Quim. Nova, Vol. 37, No. 6, 989-995, 2014 http://dx.doi.org/10.5935/0100-4042.20140162

Artigo

SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL ACTIVITY OF A NEW CLASS OF DIALKYLPHOSPHORYLHYDRAZONE DERIVATIVES OF ISATIN

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Recebido em 18/09/2013; aceito em 23/02/2014; publicado na web em 17/06/2014

Sixteen dialkylphosphorylhydrazones were synthesized by condensation of phosphorylhydrazines with substituted isatins. Products were characterized by FTIR, ^{1}H-NMR, ^{13}C-NMR, and ^{31}P-NMR. Fungicidal activities of these compounds against *Rhizoctonia solani* and *Fusarium oxysporum* were also evaluated. Some compounds inhibited the growth of *Rhizoctonia solani* and *Fusarium oxysporum* by 43% and 51%, respectively. These compounds exhibited no effects on germination of lettuce seeds (*Lactuca sativa* L).

Keywords: dialkylphosphorylhydrazones; isatins; biological activity.

INTRODUCTION

Natural products isolated from plants are being used in medicine and agriculture. Furthermore, these compounds are used to control insects,[1,2] nematodes,[3] fungi,[4-6] bacteria,[7-9] as well as for the stimulation of immunity in plants[10] and as anti-germination agents.[11-14]

Isatin, 1*H*-indol-2,3-dione, is found in plants of the genus Isatis,[15] such as *Calanthe discolor* Lindl.[16] and *Couroupita guianensis* Aubl.,[17] and in the parotid gland secretions of the Bufo frog.[18] Isatin was first synthesized by Erdman and Laurent in 1840 as a product of reaction between indigo and nitric or chromic acid.[19,20]

Isatin and its derivatives have a wide range of biological activities.[21-24] Recently, a number of researchers have been studying the use of these compounds in the fight against phytopathogens[25,26] and as potential herbicides.[27]

Some researchers have shown the inhibition effects of isatin hydrazones on the replication of variola virus and the virus associated with leukemia.[28-30]

As part of our ongoing studies concerning the preparation of potential biologically active compounds,[31-36] we synthesized sixteen new dialkylphosphorylhydrazones derivatives of isatin,[37] since this class of compounds is known to have antifungal activity.[26] The synthesized compounds were characterized and evaluated for their activity against two fungi: *Rhizoctonia solani* and *Fusarium oxysporum*. Finally, their effect on the germination of lettuce seeds (*Lactuca sativa* L.) was also investigated.

EXPERIMENTAL

Melting points were determined on a Buchi 510 melting point apparatus and solvents were purified and dried according to the methods described in the literature.[38]

IR spectra were recorded on a Perkin-Elmer model 1600FT spectrometer (ν in cm^{-1}) using NaCl pellets for liquid samples and KBr for solid samples. NMR spectra were acquired on a Bruker AC 200 spectrometer (^{1}H, 200 MHz; ^{13}C, 50.3 MHz; and ^{31}P, 81.0 MHz) or a Brucker AVANCE II 400 spectrometer (^{1}H, 400 MHz; ^{13}C, 100.6 MHz; and ^{31}P, 161.9 MHz) and are reported with chemical shifts in ppm along with the following abbreviations: singlet (s), doublet (d), triplet (t), quadruplet (q), double doublet (dd), sextuplet (sext), multiplet (m), and broad signal (br s). Gas chromatography (GC)-mass spectrometry (MS) analyses were carried out on a Saturn 2000-Varian column VF-5 ms ($30 \times 0.25 \times 0.25$ mm) with the following conditions: temperature = 150–180 °C/1'-10 °C/min–290 °C/10'; temperature of injector = 270 °C; temperature of MS trap = 220 °C, temperature of manifold = 60 °C, temperature of trxline = 250 °C, and an ionization energy of 70 eV.

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Antifungal activity

Dialkylphosphorylhydrazones were tested against *Rhizoctonia solani* and *Fusarium oxysporum* at a concentration of 500 mg L^{-1} in BDA medium. For the positive control, only BDA culture medium was used. For first negative control, BDA culture medium containing fungicide Mancozeb (200 mg L^{-1}) was used and for second negative control BDA culture medium was employed, and finally for the third negative control, culture medium with 1% DMSO (vehicle) was employed. A broad-spectrum antibiotic (gentamicine) was added to all culture media.

Mycelial disks of pure fungi culture (6 mm in diameter) obtained from the Phytopathology Department of Universidade Federal Rural do Rio de Janeiro and Universidade Federal de Viçosa were individually transferred to the center of each Petri plate. Plates containing *Rhizoctonia solani* were incubated for three days and those containing *Fusarium oxysporum* were incubated for seven days at 24 ± 1 °C. The inhibition was recorded relative to percent mycelial inhibition, calculated using the average orthogonal diameter and compared against controls.

Seed germination

The test for seed germination was conducted with commercial lettuce seeds (*Lactuca sativa* L.) using one concentration of dialkylphosphorylhydrazones (400 mg L^{-1}) and 50 seeds. Each test was repeated five times.

Seeds were immersed in a solution of dialkylphosphorylhydrazones (in CH_2Cl_2) until solvent evaporated. The seeds were then transferred to petri plates containing a filter paper, and water (3

mL) was added to it. The petri plates were covered and put in a germination chamber with a photoperiod of 12 h under daylight and temperature of 23 ± 1 °C for 7 days. For the negative control, only pure CH_2Cl_2 was used. Untreated seeds were used as the positive control. On the last day, germinated seeds were counted and the average recorded.

Synthesis of dialkylphosphonates

Dialkylphosphonates were synthesized by reacting alcohols (ethanol, butanol, and isobutanol) with phosphorus trichloride (PCl_3) at a 3:1 (alcohol:PCl_3) molar proportion.[31-36] The prepared dialkylphosphonates were used without further purification.

Synthesis of dialkylphosphorylhydrazines

Dialkylphosphorylhydrazines were synthesized by a modified Todd-Atherton reaction.[31-36] In this reaction, hydrazine reacts with dialkylphosphonates in a biphasic medium (H_2O/CCl_4).

Synthesis of dialkylphosphorylhydrazones (1–16)

Dialkylphosphorylhydrazones (1–16) were synthesized by acid catalyzed condensation of dialkylphosphorylhydrazines with substituted isatins as shown in Figure 1.

Isatin derivatives were added to an ethanolic solution (20 mL) of the different dialkylphosphorylhydrazines in a 50 mL round-bottom flask in a 1:1 molar ratio. Subsequently, two drops of concentrated HCl were added to the mixture. When the reaction was complete, ten drops of a 10% sodium bicarbonate solution were added. The resulting solution was washed with dichloromethane. The organic phase was dried over anhydrous sodium sulfate and then filtered. The solid products were obtained by evaporation of the dried filtrate and were recrystallized when necessary. In the case of oils, the products were obtained by evaporation of the solvent (Table 1).

The spectral characteristics of the compounds obtained in this work are described below.

Phosphorohydrazidic acid *N′*-[1,2-dihydro-2-oxo-3*H*-indol-3-ylidene]-diisobutyl ester (1). Yellow solid**.** Yield: 84 %. M.p. 110 ºC; **IR (cm^{-1}):** 3149.2 (ν_{NH}); 1619.9 ($\nu_{C=N}$); 1695.1 ($\nu_{C=O}$); 1238.1 ($\nu_{P=O}$); 1020.1 (ν_{P-O-C}). **^{1}H NMR (δ):** 11.42 and 8.47 [d, N-H, J_{HP} = 33.67 Hz e J_{HP} = 28.37 Hz, 1H]; 9.72 [s, N-H 1H]; 7.87 [d, H-4, J_{HH} = 7.53 Hz, 1H]; 6.92-7.15 [m, H-5, 1H]; 7.20-7.30 [m, H-6, 1H]; 6.99 [d, H-7, J_{HH} = 8.0 Hz, 1H]; 0.90-0.92 and 0.96-0.98 [2d ($(CH_3)_2CHCH_2O$-), 12H]; 1.89-2.09 [m ($(CH_3)_2CHCH_2O$-), 2H]; 3.82-4.01 [m ($(CH_3)_2CHCH_2O$-), 4H]. **^{13}C NMR (δ):** 18.66 [s ($(CH_3)_2CHCH_2O$-), 4C]; 29.06 [d ($(CH_3)_2CHCH_2O$-), J_{CP} = 6.97 Hz, 2C]; 73.92 [d ($(CH_3)_2CHCH_2O$-), J_{CP} = 6.61 Hz, 2C]; 140.63 [s (-N=C), 1C]; 163.17 [s (C=O), 1C]; 111.07-135.68 [(C, arom.), 6C]. **^{31}P NMR (δ):** -0.55 -0.92**.** ***m/z* (%):** 353 (14), 297 (20), 281(5), 267 (5), 241 (85), 224 (10), 160 (100), 132 (45).

Phosphorohydrazidic acid *N′*-[1,2-dihydro-1-methyl-2-oxo-3*H*-indol-3-ylidene]-diisobutyl ester (2). Yellow solid**.** Yield: 85%. M.p. 60 °C; **IR (cm^{-1}):** 3199.3 (ν_{NH}); 1616.0 ($\nu_{C=N}$); 1691.2 ($\nu_{C=O}$); 1255.4 ($\nu_{P=O}$);1018.2 (ν_{P-O-C}). **^{1}H NMR (δ):** 11.41 and 8.29 [d, N-H, J_{HP} = 33.79 Hz and J_{HP} = 28.37 Hz, 1H]; 3.23 [s, N-CH_3, 3H]; 7.56 [d, H-4, J_{HH} = 7.62 Hz, 1H]; 7.08 [t, H-5, J_{HH} = 7.62 Hz, 1H]; 7.33 [t, H-6, J_{HH} = 7.85 Hz, 1H]; 6.84 [d, H-7, J_{HH} = 7.85 Hz, 1H]; 0.93 [d (($CH_3)_2CHCH_2O$-), J_{HH} = 6.69 Hz, 12H]; 1.88-2.08 [m ($(CH_3)_2CHCH_2O$-), 2H]; 3.80-3.99 [m ($(CH_3)_2CHCH_2O$-), 4H]. **^{13}C NMR (δ):** 18.53 [s ($(CH_3)_2CHCH_2O$-), 4C]; 28.90 [d ($(CH_3)_2CHCH_2O$-), J_{CP} = 7.34 Hz, 2C]; 73.58 and 74.10 [d ($(CH_3)_2CHCH_2O$-), J_{CP} = 6.60 Hz, 2C]; 142.48 and 144.39 [s(-N=C), 1C]; 161.05 and 163.69 [s (C=O), 1C] 108.60-134.80 [C, arom.), 6C]; 25.39 [s (CH_3), 1C]. **^{31}P NMR (δ):** -0.43 and 1.01. ***m/z* (%):** 368 (70), 311 (53), 255(60), 174 (100), 146 (54), 117 (28), 91 (25).

Phosphorohydrazidic acid *N′*-[1-butyl-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene]-diisobutyl ester (3). Orange oil. Yield: 75%. **IR (cm^{-1}):** 3212.8 (ν_{NH}); 1614.15 ($\nu_{C=N}$); 1685.5 ($\nu_{C=O}$); 1276.6 ($\nu_{P=O}$); 1022.1 (ν_{P-O-C}). **^{1}H NMR (δ):** 11.44 [d, -N-H, J_{HP} = 33.67 Hz, 1H]; 3.71 [t, N-$CH_2CH_2CH_2CH_3$, J_{HH} = 7.32 Hz, 2H]; 1.52-1.70 [m, N-$CH_2CH_2CH_2CH_3$, 2H]; 1.30-1.50[m, N-$CH_2CH_2CH_2CH_3$, 2H]; 0.93 [t, N-$CH_2CH_2CH_2CH_3$, J_{HH} = 7.19 Hz, 3H]; 7.58 [d, H-4, J_{HH} = 7.0 Hz, 1H]; 7.06 [t, H-5, J_{HH} = 7.0 Hz, 1H]; 7.31 [t, H-6, J_{HH} = 7.0 Hz, 1H]; 6.85 [d, H-7, J_{HH} = 7.0 Hz, 1H]; 0.93 [d (($CH_3)_2CHCH_2O$-), J_{HH} = 6.69 Hz, 12H]; 1.92-2.02 [m ($(CH_3)_2CHCH_2O$-), 2H]; 3.80-4.00 [m ($(CH_3)_2CHCH_2O$-), 4H]. **^{13}C NMR (δ):** 18.67 [s ($(CH_3)_2CHCH_2O$-), 4C]; 29.05 [d ($(CH_3)_2CHCH_2O$-), J_{CP} = 6.61 Hz, 2C]; 73.72 [d ($(CH_3)_2CHCH_2O$-), J_{CP} = 6.60 Hz, 2C]; 141.99 [s(-N=C), 1C]; 161.15 [(C=O), 1C]; 108.97-134.97 [(C, arom.), 6C]; 39.40 [s ($NCH_2CH_2CH_2CH_3$), 1C]; 29.63 [s ($NCH_2CH_2CH_2CH_3$), 1C]; 20.15 [s ($NCH_2CH_2CH_2CH_3$), 1C]; 13.68 [s ($NCH_2CH_2CH_2CH_3$), 1C]. **^{31}P NMR (δ): -** 0.50 dq. ***m/z* (%):** 409 (32), 388 (2), 353 (50), 297 (40), 269 (12), 216 (100), 170 (5), 146 (32), 117 (10), 77 (5), 57 (5).

Phosphorohydrazidic acid *N′*-[1,2-dihydro-2-oxo-1-(benzyl)-3*H*-indol-3-ylidene]-diisobutyl ester (4). Orange oil. Yield: 38%. **IR (cm^{-1}):** 3209.0 (ν_{NH}); 1616.0 ($\nu_{C=N}$); 1687.4 ($\nu_{C=O}$); 1261.2 ($\nu_{P=O}$); 1022.1 (ν_{P-O-C}). **^{1}H NMR (δ):** 11.40 [d, -N-H, J_{HP} = 34.29 Hz, 1H]; 4.93 [s, N-CH_2, 2H]; 6.73-7.23 [m, H-9 to H-13, 5H]; 7.60 [d, H-4, J_{HH} = 7.0 Hz, 1H]; 7.08 [t, H-5, J_{HH} = 7.0 Hz, 1H]; 7.21-7.23 [m**,** H-6, 1H]; 6.78 [d, H-7, J_{HH} = 7.0 Hz, 1H]; 0.95 [d (($CH_3)_2CHCH_2O$-), J_{HH} = 6.68 Hz, 12H]; 1.94-2.07 [m ($(CH_3)_2CHCH_2O$-), 2H]; 3.78-4.03 [m ($(CH_3)_2CHCH_2O$-), 4H]. **^{13}C NMR (δ):** 18.60 [s ($(CH_3)_2CHCH_2O$-), 4C]; 28.97 [d ($(CH_3)_2CHCH_2O$-), J_{CP} = 7.33 Hz, 2C]; 73.78[d ($(CH_3)_2CHCH_2O$-), J_{CP} = 6.60 Hz, 2C]; 141.70 [s(-N=C), 1C]; 161.11 [(C=O), 1C] 109.61-135.10 [(C, arom.), 6C]; 43.17 [s ($CH_2C_6H_5$),

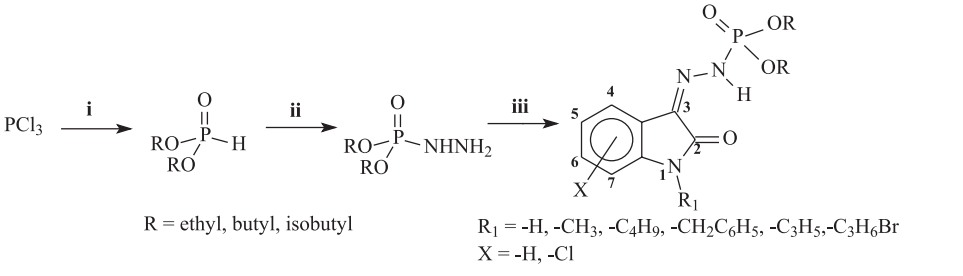


Figure 1. *Synthetic route to dialkylphosphorylhydrazones*

1C]; 127.29-134.64 [s ($CH_2\underline{C}_6H_5$), 6C]. **^{31}P NMR (δ): -** 0.55 dq. ***m/z*(%):** 444 (70), 388 (60), 332 (30), 251(100), 222 (15), 91(73).

Phosphorohydrazidic acid *N′*-[5-chloro-1,2-dihydro-2-oxo-1-(benzyl)-3*H*-indol-3-ylidene]-diisobutyl ester (5). Orange oil**.** Yield: 91%. **IR (cm^{-1}):** 3210.0 (ν_{NH}); 1614.1 ($\nu_{C=N}$); 1691.2 ($\nu_{C=O}$); 1247.3 ($\nu_{P=O}$); 1020.1 (ν_{P-O-C}). **^{1}H NMR (δ):** 11.44 [d, -N-H, J_{HP} = 33.63 Hz, 1H]; 4.92 [s, N-C$\underline{H}_2$C$_6$H$_5$, 2H]; 7.29-7.57 [m, H-9 - H-13, 5H]; 7.36 [d, H-4, J_{HH} = 8.0 Hz, 1H]; 7.30 [d, H-6, J_{HH} = 8.0 Hz, 1H]; 6.68 [d, H-7, J_{HH} = 8.0 Hz, 1H]; 0.96 [d ((C$\underline{H}_3$)$_2$CHCH$_2$O-), J_{HH} = 6.78 Hz, 12H]; 1.94-2.06 [m ((CH$_3$)$_2$C$\underline{H}$CH$_2$O-), 2H]; 3.87-4.00 [m ((CH$_3$)$_2$CHC$\underline{H}_2$O-), 4H].**^{13}C NMR (δ):** 18.64 [s ((C$\underline{H}_3$)$_2$CHCH$_2$O-), 4C]; 29.07 [d ((CH$_3$)$_2$C$\underline{H}$CH$_2$O-), J_{CP} = 6.60 Hz, 2C]; 73.99[d ((CH$_3$)$_2$CH$\underline{C}$H$_2$O-), J_{CP} = 5.87 Hz, 2C]; 141.11 [s(-N=$\underline{C}$), 1C]; 160.90 [($\underline{C}$=O), 1C]; 110.76-135.55 [($\underline{C}$, arom.), 6C]; 43.38 [s ($\underline{C}H_2C_6H_5$), 1C]; 127.44-139.99 [s ($CH_2\underline{C}_6H_5$), 6C]. **^{31}P NMR (δ): -** 0.97 dq. ***m/z* (%):** 477(47), 421 (100), 365 (62), 348 (7), 285 (87), 256 (27), 193 (7), 150 (5), 118 (7), 91 (100), 65 (20).

Phosphorohydrazidic acid *N′*-[5-chloro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene]-diisobutyl ester (6). Yellow solid. Yield: 90%. M.p. 150 °C; **IR (cm^{-1}):** 3210.9 (ν_{NH}); 1614.2 ($\nu_{C=N}$); 1691.2 ($\nu_{C=O}$); 1259.3 ($\nu_{P=O}$); 1020.2 (ν_{P-O-C}). **^{1}H NMR (δ):** 11.36 [d, -N-H, J_{HP} = 33.42 Hz, 1H]; 8.86 [s, N-H, 1H]; 7.51 [d, H-4, 1H]; 7.24 [d, H-6, J_{HH} = 8.0 Hz, 1H]; 6.87 [d, H-7, J_{HH} = 8.0 Hz, 1H]; 0.96 [d ((C$\underline{H}_3$)$_2$CHCH$_2$O-), J_{HH} = 6.68 Hz, 12H]; 1.94-2.08 [m ((CH$_3$)$_2$C$\underline{H}$CH$_2$O-), 2H]; 3.80-4.03 [m ((CH$_3$)$_2$CHC$\underline{H}_2$O-), 4H]. **^{13}C NMR (δ):** 18.62 [s ((C$\underline{H}_3$)$_2$CHCH$_2$O-), 4C]; 29.00 [d ((CH$_3$)$_2$C$\underline{H}$CH$_2$O-), J_{CP} = 6.61 Hz, 2C]; 74.22 and 73.97 [d ((CH$_3$)$_2$CH$\underline{C}$H$_2$O-), J_{CP} = 6.60 Hz, 2C]; 140.88 and 139.24 [s(-N=$\underline{C}$), 1C]; 166.15 and 163.04 [s ($\underline{C}$=O), 1C]; 116.90-136.25 [($\underline{C}$, arom.), 6C]. **^{31}P NMR (δ): -** 0.93 dq. ***m/z* (%):** 388 (12), 331 (20), 275 (100), 258 (12), 194 (82), 165 (24), 138 (15), 102 (12), 111 (13), 75 (7), 57 (7).

Phosphorohydrazidic acid *N′*-[1,2-dihydro-2-oxo-1-(2-propenyl)-3*H*-indol-3-ylidene]-diisobutyl ester (7). Orange oil. Yield: 88%. **IR (cm^{-1}):** 3214.8 (ν_{NH}); 1614.1 ($\nu_{C=N}$); 1689.3 ($\nu_{C=O}$); 1272.8 ($\nu_{P=O}$); 1020.1 (ν_{P-O-C}). **^{1}H NMR (δ):** 11.40 [d, -N-H, J_{HP} = 33.63 Hz, 1H]; 4.36 [dt, N-C$\underline{H}_2$CH=CH$_2$, 2H]; 5.80-5.88 [m, N-CH$_2$C$\underline{H}$=CH$_2$, 2H]; 5.24 [dd, N-CH$_2$CH=C$\underline{H}_2$, 2H]; 7.60 [d, H-4, J_{HH} = 7.0 Hz, 1H]; 7.09 [t, H-5, J_{HH} = 7.0 Hz, 1H]; 7.30 [t, H-6, J_{HH} = 8.0 Hz, 1H]; 6.85 [d, H-7, J_{HH} = 8.0 Hz, 1H]; 0.95 [d ((C$\underline{H}_3$)$_2$CHCH$_2$O-), J_{HH} = 6.78 Hz, 12H]; 1.97-2.05 [m ((CH$_3$)$_2$C$\underline{H}$CH$_2$O-), 2H]; 3.85-3.95 [m ((CH$_3$)$_2$CHC$\underline{H}_2$O-), 4H]. **^{13}C NMR (δ):** 18.64 [s ((C$\underline{H}_3$)$_2$CHCH$_2$O-), 4C]; 29.09 [d ((CH$_3$)$_2$C$\underline{H}$CH$_2$O-), J_{CP} = 7.34 Hz, 2C]; 73.75 [d ((CH$_3$)$_2$CH$\underline{C}$H$_2$O-), J_{CP} = 5.87 Hz, 2C]; 141.73 [s(-N=$\underline{C}$), 1C]; 160.86 [($\underline{C}$=O), 1C]; 109.57-134.72 [s ($\underline{C}$, arom.), 6C]; 41.76 [s ($\underline{C}H_2$-CH=CH$_2$), 1C]; 130.22 [s (CH$_2$-$\underline{C}$H=CH$_2$), 1C]; 118.08 [s (CH$_2$-CH=$\underline{C}H_2$), 1C]. **^{31}P NMR (δ): -** 0.50 dq. ***m/z* (%):** 394 (87), 356 (5), 337 (52), 311(10), 281 (42), 253 (30), 200 (100), 172 (35), 143(27),117 (15), 91 (5), 57 (10).

Phosphorohydrazidic acid *N′*-[1-(3-bromopropyl)-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene]-diisobutyl ester (8). Orange oil. Yield: 75%. **IR (cm^{-1}):** 3212.8 (ν_{NH}); 1614.1 ($\nu_{C=N}$); 1687.4 ($\nu_{C=O}$); 1267.0 ($\nu_{P=O}$); 1024.0 (ν_{P-O-C}). **^{1}H NMR (δ):** 11.37 [d, -N-H, J_{HP} = 33.42 Hz, 1H]; 3.78-4.01 [m, N-C$\underline{H}_2$CH$_2$CH$_2$Br, 2H]; 2.19-2.29 [m, N-CH$_2$C$\underline{H}_2$CH$_2$Br, 2H]; 3.44 [t, N-CH$_2$CH$_2$C$\underline{H}_2$Br, J_{HH} = 6.30 Hz, 2H]; 7.60 [d, H-4, J_{HH} = 7.0 Hz, 1H]; 7.10 [t, H-5, J_{HH} = 7.0 Hz, 1H]; 7.35 [t, H-6, J_{HH} = 7.0 Hz, 1H]; 6.96 [d, H-7, J_{HH} = 7.0 Hz, 1H]; 0.94 [d ((C$\underline{H}_3$)$_2$CHCH$_2$O-), J_{HH} = 6.68 Hz, 12H]; 1.90-2.06 [m ((CH$_3$)$_2$C$\underline{H}$CH$_2$O-), 2H]; 3.78-4.01 [m ((CH$_3$)$_2$CHC$\underline{H}_2$O-), 4H]. **^{3}C NMR (δ):** 18.66 [s ((C$\underline{H}_3$)$_2$CHCH$_2$O-), 4C]; 29.07 [d ((CH$_3$)$_2\underline{C}$HCH$_2$O-), J_{CP} = 6.60 Hz, 2C]; 73.83 [d ((CH$_3$)$_2$CH$\underline{C}$H$_2$O-), J_{CP} = 5.87 Hz, 2C]; 141.66 [s(-N=$\underline{C}$), 1C]; 161.29 [s ($\underline{C}$=O), 1C]; 108.84-134.59 [($\underline{C}$, arom.), 6C]; 38.11 [s ($\underline{C}H_2$-CH$_2$CH$_2$Br), 1C]; 30.71 [s (CH$_2$-$\underline{C}$H$_2$CH$_2$Br), 1C]; 30.17[s ($\underline{C}H_2$-CH$_2$CH$_2$Br), 1C]. **^{31}P NMR (δ):** -0.56. ***m/z* (%):** 475 (10), 419 (27), 395 (10), 363(45), 340 (15), 282 (100), 226 (10), 201 (27), 174 (25), 144 (25), 90 (12), 51 (7).

Phosphorohydrazidic acid *N′*-[5-chloro-1,2-dihydro-1-methyl-2-oxo-3*H*-indol-3-ylidene]-diisobutyl ester (9). Orange oil. Yield: 73%. **IR (cm^{-1}):** 3214.8 (ν_{NH}); 1614.1 ($\nu_{C=N}$); 1691.2 ($\nu_{C=O}$); 1268.9 ($\nu_{P=O}$); 1014.3 (ν_{P-O-C}). **^{1}H NMR (δ):** 11.41 [d, -N-H, J_{HP} = 33.63 Hz, 1H]; 3.25 [s, N-C$\underline{H}_3$, 3H]; 7.59 [d, H-4, 1H]; 7.32 [d, H-6, J_{HH} = 8.0 Hz, 1H]; 6.81 [d, H-7, J_{HH} = 8.0 Hz 1H]; 0.96 [d ((C$\underline{H}_3$)$_2$CHCH$_2$O-), J_{HH} = 6.78 Hz, 12H]; 1.98-2.01 [m ((CH$_3$)$_2$C$\underline{H}$CH$_2$O-), 2H]; 3.84-4.01 [m ((CH$_3$)$_2$CHC$\underline{H}_2$O-), 4H]. **^{13}C NMR (δ):** 18.67 [s ((C$\underline{H}_3$)$_2$CHCH$_2$O-), 4C]; 29.07 [d ((CH$_3$)$_2\underline{C}$HCH$_2$O-), J_{CP} = 7.34 Hz, 2C]; 73.88 [d ((CH$_3$)$_2$CH$\underline{C}$H$_2$O-), J_{CP} = 5.87 Hz, 2C]; 148.73 [s(-N=$\underline{C}$), 1C]; 160.96 [($\underline{C}$=O), 1C]; 109.70-133.66 [($\underline{C}$, arom.), 6C]; 25.68 [s ($\underline{C}H_3$), 1C]. **^{31}P NMR (δ): -** 0.25 dq. ***m/z* (%):** 401(100), 373 (5), 346 (57), 290 (57), 272 (10), 250 (5), 235 (1), 208(60), 180 (30), 151 (10), 117 (20), 89 (10), 75 (5), 57 (7).

Phosphorohydrazidic acid *N′*-[1,2-dihydro-2-oxo-1-benzyl-3*H*-indol-3-ylidene]-dibutyl ester (10). Yellow oil. Yield: 88%. **IR (cm^{-1}):** 3216.7 (ν_{NH}); 1612.2 ($\nu_{C=N}$); 1712.5 ($\nu_{C=O}$); 1253.5 ($\nu_{P=O}$); 1027.8 (ν_{P-O-C}). **^{1}H NMR (δ):** 11.47 and 8.74[2d, N-H-, J_{HP} = 33.16 Hz e J_{HP} = 28.12 Hz, 1H]; 4.92 and 4.94 [2s, N-C$\underline{H}_2$-, 2H]; 7.23-7.93 [m, H-9 a H-13, 5H]; 7.97 [d, H-4, J_{HH} = 7.44 Hz, 1H]; 7.05 [t, H-5, J_{HH} = 7.44, 1H]; 7.23 [d, H-6, 1H]; 6.75 [d, H-7, J_{HH} = 7.82 Hz, 1H]; 0.90-0.91 [2t (C$\underline{H}_3$CH$_2$CH$_2$CH$_2$O-), 6H]; 1.37-1.44 [m, (CH$_3\underline{C}$H$_2$CH$_2$CH$_2$O-) 4H]; 1.63-1.77 [m (CH$_3$CH$_2\underline{C}$H$_2$CH$_2$O-), 4H]; 4.07-4.29 [m (CH$_3$CH$_2$CH$_2\underline{C}$H$_2$O-), 4H]. **^{13}C NMR (δ):** 13.59 [s ($\underline{C}$H$_3$CH$_2$CH$_2$CH$_2$O-), 4C]; 18.65 [d (CH$_3\underline{C}$H$_2$CH$_2$CH$_2$O-), J_{CP} = 6.60 Hz, 2C]; 32.28 (CH$_3$CH$_2\underline{C}$H$_2$CH$_2$O-), J_{CP} 67.75 [d (CH$_3$CH$_2$CH$_2\underline{C}$H$_2$O-), J_{CP} = 5.86 Hz, 2C]; 143.58 [s(-N=$\underline{C}$), 1C]; 161.21 [($\underline{C}$=O), 1C]; 109.67-135.52 [($\underline{C}$, arom.), 6C]; 25.68 [s ($\underline{C}H_3$), 1C]. **^{31}P NMR (δ):** -0.27 e -1.30. ***m/z* (%):** 444 (78), 387 (10), 357 (5), 292 (26), 251(48), 222(45), 144(10), 91 (100).

Phosphorohydrazidic acid *N′*-[1,2-dihydro-2-oxo-3*H*-indol-3-ylidene]-dibutyl ester (11). Yellow oil. Yield: 83%. **IR (cm^{-1}):** 3185.8 (ν_{NH}); 1621.8 ($\nu_{C=N}$); 1700.9 ($\nu_{C=O}$); 1249.6 ($\nu_{P=O}$); 1022.1 (ν_{P-O-C}). **^{1}H NMR (δ):** 11.46 [d, P-N-H, J_{HP} = 33.63 Hz, 1H]; 9.79 [s, N-H, 1H]; 7.52 [d, H-4, J_{HH} = 7.53 Hz, 1H]; 7.05 [t, H-5, J_{HH} = 7.53 Hz, 1H]; 7.25 [t, H-6, J_{HH} = 7.78 Hz, 1H]; 6.94 [d, H-7, J_{HH} = 7.78 Hz, 1H]; 0.91 [t (C$\underline{H}_3$CH$_2$CH$_2$CH$_2$O-), J_{HH} = 7.28 Hz, 6H]; 1.38-1.44 [m, (CH$_3\underline{C}$H$_2$CH$_2$CH$_2$O-) 4H]; 1.67-1.74 [m (CH$_3$CH$_2\underline{C}$H$_2$CH$_2$O-), 4H]; 4.14-4.22 [m (CH$_3$CH$_2$CH$_2\underline{C}$H$_2$O-), 4H]. **^{13}C NMR (δ):** 13.50 [s ($\underline{C}$H$_3$(CH$_2$)$_3$O-), 2C]; 18.60 [s (CH$_3\underline{C}$H$_2$(CH$_2$)$_2$O-), 2C]; 32.16 [d (CH$_3$CH$_2\underline{C}$H$_2$CH$_2$O-), J_{CP} = 6.60 Hz, 2C]; 67.80 [d (-CH$_3$(CH$_2$)$_2\underline{C}$H$_2$O-), J_{CP} = 5.87 Hz, 2C]; 140.99 [s (-N=$\underline{C}$), 1C]; 163.17 [($\underline{C}$=O), 1C]; 111.15-135.92 [($\underline{C}$, arom.), 6C]. **^{31}P NMR (δ):** - 0.22 dq. ***m/z* (%):** 354 (100), 339 (5), 323 (7), 298 (10), 280 (7), 267 (5), 241 (12), 213 (25), 187 (5), 160 (77), 131 (65), 104 (32), 77 (21), 51 (10).

Phosphorohydrazidic acid *N′*-[1,2-dihydro-1-methyl-2-oxo-3*H*-indol-3-ylidene]-dibutyl ester (12). Orange oil. Yield: 58%. **IR (cm^{-1}):** 3210.9 (ν_{NH}); 1614.1 ($\nu_{C=N}$); 1689.3 ($\nu_{C=O}$); 1272.8 ($\nu_{P=O}$); 1029.8 (ν_{P-O-C}). **^{1}H NMR (CDCl$_3$):** 11.35 and 8.33 [d, P-N-H, J_{HP} = 33.55 Hz e J_{HP} = 28.62 Hz, 1H]; 3.20 [s, N-C$\underline{H}_3$, 3H]; 7.54 [2d, H-4, J_{HH} = 6.93 Hz, 1H]; 7.04 [t, H-5, J_{HH} = 6.80 Hz, 1H]; 7.32

[t, H-6, J_{HH} = 7.69 Hz, 1H]; 6.82 [d, H-7, J_{HH} = 7.95 Hz, 1H]; 0.87 [t ($C\underline{H}_3CH_2CH_2CH_2O$-), J_{HH} = 7.31 Hz, 6H]; 1.29-1.44 [m, ($CH_3C\underline{H}_2CH_2CH_2O$-) 4H]; 1.57-1.73 [m ($CH_3CH_2C\underline{H}_2CH_2O$-), 4H]; 4.03-4.19 [m ($CH_3CH_2CH_2C\underline{H}_2O$-), 4H]. **^{13}C NMR (δ):** 13.46 [s ($\underline{C}H_3(CH_2)_3O$-), 2C]; 18.54 [s ($CH_3\underline{C}H_2(CH_2)_2O$-), 2C]; 32.12 [d ($CH_3CH_2\underline{C}H_2CH_2O$-), J_{CP} = 6.61 Hz, 2C]; 67.52 e 67.99 [d (-$CH_3(CH_2)_2\underline{C}H_2O$-), J_{CP} = 6.60 Hz, 2C]; 142.43 [s (-N=C), 1C]; 161.06 [(C=O), 1C]; 108.70-134.97 [(C, arom.), 6C]; 25.42 and 25.96 [s ($\underline{C}H_3$), 1C]. **^{31}P NMR (δ):** - 0.25 dq. ***m/z* (%):** 368 (100), 353(10), 337 (5), 312 (10), 208 (23), 174 (87), 146 (76), 117 (48), 91 (30).

Phosphorohydrazidic acid *N′*-[5-chloro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene]-dibutyl ester (13). Yellow solid. Yield: 35%. M.p. 148 °C. **IR (cm^{-1}):** 3129.9 (ν_{NH}); 1623.8 ($\nu_{C=N}$); 1704.7 ($\nu_{C=O}$); 1247.7 ($\nu_{P=O}$); 1016.8 (ν_{P-O-C}). **^{1}H NMR (δ):** 11.41 [d, P-N-H, J_{HP} = 33.63 Hz, 1H]; 9.63 [s, N-H, 1H]; 7.49 [d, H-4, J_{HH} = 2.01, 1H]; 7.24 [dd, H-6, J_{HH} = 8.38 Hz, 1H]; 6.89 [d, H-7, J_{HH} = 8.28 Hz, 1H]; 0.91 [t ($C\underline{H}_3CH_2CH_2CH_2O$-), J_{HH} = 7.28 Hz, 6H]; 1.42 [sex, ($CH_3C\underline{H}_2CH_2CH_2O$-), J_{HH} = 7.53 Hz 4H]; 1.70 [qui ($CH_3CH_2C\underline{H}_2CH_2O$-), J_{HH} = 8.03 Hz, 4H]; 4.12-4.21 [m ($CH_3CH_2CH_2C\underline{H}_2O$-), 4H]. **^{13}C NMR** (δ): 13.52 [s ($\underline{C}H_3(CH_2)_3O$-), 2C]; 18.63 [s ($CH_3\underline{C}H_2(CH_2)_2O$-), 2C]; 32.22 [d ($CH_3CH_2\underline{C}H_2CH_2O$-), J_{CP} = 7.33 Hz, 2C]; 68.01 [d (-$CH_3(CH_2)_2\underline{C}H_2O$-), J_{CP} = 5.87 Hz, 2C]; 139.08 [s (-N=C), 1C]; 162.86 [(C=O), 1C]; 120.60-134.92 [(C, arom.), 6C]. **^{31}P NMR (δ):** - 0.75. ***m/z* (%):** 388(100), 357(10), 332 (13), 301 (7), 275 (25), 236 (20), 194 (60), 165 (40), 138 (28), 102 (12), 83 (15), 57 (7).

Phosphorohydrazidic acid, *N′*-[1,2-dihydro-2-oxo-1-(benzyl)-3*H*-indol-3-ylidene]-diethyl ester (14). Orange oil. Yield: 86%. **IR (cm^{-1}):** 3214.8 (ν_{NH}); 1614.1 ($\nu_{C=N}$); 1685.5 ($\nu_{C=O}$); 1267.0 ($\nu_{P=O}$); 1025.9 (ν_{P-O-C}). **^{1}H NMR (CDCl$_3$):** 11.47 [d, P-N-H, J_{HP} = 33.38 Hz, 1H]; 4.93 [s, N-$C\underline{H}_2C_6H_5$); 7.22-7.38 [m, H-9 a H-13, 5H]; 7.65[d, H-4, J_{HH} = 7.0 Hz, 1H]; 7.08 [t, H-5, J_{HH} = 7.0 Hz, 1H]; 7.25 [t, H-6, J_{HH} = 7.0 Hz, 1H]; 6.78 [d, H-7, J_{HH} = 7.0 Hz, 1H]; 1.38 [t (CH_3CH_2O-), J_{HH} = 7.03 Hz, 6H]; 4.18-4.30 [m (CH_3CH_2O-), 4H]. **^{13}C NMR** (δ)**:** 16.22 [d ($\underline{C}H_3CH_2O$-), J_{CP} = 6.60 Hz, 2C]; 64.09 [d ($\underline{C}H_2O$-), J_{CP} = 5.87 Hz , 2C]; 141.73 [s (-N=C), 1C]; 161.23 [(C=O), 1C]; 109.70-135.16 [(C, arom.), 6C]; 43.26 [s ($\underline{C}H_2C_6H_5$), 1C]; 127.37-134.99 [s ($CH_2\underline{C}_6H_5$), 6C]. **^{31}P NMR (δ):** - 0.35 dq. ***m/z* (%):** 388 (100), 360 (1), 250 (20), 222 (8), 194 (6), 144 (3), 109 (5), 91 (32).

Phosphorohydrazidic acid, *N′*-[1,2-dihydro-2-oxo-3*H*-indol-3-ylidene]-diethyl ester (15). Brown solid. Yield: 86%. M.p. 120 °C. **IR (cm^{-1}):** 3137.6 (ν_{NH}); 1621.8 ($\nu_{C=N}$); 1697.0 ($\nu_{C=O}$); 1240.0 ($\nu_{P=O}$); 1018.2 (ν_{P-O-C}). **^{1}H NMR (CDCl$_3$):** 11.43 [d, -N-H, J_{HP} = 33.63 Hz, 1H]; 9.63[s, N-H]; 7.54[d, H-4, J_{HH} = 8.0 Hz, 1H]; 7.02 [t, H-5, J_{HH} = 8.0 Hz, 1H]; 7.25 [t, H-6, J_{HH} = 8.0 Hz, 1H]; 6.92 [d, H-7, $_{HH}J$ = 8.0 Hz, 1H]; 1.36 [t ($C\underline{H}_3CH_2O$-), J_{HH} = 7.03 Hz, 6H]; 4.16-4.27 [m ($CH_3C\underline{H}_2O$-), 4H]. **^{13}C NMR** (δ)**:** 16.10 [d ($\underline{C}H_3CH_2O$-), J_{CP} = 6.61 Hz, 2C]; 64.20 [d ($\underline{C}H_2O$-), J_{CP} = 5.13 Hz , 2C]; 140.74 [s (-N=C), 1C]; 163.19 [(C=O), 1C]; 11.15-136.06 [(C, arom.), 6C]. **^{31}P NMR (δ):** - 0.29 dq. ***m/z* (%):** 297 (100), 276 (8), 252 (5), 241 (8), 224 (7), 213 (18), 195 (5), 173 (10), 159 (55), 131 (85), 104 (35), 77 (27), 65 (5), 51 (12).

Phosphorohydrazidic acid, *N′*-[1-butyl-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene]-diethyl ester (16). Orange oil. Yield: 65%. **IR (cm^{-1}):** 3209.0 (ν_{NH}); 1614.1 ($\nu_{C=N}$); 1685.5 ($\nu_{C=O}$); 1267.0 ($\nu_{P=O}$);1025.9 (ν_{P-O-C}). **^{1}H NMR (δ):** 11.48 [d, -N-H, J_{HP} = 33.38 Hz, 1H]; 3.72 [t, N-$C\underline{H}_2CH_2CH_2CH_3$, J_{HH} = 7.28 Hz, 2H]; 1.66 [quint, N-$CH_2C\underline{H}_2CH_2CH_3$, J_{HH} = 7.28 Hz, 2H]; 1.16-1.34 [m, N-$CH_2CH_2C\underline{H}_2CH_3$, 2H]; 0.95 [t, N-$CH_2CH_2CH_2C\underline{H}_3$, J_{HH} = 7.28 Hz, 2H]; 7.62 [d, H-4, J_{HH} = 7.0 Hz, 1H]; 7.08 [t, H-5, J_{HH} = 7.0 Hz, 1H]; 7.32 [t, H-6, J_{HH} = 7.0 Hz, 1H]; 6.86 [d, H-7, J_{HH} = 7.0 Hz, 1H]; 1.37 [t ($C\underline{H}_3CH_2O$-), J_{HH} = 7.03 Hz, 6H]; 4.21-4.26 [m ($CH_3C\underline{H}_2O$-), 4H]. **^{13}C NMR** (δ)**:** 16.13 [d ($\underline{C}H_3CH_2O$-), J_{CP} = 6.60 Hz, 2C]; 63.97 [d ($\underline{C}H_2O$-), J_{CP} = 5.13 Hz , 2C]; 142.03 [s (-N=C), 1C]; 161.20 [(C=O), 1C]; 108.97-135.25 [(C, arom.), 6C]; 39.33 [s ($N\underline{C}H_2CH_2CH_2CH_3$), 1C]; 29.64 [s ($NCH_2\underline{C}H_2CH_2CH_3$), 1C]; 20.18 [s ($NCH_2CH_2\underline{C}H_2CH_3$), 1C]; 13.70 [s ($NCH_2CH_2CH_2\underline{C}H_3$), 1C]. **^{31}P NMR (δ): -** 0.20 dq. ***m/z* (%):** 353 (100), 339 (2), 297 (2), 280 (3), 216 (52), 201 (10), 187 (18), 170 (12), 146 (30), 117 (15), 104 (7), 91 (12).

RESULTS AND DISCUSSION

Physical characteristics and yields of dialkylphosphorylhydrazones synthesized in this work (**1**–**16**) are shown in Table 1.

Infrared spectroscopy

Dialkylphosphonates

The most characteristic absorption band of dialkylphosphonates is the axial vibration of the P–H bond, which occurs between 2222 cm^{-1} and 2505 cm^{-1}.[39] Other important absorption bands are observed at 1210 cm^{-1} and 1261 cm^{-1}, which correspond to P=O axial vibrations, and at 950 cm^{-1} and 1018 cm^{-1}, which are attributed to P-O-C angular vibrations.

The infrared spectra of all dialkylphosphonates present absorptions bands in the ranges from 2426 cm^{-1} to 2434 cm^{-1}, from 1216 cm^{-1} to 1255 cm^{-1}, and from 1006 cm^{-1} to 983 cm^{-1}, which are attributed to axial deformations of P–H, P=O, and P–O bonds, respectively.

Dialkylphosphorylhydrazines

An absorption band in the region from 1031 cm^{-1} to 1166 cm^{-1} corresponding to P–N vibration is the main evidence for the formation of a dialkylphosphorylhydrazine.[39] Furthermore, no absorption bands in the region of 2425 cm^{-1} are observed, which shows the absence of the P–H bond, a characteristic of the starting dialkylphosphonates.

*Dialkylphosphorylhydrazones (**1–16**)*

The main absorption bands observed in the IR spectra correspond to stretching frequencies of the P=O, P–O–C, and C=N bonds of the dialkylphosphorylhydrazones, which range from 1238 cm^{-1} to 1272 cm^{-1}, from 1012 cm^{-1} to 1029 cm^{-1}, and from 1612 cm^{-1} to 1623 cm^{-1}, respectively. The presence of the C=N bond absorption confirms the formation of the desired products from the reaction between the various isatins and dialkylphosphorylhydrazines.

^{1}H NMR

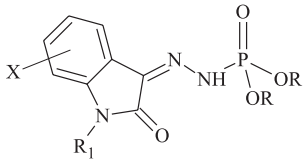
Dialkylphosphonates

A characteristic signal of the dialkylphosphonates is that of the P–H proton, which is a doublet at 6.70 ppm with J_{HP} = 660–750 Hz.[40] A further characteristic signal is a multiplet at 4.00 ppm, corresponding to the hydrogen atoms of the alkoxy group linked to the phosphoryl group.

Dialkylphosphorylhydrazines

A characteristic ^{1}H NMR signal of dialkylphosphorylhydrazine is the presence of a broad singlet at ~3.6 ppm, ascribed to the hydrogens linked to nitrogen atoms. An evidence for the occurrence of the reaction is the disappearance of doublet centered at ~6.70 ppm, indicating a complete consumption of dialkylphosphonate.

Table 1. Data for the synthesized compounds



Compd.	R	R_1	X	Yield (%)	M.p (ºC)	Purification method	Reaction Time (h)
1	*iso*-butyl	H	H	84	110	column filtration C_6H_{12}/CH_3COOEt (85:15)	3
2	*iso*-butyl	CH_3	H	85	60	recryst from EtOH.	4
3	*iso*-butyl	C_4H_9	H	75	*	used as obtained	4
4	*iso*-butyl	$CH_2C_6H_5$	H	38	*	column filtration C_6H_{12}/CH_3COOEt (70:30)	4
5	*iso*-butyl	$CH_2C_6H_5$	Cl	91	*	used as obtained	3
6	*iso*-butyl	H	Cl	90	150	recryst. from EtOH	3
7	*iso*-butyl	C_3H_5	H	88	*	used as obtained	4
8	*iso*-butyl	C_3H_6Br	H	75	*	"	5
9	*iso*-butyl	CH_3	Cl	73	*	"	3
10	butyl	$CH_2C_6H_5$	H	88	*	"	3
11	butyl	H	H	83	*	"	3
12	butyl	CH_3	H	58	*	"	4
13	butyl	H	Cl	35	148	recryst. from EtOH	4
14	ethyl	$CH_2C_6H_5$	H	86	*	used as obtained	4
15	ethyl	H	H	86	120	"	3
16	ethyl	C_4H_9	H	65	*	"	4

*Oil

*Dialkylphosphorylhydrazones (**1–16**)*

In the ^{1}H NMR analyses, a typical signal that confirms the formation of dialkylphosphorylhydrazones corresponds to the phosphoramidic hydrogen (11.36–11.47 ppm), which is linked to the nitrogen atom α to the phosphorus atom. This hydrogen is observed as a doublet (J = 33–34 Hz) due to its coupling with the P atom. This chemical shift is characteristic of compounds containing an internal hydrogen bond; therefore, confirming that all compounds exhibit a hydrogen bond between the amide hydrogen and the carbonyl of isatin, forming a six-membered ring favoring the ***Z*** configuration (Figure 2).

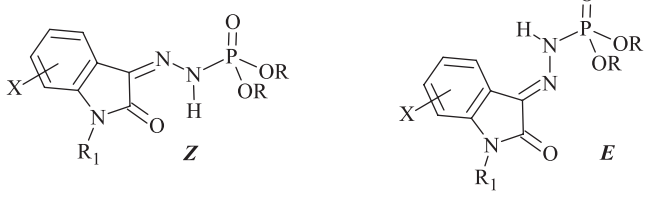


Figure 2. *The two possible diastereoisomers*

^{1}H NMR spectra of **3**, **4**, **5**, **7**, **8**, **9,** and **11** indicate that the reaction is 100% diastereoselective. However, analysis of spectra of **1**, **2**, **6**, **10**, and **12** shows that the two possible isomers ***E*** and ***Z*** coexist, as indicated by the presence of a second doublet (J = 28 Hz) at 8.45 ppm.

^{13}C NMR and ^{31}P NMR

The carbon atoms of compounds **1–10,** which are most affected by substitution, are the carbonyl and the hydrazidic carbons of the isatin moiety. The chemical shifts for the carbonyl carbon and hydrazidic carbon of the synthesized compounds varies from 160.88 ppm to 166.15 ppm and from 140.74 ppm to 148.73 ppm, respectively. The ^{31}P NMR spectra of compounds **1**, **2,** and **10** show two signals each at –0.55/–0.92 ppm, 1.01/–0.43 ppm, and–0.27/–1.30 ppm, respectively, indicating that there are two different ^{31}P atoms, which in turn suggests the presence of both ***E*** and ***Z*** isomers. This observation is corroborated by the presence of two C=O and C=N carbon signals in the ^{13}C NMR spectrum of **2**, i.e., 163.69 and 161.05 ppm for C=O and 144.28 and 142.37 ppm for C=N. The same observation can be made from the ^{13}C NMR spectrum of **10** (163.85 and 161.21 ppm for C=O and 143.58 and 141.71 ppm for C=N). Because of the poor resolution of the ^{13}C NMR spectrum of **1**, the doubling of these signals is not readily visible. However, as mentioned before, the doubling of signals in its ^{31}P NMR spectrum is proof of the presence of the two diastereoisomers.

Biological activity

Compounds **1**, **2**, **11,** and **12** exhibit higher inhibition potential for the development of *Fusarium oxysporum*. These four compounds have small groups (H and CH_3) linked to the amide nitrogen, while the rest of the molecules, except **6** and **9**, in this series have larger substituents (Table 2). The smaller inhibitory effects associated with compounds **6** and **9** can be attributed to the presence of electron withdrawing (chlorine) substituent on the aromatic ring. Further studies will be conducted to prove this hypothesis.

The growth of *Rhizoctonia solani* is inhibited by dialkylphosphorylhydrazones **9**, **11,** and **12**, the latter showing the highest inhibition potential. In this case as well, the compounds contain small groups (H and CH_3) substituted on the amide nitrogen (Table 2).

Seed germination

Use of lettuce or tomato seeds as a means to assess the effect of chemicals on germination has been shown to be a good method since the seeds germinate in a few days; between 4 and 6 days for lettuce and between 5 and 10 days for tomatoes, thereby providing faster results. Secondly, the tests can be performed in Petri dishes and are easily manipulated in a germination chamber. Finally, the cost of implementation of the assay is very low.

Table 2. Antifungal activity of dialkylphosphorylhydrazones

Compounds	Inhibition Percentage/standard deviation	
	Fusarium oxysporum	*Rhizoctonia solani*
1	57.9 ± 1.5	--
2	54.9 ± 2.9	--
3	27.2 ± 4.9	--
4	24.3 ± 1.5	--
6	27.7 ± 2.7	11.1 ± 8.8
7	34.7 ± 1.3	--
9	43.1 ± 0.8	58.1 ± 1.5
11	59.4 ± 0.9	58.7 ± 1.7
12	52.0 ± 1.5	72.1 ± 3.3
15	41.6 ± 2.0	--
16	--	49.4 ± 3.7
Mancozeb	93.7 ± 0.0	13.4 ± 5.9

The effect of dialkylphosphorylhydrazones (400 mg L^{-1}) on lettuce germination is low for all compounds (Table 3). These results indicate that these compounds can be used for protection of lettuce seeds against *Fusarium oxysporum* and *Rhizoctonia solani*. However, it is important to note that investigations with seeds of other cultivated plants must be carried out, since these results cannot be extrapolated to all types of seeds.

Table 3. Anti-germinative activity of dialkylphosphorylhydrazones

Compounds	Germination(%)/standard deviation
1	96.3 ± 3.0
2	95.0 ± 3.3
5	89.2 ± 9.8
6	98.3 ± 2.3
9	88.3 ± 3.3
11	94.2 ± 2.4
12	92.1 ± 3.0
16	99.2 ± 2.1

CONCLUSION

New dialkylphosphorylhydrazones derived from isatin were synthesized with good yields. These interesting compounds can be synthesized by relatively simple and reproducible methodologies.

Evaluation of fungicidal activity of dialkylphosphorylhydrazones against *Rhizoctonia solani* and *Fusarium oxysporum* showed that compounds **1**, **2**, **9**, **11**, and **12** can inhibit their growth. In addition, the biologically active compounds did not inhibit germination of lettuce seeds, making them potential leads in the search for new fungicides.

SUPLEMENTARY MATERIAL

All Figures and spectra in this section are available at http://quimicanova.sbq.org.br (.pdf format) with free access.

ACKNOWLEDGMENTS

We thank for CAPES and CNPq for financial support.

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SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL ACTIVITY OF A NEW CLASS OF DIALKYLPHOSPHORYLHYDRAZONE DERIVATIVES OF ISATIN

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Spectra: IR, ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR and mass.

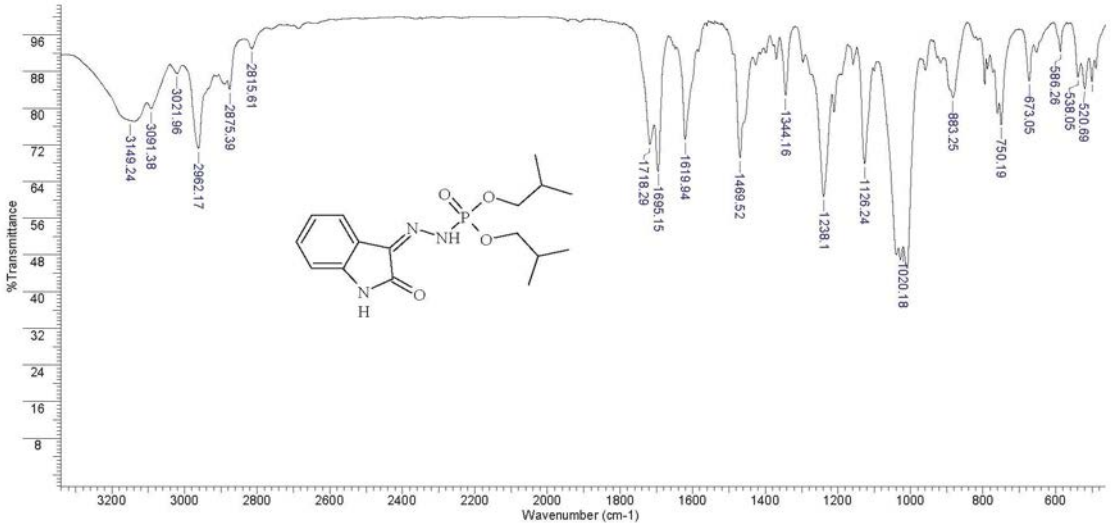

Figure 1S. *IR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**1**)*

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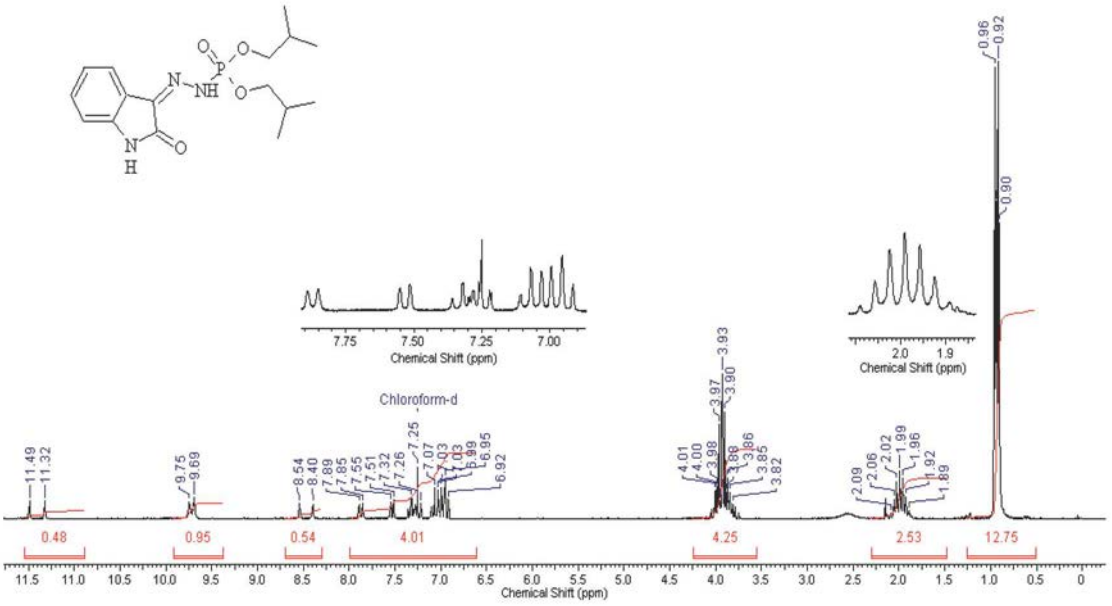

Figure 2S. *[1]H NMR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**1**) ($CDCl_3$ - 200 MHz)*

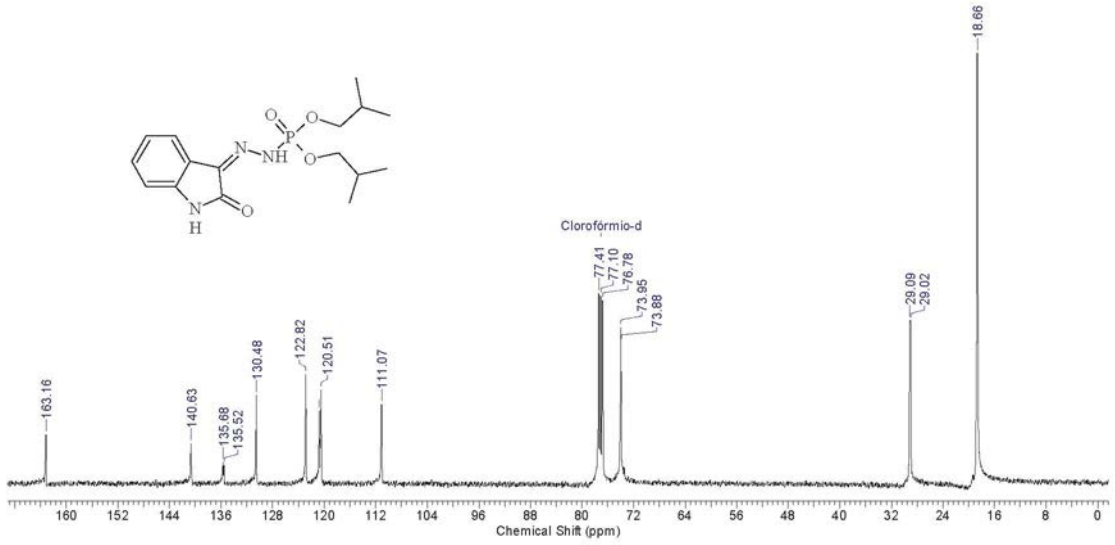

Figure 3S. *[13]C NMR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**1**) ($CDCl_3$ – 50.3 MHz)*

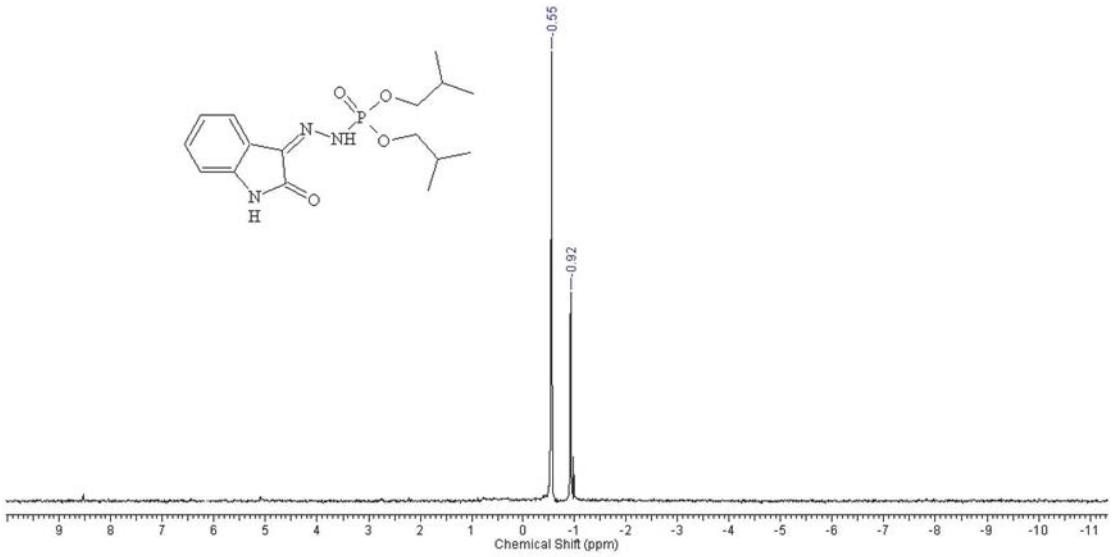

Figure 4S. *^{31}P NMR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**1**) ($CDCl_3$ – 81.0 MHz)*

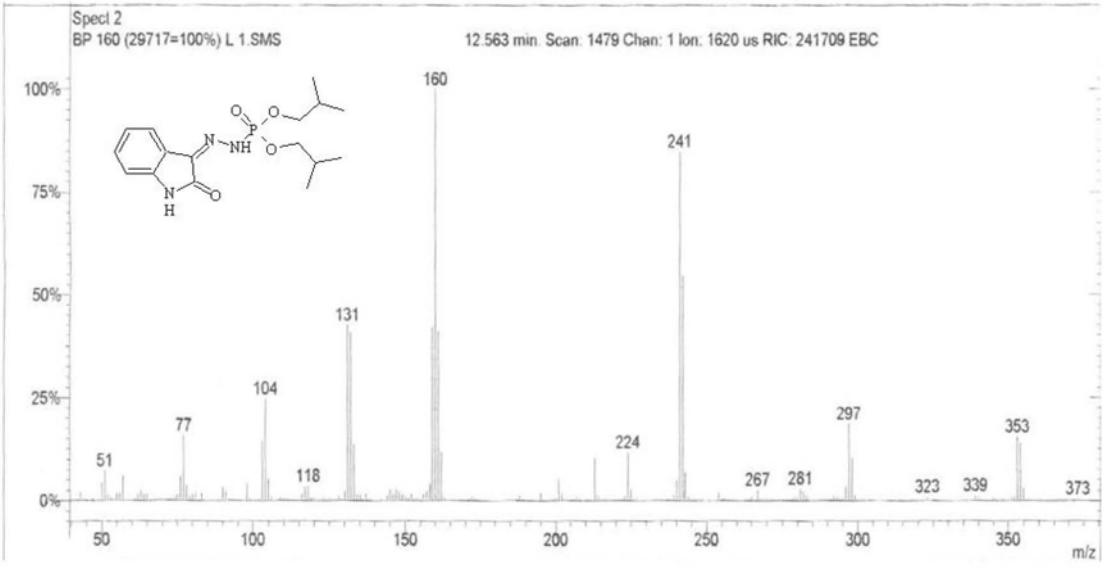

Figure 5S. *Mass spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**1**)*

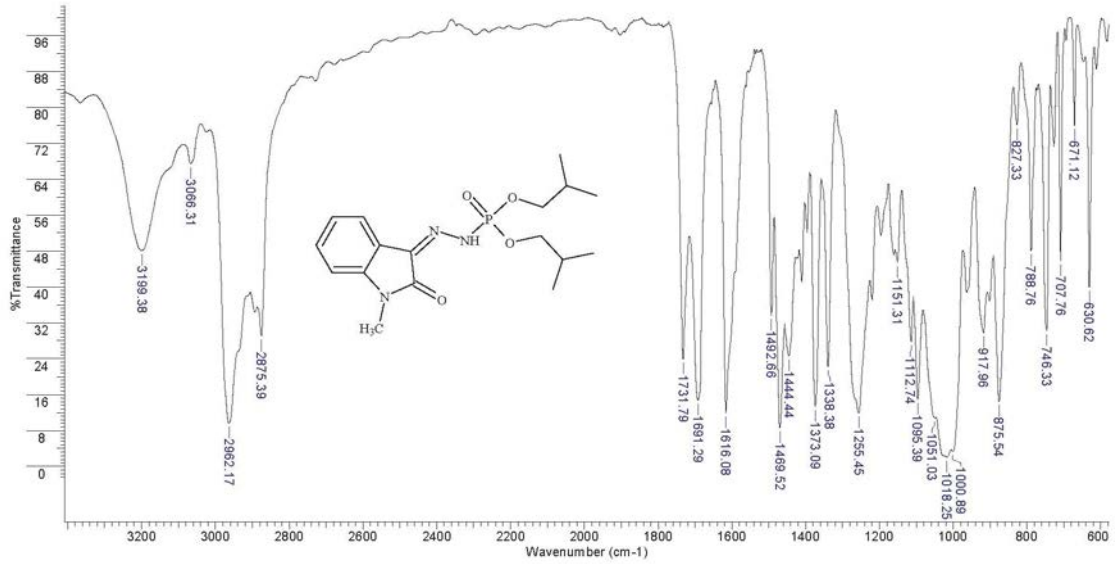

Figure 6S. *IR spectrum of Phosphorohydrazidic acid N'-[1,2-dihydro-1-methyl-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**2**)*

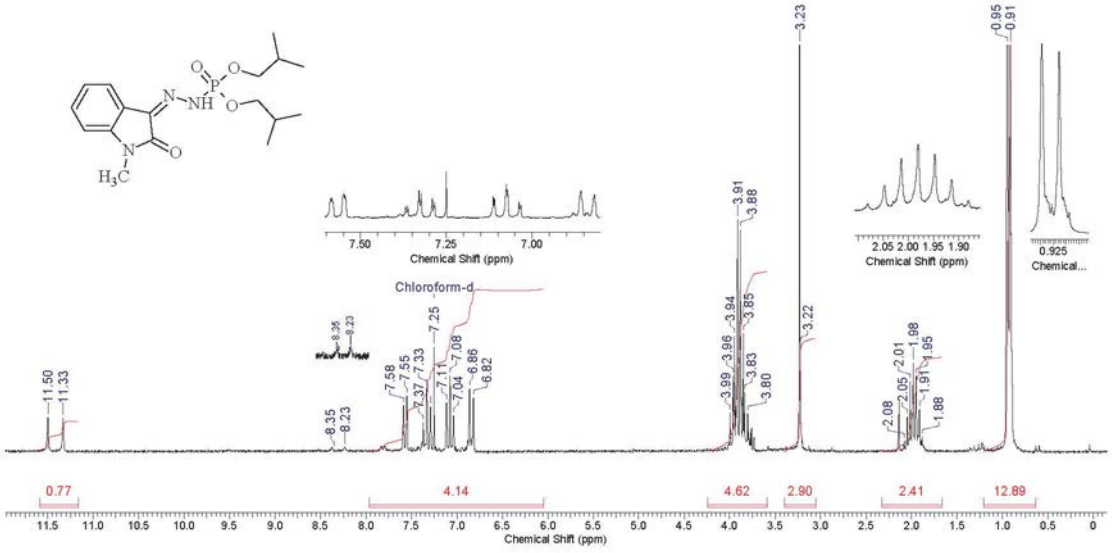

Figure 7S. *^{1}H NMR spectrum of Phosphorohydrazidic acid N'-[1,2-dihydro-1-methyl-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**2**) ($CDCl_3$ - 400 MHz)*

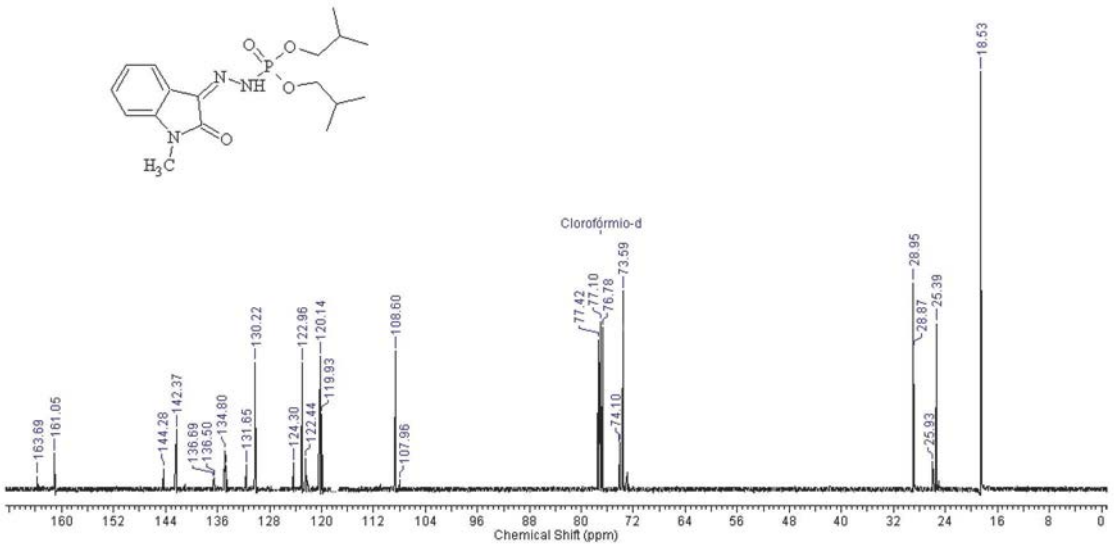

Figure 8S. *^{13}C NMR spectrum of Phosphorohydrazidic acid N'-[1,2-dihydro-1-methyl-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**2**) ($CDCl_3$ - 100.6 MHz)*

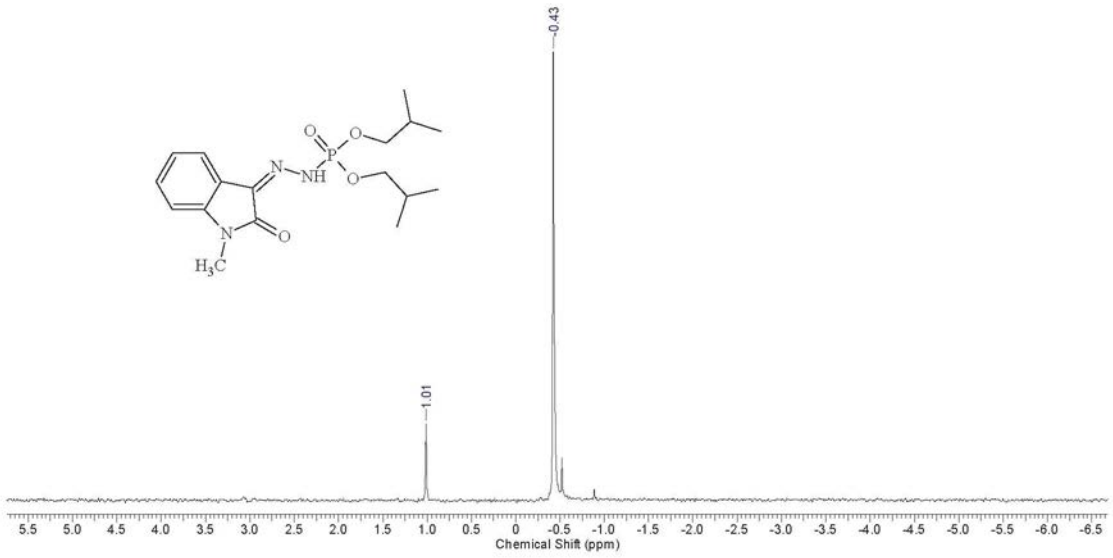

Figure 9S. *^{31}P NMR spectrum of Phosphorohydrazidic acid N'-[1,2-dihydro-1-methyl-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**2**) ($CDCl_3$ - 161.9 MHz)*

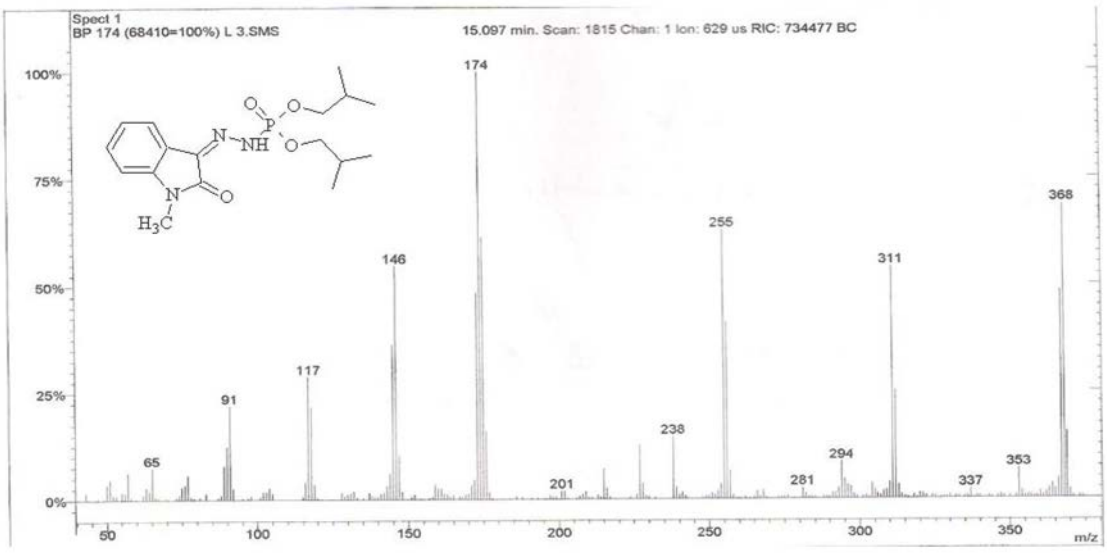

Figure 10S. *Mass spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-1-methyl-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**2**)*

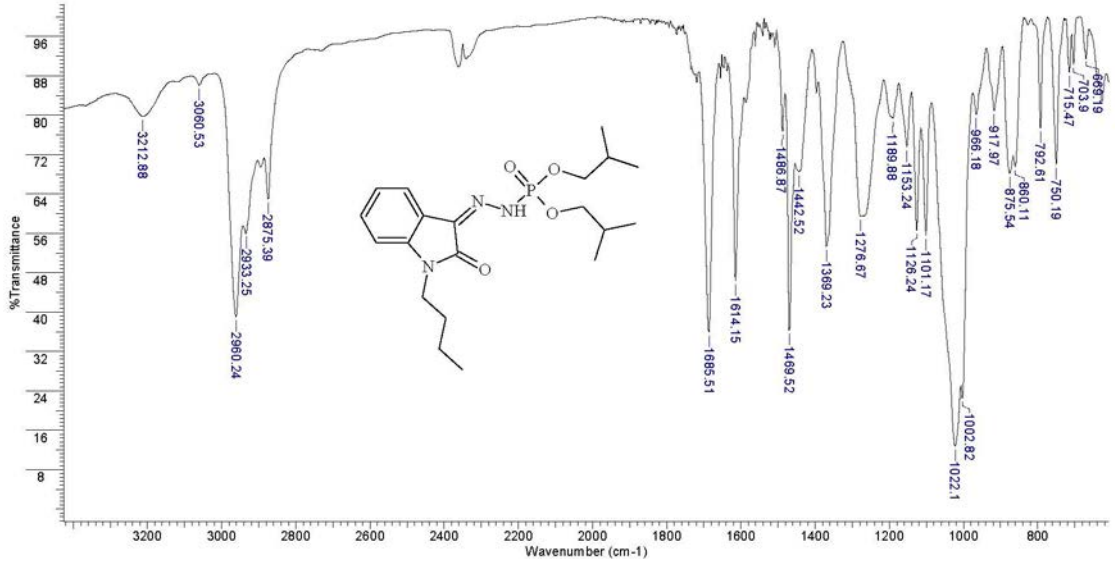

Figure 11S. *IR spectrum of phosphorohydrazidic acid N'-[1-butyl-1,2-dihydro-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**3**)*

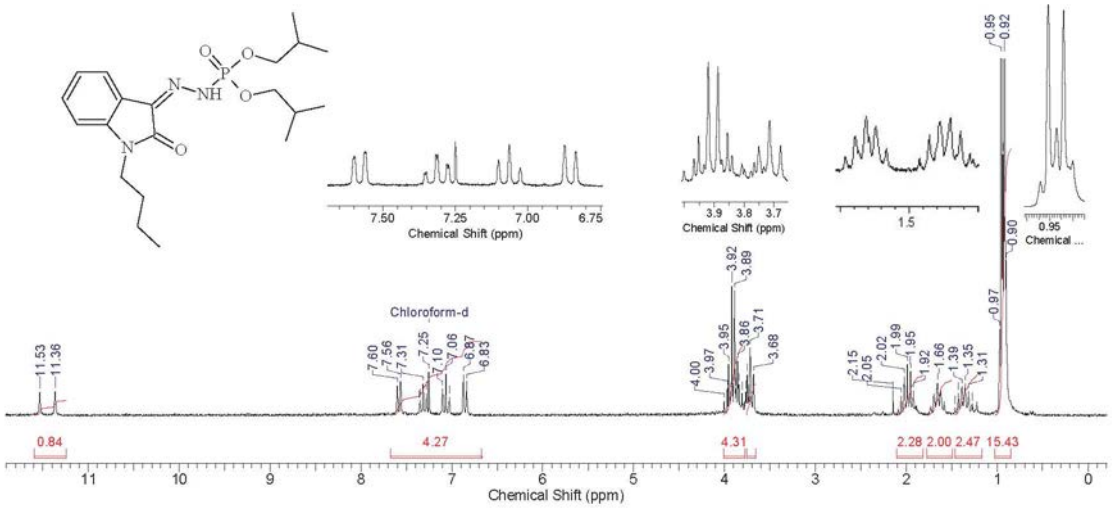

Figure 12S. *^{1}H NMR spectrum of phosphorohydrazidic acid N'-[1-butyl-1,2-dihydro-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**3**) ($CDCl_3$ - 200 MHz)*

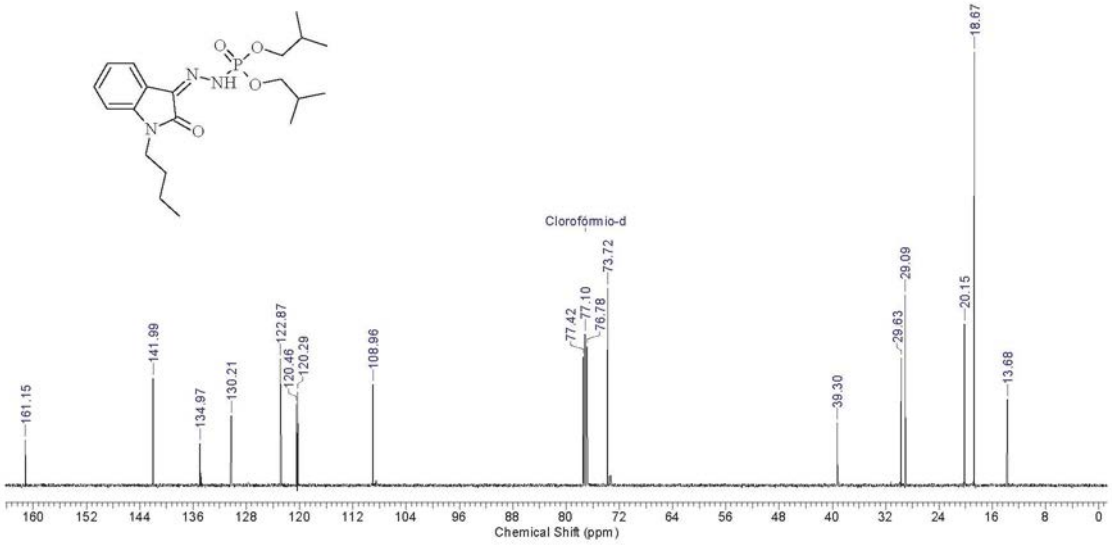

Figure 13S. *^{13}C NMR spectrum of phosphorohydrazidic acid N'-[1-butyl-1,2-dihydro-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**3**) ($CDCl_3$ - 50.3 MHz)*

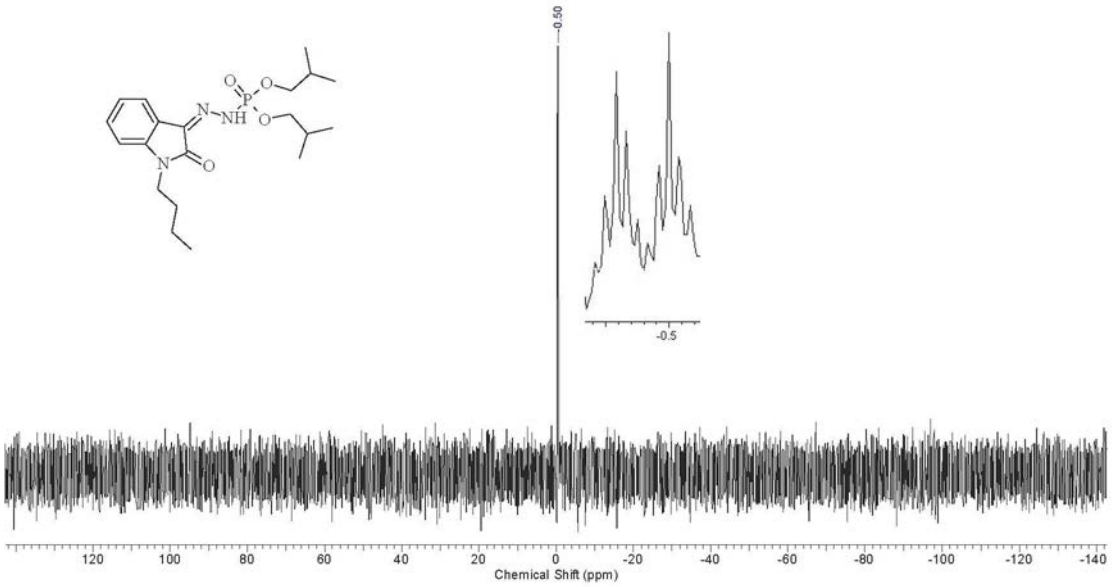

Figure 14S. *^{31}P NMR spectrum of phosphorohydrazidic acid N'-[1-butyl-1,2-dihydro-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**3**) ($CDCl_3$ - 81.0 MHz)*

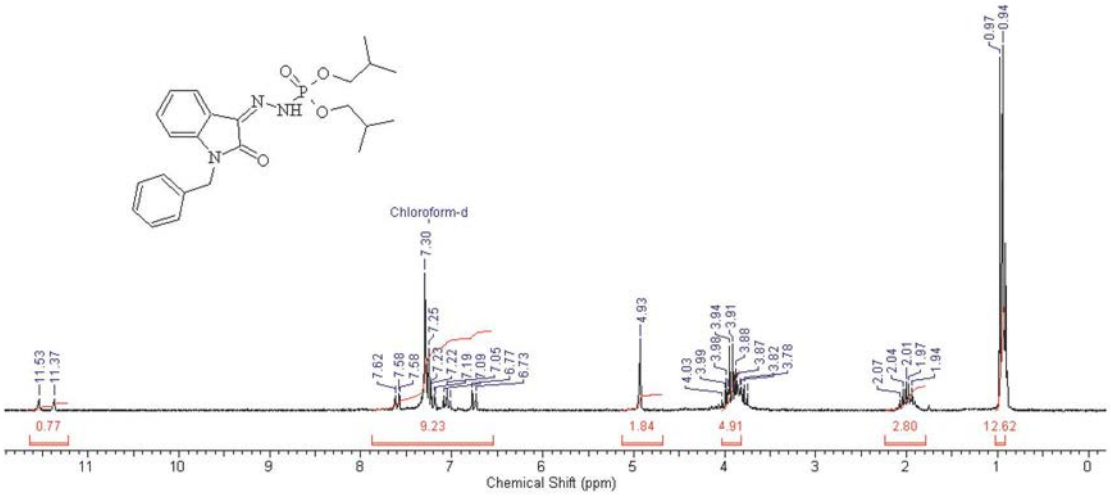

Figure 15S. *^{1}H NMR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-1-(benzyl)-3H-indol-3-ylidene] - diisobutyl ester (**4**) ($CDCl_3$ - 200 MHz)*

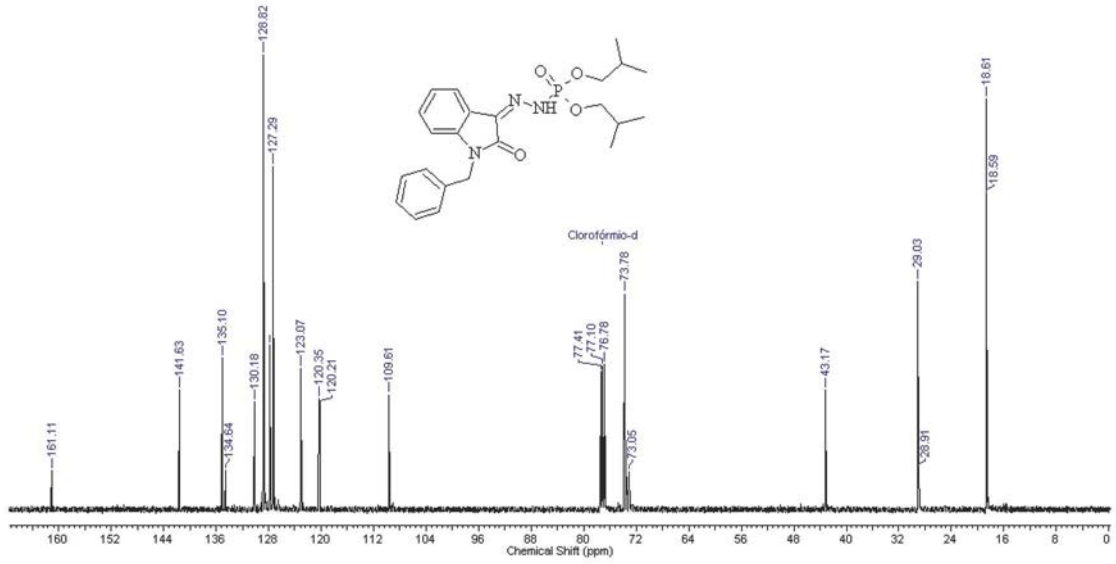

Figure 16S. *^{13}C NMR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-1-(benzyl)-3H-indol-3-ylidene] - diisobutyl ester (**4**) ($CDCl_3$ - 50.3 MHz)*

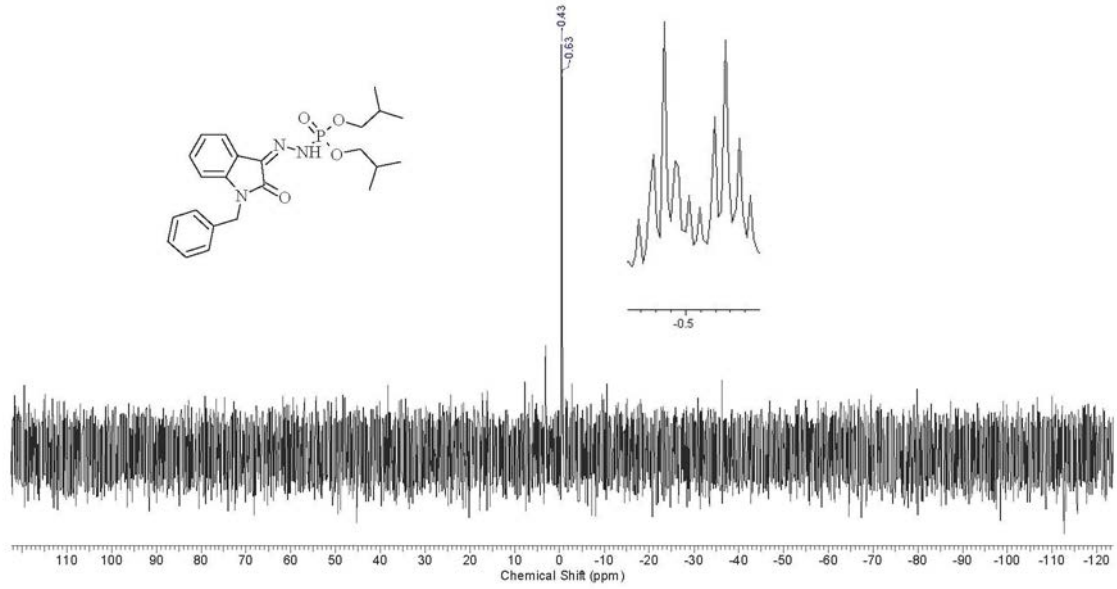

Figure 17S. *^{31}P NMR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-1-(benzyl)-3H-indol-3-ylidene] - diisobutyl ester (**4**) ($CDCl_3$ - 81.0 MHz)*

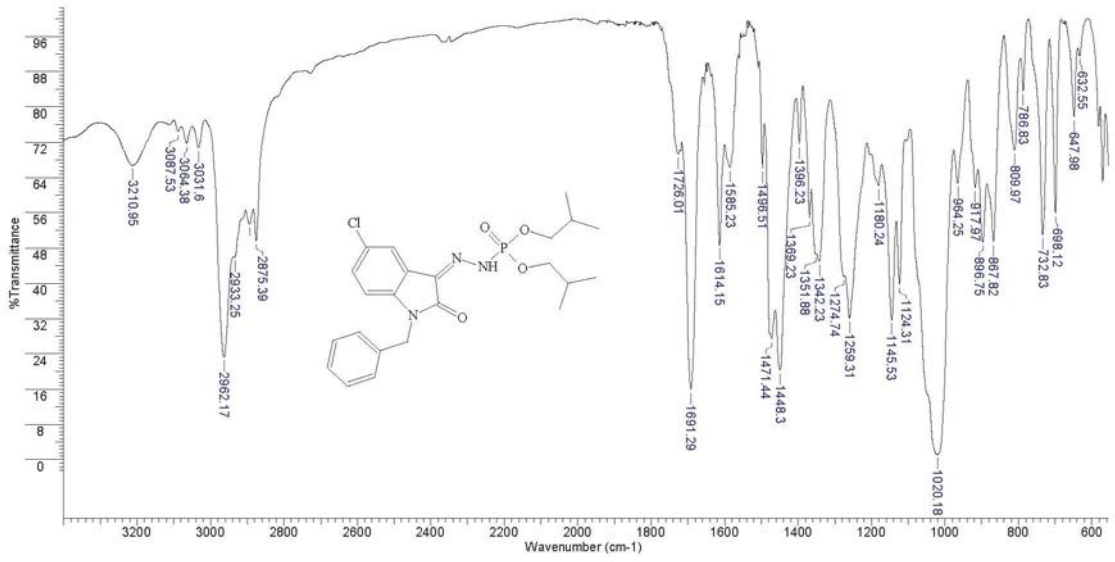

Figure 18S. *IR spectrum of phosphorohydrazidic acid, N'-[5-chloro-1,2-dihydro-2-oxo-1-(benzyl)-3H-indol-3-ylidene] - diisobutyl ester (**5**)*

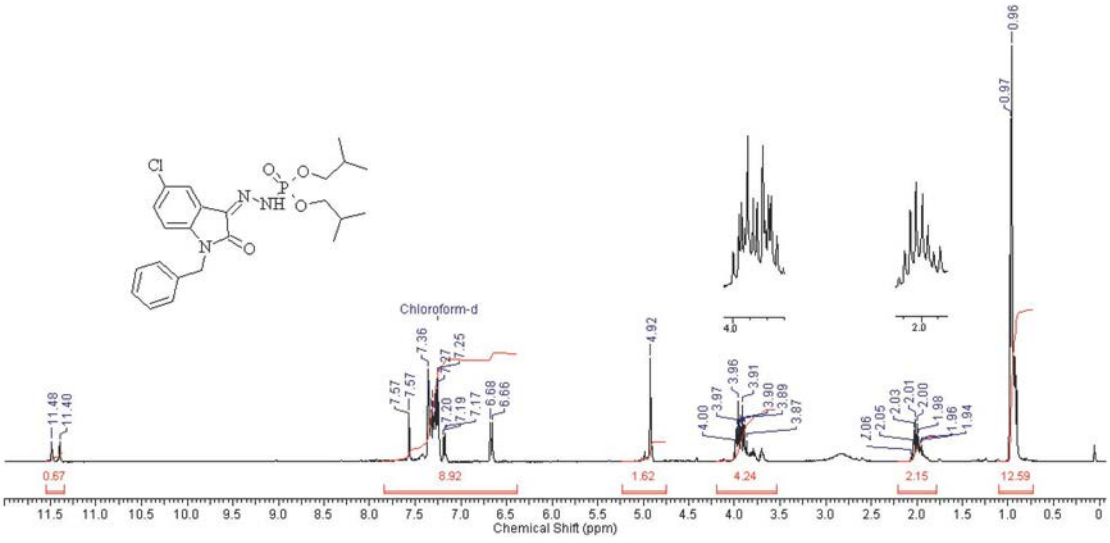

Figure 19S. *^{1}H NMR spectrum of phosphorohydrazidic acid, N'-[5-chloro-1,2-dihydro-2-oxo-1-(benzyl)-3H-indol-3-ylidene] - diisobutyl ester (**5**) ($CDCl_3$ - 400 MHz)*

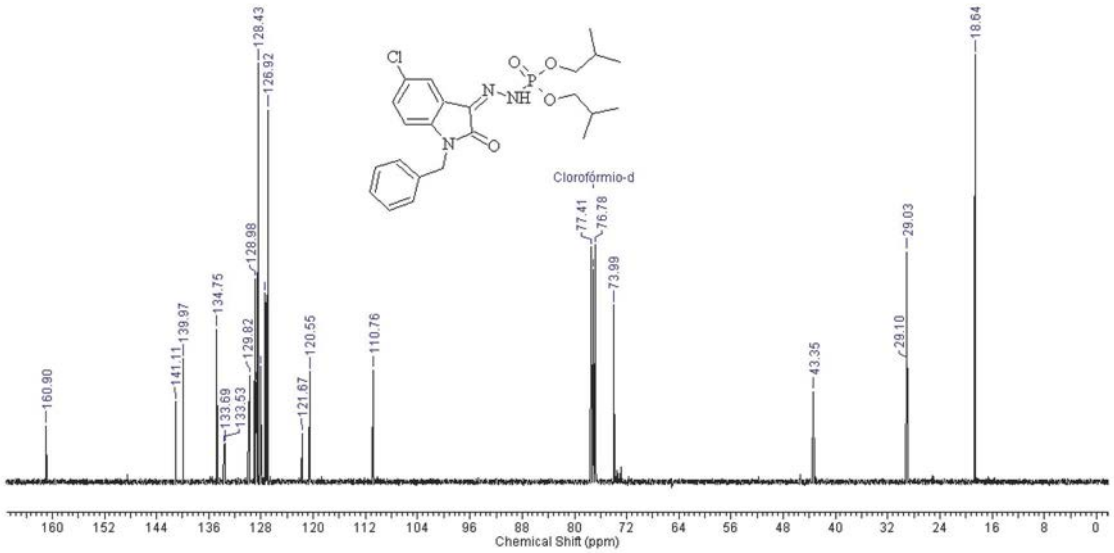

Figure 20S. *^{13}C NMR spectrum of phosphorohydrazidic acid, N'-[5-chloro-1,2-dihydro-2-oxo-1-(benzyl)-3H-indol-3-ylidene] - diisobutyl ester (**5**) ($CDCl_3$ – 100.6 MHz)*

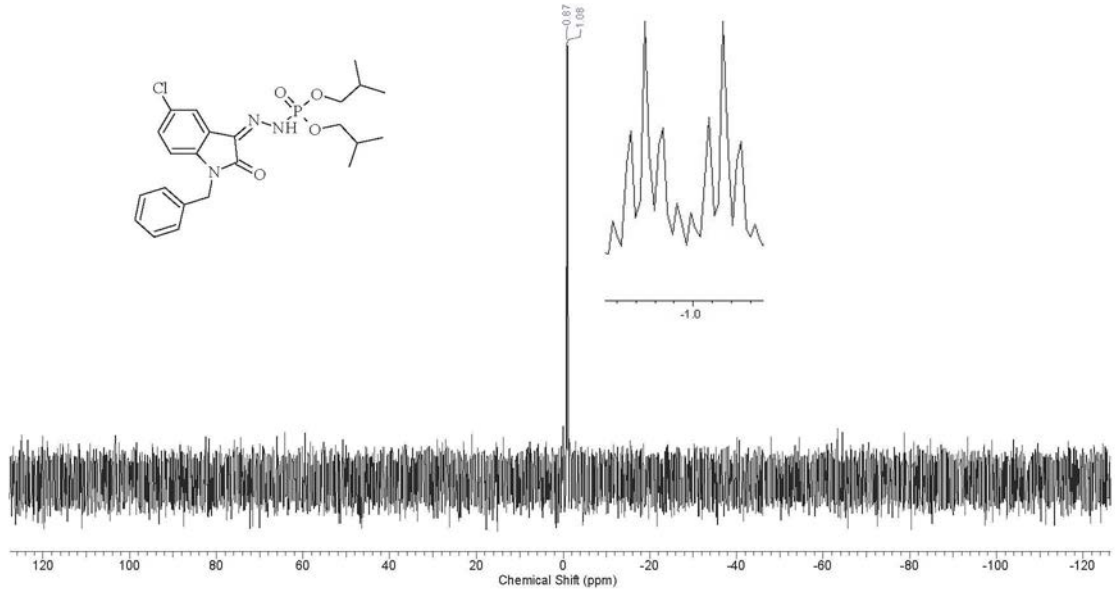

Figure 21S. *^{31}P NMR spectrum of phosphorohydrazidic acid, N'-[5-chloro-1,2-dihydro-2-oxo-1-(benzyl)-3H-indol-3-ylidene] - diisobutyl ester (**5**) ($CDCl_3$ - 161.9 MHz)*

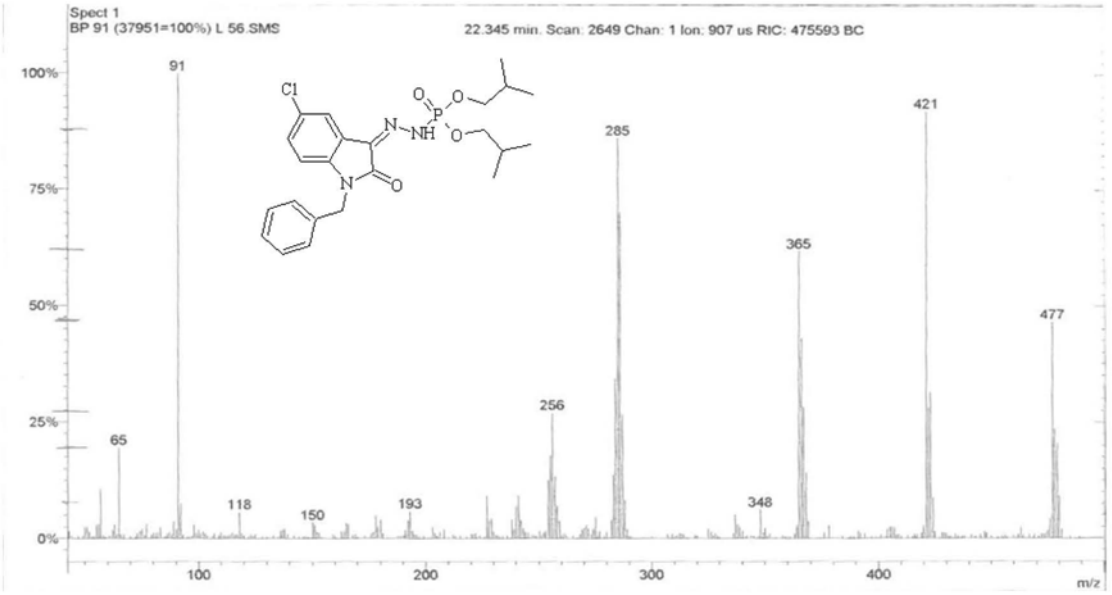

Figure 22S. *Mass spectrum of phosphorohydrazidic acid, N'-[5-chloro-1,2-dihydro-2-oxo-1-(benzyl)-3H-indol-3-ylidene] - diisobutyl ester (**5**)*

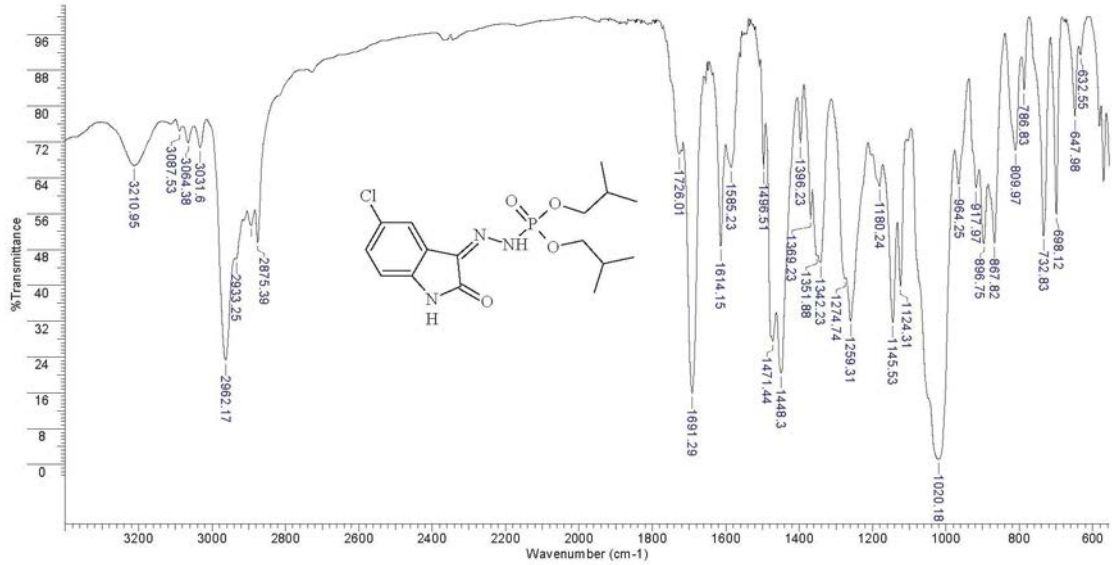

Figure 23S. *IR spectrum of phosphorohydrazidic acid, N'- [5-chloro-1,2-dihydro-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**6**)*

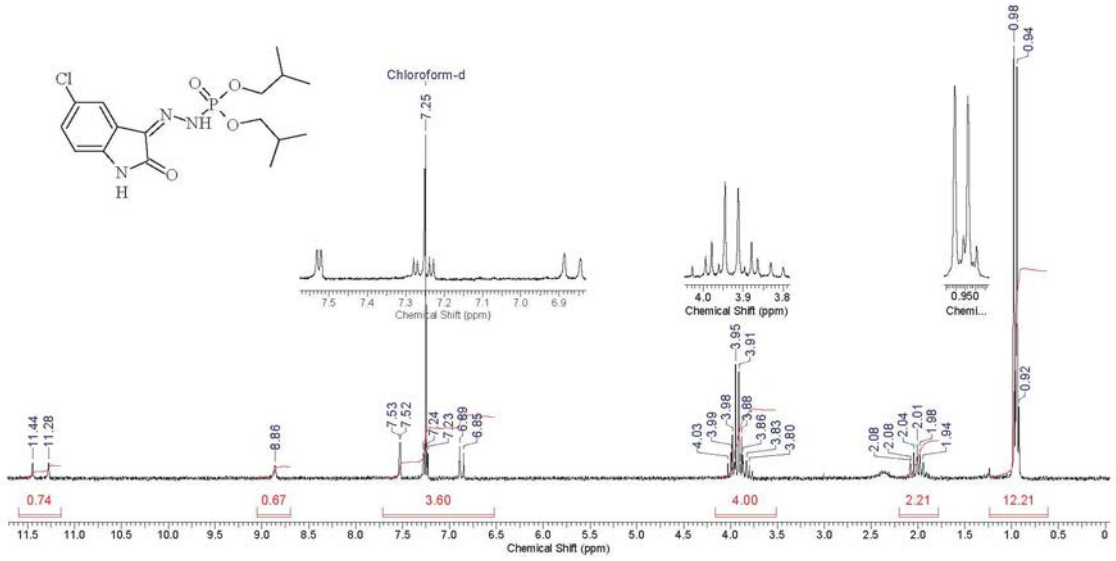

Figure 24S. *[1]H NMR spectrum of phosphorohydrazidic acid, N'- [5-chloro-1,2-dihydro-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**6**) ($CDCl_3$ - 400 MHz)*

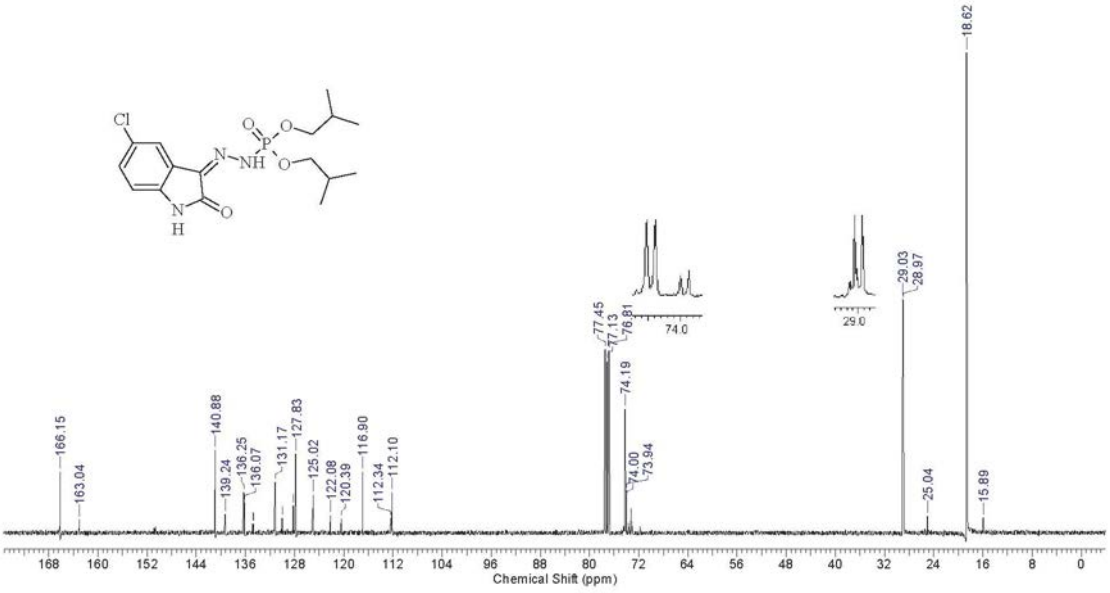

Figure 25S. *[13]C NMR spectrum of phosphorohydrazidic acid, N'- [5-chloro-1,2-dihydro-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**6**). ($CDCl_3$ - 100.6 MHz)*

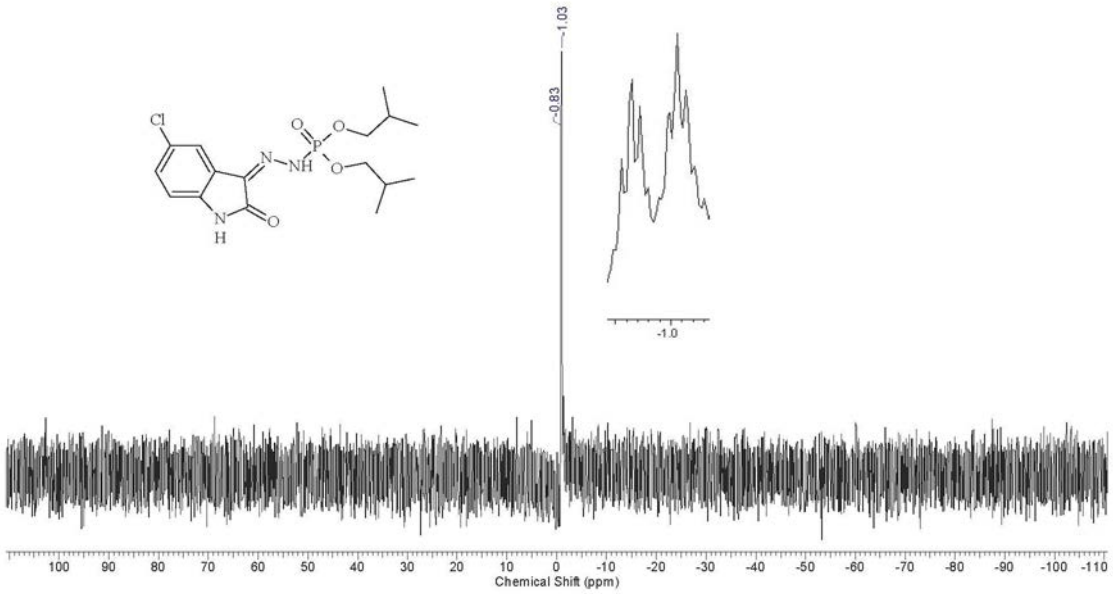

Figure 26S. *^{31}P NMR spectrum of phosphorohydrazidic acid, N'- [5-chloro-1,2-dihydro-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**6**). ($CDCl_3$ - 161.9 MHz)*

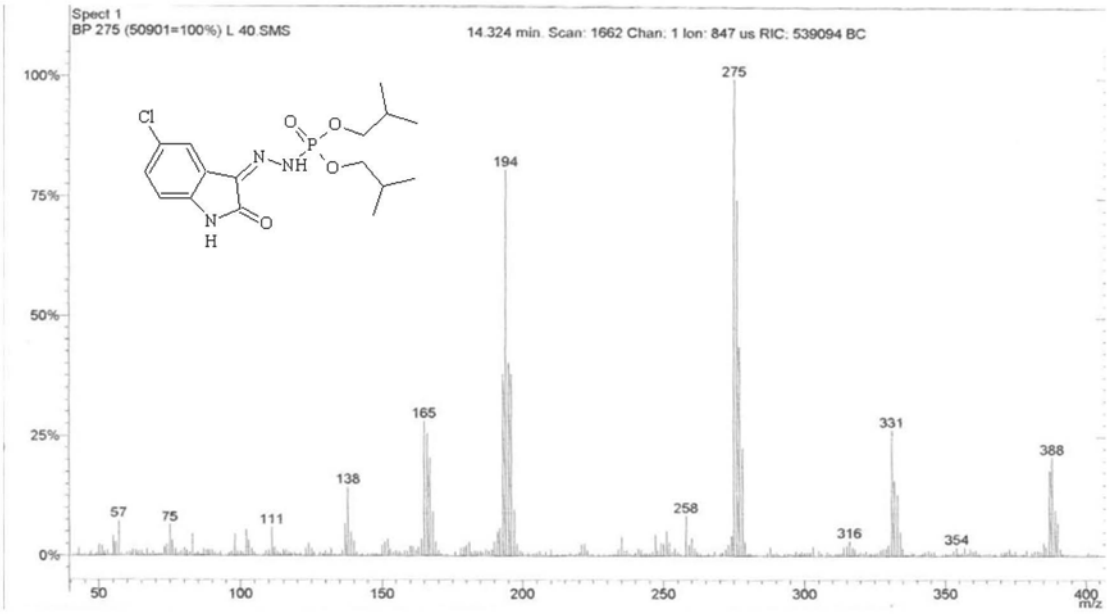

Figure 27S. *Mass spectrum of phosphorohydrazidic acid, N'- [5-chloro-1,2-dihydro-2-oxo-3H-indol-3-ylidene] - diisobutyl ester (**6**)*

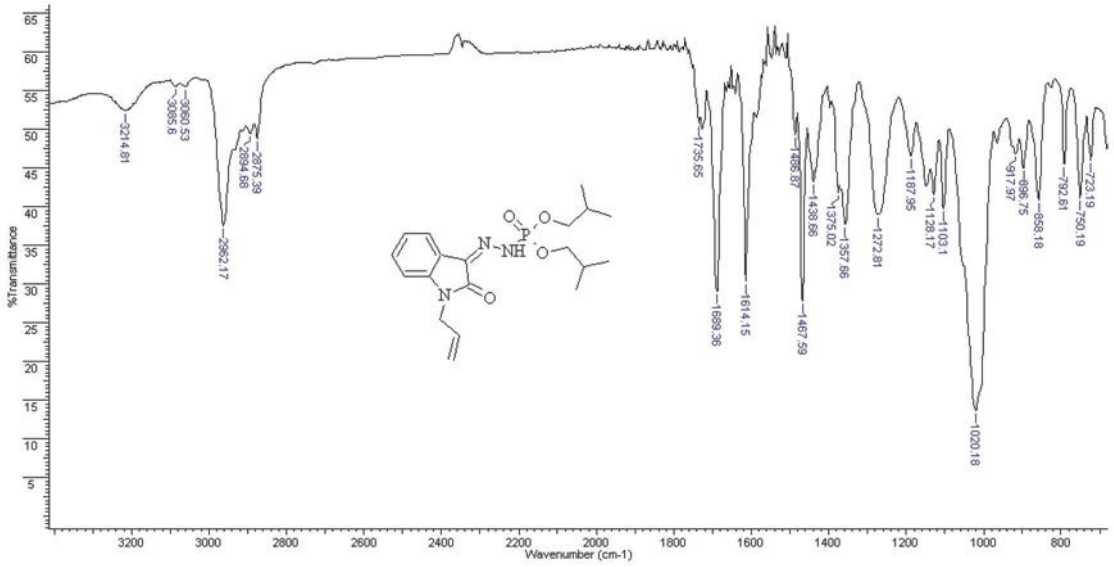

Figure 28S. *IR spectrum of phosphorohydrazidic acid, N'- [1,2-dihydro-2-oxo-1-(2-propenyl)-3H-indol-3-ylidene] - diisobutyl ester (**7**)*

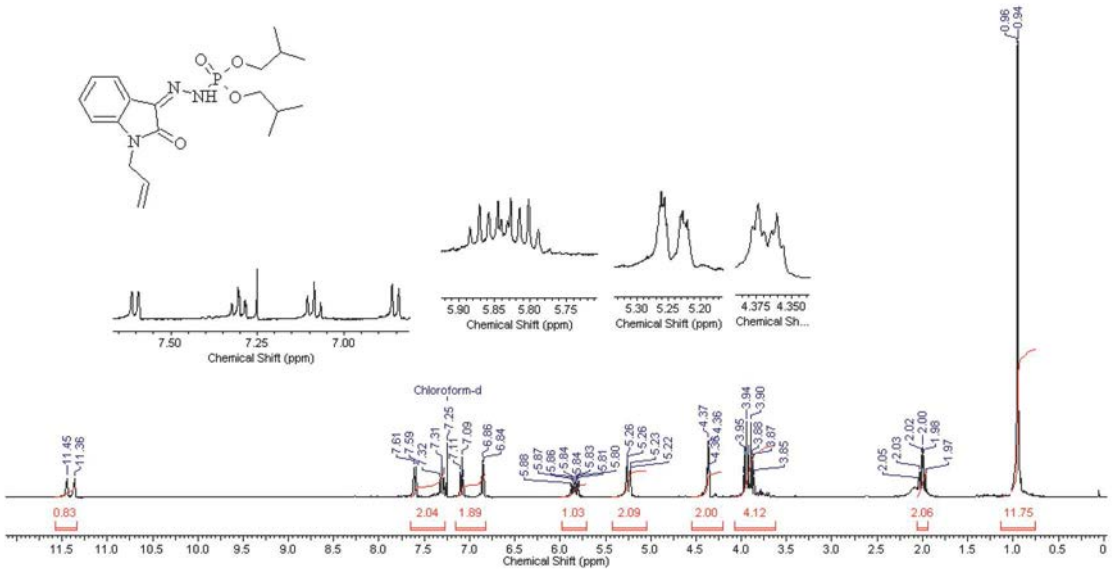

Figure 29S. *^{1}H NMR spectrum of phosphorohydrazidic acid, N'- [1,2-dihydro-2-oxo-1-(2-propenyl)-3H-indol-3-ylidene] - diisobutyl ester (**7**). ($CDCl_3$ - 400 MHz)*

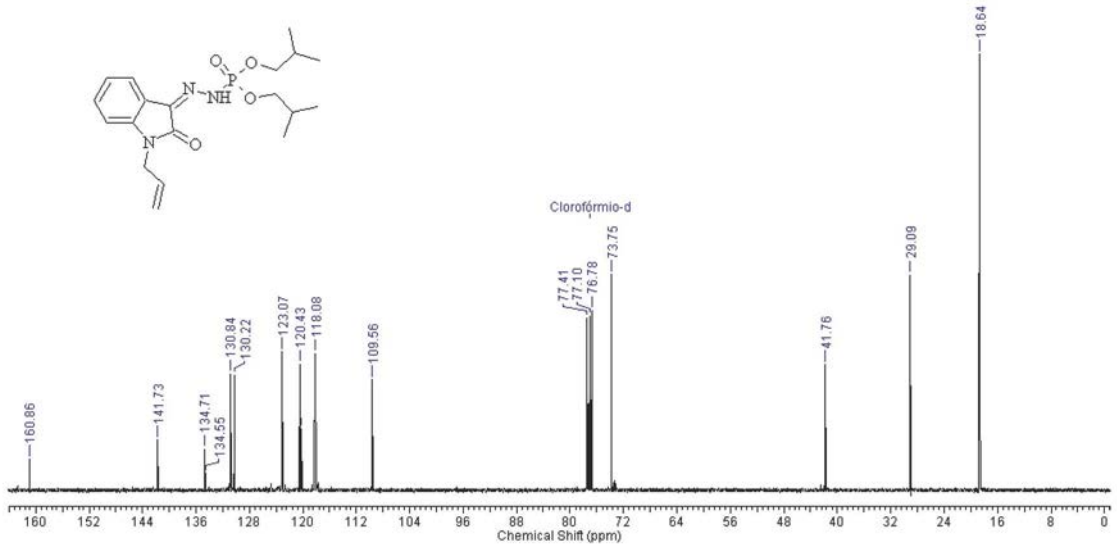

Figure 30S. *[13]C NMR spectrum of phosphorohydrazidic acid, N'- [1,2-dihydro-2-oxo-1-(2-propenyl)-3H-indol-3-ylidene] - diisobutyl ester (**7**). ($CDCl_3$ - 100.6 MHz)*

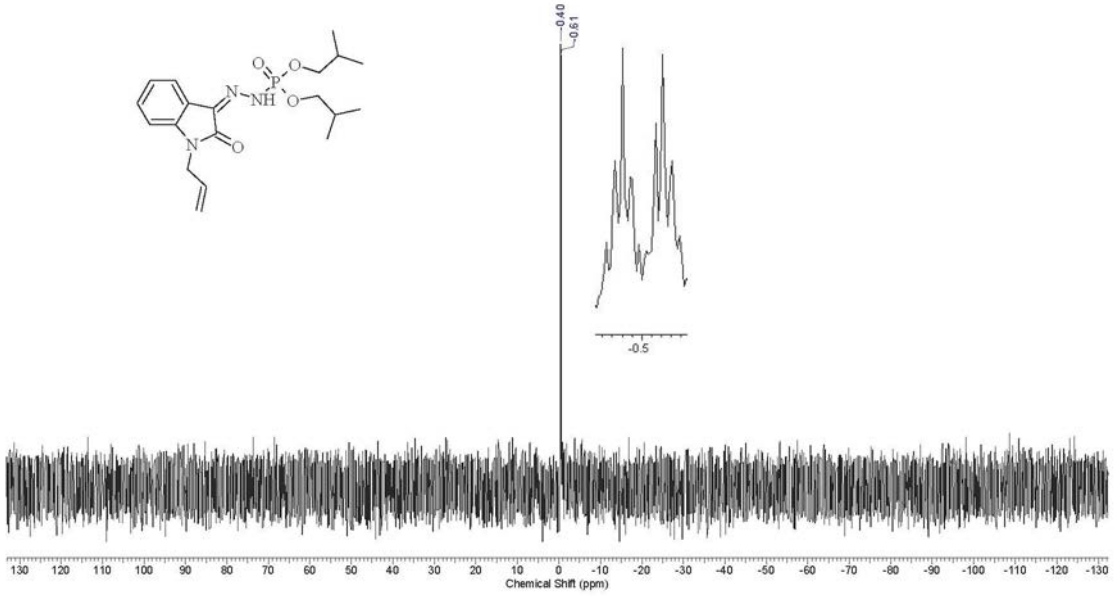

Figure 31S. *[31]P NMR spectrum of phosphorohydrazidic acid, N'- [1,2-dihydro-2-oxo-1-(2-propenyl)-3H-indol-3-ylidene] - diisobutyl ester (**7**). ($CDCl_3$ - 161.9 MHz)*

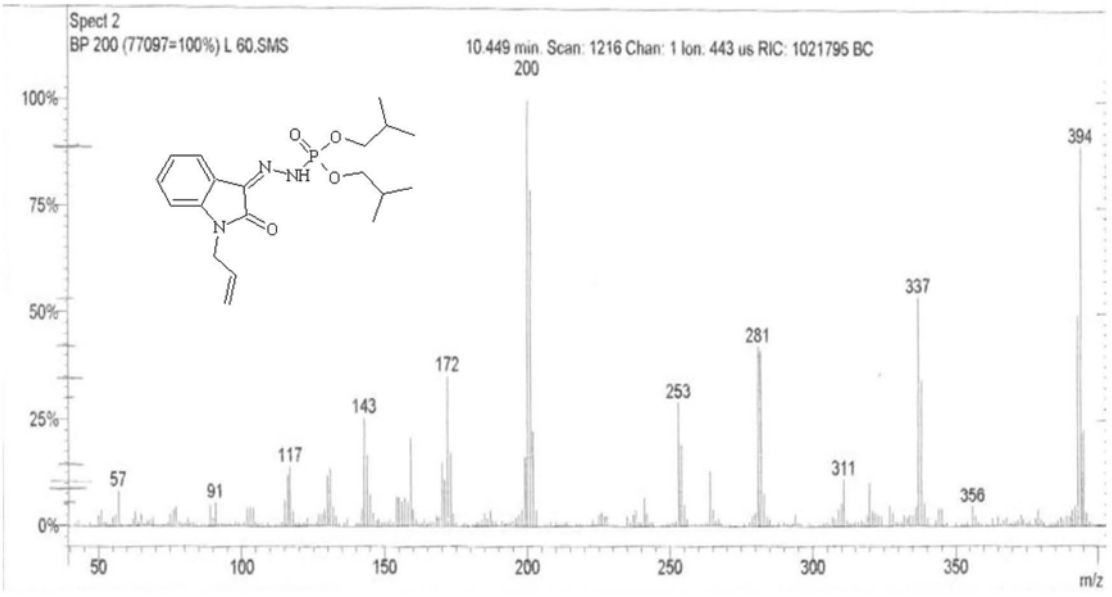

Figure 32S. *Mass spectrum of phosphorohydrazidic acid, N'- [1,2-dihydro-2-oxo-1-(2-propenyl)-3H-indol-3-ylidene] - diisobutyl ester (**7**)*

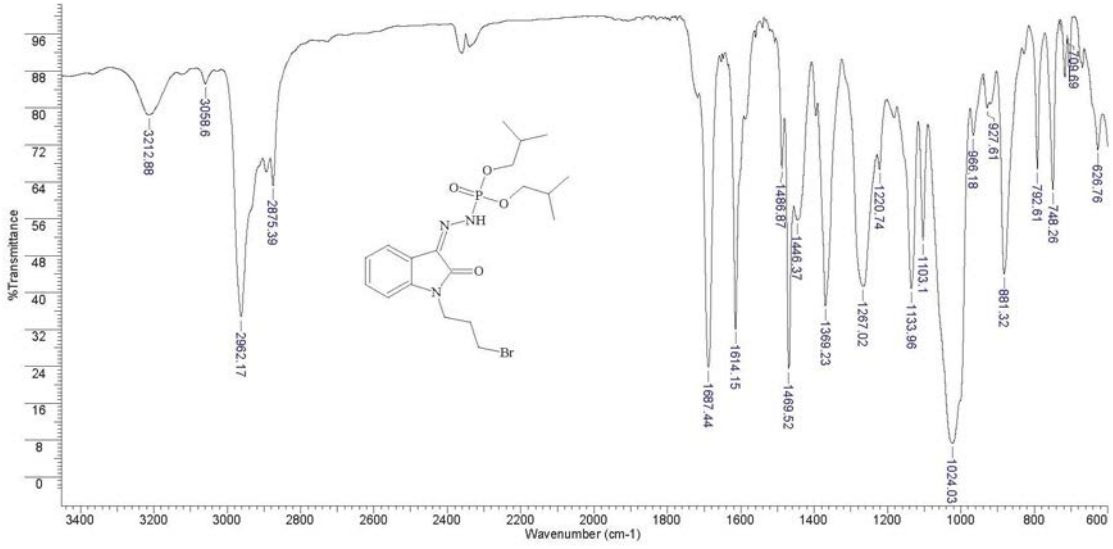

Figure 33S. *IR spectrum of phosphorohydrazidic acid, N'- [1-(3-bromopropyl)-1,2-dihydro-2-oxo-3H-indol-3-ylidene]-, diisobutyl ester (**8**)*

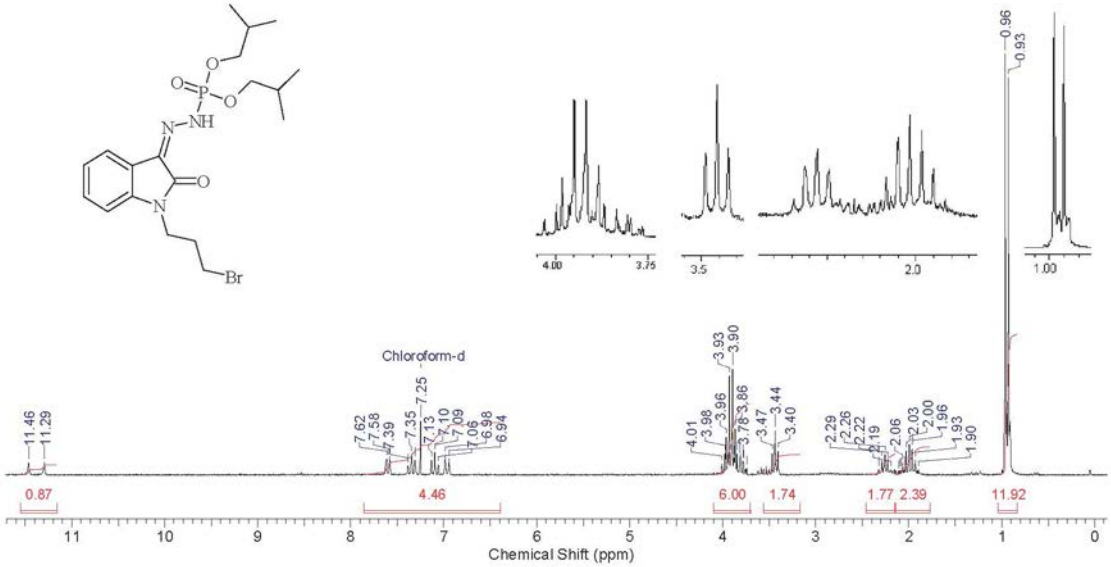

Figure 34S. *[1]H NMR spectrum of phosphorohydrazidic acid, N'- [1-(3-bromopropyl)-1,2-dihydro-2-oxo-3H-indol-3-ylidene]-, diisobutyl ester (**8**). ($CDCl_3$ - 200 MHz)*

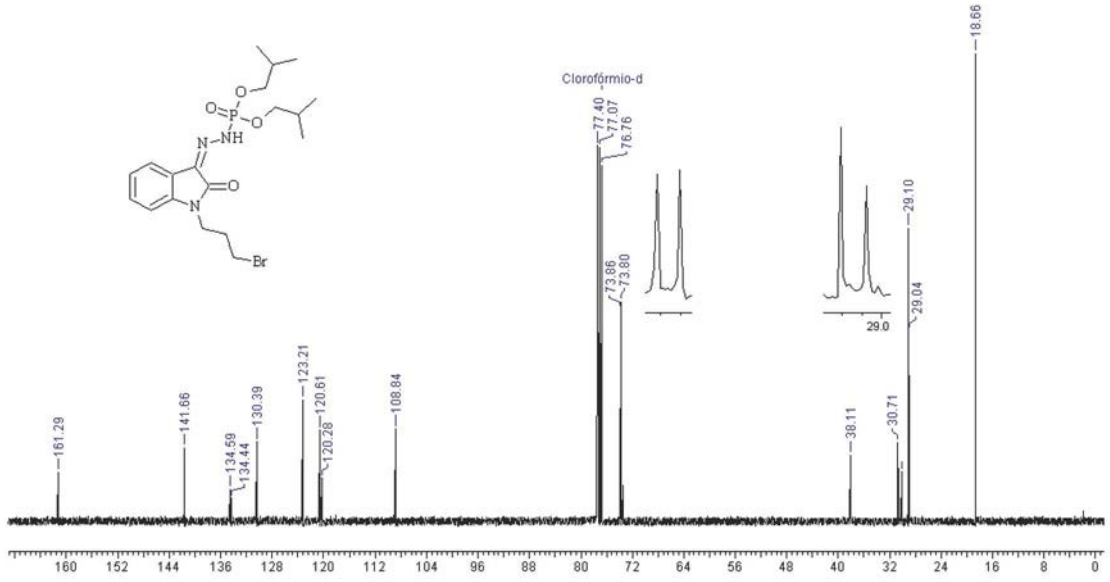

Figure 35S. *[13]C NMR spectrum of phosphorohydrazidic acid, N'- [1-(3-bromopropyl)-1,2-dihydro-2-oxo-3H-indol-3-ylidene]-, diisobutyl ester (**8**). ($CDCl_3$ - 50.3 MHz)*

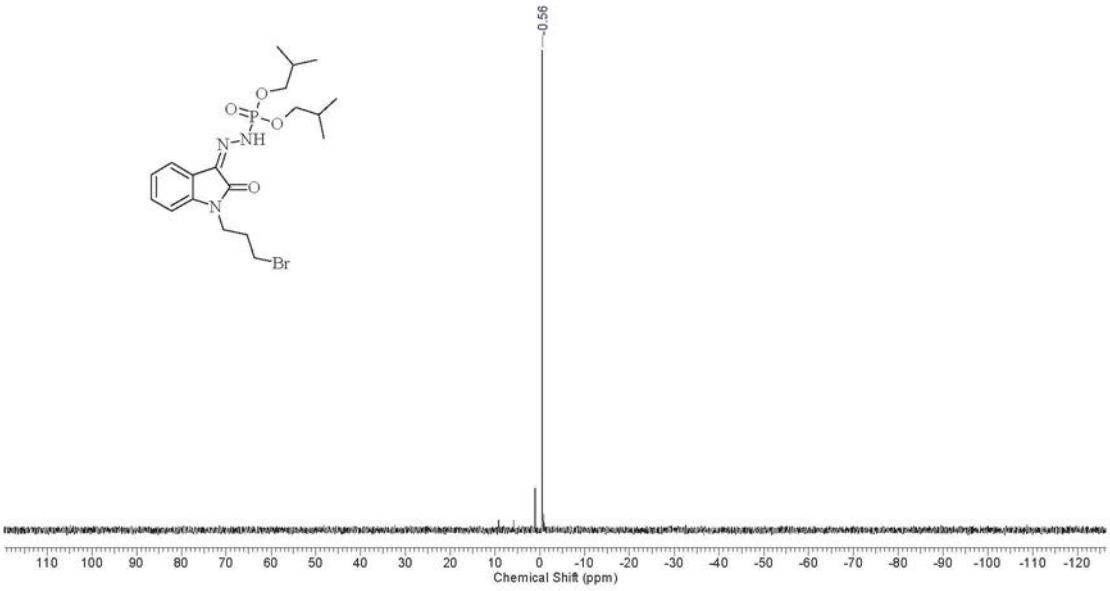

Figure 36S. *^{31}P NMR spectrum of phosphorohydrazidic acid, N'- [1-(3-bromopropyl)-1,2-dihydro-2-oxo-3H-indol-3-ylidene]-, diisobutyl ester (**8**). ($CDCl_3$ - 81.0 MHz)*

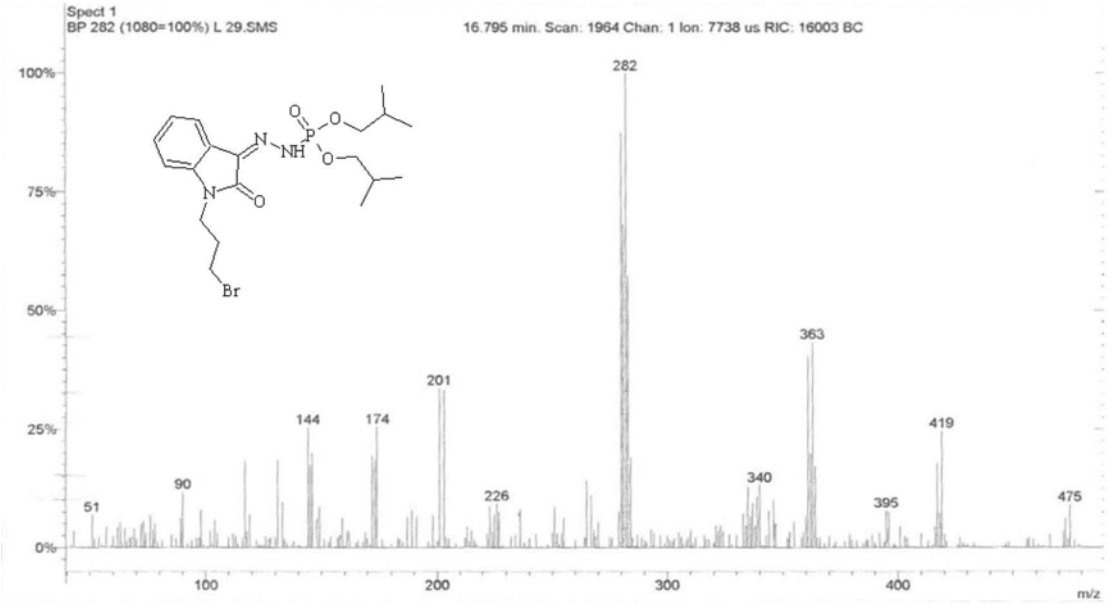

Figure 37S. *Mass spectrum of phosphorohydrazidic acid, N'- [1-(3-bromopropyl)-1,2-dihydro-2-oxo-3H-indol-3-ylidene]-, diisobutyl ester (**8**)*

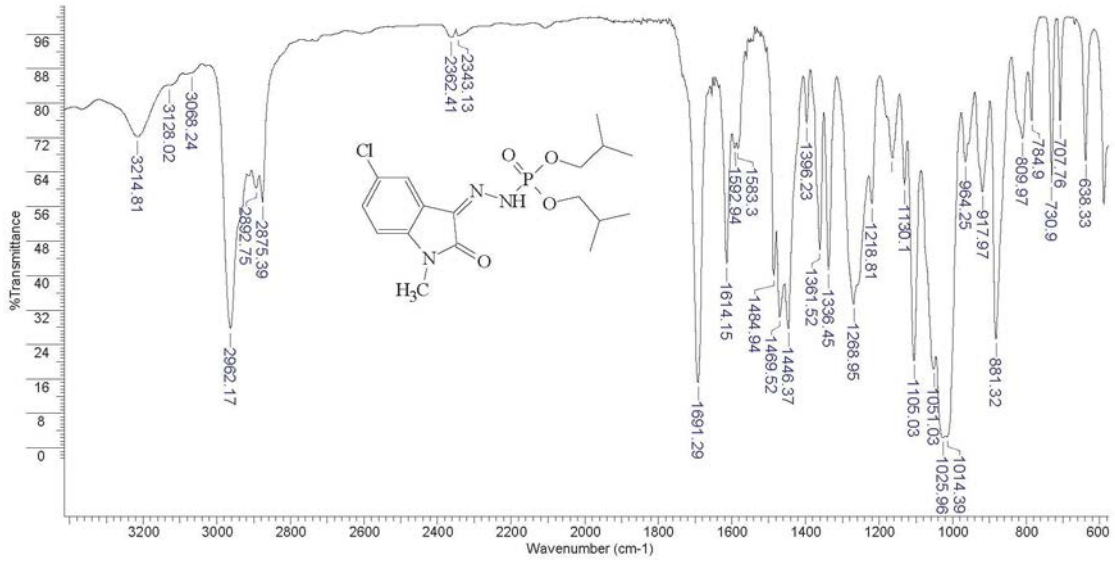

Figure 38S. *IR spectrum of phosphorohydrazidic acid, N'-[5-cloro-1,2-dihydro-1-metil-2-oxo-3H-indol-3-ylidene]-diisobutyl ester (**9**)*

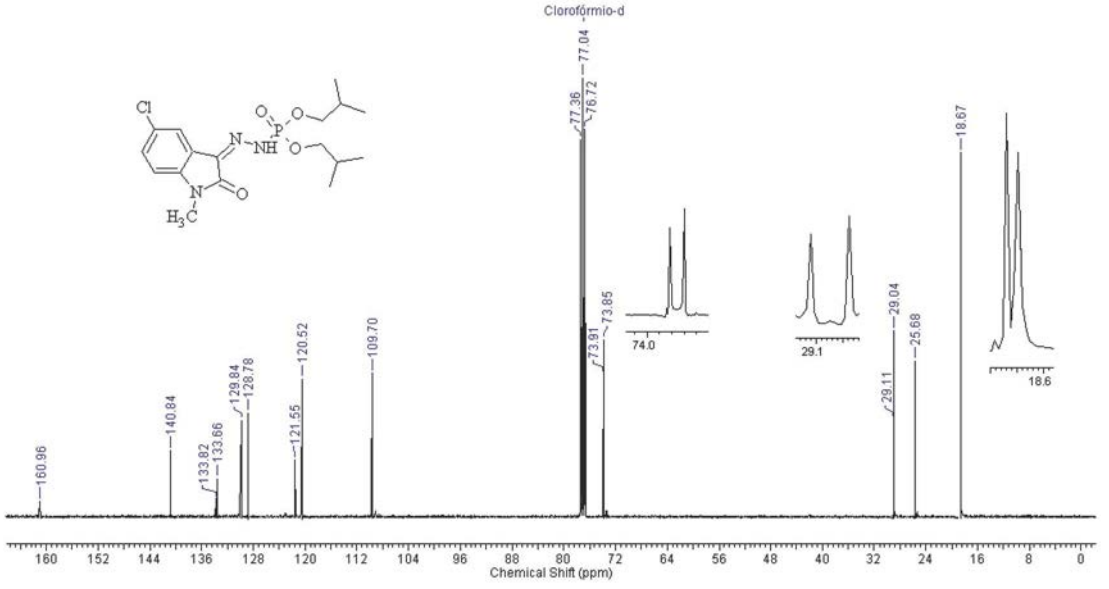

Figure 39S. *^{13}C NMR spectrum of phosphorohydrazidic acid, N'-[5-cloro-1,2-dihydro-1-metil-2-oxo-3H-indol-3-ylidene]-diisobutyl ester (**9**). ($CDCl_3$ - 50.3 MHz)*

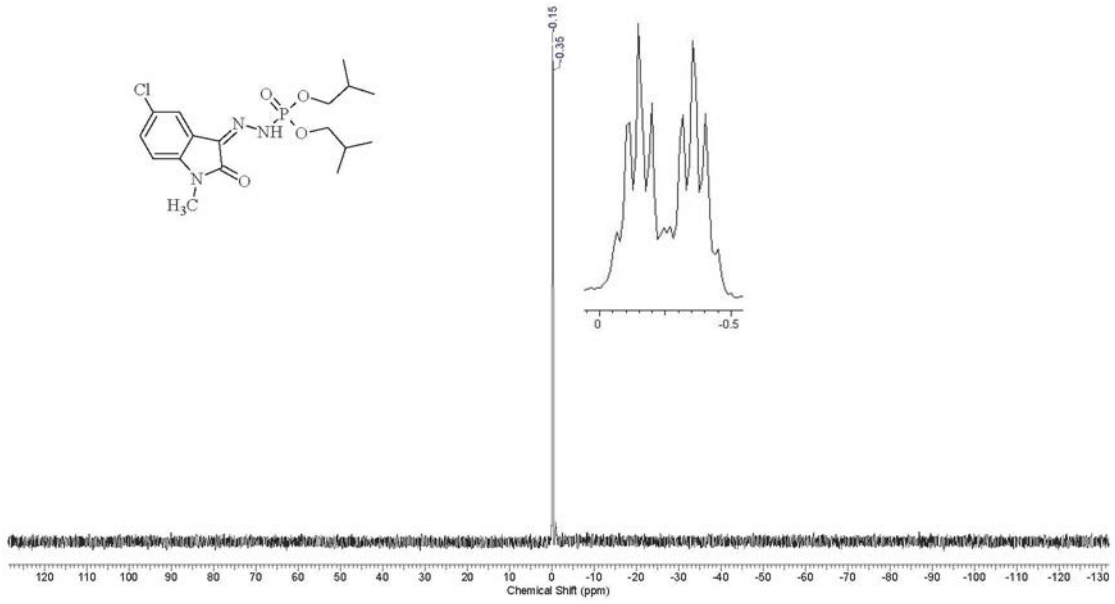

Figure 40S. *^{31}P NMR spectrum of phosphorohydrazidic acid, N'-[5-cloro-1,2-dihydro-1-metil-2-oxo-3H-indol-3-ylidene]-diisobutyl ester (**9**). ($CDCl_3$ - 81.0 MHz)*

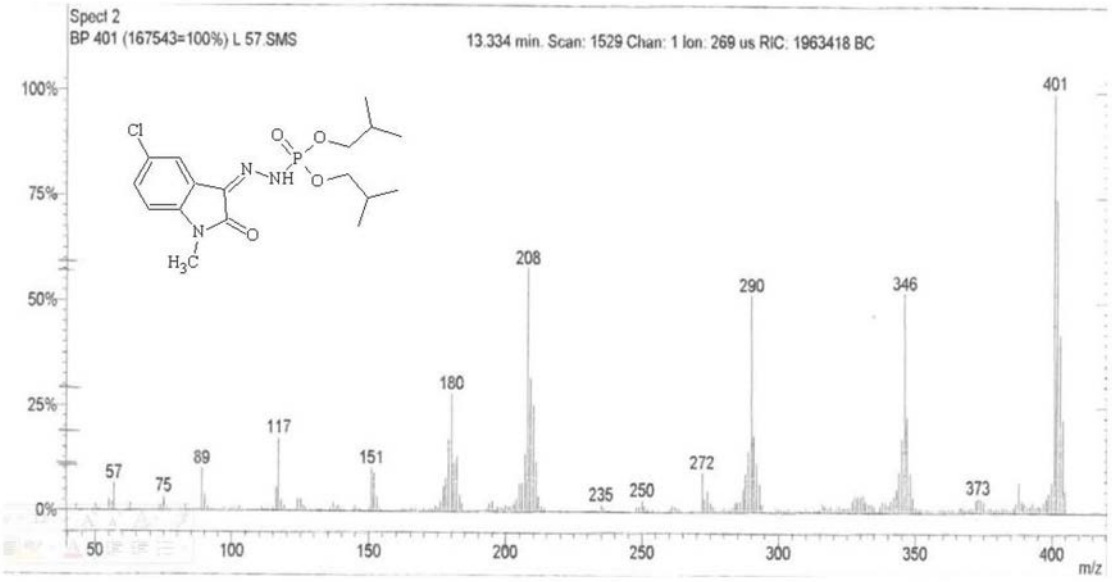

Figure 41S. *Mass spectrum of phosphorohydrazidic acid, N'-[5-cloro-1,2-dihydro-1-metil-2-oxo-3H-indol-3-ylidene]-diisobutyl ester (**9**)*

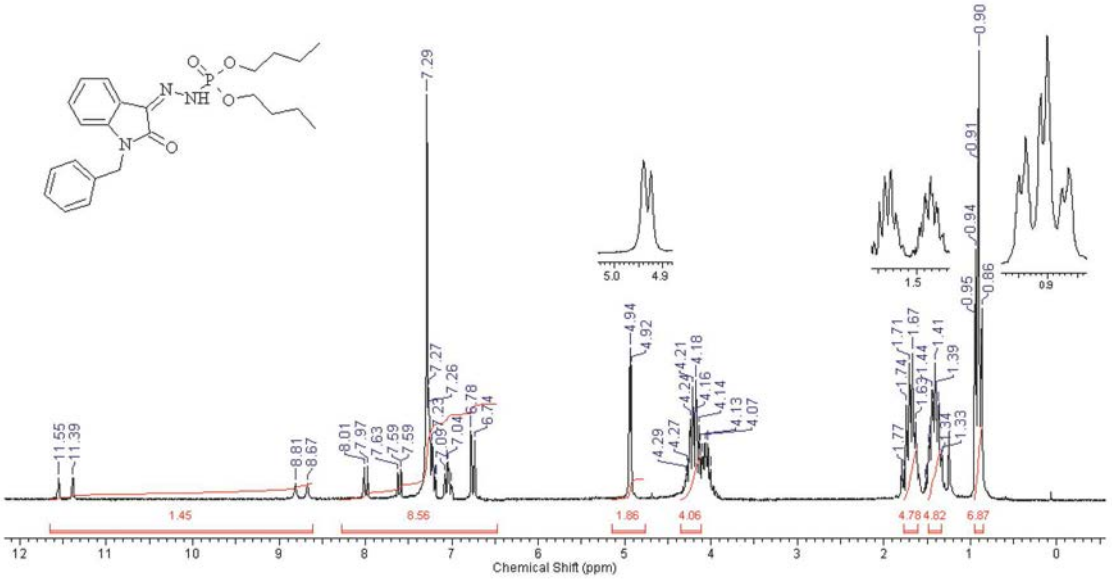

Figure 42S. *[1]H NMR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-1-benzyl-3H-indol-3-ylidene]- dibutyl ester (**10**). ($CDCl_3$ - 200 MHz)*

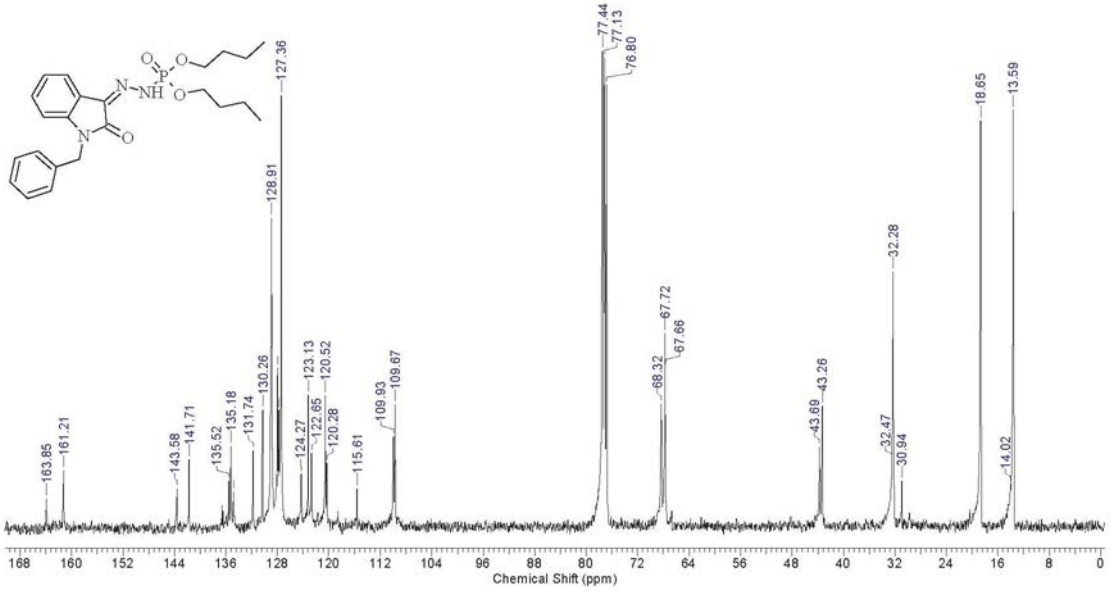

Figure 43S. *[13]C NMR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-1-benzyl-3H-indol-3-ylidene]- dibutyl ester (**10**). ($CDCl_3$ - 50,3 MHz)*

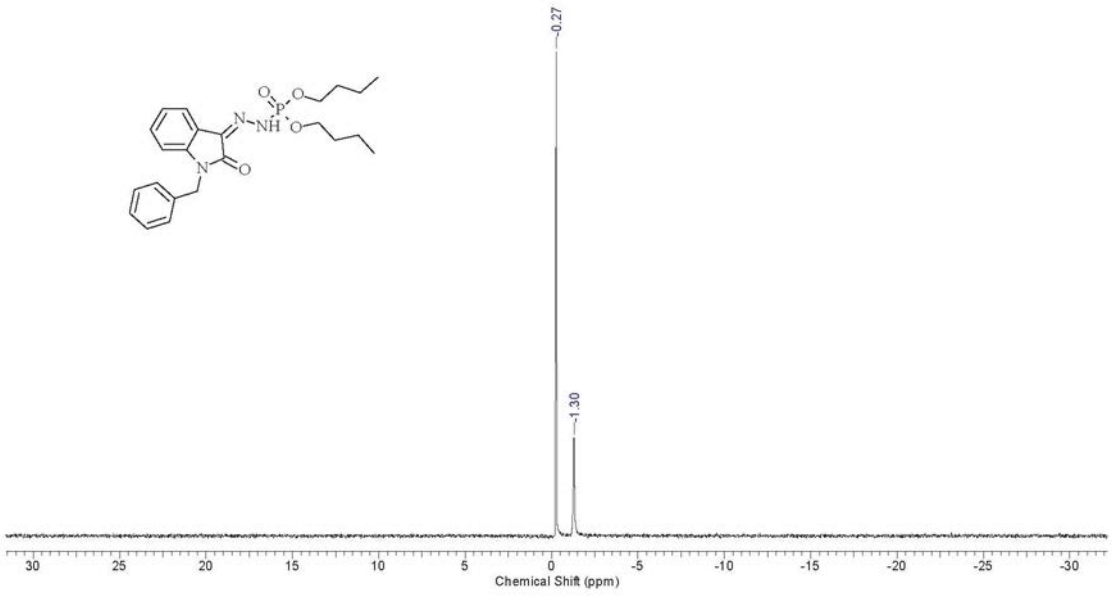

Figure 44S. *^{31}P NMR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-1-benzyl-3H-indol-3-ylidene]- dibutyl ester (**10**). ($CDCl_3$ - 81.0 MHz)*

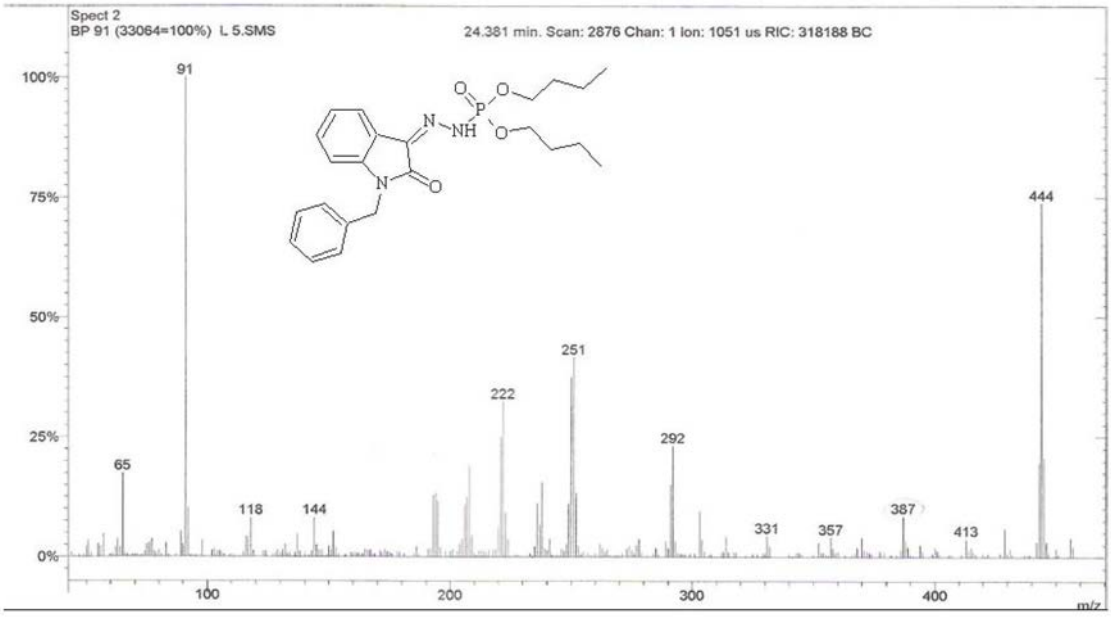

Figure 45S. *Mass spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-1-benzyl-3H-indol-3-ylidene]- dibutyl ester (**10**)*

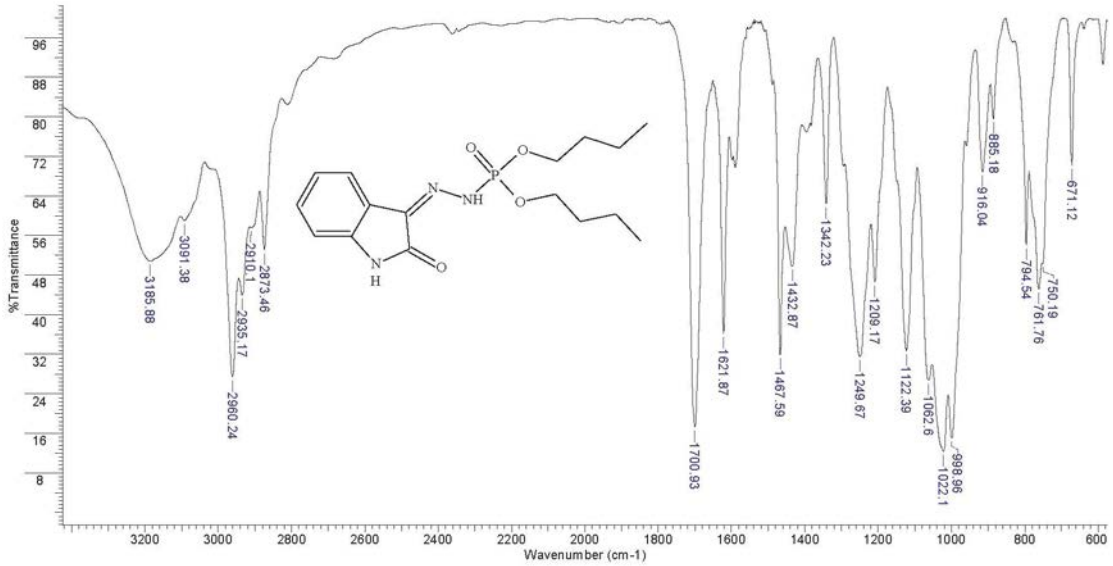

Figure 46S. *IR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-3H-indol-3-ylidene] dibutyl ester (**11**)*

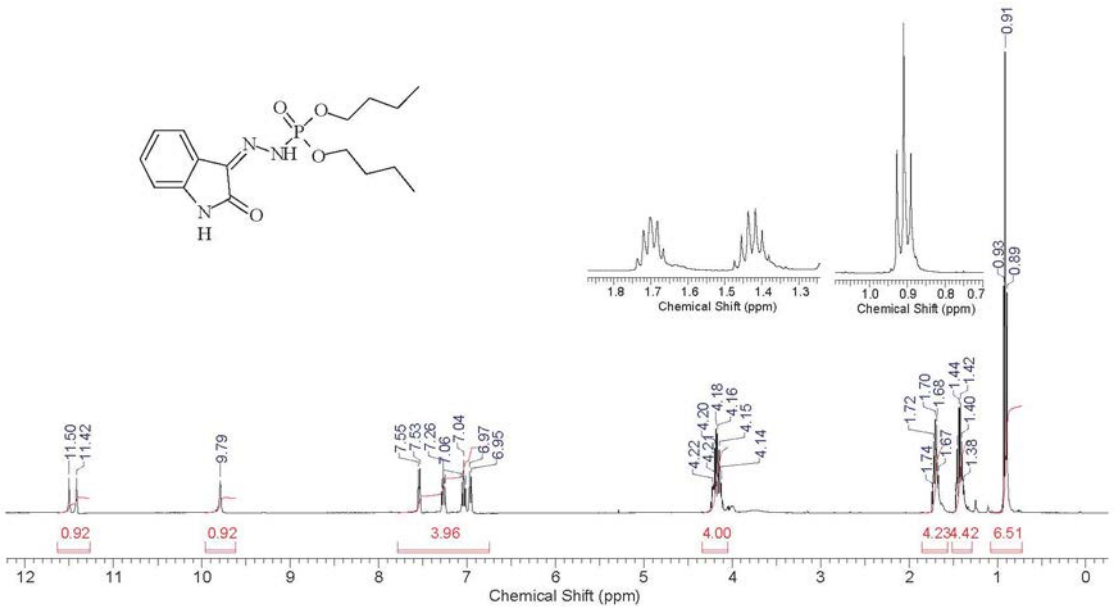

Figure 47S. *^{1}H NMR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-3H-indol-3-ylidene] dibutyl ester (**11**). ($CDCl_3$ - 200 MHz)*

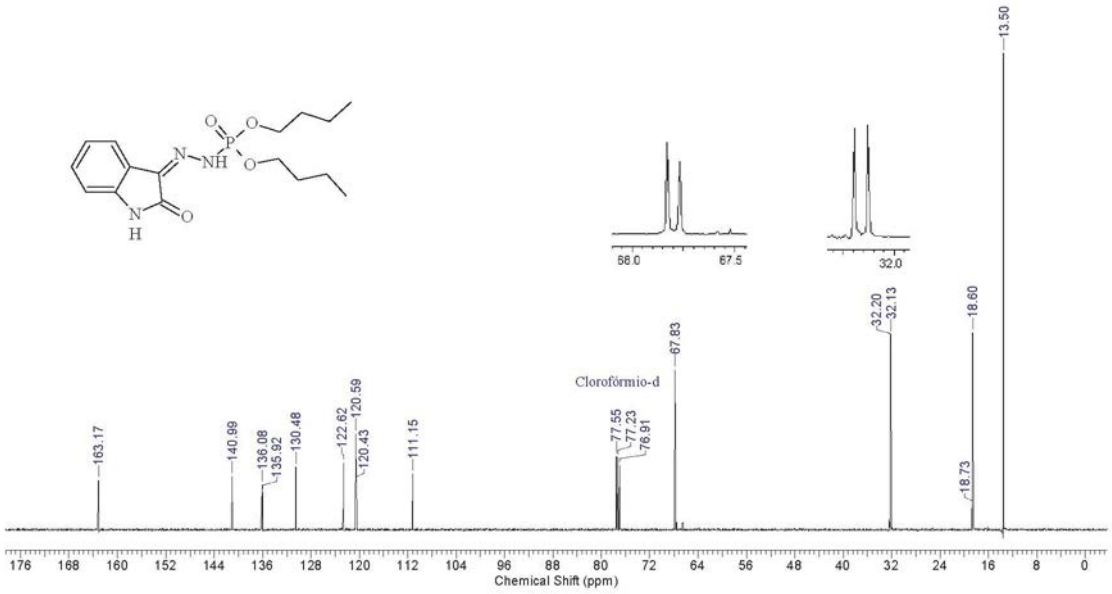

Figure 48S. *^{13}C NMR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-3H-indol-3-ylidene] dibutyl ester (**11**). ($CDCl_3$ - 50.3 MHz)*

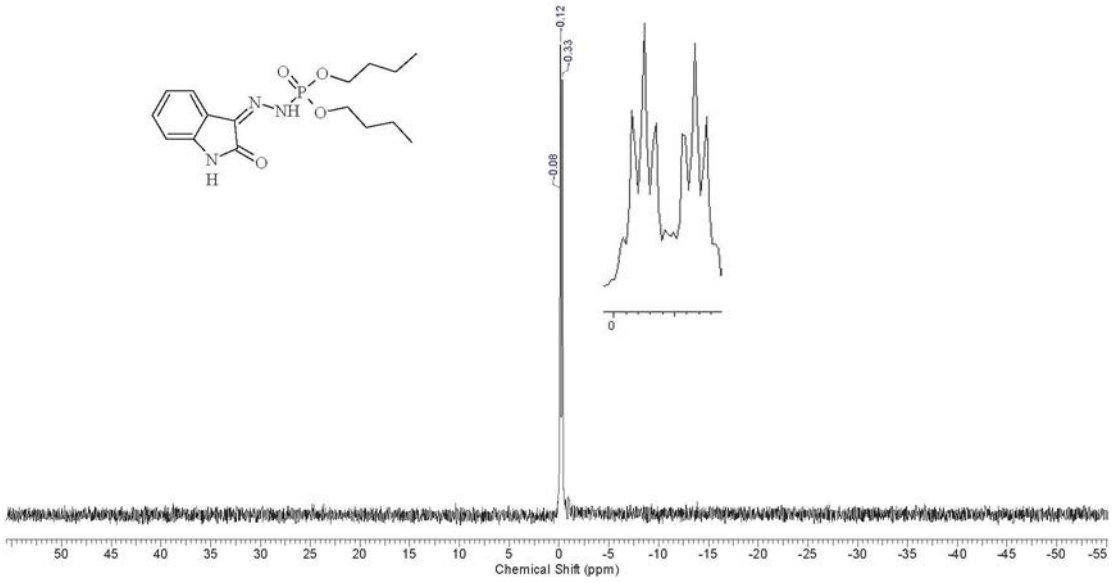

Figure 49S. *^{31}P NMR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-3H-indol-3-ylidene] dibutyl ester (**11**). ($CDCl_3$ - 81.0 MHz)*

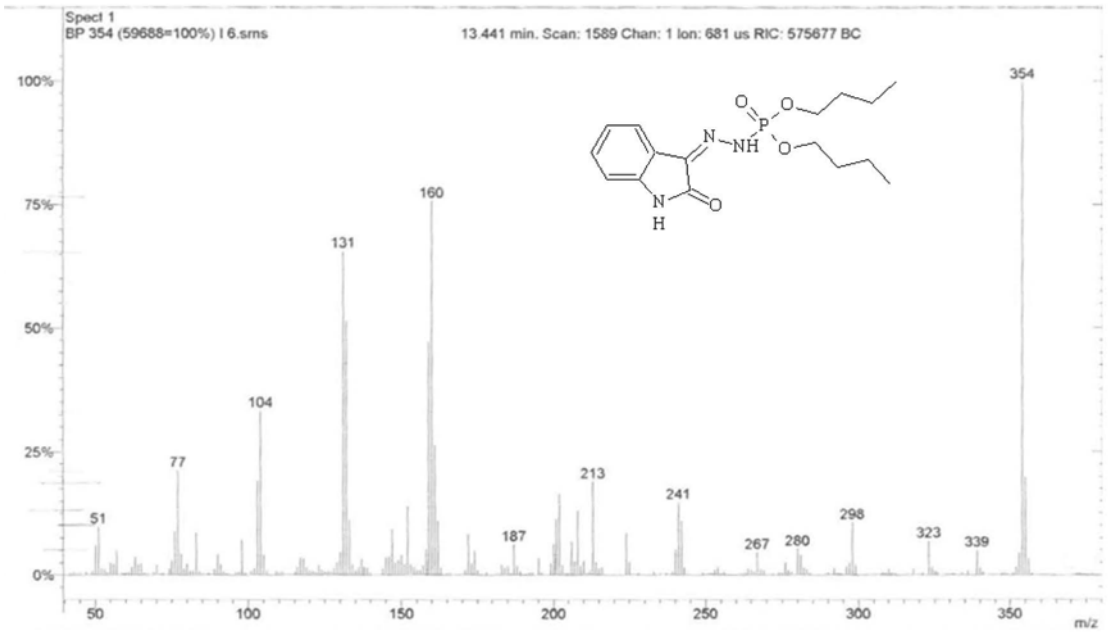

Figure 50S. *Mass spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-2-oxo-3H-indol-3-ylidene] dibutyl ester (**11**)*

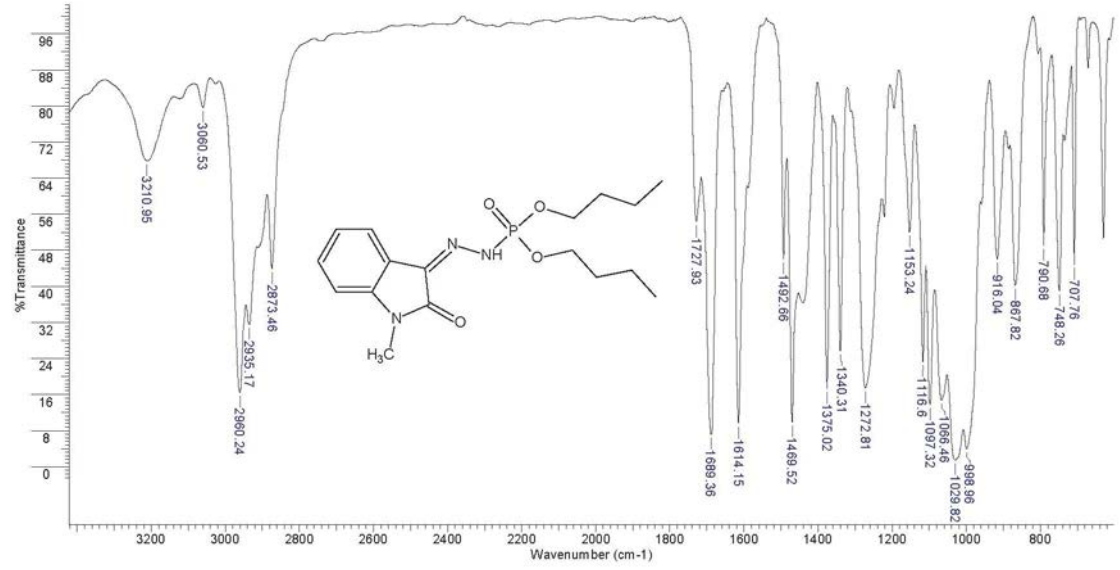

Figure 51S. *IR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-1-methyl-2-oxo-3H-indol-3-ylidene] dibutyl ester (**12**)*

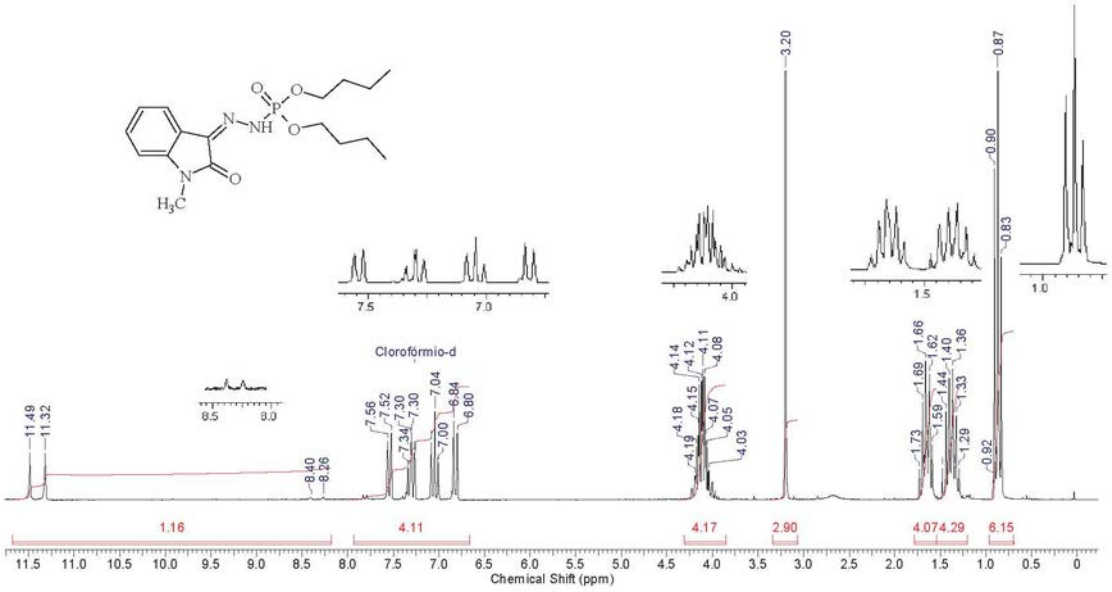

Figure 52S. *^{1}H NMR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-1-methyl-2-oxo-3H-indol-3-ylidene] dibutyl ester (**12**). ($CDCl_3$ - 400 MHz)*

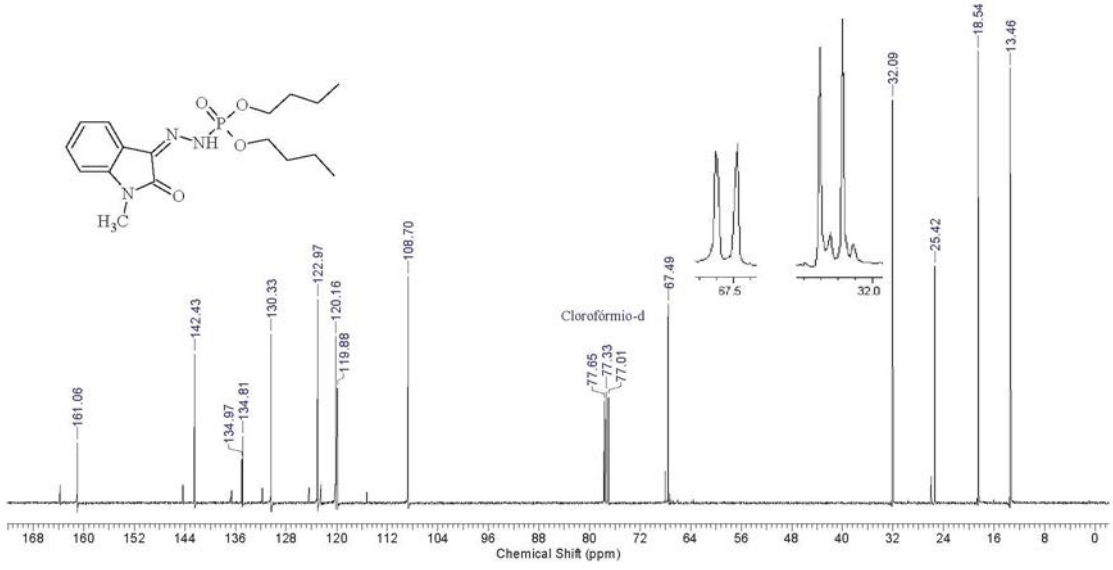

Figure 53S. *^{13}C NMR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-1-methyl-2-oxo-3H-indol-3-ylidene] dibutyl ester (**12**). ($CDCl_3$ - 100.6 MHz)*

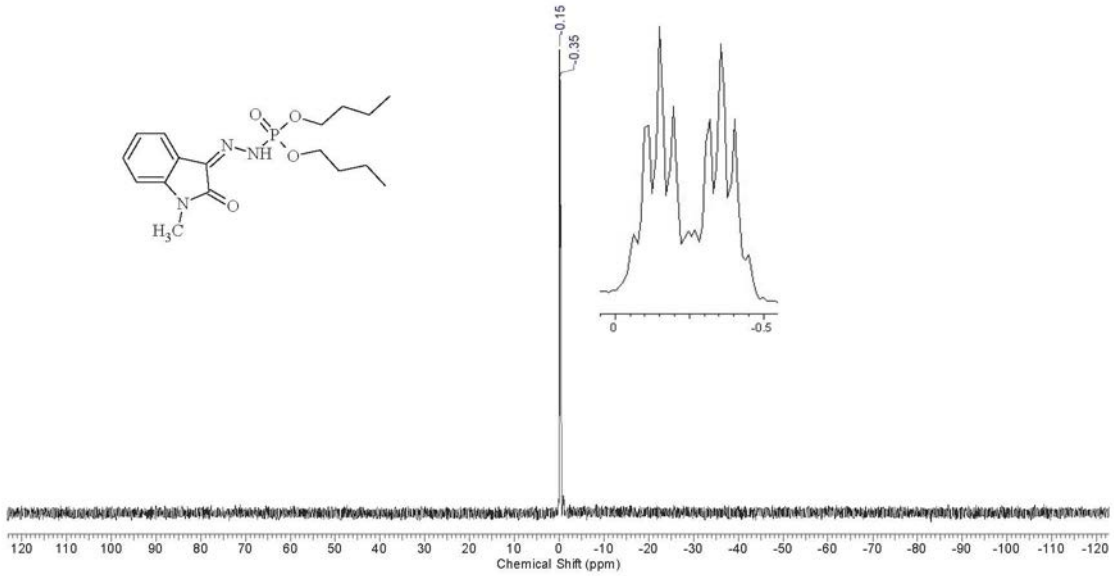

Figure 54S. *^{31}P NMR spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-1-methyl-2-oxo-3H-indol-3-ylidene] dibutyl ester (**12**). ($CDCl_3$ - 161.9 MHz)*

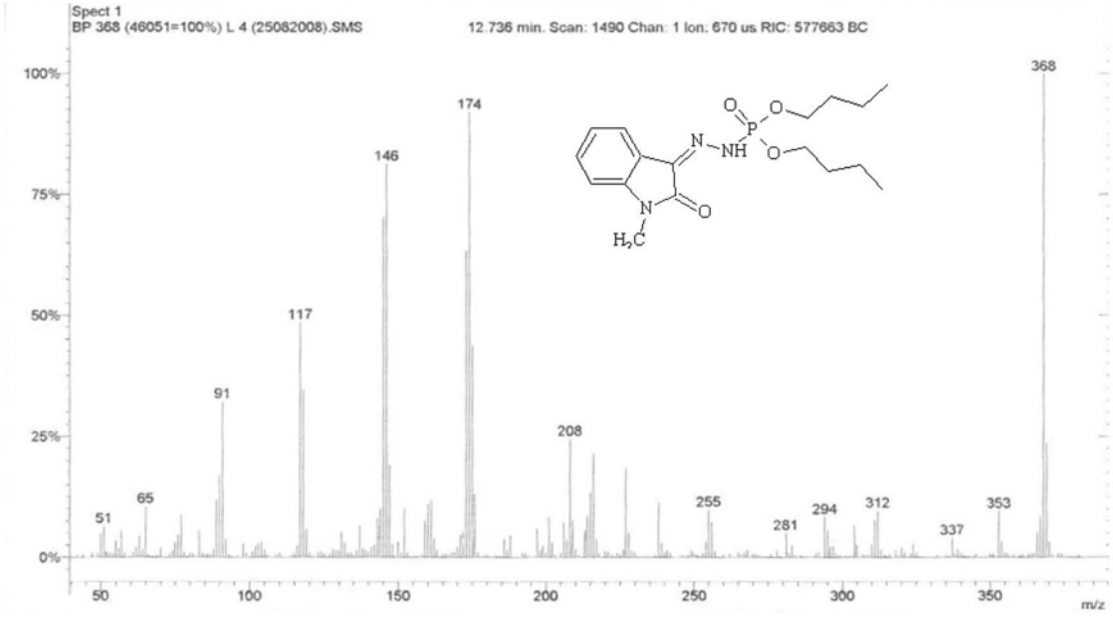

Figure 55S. *Mass spectrum of phosphorohydrazidic acid N'-[1,2-dihydro-1-methyl-2-oxo-3H-indol-3-ylidene] dibutyl ester (**12**)*

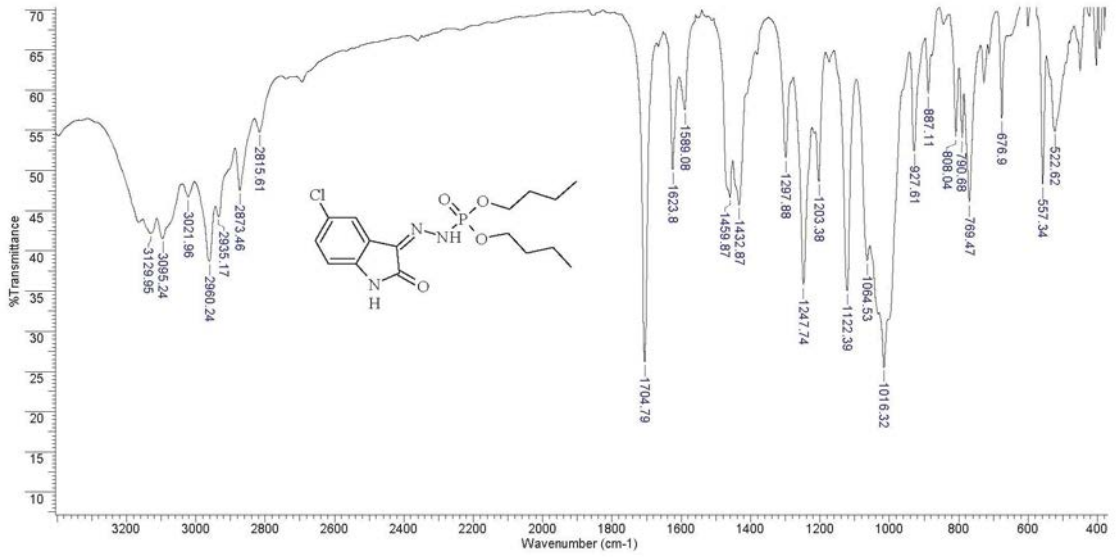

Figure 56S. *IR spectrum of phosphorohydrazidic acid, N'- [5-cloro-1,2-dihydro-2oxo-3H-indol-3-ylidene]-, dibutyl ester (**13**)*

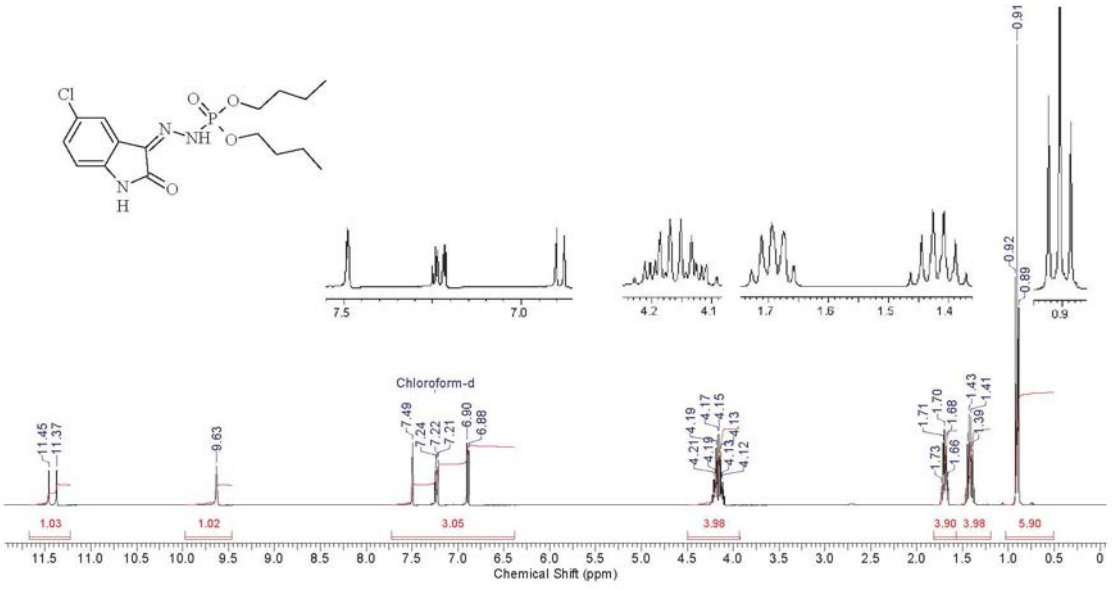

Figure 57S. *^{1}H NMR spectrum of phosphorohydrazidic acid, N'- [5-cloro-1,2-dihydro-2oxo-3H-indol-3-ylidene]-, dibutyl ester (**13**). ($CDCl_3$ - 400 MHz)*

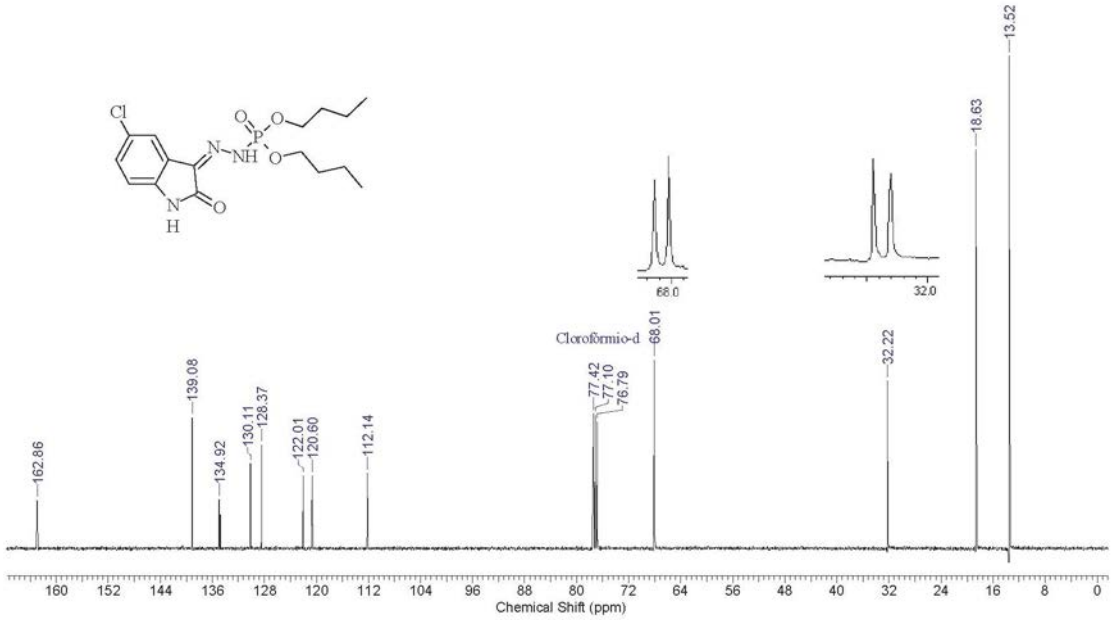

Figure 58S. *^{13}C NMR spectrum of phosphorohydrazidic acid, N'- [5-cloro-1,2-dihydro-2oxo-3H-indol-3-ylidene]-, dibutyl ester (**13**). ($CDCl_3$ - 100.6 MHz)*

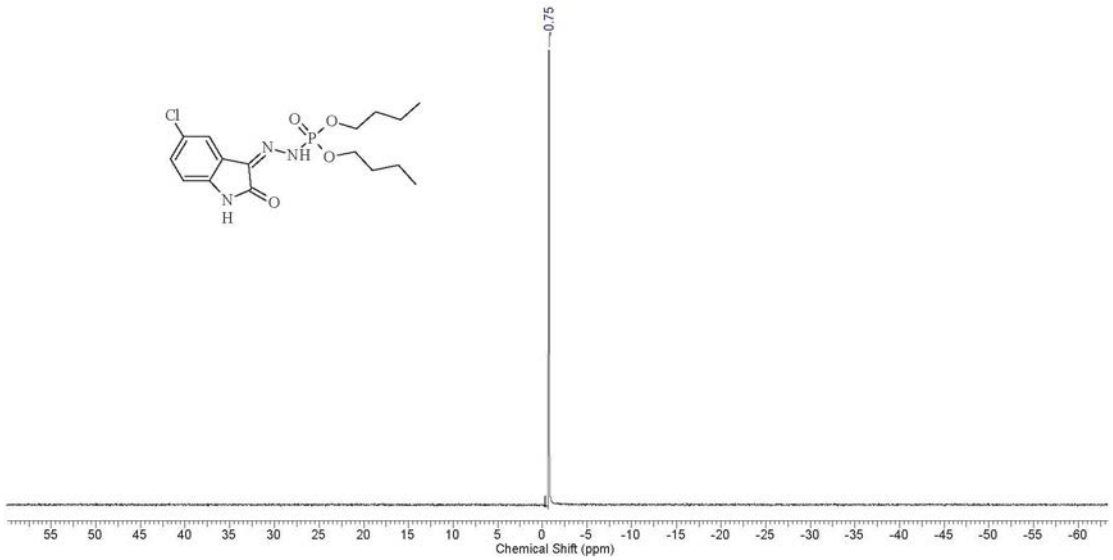

Figure 59S. *^{31}P NMR spectrum of phosphorohydrazidic acid, N'- [5-cloro-1,2-dihydro-2oxo-3H-indol-3-ylidene]-, dibutyl ester (**13**). ($CDCl_3$ - 161.9 MHz)*

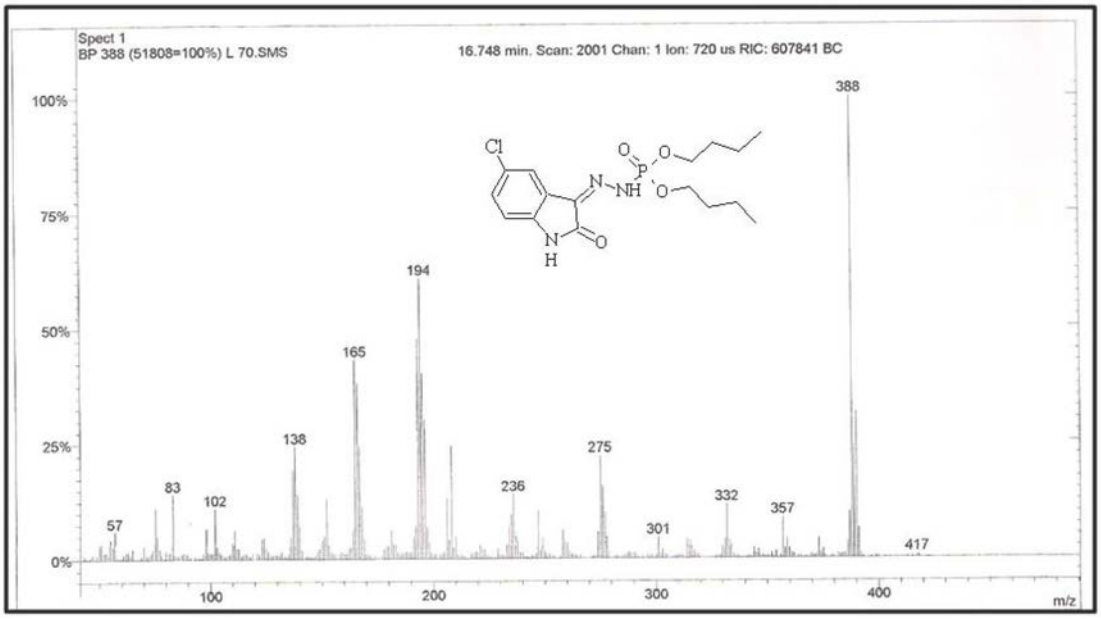

Figure 60S. *Mass spectrum of phosphorohydrazidic acid, N'- [5-cloro-1,2-dihydro-2oxo-3H-indol-3-ylidene]-, dibutyl ester (**13**)*

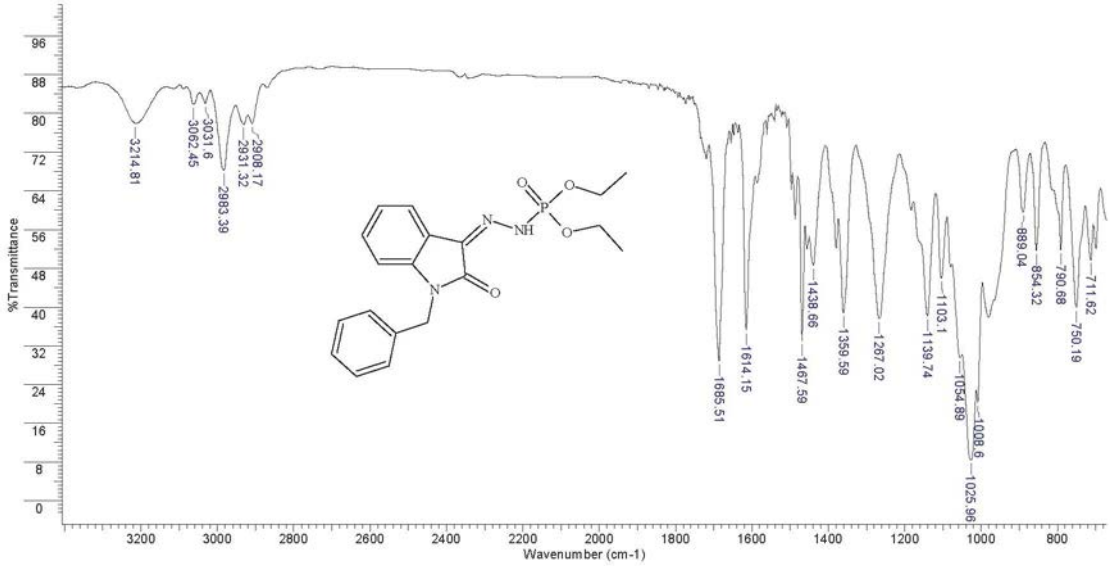

Figure 61S. *IR spectrum of phosphorohydrazidic acid, N'- [1,2-dihydro-2-oxo-1-(benzyl)-3H-indol-3-ylidene]-, diethyl ester (**14**)*

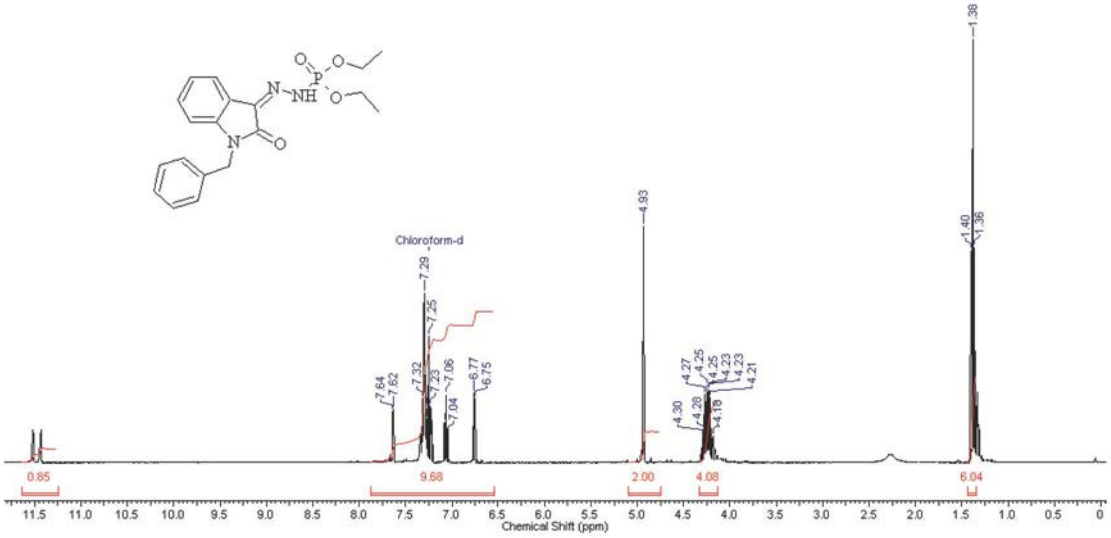

Figure 62S. *[1]H NMR spectrum of phosphorohydrazidic acid, N'- [1,2-dihydro-2-oxo-1-(benzyl)-3H-indol-3-ylidene]-, diethyl ester (**14**). ($CDCl_3$ - 400 MHz)*

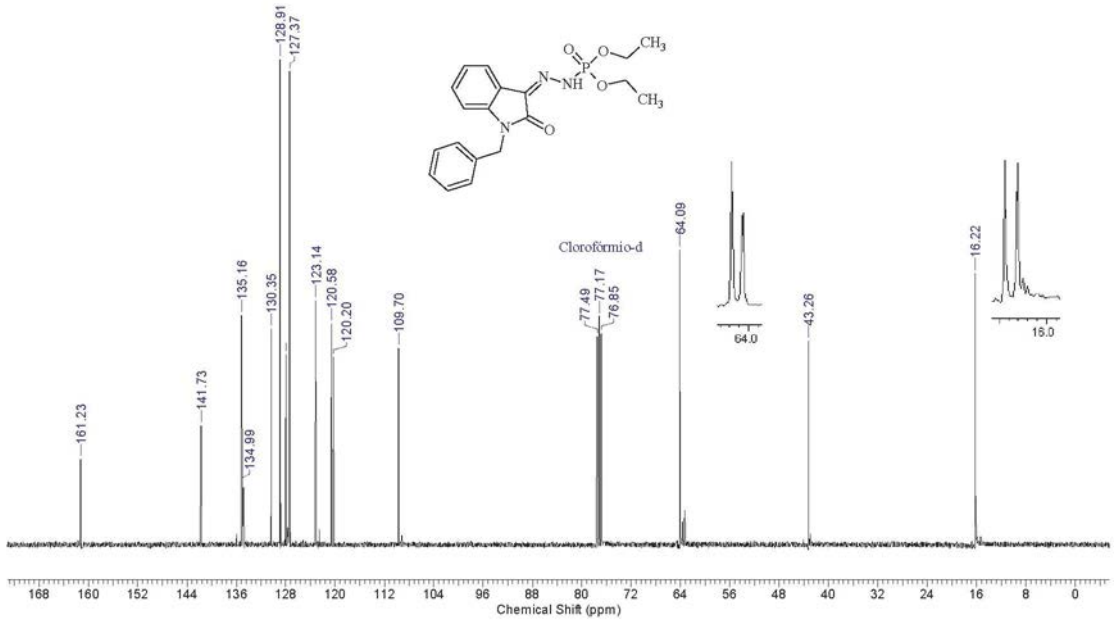

Figure 63S. *[13]C NMR spectrum of phosphorohydrazidic acid, N'- [1,2-dihydro-2-oxo-1-benzyl-3H-indol-3-ylidene]-, diethyl ester (**14**). ($CDCl_3$ - 100.6 MHz)*

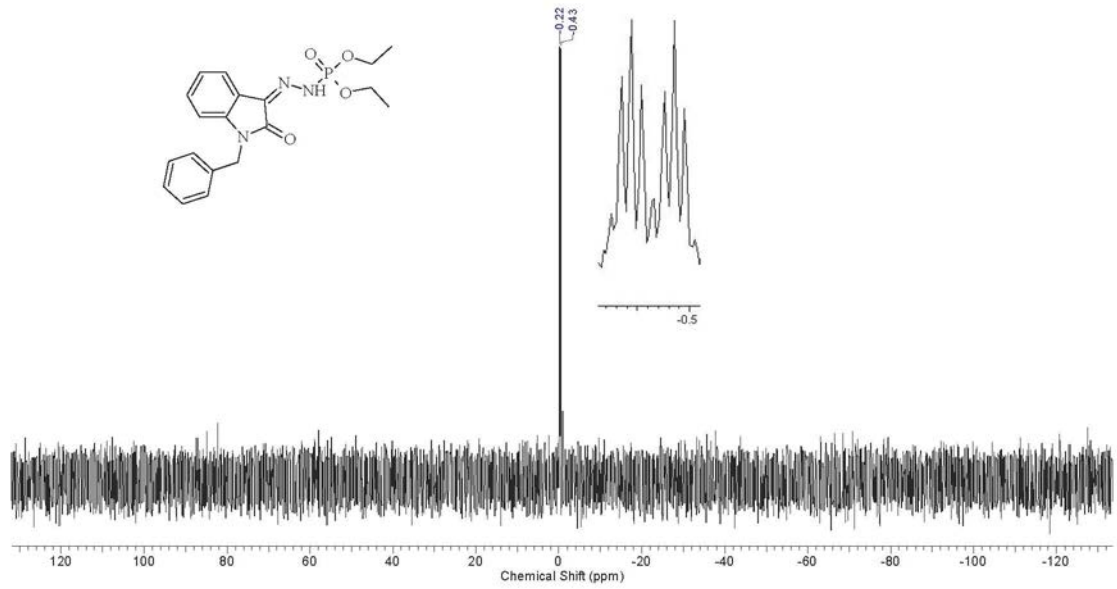

Figure 64S. *^{31}P NMR spectrum of phosphorohydrazidic acid, N'- [1,2-dihydro-2-oxo-1-benzyl-3H-indol-3-ylidene]-, diethyl ester (**14**). ($CDCl_3$ - 161.9 MHz)*

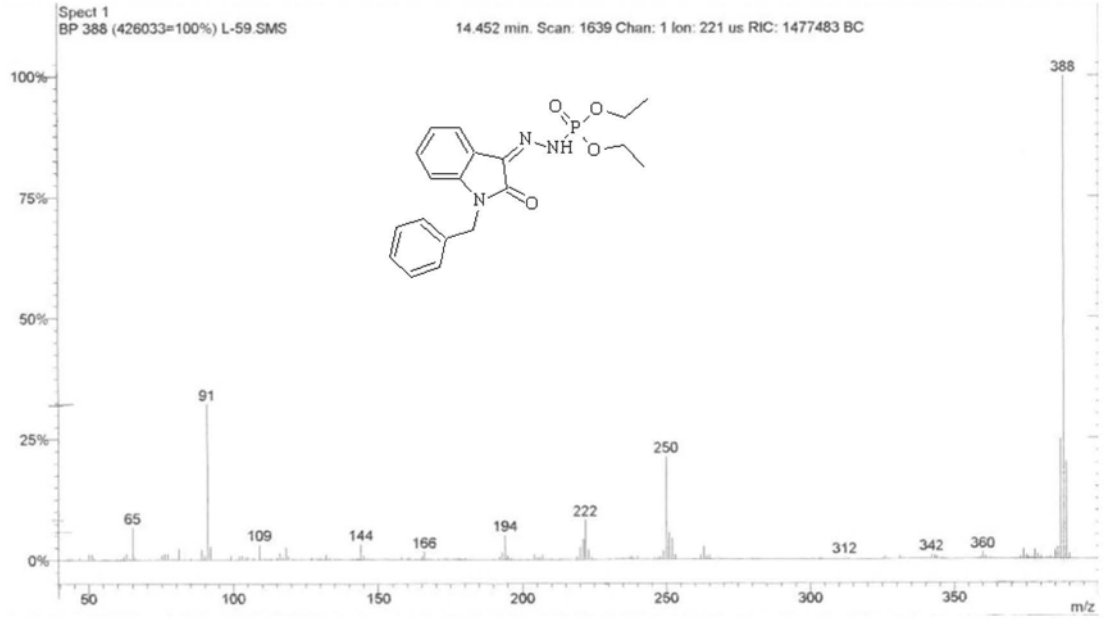

Figure 65S. *Mass spectrum of phosphorohydrazidic acid, N'- [1,2-dihydro-2-oxo-1-benzyl-3H-indol-3-ylidene]-, diethyl ester (**14**)*

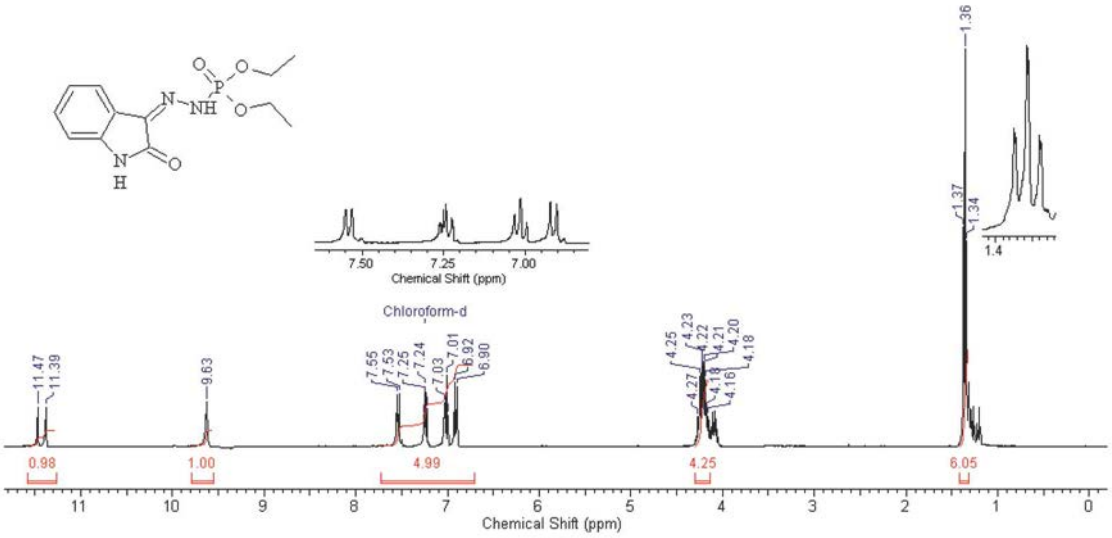

Figure 66S. *[1]H NMR spectrum of phosphorohydrazidic acid, N'- [1,2-dihydro-2-oxo-3H-indol-3-ylidene]-, diethyl ester (**15**). ($CDCl_3$ - 400 MHz)*

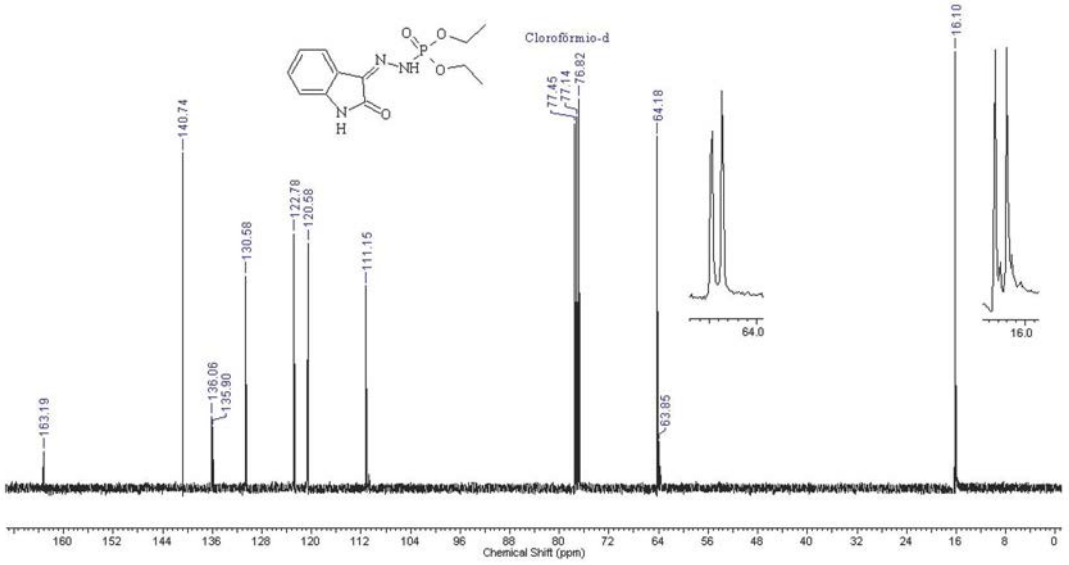

Figure 67S. *[13]C NMR spectrum of phosphorohydrazidic acid, N'- [1,2-dihydro-2-oxo-3H-indol-3-ylidene]-, diethyl ester (**15**). ($CDCl_3$ - 100.6 MHz)*

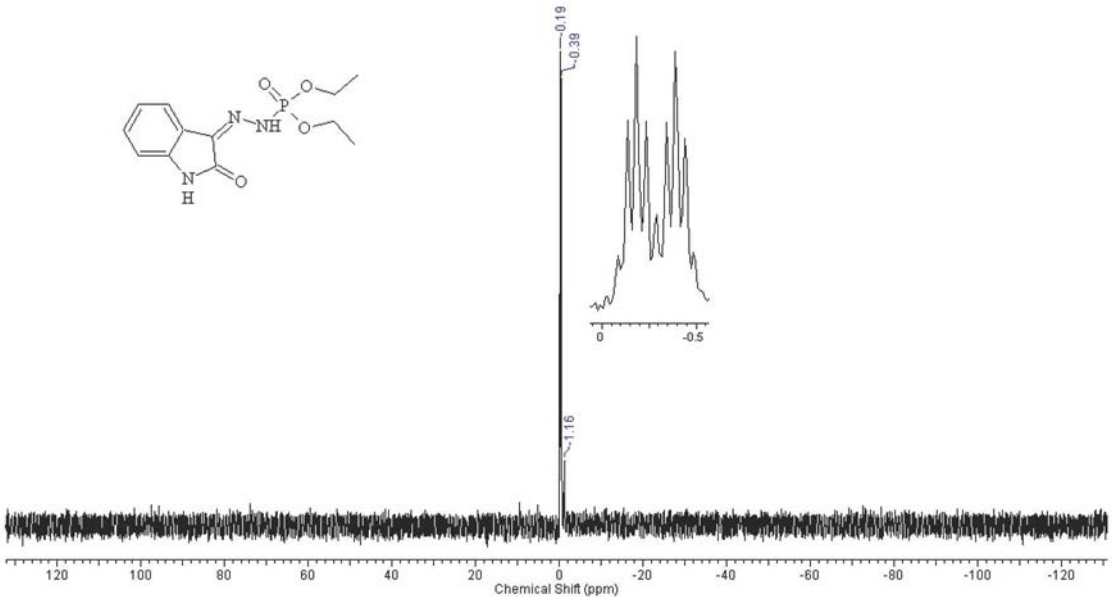

Figure 68S. *^{31}P NMR spectrum of phosphorohydrazidic acid, N'- [1,2-dihydro-2-oxo-3H-indol-3-ylidene]-, diethyl ester (**15**). ($CDCl_3$ - 161.9 MHz)*

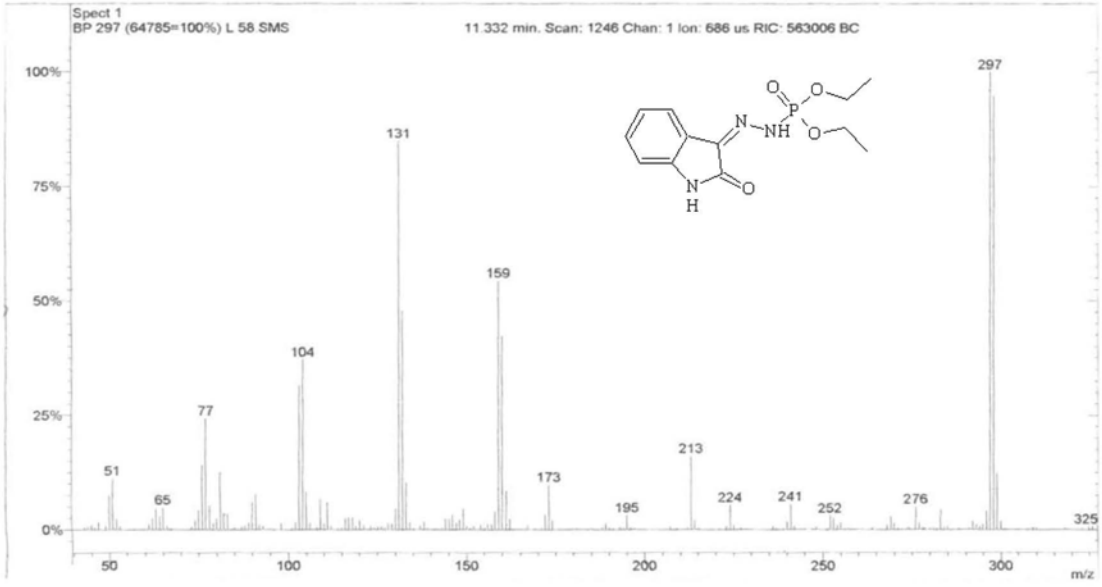

Figure 69S. *Mass spectrum of phosphorohydrazidic acid, N'- [1,2-dihydro-2-oxo-3H-indol-3-ylidene]-, diethyl ester (**15**)*

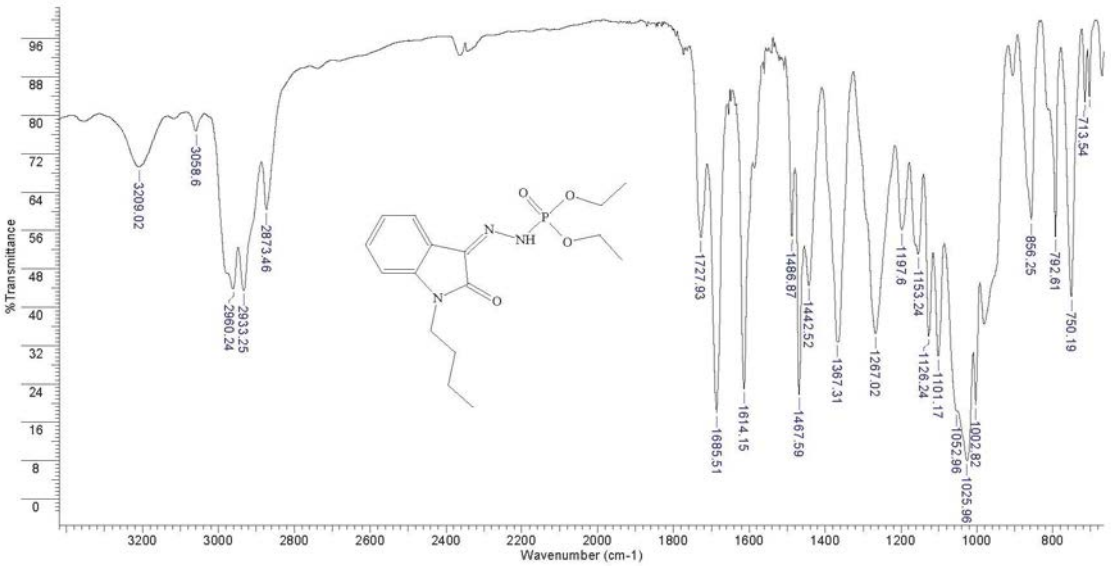

Figure 70S. *IR spectrum of phosphorohydrazidic acid, N'- [1-butyl-1,2-dihydro-2-oxo-3H-indol-3-ylidene]-, diethyl ester (**16**)*

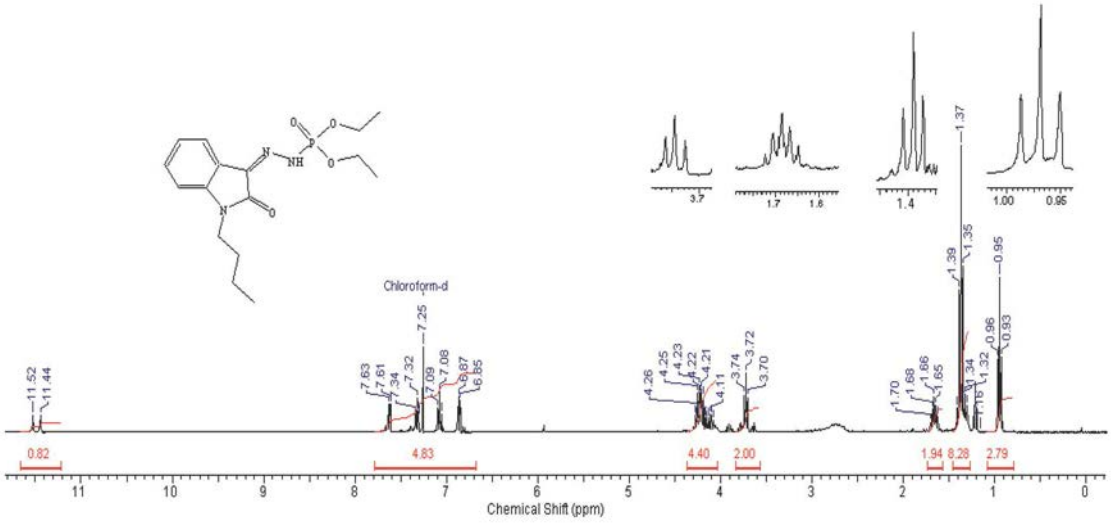

Figure 71S. *^{1}H NMR spectrum of phosphorohydrazidic acid, N'- [1-butyl-1,2-dihydro-2-oxo-3H-indol-3-ylidene]-, diethyl ester (**16**). ($CDCl_3$ - 400 MHz)*

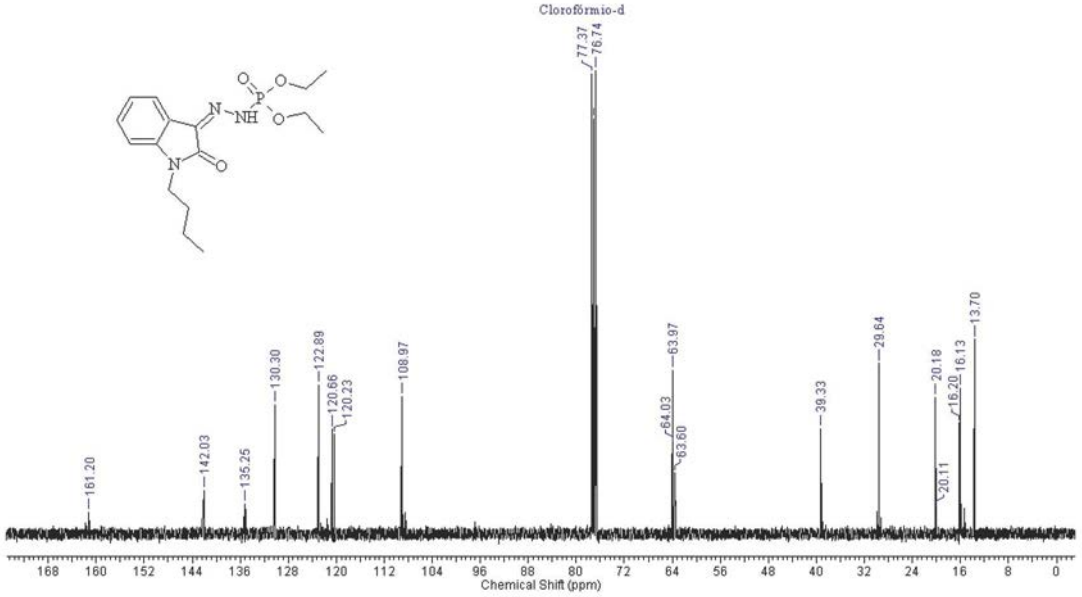

Figure 72S. *^{13}C NMR spectrum of phosphorohydrazidic acid, N'- [1-butyl-1,2-dihydro-2-oxo-3H-indol-3-ylidene]-, diethyl ester (**16**). ($CDCl_3$ - 100.6 MHz)*

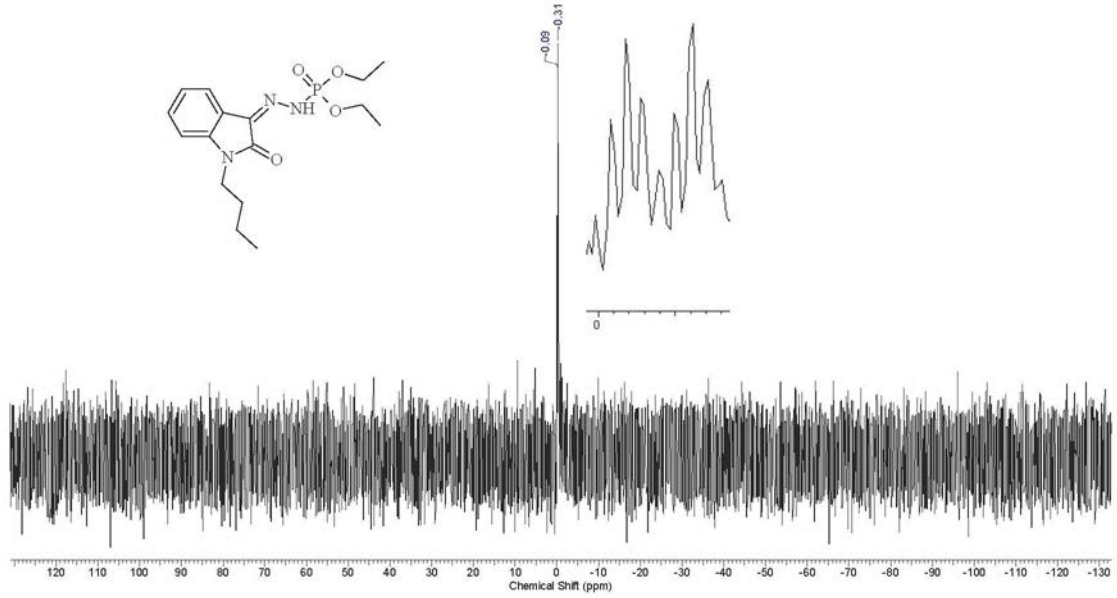

Figure 73S. *^{31}P NMR spectrum of phosphorohydrazidic acid, N'- [1-butyl-1,2-dihydro-2-oxo-3H-indol-3-ylidene]-, diethyl ester (**16**). ($CDCl_3$ - 161.9 MHz)*

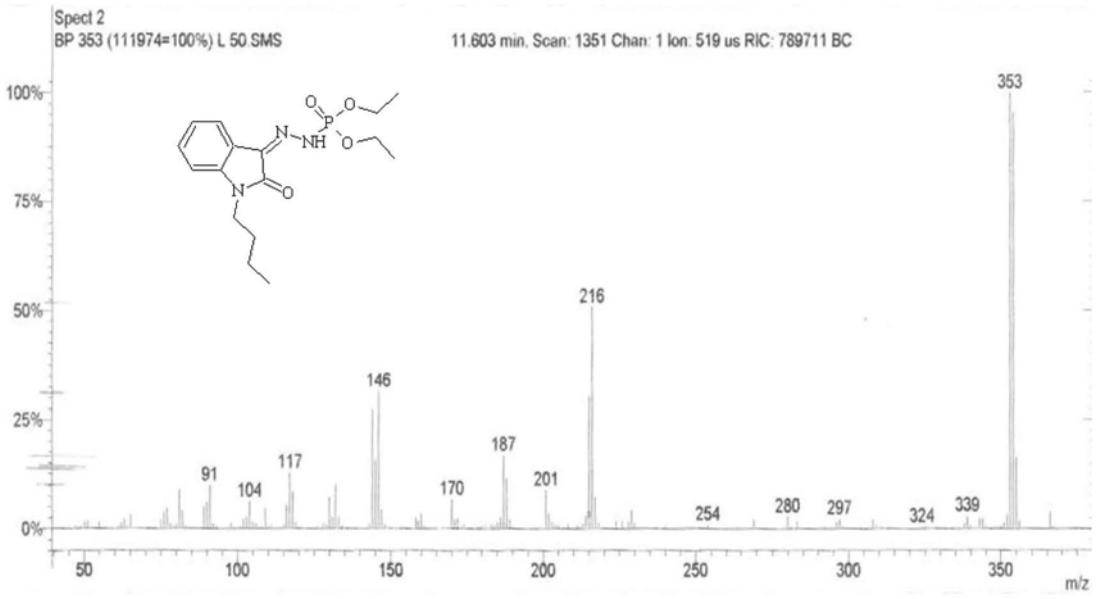

Figure 74S. *Mass spectrum of phosphorohydrazidic acid, N'- [1-butyl-1,2-dihydro-2-oxo-3H-indol-3-ylidene]-, diethyl ester (**16**)*

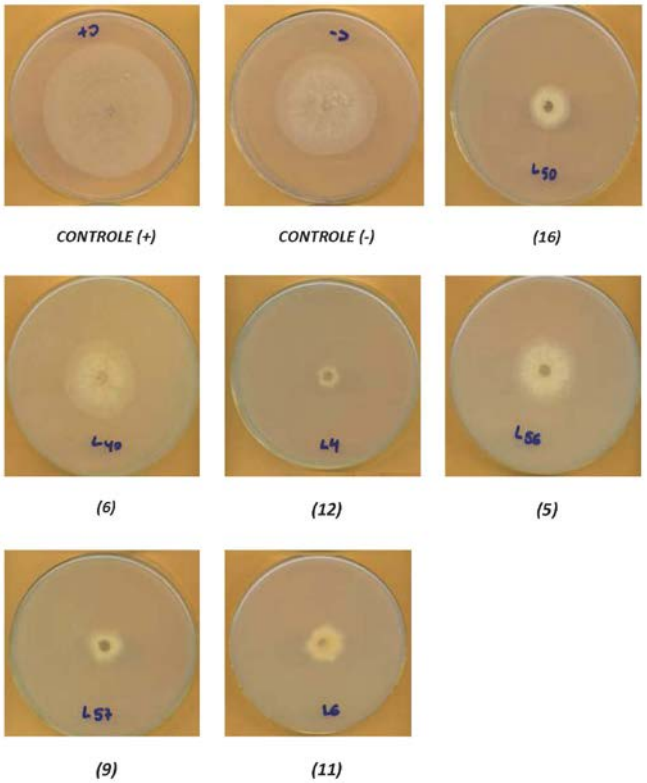

Figure 75S. *Images of the fungus Rhizoctonia solani on potato dextrose agar (PDA) medium containing derivatives of isatin solution in dimethylsulfoxide – DMSO (1 % v/v) at a concentration of 0.5 gL^{-1}. Image captured at the end of the plate assay containing the positive and negative controls and compounds **5**, **6**, **9**, **11**, **12** and **16***

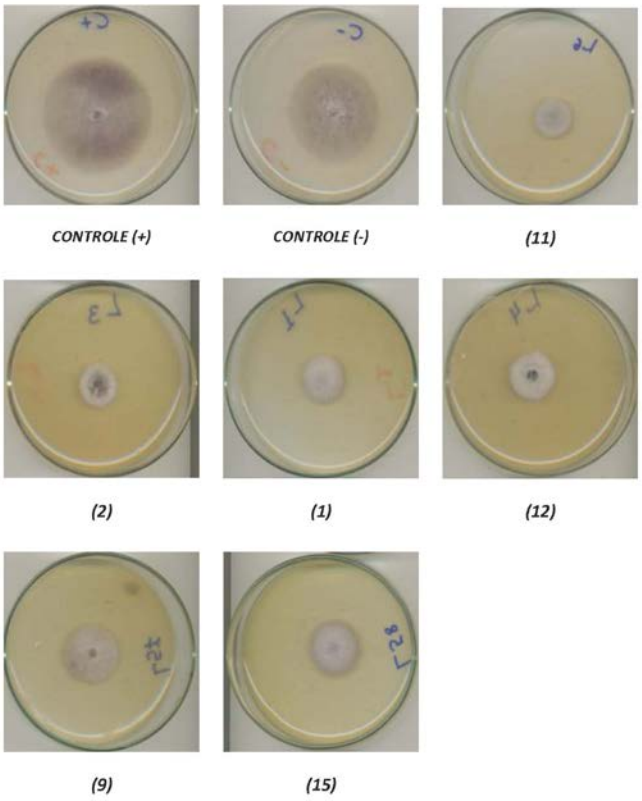

Figure 76S. *Images of Fusarium oxysporum in medium potato dextrose agar (PDA), containing the isatin derivatives in solution with DMSO - 1-mL.50 mL 0.5) at a concentration of 0.5 gL^{-1}. Image captured at the end of the plate assay containing the positive and negative controls and compounds **1**, **2**, **9**, **11**,**12** and **15***

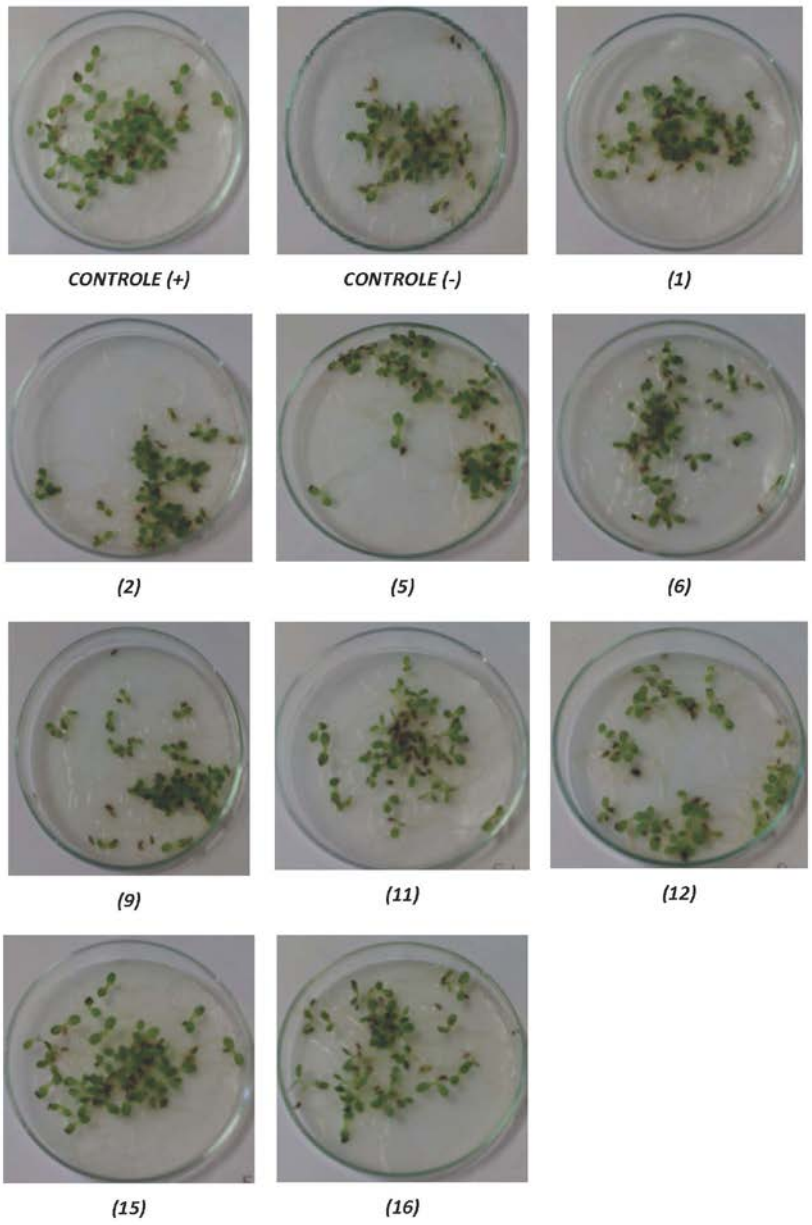

Figure 77S. *Image of Petri dishes containing lettuce seeds undergoing treatment with the compounds in dichloromethane at a concentration of 0.4 gL^{-1}*