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ORIGINAL ARTICLE

Anticancer activity, spectroscopic and molecular docking of some new synthesized sugar hydrazones, Arylidene and α-Aminophosphonate derivatives

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Abstract In this work, we design and then synthesis of new derivatives of nucleosides, oxadiazoline derivatives containing acetylated sugars and α-aminophosphonate derivatives. The synthesized compounds have been elucidated by different spectroscopic analysis, such as, elemental analysis, ^{13}C NMR, infrared (IR) and ^{1}H NMR. The compounds previously synthesized were purified and then tested against breast cancer cells (MCF-7). The results showed that the compounds **5d**, **6b**, **7a** and **9h** showed moderate to very high activity against breast cancer, with incidence rates of 78.45%, 84.60%, 93.45% and 95.78%, respectively. The reference ratio of 5-fluorouracil was inhibitory of 96.02%. The binding potential of synthesized compounds against thymidylate synthase (TS) and Cathepsin B (CB) has been investigated and the compounds **7a** and **9h** exhibits highly binding with thymidylate synthase (TS) and Cathepsin B (CB).

1. Introduction

It is noticeable in recent times that a large number of researchers in the field of organic and medical chemistry have tended to prepare new derivatives of nucleosides that contain monosaccharaides in their composition as well as contain heterocyclic rings known for their biological activity (Ayoup et al., 2016). A study has been conducted on sugars hydrazones derived from the compounds oxadiazole and oxadiazoline against

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cancer cells, and the results showed the effectiveness of these compounds compared to 5-fluorouracil (Amer et al., 2018; Alotaibi, 2020). It is known that heterocycles containing one or more nitrogen atoms have multiple biological activity, so thienyl and thiazolopyrimidine derivatives associated with monosaccharaides were synthesized and showed high results as anti-cancer (Basiony et al., 2020; Dwivedi et al., 2020). Scientists working in the field of discovering new drugs seek to synthesize simple compounds with high biological activity. Among these compounds are Schiff bases known for their high effectiveness as anti-cancer (Ejidike and Ajibade, 2016), as well as found that they work as antioxidants (Kumar et al., 2017), anti-viral (Seley-Radtke, 2020) and antibacterial (Amer et al., 2017; Mesbah et al., 2018; Alotaibi and Amer, 2020). Schiff's bases associated with hydrazone have been linked to copper (II), zinc (II), and cobalt (II) ions to form complexes that have been found to have effective anticancer effects (Fekri et al., 2019; Abd-Elzaher et al., 2016). In this work, we synthesized new nucleosides and shiff bases derived from 4-Hydroxybenzaldehyde and the synthesized compounds were tested against brest cancer cells.

2. Material and methods

2.1. Chemistry

Melting points were determined with a Kofler block apparatus and are uncorrected. The nuclear magnetic resonance analysis for hydrogen ^{1}HNMR was measured on spectrometer (400 MHz) using $CDCl_3$ as a solvent and TMS (δ) as the internal standard in King Saud University, Riyadh, Saudi Arabia. The IR spectra were recorded on a by ic50 model FTIR (Thermo) using KBr disks in University College of Turbah, Taif University, Turbah, Taif, Saudi Arabia. TLC using aluminum silica gel plates 60 F245 monitored the progress of the reactions. The anticancer activity of the synthesized compounds was carried out in Al-Azhar University, Cairo, Egypt.

2.1.1. Ethoxycarbonylmethyl parahydroxybenzaldhyde (2)

In a dry acetone (250 ml), a mix of *p*-hydroxybenzaldhyde **1** (10 mmole) and ethylchloroacetate (10 mmole) was boiled in the presence of condenser for 12 h in the presence of anhydrous K_2CO_3 (10 mmole). After we make sure that the reaction has ended, the mixture is filtered, and the filtrate is evaporated under pressure to give the yellow oil in 85% yield. R_f = 0.50 (3% Ethylacetate in Diethyl ether). ^{1}H NMR (DMSO d_6): δ = 1.22 (t, 3H, J = 8.1 Hz, CH_3CH_2), 4.11 (q, 2H, J = 8.1 Hz, CH_3CH_2), 4.63 (s, 2H, CH_2), 6.93 (d, 1H, J = 5.5 Hz, H-2), 7.56 (d, 1H, 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, 63.49; H, 5.85.

2.1.2. 2-(4-formylphenoxy)acetohydrazide (3)

A mixture of **2** (10 mmol) and hydrazine hydrate (30 mmole) in ethanol (30 ml) was refluxed for 5 h. The separated product is recrystallized by ethanol to yield a white powder in 90% yield, m.p. 203–205 °C, R_f = 0.31 (3% Ethylacetate in Diethyl ether). ^{1}H NMR (DMSO d_6): δ = 4.35 (brs, 2H, NH_2), 4.63 (s, 2H, CH_2), 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, 55.72; H, 5.23; N, 14.38.

2.1.3. General procedure for the preparation of nucleosides derived from 2-(4-formylphenoxy) acetohydrazide 5a-d

To a solution of hydrazide **1** (10 mmole) in absolute ethanol (40 ml), appropriate sugar derivatives (D-(+)-mannose, D-(+)-galactose, D-(+)-glucose and L-(+)-arabinose) (10 mmole) respectively and acetic acid few drops were added. The mixture was heated for 8–12 h in the presence of a condenser. After the reaction finished the solvent was removed by evaporation and the formed precipitate was filtered off, washed by absolute ethanol, dried to give the nucleosides **5a-d** in 80–85% yields respectively.

*2.1.3.1. 2-(4-formylphenoxy)-N'–2,3,4,5,6-pentahydroxyhexylidene)acetohydrazide (**5a**).* White powder, 85% yield, m.p. = 252–254 °C, Rf = 0.68 (5% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3370 (NH), 3310 (OH), 3050 (Ar-H), 1787 (CO of aldehyde), 1640 (CO of amide); ^{1}H NMR (400 MHz, DMSO d_6) δ: 2.75 (1H, dd, J = 5.2 Hz, OH), 3.55 (3H, dd, J = 5.2 Hz, 3xOH), 3.63 (1H, t, J = 5.2 Hz, OH), 3.59 (1H, d, J = 2.50 Hz, H-1), 3.35–3.80 (6H, m, H-2, H-3, H-4, H-5, H-6), 4.60 (2H, s, CH_2), 7.43 (1H, s, CH), 7.17 (2H, dd, J = 6.00 Hz, CH-aromatic), 7.85 (2H, dd, J = 6.2 Hz, CH-aromatic), 8.00 (H, brs, NH), 9.85 (1H, s, CHO); ^{13}C NMR (100 MHz, CDCl3): δ: 64.42 (CH_2OH of sugar), 69.21 (CH_2), 60.75, 70.62, 71.73, 72.33 (CH-Sugar), 114.82, 129.21, 132.33, 163.92 (Ar-CH), 153.52 (CH═N), 171.12 (CONH), 191.06 (CHO); Anal. Calcd for $C_{15}H_{20}N_2O_8$: C, 50.56; H, 5.66; N, 7.86. Found C, 50.51; H, 5.72; N, 7.90.

*2.1.3.2. D-(+)-Galactose-2-(4-formylphenoxy) acetohydrazide (**5b**).* White powder, 82% yield, m.p. = 283–285 °C, Rf = 0.65 (5% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3370 (NH), 3310 (OH), 3050 (Ar-H), 1787 (CO of aldehyde), 1640 (CO of amide); ^{1}H NMR (400 MHz, DMSO d_6) δ: 2.75 (1H, dd, J = 5.2 Hz, OH), 3.57 (3H, dd, J = 5.2 Hz, 3xOH), 3.65 (1H, t, J = 5.2 Hz, OH), 3.58 (1H, d, J = 2.50 Hz, H-1), 3.37–3.82 (6H, m, H-2, H-3, H-4, H-5, H-6), 4.63 (2H, s, CH_2), 7.40 (1H, s, CH), 7.18 (2H, dd, J = 6.00 Hz, CH-aromatic), 7.83 (2H, dd, J = 6.2 Hz, CH-aromatic), 8.00 (H, brs, NH), 9.85 (1H, s, CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 64.45 (CH_2OH of sugar), 69.24 (CH_2), 60.65, 70.67, 71.75, 72.33 (CH-Sugar), 114.81, 129.21, 132.33, 163.92 (Ar-CH), 153.52 (CH═N), 171.12 (CONH), 191.06 (CHO); Anal. Calcd for $C_{15}H_{20}N_2O_8$: C, 50.56; H, 5.66; N, 7.86. Found C, 50.61; H, 5.71; N, 7.91.

*2.1.3.3. D-(+)-Glucose-2-(4-formylphenoxy) acetohydrazide (**5c**).* White powder, 80% yield, m.p. = 225–227 °C, Rf = 0.55 (5% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3370 (NH), 3310 (OH), 3050 (Ar-H), 1787 (CO of aldehyde), 1640 (CO of amide); ^{1}H NMR (400 MHz, DMSO d_6) δ: 2.75 (1H, dd, J = 5.2 Hz, OH), 3.57 (3H, dd, J = 5.2 Hz, 3xOH), 3.65 (1H, t, J = 5.2 Hz, OH), 3.58 (1H, d, J = 2.50 Hz, H-1), 3.37–3.82 (6H, m, H-2, H-3, H-4, H-5, H-6), 4.63 (2H, s, CH_2), 7.40 (1H, s, CH), 7.18 (2H, dd, J = 6.00 Hz, CH-aromatic), 7.83 (2H, dd, J = 6.2 Hz, CH-aromatic), 8.00 (H, brs, NH), 9.85 (1H, s, CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 64.45 (CH_2OH of sugar), 69.24

(CH_2), 60.65, 70.67, 71.75, 72.33 (CH-Sugar), 114.81, 129.21, 132.33, 163.92 (Ar-CH), 153.52 (CH═N), 171.12 (CONH), 191.06 (CHO); Anal. Calcd for $C_{15}H_{20}N_2O_8$: C, 50.56; H, 5.66; N, 7.86. Found C, 50.60; H, 5.70; N, 7.91.

*2.1.3.4. L-(+)-Arabinose-2-(4-formylphenoxy) acetohydrazide (**5d**)*. White powder, 84% yield, m.p. = 298–300 °C, Rf = 0.78 (5% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3370 (NH), 3310 (OH), 3050 (Ar-H), 1787 (CO of aldehyde), 1640 (CO of amide); ^1H NMR (400 MHz, DMSO d_6) δ: 2.75 (1H, dd, J = 5.2 Hz, OH), 3.57 (2H, dd, J = 5.2 Hz, 2xOH), 3.65 (1H, t, J = 5.2 Hz, OH), 3.58 (1H, d, J = 2.50 Hz, H-1), 3.37–3.82 (4H, m, H-2, H-3, H-4, H-5), 4.63 (2H, s, CH_2), 7.40 (1H, s, CH), 7.18 (2H, dd, J = 6.00 Hz, CH-aromatic), 7.83 (2H, dd, J = 6.2 Hz, CH-aromatic), 8.00 (H, brs, NH), 9.85 (1H, s, CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 64.15 (CH_2OH of sugar), 69.09 (CH_2), 60.55, 72.15, 74.13 (CH-Sugar), 114.81, 129.21, 132.33, 163.92 (Ar-CH), 153.52 (CH═N), 171.12 (CONH), 191.06 (CHO); Anal. Calcd for $C_{14}H_{18}N_2O_7$: C, 51.53; H, 5.56; N, 8.59. Found C, 51.48; H, 5.60; N, 8.54.

*2.1.4. General procedure for the synthesis of acetylated nucleosides **6a-d***

A solution of nucleoside derivatives **5a-d** (10 mmole) in 20 ml of anhydrous pyridine, then the acetic anhydride (60 mmole) was added and The mixture was stirred at room temperature overnight (TLC). Then the product was poured over 80 gm of ice to give precipitates. The resultant precipitates were filtered, washed with water and dried to give the acetylated nucleosides **6a-d** in 83 = 91% yields.

*2.1.4.1. 2,3,4,5,6-Penta-O-acetyl-D-(+)-Mannose-2-(4-formylphenoxy)acetohydrazone (**6a**)*. Pale brown powder, 87% yield, m.p. = 198–200 °C, Rf = 0.55 (7% ethyl acetate in $CHCl_3$). IR sp-ectra (KBr) (v, cm^{-1}):, 3370 (NH), 3050 (Ar-H), 1735 ($COCH_3$), 1787 (CO of aldehyde), 1640 (CO of amide), 1375 (CH_3); ^1H NMR (400 MHz, DMSO d_6) δ: 2.11. 2.14. 2.15, 2.17, 2.20 (15H, 5 s, 5xCOCH3), 4.53 (1H, d, J = 2.50 Hz, H-1), 4.45–5.14 (5H, m, H-2, H-3, H-4, H-5, H-6), 4.63 (2H, s, CH_2), 7.48 (1H, s, CH), 7.19 (2H, dd, J = 6.00 Hz, CH-aromatic), 7.86 (2H, dd, J = 6.2 Hz, CH-aromatic), 8.08 (H, brs, NH), 9.91 (1H, s, CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.73 ($2CH_3$ of $2COCH_3$), 21.08 ($3CH_3$ of $3COCH_3$), 69.18 (CH_2), 58.67, 61.65, 67.54, 70.22, 71.37 (CH-Sugar), 114.79, 129.23, 132.33, 163.90 (Ar-CH), 153.43 (CH═N), 170.31 (CO of acetyl groups of sugar), 171.03 (CONH), 191.12 (CHO); Anal. Calcd for $C_{25}H_{30}N_2O_{13}$: C, 53.00; H, 5.34; N, 4.49. Found C, 52.99; H, 5.29; N, 4.45.

*2.1.4.2. 2,3,4,5,6-Penta-O-acetyl-D-(+)-Galactose-2-(4-formylphenoxy)acetohydrazone (**6b**)*. Pale brown powder, 85% yield, m.p. = 234–236 °C, Rf = 0.45 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3370 (NH), 3050 (Ar-H), 1735 ($COCH_3$), 1787 (CO of aldehyde), 1640 (CO of amide), 1375 (CH_3); ^1H NMR (400 MHz, DMSO d_6) δ: 2.11. 2.14. 2.15, 2.17, 2.20 (15H, 5 s, 5xCOCH3), 4.53 (1H, d, J = 2.50 Hz, H-1), 4.45–5.14 (5H, m, H-2, H-3, H-4, H-5, H-6), 4.63 (2H, s, CH_2), 7.48 (1H, s, CH), 7.19 (2H, dd, J = 6.00 Hz, CH-aromatic), 7.86 (2H, dd, J = 6.2 Hz, CH-aromatic), 8.08 (H, brs, NH), 9.91 (1H, s, CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.73 ($2CH_3$ of $2COCH_3$), 21.08 ($3CH_3$ of $3COCH_3$), 69.18 (CH_2), 58.67, 61.65, 67.54, 70.22, 71.37 (CH-Sugar), 114.79, 129.23, 132.33, 163.90 (Ar-CH), 153.43 (CH═N), 170.31 (CO of acetyl groups of sugar), 171.03 (CONH), 191.12 (CHO); Anal. Calcd for $C_{25}H_{30}N_2O_{13}$: C, 53.00; H, 5.34; N, 4.49. Found C, 52.99; H, 5.29; N, 4.45.

*2.1.4.3. 2,3,4,5,6-Penta-O-acetyl-D-(+)-Glucose-2-(4-formylphenoxy)acetohydrazone (**6c**)*. Pale brown powder, 83% yield, m.p. = 291–293 °C, Rf = 0.50 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3370 (NH), 3050 (Ar-H), 1735 ($COCH_3$), 1787 (CO of aldehyde), 1640 (CO of amide), 1375 (CH_3); ^1H NMR (400 MHz, DMSO d_6) δ: 2.11. 2.14. 2.15, 2.17, 2.20 (15H, 5 s, 5xCOCH3), 4.53 (1H, d, J = 2.50 Hz, H-1), 4.45–5.14 (5H, m, H-2, H-3, H-4, H-5, H-6), 4.63 (2H, s, CH_2), 7.48 (1H, s, CH), 7.19 (2H, dd, J = 6.00 Hz, CH-aromatic), 7.86 (2H, dd, J = 6.2 Hz, CH-aromatic), 8.08 (H, brs, NH), 9.91 (1H, s, CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.73 ($2CH_3$ of $2COCH_3$), 21.08 ($3CH_3$ of $3COCH_3$), 69.18 (CH_2), 58.67, 61.65, 67.54, 70.22, 71.37 (CH-Sugar), 114.79, 129.23, 132.33, 163.90 (Ar-CH), 153.43 (CH═N), 170.31 (CO of acetyl groups of sugar), 171.03 (CONH), 191.12 (CHO); Anal. Calcd for $C_{25}H_{30}N_2O_{13}$: C, 53.00; H, 5.34; N, 4.49. Found C, 52.99; H, 5.29; N, 4.45.

*2.1.4.4. 2,3,4,5,6-Penta-O-acetyl-D-(+)-Arabinose-2-(4-formylphenoxy)acetohydrazone (**6d**)*. Pale brown powder, 91% yield, m.p. = 258–260 °C, Rf = 0.60 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3370 (NH), 3050 (Ar-H), 1735 ($COCH_3$), 1787 (CO of aldehyde), 1640 (CO of amide), 1375 (CH_3); ^1H NMR (400 MHz, DMSO d_6) δ: 2.08. 2.13. 2.16, 2.18, (12H, 4 s, 4xCOCH3), 4.53 (1H, d, J = 2.50 Hz, H-1), 4.21–5.09 (4H, m, H-2, H-3, H-4, H-5), 4.63 (2H, s, CH_2), 7.48 (1H, s, CH), 7.20 (2H, dd, J = 6.00 Hz, CH-aromatic), 7.90 (2H, dd, J = 6.2 Hz, CH-aromatic), 8.12 (H, brs, NH), 9.78 (1H, s, CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.75 ($2CH_3$ of $2COCH_3$), 21.11 ($2CH_3$ of $2COCH_3$), 58.34, 61.30, 67.05, 68.47 (CH-Sugar), 69.20 (CH_2), 114.79, 129.23, 132.33, 163.90 (Ar-CH), 153.48 (CH═N), 170.22 (CO of acetyl groups of sugar), 171.07 (CONH), 191.08 (CHO); Anal. Calcd for $C_{22}H_{26}N_2O_{11}$: C, 53.44; H, 5.30; N, 5.67. Found C, 53.49; H, 5.36; N, 5.70.

*2.1.5. General procedure for the synthesis of Oxadiazoline derivatives **7a-d***

A mixture of acetylated nucleoside derivatives **6a-d** (10 mmole) was dissolved in acetic anhydride (60 mmole), then the reaction mixture was heated in the presence of condenser at 80–90 °C for 1–2 h (TLC). The resultant mixture was poured into 80 gm of ice, and then chloroform was added to the aqueous solution in separating funnel. We then obtained the separated organic layer dissolved in the solvent and then evaporated the solvent to give the corresponding oxadiazoline derivatives **7a-d** in 70–75% yields.

*2.1.5.1. 3-Acetyl-2-(2,3,4,5,6-Penta-O-acetyl-D-(+)-Mannose-2-(4-formylphenoxy)methyl – 1,3,4- Oxadiazoline (7**a**)*. Brown gum, 75% yield, Rf = 0.63 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3050 (Ar-H), 1738 ($COCH_3$), 1785 (CO of aldehyde), 1375 (CH_3), 1450 (CH_2); ^1H NMR (400 MHz, DMSO d_6) δ: 2.05 (3H, s, $COCH_3$ of

Oxadiazoline), 2.10, 2.13, 2.15, 2.18, 2.22 (15H, 5 s, 5x$COCH_3$), 4.60 (2H, s, CH_2), 4.24–5.12 (5H, m, H-2, H-3, H-4, H-5, H-6), 5.25 (1H, d, J = 2.50 Hz, H-1), 5.94 (1H, s, CH of Oxadiazoline), 7.20 (2H, dd, J = 6.00 Hz, CH-aromatic), 7.88 (2H, dd, J = 6.2 Hz, CH-aromatic), 9.87 (1H, s, CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.71 (CH_3 of $COCH_3$), 21.04 (4CH_3 of 4$COCH_3$), 23.38 (CH_3 of $COCH_3$ of Oxadiazoline), 69.99 (CH_2), 61.60, 62.17, 67.78, 68.75, 75.82 (CH-Sugar), 76.48 (CH-N-Ac of Oxadiazoline), 114.79, 129.23, 132.33, 163.90 (Ar-CH), 158.25 (CH═N of Oxadiazoline), 168.73 (CO of N-acetyl of Oxadiazoline), 170.20 (CO of acetyl groups of sugar), 190.43 (CHO); Anal. Calcd for $C_{27}H_{32}N_2O_{14}$: C, 53.29; H, 5.30; N, 4.60. Found C, 53.33; H, 5.36; N, 4.65.

*2.1.5.2. 3-Acetyl-2-(2,3,4,5,6-Penta-O-acetyl-D-(+)-Galactose-2-(4-formylphenoxy)methyl – 1,3,4- Oxadiazoline (**7b**).* Brown gum, 73% yield, Rf = 0.67 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3050 (Ar-H), 1738 ($COCH_3$), 1785 (CO of aldehyde), 1375 (CH_3), 1450 (CH_2); ^{1}H NMR (400 MHz, DMSO d_6) δ: 2.05 (3H, s, $COCH_3$ of Oxadiazoline), 2.10, 2.13, 2.15, 2.18, 2.22 (15H, 5 s, 5x$COCH_3$), 4.60 (2H, s, CH_2), 4.24–5.12 (5H, m, H-2, H-3, H-4, H-5, H-6), 5.25 (1H, d, J = 2.50 Hz, H-1), 5.94 (1H, s, CH of Oxadiazoline), 7.20 (2H, dd, J = 6.00 Hz, CH-aromatic), 7.88 (2H, dd, J = 6.2 Hz, CH-aromatic), 9.87 (1H, s, CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.71 (CH_3 of $COCH_3$), 21.04 (4CH_3 of 4$COCH_3$), 23.38 (CH_3 of $COCH_3$ of Oxadiazoline), 69.99 (CH_2), 61.60, 62.17, 67.78, 68.75, 75.82 (CH-Sugar), 76.48 (CH-N-Ac of Oxadiazoline), 114.79, 129.23, 132.33, 163.90 (Ar-CH), 158.25 (CH═N of Oxadiazoline), 168.73 (CO of N-acetyl of Oxadiazoline), 170.20 (CO of acetyl groups of sugar), 190.43 (CHO); Anal. Calcd for $C_{27}H_{32}N_2O_{14}$: C, 53.29; H, 5.30; N, 4.60. Found C, 53.33; H, 5.36; N, 4.65.

*2.1.5.3. 3-Acetyl-2-(2,3,4,5,6-Penta-O-acetyl-D-(+)-Glucose-2-(4-formylphenoxy)methyl – 1,3,4- Oxadiazoline (**7c**).* Brown gum, 70% yield, Rf = 0.60 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3050 (Ar-H), 1738 ($COCH_3$), 1785 (CO of aldehyde), 1375 (CH_3), 1450 (CH_2); ^{1}H NMR (400 MHz, DMSO d_6) δ: 2.05 (3H, s, $COCH_3$ of Oxadiazoline), 2.10, 2.13, 2.15, 2.18, 2.22 (15H, 5 s, 5x$COCH_3$), 4.60 (2H, s, CH_2), 4.24–5.12 (5H, m, H-2, H-3, H-4, H-5, H-6), 5.25 (1H, d, J = 2.50 Hz, H-1), 5.94 (1H, s, CH of Oxadiazoline), 7.20 (2H, dd, J = 6.00 Hz, CH-aromatic), 7.88 (2H, dd, J = 6.2 Hz, CH-aromatic), 9.87 (1H, s, CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.71 (CH_3 of $COCH_3$), 21.04 (4CH_3 of 4$COCH_3$), 23.38 (CH_3 of $COCH_3$ of Oxadiazoline), 69.99 (CH_2), 61.60, 62.17, 67.78, 68.75, 75.82 (CH-Sugar), 76.48 (CH-N-Ac of Oxadiazoline), 114.79, 129.23, 132.33, 163.90 (Ar-CH), 158.25 (CH═N of Oxadiazoline), 168.73 (CO of N-acetyl of Oxadiazoline), 170.20 (CO of acetyl groups of sugar), 190.43 (CHO); Anal. Calcd for $C_{27}H_{32}N_2O_{14}$: C, 53.29; H, 5.30; N, 4.60. Found C, 53.33; H, 5.36; N, 4.65.

*2.1.5.4. 3-Acetyl-2-(2,3,4,5,6-Penta-O-acetyl-D-(+)-Arabinose-2-(4-formylphenoxy)methyl – 1,3,4- Oxadiazoline (**7d**).* Brown gum, 74% yield, Rf = 0.65 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3050 (Ar-H), 1738 ($COCH_3$), 1785 (CO of aldehyde), 1375 (CH_3), 1450 (CH_2); ^{1}H NMR (400 MHz, DMSO d_6) δ: 2.06 (3H, s, $COCH_3$ of Oxadiazoline), 2.10, 2.13, 2.15, 2.18, 2.20 (12H, 5 s, 4x$COCH_3$), 4.60 (2H, s, CH_2), 4.21–5.15 (4H, m, H-2, H-3, H-4, H-5), 5.27 (1H, d, J = 2.50 Hz, H-1), 5.99 (1H, s, CH of Oxadiazoline), 7.18 (2H, dd, J = 6.00 Hz, CH-aromatic), 7.87 (2H, dd, J = 6.2 Hz, CH-aromatic), 9.87 (1H, s, CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.71 (CH_3 of $COCH_3$), 21.04 (3CH_3 of 3$COCH_3$), 23.25 (CH_3 of $COCH_3$ of Oxadiazoline), 70.00 (CH_2), 61.62, 64.65, 68.73, 75.63 (CH-Sugar), 76.79 (CH-N-Ac of Oxadiazoline), 114.79, 129.23, 132.33, 163.90 (Ar-CH), 158.30 (CH═N of Oxadiazoline), 168.55 (CO of N-acetyl of Oxadiazoline), 170.23 (CO of acetyl groups of sugar), 191.12 (CHO); Anal. Calcd for $C_{24}H_{28}N_2O_{12}$: C, 53.73; H, 5.26; N, 5.22. Found C, 53.77; H, 5.31; N, 5.36.

*2.1.6. Preparation of α-aminophosphonates **9a-l***

Equivalent amounts of Oxadiazoline derivatives **7a-d** (10 mmole) triphenylphosphite (10 mmole) and different aromatic amines (1-Napthyl amine (10 mmole), 2-Nitro aniline (10 mmole) and 4-Methyl aniline (10 mmole) respectively were dissolved in CH_3CN, the addition of few drops of perchloric acid occurred, later the stirring was occurred at 25 °C overnight. The solvent evaporated and then the residues of a-aminophosphonate derivatives were treated with diethyl ether to afford the corresponding α-Aminophosphonates **9a-l** in 85–92% yields.

*2.1.6.1. 5-((4-((diphenoxyphosphoryl)(naphthalen-1-ylamino)methyl)phenoxy)methyl)-3-Acetyl-2-(2,3,4,5,6-Penta-O-acetyl-D-(+)-Mannose-2-phenoxy)methyl – 1,3,4- Oxadiazoline (**9a**).* Pale yellow powder, 85% yield, m.p. = 278–280 °C, Rf = 0.75 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3350 (NH), 3050 (Ar-H), 1738 ($COCH_3$), 1375 (CH_3), 1450 (CH_2); ^{1}HNMR (400 MHz, DMSO d_6) δ: 2.03 (3H, s, $COCH_3$ of Oxadiazoline), 2.08, 2.11, 2.14, 2.17, 2.21 (15H, 5 s, 5x$COCH_3$), 4.60 (2H, s, CH_2), 4.22–5.12 (5H, m, H-2, H-3, H-4, H-5, H-6), 5.27 (1H, d, J = 2.50 Hz, H-1), 5.65 (1H, t, J = 5.5 Hz, CH-P-), 5.90 (1H, s, CH of Oxadiazoline), 6.85–8.10 (21H, m, Ar-H), 8.98 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.73 (CH_3 of $COCH_3$), 21.08 (4CH_3 of 4$COCH_3$), 23.41 (CH_3 of $COCH_3$ of Oxadiazoline), 69.65 (CH-P-), 72.31 (CH_2) 61.67, 62.27, 67.72, 68.43, 75.76 (CH-Sugar), 76.48 (CH-N-Ac of Oxadiazoline), 109.34, 114.09, 119.12, 120.33, 121.23, 124.65, 124.76, 125.06, 126.11, 127.66, 127.92, 128.4, 128.6, 10.1, 134.34, 14748, 150.42, 156.18 (Ar-CH), 158.27 (CH═N of Oxadiazoline), 168.55 (CO of N-acetyl of Oxadiazoline), 170.25 (CO of acetyl groups of sugar); Anal. Calcd for $C_{49}H_{50}N_3O_{16}P$: C, 60.80; H, 5.21; N, 4.34. Found C, 60.86; H, 5.25; N, 4.40.

*2.1.6.2. 5-((4-((diphenoxyphosphoryl)((2-nitrophenyl)amino)methyl)phenoxy)methyl)-3-Acetyl-2-(2,3,4,5,6-Penta-O-acetyl-D-(+)-Mannose-2-phenoxy)methyl – 1,3,4- Oxadiazoline (**9b**).* Pale yellow powder, 90% yield, m.p. = > 300 °C, Rf = 0.80 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3350 (NH), 3050 (Ar-H), 1738 ($COCH_3$), 1570, 1380 (NO_2), 1375 (CH_3), 1450 (CH_2); ^{1}HNMR (400 MHz, DMSO d_6) δ: 2.03 (3H, s, $COCH_3$ of Oxadiazoline), 2.08, 2.11, 2.14, 2.17, 2.21 (15H, 5 s, 5x$COCH_3$), 4.60 (2H, s, CH_2), 4.22–5.12 (5H, m, H-2, H-3, H-4, H-5, H-6), 5.27 (1H, d, J = 2.50 Hz, H-1), 5.65 (1H, t, J = 5.5 Hz, CH-P-), 5.90 (1H, s, CH of Oxadiazoline), 6.85–8.10 (18H, m, Ar-H), 8.98

(1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.73 (CH_3 of $COCH_3$), 21.08 ($4CH_3$ of $4COCH_3$), 23.41 (CH_3 of $COCH_3$ of Oxadiazoline), 69.65 (CH-P-), 72.31 (CH_2) 61.67, 62.27, 67.72, 68.43, 75.76 (CH-Sugar), 76.48 (CH-N-Ac of Oxadiazoline), 109.34, 114.09, 119.12, 120.33, 121.23, 124.65, 124.76, 125.06, 126.11, 127.66, 127.92, 128.4, 128.6, 10.1, 134.34, 14748, 150.42, 156.18 (Ar-CH), 158.27 (CH═N of Oxadiazoline), 168.55 (CO of N-acetyl of Oxadiazoline), 170.25 (CO of acetyl groups of sugar); Anal. Calcd for $C_{45}H_{48}N_4O_{18}P$: C, 56.13; H, 4.92; N, 5.82. Found C, 56.08; H, 5.00; N, 5.78.

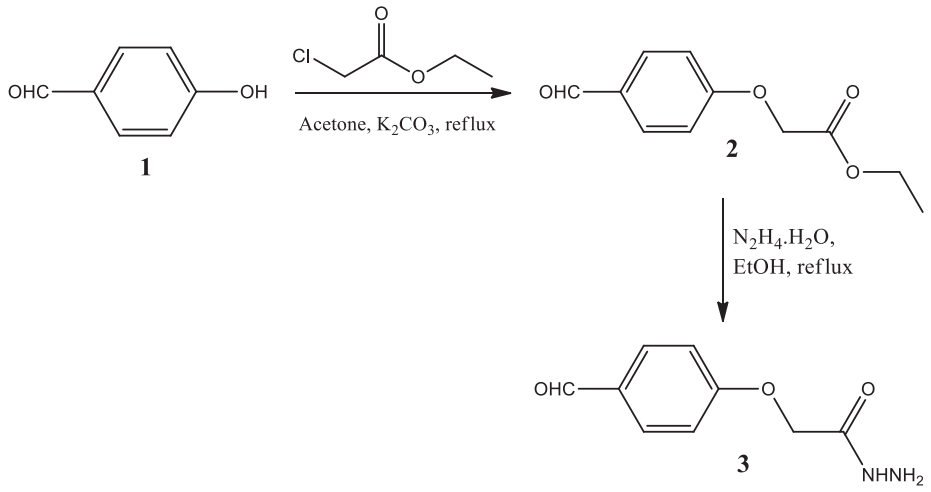
*2.1.6.3. 5-((4-((diphenoxyphosphoryl)(p- tolylamino)methyl) phenoxy)methyl)-3-Acetyl-2-(2,3,4,5,6-Penta-O-acetyl-D-(+)-Mannose-2-phenoxy)methyl – 1,3,4- Oxadiazoline (**9c**)*. Pale yellow powder, 87% yield, m.p. = > 300 °C, Rf = 0.70 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3350 (NH), 3050 (Ar-H), 1738 ($COCH_3$), 1375 (CH_3), 1450 (CH_2); ^{1}HNMR (400 MHz, DMSO d_6) δ: 2.03 (3H, s, $COCH_3$ of Oxadiazoline), 2.08, 2.11, 2.14, 2.17, 2.21 (15H, 5 s, 5x$COCH_3$), 2.35 (3H, s, CH_3), 4.60 (2H, s, CH_2), 4.22–5.12 (5H, m, H-2, H-3, H-4, H-5, H-6), 5.27 (1H, d, J = 2.50 Hz, H-1), 5.65 (1H, t, J = 5.5 Hz, CH-P-), 5.90 (1H, s, CH of Oxadiazoline), 6.85–8.10 (18H, m, Ar-H), 8.98 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.73 (CH_3 of $COCH_3$), 21.08 ($4CH_3$ of $4COCH_3$), 21.41 (CH_3), 23.41 (CH_3 of $COCH_3$ of Oxadiazoline), 69.65 (CH-P-), 72.31 (CH_2) 61.67, 62.27, 67.72, 68.43, 75.76 (CH-Sugar), 76.48 (CH-N-Ac of Oxadiazoline), 109.34, 114.09, 119.12, 120.33, 121.23, 124.65, 124.76, 125.06, 126.11, 127.66, 127.92, 128.4, 128.6, 10.1, 134.34, 14748, 150.42, 156.18 (Ar-CH), 158.27 (CH═N of Oxadiazoline), 168.55 (CO of N-acetyl of Oxadiazoline), 170.25 (CO of acetyl groups of sugar); Anal. Calcd for $C_{46}H_{50}N_3O_{16}P$: C, 59.29; H, 5.41; N, 4.51. Found C, 59.33; H, 5.37; N, 4.55.

*2.1.6.4. 5-((4-((diphenoxyphosphoryl)(naphthalen-1-ylamino) methyl)phenoxy)methyl)-3-Acetyl-2-(2,3,4,5,6-Penta-O-acetyl-D-(+)-Galactose-2-phenoxy)methyl – 1,3,4- Oxadiazoline (**9d**)*. Pale yellow powder, 92% yield, m.p. = 267–269 °C, Rf = 0.72 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3350 (NH), 3050 (Ar-H), 1738 ($COCH_3$), 1375 (CH_3), 1450 (CH_2); ^{1}HNMR (400 MHz, DMSO d_6) δ: 2.03 (3H, s, $COCH_3$ of Oxadiazoline), 2.08, 2.11, 2.14, 2.17, 2.21 (15H, 5 s, 5x$COCH_3$), 4.60 (2H, s, CH_2), 4.22–5.12 (5H, m, H-2, H-3, H-4, H-5, H-6), 5.27 (1H, d, J = 2.50 Hz, H-1), 5.65 (1H, t, J = 5.5 Hz, CH-P-), 5.90 (1H, s, CH of Oxadiazoline), 6.85–8.10 (21H, m, Ar-H), 8.98 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.73 (CH_3 of $COCH_3$), 21.08 ($4CH_3$ of $4COCH_3$), 23.41 (CH_3 of $COCH_3$ of Oxadiazoline), 69.65 (CH-P-), 72.31 (CH_2) 61.67, 62.27, 67.72, 68.43, 75.76 (CH-Sugar), 76.48 (CH-N-Ac of Oxadiazoline), 109.34, 114.09, 119.12, 120.33, 121.23, 124.65, 124.76, 125.06, 126.11, 127.66, 127.92, 128.4, 128.6, 10.1, 134.34, 14748, 150.42, 156.18 (Ar-CH), 158.27 (CH═N of Oxadiazoline), 168.55 (CO of N-acetyl of Oxadiazoline), 170.25 (CO of acetyl groups of sugar); Anal. Calcd for $C_{49}H_{50}N_3O_{16}P$: C, 60.80; H, 5.21; N, 4.34. Found C, 60.86; H, 5.26; N, 4.40.

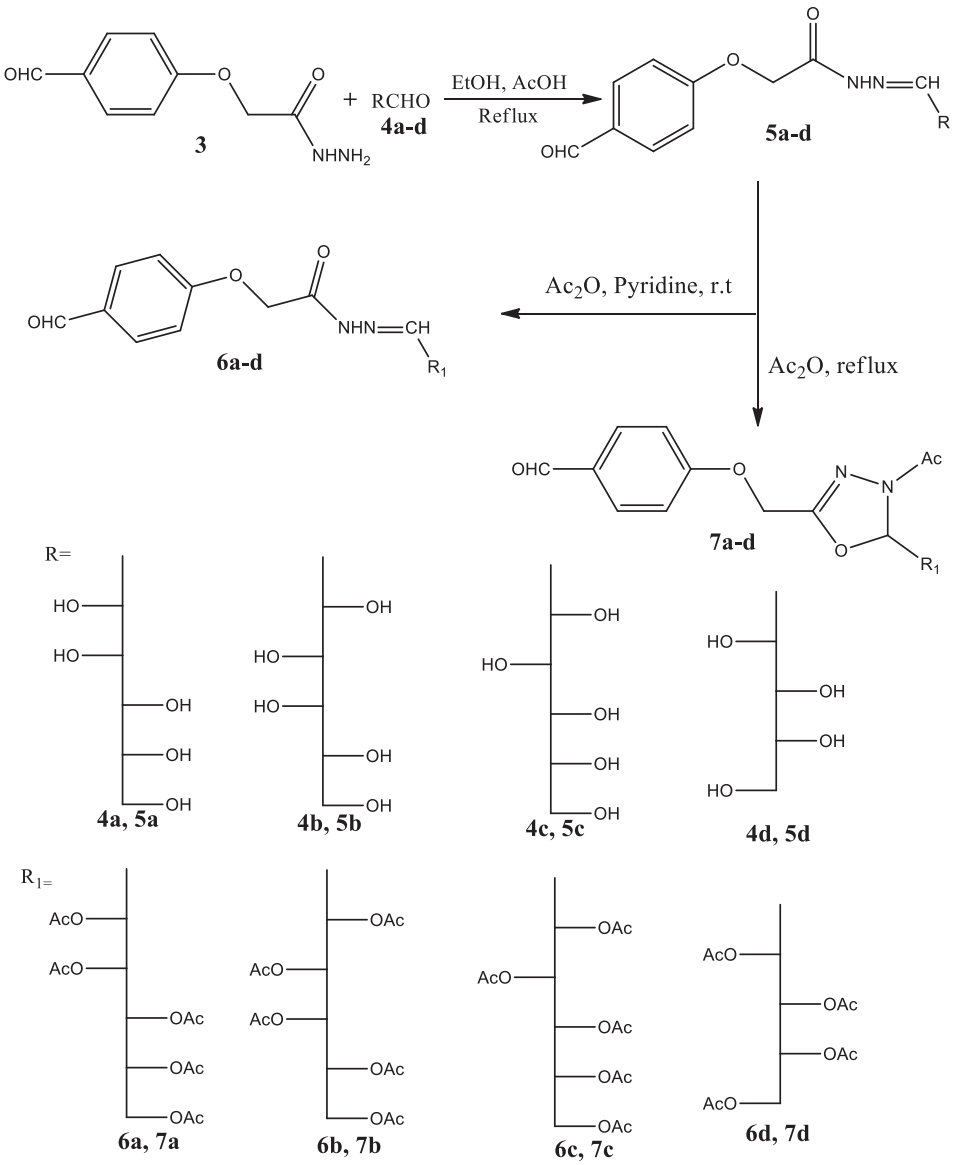
*2.1.6.5. 5-((4-((diphenoxyphosphoryl)((2-nitrophenyl)amino) methyl)phenoxy)methyl)-3-Acetyl-2-(2,3,4,5,6-Penta-O-acetyl-D-(+)-Galactose-2-phenoxy)methyl – 1,3,4- Oxadiazoline (**9e**)*. Pale brown powder, 88% yield, m.p. = 290–292 °C, Rf = 0.65 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3350 (NH), 3050 (Ar-H), 1738 ($COCH_3$), 1570, 1380 (NO_2), 1375 (CH_3), 1450 (CH_2); ^{1}HNMR (400 MHz, DMSO d_6) δ: 2.03 (3H, s, $COCH_3$ of Oxadiazoline), 2.08, 2.11, 2.14, 2.17, 2.21 (15H, 5 s, 5x$COCH_3$), 4.60 (2H, s, CH_2), 4.22–5.12 (5H, m, H-2, H-3, H-4, H-5, H-6), 5.27 (1H, d, J = 2.50 Hz, H-1), 5.65 (1H, t, J = 5.5 Hz, CH-P-), 5.90 (1H, s, CH of Oxadiazoline), 6.85–8.10 (18H, m, Ar-H), 8.98 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.73 (CH_3 of $COCH_3$), 21.08 ($4CH_3$ of $4COCH_3$), 23.41 (CH_3 of $COCH_3$ of Oxadiazoline), 69.65 (CH-P-), 72.31 (CH_2) 61.67, 62.27, 67.72, 68.43, 75.76 (CH-Sugar), 76.48 (CH-N-Ac of Oxadiazoline), 109.34, 114.09, 119.12, 120.33, 121.23, 124.65, 124.76, 125.06, 126.11, 127.66, 127.92, 128.4, 128.6, 10.1, 134.34, 147.48, 150.42, 156.18 (Ar-CH), 158.27 (CH═N of Oxadiazoline), 168.55 (CO of N-acetyl of Oxadiazoline), 170.25 (CO of acetyl groups of sugar); 31P NMR (162 MHz, $CDCl_3$): d = 17.23 (O = P-CH-); Anal. Calcd for $C_{45}H_{48}N_4O_{18}P$: C, 56.13; H, 4.92; N, 5.82. Found C, 56.19; H, 5.00; N, 5.89.

*2.1.6.6. 5-((4-((diphenoxyphosphoryl)(p- tolylamino)methyl) phenoxy)methyl)-3-Acetyl-2-(2,3,4,5,6-Penta-O-acetyl-D-(+)-Galactose-2-phenoxy)methyl – 1,3,4- Oxadiazoline (**9f**)*. White powder, 88% yield, m.p. = > 300 °C, Rf = 0.55 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3350 (NH), 3050 (Ar-H), 1738 ($COCH_3$), 1375 (CH_3), 1450 (CH_2); ^{1}HNMR (400 MHz, DMSO d_6) δ: 2.03 (3H, s, $COCH_3$ of Oxadiazoline), 2.08, 2.11, 2.14, 2.17, 2.21 (15H, 5 s, 5x$COCH_3$), 2.35 (3H, s, CH_3), 4.60 (2H, s, CH_2), 4.22–5.12 (5H, m, H-2, H-3, H-4, H-5, H-6), 5.27 (1H, d, J = 2.50 Hz, H-1), 5.65 (1H, t, J = 5.5 Hz, CH-P-), 5.90 (1H, s, CH of Oxadiazoline), 6.85–8.10 (18H, m, Ar-H), 8.98 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.73 (CH_3 of $COCH_3$), 21.08 ($4CH_3$ of $4COCH_3$), 21.41 (CH_3), 23.41 (CH_3 of $COCH_3$ of Oxadiazoline), 69.65 (CH-P-), 72.31 (CH_2) 61.67, 62.27, 67.72, 68.43, 75.76 (CH-Sugar), 76.48 (CH-N-Ac of Oxadiazoline), 109.34, 114.09, 119.12, 120.33, 121.23, 124.65, 124.76, 125.06, 126.11, 127.66, 127.92, 128.4, 128.6, 10.1, 134.34, 14748, 150.42, 156.18 (Ar-CH), 158.27 (CH═N of Oxadiazoline), 168.55 (CO of N-acetyl of Oxadiazoline), 170.25 (CO of acetyl groups of sugar); Anal. Calcd for $C_{46}H_{50}N_3O_{16}P$: C, 59.29; H, 5.41; N, 4.51. Found C, 59.34; H, 5.37; N, 4.58.

*2.1.6.7. 5-((4-((diphenoxyphosphoryl)(naphthalen-1-ylamino) methyl)phenoxy)methyl)-3-Acetyl-2-(2,3,4,5,6-Penta-O-acetyl-D-(+)-Glucose-2-phenoxy)methyl – 1,3,4- Oxadiazoline (**9g**)*. Brown gum, 85% yield, Rf = 0.73 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3350 (NH), 3050 (Ar-H), 1738 ($COCH_3$), 1375 (CH_3), 1450 (CH_2); ^{1}HNMR (400 MHz, DMSO d_6) δ: 2.03 (3H, s, $COCH_3$ of Oxadiazoline), 2.08, 2.11, 2.14, 2.17, 2.21 (15H, 5 s, 5x$COCH_3$), 4.60 (2H, s, CH_2), 4.22–5.12 (5H, m, H-2, H-3, H-4, H-5, H-6), 5.27 (1H, d, J = 2.50 Hz, H-1), 5.65 (1H, t, J = 5.5 Hz, CH-P-), 5.90 (1H, s, CH of Oxadiazoline), 6.85–8.10 (21H, m, Ar-H), 8.98 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.73 (CH_3 of $COCH_3$), 21.08 ($4CH_3$ of $4COCH_3$), 23.41 (CH_3 of $COCH_3$ of Oxadiazoline), 69.65 (CH-P-), 72.31 (CH_2) 61.67, 62.27, 67.72, 68.43, 75.76 (CH-Sugar), 76.48 (CH-N-Ac of Oxadiazoline), 109.34, 114.09, 119.12, 120.33, 121.23, 124.65, 124.76, 125.06, 126.11, 127.66, 127.92, 128.4, 128.6, 10.1, 134.34, 14748, 150.42, 156.18 (Ar-CH), 158.27 (CH═N of Oxadiazoline), 168.55 (CO of N-acetyl of Oxadiazoline), 170.25 (CO of



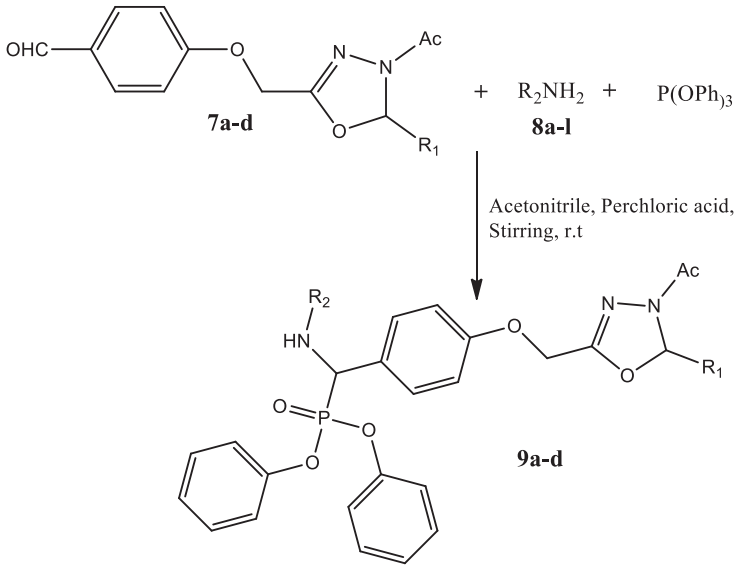
Scheme 1



Scheme 2

acetyl groups of sugar); Anal. Calcd for $C_{49}H_{50}N_3O_{16}P$: C, 60.80; H, 5.21; N, 4.34. Found C, 60.86; H, 5.25; N, 4.40.

*2.1.6.8. 5-((4-((diphenoxyphosphoryl)((2-nitrophenyl)amino) methyl)phenoxy)methyl)-3-Acetyl-2-(2,3,4,5,6-Penta-O-acetyl-D-(+)-Glucose-2-phenoxy)methyl – 1,3,4- Oxadiazoline (**9h**)*. Brown gum, 87% yield, Rf = 0.72 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3350 (NH), 3050 (Ar-H), 1738 ($COCH_3$), 1570, 1380 (NO_2), 1375 (CH_3), 1450 (CH_2); ^{1}-HNMR (400 MHz, DMSO d_6) δ: 2.03 (3H, s, $COCH_3$ of Oxadiazoline), 2.08, 2.11, 2.14, 2.17, 2.21 (15H, 5 s, 5x$COCH_3$), 4.60 (2H, s, CH_2), 4.22–5.12 (5H, m, H-2, H-3, H-4, H-5, H-6), 5.27 (1H, d, J = 2.50 Hz, H-1), 5.65 (1H, t, J = 5.5 Hz, CH-P-), 5.90 (1H, s, CH of Oxadiazoline), 6.85–8.10 (18H, m, Ar-H), 8.98 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.73 (CH_3 of $COCH_3$), 21.08 (4CH_3 of 4$COCH_3$), 23.41 (CH_3 of $COCH_3$ of Oxadiazoline), 69.65 (CH-P-), 72.31 (CH_2) 61.67, 62.27, 67.72, 68.43, 75.76 (CH-Sugar), 76.48 (CH-N-Ac of Oxadiazoline), 109.34, 114.09, 119.12, 120.33, 121.23, 124.65, 124.76, 125.06, 126.11, 127.66, 127.92, 128.4, 128.6, 10.1, 134.34, 14748, 150.42, 156.18 (Ar-CH), 158.27 (CH═N of Oxadiazoline), 168.55 (CO of N-acetyl of Oxadiazoline), 170.25 (CO of acetyl groups of sugar); Anal. Calcd for $C_{45}H_{48}N_4O_{18}P$: C, 56.13; H, 4.92; N, 5.82. Found C, 56.18; H, 4.86; N, 5.76.



9	R_1	R_2
a	2,3,4,5,6-Pentaacetylmannose	1-Naphthyl
b	2,3,4,5,6-Pentaacetylmannose	2-Nitrophenyl
c	2,3,4,5,6-Pentaacetylmannose	4-Methylphenyl
d	2,3,4,5,6-Pentaacetylgalactose	1-Naphthyl
e	2,3,4,5,6-Pentaacetylgalactose	2-Nitrophenyl
f	2,3,4,5,6-Pentaacetylgalactose	4-Methylphenyl
g	2,3,4,5,6-Pentaacetylglucose	1-Naphthyl
h	2,3,4,5,6-Pentaacetylglucose	2-Nitrophenyl
i	2,3,4,5,6-Pentaacetylglucose	4-Methylphenyl
j	2,3,4,5-Tetraacetylarabinose	1-Naphthyl
k	2,3,4,5-Tetraacetylarabinose	2-Nitrophenyl
l	2,3,4,5-Tetraacetylarabinose	4-Methylphenyl

Scheme 3

*2.1.6.9. 5-((4-((diphenoxyphosphoryl)(p- tolylamino)methyl) phenoxy)methyl)-3-Acetyl-2-(2,3,4,5,6-Penta-O-acetyl-D-(+)-Glucose-2-phenoxy)methyl – 1,3,4- Oxadiazoline (**9i**)*. Brown powder, 88% yield, m.p. = > 300 °C, Rf = 0.66 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3350 (NH), 3050 (Ar-H), 1738 ($COCH_3$), 1375 (CH_3), 1450 (CH_2); ^{1}HNMR (400 MHz, DMSO d_6) δ: 2.03 (3H, s, $COCH_3$ of Oxadiazoline), 2.08, 2.11, 2.14, 2.17, 2.21 (15H, 5 s, 5x$COCH_3$), 2.35 (3H, s, CH_3), 4.60 (2H, s, CH_2), 4.22–5.12 (5H, m, H-2, H-3, H-4, H-5, H-6), 5.27 (1H, d, J = 2.50 Hz, H-1), 5.65 (1H, t, J = 5.5 Hz, CH-P-), 5.90 (1H, s, CH of Oxadiazoline), 6.85–8.10 (18H, m, Ar-H), 8.98 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.73 (CH_3 of $COCH_3$), 21.08 (4CH_3 of 4$COCH_3$), 21.41 (CH_3), 23.41 (CH_3 of $COCH_3$ of Oxadiazoline), 69.65 (CH-P-), 72.31 (CH_2) 61.67, 62.27, 67.72, 68.43, 75.76 (CH-Sugar), 76.48 (CH-N-Ac of Oxadiazoline), 109.34, 114.09, 119.12, 120.33, 121.23, 124.65, 124.76, 125.06, 126.11, 127.66, 127.92, 128.4, 128.6, 10.1, 134.34, 14748, 150.42, 156.18 (Ar-CH), 158.27 (CH═N of Oxadiazoline), 168.55 (CO of N-acetyl of Oxadiazoline), 170.25 (CO of acetyl groups of sugar); Anal. Calcd for $C_{46}H_{50}N_3O_{16}P$: C, 59.29; H, 5.41; N, 4.51. Found C, 59.35; H, 5.36; N, 4.57.

*2.1.6.10. 5-((4-((diphenoxyphosphoryl)(naphthalen-1-ylamino)methyl)phenoxy)methyl)-3-Acetyl-2-(2,3,4,5,6-Penta-O-acetyl-D-(+)-Arabinose-2-phenoxy)methyl – 1,3,4- Oxadiazoline (**9j**)*. White powder, 92% yield, m.p. = 212–214 °C, Rf = 0.63 (7% methanol in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3350 (NH), 3050 (Ar-H), 1738 ($COCH_3$), 1375 (CH_3), 1450 (CH_2); ^{1}HNMR (400 MHz, DMSO d_6) δ: 2.03 (3H, s, $COCH_3$ of Oxadiazoline), 2.08, 2.13, 2.17, 2.21 (12H, 4 s, 4x$COCH_3$), 4.60 (2H, s, CH_2), 4.22–5.12 (4H, m, H-2, H-3, H-4, H-5), 5.27 (1H, d, J = 2.50 Hz, H-1), 5.65 (1H, t, J = 5.5 Hz, CH-P-), 5.90 (1H, s, CH of Oxadiazoline), 6.85–8.10 (21H, m, Ar-H), 8.98 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.73 (CH_3 of $COCH_3$), 21.08 (3CH_3 of 3$COCH_3$), 23.41 (CH_3 of $COCH_3$ of Oxadiazoline), 69.65 (CH-P-), 72.31 (CH_2) 61.67, 67.72, 68.43, 75.76 (CH-Sugar), 76.48 (CH-N-Ac of Oxadiazoline), 109.34, 114.09, 119.12, 120.33, 121.23, 124.65, 124.76, 125.06, 126.11, 127.66, 127.92, 128.4, 128.6, 10.1, 134.34, 14748, 150.42, 156.18 (Ar-CH), 158.27 (CH═N of Oxadiazoline), 168.55 (CO of N-acetyl of Oxadiazoline), 170.25 (CO of acetyl groups of sugar); Anal. Calcd for $C_{46}H_{46}N_3O_{14}P$: C, 61.67; H, 5.18; N, 4.69. Found C, 61.73; H, 5.25; N, 4.74.

*2.1.6.11. 5-((4-((diphenoxyphosphoryl)((2-nitrophenyl) amino)methyl)phenoxy)methyl)-3-Acetyl-2-(2,3,4,5,6-Penta-O-acetyl-D-(+)-Arabinose-2-phenoxy)methyl – 1,3,4- Oxadiazoline (**9k**)*. White powder, 89% yield, m.p. = 277–279 °C, Rf = 0.56 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3350 (NH), 3050 (Ar-H), 1738 ($COCH_3$), 1570, 1380 (NO_2), 1375 (CH_3), 1450 (CH_2); ^{1}HNMR (400 MHz, DMSO d_6) δ: 2.03 (3H, s, $COCH_3$ of Oxadiazoline), 2.11, 2.14, 2.17, 2.21 (12H, 4 s, 4x$COCH_3$), 4.60 (2H, s, CH_2), 4.22–5.12 (4H, m, H-2, H-3, H-4, H-5), 5.27 (1H, d, J = 2.50 Hz, H-1), 5.65 (1H, t, J = 5.5 Hz, CH-P-), 5.90 (1H, s, CH of Oxadiazoline), 6.85–8.10 (18H, m, Ar-H), 8.98 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.73 (CH_3 of $COCH_3$), 21.08 (3CH_3 of 3$COCH_3$), 23.41 (CH_3 of $COCH_3$ of Oxadiazoline), 69.65 (CH-P-), 72.31 (CH_2) 61.67, 67.72, 68.43, 75.76 (CH-Sugar), 76.48 (CH-N-Ac of Oxadiazoline), 109.34, 114.09, 119.12, 120.33, 121.23, 124.65, 124.76, 125.06, 126.11, 127.66, 127.92, 128.4, 128.6, 10.1, 134.34, 14748, 150.42, 156.18 (Ar-CH), 158.27 (CH═N of Oxadiazoline), 168.55 (CO of N-acetyl of Oxadiazoline), 170.25 (CO of acetyl groups of sugar); Anal. Calcd for $C_{42}H_{43}N_4O_{16}P$: C, 56.63; H, 4.87; N, 6.29. Found C, 56.68; H, 4.94; N, 6.35.

*2.1.6.12. 5-((4-((diphenoxyphosphoryl)(p- tolylamino)methyl) phenoxy)methyl)-3-Acetyl-2-(2,3,4,5,6-Penta-O-acetyl-D-(+)-Arabinose-2-phenoxy)methyl – 1,3,4- Oxadiazoline (**9l**)*. Brown gum, 88% yield, Rf = 0.57 (7% ethyl acetate in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3350 (NH), 3050 (Ar-H), 1738 ($COCH_3$), 1375 (CH_3), 1450 (CH_2); ^{1}HNMR (400 MHz, DMSO d_6) δ: 2.03 (3H, s, $COCH_3$ of Oxadiazoline), 2.11, 2.14, 2.17, 2.21 (12H, 4 s, 4x$COCH_3$), 2.35 (3H, s, CH_3), 4.60 (2H, s, CH_2), 4.22–5.12 (4H, m, H-2, H-3, H-4, H-5), 5.27 (1H, d, J = 2.50 Hz, H-1), 5.65 (1H, t, J = 5.5 Hz, CH-P-), 5.90 (1H, s, CH of Oxadiazoline), 6.85–8.10 (18H, m, Ar-H), 8.98 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 20.73 (CH_3 of $COCH_3$), 21.08 (3CH_3 of 3$COCH_3$), 21.41 (CH_3), 23.41 (CH_3 of $COCH_3$ of Oxadiazoline), 69.65 (CH-P-), 72.31 (CH_2) 62.27, 67.72, 68.43, 75.76 (CH-Sugar), 76.48 (CH-N-Ac of Oxadiazoline), 109.34, 114.09, 119.12, 120.33, 121.23, 124.65, 124.76, 125.06, 126.11, 127.66, 127.92, 128.4, 128.6, 10.1, 134.34, 14748, 150.42, 156.18 (Ar-CH), 158.27 (CH═N of Oxadiazoline), 168.55 (CO of N-acetyl of Oxadiazoline), 170.25 (CO of acetyl groups of sugar); Anal. Calcd for $C_{43}H_{46}N_3O_{14}P$: C, 60.07; H, 5.39; N, 4.89. Found C, 60.00; H, 5.43; N, 4.94.

2.2. Anticancer activity

2.2.1. Cell line propagation

At the start of pretreatment, cell propagation was established via Dulbecco modified Eagle Medium (DMEM), consisting of 1% L-glutamine, 50 μg/mL gentamicin, and 10% heat inactivated fetal bovine serum and HEPES buffer. Cells were placed in an incubation at 37 °C. Hence, they were promoted twice a week. (Mosmann, 1983). Cytotoxicity was determined via a viability assay in which a 96-well plate was used to prepare cells to accommodate a concentration of 1x 104 per well in 100 μl growth medium. After 24 h, different concentrations of the medium were applied and then added using a multichannel pipette, the serial double dilution of the tested chemical compounds was assigned to the confluent cell. Monolayers were distributed in 96 well plates. Adjustment of microtiter plate incubation for 48 h with moisture at 37 °C and 5% CO2 was performed. The test sample was concentrated with three wells. Then, each control cell was incubated without test containment as well as with the addition of dimethylsulphoxide and without. The maximum inactive concentration of dimethylsulphoxide was 0.1%. After the cell incubation at 37 °C, and with a 24-hour incubation setting, different concentrations of samples were produced. A colorimetric technique was used to determine the cell productivity. After incubation, 1% violet crystalline solution was placed into the remaining cell media in each well for 30 min. The remaining stains were washed by distilled water. 30% glacial acetic acid was added in the wells followed by absorption measurements at 490 nm followed by a spectral background correction across the well without spots. By the absence of the compounds tested. Sam-

Table 1 Inhibitory activity of the nucleoside derivative **5d** against breast carcinoma cells. IC50 (165 ± 4.4 μM/mL).

Sample Concentration (μg/ml)	Inhibition	Viability (%)	SD (±)
500	78.45	21.55	0.73
250	63.56	36.44	2.01
125	42.14	57.86	1.27
62.5	20.50	79.50	0.50
31.25	8.23	91.77	0.38
15.6	1.78	98.22	0.21
7.8	0.55	99.45	0.14
3.9	0	100	–
0	0	100	–

Table 2 Inhibitory activity of the acetylated nucleoside derivative **6b** against breast carcinoma cells. (IC50 = 88.25 ± 7.2 μM/mL).

Sample Concentration (μg/ml)	Viability (%)	Inhibition	SD (±)
500	15.40	84.60	0.25
250	29.80	70.20	1.11
125	41.85	58.15	1.95
62.5	63.76	36.24	2.08
31.25	81.75	18.43	0.65
15.6	92.36	7.64	0.26
7.8	97.45	2.55	0.28
3.9	99.80	0.20	0.05
0	100	0	–

Table 3 Inhibitory activity of the oxadiazoline derivative **7a** against breast carcinoma cells. (IC50 = 40.7 ± 4.7 μM/mL).

Sample Concentration (μg/ml)	Viability (%)	Inhibition	SD (±)
500	6.55	93.45	0.28
250	15.57	84.43	0.55
125	24.44	75.56	0.85
62.5	36.28	63.72	2.55
31.25	47.42	52.58	2.25
15.6	74.35	25.65	1.76
7.8	92.90	7.10	0.80
3.9	99.33	0.77	0.14
0	100	0	–

ples were compared with cellular controls as the experiment was conducted in triplicate; the efficacy of cytotoxicity was then calculated. Hence, a microplate reader was used to detect the optical density of the samples. The viable cells were mapped together with the percentage survival by the following equation: [(ODt/ODc)] × 100%, where ODc is the average optical density of untreated cells and ODt is the average optical density of all wells treated with samples tested; A graph of live cells and drug concentration was made to understand the degree of survival of tumor cells alive after treatment (Gomha et al., 2015). Graphic plots of the dose–response-curve for all the concentration help to determine the estimation of the IC50 of intact cells (GraphPad Prism software; San Diego, CA USA).

Table 4 Inhibitory activity of the α-aminophosphonate derivative **9h** against breast carcinoma cells. (IC50 = 32.4 ± 3.75 μM/mL).

Sample Concentration (μg/ml)	Viability (%)	Inhibition	SD (±)
500	4.22	95.78	0.15
250	11.24	88.76	0.31
125	21.60	78.40	0.42
62.5	31.53	68.47	1.00
31.25	39.55	60.45	2.30
15.6	71.24	28.76	2.00
7.8	89.76	10.24	0.34
3.9	98.50	1.50	0.18
0	100	0	–

Table 5 Inhibitory activity of 5-Flurouracil against breast carcinoma cells. (IC50 = 14 ± 0.8 μM/mL).

Sample Concentration (μg/ml)	Viability (%)	Inhibition	SD (±)
500	3.98	96.02	0.16
250	8.12	91.88	0.24
125	14.91	95.09	0.33
62.5	27.84	72.16	0.18
31.25	39.58	60.42	0.62
15.6	46.79	53.21	2.31
7.8	62.43	37.57	1.69
3.9	78.15	21.58	0.41
0	100	0	–

Table 6 The docking binding free energies of the examined compounds and the co-crystallized ligands against TS and CB (ΔG in Kcal/mole).

	Comp.	Thymidylate Synthase	Cathepsin-B
1	**5a**	−22.66	−14.78
2	**5b**	−22.27	−16.64
3	**5c**	−22.65	−15.08
4	**5d**	−21.62	−15.36
5	**6a**	−31.57	−18.66
6	**6b**	−30.17	−17.57
7	**6c**	−30.05	−18.27
8	**6d**	−28.29	−18.10
9	**7a**	−33.54	−19.39
10	**7b**	−30.91	−15.96
11	**7c**	−32.07	−17.25
12	**7d**	−29.58	−17.62
13	**9a**	−35.53	−18.68
14	**9b**	−35.31	−17.20
15	**9c**	−37.25	−19.89
16	**9d**	−34.22	−17.07
17	**9e**	−36.31	−16.18
18	**9f**	−35.74	−16.90
19	**9g**	−37.23	−18.88
20	**9h**	−38.22	−20.02
21	**9i**	−39.47	−18.17
22	**9j**	−33.78	−18.74
23	**9k**	−33.31	−18.68
24	**9l**	−32.63	−17.15
25	co-crystallized ligand (LYA)	−23.30	–
25	co-crystallized ligand(78A)	–	−18.59

2.3. Docking studies

Into TS binding site (PDB ID: 1JU6, resolution: 2.89 Å) and CB (PDB ID: 2DCD, resolution: 2.50 Å). The co-crystallized ligands (LYA and 78A) were used as reference molecules against TS and CB, respectively. The binding free energies (ΔG) were reported in (Table 6) against thymidylate synthase (TS) and Cathepsin B (CB). The crystal structures of the target proteins; thymidylate synthase (TS) (PDB ID: JU6, resolution: 2.89 Å) and Cathepsin B (CB) (PDB ID: 2DCD, resolution: 2.50 Å) were downloaded from Protein Data Bank (http://www.pdb.org). Molecular Operating Environment (MOE) was used for the docking analysis (Nasser et al., 2020). In these

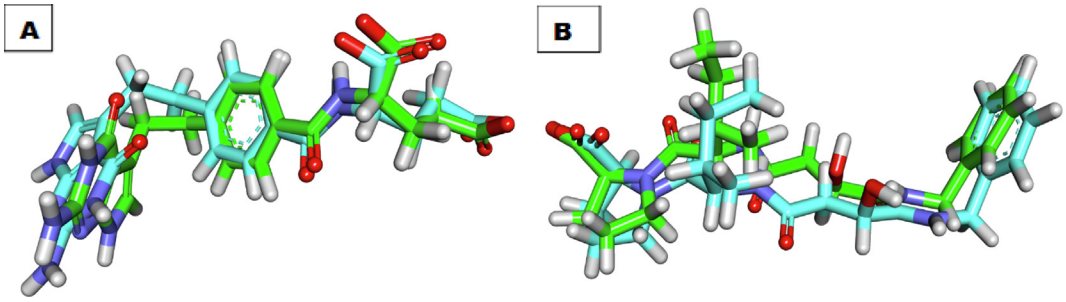


Fig. 1 Superimposition of the co-crystallized pose (green) and the docking pose (maroon) of the same ligands, **A**) LYA, **B**) 78A.

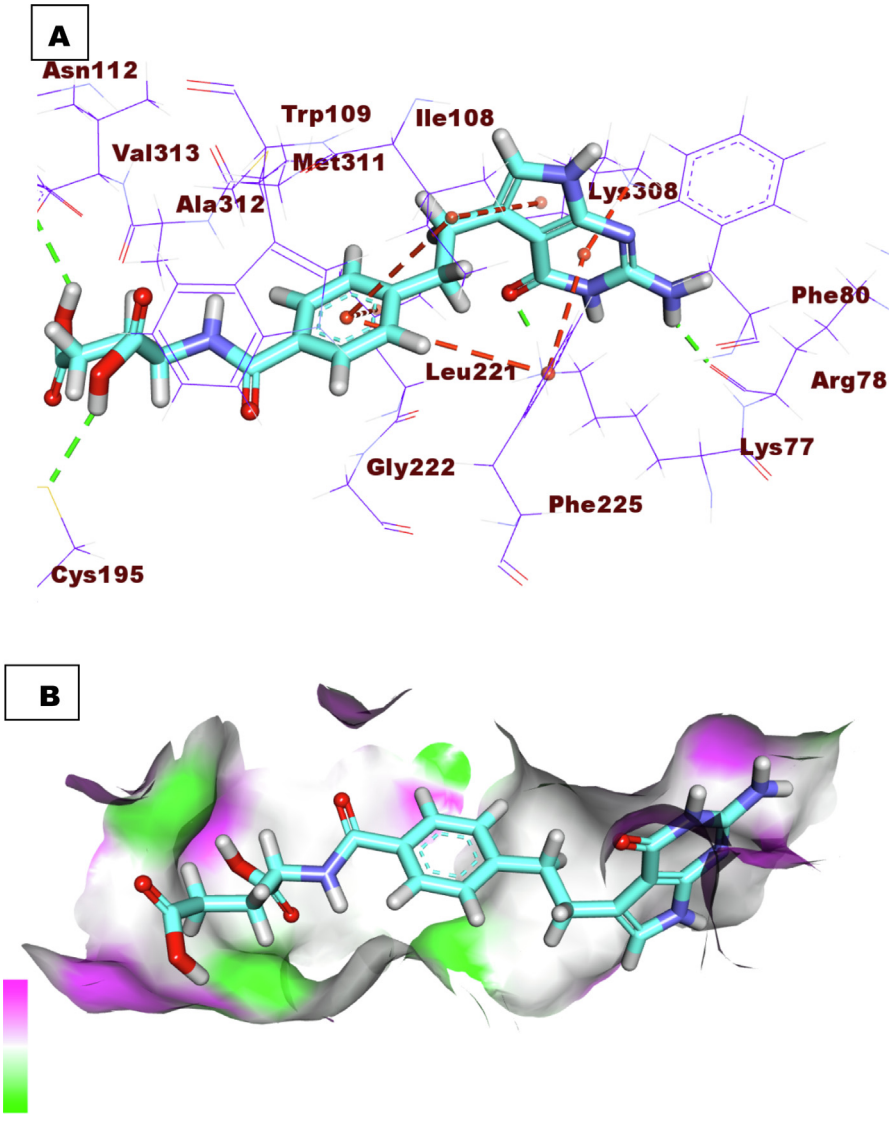


Fig. 2 **A)** Co-crystallized ligand **(**LYA**)** docked into the active site of TS. **B)** Mapping surface showing co-crystallized ligand **(**LYA**)** occupying the active pocket of TS.

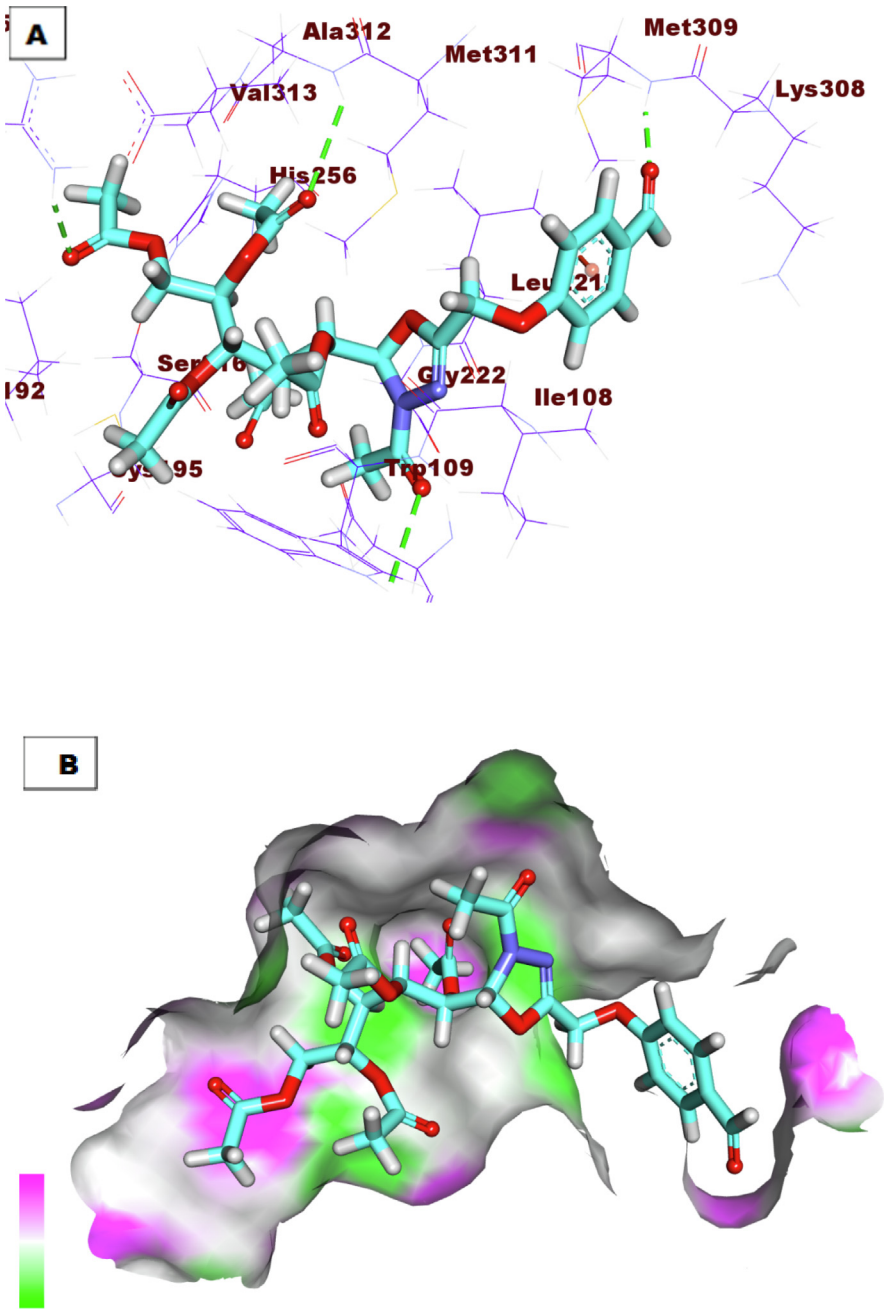


Fig. 3 **A)** Compound **7a** docked into the active site of TS. **B)** Mapping surface showing compound **7a** occupying the active pocket of TS.

studies, the free energies and binding modes of the examined molecules against target proteins were determined. At first, the water molecules were removed from the crystal structures of target proteins, retaining only one chain which essential for binding. The co-crystallized ligands (LYA and 78A for TS and CB, respectively) were used as reference ligands. Then, the protein structures were protonated and the hydrogen atoms were hided. Next, the energy was minimized and the binding pockets of each protein was defined (Li et al., 2020; El-Gamal et al., 2018). The structures of the examined compounds and the co-crystallized ligands were drawn using ChemBioDraw Ultra 14.0 and saved as SDF formats. Then, the saved files were opened using MOE software and 3D structures were protonated. Next, the energy of the molecules was minimized. Validation processes were performed for each target receptor by running the docking process for only the co-crystallized ligand. Low RMSD values between docked and crystal conformations indicate valid performance (Ibrahim, 2017; Elmetwally et al., 2019). The docking procedures were carried out utilizing a default protocol. In each case, 20 docked structures were generated using genetic algorithm searches. The output from of MOE software was further analyzed and visualized using Discovery Studio 4.0 software (Nasser et al., 2020; Elmetwally et al., 2019; Mahdy, 2020; El-Zahabi et al., 2019; El-Naggar, 2020).

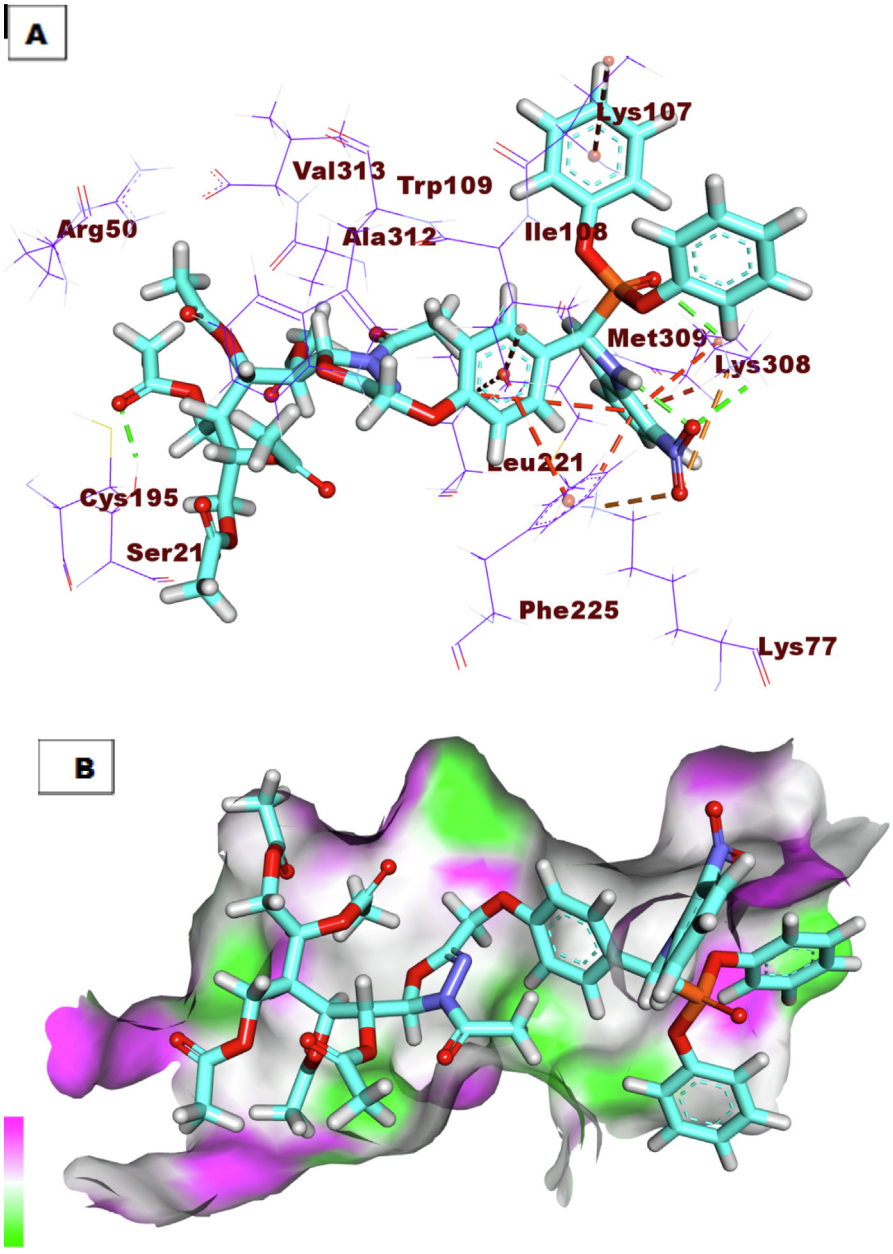


Fig. 4 **A)** Compound **9i** docked into the active site of TS. **B)** Mapping surface showing compound **9i** occupying the active pocket of TS.

2.4. In silico toxicity

The toxicity parameters of the synthesized compounds were calculated using Discovery studio 4.0. Sorafenib was used as a reference drug. At first, the CHARMM force field was applied then the compounds were prepared and minimized according to the preparation of small molecule protocol. Then different parameters were calculated from the toxicity prediction (extensible) protocol.

3. Results and discussion

3.1. Chemistry

4-Hydroxybenzaldehyde (**1**) was reacted with ethylchloroacetate and potassium carbonate in dry acetone under reflux to give Ethoxycarbonylmethyl parahydroxybenzaldhyde (**2**) in 85% yield. Ethoxycarbonylmethyl parahydroxybenzaldhyde (**2**) was hydrazinolysis by hydrazine hydrate in absolute ethanol to give 2-(4-formylphenoxy) acetohydrazide (**3**) (Scheme 1). The elucidation of compounds **2** by ^{1}HNMR spectra appears that triplet peak at 1.22 for $\underline{CH_3}CH_2$, quartet at 4.11 for CH_3-$\underline{CH_2}$ and 10.49 for CHO group; on the other hand, the elucidation of **3** by ^{1}HNMR spectra appears that broad peak at 4.35 for NH_2 group, broad peak at 9.33 for NH and singlet at 10.42 for CHO group.

Hydrazide **3** was reacted with sugar moieties (D-(+)-Mannose, D-(+)-Galactose, D-(+)-Glucose and D-(+)-arabinose respectively) **4a-d** in absolute ethanol and in the presence of acetic acid as catalyst under reflux to give the corresponding nucleosides **5a-d** in 80–85% yields which were acetylated by acetic anhydride in pyridine at room temperature

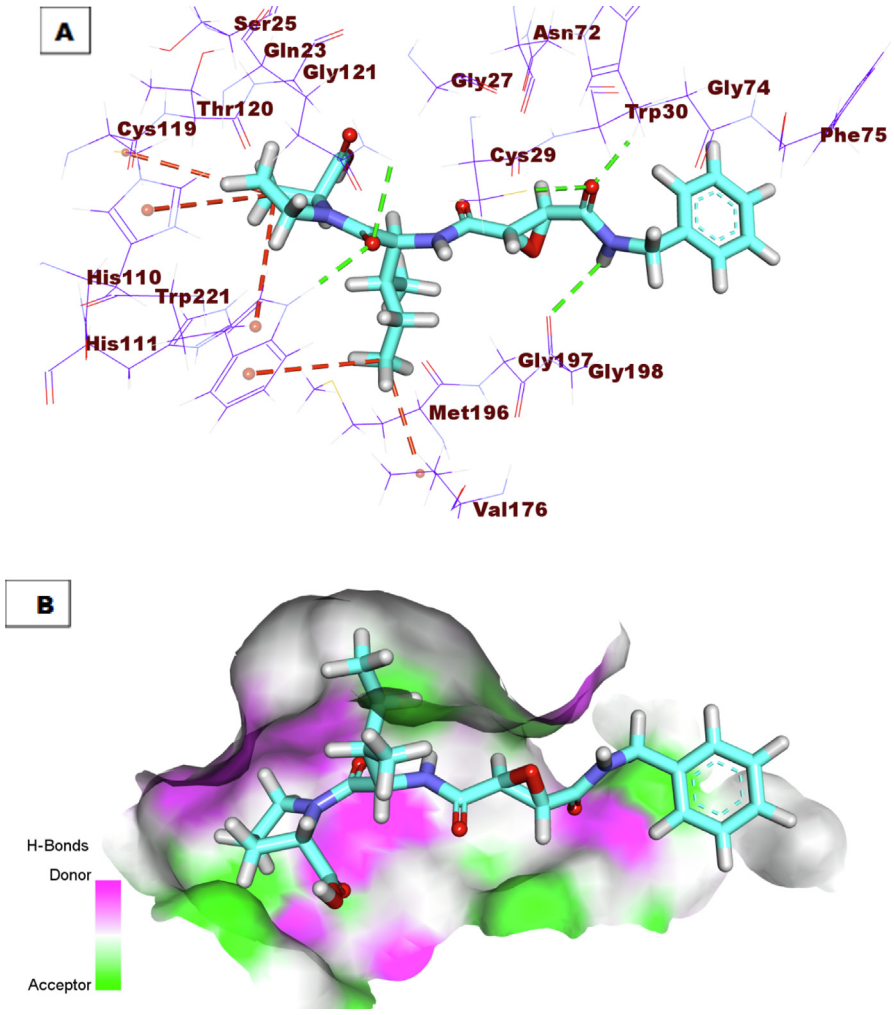


Fig. 5 **A)** Co-crystallized ligand (78A) docked into the active site of Cathepsin B. **B)** Mapping surface showing co-crystallized ligand (78A) occupying the active pocket of Cathepsin B.

to give the acetylated nucleosides **6a-d** in yields, on the other hand nucleosides **5a-d** were reacted with acetic anhydride at **80–90** °C to give oxadiazoline derivatives **7a-d** in yields (Scheme 2). The elucidation of nucleosides **5a-d** by IR spectra showed that the disappearance of NH_2 and appearance of 1640 of CONH, 1778 for CHO, 3310 for OH and 3370 for NH; 1HNMR spectra appears that the disappearance of NH_2 and appearance of peaks around 3.35–3.80 for ((CH) groups of sugar moieties), singlet peaks around 2.75–3.63 for (OH) groups of sugar moieties), doublet of doublet 7.17 and 7.85 for CH-aromatic, broad peak around 8.00 for (NH group) and singlet peak around 9.85 for CHO group; ^{13}CNMR spectra appears that peaks around 60.75 to 72.53 for (CH of sugar moieties), 64.42 for hydroxyl groups of sugar moieties, the peaks of (CH– aromatics) appears around 114.82 to 163.92, peak around 153.52 for (CH═N), peak around 171.12 for (CONH) and peak around 191.06 for formyl group. The elucidation of nucleosides **6a-d** by IR spectra showed that the disappearance of hydroxyl group and appearance of 3370 for NH, 1735 for ($COCH_3$) and 1640 of CONH, 1778 for CHO; ^{1}HNMR spectra appears that the disappearance of hydroxyl groups of sugar moieties and appearance of singlet peaks around 2.08 to 2.20 for ($COCH_3$ groups of sugar moieties), singlet peaks around 4.45–5.14 for ((CH) groups of sugar moieties), doublet of doublet 7.19 and 7.86 for CH-aromatic, singlet peak around 7.84 for (CH═N), broad peak around 8.08 for (NH group) and singlet peak around 9.91 for CHO group; ^{13}CNMR spectra appears that peaks around 20.73 to 21.08 for (CH_3 of acetyl groups of sugar moieties), peaks around 58.67 to 71.37 for (CH of sugar moieties), peaks of (CH– aromatics) appears around 114.79 to 163.90, peak around 153.43 for (CH═N), peak around 170.31 for (CO of acetyl groups of sugar moieties), peak around 171.03 for (CONH) and peak around 191.12 for formyl group. The elucidation of nucleosides **7a-d** by IR spectra showed that the appearance of 1785 for CHO and 1735 for ($COCH_3$); ^{1}HNMR spectra appears that the appearance of singlet peak around 2.05 for acetyl group of oxadiazoline, singlet peaks around 2.10 to 2.22 for (acetyl groups of sugar moieties), singlet peaks around 4.24–5.25 for ((CH) groups of sugar moieties), doublet of doublet 7.20 and 7.88 for CH-aromatic, singlet peak around 5.94 for (CH═N of oxadiazoline), and singlet peak around 9.87 for CHO group; ^{13}CNMR spectra appears that peaks around 20.71 to 21.04 for (CH_3 of acetyl groups of sugar moieties), singlet peak around 23.38 for acetyl group of oxadiazoline ring, peaks around 61.60 to 75.82 for (CH of sugar

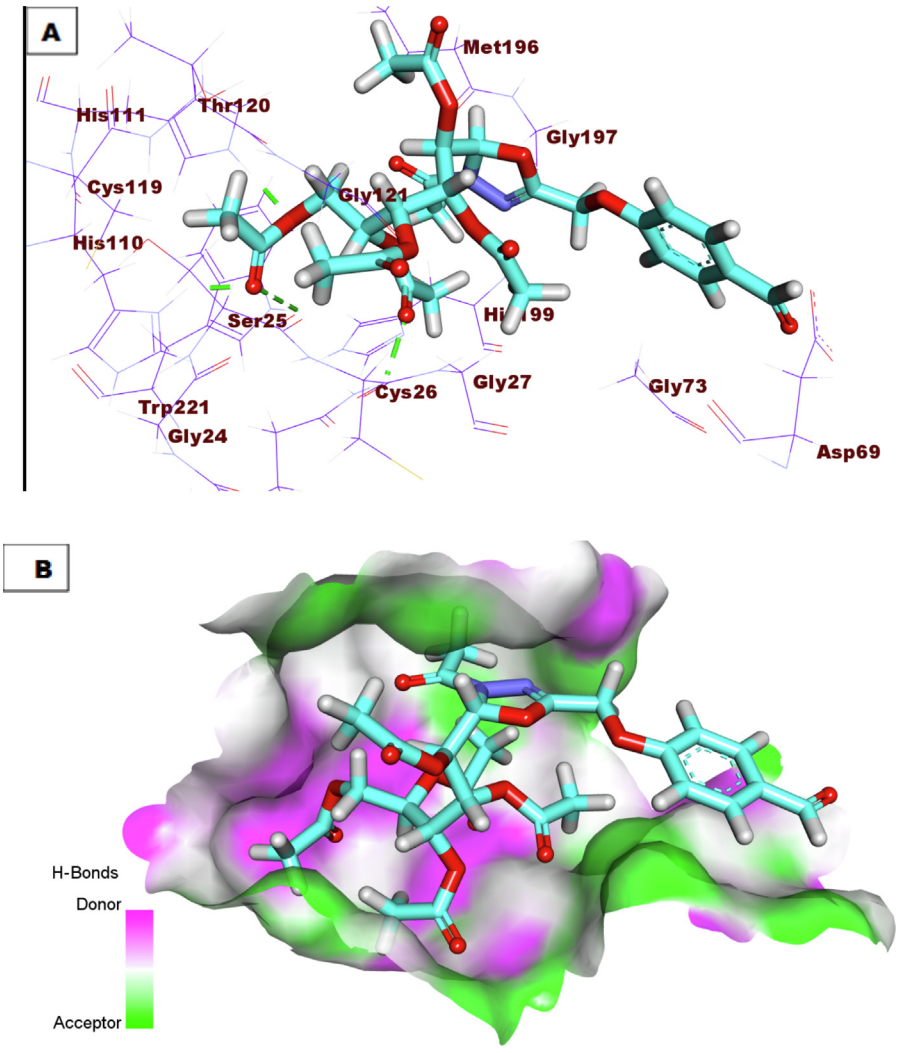


Fig. 6 **A)** Compound **7a** docked into the active site of Cathepsin B. **B)** Mapping surface showing compound **7a** occupying the active pocket of Cathepsin B.

moieties), peak around 76.48 for (CH-N-Ac of oxadiazoline), peaks of (CH– aromatics) appears around 114.79 to 163.90, peak around 158.25 for (CH═N), peak around 168.73 for (CO of N-acetyl of oxadiazoline), peak around 170.20 for (CO of acetyl groups of sugar moieties), peak around 171.03 for (CONH) and peak around 190.43 for formyl group.

Oxadiazoline derivatives **7a-d** were reacted with primary amine derivatives **8a-l** and triphenylphosphite in acetonitrile and in the presence of perchloric acid as catalyst at room temperature to give α-aminophosphonate derivatives **9a-l** in 85–92% yields (Scheme 3). The elucidation of nucleosides **9a-l** by IR spectra showed that the disappearance of formyl group and appearance of peak around 3350 for (NH), peak around 3050 for (Ar-H) and peak around 1738 for ($COCH_3$); ^{1}HNMR spectra appears that the appearance of singlet peak around 2.03 for acetyl group of oxadiazoline, singlet peaks around 2.08 to 2.21 for (acetyl groups of sugar moieties), singlet peaks around 4.22–5.27 for ((CH) groups of sugar moieties), peak around 5.65 for (CH-P-), peak around 5.90 for (CH═N of oxadiazoline), multiplet peaks around 6.85 to 8.00 for (CH-aromatic) and broad peak around 8.98 for NH group; ^{13}-CNMR spectra appears that peaks around 20.73 to 21.08 for (CH_3 of acetyl groups of sugar moieties), singlet peak around 23.41 for acetyl group of oxadiazoline ring, peaks around 61.67 to 75.76 for (CH of sugar moieties), peak around 69.65 for (CH-P-) peak around 76.48 for (CH-N-Ac of oxadiazoline), peaks of (CH– aromatics) appears around 109.34 to 156.18, peak around 158.27 for (CH═N of oxadiazoline), peak around 168.55 for (CO of N-acetyl of oxadiazoline) and peak around 170.25 for (CO of acetyl groups of sugar moieties).

3.2. Anticancer activity

In this work, the compounds synthesized as anti-breast cancer compounds were tested. These compounds were nucleosides, acetylated nucleosides, and oxadiazoline derivatives, alpha-aminophosphonates **6d**, **4d**, **6b** and **4c** against the line cells of MCF-7. The well-known drug as the anticancer 5-fluorouracil (5-FU) was used as a reference, which is an important analog of Pyrimidines. Table 5 and Fig. 5. The results showed that the compound **9h** containing phosphonate group had very high inhibitory activity against MCF-7cell line, with 95.78% inhibition against breast cancer cells; Compared to the

inhibition by the standard 5-FU of 96.02% (Table 4). The results also showed that the compound **7a** containing the sugar acetylated and oxadiazoline ring (Table 3) had high activity against the MCF-7 cell line, with 93.45% inhibition. On the other hand, the results showed that the two compounds **5d** and **6b** had moderate activity against breast cancer (Tables 1, 2), with 78.45% and 84.60% inhibition, respectively.

3.3. Docking study

In this work, the binding potential of twenty-four compounds against thymidylate synthase (TS) and Cathepsin B (CB) has been investigated. This investigational work was performed to get further insight into the binding modes into TS binding site (PDB ID: 1JU6, resolution: 2.89 Å) and CB (PDB ID: 2DCD, resolution: 2.50 Å) (Nasser et al., 2020). The co-crystallized ligands (LYA and 78A) were used as reference molecules against TS and CB, respectively. The binding free energies (ΔG) were reported in (Table 6). To validate the docking process, the co-crystallized ligands (LYA and 78A) were re-docked against the isolated pockets of the target proteins. It was found that, the RMSD values between the re-docked conformer and the co-crystallized conformers of LYA and 78A were 1.34 and 1.51, respectively, which confirms the validity of the used docking protocols (Fig. 1).

The binding mode of the co-crystallized ligand (LYA) against TS showed an affinity value of −23.30 Kcal/mole. It formed four hydrogen bonds and six hydrophobic interactions. The 2-oxopyrrolidin-3-yl moiety occupied the first pocket of TS forming two hydrogen bonds with Arg78 and Lys77. Furthermore, it formed three hydrophobic interactions with Lys308, Phe225, and Ile108. The central phenyl ring occupied the spacer region of TS forming three hydrophobic interactions with Ile108, Phe225, and Leu225. Besides, the glutamic acid moiety occupied the 2nd pocket of the receptor and was incorporated in two hydrogen-bonding interactions with Val313 and Cys195 (Fig. 2). Compound **7a** exhibited a binding mode like that of the co-crystallized ligands against TS with an affinity value of −33.54 Kcal/mole. It formed four hydrogen bonds. The benzaldehyde moiety occupied the first pocket of TS forming a hydrogen bond with Met309. Additionally, the 3-acetyl-2, 3-dihydro-1, 3, 4-oxadiazole moiety occupied the spacer region of the TS forming a hydrogen bond with Trp109. The pentane-1, 2, 3, 4, 5-pentayl pentaacetate occu-

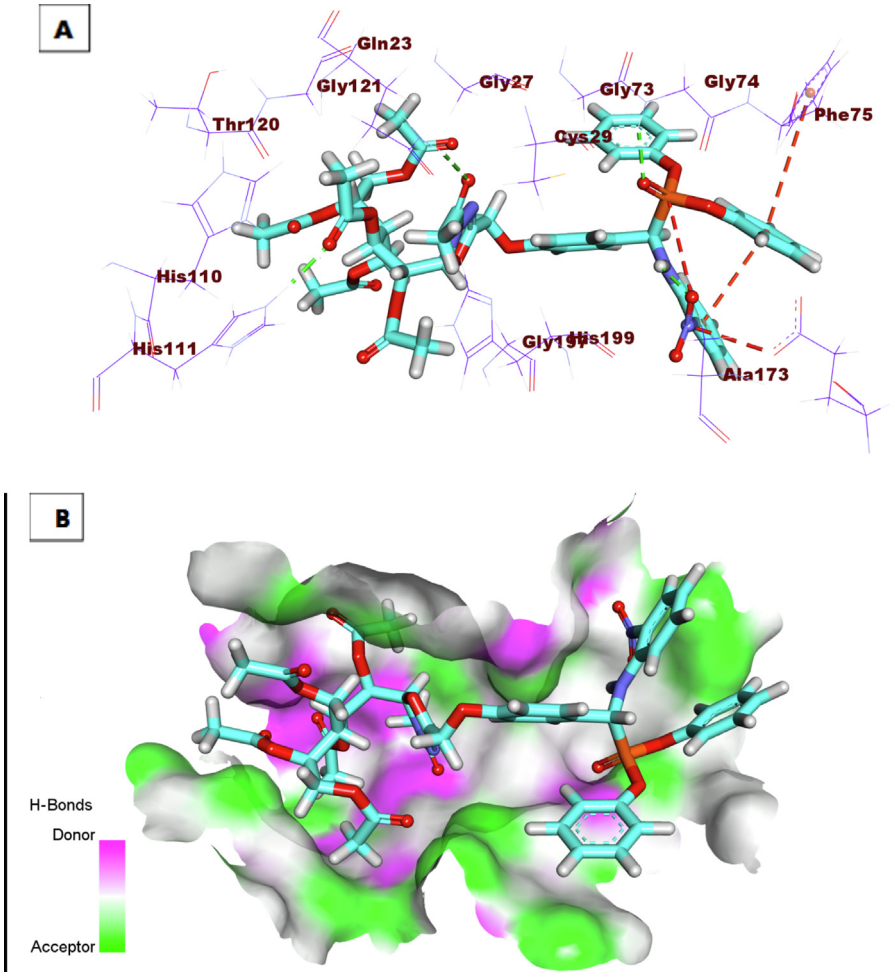


Fig. 7 A) Compound **9h** docked into the active site of Cathepsin B. B) Mapping surface showing compound **9h** occupying the active pocket of Cathepsin B.

pied the 2nd pocket of the receptor forming two hydrogen bonds with Ala312 and Arg50 (Fig. 3). Compound **9i** exhibited a binding mode like that of the co-crystallized ligands against TS with an affinity value of −39.47 Kcal/mole. It formed four hydrogen bonds and ten hydrophobic interactions. The diphenyl (((2-nitrophenyl) amino)(phenyl)methyl)phosphonate moiety occupied the first pocket of TS forming two hydrogen bonds with Lys309. In addition, it formed seven hydrophobic interactions with Lys308, Leu221, Lys77, and Lys107. The 3-acetyl-2, 3-dihydro-1, 3, 4-oxadiazole moiety occupied the spacer region of TS forming Three hydrophobic interactions with Leu221, Ile108, and Phe225. The 3-(acetoxymethyl) pentane-1, 2, 4, 5-tetrayl tetraacetate occupied the 2nd pocket of the receptor. It formed one hydrogen bond with Ser216 (Fig. 4).

3.3.1. Docking against Cathepsin-B (CB)

The proposed binding mode of the co-crystallized ligand (78A) showed an affinity value of −18.59 kcal/mole. The benzylcarbamoyl was involved in hydrogen bonding interaction forming three bonds with Gly74, Cys29, and Gly198. In addition, the L-isoleucyl moiety formed two hydrogen bonds with Trp221 and Gln23. In addition, it formed two hydrophobic interactions with Val176 and Trp221. Moreover, the L-proline moiety formed three hydrophobic interactions with Cys119, His110, and Trp221 (Fig. 5). Compound **7a** exhibited a binding mode like that of the co-crystallized ligands against CB with an affinity value of −19.39 Kcal/mole. The pentane-1,2,3,4,5-pentayl pentaacetate formed four hydrogen bonds with the key amino acid residues in the active site including Gln23, Trp221, His110, and His111(Fig. 6). Compound **9h** exhibited a binding mode like that of the co-crystallized ligands against CB with an affinity value of −20.02 Kcal/mole. In detail, the diphenyl (((2-nitrophenyl) amino)(phenyl) methyl)phosphonate moiety formed a hydrogen bond with Gly74. Additionally, it formed one hydrophobic interaction with Phe75, and one electrostatic interaction with Gln245. The 3-acetyl-2, 3-dihydro-1, 3, 4-oxadiazole moiety formed one hydrogen bond with Gln23. Finally, the 3-(acetoxymethyl) pentane-1, 2, 4, 5-tetrayl tetraacetate formed one hydrogen bond with His111 (Fig. 7).

3.4. In silico toxicity studies

The toxicity profile of the synthesized compounds was determined according to the validated and constructed models in Discovery studio software (Xia et al., 2004). These models include i) FDA rodent carcinogenicity, which computes the probability of a submitted chemical structure being a carcinogen. ii) Carcinogenic potency TD50 which predicts the tumorigenic dose rate 50 (TD50) of a chemical in a rodent chronic exposure toxicity test of carcinogenic potency (Venkatapathy et al., 2011). iii) Rat maximum tolerated dose, which predicts

Table 7 Toxicity properties of the synthesized compounds.

No.	FDA rodent carcinogenicity (Mouse)	Carcinogenic Potency TD50 (Rat)[a]	Rat Maximum Tolerated Dose (Feed)[b]	Developmental Toxicity Potential	Rat Oral LD50 [b]	Chronic LOAEL [b]	Ocular Irritancy	Skin Irritancy
5a	Non-Carcinogen	55.062	0.888	Toxic	0.323	0.893	Irritant	Non-Irritant
5b	Non-Carcinogen	55.062	0.888	Toxic	0.323	0.893	Irritant	Non-Irritant
5c	Non-Carcinogen	55.062	0.888	Toxic	0.323	0.893	Irritant	Non-Irritant
5d	Non-Carcinogen	53.248	0.620	Toxic	0.237	0.606	Irritant	Non-Irritant
6a	Non-Carcinogen	327.059	0.048	Non-Toxic	0.129	1.448	Irritant	Non-Irritant
6b	Non-Carcinogen	0.048	327.059	327.059	0.129	1.448	Irritant	Non-Irritant
6c	Non-Carcinogen	0.048	327.059	0.048	0.129	1.448	Irritant	Non-Irritant
6d	Non-Carcinogen	0.059	261.443	Non-Toxic	0.120	1.357	Irritant	Non-Irritant
7a	Non-Carcinogen	0.023	64.843	Toxic	0.027	6.335	Irritant	Irritant
7b	Non-Carcinogen	0.023	64.843	Toxic	0.027	6.335	Irritant	Irritant
7c	Non-Carcinogen	0.023	64.843	Toxic	0.027	6.335	Irritant	Irritant
7d	Non-Carcinogen	0.028	52.365	Toxic	0.025	5.996	Irritant	Irritant
9a	Non-Carcinogen	0.016	2.581	Non-Toxic	0.003	0.420	Non-Irritant	Non-Irritant
9b	Non-Carcinogen	0.013	4.359	Non-Toxic	0.027	6.335	Irritant	Non-Irritant
9c	Non-Carcinogen	0.016	2.581	Non-Toxic	0.027	6.335	Irritant	Non-Irritant
9d	Non-Carcinogen	0.013	4.359	Non-Toxic	0.027	6.335	Non-Irritant	Non-Irritant
9e	Non-Carcinogen	0.015	7.344	Non-Toxic	0.005	0.606	Irritant	Non-Irritant
9f	Non-Carcinogen	0.016	3.150	Non-Toxic	0.004	0.928	Irritant	Non-Irritant
9g	Non-Carcinogen	0.013	5.319	Non-Toxic	0.005	0.606	Non-Irritant	Non-Irritant
9h	Non-Carcinogen	0.013	5.319	Non-Toxic	0.005	0.606	Irritant	Non-Irritant
9i	Non-Carcinogen	0.021	2.189	Non-Toxic	0.005	0.606	Irritant	Non-Irritant
9j	Non-Carcinogen	0.017	3.694	Non-Toxic	0.003	0.417	Non-Irritant	Non-Irritant
9k	Non-Carcinogen	0.019	6.207	Non-Toxic	0.005	0.601	Irritant	Non-Irritant
9l	Non-Carcinogen	0.133	2.437	Non-Toxic	0.004	0.919	Irritant	Non-Irritant
Raltitrexed	Non-Carcinogen	0.021	2.189	Non-Toxic	0.175	8.753	Irritant	Non-Irritant

[a] Unit: mg/kg body weight/day.
[b] Unit: g/kg body weight.

the rat maximum tolerated dose (MTD) of a chemical (Shlyakhter et al., 1992). iv) Developmental toxicity potential which predicts whether a particular compound is likely to be toxic in a developmental toxicity potential assessment (Louisse et al., 2015). v) Rat oral LD50 which predicts the rat oral acute median lethal dose (LD50) in the toxicity test of a chemical (Diaza et al., 2015). vi) Rat chronic LOAEL which predicts the rat chronic lowest observed adverse effect level (LOAEL) value of a chemical (Pizzo and Benfenati, 2016; Venkatapathy et al., 2004). vii) Ocular irritancy predicts whether a particular compound is likely to be an ocular irritant and how severe the irritation is in the Draize test (Wilhelmus, 2001). viii) Skin irritancy predicts whether a particular compound is likely to be a skin irritant and how severe it is in a rabbit skin irritancy test [13]. As shown in Table 7, most compounds showed in silico low toxicity profile against the tested models. All compounds were predicted to be non-carcinogenic against the FDA rodent carcinogenicity model. Moreover, all compounds showed carcinogenic potency TD50 more than the reference compounds; raltitrexed (carcinogenic potency TD50 = 2.437 mg/kg body weight/day) except compound **7j**. The tested compound had carcinogenic potency TD50 values ranging from 2.581 to 327.59 mg/kg body weight/day). Besides, the rat maximum tolerated doses of compounds 4a-d were estimated to be between 0.620 and 0.888 g/kg body weight, which were higher than that of raltitrexed (rat maximum tolerated dose = 0.188 g/kg body weight). The other derivatives were predicted to have less rat maximum tolerated doses. Moreover, all compounds with an exception of 4a-d were predicted to be non-toxic against the developmental toxicity potential model. For the rat oral LD50 model, the tested compounds showed oral LD50 values ranging from 0.389 to 6.385 mg/kg body weight/day. Such values less than that of raltitrexed (oral LD50 = 8.753 mg/kg body weight/day). Moreover, all the tested compounds were predicted to be irritant against the ocular irritancy model except compounds **9a**, **9d**, **9g**, and **9j**. Finally, all compounds were predicted to be non-irritant against the skin irritancy model except compounds **7a-d**.

4. Structure-Activity relationships (SAR)

The activity of synthesized compound determined by the activity of functional groups in the composition. The theoretical study showed that compounds **9h** and **7a** have high activity of binding with TS and CB respectively, when we make comparison with biological study we found that compounds **9h** and **7a** have high activity against breast cancer. These high activities refers to the presence of phosphonate group and acetylated sugar in compound **9h** while refers to the presence of oxadiazoline ring and acetylated sugar in compound **7a**.

5. Conclusions

In our study we were designed a new compounds as nucleosides, acetylated nucleosides, oxadiadiazoline and α-aminophosphonate derivatives and then these compounds were purified and elucidated by different spectroscopic analysis. The synthesized compounds were tested against breast cancer cells (MCF-7). The binding potential of synthesized compounds against thymidylate synthase (TS) and Cathepsin B (CB) has been investigated and the compounds **7a** and **9h** exhibits highly binding with thymidylate synthase (TS) and Cathepsin B (CB).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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