J. Chem. Sci. Vol. 125, No. 6, November 2013, pp. 1419–1428.

Chemistry of phosphorus ylides 31: Reaction of azidocoumarin with active phosphonium ylides, synthesis and antitumour activities of chromenones

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MS received 26 April 2012; revised 21 November 2012; accepted 7 December 2012

Abstract. The reaction of 4- azidochromen-2-one (**1**) with the nucleophilic phosphacumulene ylides **2**, **8**, and **12** afforded the new heterocyclic triazoles, triazepines, aziridine, pyrrolone containing a coumarin moiety. Cycloaddition reactions took place first to give triazoline **3** and **9**. The triazolines rearranged to the triazepines **4**, **10**, and **13** accompanied by elimination of triphenylphosphine leading to the phosphorus-free triazepines **5**, **11**, and moreover, aziridine **6** was produced via nitrogen extrusion from the triazoline **3**, followed by ring expansion to the pyrrolone **7**. On the other hand, the reaction of the azidocoumarin **1** with the phosphallene yield **15** behaves differently and afforded the triazine **17** and azetone **18**. The antitumour activity of compounds **3**, **4**, **6**, and **11** was evaluated, *in vitro*, against (breast: MCF-7 and liver: HPEG2) human solid tumour cell lines. They showed values closed to that recorded by the reference drug doxorubicin.

Keywords. Phosphacumulenes; phosphallene ylide; triazoles; triazepines; triazine-phosphanylidenes and antitumour activity.

1. Introduction

Coumarin and its derivatives are found in many plants and exhibit various biological activities.[1] They have long been recognized to possess antiinflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities.[2–5] Moreover, coumarins have important effects in plant biochemistry and physiology, acting as enzyme inhibitors and precursor of toxic substances. In addition, these compounds are involved in the actions of plant growth hormones and growth regulators, the control of respiration, photosynthesis as well as defense against infection.[2,6] In recent years nitrogen heterocycles containing a coumarin nucleus have received increasing attention due to their potential biological properties and considerable effects have been undertaken to exploit synthetic routes to these compounds.[4–9] The biological importance of coumarin and triazole prompted us to synthesize new triazole derivatives containing coumarin backbone, which would be favourable in achieving some specificity of pharmacological action in view of the development of effective clinical anticancer and anti HIV drugs.

2. Experimental

2.1 *Chemistry*

Melting points were determined with an electrothermal digital melting point apparatus (Electro-thermal Engineering Ltd., Essex, United Kingdom). 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, England and Shimadzu, Tokyo, Japan, respectively). ^{1}H and ^{13}C 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 an internal reference at 500 and 125 MHz, respectively and ^{31}P NMR spectra were obtained from a Jeol ECA 500 MHz NMR spectrometer at 200 MHz. 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

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(Germany), phosphorus was measured by spectrophotometric methods and all of them agreed satisfactory with the calculated values. The reported yields are of pure isolated materials obtained by column chromatography silica gel 60 (Merck).

2.1a *Reaction of (N-phenyliminovinylidene)triphenylphosphorane **2** with 4-azido chromen-2-one (**1**)*: A solution of (*N*-phenyliminovinylidene) triphenylphosphorane **2**[10] (377 mg, 1 mmol) in 20 mL of tetrahydrofuran, was added drop by drop with stirring at room temp, to a solution of 4-azidochromen-2-one **1**[11] (187 mg, 1 mmol) in 20 mL of tetrahydrofuran (THF). The reaction mixture was stirred for 10 h during which the colour changed from white to pink (the progress of the reaction was monitored by TLC). THF was distilled off under reduced pressure and the residue was chromatographed on silica gel column using petroleum ether (60–80 °C): ethyl acetate (50:50, *v*/*v*) as eluent. Three products **3, 4** and **5** were isolated along with triphenylphosphine (m.p. and mixed m.p. 78 °C).

2.1b *4-[5-(Phenylimino)-4-(triphenyl-λ^5-phosphanylidene)-4,5-dihydro-1H-1,2,3-triazol-1-yl]-2H-chromen-2-one (**3**)*: Yield, 214 mg (38 %), pink crystal, m.p.: 231–233°C, Analysis for $C_{35}H_{25}N_4O_2P$(564.5): Calcd. C, 74.46; H, 4.46; N, 9.92; P, 5.49. Found: C, 74.64; H, 4.55; N, 9.89; P, 5.99.

2.1c *5-(Phenylimino)-4-(triphenyl-λ^5-phosphanylidene)-4,5-dihydrochromeno[4,3-d][1,2,-3]triazepine-6(1H) one (**4**)*: Yield, 118 mg (21%), colorless crystal, m.p.: 331–333 °C, IR (KBr,$\tilde{\nu}$, cm^{-1}): 3445 (NH), 1699 (C=O, lactone), 1601 (C=N), 1524 (C=P), 1438, 1390 (P-aryl). ^{1}H NMR (500 MH_Z, d_6-DMSO, δ, ppm): 6.93 (s, 1H, NH, exchangeable with D_2O), 7.32–7.83 (m, 24H, Ar H); ^{13}C NMR (125 MH_Z, d_6-DMSO, δ, ppm): 162.03 (C=N), 157.9 (C=O, lactone), 153.30 (C=P). ^{31}P NMR: δ 30.33 ppm.[12] MS *m*/*z* 535 $[M-N_2]^+$. Analysis for $C_{35}H_{25}N_4O_2P$(564.5): Calcd. C, 74.46; H, 4.46; N, 9.92; P, 5.49. Found: C,74.03; H, 4.65; N, 9.84; P, 5.49.

2.1d *5-(Phenylimino)chromeno[4,3-d][1,2,3]triazepin-6-(5H)-one (**5**)*: Yield, 45 mg (15 %), colorless crystal, m.p.: 220–222 °C, IR (KBr,$\tilde{\nu}$, cm^{-1}): 1730 (C=O, lactone), 1600 (C=N). ^{1}H NMR (500 MHz, d_6-DMSO, δ, ppm): 7.22–7.78 (m, 9H, Ar H), 8.75 (s, 1H, azomethine); MS *m*/*z* 301$[M-H]^+$, 273$[M-N_2]^+$, 245$[M-(N_2+CO)]^+$, 229$[M-(N_2+CO_2)]^+$. Analysis for $C_{17}H_{10}N_4O_2$(302.2): Calcd. C, 67.55; H, 3.33; N, 18.53. Found: C, 67.36; H, 3.02; N, 18.72.

When the reaction of (*N*-phenyliminovinylidene)-triphenylphosphorane **2** (377 mg, 1 mmol,) and 4-azidochromen-2-one **1** (187 mg, 1 mmol), was repeated in boiling toluene (40 mL) for 8 h, evolution of N_2 bubbles was observed. Toluene was distilled off under reduced pressure and the residue was subjected to silica gel column chromatography using petroleum ether (60–80°C)/ ethyl acetate as eluent (65:35, *v*/*v*), to give two products **6** and **7**.

2.1e *4-[2-(Phenylimino)-3-(triphenyl-λ^5-phosphanylidene)aziridin-1-yl]-2H–chromen 2-one (**6**)*: Yield, 321 mg (60 %), pale yellow crystals, m.p.: 132–134 °C, IR (KBr,$\tilde{\nu}$, cm^{-1}): 1648 (C=O, lactone), 1600 (C=N), 1526 (C=P), 1439, 1392 (P-aryl). ^{1}H NMR (500 MHz, d_6-DMSO, δ, ppm) 6.93–7.74 (m, 25H, Ar H); ^{13} C NMR (125 MH_Z, d_6-DMSO, δ, ppm): 164.35 (C=N), 159.65 (C=O, lactone), 153.83 (C=P); ^{31}P NMR: δ 9.74 ppm.[13] MS *m*/*z* 508 $[M-CO]^+$. Analysis for $C_{35}H_{25}N_2O_2P$ (536.5): Calcd. C, 78.35; H, 4.70; N, 5.22; P, 5.77. Found: C, 78.83; H, 4.95; N, 5.02; P, 6.00.

2.1f *3-(Phenylimino)-2-(triphenyl-λ^5-phosphanylidene)-2,3-dihydrochromeno[4,3-d]pyrrol-4-(1H)-one (**7**)*: Yield, 107 mg (20 %), colourless crystals, m.p.: 271–273 °C. IR (KBr,$\tilde{\nu}$, cm^{-1}): 3423 (NH), 1738 (C=O, lactone), 1597 (C=N), 1565 (C=P), 1493, 1465 (P-aryl). ^{1}H NMR (500 MHz, d_6-DMSO, δ, ppm): 6.88–7.56 (m, 24H, Ar H), 10.99 (s, 1H, NH, exchangeable with D_2O). MS *m*/*z* 537 $[M+H]^+$: Analysis for $C_{35}H_{25}N_2O_2P$(536.5): Calcd. C, 78.35; H, 4.70; N, 5.22; P, 5.77. Found: C, 78.14; H, 4.79; N, 5.32; P, 5.40.

2.1g *Reaction of (2-oxovinylidene)triphenylphosphorane **8** with 4-azidochromen-2-one (**1**)*: A mixture of (2-oxovinylidene)triphenylphosphorane **8**[14] (302 mg, 1 mmol), and 4-azidochromen-2-one **1** (187 mg, 1 mmol), in toluene (40 mL) was refluxed for 8 h during which the colour changed to pale yellow. The precipitate was filtered off and the residue crystallized from ethanol to give **9**. The resulting liquor was distilled off and the residue was subjected to silica gel column chromatography using petroleum ether (60–80 °C)/ethyl acetate as eluent (30:70, *v*/*v*), to give two products **10** and **11** in addition to triphenylphosphine (m.p. and mixed m.p. 78 °C).

2.1h *3-(2-Oxo-2H-chromen-4-yl)-5-(triphenyl-λ^5-phosphanylidene)-3,5-dihydro-4H-1,2,3-triazol-4-one (**9**)*: Yield, 195 mg (40 %), colourless crystals m.p.: 252–254 °C. IR, (KBr,$\tilde{\nu}$, cm^{-1}): 1718 (C=O, lactone), 1645

(C=O), 1597 (C=P), 1433, 1395 (P-aryl). ^{1}H NMR (500 MHz, d_6-DMSO, δ, ppm): 7.08–8.30 (m, 20H, Ar H) ^{13}C NMR (125 MH_Z, d_6-DMSO, δ, ppm): 164.10 (C=O, triazole), 161.40 (C=O, lactone), 154.30 (C=P). ^{31}P NMR: δ 11.57 ppm. MS m/z = 461 $[M\text{-}N_2]^+$. Analysis for $C_{29}H_{20}N_3O_3P$(489.4): Calcd. C, 71.16; H, 4.12; N, 8.58; P, 6.33. Found: C, 71.64; H, 4.06; N, 8.90; P, 6.40.

2.1i *4-(Triphenyl-λ^5-phosphanylidene)-1,4-dihydrochromeno[4,3-d][1,2,3]triazepine-5,6-dione **(10)***: Yield, 146 mg (30 %), colourless crystals, M.p.: 228–230 °C. IR (KBr,$\widetilde{\nu}$,cm^{-1}): 3425 (NH), 1721 (C=O, lactone), 1647 (C=O, triazepine), 1600 (C=P), 1486, 1482 (P-aryl).^{1}H NMR (500 MHz, d_6-DMSO, δ, ppm): 7.37–7.73 (m, 19H, Ar H), 11.28 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (125 MHz, d_6-DMSO, δ, ppm): 178.80 (C=O, triazepine), 161.20 (C=O, lactone), 157.40 ppm (C=P); ^{31}P NMR: δ 16.39 ppm. MS m/z = 488$[M\text{-}H]^+$. Analysis for $C_{29}H_{20}N_3O_3P$ (489.4): Calcd. C, 71.16; H, 4.12; N, 8.58; P, 6.33. Found: C, 71.66; H, 4.16; N, 8.14; P, 6.03.

2.1j *Chromeno[4,3-d][1,2,3]triazepine-4,6-dione **(11)***: Yield, 34 mg (15 %), colourless crystals, M.p.: 157–159 °C. IR (KBr,$\widetilde{\nu}$, cm^{-1}): 1719 (C=O, lactone), 1653 (C=O, triazepine), 1601 (C=N). ^{1}H NMR (500 MHz, d_6-DMSO, δ, ppm): 7.44–7.67 (m, 5H, Ar H); ^{13}C NMR (125 MH_Z, d_6-DMSO, δ, ppm): 186.28 (C=O, triazepine), 163.27 (C=N), 159.33, (C=O, lactone). MS m/z = 227 $[M]^+$; 199 $[M\text{-}N_2]^+$; 171 $[M\text{-}N_2, CO]^+$; 155 $[M\text{-}N_2, CO_2]^+$. Analysis for $C_{11}H_5N_3O_3$(227.1): Calcd. C, 58.16; H, 2.22; N, 18.50. Found: C, 58.16; H, 2.44; N, 18.94.

2.1k *Reaction (2-thioxovinylidene)triphenylphosphorane **(12)** with 4-azidochromen-2-one **(1)***: A solution of (2-thioxovinylidene)triphenylphosphorane **12** (318 mg, 1 mmol) was added to solution of 4-azidochromen-2-one **1** (187 mg, 1 mmol), in toluene (40 mL). The reaction mixture was boiled for 10 h during which the colour was changed from colourless to dark brown. Toluene was distilled off and the residue was subjected to silica gel column chromatography using petroleum ether (60–80 °C)/ ethyl acetate as an eluent (70:30, v/v), to give two products **13**, **14** and triphenylphosphine (m.p. and mixed m.p. 78 °C).

2.1l *5-Thioxo-4-(triphenyl-λ^5-phosphanylidene)-4,5-dihydrochromeno[4,3-d][1,2,3]-triazepin-6(1H)-one **(13)***: Yield, 277 mg (55 %), pale brown crystals, m.p.: 243–245 °C, IR (KBr,$\widetilde{\nu}$, cm^{-1}) : 3367 (NH), 1696 (C=O, lactone), 1597 (C=P), 1480 (P-aryl), 1240 (C=S).^{1}H NMR (500 MHz, d_6-DMSO, δ, ppm): 7.15–7.79 (m, 19H, Ar H), 8.37 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (125 MH_Z, d_6-DMSO, δ, ppm): 207.53 (C=S), 160.74 (C=O, lactone), 153.73 (C=P); ^{31}P NMR: δ 13.40 ppm. MS m/z = 477$[M\text{-}N_2]^+$. Analysis for $C_{29}H_{20}N_3O_2PS$(505.5): Calcd. C, 68.90; H, 3.99; N, 8.31; P, 6.13; S, 6.34. Found: C, 68.70; H, 3.44; N, 8.33; P, 6.44; S, 6.05.

2.1m *5-Thioxochromeno[4,3-d][1,2,3]triazepin-6(5H)-one **(14)***: Yield: 65 mg (27 %), colorless crystals, M.p.: 147–149 °C. IR (KBr,$\widetilde{\nu}$, cm^{-1}): 1732 (C=O, lactone), 1610 (C=N), 1270 (C=S). ^{1}H NMR (500 MHz, d_6-DMSO, δ, ppm): 7.27–7.57 (m, 4H, Ar H); 8.65 (s, 1H, CH, azomethine); ^{13}C NMR (125 MHz, d_6-DMSO, δ, ppm): 206.96 (C=S), 163.03 (C=N), 159.89 (C=O, lactone). MS m/z 242 $[M]^+$. Analysis for $C_{11}H_5N_3O_2S$(243.2): Calcd. C, 54.32; H, 2.07; N, 17.28; S, 13.18. Found: C, 54.02; H, 2.09; N, 17.54; S, 13.44.

2.1n *Interaction of hexaphenylcarbodiphosphorane **(15)** with 4-azidochromen-2-one **(1)***: To a solution of 4-azidochromen-2-one **1** (187 mg, 1 mmol), in 20 mL THF was added, hexaphenylcarbodiphosphorane **15**,[15] (536 mg, 1 mmol) in 20 mL THF. The reaction mixture was stirred at room temp for 12 h during which the colour change from colourless to yellow then brown, and N_2 gas was evolved. THF was distilled off under reduced pressure and the remained residue was chromatographed on silica gel using petroleum ether (60–80°C): ethyl acetate as an eluent (60:40, v/v), to give **17**, **18** together with triphenylphosphine (m.p. and mixed m.p. 78 °C).

2.1o *4-(1,1,1-Triphenyl-λ^5-phosphan-1-ylidene)-1,4-dihydro-5H-chromeno[4,3-d][1,2,-3]triazin-5-one **(17)***: Yield, 184 mg (40 %), golden yellow crystals, m.p.: 250–252 °C, IR (KBr, $\widetilde{\nu}$, cm^{-1}): 3208 (NH), 1641 (C=O, lactone), 1546 (C=P), 1484, 1435 (P-aryl). ^{1}H NMR (500 MHz, d_6-DMSO, δ, ppm): 7.25–7.57 (m, 20H, Ar H+ NH); ^{31}P NMR δ 29.79 ppm.[16–18] MS m/z = 462 $[M+H]^+$. Analysis for $C_{28}H_{20}N_3O_2P$(461.4): Calcd. C, 72.88; H, 4.37; N, 9.11; P, 6.71. Found: C, 72.72; H, 4.12; N, 9.15; P, 6.82.

2.1p *2-(Triphenyl-λ^5-phosphanylidene)-1,2-dihydro-3H-chromeno[4,3-d]azet-3-one **(18)***: Yield, 129 mg (30 %), colourless crystals, m.p.: 132–134°C. IR (KBr,$\widetilde{\nu}$, cm^{-1}): 3374 (NH), 1695 (C=O, lactone), 1597 (C=P), 1485, 1437 (P-aryl). ^{1}H NMR (500 MHz,

d_6-DMSO, δ, ppm): 7.25–7.57 (m, 20H, Ar H + NH). ^{31}P NMR: δ 13.45 ppm.[19–21] MS m/z = 433 $[M]^+$. Analysis for $C_{28}H_{20}NO_2P$ (433.4): Calcd. C, 77.59; H, 4.65; N, 3.23; P, 7.15. Found: C, 77.40; H, 4.20; N, 3.15; P, 6.99.

2.2 *Biological screening in vitro cytotoxicity*

2.2a *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.

2.2b *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.

2.2c *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 sulphorhodmine-B (SRB) colorimetric assay according to Skehan *et al.*[22] This test is based on the sensitivity of the human tumour 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).

2.2d *Calculation*: The percentage of cell survival was calculated as follows. Survival fraction = OD (treated cells) / OD (control cells) where (OD) 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.

3. Results and discussion

3.1 *Chemistry*

As a continuation of our investigations into the synthesis of new heterocyclic compounds using active phosphacumulenes and phosphallene,[23–28] 4-azidochromen-2-one **1** was selected for the synthesis of the new triazolo-coumarin derivatives.

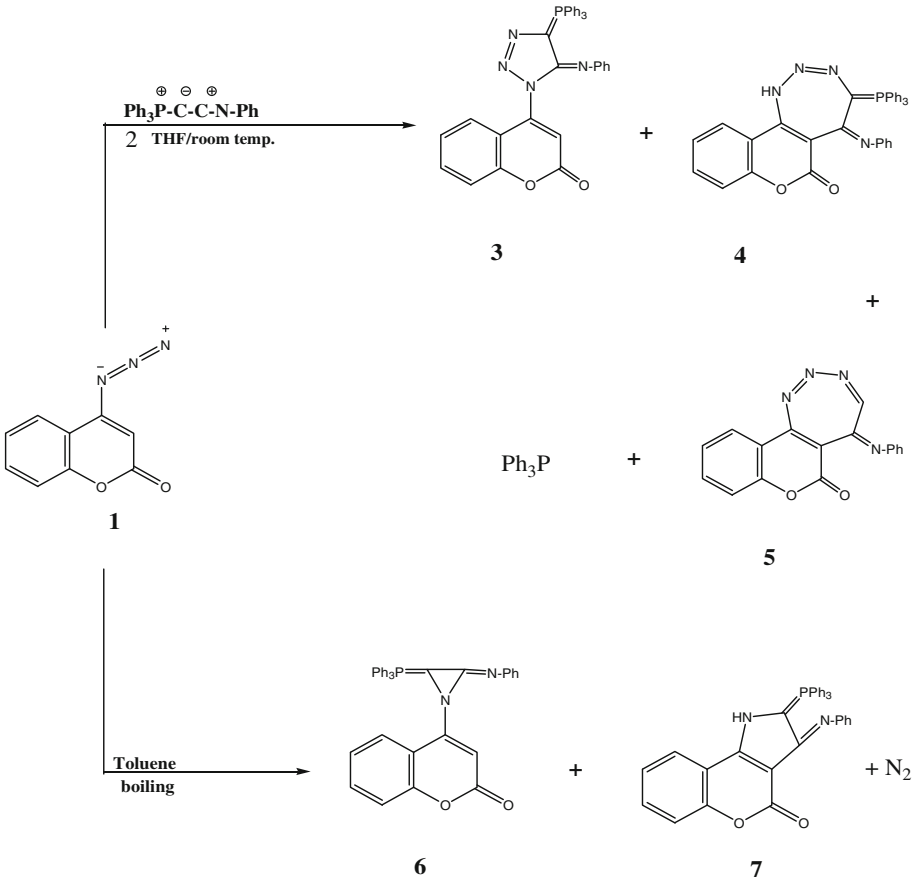
When compound **1** was allowed to react with (*N*-phenyliminovinylidene)triphenylphosphorane **2** in THF at room temperature, the reaction results in the formation of three products. A pink crystalline compound **3** was obtained as a major product. It was formulated as 4-[5-(phenylimino)-4-(triphenyl-λ^5-phosphanylidene)-4,5-dihydro-1*H*-1,2,3-triazol-1-yl]-2*H*-chromen-2-one **3**. Besides, 5-(phenylimino)-4-(triphenyl-λ^5-phosphanylidene)4,5-dihydrochromeno [4,3-*d*][1,2,3]triazepin-6(1*H*)one **4**, 5-(phenylimino)-chromeno[4,3-*d*][1,2,3]triazepin-6-(5*H*)-one **5** and triphenylphosphine were isolated from this reaction as minor products. The reaction can be visualized as a 1,3-dipolar cycloaddition of the bifunctional 4-azido-chromen-2-one **1** to the nucleophilic phosphorane **2**, to form the triazoline **3**. Due to the reactivity of position 3 in the triazoline **3**, it undergoes ring expansion[29,30] to the corresponding phosphanylidene triazepine **4**. Triphenylphosphine is a good leaving group, so *Hoffmann degradation*[31] of compound **4** afforded the triazepine **5** and triphenylphosphine. Compounds **3–5** were identified by ^{1}H, ^{13}C, ^{31}P NMR, MS and IR spectra which are in agreement with the proposed structures. The ^{1}H NMR of the phosphanylidene triazolyl chromenone **3** showed the H-3 proton as a singlet at δ 6.6 ppm. The ^{13}C NMR of compound **3**, showed signals at δ 164.26 (C=N), 159.56 (C=O, lactone) and 153.88 ppm (C=P). In the ^{31}P NMR of **3** a signal was observed at δ 9.74 ppm which is in agreement with a phosphorane on a 5-membered ring system[12,32,33], and in the mass spectrum, the M^+ was found at m/z = 564 (3.13%). The IR spectrum of compound **3** showed bands at $\tilde{\nu}$ 1664 (C=O, lactone), 1598 (C=N), 1557 (C=P), 1494, 1488 cm^{-1} (P-aryl).[34] The most important features in the spectroscopic data of the phosphanylidene triazepine **4**, is that the ^{1}H NMR spectrum lack the presence of H-3 proton which appeared in the starting material **1** at δ 6.22 ppm, and showed the presence of NH group at δ 6.93, exchangeable with

D_2O. Moreover, a signal at δ 30.33 ppm was observed in its ^{31}P NMR spectrum. When 4-azidochromen-2-one **1** was reacted with (*N*-phenyliminovinylidene)triphenylphosphorane **2**, in dry boiling toluene for 8 h, N_2 gas was evolved, and 4-[2-(phenylimino)-3-(triphenyl-λ^5-phosphanylidene)aziridin-1-yl]-2*H*-chromen-2-one **6** and 3- (phenylimino)-2-(triphenyl-λ^5-phosphanylidene)-2,3-dihydrochromeno-[4,3-*d*]pyrrol-4(1*H*)-one **7** were isolated. The triazoline **3,** was formed first by an intermolecular 1,3-dipolar cycloaddition of the azide **1** to the phosphorane **2**, followed by nitrogen extrusion and ring contraction to the aziridine **6**. Formation of aziridines from triazolines is well-known.[35] Rearrangement of the aziridine **6**, resulted in the formation of the pyrrolone **7**[36] (scheme 1).

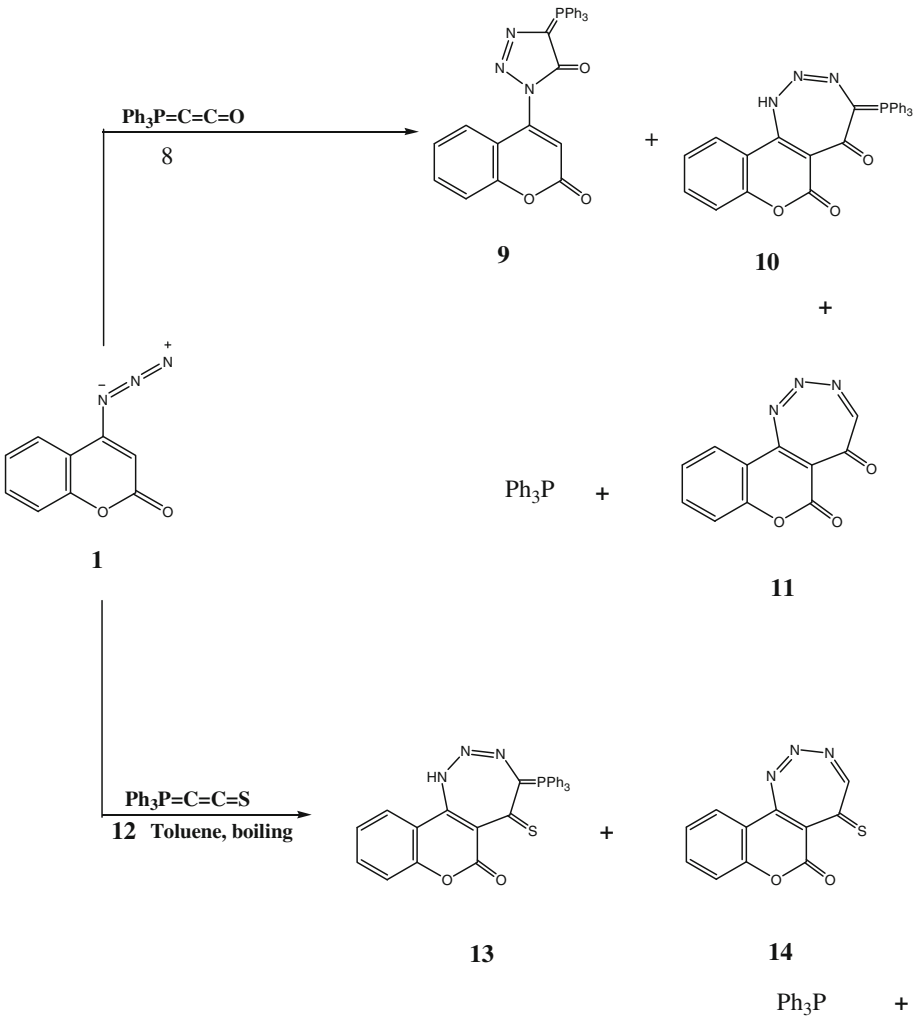
When the reaction of the azide **1** with (2-oxovinylidene)triphenylphosphorane **8** was carried out in boiling toluene for 8 h, three products were formed. Cycloaddition of the azide **1** and the phosphorane **8**, afforded 3-(2-oxo-2*H*-chromen-4-yl)-5- (triphenyl-λ^5-phosphanylidene)-3,5-dihydro-4*H*-1,2,-3-triazol-4-one **9**. Ring enlargement of **9** gave 4-(triphenyl-λ^5-phosphanylidene)-1,4-dihydrochromeno-[4,3-*d*]-[1,2,3]triazepine-5,6-dione **(10)**. Elimination of triphenylphosphine from **10** leads to chromeno[4,3-*d*] [1,2,3] triazepine-4,6-dione **11**.

In addition, the reaction of the azide **1** with (2-thioxovinylidene)triphenylphosphorane **12** was performed in boiling toluene for 10 h. The reaction proceeded with the formation of 5-thioxo-4-(triphenyl-λ^5-phosphanylidene)-4,5-dihydrochromeno[4,3-*d*][1,2,3] triazepin-6(1*H*)-one **13** and 5-thioxochromeno [4,3-*d*][1,2,3] triazepin-6(5*H*)-one **14**. Cycloaddtion of the azide **1** and the thiophosphacumulene **12** gave the phosphanylidene triazepine **13**, followed by elimination of triphenylphosphine in order to give the triazepinone **14** (scheme 2).

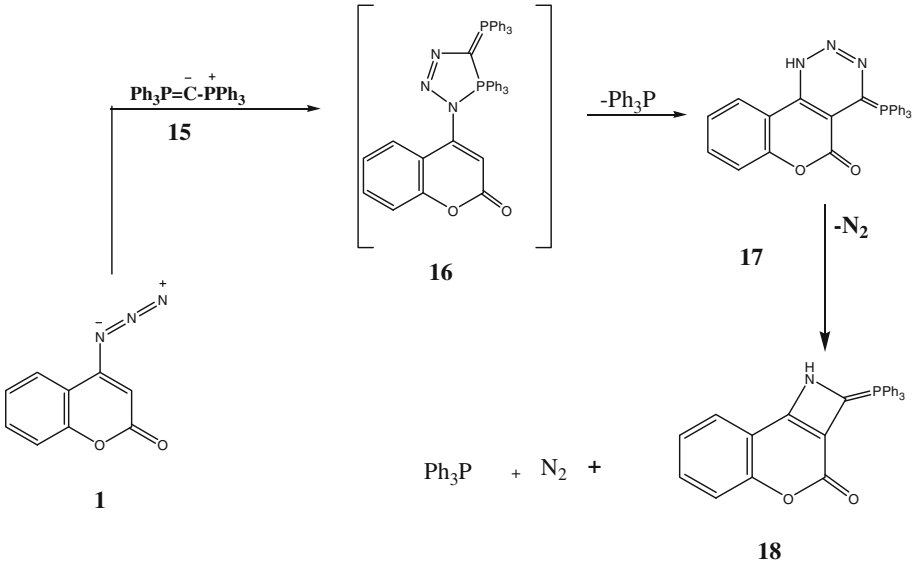
Furthermore, a novel approach to triazinone and azetone by an intermolecular [2+3]-addition of the allylic phosphonium ylide, namely, hexaphenylcarbodiphosphorane **15** to the azidocoumarine **1** was described, too. In this case, two products namely, 4-(1,-1,1-triphenyl-λ^5-phosphan-1-ylidene)-1,4-dihydro-5*H*-chromeno[4,3-*b*][1,2,3]triazin-5-one **17** and 2-(triphenyl-λ^5-phosphanylidene)-1,2-dihydro-3*H*-chromeno[4,3-*b*]azet-3-one **18** were isolated. The most important features in the spectroscopic data of the triazinone **17** and azetone **18**, is that they show only one signal in



Scheme 1. Formation of compounds **3–7**.



Scheme 2. Formation of compounds **9–14**.



Scheme 3. Formation of **17 and 18**.

their ^{31}P NMR at δ 29.79 and δ 13.45 ppm, respectively. The reaction proceeds by intermolecular cycloaddition, producing the intermediate phosphanylidene- triazaphosphol chromenone **16**. Since triphenylphosphine is a good leaving group, so fragmentation and rearrangement of **16** occurs with the formation of the triazinone **17** and triphenylphosphine. The latter triazinone **17** can loose nitrogen to give the azetone **18** (scheme 3). Generation of azetes from 1,2,3-triazine derivatives has been previously observed.[37,38] [Physical and spectroscopic data are provided in the experimental section].

3.2 *Cytotoxic assay*

Cancer diseases are a serious threat to health and development of mankind and the research for effective anticancer agents are important. 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[39] and for many years coumarin-related compounds have proved to be a significant therapeutic potential.[40] On the other hand, it is well-known that compounds containing triphenylphosphine moiety are part of a class of lipophilic cationic molecules that accumulate preferentially in mitochondria and inhibit the growth of human and rodent carcinoma cells *in vitro* and in animal models. It shows remarkable activity in a panel of cancer cell line as well as in mouse model of human breast cancer. Moreover, triphenylphosphine-treated mice showed significantly decreased tumour growth. No toxicities or organ damage were observed following triphenylphosphine treatment.[41–45] Based on these considerations 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) and human carcinoma(HEPG2) liver cell lines, in comparison with the activity of the utilized anticancer Doxorubicin (DXR) as a reference drug. However, 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[22] drugs with $IC_{50} < 30$ are active.

Table 1. Effect of the tested compounds on MCF-7 tumuor cell lines.

Compound	IC_{50} (μg /ml)
Doxorubicin (st.)	2.97
3	3.89
4	4.50
6	9.22
11	16.5

From table 1, it is evident that most of the tested compounds show antitumour activities with IC_{50} values ranging from 3.89 to 16.5 μg/mL and reaching strong correlation that of DXR (IC_{50}: 2.97 μg/mL) in the case of compound **3** (IC_{50}: 3.89 μg/mL). It is clear from the data that the comparison of the cytotoxicity against MCF-7 cells (figure 1) of prepared compounds has shown that the cells killing potency follows the order **3** > **4** > **6** > **11**. This may be attributable to presence of the phosphanylidene-triazolyl moiety in its mole-

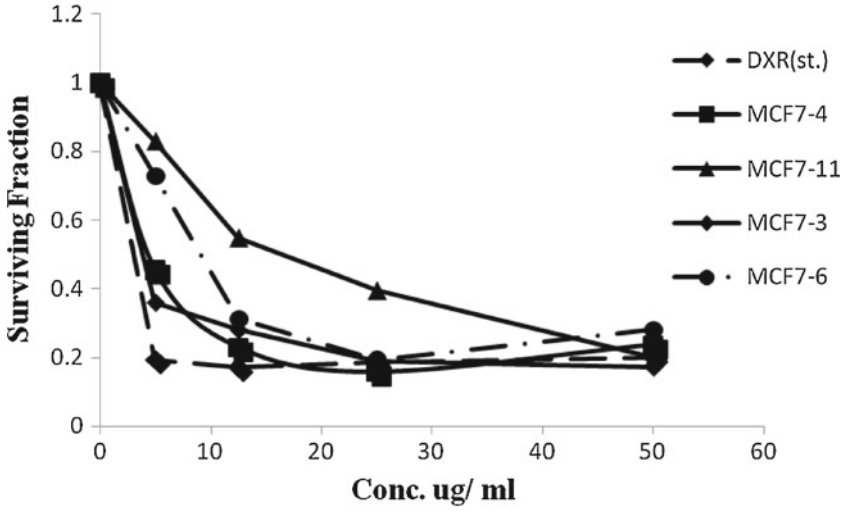


Figure 1. The surviving fraction as a function of drug concentration for the reference compared particularly with the other compounds (MCF-7).

Table 2. Effect of the tested compounds on HEPG2 tumour cell lines.

Compound	IC_{50} (μg/ml)
Doxorubicin (st.)	3.73
3	4.18
4	6.10
6	6.18
11	14.3

cular structure, the cytotoxic activity that may interact with DNA by intercalation and inhibition of macromolecular biosynthesis. This inhibits the progression of the 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,[46] suppressing agent, inhibit the formation and growth of tumours from initiated cells.[47]

On the other hand, substitution of the triazole moiety with the phosphanylidene-triazepinone moiety in one and the same structure (cf. **4**, IC_{50}: 4.5 μg/mL) also closely related the activity of the standard. The antitumour activity of the phosphanylidene-aziridine derivatives **6** recorded 9.22 μg/mL. On the other side, compound **11** marked 16.5 μg/mL due to the absence of phosphanylidene moiety in their molecular structures, 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 **3, 4, 6, 11** were screened for their *in vitro* cytotoxic and growth inhibitory activities towards liver carcinoma (HEPG2) cell line. The IC_{50} after short time exposure was 4.18, 6.10, 6.18 and 14.30 μg/mL for compounds **3, 4, 6** and **11**, respectively i.e., the cell killing potency follows the order, **3** > **4** > **6** > **11** (table 2).

Compound **3** was the best compound, exerting a significant cytotoxicity election (IC_{50}: 4.18 μg/mL) HEPG2 cells compared with Doxorubicin (IC_{50}: 3.73 μg/mL) (figure 2).

The results show that compounds **3** and **4** are better antitumour agents for MCF7 than HEPG2 and compound **11** and compound **6** may conduct to a promising pharmaco modulation for the discovery of new potential drugs.

4. Conclusion

The reaction of 4-azidochromen-2-one **(1)** with the phosphacumulene ylides **2, 8, 12** and the phosphallene ylide **15** represent an expeditious access to new heterocyclic triazoles, triazepines, aziridines, pyrroles and triazines containing a coumarin moiety. Cycloaddition reactions took place first to give triazoline adducts like compounds **3, 9** and triazaphosphol **16.** The triazoline rearranged to the triazepines **4**, **10**, and **13** accompanied by elimination of triphenylphosphine leading to the phosphorus-free triazepines **5, 11** and **14.** Moreover, aziridine **6** can be eventually produced via nitrogen extrusion from the triazolin **3**, followed by ring expansion to the pyrrolone **7**. The present evidence contradicts claim that a singlet nitrene should be an intermediating in the formation of the aziridine **6** and pyrrolone **7**. On the other hand, the reaction of the azidocoumarin **1** with the active phosphallene ylide **15** behaves differently and the triazinone **17** and azetone **18** were isolated. Moreover, the difference in the nucleophilic character and reactivity of the phosphacumulenes **2** > **8** > **12** was noticed.[48] Therefore, the

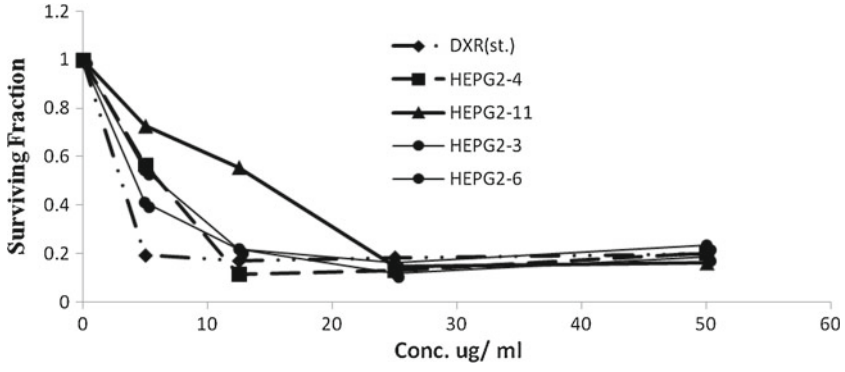


Figure 2. The surviving fraction as a function of drug concentration for the reference compared particularly with the other compounds (HEPG2).

reaction course between the active phosphacumulenes and phosphallene, is rather dependant on the nature of the reagents and the reaction conditions. The present study reports on simple and efficient approaches for the synthesis of new phosphorus derivatives of triazol-, triazepin-, aziridin-, and free phosphorus triazepin chromenone derivatives, especially azepines which rare class of highly strained compounds.[49,50]

Many of the new compounds revealed pronounced *in vitro* antitumour 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 phosphanylidene-triazolyl chromenone **3**. 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 **3** (IC_{50}: 4.18 μg/mL) was very close to that of the same reference drug (IC_{50}: 3.73 μg/mL) against human liver (HPG2) carcinoma cell lines. Moreover, the cytotoxic and growth inhibitory activity effect of mentioned compounds is more significant on liver carcinoma than breast carcinoma.

Acknowledgements

We thank the Cancer Biology Department, National Cancer Institute, Cairo University for the pharmacological evaluation.

References

1. Sivakumar K, Xie F, Cash B M, Long S, Barnhill H N and Wang Q 2004 *Org. Lett.* **6(24)** 4603
2. kostova I, Raleva S, Genova P and Argirova R 2006 *Bioorg. Chem. Appl.* 1
3. Sukdolok S, Solujic S, Vukovic N, Manojlovic N and Kristic L 2004 *J. Serb. Chem. Soc.* **69(5)** 319
4. Al-Soud Y A, Al-Masoudi I A, Saeed B, Beifub H and Al-Masoudi N A 2006 *Chem. Heterocycl. Commun.* **42(5)** 583
5. Jung J-C and Park O-S 2009 *Molecules* **14** 4090.
6. Tao L, Zhang L L, Shen S J and Han X P 2001 *Chin. Chem. Lett.* **12(9)** 763
7. Lutz J F and Zarafshani Z 2008 *Adv. Drug Delivery Rev.* **60** 958
8. Shafran E A, Bakulev V A, Rozin Yu A and Shafran Yu M 2008 *Chem. Heterocyclic Comp.* **44(9)** 1040
9. Habib P M, Raju B R, Kavala V, Kuo C W and Yao C F 2009 *Tetrahedron* **65** 5799
10. Bestmann H J and Schmid G 1975 Ger. Offen. 2409356, 1976 *Chem. Abstr.* **84** 31239
11. Stadlbauer W 1986 *Monatsh. Chem.* **117(11)** 1305
12. Albright T A, Freeman W J and Schweizer E E 1975 *J. Am. Chem. Soc.* **97** 2942
13. Schmidbauer H, Schier A, Milewski-Mahrla B and Schubert U 1982 *Chem. Ber.* **115** 722
14. Bestmann H J and Sandmeier D 1975 Angew Chem. Internt Edn 14: 634, 1976 *Chem. Abstr.* **84** 5070s.
15. Verma S, Athale M and Bokodia M M 1981 *Indian J Chem.* **20B** 1096
16. Bestmann H J, Schmid G, Sandmeier D and Geismann Ch 1980 *Tetrahedron Lett.* 2401.
17. Bestmann H J and Kloeters W 1977 *Tetrahedron Lett.* **34** 79
18. Johnson A Wm 1966 *Ylides chemistry in organic chemistry. A series of monographs*, A T Blomquist (ed) London: Academic Press
19. Bestmann H J and Zimmermann R 1972 *Organic phosphorus compounds* Vol. 3, G M Kosolapoff, L Maier (eds), New York: John Wiley and Sons, Inc. Wiley 1973 *Chem. Abstr.* **78** 84461x
20. Bestmann H J, Schmid G, Sandmeier D and Kisielowski L 1977 *Angew. Chem.* **89** 275
21. Grim S O, Mc Farlane W and Marks T J 1967 *J. Chem. Commun.* 1191
22. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren J T, Bokesch H, Kenny S and Byod M R 1990 *J. Natl. Cancer Inst.* **82** 1107
23. Maigali S S, Abdel-Malek H A and Soliman F M 2010 *Egypt J. Chem.* **53(2)** 315
24. Maigali S S, Said M M, Abd-El-Maksoud M A and Soliman F M 2008 *Monatsh. Chem.* **139** 495
25. Said M M, Maigali S S, Abd-El-Maksoud, M A and Soliman F M 2008 *Monatsh. Chem.* **139** 1299
26. Maigali S S, Abd-El-Maksoud M A and Soliman F M 2011 *J. Chem. Pharm. Res.* **3(4)** 713
27. Soliman F M, Abd-Ella I, Maigali S S and Abd-El-Naim G 2009 *J. Chem. Res.* 277
28. Maigali S S, Said M M, Abd-El-Maksoud M A and Soliman F M 2011 *Arch. Pharm. Chem.* **344(7)** 442
29. Iddon B, Cohn O M, Scriven V, Suschitzky H and Gallagher P T 2003 *Angew. Chem. Inter.* **18(12)** 900
30. Laatsch H, Erns B P and Hoffmann D 2006 *Liebig Ann.* **1995(10)** 1773
31. Nelson N and Levy R B 1979 *J. Catal.* **58** 485
32. Birum G H and Matthews C N 1967 *Chem. Commun.* 137
33. Schmidpeter A, Gebler W, Zwaschka F and Sheldrich W S 1980 *Angew. Chem.* **92** 767 1980 *Angew. Chem. Int. Ed. Engl.* **19** 722
34. Williams D H and Fleming I 1987 *Spectroscopic methods in organic chemistry*; Berkshire, United Kingdom: Mc Graw-Hill Book Company: Maidenhead p. 55
35. D'hooghe M, Kenis S, Vervisch K, Lategan C, Smith P J, Chibale K and De Kimpe N 2011 *Eur. J. Med. Chem.* **46(2)**, 579
36. Hu X E 2004 *Tetrahedron* **60** 2701
37. Chambers R D, Tamura M, Shepherd T and Ludman C J 1987 *J. Chem. Soc. Chem. Commun.* 1699
38. Soliman F M, Said M M and Maigali S S 2005 *Heteroatom. Chem.* **16(6)** 476
39. Surth Y J 2003 *Nat. Rev. Cancer* **3** 768
40. Patela M M, Malia D M and Patel S K 2010 *Bioorg. Med. Chem. Lett.* **20(21)** 6324
41. Manetta A, Gamboa G, Nasseri A, Podnos Y D, Emma D, Dorion G, Rawlings L, Carpenter P M, Bustamante

A, Patel J and Rideout D 1996 *Gynencol. Oncol.* **60(2)** 203
42. Melissa M, Divya P, Yumna S, Laleh T, Jinxia D and Nouri N 2010 *Plose ONE* **5(10)** 1
43. Modica-Napolitano J and Aprille J 2001 *Adv. Drug Deliv. Rev.* **49** 63
44. Wang J, Yang C, Kim Y, Sreerama S and Cao Q 2007 *J. Med. Chem.* **50** 5057
45. Kim Y, Yang C, Wang J, Wang L and Li Z 2008 *J. Med. Chem.* **51** 2971
46. Fornari F A, Randolph J K, Yalowich J C, Ritke M K and Gewirtz D A 1994 *Mol. Pharmcol.* **45(4)** 649
47. Hidalgo N, Almirante B and Pahissa A 2009 *Clin. Infect. Dis.* **48** 1166
48. Bestmann H J 1977 *Angew. Chem.* **89(6)** 361, 1977 *Angew. Chem. Int. Ed. Engl.* **16** 349
49. Karney W L and Borden W T 1997 *J. Am. Chem. Soc.* **119** 5061
50. Wenk H H and Sunder W 2002 *Angew. Chem.* **114** 2873 2002 *Angew. Chem. Int. Ed.* **41** 2742