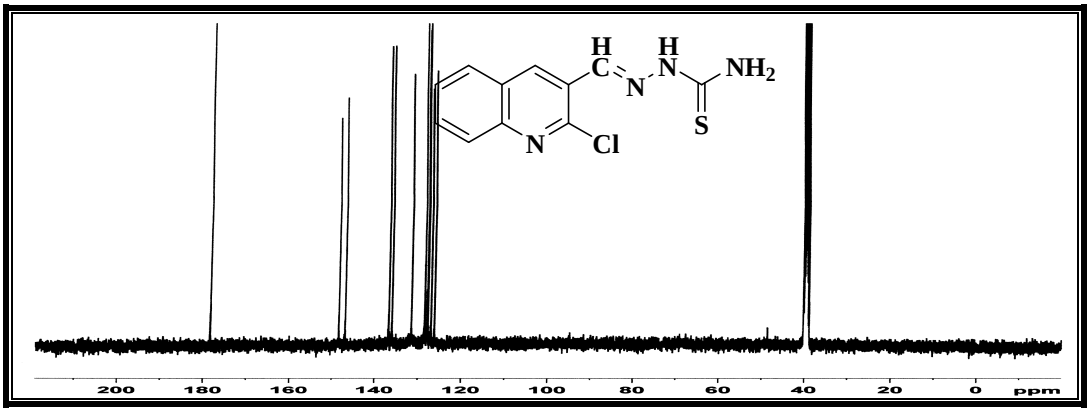
IR spectrum of RMT-3



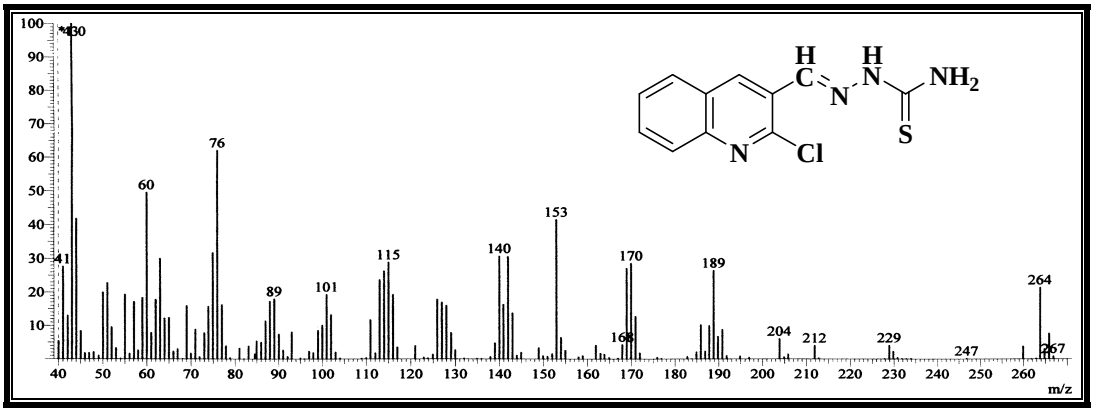
^{1}H NMR spectrum of RMT-3



^{13}C NMR spectrum of RMT-3



Mass spectrum of RMT-3



CONCLUSION

Thiosemicarbazone derivatives used as ligands to synthesize Co(II), Ni(II) and Co(II) based metal complexes. Antibacterial and antifungal study indicates that some Thiosemicarbazone have shown better activity or similar activity compared to standard drugs taken.

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