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Novel 1,2,3-triazolo phosphonate derivatives as potential antibacterial agents

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Abstract

We describe the synthesis, characterization, and in vitro antibacterial evaluation of a library of novel compounds based on 1,2,3-triazolo phosphonate framework along with the evaluation of DNA gyrase inhibitory potential of a promising molecule in silico. Preparation of these compounds was carried out via a multistep sequence comprising of the Abramov reaction followed by the Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) as the key steps. Various α-hydroxyphosphonate derivatives containing either a secondary or tertiary alcohol at the α position were prepared. When screened for their antibacterial activities in vitro using a Gram-positive (*Staphylococcus aureus*) and three Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) strains, majority of these derivatives exhibited reasonable to good effects with the analogue **5k** being active against all the strains. The SAR analysis indicated that the activity was influenced by the position of the α-hydroxyphosphonate moiety as well as the substituent present on the benzene ring attached to the 1,2,3-triazole ring. Moreover, the compound **5k** showed strong interactions with the DNA active site when docked into the DNA gyrase in silico. Thus, the 1,2,3-triazolo phosphonate derivative **5k** appeared to be a novel and promising hit molecule that deserves further study as a potential antibacterial agent.

1 | INTRODUCTION

The organophosphorus compounds have found enormous applications in medicinal chemistry in addition to synthetic chemistry as well as agriculture and plastic industry [1–3]. For example, the α-hydroxyphosphonates, a common class of organophosphorus derivatives, were explored for various biological activities [4–8], including antibacterial effects. In particular, the α-hydroxyphosphonates (**A**, Figure 1) derived from 2-chloroquinolin-3-carbaldehyde showed antibacterial activities comparable to the known antibiotic streptomycin [7]. Furthermore, the in vitro antibacterial and antifungal activities of α-hydroxyphosphonate derivatives of tetrazolo[1,5-*a*] quinoline indicated that a combination of a *N*-heterocyclic framework with the phosphonate moiety in a single molecular entity is beneficial for activities especially against bacteria [5]. Indeed, molecules (**B**, Figure 1) containing the 1,2,3-triazole and phosphonate moieties showed antibacterial effects in addition to the cytotoxic activities [9]. Apart from involving in the important interactions with the target enzyme, the phosphonate antibiotics are known to be capable of hijacking the glycerol-3-phosphate and glucose-6-phosphate importers expressed by many bacterial species [10].

The triazole moiety (a bioisostere of peptide) on the other hand is a well-explored framework for the identification of new and potent antibacterial agents [11–13].

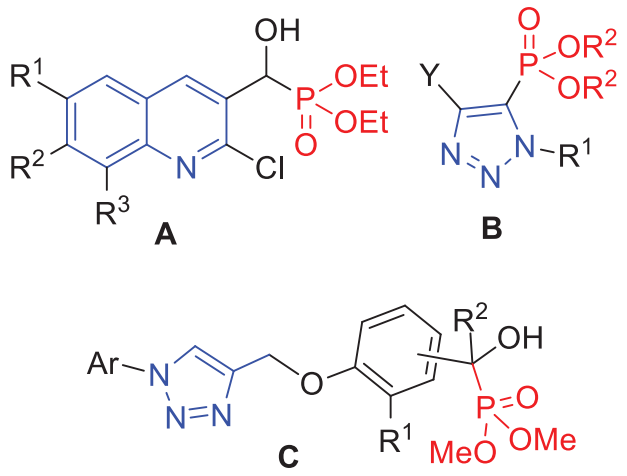


FIGURE 1 Compounds (**A** and **B**) containing an *N*-heterocyclic framework and the phosphonate moiety and the newly designed template **C** [Colour figure can be viewed at wileyonlinelibrary.com]

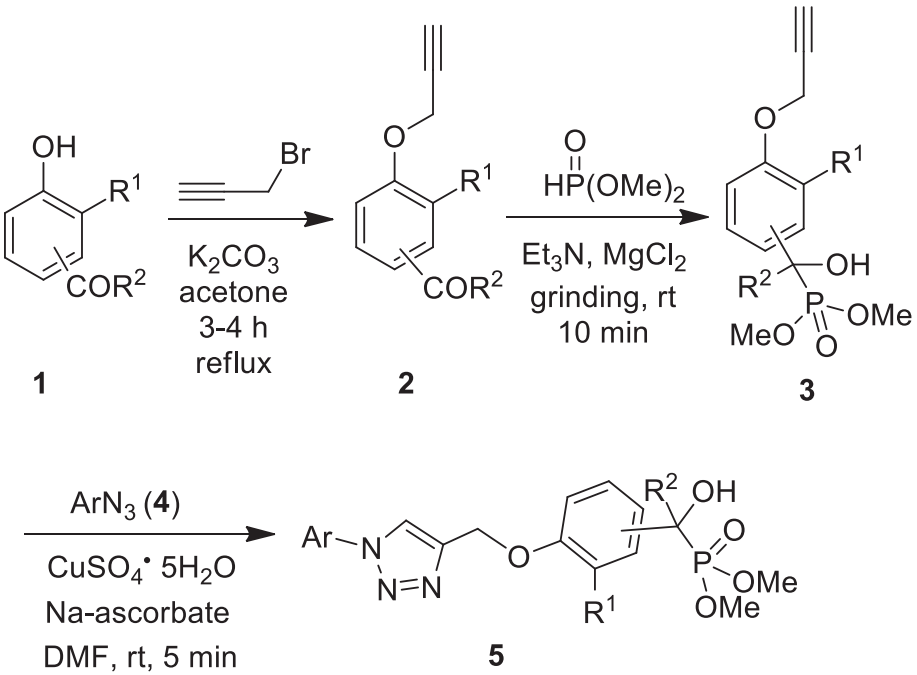
Due to our continuing interest on triazole-based antibacterial agents [14–16], we explored the novel template (**C**, Figure 1) containing 1,2,3-triazole core and the hydroxyphosphonate moiety to find new bioactive compounds possessing potential antibacterial properties. Our current initiative was further prompted by the fact that the discovery and development of new antibacterial agents with novel chemical scaffolds is one of the key approaches to combat infections due to drug-resistant strains [17,18]. Moreover, since the DNA gyrase (one of the validated targets for the identification of antibacterial agents) has been explored [19–23] to tackle the problem of antibiotic-resistant bacteria, we aimed to target this protein in our in silico studies. Herein, we report the synthesis, characterization, and in vitro antibacterial screening of a library of compounds based on template **C** along with the evaluation of DNA gyrase inhibitory potential of a promising molecule in silico. Notably, while α-hydroxyphosphonates containing a secondary alcohol at the α position is common in the literature, the compounds containing tertiary alcohol at the same position are rather scarce. Nevertheless, to the best of our knowledge, the preparation and pharmacological assessment of 1,2,3-triazolo phosphonate derivatives related to **C** have not been explored earlier.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

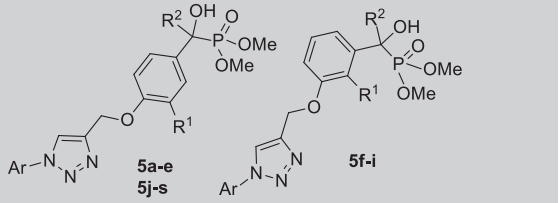
The preparation of target molecules was undertaken via a multistep sequence using the Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) [24] as the main step (Scheme 1).

One of the required substrates, that is, the terminal alkyne (**3**), was prepared as shown in Scheme 1. Thus, commercially available, appropriately substituted hydroxybenzaldehyde derivatives (**1**) were *O*-propargylated to give the corresponding terminal alkyne (**2**) that on Abramov reaction with the mixture of dimethylphosphite, $MgCl_2$, and Et_3N under neat reaction conditions afforded the desired α-hydroxyphosphonate derivatives (**3**) [25]. The organic azides (**4**) were synthesized from the primary amines via a known procedure



SCHEME 1 Preparation of compounds (**5**) based on 1,2,3-triazolo phosphonate framework (**C**)

TABLE 1 Target compounds synthesized



Azide 4; Ar =	Alkyne 3		Product 5; %yield[a]
	R^1	R^2	
4a; *o*-$NO_2C_6H_4$	H	H	**5a**; 90
4b; *m*-$NO_2C_6H_4$	H	H	**5b**; 80
4c; *o*-$MeOC_6H_4$	H	H	**5c**; 81
4d; *m*-$MeOC_6H_4$	H	H	**5d**; 85
4e; *p*-$MeOC_6H_4$	H	H	**5e**; 90
4a	H	H	**5f**; 85
4b	H	H	**5g**; 80
4d	H	H	**5h**; 78
4e	H	H	**5i**; 81
4a	H	Me	**5j**; 80
4b	H	Me	**5k**; 78
4c	H	Me	**5l**; 80
4d	H	Me	**5m**; 80
4e	H	Me	**5n**; 80
4a	OMe	H	**5o**; 90
4b	OMe	H	**5p**; 90
4c	OMe	H	**5q**; 90
4d	OMe	H	**5r**; 90
4e	OMe	H	**5s**; 90

[a]Isolated yields.

[26]. Next, both the reactants, that is, **3** and **4**, were allowed to react in the presence of $CuSO_4.5H_2O$ and Na-ascorbate at room temperature in DMF to furnish the desired products (**5**) in moderate to good yields (Scheme 1 and Table 1). A total of 18 new compounds were prepared and characterized by (NMR, IR, and MS) spectral data. Some selected ^1H and ^{13}C NMR spectral data of a representative molecule **5o** are shown in Figure 2. Briefly, the two singlets near δ 7.9 and 5.3 in the ^1H NMR spectra were due to the 1,2,3-triazole ring proton and the OCH_2 group, respectively. The singlet at δ 3.9 was due to the OCH_3 group attached to the aromatic ring, whereas two doublets at δ 3.7 and 3.6 were due to the diastereotopic OCH_3 groups in —$PO(OCH_3)_2$ moiety. A broad singlet at δ 3.2 and a double doublet at δ 5.0 were due to the —OH and the hydrogen at α to the —$PO(OCH_3)_2$ group. The ^{13}C signals appeared near 63.0, 69.4, and 119.7 ppm in the ^{13}C NMR spectra were due to the OMe (aryl), OCH_2, and C-5 of the triazole ring, respectively, whereas the signals at 55.9 and 53.8 ppm were due to the —$PO(OCH_3)_2$ moiety. The signal appeared at 71.0 ppm was due to the —CH(OH)— moiety. The presence of —$PO(OCH_3)_2$ group was further confirmed by the signal near δ 26 in the ^{31}P NMR spectra.

2.2 | Biology

2.2.1 | Antibacterial activities

Having prepared a library of new compounds based on the 1,2,3-triazolo phosphonate framework (**C**), (Figure 1) all were tested for their antibacterial effects using a Gram-positive (*Staphylococcus aureus*) and three Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) strains. The assay was performed via an agar-well diffusion method [27–29] where perfloxacin was included as a positive control. The outcome (Table 2) suggested that while most of the compounds showed activities, the compounds **5k**, **5l,** and **5q** showed better activity than the others against *S. aureus* (+ve), whereas **5b**, **5c**, **5k**, **5p, 5r,** and **5s** showed good activity against *E. coli* (−ve). Compounds **5b** and **5k** showed some mediocre activity against *K. pneumoniae* (−ve), whereas **5l** showed good activity against *P. aeruginosa* (−ve). Compounds **5c**, **5g,** and **5k** showed mediocre activity against the same strain. The structure activity relationship (SAR) overview of antibacterial effects of derivatives **5** (Figure 3) indicated that the activity was influenced by the position of the α-hydroxyphosphonate moiety on the aryl ring attached to the 1,2,3-triazole ring through the –OCH_2-group. In general, the *p*-position was found to be favorable over the *m*-position as except **5g** no other compounds having the α-hydroxyphosphonate group at *m*-position (e.g., **5f**, **5h,** and **5i**) showed good activity. The tertiary alcohol at α to the –$PO(OCH_3)_2$ moiety seemed to have somewhat better effect than that of secondary alcohol at the same position. Notably, the presence of a substituent such as OMe on the benzene ring next to the α-hydroxyphosphonate group was favorable for activity preferably against the *E. coli*. Nevertheless, the activity of compound **5** was also influenced by the place and nature of substituent, for example, NO_2 or OMe present on the benzene ring attached to the 1,2,3-triazole ring. Briefly, the analogue **5k** seemed to be the most encouraging among these derivatives examined as it showed activities with MIC of 32, 25, 35, and 36 μg/ml, respectively, against the entire

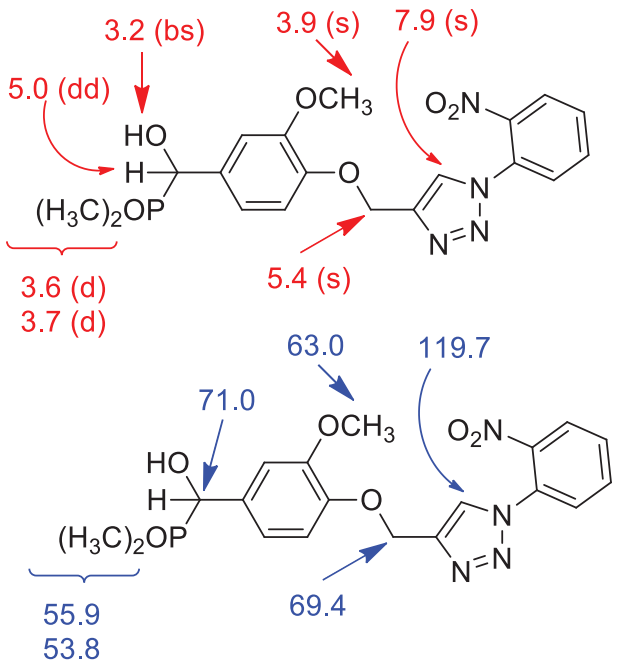


FIGURE 2 Selected ^{1}H (red) and ^{13}C NMR (blue) signals of compound **5o** [Colour figure can be viewed at wileyonlinelibrary.com]

strains, including Gram-positive and Gram-negative bacterial species. The analogues **5b**, **5c,** and **5l** though showed activities against more than one strain, **5l** is the only other compound apart from **5k** that showed activities against both Gram-positive and Gram-negative bacterial strains. Nonetheless, the present class of α-hydroxyphosphonate-1,2,3-triazole derivatives in general appeared to be effective against bacteria and

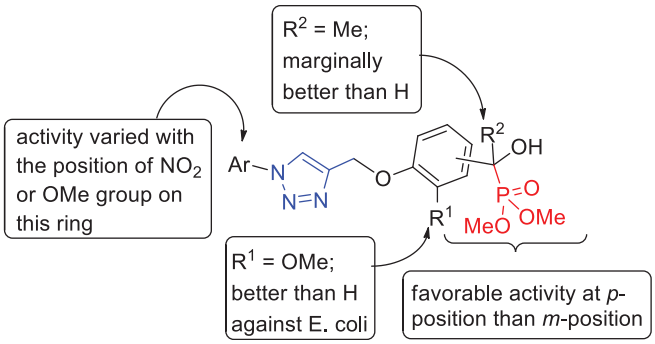


FIGURE 3 SAR overview for antibacterial effects of **5** [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Antibacterial assessment of **5**

Compound	Zone of inhibition[a] (mm)			
	S. aureus (+ve)	*E. coli* (−ve)	*K. pneumoniae* (−ve)	*P. aeruginosa* (−ve)
5a	16 ± 0.45	17 ± 0.34	14 ± 0.77	15 ± 0.63
5b	12 ± 0.71	23 ± 0.51	17 ± 0.34	16 ± 0.11
5c	16 ± 0.80	21 ± 0.40	15 ± 0.55	18 ± 0.26
5d	15 ± 0.67	19 ± 0.36	16 ± 0.83	16 ± 0.65
5e	15 ± 0.59	18 ± 0.33	16 ± 0.71	16 ± 0.86
5f	14 ± 0.46	16 ± 0.57	15 ± 0.24	14 ± 0.94
5g	14 ± 0.34	14 ± 0.42	15 ± 0.44	17 ± 0.21
5h	15 ± 0.29	14 ± 0.40	16 ± 0.63	16 ± 0.33
5i	15 ± 0.28	12 ± 0.13	16 ± 0.39	15 ± 0.43
5j	12 ± 0.18	13 ± 0.73	12 ± 0.41	14 ± 0.52
5k	18 ± 0.23	25 ± 0.42	17 ± 0.84	17 ± 0.19
5l	18 ± 0.19	17 ± 0.45	16 ± 0.92	23 ± 0.82
5m	14 ± 0.17	14 ± 0.81	15 ± 0.74	15 ± 0.53
5n	16 ± 0.24	15 ± 0.43	16 ± 0.33	14 ± 0.23
5o	16 ± 0.51	18 ± 0.56	16 ± 0.63	14 ± 0.96
5p	16 ± 0.37	24 ± 0.33	15 ± 0.91	16 ± 0.77
5q	18 ± 0.31	14 ± 0.65	16 ± 0.58	15 ± 0.43
5r	15 ± 0.70	22 ± 0.19	15 ± 0.21	16 ± 0.32
5s	16 ± 0.62	22 ± 0.26	14 ± 0.34	15 ± 0.19
pfx	28 ± 0.11	36 ± 0.24	35 ± 0.32	31 ± 0.41

Note: Data are means (n = 3) ± Standard deviation of three replicates.

Abbreviations: *E. coli, Escherichia coli; K. pneumonia, Klebsiella pneumoniae; P. aeruginosa, Pseudomonas aeruginosa; pfx, Perfloxacin*; *S. aureus, Staphylococcus aureus*.

[a]Calculation of Zone of inhibition was performed for stock solution at 0.1 mg/50 μl.

therefore are of further medicinal interest. Next, some of the active molecules, for example, **5b**, **5c**, **5g**, **5k**, **5l**, **5p**, **5q**, **5r**, and **5s** were evaluated for their potential toxicity by an MTT assay on RAW 264.7 cell line at different concentrations. None of them showed significant toxicities till 30 μM of their concentration tested. Indeed, % cell viability was found to be 100%, 96%, 89%, 88%, 75%, and 61% at the concentration of 1, 3, 10, 30, 60, and 100 μM of **5k**, respectively.

2.3 | In silico studies

The molecular docking studies of compound **5k** was performed using Autodock Vina software [30,31] to verify and understand its interactions with the target protein. Thus, the binding affinity of **5k** toward the DNA-gyrase cleavage complex of *S. aureus* and topoisomerase Top. IV of *K. pneumonia* organism's DNA binding site encircled by the protein residues were analyzed in silico separately. The strong interactions of **5k** with the DNA active site were indicated by the binding affinity of the molecule in both cases as shown in Table 3.

Initially, the crystal structure of the DNA-gyrase cleavage complex of *S. aureus* (PDB_ID: 5CDQ) was taken into consideration to dock the analogue **5k**. The crystal structure contains an inhibitor Moxifloxacin intercalation in the E and F chains of DNA. Some of the amino acid residues of A and C chains of the DNA-gyrase complex stabilized the Moxifloxacin bound DNA structure. The docking of **5k** was done in the intercalation location of DNA of the DNA-gyrase complex. The DNA bound molecule was stabilized by the H-bonds and hydrophobic as well as π–π interactions. The stabilization of *m*-nitro phenyl ring was aided by the thymine ring (DT-8) of the F chain via π–π interactions. The oxygen of nitro group formed an H-bond with the Ser-84 and also participated in the interaction with Mg^{2+} ion. These interactions are shown in Figure 4. In order to consolidate these findings, the crystal structure of K. Pneumonia topoisomerase, Top. IV (PDB_ID: 5EIX) was also taken into consideration to dock **5k**. In this case, the crystal structure contains the inhibitor Levofloxacin intercalation in the E, F, and I chains of DNA, and some of the amino acid residues of A and G chains stabilized the Levofloxacin bound DNA structure. The molecule **5k** was

TABLE 3 Binding affinity of compound **5k**

PDB_ID	Binding affinity (kcal/mol)
5CDQ	−8.1
5EIX	−8.3

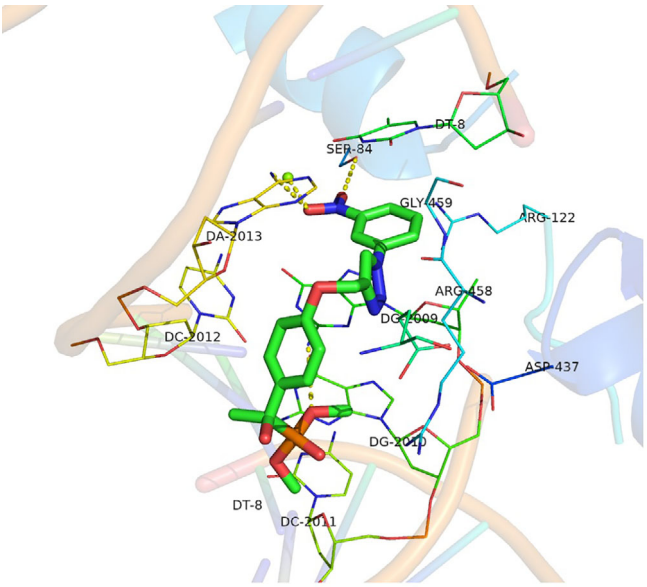


FIGURE 4 The docking of compound **5k** into the active site of the DNA-gyrase cleavage complex of *S. aureus* (PDB_ID:5CDQ). The stick style (in green) shows the molecule and the line style shows the amino acid residues and DNA bases [Colour figure can be viewed at wileyonlinelibrary.com]

docked in the intercalation location of DNA of the topoisomerase, Top. IV complex. The stabilization of DNA bound molecule was caused by the H-bonds, hydrophobic, and π–π interactions, whereas the stabilization of the nitro phenyl ring was aided by the thymine ring (DT-15) of E chain via π–π interactions. The oxygen of nitro group interacted with the Mg^{2+} ion. The hydroxyl group of the **5k** formed H-bond with the nitrogen of the adenine ring (DA-2) of the I chain. These interactions are shown in Figure 5.

3 | CONCLUSIONS

To conclude, we have presented the preparation, characterization, and in vitro antibacterial screening of a library of novel compounds based on 1,2,3-triazolo phosphonate framework along with the evaluation of DNA gyrase inhibitory potential of a promising molecule in silico. These compounds were accessed through a multistep sequence using the Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) as the main reaction step. The Abramov reaction was used to introduce the phosphonate group into one of the key reactants. Various α-hydroxyphosphonate derivatives containing either a secondary or tertiary alcohol at the α position were prepared. The characterization of the products was carried out using IR, ^{1}H, ^{13}C, and ^{31}P NMR and mass spectrometry. These compounds were then tested for their antibacterial effects using a Gram-positive (*S. aureus*) and three Gram-negative (*E. coli, K. pneumoniae* and *P. aeruginosa*) strains via an

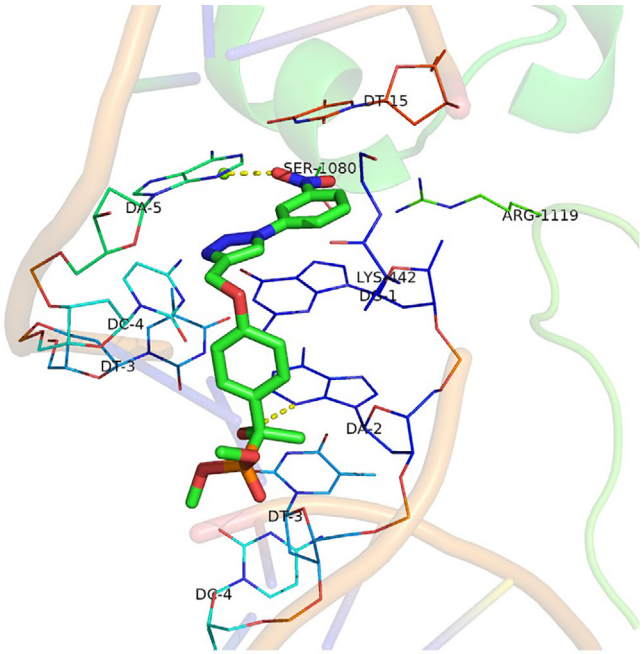


FIGURE 5 The docking of compound **5k** into the active site of the topoisomerase top. IV of K. pneumonia (PDB_ID: 5EIX). The molecule is shown in stick style (in green) and the amino acid residues and DNA bases are shown in line style [Colour figure can be viewed at wileyonlinelibrary.com]

agar-well diffusion technique. While majority of the derivatives were found to be active (with reasonable to good antibacterial effects), the analogue **5k**, that is, dimethyl (1-hydroxy-1-(4-((1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)ethyl) phosphonate exhibited good effects against the entire strains. The SAR analysis indicated that the activity was influenced by the position of the α-hydroxyphosphonate moiety as well as the substituent present on the benzene ring attached to the 1,2,3-triazole ring. To assess its potential mechanism of action (MOA), the interaction of compound **5k** with DNA gyrase was examined in silico when the molecule showed strong interactions with the DNA active site as indicated by its binding affinities. Thus, the current study not only discloses the 1,2,3-triazolo phosphonate framework as a new and promising template for the identification of potential antibacterial agents but also demonstrates the utility of the Abramov reaction followed by the CuAAC for the synthesis of compounds based on this framework.

4 | EXPERIMENTAL

4.1 | Chemistry

Determination of melting points was carried out using a Bio-technics melting point instrument (open glass capillary method). The IR spectra were recorded using a Bruker-Tensor 27 spectrometer in the form of KBr pellets. The ^{1}H and ^{13}C NMR spectra were obtained using a Bruker ACF-300 machine or a Varian 300 or 400 MHz spectrometer, and the solvent $CDCl_3$ or DMSO-d_6 was used with tetramethylsilane as an internal reference. The ^{31}P NMR spectra were obtained using a Bruker-AVANCE-III 400 MHz spectrometer, and DMSO-d_6 was used as a solvent. The mass spectra were obtained using a Jeol JMC D-300 instrument (electron ionization at 70 eV). Elemental analyses were performed using Varian 3LV analyzer series CHN analyzer. TLC (thin layer chromatography) on precoated silica gel plates were used to monitor the progress of reactions. Column chromatography was carried out by using silica gel (100–200 mesh, SRL, India) [10–20 times (by weight) of the crude compound.

4.2 | Synthesis of alkyne 3

Step 1. To a mixture of aldehyde or ketone (**1**, 8.1 mmol) and potassium carbonate (2.2 g, 16.2 mmol) in acetone (15 ml) was added propargyl bromide (1.9 g, 16.2 mmol) dropwise. The stirring of the mixture was carried out at the refluxing temperature for 3–4 h and TLC was used to monitor the progress of the reaction. Once the reaction was completed, the mixture was added to the crushed ice (30 g). The solid precipitated was filtered, collected, and dried under vacuum. The crude compound (**2**) isolated was used for the subsequent step.

When solid was not precipitated out after pouring the reaction mixture into the crushed ice, the mixture was treated with ethyl acetate (3 × 15 ml). The organic parts were collected, combined, washed with NaCl solution (2 × 15 ml), dried using Na_2SO_4 (anhyd.), and filtered. The filtrate was evaporated under low vacuum and the residue was dried under vacuum. The crude product (**2**) obtained was used for the subsequent step.

Step 2. A suspension of the crude product **2** (0.106 g, 1.0 mmol), anhydrous magnesium chloride (0.1 g, 1.0 mmol), triethylamine (0.3 g, 3.0 mmol), and dimethylphosphite (0.136 g, 1.1 mmol) were taken in a mortar. The mixture was ground for 10 min until a paste is obtained (TLC was used to monitor the reaction progress). Once the reaction was completed, the paste was treated with EtOAc (3 × 5 ml). The organic parts were collected, combined, dried using anhydrous Na_2SO_4, filtered, and evaporated under low vacuum. The purification of crude compound was carried out using column chromatography on silica gel and petroleum ether/EtOAc as eluent to furnish the expected compound (**3**).

4.3 | Spectral data of compound 3

4.3.1 | Dimethyl (hydroxy(4-(prop-2-yn-1-yloxy)phenyl)methyl)phosphonate (3a)

White solid; Yield 90%; mp 85-86 °C; R*f*: 0.56(3:1 Petroleum ether: Ethyl acetate); MS *m/z* 271.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3260.4, 3049.9, 2945.6, 2913.1, 2350.11606.4, 1539.6, 1510.1, 1135.2; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.45 (d, 2H, *J* = 10.0 Hz), 6.91 (d, 2H, *J* = 10.0 Hz), 5.50 (d, 1H, *J* = 12.3 Hz), 4.69 (d, 2H, *J* = 4.0 Hz), 3.71 (d, 3H, *J* = 12.3 Hz), 3.65 (d, 3H, *J* = 12.3 Hz), 3.62 (bs, 1H), 2.50 (t, 1H, *J* = 4.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz):δ 156.5, 131.0, 128.5, 114.1, 79.2, 78.1, 69.5, 67.9, 62.0, 55.3; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 23.6.

4.3.2 | Dimethyl(hydroxy(3-(prop-2-yn-1-yloxy)phenyl)methyl)phosphonate (3b)

Brown Liquid; Yield 90%; R*f*: 0.53(3:1 Petroleum ether: Ethyl acetate); MS *m/z* 271.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3267.4, 3050.9, 2957.6, 2913.1, 2350.1, 1606.4, 1539.6, 1510.1, 1140.2; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.51 (d, 1H, *J* = 8.0 Hz), 7.35–7.30 (m, 1H), 7.09 (t, 1H, *J* = 10.0 Hz), 7.00 (d, 1H, *J* = 8.0 Hz), 5.50 (d, 1H, *J* = 12.3 Hz), 4.53 (s, 2H), 3.89 (d, 3H, *J* = 12.3 Hz), 3.64 (d, 3H, *J* = 12.3 Hz), 3.20 (bs, 1H), 2.50 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 158.2, 143.2, 130.2, 121.2, 114.7, 112.2, 70.8, 69.2, 62.0, 55.9, 53.8; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 23.3.

4.3.3 | Dimethyl (1-hydroxy-1-(4-(prop-2-yn-1-yloxy)phenyl)ethyl) phosphonate (3c)

White solid; Yield 90%; mp 74-76 °C; R*f*: 0.70(3:1 Petroleum ether: Ethyl acetate); MS *m/z* 285.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3235.4, 3047.9, 2956.6, 2912.1, 2349.1, 1606.4, 1539.6, 1510.1, 1140.2; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.99 (d, 2H, *J* = 8.0 Hz), 6.80–6.78 (m, 2H), 4.80 (d, 2H, *J* = 4 Hz), 3.99 (d, 3H, *J* = 12.3 Hz), 3.60 (d, 3H, *J* = 12.3 Hz), 2.89 (bs, 1H), 2.50 (t, 1H, *J* = 4.0 Hz), 1.89 (d, 3H, *J* = 12.3 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 156.5, 131.0, 128.5, 114.1, 79.2, 78.1, 69.5, 67.9, 67.0, 62.0, 23.8; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 26.1.

4.3.4 | Dimethyl(hydroxy(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)methyl) phosphonate (3d)

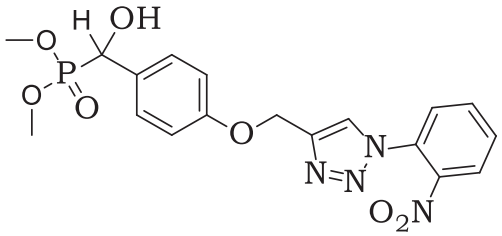
White solid; Yield 90%; mp 71-72 °C; R*f*: 0.45(3:1 Petroleum ether: Ethyl acetate); MS *m/z* 301.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3262.4, 3047.9, 2956.6, 2912.1, 2355.6, 1606.4, 1539.6, 1510.1, 1140.2; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.18–7.16 (m, 1H), 7.02–6.98 (m, 2H), 5.00 (d, 1H), 4.89 (s, 2H), 3.99 (s, 3H), 3.88 (d, 3H, *J* = 12.3 Hz), 3.65 (d, 3H, *J* = 12.3 Hz), 2.90 (bs, 1H), 2.50 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.8, 149.3, 144.4, 130.3, 122.1, 114.7, 79.9, 76.0, 70.9, 69.3, 62.8, 55.9, 53.7; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 23.6.

4.4 | General procedure for the preparation of compound 5

A mixture of azide **4** (0.53 mmol), an appropriate terminal alkyne **3** (0.53 mmol), $CuSO_4 \cdot 5H_2O$ (65 mg, 0.26 mmol), and sodium ascorbate (52 mg, 0.26 mmol) in DMF (5 ml) was stirred vigorously for 5 min, at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was poured into the crushed ice (30 g). The solid separated was filtered, dried, and purified by column chromatography on silica gel using petroleum ether/ethyl acetate as eluent.

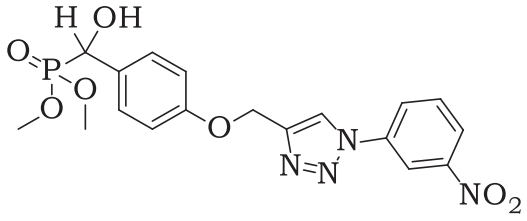
4.5 | Spectral data of compound 5

4.5.1 | Dimethy(hydroxy (4-((1-(2-nitrophenyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)methyl) phosphonate (5a)



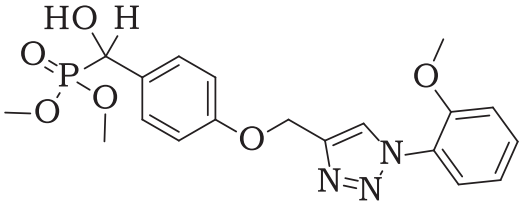
White solid; Yield 90%; mp 164–166°C; R*f*: 0.59 (3:2 Petroleum ether: Ethyl acetate); MS *m/z* 435.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3247.4, 3047.9, 2956.6, 2912.1, 1606.4, 1539.6, 1510.1, 1463.8, 1424.3, 1140.2. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.11 (d, 1H, *J* = 9.7 Hz), 7.94 (s, 1H), 7.81 (t, 1H, *J* = 7.7 Hz), 7.73 (t, 1H, *J* = 8.0 Hz) 7.65 (d, 1H, *J* = 8.0 Hz), 7.55 (dd, 2H, *J* = 9.0 Hz, 2.5 Hz), 7.03 (d, 2H, *J* = 9.2 Hz), 5.39 (s, 2H), 5.00 (dd, 1H, *J* = 12.5, 5.0 Hz), 3.75 (d, 3H, *J* = 10.4 Hz,), 3.67 (d, 3H, *J* = 10.2 Hz), 3.21 (bs,1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 143.6, 132.8, 129.9, 129.1128.0, 127.6, 127.5, 127.0, 124.6, 123.4, 113.8, 69.9, 68.3, 60.8, 52.8; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 24.1. Elemental Analysis found C, 49.59; H, 4.32; N, 12.68; Calc. for $C_{18}H_{19}N_4O_7P$; C, 49.77; H, 4.41; N, 12.90.

4.5.2 | Dimethy(hydroxy (4-((1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)methyl) phosphonate (5b)



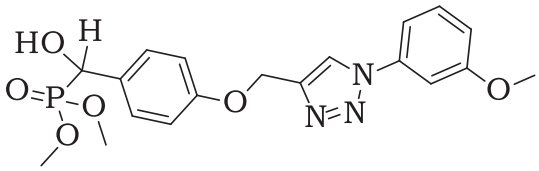
White solid; Yield 80%; mp 166–168°C; R*f*: 0.59(3:2 Petroleum ether: Ethyl acetate); MS *m/z* 435.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3287.4, 3047.9, 2956.6, 2912.1, 1606.4, 1539.6, 1510.1, 1463.8, 1424.3, 1140.2; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.62 (s, 1H), 8.33 (d, 1H, *J* = 8.0 Hz), 8.22–8.19 (m, 2H), 7.77 (t, 1H, *J* = 9.5 Hz), 7.56 (d, 1H, *J* = 9.5 Hz), 7.45 (d, 1H, *J* = 10.0 Hz), 7.04 (d, 2H, *J* = 10.0 Hz), 5.33 (s, 2H), 5.00 (dd, 1H, *J* = 12.3,4 Hz), 3.75 (d, 3H, *J* = 12.3 Hz), 3.69 (d, 3H, *J* = 12.3 Hz), 3.62 (d, 1H, *J* = 12.3 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 158.1, 144.4, 137.6, 131.0, 128.6, 127.3, 126.0, 123.3, 120.7, 115.3, 114.8, 114.3, 71.0, 69.4, 61.8, 53.9; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 24.1. Elemental Analysis found C, 49.50; H, 4.43; N, 12.99; Calc. for $C_{18}H_{19}N_4O_7P$; C, 49.77; H, 4.41; N, 12.90.

4.5.3 | Dimethyl(hydroxy (4-((1-(2-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) methyl) phosphonate (5c)



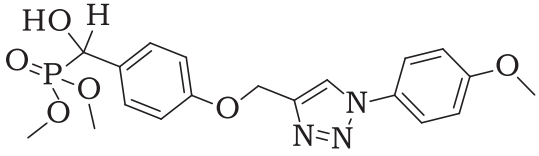
Light brown solid; Yield 81%; mp 118–120°C; R*f*: 0.48 (3:2 Petroleum ether: Ethyl acetate); MS *m/z* 420.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3262.4, 3047.9, 2956.6, 2912.1, 1606.4, 1539.6, 1510.1, 1463.8, 1424.3, 1140.2; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.19 (s, 1H), 7.79 (dd, 1H, *J* = 8.0 Hz, 1.7 Hz), 7.46–7.42 (m, 3H), 7.13–7.04 (m, 4H), 5.30 (s, 2H), 5.00 (dd, 1H, *J* = 8.0 Hz, 4.0 Hz), 3.90 (s, 3H), 3.73 (d, 3H, *J* = 8.0 Hz), 3.67 (d, 3H, *J* = 8.0 Hz), 3.14 (dd, 1H, *J* = 12.0 Hz, 4.0 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 158.2, 151.0, 143.2, 130.2, 129.2, 128.5, 126.1, 125.4, 125.1, 121.2, 114.7, 112.2, 70.8, 69.2, 61.9, 55.9, 53.8; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 24.1. Elemental Analysis found C, 54.63; H, 5.28; N, 9.87; Calc. for $C_{19}H_{22}N_3O_6P$; C, 54.42; H, 5.29; N, 10.02.

4.5.4 | Dimethyl(hydroxy (4-((1-(3-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) methyl) phosphonate (5d)



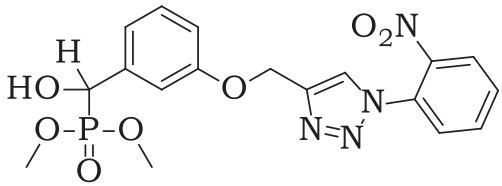
Light brown solid; Yield 85%; mp 120–122°C; R*f*: 0.44 (3:2 Petroleum ether: Ethyl acetate); MS *m/z* 420.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3262.4, 3047.9, 2956.6, 2912.1, 1606.4, 1539.6, 1510.1, 1463.8, 1424.3, 1140.2; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.05 (s, 1H), 7.44 (d, 3H, *J* = 12.0 Hz), 7.35 (s, 1H), 7.04 (d, 2H, *J* = 8.0 Hz), 6.98 (d, 2H, *J* = 8.0 Hz), 5.29 (s, 2H), 5.01 (d, 1H, *J* = 10.2 Hz), 3.89 (s, 3H), 3.73 (d, 3H, *J* = 11.0 Hz), 3.68 (d, 3H, *J* = 11.0 Hz), 3.30 (bs, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.6, 158.2, 137.9, 130.5, 129.2, 128.6, 128.5, 121.1, 114.7, 112.4, 106.4, 70.9, 69.3, 61.9, 55.6, 53.8. ^{31}P NMR (DMSO-d_6, 400 MHz): δ 24.1. Elemental Analysis found C, 54.21; H, 5.28; N, 10.17; Calc. for $C_{19}H_{22}N_3O_6P$; C, 54.42; H, 5.29; N, 10.02.

4.5.5 | Dimethyl(hydroxy (4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) methyl) phosphonate (5e)



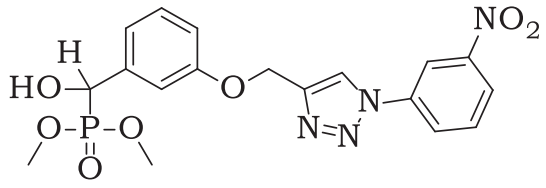
White solid; Yield 90%; mp 150–152°C; R*f*: 0.60 (3:2 Petroleum ether: Ethyl acetate) MS *m/z* 420.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3262.4, 3047.9, 2956.6, 2912.1, 1606.4, 1539.6, 1510.1, 1463.8, 1424.3, 1140.2; ^{1}H NMR ($CDCl_3$, 400 MHz):δ 7.98 (s, 1H), 7.63 (d, 2H, *J* = 9.0 Hz), 7.44 (d, 2H, *J* = 9.0 Hz), 7.02 (d, 4H, *J* = 9.5 Hz), 5.27 (s, 2H), 5.01 (dd, 1H, *J* = 12.0 Hz, 8.0 Hz), 3.87 (s, 3H), 3.84 (d, 1H, *J* = 8.0 Hz) 3.73 (d, 3H, *J* = 12.0 Hz), 3.67 (d, 3H, *J* = 12.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.8, 158.1, 144.4, 130.3, 129.4, 128.6, 128.5, 122.2, 121.2, 114.7, 70.7, 69.1, 61.8, 55.6, 53.7. ^{31}P NMR (DMSO-d_6, 400 MHz): δ 24.1. Elemental Analysis found C, 54.66; H, 5.30; N, 9.86; Calc. for $C_{19}H_{22}N_3O_6P$; C, 54.42; H, 5.29; N, 10.02.

4.5.6 | Dimethyl(hydroxy (3-((1-(2-nitrophenyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)methyl) phosphonate (5f)



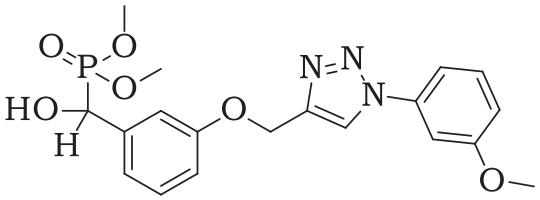
Brown liquid; Yield 85%; R*f*: 0.48 (3:2 Petroleum ether: Ethyl acetate); MS *m/z* 435.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3262.4, 3047.9, 2956.6, 2912.1, 1606.4, 1539.6, 1510.1, 1463.8, 1424.3, 1140.2;^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.15 (s,1H), 7.41 (t, 1H, *J* = 8.2 Hz), 7.37 (t, 1H, *J* = 2.5 Hz), 7.32–7.28 (m, 2H), 7.23 (m, 1H) 7.08 (d, 1H, *J* = 12.5 Hz), 6.98–6.96 (m, 2H), 5.30 (s, 2H), 5.06 (d, 1H, *J* = 11.5 Hz), 3.73 (d, 3H, *J* = 10.5 Hz), 3.65 (d, 3H, *J* = 10.5 Hz), 1.76 (1H, OH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 143.6, 132.8, 129.9, 129.1, 128.0, 127.6, 127.5, 127.0, 124.6, 123.4, 113.9, 113.8, 69.8, 68.3, 60.8, 52.8; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 24.1. Elemental Analysis found C, 49.60; H, 4.43; N, 12.73; Calc. for $C_{18}H_{19}N_4O_7P$; C, 49.77; H, 4.41; N, 12.90.

4.5.7 | Dimethyl(hydroxy (3-((1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)methyl) phosphonate (5g)



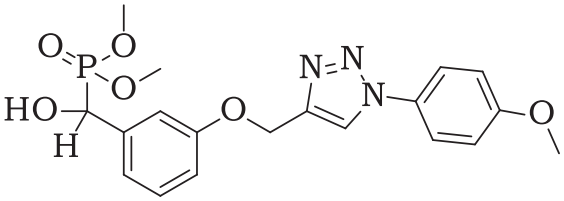
Brown solid; Yield 80%; mp 120–122°C; R*f*: 0.62 (3:2 Petroleum ether: Ethyl acetate) MS *m/z* 435.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3290.4, 3076.9, 2955.6, 2931.2, 1601.4, 1499.4, 1449.4, 1378.6, 1350.3, 1162.7; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.15 (s, 1H), 7.41 (t, 1H, *J* = 8.2 Hz), 7.37 (t, 1H, *J* = 2.5 Hz), 7.32–7.28 (m, 2H), 7.23 (m, 1H) 7.08 (d, 1H, *J* = 12.5 Hz), 6.98–6.96 (m, 2H), 5.30 (s, 2H), 5.06 (d, 1H, *J* = 11.5 Hz), 3.73 (d, 3H, *J* = 10.5 Hz), 3.65 (d, 3H, *J* = 10.5 Hz), 1.76 (bs, 1H, OH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 158.1, 157.5, 144.4, 137.6, 131.0, 128.6, 127.3, 126.0, 123.3, 120.7, 115.3, 114.8, 114.3, 71.0, 69.4, 61.8, 54.0, 53.7; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 23.8. Elemental Analysis found C, 49.89; H, 4.42; N, 12.73; Calc. for $C_{18}H_{19}N_4O_7P$; C, 49.77; H, 4.41; N, 12.90.

4.5.8 | Dimethyl(hydroxy (3-((1-(3-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl) methoxy) phenyl) methyl) phosphonate (5h)



Brown solid; Yield 78%; mp 116–118°C; R*f*: 0.44 (3:2 Petroleum ether: Ethyl acetate); MS *m/z* 420.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3262.4, 3047.9, 2956.6, 2912.1, 1606.4, 1539.6, 1510.1, 1463.8, 1424.3, 1140.2;^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.09 (d, 1H *J* = 8.0 Hz), 7.96 (s, 1H), 7.80 (t, 1H, *J* = 8.5 Hz), 7.71(t, 1H, *J* = 7.2 Hz), 7.63 (d, 1H), 7.05 (m, 2H), 6.89 (d, 1H, *J* = 8.7 Hz), 6.82 (t, 1H, *J* = 7.0 Hz), 5.37 (s, 2H), 5.27 (d, 1H, *J* = 11.3 Hz), 3.82 (s, 3H), 3.76 (d, 3H, *J* = 11.0 Hz), 3.69 (d, 3H, *J* = 11.0 Hz), 3.55 (bs, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.5, 158.1, 144.5, 138.3, 137.9130.5, 129.5, 121.4, 120.1, 115.0, 114.7, 113.1, 112.3, 106.1, 71.1, 69.5, 61.6, 55.6, 53.8; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 23.8. Elemental Analysis found C, 54.23; H, 5.27; N, 9.87; Calc. for $C_{19}H_{22}N_3O_6P$; C, 54.42; H, 5.29; N, 10.02.

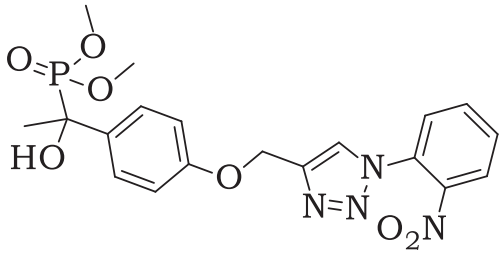
4.5.9 | Dimethyl(hydroxy (3-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-ylyl)methoxy)phenyl) methyl) phosphonate (5i)



Brown solid; Yield 81%; mp 110–112°C; R*f*: 0.60 (3:2 Petroleum ether: Ethyl acetate); MS *m/z* 420.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3262.4, 3047.9, 2956.6,

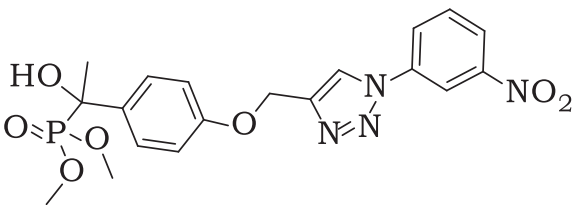
2912.1, 1606.4, 1539.6, 1510.1, 1463.8, 1424.3, 1140.2; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.05 (s, 1H), 7.65 (d, 2H, J = 9.2 Hz), 7.30 (t, 1H, J = 8.2 Hz), 7.22 (s, 1H), 7.09 (d, 1H, J = 8.7 Hz), 7.03–6.91 (m, 3H), 5.30 (s, 2H), 5.05 (d, 1H, J = 12.5 Hz), 3.87 (s, 3H), 3.73 (d, 3H, J = 10.2 Hz), 3.67 (d, 3H, J = 10.2 Hz), 3.56 (bs,1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.8, 158.1, 144.4, 130.3, 129.4, 128.6, 128.5, 122.2, 121.2, 114.7, 70.7, 69.1, 61.8, 55.6, 53.7; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 24.1. Elemental Analysis found C, 54.23; H, 5.27; N, 10.18; Calc, for $C_{19}H_{22}N_3O_6P$; C, 54.42; H, 5.29; N, 10.02.

4.5.10 | Dimethyl(1-hydroxy-1-(4-((1-(2-nitrophenyl)-1*H*-1,2,3-triazol-4-yl) methoxy) phenyl) ethyl) phosphonate (5j)



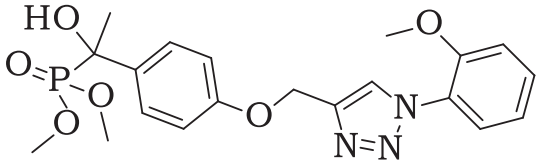
White solid; Yield 80%; mp 166–168°C; R*f*: 0.46 (3:2 Petroleum ether: Ethyl acetate); MS *m/z* 449.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3270.7, 3081.7, 2981.7, 2954.5, 1610.2, 1588.1, 1534.4, 1467.3, 1414.4, 1175.2; ^{1}H NMR ($CDCl_3$,400 MHz): δ 8.11 (d, 1H, J = 9.7 Hz), 7.94 (s, 1H), 7.81 (t, 1H, J = 7.7 Hz), 7.73 (t, 1H, J = 8.0 Hz), 7.65 (d, 1H, J = 8.0 Hz), 7.55 (dd, 2H, J = 9.0 Hz, 2.5 Hz), 7.03 (d, 2H, J = 9.2 Hz), 5.32 (s, 2H), 3.74 (d, 3H, J = 10.2 Hz), 3.61 (d, 3H, J = 10.2 Hz), 2.85 (d, 1H, J = 7.5 Hz), 1.82 (d, 3H, J = 15.3 Hz);^{13}C NMR ($CDCl_3$, 100 MHz): δ 157.5, 148.9, 145.7, 137.6, 133.8, 131.1, 127.3, 125.9, 123.3, 120.9, 115.3, 114.2, 77.4, 76.7, 74.1, 72.6, 61.8, 54.0, 25.8;^{31}P NMR (DMSO-d_6, 400 MHz): δ 26.3; Elemental Analysis found C, 50.69; H, 4.74; N, 12.69; Calc. for $C_{19}H_{21}N_4O_7P$; C, 50.90; H, 4.72; N, 12.50.

4.5.11 | Dimethyl(1-hydroxy-1-(4-((1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methoxy) phenyl)ethyl) phosphonate (5k)



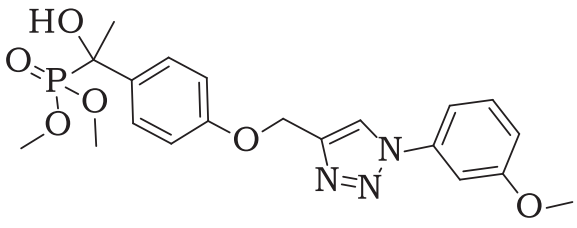
Brown solid; Yield 78%; mp 150–152°C; R*f*: 0.48 (3:2 Petroleum ether: Ethyl acetate); MS *m/z* 449.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3252.4, 3085.0, 3018.5, 2957.1, 1599.4, 1564.6, 1514.1, 1454.3, 1416.2, 1144.7;^{1}H NMR ($CDCl_3$, 400 MHz): δ8.62 (s, 1H), 8.32 (d, 1H, J = 8.5 Hz), 8.22–8.19 (m, 2H), 7.77 (t, 1H, J = 8.2 Hz), 7.56 (d, 2H, J = 9.2 Hz), 7.02 (d, 2H, J = 9.5 Hz), 5.33 (s, 2H), 3.75 (d, 3H, J = 10.2 Hz), 3.64 (d, 3H, J = 10.2 Hz), 3.52 (d, 1H, J = 7.0 Hz), 1.81(d, 3H, J = 15.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 157.4, 148.9, 145.7, 137.6, 133.8, 131.0, 127.3, 125.9, 123.3, 120.9, 115.3, 114.2, 77.4, 76.7, 74.1, 72.6, 61.8, 54.0, 25.8; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 26.2. Elemental Analysis found C, 50.69; H, 4.70; N, 12.71; Calc. for $C_{19}H_{21}N_4O_7P$; C, 50.90; H, 4.72; N, 12.50.

4.5.12 | Dimethyl(1-hydroxy-1-(4-((1-(2-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)ethyl) phosphonate (5l)



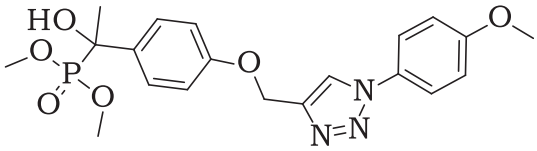
White solid; Yield 80%; mp 150–152°C; R*f*: 0.71 (3:2 Petroleum ether: Ethyl acetate); MS *m/z* 434.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3270.7, 3081.7, 2981.7, 2954.5, 1610.2, 1588.1, 1534.4, 1467.3, 1414.4, 1175.2; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.19(s,1H), 7.79 (dd, 1H, J = 8.0 Hz, 4.0 Hz), 7.54 (dd, 2H, J = 12.0 Hz, 4.0 Hz), 7.44 (td, 1H, J = 8.0 Hz, 4.0 Hz), 7.10–7.08 (m, 2H), 7.06–7.03 (m, 2H), 5.30 (s, 2H), 3.90 (s, 3H), 3.74 (d, 3H, J = 12.0 Hz), 3.61 (d, 3H, J = 8.0 Hz), 3.08 (bs,1H), 1.81 (d,3H, J = 12.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 157.7, 151.1, 143.3, 133.5, 130.2, 127.2, 126. 1, 125.4, 125.0, 121.2, 114.4, 112.2, 74.2, 72.6, 62.0, 55.9, 54.1, 25.7; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 26.2. Elemental Analysis found C, 55.23; H, 5.50; N, 9.52; Calc. for $C_{20}H_{24}N_3O_6P$; C, 55.43; H, 5.58; N, 9.70.

4.5.13 | Dimethyl(1-hydroxy-1-(4-((1-(3-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl) ethyl) phosphonate (5m)



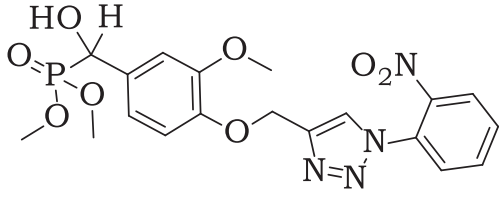
Pale yellow; Yield 80%; mp 160–162°C; R*f*: 0.71 (3:2 Petroleum ether: Ethyl acetate); MS *m/z* 434.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3270.7, 3081.7, 2981.7, 2954.5, 1610.2, 1588.1, 1534.4, 1467.3, 1414.4, 1175.2; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.05 (s, 1H), 7.56 (d, 1H, *J* = 2.5 Hz), 7.53 (d, 1H, *J* = 2.5 Hz), 7.42 (t, 1H, *J* = 8.2 Hz), 7.35 (t, 1H, *J* = 2.5 Hz), 7.28–7.25 (m, 2H), 7.03 (d, 1H, *J* = 9.0 Hz), 7.00–6.97 (m, 1H) 5.30 (s, 2H), 3.89 (s, 3H), 3.74 (d, 3H, *J* = 10.2 Hz), 3.61 (d, 3H, *J* = 10.2 Hz), 2.88 (bs, 1H), 1.81 (d, 3H, *J* = 15.3 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.6, 157.6, 144.8, 137.9, 133.5, 130.5, 127.2, 121.0, 114.7, 114.3, 112.4, 106. 4, 74.2, 72.6, 62.0, 56.6, 54.0, 25.8; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 26.2. Elemental Analysis found C, 55.22; H, 5.51; N, 9.62; Calc. for $C_{20}H_{24}N_3O_6P$; C, 55.43; H, 5.58; N, 9.70.

4.5.14 | Dimethyl(1-hydroxy-1-(4-((1-(2-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl) methoxy) phenyl) ethyl phosphonate (5n)



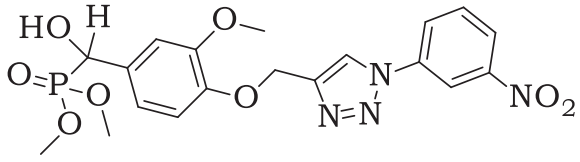
White solid; Yield 80%; mp 150–152°C; R*f*: 0.71 (3:2 Petroleum ether: Ethyl acetate) MS *m/z* 434.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3265.7, 3071.7, 2980.7, 2955, 1600.2, 1568.1, 1534.4, 1467.3, 1443.4, 1165.2;^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.98 (s, 1H), 7.64 (d, 2H, *J* = 12.0 Hz), 7.55 (dd, 2H, *J* = 8.0 Hz, 4.0 Hz), 7.03 (d, 2H, *J* = 8.0 Hz), 7.02 (d, 2H, 8.0 Hz) 5.29 (s, 2H), 3.87 (s, 3H), 3.75 (d, 3H, *J* = 12.0 Hz), 3.62 (d, 3H, *J* = 12.0 Hz), 3.20 (d,1H, *J* = 8.0 Hz), 1.82 (d, 3H, *J* = 12.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.8, 157.6, 144.6, 133.6, 130.3, 127.3, 127.2, 122.2, 121.1, 114.7, 114.3, 114.2, 74.1, 72.6, 61.9, 55.6, 53.9; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 26.3. Elemental Analysis found C, 55.21; H, 5.57; N, 9.92; Calc. for $C_{20}H_{24}N_3O_6P$; C, 55.43; H, 5.58; N, 9.70.

4.5.15 | Dimethyl(hydroxy(3-methoxy-4-((1-(2-nitrophenyl)-1*H*-1,2,3-triazol-4-yl) methoxy)phenyl)methyl) (5o)



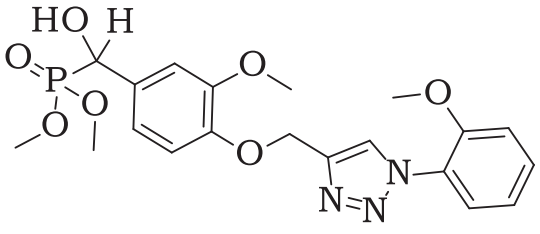
Light Yellow solid; Yield 90%; mp 148–150°C; R*f*: 0.44 (3:2 Petroleum ether: Ethyl acetate); MS *m/z* 465.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3262.4, 3047.9, 2956.6, 2912.1, 1606.4, 1539.6, 1510.1, 1463.8, 1424.3, 113.2; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10 (d, 1H, *J* = 8.2 Hz), 7.96 (s, 1H), 7.81 (t,1H, *J* = 9.2 Hz), 7.71 (t, 1H, *J* = 8.2 Hz), 7.63 (d, 1H, *J* = 9.2 Hz), 7.12 (s, 1H), 7.08 (d, 1H, *J* = 8.5 Hz), 7.00 (d, 1H, *J* = 8.0 Hz), 5.39 (s, 2H), 5.00 (dd, 1H, *J* = 12.5,5.0 Hz), 3.90 (s, 3H), 3.75 (d, 3H, *J* = 10.4 Hz,), 3.67 (d, 3H, *J* = 10.2 Hz), 3.21 (bs,1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.6, 147.4, 144.8, 144.4, 133.8, 130.8, 130.1, 127.9, 125.6, 124.6, 119.7, 114.1, 110.7, 71.0, 69.4, 63.0, 55.9, 53.7; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 23.1. Elemental Analysis found C, 48.96; H, 4.50; N, 11.98; Calc. for $C_{19}H_{21}N_4O_8P$; C, 49.14; H, 4.56; N, 12.07.

4.5.16 | Dimethyl(hydroxy(3-methoxy-4-((1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl methoxy) phenyl) methyl) phosphonate (5p)



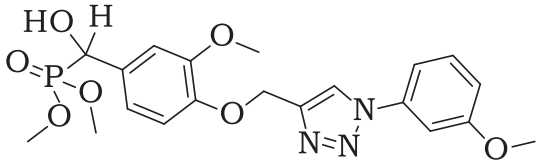
White solid; Yield 90%; mp 116–118°C; R*f*: 0.55 (3:2 Petroleum ether: Ethyl acetate); MS *m/z* 465.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3288.4, 3046.9, 2957.6, 2912.1, 1606.4, 1529.6, 1515.1, 1470.8, 1424.3, 1140.2;^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.62 (s,1H), 8.32 (d, 1H, *J* = 8.2 Hz), 8.24 (s,1H), 8.18 (d, 1H, *J* = 8.2 Hz), 7.76 (t, 1H, *J* = 10.0 Hz), 7.12 (s, 1H), 7.06–7.05 (m, 1H), 7.00 (d, 1H, *J* = 10.5 Hz), 5.39 (s, 2H), 5.00 (dd, 1H, *J* = 13.3 Hz, 4.2 Hz), 3.91 (s, 3H), 3.75 (d, 3H, *J* = 10.5 Hz), 3.69 (d, 3H, *J* = 10.5 Hz), 3.48 (bs,1H);^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.5, 148.9, 147.4, 137.6, 131.0, 130.1, 125.9, 123.3, 121.1, 119.7, 119.6, 115.3, 113.7, 110.7, 71.1, 69.5, 62.9, 55.9, 53.5; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 24.1. Elemental Analysis found C, 48.95; H, 4.49; N, 11.99; Calc. for $C_{19}H_{21}N_4O_8P$; C, 49.14; H, 4.56; N, 12.07.

4.5.17 | Dimethyl(hydroxy(3-methoxy-4-((1-(2-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy) phenyl) methyl) phosphonate (5q)



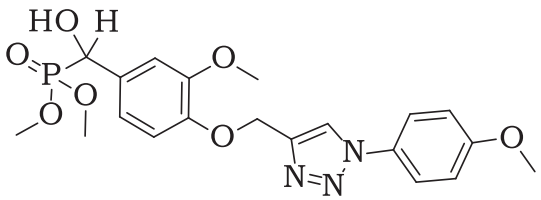
White solid; Yield 90%; mp 146–148°C; R*f*: 0.60 (3:2 Petroleum ether: Ethyl acetate); MS *m/z* 450.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3245.5, 3085.2, 2956.1, 2846.8, 1599.0, 1566.0, 1515.5, 1456.2, 1418.6, 1145.2;^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10 (d, 1H, *J* = 8.2 Hz), 7.96 (s, 1H), 7.81 (t,1H, *J* = 9.2 Hz), 7.71 (t, 1H, *J* = 8.2 Hz), 7.63 (d, 1H, *J* = 9.2 Hz), 7.12 (s,1H), 7.08 (d, 1H, *J* = 8.5 Hz), 7.00 (d, 1H, *J* = 8.0 Hz), 5.39 (s, 2H), 5.00 (dd, 1H, *J* = 12.5 Hz,5.0 Hz), 3.90 (s, 3H), 3.87 (s, 3H), 3.75 (d, 3H, *J* = 10.4 Hz), 3.67 (d, 3H, *J* = 10.2 Hz), 3.21 (bs,1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 151.0, 149.5, 147.6, 143.3, 130.2, 130.0, 126.1, 125.4, 121.1, 119.7, 119.6, 113.9, 112.2, 110.8, 110.7, 70.9, 69.3, 63.0, 55.9, 53.7; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 26.2. Elemental Analysis found C, 53.68; H, 5.30; N, 9.29; Calc. for $C_{20}H_{24}N_3O_7P$; C, 53.45; H, 5.38; N, 9.35.

4.5.18 | Dimethyl(hydroxy(3-methoxy-4-((1-(3-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy) phenyl)methyl) phosphonate (5r)



White solid; Yield 90%; mp 104–106°C; R*f*: 0.57; (3:2 Petroleum ether: Ethyl acetate); MS *m/z* 450.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3262.4, 3047.9, 2956.6, 2912.1, 1606.4, 1539.6, 1510.1, 1463.8, 1424.3, 1140.2; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.08 (s, 1H), 7.41 (t, 1H, *J* = 8.2 Hz), 7.34–7.32 (m, 1H), 7.24(1H, d, *J* = 4.0 Hz), 7.09 (d, 2H, *J* = 8.7 Hz), 7.02–6.96 (m, 2H), 5.38 (s,2H), 4.99 (dd, 1H, *J* = 12.0 Hz, 8.0 Hz), 3.91 (s, 3H), 3.88 (s, 3H, *J* = 10.5 Hz), 3.75 (d, 3H, *J* = 12.0 Hz), 3.67 (d, 3H, *J* = 12.0 Hz), 2.85 (dd, 1H, *J* = 8.0 Hz, 12.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.5, 149.5, 149.4, 147.5, 137.9, 130.5, 129.9, 121.3, 119.7, 119.6, 114.7, 113.7, 110.7, 106.3, 70.6, 69.4, 62.9, 55.9, 55.6, 53.7. ^{31}P NMR (DMSO-d_6, 400 MHz): δ 23.8 Elemental Analysis found C, 53.08; H, 5.30; N, 9.47; Calc. for $C_{20}H_{24}N_3O_7P$; C, 53.26; H, 5.29; N, 9.30.

4.5.19 | Dimethyl(hydroxy(3-methoxy-4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy) phenyl)methyl) phosphonate (5s)



White solid; Yield 90%; mp 164–166°C; R*f*: 0.44 (3:2 Petroleum ether: Ethyl acetate); MS *m/z* 450.0 (M + 1, 100%); FTIR (KBr, υ in cm^{-1}): 3245.5, 3085.2, 2956.1, 2846.8, 1599.0, 1566.0, 1515.5, 1456.2, 1418.6, 1145.29; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.01 (s, 1H), 7.62 (d, 2H, *J* = 9.2 Hz), 7.11–7.07 (m, 2H), 7.03–6.98 (m, 3H), 5.36 (s, 2H), 4.99 (d, 1H, *J* = 12.5 Hz), 3.90 (s, 3H), 3.87 (s, 3H), 3.74 (d, 3H, *J* = 10.5 Hz), 3.67 (d, 3H, *J* = 10.5 Hz), 3.40 (bs, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.8, 149.3, 147.4, 144.4, 130.3, 130.1, 122.1, 121.5, 119.7, 114.7, 113.5, 110.8, 70.9, 69.3, 62.8, 55.9, 55.6, 53.7; ^{31}P NMR (DMSO-d_6, 400 MHz): δ 23.8. Elemental Analysis found C, 53.11; H, 5.21; N, 9.22; Calc. for $C_{20}H_{24}N_3O_7P$; C, 53.26; H, 5.29; N, 9.30.

4.6 | Pharmacology

4.6.1 | Materials and conditions

Four organisms including one Gram-positive, for example, *Staphylococcus aureus*, and three Gram-negative species, for example, *K. pneumoniae*, *P. aeruginosa*, and *E. coli*, were employed for the measurement of antibacterial effects of our newly prepared derivatives. The bacterial strains were obtained from the Global Hospitals, Hyderabad, India. Standard microbiological processes were utilized to examine the purity of all bacterial strains. To store the bacterial stock culture, the Mueller Hinton Agar (MHA) slants were utilized for maintaining the temperature at 4°C with a subculture period of 15 days. The surface viable counting technique was used for the maintenance of microbial stock solution with a concentration of 108–109 colony forming units (CFU)/ml. The preparation of a 24-h old microbial stock suspension was undertaken each time, and the experimental conditions (temperature and aeration) were maintained constant prior to the antimicrobial assay was performed.

4.6.2 | The assay protocol

The Agar well diffusion procedure was employed for assessing the antimicrobial effects [22,23]. The standardized microbial stock suspension (1 × 108) (100 μl) and molten Mueller-Hinton Agar medium was mixed thoroughly and then poured into the sterile Petri plates. Three 8 mm wells were made using a sterile cork borer no 4In in each Petri plate. Then, to two of the wells was added 50 μl of solution containing 0.5 mg of compound, whereas 50 μl of solution containing 0.05 mg of "a broad spectrum" antibiotic Perfloxacin (as the control) was added to the third well. In order to allow the bacterial growth, plates were then incubated overnight at 37°C.

After incubation, the measurement of the diameter of the zones of inhibition was carried out and charted for each microorganism.

4.6.3 | Measurement of MIC

To determine the MIC, the microorganism's susceptibility tests in nutrient and potato dextrose broths were employed [24]. The stock solutions (1000 μg/ml) of the tested compounds and perfloxacin in DMSO were prepared followed by dilutions to 250–25 μg/ml concentrations. For MIC determination, the incubation of inoculated microorganism suspensions was done at 37°C for 1–5 days. Then, the inoculation of microorganism suspensions into the concentrations of corresponding compounds and control experiments was carried out.

4.6.4 | MTT assay

Initially, 20,000–25,000 cells per well were seeded in a 96-well plate and incubated with the test compound at different concentrations for 24 h. After treatment, the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reagent was added to a final concentration of 0.25 mg/ml and incubated at 37°C for 2 h. After 2 h, media was removed completely, and the intracellular purple formazan crystals were dissolved in 100 μl of DMSO. The absorbance of this solution was measured at 570 nm. Each concentration of the compound was performed in triplicate, and the relative cell viability was expressed as a percentage relative to the untreated control cells.

4.7 | Molecular docking

Molecular docking studies were carried out using Autodock Vina software [25,26] (an open-source molecular docking software). The molecule **5k** was docked into the crystal structures of DNA-gyrase cleavage complex of *S. aureus* (Gram-positive bacteria) with PDB_ID:5CDQ, and the cleavage complex of topoisomerase Top. IV of *K. pneumonia* (Gram-negative bacteria) with PDB_ID:5EIX. A grid box was generated with desired parameters around the active site of DNA-gyrase cleavage complex of *S. aureus* (PDB_ID:5CDQ) as the center: x = 40.123, y = −46.732, z = 64.933 and grid box size: x = 22, y = 36, z = 26. Another grid box was generated with desired parameters around the active site of topoisomerase Top. IV of K. pneumonia (PDB_ID:5EIX) as the center: x = 183.18, y = − 28.952, z = −7.879 and grid box size: x = 22, y = 32, z = 32. Then, advanced genetic algorithm method in Vina was used to generate 20 conformations in each docking output, whereas MGLTools-1.5.6 software was used for the protein/DNA complex as well as molecule input preparations and docking output analysis.

ACKNOWLEDGMENT

Jhonsee Rani Telu thanks DST-INSPIRE, New Delhi, India, for a Junior Research Fellowship. The authors also thank CFRD, Osmania University, India and JNTU Hyderabad, India for support.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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REFERENCES

[1] T. E. Ali, S. M. Abdel-Kariem, *ARKIVOC* **2015**, *2015*, 246.
[2] K. Moonen, I. Laureyn, C. V. Stevens, *Chem. Rev.* **2004**, *104*, 6177.
[3] R. K. Bansal Ed., *Topics in Heterocyclic Chemistry*, Vol. 21, Springer, Berlin, Germany **2010**.
[4] P. Kafarski, B. Lejczak, *J. Mol. Catal. B Enzym* **2004**, *29*, 99.
[5] A. H. Kategaonkar, R. U. Pokalwar, S. S. Sonar, V. U. Gawali, B. B. Shingate, M. S. Shingare, *Eur. J. Med. Chem.* **2010**, *45*, 1128.
[6] R. Snoeck, A. Holy, C. Dewolf-Peeters, J. Van Den Ord, E. De Clercq, G. Andrei, *Antimicrob. Agents Chemother.* **2002**, *46*, 3356.
[7] R. U. Pokalwar, R. V. Hangarge, P. V. Maske, M. S. Shingare, *Arkivoc* **2006**, *2006*(11), 196.
[8] R. M. N. Kalla, H. R. Lee, J. Cao, J. W. Yoo, I. Kim, *New J. Chem.* **2015**, *39*, 3916.
[9] A. Tripolszky, E. Tóth, P. T. Szabó, L. Hackler Jr., B. Kari, L. G. Puskás, E. Bálint, *Molecules* **2020**, *25*, 2643. https://doi.org/10.3390/molecules25112643.
[10] K. S. Ju, J. R. Doroghazi, W. W. Metcalf, *J. Ind. Microbiol. Biotechnol.* **2014**, *41*, 345. https://doi.org/10.1007/s10295-013-1375-2.
[11] K. Lal, P. Yadav, A. Kumar, A. Kumar, A. K. Paul, *Bioorg. Chem.* **2018**, *77*, 236.
[12] A. H. Kategaonkar, P. V. Shinde, A. H. Kategaonkar, S. K. Pasale, B. B. Shingate, M. S. Shingare, *Eur. J. Med. Chem.* **2010**, *45*, 3142.
[13] B. Garudachari, A. M. Isloor, M. N. Satyanarayana, H. K. Fun, G. Hegde, *Eur. J. Med. Chem.* **2014**, *74*, 324.
[14] K. Naveen, J. R. Telu, V. Banothu, S. B. Nallapati, J. S. Anireddy, S. Pal, *Med. Chem. Commun.* **2015**, *6*, 1612.
[15] S. Pal, N. Kuntala, J. R. Telu, V. Banothu, J. S. Anireddy, *Der Pharma Chem* **2017**, *9*, 1.

[16] K. Naveen, J. R. Telu, V. Banothu, S. Balasubramanian, J. S. Anireddy, S. Pal, *Mini Rev. Med. Chem.* **2018**, *18*, 803.
[17] L. Freire-Moran, B. Aronsson, C. Manz, I. C. Gyssens, A. D. So, D. L. Monnet, O. Cars, the ECDC-EMA working group, *Drug Resist. Updates* **2011**, *14*, 118.
[18] D. J. Payne, M. N. Gwynn, D. J. Holmes, D. L. Pompliano, *Nat. Rev. Drug Discov.* **2007**, *6*, 29.
[19] J. D. Bilavendran, A. Manikandan, P. Thangarasu, K. Sivakumar, *J. Heterocycl Chem.* **2020**, *57*, 1143.
[20] S. R. Ashok, M. K. Shivananda, M. S. Prakash, S. Sreenivasa, A. Manikandan, *J. Heterocycl Chem.* **2020**, *57*, 1211.
[21] A. Manikandan, V. P. Muralidharan, M. Andrew, M. H. Ahmed, K. I. Sathiyanarayanan, A. Sivakumar, *Mol. Inf.* **2018**, *37*, 1800048.
[22] A. Manikandan, A. Sivakumar, *Bioorg. Med. Chem.* **2017**, *25*, 1448.
[23] T. Ponnusamy, A. Manikandan, S. Thamaraiselvi, *Bioorg. Med. Chem.* **2018**, *26*, 3438.
[24] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.
[25] M. Tajbakhsh, S. Khaksar, Z. Tafazoli, A. Bekhradnia, *Chin. J. Chem.* **2012**, *30*, 827.
[26] F. Nemati, A. Elhampour, *J. Appl. Chem.* **2012**, *7*, 29.
[27] F. Kavanagh, *Analytical Microbiology*, Vol. II, Academic Press, New York and London **1972**, p. 11.
[28] S. S. Miles, S. S. Misra, J. O. Irwin, *J Hyg* **1938**, *38*, 732.
[29] V. Singh, D. Katiyar, *Bioorg. Chem.* **2017**, *71*, 120.
[30] O. Trott, A. Olson, *J. Comput. Chem.* **2010**, *31*, 455.
[31] G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, A. J. Olson, *J. Comput. Chem* **2009**, *16*, 2785.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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