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Utilization of Ultrasonic as an Approach of Green Chemistry for Synthesis of Hydrazones and Bishydrazones as Potential Antimicrobial Agents

Ahmed Younis [1]*, Ghada E. A. Awad [2]

[1] Green Chemistry Dept., National Research Centre, 33 El Bohouth St. (former EL Tahrir St.)-Dokki-Giza-P.O.12622 and [2] Chemistry of Natural and Microbial Products, National Research Centre, , Cairo , Egypt

Hydrazides **3,4**, Hydrazones 6a-c, bishydrazones 8a,b, N-hydroxy-N'-arylpropanehydrazonamide **9a,b** and 1-(piperidin-1-yl)-N2-arylamidrazones **10a,b** were prepared under ultrasonic waves as an approach for green chemistry. a notable good yield and short reaction time were afforded under ultrasonic waves.The structures of compounds were confirmed in terms of spectroscopic and elemental analyses. The invitro antimicrobial activity of the prepared compounds were evaluated. most of compounds exhibited an excellent growth inhibition such as compounds **2, 3** and **8b** against gram positive bacteria, while **2, 3, 8b, 9a, 10a** and **10b** against gram negative bacteria. all of tested compounds have excellent or good antifungal activity except **3**

Keywords: Ultrasonic, Green Chemistry, Hydrazide, Hydrazone, Bishydrazones, Antimicrobial

1. INTRODUCTION

We cannot deny that most of used traditional techniques in organic synthesis upset our ecosystem [1-3]. From this point of view researchers are encouraged to exert great efforts to get alternative techniques such as ultrasonic, microwave and friction forces and use it in synthesis of essential chemical substances which verify some of green chemistry principles[4-6]. Ultrasonic waves is considered from the most recent green chemistry tools which is used in different processes such as accelerate and enhance organic reactions due to its many advantages such as increase yield and decrease reaction time in addition to it is considered safe energy source[7-9].

The World Health Organization's latest report on antimicrobial agents exhibit the importance of antimicrobial agents and the bad need to discover new antimicrobial agents that is distinct from the known antimicrobial agents[10]. Hydrazones, bishydrazones, amidrazones are

*Corresponding author e-mail: younischem@gmail.com
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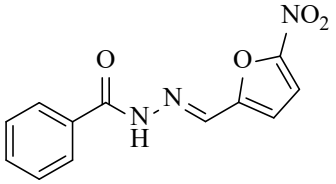
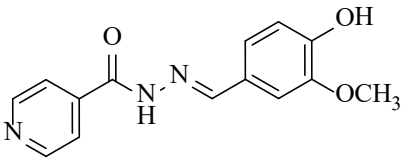
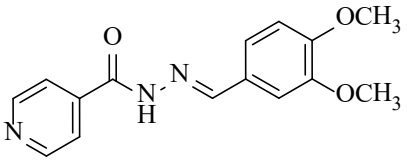
considered as an important pharmacophore for antimicrobial activity such as Nifuroxazide, Phtivazid **III**, Verazide **IV**, Salinazide **V** [11-14] (figure 1).



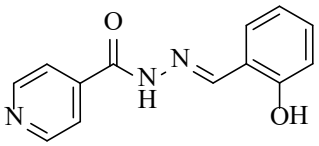
Nifuroxazide I



phtivazide II



verazide III



Salinazide IV

Fig.1 structures of antimicrobial agents I-IV

In continuation of our interest of using different green chemistry tools in organic synthesis [15-20]. Herein, we report a facile sonochemical synthesis of Ethyl 4-acetamidobenzoate **(2)**, N-(4-(hydrazinecarbonyl)phenyl)acetamide **(3)** 4-aminobenzohydrazide **(4)**, 4-amino-N-(benzylidene)benzohydrazide **(6a-c)**, 2-(2-(4-aminobenzoyl)hydrazono)-N'-(aryl) propanehydrazonoylchloride **(8a,b)** 4-amino-N'-(1-(2-arylhydrazono)-1-(piperidin-1-yl) propan-2-ylidene)benzohydrazide **(9a,b)** 2-(2-(4-aminobenzoyl)hydrazono)-N-hydroxy-N'-(aryl)propanehydrazonamide **(10a,b)** under ultrasonic waves as an approach of green chemistry [21, 22].

2. EXPRIMENTAL

2.1. Chemistry

General

All melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. The infrared spectra were recorded in potassium bromide disks on a pyeUnicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometers. The ^{1}HNMR and ^{13}C spectra were recorded on an Agilent Technologies model spectrometer NMR400-mercury 400. ^{1}H spectra were run at 300 MHz and ^{13}C spectra were run at 75.46 MHz in dimethylsulphoxide (DMSO-d6). Tetramethylsilane (TMS) was used as an internal reference and chemical shifts are quoted in δ (ppm) at the Main Chemical Warfare Laboratories, Chemical Warfare Department, Ministry of Defence, Cairo, Egypt. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer at 70 e.V. Elemental analyses were carried out at the MicroanalyticalCenter of Cairo University, Giza, Egypt. Reactions carried out under ultrasonic irradiation were performed by fischer sonicator (with frequency of 25 kHz and nominal power 600W

*Synthesis of Ethyl 4-acetamidobenzoate **(2)***

Ethyl 4-aminobenzoate **(1)** (20 mmol) was subjected to ultrasound waves in 10 ml acetic acid. the reaction was monitored by TLC and completed after one hour then poured into crushed ice, The resulting solid was collected by filtration, washed with water and recrystallized from toluene to give colourless crystals of ethyl 4-acetamidobenzoate **(2)** in 98% yield

IR (KBr) ν/cm^{-1}: 3335 (NH); 1705, 1682 (2C=O); ^{1}H NMR (DMSO): δ= 1.35 (t, 3H, CH_3), 2.05 (s, 3H, CH_3CO), 4.27 (q, 2H, CH_2), 7.68-788 (dd, 4H, Ar-H), 10.23 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ= 14, 24, 60, 118, 124,130, 144, 165, 169; MS (m/z): 207 for $C_{11}H_{13}NO_3$ (Found: C, 63.25; H, 6.27; N, 6.85% Calcd.: C, 63.76; H, 6.32; N, 6.76%

*Synthesis of N-(4-(hydrazinecarbonyl)phenyl) acetamide **(3)***

A mixture of Ethyl 4-acetamidobenzoate **(2)** (20 mmol) and 8 ml hydrazine hydrate (99%) was subjected to ultrasound waves for one hour. The separated white solid was filtered off and recrystallized from EtOH / DMF to give N-(4-(hydrazinecarbonyl)phenyl)acetamide **(3)** in 92% yield

IR (KBr) ν/cm^{-1}: 3346, 3307, 3234 (2NH, NH_2), 1603 (2C=O); ^{1}H NMR (DMSO-d6): δ= 2.04 (s, 3H, CH_3), 4.53 (br. s, ^{1}H, NH_2, D_2O exchangeable), 7.59-7.76 (dd, 4H, Ar-H), 9.61 (s, 1H, NH, D_2O exchangeable), 10.11 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ= 24, 118, 128, 130, 142, 166, 169; MS (m/z): 193 for $C_9H_{11}N_3O_2$ (Found: C, 55.83; H, 5.69; N, 21.89; % Calcd.: C, 55.95; H, 5.74; N, 21.75%.

*Synthesis of 4-aminobenzohydrazide **(4)***

A mixture of ethyl 4-aminobenzoate **(1)** (20 mmol) and 8 ml hydrazine hydrate (99%) was subjected to ultrasound waves for one hour. The separated white solid was filtered off and recrystallized from EtOH / DMF to give 4-aminobenzohydrazide **(4)**

IR (KBr) ν/cm^{-1}: 3335, 3280, 3234 (NH, $2NH_2$), 1620 (C=O); ^{1}H NMR (DMSO-d6): δ= 4.42 (br. s, ^{1}H, NH_2, D_2O exchangeable), 6.11 (s, 2H, NH_2, D_2O exchangeable), 7.43-7.96 (dd, 4H, Ar-H), 9.64 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ= 114, 122, 130, 155, 169; MS (m/z): 151 for $C_7H_9N_3O$ (Found: *C, 55.51; H, 6.09; N, 27.72; O, 10.58* % Calcd.: *C, 55.62; H, 6.00; N, 27.80; O, 10.58* %.

*Synthesis of compounds **6a-c***

Method A

A mixture of 4-aminobenzohydrazide **(4)** (1 mmol) and appropriate aromatic aldehydes **5a-c** (1 mmol) in 1 mL absolute ethanol was exposed to ultrasonic waves for appropriate time (table1). The formed solid was filtered off, dried, and recrystallized from the proper solvent to afford compounds **6a-c** in excellent yield.

Method B

N-(4-(hydrazinecarbonyl)phenyl) acetamide **(3)** (1 mmol) and appropriate aromatic aldehydes **5a-c** (1 mmol) in 1 mL absolute ethanol were subjected to ultrasonic waves for appropriate time (table1). The formed solid was filtered off, dried, and recrystallized from the proper solvent to afford compounds **6a-c** in excellent yield.

*4-amino-N-(4-nitrobenzylidene)benzohydrazide **(6a)***

IR (KBr) ν/cm^{-1}: 3393, 3246, 3219(NH, NH_2),1654 (C=O), 1585 (C=N); ^{1}H NMR (DMSO-d6): δ= 5.82 (br. s, ^{1}H, NH_2, D_2O exchangeable), 6.58-8.27 (m, 8H, Ar-H), 8.46 (s, 1H, N=CH), 11.73 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ= 113, 119,124, 128, 130, 141, 147, 158; MS (m/z): 284 for $C_{14}H_{12}N_4O_3$ (Found: C, 59.03; H, 4.17; N, 19.79% Calcd.: C, 59.15; H, 4.25; N, 19.71%

*4-amino-N-(4-cyanobenzylidene)benzohydrazide **(6b)***

IR (KBr) ν/cm^{-1}: 3392, 3231, 3226 (NH, NH_2), 2226 (CN), 1656 (C=O), 1571 (C=N); ^{1}H NMR (DMSO-d6): δ= 5.80 (br. s, ^{1}H, NH_2, D_2O exchangeable), 6.57-7.86 (m, 8H, Ar-H), 8.41 (s, 1H, N=CH), 11.66 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ= 113, 118, 119, 123, 127, 133, 139, 143, 153; MS (m/z): 264 for $C_{15}H_{12}N_4O$ (Found: C, 68.01; H, 4.32; N, 21.28; % Calcd.: C, 68.17; H, 4.58; N, 21.20; %

*4-amino-N-(4-chlorobenzylidene)benzohydrazide **(6c)***

IR (KBr) ν/cm^{-1}: 3440, 3331, 3216 (NH, NH_2),1632 (C=O), 1595 (C=N); ^{1}H NMR (DMSO-d6): δ= 5.80 (br. s, ^{1}H, NH_2, D_2O exchangeable), 6.72-8.36 (m, 8H, Ar-H), 8.40 (s, 1H, N=CH), 11.61 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ= 112, 118,124, 128, 132, 141, 147, 158; MS (m/z): 273 for $C_{14}H_{12}ClN_3O$ (Found: C, 61.30; H, 4.51; Cl, 12.82; N, 15.28 % Calcd C, 61.43; H, 4.42; Cl, 12.95; N, 15.35 %

*Synthesis of compounds **8a,b***

Method A

4-aminobenzohydrazide **(4)** (1mmol) and the appropriate 2-oxo-N-arylpropanehydrazonoylchloride **7a,b** (1mmol) in absolute ethanol (1 ml) were reacted under ultrasonic waves. The reaction was controlled by TLC and continued until the starting substrates were completely consumed after appropriate time (table 1).The solid products that formed were recrystallized from EtOH/DMF to afford compounds **8a,b** in a good yield.

Method B

A mixture of N-(4-(hydrazinecarbonyl) phenyl)acetamide **(3)** (1 mmol) and the appropriate 2-oxo-N-arylpropanehydrazonoylchloride **7a,b** (1mmol) in 1 mL absolute ethanol was exposed to ultrasonic waves for appropriate time. The formed solid was filtered off, dried, and recrystallized from the proper solvent to afford compounds **8a,b** in a good yield (table1).

*2-(2-(4-aminobenzoyl)hydrazono)-N'-(4-nitrophenyl)propanehydrazonoyl chloride **(8a)***

IR (KBr) ν/cm^{-1}: 3477, 3378, 3199(NH_2, 2NH),1660 (C=O), 1601 (C=N); ^{1}H NMR (DMSO-d6): δ= 2.14 (s, 3H, CH_3), 6.63-8.06 (m, 8H, Ar-H), 9.07 (s, 1H, NH_2, D_2O exchangeable), 10.19 (s, 1H, =NNH-, D_2O exchangeable), 10.49(s, 1H, -CONH-, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ=12 (1C of propane CH_3), 111, 113, 126, 130, 131, 138, 155, 165; MS (m/z): 374 for $C_{16}H_{15}ClN_6O_3$ (Found: : C, 51.13; H, 4.10; Cl, 9.53; N, 22.31 % calcd.: C, 51.28; H, 4.03; Cl, 9.46; N, 22.42 %

*2-(2-(4-aminobenzoyl)hydrazono)-N'-(4-chlorophenyl)propanehydrazonoyl chloride **(8b)***

IR (KBr) ν/cm^{-1}: 3448, 3338, 3211 (NH_2, 2NH),1660 (C=O), 1597 (C=N); ^{1}H NMR (DMSO-d6): δ= 2.12 (s, 3H, CH_3), 6.63-7.68 (m, 8H, Ar-H), 9.82 (s, ^{1}H, NH_2, D_2O exchangeable), 10.48 (s, 1H, =NNH-, D_2O exchangeable), 10.76(s, 1H, -CONH-, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ=12 (1C of propane CH_3), 113, 116, 122, 128, 130, 141, 143, 148, 165; MS (m/z): 363 for $C_{16}H_{15}Cl_2N_5O$ (Found: C, 52.65; H, 4.19; Cl, 19.39; N, 19.15 calcd.: C, 52.76; H, 4.15; Cl, 19.47; N, 19.23 %

*Synthesis of 2-(2-(4-aminobenzoyl)hydrazono)-N-hydroxy-N'-(aryl)propanehydrazonamide **9a,b***

A mixture of compound **8a,b** (1 mmol), hydroxylamine hydrochloride(1.5 mmol) and anhydrous potassium carbonate (1.5 mmol) in ethanol (1 ml) was subjected to ultrasonic waves for 30 and 35 minutes respectively. The reaction mixture was poured into cold water and the solid product was filtered off, washed with water, dried and finally recrystallized from EtOH/DMF to afford **9a,b**

*2-(2-(4-aminobenzoyl)hydrazono)-N-hydroxy-N'-(4-nitrophenyl)propanehydrazonamide **9a***

IR (KBr) ν/cm^{-1}: 3428 (OH), 3355, 3290, 3233(NH_2, 3NH),1603 (C=O), 1577 (C=N);^{1}H NMR (DMSO-d6): δ= 2.09 (s, 3H, CH_3), 5.82 (s, 2H, –NH_2 D_2O exchangeable), 7.41-8.35 (m, 8H, Ar-H), 9.51 (s, 1H, –NH D_2O exchangeable), 10.12(s, 1H, =NNH-, D_2O exchangeable), 10.75 (s, 1H, -CONH-, D_2O exchangeable), 11.99 (s, 1H, –OH D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ=11, 113, 121, 125, 126,127,140, 145, 149, 155, 165; MS (m/z): 371 for $C_{16}H_{17}N_7O_4$ (Found: C, C, 51.61; H, 4.66; N, 26.55 % Calcd.: C, 51.75; H, 4.61; N, 26.40 %

*2-(2-(4-aminobenzoyl)hydrazono)-N-hydroxy-N'-(4-chlorophenyl)propanehydrazonamide **9b***

IR (KBr) ν/cm^{-1}: 3434 (OH), 3343, 3298, 3233(NH_2, 3NH),1603 (C=O), 1560 (C=N); ^{1}H NMR (DMSO-d6): δ= 2.07 (s, 3H, CH_3), 5.80 (s, 2H, –NH_2 D_2O exchangeable), 6.54-7.80 (m, 8H, Ar-H), 8.44 (s, 1H, –NH D_2O exchangeable), 10.09 (s, 1H, –NH D_2O exchangeable), 11.29 (s, 1H, -CONH-, D_2O exchangeable), 11.75 (s, 1H, –OH D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ=11,113, 115, 123, 129, 131, 135, 143, 149, 152, 166; MS (m/z): 360 for $C_{16}H_{17}ClN_6O_2$ (Found: C, 53.12; H, 4.70; Cl, 9.92; N, 23.37 % Calcd.: C, 53.26; H, 4.75; Cl, 9.83; N, 23.29 %

*Synthesis of 4-amino-N'-(1-(2-arylhydrazono)-1-(piperidin-1-yl)propan-2-ylidene)benzohydrazide **(10a,b)***

To a solution of compound **8a,b** (1 mmol) in ethanol (1 ml), piperidine (2 mmol) was added. The reaction mixture was subjected to ultrasonic waves for 30 minutes then left at room temperature overnight. The precipitated

product was filtered off, washed with ethanol and dried. Recrystallization from ethanol afforded compound **10a,b**.

4-amino-N'-(1-(2-(4-nitrophenyl)hydrazono)-1-(piperidin-1-yl)propan-2-ylidene)benzohydrazide ***(10a)***

IR (KBr) ν/cm^{-1}: 3399, 3367, 3270 (NH_2, 2NH), 2932-2853 ($5CH_2$ piperidine), 1598 (C=O), 1550 (C=N);^{1}H NMR (DMSO-d6): δ= 1.56 (m, 6H, $3CH_2$ of piperidine), 2.13 (s, 3H, CH_3), 3.2 (m, 4H, $2CH_2$ of piperidine), 5.83 (s, 1H, NH_2, D_2O exchangeable), 6.56-8.35 (m, 8H, Ar-H), 10.18 (s, 1H, =NNH-, D_2O exchangeable), 10.74(s, 1H, -CONH-, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ=12, 22, 25, 50, 111, 119, 124, 129, 131, 135, 139, 143, 146, 153, 156, 167; MS (m/z): 423 of $C_{21}H_{25}N_7O_3$ (Found: C, 59.41; H, 5.90; N, 23.27 % Calcd.:C, 59.56; H, 5.95; N, 23.15 %

4-amino-N'-(1-(2-(4-chlorophenyl)hydrazono)-1-(piperidin-1-yl)propan-2-ylidene)benzohydrazide ***(10b)***

IR (KBr) ν/cm^{-1}: 3390, 3329, 3220(NH_2, 2NH), 2947-2738 ($5CH_2$ piperidine), 1604 (C=O), 1545 (C=N); ^{1}H NMR (DMSO-d6): δ= 1.58 (m, 6H, $3CH_2$ of piperidine), 2.16 (s, 3H, CH_3), 3.1 (m, 4H, $2CH_2$ of piperidine), 5.80 (s, 1H, NH_2, D_2O exchangeable), 6.88-8.22 (m, 8H, Ar-H), 10.11 (s, 1H, =NNH-, D_2O exchangeable), 10.54(s, 1H, -CONH-, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ=11, 22, 25, 50, 113, 118, 122, 129, 131, 135, 138, 143, 146, 153, 156, 168; MS (m/z): 412 of $C_{21}H_{25}ClN_6O$ (Found: C, 60.95; H, 6.04; Cl, 8.66; N, 20.24 % Calcd. C, 61.08; H, 6.10; Cl, 8.59; N, 20.35 %

Antimicrobial activity

Chemical compounds were individually tested against a panel of Gram positive, Gram negative bacterial pathogens and yeast. Antimicrobial tests were carried out by the agar well diffusion method (Perez *et al*. 1990) using 100 µL of suspension containing 1 x10^6 CFU/ mL of pathological tested bacteria and 1 x10^6 CFU/ml of yeast spread on nutrient agar (NA), Sabour and dextrose agar (SDA)respectively. After the media had cooled and solidified, wells (10 mm in diameter) were made in the solidified agar and loaded with 100 µL of tested compound solution prepared by dissolving 83µg/ml of the chemical compound in one ml of dimethyl sulfoxide (DMSO). The inculcated plates were then incubated for 24 h at 37 °C for bacteria and, 48h at 28°C for yeast. Negative controls were prepared using DMSO employed for dissolving the tested compound. (vancomycine (50 µg/ ml) and Ketoconazole (50 µg/ml) were used as standard for antibacterial and antifungal activity respectively. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard. The observed zone of inhibition is presented in Table 2.Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated.

Minimal inhibitory concentration (MIC) measurement

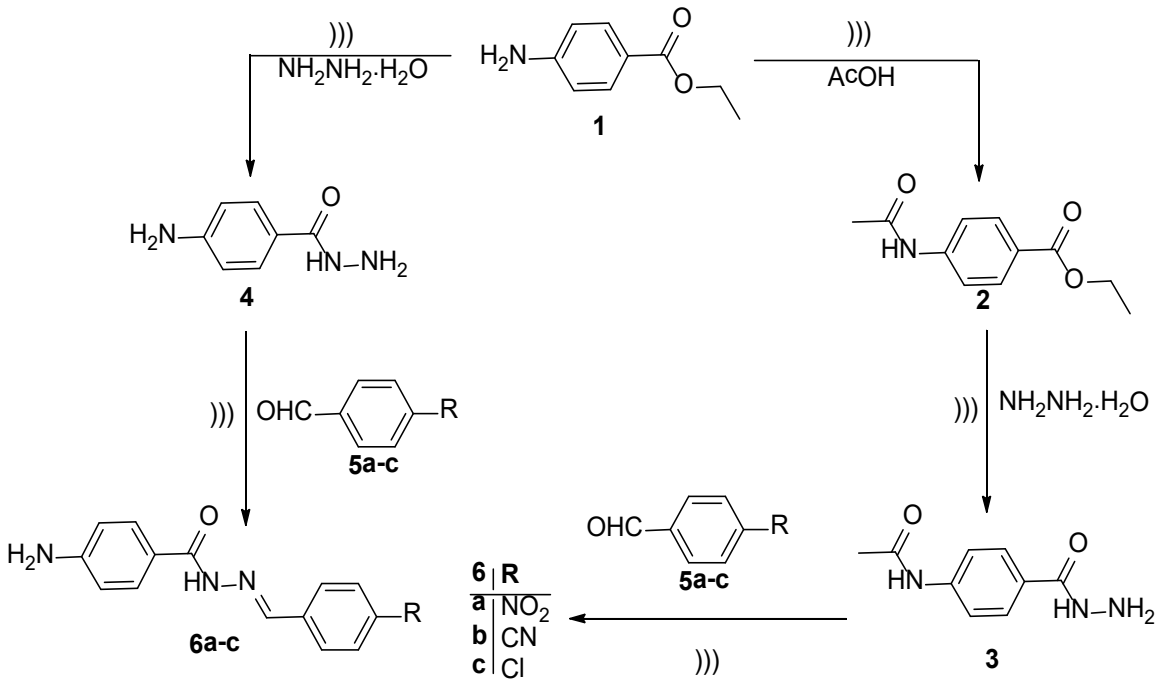
The bacteriostatic activity of the active compounds (having inhibition zones (IZ) ≥ 16 mm) was then evaluated using the two fold serial dilution technique (Scott, 1998). Two fold serial dilutions of the tested compounds solutions were prepared using the proper nutrient broth. The final concentration of the solutions was 200, 100, 50 and 25 µg/ml. The tubes were then inoculated with the test organisms, grown in their suitable broth at 37 °C for 24 hours for tested microorganisms (1×10^{6}CFU/ml for bacteria and 1 x10^6 CFU/ml of yeast), each 5 ml received 0.1 ml of the above inoculum and incubated at 37 °C for 24hours. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC).

3. Result and discussion

3.1. *Chemistry*

Ethyl 4-acetamidobenzoate **(2)** were obtained in excellent yield (98%) from the reaction of ethyl 4-aminobenzoate **(1)** and acetic acid under ultrasonic waves (Scheme 1). The structure of ethyl 4-acetamidobenzoate **(2)** were confirmed in terms of elemental and spectroscopic analyses where Its IR spectrum revealed the appearance of characteristic absorption bands at 3335 cm^{-1} due to NH and 1705, 1682 due to two carbonyl groups while characteristic absorption band of amino group disappeared. In The ^{1}HNMR spectrum of Ethyl 4-acetamidobenzoate **(2)** triplet signal appears at δ= 1.35 due to -CH_3, quartet signal at δ=

4.27 due to CH_2 while singlet signal appears at δ= 2.05 due to CH_3CO and duplet of duplets appears at δ= 7.68-7.88 due to aromatic hydrogen in addition to singlet signal at δ= 10.23 due to NH which confirm the structure (figure 2). The mass spectrum of compound 2 showed a peak corresponding to its molecular ion at m/z 207



Scheme 1

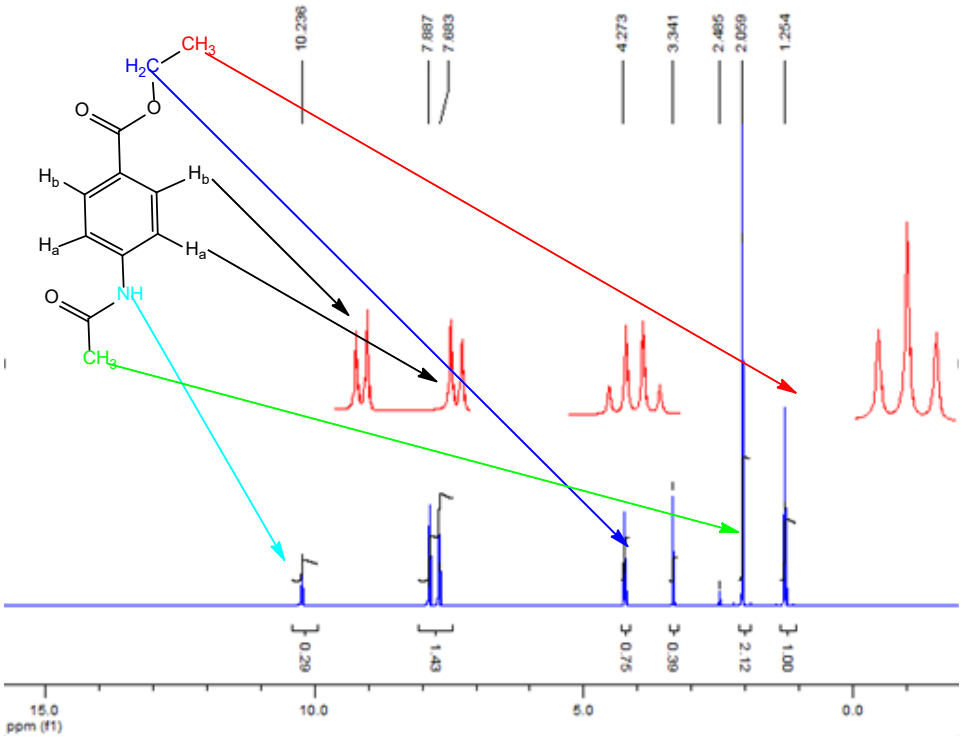
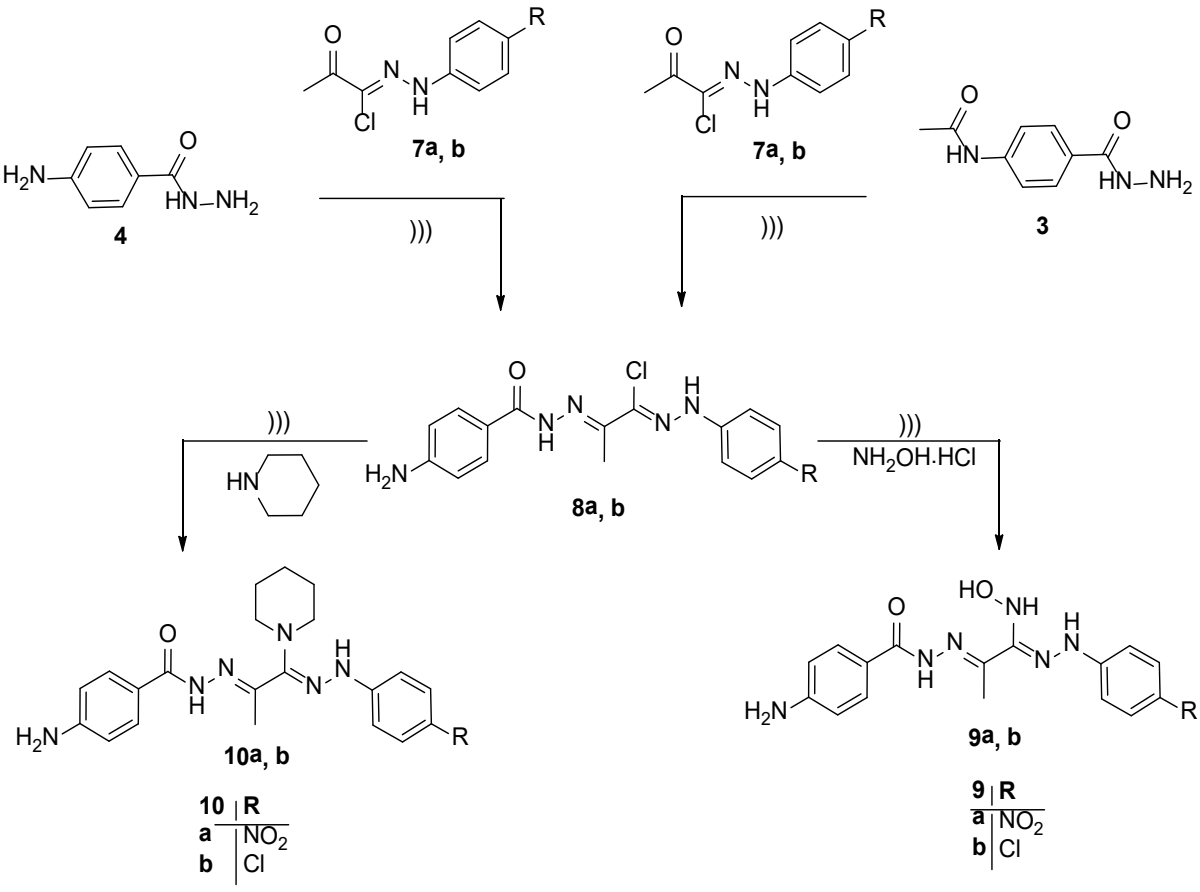


Fig. 2: ^{1}HNMR of compound 2

The reaction of Ethyl 4-acetamidobenzoate **(2)** with hydrazine hydrate under ultrasonic waves gave N-(4-(hydrazinecarbonyl)phenyl)acetamide **(3)** in a good yield (92%). the IR spectrum of compound **3** showed three absorption bands at 3346, 3307 and 3234 cm^{-1} due to 2NH and NH_2 in addition to characteristic bands at 1603 cm^{-1} due to 2CO. In The ^1H NMR spectrum of N-(4-(hydrazinecarbonyl)phenyl)acetamide **(3)** singlet signal appears at δ= 2.04 due to CH_3CO, broad singlet signal at δ= 4.53 due to amino group and duplet of duplets appears at δ= 7.59-7.76 due to aromatic hydrogen in addition to two singlet signals at δ= 9.61 and 10.11 due to two NH which confirm the formation of N-(4-(hydrazinecarbonyl)phenyl)acetamide **(3)** [23]. The absence of triplet and quartet signal due to CH_2CH_3 is considered further confirmation for hydrazide formation (scheme 1)

Ethyl 4-aminobenzoate **(1)** reacted with hydrazine hydrate under ultrasonic waves to produce 4-aminobenzohydrazide **(4)** in good yield compared to conventional method [21]. Furthermore, the reaction of 4-aminobenzohydrazide **(4)** with appropriate aldehydes **5a-c** afforded the corresponding hydrazones **6a-c** (Scheme 1). The structures of these hydrazones were confirmed on the basis of their spectral data. The IR spectrum of hydrazones **6a-c** showed characteristic absorption bands of ranges 3440-3216 cm^{-1} due to NH and NH_2 1656-1632 cm^{-1} due to CO and 1595-1571 cm^{-1} due to C=N. The appearance of characteristic band at 2226 cm^{-1} in hydrazones 6b due to CN group confirmed its structure. The ^1H NMR spectrum of all hydrazones **6a-c** showed a signal due to the -CH=N- proton at ranges δ 8.40-8.46 [24, 25]. An alternative synthesis of hydrazones 6a-c was explored where N-(4-(hydrazinecarbonyl) phenyl)acetamide (3) was mixed with appropriate aldehyde 5a-c in ethanol and subjected to ultrasonic for appropriate time to afford hydrazones 6a-c in better yield (scheme 1).



Scheme 2

The reaction of 2-oxo-N-arylpropanehydrazonoyl chlorides **7a, b** with 4-aminobenzohydrazide **(4)** in ethanol under ultrasonic waves was investigated, where 2-(2-(4-aminobenzoyl) hydrazono)-N'-arylpropanehydrazonoyl chloride **(8a,b)** were afforded in a good yield and their structures were confirmed in terms of elemental and spectroscopic analyses where their IR spectra revealed characteristic absorption band due to NH_2 and 2NH groups in the region 3477-3199 cm^{-1} and characteristic absorption band due to carbonyl group at 1601and 1660 cm^{-1} for 8a and 8b respectively in addition to characteristic absorption band due to C=N groups at 1601 and 1597 cm^{-1} for 8a and 8b respectively. The ^{1}H NMR spectra of compounds **8a,b** exhibited singlet signal of the methyl group (CH_3C=N) at δ 2.14 and 2.12 and two D_2O exchangeable signals of 2NH groups in the regions δ = 10.19 – 10.49 and δ 10.48 – 10.76 for **8a** and **8b** respectively, furthermore The sp^3 hybridized carbon of the methyl group (CH_3C=N) appeared at δ = 12 in ^{13}CNMR spectra of **8a** and **8b**. The previous spectroscopic data of the reaction products **8a,b** and their adequate elemental analyses supported the structure 2-(2-(4-aminobenzoyl)hydrazono)-N'-arylpropanehydrazonoyl chloride **(8a,b)** as postulated in Scheme 2. The structure of compounds 8 a,b were further confirmed by an independent synthesis outlined in Scheme II. Thus, treatment of 2-oxo-N-arylpropanehydrazonoyl chlorides **7a, b** with N-(4-(hydrazinecarbonyl)phenyl)acetamide **(3)** in ethanol under ultrasonic waves led to the formation of a product identical to compounds **8a,b**.

The reaction of **8a,b** with hydroxylamine hydrochloride in ethanol in presence of potassium carbonate under ultrasonic waves afforded 2-(2-(4-aminobenzoyl)hydrazono)-N-hydroxy-N'-(aryl)propanehydrazonamide **9a,b** in a good yield. The structure of compounds **9a, b** was established on the basis of elemental analysis and spectral data. The IR spectrum of compounds **9a,b** showed characteristic absorption band due to hydroxyl group at 3428 and 3434 cm^{-1} for **9a** and **9b** respectively, characteristic absorption bands in the region 3355-3233 cm^{-1} due to NH_2 and 3NH groups,1603 cm^{-1} due to carbonyl group and at 1577, 1560 due to C=N. for 10a and 10b respectively. The ^{1}HNMR spectrum of compound **9a,b** showed the appearance of singlet exchangeable signal due to the hydroxyl proton at δ =11.99 and 11.75 for **9a** and **9b** respectively (*cf* experimental) [10].

The reactivity of **8a,b** towards piperidine was examined. Reaction of each compound **8a** and **8b** in ethanol under ultrasonic led to elimination of hydrogen chloride and the formation of a single product, in each case, as confirmed by TLC analysis of the crude product. Elemental analysis, ^{1}H NMR and the mass spectral data were well-matched with the structures **10a,b** in Scheme 2 where its IR spectra revealed characteristic absorption band in the region 2947-2738 due to presence of piperidine ring. ^{1}HNMR spectra showed two multiplet signals at δ 1.65, 1.58 and 3.2, 3.1 due to methylene groups of piperidine ring , singlet signal of the methyl group (CH_3C=N) at δ= 2.13, 2.16 in addition to two D_2O exchangeable signals of 2NH groups at δ= 10.18, 10.74 and δ=10.11, 10.54 for **10a** and **10b** respectively [10]. Further evidence for **10a,b** structure was showed from the appearance of signals in the region δ= 11-25 and 50 in ^{13}C NMR due to carbon of piperidine ring (*cf* experimental part).

Table 1: reaction time, yield and melting points of the synthesized compounds

compound	Reaction time	Yield %	m.p.°C
2	1h	98	100-101
3	1h	92	270-272
4	1h	90	225-227
6a	25 min.	88[a], 93[b]	298-300
6b	20min.	85[a], 89[b]	239-241
6c	30min.	86[a], 93[b]	200-202
8a	30 min.	85[a], 92[b]	224-225
8b	30 min.	87[a], 91[b]	205
9a	30 min.	80	125-127
9b	35 min.	82	98-100
10a	30 min.	89	241-243
10b	30 min.	92	165-167

a) The yield of method A

b) The yield of method B

3.2. *Biology*

All prepared compounds were screened for their antibacterial and antifungal activities. Vancomycine and Ketoconazole were used as a standards antibacterial and antifungal agent respectively (*cf* experimental part). Most of the newly synthesized compounds showed excellent antimicrobial activities with respect to standards (table 2, 3) [26, 27].

3.2.1. *Antibacterial activity*

a) *Gram positive bacteria*

From the results of table 2 we observed that Compound **2** revealed inhibition zone against Staphelococcus Aureus larger than the reference compound Vancomycine, while in case of B. cereus compound **3** zone is larger than reference and activity of compound **8b** against B. cereus equal to reference.

b) *Gram negative bacteria*

Compounds 2, 8b, 9a, 10a and 10b have antibacterial activity against Pseudomonas Aeruginosa stronger than reference compound Vancomycine. While Compounds 3, 8b, 9a and 10b have antibacterial activity against Sarseina equal or more than standard. The antibacterial activity of Compounds 2, 8b, 9a, 10a, 10b against E. coli equal or more than standard.

3.2.2 Antifungal activity

Except compound **3** all of tested compounds have excellent or good antifungal activity especially **6a, 6b, 9a, 10a** and **10b** which have inhibition zone against Saccharomyces Cervesia equal to standard compound Ketoconazole, while compounds **2, 9a** and **10b** have inhibition zone against Candida Albicans equal to standard compound. According to above results it was not surprise for us to found that **6a** has inhibition zone against Candida Albicans more than Ketoconazole

3.2.3 Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of all compounds were measured against bacteria and fungi and many of these compounds showed low MIC (25 μg/ml) against different bacteria and fungi which is illustrated in table 3

Table 2 Antimicrobial activity expressed as inhibition diameter zones in millimeters (mm) of chemical compounds against the pathological strains based on well diffusion assay

	Gram positive bacteria			*Gram negative bacteria*			*Yeast*	
Chemical compound	*Staphelococcus aureus* ATCC 29213	*B. subtilis ATCC6633*	*B. cereus*	*Pseudomonas. aeroginosa* ATCC27953	*Sarseina*	*E. coli ATCC 25922*	*Saccharomyces cervesia*	*Candida Albicans* NRRL Y-477
2	37	35	34	38	34	37	35	38
3	32	30	38	20	38	18	N.A.	N.A.
6a	17	17	19	20	34	14	38	39
6b	13	30	16	14	35	20	38	37
6c	12	13	18	12	27	14	35	30
8a	16	27	15	16	35	12	35	33
8b	34	20	37	37	37	37	35	34
9a	30	28	32	37	38	36	38	38
9b	30	30	34	15	30	N.A.	36	35
10a	16	N.A.	34	36	34	37	38	35
10b	30	27	31	37	38	36	38	38
Vancomycine	36	36	37	35	37	34	N.A.	N.A.
Ketoconazole	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	38	38

The experiment was carried out in triplicate and the average zone of inhibition was calculated;
N.A.
(No activity).

Table (3) Minimum inhibitory concentration (µg/ml) against the pathological strains based on two fold serial dilution technique

	Gram positive bacteria			*Gram negative bacteria*			*Yeast*	
Chemical compound	*Staphelococcus aureus* ATCC 29213	*B. subtilis ATCC6633*	*B. cereus*	*Pseudomonas. aeroginosa* ATCC27953	*Sarseina*	*E. coli ATCC 25922*	*Saccharomyces cervesia*	*Candida Albicans* NRRL Y-477
2	25	25	25	25	25	25	25	25
3	50	50	25	100	25	200	-	-
6a	200	200	200	200	50	-	25	25
6b	-	50	200	-	100	200	25	25
6c	-	-	200	-	100	-	50	25
8a	200	200	-	200	25	-	50	50
8b	25	100	25	25	25	25	25	25
9a	100	100	100	25	25	25	25	25
9b	50	50	25	200	100	-	25	25
10a	200	-	25	25	25	25	25	25
10b	100	100	100	25	25	25	25	25
Vancomycine	25	25	25	25	25	25	N.A.	N.A.
Ketoconazole	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	25	25

N.A. (No activity).

4. CONCLUSION

1- Ultrasonic wave verify some of green chemistry principles

2- Ultrasonic waves increases the yield and decreases reaction time

3- Most of novel synthesized compounds have excellent antimicrobial activity equal or more than standards

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