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New furobenzopyrones: Synthesis, antimicrobial and photochemotherapeutic evaluation, QSAR and molecular docking studies

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ABSTRACT

*The synthesis of some new linear furobenzopyrone **3a-h**, **7a,b** and angular furobenzopyrone derivatives **9a-s** and **12a,b** were described on the base of being monofunctional compounds to decrease possible toxicity. All the prepared compounds were evaluated for their antimicrobial and photosensitizing activities. Compounds **2e**, **4** and **7b** were found to have good antimicrobial activity while only compound **2e** exhibited higher photosensitizing activity than xanthotoxin. In addition, photosensitizing activity increased upon increasing time of radiation and concentration of substance. Quantitative structure–activity relationship (QSAR) study was applied to find a correlation between the photosensitizing activities of the newly synthesized furobenzopyrone derivatives and their physicochemical parameter. Furthermore, docking study was undertaken to gain insight into the possible binding mode of these compounds with the binding site of the DNA gyrase (topoisomerase II) enzyme which is responsible for resolving topological problems which arise during the various processes of DNA.*

Keywords Furobenzopyrones, Antimicrobial, Photochemotherapeutic, QSAR study, Docking study.

INTRODUCTION

Furobenzopyrones are an important class of photosensitizing drugs used in combination with radiation in the interval of UV-A (320-400 nm) (PUVA) for treatment of some skin diseases such as psoriasis, vitiligo, mycosis and eczema [1-4]. These compounds are derivatives of psoralen (linear furobenzopyrones), or angelicin (its angular isomers) [5]. Psoralen tricyclic moiety constitutes the basic chromophore from which drugs employed in this therapy were developed in particular, xanthotoxin (8-methoxypsoralen), 5-bergapten (5-methoxypsoralen) and to a lesser extent trioxsalen (4,5′,8-trimethylpsoralen, TMP) [1].

The biological activity of psoralens is primarily due to intercalation between two base pairs of DNA. The process is believed to involve three major steps: a) non-covalent interactive binding to DNA helix, b) formation of monoaddition product between DNA base and psoralen upon long wavelength ultraviolet irradiation, c) absorption of a second photon by some of the monoadducts to form diadducts, result in interstrand cross linkage [6]. Therefore, 2,3 (furan side) and 5,6 (pyrone) double bonds of the linear furobenzopyrones are the two photoreactive sites responsible for the DNA photobinding and for the biological activity [7]. Linear furobenzopyrones are reported to induce bifunctional photodamage to the DNA of the cutaneous cells in a selective way, thus inhibiting DNA functions and as a consequence, cell proliferation [8]. The photodamages consist of the products of photocycloaddition between one molecule of psoralen and two pyrimidine bases (biadduct) [7]. From the biological point of view, cross linkage provokes more pronounced biological consequences, but repair of interstrand cross linkage is less effective than repair of the monofunctional adduct [9]. Skin phototoxicity is strictly connected with

the bifunctional lesions in DNA which seems to be the main cause of skin cancer. On the other hand, monoadducts are reported to lack skin phototoxicity [10].

Nowadays, most of researches are devoted to develop new photochemotherapeutic compounds endowed with photo-antiproliferative activity and lower skin phototoxicity. DNA monofunctional furobenzopyrones such as carbmethoxypsoralen [11], carbethoxypsoralen [12], pyridopsoralen [12], benzo- and tetrahydrobenzopsoralen [13,14] and phenylpsoralen [15] analogues were designed and synthesized in order to prevent DNA interstrand cross linking formation, maintain the photosensitizing activity and consequently lack skin phototoxicity.

Intercalation complex between furobenzopyrones and nucleic acid revealed that, the C5 methyl of thymidine and the C5 substitution of the furobenzopyrone are in close proximity. Thus, presence of methyl group in this position could lead to steric crowding not present in the demethyl case. The results reported about TMP and psoralen added further support to this interpretation (TMP showed ≈ 98% furan addition, while psoralen lacking methyl at 5-position, showed nearly ≈ 20% pyrone addition) [16].

Enforced by these informations, we were encouraged to design and synthesize new linear furobenzopyrone derivatives (**3a-h** and **7a,b**) with variety of peripheral substituents that may produce monofunctional adduct with nucleic acid, therefore inhibiting the genotoxicity. Moreover, linear furobenzopyrones were reported to be more phototoxic than angular furobenzopyrones [17]. Therefore, new angular furobenzopyrone derivatives (**9a-s** and **12a,b**) were also synthesized in order to optimize the biological activity. All the newly synthesized compounds were evaluated for the antimicrobial and photosensitizing activities. In addition, quantitative structure–activity relationship (QSAR) study was also performed for understanding and validating the photosensitizing activities. Furthermore, attempt to elucidate a molecular target for the antimicrobial activity was achieved via molecular docking of the prepared compounds in the active site of DNA gyrase enzyme using Molecular Operating Environment (MOE).

MATERIALS AND METHODS

1.1. Chemistry

Melting points were determined by open capillary tube method using Electrothermal 9100 melting point apparatus MFB-595-010M (Gallen Kamp, London, England) and were uncorrected. Microanalyses were 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 (cm^{-1}). The ^{1}H-NMR spectra were recorded on Varian Mercury VX-300 NMR spectrometer at 300 MHz and *JEOL-ECA500 NMR spectrometer at 500 MHz in dimethylsulphoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$). 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. TLC were carried out using Art.DC-Plastikfolien, Kieselgel 60 F254 sheets (Merck, Darmstadt, Germany), the developing solvents was chloroform/methanol 9.5:0.5 and the spots were visualized at 366, 254 nm by UV Vilber Lourmat 77202 (Vilber, Marne La Vallee, France).

Starting compounds 3,8-disubstituted-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one **(1a-d)** [18] and phenacyl bromide derivatives [19] were prepared according to reported procedures.

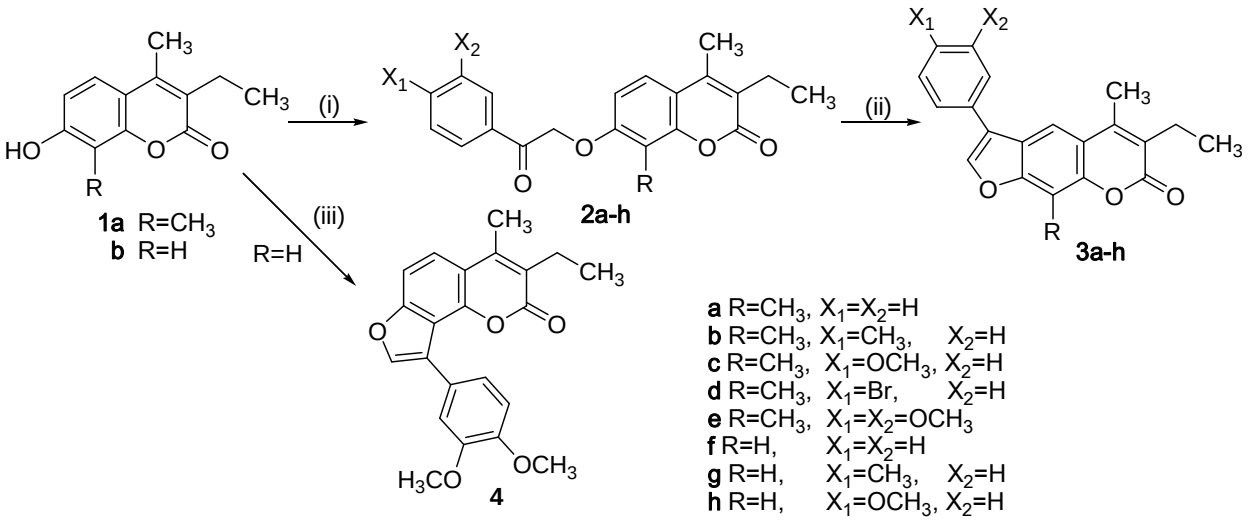
2.1.1. General Procedure for synthesis of 3-ethyl-4-methyl-8-substituted-7-((un)substituted phenacyloxy)-2*H*-1-benzopyran-2-ones (2a-h) (Scheme 1).

A solution of compound **1a,b** (0.01 mol) and appropriate phenacyl bromide derivative (0.015 mol) in acetone (50 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 isopropanol.

2.1.1.1. 4,8-Dimethyl-3-ethyl-7-phenacyloxy-2*H*-1-benzopyran-2-one (2a)

Yield 83%. mp 189-190 °C. IR (KBr) cm^{-1}: 3062 (CH Ar), 2964, 2870 (CH aliphatic), 1708, 1697 (2 C=O), 1600, 1577, 1500 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 1.04 (t, 3H, CH_2CH_3), 2.27 (s, 3H, CH_3 at C4), 2.37 (s, 3H, CH_3 at C8), 2.51 (q, 2H, CH_2CH_3), 5.77 (s, 2H, OCH_2), 6.98 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.49-7.71 (m, 4H, H-5 Ar, H-3′,4′,5′ Ar), 8.03 (d, 2H, *J*=7.5 Hz, H-2′,6′ Ar). MS (*m/z*) %: 336 (M^+) 5.48%. Anal. Calcd. for $C_{21}H_{20}O_4$ (336.38): C, 74.98; H, 5.99. Found: C, 75.04; H, 6.12.

2.1.1.2. 4,8-Dimethyl-3-ethyl-7-(4-methylphenacyloxy)-2*H*-1-benzopyran-2-one (2b)
Yield 92%. mp 181-183 °C. IR (KBr) cm^{-1}: 3020 (CH Ar), 2964, 2872 (CH aliphatic), 1700, 1695 (2 C=O), 1606, 1571, 1554 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.13 (t, 3H, CH_2CH_3), 2.31 (s, 3H, CH_3 at C4), 2.37 (s, 3H, CH_3 at C8), 2.45 (s, 3H, CH_3 at C4′), 2.67 (q, 2H, CH_2CH_3), 5.35 (s, 2H, OCH_2), 6.70 (d, 1H, *J*=8.7 Hz, H-6 Ar), 6.89 (d, 1H, *J*=9.3 Hz, H-5 Ar), 7.91 (d, 2H, *J*=7.8 Hz, H-3′,5′ Ar), 8.21 (d, 2H, *J*=8.4 Hz, H-2′,6′ Ar). MS (*m/z*) %: 350 (M^+) 13.20%. Anal. Calcd. for $C_{22}H_{22}O_4$ (350.41): C, 75.41; H, 6.33. Found: C, 75.47; H, 6.38.



Scheme 1. Reagents and conditions: (i) Appropriate phenacyl bromide derivative, K_2CO_3, dry acetone, reflux 24 h, (ii) Ethanolic KOH, reflux 18 h, (iii) 3,4-Dimethoxyphenacyl bromide, K_2CO_3, dry acetone, reflux 18 h.

2.1.1.3. 4,8-Dimethyl-3-ethyl-7-(4-methoxyphenacyloxy)-2*H*-1-benzopyran-2-one (2c)
Yield 72%. mp 191-193 °C. IR (KBr) cm^{-1}: 3055 (CH Ar), 2922, 2839 (CH aliphatic), 1710, 1695 (2 C=O), 1602, 1598, 1566, 1512 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 1.03 (t, 3H, CH_2CH_3), 2.26 (s, 3H, CH_3 at C4), 2.37 (s, 3H, CH_3 C8), 2.53 (q, 2H, CH_2CH_3), 3.86 (s, 3H, OCH_3), 5.69 (s, 2H, OCH_2), 6.94 (d, 1H, *J*=9.0 Hz, H-6 Ar), 7.09 (d, 2H, *J*=8.7 Hz, H-3′,5′ Ar), 7.55 (d, 1H, *J*=8.7 Hz, H-5 Ar), 8.01 (d, 2H, *J*=9 Hz, H-2′,6′ Ar). MS (*m/z*) %: 366 (M^+) 32.41%. Anal. Calcd. for $C_{22}H_{22}O_5$ (366.41): C, 72.12; H, 6.05. Found: C, 72.08; H, 6.13.

2.1.1.4. 7-(4-Bromophenacyloxy)-4,8-dimethyl-3-ethyl-2*H*-1-benzopyran-2-one (2d)
Yield 79%. mp 203-206 °C. IR (KBr) cm^{-1}: 3098 (CH Ar), 2977, 2860 (CH aliphatic), 1710, 1701 (2 C=O), 1604, 1585 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.14 (t, 3H, CH_2CH_3), 2.30 (s, 3H, CH_3 at C4), 2.36 (s, 3H, CH_3 at C8), 2.68 (q, 2H, CH_2CH_3), 5.31 (s, 2H, OCH_2), 6.85 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.38 (d, 1H, *J*=8.7 Hz, H-5 Ar), 7.88 (d, 2H, *J*=8.7 Hz, H-3′,5′ Ar), 8.20 (d, 2H, *J*=8.7 Hz, H-2′,6′ Ar). MS (*m/z*) %: 415 (M^+) 11.04%, 417 (M^++2) 10.28%. Anal. Calcd. for $C_{21}H_{19}BrO_4$ (415.28): C, 60.74; H, 4.61. Found: C, 60.81; H, 4.68.

2.1.1.5. 7-(3,4-Dimethoxyphenacyloxy)-4,8-dimethyl-3-ethyl-2*H*-1-benzopyran-2-one (2e)
Yield 68%. mp 215-217 °C. IR (KBr) cm^{-1}: 3020 (CH Ar), 2922, 2846 (CH aliphatic), 1705, 1700 (2 C=O), 1604, 1581, 1550 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 1.03 (t, 3H, CH_2CH_3), 2.27 (s, 3H, CH_3 at C4), 2.36 (s, 3H, CH_3 at C8), 2.56 (q, 2H, CH_2CH_3), 3.82 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 5.70 (s, 2H, OCH_2), 6.94 (d, 1H, *J*=9.0 Hz, H-6 Ar), 7.12 (d, 1H, *J*=8.7 Hz, H-5′ Ar), 7.48 (s, 1H, H-2′ Ar), 7.55 (d, 1H, *J*=8.7 Hz, H-5 Ar), 7.72 (d, 1H, *J*=9 Hz, H-6′ Ar). MS (*m/z*) %: 396 (M^+) 13.18%. Anal. Calcd. for $C_{23}H_{24}O_6$ (396.43): C, 69.68; H, 6.10. Found: C, 69.73; H, 6.16.

2.1.1.6. 3-Ethyl-4-methyl-7-phenacyloxy-2*H*-1-benzopyran-2-one (2f)
Yield 85%. mp 169-170 °C. IR (KBr) cm^{-1}: 3060 (CH Ar), 2964, 2850 (CH aliphatic), 1708, 1697 (2 C=O), 1600, 1577, 1544 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 1.03 (t, 3H, CH_2CH_3), 2.38 (s, 3H, CH_3), 2.55 (q, 2H, CH_2CH_3), 5.73 (s, 2H, OCH_2), 7.01 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.04 (s, 1H, H-8 Ar), 7.56-7.71 (m, 4H, H-5 Ar, H-3′,4′,5′ Ar), 8.04 (d, 2H, *J*=7.5 Hz, H-2′,6′ Ar). MS (*m/z*) %: 322 (M^+) 0.90%. Anal. Calcd. for $C_{20}H_{18}O_4$ (322.35): C, 74.52; H, 5.63. Found: C, 74.60; H, 5.67.

2.1.1.7. 3-Ethyl-4-methyl-7-(4-methylphenacyloxy)-2*H*-1-benzopyran-2-one (2g)
Yield 94%. mp 138-141 °C. IR (KBr) cm^{-1}: 3070 (CH Ar), 2970, 2875 (CH aliphatic), 1712, 1666 (2 C=O), 1606, 1571, 1554, 1502 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 1.03 (t, 3H, CH_2CH_3), 2.38 (s, 3H, CH_3 at C4), 2.40 (s, 3H, CH_3 at C4′), 2.55 (q, 2H, CH_2CH_3), 5.68 (s, 2H, OCH_2), 6.99 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.02 (s, 1H, H-8 Ar), 7.38 (d, 2H, *J*=8.1 Hz, H-3′,5′ Ar), 7.69 (d, 1H, *J*=8.7 Hz, H-5 Ar), 7.93 (d, 2H, *J*=8.4 Hz, H-2′,6′ Ar). MS (*m/z*) %: 336 (M^+) 10.68%. Anal. Calcd. for $C_{21}H_{20}O_4$ (336.38): C, 74.98; H, 5.99. Found: C, 75.06; H, 6.03.

2.1.1.8. 3-Ethyl-7-(4-methoxyphenacyloxy)-4-methyl-2*H*-1-benzopyran-2-one (2h)
Yield 60%. mp 136-137 °C. IR (KBr) cm^{-1}: 3060 (CH Ar), 2958, 2872 (CH aliphatic), 1708, 1695 (2 C=O), 1610, 1602, 1562, 1508 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 1.03 (t, 3H, CH_2CH_3), 2.37 (s, 3H, CH_3), 2.54 (q, 2H, CH_2CH_3), 3.86 (s, 3H, OCH_3), 5.65 (s, 2H, OCH_2), 6.98 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.00 (s, 1H, H-8 Ar), 7.09 (d, 2H, *J*=8.7 Hz, H-3′,5′ Ar), 7.68 (d, 1H, *J*=9.0 Hz, H-5 Ar), 8.01 (d, 2H, *J*=9.0 Hz, H-2′,6′ Ar). MS (*m/z*) %: 352 (M^+) 23.35%. Anal. Calcd. for $C_{21}H_{20}O_5$ (352.38): C, 71.58; H, 5.72. Found: C, 71.61; H, 5.80.

2.1.2. General Procedure for synthesis of 6-ethyl-5-methyl-9-substituted-3-((un)substituted phenyl)-7*H*-furo[3,2-g]benzopyran-7-ones (3a-h) (Scheme 1).
Compound **2a-h** (0.01 mol) was added to solution of 2% potassium hydroxide in absolute ethanol (50 ml) and the mixture was refluxed for 18 h. Solution was concentrated and acidified with a cold solution of 10% HCl. The precipitated product was filtered, washed and dried. Product was crystallized from isopropanol.

2.1.2.1. 5,9-Dimethyl-6-ethyl-3-phenyl-7*H*-furo[3,2-g]benzopyran-7-one (3a)
Yield 85%. mp 228-229 °C. IR (KBr) cm^{-1}: 3055 (CH Ar), 2974, 2870 (CH aliphatic), 1701 (C=O), 1593 (C=C). ^{1}H-NMR *($CDCl_3$) δ: 1.18 (t, 3H, CH_2CH_3), 2.50 (s, 3H, CH_3 at C5), 2.63 (s, 3H, CH_3 at C9), 2.70 (q, 2H, CH_2CH_3), 7.42-7.83 (m, 5H, Ar-H), 8.01 (s, 1H, H-4 Ar), 8.19 (s, 1H, H-2 Ar). MS (*m/z*) %: 318 (M^+) 100%. Anal. Calcd. for $C_{21}H_{18}O_3$ (318.37): C, 79.22; H, 5.70. Found: C, 79.31; H, 5.69.

2.1.2.2. 5,9-Dimethyl-6-ethyl-3-(4-methylphenyl)-7*H*-furo[3,2-g]benzopyran-7-one (3b)
Yield 80%. mp 294-296 °C. IR (KBr) cm^{-1}: 3099 (CH Ar), 2924, 2860 (CH aliphatic), 1705 (C=O), 1608, 1593, 1571 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.18 (t, 3H, CH_2CH_3), 2.45 (s, 3H, CH_3 at C5), 2.49 (s, 3H, CH_3 at C9), 2.64 (s, 3H, CH_3 at C4′), 2.74 (q, 2H, CH_2CH_3), 7.34 (d, 2H, *J*=7.8 Hz, H-3′,5′ Ar), 7.54 (d, 2H, *J*=7.5 Hz, H-2′,6′ Ar), 7.80 (s, 1H, H-4 Ar), 7.83 (s, 1H, H-2 Ar). MS (*m/z*) %: 332 (M^+) 1.08%. Anal. Calcd. for $C_{22}H_{20}O_3$ (332.39): C, 79.50; H, 6.06. Found: C, 79.58; H, 6.12.

2.1.2.3. 5,9-Dimethyl-6-ethyl-3-(4-methoxyphenyl)-7*H*-furo[3,2-g]benzopyran-7-one (3c)
Yield 60%. mp 188-190 °C. IR (KBr) cm^{-1}: 3082 (CH Ar), 2926, 2852 (CH aliphatic), 1714 (C=O), 1606, 1577 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.19 (t, 3H, CH_2CH_3), 2.50 (s, 3H, CH_3 at C5), 2.65 (s, 3H, CH_3 at C9), 2.75 (q, 2H, CH_2CH_3), 3.89 (s, 3H, OCH_3), 7.06 (d, 2H, *J*=8.1 Hz, H-3′,5′ Ar), 7.60 (d, 2H, *J*=8.1 Hz, H-2′,6′ Ar), 7.77 (s, 1H, H-4 Ar), 7.80 (s, 1H, H-2 Ar). MS (*m/z*) %: 348 (M^+) 100%. Anal. Calcd. for $C_{22}H_{20}O_4$ (348.39): C, 75.84; H, 5.79. Found: C, 75.90; H, 5.82.

2.1.2.4. 3-(4-Bromophenyl)-5,9-dimethyl-6-ethyl-7*H*-furo[3,2-g]benzopyran-7-one (3d)
Yield 75%. mp 287-289 °C. IR (KBr) cm^{-1}: 3060 (CH Ar), 2958, 2870 (CH aliphatic), 1701 (C=O), 1569, 1558 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.16 (t, 3H, CH_2CH_3), 2.38 (s, 3H, CH_3 at C5), 2.50 (s, 3H, CH_3 at C9), 2.73 (q, 2H, CH_2CH_3), 7.35 (s, 1H, H-4 Ar), 7.54 (d, 2H, *J*=8.4 Hz, H-2′,6′ Ar), 7.61 (d, 2H, *J*=9.0 Hz, H-3′,5′ Ar), 7.73 (s, 1H, H-2 Ar). MS (*m/z*) %: 397 (M^+) 0.90%, 399 (M^++2) 1.03%. Anal. Calcd. for $C_{21}H_{17}BrO_3$ (397.26): C, 63.49; H, 4.31. Found: C, 63.53; H, 4.37.

2.1.2.5. 3-(3,4-Dimethoxyphenyl)-5,9-dimethyl-6-ethyl-7*H*-furo[3,2-g]benzopyran-7-one (3e)
Yield 57%. mp 158-160 °C. IR (KBr) cm^{-1}: 3080 (CH Ar), 2947, 2841 (CH aliphatic), 1705 (C=O), 1593, 1512 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.04 (t, 3H, CH_2CH_3), 2.27 (s, 3H, CH_3 at C5), 2.37 (s, 3H, CH_3 at C9), 2.53 (q, 2H, CH_2CH_3), 3.83 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 7.06 (d, 1H, *J*=8.1 Hz, H-5′ Ar), 7.13 (s, 1H, H-2′ Ar), 7.30 (d, 1H, *J*=9.0 Hz, H-6′ Ar), 7.79 (s, 1H, H-4 Ar), 7.82 (s, 1H, H-2 Ar). MS (*m/z*) %: 378 (M^+) 6.12%. Anal. Calcd. for $C_{23}H_{22}O_5$ (378.42): C, 73.00; H, 5.86. Found: C, 73.09; H, 5.91.

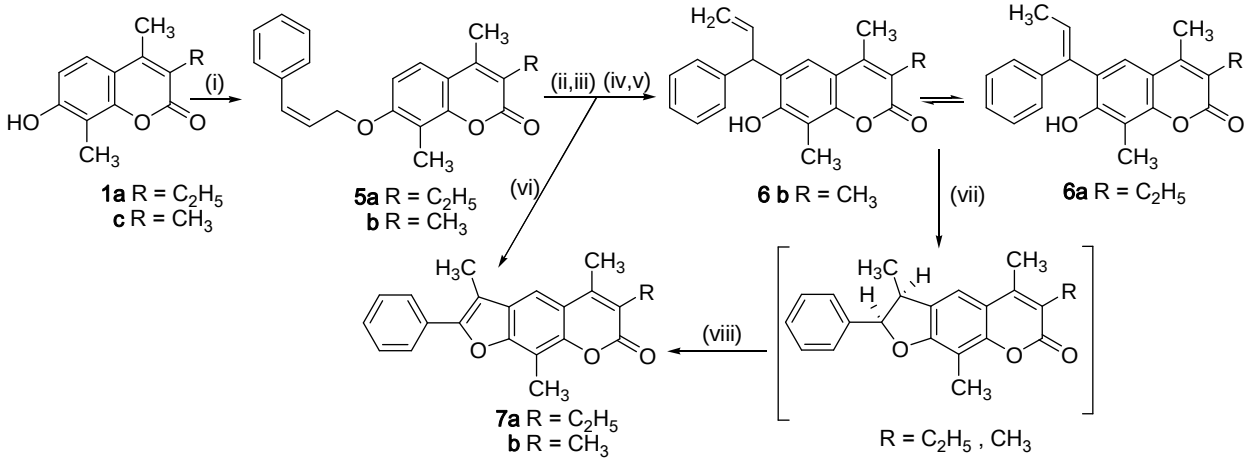
2.1.2.6. 6-Ethyl-5-methyl-3-phenyl-7*H*-furo[3,2-g]benzopyran-7-one (3f)
Yield 79%. mp 154-155 °C. IR (KBr) cm^{-1}: 3059 (CH Ar), 2970, 2873 (CH aliphatic), 1712 (C=O), 1577 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.19 (t, 3H, CH_2CH_3), 2.52 (s, 3H, CH_3), 2.75 (q, 2H, CH_2CH_3), 7.41-7.84 (m, 6H, H-9 Ar, Ar-H), 8.00 (s, 1H, H-4 Ar), 8.03 (s, 1H, H-2 Ar). MS (*m/z*) %: 304 (M^+) 14.64%. Anal. Calcd. for $C_{20}H_{16}O_3$ (304.34): C, 78.93; H, 5.30. Found: C, 78.79; H, 5.28.

2.1.2.7. 6-Ethyl-5-methyl-3-(4-methylphenyl)-7*H*-furo[3,2-g]benzopyran-7-one (3g)
Yield 74%. mp 171-173 °C. IR (KBr) cm^{-1}: 3028 (CH Ar), 2966, 2870 (CH aliphatic), 1701 (C=O), 1604, 1581, 1512 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.16 (t, 3H, CH_2CH_3), 2.48 (s, 3H, CH_3 at C5), 2.54 (q, 2H, CH_2CH_3), 2.70 (s, 3H, CH_3 at C4′), 7.29 (d, 2H, *J*=7.8 Hz, H-3′,5′ Ar), 7.52 (d, 2H, *J*=8.4 Hz, H-2′,6′ Ar), 7.67 (s, 1H, H-9 Ar), 7.80 (s, 1H, H-4 Ar), 7.83 (s, 1H, H-2 Ar). MS (*m/z*) %: 318 (M^+) 16.69%. Anal. Calcd. for $C_{21}H_{18}O_3$ (318.37): C, 79.22; H, 5.70. Found: C, 79.26; H, 5.79.

2.1.2.8. 6-Ethyl-3-(4-methoxyphenyl)-5-methyl-7*H*-furo[3,2-g]benzopyran-7-one (3h)
Yield 56%. mp 155-158 °C. IR (KBr) cm^{-1}: 3074 (CH Ar), 2966, 2873 (CH aliphatic), 1710 (C=O), 1597, 1573, 1508 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.03 (t, 3H, CH_2CH_3), 2.39 (s, 3H, CH_3), 2.50 (q, 2H, CH_2CH_3), 3.85 (s, 3H, OCH_3), 7.07 (d, 2H, *J*=8.4 Hz, H-3′,5′ Ar), 7.59 (d, 2H, *J*=8.4 Hz, H-2′,6′ Ar), 7.63 (s, 1H, H-9 Ar), 7.78 (s, 1H, H-4 Ar), 7.81 (s, 1H, H-2 Ar). MS (*m/z*) %: 334 (M^+) 0.59%. Anal. Calcd. for $C_{21}H_{18}O_4$ (334.37): C, 75.43; H, 5.43. Found: C, 75.90; H, 5.44.

2.1.3. Synthesis of 3-(3,4-dimethoxyphenyl)-6-ethyl-7-methyl-5*H*-furo[2,3-h]benzopyran-5-one (4) (Scheme 1).
Previous procedure adopted for synthesis of compounds **2a-h** was applied on reacting 3-ethyl-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one **1b** and 3,4-dimethoxyphenacyl bromide except that reaction was proceeded for 18 h instead of 24 h. Product was crystallized from isopropanol.

Yield 53%. mp 187-189 °C. IR (KBr) cm^{-1}: 3082 (CH Ar), 2966, 2873 (CH aliphatic), 1716 (C=O), 1610, 1595, 1517, 1508 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 1.02 (t, 3H, CH_2CH_3), 2.37 (s, 3H, CH_3), 2.55 (q, 2H, CH_2CH_3), 3.79 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 7.09-7.17 (m, 3H, H-2′,5′,6′ Ar), 7.55 (s, 1H, H-2 Ar), 7.75 (d, 1H, *J*=9.0 Hz, H-9 Ar), 7.83 (d, 1H, *J*=8.4 Hz, H-8 Ar). MS (*m/z*) %: 364 (M^+) 0.14%. Anal. Calcd. for $C_{22}H_{20}O_5$ (364.39): C, 72.51; H, 5.53. Found: C, 72.58; H, 5.54.



Scheme 2. Reagents and conditions: (i) Cinnamyl chloride, K_2CO_3, dry acetone, reflux 24 h, (ii) Claisen rearrangement: *N,N*-diethylaniline, 185 ºC, 3 h, (iii) 10% HCl, (iv) 5% NaOH extract, (v) 10% HCl, (vi) Ether extract, (vii) 48% HBr, glacial acetic acid, reflux 8 h, (viii) DDQ, dry benzene, refluxr 8 h.

2.1.4. General Procedure for synthesis of 7-cinnamyloxy-4,8-dimethyl-3-substituted-2*H*-1-benzopyran-2-ones (5a,b) (Scheme 2).
A mixture of compound **1a,c** (0.01 mol) and cinnamyl chloride (1.52 g, 0.01 mol) was refluxed in acetone (50 ml) in presence of anhydrous potassium carbonate (2.76 g, 0.02 mol) for 24 h. Acetone was distilled off and the residue washed with water and dried. Product was crystallized from isopropanol to give **5a,b**.

2.1.4.1. 7-Cinnamyloxy-4,8-dimethyl-3-ethyl-2*H*-1-benzopyran-2-one (5a)
Yield 67%. mp 131-133 °C. IR (KBr) cm^{-1}: 3024 (CH Ar), 2962, 2860 (CH aliphatic), 1705 (C=O), 1604, 1577, 1550 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.15 (t, 3H, CH_2CH_3), 1.59 (s, 3H, CH_3 at C4), 2.39 (s, 3H, CH_3 at C8), 2.68 (q, 2H, CH_2CH_3), 4.80 (d, 2H, *J*=6.0 Hz, OCH_2CH=CH), 6.40-6.47 (m, 1H, OCH_2-CH=CH), 6.76 (d, 1H, *J*= 16.2 Hz, OCH_2-CH=CH), 6.88 (d, 1H, *J*=9.3 Hz, H-6 Ar), 7.30-7.44 (m, 6H, H-5 Ar, Ar-H). MS (*m/z*) %: 334 (M^+) 0.53%. Anal. Calcd. for $C_{22}H_{22}O_3$ (334.41): C, 79.02; H, 6.63. Found: C, 79.13; H, 6.68.

2.1.4.2. 7-Cinnamyloxy-3,4,8-trimethyl-2*H*-1-benzopyran-2-one (5b)
Yield 72%. mp 139-140 °C. IR (KBr) cm^{-1}: 3024 (CH Ar), 2999, 2868 (CH aliphatic), 1697 (C=O), 1608, 1593, 1573 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.57 (s, 3H, CH_3 at C4), 2.20 (s, 3H, CH_3 at C3), 2.37 (s, 3H, CH_3 at C8), 4.80 (d,

2H, *J*=5.7 Hz, $OCH_2CH=CH$), 6.40-6.46 (m, 1H, $OCH_2CH=CH$), 6.73 (d, 1H, *J*=16.6 Hz, $OCH_2CH=CH$), 6.88 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.32-7.44 (m, 6H, H-5 Ar, Ar-H). MS (*m/z*) %: 320 (M^+) 1.01%. Anal. Calcd. for $C_{21}H_{20}O_3$ (320.38): C, 78.73; H, 6.29. Found: C, 78.90; H, 6.36.

2.1.5. General Procedure for synthesis of 4,8-dimethyl-7-hydroxy-6-(1-phenyl-(1 or 2)-propenyl)-3-substituted -2*H*-1-benzopyran-2-ones (6a,b) (Scheme 2).

The cinnamyloxy derivative **5a,b** (0.003 mol) in *N*,*N*-diethylaniline (5 ml) was refluxed at 185 ^oC for 3 h. The reaction mixture was poured onto cold 10% HCl and the separated solid was filtered off. Solid product was dissolved in ether and extracted with 5% NaOH then ether layer was separated from alkali aqueous layer. The alkali soluble fraction was acidified with 10% HCl and the separated product was filtered, purified by column chromatography using silica gel as stationary phase and chloroform as mobile phase. Subsequent crystallization was carried out from benzene:petroleum ether (1:1) to give **6a,b**.

2.1.5.1. 4,8-Dimethyl-3-ethyl-7-hydroxy-6-(1-phenyl-1-propenyl)-2*H*-1-benzopyran-2-one (6a)

Yield 35%. mp 201-203 °C. IR (KBr) cm^{-1}: 3329 (OH), 3064 (CH Ar), 2970, 2872 (CH aliphatic), 1700 (C=O), 1591, 1577, 1554 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 1.03 (t, 3H, CH_2CH_3), 1.59 (d, 3H, *J*=6.9 Hz, CH-CH_3), 2.23 (s, 3H, CH_3 at C4), 2.31 (s, 3H, CH_3 at C8), 2.53 (q, 2H, CH_2CH_3), 6.40 (q, 1H, $CHCH_3$), 7.17-7.29 (m, 6H, H-5 Ar, Ar-H), 9.04 (s, 1H, OH exch. D_2O). MS (*m/z*) %: 334 (M^+) 100%. Anal. Calcd. for $C_{22}H_{22}O_3$ (334.41): C, 79.02; H, 6.63. Found: C, 79.10; H, 6.67.

2.1.5.2. 7-Hydroxy-6-(1-phenyl-2-propenyl)-3,4,8-trimethyl-2*H*-1-benzopyran-2-one (6b)

Yield 36%. mp 209-210 °C. IR (KBr) cm^{-1}: 3157 (OH), 3076 (CH Ar), 2926, 2852 (CH aliphatic), 1685 (C=O), 1604, 1583, 1571, 1504 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 1.98 (s, 3H, CH_3 at C4), 2.18 (s, 3H, CH_3 at C3), 2.30 (s, 3H, CH_3 at C8), 4.89 (d, 1H, *J*=15.6 Hz, $CHCH=CH_2$), 5.12-5.18 (m, 2H, $CHCH=CH_2$), 6.39-6.50 (m, 1H, $CHCH=CH_2$), 7.14-7.35 (m, 6H, H-5 Ar, Ar-H), 9.34 (s, 1H, OH exch. D_2O). MS (*m/z*) %: 320 (M^+) 100%. Anal. Calcd. for $C_{21}H_{20}O_3$ (320.38): C, 78.73; H, 6.29. Found: C, 78.76; H, 6.41.

2.1.6. General Procedure for synthesis of 2-phenyl-6-substituted-3,5,9-trimethyl-7*H*-furo[3,2-g]benzopyran-7-ones (7a,b) (Scheme 2)

Following **procedure 1** or **2**:

Procedure 1: The ether layer from the previous extraction was evaporated and the residue was purified by column chromatography using silica gel as stationary phase and chloroform as mobile phase to give **7a,b**.

Procedure 2: The rearranged product **6a,b** (0.003 mol) was refluxed in mixture of glacial acetic acid (12 ml) and HBr (48%, 8 ml) for 8 h. The reaction mixture was poured onto crushed ice and the separated product was dried and dehydrogenated directly through refluxing with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (0.5 g) in dry benzene (15 ml) on boiling water bath for 8 h. The mixture was filtered while hot and benzene was concentrated. On standing, crystals of the same furobenzopyrones **7a,b** separated out and were purified by column chromatography using silica gel as stationary phase and chloroform as mobile phase.

2.1.6.1. 6-Ethyl-2-phenyl-3,5,9-trimethyl-7*H*-furo[3,2-g]benzopyran-7-one (7a)

Yield 40%. mp 180-183 °C. IR (KBr) cm^{-1}: 3060 (CH Ar), 2956, 2854 (CH aliphatic), 1701 (C=O), 1593, 1577, 1560 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.18 (t, 3H, CH_2CH_3), 2.44 (s, 3H, CH_3 at C5), 2.57 (s, 3H, CH_3 at C3), 2.63 (s, 3H, CH_3 at C9), 2.73 (q, 2H, CH_2CH_3), 7.40-7.54 (m, 5H, Ar-H), 7.57 (s, 1H, H-4 Ar). MS (*m/z*) %: 332 (M^+) 96.34%. Anal. Calcd. for $C_{22}H_{20}O_3$ (332.39): C, 79.50; H, 6.06. Found: C, 79.64; H, 6.15.

2.1.6.2. 2-Phenyl-3,5,6,9-tetramethyl-7*H*-furo[3,2-g]benzopyran-7-one (7b)

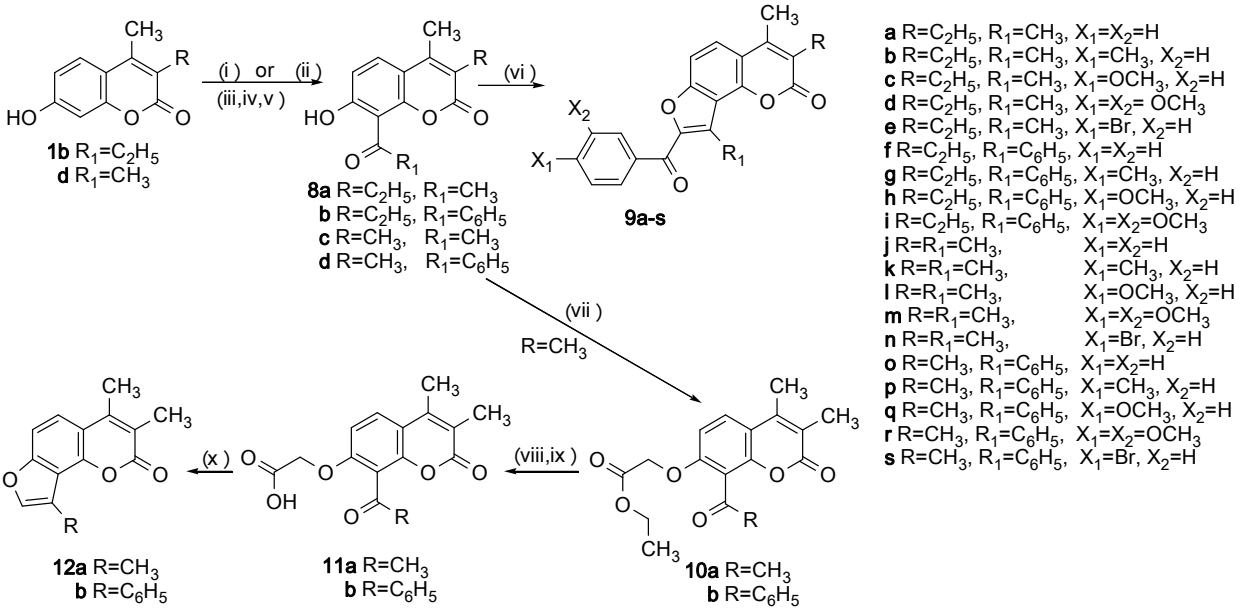
Yield 43%. mp 234-236 °C. IR (KBr) cm^{-1}: 3084 (CH Ar), 2920, 2855 (CH aliphatic), 1701 (C=O), 1593, 1573 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 2.24 (s, 3H, CH_3 at C5), 2.42 (s, 3H, CH_3 at C6), 2.57 (s, 3H, CH_3 at C3), 2.63 (s, 3H, CH_3 at C9), 7.40-7.54 (m, 5H, Ar-H), 7.57 (s, 1H, H-4 Ar). MS (*m/z*) %: 318 (M^+) 100%. Anal. Calcd. for $C_{21}H_{18}O_3$ (318.37): C, 79.22; H, 5.70. Found: C, 79.27; H, 5.73.

8-Acetyl-7-hydroxy-4-methyl-3-substituted-2*H*-1-benzopyran-2-one **(8a,c) (Scheme 3)** were repared as reported in literature [20].

2.1.7. General Procedure for synthesis of 8-benzoyl-7-hydroxy-4-methyl-3-substituted-2*H*-1-benzopyran-2-one (8b,d) (Scheme 3).

A mixture of compound **1b,d** (0.05 mol) and benzoyl chloride (10.52 g, 8.7 ml, 0.075 mol) in pyridine (5 ml) was refluxed in an oil bath at 165 ^oC for 2 h, then poured onto crushed ice with stirring. The separated product was filtered, washed with sodium bicarbonate solution, then with water and dried. The separated solid was mixed with

anhydrous aluminium chloride (20 g, 0.15 mol) and heated in an oil bath at 165 °C for 2 h. The reaction mixture was cooled and treated with 10% HCl then filtered. The product was dissolved in 5% NaOH, filtered and reprecipitated by acidification with 10% HCl. The separated solid was collected, washed with water and crystallized from isopropanol giving **8b,d**.



Scheme 3. Reagents and conditions: (i) Acetic anhydride, reflux 1h, (ii) Benzoyl chloride, pyridine, 165 °C, 2 h, (iii) Fries rearrangement: $AlCl_3$, 165 °C, 2h, (iv) 5% NaOH, (v) 10% HCl, (vi) Appropriate phenacyl bromide derivative, K_2CO_3, dry acetone, reflux 24 h, (vii) Ethyl chloroacetate, K_2CO_3, dry acetone, reflux 24 h, (viii) 5% KOH, methanol/water mixture (1:1) , reflux 30 min., (ix) 10% HCl, (x) Sodium acetate, acetic anhydride, reflux 1 h.

2.1.7.1. 8-Benzoyl-3-ethyl-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one (8b)

Yield 51%. mp 197-200 °C. IR (KBr) cm^{-1}: 3246 (OH), 3047 (CH Ar), 2970, 2868 (CH aliphatic), 1685, 1668 (2 C=O), 1595, 1577 (C=C). ^{1}H-NMR *(DMSO-d_6) δ: 0.97 (t, 3H, CH_2CH_3), 2.35 (s, 3H, CH_3), 2.46 (q, 2H, CH_2CH_3), 6.91 (d, 1H, *J*=9.2 Hz, H-6 Ar), 7.49 (t, 2H, H-3′,5′ Ar), 7.63 (t, 1H, H-4′ Ar), 7.70 (d, 1H, *J*=9.2 Hz, H-5 Ar), 7.72 (d, 2H, *J*=9.2 Hz, H-2′,6′ Ar), 10.80 (s, 1H, OH exch. D_2O). MS (*m/z*) %: 308 (M^+) 72.07%. Anal. Calcd. for $C_{19}H_{16}O_4$ (308.33): C, 74.01; H, 5.23. Found: C, 74.08; H, 5.29.

2.1.7.2. 8-Benzoyl-3,4-dimethyl-7-hydroxy-2*H*-1-benzopyran-2-one (8d)

Yield 56%. mp 230-234 °C. IR (KBr) cm^{-1}: 3421 (OH), 3062 (CH Ar), 2970, 2877 (CH aliphatic), 1708, 1693 (2 C=O), 1604, 1529, 1512 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 2.02 (s, 3H, CH_3 at C4), 2.38 (s, 3H, CH_3 at C3), 6.95 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.55 (t, 2H, H-3′,5′ Ar), 7.66 (t, 1H, H-4′ Ar), 7.76 (d, 3H, *J*=8.4 Hz, H-5 Ar, H-2′,6′ Ar), 10.75 (s, 1H, OH exch. D_2O). MS (*m/z*) %: 294 (M^+) 54.82%. Anal. Calcd. for $C_{18}H_{14}O_4$ (294.30): C, 73.46; H, 4.79. Found: C, 73.52; H, 4.82.

2.1.8. General Procedure for synthesis of 3,6-disubstituted-2-((un)substituted benzoyl)-7-methyl-5*H*-furo [2,3-h]benzopyran-5-ones (9a-s) (Scheme 3).

A mixture of compound **8a-d** (0.01 mol) and appropriate phenacyl bromide derivative (0.015 mol) in acetone (50 ml) was refluxed in presence of anhydrous potassium carbonate (2.76 g, 0.02 mol) with stirring 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 to give **9a-s**. The solid product was crystallized from isopropanol.

2.1.8.1. 2-Benzoyl-3,7-dimethyl-6-ethyl-5*H*-furo[2,3-h]benzopyran-5-one (9a)

Yield 74%. mp 221-223 °C. IR (KBr) cm^{-1}: 3070 (CH Ar), 2968, 2875 (CH aliphatic), 1703 (2 C=O), 1598, 1575, 1548 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 1.09 (t, 3H, CH_2CH_3), 2.50 (s, 3H, CH_3 at C7), 2.63 (q, 2H, CH_2CH_3), 2.80 (s, 3H, CH_3 at C3), 7.60-8.00 (m, 7H, H-8,9 Ar, Ar-H). MS (*m/z*) %: 346 (M^+) 6.68%. Anal. Calcd. for $C_{22}H_{18}O_4$ (346.38): C, 76.29; H, 5.24. Found: C, 76.61; H, 5.40.

2.1.8.2. 3,7-Dimethyl-6-ethyl-2-(4-methylbenzoyl)-5*H*-furo[2,3-h]benzopyran-5-one (9b)

Yield 65%. mp 235-236 °C. IR (KBr) cm^{-1}: 3079 (CH Ar), 2955, 2845 (CH aliphatic), 1706 (2 C=O), 1601, 1510 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 1.08 (t, 3H, CH_2CH_3), 2.42 (s, 3H, CH_3 at C7), 2.50 (s, 3H, CH_3 at C3), 2.62 (q, 2H,

CH_2CH_3), 2.77 (s, 3H, CH_3 at C4′), 7.39 (d, 1H, *J*=7.8 Hz, H-9 Ar), 7.63 (d, 1H, *J*=9.0 Hz, H-8 Ar), 7.88-7.92 (m, 4H, H-2′,3′,5′,6′ Ar). MS (*m/z*) %: 360 (M^+) 23.75%. Anal. Calcd. for $C_{23}H_{20}O_4$ (360.40): C, 76.65; H, 5.59. Found: C, 76.93; H, 5.74.

2.1.8.3. 3,7-Dimethyl-6-ethyl-2-(4-methoxybenzoyl)-5*H*-furo[2,3-h]benzopyran-5-one (9c)
Yield 68%. mp 230-232 °C. IR (KBr) cm^{-1}: 3078 (CH Ar), 2966, 2839 (CH aliphatic), 1708 (2 C=O), 1597, 1570, 1546, 1508 (C=C). ^{1}H-NMR ($CDCl_3$) *δ*: 1.19 (t, 3H, CH_2CH_3), 2.50 (s, 3H, CH_3 at C7), 2.75 (q, 2H, CH_2CH_3), 2.95 (s, 3H, CH_3 at C3), 3.92 (s, 3H, OCH_3), 7.02 (d, 2H, *J*=8.7 Hz, H-3′,5′ Ar), 7.43 (d, 1H, *J*=9.0 Hz, H-9 Ar), 7.68 (d, 1H, *J*=9.0 Hz, H-8 Ar), 8.11 (d, 2H, *J*=8.1 Hz, H-2′,6′ Ar). MS (*m/z*) %: 376 (M^+) 63.53%. Anal. Calcd. for $C_{23}H_{20}O_5$ (376.40): C, 73.39; H, 5.36. Found: C, 73.58; H, 5.52.

2.1.8.4. 2-(3,4-Dimethoxybenzoyl)-3,7-dimethyl-6-ethyl-5*H*-furo[2,3-h]benzopyran-5-one (9d)
Yield 61%. mp 252-255 °C. IR (KBr) cm^{-1}: 3086 (CH Ar), 2966, 2850 (CH aliphatic), 1701 (2 C=O), 1593, 1550, 1516 (C=C). ^{1}H-NMR ($CDCl_3$) *δ*: 1.19 (t, 3H, CH_2CH_3), 2.50 (s, 3H, CH_3 at C7), 2.72 (q, 2H, CH_2CH_3), 2.92 (s, 3H, CH_3 at C3), 3.98 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 6.98 (d, 1H, *J*=8.7 Hz, H-5′ Ar), 7.44 (d, 1H, *J*=8.7 Hz, H-9 Ar), 7.63 (s, 1H, H-2′ Ar), 7.69 (d, 1H, *J*=9.0 Hz, H-8 Ar), 7.84 (d, 1H, *J*=8.7 Hz, H-6′ Ar). MS (*m/z*) %: 406 (M^+) 34.78%. Anal. Calcd. for $C_{24}H_{22}O_6$ (406.43): C, 70.92; H, 5.46. Found: C, 70.97; H, 5.48.

2.1.8.5. 2-(4-Bromobenzoyl)-3,7-dimethyl-6-ethyl-5*H*-furo[2,3-h]benzopyran-5-one (9e)
Yield 65%. mp 234-238 °C. IR (KBr) cm^{-1}: 3068 (CH Ar), 2964, 2870 (CH aliphatic), 1707 (2 C=O), 1602, 1583, 1562, 1546 (C=C). ^{1}H-NMR (DMSO-d_6) *δ*: 1.06 (t, 3H, CH_2CH_3), 2.50 (s, 3H, CH_3 at C7), 2.64 (q, 2H, CH_2CH_3), 2.81 (s, 3H, CH_3 at C3), 7.63 (d, 1H, *J*=9.3 Hz, H-9 Ar), 7.80 (d, 1H, *J*=8.4 Hz, H-8 Ar), 7.92 (d, 2H, *J*=3.9 Hz, H-3′,5′ Ar), 7.95 (d, 2H, *J*=3.3 Hz, H-2′,6′ Ar). MS (*m/z*) %: 425 (M^+) 6.90%. Anal. Calcd. for $C_{22}H_{17}BrO_4$ (425.27): C, 62.13; H, 4.03. Found: C, 62.08; H, 4.17.

2.1.8.6. 2-Benzoyl-6-ethyl-7-methyl-3-phenyl-5*H*-furo[2,3-h]benzopyran-5-one (9f)
Yield 80%. mp 233-235 °C. IR (KBr) cm^{-1}: 3057 (CH Ar), 2966, 2873 (CH aliphatic), 1714 (2 C=O), 1600, 1577, 1544 (C=C). ^{1}H-NMR ($CDCl_3$) *δ*: 1.13 (t, 3H, CH_2CH_3), 2.50 (s, 3H, CH_3), 2.67 (q, 2H, CH_2CH_3), 7.29-7.79 (m, 12H, Ar-H). MS (*m/z*) %: 408 (M^+) 30.20%. Anal. Calcd. for $C_{27}H_{20}O_4$ (408.45): C, 79.40; H, 4.94. Found: C, 79.68; H, 5.21.

2.1.8.7. 6-Ethyl-7-methyl-2-(4-methylbenzoyl)-3-phenyl-5*H*-furo[2,3-h]benzopyran-5-one (9g)
Yield 67%. mp 213-216 °C. IR (KBr) cm^{-1}: 3034 (CH Ar), 2968, 2873 (CH aliphatic), 1714 (2 C=O), 1602, 1544 (C=C). ^{1}H-NMR (DMSO-d_6) *δ*: 1.03 (t, 3H, CH_2CH_3), 2.33 (s, 3H, CH_3 at C7), 2.49 (s, 3H, CH_3 at C4′), 2.54 (q, 2H, CH_2CH_3), 7.22 (d, 1H, *J*=7.8 Hz, H-9 Ar), 7.33-7.53 (m, 5H, H-3′,5′ Ar, H-3″,4″,5″ Ar), 7.69 (d, 1H, *J*=8.4 Hz, H-8 Ar), 7.77 (d, 2H, *J*=8.7 Hz, H-2″,6″ Ar), 7.99 (d, 2H, *J*=8.7 Hz, H-2′,6′ Ar). MS (*m/z*) %: 422 (M^+) 82.96%. Anal. Calcd. for $C_{28}H_{22}O_4$ (422.47): C, 79.60; H, 5.25. Found: C, 79.54; H, 5.29.

2.1.8.8. 6-Ethyl-2-(4-methoxybenzoyl)-7-methyl-3-phenyl-5*H*-furo[2,3-h]benzopyran-5-one (9h)
Yield 69%. mp 218-220 °C. IR (KBr) cm^{-1}: 3090 (CH Ar), 2962, 2839 (CH aliphatic), 1710 (2 C=O), 1600, 1575, 1544, 1508 (C=C). ^{1}H-NMR ($CDCl_3$) *δ*: 1.13 (t, 3H, CH_2CH_3), 2.49 (s, 3H, CH_3), 2.67 (q, 2H, CH_2CH_3), 3.83 (s, 3H, OCH_3), 6.79 (d, 3H, *J*=9.3 Hz, H-9 Ar, H-3′,5′ Ar), 7.35 (t, 1H, H-4″ Ar), 7.50 (t, 2H, H-3″,5″ Ar), 7.55 (d, 1H, *J*=8.7 Hz, H-8 Ar), 7.74 (d, 2H, *J*=9.3 Hz, H-2″,6″ Ar), 7.83 (d, 2H, *J*=9.0 Hz, H-2′,6′ Ar). MS (*m/z*) %: 438 (M^+) 99.13%. Anal. Calcd. for $C_{28}H_{22}O_5$ (438.47): C, 76.70; H, 5.06. Found: C, 76.79; H, 5.11.

2.1.8.9. 2-(3,4-Dimethoxybenzoyl)-6-ethyl-7-methyl-3-phenyl-5*H*-furo[2,3-h]benzopyran-5-one (9i)
Yield 59%. mp 215-218 °C. IR (KBr) cm^{-1}: 3090 (CH Ar), 2962, 2843 (CH aliphatic), 1720 (2 C=O), 1593, 1581, 1554, 1512 (C=C). ^{1}H-NMR ($CDCl_3$) *δ*: 1.13 (t, 3H, CH_2CH_3), 2.49 (s, 3H, CH_3), 2.67 (q, 2H, CH_2CH_3), 3.85 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.73 (d, 2H, *J*=8.4 Hz, H-9 Ar, H-5′ Ar), 7.34-7.57 (m, 6H, H-8 Ar, H-2′,6′ Ar, H-3″,4″,5″ Ar), 7.74 (d, 2H, *J*=8.7 Hz, H-2″,6″ Ar). MS (*m/z*) %: 468 (M^+) 95.11%. Anal. Calcd. for $C_{29}H_{24}O_6$ (468.50): C, 74.35; H, 5.16. Found: C, 74.42; H, 5.22.

2.1.8.10. 2-Benzoyl-3,6,7-trimethyl-5*H*-furo[2,3-h]benzopyran-5-one (9j)
Yield 75%. mp 154-156 °C. IR (KBr) cm^{-1}: 3066 (CH Ar), 2922, 2860 (CH aliphatic), 1701 (2 C=O), 1597, 1550 (C=C). ^{1}H-NMR (DMSO-d_6) *δ*: 2.14 (s, 3H, CH_3 at C7), 2.63 (s, 3H, CH_3 at C6), 2.80 (s, 3H, CH_3 at C3), 7.75 (m, 4H, H-9 Ar, H-3′,4′,5′ Ar), 7.93 (d, 1H, *J*=8.7 Hz, H-8 Ar), 7.99 (d, 2H, *J*=7.2 Hz, H-2′,6′ Ar). MS (*m/z*) %: 332 (M^+) 92.23%. Anal. Calcd. for $C_{21}H_{16}O_4$ (332.35): C, 75.89; H, 4.85. Found: C, 76.14; H, 5.02.

2.1.8.11. 2-(4-Methylbenzoyl)-3,6,7-trimethyl-5*H*-furo[2,3-h]benzopyran-5-one (9k)
Yield 64%. mp 243-246 °C. IR (KBr) cm^{-1}: 3080 (CH Ar), 2922, 2854 (CH aliphatic), 1705 (2 C=O),1602, 1566, 1548 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 2.15 (s, 3H, CH_3 at C7), 2.43 (s, 3H, CH_3 at C6), 2.50 (s, 3H, CH_3 at C3), 2.79 (s, 3H, CH_3 at C4′), 7.41 (d, 1H, *J*=8.7 Hz, H-9 Ar), 7.66 (d, 1H, *J*=9.0 Hz, H-8 Ar), 7.91 (d, 2H, *J*=4.8 Hz, H-3′,5′ Ar), 7.94 (d, 2H, *J*=5.7 Hz, H-2′,6′ Ar). MS (*m/z*) %: 346 (M^+) 0.97%. Anal. Calcd. for $C_{22}H_{18}O_4$ (346.38): C, 76.29; H, 5.24. Found: C, 76.38; H, 5.42.

2.1.8.12. 2-(4-Methoxybenzoyl)-3,6,7-trimethyl-5*H*-furo[2,3-h]benzopyran-5-one (9l)
Yield 70%. mp 238-241 °C. IR (KBr) cm^{-1}: 3070 (CH Ar), 2966, 2873 (CH aliphatic), 1708 (2 C=O), 1597, 1566, 1548 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 2.15 (s, 3H, CH_3 at C7), 2.43 (s, 3H, CH_3 at C6), 2.79 (s, 3H, CH_3 at C3), 3.91 (s, 3H, OCH_3), 7.03 (d, 2H, *J*=9.0 Hz, H-3′,5′ Ar), 7.44 (d, 1H, *J*=9.3 Hz, H-9 Ar), 7.69 (d, 1H, *J*=9.0 Hz, H-8 Ar), 8.12 (d, 2H, *J*=8.7 Hz, H-2′,6′ Ar). MS (*m/z*) %: 362 (M^+) 5.17%. Anal. Calcd. for $C_{22}H_{18}O_5$ (362.38): C, 72.92; H, 5.01. Found: C, 73.15; H, 4.97.

2.1.8.13. 2-(3,4-Dimethoxybenzoyl)-3,6,7-trimethyl-5*H*-furo[2,3-h]benzopyran-5-one (9m)
Yield 64%. mp 176-178 °C. IR (KBr) cm^{-1}: 3085 (CH Ar), 2931, 2839 (CH aliphatic), 1712 (2 C=O), 1593, 1512 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 2.19 (s, 3H, CH_3 at C7), 2.37 (s, 3H, CH_3 at C6), 2.92 (s, 3H, CH_3 at C3), 3.90 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 7.00 (d, 1H, *J*=9.0 Hz, H-5′ Ar), 7.43-7.68 (m, 4H, H-8,9 Ar, H-2′,6′ Ar). MS (*m/z*) %: 392 (M^+) 51.74%. Anal. Calcd. for $C_{23}H_{20}O_6$ (392.40): C, 70.40; H, 5.14. Found: C, 70.38; H, 5.19.

2.1.8.14. 2-(4-Bromobenzoyl)-3,6,7-trimethyl-5*H*-furo[2,3-h]benzopyran-5-one (9n)
Yield 73%. mp 252-254 °C. IR (KBr) cm^{-1}: 3085 (CH Ar), 2924, 2858 (CH aliphatic), 1714 (2 C=O), 1604, 1581, 1548 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 2.13 (s, 3H, CH_3 at C7), 2.45 (s, 3H, CH_3 at C6), 2.79 (s, 3H, CH_3 at C3), 7.62 (d, 1H, *J*=8.7 Hz, H-9 Ar), 7.80 (d, 1H, *J*=8.7 Hz, H-8 Ar), 7.91 (d, 2H, *J*=4.8 Hz, H-3′,5′ Ar), 7.93 (d, 2H, *J*=5.4 Hz, H-2′,6′ Ar). MS (*m/z*) %: 411 (M^+) 4.78%, 413 (M^++2) 4.09%. Anal. Calcd. for $C_{21}H_{15}BrO_4$ (411.25): C, 61.33; H, 3.68. Found: C, 61.41; H, 3.76.

2.1.8.15. 2-Benzoyl-6,7-dimethyl-3-phenyl-5*H*-furo[2,3-h]benzopyran-5-one (9o)
Yield 77%. mp 127-129 °C. IR (KBr) cm^{-1}: 3057 (CH Ar), 2924, 2860 (CH aliphatic), 1716 (2 C=O), 1597, 1579, 1554 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 2.19 (s, 3H, CH_3 at C7), 2.37 (s, 3H, CH_3 at C6), 7.29-7.58 (m, 10H, H-8,9 Ar, H-3′,4′,5′, H-2″,3″,4″,5″,6″ Ar), 7.80 (d, 2H, *J*=6.9 Hz, H-2′,6′ Ar). MS (*m/z*) %: 394 (M^+) 71.43%. Anal. Calcd. for $C_{26}H_{18}O_4$ (394.42): C, 79.17; H, 4.60. Found: C, 79.22; H, 4.63.

2.1.8.16. 6,7-Dimethyl-2-(4-methylbenzoyl)-3-phenyl-5*H*-furo[2,3-h]benzopyran-5-one (9p)
Yield 69%. mp 230-232 °C. IR (KBr) cm^{-1}: 3040 (CH Ar), 2919, 2850 (CH aliphatic), 1714 (2 C=O), 1604, 1544 (C=C).^{1}H-NMR (DMSO-d_6) δ: 2.09 (s, 3H, CH_3 at C7), 2.34 (s, 3H, CH_3 at C6), 2.48 (s, 3H, CH_3 at C4′), 7.22 (d, 3H, *J*=8.4 Hz, H-9 Ar, H-3′,5′ Ar), 7.33-7.53 (m, 3H, H-3″,4″,5″ Ar), 7.70 (d, 1H, *J*=8.1 Hz, H-8 Ar), 7.77 (d, 2H, *J*=9.0 Hz, H-2″,6″ Ar), 7.98 (d, 2H, *J*=8.7 Hz, H-2′,6′ Ar). MS (*m/z*) %: 408 (M^+) 45.99%. Anal. Calcd. for $C_{27}H_{20}O_4$ (408.45): C, 79.40; H, 4.94. Found: C, 79.46; H, 5.08.

2.1.8.17. 6,7-Dimethyl-2-(4-methoxybenzoyl)-3-phenyl-5*H*-furo[2,3-h]benzopyran-5-one (9q)
Yield 72%. mp 224-226 °C. IR (KBr) cm^{-1}: 3055 (CH Ar), 2966, 2839 (CH aliphatic), 1714 (2 C=O), 1600, 1573, 1544, 1508 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 2.20 (s, 3H, CH_3 at C7), 2.48 (s, 3H, CH_3 at C6), 3.83 (s, 3H, OCH_3), 6.79 (d, 3H, *J*=8.7 Hz, H-9 Ar, H-3′,5′ Ar), 7.33-7.49 (m, 3H, H-3″,4″,5″ Ar), 7.51 (d, 1H, *J*=8.7 Hz, H-8 Ar), 7.73 (d, 2H, *J*=9.3 Hz, H-2″,6″ Ar), 7.83 (d, 2H, *J*=8.7 Hz, H-2′,6′ Ar). MS (*m/z*) %: 424 (M^+) 79.00%. Anal. Calcd. for $C_{27}H_{20}O_5$ (424.44): C, 76.40; H, 4.75. Found: C, 76.53; H, 4.72.

2.1.8.18. 2-(3,4-Dimethoxybenzoyl)-6,7-dimethyl-3-phenyl-5*H*-furo[2,3-h]benzopyran-5-one (9r)
Yield 68%. mp 150-153 °C. IR (KBr) cm^{-1}: 3059 (CH Ar), 2935, 2839 (CH aliphatic), 1716 (2 C=O), 1597, 1512 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 2.20 (s, 3H, CH_3 at C7), 2.48 (s, 3H, CH_3 at C6), 3.85 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 6.72-7.75 (m, 10H, Ar-H). MS (*m/z*) %: 454 (M^+) 45.73%. Anal. Calcd. for $C_{28}H_{22}O_6$ (454.47): C, 74.00; H, 4.88. Found: C, 74.08; H, 4.91.

2.1.8.19. 2-(4-Bromobenzoyl)-6,7-dimethyl-3-phenyl-5*H*-furo[2,3-h]benzopyran-5-one (9s)
Yield 75%. mp 258-262 °C. IR (KBr) cm^{-1}: 3060 (CH Ar), 2924, 2855 (CH aliphatic), 1720 (2 C=O), 1610, 1585, 1540 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 2.20 (s, 3H, CH_3 at C7), 2.49 (s, 3H, CH_3 at C6), 7.26-7.76 (m, 11H, Ar-H). MS (*m/z*) %: 473 (M^+) 100%. Anal. Calcd. for $C_{26}H_{17}BrO_4$ (473.31): C, 65.98; H, 3.62. Found: C, 66.04; H, 3.59.

2.1.9. General Procedure for synthesis of ethyl (8-acyl-3,4-dimethyl-2-oxo-2*H*-1-benzopyran-7-yloxy)acetates (10a,b) (Scheme 3).
A mixture of compound **8c,d** (0.01 mol) and ethyl chloroacetate (2.4 ml, 0.02 mol) in acetone (50 ml) in presence of anhydrous potassium carbonate (2.76 g, 0.02 mol) was refluxed while stirring for 24 h. The solvent was distilled off and the residue was washed with water, filtered and dried to give **10a,b**. Product was crystallized from isopropanol.

2.1.9.1. Ethyl (8-acetyl-3,4-dimethyl-2-oxo-2*H*-1-benzopyran-7-yloxy)acetate (10a)
Yield 91%. mp 152-154 °C. IR (KBr) cm^{-1}: 3091 (CH Ar), 2984, 2927 (CH aliphatic), 1746, 1705 (3 C=O), 1604, 1498 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 1.20 (t, 3H, CH_2CH_3), 2.07 (s, 3H, CH_3 at C4), 2.37 (s, 3H, CH_3 at C3), 2.54 (s, 3H, $COCH_3$), 4.16 (q, 2H, CH_2CH_3), 5.01 (s, 2H, OCH_2CO), 7.08 (d, 1H, *J*=9.3 Hz, H-6 Ar), 7.78 (d, 1H, *J*=9.0 Hz, H-5 Ar). MS (*m/z*) %: 318 (M^+) 68.33%. Anal. Calcd. for $C_{17}H_{18}O_6$ (318.32): C, 64.14; H, 5.70. Found: C, 64.31; H, 5.78.

2.1.9.2. Ethyl (8-benzoyl-3,4-dimethyl-2-oxo-2*H*-1-benzopyran-7-yloxy)acetate (10b)
Yield 72%. mp 160-161 °C. IR (KBr) cm^{-1}: 3057 (CH Ar), 2987, 2870 (CH aliphatic), 1757, 1735, 1712 (3 C=O), 1597, 1531 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.22 (t, 3H, CH_2CH_3), 2.15 (s, 3H, CH_3 at C4), 2.39 (s, 3H, CH_3 at C3), 4.18 (q, 2H, CH_2CH_3), 4.64 (s, 2H, OCH_2CO), 6.79 (d, 1H, *J*=9.0 Hz, H-6 Ar), 7.41-7.91 (m, 4H, H-5 Ar, H-3′,4′,5′ Ar), 7.62 (d, 2H, *J*=7.2 Hz, H-2′,6′ Ar). MS (*m/z*) %: 380 (M^+) 53.06%. Anal. Calcd. for $C_{22}H_{20}O_6$ (380.39): C, 69.46; H, 5.30. Found: C, 69.53; H, 5.27.

2.1.10. General Procedure for synthesis of (8-acyl-3,4-dimethyl-2-oxo-2*H*-1-benzopyran-7-yloxy)acetic acid (11a,b) (Scheme 3).
A solution of **10a,b** (0.01 mol) and potassium hydroxide (2.52 g, 0.045 mol) in methanol/water mixture (1:1) (50 ml) was refluxed for 30 min. The solution was acidified with 10% HCl and the precipitated solid was filtered, washed and dried. The obtained solid was crystallized from ethyl acetate to give **11a,b**.

2.1.10.1. (8-Acetyl-3,4-dimethyl-2-oxo-2*H*-1-benzopyran-7-yloxy)acetic acid (11a)
Yield 73%. mp 252-253 °C. IR (KBr) cm^{-1}: 3059 (CH Ar), 2929, 2860 (CH aliphatic), 2800, 2560 (carboxylic OH), 1766, 1710, 1674 (3 C=O), 1600, 1500 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 2.07 (s, 3H, CH_3 at C4), 2.27 (s, 3H, CH_3 at C3), 2.53 (s, 3H, $COCH_3$), 4.90 (s, 2H, CH_2), 7.06 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.78 (d, 1H, *J*=9.3 Hz, H-5 Ar), 13.20 (s, 1H, OH exch. D_2O). MS (*m/z*) %: 290 (M^+) 31.17%. Anal. Calcd. for $C_{15}H_{14}O_6$ (290.27): C, 62.07; H, 4.86. Found: C, 62.08; H, 4.94.

2.1.10.2. (8-Benzoyl-3,4-dimethyl-2-oxo-2*H*-1-benzopyran-7-yloxy)acetic acid (11b)
Yield 61%. mp 200-202 °C. IR (KBr) cm^{-1}: 3088 (CH Ar), 2968, 2850 (CH aliphatic), 2586, 2493 (carboxylic OH), 1720, 1710, 1689 (3 C=O), 1583, 1508 (C=C). ^{1}H-NMR (DMSO-d_6) δ: 2.04 (s, 3H, CH_3 at C4), 2.40 (s, 3H, CH_3 at C3), 4.80 (s, 2H, CH_2), 7.09 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.50-7.89 (m, 6H, H-5 Ar, Ar-H), 13.19 (s, 1H, OH exch. D_2O). MS (*m/z*) %: 352 (M^+) 12.38%. Anal. Calcd. for $C_{20}H_{16}O_6$ (352.34): C, 68.18; H, 4.58. Found: C, 68.14; H, 4.63.

2.1.11. General Procedure for synthesis of 6,7-dimethyl-3-substituted-5*H*-furo[2,3-h]benzopyran-5-ones (12a,b) (Scheme 3).
A mixture of **11a,b** (0.02 mol) and anhydrous sodium acetate (1.64 g, 0.02 mol) in acetic anhydride (35 ml) was refluxed for 1 h, water was added and the mixture was refluxed for 10 min., diluted with water and extracted with ethyl acetate. The organic layer was washed with sodium bicarbonate solution (50 ml). The ethyl acetate was evaporated and the residue was crystallized from isopropanol giving **12a,b**.

2.1.11.1. 3,6,7-Trimethyl-5*H*-furo[2,3-h]benzopyran-5-one (12a)
Yield 70%. mp 244-246 °C. IR (KBr) cm^{-1}: 3101 (CH Ar), 2927, 2868 (CH aliphatic), 1701 (C=O), 1613, 1527 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 2.25 (s, 3H, CH_3 at C7), 2.47 (s, 3H, CH_3 at C6), 2.55 (s, 3H, CH_3 at C3), 7.36 (d, 1H, *J*=9.0 Hz, H-9 Ar), 7.41 (s, 1H, H-2 Ar), 7.49 (d, 1H, *J*=9.0 Hz, H-8 Ar). MS (*m/z*) %: 228 (M^+) 100%. Anal. Calcd. for $C_{14}H_{12}O_3$ (228.24): C, 73.67; H, 5.30. Found: C, 73.69; H, 5.33.

2.1.11.2. 6,7-Dimethyl-3-phenyl-5*H*-furo[2,3-h]benzopyran-5-one (12b)
Yield 65%. mp 190-191 °C. IR (KBr) cm^{-1}: 3095 (CH Ar), 2924, 2856 (CH aliphatic), 1701 (C=O), 1601 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 2.22 (s, 3H, CH_3 at C7), 2.48 (s, 3H, CH_3 at C6), 7.39-7.75 (m, 8H, Ar-H). MS (*m/z*) %: 290 (M^+) 100%. Anal. Calcd. for $C_{19}H_{14}O_3$ (290.31): C, 78.61; H, 4.86. Found: C, 78.82; H, 4.91.

1.2. Antimicrobial and photosensitizing screening

All the synthesized compounds were screened for their antimicrobial and photosensitizing activities by the paper disc diffusion method [21] and compared with xanthotoxin as reference compound. The tested organism used was *Bacillus Subtilus*.

In the preliminary test, employing strong condition (high concentration of the substance) was used for selecting the active compounds, even weakly active, from the inactive ones.

In another test, only the active compounds were tested to determine the effect of radiation time (exposure to UV-A) and concentration on their photosensitizing activity.

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: slant agar seeded with the tested organism *(Bacillus Subtilus*) and incubated overnight.

c) Paper disc: Whatman no. 1 filter paper disc (5 mm) were sterilized and impregnated with different concentrations of the tested compounds (compounds were dissolved in dimethylformamide, DMF), and allowed to dry overnight. Two concentrations were prepared for the tested compounds.

EXPERIMENTAL

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

Two groups of plates were used, one as test plates, incubated in the dark at 37 ^{o}C for 3 h before irradiation (to allow for diffusion of the tested compounds through the agar layer) and the duplicate plates were left in the incubator overnight as control to determine the antimicrobial activity.

Covers were removed from plates of first group (tested petri dishes) and exposed to UV lamp (365 nm) for 20 min. After irradiation, plates were reincubated in the dark at 37 ^{o}C overnight and examined for photosensitizing activity (antimicrobial and photosensitizing activities were determined by measuring the produced inhibition zones).

Effect of increasing time of UV-A radiation and concentration on photosensitizing activity for active benzopyrone and furobenzopyrone derivatives

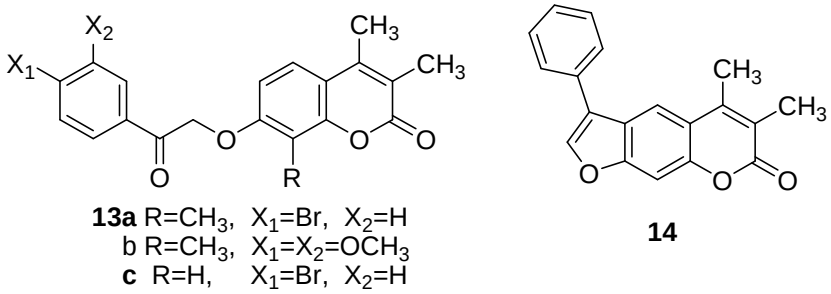
The experiment was repeated using the selected active compounds to study the effect of radiation time and concentration on the photosensitizing activity.

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).

1.3. QSAR

2.3.1. Computational method

All the computational works were performed on Molecular Operating Environment software (MOE version 2008.10.2) [22]. The structures of 17 compounds (14 new compound in addition to 3 puplished compound [23], **Figure 1**) used as training set and structures of 3 compounds used as test set (2 new compounds in addition to 1 published compound [23], **Figure 1**) were sketched using molecular builder of MOE and each structure was subjected to energy minimization up to 0.01 Kcal/mol Å using the MMFF94x force field. Optimization methods were used followed by conformational search of each energy-minimized structure. The most stable conformer of each structure was selected and saved into database to generate the common descriptors. QuaSAR descriptor module of MOE was used to calculate descriptors for each molecule. The probability density functions used are Gaussian. The RMSD tolerance was set to 0.5 Å. Regression analysis was performed using photosensitizing activity after radiation for fourty min. as dependent factor and the calculated descriptors as predictable variables.



13a R=CH_3, X_1=Br, X_2=H
b R=CH_3, X_1=X_2=OCH_3
c R=H, X_1=Br, X_2=H

14

Figure 1: Structure of published compounds used in QSAR study

In this study, the pool of descriptors was optimized using principal components analysis (PCA). The optimization started with the reduction in the number of molecular descriptors by the determination of the highly inter-correlated descriptor pairs and only one from each pair was selected then the descriptors with insignificant variance through the data set were also rejected. QSAR model was then constructed after ensuring reasonable correlation of photosensitizing activity with the individual descriptors and minimum inter-correlation among the descriptors used in the derived model. The quality of the model was assessed using the statistical parameter r^2.

2.3.2. Molecular descriptors

AM1_dipole: The dipole moment calculated using the AM1 Hamiltonian [MOPAC]. The magnitude of the dipole moment does not depend on the absolute orientation in space.

logP: Log of the octanol/water partition coefficient (including implicit hydrogens). This property is calculated from a linear atom type model.

logS: Log of the aqueous solubility (mol/l). This property is calculated from an atom contribution linear atom type model.

SMR: Molecular refractivity (including implicit hydrogens). This property is an atomic contribution model that assumes the correct protonation state (washed structures).

TPSA: Polar surface area ($Å^2$) calculated using group contributions to approximate the polar surface area from connection table information only.

2.3.3. Model evaluation:

Evaluation of the model and its trial on test set (3 compounds) was used for further assessment of predictivity for the produced model. The predictive ability of the model was expressed by the predictive r^2 value ($r^2_{pred.}$).

1.4. Docking study

2.4.1. Docking procedure

Docking studies of active antimicrobial compounds were performed by Molecular Operating Environment software (MOE version 2008.10.2) [22]. 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.

2.4.1.1. Preparation of the target DNA gyrase

The X-ray crystal structure of the enzyme with benzopyrone ligand (PDB code 1AJ6) [24] 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 novobiocin. (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 (novobiocin) with the amino acids of the active site.

2.4.1.2. Preparation of compounds for docking

The 3D structures of the synthesized 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 novobiocin.

2.4.1.3. Docking running

Prior to docking of benzopyrone and furobenzopyrone derivatives, redocking of the native ligand bound in the topoisomerase II 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 topoisomerase II. 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.

RESULTS AND DISCUSSION

1.5. Chemistry

The target compounds **3a-h**, **7a,b**, **9a-s** and **12a,b** were synthesized as depicted in **Schemes 1-3.**

The starting compounds 3,8-disubstituted-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one **1a-d** [18] and phenacyl bromide derivatives [19] were prepared according to the previously reported procedures.

Refluxing 7-hydroxy-2*H*-1-benzopyran-2-one **1a,b** with appropriate phenacyl bromide derivative in dry acetone containing anhydrous potassium carbonate yielded ether derivatives **2a-h, Scheme 1**. This mild reaction condition was adopted to avoid any probability for the opening of the sensitive pyrone ring [25]. Complete reaction was achieved with negative ferric chloride test.

Cyclization of ether derivatives **2a-h** to furo[3,2-g]benzopyran-7-ones **3a-h** were achieved through reflux with alcoholic potassium hydroxide followed by subsequent acidification**, Scheme 1**.

Attempts for etherification of 3-ethyl-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one **1b** with 3,4-dimethoxyphenacyl bromide under previous conditions gave the angular furo[2,3-h]benzopyran-5-one **4** in one step reaction**, Scheme 1**. Trials to decrease reaction time to obtain the ether derivative gave same product.

Cinnamylation of 7-hydroxybenzopyran-2-one **1a,c** with cinnamyl chloride through reflux in dry acetone in presence of anhydrous potassium carbonate yielded cinnamyl ethers **5a,b, Scheme 2**. These ether derivatives **5a,b** gave negative ferric chloride test.

Claisen rearrangement of 7-cinnamyloxybenzopyrones **5a,b** was achieved through refluxing with *N*,*N*-diethylaniline and gave mixture of products, the open rearranged product **6a,b** and the cyclized furobenzopyran-7-one **7a,b, Scheme 2**. Use of *N*,*N*-diethylaniline [26] was preferred than *N*,*N*-dimethylaniline [27] due to reduction in reaction time and lower temperature. Separation of product was obtained through alkalinization and extraction with ether. The open rearranged product **6a,b** were achieved from aquous alkaline solution after acidification with hydrochloric acid. Compounds **6a,b** gave positive result with ferric chloride test. ^{1}H-NMR spectra proved different position of double bond of propenyl group that either 1-phenyl-1-propenyl **6a** or 1-phenyl-2-propenyl **6b**. Compound **6a** spectrum showed presence of doublet signal at 1.59 ppm corresponding to CH_3-CH=C and quartet at 6.40 ppm corresponding to CH_3-CH=C. On the other hand, spectrum for compound **6b** showed presence of doublet signal at 4.89 ppm and two multiplet at 5.12-5.18 and 6.39-6.50 ppm assigned to CH_2=CH-CH, CH_2=CH-CH and CH_2=CH-CH, respectively.

Furo[3,2-g]benzopyran-7-ones **7a,b** were obtained by two different procedures**, Scheme 2**. First, the ether layer from the previous extraction was evaporated to achieve the cyclized furobenzopyran-7-ones **7a,b**. Second, the rearranged product **6a,b** underwent cyclization by refluxing in a mixture of glacial acetic acid and hydrobromic acid. Cyclization mechanism was presumably via cyclopropane intermediate to give dihydrofurobenzopyrone derivatives, **Figure 2** [28].

Dihydrofurobenzopyran-7-ones were directly dehydrogenated without further purification by refluxing with DDQ in dry benzene to achieve the target compounds **7a,b**.

8-Acetyl-7-hydroxy derivatives **8a,c** were obtained according to reported procedure [20]. Reaction of 7-hydroxybenzopyrones **1b,d** with benzoyl chloride yielded 7-benzoyloxy derivative which were subjected to Fries rearrangement by fusion with anhydrous aluminium chloride to obtain 8-benzoyl-7-hydroxy derivatives **8b,d, Scheme 3**. The rearrangement occurs either to C6 or C8, however, the majority of rearrangements take place to the 8-position as it was stabilized by the pyrone ring [29]. Positive ferric chloride test indicated free phenolic OH group. Refluxing 8-acyl-7-hydroxybenzopyrones **8a-d** with different phenacyl bromide derivatives in presence of anhydrous potassium carbonate in dry acetone afforded furo[2,3-h]benzopyran-5-ones **9a-s, Scheme 3**. Condensation and cyclization took place in one step reaction. Ferric chloride test gave negative result.

Refluxing 7-hydroxycoumarins **8c,d** with ethyl chloroacetate in dry acetone in presence of anhydrous potassium carbonate yielded the required ether derivatives **10a,b, Scheme 3.** Use of anhydrous potassium carbonate [30] is preferred than metal alcoholate [31] to keep the benzopyrone nucleus intact.

Saponification of esters **10a,b** with methanolic potassium hydroxide, followed by acidification gave compounds **11a,b, Scheme 3**. Use of methanolic potassium hydroxide [32] decreased reaction time than use of sodium ethoxide in ethanol or sodium hydroxide [33,34].

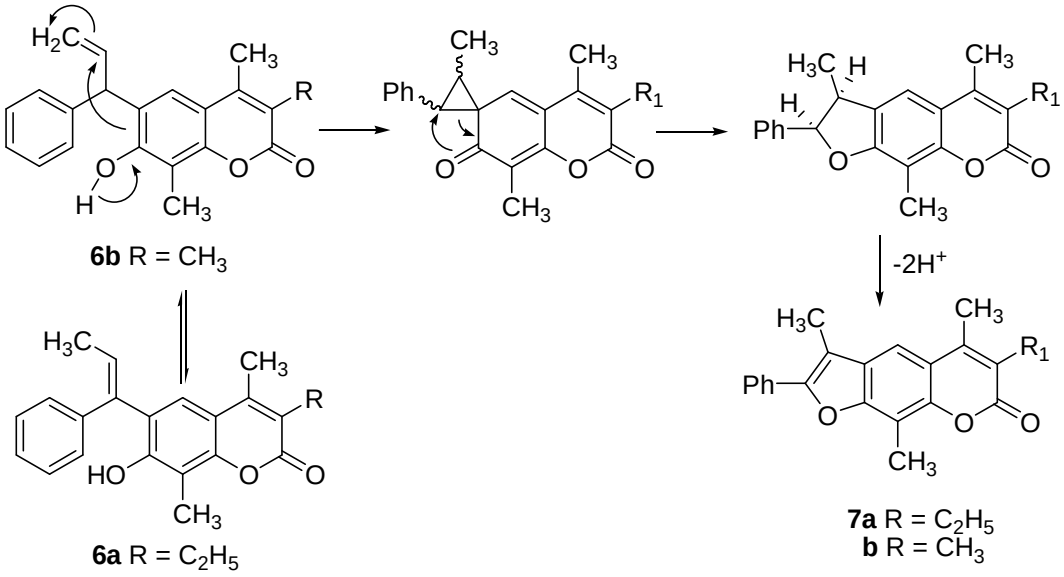


Figure 2: Mechanism of cyclization for open rearranged products 6a,b

Table 1: Preliminary screenining of tested compounds for antimicrobial and photosensitizing activities

Cpd. No.	Control	Test	Cpd. No.	Control	Test
DMF	---	---	**8c**	*6	10
Xanthotoxin	9	12	**8d**	---	---
2a	---	---	**9a**	---	---
2b	---	---	**9b**	---	7
2c	---	---	**9c**	---	---
2d	7	7	**9d**	---	---
2e	15	17	**9e**	---	---
2f	---	---	**9f**	---	---
2g	---	---	**9g**	---	---
2h	---	---	**9h**	---	---
3a	---	---	**9i**	---	---
3b	---	---	**9j**	---	---
3c	---	---	**9k**	*6	10
3d	8	8	**9l**	---	---
3e	---	6	**9m**	---	---
3f	8	10	**9n**	---	---
3g	---	---	**9o**	---	8
3h	---	---	**9p**	---	6
4	11	11	**9q**	---	---
5a	---	8	**9r**	---	---
5b	---	---	**9s**	7	7
6a	---	8	**10a**	---	---
6b	---	---	**10b**	---	---
7a	---	---	**11a**	*6	6
7b	13	13	**11b**	9	9
8a	---	---	**12a**	7	10
8b	*6	7	**12b**	8	10

Control *(disc contains 0.01 ml of the screened, the reference compound).*
Test *(disc contains 0.01 ml of the screened, the reference compound and time of radiation is 20 min.).*
* *Non significant antimicrobial agents.*

Cyclization of benzopyronoxyacetic acid derivatives **11a,b** to the corresponding angular furobenzopyrones **12a,b** were performed using acetic anhydride in presence of anhydrous sodium acetate**, Scheme 3**.

All the new synthesized compounds were characterized by spectral and elemental analyses which were in full agreement with the proposed structures.

1.6. Antimicrobial and photosensitizing screening

Screening of antimicrobial and photosensitizing activities for all the synthesized benzopyrone and furobenzopyrone analogues (linear and angular) were performed by paper disc diffusion method [21] using xanthotoxin as reference compound. The tested organism used was *Bacillus Subtilus*.

3.2.1. Antimicrobial activity

The Antimicrobial activity was compared with that of xanthotoxin "diameter of inhibition zone before UV-A radiation ", **Table 1**. Benzopyrone derivatives **2d**,**e** and **11b** showed antimicrobial activity, from which compound **2e** showed higher activity even than xanthotoxin. Linear furobenzopyrone analogues **3d,f** and **7b** exhibited antimicrobial activity. Compound **7b**, in particular, had good activity higher than xanthotoxin. Angular furobenzopyrone analogues **4**, **9s** and **12a,b** showed antimicrobial activity. Compounds **4** had reasonable antimicrobial activity better than xanthotoxin. Compounds **8b,c, 11a** and **9k** had non significant antimicrobial activity. The rest of the screened compounds showed no antimicrobial activity.

3.2.2. Photosensitizing activity

The photosensitizing activity was compared with that of xanthotoxin "diameter of inhibition zone after UV-A radiation ", when the disc contains 0.01 ml of the reference or screened compounds and the time of radiation was 20 min, **Table 1**. Benzopyrone derivatives **2e**, **5a**, **6a** and **8b,c** showed photosensitizing activity. Linear furobenzopyrones **3e**,**f** exhibited photosensitizing activity. Angular furobenzopyrones **9b**,**k**,**o**,**p** and **12a,b** proved to be active as photosensitizing. The rest of the screened compounds were devoid of photosensitizing activity.

Increasing the time of UV-A radiation up to 40 min. instead of 20 min. and using the same concentration of the screened, reference compounds, **Table 2**, **Figure 3**. Photosensitizing activity for benzopyrone derivatives **2d,e**, **5a**, **6a** and **8b,c** was affected by increasing time of radiation. Compounds **11a,b** showed no change in photosensitizing activity. Photosensitizing activity for linear furobenzopyrone analogues **3d-f** was directly proportional with time of radiation to UV-A. Otherwise, compound **7b** exhibited no photosensitizing activity. Photosensitizing activity for angular furobenzopyrone analogues **9b,k,o,p,s** and **12a,b** was directly proportional with time of radiation to UV-A. However, compound **4** had no photosensitizing activity.

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

Cpd. No.	Control	Test	•Test	••Test
DMF	----	----	----	----
2d	7	7	15	21
2e	15	17	23	27
3d	8	8	12	18
3e	----	6	10	16
3f	8	10	17	22
4	11	11	11	14
5a	----	8	10	12
6a	----	8	9	10
7b	13	13	13	18
8b	6	7	10	10
8c	6	10	13	15
9b	----	7	8	12
9k	6	10	11	14
9o	----	8	10	12
9p	----	6	11	12
9s	7	7	9	15
11a	6	6	6	9
11b	9	9	9	13
12a	7	10	12	14
12b	8	10	13	17
Xanthotoxin	9	12	15	19

Control (disc contains 0.01 ml of the screened, the reference compound).
Test (disc contains 0.01 ml of the screened, the reference compound and time of radiation is 20 min).
•Test (disc contains 0.01 ml of the screened, the reference compound and time of radiation is 40 min.).
••Test (disc contains 0.02 ml of the screened, the reference compound and time of radiation is 20 min.).

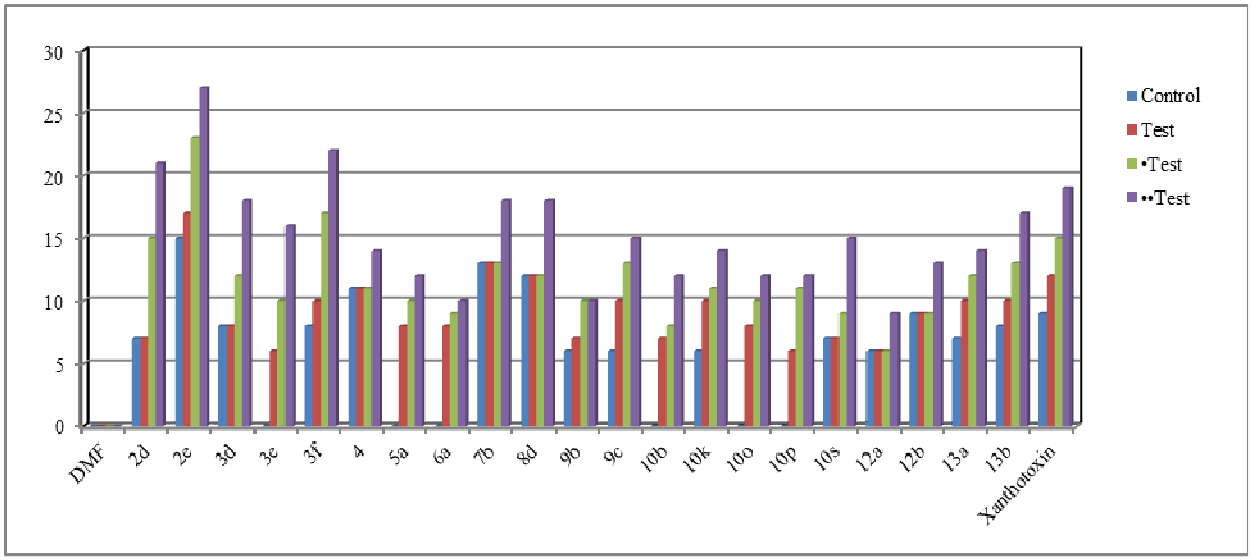


Figure 3: The bar diagram showing antimicrobial, photosensitizing activities and effect of increasing time of radiation and concentration on photosensitizing activity of the screened compounds and their comparison to solvent DMF and reference standard xanthotoxin

Increasing the concentration of the reference or screened compounds up to 1 mg/ml instead of 0.5 mg/ml and using the same period of UV-A radiation, 20 min., **Table 2**, **Figure 3**, results showed a direct correlation between photosensitizing activity and concentration for most of the compounds.

1.7. QSAR Study

In an attempt to correlate the photosensitizing activity with the structure conformation of the synthesized benzopyrone, linear and angular furobenzopyrone derivatives, QSAR study was undertaken. Descriptors of the molecular modeling software, Molecular Operating Environment (MOE version 2008.10.2) were used [22]. The structural descriptors used in the generation of these models include: The dipole moment calculated using the AM1 Hamiltonian (AM1_dipole), Log of the octanol/water partition coefficient (logP), Log of the aqueous solubility (logS), Molecular refractivity (SMR) and Polar surface area (TPSA) as shown in **Table 3**.

Table 3: The molecular descriptor values of the training set compounds

Cpd. No.	Descriptors				
	AM1_dipole	**LogP (o/w)**	**LogS**	**SMR**	**TPSA**
2d	7.1672	5.2590	-7.0341	10.3666	52.6000
2e	8.0418	4.1597	-6.0444	10.9071	71.0600
3d	3.7779	6.2240	-8.7561	10.2607	39.4400
3e	6.9207	4.7800	-7.5155	10.5898	65.7400
3f	5.8849	5.1690	-7.5053	9.0170	39.4400
5a	5.8238	5.5500	-6.1181	10.0670	35.5300
6a	6.0687	5.4560	-6.2327	9.9846	46.5300
8b	5.6644	4.0710	-5.3444	8.6501	63.6000
9b	2.9581	5.1220	-7.9390	10.4082	56.5100
9k	2.6750	4.6470	-7.4238	9.9466	56.5100
9o	2.5574	6.0110	-9.2158	11.5428	56.5100
9s	5.4731	6.8090	-10.3062	12.3127	56.5100
12a	3.6649	2.9860	-4.7242	6.4854	39.4400
12b	3.7244	4.6180	-6.9921	8.5553	39.4400
13a	7.2331	7.7840	-6.5188	9.9050	52.6000
13b	7.9229	3.6847	-5.5292	10.4453	71.0600
13c	7.2405	4.5270	-6.3584	9.4312	52.6000

The observed photosensitizing activity "expressed in the term of produced inhibition zones after UV-A irradiation for 40 min." together with the predicted activities (Pred. activity) for the training set compounds. The best derived QSAR linear model for the 17 compounds (14 new compounds in addition to 3 published compounds [23], **Figure 1**) was presented by the following estimated equation:

Photosensitizing activity = 12.92794 + 1.34495 x **AM1_dipole** - 8.60214 x **logP (o/w)**
–1.97840 x **logS** + 3.80798 x **SMR** - 0.31966 x **TPSA**

r^2 = 0.6153

From the equation, photosensitizing activity was positively correlated with AM1_dipole, SMR and negatively correlated with LogP (o/w), LogS and TPSA. The high coefficient value of logP (o/w), SMR and the comparatively lower value of LogS, AM1_dipole and TPSA suggested that the decrease in partition coefficient and increase in molecular refractivity lead to enhancement of activity. This was in good agreement with the obtained experimental data, where in case of most active compounds **2e** and **13b** showed slight increase in molecular refractivity value accompanied by decrease in partition coefficient value leading to increase in activity.

The observed activities (Obs. activity) together with the predicted activities (Pred. activity) for the tested compounds calculated using multi-linear regression (MLR) are listed in **Table 4**. All compounds showed very good results with Z-scores not exceed the value of 2.5 indicating excellent predictive ability of the model.

Table 4: The observed photosensitizing activity (Obs. activity) "expressed in the term of produced inhibition zones after UV-A irradiation for 40 min." together with the predicted activity (Pred. activity) for the training set compounds

Cpd. No.	Obs. Activity	Pred. Activity	Residual	Z-score
2d	15	13.9069	1.0932	0.4188
2e	23	18.7382	4.2618	1.6327
3d	12	8.2576	3.7424	1.4337
3e	10	15.2975	-5.2575	2.0295
3f	17	12.9560	4.0440	1.5493
5a	10	12.1004	-2.1004	0.8047
6a	9	9.6752	-0.6752	0.2587
8b	10	8.7096	1.2904	0.4943
9b	8	10.1234	-2.1234	0.8135
9k	11	11.0512	-0.0512	0.0196
9o	10	8.7832	1.2168	0.4661
9s	9	10.9296	-1.9295	0.7392
12a	12	13.8643	-1.8643	0.7142
12b	13	12.0126	0.9874	0.3783
13a	12	15.3041	-3.3041	1.2658
13b	22	19.8868	2.1132	0.8095
13c	14	15.4034	-1.4034	0.5377

The Z score method was adopted for the detection of outliers. Z score can be defined as absolute difference between the value of the model and the activity field, divided by the square root of the mean square error of the data set. Any compound which shows a value of Z score higher than 2.5, during generation of a particular QSAR model, is considered as outlier [35]. No outliers were observed in this model as showed in **Table 4**, indicating that, molecular descriptors used for the training set were good.

The observed activity was plotted against their predicted values (calculated by MLR) with a value of r^2 found to be 0.6153, **Table 4**, **Figure 3**.

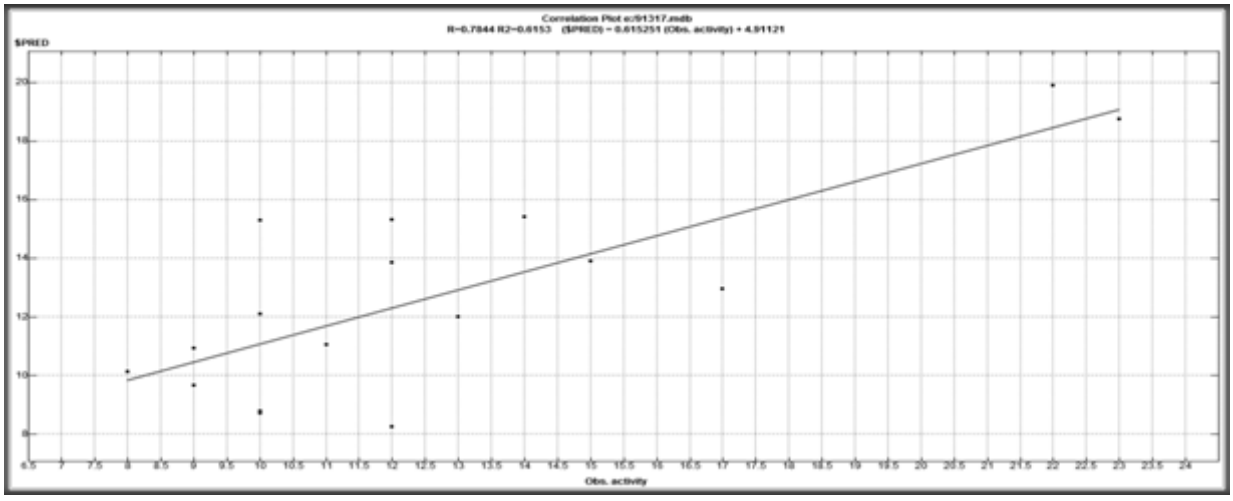


Figure 3: Correlation plot of observed and predicted activities of the training set for QSAR model, r^2 = 0.6153

Fraction of the Variance (r^2): Represent the goodness of fit. The value of r^2 may vary between 0 and 1, when multiplied by 100 gives explained variance in biological activity, where 1 means a perfect model explaining 100% of the variance in the data, and 0 means a model without any explanatory power. It has already been suggested that

the only QSAR model having $r^2 > 0.6$ will be considered for validation [36]. Value of r^2 for this QSAR model is 0.6153.

3.3.1. Model evaluation:

The true predictive power of a QSAR model was determined by comparing the predicted and observed activities of the test set compounds (2 new compounds in addition to 1 published compound [23], **Figure 1**) that not used in the QSAR model development of training set. The observed activities were plotted against their predicted values, **Table 5**, **Figure 4**.

Table 5: The observed photosensitizing activity (Obs. activity) "expressed in the term of produced inhibition zones after UV-A irradiation for 40 min." together with the predicted activity (Pred. activity) for the test set

Cpd. No.	Obs. Activity	Pred. Activity	Residual
8c	13	12.3304	0.6696
9b	11	10.2702	0.7298
14	14	14.3391	-0.3391

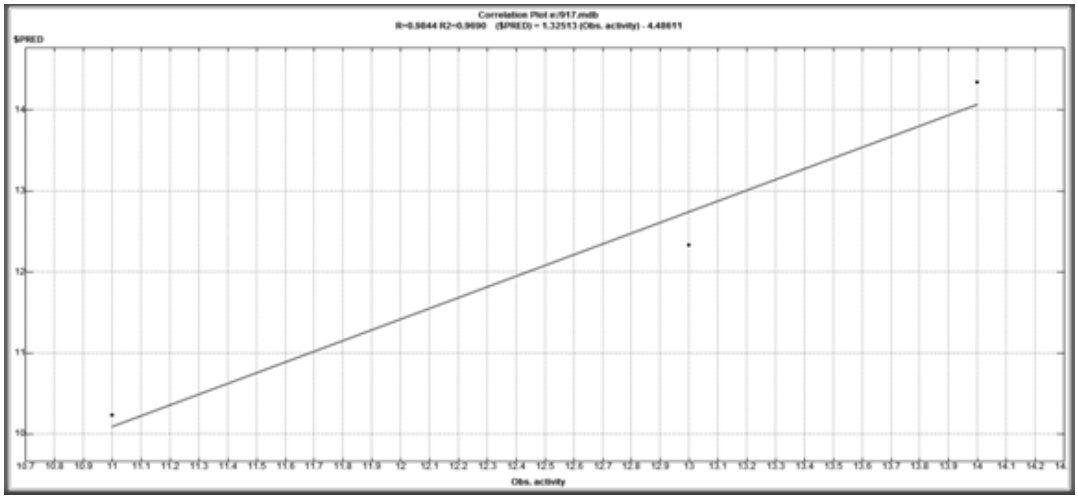


Figure 4: Correlation plot of observed and predicted activities of the test set for QSAR model, r^2_{pred} = 0.9690

The predictive ability of the model was expressed by the predictive r^2 value ($r^2_{pred.}$), the value of r^2_{pred} is 0.9690, it was calculated by the following Equation [37]:

$$r^2 pred = 1 - \frac{\sum(y\ pred(test) - y\ test)^2}{\sum(y\ test - \bar{y}\ training)^2}$$

where y pred(test) and y test are the respective predicted and observed activities of the test set compounds and ȳ training is the observed mean activity of the training set compounds.

1.8. Docking study

DNA topoisomerases are ubiquitous enzymes responsible for controlling the topological state of DNA in cells. They are charged with the task of resolving topological problems which arise during the various processes of DNA including transcription, recombination, replication and chromosome partitioning during cell division. The mechanism of these enzymes involves DNA cleavage and DNA strand passage through the break, followed by religation of the cleaved DNA. From a medical point of view, topoisomerases are important targets for a large variety of antitumor as well as antibacterial compounds [38]. Topoisomerase inhibitors are often divided into, according to which type of enzyme it inhibits, topoisomerase I and II inhibitors. Type I enzymes, which cleave a single strand of DNA during the course of the reaction and type II enzymes, which cleave both strands. In addition, there are two subclasses of type II topoisomerase, type IIA and type IIB [39]. Type IIA topoisomerases include the enzymes DNA gyrase, eukaryotic topoisomerase II (topo II) and bacterial topoisomerase IV (topo IV). Type IIB topoisomerases, comprise a single family member, topoisomerase VI (topo VI).

DNA gyrase is essential to the cell due to its unique ability to introduce negative supercoils into DNA and so involved during replication and transcription. Gyrase is present in prokaryotes and some eukaryotes, but not present in humans. This makes gyrase a good target for antibiotics. The enzyme consists of two subunits, A and B. The A

protein is responsible for DNA cleavage and rejoining, whereas the B protein contains the ATP-binding site. There are two classes of antibiotics that inhibit gyrase [24]. The aminocoumarins, including novobiocin, work by competitive inhibition of energy transduction of DNA gyrase by binding to the ATPase active site located on the GyrB subunit. The quinolones, including nalidixic acid and ciprofloxacin, act by interfering with the DNA breaking-rejoining step on the A subunit. The gyrase B subunit contains N-terminal domain which includes the site of ATP hydrolysis and C-terminal domain which interacts with the A subunit and probably DNA. The crystal structure of this N-terminal fragment complexed with novobiocin showed that, residues that make contact with bounded coumarin derivatives in the active site of DNA gyrase are Thr165, Gly146, Arg136, Ile78, Gly77, Arg76, Asp73 and Asn46 amino acids [24,40].

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 6**, **Figures 6-9**.

Table 6: Docking results

Cpd. No.	Energy score S (Kcal/mol)	Binding amino acid	Interacting function group	Hydrogen bond length Å
Novobiocin	-13.6636	Thr165(through water molecule) Gly77(through water molecule) Gly77(through water molecule) Arg76(cation-arene) Arg76(through water molecule) Asp73(through water molecule) Asp73 Asn46 Val43(through water molecule)	CO carbamate CO carbamate CO benzopyrone Benzene of benzopyrone CO amide CO carbamate NH_2 carbamate OH pyrane NH_2 carbamate	2.02 2.02 1.82 2.04 2.02 1.91 2.05 2.30
2d	-9.4339	Thr165(through water molecule) Gly77 Gly77(through water molecule) Arg76(cation-arene) Asp73(through water molecule)	CO acyloxy CO acyloxy CO acyloxy Benzene of benzopyrone CO acyloxy	1.93 2.17 1.93 1.93
2e	-12.0553	Thr165 Thr165(through water molecule) Gly77(through water molecule) Arg76(through water molecule) Asp73(through water molecule) Val43(through water molecule)	3′-OCH_3 3′-OCH_3 3′-OCH_3 CO benzopyrone 3′-OCH_3 4′-OCH_3	3.12 1.64 1.64 2.21 1.64 2.78
3d	-10.2129	Thr165 Thr165(through water molecule) Gly77(through water molecule) Asp73(through water molecule)	CO benzopyrone CO benzopyrone CO benzopyrone CO benzopyrone	2.95 1.52 1.52 1.52
3f	-9.7639	Val120(through water molecule) Arg76(cation-arene) Asn46(through water molecule)	CO benzopyrone Phenyl at C3 CO benzopyrone	2.16 2.16
4	-10.9973	Thr165 Thr165(through water molecule) Gly77(through water molecule) Asp73(through water molecule)	3′-OCH_3 3′-OCH_3 3′-OCH_3 3′-OCH_3	3.24 1.44 1.44 1.44
7b	-11.0759	Thr165 Thr165(through water molecule) Gly77(through water molecule) Arg76(cation-arene) Arg76(cation-arene) Asp73(through water molecule)	CO benzopyrone CO benzopyrone CO benzopyrone Benzene of benzopyrone Furan ring CO benzopyrone	2.91 1.44 1.44 1.44
9s	-9.4329	Thr165(through water molecule) Gly77(through water molecule) Asp73(through water molecule)	CO benzopyrone CO benzopyrone CO benzopyrone	1.85 1.85 1.85
11b	-11.8855	Arg76 Arg76 Arg76(through water molecule) Arg76(cation-arene) Gly50	CO benzoyl O ether linker CO carboxylic acid Benzene of benzopyrone CO benzopyrone	1.88 3.28 3.52 1.90
12a	-8.5277	Gly77	CO benzopyrone	1.92
12b	-9. 4829	Arg76 Arg76(through water molecule)	CO benzopyrone CO benzopyrone	1.97 2.81

Analysis of the docking results revealed that:

i- The novobiocin-DNA gyrase complex was precisely reproduced by the docking procedure as demonstrated by low root mean square deviation, rmsd (0.6204) and dock score (-13.6636 Kcal/mol, **Table 6**), i.e. the docking protocol was valid. Novobiocin nearly fits in the active site forming various hydrogen bonding interactions with the active site residues: CO carbamate with Thr165, Gly77 and Asp73 (2.02 Å) through water molecule, CO benzopyrone with Gly77 (1.82 Å) through water molecule, CO amide with Arg76 (2.04 Å) through water molecule, NH_2 carbamate with Asp73 (1.91 Å) and Val43 (2.30 Å) through water molecule and OH pyrane with Asn46 (2.05 Å). Also novobiocin formed arene cation interaction of benzene of benzopyrone with Arg76, **Figure 6**.

ii-

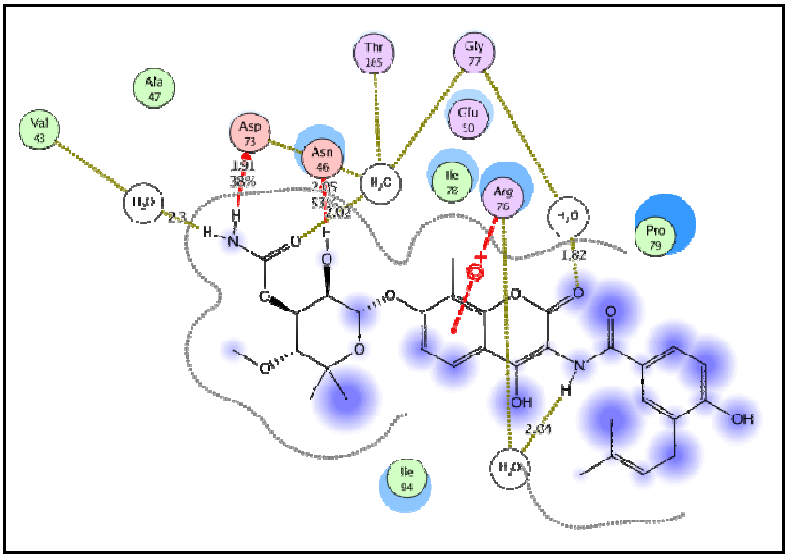


Figure 6: 2D interactions of Novobiocin on the active site of Topoisomerase II

iii- Dock scores for significantly active antimicrobial compounds (**2d**,**e**, **11b**, **3d**,**f**, **4**, **7b**, **9s**, **12a**,**b**) were found to have dock score in the range (-12.0553 to -8.5277 Kcal/mol). A significant correlation between dock scores and antimicrobial activity (diameters of inhibition zones produced before UV-A radiation) of compounds was observed. Benzopyrone derivatives **2d**,**e** and **11b**, which had antimicrobial activity (inhibition zones were 7, 15 and 9 mm, respectively), showed strong binding affinity with the active site of the DNA gyrase enzyme (dock score, -9.4339, -12.0553 and -11.8855 Kcal/mol, respectively).

Linear furobenzopyrone derivatives **3d**,**f** and **7b**, which have antimicrobial activity (inhibition zones were 8, 8 and 13 mm, respectively), showed strong binding affinity with the active site of the enzyme (dock score, -10.2129, -9.7639 and -11.0759 Kcal/mol, respectively).

Angular furobenzopyrone derivatives **4**, **9s** and **12a**,**b**, which have potential antimicrobial activity (inhibition zones were 11, 7, 7 and 8 mm, respectively), showed strong binding affinity with the active site of the enzyme (dock score, -10.9973, -9.4329, -8.5277 and -9.4829 Kcal/mol, respectively).

The highest negative dock score among all tested compounds was estimated for the derivative **2e** (with dimethoxy substitution) that exhibited higher antimicrobial activity than xanthotoxin. This result was attributed to envolvement of 3′,4′-dimethoxy groups in hydrogen bonding with amino acids in active site of the enzyme.

iv- Inspection of the binding mode also demonstrated that all compounds showed one to six hydrogen bonds and arene cation interaction with the enzyme active site residue. Thr165, Val120, Gly77, Arg76, Asp73, Gly50, Asn46 and Val43 are the amino acid residues involved in this interaction, **Table 6**, **Figures 7-9**.

The benzopyrone **2e** with low energy score (-12.0553 Kcal/mol), the most active compound mediated six strong hydrogen bonds with Thr165 (3.12 Å), Thr165 through water molecule (1.64 Å), Gly77 through water molecule (1.64 Å) and Asp73 through water molecule (1.64 Å) with 3′-methoxy group, Val43 through water molecule (2.78 Å) with 4′-methoxy group and Arg76 (1.95 Å) and through water molecule (2.21 Å) with CO benzopyrone, **Figure 7**.

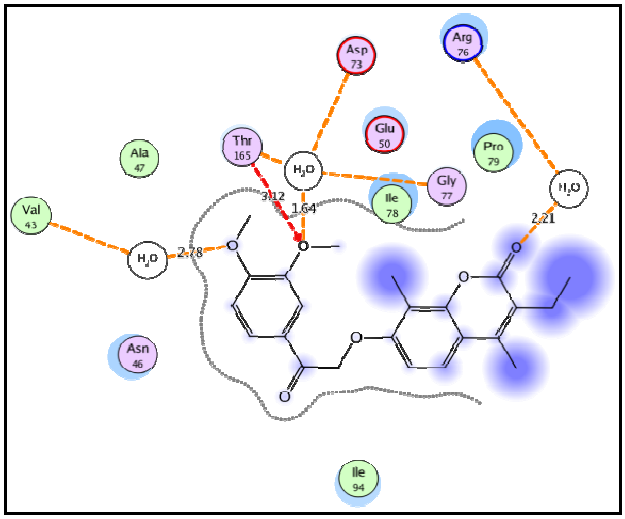


Figure 7: 2D interactions of 2e on the active site of Topoisomerase II

The angular furobenzopyrone **4** with energy score (-10.9973Kcal/mol), the most active compound, mediated four strong hydrogen bonds with Thr165 (3.24 Å) and through water molecule (1.44 Å), Gly77 through water molecule (1.44 Å) and Asp73 through water molecule (1.44 Å) with 3′-methoxy group, **Figure 8**.

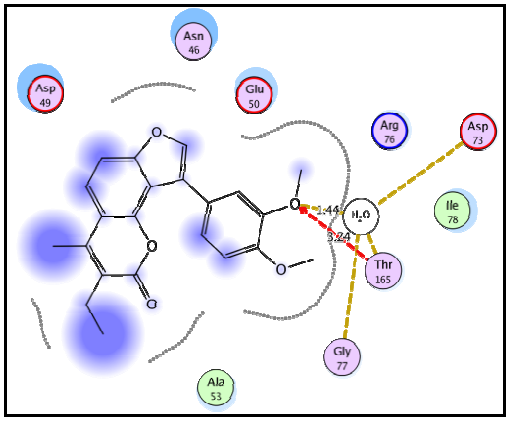


Figure 8: 2D interactions of 4 on the active site of Topoisomerase II

The linear furobenzopyrone **7b** with energy score equal -11.0759 Kcal/mol showed four strong hydrogen bonds with Thr165 (2.91 Å) and through water molecule (1.44 Å), Gly77 through water molecule (1.44 Å) and Asp73 through water molecule (1.44 Å) with CO benzopyrone, in addition, two cation arene were mediated through Arg76 with benzene ring of benzopyrone and furan ring, **Figure 9**.

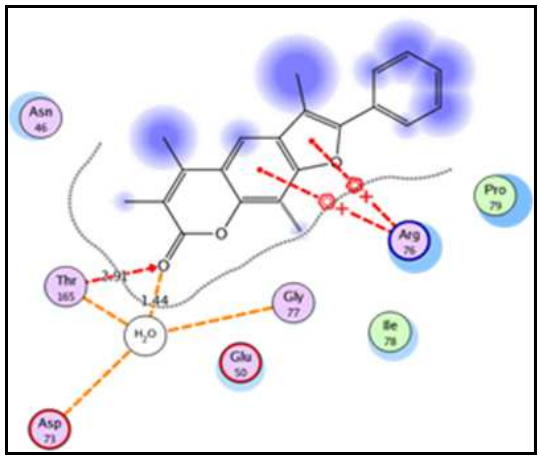


Figure 9: 2D interactions of 7b on the active site of Topoisomerase II

CONCLUSION

According to photosensitizing activity of tested compounds, it was apparent from the results that:

For benzopyrone derivatives: 4-bromophenacyloxy substituent had strong photosensitizing activity after increasing exposure time (40 min.) for UV-A radiation. In addition, 7-cinnamyloxy derivatives and its rearranged product with ethyl group at C3 had photosensitizing activity, but were completely abolished by replacing ethyl with methyl group.

For linear furobenzopyrones: C3 substitution with 4-methylphenyl or 4-methoxyphenyl substituents completely abolished the photosensitizing activity. On the other hand, 3,4-dimethoxyphenyl substituents showed photosensitizing activity after UV-A radiation for 20 min. and increased after exposure for 40 min. Photosensitizing activity of 4-bromophenacyloxy substituent increased by increasing exposure time for UV-A radiation. Moreover, substitution at C2 and C3 with aromatic and alkyl groups, respectively, produced compounds devoid of photosensitizing activity.

For angular furobenzopyrones: substitution at C3 with methyl, phenyl and substituted phenyl moieties, respectively, led to descending order of photosensitizing activity.

Briefly, photosensitizing activity is time dependant (needed long exposure time for UV-A light to exhibit their activity) and directly proportional with concentration for most compounds. Physicochemical properties are dependant factors for activity as expressed from QSAR study. Concerning the Antimicrobial activity, presence of 3,4-dimethoxyphenyl substituent at C7 of benzopyrone or C3 of angular furobenzopyrone, exhibited good antimicrobial activity. This was attributed to possible hydrogen bonding with amino acid residues in the active site of DNA gyrase enzyme as showed by molecular docking.

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