Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 43 (2008) 1336–1343
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Preliminary communication

Synthesis and antiproliferative activity of aryl- and heteroaryl-hydrazones derived from xanthone carbaldehydes

Martine Varache-Lembège [a], Stéphane Moreau [a], Stéphane Larrouture [a,✠], Danièle Montaudon [b], Jacques Robert [b], Alain Nuhrich [a,*]

[a] *Laboratoire de Chimie Thérapeutique, EA2962, Faculté de Pharmacie, Université Victor Segalen Bordeaux 2, 146 Rue Léo-Saignat, 33076 Bordeaux Cedex, France*

[b] *Laboratoire de Pharmacologie des Médicaments Anticancéreux, Université Victor Segalen Bordeaux 2, Institut Bergonié, 229 cours de l'Argonne, 33076 Bordeaux Cedex, France*

Received 29 January 2007; received in revised form 27 July 2007; accepted 6 September 2007
Available online 15 September 2007

Abstract

In order to explore the antiproliferative effect associated with the xanthone framework, several arylhydrazonomethyl derivatives were synthesized from various isomeric 1,3-dihydroxyxanthone carbaldehydes. Variation in the position of the aldehydic function led to three sets of compounds, bearing the hydrazonomethyl chain at positions 5, 6 or 7 on the xanthone nucleus, respectively.

The antiproliferative effect of the compounds was evaluated in vitro using the MTT colorimetric method against two human cancer cell lines (MCF-7, breast adenocarcinoma, and KB 3.1, squamous cell oral carcinoma) for two time periods (24 h and 72 h).

Among the series, four compounds exhibited interesting growth inhibitory effects against both the cell lines, with IC_{50} values in the micromolar concentration range. When compared with doxorubicin, the xanthone derivatives showed moderate cytotoxic effects. Surprisingly, unlike doxorubicin, these compounds displayed no significant time-dependent change in the concentration causing 50% inhibitory effect in proliferation.

This unusual cytotoxicity profile led to the hypothesis that these molecules could be endowed with a mechanism of action distinct to that of doxorubicin.

Keywords: Xanthones; Hydrazones; Antiproliferative activity

1. Introduction

During the past several years, the xanthone template has generated a growing interest in the search for new antitumor agents [1–6]. Thus, noticeable antiproliferative effects were discovered in various naturally occurring xanthones, as exemplified by psorospermin and mangostin derivatives [7–9]. On the other hand, within synthetic molecules, 2-(5,6-dimethyl-9-oxo-9*H*-xanthen-4-yl)acetic acid (DMXAA) developed at the Auckland Cancer Society Research Centre in New Zealand [10] emerged as an interesting compound exhibiting both excellent experimental antitumor activity and antivascular effects. Several biochemical pathways explaining the activity of DMXAA have been established. This drug induces apoptosis of tumoral vascular endothelial cells via an up-regulation of the nuclear transcription factor, NFκB, leading to the production of TNFα and other cytokines [11,12].

Due to this particular therapeutic profile, DMXAA is considered as a very promising molecule among the novel classes of anti-cancer drugs named vascular disrupting agents (VDAs) [13]. Antitumor activity of DMXAA appears to be due to indirect effects more than direct action on DNA. In combination with standard chemotherapy this substance is currently

* Corresponding author. Tel.: +33 05 57 57 46 98; fax: +33 05 57 57 47 05.
E-mail address: alain.nuhrich@chimthera.u-bordeaux2.fr (A. Nuhrich).
✠ Deceased on 7 July 2006.

0223-5234/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2007.09.003

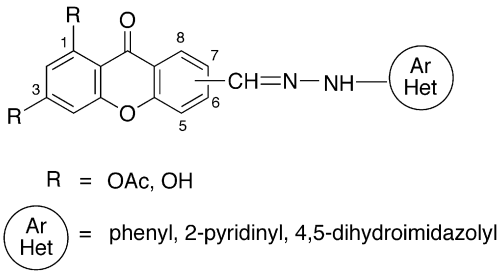


Fig. 1. General structure of the investigated xanthones.

investigated in the clinic (phase III trials) under the code AS1404 and seems of particular interest for the treatment of solid tumors [14].

These findings allow us to think that the xanthone chromophore is an attractive lead for the design of novel antitumor drugs. On the other hand, hydrazino system has revealed good inhibitory effects on cancer cells' proliferation [15–17]. With the aim of further exploring the therapeutic potential of this tricyclic framework, we describe in this paper the synthesis and a preliminary investigation of the cytotoxic profile of a series of new compounds whose general structure is outlined in Fig. 1.

2. Chemistry

The synthetic approach (Fig. 2) for the construction of the xanthone nucleus is based on the method of Grover et al. [18] and involves the condensation between phloroglucinol **1** and 3-, 4- or 5-methylsalicylic acid **2**. The use of Eaton's reagent (phosphorus pentoxide and methanesulfonic acid: P_2O_5/CH_3SO_3H) [19] as the coupling agent afforded the xanthones **3** in high yield (90%). The strategy used to protect the phenolic groups is of primordial importance for governing the regiospecificity of the following bromination step [20]. Thus, the *O*-acetyl derivatives **4**, when treated with *N*-bromosuccinimide/benzoyl peroxide in CCl_4 afforded in all cases the desired bromomethyl compounds **5** as major products.

The xanthone carbaldehydes **6** were obtained from the bromomethyl derivatives by smooth oxidation with bis-tetrabutylammonium dichromate (prepared by reacting tetrabutylammoniumhydrogensulfate with potassium dichromate) [21].

Condensation of **6** with appropriate aryl- or heteroarylhydrazine salts gave the corresponding hydrazones **7a–i**. Deprotection by alkaline hydrolysis in mild conditions then afforded the final 1,3-diphenolic compounds (**8a–i**).

3. Results and discussion

In the first experiment, the selected arylhydrazones **7** and **8** were tested for their effect on cellular viability against two human tumor cell lines (MCF-7, breast carcinoma, and KB 3.1, oral squamous cell carcinoma). Assays were performed in vitro on exponentially growing cells. The activity was evaluated by measuring the levels of surviving cells after incubation for 24 h with the test samples, using the MTT colorimetric assay [22], based on the ability of metabolically active cells to convert the pale yellow MTT to a blue formazan product, which is quantifiable spectrophotometrically.

The results of this primary screening are reported in Table 1.

Most of the hydrazone derivatives exhibited a rather moderate cytotoxicity or were inactive.

However, within the series studied, four xanthones revealed a significant activity (**7g**, **8e**, **7h**, and **8f**). In terms of cell line sensitivity, comparable responses were observed against both the MCF-7 and the KB 3.1 cells.

For these active compounds, results appeared to be indicative of a dose–response relationship, with a clear inhibition of the percentage of living cells after exposure to 10 μM of substance.

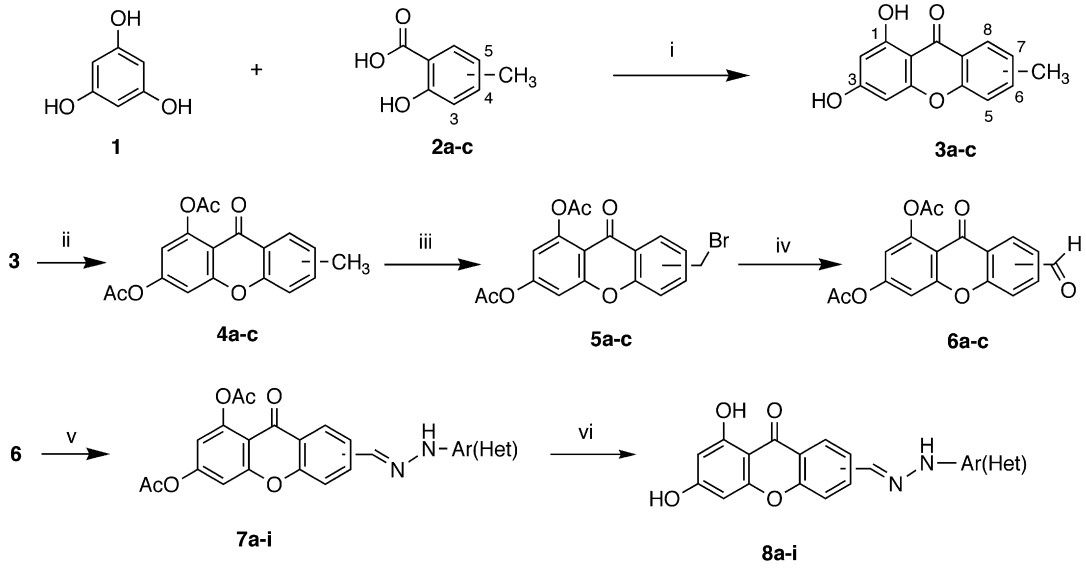
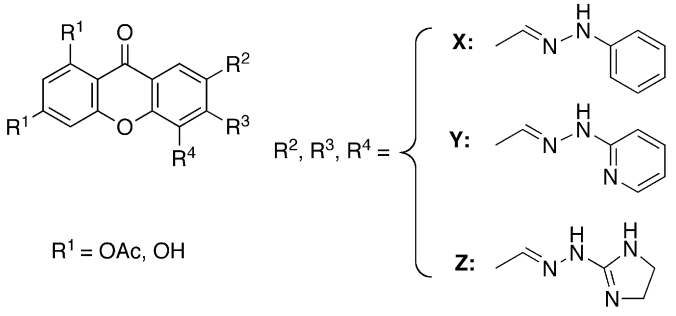


Fig. 2. Synthetic pathway for arylhydrazonomethylxanthones. Reagents: (i) P_2O_5/CH_3SO_3H; (ii) Ac_2O/pyridine; (iii) NBS/DBP/CCl_4; (iv) $(Bu_4N)_2Cr_2O_7/CHCl_3$; (v) Ar(Het))–NH–NH_2/EtOH; (vi) $NaHCO_3/H_2O$.

Table 1
Effects of studied xanthones on the viability of MCF-7 and KB 3.1 cells



Comp.	R^1	R^2	R^3	R^4	MCF-7		KB 3.1	
					10 μM	1 μM	10 μM	1 μM
7a	OAc	**X**	H	H	100	100	100	100
8a	OH	**X**	H	H	72	100	82	100
7b	OAc	H	**X**	H	100	100	100	100
8b	OH	H	**X**	H	100	100	61	100
7c	OAc	H	H	**X**	100	100	82	100
8c	OH	H	H	**X**	100	100	96	100
7d	OAc	**Y**	H	H	70	85	60	98
8d	OH	**Y**	H	H	41	58	90	95
7e	OAc	H	**Y**	H	100	100	87	100
8e	OH	H	**Y**	H	3	100	2	77
7f	OAc	H	H	**Y**	79	80	90	95
8f	OH	H	H	**Y**	12	86	3	96
7g	OAc	**Z**	H	H	14	64	14	100
8g	OH	**Z**	H	H	64	90	92	99
7h	OAc	H	**Z**	H	13	35	13	85
8h	OH	H	**Z**	H	70	73	80	93
7i	OAc	H	H	**Z**	48	80	66	95
8i	OH	H	H	**Z**	82	97	75	90

Viability was estimated as the percentage of living cells after incubation of the cells for 24 h with two concentrations (1 μM and 10 μM) of the molecules tested (100 indicates no activity; 0 indicates complete cell death) with final DMSO concentration of 0.1%.

From the data obtained, it is obvious that the nature of the terminal ring on the hydrazono chain exerts a striking effect on the antiproliferative activity. Within the few variations considered in this study, the presence of a nitrogen heterocycle afforded a clear beneficial effect with regard to antiproliferative properties. With the exception of **7g**, the dihydroimidazolyl derivatives were slightly less active than their pyridinyl counterpart (compare **8e** to **8h**, and **8f** to **8i**). Introduction of a non-nitrogen ring resulted in compounds completely devoid of activity (in the case of phenyl derivatives **7a–c** and **8a–c**).

Intriguingly, position of the hydrazonomethyl functionality on the third ring of the xanthone nucleus did not allow to delineate clear structure–activity relationships. In this context, it is worth noting that similar activities are observed for **7g** (5-substituted), **8e** (6-substituted) and **8f** (7-substituted).

The role of the oxygenated groups in the 1- and 3-position on the xanthone system seems rather difficult to clarify since significant activities were shown both among acetylated derivatives and in free phenolic compounds. A plausible explanation is that tumoral drug-metabolizing enzymes (DMEs), which are capable of biotransforming a variety of xenobiotics [23], could be involved in the hydrolysis of diacetylated compounds, thus releasing dihydroxyxanthone in the cellular medium. The extended incubation periods used in our assays are consistent with this hypothesis. Without further investigation, it was not possible to decide if the biological response was due to the ester itself or attributable to its hydrolyzed product.

Since the inhibitory effects of xanthones on the cell viability were established, we then examined the impact of exposure time on antiproliferative activity. In a further experiment, we first exposed KB 3.1 and MCF-7 cells to the test compound for 24 h or 72 h and we then explored the growing capacity of the treated cells when reincubated on fresh culture medium for a further 96-h period. Doxorubicin was used as reference substance.

Results of these assays are presented in Table 2.

In our experiments, data for antiproliferative effect of doxorubicin on MCF-7 cells showed a striking evolution of the drug concentration required to decrease cell survival by 50% after 24-h or 72-h treatment. This proceeds from the corresponding IC_{50} ratio values, $R_{(24/72)} = 25$. This observation is in accordance with the time-dependent cytotoxic response usually reported for doxorubicin [24,25].

In contrast, evaluation of the cytotoxicity induced by the tested hydrazonoxanthones yielded an apparently different pattern. The doses causing 50% inhibitory effect did not change significantly after 24 h and 72 h, as indicated by the $R_{(24/72)}$ value

Table 2
Effect of time of incubation on antiproliferative activity against KB 3.1 and MCF-7 cells (IC_{50}, μM)

	KB 3.1			MCF-7		
	24 h	72 h	$R_{(24/72)}$	24 h	72 h	$R_{(24/72)}$
7g	11.3 ± 1.1	4.7 ± 0.9	2.4	9.3 ± 1.3	7.0 ± 1.0	1.3
7h	1.6 ± 0.3	1.8 ± 0.4	0.9	1.9 ± 0.4	2.4 ± 0.2	0.8
7i	6.8 ± 0.6	6.5 ± 0.3	1.05	5.1 ± 0.6	5.6 ± 0.5	0.9
Doxorubicin	0.010 ± 0.003	0.0004 ± 0.0001	25.0	0.018 ± 0.004	0.0007 ± 0.0001	25.7

Cell viability after a further 96-h period in fresh culture medium was estimated as the compound concentration required for 50% growth inhibition. For each drug, a $R_{(24/72)}$ value was calculated and represents the ratio of the IC_{50}s (μM) obtained after 24-h and 72-h treatment, respectively. Data represent mean values (±S.D.) for three independent experiments.

which is always near to 1.This would indicate that the cytotoxicity of these compounds is not cell cycle phase-dependent.

4. Conclusion

According to these preliminary results, it appears that the introduction of a heteroarylhydrazonomethyl moiety on the xanthone system is potentially of interest to obtain antiproliferative compounds.

This work allowed us to identify a few active molecules able to inhibit in vitro the growth of two human cancer lines. Compared to doxorubicin, these compounds displayed both a relatively weak cytotoxicity and a phase-independent cytotoxic mechanism. Our results suggest that the antiproliferative properties induced by the xanthone framework might involve distinct mechanisms to that of classical topoisomerase inhibitors.

All these findings support the need for further investigations to clarify the features underlying the antitumor potential of these new xanthone derivatives.

5. Experimental

5.1. Materials and methods

Melting points (mp) were determined using an electrothermal capillary melting point apparatus (Digital Mel-Temp 3.0, Model 1402) and are uncorrected. IR spectra were recorded as potassium bromide discs on a Shimadzu IR 470 spectrometer. NMR spectra were recorded, in DMSO-d_6 solution, on a Bruker AMX 500 instrument (^{1}H: 500 MHz, ^{13}C: 125 MHz). Chemical shifts are reported as δ (ppm) relative to TMS as internal standard. Multiplicities in ^{13}C NMR spectra were derived from JMOD experiments. The 2D experiments (Homonuclear Correlation {^{1}H–^{1}H COSY} and Heteronuclear Correlations {HMQC and HMBC}) were used to assign the signals. Thin-layer chromatography (TLC) was performed using aluminium precoated plates (silica gel SDS 60F 254 Whatman, 0.2 mm thickness). Column chromatography was carried out on silica gel 60–200 μm (Merck). Elemental analyses performed on the terminal products were within ±0.4% of the calculated values (for element indicated in brackets).

5.1.1. General procedure for the preparation of hydroxylated xanthones (**3**)

To a mixture containing phloroglucinol dihydrate **1** (9.73 g, 60 mmol, dried at 120 °C overnight) and appropriate methylated salicylic acid **2** (9.13 g, 60 mmol) was added slowly 100 ml of Eaton's reagent (P_2O_5/CH_3SO_3H, Aldrich). The mixture was warmed up to 80 °C for 20 min, under stirring. After cooling to room temperature, the reaction mixture was poured onto ice (300 g) and stirred for 2 h. The resulting solid was collected by filtration, washed with water until pH 6, and dried at 60 °C to give a reddish brown solid used in the following step without further purification.

*5.1.1.1. 1,3-Dihydroxy-5-methyl-9H-xanthen-9-one (**3a**).* Mp > 260 °C; yield: 96% [20].

*5.1.1.2. 1,3-Dihydroxy-6-methyl-9H-xanthen-9-one (**3b**).* Mp > 260 °C; yield: 95% [20].

*5.1.1.3. 1,3-Dihydroxy-7-methyl-9H-xanthen-9-one (**3c**).* Mp > 260 °C; yield: 91% [20].

5.1.2. General procedure for the methyl-9-oxo-9H-xanthene-1,3-diyl diacetates (**4**)

A suspension of dihydroxylated derivative **3** (14 g, 57.8 mmol) in a mixture of acetic anhydride (130 ml) and pyridine (10 ml) was stirred at reflux for 3 h. After cooling to room temperature, the reaction mixture was poured into ice-water (400 g) and then acidified with hydrochloric acid. The resulting precipitate was separated and washed with water until pH 7. The expected acetylated compound **4** was recrystallized from ethanol.

*5.1.2.1. 5-Methyl-9-oxo-9H-xanthene-1,3-diyl diacetate (**4a**).* Mp: 173 °C; yield: 62% [20].

*5.1.2.2. 6-Methyl-9-oxo-9H-xanthene-1,3-diyl diacetate (**4b**).* Mp: 156 °C; yield: 56%; IR (cm^{-1}) 1770 (CO ester), 1650 (CO ketone); ^{1}H NMR (δ) 2.33 and 2.38 {2(s, 3H, CO–CH_3)}, 2.48 (s, 3H, CH_3), 7.05 (d, 1H, H2, $^4J = 2.2$ Hz), 7.29 (dd, 1H, H7, $^3J = 8.1$ Hz and $^4J = 1.3$ Hz), 7.44 (d, 1H, H4, $^4J = 2.2$ Hz), 7.45 (s, 1H, H5), 7.99 (d, 1H, H8, $^3J = 8.1$ Hz); ^{13}C NMR (δ) 20.8 2 × CH_3 (Ac), 21.2 CH_3, 109.1 C4, 112.3 C1a, 113.1 C2, 117.3 C5, 119.11 C8a, 125.5 C7, 126.0 C8, 146.8 C6, 150.4 C1, 154.7 C3, 154.8 C5a, 156.9 C4a, 168.1 and 168.6 C=O (Ac), 173.0 C=O (xanthone). Anal. $C_{18}H_{14}O_6$ (C, H).

*5.1.2.3. 7-Methyl-9-oxo-9H-xanthene-1,3-diyl diacetate (**4c**).* Mp: 163 °C; yield: 42%; IR (cm^{-1}) 1770 (CO ester), 1660

(CO ketone); ^{1}H NMR (δ) 2.34 and 2.38 {2(s, 3H, CO–CH_3)}, 2.42 (s, 3H, CH_3, 7.05 (d, 1H, H2, $^4J = 2.3$ Hz), 7.44 (d, 1H, H4, $^4J = 2.3$ Hz), 7.53 (dd, 1H, H6, $^3J = 8.5$ Hz and $^4J = 2.7$ Hz), 7.67 (d, 1H, H5, $^3J = 8.5$ Hz), 7.90 (s, 1H, H8); ^{13}C NMR (δ) 20.1 CH_3, 20.7 CH_3 (Ac), 109.0 C2, 112.1 C1a, 112.9 C4, 117.4 C5, 120.9 C8a, 125.0 C8, 134.1 C7, 136.4 C6, 150.3 C1, 152.9 C5a, 154.7 C3, 156.9 C4a, 168.0 and 168.6 C=O (Ac), 173.9 C=O (xanthone). Anal. $C_{18}H_{14}O_6$ (C, H).

5.1.3. General procedure for the bromomethyl-9-oxo-9H-xanthene-1,3-diyl diacetates (**5**)

A mixture of diacetate derivative **4** (5.55 g, 17 mmol), *N*-bromosuccinimide (3.03 g, 17 mmol) and dibenzoylperoxide (412 mg, 1.7 mmol) in carbon tetrachloride (110 ml) was refluxed for 3–5 h under light (2 × 60 W). After cooling at 0 °C and stirring for 2 h, the precipitate was filtered, washed with water (3 × 15 ml), acetone (2 × 15 ml) and diethyl ether (20 ml) to give a solid which was recrystallized from butan-2-one.

5.1.3.1. 5-Bromomethyl-9-oxo-9H-xanthene-1,3-diyl diacetate (**5a**).

Mp: 198 °C (butan-2-one); yield: 50% [20].

5.1.3.2. 6-Bromomethyl-9-oxo-9H-xanthene-1,3-diyl diacetate (**5b**).

Mp: 198 °C (butan-2-one); yield: 40%; IR (cm^{-1}) 1780 (CO ester), 1660 (CO ketone); ^{1}H NMR (δ) 2.34 and 2.38 {2(s, 3H, CO–CH_3)}, 4.84 (s, 2H, CH_2Br), 7.07 (d, 1H, H2, $^4J = 2.1$ Hz), 7.47 (d, 1H, H4, $^4J = 2.2$ Hz), 7.52 (dd, 1H, H7, $^3J = 7.6$ Hz and $^4J = 2.2$ Hz), 7.72 (d, 1H, H5, $^4J = 1.3$ Hz), 8.09 (d, 1H, H8, $^3J = 8.2$ Hz); ^{13}C NMR (δ) 20.7 and 20.7 CH_3 (Ac), 32.2 CH_2Br, 109.1 C4, 112.3 C1a, 113.3 C2, 117.9 C5, 120.8 C8a, 125.6 C7, 126.3 C8, 145.8 C6, 150.3 C1, 154.5 C3, 154.9 C5a, 156.9 C4a, 168.0 and 168.7 C=O (Ac), 173.6 C=O (xanthone). Anal. $C_{18}H_{13}BrO_6$ (C, H).

5.1.3.3. 7-Bromomethyl-9-oxo-9H-xanthene-1,3-diyl diacetate (**5c**).

Mp: 156 °C (butan-2-one); yield: 73%; IR (cm^{-1}) 1770 (CO ester), 1660 (CO ketone); ^{1}H NMR (δ) 2.35 and 2.45 {2(s, 3H, CO–CH_3)}, 4.52 (s, 2H, CH_2Br), 6.93 (d, 1H, H2, $^4J = 2.2$ Hz), 7.32 (d, 1H, H4, $^4J = 2.3$ Hz), 7.47 (d, 1H, H6, $^3J = 8.6$ Hz), 7.68 (d, 1H, H5, $^3J = 8.6$ Hz), 8.26 (s, 1H, H8). Anal. $C_{18}H_{13}BrO_6$ (C, H).

5.1.4. General procedure for the formyl-9-oxo-9H-xanthene-1,3-diyl diacetates (**6**)

The convenient bromomethyl derivatives **5a–c** (2.03 g, 5 mmol) were added to the solution of bis-tetrabutylammonium dichromate [21] in 100 ml of chloroform. The reaction mixture was heated under reflux for 5 h. After cooling at room temperature, the crude product was filtered through silica gel (40–63 μm, 20 g) to eliminate inorganic and tetrabutylammonium salts. Silica was washed with ethyl acetate (2 × 50 ml). Evaporation of the combined organic solvents gave a greenish brown oil. The residue was further purified on a silica gel (63–200 μm, 30 g) column (1.6 cm in diameter, 47 cm in length) with methylene chloride–ethyl acetate (9:1) as eluent. The desired product **6**, obtained as a yellowish white solid, was further recrystallized from ethanol.

5.1.4.1. 5-Formyl-9-oxo-9H-xanthene-1,3-diyl diacetate (**6a**).

Mp: 172 °C (ethanol); yield: 29% [20].

5.1.4.2. 6-Formyl-9-oxo-9H-xanthene-1,3-diyl diacetate (**6b**).

Mp: 196 °C (ethanol); yield: 43%; IR (cm^{-1}) 1780 (CO ester), 1700 (CO aldehyde), 1660 (CO ketone); ^{1}H NMR (δ) 2.36 and 2.40 {2(s, 3H, CO–CH_3)}, 7.11 (d, 1H, H7, $^4J = 2.2$ Hz), 7.52 (d, 1H, H5, $^4J = 2.2$ Hz), 7.90 (dd, 1H, H2, $^3J = 8.1$ Hz and $^4J = 1.35$ Hz), 8.14 (d, 1H, H4, $^4J = 1.3$ Hz), 8.27 (d, 1H, H1, $^3J = 8.3$ Hz), 10.17 (s, 1H, CHO); ^{13}C NMR (δ) 20.6 and 20.7 CH_3 (Ac), 109.2 C5, 112.4 C8a, 113.5 C7, 119.8 C4, 123.2 C2, 124.6 C1a, 126.9 C1, 140.6 C3, 151.4 C8, 154.8 C4a, 155.1 C6, 157.1 C5a, 167.9 and 168.5 C=O (Ac), 173.7 C=O (xanthone), 192.0 CHO. Anal. $C_{18}H_{12}O_7$ (C, H).

5.1.4.3. 7-Formyl-9-oxo-9H-xanthene-1,3-diyl diacetate (**6c**).

Mp: 175 °C (ethanol); yield: 51%; IR (cm^{-1}) 1780 (CO ester), 1690 (CO aldehyde), 1670 (CO ketone); ^{1}H NMR (δ) 2.34 and 2.40 {2(s, 3H, CO–CH_3)}, 7.13 (d, 1H, H7, $^4J = 2.2$ Hz), 7.51 (d, 1H, H5, $^4J = 2.2$ Hz), 7.77 (d, 1H, H4, $^3J = 8.6$ Hz), 8.27 (dd, 1H, H3, $^3J = 8.7$ Hz and $^4J = 2.0$ Hz), 8.66 (d, 1H, H1, $^4J = 1.9$ Hz), 10.11 (s, 1H, CHO); ^{13}C NMR (δ) 20.7 CH_3 (Ac), 109.3 C7, 112.3 C8a, 113.8 C5, 119.0 C4, 121.4 C1a, 129.6 C1, 132.4 C2, 133.7 C3, 150.3 C8, 155.2 C6, 156.8 C5a, 158.0 C4a, 167.9 and 168.5 C=O (Ac), 173.6 C=O (xanthone), 191.2 CHO. Anal. $C_{18}H_{12}O_7$ (C, H).

5.1.5. General procedure for the 9-oxo-((2-arylhydrazono)methyl)-9H-xanthene-1,3-diyl diacetates (**7**)

A mixture of the appropriate carboxaldehyde **6** (340 mg, 1 mmol) and the convenient aryl or heteroaryl-hydrazine (1.2 mol) was stirred at room temperature in 15 ml of 1-propanol for 15 min and then acetic acid (about 0.5 ml) was added dropwise to the reaction mixture. The suspension was stirred for an additional period of 15 min and heated under reflux for 2 h. The mixture was cooled to room temperature and filtered to give hydrazone **7**. The different ways of purification are given for each compound.

5.1.5.1. 9-Oxo-7-((2-phenylhydrazono)methyl)-9H-xanthene-1,3-diyl diacetate (**7a**).

Mp: 214 °C (ethanol); yield: 63% [20].

5.1.5.2. 9-Oxo-6-((2-phenylhydrazono)methyl)-9H-xanthene-1,3-diyl diacetate (**7b**).

Mp: 210 °C (acetone–water, 1:2); yield: 42% [20].

5.1.5.3. 9-Oxo-5-((2-phenylhydrazono)methyl)-9H-xanthene-1,3-diyl diacetate (**7c**).

Mp: 196 °C (ethanol–acetone, 9:1); yield: 61% [20].

5.1.5.4. 9-Oxo-7-((2-(pyridin-2-yl)hydrazono)methyl)-9H-xanthene-1,3-diyl diacetate (**7d**).

Mp: 211 °C; yield: 83%; IR (cm^{-1}) 3400 (NH), 1770 (CO ester), 1660 (CO ketone), 1620 (CN), 1570 (N–H); ^{1}H NMR (δ) 2.36 and 2.40

{2(s, 3H, CO–CH_3)}, 6.82 (t, 1H, H5′, $^3J = 5.8$ Hz), 7.10 (s, 1H, H7), 7.34 (d, 1H, H6′, $^3J = 7.9$ Hz), 7.44 (s, 1H, H5), 7.51 (t, 1H, H2, $^3J = 7.6$ Hz), 7.68 (t, 1H, H4′, $^3J = 7.6$ Hz), 8.08 (d, 1H, H1, $^3J = 7.9$ Hz), 8.16 (d, 1H, H3′, $^3J = 7.3$ Hz), 8.39 (d, 1H, H3, $^3J = 7.3$ Hz), 8.66 (s, 1H, CH═), 11.16 (s, 1H, NH). Anal. $C_{23}H_{17}N_3O_6$ (C, H, N).

*5.1.5.5. 9-Oxo-6-((2-(pyridin-2-yl)hydrazono)methyl)-9H-xanthene-1,3-diyl diacetate (**7e**).* Mp: 254 °C; yield: 77%; IR (cm^{-1}) 3200 (NH), 1780 (CO ester), 1650 (CO ketone), 1620 (CN), 1540 (N–H); ^{1}H NMR (δ) 2.34 and 2.39 {2(s, 3H, CO–CH_3)}, 6.84 (td, 1H, H5′, $^3J = 7.1$ Hz and $^4J = 0.9$ Hz), 7.07 (d, 1H, H7, $^3J = 2.2$ Hz), 7.37 (d, 1H, H6′, $^3J = 8.4$ Hz), 7.45 (d, 1H, H5, $^4J = 2.2$ Hz), 7.69 (td, 1H, H4′, $^3J = 6.9$ Hz and $^4J = 1.8$ Hz), 7.79 (s, 1H, H4), 7.80 (dd, 1H, H2, $^3J = 9.9$ Hz and $^4J = 1.6$ Hz), 8.10 (d, 1H, H1, $^3J = 8.2$ Hz), 8.15 (s, 1H, CH═), 8.16 (dd, 1H, H3′, $^3J = 4.9$ Hz and $^4J = 0.9$ Hz), 11.27 (s, 1H, NH); ^{13}C NMR (δ) 20.7 and 20.7 CH_3 (Ac), 106.8 C6′, 109.0 C5, 112.4 C8a, 113.2 C7, 114.2 C4, 115.7 C5′, 120.6 C1a, 121.6 C2, 126.2 C1, 136.2 CH═, 137.9 C4′, 142.4 C6, 147.5 C3′, 150.3 C8, 154.7 C6, 155.1 C4a, 156.4 C_{ipso}, 157.0 C5a, 168.1 and 168.6 C═O (Ac), 173.5 C═O (xanthone). Anal. $C_{23}H_{17}N_3O_6$ (C, H, N).

*5.1.5.6. 9-Oxo-5-((2-(pyridin-2-yl)hydrazono)methyl)-9H-xanthene-1,3-diyl diacetate (**7f**).* Mp: 228 °C; yield: 88%; IR (cm^{-1}) 3300 (NH), 1780 (CO ester), 1660 (CO ketone), 1620 (CN), 1540 (N–H); ^{1}H NMR (δ) 2.35 and 2.41 {2(s, 3H, CO–CH_3)}, 6.79 (t, 1H, H5′, $^3J = 5.7$ Hz), 7.10 (s, 1H, H7), 7.29 (d, 1H, H6′, $^3J = 8.2$ Hz), 7.50 (s, 1H, H5), 7.67 (t, 1H, H4′, $^3J = 8.7$ Hz), 7.69 (d, 1H, H4, $^3J = 8.2$ Hz), 8.11–8.15 (m, 2H, H3′ and CH═), 8.22 (d, 1H, H3, $^3J = 9.3$ Hz), 8.25 (s, 1H, H1), 10.97 (s, 1H, NH). Anal. $C_{23}H_{17}N_3O_6$ (C, H, N).

*5.1.5.7. 7-((2-(4,5-Dihydro-1H-imidazol-2-yl)hydrazono)-methyl)-9-oxo-9H-xanthene-1,3-diyl diacetate (**7g**).* Mp: 260 °C (ethanol); yield: 20%; IR (cm^{-1}) 3400 (NH), 1770 (CO ester), 1660 (CO ketone), 1620 (CN), 1560 (N–H); ^{1}H NMR (δ) 2.36 and 2.39 {2(s, 3H, CO–CH_3)}, 3.78 (s, 4H, CH_2–CH_2), 7.09 (s, 1H, H7), 7.41 (s,1H, H5), 7.54 (t, 1H, H2, $^3J = 7.7$ Hz), 8.19 (d, 1H, H1, $^3J = 7.7$ Hz), 8.56 (d, 1H, H3, $^3J = 7.2$ Hz), 8.86 (m, 2H, CH═ and NH), 11.32 (m, 1H, NH); ^{13}C NMR (δ) 20.7 and 20.9 CH_3 (Ac), 42.7 CH_2–CH_2, 108.9 C5, 112.1 C8a, 113.4 C7, 119.3 C_{para}, 121.8 C1a, 124.2 C2, 128.0 C1, 122.2 C4, 131.6 C3, 140.8 CH═, 150.4 C_{ipso}, 152.7 C8, 154.9 C4a, 156.4 C6, 157.7 C5a, 168.1 and 168.6 C═O (Ac), 173.6 C═O (xanthone). Anal. $C_{22}H_{19}N_3O_6$ (C, H, N).

*5.1.5.8. 6-((2-(4,5-Dihydro-1H-imidazol-2-yl)hydrazono)-methyl)-9-oxo-9H-xanthene-1,3-diyl diacetate (**7h**).* Mp: 241 °C (ethanol); yield: 86%; IR (cm^{-1}) 3200 (NH), 1770 (CO ester), 1660 (CO ketone), 1560 (N–H); ^{1}H NMR (δ) 2.34 and 2.39 {2(s, 3H, CO–CH_3)}, 3.78 (s, 4H, CH_2–CH_2), 7.09 (d, 1H, H7, $^4J = 2.1$ Hz), 7.45 (d, 1H, H5, $^4J = 2.1$ Hz), 7.93 (d, 1H, H2, $^3J = 8.3$ Hz), 8.12 (s, 1H, H4), 8.15 (d, 1H, H1, $^3J = 8.3$ Hz), 8.33 (s, 1H, CH═), 8.85 (m, 1H, NH) 12.57 (s, 1H, NH); ^{13}C NMR (δ) 20.7 and 20.8 CH_3 (Ac), 42.7 CH_2–CH_2, 109.0 C5, 112.4 C8a, 113.4 C7, 116.4 C4, 122.1 C1a, 123.1 C2, 126.2 C1, 139.8 C3, 147.2 CH═, 150.4 C_{ipso}, 154.8 C8, 154.9 C4a, 157.0 C6, 157.8 C5a, 168.0 and 168.6 C═O (Ac), 173.6 C═O (xanthone). Anal. $C_{22}H_{19}N_3O_6$ (C, H, N).

*5.1.5.9. 5-((2-(4,5-Dihydro-1H-imidazol-2-yl)hydrazono)-methyl)-9-oxo-9H-xanthene-1,3-diyl diacetate (**7i**).* Mp: 259 °C dec. (acetone–water, 1:2); yield: 52%; IR (cm^{-1}) 3200 (NH), 1770 (CO ester), 1660 (CO ketone), 1620 (CN); ^{1}H NMR (δ) 2.34 and 2.39 {2(s, 3H, CO–CH_3)}, 3.76 (s, 4H, CH_2–CH_2), 7.11 (s, 1H, H7), 7.49 (s, 1H, H5, $^4J = 2.1$ Hz), 7.74 (d, 1H, H4, $^3J = 8.8$ Hz), 8.33 (s, 1H, CH═), 8.37 (d, 1H, H3, $^3J = 9.2$ Hz), 8.50 (s, 1H, H1), 8.72 (m, 1H, NH) 11.32 (s, 1H, NH); ^{13}C NMR (δ) 20.7 and 20.7 CH_3 (Ac), 42.6 CH_2–CH_2, 109.2 C5, 112.3 C8a, 113.5 C7, 118.5 C4, 121.4 C1a, 125.6 C1, 129.8 C2, 133.4 C3, 146.6 CH═, 150.3 C8, 154.9 C6, 155.8 C4a, 156.8 C5a, 157.7 C_{ipso}, 168.0 and 168.5 C═O (Ac), 173.7 C═O (xanthone). Anal. $C_{22}H_{19}N_3O_6$ (C, H, N).

*5.1.6. General procedure for the 1,3-dihydroxy-((2-arylhydrazono)methyl)-9H-xanthen-9-ones (**8**)*

The diacetate derivative **7** (0.3 mmol) was treated with sodium bicarbonate (252 mg, 3 mmol) in 20 ml of 1-propanol–water (1:1). The alkaline hydrolysis was processed by heating under reflux for 3 h and then cooling to 0 °C overnight. The phenolic hydrazone derivative **8** was collected and purified as described below.

*5.1.6.1. 1,3-Dihydroxy-7-((2-phenylhydrazono)methyl)-9H-xanthen-9-one (**8a**).* Mp: 256 °C dec. (acetone–water, 1:2); yield: 58% [20].

*5.1.6.2. 1,3-Dihydroxy-6-((2-phenylhydrazono)methyl)-9H-xanthen-9-one (**8b**).* Mp: 260 °C dec. (ethanol–water, 1:1); yield: 81% [20].

*5.1.6.3. 1,3-Dihydroxy-5-((2-phenylhydrazono)methyl)-9H-xanthen-9-one (**8c**).* Mp: 272 °C dec. (acetone–water, 1:2); yield: 48% [20].

*5.1.6.4. 1,3-Dihydroxy-7-((2-(pyridin-2-yl)hydrazono)methyl)-9H-xanthen-9-one (**8d**).* Mp: 286 °C dec.; yield: 92%; IR (cm^{-1}) 3500 (OH), 3200 (NH), 1650 (CO ketone), 1610 (CN), 1560 (N–H); ^{1}H NMR (δ) 5.62 (s, 1H, H7), 5.80 (s, 1H, H5), 6.79 (t, 1H, H5′, $^3J = 5.8$ Hz), 7.31 (d, 1H, H6′, $^3J = 7.5$ Hz), 7.34 (t, 1H, H2, $^3J = 7.7$ Hz), 7.66 (t, 1H, H4′, $^3J = 7.7$ Hz), 7.95 (dd, 1H, H1, $^3J = 7.5$ Hz and $^4J = 1.5$ Hz), 8.13 (d, 1H, H3′, $^3J = 4.5$ Hz), 8.20 (d, 1H, H3, $^3J = 8.0$ Hz), 8.60 (s, 1H, CH═), 11.08 (s, 1H, NH), 12.91 (s, 1H, OH). Anal. $C_{19}H_{13}N_3O_4$ (C, H, N).

*5.1.6.5. 1,3-Dihydroxy-6-((2-(pyridin-2-yl)hydrazono)methyl)-9H-xanthen-9-one (**8e**).* Mp: 319 °C dec.; yield: 98%; IR

(cm^{-1}) 3450 (OH), 3200 (NH), 1650 (CO ketone), 1540 (N–H); ^{1}H NMR (δ) 5.88 (s, 1H, H7), 6.06 (s, 1H, H5), 6.83 (m, 1H, H5′), 7.36 (d, 1H, H6′, $^3J = 8.7$ Hz), 7.67–7.70 (m, 3H, H4′, H4 and H2), 8.04 (d, 1H, H1, $^3J = 8.1$ Hz), 8.10 (s, 1H, CH═), 8.15 (m, 1H, H3′), 11.19 (s, 1H, NH), 12.92 (s, 1H, OH). Anal. $C_{19}H_{13}N_3O_4$ (C, H, N).

*5.1.6.6. 1,3-Dihydroxy-5-((2-(pyridin-2-yl)hydrazono)methyl)-9H-xanthen-9-one (**8f**).* Mp: 307 °C dec.; yield: 96%; IR (cm^{-1}) 3400 (OH), 3300 (NH), 1660 (CO ketone), 1530 (N–H); ^{1}H NMR (δ) 6.21 (s, 1H, H7), 6.39 (s, 1H, H5), 6.79 (m, 1H, H5′), 7.28 (d, 1H, H6′, $^3J = 8.4$ Hz), 7.62 (d, 1H, H4 $^3J = 8.4$ Hz), 7.67 (m, 1H, H4′), 8.13–8.15 (m, 2H, H3′ and CH═), 8.19 (d, 1H, H3, $^3J = 7.37$ Hz), 8.25 (s, 1H, H1), 10.96 (s, 1H, NH), 12.80 (s, 1H, OH). Anal. $C_{19}H_{13}N_3O_4$ (C, H, N).

*5.1.6.7. 7-((2-(4,5-Dihydro-1H-imidazol-2-yl)hydrazono)-methyl)-1,3-dihydroxy-9H-xanthen-9-one (**8g**).* After heating under reflux for 20 h, the solution was cooled to 0 °C, concentrated to half volume under vacuum and extracted with three portions of ethyl acetate. The organic layer was dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residual solid was triturated with hexane, filtered, recrystallized from methylethylcetone and dried at 60 °C.

Mp: 327 °C dec.; yield: 89%; IR (cm^{-1}) 3450 (OH), 3300 (NH), 1640 (CO ketone), 1560 (N–H); ^{1}H NMR (δ) 3.77 (s, 4H, CH_2–CH_2), 6.22 (s, 1H, H7), 6.38 (s, 1H, H5), 7.48 (t, 1H, H2, $^3J = 7.5$ Hz), 8.14 (d, 1H, H1, $^3J = 7.5$ Hz), 7.51 (d, 1H, H3, $^3J = 7.6$ Hz), 8.77 (m, 2H, CH═ and NH), 11.19 (m, 1H, NH), 12.63 (s, 1H, OH); ^{13}C NMR (δ) 42.7 CH_2–CH_2, 94.1 C5, 98.4 C7, 101.9 C8a, 120.3 C1a, 122.0 C4, 123.8 C2, 127.3 C1, 131.5 C3, 140.7 CH═, 153.0 C4a, 156.7 C5a, 157.8 C_{ipso}, 162.8 C8, 166.0 C6, 179.1 C═O (xanthone). Anal. $C_{17}H_{14}N_4O_4$ (C, H, N).

*5.1.6.8. 6-((2-(4,5-Dihydro-1H-imidazol-2-yl)hydrazono)-methyl)-1,3-dihydroxy-9H-xanthen-9-one (**8h**).* The mixture was refluxed for 3 h, cooled to 0 °C, and filtered. The solid was washed with water and dried at 60 °C.

Mp: 273 °C dec.; yield: 95% IR (cm^{-1}) 3450 (OH), 3200 (NH), 1650 (CO ketone), 1540 (N–H); ^{1}H NMR (δ) 3.47 (s, 4H, CH_2–CH_2), 6.17 (d, 1H, H7, $^4J = 2.1$ Hz), 6.34 (d, 1H, H5, $^4J = 2.1$ Hz), 6.80 (m, 1H, NH), 7.22 (m, 1H, NH), 7.75 (dd, 1H, H2, $^3J = 8.3$ Hz and $^4J = 1.2$ Hz), 7.83 (d, 1H, H4, $^4J = 1.0$ Hz), 8.01 (d, 1H, H1, $^3J = 8.2$ Hz), 8.06 (s, 1H, CH═), 12.92 (s, 1H, OH); ^{13}C NMR (δ) 42.5 CH_2–CH_2, 93.9 C5, 98.1 C, 101.9 C8a, 113.6 C4, 118.5 C1a, 122.0 C2, 124.9 C1, 141.9 CH═, 144.2 C3, 148.6 C_{ipso}, 155.6 C4a, 157.4 C5a, 162.8 C8, 166.2 C6, 179.1 C═O (xanthone). Anal. $C_{17}H_{14}N_4O_4$ (C, H, N).

*5.1.6.9. 5-((2-(4,5-Dihydro-1H-imidazol-2-yl)hydrazono)-methyl)-1,3-dihydroxy-9H-xanthen-9-one (**8i**).* The mixture was heated under reflux for 5 h. The resulting solution was cooled to 0 °C and then allowed to stand at 0 °C for 1 h. The precipitate was collected by filtration, washed with water and dried at 60 °C.

Mp: 262 °C dec.; yield: 52%; IR (cm^{-1}) 3400 (OH), 3200 (NH), 1660 (CO ketone), 1540 (NH); ^{1}H NMR (δ) 3.54 (s, 4H, CH_2–CH_2), 6.20 (d, 1H, H7, $^4J = 1.9$ Hz), 6.38 (d, 1H, H5, $^4J = 1.9$ Hz), 7.57 (d, 1H, H4, $^3J = 8.7$ Hz), 7.68 (m, 1H, NH), 8.18 (s, 1H, CH═), 8.26 (s, 1H, H1), 8.29 (dd, 1H, H3, $^3J = 8.8$ Hz and $^4J = 1.7$ Hz), 8.32 (s, 1H, NH), 12.75 (s, 1H, OH); ^{13}C NMR (δ) 47.5 CH_2–CH_2, 99.5 C5, 103.6 C7, 107.4 C8a, 123.2 C4, 125.1 C1a, 128.6 C1, 137.2 C2, 138.2 C3, 149.4 CH═, 154.2 C_{ipso}, 160.7 C4a 162.5 C5a, 168.1 C8, 171.4 C6, 184.7 C═O (xanthone). Anal. $C_{17}H_{14}N_4O_4$ (C, H, N).

5.2. Biological assays

Stock solutions (20 mM) of test compounds were first prepared in dimethylsulfoxide (DMSO) and stored at −20 °C. On the day of experiment, these concentrated solutions were diluted to the desired final concentrations immediately prior addition to cell culture wells. The final DMSO concentration was 0.1% in each well and showed no interference with the biological activities tested.

5.2.1. Cell culture

The human tumor cell lines KB 3.1 (oral squamous cell carcinoma) and MCF-7 (breast adenocarcinoma) were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Cells were routinely grown with either DMEM (KB 3.1) or RPMI 1640 medium (MCF-7), both supplemented with 10% fetal calf serum, and a mixture of antibiotics, all obtained from Biochrom AG (Berlin, Germany). They were grown on Petri dishes (Nunc, Denmark) at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were replicated every 4–5 days and the medium changed once in-between.

5.2.2. Viability assay

The potential effects on cell viability were investigated according to our previously reported conditions [26], using the MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma–Aldrich Chimie, Saint-Quentin-Fallavier, France] as an indicator of metabolically active cells.

Known number of MCF-7 or KB 3.1 cells (10^3) were transferred into 96-well plates in a volume of 200 μl of culture medium and incubated for 48 h before addition of test compounds. Cells were then exposed for 24 h at 37 °C to known concentrations of the compound to be tested (1 or 10 μM, expressed as final concentration). After drug exposure, the culture medium was removed and 200 μl of MTT reagent (diluted in culture medium, 1 mg/ml) was added. Following incubation for 4 h, the MTT/medium was removed and DMSO (200 μl) was added to dissolve the formazan crystals. Absorbance of the colored solution was measured on a microplate photometer (Bio-Tek Instruments) using a test wavelength of 570 nm and a reference wavelength of 630 nm. Results were evaluated by comparing the absorbance of the wells containing compound-treated cells with the absorbance of wells containing 0.1%

DMSO alone (solvent control). Conventionally, cell viability was estimated to be 100% in the solvent control. All experiments were performed at least twice in triplicate.

5.2.3. Effect of exposure time on antiproliferative activity

Tumor cells in exponential growth phase were exposed to the test compounds for 24 or 72 h, respectively, using a series of drug concentration between 1 μM and 100 μM. After drug exposure, the cells were washed twice with phosphate-buffered saline and then reincubated in fresh culture medium for a further 96 h.

The reduction in cell proliferation was measured using the MTT assay according to the same protocol as indicated in Section 5.2.2. Doxorubicin hydrochloride (purchased from Sigma) was used as positive control. The concentration of substance required for 50% growth inhibition (IC_{50}) was estimated as that giving a 50% decrease in absorbance as compared to controls incubated simultaneously without substance.

Acknowledgements

The authors wish to thank Mrs. Annie Lagardère and Mrs. Christiane Chapey for their collaboration. This work was supported by grants from the University Victor Segalen Bordeaux 2 and the Ligue Nationale Contre le Cancer.

References

[1] For a general review on naturally occurring oxygenated xanthones, see: V. Peres, T.J. Nagem, F. Faustino de Oliveira Phytochemistry 55 (2000) 683–710.
[2] M. Pedro, F. Cerqueira, M.E. Sousa, M.S. Nascimento, M. Pinto, Bioorg. Med. Chem. 10 (2002) 3725–3730.
[3] J. Mutanyatta, B.G. Matapa, D.D. Shushu, B.M. Abegaz, Phytochemistry 62 (2003) 797–804.
[4] M.C. Yimdjo, A.G. Azebaze, A.E. Nkengfack, M. Meyer, B. Bodo, Z.T. Fomum, Phytochemistry 65 (2004) 2789–2795.
[5] M.M.M. Pinto, M.E. Sousa, M.S.J. Nascimento, Curr. Med. Chem. 12 (2005) 2517–2538.
[6] L.M.M. Vieira, A. Kijjoa, Curr. Med. Chem. 12 (2005) 2413–2446.
[7] Y. Kwork, Q. Zeng, L.H. Hurley, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 13531–13536.
[8] K. Matsumoto, Y. Akao, H. Yi, K. Ohguchi, T. Ito, T. Tanaka, E. Kobayashi, M. Iinuma, Y. Nozawa, Bioorg. Med. Chem. 12 (2004) 5799–5806.
[9] K. Matsumoto, Y. Akao, K. Ohguchi, T. Ito, T. Tanaka, M. Iinuma, Y. Nozawa, Bioorg. Med. Chem. 13 (2005) 6064–6069.
[10] G.W. Rewcastle, G.J. Atwell, B.C. Baguley, M. Boyd, L.L. Thomsen, L. Zhuang, W.A. Denny, J. Med. Chem. 34 (1991) 2864–2870.
[11] B.C. Baguley, Lancet Oncol. 4 (2003) 141–148.
[12] J.W. Lippert, Bioorg. Med. Chem. 15 (2007) 605–615 and references cited therein.
[13] A.M. Gaya, G.J.S. Rustin, Clin. Oncol. 17 (2005) 277–290.
[14] G.M. Tozer, C. Kanthou, B.C. Baguley, Nat. Rev. Cancer 5 (2005) 423–435.
[15] P. Diana, P. Barraja, A. Lauria, A. Montalbano, A.M. Almerico, G. Dattolo, G. Cirrincione, Eur. J. Med. Chem. 37 (2002) 267–272.
[16] P. Vicini, M. Incerti, I.A. Doytchinova, P. La Colla, B. Busonera, R. Loddo, Eur. J. Med. Chem. 41 (2006) 624–632.
[17] K. Sztanke, J. Rzymowska, M. Niemczyk, I. Dybala, A.E. Koziol, Eur. J. Med. Chem. 41 (2006) 1373–1384.
[18] P.K. Grover, G.D. Shah, R.C. Shah, J. Chem. Soc. (1955) 3982–3985.
[19] P.E. Eaton, G.R. Carlson, J.T. Lee, J. Org. Chem. 38 (1973) 4071–4073.
[20] S. Moreau, M. Varache-Lembège, S. Larrouture, D. Fall, A. Neveu, G. Deffieux, J. Vercauteren, A. Nuhrich, Eur. J. Med. Chem. 37 (2002) 237–253.
[21] D. Landini, F. Rolla, Chem. Ind. (1979) 213.
[22] T. Mossman, J. Immunol. Methods 65 (1983) 55–63.
[23] M. Michael, M.M. Doherty, J. Clin. Oncol. 23 (2005) 205–229.
[24] S. Villamarin, S. Mansilla, N. Ferrer-Miralles, W. Priebe, J. Portugal, Eur. J. Biochem. 270 (2003) 764–770.
[25] E. Czeczuga-Semeniuk, S. Wolczynski, M. Dabrowska, J. Dzieciol, T. Anchim, Folia Histochem. Cytobiol. 42 (2004) 221–227.
[26] Y.P. Hu, J. Robert, Biochem. Pharmacol. 50 (1995) 169–175.