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RESEARCH ARTICLE

Synthesis, Characterization, and Antimicrobial Activity of New Schiff's and Mannich Bases of Isatin and Isatin Derivatives

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Abstract

Objective: Schiff's and Mannich bases of isatins are an important group of heterocyclic compounds which are of great importance in medicinal chemistry as antimicrobial agents. In the vision of these facts, new bis-Schiff bases and Mannich bases of isatins were synthesized. Methods: Three different bis-Schiff bases (3a-c) have been synthesized by reacting isatin, 5-fluoroisatin and 5-methoxy isatin with thiophene-2-carboxaldehyde using hydrazine hydrate to link between the carbonyl compounds, and then these bis-Schiff bases were condensed with two different secondary amines (piperidine and morpholine) separately, and formaldehyde to form the Mannich bases (4a-c and 5a-f), respectively. Results: The structures of the newly synthesized compounds were confirmed using (Fourier- transform infrared) FTIR, ^{1}H-nuclear magnetic resonance spectroscopy (^{1}H-NMR) and CHNS elementary analysis. All the synthesized bis-Schiff bases and Mannich bases were screened for their antimicrobial activities. Conclusion: The target compounds showed convincing antibacterial activity, whereas their antifungal activity was more potent, and the most active ones were Mannich bases of 5-methoxy isatin derivatives (4c and 5c) against *Candida albicans*.

Keywords: *Antimicrobial activity, Bis-Schiff bases, Isatin derivatives, Mannich bases, Thiophene-2-carboxaldehyde.*

Introduction

The efforts for synthesis new compounds as useful therapeutic agents in patients with bacterial or fungal infections remain a challenge for many researchers in the medicinal field [1, 2]. Many studies have been focused on heterocyclic compounds with effective antimicrobial activity. Isatin, naturally occurring compound, is an indole derivative isolated in 1988 [3] and reported to possess a wide range of biological activities, as well as isatin analogues, are important groups of heterocyclic compounds of significant importance in medicinal chemistry [4, 6]. In the last decade, Schiff's and Mannich bases of isatins have attracted considerable interest not only because of their convenient chemical synthesis but also because of their various pharmacological properties such as antibacterial, antifungal [7, 9], anti-HIV [10, 11], antiviral [12], anticonvulsant [13, 14], anti-tubercular [15, 17] and anticancer [18, 21], as well as copper (II) complexes of isatin Schiff bases affect cellular viability [22]. Different Schiff bases were synthesized from ampicillin and amoxicillin with isatin derivatives showed improvement of parent antibiotics antibacterial activity [23]. Moreover, bis-Schiff bases of isatin showed antioxidant activity [24] and various antimicrobial activities [25, 27]. A new study reported that bis-isatin Schiff base ligands and their coordination complexes showed moderate to strong antimicrobial activity [28]. Thiophene-2-carboxaldehyde Schiff bases and their metal complexes exhibit antimicrobial activity [29, 31]. Other study reveals that asymmetric thienyl- tetradentate Schiff bases and their copper (II) and zinc (II) metal complexes showed interesting antibacterial and antifungal activities [32]. The previous investigations of different studies on the antimicrobial activity of Schiff's and Mannich bases of isatins encouraged the researchers of the present study to continue the research of screening of new derivatives of isatins for their antimicrobial activity, this is achieved by synthesis of bis-Schiff bases (**3a-c**) of

isatin analogues (**1a-c**) with thiophene-2-carboxaldehyde using hydrazine hydrate, and these bis- Schiff bases were condensed with two different secondary amines (piperidine and morpholine) and formaldehyde to form the Mannich bases (**4a-c** and **5a-c**), respectively. The designated compounds were evaluated for their antimicrobial activity.

Materials and Methods

General

All chemicals and solvents used were of the analytical grade used without further purification. Isatin (**1a**) was purchased from Hi Media Laboratories/ India, 5-fluoroisatin (**1b**), 5-methoxy isatin (**1c**), and thiophene-2-carboxyaldehyde were obtained from Hangzhou Hyper Chemicals Limited/ China. Hydrazine hydrate 80% was from Alpha Chemika/ India. Uncorrected melting points were determined on Stuart SMP30 Electronic Melting Point Apparatus. The FT-IR spectra were recorded on FTIR spectrophotometer/ Shimadzu, Japan, supplied by Specac® Quest ATR (diamond)-UK. CHNS elemental analyses were performed by Vario MICRO CUBE elemental analyzer/ Germany. ^{1}H-NMR spectra were recorded in DMSO-*d6* on NMReady-60 PRO, Canada.

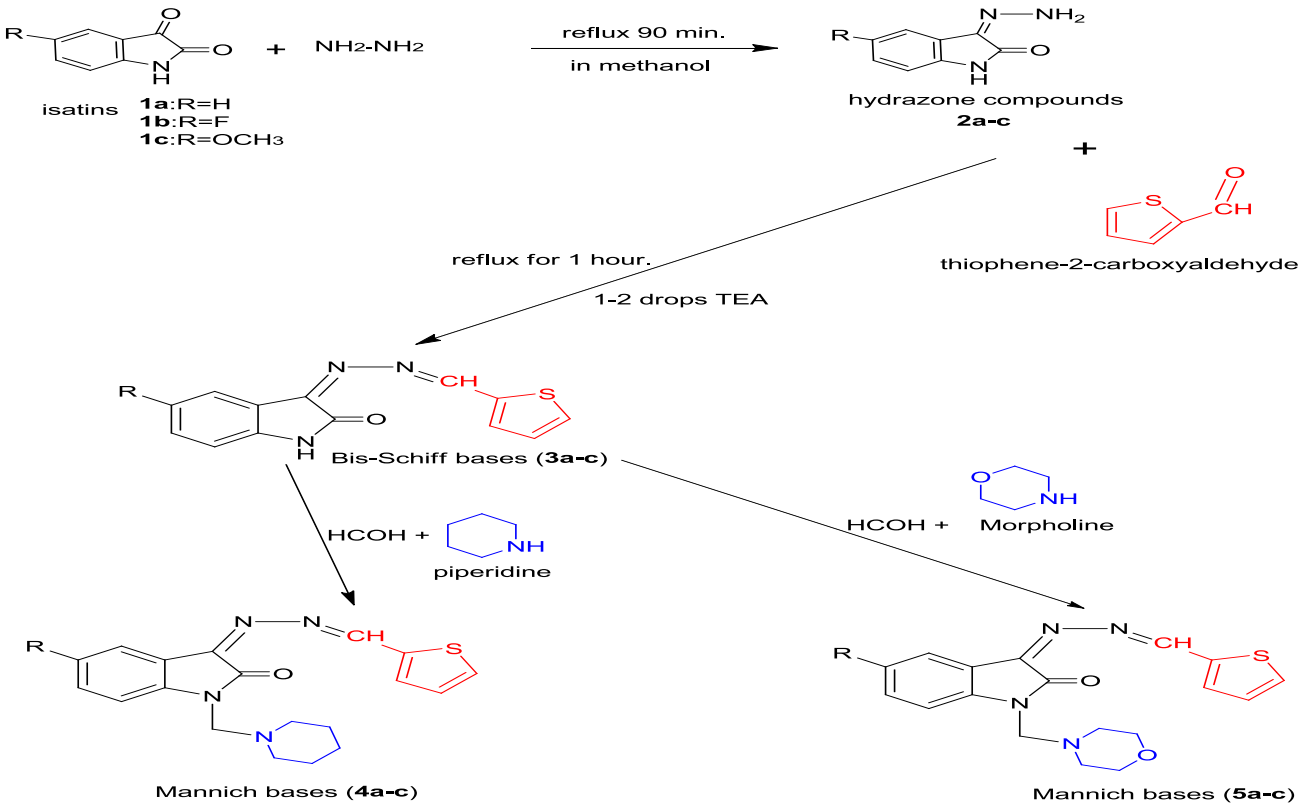
Chemical Synthesis

General Procedure for the Synthesis of 3-hydrazono-indolin-2-one Compounds (2a-c)

The hydrazone compounds were prepared by mixing isatin (**1a**) or its derivatives (5-fluoroisatin (**1b**) and 5-methoxyisatin (**1c**), 13.6 mmol) with an excess amount of hydrazine hydrate 80% in 20ml of methanol and refluxed for 90 minutes. The obtained product was poured on crushed ice water; the separated precipitate was filtered and washed with distilled water for several times.

3-hydrazono-indolin-2-one (2a)

Isatin (**1a**) (13.6mmol, 2gm) was reacted with 20 mL of hydrazine hydrate as previously described, as showed in scheme 1, for purification; the separated precipitate was washed with hot distilled water and then methanol, dried and recrystallized from ethanol. Yellow solid; Yield: (75%); m.p. 230-233°C (Literature [33] 231-232 °C); FTIR (υ, cm^{-1}): 3437 (N-H, hydrazone), 3140 (N-H, isatin), 1689 (C=O, isatin), 1585 (C=N, imine).



Scheme 1: Synthesis of Bis-Schiff bases and Mannich bases

5- Fluoro-3- Hydrazonoindolin-2-one (2b)

5-fluoroisatin (**1b**) (13.6mmol, 2.25gm) was reacted with 20mL of hydrazine hydrate as previously described, as showed in scheme 1, for purification; the separated precipitate was washed with hot distilled water and then methanol, dried and recrystallized from ethanol. Dark yellow solid; Yield: (76.5%); m.p. 236-238°C (Literature [33] 239-241°C); FTIR (υ, cm^{-1}): 3352 (N-H, hydrazone), 3140 (N-H, 5-fluoroisatin), 1681 (C=O, 5-fluoroisatin), 1647 (C=N, imine).

5-Methoxy- 3- Hydrazonoindolin-2-one (2c)

5-methoxyisatin (**1c**) (13.6mmol, 2.41gm) was reacted with 20mL of hydrazine hydrate as previously described, as shown in scheme 1, for purification; the separated precipitate was washed with cold distilled water many times, dried and recrystallized from ethanol. Yellowish-orange solid; Yield: (80%); m.p. 188-191°C (Literature [33] 186-188 °C); FTIR (υ, cm^{-1}): 3387 (N-H, hydrazone), 3151 (N-H, 5-methoxyisatin), 1685 (C=O, 5-methoxyisatin), 1666 (C=N, imine).

General Procedure for the Synthesis of bis- Schiff bases (3a-c)

Hydrazone compounds (**2a-c)** (6.2 mmol) were dissolved in 30mL of methanol (dried with anhydrous $CaCl_2$) to this solution (6.82 mmol) of thiophene-2-carboxaldehyde was added, the pH of the solution was adjusted from 7-8 by adding 1-2 drops of trimethylamine (TEA), then the mixture was refluxed for 1hour then stirring for another 1hour at room temperature, the progress of reaction was monitored by TLC. The separated product was filtered and washed with warm methanol, twice. After drying, it was recrystallized from hexane: ethanol (8:1) to give compounds **3a-c**.

3- ((Thiophen-2- ylmethylene) Hydrazono) indolin-2-one (3a)

3-hydrazono-indolin-2-one (**2a**) (6.2 mmol, 1gm) was reacted with thiophene-2-carboxaldehyde (6.82 mmol, 0.765gm) as previously described, as shown in scheme 1. Faint brown solid; Yield: (70%); m.p. 253-255°C; FTIR (υ, cm^{-1}): 3136 (N-H, Isatin), 1710 (C=O, Isatin), 1597 (C=N, imine), 1450 (aromatic-C=N-N=C, conjugation), 709 (C-S, thiophene); ^{1}H-NMR δ ppm: 7.26 (s, 1H, N-N=C<u>H</u>), 7.19- 7.86(m, Ar-<u>H</u>), 8.01 (s, 1H, amide). CHNS analysis for $C_{13}H_9N_3OS$, Calcd.: C, 61.16; H, 3.55; N, 16.46; S, 12.56%. Found: C, 60.19; H, 3.23; N, 16.06; S, 12.86%.

5- Fluoro-3- ((thiophen- 2-ylmethylene) hydrazono) indolin-2-one (3b)

5-fluoro-3-hydrazonoindolin-2-one (**2b**) (6.2 mmol, 1.11gm) was reacted with thiophene-2-carboxaldehyde (6.82 mmol, 0.765gm) as previously described, as shown in scheme 1. Dark brown solid; Yield: (81.8%); m.p. 159-162°C; FTIR (υ, cm^{-1}): 3147 (N-H, 5-fluoroisatin), 1685(C=O, 5-fluoroisatin), 1581 (C=N, imine), 1470 (aromatic -C=N-N=C, conjugation), 680 (C-S, thiophene); ^{1}H-NMR δ ppm: 6.79 (s, 1H, N-N=C<u>H</u>), 6.88- 7.78(m, Ar-<u>H</u>), 7.91 (s, 1H, amide). CHNS analysis for $C_{13}H_8FN_3OS$, Calcd.: C, 57.13; H, 2.95; N, 15.38; S, 11.73%. Found: C, 57.82; H, 3.05; N, 15.09; S, 11.98%.

5-methoxy-3-((thiophen-2-ylmethylene) hydrazono)indolin-2-one (3c)

5-methoxy-3-hydrazonoindolin-2-one (**2c**) (6.2 mmol, 1.18gm) was reacted with thiophene-2-carboxaldehyde (6.82 mmol, 0.765gm) as previously described, as shown in scheme 1. Dark brown solid; Yield: (81.6%); m.p. 137-139°C; FTIR (υ, cm^{-1}): 3163 (N-H, 5-methoxyisatin), 2850(-OCH_3), 1681(C=O, 5-methoxyisatin), 1597 (C=N, imine), 1480 (aromatic-C=N-N=C, conjugation), 709 (C-S, thiophene); ^{1}H-NMR δ ppm: 6.71 (s, 1H, N-N=C<u>H</u>), 6.84 to 7.80(m, Ar-<u>H</u>), 8.03 (s, 1H, amide), 3.89 (s, 3H, O<u>CH</u>$_3$). CHNS analysis for $C_{14}H_{11}N_3O_2S$, Calcd.: C, 58.93; H, 3.89; N, 14.73; S, 11.24%. Found: C, 59.55; H, 3.76; N, 14.88; S, 11.45%.

General Procedure for the Synthesis of Mannich bases (4a-c) and (5a-c)

A suspended mixture consisting of 2.12 mmol of bis-Schiff bases (**4a-c**), separately, 5 ml of ethanol and 1mL of 37% formaldehyde was made. To this mixture, a secondary amine, piperidine or morpholine (2.33mmol), was added dropwise with cooling and shaking. The reaction mixture was allowed to stand at room temperature for 1 hour with stirring after that the mixture was warmed on a steam bath for 30 minutes, then leaving the mixture over two nights in the refrigerator. The products were separated, washed with distilled water several times, dried, washed with ether and petroleum ether and then recrystallized from ethanol.

1-(piperidin-1-ylmethyl)-3-((thiophen-2-ylmethylene) hydrazono) indolin-2-one (4a)

3-((thiophen-2-ylmethylene)hydrazono)indolin-2-one (**3a**) (2.12mmol, 0.54g) was reacted with 1mL of formaldehyde and piperidine (2.33mmol, 0.2gm) as mentioned previously, as shown in scheme 1. Dark brown solid; Yield: (53.5%); m.p. 281-284°C; FTIR (υ, cm^{-1}): 2974 (C-H, Stretching, CH_2, asy.), 2843 (C-H, Stretching, CH_2, sy.), 1705(C=O, isatin), 1597 (C=N, imine); ^{1}H-NMR δ ppm: 7.23 (s, 1H, N-N=C<u>H</u>) 7.19 to 7.90 (m, Ar-<u>H</u>), 4.23(s,2H, CH_2), 1.23-2.36(m, 10H, piperidine); CHNS analysis for $C_{19}H_{20}N_4OS$, Calcd.: C, 64.75; H, 5.72; N, 15.90; S, 9.10%. Found: C, 65.09; H, 5.54; N, 16.21; O, 4.54; S, 9.72%.

5-fluoro-1-(piperidin-1-ylmethyl)-3-((thiophen-2-ylmethylene) hydrazono) indolin-2-one (4b)

5-fluoro-3-((thiophen-2-ylmethylene)hydrazono)indolin-2-one(**3b**) (2.12mmol, 0.54g) was reacted with 1mL of formaldehyde and piperidine (2.33mmol, 0.2gm) as mentioned previously and as shown in scheme 1. Faint yellow solid; Yield: (50.2%); m.p. 124-126°C; FTIR (υ, cm^{-1}): 2935 (C-H, Stretching, CH_2, asy.), 2854(C-H, Stretching, CH_2, sy.), 1697(C=O, isatin), 1604 (C=N, imine); ^{1}H-NMR δ ppm: 7.00 (s, 1H, N-N=C<u>H</u>), 7.18 to 7.89 (m, Ar-<u>H</u>), 4.44 (s,2H, CH_2), 1.42-2.39(m, 10H, piperidine); CHNS analysis for $C_{19}H_{19}FN_4OS$, Calcd.: C, 61.6; H, 5.17; N, 15.12; S, 8.66%. Found: C, 62.51; H, 5.23; N, 15.46; S, 8.85%.

5-methoxy-1-(piperidin-1-ylmethyl)-3-((thiophen-2-ylmethylene) hydrazono) indolin-2-one (4c)

5-methoxy-3-((thiophen-2-ylmethylene)hydrazono)indolin-2-one(**3c**) (2.12mmol, 0.54g) was reacted with 1mL of formaldehyde and piperidine (2.33mmol, 0.2gm) as mentioned previously, as shown in scheme 1. Dark brown solid; Yield: (66%); m.p. 109-112°C; FTIR (υ, cm^{-1}): 2931 (C-H, Stretching, CH_2, asy.), 2850(C-H, Stretching, CH_2, sy.), 1685(C=O, 5-methoxyisatin), 1597 (C=N, imine); ^{1}H-NMR δ ppm: 7.10 (s, 1H, N-N=C<u>H</u>), 7.11 to7.78 (m, Ar-<u>H</u>), 4.43(s,2H, CH_2), 3.70 (s, 3H, OC<u>H</u>$_3$), 1.20 -2.44 (m, 10H, piperidine); CHNS analysis for $C_{20}H_{22}N_4O_2S$, Calcd.: C, 62.80; H, 5.80; N, 14.65; S, 8.38%. Found: C, 63.09; H, 5.66; N, 15.07; S, 8.79%.

1- (morpholinomethyl)- 3- ((thiophen- 2-ylmethylene) hydrazono) indolin-2-one (5a)

3-((thiophen-2-ylmethylene)hydrazono)indolin-2-one (**3a**) (2.12mmol, 0.54g) was reacted with 1mL of formaldehyde and morpholine (2.33mmol, 0.203gm) as mentioned previously, as shown in scheme 1. Faint brown solid; Yield: (52%); m.p. 242-244°C; FTIR (υ, cm^{-1}): 2947 (C-H, Stretching, CH_2, asy.), 2846 (C-H, Stretching, CH_2, sy.), 1708(C=O, isatin), 1616 (C=N, imine); ^{1}H-NMR δ ppm: 7.05 (s, 1H, N-N=C<u>H</u>), 7.19 to7.87 (m, Ar-<u>H</u>), 4.33(s,2H, CH_2), 2.55 to 3.77 (m, 8H, morpholine); CHNS analysis for $C_{18}H_{18}N_4O_2S$, Calcd.: C, 61.00; H, 5.12; N, 15.81; S, 9.05%. Found: C, 61.81; H, 5.23; N, 16.21; S, 9.41%.

5-fluoro-1-(morpholinomethyl)-3-((thiophen-2-ylmethylene) hydrazono) indolin-2-one (5b)

5-fluoro-3-((thiophen-2-ylmethylene)hydrazono)indolin-2-one(**3b**) (2.12mmol,0.54g) was reacted with 1mL of formaldehyde and morpholine (2.33mmol, 0.203gm) as mentioned previously and as shown in scheme 1. Yellow solid; Yield: (50.6%); m.p. 182-184°C; FTIR (υ, cm^{-1}): 2954 (C-H, Stretching, CH_2, asy.), 2854 (C-H, Stretching, CH_2, sy.), 1705(C=O, isatin), 1608 (C=N, imine); ^{1}H-NMR δ ppm: 6.99 (s, 1H, N-N=C<u>H</u>), 7.22 to7.88(m, Ar-<u>H</u>), 4.24(s,2H, CH_2), 2.45-3.65(m, 8H, morpholine); CHNS analysis for $C_{18}H_{17}FN_4O_2S$, Calcd.: C, 58.05; H, 4.60; N, 15.04; S, 8.61%. Found: C, 58.87; H, 4.43; N, 15.76; S, 8.85%.

5-methoxy-1-(morpholinomethyl)-3-((thiophen-2-ylmethylene) hydrazono) indolin-2-one (5c)

5-methoxy-3-((thiophen-2-ylmethylene)hydrazono)indolin-2-one(**3c**) (2.12mmol, 0.54g) was reacted with 1mL of formaldehyde and morpholine (2.33mmol, 0.203gm) as mentioned previously, as shown in scheme 1. Dark brown solid; Yield: (63%); m.p. 83-86°C; FTIR (υ, cm^{-1}): 2954 (C-H, Stretching, CH_2, asy.), 2850 (C-H, Stretching, CH_2, sy.), 1693(C=O, isatin), 1597 (C=N, imine); ^{1}H-NMR δ ppm: 7.04 (s, 1H, N-N=C<u>H</u>), 6.97 to7.66 (m, Ar-<u>H</u>), 4.44(s,2H, CH_2), 3.74 (s, 3H, OC<u>H</u>$_3$) 2.80 to 3.61 (m, 4H, morpholine); CHNS analysis for $C_{19}H_{20}N_4OS$, Calcd.: C, 59.36; H, 5.24; N, 14.57; S, 8.34%. Found: C, 60.16; H, 5.11; N, 14.92; S, 8.61%.

In Vitro Antimicrobial Screening

The newly synthesized compounds (bis-Schiff bases and Mannich bases) were evaluated for antimicrobial activity as primary screening in two different concentrations against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia*) bacteria and (*Candida albicans*) fungi by using well diffusion technique [34]. The inhibition zone (IZ) was measured in mm and compared with that of standard drugs amoxicillin, sulfamethoxazole, and fluconazole. All the tested compounds and standard drugs were dissolved in dimethyl sulfoxide (DMSO) to give two different concentrations of 250 and 500µg/mL. The *in vitro* antimicrobial activity was evaluated in the National Center for Drug Control and Research / University of Baghdad /College of Education for Pure Sciences Ibn Al-Haitham.

Results and Discussion

Chemical Synthesis

The designed bis-Schiff bases (**3a-c**) were obtained through 2 steps reaction as described in Scheme 1. The first step, the treatment of isatins (**1a-c**) with 80% hydrazine hydrate in methanol resulted in the formation of hydrazone compounds (**2a-c**) [30]. The second step, the nucleophilic addition of the primary amino group of hydrazone compounds (**2a-c**) to thiophene-2-carboxaldehyde in slightly basic media (pH 7-8) in methanol with reflux for 1hr. Mannich bases (**4a-c**) and (**5a-c**) were formed by condensing the acidic imino group of the synthesized bis-Schiff bases (**3a-c**) with formaldehyde and piperidine or morpholine, as illustrated in Scheme 1. The chemical structures of the newly synthesized bis-Schiff bases and Mannich bases were confirmed by FTIR and ^{1}H-NMR and CHNS elemental analysis. The FTIR spectra; Hydrazone compounds **2a**, **2b** and **2c** showed characteristic absorption bands at 3437, 3352 and 3387cm^{-1} region, respectively, resulting from the stretching vibration of NH_2 group of hydrazones, other IR bands for these compounds displayed at 1585-1685cm^{-1} was due to imine group (C=N) stretching vibration.

For bis-Schiff bases **3a**, **3b**, and **3c** showed IR absorption peaks attributed to (C=N) stretching vibrations at 1597, 1581, and 1597 cm^{-1}, respectively, while the IR bands for the (-NH_2, hydrazones) stretching vibration were disappeared, other characteristic IR bands due to highly conjugation of these compounds were around 1450-1480cm^{-1}, the (C-S) IR bands for these bis- Schiff bases were around 680-709 cm^{-1}. In the IR spectra of Mannich bases (**4a-c**) and (**5a-c**), the isatins N-H functions of bis-Schiff bases found in the 3136-3163cm^{-1} region were disappeared, while the new IR bands for (**4a-c**) compounds displayed at 2931-2974cm^{-1} and for (**5a-c**) were found in 2947-2954cm^{-1}region were assigned to (N-CH_2-N) asymmetric vibration, while the symmetric vibration(-CH_2) for these compounds (**4a-c** and **5a-c**) appeared in 2843-2854cm^{-1} which confirmed the formation of Mannich bases. The ^{1}H-NMR spectra of these synthesized bis-Schiff bases (**3a**, **3b**, and **3c**) showed a single peak was assigned to one proton of (N-N=CH) appeared at 6.71, 6.79 and 7.26 ppm, respectively, confirming the formation of azomethine compounds. Mannich bases; compounds (**4a-c** and **5a-c**) displayed characteristic (N-CH_2-N) signals around 4.23- 4.44 ppm of two protons as one singlet represents the formation of methylene derivatives. The elemental analysis achieved good agreement with the calculated percentages and the percent deviations of the found/calculated values conform to the accepted limits.

In Vitro Antimicrobial Evaluation

From the results in table 1, all the synthesized compounds showed moderate antibacterial activity against *E. coli* at 500µg/mL and most of them moderately active against *K. pneumonia* of the same concentration comparable to the standard drugs (amoxicillin and sulfamethoxazole), furthermore the 5-methoxy isatin derivatives (**3c**, **4c**, and **5c**) at 500µg/mL showed potent activity against *S. aureus* compared to amoxicillin reference drug. All the targeting compounds were not effective against Gram-negative bacteria (*P. aeruginosa*) even the standard drugs. Whereas, antifungal activity against *C. albicans*; the tested compounds exhibited moderate to high activity at 250 and 500µg/mL, respectively, but less effective than fluconazole drug (standard).

The Mannich bases of 5- substituted isatin derivatives (**4b**, **4c**, **5b**, and **5c**) showed good antifungal activity and the most potent ones were Mannich bases of 5-methoxy isatin derivatives (**4c** and **5c**) with an inhibition zone of 21mm at 500µg/mL, these results

seem rational because a previous study was revealed that the substitution at 5th position of isatin would increase its antimicrobial activity [35], as well as, *N*-Mannich bases of isatins were effective in causing a marked enhancement in their antibacterial activity [17]. Finally, the obtained data in Table 1 proved that these isatin derivatives showed better antifungal activity than their antibacterial one.

Table 1: Antimicrobial activities of the bis-Schiff bases (3a-c) and Mannich bases (4a-c and 5a-c), in two different concentrations (250 and 500 µg/mL)

Compound	Inhibition zone (IZ) in mm									
	Gram-positive		Gram-negative						Fungi	
	S. aureus		*E. coli*		*K. pneumonia*		*P. aeruginosa*		*C. albicans*	
	250	500	250	500	250	500	250	500	250	500
3a	-	-	-	11	-	-	-	-	18	18
3b	-	-	11	13	-	-	-	-	12	17
3c	16	25	-	13	-	12	-	-	15	19
4a	-	-	12	12	12	12	-	-	16	16
4b	-	-	11	12	-	12	-	-	12	20
4c	12	18	11	12	-	-	-	-	14	21
5a	14	17	12	13	-	12	-	-	15	15
5b	-	-	-	13	13	13	-	-	15	18
5c	13	24	-	11	-	11	-	-	14	21
Amoxicillin*	19	26	12	12	11	11	-	-	-	-
Sulfamethoxazole*	-	-	11	11	11	12	-	-	-	-
Fluconazole**	-	-	-	-	-	-	-	-	30	36
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) [36].

Conclusion

New bis- Schiff bases and Mannich bases of isatins have been synthesized and in good yield. The chemical structures of these synthesized compounds (**3a-c**, **4a-c**, and **5a-c**) have been confirmed spectroscopically. The antimicrobial evaluation of the target compounds was performed against Gram-positive and Gram-negative bacteria, as well as fungi using well diffusion technique. 5-methoxy isatin derivatives (**5a-c**) showed interesting activity against *S. aureus* and *C. albicans*. The antifungal activity of all tested compounds seemed to be better than their antibacterial activity. The results of the present study might be helpful in developing new bioactive isatin derivatives as antimicrobial agents.

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