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Original article

Synthesis of 4-hydroxycoumarin and 2,4-quinolinediol derivatives and evaluation of their effects on the viability of HepG2 cells and human hepatocytes culture

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Abstract

We report here the synthesis of aromatic coumarins and aromatic α-quinolones which were evaluated in vitro for their protective potentialities against *tert*-butyl hydroperoxide (*t*-BHP)-induced oxidative damage on human liver cell death, i.e., human hepatoma HepG2 cell line and human hepatocytes in primary culture. We found that the presence of a benzylidene at the 3-position or a heterocycle with N and S heteroatoms on the benzopyrone or quinolone system was essential for the protective effect of these compounds against *t*-BHP-induced decrease in viability of cells. We found also that a methoxy group on the aromatic ring systems decreased this potential. *t*-BHP-induced cytotoxicity in primary cultures of human hepatocytes could be therefore prevented by these compounds suggesting that they could display hepatoprotective effects in humans.

Keywords: Coumarins; Quinolones; Human liver cells; Cell viability

1. Introduction

During these last 30 years, a great number of heterocyclic coumarins or quinolones have been synthesized and their biological activities extensively evaluated. Indeed, heterocyclic coumarins and quinolones display some interesting pharmacological activities, particularly as anticoagulants, photosensibilizing drugs, estrogen mimics or antiproliferative compounds [1–9].

Tert-butyl hydroperoxide (*t*-BHP) is a well known inducer of oxidative stress in biological systems and it is believed that reactive free radical intermediates including peroxy, alkoxy and carbon centred radicals are mediators of peroxide-dependant injury [10,11]. The human hepatoma cell line HepG2 is classically used [12–18] to investigate the mechanisms of the cytotoxic or cytoprotective effects of xenobiotics. In particular, *t*-BHP-induced oxidative stress, its consequent cytotoxicity, and the protective effects of various compounds against this toxicity have been recently described using this specific cell line [19–21].

We report here the synthesis of benzothiazinic derivatives of coumarin (**2a–b**) and quinolones (**2c–d**) (Fig. 1) as well as 3-benzylidene derivatives of coumarins (**5a–b**) and quinolo-

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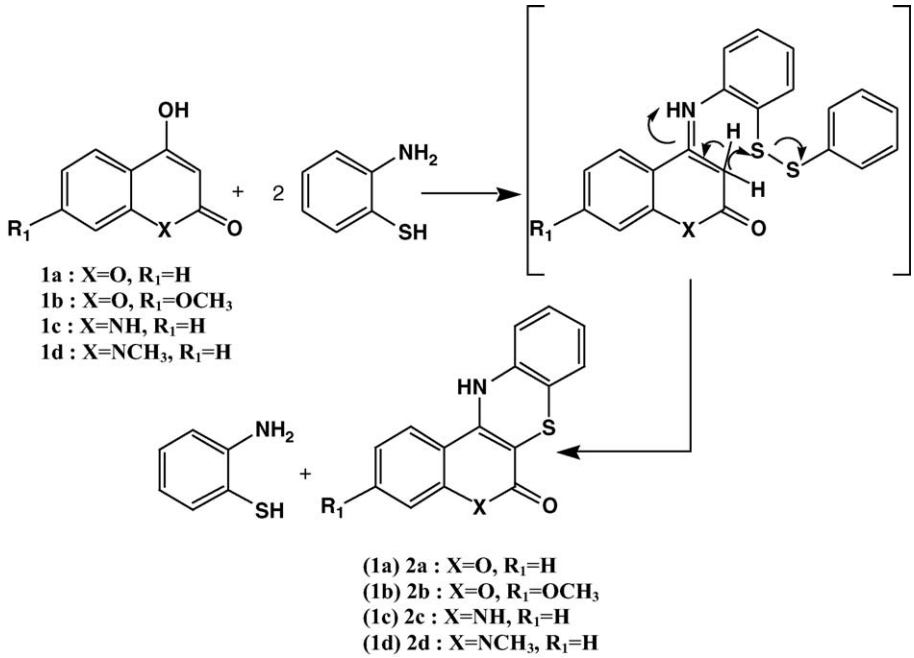

Fig. 1. Synthesis of quadricyclic coumarins and quinolones.

nes (**5c–d**) (Fig. 2) [22–26]. We were investigated their effect on *t*-BHP-induced cytotoxicity in HepG2 cells and primary cultures of human hepatocytes, widely used for the evaluation of hepatotoxic and hepatoprotective compounds in humans [27–29]. We were also tested the potential hepatoprotective activity of the reported derivatives and their effects on *t*-BHP-induced cytotoxicity in primary cultures of human hepatocytes.

2. Chemistry

The 6,12-dihydrobenzopyrano[3,4-*b*][1,4]benzothiazin-6-ones **2a–b** and 6,12-dihydroquinolo [3,4-*b*][1,4]benzothiazin-6-ones **2c–d** were prepared as shown in Fig. 1. The quadricyclic system was synthesized according to the method of Tabakovic [3,22,25]. The addition of 2-aminothiophenol on the substituted 4-hydroxycoumarins **1a–b** or on the substituted 2,4-quinolinediols **1c–d** was made in polar aprotic solvents such as DMSO or DMF. This addition was accompanied by the elimination of one molecule of water and conducted to a non-isolable enaminone intermediate [26]. Subject to a nucleophilic attack at the 3-position of the coumarin or the quinolone, the bis(o-aminophenyl)disulfde (DAPDS) motif was cleaved and the enaminone led to the desired products **2a–d** by an intramolecular cyclisation. The expected compounds were crystallized from DMSO or DMF on cooling at room temperature and were isolated by filtration under vacuum. Their structures were assigned from FTIR and ^{1}H-NMR spectroscopy, EI-MS spectrometry and elemental analysis.

The 3-benzylidenecoumarins **5a–b** and 3-benzylidenequinolones **5c–d** were prepared as shown in Fig. 2. The Knovenagel condensation of 4-hydroxycoumarins **1a–b** or 2,4-quinolinediols **1c–d** on the substituted benzaldehydes (**3, 4**) carried out in pyridine gave compounds **5a–d** in only one diastereoisomeric form (*Z*). These compounds were crystallized from alcohol and were isolated by filtration under vacuum.

The structural determination of compounds **5a–d** was realised on the basis of NMR studies. The stereochemistry (*Z*) of compounds (**5a–d**) was easily determined by simple

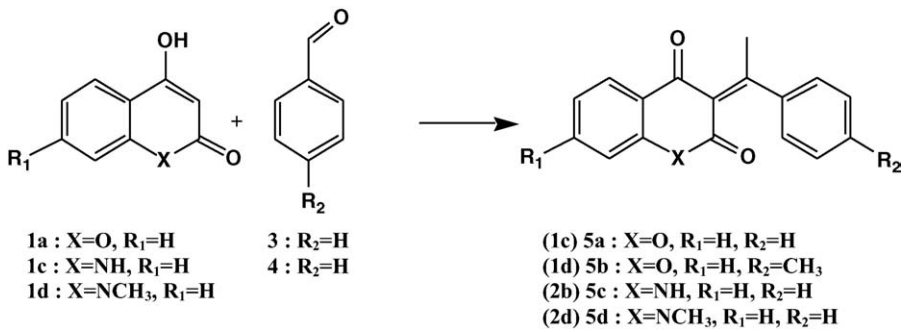

Fig. 2. Synthesis of benzylidene coumarins or quinolones.

examination of ^{1}H-NMR spectra and comparison with the data reported in the literature [30,31]. For compounds **5a–d** the (*Z*) configuration has been characterised by the presence of an intramolecular hydrogen bond.

3. Results and discussion

Among the synthesized products, the coumarin **2a** was particularly interesting for its protective activity against *t*-BHP-induced cytotoxicity in the cultured human hepatoma HepG2 cell line. Actually, it was shown that a concentration-dependent protective effect on 3 mM *t*-BMP-induced cytotoxicity in HepG2 cells (Fig. 3b). Of note, **2a** displayed a complete protection at 10 μM comparable to that of quercetin (tested as a reference antioxidant compound) at the same concentration (Fig. 3c) [32–35]. A significant effect on HepG2 cell line viability was also observed with *t*-BHP from 1 mM and about 50% cell mortality was observed at 3 mM *t*-BHP (Fig. 3a).

Table 1 shows the percentage of HepG2 cells viability co-incubated for 3 h with 3 mM *t*-BHP in the absence or in the presence of the tested compounds at various concentrations.

As shown in this table, the presence of a benzylidene group at the 3-position (compounds **5a–d**) or a heterocycle with two heteroatoms on the coumarin or the quinolone (**2a–d**) system appeared essential for the protective effect of coumarin and quinolone derivatives. Note that compounds with a methoxy group on the aromatic ring (**2b, 5b**) are totally devoid of cytoprotective activity. Our data confirm also recent studies from other groups [19–21], which were reported that the human hepatoma cell line HepG2 provides a useful model to study compounds with antagonist effects on *t*-BHP-induced oxidative stress and cell damage.

In order to assess how the data obtained with the human hepatoma cell line HepG2 correlate with those obtained with primary cultures of human hepatocytes, the protective effects of compound **2a** were also assessed on primary cultures of human hepatocytes isolated from three different donors. Fig. 4a shows that *t*-BHP-induces a significant effect on the cell viability of human hepatocytes with 50% cell viability at 0.3 mM *t*-BHP. It thus appeared that primary cultures of human hepatocytes were much more sensitive to *t*-BHP-induced oxidative stress than the cell line HepG2. Actually, we have previously reported that HepG2 cells express only in part some fundamental metabolic pathways [13] and mimic in part the pleiotropic responses to xenobiotics [14] of primary cultures of human hepatocytes. It could be possible that the metabolic pathways potentially involved in the production of reactive free radical intermediates [10–11] are only partly expressed in HepG2 cells. Another possibility could be that the HepG2 cell line of human liver origin is more resistant to oxidative stress-induced damages than their primary human hepatocyte counterparts, due to a higher expression of defence systems against oxidative stress. As we recently described that the antioxidant status in primary cultures of rat hepatocytes was depressed compared to livers [36], a similar pattern in primary cultures of human hepatocytes could explain their greater sensitivity to oxidative stress. Further studies are required to explore this hypothesis.

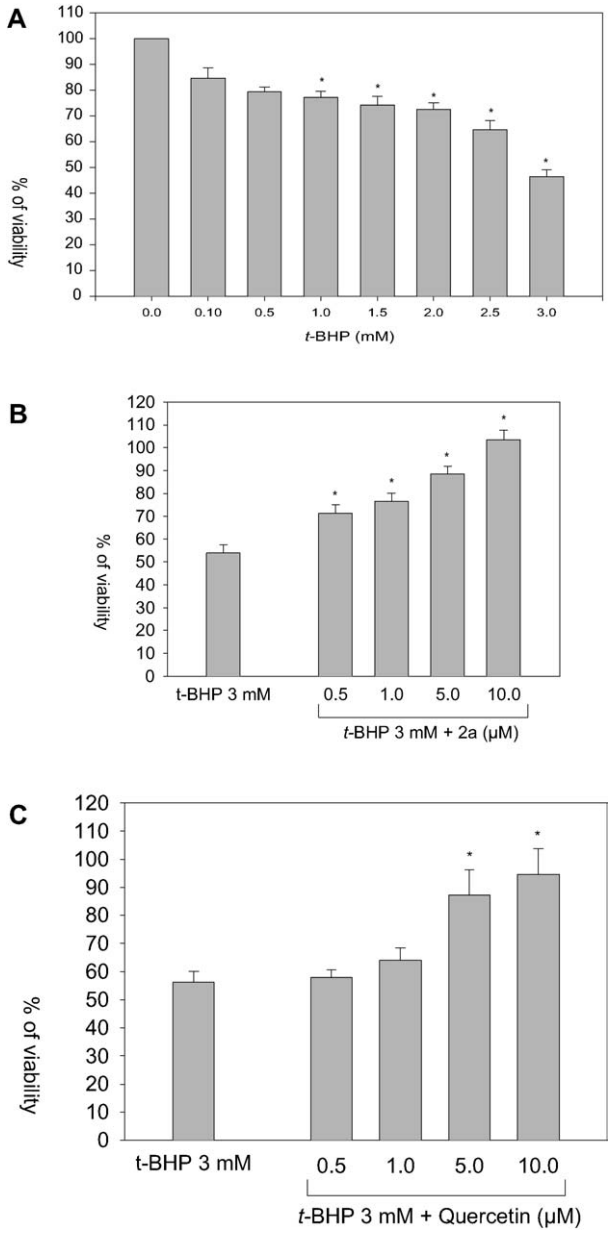

Fig. 3. Cytotoxic effect of *tert*-butylhydroperoxide in HepG2 cells. Results are reported as mean ± SEM of three independent experiments. * Values significantly different from control, $P < 0.05$ (Tuckey's test). (a) Of 3 mM *t*-BPH-induced cytotoxicity in HepG2 cells (b) Protective effect of compound **2a** (c) of quercetin. Results are reported as mean ± SEM of three independent experiments. * Values significantly different from control, $P < 0.05$ (Tuckey's test).
[a] Values significantly different from (*t*-BHP 3 + 10 mM **2a** or quercetin).

The well-known antioxidant compound quercetin [32–35] displayed a concentration-dependent protective effect against *t*-BHP (0.3 mM) induced cytotoxicity in primary cultures of human hepatocytes with a complete protection at 10 μM (Fig. 4c). The benzopyrone derivative **2a**, by achie-

Table 1
Protective effect of synthesized compounds at different concentrations on 3 mM *t*-BHP-induced cytotoxicity in HepG2 cells

Compounds	X	R_1	R_2	Concentrations of tested compounds (μM)				
				0	0.5	1	5	10
2a	O	H	–	54 ± 4	71 ± 4 [a]	76 ± 4 [a]	89 ± 3 [a]	104 ± 4 [a]
2b	O	OCH_3	–	53 ± 6	42 ± 6	44 ± 2	48 ± 9	45 ± 8
5a	O	H	H	57 ± 1	63 ± 2	72 ± 4	80 ± 3	73 ± 6
5b	O	H	OCH_3	58 ± 2	51 ± 2	43 ± 4 [a]	40 ± 2 [a]	45 ± 2 [a]
2c	NH	H	–	61 ± 4	76 ± 2	88 ± 7 [a]	78 ± 1	82 ± 4 [a]
5c	NH	H	H	63 ± 7	93 ± 3 [a]	81 ± 4	83 ± 1	80 ± 3
2d	NCH_3	H	–	54 ± 2	58 ± 5	74 ± 3 [a]	83 ± 4 [a]	79 ± 5 [a]
5d	NCH_3	H	H	41 ± 2	63 ± 2 [a]	77 ± 3 [a]	62 ± 4 [a]	65 ± 6 [a]

HepG2 cells were exposed to 3 mM *t*-BHP together with various concentrations of a given tested compound. Cell viability was expressed as percentage of HepG2 cells viability in the absence of *t*-BHP (0 mM). Results are reported as mean ± SEM of two or three independent experiments.
[a] Significantly different from 3 mM *t*-BPH alone, $P < 0.05$ (Tuckey's test).

ving considerable protection from concentrations as low as 0.5 μm (Fig. 4b), appeared to display even a higher protecting potential.

4. Conclusion

In conclusion, the present work confirms that the human hepatoma cell line HepG2 constitutes a reliable model to study compounds with protective potentialities against *t*-BHP-induced cytotoxicity. Our results suggest that the presence of a benzylidene group at the 3-position or a heterocycle with two heteroatoms on the coumarin or on the quinolone system is essential for a protective potential and that a methoxy group on the aromatic ring system decreases dramatically this protection. The observation that those compounds also antagonised *t*-BHP-induced cytotoxicity in primary cultures of human hepatocytes suggests that they could display interesting hepatoprotective effects in humans. The discovery of 6,12-dihydro-1-benzopyrano[3,4-*b*][1,4]benzothiazin-6-one **2a** as a cytoprotective compound against hepatic oxidative stress at 0.5 μM may conduct to a promising pharmacomodulation for the discovery of new potential drugs.

5. Experimental protocols

5.1. Chemistry

All compounds were characterized using the methods of elemental analyses, IR spectroscopy, NMR spectroscopy and MS spectrometry. Infrared spectra were recorded on a Shimadzu FTIR-8201 PC spectrometer in potassium bromide pellets for solids and as liquid films for oils (ν in cm^{-1}). Proton NMR spectra were recorded on a Bruker AC 200 spectrometer. The samples were dissolved in DMSO-d_6 or in $CDCI_3$. All measurements were performed at 293 K and the chemical shift values were referenced to tetramethylsilane (CEA Saclay, France) as internal standard. Chemical shift values and IR data for all compounds are summarized in the experimental part and are in agreement with the proposed structures.

Melting points were determined on a Büchi N°510 apparatus and were uncorrected. Thin layer chromatography (TLC) was carried out on an Alugram Sil G/UV_{254} plate with appropriate solvents. Microanalyses were carried out by the Service Central d'Analyses, Centre National de la Recherche Scientifique, Vernaison (France).

*5.1.1. 6,12-Dihydrobenzopyrano[3,4b][1,4]benzothiazin-6-one (**2a**)*

The 4-hydroxycoumarin (**1a**) (50 mmol) and 2-aminothiophenol (60 mmol) were added to 20 ml of DMSO and heated to 140 °C for 12 h. On cooling at room temperature, the product crystallized and was filtered under vacuum. Recrystallization from ethanol gave **2a** in 60% yield; mp > 300 °C. IR (KBr) (cm^{-1}): 3335, 3010, 1668, 1618, 1015, 737; ^{1}H-NMR (DMSO-d^6) δ (ppm): 6.90–7.10 (4H, m, Ar–H), 7.50 (2H, m Ar), 7.65 (1H, dd, $_4J$ = 6.5 Hz, $_3J$ = 7.5 Hz, H-2), 8.20 (1H, m, $_3J$ = 7.5 Hz, H-1), 9.00 (1H, s, NH). MS (*m/z*): 267 [M^+]; $C_{15}H_9NO_2S$.

*5.1.2. 6,12-Dihydro-3-methoxybenzopyrano[3,4-b][1,4]benzothiazin-6-one (**2b**)*

This compound was obtained in 65% yield from 7-methoxy-4-hydroxycoumarin (**1b**) according to the general procedure used for the synthesis of **2a**; mp > 300°C. IR (KBr) (cm^{-1}): 3335, 3010, 2934, 1670, 1616, 1028, 750; ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.85 (3H, s, OCH_3), 6.85 (2H, m, Ar-H), 6.98 (1H, d, $_3J$ = 2.5 Hz, H-4), 6.95 (2H, m, Ar-H), 7.02 (1H, dd, $_4J$ = 2.5 Hz, $_3J$ = 9.1 Hz, H-2), 8.10 (1H, d, H-1), 9.01 (1H, s, NH). MS (*m/z*) = 297 [M^+]. $C_{16}H_{11}NO_3S$.

*5.1.3. 6,12-Dihydroquinolo[3,4-b][1,4]benzothiazin-6-one (**2c**)*

Quinolin-2,4-diol (**1c**) (50 mmol) and 2-aminothiophenol (60 mmol) were added to 15 ml of DMF and heated at 120 °C for 12 h. On cooling at room temperature, the product crystallized and was filtered under vacuum. Recrystallization from ethanol gave (**2c**) in 65% yield: mp > 300 °C. IR (KBr) (cm^{-1}): 3335, 2820, 1668, 1654, 1606, 748; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.05–7.70 (7H, m, Ar–H), 8.10 (1H, m, H–1), 9.10 (1H, s, NH), 12.00 (1H, s, NH). MS (*m/z*): 266 [M^+]. $C_{15}H_{10}N_2OS$.

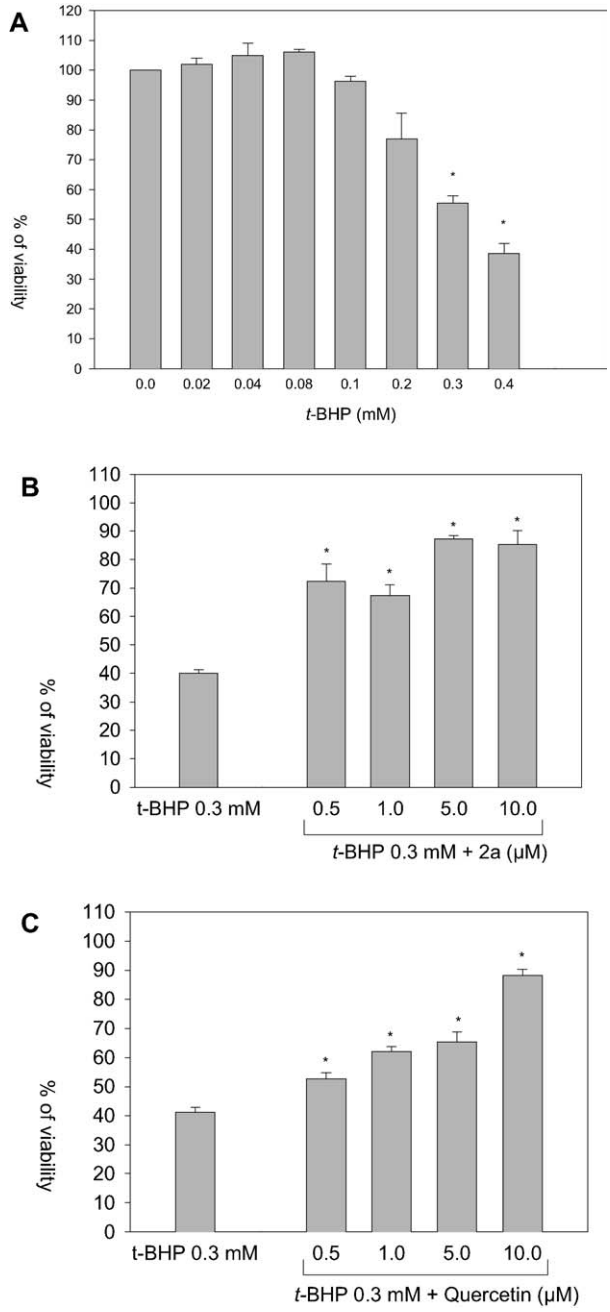

Fig. 4. Cytotoxic effect of *tert*-butylhydroperoxide in human hepatocytes in primary culture. Results are reported as mean ± SEM of three independent experiments. *Values significantly different from control, $P < 0.05$ (Tuckey's test). (a) Hepatoprotective effect of 0.3 mM *t*-BPH-induced cytotoxicity in human hepatocytes in primary culture (b) of compound **2a** (c) of quercetin. Results are reported as mean ± SEM of three independent experiments. [a] Values significantly different from (*t*-BHP 0.3 + 10 mM **2a** or quercetin). [b] Values significantly different from (*t*-BHP 0.3 + 5 mM **2a** or quercetin).

*5.1.4. 5-Methyl-6,12-dihydroquinolo[3,4-b][1,4]benzothiazin-6-one (**2d**)*

This compound was obtained in 65% yield from *N*-methylquinolin-2,4-diol (**1d**) according to the general procedure used for the synthesis of **2a**: mp > 300 °C. IR (KBr) (cm^{-1}): 3325, 3006, 2860, 1655, 1604, 1093, 758; ^{1}H-NMR (DMSO-d_6). δ (ppm): 3.55 (3H, s, N-CH_3), 7.00–7.70 (7H, m, Ar–H), 8.05 (1H, d, H–1), 9.00 (1H, s, NH), 12.00 (1H, s, NH); MS (*m/z*) = 280 [M+]. Anal. calculated for $C_{16}H_{12}N_2OS$: C, 68.57, H, 4.29, N, 10.00, S, 11.43. Found: C, 68.66, H, 4.22, N, 9.95, S, 11.37.

*5.1.5. (Z)-2,4-Dihydro-3-benzylidenebenzopyran-2,4-dione (**5a**)*

The 4-hydroxycoumarin (**1a**) (0.01 mol) and 0.012 mole of benzaldehyde (**3**) were added to 10 ml of pyridine and refluxed for 3 h. Once cooling at room temperature, the mixture was rapidly added to 100 ml of cold alcohol and vigorously stirred. The product crystallized and was filtered under vacuum. Recrystallization from ethanol gave **5a** in 75% yield; mp > 230 °C. IR (KBr) (cm^{-1}): 3030, 1654, 1605, 1164, 748; ^{1}H-NMR (DMSO-d_6) δ (pm): 7.10–7.30 (10H, m, Ar–H), 6.90 (1H, s), 9.10 (1H, s); MS (*m/z*): 218 [M+]. $C_{16}H_{10}O_3$.

*5.1.6. (Z)-2,4-Dihydro-3-(p-methoxybenzylidene)benzopyran-2,4-dione (**5b**)*

This compound was obtained in 77% yield from benzaldehyde (**4**) according to the general procedure used for the synthesis of **5a**; mp = 212 °C. IR (KBr) (cm^{-1}): 3030, 1652, 1607, 1160, 752. ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.10–7.30 (9H, m, Ar–H), 6.90 (1H, s), 3.85 (1H, s) MS (*m/z*) = 280 [M^+]. $C_{17}H_{12}O_4$.

*5.1.7. (Z)-2,4-Dihydro-3-benzylidenquinolin -2,4-dione (**5c**)*

This compound was obtained in 75% yield from *p*-methoxybenzaldehyde (**3**) according to the general procedure used for the synthesis of **5a**; mp = 195 °C. IR (KBr) (cm^{-1}): 3390, 3030, 2820, 1654, 1606, 1164, 748. ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.00–7.30 (10H, m, Ar–H), 6.85 (1H, s). MS (*m/z*) = 249 [M^+]. $C_{16}H_{11}NO_2$.

*5.1.8. (Z)-2,4-Dihydro-1-methyl-3-benzylidenquinolin-2,4-dione (**5d**)*

This compound was obtained in 78% yield from benzaldehyde (**3**) according to the general procedure used for the synthesis of **5a**; mp = 192 °C. IR (KBr) (cm^{-1}): 3390, 3020, 2830, 1627, 1608, 1087, 754. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.00–7.30 (10H, m, Ar-H), 6.85 (1H, s), 3.50 (3H, s); MS (*m/z*) = 263 [M^+]. $C_{17}H_{13}NO_2$.

5.2. Pharmacology

Collagenase and all products for cell culture were purchased from Life Technologies (Cergy Pontoise, France) and foetal calf serum (FCS) from Eurobio (Les Ulis, France). All other chemicals were purchased from Sigma Chemicals (St Quentin Fallavier, France) and were of analytical grade. Culture plastic plates were obtained from Costar (Dutscher, France).

5.2.1. HepG2 cell culture

The HepG2 cell line was derived from a human hepatoblastoma and was obtained from Dr. J.P. Beck (Strasbourg).

Cells were cultured in William's medium containing 10% foetal calf serum (FCS) (Gibco, France), 2 mM glutamine and 50 μg/ml gentamycin. Cultures were maintained in 5% CO_2 in air at 37 °C. HepG2 cells were routinely maintained in 25 cm^2 flasks, the medium was renewed every 48 h, and cells subcultured every 7 days. To study the effects of the various tested compounds, HepG2 cells were plated in 96-multiwell culture plates at a concentration of 0.6 10^4 cells/100 μl medium.

5.2.2. Human hepatocyte isolation and culture

Adult normal liver samples were obtained from patients undergoing partial hepatectomy for primary or secondary tumours. All experimental procedures were done in compliance with French laws and regulations and were approved by the National Ethics Committee. Hepatocytes were isolated by a two-step collagenase perfusion as previously described by David et al. [28].

Viable hepatocytes were seeded in 96-multiwell culture plates at a concentration of 0.3 10^4 cells/100 μl DMEM medium as previously described by Goll et al. [29].

5.2.3. Assay for t-BHP cytotoxicity and protection by various compounds

5.2.3.1. Assay of t-BHP cytotoxicity. HepG2 cells and primary cultures of human hepatocytes were plated in 96-multiwell culture plates. Twenty-four hours after plating, the medium was discarded and fresh medium containing *t*-BHP 0.3 mM was added to cultures. After 3 hours, cellular viability was determined by measuring reduction of the tetrazolium-blue compound MTT to a blue formazan product according to Carmichael et al. [37].

5.2.3.2. Assay of protection against t-BHP cytotoxicity. The various compounds were first tested for their effects on the viability of HepG2 cells and primary cultures of human hepatocytes. HepG2 cells and primary cultures of human hepatocytes were plated in 96-multiwell culture plates. Twenty-four hours after plating, the medium was discarded and fresh medium containing the compounds at 0–10 μM were added to cell cultures. After 24 h, cellular viability was determined by measuring the reduction for the tetrazolium-blue compound MTT to formazan. All tested compounds did not affect HepG2 cell viability and primary cultures of human hepatocytes up to 10 μM (data not shown). For this reason, the protective effects of the various compounds at concentrations 0–10 μM were evaluated.

The potential protective effect was tested by co-incubating for 3 h a given tested compound at 0.5, 1.0, 5.0 or 10 μM with *t*-BHP (3 mM) for HepG2 cells and *t*-BHP (0.3 mM) for primary cultures of human hepatocytes. Cellular viability was then determined by measuring reduction of the tetrazolium-blue compound MTT to formazan blue.

5.2.4. Statistical analysis

The statistical significance of the difference between the mean results of the least two to three independent experiments was calculated using the one-way analysis of variance followed by Thuckey's test for multiple comparisons versus pairwise. Values were considered statistically significant at $P < 0.05$.

References

[1] R.A. Micheli, A.N. Booth, A.L. Livingston, E.M. Bickoff, J. Med. Chem. 5 (1962) 321–329.
[2] S. Nishimura, M. Taki, S. Takaishi, Y. Lijima, T. Akiyama, Chem. Pharm. Bull. 48 (2000) 505–508.
[3] Y. Jacquot, L. Bermont, H. Giorgi, B. Refouvelet, G.L. Adessi, E. Daubrosse, A. Xicluna, Eur. J. Med. Chem. 36 (2001) 127–136.
[4] S.-G. Cao, X.-H. Wu, K.-Y. Sim, B.H.K. Tan, J.J. Vittal, J.T. Pereira, S.-H. Goh, Helv. Chim. Acta 81 (1998) 1404–1407.
[5] T. Fujioka, K. Furumi, H. Fujii, H. Okabe, K. Mihashi, Y. Nakano, H. Matsunaga, M. Katano, M. Mori, Chem. Pharm. Bull 47 (1999) 96–100.
[6] L.W.L. Woo, A. Purohit, B. Malini, M.J. Reed, B.V.L. Potter, Chem. Biol. 7 (2000) 773–791.
[7] A. Purohit, L.W.L. Woo, D. Barrox, H.A.M. Hejaz, R.I. Nicholson, B.V.L. Potter, M.J. Reed, Mol. Cell. Endocrinol. 171 (2001) 129–135.
[8] Y. Jacquot, A. Cleeren, J. Laios, Y. Ma, A. Boulahdour, L. Bermont, B. Refouvelet, G. Adessi, G. Leclercq, A. Xicluna, Biol. Pharm. Bull. 25 (2002) 335–341.
[9] Y. Jacquot, B. Refouvelet, L. Bermont, G.L. Adessi, G. Leclercq, A. Xicluna, Pharmazie 57 (2002) 233–237.
[10] A. Iannone, A. Bini, G. Jin, V. Vannini, A. Tomasi, Free Radical Res. Comm. 19 (1993) 141–147.
[11] H. Sies, K.H.H. Summer, Eur. J. Biochem. 57 (1975) 503–512.
[12] L. Nicod, C. Viollon, A. Regnier, A. Jacqueson, L. Richert, Hum. Exp. Toxicol. 16 (1997) 28–34.
[13] E. Alexandre, P. David, C. Viollon-Abadie, A. Azimzadeh, P. Wolf, D. Jaeck, L. Nicod, K. Boudjema, L. Richert, Toxicol. In Vitro 13 (1999) 427–435.
[14] V. Goll, C. Viollon-Abadie, L. Nicod, L. Richert, Hum. Exp. Toxicol. 19 (2000) 193–202.
[15] M. Colombain, V. Goll, F. Muyard, C. Girard, F. Bévalot, L. Richert, Planta Med. 67 (2001) 644–646.
[16] M.A. Gore, M.M. Morshedi, J.F. Reidhaar-Olson, Funct. Integ. Genomics 1 (2000) 114–126.
[17] Y. Yamamoto, M. Bnakajima, H. Yamazaki, T. Yokoi, Life Sci. 70 (2001) 471–482.
[18] F. Carubbi, M.E. Guicciardi, M. Concazri, P. Loria, M. Bertolotti, N. Carilli, Bicochem. Biophys. Acta 1580 (2002) 31–39.
[19] K.R. Martin, M.L. Failla, J.C. Smith, J. Nutr. 126 (1996) 2098–2106.
[20] J.A. Kim, Y.S. Kang, S.H. Lee, Y.S. Lee, Free Radical Res. 33 (2000) 267–277.
[21] Q. Feg, Y. Torii, K. Uchda, Y. Nakamura, Y. Hara, T. Osawa, J. Agric. Food Chem. 2 (2002) 213–220.
[22] A. Jayashree, V.S. Rao, M. Darbarwar, Synth. Commun. 20 (7) (1990) 919–924.
[23] N.P. Buu-Hoï, M. Mangane, P. Jacquignon, J. Chem. Soc. (C) (1966) 50–52.
[24] M. Vanhaelen, R. Vanhaelen-Fastré, Pharm. Acta Helv. 51 (1976) 307–312.
[25] K. Tabakovic, I. Tabakovic, M. Trkovnik, A. Juric, N. Trinajstic, J. Heterocyclic Chem. 17 (1980) 801–803.
[26] B.S. Reddy, M. Darbarwar, J. Indian Chem. Soc. 62 (1985) 377–379.
[27] P. Maurel, Drug Delivery Rev. 22 (1996) 105–132.
[28] P. David, C. Viollon-Abadie, E. Alexandre, L. Nicod, P. Wolf, D. Jeack, K. Boudjema, L. Richert, Hum. Exp. Toxicol. 17 (1998) 544–553.

[29] V. Goll, E. Alexandre, C. Viollon-Abadie, L. Nicod, D. Jaeck, L. Richert, Toxicol. Appl. Pharmacol. 160 (1999) 21–32.
[30] A. Da Settimo, A.-M. Marini, G. Primofiore, F. Da Settimo, S. Salerno, C. La Mota, G. Pardit, P.L. Ferrarini, C. Mori, J. Heterocyclic Chem. 37 (2000) 379–382.
[31] W. Davey, J.E. Gwilt, J. Chem. Soc. (1957) 1008–1019.
[32] A.M. Clement, J.K. Chipman, Carcinogenesis 19 (9) (1998) 1583–1589.
[33] H. Wang, J.A. Joseph, Free Radical Biol. Med. 27 (5 and 6) (1999) 683–694.
[34] N. Sugihara, T. Arakawa, M. Onhnishi, K. Furuno, Free Radical Biol. Med. 27 (11 and 12) (1999) 1313–1323.
[35] H. Nagata, S. Takekoshi, T. Takagi, T. Honma, K. Watanabe, J. Tokai, Exp. Clin. Med. 24 (1) (1999) 1–11.
[36] L. Richert, D. Binda, G. Hamilton, C. Viollon-Abadie, E. Alexandre, D. Bigot-Lasserre, R. Bars, P. Cassolo, E. LeCluyse, Toxicol. In Vitro 16 (2002) 89–99.
[37] J. Carmichael, W.G. DeGraff, A.F. Gazdar, J.D. Minna, J.B. Mitchell, Cancer Res. 47 (1987) 936–942.