SYNTHESIS OF NEW INTERCALATED QUINONES AND THEIR CYTOTOXIC EFFECTS ON CANCER CELL LINES

Feyriel Dridi, Nathalie Bar, Odile Sainte-Catherine, Messaoud Hachemi, Marc Lecouvey, Didier Villemin

SYNTHESIS OF NEW INTERCALATED QUINONES AND THEIR CYTOTOXIC EFFECTS ON CANCER CELL LINES

Feyriel Dridi,[a,b] Nathalie Bar,[a] Odile Sainte-Catherine,[c] Messaoud Hachemi,[b] Marc Lecouvey,[c] Didier Villemin[a]*

Keywords: naphthoquinone; benzofurane; benzodioxine; antitumor activity; cytotoxicity

Naphthoquinones with benzofuran or benzodioxan ring were obtained from dichloronaphthoquinone and were fully characterized. The new benzodioxanes were tested on 4 cancer cells and one of them, a derivative from methyl pyrogallate was found very cytotoxic for cancer cells.

* Corresponding Authors

E-Mail: villemin@ensicaen.fr

[a] Laboratoire de Chimie Moléculaire et Thio-organique, UMR CNRS 6507, INC3M, FR 3038, Labex EMC[3], Labex Synorg, ENSICAEN & Université de Caen, 14050 Caen, France

[b] Laboratoire de Chimie Moléculaire et Composites, Faculté des Sciences de l'Ingénieur, Université M' Hamed, Boumerdes, Algérie

[c] Université Paris 13, Sorbonne Paris Cité, Laboratoire de Chimie, Structure, Propriétés de Biomatériaux et d'Agents Thérapeutiques (CSPBAT), CNRS UMR 7244, 74, Rue Marcel Cachin F-93017

Introduction

Quinones are one of the largest classes of antitumor agents.[1] For example, among the drugs the most potent in cancer chemotherapy, there are the anthracycline antibiotics, Daunorubicin or Doxorubicin.[2,3]

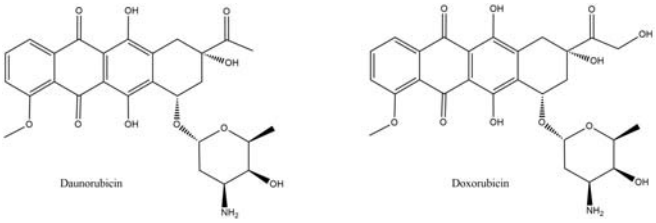


Figure 1. Structures of anthracycline Daunorubicin and Doxorubicin.

Quinoid antitumor agents generally give intercalation into DNA,[1,4] fitting themselves between base pairs of DNA which induce structural distortions due to the formation of stable intercaled complex (for example by alkylation) and it results in inhibition of human topoisomerases. The DNA topoisomerases do not indeed recognize DNA sequences anymore conducting to reading errors during the replication process, followed by cells apoptosis. The inhibition can be also due to the cleavage of DNA, which is sometimes induced by a proton-coupled electron transfer generally when photoexcitation takes place,[5,6] this is particularly the case when quinone or Psoralen derivatives are involved.[7]

DNA-intercalating molecules are usually aromatic, polycyclic and planar such as anthraquinones (as Doxorubicin, Saintopin),[8,9] coumarins[10] (as Elsamicin A[11]), or furanocoumarins (as Psoralen, Angelicin, Bergapten)[12-15] and benzodioxins.[16,17]

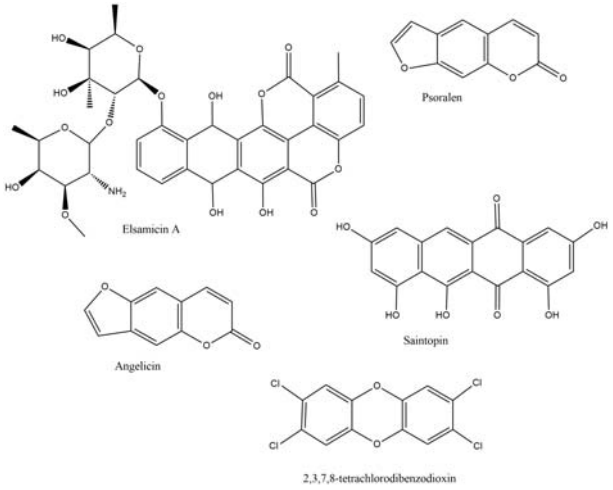


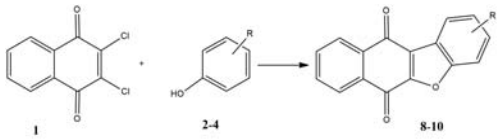
Figure 2. Structures of Psoralen, Saintopin, Elsamicin A, TCDD and Angelicin.

Results and Discussion

We have chosen to study naphthoquinone derivatives containing a naphthobenzofuran or a benzodioxin ring. These molecules are easily available from the reaction of dichloronaphthoquinone with phenols according to Lieberman reaction[18,19] and are well reported in literature.[20]

Synthesis

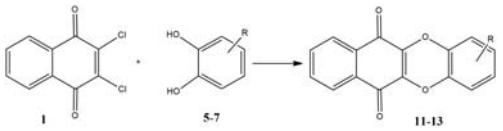
The reactions between a wide range of structurally varied phenols and 2,3-dichloro-1,4-naphthoquinone (2,3-DCNQ) **1** led to the formation of C-O and C-C bonds affording the derivatives of a variety of polycyclic quinones in good yields (Scheme 1).



Scheme 1. Formation of benzofuranonaphthoquinones from DCNQ **1** and phenols **2-4**.

The products were obtained by the reaction of phenol derivatives with commercially available 2,3-DCNQ **1** under basic conditions. Resorcinol **2**, phloroglucinol **3** and Sesamol **4**, furnished [2,3]furan-4,9-dione (benzofurano-naphthaquinones) **8**, **9** and **10** respectively. The compounds **8**, **9** and **10** were previously prepared and described as cytotoxic for tumoral cells but not well characterised. In order to test in the future these compounds, we have fully characterised them by NMR (^{1}H, ^{13}C) and mass spectroscopy.

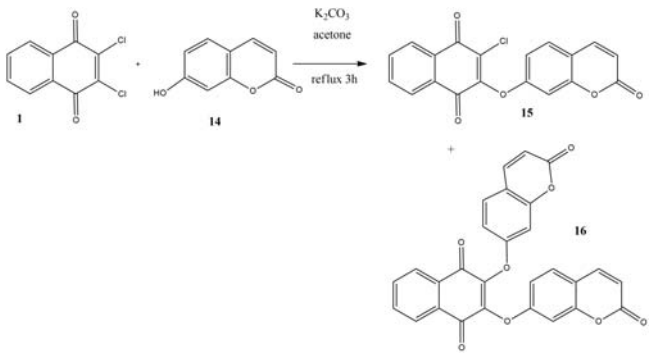
The reactions of 2,3-DCNQ **1** under basic conditions with catechol derivatives permit the formation of benzodioxin derivatives, through the formation of two C-O bonds.



Scheme 2. Formation of benzodioxins from DCNQ **1** and catechols **5-7**.

With catechols like dihydroxycoumarin **5**, pyrogallate derivates **6** and **7**, we have obtained naphthoquinone benzodioxins respectively **11**, **12** and **13**. These compounds are not described in the literature. The reactions were simply performed by mixing reactants in the presence of potassium carbonate and acetone under reflux during several hours. All structures were fully characterized by standard spectroscopic methods (^{1}H, ^{13}C NMR, IR and MS data).

The results and the conditions of these reactions are reported in Table 1.

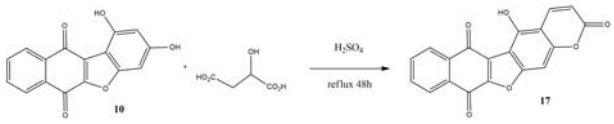


Scheme 3. Reaction of 2,3-DCNQ 1 with Umbelliferon **14**.

With 7-hydroxycoumarin (Umbelliferon) **14**, we are unable to obtain benzofuranonaphthoquinones but we have observed the formation of two products **15** and **16** (Scheme 3).

Clearly the phenol of Umbelliferon is desactivated and the formation of C-C bond does not occur. The attempts to obtain benzofuranonaphthoquinones with a Psoralen substructure, by ring closing of **15**, in the presence of hard or soft Lewis acid ($AlCl_3$ or $BiCl_3$) or palladium acetate oxidative coupling conditions were unsuccessful.

Finally, in order to raise the Psoralen pattern, we have performed the reaction between the compound **9** and malic acid under acidic conditions, according to Pechmann conditions.[21]



Scheme 4. Reaction of **9** in the Pechmann conditions.

We have obtained a mixture of three lactone compounds which we were not able to break up (**17**, **18**, **19**).

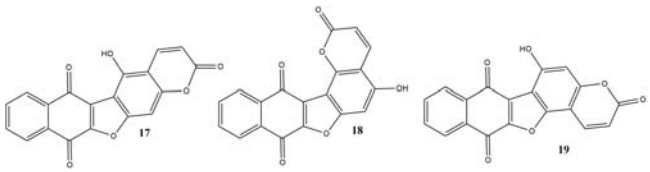


Figure 3. Mixture of products obtained from **9** in the Pechmann conditions.

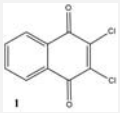
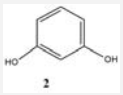
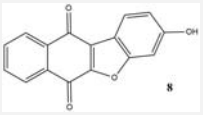
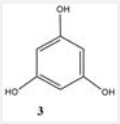
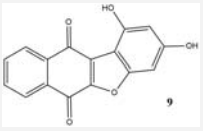
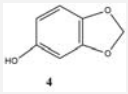
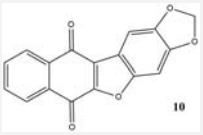
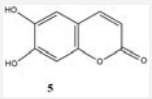
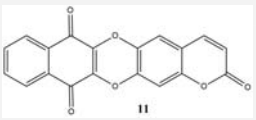
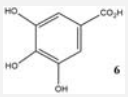
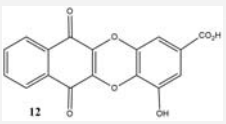
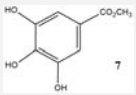
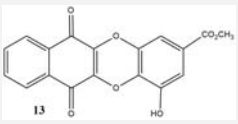
Cytotoxic effects

Compounds **8,**[22] **9**[23] and **10**[22] have been already synthetized and were evaluated in vitro by Cheng et al.[22,24] for their inhibitory actions against cells line panel such as HL-60 (human promyelocytic leukemia) and SCLC (small cell lung cancer) cell lines. Compounds **8** and mainly **9** displayed the better cytotoxicity. The authors attributed this activity to the presence of hydroxyl group on aromatic cycle.

In this study, naphthoquinone benzodioxins were screened on a panel of four other cancer cell types corresponding to four types of cancer and isolated from four different cancer tissues.

The panel comprises human GBM cell line (U87MG); mouse melanoma cell line (B16F10); human epidermoïd cell line (A431); human breast adenocarcinoma (pleural metastasis) cell line (MDAMB231). Throughout our goal to identify new compounds active against cancer cell, three new compounds were evaluated for their antiproliferative activity using a MTT test.

Table 1. Reactions of 2,3-dichloronaphthoquinone **1** with phenols **2-4** and catechols **5-7** under basic conditions

DCNQ	Phenols, catechols	Reactions conditions	Products	Yield[a] %
		EtONa, RT, 12h		65
		KOH, MeOH 30°C, 3h		55
		C_6H_5N, reflux 3h		86
		K_2CO_3, acetone 60°C, 14h		90
		K_2CO_3, acetone 60°C, 14h		40
		K_2CO_3, acetone 60°C, 14h		90

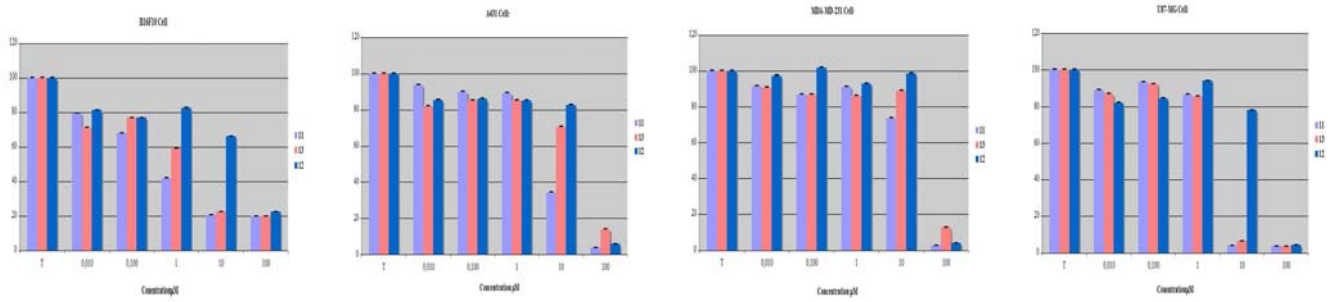


Figure 4. Comparative effects of naphthoquinone benzodioxins on B16F10 cell proliferation (A), A431 cell proliferation (B), MDAMB231 cell proliferation (C) and U87MG cell proliferation (D). B16F10, A431, MDAMB231, U87MG cells were incubated with different concentrations of each compound. After 72 h, B16F10, A431, MDAMB231 and U87MG cell proliferation were assessed as described in "Experimental protocols". Data represent the mean value + SD of three independent experiments.

Naphthoquinone benzodioxins **11**, **12**, **13** were evaluated on the U87MG, B16F10, A431, and MDAMB 231 cell viability (Figure 3). Cells were treated at concentrations ranging from 0.01 µM to 100 µM. Two compounds **11**, **13**, (Table 2) were identified with EC50 inferior to 10 µM on the GBM cell line (U87MG) and the mouse melanoma cell line (B16F10). Among the compounds showing an extended antiproliferative activity **11** was the only one to be active against cancer cell lines derived from epidermoïd cancer cell line (A431).

The differences of structure allowed us to establish structure activity relationships. Results showed that naphthoquinones benzodioxins have limited effect on MDAMB cell proliferation. In the other hand, the cytotoxicity is increased for the other cell models but depends on the structure of the heterocycles. The presence of free carboxylic group and hydroxyl group on the phenyl ring diminished drastically the cytotoxicity (compound **12**). Replacement by an ester group increased significantly the biological activity for three cell lines such as human GBM cell line (U87MG), mouse melanoma cell line (B16F10), human epidermoïd cell line (A431) (compound **11)** and two cell lines human GBM cell line (U87MG); mouse melanoma cell line (B16F10) (compound **13**). The nature of the ester function is an important factor to explain the biological activity differences. The presence of the ester with the free hydroxyl group on the phenyl ring did not increase the cytotoxicity. The lactone introduction leaded to the better results of cytotoxicity but the difference is weak.

Table 2. Antiproliferative values (EC50 µM) of the naphthoquinone benzodioxins on: A431- B16F10 (skin tissue), MDAMB231 (breast tissue), U87MG (brain tissue).

Compounds	U87MG	B16F10	A431	MDA-MB231
11	2 ± 0.9	0.4 ±0.2	9 ± 1	34 ± 2
12	20 ± 5	22 ± 5	18 ± 2	34± 5
13	2 ± 0.9	1.1 ±0.5	18 ± 2	35± 2

*Values are calculated from at least three independent experiments and for each set of experiments each point was repeated 3 times. **EC50 estimation was determined with XLSTAT software.

In conclusion, the compound **11** has given the best biological activity but the compound **13** could be interesting for in vivo biological evaluation. Naphthoquinones benzodioxins are very lipophilic. The presence of a free hydroxyl group should allow to increase the hydrophilicity and the solubilization in biocompatible medium.

Experimental

General

All commercial reagents were purchased from Acros, Aldrich, and Sigma and were used as recieved without further purification. Reaction times were monitored by TLC until no starting material remained. TLC was performed using Silica gel 60 F254 precoated aluminium sheets. Column chromatography was performed using Silica gel Si 60 (40-63 µm). ^{1}H,^{13}C, HMBC and HSQC NMR spectra were recorded on a Bruker AC 400 or Bruker AC 500 spectrometers. Chemical shifts (δ) are expressed in parts per million (ppm) and are referenced to the internal deuterated solvents with tetramethylsilane as the internal standard. Data are reported as follows: multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, dt = doublet of triplet, m = multiplet, brs = broad signal). Coupling constants are expressed in Hertz (Hz). Mass spectra were recorded on a QTOF Micro (Waters) spectrometer with electrospray ionization (ESI, positive mode), lockspray orthophosphoric acid, infusion introduction at 10 µL/min, a source temperature of 80°C and desolvation temperature of 120 °C.

Organic synthesis

Synthesis of 3-hydroxybenzo[d]naphto[2,3-b]furan-6,11-dione (8)

Sodium (1.6 g) is slowly added to ethanol (50 mL) into a 100 mL flask fitted with a reflux condenser, the mixture is stirred until total dissolution of sodium. The flask is then cooled to 0°C and 2,3-dichloro-1,4-naphthoquinone (M=227, 2.27 g, n=0.01 mol) is first introduced by small portions, followed by dropwise addition of resorcinol (M=110, 2.20 g, n = 0.02 mol) dissolved in 25 mL ethanol. The reaction is allowed to proceed under stirring overnight at room temperature. The next day, the mixture is acidified using a solution of HCl (1M). The formed precipitate is collected by suction filtration, washed successively with water, methanol and with diethylether affording the compound 8 as an orange solid. Yield = 65%. mp = 325°C (litt = 320°C [23]). IR ν (cm-1) = 1658, 1578, 1247, 993. NMR 1H (400 MHz, CDCl3) δ = 10.5 (s, 1H, OH), 8.09-8.13 (m, 2H, Hnapht), 7.99 (d, J = 8.5 Hz, 1H, CH-CH-COH), 7.87-7.91 (m, 2H, Hnapht), 7.19 (d, J = 2.0 Hz, 1H, CH-COH), 7.07 (dd, J = 2.0 Hz and J = 8.5 Hz, 1H, CH-CH-COH). NMR 13C (125 MHz, CDCl3) δ = 181.4, 174.0, 160.1, 157.6, 152.3, 134.2, 134.1, 132.7, 132.2, 126.2, 126.1, 123.1, 123.7, 116.5, 114.1, 98.4. MS m/z (% relative abundance): 263 (M-H, 100), 235 (10), 219 (20), 191 (18). Exact mass (ESI-TOF) calculated for $C_{16}H_7O_4$ [M-H] = 263.0344, found 263.0344.

Synthesis of 1,3-dihydroxybenzo[d]naphtho[2,3-b]furan-6,11-dione (9)

Potassium hydroxide (2.0 g; M = 56, n = 0,036 mol) is dissolved in methanol (50 mL) in a 100 mL flask fitted with a reflux condenser, at room temperature, under stirring until total dissolution of potassium hydroxide. The mixture is then heated under nitrogen to 30°C, 2,3-dichloro-1,4-naphthoquinone (M=227, 1.70 g, n = 7.5 10^{-3} mol) is introduced and the medium becomes turbid. 1.4 g of phloroglucinol (M=126, n=11 10^{-3} mole) previously dissolved in 20 mL of methanol, is added dropwise and the reaction is allowed to proceed under stirring during 3h. The formed precipitate is collected by suction filtration, washed with methanol. It is then dispersed in 50 mL of HCl (0.2 M) at 0°C, filtered, washed with ethanol affording **9** as a brown solid. Yield = 55 %. mp = 348°C (litt = 340-342°C [**Error! Bookmark not defined.**]). IR ν (cm^{-1}) = 3391, 1622, 1576, 1562, 1269, 1187, 1003. NMR ^{1}H (400 MHz, DMSO-d6): δ = 8.11-8.17 (m, 2H, Hnapht), 7.88-7.97 (m, 2H, Hnapht), 6.69 (d, *J*=1.8 Hz, 1H, CH-C-Ofuryl), 6.40 (d, *J*=1.8 Hz, 1H, COH-CH-COH), 3.32 (s, 2H, 2 OH). NMR ^{13}C (DMSO-d6) δ = 183.3, 173.1, 162.4, 158.2, 152.6, 150.9, 135.1, 134.0, 132.3, 131.7, 126.7, 126.5, 125.7, 104.5, 99.9, 90.5. MS *m/z* (% relative abundance): 281 (M+H, 100), 253 (10), 225

(29), 183 (38). Exact mass (ESI-TOF) calculated for $C_{16}H_9O_5$ [M+H] = 281.0450, found 281.0439.

Synthesis of (methylenedioxy)benzo[b]naphtho[2,3-d]furan-7,11-dione (10)

0.73 g of Sesamol (M=138, n=5.28 10^{-3} mol), 1 g of 2,3-dichloro-1,4-naphthoquinone (M = 227, n=4.4 10^{-3} mol) and 10 mL of pyridine are introduced in a 50 mL flask fitted with a reflux condenser and the mixture is stirred and heated under reflux during 3 h. After cooling, acetic acid (6M) is added and the neutralisation reaction is allowed to proceed during 12h. Finally, the formed precipitate is collected by suction filtration, washed with water affording the compound **10** as a red-orange solid. Yield = 86%. mp = 309-310°C (lit. = 310°C[22]). IR (ν, cm^{-1}) = 3085, 3056, 2897, 1659. NMR ^{1}H (400 MHz, DMSO-d6) δ = 5.95 (s, 2H, OCH_2O), 6.72 (m, 1H, CH-C-Ofuryl), 6.85 (m, 1H, CH), 7.88 (m, 2H, Hnapht), 8.10 (m, 2H, Hnapht). NMR ^{13}C (125 MHz, $CDCl_3$) δ = 180.8, 174.0, 161.2, 153.0, 152.3, 144.0, 143.3, 134.3, 126.2, 118.6, 117.4, 106.6, 101.3, 97.8. Exact mass (ESI-TOF) calculated for $C_{17}H_8O_5$ [M] = 292.0346, found 292.0350.

Synthesis of 1H-benzo[b]pyrano[3,2-i]dibenzo[b,e][1,4]dioxine-2,7,12-trione (11)

2,3-dichloro-1,4-naphthoquinone (0.5 g, M=227, n = 2.2 10^{-3} mol), 4,5-dihydroxycoumarine (0.35 g, M=178, n = 2.10^{-3} mol), K_2CO_3 (0.61 g, M = 138, n = 4.4 10^{-3} mol) and 4 mL of anhydrous acetone are introduced in a 50 mL flask fitted with a reflux condenser and a $CaCl_2$ dying tube. The medium is under nitrogen stream and heated at 60°C using an oil bath during 14 hours. After cooling, the formation of a red precipitate is observed. It is then filtered, washed with sodium carbonate solution and water affording the compound **11** as a red solid. Yield = 90 %. mp > 399°C. IR ν (cm^{-1}) = 1668, 1656, 1567, 1284, 1265, 979. NMR ^{1}H (500 MHz, DMSO-d6, 40°C): δ = 8.04 -8.03 (m, 2H, Hnapht), 7.93 (d, *J* = 9.5 Hz, CH=CH-C=O), 7.90-7.88 (m, 2H, Hnapht), 7.53 (s, 1H, CH-C-CH=CH-C=O), 7.30 (s, 1H, CH-C-OCO), 6.46 (d, *J* = 9.5 Hz, CH=CH-C=O). NMR ^{13}C (125 MHz, DMSO-d_6, 40 °C): δ = 177.0 (1), 176.4 (2), 159.4 (3), 151.5 (4), 143.1 (5), 139.0 (6), 138.0 (7), 136.9 (8), 134.5 (9), 134.4 (10), 129.7 (11-12), 125.8 (13-14), 115.9 (15), 115.6 (16), 115.2 (17), 105.6 (18). MS *m/z* (% relative abundance): 333 (M+H, 100), 305 (8), 277 (28), 249 (20), 221 (10). Exact mass (ESI) calculated for $C_{19}H_9O_6$ [M+H] = 333.0399, found 333.0414.

Synthesis of 4-hydroxy-6,11-dioxo-6,11-dihydrobenzo[b]dibenzo[b,e][1,4]dioxine-2-carboxylic acid (12)

2,3-dichloro-1,4-naphthoquinone (0.5 g, M=227, n = 2.2 10^{-3} mol), gallic acid (0.37 g, M=170, n = 2.1 10^{-3} mol), K_2CO_3 (0.61 g, M = 138, n = 4.4 10^{-3} mol) and 6 mL of anhydrous acetone are introduced in a 50 mL flask fitted with a reflux condenser and a $CaCl_2$ dying tube. The medium is stirred under nitrogen stream and heated at 60°C using an oil bath during 14 hours. The formation of a precipitate is observed. It is then filtered, washed with sodium carbonate solution and the washing water layers are then acidified to pH = 3. They are then filtered, affording compound **12** as an orange solid. Yield = 40 %. mp > 399°C. IR ν (cm^{-1}) 3394, 1654, 1601, 1440, 1339, 1223, 1196. NMR ^{1}H (125 MHz, DMSO-d6, 40°C): δ = 10.38 (s, 1H, CO_2H), 8.05-8.01 (m, 2H, Hnapht), 7.89-7.85 (m, 2H, Hnapht), 7.28 (d, *J* = 2.0 Hz, 1H, CH-COH), 6.96 (d, *J* = 2.0 Hz, 1H, CH-C-CO_2H). NMR ^{13}C (500 MHz, DMSO-d6, 40°C): δ = 176.8 (CO), 176.7 (CO), 165.7 (CO_2H), 146.1 (COH), 141.1 (5), 138.9 (6), 138.8 (7), 134.3 (8), 134.3 (9), 132.5 (10), 129.7 (11-12), 127.4 (13), 125.7 (14-15), 115.4 (16), 107.8 (17). MS *m/z* (% relative abundance): 323 (M-H, 100), 279 (74), 251 (62), 235 (12). Exact mass (ESI) calculated for $C_{17}H_7O_7$ [M-H] = 323.0192, found 323.0204.

Synthesis of methyl 4-hydroxy-6,11-dioxo-6,11-dihydrobenzo[b]dibenzo[b,e][1,4]dioxine-2-carboxylate (13)

2,3-dichloro-1,4-naphthoquinone (0.5 g, M=227, n = 2.2 10^{-3} mol), methyl gallic ester (0.37 g, M=184, n = 2.10^{-3} mol), K_2CO_3 (0.61 g, M = 138, n = 4.4 10^{-3} mol) and 6 mL of anhydrous acetone are introduced in a 50 mL flask fitted with a reflux condenser and a $CaCl_2$ dying tube. The medium is stirred under nitrogen stream and heated at 60°C using an oil bath during 14 hours. After cooling, the formed precipitate is collected by suction filtration, and then recrystalised in glacial acetic acid affording compound **13** as a red solid. Yield = 90 %. mp > 399°C. IR ν (cm^{-1}) = 3312, 1717, 1676, 1665, 1649, 1595, 1507, 1449, 1370, 1351, 1184, 1004. NMR ^{1}H (500 MHz, DMSO-d6, 60°C): δ = 8.03-8.00 (m, 2H, Hnapht), 7.87-7.85 (m, 2H, Hnapht), 7.31 (d, *J* = 2.0 Hz, 1H, CH-COH), 6.97 (d, *J* = 2.0 Hz, 1H, CH-C-CO_2Me), 3.83 (s, 3H, Me), 3.10 (s, 1H, OH). NMR ^{13}C (125 MHz, DMSO-d6, 60°C): δ = 176.7 (1), 176.6 (2), 164.7 (3), 146.3 (4), 141.3 (5), 138.9 (6), 138.8 (7), 134.3 (8), 134.2 (9), 133.0 (10), 129.8 (11-12), 126.3 (13), 125.7 (14-15), 115.5 (16), 107.7 (17), 52.2 (18). MS *m/z* (% relative abundance): 339 (M+H (100), 243 (8), 214 (15). Exact mass (ESI) calculated for $C_{18}H_{11}O_7$ (M+H) 339.0505, found 339.0515.

Synthesis of 2-chloro-3-(2-oxo-2H-chromen-7-yloxy)naphtalene-1,4-dione (15)

2,3-dichloro-1,4-naphthoquinone (0.5 g, M=227, n = 2.2 10^{-3} mol), 7-hydroxycoumarine (0.39 g, M=162, n = 2.4 10^{-3} mol), K_2CO_3 (0.48 g, M = 138, n = 3.5 10^{-3} mol) and 15 mL of anhydrous acetone are introduced in a 100 mL flask fitted with a reflux condenser and a $CaCl_2$ dying tube. The medium is weakly stirred under a stream of nitrogen and heated under reflux with an oil bath, during 3 hours. After cooling, the formed precipitate is collected by suction filtration. A purification by chromatography (silica,) furnished the compound **15** as a yellow solid. Yield = 53 %. mp = 224°C (litt = 207°C.[25] IR ν (cm^{-1}) = 1724, 1668, 1575, 1251. NMR ^{1}H (400 MHz, $CDCl_3$) δ = 8.23-8.27 (m, 1H, Hnapht), 8.06-8.09 (m, 1H, Hnapht), 7.78-7.87 (m, 2H, Hnapht), 7.67 (d, *J = 9.5 Hz,* 1H, CH=CH-C=O), 7.47 (d, *J = 8.6 Hz,* 1H, CH-CH-C-Onapht), 6.98 (dd, *J = 2.5 Hz, J = 8.6 Hz,* 1H, CH-CH-C-Onapht), 6.92 (d, *J = 2.5 Hz,* 1H, CH-C-Onapht), 6.35 (d, *J = 9.5 Hz,* 1H, CH=CH-C=O). NMR ^{13}C (125 MHz, $CDCl_3$) δ = 178.1, 177.6, 160.4, 158.9, 155.5, 152.8, 142.9, 135.0, 134.9, 134.9, 131.3, 130.5, 129.4, 127.8, 127.5, 115.4, 115.3, 113.6, 104.9. MS *m/z* (% relative abundance): 375, (M+Na, 14), 355 (35), 353 (M+1, 100), 325 (39), 309 (12). Exact mass (ESI-TOF) calculated for $C_{19}H_{10}O_5Cl$ [M+H] = 353.0217, found 353.0232.

Synthesis of 2,3-bis(2-oxo-2H-chromen-7-yloxy)naphtalene-1,4-dione (16)

The compound **16** is isolated after chromatography as a second fraction. NMR ^{1}H (400 MHz, $CDCl_3$): δ = 8.12-8.16 (m, 2H), 7.82-7.87 (m, 2H), 7.63 (d, *J = 9.6 Hz,* 2Hb), 7.41 (d, *J = 8.5 Hz,* 2Hc), 6.91 (dd, J_1 *= 2.4 Hz,* J_2 *= 8.5 Hz,* 2Hd), 6.87 (d, *J = 2.5 Hz,* 2He), 6.32 (d, *J = 9.6 Hz,* 2Ha). Exact mass (ESI-TOF) calculated for $C_{28}H_{14}O_8Na$ [M+Na] = 501.0586, found 501.0582.

MTT assay

Cell lines

A431, MDA-MB-231, B16F10, and U87MG cells were purchased from American Tissue Culture Collection (Rockville, MD, USA). The cells were routinely grown in DMEM (Life Technologies Inc., Gaithersburg, MD, USA), supplemented with 10% FCS, 2 mM L-glutamine, 1 mM sodium pyruvate, 50U mL^{-1} penicillin and 50 mL^{-1} streptomycin (all obtained from Life Technologies Inc.), at 37°C in a 5% CO_2-humidified atmosphere.

Cell proliferation assay

Cell proliferation was assessed using a MTT-microculture assay[26] which is based on the ability of mitochondrial enzymes to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MMT) (Sigma, LO, USA) into purple formazan crystals. Briefly, the cells were seeded in 10 % FCS-DMEM at a density of $5x10^3$ cells/well in 96-well tissue culture plates (Falcon, Strasbourg, France) and allowed to adhere for 24 h. Cells were washed and incubated in DMEM-2% FCS with various concentrations of naphthoquinone benzodioxins varying from 0.1 μM to 100 μM. After a 72 h incubation, cells were washed with PBS and incubated with 0.1 ml of MTT (2 mg mL^{-1}) for 4 h. Cells were lysed in 200 μL DMSO and absorbance corresponding to solubilized formazan pellet (which reflects the relative viable cell number) was measured in a Labsystem plate reader at 570 nm. Concentration-response curves were constructed and the EC_{50} values (concentration of the compound inhibiting 50% of cell proliferation) were determined using the XL STAT software (Addinsoft).

Conclusions

Benzofuran napthoquinones previously reported as cytotoxin on tumor cells were synthetized and fully characterized. The new benzodioxan naphthoquinones were tested on 4 cancer cell lines, two of them displayed antiproliferative activity, and the ester **11** was found to be the most promising. According to our knowledge the cytotoxicity on cancer cells of benzodioxan naphthoquinones has not been reported in literature yet.

Acknowledgments

We gratefully acknowledge the CNRS (National Center for ScientificResearch), the "Region Basse Normandie", the University of Boumerdes (Algeria), the Franco-Algerian program for the Superior Education (PROFS), and the Algerian-French cooperation for a BAF grant for Feyriel Dridi. Also the authors thank Rémy Legay and Baptiste Rigaud for NMR spectra and Mrs. Karine Jarsalé for ESIMS and HRMS analysis.

References

[1]Asche, C., *Mini-Rev. Med. Chem.*, **2005**, *5(5),* 449-67.

[2]Aubel-Sadron, G., Londos-Gagliardi, D., *Biochimie,* **1984**, *66(5)*, 333-352.

[3]Preobrazhenskaya, M. N., Tevyashova, A. N., Olsufyeva, E. N., Huang Kuo-Feng, Huang Hsu-Shan, *J. Med. Sci.,* **2006**, *26(4),* 119-128.

[4]Campanelli, A. R., D'Alagni, M., Marini-Bettolo, G. B., *FEBS Letters*, **1980**, *122*, 256-260.

[5]Koch, T., Ropp, J. D., Sligar, S. G., Schuster, G. B., *Photochem. Photobiol.,* **1993**, *58(4),* 554-8.

[6]Ou, C. N., Tsai, C. H., Song, P.-S., In: *Research in Photobiology,* ed. A. Castellani. New York Plenum, **1977**, 257.

[7]Sage, E., Le Doan, T., Boyer, V., Helland, D. E., Kittler, L., Moustacchi, H. C., *J. Mol. Biol.,* **1989**, *209(2)*, 297-314.

[8]Kellogg, G. E., Scarsdale J. N., Fornari Jr, F.A., *Nucleic Acids Res.,* **1998**, *26(20)*, 4721-32.

[9]Nabiev, I., Chourpa, I., Manfait, M., *J. Phys. Chem.*, **1994**, *98*, 1344-50.

[10]Thati, B., Noble, A., Creaven, B. S., Walsh, M., McCann, M., Kavanagh, K., Devereux, M., Egan, D. A., *Cancer Lett.*, **2007**, *248(2)*, 321-331.

[11]Barcelo, F., Portugal, J., *FEBS Letters*, **2004**, *576*, 68-72.

[12]Al-Sehemi, A. G., El-Gogary, S. R., *Chin. J. Chem.,* **2012**, *30(2)*, 316-20.

[13]Demaret, J. P., Brunie, S., Ballini, J. P., Vigny, P., *Photochem. Photobiol.*, **1989**, *50(1)*, 7-21.

[14]Rocha, M. S., Lúcio, A. D., Alexandre, S. S., Nunes, R. W., Mesquita, O. N., *Appl. Phys. Lett.*, **2009**, *95*, 253703.

[15]Muniandy, P. A., Thapa, D., Thazhathveetil A. K., Liu, S-T., *J. Biol. Chem.*, **2009**, *284(41)*, 27908-17.

[16]Csík, G., Rontó, G., Nocentini, S., Averbeck, S., Averbeck, D., Besson, T., Coudert, G., Guillaumet, G., *J. Photochem. Photobiol. B.*, **1994**, *24(2),* 129-39.

[17]Lee, H. H., Palmer, B. D., Boyd, M., Baguley, B. C., Denny, W. A., *J. Med. Chem.,* **1992**, *35(2)*, 258-66.

[18]Liebermann, C., *Chem. Ber.*, **1899**, *32*, 916-25.

[19]Liebermann, C., *Chem. Ber.*, **1900**, *33*, 566-78.

[20]Satori, M. F., *Chem. Rev.*, **1963**, *63*, 279-296.

[21]Pechmann, H., *Chem. Ber.*, **1884**, *17(1)*, 929-36.

[22]Cheng, C. C., Dong, Q., Liu, D. F., Luo, Y. L., Liu, L.F., Chen, A. Y., Yu, C., Savaraj, N., Chou, T. C., *J. Med. Chem.*, **1993**, *36*, 4108-12.

[23]Kostanecki, S., von Lampe, V., *Chem. Ber.*, **1908**, *41,* 2373.

[24]Chang, H. X., Chou, T. C., Savaraj, N., Liu L. F., Yu C., Cheng, C.C., *J. Med. Chem*., **1999**, *42*, 405-08.

[25]Tandon, V. K., Maurya, H. K., *Heterocycles*, **2009**, *77*, 611-615.

[26]Mosmann T., *J. Immunol. Methods*, **1983**, *65*, 55-63.

Received: 12.10.2014.
Accepted: 16.11.2014.