RESEARCH PAPER ISSN: 2249 –4820

CHEMISTRY & BIOLOGY INTERFACE

An official Journal of ISCB, Journal homepage; www.cbijournal.com

DESIGN AND SYNTHESIS OF THIOSEMICARBAZONE METAL COMPLEX DERIVATIVE AS ANTIMICROBIAL AGENT

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Received; 29 October 2022, Accepted; 6December 2022

Abstract: Synthesis of cyanuric chloride based varied chalcones **D1-D5** by condensation reaction of 1, 3, 5-triazine with 4'-Amino Acetophenone to yields product which upon condensation with varied aromatic aldehyde **a-e**. These chalcones on further condensation with Thiosemicarbazide to produced heterocyclic compounds **E1-E5** which were used as the ligands. Two moles of this prepared ligand were reacted with three different metal chloride such as $MnCl_2$, $CuCl_2$ and $ZnCl_2$ to produced complexes **F1-F15.** Characterizations of all synthesized complexes were done using ^{1}HNMR and IR. Biological evaluation of all prepared complexes was done using against two-gram positive bacteria such as *Staphylococcus aureus*, *Bacillus megaterium* and two-gram negative bacteria *Escherichia coli*, *Proteus vulgaris*. Most of the synthesized products exhibited moderate to good potency against bacteria as compared to standard drugs.

Keywords: 1, 3, 5-triazine, 4'- Amino Acetophenone, Metal complexes, Antimicrobial activity, Thiosemicarbazone, Spectroscopy.

1. Introduction

Transition metal complexes are molecule that contains varied groups arranged around a central metal ion. Metal complexes are easily assembled from smaller parts, and sometimes they are easily transformed into in to new moiety by switching out old parts for new ones. That rapid assembly and disassembly is part of what makes these compounds very useful in biological and industrial as catalysis. The interaction between transition or inner transition metal ions and other heterocyclic ligand molecules has received greater study in recent years. Transition metal complexes open up a world of possibilities for designing and developing new biological active chemicals that outperform the present metal salts in terms of biological efficacy. By changing the geometry of metal complexes with different heterocyclic ligands, they can be made more reactive and physiologically active. Heterocyclic ligands often contain a functional group such as -OH, -NH_2, -SH, -C=N (Schiff base) etc., which forms

five or six membered chelating rings when complexes with metal ions [1].

Coordination compounds also find many applications in electroplating, textile dyeing and medicinal chemistry. Savina Savir et al. have studied on Nickel (II) Complexes with Polyhydroxybenzaldehyde and O, N, S tridentate Thiosemicarbazone ligands: Synthesis, Cytotoxicity, Antimalarial Activity, and Molecular Docking [2].

Roksana Rzycka-Korzec et al. have examined Effect of the complex-formation ability of thiosemicarbazones containing (aza) benzene or 3-nitro-1, 8-naphthalimide unit towards Cu (II) and Fe (III) ions on their anticancer activity [3].

Nurin Sakinatul Hayati Haji Damit et al. have studies on Synthesis, structural characterisation and antibacterial activities of lead (II) and some transition metal complexes derived from quinoline-2-carboxaldehyde 4-methyl-3-thiosemicarbazone [4].

Franco Bisceglie et al. have studied on Antibacterial activity of metal complexes based on cinnamaldehyde thiosemicarbazone analogues [5].

Ali A. A. Al-Riyahee et al. have studied on Ni(II), Cu(II) and Zn(II) complexes of functionalised thiosemicarbazone ligands and also examine its reactivity, characterization and structural studies [6].

T. A. Nibila have synthesized transition metal complexes from 2, 4-dihydroxybenzaldehyde n(4)-methyl (phenyl)thiosemicarbazone and studied its structural characterization and biological activities [7].

Fekadu Muleta et al. have synthesized Metal-complexes from natural product derivatives of (thio)semicarbazone and studied its antibacterial and antioxidant activities [8].

Mansura Huseynova et al. have carried out synthesis, biological and theoretical properties of crystal zinc complex with thiosemicarbazone of glyoxylic acid [9].

Sakshi Gupta a, Nidhi Singh a, Tahmeena Khan b, Seema Joshi reviewed Thiosemicarbazone derivatives of transition metals as multi-target drugs [10].

Monika Pitucha et al. have studied Influence of Complexation of Thiosemicarbazone Derivatives with Cu (II) Ions on Their Antitumor Activity against Melanoma Cells [11].

P. T. da Silva et al. have studied on chalcones and their derivatives, all structures were determined by NMR and observed its Antimicrobial avtivity [12].

Ravi KumarMarella et al. have founded that highly active biomorphic MgO/C supported CuNPs direct catalytic coupling of 1,4-butanediol dehydrogenation and acetophenone hydrogenation using in-situ liberated H_2 [13].

Harbi Tomah Al-Masri et al. have studied on Hg(II) and Ru(II) complexes of mono- and dichalcogenides of bis(diphenylphosphino)amine chelating ligands also examined its catalytic activity in transfer hydrogenation of

acetophenone derivatives [14].

Fatima Kanso et al. have founded that thiosemicarbazones derivatives showed in *vitro*/*in vivo* anti-inflammatory activities. And also studied thiosemicarbazones (TSC) compounds and their metals complexes attracted high interest due to their wide range of biological activities and interestingly [15].

Jai Devi et al. studied on, Recent advancements of organotin(IV) complexes, its derived from hydrazone and thiosemicarbazone ligands they also examined its ligands act as a potential Anticancer agents [16].

Alireza Akbari et al. have synthesized new structure of Ni(II) complexes of thiosemicarbazone and isothiosemicarbazone-based ligands and studied about Antimicrobial activity [17].

Mauro Carcelli et al. have synthesized Salicylaldehyde thiosemicarbazone ligands and their copper(II) complexes and examined anticancer activity of tridentate thiosemicarbazone copper complexes [18].

Narendra Kumar Singh et al. have studied on thiosemicarbazone based metal complexes in complexes formation Cu metal used and studied Antitumor and Anticancer also examined Chelated thiosemicarbazones to copper(II) reduces side effects [19].

Vasile Gutsanu et al. have studied on coordination compounds of thiosemicarbazone with transition metal and examined its thermal, antimicrobial and antifungal properties [20].

Sebastian Kallus et al. have synthesized biotin-conjugated anticancer thiosemicarbazones and their iron(III) and copper(II) complexes and biological evaluation of metal complexes [21].

In continuance to metal complexes, the present article expressed synthesis of metal complexes from 4'- Amino Acetophenone based Thiosemicarbazone ligand and its complexation with transition metal such as Mn, Cu and Zn.

2. Methods and Materials

2.1 Chemicals and Reagents

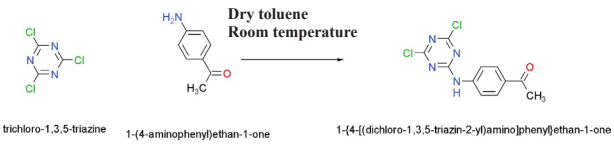
All chemicals used were of laboratory reagent grade and used without further purification**.** Various aldehydes, 1, 3, 5-triazine, 4'- Amino Acetophenone, Thiosemicarbazide, KOH, $CuCl_2$, $MnCl_2$, $ZnCl_2$, DMF, Toluene and Ethanol were used as received from Merck, Mumbai, India.

2.2 Experimental

Bruker Avance-400 instrument was used for Proton NMR study and 100MHZ frequency instrument was used for ^{13}C NMR. Parts per million unit was used to express chemical shift value. ABB Bomem Inc. FT-IR 3000 Spectrophotometer was used for Infrared Spectral study. Data obtained was expressed in cm^{-1} unit. Shimadzu LCMS-2010 was used for MASS spectral analysis. Perkin Elmer-2400 Series II CHNS/O Elemental Analyzer was used for Composition measurement.

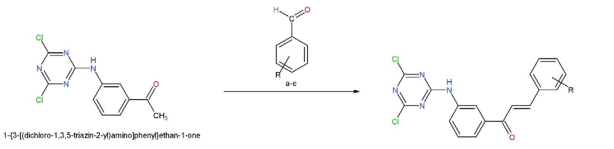
2.3 Method of Synthesis

2.3.1 Synthesis of Chalcones D1-D5



SCHEME 1

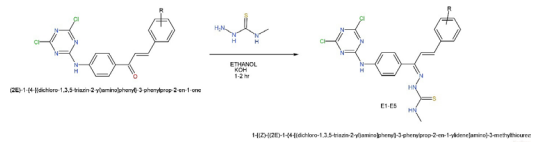
In a 250 ml round bottom flask, 1, 3, 5-triazine (0.1 mole) and 4'- Amino Acetophenone dissolved in dry toluene (50 ml) with constant shaking maintaining the temperature below 25°C. After the completion of dissolution, the mixture was refluxed for 1.5 hour then it was cooled and poured into crushed ice. Solid was separated by filtration and crystalline from ethanol. Prepared product was treated with aldehyde **a-e** (0.1 mole) to produced chalcones **D1-D5 (Scheme 1 & 2)**



SCHEME 2

2.3.2 Synthesis of Ligands E1-E5

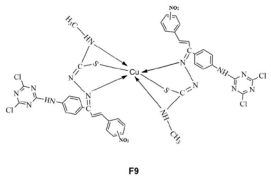
To a well stirred solution of chalcones (0.01 mole) in 250 ml round bottom flask, add 0.01 mole Thiosemicarbazide, 40 ml ethanol and 40 ml 40% KOH to this mixture solution. Reflux the entire mixture for 1-2 hours to produce **E1-E5.** Completion of reaction was monitored by TLC (**Scheme 3)**.

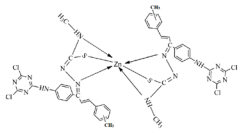


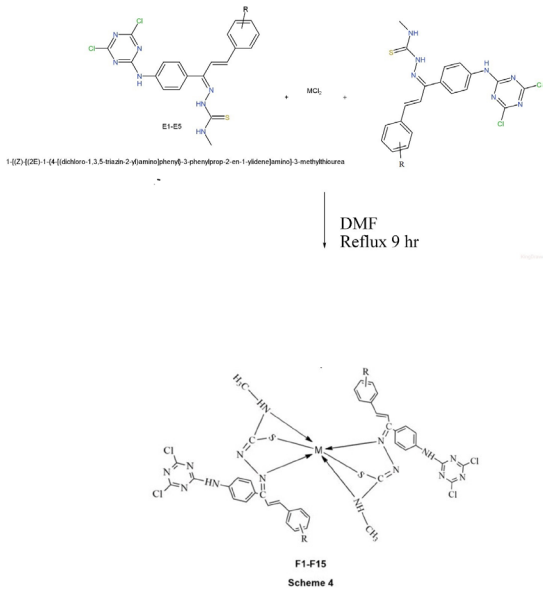
SCHEME 3

2.3.3 Synthesis of Metal Complexes F1-F15

To a well stirred solution of ligands **E1-E5** in 250 ml round bottom flask, add 0.01 mole MCl_2 (M = Cu, Zn and Mn), 30 ml DMF. Reflux the mixture in the presence of HCl for 9 hour to produce metal complexes **F1-F15**. Completion of reaction was monitored by TLC (**Scheme4)**.

Compound code: F9	 F9
Molecular formula: $C_{40}H_{30}Cl_4CuN_{16}O_4S_2$	
M. P. (°C): >310	
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:	^{1}H NMR: δ 3.04 (3H S), 6.99 (1H, d , j=15.6Hz), 7.17 (1H, d j=15.6Hz),7.44(2H, ddd J=8.2,1.8,0.5Hz), 7.56-7.69 (4H 7.63(ddd J=8.6,1.9,0,5Hz),7.62(4H ddd J= 8.2,1.4, 0.5 Hz), 8.28(2H, ddd, J=8.6,1.7 ,0.5Hz).
IR cm^{-1} (KBr):	3425 (-NH stretching), 3010 (Aromatic C-H stretch.), 2940 (Aliphatic C-H Stretch.), 1660 (C=C stretching), 1550 (N-O Streching), 1330 (C-S stretching), 740 (C-H bending in substituted ring), 1680 (C=C Streching di substituted (trans) Alkene. 755 (C-H stretching Di substituted ring), 855 (C-Cl Streching).
Mass (M+1):	1090.5
Elemental analysis:	**Calculated (%):** C: 48.40; H: 3.60; N: 15.45. **Found (%):** C: 48.35; H: 3.54; N: 15.35

Compound code: F12	 F12
Molecular formula: $C_{42}H_{36}Cl_4ZnN_{14}S_2$	
M. P. (°C): >300	
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:	^{1}H NMR: δ2.05 (3H s), 3.01 (3H, s) 6.71-6.96(2H, 6.78(d,J=15.6Hz), 6.89(d J=15.6Hz), 7..10-7.33(4H, 7.16(ddd, J=7.8, 1.6, 0.5Hz), 7.27(4H ddd,J=7.8,1.9,0.5 Hz), 7.42(2H,ddd, J=8.2,1.8,0.6 Hz), 7.65(2H, ddd, J=8.2,1.4,0.6 Hz).
IR cm^{-1} (KBr):	3425 (-NH stretching), 3010 (Aromatic C-H stretch.), 2940 (Aliphatic C-H Stretch.), 1660 (C=C stretching), 1330 (C-S stretching), 740 (C-H bending in substituted ring), 1680 (C=C Streching di substituted (trans) Alkene. 755 (C-H stretching Di substituted ring), 855 (C-Cl Streching), 1450 (C-H Bending).
Mass (M+1):	1100.8
Elemental analysis:	**Calculated (%):** C: 47.40; H: 3.78; N: 16.76. **Found (%):** C: 47.35; H: 3.62; N:16.68



Scheme 4

M = Cu, Zn, Mn

R = -H, -4-CH_3,-4Cl, -NO_2, -4-OCH_3

3. Characterization

F9 and **F12** compounds of the series are taken as the representative compound. In the ^{1}H NMR spectrum the characteristic signals due to each proton and functional groups with protons are well described on the basis of shielding and deshielding effect. From the ^{1}HNMR spectra it shows that the aromatic protons are comes in the region of 6-8.5 δppm in downfield region where as the NH protons comes in more down field region at chemical shift value around 11.3 δppm. From IR spectroscopy it clearly confirms the presence of the –NH and –NO_2 groups in the structure. It also confirms the S-C linkage, Aliphatic C-H Stretching, C=C stretching and Aromatic C-H stretching present in the metal complex

4. Result and Discussion

Table 1 Data showing synthesis of Complexes F1-F15

Sr. No.	Compounds Code	M	R[c]	Reaction Time[a] (hr)	% Yiled[b]
1	**F1**	Mn	H	6.5	75
2	**F2**	Mn	4-CH_3	6.5	77
3	**F3**	Mn	4-Cl	7.5	86
4	**F4**	Mn	4-NO_2	6.5	83
5	**F5**	Mn	4-OCH_3	6.5	76
6	**F6**	Cu	H	5.5	82
7	**F7**	Cu	4-CH_3	6.5	78
8	**F8**	Cu	4-Cl	7	84
9	**F9**	Cu	4-NO_2	6.5	86
10	**F10**	Cu	4-OCH_3	5.5	76
11	**F11**	Zn	H	6	82
12	**F12**	Zn	4-CH_3	5	78
13	**F13**	Zn	4-Cl	7.5	86
14	**F14**	Zn	4-NO_2	7.5	86
15	**F15**	Zn	4-OCH_3	7	76

[a]Reaction is monitored by TLC, [b]Isolated yield and [c]Names of aldehyde groups

From the Table 1 show the various complex prepared by reaction between chalcone based ligands which having thiosemicarbazone moiety with transition metal such as Mn, Cu and Zn chlorides in the presence of DMF as the basic reagent by stirring the mixture for further 8-9 hour to produced various metal complexes F1-F15. Results shows that the metal complexes bearing electron withdrawing groups are synthesized in the shorter reaction time with good yields of the product (F3, F4, F8, F9, F13 and F14) as compare to the metal complexes bearing electron releasing group such as complexes F2, F5, F6, F7, F10, F12 and F15.

5. Antimicrobial Activity

5.1 Preparation of Media:

For bacterial activity nutrient agar is used. Nutrient agar is prepared as follows:

5gm Peptone, 3gm Metal Extract, 5gm NaCl and 15gm Agar-Agar Peptone were mixed in one litre distilled water and heated to dissolve all the ingredients. The medium was stabilized in autoclave at 15 pound pressure at 125°C for 20 minutes. The medium was cooled down to 45°C and 20 ml poured in sterilized Petri-dish. The pH of the medium was adjusted between 7.0 to 7.5.The culture of the above organism was prepared in nutrient broth dissolved in distilled water. The content of nutrient broth is:

1) Beef extract : 10 gm
2) Peptone : 10 gm
3) Sodium chloride : 5 gm

After sterilizing the above media, it was used for the culture purpose.

The culture was ground at 37°C in incubator. With the help of swab, the culture was spread over the agar plates, under specific condition 5 mm diameter paper discs were prepared and were sterilized in autoclave.

The solution of the test compound was kept over these paper discs with the help of micropipette. These discs were dried to remove the solvent.

Sterile test compound coated by discs were kept in Petri dish containing culture media. The discs was pressed to sterile on media and Petri dishes were incubated for 24 hours at 37°C.

After the incubations the zone of inhibition was measured.

5.2 Experimental Data of Antimicrobial Study.

Table 2 Antibacterial Activities of complexes F1-F15

Samples	*S.aureus* (+Ve)	*B.megaterium* (+Ve)	*E.coli*(-Ve)	*P.vulgaris*(-Ve)
F1	11	18	12	15
F2	13	20	17	18
F3	7	17	12	11
F4	15	18	13	12
F5	12	16	12	12
F6	11	14	17	9
F7	9	12	15	12
F8	9	16	12	14
F9	15	14	16	16
F10	6	18	13	13
F11	9	14	16	11
F12	13	16	17	16
F13	15	13	16	14
F14	12	9	14	12
F15	6	12	13	11
Ampicillin	**25**	**24**	**18**	**22**
Pencillin-G	**11**	**11**	**8**	**9**

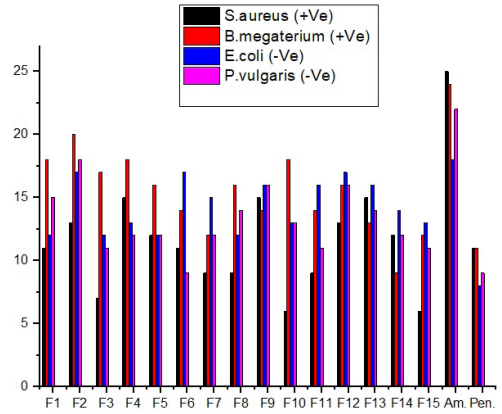


Figure-1 Antimicrobial activities of Complexes F1-F15

(I) **Against *Staphylococcus aureus*:**

Maximum activity were found in compounds (F4, F9, and F13) zone of inhibition 15.0 mm whereas minimum activity were found in compound (F4 and F13) zone of inhibition 6.0 mm

(II) **Against *Bacillus megaterium*:**

Maximum activity was found in compounds (F1, F2, F4, and F9) zone of inhibition 18.0 - 20.0 mm and minimum

activity were found in compounds (F14) zone of inhibition 9.0 mm

(III) **Against *Escherichia coli*:**

Maximum activity were found in compound (F2, F6 and F12) zone of inhibition 17.0 mm (near to standard drug) and minimum activity were found in compounds (F1, F3, F5 and F8) zone of inhibition 12.0 mm

(IV) **Against *Proteus vulgaris*:**
Maximum activity were found in compounds (F2, F9, and F12) zone of inhibition 16.0 - 18.0 mm and minimum activity were found in compounds (F3, F6,F11 and F15) zone of inhibition 9.0 - 11.0 mm

6. Conclusion

In conclusion the highly functionalized ligand-based metal complexes F1-F15 were synthesized from various thiosemicarbazide based chalcones and transition metal chloride in the presence of basic condition. All the prepared complexes were characterized by different ^{1}HNMR and IR spectroscopic techniques and screened for antimicrobial activity against gram positive and gram negative bacteria. Satisfactory results of antimicrobial activity were obtained with most of the compounds.

7. Acknowledgements

We are very thankful to Principal and Head of Chemistry Department of KKSJ Maninagar Science College Ahmedabad for providing required research facility during research work, we are also grateful to Authority of Gujarat University, Ahmedabad for all kind of support during research work.

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