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Synthesis and Antimicrobial Efficacy of Some Novel Heterocyclic Acetyl Istain Derivatives

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Abstract

A series of novel heterocyclic compounds containing N-acetyl isatin moiety were synthesized. Herein, reaction of N-acetyl isatin with water in the presence of a catalytic amount of glacial acetic acid, via a ring opening route, yielded 2-(2-acetamidophenyl)-2-oxoacetic acid, which acts as an adjustable precursor with different heteroaryl amines such as aniline, 2-amino pyridine, 5-amino-1,3,4-thiadiazole-2-thiol and phenylhydrazeine for the preparation of different Schiff bases. Furthermore, acetamide derivatives were prepared via the ring opening path of N-acetyl isatin, by their interaction with 2-aminomethylpyridine, aniline, and hydrazine hydrate. All the prepared samples were confirmed by their spectral data such as FT-IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectrometry, and elemental analysis. Furthermore, all the obtained compounds were evaluated as antibacterial agents against E. coli and S. aureus strains. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations were 0.5-1.8 mlgm-1. The results were promising where the colorimetric INT- formazon proved that the novel synthesized compounds 2d, 3a, 3c and 2b possess potent antibacterial activity against the tested E. coli strain and Staphylococcus aureus (MRSA).

Key words: N-acetylisatin, Schiff bases, NMR, azomethine group, antimicrobial

1. Introduction

Heterocyclic compounds considered one of the most important classes in organic chemistry [1], many of heterocyclic compounds have biological activity against common diseases such as; triazine derivatives have been used as antimicrobial agents [2], anti-inflammatory [3] and urinary antiseptics agents [4].

Isatin derivatives has a large significant as substrates in many chemical reactions due to its utilize for the synthesis of a great variety of heterocyclic compounds, in addition to using as a raw material for drug synthesis [5]. Recently, isatin derivatives have attracted strong interest in organic and medicinal chemistry due to their potent biological and pharmacological activities [6], where the heterocyclic compound of isatin derivatives can be used as antimicrobial and antiviral [7], antifungal [8], antitumor [9], anticancer [10], act as ant proliferative agents [11], antibacterial [12,13], antimalarial [14], and antipyretic properties [15]. Schiff bases are generated by the condensation of a primary amine with a carbonyl compound at variety conditions [16]. Schiff bases of isatin derivatives are reported to show variety of biological activities like antimicrobial [17], anticonvulsant [18], antidepressant [19], and anti-inflammatory activities [20,21], Several of Schiff bases and their complexes have been reported because of their various chemical and biological properties. Additionally, Schiff bases are considered as privileged ligands in the novel preparation of transition metal complexes due to their ability to stabilize the metal ions in various oxidation states and as the property of reversibly bind with oxygen [22]. In view of these facts, this study aimed to synthesis of glycoxalic acid derivative (1) by facile method under green chemistry conditions and using it as precursor for synthesis of a novel series of isatin derivatives, as well as investigation of the antibacterial activity for all synthesized compounds against E. coli strain and Staphylococcus aureus (MRSA) .

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2. Experimental

2.1 Characterization of the synthesized compounds:

All solvents used were of analytical grade. Melting point measurements were done on a Stuart SMP11 and were uncorrected. Thin layer chromatography (TLC) was done on aluminum plates coated with silica gel 60 F (Merck) and was seen using ultraviolet light. FTIR spectra were recorded for KBr disks FT/IR-4100 kind A, and serial Number B117761016. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in ppm at 500 MHz, respectively, using Bruker instrument in DMSO as solvent and using TMS as an internal standard, and chemical shifts are expressed as δ. Mass spectrometry (EI and ESI routes) was determined. At Cairo University, Giza, Egypt, analytical data were achieved from the micro analytical data unit.

2.2. Synthesis of 2-(2-acetamidophenyl)-2-oxoaceticacid (1)

Stirring of a mixture N-acetylisatin (1 gm) and 10 ml H_2O in the presence of glacial acetic acid as a catalyst for 48 h at 30 ^{o}C. the formed solid product was collected by filtration. The final product precipitated was crystallized from mixed solvent ethanol-pet.ether (1:5) to give (1) as white crystals. Yield: 90%, m.p.=155-157^{o}C.^{1}H-NMR(DMSO) δ(ppm)=2.08(S,3H), 7.84-7.27 (m, 4H,Ar-H), 10.56 (S,1H,COOH); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 24.34, 118.29, 122.43, 123.21, 124.23, 124.39, 125.15, 131.65, 134.97, 138.82, 138.99, 165.035, 169.55, 189.24; MS (EI, 70 eV): m/z (%) = 207.05 (M+, 20%); Chemical formula:$C_{10}H_9NO_4$: (207.18); Calcd: C, 57.97; H, 4.38; N, 6.76; O,30.89 Found: C, 57.96; H, 4.37; N, 6.75.

2.3. General procedure for the synthesis of the Schiff bases.

Stirring at room temperature an equimolar ratio mixture (0.001 mol) of 2-(2-acetamidophenyl)-2-oxoaceticacid compound (1) and various arylamines such as aniline, phenyl hydrazine, 2-amino pyridine, p-methoxy aniline, 5-amino-1,3,4-thiadiazole-2-thiol, in CH_2Cl_2without catalyst for 10 to 30 minutes. The reaction was monitored by TLC. The corresponding products precipitated out of solution, filtered and crystallized from ethanol and petroleum ether (1:1) to afford compounds 2(a-e).

2-(2-acetamidophenyl)-2(phenylimino)acetic acid compound (2a)

White crystals; Yield 95%, m.p.105-107^{o}C. ^{1}H-NMR ($CDCl_3$) δ (ppm), 2.27 (S,3H), 2.88 (S,NH), 8.12-6.73 (m,9H,Ar-H), 10.96 (S,COOH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 25.25, 117.76, 120.39, 120.72, 122.77, 125.29, 129.62, 133.80, 136.31, 144.95, 169.02, 169.85, 195.49; MS (EI, 70 eV): m/z (%) = 282.10 (M+, 20%); Chemical formula:$C_{16}H_{14}N_2O_3$: (282.29); Calcd: C, 68.07; H, 5.00; N, 9.29; O,17.00 Found: C, 67.90; H, 4.99; N, 9.19.

2-(2-acetamidophenyl)-2-phenylhydrazono)acetic acid compound (2b)

White crystals; Yield 95%, m. p. 150-152 ^{o}C.^{1}H-NMR (DMSO-d_6) δ (ppm), 2.09 (S,-$COCH_3$), 7.85-7.12 (m, 4H, Ar-H), 10.56 (S,COOH), 13.21 (S,NH), ^{13}C-NMR (100 MHz, DMSO-d_6): δ 23.58, 114.06, 115.69, 122.29, 124.66, 125.99, 129.80, 132.43, 144.11, 165.78, 168.56, 169.45; MS (EI, 70 eV): m/z (%) = 297,11 (M+, 20%); Chemical formula:$C_{16}H_{15}N_3O_3$: (297,31); Calcd: C, 64.64; H, 5.09; N, 14.13; O,16.14; Found: C, 64.61; H, 4.59; N, 14.11.

2-(2-acetamidophenyl)-2-(pyridine-2 ylimino)acetic acid compound (2c)

White crystals; Yield 90%, m. p. 120-122^{o}C. ^{1}H-NMR (DMSO-d_6) δ (ppm), 2.14 (S,$COCH_3$), 7.12-8.50 (m, 8H, Ar-H), 11.60 (S,COOH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 25.19, 25.38, 42.70, 56.52, 57.64, 122.43, 123.18, 127.75, 128.26, 128.87, 129.98, 131.91, 133.82, 134.75, 140.62, 165.68, 168.84, 169.33, 200.48; MS (EI, 70 eV): m/z (%) = 283,10 (M+, 20%); Chemical formula:$C_{15}H_{13}N_3O_3$: (283,28); Calcd: C, 63.60; H, 4.63; N, 14.83; O,16.94; Found: C, 63.50; H, 4.53; N, 14.73.

(Z)-2-(2-acetamidophenyl)-2-((4 methoxyphenyl)imino)acetic acidcompound (2d)

White crystals; Yield 96%, m. p. 90-95 ^{o}C ^{1}H-NMR (DMSO-d_6) δ (ppm) = 2.11(S,$COCH_3$), 3.77(S,-OCH_3), 6.88-8.56 (m, 8H, Ar-H), 11.02(S, COOH). ^{13}C -NMR (100 MHz, DMSO-d_6): δ 20.72, 24.70, 25.39, 117.46, 119.85, 121.91, 123.90, 129.42, 130.02, 134.93, 139.76, 140.42, 145.97, 162.30, 165.76, 169.22, 169.49, 192.69; MS (EI, 70 eV): m/z (%) = 312,11 (M+, 20%); Chemical formula:$C_{17}H_{16}N_2O_4$: (312,32); Calcd: C, 65.38; H, 5.16; N, 8.97; O,20.49; Found: C, 65.26; H, 5.03; N, 8.73.

2-(2-acetamidophenyl)-2-((5-mercapto-1,3,4-thiazol-2-yl)imino)aceticacid compound (2e)

White crystals; Yield 92%, m. p. 150-152 °C. ^{1}H-NMR (DMSO-d_6) δ (ppm), 2.09(S,-$COCH_3$), 7.12-7.85 (m,4H,Ar-H), 10.56 (S,COOH), 13.21 (S,NH), ^{13}C-NMR (100 MHz, DMSO-d_6): δ 24.36, 122.45, 124.27, 124.42, 131.64, 134.97, 138.97, 161.97, 165.05, 169.58, 181.35, 189.28; MS (EI, 70 eV): m/z (%) = 322,02 (M+, 20%); Chemical formula:$C_{12}H_{10}N_4O_3S_2$: (323,02); Calcd: C, 44.71; H, 3.13; N, 17.38; O,14.89; S,19.89; Found: C, 44.61; H, 3.09; N, 17.28.

3.8. General procedure for the synthesis of acetamido compound.

Stirring at room temperature an equimolar ratio mixture (0.001 mol) of N-Acetyl isatin and various aryl amines such as aniline, 2-aminomethyl pyridine, and hydrazine hydrate in CH_2Cl_2 in the presence of glacial acetic acid for 12hrs. The reaction was monitored by TLC. The corresponding products precipitated out of solution, filtered and crystallized from ethanol and petroleum ether (1:1) to afford compounds 3(a-c).

2-(2-acetamidophenyl)-2-oxo-N-(pyridine-2-ylmethyl)acetamide compound(3a)

White crystals; Yield 92%, m. p. 150-152°C.^{1}H-NMR ($CDCl_3$) δ(ppm), 2.23(S,-$COCH_3$), 7.11-8.68 (m,8H,Ar-H), 11.04 (S,NH), ^{13}C-NMR (100 MHz, DMSO-d6): δ 25.48, 44.25, 118.63, 120.68, 121.73, 122.75, 127.88, 134.41, 136.54, 137.02, 142.19, 149.23, 150.22, 155.12, 163.09, 169.30, 191.97; MS (EI, 70 eV): m/z (%) = 297,11 (M+, 20%); Chemical formula:$C_{16}H_{15}N_3O_3$: (297,31); Calcd: C, 64.64; H, 5.09; N, 14.13; O,16.14; Found: C, 64.54; H, 4.59; N, 14.03.

2-(2-acetamidophenyl)-2-oxo-N-phenylacetamide compound (3b)

White crystals; Yield 92%, m. p. 150-152°C.^{1}H-NMR ($CDCl_3$) δ (ppm), 2.27(S,-$COCH_3$), 7.15-8.87(m, 8H, Ar-H), 10.91 (S,NH), ^{13}C-NMR (100 MHz, DMSO-d6): δ 25.48, 118.76, 120.04, 122.71, 125.54, 129.30, 134.48, 136.25, 142.10, 159.94, 169.35, 191.03; MS (EI, 70 eV): m/z (%) = 282,10 (M+, 20%); Chemical formula:$C_{16}H_{14}N_2O_3$: (282,29); Calcd: C, 68.07; H, 5.00; N, 9.92; O,17.00; Found: C, 68.01; H, 4.09; N, 16.78.

2-N-(2-(2-hydraziyl-2-oxoacetyl)phenyl)acetamide compound (3c)

White crystals; Yield 92%, m. p. 150-152°C.^{1}H-NMR (DMSO-d_6) δ (ppm), 2.15(S,-$COCH_3$), 7.28-8.00 (m, 4H, Ar-H), 10.67 (S,NH), 10.99(S, NH), ^{13}C-NMR (100 MHz, DMSO-d_6): δ 24.47, 25.34, 121.72, 123.08, 123.91, 132.96, 134.34, 135.41, 139.54, 141.39, 163.32, 169.37, 169.63, 191.22 MS (EI, 70 eV): m/z (%) = 221,08 (M+, 20%); Chemical formula:$C_{10}H_{11}N_3O_3$: (221,21); Calcd: C,54.29; H, 5.01; N, 19.00; O,21.70; Found: C,54.20; H, 5.00; N, 18.28.

2.4. Biological activity

A weight of 0.3 gm of each compound was dissolved in 1 ml dimethyl sulfoxide (DMSO) and completed with sterile tryptic soy broth medium to 10 ml to avoid the inhibitory effect of DMSO (10-fold dilution) [23].Serial dilutions of all target compounds were prepared overnight cultures of E. Coli and Staphylococcus aureus (MRSA) strains were diluted to 1:10.000 into tryptic soy broth (TSB). 100 µl of bacterial growth was placed into 96-well plates plus 20 µl of each dilution of all compounds. After 24 h incubation at 37°C, the MIC was the lowest concentration that inhibited bacterial growth. To confirm bacterial growth inhibition and lack of metabolic activity, 40 µl of p-iodonitro tetrazolium violet (INT) (0.2 mg ml-1, Sigma-Aldrich) was added to the microplate wells and re-incubated at 37oC for 30 min [24]. The MIC in the INT assay was defined as the lowest concentration that prevented color change as described earlier by [25]. The minimum bactericidal concentration (MBC) test was also performed. The bactericidal effect was defined as a 99.9% decrease in CFU (3 logs) in the starting inoculum during 24 h of incubation. The MBC was determined by transferring 50 µl from each well of overnight MIC plates to sterile tryptic soy agar (TSA) fresh plates. Viable colonies were counted after 24 h at 37°C. The limit of detection for this assay was 10 cfu ml-1[26].

3. Results and discussion

3.1. Chemistry

In this part, novel of glycoxalic acid derivative was synthesized as a precursor of Schiff bases. Treatment of N-acetyl isatin with water as a weak nucleophile in the presence of a catalytic amount of glacial acetic acid at room temperature (30°C) for 48 hrs. afforded 2-(2-acetamidophenyl)-2-oxoaceticacid in a 90 % yield, (Scheme 1). Compound (1) was confirmed based on

its spectral analysis, ^{1}H-NMR, ^{13}C-NMR, mass spectra, and analytical data.^{1}H-NMR(DMSO) δ(ppm), 2.08(S,3H), 7.26-7.83 (m, 4H,Ar-H), 10.56(S,1H, COOH) (Figure 1). Furthermore, 13C-NMR (100 MHz, DMSO-d_6) gave three peaks at 165.03, 165.55 and 189.24 cm-1 due to the three carbonyl groups and one peak at 24.357 due to the methyl group (Figure 2). Additionally, the mass spectrum showed the molecular ion peak at m/z = 207.05 (M+), corresponding to the molecular formula $C_{10}H_9NO_4$. (Scheme 2) shows the mechanism for the formation of compound (1).

It was worth mentioning that the reaction of generated compound (1) with aniline in CH_2Cl_2 at room temperature for 10 minutes without catalyst gave the corresponding imino compound 2-(2-acetamidophenyl)-2-(phenylimino)acetic acid compound (2a) in excellent yield (95%), (Scheme 3). Compound (2a) was confirmed based on its spectral analysis, ^{1}H-NMR, ^{13}C- NMR, mass spectra, and analytical data. ^{1}H-NMR ($CDCl_3$) δ (ppm), 2.26 (S,3H), 2.88 (S,NH), 8.12-6.73 (m, 9H, Ar-H), 10.96 (S, COOH); ^{13}C-NMR (100 MHz, DMSO-d_6) gave two peaks at 169.58, 195.49 cm^{-1} due to the two carbonyl groups other peak at 25.25 corresponding to the methyl group. Also, the mass spectrum indicated the molecular ion peak at m/z = 282.10 (M+), corresponding to the molecular formula $C_{16}H_{14}N_2O_3$.

Stirring an equimolar ratio, a mixture of compound (1) with phenyl hydrazine in CH_2Cl_2 at room temperature for 10minutes without catalyst afforded 2-(2-acetamidophenyl)-3-(2-phenylhydrazono) propanoic acid (2b) in a 98% yield, (Scheme 3). Compound (2b) was confirmed based on their spectral analysis.^{1}H-NMR, ^{13}C NMR, mass spectra, and analytical data. ^{1}H-NMR (DMSO-d_6) δ (ppm) 1.94(S,-$COCH_3$), 6.88-7.68 (m, 9H, Ar-H), 12.43(S, COOH). Additionally, ^{13}C-NMR (100 MHz, DMSO-d_6) for compound (2b) gave two peaks at 169.33, 200.48 cm-1 due to the carbonyl groups.

Moreover, reaction of compound (1) with 2-aminopyridine in CH_2Cl_2 at room temperature for 10minutes without catalyst gave 2-(2-acetamidophenyl)-2-(pyridine-2-ylimino)acetic acid compound (2c)in a 95 % yield, (Scheme 3).Compound (2c) was confirmed based on their spectral analysis,^{1}H-NMR, ^{13}C-NMR, mass spectra, and analytical data. ^{1}H-NMR (DMSO-d6) δ (ppm) 2.15(S,-$COCH_3$), 8.51-7.13(m,4H,Ar-H), 11.61 (S,COOH). Additionally,13C-NMR (100 MHz, DMSO-d_6) for compound (2c) gave two peaks at 169.33, 200.48 cm^{-1} due to the carbonyl groups other peak at 25.38 corresponding to the methyl group. Also, the mass spectrum showed the molecular ion peak at m/z = 283.10 (M+), corresponding to the molecular formula $C_{15}H_{13}N_3O_3$.

Similarly, reaction of compound (1) with p-methoxy aniline in CH_2Cl_2 at room temperature for 10 minutes without catalyst gave (Z)-2-(2-acetamidophenyl)-2-((4-methoxyphenyl)imino)acetic acid compound (2d) in 96 % yield, (Scheme 3). Compound (2d) was confirmed based on their spectral analysis, ^{1}H-NMR, ^{13}C NMR, mass spectra, and analytical data. 1H-NMR (DMSO-d_6) δ (ppm) 2.10(S,-$COCH_3$),6.53-7.96(m,8H,Ar-H), 11.02(S,COOH).Additionally,^{13}C-NMR (100 MHz, DMSO-d_6) for compound (2d) gave two peaks at 169.33, 200.48 cm-1 due to the carbonyl groups other peak at 25.38 corresponding to the methyl group. Also, the mass spectrum showed the molecular ion peak at m/z = 312.11 (M+), corresponding to the molecular formula $C_{17}H_{16}N_2O_4$.

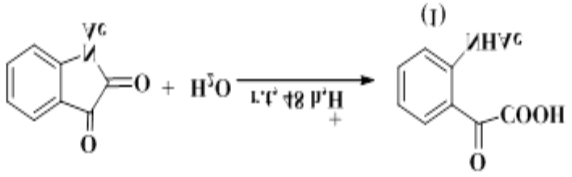
Also the reaction of compound (1) with 5-amino-1,3,4-thiadiazole-2-thiol as a huge bulky in CH_2Cl_2 at room temperature for 30 minutes without catalyst gave 2-(2-acetamidophenyl)-2-((5-mercapto-1,3,4-thiazol-2-yl)imino)acetic acid compound(2e) in a high yield (92%), Scheme 3. Compound (2e) was established based on their spectral analysis, 1H-NMR, ^{13}C- NMR, mass spectra, and analytical data. 1H-NMR (DMSO-d6) δ (ppm) 2.08 (S,-$COCH_3$), 7.84-7.12 (m,4H, Ar-H), 10.56 (S,COOH), 13.21(S,NH) (Figure 3). In addition to the ^{13}C NMR (100 MHz, DMSO-d_6) for compound (2e) gave two peaks at181.35, 189.28, cm-1due to the carbonyl groups other peak at 24.36 cm^{-1} corresponding to the methyl group (Figure 4). The mass spectrum showed the molecular ion peak at m/z = 322.02 (M+), corresponding to the molecular formula $C_{12}H_{10}N_4O_3S_2$.

On the other hand, the reaction of an equimolar ratio mixture of N-acetyl isatin with 2-aminomethylpyridine in CH_2Cl_2 in the presence of a catalytic amount of glacial acetic acid at room temperature (30°C) for 12h gave 2-(2-acetamidophenyl)-2-oxo-N-(pyridine-2-ylmethyl)acetamidecompound (3a) in a good yield (85%), (Scheme 4).Compound (3a) was established based on their spectral analysis, ^{1}H-NMR, ^{13}C- NMR, mass spectra, and analytical data. 1H-NMR (DMSO-d6) δ (ppm) 2.08 (S,-$COCH_3$), 7.84-7.12 (m, 4H, Ar-H), 10.56 (S, COOH), 13.21(S, NH). In addition, the ^{13}C-NMR (100 MHz, DMSO-d_6) for compound (3a) gave two peaks at181.35, 189.28, cm-1 due to the

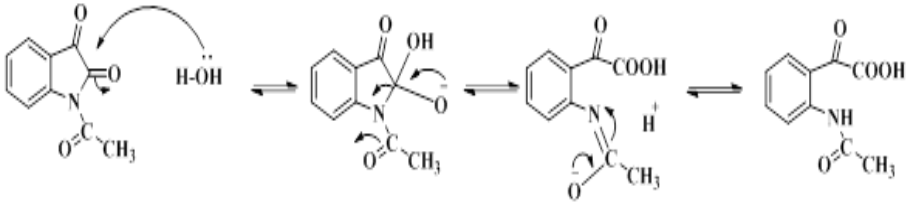
carbonyl groups other peak at 24.36 cm^{-1} corresponding to the methyl group.

Stirring at room temperature equimolar ratio mixture of N-acetyl isatin with aniline in CH2Cl2 in the presence of a catalytic amount of glacial acetic acid at room temperature (30ºC) for 12h gave 2-(2-acetamidophenyl)-2-oxo-N-phenylacetamide compound (3b) in excellent yield (85%), (Scheme 4).Compound (3b) was established based on their spectral analysis, ^{1}H-NMR, ^{13}C-NMR, mass spectra, and analytical data.^{1}H-NMR (DMSO-d_6) δ (ppm), 2.08(S,-$COCH_3$), 7.84-7.12 (m, 9H, Ar-H), 10.56(S, COOH), 13.21 (S, NH). In addition, the ^{13}C-NMR (100 MHz, DMSO-d_6) for compound (3b) gave two peaks at 181.35, 189.28, cm^{-1} due to the carbonyl groups other peak at 24.36 cm^{-1} corresponding to the methyl group. In addition, the mass spectrum showed the molecular ion peak at m/z = 282.10 (M+), corresponding to the molecular formula $C_{16}H_{14}N_2O_3$.

Reaction of an equimolar ratio mixture of N-acetyl isatin with hydrazine hydrate in CH_2Cl_2 in the presence of a catalytic amount of glacial acetic acid at room temperature (30ºC) for 12h gave N-(2-(2-hydraziyl-2-oxoacetyl)phenyl)acetamide compound (3c) in high yield (80%), (Scheme 4).Compound (3c) was established based on their spectral analysis, ^{1}H-NMR, ^{13}C- NMR, mass spectra, and analytical data.^{1}H-NMR (DMSO-d_6) δ (ppm), 2.08 (S,-$COCH_3$), 7.84-7.12 (m, 4H, Ar-H), 10.56 (S, COOH), 13.21 (S, NH). In addition, the ^{13}C-NMR (100 MHz, DMSO-d_6) for compound (3c) gave two peaks at181.35, 189.28, cm^{-1}due to the carbonyl groups other peak at 24.36 cm^{-1} corresponding to the methyl group (Figure 5). In addition to the mass spectrum showed the molecular ion peak at m/z = 221.08 (M+), corresponding to the molecular formula $C_{10}H_{11}N_3O_3$.



Scheme 1 Synthesis of compound (1)



Scheme 2 Mechanism of synthesis of compound (1)

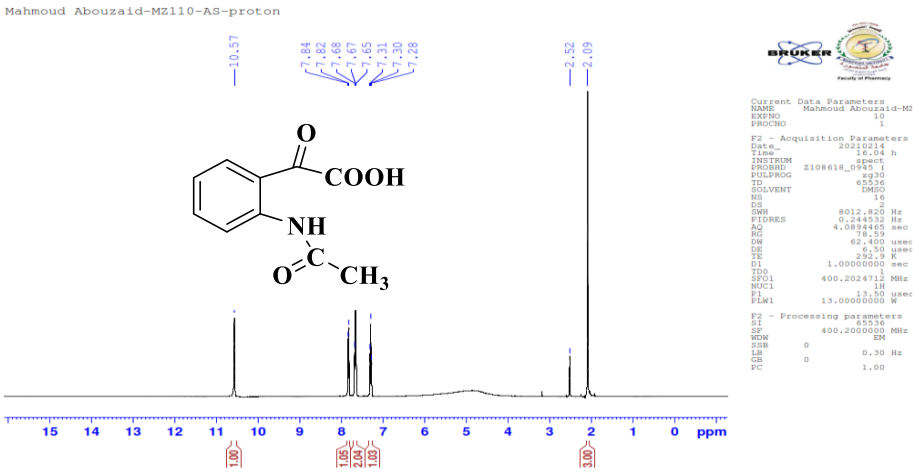


Fig.1. ^{1}H-NMR spectra of compound **(1)**

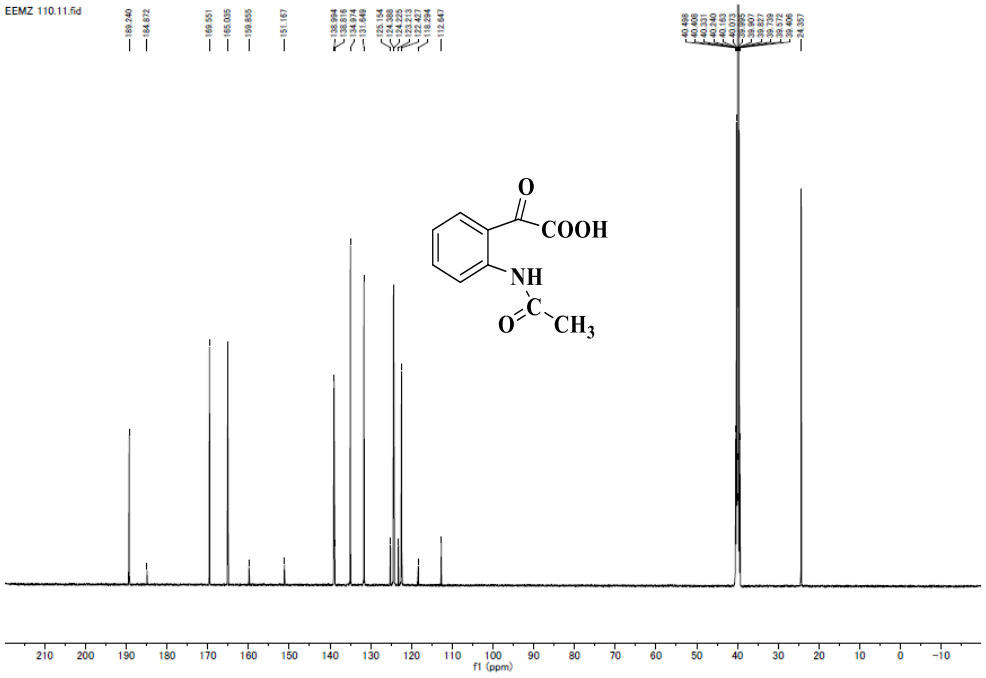


Fig.2. ^{13}C-NMR spectra of compound **(1)**

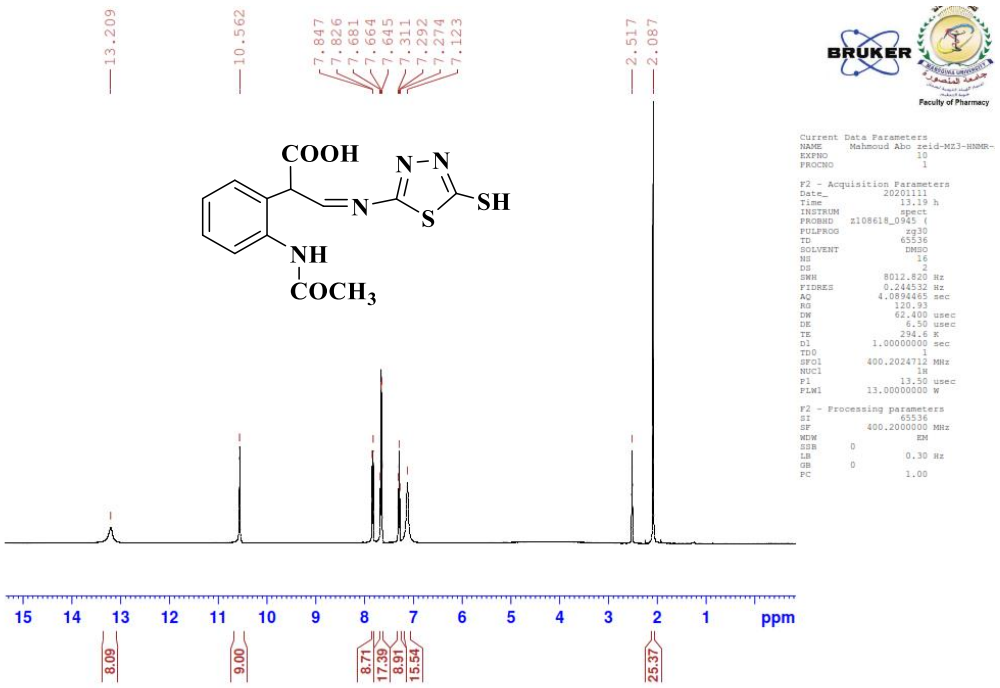


Fig.3. ^{1}H-NMR of compound **(2e)**

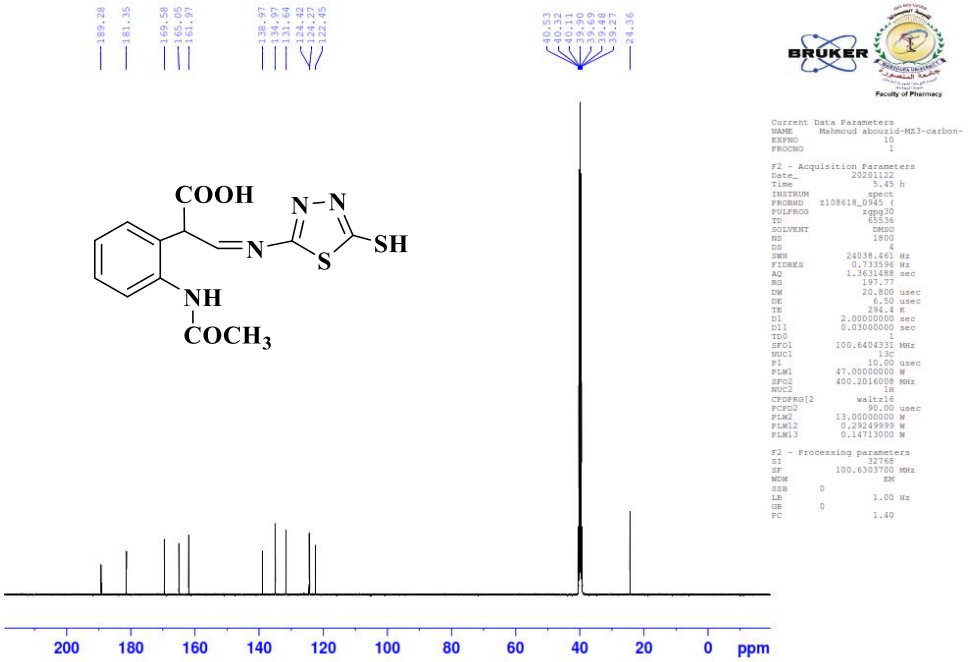


Fig.4. ^{13}C-NMR of compound **(2e)**

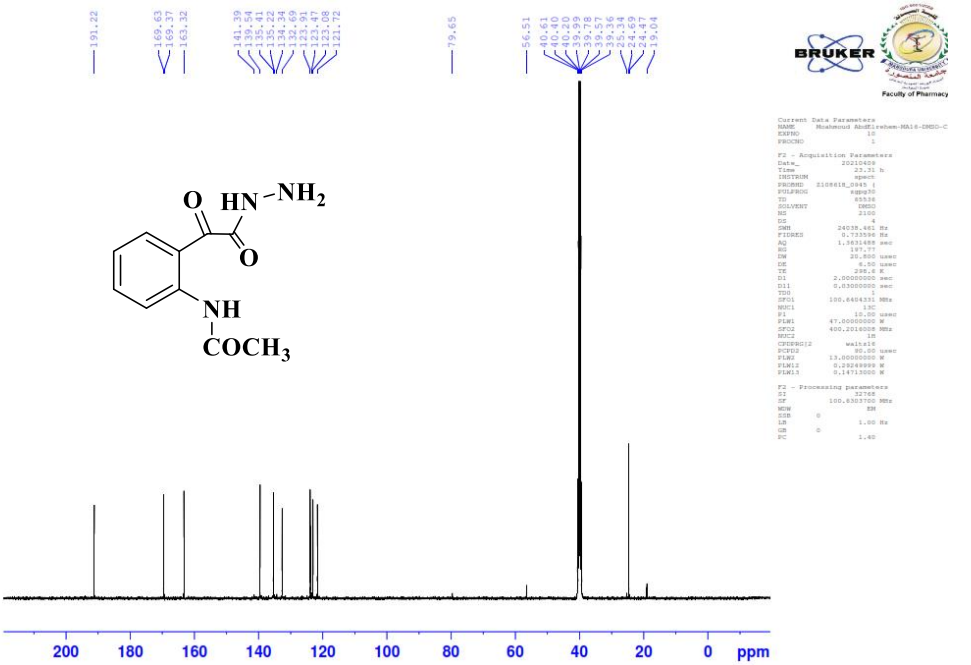
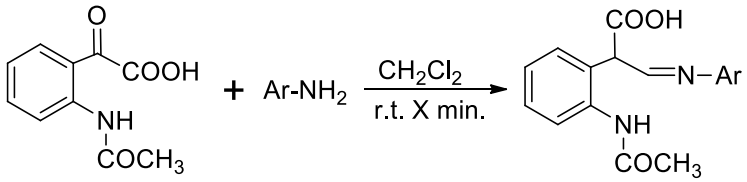
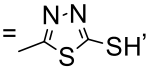


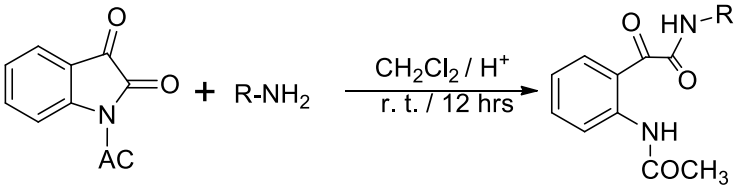
Fig.5. ^{13}C-NMR of compound **(3c)**



(2a-e)

a) Ar = C_6H_5, x = 10 minuts
b) Ar = C_6H_5NH, x = 10 minuts
c) Ar = Pyridyl, x = 10 minuts
d) Ar = 4- $OCH_3C_6H_5$, x = 10 minuts
e) Ar =  , x = 30 minuts

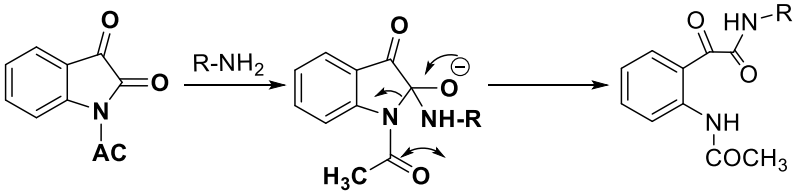
Scheme 3. Synthesis of compound (2a-e)



(3a-c)

a) R = CH_2- pyridyl
b) R = C_6H_5
c) R = $NH_2.H_2O$

Scheme 4 Synthesis of compounds (3a-c)



Scheme 5. Mechanism of synthesis of compounds (3a-c)

TABLE 1 Antibacterial activity of target compounds compared with different standard antibiotics against Escherichia coli and Staphylococcus aureus

strains	*E. coli*		*S. aureus*	
compound	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
1	-ve	-ve	-ve	-ve
2a	-ve	-ve	-ve	-ve
2c	4	4	-ve	-ve
2e	1.8	1.8	1.8	1.8
3c	1.4	1.4	1.4	1.4
3a	0. 7	0.7	0.7	0.7
2b	1.4	1.4	1.4	1.4
2d	0.5	0.5	0.5	0.5
3b	1.5	1.5	1.5	1.5

Antibacterial profile of the target compounds against *Escherichia coli (E. coli)*, and *Staphylococcus aureus* (*S. aureus)*. MIC (minimum inhibitory concentration expressed in mg ml^{-1}); MBC (Minimum bactericidal concentration expressed in mg ml^{-1}).–ve: means the effect was negative.The used standard antibiotic (control)[a]. Chloramphenicol (30 µg), Cefotaxime (30 µg), Gentamycin (10 µg), Vancomycin (30 µg), Ampicillin/sulbactam (10/10 µg), Piperacillin (100 µg) ,Oxacillin (1 µg)

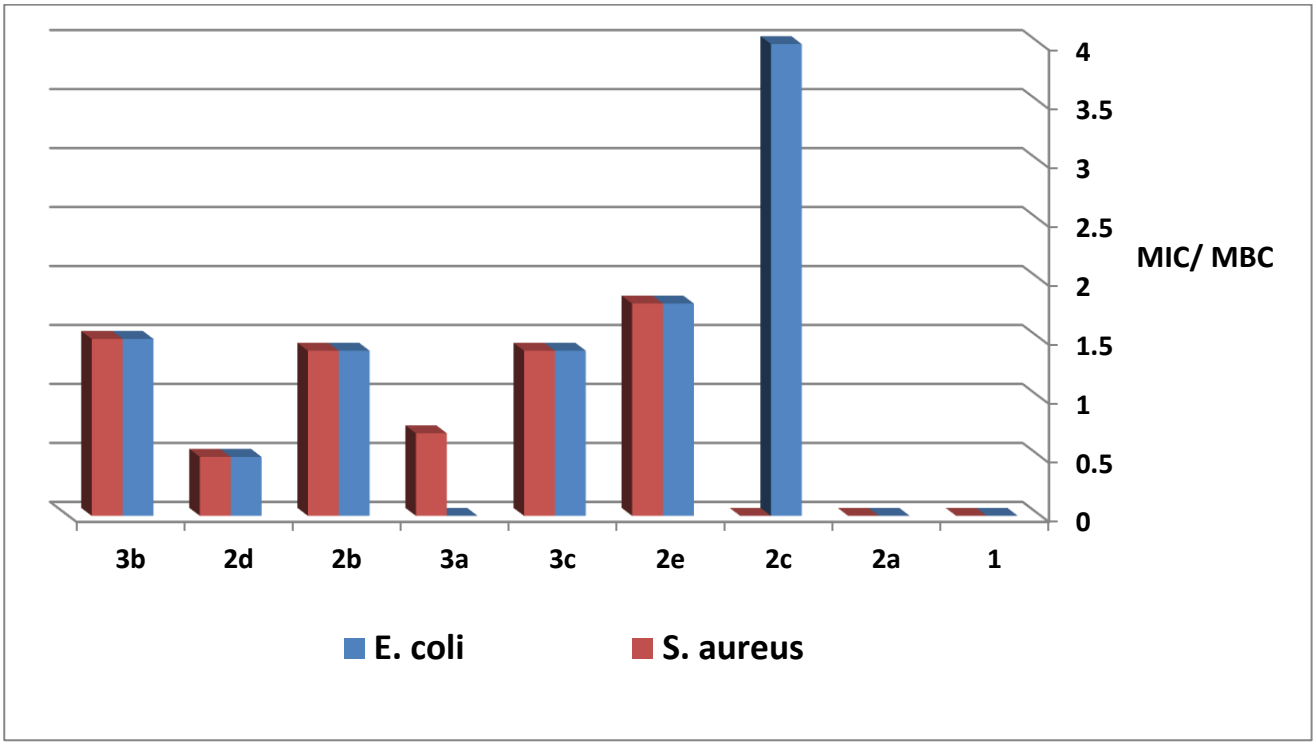


Fig. 6 the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the Schiff bases against the tested bacteria

3.2 Antibacterial part

3.3. Determination of the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) of the target compounds and Structure-activity correlation.

The colorimetric INT- formazon assay of compounds (2d), (3a), (3c) and (2b) showed reproducible, effective antibacterial activity against the tested E. coli strain and Staphylococcus aureus (MRSA).The concentrations of 0.5-1.8 mlgm-1 was sufficient as minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations for the tested E. coli and S. aureus strains. The highest efficacy recorded by (2d) and the lowest effect was for (2c) and (2e) inhibit E. coli only with MIC and MBC of 4 mgml-1 and no effect recorded on S.aureus.

Generally, the presence of some cross functional side chain of tested compounds results in high inhibitory and bactericidal effect against the tested bacteria. Compound 1 does not have antibacterial effect against the tested bacteria. The activity of glycoxalic acid derivative 1 increased due to substituents entered on its structure. Thus, compound (2d) showed bactericidal effect against the tested strains due to the presence of methoxy phenyl group [27] while the presence of the phenyl group alone in compound (2a) did not affect its antibacterial activity. Additionally, the inhibitory effect of (3a),(3b) and (2c) attributed to the presence of adjacent two carbonyl groups and pyridine rings [28] which facilitate the formation of hydrogen bonds with the protein amino acid reside of cell wall of bacteria while the antibacterial effect of compounds (2b) and (3c) may be attributed to the binding of their three (NH) groups with the carboxylic group of the protein amino acid residue [29].The presence of thiadiazole ring in compound (2e) result in antibacterial activity [30].

Conclusion

and consistently. Where they first appear in the text, they should be defined; authors may also explain large numbers of abbreviations and acronyms after the conclusion part.

Conflicts of interest

The authors declare no potential conflicts of interest

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