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SYNTHESIS AND ANTI-PROLIFERATIVE ACTIVITY OF NEW α-AMINO PHOSPHONATE DERIVATIVES BEARING HETEROCYCLIC MOIETY

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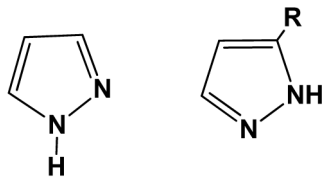
Condensation of 4-chloroacetophenone **1** with phenyl hydrazine **2** afforded hydrazone **3**. Further reaction of **3** with Vilsmeier reagent yielded the pyrazolaldehyde **4** in excellent yield**.** A one-pot, three-component reaction between aldehyde **4**, triphenylphosphite **5**, and appropriate amines in the presence of lithium perchlorate as Lewis acid catalyst gave the corresponding α-amino phosphonates **7-12(a-d)** in good yields. The chemical structures of all new compounds were established by IR, ^{1}H NMR, and mass spectroscopy analysis. The anti-proliferative activity of the synthesized compounds against HCT-116, HepG2 and MCF-7 human cancer cells using the MTT assay was evaluated, and revealed higher anticancer activity when compared with reference drug doxorubicin. Among the tested compounds, pyrazole derivatives **4** and **9** exhibited the highest anticancer activity against breast (MCF-7), and colon (HCT-116) cancer cell lines with IC_{50} = 2.7 and 3.3 μM, respectively.

Keywords: acetophenone; phenylhydrazine; Vilsmeier-Haack reaction; triphenylphosphite; anti-proliferative activity.

1. INTRODUCTION

Cancer constitutes a major public health problem worldwide, since it is the second leading cause of death globally, with 9.6 million deaths estimated in 2018. Due to the limitations and side effects associated with available cancer treatments now days, it is an urgent challenge for medicinal researchers to develop more safe and selective anticancer drugs [1]. Towards that end, pyrazole **I** and its derivatives **II** are considered a pharmacologically important active scaffold that possesses almost all types of pharmacological activities. The presence of the pyrazole nucleus in different structures leads to diversified applications in different areas such medicine [2, 3]. In particular, they are described as inhibitors of protein glycation, antibacterial [4], antifungal, anticancer [5], antidepressant, anti-inflammatory [6], anti-tuberculosis, antioxidant [7] as well as antiviral agents [8]. Owing to this diversity in the biological field, this nucleus has attracted the attention of many researchers to study its skeleton chemically and biologically [9–11].



I **II**

Moreover, α-amino phosphonates **III** are versatile class of organophosphorus compounds because they are analogues of the corresponding α-amino acids **IV** and possess high metabolic stability as well as insignificant toxicity [12-15]. Furthermore, incorporation of α-amino phosphonate moiety into different molecules have demonstrated to be a novel and promising approach to design more potent and safer anti-cancer drugs [16].

In continuation of our program on the utilization of nitrogen-containing heterocyclic scaffolds in multicomponent reactions for one-pot synthesis of new hybrid molecules incorporating α-amino phosphonate scaffold, we would like to report the synthesis of new α-amino phosphonate derivatives containing pyrazole heterocycle [17, 18]. This hybrid structure is important due to a range of biological activities and

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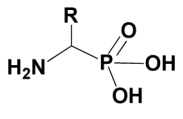
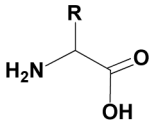
medicinal applications associated with them by combining both moieties with the aim to have synergistc pharmacological properties [19–21].



III



IV

2. EXPERIMENTAL

2.1. Materials and Methods

^{1}HNMR spectra were recorded on Varian 400 MHz spectrophotometer using DMSO-d_6 solvent at the University of Ulm (Germany) and the chemical shifts were expressed in part per million (δ, ppm) relative to the internal slandered TMS (0PPM) for the center peak of residual DMSO (2.49 ppm). The peak patterns are indicated as follows: s, singlet; d, doublet; t, triplet. The coupling constants, J, are reported in Hertz (Hz|). IR spectra were recorded on Shimadzu, Japan spectrophotometer, Cairo University using anhydrous KBr disc. Melting point (mp) was recorded on Stuart-scientific melting point apparatus and was uncorrected. The biological activity was carried out at the division of Pharmaceutical Industries, National Research Center, and Cairo, Egypt. All reactions were followed by thin layer chromatography (TLC) on kiesel gel F254 pre-coated plates (Merck). Starting materials and solvents were purchased and used as received without further purification.

2.2. Chemistry

Synthesis of 3-(4-chlorophenyl)-1-phenyl-1H pyrazole-4-carbaldehyde (4). To a mixture of p-chloroacetophenone **1** (1.55 g, 0.01 mole), and phenyl hydrazine (1.08 g., 0.01 mole) of **2** in 10 mL ethanol was refluxed in water bath for 4 hrs. The reaction mixture was cooled, and the solid formed was filtered and crystallized from diethyl ether to form hydrazone **3**. Further dropwise addition of a mixture of DMF (0.73 g, 0.01 mole) and $POCl_3$ (1.53 g, 0.01 mole) with cooling under mechanical stirring for 5 h. The reaction mixture was further refluxed for 6 h. at 70 – 80°C, then hydrolyzed on ice/water mixture , and neutralized by 5% NaOH solution till pH= 4, then cooled. The solid formed was filtered off, washed with water, dried and crystallization from isopropanol to yield: (2.55 g, 90%), yellow solid, mp: 252 – 253°C. IR (cm^{-1}): $\nu_{C=O}$ 1667, $\nu_{C=N}$ 1603, $\nu_{C=C}$ 1511 and ν_{C-H} 731, ^{1}HNMR (δ, ppm): 10.13 (s, 1H, CHO), 9.31 (C-H Pyrazole), 6.54 – 7.95 (m, 8H, Ar-H), MS: m/z = 282 (M^+, $C_{16}H_{11}ClN_2O$, 78%), m/z = 77(M^+-$C_8H_5N_2O$, 100%), m/z = 209 (M^+-CHO, 15%), m/z = 237(M^+-H, 74%), m/z = 210(M^+-CO, 20%).

Synthesis of α-amino phosphonate derivatives (7 – 12). General Procedure: To a mixture of aldehyde **4** (0.23 g, 0.8 mmole), triphenylphosphite **5** (0.25 g., 0.8 mmole), and appropriate amine **6a-6d** and **11a-11d** (0.8 mmole) in acetonitrile (3 mL), lithium perchlorate (10 mole %) was added. The reaction mixture was stirred at room temperature until the starting materials were consumed as monitored by TLC (**3d**). After the completion of the reaction, the precipitated product was filtered off and crystallized by using diethyl ether to give the desired product in good yield.

Synthesis of diphenyl ((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl) (pyridin-4-ylamino) methyl) phosphonate 7: Yield: (0.83 g., 70%), dark green solids, mp. 198 – 200°C, IR (cm^{-1}): ν_{NH} 3395, $\nu_{C=N}$ 1670, $\nu_{P=O}$ 1227, ν_{POC} 1076, ν_{C-N} 752 and ν_{C-Cl} 671. ^{1}HNMR (δ, ppm): 5.45 – 5.53 (dd, 1H, CHP), 5.9 (s, 1H, NH), 7.22 – 9.31 (m, 24H, Ar.). MS: m/z = 593 (M^+, $C_{33}H_{26}ClN_4O_3P$, 75%,), m/z = 328 (M^+-$C_{16}H_{16}N_3O_3P$, 100%), m/z = 77 (M^+-$C_{27}H_{21}ClN_4O_3P$, 34%), m/z = 187 (M^+-$C_{11}H_{17}NO_5P$, 6%), m/z = 406 (M^+-$C_{21}H_{19}N_4O_3P$, 63%).

Synthesis of diphenyl ((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)amino) methyl) phosphonate 8: Yield: (0.96 g, 68%), orange solids, mp. 168 – 170°C; IR (cm^{-1}): ν_{NH} 3143, $\nu_{C=o}$ 2411, $\nu_{C=N}$ 1645, $\nu_{P=O}$ 1294, ν_{POC} 1137, ν_{C-N} 762 and ν_{C-Cl} 693. ^{1}HNMR (δ, ppm): 2.39 (s, 3H, CH_3), 3.13 (s, 3H, N-CH_3), 3.94 (s, 1H, NH), 5.88 (dd, 1H, CHP), 6.72 – 7.99 (m, 25H, Ar.). MS: m/z = 702 (M^+ $C_{39}H_{33}ClN_5O_4P$, 33%), m/z = 388 (M^+-$C_{22}H_{17}N_2O_4P$, 100%), m/z = 77 (M^+-$C_{33}H_{27}ClN_5O_4P$, 55%), m/z = 314 (M^+-$C_{17}H_{16}ClN_3OP$, 5%).

Synthesis of diphenyl ((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)((4-oxo-2-thioxothiazoldin-3-yl)amino) methyl)phosphonate 9: Yield: (1.1g, 85%), yellow solids, mp.173 – 175°C; IR (cm^{-1}): ν_{NH} 3436, $\nu_{C=O}$ 1739, $\nu_{C=N}$ 1601, $\nu_{P=O}$ 1288, ν_{POC} 1117, ν_{C-N} 760 and ν_{C-Cl} 647 cm^{-1}, ^{1}HNMR (δ, ppm): 4.3(s, 2H, CH_2), 5.88 (s, 1H, CHP), 7.4 – 9.27 (m, 21H, Ar.), MS: m/z = 647 (M^+-$C_{14}H_{10}N_2O_2$, 14%), m/z = 347 (M^+-$C_{15}H_{10}N_2O_4PS_2$, 100%), m/e = 77 (M^+-$C_{25}H_{18}ClN_4O_4PS_2$, 33%).

Synthesis of benzyl ((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)(diphenoxyphosphoryl)methyl)carbamate 10: Yield: (1.13 g, 87%), orange solids, mp. 138 – 140°C; IR (cm^{-1}): ν_{NH} 3319, $\nu_{C=O}$ 1732, $\nu_{C=N}$ 1656, $\nu_{P=O}$ 1271, ν_{POC} 1091, ν_{C-N} 756 and ν_{C-Cl} 685. ^{1}HNMR (δ, ppm): 4.57(s, 2H, CH_2), 4.71 – 4.78 (dd, 1H, CHP), 5.9 (s, 1H, -NH), 6.68 – 9.17 (m, 25H, Ar.), MS: m/z = 650 ($M^+C_{23}H_{15}N_3O_3$, 45%), m/z = 350 (M^+-$C_{20}H_{15}NO_5$, 100%), m/z = 77 (M^+-$C_{30}H_{23}ClN_3O_5P$, 42%), m/z = 226 (M^+-$C_{22}H_{18}ClN_2O_3P$, 26%).

Synthesis 4-(((3-(4-chlorophenyl)-1-phenyl-1H- pyrazol-4yl)(diphenoxyphosphoryl)methyl) amino)benzoic acid 12a: Yield: (0.76 g, 60%), white solids, mp. 198 – 200°C; IR (cm^{-1}): ν_{NH} 3291, $\nu_{C=O}$ 1794, $\nu_{C=N}$ 1674, $\nu_{P=O}$ 1254, ν_{POC} 1092, ν_{C-N} 764 and ν_{C-Cl} 691, ^{1}HNMR (δ, ppm): 5.49 (s, 1H, CHP), 6.73 – 7.74 (m, 15H, Ar.), 9.66 (S,

1H, -COOH). MS: m/z = 636 (M^+-$C_{34}H_{26}N_4O_3$, 33%), m/z = 382 (M^+-$C_{15}H_{10}N_2P$, 100%), m/z = 77 (M^+-$C_{29}H_{22}ClN_3O_3P$, 22%), m/z = 253 (M^+-$C_{20}H_{17}NO_5P$, 6%).

Synthesis of diphenyl ((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl) (p-tolylamino)methyl) phosphonate 12b: Yield: (0.92 g, 76%), black solids, mp. 103 – 105°C; IR (cm^{-1}): ν_{NH} 3119, $\nu_{C=N}$ 1670, $\nu_{P=O}$ 1223, ν_{POC} 1093, ν_{C-N} 752 and ν_{C-Cl} 686. ^{1}HNMR (δ, ppm): 3.34 (s, 3H, CH_3), 5.40 – 5.45 (dd, 1H, CHP), 5.72 (s, 1H, NH),6.73 – 9.16(m, 24H, Ar). MS: m/z = 606 (M^+-$C_{35}H_{29}ClN_3O_3P$, 11%), m/z = 352 (M^+-$C_{15}H_{10}ClN_2$, 100%), m/z = 77 (M^+-$C_{29}H_{24}ClN_3O_3P$, 33%), m/z = 219 (M^+-$C_{20}H_{18}ClNO_3P$, 86%), m/z = 341 (M^+-$C_{18}H_{20}N_3O_2P$, 87%).

Synthesis of diphenyl ((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)(mesitylamino)methyl)phosphonate 12c: Yield: (0.97 g, 76%), orange solids, mp. 183 – 185°C; IR (cm^{-1}): ν_{NH} 3329, ν_{C-C} 2862, $\nu_{C=N}$ 1652, $\nu_{P=O}$ 1269, ν_{POC} 1088, ν_{C-N} 752 and ν_{C-Cl} 686.66, ^{1}HNMR (δ, ppm): 4.95 (s, 9H, CH_3), 5.04 – 5.08 (dd, 1H, CHP), 6.57 (s, 1H, -NH), 6.93 – 8.35 (m, 22H, Ar.), MS: m/z = 634 (M^+-$C_{23}H_{17}N_3O_4$, 33%), m/z = 382 (M^+-$C_{15}H_{10}N_2P$, 100%), m/z = 77 (M^+-$C_{29}H_{22}ClN_3O_3P$, 22%), m/z = 253 (M^+-$C_{20}H_{17}NO_5P$, 6%).

Synthesis of diphenyl (((4-aminophenyl)amino)(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methyl)phosphonate 12d: Yield: (0.90 g, 73%), black solids, mp. 218 – 220°C; IR (cm^{-1}): ν_{NH2} 3422, ν_{NH} 3321, $\nu_{C=N}$ 1625, $\nu_{P=O}$ 1269, ν_{POC} 1092, ν_{C-N} 756 and ν_{C-Cl} 687. ^{1}HNMR (δ, ppm): 5.38 (dd, 1H, CHP), 6.06 (s, 1H, -NH),), 6.8 (s, 2H, $-NH_2$), 7.04 – 8.34 (m, 24H, Ar.). MS: m/z = 607 ($M^+C_{24}H_{19}N_3O_4$, 52%), m/z = 293 (M^+-$C_{17}H_{16}ClN_2P$, 100%), m/z = 78 (M^+-$C_{28}H_{22}ClN_4O_3P$, 63%), m/z = 254 (M^+-$C_{19}H_{17}N_2O_3P$, 13%).

2.3. Antiproliferative Activity

***In-vitro* cytotoxic activity.** Cell culture of HCT-116 (human colorectal carcinoma), HepG2 (human liver carcinoma) and MCF-7 (human breast adenocarcinoma) cell lines were purchased from the American Type Culture Collection (Rockville, MD) and maintained in DMEM medium which was supplemented with 10% heat-inactivated FBS (fetal bovine serum), 100U/ml penicillin and 100U/ml streptomycin. The cells were grown at 37°C in a humidified atmosphere of 5% CO_2.

MTT cytotoxicity assay. The cytotoxicity activity against HCT-116, HepG2 and MCF-7 human cancer cell lines was estimated using the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, which is based on the reduction of the tetrazolium salt by mitochondrial dehydrogenases in viable cells [22 – 24]. Cells were dispensed in a 96 well sterile microplate (5×10^4 cells/well), and incubated at 37°C with series of different concentrations, in DMSO, of each tested compound or Doxorubicin (positive control) for 48 h. In a serum free medium prior to the MTT assay. After incubation, media were carefully removed, 40 μL of MTT (2.5 mg/mL) were added to each well and then incubated for an additional 4 h. The purple formazan dye crystals were solubilized by the addition of 200 μL of DMSO. The absorbance was measured at 570 nm using a Spectra Max Paradigm Multi-Mode micro plate reader. The relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells.

2.4. Statistical Analysis

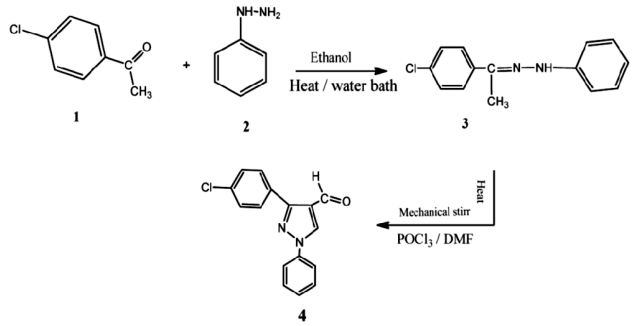
All experiments were conducted in triplicate and repeated on three different days. All values were represented as mean ± SD. The values of IC_{50} were determined by probit analysis using SPSS Inc. probit analysis software (IBM Corp., Armonk, NY, USA).

3. RESULTS AND DISCUSSION

3.1. Chemistry

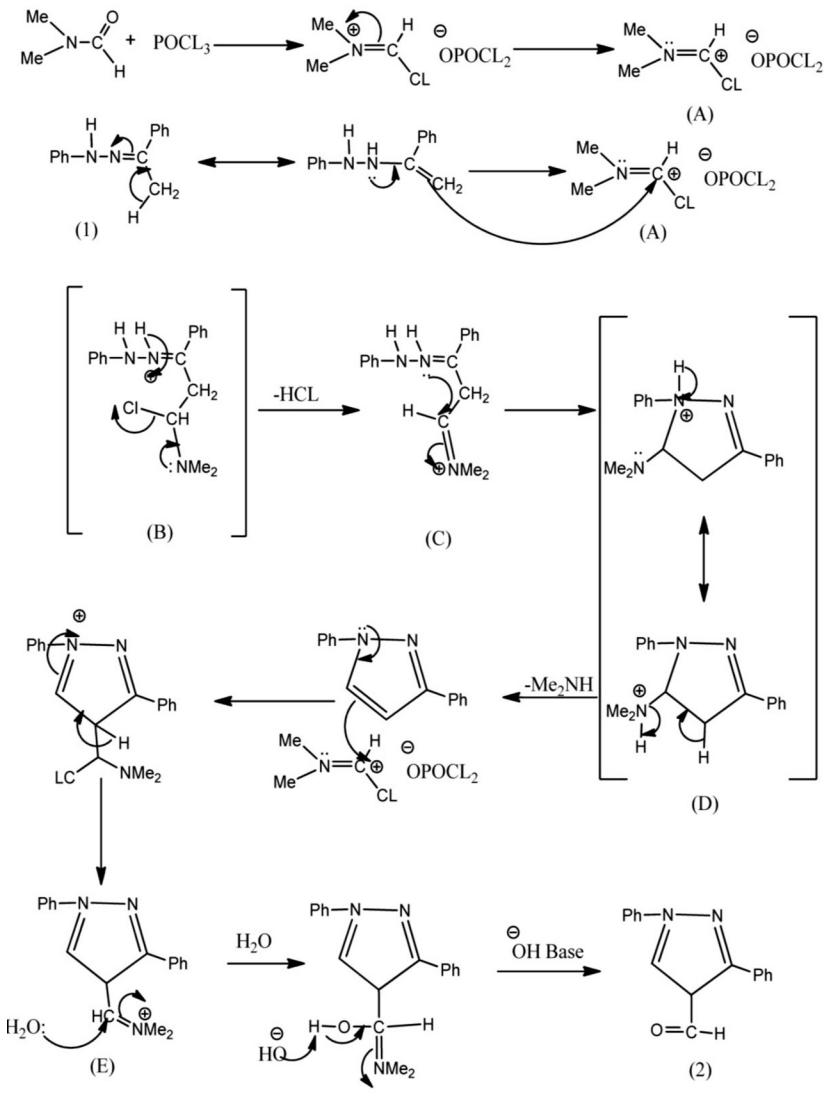
Aacetophenone hydrazone **3** was prepared in good yield by heating p-chloroacetophenone **1** with phenylhydrazine **2** in methanol under reflux for 4 – 5 h. Then, Vilsmeier-Haack (FH) reaction of hydrazone **3** using DMF and $POCl_3$ afforded the corresponding pyrazole-4-carbaldehyde **4** in good yield and high purity as depicted in Scheme 1.

Scheme 1



The structure of compound **4** was elucidated on the basis of IR, ^{1}HNMR and mass spectral analysis. In the structure prove of the aldehyde **4** a characteristic signal appeared in HNMR at delta 10.13 ppm which corresponds to the aldehydic proton (CHO), furthermore; a broad peak appeared in IR spectrum at 1667 cm^{-1} corresponds to carbonyl absorption of the aldehyde group. This in addition to the mass spectrum analysis showed the molecular ion peak of **4** at m/z 282. A plausible mechanism for cyclization along with formylation of pyrazole is outlined in (**scheme 2**)**.** The mechanism involves an initial electrophilic attack of VH reagent **A** on hydrazone **1** yielded the intermediate **B** which subsequently losses amolecule of HCl to provide intermediate **C**, the nucleophilic attack by N-H group initiates the cyclisation and the resulting pyrazole intermediate losses Me_2NH to give the more stable pyrazole derivative **D**. The pyrazole **D** react with another molecule of VH reagent **A** in an electro-

Scheme 2

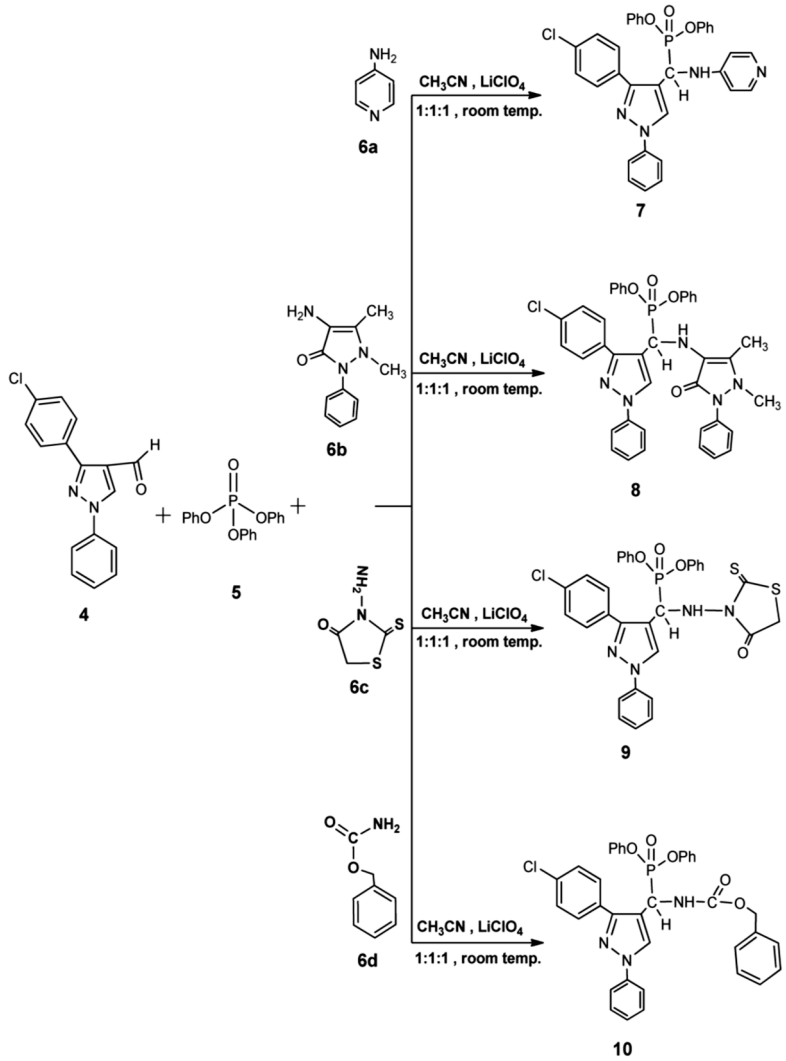


philic substitution process giving an iminium salt **E**, which is hydrolysed to corresponding 4-formyl pyrazole **2** as depicted in Scheme 2. In summary the electrophilic attack of first VH complex at the probable attacking site of hydrazones results into cyclisation. While electrophilic attack of second complex forms formyl product after hydrolysis. Finally, intramolecular (1, 5) hydrogen shift, cyclisation and elimination of $NHMe_2$ to give the corresponding aldehyde **4**.

Furthermore, the three one-pot reaction of aldehyde **4**, triphenylphosphite **5**, appropriate amine such as 4-amino-pyridine **6a**, 4-amino-antipyrine **6b**, 3-amino-Rhodanine **6c** and benzyl carbamate **6d**, in acetonitrile (3 mL), in the presence of lithium perchlorate (10 mole%) as a Lewis acid catalyst. The reaction mixture was stirred at room temperature until the starting materials were consumed as monitored by TLC (3 days). After the completion of the reaction, the precipitated product was filtered off and crystallized by using diethyl ether, to product the compound **7, 8, 9**and **10** in good yield and high purity as depicted in (Scheme 3). The structure of **7, 8, 9** and **10** was elucidated on the basis of IR, ^{1}HNMR and mass spectral analysis. In the structure prove of α-amino phosphonates **7, 8, 9** and **10** exhibited characteristic IR stretching frequencies in the region 1288 – 1291, 1117 – 1137 and 3436 – 3143 cm^{-1} for P=O, POC and N-H respectively. The P-C-H proton signal appeared as multiplets at δ 5.45 – 5.53 and the N-H proton signal appeared at δ 5.9 for N-protected α-amino phosphonates **7**. While the P-C-H proton signal appeared at δ 5.88 and the N-H proton signal appeared at δ 3.94, -CH_2 proton signal appeared at δ 4.3 for the free α-amino phosphonates **8, 9**. The P-C-H proton signal appeared as multiplets at δ 4.71 – 4.78 and the N-H proton signal appeared at δ 5.9, -CH_2 proton signal appeared at δ 5.57 for the free α-amino phosphonates **10**. This, in addition to the mass spectrum analysis, showed the molecular ion peaks of **7, 8, 9** and **10** at m/z 593,702,647 and 650.

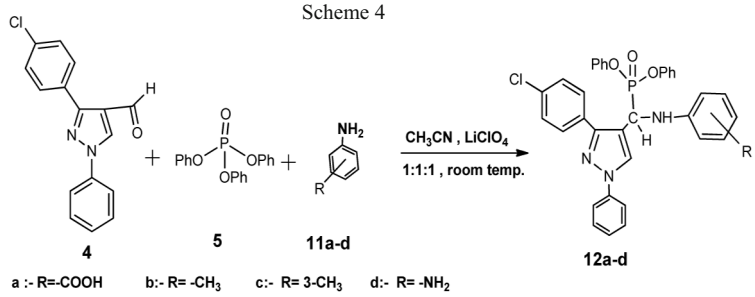
Moreover, aldehyde **4 and** triphenylphosphite **5** were converted to compounds **12a-d** when reacted with 4-amino-

Scheme 3

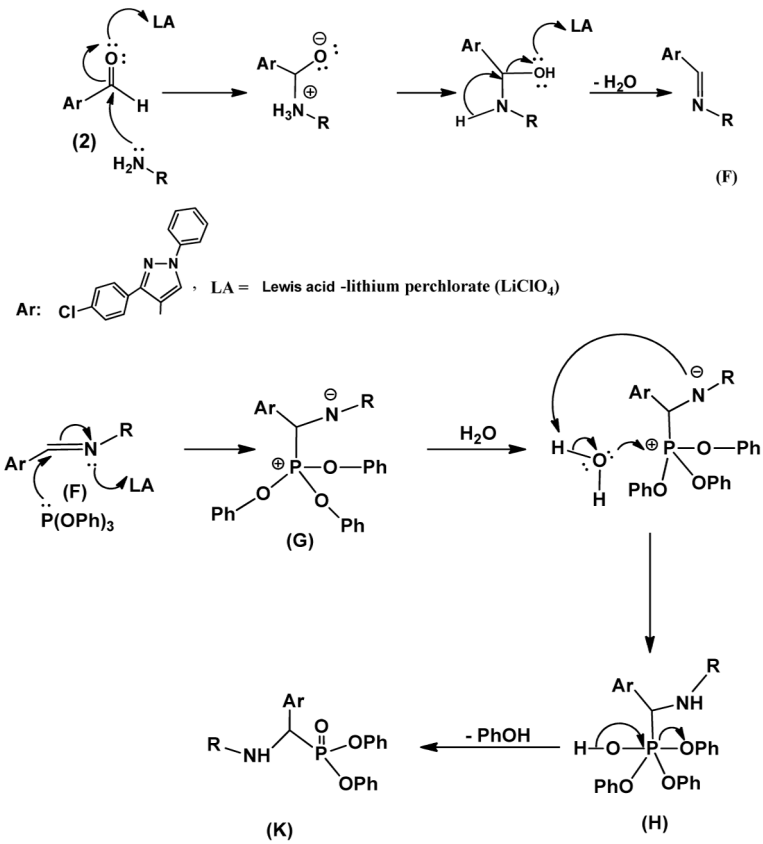


benzoic acid **11a**, p-toluidine **11b**, 2,4,6-trimethylaniline **11c** and benzene-1,4-diamine**11d**, Dissolve all the mixture in acetonitrile (3 mL), in the presence of lithium perchlorate (10 mole%) as a Lewis acid catalyst. The reaction mixture was stirred at room temperature until the starting materials were consumed as monitored by TLC (3 days). After the completion of the reaction the precipitated product was filtered off and crystallized by using diethyl ether, The structures were confirmed on the basis of IR, ^{1}H NMR and M.S spectral data according to Scheme 4**.** The structures of **12a-d** were elucidated on the basis of IR, ^{1}HNMR and mass spectral analysis. In the structure prove of α-amino phosphonates **12a, 12b, 12c** and **12d** exhibited characteristic IR stretching frequencies in the region 1254 – 1269, 1088 – 1093 and 3422 – 3291 cm^{-1} for P=O, POC and N-H respectively. The P-C-H proton signal appeared at δ 5.49 and the -COOH proton signal appeared at δ 9.66 for α-amino phosphonates **12a**. While the P-C-H proton signal appeared as multiplet at δ 5.40 – 5.45, 5.04 – 5.08 and the N-H proton signal appeared

Scheme 4



Scheme 5



at δ 5.72, 6.57 for the free α-amino phosphonates **12b, 12c**. The P-C-H proton signal appeared at δ 5.38 and the N-H proton signal appeared at δ 6.06 for the free α-amino phosphonates **12d**. This in addition to the mass spectrum analysis showed the molecular ion peak of **12a, 12b, 12c** and **12d** at m/z 636, 606, 634 and 607.

A plausible mechanism for the formation of α-amino phosphonates compounds is outlined in (**Scheme 5**). The mechanism of the reaction is believed to be following the path of activation of aldehyde **4** as well as the in situ generated imine (Schiff base) **F** by Lewis acid in the first step. This facilitates nucleophilic addition of the amino group of the carbamate component to adehyde **4** as well as the nucleophilic addition of trimethyl- or triphenyl phosphite to the polar C=N bond of imine **F** to give the phosphonium salt **G** as an intermediate. In final step, this phosphonium salt get decomposed by water liberating product **K** through the hydroxyl phosphite intermediate **H** as shown in **Scheme 5**.

3.2. In Vitro Anti-Proliferative Activity

Nine compounds were examined *in vitro* for their activity against HCT-116, HepG2 and MCF-7 human cancer cells using the MTT assay. The percentages of viable cells were calculated and compared to those of the control. Activities of these compounds against the three carcinoma cell lines were compared to the activity of doxorubicin as well. All compounds suppressed the three cancer cells in a dose-dependent manner (Figs. 1 – 3). In case of HCT-116 human colorectal carcinoma cells, both (Fig. 1 and Table 1) show that, six compounds (**7**, **12b, 9, 12a, 8,** and **12c**) were more potent cytotoxic compounds; three compounds (**10, 12d, and 4**) had significant comparable cytotoxic activities to that of doxorubicin. In case of MCF-7 human breast cancer cells, four compounds (**4, 9, 12a** and **12c**, respectively) were more potent cytotoxic compounds; two compounds (**12d** and **7**) had significant comparable cytotoxic activity to that of doxorubicin (Fig. 2 and Table 1). The three other compounds were slightly less active against MCF-7 cancer cells. In case of HepG2 human liver cancer cells, seven compounds (**9, 12b, 8, 12a, 10, 12c** and **7**) were more potent cytotoxic compounds; the two other compounds (**12d** and **4**) had significant comparable cytotoxic activity to that of doxorubicin (Fig. 3 and Table 1)**.**

3.3. Structure-Activity Relationships (SARs)

Results of the anti-proliferative activity assay of α-amino phosphonate derivatives are summarized in Table 1, along with the data of the anticancer drug doxorubicin. The cytotoxicity test of the α-amino phosphonate derivatives and their chemically modified analogues indicated a synergistic effect of the substituents at the pyrazole core structure. In case of human colon cancer and human liver cancer, one can conclude that, the anti-proliferative activity increased by the introducing the following groups: amino-pyridine, amino-an-

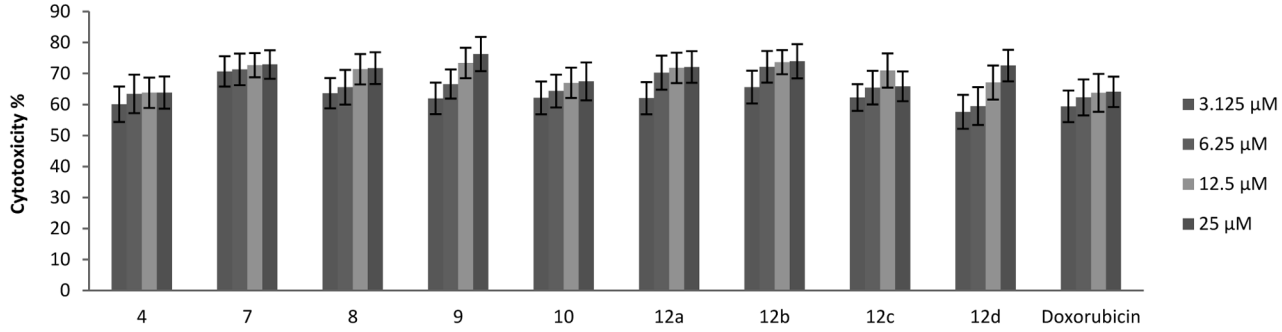


Fig. 1. Dose dependent cytotoxic activities of nine compounds against HCT-116 cancer cells according to the MTT assay.

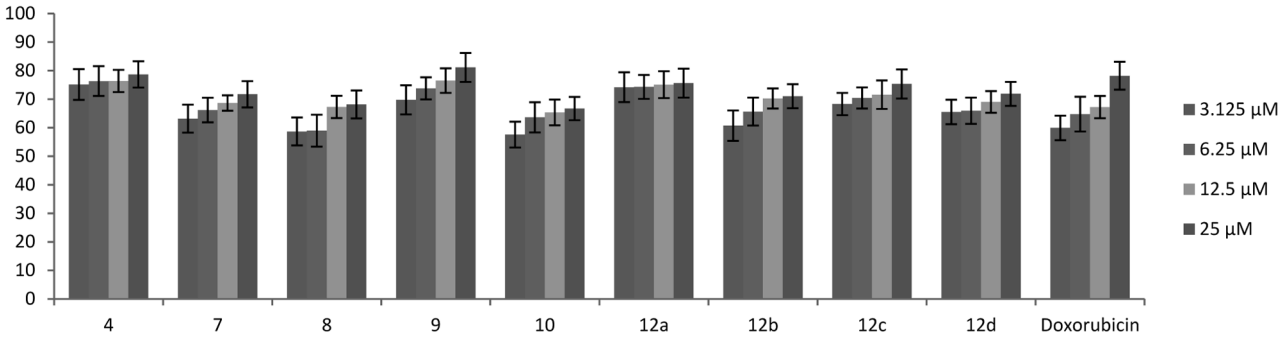


Fig. 2. Dose dependent cytotoxic activities of nine compounds against MCF-7 cancer cells according to the MTT assay.

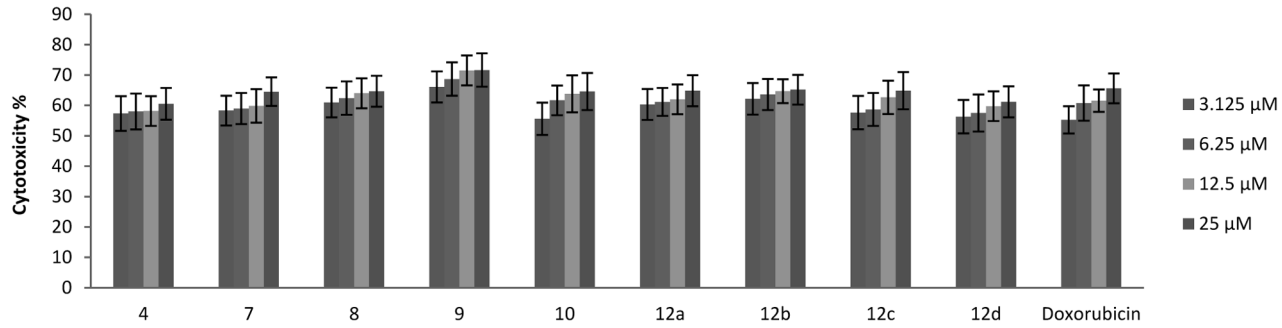
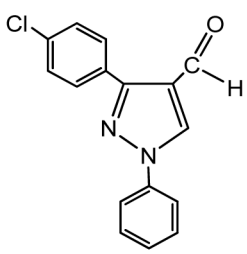


Fig. 3. Dose dependent cytotoxic activities of nine compounds against HepG2 cancer cells according to the MTT assay.

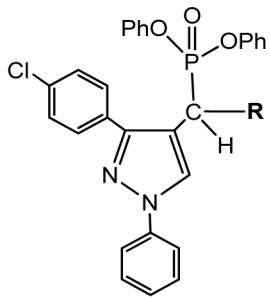
tipyrine, amino-Rhodanine, benzyl carbamate, aminobenzoic acid, p-toluidine and 2,4,6-trimethylaniline, respectively. However, introducing benzene-1,4-diamine moiety did not cause any change in the activity compared to compound **4**. Surprisingly, in case of human lung cancer, introducing the same groups caused decrease in the anti-proliferative activity compared to compound **4**. On the other hand, compound **9** showed a strong anticancer activity against all three cancer cell lines tested in this study with IC_{50} = 5.6, 3.3 and 7.3 µM for HCT-116, MCF-7 and HePG-2 cancer cell lines respectively. Moreover, products **7, 12b, 9, 12a, 8, 12c, 10,** and **12d** showed a higher activity with IC_{50} = 4.4, 4.7, 5.6, 5.9, 6.4, 7.5, 7.9 and 8.4 µM against colorectal carcinoma (HCT-116) in addition, compounds **9, 12b, 8, 12a, 10, 12c, 7** and **12d** also exhibited a strong potency with IC_{50} = 7.3, 8.6, 8.7, 9, 9.4, 9.6, 9.8 and 11.3 µM against (HePG-2) cell lines. From the above mentioned results, it is obtained that all the nine tested compounds are strong cytotoxic compounds on both human colon cancer and human liver cancer. Further variations in substituents and substitution pattern may be necessary to obtain more potent and selective anticancer lead compounds.

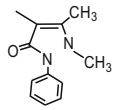
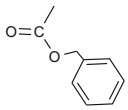
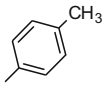
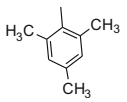
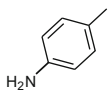
In conclusion, the present study fosters a simple and easily scalable approach for the preparation of a new series of α-amino phosphonate derivatives containing pyrazole heterocycle. All the newly synthesized compounds were

TABLE 1. IC_{50} of Nine Compounds against Three Cancer Cell Lines According to MTT assay



Aldehyde (**4**)



Compound	R	IC_{50} (μM) ± SD		
		HCT-116	MCF-7	HepG2
7		4.4 ± 2.5	7.0 ± 2.5	9.8 ± 4.1
8		6.4 ± 3.1	9.2 ± 4.1	8.7 ± 4.5
9		5.6 ± 2.4	3.3 ± 1.1	7.3 ± 2.9
10		7.9 ± 3.5	9.0 ± 3.5	9.4 ± 3.1
12a		5.9 ± 2.4	3.4 ± 2.1	9.0 ± 3.5
12b		4.7 ± 2.6	7.2 ± 3.8	8.6 ± 3.2
12c		7.5 ± 3.1	4.8 ± 2.3	9.6 ± 3.5
12d		8.4 ± 4.1	6.6 ± 3.0	11.0 ± 5.1
Aldehyde **4**		9.2 ± 3.9	2.7 ± 1.1	11.3 ± 6.5
Doxorubicin		9.4 ± 3.9	6.7 ± 2.1	10.4 ± 3.1

screened for their *in vitro* antitumor activity against three human carcinoma cell lines, namely colorectal carcinoma (HCT-116), breast carcinoma (MCF-7) and liver carcinoma (HepG-2) using MTT cytotoxicity assay at different concentrations. The results showed that all compounds possessed good antitumor activity against all cell lines. Accordingly, this class of compounds could be considered as excellent templates for future optimization or modification to obtain potent antitumor agents.

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