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Original article

Synthesis, spectroscopic and molecular docking studies on new schiff bases, nucleosides and α-aminophosphonate derivatives as antibacterial agents

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ABSTRACT

New Nucleosides, analogues derived from 1, 3, 4-oxadiazole, arylidene analogues and α-aminophosphonate were prepared. Infrared (IR), elemental analysis and ^{1}HNMR elucidated nucleosides; arylidines and phosphonate derivatives. The prepared derivatives were purified and allowed to test against bacteria strains. Phosphonate derivative **12a** showed the higher antibacterial against *E. coli* with inhibition zone 35 mm, P. aeruginosa with inhibition zone 30 and S. aureus with inhibition zone 22 while compounds **4**, **6d**, **9a**, **9c** and **12c** showed moderate to weak activity against these bacteria species with inhibition zones ranged from 12 mm to 24 mm. The molecular docking studies was applied on compound **12a**, which showed the binding at the active DNA Gyrase.

1. Introduction

Analogues of nucleoside are used as antibiotics as antiviral strains to treat cancer have been source of investigation for therapies and drug development to treat cancer and other pathogens (Jordheim et al., 2013). They possess properties of anticancer, antiviral and antibacterial (Ding et al., 2010). The main components of deoxyribonucleic acid and ribonucleic acid are nucleotides, It consists of nucleosides and phosphate group, also responsible for the production of proteins, also responsible for the production of proteins, acts as cofactor in many metabolic pathways; lipid and polyamine biosynthesis (Yssel et al., 2017). Nucleosides are metabolites excreted from RNA, composed of nucleobases which are covalently attached to ribose or deoxyribose. The aromatic heterocyclic nucleobases contain nitrogen in the ring with purine or pyrimidine. (Dudley, 2009). All pro-drugs derived from nucleoside are undergo chemical modification, to add phosphorous group to activate sites in many cellular processes during nucleotide and nucleic acid metabolism (Tsesmetzis et al., 2018). Development of nucleoside-analogue; Acyclic nucleosides such as zidovudine (AZT), Didanosine (DDL) and Zalcitabine (ddC) were effective antiviral analogues used for treating human immunodeficiency virus (HIV) infection but due to its toxicity has led to desertion (Seley-Radtke & Yates, 2018). Phthalazines heterocycle containing nitrogen is a novel drug possess antitumor, antihypertensive and antidiabetic activities (Sangshetti et al., 2019; EL-Hashasha et al., 2017; Haikal et al., 2003; Demirayak et al., 2004; Lenz et al., 2002; Dogruer et al., 2004; Watanabe et al., 1998). Purine and Pyrimidines as nucleotides and nucleosides are energy carriers show various bioactivity such as bactericidal, fungicidal, insecticidal properties (Thomson & Lamont, 2019).

2. Material and methods

2.1. Chemistry

Melting points were analyzed on kofler block apparatus and were uncorrected ^{1}HNMR was recorded on spectrometer (400 MHz), chemical shifts are referenced from tetramethylsilane

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(TMS) δ 0.00, as internal standard, using $CDCl_3$ as a solvent for ^1H. Fourier transform infrared (FT- IR) Spectroscopy were performed by means of ic50 model FTIR (Thermo) using KBr disks. Mass spectra were obtained using GC–MS, TLC using aluminum silica gel plates 60 F245 monitored the progress of the reactions. The antibacterial activity of the synthesized compounds was carried out at well- maintained Biology Lab.

*2.1.1. Ethyl 2-(4-nitrophenoxy) acetate (**2**) (Amer et al., 2018)*

Equivalent amounts of 4-nitrophenol (**1**) (1.39 gm, 0.01 mol), ethylchloroacetate (1.225 gm, 0.01 mol) and K_2CO_3 (1.38 gm, 0.01 mol) were dissolved in 50 ml of acetone and then, refluxed for 6 h. The solvent evaporation took place at low pressure. The final product was collected, dried and cleaned to yield 92% of a yellow powder. m.p. 221–222 °C. Rf = 0.45 (3% CH_3OH: $CHCl_3$).^1H NMR ($CDCl_3$): d = 1.18 (3H, t, *J* = 7.2 Hz, $\underline{CH_3}CH_2$), 4.33 (2H, q, *J* = 7.2 Hz, CH_3CH_2), 4.75 (2H, s, CH_2), 7.55 (2H, d, *J* = 5.5 Hz, H-2), 8.30 (2H, d, *J* = 4.9 Hz, H-3); MS: *m/z*(%) 226 (M^++ H).

*2.1.2. 2-(4-Nitrophenoxy) acetohydrazide (**3**) (Amer et al., 2018)*

Equivalent amounts of **2** (2.25 gm, 0.01 mol) and hydrazine hydrate (1.5 gm, 0.03 mol) were dissolved in 40 ml ethanol and then, refluxed for 10 h. Solvent has been evaporated by halving it and put in the fridge one day; A white powder was collected in order to yield 95% of the resulting product, m.p. 186–188 °C. Rf = 0.45 (5% CH_3OH in $CHCl_3$). IR spectra (KBr) (v,cm^{-1}): 3460, 3505 (NH_2), 3345 (NH), 1645 (CO); ^1HNMR ($CDCl_3$): d = 2.65 (2H, brs, NH_2), 4.63 (2H, s, CH_2),7.35 (2H, d, *J* = 5.5 Hz, Ar-AH), 8.22 (2H, d, *J* = 4.9 Hz, Ar-H), 8.53 (1H, brs, NH); MS: *m/z* (%) 211 (M^+).

*2.1.3. 4-(5-((4-nitrophenoxy) methyl)-1, 3, 4-oxadiazol-2-yl) aniline (**4**)*

Equivalent amounts of hydrazide **3** (2.11 gm, 0.01 mol) and p-aminobenzoic acid (1.37 gm, 0.01 mol) were dissolved in 20 ml phosphorousoxychloride and then heated in the presence of condenser for 20 h. The final blend has been poured on 100 g ice; the sample was collected and dried for 70% yield of brown crystal, m.p. 245–247 °C. Rf = 0.75 (10% CH_3OH in $CHCl_3$). IR spectra (KBr) (v,cm^{-1}): 3460, 3505 (NH_2), 1645 (CO); ^1HNMR ($CDCl_3$): d = 4.60 (2H, s, CH_2), 6.05 (2H, brs, NH_2), 6.56 (2H, d, *J* = 5.5 Hz, Ar-H), 7.20 (2H, d, *J* = 4.9 Hz, Ar-H), 7.50 (2H, d, *J* = 5.5 Hz, Ar-H), 8.10 (2H, d, *J* = 5.5 Hz, Ar-H), 8.53 (1H, brs, NH); MS: *m/z* (%) 312 (M^+).

*2.1.4. Synthesis of arylidene derivatives **6a-d***

Equivalent amounts of **4** (3.12 gm, 0.01 mol) and aromatic aldehydes (1.56 gm, 1.22 gm, 1.40 gm and 0.96 gm, 0.01 mol respectively) were dissolved in 40 ml ethanol; the addition of few drops of AcOH catalyst was occurred directly and then the blend was refluxed for 10 h. The resulting product has been purified to produce a yield of 6a-d (90–93%).

*2.1.4.1. N-(naphthalen-1-ylmethylene)-4-(5-((4-nitrophenoxy) methyl)-1, 3, 4-oxadiazol-2-yl) aniline (**6a**).* Brown powder, 90% yield, m.p. 175–177 °C Rf = 0.55 (5% CH_3OH: $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3070 (aromatic), 2995 (aliphatic), 1572, 1380 (NO_2), 1465 (CH_2); ^1HNMR (400 MHz, $CDCl_3$): d = 4.82 (2H, s, CH_2), 7.22–8.14 (15H, m, CH aromatic), 8.50 (1H, s, CH); MS: *m/z* (%) 450 (M^+).

*2.1.4.2. N-(2-hydroxybenzylidene)-4-(5-((4-nitrophenoxy)methyl)-1,3,4-oxadiazol-2-yl)aniline (**6b**).* White powder, 92% yield, m.p. 205–207 °C Rf = 0.45 (5% CH_3OH : $CHCl_3$). IR spectra (KBr) (v, cm^{-1}): 3250 (OH), 3050 (Ar-H), 2990 (CH aliphatic),1575, 1385 (NO_2), 1650 (CH_2); ^1HNMR (400 MHz, $CDCl_3$): d = 4.82 (2H, s, CH_2), 5.40 (1H, brs, OH), 7.00–8.12 (12H, m, CH aromatic), 8.65 (1H, s, CH); MS: *m/z* (%) 416 (M+).

*2.1.4.3. N-(4-chlorobenzylidene)-4-(5-((4-nitrophenoxy) methyl)-1, 3, 4-oxadiazol-2-yl) aniline (**6c**).* Yellow powder, 93% yield, m.p. 190–192 °C Rf = 0.72 (5% CH_3OH: $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3060 (Ar-H), 2985 (CH aliphatic), 1570, 1382 (NO_2), 1465 (CH_2); ^1HNMR (400 MHz, $CDCl_3$): d = 5.00 (2H, s, CH_2), 7.20–8.10 (12H, m, CH aromatic), 8.62 (1H, s, CH); MS: *m/z* (%) 436 ($M+2H)^+$. Anal. Calcd for $C_{22}H_{15}ClN_4O_4$: C, 60.77; H, 3.48; N, 12.88. Found: C, 60.34; H, 4.15; N, 13.14.

*2.1.4.4. N-(furan-2-ylmethylene)-4-(5-((4-nitrophenoxy) methyl)-1, 3, 4-oxadiazol-2-yl) aniline (**6d**).* Orange powder, 90% yield, m.p. 220–222 °C Rf = 0.55 (5% CH_3OH in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}), 3062 (Ar-H), 2990 (CH aliphatic), 1572, 1385 (NO_2), 1460 (CH_2); ^1HNMR (400 MHz, $CDCl_3$): d = 4.92 (2H, s, CH_2), 6.60–8.15 (11*H*, m, CH aromatic), 7.65 (1H, s, CH); MS: *m/z* (%) 401 (M +Na)$^+$. Anal. Calcd for $C_{20}H_{14}N_4O_5$: C, 61.54; H, 3.62; N, 14.35. Found C, 60.85; H, 4.07; N, 14.92.

*2.1.5. General procedure for the preparation of 4 – ((2-(4-nitrophenoxy) methyl) – 1, 3, 4-oxadiazole nucleosides (**8a-d**)*

Appropriate moieties of different monosaccharaides (1.5 gm, 1,8 gm, 1.8 gm and 1.8 gm, 0.01 mol respectively) and glacial acetic acid (1 ml) have been applied to the derivative of oxadiazole **4** (3.12 gm, 0.01 mol) in 35 ml EtOH. The resultant solution was heated in the presence of condenser for 5–8 h. The resultant product was purified by recrystallized by absolute ethyl acetate: methanol (1:1) in order to yield respectively 80–90% of the respective nucleosides.

*2.1.5.1. 4-(4-N-Arabinfuranosylamino-phenyl) – 4 – ((2-(4-nitrophenoxy) methyl) – 1, 3, 4-oxadiazole (**8a**).* Pale orange crystal, 90% yield, m.p. = 265–267 °C, Rf = 0.55 (7% CH_3OH: $CHCl_3$). ^1H NMR (400 MHz, DMSO d_6) δ: 2.85 (1H, d, *J* = 2.5 Hz, H-1), 3.49 (2H, d, 2xOH,), 3.70 (1H, d, OH), 4.10 (1H, brs, NH), 4.63 (2H, s, 2H), 3.65–5.40 (4H, m, H-2, H-3, H-4, H-5), 6.45 – 7.60 (4H, m, Ar-H), 7.30 (2H, dd, *J* = 7.2 Hz, CH), 8.18 (2H, dd, *J* = 5.00 Hz, CH); MS: *m/z* (%) 444 ($M)^+$.

*2.1.5.2. 4-(4-N-Glactopyranosylamino-phenyl) – 4 – ((2-(4-nitrophenoxy) methyl) – 1, 3, 4-oxadiazole (**8b**).* Pale orange crystal, 88% yield, m.p. = 227–229 °C, Rf = 0.48 (7% CH_3OH in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3343 (OH), 3060 (Ar-H), 1630 (C=N), 1570, 1382 (NO_2), 1450 (CH_2); ^1H NMR (400 MHz, DMSO d_6) δ: 3.65 (2H, d, 2xOH,), 3.59 (2H, d, 2xOH), 4.00 (1H, d, *J* = 2.5 Hz, H-1), 4.10 (1H, brs, NH), 4.60 (2H, s, 2H), 3.50–3.95(6H, m, H-2, H-3, H-4, H-5, H-6), 6.50 – 7.72 (4H, m, Ar-H), 7.22 (2H, dd, *J* = 7.2 Hz, CH), 8.10 (2H, dd, *J* = 4.9 Hz, CH); MS: *m/z* (%) 475 $(M+H)^+$. Anal. Calcd for $C_{21}H_{22}N_4O_9$: C, 53.16; H, 4.67; N, 11.81. Found C, 53.34; H, 4.85; N, 11.63.

*2.1.5.3. 4-(4-N-Glucopyranosylamino-phenyl) – 4 – ((2-(4-nitrophenoxy) methyl) – 1, 3, 4-oxadiazole (**8c**).* Pale brown crystal, 80% yield, m.p. = 294–296 °C, Rf = 0.75 (7% CH_3OH in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3347 (OH), 3045 (Ar-H), 1625 (C=N), 1572, 1384 (NO_2), 1470 (CH_2); ^1H NMR (400 MHz, DMSO d_6) δ: 3.55 (2H, d, 2xOH,), 3.58 (2H, d, 2xOH), 3.92 (1H, d, *J* = 2.5 Hz, H-1), 4.05 (1H, brs, NH), 4.65 (2H, s, 2H), 3.53–3.91(6H, m, H-2, H-3, H-4, H-5, H-6), 6.52 – 7.75 (4H, m, Ar-H), 7.20 (2H, dd, *J* = 7.2 Hz, CH), 8.00 (2H, dd, *J* = 4.9 Hz, CH); MS: *m/z* (%) 476 $(M+2H)^+$. Anal. Calcd for $C_{21}H_{22}N_4O_9$: C, 53.16; H, 4.67; N, 11.81. Found C, 53.25; H, 4.90; N, 11.93.

*2.1.5.4. 4-(4-N-Mannopyranosylamino-phenyl) – 4 – ((2-(4-nitrophenoxy) methyl) – 1, 3, 4-oxadiazole (**8d**).* Pale orange powder, 85%

yield, m.p. = 227–229 °C, Rf = 0.75 (7% CH_3OH in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3345 (OH), 3052 (Ar-H), 1632 (C═N), 1570, 1380 (NO_2), 1445 (CH_2); ^1H NMR (400 MHz, DMSO d_6) δ: 3.52 (2H, d, 2xOH,), 3.60 (2H, d, 2xOH), 3.97 (1H, d, *J* = 2.5 Hz, H-1), 4.13 (1H, brs, NH), 4.62 (2H, s, 2H), 3.55–3.90 (6H, m, H-2, H-3, H-4, H-5, H-6), 6.49 – 7.62 (4H, m, Ar-H), 7.23 (2H, dd, *J* = 7.2 Hz, CH), 8.10 (2H, dd, *J* = 4.9 Hz, CH); MS: *m*/*z* (%) 476 $(M+2H)^+$. Anal. Calcd for $C_{21}H_{22}N_4O_9$: C, 53.16; H, 4.67; N, 11.81. Found C, 53.45; H, 4.17; N, 12.02.

2.1.6. General procedure for the synthesis of acetylated nucleosides **9a-d**

Equivalent amounts of nucleoside derivatives **8a-d** (4.44 gm, 0.01 mol, 4.44 gm, 0.01 mol, 4.44 gm, 0.01 mol and 4.44 gm, 0.01 mol respectively) and acetic anhydride (6.02 gm, 0.01 mol) were dissolved in appropriate amount of anhydrous pyridine and the stirring of produced solution was occurred at 25 °C for 24 h. The blend is poured onto ice to create a light brown precipitates. The resulting products were purified to produce 80–85% of acetylated nucleosides of **9a-d**.

*2.1.6.1. 4-(2, 3, 5-Tri-O-acetyl)-N-arabinfuranosylamino-phenyl)- 4 – ((2-(4-nitrophenoxy) methyl) – 1, 3, 4-oxadiazole (**9a**).* Pale brown powder, 82% yield, m.p. = 194–196 °C, IR spectra (KBr) (v, cm^{-1}):, 3400 (NH), 3050 (Ar-H), 1735 ($COCH_3$), 1625 (C═N), 1575, 1383 (NO_2), 1445 (CH_2); ^1H NMR (400 MHz, DMSO d_6) δ: 2.02–2.19 (9H, m, 3x $COCH_3$), 4.05 (1H, brs, NH), 2.95–4.75 (5H, m, H-2, H-3, H-4, H-5), 4.76 (2H, s, CH_2), 4.94 (1H, d, *J* = 5.5 Hz, H-1), 6.90–7.45 (8H, m, Ar-H); Anal. Calcd for $C_{26}H_{26}N_4O_{11}$: C, 54.74; H, 4.59; N, 9.82. Found C, 54.95; H, 4.83; N, 9.65.

*2.1.6.2. 4-(2, 3, 5, 6-Tetra-O-acetyl)-N-galactopyranosylamino-phenyl)- 4 – (2-(4-nitrophenoxy) methyl) – 1, 3, 4-oxadiazole (**9b**).* Pale brown powder, 85% yield, m.p. = 201–203 °C, IR spectra (KBr) (v, cm^{-1}):, 3400 (NH), 3050 (Ar-H), 1735 ($COCH_3$), 1625 (C═N), 1575, 1383 (NO_2), 1445 (CH_2); ^1H NMR (400 MHz, DMSO d_6) δ: 2.10–2.27 (12H, m, 4x$COCH_3$), 4.10 (1H, brs, NH), 2.92–4.78 (6H, m, H-2, H-3, H-4, H-5, H-6), 4.63 (2H, s, CH_2), 5.10 (1H, d, *J* = 5.5 Hz, H-1), 6.96–7.41 (8H, m, Ar-H); MS: *m*/*z* (%) 642 $(M)^+$. Anal. Calcd for $C_{29}H_{30}N_4O_{13}$: C, 54.21; H, 4.71; N, 8.72. Found C, 54.41; H, 4.87; N, 8.45.

*2.1.6.3. 4-(2, 3, 5, 6-Tetra-O-acetyl)-N-glucoopyranosylamino-phenyl)- 4 – (2-(4-nitrophenoxy) methyl) – 1, 3, 4-oxadiazole (**9c**).* Pale brown powder, 80% yield, m.p. = 230–232 °C, IR spectra (KBr) (v, cm^{-1}):, 3400 (NH), 3050 (Ar-H), 1735 ($COCH_3$), 1625 (C═N), 1575, 1383 (NO_2), 1445 (CH_2); ^1H NMR (400 MHz, DMSO d_6) δ: 2.12–2.30 (12H, m, 4x$COCH_3$), 4.05 (1H, brs, NH), 2.85–4.68 (6H, m, H-2, H-3, H-4, H-5, H-6), 4.63 (2H, s, CH_2), 5.00 (1H, d, *J* = 5.5 Hz, H-1), 6.48–7.50 (8H, m, Ar-H); MS: *m*/*z* (%) 642 $(M)^+$. Anal. Calcd for $C_{29}H_{30}N_4O_{13}$: C, 54.21; H, 4.71; N, 8.72. Found C, 54.65; H, 4.92; N, 8.85.

*2.1.6.4. 4-(2, 3, 5, 6-Tetra-O-acetyl)-N-mannopyranosylamino-phenyl)- 4 - (2-(4-nitrophenoxy) methyl) – 1, 3, 4-oxadiazole (**9d**).* Pale brown powder, 85% yield, m.p. = 180–182 °C, IR spectra (KBr) (v, cm^{-1}):, 3400 (NH), 3055 (Ar-H), 1735 ($COCH_3$), 1630 (C═N), 1575, 1383 (NO_2), 1445 (CH_2); ^1H NMR (400 MHz, DMSO d_6) δ: 2.07–2.24 (12H, m, 4x$COCH_3$), 4.00 (1H, brs, NH), 2.90–4.72 (6H, m, H-2, H-3, H-4, H-5, H-6), 4.60 (2H, s, CH_2), 5.12 (1H, d, *J* = 5.5 Hz, H-1), 6.50–7.62 (8H, m, Ar-H); MS: *m*/*z* (%) 642 $(M)^+$.

2.1.7. Preparation of α-Aminophosphonates **12 (a-d)**

Equivalent amounts of hydrazide **3** (2.11 gm, 0.01 mol) triphenylphosphite (3.10 gm, 0.01 mol) and various aromatic aldehydes (pyridine-3-carboxaldehyde (1.07 gm, 0.01 mol), salisaldehyde (1.22 gm, 0.01 mol), 4-chlorobenzaldehyde (1.40 gm, 0.01 mol) and 5-methylfurfuraldehyde (1.10 gm, 0.01 mol) respectively were dissolved in CH_3CN, the addition of few drops of perchloric acid occurred, later the stirring was occurred at 25 °C for 12 h. The solvent evaporated and then the residues of α-aminophosphonate derivatives were treated with diethyl ether and dried up at yields of 70–82%,**12 (a-d)**.

*2.1.7.1. Diphenyl ((2-(2-(4-nitrophenoxy) acetyl) hydrazinyl) (pyridin-3-yl) methyl) phosphonate (**12a**).* Dark brown gum, 80% yield, Rf = 0.45 (5% CH_3OH: $CHCl_3$). ^1H NMR (400 MHz, DMSO d_6) δ: = 3.85 (1H, brs, NH), 4.65 (2H, s, CH_2), 5.88 (1H, s, CH), 7.21–8.22 (18H, m, Ar-H), 9.05 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): d = 66.54 (CH_2), 67.20 (CH—P—), 115.23, 120.35, 121.40, 123.67, 125.85, 130.15, 131.55, 134.33, 140.45, 148.22, 149.47, 150.37, 164.67 (Ar-CH), 167.55 (CONH); ^{31}P NMR (162 MHz, $CDCl_3$): δ: 19.73 (O═P—CH—); EI-MS: *m*/*z* = 534 $[M^+]$

*2.1.7.2. Diphenyl ((2-hydroxyphenyl) (2-(2-(4-nitrophenoxy) acetyl) hydrazinyl) methyl) phosphonate (**12b**).* Brown gum, 82% yield, Rf = 0.45 (5% CH_3OH in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3290 (NH), 3420 (OH), 3050 (Ar-H), 2930 (CH aliphatic),1705 (C═O), 1572, 1383 (NO_2), 1445 (CH_2); ^1H NMR (400 MHz, DMSO d_6) : δ = 3.78 (1H, brs, NH), 4.60 (2H, s, CH_2), 5.10 (1H, s, OH), 5.85 (1H, s, CH), 7.22–8.18 (18H, m, Ar-H), 9.10 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 61.43 (CH—P—), 66.63 (CH_2), 115.27, 115.63, 120.36, 121.11, 121.32, 121.45, 125.87, 128.15, 128.29, 130.13, 134.33, 140.48, 150.24, 155.73, 164.26 (Ar-CH), 166.48 (CONH); ^{31}P NMR (162 MHz, $CDCl_3$): δ = 18.86 (O═P—CH—); EI-MS: *m*/*z* = 550 $[M+H]^+$.

*2.1.7.3. Diphenyl ((4-chlorophenyl) (2-(2-(4-nitrophenoxy) acetyl) hydrazinyl) methyl) phosphonate (**12c**).* Brown gum, 70% yield, Rf = 0.45 (5% methanol in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3295 (NH), 3050 (Ar-H), 2905 (CH aliphatic)1700 (C═O), 1572, 1383 (NO_2), 1445 (CH_2); ^1H NMR (400 MHz, DMSO d_6) δ = 3.80 (1H, brs, NH), 4.60 (2H, s, CH_2), 5.70 (1H, s, CH), 7.25–8.30 (18H, m, Ar-H), 8.97 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ: = 66.63 (CH_2), 68.02 (CH—P—), 115.27, 115.33, 120.45, 121.21, 121.25, 121.49, 125.98, 128.17, 128.40, 130.15, 134.41, 140.53, 150.26, 154.63, 164.25 (Ar-CH), 166.88(CONH); ^{31}P NMR (162 MHz, $CDCl_3$): δ = 19.22 (O═P—CH—); EI-MS: *m*/*z* = 567 $[M]^+$ Anal. Calc. for $C_{27}H_{23}ClN_3O_7P$: C, 57.10; H, 4.08; N, 7.40. Found C, 57.33; H, 4.59; N, 7.56.

*2.1.7.4. Diphenyl ((5-methylfuran-2-yl) (2-(2-(4-nitrophenoxy) acetyl) hydrazinyl) methyl) phosphonate (**12d**).* Yellow gum, 75% yield, Rf = 0.45 (5% methanol in $CHCl_3$). IR spectra (KBr) (v, cm^{-1}):, 3295 (NH), 3050 (Ar-H), 2950 (CH aliphatic)1700 (C═O), 1572, 1383 (NO_2), 1445 (CH_2), 1375 (CH_3); ^1H NMR (400 MHz, DMSO d_6) δ = 2.2 (3H, s, CH_3), 3.80 (1H, brs, NH), 4.65 (2H, s, CH_2), 5.80 (1H, s, CH), 6.30–8.15 (16H, m, Ar-H), 8.85 (1H, brs, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.96 (CH_3), 66.55 (CH_2), 69.12 (CH—P—), 106.34, 108.14, 115.18, 115.22, 120.52, 121.32, 125.59, 130.23, 140.66, 150.28, 150.58, 150.75, 164.19 (Ar-CH), 166.38(CONH); ^{31}P NMR (162 MHz, $CDCl_3$): δ = 18.53 (O═P—CH—); EI-MS: *m*/*z* = 539 $[M]^+$ Anal. Calc. for $C_{26}H_{24}N_3O_8P$: C, 58.10; H, 4.50; N, 7.82. Found C, 58.41; H, 4.73; N, 7.63.

2.2. Microbiology

2.2.1. Bacterial isolates

Ethically approved microbes were used in this research, ethical committee of Taif Directorate of Health Affairs by the members in King Faisal Hospital in Taif. Taif, Saudi Arabia. Three multi-drug

resistant bacterial strains. All strains were freshly subcultured on suitable media before beginning of the experiment.

2.2.2. Antimicrobial assay

The antimicrobial assay was conducted on synthesized compounds **4, 6b, 6d, 8a, 8c, 9b, 9d, 12a, 12b, 12c** and **12d** by using minor modifications on agar well diffusion method (Valgas et al., 2007). The inoculations in the Mueller agar plate of the measured bacteria (1 ml), which are inoculated at a standard 0.5 McFarland. The bottom of the wells was sealed with Muller Eight millimeters diameter sterile cork borer. A 20 μl of the synthesized compounds (50 mg/ml dimethylsulphoxide) were cultivated and put in incubator for 24 h at 37 °C. Amoxicillin (10 μg/ml) and Ciprofloxacin (5 μg/ml) for Pseudomonas auroginosa and *E. coli*. Vancomycin (10 μg/ml) and Amoxicillin (10 μg/ml) for S. aureus were included in the test. Finally, the experiment was repeated three times with duplicates and the inhibition zone was measured.

2.3. Computational study

2.3.1. Preparation of small molecule

Synthesized compounds to reduce their energy PM3 was used via MOPAC then DFT through B3LYP/6-311 G. The semi-empirical Hamiltonian molecular orbital calculation MOPAC16 kit was used for all Quantum chemical calculations were done. (Stewart, 2013), basis on implementation of MOE 2015 package (Molecular Operating Environment (MOE), density function theory in Gaussian 09 W program with the Becke3-Lee-Yang-parr (B3LYP) level using 6-311G* was employed. (Frisch et al., 2013). Geometry for molecular structures has been optimized to understand of the chemical structures, geometry for molecular structures has been optimized.

2.3.1.1. Selection of proteins structures. Docking tests for the active target site in DNA Gyrase B (ID: 4uro) and and Cathepsin B (ID: 1gmy) were evaluated using MOE 2015 (Version 2009.10). (Dale et al., 1999, Greenspan et al., 2003). Later, MOE was corrected for the structures with the errors of active sites. Subsequently hydrogens were added and partial charges (Amber12: EHT) were calculated. MOE Site Finder program was implied to identify the binding site of each receptors in protein; method based on alpha spheres (convex hulls) (Soga et al., 2007).

2.3.2. MOE stepwise docking methods

Crystal structures of enzymes were obtained by removal of Water and inhibitors molecule, addition of hydrogen atoms. The site finder module of MOE was used to generate alpha-site spheres. Triangular matcher placement method was used to generate optimized 3D structures of ligands. A random triplet of alpha sphere center was used to decide the pose in each iteration to generate by aligning ligand triplets of atoms in three alpha spheres defined at the receptor level. The poses developed by MMFF94x forcefield with treating solvation effects.

3. Results and discussions

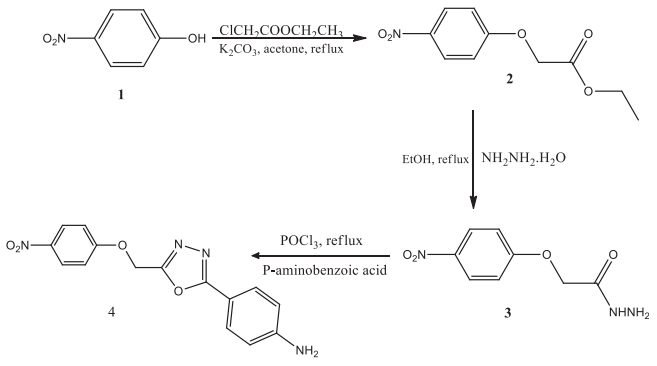
3.1. Chemistry

The ester derivative **2** in 92% yield was obtained when P-nitrophenol was reacted with ethylchloroacetate and KOH in dry acetone under reflux. 95% acid hydrazide 3 yield was obtained, when synthesized ester 2 was reacted in ethanol reflux with hydrazine hydrate. The derivative of 1, 3, 4-oxadiazole **4** in 70% yield was prepared with reaction of hydrazide **3,** p-aminobenzoic acid and phosphorous oxychloride under reflux. Compound **4** IR spectra showed the appearance of (NH_2) group at 3460, 3505 cm^{-1} and (CO) group at 1645 cm^{-1} and disappearance of (NH) group. ^{1}HNMR spectra peaks at 4.60, 6.05, 6.56–8.50 and 8.53 for CH_2, NH_2, eight protons of aromatic system and NH respectively; mass spectra with the molecular ion peak at 312 [M^+] (Scheme 1).

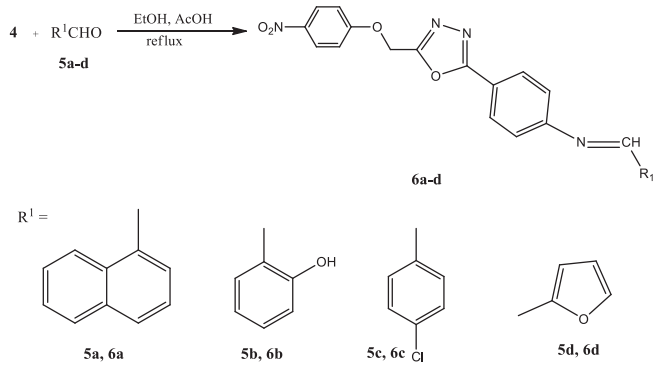
Oxadiazole derivative with amino group **4** was reacted under reflux in ethanol with different aldehyde derivatives **5a-d** and acetic acid under heat in the presence of condenser to give schiff bases **6a-d** in 90–93% yields, the IR spectra of compounds **6a-d** showed NO_2 (1572, 1380 cm^{-1}), CH_2 (1465 cm^{-1}), CH (aromatic) (3050–3070 cm^{-1}) and OH group (3250 cm^{-1}), ^{1}HNMR spectra peaks around 4.60 for CH_2 group, broad peak at 5.40. OH group had (CH) group of shiff bases around 8; the molecular ion peaks showed by mass spectra at 450 and 416 [M^+] for **6a** and **6b**, 615 [M+2H]$^+$ for **6c** and 401 [M+Na]$^+$ for **6d** (Scheme 2) with no NH_2 group.

Oxadiazole with amino group **4** reacted with different sugar (aldohexoses) derivatives **7a-d** and acetic acid in absolute ethanol under reflux to give nucleosides **8a-d** in 80–90% yields, the IR spectra of compounds **8a-d;** (NO_2) at 1570, 1380 cm^{-1}, (CH_2) at 1450 cm^{-1}, (CH aromatic) at the range of 3050 to 3060 cm^{-1} and (OH) group at 3330 cm^{-1}; ^{1}HNMR spectra peaks for (NH) at 4.05–4.13, (CH_2) at 4.60, the disappearance of (NH_2), peaks for (OH) at 3.52 to 3.60 peaks (CH-Sugars) at 3.55–3.92, (CH-aromatics) at 6.52 to 7.75; the molecular ion peaks for **8a** showed by mass spectra at 444 [M^+], for **8b** at 475 [M+H]$^+$ and for **8c and 8d** at 476 [M+2H]$^+$. The nucleosides **8a-d** were acetylated to give acetylated nucleosides **9a-d** in 80–85% yields, the disappearance of peak for (OH) around 330 cm^{-1} and the appearance of peak for ($COCH_3$) at 1735 cm^{-1} showed by IR spectra of compounds **9a-d**, this change is due to that acetylation of the nucleosides **8a-d** by acetic anhydride to the corresponding acetylated nucleosides **9a-d**; the ^{1}HNMR spectra showed that the disappearance of peaks for (OH) groups of nucleosides around 3.52 to 3.60 and appearance of peaks for ($COCH_3$) groups at the range from 2.02 to 2.30 which were acetylated nucleosides **9a-d**; mass spectra molecular ion peaks at 581 [M+Na]$^+$ for **9a** and 642 [M]$^+$ for **9b, 9c and 9d** (Scheme 3).

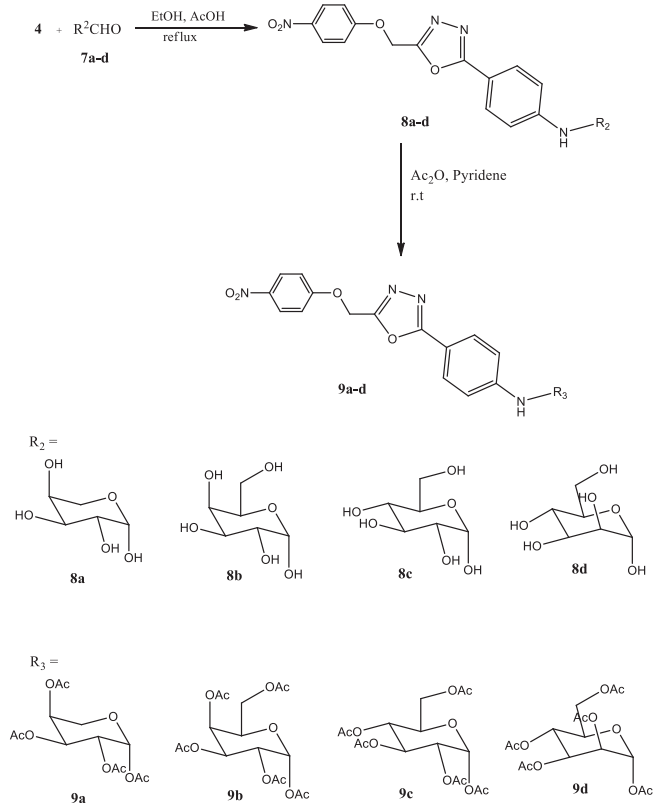
2-(4-Nitrophenoxy) acetohydrazide (**3**) reacted with triphenylphosphite (**10**), aldehyde derivatives **11a-d** (pyridine-3-carboxaldehyde, salisaldehyde, p-chlorobenzaldehyde and 5-methylfurfuraldehyde respectively) and perchloric acid in acetonitrile gives α-aminophosphonate derivatives **12a-d** in 70–82% yields. the IR spectra of **12a-d** showed (CO) at 1705 cm^{-1}, (NO_2) at 1572, 1380 cm^{-1}, (CH_2) at1460 cm^{-1}, and (OH) at 3420 cm^{-1}; ^{1}HNMR spectra peaks at 4.60 for CH_2 group, (OH) at 5.10 group, (CH) group of shiff bases around 5.88, the appearance of aromatic protons around 6.30 to 8.22 and (NH) group around 9.05 to 9.10; ^{13}C disappearance of (NH_2) group. NMR spectra of the synthesized phosphonates **12a-d;** (CH—P—) at 61.43 to 68.02; ^{31}P NMR spectra



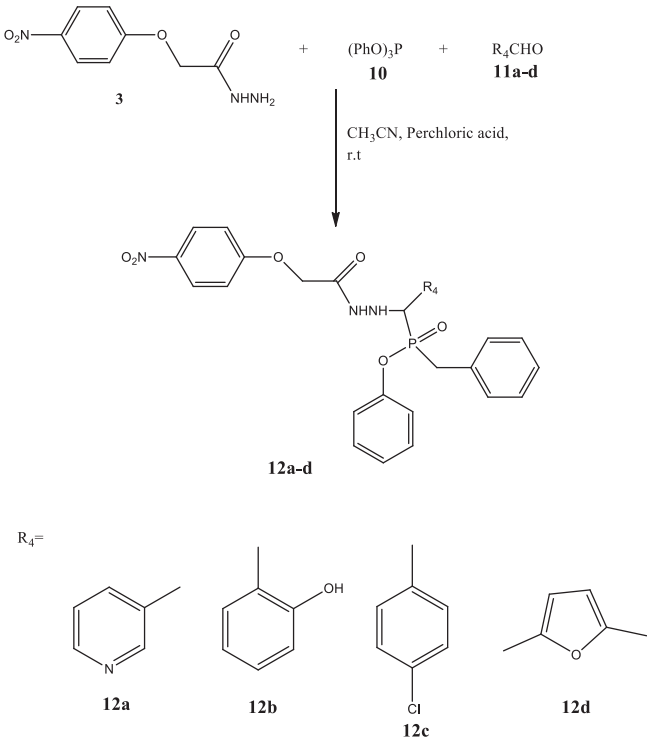
Scheme 1.



Scheme 2.



Scheme 3.



Scheme 4.

of the synthesized derivatives **12a-d** showed that the appearance of peaks for phosphorous of phosphonates (O═P-CH–) at 19.73, 18.86, 19.22 and 18.53 for **12a**, **12b**, **12c** and **12d** respectively; mass spectra; molecular ion peaks at 534 $[M^+]$ for **12a,** 550 $[M+H]^+$ for **12b,** 567 $[M]^+$ for **12c** and 539 $[M]^+$ for **12d** (scheme 4).

3.2. Microbiology

There is increasing high mortality rate related to increasing infections due to multidrug-resistant bacteria. When treating such infections, the therapeutical impact of widely used antibiotics frequently fails because of their transition to the antibiotics used by mechanisms of inductible resistance to antibiotics and regulatory bacterial gene mutations (Fowler et al., 2004). Twelve synthesized compounds (**4, 6a, 6b, 8c, 8d, 9a, 9b, 9c, 12a, 12b, 12c and 12d**) were subjected to antibiogram activity against three bacterial strains. The results showed that the substance **12a** used in concentration (50 mg / ml dimethylsulphoxide). It was the most important destructive compound against *E. coli*, P. aeruginosa and S. Aureous with inhibition zone (35, 30 and 22 mm) respectively. *E. coli* isolates were sensitive to **4, 6a, 6b, 9a, 9b, 9c, 12b, 12c** and **12d** with zone size **20, 15, 13, 18, 22, 10, 24, 17** and **22** mm respectively. *P. aeruginosa* was sensitive to **4, 9a, 9b, 9c, 12b, 12c** and **12d** with zone size **16, 16, 18, 14, 20, 12**, and **10** mm respectively. *S. aureus* was sensitive to **6a, 6b, 8c, 8d, 9a, 9b, 9c, 12b, 12c** and **12d** with inhibition zone size **12, 12, 16, 10, 24, 14, 18, 16, 15** and **18** mm respectively meanwhile, **12a** showed the highest effect against *three bacterial strains* (Table 1). Previous studies show that multiple classes of flavonoids have antimicrobial activity and have been reviewed extensively (Batovska & Todorova, 2010). The activity of synthesized derivatives is most likely attributed to the existence of hydroxy groups in different nucleoside positions and the phosphorous movement. (Lewis & Jorgensen,

Table 1
Antimicrobial activity of tested synthesized compounds and antibiotics on three clinical isolated bacteria. Nz: no zone. Nt: not tested.

Inhibition zone (mm) of 50 mg/ml			
Compound no.	*E. coli*	*P. aeruginosa*	*S. aureus*
4	20	16	nz
6a	15	nz	12
6b	13	nz	12
8c	nz	nz	16
8d	nz	nz	10
9a	18	16	24
9b	22	18	14
9c	10	14	18
12a	35	30	22
12b	24	20	16
12c	17	12	15
12d	22	10	18
Ciprofloxacin	Nz	<6	24
Amoxicillin	Nz	Nz	<6
Vancomycin	Nt	Nt	18

2005). Phosphonate qualities that are better suited to therapeutic use than widely used antibiotics. (Anandhi et al., 2014).

3.3. Docking studies

Docking experiment is most commonly used to design drug due to its efficiency in predicting the potency of molecule towards the reaction energy associated with potential binding conformations of an active site of biological strains; evaluation of target enzyme for evaluating the binding convergence at its catalytic site. The largest variation in the synthesized α-aminophosphonate derivative of Gibbs-free energy (after G) is the strongest binding convergence with the penicillin binding protein, involved in bacterial-cell wall maturation and cell-form development. The crystallographic structure of 2EX6 contained the amino acid residues with ampicillin as the attached ligand at the binding pocket. The synthesized compound **12a** molecular docking research revealed (ΔG = −5.645 Kcal/mol) (Table 2). Amino acid residues; Ser420 and Ser398 has H-bond with target Compound (see Table 3).

3.3.1. Structure activity relationship (SAR)

Compound **12a** molecular docking results showed as antimicrobial. Due to pyridine moiety core in parent phosphonate derivative. Perpendicular arrangement of phosphonate and pyridine with Ser 420, this compound is stabilized in binding pocket. The ampicillin was used as refrence for binding into the active DNA Gyrase (Fig. 1). Hydrophilic amino acids serve as backbone in binding site (Figs. 2, 3, and 4). Due to hydrophobicity in nature is

4. Conclusion

The nucleoside derivatives, α-aminophosphonates and arylidene analogues derived from 4-aminophenol were designed, prepared and elucidated by different spectroscopic analysis, physically studied by molecular docking of α-aminophosphonate derivative **12a** showed most effective antibacterial activity against all bacterial strains in this study with inhibition zones (35, 30 and 22 mm) respectively and the theoretical binding with DNA of bacteria.

Table 2
Computational study by energy scores (kcal/mol) derived from the MOE for synthesized compound 12 a.

PDB: 4uro						
mol	E.d_{GE}	E_conf	E_place	$E_{Int.}$	Eele	rmsd_
12 a	−5.645	183.702	−84.595	−10.953	−18.486	3.006
PDB:1gmy						
mol	E.d_{GE}	E_conf	E_place	$E_{Int.}$	Eele	rmsd_
12a	−6.657	210.339	−82.132	−10.086	−21.099	1.889

Table 3
Key interactions of the newly designed Ligand 12 a with active sites.

Ligand	Atom	Receptor	Amino acid residues	Interaction	Distance (A°)	E (kcal/mol)
PDB: 4uro						
12 a	N12	OD2	ASP57	H-donor	3.01	−4.8
	O10	N	GLY125	H-acceptor	3.18	−8.2
	O20	N	GLY125	H-acceptor	3.45	−1.1
	O39	N	GLY85	H-acceptor	3.12	−1.8
PDB:1gmy						
12 a	O10	SG	CYS29	H-donor	3.44	−1.2
	N12	O	GLY74	H-donor	3.06	−1.9

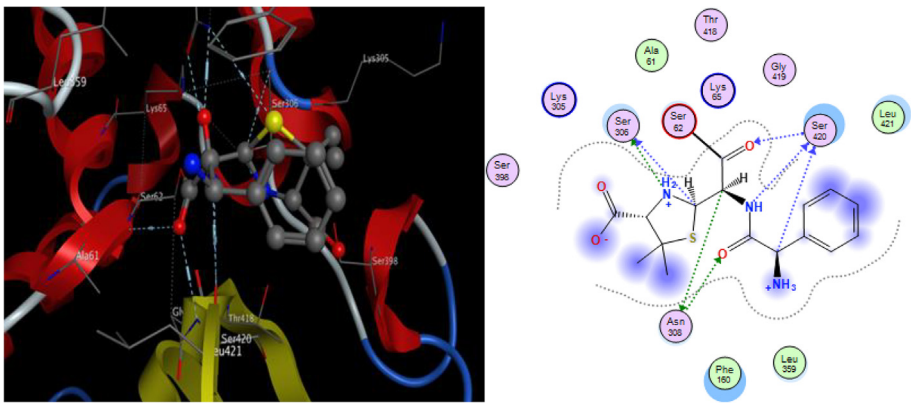


Fig. 1. The binding of ampicillin into the active DNA Gyrase.

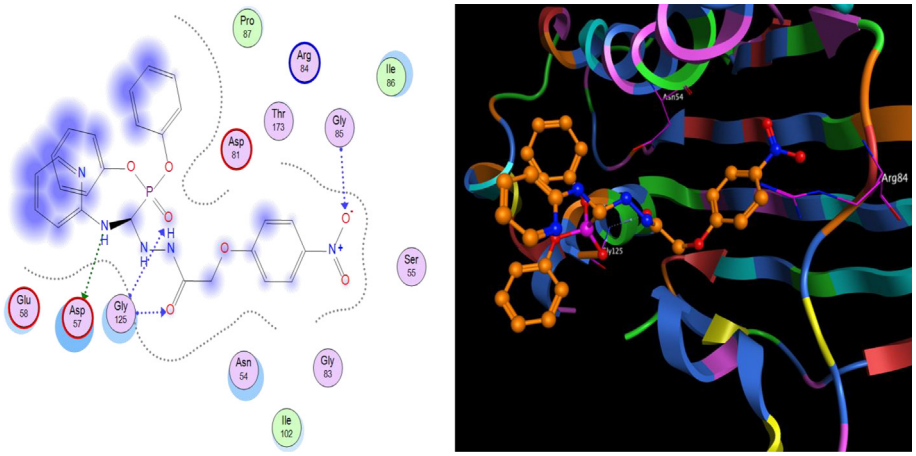


Fig. 2. The binding mode of 12a into the active DNA Gyrase.

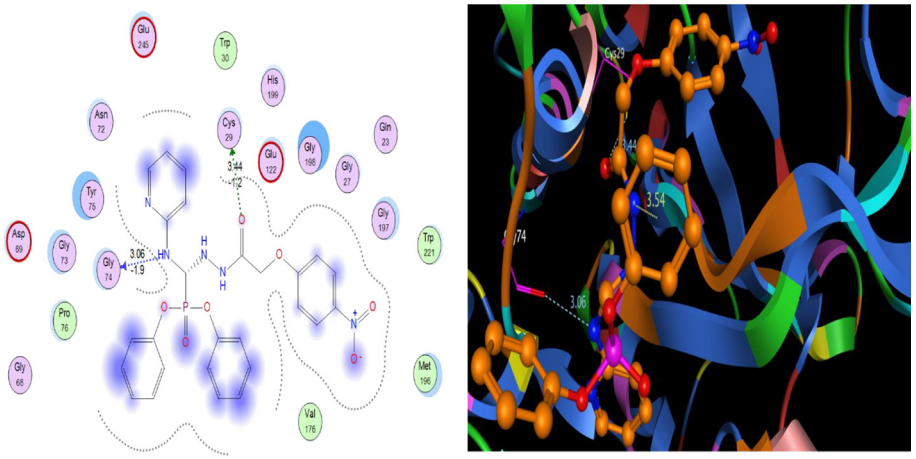


Fig.3. The binding mode of 12a into the active 1GMY.

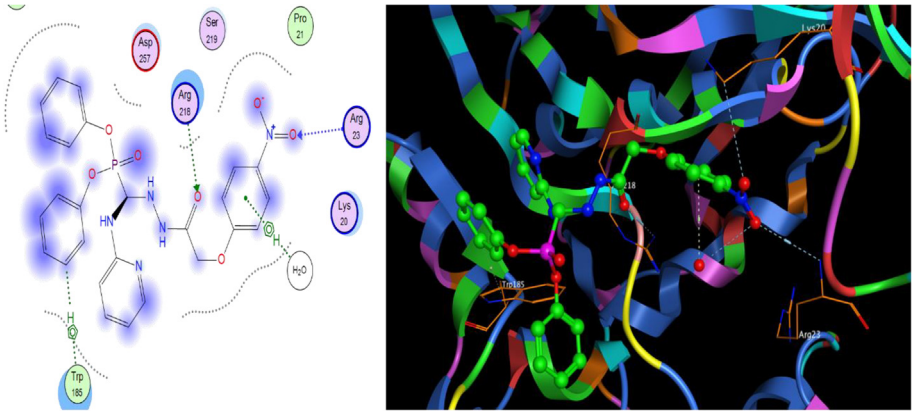


Fig. 4. The binding mode of 12a into the active 1NJE.

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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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