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Efficient Synthesis and Antimicrobial Evaluation of New Organophosphorus Dioxaspirodecanone Derivatives

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Abstract

Claisen-Schmidt condensation reaction of 1,4-cyclohexanedione monoethylene ketal **(1)** with p-methoxybenzaldehyde, under base catalyzed conditions, has afforded 7,9 bis (4-methoxybenzylidene)-1,4-dioxaspiro[4,5]decan-8-one **(2)**. The bis-benzylidene **2** was functionalized with some selected organophosphorus reagents. Thus, compound **2** was reacted with tris-(dialkylamino) phosphines **3a,b**, in refluxing toluene and in presence of a catalyst, to give a product mixture of the corresponding oxaphospholedioxolane oxides **8a,b** and tetraalkylphosphonicdiamides **9a,b**. Moreover, the reaction of bis-benzylidene **2** with the trialkylphosphite reagents **4a,b** afforded the corresponding dialkylphosphonate derivatives **10a,b**. The phosphonate **10b** could be also obtained upon reacting bis-bezylidene **2** with diisopropylphosphite **(5)** under the same experimental conditions. The reaction of bis-benzylidene **2** with the stable phosphonium ylides, namely, (carbmethoxymethylene)- **(6a)** or (carbethoxymethylene) -triphenylphosphorane **(6b)** , afforded the dihydrospiroindenedioxolanone derivative **11** under the given reaction conditions. Moreover, compound **11** was also obtained from reaction of the dialklyphosphonoacetate Wittig-Horner reagents **7a,b** with compound **2**. On the other hand, reaction of diethyl(cyanomethylene) phosphonate **7c** with bis-bezylidene **2**, in an ethanolic sodium ethoxide solution, gave the 1,4-dispiroylidene acetonitrile derivative **12**. Possible reaction mechanisms are discussed and the structures of the new products are confirmed from their analytical and spectroscopic data. The antimicrobial activity of the synthesized compounds was also investigated.

Keywords: Bis- benzylidene,Organophosphorus reagents, Antimicrobial agents

1. Introduction

α,β- Unsaturated carbonyl groups have disparate pharmacological characteristics, including cytotoxicity toward various cancer cell lines [1, 2], hepatoprotective activities [3], antitumor, antimalarial and antiulcer properties [4]. Moreover, chalcone or benzylidene derivatives possess immense domain of reactivity due to the existence of unsaturated carbonyl system. As a result, nucleophilic reagents can react with substituted chalcone at the carbonyl group and double bond [1]. So, these reactions with binucleophiles producing different cyclized compounds with particular importance [1, 5]. Moreover, the chemistry of diverse benzylidene derivatives has produced intense scientific studies in all the world [6]. From another side, phosphorus reagents are used in assorted kinds of reactions of advantage to synthetic chemists, particularly in the preparation of naturally occurring compounds with biological and pharmacological interests [7, 8]. Based on the previous findings and as continuation in organophosphorus chemistry [9-14], the behavior of 7,9-bis(4-methoxybenzylidene)-1,4-dioxaspiro[4.5] decan-8-one **(2)** towards a various of organophosphorus reagents namely, tris-(dialkylamino)phosphine derivatives **3**, trialkylphosphite derivatives **4**, dialkylphosphite **5**, Wittig reagents **6** and Wittig-Horner reagents **7** was discussed (Scheme 1).

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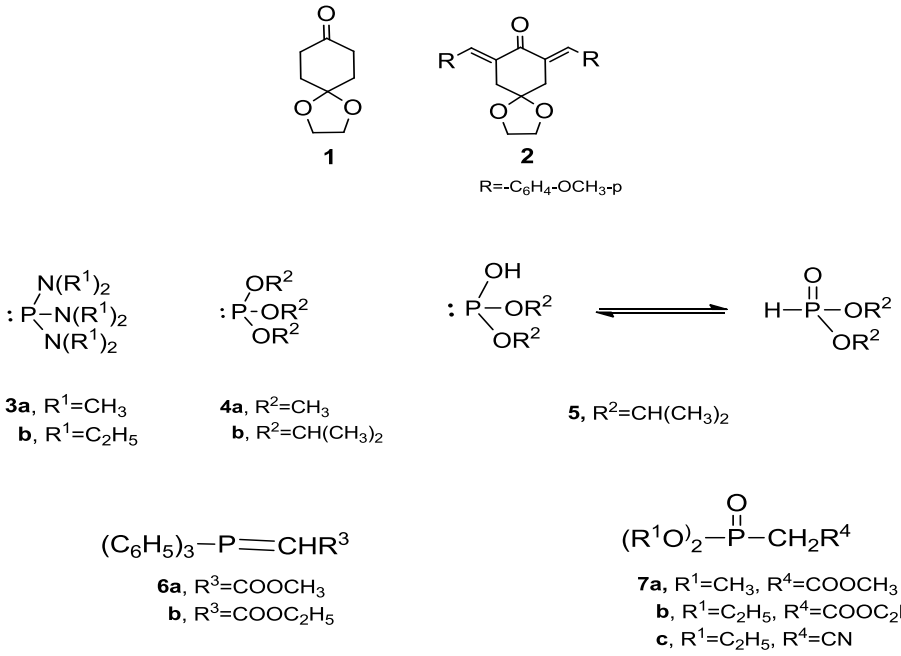


Scheme 1

2. Experimental

2.1. Chemistry

All chemicals were supplied by either Fluka or Aldrich chemical companies and were used without further purification. Starting material was prepared according to the reported method [15]. All melting points are uncorrected and were taken in open capillary tubes using Electrothermal digital melting point apparatus 9100(Electro-Thermal Engineering Ltd., Essex, United Kingdom). Elemental microanalyses were carried out at Microanalytical Unit, Central Services Laboratory, National Research Centre, Dokki, Giza, Egypt, using Vario Elementar and were found within ±0.4% of the theoretical values. **FT-IR** spectra were recorded with a Perkin-Elmer Frontier. Routine **NMR** spectra were recorded at room temperature on a Bruker Avance TM 500 and Bruker Avance TM 400 spectrometer as solutions in dimethyl sulfoxide (DMSO-d_6) or in chloroform ($CDCl_3$). All chemical shifts are quoted in δ relative to the trace resonance of protonated chloroform (δ 7.25 ppm) for ^{1}H NMR, $CDCl_3$ (δ 77.0 ppm) for ^{13}C NMR or dimethyl sulfoxide (δ 2.50 ppm) for ^{1}H NMR , DMSO (δ 39.51 ppm) for ^{13}C NMR and external 85% aqueous H_3PO_4 (δ 0.0 ppm) for ^{31}P NMR. The mass spectra were measured with a GC Finnigan MAT SSQ-7000 mass spectrometer. Follow up of the reactions and checking the purity of the compounds were made by Thin Layer Chromatography (TLC) on silica gel-precoated aluminum sheets (Type 60, F 254, Merck, Darmstadt, Germany) with eluent of petroleum ether (b.r. 60-80 ^{o}C) /ethyl acetate or acetone and the spots were detected by exposure to UV lamp at λ_{254} nanometer for a few seconds. The chemical names given for the prepared compounds are according to the IUPAC system. The reported yields are based upon pure materials isolated by column chromatography with eluent of petroleum ether (b.r. 60-80^{o}C)/ethyl acetate or acetone. Solvents were dried/purified according to conventional procedures.

*General procedure for the reaction of bis-benzylidene **2** with tris(dialkylamino)phosphine drivatives **3***

A mixture of benzylidene **2** [15] (0.39 g, 0.01 mol), tris(dimethylamino)phosphine (0.32 g, 0.02 mol) **(3a)** or tris(diethylamino)phosphine **(3b)** (0.5 g, 0.02 mol),drops of morhpoline was added and refluxed in dry toluene (20 mL) for 6-8 hrs. The reaction was monitored by (TLC), until no more of the starting material (compound **2**) could be detected. The reaction mixture was evaporated under reduced pressure and the residue was applied to silica gel column chromatography, to afford compounds **8** and **9**, respectively.

2-(Dimethylamino)-7-(4-methoxybenzylidene)-3-(4-methoxyphenyl)-3,4,6,7-tetrahydro-*2H*-spiro[benzo[d][1,2]oxaphosphole-5,2'-[1,3]dioxolane] 2-oxide (8a)

Eluent: petroleum ether (60-80^{o}C) / EtOAc (50/50, v/v), a colorless powder, yield 35%, m.p 202-203^{o}C. IR (KBr, cm^{-1}): 1345, 850 (P-N$(CH_3)_2$), 1240 (P=O). ^{1}H NMR (500.14 MHz, $CDCl_3$) δ 7.27 (d, 2 H, H

arom), 6.94 (s, 1 H, C=CH, benzylidene), 6.90 – 6.87 (m, 6 H, H arom), 3.92 - 3.90 (m, 4 H, 2 OCH_2), 3.87 (s, 6 H, 2 OCH_3), 3.78 (d, 1 H, CH-P, $^2J_{HP}$=18.1 Hz), 2.78 (d, 6 H, P-N$(CH_3)_2$, $^3J_{HP}$ = 10.5 Hz), 2.67 (d, 2 H, CH_2), 2.27 ppm (m, 2 H,CH_2). ^{13}CNMR (125 MHz, $CDCl_3$) δ 173.60 (C-O), 158.91 (2 C-OCH_3), 130.76 – 113.88 (C arom), 107.75 (C=C-CH), 64.78, 64.66 (2 OCH_2), 55.35 (2 OCH_3), 45.45 (d, P-CH, $^1J_{CP}$=107.2 Hz), 37.09 (P-N $(CH_3)_2$), 35.9, 34.20 ppm (2 CH_2).^{31}P NMR δ 47.30 ppm. MS (*m/z*, %): 483 (M^+, 77), 438 (M^{-1}– (N$(CH_3)_2$), 14), 392 (M^+ – (OP (N $(CH_3)_2$, 5). Analysis for $C_{26}H_{30}NO_6P$ (483.49). Calcd.: % C, 64.59; H, 6.25; N, 2.90; P, 6.41. Found: % C, 64.31; H, 6.15; N, 2.66; P, 6.14.

((9-(4-Methoxybenzylidene)-8-oxo-1,4-dioxaspiro [4.5]decan-7-yl)(4-methoxyphenyl) methyl) N,N,N',N'-tetramethylphosphonicdiamide (9a)

Eluent: petroleum ether (60-80°C) / EtOAc (45/55, v/v) a colorless powder, yield 45%, m.p 154-155°C. IR (KBr, cm^{-1}): 1674 (C=O), 1344, 856 (P-2(N$(CH_3)_2$), 1241 (P=O).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (d, 2 H, H arom), 7.12 (d, 2 H, H arom), 6.91 (d, 2 H, H arom), 6.82 (m, 3 H, H arom+ 1 H, C=CH benzylidene), 3.86-3.77 (m, 10 H, 2 OCH_2, 2 OCH_3), 3.64 (dd, 1 H, HC-P, $^2J_{HP}$= 18.3 Hz, J_{HH} = 10.4 Hz), 2.92 (dd, 1 H, HC-C=O, $^3J_{HP}$ = 12.4 Hz, J_{HH} = 10.4 Hz), 2.69 (d, 12 H, $^3J_{HP}$ = 10.0 Hz, [P-(N$(CH_3)_2)_2$], 2.35, 1.71 ppm (m, 4 H, 2 CH_2). ^{13}CNMR (125 MHz, $CDCl_3$) δ 189.97 (C=O), 159.64, 157.87 (2 C-OCH_3), 140.54 (C=C-H), 130.63 - 114.08 (C arom), 64.60 (2 OCH_2), 55.34, 55.12 (2 OCH_3), 47.79 (C-P), 37.10 (C-C=O), 35.69 (P-(N$(CH_3)_2)_2$), 35.43, 34.31 ppm (2 CH_2). ^{31}P NMR δ 40.37 ppm. MS (*m/z*, %): 483 (M^{-1} – (N$(CH_3)_2$), 100), 438 (M^{-2}-[N$(CH_3)_2$)]$_2$), 17), 396 (M^+ - [HO-PO(N$(CH_3)_2)_2$], 24). Analysis for $C_{28}H_{37}N_2O_6P$ (528.58).Calcd.: % C, 63.62; H, 7.06; N, 5.30; P, 5.86. Found: %C, 64.02; H, 7.32; N, 5.68; P, 6.42.

2-(Diethylamino)-7-(4-methoxybenzylidene)-3-(4-methoxyphenyl)-3,4,6,7-tetrahydro-*2H*-spiro [benzo[d][1,2]oxaphosphole-5,2'-[1,3]dioxolane] 2-oxide (8b)

Eluent: petroleum ether (60-80°C) / EtOAc (70/30, v/v) a colorless crystals, yield 35%, m.p 200-202°C. IR (KBr, cm^{-1}): 1342, 833 (P-N$(C_2H_5)_2$), 1246 (P=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30- 7.27 (m, 4 H, H arom), 6.96 (s, 1 H, C=CH, benzylidene), 6.93- 6.89 (m, 4 H, H arom), 3.95 (m, 4 H, 2 OCH_2), 3.90, 3.89 (d, 6 H, 2 OCH_3), 3.84 (d, 1 H, PC-H, $^2J_{HP}$=14.8 Hz), 3.26, 3.12 (2q, 4 H, [N(CH_2-CH_3)$_2$]), 2.35 - 2.42 (m, 4 H, 2 CH_2), 1.18 ppm (t, 6 H, N(CH_2-CH_3)$_2$). ^{13}C NMR (100.63 MHz, $CDCl_3$) δ 175.6 (C-O), 158.79 (2 C-OCH_3), 130.68 – 113.79 (C arom), 107.69 (C=C-CH), 64.72, 64.58 (2 OCH_2), 55.30 (2 OCH_3), 46.83 (d, P-CH, *Jcp* = 107.6 Hz), 42.36 (P-N(CH_2-CH_3)$_2$), 37.03, 34.20 (2 CH_2), 14.56 ppm (P-N(CH_2-CH_3)$_2$). ^{31}P NMR δ 45.88 ppm. Analysis for $C_{28}H_{34}NO_6P$ (511.55). Calcd: % C, 65.74; H, 6.70; N, 2.74; P, 6.05. Found: %C, 65.36; H, 7.03; N, 2.46; P, 5.81.

((9-(4-Methoxybenzylidene)-8-oxo-1,4-dioxaspiro [4.5]decan-7-yl)(4-methoxyphenyl) methyl) N,N,N',N'-tetraethylphosphonicdiamide (9b)

Eluent: petroleum ether (60-80°C) / EtOAc (45/55, v/v) a colorless powder, yield 40%, m.p 167-169°C. IR (KBr, cm^{-1}): 1676 (C=O), 1344, 856 (P-N$(CH_3)_2$), 1241 (P=O).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47(d, 2 H, H arom), 7.16 – 7.10 (m, 4H, H arom), 6.90-6.77 (m, 3 H, H arom + 1 H, C=CH benzylidene), 3.88 – 3.77 (m, 4 H, 2 OCH_2 + 6H, 2 OCH_3), 3.74 (d, 1 H, HC-P, $^2J_{HP}$ = 25.7 Hz), 3.71 (dd, 1 H, HC-C=O, $^3J_{HP}$ = 12.2 Hz), 3.32, 3.12 (m, 8 H, P-[(N(C$H_2$$CH_3$) $_2$)$_2$], 2.83 (m, 2 H, CH_2), 2.29 (m, 2 H, CH_2), 1.21(t, 12 H, P-[(N(CH_2CH_3)$_2$)$_2$]) ppm. ^{13}CNMR (125 MHz, $CDCl_3$) δ 189.97 (C=O), 159.64, 157.87 (2 C-OCH_3), 140.54 (C=C-H), 129.81 - 113.91 (C arom), 64.60, 64.30 (2 OCH_2), 55.34 (2 OCH_3), 46.12 (C-P), 43.67 (P-(N(C$H_2$$CH_3$)$_2$)$_2$), 37.95 (C-C=O), 31.92, 29.77 (2 CH_2), 14.16 ppm P-(N(CH_2CH_3)$_2$)$_2$). MS (*m/z*, %): 483 (M^{+2} – P(N(CH_2CH_3)$_2$), 100), 438 (M^{-2}-([N(CH_2CH_3)$_2$)$_2$), 17). Analysis for $C_{32}H_{45}N_2O_6P$ (584.68). Calcd.: % C, 65.74; H, 7.76; N, 4.79; P, 5.30. Found: % C, 65.33; H, 7.36; N, 4.49; P, 5.69.

*General procedure for the reaction of bis-benzylidene **2** with trialkylphosphite derivatives* **4**

A mixture of benzylidene **2** (0.39 g, 0.01 mol) and excess of trimethylphosphite **(4a)** or triisopropylphosphite **(4b)** in dry toluene (20 mL) was refluxed for 5-6 hr after addition of few drops of morpholine. The reaction was monitored by (TLC) until no more of the starting materials could be detected. The reaction mixture was evaporated under reduced pressure and purified by column chromatography, to isolate compounds **10a** and **10b**, respectively.

Dimethyl((9-(4-methoxybenzylidene)-8-oxo-1,4-dioxaspiro[4.5]decan-7-yl)(4-methoxyphenyl)methyl)phosphonate (10a)

Eluent: petroleum ether (60-80°C) / EtOAc (70/30, v/v) a colorless powder, yield 45%, m.p 184-185°C. IR (KBr, cm^{-1}): 1676 (C=O), 1245 (P=O), 1094 (P-OCH_3). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.41(m, 8 H, H arom), 7.36 (s, 1 H, C=CH benzylidene), 3.79 – 3.69 (m, 4 H, 2 OCH_2 + 6 H, 3 OCH_3), 3.63, 3.52 (2 d, 6 H, [P (OCH_3)$_2$], $^3J_{HP}$ =11.3 Hz), 3.57 (dd, 1 H, CH-P, $^2J_{HP}$ = 18.8 Hz, J_{HH} = 6.9 Hz), 3.03 (dd, 1 H, CH-CO, $^3J_{HP}$ = 12.6 Hz, J_{HH} = 6.9 Hz), 2.47 (d, 2 H, CH_2), 2.35 ppm (m, 2 H, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ 189.00 (C=O), 159.71, 158.45 (2 C-OCH_3), 142.96 - 113.96 (C arom), 64.64 (2 OCH_2), 55.07 (OP(OCH_3)$_2$), 54.26 (2 OCH_3), 43.61(HC-P, $^1J_{CP}$ = 100 Hz), 45.48 (HC-C=O, $^2J_{CP}$ = 20.2 Hz), 37.51, 36.12 ppm (2 CH_2).^{31}P NMR δ 36.00 ppm. MS (*m/z*, %): 483 (M^{-1} – H_2O, 43), 393 (M^{+2} –

($OP(OCH_3)_2$, 4), 395 (M^{+2}- ($PO(OCH_3)_2$, 9). Analysis for $C_{26}H_{31}O_8P$ (502.49). Calcd.: % C, 62.15; H, 6.22; P, 6.16. Found: % C, 62.05; H, 6.12; P, 6.10.

Diisopropyl((9-(4-methoxybenzylidene)-8-oxo-1,4-dioxaspiro[4.5]decan-7-yl)(4-methoxyphenyl) methyl)phosphonate (10b)

Eluent: petroleum ether (60-80^{o}C) / EtOAc (85/15, v/v) a Pale yellow crystals, yield 85%, m.p 195-197^{o}C. IR (KBr, cm^{-1}): 1671 (C=O), 1246 (P=O), 1033 (P-O-CH). ^{1}H NMR (400.19 MHz, $DMSOd_6$) δ 7.47 - 6.86 (m, 8 H, H arom + 1 H, C=CH benzylidene), 4.61, 4.44 (2 m, 2 CH, isopropyl), 3.80 (m, 4 H, 2 OCH_2), 3.70 (s, 6 H, 2 OCH_3), 3.59 (dd, 1 H, CH-P, $^2J_{HP}$=18.0 Hz, J_{HH} = 7.6 Hz), 3.09 (dd, 1 H, HC-C=O, $^3J_{HP}$=10.2 Hz, J_{HH} = 7.6 Hz), 2.40, 1.88 (2 m, 4 H, 2 CH_2), 1.62 ppm (m, 12 H, 4 CH_3, isopropyl). ^{13}C NMR (100.63 MHz, DMSO-d_6) δ189.01 (C=O), 159.78, 158.05 (2 C- OCH_3), 132.04 - 114.32 (C arom), 71.26, 70.99 (2 $CH(CH_3)_2$), 64.66 (2 OCH_2), 55.62, 55.42 (2 OCH_3), 46.94 (HC-P, $^1J_{CP}$= 100.1 Hz), 37.51, 36.99 (2 CH_2), 35.88 (HC-C=O, $^2J_{CP}$ = 33.3 Hz),24.02 ppm (4 CH_3, isopropyl). ^{31}P NMR δ 33.41 ppm. MS (*m/z*, %): 482 (M^{-1} – $(OCH(CH_3)_2)_2$+OH, 84), 394 (M^{+2}-$(OP(OCH(CH_3)_2)_2$, 11). Analysis for $C_{30}H_{39}O_8P$ (558.60). Calcd.: %C, 64.50; H, 7.04; P, 5.54. Found: % C, 64.53; H, 7.00; P, 5.51.

*Reaction of bis-benzylidene **2** with diisopropyl phosphite* **5**

When the same reaction was carried out using benzylidene **2** (0.39 g, 0.01 mol) and excess of diisopropyl phosphite **(5)** under the same reaction conditions, yielded the phosphonate **10b.**

*General procedure for the reaction of bis-benzylidene **2** with Wittig reagents **6a** and **6b**.*

A mixture of compound **2** (0.39 g, 0.01 mol), (carbmethoxymethylene)triphenylphosphorane **(6a)** (0.66g, 0.02 mol) or (carbethoxymethylene)triphenylphosphorane **(6b)** (0.68 g, 0.02 mol) and few drops of mopholine was refluxed in dry toluene (20 mL) for 8-10 hr. The completion of the reaction was monitored by (TLC). The reaction was left to cool and precipitated. The precipitated material was collected and purified by column chromatography to yield compound **11**. Triphenylphosphane oxide was also isolated from the reaction medium and identified (mix m.p., MS).

7-(4-Methoxybenzylidene)-3-(4-methoxyphenyl)-6,7-dihydrospiro[indene-5,2'[1,3]dioxolan]-2*(4H)*-one (11)

Eluent: petroleum ether (60-80^{o}C) / EtOAc (35/65, v/v) a colorless powder, yield 45 %, m.p 220-222 ^{o}C.^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (s, 1 H, HC-C=O), 7.37 (d, 2 H, H arom), 7.17 (d, 2 H, H arom), 6.96 – 6.86 (m, 4 H, H arom), 6.27 (s, 1 H, C=CH benzylidene), 3.95 - 3.88 (m, 4 H, 2 OCH_2), 3.84, 3.82 (2 s, 6 H, 2 OCH_3), 2.53 (m, 2 H, CH_2), 2.02 ppm (m, 2 H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 187.27 (C=O), 160.05 (C=CH-C=O), 158.06, 158.15 (2 C-OCH_3), 143.31 – 113.71 (C arom), 64.95 (2 OCH_2), 55.61 (2 OCH_3), 37.91, 35.20 (2 CH_2). Analysis for $C_{26}H_{24}O_5$ (416.47). Calcd: % C, 74.98; H, 5.81. Found: % C, 74.90; H, 5.71.

*General procedure for the reaction of benzylidene **2** with Wittig- Horner reagents **7a**, **7b** and **7c***

A solution of sodium ethoxide (0.136 g, 0.02 mol) in absolute ethanol (20 mL) was treated with dialkoxyphosphonate **7a**, **7b** (0.02 mol) or diethyl (cyanomethylene)phosphonate (0.35 g, 0.02 mol) then benzylidene **2** (0.39 g, 0.01 mol) was added, the resulting reaction mixture was refluxed for 5 hr. Then it was poured on a small amount of water, extracted with ethyl acetetae, and dried, the extracts were evaporated under reduced pressure, then purified by column chromatography to produce compounds **11** and **12** respectively.

7-(4-Methoxybenzylidene)-3-(4-methoxyphenyl)-6,7-dihydrospiro[indene-5,2'[1,3]dioxolan]-2*(4H)*-one (11)

Eluent: petroleum ether (60-80^{o}C) / EtOAc (35/65, v/v) a colorless powder, yield 30 %. It was characterized by comparing TLC analyses, its m.p. and ^{1}H NMR spectrum as the authentic specimen.

7,9-Bis(4-methoxybenzylidene)-1,4-dioxaspiro[4.5]decan-8-ylideneacetonitrile (12)

Eluent: petroleum ether (60-80^{o}C) / EtOAc (90/10, v/v) a colorless crystals, yield 45%, m.p 112-114^{o}C.^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 – 7.27 (m, 2 H, H arom), 7.20 (d, 2 H, H arom), 7.15 (d, 2 H, H arom), 6.91 (m, 2 H, H arom, 1 H, C=CH benzylidene), 6.76 (s, 1 H, C=CH benzylidene), 5.41 (s, 1 H, HC-CN), 3.91 (m, 4 H, 2 OCH_2), 3.83 (s, 6 H, 2 OCH_3), 2.50 (d, 2 H, CH_2), 1.94 (d, 2 H, CH_2), ppm . ^{13}CNMR (125 MHz, $CDCl_3$) δ 164.40 (C=CH), 159.39 (2 C-OCH_3), 148.09 – 113.92 (C arom), 117.49 (C≡N), 92.82 (HC-C≡N), 64.71 (2 OCH_2), 55.36 (2 OCH_3), 45.88 (CH_2), 38.90 (CH_2) ppm. Analysis for $C_{26}H_{25}NO_4$ (415.48). Calcd.: % C, 75.16; H, 6.06; N, 3.37. Found: %C, 75.06; H, 6.00; N, 3.30.

2.2. Antimicrobial Activity

The samples were prepared by dissolving 10 mg of products under investigation (**8a, 8b, 9a, 9b, 10a, 10b, 11** and **12**) in 2 mL of methanol and 100µl of solution (containing 500µg of desired product) was used in this test. The antimicrobial activity of different samples was investigated by the agar cup plate method.

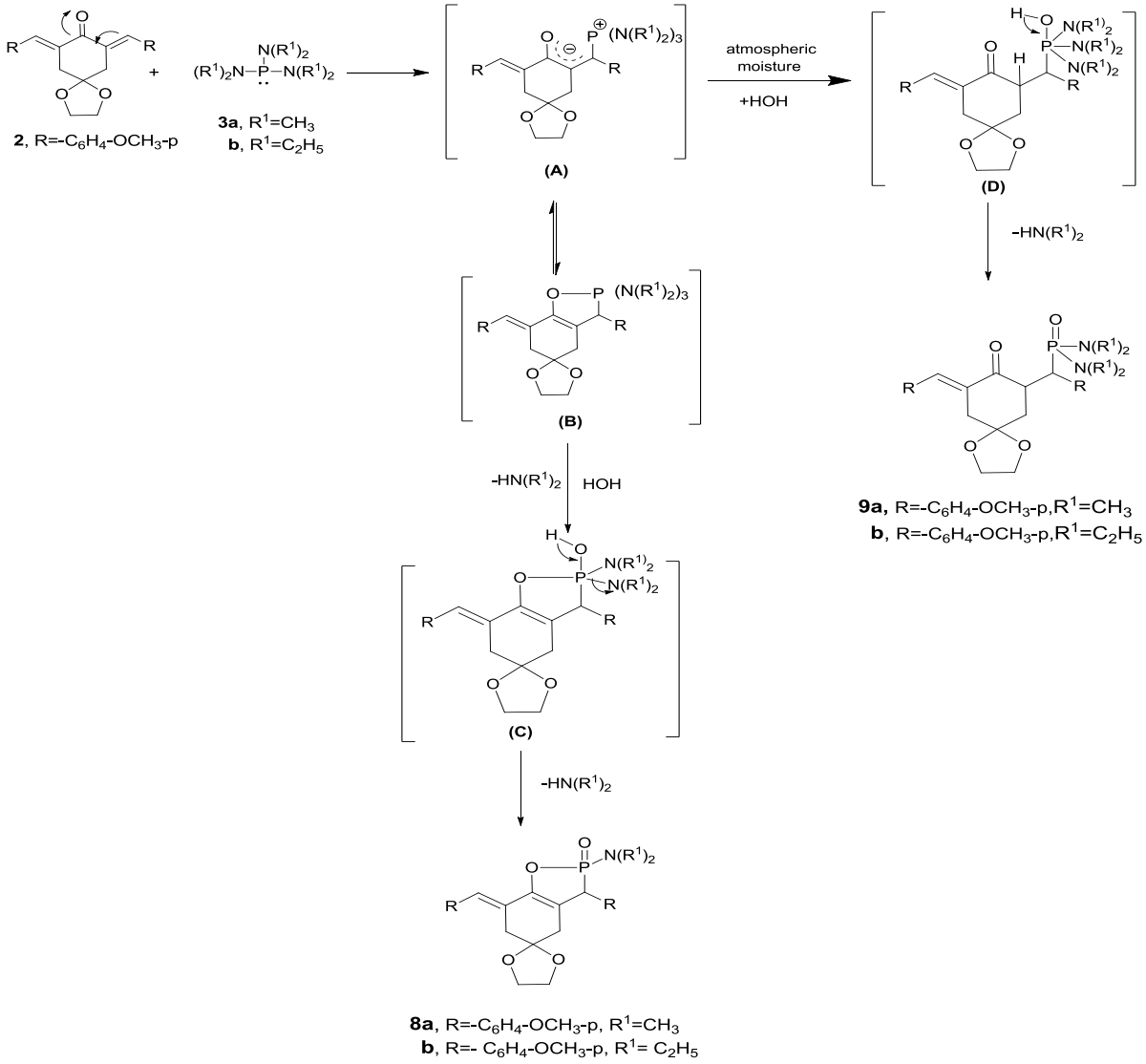
Four different test microbes namely: *Staphylococcus aureus* (Gram +ve), *Escherichia coli* (Gram -ve), *Candida albicans* (yeast) and *Aspergillus niger* (fungus) were used. Nutrient agar plates were heavily seeded uniformly with 0.1mL of 105-106 cells/mL in

case of bacteria and yeast. A Czapek-Dox agar plate seeded by 0.1mL of the fungal inoculums was used to evaluate the antifungal activities. Then a hole (1cm diameter) was made in media by gel cutter (Cork borer) in sterile condition. Then one drop of melted agar was poured into the hole and allowed to solidify to make a base layer. After that specific amount of tested sample (0.1 mL) was poured into the hole. Then plates were kept at low temperature (4°C) for 2-4 hours to allow maximum diffusion. The plates were then incubated at 37°C for 24 hours for bacteria and at 30°C for 48 hours for fungi in upright position to allow maximum growth of the organisms. The antimicrobial activity of the tested agents was determined by measuring the diameter of the zone of inhibition expressed in millimeter (mm). The experiment was carried out more than once and means of reading was recorded [16-18].

3. Result and Discussion.

3.1. Chemistry

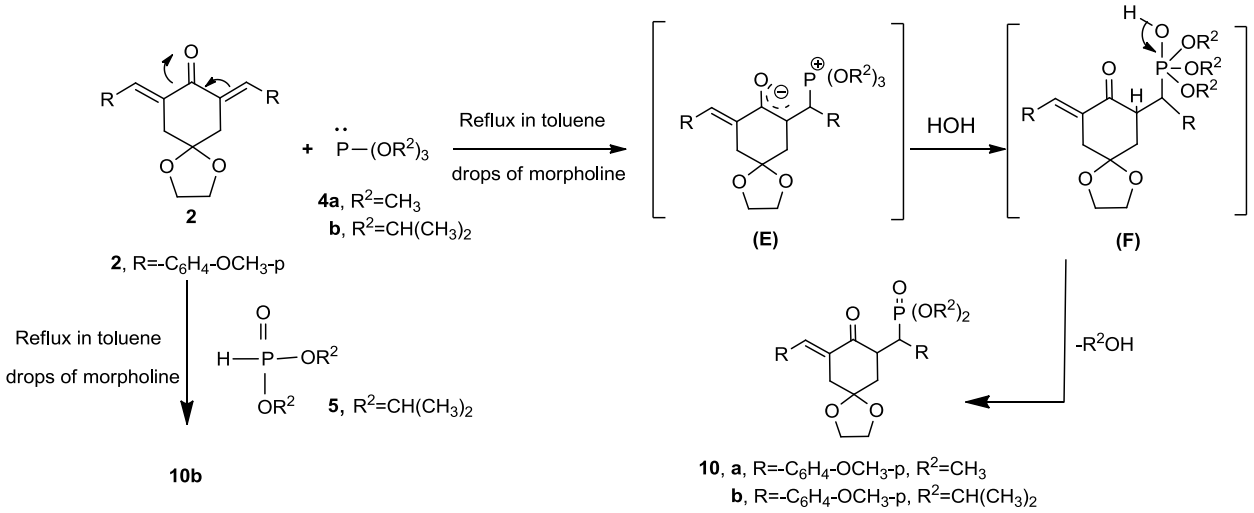
7,9-Bis-(4-methoxybenzylidene)-1,4-dioxaspiro [4.5]decan-8-one (**2**) was obtained from the interaction of 1,4-cyclohexanedione mono-ethylene ketal (**1**) with 4-methoxybenzaldhyde via Claisen-Schmidt condensation reaction using sodium hydroxide as a catalyst [19, 20]. The behavior of bis(4-methoxybenzylidene) dioxaspirodecanone **2** with tris (dialkylamino)- phosphine derivatives **3a** and **3b** was investigated. When benzylidene **2** was treated with tris(dimethylamino)phosphine **3a** in refluxing toluene in presence of a few drops of morpholine for 8 h, a mixture of two products was obtained, which could be isolated by silica gel column chromatography (using petroleum ether (60 - 80 °C) / EtOAc as an eluent) to give oxaphospholedioxolane oxide **8a** (50/50, v/v) and tetramethylphosphonicdiamide **9a** (45/55, v/v). The chemical structure of **8a** was confirmed by different spectral data and microanalyses. The IR spectrum of oxaphospholedioxolaneoxide **8a** showed strong absorption bands at 1345, 850 cm^{-1} attributed to the bands of (P-$N(CH_3)_2$) and at 1240 cm^{-1} for (P=O)[12]. Its ^{1}H NMR spectrum exhibited a signal as doublet centered at δ 3.78 ppm with ($^2J_{HP}$ =18.1 Hz) for the methine proton attached to the phosphorus atom, and a doublet signal centred at δ 2.78 ppm with ($^3J_{HP}$=10.5 Hz) attributed to the six protons of the dimethyl amino groups. ^{13}C NMR spectrum of compound **8a** also added a good substantiation for the expected structure, which revealed the presence of a doublet signal at δ 45.45 ppm with (^1Jcp = 107.2 Hz) owing to the methine carbon attached to the phosphorus atom, and a signal at δ 37.09 ppm for the methyl of (P-$N(CH_3)_2$). Moreover, the ^{31}P NMR spectrum of **8a** affirmed the cyclic oxaphospholedioxolane structure, and showed a signal at δ 47.30 ppm [12, 21]. The mass spectrum of compound **8a** exhibited the molecular ion peak at m/z =483 (M^+, 77 %), a peak at 438(M^{-1}- ($N(CH_3)_2$), 14 %) and a peak at 392 (M^{+1} - (OP-$N(CH_3)_2$, 5 %). Structural support for the second product **9a** (45%) was deduced from its spectroscopic data. The IR spectrum of compound **9a**, showed the presence of an intensive absorption bands at 1674 cm^{-1} for (C=O), two bands at 1344, 856 cm^{-1} due to the band of [$P(N(CH_3)_2)_2$], and at 1241 cm^{-1} for (P=O). Its ^{1}H NMR spectrum revealed signals at δ 3.64 (dd, 1 H, HC-P, $^2J_{HP}$=18.3 Hz) for methine proton attached to phosphorus atom, and at δ 2.92 (dd, 1 H, HC-C=O, $^3J_{HP}$=12.4 Hz), a doublet signal centered at δ 2.69 ppm for (12 H, $P(N(CH_3)_2)_2$, $^3J_{HP}$=10.0 Hz). The ^{13}C NMR spectrum proves intense evidence for the structure of tetramethylphosphonic diamide **9a**, and showed signal at δ 189.97 for (C=O), two doublet signals at δ 47.79 ppm, and at δ 37.10 ppm ascribed to two methine carbons attached to phosphorus atom CH-P and CH-CH-P, respectively. ^{31}P NMR spectrum of compound **9a** showed signal at δ 40.37 ppm. Its mass spectrum showed the molecular ion peak at m/z 483 (M^{-1} -$N(CH_3)_2$, 100%), 438 (M^{-2} – $(N(CH_3)_2)_2$, 17%). In a similar way, bis-benzylidene **2** reacted with tris(diethylamino)phosphine (**3b**) (1:2 mol equivalents) at reflux temperature to produce oxaphospholedioxolaneoxide **8b** and tetraethylphosphonicdiamide **9b**, which were confirmed from their spectral data. A possible mechanism for the reaction of bis-benzylidene **2** with tris(dialkylamino)phosphine derivatives **3** is demonstrated in (Scheme 2). The formation of the oxaphosphole derivatives **8** was occurred via a nucleophilic attack of the phosphorus atom of aminophosphines **3** on the methine carbon of benzylidene **2** which is the most interacting center, then the dipolar adduct (A) was formed as intermediate which undergoes ring closure to yield the intermediate (B). Due to the structural characteristics of (B) and under atmospheric moisture, the rapid hydrolysis occurred for the latter, to give the intermediate (C), which undergoes further decomposition affording compounds **8** [12, 22]. While, formation of compounds **9** could be explained through addition of a molecule of water (atmospheric moisture) to the intermediate (A), yields a transient intermediate (D). Products **9** were formed by expulsion of $HN(R^1)_2$ molecule (Scheme 2).



Scheme 2

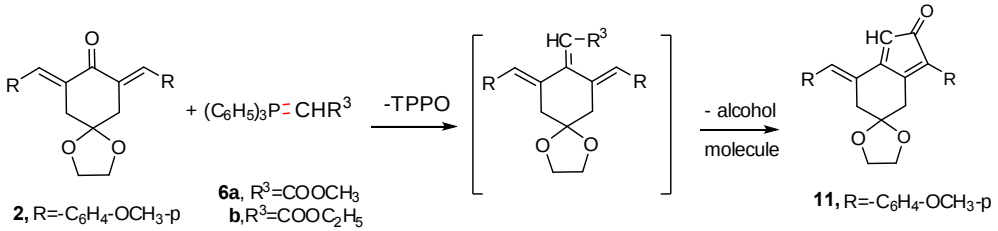
Moreover, the interaction of bis-(4-methoxybenzylidene)decanone **2** with substituted trialkyl phosphites **4a** and **4b** was also studied. We have found that the treatment of benzylidene **2** with excess of trimethyl phosphite **(4a)** or triisopropyl phosphite **(4b)** in presence of morpholine as a catalyst, in dry toluene under reflux temperature, yields chromatography pure compounds **10a** and **10b**, respectively (Scheme 3). Structure explanation for dimethyl phosphonate **10a**, taken as an example, and was based on elemental analysis and spectral data. The IR spectrum of dimethyl phosphonate **10a**, exhibited strong absorption band at 1676 cm^{-1} due to (C=O), at 1245 cm^{-1} due to (P=O), and at 1094 cm^{-1} for the absorption of $PO(OCH_3)_2$ [12]. Its ^1H NMR spectrum revealed a signal at δ 3.63, 3.52 ppm as two doublet with $^3J_{HP}$ = 11.3 Hz due to six protons of $[P(OCH_3)_2]$, a signal at δ 3.57 ppm as doublet of doublet for CH-P, with ($^2J_{HP}$ = 18.8 Hz) for the methine proton attached to phosphorus atom, and a signal at δ 3.03ppm as a doublet of doublet for HC-C=O with ($^3J_{HP}$ = 12.6 Hz). The ^{13}C NMR spectrum of compound **10a** gave signals at δ 189.00 ppm for (C=O), 55.07 ($PO(OCH_3)_2$), 43.61 (CH-P), 45.48 ppm (HC-C=O). The mass spectrum of **10a** exhibited the peak at 483 (M^{-1} – (H_2O), 43%), and at 395 for [M^{+2} - $PO(OCH_3)_2$, 9 %]. Moreover, its ^{31}P NMR spectrum affirmed the phosphonate structure and showed a signal at δ 36.00 ppm. Likewise, the reaction of bis-benzylidene **2** with excess of triisopropyl phosphite **4b** in dry boiling toluene to give the diisopropyl phosphonate **10b**, only one product was yielded whether use one mol or excess of the reagent **4b** were employed. Phosphonate **10b** was deduced from its analysis, IR, ^1H NMR, ^{13}C

NMR, ^{31}P NMR and mass spectrum data. The formation of phosphonates **10a** and **10b** were obtained probably by nucleophilic attack of the tertiary phosphite ester on the methine carbon which is the reactive center of α, β-unsaturated carbonyl system, the dipolar intermediate (E) was formed. Then under the influence of atmospheric moisture (unavoidable moisture) the unstable intermediate (F) was produced with pentacovalent phosphorus [23]. The latter could convert to dialkyl phosphonates **10a** and **10b** via expulsion of an alcohol molecule (Scheme 3). On the other hand, the study was extended to comprise the reaction of bis-benzylidene **2** with diisopropyl phosphite **5** to confirm the structure of the diisopropyl phosphonate **10b**.We have found that the reaction proceeds in the same mechanism and afforded compound **10b** (Scheme 3). Its structure was assigned from correct elemental analysis and spectral data.



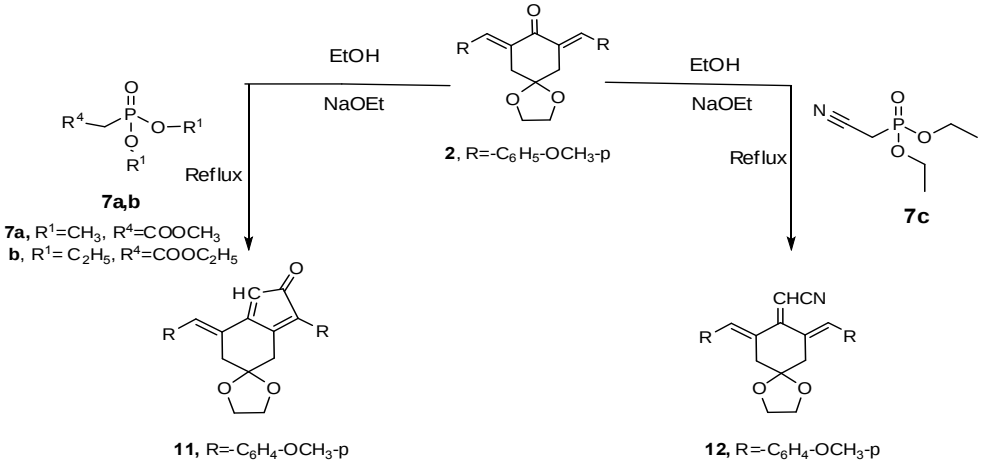
Scheme 3

When bis(4-methoxybenzylidene)cyclohexanone **2** was reacted with stable phosphonium ylides namely (carbmethoxymethylene)- **(6a)** or (carbethoxymethylene) –triphenylphosphorane **(6b)** in dry boiling toluene, afforded dihydrospiroindenedioxolanone derivative **11** and triphenylphosphane oxide. The reaction of bis-benzylidene **2** with phosphonium ylide **6a** in boiling toluene (1:2) molar ratio, give the olefinic compound **11** together with triphenylphosphane oxide. Structure illustration of adduct **11** is deduced from its elemental analysis and spectral data. The ^{1}H NMR spectrum of compound **11** showed a signal at δ 7.74 ppm owing to the methine proton attached to carbonyl group, and two doublet signals at δ 7.37 and 7.17 ppm for (4 H, H arom), at δ 6.96 – 6.86 ppm as multiplet for (4 H, H arom), a singlet signal at δ 6.27 ppm for (1 H, C=CH benzylidene), at δ 3.95 – 3.88 ppm as multiplet for (4 H, 2 OCH_2), two singlet signals at δ 3.84, 3.82 ppm owing to (6 H, 2 OCH_3), and two multiplet signals at δ 2.53 and 2.02 ppm for (2 CH_2). Actually, the structure assigned for compound **11** is based on ^{13}C NMR spectroscopy, which confirmed the presence of signal at δ 187.27 ppm ascribed to C=O group attached to the methine carbon, a signal at δ 160.05 ppm for (C=CH-C=O), 158.06, 158.15 (2 C-OCH_3), 64.95 (2 OCH_2), 55.61 (2 OCH_3), 37.91, 35.20 (2 CH_2) (Scheme 4). Moreover, the treatment of benzylidene **2** with (carbethoxymethylene)triphenylphosphorane **(6b)**, compound **11** was also obtained together with triphenylphosphane oxide. Its structure was deduced from its spectral data (Scheme 4).



Scheme 4

It worthy to mention that, when compound **2** was allowed to react with dialkoxyphosphonate **7a** or **7b**, in alchoholic sodium alkoxide solution, adduct **11** was isolated in 30 % yield [24]. While the reaction of compound **2** with diethyl(cyanomethylene)phosphonate **7c** under the same reaction condition yielded compound **12** in a good yield (Scheme 5). The structure assignment for dispiroylideneacetonitrile **12** is based on different spectroscopic data and elemental analysis. The ^{1}H NMR spectrum of adduct **12** showed a characteristic singlet signal owing to olefinic methine proton attached to cyano group at δ 5.41ppm.^{13}C NMR spectrum of compound **12** showed signals at δ 164.40, 117.49, 92.82, and 45.88, 38.90 ppm corresponding to C=CH, CN, HC-CN, and ($2CH_2$), respectively. Compound **12** was produced via carbonyl olefination by two moles of reagent **7c** to give dispiroylideneacetonitrile **12**. Due to the presence of sodium ethoxide as a base catalyst in the reaction condition (Scheme 5).



Scheme 5

3.2. Antimicrobial Activity

The antimicrobial and antifungal activities were carried out in the Microbial Chemistry Department, National Research Centre, using agar cup plate method [25].

The obtained results are compared with the reference antibiotics namely, Neomycin (bactericide) and Cyclohexamide (fungicide) that were purchased from Egyptian markets. The microbiological assay of new compounds is done by comparing the zone of inhibition formed by the microorganisms to a specific concentration of antibiotics having a known activity. All the preparing compounds **8a, 8b, 9a, 9b, 10a, 10b, 11,** and **12** were evaluated as antimicrobial and antifungal activity in vitro against, *Staphylococcus aureus* (Gram positive) bacteria, *Escherichia coli* (Gram negative) bacteria, *Candida albicans* (yeast) and *Aspergillus niger* (fungus). The obtained results were demonstrated in Table (1). They showed that most of them had different responses. The tested compounds revealed relatively good results as antifungal activity against *Candida albicans* (yeast). The activity of these compounds is arranged in descending order as **8a**, **9b**>**8b**>**11**>**10a**,**10b**>**12**>**9a** according to their inhibition zone values 19, 19, 18, 17, 16, 16, 16 and 15 mm, respectively. They also showed good antibacterial activity against *Staphylococcus aureus* (G+ve) bacteria. The descending order of the activity of them was **8a**>**11** >**8b, 10b** >**9b, 12** > **9a**>**10a**, by revealing different inhibition zone values of 18, 17, 16, 16, 15, 15, 14 and 13 mm, respectively. Some of the tested compounds exhibited activity against *Aspergillus niger* (fungus). The clear inhibition zone for compounds **9b**, **10b, 11** and **12** reached 12, 13, 14 and 16 mm, respectively, and it is considered a moderate results. Moreover, compounds **9b, 10b** and **12** showed also moderate effect against *Escherichia coli* (G-ve) bacteria. Some of the screened compounds did not exhibit any promising activity against the tested bacteria and fungi.

Sample Name	Clear zone (ϕmm)			
	Staphylococcus aureus	*Escherichia coli*	*Candida albicans*	*Aspergillus niger*
8a	**18**	0	**19**	0
8b	16	0	18	0
9a	14	0	15	0
9b	15	14	**19**	12
10a	13	0	16	0
10b	16	16	16	13
11	17	0	17	14
12	15	14	16	16
Neomycin (200µg/ml)	26	28	24	0
Cyclohexamide (200µg/ml)	0	0	0	32

Table 1. Growth inhibition zone (mm) for new synthesized tested compounds against *Staphylococcus aureus* (Gram positive), *Escherichia coli* (Gram negative), *Candida albicans* (yeast) and *Aspergillus niger* (fungus).

Conclusion

From the results of the present study, it could be concluded that the reaction of 7,9 bis (4-methoxybenzylidene)-1,4-dioxaspiro[4.5]decan-8-one **2** with different organophosphorus reagents, afforded various products depending on the nature of the reagent, reaction conditions as well as the stability of the intermediates. Thus, the reaction of benzylidene **2** with tris(dialkylamino)phospine derivatives **3a,b**, led to the formation of two products for each derivative namely dialkylaminooxaphospholedioxolane oxides **8a,b** and tetraalkylphosphonic diamides **9a,b** depending on the nature of the reagents as well as the stability of the intermediate. Moreover, dialkylphosphonates **10a,b** were produced from the reaction of benzylidene **2** with trialkylphosphite derivatives **4a,b**. **10b** was also produced from the reaction of benzylidene **2** with dialkylphosphite **5**. While, stabilized phosphonium ylides **6** proceed according to the Wittig reaction to yield the olefinic product **11** which also obtainbed from the reaction of compound **2** with trialkoxyphosphonates **7a** and **7b**, in alchoholic sodium alkoxide solution. In addition, 1,4-dioxaspiroylideneacetonitrile **12** was isolated from the reaction of benzylidene **2** with Wittig Horner reagent **7c**. Compound **12** can be obtained under the influence of the base present in the reaction condition. In addition, the synthesized compounds were screened for their antibacterial and antifungal activity. They showed good results against *Staphylococcus aureus* (G+ve) bacteria and *candida albicans* (yeast). Compound **9a** showed a good activity against yeast with inhibition zone (19mm),

compound **8a** revealed a good result against *St. aureus* (G+ve) bacteria.

4. Conflicts of interest

The authors have no conflict of interest

5. Acknowledgments

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