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MICROBIAL INVESTIGATION OF SILVER COMPLEXES

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ABSTRACT

1,2,4-Triazole-3-Thiol Compounds Were Prepared By Thiocarbohydrazide And Hydrazine Hydrate Under Acidic Condition, and The Collected Product Called 4-Amino-5-Hydrazinyl-4*h*-1,2,4-Triazole-3-Thiol and Added aromatic aldehyde, Then The 4-Amino-5-Hydrazinyl-4*h*-1,2,4-Triazole-3-Thiol Schiff Base Ligand Were Obtained By The Condensation Process. The Silver Complex of Schiff Base Ligand was Also Prepared and Characterized By elemental analysis Infra-Red and ^{1}HNMR.

KEYWORDS: 1, 2, 4-Triazole-3-Thiol, Schiff Base, Thiocarbohydrazide, aromatic aldehyde, Antimicrobial Activity.

1. INTRODUCTION

In 1864 German chemist Schiff Hugo Josef discovered Schiff bases by condensation of aldehydes and amines. The compound represented by the general formula $R_3R_2C=NR_1$ is known as azomethines or Schiff bases,[1,2–8] where R_2 and R_3 may be alkyl or aryl groups. For hundreds of years, the physical and chemical properties of Schiff bases are continuously studying[9] In modern inorganic chemistry, Schiff base metal complexes had an important position. Numerous applications are receiving considerable interest in the field of bioinorganic chemistry.[10-12] The Schiff bases are well-known as fungicides because of their biological activity. The pharmacological effects of Schiff bases, including their anti-inflammatory,[13] antibiotic,[14] and antimicrobial[15] properties, have been extensively explored. Among the Schiff bases containing triazole, the triazole moiety is the most active physiologically, and its activity increases with complexity. Numerous Schiff base complexes are quite useful in pharmacological and biological applications.[16,17] Anticancer and antitumor action has been demonstrated for metal complexes containing sulfur-containing ligands.[18] The present article discusses the synthesis and characterization of silver complexes of 1,2,4-triazole Schiff bases and their microbiological research.

2. MATERIALS AND METHODS

This part includes the details about the chemicals used, the purification of the solvents, synthesis of the ligand and complexes, analytical procedures, and instrumental techniques employed. All chemicals and solvents used were of the highest analytical grade and purity. Purchases from the retailer shop included: hydrazine hydrate, concentrated HCl, salicylaldehyde, chlorobenzaldehyde, meta-nitrobenzaldehyde, Vetraldehyde, ethanol, methanol, and dimethyl sulfoxide.

2.1 Physical measurements

The synthesis was performed on a Scientific Microwave Synthesizer Model: CTA-2R with a 32-liter capacity, a maximum output power of 850W, and a microwave frequency of 2450MHz. Through the use of TLC, we were able to determine when the reaction was complete. In order to determine the melting point, a digital melting point instrument was used. The elements C, H, and N of the produced compounds were determined using an elemental analyzer from Perkin Elmer. It was conducted at Bheemana Khandre Institute of Technology in Bhalki to conduct infrared investigations of the Schiff bases using KBr pellets in an FT-IR spectrometer by Perkin Elmer between 350 and 4000 cm-1 using KBr pellets. At SAIF IIT Cochin, 1HNMR spectra in DMSO were acquired using an NMR spectrophotometer with a 500 MHz FT NMR spectrometer frequency. It was determined that the diameter of Inhibition Zones in millimeters (mm) was obtained from the Bio-Genetics, Research and Training Centre in Biotechnology, Hubli, Karnataka, as the antibacterial and antifungal properties of synthesized compounds.

2.2 Microwave-assisted Synthesis of thiocarbohydrazide

Hydrazinolysis of CS2 is the easiest and cheapest method of synthesis of thiocarbohydrazide. It can be prepared by taking 44 ml of hydrazine hydrate and 150 ml of distilled water in a 500 ml beaker followed by a slow and dropwise addition of 12 ml of carbon disulfide over a magnetic stirrer for one hour.[19,20] The mixture was then put into a round-bottomed flask and irradiated with a microwave for 20 minutes at 595W (P-7) and 80-90 0C temperature for 20 minutes. Thin-layer chromatography was used to assess the progress and purity of the reaction carried out with silica gel (TLC). Following the completion of the reaction, the contents of the flask were allowed to cool down completely. Thin-layer chromatography was used to assess the progress and purity of the reaction carried out with silica gel (TLC). Following the completion of the reaction, the contents of the flask were allowed to cool down completely. The white material that was isolated was filtered and recrystallized using distilled water as a starting point.

2.3 Microwave-assisted Synthesis of 4-amino-5-hydrazinyl-4H-1,2,4-triazole-3-thiol

A mixture of thiocarbohydrazide (7 g) and 2 ml of Hydrazinehydrate was subjected to microwave irradiation at an interval of 2 mints for approximately 15 minutes at 425W (P-7) 90 0C for 2 mints. Within 10 minutes of irradiation, the solid started separating from the clear solution. The triazoles thus obtained were recrystallized from distilled water into shining greenish-yellow crystals. The Melting point of this tally well with the reported ones.[21,22]

2.4 Microwave-assisted Synthesis of 1,2,4-triazole Schiff bases

Mixing the equimolar ethanolic solutions of 4-amino-5-hydrazinyl-4H-1,2,4,triazole-3-thiol, and acetaldehyde with a few drops of glacial acetic acid, the 1,2,4-triazole Schiff base was produced. The reaction mixture was then heated in a microwave irradiation chamber at 595W for approximately 8-10 minutes, with 2-minute intervals between each heating cycle. A second step involved transferring the boiling solution into ice-cold distilled water and thoroughly mixing it. Solids were isolated and recrystallized from ethanol after they had been filtered out. Anhydrous CaCl2 was used to dry the product in desiccators. The synthesized ligands were stored in bottles that were yellowish-brown in color and maintained in a dark place.

2.5 Synthesis of 1,2,4-triazole Schiff bases silver complexes

Preparation of the 1,2,4-triazole Schiff bases silver complexes was accomplished by taking appropriate molar quantities of the 1,2,4-triazole Schiff bases ligand and the silver nitrate and following the process outlined below. The hot Ethanolic solution of the 1,2,4-triazole Schiff bases ligands was progressively added to the hot Ethanolic solution of the 2,4-triazole Schiff bases ligands while constantly stirring.[23] The resulting mixture was then refluxed for several minutes at 70 – 80 0C in the Microwave irradiation chamber, after which it was allowed to cool to room temperature. The resulting solid is filtered and dried before being used.

2.6 Detection of Silver in synthesized complexes

About 1 gram of each complex was dissolved in dilute nitric acid and boiled to expel brown fumes. It was then diluted with distilled water, heated & dil. HCl was added slowly with constant stirring until precipitation was complete. The appearance of precipitate confirmed the presence of silver in complexes.[24]

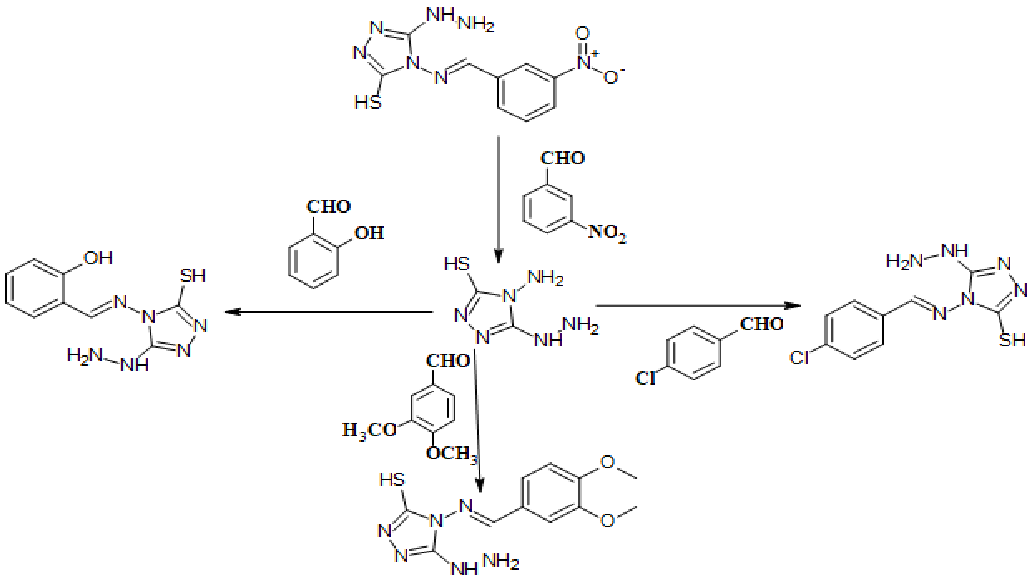
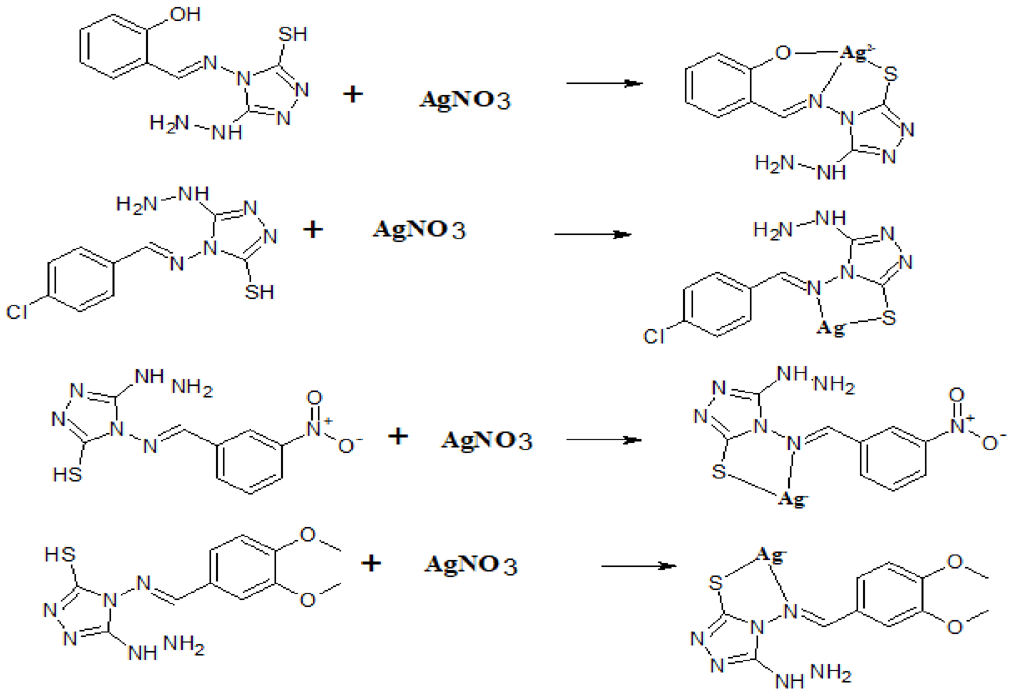


Fig 2.1: Synthesis of 4-amino-5-hydrazinyl-4H-1,2,4-triazole-3-thiol Schiff base Ligand.



2.2 Synthesis of 4-amino-5-hydrazinyl-4H-1,2,4-triazole-3-thiol Schiff base Silver complexes.

Table 2.1: Synthesis data of Mononuclear1,2,4-triazole Schiff base.

Ligand	**Color**	**Time of Refluxed**	**Yield (%)**	**Melting point (°C)**
2-{(*E*)-[(3-hydrazinyl-5-sulfanyl-4*H*-1,2,4-triazol-4-yl)imino]methyl}phenol	yellow	20 mints	72	201
(*E*)-4-((4-chlorobenzylidene)amino)-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol	Pale yellow	20 mints	78	167
5-hydrazinyl-4-{(*E*)-[(3-nitrophenyl) methylidene]amino}-4*H*-1,2,4-triazole-3-thiol	Light orange	20 mints	71	169
4-{(*E*)-[(3,4-dimethoxyphenyl) methylidene]amino}-5-hydrazinyl-4*H*-1,2,4-triazole-3-thiol	Creamy white	20 mints	68	165

3. Biological evaluation

3.1 Antimicrobial activity

Because the coordination complexes have interesting antibacterial activities, the compounds synthesized in this study were screened for antibacterial activity. Using the Paper disc process, the ligand and its complexes were examined against pathogenic bacteria.

3.2 Materials required

- Nutrient agar”
- Sterilized Petri dishes and micropipettes”.
- 20-24 hours old sub-cultures in nutrient agar medium”.
- Sterilized test tube containing a solution of the test compounds in the desired concentration”.

3.3 Preparation of agar plate’s

“For the growth of specific bacterial species, minimal agar was used. Mac Conkey agar (40 g) obtained from Merck was suspended in 1L of freshly distilled water to prepare agar plates for bacteria. After soaking for 15 minutes, it was boiled on a water bath until the agar was completely dissolved. The mixture was autoclaved at 120°C for 15 minutes before being placed in previously washed and sterilized Petri dishes and stored at 40°C for incubation”.

3.4 Procedure for inoculation

“Inoculation was carried out using a platinum wire loop that was heated in a flame, cooled, and then used to apply bacterial strains”.

3.5 Preparation of discs

“A micropipette was used to apply 5 mg of the synthesized compounds in acetone to paper discs. The discs were placed in an incubator at 37 °C for 48 hours before being applied to bacteria grown on agar plates“.[25]

3.6 Application of discs

“Sterilized forceps were used to apply the paper discs to the previously inoculated agar plates. The discs were applied and incubated at 37°C for 24 hours. The zone of inhibition (diameter in mm) around the discs was then measured”.

4. RESULTS AND DISCUSSION

4.1 Elemental analysis

The elemental analysis shows that the Ligand and Silver complexes have 1:1 stoichiometry of the type ML. The analytical data of the complexes are also in good agreement.

Table 4.1: Elemental Analysis Data of 1,2,4 triazole Schiff base Ligand and Its Silver complexes.

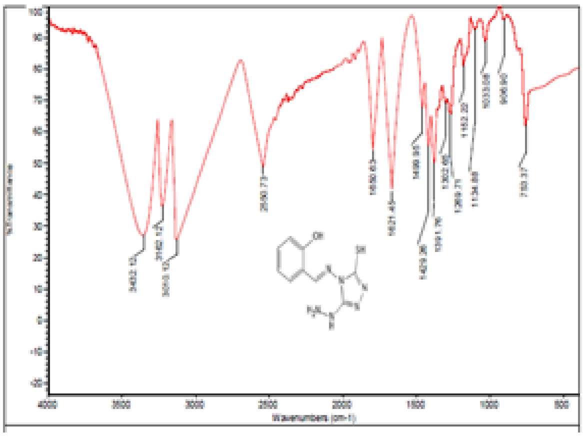
S. no.	Ligands	Molecular formula	% of elements Found (Calculated)						
			C	H	N	S	O	Cl	Ag
1	L_{13}	$C_9H_{10}N_6OS$	43.19 43.00)	4.03 (4.01)	33.58 31.70)	12.81 12.70)	6.39 (6.70)	----	----
2	C_{13}	$C_9H_8Ag_2N_6OS$	23.30 (23.17)	1.74 (1.95)	18.11 (18.03)	6.91 (7.01)	3.45 (3.39)	----	46.49 (45.93)
3	L_{14}	$C_9H_9ClN_6S$	40.23 (39.00)	3.38 (3.81)	31.27 (31.72)	11.93 (12.70)	----	13.19; (12.89)	----
4	C_{14}	$C_9H_8AgClN_6S$	28.78 (28.69)	2.15 (2.31)	22.38 (22.41)	8.54 (8.69)	----	9.44 (9.81)	28.72 (28.79)
5	L_{15}	$C_9H_9N_7O_2S$	38.71 (38.07)	3.25; (3.11)	35.11; (34.70)	11.48 (11.43)	11.46 (11.20)	----	----
6	C_{15}	$C_9H_8AgN_7O_2S$	27.99 (28.09)	2.09 (2.13)	25.39 (25.51)	8.30 (8.19)	8.29 (8.42)	----	27.94 (28.97)
7	L_{16}	$C_{11}H_{14}N_6O_2S$	44.89 43.33)	4.79 (5.01)	28.55 (28.70)	10.89 (10.57)	10.87 (10.48)	----	----
8	C_{16}	$C_{11}H_{13}AgN_6O_2S$	32.93 (32.81)	3.27 (3.53)	20.95 (20.11)	7.99 (8.07)	7.98 (8.89)	----	26.89 (27.01)

4.2 Infrared spectra

This type of spectral information is extremely important in understanding the nature of the functional group linked to the metal atom.[26] On the other hand, the 1,2,4-triazole Schiff base ligands and their respective Silver complexes exhibit notable I.R. frequencies, summarised in Table 4.12 for our convenience. The comparative infrared spectral study of the complexes with free ligands gives information regarding the ligand molecules' chelation sites. The important I.R. spectral bands for the synthesized ligands exhibit high-intensity bands in the range of 1615-1639 cm^{-1}is due to ν(HC=N), and the band appeared around 3432 cm^{-1}due to the presence of phenolic -O.H. In the complexes, the low-frequency shift (35-40 cm-1) was observed around 1574-1589 cm-1due to a decrease in electron density of an azomethine group.[27] The band around 3432 cm-1 due to phenolic O.H., which is observed in ligand but disappears in complexes. This indicates that the ligand coordinate to the Metal ion through a phenolic oxygen atom (via deprotonation).[28] Further confirmation of the coordination of the ligand through the nitrogen of azomethine and the oxygen of ν(C-O) is the emergence of non-ligand bands around 416-499 cm-1 and 456-516 cm-1, which are due to M-N and M-O bonds, respectively, at 456-516 cm-1.[29,30]

Table 4.12 IR data Spectral data of 1,2,4-triazole Schiff base Ligand silver complexes.

S. no.	**Ligand**	**ν-OH**	**νAromatic proton**	**ν S.H.**	**ν CH=N (Azomethine)**
1	L_{13}	3432	3010	2550	1621
2	C_{13}	Disappeared	3130	Disappeared	1580
3	L_{14}	----	3050	2557	1631
4	C_{14}	-----	3115	Disappeared	1584
5	L_{15}	----	3049	2560	1639
6	C_{15}	-----	3212	Disappeared	1574
7	L_{16}	----	3020	2523	1615
8	C_{16}	-----	3135	Disappeared	1589



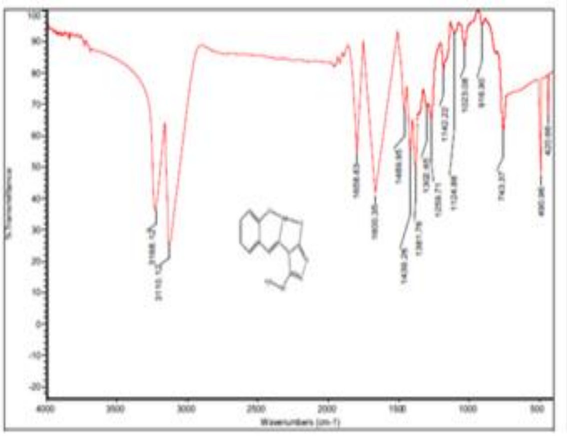


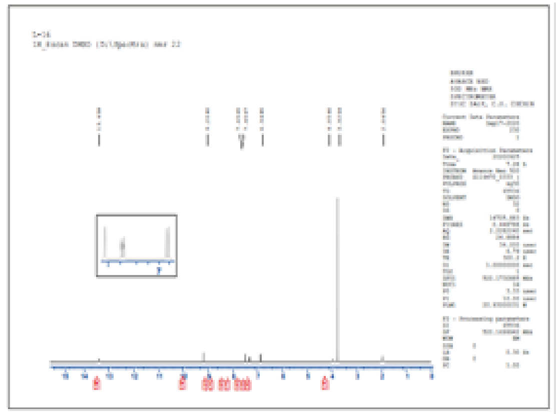
Figure 4.11: IR spectrum of Ligand 13 Figure 4.12: IR Spectrum of Complex C13

4.3 1H-NMR Studies

The 1H NMR spectra confirmed the bonding approach of the ligands. The 1H-NMR spectra of 1,2,4-triazole Schiff base ligand were recorded in DMSO-d6 at room temperature. Three different types of protons were identified i) resonance exhibits due to phenolic –O.H. protons around 10.54 ppm, ii) characteristic resonance due to azomethine proton in the Schiff base appears at 9.20-9.99.66 ppm was observed, and iii) the other signals in the region 6.95–7.89 ppm exhibits due to aromatic protons.[31] All these observations support the infrared conclusions.

Table 4.13: ^{1}HNMR Spectral data of 1,2,4-triazole schiff base ligand silver complexes.

Ligand/complex	Aromatic Hydroxyl Proton	SH	HC=N
L_9	10.54	13.56	9.26
C_9	Disappear	Disappear	8.30
L_{10}	----	13.62	9.99
C_{10}	----	Disappear	7.98
L_{11}	----	13.69	9.35
C_{11}	----	Disappear	8.67
L_{12}	----	13.45	9.20
C_{12}	----	Disappear	8.67



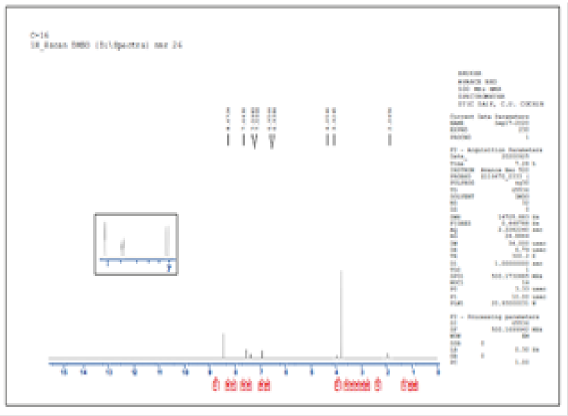


Fig. 4.13: 1HNMR spectrum of Ligand 16 Fig. 4.14: 1HNMR spectrum of Complex 16.

4.4 Antimicrobial activity

The agar well diffusion method was used to evaluate the antibacterial activity of the examined compounds against S. aureus and E. coli in vitro. Table 4.14 summarises the minimal inhibitory concentration values of the chemicals against the growth of micro-organisms and their minimum inhibitory concentration values. A comparison of the antimicrobial activity of the ligands and their silver complexes revealed that all of the complexes had either higher or lower antibacterial activity than the free ligand. Following these findings, it was shown that a majority of the complexes were more active than their 1,2,4-triazole Schiff base ligands. The antibacterial activity is either increased or decreased depending on how the chelation is carried out. It can also be neutral at times. As a result, metal chelation can either boost or diminish the therapeutic value of synthetic medicines. Because of chelation, it may be possible to keep the property intact by further stabilizing and decreasing the biodegradability/metabolic decay of the produced 1,2,4-triazole Schiff base ligand. Multidentate Schiff base ligands exhibit a better antibacterial efficacy than monodentate Schiff base ligands due to the chelate effect. So the produced ligands with their SNO donor atoms have only moderate antibacterial activity, whereas the silver complexes have significantly higher activity than the 1,2,4-triazole Schiff base ligand.

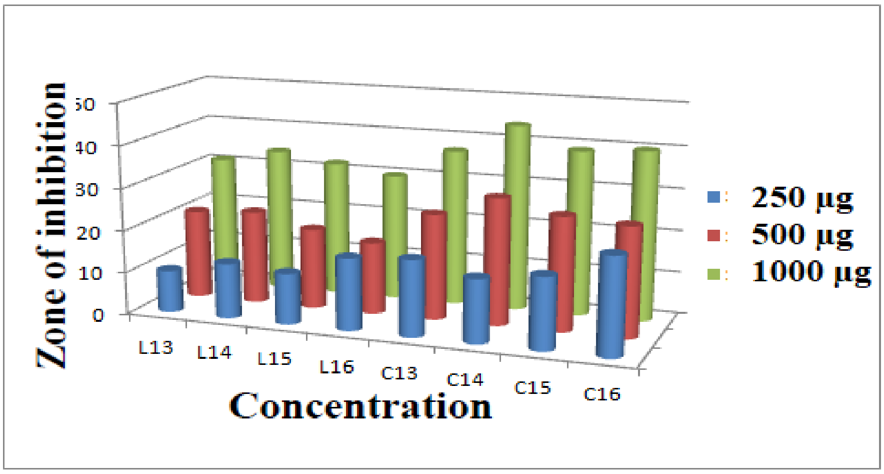


Figure 4.15: Antibacterial activities against S. Aureus.

Table 4.14: Antibacterial Activity of 1,2,4-triazole Schiff base ligand and its silver complexes against S. aureus and E. Coli.

Sample	S.aureus			E.Coli		
	250	500	1000	250	500	1000
L_{13}	10	21	31	9	16	22
L_{14}	13	22	34	10	18	25
L_{15}	12	19	32	11	17	26
L_{16}	17	17	30	14	12	25
C_{13}	18	25	37	11	19	25
C_{14}	15	30	44	12	23	30
C_{15}	17	27	39	15	22	29
C_{16}	23	26	40	13	19	31
Ciprofloxacin	34	36	*	34	36	*

5. CONCLUSION

The silver complexes containing a 1, 2, 4-triazole Schiff base ligand generated from an aromatic aldehyde were produced and studied during this investigation. A flexible tridentate ligand, the Schiff bases play an important role. It was proposed that the complexes be built in a bidentate fashion using a variety of ways. Azomethine nitrogen, phenolic oxygen, and thiol sulfur atoms are all detected in the FTIR spectrum, which indicates that the coordination is taking place. The production of the 1,2,4-triazole Schiff base ligand has been confirmed by 1HNMR spectra, confirming the ligand's creation. According to the results of the biological assay, the complexes exhibit more activity than the free ligand.

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