REVIEW ARTICLE

Synthesis, Characterization and Antimicrobial Evaluation of New Azo Compounds Derived from Sulfonamides and Isatin Schiff Base

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Received: 22th Dec, 19; Revised: 25th Jan, 20, Accepted: 13th Feb, 20; Available Online: 25th Mar, 2020

ABSTRACT

The present study deals with the synthesis of four different azo-azomethine derivatives; this is done by two steps; the first step is diazotization of sulfonamides (sulfanilamide, sulfacetamide, sulfamethoxazole, and sulfadiazine) separately, followed by the second step; the coupling reaction of diazotized compounds with isatin bis-Schiff base named 3-((4-nitrobenzylidene) hydrazono)indolin-2-one. The later one (bis-Schiff base) was synthesized by the reaction of 3-hydrazono-indolin-2-one with p-nitrobenzaldehyde. The chemical structures of newly synthesized compounds were approved on the basis of their FTIR, ^{1}H-NMR, and CHNS elemental analysis data results. The synthesized azo compounds were tested *in vitro* for their antimicrobial potential using well diffusion method. All the target compounds were clearly inhibited *Escherichia coli* and *Candida albicans*, while only compounds 2b and 2c show antibacterial activity against *Pseudomonas aeruginosa*. The most active compound among the prepared azo compounds against *Escherichia coli, Pseudomonas aeruginosa,* and *Candida albicans* is compound 2b.

Keywords: Antimicrobial activity, Azo compounds, Diazotization, Isatin bis-Schiff bases, Sulfonamides.

International Journal of Drug Delivery Technology (2020); DOI: 10.25258/ijddt.10.1.22

How to cite this article: Kamoon RA, Jawad Al-Mudhafar MM, N-A Omar T. Synthesis, Characterization and Antimicrobial Evaluation of New Azo Compounds Derived from Sulfonamides and Isatin Schiff Base. International Journal of Drug Delivery Technology. 2020; 10(1): 150-155.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Sulfonamides were the first drugs largely used as preventive and chemotherapeutic agents against many diseases.[1] Many drugs containing sulfonamide group are of medicinal and clinical importance as antibacterial, antimalarial and antileprotic agents,[2,3] they inhibit both Gram-positive and Gram-negative bacteria, and some Protozoa, as well as, enteric bacterial strains such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhi*.[4]

Recently, heterocyclic compounds and their derivatives have attracted strong interest in medicinal chemistry due to their various pharmacological properties. Isatin, chemically named as 1*H*-indole-2,3-dione, is an important class of heterocyclic compounds,[5] isatin as simple nucleus possesses many biological activities like antimicrobial, anti-HIV, antiviral, antitubercular, anti-inflammatory, antitumor, antioxidant, anticonvulsant, and CNS depressant activities.[6]

Schiff bases for many years are used as substrates in the preparation of a number of biologically active compounds.[7] Moreover, Schiff bases derived from isatins have been found to possess antiviral,[8] antibacterial,[8,9] antifungal,[8,10] antitubercular,[11,12] and anticancer activities.[13,14] Different Schiff and Mannich derivatives of 4-amino-N-carbamimidoyl benzene sulfonamide with isatins were synthesized and evaluated by the tube dilution method for their antimicrobial activity; these compounds showed better antibacterial activity than the reference drug (sulfaguanidine).[15]

With the increase in the complication of pathogenic diseases, as well as various microbes, show resistance to current antimicrobial agents, it becomes necessary to find a new area of antimicrobial agents that will counter the observed resistance.

The traditional applications of azo compounds are as dyes in many fields such as textile fibers, the coloring of different materials, biomedical studies, and organic synthesis.[16] Despite a few articles reported that azo compounds having antimicrobial activities.

Azo compounds were synthesized containing aspirin moiety showed an interesting antimicrobial activity.[17] Other azo compounds were prepared by diazotization of a primary aromatic amine with different coupling agents. The antimicrobial properties of these azo compounds were determined against six microbial organisms; *Staphylococcus aureus, Micrococcus luteus*, *Mycobacterium smegmatis*,

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Escherichia coli, Pseudomonas aureginosa, and the fungus *Candida albicans* using disc diffusion method, it was observed that the para nitro-substituted azo dye exhibit very good antimicrobial activity.[18]

Furthermore, substituted polyhydroxy azo–azomethine compounds were synthesized by chemical reaction of tris (hydroxymethyl) aminomethane with different substituted benzaldehyde compounds, the synthesized azo–azomethine compounds with 4-nitro substitution exhibited potent antimicrobial activity against Gram-positive bacteria, yeast, and mold.[19]

This article focused on the preparation of new azo sulfonamides of isatin azomethine derivative with p-nitrobenzaldehyde, and all the synthesized azo compounds were confirmed by fourier transform infrared (FT-IR), ^{1}H-NMR spectral characterization and CHNS elemental analysis, as well as their *in-vitro* antimicrobial activity, was studied.

MATERIALS AND METHODS

General

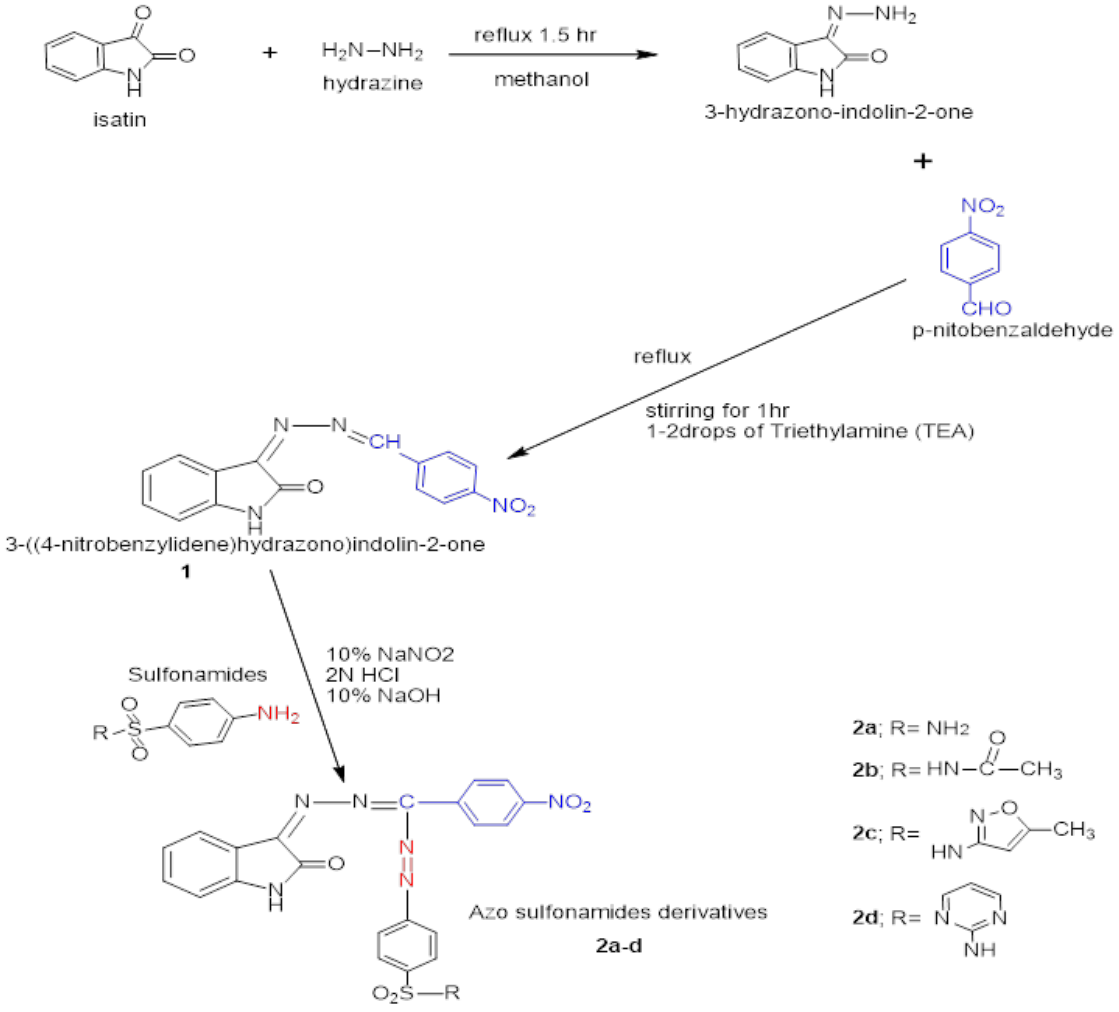
Isatin was purchased from HiMedia Laboratories, India, p-nitrobenzaldehyde was bought from Hyper Chem, China, Hydrazine hydrate 80% was obtained from Alpha Chemika, India. Sulfanilamide, sulfacetamide, sulfamethoxazole, and sulfadiazine were purchased from SDI, Iraq. All the solvents used were commercially obtained without further purification.

Melting points (uncorrected) were measured by Stuart SMP30 Electronic Melting Point Apparatus. The reactions monitoring were tested by thin-layer chromatography (TLC) on Merck silica gel 60F254 and vision with UV light. The FT-IR spectra were recorded on FTIR spectrophotometer/ Shimadzu, Japan, supplied by Specac® Quest ATR (diamond)-UK (College of Pharmacy, University of Baghdad), ^{1}H-NMR (NMReady-60 PRO, 60 MHz spectrophotometer, Canada) were done in the College of Sciences Ibn Al-Haitham, Central service laboratory, University of Baghdad. The CHNS elemental analysis was performed by Vario MICRO CUBE elemental analyzer/Germany.

Chemical synthesis

Synthesis of 3-hydrazono-indolin-2-one

30 mL of hydrazine hydrate (80%) was added to isatin (20.4 mmol, 3 gm) dissolved in 30ml of methanol and the final mixture was refluxed for 1.5 hours, as showed in Scheme 1.



Scheme 1: Synthesis of New Azo Compounds

The progress of the reaction was monitored by TLC (hexane: ethyl acetate (3:2 v/v), R_f value = 0.647). The final product was poured on crushed ice water, and then the separated precipitate was filtered and washed with hot distilled water several times and methanol two times, dried and recrystallized from ethanol. Yellow solid; Yield: (74.8%); m.p. 230–233°C (the reported melting point is 231–232°C Literature[20]); FTIR (υ, cm^{-1}): 3437.15 (N-H, hydrazone), 3136.25(N-H, isatin), 1685.79 (C=O, isatin), 1585.49 (C=N, imine).

Synthesis of 3-((4-nitrobenzylidene)hydrazono) indolin-2-one (1)

3-hydrazono-indolin-2-one (9.31 mmol, 1.5gm) was dissolved in 30mL of methanol (previously dried with anhydrous Calcium Chloride) to this solution (9.31 mmol, 1.41gm) of 4-nitrobenzaldehyde was added, the pH of the solution was adjusted around 7-8 by adding 2 drops of trimethylamine (TEA), then the mixture was refluxed for 1hour then stirring for another 1hour at room temperature, as showed in scheme 1, the progress of reaction was monitored by TLC (hexane: ethyl acetate (3:2 v/v), R_f value = 0.753). The separated product was filtered and washed with warm methanol. After drying the product, it was recrystallized from ethyl acetate: ethanol (1:4). Light brown solid; Yield: (67.6%); m.p. 201-204°C; FTIR (υ, cm^{-1}): 3051.39 (Ar-H), 3147.97 (N-H, isatin), 1681.93 (C=O, isatin), 1558.48 (C=N, azomethine), 1516.05 and 1310.00(C-NO_2);^{1}H-NMR (DMSO-d_6) δ ppm: 8.69 (s, 1H,-N-N=C<u>H</u>-), 8.03 (s, 1H, -N<u>H</u>-, isatin), 7.23-8.32 (m, 8H, Ar-<u>H</u>).

General Procedure for the Synthesis of Azo Compounds (2a-d)[21]

The formation of azo compounds was performed in two steps:

- *First step:* Sulfonamide (4.5 mmol) was dissolved in hydrochloric acid (10 mL, 50% v/v), and the solution cooled with stirring in an ice bath, the temperature of the solution was kept below 5ºC. A cold (about 0 ̊C) solution of sodium nitrite (0.324 gm, 4.7 mmol) in 3ml water was added drop by drop into the first solution in the ice bath with continuous stirring; the temperature should not exceed 10ºC. The last quantity of the sodium nitrite (about 0.5 mL) solution was added in a slower manner and after stirring for 5 minutes, the solution was checked for excess sodium nitrite (nitrous acid; HNO_2) using potassium iodide-starch paper (if nitrous acid presents a blue color on iodide paper appears). The excess amount of HNO_2 was destroyed by adding the required amount of 2% sulfamic acid with continuous stirring for 20 minutes. The resulting (diazotized) solution was utilized for the coupling reaction immediately in the next step.
- *Second step:* (4.5 mmol, 1.32 gm) of Compound (**1**) was dissolved in 8 mL methanol and 6 mL of 10% sodium hydroxide (NaOH) solution in a beaker immersed in an ice bath (the temperature of the mixture is kept to 5°C). To this well-stirred solution, the diazotized solution was added dropwise over a period of 10–15 minutes with vigorous shaking, maintaining the pH 7–7.5. The stirring was continued for 3-4 hours at 0-5ºC. The separated product was filtered, washed with distilled water several times, and recrystallized from ethanol.

3-(4-nitrophenyl)-1-(2-oxoindolin-3-ylidene)-5-(4-sulfamoylphenyl)formazan (2a)

Sulfanilamide (4.5 mmol, 0.775gm) was dissolved in hydrochloric acid (10mL, 50% v/v) and complete the preparation as mentioned in the general procedure described above and shown in scheme 1. Dark brown solid; Yield: (65%); m.p. 210-213°C; FTIR (υ, cm^{-1}): 3174.83 (N-H, isatin), 1732.08 (C=O, isatin), 1600.92 (N=N, azo group), 1516.05 and 1292.31 (C-NO_2), 1446.61(-C=N-N=C, aromatic conjugation), 1342.46 and 1165.00 (C-SO_2, sulfonamide moiety); ^{1}H-NMR (DMSO-d_6) δ ppm:1.16(s, 2H, $SO_2N\underline{H}_2$), 7.27-8.32 (m, 12H, Ar-<u>H</u>), 8.02 (s, 1H, -N<u>H</u>-, isatin); CHNS elemental Analysis for $C_{21}H_{15}N_7O_5S$, Calcd.: C, 52.83; H, 3.17; N, 20.54; S, 6.72%. Found: C, 53.22; H, 2.96; N, 21.85; S, 6.98%.

5-(4-(N-acetylsulfamoyl) phenyl)-3-(4-nitrophenyl)-1-(2-oxoindolin-3-ylidene)formazan (2b)

Sulfacetamide sodium (4.5 mmol, 1.063gm) was dissolved in hydrochloric acid (10mL, 50% v/v) and complete the preparation as mentioned in the general procedure described above and shown in scheme 1. Dark brown solid; Yield: (61.9%); m.p. 236-238°C; FTIR (υ, cm^{-1}): 3174.83 (N-H, isatin), 1735.93 (C=O, isatin), 1600.92 (N=N, azo group), 1516.05 and 1292.31 (C-NO_2), 1446.61((-C=N-N=C, aromatic conjugation), 1342.46 and 1165.00 (C-SO_2, sulfonamide moiety); ^{1}H-NMR (DMSO-d_6) δ ppm:1.71(s, 3H, C$\underline{H}_3$), 7.24-8.32 (m, 12H, Ar-<u>H</u>), 8.03 (s, 1H, -N<u>H</u>-, isatin); CHNS elemental Analysis for $C_{23}H_{17}N_7O_6S$, Calcd.: C, 53.18; H, 3.30; N, 18.87; S, 6.17%. Found: C, 53.13; H, 2.88; N, 20.03; S, 6.46%.

5-(4- (N-(5-methylisoxazol-3-yl) sulfamoyl) phenyl)-3-(4-nitrophenyl)-1-(2-oxoindolin -3- ylidene) formazan (2c)

Sulfamethoxazole (4.5 mmol, 1.14gm) was dissolved in hydrochloric acid (10mL, 50% v/v) and complete the preparation as mentioned in the general procedure described above and shown in scheme 1. Dark reddish brown solid; Yield: (60%); m.p. 213-216°C; FTIR (υ, cm^{-1}): 3174.83 (N-H, isatin), 1735.93 (C=O, isatin), 1600.92 (N=N, azo group), 1516.05 and 1288.45 (C-NO_2), 1446.61((-C=N-N=C, aromatic conjugation), 1342.46 and 1165.00 (C-SO_2, sulfonamide moiety); ^{1}H-NMR (DMSO-d_6) δ ppm:1.18(s, 3H, C$\underline{H}_3$), 6.4(s, 1H, oxazole ring), 7.25-8.33 (m, 12H, Ar-<u>H</u>), 8.03 (s, 1H, -N<u>H</u>-, isatin); CHNS elemental Analysis for $C_{25}H_{18}N_8O_6S$, Calcd.:C, 53.76; H, 3.25; N, 20.06; S, 5.74%. Found: C, 54.24; H, 2.94; N, 21.31; S, 5.97%.

3-(4-nitrophenyl)-1-(2-oxoindolin-3-ylidene)-5-(4-(N-(pyrimidin-2-yl) sulfamoyl) phenyl) formazan (2d)

Sulfadiazine (4.5 mmol, 1.126gm) was dissolved in hydrochloric acid (10mL, 50% v/v) and complete the preparation as mentioned in the general procedure described above and shown in scheme 1. Dark brown solid; Yield: (61.6%); m.p. 245-247°C; FTIR (υ, cm^{-1}): 3167.12 (N-H, isatin), 1735.93 (C=O, isatin), 1600.92 (N=N, azo group), 1516.05 and 1292.31 (C-NO_2),

1446.61((-C=N-N=C, aromatic conjugation), 1342.46 and 1165.00 (C-SO_2, sulfonamide moiety); ^{1}H-NMR (DMSO-d_6) δ ppm: 7.01 -8.55 (m, 12H, Ar-H), 8.02 (s, 1H, -NH-, isatin); CHNS elemental Analysis for $C_{25}H_{17}N_9O_5S$, Calcd.: C, 54.05; H, 3.08; N, 22.69; S, 5.77%. Found: C, 55.02; H, 2.83; N, 23.42; S, 6.09%.

Anti-microbial Activity

The four synthesized azo compounds (2a-d) were evaluated for their antimicrobial activity using well diffusion technique[22] against four Gram-negative and Gram-positive bacterial strains; *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Staphylococcus aureus* and one fungus (*Candida albicans*). The tested azo compounds and reference drugs were dissolved in dimethyl sulfoxide (DMSO) to obtain different concentrations (400, 200, 100, 50, 25 and 12.5 µg/mL). The inhibition zone (IZ) at 400µg/mL concentration was calculated in mm and compared with that of reference drugs amoxicillin, sulfamethoxazole, and fluconazole, as well as, the minimum inhibitory concentration (MIC) was determined by the lowest concentration of the sample that retarded the growth of microorganism and compared with that of reference drugs amoxicillin, sulfamethoxazole, and fluconazole. The microorganisms were isolated from humans with characteristic infectious diseases. The antimicrobial activity was screened in the National Center for Drug Control and Research/College of Education for Pure Sciences Ibn Al-Haitham/University of Baghdad.

RESULTS AND DISCUSSION

Chemistry

Isatin hydrazone named 3-hydrazono-indolin-2-one was formed by simple condensation of isatin with 80% hydrazine hydrate; an excess amount of hydrazine hydrate was used to act as a catalyst as well. Bis- Schiff base (Compound 1; 3-((4-nitrobenzylidene) hydrazono)indolin-2-one) was prepared by condensation of p-nitrobenzaldehyde with 3-hydrazono-indolin-2-one using 2 drops of TEA as a catalyst. The target azo compounds (2a-d) were synthesized by coupling of diazonium salts of four different sulfonamides (sulfanilamide, sulfacetamide, sulfamethoxazole, and sulfadiazine), separately with 3-((4-nitrobenzylidene) hydrazono)indolin-2-one (bis-Schiff base) maintaining the pH of the reaction mixture from 7 to 7.5 (Scheme 1). Excess amount of nitrous acid was destroyed by the addition of 2% sulfamic acid during diazotization process.

The structures of prepared azo compounds have been confirmed by FTIR, ^{1}HNMR, and elemental analysis. The infrared (IR) spectra of the prepared starting material (3-hydrazono-indolin-2-one) showed a sharp absorption band at 3437.15 cm^{-1} corresponding to NH_2 group stretching vibration and band at 1685.79 cm^{-1} with respect to the carbonyl of isatin amide group, strong band at 1558.48 cm^{-1} attributed to the presence of the characteristic azomethine -CH=N linkage in the isatin hydrazone compound. The disappearance of the NH band at 3437.15 cm^{-1} due to primary amine stretching vibration NH_2 in the IR spectra of compound (1) indicates the bis-Schiff base formation, moreover, the appearance of two bands at 1516.05 cm^{-1} and 1310.00cm^{-1} for compound 1 were assigned to C-NO_2 stretching vibration. The ^{1}HNMR of compound (1) showed a singlet band within 8.69 ppm represent one proton of (N-N=CH) azomethine group.

The FTIR spectra of synthesized azo compounds (2a-d) were shown a characteristic band within the 1600.92 cm^{-1} due to (N=N) stretching vibration that predominantly indicates the formation of azo group. The two sharp bands at 1342.46cm^{-1} and 1165.00cm^{-1} were assigned to (C-SO_2) stretching vibration represent the presence of sulfonamide moiety.

The ^{1}HNMR of compounds (2a-d); azo compounds (2a-c) exhibit a multiplet band in the region of 7.24-8.33 ppm due to the presence of aromatic protons, while the signals in the range (7.01–8.55 ppm) for compound 2d were assigned for multiplet H of aromatic rings. The disappearance of the signal at 8.69 ppm for the azomethine group indicates the formation of azo compounds. The elemental analysis results revealed good approval with the calculated percentages and the percent deviations of the found/calculated values within acceptable limits.

Table 1: Inhibition zone (IZ) in mm of new azo compounds at 400 µg/mL concentration for different microorganisms

	Inhibition zone(IZ) in mm				
	Bacterial strains				
	Gram-negative			*Gram-positive*	*Fungi*
Compounds	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Candida albicans*
2a	13	-	-	-	22
2b	14	14	-	-	25
2c	13	11	-	-	24
2d	15	-	-	-	24
Sulfamethoxazole*	12	20	-	-	-
Amoxicillin*	12	12	-	-	-
Fluconazole**	-	-	-	-	30
DMSO	-	-	-	-	-

* Standard for bacterial strains, ** Standard for fungi.

(-) = No activity, slightly active (IZ = 5–10 mm), moderately active (IZ = 10-15 mm), highly active (IZ = more than 15 mm).[24,25]

Table 2: The Minimum inhibitory concentration (MIC) in µg/mL of the azo compounds against different microorganisms

	Minimum inhibitory concentration(MIC)in µg/mL				
	Bacterial strains				
	Gram-negative			*Gram-positive*	*Fungi*
Compounds	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Candida albicans*
2a	100	-	-	-	25
2b	50	100	-	-	12.5
2c	50	200	-	-	25
2d	100	-	-	-	25
Sulfamethoxazole*	50	50	-	-	-
Amoxicillin*	100	100	-	-	-
Fluconazole**	-	-	-	-	12.5
DMSO	-	-	-	-	-

* Standard for bacterial strains, ** Standard for fungi, (-) =No activity

Antimicrobial Evaluation

Inhibition Zone (IZ) and Minimum Inhibitory Concentration (MIC)

The synthesized azo compounds were screened for antimicrobial activity; the data from Table 1 illustrate that all the synthesized compounds were inactive against *Klebsiella pneumoniae* and *Staphylococcus aureus*, but they clearly inhibited *Escherichia coli* with moderate activity. The most active azo compounds with the lowest MIC against *Escherichia coli* were the 2b and 2c compounds (Table 2), while only compounds 2b and 2c show good antibacterial activity against *Pseudomonas aeruginosa*, and the MIC of compound 2b (100 µg/mL) was lower than that of 2c (200 µg/mL), which means that compound 2b is more potent than 2c (Table 2). These results were compatible with a previous study that showed azo compounds derived from ethyl vanillin have moderate activity against *Escherichia coli* and less active against *Pseudomonas aeruginosa.*Moreover, the results in table 1 showed significant inhibitory effects of these four compounds as antifungal against *Candida albicans* with their MIC values 12.5-25µg/mL (Table 2), and the most potent one is compound 2b in comparison with reference drug fluconazole.

Finally, the most active compound among the prepared azo compounds against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* is compound 2b.

CONCLUSION

Four new azo compounds derivatives of isatin bis-Schiff base and sulfonamides have been synthesized successfully in good yields and tested for their antimicrobial activity against four bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae,* and *Staphylococcus aureus*) and one fungal strain(*Canadians albicans*). It was concluded that the azo compounds possess moderate activity against *Escherichia coli* and interesting activity against *Canadians albicans,* and the most potent one among the prepared azo compounds is **2b**.

ACKNOWLEDGMENT

The continuous support of Dr. Mohamed Hassan Mohammed head of Pharmaceutical Chemistry Department/College of Pharmacy/University of Baghdad is greatly acknowledged.

REFERENCES

1. Kołaczek A, Fusiarz I, Ławecka J, Branowska D. Biological activity and synthesis of sulfonamide derivatives: a brief review. Chemik. 2014; 68(7):620-628.
2. Mengelers MJ, Hougee PE, Janssen LH, Van Miert AS. Structure-activity relationships between antibacterial activities and physicochemical properties of sulfonamides. Journal of Veterinary Pharmacology and Therapeutics. 1997; 20(4):276-283.
3. Genç Y, Özkanca R, Bekdemir Y. Antimicrobial activity of some sulfonamide derivatives on clinical isolates of Staphylococus aureus. Annals of Clinical Microbiology and Antimicrobials. 2008; 7(1):17.
4. Lavanya R. Sulphonamides: A Pharmaceutical Review. International Journal of Pharmaceutical Science Invention. 2017; 6(2):1-3.
5. Manju P, Neeraj K, Sharma P, Jha KK. Synthetic and biological multiplicity of isatin: A Review. Journal of Advanced Scientific Research. 2011; 2: 35-44.
6. Visagaperumal D, Ezekwem JE, Munji H, Chandy V. Isatin Schiff Base-An Overview. PharmaTutor. 2018; 6(5):38-47.
7. Da Silva CM, da Silva DL, Modolo LV, Alves RB, de Resende MA, Martins CV, de Fátima Â. Schiff bases: A short review of their antimicrobial activities. Journal of Advanced Research. 2011; 2(1):1-8.
8. Jarrahpour A, Khalili D, De Clercq E, Salmi C, Brunel J. Synthesis, antibacterial, antifungal and antiviral activity evaluation of some new bis-Schiff bases of isatin and their derivatives. Molecules. 2007; 12(8):1720-1730.
9. Almudhafar MMJ. Synthesis, Characterization and Preliminary Antimicrobial evaluation of new Schiff bases of Ampicillin and Amoxicillin derived from isatin derivatives, International Journal of Pharmacy and Pharmaceutical Sciences. 2016; 8(5):113-116.
10. Chohan ZH, Pervez H, Rauf A, Khan KM, Supuran CT. Isatin-derived antibacterial and antifungal compounds and their transition metal complexes. Journal of Enzyme Inhibition and Medicinal Chemistry. 2004; 19(5):417-423.
11. Aboul-Fadl T, Bin-Jubair FA. Anti-tubercular activity of isatin derivatives. International Journal of Research in Pharmaceutical Sciences. 2010; 1(2): 113-126.
12. Karalı N, Gürsoy A, Kandemirli F, Shvets N, Kaynak FB, Özbey S, Kovalishyn V, Dimoglo A. Synthesis and structure–antituberculosis activity relationship of 1H-indole-2, 3-dione derivatives. Bioorganic & Medicinal Chemistry. 2007; 15(17):5888-5904.

13. Vine KL, et al. In vitro cytotoxicity evaluation of some substituted isatin derivatives. Bioorganic & Medicinal Chemistry. 2007; 15(2):931-938.
14. Yogeeswari P, Sriram D, Kavya R, Tiwari S. Synthesis and in-vitro cytotoxicity evaluation of gatifloxacin Mannich bases. Biomedicine & pharmacotherapy. 2005; 59(9):501-510.
15. Singh UK, Pandeya SN, Singh A, Srivastava BK, Pandey M. Synthesis and Antimicrobial activity of Schiff's and N-Mannich bases of isatin and its derivatives with 4-Amino-N-Carbamimidoyl benzene Sulfonamide. International Journal of Pharmaceutical Sciences and Drug Research. 2010; 2(2): 151-154.
16. Rathod KM, Thakre NS. Synthesis and antimicrobial activity of azo compounds containing m-cresol moiety. Chemical Science Transactions. 2013; 2(1):25-28.
17. Pathan RU and Borul SB. Synthesis and antimicrobial activity of Azo compounds containing drug moiety. Oriental Journal of Chemistry. 2008; 24(3):1147-1148.
18. Kofie WI, Amengor CD, Adosraku RK. Synthesis and evaluation of antimicrobial properties of azo dyes. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 7(4):398-401.
19. Odabaşoğlu M, Albayrak Ç, Özkanca R, Aykan FZ, Lonecke P. Some polyhydroxy azo–azomethine derivatives of salicylaldehyde: Synthesis, characterization, spectroscopic, molecular structure and antimicrobial activity studies. Journal of Molecular Structure. 2007; 840(1-3):71-89.
20. Coffey KE, Moreira R, Abbas FZ, Murphy GK. Synthesis of 3, 3-dichloroindolin-2-ones from isatin-3-hydrazones and (dichloroiodo) benzene. Organic & Biomolecular Chemistry. 2014; 13(3):682-685.
21. Vogel Arthur, Vogel's Text Book of Practical Organic Chemistry, 5th ed., Longman, London and New York, 1989, 948-950.
22. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 2016; 6(2):71-79.
23. Pagariya K, Pathade M, Bodkhe S. Synthesis, Characterization and Antimicrobial screening of some azo compounds derived from Ethyl vanillin. Research Journal of Chemical Sciences. 2015; 5(7):20-28.
24. Dabholkar VV, Gavande RP. Synthesis and antimicrobial activities of novel 1, 4-benzothiazine derivatives. Arabian Journal of Chemistry. 2016; 9: S225-S229.
25. Azab M, Youssef M, El-Bordany E. Synthesis and antibacterial evaluation of novel heterocyclic compounds containing a sulfonamido moiety. Molecules. 2013; 18(1):832-844.