

Synthesis and antimicrobial activities of some novel *bis*-pyrazole derivatives containing a hydrophosphoryl unit

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Abstract

Vilsmeier-Haack reaction conditions were applied on some methyl ketone aryl phosphonic dihydrazones to yield some interesting *bis*-pyrazole derivatives containing a hydrophosphoryl unit. *Bis*-{4-formyl-3-aryl-1*H*-pyrazol-1-yl}phosphine oxides (**4a,b**) were condensed with some nucleophiles such as aniline, phenacyl triphenylphosphonium bromide and 4-phenylthiosemicarbazide followed by treatment with thioglycolic acid, diethyl phosphite and/or acetic anhydride to yield a novel class of *bis*-pyrazoles containing sulfur and phosphorus derivatives. Most of the newly synthesized compounds were evaluated for their *in vitro* antimicrobial activities.

Keywords: Phosphonic dihydrazones, Hydrophosphoryl, Vilsmeier-Haack reaction, *Bis*-pyrazoles, Sulfur-phosphorus heterocycles, Antimicrobial activity,

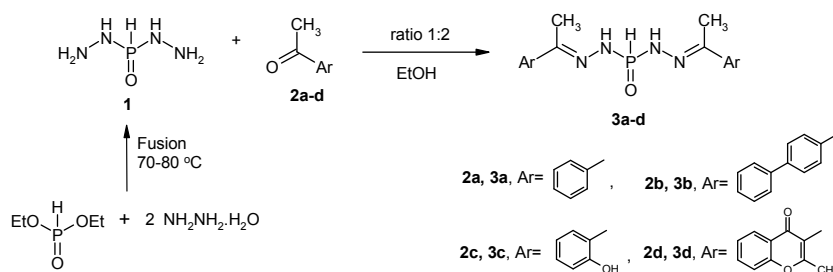
Introduction

Pyrazole and its derivatives represent one of the most active classes of compounds possessing a wide spectrum of biological activities including antibacterial [1,2], antifungal [3,4], herbicidal [5], insecticidal [6] and other biological activities [7]. Up till now, a great variety of these kinds of compounds have been synthesized, among which some commercial pesticides have been developed including Fipronil (MB46030) [8], ET-751 [9] and Pyrazolate (A-544) [10]. On the other hand, organophosphorus compounds possess insecticidal, pesticidal, acaricidal and antimicrobial properties [11-15]. These compounds exert their biological action on arthropods by attacking the system of neural transmission and inhibiting the function of acetyl cholinesterase [16,17]. In particular, organophosphorus compounds including hydrophosphoryl group (H-P=O) are widely used in industry, agriculture and medicine. It is interesting that many hydrophosphoryl compounds are also used as complexing and extracting agents, as well as corrosion and saline deposition inhibitors [18,19]. The connection of a heterocyclic moiety with organophosphorus compounds further may enhance their biological activities. In continuation of our research work on the synthesis of bioactive phosphorus containing heterocycles [13,15,20-22], it was considered valuable to integrate hydrophosphoryl unit and pyrazole rings together in novel a

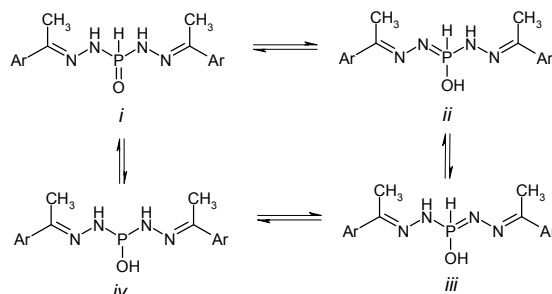
molecular frame to see the additive effect of these novel frames towards the antimicrobial activity.

Results and Discussion

The key intermediate in the present work is phosphonic dihydrazide (**1**), which was obtained from fusion of diethyl phosphite with two equivalent amounts of hydrazine hydrate at 70–80 °C [23] (Scheme 1). Normal Condensation of phosphonic dihydrazide (**1**) with acetophenone derivatives **2a-d** gave the corresponding phosphonic dihydrazones **3a-d** (Scheme 1). These reactions were carried out in absolute ethanol under mild conditions, and the products were isolated as yellow crystalline substances in 46-71% yields. Structures of **3a-d** were deduced from their spectroscopic measurements. The ¹H NMR spectra of compounds **3a-d** recorded signals of methyl protons in the region δ 2.07–2.28 ppm. Also, the signals of NH and H–P=O protons were displaced, presumably as result of rapid protons exchange in two types of tautomeric equilibriums. Firstly, the tautomeric amide-imide equilibrium (i, ii, iii). Secondly, hydrophosphoryl unit in solutions easily undergoes the tautomeric transition, providing it a unique combination of properties of pentavalent (λ^5 , σ^4 form) and trivalent (λ^3 , σ^3 form) phosphorus atom (phosphonate–phosphite i and iv) (Scheme 2) [24-27]. Also, the ¹³C NMR spectrum of compound **3a** displayed the carbon atoms of methyl and C=N groups at δ 14.7 and 157.2 ppm, respectively. Furthermore, compound **3c** exhibited a signal in its ³¹P NMR spectrum at δ 18.4 ppm due to the presence of a hydrophosphoryl unit [28]. The mass spectral data of **3a-d** were in accordance with their molecular formulas.



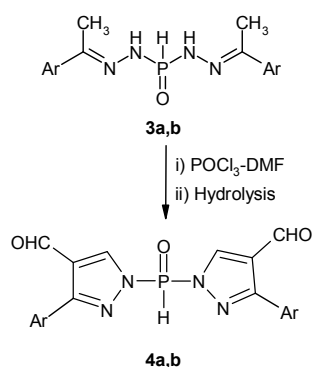
Scheme 1



Scheme 2

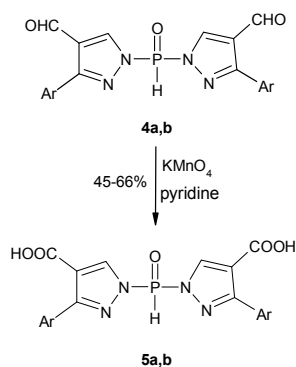
A convenient procedure for the synthesis of 4-formylpyrazole derivatives is based on the Vilsmeier-Haack reactions with methyl ketone aryl hydrazones [29-32]. Thus, application of Vilsmeier-Haack reaction on phosphonic dihydrazones **3a,b** afforded *bis*{4-formyl-3-aryl-

1*H*-pyrazol-1-yl}phosphine oxides (**4a,b**) (Scheme 3). The proposed mechanism for the formation of **4a,b** involved double formylation at each methyl group of phosphonic dihydrazones **3a,b**, followed by self cyclization then hydrolysis in basic medium [33]. The IR spectra of compounds **4a,b** showed two strong absorption bands at 1678–1671 and 1284–1241 cm^{-1} assignable to CHO and P=O functional groups, respectively. The ^1H NMR spectra of compounds **4a,b** exhibited singlet signals at δ 9.95–10.00 ppm for the formyl protons and the protons of the pyrazole rings in position 5 resonated at δ 8.69–8.84 ppm. In addition, doublet signals were present at δ 7.45–7.54 ppm ($J_{\text{PH}} = 682\text{--}733$ Hz) corresponding to proton of H–P=O units. Furthermore, the ^{13}C NMR spectrum of **4a** displayed two characteristic signals at δ 184.6 and 144.5 ppm corresponding to aldehydic carbon atom and C–5 of the pyrazole rings, respectively. Also, the ^{31}P NMR spectrum of **4b** showed a signal at δ 7.71 ppm. Mass spectra of **4a,b** revealed the molecular ion peaks at m/e 389 (M–H) and 390 (M– C_{12}H_8), respectively.



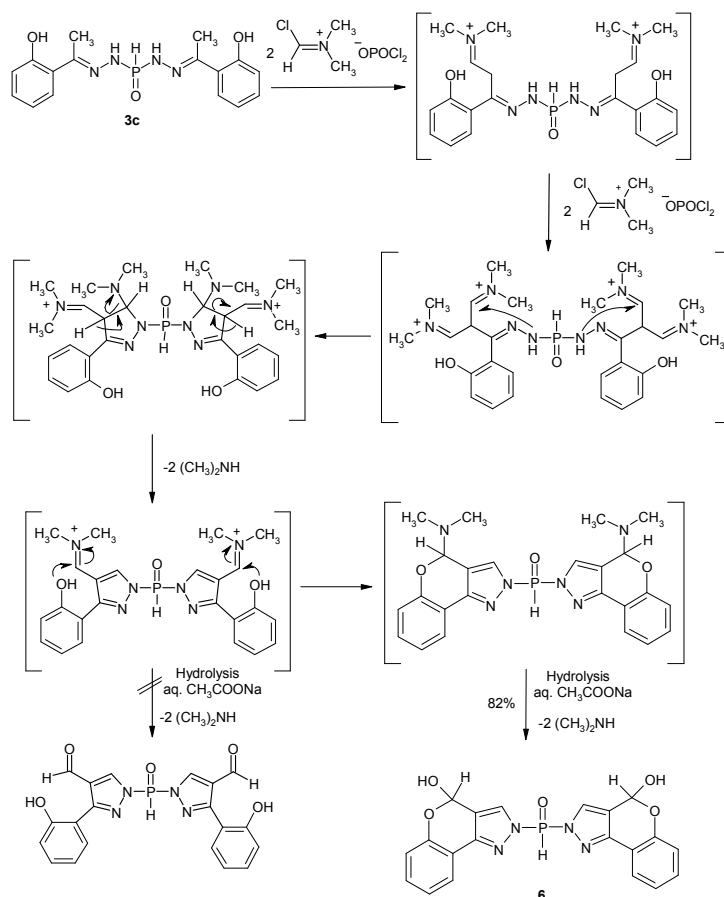
Scheme 3

Bis(4-formylpyrazolyl)phosphine oxides **4a,b** were oxidized in basic medium by potassium permanganate to give the corresponding *bis*(4-carboxypyrazolyl)phosphine oxides **5a,b**, respectively (Scheme 4). The oxidation reaction took place only on the formyl groups. This may be due to the phosphorus centers in **4a,b** are less susceptible to electrophiles, and ultimately more stable and resistant to spontaneous oxidation. Structures of products **5a,b** were established on the basis of their elemental and spectral data (See Experimental Section).

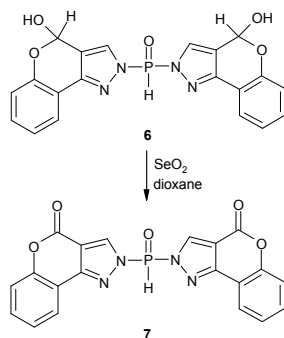


Scheme 4

The present work was also extended to apply the Vilsmeier-Haack reaction on phosphonic dihydrazones **3c,d** which contain active functional groups in *ortho* positions that led to new fused pyrazole systems. Thus, when phosphonic dihydrazone **3c** was treated with Vilsmeier reagent afforded a red crystalline product namely, *bis*{4-hydroxy-2,4-dihydrochromeno[4,3-*c*]pyrazol-2-yl}phosphine oxide (**6**). Formation of compound **6** involved double formylation at the methyl groups of **3c**, followed by nucleophilic attack of NH groups at $-\text{CH}=\text{N}^+(\text{CH}_3)_2$ moieties to eliminate two molecules of dimethylamine. Another nucleophilic attack took place by phenolic OH groups at $-\text{CH}=\text{N}^+(\text{CH}_3)_2$ moieties, followed by hydrolysis to give the final product (Scheme 5). The spectral data of **6** recommended the cyclic structure as its ^1H NMR spectrum displayed a broad singlet (D_2O exchangeable) at δ 3.72 ppm due to alcoholic OH protons and singlet signal at δ 6.53 ppm indicated to H-2 proton of hydrochromene moieties. The protons of the formed pyrazole rings were also observed in ^1H NMR spectrum at δ 7.96 ppm while proton of hydrophosphoryl unit at δ 7.44 ppm ($J_{\text{PH}} = 423$ Hz), respectively. Also, its IR spectrum showed a broad band at 3135 cm^{-1} due to alcoholic OH group. The oxidation reaction of compound **6** with selenium dioxide in dry dioxane yielded *bis*{chromeno[4,3-*c*]pyrazol-4-oxo-2-yl}phosphine oxide (**7**) (Scheme 6). The absorption band of carbonyl group appeared at 1652 cm^{-1} [34] in the IR spectrum of **7**. Also, its structure was confirmed from ^1H NMR spectrum by disappearance of OH and $\text{C}_2\text{-H}$ protons of compound **6**.

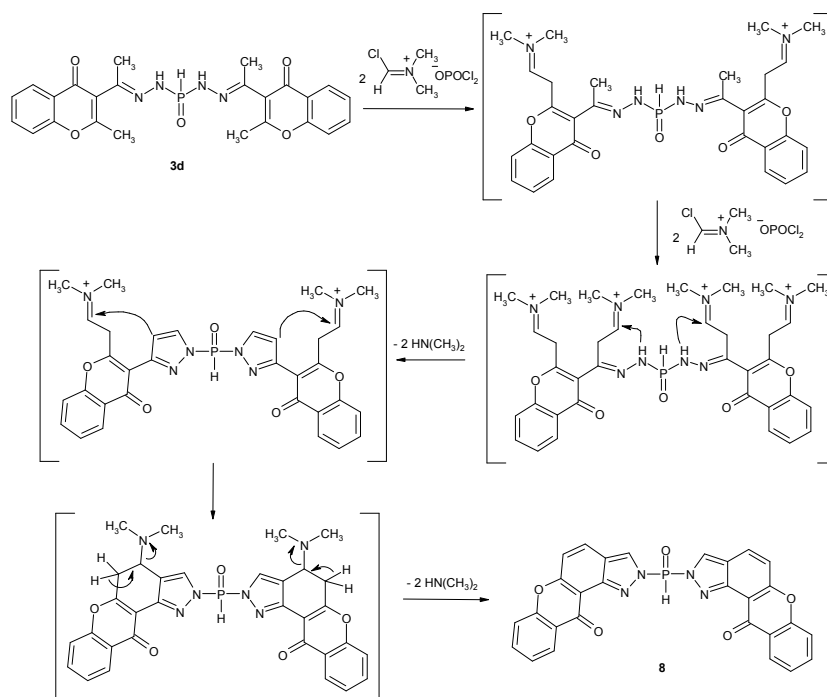


Scheme 5



Scheme 6

Consequently, the effect of Vilsmeier reagent on the phosphonic dihydrazone **3d** afforded *bis*{chromeno[2,3-*g*]indazol-11-oxo-2-yl}phosphine oxide (**8**) in moderate yield (Scheme 8). This transformation involved monoformylation at each methyl group of **3d**, followed by two steps of cyclization process. The first step is nucleophilic attack of NH groups at $-\text{CH}=\text{N}^+(\text{CH}_3)_2$ to eliminate two molecules of dimethylamine. The second step is nucleophilic attack of the C-4 of formed pyrazole rings at $-\text{CH}=\text{N}^+(\text{CH}_3)_2$ of chromone moieties to cyclize into benzoid system (Scheme 7) [35]. The IR spectrum of product **8** showed three characteristic absorption bands at 1646, 1596 and 1569 cm^{-1} assignable to $\text{C}=\text{O}$, $\text{C}=\text{N}$ and $\text{C}=\text{C}$ functional groups, respectively. Moreover, its ^1H NMR spectrum revealed a singlet signal of $\text{C}_5\text{-H}$ of pyrazole rings at δ 7.89 ppm while the aromatic protons at δ 7.05–7.80 ppm as multiplet signals. The mass spectrum of **8** showed a molecular ion peak at m/e 518 with a base peak at m/e 384.

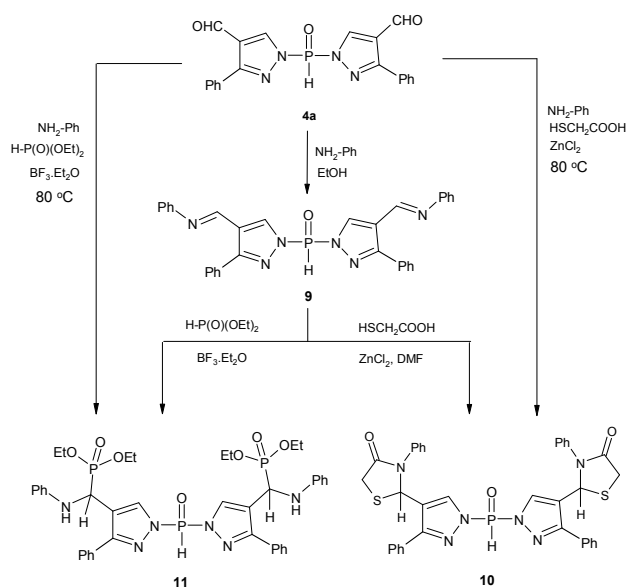


Scheme 7

Sulfur and phosphorus containing heterocyclic compounds play an important role in organic chemistry and attract strong interest due to diversity of their chemical transformations and broad spectrum of biological activity [36,37]. In this research, *bis*(4-formylpyrazolyl)phosphine oxides **4a,b** turned out to be fairly reactive compounds, and they readily condensed with nitrogen and carbon nucleophiles to give the corresponding condensation products. The reactivity of these condensation products towards sulfur and phosphorus reagents was investigated. Thus, treatment of compound **4a** with aniline, in refluxing ethanol, afforded yellow crystals of *bis*{3-phenyl-4-[(phenylimino)methyl]-1*H*-pyrazol-1-yl}phosphine oxide (**9**) (Scheme 8). The structure of *bis*-Schiff's base **9** was confirmed by both spectral and elemental analysis (See Experimental Section).

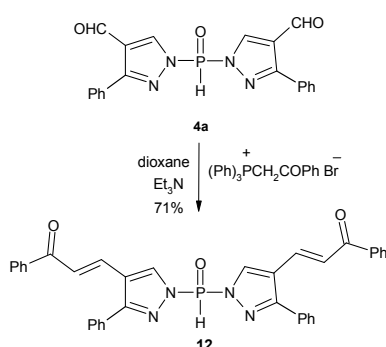
When *bis*-Schiff's base **9** was treated with thioglycolic acid, in refluxing dry DMF containing anhydrous zinc chloride as a catalyst, afforded a single product identified as *bis*{3-phenyl-4-[3-phenyl-4-oxo-1,3-thiazolidin-2-yl]-1*H*-pyrazol-1-yl}phosphine oxide (**10**). The latter compound was also obtained authentically in one-pot (four components) from the direct reaction of **4a** with aniline and thioglycolic acid in the presence of anhydrous zinc chloride (Scheme 8). The IR spectrum of **10** showed the carbonyl groups of thiazolidinone moieties at 1658 cm⁻¹. Also, its ¹H NMR spectrum exhibited two singlet signals at δ 5.91 and 4.46 ppm due to C₂-H and CH₂ of thiazolidinone protons, respectively, while the proton of H-P=O appeared as a doublet signal at δ 7.28 ppm (*J*_{PH} = 454 Hz).

Also, when *bis*-Schiff's base **9** was treated with diethyl phosphite in the presence of BF₃.Et₂O as a catalyst at 80 °C under Pudovik reaction condition [38], produced a novel type of *bis*(α-aminophosphonate) structure **11**, which was also obtained authentically in high yield in one-pot (four components) from the direct reaction of **4a** with aniline and diethyl phosphite in the presence of BF₃.Et₂O at 80 °C under Kabachnik-Fields reaction conditions [39] (Scheme 8). The absorption bands of NH and two P=O groups in IR spectrum appeared at 3300, 1287 and 1233 cm⁻¹, respectively. Also, the ¹H NMR spectrum of **11** exhibited triplet and quartet at δ 1.07 and 3.96 (*J* = 6.8 Hz) corresponding to ethoxy protons. Because of coupling with phosphorus atom, the proton of CH-P exhibited a doublet signal at δ 4.91 ppm (*J*_{PCH} = 18.4 Hz). Moreover, its ¹³C NMR spectrum displayed the ethoxy carbon atoms at δ 16.0 and 62.7 ppm, while the carbon atom of CH-P at δ 46.1 ppm (*J*_{PC} = 161 Hz). The presence of only one signal observed for CH-P and OCH₂CH₃ groups indicated that only one of the two possible diastereomers (*meso* and *racemic* forms) is formed stereospecifically. These observations were further confirmed by analysis of the ³¹P NMR spectrum of **11**, in which only one sharp phosphorus signal is observed at δ 21.56 ppm for α-aminophosphonate groups indicating that only one diastereomer contributes to the structure [40].



Scheme 8

Recently, chalcones have been reported to exhibit a wide variety of pharmacological effects [41,42]. Thus, when *bis*(4-formylpyrazolyl)phosphine oxide **4a** was allowed to react with phenacyltriphenylphosphonium bromide in dry dioxane containing drops of triethylamine as basic catalyst under Wittig reaction conditions [43], afforded a product identified as *bis*{3-phenyl-4-(1-oxo-phenylprop-2-en-3-yl)-1H-pyrazol-1-yl}phosphine oxide (**12**) (Scheme 9). The IR spectrum of **12** showed one characteristic absorption band at 1687 cm^{-1} assignable to carbonyl groups. Also, its ^1H NMR spectrum exhibited two doublet signals at δ 8.09 and 7.78 ppm ($J = 13.8\text{ Hz}$) due to olefinic protons H_α and H_β , respectively, while the carbon atoms C_α and C_β appeared at δ 138 and 118.7 ppm in its ^{13}C NMR spectrum [44]. Moreover, the mass spectrum of **12** recorded a highest ion peak at m/e 414 after losing diphenyl moieties and carbon monoxide with a base peak at m/e 57.

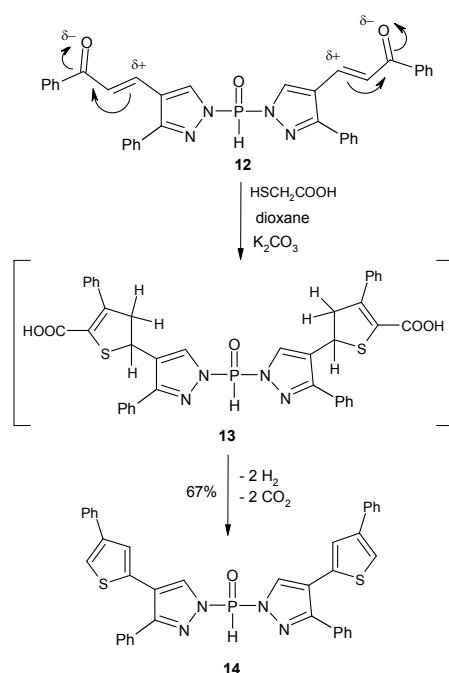


Scheme 9

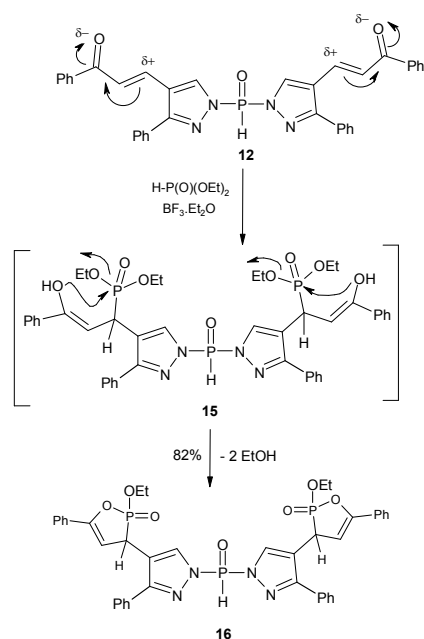
Bis-chalcone **12** is seemed to be a logical starting material for synthesis of sulfur and phosphorus heterocycles *via* its reaction with sulfur and phosphorus nucleophiles. Thus, reaction of *bis*-chalcone **12** with thioglycolic acid in dry dioxane containing anhydrous potassium carbonate furnished exclusively and reasonable good yield a product that could be formulated as *bis*{3-phenyl-4-(4-phenyl-2-thienyl)-1H-pyrazol-1-yl}phosphine oxide (**14**)

(Scheme 10). A plausible mechanism involved an initial Michael type addition of the thiol group of thioglycolic acid to the activated double bond in compound **12**, followed by cyclocondensation between active methylene and carbonyl group to give the nonisolable intermediate **13**. Decarboxylation and auto-oxidation of the intermediate **13** produced the final product **14** (Scheme 11). The chemical structure of **14** was elucidated on the basis of spectral techniques. Its IR spectrum did not record any carbonyl or hydroxyl groups which confirmed the decarboxylation process. Also, its ^1H NMR spectrum displayed resonated signals at δ 6.60 and 7.08 ppm due to the two protons $\text{C}_3\text{-H}$ and $\text{C}_5\text{-H}$ of thiophene moieties, respectively, in addition to a doublet signal at δ 7.64 ppm ($J_{\text{PH}}=594$ Hz) correspond to proton of H-P=O unit.

Moreover, it has been found that the one-pot reaction of *bis*-chalcone **12** with diethyl phosphite in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ at 80°C for 8 hours, afforded *bis*{[2-ethoxy-2-oxo-5-phenyl-2,3-dihydro-1,2-oxaphosphol-3-yl]-3-phenyl-1*H*-pyrazol-1-yl}phosphine oxide (**16**) (Scheme 11). The proposed mechanism involved an initial Michael type addition of phosphorus atom of diethyl phosphite to the activated double bond in compound **12** to give the nonisolable intermediate **15**, which underwent cyclization by elimination of ethanol molecules to give **16** (Scheme 11) [45]. The ^1H NMR spectrum of **16** displayed two doublet signals at δ 6.70 ppm ($J=28$ Hz) and 7.15 ppm ($J=5$ Hz) assignable to protons of CH-P and $\text{C}_4\text{-H}$, respectively, of oxaphosphole rings, in addition to the presence of the ethoxy protons as triplet and quartet at δ 1.34 and 4.35 ppm ($J=7.2$ Hz), respectively. Also, its IR spectrum confirmed the disappearance of carbonyl group of compound **12** which support the cyclized state.

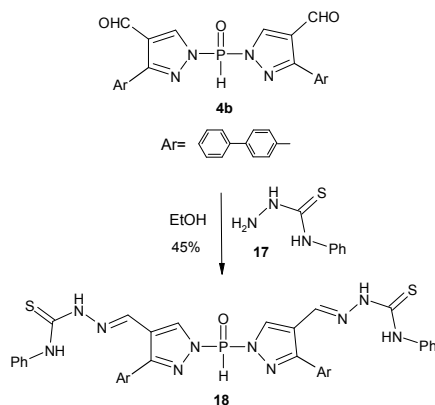


Scheme 10



Scheme 11

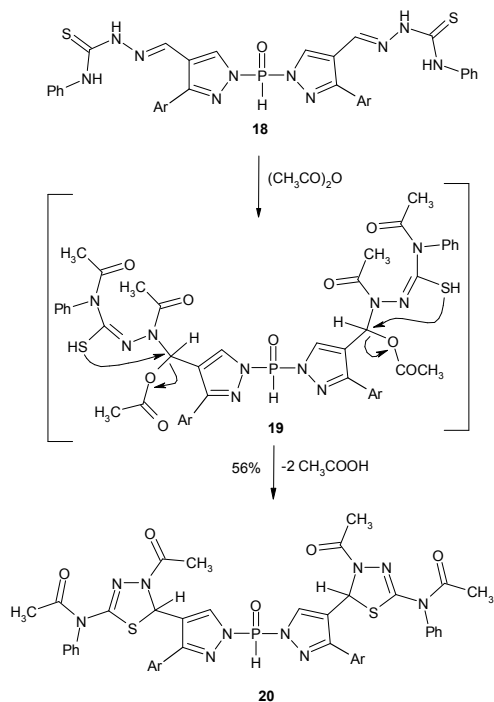
Reaction of *bis*(4-formylpyrazolyl)phosphine oxide **4b** with 4-phenylthiosemicarbazide (**17**) in refluxing ethanol produced the corresponding *bis*-thiosemicarbazone **18** in moderate yield (Scheme 12). The structure of **18** was established on the basis of its elemental analysis, IR, ^1H NMR and mass spectral data (See Experimental Section).



Scheme 12

It is known that thiosemicarbazones could be used as a precursor for the synthesis of a variety of bioactive sulfur-nitrogen heterocyclic systems [46,47]. Thus, refluxing of *bis*-thiosemicarbazone **18** in acetic anhydride for 4 hours produced the corresponding *bis*(thiadiazolylpyrazolyl)phosphine oxide **20** in 56 % yield (Scheme 13). The formation of **20** may be occurred *via* acetylation of NHPH moieties and the azomethine nitrogen atoms which favor the development of a positive charge on the carbon atoms of these groups to give the intermediate **19**, which underwent ring closure by attack of sulfur atom to eliminate two molecules of acetic acid to give the final product **20** (Scheme 13) [48]. The structure of **20**

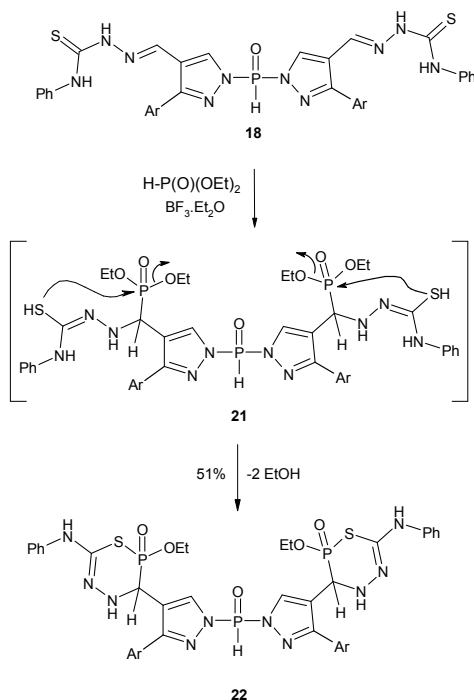
was established on the basis of spectral data. Its IR spectrum confirmed the presence of C=O of acetyl groups at 1688 and 1667 cm^{-1} , while its ^1H NMR spectrum revealed two singlet signals at δ 1.81 and 1.88 ppm assigned to protons of methyl groups, in addition to singlet signal at δ 6.54 ppm due to C₅-H of thiazazole moieties. The ^{13}C NMR spectrum of **20** was also good support for the proposed structure which exhibited characteristic signals at δ 21.0, 23.1, 60.4 and 153 ppm corresponding to carbon atoms of two methyl groups, C-5 and C-2 of thiazazole moieties, respectively. Also, the carbon atoms of carbonyl groups appeared at δ 168 and 170.1 ppm.



Scheme 13

Finally, heterocyclization of *bis*-thiosemicarbazone **18** with diethyl phosphite at 80 °C in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ for 10 hours, afforded an interesting type of phosphorus heterocycle, namely *bis*{3-(4'-biphenyl)-4-[2-ethoxy-6-phenylamino-2-oxo-3,4-dihydro-2*H*-1,4,5,2-thiadiazaphosphinin-3-yl]-1*H*-pyrazol-1-yl}phosphine oxide (**22**) (Scheme 14). The proposed mechanism for formation of **22** may occur *via* addition of phosphorus atom of diethyl phosphite to $\text{CH}=\text{N}_{\text{exocyclic}}$ groups to give the nonisolable intermediate **21**, which underwent cyclization by nucleophilic attack of SH groups at phosphonate groups to eliminate two molecules of ethanol (Scheme 14). The IR spectrum of **22** displayed characteristic absorption bands for NH and P=O groups at 3404–3276 and 1248–1228 cm^{-1} , respectively. Also, its ^1H NMR spectrum clearly indicated the presence of one ethyl ester group at each 1,4,5,2-thiadiazaphosphinine moiety as a triplet and quartet signals at δ 1.16 and 3.96 ppm ($J = 8.2$ Hz), respectively, in addition to one doublet and two singlet signals at δ 4.90 ($J_{\text{PH}} = 20.4$ Hz), 9.83 and 11.69 ppm assigned to CH–P and each two NH protons,

respectively. Moreover, its ^{31}P NMR spectrum exhibited two signals at δ 6.62 and 28.08 ppm corresponding to $\text{H}-\text{P}=\text{O}$ and $\text{EtO}-\text{P}=\text{O}$ groups, respectively [15].



Scheme 14

In vitro antimicrobial activity

All the synthesized compounds were evaluated *in vitro* for their antibacterial activity against *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 19615) as examples of Gram positive bacteria and *Pseudomonas fluorescens* (S 97) and *Pseudomonas phaseolicola* (GSPB 2828) as examples of Gram negative bacteria. They were also evaluated *in vitro* for their antifungal activity against *Fusarium oxysporum* and *Aspergillus fumigatus* fungal strains. Agar-diffusion technique was used for the determination of the preliminary antibacterial and antifungal activity [49]. The test was performed on medium potato dextrose agars (PDA) which contain infusion of 200 g potatoes, 6 g dextrose and 15 g agar [50]. Uniform size filter paper disks (3 disks per compound) were impregnated by equal volume (10 μl) from the concentrations of 1 and 2 mg/cm^3 dissolved compounds in dimethylformamide (DMF) and carefully placed on inoculated agar surface. After incubation for 36 h at 27 $^\circ\text{C}$ in the case of bacteria and for 48 h at 24 $^\circ\text{C}$ in the case of fungi. Cephalothin, Chloramphenicol and Cycloheximide were used as reference drugs at 30 $\mu\text{g}/\text{cm}^3$ for Gram positive bacteria, Gram negative bacteria and fungi, respectively. The results were recorded for each tested compound as average diameter of inhibition zones of bacterial or fungal around the disks in mm at the concentrations 1 and 2 mg/cm^3 (Tables 1-3).

- 1) The investigation of antibacterial and antifungal screening data in Tables 1-3 revealed that most of the synthesized compounds were found to possess various antimicrobial activities towards all the microorganisms.
- 2) In general, most of the synthesized compounds exhibited antibacterial activity better than antifungal activity.
- 3) Most of the synthesized compounds showed inhibitions against Gram-positive bacteria more than Gram-negative bacteria except **12** which showed also high activity against *P. phaseolicola*.
- 4) Most of the synthesized compounds showed low activity at 1 mg/cm³ and moderate inhibition at 2 mg/cm³.
- 5) Phosphonic dihydrazones **3a-c** did not exhibit any effects against all microbial strains. Only compound **3b** showed a lower inhibition against Gram-negative bacteria at 2 mg/cm³.
- 6) The products **4a,b**, **6** and **8** revealed better activities against bacterial strains in comparison with the corresponding phosphonic dihydrazones **3a-d**. This activity may be attributed to the presence of the formed bioactive pyrazole rings by Vilsmeier-Haack reaction.
- 7) Compounds **9**, **12** and **18** did not show noticeable activity in comparison with their corresponding *bis*(4-formylpyrazolyl) phosphine oxides **4a** and **4b** (Figures 1-3).
- 8) Compounds **10**, **14** and **20** showed slightly activity more than their corresponding starting material **9**, **12** and **18**, respectively, as result of sulfur atoms effects to their structures which may cause enhanced activity (Figures 1-3).
- 9) Compounds **11**, **16** and **22** showed comparatively good activity more than their corresponding starting material **9**, **12** and **18**, respectively, which may due to addition phosphorus atoms to their structures which may cause enhanced activity (Figures 1-3).
- 10) Compounds **11**, **16** and **22** (including extra phosphorus atom) showed good inhibitions against all bacterial and fungal strains, while compounds **10**, **14** and **20** (including sulfur atom) showed a degree of inhibition against only bacterial strains (Tables 1 & 2). This may due to a combination between the extra phosphorus atoms with compounds **9**, **12** and **18** leading to a biocidal effects activity more that sulfur atom moieties.
- 11) Compounds **11**, **16** and **22** exhibited good inhibitions against all microbial strains which may due to presence of acyclic or cyclic α -aminophosphonate and ethyl phosphonate moieties, previously noted for their impact on biological systems [17] (Figure 4).
- 12) In conclusion, compounds **11**, **16** and **22** are nearly as active as reference drugs against some microbial strains. However none of the test compounds show superior activity than the reference drugs.

Table 1. Antibacterial activity (Gram positive bacteria) of the synthesized compounds **3–22** at 1 and 2 mg/cm³ by disc diffusion assay.

Compd. No.	Diameter of zone of inhibition in mm			
	Gram-positive bacteria			
	<i>S. aureus</i> (ATCC 25923)		<i>S. pyogenes</i> (ATCC 19615)	
	2 mg/cm ³	1 mg/cm ³	2 mg/cm ³	1 mg/cm ³
3a	–	–	–	–
3b	–	–	–	–
3c	–	–	–	–
3d	–	–	–	–
4a	10	8	7	–
4b	10	8	8	5
6	10	8	7	6
8	10	7	8	5
9	8	5	5	–
10	14	8	18	14
11	21	12	17	14
12	8	6	7	–
14	10	7	11	9
16	24	17	19	13
18	11	7	8	5
20	16	11	20	15
22	30	24	26	20
Cephalothin	28		30	

Less active: 6–12 mm; moderately active: 13–19 mm; highly active: 20–30 mm; –: No inhibition or inhibition less than 5 mm.

Table 2: Antibacterial activity (Gram negative bacteria) of the synthesized compounds **3–22** at 1 and 2 mg/cm³ by disc diffusion assay.

Compd. No.	Diameter of zone of inhibition in mm			
	Gram-negative bacteria			
	<i>P. phaseolicola</i> (GSPB 2828)		<i>P. fluorescens</i> (S 97)	
	2 mg/cm ³	1 mg/cm ³	2 mg/cm ³	1 mg/cm ³
3a	–	–	–	–
3b	6	–	7	–
3c	–	–	–	–
3d	–	–	–	–
4a	10	8	8	6
4b	–	–	6	–
6	5	–	7	–
8	9	5	9	7
9	5	–	6	–
10	6	–	8	–
11	7	6	10	7
12	10	7	8	6
14	13	10	9	6
16	12	10	12	9
18	6	–	7	–
20	27	22	20	12
22	20	14	24	18
Chloramphenicol	25		30	

Less active: 6–12 mm; moderately active: 13–19 mm; highly active: 20–30 mm; –: No inhibition or inhibition less than 5 mm.

Table 3 Antifungal activity of the synthesized compounds **3–22** at 1 and 2 mg/cm³ by disc diffusion assay.

Compd. No.	Diameter of zone of inhibition in mm			
	Fungi			
	<i>F. oxysporum</i>		<i>A. fumigatus</i>	
	2 mg/cm ³	1 mg/cm ³	2 mg/cm ³	1 mg/cm ³
3a	–	–	–	–
3b	–	–	–	–
3c	–	–	–	–
3d	–	–	–	–
4a	–	–	–	6
4b	–	–	–	–
6	–	–	–	–
8	–	–	–	7
9	–	–	–	–
10	–	–	–	–
11	17	10	14	7
12	–	–	–	6
14	8	–	–	6
16	11	9	7	9
18	–	–	–	–
20	–	–	–	12
22	35	29	33	18
Cycloheximide	28		31	

Less active: 6–12 mm; moderately active: 13–19 mm; highly active: 20–30 mm; –: No inhibition or inhibition less than 5 mm.

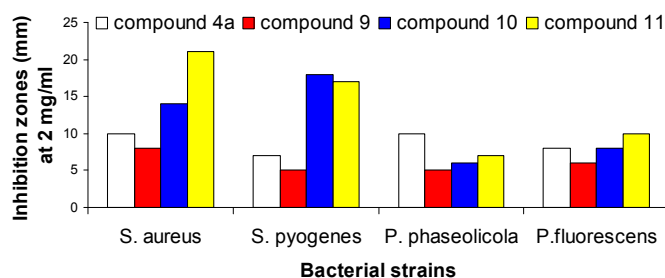


Figure 1. Relationship between inhibition zones at 2 mg/ml and bacterial strains for compounds **10** and **11** which refers to an increase in activity *via* addition sulfur and phosphorus atoms to starting materials **4a** and **9**.

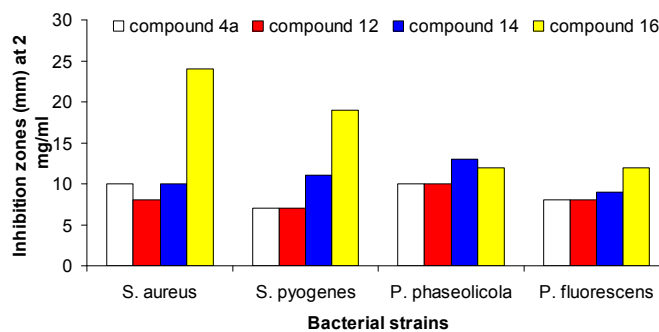


Figure 2. Relationship between inhibition zones at 2 mg/ml and bacterial strains for compounds **14** and **16** which refers to an increase in activity *via* addition sulfur and phosphorus atoms to starting materials **4a** and **12**.

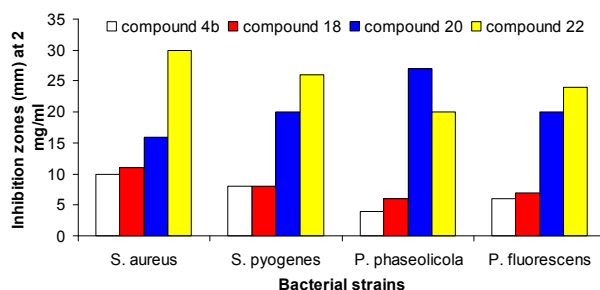


Figure 3. Relationship between inhibition zones at 2 mg/ml and bacterial strains for compounds **20** and **22** which refers to a clear increase in activity *via* addition sulfur and phosphorus atoms to starting materials **4b** and **18**.

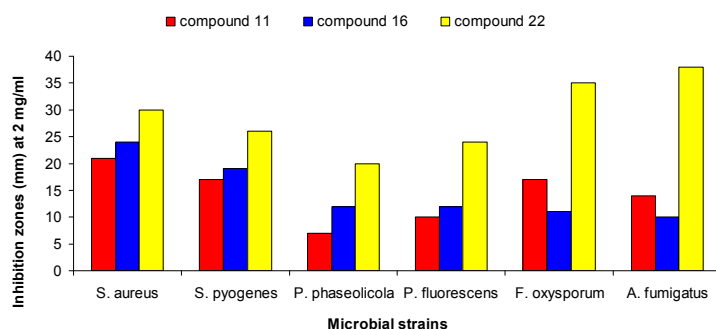


Figure 4. Relationship between inhibition zones at 2 mg/ml and microbial strains for compounds **11**, **16** and **22** (including extra phosphorus atoms in shape acyclic α -aminophosphonate, ethyl phosphonate and cyclic α -aminophosphonate moieties, respectively) which showed good inhibitions against all microbial strains. Compound **22** is the highest one because it contains cyclic α -aminophosphonate moiety in addition to sulfur element which revealed that the presence of phosphorus and sulfur in one compound enhance very clear increase in activity.

Experimental

The melting point was determined in an open capillary tube on a digital Stuart SMP-3 apparatus. Infrared spectra were measured on Perkin-Elmer 293 spectrophotometer (cm^{-1}), using KBr disks. ^1H NMR spectra were measured on Gemini-200 spectrometer (200 MHz), using $\text{DMSO-}d_6$ as a solvent and TMS (δ) as the internal standard. ^{13}C NMR spectra were measured on Mercury-300BB (75 MHz), using $\text{DMSO-}d_6$ as a solvent and TMS (δ) as the internal standard. ^{31}P NMR spectra were registered on a Varian Inova 500 MHz spectrometer at room temperature using $\text{DMSO-}d_6$ as a solvent and TMS as internal standard and 85% H_3PO_4 as external reference. Mass spectra recorded on a Gas Chromatographic GCMSqp 1000 ex Shimadzu instrument at 70 eV. Elemental microanalyses were performed at microanalysis center in National Research Center, Giza. The purity of the synthesized compounds was checked by thin layer chromatography (TLC).

Preparation of phosphonic dihydrazide (1)

A mixture of hydrazine hydrate 99% (0.1 mol, 5 cm³) and diethyl phosphite (0.05 mol, 7 cm³) was heated under reflux at 70–80 °C for two hours. The reaction mixture was cooled in an ice bath for 30 minutes. The formed solid was filtered off and washed with absolute ethanol (10 cm³). The filtrate was concentrated to its third volume under reduced pressure and cooled in an ice bath for one hour. The white solid was filtered off and dried. Total yield 80%; m.p 98–99 °C (Lit. 92–94) [23].

General procedure for preparation of phosphonic dihydrazones 3a-d

A mixture of phosphonic dihydrazide (1) (0.01 mol, 1.1 g) and acetophenone derivatives namely, acetophenone (2a), 4'-acetyl-biphenyl (2b), 2-hydroxyacetophenone (2c) and/or 3-acetyl-2-methylchromone (2d) (0.02 mol) in absolute ethanol (20 cm³) in the presence of few drops of concentrated sulfuric acid, was refluxed for 4 h. The reaction mixture was cooled; the resulting precipitate was filtered off and crystallized from the proper solvent to give the corresponding phosphonic dihydrazones 3a–d, respectively.

***N*¹,*N*⁵-bis{1-phenylethylidene}phosphonic dihydrazide (3a):** Yellow crystals from ethanol in 46% yield; m.p 128–130 °C. IR (KBr), (ν_{\max} , cm⁻¹): 3423 (br, NH), 3053 (C–H_{arom}), 2960 (C–H_{aliph}), 2339 (P–H), 1599 (C=N), 1562 (C=C), 1284 (P=O). ¹H NMR (DMSO, δ ppm): 2.28 (s, 6H, CH₃), 7.47–7.93 (m, 10H, Ph–H). ¹³C NMR (DMSO, δ ppm): 14.7 (CH₃), 126.4 (C_{3,5}), 128.3 (C_{2,6}), 129.6 (C₄), 137.8 (C₁), 157.2 (C=N). MS (*m/e*, %): 237 (M⁺–Ph, 5%), 222 (4.5), 221 (35), 159 (12), 118 (15), 103 (28), **77** (100), 51 (92). Anal. Calcd for C₁₆H₁₉N₄OP (314.33): C, 61.14; H, 6.09; N, 17.82. Found: C, 60.67; H, 5.82; N, 17.53%.

***N*¹,*N*⁵-bis{1-(4'-biphenyl)ethylidene}phosphonic dihydrazide (3b):** Pale yellow crystals from dilute dimethylsulfoxide in 71% yield; m.p 282–284 °C. IR (KBr), (ν_{\max} , cm⁻¹): 3425 (br, NH), 3054, 3032 (C–H_{arom}), 2964 (C–H_{aliph}), 2373 (P–H), 1599 (C=N), 1577 (C=C), 1294 (P=O). ¹H NMR (DMSO, δ ppm): 2.12 (s, 6H, CH₃), 7.38–8.06 (m, 18H, Ar–H). MS (*m/e*, %): 389 (M⁺–Ph, 6%), 388 (31), 373 (57), 209 (12), 194 (25), 178 (31), 153 (29), **152** (100), 76 (32), 51 (19). Anal. Calcd for C₂₈H₂₇N₄OP (466.53): C, 72.09; H, 5.83; N, 12.01. Found: C, 71.72; H, 5.59; N, 11.79%.

***N*¹,*N*⁵-bis{1-(2-hydroxyphenyl)ethylidene}phosphonic dihydrazide (3c):** Yellow crystals from dimethylformamide in 70% yield; m.p 202–204 °C. IR (KBr), (ν_{\max} , cm⁻¹): 3150 (br, OH, NH), 3050 (C–H_{arom}), 2924 (C–H_{aliph}), 2441 (br, P–H), 1604 (C=N), 1559 (C=C), 1245 (P=O). ¹H NMR (DMSO, δ ppm): 2.08 (s, 6H, CH₃), 6.97 (d, 4H, *J*=8.2 Hz, Ar–H), 7.40 (t, 2H, *J*=7.4 Hz, Ar–H), 7.76 (d, 2H, *J*=7.6 Hz, Ar–H), 12.90 (s, 2H, OH exchangeable with D₂O). ³¹P NMR (DMSO, δ ppm): 18.4. MS (*m/e*, %): 348 (M+2, 12%), 302 (9), 301 (12), 119 (14), 80 (44), 77 (14), **64** (100), 52 (42). Anal. Calcd for C₁₆H₁₉N₄O₃P (346.33): C, 55.49; H, 5.53; N, 16.18. Found: C, 55.14; H, 5.24; N, 15.68 %.

***N*¹,*N*⁵-bis{1-(2-methyl-4-oxo-4H-chromen-3-yl)ethylidene}phosphonic dihydrazide (3d):** Pale yellow crystals from dimethylformamide in 69% yield; m.p 266–268 °C. IR (KBr), (ν_{\max} ,

cm⁻¹): 3241 (NH), 3023 (C–H_{arom}), 2920 (C–H_{aliph}), 2599 (P–H), 1665 (C=O), 1609 (C=N), 1528 (C=C), 1256 (P=O), 1011 (C–O–C). ¹H NMR (DMSO, δ ppm): 2.07 (s, 6H, CH₃), 2.34 (s, 6H, CH₃), 6.88–7.74 (m, 8H, Ar–H). MS (*m/e*, %): 515 (M+2H₂O, 18%), 286 (30), 200 (30), 99 (30), 77 (70), **55** (100), 51 (26). Anal. Calcd for C₂₄H₂₃N₄O₅P (478.45): C, 60.25; H, 4.85; N, 11.71. Found: C, 59.81; H, 4.51; N, 11.29%.

Synthesis of compounds 4a,b, 6 and 8: General procedure for Vilsmeier-Haack reaction of phosphonic dihydrazones 3a-d

The Vilsmeier reagent was prepared by adding dimethylformamide (0.05 mol, 3.88 cm³) in an ice-cold condition (0–5 °C) under constant stirring. To this, phosphorus oxychloride (0.025 mol, 2.34 cm³) was added dropwise over a period of half hour and the resulting mixture was stirred for a further half hour. Each one of phosphonic dihydrazones **3a-d** (0.005 mol) was added to the Vilsmeier reagent and stirred for 5 hours at 50–60 °C. The reaction mixture was cooled and poured into crushed ice and 2 g of sodium acetate was added under constant manual stirring. The reaction mixture was kept aside overnight. The resulting precipitate was filtered off, washed with water several times and crystallized from the proper solvent to give the corresponding products **4a,b, 6** and **8**, respectively.

Bis{4-formyl-3-phenyl-1H-pyrazol-1-yl}phosphine oxide (4a): Beige crystals from ethanol in 61% yield; m.p 193–195 °C. IR (KBr), (ν_{max}, cm⁻¹): 3014 (C–H_{arom}), 2771 (P–H), 1678 (C=O), 1620 (C=N), 1535 (C=C), 1284 (P=O). ¹H NMR (DMSO, δ ppm): 7.45 (d, 1H, J_{PH}=682 Hz, P–H), 7.45–7.93 (m, 10H, Ph–H), 8.69 (s, 2H, C₅–H_{pyrazole}), 9.95 (s, 2H, CHO). ¹³C NMR (DMSO, δ ppm): 121.1 (C₄ pyrazole), 127.5–138.2 (Phenyl carbons), 144.5 (C₅ pyrazole), 152.1 (C₃ pyrazole), 184.6 (CHO). MS (*m/e*, %): 389 (M⁺–H, 15%), 388 (M⁺–2H, 30), 286 (18), 274 (22), 184 (18), 144 (52), 103 (81), 77 (85), **51** (100). Anal. Calcd for C₂₀H₁₅N₄O₃P (390.34): C, 61.54; H, 3.87; N, 14.35. Found: C, 61.27; H, 3.67; N, 13.96 %.

Bis{3-(4'-biphenyl)-4-formyl-1H-pyrazol-1-yl}phosphine oxide (4b): Orange crystals from ethanol in 62% yield; m.p 149–150 °C. IR (KBr), (ν_{max}, cm⁻¹): 3054, 3029 (C–H_{arom}), 2363 (P–H), 1671 (C=O), 1643 (C=N), 1601 (C=C), 1241 (P=O). ¹H NMR (DMSO, δ ppm): 7.54 (d, 1H, J_{PH}=733 Hz, P–H), 7.47–8.14 (m, 18H, Ar–H), 8.84 (s, 2H, C₅–H_{pyrazole}), 10.00 (s, 2H, CHO). ³¹P NMR (DMSO, δ ppm): 7.71. MS (*m/e*, %): 390 (M⁺–C₁₂H₈, 32%), 351 (21), 223 (33), 152 (6), 139 (51), **99** (100), 77 (35), 60 (89), 55 (68). Anal. Calcd for C₃₂H₂₃N₄O₃P (542.54): C, 70.84; H, 4.27; N, 10.33. Found: C, 70.53; H, 3.86; N, 9.90%.

Bis{2,4-dihydrochromeno[4,3-c]pyrazol-4-hydroxy-2-yl}phosphine oxide (6): Red crystals from ethanol in 82% yield; m.p 279–282 °C. IR (KBr), (ν_{max}, cm⁻¹): 3135 (br, OH), 3070 (C–H_{arom}), 2930 (C–H_{aliph}), 2500 (P–H), 1656 (C=N), 1615 (C=C), 1246 (P=O), 1104 (C–O–C). ¹H NMR (DMSO, δ ppm): 3.72 (br, 2H, OH exchangeable with D₂O), 6.53 (s, 2H, C₂–H_{pyran}), 6.95–7.78 (m, 8H, Ar–H), 7.44 (d, 1H, J_{PH}=423 Hz, P–H), 7.96 (s, 2H, C₅–H_{pyrazole}). Anal. Calcd for C₂₀H₁₅N₄O₅P (422.34): C, 56.88; H, 3.58; N, 13.27. Found: C, 56.56; H, 3.29; N, 12.89%.

Bis{chromeno[2,3-g]indazol-11-oxo-2-yl}phosphine oxide (8): Yellow crystals from dilute dimethylformamide in 39% yield; m.p 296–297 °C. IR (KBr), (ν_{\max} , cm^{-1}): 3050 (C–H_{arom}), 2642 (P–H), 1646 (C=O), 1596 (C=N), 1569 (C=C), 1246 (P=O), 1041 (C–O–C). ¹H NMR (DMSO, δ ppm): 6.50 (d, 1H, $J_{\text{PH}}=556$ Hz, P–H), 7.05–7.80 (m, 12H, Ar–H), 7.89 (s, 2H, C₅–H_{pyrazole}). MS (m/e , %): 518 (M⁺, 2%), 458 (3), **384** (100), 369 (83), 284 (2), 192 (74), 165 (6), 120 (10), 117 (2), 92 (7), 77 (30), 69 (35), 53 (19). Anal. Calcd for C₂₈H₁₅N₄O₅P (518.43): C, 64.87; H, 2.92; N, 10.81. Found: C, 64.63; H, 2.71; N, 10.52%.

Synthesis of bis(4-carboxypyrazolyl)phosphine oxides 5a,b: General procedure for oxidation of bis-(4-formylpyrazolyl) phosphine oxides 4a,b

A solution of potassium permanganate (0.002 mol, 0.32 g) in water (10 cm³) was added with stirring to a suspension of bis-(4-formylpyrazolyl)phosphine oxides **4a,b** (0.001 mol) in pyridine (10 cm³) at room temperature. The reaction mixture was stirred for 3 h, a solution of NaOH (1%, 10 cm³) was added, and stirring continued for 2 h at 50°C. After cooling, the inorganic precipitate was filtered off and washed with water. The filtrate was acidified with hydrochloric acid to pH 4. The formed precipitate was filtered off, washed with water, dried, and crystallized from dilute ethanol.

Bis{4-carboxy-3-phenyl-1H-pyrazol-1-yl}phosphine oxide (5a): Grey crystals from dilute ethanol in 80% yield; m.p >300 °C. IR (KBr), (ν_{\max} , cm^{-1}): 3243 (OH), 2362 (P–H), 1713 (C=O), 1550 (C=N), 1237 (P=O). ¹H NMR (DMSO, δ ppm): 7.64 (d, 1H, $J_{\text{PH}}=517$ Hz, P–H), 7.60–8.17 (m, 10H, Ph–H), 8.92 (s, 2H, C₅–H_{pyrazole}), 12.14 (s, 2H, COOH). Anal. Calcd for C₂₀H₁₅N₄O₅P (422.34): C, 56.88; H, 3.58; N, 13.27. Found: C, 56.61; H, 3.43; N, 12.97%.

Bis{3-(4'-biphenyl)-4-carboxy-1H-pyrazol-1-yl}phosphine oxide (5b): Beige crystals from ethanol in 77% yield; m.p >300 °C. IR (KBr), (ν_{\max} , cm^{-1}): 3243 (br, OH), 2360 (P–H), 1711 (C=O), 1239 (P=O). ¹H NMR (DMSO, δ ppm): 7.32 (d, 1H, $J_{\text{PH}}=468$ Hz, P–H), 6.67–7.82 (m, 18H, Ar–H), 8.13 (s, 2H, C₅–H_{pyrazole}), 11.80 (s, 2H, COOH). Anal. Calcd for C₃₂H₂₃N₄O₅P (574.54): C, 66.90; H, 4.04; N, 9.75. Found: C, 66.61; H, 3.87; N, 9.52%.

Bis{chromeno[4,3-c]pyrazol-4-oxo-2-yl}phosphine oxide (7)

A mixture of **6** (0.001 mol, 0.422 g) and selenium dioxide (0.002 mol, 0.22 g) in dry dioxane (15 cm³) was refluxed for 5 h. The reaction mixture was filtered off while hot. Some water (20 cm³) was added to the filtrate, and then left for complete precipitation. The resulting precipitate was filtered off and crystallized from dilute dimethylformamide to give deep red crystals in 75% yield; m.p >300 °C. IR (KBr), (ν_{\max} , cm^{-1}): 3054 (C–H_{arom}), 2360 (P–H), 1652 (C=O), 1624 (C=N), 1592 (C=C), 1277 (P=O), 1080 (C–O–C). ¹H NMR (DMSO, δ ppm): 7.21 (d, 1H, $J_{\text{PH}}=425$ Hz, P–H), 7.32 (t, 2H, $J=8.8$ Hz, Ar–H), 7.46 (t, 2H, $J=8.0$ Hz, Ar–H), 7.58 (d, 2H, $J=8.2$ Hz, Ar–H), 7.94 (d, 2H, $J=7.8$ Hz, Ar–H), 8.64 (s, 2H, C₅–H_{pyrazole}). Anal. Calcd for C₂₀H₁₁N₄O₅P (418.31): C, 57.43; H, 2.65; N, 13.39. Found: C, 57.09; H, 2.43; N, 13.04%.

Bis{3-phenyl-4-[(phenylimino)methyl]-1H-pyrazol-1-yl}phosphine oxide (9)

A mixture of *bis*-(4-formylpyrazolyl)phosphine oxide **4a** (0.001 mol, 0.39 g) and freshly distilled aniline (0.002 mol, 0.186 g) in absolute ethanol (10 cm³) was refluxed for 4 h. The solvent was concentrated to half its volume and left for two days for complete precipitation. The formed solid was filtered off and crystallized from dilute ethanol to give yellow crystals in 66% yield; m.p 148–150 °C. IR (KBr), (ν_{\max} , cm⁻¹): 3050 (C–H_{arom}), 2498 (P–H), 1631 (C=N_{exocyclic}), 1540 (C=C), 1230 (P=O). ¹H NMR (DMSO, δ ppm): 7.69 (d, 1H, $J_{\text{PH}} = 608$ Hz, P–H), 7.11–8.10 (m, 20H, Ph–H), 8.56 (s, 2H, C₅–H_{pyrazole}), 9.36 (s, 2H, CH=N_{exocyclic}). MS (m/e , %): 520 (M⁺–H₂O, –H₂, 1%), 423 (1), 260 (42), 246 (24), 130 (12), **77** (100), 51 (48). Anal. Calcd for C₃₂H₂₅N₄OP (540.57): C, 71.10; H, 4.66; N, 15.55. Found: C, 70.83; H, 4.34; N, 15.23%.

Bis{3-phenyl-4-[3-phenyl-4-oxo-1,3-thiazolidin-2-yl]-1H-pyrazol-1-yl}phosphine oxide (10)

Method A: a mixture of *bis*-Schiff's base **9** (0.001 mol, 0.54 g) and thioglycolic acid (0.0025 mol, 0.23 g) in dry dimethylformamide (20 cm³) in the presence of anhydrous zinc chloride (1 g), was refluxed for 8 h. The mixture was cooled, and poured into crushed ice. The resulting precipitate was filtered off and crystallized from dry benzene to give beige crystals in 45% yield; m.p 278–280 °C (dec).

Method B: a mixture of *bis*-(4-formylpyrazolyl)phosphine oxide **4a** (0.001 mol, 0.39 g), freshly distilled aniline (0.002 mol, 0.186 g), thioglycolic acid (0.0025 mol, 0.23 g) and anhydrous zinc chloride (1 g), was heated on water bath for 4 h. The mixture was cooled, and poured into crushed ice. The resulting precipitate was filtered off and crystallized from dry benzene to give beige crystals in 66% yield; m.p 277–279 °C (dec). IR (KBr), (ν_{\max} , cm⁻¹): 3059 (C–H_{arom}), 2877 (C–H_{aliph}), 2500 (br, P–H), 1658 (C=O), 1595 (C=N), 1308 (P=O). ¹H NMR (DMSO, δ ppm): 4.46 (s, 4H, CH₂), 5.91 (s, 2H, C₂–H_{thiazolidine}), 7.28 (d, 1H, $J_{\text{PH}} = 454$ Hz, P–H), 7.07–8.23 (m, 20H, Ph–H), 8.64, 8.53 (ss, 2H, C₅–H_{pyrazole}). Anal. Calcd for C₃₆H₂₉N₆O₃PS₂ (688.77): C, 62.78; H, 4.24; N, 12.20; S, 9.31. Found: C, 62.38; H, 3.94; N, 11.84; S, 9.03%.

Bis{diethyl[phenylamino(3-phenyl-1H-pyrazol-4-yl)methyl]phosphonate}phosphine oxide (11)

Method A: a mixture of *bis*-Schiff's base **9** (0.001 mol, 0.54 g) and diethyl phosphite (5 ml) in the presence of boron trifluoride etherate (0.2 cm³), was heated on water bath for 4 h. The excess of diethyl phosphite was removed under reduced pressure. Some water (5 cm³) was added to the residue to give yellow precipitate which was filtered off and crystallized from dilute ethanol to give pale yellow crystals in 68% yield; m.p 99–100 °C.

Method B: a mixture of *bis*-(4-formylpyrazolyl)phosphine oxide **4a** (0.001 mol, 0.39 g), freshly distilled aniline (0.002 mol, 0.186 g), diethyl phosphite (5 cm³), and boron trifluoride etherate (0.2 cm³), was heated on water bath for 4 h. The excess of diethyl phosphite was removed under reduced pressure. Some water (5 cm³) was added to the residue

to give yellow precipitate which was filtered off and crystallized from dilute ethanol to give pale yellow crystals in 72% yield; m.p 100–101 °C. IR (KBr), (ν_{\max} , cm^{-1}): 3300 (NH), 3057 (C-H_{arom}), 2983, 2906 ($\text{C-H}_{\text{aliph}}$), 2583 (P–H), 1599 (C=N), 1536 (C=C), 1287, 1233 (2 P=O), 1049 (P–O–C). ^1H NMR (DMSO, δ ppm): 1.07 (t, 12H, $J=6.8$ Hz, $\text{CH}_3\text{CH}_2\text{O}$), 3.96 (q, 8H, $J=6.8$ Hz, $\text{CH}_3\text{CH}_2\text{O}$), 4.91 (d, 2H, $J_{\text{PCH}}=18.4$ Hz, CH-P), 6.60–7.97 (m, 20H, Ph–H), 7.40 (d, 1H, $J_{\text{PH}}=498$ Hz, P–H), 8.25 (s, 2H, NH exchangeable with D_2O), 8.65 (s, 2H, $\text{C}_5\text{-H}_{\text{pyrazole}}$). ^{13}C NMR (DMSO, δ ppm): 16.0 ($J=6.9$ Hz, $\text{CH}_3\text{CH}_2\text{O}$), 46.1 (d, $J_{\text{PC}}=161$ Hz, CH-P), 62.7 ($J=6.9$ Hz, $\text{CH}_3\text{CH}_2\text{O}$), 118.0 ($\text{C}_4_{\text{pyrazole}}$), 126.5–139.0 (Phenyl carbons), 146.7 ($\text{C}_5_{\text{pyrazole}}$), 151.2 ($\text{C}_3_{\text{pyrazole}}$). ^{31}P NMR (DMSO, δ ppm): 8.2 (O=P–H), 21.5 (EtO–P=O). Anal. Calcd for $\text{C}_{40}\text{H}_{47}\text{N}_6\text{O}_7\text{P}_3$ (816.78): C, 58.82; H, 5.80; N, 10.29. Found: C, 58.61; H, 5.42; N, 9.79%.

Bis{3-phenyl-4-(1-oxo-1-phenylprop-2-en-3-yl)-1H-pyrazol-1-yl}phosphine oxide (12)

A mixture of *bis*-(4-formylpyrazolyl)phosphine oxide **4a** (0.001 mol, 0.39 g) and phenacyltriphenylphosphonium bromide (0.002 mol, 0.92 g) in dry dioxane (20 cm^3) in the presence of few drops of triethylamine was refluxed for 6 h. The reaction mixture was cooled; the resulting precipitate was filtered off and crystallized from dilute dimethylformamide to give pale yellow crystals in 71% yield; m.p 128–130 °C. IR (KBr), (ν_{\max} , cm^{-1}): 3052 (C-H_{arom}), 2500 (br, P–H), 1687 (C=O), 1599 (CH=CH), 1562 (C=N), 1529 (C=C), 1215 (P=O). ^1H NMR (DMSO, δ ppm): 7.41–7.73 (m, 16H, Ph–H), 7.78 (d, 2H, $J=13.8$ Hz, CH=CH-C=O), 7.83–7.96 (m, 4H, Ph–H), 8.09 (d, 2H, $J=13.8$ Hz, CH=CH-C=O), 8.36 (d, 1H, $J_{\text{PH}}=638$ Hz, P–H), 8.62 (s, 2H, $\text{C}_5\text{-H}_{\text{pyrazole}}$). ^{13}C NMR (DMSO, δ ppm): 118.7 (CH=CH-C=O), 121.6 ($\text{C}_4_{\text{pyrazole}}$), 127.9–137.0 (phenyl carbons), 138.0 (CH=CH-C=O), 139.0 ($\text{C}_5_{\text{pyrazole}}$), 153.0 ($\text{C}_3_{\text{pyrazole}}$), 188.0 (C=O). MS (m/e , %): 414 (M^+-2Ph , –CO, 0.1%), 363 (0.3), 293 (14), 231 (3), 168 (14), 149 (86), 94 (35), 85 (71), 77 (2), 71 (55), **57** (100). Anal. Calcd for $\text{C}_{36}\text{H}_{27}\text{N}_4\text{O}_3\text{P}$ (594.59): C, 72.72; H, 4.58; N, 9.42. Found: C, 72.35; H, 4.12; N, 9.53%.

Bis{3-phenyl-4-(4-phenyl-2-thienyl)-1H-pyrazol-1-yl}phosphine oxide (14)

A mixture of *bis*-chalcone **12** (0.001 mol, 0.59 g) and thioglycolic acid (0.0025 mol, 0.23 g) in dry dioxane (20 cm^3) in the presence of anhydrous potassium carbonate (1 g), was refluxed for 8 h. The reaction mixture was cooled; the resulting precipitate was filtered off, washed with water several times and crystallized from ethanol to give pale yellow crystals in 67% yield; m.p 228–230 °C. IR (KBr), (ν_{\max} , cm^{-1}): 3057 (C-H_{arom}), 2363 (br, P–H), 1614 ($\text{C=C}_{\text{thiophene}}$), 1597 (C=N), 1533 (C=C), 1209 (P=O). ^1H NMR (DMSO, δ ppm): 6.60 (t, 2H, $J=9.2$ Hz, $\text{C}_3\text{-H}_{\text{thiophene}}$), 7.08 (d, 2H, $J=8.6$ Hz, $\text{C}_5\text{-H}_{\text{thiophene}}$), 7.64 (d, 1H, $J_{\text{PH}}=594$ Hz, P–H), 8.53, 8.68 (ss, 2H, $\text{C}_5\text{-H}_{\text{pyrazole}}$), 7.75 (d, 2H, $J=6.2$ Hz, Ph–H), 8.25 (d, 1H, $J=8.2$ Hz, Ph–H), 8.02 (d, 2H, $J=7.8$ Hz, Ph–H), 7.36–7.49 (m, 14H, Ph–H), 7.53 (d, 1H, $J=6.6$ Hz, Ph–H). Anal. Calcd for $\text{C}_{38}\text{H}_{27}\text{N}_4\text{O}_2\text{PS}_2$ (650.75): C, 70.14; H, 4.18; N, 8.61; S, 9.85. Found: C, 70.32; H, 3.93; N, 8.42; S, 9.48 %.

Bis{4-[(2-ethoxy-2-oxido-5-phenyl-2,3-dihydro-1,2-oxaphosphol-3-yl)-3-phenyl-1H-pyrazol-1-yl]phosphine oxide (16)}

A mixture of *bis*-chalcone **12** (0.001 mol, 0.59 g) and diethyl phosphite (5 cm³) in the presence of boron trifluoride etherate (0.2 cm³), was heated on water bath for 8 h. The excess of diethyl phosphite was removed under reduced pressure. The formed precipitate was filtered off and crystallized from ethanol to give yellow crystals in 82% yield; m.p 189–191 °C. IR (KBr), (ν_{\max} , cm⁻¹): 3059 (C–H_{arom}), 2916, 2895 (C–H_{aliph}), 2613 (br, P–H), 1592 (C=N), 1531 (C=C), 1286, 1211 (P=O), 1064, 1015 (P–O–C). ¹H NMR (DMSO, δ ppm): 1.34 (t, 6H, $J=7.2$ Hz, CH₃CH₂O), 4.35 (q, 4H, $J=7.2$ Hz, CH₃CH₂O), 6.70 (d, 2H, $J=28$ Hz, CH–P), 7.15 (d, 2H, $J=5$ Hz, C₄–H_{oxaphosphole}), 7.73 (d, 1H, $J_{\text{PH}}=612$ Hz, P–H), 7.43–8.33 (m, 20H, Ph–H), 8.60 (s, 2H, C₅–H_{pyrazole}). ¹³C NMR (DMSO, δ ppm): 13.9 (CH₃CH₂O), 54.5 (d, $J=168$ Hz, C₃ oxaphosphole), 62.0 (CH₃CH₂O), 119.6 (C₄ oxaphosphole), 120.6 (C₄ pyrazole), 126.6–139.1 (phenyl carbons), 145.4 (C₅ pyrazole), 154.6 (C₃ pyrazole), 161.8 (C₅ oxaphosphole). MS (m/e , %): 778.66 (M⁺, not detected), 431 (0.1), 311 (2), 245 (3), 149 (72), 97 (9), 71 (19), **57** (100). Anal. Calcd for C₄₀H₃₇N₄O₇P₃ (778.66): C, 61.70; H, 4.79; N, 7.20. Found: C, 61.29; H, 4.44; N, 6.79%.

Bis{3-(4'-biphenyl)-1H-pyrazole-4-carboxaldehyde N⁴-phenylthiosemicarbazone} phosphine oxide (18)}

A mixture of *bis*(4-formylpyrazolyl)phosphine oxide **4b** (0.001 mol, 0.54 g) and 4-phenylthiosemicarbazide (**17**) (0.002 mol, 0.33 g) in absolute ethanol (10 cm³) was refluxed for 4 h. The product was precipitated on heating, filtered off and crystallized from dimethylformamide to give orange yellow crystals in 45% yield; m.p 214–216°C. IR (KBr), (ν_{\max} , cm⁻¹): 3303, 3127 (2NH), 3055, 3029 (C–H_{arom}), 2359 (br, P–H), 1621 (C=N_{exocyclic}), 1596 (C=N_{endocyclic}), 1539 (C=C), 1260 (P=O), 1193 (C=S). ¹H NMR (DMSO, δ ppm): 7.02 (d, 1H, $J_{\text{PH}}=532$ Hz, P–H), 6.97–7.79 (m, 28H, Ar–H), 8.40 (s, 2H, C₅–H_{pyrazole}), 8.80 (s, 2H, CH=N), 9.85 (s, 2H, NH exchangeable with D₂O), 11.71 (s, 2H, NH exchangeable with D₂O). MS (m/e , %): 840 (M⁺, not detected), 761 (M⁺–Ph, –2H, 5%), 701 (5), 570 (5), 413 (5), 259 (5), 153 (10), 135 (36), 93 (97), 77 (76), 66 (56), **51** (100). Anal. Calcd for C₄₆H₃₇N₁₀OPS₂ (840.98): C, 65.70; H, 4.43; N, 16.66; S, 7.63. Found: C, 65.32; H, 4.21; N, 16.31; S, 7.30%.

Bis{3-(4'-biphenyl)-4-[(4-acetyl-2-(N-phenylacetamido)-4,5-dihydro-1,3,4-thiadiazol-5-yl]-1H-pyrazol-1-yl}phosphine oxide (20)}

A solution of *bis*-thiosemicarbazone **18** (0.001 mol, 0.84 g) in acetic anhydride (10 cm³) was heated under reflux for 4 h. The excess of solvent was removed under reduced pressure and the residue was poured on ice and stirred for 10 minutes. The separated solid was filtered off and crystallized from ethanol to give deep green crystals in 56% yield; m.p 124–126 °C. IR (KBr), (ν_{\max} , cm⁻¹): 3056, 3028 (C–H_{arom}), 2930 (C–H_{aliph}), 2361 (P–H), 1688 (C=O), 1667 (C=O), 1597 (C=N), 1540 (C=C), 1297 (P=O). ¹H NMR (DMSO, δ ppm): 1.81 (s, 6H, CH₃), 1.88 (s, 6H, CH₃), 6.30 (d, 1H, $J_{\text{PH}}=327$ Hz, P–H), 6.54 (brs, 2H, C₅–H_{thiadiazole}), 7.35–7.90

(m, 28H, Ar-**H**), 8.18 (s, 2H, C₅-**H**_{pyrazole}). ¹³C NMR (DMSO, δ ppm): 21.0 (CH₃), 23.1 (CH₃), 60.4 (C₅ thiadiazole), 121.3 (C₄ pyrazole), 126.4–140.8 (aromatic carbons), 144.5 (C₅ pyrazole), 148.0 (C₃ pyrazole), 153.0 (C₂ thiadiazole), 168.0 (C=O), 170.1 (C=O). Anal. Calcd for C₅₄H₄₅N₁₀O₅PS₂ (1009.13): C, 64.27; H, 4.49; N, 13.88; S, 6.36. Found: C, 64.22; H, 4.31; N, 13.28; S, 6.01 %.

Bis{3-(4'-biphenyl)-4-[2-ethoxy-6-phenylamino-2-oxido-3,4-dihydro-2H-1,4,5,2-thiadiazaphosphinin-3-yl]-1H-pyrazol-1-yl}phosphine oxide (22)

A mixture of *bis*-thiosemicarbazone **18** (0.001 mol, 0.84 g) and diethyl phosphite (8 cm³) in the presence of boron trifluoride etherate (0.2 cm³), was heated on water bath for 10 h. The excess of diethyl phosphite was removed under reduced pressure. The residue was treated with ethyl acetate to give solid which was filtered off and crystallized from ethyl acetate to give beige crystals in 51% yield; m.p 158–160 °C. IR (KBr), (ν_{max}, cm⁻¹): 3404, 3276 (br, 2NH), 3058 (C-H_{arom}), 2978, 2850 (C-H_{aliph}), 2493 (P-H), 1600 (C=N), 1543 (C=C), 1228, 1248 (2P=O), 1046 (P-O-C). ¹H NMR (DMSO, δ ppm): 1.16 (t, 6H, *J*=8.2 Hz, CH₃CH₂O), 3.96 (q, 4H, *J*=8.2 Hz, CH₃CH₂O), 4.90 (d, 2H, *J*=20.4 Hz, C₃-**H**_{thiadiazaphosphinine}), 6.59–7.79 (m, 28H, Ar-**H**), 7.08 (d, 1H, *J*_{PH}=532.4 Hz, P-**H**), 8.65 (s, 2H, C₅-**H**_{pyrazole}), 11.69 (s, 2H, **NH** exchangeable with D₂O), 9.83 (s, 2H, **NH** exchangeable with D₂O). ³¹P NMR (DMSO, δ ppm): 6.6 (O=P-H), 28.0 (EtO-P=O). Anal. Calcd for C₅₀H₄₇N₁₀O₅P₃S₂ (1025.05): C, 58.59; H, 4.62; N, 13.66; S, 6.26. Found: C, 58.32; H, 4.34; N, 13.44; S, 5.94%.

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