European Journal of Medicinal Chemistry 44 (2009) 2967–2974

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and antitumor activity of some new xanthotoxin derivatives

Omaima M. Abdel Hafez [a,*], Kamellia M. Amin [b], Nehad A. Abdel-Latif [a], Tahia K. Mohamed [a], Eman Y. Ahmed [a], Timothy Maher [c]

[a] Chemistry of Natural Product Department, National Research Center, El Tahrir Street, Dokki, Cairo, Egypt
[b] Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, Egypt
[c] Faculty of Pharmacy and Health Science, Boston, MA, USA

ARTICLE INFO

Article history:
Received 16 October 2008
Received in revised form
30 December 2008
Accepted 9 January 2009
Available online 19 January 2009

Keywords:
Xanthotoxin
Schiff's bases
Thiazolidinones
Anhydrides
Antitumor

ABSTRACT

The condensation of 4-amino-9-methoxy psoralene (4-aminoxanthotoxin) with some aromatic aldehydes led to the formation of 4-arylimine xanthotoxin derivatives **2a**–**h**, which were cyclized with mercaptoacetic acid to afford the thiazolidinone derivatives **3a**–**h**. On the other hand, the reaction of aminoxanthotoxin **1** with some anhydrides afforded 4-imidione derivatives **3a**–**d**. When **1** reacted with some isothiocyanates, the thiourea derivatives **5a**–**c** were obtained but the thiourea derivative **6** was obtained when **1** reacted with ammonium thiocyanate. The thiourea derivative **6** was cyclized by the reaction with monochloroacetic acid in the presence of sodium acetate to give aminothiazolidinone derivative **7**, but when the same reaction is carried out in the presence of pyridine, the thioxoimidazolidinone **8** was formed. The condensation of xanthotoxin sulphonamide with aromatic aldehydes gave the aryliminosulphonyl derivatives **9a**–**e**. Xanthotoxin sulphonyl hydrazine condensed with some anhydride afforded sulphonic acid imide derivatives **10a**–**c**. The antitumor and cytotoxic activities of **9** synthesized derivatives were tested, five compounds were found to be active, they inhibited the growth of HeLa cells.

1. Introduction

Xanthotoxin is a natural compound with antileukodermal activity and antitumor properties [1–6]. On the other hand, many publications reported that many Schiff bases with azomethine linkage show interesting inhibitory activity against experimental tumor systems [7–10], they could be hydrolyzed selectively by the tumor cells to serve as alkylating agent at the same time as the active amine is freed to act as antimetabolite [11]. Indeed the ability of many candidates containing the 4-thiazolidinone being such exhibit antiproliferative activity has been documented in numerous publications and reviews [12–14]; some of thiazolidinones exhibit the increase of the interaction between Hif-1α and P300 and prevent VEGF gene production in tumor cells under hypoxia conditions, the compounds are therefore useful for the control of angiogenesis and tumor growth. In the literature, thiourea derivatives have been described as new drugs with strong cancercidal activity [15], they have been used for brain cancer treatment [16–18]and a potent inhibitors of human DNA-topoisomerase II. The significant effect of the anhydride moiety as cytotoxic agent for T-Lymphoblastic Leukemia cell line (CEM) [19] and selective cytotoxic agent for leukemia HL-60 (TB) cell line has been reported [20]. All the previous findings generated our interest for preparing new derivatives by incorporating the xanthotoxin with the above-mentioned groups with antitumor activity and study the activity of the new obtained derivatives, with the hope of optimizing the antitumor and cytotoxic activity of the xanthotoxin.

2. Results and discussion

2.1. Chemistry

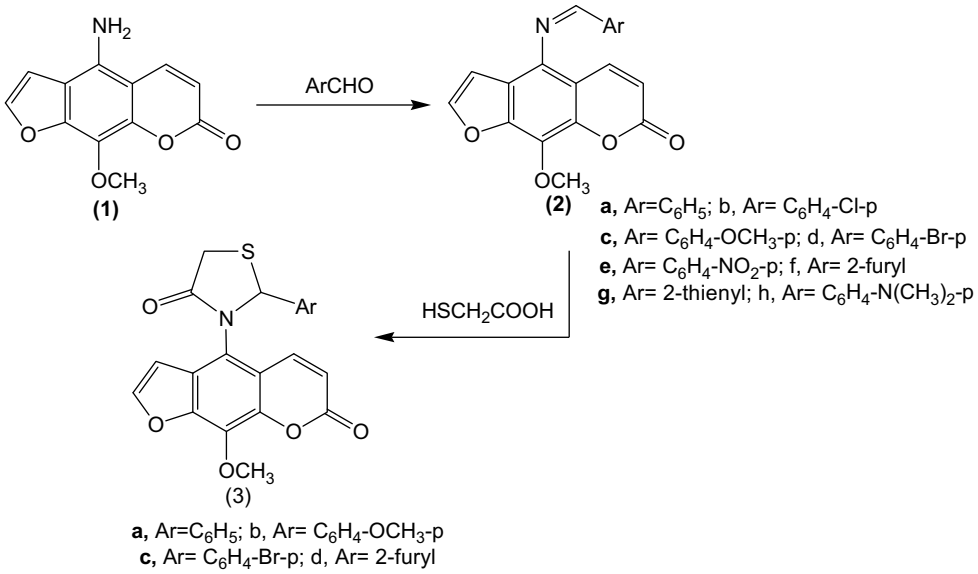
Condensation of 4-aminoxanthotoxin **1** with some aromatic aldehydes namely, benzaldehyde, *p*-chlorobenzaldehyde, *p*-methoxybenzaldehyde, *p*-bromobenzaldehyde, *p*-nitrobenzaldehyde, furfural, 2-thiophene aldehyde and *p*-*N*,*N*-dimethyl aminobenzaldehyde in chloroform in the presence of acetic acid yielded the corresponding 4-arylimine-9-methoxy psoralenes **2a**–**h** (Scheme 1).

The evidence for the formation of the 4-arylimine derivatives **2a**–**h** can be achieved by the microanalysis, ^{1}H NMR and IR spectra (Experimental part).

A facile synthesis of thiazolidin-4-ones which involves cyclocondensation of the appropriate imine with thioglycolic acid in refluxing benzene was reported [21,22]. Accordingly, reaction of the

* Corresponding author. Tel.: +20 237608284.
E-mail address: ommryan@yahoo.com (O.M. Abdel Hafez).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.01.006



Scheme 1.

Schiff bases **2a**, **c**, **d**, **f** with mercaptoacetic acid in dry benzene afforded the target thiazolidin-4-one derivatives **3a**–**d** in 69–81% yields (Scheme 1).

The structures of these compounds were confirmed with concordant microanalytical and spectral data (Experimental part).

Amr et al. [23] reported the preparation of the imide and bis-diimide by the reaction of the aminoxanthotoxin derivative with the appropriate anhydride and dianhydride in acetic acid (Scheme 2). The structures assigned to the products **4a**–**d** were substantiated by their microanalysis, ^{1}H NMR, IR and mass spectra (Experimental part).

In this work, the target thioureas **5a**–**c** were prepared by reaction of the key 4-aminoxanthotoxin with phenyl, ethyl and benzyl isothiocyanate in dry toluene under reflux (Scheme 3).

The proposed structures of the products **5a**–**c** were confirmed by elemental analysis, IR and mass spectra (Experimental part).

In this investigation, the target thiourea derivative **6** was synthesized by refluxing of 4-aminoxanthotoxin and ammonium thiocyanate in ethanol and diluted hydrochloric acid [24]. Its structure was confirmed by elemental analysis and mass spectrum (Experimental part).

In this work, the thiazolidinone derivatives **7** were prepared by the fusion of xanthotoxin thiourea **6** with chloroacetic acid in the presence of anhydrous sodium acetate [24]. The product was characterized by its physical, analytical and spectral data. When the foregoing reaction was applied using chloroacetic acid and the thiourea derivative **6** in the presence of pyridine, the target compound **8** was obtained in 60% yield.

The structural assignment for compound **8** was deduced by elemental and IR spectral analyses (Experimental part).

As previously discussed the Schiff bases **9a**–**e** were prepared by refluxing 9-methoxy-chromen-7-one-4-sulphonamide with the appropriate aldehyde in acetic acid to give the target compounds in good yield after the evaporation of the solvent (Scheme 4).

The structures of the new Schiff bases **9a**–**e** were assigned on the basis of analytical and spectral data (Experimental part).

As previously described the 4-cyclic imide dione derivatives **10a**–**c** were prepared by reaction of the xanthotoxin sulphonyl hydrazide with the appropriate anhydride in acetic acid to give the target compounds in good yield (Scheme 5).

The imides were characterized by elemental analysis, in addition to the IR spectrum (KBr, cm^{-1}) of compound (**10a**) (Experimental part).

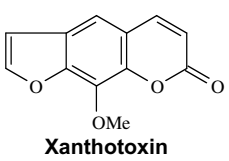
2.2. In vitro antitumor activity

Five compounds (**Xanthotoxin**, **2g**, **3c**, **5b** and **5c**) were active, inhibiting the growth of HeLa cells. In contrast, none of the compounds showed activity inhibiting the growth of (MCF-7) cells. IC_{50} values were calculated using an inhibitory model, with the sum of squares of the residuals minimized by Excel solver software. The values obtained for the five compounds against the HeLa cell line are shown in Table 1.

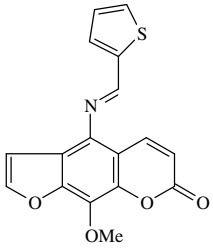
The viability data used to calculate the IC_{50} values is presented in Table 2 and the relationship between percent viability and the compound concentration is illustrated in Figs. 1–5.

Assay was run on two plates. The ratio of viability in wells containing 2.5% DMSO compared to wells containing media and cells but no DMSO in plate 1, in which compounds **Xanthotoxin**, **2g** and **3c** were tested, was 0.77 (control + DMSO/control − DMSO, %RSD 47, 32 respectively) and in plate 2 (compounds **5b** and **5c**) the ratio was 0.61 (34, 9).

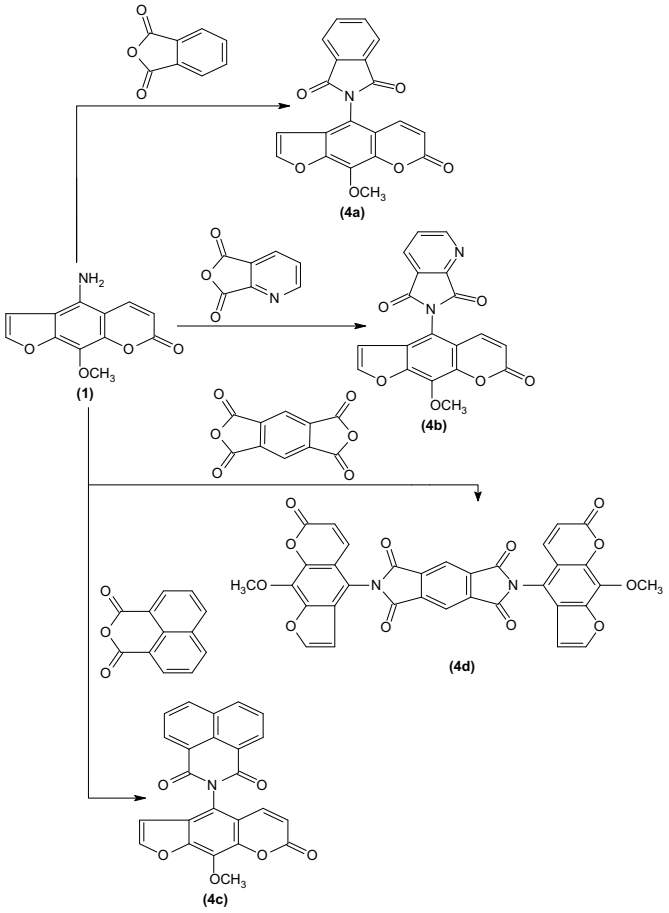
The results showed that the Schiff base (**2g**) was the most effective derivative of all the newly tested compounds, showing $IC_{50} = 7.2$ and percent viability 70 that are closely related to that of xanthotoxin, 7.6 and 62 respectively.



Xanthotoxin

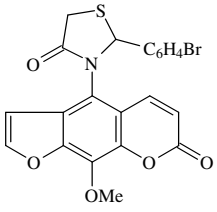


2g



Scheme 2.

In order to assess the activity of xanthotoxin bearing a heterocyclic ring at position 4, 4-thiazolidonyl xanthotoxin (**3c**) was tested and the result revealed that it was moderately active with an IC_{50} 40 and percent viability 82 at a concentration of 5 mcg/mL.



3c

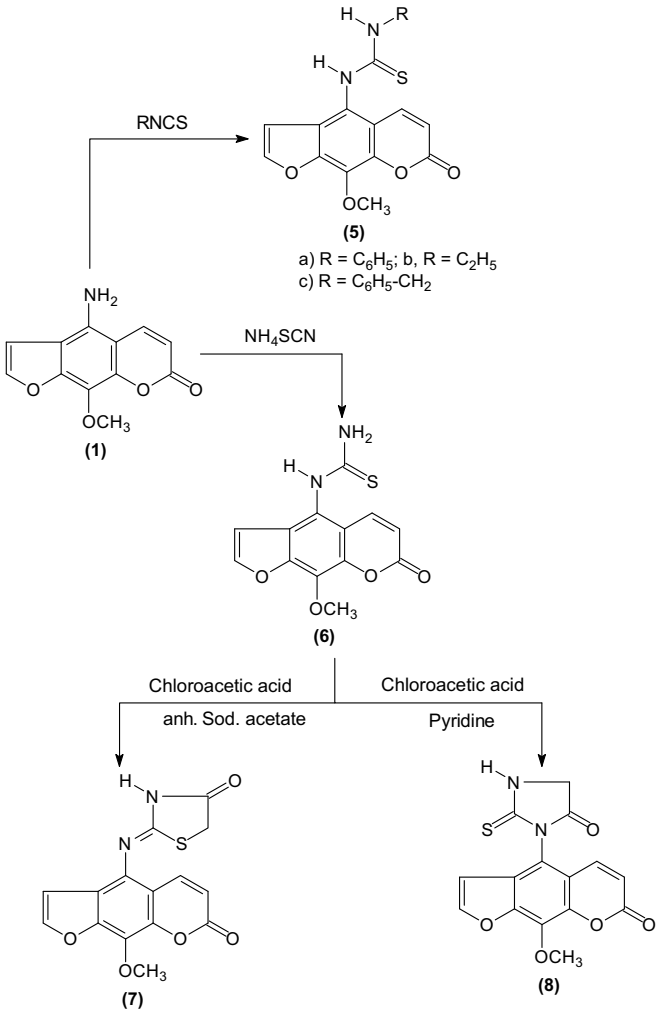
In case of xanthotoxin thiourea derivatives, the benzyl derivative (**5c**), of $IC_{50} = 7.5$, elicited higher cytotoxicity than the ethyl derivative (**5b**), of $IC_{50} = 23$, the two compounds showed % viability 56 and 70 at a concentration of 10 mcg/mL.

The survival curves obtained after treating the selected tumor cell line with the tested compounds are illustrated in Figs. 1–5.

3. Experimental

3.1. Synthesis

Melting points were determined on Electrothermal IA 9000 apparatus and were uncorrected. Elemental microanalysis were performed on Elementar, Vario EL, at the Micro Analytical Center, Faculty of Science, Cairo University. The infrared (IR) spectra were

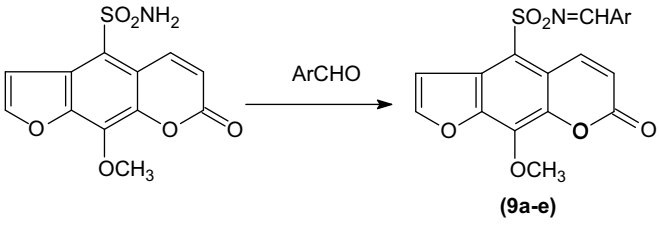


Scheme 3.

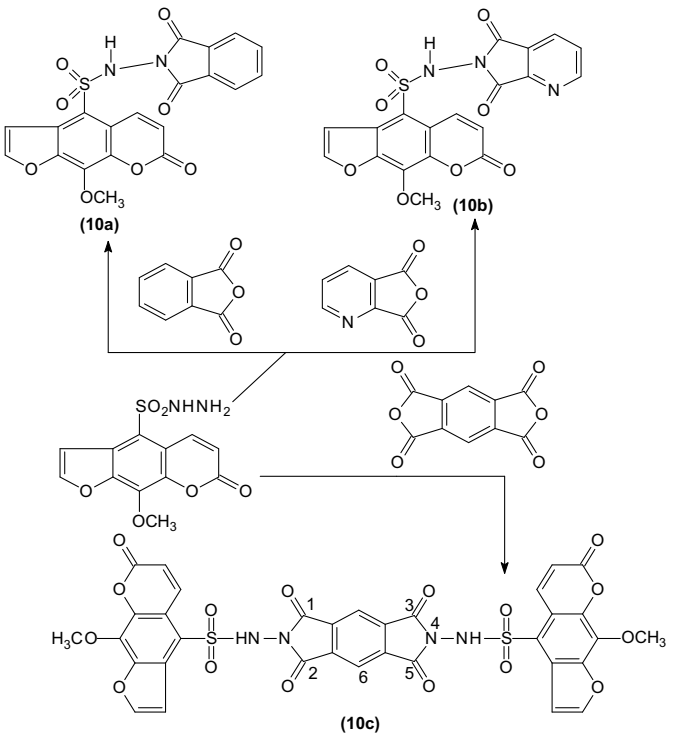
recorded on Nexus 670 FT-IR FT-Raman spectrometer as potassium bromide discs, at the National Research Centre. The proton nuclear resonance (^{1}H NMR) spectra were determined on Varian mercury 300 MHz spectrometer, using tetramethylsilane (TMS) as the internal standard, at Cairo University. The mass spectra were performed on JEOL JMS-AX500 mass spectrometer at the National Research Centre. The reactions were followed by TLC (Silica gel, aluminum sheets 60 F_{254}, Merck) using benzene:ethyl acetate (8:2 v/v) as eluent and sprayed with iodine–potassium iodide reagent.

*3.1.1. Preparation of 4-arylimine-9-methoxy[2,3-g]chromen-7-ones (Schiff's bases) (**2a–h**)*

3.1.1.1. General procedure. A mixture of 4-aminoxanthotoxin (2.31 g, 0.01 mol) and the appropriate aldehydes (0.012 mol), namely, benzaldehyde, 4-chlorobenzaldehyde, anisaldehyde, 4-bromobenzaldehyde, 4-nitrobenzaldehyde, furfural, 2-thiophene



Scheme 4.



Scheme 5.

aldehyde or 4-dimethyl aminobenzaldehyde in 20 mL of chloroform and 2 mL acetic acid was refluxed for 10–15 h. The solid obtained by evaporation of the solvent was crystallized from chloroform/methanol to afford the Schiff bases.

3.1.1.2. Microanalytical and spectral data of compounds ***10a–c*** *(Mol. For., M. wt., yield % and m.p. °C)*

*3.1.1.2.1. 4-(Benzylideneamino)-9-methoxy-7H-furo[3,2-g]chromen-7-one (**2a**) [25] ($C_{19}H_{13}NO_4$, 319.298, 79, 198–200).* Anal. Calcd.: C, 71.47; H, 4.02; N, 4.39. Found: C, 71.41; H, 4.05; N, 4.30. IR (cm^{-1}, KBr): 1749 (C=O α-pyrone), 1680 (C=N), 1230 (C–O), 1290 (C–N), ^{1}H NMR spectrum (DMSO-d_6, δ, ppm) 3.7 (3H, s, OCH_3), 6.5 (1H, d, H-6), 6.7 (1H, d, H-3), 7.2–7.7 (7H, m, 5 Ar–H, H-2, H-5), 8.5 (1H, s, N=CH).

*3.1.1.2.2. 4-(4-Clorobenzylideamino)- 9-methoxy-7H-furo[3,2-g]chromen-7-one (**2b**) [26] ($C_{19}H_{12}ClNO_4$, 353.755, 62.1, 210–212).* Anal. Calcd.: C, 64.51; H, 3.42; N, 3.96. Found: C, 64.45; H, 3.36; N, 3.91. IR (cm^{-1}, KBr): 1749 (C=O α-pyrone), 1699 (C=N), 1235 (C–O), 1295 (C–N), 760 (C–Cl). ^{1}H NMR spectrum (DMSO-d_6, δ, ppm) 4.12 (3H, s, OCH_3), 6.3 (1H, d, H-6), 7.12 (1H, d, H-3), 7.2–7.8 (6H, m, 4 Ar–H, H-2, H-5), 8.7 (1H, s, N=CH).

*3.1.1.2.3. 4-(4-Methoxybenzylideneamino)-9-methoxy-7H-furo[3,2-g]chromen-7-one (**2c**) [27] ($C_{20}H_{15}NO_5$, 349.328, 65.3, 125–127).* Anal. Calcd.: C, 68.76; H, 4.33; N, 4.00. Found: C, 68.71; H, 4.29; N, 3.96 IR (cm^{-1}, KBr): 1743 (C=O α-pyrone), 1695 (C=N), 1229 (C–O), 1295 (C–N), 760 (C–Cl). ^{1}H NMR spectrum (DMSO-d_6, δ, ppm) 3.9 (3H, s, OCH_3 xanthotoxin), 4.12 (3H, s, OCH_3 aromatic), 6.5 (1H, d, H-6), 6.7 (1H, d, H-3), 7.2–7.8 (6H, m, 4 Ar–H, H-2, H-5), 8.4 (1H, s, N=CH).

*3.1.1.2.4. 4-(4-Bromobenzylideamino)-9-methoxy-7H-furo[3,2-g]chromen-7-one (**2d**) [26] ($C_{19}H_{12}BrNO_4$, 398.214, 60.54, 280–285).* Anal. Calcd.: C, 57.30; H, 3.04; N, 3.52. Found: C, 57.27; H, 2.99; N, 3.48 IR (cm^{-1}, KBr): 1739 (C=O α-pyrone), 1685 (C=N), 1230 (C–O), 1290 (C–N) 616 (C–Br). ^{1}H NMR spectrum (DMSO-d_6, δ, ppm) 3.7 (3H, s, OCH_3), 6.5 (1H, d, H-6), 6.7 (1H, d, H-3), 7.5–7.9 (6H, m, 4 Ar–H, H-2, H-5), 8.4 (1H, s, N=CH).

*3.1.1.2.5. 4-(4-Nitrobenzylidenamino)-9-methoxy-7H-furo[3,2-g]chromen-7-one (**2e**) [26] ($C_{19}H_{12}N_2O_6$, 364.22, 77, 312–315).* Anal. Calcd.: C, 62.65; H, 3.32; N, 7.69. Found: C, 62.61; H, 3.28; N, 7.66. IR (cm^{-1}, KBr): 1742 (C=O α-pyrone), 1685 (C=N), 1240 (C–O), 1290 (C–N), 1522 (N=O). ^{1}H NMR spectrum (DMSO-d_6, δ, ppm) 3.7 (3H, s, OCH_3), 6.5 (1H, d, H-6), 6.7 (1H, d, H-3), 7.4 (1H, d, H-5), 7.5–8.2 (5H, m, 4 Ar–H, H-2), 8.4 (1H, s, N=CH).

*3.1.1.2.6. 4-(Furan-2-yl-methyleneamino)-9-methoxy-7H-furo[3,2-g]chromen-7-one (**2f**) ($C_{17}H_{11}NO_5$, 309.268, 76.7, 134–135).* Anal. Calcd.: C, 66.02; H, 3.58; N, 4.53. Found: C, 66.01; H, 3.51; N, 4.50. IR (cm^{-1}, KBr): 1748 (C=O α-pyrone), 1690 (C=N), 1240 (C–O), 1293 (C–N). ^{1}H NMR spectrum (DMSO-d_6, δ, ppm) 4.12 (3H, s, OCH_3), 6.4 (1H, d, H-6), 6.6 (1H, d, H-3), 7.5–7.8 (6H, m, 4 Ar–H, H-2, H-5), 8.6 (1H, s, N=CH).

*3.1.1.2.7. 4-(Thiophen-2-yl-methyleneamino)-9-methoxy-7H-furo[3,2-g]chromen-7-one (**2g**) ($C_{17}H_{11}NO_4S$, 325.334, 61.5, 222–223).* Anal. Calcd.: C, 62.76; H, 3.41; N, 4.31. Found: C, 62.71; H, 3.37; N, 4.29. IR (cm^{-1}, KBr): 1749 (C=O α-pyrone), 1709 (C=N), 1245 (C–O), 1284 (C–N). ^{1}H NMR spectrum (DMSO-d_6, δ, ppm) 3.7 (3H, s, OCH_3), 6.2 (1H, d, H-6), 6.7 (1H, d, H-3), 7–7.2 (3H, m, thiophene protons), 7.4 (1H, d, H-5), 7.5 (1H, d, H-2), 7.6 (1H, s, N=CH).

*3.1.1.2.8. 4-(4-(dimethylamino)benzylideneamino)-9-methoxy-7H-furo[3,2-g]chromen-7-one (**2h**) ($C_{21}H_{18}N_2O_4$, 362.37, 72,184–185).* Anal. Calcd.: C, 69.60; H, 5.01; N, 7.73. Found: C, 69.57; H, 4.94; N, 7.71. IR (cm^{-1}, KBr): 1740 (C=O α-pyrone), 1695 (C=N),

Table 1
IC_{50} of some newly synthesized compounds.

Compound no.	IC_{50} (mcg/mL)
Xanthotoxin	7.6
2g	7.2
3c	40
5b	23
5c	7.5

Table 2
Percent viability against dose concentrations.

Dose (mcg/mL)	Compound				
	Xanthotoxin	**2g**	**3c**	**5b**	**5c**
5	62 (17)[a]	70 (24)	82 (27)	101 (36)	74 (11)
10	51 (33)	53 (10)	91 (18)	70 (31)	56 (11)
50	21 (18)	32 (21)	26 (2)	35 (9)	34 (11)
100	16 (5)	28 (21)	26 (12)	41 (27)	35 (14)

[a] Values given are percent viability with %RSD of three wells/concentration in parentheses.

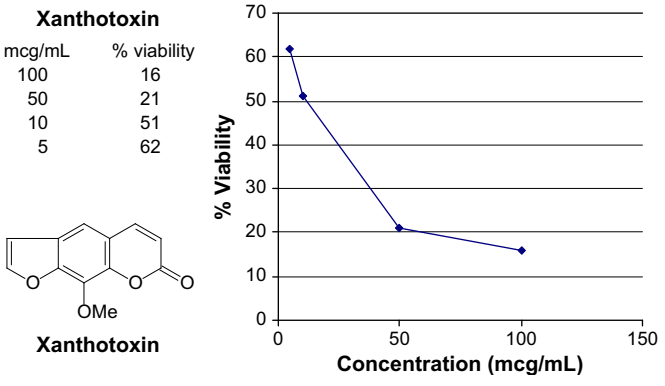


Fig. 1. Survival curve of **Xanthotoxin**.

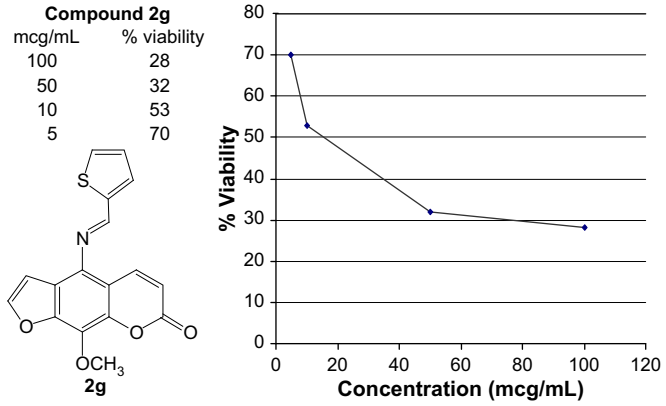


Fig. 2. Survival curve of **2g**.

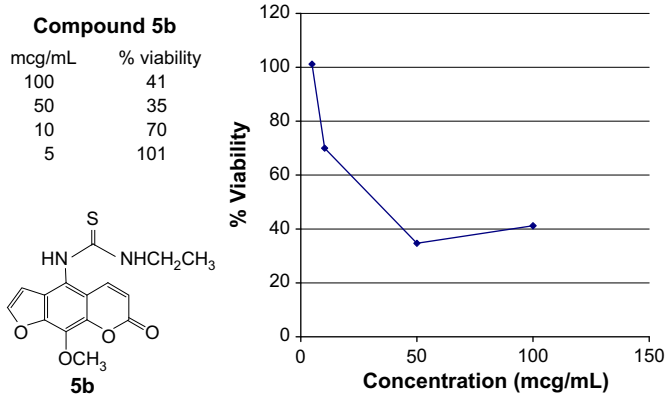


Fig. 4. Survival curve of **5b**.

1235 (C–O), 1298 (C–N). ^{1}H NMR spectrum (DMSO-d_6, δ, ppm) 2.48 (6H, s, 2CH_3), 4.1 (3H, s, OCH_3), 6.1 (1H, d, H-6), 6.7 (1H, d, H-3), 7.3 (1H, d, H-5), 7.5 (1H, d, H-2), 8.2–8.4 (4H, 2dd, Ar–H), 8.5 (1H, s, N=CH).

*3.1.2. Preparation of (7-oxo-furo[3,2-g]chromen-4-yl)-2-aryl-thiazolidin-4-ones (**3a–d**)*

3.1.2.1. General procedure. To a well-stirred suspension of the appropriate arylidene (**2a**, **c**, **d**, **f**) (0.001 mol) in dry benzene (15 mL), mercaptoacetic acid (0.42 mL, 0.006 mol) in dry benzene (5 mL) was added. The reaction mixture was refluxed for 3 h, cooled; the deposited solid was filtered off and crystallized from ethanol to give the title compounds (Table 2).

*3.1.2.2. Microanalytical and spectral data of compounds **10a–c** (Mol. For., M. wt., yield % and m.p. °C)*

*3.1.2.2.1. 3-(9-Methoxy-7-oxo-7H-furo[3,2-g]chromen-4-yl)-2-phenyl-thiazolidin-4-one (**3a**) ($C_{21}H_{15}NO_5S$, 393.404, 69, 223–225).* Anal. Calcd.: C, 64.11; H, 3.84; N, 3.56; S, 8.15. Found: C, 64.04; H, 3.79; N, 3.51; S, 8.09. IR (cm^{-1}, KBr): 1740 (C=O α-pyrone), 1775 (C=O thiazolidinone), 1223 (C–O), 1294 (C–N). ^{1}H NMR spectrum ($CDCl_3$, δ, ppm) 3.3, 3.4 (2H, dd, CH_2 thiazolidinone), 3.73 (3H, s, OCH_3), 5.7 (1H, s, CH thiazolidinone), 6.2 (1H, d, H-6), 6.7 (1H, d, H-3), 7–7.5 (7H, m, 4 Ar–H, H-2, H-5). MS: M^+ 393 (2%), 295 (10%), 230 (19%), 215 (28%), 393 (2%), 105 (100%), 77 (50%).

*3.1.2.2.2. 3-(9-Methoxy-7-oxo-7H-furo[3,2-g]chromen-4-yl)-2-(4-methoxyphenyl) thiazolidin-4-one (**3b**) ($C_{22}H_{17}NO_6S$, 423.434, 76.1, 238–240).* Anal Calcd.: C, 62.40; H, 4.05; N, 3.31; S, 7.57. Found: C, 62.37; H, 4.01; N, 3.30; S, 7.53. IR (cm^{-1}, KBr): 1740 (C=O α-pyrone), 1770 (C=O thiazolidinone), 1220 (C–O), 1295 (C–N). ^{1}H NMR spectrum ($CDCl_3$, δ, ppm) 3.3, 3.4 (2H, dd, CH_2 thiazolidinone), 3.73 (3H, s, OCH_3 xanthotoxin), 4.1 (3H, s, OCH_3 aromatic), 5.9 (1H, s, CH thiazolidinone), 6.12 (1H, d, H-6), 6.7 (1H, d, H-3), 7.1–7.5 (6H, m, 4 Ar–H, H-2, H-5). MS: 421 (8%), 231 (100%), 218 (95%), 188 (75%), 120 (70%).

*3.1.2.2.3. 2-(4-Bromophenyl)-3-(9-methoxy-7-oxo-7H-furo[3,2-g]chromen-4-yl)-thiazolidin-4-one (**3c**) ($C_{21}H_{14}BrNO_5$, 472.31, 71.7, 217–220).* Anal. Calcd.: C, 53.40; H, 2.99; N, 2.96; S, 6.79. Found: C, 53.38; H, 2.96; N, 2.92; S, 6.75. IR (cm^{-1}, KBr): 1749 (C=O α-pyrone), 1775 (C=O thiazolidinone), 1225 (C–O), 1295 (C–N), 616 (C–Br). ^{1}H NMR spectrum ($CDCl_3$, δ, ppm) 3.3, 3.4 (2H, dd, CH_2 thiazolidinone), 3.7 (3H, s, OCH_3), 5.9 (1H, s, CH thiazolidinone), 6.12 (1H, d, H-6), 6.6 (1H, d, H-3), 6.9–7.5 (6H, m, 4 Ar–H, H-2, H-5). M^+/M^{+2} 472/474 (0.56%, 0.50%), 396/398 (8.53%, 7.29%), 230 (54.76%), 215 (64.03%), 63 (100%).

*3.1.2.2.4. 2-Furan-2-yl-3-(9-methoxy-7-oxo-7H-furo[3,2-g]chromen-4-yl)-thiazolidin-4-one (**3d**) ($C_{19}H_{13}NO_6S$, 383.364, 80.7, 192–195).* Anal. Calcd.: C, 59.52; H, 3.42; N, 3.65; S, 8.36. Found: C, 59.51; H, 3.39; N, 3.61; S, 8.35. IR (cm^{-1}, KBr): 1742 (C=O α-pyrone), 1772 (C=O thiazolidinone), 1225 (C–O), 1290 (C–N). ^{1}H NMR spectrum ($CDCl_3$, δ, ppm) 3.3, 3.4 (2H, dd, CH_2 thiazolidinone), 3.7 (3H, s, OCH_3), 5.9 (1H, s, CH thiazolidinone), 6.12 (1H, d, H-6), 6.2–6.5 (3H, m, furan protons), 6.6 (1H, d, H-3), 7.3–7.5 (2H, d, H-2, H-5). M^+ 383 (10%), 349 (30%), 215 (95%), 158 (100%), 143 (80%).

*3.1.3. Preparation of 4-cyclic imide dione chromene-7-one derivatives (**4a–d**)*

To a solution of aminoxanthotoxin (**1**) (2.31 g, 0.01 mol) in acetic acid, the appropriate anhydrides (0.01 mol) namely phthalic anhydride, pyridine anhydride, 1,8-napthaleneanhydride or benzene dianhydride were added. The reaction mixture was

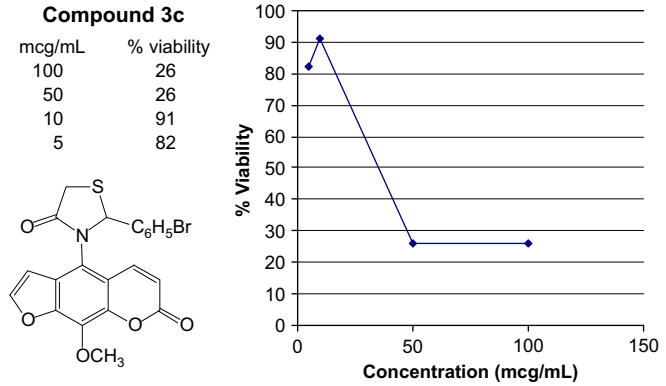


Fig. 3. Survival curve of **3c**.

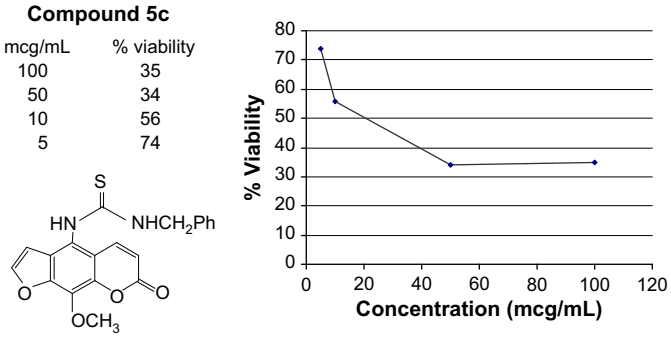


Fig. 5. Survival curve of **5c**.

refluxed for 5–10 h; the formed precipitate by cooling was filtered off and crystallized from glacial acetic acid to give the corresponding cyclic imide dione derivatives.

*3.1.3.1. Microanalytical and spectral data of compounds **10a–c** (Mol. For., M. wt., yield % and m.p. °C)*

*3.1.3.1.1. 6-(9-Methoxy-7-oxo-7*H*-furo[3,2-g]chromen-4-yl)-isoindole-1,3-dione (**4a**) ($C_{20}H_{11}NO_6$, 361.301, 88.4, 227–230).* Anal. Calcd.: C, 66.48; H, 3.07; N, 3.88. Found: C, 66.42; H, 3.11; N, 3.86. IR (cm^{-1}, KBr): 1740 (C=O α-pyrone), 1750 (C=O imide), 1225 (C–O), 1290 (C–N). ^{1}H NMR spectrum (DMSO-d_6, δ, ppm) 4.0 (3H, s, OCH_3), 6.12 (1H, d, H-6), 6.6 (1H, d, H-3), 7.3 (1H, d, H-5), 7.5 (1H, d, H-2), 8–8.2 (4H, m, 4 Ar–H). MS: M^+ 361 (5%), 330 (71%), 215 (100%).

*3.1.3.1.2. 2-(9-Methoxy-7-oxo-7*H*-furo[3,2-g]chromen-4-yl)-pyrrolo[3,4-*b*]pyridin-5,7-dione (**4b**) ($C_{19}H_{10}N_2O_6$, 362.29, 94.34, 240–243).* Anal. Calcd.: C, 62.98; H, 2.78; N, 7.73. Found: C, 62.92; H, 2.72; N, 7.71. IR (cm^{-1}, KBr): 1735 (C=O α-pyrone), 1753 (C=O imide), 1228 (C–O), 1298 (C–N). ^{1}H NMR spectrum (DMSO-d_6, δ, ppm) 3.9 (3H, s, OCH_3), 6.12 (1H, d, H-6), 6.4 (1H, d, H-3), 7.2 (1H, d, H-5), 7.4 (1H, d, H-2), 8–9 (3H, m, pyridine protons). MS: M^+ 361 (2%), 272 (100%), 229 (90%), 216 (98%), 173 (50%), 120 (62%).

*3.1.3.1.3. 2-(9-Methoxy-7-oxo-7*H*-furo[3,2-g]chromen-4-yl)-benzo[de]isoqunolin-1,3-dione (**4c**) ($C_{24}H_{13}NO_6$, 411.35, 83.09, 23-2).* Anal. Calcd.: C, 70.07; H, 3.18; N, 3.40. Found: C, 70.05; H, 3.16; N, 3.39. IR (cm^{-1}, KBr): 1743 (C=O α-pyrone), 1752 (C=O imide), 1222 (C–O), 1295 (C–N). ^{1}H NMR spectrum (DMSO-d_6, δ, ppm) 3.9 (3H, s, OCH_3), 6.12 (1H, d, H-6), 6.7 (1H, d, H-3), 7.2 (1H, d, H-5), 7.4–8 (7H, m, 6 Ar–H, H-2). MS: M^+ 411 (84.6%), 410 (50.9%), 213 (28.7%), 212 (34.7%), 180 (85.8%), 179 (100%), 170 (30.2%), 152 (36.2%).

*3.1.3.1.4. 2,6-Bis-(9-methoxy-7-oxo-7*H*-furo[3,2-g]chromen-4-yl)-pyrrolo[3,4-f]-isoindol-1,3,5,7-tetraone (**4d**) ($C_{34}H_{16}N_2O_{12}$, 644.49, 84.3, 300).* Anal. Calcd.: C, 63.36; H, 2.50; N, 4.35. Found: C, 63.33; H, 2.49; N, 4.31. IR (cm^{-1}, KBr): 1742 (C=O α-pyrone), 1752 (C=O imide), 1227 (C–O), 1298 (C–N). ^{1}H NMR spectrum (DMSO-d_6, δ, ppm) 3.8 (3H, s, OCH_3), 6.2 (1H, d, H-6), 6.7 (1H, d, H-3), 7.3 (1H, d, H-5), 7.4 (1H, d, H-2), 8.9 (2H, s, 2 Ar–H). MS: M^+ 644 (10%), 215 (100%), 187 (58%).

*3.1.4. Preparation of 7-oxo-furochromen-4-yl thiourea derivatives (**5a–c**)*

To a solution of aminoxanthotoxin (**1**) (2.31 g, 0.01 mol) in 40 mL dry toluene, the appropriate isothiocyanates (0.015 mol) namely phenyl, ethyl or benzyl isothiocyanate respectively were added. The mixture was refluxed for 3 h, cooled, filtered and crystallized from methanol to afford the title compounds.

*3.1.4.1. Microanalytical and spectral data of compounds **10a–c** (Mol. For., M. wt., yield % and m.p. °C)*

*3.1.4.1.1. 1-(9-Methoxy-7-oxo-7*H*-furo[3,2-g]chromen-4-yl)-3-phenyl thiourea (**5a**) ($C_{19}H_{14}N_2O_4S$, 366.391, 35.5, 241–244).* Anal. Calcd.: C, 62.28; H, 3.85; N, 7.65; S, 8.75. Found: C, 62.22; H, 3.81; N, 7.62; S, 8.70. IR (cm^{-1}, KBr): 1743 (C=O α-pyrone), 3478, 3384 (Two NH groups, br s), 1285 (C=S), 1290 (C–N). MS: M^+ 366 (7.78%), 277 (8.73%), 231 (62.49%), 216 (100%), 188 (43%), 231 (12%), 215 (64%).

*3.1.4.1.2. 1-Ethyl-3-(9-methoxy-7-oxo-7*H*-furo[3,2-g]chromen-4-yl)thiourea (**5b**) ($C_{15}H_{14}N_2O_4S$, 318.351, 72.2, 233–235).* Anal. Calcd.: C, 56.59; H, 4.43; N, 8.80; S, 10.07. Found: C, 56.56; H, 4.41; N, 8.79; S, 10.02. IR (cm^{-1}, KBr): 1745 (C=O α-pyrone), 3384 (NH, br s), 1260 (C=S), 1295 (C–N). MS: M^+ 318 (15%), 231 (72%), 216 (100%),188 (30%).

*3.1.4.1.3. 1-Benzyl-3-(9-methoxy-7-oxo-7*H*-furo[3,2-g]chromen-4-yl)thiourea (**5c**) ($C_{20}H_{16}N_2O_4S$, 380.421, 57.9, 238–240).* Anal. Calcd.: C, 63.14; H, 4.24; N, 7.36; S, 8.43. Found: C, 63.11; H, 4.22; N, 7.33; S, 8.41. IR (cm^{-1}, KBr): 1747 (C=O α-pyrone), 3470 (NH, br s), 1260 (C=S), 1285 (C–N). MS: 381 (5%), 252 (25%), 230 (72%), 216 (100%), 188 (27%).

*3.1.5. Preparation of 9-methoxy-7-oxo-7*H*-furo[3,2-g]chromen-4-yl thiourea (**6**)*

To a solution of 4-aminoxanthotoxin (**1**) (1 g, 0.0043 mol) in ethanol (20 mL) and dilute hydrochloric acid (20 mL), ammonium thiocyanate (0.5 g, 0.005 mol) was added. The mixture was refluxed for 10 h. The solution was cooled. The precipitated solid was filtered off, washed with water and crystallized from DMF/water, yield 78%, m.p. above 300 °C.

Analysis for $C_{13}H_{10}N_2O_4S$ (M. wt. 290.31): Calculated %: C, 53.78; H, 3.47; N, 9.65; S, 11.05. Found %: C, 53.74; H, 3.5; N, 9.67; S, 11.10.

Mass spectrum of **6**: M^+ 290 (3.99%), 259 (2.8%), 231 (64.0%), 215 (100%), 187 (22.8%), 158 (23%), 130 (3%), 102 (6%) and 77 (13%).

*3.1.6. Preparation of 2-(9-methoxy-7-oxo-7*H*-furo[3,2-g]-chromen-4-yl-amino)-thiazolidine-4-one (**7**)*

A mixture of thiourea derivative (**6**) (1 g, 3.4 mol), anhydrous sodium acetate (0.5 g) and monochloroacetic acid (0.4 g, 4 mol) was fused for 6 h and filtered off to give a solid product, which was crystallized from DMF/H_2O, yield 65%, m.p. above 300 °C.

Analysis for $C_{15}H_{10}N_2O_5S$ (M. wt. 330.3): Calculated %: C, 54.87; H, 3.07; N, 8.53; S, 9.77. Found %: C, 54.89; H, 3.10; N, 8.60; S, 9.71.

Mass spectrum: M^+ 330 (5.46%), 245 (0.9%), 231 (44.6%), 216 (72.7%), 215 (100%), 187 (16%). IR (KBr, cm^{-1}): 3451 (NH), 3123 (C–H, stretching), 1777 (C=O, thiazolidine), 1759 (C=O, α-pyrone), 1667 (C=N).

*3.1.7. Preparation of 3-(9-methoxy-7-oxo-7*H*-furo[3,2-g]-chromen-4-yl)-2-thioxo-imidazolidine-4-one (**8**)*

A mixture of thiourea derivative (**6**) (1 g, 3.4 mol), monochloroacetic acid (0.4 g, 4 mol) and drops of pyridine was fused for 6 h, poured on crushed ice then filtered off to give a solid product which was crystallized from DMF/water, yield 60%, m.p. above 300 °C.

Analysis for $C_{15}H_{10}N_2O_5S$ (M. wt. 330.3): Calculated %: C, 54.87; H, 3.07; N, 8.53; S, 9.77. Found %: C, 54.70; H, 3.15; N, 8.40; S, 9.72.

IR (KBr, cm^{-1}): 3448 (NH), 3123 (C–H, stretching), 1743 (C=O, α-pyrone), 1769 (C=O, imidazolidine).

*3.1.8. Preparation of 4-aryliminosulphonyl-furochromen-7-one derivatives (Schiff's bases) (**9a–e**)*

To a solution of xanthotoxin sulfonamide **8** (2.95 g, 0.01 mol) in acetic acid, the appropriate aldehydes (0.12 mol) namely benzaldehyde, 4-chlorobenzaldehyde, anisaldehyde, 4-bromobenzaldehyde or furfural respectively were added. The mixture was refluxed for 6 h. The solid obtained by evaporation of the solvent was crystallized from benzene to afford the Schiff bases (**9a–e**).

*3.1.8.1. Microanalytical and spectral data of compounds **10a–c** (Mol. For., M. wt., yield % and m.p. °C)*

*3.1.8.1.1. 9-Methoxy-7-oxo-7*H*-furo[3,2-g]chromen-4-sulfonic acid benzylidine-amide (**9a**) ($C_{19}H_{13}NO_6S$, 383.36, 46.2, 227–229).* Anal. Calcd.: C, 59.52; H, 3.42; N, 3.65; S, 8.36. Found: C, 59.49; H, 3.39; N, 3.61; S, 8.32. IR (cm^{-1}, KBr): 1731 (C=O α-pyrone), 1370, 1170 (SO_2–N), 1669 (C=N). MS: M^+ 383 (1%), 312 (50%), 249 (49%), 252 (100%), 161 (75%), 146 (88%).

*3.1.8.1.2. 9-Methoxy-7-oxo-7*H*-furo[3,2-g]chromen-4-sulfonica-cid-4-chlorobenzylidine-amide (**9b**) ($C_{19}H_{12}ClNO_6S$, 417.82, 48, 242–243).* Anal. Calcd.: C, 54.61; H, 2.89; N, 3.35; S, 7.67. Found: C, 54.59; H, 2.87; N, 3.29; S, 7.65. IR (cm^{-1}, KBr): 1728 (C=O α-pyrone), 1397,

1169 (SO_2–N), 1667 (C=N), 755 (C–Cl). MS: M^+/M^{+2} 417/419 (0.76%), 294 (13.49%), 215 (13.3%), 63 (100%).

*3.1.8.1.3. 9-Methoxy-7-oxo-7*H*-furo[3,2-g]chromen-4-sulfonica-cid-4-methoxybenzylidine-amide (**9c**) ($C_{20}H_{15}NO_7S$, 413.39, 58.6, 258–260).* Anal. Calcd.: C, 58.10; H, 3.66; N, 3.39; S, 7.76. Found: C, 58.08; H, 3.62; N, 3.35; S, 7.75. IR (cm^{-1}, KBr): 1730 (C=O α-pyrone), 1390, 1170 (SO_2–N), 1673 (C=N). MS: M^+ 413 (16.1%), 215 (38.3%), 177 (90.9%), 135 (100%), 187 (9%), 158 (6%), 202 (14%), 135 (100%), 74 (11%).

*3.1.8.1.4. 9-Methoxy-7-oxo-7*H*-furo[3,2-g]chromen-4-sulfonic acid furan-2-yl-methylene amide (**9d**) ($C_{19}H_{12}BrNO_6S$, 462.38, 85.7, 236–238).* Anal. Calcd.: C, 49.36; H, 2.62; N, 3.03; S, 6.94. Found: C, 49.35; H, 2.59; N, 2.99; S, 6.92. IR (cm^{-1}, KBr): 1731 (C=O α-pyrone), 1397, 1169 (SO_2–N), 1677 (C=N). MS: M^+ 462 (9%), 295 (64%), 253 (90%), 215 (40%), 205 (55%), 161 (100%).

*3.1.8.1.5. 9-Methoxy-7-oxo-7*H*-furo[3,2-g]chromen-4-sulfonica-cid-4-methoxybenzylidine-amide (**9e**) ($C_{17}H_{11}NO_7S$, 373.33, 72.6, 260–263).* Anal. Calcd.: C, 54.69; H, 2.97; N, 3.75; S, 8.59 . Found: C, 54.67; H, 2.92; N, 3.73; S, 8.54. IR (cm^{-1}, KBr): 1740 (C=O α-pyrone), 1395, 1172 (SO_2–N), 1670 (C=N). MS: M^+ 373 (2.5%), 295 (92%), 215 (100%), 202 (25%), 188 (28%), 160 (38%).

*3.1.9. Preparation of 7-oxo-furochromen sulphonic acid imide derivatives (**10a–c**)*

To a solution of xanthotoxin-4-sulfonyl hydrazide (3.14 g, 0.01 mol) in acetic acid, the appropriate anhydrides, namely, phthalic anhydride, pyridine anhydride (0.01 mol) or benzen dianhydride (0.005 mol) was added . The mixture was refluxed for 6–8 h, poured onto cold water; the formed precipitate was filtered, washed with water and crystallized from glacial acetic acid to give the cyclic imide dione derivatives (**10a–c**).

*3.1.9.1. Microanalytical and spectral data of compounds **10a–c** (Mol. For., M. wt., yield % and m.p. °C)*

*3.1.9.1.1. 9-Methoxy-7-oxo-7*H*-furo[3,2-g]chromen-4-sulfonica-cid(1,3-dioxo-1,3-dihydro-isoindol-2-yl) (**10a**) ($C_{20}H_{12}N_2O_8S$, 440.39, 70.45, 220–222).* Anal. Calcd.: C, 54.54; H, 2.75; N, 6.36; S, 7.28. Found: C, 54.50; H, 2.71; N, 6.32; S, 7.25. IR (cm^{-1}, KBr): 1765 (C=O α-pyrone), 1746, 1773 (C=O imide), 1370, 1150 (SO_2–N). MS: M^+ 440 (2.91%), 294 (4.04%), 279 (3.51%), 247 (9.09%), 216 (6.72%) and 63 (100%).

*3.1.9.1.2. 9-Methoxy-7-oxo-7*H*-furo[3,2-g]chromen-4-sulfonica-cid(5,7-dioxo-5,7-dihydro-pyrrolo[3,4-*b*]pyridin-6-yl)-amide (**10b**) ($C_{19}H_{11}N_3O_8S$, 441.37, 72.72, >250).* Anal. Calcd.: C, 51.70; H, 2.51; N, 9.52; S, 7.26. Found: C, 51.66; H, 2.49; N, 9.50; S, 7.25. IR (cm^{-1}, KBr): 1750 (C=O α-pyrone), 1740, 1772 (C=O imide), 1370, 1165 (SO_2–N). MS: M^+ 440 (2.91%), 294 (4.04%), 279 (3.51%), 247 (9.09%), 216 (6.72%) and 63 (100%).

*3.1.9.1.3. 2,6-Bis-(9-methoxy-7-oxo-7*H*-furo[3,2-g]chromen-4-sulfonicacid)-1,3,5,7-tetraoxo-3,5,6,7-tetrahydro-1*H*-pyrrolo [3,4-*b*]isoindol-2-yl)-amide (**10c**) ($C_{34}H_{18}N_4O_{16}S$, 802.64, 72.5,227–230).* Anal. Calcd.: C, 50.87; H, 2.26; N, 6.98; S, 7.99. Found: C, 50.85; H, 2.25; N, 6.96; S, 7.97. IR (cm^{-1}, KBr): 1740 (C=O α-pyrone), 1745, 1776 (C=O imide), 1372, 1160 (SO_2–N). MS: M^+ 802 (6%), 477 (8%), 276 (84%),173 (42%), 78 (74%), 62 (100%).

3.2. In vitro antitumor screening

The ten compounds (**Xanthotoxin**, **2d**, **2g**, **3c**, **4a**, **5b**, **5c**, **7**, **9d** and **10a**) were tested for their ability to inhibit the growth of the HeLa cervical cancer cell line (obtained from ATCC, Cat. No. CCL-2) and the (MCF-7) breast cancer cell line (ATCC, Cat. No. HTB-22). The cells were grown in Eagle's Minimal Essential medium with Earle's salts and 2 mM L-glutamine, that was further modified to contain 1.0 mM sodium pyruvate, 0.1 mM nonessential amino acids, and 1.5 g/L sodium bicarbonate. The medium was supplemented with 10% fetal bovine serum and the medium for MCF-7 contained additionally 0.01 mg/mL bovine insulin. Cultures were maintained in T-75 flasks in an atmosphere of 5.0% CO_2 at 37 °C. Media were changed every other day in cultures of MCF-7 cells and cells were passaged once a week at a subcultivation ratio of 1:3 after release from the flasks with 0.25% Trypsin–0.53 mM EDTA solution. HeLa cells were passaged every other day or on the third day after a weekend at a ratio of 1:8.

Cells were plated in 96-well plates at a density of 10^4 cells/well in 190 μL media. At 24 h, appropriate dilutions of test compounds were added to the wells. All dilutions were made into 50% DMSO and were added to the wells (10 μL of appropriate dilutions in 50% DMSO to 190 μL media containing 10^4 cells) to make final concentrations of 5, 10, 50 and 100 μg compound/mL with a final concentration of 2.5% DMSO in 200 μL media in each well. The 200 μL volume was used, because in earlier assays with 100 μL of media in the wells, there was significant evaporation especially of wells in the outside rows of the plates. (The outside rows were not used in subsequent assays.) The plates were incubated for 72 h and were read with a plate reader (Synergy HT Multi-Detection Microplate Reader with Gen5 software, BioTek Instruments, Winooski, VT). The Cell titer 96 Aqueous One Solution Reagent (Promega Corporation, Madison, WI) was then added to the wells (20 μL/well) and the plates were read again after 3 h. Each concentration of the test compound and controls with and without DMSO was tested in triplicate and the percent viability in the wells with test compounds was calculated by comparing absorbance to that of the control wells containing 2.5% DMSO.

The Cell Titer 96 Aqueous One Solution Reagent contains a novel tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carbomethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt; MTS] and an electron coupling reagent (phenazine ethosulfate; PES). The MTS tetrazolium compound is reduced by cells to a colored formazan product which is soluble in culture medium, so that it can be read by a plate reader with no other steps after addition of the reagent. Since some of the test compounds were highly colored, affecting the color of the medium in the wells even before the reagent was added, the plates were read immediately before addition of the reagent to the wells and the absorbance before addition of reagent was subtracted from the absorbance recorded 3 h after addition to determine the absorbance attributed to reduction of the MTS by viable cells.

References

[1] M. Sakamoto, T. Imaoka, M. Motoyama, Y. Yamomoto, H. Takasu. PCT Int. Appl. WO 94 21, 612 (Cl. C0 7D 215/38) (1994). Chem. Abstr. 123 (1995) 143655m.
[2] I.R. Fahmy, H. Abu-Shady, Q. J. Pharm. Pharmacol. (1947) 281.
[3] B.R. Scott, M.A. Pathak, G.R. Mohn, Mutat. Res. 39 (1976) 29.
[4] L. Musajo, G. Rodighiero, G. Caporale, Bull. Soc. Chim. Biol. 36 (1954) 1213.
[5] E.M. Vermel, A.L. Tsetlin, Vopr. Onkol. 10 (6) (1964) 85; Chem. Abstr. 62 (1965) 4497.
[6] T. Fujioka, K. Furumi, H. Fujii, H. Okabc, K. Mihashi, Y. Nakano, H. Matsunaga, M. Katano, M. Mori, Chem. Pharm. Bull. 47 (1) (1999) 96.
[7] F.D. Popp, J. Org. Chem. 26 (1961) 1566.
[8] F.D. Popp, W. Kirsch, J. Org. Chem. 26 (1961) 3858.
[9] J.D. Modi, S.S. Sabins, C.V. Deliwala, J. Med. Chem. 13 (1970) 935.
[10] A.Z. Abdel Hamid, O.A. Facthalla, Egypt J. Pharm. Sci. 34 (1–3) (1993) 67.
[11] J.H. Billiman, R.L. Schmidgall, J. Pharm. Sci. 59 (8) (1970) 1191.
[12] R. Ottana, S. Carotti, R. Maccari, I. Landini, G. Chiricosta, B. Caciagli, M.G. Vigrita, E. Mini, Biorg. Med. Chem. Lett. 15 (2005) 3930.
[13] V. Gududuru, E. Hurh, J.T. Dalton, D.D. Miller, J. Med. Chem. 48 (7) (2005) 2584.
[14] O.M. Abdel Hafez, E.A. Elkrisy, F. Badria, A.E.M. Fathy, Arch. Pharm. Res. 26 (8) (2003) 686.
[15] J.D. Jiang, J. Roboz, I. Weisz, L. Deng, L. Ma, J.F. Holland, J.G. Bekesi, Anticancer Drug Des. 13 (1998) 735.
[16] M. Miyahara, Y. Kashiwada, X. Guo, H.X. Chen, Y.C. Cheng, K.H. Lee, Heterocycles 39 (1994) 361.
[17] A.R. Katritzky, A. Oliferenko, A. Lomarka, M. Karelson, Biorg. Med. Chem. Lett. 12 (2002) 3453.

[18] R.G. Wilde, J.T. Bilheimer, Biorg. Med. Chem. Lett. 5 (1995) 173.
[19] M. Urban, J. Sarek, J. Klinot, G. Korkinova, M. Hajduch, J. Nat. Prod. 67 (7) (2004) 1100.
[20] Z. Brozozowski, F. Saczewski, J. Med. Chem. 45 (2002) 430.
[21] E.L. Chung, Exp. Lung Res. 27 (3) (2001) 319.
[22] A.K. El-Shafei, M. HassanKh, H.S. El-Kashef, J. Indian Chem. Soc. LIV (1977) 743.
[23] A.E. Amr, O.I. Abdelsalam, A. Attia, I. Stibor, Collect. Czech. Chem. Commun. 64 (1999) 288.
[24] F.A. Ragab, M. Seif El Nasr, Bull. Fac. Pharm. Cairo Univ. 30 (3) (1992) 209.
[25] M.E. Brokke, B.E. Christensen, J. Org. Chem. 23 (1958) 589.
[26] B.A.H. El-Tawil, F.K.A. El-Beih, A.M. Khalil, Pharmazie 36 (1981) 160.
[27] F.K.A. El-Beih, B.A.H. El-Tawil, A.M. Khalil, Pol. J. Chem. 59 (10–12) (1985) 1247.