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Synthesis of Novel Alkyl (dialkoxyphosphoryl)-1*H*-indole-3-yl)acetate, Dialkoxyphosphoryl[2,3-b]indole-3-carboxylate and Dialkyl methyl phosphonate Derivatives Using Wittig-Horner Reagents and their Antimicrobial Activity.

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ABSTRACT

The reaction of diazoisatin with five derivatives of Wittig-Horner reagents; trialkylphosphonoacetates, diethyl(pyrrolidinomethyl)phosphonate, diethyl (1,3-dioxoisoindolin-2-yl) methylphosphonate, (diethylphospho) acetonitrile and diethyl 4-methoxybenzylphosphonate has been reported. Some of the prepared products were screened for their antimicrobial activity.
Keywords: Diazoisatin; Wittig-Horner Reagents; Antimicrobial Activity.

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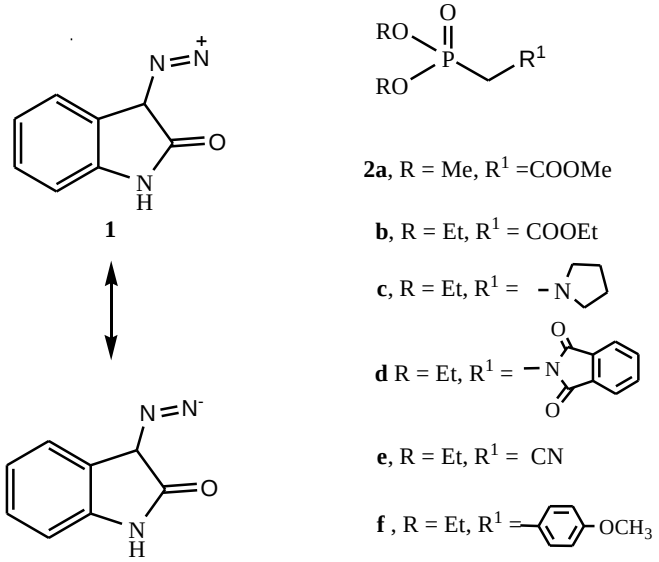
INTRODUCTION

Diazo compounds are among the most versatile intermediate in organic synthesis. Because of their inherent dipolar nature, diazo compounds can readily participate in 1,3-dipolar cycloaddition reactions with a wide range of dipolarphiles [1,2]. Moreover, C-protonation gives rise to diazonium ions, which are highly reactive alkylating agents [2]. The broad reactivity makes diazo compounds attractive for applications in chemical biology having special promise in labeling of proteins [3-13] and as tunable reactants in 1,3-dipolar cycloaddition reactions with cycloalkynes [1,2,14]. In view of this and in continuation of our work in organophosphorus chemistry [15-20], it was of considerable interest to study the reactivity of diazoisatin **1** towards Wittig-Horner reagents **2a-f** (Scheme 1).



Scheme 1

EXPERIMENTAL SECTION

MATERIALS and METHOD

Melting points were determined in open glass capillaries using Electrothermal IA 9000 series digital melting point apparatus (Electrothermal, Essex, UK) and are uncorrected. Diazoisatin **1** was easily prepared in 85% yield according to the literature [21]. The IR spectra were measured in KBr pellets with a Perkin-Elmer Infracord Spectrophotometer model 157(Grating). NMR spectra were obtained on Joel-500 MHz Spectrometer (^{1}H NMR at 500 MHz, ^{13}C NMR at 125 Hz) in $CDCl_3$/ or DMSO-d_6 using TMS as internal standard. Chemical shifts (δ) were given in ppm and coupling constants (J) in Hz. The ^{31}P NMR spectra were taken with a Varian CFT-20 (vs. external 85% H_3PO_4 standard). The mass spectra were performed at 70eV on a Shimada GCS-OP 1000 Ex Spectrometer provided with a data system. Elemental analyses were performed using Elmenter Varu EL Germany Instrument. The reported yields are based upon pure materials isolated by column chromatography. Solvents were dried/purified according to conventional procedures.

General Procedures of Reaction of 3-diazo-1,3-dihydro-2H- indol-2-one 1 with Wittig-Horner reagents 2a-f

A solution of 1 mmol of sodium alkoxide in absolute alcohol (30 mL) was treated with an equimolar amount of the Wittig-Horner reagents **2a-f** (1 mmol) then diazoisatin **1** (1 mmol, 0.15g) was added. The resulting reaction mixture was allowed to reflux for 4-8 h (TLC). Thence, the reaction mixture was poured onto a small amount of water (2mL), extracted with ethyl acetate (3*20mL), the extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was subjected to silica gel column chromatography to give products **3-9**. Product **5** was isolated as deep red crystals, M.p. 350-352 ^{o}C (lit.[22-24], M.p. 350^{o}C).

(*E*)-Methyl (2-oxoindoline-3-ylidene)acetate 3a

Eluent: petroleum ether (60-80°C)/ethyl acetate (90/10, *v/v*). Product **3a** was separated as orange crystals, yield 0.034g (10%). M.p. 177-179 °C, ref. [25,26]. IR (KBr): *v* = 3167 (NH), 1712 (ester C=O), 1628 (amide C=O), 1612 (C=C). ^{1}H NMR (500.14 MHz, $CHCl_3$): δ = 3.30 (s, 3H, OCH_3), 6.92 (s, 1H, vinyl), 7.07 (d, J_{HH} = 7.6 Hz, 1H, H_{arom} at C-7), 7.09, 7.25 (dt, J_{HH} = 7.4 and 1.2 Hz, 2H, H_{arom} at C-5, C-6), 7.52 (s, 1 H, NH, exchangeable with D_2O), 7.91 (dd, J_{HH} = 7.8 and 1.0 Hz, 1 H, H_{arom} at C-4) ppm. ^{13}C NMR (125.76 MHz, $CHCl_3$): δ = 51.3 (OCH_3), 117.2-143.8 (Ar-C), 166.1 (amide C=O), 171.4 (ester C=O) ppm. MS (EI, 70 eV): *m/z* (%) = 203 (43) $[M]^+$, 172 (25), $[M\text{-}OCH_3]^+$. Anal. for $C_{11}H_9NO_3$ (203.19): Calcd C, 65.02; H, 4.46; N, 6.89; Found C, 65.32; H, 4.22; N, 6.97.

***(E)*-Ethyl (2-oxoindoline-3-ylidene)acetate 3b**

Eluent: petroleum ether (60-80°C)/ethyl acetate (90/10, *v/v*). Product **3b** was separated as yellow crystals, yield 0.038g (10%). M.p. 163-164 °C, ref [27]. IR (KBr): *v* = 3160 (NH), 1712 (ester C=O), 1620 (amide C=O), 1613 (C=C) cm^{-1}. ^{1}H NMR (500.14 MHz, $CHCl_3$): δ = 1.36 (t, J_{HH} = 6.2Hz, 3 H, $COOCH_2\mathit{CH_3}$), 4.32 (q, J_{HH} = 6.2Hz, 2 H, $COO\mathit{CH_2}CH_3$), 6.83 (s, 1H, vinyl), 7.07 (d, J_{HH} = 7.6 Hz, 1H, H_{arom} at C-7), 7.09, 7.25 (dt, J_{HH} = 7.4 and 1.2 Hz, 2H, H_{arom} at C-5, C-6), 7.77 (br.s, 1 H, NH, exchangeable with D_2O), 8.56 (dd, J_{HH} = 7.3 and 1.1 Hz, 1 H, H_{arom} at C-4) ppm. ^{13}C NMR (125.76 MHz, $CHCl_3$): δ = 14.5 (CH_3), 61,8 (CH_2), 119.6-144.7 (Ar-C), 170.0 (ester C=O), 179.2 (amide C=O)ppm. MS (EI, 70 eV): *m/z* (%) = 217(6) $[M]^+$. Anal. for $C_{12}H_{11}NO_3$ (217.22): Calcd C, 66.35; H, 5.10; N, 6.45; Found C,66.68; H, 5.25; N, 6.73.

***Syn*-Methyl (dimethoxyphosphoryl)(2-oxo-2,3-dihydro-1*H*-indol-3-yl)acetate 4a**

Eluent: petroleum ether (60-80°C)/ethyl acetate (75/25, *v/v*). Product **4a** was separated as deep brown crystals, yield 0.05g (15%). M.p. 182-184 °C. IR (KBr): *v* = 3160 (NH), 1720 (ester C=O), 1663 (amide C=O), 1228 (P=O), 1049 (P-O-C) cm^{-1}. ^{1}H NMR (500.14 MHz, $CDCl_3$): δ = 3.29, (1s, $^3J_{HP}$ = 11.5 Hz, 6H, 2 P(O)($O\underline{CH_3}$)), 3.30 (s, 3H, $COO\underline{CH_3}$), 4.21 (dd, $^2J_{HP}$ = 21.3 Hz, J_{HH} = 7.8 Hz, 1H, CH^a), 4.93 (dd, $^3J_{HP}$ = 11.5 Hz, J_{HH} = 7.8 Hz, 1H, CH^b), 6.79 -7.70 (m, 3H, H_{arom}), 7.91 (broad, 1H, NH exchangeable with D_2O), 9.11 (dd, 1H, ArH at C-4) ppm. ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 30.3 (d, $^2J_{CP}$ = 35.37 Hz, $\underline{CH}^b$), 39.4 (d, J_{CP} = 133.20, $\underline{CH}^a$), 51.2 (s, $COOCH_3$), 53.0 (OCH_3, $^2J_{CP}$ = 37.37 Hz), 123.1-143.7 (Ar-C), 163.1 (ester C=O), 174.7 (C=O amide) ppm. ^{31}P NMR = 23 ppm. MS (EI, 70 eV): *m/z* (%) = 313 (57.77) $[M]^+$, 268 (10.31.) $[M\text{-}3\ CH_3]^+$. Anal. for $C_{13}H_{16}NO_6P$ (313.24): Calcd C, 49.85; H, 5.15; N, 4.47; P, 9.89; Found C, 50.05; H, 5.25; N, 5.07; P, 9.90.

***Syn*-Ethyl (diethoxyphosphoryl)(2-oxo-2,3-dihydro-1*H*-indol-3-yl)acetate 4b**

Eluent: petroleum ether (60-80°C)/ethyl acetate (75/25, *v/v*). Product **4b** was separated as brown crystals, yield 0.04g (13%). M.p. 191-193 °C. IR (KBr): *v* = 3155 (NH), 1732 (ester C=O), 1670 (amide C=O), 1226 (P=O), 1051 (P-O-C) cm^{-1}. ^{1}H NMR (500.14 MHz, $CDCl_3$): δ = 1.33 (t, J_{HH} = 6.2Hz, 6H, $CH_2\mathit{CH_3}$), 1.52 (t, J_{HH} = 6.2Hz, 3H, $COOCH_2\mathit{CH_3}$,), 4.11 (dd, $^2J_{HP}$ = 15.5 Hz, 1H, CH^a), 4.13 (2q, J_{HH} = 6.2Hz, 4H, P-(O-$\mathit{CH_2}$-)$_2$), 4.18 (q, J_{HH} = 6.2Hz, 2H, COO-$\mathit{CH_2}$), 4.87 (dd, $^3J_{HP}$ = 11.5 Hz, 1H, CH^b), 6.70 -7.70 (m, 3H, H_{arom}), 9.13 (dd, J_{HH} = 7.7 Hz, 1H, ArH at C-4), 7.91 (broad, 1H, NH exchangeable with D_2O) ppm. ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 13.9, 14.2 (3 CH_3), 38.1 (d, J_{CP} = 88.2 Hz, $\underline{C}H^a$), 62.5, 63.0 (3 CH_2), 33.2($\underline{C}H^b$), 127.1-142.3 (Ar-C), 170.7 (ester C=O) 178.0 (amide C=O) ppm. ^{31}P NMR: δ = 23.20 ppm. MS (EI, 70 eV): *m/z* (%) = 355 (65) $[M]^+$, 310 (15.31.) $[M\text{-}3CH_3]^+$, 268 (22.11) $[M\text{-}3\ CH_2CH_3]^+$. Anal. for $C_{16}H_{22}NO_6P$ (355.32): Calcd C, 54.08; H, 6.24; N, 3.94; P, 8.72; Found C, 53.98; H, 6.28; N, 4.01; P, 8.45.

***(E)*-3-(3-oxoindolin-2-ylidene)indolin-2-one 5**

Eluent: petroleum ether (60-80°C)/ethyl acetate (80/20, *v/v*). Product **5** was separated as dark red crystals, yield 0.068g (20%). M.p. 350 °C (lit.[25,26] m.p 350 °C). ^{1}H NMR (500.14 MHz, DMSO): δ = 6.87 – 7.01 (m, 8H, H arom.), 9.01, 10.08 (2s, 2 NH exchangeable with D_2O) ppm. MS (EI, 70 eV): *m/z* (%) = 262 (75) $[M]^+$. Compound **5** gave the correct elemental analyses was characterized by TLC analyses (one spot) and comparative IR spectra with authentic specimen [25,26].

***Syn*-Methyl 2-(dimethoxyphosphoryl)-3,3a-dihydro-2*H*-furo[2,3-b]indole-3-carboxylate 6a**

Eluent: petroleum ether (60-80°C)/ethyl acetate (60/40, *v/v*). Product **6a** was separated as yellow crystals, yield 0.1g (30%). M.p. 210-212 °C. IR (KBr): *v* = 1728 (ester C=O), 1228 (P=O), 1050 (P-O-C) cm^{-1}. ^1H NMR (500.14 MHz, $CDCl_3$): δ = 2.53 (d, $^3J_{HP}$ = 11.5 Hz, J_{HH} = 7.8 Hz, 1H, CH^b), 2.94 (s, $^3J_{HP}$ = 11.5 Hz, 6 H, 2 P(O)(OCH_3), 3.45 (s, 3 H, $COOCH_3$), 3.39 (d, J_{HH} = 7.8 Hz, 1 H, CH^a), 4.3 (dd, $^2J_{HP}$ = 21.3 Hz, J_{HH} = 7.8 Hz, 1 H, CH^c), 6.92.-7.25 (m, 3H, H_{arom}), 8.10 (d, 1 H, ArH at C-4) ppm. ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 36.1-37.8 (2s, CH^a, CH^b), 52.8 (s, $COOCH_3$), 53.4 (OCH_3, $^2J_{CP}$ = 37.37 Hz), 67.9 (d, J_{CP} = 102.20, CH^c), 123.5-150.2 (Ar-C), 164.7 (C=O), 168.1 (ester C=O) ppm. ^{31}P NMR = 8.66 ppm. MS (EI, 70 eV): *m/z* (%) = 325 (57.33) $[M]^+$, 310 (50), 294 (100) $[M\text{-}OCH_3]^+$. Anal. for $C_{14}H_{16}NO_6P$ (325.25): Calcd C, 51.70; H, 4.96; N, 4.31; P, 9.52; Found C, 51.54; H, 5.06; N, 4.01; P, 9.77.

***Syn*-Ethyl 2-(diethoxyphosphoryl)-3,3a-dihydro-2*H*-furo[2,3-b]indole-3-carboxylate 6b**

Eluent: petroleum ether (60-80°C)/ethyl acetate (60/40, *v/v*). Product **6b** was separated as yellow crystals, yield 0.11g (30%). M.p. 230-232 °C. IR (KBr): *v* = 1728 (ester C=O), 1229 (P=O), 980 (P-O-C) cm^{-1}. ^1H NMR (500.14 MHz, $CDCl_3$): δ = 1.31 (2t, J_{HH} = 6.2Hz, 6 H, CH_2CH_3), 1.34 (t, J_{HH} = 13.8 Hz, 3 H, CH_2CH_3), 2.10 (d, $^3J_{HP}$ = 11.5 Hz, J_{HH} = 7.8 Hz, 1H, CH^b), 2.92 (d, J_{HH} = 7.8 Hz, 1H, CH^a), 4.20 (dd, $^2J_{HP}$ = 15.5 Hz, J_{HH} = 7.8 Hz, 1H, CH^c), 4.39 (2q, J_{HH} = 6.2Hz, 4 H, CH_2CH_3), 4.40, (q, J_{HH} = 6.2Hz, 2 H, $COOCH_2$), 6.80-7.30 (m, 3H, H_{arom}), 8.31 (d, 1 H, ArH at C-4) . ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 13.8, 14.2 ($3CH_3$), 36.1-37.8 (2s, CH^a, CH^b), 61.2, 63.5 (3 CH_2), 68.2 (d, J_{CP} = 88.2 Hz, CH^c), 121.3-147.2 (Ar-C), 160.2 (C=N), 170.7 (ester C=O) ppm. ^{31}P NMR: δ = 8.61 ppm. MS (EI, 70 eV): *m/z* (%) = 367 (2) $[M]^+$, 352 (53) $[M\text{-}CH_3]^+$. Anal. for $C_{17}H_{22}NO_6P$ (367.33): Calcd C, 55.58; H, 6.04; N, 3.81; P, 8.43; Found C, 55.32; H, 5.94; N, 4.01; P, 8.34.

***Syn*-Diethyl [(2-oxo-2,3-dihydro-1*H*-indol-3-yl)(pyrrolidin-1-yl)methyl]phosphonate 7a**

Eluent: petroleum ether (60-80°C)/ethyl acetate (80/20, *v/v*). Product **7a** was separated as pale yellow crystals, yield 0.11g (30%). M.p. 210-213 °C. IR (KBr): *v* = 3160 (NH), 1633 (amide C=O), 1227 (P=O), 998 (P-O-C) cm^{-1}. ^1H NMR (500.14 MHz, $CDCl_3$): δ = 1.24 (t, J_{HH} = 8.8Hz, 6H, 2 CH_2CH_3), 1.38- 2.33 (8H, CH_2 pyrrolidine), 3.01 (dd, $^2J_{HP}$ = 15.5 Hz, 1H, CH^a), 4.21 (d, $^3J_{HP}$ = 11.5Hz, J_{HH} = 7.8 Hz, 1H, CH^b), 4.37 (2q, J_{HH} = 6.2 Hz, 4H, P-($O\text{-}CH_2CH_3$)$_2$), 7.01 (d, J_{HH} = 7.6 Hz, 1H, H_{arom} at C-7),7.25, 7.53 (dt, J_{HH} = 7.4 and 1.2 Hz, 2H, H_{arom} at C-5, C-6), 8.42 (dd, J_{HH} = 7.8 and 1.0 Hz, 1 H, H_{arom} at C-4), 10.55 (s, 1 H, NH, exchangeable with D_2O) ppm. ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 14.5 (2 CH_3), 26.6, 58.2 (CH_2 pyrrolidine), 33.4 (CH^a), 49.7 (d, J_{CP} = 88.2 Hz, CH^b), 63.4 (2 CH_2), 119.0-143.8 (Ar-C), 178.0 (amide C=O) ppm. ^{31}P NMR: δ = 27.4 ppm. MS (EI, 70 eV): *m/z* (%) = 352 (8.22) $[M]^+$, 280 (23) [M-pyrrolyl radical]$^+$. Anal. for $C_{17}H_{25}N_2O_4P$ (352.37): Calcd C, 57.95; H, 7.15; N, 7.95; P, 8.79; Found C, 58.05; H, 7.45; N, 8.01; P, 8.45.

***Syn*-Diethyl (2-oxoindolin-3-yl)(1,3-dioxoisoindolin-2-yl)methylphosphonate 7b**

Eluent: petroleum ether (60-80°C)/ethyl acetate (80/20, *v/v*). Product **7b** was separated as pale yellow crystals, yield 0.13g (30%). M.p. 230-231 °C. IR (KBr): *v* = 3282 (NH), 1612 (amide C=O), 1225 (P=O), 980 (P-O-C) cm^{-1}. ^1H NMR (500.14 MHz, $CDCl_3$): δ = 1.23 (t, J_{HH} = 13.8 Hz, 6 H, 2 CH_2CH_3), 4.01 (dd, $^2J_{HP}$ = 15.5 Hz, 1 H, CH^a), 4.21(d, $^3J_{HP}$ = 11.5 Hz, J_{HH} = 7.8 Hz, 1H, CH^b), 4.36 (2 q, J_{HH} = 6.2Hz, 4H, P-($O\text{-}CH_2$-)$_2$), 7.01-8.42 (m, 8 H, H_{arom}), 10.55 (s, 1 H, NH, exchangeable with D_2O) ppm. ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 14.3 (2 CH_3), 32.8 (CH^b), 49.1 (d, J_{CP} = 88.2 Hz, CH^a), 63.3 (2 CH_2), 122.0-144.8 (Ar-C), 162.0 (Phthalimido C=O), 178.0 (amide C=O) ppm. ^{31}P NMR: δ = 26.0 ppm. MS (EI, 70 eV): *m/z* (%) = 428 (8.22) $[M]^+$, 399 (23) $[M\text{-}C_2H_5]^+$. Anal. for $C_{21}H_{21}N_2O_6P$ (428.38): Calcd C, 58.88; H, 4.94; N, 6.54; P, 7.23; Found C, 58.55; H, 4.90; N, 6.63; P, 7.12.

***(2Z)*2-(2-Oxo-2,3-dihydro-1*H*-indol-3-yl)but-2-enedinitrile 8**

Eluent: petroleum ether (60-80°C)/ethyl acetate (20/80, *v/v*). Product **8** was separated as yellowish brown crystals, yield 0.16g (50%). M.p. 224-226 °C. IR (KBr): *v* = 3151 (NH), 1643 (amide C=O), 2228 (CN) cm^{-1}. ^1H NMR (500.14 MHz, $CDCl_3$): δ = 3.59 (s, $^4J_{HH}$ = 2.8 Hz, 1H, CH^b), 5.90 (s, $^4J_{HH}$ = 2.8 Hz, 1 H, CH^a), 7.06 (t, J_{HH} = 7.4 and 1.2 Hz, 1 H, H_{arom} at C-5), 7.06 (t, J_{HH} = 7.4 and 1.2 Hz, 1 H, H_{arom} at C-6), 8.01 (s, 1 H, NH, exchangeable with D_2O), 8.25 (dd, J_{HH} = 7.8 and 1.0 Hz, 1 H, H_{arom} at C-4) ppm. ^{13}C NMR (125.76 MHz, $CDCl_3$): δ = 43.9 (CH^b), 108.7, 128.9 (CH=C), 112.2 (CH^a), 112.7(C-*CN*), 127.1-147.6 (Ar-C), 168.43 (amide C=O) ppm. MS (EI, 70 eV): *m/z* (%) = 209 (5) $[M]^+$, 212.07 (29), 196 (18) $[M\text{-}OH]^+$. Anal. for $C_{12}H_7N_3O$ (209.2): Calcd C, 68.89; H, 3.37; N, 20.09; Found C, 69.03; H, 3.07; N, 19.99.

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***(E)*-3-(4-methoxybenzylidene)indolin-2-one 9**

Eluent: petroleum ether (60-80°C)/ethyl acetate (90/10, *v/v*). Product **9** was separated as pale yellow crystals, yield 0.06g (15%). M.p. 152-154 °C (lit. [28] m.p 156 °C). MS (EI, 70 eV): *m/z* (%) = 251 (65) $[M]^+$. Compound **9** was characterized by comparing its m.p. as IR spectrum with those of a reference sample [28].

BIOLOGICAL EVALUATION OF THE TESTED COMPOUNDS

Biological Screening

The antibacterial and antifungal activities were carried out in the Microbial Chemistry Department, National Research Centre, using the diffusion plate method [29-32].

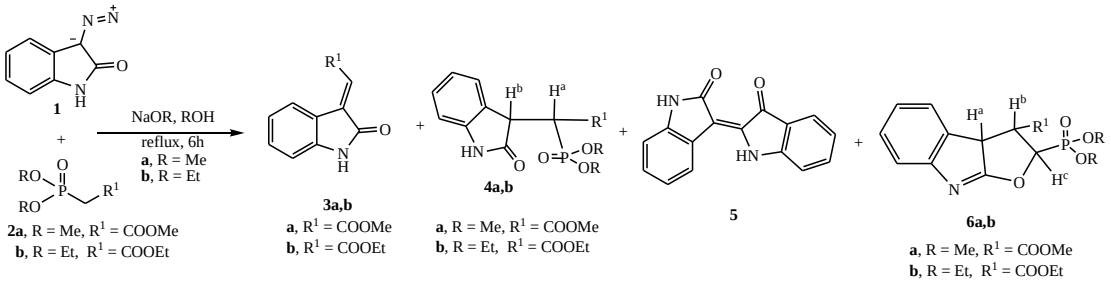
Procedure

A disc of sterilized filter paper saturated with measured quantity (25 µL) of the tested sample (1 mg/mL final concentration) was placed on a plate (9 cm diameter) containing a solid bacterial medium (nutrient agar) or a fungal medium (Dox's medium) which has been seeded with the spore suspension of the test organism. After incubation at 37 °C for 24 h for bacteria (in case of fungi, at 25 °C for 72 h), the diameter of the clear zone of inhibition surrounding the sample was taken as a measure of the inhibitory power of the sample against the particular test organism (% inhibition = sample inhibition zone (cm)/plate diameter × 100). All measurements were done in chloroform as a solvent.

RESULTS AND DISCUSSION

Chemistry

When 2-diazo-1,3-dihydro-2*H*-indole-2-one (diazoisatin) (**1**) was treated with one mol equivalent of trimethylphosphonoacetate **2a** in the presence of methanolic sodium methoxide solution at reflux temperature for 6h, *E*-methyl-2-(oxoindoline-3-ylidene)acetate (**3a**, 10 %), *Syn*-methyl(dimethoxyphosphoryl)(2-oxo-2,3-dihydro-1*H*-indole-3-yl)acetate (**4a**, 15%), indirubin (**5**, 20%, 0.068 gm) and *Syn*-methyl 2-(dimethoxyphosphoryl)-3,3a-dihydro-*2H*-furo[2,3-b]indole-3-carboxylate (**6a**, 30 %) were obtained (Scheme 2).



Scheme 2

Structural assignments for compounds **3a,b**, **4a,b**, **5**, and **6a,b** were based upon elemental and spectroscopic data. The most important features of *E*-methyl-2-(oxoindoline-3-ylidene)acetate (**3a**) were confirmed according to the following evidences: elemental and mass spectral analysis led to empirical formula $C_{11}H_9NO_3$. The ^{1}H NMR (500 MHz) spectrum of **3a** showed a singlet at δ H = 3.30 (s, 3 H, OCH_3), a singlet at δ 6.92 ppm for the exocyclic vinyl proton. The phenyl proton at C-7 appeared as a doublet centered at 7.07 ppm with coupling constant J_{HH} = 7.8 Hz whereas the chemical shift of proton at C-4 is deshielded at 7.91 ppm, due to the anisotropic effect of the carbonyl group of the ester [25,33] and split into doublet with J_{HH} = 7.8 and 1.2 Hz. The other two phenyl protons at C-5 and C-6 appeared as two di-*ortho*/*meta* triplet of doublets [34] at 7.09 and 7.25 ppm corresponding to NH group which is exchangeable with D_2O [35].

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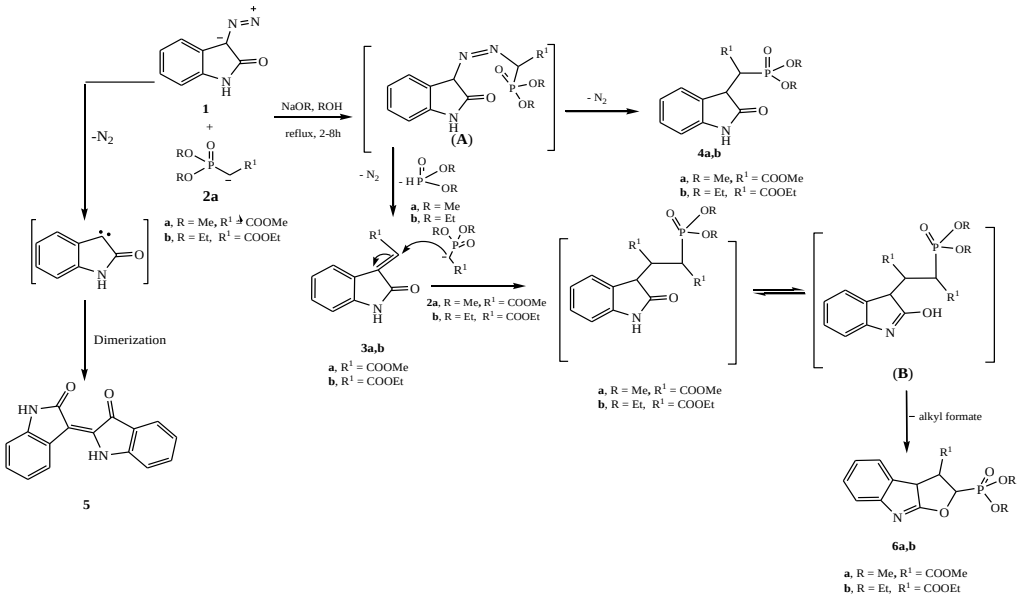
Previously, it has been reported that compound **3a** was obtained exclusively as *E*-isomer by treating isatin with methoxycarbonylmethylenetriphenylphosphorane [36].

Compound **4a** was identified as *syn*-methyl (dimethoxyphosphoryl)(2-oxo-2,3-dihydro-1*H*-indol-3-yl)acetate and its structure was also assigned on the basis of its IR, ^{1}H, ^{13}C, ^{31}P NMR (*cf*. Experimental Section). The main characteristic features of the ^{1}HNMR spectrum of *syn* **4a** (500.14 MHz) is appearance of the methine proton (CH^{a}) as two sets of different chemical shifts at δ = 4.21 (dd, $^{2}J_{HP}$ = 21.3 Hz, J_{HH} = 7.8 Hz, 1H, CH^{a}-P) and at δ = 4.93 (dd, $^{3}J_{HP}$ = 11.5 Hz, J_{HH} = 7.8 Hz, 1H, CH^{b}-CH^{a}-P). The assigned (*syn*) configuration for **4a** is supported by the chemical shifts and coupling constants of both protons (J_{HH} = 7.8 Hz) which indicate the (*syn*) form, rather than the (*anti*) configuration, which is expected to record larger coupling constants (around 16Hz). Moreover, an inspection of a model drawn Newman projection [37, 38] indicates that there is no plausible alternative structure for *syn* **4a**. In the same sense, Product **6a** (major 30% yield) was identified as *syn*-methyl 2-(dimethoxyphosphoryl)-3,3a-dihydro-2*H*-furo[2,3-*b*]indole-3-carboxylate (syn **6a**) on the basis of its IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectral data (*cf*. Experimental Section). It recorded a positive shift δ = 8.66 ppm (s) in the ^{31}P NMR spectrum which support the assigned phosphonate structure [39]. The ^{1}HNMR spectrum of *syn* **6a** (500.14 MHz) showed the methine proton on the heterocyclic ring (H^{a}) at δ = 3.39 (d, $^{4}J_{HP}$ = 7.3 Hz, J_{HH} = 7.8 Hz, 1 H). The methine protons (2H, H^{b}, H^{c}) appeared in two sets of different chemical shifts at δ = 2.53 (d, $^{3}J_{HP}$ = 11.5 Hz, J_{HH} = 7.8 Hz, 1H, CH^{b}) and 4.3 (dd, $^{2}J_{HP}$ = 21.3 Hz, J_{HH} = 7.8 Hz, 1 H, CH^{c}). Again, chemical shifts and coupling constants of both protons (H^{a}, H^{b}, J_{HH} = 7.8 Hz) support the (*syn*) configuration for compound **6a**.

Compound **5** was isolated as dark red crystals in a 20% yield and proved to be indirubin by comparing its melting point and IR spectrum with those of a reference sample [22-24]. It is possibly formed by ejection of N_2 molecule from **1** under the prevailing experimental conditions followed by dimerization of the resulting the carbene species [22-24].

Similarly, when diazoiazatin **1** was treated with one mol equivalent of triethylphosphonoacetate **2b** in the presence of alcoholic sodium ethoxide solution at reflux temperature for 8h, adducts **3b** (10%), **4b** (13%), **5** (20%, 0.07gm) and **6b** (30%) were isolated (Scheme 2). *E*-ethyl-2-(oxoindoline-3-ylidene)acetate (**3b**, 10 %) was isolated and identified by comparing its melting point (m.p 163 - 164 °C, lit. 169- 170°C) and IR spectrum with those of an authentic specimen [25-27]. The structure of **4b** and **6b** were assigned on the basis of elemental analyses, the IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectral data (*cf*. Experimental Section).



Scheme 3

A possible explanation for the course of the reaction of diazoisatin **1** with trialkylphosphonoacetate **2a,b** is depicted in Scheme 3. Initial attack of trialkylphosphonoacetate **2a,b** on the most reactive center of **1** resulted in the formation of the phosphonate intermediate (**A**). Under the influence of the base present in the reaction medium, elimination of both dialkylphosphite and N_2 gave rise to the olefinic compounds **3a,b**. The

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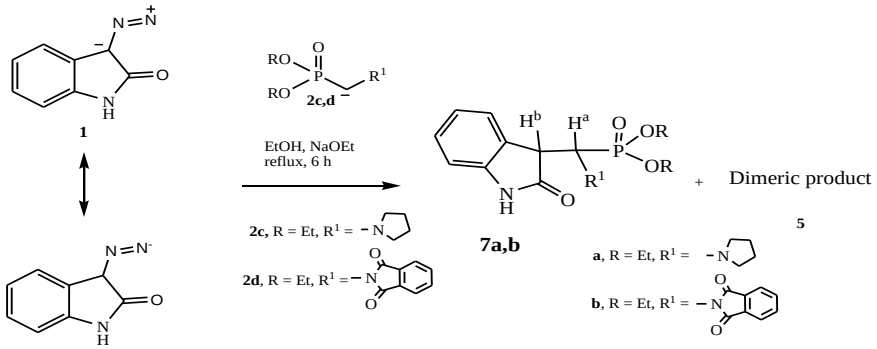
phosphonate products **4a,b** can be obtained *via* expulsion of N_2 from the intermediate (**A**). Compounds **6a,b** presumably, were formed *via* addition of another molecule of trialkylphosphonoacetate **2a,b** to the olefinic compounds **3a,b** to give the intermediate (**B**) followed by elimination of alkyl formate [40] (Scheme 3).

The reaction of diethyl(pyrrolidinomethyl)phosphonate (**2c**) with **1** was completed in ethanolic sodium ethoxide at reflux temperature for 6h to give a pure product (30% yield) for which structure *syn*-diethyl [(2-oxo-2,3-dihydro-1*H*-indol-3-yl)(pyrrolidin-1-yl)methyl]phosphonate (**7a**) was assigned on the basis of compatible IR, ^{1}H, ^{13}C, ^{31}P NMR, and mass spectral data as well as elementary analysis (*cf*. Experimental Section). The assigned (*syn*) configuration for **7a** is supported by the chemical shifts and coupling constants of methine protons (2H, H^a, H^b) in ^{1}HNMR spectrum (500.14 MHz) which showed the methine protons as two sets of different chemical shifts on the CH^a-CH^b-P axis (H^a) at δ = 3.01 (dd, J_{HH} = 7.8Hz, $^{2}J_{HP}$ = 15.5Hz, 1H, CH^a) and (H^b) at δ = 4.21 (d, $^{3}J_{HP}$ = 11.5Hz, J_{HH} = 7.8Hz, 1H, CH^b). Indirubin (**5**) was isolated in a 40% yield (0.15 gm) and identified as mentioned before (vide supra).

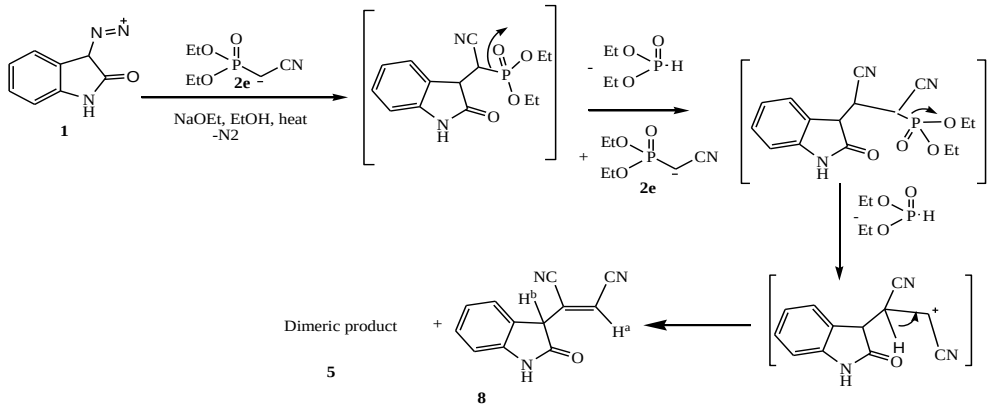
Similarly, the reaction of diethyl (1,3-dioxoisoindolin-2-yl)methylphosphonate (**2d**) with **1** proceeds in ethanolic sodium ethoxide to give a pure product (30 % yield) for which structure *syn*-diethyl (2-oxoindolin-3-yl)(1,3-dioxoisoindolin-2-yl)methylphosphonate (**7b**) was assigned on the basis of compatible IR, ^{1}H, ^{13}C, ^{31}P NMR, and mass spectral data as well as elementary analysis (*cf*. Experimental Section). Indurabin (**5**) was also isolated in a 40% yield (0.18 gm) and identified as mentioned before (vide supra) (Scheme 4).

Formation of compounds **7a**,**b** could be explained in terms of initial addition of one mol of the Wittig-Horner reagents **2c,d** to compound **1** followed by expulsion of N_2 (Scheme 4).



Scheme 4

The reaction of diazoisatin **1** with (diethylphospho)acetonitrile (**2e**) was also investigated. Performing the reaction of **1** with **2e** in the presence of alcoholic sodium ethoxide solution at reflux temperature for 8h, led to the formation of (2Z)2-(2-oxo-2,3-dihydro-1*H*-indol-3-yl)but-2-enedinitrile (**8**) as the main product in 50% yield (Scheme 5). Compound **5** was also isolated from the reaction mixture in 10% yield (0.03 gm). The structure assignments for compound **8** are based upon elemental analysis and spectroscopic (IR, ^{1}H NMR, ^{13}C NMR) as well as MS data (*cf*. Experimental Section).



Scheme 5

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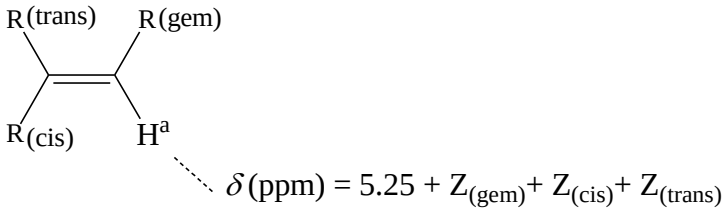
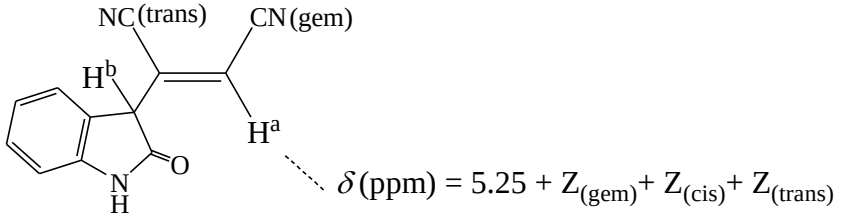
The ^{1}H NMR spectrum of compound **8** showed the proton on the heterocyclic oxindolyl ring (H^b) as a doublet (J_{HH} = 2.8Hz) at δ = 3.59 due to an allylic type of coupling with H^a. Meanwhile, proton H^b also appeared as a doublet (J_{HH} = 2.8Hz) for the same reason at δ = 5.90 ppm.

In order to determine the configuration of the methine proton (H^a) on the exocyclic ethylenic bond, use was made of the additive increment system (Eqn. 1) [41].



Eqn. 1

Applying equation 1 for compound **8** δ (ppm) = 5.25 + 0.27 + (-0.08) + 0.55
δ = 5.99 (calcd.); δ = 5.90 (found); Deviation = D = 0.09ppm

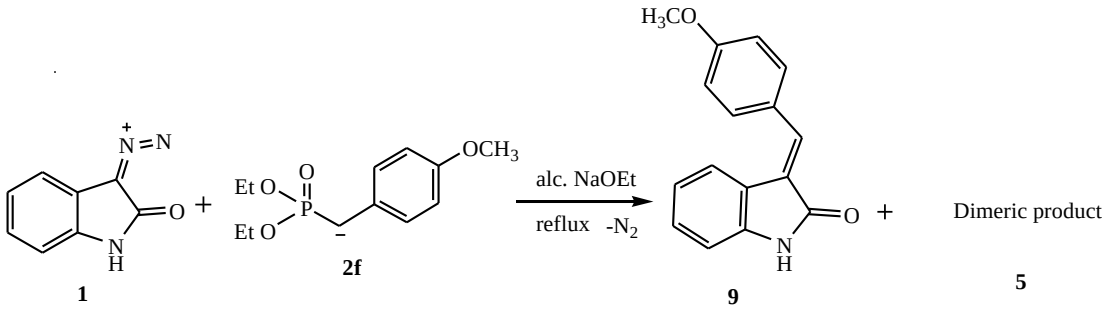


8

Compound 8

The calculated shift for H^a in compound **8** (δ = 5.99 ppm) is in good agreement with the experimental value (δ = 5.90 ppm) which deviation in only 0.09 ppm denoting that the two cyano groups in **8** are *Z* (*cis* or *syn*) with respect to one another.

Next, the reaction of **1** with diethyl 4-methoxybenzylphosphonate (**2f**) was performed in alcoholic sodium ethoxide solution to give product (**9**, 15% yield) and (**5**, 60 % yield, 0.24 gm). Compound **9** was obtained in chromatographically pure form and was found to possess a sharp melting point. Elemental analyses for compound **9** corresponded to an empirical formula $C_{16}H_{13}NO_2$. The identity of *(E)*-3-(4-methoxybenzylidene)indolin-2-one (**9**) [28] was inferred from its correct analytical and spectroscopic analyses (*cf*. Experimental Section).



1 **2f** **9** **5**

Scheme 6

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Antimicrobial Evaluation [29-32]

As shown in (Table 1). The obtained results indicated large diversity in the antimicrobial effect of the tested compounds. Thus, excellent antibacterial activity was achieved by compounds **8** followed by **6a** and **3a** against the pathogen *Pseudomonas aeruginosa* (negative Gram strain bacterium). In the same time, **3a** and **6a** exhibited also good inhibiting effect against *Staphylococcus aureus* (positive Gram strain bacterium). Compound **3a** gave the same inhibitory effect on both Gram +ve and Gram -ve pathogens while **6a** exerted double the inhibitory effect on the Gram –ve *Pseudomonas aeruginosa* reaching clear zone of 40 mm and only 20 mm clear zone of *Staphylococcus aureus* (Gram +ve pathogen). Distinct antimicrobial effect was obtained from compound **7a** against *Salmonella typhimurium* reaching 22 mm clear zone. On the other hand, **1** is completely inert with zero antimicrobial effect, followed by compound **5**. The results have high importance to biological application especially compounds **8**, **6a**, **3a** and **7a**.

Table 1: The antibacterial and Antifungal Activities of the Synthesized Compounds

	Inhibition zone diameter *mm/mg* sample Compound No.										
Microorganism	Gram Strain	**1**	**3a**	**4a**	**4b**	**5**	**6a**	**7a**	**7b**	**8**	**Ref. antib.** *
Bacillus cereus	+ve	0.0	0.0	0.0	0.0	0.0	10	0.0	12	12	30
Staphylococcus aureus	+ve	0.0	33	11	0.0	0.0	20	12	15	0.0	30
Escherichia coli	-ve	0.0	12	10	12	0.0	18	0.0	10	10	20
Pseudomonas aeruginose	-ve	0.0	32	21	14	0.0	40	20	15	52	50
Salmonella typhimurium	-ve	0.0	21	10	9	0.0	0.0	22	15	0.0	40
Candida albicans	fungus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	44

** Reference antibiotics are Nizo-arm (antifungal) and Penicillin (antimicrobial*

In the present work, we found that the dinitrile isatin derivatives showed promising activity against *Pseudomonas aeruginose* which is known to promote the biological activity. Moreover, 3-(methoxycarbonyl)methylene derivatives showed nearly equal activity with reference antibiotic against *Staphylococcus aureus*.

CONCLUSION

Diazoisatin **1** reacts with trialkoxyphosphonoacetate **2a,b** in the presence of alcoholic sodium alkoxide solution to give the *E*-alkyl-2(oxoindoline-3-ylidene)acetates **3a,b**, *Syn*-alkyl(dialkoxyphosphoryl)(2-oxo-2,3-dihydro-1*H*-indole-3-yl)acetates **4a,b**, indirubin **5** and *Syn*-alkyl-2-(dialkoxyphosphoryl)-3,3a-dihydro-2*H*-furo[2,3-*b*]indole-3-carboxylates **6a,b**. On the other hand, The reaction of **1** with **2c,d** affords the alkylphosphonate derivatives **7a,b**. (Diethylphospho)acetonitrile **2e** reacts with diazoisatin **1** to give 2-(2-oxo-2,3-dihydro-1*H*-indol-3-yl)but-2-enedinitrile (**8**). Moreover, diazoisatin **1** reacts with diethyl-1-methoxy-benzylphosphonate **2f** to give (*E*)-3-(4-methoxy-benzylidine)indolin-2-one **9**. Some of the newly synthesized compounds were selected and screened for antimicrobial activity. The tested compounds revealed antimicrobial activity against different strains.

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