



## Synthesis, characterization, identification and bioactivity of new transition-metal compounds from thiophene derivatives legend

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### Abstract

A lot of consideration and investigation have been made to legends and its metal complexes due to its bioactivity against microbes and cancer cells. The aim of the study was to synthesize new legend and five metal complexes. In this study, 2-methyl thiophene (2MT) with methyl hydrazinecarbodithioate (HC) were reacted to form the new legend and five metal salts were also used to synthesize five new metal complexes. Bioactivities to the new metal complexes were also achieved. New organic legend methyl (2E)-2-(thiophen-2-ylmethylidene) hydrazine carbodithioate (2MTHC) was successfully synthesized and five new organo-metalic compounds were also synthesized by reacting the legend with solution of metal slats of Nickel, Cadmium, Copper, Cobalt and Zinc. Characterization was achieved using various physico-chemical analyses to identify the novel organic legend and its transition-metal compounds. The melting point was ranged between 184 to 269 °C which indicate the complexation of the leeand with central positive ion of the metal, while the  $\nu(\text{N-H})$  was not fond in metal complexes which indicate the complexation of the legend with the ions of the metals as well. New Schiff base legend and five new metal complexes were synthesized and characterized, the bioactivity of the new compounds were achieved against certain bacteria and microbes and it was found that the legend is more active than its metal complexes.

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## Introduction

Considering the wide scope of the subject, present research is restricted to the 2-methyl thiophene legend with five transition metals, namely, cobalt(III), nickel(II), copper(II), zinc(II) and cadmium(II) (Subhankar *et al.*, 2016; Nora *et al.*, 2016).

Usually, organometallic compounds are very versatile and their activity is high. Hence, metal complexes are largely utilized by taking advantage of their reactivity. Furthermore, MC contains many elements in the molecule unlike organic compounds (Nanjan *et al.*, 2016). They are utilized for their spacious variety of properties since each element possesses confidential properties. Nowadays, there are many drugs in the market that are based on organic compounds or extract from natural products (Qian *et al.*, 2016), but these are generally expensive due to very low percentage capitulated of recrystallized and pure resultant and high cost of synthesis (Abeer and Al-Karkhi 2013).

Recently, consideration has been paid to the legends and their organo-metallic compounds because of the importance in understanding the effect of metal complexes (MC) in biological activity (Hamsa and Eman 2016). Modification to configuration of the MC was achieved by coordinate metals with different Schiff bases (legends) were synthesized to increase the possible use as drugs in pharmaceutical industry (Eman 2017). However, studies related to drug design are limited and awareness of the physico-chemical characteristics of potentially active compounds is required (Meriem *et al.*, 2015).

## Materials and methods

### Chemicals

Chemicals that have been used in this research were: Reagents: benzyl chloride (BDH), 1-(2-thienyl)-1-propanone (ACROS), thiophene-2-carbaldehyde, iodomethane (BDH), potassium hydroxide (BDH), hydrazine hydrate (Fluka), carbon disulphide (BDH), copper(II) acetate (UNIVAR), nickel(II) acetate tetrahydrate (Fluka), cadmium(II) acetate (BDH), cobalt(II) acetate (MERCK), zinc(II) acetate (Fluka),

RPMI-1640 (Sigma), Foetal Calf Serum (PAA), MTT Powder (Sigma), Insulin (Sigma), PBS Tablets (Sigma). All chemicals were of analytical grade and were used as supplied without further purification.

Solvents: absolute ethanol (99.8%, Scharlau), ethanol (95%, Hamburg), dimethyl sulphoxide (Scharlau), dimethylformamide (Fischer) and acetonitrile (Baker)

### *Preparation of methyl 2-methylidene hydrazine carbodithioate (2MHC)*

This compound was prepared according to the procedure developed by as reported previously. Potassium hydroxide 11.4g (0.2 mol) dissolves in ethanol 90% (70 ml). To this solution, hydrazine hydrate 1g (0.2 mol) was added and the mixture was cooled in an ice-salt bath to 0 °C. After that, carbon disulphide 15.2g (0.2 mol) was added drop by drop with constant stirring over an hour using a mechanical stirrer. During this time, two layers were formed. The lower oil layer which was light-brown in colour was separated using a separator funnel and then dissolved in 40% ethanol (60 ml) at temperature below 5°C to prevent the dithiocarbazic acid from decomposing.

The mixture was kept in an ice-bath and to it 12.5 ml methyl iodide (0.2 mol) was added drop wise with dynamic moving (continuous stirring) of the mixture using a mechanical stirrer. The white product, filtered, washed with ethanol and recrystallized after drying. The white product was kept in a desiccator. The melting point (MP) was 126.8-127.9°C (Lit. 81 °C; yield: 75%).

### *Preparation of the Legend methyl (2E)-2-(thiophene-2-ylmethylidene) hydrazine carbodithioate (2MTHC Schiff base)*

The new legend (2MTHC) was synthesized by dissolving methyl 2-methylidene hydrazinecarbodithioate (2MHC) (0.61g, 0.005 mol) in 35 ml of warm ethanol (95%), and adding to an equimolar quantity of 2-methylthiophene (2MT) (0.60 ml, ≈0.005 mol) also dissolved in hot 95% ethanol (20 ml). The mixture was heated for 15 minutes. The mixture was cooled to 0 °C in an ice-salt

bath for complete crystallization. The output was filtered off, washed using cold ethanol, recrystallized from absolute ethanol and then dehydrated over silica gel.

#### *Preparation of metal complexes*

Ethanol (95%) was used to dissolve the (0.1 mol) of the metal salts, ethanol was also used to dissolve (0.2 mole of the ligand). Both solutions were added to each other's and was mixed and warmed on a steam bath with continuous stirring.

The solid product was separated from the solution using filter paper, and washed with ice-cold ethanol. The quantity and the amount of the synthesized metal complexes varies from 53%-72%.

The metal salts used were copper(II) acetate, nickel(II) acetate tetra hydrate, cadmium(II) acetate, cobalt(II) acetate and zinc(II) acetate. Suitable crystals for X-ray diffraction analysis were obtained for Ni(2MTHC)<sub>2</sub>, Zn(2MTHC)<sub>2</sub>, Ni(2MTHC)<sub>2</sub>, Cd(2MTHC)<sub>2</sub>, Co(2MTHC)<sub>2</sub> and Zn(2MTHC)<sub>2</sub> from acetonitrile solution (Yield: 55-65%).

#### *Melting Point determination*

An electro-thermal digital melting point (M.P) apparatus was used to find out the (M.P) to the new complexes ( $\pm 1^\circ\text{C}$ ). All samples are placed in the micro tube (soda lime glass). The melting point of each sample was recorded after it has melted completely.

#### *Inductively Coupled Plasma-Atomic Emission*

##### *Spectroscopic Analyses (ICP-AES)*

Determinations of metal contents in sample were determined by means of a Perkin-Elmer Plasma 1000 Emission Spectrometer, organo-metallic compounds were digested in 5 ml of 65% sulphuric acid by heating until a clear solution was obtained.

This solution was quantitatively transferred to 25 ml volumetric flask and was made up to volume with distilled water. The calibration was carried out using different concentrations (5, 15 and 20 ppm) from a

prepared stock solution of each metal salt ( $\approx 1000$  ppm) (Elbadawy *et al.*, 2018).

#### *Fourier Transform-Infrared (FT-IR) Spectroscopic Analyses*

Infrared spectra of samples were recorded as potassium bromide (KBr) pellets on a Perkin Elmer FT-IR Spectrometer BX,

The spectrums were done in the range 400-4000  $\text{cm}^{-1}$  under room temperature (RT).

#### *Ultraviolet/Visible (UV-Vis) Spectroscopic Analyses*

The UV-Vis spectra of the complexes were measured in quartz cuvettes in the range of 200-1000 nm on spectrometer (Shimadzu UV-2501 PC).  $1 \times 10^{-3}\text{M}$  solution of each metal complex in DMSO was prepared and diluted to  $1 \times 10^{-4}$  or  $1 \times 10^{-5}\text{M}$  depending on the spectra needed.

#### *Quantitatively evaluation for antimicrobial*

Each compound shows positive (diameter  $> 15$  mm) were diluted to evaluate quantitative measurement, the lowest concentration that completely inhibited visible microbial growth was recorded as the minimum inhibitory concentration (MIC,  $\mu\text{g}/\text{cm}^3$ ).

All the samples were dissolved in DMSO and the disc diffusion technique was employed. When clear inhibition of more than 15 mm is found, the compound is considered active and then quantitative analyses are carried out (MIC values).

The MIC (minimum inhibition concentration) value is the lowest concentration that completely inhibited visible microbial growth. Microbes that have been used in this research are Methicillin resistant *Staphylococcus Aureus* (MRSA), *Bacillus subtilis*-wild type (B29), *S. Typhimurium* (S.T.), *Pseudomonas aeruginosa* (60690), *Candida albicans* (C.A.), *Aspergillus ochraceous* (398) and *Saccharomyces cerevisiae* (20341).

## Results

Table 1 shows the melting points, colours and the suggested formula of the new compounds. Table 2 below shows the percent of metals in the compound and compare the theoretically calculated with what was found in measurement. The results calculated from infrared measurements was depicted in Table 3.

**Table 1.** Analytical data and physical properties of the Schiff bases and their metal complexes.

Compound	Colour	Melting Point ( $\pm 1^\circ\text{C}$ )	Chemical formula
2MTHC	Bright Orange	184	$\text{C}_{12}\text{H}_{14}\text{N}_2\text{S}_4$
$\text{Co}(2\text{MTHC})_2$	Deep Brown	224	$\text{C}_{24}\text{H}_{28}\text{N}_4\text{S}_8\text{Co}$
$\text{Ni}(2\text{MTHC})_2$	Brown	269	$\text{C}_{24}\text{H}_{28}\text{N}_4\text{S}_8\text{Ni}$
$\text{Cu}(2\text{MTHC})_2$	Bright Brown	223	$\text{C}_{24}\text{H}_{28}\text{N}_4\text{S}_8\text{Cu}$
$\text{Zn}(2\text{MTHC})_2$	Bright Yellow	215	$\text{C}_{24}\text{H}_{28}\text{N}_4\text{S}_8\text{Zn}$
$\text{Cd}(2\text{MTHC})_2$	Yellow	249	$\text{C}_{24}\text{H}_{28}\text{N}_4\text{S}_8\text{Cd}$

### Magnetic susceptibility measurements

Each metal complex was ground in a mortar and weighed into a glass magnetic tube (5 mm diameter).

The elevation of the metallic compounds was above 1.5 cm. Magnetic susceptibility data were collected with a Sherwood Scientific MSB-AUTO magnetic susceptibility balance at 300K.

**Table 2.** ICP-AES statistics of the metal complexes.

Compound	Metal Content (ppm)	
	Calc.	Found
$\text{Co}(\text{MTHC})_3$	9.54	9.28
$\text{Ni}(\text{MTHC})_2$	12.49	12.66
$\text{Cu}(\text{MTHC})_2$	11.08	11.11
$\text{Zn}(\text{MTHC})_2$	10.00	10.46
$\text{Cd}(\text{MTHC})_2$	12.79	11.02

*A.ochraceous* – *Aspergillus ochraceous* (398)

*S.cerivisiae* – *Saccharomyces cerivisiae* (20341)

MRSA – Methicillin resistant *Staphylococcus Aureus*

*B. Subtilis* – *Bacillus subtilis*- wild type (B29) *S.*

*typhimurium* – *Salmonella typhimurium* *P.*

*aeruginosa* – *Pseudomonas aeruginosa* (60690<sup>a</sup>)

Diameter of 15 mm and above considered active;- inactive.

## Discussion

### Infra red analysis

### Molar Conductivity measurements

The molar conductivity of a  $1 \times 10^{-3}$  M solution of each metal complex in DMSO was measured at  $27.5^\circ\text{C}$  using a Jenway Conductivity Meter and a dip-cell with platinized electrode.

Table 4 shows the molar conductance data for the compounds in DMSO ( $10^{-3}\text{M}$ ) at room temperature. Electronic spectral data of legand and their metallic compounds are depicted in Table 5.

Table 6 shows the bioactivity test of the new compounds comparing with the slandered Streptomycin (standard) and Nystatin (Standard).

The IR spectral data for the NS legand and their new metallic compounds are shown in Table 3.

The major functional groups present in the Schiff bases and their metal complexes are  $\nu(\text{N-H})$ ,  $\nu(\text{N-N})$ ,  $\nu(\text{C=N})$ ,  $\nu(\text{CSS})$ ,  $\nu(\text{C-S-C})_{\text{thiophene}}$  and  $\gamma(\text{C-H})_{\text{thiophene}}$ . Proof of harmonization to the central metal ion through thiolate sulphur atom, azomethine nitrogen can be concluded based on the existence of the functional groups (Shane *et al.*, 2016).

In the spectra of the Schiff base, a sharp peak at  $\approx 3100\text{ cm}^{-1}$  indicates the presence of a secondary amine  $\nu(\text{N-H})$ .

The  $\nu(\text{N-H})$  stretching mode disappeared in the spectra of the metallic compounds which indicates de-protonation of the ligand during coordination with

the metal ions. This occurred as harmonization to the central metal ion after deprotonation can give extra stability through  $\pi$ -electron delocalization along the  $-\text{C-N-N-C-S}$  chain moieties.

**Table 3.** Infrared data of the ligands and metal complexes.

Compound	Bands (Wavelength, $\text{cm}^{-1}$ )					
	$\nu(\text{N-H})$	$\nu(\text{C=N})$	$\nu(\text{N-N})$	$\nu(\text{CSS})$	$\nu(\text{C-H})_{\text{thiophene}}$	$\nu(\text{C-S-C})_{\text{thiophene}}$
MTHC	3106	1528	1096	928	1222	856
$\text{Co}(\text{MTHC})_3$	-	1578	1058	948	1220	862
$\text{Ni}(\text{MTHC})_2$	-	1574	1060	950	1216	860
$\text{Cu}(\text{MTHC})_2$	-	1584	1060	962	1220	864
$\text{Zn}(\text{MTHC})_2$	-	1594	1058	960	1220	850
$\text{Cd}(\text{MTHC})_2$	-	1584	1056	946	1218	862

The thione group is comparatively unsteady in the mono-meric shape and tends to turn to a stable C-S single bond (thiol form) by enethiolisation, if there is at least one hydrogen atom adjacent to the C=S.

This is in agreement with many previous reports (Emad *et al.*, 2017).

A band at  $1528\text{ cm}^{-1}$  in the spectrum of the legand was assign to the  $\nu(\text{C=N})$  mode and this shifted to higher frequencies in the metallic compounds.

This evidence used to support the proposal that metallic captions were harmonized to the azomethine nitrogen. However, both shifting to lesser

wavenumber and no shifting of this band were mentioned also upon complexation. Variation in direction of the recognized shift was related to the coupling of  $\nu(\text{C=N})$  with other bands. Shifting of the hydrazinic  $\nu(\text{N-N})$  to higher frequencies metallic compounds has been reported, and this is due to decrease in the revulsion among the coordination of non-bonding electron pair on the azomethine nitrogen (Emad *et al.*, 2017).

Existence of thiophene ring in each compound can be determined by bands at around  $1234\text{ cm}^{-1}$  assigned to the  $\nu(\text{C-H})_{\text{thiophene}}$  modes. However, these bands are of slightly weaker intensity and no shifting was recorded in metal complex spectrum.

**Table 4.** Magnetic susceptibility and molar conductivity measurements of the metal Complexes.

Compounds	$\mu_{\text{eff}}$ (B.M) at 298K	$\Lambda$ ( $\Omega\text{ cm}^2\text{ mol}^{-1}$ )
$\text{Co}(\text{MTHC})_3$	Diamagnetic	2.33
$\text{Ni}(\text{MTHC})_2$	Diamagnetic	1.28
$\text{Cu}(\text{MTHC})_2$	1.89	1.66
$\text{Zn}(\text{MTHC})_2$	Diamagnetic	0.17
$\text{Cd}(\text{MTHC})_2$	Diamagnetic	6.65

These bands also occurred in  $\text{Cu}(\text{II})$  complexes resulting from thiophene-2-carbaldehyde thiosemicarbazone. The  $\nu(\text{C-S-C})_{\text{thiophene}}$  appeared at

$\approx 850\text{ cm}^{-1}$  in both the legand znd metal complexes spectra. Therefore, it was concluded that atom of sulfur ring is not involved in coordination with

central cation as there was no shifting in the positions of the bands.

#### Magnetic Susceptibility and Molar Conductivity Analysis

The conductivity measurements of the metallic compounds were below those expected of 1:1 electrolyte complexes which is around 50-70  $\Omega^{-1}$

$\text{cm}^2\text{mol}^{-1}$ . This indicates that all the complexes are non-electrolytes and legend is shows coordination to the central metal ion in DMSO. Furthermore, the non-electrolytic nature of the complexes proves that shows the coordination of legend to the central metal ion as a uninegatively charged bidentate species *via* the nitrogen atom of the azomethine and the sulphur atom.

**Table 5.** Electronic Spectral measurements of the legand and Metal Complexes.

Compounds	UV-Vis spectra ( $\lambda_{\text{max}}$ , nm) <sup>a</sup>
(MTHC)	277(7.00), 349(7.53)
Co(MTHC) <sub>3</sub>	299(5.31), 327(5.28), 393(5.29), 770(2.60), 809(2.60)
Ni(MTHC) <sub>2</sub>	274(5.29), 335(5.34), 587.5(5.76), $\approx$ 350sh, $\approx$ 400sh, $\approx$ 450sh
Cu(MTHC) <sub>2</sub> .H <sub>2</sub> O	340(5.36), 375(5.29), 785(2.65), $\approx$ 600sh, $\approx$ 250sh
Zn(MTHC) <sub>2</sub>	289(5.31), 348(5.38), 367(5.30)
Cd(MTHC) <sub>2</sub>	283(5.04), 345(5.40), 363(5.29)

<sup>a</sup>Log  $\epsilon$  ( $\text{mol}^{-1} \text{l cm}^{-1}$ ) are in parentheses.

The magnetic susceptibility readings to all metallic compounds were achieved under room temperature was listed in Table 4. Diamagnetic complex are those who does not contain unpaired electrons and they tend to shift the magnetic field while the paramagnetic contain unpaired electrons and they tend to move into a magnetic field.

All Cobalt complexes exhibit diamagnetism. This shows that Co (II) from the metal salt have been oxidized to low spin system of Co(III).

However, the oxidation of Co(II) to Co(III) occurred when the preparation of complexes using Co(II) salts with softly coordinating anions (acetate) and the mechanism wasn't proposed.

This indicates that Co(III) complexes were in an octahedral environment.

This finding was well supported by the X-ray crystal structure of Co(MTHC)<sub>3</sub>, which indicated that the complex possessed octahedral geometry.

**Table 6.** Qualitative Antimicrobial evaluate ( $100 \text{ mg cm}^{-3}$ )<sup>a</sup>.

Sample	Bacterial strains		Fungal strains				
	MRSA	B29	S. Typhimurium	60690	C.A	398	20341
MTHC	10	7	11	7	-	14	-
Co(MTHC) <sub>3</sub>	-	8	8	6	-	-	-
Ni(MTHC) <sub>2</sub>	-	-	-	7	-	-	-
Cu(MTHC) <sub>2</sub>	-	9	-	-	-	11	-
Zn(MTHC) <sub>2</sub>	-	7	-	6	-	10	-
Cd(MTHC) <sub>2</sub>	-	6	9	-	-	-	-
Streptomycin (standard)	25	15	17	20	-	-	-
Nystatin (Standard)	-	-	-	-	23	24	28

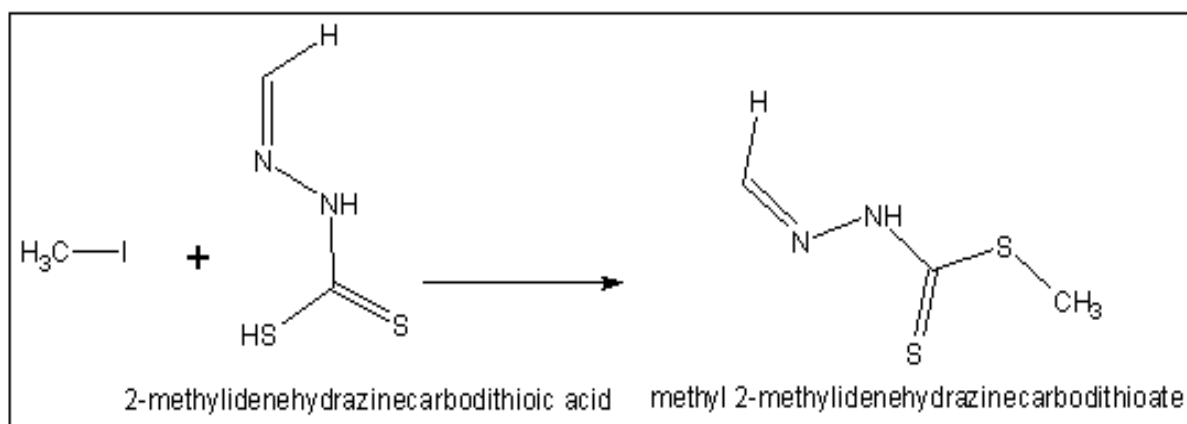
*C.albicans* – *Candida albicans* (C.A).

All the Cu(II)  $d^9$  complexes, had values of  $\mu_{eff}$  is 1.66 B.M. These values are in the normal range for a square planar Cu(II) ion.

All Ni(II), Zn(II) and Cd(II) complexes exhibit diamagnetism. This shows that the Ni(II) complexes exist in square planar geometry. In addition,  $d^{10}$  configuration of Cd(II) and Zn(II) causes them to usually exist as tetrahedral complexes (Ahlam *et al.*, 2015).

#### UV-Vis analysis

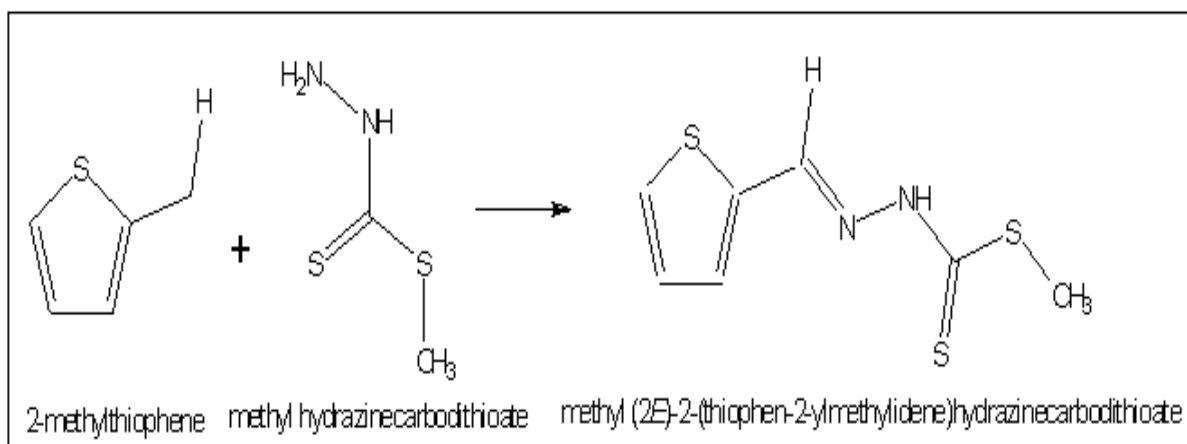
The Schiff base spectrum exhibit bands at 277 nm and 349 nm attributed to the  $n \rightarrow \pi$  and  $\pi \rightarrow \pi^*$  transitions, respectively. In the spectra of the metal complexes, these bands were sometimes shifted to higher or lower wavelengths compared with their corresponding free ligands. All Co(III) complexes exhibit octahedral environment characteristic, where shoulder bands at  $\approx 700$  nm and bands at around 400 nm were due to  ${}^1A_{1g} \rightarrow {}^3T_{2g}$  and  ${}^1A_{1g} \rightarrow T_{2g}$ , respectively (Emad *et al.*, 2017).



**Fig. 1.** Preparation of methyl 2-methylidenehydrazinecarbodithioate (2MHC).

The electronic spectra of Ni(II) complexes, in addition to showing the intra-ligand bands, also exhibits a band at 274 nm for Ni(MTHC)<sub>2</sub>, and shoulder-bands for the other Ni(II) complexes 335 nm. These bands can be assigned to S to Ni(II) charge transfer band and indicating that the Ni(II) ion is coordinated to

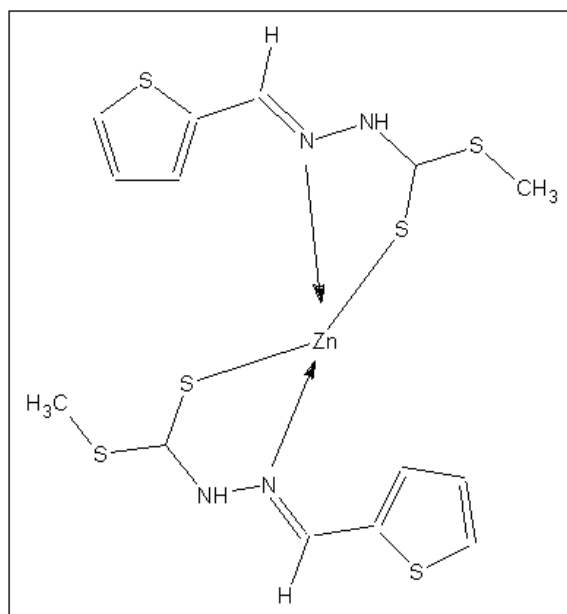
sulphur through the thio sulphur atom. Bands at 587.50, 335 and 274 nm were observed corresponding to  ${}^1A_{1g} \rightarrow {}^1A_{2g}$ ,  ${}^1A_{1g} \rightarrow {}^1B_{1g}$  and  ${}^1A_{1g} \rightarrow {}^1E_g$  transition, respectively arising a from square planar Ni(II) configuration (Padma *et al* 2016).



**Fig. 2.** Synthesis of the new Schiff base (2MTHC).

The reflectance spectrum of Cu(II) complex showed a intra-ligands bands at 340 nm. In addition to the intra-ligand transition, Cu(MTHC)<sub>2</sub> (347 nm) could be attributed to the <sup>2</sup>B<sub>1g</sub> → <sup>2</sup>E<sub>1g</sub> transition. Furthermore, peak at 375 nm for Cu(MTHC)<sub>2</sub> could be attributed to the <sup>2</sup>B<sub>1g</sub> → <sup>2</sup>A<sub>1g</sub> transition, arising from a square planar Cu(II) (Padma *et al.*, 2016) A d → d transition is observed in all Cu(II) complex, at 785 nm (Ahlam *et al.*, 2015).

The band maxima in the UV-Vis spectra of the Zn(II) and Cd(II), d<sup>10</sup> complexes showed the intra ligand transition bands at 242 nm to 370 nm. No d→d transition is observed in the spectrum of Zn(II) and Cd(II) complexes because of the fully occupied orbital of the metal cation. Appearance of only intra ligand transition bands for Zn(II) complexes was also observed in the spectra of bis[S-methyl-β-N-(2-furylmethyl) methylene dithiocarbazato] zinc(II) [3] and [S-benzyl-β-N-(2-furyl methyl ketone) dithiocarbazato] cadmium(II) [14]. This correlated well with the X-ray crystal structure of Zn(MTHC)<sub>2</sub> and Cd(MTHC)<sub>2</sub> which indicated that they had a distorted tetrahedral geometry (Mehmet *et al.*, 2012).



**Fig. 3.** Expected structure for the metal.

#### Complex

#### Bioactivity analysis

No pattern was observed for the series of metal complexes derived from ligand MTHC. They were

more active, less active or not active at all after chelation occurred, i.e before chelation, was more active against S.T and 398 with clear inhibition of 10 mm and 14 mm, respectively. However, after chelation with various transition metals, no activities were observed as shown in Table 6. A conclusion can be made that when the substituents were hydrogen atom and ethyl group at the carbon attached to the thiophene group, the bioactivity of the complexes against objective fungi and microbes (Emad *et al.*, 2017). The expected structure of the Schiff base and the metal complex is shown below.

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