# Synthesis and docking studies of furobenzopyrones of potential antimicrobial and photochemotherapeutic activities

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**Abstract:** Benzopyrone and furobenzopyrone derivatives were designed to be synthesized and screened for antimicrobial and photosensitizing activities. Synthesis of benzopyrone derivatives (**IVa-e** and **Va-d**) was proceeded via etherification of hydroxy benzopyrone **I** and **II** with ω-bromoacetophenone derivatives **IIIa-e** followed by cyclization to achieve linear furobenzopyrone analogues (**IVa-e** and **Va-d**). Surprisingly, an angular furobenzopyrone derivative **VIII** instead of the linear analogue was synthesized in one step reaction from the condensation of hydroxy benzopyrone **II** with 3,4-dimethoxy-ω-bromoacetophenone **IIIe.** This may be attributed to the presence of the two methoxy substituents which are electron donating group. All newly synthesized compounds were evaluated for their antimicrobial and photosensitizing activities by the paper disc diffusion method compared with xanthotoxin. Results showed that, compounds **IVd, IVe, Vd, VIIa** and **VIII** possessed antimicrobial and potential photosensitizing activity. Compounds **IVe** and **VIII** exhibited antimicrobial activity higher than that of xanthotoxin while the other three compounds were less active than xanthotoxin. Docking of the antimicrobial active compounds into topoisomerase II using MOE program was performed in order to predict the correlation between dock scores and antimicrobial activity of these compounds.

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## 1. Introduction

Photochemotherapy is used for treatment of hyperproliferative skin diseases, (psoriasis, mycosis fungoides or cavitary tumors). In skin diseases, a combination of psoralen **1 (Figure 1)** and radiation in the interval of UV-A (320-400 nm), is called PUVA [therapy derived from psoralens (linear furocoumarins) and UV-A] [1].

Furocoumarin and their derivatives are well known photosensitizing drugs for the treatment of some skin diseases (e.g., psoriasis, vitiligo, mycosis and eczema) [2-4], as well as bacterial, fungal [5] and viral infections [6-8]. Moreover, it was reported that psoralen derivatives had applications in the treatment of cutaneous T-cell lymphoma [9], human immunodeficiency disease [10,11] and prevention of organ transplants rejection [12].

Psoralen tricyclic moiety constitutes the basic chromophore of drugs employed in this therapy, in particular, 8-methoxy psoralen [8-MOP] **2**, 5-Methoxy psoralen [5-MOP] **3** and to a lesser extent the synthetic 4,5′,8-trimethylpsoralen [TMP] **4, Figure 1** [1].

Photosensitizing drugs [13] used in photochemotherapy were proved to act through various mechanisms. Type **I** implies substrate photo-oxidation by radical species. Both activated oxygen species (superoxide anion and hydroxyl radical) and radical species are formed by electron transfer photo exited furocoumarins. Type **II** involves generation of singlet oxygen by energy transfer process [14]. In the type **III** mechanism, the triplet photosensitizer may react directly with a substrate in an oxygen independent process [15]. The main biological and therapeutic effects of psoralens are generally attributed to type **III** mechanism, and in particular to photoreaction with DNA due to presence of olefinic double bonds [16,17].

This photo reactive process seems to take place in three phases: i Insertion between adjacent pyrimidine base pairs in the DNA duplex (occurs in the dark). ii Absorption of one photon by psoralen induces the formation of monoadducts with the neighboring pyrimidine via interaction of the respective carbon-carbon double bonds that both compounds have (two types of monoadduct, pyrone type and furan type). iii Monoadduct absorb another photon, inducing its other photo reactive double bond to interact with a thymidine on the opposite strand of DNA, therefore a diaddduct, that cross linked the DNA helix, is formed resulting in interstrand cross linkage. Cross linkage provokes more pronounced biological consequences, but repair of interstrand cross linkage is less effective than repair of the monofunctional adduct [18-20].

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, the cell proliferation. The photodamages consist of the products of photocycloaddition between one molecule of psoralen and two pyrimidine bases of (biadduct). Therefore, 2,3 (furan side) and 5,6 (pyrone) double bonds of the furobenzopyrones are the two photoreactive sites responsible for the DNA photobinding and for the biological activity [20].

The therapeutic treatment, however, is accompanied by some undesirable side effects such as skin phototoxicity and risk of skin cancer. Skin phototoxicity is strictly connected with the bifunctional lesions in DNA which seems to be the main cause of the risk of skin cancer. On the other hand, monoadducts are reported to lack skin phototoxicity.

It is reported that, genotoxicity is the undesirable side effect of linear furobenzopyrones and it is developed from the formation of DNA bifunctional adduct [21]. Therefore DNA monofunctional furobenzopyrone such as 6-carbethoxy **5** [22], pyrido **6** [22], benzo **7** [23] analogues (**Figure 1**) have been designed and synthesized in order to prevent DNA interstrand cross link formation, consequently lack skin phototoxicity and at the same time maintain the photosensitizing activity. In a previous work, a phenyl substituent at C5 in furobenzopyrones increased the photosensitizing activity [24].

Examination of the model of 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, the presence of a methyl group in this position could lead to steric crowding not present in the demethyl case [25]. The results reported about TMP **4** and psoralen **1** lend further support to this interpretation [TMP showed ≈ 98% furan furan addition, while psoralen lacking a methyl at 5-position, showed nearly ≈ 20% pyrone addition].

The previous information enforced us to design and synthesize new linear furobenzopyrone substituted at position 5 and 6 with methyl groups to act as monofunctional agents thus decrease the side effect (phototoxicity) of these novel analogues.

## 2. Experimental

### 2.1. General remarks

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. 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). The $^1$H NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer at 300 MHz and *JEOL-ECA500 NMR spectrometer at 500 MHz in dimethylsulphoxide (DMSO-$d_6$) or $CDCl_3$. 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).

### 2.2. Chemistry

Starting compounds 3,4-Dimethyl-7-hydroxy-8-substituted-2*H*-benzopyran-2-one **I, II** [26-28] and ω-bromoacetophenone derivatives **IIIa-e** [29] were prepared according to reported procedures.

**2.2.1.** General Procedure for synthesis of **3,4-dimethyl-8-substituted-7-(3,4-disubstituted phenacyloxy)-2*H*-benzopyran-2-one (IVa-e** and **Va-d):** A solution of **I** or **II** (0.01 mol) and ω-bromoacetophenone derivatives **IIIa-e** (0.015 mol) in acetone (50 mL) was refluxed in presence of anhydrous potassium carbonate (2.76 g, 0.02 mol) for 24 h. Acetone was distilled then the solid products were filtered, washed and dried to yield 57-95%.

**2.2.1.1. 7-Phenacyloxy-3,4,8-trimethyl-2*H*-benzopyran-2-one (IVa):** Yield 84%. The crude product was crystallized from isopropanol. Mp 195 – 196 °C. IR $\upsilon_{max}$/ cm$^{-1}$: 3100 (CH Ar), 2964, 2872 (CH aliphatic), 1710, 1697 (2 C=O), 1606, 1568, 1556, 1508 (C=C). $^1$H NMR (DMSO-$d_6$) δ *ppm*: 2.08 (s, 3H, $CH_3$), 2.27 (s, 3H, $CH_3$), 2.35 (s, 3H, $CH_3$), 5.77 (s, 2H, $CH_2$), 6.98 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.48-7.70 (m, 4H, H-3′,4′,5′ Ar and H-5 Ar), 8.02 (d, 2H, *J*=7.2 Hz, H-2′,6′ Ar). MS (*m/z*) %: 322 ($M^+$) 15.03%. Anal. Calcd. For $C_{20}H_{18}O_4$ (322.35): C, 74.52; H, 5.63. Found: C, 74.59; H, 5.61.

**2.2.1.2. 7-(4-Methylphenacyloxy)-3,4,8-trimethyl-2*H*-benzopyran-2-one (IVb):** Yield 91%. The crude product was crystallized from isopropanol. Mp 249 – 250 °C. IR $\upsilon_{max}$/ cm$^{-1}$: 3059 (CH Ar), 2922, 2858 (CH aliphatic), 1710, 1683 (2 C=O), 1602, 1573, 1500 (C=C). $^1$H NMR (DMSO-$d_6$) δ *ppm*: 2.07 (s, 3H, $CH_3$), 2.27 (s, 3H, $CH_3$), 2.34 (s, 3H, $CH_3$), 2.39 (s, 3H, $CH_3$), 5.71 (s, 2H, $CH_2$), 6.95 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.38 (d, 2H , *J*=7.8 Hz, H-3′,5′ Ar), 7.54 (d, 1H, *J*=8.7 Hz, H-5 Ar), 7.92 (d, 2H, *J*=8.1 Hz, H-2′,6′ Ar). MS (*m/z*) %: 336 ($M^+$) 8.99 %,. Anal. Calcd. For

$C_{21}H_{20}O_4$ (336.38): C, 74.98; H, 5.99. Found: C, 75.03; H, 6.06.

**2.2.1.3. 7-(4-Methoxyphenacyloxy)-3,4,8-trimethyl-2*H*-benzopyran-2-one (IVc):** Yield 75%. The crude product was crystallized from isopropanol. Mp 169 – 170 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3070 (CH Ar), 2922, 2843 (CH aliphatic), 1708, 1693 (2 C=O), 1602, 1575, 1520, 1500 (C=C). $^1$H NMR (DMSO-$d_6$) δ *ppm*: 2.07 (s, 3H, $CH_3$), 2.26 (s, 3H, $CH_3$), 2.34 (s, 3H, $CH_3$), 3.85 (s, 3H, $OCH_3$), 5.68 (s, 2H, $CH_2$), 6.94 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.09 (d, 2H, *J*=9 Hz, H-3′,5′ Ar), 7.54 (d, 1H, *J*=8.7 Hz, H-5 Ar), 8.00 (d, 2H, *J*=8.7 Hz, H-2′,6′ Ar). MS (*m/z*) %: 352 ($M^+$) 12.28 %. Anal. Calcd. For $C_{21}H_{20}O_5$ (352.38): C, 71.58; H, 5.72. Found: C, 71.58; H, 5.76.

**2.2.1.4. 7-(4-Bromophenacyloxy)-3,4,8-trimethyl-2*H*-benzopyran-2-one (IVd):** Yield 80%. The crude product was crystallized from isopropanol. Mp 240 – 242 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3060 (CH Ar), 2964, 2872 (CH aliphatic), 1710, 1695 (2 C=O), 1606, 1579, 1554 (C=C). $^1$H NMR ($CDCl_3$) δ *ppm*: 2.19 (s, 3H, $CH_3$), 2.31 (s, 3H, $CH_3$), 2.36 (s, 3H, $CH_3$), 5.31 (s, 2H, $CH_2$), 6.70 (d, 1H, *J*=8.7 Hz, H-6 Ar), 6.88 (d, 1H, *J*=8.7 Hz, H-5 Ar), 7.67 (d, 2H, *J*=8.7 Hz, H-3′,5′ Ar), 7.88 (d, 2H, *J*=8.7 Hz, H-2′,6′ Ar). MS (*m/z*) %: 401 ($M^+$) 5.95%, 403 ($M^+$+2) 5.52%. Anal. Calcd. for $C_{20}H_{17}BrO_4$ (401.25): C, 59.87; H, 4.27. Found: C, 59.92; H, 6.04.

**2.2.1.5. 7-(3,4-Dimethoxyphenacyloxy)-3,4,8-trimethyl-2*H*-benzopyran-2-one (IVe):** Yield 71%. The crude product was crystallized from isopropanol. Mp 208 – 209 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3060 (CH Ar), 2960, 2860 (CH aliphatic), 1710, 1697 (2 C=O), 1602, 1571 (C=C). $^1$H NMR (DMSO-$d_6$) δ *ppm*: 2.07 (s, 3H, $CH_3$), 2.27 (s, 3H, $CH_3$), 2.34 (s, 3H, $CH_3$), 3.82 (s, 3H, $OCH_3$), 3.86 (s, 3H, $OCH_3$), 5.70 (s, 2H, $CH_2$), 6.93 (d, 1H, *J*=9.3 Hz, H-6 Ar), 7.11 (d, 1H, *J*=9 Hz, H-5′ Ar), 7.48 (s, 1H, H-2′ Ar), 7.54 (d, 2H, *J*=9 Hz, H-5 Ar), 7.72 (d, 1H, *J*=8.4 Hz, H-6′ Ar). MS (*m/z*) %: 382 ($M^+$) 36.11%. Anal. Calcd. For $C_{22}H_{22}O_6$ (382.41): C, 69.10; H, 5.80. Found: C, 69.08; H, 5.83.

**2.2.1.6. 3,4-Dimethyl-7-phenacyloxy-2*H*-benzopyran-2-one (Va):** Yield 85%. The crude product was crystallized from isopropanol. Mp 178 – 179 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3057 (CH Ar), 2912, 2860 (CH aliphatic), 1703, 1693 (2 C=O), 1624, 1614, 1581, 1566 (C=C). $^1$H NMR (DMSO-$d_6$) δ *ppm*: 2.07 (s, 3H, $CH_3$), 2.37 (s, 3H, $CH_3$), 5.73 (s, 2H, $CH_2$), 7.01 (d, 1H, *J*=9 Hz, H-6 Ar), 7.05 (s, 1H, H-8 Ar), 7.58 (t, 3H, H-3′,4′,5′ Ar), 7.70 (d, 1H, *J*=9 Hz, H-5 Ar), 8.04 (d, 2H, *J*=10.2 Hz, H-2′,6′ Ar). MS (*m/z*) %: 308 ($M^+$) 18.14% . Anal. Calcd. For $C_{19}H_{16}O_4$ (308.33): C, 74.01; H, 5.23. Found: C, 74.13; H, 5.28.

**2.2.1.7. 3,4-Dimethyl-7-(4-methylphenacyloxy)-2*H*-benzopyran-2-one (Vb):** Yield 95%. The crude product was crystallized from isopropanol. Mp 173 – 176 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3007 (CH Ar), 2958, 2872 (CH aliphatic), 1708, 1695 (2 C=O), 1602, 1562, 1508 (C=C). $^1$H NMR ($CDCl_3$) δ *ppm*: 2.18 (s, 3H, $CH_3$), 2.36 (s, 3H, $CH_3$), 2.45 (s, 3H, $CH_3$), 5.34 (s, 2H, $CH_2$), 6.78 (s, 1H, H-8 Ar), 6.94 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.32 (d, 1H, *J*=8.4 Hz, H-3′,5′ Ar), 7.51 (d, 1H, *J*=9 Hz, H-5 Ar), 7.89 (d, 2H, *J*=8.4 Hz, H-2′,6′ Ar). MS (*m/z*) %: 322 ($M^+$) 13.21%. Anal. Calcd. For $C_{20}H_{18}O_4$ (322.35): C, 74.52; H, 5.63. Found: C, 74.49; H, 5.65.

**2.2.1.8. 3,4-Dimethyl-7-(4-methoxyphenacyloxy)-2*H*-benzopyran-2-one (Vc):** Yield 57%. The crude product was crystallized from isopropanol. Mp 167 – 170 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3072 (CH Ar), 2960, 2839 (CH aliphatic), 1710, 1689 (2 C=O), 1604, 1577, 1566, 1508 (C=C). $^1$H NMR (DMSO-$d_6$) δ *ppm*: 2.06 (s, 3H, $CH_3$), 2.36 (s, 3H, $CH_3$), 3.86 (s, 3H, $OCH_3$), 5.64 (s, 2H, $CH_2$), 6.99 (d, 1H, *J*=8.7 Hz, H-6 Ar), 7.01 (s, 1H, H-8 Ar), 7.09 (d, 1H, *J*=9 Hz, H-3′,5′ Ar), 7.69 (d, 1H, *J*=8.4 Hz, H-5 Ar), 8.01 (d, 2H, *J*=8.7 Hz, H-2′,6′ Ar). MS (*m/z*) %: 338 ($M^+$) 1.07%. Anal. Calcd. For $C_{20}H_{18}O_5$ (338.35): C, 70.99; H, 5.36. Found: C, 71.08; H, 5.38.

**2.2.1.9. 7-(4-Bromophenacyloxy)-3,4-dimethyl-2*H*-benzopyran-2-one (Vd):** Yield 85%. The crude product was crystallized from isopropanol. Mp 219 – 220 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3064 (CH Ar), 2920, 2866 (CH aliphatic), 1710, 1697 (2 C=O), 1610, 1583, 1568, 1535 (C=C). $^1$H NMR ($CDCl_3$) δ *ppm*: 2.19 (s, 3H, $CH_3$), 2.37 (s, 3H, $CH_3$), 5.30 (s, 2H, $CH_2$), 6.80 (s, 1H, H-8 Ar), 6.93 (d ,1H, *J*=8.7 Hz, H-6 Ar), 7.52 (d, 1H, *J*=8.7 Hz, H-5 Ar), 7.67 (d, 2H, *J*=7.8 Hz, H-3′,5′ Ar), 7.87 (d, 1H, *J*=7.8 Hz, H-2′,6′ Ar). MS (*m/z*) %: 387 ($M^+$) 0.15%. Anal. Calcd. for $C_{19}H_{15}BrO_4$ (387.22): C, 58.93; H, 3.90. Found: C, 59.02; H, 3.94.

**2.2.2.** General Procedure for synthesis of **5,6-dimethyl-9-substituted-3-(3,4-disubstituted phenyl)-7*H*-furo[3,2-g]benzopyran-7-one (VIa-e** and **VIIa-d):** Compound **IVa-e** or **Va-d** (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 to yield 55-87%.

**2.2.2.1. 3-Phenyl-5,6,9-trimethyl-7*H*-furo[3,2-g]benzopyran-7-one (VIa):** Yield 87%. The crude product was crystallized from isopropanol. Mp 213 – 215 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3026 (CH Ar), 2920, 2825 (CH aliphatic), 1701 (C=O), 1629, 1593, 1566 (C=C). $^1$H NMR (DMSO-$d_6$) δ *ppm*: 2.11 (s, 3H, $CH_3$), 2.45 (s, 3H, $CH_3$), 2.49 (s, 3H, $CH_3$), 7.42 (t, 1H, H-4′ Ar), 7.53 (t, 2H, H-3′,5′ Ar), 7.76 (d, 2H, *J*=7.8 Hz, H-2′,6′ Ar), 7.90 (s, 1H, H-4 Ar), 8.39 (s, 1H, H-2 Ar). MS

(*m/z*) %: 304 ($M^+$) 100%. Anal. Calcd. For $C_{20}H_{16}O_3$ (304.34): C, 78.93; H, 5.30. Found: C, 79.15; H, 5.36.

**2.2.2.2. 3-(4-Methylphenyl)-5,6,9-trimethyl-7*H*-furo[3,2-g]benzopyran-7-one (VIb):** Yield 83%. The crude product was crystallized from isopropanol. Mp 222 – 225 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3113 (CH Ar), 2966, 2873 (CH aliphatic), 1708 (C=O), 1612, 1591, 1566, 1510 (C=C). $^1$H NMR* ($CDCl_3$) δ *ppm*: 2.24 (s, 3H, $CH_3$), 2.43 (s, 3H, $CH_3$), 2.45 (s, 3H, $CH_3$), 2.62 (s, 3H, $CH_3$), 7.32 (d, 2H, *J*=7.65 Hz, H-3′,5′ Ar), 7.52 (d, 2H, *J*=8.4 Hz, H-2′,6′ Ar), 7.78 (s, 1H, H-4 Ar), 7.80 (s, 1H, H-2 Ar). MS (*m/z*) %: 318 ($M^+$) 0.4%. Anal. Calcd. For $C_{21}H_{18}O_3$ (318.13): C, 79.22; H, 5.70. Found: C, 79.28; H, 5.79.

**2.2.2.3. 3-(4-Methoxyphenyl)-5,6,9-trimethyl-7*H*-furo[3,2-g]benzopyran-7-one (VIc):** Yield 58%. The crude product was crystallized from isopropanol. Mp 236 – 238 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3007 (CH Ar), 2926, 2829 (CH aliphatic), 1710 (C=O), 1616, 1591, 1570, 1508 (C=C). $^1$H NMR* ($CDCl_3$) δ *ppm*: 2.23 (s, 3H, $CH_3$), 2.45 (s, 3H, $CH_3$), 2.64 (s, 3H, $CH_3$), 3.87 (s, 3H, $OCH_3$), 7.05 (d, 2H, *J*=8.4 Hz, H-3′,5′ Ar), 7.55 (d, 2H, *J*=8.45 Hz, H-2′,6′ Ar), 7.74 (s, 1H, H-4 Ar), 7.76 (s, 1H, H-2 Ar). MS (*m/z*) %: 334 ($M^+$) 1.19%. Anal. Calcd. For $C_{21}H_{18}O_4$ (334.37): C, 75.43; H, 5.43. Found: C, 75.48; H, 5.50.

**2.2.2.4. 3-(4-Bromophenyl)-5,6,9-trimethyl-7*H*-furo[3,2-g]benzopyran-7-one (VId):** Yield 72%. The crude product was crystallized from isopropanol. Mp 265 – 268 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3107 (CH Ar), 2928, 2838 (CH aliphatic), 1705 (C=O), 1590 (C=C). $^1$H NMR ($CDCl_3$) δ *ppm*: 2.26 (s, 3H, $CH_3$), 2.47 (s, 3H, $CH_3$), 2.63 (s, 3H, $CH_3$), 7.51 (d, 2H, *J*=8.4 Hz, H-2′,6′ Ar), 7.65 (d, 2H, *J*=8.4 Hz, H-3′,5′ Ar), 7.76 (s, 1H, H-4 Ar), 7.82 (s, 1H, H-2 Ar). MS (*m/z*) %: 383 ($M^+$) 0.58%. Anal. Calcd. For $C_{20}H_{15}BrO_3$ (383.24): C, 62.68; H, 3.95. Found: C, 62.71; H, 3.93.

**2.2.2.5. 3-(3,4-Dimethoxyphenyl)-5,6,9-trimethyl-7*H*-furo[3,2-g]benzopyran-7-one (VIe):** Yield 63%. The crude product was crystallized from isopropanol. Mp 203 – 206 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3092 (CH Ar), 2942, 2843 (CH aliphatic), 1697 (C=O), 1620, 1593, 1560 (C=C). $^1$H NMR ($CDCl_3$) δ *ppm*: 2.26 (s, 3H, $CH_3$), 2.47 (s, 3H, $CH_3$), 2.65 (s, 3H, $CH_3$), 3.97 (s, 6H, 2x$OCH_3$), 7.03 (d, 1H, *J*=8.1 Hz, H-5′ Ar), 7.13 (s, 1H, H-2′ Ar), 7.21 (d, 1H, *J*=9 Hz, H-6′ Ar), 7.78 (s, 1H, H-4 Ar), 7.81 (s, 1H, H-2 Ar). MS (*m/z*) %: 364 ($M^+$) 2.05%. Anal. Calcd. For $C_{22}H_{20}O_5$ (364.39): C, 72.51; H, 5.53. Found: C, 72.49; H, 5.58.

**2.2.2.6. 5,6-Dimethyl-3-phenyl-7*H*-furo[3,2-g]benzopyran-7-one (VIIa):** Yield 77%. The crude product was crystallized from isopropanol. Mp 215 – 216 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3082 (CH Ar), 2929, 2860 (CH aliphatic), 1714 (C=O), 1627, 1606, 1577 (C=C). $^1$H NMR* ($CDCl_3$) δ *ppm*: 2.24 (s, 3H, $CH_3$), 2.47 (s, 3H, $CH_3$), 7.43 (t, 1H, H-4′ Ar), 7.46 (s, 1H, H-9 Ar), 7.52 (t, 2H, H-3′,5′ Ar), 7.63 (d, 1H, *J*=6.9 Hz, H-2′,6′ Ar), 7.80 (s, 1H, H-4 Ar), 7.90 (s, 1H, H-2 Ar). MS (*m/z*) %: 290 ($M^+$) 98.64%. Anal. Calcd. For $C_{19}H_{14}O_3$ (290.31): C, 78.61; H, 4.86. Found: C, 78.68; H, 4.91.

**2.2.2.7. 5,6-Dimethyl-3-(4-methylphenyl)-7*H*-furo[3,2-g]benzopyran-7-one (VIIb):** Yield 76%. The crude product was crystallized from isopropanol. Mp 251 – 253 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3080 (CH Ar), 2960, 2860 (CH aliphatic), 1690 (C=O), 1640, 1580, 1550 (C=C). $^1$H NMR ($CDCl_3$) δ *ppm*: 2.26 (s, 3H, $CH_3$), 2.45 (s, 3H, $CH_3$), 2.49 (s, 3H, $CH_3$), 7.35 (d, 1H, *J*=8.4 Hz, H-3′,5′ Ar), 7.49 (s, 1H, H-9 Ar), 7.54 (d, 1H, *J*=7.8 Hz, H-2′,6′ Ar), 7.79 (s, 1H, H-4 Ar), 7.97 (s, 1H, H-2 Ar). MS (*m/z*) %: 304 ($M^+$) 100%. Anal. Calcd. For $C_{20}H_{16}O_3$ (304.34): C, 78.93; H, 5.30. Found: C, 79.04; H, 5.31.

**2.2.2.8. 5,6-Dimethyl-3-(4-methoxyphenyl)-7*H*-furo[3,2-g]benzopyran-7-one (VIIc):** Yield 55%. The crude product was crystallized from isopropanol. Mp 197 – 198 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3060 (CH Ar), 2931, 2839 (CH aliphatic), 1697 (C=O), 1598, 1570, 1508 (C=C). $^1$H NMR* ($CDCl_3$) δ *ppm*: 2.17 (s, 3H, $CH_3$), 2.41 (s, 3H, $CH_3$), 3.89 (s, 3H, $OCH_3$), 6.77 (s, 1H, H-9 Ar), 6.92 (d, 2H, *J*=8.4 Hz, H-3′,5′ Ar), 7.50 (d, 2H, *J*=9.1 Hz, H-2′,6′ Ar), 7.96 (s, 1H, H-4 Ar), 7.98 (s, 1H, H-2 Ar),. MS (*m/z*) %: 320 ($M^+$) 0.95%. Anal. Calcd. For $C_{20}H_{16}O_4$ (320.34): C, 74.99; H, 5.03. Found: C, 75.03; H, 5.08.

**2.2.2.9. 3-(4-Bromophenyl)-5,6-dimethyl-7*H*-furo[3,2-g]benzopyran-7-one (VIId):** Yield 83%. The crude product was crystallized from isopropanol. Mp 242 – 245 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3074 (CH Ar), 2924, 2860 (CH aliphatic), 1708 (C=O), 1608, 1585, 1571, 1508 (C=C). $^1$H NMR ($CDCl_3$) δ *ppm*: 2.21 (s, 3H, $CH_3$), 2.39 (s, 3H, $CH_3$), 6.99 (s, 1H, H-9 Ar), 7.41 (s, 1H, H-4 Ar), 7.54 (d, 2H, *J*=8.4 Hz, H-2′,6′ Ar), 7.62 (d, 2H, *J*=8.1 Hz, H-3′,5′ Ar), 7.87 (s, 1H, H-2 Ar). MS (*m/z*) %: 369 ($M^+$) 3.92%. Anal. Calcd. For $C_{19}H_{13}BrO_3$ (369.21): C, 61.81; H, 3.55. Found: C, 61.90; H, 3.49.

**2.2.3.** Synthesis of **3-(3,4-dimethoxyphenyl)-6,7-dimethyl-5*H*-furo[2,3-h]benzopyran-5-ones (VIII):** Previous procedure adopted for synthesis of **3,4-dimethyl-8-substituted-7-(3,4-disubstituted phenacyloxy)-2*H*-benzopyran-2-one (IVa-e** and **Va-d)** was applied on reacting 3,4-dimethyl-7-hydroxy-2*H*-benzopyran-2-one **II** and 3,4-dimethoxy-ω-bromoacetophenone **IIIe** except that reaction was proceeded for 18 h instead of 24 h.

Yield 59%. The crude product was crystallized from isopropanol. Mp 169 – 171 °C. IR $\upsilon_{max}$/ $cm^{-1}$: 3080 (CH Ar), 2922, 2852 (CH aliphatic), 1691 (C=O), 1612, 1550 (C=C). $^1$H NMR (DMSO-$d_6$) δ *ppm*: 2.07 (s, 3H, $CH_3$), 2.36 (s, 3H, $CH_3$), 3.79 (s, 3H, $OCH_3$), 3.86 (s, 3H, $OCH_3$), 7.08-7.17 (m, 3H, H-2′,5′,6′ Ar), 7.55 (s, 1H , H-2 Ar), 7.76 (d, 1H, *J*=8.7 Hz, H-9 Ar),

7.84 (d, 1H, *J*=8.4 Hz, H-8 Ar). MS (*m/z*) %: 350 ($M^+$) 16.72%. Anal. Calcd. For $C_{21}H_{18}O_5$ (350.36): C, 71.99; H, 5.18 . Found: C, 71.96; H, 5.22.

**2.3. Antimicrobial and photosensitizing activity:**

Nine benzopyrone derivatives and ten furobenzopyrone analogues (nine linear and one angular) were screened for antimicrobial and photosensitizing activity by the paper disc diffusion method [30] compared with xanthotoxin, as a reference compound, that has been clinically investigated. 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 if weakly active, from the inactive ones.

In another test, only active compounds were tested to determine the effect of concentration and the time of radiation (exposure to UV-A) on their photosensitizing activity, which was 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: slant agar seeded with the tested organism (*Bacillus subtilus*), incubated overnight and the broth culture of the organism was prepared.

c) Paper disc: Whattman no. 1 filter paper disc (5mm) were sterilized and impregnated with the different concentrations of the tested compounds which were dissolved in dimethylformamide (DMF), and allowed to dry overnight. Two concentrations were prepared from each of 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 was incubated in the dark at 37 $^oC$ 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 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 $^oC$ overnight and examined for antimicrobial and photosensitizing activity by measuring the produced inhibition zones. Results are presented in **Table 1, Figure 2**.

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

Two groups of discs were prepared. One group of the 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 g of the tested compounds). Results are presented in **Table 2, Figure 3**.

**2.4. Molecular docking**

**Docking procedure**

Docking studies of all the synthesized compounds were performed by molecular operating Environment (MOE) 2008.10 release of Chemical Computing Group, Canada. [31] 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 the evaluation of the binding affinity of the ligand.

**Preparation of the target topoisomerase II**

The X-ray crystal structure of the enzyme with benzopyrone ligand (PDB code 1AJ6) [32] was obtained from the protein data bank in PDB formate. The enzyme was prepared for docking studies.

(i) 3D protonation for the amino acid side chain and Novobiocin. (ii) Deleting all water of crystallization away from the active site. (iii) Isolation of the active site, fixation to be dealt with as rigid structure and recognition of the amino acids. (iv) Creation of dummies around the active site. (v) Studying the interactions of the ligand (Novobiocin) with the amino acids of the active site.

**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.

**Docking running**

Prior to the docking of the benzopyrone 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.

## 3. Results and Discussion

### 3.1. Chemistry

The targeted compounds **VIa-e, VIIa-d,** and **VIII** were synthesized as illustrated in **Scheme 1.** The intermediate compounds 3,4-dimethyl-8-substituted-7-(3,4-disubstituted phenacyloxy)-2*H*-benzopyran-2-one **IVa-e** and **Va-d** were prepared following procedure adopted by Musajo *et al.* [33] to avoid any probability for the opening of the sensitive pyrone ring. Reaction was processed via refluxing 7-hydroxy-2*H*-benzopyran-2-one **I** or **II** with ω-bromoacetophenone derivatives **IIIa-e** in dry acetone containing anhydrous potassium carbonate. The new ether derivatives gave negative ferric chloride test. The proposed structures were confirmed by spectral and analytical data. The IR spectrum revealed absence of band corresponding to phenolic OH at 3300 $cm^{-1}$ and presence of two bands at 1710-1703 and 1697-1683 $cm^{-1}$ corresponding to C=O of pyrone ring and ketone C=O, respectively. $^1$H NMR revealed presence of a singlet signal at δ = 5.30-5.77 ppm assigned to $CH_2$ group confirming ether formation in addition to increased number of aromatic protons. MS spectra showed appearance of their molecular ion peaks.

Cyclization of ether derivatives **IVa-e** and **Va-d** to the corresponding furo[3,2-g]benzopyran-7-ones **VIa-e** and **VIIa-d** were achieved through reflux with alcoholic potassium hydroxide followed by subsequent acidification. The structures of the synthesized compounds were confirmed by spectral and analytical data. IR spectrum showed only one band at 1714-1690 $cm^{-1}$ corresponding to C=O of pyrone ring. $^1$H NMR revealed disappearance of singlet peak assigned to $CH_2$, two doublet peaks of the H-6 and H-5 aromatic protons, respectively and appearance of two singlet peak corresponding to H-4 and H-2 at 7.41-7.96 and 7.76-8.39 ppm, respectively. Presence of these two singlet peaks instead of two doublets and one singlet peaks (in case of derivatives with unsubstituted 8- position) confirmed formation of linear furobenzopyranone. MS spectra showed appearance of their molecular ion peaks.

Attempts for etherification of 3,4-dimethyl-7-hydroxy-2*H*-benzopyran-2-one **II** with 3,4-dimethoxy-ω-bromoacetophenone **IIIe** under previous conditions gave the angular 3-(3,4-dimethoxyphenyl)-6,7-dimethyl-5*H*-furo[2,3-h]benzopyran-5-one **VIII** in one step reaction. Trials to decrease reaction time to obtain the ether derivative gave same product. The structure was deduced by spectral and analytical data. IR showed absence of band corresponding to phenolic OH present in starting compound. $^1$H NMR revealed presence of two singlets at 3.79 and 3.86, one singlet at 7.55 and two doublets at 7.76 and 7.84 assigned to 3′,4′ methoxy groups, H-2, H-9 and H-8, respectively. In addition, absence of singlet signal assigned to $CH_2$ protons confirmed etherification followed by subsequent cyclization and formation of angular furobenzopyranone. MS spectra showed presence of molecular ion peak at 350.

### 3.2. Antimicrobial and photosensitizing activity:

The result of preliminary experiment showed that, linear furobenzopyrone derivative **VIIa** and benzopyrone derivatives **IVe** and **Vd** possessed antimicrobial and potential photosensitizing activity while compounds such as benzopyrone derivative **IVd** and angular furobenzopyrone **VIII** had antimicrobial activity only. Moreover, compounds **IVe** and **VIII** (both had dimethoxy substitution) exhibited antimicrobial activity higher than that of xanthotoxin while the other three compounds were less active than xanthotoxin. The rest of the prepared new compounds were inactive.

The study of time and concentration effect on photosensitizing activity revealed that, increase in time of exposure to light increased photosensitizing activity in all tested compounds except for compound **VIII** while increase in concentration increased photosensitizing activity in all tested compounds. Benzopyrone derivatives (containing bromo substituent) **IVd** and **Vd** and linear furobenzopyrone derivative **VIIa** (contained unsubstituted phenyl at C3) possessed photosensitizing activity greater than xanthotoxin.

### 3.3. Molecular docking

Topoisomerases are enzymes that control the changes in DNA structure by catalyzing the breaking and rejoining of the phosphdiester backbone of DNA strands during the normal cell cycle. Topoisomerases became targets for cancer chemotherapy treatments as topoisomerase inhibitors block the ligation step of the cell cycle, generating single and double stranded breaks in DNA and subsequently lead to apoptosis and cell death. Topoisomerase inhibitors can also function as antibacterial agents [34]. Topoisomerase inhibitors are often divided according to which type of enzyme they inhibit to Topoisomerase I inhibitors and Topoisomerase II inhibitors. There are two subclasses of type II topoisomerases, type IIA and IIB. Type IIA topoisomerases form double-stranded breaks with four-base pair overhangs and able to simplify DNA topology, while type IIB topoisomerases form double-stranded breaks with two base overhangs and do not simplify DNA topology [35]. Small molecules that target type II topoisomerase are divided into two classes: inhibitors and poisons. Inhibitors of type II topoisomerase as mitindomide work by inhibiting the ATPase activity by acting as a non-competitive

inhibitor of ATP. Poisons of type II topoisomerases such as Novobiocin target the DNA-protein complex and lead to increased cleavage, whereas others, such as etoposide, inhibit religation. Topoisomerase poisons are used as both anticancer and antibacterial therapies [36].

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 topoisomerase II active site amino acid interacting ligand moieties and hydrogen bond length for each compound and ligand are listed in **Table 3, Figures 4-8.**

Analysis of the docking results revealed that:

i) Novobiocin-topoisomerase II complex was precisely reproduced by the docking procedure as demonstrated by low root mean standard deviation, rmsd (0.6204) and dock score (-13.6636 Kcal/mol, **Table 3**), i.e. the docking protocol was valid. As shown in **Figure 4**, Novobiocin nearly fits in the active site forming various hydrogen bonding interactions with the active site residues: CO carbamate with **Thr 165, Gly 77** and **Asp 73** (2.02 Å) through a water molecule, CO benzopyrone with **Gly 77** (1.82 Å) through a water molecule, CO amide with **Arg 76** (2.04 Å) through a water molecule, $NH_2$ carbamate with **Asp 73** (1.91 Å) and **Val 43** (2.30 Å) through a water molecule and OH pyrane with **Asn 46** (2.05 Å). Also Novobiocin forms arene cation interaction of benzene of benzopyrone with **Arg 76**.

ii) From the dock scores, all compounds were found to have negative dock score ranging from -12.7145 to -11.0298 Kcal/mol. It means that most of compounds formed stable complex with enzyme. A significant correlation between dock scores and antimicrobial activity of the compounds was observed. For benzopyrone derivatives **IVd, IVe** and **Vd** (dock score, -11.0737, -12.0769 and -11.0759 Kcal/mol, respectively), linear furobenzopyrone derivative **VIIa** (dock score, -11.0298 Kcal/mol) and angular furobenzopyrone **VIII** (dock score, -12.7145 Kcal/mol), the highest negative dock score among all tested compounds was estimated for the derivatives **VIII** and **IVe** (with dimethoxy substitution) that exhibited higher antimicrobial activity than xanthotoxin. Other tested compounds exhibited antimicrobial activity lower than xanthotoxin. These results were attributed to envolvement of 3′,4′-dimethoxy groups in hydrogen bonding with amino acids in active site of the enzyme.

iii) Inspection of the binding mode also demonstrated that all compounds showed from one to six hydrogen bonds and arene cation interaction with the enzyme active site residue. **Thr 165**, **Gly 77**, **Arg 76**, **Asp 73** and **Val 43** are the amino acid residues involved in this interaction and **Gly 77** is the common residue involved in this interaction (**Table 3** and **Figures 5-8**).

Regarding the angular furobenzopyrone **VIII** with lowest energy score (-12.7145 Kcal/mol), the most active compound, mediated four strong hydrogen bonds with **Thr 165** (2.92 Å), **Gly 77** through a water molecule (1.74 Å) and **Asp 73**, through a water molecule (1.74 Å) through 3′-methoxy group and **Thr 165** through a water molecule (2.48 Å), through 4′-methoxy group (**Table 3, Figures 5** and **6)**.

Regarding the benzopyrone **IVe** with low energy score (-12.0769 Kcal/mol), the second most active compound mediated six strong hydrogen bonds with **Thr 165** (1.84 Å) through a water molecule, **Gly 77** (1.84 Å) through a water molecule and **Asp 73** (1.84 Å) through a water molecule through 3′-methoxy group, **Val 43** through water molecule (3.61 Å) through 4′-methoxy group and **Arg 76** (1.95 Å) and through a water molecule (2.57 Å) through CO benzopyrone (**Table 3, Figures 7** and **8)**.

**Table 1: Preliminary screenining of substituted benzopyrone and furobenzopyrone derivatives as antimicrobial and photosensitizing agents.**

| Compound | Control* | Test** |
|---|---|---|
| **DMF** | --- | --- |
| **IVa** | --- | --- |
| **IVb** | --- | --- |
| **IVc** | --- | --- |
| **IVd** | 6 | 6 |
| **IVe** | 17 | 18 |
| **Va** | --- | --- |
| **Vb** | --- | --- |
| **Vc** | --- | --- |
| **Vd** | 8 | 10 |
| **VIa** | --- | --- |
| **VIb** | --- | --- |
| **VIc** | --- | --- |
| **VId** | --- | --- |
| **VIe** | --- | --- |
| **VIIa** | 6 | 10 |
| **VIIb** | --- | --- |
| **VIIc** | --- | --- |
| **VIId** | --- | --- |
| **VIII** | 21 | 21 |
| **Xanthotoxin** | 9 | 12 |

*Disk contains 0.01 mL of the tested and reference compounds.

**Disk contains 0.01 mL of the tested and reference compounds and time of radiation is 20 min.

**Table 2: Antimicrobial and photosensitizing activity of substituted benzopyrone and furobenzopyrone derivatives.**

| Comp. No. | Control* | Test** | Test*** | Test**** |
|---|---|---|---|---|
| **DMF** | --- | --- | --- | --- |
| **IVd** | 6 | 6 | 12 | 18 |
| **IVe** | 17 | 18 | 22 | 25 |
| **Vd** | 8 | 10 | 14 | 19 |
| **VIIa** | 6 | 10 | 14 | 17 |
| **VIII** | 21 | 21 | 21 | 26 |
| **Xanthotoxin** | 9 | 12 | 15 | 19 |

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

**Table 3: Docking results**

| Compound | Energy score S (Kcal/mol) | Binding amino acid | Interacting function group | Hydrogen bond length Å |
|---|---|---|---|---|
| **Novobiocin** | -13.6636 | Thr 165 (through water molecule) | CO carbamate | 2.02 |
| | | Gly 77 (through water molecule) | CO carbamate | 2.02 |
| | | Gly 77 (through water molecule) | CO benzopyrone | 1.82 |
| | | Arg 76 (cation-arene) | Benzene of benzopyrone | |
| | | Arg 76 (through water molecule) | CO amide | 2.04 |
| | | Asp 73 (through water molecule) | CO carbamate | 2.02 |
| | | Asp 73 | $NH_2$ carbamate | 1.91 |
| | | Asn 46 | OH pyrane | 2.05 |
| | | Val 43 (through water molecule) | $NH_2$ carbamate | 2.30 |
| **IVd** | -11.0737 | Thr 165 (through water molecule) | CO acyloxy | 1.82 |
| | | Gly 77 (through water molecule) | CO acyloxy | 1.82 |
| | | Arg 76 (cation-arene) | Benzene of benzopyrone | |
| | | Asp 73 (through water molecule) | CO acyloxy | 1.82 |
| **IVe** | -12.0769 | Thr 165 (through water molecule) | 3′-$OCH_3$ | 1.84 |
| | | Gly 77 (through water molecule) | 3′-$OCH_3$ | 1.84 |
| | | Arg 76 | CO benzopyrone | 1.95 |
| | | Arg 76 (through water molecule) | CO benzopyrone | 2.57 |
| | | Asp 73 (through water molecule) Val | 3′-$OCH_3$ | 1.84 |
| | | 43 (through water molecule) | 4′-$OCH_3$ | 3.61 |
| **Vd** | -11.0759 | Thr 165 | CO benzopyrone | 3.44 |
| | | Gly 77 (through water molecule) | O acyloxy | 2.06 |
| | | Gly 77 (through water molecule) | CO acyloxy | 1.49 |
| | | Arg 76 (cation-arene) | Benzene of benzopyrone | |
| **VIIa** | -11.0298 | Arg 76 (cation-arene) | Phenyl at $C_2$ | |
| | | Val 43 (through water molecule) | CO benzopyrone | 3.69 |
| **VIII** | -12.7145 | Thr 165 | 3′-$OCH_3$ | 2.92 |
| | | Thr 165 (through water molecule) | 4′-$OCH_3$ | 2.48 |
| | | Gly 77 (through water molecule) | 3′-$OCH_3$ | 1.74 |
| | | Asp 73 (through water molecule) | 3′-$OCH_3$ | 1.74 |

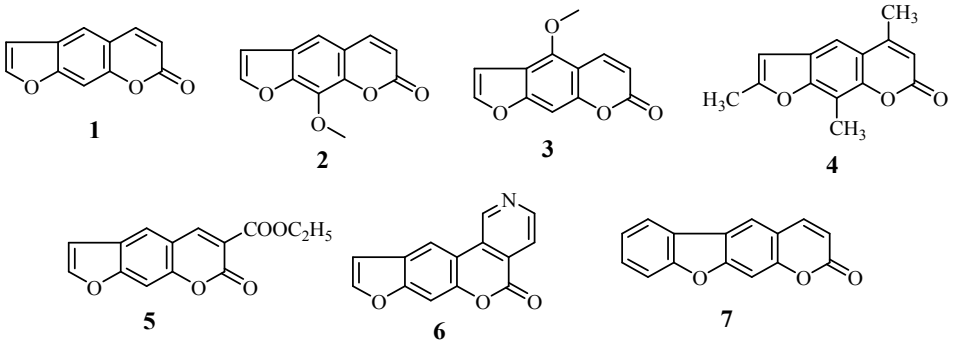



**Figure 1**: Furobenzopyrane compounds

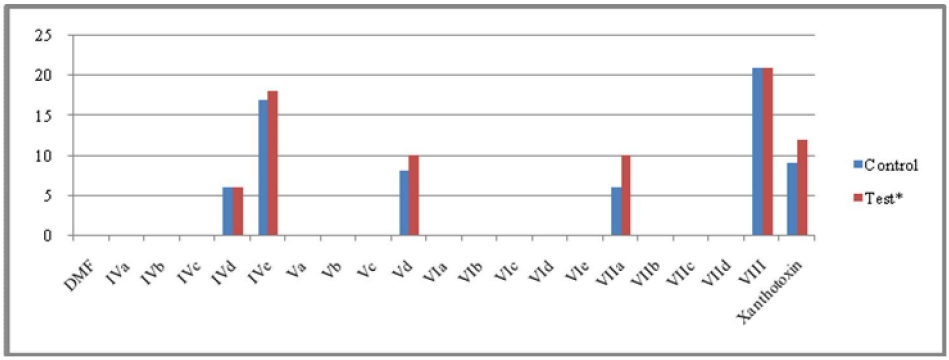



**Figure 2: The bar diagram showing antimicrobial and photosensitizing activity of the tested compounds and their comparison to solvent DMF and Xanthotoxin.**

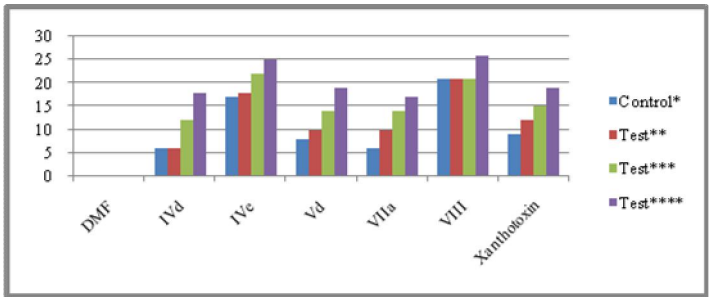



**Figure 3: The bar diagram showing effect of increase in time of radiation and concentration of solution on photosensitizing activity of the tested compounds and their comparison to solvent DMF and Xanthotoxin.**

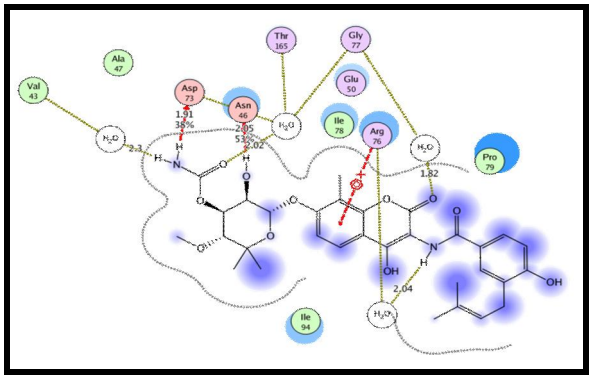



**Figure 4: 2D interactions of Novobiocin on the active site of Topoisomerase II**

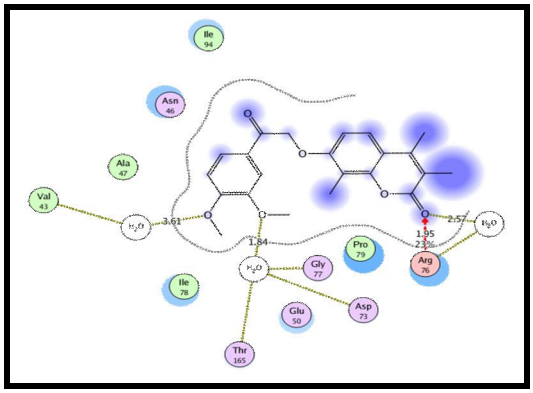



**Figure 5: 2D interactions of compound IVe on the active site of Topoisomerase II**

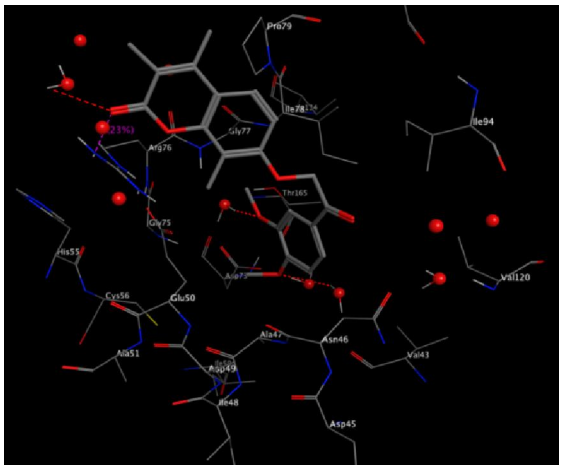



**Figure 6: 3D interactions of compound IVe on the active site of Topoisomerase II**

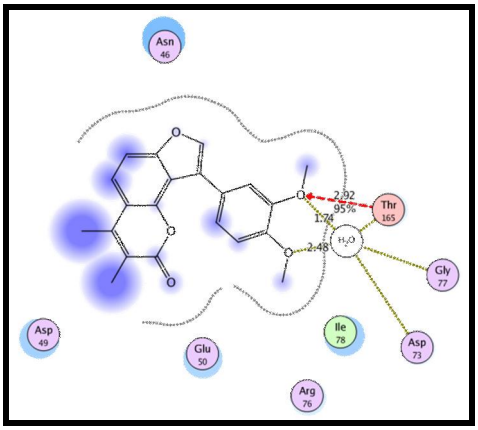



**Figure 7: 2D interactions of compound VIII on the active site of Topoisomerase II**

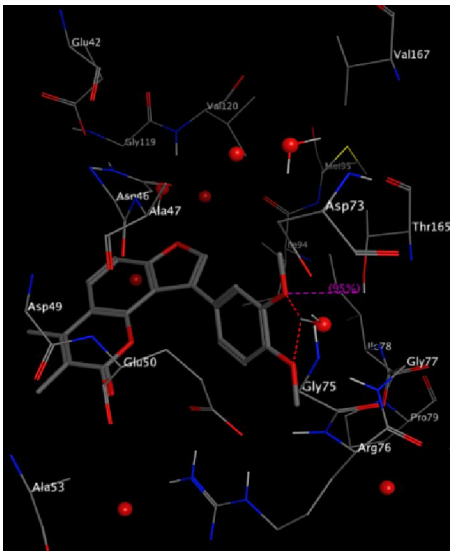
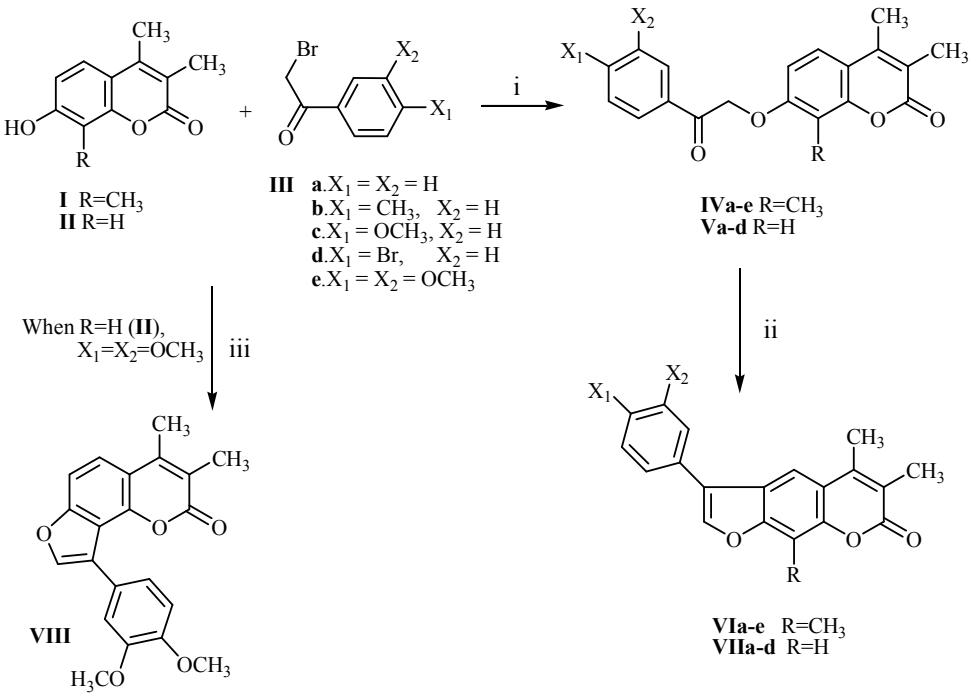



**Figure 8: 3D interactions of compound VIII on the active site of Topoisomerase II**




**Scheme 1**. Reagents and conditions: (i) $K_2CO_3$, dry acetone, reflux 24 h, (ii) Ethanolic KOH, reflux 18 h, (iii) $K_2CO_3$, dry acetone, reflux 18 h

## 4. Conclusion

From the previous results of antimicrobial and photosensitizing screening we concluded that:

The two methoxy groups (compounds **IVe** and **VIII** were active and even higher than xanthtoxin as antimicrobial) and their orientation towards carbonyl of benzopyrone (**VIe** was inactive) are important factors that enhanced antimicrobial activity.

Benzopyrone derivatives containing bromo substitution needed longer time of exposure to light or high concentration to exhibit photosensitizing activity (compounds **IVd** and **Vd** were active as antimicrobial lower than xanthtoxin but exhibited photosensitizing activity higher than xanthotoxin on increasing time of exposure to radiation or increasing concentration of solution used).

Substitution on phenyl ring present at C3 of linear furobenzopyrone derivative abolished activity as only compound **VIIa** (contained unsubstituted phenyl at C3) possessed antimicrobial activity less than xanthotoxin although the photosensitizing activity was good.

Therefore, docking of the antimicrobial active compounds into topoisomerase II using MOE program revealed a correlation between dock scores and experimental antimicrobial activity of these compounds. Presence of two methoxy groups in compounds **IVe** and **VIII** provided hydrogen bonds to amino acids in active site of topoisomerase II enzyme and this may be the reason for their high antimicrobial activity.

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