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Novel Synthesis, Docking studies and Antitumor Evaluation of Pyrazolo- and Pyrazolo Aminophosphonate Derivatives Derived from *N*-Heterocyclic Amines

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Abstract

Amino thiophene carbonitrile **1** reacted with chloropyrazole carbaldehyde **2a,b** to afford Schiff bases **3a,b** in good yield *via* condensation reaction. Compounds **3a,b** reacted with different primary and secondary amines to afford pyrazolo derivatives **4-10**. Also, one-pot Kabachnic-Fields reaction invloved the synthesis of α-aminophosphonates by condensation of pyrazole-5-carbaldehyde derivatives, *N*-heterocyclic amines and trialkylphosphites in moderate yields. α-Aminophosphonates were also produced in high yields (75%) by reacting Schiff bases directly with dialkylphosphites. In addition, hexaalkyl triamidophosphites were applied to the Schiff bases in THF to afford the amino derivatives in high yields. Molecular modelling investigations were studied to explore the possible binding interactions which revealed that compounds **8**, **7**, and **3b** were the most promising candidates comparing with Doxorubicin. Antitumor activity of new compounds was tested on three cell lines of liver (HepG2), breast (MCF7) carcinoma cell lines and HCT-116 cancer cells together with human healthy cell line (BJ-1) using the MTT assay.

Keywords: α-Aminophosphonate; Antitumor activity; Docking studies; Kabachnik–Fields; Pyrazole-5-carbaldehyde; Tetrahydrobenzo[*b*]thiophene

1. Introduction

Nitrogen heterocycles are a key component of biologically active natural compounds, including azoles and pyrimidines which have been among the most well-known nitrogen heterocycles. Due to their reactions with biological macromolecules such as receptors, enzymes, and nucleic acids, they are widely employed in the synthesis of biologically relevant substances. Uracil is a pyrimidine that occurs naturally and is an active component of ribonucleic acid (RNA). Uracil derivatives are the most well-known pharmacophores in medicinal chemistry especially as anti-cancer, anti-viral and anti-diabetes properties [1-3].

Pyrazoles are a family of five-membered heterocyclic compounds that are particularly valuable in organic synthesis [4, 5]. Among the azole family, they are one of the most investigated groups of chemicals. The existence of the pyrazole nucleus in various structures causes a variety of applications in different fields such as technology, medicine, and agriculture [6, 7]. They are classified as anticancer, antifungal, antibacterial, antidepressant [8], anti-inflammatory, antioxidant, antiviral, as well as anti-tuberculosis agents [9, 10].

Thiophene derivatives are widely recognized as biologically active chemicals, and many of them are used in cancer chemotherapy [11, 12]. Thiophene and its substituted derivatives are a very prominent class of heterocyclic compounds with promising medicinal chemistry applications [13, 14]. It has become an essential cornerstone for medicinal chemists in their efforts to create combinatorial library and conduct comprehensive searches for lead compounds. It has been found to have a wide spectrum of therapeutic activities with numerous applications in biomedicinal chemistry and material science, producing a considerable interest in both industry and academia [15]. It has been demonstrated that these drugs are effective in the current clinical context. They have

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considerable effective biological and physiological activities as anti-inflammatory, anti-anxiety, anti-fungal, estrogen receptor modulating, anti-cancer, anti-microbial, antioxidant, anti-mitotic, and kinases inhibiting agents[16, 17].

In the same manner, α-Aminophosphonate derivatives are important class of organophosphorus compounds that have gained a significant interest in medicinal chemistry due to their unique structural features and different applications as potent enzyme inhibitors [18], antimicrobial [19], antiviral [20], antitumor [21], and peptide mimetics agents [22]. Introducing aminophosphonate group which is considered a potent antitumor drug to a pharmacy core, could enhance the antitumor activity [23, 24]. Several aminophosphonates derivatives have revealed to be effective inhibitors of human tumors [25].

In perspective of the aforementioned considerations, the combination of a pyrazole fragment with a thiophene or uracil heterocycle and a bioactive α-aminophosphonate moiety in one molecular frame could lead to a valuable development in biological properties [26, 27].

By keeping with our interest in the synthesis of *N*-heteroaromatic Schiff bases and α-aminophosphonates [28, 29], we proposed applying pyrazolo-carbaldehyde as the aldehyde component, amino-thiophene and -uracil as the amino component with trialkyl phosphite in the one pot three-component Kabachnik-Field reaction [30] to afford a novel α-aminophosphonate derivatives.

2. Experimental

Melting points were determined on a Stuart SMP30 melting point apparatus with open capillary tubes. Analytical thin layer chromatography (TLC) was performed on precoated silica gel 60 F_{245} aluminum plates (Merck) with visualization under UV light. IR spectra (4000-400 cm^{-1}) were recorded using KBr pellets in a Jasco FT/IR 300E Fourier transform infrared spectrophotometer on a Perkin Elmer FTIR 1650 (spectrophotometer). The mass spectra were performed at 70 eV on an MS-50 Kratos (A.E.I.) spectrometer provided with a data system spectrometer (Kratos, UK). Elemental analysis was carried out at the Microanalysis Laboratory, Cairo University, Cairo, Egypt, using elementary Analysen-systeme GmbH-vario EL III Element Analyzer, Germany. NMR spectra were registered at (^{1}H: 500 MHz, ^{13}C: 125 MHz) on JEOL instruments using DMSO as solvent. Chemical shifts are given in parts per million (ppm) compared with TMS as internal standards. Coupling constants are described in Hertz (Hz).

General procedures of synthesis of *2-(((4-chloro-3-methyl-(1-phenyl)-1H-pyrazol-5-yl)methylene)amino)*-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (3a,b)

A mixture of (1 mmol, 0.178g) 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (**1**) and (1mmol) 4-chloro-3-methyl-1H-pyrazole-5-carbaldehyde (**2a,b**) in glacial acetic acid (20mL) was refluxed with stirring for 14h (TLC monitoring). After evaporation of acetic acid, the residue poured onto ice, the precipitate was filtered and recrystallized from ethanol to give **3a,b**.

2-(((4-Chloro-3-methyl-1H-pyrazol-5-yl)methylene)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (**3a**)

Product **3a** was separated as brown substance. Yield: 62%; m.p.: 87-89 ˚C (EtOH); IR (KBr) ύ$_{max}$: 3217 (NH), 2230 (CN), 1624 (N=CH) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ = 1.68-1.70 (m, 4H, 2H_2C-hexyl), 2.27 (s, 3H, H_3C), 2.62-2.83 (m, 4H, 2H_2C-hexyl), 7.15 (s, 1H, *H*C=N), 9.81 (br, s, 1H, *H*N exchangeable with D_2O) ppm; ^{13}C NMR (125 MHz, DMSO): δ 147.0 (*C*H=N), 143.3 (*C*=N, pyrazole), 142.2, 115.8 (*C*=*C*, pyrazole), 136.5,124.1 (*C*=*C*, thiophene), 110.3 (*C*N, thiophene), 136.4, 91.9 (*C*=*C*, thiophene), 25.8, 25.1, 24.2, 23.3 (*C*H_2-hexyl), 10.2 (*C*H_3) ppm. MS (EI, 70 eV): m/z (%) = 304 (40) [M^+]. Anal. for $C_{14}H_{13}ClN_4S$ (304.05): Calcd. C, 55.17; H, 4.30; N, 18.38. Found C, 55.29; H, 4.21; N, 18.26.

2-(((4-Chloro-3-methyl-1-phenyl-1H-pyrazol-5-yl)methylene)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (**3b**)

Product **3b** was separated as dark brown substance. Yield: 68%; m.p.: 102-104 ˚C (EtOH); IR (KBr) ύ$_{max}$: 3090 (CH, Ar), 2235 (CN), 1626 (N=CH) cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ = 1.69-1.71 (m, 4H, 2 H_2C-hexyl), 2.35 (s, 3H, H_3C), 2.62-2.80 (m, 4H, 2 H_2C-hexyl), 7.15-7.65 (m, 6H, H_{arom}, *H*C=N) ppm. ^{13}C NMR (125 MHz, DMSO): δ 163.5 (*C*H=N), 143.3 (*C*=N, pyrazole), 142.2, 115.8 (*C*=*C*, pyrazole), 139.9, 129.2, 128.1, 127.8, 123.1 (*C*H_{arom}), 136.5, 124.1 (*C*=*C*, thiophene), 110.3 (*C*N, thiophene), 137.0, 91.8 (*C*=*C*, thiophene), 26.5, 25.3, 24.2, 23.6 (*C*H_2-hexyl), 10.0 (*C*H_3) ppm. MS (EI, 70 eV): m/z (%) = 380 (48) [M^+]. Anal. for $C_{20}H_{17}ClN_4S$ (380.09): Calcd. C, 63.07; H, 4.50; N, 14.71. Found C, 63.18; H, 4.59; N, 14.80.

General procedures of synthesis of compounds 4-7

A mixture of equimolar amounts of compound **3a** and various amines namely; piperazine, 2-aminothiazole, 3-amino-5-methylisooxazole, or 4-aminopyridine together with 1 mmol Na_2CO_3 in 20mL DMF was refluxed for 10h (TLC monitoring). After cooling, the

mixture was poured onto ice water to afford a precipitate which was filtered and crystallized to give compounds **4-7** respectively.

2-(((3-Methyl-4-(piperazin-1-yl)-1H-pyrazol-5-yl)methylene)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (4)
Product **4** was separated as brown substance. Yield: 50%; m.p.: 246-248 ˚C (EtOH); IR (KBr) ύ$_{max}$ 3210, 3190 (2NH), 2227 (CN), 1620 (N=CH) cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ = 1.59-1.77 (m, 4H, 2H_2C-hexyl), 2.25 (s, 3H, H_3C), 2.75-2.80 (m, 2H, H_2C-hexyl), 2.81-2.86 (m, 6H, 3H_2C-piperazine, cyclohexyl), 3.29-3.31(m, 2H, H_2C-piperazine), 3.40 - 3.49 (m, 2H, H_2C-piperazine), 7.16 (s, 1H, *H*C=N), 9.8, 10.5 (br,s, 2H, 2*H*N, exchangeable with D_2O) ppm. ^{13}C NMR (125 MHz, DMSO): δ 158.1 (*C*H=N), 148.5 (=*C*-N), 147.0 (*C*-N=), 145.1 (*C*=N, pyrazole), 136.5, 124.3 (*C*=*C*, thiophene), 130.3 (*C*=*C*, pyrazole), 110.3 (*C*N, thiophene), 91.9 (*C*-C=N, thiophene), 51.9, 50.8, 45.0, 44.9 (*C*-piperazine), 26.5, 25.3, 24.2, 23.6 (*C*H_2-hexyl), 13.6 (*C*H_3) ppm. MS (EI, 70 eV): m/z (%) = 354 (48) [M$^+$]. Anal. for $C_{18}H_{22}N_6S$ (354.16): Calcd. C, 60.99; H, 6.26; N, 23.71. Found C, 60.95; H, 6.30; N, 23.75.

2-(((3-Methyl-4-(thiazol-2-ylamino)-1H-pyrazol-5-yl)methylene)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (5)
Product **5** was separated as pale yellow crystals. Yield: 60%; m.p.: 117-120 ˚C (EtOH); IR (KBr) ύ$_{max}$: 3200, 3180 (2NH), 2228 (CN), 1623 (N=CH) cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ = 1.65-1.70 (m, 4H, 2H_2C-hexyl), 2.19 (s, 3H, H_3C), 2.52-2.55 (m, 4H, 2H_2C), 6.91-7.62 (m, 3H, 3*H*C=N, H thiazole), 8.50, 10.20 (2 br, s, 2H, 2 *H*N, exchangeable with D_2O) ppm. ^{13}C NMR (125 MHz, DMSO): δ = 161.7 (*C*=N thiazole), 158.1 (*C*H=N), 148.1 (=*C*-N), 147.0 (*C*-N=), 145.1 (*C*=N, pyrazole), 137.4, 114.2 (*C*=*C* thiazole), 136.5, 124.3 (*C*=*C*, thiophene), 130.3(*C*=C, pyrazole), 110.3 (*C*N, thiophene), 91.9 (*C*-CN, thiophene), 26.5, 25.3, 24.2, 23.6 (*C*H_2-hexyl), 13.6 (CH_3) ppm. MS (EI, 70 eV): m/z (%) = 368 (43) [M$^+$]. Anal. for $C_{17}H_{16}N_6S_2$ (368.09): Calcd. C, 55.51; H, 4.38; N, 22.81. Found C, 55.35; H, 4.49; N, 22.99.

2-(((3-Methyl-4-((5-methylisoxazol-3-yl)amino)-1H-pyrazol-5-yl)methylene)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (6)
Product **6** was separated as brown powder. Yield 67%.; m.p. 198-200 ˚C (EtOH); IR (KBr) ύ$_{max}$: 3320, 3212 (2 NH), 2218 (CN), 1622 (N=CH) cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ = 1.63-1.66 (m, 4H, 2 H_2C-hexyl), 2.18 (s, 3H, H_3C), 2.28 (s, 3H, CH_3), 2.50-2.56 (m, 4H, 2 H_2C), 6.50 (s, 1H, *H*-isoxazole), 7.41(s, 1H, *H*C=N), 9.30, 10.03 (2 br, s, 2H, 2*H*N, exchangeable with D_2O). ^{13}C NMR (125 MHz, DMSO): δ 163.7 (*C*H=N), 161.1 (*C*-N, thiophene), 159.2 (*C*-Me), 148.5 (*C*-NH, isoxazole), 137.5, 126.9 (*C*=*C*, thiophene), 131.3 (*C*-Me), 124.0, 121.1 (*C*=*C*), 116.3 (*C*N, thiophene), 110.2 (*C*-CN, thiophene), 90.5 (*C*H,isoxazole) 26.5, 25.3, 24.3, 23.6 (*C*H_2- hexyl), 14.1, 12.5 (2*C*H_3) ppm. MS (EI, 70 eV): m/z (%) = 366 (36) [M$^+$]. Anal. for $C_{18}H_{18}N_6OS$ (366.13): Calcd. C, 59.00; H, 4.95; N, 22.93. Found C, 59.15; H, 4.88; N, 22.88.

2-(((3-Methyl-4-(pyridin-4-ylamino)-1H-pyrazol-5-yl)methylene)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (7)
Product **7** was separated as brown powder. Yield: 65%; m.p.: 136-138 ˚C (EtOH). IR (KBr) ύ$_{max}$: 3227, 3200 (2NH), 3090 (CH arom), 2216 (CN), 1619 (N=CH) cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ = 1.63 -1.68 (m, 4H, 2H_2C-hexyl), 2.19 (s, 3H, H_3C), 2.53 - 2.58 (m, 4H, 2H_2C-hexyl), 6.51- 6.53 (m, 2H, *H*-pyridine), 7.43(s, 1H, *H*C=N), 8.25-8.27 (m, 2H, *H*-pyridine), 9.40, 10.31 (2 br, s, 2H, 2*H*N, exchangeable with D_2O). ^{13}C NMR (125 MHz, DMSO): δ 161.8 (*C*H=N), 152.9 (*C*=N), 152.9, 147.5, 141.0, 140.4, 135.4, 130.0, 124.0, 114.1, 113.3, 91.9 (*C*=*C* pyridine), 140.3 (*C*=N), 26.2, 25.1, 24.3, 23.6 (*C*H_2-hexyl), 12.7 (*C*H_3) ppm. MS (EI, 70 eV): m/z (%) = 362 (50) [M$^+$]. Anal. for $C_{19}H_{18}N_6S$ (362.13): Calcd. C, 62.96; H, 5.01; N, 23.19. Found C, 62.88; H, 5.13; N, 23.25.

General procedures of synthesis of compounds 8-10
A mixture of 1 mmol of compound **3b** and 1 mmol of various amines namely; methyl piperazine, piperidine, or morpholine together with 1 mmol Na_2CO_3 in 20 mL DMF was refluxed for 11h (TLC monitoring). After cooling, the mixture was poured onto ice water to afford a precipitate which was filtered and crystallized to give compounds **8-10**.

2-(((3-Methyl-4-(4-methylpiperazin-1-yl)-1-phenyl-1H-pyrazol-5-yl)methylene)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (8)
Product **8** was separated as dark brown powder. Yield: 71%; m.p.: 181-183 ˚C (EtOH); IR (KBr) ύ$_{max}$: 3090 (CH, aromatic), 2218 (CN), 1625 (N=CH) cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ = 1.68-1.70 (m, 4H, 2H_2C-hexyl), 2.23 (s, 3H, H_3C), 2.35 (s, 3H, CH_3), 2.61-2.82 (m, 4H, 2H_2C-hexyl), 2.87 (m, 4H, 2H_2C-piperazine), 3.43 –3.49 (m, 4H, 2H_2C-piperazine), 7.17-7.72 (m, 6H, H_{arom}, & *H*C=N). ^{13}C NMR (125 MHz, DMSO-d_6): δ 158.8 (*C*H=N), 145.4 (*C*=N pyrazole), 147.0, 142.2, 139.9, 136.5, 130.3, 129.2, 127.8, 123.1, 124.3, 115.9, 91.9 (*C*=*C*, thiophene, pyrazole, and CH aromatic), 110.0 (*C*N, thiophene), 91.9 (*C*-CN, thiophene), 52.5, 51.3, 45.0, 44.3 (*C*-piperazine), 26.5, 25.3, 24.2, 23.6 (*C*H_2-hexyl), 12.1, 10.2 (2*C*H_3) ppm. MS (EI, 70 eV): m/z (%) = 444 (40)

[M$^+$]. Anal. for $C_{25}H_{28}N_6S$ (444.21): Calcd. C, 67.54; H, 6.35; N, 18.90. Found C, 67.44; H, 6.24; N, 18.99.

2-(((3-Methyl-1-phenyl-4-(piperidin-1-yl)-1H-pyrazol-5-yl)methylene)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (9)

Product **9** was separated as dark brown powder. Yield: 76%; m.p.: 153-155 ˚C (EtOH); IR (KBr) ύ$_{max}$: 3095 (CH, arom.), 2221 (CN), 1623 (N=CH) cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ = 1.53-1.59 (m, 4H, 2H2C-pyridine), 1.65-1.76 (m, 4H, 2*H*$_2$C-hexyl), 2.26 (s, 3H, *H*$_3$C), 2.61-2.83 (m, 4H, 2*H*$_2$C-hexyl), 3.88-3.53 (m, 6H, 3*H*$_2$C-pyridine),7.79-7.18 (m, 6H, H$_{arom}$, *H*C=N). ^{13}C NMR (125 MHz, DMSO): δ 158.8 (*C*H=N), 145.4 (*C*=N pyrazole), 147.0, 142.2, 139.9, 136.5, 130.3, 129.2, 127.8, 123.1, 124.3, 115.9, 91.9 (*C*=*C*, thiophene, pyrazole, and CH aromatic), 110.3 (*C*N, thiophene), 91.9 (*C*-CN, thiophene), 49.4, 49.3, 24.4, 23.4, 23.1 (*C*-pyridine), 26.5, 25.3, 24.2, 23.6 (*C*H$_2$-hexyl), 13.5 (*C*H$_3$) ppm. MS (EI, 70 eV): m/z (%) = 429 (60) [M$^+$]. Anal. for $C_{25}H_{27}N_5S$ (429.20): Calcd. C, 69.90; H, 6.34; N, 16.30. Found C, 69.70; H, 6.44; N, 16.19.

2-(((3-Methyl-4-morpholino-1-phenyl-1H-pyrazol-5-yl)methylene)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (10)

Product **10** was separated as brown powder. Yield: 62%; m.p.: 222-224 °C (EtOH); IR (KBr) ύ$_{max}$: 3092 (CH, aromatic), 2221 (CN), 1628 (N=CH) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ =1.66-1.75 (m, 4H, 2 *H*$_2$C-hexyl), 2.25 (s, 3H, *H*$_3$C), 2.60-2.83 (m, 4H, 2 *H*$_2$C -hexyl), 3.55-3.79 (m, 8H, 4*H*$_2$C-morpholine), 7.19-7.79 (m, 6H, H(arom), *H*C=N). ^{13}C NMR (125 MHz, DMSO): δ 158.8 (*C*H=N), 145.1 (*C*=N pyrazole), 147.0, 142.2, 139.9, 136.5, 130.3, 129.2, 127.8, 124.1, 123.2, 115.9, 91.9 (*C*=*C*, thiophene, pyrazole, and *C*H aromatic), 110.3 (*C*N, thiophene), 66.1, 66.0, 49.4, 25.4, 23.6 (*C*-morpholine), 91.9 (*C*-CN, thiophene), 26.5, 25.3, 24.2, 23.6 (*C*H$_2$-hexyl), 13.6 (*C*H$_3$) ppm. MS (EI, 70 eV): m/z (%) = 431 (49) [M$^+$]. Anal. for $C_{24}H_{25}N_5OS$ (431.18): Calcd. C, 66.80; H, 5.84; N, 16.23. Found C, 66.60; H, 5.75; N, 16.44.

General procedure for the one-pot preparation of α-aminophosphonates 11a-c

A mixture of 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (**1**) (0.48 g, 2.7 mmol), 4-chloro-3-methyl-1-phenyl-1*H*-pyrazole-5-carbaldehyde (**2b**) (0.59 g, 2.7 mmol) and trialkylphosphite (3.2 mmol) in 10 mL tetrahydrofuran (THF) containing 10 % $FeCl_3$ (or 2 mL glacial AcOH; best results with $FeCl_3$) was heated under reflux for 8-10 h. After completion of the reaction (TLC), 10 mL AcOEt was added to the mixture. The organic phase was separated, washed with 20 mL distilled water, and dried over anhydrous sodium sulfate. Solvents were evaporated under vacuum, and the residue was crystallized from the proper solvent to give compounds **11a-c**.

Dimethyl(4-chloro-3-methyl-1-phenyl-1H-pyrazol-5-yl)(3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-ylamino)methylphosphonate (11a)

Product **11a** was obtained as yellow substance. Yield: 0.87 g (64%); m.p.: 190-192 ºC (EtOH); IR (KBr) ύ$_{max}$: 3340 (NH), 2195 (CN), 1221 (P=O), 1045 (P–O–C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ = 1.81, 2.89 (2m, 8H, 4*H*$_2$C-hexyl), 2.18 (s, 3H, *H*$_3$C), 3.65 (d, $^3J_{PH}$ = 13.4 Hz, 6H, (*H*$_3$CO)$_2$P), 5.62 (dd, J_{HH} = 8.4, $^2J_{PH}$ = 16.9 Hz, 1H, *H*C–P), 7.24-7.47 (m, 5H, *H*–Ph), 9.53 (br, 1H, *H*N) ppm; ^{13}C NMR (125 MHz, DMSO): δ = 166.3 (d, $^3J_{PC}$ = 10.6 Hz, *C*-S), 150.2 (*C*=N-pyrazole), 137.8, 129.6, 126.3, 124.2 (*C*–Ph), 132.4, 126.9 (*C*=*C*-hexyl), 123.4 (d, $^2J_{PC}$ = 14.6 Hz, *C*=CCl), 115.2 (*C*N, thiophene), 113.5 (d, $^3J_{PC}$ = 10.2 Hz, *C*-Cl), 93.5 (*C*-CN, thiophene), 59.4 (d, $^1J_{PC}$ = 166.3 Hz, *C*H–P), 52.6 (d, $^2J_{PC}$ = 14.8 Hz, (*C*H$_3$O)$_2$P), 26.4, 25.1, 23.6, 22.3 (*C*H$_2$-hexyl), 15.5 (*C*H$_3$) ppm; MS (EI, 70 eV): *m/z* (%) = 490 (11) [M$^+$]. Anal. Calcd. for $C_{22}H_{24}ClN_4O_3PS$ (490.94) C, 53.82; H, 4.93; N, 11.41. Found: C, 53.98; H, 4.76; N, 11.28.

Diethyl(4-chloro-3-methyl-1-phenyl-1H-pyrazol-5-yl)(3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-ylamino)methylphosphonate (11b)

Product **11b** was obtained as yellow powder. Yield: 0.90 g (66%); m.p.: 185-187 ºC (EtOH); IR (KBr) ύ$_{max}$: 3343 (NH), 2192 (CN), 1225 (P=O), 1048 (P–O–C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ = 1.18 (dt, J_{HH} = 6.6, $^4J_{PH}$ = 4.5 Hz, 6H, (*H*$_3$CCO)$_2$P), 1.83, 2.91 (2m, 8H, 4*H*$_2$C-hexyl), 2.16 (s, 3H, *H*$_3$C), 4.23 (dq, J_{HH} = 6.6, $^3J_{PH}$ = 12.6 Hz, 4H, (*H*$_2$CO)$_2$P), 5.59 (dd, J_{HH} = 9.3, $^2J_{PH}$ = 17.8 Hz, 1H, *H*C–P), 7.26-7.46 (m, 5H, *H*–Ph), 9.65 (br, 1H, *H*N) ppm; ^{13}C NMR (125 MHz, DMSO): δ = 167.5 (d, $^3J_{PC}$ = 11.7 Hz, *C*-S), 151.9 (*C*=N-pyrazole), 138.6, 129.8, 128.2, 124.5 (*C*–Ph), 130.7, 129.1 (*C*=*C*-hexyl), 123.9 (d, $^2J_{PC}$ = 16.8 Hz, *C*=CCl), 115.9 (*C*N, thiophene), 114.2 (d, $^3J_{PC}$ = 11.6 Hz, *C*-Cl), 96.3 (*C*-CN), 62.5 (d, $^2J_{PC}$ = 14.7 Hz, (*C*H$_2$O)$_2$P), 55.8 (d, $^1J_{PC}$ = 164.7 Hz, *C*H–P), 26.2, 25.4, 23.4, 21.6 (*C*H$_2$-hexyl), 16.8 (d, $^3J_{PC}$ = 9.8 Hz, (*C*H$_3$CO)$_2$P), 14.9 (*C*H$_3$) ppm; MS (EI, 70 eV): *m/z* (%) = 519 (17) [M$^+$]. Anal. Calcd. for $C_{24}H_{28}ClN_4O_3PS$ (519.00) C, 55.54; H, 5.44; N, 10.80. Found: C, 55.65; H, 5.32; N, 10.66.

Diisopropyl(4-chloro-3-methyl-1-phenyl-1H-pyrazol-5-yl)(3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-ylamino)methylphosphonate (11c)

Product **11c** was obtained as green substance. Yield: 0.92 g (63.5%); m.p.: 165-167 ºC (EtOH); IR (KBr) ύ$_{max}$: 3349 (NH), 2196 (CN), 1227 (P=O), 1043 (P–O–C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ = 1.12, 1.33 (2dd, J_{HH} = 7.2, $^4J_{PH}$ = 5.2 Hz, 12H, ((*H*$_3$C)$_2$CHO)$_2$P), 1.85, 2.92 (2m, 8H, 4*H*$_2$C-hexyl), 2.18 (s, 3H, *H*$_3$C), 4.21 (m, 2H, (*H*CO)$_2$P), 5.68 (dd, J_{HH} = 7.9, $^2J_{PH}$ = 16.5 Hz, 1H, *H*C–P), 7.31-7.49 (m, 5H, *H*–Ph), 9.57 (br, 1H, *H*N) ppm; ^{13}C NMR (125 MHz, DMSO): δ = 169.2 (d, $^3J_{PC}$ = 10.4 Hz, *C*-S), 149.6 (*C*=N-pyrazole), 138.1, 129.5, 128.2, 124.9 (*C*–Ph), 130.4, 128.9 (*C*=*C*-hexyl), 123.1 (d, $^2J_{PC}$ = 17.5 Hz, *C*=CCl), 116.4 (*C*N, thiophene), 114.9 (d, $^3J_{PC}$ = 10.6 Hz, *C*-Cl), 95.3 (*C*-CN), 77.5 (d, $^2J_{PC}$ = 15.4 Hz, (*C*HO)$_2$P), 57.3 (d, $^1J_{PC}$ = 167.4 Hz, *C*H–P), 26.6, 25.8, 23.2, 21.8 (*C*H$_2$-hexyl), 23.9 (d, $^3J_{PC}$ = 8.9 Hz, ((*C*H$_3$)$_2$CO)$_2$P), 14.2 (*C*H$_3$) ppm; MS (EI, 70 eV): *m/z* (%) = 546 (11) [M$^+$ - 1]. Anal. Calcd. for $C_{26}H_{32}ClN_4O_3PS$ (547.05) C, 57.08; H, 5.90; N, 10.24. Found: C, 57.24; H, 5.71; N, 10.07.

Synthesis of 11a–c by reaction of 3b with dialkyl phosphites

A mixture of 0.8 g Schiff base **3b** (1.8 mmol) and 2.5 mmol dialkyl phosphites was added dropwise at 26°C on intensive stirring. After complete addition, the mixture was heated gradually from 26°C by a water bath. After completion of the reaction 6-8 h (TLC), the excess of dialkyl phosphites was evaporated under vacuum and the solid was collected, then crystallized from the proper solvent to give the corresponding phosphonates **11a–c** in higher yields: **11a**: 1.04 g (76%); **11b**: 1.02 g (74%); **11c**: 1.12 g (76.5%). Compounds **11a–c** were characterized by m.p., mixed m.p., and comparable IR spectra with the material previously obtained.

S

ynthesis of α-aminophosphonates 13a–c

A mixture of 5-aminopyrimidine-2,4(1*H*,3*H*)-dione (**12**) (0.32 g, 2.5 mmol), 4-chloro-3-methyl-1*H*-pyrazole-5-carbaldehyde (**2a**) (0.36 g, 2.5 mmol) and trialkylphosphites (3 mmol) in 10 mL tetrahydrofuran (THF) containing 10% $FeCl_3$ (or 2 mL glacial AcOH) was heated under reflux for 10–12 h. After completion of the reaction (TLC), 10 mL AcOEt was added to the mixture. The organic phase was separated, washed with 20 mL distilled water, and dried over anhydrous sodium sulfate. Solvents were evaporated under vacuum, and the residue was crystallized from the proper solvent to give compounds **13a–c**.

Dimethyl(4-chloro-3-methyl-1H-pyrazol-5-yl)(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-ylamino)methylphosphonate (13a)

Product **13a** was obtained as yellow substance. Yield: 0.56 g (61.5%); m.p.: 225-227 ºC (EtOH); IR (KBr) ύ$_{max}$: 3342-3325 (NH), 1687, 1645 (2C=O), 1225 (P=O), 1046 (P–O–C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ = 2.13 (s, 3H, *H*$_3$C), 3.56 (d, J_{HH} = 7.2, $^3J_{PH}$ = 6.3 Hz, 6H, (*H*$_3$CO)$_2$P), 4.61 (dd, J_{HH} = 8.3, $^2J_{PH}$ = 17.9 Hz, 1H, *H*C–P), 7.19, 9.51 (s, 2H, 2*H*N), 7.91 (s, 1H, *H*C), 11.25,12.31 (br, 2H, *2H*N) ppm; ^{13}C NMR (125 MHz, DMSO): δ = 165.2, 154.4 (2*C*=O), 142.8 (d, $^3J_{PC}$ = 11.6 Hz, *C*-NH), 133.2 (d, $^3J_{PC}$ = 10.7 Hz, *C*-Cl), 127.5 (*C*-CH$_3$), 119.5 (*C*H), 110.3 (d, $^2J_{PC}$ = 17.5 Hz, *C*=CCl), 53.9 (d, $^2J_{PC}$ = 15.4 Hz, (*C*H$_3$O)$_2$P), 50.3 (d, $^1J_{PC}$ = 175.4 Hz, *C*H–P), 13.9 (*C*H$_3$) ppm; MS (EI, 70 eV): *m/z* (%) = 363 (16) [M$^+$]. Anal. Calcd. for $C_{11}H_{15}ClN_5O_5P$ (363.69) C, 36.33; H, 4.16; N, 19.26. Found: C, 36.46; H, 4.01; N, 19.09.

Diethyl(4-chloro-3-methyl-1H-pyrazol-5-yl)(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-ylamino)methylphosphonate (13b)

Product **13b** was obtained as yellow substance. Yield: 0.62 g (63.3%); 247-249ºC (EtOH); IR (KBr) ύ$_{max}$: 3348-3320 (NH), 1684, 1643 (2C=O), 1229 (P=O), 1042 (P–O–C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ = 1.24 (2dt, J_{HH} = 6.6, $^4J_{PH}$ = 5.4 Hz, 6H, (*H*$_3$CCH$_2$O)$_2$P), 2.16 (s, 3H, *H*$_3$C), 4.13 (dq, J_{HH} = 6.6, $^3J_{PH}$ = 12.6 Hz, 4H, (*H*$_2$CO)$_2$P), 4.59 (dd, J_{HH} = 7.1, $^2J_{PH}$ = 18.4 Hz, 1H, *H*C–P), 7.13, 9.45 (s, 2H, 2*H*N), 7.85 (s, 1H, *H*C), 11.21,12.35 (br, 2H, *2H*N) ppm; ^{13}C NMR (125 MHz, DMSO): δ = 166.5, 155.1 (2*C*=O), 143.6 (d, $^3J_{PC}$ = 10.8 Hz, *C*-NH), 133.8 (d, $^3J_{PC}$ = 11.9 Hz, *C*-Cl), 128.2 (*C*-CH$_3$), 120.1 (*C*H), 111.8 (d, $^2J_{PC}$ = 16.2 Hz, *C*=CCl), 65.5 (d, $^2J_{PC}$ = 18.4 Hz, (*C*H$_2$O)$_2$P), 51.8 (d, $^1J_{PC}$ = 180.2 Hz, *C*H–P), 17.2 (d, $^3J_{PC}$ = 8.4 Hz, (*C*H$_3$O)$_2$P), 13.2 (*C*H$_3$) ppm; MS (EI, 70 eV): *m/z* (%) = 391 (16) [M$^+$]. Anal. Calcd. for $C_{13}H_{19}ClN_5O_5P$ (391.75) C, 39.86; H, 4.89; N, 17.88. Found: C, 40.03; H, 4.71; N, 17.69.

Diisopropyl(4-chloro-3-methyl-1H-pyrazol-5-yl)(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-ylamino)methylphosphonate (13c)

Product **13c** was obtained as yellow substance. Yield: 0.67 g (64.2%); m.p.: 235-237 ºC (EtOH); IR (KBr) ύ$_{max}$: 3348-3324 (NH), 1689, 1647 (2C=O), 1235 (P=O), 1037 (P–O–C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ = 1.11, 1.34 (2dd, J_{HH} = 7.2, $^4J_{PH}$ = 5.2 Hz, 12H, ((*H*$_3$C)$_2$CHO)$_2$P), 2.13 (s, 3H, *H*$_3$C), 4.11 (m, 2H, (*H*CO)$_2$P), 4.55 (dd, J_{HH} = 7.6, $^2J_{PH}$ = 17.8 Hz, 1H, *H*C–P), 7.16, 10.11 (s, 2H, 2*H*N), 7.79 (s, 1H, *H*C), 11.23,12.39 (br, 2H, *2H*N) ppm; ^{13}C NMR (125 MHz, DMSO): δ = 165.2, 153.4 (2*C*=O), 143.5 (*C*-CH$_3$), 134.1 (d, $^3J_{PC}$ = 10.4 Hz, *C*-NH), 131.8 (d, $^2J_{PC}$ = 17.5 Hz, *C*=CCl),119.5 (*C*H), 110.4 (d, $^3J_{PC}$ = 9.9 Hz, *C*-Cl), 78.5 (d, $^2J_{PC}$ = 19.8 Hz, (*C*HO)$_2$P), 53.3 (d, $^1J_{PC}$ = 166.4 Hz, *C*H–P), 24.3 (d, $^3J_{PC}$ = 10.4 Hz, ((*C*H$_3$)$_2$CO)$_2$P), 13.2 (*C*H$_3$) ppm; MS (EI, 70 eV): *m/z* (%) = 419 (13) [M$^+$]. Anal. Calcd. for $C_{15}H_{23}ClN_5O_5P$ (419.80) C, 42.92; H, 5.52; N, 16.68. Found: C, 42.76; H, 5.39; N, 16.54.

5-((4-chloro-3-methyl-1H-pyrazol-5-yl)methyleneamino)pyrimidine-2,4(1H,3H)-dione (14)

To a mixture of 2.5 g of 4-chloro-3-methyl-1*H*-pyrazole-5-carbaldehyde (**2a**, 17.4 mmol) and 2.2 g of 5-aminopyrimidine-2,4(1*H*,3*H*)-dione (**12**, 17.4 mmol) in 50 mL ethanol was added 0.5 mL acetic acid. The reaction mixture was refluxed for 8 h. The product mixture was concentrated to its half, followed by filtration. The collected material was crystallized from ethanol to give the Schiff base **14** as yellowish brown crystals. Yield 3.7 g (86%); m.p.: 265-267ºC (EtOH); IR (KBr) ύ$_{max}$: 3342-3325 (NH), 1687, 1645 (2C=O) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ = 2.56 (s, 3H, H_3C), 7.53, 10.56 (s, 2H, 2*H*N), 7.97 (s, 1H, *H*C), 11.85 (s, 1H, *H*C),13.45 (br, 1H, *H*N) ppm; ^{13}C NMR (125 MHz, DMSO): δ = 163.5, 152.4 (2*C*=O), 143.8 (*C*H=N), 140.7 (*C*-CH_3), 134.5 (*C*H-NH), 132.3 (*C*-Cl), 116.5 (*C*-N), 109.7 (*C*=CCl), 13.9 (*C*H_3) ppm; MS (EI, 70 eV): *m/z* (%) = 253 (20) [M$^+$]. Anal. Calcd. for $C_9H_8ClN_5O_2$ (253.65) C, 42.62; H, 3.18; N, 27.61. Found: C, 42.78; H, 2.99; N, 27.46.

Synthesis of 13a–c by reaction of Schiff base 14 with dialkyl phosphites

A mixture of 0.8 g Schiff base **14** (1.8 mmol) and 2.5 mmol dialkyl phosphites was added dropwise at 26°C on intensive stirring. After complete addition, the mixture was heated gradually from 26°C by a water bath. After completion of the reaction (TLC), the excess of dialkyl phosphites were evaporated under vacuum and the solid was collected, then crystallized from the proper solvent to give the corresponding phosphonate **13a–c** in higher yields: **13a**: 0.74 g (76%); **13b**: 0.67 g (74%); **13c**: 0.77 g (73.5%). Compounds **13a–c** were characterized by m.p., mixed m.p., and comparable IR spectra with the material previously obtained.

Synthesis of 15, 16a,b by reaction of the Schiff base 3b,14 with hexaalkyltriamidophosphites

Hexalkylphosphorus triamide (5.2 mmol) in 10 mL dry THF was added dropwise to 1.48, 0.98 g of Schiff base **3b**, or **14** (3.9 mmol) in 10 mL dry THF. The reaction mixture was stirred at r.t. for 4 h (TLC). The precipitate was collected and washed several times with light petroleum (40–60 °C) and crystallized from the proper solvent to give compounds **15a,b** and **16a,b**.

2-((4-(dimethylamino)-3-methyl-1-phenyl-1H-pyrazol-5-yl)methyleneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (15a)

Product **15a** was obtained as yellowish green substance. Yield: 1.2 g (78.5%); m.p.: 210-212ºC (EtOH); IR (KBr): ύ$_{max}$: 2199 (CN) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ = 1.75, 2.89 (2m, 8H, 4H_2C-hexyl), 2.55 (s, 3H, H_3C), 3.11 (s, 6H, 2H_3C), 7.24–7.47 (m, 5H, *H*–Ph), 9.26 (s, 1H, *H*C) ppm; ^{13}C NMR (125 MHz, DMSO): δ = 164.7 (*C*-N), 163.3 (*C*H=N), 153.6 (*C*-N(CH_3)$_2$), 151.2 (*C*-CH_3), 140.1, 129.8, 127.5, 124.6 (*C*–Ph), 135.2, 126.2 (*C*=*C*-hexyl), 116.5 (*C*N), 110.1 (*C*-CN), 102.4 (*C*-C=N), 43.1 ((*C*H_3)$_2$N), 26.3, 25.5, 23.6, 21.4 (*C*H_2-hexyl), 13.2 (*C*H_3) ppm; MS (EI, 70 eV): *m/z* (%) = 389 (25) [M$^+$]. Anal. Calcd. for $C_{22}H_{23}N_5S$ (389.52) C, 67.84; H, 5.95; N, 17.98. Found: C, 67.84; H, 5.77; N, 17.83.

2-((4-(diethylamino)-3-methyl-1-phenyl-1H-pyrazol-5-yl)methyleneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (15b)

Product **15b** was obtained as yellowish green substance. Yield: 1.3 g (81.5%); m.p.: 225-227 ºC (EtOH); IR (KBr): ύ$_{max}$: 2193 (CN) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ = 1.09 (2dt, J_{HH} = 6.9, 6H, 2H_3C-CH_2),1.74, 2.92 (2m, 8H, 4H_2C-hexyl), 2.56 (s, 3H, H_3C), 2.68 (2dq, J_{HH} = 6.9, 4H, 2H_2CCH_3), 7.24–7.43 (m, 5H, *H*–Ph), 9.23 (s, 1H, *H*C) ppm; ^{13}C NMR (125 MHz, DMSO): δ = 163.9 (*C*-N), 159.1 (*C*H=N), 152.4 (*C*-N(CH_3)$_2$), 149.7 (*C*-CH_3), 140.5, 129.7, 127.2, 124.1 (*C*–Ph), 134.7, 126.7 (*C*=*C*-hexyl), 116.8 (*C*N), 110.5 (*C*-CN), 102.7 (*C*-C=N), 45.2 (*C*H_2N), 26.5, 25.3, 23.8, 21.4 (*C*H_2-hexyl), 13.5 (*C*H_3), 12.1((*C*H_3CH_2)N) ppm; MS (EI, 70 eV): *m/z* (%) = 417 (19) [M$^+$]. Anal. Calcd. for $C_{24}H_{27}N_5S$ (417.57) C, 69.03; H, 6.52; N, 16.77. Found: C, 69.15; H, 6.36; N, 16.64.

5-((4-(dimethylamino)-3-methyl-1H-pyrazol-5-yl)methyleneamino)pyrimidine-2,4(1H,3H)-dione (16a)

Product **16a** was separated as yellow crystals. Yield: 0.84 g (83%); m.p.: 185-187ºC (EtOH); IR (KBr) ύ$_{max}$: 3342, 3325 (NH), 1687, 1645 (2C=O) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ = 2.43 (s, 3H, H_3C), 3.15 (s, 6H, 2H_3C), 7.34, 10.46 (s, 2H, 2*H*N), 7.94 (s, 1H, *H*C), 11.73 (s, 1H, *H*C),13.35 (br, 1H, *H*N) ppm; ^{13}C NMR (125 MHz, DMSO): δ = 161.6, 152.7 (2*C*=O), 149.5 (*C*NMe_2), 145.7 (*C*-CH_3), 143.9 (*C*H=N), 134.8 (*C*H-NH), 119.5 (*C*-N), 93.7 (*C*-CMe), 41.5 ((*C*H_3)$_2$N), 13.3 (*C*H_3) ppm; MS (EI, 70 eV): *m/z* (%) = 262 (11) [M$^+$]. Anal. Calcd. for $C_{11}H_{14}N_6O_2$ (262.27) C, 50.38; H, 5.38; N, 32.04. Found: C, 50.54; H, 5.26; N, 31.89.

5-((4-(diethylamino)-3-methyl-1H-pyrazol-5-yl)methyleneamino)pyrimidine-2,4(1H,3H)-dione (16b)

Product **16b** was separated as yellow crystals. Yield: 0.95 g (85%); m.p.: 198-200ºC (EtOH); IR (KBr) ύ$_{max}$: 3347, 3326 (NH), 1690, 1651 (2C=O) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ = 1.12 (2dt, J_{HH} = 7.5, 6H,

2H_3C-CH_2), 2.47 (s, 3H, H_3C), 2.83 (2dq, J_{HH} = 7.5, 4H, 2H_2CCH_3), 7.38, 10.66 (s, 2H, 2*H*N), 7.86 (s, 1H, *H*C), 11.37 (s, 1H, *H*C),13.24 (br, 1H, *H*N) ppm; ^{13}C NMR (125 MHz, DMSO): δ = 161.3, 152.5 (2*C*=O), 149.3 (*C*N(CH_3)$_2$), 138.6 (*C*-CH_3), 137.5 (*C*H=N), 135.1 (*C*H-NH), 119.1 (*C*-N), 93.1 (*C*-C-CH_3), 46.5 (CH_2N), 13.5 (CH_3), 12.1 ((CH_3CH_2)$_2$N), ppm; MS (EI, 70 eV): m/z (%) = 290 (16) [M^+]. Anal. Calcd. for $C_{13}H_{18}N_6O_2$ (290.32) C, 53.78; H, 6.25; N, 28.95. Found: C, 53.64; H, 6.08; N, 28.82.

Materials and Methods

Cell culture conditions

HCT-116 (human colorectal carcinoma), human liver carcinoma (HepG-2), MCF-7 (human breast adenocarcinoma) and the normal human skin fibroblast (BJ-1) cell lines were purchased from the American Type Culture Collection (Rockville, MD, USA) and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U mL^{-1} penicillin, and 100 U mL^{-1} streptomycin. The cells were grown at 37 °C in a humidified atmosphere of 5% CO_2.

MTT antiproliferative assay

The antiproliferative activities on the HepG-2, HCT-116, MCF-7 and BJ-1 was estimated by the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. This test is based on MTT cleavage by mitochondrial dehydrogenases form viable cells [31,32]. Cells were placed in a 96 well sterile microplate (5 x 10^4 cells $well^{-1}$) and incubated at 37 °C in serum-free media containing dimethyl sulfoxide (DMSO) and either a series of various concentrations of each compound or doxorubicin (positive control) for 48 h before the MTT assay. After incubation, the media were removed and 40 µL MTT (2.5 mg mL^{-1}) was added to each well. Incubation was resumed for an additional 4 h. The purple formazan dye crystals were solubilized with 200 µL DMSO. Absorbance was measured at 590 nm in a Spectra Max Paradigm Multi-Mode microplate reader (Molecular Devices, LLC, San Jose, CA, USA). Relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells. All experiments were conducted in technical triplicate and three biological replicates. All values were reported as mean ± SD. IC_{50} were determined by SPSS Inc probit analysis (IBM Corp., Armonk, NY, USA).

3. Results and Discussion

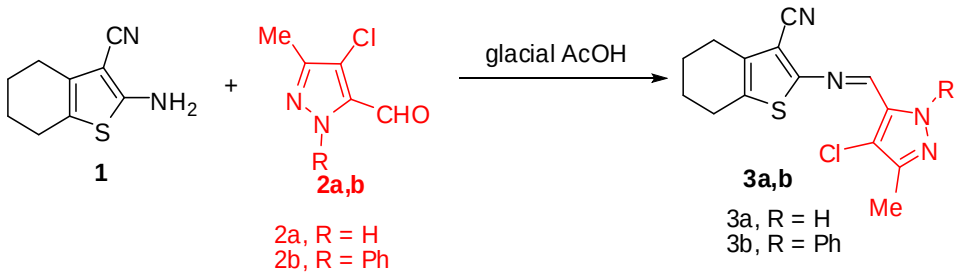
Compound **3a,b** were simply synthesized *via* one pot reaction of an equimolar amount of 2-amino benzo[*b*]thiophene-3-carbonitrile **1** and pyrazole-5-carbaldehydes **2a,b** in glacial acetic acid as illustrated in Scheme 1. Compounds **3a,b** were elucidated according to the elemental and spectroscopic analyses (*cf.* Experimental).

The most important feature of compound **3a** as an example, is the presence of signals at 3217 (NH), 2230 (CN), 1624 (N=CH) cm^{-1} in its IR spectra. Also, the ^{1}H NMR spectrum of compound **3a** (at 400 MHz) revealed signals at δ_H = 9.81 (br, 1H, exchangeable NH), 7.15 (s, 1H, CH=N), 2.62-2.83 (m, 4H, CH_2 cyclohexyl), 2.27 (s, 3H, CH_3), 1.68-1.70 (m, 4H, CH_2 cyclohexyl) ppm. Also, the structure of compound **3a** has been assigned by ^{13}C NMR, elemental analysis, and mass spectral data (Experimental section).

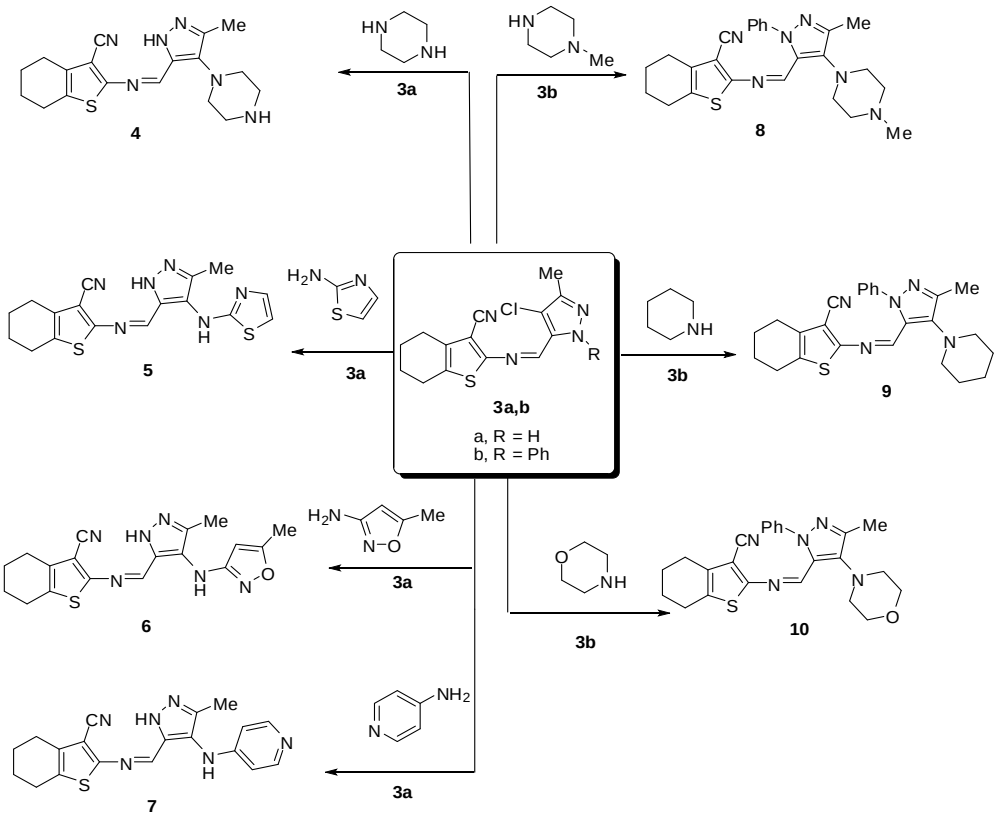
The reactions of **3a** with piperazine, 2-amino-thiazole, 2-amino-5-methylisooxazole, and 4-aminopyridine at DMF in the presence of sodium carbonate were also examined. When **3a** was treated with equivalent moles of amino derivatives in boiling DMF, products **4-7** were isolated (Scheme 2). Compounds **4–7** have assigned based on spectroscopic data (IR, ^{1}H, ^{13}C NMR, and mass spectrum (MS)) and elemental analyses (cf. Experimental section).

In the same manner, compound **3b** reacted with *N*-methylpiperazine, piperidine or morpholine under the same conditions to afford products **8-10**. The elemental and spectroscopic data are agreed with the expected structures **8-10** (Scheme 2).

The IR spectrum of **10** revealed the presence of strong absorption bands at 3092 (CH, aromatic), 2221 (CN), 1628 (N=CH). ^{1}H NMR (500 MHz, DMSO) of compound **10** revealed signals at δ = 7.79-7.19 (m, 6H, H_{arom}, N=CH), 3.79 – 3.55 (m, 8H, CH_2 morpholine), 2.83–2.60 (m, 4H, CH_2 cyclohexane), 2.25 (s, 3H, CH_3), 1.75-1.66 (m, 4H, CH_2 cyclohexane). Also, ^{13}C NMR, and mass spectral data as well as elementary analysis were compatible with the suggested products (*cf.* Experimental Section).



Scheme 1: Synthesis of Schiff bases 3a,b



Scheme 2: Synthesis of Schiff bases 4-10

On the other hand, the required aminophosphonates **11a-c** were obtained from mixing three components *via* Kabachnic-Fields reaction; aldehyde **2b** with amine **1** in dry THF in the presence of 10% $FeCl_3$, then trialkyl phosphites (TAPs) were added at r.t., followed by heating under reflux for ~10h, (Mannich type reaction) (Scheme 3). Compounds **11a-c** were elucidated by ^1H, ^{13}C and IR spectroscopic data.

IR spectum (KBr, cm^{-1}) of dimethyl(4-chloro-3-methyl-1-phenyl-1*H*-pyrazol-5-yl)(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-ylamino)methylphosphonate (**11a**) is showed the P=O stretching band at 1221 cm^{-1} . The ^1H NMR of **11a** exhibited a doublet of doublet at 5.62 ppm (J_{HH} = 8.4, $^2J_{PH}$ = 16.9 Hz) refered to the proton at exocyclic asymmetric chiral carbon. (*cf.* Experimental).

Mechanism of this reaction is proposed *via* the Kabachnik- Fields reaction [33-35]; the first stage is the codensation of aldehyde with amine in the presence of $FeCl_3$ to afford Schiff base intermediate, at second stage, addition of TAPs to obtian the aminophosphonates **11a-c**. Acidic medium of $FeCl_3$ hydrolyzed TAPs to their dialkyl phosphite (DAPs) reagents.

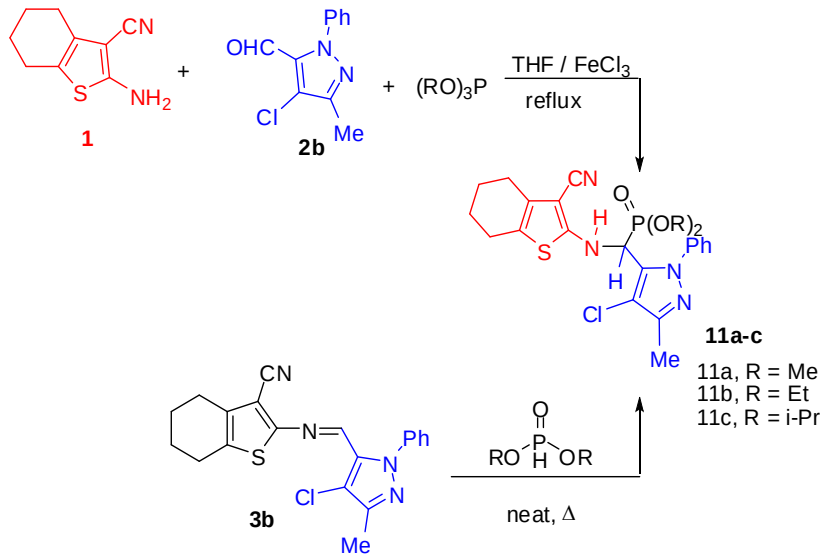
According to this mechanism, compounds **11a-c** were also obtained in higher yields (~76%) by treating Schiff base **3b** with DAPs at 100 °C in neat condition to give the required α-aminophosphonate.

Furthermore, α-aminophosphonates **13a-c** were prepared in moderate yield (~ 60%) by combining the amine **12** with the aldehyde **2a** in the same previous conditions, then TAPs were added at r.t., followed by heating for ~ 12 hours (Scheme 4).

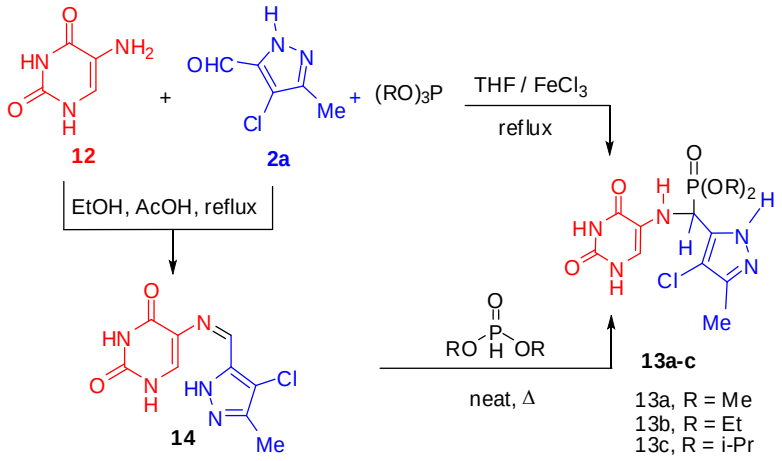
Similarly, compounds **13a-c** were also obtained in good yields and identified by treating **14** with DAPs at 100 °C in absence of solvent. The suggested structures are in good agreement with their spectral and analytical data (Scheme 4).

By using hexaalkyltriamidophosphites on the Schiff base **3b** or **14**, the experiment was expanded to create further substituted heterocyclic derivatives. So when Schiff base **3b** or **14** was reacted with tris(dialkylamino)phosphines in THF at ambient temperature, phosphorus-free compounds **15a,b** and **16a,b** were obtained (Scheme 5).

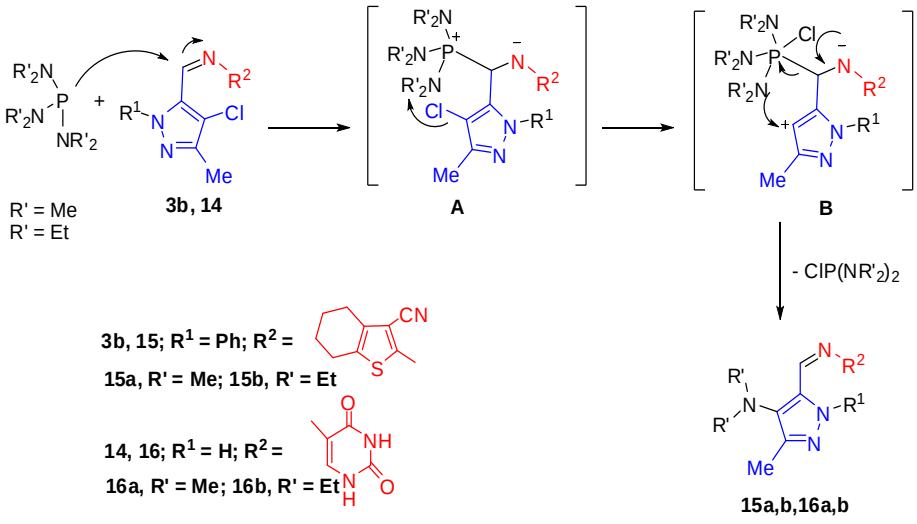
The mechanism of this reaction is represented in (Scheme 5). Anils **3b** or **14** are initially attacked by the nucleophilic phosphine-phosphorus atom which afford the structure of betaine [**A**][36]. Because phosphonium ions have a high affinity for halides, the synthesis of transitory betaine of type [**B**] would be facilitated. Because of its bulkiness, the phosphorus reagent can be acted as a good leaving group, [**B**] is dissociated to produce **15**, **16a,b**. Compounds **15**, **16a,b** have spectroscopic and analytical data to back up the proposed mechanism.



Scheme 3: Synthesis of α-aminophosphonates 11a-c via TAPs and DAPs



Scheme 4: Synthesis of α-aminophosphonates 13a-c via TAPs and DAPs



Scheme 5: Synthesis of Dialkylamino derivatives 15, 16a,b

Molecular Modelling Studies

To explore possible binding interactions, studies of molecular modelling were carried out by randomly docking the most active compounds to the Protein quinone reductase-2 (4ZVM) using AutoDock v4.2 as indicated in **Table1**. The results indicated that all tested compounds displayed good to excellent binding affinities toward the target protein 4ZVM with values ranging from -9.66 to -7.75 kcal/mol. The lowest binding energies of -9.66, -8.99, and -8.83 kcal/mol, respectively, indicated that compounds **8**, **7**, and **3b** were the most promising choices when compared with Doxorubicin (Table 1).

The standard Doxorubicin demonstrated hydrogen bond interactions with GLY149, GLU193 and ASN161 as well as four hydrophobic interactions with TRP105, PHE126 and PHE178 residues, according to docking analysis (Table 1). The most active compound **8**, exhibited one hydrogen bond interactions with GLY149 (1.95 Å bond length) and five hydrophobic interactions with TRP105, PHE106, PHE178, TYR104 and PRO102 residues (Table 1). Also compound **3b** showed one hydrogen bond interactions with ASN161 (2.13 Å bond length) and eight hydrophobic interactions with six different amino acid residues among them Pi-Pi T-shaped interaction with PHE178 like the Doxorubicin (Table 1). Compared with Doxorubicin binding mode, compound **7** established a distinct network of interactions at the molecular level, including H-bonds, π−σ interactions, and atomic charge with the receptor 4ZVM residues when studied in 2D plots this could explain the high binding affinity as depicted in Figure 7. The other tested compounds also revealed good and comparable binding interactions within the target protein active site 4ZVM (Figures 1-6).

The Protein Data Bank (PDB ID 4ZVM) was used to obtain the X-ray crystal structures of Protein quinone reductase-2 in interaction with Doxorubicin [37]. The selected protein was prepared using Discovery Studio v3.1 by eliminating the Ligands and water molecules [38]. AutoDock Tools v4 was used to add hydrogen atoms, merge non-polar hydrogen atoms, and repair lost atoms [39]. The structures of synthesized compounds were built and saved in their three-dimensional structural conformation using Chem Bio-3D v13 (CambridgeSoft, Cambridge, MA, USA). To achieve a lowest energy conformation, compounds were subsequently minimized using Hyperchem Pro V6.0 (MM+ geometry optimization). Selected compounds were docked on the target receptor 4ZVM randomly with AutoDock v4.0 by using a hybrid Lamarckian Genetic Algorithm. The complex of Ligand-protein interactions were visualized using Discovery Studio v3.1.

Table 1 Molecular docking analysis

Compound	binding affinity kcal/mol	Interacting Amino Acids
3b	-8.83	PHE126 , PHE131, ILE128, PHE178, MET154, ASN161
5	-7.75	TRP105, PHE106, PHE126, PHE178, GLN122, CYS121, HIS72,VAL69
7	-8.99	TYR104, TRP105, PHE106, LEU103, PRO102, GLY68, ASN66, TYR67, GLU193, PHE178, THR147, THR148, TYR155, GLY150
8	-9.66	PRO102, TRP105, PHE106, GLY149, TYR104, PHE178
10	-8.44	ASN161, VAL160, MET154, PHE178, PHE126, PHE131, ILE128, TRP105
11a	-8.13	ASN161, PHE178, PHE126, PHE131, ILE128, VAL160, MET154
11b	-8.19	TYR155, ILE128, PRO102, TRP105, PHE126, PHE178
11c	-8.66	GLU193, ILE128, PRO102, TRP105, PHE126, PHE178
Doxorubicin	-8.68	TRP105, PHE126, GLY149, GLY68, GLU193, ASN161, GLY150, PHE178

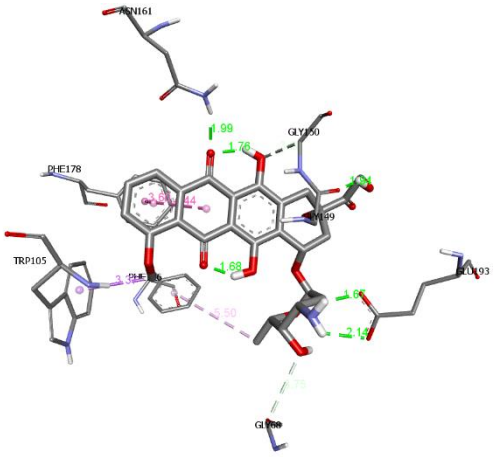


Figure 1: Docking poses of Doxorubicin on the active site of 4ZVM

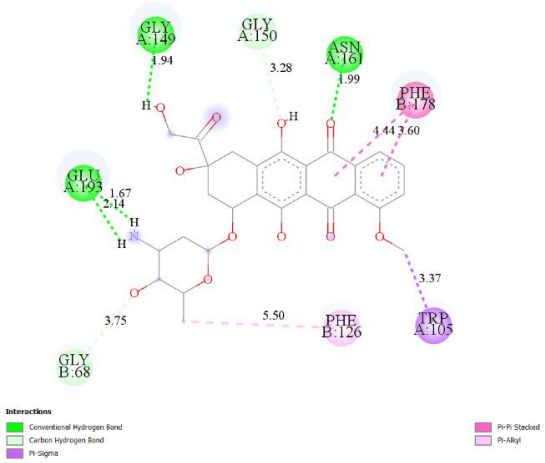


Figure 2: 2D interaction representation of Doxorubicin complex with 4ZVM

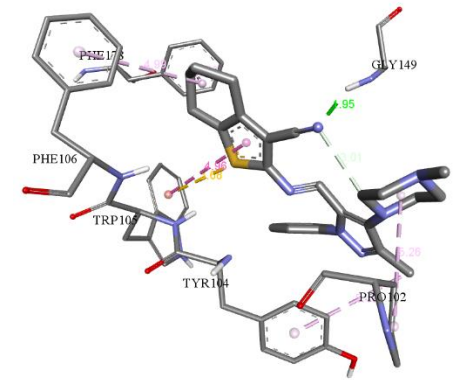


Figure 3: Docking poses of compound **8** on the active site of 4ZVM

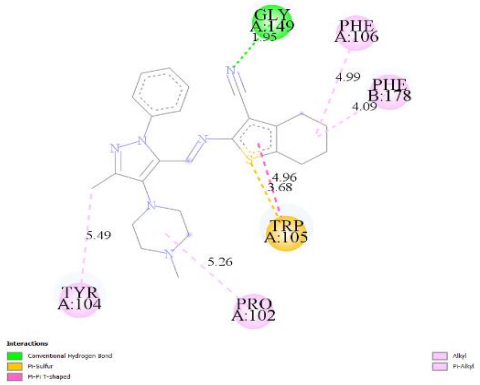


Figure 4: 2D interaction representation of **8**-complex with 4ZVM

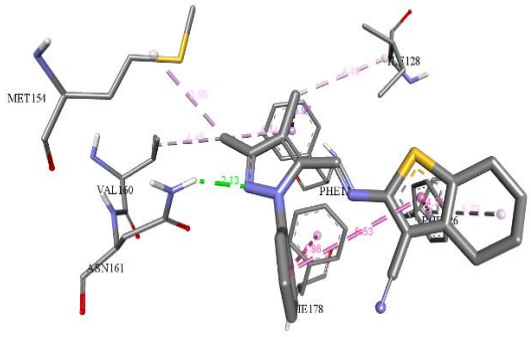


Figure 5: Docking poses of compound **3b** on the active site of 4ZVM

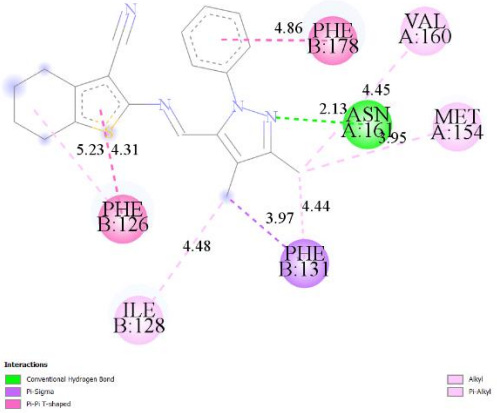


Figure 6: 2D interaction representation of **3b**-complex with 4ZVM

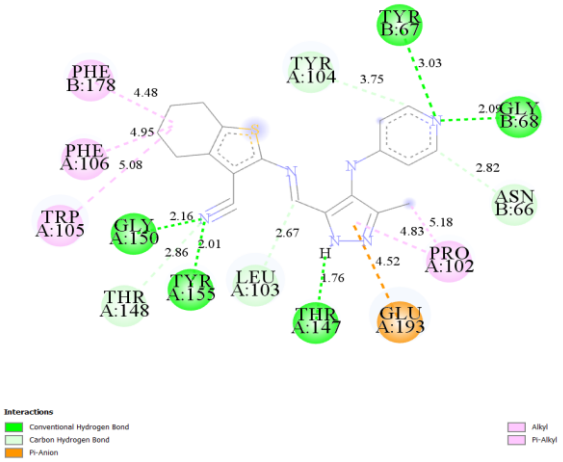


Figure 7: 2D interaction representation of **7**-complex with 4ZVM

Antiproliferative activity

Twelve compounds were tested *in vitro* for their activity against MCF-7, HepG2 and HCT-116 human cancer cells together with one human healthy cell line (BJ-1) using the MTT assay. The percentages of intact cells were measured and compared to the control. The activities of these compounds were compared with Doxorubicin's activity against three cancer cell lines. In a dose-dependent manner, all compounds inhibited both cancer cells (Figures 8-10).

In case of HCT-116 human colorectal carcinoma cells: both Figure 8 and Table 2 exhibited that six compounds (**8**, **11a**, **10**, **11b**, **5** and **7**, respectively) have more potent cytotoxic activity compared with Doxorubicin as standard drug; compounds **11c** and **3b** have moderate with insignificant cytotoxic activity; while compounds **13a,b, 15a**, and **16b** have significantly less cytotoxic activity against HCT-116 relative to that of doxorubicin.

Compounds **8**, and **5** is more potent than Doxorubicin on MCF-7 human breast cancer cells: while **7**, **3b** and **11a**, respectively have insignificantly less cytotoxic activity; and seven compounds **11b,c, 10, 16b, 15a,** and **13a,b** have less cytotoxic activity against MCF-7 relative to the reference drug (**Figure 9** and **Table 2**).

Seven compounds **11c**, **10**, **5**, **11b**, **11a**, **8** and **3b**, respectively have more potent cytotoxic activities on HepG2 human liver cancer cells, while compounds **16b, 7, 15a,** and **13a,b** have significantly less cytotoxic activity relative to that of doxorubicin (**Figure 10** &**Table 2**).

Structure Activity Relationship

In case of HCT-116 human cancer cells: six compounds (**8, 11a, 10, 11b, 5** and **7**) have more potent cytotoxic activities (6.7, 10.1, 11.1, 11.3, 18.4 and 21.1 respectively) in comparison with Doxorubicin (21.8), as a standard drug, compound **8** that is more active than compound **10** may be related to *N*-methyl pipredine group [40]. Also, compound **5** more active than compound **7** may be related to thiazole ring [41].

In case of MCF-7 human breast cancer cells: compounds **8**, **5** is more potent than Doxorubicin human breast cancer cells (15.9, 16.7 and 16.7, respectively).

In case of HepG2 human liver cancer cells: compounds (**11c, 10, 5, 11b, 11a, 8** and **3b**) have more potent activities (38.8, 39.9, 42.5, 45.6, 46.9, 49.4 and 52.2, respectively) on HepG2 human liver cancer cells as compared with Doxorubicin (63.2). **11c** is slightly more active than **11b** may be due to the presence of isopropyl group [42]. Three compounds **11b, c** and **10** are selectively active on HCT-116 and HepG2 human cancer cells, but not active on MCF-7 human breast cancer cells. Compound **7** is selectively active on human colon and breast cancer cells, but not active on human liver cancer cells. Four compounds **11a, 3b, 5** and **8** are selectively active on all three human cancer types.

So, we can conclude that:

- Three compounds **10**, and **11b,c** are selectively active on both human liver and colon cancer types but not active on human breast cancer type.
- Compound **7** is selectively active on human colon and breast cancer types but not active on human liver cancer type.
- Four compounds **3b**, **5**, **8**, and **11a** are not selectively active on all the three human cancer types.
- All compounds were tested against non-tumor fibroblast derived cell line (BJ) and demonstrated very low cytotoxicity.

Table 2: The antiproliferative IC_{50} of the twelve compounds against the three cancer cell lines according to the MTT assay

Compound Code	IC_{50} (µM) ± SD HCT-116	HepG-2	MCF-7
11a	10.1 ± 1.5	46.9 ± 4.6	19.8 ± 2.3
11b	11.3 ± 1.9	45.7 ± 3.9	20.5 ± 2.1
11c	24.8 ± 2.4	38.8 ± 3.2	20.6 ± 3.1
15a	38.4 ± 3.7	92.7 ± 5.9	25.1 ± 3.5
13a	49.1 ± 4.1	105.1 ± 6.8	27.2 ± 3.1
16b	40.3 ± 5.1	81.4 ± 5.9	21.2 ± 2.7
13b	33.9 ± 3.5	109.1 ± 6.8	25.3 ± 3.3
3b	27.3 ± 2.1	52.2 ± 4.7	18.8 ± 1.9
5	18.4 ± 1.9	42.5 ± 3.5	16.7 ± 1.5
7	21.1± 2.1	88.9 ± 5.1	17.3 ± 1.4
8	6.7 ± 0.7	49.4 ± 3.7	15.9 ± 2.1
10	11.1 ± 1.1	39.9 ± 4.1	20.6 ± 3.1
Doxorubicin	21.8 ± 2.9	63.2 ± 5.8	16.7 ± 1.5

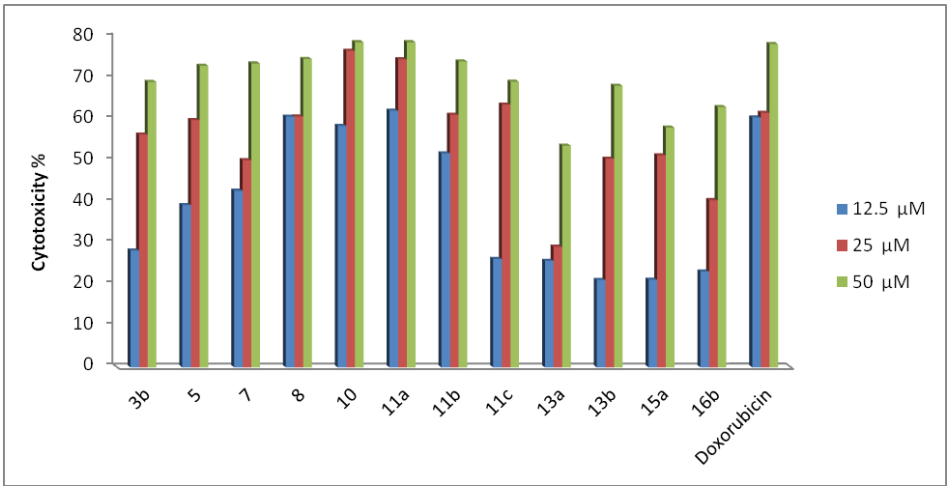


Figure 8: Dose dependent antiproliferative data of the twelve compounds against HCT-116 cancer cells according to the MTT assay after 48 h of exposure

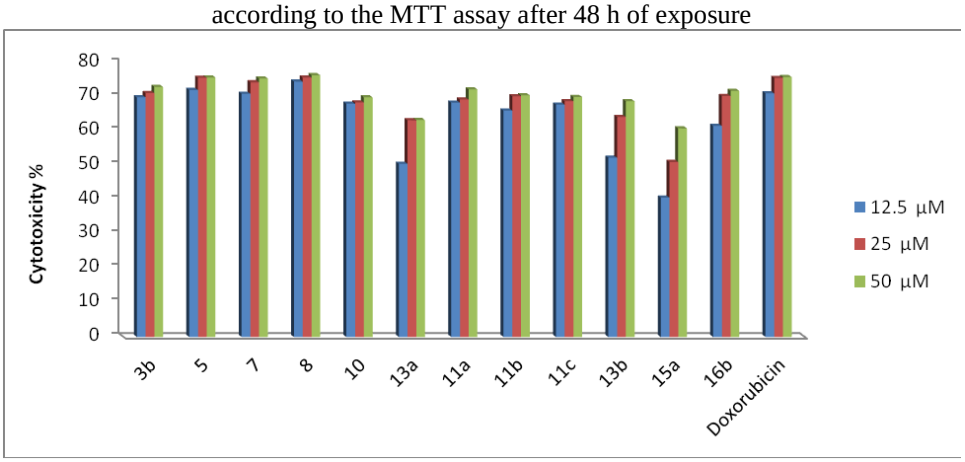


Figure 9: Dose dependent antiproliferative data of the twelve compounds against MCF-7 cancer cells according to the MTT assay after 48 h of exposure

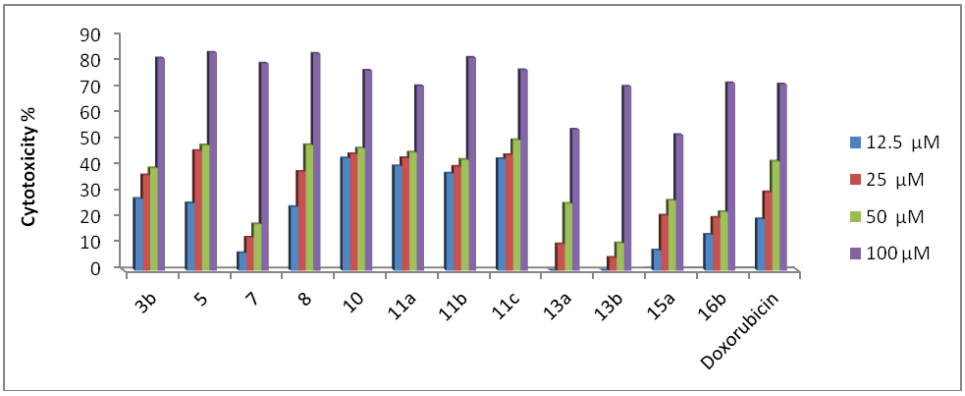


Figure 10: Dose dependent antiproliferative data of the twelve compounds against HepG2 cancer cells according to the MTT assay after 48 h of exposure

4. Conclusions

In this text, a series of Schiff bases were synthesized and reacted with various amino derivatives to afford a variety of *N*-heterocyclic compounds. α-aminophosphonates are prepared from the reaction of *N*-heterocyclic amines and aldehyde derivatives in the presence of phosphorus reagents. Also, tris(dialkylamino)phosphines are used as aminating agent and chlorine displacement was occurred to give the respective dialkylamino derivatives. The molecular docking experiments revealed that compounds **8**, **7**, and **3b** were the most promising candidates comparing with Doxorubicin. All new compounds have potential synthetic and pharmaceutical interest.

5. Conflicts of interest

"There are no conflicts to declare".

6. Acknowledgments

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