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Synthesis, antimicrobial, photochemotherapeutic evaluation and molecular docking of novel furobenzopyrones, tetrahydrobenzo- and benzofurobenzopyrones

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ABSTRACT

*New series of tricyclic compounds (linear furobenzopyrones **3a-d** and angular furobenzopyrones **11a-h**) and tetracyclic compounds (tetrahydrobenzofurobenzopyrone **5a,b** and benzofurobenzopyrone **6a,b**) derivatives were synthesized to ensure mono functional photoreaction with DNA in order to decrease toxicity. All prepared compounds were evaluated for antimicrobial and photochemotherapeutic activities. Compounds **2d**, **3d**, **5a**, **10c** and **11d** were found to have good antimicrobial activity while only compound **11e** exhibited good photosensitizing activity. In addition, molecular docking study was applied to elucidate a molecular target for the antimicrobial activity and gain insight into the possible binding mode of these compounds with the active site of the DNA gyrase.*

Key words: Furobenzopyrones, Tetrahydrobenzofurobenzopyrones, Benzofurobenzopyrone, Antimicrobial, Photochemotherapeutic.

INTRODUCTION

PUVA is a type of photochemotherapy where treatment of some skin diseases were achieved using psoralen (linear furobenzopyrone) **A (**Fig. 1) in combination with ultraviolet light (UV-A, 320-400 nm) presuming that DNA is the major site of action [1-4]. Furobenzopyrones are considered to be photosensitizing drugs for the treatment of some skin diseases such as vitiligo, psoriasis, cutaneous lymphomas and in autoimmune diseases [5-8]. In addition, furobenzopyrones were used in some cases of bacterial and viral infections [9].

The biological effects produced by furobenzopyrone after radiation with UV-A were reported in terms of a photocycloaddition reaction of furobenzopyrone and DNA [10-14]. The photo reactive process is believed to involve three major steps: non covalent intercalative binding to DNA helix [11], formation of a number of mono additional products between the furobenzopyrone and DNA based upon long wave length UV-irradiation [13] and finally, absorption of a second photon by some of the mono-adducts to form bi-adducts resulting in inter strand cross-linkages [13]. Biadducts resulted from photocycloaddition between two pyrimidine bases and the two photo reactive sites of furobenzopyrone (furan and pyrone double bonds). Skin photo toxicity and skin cancer side effects were attributed to cross linkage biadducts [13,15-17].

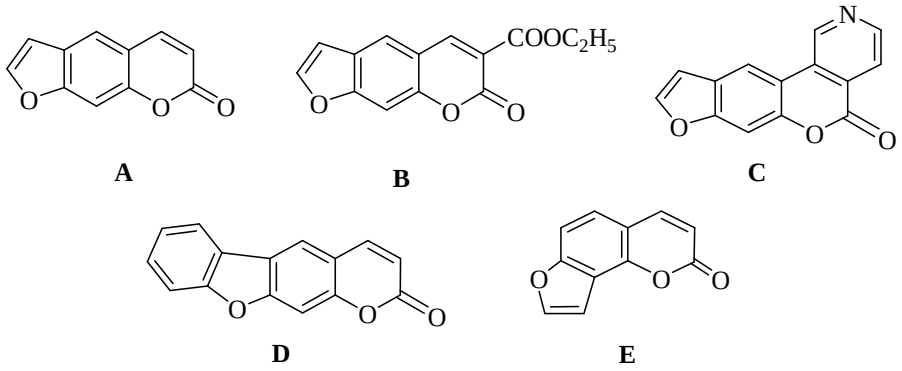


Fig. 1. Active photochemotherapeutic furobenzopyrones

Mono functional furobenzopyrones such as carbethoxypsoralen **B** [18], pyridopsoralens **C** [19-22] and benzofurobenzopyrones **D** [23], (**B-D,** Fig. 1) were reported to lack skin phototoxicity due to inhibition of interstrand cross linkage [20-22]. Moreover, angelicin (angular furobenzopyrone) **E (**Fig. 1) had lower genotoxicity and skin erythemogenic effects than psoralens [18,24,25]. This is strictly connected with the molecular complex formed with DNA, which undergoes intercalation between base pairs of DNA but for geometric reasons cannot form inter strand cross-links [19,24,26].

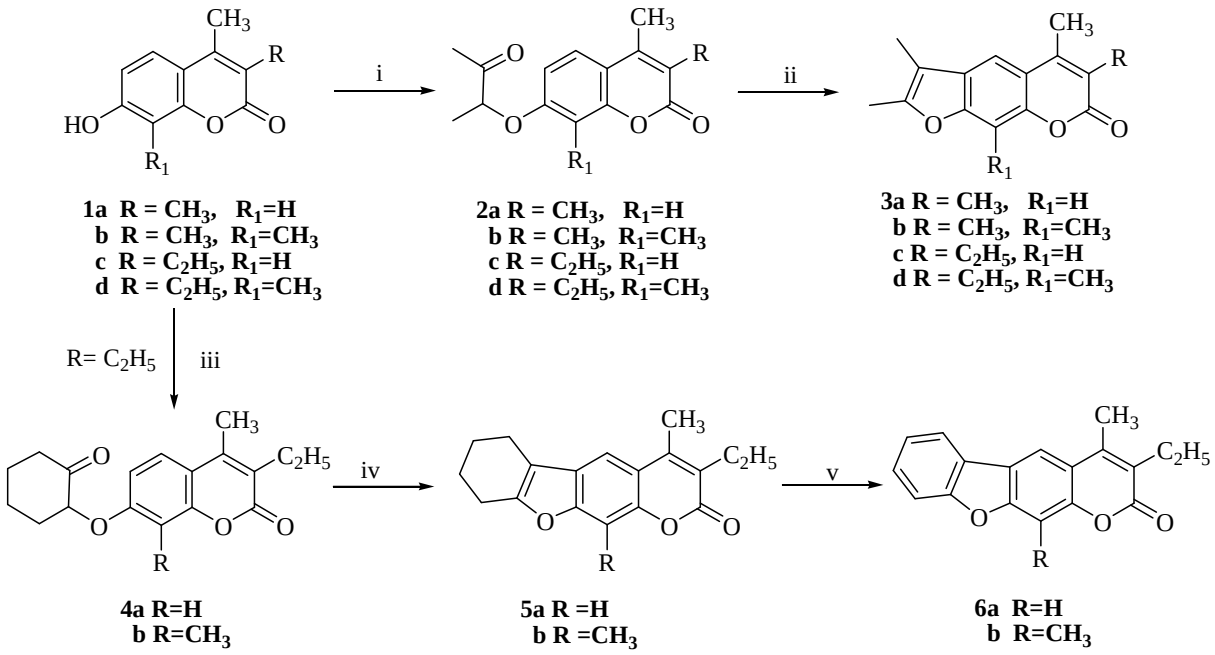
Accordingly, new series of tricylcic (linear and angular furobenzopyrones) and tetracyclic (tetrahydrobenzo- and benzofurobenzopyrone) derivatives were designed and synthesized to ensure mono functional photoreaction with DNA. Moreover, all prepared compounds were evaluated for antimicrobial and photochemotherapeutic activities. In addition, molecular docking study for possible binding of active compounds with DNA gyrase active site was applied to elucidate a molecular target for the antimicrobial activity.

MATERIALS AND METHODS

2.1. Chemistry

Melting points were determined by an open capillary tube method using Stuart SMP10 melting point apparatus and were uncorrected. Microanalysis was carried out at The Regional Center for Mycology and Biotechnology, Al-Azhar University. Infrared Spectra were recorded as potassium bromide discs on Schimadzu FT-IR 8400S spectrophotometer (Shimadzu, Kyoto, Japan) and expressed in wave number υ_{max} (cm^{-1}). The ^{1}H-NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer at 300 MHz in chloroform ($CDCl_3$) or dimethylsulphoxide (DMSO-d_6). Chemical Shifts are quoted in δ as parts per million (ppm) downfield from tetramethylsilane (TMS) as internal standard and *J* values are reported in Hz. Mass spectra were performed as EI at 70eV on Hewlett Packard Varian (Varian, Polo, USA) and Shimadzu Gas Chromatograph Mass spectrometer-QP 1000 EX and TSQ quantum (Thermo Electron Corporation) instrument prepared with a triple quadrupole mass detector (Thermo Finnigan) and an ESI source. TLC was carried out using Macherey-Nagel AlugramSil G/UV254 silica gel plates with fluorescent indicator UV254 and chloroform/methanol 9.5:0.5 as the eluting system and the spots were visualized at 366, 254 nm by UV Vilber Lourmat 77202 (Vilber, Marne La Vallee, France).

Starting and intermediate compounds, 3,8-disubstituted-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-ones **1a-d** [27], ethyl 3-(7-hydroxy-4-methyl-2-oxo-2*H*-benzopyran-3-yl)propanoate **7** [28], ethyl 3-(7-acetyloxy-4-methyl-2-oxo-2*H*-benzopyran-3-yl)propanoate **8a** [29] and (un)substituted phenacyl bromide derivatives [30] were prepared according to reported procedures.



Scheme 1. Reagents and conditions: (i)3-Chlorobutan-2-one, acetone, K_2CO_3, reflux, 24 h, (ii) 1N NaOH, isopropanol, reflux, 4h, (iii) 2-Chlorocyclohexanone, acetone, K_2CO_3 reflux, 24 h, (iv) 1N NaOH, isopropanol, reflux, 4h, (v) DDQ, benzene, reflux, 20 h.

2.1.1. General Procedure for synthesis of 3,4,8-trisubstituted-7-(3-oxobutan-2-yloxy)-2*H*-1-benzopyran-2-one (2a-d), (Scheme 1)

A solution of compound **1a-d** (0.01 mol) and 3-chlorobutan-2-one (1.06 g, 0.01 mol) in acetone (30 ml) was refluxed in presence of anhydrous potassium carbonate (2.76 g, 0.02 mol) for 24 h. The solution was filtered and the remaining residue was washed with acetone. The combined filtrates and washings were distilled under reduced pressure. The product was crystallized from ethanol.

2.1.1.1. 3,4-Dimethyl-7-(3-oxobutan-2-yloxy)-2*H*-1-benzopyran-2-one (2a)

Yield 59%. mp 126-127 °C. IR υ_{max}/ cm^{-1}: 3084 (CH Ar), 2987, 2929 (CH aliphatic), 1697 (2C=O), 1612, 1581, 1508 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.55 (d, 3H, *J*=6.6 Hz, OCHC$\underline{H}_3$), 2.19 (s, 6H, 2xCH_3 at C3,4), 2.37 (s, 3H,$COCH_3$), 4.69 (q, 1H, OC$\underline{H}CH_3$), 6.74 (s, 1H, H-8 Ar), 6.82 (d, 1H, *J*=9.0 Hz, H-6 Ar), 7.51 (d, 1H, *J*=9.0 Hz, H-5 Ar). Anal. Calc. for $C_{15}H_{16}O_4$ (260.29): C, 69.22; H, 6.20. Found: C, 69.31; H, 6.18.

2.1.1.2. 7-(3-Oxobutan-2-yloxy)-3,4,8-trimethyl-2*H*-1-benzopyran-2-one (2b)

Yield 79%. mp 121-122 °C. IR υ_{max}/ cm^{-1}: 3059 (CH Ar), 2929, 2877 (CH aliphatic), 1722, 1705 (2C=O), 1608, 1575 (C=C). ^{1}H-NMR (DMSO-d_6) δ ppm: 1.47 (d, 3H, *J*=6.9 Hz, OCHC$\underline{H}_3$), 2.07 (s, 3H, CH_3 at C4), 2.19 (s, 3H, CH_3 at C3), 2.24 (s, 3H, CH_3 at C8), 2.33 (s, 3H, $COCH_3$), 5.08 (q, 1H, OC$\underline{H}CH_3$), 6.84 (d, 1H, *J*=9.0 Hz, H-6 Ar), 7.53 (d, 1H, *J*=9.0 Hz, H-5 Ar). MS *m/z*: 275 (M^++1). Anal. Calc. for $C_{16}H_{18}O_4$ (274.31): C, 70.06; H, 6.61. Found: C, 70.12; H, 6.64.

2.1.1.3. 3-Ethyl-4-methyl-7-(3-oxobutan-2-yloxy)-2*H*-1-benzopyran-2-one (2c)

Yield 56%. mp 84-85 °C. IR υ_{max}/ cm^{-1}: 3080 (CH Ar), 2964, 2860 (CH aliphatic), 1714, 1697 (2C=O), 1608, 1566, 1535, 1508 (C=C). ^{1}H-NMR (DMSO-d_6) δ ppm: 1.03 (t, 3H, $CH_2C\underline{H}_3$), 1.46 (d, 3H, *J*=6.9 Hz, OCHC$\underline{H}_3$), 2.19 (s, 3H, CH_3 at C4), 2.37 (s, 3H, $COCH_3$), 2.55 (q, 2H, $C\underline{H}_2CH_3$), 5.11 (q, 1H, OC$\underline{H}CH_3$), 6.86 (s, 1H, H-8 Ar), 6.91 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.68 (d, 1H, *J*=8.7 Hz, H-5 Ar). MS *m/z*: 275 (M^++1). Anal. Calc. for $C_{16}H_{18}O_4$ (274.31): C, 70.06; H, 6.61. Found: C, 70.12; H, 6.64.

2.1.1.4. 4,8-Dimethyl-3-ethyl-7-(3-oxobutan-2-yloxy)-2*H*-1-benzopyran-2-one (2d)
Yield 49%. mp 86-87 °C. IR υ_{max}/ cm^{-1}: 3057 (CH Ar), 2939, 2875 (CH aliphatic), 1730, 1701 (2C=O), 1604, 1593, 1570 (C=C). ^{1}H-NMR (DMSO-d_6) δ ppm: 1.03 (t, 3H, $CH_2C\underline{H_3}$), 1.47 (d, 3H, *J*=6.6 Hz, $OCHC\underline{H_3}$), 2.18 (s, 3H, CH_3 at C4), 2.23 (s, 3H, CH_3 at C8), 2.34 (s, 3H, $COCH_3$), 2.52 (q, 2H, $C\underline{H_2}CH_3$), 5.07 (q, 1H, $OC\underline{H}CH_3$), 6.83 (d, 1H, *J*=9.0 Hz, H-6 Ar), 7.52 (d, 1H, *J*=8.4 Hz, H-5 Ar). MS *m/z*: 289 (M^++1). Anal. Calc. for $C_{17}H_{20}O_4$ (288.34): C, 70.81; H, 6.99. Found: C, 70.86; H, 7.04.

2.1.2. General Procedure for synthesis of 6,9-disubstituted-2,3,5-trimethyl-7*H*-furo[3,2-g]benzopyran-7-one (3a-d), (Scheme 1)
1N NaOH (15 ml) was added to a suspension of compound **2a-d** (0.002 mol) in isopropanol (15 ml) and refluxed for 4h (complete dissolution of starting compound). Solution was filtered while hot then cooled. 1N H_2SO_4 was added till the solution became acidic. The precipitate formed was filtered, dried and crystallized from isoprpanol.

2.1.2.1. 2,3,5,6-Tetramethyl-7*H*-furo[3,2-g]benzopyran-7-one (3a)
Yield 68%. mp 146-147 °C. IR υ_{max}/ cm^{-1}: 3053 (CH Ar), 2924, 2860 (CH aliphatic), 1703 (C=O), 1583, 1543, 1516 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.20 (s, 3H, CH_3 at C5), 2.24 (s, 3H, CH_3 at C6), 2.40 (s, 3H, CH_3 at C3), 2.49 (s, 3H, CH_3 at C2), 7.31 (s, 1H, H-9 Ar), 7.54 (s, 1H, H-4 Ar). Anal. Calc. for $C_{15}H_{14}O_3$ (242.27): C, 74.36; H, 5.82. Found: C, 74.51; H, 5.89.

2.1.2.2. 2,3,5,6,9-Pentamethyl-7*H*-furo[3,2-g]benzopyran-7-one (3b)
Yield 54%. mp 166-167 °C. IR υ_{max}/ cm^{-1}: 3010 (CH Ar), 2924, 2850 (CH aliphatic), 1701 (C=O), 1595 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.17 (s, 3H, CH_3 at C5), 2.22 (s, 3H, CH_3 at C6), 2.40 (s, 3H, CH_3 at C3), 2.46 (s, 3H, CH_3 at C2), 2.55 (s, 3H, CH_3 at C9), 7.36 (s, 1H, H-4 Ar). Anal. Calc. for $C_{16}H_{16}O_3$ (256.30): C, 74.98; H, 6.29. Found: C, 75.07; H, 6.33.

2.1.2.3. 6-Ethyl-2,3,5-trimethyl-7*H*-furo[3,2-g]benzopyran-7-one (3c)
Yield 74%. mp 190-191°C. IR υ_{max}/ cm^{-1}: 3059 (CH Ar), 2958, 2866 (CH aliphatic), 1701 (C=O), 1581, 1558 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.18 (t, 3H, $CH_2C\underline{H_3}$), 2.20 (s, 3H, CH_3 at C5), 2.40 (s, 3H, CH_3 at C3), 2.51 (s, 3H, CH_3 at C2), 2.72 (q, 2H, $C\underline{H_2}CH_3$), 7.30 (s, 1H, H-9 Ar), 7.54 (s, 1H, H-4 Ar). MS *m/z*: 257 (M^++1). Anal. Calc. for $C_{16}H_{16}O_3$ (256.30): C, 74.98; H, 6.29. Found: C, 75.05; H, 6.32.

2.1.2.4. 6-Ethyl-2,3,5,9-tetramethyl-7*H*-furo[3,2-g]benzopyran-7-one (3d)
Yield 60%. mp 187-188 °C. IR υ_{max}/ cm^{-1}:3050 (CH Ar), 2960, 2868 (CH aliphatic), 1691 (C=O), 1593 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.17 (t, 3H, $CH_2C\underline{H_3}$), 2.19 (s, 3H, CH_3 at C5), 2.43 (s, 3H, CH_3 at C3), 2.48 (s, 3H, CH_3 at C2), 2.56 (s, 3H, CH_3 at C9), 2.73 (q, 2H, $C\underline{H_2}CH_3$), 7.40 (s, 1H, H-4 Ar). MS *m/z*: 271 (M^++1). Anal. Calc. for $C_{17}H_{18}O_3$ (270.32): C, 75.53; H, 6.71. Found: C, 75.58; H, 6.77.

2.1.3. General Procedure for synthesis of 3-ethyl-4-methyl-7-(2-oxocyclohexyloxy)-8-(un)substituted-2*H*-1-benzopyran-2-one (4a,b), (Scheme 1)
A solution of compound **1a-d** (0.01 mol) and 2-chlorocyclohexanone (1.32 g, 0.01 mol) in acetone (30 ml) was refluxed in presence of anhydrous potassium carbonate (2.76 g, 0.02 mol) for 24 h. The solution was filtered and the remaining residue was washed with acetone. The combined filtrates and washings were distilled under reduced pressure. The product was crystallized from ethanol.

2.1.3.1. 3-Ethyl-4-methyl-7-(2-oxocyclohexyloxy)-2*H*-1-benzopyran-2-one (4a)
Yield 68%. mp 179-180 °C. IR υ_{max}/ cm^{-1}: 3064 (CH Ar), 2949, 2870 (CH aliphatic), 1718, 1705 (2C=O), 1618, 1566, 1510 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.14 (t, 3H, $CH_2C\underline{H_3}$), 1.81-1.83 (m, 4H, CH_2 cyclohexanone), 2.01-2.08 (m, 2H, CH_2 cyclohexanone), 2.37 (s, 3H, CH_3 at C4), 2.59-2.70 (m, 4H, $C\underline{H_2}CH_3$, CH_2 cyclohexanone), 4.72 (t, 1H, CH cyclohexanone), 6.68 (s, 1H, H-8 Ar), 6.84 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.49 (d, 1H, *J*=8.7 Hz, H-5 Ar). MS *m/z*: 301 (M^++1). Anal. Calc. for $C_{18}H_{20}O_4$ (300.35): C, 71.98; H, 6.71. Found: C, 72.07; H, 6.74.

2.1.3.2. 4,8-Dimethyl-3-ethyl-7-(2-oxocyclohexyloxy)-2*H*-1-benzopyran-2-one (4b)
Yield 72%. mp 139-140 °C. IR υ_{max}/ cm^{-1}: 3055 (CH Ar), 2943, 2872 (CH aliphatic), 1726, 1681 (2C=O), 1598, 1577, 1538 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.13 (t, 3H, $CH_2C\underline{H_3}$), 1.75-1.86 (m, 4H, CH_2 cyclohexanone), 2.00-2.20 (m, 2H, CH_2 cyclohexanone), 2.33 (s, 3H, CH_3 at C4), 2.37 (s, 3H, CH_3 at C8), 2.60-2.70 (m, 4H, $C\underline{H_2}CH_3$,

CH_2 cyclohexanone), 4.71 (t, 1H, CH cyclohexanone), 6.62 (d, 1H, *J*=9.0 Hz, H-6 Ar), 7.32 (d, 1H, *J*=9.0 Hz, H-5 Ar). MS *m/z*: 315 (M^++1). Anal. Calc. for $C_{19}H_{22}O_4$ (314.38): C, 72.59; H, 7.05. Found: C, 72.67; H, 7.12.

2.1.4. General procedure for synthesis of 3-ethyl-4-methyl-11-(un)substituted-6,7,8,9-tetrahydro-2*H*-benzofuro[3,2-g]benzopyran-2-one (5a,b), (Scheme 1)
1N NaOH (15 ml) was added to a suspension of compound **4a,b** (0.002 mol) in isopropanol (15 ml) and refluxed for 4h (complete dissolution of starting compound). Solution was filtered while hot then cooled. 1N H_2SO_4 was added till the solution became acidic. The precipitate formed was filtered, dried and crystallized from isoprpanol.

2.1.4.1. 3-Ethyl-4-methyl-6,7,8,9-tetrahydro-2*H*-benzofuro[3,2-g]benzopyran-2-one (5a)
Yield 45%. mp 183-184 °C. IR υ_{max}/ cm^{-1}: 3057 (CH Ar), 2935, 2872 (CH aliphatic), 1701 (C=O), 1579 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.20 (t, 3H, $CH_2C\underline{H_3}$), 1.86-1.99 (m, 4H, tetrahydrobenzo H), 2.47 (s, 3H, CH_3 at C4), 2.64-2.78 (m, 6H, $C\underline{H_2}CH_3$, tetrahydrobenzo H), 7.34 (s, 1H, H-11 Ar), 7.55 (s, 1H, H-5 Ar). MS *m/z*: 282 (M^++1). Anal. Calc. for $C_{18}H_{18}O_3$ (282.33): C, 76.57; H, 6.43. Found: C, 76.57; H, 6.43.

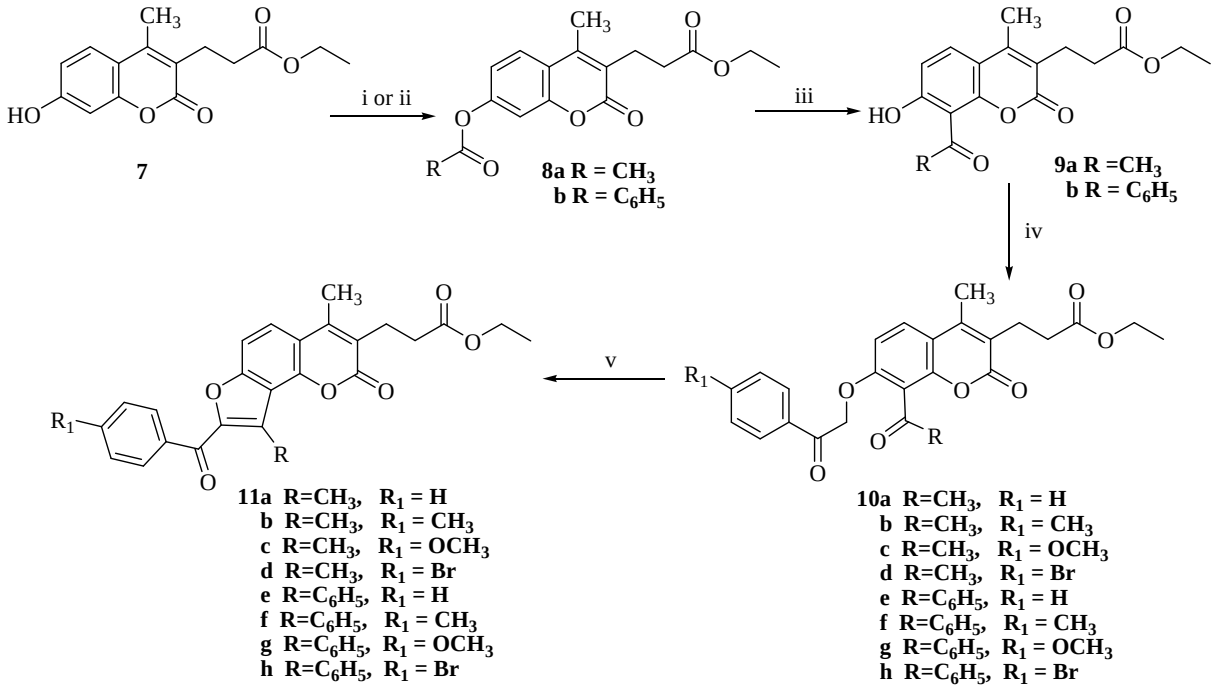
2.1.4.2. 4,11-Dimethyl-3-ethyl-6,7,8,9-tetrahydro-2*H*-benzofuro[3,2-g]benzopyran-2-one (5b)
Yield 50%. mp 192-193 °C. IR υ_{max}/ cm^{-1}: 3040 (CH Ar), 2933, 2868 (CH aliphatic), 1693 (C=O), 1593 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.18 (t, 3H, $CH_2C\underline{H_3}$), 1.88-1.99 (m, 4H, tetrahydrobenzo H), 2.49 (s, 3H, CH_3 at C4), 2.58 (s, 3H, CH_3 at C11), 2.63-2.80 (m, 6H, $C\underline{H_2}CH_3$, tetrahydrobenzo H), 7.42 (s, 1H, H-5 Ar). MS *m/z*: 297 (M^++1). Anal. Calc. for $C_{19}H_{20}O_3$ (296.36): C, 77.00; H, 6.80. Found: C, 77.12; H, 6.83.

2.1.5. General procedure for synthesis of 3-ethyl-4-methyl-11-(un)substituted-2*H*-benzofuro[3,2-g]benzopyran-2-one (6a,b), (Scheme 1)
2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (0.5 g) was added to a solution of compound **5a,b** (0.003 mol) in benzene (30 ml) and refluxed for 20 h. The mixture was filtered while hot and benzene was concentrated. The residue was crystallized from ethanol.

2.1.5.1. 3-Ethyl-4-methyl-2*H*-benzofuro[3,2-g]benzopyran-2-one (6a)
Yield 90%. mp 158-159 °C. IR υ_{max}/ cm^{-1}: 3057 (CH Ar), 2926, 2872 (CH aliphatic), 1693 (C=O), 1606, 1575 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.23 (t, 3H, $CH_2C\underline{H_3}$), 2.57 (s, 3H, CH_3 at C4), 2.75 (q, 2H, $C\underline{H_2}CH_3$), 7.42 (t, 1H, H-7 Ar), 7.50 (t, 2H, H-8,11 Ar), 7.59 (d, 1H, *J*=9.0 Hz, H-9 Ar), 7.98 (d, 1H, *J*=9.6 Hz, H-6 Ar), 8.14 (s, 1H, H-5 Ar). Anal. Calc. for $C_{18}H_{14}O_3$ (278.30): C, 77.68; H, 5.07. Found: C, 77.86; H, 5.04.

2.1.5.1. 4,11-Dimethyl-3-ethyl-2*H*-benzofuro[3,2-g]benzopyran-2-one (6b)
Yield 82%. mp 169-170 °C. IR υ_{max}/ cm^{-1}: 2924, 2852 (CH aliphatic), 1695 (C=O), 1583, 1564, 1508 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.21 (t, 3H, $CH_2C\underline{H_3}$), 2.57 (s, 3H, CH_3 at C4), 2.68 (s, 3H, CH_3 at C11), 2.77 (q, 2H, $C\underline{H_2}CH_3$), 7.41 (t, 1H, H-7 Ar), 7.49 (t, 1H, H-8 Ar), 7.61 (d, 1H, *J*=7.8 Hz, H-9 Ar), 7.98 (d, 1H, *J*=7.50, H-6 Ar), 8.01 (s, 1H, H-5 Ar). Anal. Calc. for $C_{19}H_{16}O_3$ (292.33): C, 78.06; H, 5.52. Found: C, 78.23; H, 5.58.

2.1.6. Ethyl 3-(7-benzoyloxy-4-methyl -2-oxo-2*H*-1-benzopyran-3-yl)propanoate (8b), (Scheme 2)
A mixture of compound **7** (5.52 g, 0.02 mol) and benzoyl chloride (4.21 g, 3.5 ml, 0.03 mol) was refluxed in an oil bath at 165 °C for 2 h. Reaction mixture was poured onto crushed ice with stirring. The separated product was filtered, washed with sodium bicarbonate solution, then with water and dried. The crude product was crystallized from ethanol. Yield 86%. mp 82-83 °C. IR υ_{max}/ cm^{-1}: 3043 (CH Ar), 2954, 2924 (CH aliphatic), 1732, 1707 (3C=O), 1571, 1527 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.25 (t, 3H, $OCH_2C\underline{H_3}$), 2.51 (s, 3H, CH_3 at C4), 2.63 (t, 2H, $COC\underline{H_2}CH_2$), 3.01 (t, 2H, $COCH_2C\underline{H_2}$), 4.14 (q, 2H, $OC\underline{H_2}CH_3$), 7.20 (d, 1H, *J*=8.4 Hz, H-6 Ar), 7.51-7.56 (m, 4H, H-8 Ar, H-3′, 4′, 5′ Ar), 7.68 (d, 1H, *J*=7.5 Hz, H-5 Ar), 8.21 (d, 2H, *J*=9.6 Hz, H-2′, 6′Ar). Anal. Calc. for $C_{22}H_{20}O_6$ (380.39): C, 69.46; H, 5.30. Found: C, 69.53; H, 5.36.



Scheme 2. Reagents and conditions: (i) Acetic anhydride, reflux, 2h, (ii) benzoyl chloride, 165 °C, 2h, (iii) anhydrous $AlCl_3$, 165 °C, 2h, (iv) appropriate phenacyl bromide, acetone, K_2CO_3, reflux, 24h, (v) 1N NaOH, isopropanol, 10h.

2.1.7. General Procedure for synthesis of ethyl 3-(8-acyl-7-hydroxy-4-methyl-2-oxo-2*H*-1-benzopyran-3-yl)propanoate (9a,b), (Scheme 2)

Finely powdered compound **8a,b** (0.046 mol) was thoroughly mixed with anhydrous aluminum chloride (20.2 g, 0.152 mol) and heated to 165 °C (in an oil bath) for 2 h. The melt was cooled, 10% HCl (50 ml) was added and refluxed for 30 min. The separated solid was filtered, washed with cold water then dissolved in 5% NaOH, filtered and acidified with 10% HCl. The precipitate formed was filtered, dried and crystallized from ethanol.

2.1.7.1. Ethyl 3-(8-acetyl-7-hydroxy-4-methyl-2-oxo-2*H*-1-benzopyran-3-yl)propanoate (9a)

Yield 52%. mp 135-136 °C. IR υ_{max}/ cm^{-1}: 3460 (OH), 2929 (CH aliphatic), 1714, 1700 (3C=O), 1591 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.25 (t, 3H, $OCH_2C\underline{H_3}$), 2.51 (s, 3H, CH_3 at C4), 2.69 (t, 2H, $COC\underline{H_2}CH_2$), 2.95 (s, 3H, $COCH_3$), 3.07 (t, 2H, $COCH_2C\underline{H_2}$), 3.64 (q, 2H, $OC\underline{H_2}CH_3$), 6.93 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.71 (d, 1H, *J*=7.8 Hz, H-5 Ar), 13.48 (s, 1H, OH exch. D_2O). Anal. Calc. for $C_{17}H_{18}O_6$ (318.32): C, 64.14; H, 5.70. Found: C, 64.19; H, 5.73.

2.1.7.2. Ethyl 3-(8-benzoyl-7-hydroxy-4-methyl-2-oxo-2*H*-1-benzopyran-3-yl)propanoate (9b)

Yield 44%. mp 79-80 °C. IR υ_{max}/ cm^{-1}: 3412 (OH), 3080 (CH Ar), 2933, 2875 (CH aliphatic), 1705 (3C=O), 1604 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.26 (t, 3H, $OCH_2C\underline{H_3}$), 2.41 (s, 3H, CH_3 at C4), 2.66 (t, 2H, $COC\underline{H_2}CH_2$), 3.05 (t, 2H, $COCH_2C\underline{H_2}$), 4.12 (q, 2H, $OC\underline{H_2}CH_3$), 6.77 (d, 1H, *J*=7.8 Hz, H-6 Ar), 7.35-7.92 (m, 4H, H-5 Ar, H-3′, 4′, 5′ Ar), 8.10 (d, 2H, *J*= 8.7 Hz, H-2′, 6′Ar), 13.20 (s, 1H, OH exch. D_2O). Anal. Calc. for $C_{22}H_{20}O_6$ (380.39): C, 69.46; H, 5.30. Found: C, 69.62; H, 5.31.

2.1.8. General Procedure for synthesis of ethyl 3-(8-acyl-4-methyl-2-oxo-7-(un)substituted phenacyloxy-2*H*-1-benzopyran-3-yl)propanoate (10a-h), (Scheme 2)

A solution of compound **9a,b** (0.01 mol) and appropriate phenacyl bromide derivative (0.015 mol) in acetone (30 ml) was refluxed in presence of anhydrous potassium carbonate (2.76 g, 0.02 mol) for 24 h. The solution was filtered and the remaining residue was washed with acetone. The combined filtrates and washings were distilled under reduced pressure. The product was crystallized from ethanol.

2.1.8.1. Ethyl 3-(8-acetyl-4-methyl-2-oxo-7-phenacyloxy-2*H*-1-benzopyran-3-yl)propanoate (10a)
Yield 86%. mp 105-106 °C. IR υ_{max}/ cm^{-1}: 3062 (CH Ar), 2933 (CH aliphatic), 1743, 1705 (4C=O), 1598, 1579, 1562, 1554, 1508 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.24 (t, 3H, $OCH_2C\underline{H_3}$), 2.45 (s, 3H, CH_3 at C4), 2.61 (s, 3H, $COCH_3$), 2.78-3.12 (m, 4H, $COCH_2CH_2$), 3.72 (q, 2H, $OC\underline{H_2}CH_3$), 5.35 (s, 2H, OCH_2CO), 6.96 (d, 1H, *J*=10.8 Hz, H-6 Ar), 7.43-7.74 (m, 3H, H-3′, 4′, 5′Ar), 7.91 (d, 1H, *J*= 11.2 Hz, H-5 Ar), 8.06 (d, 2H, *J*=6.9 Hz, H-2′, 6′ Ar). MS *m/z*: 436 (M^+). Anal. Calc. for $C_{25}H_{24}O_7$ (436.45): C, 68.80; H, 5.54. Found: C, 68.93; H, 5.57.

2.1.8.2. Ethyl 3-[8-acetyl-4-methyl-7-(4-methylphenacyloxy)-2-oxo-2*H*-1-benzopyran-3-yl]propanoate (10b)
Yield 62%. mp 110-111 °C. IR υ_{max}/ cm^{-1}: 3030 (CH Ar), 2926, 2872 (CH aliphatic), 1743, 1703 (4C=O), 1606, 1568, 1550 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.27 (t, 3H, $OCH_2C\underline{H_3}$), 2.57 (s, 3H, CH_3 at C4), 2.62 (s, 3H, CH_3 at C4′), 2.86 (t, 2H, $COC\underline{H_2}CH_2$), 2.93 (s, 3H, $COCH_3$), 3.09 (t, 2H, $COCH_2C\underline{H_2}$), 3.72 (q, 2H, $OC\underline{H_2}CH_3$), 5.32 (s, 2H, OCH_2CO), 6.93 (d, 1H, *J*= 8.1 Hz, H-6 Ar), 7.34 (d, 2H, *J*= 8.1 Hz, H-3′, 5′ Ar), 7.79 (d, 2H, *J*= 8.4 Hz, H-2′, 6′Ar), 7.98 (d, 1H, *J*= 8.1 Hz, H-5 Ar). Anal. Calc. for $C_{26}H_{26}O_7$ (450.48): C, 69.32; H, 5.82. Found: C, 69.47; H, 5.84.

2.1.8.3. Ethyl 3-[8-acetyl-7-(4-methoxyphenacyloxy)-4-methyl-2-oxo-2*H*-1-benzopyran-3-yl]propanoate (10c)
Yield 94%. mp 144-145 °C. IR υ_{max}/ cm^{-1}: 2933, 2839 (CH aliphatic), 1712, 1703 (4C=O), 1600, 1571, 1554, 1510 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.25 (t, 3H, $OCH_2C\underline{H_3}$), 2.56 (s, 3H, CH_3 at C4), 2.66 (t, 2H, $COC\underline{H_2}CH_2$), 2.93 (s, 3H, $COCH_3$), 3.01 (t, 2H, $COCH_2C\underline{H_2}$), 3.90 (s, 3H, OCH_3), 4.14 (q, 2H, $OC\underline{H_2}CH_3$), 5.30 (s, 2H, OCH_2CO), 6.96 (d, 1H, *J*= 9.0 Hz, H-6 Ar), 7.03 (d, 2H, *J*= 9.0 Hz, H-3′, 5′ Ar), 7.70 (d, 1H, *J*= 9.0 Hz, H-5 Ar), 8.11 (d, 2H, *J*= 9.0 Hz, H-2′, 6′ Ar). Anal. Calc. for $C_{26}H_{26}O_8$ (466.48): C, 66.94; H, 5.62. Found: C, 67.11; H, 5.68.

2.1.8.4. Ethyl 3-[8-acetyl-7-(4-bromophenacyloxy)-4-methyl-2-oxo-2*H*-1-benzopyran-3-yl]propanoate (10d)
Yield 58%. mp 93-94 °C. IR υ_{max}/ cm^{-1}: 2929 (CH aliphatic), 1715, 1705 (4C=O), 1585 (C=C), ^{1}H-NMR ($CDCl_3$) δ ppm: 1.25 (t, 3H, $OCH_2C\underline{H_3}$), 2.45 (s, 3H, CH_3 at C4), 2.87 (t, 2H, $COC\underline{H_2}CH_2$), 2.99 (s, 3H, $COCH_3$), 3.05 (t, 2H, $COCH_2C\underline{H_2}$), 3.75 (q, 2H, $OC\underline{H_2}CH_3$), 5.29 (s, 2H, OCH_2CO), 6.93 (d, 1H, *J*= 9.3 Hz, H-6 Ar), 7.63 (d, 2H, *J*= 8.7 Hz, H-3′, 5′ Ar), 7.75 (d, 2H, *J*= 8.7 Hz, H-2′, 6′ Ar), 8.01 (d, 1H, *J*=9.0 Hz, H-5 Ar). Anal. Calc. for $C_{25}H_{23}BrO_7$ (515.35): C, 58.26; H, 4.50. Found: 58.52; H, 4.56.

2.1.8.5. Ethyl 3-[8-benzoyl-4-methyl-2-oxo-7-phenacyloxy-2*H*-1-benzopyran-3-yl]propanoate (10e)
Yield 36%. mp 88-90 °C. IR υ_{max}/ cm^{-1}: 3064 (CH Ar), 2929 (CH aliphatic), 1705 (4C=O), 1606, 1579, 1550, 1508 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.26 (t, 3H, $OCH_2C\underline{H_3}$), 2.45 (s, 3H, CH_3), 2.81 (t, 2H, $COC\underline{H_2}CH_2$), 3.04 (t, 2H, $COCH_2C\underline{H_2}$), 3.74 (q, 2H, $OC\underline{H_2}CH_3$), 5.34 (s, 2H, OCH_2CO), 6.95 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.30-7.68 (m, 7H, H-5, 3′,4′,5′,3″,4″, 5″Ar), 7.78 (d, 2H, *J*=9.0 Hz, H-2″, 6″Ar), 7.99 (d, 2H, *J*=8.4 Hz, H-2′, 6′Ar). Anal. Calc. for $C_{30}H_{26}O_7$ (498.52): C, 72.28; H, 5.26. Found: C, 72.37; H, 5.30.

2.1.8.6. Ethyl 3-[8-benzoyl-4-methyl-7-(4-methylphenacyloxy)-2-oxo-2*H*-1-benzopyran-3-yl]propanoate (10f)
Yield 34%. mp 115-116 °C. IR υ_{max}/ cm^{-1}: 2929 (CH aliphatic), 1715, 1703 (4C=O), 1606 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.25 (t, 3H, $OCH_2C\underline{H_3}$), 2.45 (s, 3H, CH_3 at C4), 2.47 (s, 3H, CH_3 at C4′), 3.04 (t, 2H, $COC\underline{H_2}CH_2$), 3.64 (t, 2H, $COCH_2C\underline{H_2}$), 3.73 (q, 2H, $OC\underline{H_2}CH_3$), 5.31 (s, 2H, OCH_2CO), 6.91 (d, 1H, *J*=9.6 Hz, H-6 Ar), 7.28-7.91 (m, 10H, Ar H). Anal. Calc. for $C_{31}H_{28}O_7$ (512.55): C, 72.64; H, 5.51. Found: C, 72.76; H, 4.38.

2.1.8.7. Ethyl 3-[8-benzoyl-7-(4-methoxyphenacyloxy)-4-methyl-2-oxo-2*H*-1-benzopyran-3-yl]propanoate (10g)
Yield 35%. mp 174-176 °C. IR υ_{max}/ cm^{-1}: 2927 (CH aliphatic), 1714, 1705 (4C=O), 1602 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.25 (t, 3H, $OCH_2C\underline{H_3}$), 2.49 (s, 3H, CH_3), 3.09 (t, 2H, $COC\underline{H_2}CH_2$), 3.59 (t, 2H, $COCH_2C\underline{H_2}$), 3.73 (q, 2H, $OC\underline{H_2}CH_3$), 3.88 (s, 3H, OCH_3), 5.28 (s, 2H, OCH_2CO), 6.89-7.04 (m, 3H, H-6, 3′, 5′ Ar), 7.45-7.97 (m, 8H, H- 5, 2′, 6′ and 2″,3″,4″,5″, 6″ Ar). MS *m/z*: 528 (M^+). Anal. Calc. for $C_{31}H_{28}O_8$ (528.55): C, 70.44; H, 5.34. Found: C, 70.62; H, 5.41.

2.1.8.8. Ethyl 3-[8-benzoyl-7-(4-bromophenacyloxy)-4-methyl-2-oxo-2*H*-1-benzopyran-3-yl]propanoate (10h)
Yield 32%. mp 97-99 °C. IR υ_{max}/ cm^{-1}: 3084 (CH Ar), 2953, 2933 (CH aliphatic), 1697 (4C=O), 1606, 1585, 1543 (C=C), ^{1}H-NMR ($CDCl_3$) δ ppm: 1.25 (t, 3H, $OCH_2C\underline{H_3}$), 2.49 (s, 3H, CH_3), 3.06 (t, 2H, $COC\underline{H_2}CH_2$), 3.62 (t, 2H, $COCH_2C\underline{H_2}$), 3.73 (q, 2H, $OC\underline{H_2}CH_3$), 5.29 (s, 2H, OCH_2CO), 6.60-7.99 (m, 11H, Ar H). MS *m/z*: 577 (M^+). Anal. Calc. for $C_{30}H_{25}BrO_7$ (577.42): C, 62.40; H, 4.36. Found: C, 62.51; H, 4.58.

2.1.9. General Procedure for synthesis of ethyl 3-(7-methyl-5-oxo-3-substituted-2-(un)substituted benzoyl-5*H*-furo[2,3-h]benzopyran-6-yl)propanoate (11a-h), (Scheme 2)
1N NaOH (10 ml) was added to a solution of compound **10a-h** (0.001 mol) in isopropanol (10 ml) and refluxed for 10h. Solution was poured onto 1N HCl (50 ml). The precipitate formed was filtered, dried and crystallized from ethanol.

2.1.9.1. Ethyl 3-(2-benzoyl-3,7-dimethyl-5-oxo-5*H*-furo[2,3-h]benzopyran-6-yl)propanoate (11a)
Yield 25%. mp 118-120 °C. IR υ_{max}/ cm^{-1}: 3070 (CH Ar), 2915 (CH aliphatic), 1709, 1689 (3C=O), 1605, 1570 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.25 (t, 3H, $OCH_2C\underline{H_3}$), 2.56 (s, 3H, CH_3 at C7), 2.66 (t, 2H, $COC\underline{H_2}CH_2$), 2.94 (s, 3H, CH_3 at C3), 3.04 (t, 2H, $COCH_2C\underline{H_2}$), 4.14 (q, 2H, $OC\underline{H_2}CH_3$), 7.36-8.10 (m, 7H, Ar H). MS *m/z*: 419 (M^++1). Anal. Calc. for $C_{25}H_{22}O_6$ (418.44): C, 71.76; H, 5.30. Found: C, 71.89; H, 5.34.

2.1.9.2. Ethyl 3-[3,7-dimethyl-2-(4-methylbenzoyl)-5-oxo-5*H*-furo[2,3-h]benzopyran-6-yl]propanoate (11b)
Yield 22%. mp 215-216 °C. IR υ_{max}/ cm^{-1}: 2924 (CH aliphatic), 1714, 1691 (3C=O), 1606, 1550 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.25 (t, 3H, $OCH_2C\underline{H_3}$), 2.43 (s, 3H, CH_3 at C7), 2.47 (s, 3H, CH_3 at C3), 2.73 (t, 2H, $COC\underline{H_2}CH_2$), 2.92 (s, 3H, CH_3 at C4′), 3.74 (t, 2H, $COCH_2C\underline{H_2}$), 4.10 (q, 2H, $OC\underline{H_2}CH_3$), 7.34 (d, 2H, *J*= 8.7 Hz, H-3′, 5′ Ar), 7.43 (d, 1H, *J*= 9.0 Hz, H-9 Ar), 7.71 (d, 1H, *J*= 9.0 Hz, H-8 Ar), 7.97 (d, 2H, *J*= 7.8 Hz, H- 2′, 6′ Ar). Anal. Calc. for $C_{26}H_{24}O_6$ (432.47): C, 72.21; H, 5.59. Found: C, 72.36; H, 5.63.

2.1.9.3. Ethyl 3-[3,7-dimethyl-2-(4-methoxybenzoyl)-5-oxo-5*H*-furo[2,3-h]benzopyran-6-yl]propanoate (11c)
Yield 24%. mp 113-114 °C. IR υ_{max}/ cm^{-1}: 3046 (CH Ar), 2978, 2932 (CH aliphatic), 1682 (3C=O), 1610, 1556 (C=C), ^{1}H-NMR ($CDCl_3$) δ ppm: 0.93 (t, 3H, $OCH_2C\underline{H_3}$), 2.57 (s, 3H, CH_3 at C7), 2.93 (s, 3H, CH_3 at C3), 3.06 (t, 2H, $COC\underline{H_2}CH_2$), 3.88 (t, 2H, $COCH_2C\underline{H_2}$), 3.92 (s, 3H, OCH_3), 4.10 (q, 2H, $OC\underline{H_2}CH_3$), 7.03 (d, 2H, *J*= 9.0 Hz, H-3′, 5′ Ar), 7.47 (d, 1H, *J*= 9.0 Hz, H-9 Ar), 7.70 (d, 1H, *J*= 9.0 Hz, H-8 Ar), 8.11 (d, 2H, *J*= 8.7 Hz, H-2′, 6′ Ar). Anal. Calc. for $C_{26}H_{24}O_7$ (448.46): C, 69.63; H, 5.39. Found: C, 69.81; H, 5.45.

2.1.9.4. Ethyl 3-[2-(4-bromobenzoyl)-3,7-dimethyl-5-oxo-5*H*-furo[2,3-h]benzopyran-6-yl]propanoate (11d)
Yield 23%. mp 102-105 °C. IR υ_{max}/ cm^{-1}: 3066 (CH Ar), 2931 (CH aliphatic), 1700, 1681 (3C=O), 1612, 1587, 1571 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.25 (t, 3H, $OCH_2C\underline{H_3}$), 2.43 (s, 3H, CH_3 at C7), 2.90 (t, 2H, $COC\underline{H_2}CH_2$), 2.97 (s, 3H, CH_3 at C3), 4.13 (t, 2H, $COCH_2C\underline{H_2}$), 4.20 (q, 2H, $OC\underline{H_2}CH_3$), 7.37-7.96 (m, 6H, Ar H). Anal. Calc. for $C_{25}H_{21}BrO_6$ (497.33): C, 60.38; H, 4.26. Found: C, 60.43; H, 4.30.

2.1.9.5. Ethyl 3-[2-benzoyl-7-methyl-3-phenyl-5-oxo-5*H*-furo[2,3-h]benzopyran-6-yl]propanoate (11e)
Yield 25%. mp 99-101 °C. IR υ_{max}/ cm^{-1}: 3090 (CH Ar), 2960, 2933 (CH aliphatic), 1701 (3C=O), 1610, 1566 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.24 (t, 3H, $OCH_2C\underline{H_3}$), 2.55 (s, 3H, CH_3), 2.96 (t, 2H, $COC\underline{H_2}CH_2$), 3.72 (t, 2H, $COCH_2C\underline{H_2}$), 4.12 (q, 2H, $OC\underline{H_2}CH_3$), 7.26-8.11 (m, 12H, Ar H). Anal. Calc. for $C_{30}H_{24}O_6$ (480.51): C, 74.99; H, 5.03. Found: C, 75.13; H, 5.09.

2.1.9.6. Ethyl 3-[7-methyl-2-(4-methylbenzoyl)-3-phenyl-5-oxo-5*H*-furo[2,3-h]benzopyran-6-yl]propanoate (11f)
Yield 24%. mp 90-93 °C. IR υ_{max}/ cm^{-1}: 3045 (CH Ar), 2971, 2925 (CH aliphatic), 1696 (3C=O), 1601, 1575 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.26 (t, 3H, $OCH_2C\underline{H_3}$), 2.44 (s, 6H, 2xCH_3), 3.00 (t, 2H, $COC\underline{H_2}CH_2$), 3.60 (m, 2H, $COCH_2C\underline{H_2}$), 4.10 (q, 2H, $OC\underline{H_2}CH_3$), 7.46-8.00 (m, 11H, Ar H). Anal. Calc. for $C_{31}H_{26}O_6$ (494.53): C, 75.29; H, 5.30. Found: C, 75.42; H, 5.28.

2.1.9.7. Ethyl 3-[2-(4-methoxybenzoyl)-7-methyl-3-phenyl-5-oxo-5*H*-furo[2,3-h]benzopyran-6-yl]propanoate (11g)
Yield 28%. mp 129-132 °C. IR υ_{max}/ cm^{-1}: 3066 (CH Ar), 2920, 2866 (CH aliphatic), 1705 (3C=O), 1610, 1580 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.24 (t, 3H, $OCH_2C\underline{H_3}$), 2.11 (s, 3H, CH_3), 2.45 (t, 2H, $COC\underline{H_2}CH_2$), 3.00 (t, 2H, $COCH_2C\underline{H_2}$), 3.88 (s, 3H, OCH_3), 4.10 (q, 2H, $OC\underline{H_2}CH_3$), 6.90-7.70 (m, 11H, Ar H). MS *m/z*: 511 (M^++1). Anal. Calc. for $C_{31}H_{26}O_7$ (510.53): C, 72.93; H, 5.13. Found: C, 73.07; H, 5.17.

2.1.9.8. Ethyl 3-[2-(4-bromobenzoyl)-7-methyl-3-phenyl-5-oxo-5*H*-furo[2,3-h]benzopyran-6-yl]propanoate (11h)
Yield 30%. mp 107-109 °C. IR υ_{max}/ cm^{-1}: 3100 (CH Ar), 2927, 2856 (CH aliphatic), 1705, 1681 (3C=O), 1606, 1587 (C=C). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.26 (t, 3H, $OCH_2C\underline{H_3}$), 2.62 (s, 3H, CH_3 at C7), 2.95 (t, 2H, $COC\underline{H_2}CH_2$), 4.14 (t, 2H, $COCH_2C\underline{H_2}$), 4.32 (q, 2H, $OC\underline{H_2}CH_3$), 7.36-8.09 (m, 11H, Ar H). MS *m/z*: 559 (M^+). Anal. Calc. for $C_{30}H_{23}BrO_6$ (559.40): C, 64.41; H, 4.14. Found: C, 64.54; H, 4.16.

2.2. Antimicrobial and photosensitizing activity:
All newly synthesized compounds were screened for antimicrobial and photosensitizing activity by applying the paper disc diffusion method [31] compared with xanthotoxin, as a reference compound. The tested organism used was *Bacillus subtilis*.

First, high concentration of the substances were used for picking out the active compounds, even if weakly active, from the inactive ones. Then, only active compounds were tested to assign the effect of concentration and time of exposure to UV-A on their photosensitizing activity. The results were compared with xanthotoxin as a reference compound.

Pre-experimental preparations:
a) Nutrient agar medium: 0.3% of the beef extract, 0.5% of peptone, 0.1% of dipotassium hydrogen phosphate and 1.5% agar.

b) Broth culture of the organism, *Bacillus subtilis*, was prepared through incubation of slant agar seeded with the organism overnight.

c) Paper disc: Whatman no. 1 filter paper discs (6 mm) were sterilized and impregnated with different concentrations of the tested compounds dissolved in dimethylformamide (DMF), and allowed to dry overnight. Two concentrations were prepared for each of the tested compounds.

Experimental
In the sterile petri dishes, the prepared broth culture (0.02 ml) was added carefully. Then, 10 ml of the liquefied nutrient agar medium was added, allowed to be mixed uniformly and solidified. The impregnated discs, 6 mm diameter, were placed uniformly on the solidified agar layer. Each plate contained disc impregnated with DMF, to neglect the effect of solvent, and another disc impregnated with xanthotoxin as a reference compound.

The plates were divided into two groups, one as test plate, was incubated in the dark at 37 °C for 3 h before irradiation to allow the diffusion of the tested compounds through the agar layer, and the duplicate plates were left in the incubator overnight as a control to determine the antimicrobial activity.

The covers were removed from the plates of the first group (tested petri dishes) and the dishes were exposed to U.V. lamp (365 nm) for 20 min. After irradiation, the plates were reincubated in the dark at 37 °C overnight. The inhibition zones around each disc were measured, in both control and test, which was indicative for antimicrobial and photosensitizing activity of the tested compounds respectively. Results were recorded in Table 1.

Effect of increasing time of UV-A radiation and concentration on photosensitizing activity for active compounds
The experiment was repeated using the selected active compounds to study the effect of radiation time and concentration on the photosensitizing activity.

Two groups of discs were prepared. One group of discs was impregnated with 0.01 ml (each disc contained 0.5 mg of the tested compounds) and the other group was impregnated with 0.02 ml (each disc contained 1 mg of the tested compounds). Results were recorded in Table 2.

2.3. Molecular Docking
Docking studies of active antimicrobial compounds were performed by Molecular Operating Environment software (MOE version 2008.10.2) [32]. The program operated under “Window XP” operating system installed on an Intel Pentium IV PC with a 2.8 MHz processor and 512 RAM. All minimizations were performed with MOE until a RMSD gradient of 0.05 Kcal mol^{-1} $Å^{-1}$ with MMFF94 force field and the partial charges were automatically calculated. The score function, dock function (S, Kcal/mol) developed by MOE program was used for evaluation of the binding affinity of the ligand.

Preparation of the target DNA gyrase
The X-ray crystal structure of the enzyme with benzopyrone ligand, clorobiocin (PDB code 1KZN) [33] was obtained from the protein data bank in PDB formate. The enzyme was prepared for docking. (i) 3D protonation for

the amino acid side chain and clorobiocin. (ii) Isolation of the active site, fixation to be dealt with as rigid structure and recognition of amino acids. (iii) Creation of dummies around active site. (iv) Studying the interactions of the ligand (clorobiocin) with the amino acids of the active site.

Preparation of compounds for docking

The 3D structures of the active compounds were built using MOE and subjected to the following procedure: (i) 3D protonation of the structures. (ii) Running conformational analysis using systemic search. (iii) Selecting the least energetic conformer. (iv) Applying the same docking protocol used with clorobiocin.

Docking running

Prior to docking of active compounds, redocking of the native ligand bound in the DNA gyrase active site was performed to validate the docking protocol. The generated most stable conformer of each compound was virtually docked into the predefined active site of gyrase. The developed docked models were energetically minimized and then used to predict the interaction of the ligand with the amino acids in the active site of the enzyme, Table 3 and Fig. 2-5.

RESULTS AND DISCUSSION

3.1. Chemistry

The synthesis of the desired furobenzopyrones, tetrahydrobenzo- and benzofurobenzopyrones was carried out as illustrated in Schemes 1,2 .

Starting compounds, 3,8-disubstituted-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-ones **1a-d** [27] were prepared following reported procedure.

Refluxing 7-hydroxybenzopyrones **1a-d** with appropriate α-haloketone in dry acetone containing anhydrous potassium carbonate yielded ether derivatives **2a-d** and **4a,b** (Scheme 1). Negative ferric chloride test confirmed complete reaction. The proposed structures were confirmed by spectral and analytical data. The IR spectra revealed absence of band corresponding to phenolic OH. ^{1}H-NMR spectra of compounds **2a-d** showed appearance of doublet, singlet and quartet signals at δ = 1.46-1.55, 2.33-2.37 and 4.69-5.11 ppm assigned to OCHC$\underline{H}_3$, COCH_3 and OC$\underline{H}$CH_3, respectively of the formed 3-oxobutan-2-yloxy moiety. ^{1}H-NMR spectra of compounds **4a,b** showed appearance of three multiplet and one triplet signals at δ = 1.75-1.86, 2.00-2.20, 2.59-2.70 and 4.71-4.72 ppm assigned to cyclohexanone protons.

Cyclization of ether derivatives **2a-d** to the corresponding furo[3,2-g]benzopyran-7-ones **3a-d** or **4a,b** to the corresponding tetrahydrobenzofurobenzopyrones **5a,b** were achieved through reflux of isopropanol suspension of ether derivative with sodium hydroxide followed by subsequent acidification (Scheme 1), [34,35]. The structures of the synthesized compounds were confirmed by spectral and analytical data. ^{1}H-NMR spectra revealed disappearance of two doublet signals of the H-6 and H-5 aromatic protons at 6.62-6.91 and 7.32-7.68 ppm, respectively. Instead, appearance of one singlet signal corresponding to H-4 in compounds **3a-d** or H-5 in compounds **5a,b** at 7.36-7.54 or 7.42-7.55 ppm, respectively confirmed cyclization.

Refluxing the tetrahydrobenzo- compounds **5a,b** with DDQ in dry benzene [36] provided dehydrogenation to the corresponding benzofurobenzopyrones **6a,b** (Scheme 1). The structures of the synthesized compounds were deduced from spectral and analytical data. ^{1}H-NMR spectra revealed disappearance of two multiplet signals at 1.86-1.99 and 2.63-2.80 ppm corresponding to tetrahydrobebzo- protons. Instead, appearance of two triplet and two doublet signals at 7.41-7.42, 7.49-7.50, 7.59-7.61 and 7.98 ppm, respectively corresponding to H-7, H-8, H-9, H-6 aromatic protons, respectively, confirmed dehydrogenation.

Ethyl 3-(7-hydroxy-4-methyl-2-oxo-2*H*-benzopyran-3-yl)propanoate **7** [28] and phenacyl bromide derivatives [30] were prepared as reported in literature.

Acylation of 7-hydroxy compound **7** with acid anhydride [29] or acid chloride [29,37] yielded compounds **8a** [29] and **8b**, respectively (Scheme 2). Fries rearrangement of esters **8a**,**b** provided both 6-acyl and 8-acyl derivatives. However, the majority of rearrangements take place to the 8-position as it was stabilized by the pyrone ring [38,39] and the 8-acyl derivatives **9a,b** were the achieved compounds (Scheme 2). Positive ferric chloride test indicated

presence of phenolic OH group. Moreover, the IR spectra revealed presence of band at 3460-3412 cm^{-1} corresponding to phenolic OH group. ^{1}H-NMR spectra of **9a,b** showed presence of doublet signal at 6.77-6.93 ppm assigned to H-6 aromatic proton in addition to singlet signal at 13.20-13.48 ppm exchanged with D_2O corresponding to OH confirmed rearrangement to 8- position.

Reaction of 8-acyl-7-hydroxy compounds **9a,b** with appropriate phenacyl bromide derivative in presence of anhydrous potassium carbonate resulted in ether derivatives **10a-h** (Scheme 2). The new ether derivatives gave negative ferric chloride test and the proposed structures were confirmed by spectral and analytical data. ^{1}H-NMR revealed presence of singlet signal at δ = 5.28-5.35 ppm assigned to OCH_2CO protons in addition to increased number of aromatic protons confirmed etherification.

Cycliztion of ether derivatives **10a-h** applied in alkaline medium using sodium hydroxide followed by subsequent acidification yielded angular furobenzopyrones **11a-h** (Scheme 2). The structures of the synthesized compounds were confirmed by spectral and analytical data. ^{1}H-NMR spectra revealed the disappearance of singlet peak assigned to OCH_2CO in addition to presence of two doublet peaks of the at 7.43-7.47 and 7.70-7.71 ppm assigned to H-9 and H-8, respectively, for compounds **11b,c** confirmed formation of angular furobenzopyranone..

Table 1: Preliminary screenining for antimicrobial and photosensitizing activities

Cpd. No.	Control	Test	Cpd. No.	Control	Test
DMF	-	-	**10a**	-	-
Xanthotoxin	9	12	**10b**	-	12
2a	7	7	**10c**	10	10
2b	-	12	**10d**	8	8
2c	-	7	**10e**	-	10
2d	10	10	**10f**	7	7
3a	-	10	**10g**	-	-
3b	-	12	**10h**	-	8
3c	-	7	**11a**	7	7
3d	10	10	**11b**	8	8
4a	-	-	**11c**	-	10
4b	-	-	**11d**	10	10
5a	10	10	**11e**	7	12
5b	-	-	**11f**	-	-
6a	-	-	**11g**	7	7
6b	-	-	**11h**	-	-

Control *Disk contains 0.01 ml of the tested and reference compounds.*
Test*: Disk contains 0.01 ml of the tested and reference compounds and time of radiation is 20 min.*

3.2. Antimicrobial and photosensitizing activity:

Results of antimicrobial evaluation (Table 1) showed that, benzopyrones **2d** and **10c** had activity higher than xanthotoxin while compounds **2a**, **10d,f** were less active and rest of compounds were inactive. Examination of furobenzopyrones results revealed that only compound **3d** (linear furobenzopyrone) and compound **11d** (angular furobenzopyrone) were more active than xanthotoxin. Compounds **11a,b,e,g** were less active and rest of compounds were inactive. Tetrahydrobenzofurobenzopyrone **5a** was also more active than xanthotoxin while rest of other tetrahydrobenzo-, and benzofurobenzopyrones were inactive.

Results of photosensitizing activity (Table 1,2) revealed that compound **11e** showed good photosensitizing activity and increased upon increasing radiation time to 40 min. It was interesting to find photosensitizing activity to antimicrobial inactive compounds **2b,c**, **3a-c**, **10b,e,h** and **11c**. Photosensitizing activity increased for compounds **3a,c**, **10e** and **11c** upon increasing radiation time to 40 min.

Active compounds **2a,d**, **3d**, **5a**, **10c,d,f** and **11a,b,d,g** (with antimicrobial activity) showed no photosensitizing activity after irradiation for 20 min. Photosensitizing activity of compounds **2d**, **10d,f** and **11b** increased upon increasing radiation time to 40 min.

The study of concentration effect on photosensitizing activity showed increase in activity for all photosensitizing compounds except compound **10b**.

Table 2: Effect of increasing time of radiation and concentration on photosensitizing activity for the active derivatives

Cpd. No.	Control	Test	Test*	Test**
DMF	-	-	-	-
2a	7	7	7	7
2b	-	12	12	14
2c	-	7	7	8
2d	10	10	12	15
3a	-	10	11	14
3b	-	12	12	12
3c	-	7	12	12
3d	10	10	10	13
5a	10	10	10	12
10b	-	12	12	12
10c	10	10	10	10
10d	8	8	9	13
10e	-	10	13	13
10f	7	7	8	8
10h	-	8	8	9
11a	7	7	7	8
11b	8	8	10	10
11c	-	10	12	12
11d	10	10	10	13
11e	7	12	14	15
11g	7	7	7	7
Xanthotoxin	9	12	15	19

Control *Disk contains 0.01 ml of the tested and reference compounds.*
Test *Disk contains 0.01 ml of the tested and reference compounds and time of radiation is 20 min.*
Test* *Disk contains 0.01 ml of the tested and reference compounds and time of radiation is 40 min.*
Test* *Disk contains 0.02 ml of the tested and reference compounds and time of radiation is 20 min.*

3.3. Molecular docking

Four main classes of bacterial topoisomerases, designated I-IV have been identified. Bacterial topoisomerases I and III are type IA topoisomerases that interact with single-stranded DNA, while bacterial topoisomerases II and IV are type IIA topoisomerases that interact with double-stranded DNA [40]. DNA gyrase is a unique enzyme among the type IIA topoisomerases, as it can introduce negative super helical turns into DNA and removes positive supercoils. This function is essential for bacterial DNA replication [41,42].

Over the past decades, inhibition of bacterial DNA gyrase was considered a good target for potent antibacterial agents [43]. Quinolones (particularly fluoroquinolones), quinazolinediones, pyrazoles, indazoles, benzimadazoles and benzothiazoles have been shown to be highly successful inhibitors of bacterial DNA gyrase [42]. In addition, naturally occurring bacterial DNA gyrase inhibitors such as aminobenzopyrone derivatives, which include clorobiocin and novobiocin were also known as antibacterial agents. They inhibit ATPase activity of DNA gyrase by competing with ATP for binding to the subunit B of the enzyme [14,43].

The binding affinity of the ligand was evaluated with energy score (S, Kcal/mol). The compound which revealed the highest binding affinity, minimum dock score, is the one forming the most stable ligand-enzyme complex. Length of the hydrogen bond and arene cation interaction were also used to assess the binding models. The results of docking studies; dock score, involved DNA gyrase active site amino acid interacting ligand moieties and hydrogen bond length for each compound and ligand are listed in Table 3, Fig. 2-5.

Table 3: Docking Results

Cpd.	Energy score S (Kcal/mol)	Binding amino acid	Interacting function group	Hydrogen bond length Å
Clorobiocin	-44.8228	Asn 46 Asp 49 (through water molecule) Glu 50 (through water molecule) Asp 73 Asp 73 (through water molecule) Arg 76 (cation-arene) Gly 77 (through water molecule) Thr 80 (through water molecule) Arg136 Thr 165 (through water molecule)	OH pyrane OH pyrane O ether NH pyrrole CO ester Benzene of benzopyrone CO ester OH phenyl CO benzopyrone CO ester	1.88 2.80 3.34 1.81 3.16 3.16 2.57 2.96 3.16
2a	-15.4849	Asp 73 (through water molecule) Arg 76 Arg 76 (cation-arene) Gly 77 (through water molecule) Thr 165 (through water molecule)	CO benzopyrone CO butyloxy Benzene of benzopyrone CO benzopyrone CO benzopyrone	3.13 2.13 3.13 3.13
2d	-16.6778	Asp 49 (through water molecule) Arg 76 (cation-arene) Arg136	CO butyloxy Benzene of benzopyrone CO benzopyrone	2.96 3.05
3d	-14.0501	Asp 73 (through water molecule) Gly 77 (through water molecule) Thr 165 (through water molecule)	CO benzopyrone CO benzopyrone CO benzopyrone	2.83 2.83 2.83
5a	-18.3565	Asp 73 (through water molecule) Arg 76 (cation-arene) Arg 76 (cation-arene) Gly 77 (through water molecule) Thr 165 (through water molecule)	CO benzopyrone Benzene of benzopyrone Furan of fruobenzopyrone CO benzopyrone CO benzopyrone	3.13 3.13 3.13
10c	-23.3971	Asp 73 (through water molecule) Gly 77 (through water molecule) Thr 165 (through water molecule)	CO benzopyrone CO benzopyrone CO benzopyrone	2.96 2.96 2.96
10d	-18.2528	Asp 73 (through water molecule) Arg 76 (cation-arene) Gly 77 (through water molecule) Thr 165 (through water molecule)	CO benzopyrone Benzene of benzopyrone CO benzopyrone CO benzopyrone	2.60 2.60 2.60
10f	-20.9931	Asn 46 (through water molecule) Arg 76 (cation-arene) Val 120 (through water molecule)	CO benzopyrone Benzene of benzopyrone CO benzopyrone	2.28 2.28
11a	-21.2338	Asp 73 (through water molecule) Gly 77 (through water molecule) Thr 165 (through water molecule)	CO benzopyrone CO benzopyrone CO benzopyrone	2.06 2.06 2.06
11b	-18.6683	Asp 73 (through water molecule) Gly 77 (through water molecule) Thr 165 (through water molecule)	CO benzopyrone CO benzopyrone CO benzopyrone	2.33 2.33 2.33
11d	-24.3583	Asp 49 (through water molecule) Arg 76 (cation-arene) Arg 136	CO benzoyl Benzene of benzopyrone CO benzopyrone	2.91 2.82
11e	-15.6949	Asp 49 (through water molecule)	CO benzopyrone	3.19
11g	-10.9455	Asp 49 (through water molecule)	OCH_3	3.47

Analysis of the docking results revealed that:

i. Clorobiocin- DNA gyrase complex was reproduced by the docking procedure as demonstrated by low root mean square deviation, rmsd (0.4152) and dock score (-44.8228 kcal/mol, Table 3) that indicated the docking protocol was valid. Moreover, clorobiocin nearly fits into the active site forming various hydrogen bonding interactions with the active site residues: OH pyrane with Asn46 (1.88 Å) and Asp49 (2.80 Å) through water molecule, O ether with Glu50 (3.34 Å) through water molecule, NH pyrrole with Asp73 (1.81 Å), CO ester with Asp73, Gly77 and Thr165 (3.16 Å) through water molecule, OH phenyl with Thr80 (2.57 Å) through water molecule and CO benzopyrone with Arg136 (2.96 Å). Also clorobiocin formed arene cation interaction of benzene ring of benzopyrone with Arg76, Fig. 2.

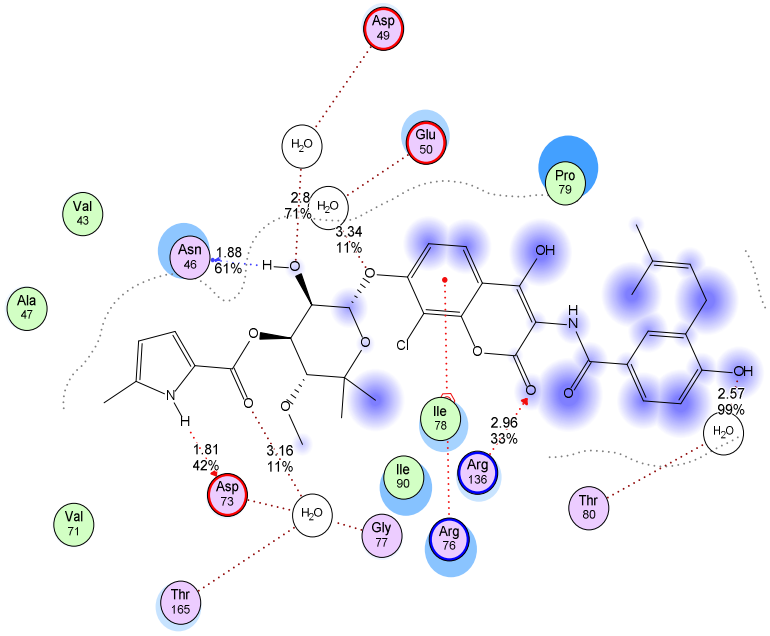


Fig. 2. 2D interactions of native ligand Clorobiocin in the active site of DNA gyrase

ii. Dock scores for significantly active antimicrobial compounds (**2a,d**, **3d**, **5a**, **10c,d,f**, **11a,b,d,e,g**) were found to have dock score in the range (-24.3583 to -10.9455 Kcal/mol, Table 3). A correlation between dock scores and antimicrobial activity (diameters of inhibition zones produced before UV-A radiation) of compounds was observed. Benzopyrone derivatives **2a,d**, and **10c,d,f** which had antimicrobial activity (inhibition zones were 7, 10, 10, 8 and 7 mm, respectively, Table 1), showed binding affinity with the active site of the DNA gyrase enzyme (dock score, -15.4849, -16.6778, -23.3971, -18.2528 -20.9931 Kcal/mol, respectively, Table 3).

Linear furobenzopyrone derivative **3d** and angular furobenzopyrone derivatives **11a,b,d,e,g** which had antimicrobial activity (inhibition zones were 10, 7, 8, 10, 7 and 7 mm, respectively, Table 1), showed binding affinity with the active site of the enzyme (dock score -14.0501, -21.2338, -18.6683, -24.3583, -15.6949 and -10.9455 Kcal/mol, respectively, Table 3).

Tetrahydrobenzofuroenzopyrone derivative **5a** which had antimicrobial activity (inhibition zones was 10 mm, Table 1), showed binding affinity with the active site of the DNA gyrase enzyme (dock score, -18.3565 Kcal/mol, Table 3).

iii. Inspection of the binding mode also demonstrated that all compounds showed one to four hydrogen bonds and arene cation interaction with the enzyme active site residue Asn46, Asp49, Asp73, Arg76, Gly77, Val120, Arg136 and Thr165.

iv. The benzopyrone **2d** with energy score (-16.6778 Kcal/mol) mediated two hydrogen bonds with the active site residues: Asp49 through water molecule (2.96 Å) with CO butyloxy and Arg136 (3.05 Å) with CO benzopyrone in addition to arene cation interaction of benzene ring of benzopyrone with Arg76, Fig. 3 (left). The benzopyrone **10c** with energy score (-23.3971 Kcal/mol) mediated three hydrogen bonds with the active site residues: Asp73, Gly77 and Thr165 (2.96 Å) through water molecule with CO benzopyrone, Fig. 3 (right).

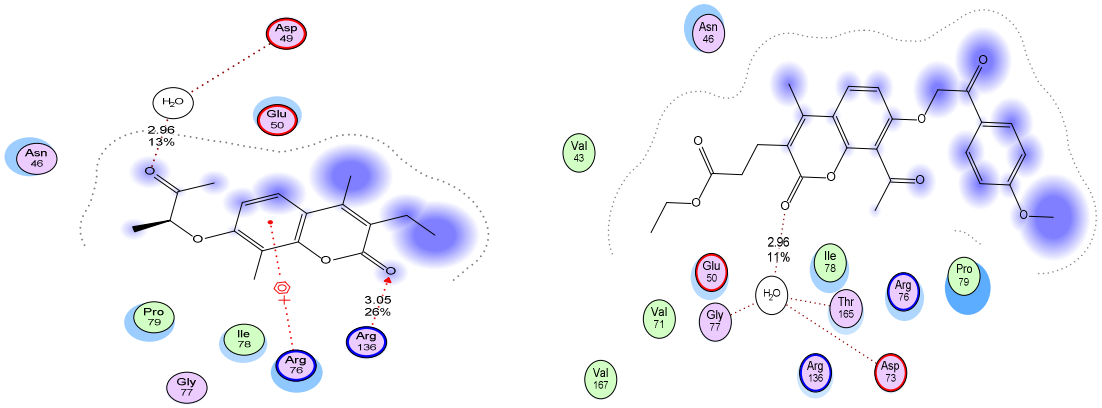


Fig. 3. 2D interactions of compound 2d (left) and 10c (right) in the active site of DNA gyrase

The furobenzopyrone **3d** (linear) with energy score (-14.0501 Kcal/mol) mediated three hydrogen bonds with the active site residues: Asp73, Gly77 and Thr165 (2.83 Å) through water molecule with CO benzopyrone, Fig. 4 (left). The furobenzopyrone **11d** (angular) with energy score (-24.3583 Kcal/mol) mediated two hydrogen bonds with the active site residues: Asp49 through water molecule (2.91 Å) with CO benzoyl and Arg136 (2.82 Å) with CO benzopyrone in addition to arene cation interaction of benzene ring of benzopyrone with Arg76, Fig. 4 (right).

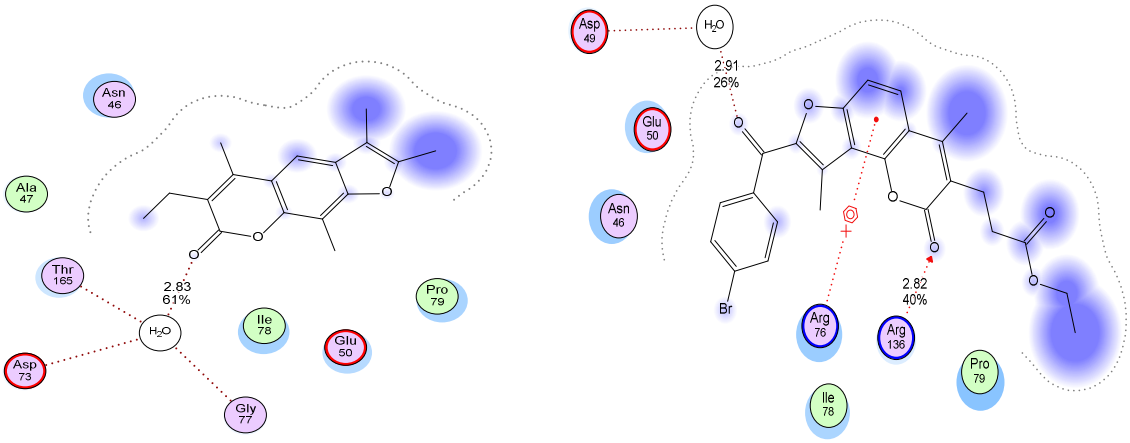


Fig. 4. 2D interactions of compound 3d (left) and 11d (right) in the active site of DNA gyrase

Tetrahydrobenzofurobenzopyrone **5a** with energy score (-18.3565 Kcal/mol) showed three hydrogen bonds with the active site residues: Asp73, Gly77 and Thr165 (3.13 Å) through water molecule with CO benzopyrone in addition to two arene cation interaction of benzene ring of benzopyrone and furan ring with Arg76, Fig. 5.

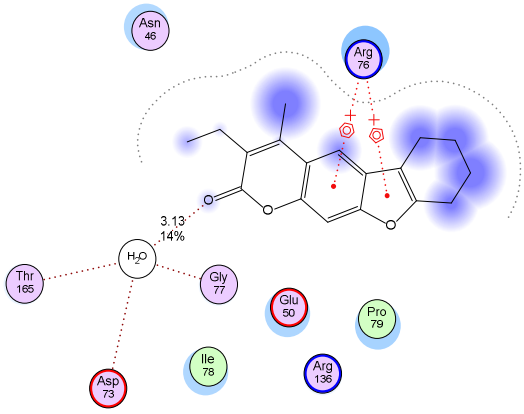


Fig. 5. 2D interactions of compound 5a in the active site of DNA gyrase

CONCLUSION

The newly synthesized compounds of benzopyrones **2a-d**, **4a,b**, **10a-h**, furobenzopyrones (linear) **3a-d**, (angular) **11a-h**, tetrahydrobenzofurobenzopyrones **5a,b** and benzofurobenzopyrones **6a,b** derivatives were synthesized and evaluated for antimicrobial and photochemotherapeutic activities. Compounds **2d**, **3d**, **5a**, **10c** and **11d** were found to have good antimicrobial activity. Compound **11e** exhibited good photosensitizing activity comparable to reference standard xanthotoxin after radiation for 20 and 40 min. Compounds **2b**, **3b** and **10b** exhibited good photosensitizing activity similar to xanthotoxin after radiation for 20 min. but not increased upon increasing time to 40 min. Compounds **3a**, **10e** and **11c** exhibited good photosensitizing activity (less than xanthotoxin) after radiation for 20 min. and increased upon increasing time to 40 min. Docking studies of antimicrobial active compounds were performed with DNA gyrase enzyme in order to gain insight into their possible binding mode. Although a correlation between dock score and observed antimicrobial activity (expressed as diameter of inhibition zone) was routinely observed, most of the docked compounds shared some binding interactions with DNA gyrase similar to those of the native ligand inhibitor clorobiocin. This suggests that these compounds might possibly act as DNA gyrase inhibitors, and this may contribute at least in part to their antimicrobial activity.

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REFERENCES

[1] F. Almutawa, N. Alnomair, Y. Wang, I. Hamzavi, H.W. Lim, *Am. J. Clin. Dermatol.*, **2013** 14(2), 87.
[2] Z. Zarebska, E. Waszkowska, S. Caffieri, F. Dall'Acqua, *Farmaco*, **2000**, 55(8), 515.
[3] J.D. Laskin, E. Lee, E.J. Yurkow, D.L. Laskin, M.A. Gallo, *Proc. Natl. Acad. Sci. USA*, **1985**, 82(18), 6158.
[4] N.A.H. Farag, *Eur. J. Med. Chem.*, **2009**, 44, 18.
[5] M. Grundmann-Kollmann, R. Ludwig, T.M. Zollner, F. Ochsendorf, D. Thaci, W.H. Boehncke, J. Krutmann, R. Kaufmann, M. Podda, *J. Am. Acad. Dermatol.*, **2004**, 50(5),734.
[6] J.H. Park, M.H. Lee, *Int. J. Dermatol.*, **2004**, 43(2), 138.
[7] H. Petering, C. Breuer, R. Herbst, A. Kapp, T. Werfel, *J. Am. Acad. Dermatol.*, **2004**, 50(1),68.
[8]C.S. Francisco, L.R. Rodrigues, N.M. Cerqueira, A.M. Oliveira-Campos, A.P. Esteves, *Eur. J. Med. Chem.*, **2014**, 87C, 298.
[9] S.-H. Shim, J.-C. Kim, K.S. Jang, G.J. Choi, *Plant Pathol. J.*, **2009**, 25(1), 103.
[10] S. Cannistraro, A. Van de Vorst, *Biochim. Biophys. Acta*, **1977**, 476(2), 166.
[11] F. Dall'Acqua, *Res. Photobiol.*, **1977**, 245.
[12] K. Straub , D. Kanne , J.E. Hearst, H. Rapoport, *J. Am. Chem. Soc.*, **1981**, 103 (9), 2347.
[13] F. Dall'Acqua, G. Jori, In: W.O. Foye, T.L. Lemke, D.A. Williams (Eds.), Principle of medicinal chemistry, (**1995**) 898.

[14] M.L. Panno, F. Giordano, *World J. Clin. Oncol.*, **2014**, 5(3), 348.
[15] F. Dall'Acqua, S. Marciani, L. Ciavatta, G. Rodighiero, *Z. Naturforsch. B.*, **1971**, 26(6), 561.
[16] J.J. Serrano-Pérez, L .Serrano-Andrés, M. Merchán, *J. Chem. Phys.*, **2006**, 124(12), 124502.
[17] D. Kanne, K. Straub, H. Rapoport, J.E. Hearst, *Biochemistry*, **1982**, 21(5), 861.
[18] X. Chen, J. Kagan, G. Miolo, F. Dall'Acqua, D. Averbeck, E. Bisagni, *J. Photochem. Photobiol. B.*, **1994**, 22(1), 51.
[19] D. Averbeck, *Mutat. Res.*, **1985**, 151(2), 217.
[20] J. Blais, P. Vigny, J. Moron, E. Bisagni, *Photochem. Photobiol.*, **1984**, 39(2), 145.
[21] J.P. Demaret, S. Brunie, J.P. Ballini, J. Cadet, P. Vigny, *J. Photochem. Photobiol. B.*, **1990**, 6(1-2), 207.
[22] R. Costalat, J. Blais, J.P. Ballini, A. Moysan, J. Cadet, O. Chalvet, P. Vigny, *Photochem. Photobiol.*, **1990**, 51(3), 255.
[23] O. Gia, S. Mobilio, M. Palumbo, M.A. Pathak, *Photochem. Photobiol.*, **1993**, 57(3), 497.
[24] F. Dall'Acqua, D. Vedaldi, S. Caffieri, A. Guiotto, P. Rodighiero, F. Baccichetti, F. Carlassare, F. Bordin, *J. Med. Chem.*, **1981**, 24(2), 178.
[25] D. Vedaldi, S. Caffieri, G. Miolo, A. Guiotto, F. Dall'Acqua, G. Bombieri, F. Benetollo, *J. Photochem. Photobiol. B.*, **1992**, 14(1-2), 81.
[26] F. Bordin, S. Marciani, F.R. Baccichetti, F. Dall'Acqua, G. Rodighiero, *Ital. J. Biochem.*, **1975**, 24(5), 258.
[27] F.W. Canter, F.H. Curd, A. Robertson, *J. Chem. Soc.*, **1931**, 1255.
[28] N.M. Shah, R.C. Shah, *Ber*., **1938**, 71(10), 2075.
[29] V.S. Parmar, S. Singh, P.M. Boll, *Magnetic Resonance Chem.*, **1988**, 26(5), 430.
[30] M.I. Shevchuk, A.V. Dombrovskii, *Zh. Obshch. Khim.*, **1963**, 33, 1135. [*Chem. Abstr.*, **1963**, 57, 9921].
[31] R. Cruickshank, I.P. Duguid, R.H.A. Swain, Medical microbiology. A guide to the laboratory diagnosis and control of infection, Livingstone Limited, London, **1968,** 11 Edition.
[32] MOE. Chemical Computing Group, Inc., Montréal. http://www.chemcomp.com.
[33] D. Lafitte, V. Lamour, P.O. Tsvetkov, A.A. Makarov, M. Klich, P. Deprez, D. Moras, C. Briand, R. Gilli, *Biochemistry*, **2002**, 41(23), 7217.
[34] R.C. Esse, B.E. Cristensen, *J. Org. Chem.*, **1960**, 25 (9), 1565.
[35] F.N. Lahey, J.K. Macleod, *Aust. J. Chem.*, **1967**, 20(9), 1943.
[36] J.K. MacLeod, B.R. Worth, *Tetrahedron Lett.*, **1972**, 13(3), 237.
[37] H. Abu Shady, K. Amin, S. El-Ansary, *Egypt J. Pharm. Sci.*, **1985**, 26, 195.
[38] D.B. Limaye, *Ber*., **1934**, 67, 12.
[39] D.N. Shah, N.M. Shah, *J. Org. Chem.*, **1954**, 19(10), 1681.
[40] Y.C., Tse-Dinh, *Infect. Disord. Drug Targets*, **2007**, 7(1), 3.
[41] C. Levine, H. Hiasa, K.J. Marians, *Biochim. Biophys. Acta*, **1998**, 1400(1-3), 29.
[42] B.J. Bradbury, M.J. Pucci, *Curr. Opin. Pharmacol.*, **2008**, 8(5), 574.
[43] A. Tanitame, Y. Oyamada, K. Ofuji, M. Fujimoto, N. Iwai, Y. Hiyama, K. Suzuki, H. Ito, H. Terauchi, M. Kawasaki, K. Nagai, M. Wachi, J. Yamagishi, *J. Med. Chem.*, **2004**, 47(14), 3693.