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Synthesis of novel pyrazole derivatives using organophosphorus, stibine, and arsine reagents and their antitumor activities

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Abstract: The reactions of 5-azido-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (azidopyrazole) with several classes of organophosphorus reagents: phosphonium ylides, Wittig-Horner reagents, dialkylphosphonates, trialkylphosphites, tris(dialkylamino)phosphanes, triphenylstibane, triphenylarsane, and Lawesson's reagent are reported. Structural reasoning for the new products was based on compatible analytical and spectral data. The cytotoxic activity of most of the new products was evaluated against human breast carcinoma cell line (MCF7) and human hepatocellular carcinoma cell line(HepG2). Certain tested compounds showed promising results.

Keywords: antitumor; azidopyrazole; crystal structure; organophosphorus; stibine.

1 Introduction

The easily prepared pyrazole ring is an interesting template for combinatorial [1, 2] and medicinal chemistry [3, 4] as well as a prominent structural motif found in numerous pharmaceutically active compounds [5]. Moreover, the pyrazole derivatives are known to have pharmacological, antimicrobial, antifungal [6], and antitumor [7] activities. In view of this and in continuation of our work in organophosphorus chemistry [8–12], it was of considerable interest to investigate the behavior of azidopyrazole **1** toward phosphonium ylides **2a–c**, Wittig-Horner reagents **3a**,**b**, diethylphosphonate **4**, trialkylphosphites **5a**,**b**, tris(dialkylamino)phosphanes **5c**,**d**, triphenylstibane **6a**, triphenylarsane **6b**, and Lawesson's reagent (LR) **7** (Fig. 1).

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2 Results and discussion

2.1 Chemistry

When azidopyrazole **1** was treated with two molar equivalents of (cyanomethylene)triphenylphosphorane (**2a**) in dry THF at ambient temperature for 1 h, the triphenyl phosphoranylidenesuccinonitrile **8a** was obtained (85% yield). Triphenylphosphane oxide (TPPO) was also isolated and identified (Scheme 1). Structure **8a** is confirmed by a correct elemental analysis, IR, ^{1}H, ^{13}C, ^{31}P NMR, and mass spectra. Its IR spectrum revealed the presence of a strong band at 2182 cm^{-1}, which is ascribed to the N_3 absorption [13]. A possible explanation for the course of the formation of compound **8a** is shown in Scheme 1. Azidopyrazole **1** reacted with P-ylide **2a** to give the intermediate **A** with expulsion of TPPO. Moreover, intermediate **A** reacted with another molecule of the ylide **2a** yielding intermediate **B**, which cyclized to intermediate **C**. Rearrangement of the latter gave the new ylide **8a** (Scheme 1). Furthermore, when azidopyrazole **1** was allowed to react with (acetylmethylene)triphenylphosphorane (**2b**) in dry THF at room temperature, product **8b** was separated as colorless crystals with 75% yield (Scheme 1). Elemental and mass spectral analysis of **8b** led to an empirical formula $C_{14}H_{13}N_5O$. The IR spectrum displays absorption bands at ν = 1682 (C=O) and 1593 (cyclic C=N) cm^{-1} but lacks the azido function group around at 2180 cm^{-1} [13]. The ^{1}H NMR spectrum of **8b** gave signals at δ = 1.96 (s, 3H, CH_3), at 2.64 ppm (s) for a methyl group attached to the triazole ring, at 7.34–7.54 ppm (m, 6H, H_{arom}, =CH), and a singlet at 9.66 ppm for the aldehydic proton. The ^{13}C NMR spectrum of **8b** added a good support for the proposed structure and revealed signals at 8.3 and

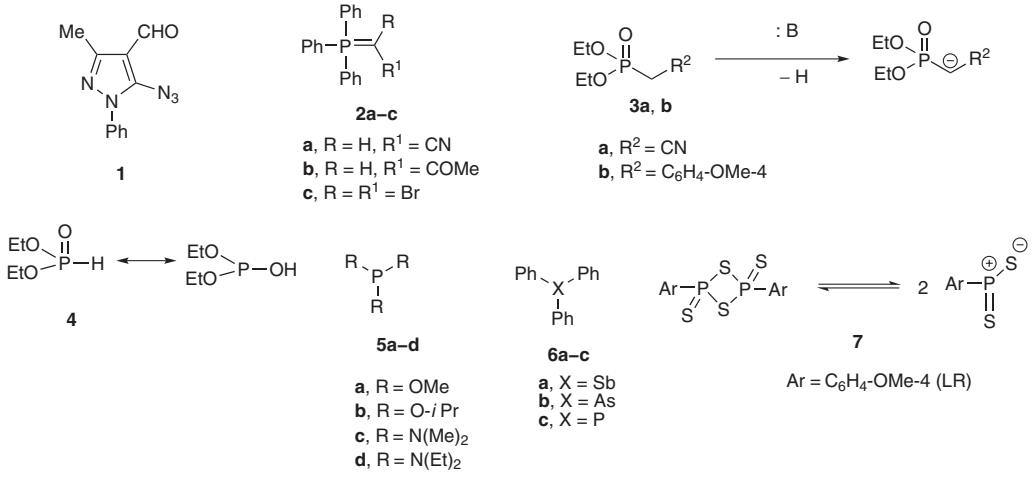
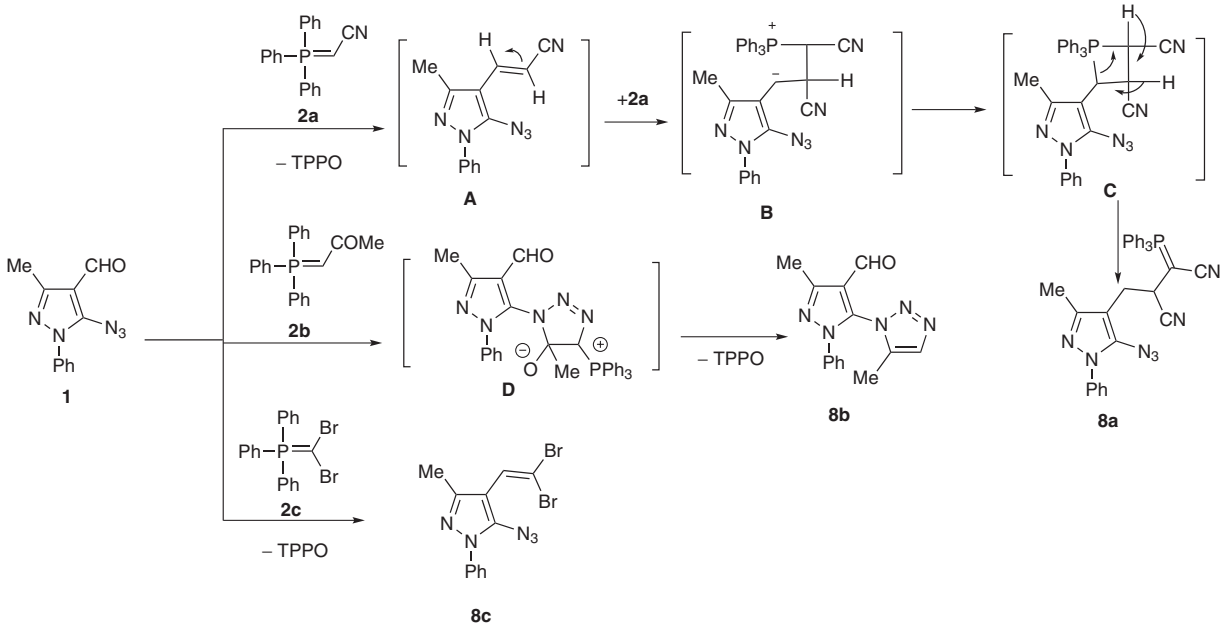


Fig. 1: Azidopyrazole **1**, organophosphorus reagents **2–6** and LR **7**.

The purpose of this study was to determine the preferential site of attack by these reagents and synthesize new P-containing pyrazole derivatives with anticipated biological activities.



Scheme 1: The behavior of azidopyrazole **1** toward stabilized phosphonium ylides **2a–c**.

13.7 ppm for the two methyl groups, 136.3 and 136.6 ppm (2C, triazole), 123.2–133.2 ppm (aromatic CH), 117.4, 136.7, and 151.6 ppm (quaternary carbon atoms C-4, C-3, C-5 of pyrazole), and 183.4 ppm for a carbonyl group. The reaction presumably proceeds *via* 1,3-dipolar cycloaddition of the azide group of azidopyrazole **1** to the C=C bond of the ylide **2b** followed by loss of TPPO from the cyclic intermediate **D** to give 1,2,3-triazole derivative **8b** [14–16] (Scheme 1). The molecular structure of **8b** was also confirmed by X-ray crystallographic analysis. An ORTEP diagram of compound **8b** is shown in Fig. 2. The crystal data and experimental parameters used for intensity data collection and the final results of the structure refinement are presented in Table 2 (cf. Experimental Section).

Similarly, the Wittig reaction of (dibromomethylene) triphenylphosphorane (**2c**) with azidopyrazole **1** afforded 4-(2,2-dibromovinyl)pyrazole **8c** (Scheme 1). The IR spectrum of **8c** revealed the presence of absorption bands at ν = 2125 (N_3) [13] and 626 (C–Br) [17] cm^{-1}. The structure of **8c** was also deduced from its NMR (^1H, ^{13}C) and mass spectra together with correct microanalyses (cf. Experimental Section).

Next, when azidopyrazole **1** was allowed to react with two molar equivalents of diethyl(cyanomethyl)

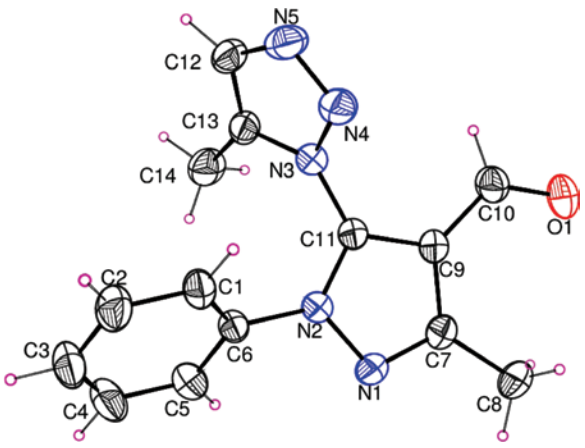
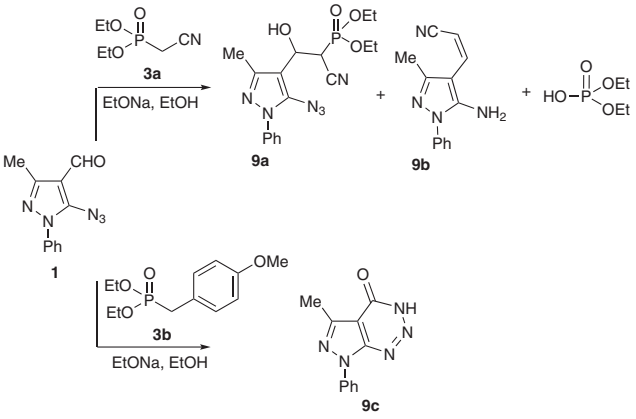


Fig. 2: ORTEP view of **8b** with crystallographic atom numbering. Displacement parameters are drawn at the 30% probability level, H as spheres with arbitrary radii. Selected bond lengths (Å) and angles (deg): N1–N2 1.372(3), N3–N4 1.374(4), N4–N5 1.294(4); N1–N2–C6 120.2(2), N1–N2–C11 111.1(2), N3–N4–N5 106.8(3).

phosphonate (**3a**) in an ethanolic sodium ethoxide solution at room temperature, products **9a** and **9b** were obtained (Scheme 2). The most important feature of structure **9a** is the presence of a signal at δ = 23.03 (s) ppm in its ^{31}P NMR spectrum and the presence of an IR band at 2114 cm^{-1}, which is ascribed to the N_3 absorption [13]. Moreover, the ^{1}H NMR spectrum of **9a** revealed a triplet at δ = 1.32 ppm, which indicates CH_3 protons of ethyl group with J_{HH} = 13.4 Hz, singlet at 2.63 ppm (3H, CH_3), doublet at 2.72 ppm with $^2J_{HP}$ = 22.89 Hz, doublet at 3.47 ppm with $^3J_{HP}$ = 12.5 Hz (C*H*OH), and two quartet at 4.22 ppm (4H, $^3J_{HP}$ = 12.5 Hz) for CH_2 protons of ethyl group.

A possible explanation for the course of the reaction of **1** with Wittig-Horner reagent **3a** is shown in Scheme 3. The initial attack of the anion derived from phosphonic ester **3a** on the most reactive center of **1** gave product **9a** *via* intermediate **E**. The assigned product **9b** presumably was formed *via* intermediate **F**. Under the influence of the base present in the reaction medium, elimination of N_2 was followed by hydrolysis **F** to give **9b** and dialkyl phosphite (Scheme 2). The dialkyl phosphite was detected in the water layer by the development of a violet color on addition of 3,5-dinitrobenzoic acid [18]. The assigned *cis* configuration for **9b** is supported by the chemical shift and coupling constant of the two protons CH=CHCN (J_{HH} = 7.6 Hz) (Scheme 2).



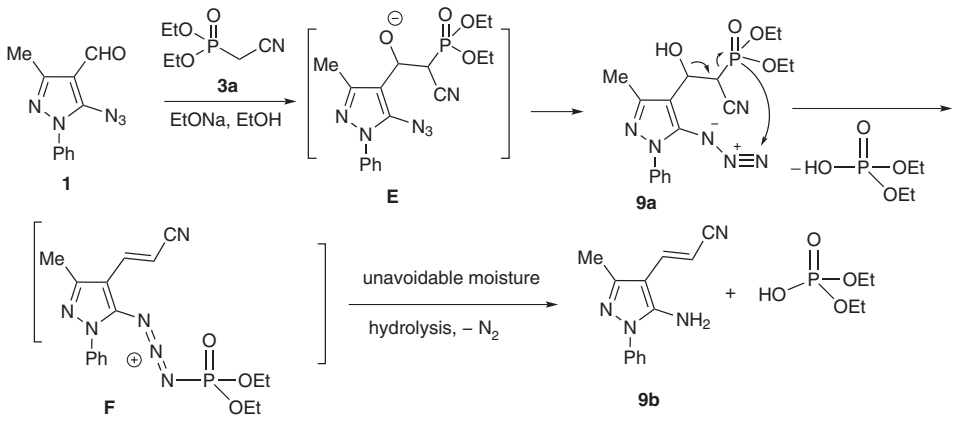
Scheme 2: The behavior of azidopyrazole **1** toward W-H reagents **3a,b**.

The reaction of azidopyrazole **1** with 4-methoxybenzylphosphonate **3b** was also investigated. We have found that the reaction of **1** with a molar equivalent of Wittig-Horner reagent **3b** in the presence of sodium ethoxide in ethanol afforded the pure **9c** (Scheme 2). The structural assignment for compound **9c** was based upon elemental analysis and spectroscopic data (cf. Experimental Section). Product **9c** can be obtained *via* intracyclization of azide **1.** It has been reported that *o*-azido-carbaldehyde compounds can be intracyclized with good yield under the influence of a basic medium [19, 20].

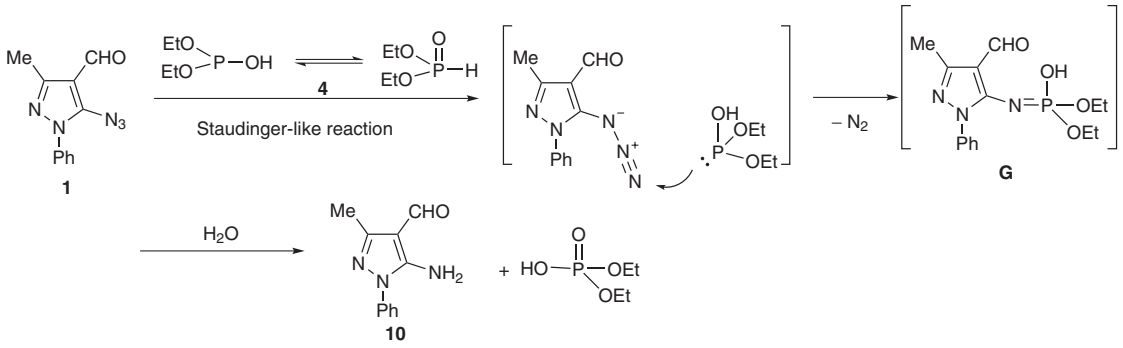
The reaction of azidopyrazole **1** with diethyl phosphonate (diethyl hydrogen phosphite) (**4**) in dry toluene at room temperature gave 5-amino-pyrazole-4-carbaldehyde **10** with 75% yield (Scheme 4). The assignment of structure **10** was based on correct elemental analysis and molecular weight determination (MS), ^{1}H NMR, and IR comparison with an authentic specimen [21].

A mechanism that accounts for the reaction of azidopyrazole **1** with diethyl phosphonate (**4**) is depicted in Scheme 4. The initial attack of the phosphorus lone pair at the azide group with expulsion of a nitrogen molecule gives intermediate **G,** which decomposes due to the unavoidable moisture to give the aminopyrazole **10** and diethylphosphite (Scheme 4) [21].

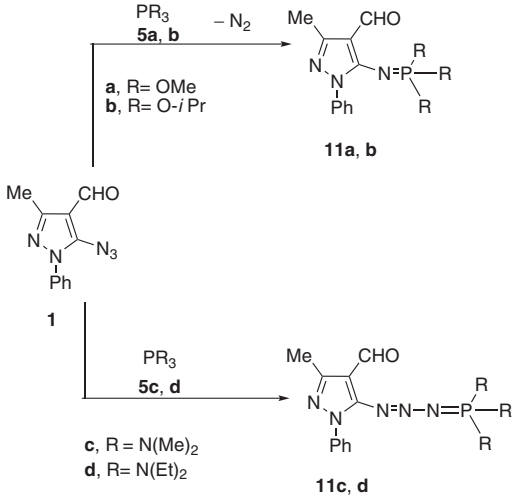
We have found that the reaction of trimethyl phosphite (**5a**) with azidopyrazole **1** in dry toluene proceeded at room temperature to give chromatographically pure adduct **11a** (Scheme 5), which was separated as brown crystals (75% yield). The results of elemental analysis and molecular weight determination (MS) of this product correspond to $C_{14}H_{18}N_3O_4P$. The ^{31}P NMR spectrum has one signal at δ = 23.2 ppm. The IR spectrum reveals the absence of an N_3 band [13] and the presence of both the (C=N) band at 1558 and C=O band at 1658 cm^{-1}. The structure of **11a** has also been assigned on the basis of ^{1}H and ^{13}C NMR spectra (cf. Experimental Section). Similarly, when **1** was reacted with **5b** in dry toluene at room temperature, the phosphorane imine product **11b** was obtained with 85% yield (Scheme 5). Structure elucidation of **11b** was derived from its spectral data (cf. Experimental Section).



Scheme 3: Formation mechanism of **9a,b**.



Scheme 4: The behavior of azidopyrazole **1** toward diethylphosphonate **4**.



Scheme 5: The behavior of azidopyrazole **1** toward trialkylphosphites **5a,b** and trisdialkylaminophosphines **5c,d**.

Meanwhile, performing the reaction of **1** with tris(dialkylamino)phosphanes **5c**,**d** led to the formation of the [tris(dialkylamino)phosphoranylidene]triazenes **11c**,**d** (Scheme 5). The structures of **11c**,**d** have also been assigned on the basis of elemental analysis, MS, IR, ^{1}H, ^{13}C, ^{31}P NMR spectral data (cf. Experimental Section). The structure of **11c** was also confirmed by X-ray crystallographic analysis. An ORTEP diagram of the molecular structure of compound **11c** in the crystal is shown in Fig. 3. Furthermore, this study has been extended to include the reaction of azidopyrazole **1** with triphenylstibane (**6a**) and triphenylarsane (**6b**) to establish whether they would behave in a similar manner as triphenylphosphane (**6c**) [22–25]. A different behavior is observed in the reaction of **1** with **6a** and **6b**, yielding 5-azido-4-(triphenylstiboranylidene)methyl-1*H*-pyrazole **12a** in 75% yield and 4-(triphenylarsoranylidene)methyl-1*H*-pyrazole **12b** in 45% yield with extrusion triphenylstibane oxide and triphenylarsane oxide, respectively (Scheme 6).

When the pyrazole-4-carbaldehyde **1** was stirred at ambient temperature with LR, the 5-sulfido-1,2,3,4,5-thiatriazaphosphole **13** was obtained in good yield through cycloaddition reaction between the monomeric form of LR **7** and the azido group of **1** [26, 27] (Scheme 7). The elemental microanalysis, ^{1}H, ^{13}C, ^{31}P NMR, and MS data agreed with structure **13**.

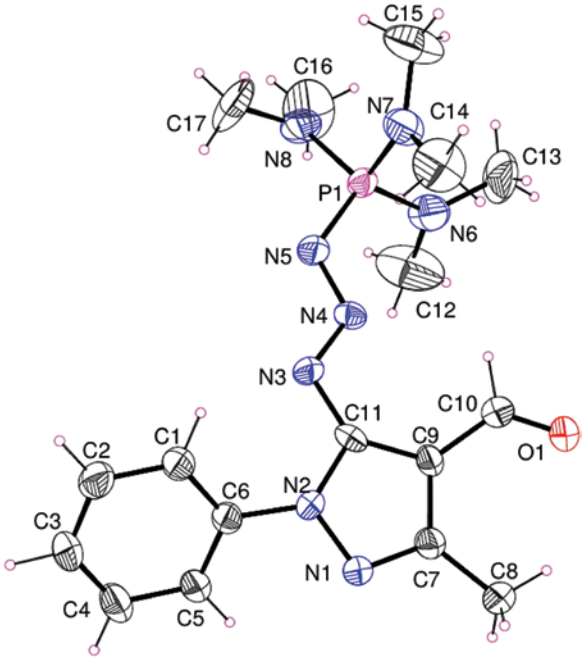


Fig. 3: ORTEP view of **11c** with crystallographic atom numbering. Displacement parameters are drawn at the 30% probability level, H atoms as spheres with arbitrary radii. Selected bond lengths (Å) and angles (deg): P1–N5 1.579(4), P1–N6 1.709(4), P1–N7 1.714(4), P1–N8 1.704(4); N5–P1–N6 113.2(4), N5–P1–N7 110.8(4), N6–P1–N7 110.3(4), N5–P1–N8 106.1(4), N6–P1–N8 109.9(4), N7–P1–N8 106.3(4).

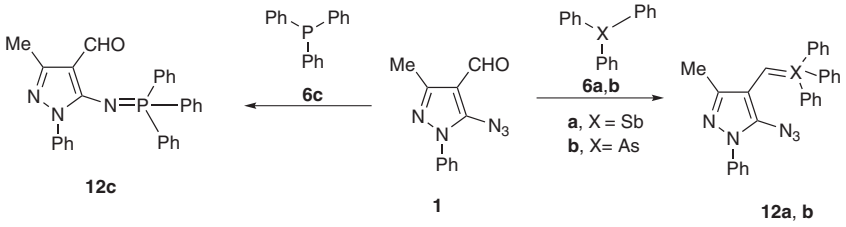
The IR spectrum of structure compound **13** revealed the absence of an N_3 absorption band [13] and showed bands at 625 cm^{-1} (P=S), 1585 cm^{-1} (C=N), 1696 cm^{-1} (C=O). The ^1H NMR shifts of compound **13** were at δ = 2.46 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), and 6.95–7.65 (m, 9H aromatic). The structure assigned to **13** was also based on a ^{13}C NMR spectrum that shows signals of 55.7 (OCH_3), 105.0 (quaternary C-4, pyrazole), 113.9–145.5 (aromatic C-H, C-3, and C-5 of pyrazole), and 161.6, 161.7 (C=O) ppm.

2.2 Description of the crystal and molecular structures of 8b and 11c

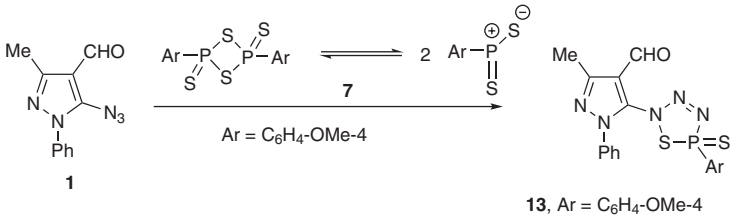
ORTEP plots of the molecular structures of compounds **8b** and **11c** are shown in Figs. 2 and 3, respectively. Both compounds crystallize in the monoclinic system possessing four molecules in the unit cell with space groups *Cc* for **8b** and *P2/n* for **11c**. The asymmetric unit in the two structures contains only one molecule. In **8b**, there is a significant twist between the pyrazole ring and both attached triazole and benzene rings with the dihedral angles being 49.9(2)° and 62.3(2)°, respectively. The same is observed in compound **11c**, with a significant twist between the pyrazole ring and the attached benzene ring with the dihedral angle being 38.4(2)°. An important feature to note in **8b** is that the molecules are linked into chains by one weak hydrogen bond of the C–H···O type and a set of H···π and O···π interactions as listed in Table 1 and illustrated in Fig. 4. Molecules **11c** are linked into chains by one hydrogen bond of the C–H···O type listed in Table 2 and illustrated in Fig. 5.

2.3 Anticancer screening

Cancer is one of the diseases with a high mortality rate [28]. The current trend of research focuses on investigating the



Scheme 6: The behavior of azidopyrazole **1** toward TPP, TPSb, TPAs **7a–c**.



Scheme 7: The behavior of azidopyrazole **1** toward LR **8**.

Table 1: Hydrogen bond geometry and non-bonding contacts (Å, deg) of compound **8b**.[a]

D–H···A	D–H	H···A	D···A	D–H···A
C14–H142···O1[i]	0.95	2.55	3.499(5)	175
C1–H11···Cg2	0.95	2.99	3.469(4)	113
Y–X···Cg	Y–X	X···Cg	Y···Cg	Y–X···Cg
C10–O1··· Cg1[i]	1.207(4)	3.195(3)	3.430(4)	90.7(2)

[a]Symmetry codes: (i) x, $1-y$, $-1/2+z$; Cg1 = N1–N2–C11–C9–C7; Cg2 = N3–N4–N5–C12–C13.

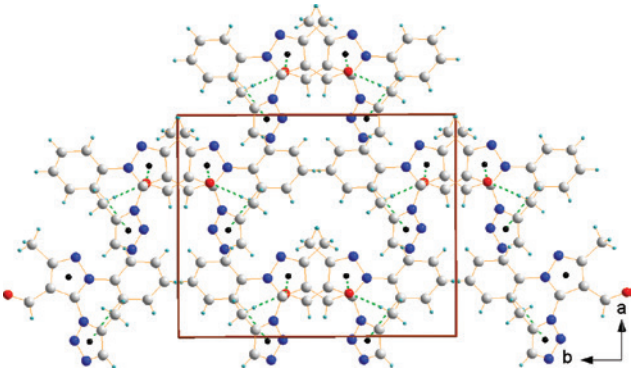


Fig. 4: Packing of the molecules in the unit cell of compound **8b** showing various types of intermolecular interactions as dashed lines.

Table 2: Hydrogen bond geometry and non-bonding contacts (Å, deg) of compound **11c**.[a]

D–H···A	D–H	H···A	D···A	D–H···A
C17–H173···O1[i]	**0.95**	**2.34**	**3.273(14)**	**165**

[a]Symmetry code: (i) $3/2-x$, $1/2+y$, $3/2-z$.

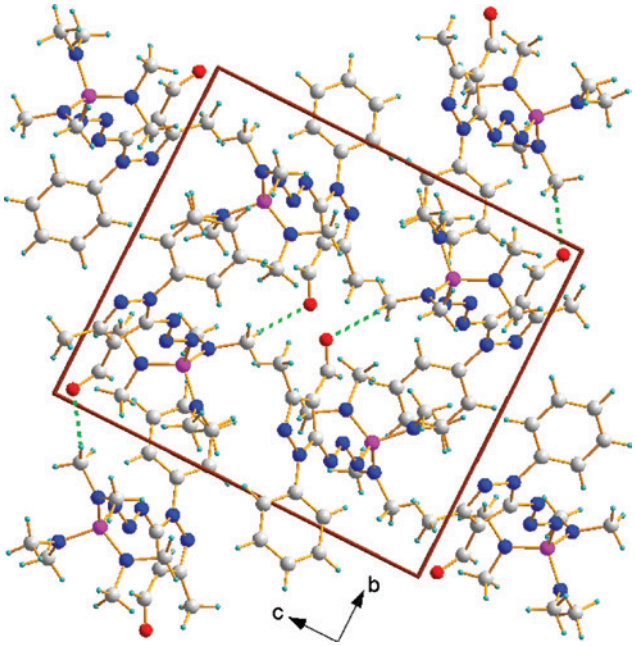


Fig. 5: Packing of the molecules in the unit cell of compound **11c** showing weak hydrogen bonds as dashed lines.

Table 3: Cytotoxic activity of azidopyrazole derivatives against MCF7 and HepG2.

Compounds	MCF7 IC_{50} (μM)	HepG2 IC_{50} (μM)[a]
Doxorubicin[b]	10.2 ± 0.13	5.61 ± 0.037
1	21.27 ± 0.85	18.94 ± 2.14
8a	10.12 ± 0.65	12.52 ± 0.220
8b	1.012 ± 0.13	24.1 ± 1.78
9a	8.56 ± 0.23	20.1 ± 0.42
9b	8.36 ± 0.11	16.88 ± 0.29
11a	19.31 ± 1.65	14.85 ± 0.39
11b	13.33 ± 0.28	25.96 ± 1.39
11c	24.33 ± 0.17	12.99 ± 4.81
11d	24.78 ± 1.75	19.42 ± 1.29
12a	15.22 ± 0.92	16.31 ± 0.23
12b	26.23 ± 0.88	36.46 ± 0.86
12c	24.09 ± 0.60	12.58 ± 5.34
13	32.23 ± 0.38	37.6 ± 0.13

[a]IC_{50} value was defined as the concentration at which 50% survival of cells was observed and it is expressed as mean ± SE. DMSO was used as negative control. [b]Doxorubicin was used a reference drug.

biological activity of newly synthesized compounds, aiming to develop new anticancer drugs. Derivatives of pyrazole have received considerable attention owing to their diverse chemotherapeutic potentials including their versatile anticancer activities [7]. Thus, the current study was tailored to screen *in vitro* cytotoxic and growth inhibitory activities of most of the newly synthesized azidopyrazole compounds on human breast carcinoma cell line (MCF7) and human hepatocellular carcinoma cell line (HepG2). MCF7 and HepG2 cells were cultured as monolayers and treated with our compounds for 48 h. The rate of proliferation was assessed by the Alamar blue reduction assay. According to current data, compounds **8a**, **8b**, **9a**, and **9b** demonstrate a higher antitumor activity against MCF7 cells with low cytotoxic activity (IC_{50} = 10.12, 1.012, 8.56, and 8.36 μM, respectively) than that of the reference drug (doxorubicin, IC_{50} = 10.2 μM) (Table 3). Meanwhile, azidopyrazole derivatives showed less antitumor activity against HepG2 cells with higher IC_{50} than doxorubicin (IC_{50} = 5.61 μM). The study revealed that azidopyrazole derivatives have a potential antitumor activity against MCF7 cells; however, those derivatives are less potent against HepG2 cells. In addition, the current data stressed that compounds **9a**, **9b**, and **8b** have the most effective antitumor activity. Compound **8b** has a triazole ring that was previously reported to have a

potentially broad range of biological and pharmacological activities [29]. Additionally, compounds **9a** and **9b** have a significant antitumor activity that may be associated with presence of the nitrile (CN) group, which has previously been reported to have a strong biological activity due to their strong hydrogen bond acceptor leading to strong versatile interactions with different proteins [30]; therefore, compounds **9a**, **9b**, and **8b** have promising therapeutic effects that can be further studied *in vivo* and *in vitro*.

3 Conclusion

The reaction of 5-azido-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**1**) with phosphonium ylides **2a–c** led to different products **8a–c** depending on the nature of the phosphonium ylides as well as the stability of the addition products. The reaction of **1** with Wittig-Horner reagents **3a** and **3b** gave different product **9a**,**b** and **9c** depending on the nature of the methane phosphonate anion as well as the stability of the addition products. In the reaction of **1** with diethyl phosphonate, 4,5-amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**10**) and dialkyl phosphite were the sole reaction products. The reaction of azidopyrazole **1** with trialkyl phosphites **5a,b** and tris(dialkylamino) phosphines **5c,d** yielded different products. These processes can be considered as new and simple routes for the preparation of various organophosphorus and heterocyclic compounds that cannot be obtained by other conventional methods. The compounds have shown promising anticancer activities against the human breast cancer cell lines (MCF7) at very low concentrations.

4 Experimental section

4.1 Chemistry

Melting points were determined in open glass capillaries using an Electrothermal IA 9100 series digital melting point apparatus (Electorthermal, Essex, UK), IR spectra were measured as KBr pellets with a Perkin-Elmer infracord spectrophotometer model 157. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or [D_6]dimethyl sulfoxide (DMSO) as solvents on a Jeol-500 spectrometer (^{1}H: 500 MHz; ^{13}C: 125 MHz), and the chemical shifts are recorded in δ values relative to TMS as internal reference. The ^{31}P NMR spectra were taken with a Varian CFT-20 spectrometer (*vs*. external 85% H_3PO_4 as standard). The mass spectra were recorded at 70 eV with a Kratos MS equipment or Varian MAT 311A spectrometer. Elemental analyses were performed using an Elementar Vario E1 instrument. The reported yields are of pure isolated materials obtained by column chromatography on silica gel 60 (Merk). 5-Azido-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**1**) was prepared according to a reported method [31, 32].

4.2 Reaction of (cyanomethylene)triphenylphosphorane (2a) with 1

A mixture of **2a** (0.60 g, 2 mmol) and 0.27 g (1 mmol) of **1** in dry THF (30 mL) was stirred for 1h. The volatile material was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography to give product **8a**. TPPO was also isolated and identified.

4.2.1 2-((5-Azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methyl)-3-(triphenylphosphoranylidene) succinonitrile (8a)

Eluent: petroleum ether-ethyl acetate (70:30, *v/v*). Product **8a** was separated as deep red crystals, yield 85%; m.p. 262°C–263°C. – IR (KBr): ν = 1587 (cyclic C=N), 2182 (N_3), 2235, 2364 (2 CN) cm^{-1}. – ^{1}H NMR (300 MHz, DMSO): δ = 1.76 (s, 3 H, CH_3), 1.99 (t, 1H, CH), 3.60 (d, 2 H, CH_2), 7.65–7.84 (m, 20H, H_{arom}) ppm. – ^{13}C NMR (75 MHz, DMSO): δ = 14.6 (CH_3), 25.6 (CH_2), 67.5 (CH), 99.9 (C-4, pyrazole), 115.8, 118.21 (2 CN), 121.5, 121.6 (C=P, $J_{c,p}$ = 98.5 Hz),118.4–144.2 (aromatic C–H, C-3, C-5 of pyrazole) ppm. – ^{31}P NMR: δ = 21.88 ppm. – MS (EI, 70 eV): *m/z* (%) = 551(5) $[M]^+$, 384 (100) $[M–167]^+$. – $C_{33}H_{26}N_7P$ (551.58): calcd. C 71.86, H 4.75, N 17.78, P 5.62; found C 71.89, H 4.77, N 17.98, P 5.72.

4.3 Reaction of (acetylmethylene)triphenylphosphorane (2b) with 1

A mixture of 1 mmol of **2b** (0.31 g) and 1 mmol of **1** (0.27 g) in dry THF (30 mL) was stirred for 2 h. The volatile material was evaporated under reduced pressure. The residue was subjected to silica gel column chromatography to give product **8b.** TPPO was also isolated and identified.

4.3.1 3-Methyl-5-(5-methyl-1*H*-1,2,3-triazol-1-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (8b)

Eluent: petroleum ether-ethyl acetate (80:20, *v/v*). Product **8b** was separated as colorless crystals, yield 75%; m.p.

99°C–101°C. – IR (KBr): ν = 1593 (C=N), 1682 (C=O) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.96 (s, 3H, CH_3), 2.64 (s, 3 H, CH_3), 7.34–7.54 (m, 6H, H_{arom}, =CH), 9.66 (s, 1H, CHO) ppm. – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 8.3 (CH_3), 13.7 (CH_3), 136.3, 136.6 (2C, triazole), 123.2–133.2 (aromatic CH), 117.4, 136.7, 151.6 (C-4, C-3, C-5of pyrazole), 183.4 (CHO) ppm. – MS (EI, 70 eV): *m/z* (%) = 268 (10) $[M+1]^+$, 267(8) $[M]^+$. – $C_{14}H_{13}N_5O$ (267.29): calcd. C 62.91, H 4.90, N 26.20; found C 62.95, H 4.87, N 26.18.

4.4 Reaction of (dibromomethylene)triphenylphosphorane (2c) with 1

Triphenylphosphane (0.5 g, 0.1 mmol) was added to a well-stirred solution of carbon tetrabromide (0.3 g, 0.05 mL) in DCM (3 mL). When the solution became orange (i.e. **2c** was formed) [33], pyrazole **1** (0.27 g, 0.1 mmol) was added, and the mixture was stirred at room temperature for 8 h. After evaporation of the volatile material, the buff residual substance was chromatographed on a silica gel column. TPPO was also isolated and identified.

4.4.1 5-Azido-4-(2,2-dibromovinyl)-3-methyl-1-phenyl-1*H*-pyrazole (8c)

Eluent: petroleum ether-ethyl acetate (80:20, *v/v*). Product **8c** was separated as buff powder, yield 35%. – m.p. 158°C–161°C. – IR (KBr): ν = 626 (C–Br), 1593 (C=N), 2125 (N_3) cm^{-1}. – ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 2.04 (s, 3H, CH_3), 7.58–7.99 (m, 6H, H_{arom}), 8.59 (s, 1H, CH) ppm. – ^{13}C NMR (125 MHz, [D_6]DMSO): δ = 13.8 (CH_3), 99.8 (=$C(Br)_2$), 100.2 (C-4, pyrazole), 124.8–140.3 (aromatic C–H), 135.2, 150.3 (C-3, C-5 of pyrazole) ppm. – MS (EI, 70 eV): *m/z* (%) = 380, 382, 384 [1:2:1] (15). – $C_{12}H_9Br_2N_5$ (383.04): calcd. C 37.63, H 2.37, Br 41.72, N 18.28; found C 37.71, H 2.23, Br 41.64, N 18.19.

4.5 Reaction of diethyl cyanomethylphosphonate (3a) or diethyl 4-methoxybenzylphosphonate (3b) with 1

A solution of sodium ethoxide (0.14 g, 2 mmol) in absolute ethanol (30 mL) was treated with an equimolar amount of diethyl (cyanomethyl)phosphonate (**3a**) or diethyl 4-(methoxybenzyl)phosphonate (**3b**) (2 mmol). Azidopyrazole **1** (0.27 g, 1 mmol) was added, and the reaction mixture was stirred at room temperature for 4 h (TLC). The mixture was poured on a small amount of water, extracted with ethyl acetate, dried over anhydrous sodium sulfate, and the extracts were evaporated under reduced pressure. The residue was applied to silica gel column chromatography to give compounds **9a**,**b** and/or **9c**.

4.5.1 Diethyl 2-(5-azido-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1-cyano-2-hydroxyethylphosphonate (9a)

Eluent: petroleum ether-ethyl acetate (80:20, *v/v*). Product **9a** was separated as pale yellow crystals, yield 20%; m.p. 86°C–87°C. – IR (KBr): ν = 1024 (P=O), 1592 (C=N), 2114 (N_3), 2359 (CN), 3441 (OH) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.32 (t, 6H, J_{HH} = 13.4 Hz, P–OCH_2CH_3), 2.63 (s, 3H, CH_3), 2.72 (d, 1H, CH–P, $^2J_{HP}$ = 22.9 Hz), 3.47 (d, 1H, $^3J_{HP}$ = 12.5 Hz, C*H*OH), 4.22 (2q, 4H, $^3J_{HP}$ = 12.5 Hz, P–O–CH_2),7.92–8.72 (m, 5H, H_{arom}), 8.96 (s, 1H, OH) ppm. – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.6 (CH_3), 16.4 (O–CH_2–CH_3), 40.5 (C–P, J_{C-P} = 125 Hz), 63.58, 64.49 (CH_2), 69.73 (*CH*–OH), 101.9 (CN), 104.5 (C-4, pyrazole), 124.2–144.6 (aromatic C–H, C-3, C-5 of pyrazole) ppm. – ^{31}P NMR: δ = 23.03 ppm. – MS (EI, 70 eV): *m/z* (%) = 386 (100) $[M-OH]^+$. – $C_{17}H_{21}N_6O_4P$ (404.36): calcd. C 50.50, H 5.23, N 20.78, P 7.66; found C 50.48, H 5.11, N 20.66, P 7.93.

4.5.2 (*Z*)-3-(5-Amino-3-methyl-1-phenyl-1*H*-pyrazol-4-yl) acrylonitrile (9b)

Eluent: petroleum ether-ethyl acetate (70:30, *v/v*). Product **9b** was separated as buff crystals, yield 40%; m.p. 165°C–166°C. – IR (KBr): ν = 1599 (C=N), 2362 (CN), 3362, 3302, 3205 (NH_2) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.55 (s, 3H, CH_3), 6.47 (d, 1H, *J* = 7.6 Hz, CHCN), 7.76 (1H, C*H*=CH, J_{HH} = 7.6 Hz), 7.92–8.72 (m, 7H, H_{arom}, NH_2) ppm. – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.8 (CH_3), 98.5 (*C*–CN), 112.5 (C-4, pyrazole), 123.2–149.6 (aromatic C–H, C-3, C-5 of pyrazole) ppm. – MS (EI, 70 eV): *m/z* (%) = 224(5) $[M]^+$. – $C_{13}H_{12}N_4$ (224.26): calcd. C 69.62, H 5.39, N 24.98; found C 69.73, H 5.42, N 25.01.

4.5.3 5-Methyl-7-phenyl-3*H*-pyrazolo[3,4-*d*][1,2,3] triazin-4(7*H*)-one (9c)

Eluent: petroleum ether-ethyl acetate (95:5, *v/v*). Product **9c** was separated as orange crystals, yield 65%; m.p. 160°C–162°C. – IR (KBr): ν = 3334 (NH), 1672 (C=O), 1665 (N=N), 1619 (C=C), 1596 (C=N) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.36 (s, 3 H, CH_3), 4.51 (s, 1 H, NH, exchangeable with D_2O), 7.45–7.57 (m, 5 H, CH_{arom}) ppm. – ^{13}C NMR ($CDCl_3$): δ = 14.3 (CH_3), 96.3, 152.5 (cyclic C=C), 124.9–138.7

(aromatic, C–H), 149.2 (C=N), 162.4 (C=O) ppm. – MS (EI, 70 eV): *m/z* (%) = 227 (50) $[M]^+$. – $C_{11}H_9N_5O$ (227.22): calcd. C 58.14, H 3.99, N 30.82; found: C 58.12, H 4.01, N 30.74.

4.6 Reaction of diethyl hydrogenphosphite (4) with 1

A mixture of 1 mmol of **4** (0.17g) and 1 mmol of **1** (0.27 g) in dry toluene (30 mL) was stirred for 30 min. After evaporation of the volatiles under reduced pressure, the residue was subjected to silica gel column chromatography to yield structure **10**.

4.6.1 5-Amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (10)

Eluent: petroleum ether-ethyl acetate (80:20, *v/v*). Product **10** was separated as colorless crystals, yield 75%; m.p. 96°C–98°C. – IR (KBr): ν = 1543 (C=N), 1606 (C=C), 1644 (C=O), 3398, 3288, 3187 (NH_2) cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 2.64 (s, 3 H, CH_3), 5.32 (s, 2H, NH_2 exchangeable with D_2O), 7.34–7.54 (m, 5H, H_{arom}), 9.66 (s, 1H, CHO) ppm. – MS (EI, 70 eV): *m/z* (%) = 201.22 (100) $[M]^+$. Compound **10** gave a correct elemental analysis and it was characterized by TLC analysis (one spot) and IR comparison with an authentic specimen [21].

4.7 Reaction of trialkylphosphite (5a,b) with 1

A mixture of 1 mmol of **5a** or **5b** and 1 mmol of **1** in dry toluene (30 mL) was stirred for 1–2 h (TLC). After evaporation of the volatile material, the residue was recrystallized from a proper solvent to yield compound **11a**,**b**.

4.7.1 Trimethyl (4-formyl-3-methyl-1-phenyl-1*H*-pyrazol-5-yl)phosphoramidate (11a)

Product **11a** was separated as brown crystals (benzene), yield 75%; m.p. 238°C–239°C. – IR (KBr): ν = 1063 (P–O–alkyl), 1558 (C=N), 1658 (C=O) cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 2.66 (s, 3H, CH_3), 3.36–3.52 (d, $^3J_{HP}$ = 11.0 Hz, 9H, $P(OCH_3)_3$), 7.25–7.97 (m, 5H, H_{arom}), 10.24 (s, H, CHO) ppm. – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9 (CH_3), 54.9, 55.1 (d, $^2J_{CP}$ = 11 Hz, P–$(OCH_3)_3$), 104.5 (C-4, pyrazole), 123.2–152.6 (aromatic C–H, C-3, C-5 of pyrazole), 179.4 (C=O) ppm. – MS (EI, 70 eV): *m/z* (%) = 323 (35) $[M]^+$. – ^{31}P NMR δ = 23.2 ppm. – $C_{14}H_{18}N_3O_4P$ (323.28): calcd. C 52.01, H 5.61, N 13.00, P 9.58; found C 52.21, H 5.66, N 13.11, P 9.32.

4.7.2 Triisopropyl (4-formyl-3-methyl-1-phenyl-1*H*-pyrazol-5-yl)phosphorimidate (11b)

Product **11b** was separated as brown crystals (benzene), yield 85%; m.p. 257°C–259°C. – IR (KBr): ν = 1082 (P–O–alkyl), 1592 (C=N), 1655 (C=O) cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ =1.26 (m, 18H, 3 $CH(CH_3)_2$), 2.46 (s, 3H, CH_3), 3.96 (m, 3H, CH, 3 $CH(CH_3)_2$), 7.22–7.83 (m, 5H, H_{arom}), 10.11 (s, H, CHO) ppm. – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9 (CH_3), 22.8 (CH_3, 3 $CH(CH_3)_2$), 74.2 (d, $^2J_{CP}$ = 11 Hz, P–O–CH), 104.5 (C-4, pyrazole), 123.3–152.5 (aromatic C–H, C-3, C-5 of pyrazole), 179.4 (C==O) ppm. – ^{31}P NMR: δ = 23.7 ppm. – MS (EI, 70 eV): *m/z* (%) = 407 (100) $[M]^+$. – $C_{20}H_{30}N_3O_4P$ (407.44): calcd. C 58.96, H 7.42, N 10.31, P 7.60; found C 59.00, H, 7.22, N, 10.52, P7.61.

4.8 Reaction of tris(dialkylamino)phosphanes 5c,d with 1

A mixture of **5c** or **5d** (1 mmol) and 0.27 g (1 mmol) of **1** in dry benzene (30 mL) was stirred for 3 h. After evaporation of the volatile material under reduced pressure, the residue was subjected to silica gel column chromatography to yield **11c,d**.

4.8.1 3-Methyl-1-phenyl-5-[(tris(dimethylamino) phosphoranylidene]triaz-1-en-1-yl)-1*H*-pyrazole-4-carbaldehyde (11c)

Eluent: petroleum ether-ethyl acetate (80:20, *v/v*). Product **11c** was separated as yellow crystals, yield 75%; m.p. 140°C–142°C. – IR (KBr): ν =1599 (C=N), 1651 (C=O), 1665 (N=N) cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$): δ = 2.36 (s, 3 H, CH_3), 2.50, 2.64, 3.36 (d, $^3J_{HP}$ = 9.3 Hz, 18H, $P[N(CH_3)_2]_3$), 7.33–7.69 (m, 5H, H_{arom}), 9.71 (s, H, CHO) ppm. – ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.8 (CH_3), 37.0 ($N(CH_3)_2$), 110.8 (C-4, pyrazole), 124.7–149.6 (aromatic C–H, C-3, C-5 of pyrazole), 186.9 (C=O) ppm. – ^{31}P NMR: δ = 29.8 ppm. – MS (EI, 70 eV): *m/z* (%) = 390 (10) $[M]^+$. – $C_{17}H_{27}N_8OP$ (390.42): calcd. C 52.30, H 6.97, N 28.70, P 7.93; found C 52.23, H 6.82, N 28.73, P 7.87.

4.8.2 3-Methyl-1-phenyl-5-[(tris(diethylamino) phosphoranylidene]triaz-1-en-1-yl)-1*H*-pyrazole-4-carbaldehyde (11d)

Eluent: petroleum ether-ethyl acetate (80:20, *v/v*). Product **11d** was separated as colorless crystals, yield 60%; m.p.

153°C–155°C. – IR (KBr): ν = 1599 (C=N), 1664 (N=N), 1728 (C=O) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.06–1.19 (m, 18H, CH_3), 2.48 (s, 3 H, CH_3), 2.99–3.03 (q, 12H, CH_2), 7.47–7.96 (m, 5H, H_{arom}), 9.93 (s, H, CH=O) ppm. – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.21 (CH_3), 19.34 ($N(CH_3)_2$), 46.73 ($N(CH_2)_2$), 118.9 (C-4, pyrazole), 126.0–152.6 (aromatic C–H, C-3, C-5 of pyrazole), 167.7 (C=O) ppm. – ^{31}P NMR: δ = 25.8 ppm. – MS (EI, 70 eV): *m/z* (%) = 474 (15) $[M]^+$. – $C_{23}H_{39}N_8OP$ (474.58): calcd. C 58.21, H 8.28, N 23.61, P 6.53; found C 58.31, H 8.31, N 23.54, P 6.23.

4.9 Reaction of triphenylstibane/triphenylarsane 6a,b with 1

A mixture of **6a** or **6b** (1 mmol) and 0.27 g (1 mmol) of **1** in dry toluene (30 mL) was warmed for 1–4 h (TLC). After evaporation of the volatile material, the buff residual substance was recrystallized from benzene to yield **12a**,**b** and triphenylstibane oxide, triphenylarsane oxide were isolated and identified (m.p. and mixed m.p.).

4.9.1 5-Azido-3-methyl-1-phenyl-4-[(triphenylstiboranylidene)methyl]-1*H*-pyrazole (12a)

Product **12a** was separated as buff crystals (benzene), yield 75%; m.p. 236°C–238°C. – IR (KBr): ν =1593 (C=N), 2195 (N_3) cm^{-1}. – ^{1}H NMR (500 MHz, [D_6]DMSO):δ = 1.99 (s, 3 H, CH_3), 6.90 (s, 1H, CH), 7.33–7.68 (m, 20H, H_{arom}) ppm. – ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 14.5 (CH_3), 70.0 (CH), 117.8 (C-4, pyrazole), 120.5–148.6 (aromatic C–H, C-3, C-5 of pyrazole), 166.7 (C=O) ppm. – MS (EI, 70 eV): *m/z* (%) = 564(5) $[M]^+$, 429 (15) $[M–135]^+$. – $C_{29}H_{24}N_5Sb$ (564.29): Sb 21.58, Sb 21.60.

4.9.2 5-Azido-3-methyl-1-phenyl-4-((triphenylarsoranylidene)methyl)-1*H*-pyrazole (12b)

Product **12b** was separated as buff crystals (benzene), yield 45%; m.p. 211°C–213°C. – IR (KBr): ν =1592 (C=N), 2196 (N_3) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.36(s, 3 H, CH_3), 7.25–7.57 (m, 21H, H_{arom}, CH) ppm. – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.2 (CH_3), 70.07 (CH), 117.9 (C-4, pyrazole), 122.5–149.6 (aromatic C–H, C-3, C-5 of pyrazole), 169.7 (C=O) ppm. – MS (EI, 70 eV): *m/z* (%) = 517(5) $[M]^+$. – $C_{29}H_{24}AsN_5$ (517.46): As 14.48, As 14.50.

4.10 Reaction of LR 7 with 1

A mixture of 0.20 g (0.5 mmol) of **7** and 0.27 g (1 mmol) of **1** was stirred for 15 min in dry benzene. The volatile material was evaporated under reduced pressure. The precipitate was filtered off and washed with diethyl ether, then recrystallized from benzene to afford product **13**.

4.10.1 5-[5-(4-Methoxyphenyl)-5-sulfido-1,2,3,4,5-thiatriazaphosphol-2(5*H*)-yl]-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (13)

Compound **13** was isolated as buff powder, yield 65%; m.p. 144°C–146°C. – IR (KBr): ν = 625 (P=S), 1585 (C=N), 1696 (C=O) cm^{-1}. – ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 2.46 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 6.95–7.65 (m, 9H, H_{arom}) ppm. – ^{13}C NMR (125 MHz, [D_6]DMSO): δ = 12.1 (CH_3), 55.7 (OCH_3), 105.0 (C-4, pyrazole), 113.9–145.5 (aromatic C–H, C-3, C-5 of pyrazole), 161.6, 161.7 (C=O) ppm. – ^{31}P NMR: δ = 15.0 ppm. – MS (EI, 70 eV): *m/z* (%) = 429(5) $[M]^+$. – $C_{18}H_{16}N_5O_2PS_2$ (429.46): calcd C 50.34, H 3.76, N, 16.31, P 7.21, S 14.93; found: C 55.30, H 3.78, N 16.32, P 7.23, S 14.91.

4.11 Crystal structure determinations of 8a and 11c

Single crystals of **8a** and **11c** were grown by crystallization from benzene. The diffraction data were collected on an Enraf-Nonius 590 diffractometer with a Kappa CCD detector using graphite-monochromatized MoK_α (λ = 0.71073 Å) radiation at National Research Center of Egypt [34, 35]. Reflection data have been recorded in the rotation mode using the ϕ and ω scan technique with θ_{max} = 34.98° for **8a** and 30.04° for **11c**. Unit cell parameters were determined from least-squares refinement with θ in the range $4 \le \theta \le 34°$ for **8a** and $3 \le \theta \le 30°$ for **11c**. The structures of **8a** and **11c** were solved by Direct Methods using SUPERFLIP [36] implemented in the program suit CRYSTALS [37]. The refinement was carried out by full-matrix least-squares method on the positional and anisotropic temperature parameters of all non-hydrogen atoms based on F^2 using CRYSTALS. All hydrogen atoms were positioned geometrically and were initially refined with soft restraints on the bond lengths and angles to regularize their geometry (C–H in the range 0.93–0.98 Å and N–H in the range 0.86–0.89 Å) and U_{iso}(H) = 1.2–1.5 × U_{eq} of the parent atom. Then, the positions were refined with riding constraints [38]. The general-purpose crystallographic tool PLATON [39] was used for the structure analysis and presentation of the results. The molecular graphics were done using ORTEP-III for Windows [40] and DIAMOND [41]. Details of the data collection conditions and the parameters of the refinement process for **8a** and **11c** are given in Table 4.

CCDC 1415329 (**8a**) and 1415326 (**11c**) contain the supplementary crystallographic data for this paper. These

data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

4.12 Anticancer evaluation

4.12.1 Cell culture and treatment

All reagents were handled as previously reported [42–44] in a sterile fume hood. DMEM medium and fetal bovine serum (FBS) were purchased from Gibco; phosphate-buffered saline (PBS), pH 7.4, and trypsin-EDTA were obtained from Sigma-Aldrich. Alamar blue or Resazurin (Promega, Mannheim, Germany) reduction assay was used to assess the cytotoxicity of the studied samples. The growth medium (DMEM medium with 10% FBS, 100 U/mL penicillin, and 100 mg/L streptomycin) and Alamar blue were stored at 48°C, whereas trypsin-EDTA and FBS were stored frozen at −80°C and thawed before use; PBS was stored at room temperature. The MCF7 and HepG2 were obtained from the German Cancer Research Center (DKFZ).Cells were cultured in 50-cm^2 culture flasks (Corning) using DMEM medium supplemented with 10% FBS, penicillin (100 IU/ml), and streptomycin (100 mg/ml). The culture was maintained at 37°C in an atmosphere of 5% CO_2 and 95% relative humidity. The cells were transferred to a new flask every 2 days and treated with trypsin–EDTA to detach them from the flask. Cells were counted under a microscope using a hemacytometer (Hausser Scientific). Cell solutions were diluted with growth medium to a concentration of 1×10^5 cells/mL, transferred to a 96-well plate, and treated with gradient concentrations of test compounds.

4.12.2 Resazurin cell growth inhibition assay

Alamar blue or Resazurin (Promega) reduction assay was used to assess the cytotoxicity of the studied samples. The assay tests the cellular viability and mitochondrial function. Briefly, adherent cells were grown in tissue culture flasks, and then harvested by treating the flasks with 0.025% trypsin and 0.25 mM EDTA for 5 min. Once detached, cells were washed and counted, and an aliquot (5×10^3 cells) was placed in each well of a 96-well cell culture plate with a total volume of 100 µL. Cells were allowed to attach overnight and then treated with samples. The final concentration of samples ranged from 0 to 100 µM. After 48 h,

Table 4: Crystal data and experimental parameters of data collection and structure refinement of compounds **8b** and **11c**.

	8b	**11c**
Crystal data		
Chemical formula	$C_{14}H_{13}N_5O$	$C_{17}H_{27}N_8OP$
M_r	267.292	390.43
Temperature, K	298	298
Crystal size, mm^3	0.12 × 0.13 × 0.15	0.14 × 0.15 × 0.18
Crystal system, space group	Monoclinic, *Cc*	Monoclinic, $P2_1/n$
a, Å	11.9497(10)	10.8101(4)
b, Å	14.8959(13)	13.3926(5)
c, Å	7.7132(8)	15.6394(8)
β, deg	96.245(5)	107.0286(12)
V, Å^3	1364.8(2)	2164.93(16)
Z	4	4
Radiation type	MoK_α	
μ, mm^{-1}	0.09	0.15
Data collection		
Diffractometer	Nonius Kappa CCD diffractometer	
Absorption correction	Multi-scan	
T_{min}/T_{max}	0.85/1.00	0.83/1.00
Refl. measured/unique	2843/2621	6100/3100
Refl. obeserved [$I > 2.0\,\sigma(I)$]	1097	1425
$(\sin\theta/\lambda)_{max}$, Å^{-1}	0.807	0.704
Refinement		
No. of reflections/parameters	1097/153	1425/142
$R[F^2 > 2\,\sigma(F^2)]/wR\,(F^2)$	0.043/0.090	0.056/0.131
x (Flack)	−3(6)	–
S	0.95	0.90
$\Delta\rho_{max/min}$, e Å^{-3}	0.16/−0.15	0.25/−0.29

20 µL of Resazurin 0.01% *w*/*v* solution was added to each well, and the plates were incubated at 37°C for 1–2 h. Fluorescence was measured on an automated 96-well Infinite M2000 Pro™ plate reader (Tecan, Crailsheim, Germany) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. After 48 h incubation, plates were treated with Resazurin solution as mentioned above. Doxorubicin was used as a positive control, and its IC_{50} was 10.2 ± 0.13 µM against MCF7 cells and 5.61 ± 0.037 µM against HepG2 cells. Each assay was done at least three times, with two replicates each. The viability was compared based on a comparison with untreated cells. IC_{50} (on cancer cells) was the concentration of the sample required to inhibit 50% of cell proliferation and were calculated from a calibration curve by a linear regression using Microsoft Excel.

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