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Synthesis, in vitro-in vivo and Pharmacological Study of Novel 1,2-substituted benzimidazoles

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Abstract

In this paper, new 11-azo substituted benzimidazole derivatives(1a-k) have been obtained under appropriate conditions and in good yields, by reaction between a novel [1,3]thiazolo[3,2-a]benzimidazol-2(3H)-one (TBIO) substrate and a series of aryldiazonium salts bearing groups with different electronic effect. The synthesized compounds were identifying by ^{1}H NMR spectroscopy, FT-IR and mass spectrum. Evaluated the synthesis compounds in vitro antimicrobial activity have been a completion, four types of tested bacterial strains; *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella sp.* and *Escherichia coli* used in this work. Compound (1j) was found to be the higher antibacterial activity against each species used in this study; all ether synthesized compounds showed good activity comparing with standard antibiotic streptomycin. In vivo study for compound (1j) has been determined by finding the cytotoxicity LD50 (1895 mg/Kg b wt.) in albino mice, in addition to the histological study of albino mice liver. The evaluation of antibacterial activity (*Staphylococcus aureus*) in vivo on the infected rabbit have been studied.

Keywords: Antibacterial**,** Aryldiazonium, Benzimidazole, *Staphylococcus aureus*, LD50, Streptomycin, TBIO

Introduction

Antibiotic resistance by different types of bacteria led to continuous research by researchers to find new antibiotics less side effects and more effective[1]. However, various diseases can cause by *Staphylococcus aureus*; respiratory infections, food poisoning, and the main reason in skin infections including abscesses [2, 3]. Benzimidazole moiety and especially the benzimidazole derivatives was in recent decades the source in pharmaceutical chemistry to develop different drugs for various diseases; antifungal with high activity against *Candida glabrata* and *Candida krusei* which have extremely resistant to antifungals [4], anticancer for breast cancer [5], Unique and high efficiency as antibacterial and antioxidant activity [6]. However, many different applications for benzimidazole derivatives can have to be obtained, liquid crystal [7], corrosion inhibition effects[8], organic light emitting diode (OLED) devices[9] extremely sensitive probe for the detection of (HClO)[10] The need to increase biological efficiency in benzimidazole compounds led to design and synthesis various species with different moieties as in benzimidazole Schiff bases contain [11, 12], benzimidazole chalcones[13], and compounds of benzimidazole that have antimicrobial and anti tubercular activities as in azo derivatives of benzimidazole [14]. From this point, we encouraged and motivated to synthesis new benzimidazole derivatives with the azo group (N=N) by exploited the new benzimidazole moiety (TBIO). Aryl diazonium salt can normally react either with aromatic organic compounds [15, 16] or with other active groups as active methylenes groups [17]. The new (TBIO) have very active methylenes groups can readily react with diazonium salt by coupling reaction to give azo compounds. Preliminary pharmacological study of new benzimidazole derivatives in this work has been done, and this study includes determination several factors such as antibacterial activity, LD50 and histological effect of synthesized compound (1j) in the liver, in addition to the activity of (1j) synthesized compound as an antibiotic against *Staphylococcus aureus* in vivo.

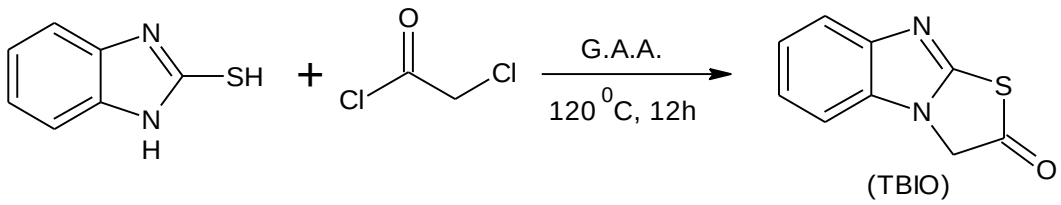
Materials and Methods

Sigma-Aldrich the company that was the mine source of all chemical compounds used in synthesis the new targets. Melting point was diagnosed by (Gallenkamp) apparatus using open capillary tube method. The purity and ending point of reactions were determined by TLC technique using (n-hexane/Ethylacetate) as eluent. spectroscopic methods use in identify the synthesis compounds includes the following devices;(^{1}H-NMR 300 MHz, DMSO, $CDCl_3$, ppm) to record the ^{1}H NMR spectra , (FT-IR-8400S Fourier Transform infrared spectrophotometer–SHIMADZU, 600-4000 cm^{-1}) for FT-IR spectra and two different devices (Shimadzu model GCMSQP 1000 EX and VARIAN 3900) for mass spectra. The liver tissue checked by using optical microscope type (LABOMED) at 400X magnification.

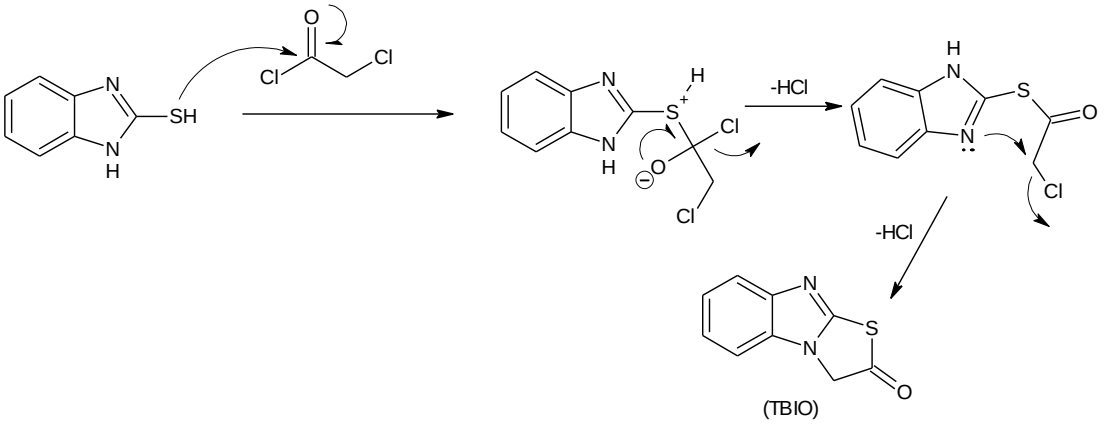
1. Synthesis of [1,3]thiazolo[3,2-a]benzimidazol-2(3H)-one (TBIO) (A).

To solution of (0.1mol, 15gm) 2-mercaptobenzimidazole, (100ml) glacial acetic acid in a 250ml RBF was add dropwise (0.15mol, 11.9ml) Chloroacetylchloride by using dropping funnel in 15 ^{0}C with good stirring by magnetic stirrer for 10min. After 1h of stirring the temperature gradually raise to 120 ^{0}C for 12h. Completion of the reaction was detected by (TLC), after cooling to R.T. the reaction mixture was poured onto (500ml) crushed -ice and stirred for 1 h. The separated

precipitate were filtered and washed with distilled water, dried, and crystallized from benzene to afford the white crystals; yield: 89%. m. p.: 150– 152 ^{0}C; FT-IR : 1737.92 (C=O)[18], 698.25 (C-S), 1610.61 (C=N) and 2924.18, 2966.62 (CH_2) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 7.876, 7.852 (d, 1H, ArH), 7.606, 7.581 (d, 1H, ArH), 7.401-7.295 (m, 2H, ArH), 4.615 (s, 2H, N–CH_2-C=O) ppm. Mass spectrum (MS, HI, 70 eV): m/z = (190) 100%.



Scheme (1): synthesis of [1,3]thiazolo[3,2-a]benzimidazol-2(3H)-one (TBIO)



Scheme (2): mechanism of [1,3]thiazolo[3,2-a]benzimidazol-2(3H)-one (TBIO)

2. General procedures for azo compounds of TBIO **(1a–k)**.

The first solution (1.9 g, 0.01 mol) of the TBIO in (30ml) pyridine was stirring for 1/2h at 0-5 ^{0}C. The second solution contains appropriate amine, diazotized by using HCl/H_2O in (1.05: 1.0) ratio followed by (0.01mol) $NaNO_2$ in good stirring at 0-5 ^{o}C. The second solution was added progressively to a first solution while maintaining temperature within limits. The mixture keep stirring for 4 hours, retained in ice chest for 12h. Mixture diluted with water and the precipitated was filtered, wash frequently with water until filtrate becomes neutral and free from pyridine, dried and recrystallized from EtOH/DMF to give pure azo compounds.

2.1. Synthesis of (3*E*)-3-(2-phenylhydrazinylidene)[1,3]thiazolo[3,2-*a*] benzimidazol-2(3*H*)-one (**1a**). Yellow crystals, Yield, 91% mp 240-242 ^{o}C ; FT-IR : 1730.21 (C=O), 1614.47 (C=N) and 3173.01 (NH) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d$_6$): δ= 7.975-7.947 (d, 1H, ArH), 7.709-7.680 (d, 1H, ArH), 7.446, 7.421 (d, 2H, ArH), 11.199-11.098 (s, 1H, NH), 7.407, 7.392 (d, 2H, ArH), 7.369 (m, 2H, ArH), 7.059-7.038 (m, 1H, ArH) ppm. Mass spectrum (MS, HI, 70 eV): m/z = 294.

2.2. Synthesis of (3*Z*)-3-[2-(2,4-dimethylphenyl)hydrazinylidene][1,3] thiazolo[3,2-*a*]benzimidazol-2(3*H*)-one(**1b**). Yellow crystals, Yield, 67% mp 285-287 ^{o}C ; FT-IR : 1720.56 (C=O), 1608.69 (C=N) and 3286.81 (NH) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d$_6$): δ= 7.669-7.654 (d, 1H, ArH), 7.636-7.625 (d, 1H, ArH), 7.276, 7.210 (m, 1H, ArH), 11.075 (s, 1H, NH), 7.218, 7.139 (m, 1H, ArH), 7.089 (s, 1H, ArH), 7.488, 7.462 (d, 1H, ArH), 7.393, 7.338 (d, 1H, ArH), 2.126 (s, 3H, *o*-CH$_3$), 2.249 (s, 3H, *p*-CH*3*) ppm. Mass spectrum (MS, HI, 70 eV): m/z = 322.

2.3. Synthesis of (3*Z*)-3-[2-(2-chlorophenyl)hydrazinylidene][1,3] thiazolo[3,2-*a*]benzimidazol-2(3*H*)-one (**1c**). Yellow crystals, Yield, 89 % mp 200-202 ^{o}C ; FT-IR : 1730.21 (C=O), 1614.47 (C=N) and 3169.15 (NH) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d$_6$): δ= 7.446, 7.441 (d, 1H, ArH), 7.516, 7.502 (m, 1H, ArH), 7.390, 7.385 (m, 1H, ArH), 11.079 (s, 1H, NH), 7.838, 7.839 (d, 1H, ArH), 7.592, 7.585 (d, 1H, ArH), 7.547, 7.542 (d, 1H, ArH), 7.249, 7.237 (m, 1H, ArH, benzi), 7.218, 7.201 (m, 1H, ArH) ppm. Mass spectrum (MS, HI, 70 eV): m/z = (328), 329 (M+1).

2.4. Synthesis of (3*Z*)-3-[2-(4-nitrophenyl)hydrazinylidene][1,3] thiazolo[3,2-*a*]benzimidazol-2(3*H*)-one(**1d**). Slight green crystals, Yield, 94% mp 255-257 ^{o}C ; FT-IR : 1728.28 (C=O), 1616.40 (C=N) and 1548.89, 1330.93 (NO$_2$) and 3188.44 (NH) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d$_6$): δ= 8.75 (d, 1H, ArH), 8.264-8.129 (d, 1H, ArH), 8.038, 8.005 (d, 1H, ArH), 11.648 (s, 1H, NH), 7.974, 7.954 (d, 2H, ArH), 7.565, 7.496 (d, 2H, ArH), 7.430, 7.418 (m, 1H, ArH) ppm. Mass spectrum (MS, HI, 70 eV): m/z = 339 100%.

2.5. Synthesis of (3*Z*)-3-[2-(4-bromophenyl)hydrazinylidene][1,3] thiazolo[3,2-*a*]benzimidazol-2(3*H*)-one (**1e**). Yellow crystals, Yield, 93% mp 267-269 ^{o}C ; FT-IR : 1726.35 (C=O), 1612.54 (C=N) and 3161.43 (NH) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d$_6$): δ = 8.064, 8.036 (d, 1H, ArH), 7.999-7.944 (d, 1H, ArH), 7.703, 7.678 (d, 2H, ArH), 11.264 (s, 1H,

NH), 7.574, 7.545 (d, 2H, ArH), 7.417, 7.391 (m, 1H, ArH), 7.306, 7.277 (d, 1H, ArH) ppm. Mass spectrum (MS, HI, 70 eV): m/z = (373) 100%, 374 (M+1), 375(M+2).

2.6. Synthesis of (3*Z*)-3-[2-(2-nitrophenyl)hydrazinylidene][1,3] thiazolo[3,2-*a*]benzimidazol-2(3*H*)-one (**1f**). Brown crystals, Yield, 86% mp 322-324 ^{o}C ; FT-IR : 1737.92 (C=O), 1608.69 (C=N), 3242.45 (NH) and 1371.43, 1539.25 (*o*-NO_2) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 8.285, 8.257 (d, 1H, ArH), 8.100, 7.071 (d, 1H, ArH), 8.054, 8.047 (d, 1H, ArH), 11.034 (s, 1H, NH), 7.732 (d, 1H, ArH), 7.713 (d, 1H, ArH), 7.579, 7.562 (m, 1H, ArH), 7.542, 7.416 (m, 1H, ArH), 7.170, 7.044 (m, 1H, ArH) ppm. Mass spectrum (MS, HI, 70 eV): m/z = (339) 100% .

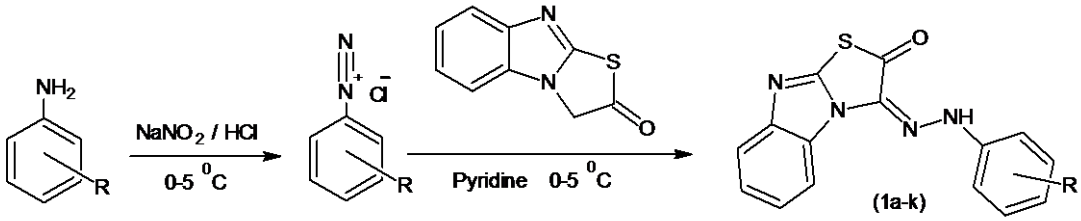
2.7. Synthesis of (3*Z*)-3-[2-(3-chlorophenyl)hydrazinylidene][1,3] thiazolo[3,2-*a*]benzimidazol-2(3*H*)-one (**1g**). Yellow crystals, Yield, 74% mp 244-246 ^{o}C ; FT-IR : 1705.13 (C=O), 1610.61 (C=N) and 3155.35 (NH) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 7.775-7.770 (s, 1H, ArH), 7.299-7.291 (d, 1H, ArH), 7.675, 7.671 (m, 1H, ArH), 11.061 (s, 1H, NH), 7.327, 7.322 (d, 1H, ArH), 7.578, 7.569 (d, 1H, ArH), 7.546-7.538 (d, 1H, ArH), 7.249, 7,243 (m, 1H, ArH), 7.217, 7.206 (m, 1H, ArH) ppm. Mass spectrum (MS, HI, 70 eV): m/z = (328), 329 (M+1).

2.8. Synthesis of (3*Z*)-3-[2-(4-methylphenyl)hydrazinylidene][1,3] thiazolo[3,2-*a*]benzimidazol-2(3*H*)-one(**1h**). Yellow crystals, Yield, 90 % mp 287-289 ^{o}C ; FT-IR : 1728.28 (C=O), 1610.61 (C=N), 3165.29 (NH) and 2864.39 , 2922.25 (CH_3) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 7.970-7.943 (d, 1H, ArH), 7.699-7.677 (d, 1H, ArH), 7.443, 7.418 (m, 1H, ArH), 11.154 (s, 1H, NH), 7.403, 7.387 (m, 1H, ArH), 7.273, 7.245 (d, 2H, ArH), 7.209, 7.181 (d, 2H, ArH), 2.284 (s, 3H, CH_3) (m, 2H, ArH), 7.059-7.038 (m, 1H, ArH) ppm. Mass spectrum (MS, HI, 70 eV): m/z = 308.

2.9. Synthesis of 4-[(2*Z*)-2-(2-oxo[1,3]thiazolo[3,2-*a*]benzimidazol-3(2*H*)-ylidene)hydrazinyl]benzoic acid(**1i**). Yellow crystals, Yield, 84 % mp 309-311 ^{o}C ; FT-IR : 1734.06 (C=O), 1600.97 (C=N), 3169.15 (NH), 2600-3213.51 (OH) and 1678.13 (C=O acid) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 8.157, 7.976 (d, 2H, ArH), 7.788-7.705 (d, 2H, ArH), 7.422, 7.393 (d, 1H, ArH), 11.440 (s, 1H, NH), 12.577, 12.364 (s, 1H, OH), 7.422, 7.393 (d, 1H, ArH), 7.422, 7.393 (m, 1H, ArH), 7.422, 7.393 (m, 1H, ArH) ppm. Mass spectrum (MS, HI, 70 eV): m/z = (338) 100%.

2.10. Synthesis of ethyl 4-[(2*Z*)-2-(2-oxo[1,3]thiazolo[3,2-*a*] benzimidazol-3(2*H*)-ylidene)hydrazinyl]benzoate (**1j**). Yellow crystals, Yield, 94 % mp 260-262 ^{o}C ; FT-IR : 1710.92 (C=O), 1608.69 (C=N) and 3171.08 (NH), 1697.41 (C=O benzocain), 2943.47, 2980.12 (CH_2CH_3) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 8.001, 7.972 (d, 2H, ArH), 7.949, 7.873 (d, 1H, ArH), 7.686, 7.615 (d, 1H, ArH), 11.453 (s, 1H, NH), 7.579, 7.569 (d, 2H, ArH), 7.369-7.377 (m, 2H, ArH), 4.289-4.188 (q, 2H, CH_2), 1.326-1.240 (t, 3H, CH_3) ppm. Mass spectrum (MS, HI, 70 eV): m/z = 366.

2.11. Synthesis of (3*E*)-3-[2-(4-chlorophenyl)hydrazinylidene][1,3] thiazolo[3,2-*a*]benzimidazol-2(3*H*)-one (**1k**). Yellow crystals, Yield, 83% mp 282-284 ^{o}C ; FT-IR : 1728.28 (C=O), 1610.61 (C=N) and 3161.43 (NH) cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 7.957, 7.937 (d, 1H, ArH), 7.698-7.677 (d, 2H, ArH), 7.443 (d, 2H, ArH), 11.274 (s, 1H, NH), 7.415, 7.353 (d, 2H, ArH), 7.324 (m, 1H, ArH) ppm. Mass spectrum (MS, HI, 70 eV): m/z = (328) 100%, 329 (M+1).



	1a	1b	1c	1d	1e	1f	1g	1h	1i	1j	1k
R	H	2,4-$(CH_3)_2$	2-Cl	4-NO_2	4-Br	2-NO_2	3-Cl	4-CH_3	4-COOH	4-$COOC_2H_5$	4-Cl

Scheme (3): synthesis of azo-substituted benzimidazole derivatives (1a-k).

Evaluation of antimicrobial activity

The synthesized azo-substituted benzimidazole derivatives were evaluated the antibacterial activity by using different types of bacteria strains; (Gram-positive; *Staphylococcus aureus, Staphylococcus epidermidis* and *Gram-negative; Klebsiella sp, Escherichia coli*). By using the agar diffusion method [19]. The bacteria strains (pathogenic bacteria) used in this study equipped by Bacteriology lab. Imamein Kadhimein Medical City. The concentrations 500,250, 125, 62.5 µg cm^{-3} and DMSO as control/solvent for synthesis compounds was used to test their antimicrobial activity. Positive control Streptomycin was used for comparison with antibacterial activities of

synthetic compounds. The limitations of (MIC) Minimum inhibitory concentration for all compounds have been done. After spread the bacteria *Klebsiella sp., Escherichia coli, Staphylococcus aureus,* and *Staphylococcus epidermidis* on agar plates with a specified quantity of test compounds they incubated at 37 ^{0}C for 24 h. The growth of bacterial cells and zone size inhibition was observed on agar plates and measured.

Measurement LD50 for compound [1j]:

The median lethal dose (LD50) which is an acute toxicity test was measured for (1j) synthesized compound by using laboratory albino mice [Females, eight weeks, average weight 25 g]. This part of the work has been completed in the Iraqi Center for Genetics and Cancer Research. Five groups each group contains six albino mice were used to obtain the best result. Intraperitoneal injection used as a method to give the dose to the albino mice with 0.5 ml of compound (1j) in concentrations 1000, 500, 250 and 125 mg/Kg body weight (b. wt.). However, one group of albino mice was used to test the solvent (DMSO) with 0.5 ml a single intraperitoneal injection. After 24 hours LD50 was determined and measured by observing the number of lifeless albino mice. [20]

Study the histological effect on albino mice:

The albino mice of each group were sacrificed after treatment with (1j) compound. Histological section was produced to compatible with procedure [21]. Liver tissues from all groups were removed to dissecting the liver for histopathological changes study and quickly fixed in 10% formalin (10 ml formalin + 90 ml NaCl 0.9%), then treatment the tissues with different concentrations of ethanol (50%, 70%, 80%, 90% and 100%) for several hours. After wiping by xylol thy embedding in paraffin at limited time and temperature. The model then was cut into 5-µm. The slices are embedding in glass slides using Meyer's album, dry at 37 ^{0}C. The stains were used to processing slides for study under a microscope; hematoxylin stain was used first for 10 min. The second stain was used is eosin and for less than one minute. The distilled water then applied to wash the slides, a series of alcohol (70%, 90%, and 100%) were used, covering the slides by cover slides and studied through an optical microscope type (LABOMED) at 400X magnification.

Results and Discussion

The newly substrate (TBIO) was synthesis by using chloroacetylchloride and 2-mercaptoimidazole in an acidic environment (Scheme 1), the mechanism of reaction clarified in (Scheme 2). Novel azo-substituted benzimidazole derivatives (1a-k) were synthesized in excellent yield by the coupling reaction between aryldiazonium salts and (TBIO) in the presence of pyridine as summarized in (Scheme 3). The synthesis compounds were identified and characterized after purified by spectral methods includes; FT-IR, ^{1}H NMR, and GC-Ms spectra. The FT-IR spectrum of (TBIO) shows the absorption at 1737.92 cm^{-1} which related to C=O stretching in the five-membered heterocyclic ring [18], while the absorption band of C=N showed at 1610.61 cm^{-1}. The ^{1}H NMR of the substrate (TBIO) showed a singlet signal at δ 4.615 due to 2H of the methylene group in a five-membered ring. The Mass spectrum of (TBIO) gives the molecular ion and fragments identical exactly with molecular weight and a molecular formula of (TBIO). The spectroscopic data of synthesis compounds (1a-k) give satisfying results which

confirm the success of the reactions and required structures. However, the FT-IR of all new compounds showed disappearance the frequency of methylene group and shift occur towards low frequency in C=O stretching of (TBIO) compound due to increase in conjugation, for example, The C=O stretching of (1a, 1b, and 1c) compounds appeared at 1730.21, 1720.56 and 1730.21 cm-1 respectively. The ^{1}H NMR of all synthesis compounds (1a-k) showed disappearance the singlet signal at δ 4.615 in (TBIO) and appeared new signals related to new aromatic protons. The mass analysis, spectral data and physical properties of all newly synthesis compounds were given in the experimental section.

Evaluation of antimicrobial activity

The antibacterial activity of synthesis compounds was measured against Gram-positive; *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Gram-negative; Klebsiella sp*, *Escherichia coli*. The minimum inhibition concentration (MIC, μg cm^{-3}) values of all compounds are mention in (Table 1). In general, all compounds showed excellent activity comparing with standard drug streptomycin. The (1j) compound has broad-spectrum activity, give higher and excellent activity against gram-positive and gram-negative; this is due to containing the compound on $COOC_2H_5$ group in *p*-positions. The compounds (1i and 1h) have *p*-COOH and *p*-CH_3 groups respectively showed high activity against all types of bacteria. However, the activity of compounds against gram-negative; *Klebsiella sp, Escherichia coli* was less than activity against Gram-positive; *Staphylococcus aureus, Staphylococcus epidermidis* especially in (1d, 1e, and 1f) compounds (Figure 1).

Table (1): MIC (μg cm^{-3}) values of synthesis compounds (TBIO, 1a-k) against various bacterial strains.

Compound	Gram-positive		Gram-negative	
	Staphylococcus aureus	*Staphylococcus epidermidis*	*Klebsiella sp*	*Escherichia coli*
TBIO	13.5	10	4.8	5.2
1a	5.5	3.5	3.5	1.5
1b	13.5	0	2.7	0
1c	13	0	5.9	1.4
1d	18.5	8.5	0	5
1e	11	5	0	2
1f	6	5	1.5	0
1g	16	13.5	3.5	0
1h	14	18	13	15.5
1i	25	20	18	15
1j	٣٠	٢٥	٢٠	٢٠
1k	13.5	4.5	7.9	0
Streptomycin	2.15	2.27	4.66	4.38

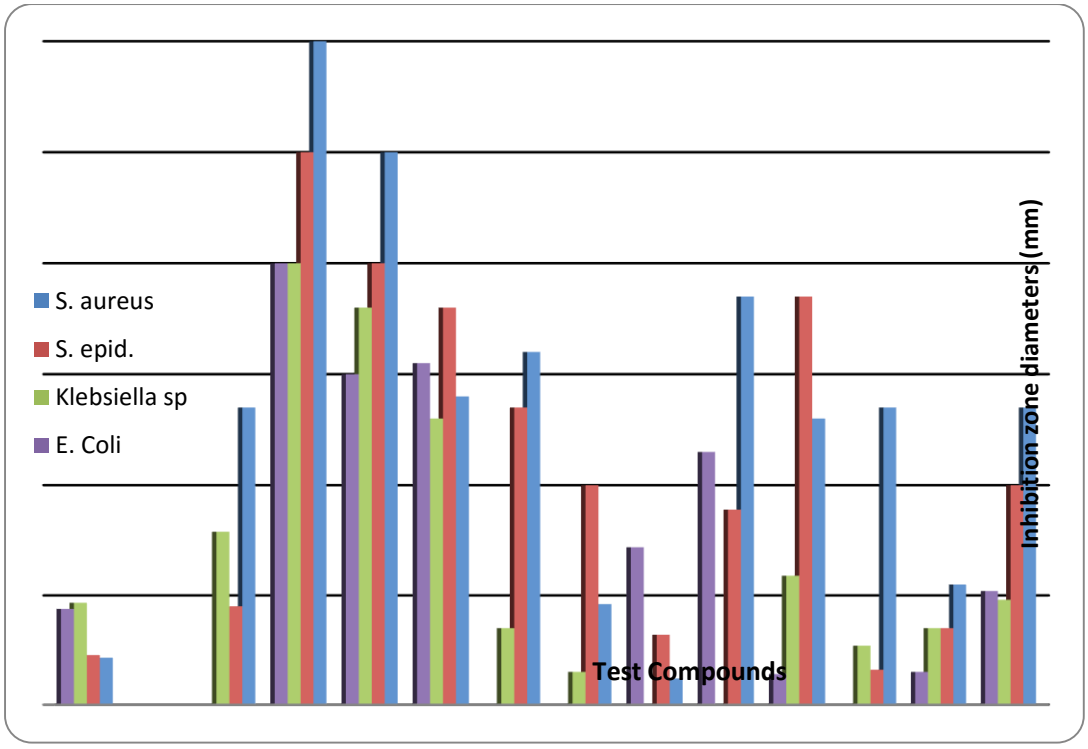


Figure (1) Antibacterial activity of synthesized compounds against *S. aureus, S. epidermidis, Klebsiella sp* and *E. coli*

Measurement lethal dose (LD50) for compound [1j]

LD50 the lethal dose or median lethal dose can be defined as the amount of standard measurement of a solid or liquid material that will kill the half of the test animals (exp. mice, rats) within a specified period. The administered methods can be done either by mouth (orally), injection (intraperitoneally or intravenously) or by dermally (placed directly on the skin). In this work synthesized compound (1j) his Cytotoxicity (LD50) has been determined. Different dosage (1000, 500, 250 and 125 mg/Kg b. wt.) for compound (1j) were injected intraperitoneally by using albino mice. The albino mice were observed at varying times (2, 6, 12 and 24 hours) to watch her behavior and survive, after having spent the specified period (24h) the number of died mice were counted in each group, and the cytotoxicity of the liver organ in survived mice was determined. However, the group of albino mice with (1000 mg/Kg b. wt.) have died after 6 hours, the group with (500 mg/Kg b. wt.) death was (4, 66 %), while (3, 50%) of (250 mg/Kg b. wt.) group have died after 24 hours. The ratio of less than 50% was observed (1, 16%) in the group of (125 mg/Kg b. wt.). From the above study the value of LD50 for (1j) compound in female albino mice was calculated to be (1895 mg/Kg b. wt.).All data illustrated in Table (2)

Table (2) Determination of the LD50 of (1j) compound after intraperitoneal injection in female mice (n=6)

group	Dose mg/Kg	No. of animal dead	Dose difference (a)	Mean mortality (b)	Probity (a*b)
1	control	0			
2	125	1	0	0	
3	250	3	125	2	250
4	500	4	250	3.5	875
5	1000	6	500	5	2500

LD50= the apparent least dose lethal to all in a group – sum (a*b)/n

Were n = number of animal in each group, (a) = dose different between two group, (b) = mean mortality = mortality in second group + first group /2

The lethal dose LD50 = 2500 – 3625 / 6 = 2500 – 604 = 1895 mg/Kg body weight. It is important to note that the elicitation value is represented the LD50 by using intraperitoneal injection, and it is possible to slightly change when the method of administration different, as in oral or intravenous administration

The histological effect of compound [1j]

This part of the work has been completed in the Iraqi Center for Genetics and Cancer Research. The histological study has been completed as procedure mention in reference [21]. After removal of the liver of survived albino mice and fixed by 10% formalin, the member has been laboratory-treated with all solutions and organic compounds mentioned in the experimental section. The liver of group (250 mg/Kg b. wt.) and control group have been examined under a (400X) microscope to ascertain the physiological condition of the liver and study the histological effect of compound (1j). Depending on the microscopic descriptions of the slides (Figure 2) for group (1j, 250 mg/Kg b. wt.) and control, shows the normal structure of liver with no pathological changes and increase in the kupffer cell, normal arrangement of the hepatic cells of the liver tissue and the central vein[22].

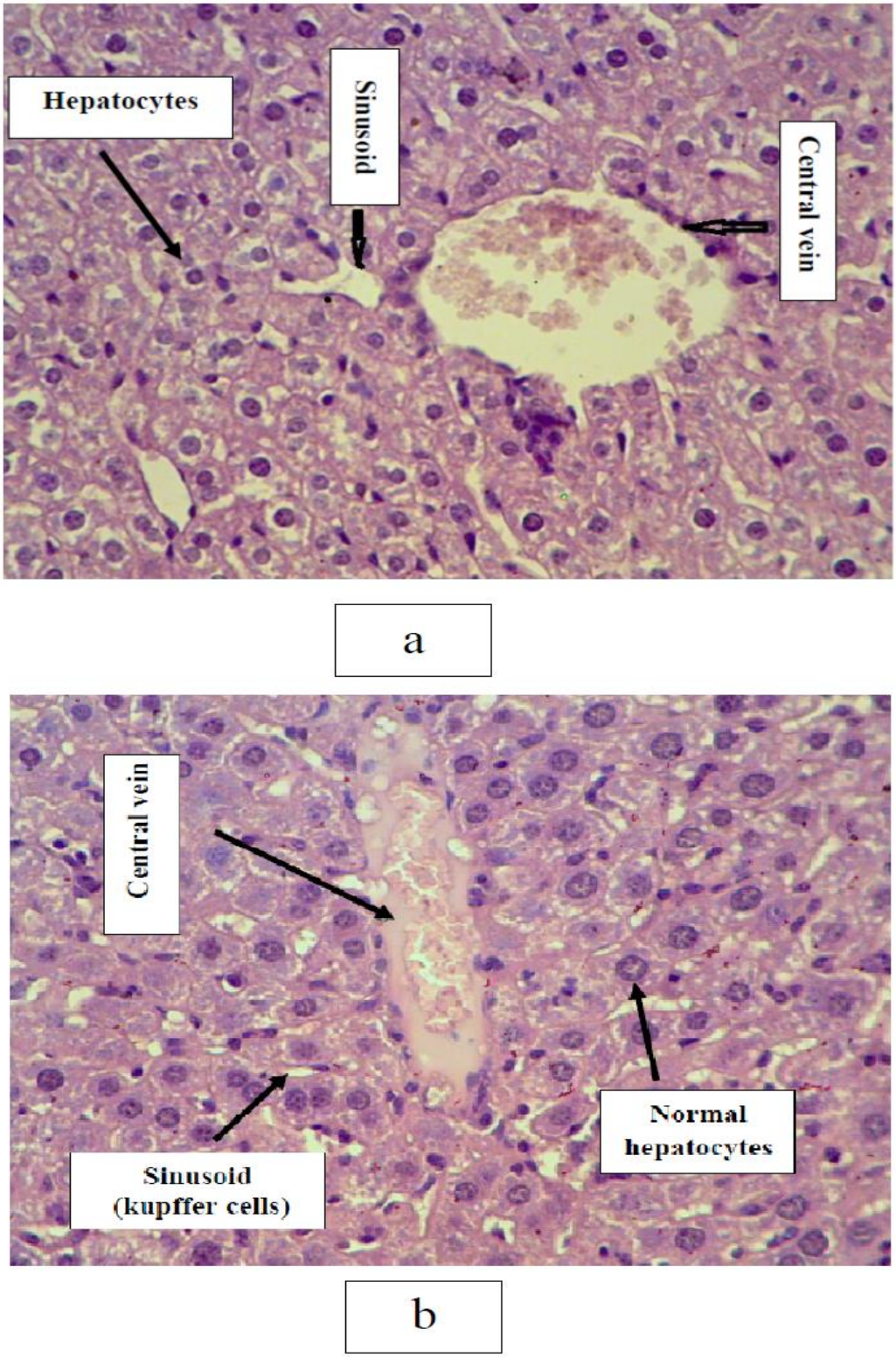


Figure (2) Histological section of the mice liver (a) control (b) treated group

Evaluation of antibacterial activity *in vivo*:-

The *Staphylococcus aureus* can cause a broad spectrum of pathologies and considered the main reason for suppurative dermatitis and skin abscesses [23]. *S. aureus* strains (pathogenic bacteria) used in this experiment obtained from (Bacteriology lab. Imamein Kadhimein Medical City) and this part of the work has been completed in Iraqi Center for Genetics and Cancer Research. The culture of the bacterial inoculate during18 hours was synthesis by inoculating a single colony infusion agar plate for 9 ml of tryptic soy broth (highly nutrient liquid medium). The resulting culture was treated by centrifuge at 3000 rpm for ten minutes. By using the normal saline as a suspending agent, the bacterial sediment is converted to suspension in a volume equal to the discarded supernatant. The dilution was performed ten times in a normal saline solution to the inoculums. The bacteria incubated for 24 hours, and according to a procedure in the reference [24] a specific volume of suspending cells with dose (30 * 1010 CFU) was used to infect the animals. Four male albino rabbits with (1.5 Kg on average) were used for a pathological test. To two groups the male albino rabbits were divided, a first group (control group) and a second group (treated group), both groups were inoculated subcutaneously with (0.5 ml) of bacterial inocula (*S. aureus*). The rabbits were moderately wounded and left in isolated cages with two meals of vegetables a day for three days, and the male albino rabbits were observed at different times to watch their behavior and clinical signs. At the end of three days, ulcers and abscesses were seen in and around the wounds in all groups of male albino rabbits (control and treated groups). However, apathy, mild fever, and a decrease in appetite were observed. The ointment treatment method was used for the wounds of the infected rabbit with *S. aureus* (treated group), the compounds (1j) in concentration (250 mg/Kg b. wt.) mixed with vaseline was placed on the wounds three times a day. Six days later, the affected area was wholly healed (Figure 3), in general, clinical signs became normal and animal appetite increased. While the affected area of (control group) was still infected, this shows the compound (1j) efficiency as an antibacterial against *S. aureus* strains.

Figure (3) Antibacterial activity of (1j, 250 mg/Kg b. wt.) in vivo

Conclusion

In this study novel substrate and newer azo-benzimidazole compounds was synthesis by a straightforward and efficient method, the chloroacetylchloride and 2-mercptobenzimidazole in acidic PH was the requirement to get the target (substrate). Antibacterial study for all synthesis compounds was complete with a presence of standard antibiotic streptomycin, the result of antibacterial evaluation has shown that the synthesis compounds possess mild to high activity against selected bacteria. However, the (1j) compound show excellent and higher antibacterial activity. Study of the LD50 for (1j) compound by using albino mice has been accomplished and the value of the LD50 determined by (1895 mg/Kg b. wt.). The histological study showed no physiological changes in the liver of albino mice after treated with a specific value of compound

(1j) (250 mg/Kg b. wt.). The evaluation of antibacterial activity in vivo for (1j) compound and by using male albino rabbits showed excellent activity against *Staphylococcus aureus* strain

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