Novel 1,2,3-Triazole Derivatives as Antimicrobial Agents

Bakr F. Abdel-Wahab[1,*], Hanan A. Mohamed[1] and Ghada E.A. Awad[2]

[1]*Applied Organic Chemistry Department, National Research Centre, Dokki, 12622 Giza, Egypt*

[2]*Chemistry of Natural and Microbial Products, National Research Center, Dokki, 12622 Giza, Egypt*

Abstract: 2-Substituted benzimidazoles **3a-c** was prepared *via* condensation of ethyl pyruvates **2a-c** with 2-phenylenediamine in glacial acetic acid. Pyrrolo[1,2-*a*]benzimidazole derivatives **5** and **8** were prepared *via* cyclocondensation of compounds **3a,b** with phosphorus oxychloride or acetic anhydride in presence of sodium acetate respectively. Reactivity of pyruvate **2** towards 2-aminophenol, hydroxylamine and cyanothioacetamide was also investigated. They exhibited potent antimicrobial activity comparable to clinical drugs such as ciprofloxacin and ketoconazole. Compounds **3a**, **11** and **13** showed excellent antimicrobial activity against all the tested microbes, and Compound **11** showed lower MIC values against all the tested organisms.

Keywords: Triazole, benzimidazole, pyruvate, antimicrobial activity, MIC, structure-activity relationship.

1. INTRODUCTION

Pyruvic acid is a natural metabolite, which plays an important role in biochemistry. In addition, derivatives of pyruvic acids are convenient synthons for the synthesis of various heterocyclic compounds [1-5]. For example, they have been utilized for the synthesis of benzimidazole [2] and 1,4-benzoxazine-2-one [3-5]. Also, 1,2,3-triazoles display significant biological activities such as antimicrobial activity against Gram positive bacteria [6]. In addition, the derivatives of 1,2,3-triazole also are applied as insecticides [7], fungicides [8] and plant growth regulators [9, 10]. Continuing our work in this research field [11-13] and in an attempt to identify new and potent antimicrobial agents, we tried here to generate new 1,2,3-triazole derivatives bearing benzimidazole, benzo[*b*][1,4] oxazin-2-one, isonicotine, isoxazole substituent as potential antimicrobial agents.

2. EXPERIMENTAL

2.1. Chemistry

All melting points were taken on Electrothermal IA 9000 series digital melting point apparatus. Elemental analytical data were carried from the micro-analytical unit, Cairo University, Giza, Egypt. The IR spectra were recorded in potassium bromide disks on a JASCO FT/IR-6100. ^{1}H-NMR and ^{13}C NMR spectra were run on JOEL-ECA 500MHz in deuterated dimethylsulphoxide (DMSO-d_6). Chemical shifts values (δ) are given in parts per million (ppm). The mass spectra were performed using mass Varian MAT CH-5 spectrometer at 70eV.

*Address correspondence to this author at the Applied Organic Chemistry Department, National Research Centre, Dokki, 12622 Giza, Egypt; Tel/Fax: +20227601877; E-mail: bakrfatehy@yahoo.com

Ethyl 4-(5-methyl-1-subs. phenyl-1H-1,2,3-triazol-4-yl)-2,4-dioxobutanoates (2a-c)

A mixture of appropriate 4-acetyl-5-methyl-1,2,3-triaozles **1a-c** (1 mmol) and diethyl oxalate (2 mmol) in 50 cm^3 sodium methoxide solution (0.005 g Na/25 cm^3 methanol) was refluxed for 30 min, and then cooled. The solid that separated was washed with dilute hydrochloric acid and recrystallized from ethanol to give **2a-c**.

Ethyl 4-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)-2,4-dioxobutanoate (2a)

Yield 70 %; m.p. 120-2°C; IR (KBr) νmax/cm^{-1}, 1662, 1745 (2 C=O), 3198 (OH); ^{1}H NMR (DMSO-d_6) δ 1.30 (t, 3H, CH_3), 2.47 (s, 3H, CH_3), 4.56 (q, 2H, CH_2), 7.34 (s, 1H, enolic-CH), 7.46-7.55 (m, 5H, Ar-H), 12.41 (s, 1H, OH, D_2O exchangeable); MS m/z (%): 301 (M^+, 15), 91 (100); Anal. Calcd for$C_{15}H_{15}N_3O_4$ (301.30): **C,** 59.79; H, 5.02; N, 13.95%. Found: C, 59.83; H, 5.18; N, 14.12%.

Ethyl 4-(5-methyl-1-4-tolyl-1H-1,2,3-triazol-4-yl)-2,4-dioxobutanoate (2b)

Yield 72 %; m.p. 128-9°C; IR (KBr) νmax/cm^{-1}, 1666, 1747 (2 C=O), 3222 (OH); ^{1}H NMR (DMSO-d_6) δ 1.31 (t, 3H, CH_3), 2.31 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 4.56 (q, 2H, CH_2), 7.36 (s, 1H, enolic-CH), 7.46-7.53 (m, 4H, Ar-H), 12.46 (s, 1H, OH, D_2O exchangeable); MS m/z (%): 315 (M^+, 9), 91 (100); Anal. Calcd for$C_{16}H_{17}N_3O_4$ (315.32): C, 60.94; H, 5.43; N, 13.33%. Found: C, 61.10; H, 5.51; N, 13.43%.

Ethyl 4-(1-(4-fluorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)-2,4-dioxobutanoate (2c)

Yield 75 %; m.p. 140-1°C; IR (KBr) νmax/cm^{-1}, 1670, 1750 (2 C=O), 3220 (OH); ^{1}H NMR (DMSO-d_6) δ 1.31 (t, 3H, CH_3), 2.43 (s, 3H, CH_3), 4.58 (q, 2H, CH_2),

E-ISSN: 2308-8044/14

7.33 (s, 1H, enolic-CH), 7.44-7.54(m, 4H, Ar-H), 12.43 (s, 1H, OH, D_2O exchangeable); MS m/z (%): 319 (M^+, 4), 91 (100); Anal. Calcd for $C_{15}H_{14}FN_3O_4$ (319.29): C, 56.43; H, 4.42; N, 13.16 %. Found: C, 56.61; H, 4.53; N, 13.20%.

1-(1H-Benzo[d]imidazol-2-yl)-3-(5-methyl-1-4-subs.phenyl-1H-1,2,3-triazol-4-yl)propane-1,3-diones (3)

A mixture of appropriate **2** (0.01 mol) with 2-phenylenediamine (0.01 mol) in acetic acid (15 cm^3) was heated under reflux for 1 h. The product obtained was collected by filtration, washed with ethanol and dried.

1-(1H-Benzo[d]imidazol-2-yl)-3-(5-methyl-1-phenyl-1H-1,2,3-triazol-4-yl)propane-1,3-dione (3a)

Yield 68 %; m.p. 290-1°C; IR (KBr) νmax/cm^{-1}, 1680(C=O), 3161 (NH); ^{1}H NMR (DMSO-d_6) δ 2.44 (s, 3H, CH_3), 7.10 (s, H, CH), 7.21-7.67(m, 9H, Ar-H), 11.97 (s, 1H, OH, D_2O exchangeable), 13.23 (s, 1H, NH, D_2O exchangeable); MS m/z (%): 345 (M^+, 17), 91 (100); Anal. Calcd for $C_{19}H_{15}N_5O_2$ (345.35): C, 66.08 ; H, 4.38; N, 20.28 %. Found: C, 66.13; H, 4.41; N, 20.20%.

1-(1H-Benzo[d]imidazol-2-yl)-3-(5-methyl-1-4-tolyl-1H-1,2,3-triazol-4-yl)propane-1,3-dione (3b)

Yield 73 %; m.p. 296-8°C; IR (KBr) νmax/cm^{-1}, 1680(C=O), 3168 (NH); ^{1}H NMR (DMSO-d_6) δ 2.44, 2.56 (2s, 6H, $2CH_3$), 7.09 (s, H, CH), 7.21-7.67(m, 8H, Ar-H), 11.89 (s, 1H, OH, D_2O exchangeable), 13.22 (s, 1H, NH, D_2O exchangeable); MS m/z (%): 359 (M^+, 20), 91 (100); Anal. Calcd for $C_{20}H_{17}N_5O_2$ (359.38): C, 66.84; H, 4.77; N, 19.49 %. Found: C, 66.90; H, 4.83; N, 19.59%.

1-(1H-Benzo[d]imidazol-2-yl)-3-(1-(4-fluorophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)propane-1,3-dione (3c)

Yield 72 %; m.p. 301-2°C; IR (KBr) νmax/cm^{-1}, 1687(C=O), 3180 (NH); ^{1}H NMR (DMSO-d_6) δ 2.44 (s, 3H, CH_3), 7.10 (s, H, CH), 7.22-7.69 (m, 8H, Ar-H), 11.93 (s, 1H, OH, D_2O exchangeable), 13.31 (s, 1H, NH, D_2O exchangeable); MS m/z (%): 363 (M^+, 17), 91 (100); Anal. Calcd for $C_{19}H_{14}FN_5O_2$ (363.35): C, 62.81; H, 3.88; N, 19.27%. Found: C, 62.92; H, 3.89; N, 19.36%.

1-(5-Methyl-1-4-subs.phenyl-1H-1,2,3-triazol-4-yl)-3H-benzo[d]pyrrolo[1,2-a]imidazol-3-ones (5a,b)

A solution of **3** (0.01 mol) in phosphorus oxychloride (30 cm^3) was heated on a under reflux for 3 h. The reaction mixture was cooled and poured into water. The product obtained was filtered off, washed with water and dried to give **5** as pale yellow crystals.

1-(5-Methyl-1-phenyl-1H-1,2,3-triazol-4-yl)-3H-benzo[d]pyrrolo[1,2-a]imidazol-3-one (5a)

Yield 30 %; m.p. 202-3°C; IR (KBr) νmax/cm^{-1}, 1650 (C=O); ^{1}H NMR (DMSO-d_6) δ 2.67 (s, 3H, CH_3), 7.60 (s, 1H, CH), 7.67-7.86 (m, 9H, Ar-H); ^{13}C NMR(DMSO-d_6) δ 10.1, 107, 114, 115.2, 123.1, 128, 125, 130, 133.2, 138, 139, 141, 149, 172; MS m/z (%): 327 (M^+, 7), 169 (100); Anal. Calcd for $C_{19}H_{13}N_5O$ (327.34): C, 69.71; H, 4.00; N, 21.39 %. Found: C, 69.88; H, 3.96; N, 21.22%.

1-(5-Methyl-1-4-tolyl-1H-1,2,3-triazol-4-yl)-3H-benzo[d]pyrrolo[1,2-a]imidazol-3-one (5b)

Yield 32 %; m.p. 208-9°C; IR (KBr) νmax/cm^{-1}, 1647 (C=O); ^{1}H NMR (DMSO-d_6) δ 2.43, 2.67 (2s, 6H, $2CH_3$), 7.61 (s, 1H, CH), 7.66-7.83 (m, 8H, Ar-H); MS m/z (%): 341 (M^+, 6), 169 (100); Anal. Calcd for $C_{20}H_{15}N_5O$ (341.37): C, 70.37; H, 4.43; N, 20.52%. Found: C, 70.44; H, 4.60; N, 20.68%.

1-Methyl-2-(5-methyl-1-subs. phenyl-1H-1,2,3-triazole-4-carbonyl)-3H-benzo[d]pyrrolo[1,2-a]imidazol-3-one (8a,b)

A solution of **3** (0.01 mol) in acetic anhydride (30 cm^3) and sodium acetate (3g) was heated under reflux for 3 h. The product formed after being cooled was filtered off dried and purified by recrystallization with ethanol to give **8** as yellow crystals.

1-Methyl-2-(5-methyl-1-phenyl-1H-1,2,3-triazole-4-carbonyl)-3H-benzo[d]pyrrolo[1,2-a]imidazol-3-one (8a)

Yield 48 %; m.p. 162-3°C; IR (KBr) νmax/cm^{-1}, 1680, 1701 (2C=O); ^{1}H NMR (DMSO-d_6) δ 1.83, 2.34 (2s, 6H, $2CH_3$), 7.22-7.67 (m, 9H, Ar-H); MS m/z (%): 369 (M^+, 7), 312 (100); Anal. Calcd for $C_{21}H_{15}N_5O_2$ (369.38): C, 68.28; H, 4.09; N, 18.96%. Found: C, 68.31; H, 4.16; N, 19.08%.

1-Methyl-2-(5-methyl-1-4-tolyl-1H-1,2,3-triazole-4-carbonyl)-3H-benzo[d]pyrrolo[1,2-a]imidazol-3-one (8b)

Yield 48 %; m.p. 210-2°C; IR (KBr) νmax/cm^{-1}, 1678, 1713 (2 C=O); ^{1}H NMR (DMSO-d_6) δ 1.81, 2.35, 3.52 (3s, 9H, $3CH_3$), 7.19-7.62 (m, 8H, Ar-H); ^{13}C NMR(DMSO-d_6) δ 10, 17, 21, 114, 115, 116, 123, 125, 129, 130, 132, 133, 138, 139, 141, 153, 172,175; MS m/z (%): 383 (M^+, 6), 312 (100); Anal. Calcd for

$C_{22}H_{17}N_5O_2$ (383.40): C, 68.92; H, 4.47; N, 18.27%. Found: C, 68.99; H, 4.52; N, 18.36%.

3-(2-Hydroxy-2-(5-methyl-1-p-tolyl-1H-1,2,3-triazol-4-yl)vinyl)-2H-benzo[b][1,4]oxazin-2-one (9)

A mixture of **2b** (0.01 mol) with 2-aminophenol (0.01 mol) in acetic acid (15 cm^3) was heated under reflux for 1 h. The product obtained was collected by filtration, washed with ethanol and dried.

Yield 54 %; m.p. 208-9°C; IR (KBr) νmax/cm^{-1}, 1648, 1660 (C=O), 3280 (OH); ^{1}H NMR (DMSO-d_6) δ 2.35, 3.48 (2s, 6H, $2CH_3$), 7.12-7.68 (m, 9H, Ar-H + olefinic-H), 12.44 (s, 1H, OH, D_2O exchangeable); MS m/z (%): 360 (M^+, 5), 91 (100); Anal. Calcd for $C_{20}H_{16}N_4O_3$ (360.37): C, 66.66; H, 4.48; N, 15.55%. Found: C, 66.73; H, 4.56 N, 15.69%.

Ethyl 5-(5-methyl-1-4-tolyl-1H-1,2,3-triazol-4-yl)isoxazole-3-carboxylate (10)

A mixture of compound **2b** (2 mmol) in ethanol (20 cm^3), hydroxylamine·HCl (0.14 g, 2 mmol) and fused sodium acetate (0.16 g, 2 mmol) was refluxed for 2 h, then left to stand at room temperature. The product obtained was filtered off and dried.

Yield 39 %; m.p. 205-6°C; IR (KBr) νmax/cm^{-1}, 1750 (C=O); ^{1}H NMR (DMSO-d_6) δ 1.42 (t, 3H, CH_3), 2.33, 2.42 (2s, 6H, $2CH_3$), 4.39 (q, 2H, CH_2), 7.44-7.64(m, 5H, $1H_4$-oxazole + 4Ar-H); MS m/z (%): 312 (M^+, 3), 91 (100); Anal. Calcd.for $C_{16}H_{16}N_4O_3$ (312.32): C, 61.53; H, 5.16; N, 17.94%. Found: C, 64.62; H, 5.22; N, 18.10%.

5-(5-methyl-1-4-tolyl-1H-1,2,3-triazol-4-yl)isoxazole-3-carboxylic acid (11)

A suspension of appropriate of isoxazole-3-carboxylates **10** (1 mmol) in ethanol (10 cm^3) and 5% NaOH (10 cm^3) was refluxed for 4 h. Left to cool to room temperature, and the white solid product was filtered and washed with ethanol.

Yield 38 %; m.p. 235-6°C; IR (KBr) νmax/cm^{-1}, 1768 (C=O), 3345 (OH); ^{1}H NMR (DMSO-d_6) δ 2.33, 2.42 (2s, 6H, $2CH_3$) 7.44-7.64 (m, 5H, $1H_4$-oxazole + 4Ar-H), 12.34 (s, 1H, OH, D_2O exchangeable); MS m/z (%): 284 (M^+, 3), 91 (100); Anal. Calcd.for $C_{14}H_{12}N_4O_3$ (284.27): C, 59.15; H, 4.25; N, 19.71 %. Found: C, 59.26; H, 4.32; N, 19.83%.

Ethyl 3-cyano-2-mercapto-6-(5-methyl-1-4-tolyl-1H-1,2,3-triazol-4-yl)isonicotinate (13)

A mixture of **2b** (2 mmol) in ethanol (20 cm^3), cyanothioacetamide (2 mmol), and a few drops of piperidine were refluxed for 4 h and left to cool. The resulting solid product was filtered off and dried.

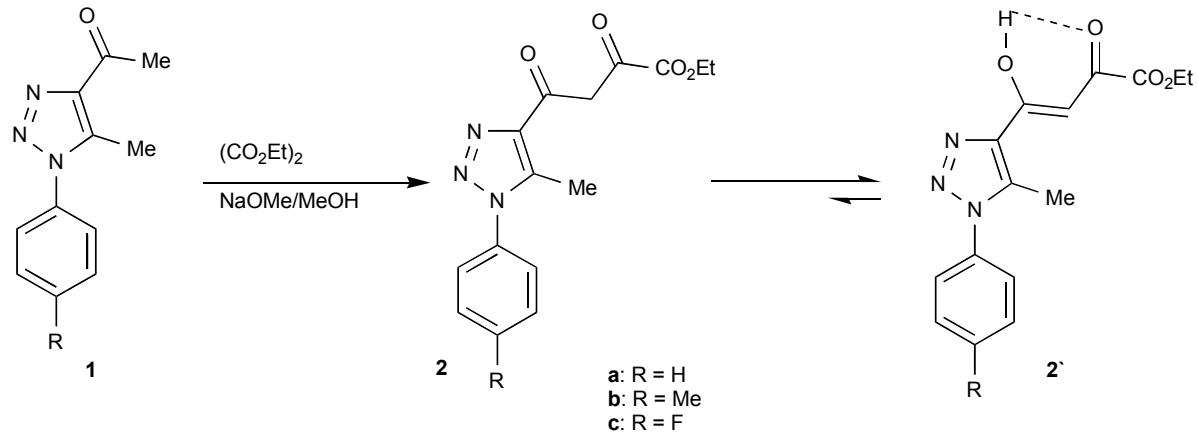
Yield 42 %; m.p. 218-20°C; IR (KBr) νmax/cm^{-1}, 1745 (C=O), 2223 (CN); ^{1}H NMR (DMSO-d_6) δ 1.50 (t, 3H, CH_3), 2.33, 2.42 (2s, 6H, $2CH_3$), 4.34 (q, 2H, CH_2), 7.33 (s, 1H, CH-pyridine) 7.41-7.60 (m, 4H, Ar-H), 12.96 (s, 1H, SH, D_2O exchangeable); MS m/z (%): 379 (M^+, 13), 65 (100); Anal. Calcd for $C_{19}H_{17}N_5O_2S$ (379.44): C, 60.14; H, 4.52; N, 18.46 %. Found: C, 60.24; H, 4.64; N, 18.57%.

2.2. Antimicrobial Activity

Chemical compounds were individually tested against a panel of gram positive and gram negative bacterial pathogens, yeast and fungi. Antimicrobial tests were carried out by the agar well diffusion method [14] using 100 µL of suspension containing $1x10^8$ CFU/mL of pathological tested bacteria and $1 x10^6$ CFU/ml of yeast spread on nutrient agar (NA) and 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 100 mg 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 fungi. Negative controls were prepared using DMSO employed for dissolving the tested compound. Ciprofloxacin (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 **1**. 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.

2.2.1. Minimal Inhibitory Concentration (MIC) Measurement

The bacterio-static activity of the active compounds (having inhibition zones (IZ) ≥ 16 mm) was then evaluated using the two fold serial dilution technique [15]. 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 bacteria (about



Scheme 1:

1×10^8 CFU/ml), each 5 ml received 0.1 ml of the above inoculums and incubated at 37 °C for 24 hours. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC).

3. RESULTS AND DISCUSSION

3.1. Chemistry

2,4-Dioxoesters **2** were prepared by Claisen condensation of the appropriate 4-acetyl-5-methyl-1,2,3-triazoles **1** with diethyl oxalate in the presence of sodium ethoxide. Acidification of the resulting solution with dilute acid to give excellent yields of the precursor **2** which present in the enolic form **2`**(Scheme **1**).

Condensation of **2a-c** with 2-phenylenediamine in glacial acetic acid under reflux, gave the corresponding tautomeric benzimidazole **3a-c** and **3`a-c**. 1-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(5-methyl-1-*p*-subs. phenyl-1*H*-1,2,3-triazol-4-yl)propane-1,3-dione **3a,b** reacted with phosphorus oxychloride under reflux on a water bath affording 1-(5-methyl-1-subs. phenyl-1*H*-1,2,3-triazol-4-yl)-3*H*-benzo[*d*]pyrrolo[1,2-*a*]imidazol-3-one **5a,b** *via* the intermediate **4a,b**. 1-Methyl-2-(5-methyl-1-subs. phenyl-1*H*-1,2,3-triazole-4-carbonyl)-3*H*-benzo [*d*]pyrrolo[1,2-*a*]imidazol-3-one **8a,b** was prepared by the cyclocondensation of benzimidazoles **3a,b** with acetic anhydride in the presence of fused sodium acetate under reflux through the intermediates **6a,b** and **7a,b** (Scheme **2**).

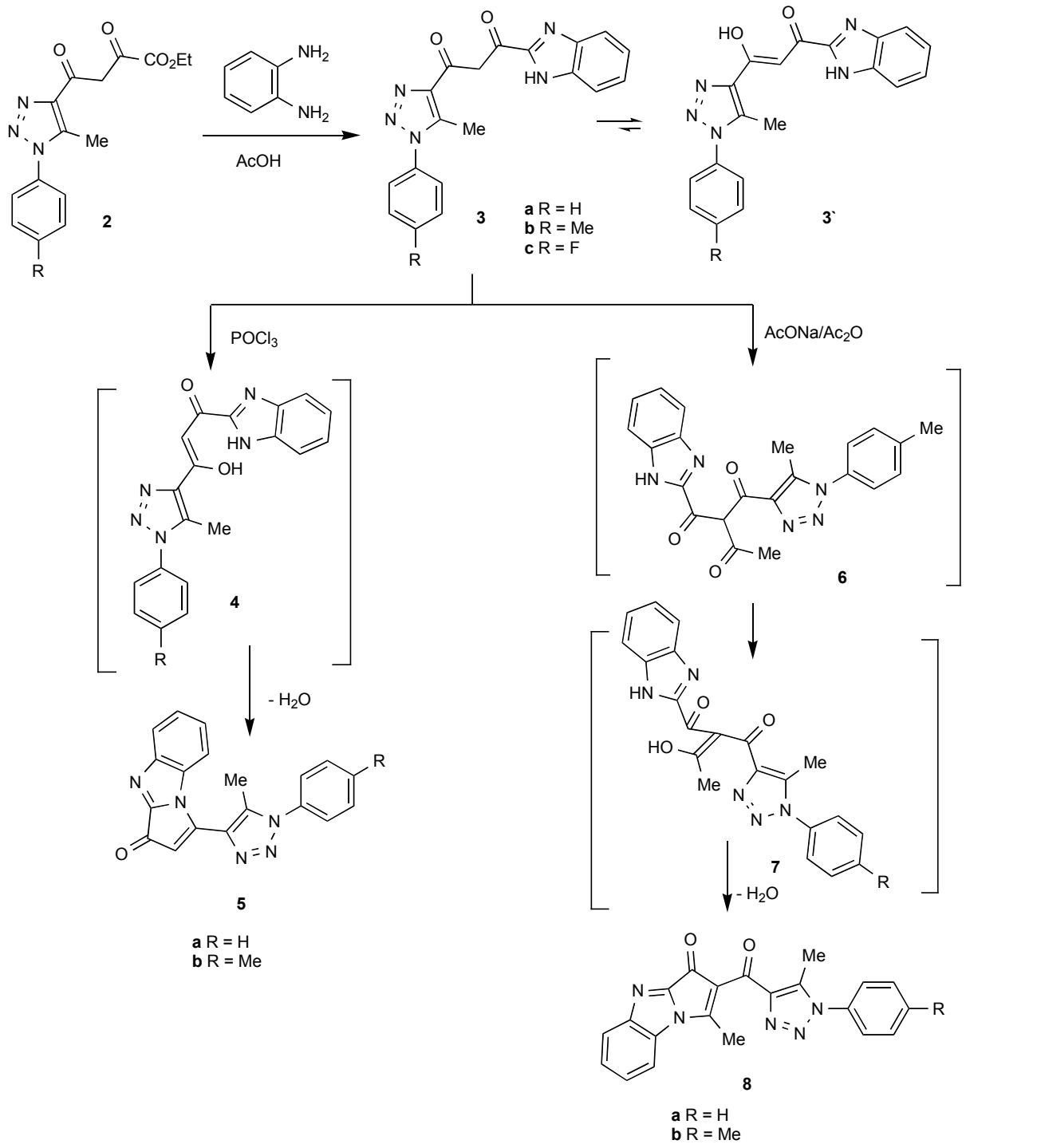
The structures of **3-8** were fully supported by elemental analysis and spectral analysis. In the ^{1}H NMR spectrum of **3b** the NH proton of benzimidazole ring appears at δ 13.22 ppm. In the mass spectra of **3a**, **5a** and **8b** showed the molecular ion peaks at *m/z* 345, 327 and 383 respectively in agreement with the calculated masses.

The condensation of 2,4-dioxoesters **2b** with 2-aminophenol in acetic acid afforded 3-(2-(5-methyl-1-*p*-tolyl-1*H*-1,2,3-triazol-4-yl)-2-oxoethyl)-2*H*-benzo[*b*][1,4] oxazin-2-one **9**. When ethyl 2,4-dioxobutanoate **2b** was treated with hydroxylamine hydrochloride gave ethyl 5-(5-methyl-1-*p*-tolyl-1*H*-1,2,3-triazol-4-yl)isoxazole-3-carboxylates **10**, which then hydrolyzed with 5% sodium hydroxide to give the corresponding acids **11**. In addition, ethyl 2,4-dioxo-pentanoate **2b** was condensed with 2-cyano-thioacetamide in the presence of piperidine to give ethyl 3-cyano-2-mercapto-6-(5-methyl-1-*p*-tolyl-1*H*-1,2,3-triazol-4-yl)isonicotinate **13** *via* the intermediate **12** (Scheme **3**).

The structures of compounds **9-13** were confirmed by ^{1}H NMR, IR, MS, spectroscopic data, and elemental analysis. In the ^{1}H NMR spectrum of **11** the OH proton of carboxylic acid appears at δ 12.34 ppm and presence of SH proton in compound **13** at δ 12.96 ppm. In the mass spectra of **9**, **11** and **13** showed the molecular ion peaks at *m/z* 360, 284 and 379 respectively in agreement with the calculated masses.

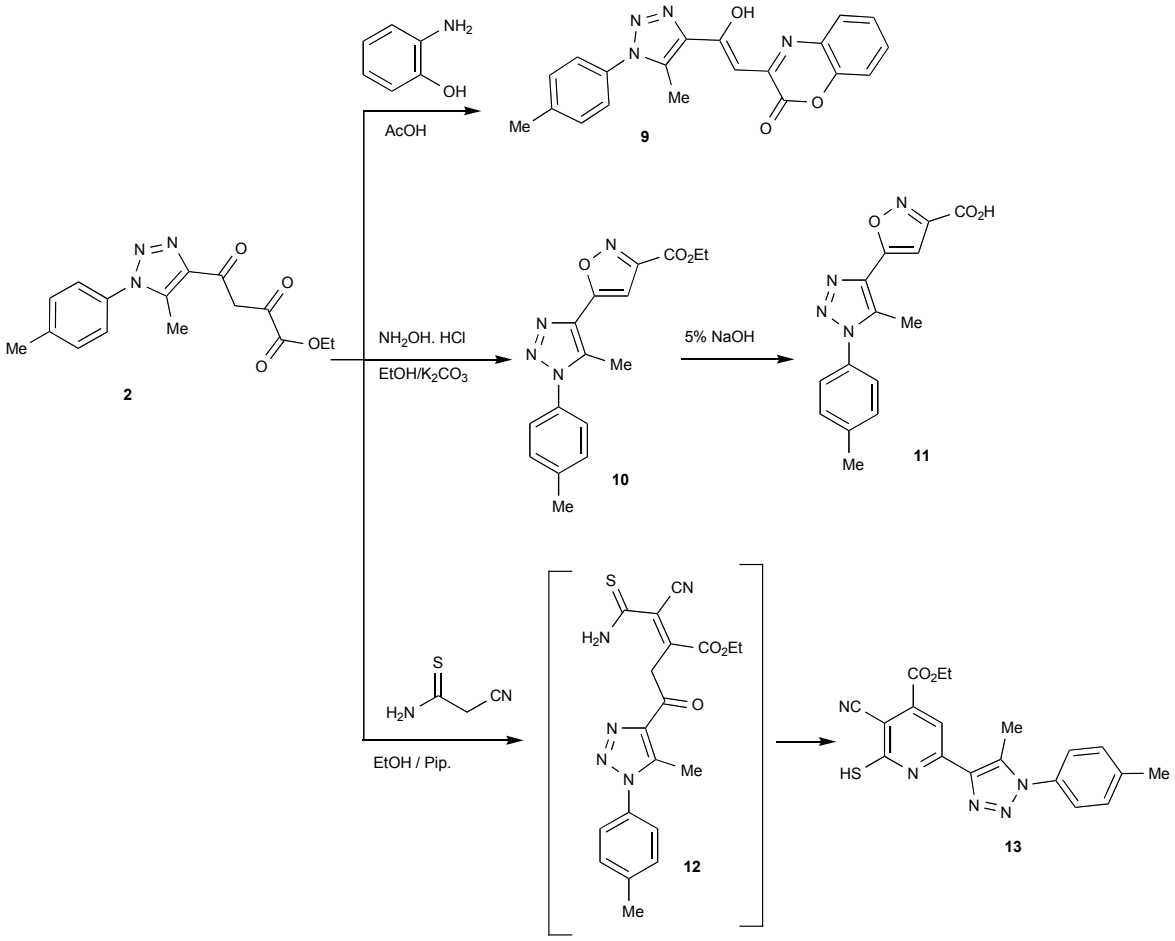
3.2. Antimicrobialactivity

The antimicrobial activity of the synthesized compounds has been evaluated by filter paper disc method [14]. The synthesized compounds have been tested for their antibacterial activity against Gram positive bacteria (*Staphylococcus aureus* ATCC 29213, Bacillus subtilis *ATCC663*, and Bacillus *megaterium ATCC* 9885); Gram negative bacteria (*Klebseilla peneumoniae* ATCC13883, *Pseudomonas. Aeroginosa* ATCC27953 and Echerichia coli *ATCC 2592*2) and fungal (*Saccharomyces cervesia*, *Candida Albicans* NRRL Y-477 and Aspergillus niger) at a concentration of 100 µg/mL in DMSO. Ciprofloxacin and Ketoco-



Scheme 2:

nazole were respectively used as standard antibacterial and antifungal reference, respectively. The inhibitory effects of compounds **2-13** against these organisms are given in Table **1**. The screening data indicate that compounds **3a**, **11** and **13** showed excellent antimicrobial activity against all the tested microbes, Also, compounds **2c**, **3c**, and **8a** showed good activities against the majority of the tested organisms. Compounds **5b** and **8a** showed no activity *B. subtilis ATCC6633* and *B. megaterium ATCC* 9885; compounds **8b** and **9** showed no activity against Staphylococcus aureus ATCC and Bacillus subtilis*ATCC6633* respectively. From the inhibition zone diameter analysis the diketo-ester **3**, isoxazole **11** and pyridine **13** were identified the more active compounds compared with the other synthetic compounds.



Scheme 3:

Table 1: Antimicrobial Activity Expressed as Inhibition Diameter Zones in Millimeters (mm) of Chemical Compounds Against the Pathological Strains Based on well Diffusion Assay

Chem. Cpds.	Gram positive bacteria			Gram negative bacteria			Yeast		Fungi
	Staphelococcus aureus ATCC 29213	*B. subtilis ATCC6633*	*B. megaterium ATCC* 9885	*Klebseilla peneumoniae* ATCC13883	*Pseudomonas. Aeroginosa* ATCC27953	*E. coli ATCC 25922*	*Saccharomyces cervesia*	*Candida Albicans* NRRL Y-477	*A.niger*
2a	26	22	21	23	21	22	15.	15	30
2b	15	20	21	18	17	15	15	12	25
2c	26	19	17	24	22	21	22	21	35
3a	32	29	30	25	29	27	22	21	38
3b	17	12	15	22	21	20	15	12	18
3c	25	15	16	16	17	18	15	12	35
5a	28	15	14	27	28	26	19	17	38
5b	21	N.A.	N.A.	24	25	24	12	13	20
8a	22	N.A.	N.A.	30	29	30	13	12	34
8b	N.A.	23	24	15	19	20	19	17	37
9	17	N.A.	12	27	25	24	14	14	38
10	21	15	18	26	29	27	18	14	38
11	35	32	35	33	36	34	32	30	38
13	32	23	24	22	21	23	19	15	34
Ciprofloxacin	20	22	24	25	24	23	N.A.	N.A.	N.A.
Ketoconazole	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	30	29	30

The experiment was carried out in triplicate and the average zone of inhibition was calculated.

Table 2: Minimum Inhibitory Concentration (µg/ml) Against the Pathological Strains Based on Two Fold Serial Dilution Technique

Chem. Cpds.	Gram positive bacteria			Gram negative bacteria			Yeast		Fungi
	Staphelococcus aureus ATCC 29213	*B. subtilis ATCC6633*	*B. megaterium ATCC* 9885	*Klebseillape neumoniae*A TCC13883	*Pseudomonas Aeroginosa* ATCC27953	*E. coli ATCC 25922*	*Saccharomyces cervesia*	*Candida Albicans* NRRL Y-477	*A.niger*
2a	100	100	200	200	200	200	N.A.	N.A.	50
2b	N.A.	200	200	200	200	N.A.	N.A.	N.A.	50
2c	50	200	200	100	200	200	200	200	50
3a	25	50	50	100	100	100	200	200	25
3b	200	N.A.	N.A.	200	200	200	N.A.	N.A.	200
3c	100	N.A.	N.A.	N.A.	200	200	N.A.	N.A.	50
5a	50	N.A.	N.A.	100	100	100	200	200	25
5b	200	N.A.	N.A.	100	100	100	N.A.	N.A.	200
8a	100	N.A.	N.A.	50	50	50	N.A.	N.A.	25
8b	N.A.	100	100	N.A.	200	200	200	200	25
9	100	N.A.	N.A.	50	100	100	N.A.	N.A.	25
10	100	N.A.	200	100	50	100	200	N.A.	25
11	25	25	25	50	50	50	25	50	25
13	50	100	100	200	200	100	200	N.A.	25
Ciprofloxacin	25	25	25	25	25	25	N.A.	N.A.	N.A.
Ketoconazole	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	25	25	25

The experiment was carried out in triplicate and the average zone of inhibition was calculated.

3.2.1. Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC, µg/ml) of compounds **2-13** in comparison to Ciprofloxacin and Ketoconazole against antibiotic susceptible strains of both Gram positive bacteria, Gram negative bacteria and fungal were determined (Table **2**). Amongst all the tested compounds, Compound **11** showed lower MIC values against all the tested organisms, also benzimidazole **3a** showed lower MIC level against Gram positive bacteria. All the synthetic compounds exhibited lower MIC values against *A.niger* except compounds **3b** and **5b**.

3.3. Structure-Activity Relationship

The structure-antimicrobial activity relationship of the synthesized compounds against the pathological strains of bacteria and fungi revealed that, benzimidazole **3a**, isoxazole-3-carboxylic acid **11**,and ethyl isonicotinate **13** have superior antimicrobial (i.e. biologically more active) than 2,4-dioxoesters **2c**, benzimidazole **3c**, and pyrrolo[1,2-*a*]imidazol-3-one **8a**. The compounds which have flouro function in para position of phenyl group in 1,2,3-triazoles **2c**, and **3c** have less antimicrobial activity than paramethyl compounds **11** and **13**. It is obvious from our antimicrobial data the compounds incorporating isoxazole and isonicotine moieties showed higher antimicrobial activity.

4. CONCLUSION

The present investigation offers rapid and effective procedures for the synthesis of the poly condensed new heterocyclic ring systems incorporating 1,2,3-triazole nucleus as bezoimidazole **3**, pyrrolo[1,2-*a*] benzimidazole derivatives **5** and **8**, benzoxazinone **9**, isoxazole-3-carboxylates **10**, and isonicotinate **13**. The antibacterial and antifungal activities of the prepared compounds were evaluated showing moderate to excellent activities. 1,2,3-triazole derivatives **3a**, **11** and, **13** exhibited the highest antibacterial and antifungal activities comparable to standard antibiotics.

REFERENCES

[1] Dawood KM, Abdel-Gawad H, Mohamed HA, Abdel-Wahab BF. Synthetic accesses to azolylthiazoles. Heterocycles 2010; 81: 1-55.

[2] Ibrahim HK, El-Tamany SH, El-Shaarawy RF, El-Deen IM. Synthesis and investigation of mass spectra of some novel benzimidazole derivatives. Macedonian J Chem Eng 2008; 27: 65-79.

[3] Orphanos DG, Taurins A. The synthesis of some 6,7-methylenedioxy- and 6,7-dimethoxy-1,4-benzoxazin-2-ones. Can J Chem 1966; 44: 1795-800. http://dx.doi.org/10.1139/v66-270

[4] Aliev ZG, Krasnykh OP, Maslivets AN, Atovmyan LO. Reaction of 3(Z)-benzoylmethylidene-6-nitro-3,4-dihydro-2H-1,4-benzooxazin-2-one with oxalyl chloride. Russ Chem Bull 2000; 49: 2045-7. http://dx.doi.org/10.1023/A:1009532227461

[5] Gein VL, Rassudikhina NA, Voronina EV. Synthesis and antibacterial activity of 6-ethylsulfonyl-3-acylmethylene-1,4-benzoxazin-2-ones. Pharm Chem J 2006; 40: 554-556. http://dx.doi.org/10.1007/s11094-006-0192-6

[6] Genin MJ, Allwine DA, Anderson DJ, Barbachyn MR, Emmert DE, Garmon SA, Graber DR, Grega KC, Hester JB, Hutchinson DK, Morris J, Reischer RJ, Ford CW, Zurenko GE, Hamel JC, Schaadt RD, Stapert D, Yagi BH. Substituent effects on the antibacterial activity of nitrogen-carbon-linked (azolylphenyl)oxazolidinones with expanded activity against the fastidious gram-negative organisms Haemophilus influenzae and Moraxella catarrhalis. J Med Chem 2000; 43: 953-70. http://dx.doi.org/10.1021/jm990373e

[7] Boddy IK, Briggs GG, Harrison RP, Jones TH, O'Mahony MJ, Marlow ID, Roberts BG, Willis RJ, Bardsley R, Reid J. The Synthesis and insecticidal activity of a series of 2-aryl-1,2,3-triazoles. Pest Sci 1996; 48: 189-96. http://dx.doi.org/10.1002/(SICI)1096-9063(199610)48:2<189::AID-PS461>3.0.CO;2-#

[8] Buechel KH, Gold H, Frohberger PE, Kaspers H. Triphenyl-1,2,3-triazolyl-(1')-methane, process for their production and their use as fungicides. German Patent 2407305,1975, Chem Abstr 1975; 83: 206290.

[9] Fletcher RA, Gilley A, Sankhla N, Davis TD. Triazoles as plant growth regulators and stress protectants. Horticultural Rev 2000; 24: 55-138.

[10] Krueger HR, Schroeer U, Baumert D, Joppien H. Trialkyl-(1,2,3-triazolyl)-tin compounds, process for preparing these compounds and compositions comprising them biocidal medium.German Patent 2936951, 1981, Chem Abstr 1981, 96, 52509.

[11] Abdel-Wahab BF, Abdel-Latif E, Mohamed HA, Awad GEA. Eur J Med Chem 2012; 52: 263. http://dx.doi.org/10.1016/j.ejmech.2012.03.023

[12] Abdel-Wahab BF, Sediek A, Mohamed HA, Awad GEA. Novel 2-pyrazolin-1-ylthiazoles as potential antimicrobial agents. Lett Drug Des Discov 2013; 10: 111-8. http://dx.doi.org/10.2174/157018013804725143

[13] Abdel-Wahab BF, Abdel-Gawad H, Awad GEA, Badria FA. Synthesis, antimicrobial, antioxidant, anti-inflammatory, and analgesic activities of some new 3-(2′-thienyl)pyrazole-based heterocycles. Med Chem Res 2012; 21: 1418-26. http://dx.doi.org/10.1007/s00044-011-9661-x

[14] Scott AC. Laboratory Control of Antimicrobial Therapy. In: Collee JG, Duguid JP, Fraser AG, Marmion BP, Mackie and MacCartney Practical Medical Microbiology. 13th Ed, Churchill Livingstone 1989; 2: 161.

[15] Perez C, Pauli M, Bazevque P. An antibiotic assay by the agarwell diffusion method. Acta Biol Med Exp 1990; 15: 113-5.

Received on 30-06-2014 Accepted on 25-08-2014 Published on 05-11-2014

DOI: http://dx.doi.org/10.12970/2308-8044.2014.02.02.3