ORIGINAL RESEARCH

Design, Synthesis, and Spectroscopic Studies of Some New α-Aminophosphonate Analogues Derived from 4-Hydroxybenzaldehyde with Special Reference to Anticancer Activity

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Introduction: As biological activity components, α-aminophosphonates and their moieties play important roles in medicinal chemistry. Alpha-phosphonic acids are significant α-amino acid counterparts. Due to its strong biological activity, this class of molecule has recently been discovered to have numerous medical applications.

Results and Discussion: A new class of α-aminophosphonates and arylidene derivatives was synthesized. Various spectroscopic and elemental analyses were used to confirm the prepared products. The produced materials were tested as anticancer against breast carcinoma cells and normal human cells (PBMC). Besides the analysis results, it was found that (**7b, 4c, 5k, 6, 5a, 7c, 5f, 5b,** and **5g**) against MCF-7 line cells. As a reference anticancer drug, 5-fluorouracil was used. The anticancer activities showed that the compounds **7b, 4c**, containing α-aminophosphonate and Schiff base groups, respectively, showed high inhibition activity against the MCF-7 cell line, with 94.32% and 92.45% inhibition compared to the inhibition by 5-FU with 96.02% inhibition. The results showed that the compounds **5k, 7b, 6**, and **5a**, respectively, had very low activity against normal human cells PBMC, with 12.77%, 13%, 13.13%, and 17.88% inhibition compared to the inhibition by 5-FU with 12.50% inhibition. The binding energy for non-bonding interactions between the ligand (studied compounds) and receptor, thymidylate synthase, was determined using molecular docking (pdb code: 1AN5).

Conclusion: α-aminophosphonate derivatives, arylidines, and disphosphonate derivatives derived from 4-hydroxybenzaldehyde were synthesized, purified, elucidated by spectroscopic analysis, and finally tested against carcinoma breast cancer to give high to moderate to low activity.

Keywords: synthesis, phosphonates, arylidene derivatives, 4-hydroxybenzaldehyde, anticancer activity

Introduction

α-Aminophosphonates and their moieties have important roles in medicinal chemistry as biological activity components.[1] Alpha-phosphonic acids are important analogues of the α-amino acids. It has been found in recent years that this type of compound has many medical applications due to its high biological activity.[2] α-Aminophosphonates are similar amino acids in their structure and are characterized by their high cell permeability.[3] Recently, the interest in phosphonate synthesis through the three-component reaction with a natural iodine-coated catalyst has been observed and has already been tested and yielded results as an anti-HIV.[4] Heterocyclic compounds that contain a pentagonal ring are called oxadiazole. When many of the α-aminophosphonates derived from them were synthesized, they were found to have very high activity as anti-liver cancer.[5,6]

On the other hand, it was found that α-aminophosphonates also have an anti-cancer effect, and they have an effect as antimicrobials in general and as anti-bacterial positive and gram-negative in particular.[7–12] Schiff's bases are considered

Graphical Abstract

Schiff Base Dervatives

Acid Hydrazide

Alpha-Aminophosphonates

Alpha-Aminophosphonates
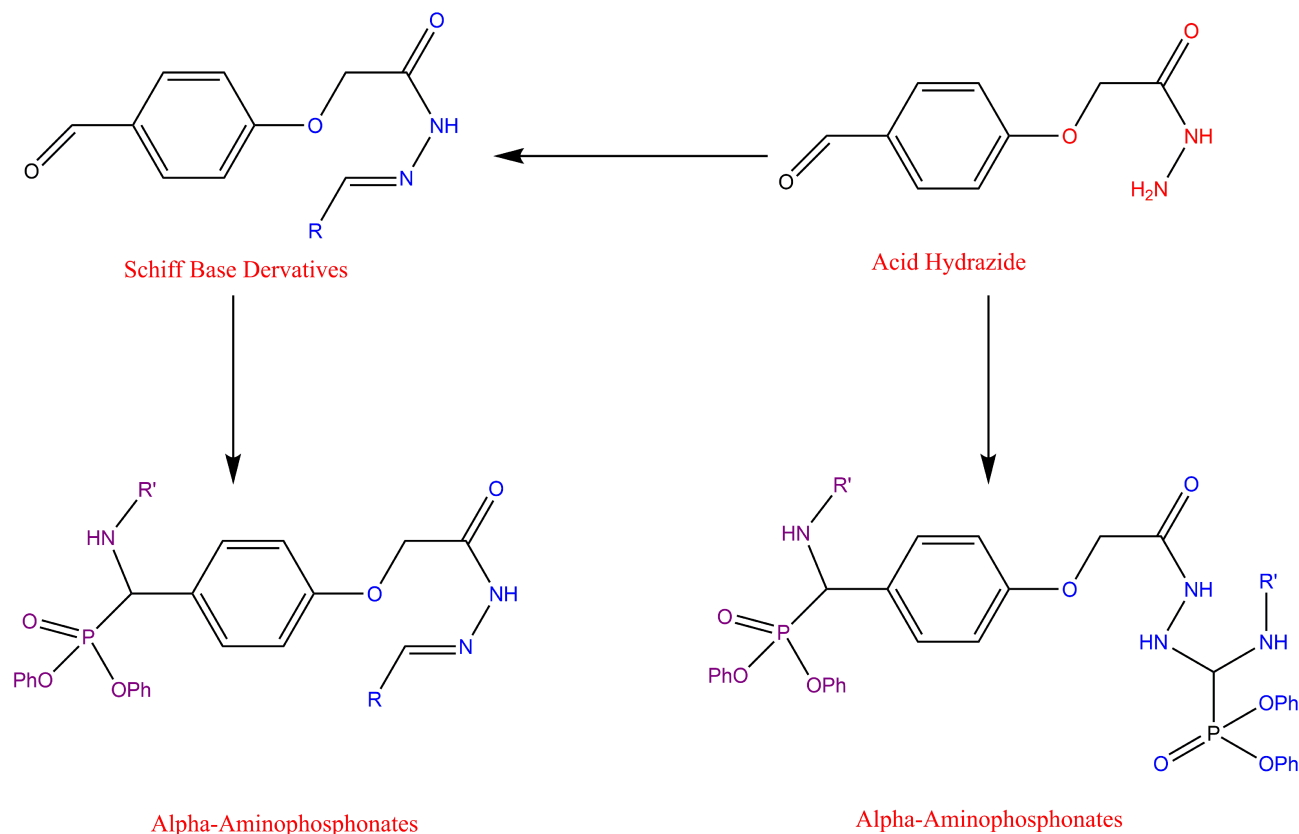
one of the compounds known in the field of medicinal chemistry for their strong effects as new drugs, and it was found that when prepared with heterocyclic aldehydes, they showed high anti-fungal and bacterial activity.[13–16]

Molecular docking describes the proper orientation of any substance that binds to a specific protein and is critical in predicting the structure of a complex formed by two or more molecules.[17] Because of its applications in medicine, the protein ligand interaction is the most interesting and entertaining case.[18] A ligand is a tiny molecule that interacts with a protein's binding site.[19] Molecular docking is important because it is useful for learning about drug receptor interactions and is widely used to learn how a small molecule binds. Its activity filters drugs to their protein targets and leads to the prediction of small molecule affinity.[20] We describe here the synthesis of α-aminophosphonates derivatives, which were evaluated against carcinoma breast cancer and normal human PBMC cells, as a continuation of our prior work in the synthesis of biologically active heterocycles.[21–27] New alpha-aminophosphonate derivatives have been designed and manufactured. Hence, the chemical composition of the synthesized compounds were elucidated by spectroscopy, such as ^{13}C NMR, elemental analysis, infrared (IR) and ^{1}HNMR. After confirming the chemical composition of these compounds and their purification, they were tested against breast cancer cells (MCF-7), which results showed that the synthesized compounds showed moderate to high activity compared to 5-fluorouracil.[28] It was found that breast cancer is the leading cause of death among women all over the world. In a very recent study, 60 female rats were divided into 6 groups. Negative control. The novel α-aminophosphonates and arylidene derivatives of 3-acetyl-1-aminoquinolin-2(1H)-one were synthesized and tested against infected breast cancer in rats. Histopathological examination showed a significant proliferation of tumor cells in the DMBA group. Treatment with alpha-aminophosphonate mainly reduced tumor mass. Bcl2 expression increased in DMBA-administered mice and then decreased in the treated groups, mostly with α-aminophosphonates. The level of CA15-3 was significantly decreased in the DMBA groups treated with α-aminophosphonates and arylidine derivatives of 3-acetyl-1-aminoquinolin-2(1H)-one. Gene

expression of GST-P, PCNA, PDK and PIK3CA was decreased in the DMBA group treated with α-aminophosphonates.[29] The binding energy for non-bonding interactions between the ligand (studied compounds) and the receptor, thymidylate synthase, was determined using a molecular docking study (pdb code: 1AN5).

Results and Discussion
Chemistry
P-Hydroxybenzaldhyde (**1**) was permitted for the reaction with ethylchloroacetate and K_2CO_3 in acetone to provide ethyl 2-(4-formylphenoxy)acetate (**2**) in an 87% yield. The addition of hydrazine hydrate to a dissolved ethyl ester **2** in absolute ethanol at a boiling temperature in the presence of a condenser resulted in the administration of hydrazide **3**, which when reacting with some aromatic aldehydes and a trace amount of AcOH yielded the arylidene derivatives **4a-d** in 87–90% yields (Scheme 1 in the Supplementary Materials).

At room temperature, the reaction of **4a-d** with the appropriate amines (1-naphthyl amine, 2-nitro aniline, or *p*–toluidine) in acetonitrile with the addition of triphenylphosphite and in the absence of perchloric acid as a catalytic agent gave the corresponding phosphonates **5a-l** in 75–92% yields (Scheme 2 in the Supplementary Materials). The elucidation of **4a-d** by ^{1}H NMR spectra appears to be the disappearance of the NH_2 group and the appearance of peaks in a broad peak around 9.32 for (NH) and 11.88 for (CHO group).

The elucidation of phosphonates **5a-l** by ^{1}H NMR spectra appear to be the disappearance of CHO groups and the appearance of peaks around 3.75 for (NH), a singlet peak around 6.00 for the (CH) group, a multiplet around 7.98 to 8.99 for CH-aromatic, and a broad peak around 9.22 for (NH group) and 8.71 for (CH) group. ^{13}C NMR spectra appear to have peaks around 69.42 for (-CH-P-of phosphonates), the peaks of (CH-aromatics) appear around 109.54 to 157.24, a peak around 145.32 for (CH=N) and a peak around 171.12 for (CONH). The reaction of **3** with HCOOH at a boiling temperature in the presence of a condenser afforded diformyl-compound **6** in a 90% yield (Scheme 3 in the Supplementary Materials). In the presence of perchloric acid, the treatment of **6** in acetonitrile with the appropriate amines (1-naphthylamine, 2-nitroaniline, or *p*–toluidine) and the addition of triphenylphosphite yield the corresponding phosphonates **7a-c** in 85–88% yields.

The elucidation of phosphonates **7a-c** by ^{1}H NMR spectra appear to be the disappearance of CHO groups and the appearance of peaks around 2.40 and 4.00 for (NH), a singlet peak around 6.00 for the (CH) group, a multiplet around 7.18 to 8.22 for CH-aromatic, 8.71 for the (CH) group, and a broad peak around 9.22 for the (NH group); ^{13}C NMR spectra appears to have peaks around 69.07 and 85.78 for (-CH-P-of phosphonates), the peaks of (CH-aromatics) appear around 114.22 to 156.55 for (CH=N) and 165.83 for (CONH).

Anticancer Activity
The newly developed and synthesized compounds were examined for their ability to fight breast cancer. MCF-7 was inhibited by Schiff bases and α-aminophosphonate analogues generated from 4-Hydroxybenzaldehyde (**7b, 4c, 5k, 6, 5a, 7c, 5f, 5b**, and **5g**). As a control, the medication 5-fluorouracil (5-FU) was used. According to the findings, compounds **7b** and **4c**, having α-aminophosphonate and Schiff base groups, respectively, displayed extremely strong inhibitory activity against the MCF-7 cell line, with 94.32% and 92.45% inhibition, respectively. Following that, **5k, 6** and **5a** demonstrated strong action against breast cancer cells, with 83.14%, 82.65%, and 80.55% inhibition, respectively. Following that, **3, 7c**, and **5f** displayed moderate activity with 77.73%, 70.65%, and 67.34% inhibition, respectively. Finally, **5b** and **5g** demonstrated minimal activity, with 57.67% and 50.50% inhibition, respectively. In comparison to the suppression by 5-FU, which had a 96.02% inhibition (Table 1).

On the other hand, the same compounds were tested against normal human cells (PBMC). According to the findings, the compounds **5k, 7b, 6**, and **5a** showed extremely poor action against normal human cells PBMC, with 12.77%, 13%, 13.13%, and 17.88% inhibition, respectively. Following that, **7c, 4c**, and **3** showed minimal activity with 24.84%, 25.35%, and 29.55% inhibition, respectively. Compounds **5f, 5b**, and **5g**, on the other hand, displayed strong action against normal human cell PBMC, with inhibition rates of 32.85%, 39.22%, and 40.13%, respectively. In comparison to the suppression by 5-FU, which has a 12.50% inhibition (Tables 2).

Table 1 Inhibition Activity of the Synthesized Compounds Against MCF-7

No.	Inhibitory Activity (IC$_{50}$) mg/mL	Sample Concentration (µg/mL)							
		500		250		125		62.5	
		Viability (%)	Inhibition	Viability (%)	Inhibition	Viability (%)	Inhibition	Viability (%)	Inhibition
3	93 ± 6.5	22.17	77.73	29.85	70.13	47.24	48.87	61.44	38.56
4c	38.8 ± 5.1	7.55	92.45	18.54	81.46	27.57	72.43	39.09	60.91
5a	91 ± 6.2	19.45	80.55	26.88	73.12	47.24	52.76	68.67	31.33
5b	101 ± 4.7	42.33	57.67	45.13	54.87	40.87	59.13	70.52	29.48
5f	87 ± 6.5	32.66	67.34	36.65	63.35	42.12	57.88	67.65	32.35
5g	105 ± 7.8	49.50	50.50	38.57	61.43	30.12	69.88	81.76	18.24
5k	68.32 ± 4.9	16.86	83.14	29.90	70.10	43.63	56.37	65.49	34.51
6	100 ± 4.1	17.35	82.65	32.87	67.13	45.44	54.56	71.60	28.40
7b	29.5 ± 3.85	5.68	94.32	13.89	86.11	24.50	75.50	26.06	73.94
7c	75 ± 7.3	29.44	70.56	32.03	67.97	45.64	54.36	65.89	34.11
5-FU	14 ± 0.8	3.98	96.02	8.12	91.88	14.91	95.09	27.84	72.16

Table 2 Inhibition Activity Against Normal Human Cells PBMC

No.	Inhibitory activity (IC$_{50}$) mg/mL	Sample Concentration (µg/mL)							
		500		250		125		62.5	
		Viability (%)	Inhibition	Viability (%)	Inhibition	Viability (%)	Inhibition	Viability (%)	Inhibition
3	44.56 ± 1.7	70.45	29.55	73.87	26.13	78.12	21.88	82.37	17.63
4c	38.10 ± 1.6	74.65	25.35	77.45	22.55	80.22	19.78	90.05	9.95
5a	35.14 ± 1.8	82.12	17.88	84.45	15.55	87.17	12.13	90.15	9.85
5b	30.56 ± 2.5	60.78	39.22	65.13	34.87	67.34	32.66	77.56	22.44
5f	36.26 ± 2.3	67.15	32.85	68.34	31.66	70.57	29.63	73.27	26.73
5g	31.88 ± 1.9	59.87	40.13	65.14	34.86	70.19	29.81	71.20	28.80
5k	43.23 ± 3.8	87.23	12.77	90.78	9.22	91.21	9.79	95.24	4.76
6	43.14 ± 2.6	86.17	13.13	88.35	11.65	90.19	9.81	92.50	7.50
7b	34.22 ± 2.9	87.00	13.00	89.52	10.48	91.21	8.79	93.32	6.68
7c	32.56 ± 2.4	75.16	24.84	77.14	22.68	76.67	23.33	80.45	19.55
5-FU	08.91 ± 1.9	87.50	12.50	91.88	8.12	92.10	7.90	92.83	7.17

Molecular Docking Simulation

For each synthesized compound, the docking simulation process was completed, and the best conformation was chosen as the compound with the highest negative binding energy value. Figures S1 and S2 in the Supplementary Materials illustrate the 3D structures, and Figures S3 and S4 in the Supplementary Materials illustrate the 2D structures of the ligand–receptor structures of all compounds studied. Table 3 displays the estimated binding energies and the interacting residues produced by

Table 3 The Binding Energies and the Interacting Residues Produced by Docking for All Compounds Studied

Compound	Binding Energy (k.cal/mol)	Interacting Residues
7b	−10.31	LEU 144, VAL 263, SER 55, ASP 170, GLU 83, PHE 177, GLY 174, CYS 147, ILE 80, and TYR 210
4c	−10.22	ASN 178, GLY 174, ASP 170, PHE 177, CYS 147, TRP 81, and ILE 80
5k	−10.01	VAL 263, PHE 177, LEU 173, CYS 147, LEU 144, TRP 84, TRP 81, and LYS 49
6	−9.82	ARG 127, TRP 84, TRP 81, and ARG 22
5a	−9.71	VAL 263, LYS 260, ASN 178, PHE 177, and ILE 80
3	−9.64	With ASN 178, CYS 147, LEU 144, and GLU 59
7c	−9.61	VAL 263, LYS 260, HS 208, GLY 174, LEU 173, CYS 147, TRP 81, ILE 80, HS 52, and LYS 49
5f	−7.52	TRY 210, HS 208, ASN 178, PHE 177, LEU 173, ASP 170, CYS 147, LEU 144, TRP 84, and ILE 80
5b	−6.38	VAL 263, PHE 177, LEU 173, ASP 170, CYS 147, TRP 84, TRP 81, ILE 80, and CYS 51
5g	−6.22	VAL 263, PHE 177, LEU 173, TRP 84, GLU 83, TRP 81, ILE 80, THR 79, VAL 78, SER 55, and HS 52

docking for all compounds studied. All of the compounds studied formed stable complexes with receptors that had a high binding energy. Compound **7b** had the best docking energy (highest binding energy) according to our findings, with a binding affinity of 10.31 kcal/mol, followed by compound **4c** (−10.22 kcal/mol) (Table 3). This is consistent with the biological evidence acquired. As a result, the compounds studied, particularly compounds **7b** and **4c**, have the potential to be used as anti-Brest cancer. The most interacting residues in the **7b** compound active site, according to molecular docking, were LEU 144, VAL 263, SER 55, ASP 170, GLU 83, PHE 177, GLY 174, CYS 147, ILE 80, and TYR 210.

Compound **4c** interacted with ASN 178, GLY 174, ASP 170, PHE 177, CYS 147, TRP 81, and ILE 80. Compound **5k** was seen to interact with VAL 263, PHE 177, LEU 173, CYS 147, LEU 144, TRP 84, TRP 81, and LYS 49. Compound **6** showed interaction with ARG 127, TRP 84, TRP 81, and ARG 22, as well as compound **5a** interacted with VAL 263, LYS 260, ASN 178, PHE 177, and ILE 80. And compound **3** with ASN 178, CYS 147, LEU 144, and GLU 59. While compound **7c** interacted with VAL 263, LYS 260, HS 208, GLY 174, LEU 173, CYS 147, TRP 81, ILE 80, HS 52, and LYS 49. And compound **5f** interacted with TRY 210, HS 208, ASN 178, PHE 177, LEU 173, ASP 170, CYS 147, LEU 144, TRP 84, and ILE 80. Compound **5b** was shown to interact with VAL 263, PHE 177, LEU 173, ASP 170, CYS 147, TRP 84, TRP 81, ILE 80, and CYS 51. And compound **5g** interacted with VAL 263, PHE 177, LEU 173, TRP 84, GLU 83, TRP 81, ILE 80, THR 79, VAL 78, SER 55, and HS 52 as shown in Table 3.

Materials and Methods
General Information
Melting points were measured using a Kofler mass machine and were not corrected. Nuclear Magnetic Resonance for proton spectra were mapped onto the Varian Gemini Nuclear Magnetic Resonance for proton spectrometer at 500 MHz. Interactions were followed up by TLC using a 60 °F 245 aluminum silica plate. Primary analyses were carried out at Cairo University's Microanalysis Center, Faculty of Science.

Experimental Procedures
Synthesis ethyl 2-(4-formylphenoxy)acetate (**2**).[28]

In the reaction of *p*-hydroxybenzaldhyde **1** (10.6 g, 0.1 mol) dissolved in acetone (250 mL) with the slow addition of ethylchloroacetate (12.25 g, 0.1 mol) and in the presence of anhydrous K_2CO_3 (13.8 g, 0.1 mol), the mix was boiled under reflux for 12h. After ensuring that the reaction was complete, the filtrate was evaporated under pressure and recrystallized with ethanol to yield yellow oil with an 87% yield. Rf = 0.48 (3% EtOAc in $CHCl_3$). ^{1}H NMR (DMSO-d_6):

δ = 1.19 (t, 3H, *J* = 8.1 Hz, C*H₃*CH₂), 4.10 (q, 2H, *J* = 8.1 Hz, CH₃C*H₂*), 4.65 (s, 2H, CH₂), 6.98 (d, 2H, *J* = 5.5 Hz, H-2), 7.56 (d, 2H, J = 5.5 Hz, H-3), 10.49 (s, 1H, CHO). Anal. Calc. for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81; Found C, 63.60; H, 5.95.

Synthesis of 2-(4-formylphenoxy) acetohydrazide (**3**).[28]

Compound **2** (2.08 g, 0.01 mol) was dissolved in absolute ethanol (30 mL), and then hydrazine hydrate (1.5 g, 0.03 mol) was added, and the reaction was heated under reflux for 5h. After completing the reaction and separating the resulting compound, it was purified by recrystallizing ethanol to obtain a high purity white product with a yield of 90%., m.p. 203–205°C, R_f = 0.31 (3% EtOAc in CHCl₃). ¹H NMR (DMSO-d₆): δ = 4.32 (brs, 2H, NH₂), 4.55 (s, 2H, CH₂), 6.92 (d, 2H, *J*= 5.5 Hz, Ar-H), 7.49 (d, 2H, *J*= 5.5 Hz, Ar-H), 9.33 (brs, 1H, NH), 10.42 (s, 1H, CHO); Anal. Calc. for $C_9H_{10}N_2O_3$: C, 55.67; H, 5.19; N, 14.43. Found C, 55.83; H, 5.34; N, 14.57.

Synthesis of arylidene derivatives **4a-d**.

In absolute ethanol, different aromatic aldehydes (5 mmol) are allowed to react with compound **3** (5 mmol). After that, a catalytic quantity of acetic acid (glacial) was added to the mixture and it was refluxed for 15h. The product was separated to provide **4a-d** (87–90%) yields. ([Figures S5] and [S6] in the [Supplementary Materials])

(E)-N'-benzylidene-2-(4-formylphenoxy)acetohydrazide (4a).

Yellow powder (87%), m.p. 267–269 °C. R_f = 0.80 (3% EtOAc in CHCl₃), ¹H NMR (DMSO-d₆): δ = 4.45 (s, 2H, CH₂), 7.12–8.00 (m, 9H, Ar-H), 8.55 (s, 1H, CH), 9.32 (brs, 1H, NH), 11.88 (s, H, CHO); Anal. Calc. for $C_{16}H_{14}N_2O_3$: C, 68.07; H, 5.00; N, 9.92. Found C, 68.19; H, 5.16; N, 9.81.

N'-(4-(dimethylamino) benzylidene)-2-(4-formylphenoxy)-acetohydrazide (4b).

White powder (88%), m.p. > 300 °C. R_f = 0.45 (3% EtOAc in CHCl₃). ¹H NMR (DMSO-d₆): δ = 3.10 (s, 6H, 2CH₃), 4.45 (s, 2H, CH₂), 7.20–8.05 (m, 8H, Ar-H), 8.45 (s, 1H, CH), 9.32 (brs, 1H, NH), 11.80 (s, 1H, CHO); Anal. Calc. for $C_{18}H_{19}N_3O_3$: C, 66.45; H, 5.89; N, 12.91. Found C, 66.57; H, 6.03; N, 13.06.

2-(4-formylphenoxy)-N'-(3, 4, 5-trimethoxybenzylidene) acetohydrazide (4c).

White powder (90%), m.p. = 212–214 °C. R_f = 0.50 (5% EtOAc in CHCl₃). ¹H NMR (DMSO-d₆): δ = 3.92 (s, 9H, 3CH₃), 4.65 (s, 2H, CH₂), 7.20–7.95 (m, 6H, Ar-H), 8.22 (s, 1H, CH), 8.62 (brs, 1H, NH), 10.52 (s, 1H, CHO); Anal. Calc. for $C_{19}H_{20}N_2O_6$: C, 61.28; H, 5.41; N, 7.52. Found C, 61.43; H, 5.56; N, 7.37.

2-(4-formylphenoxy)-N'-(4-nitrobenzylidene) acetohydrazide (4d).

White powder (90%), m.p. = 250–252 °C. R_f = 0.65 (3% EtOAc in CHCl₃). ¹H NMR (DMSO-d₆): δ = 4.63 (s, 2H, CH₂), 7.10–7.85 (m, 8H, Ar-H), 8.30 (s, 1H, CH), 8.52 (brs, 1H, NH), 10.95 (s, 1H, CHO); Anal. Calc. for $C_{16}H_{13}N_3O_5$: C, 58.72; H, 4.00; N, 12.84. Found: C, 58.88; H, 3.89; N, 12.98.

General procedure for the synthesis of α-aminophosphonates 5a- l.

In 20 mL MeCN, triphenylphosphite (5 mmol) was added to a mixture of Schiff bases **4 (a-d)** (5 mmol) and different amines (5 mmol). 1 mL of perchloric acid was added dropwise, and the reaction mixture was stirred at room temperature for 20h. The solvent was evaporated under low pressure, and the gum was tutrated with diethyl ether and dried to give **5 (a- l)** in 75–92% yields. ([Figures S7]–[S18] in the Supplementary Materials).

Diphenyl ((4-(2-(2-benzylidenehydrazinyl)-2-oxoethoxy)phenyl)-(naphthalen-1-yl-amino)methyl)phosphonate (5a).

Yellow gum (75%), R_f = 0.60 (3% EtOAc in CHCl₃). ¹H NMR (DMSO-d₆): δ = 3.75 (brs, 1H, NH), 4.63 (s, 2H, CH₂), 6.00 (s, 1H, CH), 6.98–7.99 (m, 26H, Ar-H), 8.00 (brs, 1H, NH), 8.45 (s, 1H, CH); ¹³C NMR (100 MHz, CDCl₃): δ = 68.77 (CH₂), 69.42 (CH-P-), 109.54, 114.34, 118.98, 120.41, 124.82, 125.05, 126.11, 127.65, 127.89, 128.32, 128.87, 129.22, 130.25, 131.11, 133.65, 134.37, 141.55, 147.22, 150.23, 157.24 (C-Aromatic), 145.32 (CH=N), 171.12 (CONH). Anal. Calc. for $C_{38}H_{32}N_3O_5P$: C, 71.13; H, 5.03; N, 6.55. Found C, 71.24; H, 5.15; N, 6.68.

Diphenyl ((4-(2-(2-benzylidenehydrazinyl)-2-oxoethoxy)phenyl)((2-nitrophenyl)-amino)methyl)phosphonate (5b).

Yellow gum (75%), R_f = 0.55 (3% EtOAc in CHCl₃). ¹H NMR (DMSO-d₆): δ = 3.75 (brs, 1H, NH), 4.60 (s, 2H, CH₂), 6.00 (s, 1H, CH), 6.98–7.99 (m, 23H, Ar-H), 8.12 (brs, 1H, NH), 8.55 (s, 1H, CH); ¹³C NMR (100 MHz, CDCl₃): δ = 69.00 (CH₂), 69.42 (CH-P-), 114.65, 118.73, 120.39, 121.41, 125.94, 127.56, 128.76, 129.23, 130.35, 131.31, 131.77, 133.38, 135.27, 146.37, 150.43, 156. 94 (C-Aromatic), 144.72 (CH=N), 170.52 (CONH). Anal. Calc. for $C_{34}H_{29}N_4O_7P$: C, 64.15; H, 4.59; N, 8.80. Found: C, 64.27; H, 4.43; N, 8.66.

Diphenyl ((4-(2-(2-benzylidenehydrazinyl)-2-oxoethoxy)phenyl)(p-tolylamino)-methyl)phosphonate (5c).
Yellow gum (77%), R_f = 0.60 (6% EtOAc in CHCl$_3$). ^{1}H NMR (DMSO-d$_6$): δ = 2.20 (s, 3H, CH$_3$), 3.75 (brs, 1H, NH), 4.60 (s, 2H, CH$_2$), 6.00 (s, 1H, CH), 6.98–7.99 (m, 23H, Ar-H), 8.12 (brs, 1H, NH), 8.55 (s, 1H, CH); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 20.98 (CH$_3$), 69.08 (CH$_2$), 69.50 (CH-P-), 113.45, 114.13, 120.29, 121.45, 127.36, 128.43, 128.76, 129.17, 129.61, 130.13, 131.45, 133.77, 144.62, 150.55, 156.78 (C-Aromatic), 145.30 (CH=N), 171.00 (CONH). Anal. Calc. for C$_{35}$H$_{32}$N$_3$O$_5$P: C, 69.41; H, 5.33; N, 6.94. Found: C, 69.55; H, 5.48; N, 7.06.

Diphenyl ((4-(2-(2-(4-(dimethylamino)benzylidene)hydrazinyl)-2-oxoethoxy)-phenyl)(naphthalen-1-ylamino)methyl) phosphonate (5d).
Brown gum (82%), R_f = 0.35 (5% EtOAc in CHCl$_3$). ^{1}H NMR (DMSO-d$_6$): δ = 3.10 (s, 6H, 2CH$_3$), 4.11 (brs, 1H, NH), 4.45 (s, 2H, CH$_2$), 6.23 (s, 1H, CH), 6.82–8.12 (m, 25H, Ar-H), 8.00 (brs, 1H, NH), 8.32 (s, 1H, CH); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 40.35 (-N(CH$_3$)$_2$), 68.76 (CH$_2$), 69.53 (CH-P-), 108.46, 110.67, 113.95, 119.00, 120.22, 121.31, 123.00, 124.19, 125.36, 126.32, 127.11, 127.65, 128.16, 128.48, 128.77, 130.12, 134.66, 146.55, 150.50, 153.09, 154.60, 157. 51 (C-Aromatic), 144.53 (CH=N), 171.07 (CONH). Anal. Calc. for C$_{40}$H$_{37}$N$_4$O$_5$P: C, 70.16; H, 5.45; N, 8.18. Found C, 70.03; H, 5.58; N, 8.29.

Diphenyl ((4-(2-(2-(4-(dimethylamino)benzylidene)hydrazinyl)-2-oxoethoxy)phenyl)((2-nitrophenyl)amino)methyl) phosphonate (5e).
Brown gum (80%), R_f = 0.75 (5% EtOAc in CHCl$_3$). ^{1}H NMR (DMSO-d$_6$): δ = 3.10 (s, 6H, 2CH$_3$), 4.11 (brs, 1H, NH), 4.45 (s, 2H, CH$_2$), 6.23 (s, 1H, CH), 6.82–8.12 (m, 22H, Ar-H), 8.00 (brs, 1H, NH), 8.32 (s, 1H, CH); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 41.00 (-N(CH$_3$)$_2$), 69.11 (CH$_2$), 70.04 (CH-P-), 111.48, 114.22, 114.76, 118.13, 120.15, 121.27, 123.42, 125.45, 127.34, 128.17, 128.55, 130.25, 131.44, 134.97, 146.72, 150.10, 153.17, 156.53 (C-Aromatic), 145.07 (CH=N), 172.11 (CONH). Anal. Calc. for C$_{36}$H$_{34}$N$_5$O$_7$P: C, 63.62; H, 5.04; N, 10.30. Found C, 63.75; H, 5.15; N, 10.43.

Diphenyl ((4-(2-(2-(4-(dimethylamino)benzylidene)hydrazinyl)-2-oxoethoxy)-phenyl)(p-tolylamino)methyl)phosphonate (5f).
Brown oil (80%), R_f = 0.57 (5% EtOAc in CHCl$_3$). ^{1}H NMR (DMSO-d$_6$): δ =2.24 (s, 3H, CH$_3$), 3.10 (s, 6H, 2CH$_3$), 4.13 (brs, 1H, NH), 4.45 (s, 2H, CH$_2$), 6.23 (s, 1H, CH), 6.82–8.10 (m, 22H, Ar-H), 8.12 (brs, 1H, NH), 8.22 (s, 1H, CH); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 22.00 (CH$_3$), 41.97 (-N(CH$_3$)$_2$), 68.76 (CH$_2$), 69.53 (CH-P-), 112.06, 113.40, 114.46, 120.32, 121.45, 123.09, 127.17, 128.00, 128.33, 128.86, 129.34, 129.56, 131.00, 144.39, 150.24, 153.12, 156. 66 (C-Aromatic), 145.14 (CH=N), 170.23 (CONH). Anal. Calc. for C$_{37}$H$_{37}$N$_4$O$_5$P: C, 68.51; H, 5.75; N, 8.64. Found: C, 68.39; H, 5.88; N, 8.77.

Diphenyl ((naphthalen-1-ylamino)(4-(2-oxo-2-(2-(3,4,5-trimethoxy-benzylidene)-hydrazinyl)ethoxy)phenyl)methyl) phosphonate (5g).
Brown oil (85%), R_f = 0.63 (5% EtOAc in CHCl$_3$). ^{1}H NMR (DMSO-d$_6$): δ =3.90 (s, 9H, 3CH$_3$), 4.15 (brs, 1H, NH), 4.55 (s, 2H, CH$_2$), 6.20 (s, 1H, CH), 6.72–8.10 (m, 23H, Ar-H), 8.12 (brs, 1H, NH), 8.24 (s, 1H, CH); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 56.22 (2xOCH$_3$), 61.12 (OCH$_3$), 69.00 (CH$_2$), 69.57 (CH-P-), 104.00, 110.02, 114.34, 119.13, 120.30, 121.39, 124.73, 125.09, 126.24, 127.45, 127.68, 128.07, 128.38, 128.99, 130.61, 134.65, 141.43, 147.57, 150.26, 153.00, 156.84 (C-Aromatic), 147.43 (CH=N), 171.77 (CONH). Anal. Calc. for C$_{41}$H$_{38}$N$_3$O$_8$P: C, 67.30; H, 5.23; N, 5.74. Found: C, 67.44; H, 5.11; N, 5.61.

Diphenyl (((2-nitrophenyl)amino(4-(2-oxo-2-(2-(3,4,5-trimethoxybenzylidene)hydrazinyl)ethoxy)phenyl)methyl)phos-phonate (5h).
Brown oil (85%), R_f = 0.55 (5% EtOAc in CHCl$_3$). ^{1}H NMR (DMSO-d$_6$): δ = 3.90 (s, 9H, 3CH$_3$), 4.13 (brs, 1H, NH), 4.45 (s, 2H, CH$_2$), 6.23 (s, 1H, CH), 6.82–8.10 (m, 20H, Ar-H), 8.15 (brs, 1H, NH), 8.22 (s, 1H, CH); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 55.46 (2xOCH$_3$), 60.67(OCH$_3$), 68.15 (CH$_2$), 69.00 (CH-P-), 105.13, 114.04, 114.43, 118.63, 120.29, 121.45, 125.47, 127.36, 128.45, 128.78, 130.22, 131.50, 135.71, 141.25, 146.56, 150.43, 153.21, 156.47(C-Aromatic), 147.54 (CH=N), 172.07 (CONH). Anal. Calc. for C$_{37}$H$_{35}$N$_4$O$_{10}$P: C, 61.16; H, 4.85; N, 7.71. Found: C, 61.25; H, 4.72; N, 7.83.

Diphenyl ((4-(2-oxo-2-(2-(3,4,5-trimethoxybenzylidene)hydrazinyl)-ethoxy)phenyl)(p-tolylamino)methyl)phosphonate (5i).
Brown oil (80%), R_f = 0.60 (5% EtOAc in CHCl$_3$). ^{1}H NMR (DMSO-d$_6$): δ =2.24 (s, 3H, CH$_3$), 3.90 (s, 9H, 3CH$_3$), 4.20 (brs, 1H, NH), 4.65 (s, 2H, CH$_2$), 6.11 (s, 1H, CH), 7.11–7.42 (m, 20H, Ar-H), 8.10 (brs, 1H, NH), 8.45 (s, 1H, CH);

[^13]C NMR (100 MHz, CDCl$_3$): δ = 22.12 ($_{CH3}$), 55.46 (2xOCH$_3$), 60.67(OCH$_3$), 69.12 (CH$_2$), 69.32 (CH-P-), 104.43, 113.47, 114.87, 118.63, 120.33, 121.54, 127.48, 128.07, 128.33, 129.00, 129.56, 129.91, 130.66, 141.45, 144.05, 150.75, 153.61, 156.78 (C-Aromatic), 146.59 (CH=N), 171.63 (CONH). Anal. Calc. for C$_{38}$H$_{38}$N$_3$O$_8$P: C, 65.60; H, 5.51; N, 6.04. Found: C, 65.49; H, 5.39; N, 5.92.

Diphenyl ((naphthalen-1-ylamino)(4-(2-(2-(4-nitrobenzylidene)-hydrazinyl)-2-oxo-ethoxy)phenyl)methyl)phosphonate (5j).
Yellow gum (86%), R$_f$ = 0.66 (5% EtOAc in CHCl$_3$). [^1]H NMR (DMSO-d$_6$): δ =3.98 (brs, 1H, NH), 4.55 (s, 2H, CH$_2$), 6.00 (s, 1H, CH), 6.62–8.00 (m, 25H, Ar-H), 8.20 (s, 1H, CH), 8.42 (brs, 1H, NH); [^13]C NMR (100 MHz, CDCl$_3$): δ = 69.15 (CH$_2$), 69.70 (CH-P-), 109.11, 114.14, 119.36, 120.29, 121.45, 124.11, 124.36, 124.67, 125.18, 126.43, 127.36, 128.32, 128.65, 130.22, 134.30, 139.55, 147.74, 150.65, 156.36 (C-Aromatic), 144.98 (CH=N), 170.83 (CONH). Anal. Calc. for C$_{38}$H$_{31}$N$_4$O$_7$P: C, 66.47; H, 4.55; N, 8.16. Found: C, 66.60; H, 4.66; N, 8.29.

Diphenyl ((4-(2-(2-(4-nitrobenzylidene)hydrazinyl)-2-oxoethoxy)-phenyl)((2-nitro-phenyl)amino)methyl)phosphonate (5k).
Yellow gum (88%), R$_f$ = 0.42 (5% EtOAc in CHCl$_3$). [^1]H NMR (DMSO-d$_6$): δ =3.98 (brs, 1H, NH), 4.55 (s, 2H, CH$_2$), 6.00 (s, 1H, CH), 6.62–8.00 (m, 22H, Ar-H), 8.20 (s, 1H, CH), 8.42 (brs, 1H, NH); [^13]C NMR (100 MHz, CDCl$_3$): δ = 68.67 (CH$_2$), 69.13 (CH-P-), 114.12, 114.56, 118.56, 120.29, 121.45, 124.13, 124.46, 125.09, 127.91, 128.45, 130.22, 135.87, 139.39, 146.00, 150.67, 156.78 (C-Aromatic), 144.87 (CH=N), 171.00 (CONH). Anal. Calc. for C$_{34}$H$_{28}$N$_5$O$_9$P: C, 59.91; H, 4.14; N, 10.28. Found: C, 59.77; H, 4.25; N, 10.15.

Diphenyl ((4-(2-(2-(4-nitrobenzylidene)hydrazinyl)-2-oxoethoxy)-phenyl)(p-tolylamino) methyl)phosphonate (5l).
Yellow gum (92%), R$_f$ = 0.70 (5% EtOAc in CHCl$_3$). [^1]H NMR (DMSO-d$_6$): δ =2.22 (s, 3H, CH$_3$), 3.98 (brs, 1H, NH), 4.55 (s, 2H, CH$_2$), 6.00 (s, 1H, CH), 6.62–8.00 (m, 22H, Ar-H), 8.20 (s, 1H, CH), 8.42 (brs, 1H, NH); [^13]C NMR (100 MHz, CDCl$_3$): δ = 21.75 (CH$_3$), 69.17 (CH$_2$), 69.63 (CH-P-), 113.10, 114.23, 120.15, 121.38, 124.40, 124.58, 127.65, 128.78, 129.45, 129.86, 130.00, 140.07, 145.51, 150.72, 156.63 (C-Aromatic), 145.60(CH=N), 171.43 (CONH). Anal. Calc. for C$_{35}$H$_{31}$N$_4$O$_7$P: C, 64.61; H, 4.80; N, 8.61. Found: C, 64.72; H, 4.68; N, 8.48.

Synthesis of *N'*-Formyl-2-(4-formylphenoxy) acetohydrazide (6).
For 14h, hydrazide **3** (10 mmol) and formic acid (30 mL) were refluxed. The excess of formic acid was evaporated under low pressure to give **6** in (90%) yields. ([Figure S19] in the [Supplementary Materials])
White crystals (90%), m.p. 158–160 °C. R$_f$ = 0.38 (3% EtOAc in CHCl$_3$). [^1]H NMR (DMSO-d$_6$): δ = 2.21 (brs, 1H, NH), 4.35 (s, 2H, CH$_2$), 7.22 (d, 2H, *j* = 5.5 *Hz*), 7.92 (d, 2H, *j*= 5.5 *Hz*), 8.12 (brs, 1H, NH), 8.72 (s, 1H, CHO), 10.00 (s, 1H, CHO); Anal. Calc. for C$_{10}$H$_{10}$N$_2$O$_4$: C, 54.05; H, 4.54; N, 12.61. Found: C, 54.17; H, 4.49; N, 12.48.

General procedure for the synthesis of phosphonates 7a-c.
A mixture of the aromatic dialdehyde **6** (5 mmol), triphenylphosphite (5 mmol) and different amines (5 mmol) was dissolved in acetonitrile. A catalytic quantity of perchloric acid (0.5 mL) was added dropwise, and the reaction was stirred at room temperature for 24 h. The solvent was withdrawn under decreased pressure, and the residue of the gum was tutrated with diethyl ether and dried to provide **7a-c** in 85–88% yields. ([Figures S20]–[S25] in the [Supplementary Materials])

Diphenyl((naphthalen-1-ylamino)(2-(2-(p-tolyloxy)acetyl)hydrazinyl)methyl)phosphonatediphenyl((naphthalen-1-yla-mino)(phenyl)methyl)phosphonate (7a).
Yellow powder (85%) m.p. 152–154 °C. R$_f$ = 0.56 (5% EtOAc in CHCl$_3$). [^1]H NMR (DMSO-d$_6$): δ = 2.40 (brs, 1H, NH), 4.00 (brs, 2H, 2NH), 4.45 (s, 2H, CH$_2$), 6.00 (s, 1H, CH), 7.18–8.22 (m, 38H, Ar-H), 8.71 (s, 1H, CH), 9.22 (brs, 1H, NH); [^13]C NMR (100 MHz, CDCl$_3$): δ = 66.45 (CH$_2$), 69.07 (CH-P-), 85.78 (CH-P-), 114.22, 114.34, 118.35, 120.23, 121.46, 124.44, 125.34, 127.56, 128.35, 128.76, 129.00, 129.19, 130.69, 133.43, 146.44, 150.10, 156.55 (C-Aromatic), 165.83 (CONH). Anal. Calc. for C$_{54}$H$_{46}$N$_4$O$_8$P$_2$: C, 68.93; H, 4.93; N, 5.95. Found: C, 68.79; H, 5.04; N, 6.07.

Diphenyl((2-nitrophenyl)amino)(2-(2-(p-tolyloxy)acetyl)hydrazinyl)methyl)phosphonatediphenyl((2-nitrophenyl) amino)(phenyl)methyl)phosphonate (7b).
Yellow powder (87%) m.p. 142–144 °C. R$_f$ = 0.37 (5% EtOAc in CHCl$_3$). [^1]H NMR (DMSO-d$_6$): δ = 2.40 (brs, 1H, NH), 4.00 (brs, 2H, 2NH), 4.45 (s, 2H, CH$_2$), 6.00 (s, 1H, CH), 7.18–8.22 (m, 32H, Ar-H), 8.71 (s, 1H, CH), 9.22 (brs, 1H, NH); [^13]C NMR (100 MHz, CDCl$_3$): δ = 66.45 (CH$_2$), 69.07 (CH-P-), 85.78 (CH-P-), 114.54, 114.78, 118.65, 120.54, 121.30, 125.13, 127.70, 128.51, 130.00, 131.60, 135.80, 146.73, 150.00, 156.90 (C-Aromatic), 166.72 (CONH). Anal. Calc. for C$_{46}$H$_{40}$N$_6$O$_{12}$P$_2$: C, 59.36; H, 4.33; N, 9.03. Found: C, 59.49; H, 4.45; N, 8.88.

Diphenyl((4-methylphenyl)amino)(2-(2-(p-tolyloxy)acetyl)hydrazinyl)methyl)phosphonatediphenyl((4-methylphenyl) amino)(phenyl)methyl)phosphonate (7c).

Yellow powder (88%) m.p. 132–134 °C. R_f = 0.45 (5% EtOAc in CHCl$_3$). ^{1}H NMR (DMSO-d$_6$): δ = 2.00 (s, 6H, 2CH$_3$), 2.40 (brs, 1H, NH), 4.00 (brs, 2H, 2NH), 4.45 (s, 2H, CH$_2$), 6.00 (s, 1H, CH), 7.18–8.22 (m, 32H, Ar-H), 8.71 (s, 1H, CH), 9.22 (brs, 1H, NH); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 21.56 (CH$_3$), 65.78 (CH$_2$), 68.15 (CH-P-), 86.00 (CH-P-), 113.14, 114.65, 120.54, 121.79, 127.65, 128.74, 129.40, 129.80, 130.44, 144.20, 150.23, 156.72 (C-Aromatic), 164.93 (CONH). Anal. Calc. for C$_{48}$H$_{46}$N$_4$O$_8$P$_2$: C, 66.35; H, 5.34; N, 6.45. Found: C, 66.47; H, 5.45; N, 6.56.

Anticancer Activity

Cell Line Propagation

Cell propagation was started with Dulbecco's modified Eagle Medium (DMEM), which contained 1% L-glutamine, 50 g/mL gentamicin, 10% heat inactivated fetal bovine serum, and HEPES buffer at the start of pretreatment. At 37 degrees Celsius, the cells were incubated. As a result, they were promoted twice in week.[30] Viability assays were used to measure cytotoxicity, with the cells being prepared in a 96-well plate to accommodate a concentration of 1×104 cells per well in 100 L of growth media. After 24 hours, various medium concentrations were administered and then supplemented using a multi-channel pipette. 96-well plates were used to spread the monolayers.

Modified microtiter plate incubation was done for 48 hours at 37 degrees Celsius and 5% CO. Three wells were used to concentrate the test material. The control cells were then cultivated with and without the test confinement, as well as with and without the addition of dimethyl sulfoxide. Dimethyl sulfoxide has a maximum inactivation concentration of 0.1%. When the cell was cultured at 37 °C for 24 hours, different concentrations of samples were obtained. A colorimetric approach was used to calculate cell yield. Following incubation, a 30-minute period of 1% crystal violet solution was applied to each well's remaining cell medium. The patches that remained were cleaned with distilled water.

The wells were filled with 30% glacial acetic acid, and then absorption measurements at 490 nm and a spectroscopic background correction were performed on the wells that did not have spots. Because there are not any substances that have been evaluated, Because the experiment was done in triplicate, samples were compared to cellular controls. The cytotoxicity efficacy was then determined. As a result, the optical density of the samples was determined using a microplate reader. The following equation was used to determine the number of viable cells as well as the percentage of cells that survived: [(ODt/ODc)] x 100%, where ODc is the average optical density of untreated cells and ODt is the average optical density of all treated wells in all tested samples. To determine the degree of cancer cell survival following therapy, histograms of live cells and medication concentrations were created.[31] The IC estimate of healthy cells can be determined using graphical displays of the dose-response curve for all concentrations (GraphPad Prism Software; San Diego, CA, USA).[32]

Molecular Docking

Materials

AutoDockTools 1.5.6, PyRx and BIOVIA Discovery Studio programs were used in the molecular docking study. ChemDraw3D Ultra software was used to draw all the chemical structures of all studied compounds and the PDB (Protein Data Bank) site.

Ligand Preparation

In order to avoid repetition, ChemDraw3D Ultra was used to refine the structures of all the compounds studied. Using the open source babel program, the structures were then transformed into PDBQT format.

Protein Preparation

The PDB (Protein Data Bank) site was used to obtain the 3D crystal structure of thymidylate synthase (PDB ID: 1AN5). The BIOVIA Discovery Studio software was used to remove small molecules from the crystal structures of (1AN5).[30,33]

Molecular Docking Study

Polar hydrogens and Kollman charges were applied to the protein, and a PDBFQT format file was generated using the AutoDockTools 1.5.6 program. The protein was created using the protein preparation wizard in AutoDockTools 1.5.6.

Polar hydrogens and Kollman charges were applied to the protein, and a PDBFQT format file was generated using AutoDockTools. (1AN5) was completely devoid of water molecules. The ligand torsions were calculated by first detecting the roots in AutoDockTools 1.5.6 and then setting the aromaticity parameters to 7.5.

The receptor was given a grid size of 60 Å × 60 Å × 60 Å, and the molecular docking operation was assigned to the Lamarckian genetic algorithm (LGA). After docking, the best pose was chosen based on binding energy, ligand–receptor interactions, and active site residues. The docked posture was simply compared to the cocrystallised structure, and the root mean square deviation (RMSD) was less than 1.0 Å. All torsions were allowed to rotate during docking. The traditional docking procedure for rigid and fluid ligand docking included ten separate runs per ligand, 2.5×106 energy measurements, a total of 27,000 iterations, a mutation rate of 0.02, a crossover rate of 0.80, and an elitism value of 1.

The likelihood of conducting a local search on a person in the population was 0.06 using a limit of 300 iterations per local search. Following docking, the ten solutions were classified as having RMS differences of less than 1.0. The clusters were sorted based on the cluster's lowest energy representation. The effects of the docking process were visualized using the BIOVIA Discovery Studio program.

Conclusions

In this study, α-aminophosphonate derivatives, arylidines, and disphosphonate derivatives derived from 4-hydroxybenzaldehyde were synthesized, purified, elucidated by spectroscopic analysis, and finally tested against carcinoma breast cancer to give high to moderate to low activity. A molecular docking study was used to determine the binding energy for non-bonding interactions between the ligand (studied compounds) and receptor, thymidylate synthase (pdb code: 1AN5). All of the compounds studied formed stable complexes with receptors that had a high binding energy. Compound **7b** had the best docking energy (highest binding energy) according to our findings, with a binding affinity of 10.31 kcal/mol, followed by compound **4c** (−10.22 kcal/mol) (Table 3).

Data Sharing Statement

The figures/data presented in this study are available on request from the corresponding author.

Ethics

The cell lines were purchased commercially from the ATCC https://www.lgcstandards-atcc.org/search#q=RH30&sort=relevancy; no further approval is required.

Acknowledgments

Disclosure

The authors declare no conflicts of interest in relation to this work.

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