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ORIGINAL PAPER

Chemistry of phosphorus ylides 32: synthesis of phosphoranylidene-pyrano- and -cyclobutyl-xanthenones with potential antitumor activity

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Abstract The reaction of active phosphacumulenes with xanthenones and anthrone yields xanthene(anthracene)- and/or phosphoranylidene xanthene(anthracene)- depending upon the nature of reactants and reaction conditions. Pertinent reaction mechanisms were considered and compatible spectroscopic measurements were recorded for all new compounds. The cytotoxic activity of some new products was evaluated against human cervical and breast carcinoma cell lines. Certain tested compounds showed promising results.

Keywords Active phosphacumulenes · Xanthenones · Cyclobutanes · Pyranoxanthenones · Antitumor activity

Introduction

Natural and synthetic xanthenones are an important class of compounds [1, 2]. They have demonstrated multiple pharmacological properties such as antioxidant [3, 4], anti-inflammatory [5], and antimalarial activities [6] as well as inhibition of a variety of tumor cell lines [7–10] and modulation of protein kinase C (PKC) isoforms [11, 12]. Moreover, they have found broad applications in medicine as antimicrobial [13], antidepressive [14], and vasorelaxant [15] agents. On the other hand, xanthenone derivatives are capable of inhibiting the enzyme α-glycosidase which plays an important role in digestion of carbohydrates, glycoproteins, and glycolipids [16]. Therefore, the synthesis of novel bioactive xanthenones is important. Of particular interest are antitumor products with high efficiency and low side effects.

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Results and discussion

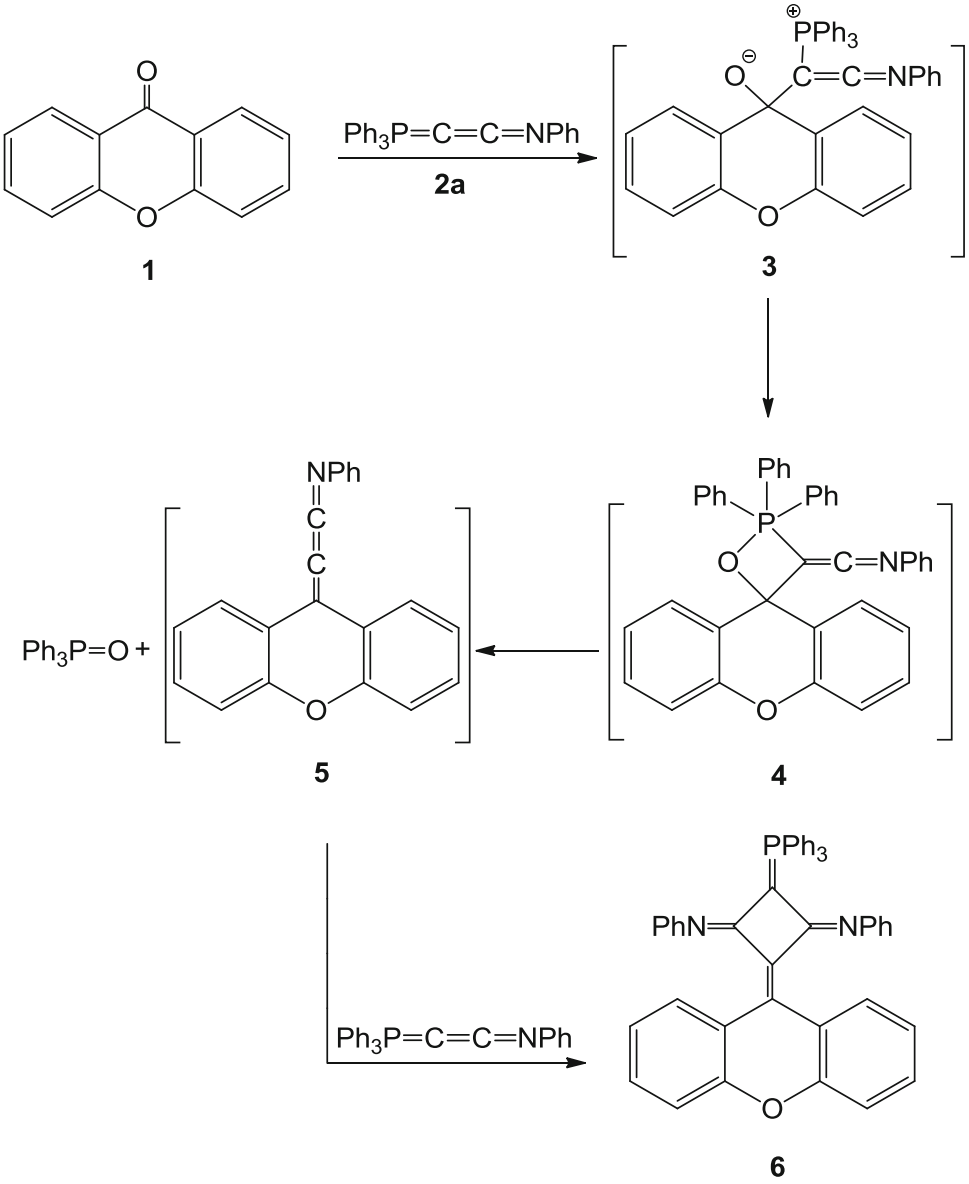
On the basis on these considerations and in continuation of our work on active phosphacumulenes [17–20], we have synthesized a series of new cyclic xanthenone derivatives with anticipated antitumor activity. Therefore, we studied the reaction of an active nucleophilic phosphacumulene (*N*-phenyliminovinylidene)triphenylphosphorane (**2a**), with 9*H*-xanthen-9-one (**1**). The corresponding *N*,*N*′-[2-(triphenylphosphoranylidene)-4-(9*H*-xanthen-9-ylidene)cyclobutane-1,3-diylidene]dianiline (**6**) was obtained together with triphenylphosphine oxide. On the basis of analytical and spectroscopic data the structure of **6** was deduced. The distinguishing feature in the IR of **6** is the absence of a carbonyl signal which was recorded at 1,657 cm^{-1} in the starting xanthenone **1**. Moreover, a signal at $\delta = 15.23$ ppm was recorded in the ^{31}P NMR of **6** which fits with phosphorane with a four-membered ring [21]. Formation of the phosphoranylidene cyclobutane **6** by the reaction of phosphacumulene **2a** with xanthenone **1** occurs by a [2+2] cycloaddition of the carbonyl group in **1** to the ylidic C–P bond of the phosphacumulene **2a** to give the oxaphosphetane **4**, through the dipolar intermediate **3** [22–24]. Expulsion of triphenylphosphine oxide from **4** affords the unstable ketene **5** [25], which is followed immediately by

[2+2] cycloaddition to a second molecule of **2a**, giving the four-membered ring phosphoranylidene cyclobutane **6** (Scheme 1).

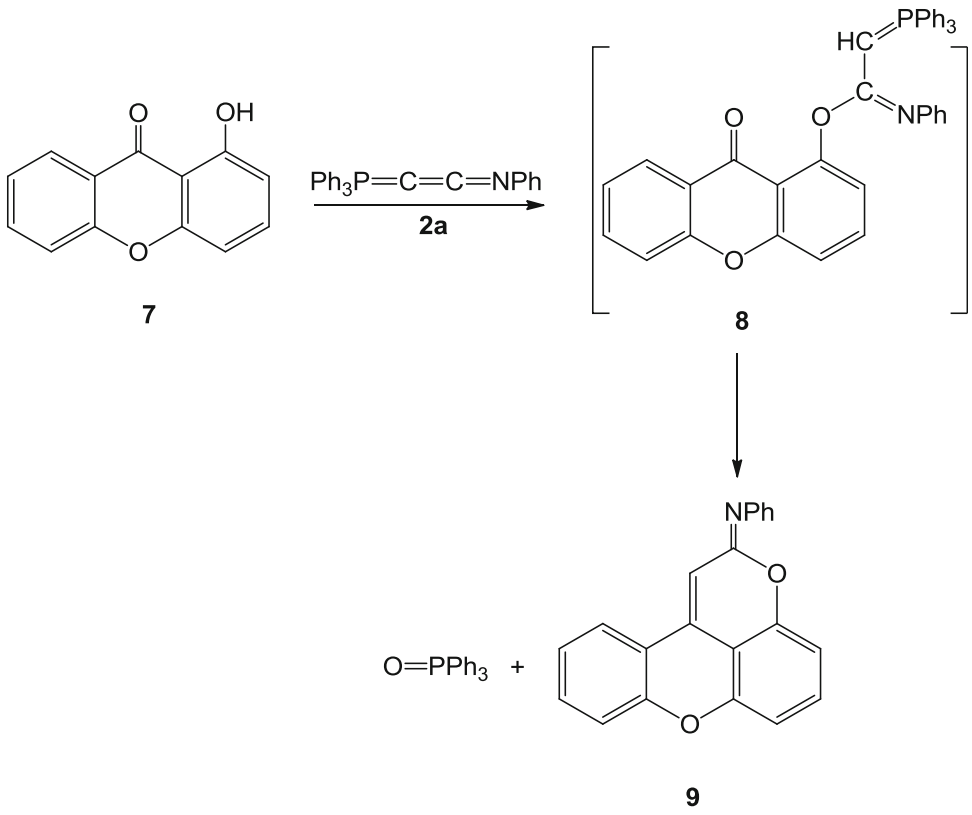
When 1-hydroxy-9*H*-xanthen-9-one (**7**) was treated with equimolar amounts of (*N*-phenyliminovinylidene)triphenylphosphorane (**2a**) in toluene at reflux temperature, *N*-(2*H*-pyrano[2,3,4-*kl*]xanthen-2-ylidene)aniline (**9**) was obtained in fairly good yield, together with triphenylphosphine oxide. The most important feature in the mass spectrum of **9** is that the M^+ was found at $m/z = 311$. Moreover, no carbonyl group was observed in the IR and ^{13}C NMR spectra of compound **9**. Formation of the pyranoxantheneylidene aniline **9** from the reaction of **2a** and **7** can be explained by the addition of the hydroxyxanthenone to the phosphacumulene to give first the complicated phosphonium ylide **8**, which undergoes cyclization by an intramolecular Wittig reaction, leading to the pyrano compound **9** and triphenylphosphine oxide (Scheme 2).

The reaction of 2-acetyl-1-hydroxy-9*H*-xanthen-9-one (**10a**) with (*N*-phenyliminovinylidene)triphenylphosphorane (**2a**) is of particular interest. Compound **10a** reacted with **2a** in boiling toluene for 10 h to afford 4-methyl-2-(phenylimino)-2*H*,12*H*-pyrano[2,3-*a*]xanthen-12-one (**12a**) along with triphenylphosphine oxide. Addition of the hydroxyxanthenone **10a** to the phosphonium ylide **2a** afforded the intermediate phosphorane **11a**, which cyclized by Wittig reaction with the active side chain carbonyl rather than the xanthenone carbonyl, leading to the pyranoxanthenone **12a**. The IR spectrum of **12a** shows the xanthenone carbonyl group at 1,667 cm^{-1} and in the ^{13}C NMR it appeared at $\delta = 174.20$ ppm. 2-Acetyl-1-hydroxy-9-xanthen-9*H*-one (**10a**) reacted with (oxovinylidene)triphenylphosphorane

Scheme 1



Scheme 2



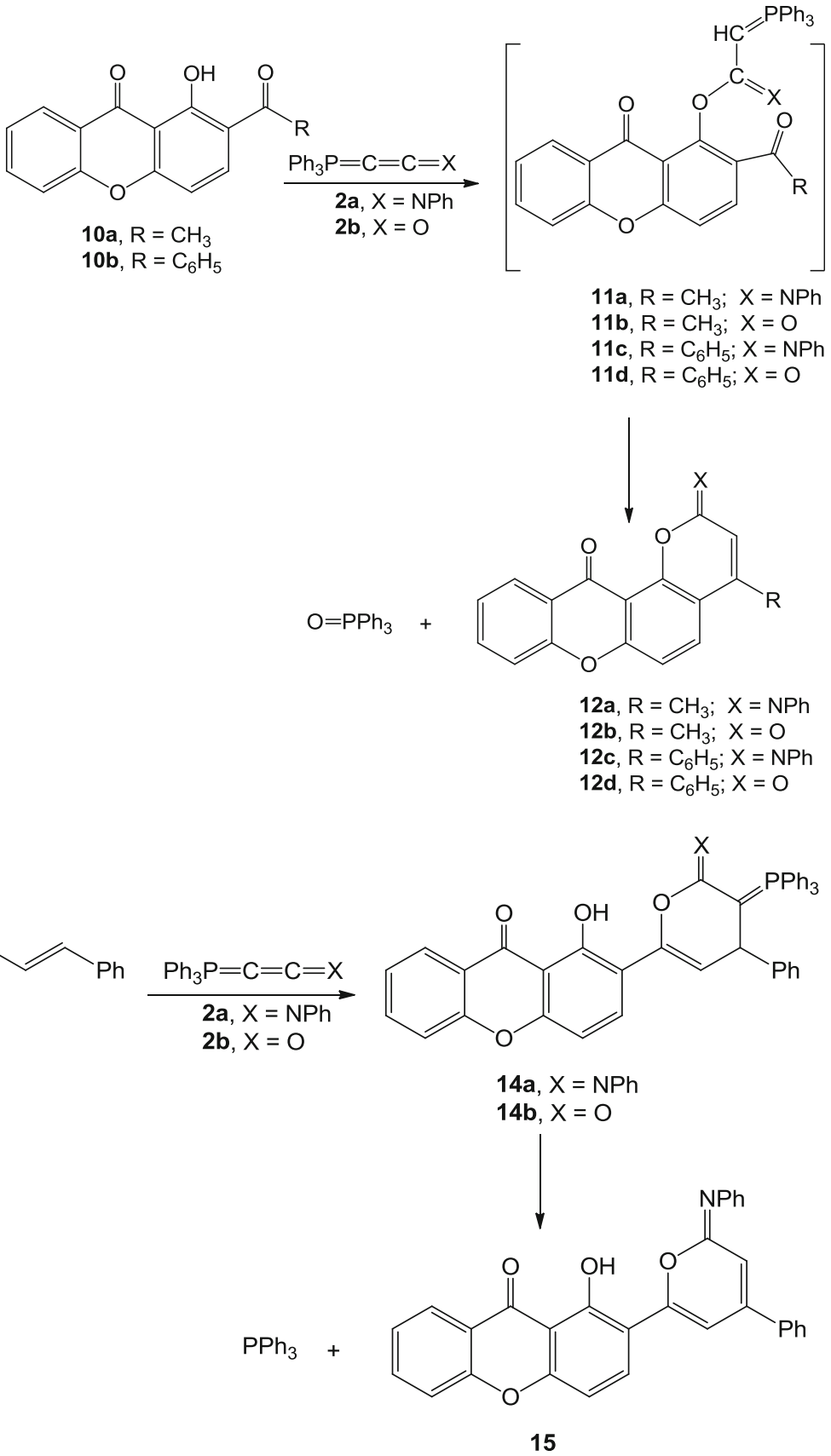
(**2b**) to afford the new 4-methyl-2*H*,12*H*-pyrano[2,3-*a*]xanthene-2,12-dione (**12b**). On the other hand, 2-benzoyl-1-hydroxy-9*H*-xanthen-9-one (**10b**) reacted with the phosphacumulenes **2a** and **2b** under the same experimental conditions to give 4-phenyl-2-(phenylimino)-2*H*,12*H*-pyrano[2,3-*a*]xanthen-12-one (**12c**) and 4-phenyl-2*H*,12*H*-pyrano[2,3-*a*]xanthene-2,12-dione (**12d**) and triphenylphosphine oxide, respectively (Scheme 3).

We have found that the reaction of 2-cinnamoyl-1-hydroxy-9*H*-xanthen-9-one (**13**) with (*N*-phenyliminovinylidene)triphenylphosphorane (**2a**) and (oxovinylidene) triphenylphosphorane (**2b**) proceeds in boiling toluene for 10 h in the case of **2a** or 15 h in the case of **2b** by addition with subsequent cyclization to give 1-hydroxy-2-[4-phenyl-2-(phenylimino)-2*H*-pyran-6-yl]-9*H*-xanthen-9-one (**15**) and 2-[3,4-dihydro-2-oxo-4-phenyl-3-(triphenylphosphoranylidene)-2*H*-pyran-6-yl]-1-hydroxy-9*H*-xanthen-9-one (**14b**), respectively. The intermediate **14a** was not isolated, it changed directly via Hofmann degradation to compound **15** and triphenylphosphine. Compounds **14b** and **15** were obtained in the same yields irrespective of whether 1 or 2 molar equivalents of the phosphacumulenes **2a**, **2b** were used. The IR spectrum of **15** showed strong absorption bands at 3,430 cm^{-1} (OH) and 1,647 cm^{-1} (xanthenone C=O). Its ^{1}H NMR spectrum showed the OH absorption band at $\delta = 14.14$ ppm (D_2O), and in the mass spectra the M^+ was found at $m/z = 457$. Moreover, a signal at $\delta = 13.88$ ppm was observed in the ^{31}P NMR spectrum of **14b** (Scheme 4).

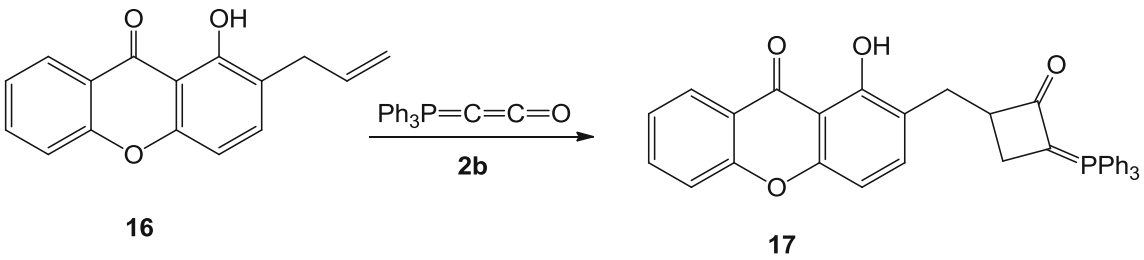
When 2-allyl-1-hydroxy-9*H*-xanthen-9-one (**16**) was treated with (oxovinylidene)triphenylphosphorane (**2b**), the 1:1 aduct 1-hydroxy-2-[(2-oxo-3-(triphenylphosphoranylidene)-cyclobutyl)methyl]-9*H*-xanthen-9-one (**17**) was isolated. Formation of **17** is enhanced by preferential nucleophilic [2+2→4] attack by the carbanionic center in **2b** on the electron-deficient center of the exocyclic ethylenic bond in **16**. Compound **17** was equally obtained whether 1 or 2 molar equivalents of the phosphorane **2b** were used with respect to 1 molar equivalent of **16**. The most important reason for assigning structure **17** is the sharp band at 3,417 cm^{-1} (OH) and broad band at 1,625 cm^{-1} (2 C=O, xanthenone and cyclobutane) in its IR spectrum. In the ^{31}P NMR spectrum of **17** a signal at $\delta = 22.31$ ppm was observed, which supported the ylidene-phosphorane structure [26, 27] (Scheme 5).

In addition, the reaction of anthracen-9(10*H*)-one (**18**) with the active phosphacumulenes **2a**, **2b** was investigated. When the anthrone **18** was treated with (*N*-phenyliminovinylidene)triphenylphosphorane (**2a**), *N,N′*-[2,4-di(anthracen-9(10*H*)ylidene)cyclobutane-1,3-diylidene]dianiline (**21**), *N,N′*-[2-(anthracen-9(10*H*)-ylidene)-4-(triphenylphosphoranylidene)cyclobutane-1,3-diylidene]dianiline (**22a**), and triphenylphosphine oxide were isolated. On the other hand, the reaction of (oxovinylidene)triphenylphosphorane (**2b**) with **18** afforded only 2-(anthracen-9(10*H*)-ylidene)-4-(triphenylphosphoranylidene)cyclobutane-1,3-dione (**22b**) and triphenylphosphine oxide. This reaction proceeds via

Scheme 3



Scheme 4



Scheme 5

[2+2] cycloaddition of the carbonyl group to the ylidic C–P bond of **2a**, **2b** to furnish the oxaphosphetane **19**. Elimination of triphenylphosphine oxide from **19** forms the unstable ketene **20** which dimerizes in the case of using **2a** to give **21** and/or adds another molecule of **2a** to **20** to give the cyclobutane **22a**. But in the case of using the phosphacumulene **2b**, only the cyclobutane **22b** was isolated (Scheme 6).

Cytotoxicity assay

Cancerous diseases are a serious threat to the health and development of mankind and the research for effective anticancer agents continues. Considerable progress has been made in recent years in the field of drug development against different types of cancer. Moreover, chemotherapy is a major approach for both localized and metastasized cancers [28], and for many years xanthenone-related compounds have proved to have significant therapeutic potential [7–10]. On the basis of these considerations, certain of the newly synthesized compounds were screened for their in vitro cytotoxic and growth inhibitory activities against human breast carcinoma cell line (MCF7) and human carcinoma cervical cells (HeLa). In order to determine the possible anticancer activity of our compounds, MCF7 and HeLa cells were cultured in a monolayer and treated with our compounds for 72 h. The sulforhodamine B (SRB) assay was performed to assess the rate of proliferation, and the resulting growth curves showed that our molecules exhibited cytotoxicity against the aforementioned cell lines with very low IC_{50} values. From the data presented in Table 1, it can be seen that compounds **6** and **12a** exhibited high activity against MCF7 and HeLa with $IC_{50} = 0.07$ μg/cm^3, 5.55 μg/cm^3 (HELA) and 0.10 μg/cm^3, 5.99 μg/cm^3 (MCF7), respectively. Moreover, compounds **9**, **12b**, **12d**, **15**, and **17** displayed certain activity against MCF7 and HeLa cells.

Conclusion

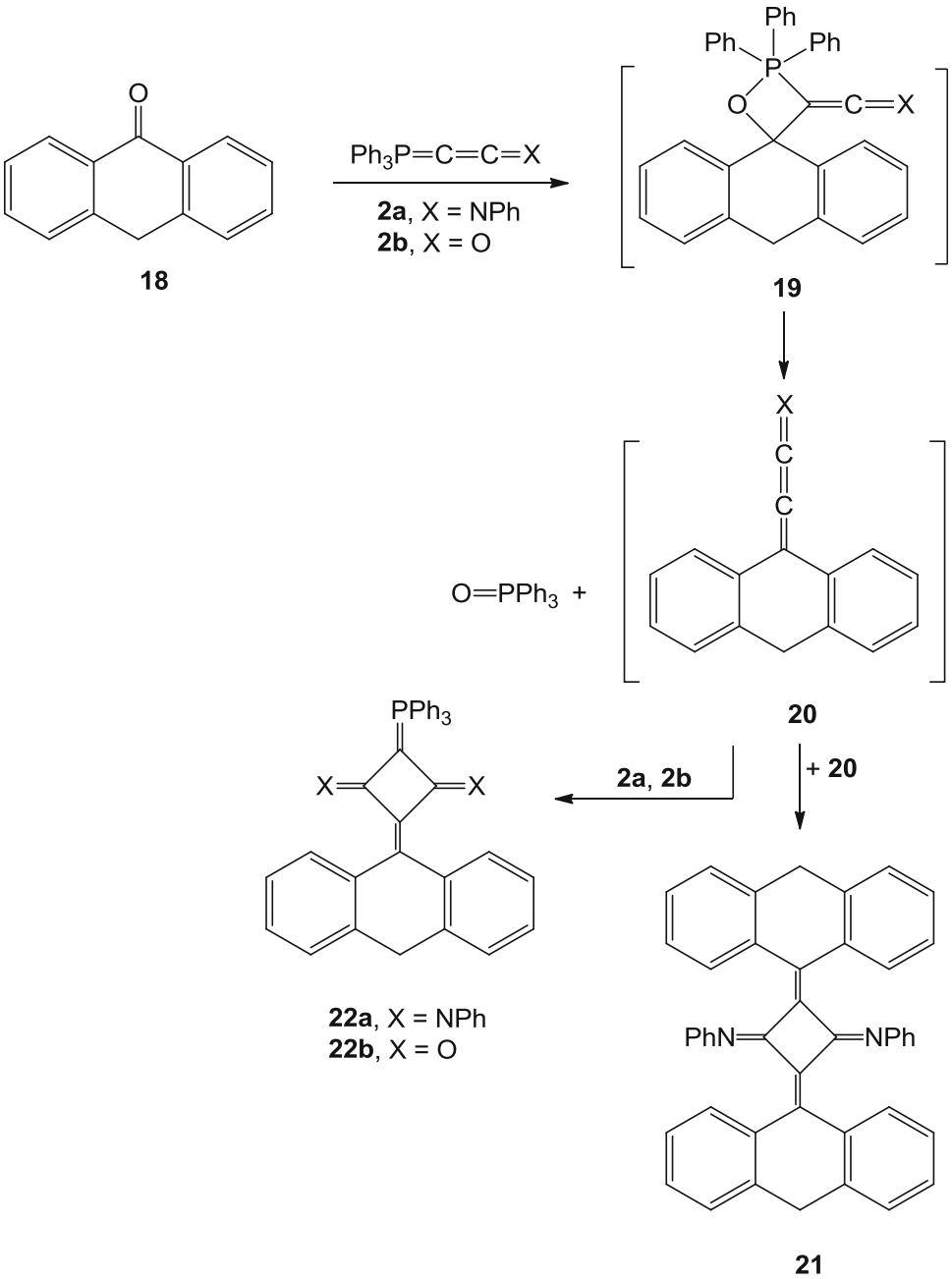
The reactions of the active phosphacumulenes **2a**, **2b** represent an interesting approach to the synthesis of new cyclic bioactive xanthenone derivatives. Moreover, the difference in the nucleophilic character and reactivity of the phosphacumulenes (**2a** > **2b**) [29] is also demonstrated in this study. Therefore, the reaction course between the active phosphacumulenes and xanthenone derivatives is rather dependent on a number of parameters, including the nature of the reactants and the reaction conditions. Our tested compounds have shown promising anticancer activities against the human breast cancer cell line (MCF7) and the human cervical cancer cell line (HeLa cells) at very low concentrations.

Experimental

Melting points were determined with an electrothermal digital melting point apparatus. The IR spectra were recorded in KBr disks on a Jasco FT-IR spectrophotometer model FT/IR-3000E. ^{1}H, ^{13}C, and ^{31}P NMR spectra were obtained from a Jeol EcA 500 MHz NMR spectrometer using $CDCl_3$ as a solvent and TMS as an internal reference at 500.1, 125.8, and 202.4 MHz, respectively. Mass spectra (EI-MS) were obtained at 70 eV with a Finnigan MAT SSQ 7000 spectrometer. Elemental analysis results agreed satisfactorily with the calculated values. The reported yields are based upon pure materials isolated by column chromatography on silica gel 60 (Merck) and thin-layer chromatography (TLC) which was performed on Merck Kieselgel F254 precoated plates (Merck). Solvents were dried/purified according to literature procedures.

N,N′-[2-(Triphenylphosphoranylidene)-4-(9H-xanthen-9-ylidene)cyclobutane-1,3-diylidene]dianiline (**6**, $C_{47}H_{33}N_2OP$)
A mixture of 9*H*-xanthen-9-one (**1**, 196 mg, 1 mmol) and (*N*-phenyliminovinylidene)triphenylphosphorane (**2a**, 754 mg, 2 mmol) [30] in 50 cm^3 dry toluene was refluxed for 20 h. The solvent was evaporated under reduced pressure to give a brown precipitate of compound **6**, which was chromatographed on silica gel using petroleum ether (60–80)/chloroform (7:3, v/v) as an eluent to afford 450 mg (67%) of **6**. M.p.: 312 °C; IR (KBr): $\bar{\nu} = 1{,}612$ (C=N), 1,431 (P–Ar) cm^{-1}; ^{1}H NMR ($CDCl_3$): $\delta = 6.93$-7.50 (m, 33H, ArH) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 105.52$ (*C*–C=N), 115.52, 119.66, 123.20, 125.79, 128.08, 129.32,

Scheme 6



132.97, 133.52, 135.73, 139.08, 143.31, 147.04 (Ar C), 152.67 (C=P), 164.67 (2C=N) ppm; ^{31}P NMR ($CDCl_3$): $\delta = 15.23$ ppm.

Triphenylphosphine oxide was isolated, m.p. and mixed m.p.: 151 °C. When the reaction was performed using (oxovinylidene)triphenylphosphorane (**2b**) instead of **2a**, no reaction was observed.

N-(2H-Pyrano[2,3,4-kl]xanthen-2-ylidene)aniline (**9**, $C_{21}H_{13}NO_2$)

A mixture of 1-hydroxy-9*H*-xanthen-9-one (**7**, 212 mg, 1 mmol) [31] and (*N*-phenyliminovinylidene)triphenylphosphorane (**2a**, 377 mg, 1 mmol) was refluxed for 20 h in 50 cm^3 dry toluene. The solvent was evaporated under reduced pressure to afford a brown precipitate which was purified by preparative TLC using petroleum ether (60–80)/chloroform (1:1, v/v) as an eluent to afford 160 mg (52%) of **9**. M.p.: 235 °C; IR (KBr): $\bar{\nu} = 1{,}660$ (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): $\delta = 6.90$ (s, 1H, CH), 7.25–7.70 (m, 12H, ArH) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 106.66$ (*C*H–C=N), 116.52, 117.94, 124.25, 128.85, 129.00, 130.42, 131.50, 132.65, 137.87, 141.63, 143.08, 143.98, 148.67, 150.00, 152.08 (Ar C), 162.97 (C=N) ppm; MS (EI): $m/z = 311.20$ (M^+).

Table 1 Cytotoxic activity of the synthesized xanthenone derivatives against MCF7 and HELA

Compound	IC_{50}/μg cm^{-3}	
	HeLa	MCF7
6	0.07	0.10
9	17.12	13.60
12a	5.55	5.99
12b	18.27	10.30
12d	7.95	18.3
15	15.47	31.20
17	7.81	15.40

IC_{50} value was defined as the concentration at which 50% survival of cells was observed. Dimethyl sulfoxide (DMSO) was used as negative control

No reaction was observed with (oxovinylidene)triphenylphosphorane (**2b**) instead of **2a**, even when the reaction was carried out in boiling xylene and using triethylamine as a base.

4-Methyl-2-(phenylimino)-2H,12H-pyrano[2,3-a]xanthen-12-one (**12a**, $C_{23}H_{15}NO_3$)

A mixture of 2-acetyl-1-hydroxy-9*H*-xanthen-9-one (**10a**, 254 mg, 1 mmol) [32] and (*N*-phenyliminovinylidene)triphenylphosphorane (**2a**, 377 mg, 1 mmol) in 20 cm^3 dry toluene was refluxed for 10 h. The solvent was evaporated under reduced pressure to give **12a** as a brown precipitate which was chromatographed on silica gel using petroleum ether (60–80)/ethyl acetate (8:2, v/v) as an eluent to afford 240 mg (68%) of **12a**. M.p.: 285 °C; IR (KBr): $\bar{\nu}$ = 1,667 (C=O), 1,604 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 2.49 (s, 3H, CH_3), 6.67 (s, 1H, CH), 7.18–7.93 (m, 11H, ArH) ppm; ^{13}C NMR ($CDCl_3$): δ = 19.00 (CH_3), 113.69 (*C*H–C=N), 117.88, 119.52, 121.33, 123.11, 123.45, 125.69, 126.28, 128.21, 128.47, 128.97, 129.76, 130.64, 133.33, 141.93, 147.07, 154.00 (Ar C), 163.20 (C=N), 174.20 (C=O) ppm; MS (EI): m/z = 352.35 ($[M-H]^+$).

4-Methyl-2H,12H-pyrano[2,3-a]xanthene-2,12-dione (**12b**, $C_{17}H_{10}O_4$)

A mixture of 2-acetyl-1-hydroxy-9*H*-xanthen-9-one (**10a**, 254 mg, 1 mmol) and (oxovinylidene)triphenylphosphorane (**2b**, 302 mg, 1 mmol) [33] in 20 cm^3 dry toluene was refluxed for 15 h. The solvent was evaporated under reduced pressure to give a yellow precipitate which was crystallized from petroleum ether to afford 180 mg (65%) of **12b**. M.p.: 114 °C; IR (KBr) showed the xanthenone and pyranone carbonyls as a broad band centered at $\bar{\nu}$ = 1,581 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 2.20 (s, 3H, CH_3), 6.90 (s, 1H, CH), 7.25–8.92 (m, 6H, ArH) ppm; ^{13}C NMR ($CDCl_3$): δ = 23.05 (CH_3), 106.50 (*C*H–C=O), 116.20, 117.90, 120.61, 124.13, 125.09, 126.14, 132.22, 135.62, 136.70, 141.70 (Ar C), 159.14 (pyranone C=O), 182.81 (xanthenone C=O) ppm; MS (EI): m/z = 279.09 ($[M+H]^+$).

4-Phenyl-2-(phenylimino)-2H,12H-pyrano[2,3-a]xanthen-12-one (**12c**, $C_{28}H_{17}NO_3$)

A mixture of 2-benzoyl-1-hydroxy-9*H*-xanthen-9-one (**10b**, 316 mg, 1 mmol) [32] and (*N*-phenyliminovinylidene)triphenylphosphorane (**2a**, 377 mg, 1 mmol) in 20 cm^3 dry toluene was refluxed for 8 h. The solvent was evaporated under reduced pressure to give a pale yellow precipitate which was crystallized from ether to afford 250 mg (64%) of **12c**. M.p.: 222 °C; IR (KBr): $\bar{\nu}$ = 1,667 (C=O), 1,644 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 6.23 (s, 1H, CH), 6.92–8.30 (m, 16H, ArH) ppm; ^{13}C NMR ($CDCl_3$): δ = 110.18 (*C*H–C=N), 120.64, 121.33, 122.63, 124.16, 127.33, 127.60, 128.19, 128.57, 129.00, 129.25, 131.10, 134.48, 134.71, 135.59, 144.32, 145.71, 146.50, 147.81 (Ar C), 157.71 (C=N), 174.46 (C=O) ppm.

4-Phenyl-2H,12H-pyrano[2,3-a]xanthene-2,12-dione (**12d**, $C_{22}H_{12}O_4$)

A mixture of 2-benzoyl-1-hydroxy-9*H*-xanthen-9-one (**10b**, 316 mg, 1 mmol) and (oxovinylidene)triphenylphosphorane (**2b**, 302 mg, 1 mmol) in 20 cm^3 dry toluene was refluxed for 10 h. The solvent was evaporated under reduced pressure to a give brown precipitate which was chromatographed on silica gel using petroleum ether (60–80)/chloroform (9:1, v/v) as an eluent, affording 240 mg (70%) of **12d**. M.p.: 240 °C; IR (KBr): $\bar{\nu}$ = 1731 (pyranone C=O), 1,666 (xanthenone C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 6.24 (s, 1H, CH), 7.52–8.38 (m, 11H, ArH) ppm; ^{13}C NMR ($CDCl_3$): δ = 114.23 (*C*H–C=O), 117.59, 123.31, 124.94, 128.53, 129.94, 132.22, 134.92, 135.16, 135.96, 139.08, 146.34, 147.84, 154.95, 155.16 (Ar C), 159.65 (pyranone C=O), 174.60 (xanthenone C=O) ppm.

2-[3,4-Dihydro-2-oxo-4-phenyl-3-(triphenylphosphoranylidene)-2H-pyran-6-yl]-1-hydroxy-9H-xanthen-9-one (**14b**, $C_{42}H_{29}O_5P$)

A mixture of 2-cinnamoyl-1-hydroxy-9*H*-xanthen-9-one (**13**, 342 mg, 1 mmol) [32] and (oxovinylidene)triphenylphosphorane (**2b**, 302 mg, 1 mmol) in 50 cm^3 dry toluene was refluxed for 15 h. The solvent was evaporated under reduced pressure to give a yellow precipitate which was purified by preparative TLC using petroleum ether (60–80)/acetone (10:2, v/v) as an eluent to afford 350 mg (55%) of **14b**. M.p.: 312 °C; IR (KBr): $\bar{\nu}$ = 3,403 (OH), 1,636 (xanthenone, pyranone broad band C=O), 1,431 (P–Ar) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 7.52–8.37 (m, 28H, ArH), 14.58 (s, 1H, OH, D_2O-exchangeable) ppm; ^{13}C NMR ($CDCl_3$): δ = 29.77 (CH–Ph), 96.47 (CH), 117.64, 123.11, 124.90, 126.94, 129.09, 131.06, 132.90, 133.85, 133.97, 136.11, 138.03, 142.85, 148.66, 150.32, 153.92 (Ar C), 155.03 (C=P), 157.36 (pyranone C=O), 180.00 (xanthenone C=O) ppm; ^{31}P NMR ($CDCl_3$): δ = 13.88 ppm.

1-Hydroxy-2-[4-phenyl-2-(phenylimino)-2H-pyran-6-yl]-9H-xanthen-9-one (**15**, $C_{30}H_{19}NO_4$)
A mixture of 2-cinnamoyl-1-hydroxy-9*H*-xanthen-9-one (**13**, 342 mg, 1 mmol) and (*N*-phenyliminovinylidene)triphenylphosphorane (**2a**, 377 mg, 1 mmol) in 50 cm^3 dry toluene was refluxed for 10 h. The solvent was evaporated under reduced pressure to give an orange precipitate which was crystallized from acetone to afford 330 mg (72%) of **15**. M.p.: 258 °C; IR (KBr): $\bar{\nu}$ = 3,430 (OH), 1,647 (C=O), 1,612 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 6.50 (s, 1H, CH), 6.80 (s, 1H, CH), 7.25–8.30 (m, 16H, ArH), 14.40 (s, 1H, OH, D_2O) ppm; ^{13}C NMR ($CDCl_3$): δ = 112.69 (*C*H–C=N), 118.37, 124.99, 127.12, 127.26, 128.54, 128.94, 129.04, 129.16, 129.30, 129.39, 135.98, 138.67, 141.21, 145.65, 147.44, 147.30, 149.79, 150.14, 153.55 (Ar C), 166.85 (C=N), 185.20 (C=O) ppm; MS (EI): *m*/*z* = 457.09 (M^+).

1-Hydroxy-2-[[2-oxo-3-(triphenylphosphoranylidene)-cyclobutyl]methyl]-9H-xanthen-9-one (**17**, $C_{36}H_{27}O_4P$)
A mixture of 2-allyl-1-hydroxy-9*H*-xanthen-9-one (**16**, 252 mg, 1 mmol) [34] and (oxovinylidene)triphenylphosphorane (**2b**, 302 mg, 1 mmol) in 50 cm^3 dry toluene was refluxed for 20 h. The solvent was evaporated under reduced pressure to give a brown precipitate which was crystallized from ether to afford 360 mg (65%) of **17**. M.p.: 105 °C; IR (KBr): $\bar{\nu}$ = 3,417 (OH), 1,625 (xanthenone, cyclobutanone broad band C=O), 1,434 (P–Ar) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 2.02 (m, 3H, CH_2, CH), 3.26 (m, 2H, CH_2), 7.42–7.70 (m, 21H, ArH) ppm; ^{13}C NMR ($CDCl_3$): δ = 18.54 (cyclobutanone CH_2), 29.32 (CH_2), 48.83 (cyclobutanone CH), 118.95, 119.73, 122.59, 123.52, 126.44, 127.65, 130.45, 130.56, 133.34, 135.09, 135.12, 137.04, 139.12, 143.91, 147.02, 147.86, 149.55 (Ar C), 154.03 (C=P), 175.00 (xanthenone C=O), 201.00 (cyclobutanone C=O) ppm; ^{31}P NMR ($CDCl_3$): δ = 22.31 ppm; MS (EI): *m*/*z* = 554.20 (M^+).

N,N′-[2,4-Di(anthracen-9(10H)-ylidene)cyclobutane-1,3-diylidene]dianiline (**21**, $C_{44}H_{30}N_2$)
A mixture of anthracen-9(10*H*)-one (**18**, 194 mg, 1 mmol) and (*N*-phenyliminovinylidene)triphenylphosphorane (**2a**, 377 mg, 1 mmol) in 50 cm^3 dry toluene was refluxed for 40 h. The solvent was evaporated under reduced pressure to give a brown precipitate which was crystallized from methylene chloride/ether (4:1), affording 320 mg (55%) of **21**. M.p.: 285 °C; IR (KBr): $\bar{\nu}$ = 1,649 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 3.50 (4H, 2 CH_2), 6.95–7.90 (m, 26H, ArH) ppm; ^{13}C NMR ($CDCl_3$): δ = 38.88 ($2CH_2$), 103.20 (cyclobutane 2C), 124.66, 128.53, 128.63, 132.14, 132.23, 137.09, 145.80, 146.09, 148.02 (Ar C), 163.20 (2C=N) ppm.

N,N′-[2-(Anthracen-9(10H)-ylidene)-4-(triphenylphosphoranylidene)cyclobutane-1,3-diylidene]dianiline (**22a**, $C_{48}H_{35}N_2P$)
A mixture of anthracen-9(10*H*)-one (**18**, 194 mg, 1 mmol) and (*N*-phenyliminovinylidine)triphenylphosphorane (**2a**, 754 mg, 2 mmol) in 30 cm^3 dry toluene was refluxed for 40 h. The solvent was evaporated under reduced pressure to give a red precipitate which was crystallized from ether to afford 450 mg (67%) of **22a**. M.p.: 215 °C; IR (KBr): $\bar{\nu}$ = 1,639 (C=N), 1,439 (P–Ar) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 3.38, 3.40 (2H, CH_2), 7.08–7.66 (m, 33H, ArH) ppm; ^{13}C NMR ($CDCl_3$): δ = 31.27 (CH_2), 104.73 (C=*C*–C=N), 115.57, 119.18, 127.03, 127.25, 128.61, 128.76, 129.04, 129.58, 130.13, 130.20, 136.60, 137.11, 141.35, 142.90, 148.12, 153.20 (Ar C), 154.30 (C=P), 156.56 (2C=N) ppm; ^{31}P NMR ($CDCl_3$): δ = 15.74 ppm.

2-(Anthracen-9(10H)-ylidene)-4-(triphenylphosphoranylidene)-cyclobutane-1,3-dione (**22b**, $C_{36}H_{25}O_2P$)
A mixture of anthracen-9(10*H*)-one (**18**, 194 mg, 1 mmol) and (oxovinylidene)triphenylphosphorane (**2b**, 604 mg, 2 mmol) in 50 cm^3 dry toluene was refluxed for 50 h. The solvent was evaporated under reduced pressure to give a brown precipitate which was crystallized from petroleum ether (60–80) to afford 300 mg (58%) of **22b**. M.p.: 200 °C; IR (KBr): $\bar{\nu}$ = 1,661 (C=O), 1,435 (P–Ar) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ = 3.49, 3.53 (2H, CH_2), 7.22–8.45 (m, 23H, ArH) ppm; ^{13}C NMR ($CDCl_3$): δ = 37.20 (CH_2), 119.02, 123.53, 127.30, 128.63, 130.50, 132.20, 134.21, 135.05, 138.13, 140.27, 144.39, 146.85, 150.05 (Ar C), 153.78 (C=P), 192.10 (2C=O) ppm; ^{31}P NMR ($CDCl_3$): δ = 19.70 ppm; MS (EI): *m*/*z* = 522.20 ($[M + 2H]^+$).

Cytotoxicity screening

Cell lines and treatments

The human breast cancer cell line (MCF7) and the human cervical carcinoma cells (HeLa) cells were obtained from the American Type Culture Collection and grown in DMEM medium with 10% foetal bovine serum, 100 units/cm^3 penicillin, and 100 μg/cm^3 streptomycin (Invitrogen). Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2. Stock solutions of our compounds were prepared in DMSO and stored at −20 °C. All controls were exposed to DMSO alone; DMSO concentration was always <0.1%.

Cell viability assay (SRB assay)

Cells were plated in 96-well plates and incubated for 48 h. After treatment, cell medium was aspirated, and 100 mm^3/well of 10% trichloroacetic acid was added. After fixation at 4 °C and washing, 50 mm^3 of 0.4% (w/v) sulforhodamine B (SRB; Sigma-Aldrich) was added. Plates were incubated at room temperature for 30 min. Unbound SRB was removed with 1% acetic acid. Bound SRB was solubilized with 100 mm^3 of 10 mM Tris-base solution. Absorbance was determined in a plate reader (TECAN Infinite) at 570 and 650 nm (background). Cell viability

was expressed as the percentage of cell survival relative to untreated cells.

Data analysis

The dose–response curve of compounds was analyzed using the E_{max} model.

$$\text{Cell viability}(\%) = (100 - R) \times \left(1 - \frac{[\text{D}]^m}{K_\text{d}^m + [\text{D}]^m}\right) + R$$

where R is the residual unaffected fraction (the resistance fraction), [D] is the drug concentration used, K_d is the drug concentration that produces a 50% reduction of the maximum inhibition rate and m is a Hill-type coefficient. IC_{50} was defined as the drug concentration required to reduce fluorescence to 50% of that of the control (i.e., $K_\text{d} = IC_{50}$ when $R = 0$ and $E_{max} = 100 - R$). Results are represented as mean ± standard error of the mean (SEM) of three independent experiments. Data analysis was done using SigmaPlot 11 software.

References

1. Hay AE, Aumond MC, Mallet S, Dumontet V, Litaudon M, Rondeau D, Richomme P (2004) J Nat Prod 67:707
2. Mondal M, Puranik VG, Argade NP (2006) J Org Chem 71:4992
3. Jung HA, Su BN, Keller WJ, Mehta RG, Kinghorn AD (2006) J Agric Food Chem 54:2077
4. Lee BW, Lee JH, Lee ST, Lee HS, Lee WS, Jeong TS, Park KH (2005) Bioorg Med Chem Lett 15:5548
5. Lin CN, Chung MI, Liou SJ, Lee TH, Wang TH (1996) J Pharm Pharmacol 48:532
6. Dua VK, Ojha VP, Roy R, Joshi BC, Velacha N, Devi CU, Bhatnagar MC, Sharma VP, Subbarao SJ (2004) Ethnopharmacology 95:274
7. Giri R, Goodell JR, Xing C, Benoit A, Kaur H, Hiasa H, Ferguson DM (2010) Bioorg Med Chem 18:1456
8. Pedro M, Cerqueira F, Sousa ME, Nascimento MS, Pinto M (2002) Bioorg Med Chem 10:3725
9. Valenti P, Bisi A, Rampa A, Belluti F, Gobbi S, Zampiron A, Carrara M (2002) Bioorg Med Chem 8:239
10. Yoshimi N, Matsunaga K, Katayama M, Yamada Y, Kuno T, Qiao Z, Hara A, Yamahara J, Mori H (2001) Cancer Lett 163:163
11. Saraiva L, Fresco P, Pinto E, Sousa E, Pinto M, Goncalves J (2002) Bioorg Med Chem 10:3219
.12. Saraiva L, Fresco P, Pinto E, Sousa E, Pinto M, Goncalves J (2003) Bioorg Med Chem 11:1215
13. Nguemeving JR, Azebaze AGB, Kuete V, Carly NNE, Beng VP, Meyer M, Bland A, Bodo B, Nkengfack AE (2006) Phytochemistry 67:1341
14. Demirkiran O (2007) Top Heterocycl Chem 9:139
15. Wang Y, Shi JG, Wang MZ, Che CT, Yeung JH (2007) Life Sci 81:2016
16. Liu Y, Ma L, Chen WH, Wang B, Xu ZL (2007) Bioorg Med Chem 15:2810
17. Soliman FM, Said MM, Maigali SS (2005) Heteroatom Chem 16:476
18. Maigali SS, Said MM, Abd El-Maksoud MA, Soliman FM (2008) Monatsh Chem 139:495
19. Said MM, Maigali SS, Abd El-Maksoud MA, Soliman FM (2008) Monatsh Chem 139:1299
20. Soliman FM, Abd-Ellah I, Maigali SS, Abd-El-Naim G (2009) J Chem Res 277
21. Bestmann HJ, Schmid G, Sandmeier D, Kisielowski L (1977) Angew Chem 89:275
22. Matthews CN, Birum GH (1969) Acc Chem Res 2:373
23. Vedejs E, Snoble KA (1973) J Am Chem Soc 95:5778
24. Schlosser M, Piskala A, Tarchini C, Tuong HB (1975) Chimia 29:341
25. Bestmann HJ, Schmid G (1974) Angew Chem Int Ed 13:273
26. Johnson AW (1993) Ylides and imines of phosphorus. Wiley, New York
27. Grim SO, McFarlane W, Marks T (1967) Chem Commun 1191
28. Surth YJ (2003) Nat Rev Cancer 3:750
29. Bestmann HJ (1977) Angew Chem Int Ed 16:349
30. Bestmann HJ, Schmid G (1975) Tetrahedron Lett 4025
31. Desai BM, Desai PR, Desai RD (1960) J Indian Chem Soc 37:53
32. Mustafa A, Hishmat OH (1975) J Am Chem Soc 79:2225
33. Bestmann HJ, Sandmeier D (1975) Angew Chem Int Ed 14:634
34. Scheinmann F, Suschitzky H (1959) Tetrahedron 7:31