Synthesis and Evaluation the Activity of 1, 3, 4-Thiadiazole Derivatives as Antibacterial Agent Against Common Pathogenic Bacteria

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Abstract

Schiff base compound 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol was prepared from condensation reaction of 2-amino-5-mercapto-1,3,4-thiadiazole with benzaldehyde. Schiff bases react with Copper(II), Ferric(III), Cobalt(II) and Zinc(II) to form four complexes. The Schiff base complexes were identification by using FTIR and UV-VIS. The antibacterial activity of complexes (Copper(II), Ferric(III), Cobalt(II) and Zinc(II)) were studied against *S. aureus* and *S . epidermidis* as a model of Gram positive, *E. coli*, *P. mirabilis*, *C. freundii* and *P. aeruginosa* as a Gram negative model to determine activity of synthesized complexes, after subjected them to some tests to confirm the identity of the pathogenic bacteria. Ten antibiotics (Ampicillin and Amoxicillin) have been chosen to investigate the ability of bacterial isolates to resistant the conventional antibiotic. Imipenem have been selected to contrast its efficiency with those of the new compounds because of its high efficiency. The results exhibited higher activity of the new compounds proportional to the chosen antibiotics. [DOI: 10.22401/ANJS.22.1.04]

Keywords: Synthesis, 1, 3, 4-thiadiazole, antibiotic, antibacterial.

Introduction

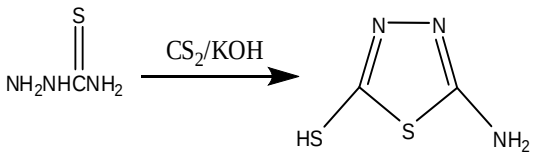
Thiadiazoles was five membered aromatic ring compounds with three hetero atoms one sulfur atom and two nitrogen atoms[1].There were four isomeric types of thiadiazoles[2]: (a)1,2,3-thiadiazole; (b)1,3,4-thiadiazole; (c) 1,2,4-thiadiazole; and (d) 1,2,5-thiadiazole. The most thermally stable isomer were 1,3,4-thiadiazole and its stability were controlled in general by the electron density at the C_2 and C_5 atoms which was largely dependent on the substituents. The stability of 1, 3, 4-thiadiazole is especially enhanced by alkyl and aryl substituents on positions 2 and 5. Thiadiazoles like all other compounds containing (-NH-CH=X) moiety (X=N, O and S) exist in two tautomeric forms [3]. 1,3,4-thiadiazol ring system shown antifungal, bacteriostatic and containing compounds perform substantial class of heterocyclic nitrogen compounds and their derivatives were recognized with a broad spectrum of biological activity in both agrochemical[4] as well as anthelmintic effects.

The growing numbers of antimicrobial-resistant pathogens place a significant burden on healthcare systems and have important global economic costs. Resistance was realized as bacteria that are not inhibited by commonly achievable systemic concentration of an agent with ordinary dosage schedule; multiple drug resistance was acquainted as the resistance to two or more drugs or drug portion [5]. Both Schiff bases and azo compounds were important structures in the medicinal and pharmaceutical fields and it had been proposed that the azomethine linkage might be responsible for biological activities displayed by Schiff bases [6]. Hence the current work was the need for the development of novel antimicrobial agents to combat the bacterial infections [7].

Experimental

A. Preparation of 2-amino-5-mercapto-1,3,4-thiadiazole

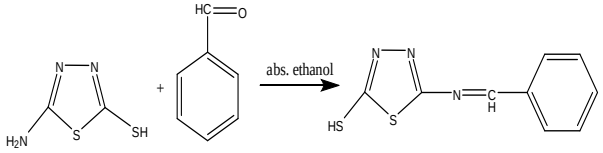
2-amino-5-mercapto-1,3,4-thiadiazole Scheme (1) was synthesized as previously reported[8], yellow needle crystal form, melting point was reported (180-185)ºC.



Scheme (1): Preparation of 2-amino-5-mercapto-1,3,4-thiadiazole. Preparation of 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol

B. Preparation of 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol

5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol scheme (2) was synthesized as previously reported [9], white crystal precipitate, melting point was reported (140-145)ºC.



Scheme (2): Preparation of 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol.

C. Preparation of 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol complexes

All 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol complexes were prepared according to previously papers [10], [11].

Copper (II) complexes (A_1)

Add (0.219g, 0.002mole) of 5-(benzylideneamino)-1,3,4 thiadiazole-2-thiol to alcoholic solution [$CuCl_2.2H_2O$ (0.341g, 0.001 mole) in 10 ml of abs. ethanol], then the mixture was refluxed for 3hrs. The gray precipitate was product and then filtered and washed with absolute ethanol and dried it in room temperature.

Ferric(III) complexes (A_2)

Add (0.219g, 0.002mole) of 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol to alcoholic solution [$FeCl_3.6H_2O$ (0.273g, 0.001 mole) in 10 ml of abs. ethanol], then the mixture was refluxed for 3hrs. The gelatinous olive precipitate was product and then filtered and washed with absolute ethanol and dried it in vacuum.

Cobalt (II) complexes (A_3)

Add (0.219g, 0.002 mole) of 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol to alcoholic solution [$CoCl_2.6H_2O$ (0.23g, 0.001 mole) in 10 ml of abs. ethanol], the mixture was refluxed for 3hrs. The brown precipitate was product and then filtered and washed it with abs. ethanol and dried it in room temperature.

Zinc (II) complexes (A_4)

Add (0.219 g, 0.002 mole) of 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol to alcoholic solution [$ZnCl_2.2H_2O$ (0.237g, 0.001 mole) in 10 ml of abs. ethanol], the mixture was refluxed for 3hrs. The yellow gelatinous precipitate was product and then filtered and washed it with abs. ethanol and dried it in room temperature.

D. Collection of pathogenic bacterial isolates

All pathogenic bacteria were obtained from the Salah El Din Hospitals. The identification of bacteria was performed using VITEK2 compact system. The pathogenic bacterial isolate were cultivated on selective media in the laboratory and stained by Gram stain and some biochemical tests were done as confirmation diagnostic tests [12]. All the collected isolates were isolated from different site of infection.

Antimicrobial susceptibility patterns

The disc diffusion method was used to determine antibiotic sensitivity of the isolates [13]. pure colony transferred to clean tube contain 4 ml brain heart infusion broth and incubated at 37 ºC for 4-5 hours and compared to 0.5 McFarland standards (1.5×10^8). Then spread on Mueller Hinton agar plates (Himedia/ India). The plates were dried at room temperature for 2h. Antibiotic discs were placed at equi-distances. The plates were incubated for 24h at 37°C and organisms were categorized as sensitive or resistant, based on the standards [14]. (A total of 10 antibiotics used in this study: Amikacin, Amoxicillin/Clavulanic acid, Azithromycin, Carbencillin, Ceftazidime, Clarithromycin, Imipenem, Levofloxacin, Norfloxacin, Tigecycline/ Bioanalyse/ India).

Antibacterial activity of complexes

The antibacterial activities of synthesized complexes were evaluated against some pathogenic bacteria (E.coli, P.mirabilis.

C.freundii, P.aeruginosa, S.aureus and S.epidermidis) using well diffusion method. 0.2 ml of fresh cultures of each organism was inoculated into 5 ml of sterile nutrient broth (Himedia/ India) and incubated for 3–5 h to standardize the culture to McFarland standards (10^6 CFC/ml). 0.1 ml of each culture of microorganism was spreading on Mueller Hinton Agar. Wells were made using gel puncture (6mm) according to [15] with modifications, then 0.1 mL of different dilutions (0.1, 0.03. 0.05 M) in case of Copper(II) complexes: (Dissolved Copper(II) complexes (0.1g, 0.2g and 0.3g) in 2ml DMSO), while the dilutions (0.1, 0.2, 0,3 M) in case of Ferric(III) complexes: (Dissolved (0.1 g, 0.2 g and 0.3 g) of Ferric(III) complexes in 2ml abs. ethanol), Cobalt(II) complexes: (0.1g, 0.3g and 0.5g of Cobalt(II) complexes and dissolved in 2ml DMSO), and Zinc(II) complexes: (Add 2ml ethanol to each of (0.1g, 0.2g and 0.3g) Zinc(II) complexes) . The petri plates were incubated at 37ºC for 24 h in incubator midst which activity was appeared by the presence of a zone of inhibition (mm) surrounding the well.

Results and Discussion

Characterization of complexes synthesis

5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol Schiff bases compound prepared through refluxing the aldehyde with 2-amino-5-mercapto-1,3,4-thiadiazole and from this compound prepared complexes to improve biological activity all prepared compounds identification and characterizations by analytical and spectral methods. The FTIR spectrum for 5-(benzylideneamino)-1,3,4-thiadiazole-2-thiol (Schiff bases compound), showed vibrational band at (3407 cm^{-1}) due to N-H_{str} [16] and band at (1250.12cm^{-1}) due to C=S_{str} this bands appearance because tautomerism phenomenon occur in 1,3,4-thiadiazole-2-thiol ring at (HS–C=N-) in position 4 of the thiadiazole ring to form (S=C-NH). The absorption bands at (1642.11cm^{-1}) due to -C=N_{str} and appearance band at (756.04 cm^{-1}) due to the C-S vibrational. In complexes appearance vibrational bands of N-H_{str} between (3309.26-3147.6) cm^{-1}, vibrational bands of C=N_{str} between (1634.92- 1618.74)cm^{-1} and vibrational bands of C=S_{str} between (1152.28-1043.26)cm^{-1} are shift less than the vibrational bands of same groups in schiff bases compound this indicate the coordination occur and the vibrational bands of C-S_{str} between (865.05- 801.43)cm^{-1} this value increase Compared to C-S value in schiff bases compound. In complexes appearance vibrational bands of metal-N between (651.60-589.50)cm^{-1} this indicated the coordination occurs between N in Schiff bases compound and metal.

Table (1)
Infrared Spectroscopy (FTIR) wave number cm^{-1} for Schiff base and complexes compounds.

Groups	Schiff base	A_1	A_2	A_3	A_4
N-H_{str}	3407	3308.47	3220.10	3147.6	3309.26
C=N_{str}	1642.11	1630.11	1618.74	1625.67	1634.92
C=S_{str}	1250.12	1152.28	1044.32	1138.2	1043.26
C-S_{str}	756.04	865.05	801.43	860.10	802.49
Metal-N	-	651.60	646.56	589.50	646.06
Metal-Cl	-	427.94	430.78	372.13	432.79

UV-visible spectrum of Schiff base compound (C=N) and (C=S) showed intense at (224nm) and (313nm) which refer to π→π* and n→π* electronic transition respectively, however its complexes occurs shifting the absorption band at (307nm), (292nm), (272nm), (320nm) due to A_1, A_2, A_3, A_4 respectively that refer to electronic transition at π→π* and n→π* shown Table (2).

Table (2)
Ultraviolet spectroscopy (UV-VIS) wave length λ nm for Schiff base and complexes compounds.

Compound	wave length λ (nm)
Schiff base compound	224 313
A_1	307
A_2	292
A_3	272
A_4	320

Identification of bacterial isolates

The conformation tests of diagnostic bacterial isolate that depends on biochemical and phenotypic properties were described in Table (3).

Table (3)
Biochemical and morphological properties results of bacterial isolates.

bacteria / tests		*E. coli*	*P. mirabilis*	*C. freundii*	*P. aeruginosa*	*S. aureus*	*S . epidermidis*
Gram stain		G^- ve	G^- ve	G^- ve	G^- ve	G^+ ve	G^+ ve
catalase		+	+	+	+	+	+
oxidase		-	-	-	+	-	-
pigment production		-	-	-	+	-	-
Urease production		-	+	V	-	-	+
Hemolysis		-	-	_	+	+	-
mannitol fermentation		-	-	-	-	+	-
IMVIC	IND	+	-	-	-	ND	ND
	MR	+	+	+	-	ND	ND
	VP	-	-	-	-	ND	ND
	C	-	+	+	+	ND	ND
lactose fermentation		+	+	+	-	-	-
coagulase production		-	-	-	-	+	-

G^- ve: Gram negatine bacteria, G^+ ve: Gram positive bacteria, IND: indole, MR:methyl red, VP: Voges proskaur, C: Citrate utilization, ND: not done.

Sensitivity of bacterial isolates to antibiotics

The present study has displayed that the bacteria isolated from different site of infection have a high degree of resistance to most of the antibiotics under investigation. Table (4) showed that most of Gram positive and negative bacteria were sensitive to fluoroquinolones antibiotic such as Norfloxacin and Levofloxacin. This resistance may be due to bacteriocidal agents inhibit bacterial DNA by inhibiting the enzyme Isomerase I one of the enzymes of DNA Gyrase [17]. The antibiotic inhibits the process of dividing the cells during the process of binary fission, these antibiotic have two target sites in the bacterial cell. In other words, the resistant bacteria require two mutations, one of them in the site of the subunit A of DNA Gyrase and the other in the site of Topoisomerase IV. This reduces the bacterial

resistance of this group of antibiotic, but the emergence of bacterial resistance against these antibiotics may be due to shifts in the target area or an increase in efflux pump system [18]. In the case of the antibiotic Amikacin, *E.coli* and *P.mirabilis,* exhibit intermediate resistance, *S.aureus* and *S.epidermidis* were sensitive while *P. aeruginosa C.freundii* were resistant to this, antibiotic. Amikacin is semi-manufactured and derived from the antibiotic Kanamycin and effective against a large number of intestinal families and effective against the Gram-positive, because of its ability to inhibit protein synthesis [19]. The results of the present study show that the majority of bacterial isolates had a relatively high resistance to beta lactam antibiotic, such as the third generation of cephalosporins Ceftazidime. While Imipenem, a group of Carbapenems showed a high effectiveness against all types of bacterial isolates. The bacterial sensitivity to the beta lactam group is due to the ability of these antibodies to bind to the PBPs on the bacterial cell wall, thus inhibiting the manufacture of the bacterial cell wall. The bacteria's resistance to these antibodies is due to the ability of the bacteria to change the target sites targeted by the antibodies. These sites, such as penicillin G, are resisted by most of the Gram-positive bacteria, which produce the beta lactamase enzymes that break the beta lactam ring, change the permeability barrier or weaken the ligament between the antibiotic and target sites (PBPs) [20]. Also it can be seen that all Gram negative isolates were resistant to Carbencillin, whereas *S. aureus* and *S. epidermidis* showed high resistance against Azithromycin and Clarithromycin. The results in Table (4) showed that the bacterial isolates were sensitive to the Augmentin antibiotic this may be due To the production of chromosomal stimulated enzymes that are not inhibited by Clavulanic acid produced by *E.coli* and may be transmitted to other intestinal species such as *P.mirabilis* and *C.freundii* by bacterial conjugation [21]. Tigecycline antibiotic showed a relatively moderate resistance ratio, as shown in Table (4). This antibiotic is bacteriostatic inhibitors that inhibit the binding of aminoacyl-tRNA to the subunit (30S) of the bacterial ribosome. Bacteria resistance to tigecycline resistance results from the loss of bacterial outer membrane proteins, which reduces the permeability of the antibody into the bacteria [22].

Table (4)
resistance of bacterial isolates to antibiotics.

Antibiotic / Bacteria	TGC	NOR	LEV	IMP	CLA	CAZ	PY	ATH	AMC	AK
E. coli	R 14	S 18	S 20	S 22	ND	R 10	R 8	ND	R 12	S 17
P. mirabilis	R 14	S 17	S 22	S 20	ND	R 10	R 8	ND	R 12	S 18
C. freundii	R 10	R 15	S 18	S 20	ND	R 8	R 8	ND	R 10	R 14
P. aeruginosa	R 8	R 14	S 18	S 20	ND	R 8	R 6	ND	R 10	R 12
S. aureus	R 18	S 18	S 20	S 24	R 12	R 12	ND	R 10	S 20	S 18
S. epidermidis	R 16	S 18	S 20	S 24	R 14	R 14	ND	R 10	S 22	S 20

Ak: Amikacin, AMC: Augmentin, ATH: Azithromycin, PY: Carbencillin, CAZ: Ceftazidime, CLA: Clarithromycin, IMI: Imipenem, LEV: Levofloxacin, NOR: Norfloxacin, TGC: Tigecycline.

Antibacterial activity of complexes

The growing numbers of antimicrobial-resistant pathogens, which were increasingly linked with nosocomial contagion, place a considerable burden on healthcare systems and have important global economic costs. [23]. The complexes Cobalt(II) complexes, Ferric(III) complexes, Copper(II) complexes and Zinc(II) complexes were screened in vitro for their ability to inhibit the growth of some Gram negative pathogenic bacteria (*E.coli*, *P.mirabilis*, *C.freundii* and *P.aeruginosa*) and *S.aureus* and *S.epidermidis* as gram positive bacteria. It can be observed from Table (5) that the Cobalt (II) complexes exhibit high activity of inhibition at concentration (0.1M) against the bacterium *E. coli* clear zone of inhibition about 26 mm. *P. mirabilis*, *C.freundii*, *S.aureus* and *S.epidermidis* with 20, 22, 22, 20 mm respectively. The zone of inhibition (ZOI) represent the activity of this complex on *P.aeruginosa* with less value 18mm, when compared with Imipenem antibiotic as an antibiotic with higher inhibitory efficacy than other antibiotics. Ferric(III) complexes exhibit less activity than Cobalt (II) complexes with the zone of inhibition (24, 16, 18, 20, 18 mm) for *E.coli*, *P.mirabilis*, *C.freundii*, *S.aureus*, and *S. epidermidis* respectively. Whereas the *P aeruginosa.* exhibit less sensitivity with ZOI about 15 mm in concentration 0.3 M compared with imipenem which have broad spectrum of activity against Gram positive and negative bacteria. Copper(II) complexes displayed a greater activity against all the studied pathogenic bacteria contrasted with Cobalt(II) complexes and Ferric(III) complexes as showed in Table (5). The highest antimicrobial activity observed against S.aureus and S.epidermidis with zone of inhibition about 26, 30 mm respectively at 0.3 M concentration compared with (24,24 22,22) for E.coli, P.mirabilis, C.freundii and P.aeruginosa respectively at the same concentration. The results of antibacterial activity of Zinc(II) complexes in concentration 0.3 M revealed a broad spectrum of activity against various pathogens compared with Imipenem as showed in Table (4,5). The higher value was 35 mm the ZOI of C.freundii, followed by 30 mm ZOI of E.coli and S. epidermidis while P.mirabilis and P. aeruginosa have 22 mm showed the zone of inhibition against zone of inhibition. (Fig. 1,2,3 and 4 some pathogenic bacteria). Several studies were investigate the effectiveness of Thiadiazoles and its derivatives on varies pathogenic microbes as a new treatment, so this results was similar to [24] who demonstrated that the [3-dicyclohexyl amino methyl -2-mercaptobenzothiazole] [L] and its derivatives have high activity against E.coli and S.aureus. Also our results come in consistence with [25] who synthesized N-benzylidene- 5-phenyl-1,3,4-thiadiazole derivatives.

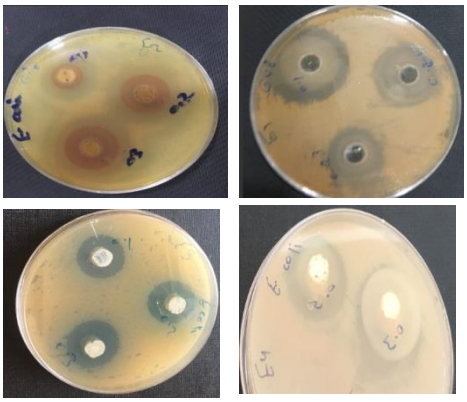


Fig.(1): Activity of A_1,A_2, A_3 and A_4 on E.coli.

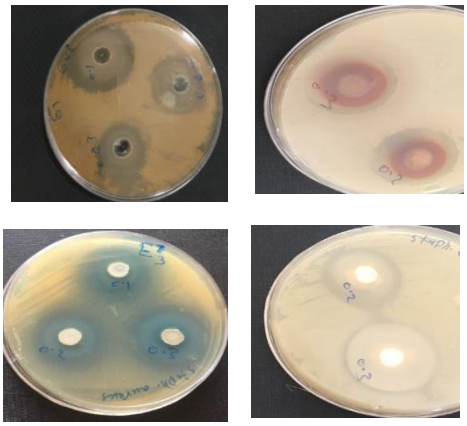


Fig.(2): Activity of A_1, A_2, A_3 and A_4 on S.aureus.

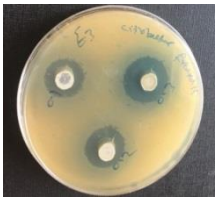


Fig.(3): Activity A_1 on C.freundii.

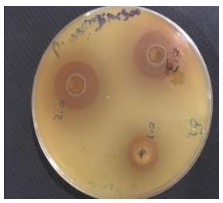


Fig.(4): Activity of A_2 on P. aeruginosa

Conclusion.

Zinc (II) complexes have the greater activity against pathogenic bacteria compared with other complexes and Imipenem antibiotic, followed by Copper (II) complexes which exhibit a higher effectiveness toward Gram positive compared with Gram negative bacteria. Cobalt (II) complexes revealed moderate activity when compared with other complexes (Zinc(II) complexes and Copper(II) complexes) but in compared with Imipenem, P aeruginosa showed a slight resistance toward Cobalt (II) complexes. Ferric (III) complexes was exhibit less activity compared with other complexes and imipenem, but when compared with other antibiotic this complex showed a good activity as showed in Table (4,5).

Table (5)
Antibacterial activities of complexes.

Bacteria	con./M	A_3	con./M	A_2	con./M	A_1	con./M	A_4
E.coli	0.05	16	0.1	18	0.1	20	0.1	20
	0.03	20	0.2	22	0.2	22	0.2	25
	0.1	26	0.3	24	0.3	24	0.3	30
P.mirabilis	0.05	12	0.1	10	0.1	18	0.1	12
	0.03	16	0.2	14	0.2	22	0.2	20
	0.1	20	0.3	16	0.3	24	0.3	22
C.freundii	0.05	12	0.1	16	0.1	12	0.1	22
	0.03	16	0.2	22	0.2	16	0.2	26
	0.1	22	0.3	18	0.3	22	0.3	35
P.aeruginosa	0.05	10	0.1	10	0.1	16	0.1	15
	0.03	14	0.2	12	0.2	20	0.2	20
	0.1	18	0.3	15	0.3	22	0.3	22
S.aureus	0.05	16	0.1	12	0.1	20	0.1	16
	0.03	20	0.2	15	0.2	22	0.2	22
	0.1	22	0.3	20	0.3	26	0.3	30
S.epidermidis	0.05	14	0.1	10	0.1	22	0.1	14
	0.03	18	0.2	15	0.2	26	0.2	18
	0.1	20	0.3	18	0.3	30	0.3	26

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