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## SYNTHESIS OF SOME NOVEL ANTIMICROBIAL AND ANTIOXIDANT AGENTS OF FUNCTIONALIZED PYRAZOLO[4',3':5,6]PYRANO[3,2-*d*]-[1,2]AZAPHOSPHOLES AND PYRAZOLO[4',3':5,6]PYRANO[2,3-*d*]-[1,3,2]DIAZAPHOSPHININES

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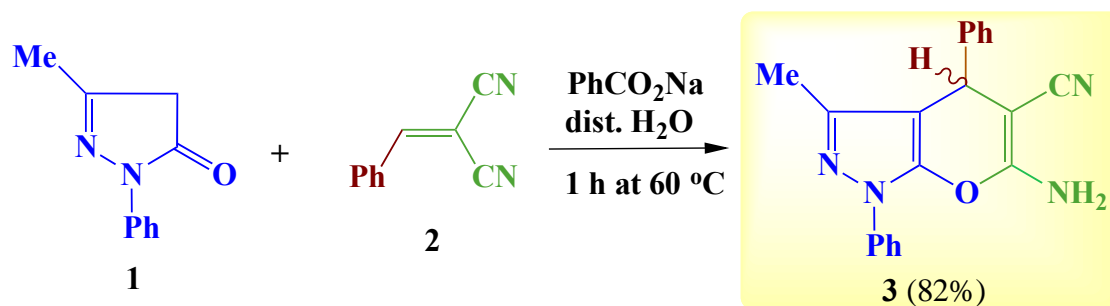
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**Abstract** – We have demonstrated facile synthetic approach for some novel functionalized pyrazolo[4',3':5,6]pyrano[3,2-*d*][1,2]azaphospholes and pyrazolo[4',3':5,6]pyrano[2,3-*d*][1,3,2]diazaphosphinines *via* treatment of 6-amino-3-methyl-1,4-diphenyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile with some phosphorus halides. The structure of the products was characterized by elemental analysis and spectral tools. The antimicrobial and antioxidant activities of the products were also evaluated.

Pyrano[2,3-*c*]pyrazole compounds gained major attention and represent an interesting template for medicinal chemistry due to their potent biological activities such as anti-inflammatory,<sup>1</sup> antimicrobial,<sup>2</sup> molluscicidal,<sup>3</sup> analgesic<sup>4</sup> and anticancer activity.<sup>5</sup> Furthermore, they are well known as biodegradable agrochemicals<sup>6</sup> and pharmaceutical ingredients.<sup>7</sup> On the other hand, there is a considerably growing interest in organophosphorus compounds due to their pharmaceutical applications and pivotal role in the field of organic synthesis, as well as their ubiquitous applications in enhancing the biological activity.<sup>8,9</sup> Especially, some nitrogen-phosphorus heterocycles are used as antitumor,<sup>10</sup> anti-inflammatory,<sup>11</sup> herbicides<sup>12</sup> and antimicrobial agents.<sup>13</sup> We have recently focused our attention on synthesis of new bioactive phosphorus compounds containing pyran ring.<sup>14-18</sup> Depending on the above, we have been motivated to construct phosphorus heterocycles such as 1,2-azaphosphole and 1,3,2-diazaphosphinine fused with the bioactive pyrano[2,3-*c*]pyrazole moiety in one novel molecular frame. To achieve these

novel chemical frames, we treated 6-amino-3-methyl-1,4-diphenyl-1,4-dihydropyran[2,3-*c*]pyrazole-5-carbonitrile (**3**) with some phosphorus halides under different reaction conditions. The antimicrobial and antioxidant properties of the separated products were evaluated.

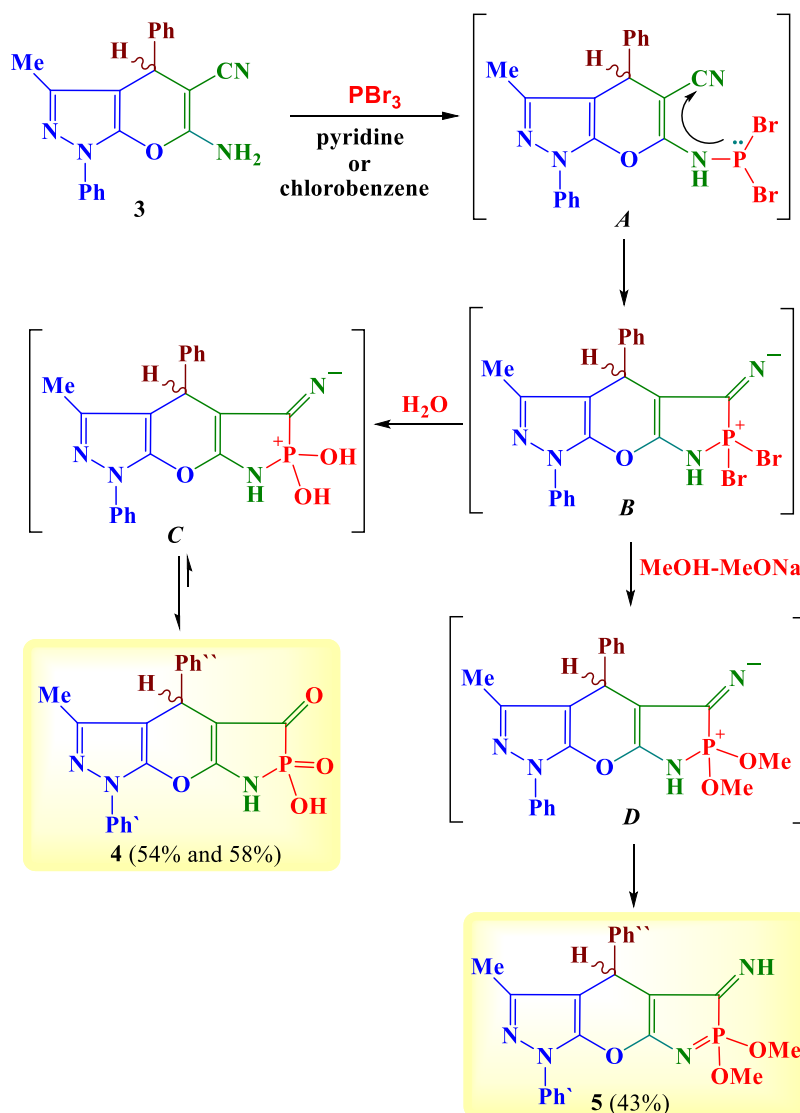
The known starting material 6-amino-3-methyl-1,4-diphenyl-1,4-dihydropyran[2,3-*c*]pyrazole-5-carbonitrile (**3**) was prepared in good yield by the treatment of 3-methyl-1-phenyl-5-pyrazolone (**1**) with 2-benzylidenemalononitrile (**2**) in distilled water containing sodium benzoate as a catalyst according to the reported method in literature (Scheme 1).<sup>19</sup>



Scheme 1

The present research studies the chemical reactivity of compound **3** towards some phosphorus halides such as phosphorus tribromide, phosphorus oxychloride and phosphorus pentachloride under different reaction conditions. Thus, reaction of the substrate **3** with phosphorus tribromide in dry pyridine or chlorobenzene followed by treatment with water furnished 6-hydroxy-3-methyl-1,4-diphenyl-6-oxido-1,4,7-trihydropyrazolo[4',3':5,6]pyrano[3,2-*d*][1,2]azaphosphol-5-one (**4**) in 54% and 58% yields, respectively (Scheme 2). When the above reaction was carried out in chlorobenzene and the oily product was treated with a solution of methanolic methoxide, it produced 6,6-dimethoxy-5-imino-3-methyl-1,4-diphenyl-1,4-dihydro-5*H*-6λ<sup>5</sup>-pyrazolo[4',3':5,6]pyrano[3,2-*d*][1,2]azaphosphole (**5**) (Scheme 2). The isolated products **4** and **5** were formed through condensation of NH<sub>2</sub> group of compound **3** with PBr<sub>3</sub> to form the phosphorus dibromide intermediate **A**. The latter intermediate underwent a nucleophilic addition of phosphorus atom at the electrophilic C≡N group to give the 1,2-azaphospholidine intermediate **B**. The labelled bromine atoms can be replaced with water and alcohol. Thus, the intermediate **B** was stirred in ice-water for 30 minutes to give the final product **4**, while its stirring under reflux in a solution of methanolic sodium methoxide afforded the product **5** (Scheme 2).<sup>20</sup> The expected molecular ion peaks of compounds **4** and **5** were showed in their mass spectra confirming the proposed structures. Their IR spectra did not show the NH<sub>2</sub> and C≡N groups, however, they displayed broad bands around 3433–3179 cm<sup>-1</sup> for OH and NH groups and new absorption band at 1663 cm<sup>-1</sup> for C=O function in the product **4**. The <sup>1</sup>H-NMR spectrum of the product **4** showed a characteristic singlet at δ 3.36 ppm attributable to OH

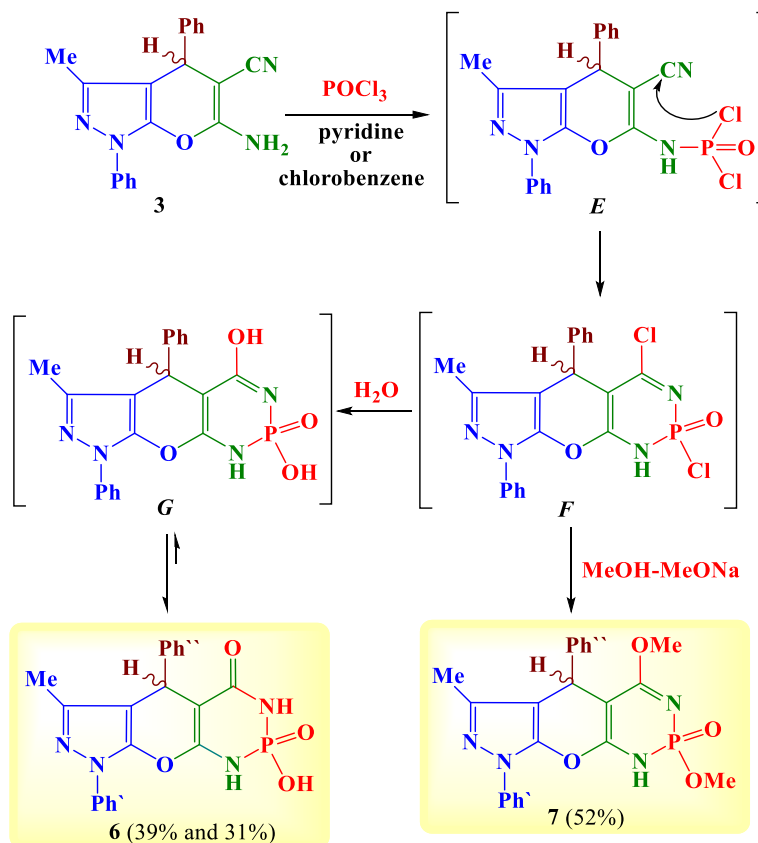
proton and a broad singlet at  $\delta$  13.99 ppm relative to NH proton, while the product **5** showed two singlets relevant to the two MeO groups and broad signal for NH proton at  $\delta$  3.92, 3.93 and 10.95 ppm, respectively. Their  $^{13}\text{C}$ -NMR spectra revealed no signals for  $\text{C}\equiv\text{N}$  group and showed new two doublets at 162.4 ( $J_{\text{PC}}=111$  Hz) and 159.7 ( $J_{\text{PC}}=165$  Hz) for  $\text{C}=\text{O}$  and  $\text{C}=\text{NH}$  groups, respectively.<sup>20</sup> In addition, the  $^{13}\text{C}$ -NMR spectrum of compound **5** displayed the carbon atoms of the two MeO groups at  $\delta$  50.5 and 51.0 ppm.



Scheme 2

Interestingly, compound **3** could react with phosphorus oxychloride in dry pyridine or chlorobenzene to give the nonisolable intermediate **E**. The nucleophilic addition of chlorine atom at  $\text{C}\equiv\text{N}$  group in the latter intermediate afforded the 2,4-dichloro-1,3,2-diazaphosphinine intermediate **F**. When the intermediate **F** was treated with ice-water for 30 minutes, gave the product **G** that underwent *Dimroth* rearrangement to separate 2-hydroxy-6-methyl-5,8-diphenyl-2-oxido-3,5,8-trihydropyrazolo[4',3':5,6]pyrano[2,3-*d*][1,3,2]-

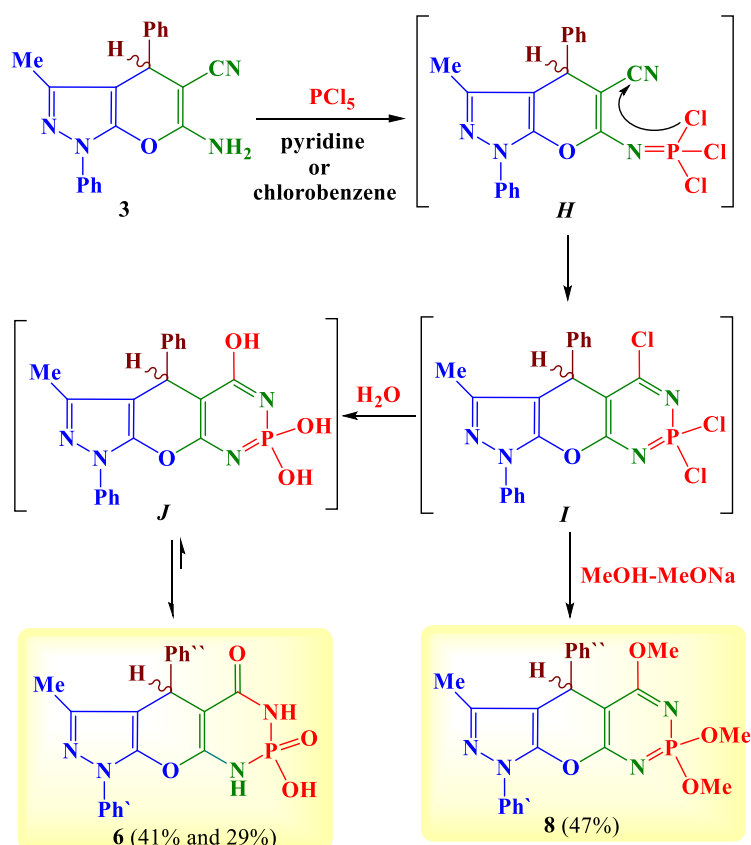
diazaphosphinin-4(1*H*)-one (**6**) in moderate yields (Scheme 3). Additionally, heating of the intermediate **F** (oily product) in a solution of methanolic sodium methoxide for 6 hours furnished 2,4-dimethoxy-6-methyl-5,8-diphenyl-2-oxido-1,5,8-trihydropyrazolo[4',3':5,6]pyrano[2,3-*d*][1,3,2]diazaphosphinine (**7**) (Scheme 3).<sup>21</sup> The IR spectrum of compound **6** recorded absorption bands for OH, two NH and C=O groups at 3419, 3182 and 1692  $\text{cm}^{-1}$ , respectively, while compound **7** recorded absorption bands at 3110 (NH) and 1029 (C–O)  $\text{cm}^{-1}$ . The chemical shifts of the specific OH and two NH protons in compound **6**, were resonated at  $\delta$  3.39, 11.41 and 13.98 ppm in its  $^1\text{H}$ -NMR spectrum. Besides, the  $^1\text{H}$ -NMR spectrum of system **7** revealed the existence of two MeO groups as two singlets at  $\delta$  3.84 and 3.97 ppm and one singlet for NH proton at  $\delta$  10.01 ppm. Moreover, the  $^{13}\text{C}$ -NMR spectra of both **6** and **7** showed characteristic signals at  $\delta$  162.9 (C=O) and 53.7 and 55.0 (2 MeO) ppm, respectively. Mass spectra of compounds **6** and **7** showed their molecular ion peaks at  $m/z$  391 ( $\text{M}^+-\text{OH}$ , 14%) and 389 ( $\text{M}^+-\text{OMe}$ , 30%), respectively.



Scheme 3

In the same manner, when compound **3** was reacted with phosphorus pentachloride in either dry pyridine or chlorobenzene then treated with distilled water, it furnished the product **6** in 41% and 29% yields, respectively (Scheme 3). While treatment of compound **3** with phosphorus pentachloride in chlorobenzene and refluxing the obtained oily product in a solution of methanolic sodium methoxide for

3 hours, furnished 2,2,4-trimethoxy-6-methyl-5,8-diphenyl-5,8-dihydro-2 $\lambda^5$ -pyrazolo[4',3':5,6]pyrano[2,3d][1,3,2]diazaphosphinine (**8**) as a sole product in 47% yield (Scheme 4). The formation of compound **8** was similar to the formation of compound **7** (Scheme 4). The  $^1\text{H-NMR}$  spectrum of product **8** revealed the presence of two singlets for MeO protons at  $\delta$  3.82 (6H, 2 MeO) and 4.01 (3H, MeO) ppm. The carbon atoms of these three MeO groups were resonated at  $\delta$  50.0 (2 MeO) and 52.0 (MeO) ppm in its  $^{13}\text{C-NMR}$  spectrum. Additionally, the IR and mass spectra of this product were in accordance with the suggested structure (See experimental section).



**Scheme 4**

## BIOLOGICAL EVALUATIONS

### Antimicrobial activities

The *in vitro* antibacterial activities of the synthesized compounds were screened against three organisms namely, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Escherichia coli*. Moreover, all the synthesized compounds were also screened for their *in vitro* antifungal activity against three organisms namely, *Aspergillus niger*, *Aspergillus clavatus* and *Candida albicans*.<sup>22,23</sup> Minimum inhibitory concentration (MIC) of all synthesized compounds was determined and given in Table 1. MIC is defined as the lowest concentration of inhibitor at which organism growth was not visually apparent. Ciprofloxacin and Ketoconazole were used as standard drugs for the antibacterial and antifungal activities,

respectively. Variable antimicrobial activities towards the used microorganisms were recorded for the synthesized compounds. Compound **6** did not show any acceptable inhibitory activities towards all bacteria and fungi organisms in comparison with the starting material **3**. Moreover, compounds **4** and **8** exhibited relatively moderate effects against all organisms. On the other hand, the product **7** showed good antibacterial and antifungal effects, while compound **5** recorded good antibacterial activities and excellent antifungal activities equal to the standard drugs. The presence of 1,2-azaphosphole or 1,3,2-diazphosphinine rings bearing MeO groups in fusion with the pyranopyrazole system in one molecular frame exhibited extremely good acceptable antibacterial and antifungal activities.

**Table 1.** The *in vitro* antimicrobial activities as minimum inhibitory concentration (MIC,  $\mu\text{g/mL}$ ) for the synthesized compounds

Compound	Bacterial strains			Fungal strains		
	<i>Streptococcus pyogenes</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Aspergillus niger</i>	<i>Candida albicans</i>	<i>Aspergillus clavatus</i>
<b>3</b>	250	250	250	250	250	500
<b>4</b>	125	62.5	62.5	125	125	125
<b>5</b>	62.5	62.5	62.5	31.25	31.25	31.25
<b>6</b>	250	250	250	250	250	250
<b>7</b>	62.5	62.5	62.5	62.5	62.5	125
<b>8</b>	125	125	125	125	125	125
Ciprofloxacin	31.25	31.25	31.25	--	--	--
Ketoconazole	--	--	--	31.25	31.25	31.25

### Antioxidant activities

The *in vitro* antioxidant activity of the synthesized compounds was investigated for by DPPH and  $\text{H}_2\text{O}_2$  methods.<sup>24-26</sup> The ascorbic acid was used as standard control. The lower  $\text{IC}_{50}$  value indicated to a higher antioxidant activity. The measured antioxidative properties of the synthesized compounds showed promising radical scavenging abilities (Table 2). The results revealed that compounds **6** and **7** exhibited poor radical scavenging abilities. However, the moderate activities were observed with the products **3** and **5**. Among all the investigated compounds by DPPH and  $\text{H}_2\text{O}_2$  methods, the compounds **4** and **8** exhibited extremely good antioxidant activities. The presence of OH and NH groups and extended conjugation in the latter compounds caused the better antioxidant activities.

**Table 2.** The *in vitro* antioxidant activities as inhibitory concentration (IC<sub>50</sub>, µg/mL) for the synthesized compounds by using DPPH and H<sub>2</sub>O<sub>2</sub> methods

Compound	Inhibitory Concentration (IC <sub>50</sub> , µg/mL)	
	DPPH method	H <sub>2</sub> O <sub>2</sub> method
<b>3</b>	16.21 ± 0.29	22.53 ± 0.51
<b>4</b>	13.78 ± 0.72	22.19 ± 0.42
<b>5</b>	18.23 ± 0.32	27.49 ± 0.69
<b>6</b>	21.19 ± 0.45	31.78 ± 0.27
<b>7</b>	22.36 ± 0.44	31.67 ± 0.34
<b>8</b>	14.29 ± 0.51	23.68 ± 0.26
<b>Ascorbic acid</b>	10.23 ± 0.23	18.62 ± 0.52

## EXPERIMENTAL

The melting points were measured on a digital Stuart SMP-3 apparatus in an open capillary tube. Infrared spectra were measured on FT-IR spectrophotometer (Nicolet iS10) using KBr disks. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were determined on Gemini-300BB (400 and 100 MHz) spectrometer, 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 direct probe controller inlet part to single quadropole 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. The antimicrobial and antioxidant evaluations were measured in Faculty of Science, King Khalid University, Abha, KSA.

**Synthesis of 6-amino-3-methyl-1,4-diphenyl-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (3):** a mixture of 3-methyl-1-phenyl-5-pyrazolone (**1**) (1.74 g, 10 mmol) and 2-benzylidenemalononitrile (**2**) (1.54 g, 10 mmol) in distilled water (40 mL) containing a catalytic amount of sodium benzoate (0.2 g), was stirred for 1 h at 60 °C. The formed solid was filtered off, washed with water, and crystallized from EtOH to give yellow crystalline in 82% yield (2.7 g); mp 179–180 °C (*Lit.*<sup>19</sup> 169–170 °C). IR (KBr), (ν<sub>max</sub>, cm<sup>-1</sup>): 3472, 3312 (NH<sub>2</sub>), 3062 (C–H<sub>arom</sub>), 2880 (C–H<sub>aliph</sub>), 2199 (C≡N), 1660, 1597 (C=C), 1516 (C=N). <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>): 2.19 (s, 3H, CH<sub>3</sub>), 4.66 (s, 1H, H-4), 5.92 (br, 2H, NH<sub>2</sub>), 7.18–7.95 (m, 10H, Ph–H).

**Synthesis of 6-hydroxy-3-methyl-1,4-diphenyl-6-oxido-1,4,7-trihydropyrazolo[4',3':5,6]pyrano-[3,2-*d*][1,2]azaphosphol-5-one (4). Method A:** a solution of phosphorus tribromide (0.5 mL, 5 mmol) in dry pyridine (5 mL) was added dropwise to a solution of compound **3** (1.64 g, 5 mmol) in dry pyridine (30 mL) at room temperature. The mixture was heated under reflux for 10 h. The solution was poured

onto ice-water and drops of diluted HCl (30%). The formed solid was filtered off and crystallized from diluted EtOH to give brown solid in 54% yield (1.06 g); mp 140–142 °C. **Method B:** a solution of phosphorus tribromide (0.2 mL, 2 mmol) in chlorobenzene (5 mL) was added dropwise to a solution of compound **3** (0.65 g, 2 mmol) in chlorobenzene (15 mL) at room temperature. The mixture was heated under reflux for 10 h. The filtrate was concentrated under vacuum. The oily product was poured onto ice-water under stirring for 30 min. The formed solid was filtered off and crystallized from diluted EtOH to give brown solid in 58% yield (0.45 g); mp 140–142 °C. IR (KBr), ( $\nu$  max,  $\text{cm}^{-1}$ ): 3433 (br, OH and NH), 3061 (C-H<sub>arom</sub>), 2923 (C-H<sub>aliph</sub>), 1663 (C=O), 1597 (C=C), 1579 (C=N), 1284 (P=O). <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>): 2.10 (s, 3H, CH<sub>3</sub>), 3.36 (s, 1H, OH), 5.00 (s, 1H, H-4), 6.98–7.81 (m, 10H, Ph-H), 13.99 (br, 1H, NH). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>): 12.5 (CH<sub>3</sub>), 32.6 (C-4), 90.1 (C-4a), 119.6 (C-3a), 121.2 (C-2',6'<sub>phenyl</sub>), 122.9 (C-4''<sub>phenyl</sub>), 125.6 (C-4'<sub>phenyl</sub>), 127.5 (C-3'',5''<sub>phenyl</sub>), 129.1 (C-2'',6''<sub>phenyl</sub>), 130.0 (C-3',5'<sub>phenyl</sub>), 132.2 (C-1''<sub>phenyl</sub>), 139.2 (C-1'<sub>phenyl</sub>), 142.7 (C-3), 150.9 (C-8a), 155.0 (C-7a), 162.4 (d,  $J=111$  Hz, C-5). MS ( $m/z$ , I%): 393 (M<sup>+</sup>, 67%). Anal. Calcd for C<sub>20</sub>H<sub>16</sub>N<sub>3</sub>O<sub>4</sub>P (393.34): C, 61.07%; H, 4.10%; N, 10.68%. Found: C, 60.82%; H, 3.88%; N, 10.34%.

**Synthesis of 6,6-dimethoxy--5-imino-3-methyl-1,4-diphenyl-1,4-dihydro-5H-6 $\lambda$ <sup>5</sup>-pyrazolo[4',3':5,6]-pyrano[3,2-*d*][1,2]azaphosphole (5):** a solution of phosphorus tribromide (0.2 mL, 2 mmol) in chlorobenzene (5 mL) was added dropwise to a solution of compound **3** (0.65 g, 2 mmol) in chlorobenzene (15 mL) at room temperature. The mixture was heated under reflux for 10 h. The filtrate was concentrated under vacuum. A solution of methanolic sodium methoxide (0.15 g Na in 10 mL MeOH) was added, and then heated under reflux for 4 h. The solvent was removed under vacuum. The formed oily product was treated with CH<sub>2</sub>Cl<sub>2</sub> to give beige solid in 43% yield (0.23 g); mp 101–103 °C. IR (KBr), ( $\nu$  max,  $\text{cm}^{-1}$ ): 3179 (br, NH), 1638 (C=N), 1594 (C=C), 1522 (C=N), 1024 (C-O). <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>): 1.91 (s, 3H, CH<sub>3</sub>), 3.92 (s, 3H, OCH<sub>3</sub>), 3.93 (s, 3H, OCH<sub>3</sub>), 4.97 (s, 1H, H-4), 6.94–7.98 (m, 10H, Ph-H), 10.95 (br, 1H, NH). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>): 12.9 (CH<sub>3</sub>), 34.4 (C-4), 50.5 (OCH<sub>3</sub>), 51.0 (OCH<sub>3</sub>), 83.2 (C-4a), 118.3 (C-3a), 123.3 (C-2',6'<sub>phenyl</sub>), 126.3 (C-4''<sub>phenyl</sub>), 127.4 (C-4'<sub>phenyl</sub>), 128.6 (C-3'',5''<sub>phenyl</sub>), 128.9 (C-2'',6''<sub>phenyl</sub>), 129.8 (C-3',5'<sub>phenyl</sub>), 131.2 (C-1''<sub>phenyl</sub>), 135.4 (C-1'<sub>phenyl</sub>), 145.2 (C-3), 154.7 (C-8a), 156.6 (C-7a), 159.7 (d,  $J=165$  Hz, C-5). MS ( $m/z$ , I%): 389 (M<sup>+</sup>-OMe, 30%). Anal. Calcd for C<sub>22</sub>H<sub>21</sub>N<sub>4</sub>O<sub>3</sub>P (420.41): C, 62.85%; H, 5.03%; N, 13.33%. Found: C, 62.51%; H, 4.891%; N, 13.02%.

**Synthesis of 2-hydroxy-6-methyl-5,8-diphenyl-2-oxido-3,5,8-trihydropyrazolo[4',3':5,6]pyrano[2,3-*d*][1,3,2]diazaphosphinin-4(1H)-one (6).** **Method A:** a solution of phosphorus oxychloride (0.5 mL, 5 mmol) or phosphorus pentachloride (1.0 g, 5 mmol) in dry pyridine (10 mL) was added dropwise to a solution of compound **3** (1.64 g, 5 mmol) in dry pyridine (30 mL) at room temperature. The mixture was heated under reflux for 15 h. The solution was poured onto ice-water and drops of diluted HCl (30%).

The formed oily product was treated with petroleum ether. The formed solid was filtered off and crystallized from diluted EtOH to give yellow solid in 39% (0.79 g) and 41% (0.83 g) yields; mp 123–125 °C. **Method B:** a solution of phosphorus oxychloride (0.2 mL, 2 mmol) or phosphorus pentachloride (0.4 g, 2 mmol) in chlorobenzene (5 mL) was added dropwise to a solution of compound **3** (0.65 g, 2 mmol) in chlorobenzene (15 mL) at room temperature. The mixture was heated under reflux for 10 h. The filtrate was concentrated under vacuum. The oily product was poured onto ice-water under stirring for 30 min. The formed solid was filtered off and crystallized from diluted EtOH to give yellow solid in 31% (0.25 g) and 29% (0.23 g) yields; mp 122–124 °C. IR (KBr), ( $\nu$  max,  $\text{cm}^{-1}$ ): 3419 (br, OH), 3182 (br, 2 NH), 3064 (C–H<sub>arom</sub>), 2921 (C–H<sub>aliph</sub>), 1692 (C=O), 1597 (C=C), 1283 (P=O). <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>): 1.93 (s, 3H, CH<sub>3</sub>), 3.39 (s, 1H, P–OH), 5.35 (s, 1H, H–5), 6.97–7.80 (m, 10H, Ph–H), 11.41 (s, 1H, NH), 13.98 (br, 1H, NH). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>): 12.7 (CH<sub>3</sub>), 33.8 (C–5), 89.4 (C–4a), 117.8 (C–5a), 119.5 (C–2',6'<sub>phenyl</sub>), 122.3 (C–4''<sub>phenyl</sub>), 126.8 (C–4'<sub>phenyl</sub>), 127.9 (C–3'',5''<sub>phenyl</sub>), 128.8 (C–2'',6''<sub>phenyl</sub>), 130.7 (C–3',5'<sub>phenyl</sub>), 132.2 (C–1''<sub>phenyl</sub>), 142.7 (C–1'<sub>phenyl</sub>), 144.9 (C–6), 150.9 (C–8a), 158.0 (C–9a), 162.9 (C–4). <sup>31</sup>P-NMR (162 MHz, DMSO-*d*<sub>6</sub>): 7.52 ppm. MS (*m/z*, I%): 391 (M<sup>+</sup>–OH, 14%). Anal. Calcd for C<sub>20</sub>H<sub>17</sub>N<sub>4</sub>O<sub>4</sub>P (408.36): C, 58.83%; H, 4.20%; N, 13.72%. Found: C, 58.49%; H, 4.01%; N, 13.46%.

**Synthesis of 2,4-dimethoxy-6-methyl-5,8-diphenyl-2-oxido-1,5,8-trihydropyrazolo[4',3':5,6]pyrano-[2,3-*d*][1,3,2]diazaphosphinine (7):** a solution of phosphorus oxychloride (0.2 mL, 2 mmol) in chlorobenzene (5 mL) was added dropwise to a solution of compound **3** (0.65 g, 2 mmol) in chlorobenzene (15 mL). The mixture was heated under reflux for 10 h. The filtrate was concentrated under vacuum. A solution of methanolic sodium methoxide (0.15 g Na in 10 mL MeOH) was added, and then heated under reflux for 6 h. The solvent was removed under vacuum to give yellow solid in 52% yield (0.29 g); mp 105–106 °C. IR (KBr), ( $\nu$  max,  $\text{cm}^{-1}$ ): 3110 (br, NH), 2970, 2940 (C–H<sub>aliph</sub>), 1638 (C=N), 1595 (C=C), 1236 (P=O), 1029 (C–O). <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>): 2.02 (s, 3H, CH<sub>3</sub>), 3.84 (s, 3H, OCH<sub>3</sub>), 3.97 (s, 3H, OCH<sub>3</sub>), 5.00 (s, 1H, H–5), 6.97–7.89 (m, 10H, Ph–H), 10.01 (s, 1H, NH). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>): 13.7 (CH<sub>3</sub>), 35.2 (C–5), 53.7 (OCH<sub>3</sub>), 55.0 (OCH<sub>3</sub>), 90.9 (C–4a), 120.7 (C–5a), 123.4 (C–2',6'<sub>phenyl</sub>), 126.4 (C–4''<sub>phenyl</sub>), 127.6 (C–4'<sub>phenyl</sub>), 128.6 (C–3'',5''<sub>phenyl</sub>), 128.8 (C–2'',6''<sub>phenyl</sub>), 129.3 (C–3',5'<sub>phenyl</sub>), 131.7 (C–1''<sub>phenyl</sub>), 138.9 (C–1'<sub>phenyl</sub>), 142.7 (C–6), 152.8 (C–8a), 157.7 (C–9a), 161.9 (C–4). MS (*m/z*, I%): 436 (M<sup>+</sup>, 32%). Anal. Calcd for C<sub>22</sub>H<sub>21</sub>N<sub>4</sub>O<sub>4</sub>P (436.41): C, 60.55%; H, 4.85%; N, 12.84%. Found: C, 60.23%; H, 4.71%; N, 12.59%.

**Synthesis of 2,2,4-trimethoxy-6-methyl-5,8-diphenyl-5,8-dihydro-2 $\lambda^5$ -pyrazolo[4',3':5,6]pyrano-[2,3-*d*][1,3,2]diazaphosphinine (8):** A solution of phosphorus pentachloride (0.4 g, 2 mmol) in chlorobenzene (5 mL) was added dropwise to a solution of compound **3** (0.65 g, 2 mmol) in chlorobenzene (15 mL) at room temperature. The mixture was heated under reflux for 10 h. The filtrate

was concentrated under vacuum. A solution of methanolic sodium methoxide (0.15 g Na in 10 mL MeOH) was added, and then heated under reflux for 3 h. The solvent was removed under vacuum. The formed oily product was treated with Et<sub>2</sub>O to give orange solid in 47% yield (0.27 g); mp 98–100 °C. IR (KBr), ( $\nu$  max, cm<sup>-1</sup>): 3050 (C–H<sub>arom</sub>), 2928 (C–H<sub>aliph</sub>), 1638 (C=N), 1600 (C=C), 1027 (C–O). <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>): 1.95 (s, 3H, CH<sub>3</sub>), 3.82 (s, 6H, 2 OCH<sub>3</sub>), 4.01 (s, 3H, OCH<sub>3</sub>), 4.83 (s, 1H, H–5), 6.98–7.96 (m, 10H, Ph–H). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>): 14.3 (CH<sub>3</sub>), 34.1 (C–5), 50.0 (2 OCH<sub>3</sub>), 52.0 (OCH<sub>3</sub>), 101.4 (C–4a), 120.6 (C–5a), 121.5 (C–2',6'<sub>phenyl</sub>), 126.3 (C–4''<sub>phenyl</sub>), 126.6 (C–4'<sub>phenyl</sub>), 128.6 (C–3'',5''<sub>phenyl</sub>), 129.4 (C–2'',6''<sub>phenyl</sub>), 129.7 (C–3',5'<sub>phenyl</sub>), 131.6 (C–1''<sub>phenyl</sub>), 136.5 (C–1'<sub>phenyl</sub>), 138.9 (C–6), 151.6 (C–8a), 159.8 (C–9a), 163.2 (C–4). MS (*m/z*, I%): 450 (M<sup>+</sup>, 29%). Anal. Calcd for C<sub>23</sub>H<sub>23</sub>N<sub>4</sub>O<sub>4</sub>P (450.44): C, 61.33%; H, 5.15%; N, 12.44%. Found: C, 61.09%; H, 4.89%; N, 12.16%.

### EVALUATION OF ANTIMICROBIAL ACTIVITY

All the synthesized products were investigated for their *in vitro* antimicrobial activity against bacterial strains namely, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Escherichia coli* and fungal strains namely, *Aspergillus niger*, *Aspergillus clavatus* and *Candida albicans* by disc diffusion method.<sup>22,23</sup> Ciprofloxacin and Ketoconazole were used as standard drugs for bacteria and fungi, respectively. The inhibitions were recorded by measuring the diameter of the inhibition zone at the end of 24 h for bacteria and 72 h for fungi. Each experiment was repeated twice. Based on the results of zone of inhibition, the minimum inhibitory concentration (MIC) of the synthesized compounds against all bacterial and fungal strains was determined by liquid dilution method. Stock solutions of tested compounds with 500, 250, 125, 62.5, 31.25, 15.62, and 7.84 µg/mL concentrations were prepared with DMSO solvent. The solutions of standard drugs, Ciprofloxacin and Ketoconazole were prepared in the same concentrations. Inoculums of the bacterial and fungal culture were also prepared. To a series of tubes containing 1 mL each of the used compound solution was added with different concentrations and 0.2 mL of the inoculums. Further 3.8 mL of the sterile water was added to each of the test tubes. These tubes were incubated for 24 h at 37 °C and observed for the presence of turbidity. The minimum inhibitory concentration at which no growth was observed was taken as the MIC values (Table 1).

### EVALUATION OF ANTIOXIDANT ACTIVITY

#### *DPPH radical scavenging activity*

According to the reported method,<sup>24,25</sup> the scavenging activity of the synthesized compounds was performed against DPPH radical. To a medium consisting of different title compounds, 85 µM of DPPH was affixed. Compounds of different concentrations were prepared in distilled EtOH; 1 mL of each compound solution (25, 50, 100, 200, 300 and 400 µmol/L) was taken in different test tubes, and 4 mL of 100 µmol/L EtOH solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark at rt for 20 min. A DPPH blank was prepared without a compound, and EtOH was used for the

baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using an ultraviolet-visible spectrophotometer. The values were articulated in the inhibition of absorbance percentage of DPPH radical with the values of standard without the synthesized compounds (ascorbic acid maximum inhibition was treated 100% of inhibition). 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  $AA$  indicates the control reaction's absorbance and  $AB$  indicates the sample's absorbance. The compound concentration providing 50% inhibition ( $IC_{50}$ ) was calculated from the graph of percentage against compound concentrations.

### **Hydrogen peroxide scavenging activity**

According to the reported method,<sup>26</sup> the scavenging activity of the synthesized compounds against hydrogen peroxide radical was determined. In PBS (phosphate-buffered saline, pH 7.4), 4  $\mu$ M solution of hydrogen peroxide was prepared, and its concentration was examined from the absorbance at 230 nm spectrophotometrically using molar absorptivity 81/M/cm. A dissolved solution of 100  $\mu$ M compounds in 4 mL distilled water was added to  $H_2O_2$ -PBS (0.6 mm<sup>3</sup>) solution. After 20 min, the absorbance of  $H_2O_2$  at 230 nm was resolved against a blank solution having parent compound with PBS and without  $H_2O_2$ . Later, a dissolved solution of ascorbic acid in 4 mL distilled water was added to  $H_2O_2$  solution in PBS (0.6 mm<sup>3</sup>). After 10 min, the absorbance was analyzed against a blank solution in the same manner. The hydrogen peroxide scavenging activities were expressed as the inhibition percentage and were calculated using the formula: %  $H_2O_2$  scavenging activity =  $(AB-AA)/AB \times 100$ , where  $AA$  indicates the control reaction's absorbance and  $AB$  indicates the sample's absorbance. The compound concentration providing 50% inhibition ( $IC_{50}$ ) was calculated from the graph of percentage against compound concentrations.

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