Synthesis of Novel Benzosuberone Derivatives using Organophosphorus Reagents and their Antitumor Activities

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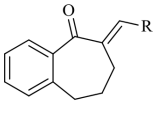
2-Arylidenebenzosuberones react with a Wittig–Horner reagent in the presence of sodium hydride as a base to give the novel dimethyl (4-(4-methoxyphenyl)-2-oxa-2,3,4,5,6,7-hexahydrobenzo-[6,7]cyclohepta[1,2-*b*]pyran-3-yl)phosphonate. On the other hand, 6,7-dihydrobenzo[6,7]cyclohepta-[1,2-*b*]pyran-2(5*H*)-ones were isolated from the reaction of 2-arylidenebenzosuberones with Wittig–Horner reagents using alcoholic sodium alkoxide. The reaction of 2-arylidenebenzosuberones with trialkyl phosphites affords the alkyl phosphonate derivatives. Tris(dialkylamino)phosphines react with 2-arylidenebenzosuberones to give the oxaphospholanoxide products. 2-Arylidenebenzosuberones react with Lawesson's reagent to yield the corresponding dimers. Some of the prepared products were screened for antitumor activity.

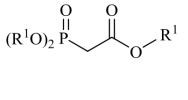
Key words: Benzosuberone Derivatives, Organophosphorus Reagents, Antitumor Activity

Introduction

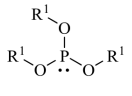
Benzosuberone derivatives are known as cytotoxic and antitumor agents against L1210 murine leukemia and HT2g cell lines [1 – 3], as potent inhibitors of tubulin polymerization [4] and as blood platelet aggregation inhibitors [5]. This together with our interest in organophosphorus chemistry [6 – 11] has encouraged the synthesis of new organophosphorus compounds incorporating such structural units that may possibly lead to further biological activity. The present study deals with the reaction of 6-arylidene-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-5-ones **1** with Wittig-Horner reagents **2**, trialkyl phosphites **3**, tris(dialkylamino)-phosphines **4**, and Lawesson's reagent **5** (Scheme 1).



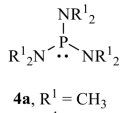
1a, R = C_6H_4-OCH_3
b, R = C_6H_5



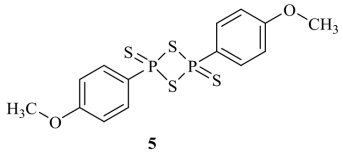
2a, R^1 = CH_3
b, R^1 = C_2H_5



3a, R^1 = CH_3
b, R^1 = CH $(CH_3)_2$



4a, R^1 = CH_3
b, R^1 = C_2H_5



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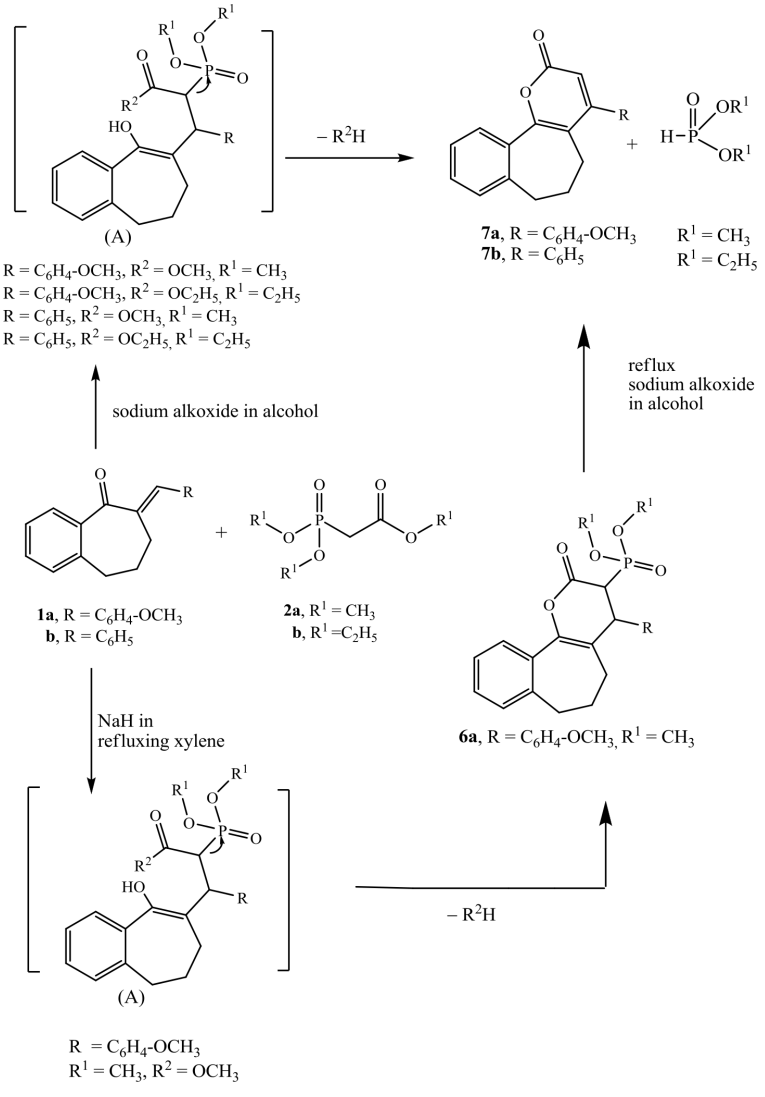
Scheme 1.

Results and Discussion

Chemistry

When benzosuberone **1a** was treated with one equivalent of trimethyl phosphonoacetate **2a** in dry xylene in the presence of sodium hydride at reflux temperature for 10 h, compound **6a** was obtained (75 % yield) (Scheme 2). Compound **6a** was formulated as lactone **6a**. Elemental and mass spectral analysis of **6a** led to an empirical formula $C_{23}H_{25}O_6P$. The IR spectrum of **6a** in KBr revealed of strong absorption bands at 1230 cm^{-1} (P=O, bonded) [12] and at 1050 cm^{-1} (P-O-C) [12]. Moreover, the IR spectrum showed a carbonyl absorption band at 1726 cm^{-1} (CO, lactone). The spectrum of **6a** lacked the carbonyl absorption which is recorded [13] for **1a** at 1660 cm^{-1}. The ^{1}H NMR spectrum of **6a** gave signals at δ = 1.23 ppm (m, 2H, CH_2), 1.79 ppm (m, 2H, CH_2), 2.6 ppm (m, 2H, CH_2). The two *ortho*-methine protons appeared as a pair of doublets at 2.7 ppm (dd, $^2J_{HP}$ = 15 Hz, J_{HH} = 7.5 Hz), and 5.6 ppm (dd, $^3J_{HP}$ = 10 Hz,J_{HH} = 7.5 Hz). Moreover, the ^{1}H NMR of **6a** exhibited signals at 3.4 ppm (s, 3H, C_6H_4-OCH_3) and 3.5, 3.6 ppm (2d, 6H, $^3J_{HP}$ = 11 Hz, $PO(OCH_3)_2$) for the two methoxy groups attached to the phosphorus and a multiplet at 6.9 – 7.1 ppm (8H, aromatic). The ^{31}P NMR measurement of **6a** supported the phosphonate structure. It exhibited a sharp signal at δ =

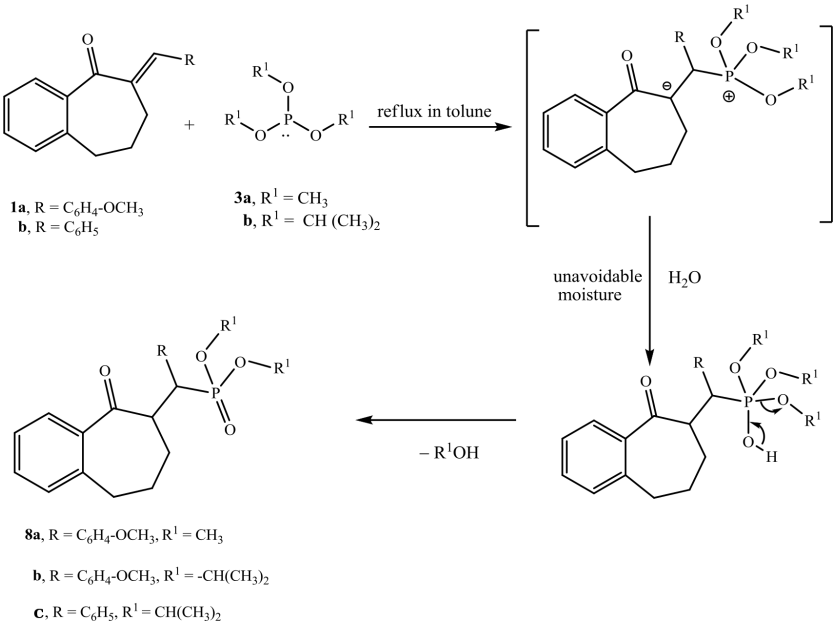


Scheme 2.

23.03 ppm [14]. The ^{13}C NMR spectrum of **6a** added good support for the proposed structure and revealed the methine proton attached to the phosphorus as a doublet at 33.4 ppm with $^{1}J_{CP}$ = 168.8 Hz.

When **1a** was allowed to react with one equivalent of trimethyl phosphonoacetate **2a**, in a methanolic sodium methoxide solution, adduct **7a** was isolated in 80 % yield (Scheme 2). The structure **7a** was established from its elemental analysis and its IR, ^{1}H, ^{13}C NMR and mass spectral data (*cf.* Experimental Section). Similarly, when **1b** was allowed to react with trimethyl phosphoacetate **2a** in a methanolic sodium methoxide solution, **7b** was obtained (78 % yield) (Scheme 2, *cf.* Experimental Section). When 2-benzylidene-1-benzosuberones **1a** and **1b** were allowed to react with phosphonate **2b** in an alcoholic sodium ethoxide solution, pyranones **7a** and **7b** were also isolated (Scheme 2).

A possible explanation for the reaction course of **1a** with Wittig–Horner reagent **2a** in the presence of sodium hydride as base is shown in Scheme 2. Initial attack of trimethyl phosphonacetatete **2a** on the most reactive center of **1a** gave the intermediate A. Elimination of one molecule of alcohol followed by cyclization result in the formation of the phosphonate derivative **6a**. Compounds **7a**,**b** presumably are also formed *via* intermediate A. Under the influence of the base present in the reaction medium, elimination of dialkyl

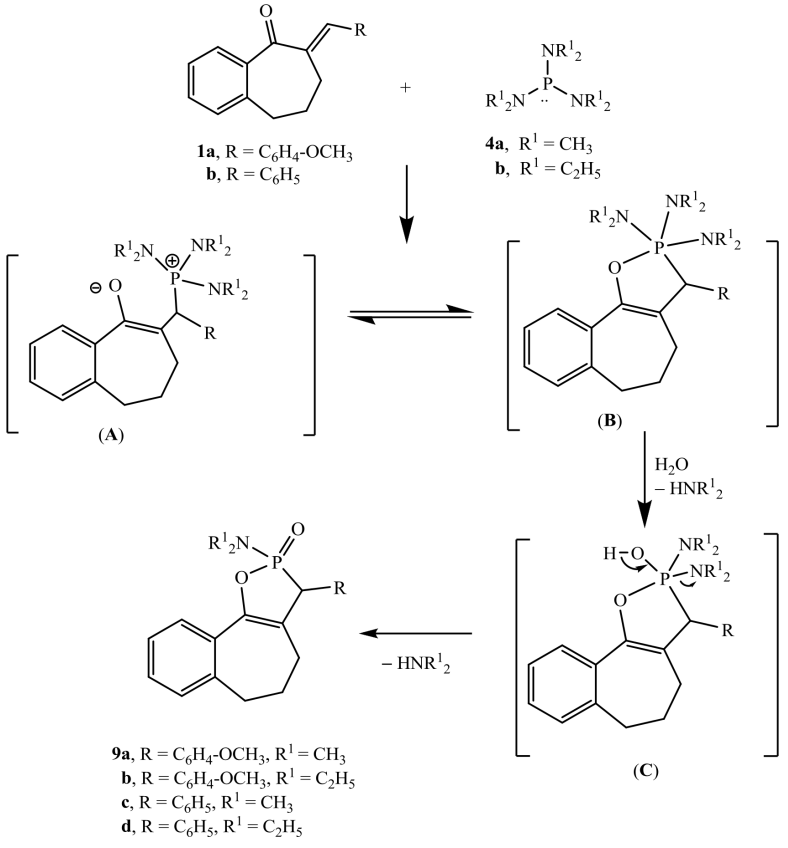


Scheme 3.

phosphite together with the loss of one molecule of alcohol and cyclization after a suitable proton transfer gives the final products **7a** and/or **7b** (Scheme 2). The dialkyl phosphite was detected in the water layer by the development of a violet color on addition of 3,5-dinitrobenzoic acid [15]. Moreover, when **6a** was allowed to react with sodium methoxide in an alcoholic solution under reflux for 6 h, compound **7a** was obtained (60 % yield). The dialkyl phosphite was again detected in the water layer.

Furthermore, this study was extended to include the behavior of benzosuberone **1a** towards trimethyl phosphite **3a** to determine the preferential site of attack. We have found that the reaction of **1a** with **3a** was successfully completed (TLC) by using an excess of phosphite as solvent and heating the mixture for 4 h at 105 °C. Separation of the product mixture yielded phosphonate **8a** (75 % yield) (Scheme 3). Compound **8a** was chromatographically pure and shows a sharp melting point. The assigned methylphosphonate structure **8a** is based on correct microanalysis and IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectral data.

The IR spectrum of **8a** revealed the presence of the carbonyl absorption band at 1660 cm^{-1}. In addition, it exhibited intense bands corresponding to the P=O (1256 cm^{-1}) and P-O-alkyl (1082 cm^{-1}) stretching vibrations [12]. The ^{1}H NMR spectrum (in DMSO) of the adduct showed signals at δ = 3.8 ppm (s, 3H, H_3CO-C_6H_4), 3.41, 3.46 (2d, $^3J_{HP}$ = 11.2 Hz, 6H, $(H_3CO)_2P$), 3.3 (dd, $^2J_{HP}$ = 18.7 Hz, J_{HH} = 7.5 Hz, 1H, CH-P), 3.5 (dd, $^3J_{HP}$ = 10.7 Hz, J_{HH} = 7.5 Hz, C*H*-CH-P), 1.7, 2.5, 2.7 (m, 6H, $3CH_2$) and 6.7 – 7.3 ppm (m, 8H, aromatic). The ^{31}P NMR measurement of **8a** supported the phosphonate structure. It exhibited a sharp signal at δ = 23.10 ppm. The ^{13}C NMR spectrum of **8a** gave signals at δ = 25.4, 29.3, 32.6 ($3CH_2$), 31.6 (CH-P=O, $^1J_{CP}$ = 100 Hz), 39.2 (*C*H-C=O, $^2J_{CP}$ = 21.72 Hz), 52.1 (C_6H_4-O*C*H_3), 54.6 ($PO(OCH_3)_2$), 157 (*C*-OCH_3), and 205.6 ppm (C=O). The mass spectrum of **8a** showed the molecular ion peak at m/z = 386 (30 %), and the peak for $[M–P(O)(OCH_3)_2]^+$ at m/z = 278 (90 %). Similarly, 2-benzylidene-1-benzosuberone (**1a**) reacts with an excess of triisopropyl phosphite **3b** as solvent to give **8b** (70 % yield). The structure of **8b** is derived from its spectral data (*cf.* Experimental Section). When benzosuberone **1b** reacted with an excess of (**3b**) as solvent, product **8c** was obtained (65 % yield). Methyl phosphonate **8c** has been identified on the basis of its elemental analysis and its IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectral data (Scheme 3, *cf.* Experimental Section). Worthy to mention is that only one isomer of phosphonate **6a** and compound **8** were isolated, which are assumed to have a *cis* configuration. The assigned *cis* configuration for these products, although not established with certainty, is supported by an inspection of Newman projections [16] as well as by the ^{1}H NMR chemical shifts and coupling constants of the two *ortho* me-

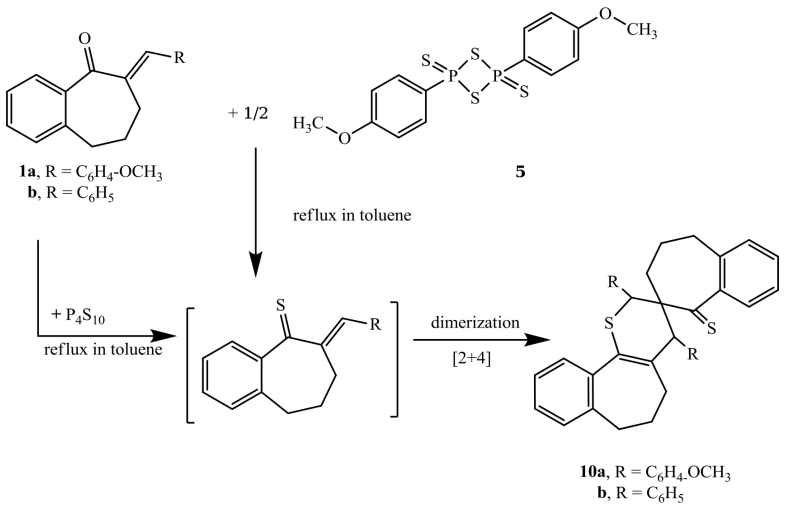


Scheme 4.

thine protons attached to phosphorus. The observed coupling constants of ($J_{H^aH^b} = J_{H^bH^a}$, *ca.* 6–7.5 Hz) indicate a *cis* configuration, rather than a *trans* configuration which would give rise to larger coupling constants (9–15 Hz).

We have found that **1a** reacted with tris(dimethylamino)phosphine (**4a**) in refluxing toluene to give a chromatographically pure adduct formulated as **9a** (Scheme 4). The structure elucidation of product **9a** is based on the following evidences. Elemental analysis and molecular weight determination (MS) of **9a** supported the molecular formula $C_{21}H_{24}NO_3P$ (369.39). The IR spectrum, in KBr, exhibited an intense band at 1240 cm^{-1} corresponding to the P=O absorption, and two bands at 1320 cm^{-1} and 860 cm^{-1} due to the absorption of P-N$(CH_3)_2$ [17]. Moreover, the IR spectrum of the oxaphosphole-2-oxide **9a** revealed the absence of the carbonyl absorption which is recorded for **1a** at 1660 cm^{-1}. The ^{1}H NMR spectrum of **9a** showed signals at δ = 3.5 (d, 1H, $^2J_{HP}$ = 18.5 Hz) for the methine proton attached to the phosphorus, 2.8 (d, 6H, $^3J_{HP}$ = 11.10 Hz) for the dimethyl amino group, a singlet at 3.8 (3H, C_6H_4-OCH_3), and multiplets at 1.8, 2.3, 2.8 ppm ($3CH_2$). The aromatic protons appeared as multiplets at δ = 6.9–7.9 ppm (8H). The ^{31}P NMR spectrum of **9a** gave one signal at δ = 43.7 ppm that supported the cyclic oxaphospholanoxide [14, 18]. Similarly, tris(diethylamino)phosphine (**4b**) reacts with **1a** to give **9b** (70 % yield). When 2-benzylidene-1-benzosuberone **1b** reacted with **4a** and **4b**, the corresponding oxaphospholoxide derivatives **9c** and **9d** were obtained (Scheme 4).

The structure of the new products **9b–d** was deduced from their spectral data (*cf.* Experimental Section). These compounds were formed presumably through nucleophilic attack of the phosphite-phosphorus on the most reactive center of **1** leading to the dipolar adduct **A**, which undergoes ring closure giving structure **B**. The latter, due to its structural features, could transform into the most stable form **9** through rapid hydrolysis (by the presence of unavoidable moisture) to give intermediate **C**, which undergoes further decomposition yielding com-



Scheme 5.

pound **9** [8, 18, 19]. Similar results have been reported previously [8, 19].

Furthermore, this study was extended to include the behavior of 2-benzyliene-1-benzosuberones **1a**, **b** toward Lawesson's reagent (**5**). We have found that **1a** reacted with half a molar equivalent of **5** in refluxing toluene for 1 h to give the dimeric product **10a** (65 % yield). Similarly, when **1b** reacted with half an equivalent of **5** in refluxing toluene, the dimeric product **10b** was obtained (60 % yield). The structures of **10a** and **10b** were confirmed by analytical and spectral data (Scheme 5, *cf.* Experimental Section).

The dimeric compounds **10a**, **b** were formed through the straightforward thionation of carbonyl group of **1a**, **b** to yield the thione monomer which immediately underwent cyclization with another thione monomer [2+4] to yield the corresponding thione dimers **10a** and **10b** (Scheme 5) [20]. It is worthy to note that when **1a** and **1b** react with phosphorus pentasulfide (P_4S_{10}) in refluxing toluene, the dimeric compounds **10a** and **10b** were also obtained (Scheme 5).

Pharmacological evaluation

Cancer diseases are a serious threat to health and development of mankind, and searching for effective anti-cancer agents remain actual. 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 [21], and benzosuberone derivatives have proved to have a significant therapeutic potential [1 – 3]. Based on these considerations, six of the newly synthesized compounds were screened for their *in vitro* cytotoxic and growth inhibitory activities against human breast adenocarcinoma cells (MCF 7) and/or human hepatocellular carcinoma (HEPG2). The cytotoxicity of the tested extracts was measured against MCF 7 cells and/or HEPG2 cells using the MTT Cell Viability Assay [22]. According to the American National Cancer Institute guidelines [23] drugs with $IC_{50} < 30$ are active. The cytotoxicity of the extracts was tested in the Cancer Biology laboratory, Center of Excellence for Advanced Sciences, National Research Center.

Treatment of MCF 7 cells with sample **7a** led to a high inhibition in the cell proliferation as concluded by the low IC_{50} value of 13.32 μg mL^{-1} (Fig. 1). On the other side, the other tested samples **6a**, **8a**, **9b**,

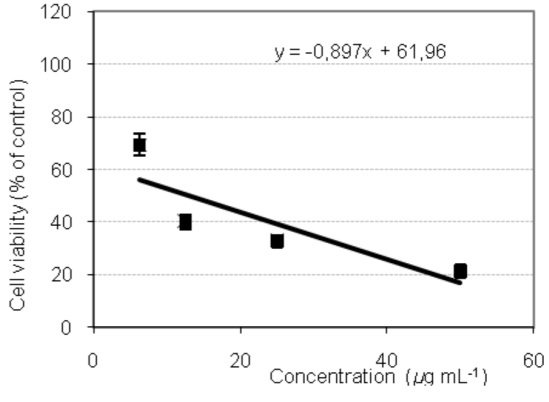


Fig. 1. Effect of compound **7a** on MCF 7 tumor cell lines (breast cancer).

Table 1. Effect of the tested compounds on HEPG2 tumor cell lines (liver cancer).

Compound	IC_{50} ($\mu g\,mL^{-1}$)
Paclitaxel	0.517
7a	27.89
9a	28.56
10b	29.27

10a, and **10b** revealed a non-cytotoxic effect against of MCF 7 cells. Paclitaxel, a known anti-cancer drug, was used as a positive control, and its IC_{50} was 0.452 $\mu g\,mL^{-1}$ against MCF 7 cells.

As shown in Table 1, the treatment of HEPG2 cells with **7a**, **9a**, and **10b** led to a high inhibition in the cell proliferation as concluded by the low IC_{50} values of 27.89, 28.56, and 29.27 $\mu g\,mL^{-1}$, respectively, whereas the other samples **6a**, **8a** and **10a** revealed a non-cytotoxic effect against HEPG2 cells. Paclitaxel, a known anti-cancer drug, was used as a positive control and its IC_{50} was 0.517 $\mu g\,mL^{-1}$ against HEPG2 cells.

Conclusion

The reaction of 2-benzylidene-1-benzosuberones **1** with Wittig–Horner reagents, trialkyl phosphites, tris(dialkylamino)phosphines and Lawesson's reagent leads to different products, depending on the reaction conditions as well as on the stability of the addition products. As a result pyran phosphonates, cycloheptapyranone, phosphonates benzocycloheptenes and oxaphospholoxide derivatives with anticipated biological activity could be obtained.

Experimental Section

Melting points were determined in open glass capillaries using an Electrothermal IA 9100 series digital melting point apparatus (Electorthermal, Essex, UK), and IR spectra were measured as KBr pellets with a Perkin-Elmer spectrophotometer model 157. The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or [D_6]DMSO as solvents on a Joel 500 (500/125 MHz) spectrometer. The chemical shifts δ (in ppm) are given relative to TMS as internal reference. The ^{31}P NMR spectra were taken with a Varian CFT-20 spectrometer (*vs.* external 85 % H_3PO_4 as standard). The mass spectra were recorded at 70 eV with a Kratos MS equipment or a Varian MAT311A spectrometer. Elemental analyses were performed using an Elementar Vario E1 instrument. The reported yields correspond to pure isolated materials obtained by column chromatography on silica gel 60 (Merck). 2-benzylidene-1-benzosuberones **1a** and **1b** were prepared according to a reported method [24].

*Reaction of trimethyl phosphonoacetate **2a** with 6-(4-methoxybenzylidene)-6,7,8,9-tetrahydro-5H-benzocyclohepten-5-one **1a** in the presence of sodium hydride*

Trimethyl phosphonoacetate **2a** (0.18 g, 1 mmol) was dissolved in dry xylene (25 mL), and sodium hydride (0.024 g, 1 mmol) was added carefully with stirring. Then the arylidene suberone **1a** (0.27 g, 1 mmol) was added, and the mixture was refluxed for 10 h. After evaporation of the volatile material under reduced pressure, the residue was subjected to silica gel column chromatography to give compound **6a**.

*Dimethyl-(4-(4-methoxyphenyl)-2-oxo-2,3,4,5,6,7-hexahydrobenzo[6,7]cyclohepta[1,2-b]pyran-3-yl)phosphonate (**6a**)*

Eluent: petroleum ether-ethyl acetate (50/50, *v*/*v*). Product **6a** was separated as colorless crystals, yield 75 %. – M. p. 216 – 217 °C. – IR (KBr): ν = 1050 (P-O-C), 1230 (P=O, bonded), 1726 (C=O, lactone) cm^{-1}. – ^1H NMR (500.14 MHz, $CDCl_3$): δ = 1.23 (m, 2 H, CH_2), 1.79 (m, 2 H, CH_2), 2.6 (m, 2 H, CH_2), 2.7 (dd, $^2J_{HP}$ = 15 Hz, J_{HH} = 7.5 Hz, CH-P=O), 3.4 (s, 3 H, OCH_3-C_6H_4), 3.5, 3.6 (2 d, 6 H, $^3J_{HP}$ = 11 Hz, P=O (OCH_3)$_2$), 5.6 (dd, $^3J_{HP}$ = 10 Hz, J_{HH} = 7.5 Hz , C*H*-C_6H_4-OCH_3), 6.9 – 7.1 (m, 8H, H_{arom}). – ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 20.0, 22.01, 32.12 (3 C, 3 CH_2), 21.4 (*C*H-C_6H_4-OCH_3, $^2J_{CP}$ = 17.5 Hz), 33.4 ($^1J_{CP}$ = 168.8 Hz, CH-P=O), 50.8 (C_6H_4-O*C*H_3), 52.5 (2 C, P=O(O*C*H_3)$_2$), 125.4 (*C*=C-O), 125.8 – 137.0 (aromatic C-H), 140.9 (C=*C*-O), 164.0 (*C*-OCH_3), 165.0 (C=O). – ^{31}P NMR: δ = 23.03. – MS (EI, 70 eV):m/z = 428 $[M]^+$. – Anal. for $C_{23}H_{25}O_6P$ (428.41): calcd. C 64.48, H 5.88, P 7.23; found C 64.53, H 6.1, P 7.30.

*Reaction of trimethyl phosphonoacetate **2a** with 6-(4-methoxybenzylidene)-6,7,8,9-tetrahydro-5H-benzocyclohepten-5-one **1a** in the presence of sodium methoxide*

A solution of sodium methoxide (0.054 g, 1 mmol) in absolute methanol (30 mL) was treated with an equimolar amount of trimethyl phosphonoacetate **2a** (0.18 g, 1 mmol). Then 2-arylidenebenzosuberone **1a** (0.27 g, 1 mmol) was added, and the reaction mixture was refluxed for 12 h (TLC). The mixture was poured on a small amount of water, extracted with ethyl acetate, and dried, and the extracts were evaporated under reduced pressure. The residue was subjected to silica gel column chromatography to give compound **7a**. When compound **6a** was reacted with sodium methoxide in alcoholic solution under reflux for 6 h, compound **7a** was obtained in 60 % yield.

*4-(4-Methoxyphenyl)-6,7-dihydrobenzo[6,7]cyclohepta[1,2-b]pyran-2(5H)-one (**7a**)*

Eluent: petroleum ether-ethyl acetate (95/5, *v*/*v*). Product **7a** was obtained as yellow crystals, yield 80 %. – M. p.

148 – 149 °C. – IR (KBr):ν = 1627 (C=O) cm^{-1}. – ^1H NMR (500.14 MHz, $CDCl_3$): δ = 2.0, 2.5, 2.8 (m, 6 H, 3CH_2), 3.8 (s, 3 H, OCH_3), 7.2 (s, 1 H, CH), 7.4 – 7.8 (m, 8H, H_{arom}). – ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 24.7, 32.4, 38.7 (3 CH_2), 55.0 (OCH_3), 102.0 (*C*H-C=O), 114.0 – 130.0 (aromatic C-H), 136.0 (O-*C*=C), 158.0 (C=*C*-C_6H_4-OCH_3), 160.0 (*C*-OCH_3), 161.0 (C=O). – MS (EI, 70 eV): m/z = 303 $[M–16]^+$. – Anal. for $C_{21}H_{18}O_3$(318.37): calcd. C 79.22, H 5.70; found C 79.49, H 5.92.

*Reaction of trimethyl phosphonoacetate **2a** with 6-benzylidene-6,7,8,9-tetrahydro-5H-benzocyclohepten-5-one **1b** in the presence of sodium methoxide*

A solution of sodium methoxide (1 mmol) in absolute methanol (30 mL) was treated with an equimolar amount of trimethyl phosphonoacetate **2a** (0.18 g, 1 mmol). Then **1b** (0.24 g, 1 mmol) was added, and the resulting reaction mixture was refluxed for 10 h (TLC). Then it was poured on a small amount of water, extracted with ethyl acetate, and dried, and the extracts were evaporated under reduced pressure. The residue was subjected to silica gel column chromatography to afford compound **7b**.

*4-Phenyl-6,7-dihydrobenzo[6,7]cyclohepta[1,2-b]pyran-2(5H)-one (**7b**)*

Eluent: petroleum ether-ethyl acetate (95/5, v/v). Product **7b** was obtained as pale-yellow crystals, yield 78 %. – M. p. 136 – 137 °C. – IR (KBr):ν = 1620 (C=O) cm^{-1}. – ^1H NMR (500.14 MHz, $CDCl_3$): δ = 2.0, 2.6, 2.8 (m, 6 H, 3 CH_2), 7.2 (s, 1 H, CH), 7.3 – 7.5 (m, 9 H_{arom}). – ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 24.7, 33.4, 39.1 (3 CH_2), 102.1 (*C*H-C=O), 126.0 – 137.0 (aromatic C-H), 137.13 (O-*C*=C), 159.0 (C=*C*-C_6H_5), 162.0 (C=O). – MS (EI, 70 eV): m/z = 273 $[M–16]^+$. – Anal. for $C_{20}H_{16}O_2$(288.34): calcd. C 83.31, H 5.59; found C 83.69, H 5.97.

*General procedure for the reaction of triethyl phosphonoacetate (**2b**) with **1a,1b***

Sodium ethoxide (0.068 g, 1 mmol) in absolute alcohol (30 mL) was added to a solution of an equimolar amount of triethyl phosphonoacetate (**2b**) (0.22 g, 1 mmol), and then **1a** or **1b** (1 mmol) was added. The resulting mixture was refluxed for 4 h, then poured on a small amount of water, extracted with ethyl acetate, and dried, and the extracts were evaporated under reduced pressure. The residue was subjected to silica gel column chromatography. Elution of the column with petroleum ether (60 – 80 °C)-ethyl acetate (95/5, v/v) to give products **7a**, **7b** (mixed melting points and comparative IR spectra with authentic samples).

*Reaction of trimethyl phosphite (**3a**) with **1a***

Excess trimethyl phosphite (**3a**) was added to **1a** (0.27 g, 1 mmol) without solvent, and the reaction mixture was refluxed for 4 h at 105 °C (TLC). After evaporation of the volatile material under reduced pressure, the residue was washed several times with petroleum ether (60 – 80 °C) to give compound **8a**.

*Dimethyl-(6,7,8,9-tetrahydro-5-oxo-5H-benzocyclohepten-6-yl)(4-methoxyphenyl)methyl-phosphonate (**8a**)*

Solvent of crystallization: petroleum ether-ethyl acetate. Product **8a** was obtained as colorless crystals, yield 75 %. – M. p. 202 – 203 °C. – IR (KBr):ν = 1082 (P-O-alkyl), 1256 (P=O), 1660 (C=O) cm^{-1}. – ^1H NMR (500.14 MHz, DMSO): δ = 1.7, 2.5, 2.7 (m, 6 H, 3 CH_2), 3.3 (dd, $^2J_{HP}$ = 18.7 Hz, J_{HH} = 7.5 Hz, 1 H, CH-P), 3.4, 3.46 (2 d, $^3J_{HP}$ = 11.2 Hz, 6 H, $PO(OCH_3)_2$, 3.5 (dd, $^3J_{HP}$ = 10.7 Hz, J_{HH} = 7.5 Hz, C*H*-CH-P), 3.8 (s, 3 H, OCH_3), 6.7 – 7.3 (m, 8H, H_{arom}). – ^{13}C NMR (125.76 MHz, DMSO): δ = 25.4, 29.3, 32.6 (3 CH_2), 31.6 (CH-P=O, $^1J_{CP}$= 100 Hz), 39.2 (*C*H-C=O, $^2J_{CP}$ =21.72 Hz), 52.1 (C_6H_4-OCH_3), 54.6 (PO-$(OCH_3)_2$), 126.0 – 130.0 (aromatic C-H), 157 (*C*-OCH_3), 205.6 (C=O). – ^{31}P NMR: δ = 23.1. – MS (EI, 70 eV): m/z = 386 $[M–2]^+$. – Anal. for $C_{21}H_{25}O_5P$ (388.39): calcd. C 64.94, H 6.49, P 7.97; found C 64.83, H 6.50, P 7.82.

*Reaction of triisopropyl phosphite (**3b**) with **1a***

Excess triisopropyl phosphite (**3b**) was added to **1a** (0.27 g, 1 mmol) without solvent. The reaction mixture was refluxed for 4 h. After evaporation of the volatile material under reduced pressure, the residue was subjected to silica gel column chromatography to give product **8b**.

*Diisopropyl-(6,7,8,9-tetrahydro-5-oxo-5H-benzocyclohepten-6-yl)(4-methoxyphenyl)methyl phosphonate (**8b**)*

Eluent: petroleum ether-ethyl acetate (80/20, v/v). Product **8b** was obteined as colorless crystals, yield 70 %. – M. p. 210 – 211 °C. – IR (KBr): ν = 1080 (P-O-alkyl), 1252 (P=O), 1667 (C=O) cm^{-1}. – ^1H NMR (500.14 MHz, DMSO): δ = 1.3 (m, 12 H, 4 CH_3), 1.6, 1.9, 2.9 (m, 6 H, 3 CH_2), 3.1 (dd, $^2J_{HP}$ = 19.3 Hz, J_{HH} = 7.5 Hz, 1H, CH-P), 3.3 (dd, $^3J_{HP}$ = 10.70 Hz, J_{HH} = 7.5 Hz, 1 H, C*H*-CH-P), 3.8 (s, 3 H, OCH_3), 4.6, 4.8 (2 m, 2 CH, isopropyl), 7.1 – 7.3 (m, 8 H, H_{arom}). – ^{13}C NMR (125.76 MHz, DMSO): δ = 24.2 (4 CH_3, isopropyl), 25.5, 29.8, 31.1 (3 CH_2), 35.0 (*C*H-C=O, $^2J_{CP}$ = 35 Hz), 44.2 (CH-P=O, $^1J_{CP}$ = 98 Hz), 55.8 (OCH_3), 72.0 (*C*H-$(CH_3)_2$), $^2J_{CP}$ = 32.4 Hz), 114.2 – 141.9 (aromatic C-H), 159.5 (*C*-OCH_3), 205.0 (C=O). – ^{31}P NMR δ = 23.2. – MS (EI, 70 eV): m/z = 444 $[M]^+$. – Anal. for $C_{25}H_{33}O_5P$ (444.50): calcd. C 67.55, H 7.48, P 6.97; found C 67.50, H 7.50, P 7.20.

*Reaction of triisopropyl phosphite (**3b**) with **1b***

Excess triisopropyl phosphite (**3b**) was added to **1b** (0.24 g, 0.001 mol) without solvent. The reaction mixture

was refluxed for 4 h. After evaporation of the volatile material under reduced pressure, the residue was subjected to silica gel column chromatography to give compound **8c**.

*Diisopropyl((5-oxo-6,7,8,9-tetrahydro-5H-benzocyclohepten-6-yl)(phenyl)methyl)phosphonate (**8c**)*

Eluent: petroleum ether-ethyl acetate (80/20, *v*/*v*). Product **8c** was obtained as colorless crystals, yield 65 %. – M. p. 190 – 191 °C. – IR (KBr): ν = 1081 (P-O-alkyl), 1250 (P=O), 1669 (C=O) cm^{-1}. – ^{1}H NMR (500.14 MHz, $CDCl_3$): δ = 1.3 (m, 12 H, 4 CH_3), 1.6, 2, 2.9 (m, 6 H, 3 CH_2), 3.2 (dd, $^2J_{HP}$ = 18.3 Hz, J_{HH} = 7.5 Hz, 1 H, CH-P), 3.4 (dd, $^3J_{HP}$ = 11.20 Hz, J_{HH} = 7.5 Hz, 1 H, C*H*-CH-P), 4.7, 4.8 (2 m, 2 CH, isopropyl), 7.2 – 7.3 (m, 9 H, H_{arom}). – ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 24.7 (4 CH_3, isopropyl), 26.2, 28.02, 35.1 (3 CH_2), 31.0 ($^1J_{CP}$ = 99.0 Hz, CH-P=O), 36.6 (*C*H-C=O, $^2J_{CP}$ = 20.0 Hz), 72.6 (CH-(CH_3)$_2$), 114.1 – 140 (aromatic C-H). – ^{31}P NMR: δ = 23.4. – MS (EI, 70 eV): m/z = 399 $[M–Me]^+$. – Anal. for $C_{24}H_{31}O_4P$ (414.47): calcd. C 69.55, H 7.54, P 7.47; found C 69.54, H 7.60, P 7.1.

*General procedure for the reaction of 2-arylidenebenzosuberones **1a**,**b** with tris(dialkylamino)phosphines **4a**,**b***

Tris(dialkylamino)phosphine **4** (1 mmol) was added to a solution of compound **1** (1 mmol) in dry toluene (30 mL), and the reaction mixture was refluxed for 7 h (TLC). After evaporation of the volatile material under reduced pressure, the residue was subjected to silica gel column chromatography to give the products **9a** – **d**.

*2-(Dimethylamino)-3-(4-methoxyphenyl)-3,4,5,6-tetrahydro-2H-benzo[3,4]cyclohepta[1,2-d][1,2]oxaphosphole-2-oxide (**9a**)*

Eluent: petroleum ether-acetone (80/20, *v*/*v*). Product **9a** was obtained as colorless crystals, yield 60 %. – M. p. 148 – 149 °C. – IR (KBr): ν = 860, 1320 (P-N(CH_3)$_2$), 1240 (P=O) cm^{-1}. – ^{1}H NMR (500.14 MHz, $CDCl_3$): δ = 1.8, 2.3, 2.8 (m, 6 H, 3 CH_2), 2.8 (d, $^3J_{HP}$ = 11.10 Hz, 6 H, 2 CH_3), 3.5 (d, $^2J_{HP}$ = 18.5 Hz, 1 H, CH), 3.8 (s, 3 H, OCH_3), 6.9 – 7.8 (m, 8 H, H_{arom}). – ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 24.1, 27.6, 35.9 (3CH_2), 43.7 (2C, N (CH_3)$_2$), 48.0 (CH), 50.8 (OCH_3), 108.0 (C=*C*-CH), 114.0 – 130.3 (aromatic C-H), 158.2 (*C*-OCH_3) 185.0 (C-O). – ^{31}P NMR: δ = 43.7. – MS (EI, 70 eV): m/z = 369 $[M]^+$. – Anal. for $C_{21}H_{24}NO_3P$ (369.39): calcd. C 68.28, H 6.55, N 3.79, P 8.39; found C 68.2, H 6.6, N 4.1, P 8.3.

*2-(Diethylamino)-3-(4-methoxyphenyl)-3,4,5,6-tetrahydro-2H-benzo[3,4]cyclohepta[1,2-d][1,2]oxaphosphole-2-oxide (**9b**)*

Eluent: petroleum ether-acetone (90/10, *v*/*v*). Product **9b** was obtained as colorless crystals, yield 70 %. – M. p. 193 – 194 °C. – IR (KBr): ν = 863, 1242 (P-N(C_2H_5)$_2$), 1328 (P=O) cm^{-1}. – ^{1}H NMR (500.14 MHz, $CDCl_3$): δ = 1.1 (t, 6 H, 2 CH_3, ethyl), 1.7, 1.9, 2.1 (m, 6 H, 3 CH_2), 2.8 (q, $^3J_{HP}$ = 10 Hz, 4 H, 2 CH_2, ethyl), 3.2 (d, $^2J_{HP}$ = 18.5 Hz, 1 H, CH), 3.8 (s, 3 H, OCH_3), 6.9 – 7.8 (m, 8 H, H_{arom}). – ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 14.1 (2 CH_3, ethyl), 25.9, 30.1, 35.7 (3 CH_2), 39.0 (2 CH_2, ethyl), 48.8 (CH, $^1J_{CP}$ = 107.30 Hz), 55.3 (OCH_3), 107.8 (C=*C*-CH), 114.0 – 130.0 (aromatic C-H), 158.0 (*C*-OCH_3), 184.0 (C-O). – ^{31}P NMR: δ = 42.5. – MS (EI, 70 eV): m/z = 397 $[M]^+$. – Anal. for $C_{23}H_{28}NO_3P$ (397.45): calcd. C 69.51, H 7.10, N 3.52, P 7.79; found C 69.47, H 7.17, N 3.5, P 7.70.

*2-(Dimethylamino)-3-phenyl-3,4,5,6-tetrahydro-2H-benzo[3,4]cyclohepta[1,2-d][1,2]oxaphosphole-2-oxide (**9c**)*

Eluent: petroleum ether-acetone (85/15, *v*/*v*). Product **9c** was obtained as colorless needles, yield 60 %. – M. p. 143 – 144 °C. – IR (KBr): ν = 862, 1240 (P-N(CH_3)$_2$), 1323 (P=O) cm^{-1}. – ^{1}H NMR (500.14 MHz, $CDCl_3$): δ = 2.2, 2.3, 2.33 (m, 6 H, 3 CH_2), 2.8 (d, $^3J_{HP}$ = 10.8 Hz, 6 H, 2 CH_3), 3.8 (d, $^2J_{HP}$ = 18.2 Hz, 1 H, CH), 7.2 – 7.8 (m, 9 H, H_{arom}). – ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 23.9, 27.1, 35.8 (3 CH_2), 43.7 (2 C, N(CH_3)$_2$), 48.1 (CH), 115.0 – 131.0 (aromatic C-H), 108.0 (C=*C*-CH), 184.0 (C-O). – ^{31}P NMR: δ = 43.7. – MS (EI, 70 eV): m/z = 339 $[M]^+$. – Anal. for $C_{20}H_{22}NO_2P$ (339.37): calcd. C 70.78, H 6.53, N 4.13, P 9.13; found C 70.80, H 6.56, N 4.1, P 9.1.

*2-(Diethylamino)-3-phenyl-3,4,5,6-tetrahydro-2H-benzo[3,4]cyclohepta[1,2-d][1,2]oxaphosphole-2-oxide (**9d**)*

Eluent: petroleum ether-acetone (90:10, *v*/*v*). Product **9d** was obtained as a colorless powder, yield 70 %. – M. p. 163 – 164 °C. – IR (KBr): ν = 862, 1242 (P-N(C_2H_5)$_2$), 1323 (P=O) cm^{-1}. – ^{1}H NMR (500.14 MHz, $CDCl_3$): δ = 1.4 (t, 6 H, 2 CH_3, ethyl), 1.7, 1.8, 2.2 (m, 6 H, 3 CH_2), 3.0 (q, 4 H, 2 CH_2, ethyl), 3.5 (d, $^2J_{HP}$ = 17.9 Hz, 1 H, CH), 6.7 – 7.1 (m, 9 H, H_{arom}). – ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 14 (2 CH_3, ethyl), 26, 30.5, 35.5 (3 CH_2), 38.8 (2 CH_2, ethyl), 48.5 (CH), 107.1 (C=*C*-CH), 114.0 – 130.0 (aromatic C-H), 185.0 (C-O). – ^{31}P NMR: δ = 42.5. – MS (EI, 70 eV): m/z = 367 $[M]^+$. – Anal. for $C_{22}H_{26}NO_2P$ (367.42): calcd. C 71.92, H 7.13, N 3.81, P 8.43; found C 71.90, H 7.18, N 3.14, P 8.4.

*Reaction of Lawesson's reagent (**5**) with **1a***

A mixture of 2-arylidene-benzosuberone **1a** (0.27 g, 1 mmol) and Lawesson's reagent (**5**) (0.2 g, 0.5 mmol) was refluxed for 1 h in (30 mL) of dry toluene. The volatile material was evaporated under reduced pressure and the residue subjected to silica gel column chromatography to give product **10a**.

*Dimeric compound **10a***

Eluent: Petroleum ether-ethyl acetate (95/5, *v*/*v*). Product **10a** was obtained as yellow crystals; yield 65 %. – M. p. 202 – 203 °C. – IR (KBr): ν =1210 (C=S) cm^{-1}. – ^{1}H NMR (500.14 MHz, $CDCl_3$): δ = 1.7, 2.3, 2.6 (m, 12 H, 6 CH_2), 3.8 (2 s, 6 H, 2 OCH_3), 4.9 (s, *C*H-C_6H_4-OCH_3), 5.6 (s, 1 H, S-C*H*-C_6H_4-OCH_3), 6.9 – 7.7 (m, 16 H, H_{arom}). – ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 23.2 – 35.9 (6 C, 6 CH_2), 43.3 (*C*H-C_6H_4-OCH_3), 49.2 (S-*C*H-C_6H_4-OCH_3), 53.2 (2 C, 2 OCH_3), 73.0 (C_6H_4-C-*C*-C=S), 114.0 – 142.9 (aromatic C-H), 241.0 (C=S). – MS (EI, 70 eV): m/z = 468 [M–(C_6H_4-OCH_3-CH)]$^+$. – Anal. for $C_{38}H_{36}O_2S_2$ (588.82): calcd. C 77.51, H 6.16, S 10.89; found C 77.55, H 6.2, S 11.

*Reaction of Lawesson's reagent (**5**) with **1b***

A mixture of (0.24 g, 1 mmol) of 2-arylidenebenzosuberone **1b** and (0.20 g, 0.5 mmol) of Lawesson's reagent (**5**) was refluxed for 1 h in 30 mL of dry toluene. The solvent was evaporated under reduced pressure. The residue was washed several times with acetone to give product **10b**.

*Dimeric compound **10b***

Solvent of crystallization: benzene. Product **10b** was obtained as colorless crystals; yield 60 %. – M. p. 230 – 232 °C. – IR (KBr): ν =1212 (C=S) cm^{-1}. – ^{1}H NMR (500.14 MHz, $CDCl_3$): δ = 1.7, 2.3, 2.8 (m, 12 H, 6 CH_2), 4.9 (s, 1 H, CH-C_6H_4-OCH_3), 5.7 (s, 1 H, S-C*H*-C_6H_4-OCH_3), 6.8 – 7.6 (m, 18 H, H_{arom}). – ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 23.0 – 35.7 (6 C, 6 CH_2), 42.9 (C*H*-C_6H_5), 48.9 (S-*C*H-C_6H_5), 73.2 (C_6H_5-C-*C*-C=S), 113.9 – 140.1 (aromatic C-H), 241.1 (C=S). – MS (EI, 70 eV): m/z = 406 [M–(C_6H_5-CH-S)]$^+$. – Anal. for $C_{36}H_{32}S_2$(528.77): calcd. C 81.77, H 6.10, S 12.13; found C 81.9, H 6.22, S 12.19.

*Reaction of **1a** with phosphorus pentasulfide*

A mixture of **1a** (0.27g, 1 mmol) and P_4S_{10} (0.02 g, 0.5 mmol) was refluxed for 2 h in 30 mL of dry toluene. A yellow powder precipitated after the evaporation of the volatile material. The solid material was collected and crystallized from methanol to give **10a** (mixed melting point and comparative IR spectrum).

*Reaction of **1b** with phosphorus pentasulfide*

A mixture of **1b** (0.24 g, 1 mmol) and P_4S_{10} (0.02 g, 0.5 mmol) was refluxed for 2 h in 30 mL of dry toluene. The volatile material was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography. The dimeric compound **10b** was separated using petroleum ether-ethyl acetate (95/5, *v*/*v*) as an eluent (mixed melting point and comparative IR spectrum).

Pharmacological activity
Material and methods

Chemicals: All cell culture material was obtained from Cambrex BioScience (Copenhagen, Denmark). All chemicals were from Sigma/Aldrich, USA, unless otherwise indicated. All experiments were repeated three times.

Cell culture: Cells were routinely cultured in DMEM (Dulbeco's Modified Eagle's Medium), which was supplemented with 10 % fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 units/mL penicillin G sodium, 100 units mL^{-1} streptomycin sulfate and 250 mg mL^{-1} amphotericin B. Cells were maintained at sub-confluence at 37 °C in humidified air containing 5 % CO_2.

In vitro cytotoxicity assay: Cells (0.5×105 cells per well), in serum-free media, were plated in a flat bottom 96-well microplate, and treated with 20 μL of different concentrations of the tested extract for 48 h at 37 °C in a humidified 5 % CO_2 atmosphere. After incubation, media were removed, and 40 μL MTT solution per well was added and incubated for an additional 4 h. MTT crystals were solubilized by adding 180 μL of acidified isopropanol per well, and the plate was shaken at r. t., followed by photometric determination of the absorbance at 570 nm using a microplate ELISA reader. Triplicate repeats were performed for each concentration, and the average was calculated. Data were expressed as the percentage of relative viability compared with the untreated cells and the vehicle control, with cytotoxicity indicated by < 100 % relative viability.

Calculation: The percentage of relative viability was calculated using the following equation: [absorbance of treated cells/absorbance of control cells)] X 100.

Then the half-maximum inhibitory concentration (IC_{50}) was calculated from the equation of the dose response curve.

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