Full Paper

Chemistry of Phosphorus Ylides. Part 33. Synthesis and Antitumor Activities of Some New Chromenone Derivatives

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The reaction of nucleophilic phosphacumulene and phosphallene ylides with different chromenone derivatives was investigated. Heterocycles and carbocycles of various ring sizes and heteroatom patterns such as pyrano-, oxaphosphino-, cyclopenta-, phosphoranylidene cyclobutane-, and phospholo-chromenones were obtained. The antitumor (breast and liver) properties of some new compounds were performed *in vitro*. Some of these compounds were more potent than the comparative standard.

Keywords: Oxaphosphino-chromenones / Phosphacumulenes / Phosphallene / Phospholo-chromenones Pyrano-chromenones

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Introduction

Coumarins are very well known for their biological activity [1]. They exist in a variety of forms due to the various substitutions possible in their basic structure which modulate their biological activity [2, 3]. Coumarins possess anti-inflammatory, antioxidant, anti-allergic, hepatoprotective, antithrombotic, antiviral, antimicrobial, and anticarcinogenic properties. Moreover, the function of plant growth and growth regulators depends on them, as well as the control of respiration, photosynthesis and defense against infection [4, 5]. Evolution developed a very long association of plant coumarins with other organisms which many account for the extraordinary range of their biochemical and pharmacological activities in mammals and other biological systems. Owing to our interest in the field of synthesis of naturally occurring analogues containing the phosphorus moieties [6–10], we planned to study the reactions of active phosphonium ylides with coumarin derivatives to prepare new heterocycles of various ring sizes and heteroatom patterns which present the pivotal core of many biologically or pharmaceutically interesting compounds.

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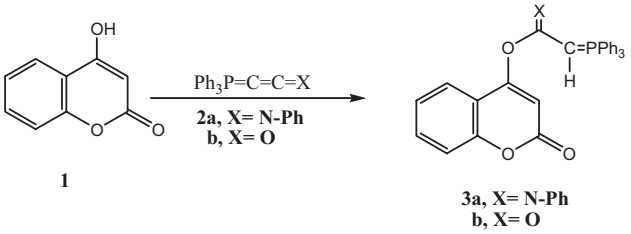
Results and discussion

Chemistry

Now, we found that reactions 4-hydroxy-2*H*-chromen-2-one **1** with (*N*-phenyliminovinylidene)-**2a** or (2-oxovinylidene)-triphenylphosphorane **2b** afforded the corresponding 2-oxo-2*H*-chromen-4-yl-1-*N*-phenyl-2-(triphenyl-λ^5-phosphanylidene) ethanimidoate **3a** and 2-oxo-2*H*-chromen-4-yl(triphenyl-λ^5-phosphanylidene) acetate **3b**. The reaction proceeded by addition of the OH group of **1** to C=C bond of **2**. The most important features are the disappearance of the OH group of compound **3a** in its IR and ^{1}H-NMR spectra, which appeared in the starting material **1** at 3444 cm^{-1} and δ 12.52 ppm spectra, respectively. A signal at 13.6 ppm in its ^{31}P-NMR spectrum was found which support a phosphorane structure [11–13] (Scheme 1).

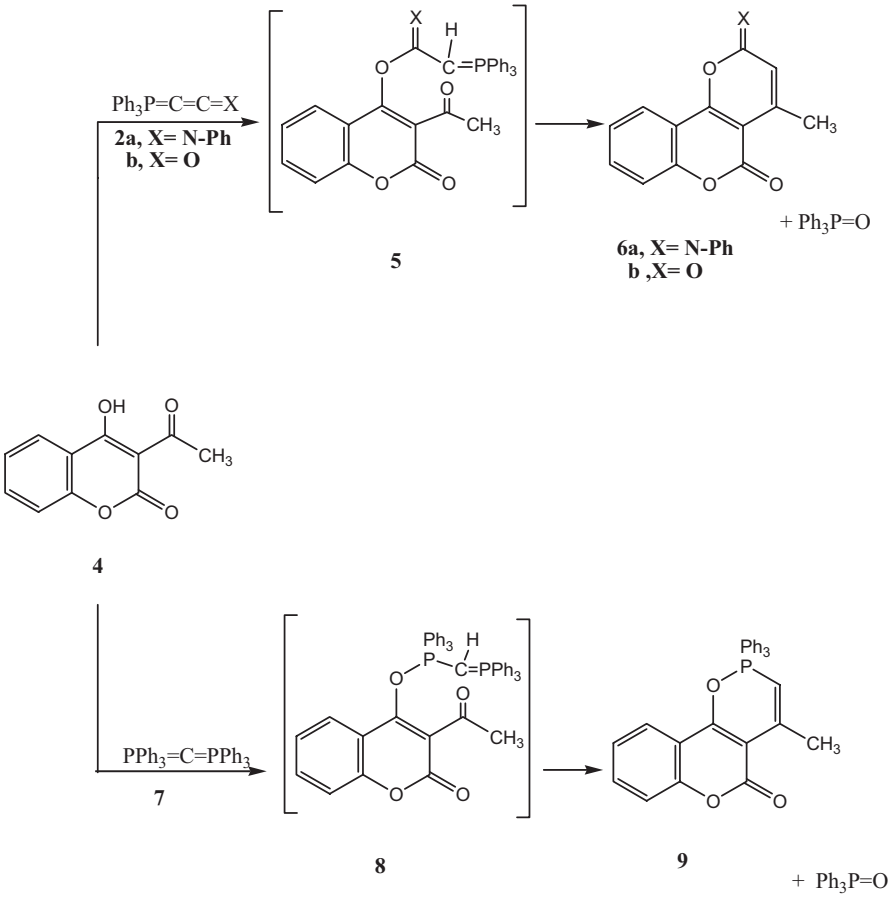
An efficient way for the synthesis of pyranochromones has been developed in this work. When 3-acetyl-4-hydroxy-2*H*-chromen-2-one **4** was allowed to react with the active phosphoranes **2a** and **2b** in boiling toluene for 6 h in case of **2a** and 10 h when **2b** was used, the corresponding 4-methyl-2-(phenylimino)pyrano[3,2-*c*]chromen-5(2*H*)-one **6a** and 4-methyl-2*H*,5*H*-pyrano-[3,2-*c*]chromene-2,5-dione **6b** were obtained, respectively, together with triphenylphosphine oxide. Compounds **6a** and **6b** were formed via the formation of the complex phosphoranes **5**, followed by intramolecular Wittig reaction affording **6a** and **6b**. The reaction of hexaphenylcarbodiphosphorane **7**, with 3-acetyl-4-hydroxy-2*H*-chromen-2-one **4** was studied, too. When the chromenone **4** was reacted with the phosphorane **7**, 4-methyl-2,2,2-tri-



Scheme 1. Reaction of 4-hydroxy-2*H*-chromen-2-one **1** with (*N*-phenyliminovinylidene)-**2a** or (2-oxovinylidene)-triphenylphosphorane **2b** to 2-oxo-2*H*-chromen-4-yl-1-*N*-phenyl-2-(triphenyl-λ^5-phosphanylidene) ethanimidoate **3a** or 2-oxo-2*H*-chromen-4-yl(triphenyl-λ^5-phosphanylidene) acetate **3b**.

phenyl-2*H*,5*H*-2λ^5-[1,2]oxaphosphinino[5,6-*c*]chromen-5-one **9** was isolated. Compound **9** was obtained through intramolecular cyclization of the intermediate **8** (Scheme 2). The IR and ^{1}H-NMR spectra of the compound **9** indicated the absence of the phenolic OH group. Its ^{31}P-NMR spectrum showed signal at δ 21.26 ppm which support the oxaphosphinin structure [14, 15] and in MS the *m*/*z* was found at 462 $[M]^+$.

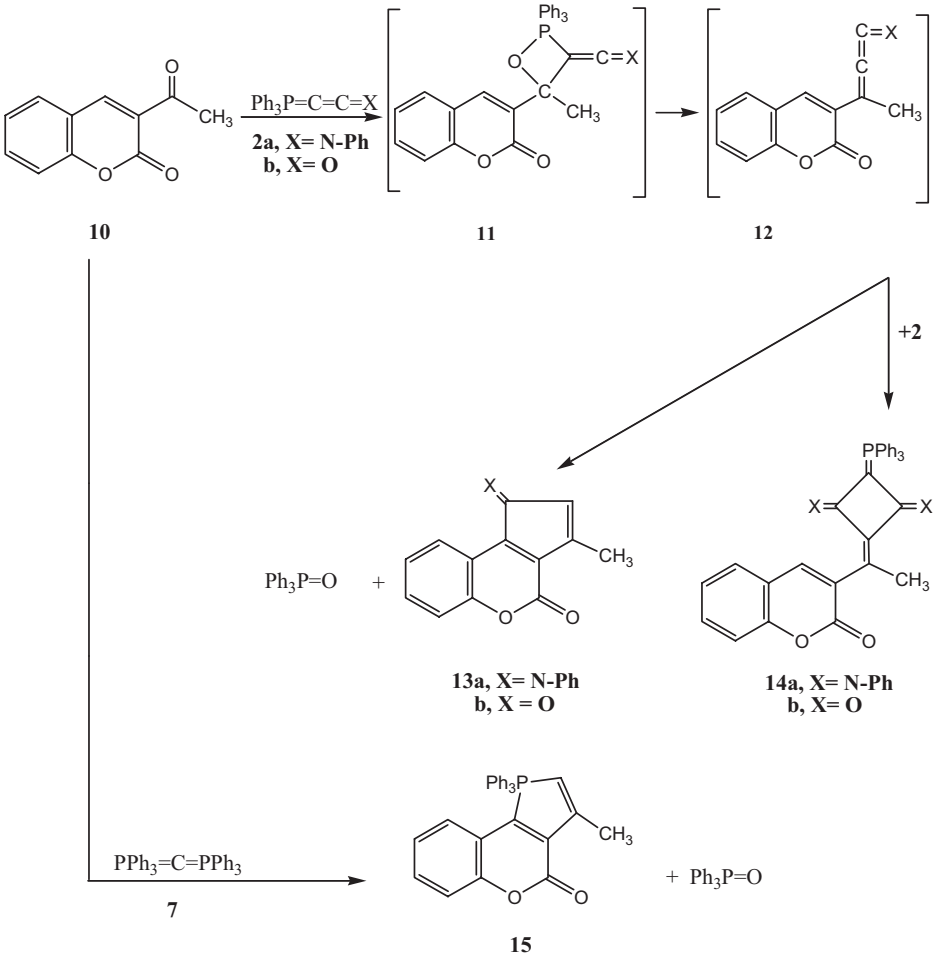
We have found that the reaction of 3-acetyl-2*H*-chromen-2-one **10** with (*N*-phenyliminovinylidene)triphenylphosphorane **2a** proceeded in tetrahydrofuran at room temperature for 10 h gave 3-methyl-1-(phenylimino)-cyclopenta[*c*]chromen-4(1*H*)-one **13a** and 3-{1-[2,4-bis(phenylimino)-3-(triphenyl-λ^5-phosphanylidene)cyclobutylidene]ethyl}-2*H*-chromen-2-one **14a**. A [2 + 2] cycloaddition of the C=O bond of **10** to the ylidic C-P bond of **2a** furnishes the oxaphosphetane **11** [16, 17]. The possible reaction route consists in elimination of triphenylphosphine oxide from **11** to form the ketimine **12**, which in turn undergoes intramolecular cyclization to give **13a** or cycloaddition to **2a**, giving the four-membered ring **14a**. The most important features in the spectroscopic data of the chromenone **13a** and phosphoranylidene-cyclobutylidene **14a** is that they show the *m*/*z* at 286 $[M - H]^+$ and ^{31}P-NMR at δ 30.03 ppm, respectively. When 3-acetylchromenone **10** was allowed to react with (2-oxovinylidene)triphenylphosphorane **2b** in boiling toluene for 8 h, the corresponding compounds, 3-methylcyclopenta[c]chromene-1,4-dione **13b** and 2-[1-(2-oxo-2*H*-chromen-3-yl)ethyl-



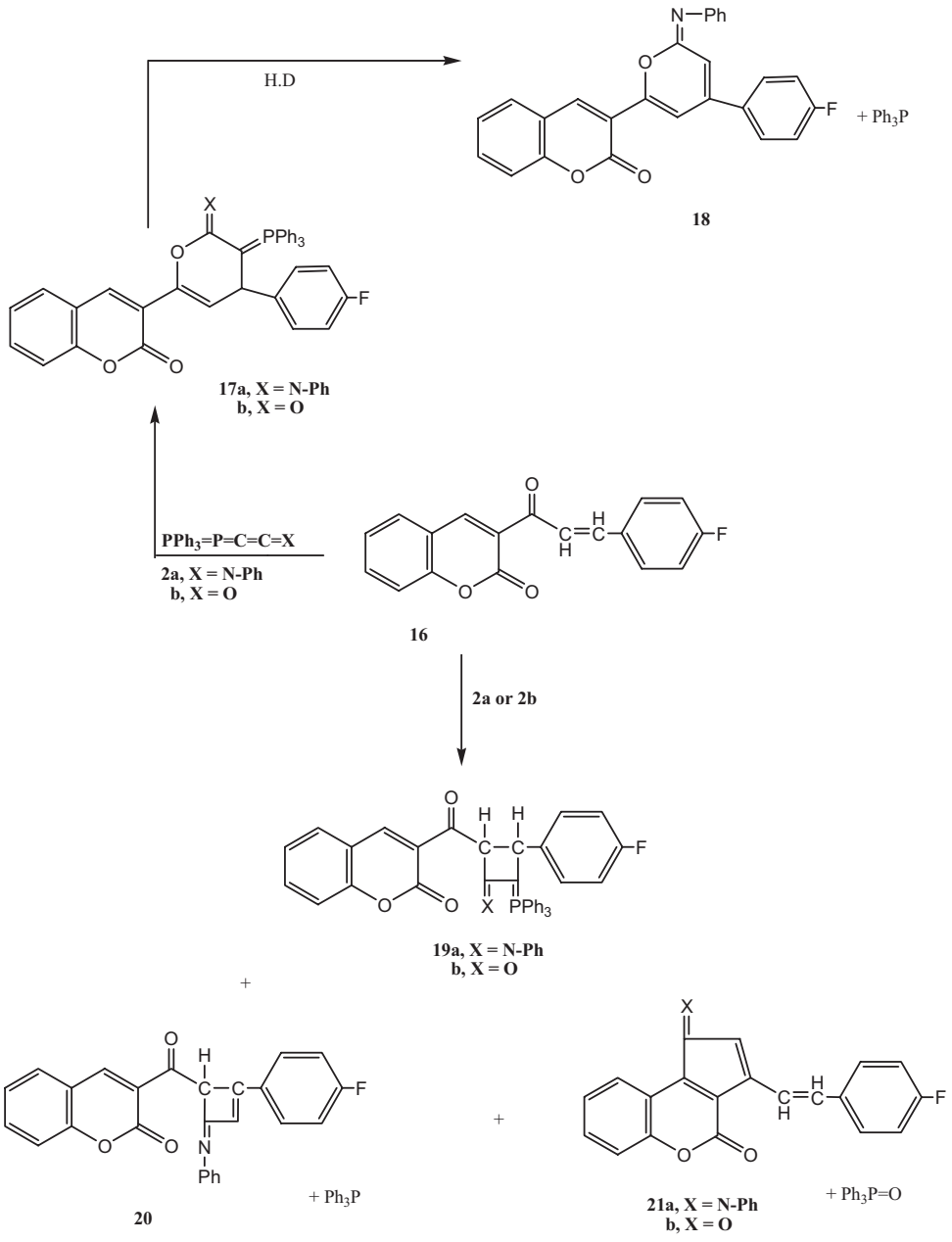
Scheme 2. Reaction of 3-acetyl-4-hydroxy-2*H*-chromen-2-one via the complex phosphoranes **5** to give compound **6a** and **6b** or to react with the phosphorane **7** to afford compound **9**.

idene]-4-(triphenyl-λ^5-phosphanylidene)cyclobutane-1,3-dione **14b** were obtained. Furthermore, a novel approach to phospholochromenone was performed by interaction of hexaphenylcarbodiphosphorane **7** and compound **10** in boiling tetrahydrofuran. 3-Methyl-(1,1,1-triphenyl-λ^5-phospholo)[3,2-*c*]-chromen-4(1*H*)-one **15**, together with triphenylphosphine oxide were obtained. The ^{31}P-NMR shift recorded for **15** was δ 30.65 ppm and the *m*/*z* was found at 446 $[M]^+$ in the mass spectra (Scheme 3).

When 3-[3-(4-fluorophenyl)acryloyl]-2*H*-chromen-2-one (**16**) was treated with (*N*-phenyliminovinylidene)triphenylphosphorane **2a** in THF at room temperature for 10 h. Three products were obtained, namely, 3-[4-(4-fluorophenyl)-2-(phenylimino)-2*H*-pyran-6-yl]-2*H*-chromen-2-one (**18**), 3-{[2-(4-fluorophenyl)-4-(phenylimino)cyclobut-2-enecarbon]-1-yl}-2*H*-chromen-2-one **20** and 3-(4-fluorostyryl)-1-(phenylimino)cyclopenta[*c*]chromen-4-(1*H*)-one **21a** together with triphenylphosphine and triphenylphosphine oxide. The α,β-unsaturated ketone **16** reacted with the phosphacumulene **2a** by [4 + 2]-cycloaddition to give the corresponding pyran **17a**, which looses triphenylphospine by Hoffmann degradation reaction, due to the presence of hydrogen atom in the α-position to give compound **18**. Besides, the phosphacumulene **2a** undergoes [2 + 2]-cycloaddtion at the C=C of **16** to give the cyclobutylidene **19a**, which looses triphenylphosphine by Hoffmann degradation to give compound **20**. Moreover, the Wittig reaction between the carbonyl group of compound **16** and the phosphorane **2a** afforded triphenylphosphine oxide and the intermediate ketene, which by intramolecular cyclization afforded the third product **21a**. The most important feature in the spectroscopic data of the three products **18**, **20**, and **21a**, is that compound **18** lacks the presence of carbonyl group in its IR and ^{13}C-NMR and showed the $[M]^+$ at $m/z = 409$ in its mass spectra, while compound **20**



Scheme 3. 3-Acetylchromenone **10** reacts with compound **2a** via [2 + 2] cycloaddition to form oxaphosphetane **11**. Elimination of triphenylphosphine oxide gives **12** which undergoes intramolecular cyclization resulting in **13a**. Addition of compound **2a,b** leads to **13a,b**. The reaction of compound **10** with **7** results in compound **15**.



Scheme 4. The reaction of compound **16** with phosphacumulene **2b** results in compounds **17b**, **19b** and **21b**.

showed the presence of carbonyl group in its IR and ^{13}C-NMR spectra. Besides, its MS showed the peak at $m/z = 409$ $[M]^+$. The last product lack the presence of the carbonyl group and in its ^{1}H-NMR a doublet was observed at 5.75 ($^2J_{HH} = 30$ Hz). Its MS showed the peak at $m/z = 393[M]^+$.

The reaction of 3-[3-(4-fluorophenyl)acryloyl]-2*H*-chromen-2-one **16** with (2-oxovinylidene)-triphenylphosphorane **2b** was also investigated. When compound **16** was allowed to react with the phosphacumulene **2b** in boiling toluene for 8 h, the corresponding, 3-[4-(4-fluorophenyl)-2-oxo-3-(triphenyl-λ^5-phosphanylidene)-3,4-dihydro-2*H*-pyran-6-yl]-2*H*-chromen-2-one **17b**, 3-[2-(4-fluorophenyl)ethenyl]cyclopenta[*c*]chromene-1,4-dione **19b**, and 3-{[2-(4-fluorophenyl)-4-oxo-3-(triphenyl-λ^5-phosphanylidene)cyclobutyl]carbonyl}-2*H*-chromen-2-one **21b** accompanied by triphenylphospine oxide were obtained. The signals at δ 21.50 and 29.50 ppm were obtained in the ^{31}P-NMR spectra of compounds **17b** and **19b** respectively (Scheme 4).

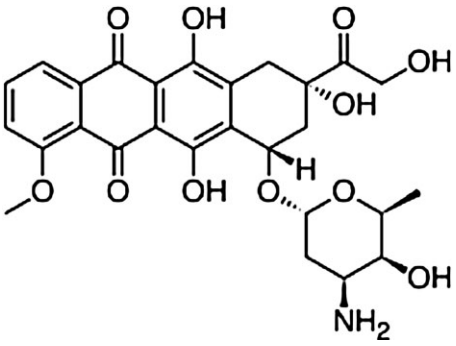


Figure 1. Doxorubicin (DXR).

Pharmacological evaluation

Chemotherapy is a major approach for both localized and metastasized cancers [18] and for many years ago coumarin-related compounds have proved to be a significant therapeutic potential [19]. Therefore, four of the newly synthesized compounds were screened for their *in-vitro* cytotoxic and growth inhibitory activities against human breast carcinoma cell line (MCF-7), in comparison with the activity of the utilized anticancer doxorubicin (DXR) (Fig. 1) as a reference drug. The cytotoxic activities of the tested compounds were expressed as the median growth inhibitory concentration (IC_{50}) which is the dose that reduces survival to 50%. The screening results are compiled in Table 1. According to the American National Cancer Institute guidelines [20] drugs with $IC_{50} < 30$ are active. From Table 1, it is evident that most of the tested compounds show antitumor activities with IC_{50} values ranging from 3.89 to 12.7 μg/mL and reaching strong correlation that of DXR (IC_{50}: 2.97 μg/mL) in the case of compound **9** (IC_{50}: 3.89 μg/mL).

It is clear from the data that the comparison of the cytotoxicity against MCF-7 cells (Fig. 2) of prepared compounds has shown that the cells killing potency follows the order **9 > 6a > 14b > 17b**. This may be attributable to presence of the phospholo-chromenone moiety in its molecular structure which may contribute to the cytotoxic activity that may interact with DNA by intercalation and inhibition of macromolecular biosynthesis. This inhibits the progression of the

Table 1. Effect of the tested compounds on MCF-7 and HEPG2 tumor cell lines.

Compound No.	MCF-7	HEPG2
Doxorubicin (st.)	2.97	3.73
9	3.89	3.58
6a	9.99	7.04
14b	12.60	9.04
17b	12.70	14.90

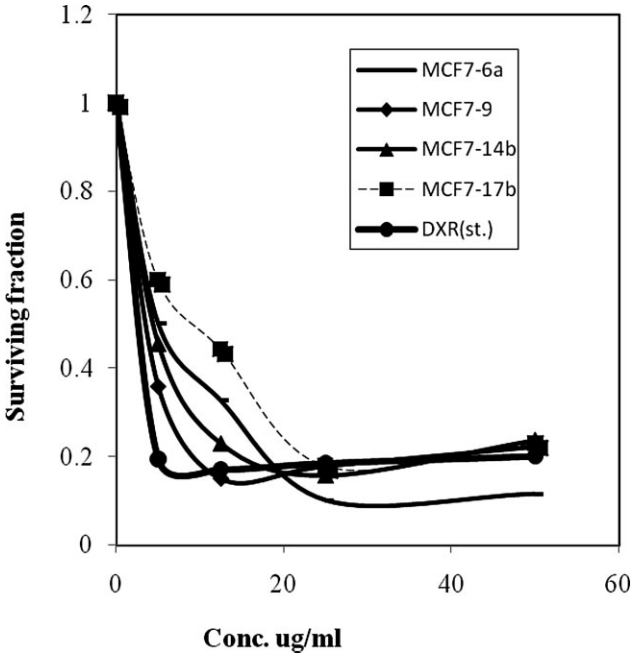


Figure 2. The surviving fraction as a function of drug concentration for the reference compared particularly with the other compounds (MCF-7).

enzyme topoisomerase II, which unwinds DNA for transcription and otherwise stabilizes the topoisomerase II complex after it has broken the DNA chain for replication, preventing the DNA double helix from being resealed and thereby stopping the process of replication as act Doxorubicin [21], suppressing agent, inhibit the formation and growth of tumors from initiated cells [22].

On the other hand, substitution of the phosphanylidene moiety with the pyran-imine moiety in this compound (cf. **6a**, IC_{50}: 9.99 μg/ml) also closely related the activity of the standard. The antitumor activity of compound **14b** recorded 12.60 μg/ml, this may be due to the presence of phosphoranylidene-cyclobutylidene moiety in its molecular structure. On the other side, compound **17b** marked 12.70 μg/mL and also good result. Therefore, the marked difference in their activities can be correlated with the difference in biological activities in different phosphorus and ring systems.

Moreover, the same four compounds (**9**, **6a**, **14b**, **17b**) were screened *in vitro* for their cytotoxic and growth inhibitory activities towards liver carcinoma (HEPG2) cell line (Fig. 3). The IC_{50} after short time exposure was 3.58, 7.04, 9.04, and 14.9 μg/mL for compounds **9**, **6a**, **14b**, and **17b**, respectively, i.e. the cell killing potency follows the order, **9 > 6a > 14b > 17b** Table 1. Compound **9** was the best compound, exerting more significant cytotoxicity election (IC_{50}: 3.58 μg/mL) HEPG2 cells compared with Doxorubicin (IC_{50}:

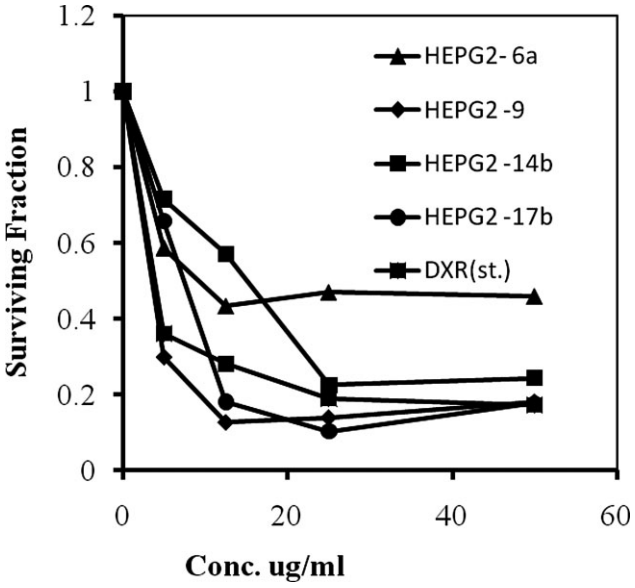


Figure 3. The surviving fraction as a function of drug concentration for the reference compared particularly with the other compounds (HEPG2).

3.73 μg/mL). The results show that compounds **6a** and **14b** are better antitumor agents for HEPG2 than MCF-7 and compound **9** may conduct to a promising pharmacomodulation for the discovery of new potential drugs.

Conclusion

The nucleophilic phosphacumulene (**2a**, **b**) and phosphallene (**7**) ylides added reactants in a variety of ways. Cycloaddition occurred at the polar C-P ylide bond or the C=C bond. Carbocycles and heterocycles of various ring sizes and heteroatom patterns (**6**, **9**, **13**, **14**, **17**, **18**, **20**, **21**), were prepared which present the pivotal core of many biologically or pharmaceutically interesting compounds. Moreover, the difference in the nucleophilic character of the phosphorus reagents was noticed **2a** > **2b** > **7** [23]. While the phosphorane **2a** reacts smoothly with the reactants, the phosphorane **2b** and **7** react less rapidly.

Many of the new compounds revealed pronounced *in-vitro* antitumor activities when tested against human MCF-7 and HEPG2 carcinoma cell lines. The most promising result against breast carcinoma (MCF-7) was recorded by the phospholo-chromenone **9**. It showed IC_{50} value of (3.89 μg/mL) which is the closest in value to that recorded by the reference drug doxorubicin (IC_{50}: 2.97 μg/mL). Similarly, the cytotoxic and growth inhibitory activity of the same compound **9** (IC_{50}: 3.58 μg/mL) was more potent than the same reference drug (IC_{50}: 3.73 μg/mL) against human liver (HPG2) carcinoma cell lines. It is concluded that coumarin-related compounds are a plentiful source of potential anti-cancer drugs deserving further study.

Experimental

General

Melting points were determined with an electrothermal digital melting point apparatus (electrothermal Engineering Ltd., Essex, UK) and are uncorrected. The IR spectra were recorded in KBr disks on a Pye Unicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometers (Pye Unicam Ltd. Cambridge, UK, and Shimadzu, Tokyo, Japan, respectively). ^{1}H-, ^{13}C-, and ^{31}P-NMR spectra were obtained from a Jeol ECA 500 MHz NMR spectrometer (Tokyo, Japan) using deuterated dimethylsulphoxide (d_6-DMSO) as a solvent and (TMS) as internal reference at 500, 125, and 200 MHz, respectively. Mass spectra (EI-MS) were obtained at 70 eV with a Finnigan MAT SSQ 7000 spectrometer (England). Elemental analyses (C, H, N) results were recorded with Elementar Vario EL Germany (Germany), P was measured by spectrophotometric methods and both of them agreed satisfactory with the calculated values. The using reported yields are of pure isolated materials obtained by column chromatography silica gel 60 (Merck). Starting materials, **1** [24], **2a** [25], **2b** [26], **4** [27], **7** [28], **10** [29], and **16** [30] were prepared according to the procedures reported in the literature.

Chemistry

*Reaction of (N-phenyliminovinylidene)-**2a** and (2-oxovinylidene)-triphenylphosphorane **2b** with 4-hydroxy-2H-chromen-2-one **1***

A mixture of **2a** (0.01 mol, 0.377 g) or **2b** (0.01 mol, 0.302 g) in 20 mL of THF was added drop by drop, with stirring at room temperature, to a solution of 4-hydroxychromen-2-one **1** (0.01 mol, 0.162 g) in 20 mL of THF. The reaction mixture was stirred for 6 h in case of **2a** and 10 h in case of **2b** during which the color was changed from white to yellow (the progress of the reaction was controlled by TLC). The precipitate that formed was filtered off and crystallized from benzene to give the phosphoranylidenes **3a** and **3b**, respectively.

*2-Oxo-2H-chromen-4-yl-1-N-phenyl-2-(triphenyl-λ^5-phosphanylidene)ethanimidoate **3a***

Colorless crystals, yield 90%, mp: 252–254°C, IR (KBr, ν, cm^{-1}) 1650 (C=O, lactone), 1598 (C=N), 1579 (C=P), 1486, 1471 (P-aryl). ^{1}H-NMR (500 MHz, d_6-DMSO, δ, ppm): 4.5 (d, 1H, $^{2}J_{HP}$ = 25 Hz, CH=P) 6.86–7.83 (m, 25H, arom.); ^{31}P-NMR at δ = 13.6 ppm. MS *m/z* 538 $[M - H]^+$. Anal. calcd. for $C_{35}H_{26}NO_3P$ (539.5): C, 77.91; H, 4.86; N, 2.60; P, 5.74; Found: C, 77.64; H, 4.55; N, 2.89, P, 5.99.

*2-Oxo-2H-chromen-4-yl(triphenyl-λ^5-phosphanylidene)acetate **3b***

Colorless crystals, yield 85%, mp: 231–233°C, IR (KBr, ν, cm^{-1}) 1693 (C=O, lactone), 1611 (C=O), 1512 (C=P), 1484, 1464 (P-aryl). ^{1}H-NMR (500 MHz, d_6-DMSO, δ, ppm): 6.06 (d, 1H, $^{2}J_{HP}$ = 22 Hz, CH=P) 7.14–7.69 (m, 20H, arom.); ^{13}C-NMR: 179.63 (C=O), 163.27 (C=O, lactone), 153.64 (C=P); ^{31}P-NMR at δ = 16.8 ppm. MS *m/z*: 465 $[M + H]^+$, 421 $[M - CO_2]^+$. Anal. calcd. for $C_{29}H_{21}O_4P$ (464.4): C, 74.99; H, 4.56; P, 6.67; Found: C, 74.64; H, 4.55, P, 6.09.

*Reaction of (N-phenyliminovinylidene)-**2a** or (2-oxovinylidene)-triphenylphosphorane **2b** with 3-acetyl-4-hydroxy-2H-chromen-2-one **4***

A solution of **2a** (0.01 mol, 0.377 g) or **2b** (0.01 mol, 0.302 g) was added to solution of 3-acetyl-4-hydroxy-2*H*-chromene-2-one **4** (0.01 mol, 0.204 g), in toluene (40 mL). The reaction mixture was boiled for 6 h in case of using **2a** and 10 h in case of **2b**, during which the color was changed from colorless to dark brown. Toluene was distilled off and the residue was subjected to silica gel column chromatography using petrol ether (60:80)/ethyl acetate as eluent (65:35, v/v) to give **6a** and **6b** respectively together with triphenylphosphine oxide (m.p. and mixed m.p. 151°C).

*4-Methyl-2-(phenylimino)pyrano[3,2-c]chromen-5(2H)-one **6a***

Colorless crystals, yield 80%, mp: 210–212°C, IR (KBr, ν, cm^{-1}) 1712 (C=O, lactone), 1608 (C=N). ^{1}H-NMR (500 MHz, d_6-DMSO, δ, ppm): 2.19 (s, 3H, CH_3), 5.89 (s, 1H, CH), 6.97–7.92 (m, 9H, arom.). ^{13}C-NMR: 163.58 (C=N), 162.18 (C=O, lactone), 25.92 (CH_3). MS *m/z*: 304 $[M + H]^+$. Anal. calcd. for $C_{19}H_{13}NO_3$ (303.31): C, 75.24; H, 4.32; N, 4.62; Found: C, 75.70; H, 4.44; N, 4.33.

*4-Methyl-2H, 5H-pyrano-[3,2-c]chromene-2,5-dione **6b***

Pale yellow crystals, yield 35%, mp: 147–149°C. IR (KBr, ν, cm^{-1}) 1704 (C=O, lactone), 1671 (C=O). ^{1}H-NMR (500 MHz, d_6-DMSO, δ, ppm): 2.30 (s, 3H, CH_3), 6.36 (s, 1H, CH), 7.25–7.66 (m, 4H, arom.). ^{13}C-NMR: 162.00 (C=O, lactone), 161.14 (C=O, lactone), 22.21 (CH_3). MS *m/z*: 228 $[M]^+$, 200 $[M - CO]^+$, 184 $[M - CO_2]^+$. Anal. calcd. for $C_{13}H_8O_4$ (228.2): C, 68.42; H, 3.53; Found: C, 68.02; H, 3.29.

*Interaction of hexaphenylcarbodiphosphorane **7** with 3-acetyl-4-hydroxy-2H-chromen-2-one **4***

To a solution of **4** (0.01 mol, 0.204 g) in 20 mL THF was added **7** (0.01 mol, 0.536 g) in 20 mL THF. The reaction mixture was refluxed for 12 h during which the color change from colorless to yellow then brown. THF was distilled off under reduced pressure and the remained residue was chromatographed on silica gel using petrol ether (60:80)/ethyl acetate as eluent (60:40, v/v) to give **9** together with triphenylphosphine oxide (m.p. and mixed m.p. 151°C).

*4-Methyl-2,2,2-triphenyl-2H,5H-2λ^5-[1,2]oxaphosphinino[5,6-c]chromen-5-one **9***

Colorless crystals, yield 75%, mp: 233–235°C. IR (KBr, ν, cm^{-1}) 1690 (C=O, lactone), 1561 (C=P), 1480, 1434 (P-aryl). ^{1}H-NMR (500 MHz, d_6-DMSO, δ, ppm): 1.94 (s, 3H, CH_3), 7.25–7.58 (m, 20H, arom.). ^{13}C-NMR: 177 (C=O), 161.14 (C=O, lactone), 24.71 (CH_3). ^{31}P-NMR: 21.26. MS *m/z*: 462 $[M]^+$, 463 $[M + H]^+$. Anal. calcd. for $C_{30}H_{23}O_3P$ (462.4): C, 77.91; H, 5.01; P, 6.70; Found: C, 77.02; H, 4.99; P, 6.06.

*Reaction of (N-phenyliminovinylidene) triphenylphosphorane **2a** with 3-acetyl-2H-chromen-2-one **10**.*

A solution of **2a** (0.377 g, 0.01 mol) in 20 mL of THF was added drop by drop, with stirring at room temperature, to a solution of **10** (0.01 mol, 0.188 g) in 20 mL of THF for 10 h, during which the color changed from yellow to brown (the progress of the reaction was controlled by TLC). THF was distilled off under reduced pressure and the residue was subjected to silica gel column chromatography using petrol ether (60:80)/ethyl acetate as eluent (7:3, v/v) to give **13a** and **14a**, in addition to triphenylphosphine oxide (m.p. and mixed m.p. 151°C).

*3-Methyl-1-(phenylimino)cyclopenta[c]chromen-4(1H)-one **13a***

Orange crystals, yield 55%, mp: 333–335°C. IR (KBr, ν, cm^{-1}) 1726 (C=O, lactone), 1618 (C=N). ^{1}H-NMR (500 MHz, d_6-DMSO, δ, ppm): 2.16 (s, 3H, CH_3), 4.75 (s, 1H, CH), 7.22–7.30 (m, 9H, arom.): MS *m/z*: 286 $[M - H]^+$; 241 $[M - CO_2]^+$. Anal. calcd. for $C_{19}H_{13}NO_2$ (287.3): C, 79.43; H, 4.56; N, 4.88; Found: C, 79.16; H, 4.44; N, 4.13.

*3-{1-[2,4-Bis(phenylimino)-3-(triphenyl-λ^5-phosphanylidene)cyclobutylidene]ethyl}-2H-chromen-2-one **14a***

Yellow crystals, yield 40%, mp: 178–180°C, IR (KBr, ν, cm^{-1}) 1729 (C=O, lactone), 1682, 1598 (2C=N), 1563 (C=P), 1490, 1458 (P-aryl). ^{1}H-NMR (500 MHz, d_6-DMSO, δ, ppm): 2.72 (s, 3H, CH_3), 7.30–7.85 (m, 30H, arom.). ^{13}C-NMR: 163.03 (2 C=N), 159.89 (C=O, lactone), 153.75 (C=P), 22.12 (CH_3). ^{31}P-NMR δ = 30.03 ppm. MS *m/z*: 663 $[M - H]^+$, 648 $[M - CH_3]^+$. Anal. calcd. for $C_{45}H_{33}N_2O_2P$ (664.7): C, 81.31; H, 5.00; N, 4.21; P, 6.44; Found: C, 80.92; H, 4.88; N, 4.05; P, 6.28.

*Reaction of (2-oxovinylidene)triphenylphosphorane **2b** with 3-acetyl-2H-chromen-2-one **10***

A mixture of **2b** (0.01 mol, 0.302 g), **10** (0.01 mol, 0.188 g), and toluene (40 mL) was refluxed for 8 h during which the color changed from yellow to dark brown. Toluene was distilled off and the residue was subjected to silica gel column chromatography using petrol ether (60:80)/ethyl acetate as eluent (3:7, v/v) to give **13b** and **14b** in addition to triphenylphosphine oxide (m.p. and mixed m.p. 151°C).

*3-Methylcyclopenta[c]chromene-1,4-dione **13b***

Colorless crystals, yield 49%, mp: 110–112°C. IR, (KBr, ν, cm^{-1}) 1727 (C=O, lactone), 1603 (C=O). ^{1}H-NMR (500 MHz, d_6-DMSO, δ, ppm): 2.21 (s, 3H, CH_3), 5.93 (s, 1H, CH), 7.26–7.56 (m, 4H, arom.). ^{13}C-NMR: 180.00 (C=O), 159.70 (C=O, lactone), 22.06 (CH_3). MS *m/z*: 213 $[M + H]^+$, 211 $[M - H]^+$. Anal. calcd. for $C_{13}H_8O_3$ (212.20): C, 73.58; H, 3.80; Found: C, 72.99; H, 3.76.

*2-[1-(2-Oxo-2H-chromen-3-yl)ethylidene]-4-(triphenyl-λ^5-phosphanylidene)cyclobutane-1,3-dione **14b***

Pale yellow crystals, yield 45%, mp: 263–265°C. IR, (KBr, ν, cm^{-1}) 1722 (C=O, lactone), 1633, 1620 (2 C=O), 1590 (C=P), 1482, 1436 (P-aryl). ^{1}H-NMR (500 MHz, d_6-DMSO, δ, ppm): 2.52 (s, 3H, CH_3), 7.55–7.71 (m, 20H, arom.). ^{13}C-NMR: 186.29 (2 C=O), 159.90 (C=O, lactone), 153.66 (C=P), 16.67 (CH_3). ^{31}P-NMR δ = 29.79 ppm. MS *m/z*: 515 $[M + H]^+$, 514 $[M]^+$, 513 $[M - H]^+$, 486 $[M - CO]^+$, 469 $[M - CO_2]^+$, 457 $[M - 2\,CO]^+$. Anal. calcd. for $C_{33}H_{23}O_4P$ (514.2): C, 77.04; H, 4.51; P, 6.02; Found: C, 76.92; H, 4.06; P, 6.03.

*Interaction of hexaphenylcarbodiphosphorane **7** with 3-acetyl-2H-chromen-2-one **10***

To a solution of **10** (0.01 mol, 0.188 g) in 20 mL THF was added **7** (0.01 mol, 0.536 g) in 20 mL THF. The reaction mixture was

refluxed for 12 h during which the color change from colorless to yellow then brown. THF was distilled off under reduced pressure and the remained residue was chromatographed on silica gel using petrol ether (60:80)/ethyl acetate as eluent (60:40, v/v) to give **15** and together with triphenylphosphine oxide (m.p. and mixed m.p. 151°C).

*3-Methyl-(1,1,1-triphenyl-λ^5-phospholo)[3,2-c]chromen-4(1H)-one **15***

Colorless crystals, yield 70%, mp: 103–105°C. IR, (KBr, ν, cm^{-1}) 1715 (C=O, lactone), 1596 (C=P), 1481, 1432 (P-aryl). ^{1}H-NMR (500 MHz, d_6-DMSO, δ, ppm): 2.02 (s, 3H, CH_3), 7.24–7.73 (m, 20H, arom.). ^{31}P-NMR: 30.65. MS m/z: 446 $[H]^+$, 444 $[M - 2\ H]^+$. Anal. calcd. for $C_{30}H_{23}O_2P$ (446.4): C, 80.70; H, 5.19; P, 6.94; Found: C, 80.14; H, 5.06; P, 6.20.

*Reaction of (N-phenyliminovinylidene) triphenylphosphorane **2a** with 3-(3-(4-fluorophenyl) acryloyl)-2H-chromen-2-one **16***

A solution of **2a** (0.377 g, 0.01 mol) in 20 mL of THF was added drop by drop, with stirring at room temperature, to a solution of **16** (0.01 mol, 0.294 g) in 20 mL of THF for 10 h, during which the color changed from yellow to brown (the progress of the reaction was controlled by TLC). THF was distilled off under reduced pressure and the residue was subjected to silica gel column chromatography using petrol ether (60:80)/ethyl acetate as eluent (7:3, v/v), to give **18**, **20**, and **21a** in addition to triphenylphosphine (m.p. and mixed m.p. 78°C) and triphenylphosphine oxide (m.p. and mixed m.p. 151°C).

*3-[4-(4-Fluorophenyl)-2-(phenylimino)-2H-pyran-6-yl]-2H-chromen-2-one **18***

Yellow crystals, yield 35%, mp: 245–247°C. IR (KBr, ν, cm^{-1}) 1715 (C=O, lactone), 1622 (C=N). ^{1}H-NMR (500 MHz, d_6-DMSO, δ, ppm): 7.08–7.50 (m, 15H, arom.); 8.65 (s, 1H, CH, methine); ^{13}C-NMR: 168.65 (C=N), 162.54 (C-F), 161.36 (C=O, lactone). MS m/z: 409 $[M]^+$, 408 $[M - H]^+$, 380 $[M - CO]^+$. Anal. calcd. for $C_{26}H_{16}FNO_3$ (409.4): C, 76.42; H, 3.94; F, 4.64; N, 3.42; Found: C, 76.02; H, 3.49; F, 4.04; N, 3.22.

*3-{[2-(4-Fluorophenyl)-4-(phenylimino)cyclobut-2-enecarbon]-1-yl}-2H-chromen-2-one **20***

Orange crystals, yield 30%, mp: 237–239°C. IR (KBr, ν, cm^{-1}) 1729 (C=O, lactone), 1671 (C=O), 1603 (C=N). ^{1}H-NMR (500 MHz, d_6-DMSO, δ, ppm): 4.17 (s, 1H, CH), 6.07 (s, 1H, CH), 7.10–7.85 (m, 13H, arom.); 8.28 (s, 1H, CH, methine); ^{13}C-NMR: 186.28 (C=O), 163.27 (C=N), 159.98 (C–F), 159.33 (C=O, lactone). MS m/z: 409 $[M]^+$. Anal. calcd. for $C_{26}H_{16}FNO_3$ (409.4): C, 76.42; H, 3.94; F, 4.64; N, 3.42; Found: C, 76.02; H, 3.49; F, 4.04; N, 3.22.

*3-(4-Fluorostyryl)-1-(phenylimino)cyclopenta[c]chromen-4(1H)-one **21a***

Yellow crystals, yield 30%, mp: 147–149°C. IR (KBr, ν, cm^{-1}) 1718 (C=O, lactone), 1586 (C=N). ^{1}H-NMR (500 MHz, d_6-DMSO, δ, ppm): 5.76 (d, 1H, $^2J_{HH} = 30$ Hz, CH), 5.83 (s, 1H, CH), 7.01–7.71 (m, 14H, arom.). MS m/z: 393 $[M]^+$. Anal. calcd. for $C_{26}H_{16}FNO_2$ (393.4): C, 79.38; H, 4.10; F, 3.83; N, 3.56; Found: C, 79.12; H, 3.99; F, 3.64; N, 3.22.

*Interaction of (2-oxovinylidene)triphenylphosphorane **2b** and 3-(3-(4-fluorophenyl)acryloyl)-2H-chromen-2-one **16***

A mixture of **2b** (0.01 mol, 0.302 g), **16** (0.01 mol, 0.294 g), and toluene (40 mL) was refluxed for 8 h during which the color changed from yellow to dark brown. Toluene was distilled off and the residue was subjected to silica gel column chromatography using petrol ether (60:80)/ethyl acetate as eluent (6:4, v/v), to give **17b**, **19b**, and **21b** in addition to triphenylphosphine oxide (m.p. and mixed m.p. 151°C).

*3-[4-(4-Fluorophenyl)-2-oxo-3-(triphenyl-λ^5-phosphanylidene)-3,4-dihydro-2H-pyran-6-yl]-2H-chromen-2-one **17b***

Yellow crystals, yield 40%, mp: 247–249°C. IR (KBr, ν, cm^{-1}) 1716 (C=O, lactone), 1632 (C=O, lactone), 1502 (C=P), 1434, 1356 (P-aryl). ^{1}H-NMR (500 MHz, d_6-DMSO, δ, ppm): 6.72–7.55 (m, 25H, arom.); 8.44 (s, 1H, CH, methine); ^{13}C-NMR: 165.82 (C=O, lactone), 160.24 (C=O, lactone), 152.79 (C=P). ^{31}P-NMR: $\delta = 21.50$ ppm. MS m/z: 598 $[M + 2\ H]^+$, 596 $[M]^+$. Anal. calcd. for $C_{38}H_{26}FO_4P$ (596.5): C, 75.50; H, 4.39; F, 3.18; P, 5.19; Found: C, 75.02; H, 4.29; F, 3.04; P, 5.11.

*3-{[2-(4-Fluorophenyl)-4-oxo-3-(triphenyl-λ^5-phosphanylidene)cyclobutyl]carbonyl}-2H-chromen-2-one **19b***

Yellow crystals, yield 35%, mp: 147–149°C. IR (KBr, ν, cm^{-1}) 1715 (C=O, lactone), 1620 (C=O), 1599 (C=P), 1437, 1300 (P-aryl). ^{1}H-NMR (500 MHz, d_6-DMSO, δ, ppm): 2.21 (m, 1H, CH); 3.03 (d, 1H, $^2J_{HH} = 15$ Hz, CH), 6.28–7.66 (m, 24H, arom.); ^{13}C-NMR: 200.10 (C=O), 159.24 (C=O, lactone), 152.79 (C=P). ^{31}P-NMR: $\delta = 29.50$ ppm. MS m/z: 597 $[M + H]^+$, 596 $[M]^+$. Anal. calcd. for $C_{38}H_{26}FO_4P$ (596.5): C, 75.50; H, 4.39; F, 3.18; P, 5.19; Found: C, 75.02; H, 4.19; F, 3.04; P, 4.99.

*3-[2-(4-Fluorophenyl)ethenyl]cyclopenta[c]chromene-1,4-dione **21b***

Yellow crystals, yield 25%, mp: 185–187°C. IR (KBr, ν, cm^{-1}) 1717 (C=O, lactone), 1633 (C=O). ^{1}H-NMR (500 MHz, d_6-DMSO, δ, ppm): 6.44 (d, 1H, $^2J_{HH} = 15$ Hz, CH), 7.49–769 (m, 10H, arom.); ^{13}C-NMR: 187.44 (C=O), 161.70 (C=O, lactone). MS m/z: 275 $[M - CO_2]^+$. Anal. calcd. for $C_{20}H_{11}FO_3$ (318.3): C, 75.47; H, 3.48; F, 5.97; Found: C, 75.02; H, 3.40; F, 5.24.

Material and methods

Chemicals

All the chemicals and reagents used in this study were of analytical grade and purchased from (Sigma Chemical Co., St. Louis, MO, USA): These were used in cryopreservation of cells.

Cells culture

The cells of MCF-7 human breast cancer and HEPG2 liver carcinoma were maintained and grown in RPMI-1640 medium supplemented with 10% heat inactivated fetal bovine serum (Sigma Chemical Co., St. Louis, MO, USA), penicillin and streptomycin at 37°C in humidified atmosphere containing 5% CO_2.

In-Vitro cytotoxicity assay

For *in-vitro* short term cytotoxicity evaluation of prepared compounds, MCF-7 and HEPG2 cells were plated a concentration of 5×10^4–10^5 cells per well, in complete culture medium in 96-well flat-bottomed culture plates (Falcon) for 24 h to assure total attachment. Then various concentration of test compounds were added to the cell suspended in 0.10 ml of phosphate buffered saline (FBS) (0.20 M, pH 7.4), the control cells without the test compounds were also cultured, then the plate was incubated for 24 h at 40°C and 72 h at 37°C, in a humidified 5% CO_2 atmosphere. Cell survival was evaluated at the end of the incubation period with sulphorhodamine-B (SRB) colorimetric assay according to Skehan et al. [20]. This test is based on the sensitivity of the human tumor cell lines to thymoquinone was determined by the SRB assay. SRB is a bright pink aminoxanthrene dye with two sulphonic groups. It is a protein stains that binds to the amino groups of intracellular proteins under mildly acidic conditions to provide a sensitive index of cellular protein content. After incubation, media were removed and 50 μL of 0.4% SRB dissolved in 1% acetic acid solution well were. The wells were then washed 4 times with 1% acetic acid. The absorbance was determined photometrically at 564 nm with ELISA microplate reader (Meter tech. Σ 960, USA.).

Calculation

The percentage of cell survival was calculated as follows:

Survival fraction = O.D. (treated cells)/O.D. (control cells) where (O. D.) is the optical density. The IC_{50} values (the concentrations of thymoquinone required to produce 50% inhibition of cell growth). The experiment was repeated 3 times for each cell line.

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