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## REACTION OF 2-IMINO-2*H*-CHROMENE-3-CARBOXAMIDE WITH PHOSPHORUS HALIDES: SYNTHESIS OF SOME NOVEL CHROMENO-[2,3-*d*][1,3,2]DIAZAPHOSPHININES AND CHROMENO[4,3-*c*][1,2]-AZAPHOSPHOLE AND THEIR ANTIOXIDANT AND CYTOTOXICITY PROPERTIES

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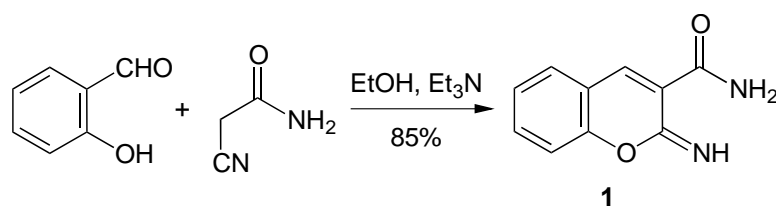
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**Abstract** – Novel 1,2-azaphosphole and 1,3,2-diazaphosphinines containing a chromene ring have been obtained from the treatment of 2-imino-2*H*-chromene-3-carboxamide (**1**) with some phosphorus halides. The compounds were evaluated for antioxidant and cytotoxic activities. Among the synthesized compounds, compound **4** exhibited the most potent antioxidant agents in comparison with ascorbic acid as standard antioxidant. Also, compound **4** recorded potentially cytotoxicity against all four cell lines.

Phosphorus-containing heterocycles have been known since the late 19th century. But a rapid development of studies, related to a variety of functionalized phosphorus-containing heterocycles, especially structures having phosphorus-nitrogen bonds, began only in the middle of the last century when the importance of phosphorus-containing substances in biological processes was fully recognized.<sup>1-5</sup> Some fused polycyclic chromene compounds have received much synthetic attention because of their important dyeing properties<sup>6</sup> and biological activities such as antioxidant,<sup>7</sup> anticancer,<sup>8</sup> and antimicrobial.<sup>9</sup> Considering the above facts and our program research on the development of new biologically active heterocyclic organophosphorus compounds,<sup>10-14</sup> we herein reported the synthesis of 1,2-azaphosphole and 1,3,2-diazaphosphinines containing a chromene ring *via* treatment of 2-imino-2*H*-chromene-3-carboxamide (**1**) with some variable phosphorus halides and evaluated their antioxidant and

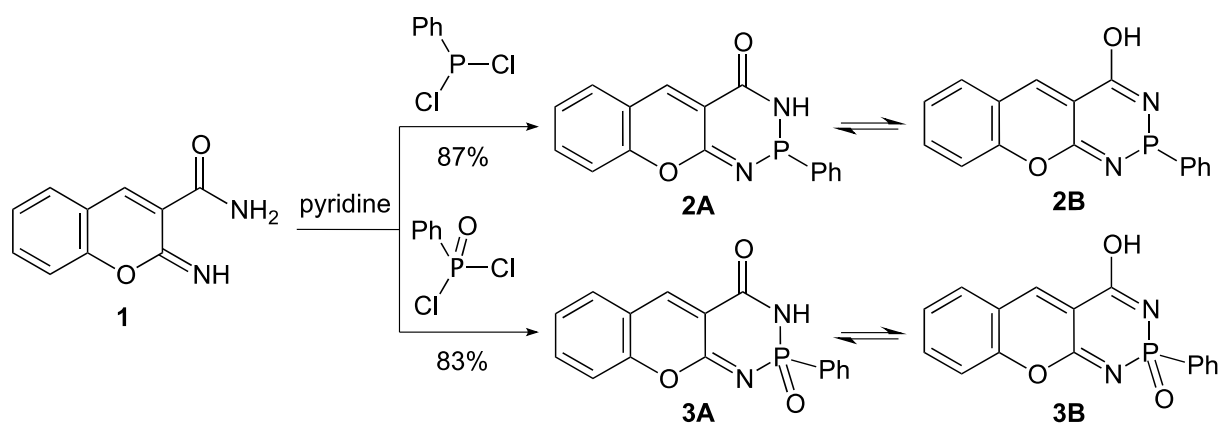
cytotoxicity properties.

The starting material 2-imino-2*H*-chromene-3-carboxamide (**1**) was prepared according to the reported method by a Knoevenagel condensation and an intramolecular cyclization of salicylaldehyde with cyanoacetamide in absolute ethanol containing a few drops of triethylamine (Scheme 1).<sup>15</sup> The presence of two active nucleophilic sides in the molecule of 2-imino-2*H*-chromene-3-carboxamide (**1**) provides alternative opportunities in the direction of the reaction with electrophilic phosphorus halides. Its synthetic precursor opens wide opportunities for the use of the chromene system in the synthesis of diverse fused phosphorus heterocyclic compounds.



**Scheme 1**

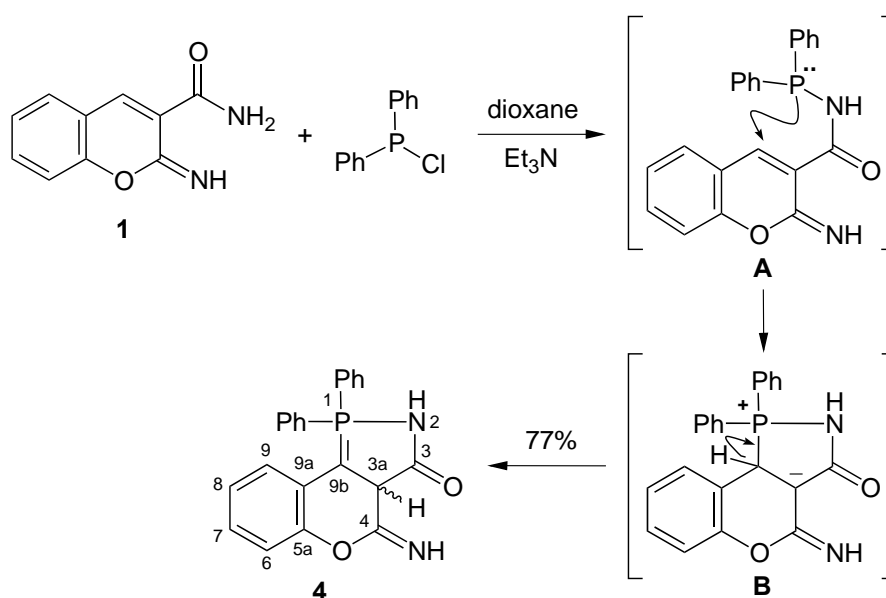
This work studies the behavior of compound **1** towards some phosphorus halides. Thus, cyclization of compound **1** with P,P-dichloro(phenyl)phosphine and phenylphosphonic dichloride in dry pyridine afforded the chromeno[2,3-*d*][1,3,2]diazaphosphinines **2** and **3**, respectively (Scheme 2). The spectral data indicated the presence of both compounds in two tautomeric forms **A** and **B**. The IR spectra of both compounds displayed absorption bands at 3361–3339, 3209–3170 and 1738–1710  $\text{cm}^{-1}$  which corresponded to the functional groups OH, NH and C=O, respectively. The  $^1\text{H-NMR}$  spectrum for each compound showed two distinct singlets in the regions  $\delta$  10.17–10.24 (NH) and 12.80–11.04 (OH) ppm. The carbon atoms of compound **3A,B** for the C=O and C–OH groups appeared at  $\delta$  164.3 and 158.4 ppm, respectively. Also, the  $^{31}\text{P-NMR}$  spectrum recorded two signals at  $\delta$  40.1 and 43.3 ppm for compound **3** which confirmed its presence in two tautomeric forms. The mass spectra of compounds **2** and **3** were



**Scheme 2**

recorded and interpreted in support of the structures proposed based on their synthesis.

Compound **1** was allowed to react with P-chlorodiphenylphosphine in dry 1,4-dioxane containing a few drops of triethylamine gave the nonisolable intermediate **A**, followed by a nucleophilic addition of phosphorus atom at the electrophilic carbon atom C-4 to form the intermediate **B**<sup>16</sup> which underwent rearrangement to isolate the product 1,1-diphenyl-4-imino-3a,4-dihydro-1 $\lambda^5$ -chromeno[4,3-*c*][1,2]-azaphosphol-3(2*H*)-one (**4**) (Scheme 3). The <sup>1</sup>H-NMR spectrum of compound **4** supported the proposed structure by the absence of H-4 of the starting material **1** and appearance of a new doublet at  $\delta$  5.01 (H-3a) ppm. Also, its <sup>13</sup>C-NMR spectrum recorded the characteristic carbon atom C-3a at  $\delta$  69.7 ppm while and the atom C-9b was observed as doublet at  $\delta$  111.0 ppm with  $J_{PC}$ =214.3 Hz due to its connection via double bond with the phosphorus atom.<sup>17</sup> Furthermore, its <sup>31</sup>P-NMR spectrum displayed a singlet at  $\delta$  31.1 ppm.<sup>18</sup> Its mass spectrum presented the molecular ion peak at  $m/z$  372 which confirmed its structure.

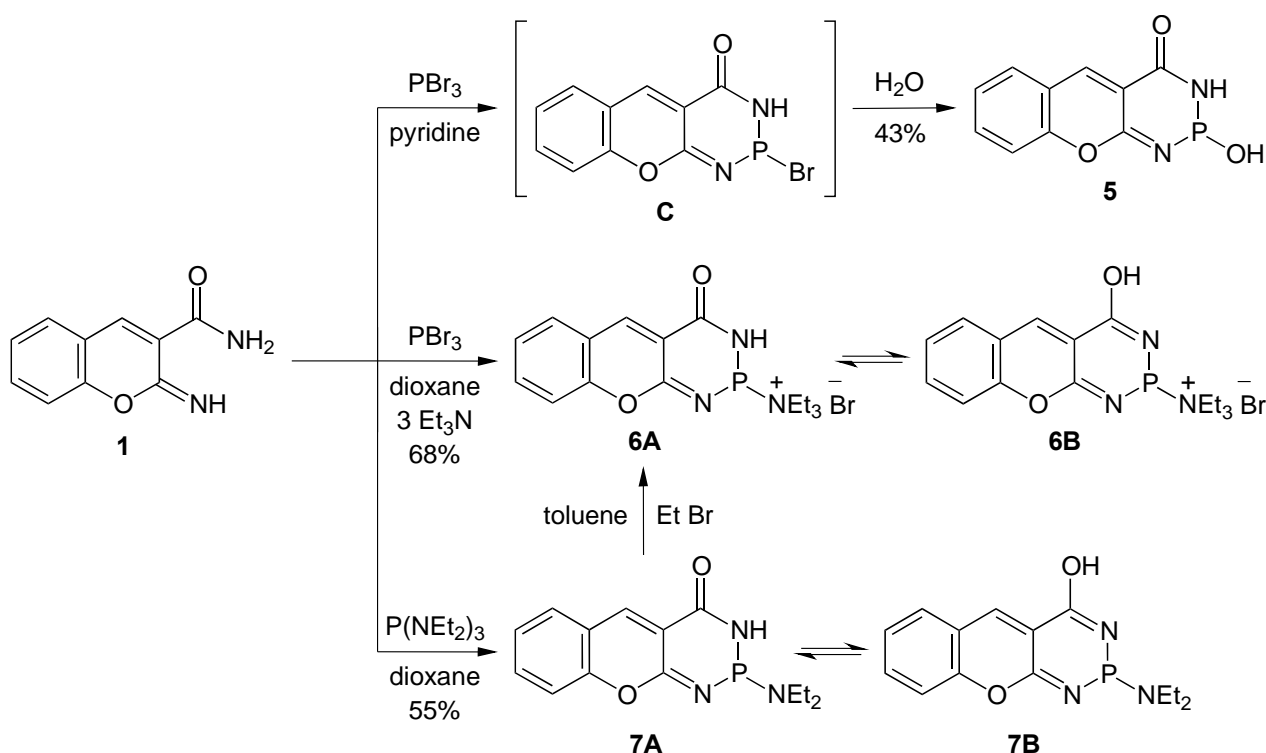


**Scheme 3**

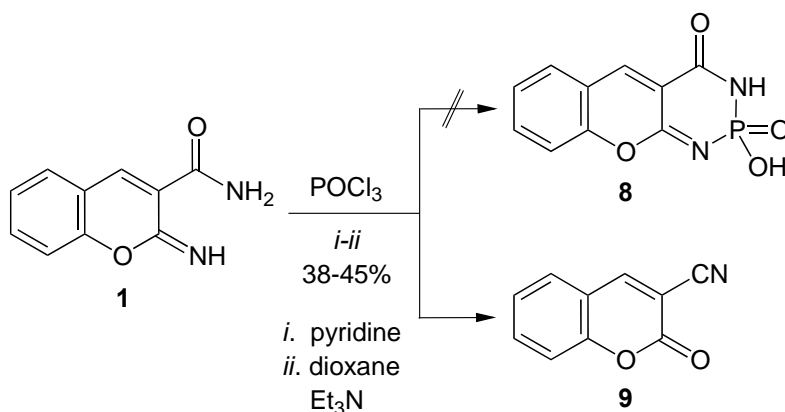
Similarly, the carboxamide **1** was reacted with phosphorus tribromide in dry pyridine and 1,4-dioxane to give different products. Thus, the 2-hydroxychromeno[2,3-*d*][1,3,2]diazaphosphinine **5** was obtained from pyridine medium, while the chromeno[2,3-*d*][1,3,2]diazaphosphinyl ammonium bromide **6** was isolated from dry 1,4-dioxane and triethylamine (Scheme 4). On the other hand, treatment of compound **1** with hexaethylphosphoramidate in dry 1,4-dioxane yielded the 2-(diethylamino)chromeno[2,3-*d*][1,3,2]-diazaphosphinine **7**, which was treated with ethyl bromide in dry toluene to give the product **6** (Scheme 4). The IR spectrum of compound **5** revealed the characteristic absorption bands at 3387 (br, OH and NH) and 1690 (C=O)  $\text{cm}^{-1}$ , while its <sup>1</sup>H-NMR spectrum represented the specific OH and NH at  $\delta$  4.56 and 7.21

ppm, respectively. The spectral data of compounds **6** and **7** confirmed their existence in two tautomeric forms (Scheme 4). Their IR spectra supported the presence of OH and NH functions. Also, their  $^1\text{H-NMR}$  spectra displayed multiplets for the ethyl protons at region  $\delta$  0.98–1.15 and 2.87–2.99 ppm, while the  $\text{D}_2\text{O}$ -exchangeable NH and OH were appeared at  $\delta$  7.89, 8.05 and 10.36, 11.59 ppm, respectively. The mass spectra of compounds **5–7** represented their molecular ion peaks at  $m/z$  234, 397 and 289, respectively.

When compound **1** was treated with phosphoryl chloride in dry pyridine or 1,4-dioxane containing triethylamine, furnished the unexpected 2-oxo-2*H*-chromene-3-carbonitrile (**9**)<sup>19</sup> (Scheme 5). Clearly,



Scheme 4



Scheme 5

compound **1** underwent dehydration followed by hydrolysis under different conditions by the effect of  $\text{POCl}_3$ .

## BIOLOGICAL EVALUATIONS

### 1. ANTIOXIDANT ACTIVITIES

The antioxidant activities of the synthesized compounds were evaluated by *in vitro* DPPH method to compare the results and to establish some structure-antioxidant activity relationships. The evaluation study was carried out at various concentrations at 50, 75 and 100  $\mu\text{g/mL}$ . DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity evaluation is a standard assay in antioxidant activity studies and offers a rapid technique for screening the radical scavenging activity of specific compounds or extracts.<sup>20,21</sup> A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally disappears when an antioxidant is present in the medium. Thus, the antioxidant molecule can quench DPPH free radical (*i.e.*, by providing hydrogen atoms or by electron donating, conceivable) and convert them to a colorless product (*i.e.*, 2,2-diphenyl-1-picrylhydrazine), resulting in a decrease in absorbance. Hence, more rapidly the absorbance decrease, the more potent the antioxidant activity of the compound. Percentage activity of DMF solutions of the synthesized compounds was examined and compared (Table 1). We can conclude from the obtained results that the synthesized compounds showed promising radical scavenging abilities by the used method and compared with ascorbic acid as a standard antioxidant. The results displayed that most of the synthesized compounds exhibited good radical scavenging abilities at lower concentrations. However, the gradual increase in the activity in all cases was observed with an increase in the concentrations of the tested compounds. It was perceived that compound **6** exhibited low activity, whereas **3**, **5** and **7** displayed moderate antioxidant properties. Also, compound **2** showed good antioxidative activity, while compound

**Table 1.** DPPH radical scavenging ability of the synthesized compounds **1–7** relative to the standard antioxidant ascorbic acid

Compd. No.	The percentage (%) antioxidant activity $\pm$ SD			IC <sub>50</sub> ( $\mu\text{g/mL}$ )
	50 ( $\mu\text{g/mL}$ )	75 ( $\mu\text{g/mL}$ )	100 ( $\mu\text{g/mL}$ )	
<b>1</b>	24.32 $\pm$ 0.21	32.23 $\pm$ 0.22	40.63 $\pm$ 0.20	128.97
<b>2</b>	59.95 $\pm$ 0.09	63.92 $\pm$ 0.11	72.36 $\pm$ 0.12	12.91
<b>3</b>	59.59 $\pm$ 0.11	75.62 $\pm$ 0.12	85.63 $\pm$ 0.13	29.66
<b>4</b>	69.12 $\pm$ 0.32	78.45 $\pm$ 0.33	89.23 $\pm$ 0.34	3.06
<b>5</b>	40.31 $\pm$ 0.18	52.41 $\pm$ 0.19	59.88 $\pm$ 0.18	72.78
<b>6</b>	1.34 $\pm$ 0.27	17.12 $\pm$ 0.27	33.23 $\pm$ 0.28	126.37
<b>7</b>	48.72 $\pm$ 0.29	64.98 $\pm$ 0.26	73.68 $\pm$ 0.28	50.04
<b>Ascorbic acid</b>	67.35 $\pm$ 0.19	73.01 $\pm$ 0.18	84.16 $\pm$ 0.21	1.11

**4** recorded the most potent antioxidant agents in comparison with ascorbic acid as standard antioxidant. It seemed that the presence of 1,3,2-diazaphosphinine and 1,2-azaphosphole rings that have NH groups and conjugated systems created a noticeable increase of antioxidative properties of compounds **2–7** in comparison with the starting material **1**. The higher antioxidant activities of compound **4** were explained by the existence of functionalized 1,2-azaphosphole rings which had free radicals at position 3a and 9a beside the original active amino groups. The conjugation between free radicals at positions 3a and 9a with carbonyl groups and  $\pi$  electrons of the aromatic rings represented the main factor for increasing the stability of the radical structures.

## 2. CYTOTOXICITY EVALUATIONS

The *in vitro* cytotoxicity of the synthesized compounds **1–7** was evaluated against human colon carcinoma HCT-116, hepatocellular carcinoma Hep-G2, lung carcinoma A-549 and breast adenocarcinoma MCF-7, using the crystal violet viability assay.<sup>22-24</sup> The  $IC_{50}$  values of the synthesized compounds ( $IC_{50} = 11.2\text{--}500\ \mu\text{g/mL}$ ) were comparable to that of doxorubicin and summarized in Table 2. Compounds **1**, **5**, **6** and **7** were found to be less active while compounds **2**, **3** and **4** showed good cytotoxicity against all the investigated cancer cell lines. Compound **2** had the acceptable  $IC_{50}$  values of 25.3 and 20.3  $\mu\text{g/mL}$  against Hep-G2 and A-549 cancer cells, respectively, while compound **3** exhibited its best cytotoxicity against the Hep-G2 cell line, with  $IC_{50} = 32.8\ \mu\text{g/mL}$  that employed about doxorubicin. Based on the results in Table 2, it can be concluded that the type of substituent attached to the phosphorus atom is a major determinant of the pharmacological properties of the parent structure chromeno[2,3-*d*][1,3,2]diazaphosphinines **2**, **3** and **5–7**. Therefore, the presence of phenyl moiety attached to phosphorus atom in compounds **2** and **3** could be considered to be the factor responsible for the high cytotoxicity against cancer cells in comparison with the phosphorus atom in compounds **5**, **6** and

**Table 2.** Cytotoxicity ( $IC_{50}$ ,  $\mu\text{g/mL}$ ) of the synthesized compounds **1–7** against human cancer cell lines in comparison with Doxorubicin

Compound	$IC_{50}$ ( $\mu\text{g/mL}$ )			
	HCT-116	Hep-G2	A-549	MCF-7
<b>1</b>	>500	>500	>500	>500
<b>2</b>	43.6	25.3	20.3	39.8
<b>3</b>	57.2	32.8	86.2	55.8
<b>4</b>	28.8	12.4	11.2	21.8
<b>5</b>	482	392	487	468
<b>6</b>	>500	469	>500	>500
<b>7</b>	243	132.6	211	285
<b>Doxorubicin</b>	0.493	0.467	0.426	0.469

7. These results provide evidence that the characteristic chemical features of phenylphosphoryl groups are key factors for their cytotoxicity and play a useful role in elucidating the mechanisms of action in relation to the synthesized compounds in future research programs. Additionally, the chromeno[4,3-*c*][1,2]-azaphosphol-3(2*H*)-one derivative **4** displayed the best activity as a system with potentially cytotoxicity against all four cell lines.

## EXPERIMENTAL

The melting points were determined in an open capillary tube on a digital Stuart SMP-3 apparatus. Infrared spectra were measured on FT-IR (Nicolet IS10) spectrophotometer using KBr disks and Perkin-Elmer 293 spectrophotometer using KBr disks. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were measured on Gemini-300BB spectrometer (400 and 100 MHz), using DMSO-*d*<sub>6</sub> as a solvent and TMS (δ) as an internal standard. <sup>31</sup>P-NMR spectra were measured on a Bruker (162 MHz) spectrophotometer using DMSO-*d*<sub>6</sub> as a solvent, TMS as an internal standard and 85% H<sub>3</sub>PO<sub>4</sub> as an external reference. Mass spectra were recorded on a Gas Chromatographic GCMSqp 1000 ex Shimadzu instrument at 70 eV and direct probe controller inlet part to single quadrupole mass analyzer in (Thermo Scientific GCMS). Elemental microanalysis was performed on Perkin-Elmer 2400II at the Chemical War department, Ministry of Defense. The purity of the synthesized compounds was checked by thin layer chromatography (TLC) and elemental microanalysis.

**Synthesis of 4-oxo-2-phenyl-2,3-dihydro-4*H*-chromeno[2,3-*d*][1,3,2]diazaphosphinine (2A) and 4-hydroxy-2-phenyl-2*H*-chromeno[2,3-*d*][1,3,2]diazaphosphinine (2B):** A solution of P,P-dichloro-(phenyl)phosphine (0.7 mL, 5 mmol) in dry pyridine (5 mL) was added to a solution of compound **1** (0.94 g, 5 mmol) in dry pyridine (30 mL) under stirring for 30 min at 10 °C then heated under reflux for 4 h. The solution was cooled, poured into cold water and neutralized with drops of concentrated HCl. The formed solid was filtered off, washed with water and crystallized from diluted EtOH to give greenish solid in 87% yield; mp 223–225 °C. IR (KBr), ( $\nu$  max, cm<sup>-1</sup>): 3361 (br, OH), 3170 (br, NH), 3071 (C-H<sub>arom</sub>), 1710 (C=O), 1670 (C=N), 1610, 1580 (C=C). <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>): 6.70 (d, 1H, *J*=8.0 Hz, Ph-H), 7.01, 7.28 (two d, 1H, *J*=8.0 Hz, H-9), 7.42–7.55 (m, 4H, H-7 and Ph-H), 7.65–7.70 (m, 1H, H-8), 7.78–7.81 (m, 1H, Ph-H), 7.96, 8.04 (two d, 1H, *J*=8.0 Hz, H-6), 8.85, 8.94 (two s, 1H, H-5), 10.17, 12.80 (s, 1H, NH and OH exchangeable with D<sub>2</sub>O). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>): 114.6, 116.1 (C-9), 118.6, 119.2 (C-4a), 119.8, 120.3 (C-5a), 120.8, 120.9 (C-3',5'<sub>phenyl</sub>), 124.6, 125.3 (C-7), 126.2, 126.4 (C-2',6'<sub>phenyl</sub>), 128.3, 128.7 (C-6), 130.8, 131.1 (C-1'<sub>phenyl</sub>), 135.1, 136.2 (C-8), 134.5, 134.8 (C-4'<sub>phenyl</sub>), 142.3, 144.5 (C-5), 152.1, 153.4 (C-9a), 158.3, 159.6 (C-10a), 157.2, 165.3 (C-4). <sup>31</sup>P-NMR (162 MHz, DMSO-*d*<sub>6</sub>): 31.2 and 32.6 ppm. MS (*m/z*, I%): 296 (M+2, 5%), 295 (M+1, 6%), 294 (M<sup>+</sup>, 10%). Anal. Calcd for C<sub>16</sub>H<sub>11</sub>N<sub>2</sub>O<sub>2</sub>P (294.24): C, 65.31%; H, 3.77%; N, 9.52%. Found: C, 64.95%;

H, 3.52%; N, 9.14%.

**Synthesis of 2-oxido-4-oxo-2-phenyl-2,3-dihydro-4*H*-chromeno[2,3-*d*][1,3,2]diazaphosphinine (3A) and 4-hydroxy-2-oxido-2-phenyl-2,3-dihydro-4*H*-chromeno[2,3-*d*][1,3,2]diazaphosphinine (3B):**

A solution of phenylphosphonic dichloride (0.7 mL, 5 mmol) in dry pyridine (5 mL) was added to a solution of compound **1** (0.94 g, 5 mmol) in dry pyridine (30 mL) under stirring for 30 min at 10 °C then heated under reflux for 7 h. The solution was cooled, poured into water and neutralized with drops of concentrated HCl. The formed solid was filtered off, washed with water and crystallized from DMF/EtOH to give brownish red solid in 83% yield; mp 256–258 °C. IR (KBr), ( $\nu$  max,  $\text{cm}^{-1}$ ): 3339 (br, OH), 3209 (br, NH), 3063 (C–H<sub>arom</sub>), 1738 (C=O), 1640, 1629 (C=N), 1606, 1558 (C=C), 1240, 1218 (P=O). <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>): 6.85 (d, 1H, *J*=8.4 Hz, Ph–H), 7.07, 7.21 (two d, 1H, *J*=8.0 Hz, H–9), 7.40–7.57 (m, 4H, H–7 and Ph–H), 7.66, 7.73 (two t, 1H, *J*=7.2 Hz, H–8), 7.80–7.83 (m, 1H, Ph–H), 7.94, 8.02 (two d, 1H, *J*=9.2 and 8.0 Hz, H–6), 9.00, 9.09 (two s, 1H, H–5), 10.24, 11.04 (two brs, 1H, NH and OH exchangeable with D<sub>2</sub>O). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>): 116.5, 116.9 (C–9), 117.3, 117.9 (C–4a), 119.2, 119.7 (C–5a), 120.1 (C–3',5'<sub>phenyl</sub>), 124.5, 125.4 (C–7), 126.0, 126.6 (C–2',6'<sub>phenyl</sub>), 128.7, 129.8 (C–6), 130.5 (C–1'<sub>phenyl</sub>), 134.2, 136.3 (C–8), 136.7 (C–4'<sub>phenyl</sub>), 147.7, 148.0 (C–5), 153.8, 154.7 (C–9a), 157.3, 157.6 (C–10a), 158.4, 164.3 (C–4). <sup>31</sup>P-NMR (162 MHz, DMSO-*d*<sub>6</sub>): 40.1 and 43.3 ppm. MS (*m/z*, I%): 310 (M<sup>+</sup>, 3%). Anal. Calcd for C<sub>16</sub>H<sub>11</sub>N<sub>2</sub>O<sub>3</sub>P (310.24): C, 61.94%; H, 3.57%; N, 9.03%. Found: C, 61.67%; H, 3.21%; N, 8.78%.

**Synthesis of 1,1-diphenyl-4-imino-3a,4-dihydro-1 $\lambda^5$ -chromeno[4,3-*c*][1,2]azaphosphol-3(2*H*)-one (4):**

A solution of P-chlorodiphenylphosphine (0.6 mL, 5 mmol) in dry pyridine (5 mL) was added to a solution of compound **1** (0.94 g, 5 mmol) in dry 1,4-dioxane (30 mL) in the presence of triethylamine (0.7 mL, 5 mmol) as a catalyst, under stirring for 30 min at 10 °C. The mixture was heated under reflux for 7 h. The solution was concentrated to its half volume and left to cool. The formed solid was filtered off, washed with water and crystallized from diluted MeOH to give yellow solid in 77% yield; mp 200–202 °C. IR (KBr), ( $\nu$  max,  $\text{cm}^{-1}$ ): 3450, 3335, 3201 (NH), 1655 (C=O), 1607 (C=N), 1576 (C=C). <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>): 5.01 (d, 1H, *J*<sub>PCH</sub>=6.8 Hz, H–3a), 6.38 (s, 1H, NH exchangeable with D<sub>2</sub>O), 6.67 (d, 1H, *J*=7.6 Hz, H–6), 6.81 (t, 1H, *J*=7.6 Hz, H–8), 6.86 (d, 1H, *J*=8.0 Hz, H–9), 7.13 (t, 1H, *J*=7.6 Hz, H–7), 7.38–7.42 (m, 2H, Ph–H), 7.45–7.49 (m, 2H, Ph–H), 7.52–7.57 (m, 1H, Ph–H), 7.68 (s, 1H, NH exchangeable with D<sub>2</sub>O), 7.69–7.79 (m, 5H, Ph–H). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>): 69.7 (C–3a), 111.0 (d, *J*=214.3 Hz, C–9b), 115.9 (C–6), 120.7 (C–9a), 120.9 (C–8), 122.2 (C–3',5'<sub>phenyl</sub>), 122.7 (C–3'',5''<sub>phenyl</sub>), 123.6 (C–9), 128.3 (d, *J*=10.9 Hz, C–1'<sub>phenyl</sub>), 128.5 (d, *J*=10.7 Hz, C–1''<sub>phenyl</sub>), 129.0 (C–4'<sub>phenyl</sub>), 130.8 (C–4''<sub>phenyl</sub>), 132.2 (d, *J*=8.4 Hz, C–2'',6''<sub>phenyl</sub>), 133.0 (C–7), 151.9 (C–5a), 161.9 (C–4), 170.9 (C–3). <sup>31</sup>P-NMR (162 MHz, DMSO-*d*<sub>6</sub>): 31.1 ppm. MS (*m/z*, I%): 373 (M+1, 11%), 372 (M<sup>+</sup>, 35%). Anal. Calcd for C<sub>22</sub>H<sub>17</sub>N<sub>2</sub>O<sub>2</sub>P (372.35): C, 70.96%; H, 4.60%; N, 7.52%. Found: C, 70.62%; H, 4.31%; N,

7.13%.

**Synthesis of 2-hydroxy-2,3-dihydro-4*H*-chromeno[2,3-*d*][1,3,2]diazaphosphinin-4-one (5):** A solution of phosphorus tribromide (0.5 mL, 5 mmol) in dry pyridine (5 mL) was added dropwise to a solution of compound **1** (0.94 g, 5 mmol) in pyridine (25 mL) for 15 min at 10 °C. The mixture was heated under reflux for 7 h. The solution was poured onto ice-water and drops of concentrated HCl. The formed solid was filtered off and crystallized from diluted EtOH to give brown solid in 43% yield; mp >300 °C. IR (KBr), ( $\nu$  max,  $\text{cm}^{-1}$ ): 3387 (br, OH, NH), 1690 (C=O), 1638 (C=N), 1606, 1560 (C=C).  $^1\text{H-NMR}$  (400 MHz,  $\text{DMSO-}d_6$ ): 4.56 (br, 1H, OH, exchangeable with  $\text{D}_2\text{O}$ ), 7.21 (br, 1H, NH, exchangeable with  $\text{D}_2\text{O}$ ), 7.37 (t, 1H,  $J=7.6$  Hz, H-7), 7.44 (d, 1H,  $J=7.2$  Hz, H-9), 7.77 (t, 1H,  $J=7.6$  Hz, H-8), 7.95 (d, 1H,  $J=8.0$  Hz, H-6), 8.56 (s, 1H, H-5).  $^{13}\text{C-NMR}$  (100 MHz,  $\text{DMSO-}d_6$ ): 114.8 (C-9), 118.8 (C-4a), 119.8 (C-5a), 124.1 (C-7), 128.8 (C-6), 133.5 (C-8), 144.6 (C-5), 153.4 (C-9a), 158.2 (C-10a), 166.3 (C-4).  $^{31}\text{P-NMR}$  (162 MHz,  $\text{DMSO-}d_6$ ): 10.2 ppm. MS ( $m/z$ , I%): 234 ( $\text{M}^+$ , 8%). Anal. Calcd for  $\text{C}_{10}\text{H}_7\text{N}_2\text{O}_3\text{P}$  (234.14): C, 51.30%; H, 3.01%; N, 11.96%. Found: C, 50.97%; H, 2.88%; N, 11.62%.

**Synthesis of 2-(*N,N,N*-triethylammonium)-4-oxo-3,4-dihydro-2*H*-chromeno[2,3-*d*][1,3,2]-diazaphosphinine bromide (6A) and 2-(*N,N,N*-triethylammonium)-4-hydroxy-2*H*-chromeno[2,3-*d*][1,3,2]diazaphosphinine bromide (6B):** A solution of phosphorus tribromide (0.5 mL, 5 mmol), in dry 1,4-dioxane (5 mL) was added dropwise to a solution of compound **1** (0.94 g, 5 mmol) in dry 1,4-dioxane (25 mL) in the presence of catalytic amount of triethylamine (2.1 mL, 15 mmol) for 15 min at 10 °C. The mixture was heated under reflux for 7 h. The formed solid on heating was filtered off, washed with water and crystallized from diluted EtOH to give yellow solid in 68% yield; mp 130–132 °C. IR (KBr), ( $\nu$  max,  $\text{cm}^{-1}$ ): 3409 (br, OH), 3190 (br, NH), 3050 (C- $\text{H}_{\text{arom}}$ ), 2973, 2938, 2936 (C- $\text{H}_{\text{aliph}}$ ), 1680 (br, C=O), 1638 (C=N), 1609, 1583 (C=C).  $^1\text{H-NMR}$  (400 MHz,  $\text{DMSO-}d_6$ ): 1.02–1.13 (m, 9H, 3  $\text{CH}_3$ ), 2.98–2.99 (m, 6H, 3  $\text{NCH}_2$ ), 6.66, 7.09 (two d, 1H,  $J=7.6$  and 7.6 Hz, H-9), 6.90–6.97 (m, 1H, H-7), 7.42, 7.73 (two t, 1H,  $J=7.2$  and 8.0 Hz, H-8), 7.89, 8.05 (two s, 1H, NH and OH exchangeable with  $\text{D}_2\text{O}$ ), 7.48, 7.95 (two d, 1H,  $J=7.6$  and 8.0 Hz, H-6), 8.85, 8.95 (two s, 1H, H-5). MS ( $m/z$ , I%): 399 ( $\text{M}+2$ , 11%), 397 ( $\text{M}^+$ , 15%). Anal. Calcd for  $\text{C}_{16}\text{H}_{21}\text{BrN}_3\text{O}_2\text{P}$  (398.23): C, 48.26%; H, 5.32%; N, 10.55%. Found: C, 47.89%; H, 5.03%; N, 10.19%.

**Synthesis of 2-(diethylamino)-4-oxo-2,3-dihydro-4*H*-chromeno[2,3-*d*][1,3,2]diazaphosphinine (7A) and 2-(diethylamino)-4-hydroxy-2*H*-chromeno[2,3-*d*][1,3,2]diazaphosphinine (7B):** A mixture of hexaethylphosphoramidate (1.5 mL, 5 mmol) and compound **1** (0.94 g, 5 mmol) in dry 1,4-dioxane (30 mL) was heated under reflux for 5 h. The solution was poured onto ice-water containing three drops of diluted HCl. The formed solid was filtered off and crystallized from diluted EtOH to give brown solid in 55% yield; mp >300 °C. IR (KBr), ( $\nu$  max,  $\text{cm}^{-1}$ ): 3420 (br, OH, NH), 3050 (C- $\text{H}_{\text{arom}}$ ), 2970, 2950

(C-H<sub>aliph</sub>), 1690 (C=O), 1655 (C=N), 1611, 1578 (C=C). <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>): 0.98–1.15 (m, 6H, 2 CH<sub>3</sub>), 2.87–2.93 (m, 4H, 2 CH<sub>2</sub>), 6.96, 7.14 (two d, 1H, *J*=8.0 and 8.4 Hz, H-9), 7.23–7.40 (m, 1H, H-7), 7.47–7.56, 7.66–7.81 (two m, 1H, H-8), 7.95, 8.10 (two d, 1H, *J*=7.6 and 8.4 Hz, H-6), 8.85, 9.02 (two s, 1H, H-5), 10.36, 11.59 (two s, 1H, NH and OH exchangeable with D<sub>2</sub>O). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>): 13.6, 14.1 (CH<sub>3</sub>), 57.3, 58.9 (CH<sub>2</sub>), 114.9, 115.6 (C-9), 117.9, 118.3 (C-4a), 119.3, 119.8 (C-5a), 123.7, 124.9 (C-7), 128.5, 129.4 (C-6), 133.4, 135.2 (C-8), 146.1, 147.3 (C-5), 153.6, 153.9 (C-9a), 157.1, 157.3 (C-10a), 158.2, 162.6 (C-4). <sup>31</sup>P-NMR (162 MHz, DMSO-*d*<sub>6</sub>): 9.3 and 9.6 ppm. MS (*m/z*, I%): 290 (M+1, 9%), 289 (M<sup>+</sup>, 12%). Anal. Calcd for C<sub>14</sub>H<sub>16</sub>N<sub>3</sub>O<sub>2</sub>P (289.26): C, 58.13%; H, 5.58%; N, 14.53%. Found: C, 57.78%; H, 5.23%; N, 14.17%.

**Synthesis of 2-oxo-2H-chromene-3-carbonitrile (9):** A solution of phosphoryl chloride (0.5 mL, 5 mmol) in dry 1,4-dioxane (5 mL) was added dropwise to compound **1** (0.94 g, 5 mmol) in dry 1,4-dioxane (25 mL) in the presence of catalytic amount of triethylamine (1.91 mL, 10 mmol) or in pyridine (25 mL) for 15 min at 10 °C. The mixture was heated under reflux for 10 h. The reaction mixture was cooled and poured into cold water. The formed solid was filtered off, washed with water and crystallized from diluted EtOH to give white solid in 38–45% yield; mp 140–142 °C (Lit.<sup>19</sup> 144–146 °C). IR (KBr), (*v* max, cm<sup>-1</sup>): 3051 (C-H<sub>arom</sub>), 2240 (C≡N), 1734 (C=O), 1607, 1562 (C=C), 1049 (C-O). <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>): 7.45 (t, 1H, *J*=7.6 Hz, H-6), 7.49 (d, 1H, *J*=8.4 Hz, H-8), 7.76–7.80 (m, 2H, H-7 and H-5), 8.93 (s, 1H, H-4).

#### Antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging effect was carried out according to the reported method.<sup>20,21</sup> One milliliter of various concentrations of the test compounds (50, 75, and 100 µg/mL) in EtOH were added to 4 mL of 0.004% (w/v) EtOH solution of DPPH. The tubes were then incubated in the dark room at rt for 30 min. A DPPH blank was prepared without compound, and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-visible spectrophotometer. The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula: % Radical scavenging activity =  $(AB-AA)/AB \times 100$  where *AB* = absorption of blank and *AA* = Absorption of the tested compound. The radical scavenging activity of ascorbic acid was also measured and compared with that of the different synthesized compound.

#### Cell lines

Colon (HCT-116), Human hepatocellular (Hep-G2), lung (A-549) and breast (MCF-7) carcinoma cells were obtained from VACSERA Tissue Culture Unit. The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50 µg/mL gentamycin. All cells were maintained at 37 °C in a humidified atmosphere

with 5% CO<sub>2</sub> and were sub-cultured two times a week.

### **Evaluation of cytotoxicity activities**

Cytotoxicity of all compounds was tested in HCT-116, Hep-G2, A-549 and MCF-7 cells. For cytotoxicity assay,<sup>22-24</sup> the cells were seeded in 96-well plate at a cell concentration of  $1 \times 10^4$  cells per well in 100  $\mu$ L of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested compounds were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37 °C in a humidified incubator with 5% CO<sub>2</sub> for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37 °C, various concentrations of the sample were added, and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method. In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 min. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates was measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated. The optical density was measured with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as: The percentage of cell viability =  $[1 - (OD_t/OD_c)] \times 100\%$ , where OD<sub>t</sub> is the mean optical density of wells treated with the tested sample and OD<sub>c</sub> is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC<sub>50</sub>), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each concentration, using Graphpad Prism software (San Diego, CA, USA).

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