Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 1102–1105
Bioorganic & Medicinal Chemistry Letters

Synthesis and protective effects of coumarin derivatives against oxidative stress induced by doxorubicin

Adeline Beillerot, Juan-Carlos Rodríguez Domínguez, Gilbert Kirsch and Denyse Bagrel*

Laboratoire d'Ingénierie Moléculaire et Biochimie Pharmacologique, Université Paul Verlaine – Metz, Institut Jean Barriol FR CNRS 2843, UFR SciFA, Campus Bridoux, rue du Général Delestraint, 57070 Metz, France

Received 18 October 2007; revised 2 December 2007; accepted 3 December 2007
Available online 27 December 2007

Abstract—The use of doxorubicin (DOX) in the treatment of solid tumors is limited by cardiotoxicity essentially due to oxidative stress generation. The aim of this study was to identify coumarin derivatives displaying a protective antioxidant activity without affecting DOX antitumoral efficiency. A set of eighteen coumarinic derivatives was synthesized. Their antioxidant power was evaluated in vitro with the FRAP (ferric reducing ability of plasma) method and in human breast adenocarcinoma MCF7 cells using H_2DCFDA (2′,7′-dichlorodihydrofluorescein diacetate) in a cytometric analysis. 4-Methyl-7,8-dihydroxycoumarin was found to exhibit an important antioxidant strength, a low cytotoxicity, and could decrease ROS (reactive oxygen species) production generated by DOX treatment without affecting DOX cytotoxicity in MCF7 cells.
© 2007 Elsevier Ltd. All rights reserved.

Coumarins are a vast group of natural compounds essentially found in green plants. As substitutions can occur at any of the six available sites of their basic molecular moiety (1,2-benzopyrone), these compounds are extremely variable in structure. This structural diversity leads to compounds displaying multiple biological properties that promote human health and help reducing the risk of diseases. In a recent review, Kostova[1] reports that coumarin itself and 7-hydroxycoumarin have provided evidence of in vitro antiproliferative and in vivo antitumor activities. Moreover, coumarins possess, among others, anti-oxidant activities, probably due to their structural analogy with flavonoids and benzophenones.[2] Indeed, this structure type could bind transition metal ions, such as Fe(III), and thus inhibit hydroxyl radical and hydrogen peroxide formation produced by Fenton's reactions. Furthermore, their hydroxy functions are potent H· donors for free radical acceptors, due to electron delocalization across the molecule.[3,4] Thus, coumarin derivatives could be potent ROS scavengers and metal chelating agents. A large number of structurally novel coumarin derivatives have been synthesized in order to improve this antioxidant activity.

Doxorubicin (DOX), an anthracycline antibiotic, is a potent chemotherapeutic agent, often used in the treatment of solid tumors, especially breast and ovarian cancer. However, its therapeutic use is limited by its cardiotoxicity. DOX-induced cardiomyopathy mechanisms are not completely understood but they include formation of oxygen free radicals.[5] The semiquinone form, produced by the DOX reduction by several endogenous enzymes, generates superoxide radicals which rapidly generate other ROS species, such as hydroxyl radical and hydrogen peroxide; these radicals are able to induce apoptosis in cardiomyocytes.[6] Several strategies, which decrease ROS quantity, have been studied to reduce the cardiac toxicity of anthracyclines without affecting their therapeutic efficacy. Different antioxidants such as vitamins A, E, and C, probucol or *N*-acetylcystein, and *S*-allylcystein do not interfere with anthracycline activity in tumor cells but their cardioprotective efficacy was limited.[7] The iron chelator IRCF-187 was found to be effective against DOX cardiotoxicity but its use is limited by an hematological toxicity.[8] Flavonoids have also showed cardioprotective effects[9] but it is well established that there is, at the moment, no universal ideal antioxidant.

The purpose of this study was to investigate the antioxidant effects of some known and new coumarin derivatives on oxidative stress induced by DOX in MCF7 cells.

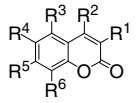
Keywords: Coumarins; Organic synthesis; Oxidative stress; Breast cancer.
* Corresponding author. Tel.: +33 387378404; fax: +33 387378423; e-mail: bagrel@univ-metz.fr

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.12.004

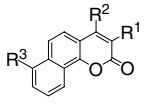
The synthesis of these compounds was carried out as described earlier using sulfated zirconia (compounds **1**–**5**)[10] or zirconyl chloride octahydrate (compounds **6**–**9**)[11] as catalysts. Compound **10** was synthesized in 69% yield in the same manner as **6**–**9**.[12]

Preparations of **11** and **12** were carried out using **3** as starting material; in the first case as reported by Campos et al. with results matching those reported in the literature.[13] In the second one, previous acetylation of the hydroxy groups of **3**[14] allowed us to obtain the diacetyl derivative (**3a**) in 58.4% yield. Subsequent substitution of chlorine by the mercaptan group gave **12** in 38.8%[15] yield.

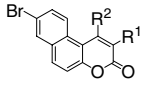
New compounds **13**–**15**, **17**, and **18** were prepared by the classical Pechmann's reaction[16] from the corresponding naphthol derivatives with yields between 25 and 85%,[17] while **16**[18] was prepared in 97% yield from **13** by the procedure of Campos et al.[13]



1: R^1, R^3, R^4, R^6= H; R^2= CH_3; R^5= OH
2: R^1, R^3, R^4= H; R^2= CH_3; R^5, R^6= OH
3: R^1, R^3, R^4= H; R^2= CH_2Cl; R^5, R^6= OH
4: R^1= Cl; R^2= CH_3; R^3, R^4= H; R^5, R^6= OH
5: R^1, R^4, R^6= H; R^2= CH_3; R^3, R^5= OH
6: R^1, R^4, R^6= H; R^2= CH_2Cl; R^3, R^5= OH
7: R^1= Cl; R^2= CH_3; R^3, R^5= OH; R^4, R^6= H
8: R^1, R^3, R^4= H; R^2= CH_3; R^5= OH; R^6= COOH
9: R^1, R^3, R^5, R^6= H; R^2= CH_3, R^4= OCH_3
10: R^1, R^4= H; R^2= CH_3; R^3, R^5= OH; R^6= $COOCH_3$
11: R^1, R^3, R^4= H; R^2= CH_2OH, R^5, R^6 = OH
12: R^1, R^3, R^4= H; R^2= $CH_2S(CH_2)_{11}CH_3$
R^5, R^6 = $OCOCH_3$



13: R^1, R^3= H; R^2= CH_2Cl
14: R^1= H; R^2= CH_2Cl; R^3= OH
15: R^1, R^3= H; R^2= $CH_2S(CH_2)_{11}CH_3$
16: R^1, R^3= H; R^2= CH_2OH



17: R^1= H; R^2= CH_2Cl
18: R^1= H; R^2= Ph

First, these eighteen compounds were characterized for their antioxidative strength and for their cytotoxicity. Their ability to reduce ferric ions in vitro (FRAP test)[19] was compared to that of Trolox, used as reference. Only four derivatives, **2**, **3**, **4**, and **11**, were more potent than Trolox, with, respectively, 4.52 ± 0.13; 5.53 ± 0.13; 5.76 ± 0.04; 5.19 ± 0.21 FRAP units (moles of Fe^{2+} produced by mole of compound) versus 1.84 ± 0.06 for Trolox (Fig. 1a).

These results were confirmed by assessing the ROS production in MCF7 cells by flow cytometric analysis using

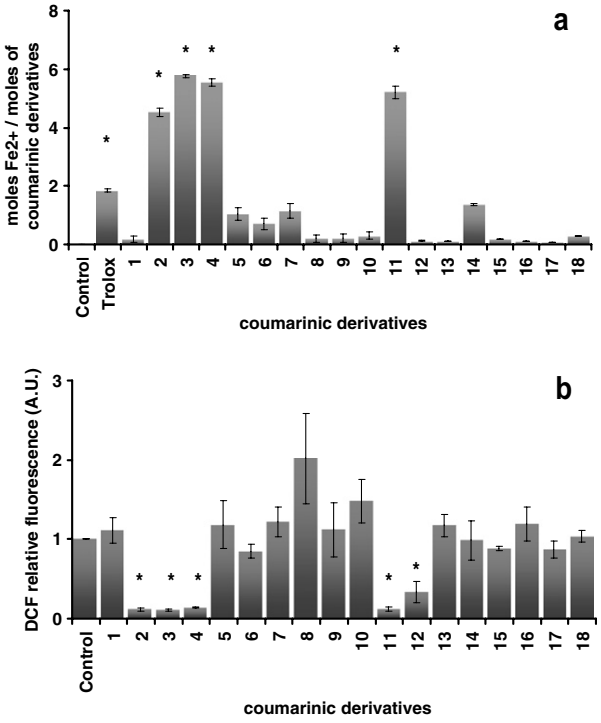


Figure 1. Antioxidant power of coumarinic derivatives. Antioxidant strength of coumarinic derivatives was evaluated, in vitro, with FRAP method (a) and, in MCF7 cells, by intracellular ROS detection using H_2DCFDA detection by flow cytometry (b). (b) MCF7 cells were treated for 24 h with the compounds at the concentration of 100 μM. The final DMSO concentration (0,5% v/v) was the same in all treatments. *Significant differences between control and treated samples: $P < 0.05$, ($n = 3$).

H_2DCFDA (2′,7′-dichlorodihydrofluorescein diacetate).[20] Five compounds, **2**, **3**, **4**, **11**, and **12**, used at the concentration of 100 μM for 24 h, were able to significantly reduce (more than 3-fold) the H_2O_2 production in these cells (Fig. 1b).

Interestingly, derivative **12** did not show in vitro antioxidant properties whereas it displayed this activity in MCF7 cells. We postulate that it could be metabolized by intracellular enzymes to an active antioxidant form. Indeed, the $OCOCH_3$ functions in R5 and R6 positions could be cleaved in hydroxy functions which seem to be more reactive with ROS. Hoult and Paya[21] have shown that 7,8-dihydroxy coumarinic derivatives can trap superoxide radical. Our experiments confirm these results because the most active compounds of our series, **2**, **3**, **4**, and **11**, bear this 7,8-dihydroxy function. Moreover, we synthesized a derivative of **12** in which the two $OCOCH_3$ functions in R_5 and R_6 were replaced by hydroxy groups. Using the FRAP method, this compound displayed an antioxidant power comparable to Trolox (1.88 ± 0.08 for the derivative of **12**).

Next, the cytotoxicity of the antioxidant compounds previously identified was tested in MCF7 cells using a flow cytometric analysis with H_2DCFDA and PI (propidium iodide). PI is a polar, highly fluorescent (red color) compound which can only enter cells that lack membrane integrity. H_2DCFDA is nonpolar and enters via-

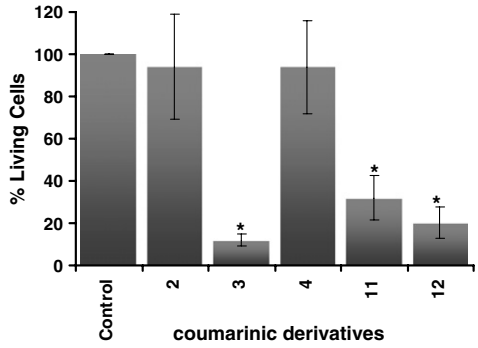


Figure 2. Cytotoxicity of antioxidant coumarinic derivatives. MCF7 cells were treated for 24 h with coumarinic derivatives at the concentration of 100 μM. Cells were stained by PI (propidium iodide) and H_2DCFDA. The viability was assessed by flow cytometry. The final DMSO concentration (0.5% v/v) was the same in all treatments. *Significant differences between control and treated cells: $P < 0.05$; $n = 3$.

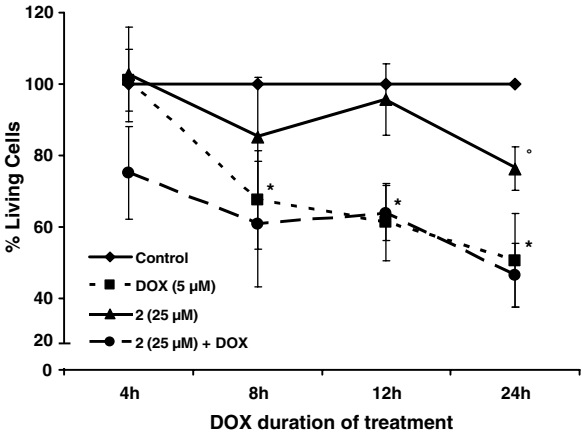


Figure 4. DOX and 2 toxicity in MCF7 cells. Cell viability was assessed by flow cytometry with PI and H_2DCFDA. Significant differences between control and treated cells: *$P < 0.002$, °$P < 0.05$; $n = 3$.

ble cells freely where it is converted to fluorescent dichlorofluorescein (green color). Thus, when using both substances, viable cells are identified with a high green and a low red fluorescence.[22] Figure 2 shows that **3**, **11**, and **12** used at the concentration of 100 μM for 24 h, led to nearly 70% of cell death, while **2** and **4** were poorly cytotoxic. Thus, **2** and **4** could be selected for further studies, and **2** was chosen because of its easy synthesis.

In the second part of this biological evaluation, we tested the protective potentiality of **2** against oxidative stress induced by doxorubicin (DOX) in MCF7 cells. At the concentration of 5 μM (IC_{50}), DOX generated a significant production of ROS in MCF7 cells treated for 24 h. A pre-treatment with **2** at the concentration of 25 μM lowered the ROS levels generated by DOX to that of control cells (Fig. 3). This effect was not obvious with short treatments (up to 12 h). This protective action of **2** was also shown in similar experiments using H_2O_2 instead of DOX (not shown).

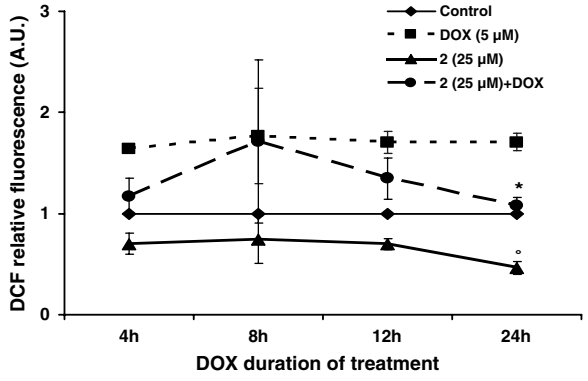


Figure 3. Effects of DOX and 2 on ROS generation in MCF7 cells. The cells were pre-treated with compound **2** at the concentration of 25 μM for 24 h and then DOX at the concentration of 5 μM (IC_{50}) was added for the indicated times. Cells were stained with H_2DCFDA and analyzed by flow cytometry. *Significant differences between DOX and **2** + DOX treated cells: $P < 0.002$; $n = 3$ °Significant differences between control and **2** treated cells: $P < 0.05$; $n = 3$.

Finally, as several mechanisms, including oxidative stress, underlie DOX efficacy in cancer treatments, it was important to know if the antioxidant properties of **2** were able to impair DOX toxicity in MCF7 cells. Figure 4 shows that it is not the case from a 12-h treatment.

In conclusion, the new coumarinic derivative **2** was synthesized and characterized in this work. This compound displayed evident antioxidant properties which were evaluated in vitro and in MCF7 cells. Interestingly, this compound was able to reduce the oxidative stress induced by DOX in the cancerous MCF7 cells without affecting the cytotoxic efficacy of this anthracycline. Further studies are needed to evaluate the protective effects of **2** in non-cancerous cells, and especially in cardiac cells, in order to determine its relevance as an adjuvant in DOX treatments.

Acknowledgments

This study was supported by grants from the Ligue contre le Cancer (Comités de Moselle, de Meurthe & Moselle et de Meuse) and the Conseil Régional de Lorraine. A.B. is recipient of a fellowship provided by the Ligue contre le Cancer (Comité de Moselle).

References and notes

1. Kostova, I. *Curr. Med. Chem. AntiCancer Agents* **2005**, *5*, 29.
2. Farombi, E. O.; Nwaokeafor, I. A. *Clin. Exp. Pharmacol. Physiol.* **2005**, *32*, 667.
3. Sharma, S. D.; Rajor, H. K.; Chopra, S.; Sharma, R. K. *Biometals* **2005**, *18*, 143.
4. Martins, H. F.; Leal, J. P.; Fernandez, M. T.; Lopes, V. H.; Cordeiro, M. N. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 848.

5. Takemura, G.; Fujiwara, H. *Prog. Cardiovasc Dis.* **2007**, *49*, 330.
6. Menna, P.; Minotti, G.; Salvatorelli, E. *Cell. Biol. Toxicol.* **2007**, *23*, 49.
7. Minotti, G.; Menna, P.; Salvatorelli, E.; Cairo, G.; Gianni, L. *Pharmacol. Rev.* **2004**, *56*, 185.
8. Speyer, J. L.; Green, M. D.; Zeleniuch-Jacquotte, A.; Wernz, J. C.; Rey, M.; Sanger, J.; Kramer, E.; Ferrans, V.; Hochster, H.; Meyers, M., et al. *J. Clin. Oncol.* **1992**, *10*, 117.
9. Van Acker, F. A.; Hulshof, J. W.; Haenen, G. R.; Menge, W. M.; Van der Vijgh, W. J.; Bast, A. *Free Radic. Biol. Med.* **2001**, *31*, 31.
10. Rodríguez-Domínguez, J. C.; Kirsch, G. *Tetrahedron Lett.* **2006**, *47*, 3279.
11. Rodríguez-Domínguez, J. C.; Kirsch, G. *Synthesis* **2006**, 1895.
12. 5,7-Dihydroxy-4-methyl-2-oxo-2H-1-benzopyran-8-carboxylic acid methyl ester (**10**): Yield: 68%, mp 225–226 °C. ^{1}H NMR (DMSO-d_6): δ: 12.35 (s, 1H, OH), 10.96 (s, 1H, OH), 6.28 (s, 1H), 5.94 (s, 1H), 3.88 (s, 3H, OCH_3), 3.4 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ: 170.78, 162.15, 162.10, 159.60, 159.05, 155.12, 110.75, 102.80, 99.80, 96.09, 53.35, 24.06.
13. Campos-Toimil, M.; Orallo, F.; Santana, L.; Uriarte, E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 783.
14. 7,8-Diacetoxy-4-chloromethyl-2-oxo-2H-1-benzopyran (**3a**): To a solution of 4.85 g (21.4 mmol) of **3** in 10 mL of pyridine was cooled and 10 mL of acetic anhydride was dropped and left at room temperature, for 1 h. The mixture was poured onto cold water and left to stir for 30 min. The suspension was filtered and the solid was dried at room temperature, protected from light, until constant weight to obtain 5.85 g (88 %) of the diacetate as a light-cream powder (**3a**). Mp 153–155 °C. ^{1}H NMR ($CDCl_3$): δ: 7.53 (d, 1H, H-Ph, J = 8.75), 7.17 (d, 1H, H-Ph, J = 8.75), 6.52 (s, 1H, H-3), 4.61 (s, 2H, CH_2), 2.38 (s, 3H, AcO), 2.32 (s, 3H, AcO). ^{13}C NMR ($CDCl_3$): δ: 167.72, 167.34, 158.78, 149.26, 147.19, 145.68, 130.79, 121.51, 119.04, 116.25, 115.66, 41.14, 20.66, 20.28.
15. 7,8-Diacetoxy-4-dodecylsulfanyl methyl-2-oxo-2H-1-benzopyran (**12**): A suspension of 0.16 g (6.76 mmol) of NaH, previously washed with petroleum ether, in 10 mL of dry dimethylformamide was cooled at 0 °C and 1.62 mL (6.75 mmol) of 1-dodecanthiol was added. The reaction mixture was left to stir for 2 h at room temperature. The mixture was cooled and 2 g (6.44 mmol) of the diacetyl derivative obtained previously was added at once and the reaction mixture was left to stir at room temperature for 60 min. Once all the starting material has disappeared (TLC) the mixture was poured into 100 mL of HCl (6 mol/L) and extracted with dichloromethane. The organic layer was washed with water and dried. The solvent was removed under reduced pressure and the remaining solid was triturated in ethyl ether and filtered to obtain 1.19 g (38.8%) of **12**. Mp 98 °C (from cyclohexane). ^{1}H NMR (DMSO-d_6): δ: 7.82 (d, 1H, H-Ph, J = 10.0), 7.31 (d, 1H, H-Ph, J = 7.5), 6.49 (s, 1H, H-3), 3.95 (s, 2H, CH_2), 2.39 (s, 3H, AcO), 2.33 (s, 3H, AcO), 1.49 (m, 2H, CH_2), 1.20 (s, 20H, CH_2-chain), 0.83 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ: 162.36, 161.81, 157.34, 153.17, 146.57, 141.64, 139.75, 124.87, 117.62, 113.44, 111.68, 108.71, 73.75, 26.58, 26.31, 24.03, 24.00, 23.94, 23.73, 23.63, 23.46, 23.23, 17.11, 15.24, 14.85, 8.83.
16. Pechmann, V. H.; Duisberg, C. *Ber. Dtsch Chem. Ges.* **1884**, *17*, 929.
17. 4-Chloromethyl-naphtho [1,2-*b*] pyran-2-one (**13**): Yield: 85%, mp 171–174 °C. ^{1}H NMR (DMSO-d_6): δ: 8.33 (m, 1H), 8.03 (m, 1H), 7.85 (m, 2H), 7.71 (m, 2H), 6.77 (s, 1H, CH, H-3), 5.08 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ: 160.04, 151.99, 150.86, 134.89, 129.51, 128.51, 128.07, 124.61, 122.75, 122.13, 121.43, 115.37, 113. 32, 42.17.
4-Chloromethyl-7-hydroxy-naphtho [1,2-*b*] pyran-2-one (**14**): Yield: 70%, mp 242–243 °C. ^{1}H NMR (DMSO-d_6): δ: 10.59 (s, 1H, OH), 8.02 (d, 1H, H-Ph, J = 7.5), 7.76–7.68 (m, 2H, H-Ph), 7.47 (t, 1H, H-Ph), 7.06 (d, 1H, H-Ph, J = 7.5), 6.72 (s, 1H, H-Pyr), 5.05 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ: 160.46, 150.50, 150.37, 134.93, 129.11, 127.79, 127.48, 125.21, 125.13, 124.36, 119.58, 115.35, 112.77, 42.03.
4-Dodecylsulfanylmethyl-naphtho [1,2-*b*] pyran-2-one (**15**): Yield: 48.7%, mp 75–76 °C. ^{1}H NMR ($CDCL_3$): δ: 8.57 (m, 1H), 7.88 (m, 1H), 7.71 (s, 2H), 7.65 (m, 2H), 6.42 (s, 1H, CH, H-3), 3.85 (s, 2H, CH_2), 2.54 (t, 2H, CH_2-chain), 1.60 (m, 4H, CH_2-chain), 1.13 (m, 16H, CH_2-chain), 0.88 (m, 3H, CH_3). ^{13}C NMR ($CDCL_3$): δ: 160.69, 152.36, 151.51, 134.90, 128.86, 127.72, 127.24, 124.19, 123.42, 122.69, 122.47, 114.07, 113.69, 33.06, 32.28, 31.97, 29.68, 29.66, 29.61, 29.53, 29.39, 29.25, 29.03, 28.87, 22.74, 14.17.
8-Bromo-1-chloromethyl-naphtho[2,1-*b*] pyran-3-one (**17**): Yield: 66.5%, mp 238–240 °C. ^{1}H NMR (DMSO-d_6): δ: 8.45 (d, 1H, H-Ph, J = 9.25), 8.37 (d, 1H, H-Ph, J = 2.00), 8.22 (d, 1H, H-Ph, J = 9.00), 7.84 (dd, 1H, H-Ph, J = 9.25 and J = 9.25), 7.64 (d, 1H, H-Ph, J = 8.75), 6.88 (s, 1H, H-3), 5.37 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ: 159.46, 155.22, 151.93, 133.91, 132.86, 131.67, 131.36, 128.30, 127.47, 119.38, 119.31, 117.94, 112.54, 46.55.
8-Bromo-1-phenyl-naphtho [2,1-*b*] pyran-3-one (**18**): Yield: 25.4%, mp 166–168 °C. ^{1}H NMR (DMSO-d_6): δ: 8.08 (s, 1H, H-Ph), 7.85 (d, 1H, H-Ph, J = 7.5), 7.69 (d, 2H, H-Ph, J = 7.5), 7.47–7.11 (m, 6H, H-Ph), 5.47 (s, 1H, CH, H-3). ^{13}C NMR (DMSO-d_6): δ: 185.76, 149.75, 149.02, 142.28, 133.50, 133.14, 131.17, 129.98, 129.37, 129.15, 129.02, 128.40, 127.32, 126.51, 124.62, 119.97, 119.33, 117.29, 109.31.
18. 4-Hydroxymethyl-naphtho [1,2-*b*] pyran-2-one (**16**): Yield: 97%, mp 165–167 °C. ^{1}H NMR (DMSO-d_6): δ: 8.26–8.23 (m, 1H, H-Ph), 7.97–7.94 (m, 1H, H-Ph), 7.81–7.61 (m, 5H, H-Ph), 6.70 (s, 1H, H-Pyr), 5.02 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ: 160.07, 151.89, 150.74, 134.79, 129.44, 128.42, 127.99, 124.59, 122.65, 122.03, 121.24, 115.23, 113. 18, 42.10.
19. Benzie, I. F.; Strain, J. *J. Anal. Biochem.* **1996**, *239*, 70.
20. Brault, L.; Denancé, M.; Banaszak, E.; El Maadidi, S.; Battaglia, E.; Bagrel, D.; Samadi, M. *Eur. J. Med. Chem.* **2007**, *42*, 243.
21. Hoult, J. R.; Forder, R. A.; de las Heras, B.; Lobo, I. B.; Paya, M. *Agents Actions* **1994**, *42*, 44.
22. Ross, D. D.; Joneckis, C. C.; Ordonez, J. V.; Sisk, A. M.; Wu, R. K.; Hamburger, A. W.; Nora, R. E. *Cancer Res.* **1989**, *49*, 3776.