Novel Synthesis, Antimicrobial Evaluation and Reactivity of Dehydroacetic Acid with N, C-Nucleophiles

[1]A.A. Fadda, [2]M.S. Amine, [2]M.M.H. Arief and [1]E. Kh. Farahat

[1]Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, Egypt
[2]Department of Chemistry, Faculty of Science, Benha University, Benha, Egypt

ABSTRACT

Background: Hydroxy-2-pyrones derivatives are considered as one important class of anti-HIV agents and exhibit a wide range of antifungal, phytotoxic, antimicrobial, cytotoxic and neurotoxic activities. Therefore, these studies an efficient synthetic methodology allowing a simple introduction of a plethora of substituents into the structures of these 2-pyrones still constitutes a challenge for the scientific community. **Materials and Methods:** Enaminone of dehydroacetic acid was subjected to react with N and C-nucleophiles in refluxing ethanol or acetic acid to give different heterocyclic compounds. **Results:** The selective enaminone of DHA 2 which has been attracting attention due to its high reactivity as building blocks for the preparation of several classes of compounds and its selective transformations with various nucleophiles. Isoxazole, pyrazole, pyrimidinthione, pyrrole, pyranopyrimidine, pyranopyrimidinthione, pyran, benzothiazolopyridine, chromen, benzochromen, benzopyran and formyl benzopyran derivatives containing pyrone moiety were synthesized via reaction of dehydroacetic acid enaminone with bifunctional reagents. **Conclusion:** All compounds were screened for their antimicrobial activity which showed promising results.

Key words: Isoxazole, pyrazole, pyrimidinthione, benzothiazolopyridine, dehydroacetic acid

INTRODUCTION

Pyran-2-one, a six-membered cyclic unsaturated ester its derivatives constituents a large family of biologically active natural products abundantly found in animals, insects, plants, bacteria microbial systems (Culter and Jacyno, 1991; Rasoanaivo *et al.*, 1993; Steyn and Heerden, 1998).

Small changes in the substitution pattern on the 2-pyrone ring often lead to diverse biological properties. For instance, 4-hydroxy-2-pyrones are considered as one important class of anti-HIV agents and exhibit a wide range of antifungal, phytotoxic, antimicrobial, cytotoxic and neurotoxic activities (Altomare *et al.*, 2000, 2004).

These two natural occurring 2-pyrones have been extensively studied as building blocks for a wide range of important biologically active heterocyclic compounds, such as phenomones, pyridones, styrylpyrones, pyrazoles, benzodiazpines and coumarins (Hernadez-Galan *et al.*, 1993; Bendaas *et al.*, 1999; Boutemeur-Kheddis *et al.*, 2001; Makhloufi-Chebli *et al.*, 2009). Therefore, these studies an efficient synthetic methodology allowing a simple introduction of a plethora of substituents into the structures of these 2-pyrones still constitutes a challenge

for the scientific community. It was interesting to use dehydroacetic acid (DHA) 1 as a building block for the synthesis of other heterocyclic compounds, the selective enaminone of DHA 2 (Kumar *et al.*, 2006) which has been attracting attention due to its high reactivity as building blocks for the preparation of several classes of compounds and its selective transformations with various nucleophiles. Inspection of the structure 2 suggests that it would be susceptible for the attack of amines at five sites (a, b, c, d and e) leading to quiet different products (Fig. 1).

Transformations involving the three reactive sites of the pyrone ring and the direct condensation in the enamine group, in particular with bi-nucleophilic reagents, offer a versatile approach to synthesize a new heterocyclic compounds. In that way, it was decided to study the reactivity of EDHA 2 with several bi-nucleophilic amines.

MATERIALS AND METHODS

Melting points were measured with a Gallenkamp apparatus and are uncorrected. IR spectra were recorded for KBr disc on a Mattson 5000 FTIR spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Bruker AC 300 (300 MHz) in CDCl₃ or DMSO-d₆ as solvent, using TMS as an internal

Corresponding Author: A.A. Fadda, Faculty of Science, Mansoura University, Mansoura, Egypt
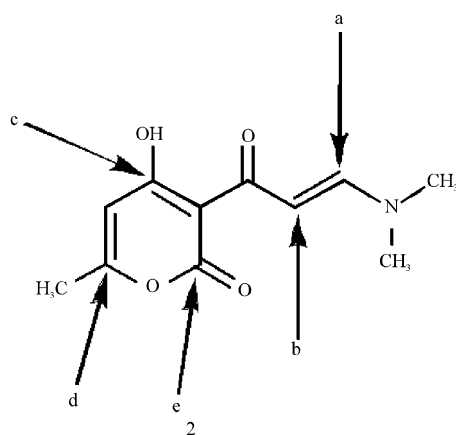

Fig. 1: Susceptible positions for the attack of amines at five sites in structure 2

standard chemical shifts are expressed as δ_{ppm}. Mass spectra were determined on Finnigan Incos 500 (70 ev). Elemental analyses were carried out at the Microanalytical Center of Cairo University. All reactions were followed by TLC (Silica gel, aluminum sheets 60 F_{254}, Merck.

General procedure for the synthesis of compounds 3 and 6: A mixture of 2 (0.40 g, 1.79 mmol), hydroxyl amine hydrochloride (0.13 g, 1.82 mmol) or thiourea (0.14 g, 1.84 mmol) in ethanol (30 mL) was refluxed in the presence of few drops of triethyl amine for 8 h. After cooling to room temperature the obtained solid material was recrystallized to give compounds 3 and 6, respectively.

3-(Isoxazol-5-yl)-6-methyl-2H-pyran-2, 4-(3H)-dione (3): Yellow powder; yield (80%); m.p. 181 °C; IR (KBr): v/cm^{-1} = 3436 (NH), 1709, 1609 (2C = O groups), 1569 (C =C) and 1194 (C-O-C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.22 (s, 3H, CH$_3$), 4.45 (s, 1H, C$_3$-H pyrone), 5.40 (s, 1H, C$_5$-H pyrone), 5.66 (d, 1H, C$_3$-H isoxazole) 7.97 (d, 1H, C$_4$-H isoxazole); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 20 (CH$_3$), 62.6 (C$_3$-pyrone), 101.2 (C$_3$-isoxazole), 115.9 (C$_5$-pyrone), 150 (C$_4$-isoxazole), 158 (C$_2$-isoxazole), 167.00 (C$_2$-pyrone), 174.00 (C$_6$-pyrone), 196.5 (C$_4$-pyrone); MS (EI, 70 ev): m/z (%) = 193 (M$^+$, 45). Anal. Calcd. for C$_9$H$_7$NO$_4$ (193.16): C, 55.96; H, 3.65; N, 7.25%. Found: C, 55.93; H, 3.61; N, 7.20%.

6-Methyl-3-(2-thioxo-1, 2-dihydropyrimidine-4-yl)-2H-pyran-2, 4(3H)-dione (6): Yellow crystals; yield (82%); m.p. 179 °C; IR (KBr): v/cm^{-1}= 3265, 3137 (NH), 1684, 1612 (2C=O groups), 1382 (C = S); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.20 (s, 3H, CH$_3$), 3.87 (s, 1H, C$_3$-H pyrone), 4.12 (d, 2H, CH$_2$ pyrimidine) 5.40 (s, 1H, C$_5$-H pyrone), 5.83 (t, 1H, CH pyrimidine), 8.89 (s, 1H, NH), 9.84 (s, 1H, NH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 20.00 (CH$_3$), 48.70 (C$_4$-pyrimidine), 66.00 (C$_3$-pyrone), 103.00 (C$_3$-pyrimidine), 115.00 (C$_5$-pyrone), 145.00 (C$_2$-pyrimidine), 167.00 (C$_2$-pyrone), 174.00 (C=S), 174.00 (C$_6$-pyrone), 186.00 (C$_4$-pyrone); MS (EI, 70 ev): m/z (%) = 238.00 (M$^+$, 5.00) Anal. Calcd. for C$_{10}$H$_{10}$N$_2$O$_3$S (238.26): C, 50.41; H, 4.23; N, 11.76%. Found: C, 50.38; H, 4.19; N, 11.70%.

General procedure for the synthesis of compounds 4 and 12: A mixture of 2 (0.40 g, 1.79 mmol), hydrazine hydrate (0.08 g, 2.00 mmol) or ethyl glycinate hydrochloride (0.27 g, 1.92 mmol) in ethanol (30 mL) was refluxed for 8 h. After cooling to room temperature the obtained solid material was recrystallized to give compounds 4 and 13, respectively.

6-Methyl-3-(1H-pyrazol-3-yl)-2H-pyran-2, 4(3H)-dione (4): Buff powder; yield (82%); m.p. 200°C; IR (KBr): v/cm^{-1}= 3184 (NH), 1693, 1640 (2C=O groups), 1590 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.21 (s, 3H, CH$_3$), 4.47 (s, 1H, C$_3$-H pyrone), 5.41 (s, 1H, C$_5$-H pyrone), 6.66 (d, 1H, C$_3$-H pyrazole), 7.48 (d, 1H, C$_4$-H pyrazole), 13.70 (s, 1H, NH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 20.00 (CH$_3$), 62.50 (C$_3$-pyrone), 103.00 (C$_3$-pyrazole), 115.00 (C$_5$-pyrone), 137.00 (C$_4$-pyrazole), 144.00 (C$_2$-pyrazole), 167.00 (C$_2$-pyrone), 174.00 (C$_6$-pyrone), 195.00 (C$_4$-pyrone); MS (EI, 70 ev): m/z (%) = 192.00 (M$^+$, 5.00) Anal. Calcd. for C$_9$H$_8$N$_2$O$_3$ (192.17): C, 56.25; H, 4.20; N, 14.58%. Found: C, 56.00; H, 4.10; N, 14.52%.

Ethyl-3-(2-hydroxy-6-methyl-4-oxo-4H-pyran-3-yl)-2, 5-dihydro-1H-pyrrole-2-carboxylate (12): Pink powder; yield (80%); m.p. 143°C; IR (KBr): v/cm^{-1} = 3306 (NH), 1740, 1651, 1626 (3C=O groups); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 1.23 (t, 3H, CH$_3$), 2.20 (s, 3H, CH$_3$), 3.27-3.17 (dd, 2H, CH$_2$ pyrrole), 3.87 (s, 1H, C$_3$-H pyrone), 4.14 (s, 1H, C$_2$-H pyrrole), 4.21 (q, 2H, CH$_2$), 5.40 (s, 1H, C$_5$-H pyrone), 5.67 (t, 1H, CH pyrrole), 7.10 (s, 1H, NH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 14.00, 20.00, 56.00, 58.00, 61.00, 67.00, 115.00, 130.00, 135.00, 167.00, 168.00, 174.00, 186.00; MS (EI, 70 ev): m/z (%) = 265.00 (M$^+$, 5.00). Anal. Calcd. for C$_{13}$H$_{15}$NO$_5$ (265.26): C, 58.86; H, 5.70; N, 5.28%. Found: C, 58.81; H, 5.65; N, 5.21%.

General procedure for the synthesis of compounds 13, 14, 15, 16 and 17: A mixture of 2 (0.40 g, 1.79 mmol), the appropriate active methylene compound namely barbituric acid (0.24 g, 1.79 mmol), thiobarbituric acid

(0.28 g, 1.94 mmol), acetyl acetone (0.20 g, 2 mmol), 2-cyanomethylbenzothiazole (0.23 g, 1.32 mmol) dimedone (0.28 g, 2 mmol) in glacial acetic acid (30 mL) was refluxed for 12 h. The reaction mixture was proud into ice cold water (50 g). The obtained solid was filtered and recrystallized from ethanol.

7-(6-Methyl-2, 4-dioxo-3, 4-dihydro-2H-pyran-3-yl)-1H-pyrano[2, 3-d]pyrimidine-2, 4(3H, 5H)-dione (13): Red powder; yield (82%); m.p. 250 °C; IR (KBr): v/cm^{-1} = 3169 (NH), 1659, 1613 (3C = O) and 1174 (C-O-C); ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ (ppm): 2.20 (s, 3H, CH$_3$), 2.66 (d, 2H, CH$_2$), 3.87 (s, 1H, C$_3$-H pyrone), 4.55 (t, 1H, C$_3$-H pyrane), 5.40 (s, 1H, C$_5$-H pyrone), 10.90 (s, 1H, NH), 10.80 (s, 1H, NH); ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ (ppm): 20.00, 20.20, 65.00, 79.50, 99.10, 115.90, 150.80, 150.70, 160.30, 163.70, 167.00, 174.30, 186.20; MS (EI, 70 ev): m/z (%) = 290.00 (M$^+$, 45.00. Anal. Calcd. for C$_{13}$H$_{10}$N$_2$O$_6$ (290.23): C, 53.80; H, 3.47; N, 9.65%. Found: C, 53.75; H, 3.41; N, 9.61%.

6-Methyl-3-(4-oxo-2-thioxo-1, 3, 4, 5-tetrahydro-2H-pyrano[2, 3-d]pyrimidin-7-yl)-2H-pyran-2, 4(3H)-dione (14): Brownish powder; yield (75%); m.p. >300°C; IR (KBr): v/cm^{-1}= 3150 (NH), 1695, 1680 (3C=O groups), 1340 (C=S); ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ (ppm): 2.21 (s, 3H, CH$_3$), 2.64 (d, 2H, CH$_2$), 3.85 (s, 1H, C$_3$-H pyrone), 4.51 (d, 1H, C$_3$-H pyrane), 5.41 (s, 1H, C$_5$-H pyrone), 9.77 (s, 1H, NH), 13.10 (s, 1H, NH); ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ (ppm): 20.00, 20.20, 65.00, 82.00, 99.00, 115.00, 150.80, 162.20, 167.00, 168.20, 170.10, 174.30, 186.20; MS (EI, 70 ev): m/z (%) = 306.00 (M$^+$, 70.00). Anal. Calcd. for C$_{13}$H$_{10}$N$_2$O$_5$S (306.29): C, 5.98; H, 3.29; N, 9.15%. Found: C, 50.92; H, 3.22; N, 9.00%.

5-Acetyl-6, 6'-dimethyl-2'H, 4H-[2, 3'-bipyran]-2', 4'(3'H)-dione (15): Brownish powder; yield (65%); m.p. 216 °C; IR (KBr): v/cm^{-1} = 1715, 1644 (3C = O groups); ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ (ppm): 2.12 (s, 3H, CH$_3$), 2.24 (s, 3H, CH$_3$), 2.65 (s, 1H, COCH$_3$), 2.88 (d, 2H, CH$_2$ pyrane), 3.71 (s, 1H, C$_3$-H pyrone), 4.55 (t, 1H, C-H pyrane), 5.46 (s, 1H, C$_5$-H pyrone); ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ (ppm): 17.80, 20.00, 20.70, 22.90, 65.40, 99.10, 115.20, 115.20, 150.80, 154.20, 167.00, 174.30, 186.20, 196.50; MS (EI, 70 ev): m/z (%) = 262.00 (M$^+$, 5.00). Anal. Calcd. for C$_{14}$H$_{14}$O$_5$ (262.26): C, 64.12; H, 5.38%. Found: C, 64.08; H, 5.31%.

1-(6-Methyl-2, 4-dioxo-3, 4-dihydro-2H-pyran-3-yl)-3H-benzo[4, 5]thiazolo[3, 2-a]-4-carbonitrile (16): Dark brown powder; yield (80%); m.p.> 300 °C; IR (KBr): v/cm^{-1}= 2220 (CN),1681, 1661 (2C=O); ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ (ppm): 2.22 (s, 3H, CH$_3$), 3.15 (d, 2H, CH$_2$), 3.87 (s, 1H, C$_3$-H pyrone), 4.34 (t, 1H, CH), 5.40 (s, 1H, C$_5$-H pyrone), 7.00-7.79 (m, 4H, Ar-H); ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ (ppm): 20.00, 25.70, 63.30, 68.10, 95.30, 115.90, 117.30, benzene ring (121.10, 119.00, 122.90, 123.70, 126.30), 145.60, 141.70, 162.50, 167.00, 174.30, 186.20; MS (EI, 70 ev) m/z (%) = 338.00 (M$^+$, 25.00). Anal. Calcd. for C$_{18}$H$_{12}$N$_2$O$_3$S (336.37): C, 64.27; H, 3.60; N, 3.38%. Found: C, 64.21; H, 3.55; N, 8.30%.

3-(7, 7-Dimethyl-5-oxo-5, 6, 7, 8-tetrahydro-4, 4-chromen-2-yl)-6-methyl-2H-pyran-2, 4(3H)-dione (17): Orange powder; yield (70%); m.p. 123 °C; IR (Kbr): v/cm^{-1} = 1725, 1659 (3C =O); ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ (ppm): 1.00 (s, 6H, 2CH$_3$), 1.82 (s, 2H, CH$_2$), 1.99 (s, 2H, CH$_2$), 2.22 (s, 3H, CH$_3$), 2.66 (d, 2H, CH$_2$), 3.85 (s, 1H, C$_3$-H pyrone), 4.55 (t, 1H, CH), 5.40 (s, 1H, C$_5$-H pyrone); ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ (ppm): 20.00, 21.00, 27.50, 27.50, 32.30, 38.60, 46.50, 65.40, 99.10, 113.80, 115.90, 150.80, 155.00, 167.00, 174.00, 148.30, 186.20; MS (EI, 70 ev): m/z (%) = 302 (M$^+$, 2.00). Anal. Calcd. for C$_{17}$H$_{18}$O$_5$ (302.33): C, 67.54; H, 6.00%. Found: C, 67.50; H, 5.55%.

General procedure for the synthesis of compounds 18, 19, 20, 21 and 22: A mixture of 2 (0.40 g, 1.79 mmol), the appropriate phenol namely (α-naphthol (0.28 g, 2 mmol), β-naphthol (0.28 g, 2 mmol), resorcinol (0.22 g, 2 mmol), salicaldehyde (0.24 g, 1.87 mmol) 2-hydroxy-1-naphthaldehyde (0.34 g, 1.98 mmol)) in glacial acetic acid (30 mL) was refluxed for 12 h. The reaction mixture was proud into ice cold water (50 g). The obtained solid was filtered and recrystallized from ethanol.

3-(4H-Benzo[h]chromen-2-yl)-6-methyl-2H-pyran-2, 4(3H)dione (18): Dark brown powder; yield (70%); m.p. 127 °C; IR (KBr): v/cm^{-1} = 1690, 1643 (2C = O groups); ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ (ppm): 2.20 (s, 3H, CH$_3$), 3.66 (d, 2H, CH$_2$), 3.85 (s, 1H, C$_3$-H pyrone), 5.37 (t, 1H, C$_3$-H chromen), 5.40 (s, 1H, C$_5$-H pyrone), 7.15-7.91 (m, 6H, Ar-H); ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ (ppm): 20.00, 24.70, 64.60, 96.90, 112.60, 115.90, 118.90, 123.80, 123.20, 126.30, 128.80, 128.30, 128.30, 133.50, 151.80, 151.30, 167.00, 174.00, 186.20; MS (EI, 70 ev): m/z (%) = 306 (M$^+$, 26.00). Anal. Calcd. for C$_{19}$H$_{14}$O$_4$ (306.32): C, 74.50; H, 4.61%. Found: C, 74.45; H, 4.57%.

3-(1H-benzo[f]chromen-3-yl)-6-methyl-2H-pyran-2, 4(3H)dione (19): Brown powder; yield (81%); 124 °C; IR (KBr): v/cm^{-1} = 1690, 1643 (2C = O groups); ^{1}H-NMR and ^{13}C-NMR spectra gave a similar picture to that obtained for α-naphthol. MS (EI, 70 ev): m/z (%) = 306 (M$^+$, 40.00). Anal. Calcd. for C$_{19}$H$_{14}$O$_4$ (306.32): C, 74.50; H, 4.61%. Found: C, 74.41; H, 4.53%.

3-(7-hydroxy-4H-chromen-2-yl)-6-methyl-2H-pyran-2, 4-dione (20): Brown powder; yield (80%); m.p. 130 °C; IR (KBr): v/cm^{-1} = 3447 (broad OH), 1683, 1645 (2C = O groups); ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ (ppm): 2.21 (s, 3H, CH$_3$), 3.22 (d, 2H, CH$_2$), 3.85 (s, 1H, C$_3$-H pyrone), 5.37 (t, 1H, CH), 5.40 (s, 1H, C$_5$-H pyrone), 6.24 (d, 1H, Ar-H), 6.27 (s, 1H, Ar-H), 7.10 (d, 1H, Ar-H), 9.89 (s, 1H, OH); ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ (ppm): 20.00, 26.40, 64.60, 96.90, 99.50, 110.00, 115.80, 123.30, 131.10, 151.30, 155.30, 162.40, 167.00, 174.30, 185.20; MS (EI, 70 ev) m/z (%) = 272 (M$^+$, 16.00). Anal. Calcd. for C$_{15}$H$_{12}$O$_5$ (272.26): C, 66.17; H, 4.44%. Found: C, 66.10; H, 4.41%.

2-(6-methyl-2, 4-dioxo-3, 4-dihydro-2H-pyran-3-yl)-4H-chromen-8-carbaldehyde (21): Brown powder; yield (90%); m.p. 135 °C; IR (KBr): v/cm^{-1} = 3441 (broad OH), 1727 (C = O aldehyde), 1641 (C = O pyrone); ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ (ppm): 2.18 (s, 3H, CH$_3$), 3.26 (d, 2H, CH$_2$ pyrone), 5.37 (t, 1H, CH pyrone), 5.37 (s, 1H, C$_5$-H pyrone), 7.57-7.93 (m, 3H, Ar-H), 10.37 (s, 1H, CHO); ^{13}C-NMR (75 MHz, DMSO-d$_6$) δ (ppm): 21.80, 26.80, 88.70, 97.30, 104.30, 123.30, 127.60, 130.40, 131.20, 137.50, 155.50, 157.50, 161.40, 174.30, 182.70, 191.00; MS (EI, 70 ev): m/z (%) = 284.00 (M$^+$, 25.00). Anal. Calcd. for C$_{16}$H$_{12}$O$_5$ (284.27): C, 67.60; H, 4.26%. Found: C, 67.57; H, 4.00%.

2-(6-methyl-2,4-dioxo-3, 4-dihydro-2H-pyran-3-yl)-4H-benzo[g]chromen-10-carbaldehyde (22): Yellowish powder; yield (93%); m.p. 86 °C; IR (KBr): v/cm^{-1} = 3442 (broad OH), 1732 (C=O aldehyde), 1644 (C=O pyrone); ^{1}H-NMR and ^{13}C-NMR spectrum gave a similar picture to that obtained for compound 21; MS (EI, 70 ev): m/z (%) = 334 (M$^+$, 25.00). Anal. Calcd. for C$_{20}$H$_{14}$O$_5$ (334.33): C, 71.85; H, 4.22%. Found: C, 71.79; H, 4.00%.

Antimicrobial evaluation and Minimal Inhibitory Concentration (MIC) measurement: The antimicrobial and minimal inhibitory concentration measurements (MIC) were evaluated according to the previously reported work (Bondock *et al.*, 2008). Standard sterilized filter paper disks (5 mm diameter) impregnated with a solution of the tested compound in DMF (1 mg mL^{-1}) was placed on an agar plate seeded with the appropriate test organism in triplicates. The utilized test organisms were: *Bacillus subtilis* and *Bacillus thuringiensis* as examples of Gram-positive bacteria and *Escherichia coli* and *Pseudomonas aeruginosa* as example of Gram-negative. They were also evaluated for their *in vitro* antifungal potential against *Botrytis fabae* fungal strains. Chloramphenicol, cephalothin and cyclohiximide were

used as standard antibacterial and antifungal agents, respectively. DMF alone was used as control at the same above mentioned concentration.

RESULTS AND DISCUSSION

The DHA 1 is converted into the corresponding (E)-3-(dimethyl amino)acryloyl)-6-methyl-2H-pyran-2, 4-dione (2). (Kumar *et al.*, 2006).

Enaminone (Hammouda *et al.*, 1995; Fadda and Khalil, 2012; Fadda *et al.*, 2013) are versatile reagents and their chemistry has recently received a considerable attention as precursors too, otherwise not readily obtainable heteroaromatics.

Several novel syntheses of azoles azeines azoloazeines utilizing enaminone as starting compounds have been reported (Bondock *et al.*, 2011; Fadda *et al.*, 2012a, b, c; Abdelrazek *et al.*, 2011). In continuation of our interest in the synthesis of heterocycles containing a pyrone moiety (Fadda *et al.*, 1991, 1992, 1996, 1998, 2000) the authors studied the reactions of enaminone 2 with several nitrogen nucleophiles aiming of synthesis of novel heteroaryl pyrones which have not been reported hitherto.

Thus, treatment of 2 with hydroxylamine hydrochloride in refluxing ethanol containing a catalytic amount of triethyl amine afforded an orange product identified as 3-(isoxazol-5-yl)-6-methyl-2H-pyran-2, 4-(3H)-dione (3) (Fig. 2).

The spectral data of the isolated product was in a complete agreement with structure 3. The IR spectrum revealed the lack of an absorption band corresponding to a conjugated C = O function at 1708 cm^{-1} and showed absorption band at 1620 cm^{-1} corresponding to C = N. The ^{1}H-NMR spectrum showed three singlet signals at δ 1.98, 4.47 and 5.40 ppm attributable to CH$_3$ two methine protons of pyrone ring, in addition to two doublets at δ 5.86, 8.03 ppm of two CH protons of isoxazole ring. Moreover, the mass spectrum showed a molecular ion peak at m/z = 193 corresponding to a molecular formula C$_9$H$_7$NO$_4$ (Fig. 2).

The pyrazole derivative 4 was achieved as a sole product by heating the enaminone 2 with hydrazine hydrate in ethanol. The structure of 4 was established on the bases of its elemental analysis and spectral data. The IR spectrum of compound 4 showed NH function at 3350 cm^{-1}. The ^{1}H-NMR spectrum displayed four singlet signals at δ 1.98, 4.48, 5.38 12.50 ppm due to CH$_3$, two methine protons of pyrone ring and NH proton besides two doublets at δ 6.06 and 7.48 attributable to two methine protons of pyrazole ring. The mass spectrum showed a molecular ion peak at m/z = 192 corresponding to molecular formula C$_9$H$_8$N$_2$O$_3$.
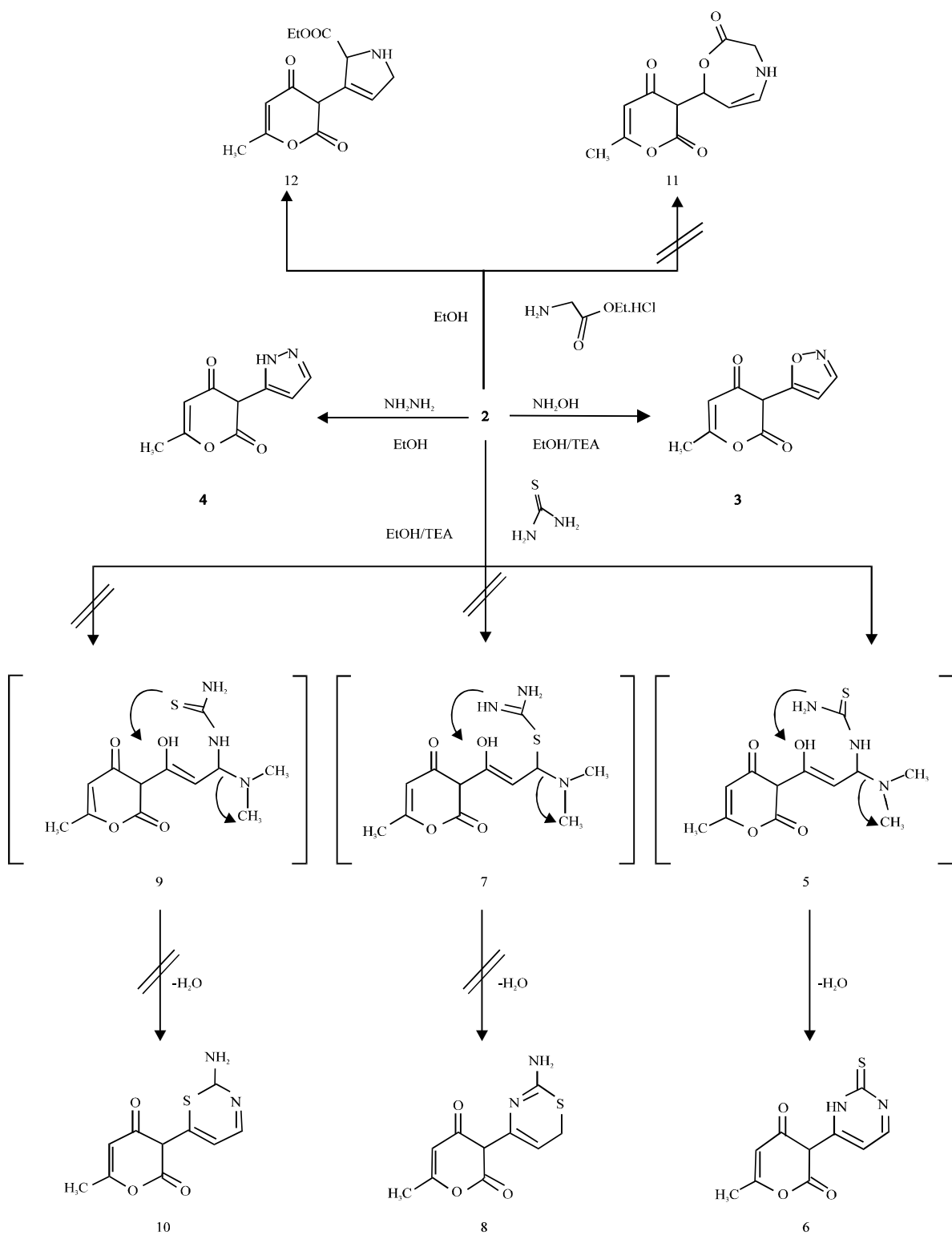
Fig. 2: Reactions of enaminone 2 with different N-nucleophiles

The site selectivity in cycloaddition of some nitrogen ampident nucleophiles with enaminones was also studied. Thus, the reaction of enaminone 2 with thiourea in refluxing ethanol in presence of a catalytic amount of triethylamine afforded a single product (as examined by TLC) for which three isomeric cycloadducts 6, 8 and 10 seemed possible.

However, the pyrimidinthione isomer 6 was assumed for the reaction product on the basis of its elemental analysis and spectral data. The IR spectrum lacked an absorption band due to carbonyl function at 1708 cm^{-1} and showed absorption band at 1320 cm^{-1} corresponding to C = S beside broad band at 3160 cm^{-1} due to NH group. The ^{1}H-NMR exhibited four singlet signals and two doublets signals at δ 5.30 and 6.30 ppm with coupling constant (J = 6.80 Hz) assignable to pyrimidine protons which could only be obtained from structure 6. Moreover, ^{13}C-NMR spectrum revealed ten carbon types, the most important signals being displayed at δ 176, 166 and 165 ppm characteristic for C = S and carbonyl carbons, respectively. The mass spectrum showed a molecular ion peak at m/z = 236 which agree with its molecular formula $C_{10}H_8N_2O_3S$ (Fig. 2).

It is interesting in this connection that the reaction of 2 with ethyl glycinate hydrochloride in a boiling ethanol does not afford the oxazipene derivative 11, as could have been expected in analogy to the formation of 6. Actually, the product of this reaction was identified on the basis of its analytical and spectral data as ethyl-3-(2-hydroxy-6-methyl-4-oxo-4H-pyran-3-yl)-2, 5-dihydro-1H-pyrole-2-carboxylate (12) (Fig. 2).

The IR spectrum has absorption band characteristic to ester group and revealed the presence of carbonyl stretching bands at 1741, 1707 and 1651 cm^{-1} corresponding to carbonyl stretching functions. ^{1}H-NMR spectrum assigned bands at δ 1.30 ppm as triplet signals corresponding to methyl group of ester, singlet signal at δ 2.00 ppm due to methyl group of pyrone ring, multiplet at δ 3.17-3.27 ppm due to CH$_2$ of pyrrole ring, singlet signal at δ 3.87 ppm of C$_3$-H protons of pyrone ring, 4.14 ppm singlet signal of C$_2$-H of pyrrole ring, 5.40 ppm as singlet signal of C$_5$-H of pyrone ring 5.67 ppm multiplet C$_4$-H of pyrrole ring. The mass spectrum showed the molecular ion peak m/z = 265 corresponding to the molecular formula $C_{13}H_{15}NO_5$.

The behavior of enaminone 2 towards an active methylene group incorporated into heterocyclic rings has been also studied. Cycloaddition of 2 with barbituric acid in glacial acetic acid afforded pyrano (2, 3-d) pyrimidine derivative 13 as 7-(6-methyl-2, 4-dioxo-3, 4-dihydro-2H-pyran-3-yl)-1H-pyrano[2, 3-d] pyrimidine-2, 4(3H,5H)-dione (13).

The structure of 13 was established for the reaction product based on its elemental analysis and spectral data. (cf. experimental part).

In a similar manner, when the enaminone 2 reacted with thiobarbituric acid in glacial acetic acid afforded pyrano (2, 3-d) pyrimidine derivative 14. Structure of 14 was established for the reaction product based on its elemental analysis and spectral data.

As an extension of this work the reactivity of enaminone 2 towards various C-nucleophiles, having an active or potentially active methylene group was studied. Thus, when 2 was treated with acetyl acetone, as a C, O-binucleophiles, in a glacial acetic acid under reflux, it afforded 15.

Structure of 15 was inferred from correct elemental analysis and spectral data. The IR spectrum revealed the absorption bands at 3447, 1715 and 1644 cm^{-1} assignable to OH and carbonyl functions, respectively. The structure of 15 was assumed to take place via the addition of the active methylene group of acetyl acetone to the activated double bond in the enaminone 2 and lose a molecule of dimethyl amine to give the a cyclic non-isolable intermediate which undergoes intramolecular cyclization to form 15 (Fig. 3).

Moreover, heating enaminone 2 with 2-cyanomethylbenzothiazole in refluxing glacial acetic acid afforded the benzothiazolopyridinylpyrone derivative 16. The structure of 16 was confirmed on the basis of elemental analysis and spectral data. The IR spectrum showed absorption band at 2220 cm^{-1} due to CN group and absorption band at 1100 cm^{-1} due to C-O-C stretching frequency.

^{1}H-NMR of compound 16 showed singlet signals at δ 1.98 due to CH$_3$ protons, 3.15 ppm as doublet due to C$_4$-H of pyridine ring, singlet signal at δ 3.87 ppm due to C$_3$-H of pyrone ring, triplet at δ 4.34 ppm for C$_5$H of pyridine ring, singlet signal at δ 5.40 ppm due to C$_5$-H of pyrone ring besides multiplet at δ 6.93-7.56 ppm due to aromatic protons. The mass spectrum of this compound provides a more confirmation of its structure which showed its molecular ion peak at m/z = 336 corresponding to the molecular formula $C_{18}H_{12}N_2O_3S$ (Fig. 3).

It was of interest to investigate the reactivity of enaminone 2 towards dimedone as candidates for a facile synthetic route to coumarin derivatives. Thus, treatment of 2 with dimedone in refluxing glacial acetic acid yielded coumarin derivative: 3-(7, 7-dimethyl-5-oxo-5, 6, 7, 8-tetrahydro-4, 4-chromen-2-yl)-6-methyl-2H-pyran-2, 4(3H)-dione (17) (Fig. 3).

The assignment of structure 17 was based on analytical and spectral data. The IR spectrum revealed the lack of absorption band at 1715 cm^{-1} characteristic to C=O of acetyl group function and the appearance of absorption bands at 1690 and 1625 cm^{-1} assignable to C=O functions of chromone and pyrone rings, respectively. ^{1}H-NMR spectrum displayed singlet signals at δ 1.11 ppm corresponding to two geminal methyl
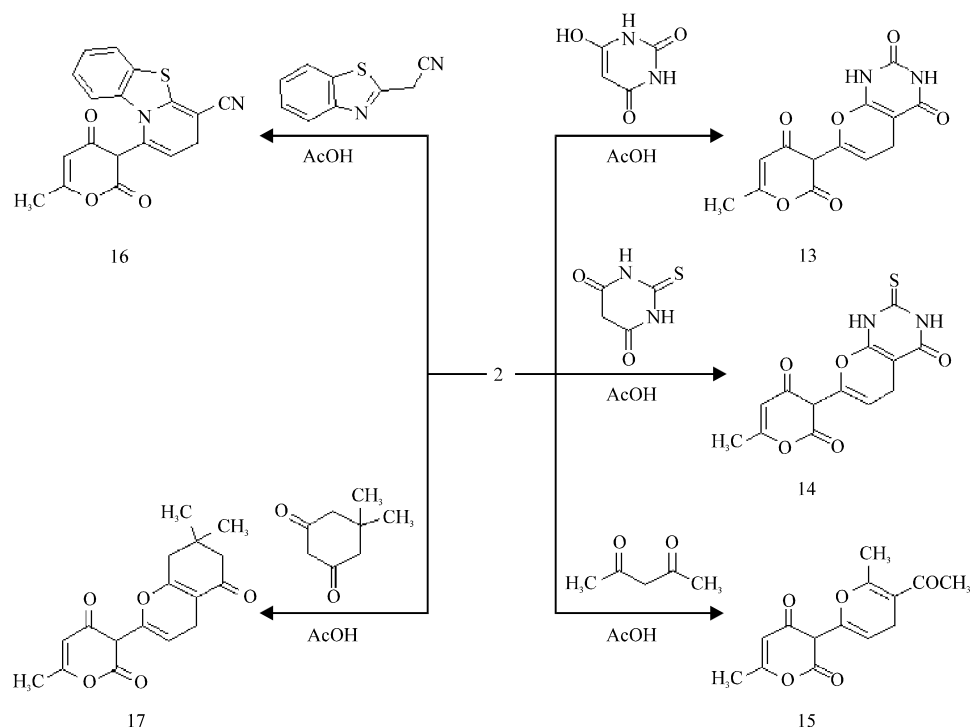
Fig. 3: Reactions of enaminone 2 with active methylene compounds

protons at δ 1.71 ppm due to methyl protons at C$_6$ of pyrone ring, three singlet signals at δ 1.89,1.90 and 2.66 ppm due to CH$_2$ protons at C$_4$, C$_6$ and C$_8$ of chromone ring, two singlet signals at 3.86 and 5.40 ppm due to C$_3$-H and C$_5$-H protons of pyrone ring. The mass spectrum showed a molecular ion peak at m/z = 302 beside fragments at m/z = 287 and 275 due to lose of one and two methyl fragments.

Also, enaminones reacted with phenols to give coumarin derivatives. Thus, enaminone 2 reacted with α-naphthol in boiling glacial acetic acid and furnished a single product identified as: 3-(4H-benzo[h]chromen-2-yl)-6-methyl-2H-pyran-2, 4(3H)dione (18).

The structure of 18 was supported on the basis of elemental analysis and spectral data. The IR spectrum appeared no absorption band at 1148 cm^{-1} attributable to C-O-C function. The ^{1}H-NMR spectrum exhibited two singlet signals at δ 1.79 and 5.50 ppm corresponding to methyl protons and C$_5$-H of pyrone ring, doublet signal at δ 3.34 ppm and triplet signal δ 5.47 corresponding to C$_4$-H and C$_3$-H of coumarin ring. The aromatic protons C$_5$-H and C$_6$-H appeared as two doublets at δ 7.03 and 7.25 ppm. The rest of aromatic protons appeared as multiplet at δ 7.46-8.19 ppm (Fig. 4).

In a similar manner, 3-(1H-benzo[f]chromen-3-yl)-6-methyl-2H-pyran-2, 4(3H)dione (19) had been prepared by the reaction of enaminone 2 with β-naphthol under the same experimental conditions described above. The structure of 19 was inferred from correct elemental analysis and spectral data. The IR spectrum showed the lack of absorption band at 1718 cm^{-1} due to the C=O function and the presence of a band at 1170 cm^{-1} corresponding to C-O-C group. The ^{1}H-NMR spectrum revealed two singlet signals at δ 1.70 and 6.20 ppm corresponding to methyl protons and C$_5$-H of pyrone ring, doublet and triplet signals at δ 3.66 and 5.45 ppm corresponding to C$_4$-H and C$_3$-H of coumarin ring, respectively, the aromatic protons appeared at δ 7.32-8.13 ppm as multiplet signals. The mass spectrum provide an additional confirmation for the formation of 26 which showed the molecular ion peak at m/z= 306 and peak at m/z = 263 corresponding to the loss of CO$_2$ fragment, this confirm the molecular formula C$_{19}$H$_{14}$O$_4$ (Fig. 4).

The reaction of enaminone 2 with resorcinol in refluxing glacial acetic acid yielded coumarin derivative 20. The elemental analysis and spectral data studies were used to establish the structure 20.

The IR spectrum displayed the absence of C-O stretching absorption band and the presence of absorption band characteristic to OH C-O-C at 3447 cm^{-1} and 1164 cm^{-1}, respectively. The ^{1}H NMR of 3-(7-hydroxy-4H-chromen-2-yl)-6-methyl-2H-pyran-2,
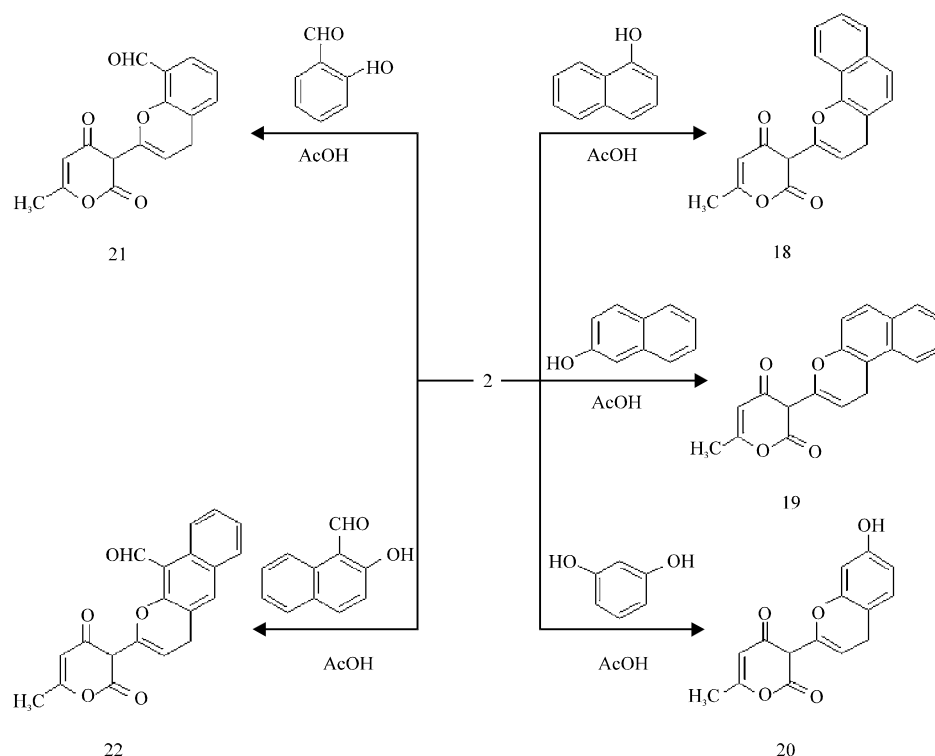
Fig. 4: Reactions of enaminone 2 with phenolic compounds

2,4-dione (20) exposed two singlet at δ 1.70 and 6.10 ppm owing to methyl and C_5-H protons of pyrone ring, doublet and triplet signals at δ 3.22 and 5.47 ppm due to C_4-H and C_3-H, respectively of chromen ring. Two singlet signals at δ 6.08 and 9.83 ppm corresponding to C_8-H and OH protons of chromen ring, two doublet at δ 6.72, 6.17 ppm due to C_5-H and C_6-H of chromen ring.

The mass spectrum showed a molecular ion peak at m/z = 272 corresponding to molecular formula $C_{15}H_{12}O_5$.

Similarly, salicaldehyde reacted with enaminone 2 in boiling glacial acetic acid to give 2-(6-methyl-2,4-dioxo-3,4-dihydro-2H-pyran-3-yl)-4H-chromen-8-carbaldehyde (21).The structure of 21 was inferred from its correct elemental analysis and spectral data. The IR spectrum showed aldehydic carbonyl group at 1725 cm^{-1}. ^{1}H-NMR spectrum gave a similar picture to these compounds of 19 and 20 in addition to singlet proton at 10.54 ppm due to aldehydic group. Moreover, the mass spectrum provide additional confirmation for this structure by showing the molecular ion peak at m/z = 284 and fragment at m/z = 255 due to lose of HCO fragment which confirm the molecular formula $C_{16}H_{12}O_5$ (Fig. 4).

Moreover, reaction of 2-hydroxy-1-naphthaldehyde with enaminone 2 in boiling glacial acetic acid afforded:

2-(6-methyl-2,4-dioxo-3,4-dihydro-2H-pyran-3-yl)-4H-benzo[g]chromen-10-carbaldehyde (22). Structure 22 was proved based on the elemental and spectral data (cf. experimental part).

Antimicrobial evaluation: Sixteen of the newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *Bacillus subtilis* and *Bacillus thuringiensis* as example of Gram-positive bacteria and *Escherichia coli* and *Pseudomonas aeruginosa* as examples of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *Botrytis fabae* fungal strains.

Agar-diffusion method was used for the determination of the preliminary antibacterial and antifungal activity. Chloramphenicol, cephalothin and cycloheximide were used as reference drugs. The results were recorded for each tested compound as the average diameter of Inhibition Zones (IZ) of bacterial or fungal growth around the disks in mm. The Minimum Inhibitory Concentration (MIC) measurement was determined for compounds showed significant growth inhibition zones (>14 mm) using two fold serial dilution method (Bondock *et al.*, 2008). The MIC (μg mL^{-1}) and inhibition zone diameters values are recorded in Table 1.

Table 1: Minimal Inhibitory Concentration and inhibition zone of some new synthesized compounds

	[a]MIC (μg mL^{-1}) and inhibition zone (mm)				
	Gram-positive bacteria		Gram-negative bacteria		Fungi
Compound No.	B. subtilis	B. thuringiensis	E. coli	P. aeruginosa	B. fabae
1	100 (15)	100 (15)	100 (14)	100 (15)	100 (15)
2	50 (19)	50 (19)	100 (15)	100 (16)	100 (14)
3	50 (17)	25 (31)	12.5 (33)	25 (27)	100 (16)
4	3.125 (45)	6.25 (36)	6.25 (38)	6.25 (38)	25 (26)
6	3.125 (43)	6.25 (37)	6.25 (37)	25 (32)	25 (27)
12	6.25 (35)	6.25 (39)	12.5 (31)	100 (16)	100 (15)
13	25 (35)	50 (20)	100 (15)	100 (15)	50 (19)
14	3.125 (45)	3.125 (44)	50 (19)	100 (14)	50 (20)
15	25 (33)	50 (20)	100 (15)	100 (15)	25 (26)
16	3.125 (44)	6.25 (39)	50 (20)	50 (19)	6.25 (37)
17	12.5 (33)	25 (30)	50 (19)	50 (20)	50 (20)
18	6.25 (37)	6.25 (36)	50 (19)	50 (18)	100 (14)
19	6.25 (38)	6.25 (38)	50 (18)	100 (17)	50 (21)
20	12.5 (32)	12.5 (37)	100 (14)	100 (16)	100 (14)
21	6.25 (38)	25 (32)	25 (27)	100 (15)	100 (16)
22	25 (31)	25 (32)	100 (15)	100 (14)	100 (16)
Chloramphenicol	3.125 (44)	3.125 (44)	6.25 (38)	6.25 (38)	[b]
Cephalothin	6.25 (37)	6.25 (38)	6.25 (37)	6.25 (38)	[b]
Cycloheximide	[b]	[b]	[b]	[b]	3.125 (42)

[a]MIC: Minimal Inhibitory Concentration values with SEM = 0.02 (The lowest concentration that inhibited the bacterial growth), [b]NT: Not tested

The results depicted in Table 1 revealed that the most of tested compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains also against antifungal strain.

In general, most of the tested compounds revealed better activity against the Gram-positive rather than the Gram-nagative bacteria. It would be also noticed that compounds belonging to pyrazole, pyrimidinthione, pyranopyrimidinthione and benzothiazolopyridine derivatives exhibited better antibacterial potentials than the rest of other compounds.

Regarding to the Structure Activity Relationship (SAR) of the tested compounds against Gram-positive bacteria, the results revealed that compounds 4, 6, 12, 14, 16, 18, 19 and 21 exhibited broad spectrum antibacterial profile against the tested organisms. Derivatives with electron withdrawing groups such as carbonyl and thiocarbonyl exist in such compounds recorded higher activity than the rest of other compounds. In this view, compounds of 4, 6, 14 and 16 were equipotent to chloramphenicol in inhibiting the growth of B. subtilis (MIC 3.125 μg mL^{-1}), while its activity was 50% lower than of chloramphenicol against B. thurigiensis exhibited compound 14 was equipotent to it. Compounds 12, 18, 19 21 showed 50% of the activity chloramphenicol (MIC 6.25 μg mL^{-1}) but they were equipotent to cephalothin in inhibiting the growth of B. subtilis and B. thurigiensis (MIC 6.25 μg mL^{-1}).

On the other hand, compounds 2, 3, 13, 15, 17 and 22 exhibited moderate growth inhibitory activity against Gram-positive bacteria as revealed from their MIC values (25-50 μg mL^{-1}).

Concerning the antibacterial activity of the compounds 2, 13, 14, 15, 16, 17, 18, 19, 20 and 22 revealed weak growth inhibitory against the tested Gram-negative bacteria (MIC 50-100 μg mL^{-1}). On the other hand, compounds 4 and 6 showed equipotent activity as chloramphenicol and cephalothin against E. coli and P. aeruginosa.

Regarding to the activity of heterocyclic rings attached to 2-pyrone ring at position 3 against antifungal of strains, the results revealed that compound 16 was 50% lower than cycloheximide in inhibitory the growth of B.fabae (MIC 6.25 μg mL^{-1}).

It is worth mentioning that conversion of compound 2 to pyrazole, thiopyrimidine, pyranopyrimidinthione benzothiazolopyridine gives an excellent and potent value of antimicrobial activity which indicates that the presence of nitrogen and sulfur atoms enhance the antimicrobial activities to be equipotent to chloramphenicol (drug reference). It is also noticed that incorporation of 2-pyrone ring to the pyrrole and chromene nucleus at position 3 showed good antimicrobial activity against Gram-positive bacteria as in compounds 12, 18, 19 and 21. In addition, compounds 18 and 19 which have polynuclear heterocyclic system showed also, increase the antimicrobial activity (MIC 6.25 μg mL^{-1}) against positive Gram bacteria. Moreover, compound 17 showed moderate antimicrobial activity may be due to the positive inductive effect of the two methyl groups attached to the tetrahydrochromene ring.

The tested compounds were more active against Gram-positive than Gram-negative bacteria, it may be concluded that the antimicrobial activity of the compounds is related to cell wall structure of the bacteria. It is possible because the cell wall is essential to survival of bacteria and some antibiotics are able to kill bacteria by inhibiting a step in the synthesis of peptidoglycan. Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids, but in contrast, Gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins. These differences in cell wall structure can produce differences in antibacterial susceptibility and some antibiotics can kill only Gram-positive bacteria and are inactive against Gram-negative pathogens (Abuo-Melha and Fadda, 2012).

CONCLUSION

In conclusion, the objective of the present study was to synthesize and investigate the antimicrobial activities of some new functionalized heterocyclic systems with the hope of discovering new structures leads serving as antimicrobial agents. Our aim has been verified by the synthesis of different heterocyclic rings. The obtained results clearly revealed that compounds derived from enamine compound 2 exhibited better antimicrobial activity than enamine 2 itself.

REFERENCES

Abdelrazek, F.M., A.A. Fadda and A.N. Elsayed, 2011. Novel Synthesis of Some New Pyridazine and Pyridazino[4,5-*d*]pyridazine Derivatives. Synth. Commun.: Int. J. Rapid Commun. Synth. Org. Chem., 41: 1119-1126.

Abuo-Melha, H. and A.A. Fadda, 2012. Synthesis, spectral characterization and *in vitro* antimicrobial activity of some new azopyridine derivatives. Spectrochim. Acta Part A: Mol. Bimolecular Spectrosc., 89: 123-128.

Altomare, C., G. Perrone, M.C. Zonno, A. Evidente, R. Pengue, F. Fanti and L. Polonelli, 2000. Biological characterization of fusapyrone and deoxyfusapyrone, two bioactive secondary metabolites of *Fusarium semitectum*. J. Nat. Prod., 63: 1131-1135.

Altomare, C., R. Pengue, M. Favilla, A. Evidente and A. Visconti, 2004. Structure-activity relationships of derivatives of fusapyrone, an antifungal metabolite of *Fusarium semitectum*. J. Agric. Food Chem., 52: 2997-3001.

Bendaas, A., M. Hamdi and N.J. Sellier, 1999. Synthesis of bipyrazoles and pyrazoloisoxazoles from 3-acetyl-4-hydroxy-6-methyl-2H-pyran-2-one. J. Heterocycl. Chem., 36: 1291-1294.

Bondock, S., R. Ramy, H.A. Etman and A.A. Fadda, 2008. Synthesis and antimicrobial activity of some new heterocycles incorporating antipyrine moiety. Eur. J. Med. Chem., 43: 2122-2129.

Bondock, S., W. Khalifa and A.A. Fadda, 2011. Synthesis and antimicrobial activity of some new 4-hetarylpyrazole and furo [2, 3-*c*] pyrazole derivatives. Eur. J. Med. Chem., 46: 2555-2561.

Boutemeur-Kheddis, B., M. Hamdi, N. Sellier and A.M.S. Silva, 2001. Study of the reactions of 3-acetoacetyl-7-methyl-2H, 5H-pyrano[4,3-b] pyran-2,5-dione with aromatic amines. J. Heterocycl. Chem., 38: 227-231.

Culter, H.G. and J.M. Jacyno, 1991. Biological activity of (-)-harzianopyridone isolated from *Trichoderma harzianum*. Agric. Biol. Chem., 55: 2629-2631.

Fadda, A.A., A.M. Khalil and M.M. El-Habbal, 1991. Synthesis of certain sulphonamides and aminopyranoquinoline derivatives from 4-hydroxyquinoline with biological interest. Pharmazie, 46: 743-744.

Fadda, A.A., M.A. Hanna and M.M. Girges, 1992. Reactions of heterocyclic β-enamino esters synthesis of pyranopyrimidine, pyranopyridine and N-(Pyran-2-yl)azetidinone ring systems. Chem. Papers, 46: 244-248.

Fadda, A.A., M.T. El-Zemaity, M.M. Girges, M. Hala Refat and E.D. Biehl, 1996. Base catalyzed condensation reaction of malononitriles and 2-Hydroxy-1-naphthaldehyde with different ketones. J. Heterocycles, 43: 23-27.

Fadda, A.A., H.A. Etman, S.I. El-Desoky and S.B. Bondon, 1998. Synthesis of substituted arylbenzopyranopyridopyrimidine ring by condensation of malononitrile, 4-hydroxyarylazosalicyaldehyde and ketones by means of ammonium acetate. Bolietino Chimico Farmaceutico, 137: 191-194.

Fadda, A.A., H.M. Refat and M.E.A. Zaki, 2000. Utility of sulphones in heterocyclic synthesis: Synthesis of some pyridine, chromene and thiophene derivatives. Molecules, 5: 701-709.

Fadda, A.A., A.A.H. Abdel-Rahman, E.A. Hamed and H.E. Khalil, 2012a. Utility of enaminonitriles in heterocyclic synthesis: Synthesis and antimicrobial activity of some New Azole and azine derivatives. Am. J. Org. Chem., 2: 7-13.

Fadda, A.A., H.A. Etman, M.Y. El-Seidy and K.M. Elattar, 2012b. Utility of enaminonitriles in heterocyclic synthesis: Synthesis of some New Pyrazole, pyridine and pyrimidine derivatives. J. Heterocycl. Chem., 49: 774-781.

Fadda, A.A., M.M. Mukhtar and H.M. Refat, 2012c. Utility of new activated nitriles in the synthesis of some new heterocyclic compounds. Am. J. Org. Chem., 2: 32-40.

Fadda, A.A. and H.E. Khalil, 2012. Utility of enaminonitriles in heterocyclic synthesis: Synthesis of some New Azepine, azocine and pyrroldione derivatives. J. Heterocycl. Chem.

Fadda, A.A., A.M. Khalil and E.H. Tawfik, 2013. Enaminonitriles in heterocyclic synthesis: Synthesis and biological evaluation of novel indeno[2,1-b]thiophene derivatives. Turk. J. Chem., 37: 134-148.

Hammouda, M., M. Mashaly and A.A. Fadda, 1995. Enaminones in heterocyclic synthesis part 2: One-pot synthesis of some new indeno [3, 2-b] pyridines. Arch. Pharm. Res., 18: 213-214.

Hernadez-Galan, R., J. Salva, G.M. Massanet and I.G. Collado, 1993. An improved synthesis of 3-(1,1-dimethylallyl)coumarins. Tetrahedron, 49: 1701-1710.

Kumar, A., O. Prakash, M. Kinger and S.P. Singh, 2006. Synthesis of some new 1-aryl-4-formyl-3-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)pyrazoles using the vilsmeier-haack reaction: Isolation of the key intermediate 1-aryl-3-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)pyrazoles. Can. J. Chem., 84: 438-442.

Makhloufi-Chebli, M., M. Hamdi, A.M.S. Silva, O. Duval and J.J. Helesbeux, 2009. 2H-Pyran-2-one-3-carbothioamide derivatives: Synthesis and reaction with hydrazine hydrate. J. Heterocycl. Chem., 46: 18-22.

Rasoanaivo, P., G. Galeffi, G. Multari, M. Nicoletti and L. Capolongo, 1993. Kalanchoside, a cytotoxic bufadienolidic glycoside from kalanchoe tomentosa baker. Gazz. Chim. Ital., 123: 539-541.

Steyn, P.S. and F.R.V. Heerden, 1998. Bufadienolides of plant and animal origin. Nat. Prod. Rep., 15: 397-413.