A new approach to design 3(3-sulfamoylbenzamido) benzoic acid containing transition metal complexes: Characterization and Biological activities

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Abstract: Zn, Cu, Co and Ni are biocompatible metals as they are active center of many enzymes in the human body. Incorporation of these biocompatible metals into 3-(*o*-Sulfamoylphenyl) carbamoylbenzoic acid (I) makes them able to prove an excellent antimicrobial agent. In the present study Ni (II), Co (II), Cu(II) and Zn (II) complexes (III-VI) were synthesized from ligand (I) derive from 3-(*o*-Sulfamoylphenyl) carbamoylbenzoic acid and zinc, nickel, cobalt acetate tetrahydrate/copper acetate monohydrate. Synthesized complexes (III-VI) were characterized by FT-IR, ^{1}H NMR and ^{13}CNMR. III-VI have 81-93% yield while melting points recorded were in the range of 209-239^{o}C. Purity of ligands and their respective complexes was confirmed by TLC. Results of antibacterial properties suggested that III, IV, V and VI were highly active against gram +ve (*S. epidermidis, B. subtilis. S. aureus*, *S. mutans*) and gram -ve bacteria (*E. coli* and *P. aruginosa*). Comparison was also performed to check whether metal complexes or ligand with its derivative exhibit best result against all tested strains. The anthelmintic activity of the complexes III-VI against tape worm, liver fluke, thread worm, and hook worm using three different concentrations (15, 30, 45mg/mL), significantly ($p<0.01$) paralyzed the worms followed by death, which was comparable with that of the standard. Overall results indicated that *S. epidermidis*, *S. mutans*, *E. coli* and *B. subtilis* are very sensitive to complex III & IV and can be used for treatment of bacterial infections whereas Complex-V, could a potent target for anti-parasite therapy.

Keywords: 3-(*o*-Sulfamoylphenyl) carbamoylbenzoic acid, transition metals, FTIR, NMR, antibacterial activities.

INTRODUCTION

In the recent era, microbial resistance against antibiotics incited scientists to search alternative compounds having therapeutically strong potential against fungal and bacterial pathogens (Haque *et al.*, 2018). The most remarkable advances in medicinal chemistry observed when amino benzoic acid had been showed a large number of therapeutic potentials as antibacterial, antineoplastic, anticonvulsant, antiemetic, gastro-kinetic, sun-screening (Gupta and Kumar, 2017). 2-[N-(2, 6-Dichloro-3-Methylphenyl) amino] Benzoic acid, an important antibacterial agent is in common use (Lam *et al*., 2003). Halogenated derivatives of amino-benzoic acids and their transition metal complexes have proved to possess the pharmacological activities such as anti-arrhythmic, antibacterial, antiviral, anti-hyperglycemic, anti-apomorphine, anti-serotonin activities (Joseph *et al*., 2013). They also exhibited endothelin receptor antagonists properties thus reduces the pulmonary hypertension and also recommended for the treatment of gastrointestinal conditions (Anthony *et al*., 2016). Synthesis of many organic compounds by using amino benzoic acid as precursor is of vital importance (Ishizuka *et al*., 2001; Kluczyk *et al*., 2002).

Compounds having benzothiazole nucleus coddled in research to synthesize new products with biological activities such as antifungal, antimicrobial, anticancer, anthelmintic, anti-diabetic, amyloid images agent etc. (Parekh *et al*., 2005). Good antitumor and anti-mycobacterial activities were shown by sulfobenzimide derivatives (Kaki *et al*., 2014). 3-(*o*-Sulfamoylphenyl) carbamoylbenzoic acid was synthesized by reacting benzisothiazole and meta-isomer of amino benzoic acid. The presence of sulfamoyl moiety is responsible for antimicrobial activity (Halli *et al*., 2012). It was thought worthwhile, to synthesize transition metal complexes and halogenated derivatives of 3-(*o*-Sulfamoylphenyl) carbamoyl benzoic acid to achieve better antibacterial activities. The synthesized compounds were subjected to purification and chemical characterization to check their antibacterial and anthelmintic potential.

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MATERIALS AND METHODS

Chemical and Instruments

All the chemicals used without any further purification were of analytical grade and were purchased from sigma-Aldrich. KBr disk was used to record FT-IR spectra on Shimadzu IR Affinity-IS FTIR Spectrophotometer. NMR spectra were collected using Benchtop NMR spectrophotometer at 300 MHz with TMS as internal standard. All chemical shift values were recorded as (δ ppm). Stuart Melting Point apparatus was used to check the melting point of all synthesized derivatives by capillary method. Purity of the compounds was determined by thin layer chromatography (TLC) while spots were detected under UV lamp.

Synthesis of 3-(o-Sulfamoylphenyl) carbamoylbenzoic acid (I)

The ligand (I) was synthesized by Benzisothiazole (2.56g) and *meta* isomer of amino benzoic acid (1.94) in a round bottom flask and reflux for 2h in the presence of xylene under optimized conditions. Molar ratio of reacting species was 1:1. Light brown powder, soluble in methanol was obtained. Product was cooled at room temperature, filtered, washed with distilled water (3 × 5mL) and recrystallized using methanol solvent system for further analysis.

Ligand I: Yield: 85%; Melting point: 209-211°C. Purity was monitored by TLC and validated by single spot (R.f. value 0.5cm) in n-hexane: ethyl acetate: methanol; 1:1:0.25 solvent. ^{1}H NMR11.00 (s, 1H, 1×COOH). ^{13}C NMR (125 MHz, d6-DMSO, 298 K): A 120.3, 121.0, 125.9, 126.8, 128.8, 129.1, 130.7, 135.8 (Ar-C) 145.5, 164.7 (CONH), 166.3 (COOH). Anal. calcd: C, 35.17; H, 2.11; N, 5.86; S, 6.71;Found: C, 35.15; H, 2.01; N, 5.81; S, 6.57.

Synthesis of 2,4-Dibromo-5-(2-sulfamoyl-benzoylamino)-benzoic acid(II)

2,4-Dibromo-5-(2-sulfamoyl-benzoylamino)-benzoic acid was synthesized by mixing methanolic solution of 3-(2-sulfamoyl-benzoylamino)-benzoic acid (1g, 3.12mM) with aqueous solution of KBr (1.11g, 9.36mM) and $KBrO_3$ (0.52g, 3.12mM). The reaction mixture was stirred for 2h and dilute solution of hydrochloric acid (HCl) was added drop-wise in the reaction mixture till reddish brown liquid at bottom was appeared. Solvent extraction method using ethyl acetate as solvent was adopted to extract product. Dark brown liquid obtained after the removal of excess organic solvent was dried over anhydrous sodium sulphate to get a desired product.

Synthesis of transition metals complexes

All the transition metal complexes i.e., Zn, Co, Cu and Ni were synthesized by following general procedure (scheme-I). Ligand-III, Na_2CO_3 and metal acetate e.g., zinc acetate dihydrate, cobalt acetate tetra hydrate, nickel acetate tetrahydrate and copper acetate monohydrate in the ratio of 1:1:1 was allowed to react for 2h in acetone (30mL) at 200^{o}C (Mounika *et al*., 2010).

Synthesis of Zinc (II) Diaqua bis-(3-o-sulfamoylphenyl carbamoylbenzoate) (III)

0.17g of zinc acetate dihydrate, 0.25g of ligand-I and 0.08g of Na_2CO_3 in the 1:1:1 ratio was reacted at 200^{o}C. $C_{29}H_{30}N_4O_{11}S_2Zn$: Yield: 93%; M.P: 222-224°C. Anal. calcd: C, 47.06; H, 4.09; N, 7.57; O, 23.78; S, 8.67; Zn, 8.83; Found: C, 47.12; H, 4.02; N, 7.59; S, 8.65.

Synthesis of Cobalt (II) Diaqua bis-(3-o-sulfamoylphenyl carbamoyl benzoate) (IV)

0.25g of ligand, 0.19g of cobalt acetate tetra hydrate and 0.082g of Na_2CO_3 were refluxed for 2hrs at 200^{o}C in 30 mL acetone.

$C_{29}H_{30}CoN_4O_{11}S_2$: Yield: 91%; M.P: 237-239°C. ^{1}H NMR (d6-DMSO, 500 MHz, 298 K): ä 1.57(d, 3H, 1×CH_3) 2.01(s, 4H, 2×NH_2), 4.01 (m, 2H) 7.17–8.11 (m, 14H, Ar 14×CH), 8.48(d, 2H, 2×CH), 9.15 (s, 1H, 1×NH).). ^{13}C NMR (125 MHz, d6-DMSO, 298 K): ä 111.8, 118.7, 120.3, 121.0 (CONH), 124.6, 125.9, 126.8, 128.8, 129.1, 130.7, 131.1, 135.8, 143.8, 145.5 (Ar-C), 164.7 (R-CH_2-N), 171.0 (COOH). Anal. calcd: C, 47.48; H, 4.12; Co, 8.03; N, 7.64; O, 23.99; S, 8.74; Found: C, 47.52; H, 4.18; N, 7.44; S, 8.69.

Synthesis of Nickle (II) Diaqua bis- (3-o-sulfamoyl phenyl carbamoyl benzoate) (V)

0.19g of Nickle acetate tetrahydrate, 0.082g of Na_2CO_3 and 0.25g of ligand 30mL in acetone soln. (30mL) was refluxed at 200^{o}C to synthesize nickel (II) diaqua bis- (3-*o* sulfamoyl phenyl carbamoyl benzoate, complex-V).

$C_{29}H_{30}N_4NiO_{11}S_2$: Yield: 89%; M.P: 235-239°C. ^{1}H NMR (d6-DMSO, 500 MHz, 298 K): ä 1.57(d, 3H, 1×CH_3) 2.01(s, 4H, 2×NH_2), 4.01 (m, 2H) 7.17-8.11 (m, 14H, Ar 14×CH), 8.48(d, 2H, 2×CH), 9.15 (s, 1H, 1×NH).). ^{13}C NMR (125 MHz, d6-DMSO, 298 K): ä 111.8, 118.7, 120.3, 121.0 (CONH), 124.6, 125.9, 126.8, 128.8, 129.1, 130.7, 131.1, 135.8, 143.8, 145.5, 164.7 (NH_2), 171.0 (COOH). Anal. calcd: C, 47.48; H, 4.12; Co, 8.03; N, 7.64; O, 23.99; S, 8.74. Found: C, 47.49; H, 4.10; N, 7.58; aS, 8.70.

Synthesis of Copper (II) diaqua bis- (3-o-sulfamoyl phenyl carbamoyl benzoate) (VI)

For the synthesis of complex-VI, 0.25g of ligand, 0.15g of copper acetate monohydrate and 0.082g of Na_2CO_3 was refluxed at same conditions mentioned above. $C_{29}H_{30}CuN_4O_{11}S_2$: Yield: 89%; M.P: 222-224°C. Anal. calcd:C, 47.18; H, 4.10; Cu, 8.61; N, 7.59; O, 23.84; S, 8.69; Found: C, 47.20; H, 4.09; N, 7.48; S, 8.66.

In-vitro determination of antibacterial activity

Disc diffusion method was used to evaluate the antibacterial activity of synthesized complexes (III-VI),

ligand (I) and derivative (II) using the concentration of 1-2mg/disc. All the synthesized complexes **(**III-VI), ligand (I) and derivative (II) were tested *in vitro* against both pathogenic and non-pathogenic bacterial strains e.g., *S. epidermidis, B. subtilis, S. aureus, E. coli, S. mutans, P. aeruginosa* to determine the minimum inhibitory concentration (MIC). The 0.1mL of culture ($3x10^8$ cells/ mL) compared to McFarland solution as standard was spreaded over Mueller Hinton Agar (Belaid *et al*., 2008). MICs of all synthesized complexes III-VI, ligand (I) and derivative (II) were determined by serially diluted method (Popiołek and Biernasiuk, 2017). Commercial antibiotics such as ciprofloxacin, and tetracycline were used as positive control.

In vitro determination of anthelmintic activity

Mature parasites of sheep like hook worms, thread worm, tape worm and liver fluke were elected for *in vitro* determination of anthelmintic activity of Complexes III-VI. The mature worms were screened from afresh scarified sheep (*Ovis aries* L) in the local slaughter house, of G. M. Abad and identified by Dept. of Pharmacology, University of Agriculture, Faisalabad. After washing and suspending in 0.9% phosphate buffer saline (PBS), parasites were kept in Hank's solution at 37 °C (Singotam *et al*., 2013). Three concentrations e.g., 15, 30 and 45mg mL^{-1} of complexes III-VI in 1% dimethyl sulphoxide (DMSO) were used against worms. A set of worms (n=4) in each category i.e., keeping without complexes in Hank's solution and 1% DMSO served as negative controls whereas Piperazine citrate ($15mgmL^{-1}$) as standard (Caballero *et al*., 2002). The anthelmintic value of complexes III-VI was adjudicated in terms of the time required for paralysis (when all signs of movements had ceased) of the test worms to death (Martin, 1985).

STATISTICAL ANALYSIS

All values are expressed as ±mean. Differences between means were determined one way analysis of variance (ANOVA) following Dunnet's test.

RESULTS

Synthesis and characterization

The primary signs for successful synthesis of the complexes III-VI were the solubility, physical states, and an obvious difference in melting points (M.P.) of ligands and complexes as the M.P. of ligand-III was in the range of 209-211°C while the M.P. of all complexes were < 211 °C. The percentage yields of these complexes were 83-91%

Spectroscopic studies

FT-IR spectra

FTIR is a valuable technique to study the binding mode of ligand (I) and transition metals in the complexes. Specific peaks in FT-IR spectrum, of ligand (I) and transition metal complexes confirm successful synthesis (fig. 1).

NMR spectra

After preliminary characterization with FTIR, successful synthesis of complexes were confirmed by NMR (fig. 2).^{1}H NMR spectrum was taken in deuterated dimethyl sulfoxide (DMSO), the protons for the two types of N-H groups clearly appear at 9.15ppm and 4ppm respectively. The difference in signal of NH group is due to difference of their substituents. signal at 4ppm, NH is attached to those carbon which have electron donating group (CH_3) that's why its signal is in up-field region while signal at 9.15ppm is due to electron withdrawing group (C=O) that's why its signal is in downfield region. Signal from 1-2ppm is due to methyl group. While signal at 2ppm is due to amide (NH_2). The remaining protons of aromatic ring appear in the range of 7-8ppm, as usual. These results further support to the mode of bonding discussed in FTIR spectra. Numbers of protons obtained from NH & CH analysis and calculated from integration curves are in accordance with each other. In ^{13}C NMR spectrum highly down-fielded signal is due to C=O at 170ppm while methyl at 17 ppm. Rest of the aromatic carbon atoms appear in the range of 120-145 ppm.

Antimicrobial activity

Biological screening of synthesized transition complexes with ligand were tested *in vitro* against gram+ve *S. epidermidis, B. subtilis, S. aureus* and gram-ve *E. coli, S. mutans, P. aeruginosa* by disc diffusion method. The values of zone of inhibitions and MIC are summated in table 1.

In vitro anthelmintic activity

A result depicted in table II illustrates the effects of complexes III-VI on paralysis time of test worms. The worms paralyzed between 85-91 min after incubation in the -ve control medium. Piperazine treated worms exhibited physical activity for 13.56–18.22 min. The complexes III-VI used in 15, 30 and $45mgmL^{-1}$ concentrations paralyzed all the worms used between 13-26 min, 9-18 min and 3-21 min respectively.

DISCUSSION

The differences in M.P. of ligand (I) and designed complexes indicated the successful synthesis (Kamal *et al.*, 2019). Furthermore, solubility of complexes in non-polar solvents e.g., diethyl ether, dichloromethane, chloroform and n-hexane compared to the ligand-III which remained soluble in polar solvents like water methanol, ethanol and remain stable in the presence of air or moisture (Habib *et al.*, 2019) indicated successful synthesis of complexes (Barrett *et al*., 2001).

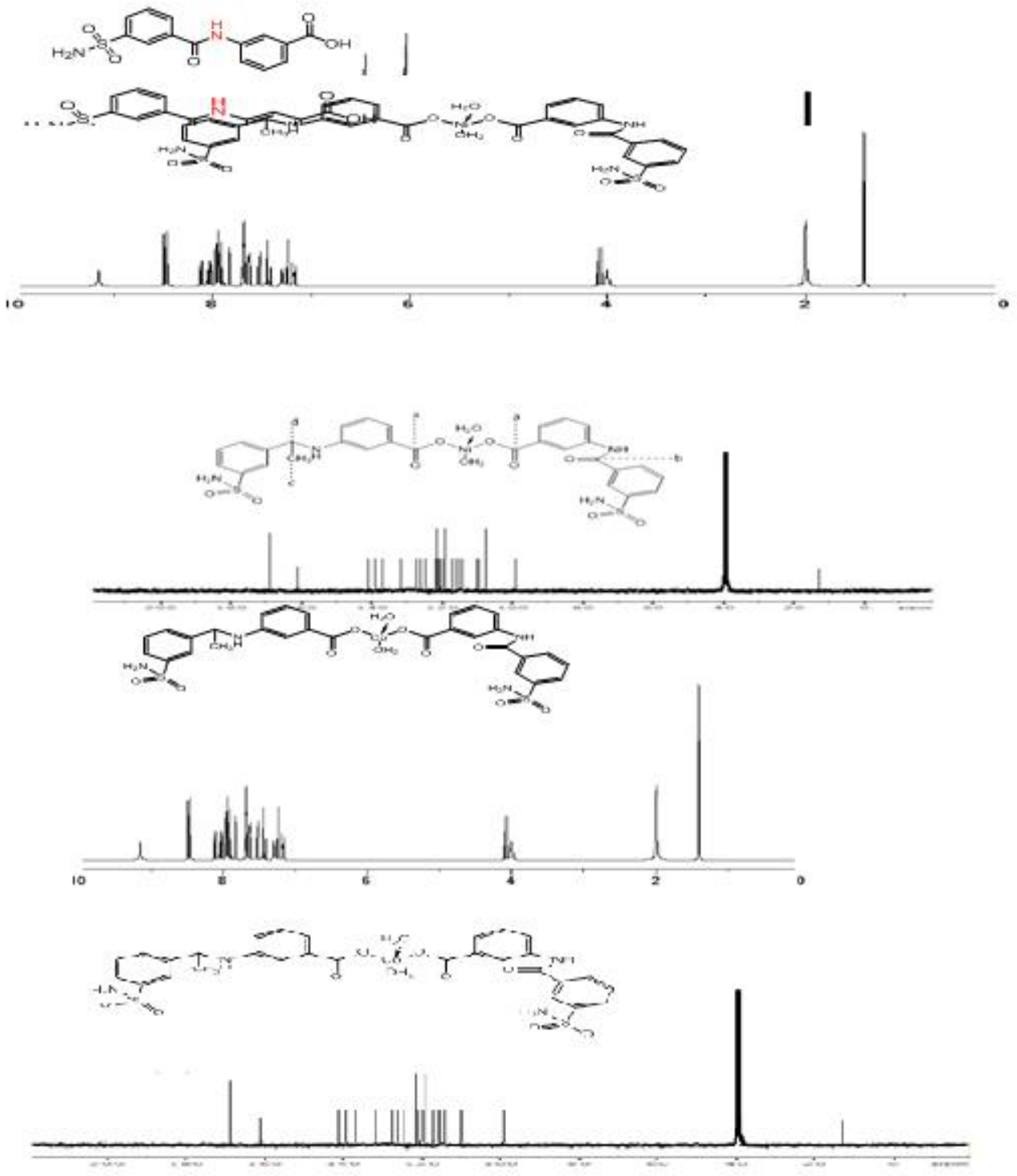


Fig. 1: ^{1}H and ^{13}C NMR of Ligand (I), IV and V

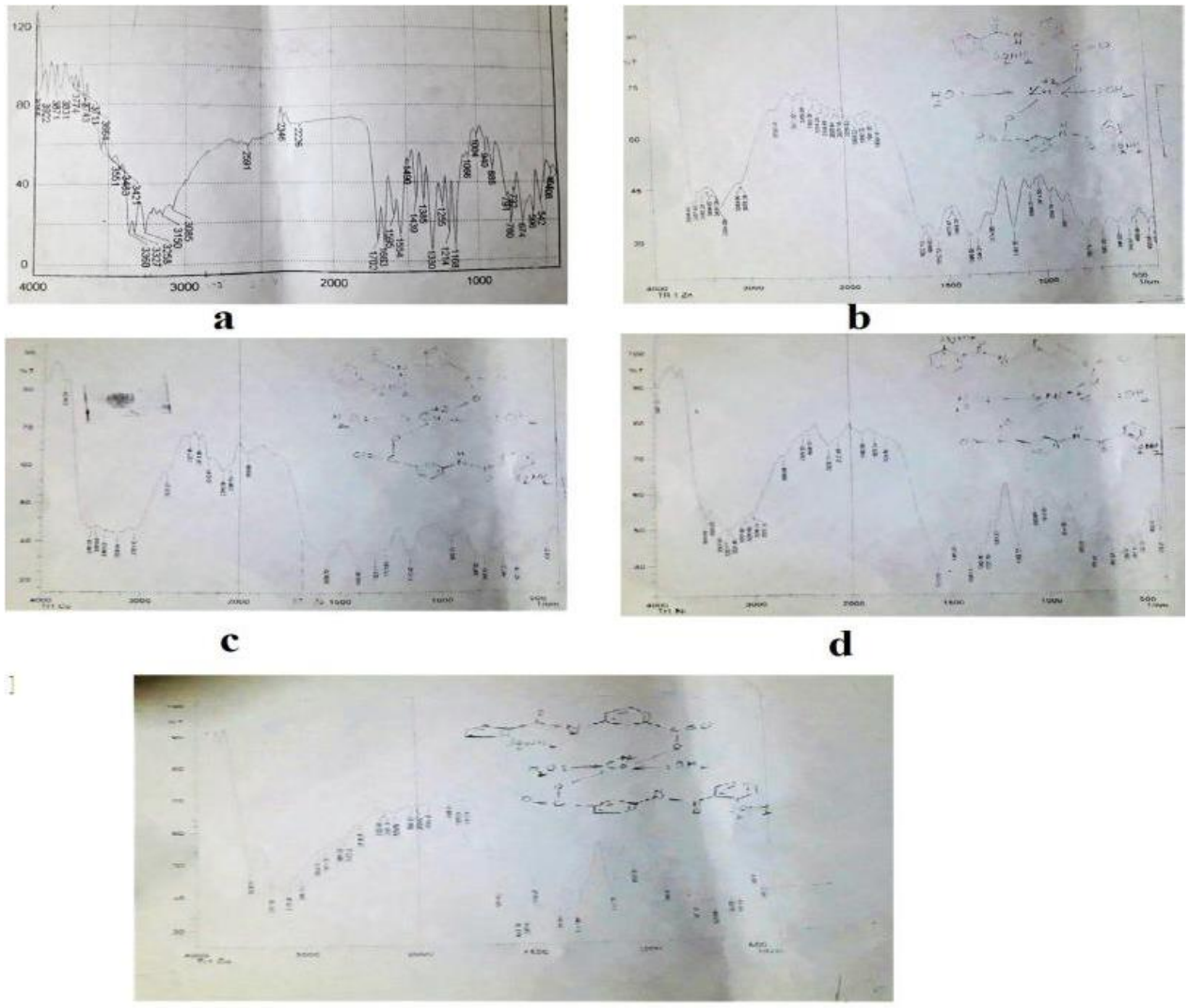
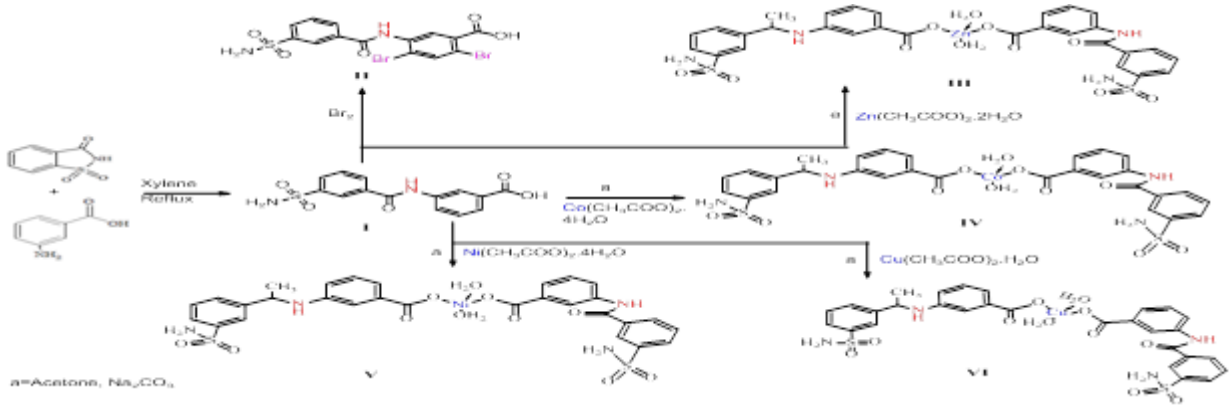


Fig. 2: FT-IR Spectra a) Ligand (I) b,c,d and e) Zn, Cu, Ni, Co metal complexes



Scheme 1: Synthesis of Co, Ni, Cu and Zn

Table 1: The antibacterial activity shown by ligand-III and its transition metal complexes

Complex 1-2mg/disc	*S. aureus*		*S. epidermidis*		*B. subtilis*		*E. coli*		*S. mutans*		*P. aeruginosa*	
	Inhibition Zone (mm)	*MIC (mg/mL)*	*Inhibition Zone (mm)*	*MIC (mg/mL)*	*Inhibition Zone (mm)*	*MIC (mg/mL)*	*Inhibition Zone (mm)*	*MIC (mg/mL)*	*Inhibition Zone (mm)*	*MIC (mg/mL)*	*Inhibition Zone (mm)*	*MIC (mg/mL)*
Ligand (I)	15±1.50	2.3±1.50	18±1.00	2.0±0.05	16±1.00	20.0±1.00	15±1.00	10.0±1.00	nd**	nd**	nd**	nd**
Derivative (II)	nd**	nd**	15±1.00	8.0±1.00	19±0.50	13.0±0.05	15±1.00	12±0.05	17±1.00	8.00±0.05	nd**	nd**
III	19±1.00	1.5±0.05	25±1.50	3.0±0.05	21±0.08	5.00±0.01	26±1.00	3.00±1.50	15±1.00	20.0±1.00	11±1.00	15.0±0.03
IV	20±0.08	1.5±1.00	21±1.08	1.0±1.00	18±1.08	10.0±0.00	25±1.050	3.00±0.05	15±1.00	15.0±0.05	21±1.00	2.00±1.00
V	14±0.02	5.0±1.00	15±1.00	8.0±1.00	14±1.50	8.0±0.05	18±1.00	11.0±1.50	17±1.00	11.0±1.00	nd**	nd**
VI	16±1.98	2.5±0.7	15±1.00	8.0±1.00	11±0.05	15.0±0.05	18±1.00	17.0±1.7	14±1.00	18.0±1.50	nd**	nd**
Ciprofloxacin	20±1.08	14±0.00	23±1.00	11±1.00	19±1.00	19±2.50	18±1.00	11±0.05	18±1.00	13±1.00	18±1.00	21±1.50

*Inactive= inhibition zone < 6mm; Slightly active = inhibition zone 7-9mm; Moderately active = inhibition zone 10-13mm; Hightly active=‘ inhibition zone>14 mm
**nd = Not determined

IR spectra of ligand-I show characteristic N-H stretching frequencies at 3258cm^{-1} and 3327-3360 cm^{-1} for carboxamide and sulfonamide functional groups, respectively. The carbonyl peaks are found at 1702 cm^{-1} and 1663 cm^{-1} for the two carboxamide and carboxylic acid stretching frequencies in the same order. Peak of carbonyl was observed at 1702 cm^{-1} by many researcher whose complexes have this group (Haque *et al.*, 2018; Habib *et al.*, 2019). The N-H deformations are evident in the range 1554-1595 cm^{-1}. Typical sulfonyl stretches (O=S=O) appear at 1330 cm^{-1} and 1168 cm^{-1}. The IR spectral data showed that two types of carbonyl stretching frequencies appeared as a broad peak in the range 1608-1622 cm^{-1} far over than these were in the ligand-**I**. A shift in the carbonyl stretching frequencies towards lower side is due to the involvement of oxygen atom of C-O with metal ion e.g, 433 (Zn-O), 455(Co-O), 532-565 (Ni-O), 442 (Cu-O) during synthesis of transition metal complexes (Lam *et al.*, 2003). Thus FTIR spectra in the region of 433, 455, 565, 442 provides the strong evidence of complexion of transition metals with ligand-I.

The comparative antibacterial activity of ligand (I) and transition complexes III-VI indicated that complexes exhibited excellent potential against both gram+ve and gram-ve bacterial strains whereas, all were found moderately to inactive against *P. aeruginosa*. The enhanced antibacterial activity of transition metal complexes III-VI than ligand (I) and derivative (II) may be due to size of metal ions, fitness of particle, high solubility, size of metal ions and presence of bulkier organic group (ligand-I). Enhanced activity could also be explained on the basis of overtone's concept that increased lipophilicity due to long p value had given the high capability to penetrate resulting in enhanced antimicrobial activity (Ramzi *et al*., 2010; Kiran *et al*., 2015). Generally it was also noticed that nickel containing complexes are more effective against gram -ve bacteria than other complexes. Our findings were in accordance with the findings of Mottaleb and Ismail, (2019). They reported that copper and nickel complexes had greater impact on *E. coli* and *P. aeruginosa* (gram -ve pathogenic bacteria) tLipophilic nature of Ni(II) and Cu(II) complexes facilitated their diffusion into bacterial cell thereby inhibiting their growths (Sripathi *et al*., 2019).

The results of *in vitro* anthelmintic activity suggest the pharmacological basis of III-VI complexes for the treatment of worm infestations comparable to standard drug “Piperazine citrate” which causes expulsion of the worm by peristalsis due to expulsion of the worm by peristalsis. Piperazine citrate results in flaccid paralysis by reducing excitability and muscle to relaxation by hyperpolarization of chloride ion (Yadav *et al*., 2011). The wormicidal effect of complexes III-VI suggests its potential against parasitic infections. Further, in future it is necessary to identify mechanism of action for the

Table 2: Anthelmintic activity of Complexes III-VI

Sr. No.	Name of worms	-Ve control	+Ve Control 15mg/mL	Conc. (mg/mL)	Time required for complete paralysis (min) Complexes			
					III	IV	V	VI
1	Liver fluke	84.76±1.02	18.22±0.83*	15	20.22±0.28*	16.80±0.90*	21.25±1.85*	15.10±0.30*
				30	17.31±1.20*	13.20±1.20*	20.18±1.20*	9.51±0.72*
				45	8.45±0.80*	9.80±0.50*	18.38±0.80*	3.48±0.50*
2	Tape worm	87.89±2.22	18.05±0.98*	15	18.48±0.50*	15.35±0.83*	18.45±0.98*	18.02±2.20*
				30	11.57±0.2*	12.20±0.75*	15.220.90*	16.15±0.20*
				45	10.18±0.96*	7.45±0.02*	13.25±1.45*	11.35±1.56*
3	Thread Worm	90.65±0.86	14.58±0.84*	15	26.48±0.82*	20.44±0.58*	12.55±0.72*	15.22±0.63*
				30	25.46±0.95*	18.05±0.64*	11.16±0.05*	13.56±0.10*
				45	21.25±070*	13.26±0.98*	11.031.02*	12.33±0.92*
4	Hook worm	84.68±2.56	13.56±0.63*	15	20.44±0.28*	17.54±0.55*	16.38±1.56*	13.42±0.96*
				30	18.56±2.50*	14.15±0.61*	11.48±1.80*	12.20±2.26*
				45	14.30±1.87*	13.55±1.02*	6.03±0.95*	9.50±1.87*

*p<0.001 vs. control

anthelmintic activity and study its pharmacological actions *in vivo*.

CONCLUSION

In the present study 3-(*o*-Sulfamoylphenyl) carbamoyl benzoic acid was used as ligand and their respective different metal complexes; Zinc (II), Cobalt (II), Copper (II) and nickel (II) were synthesized and characterized by FTIR, ^{1}H NMR and ^{13}CNMR. Presence of specific peaks (Co-O, Ni-O, Zn-O and Cu-O) confirmed the successful synthesis of desired complexes. All synthesized complexes, ligand (I) and derivative (II) were evaluated against different pathogenic and non-pathogenic bacterial strains. Result revealed that most of them exhibited strong antibacterial activity. From which complexes (III-VI) were moderately to highly active against all tested bacterial strains like *S. epidermidis, B. subtilis. S. aureus, E. coli* and *S. mutans*. However, all were found moderately to inactive against *P. aeruginosa.* The wormicidal activity of complexes III-VI recommends them effective against parasitic infections.

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