Heliyon 5 (2019) e02812

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.heliyon.com

Research article

Synthesis, antibacterial activity and molecular docking study of vanillin derived 1,4-disubstituted 1,2,3-triazoles as inhibitors of bacterial DNA synthesis

Mumtaz Hussain [a], Tahir Qadri [a], Zahid Hussain [a], Aamer Saeed [b,**], Pervaiz Ali Channar [b], Syeda Aaliya Shehzadi [c,*], Mubashir Hassan [d], Fayaz Ali Larik [b], Tarique Mahmood [a], Arif Malik [d]

[a] Department of Chemistry, University of Karachi, 75270, Karachi, Pakistan
[b] Department of Chemistry, Quaid-i-Azam University, 45320, Islamabad, Pakistan
[c] Sulaiman Bin Abdullah Aba Al-Khail-Centre for Interdisciplinary Research in Basic Sciences (SA-CIRBS), International Islamic University, 44000, Islamabad, Pakistan
[d] Institute of Molecular Biology and Biotechnology, The University of Lahore, Defence Road Campus, Lahore, Pakistan

ARTICLE INFO

Keywords:
Organic chemistry
Pharmaceutical chemistry
Click chemistry
1,4-Disubstituted 1,2,3-triazoles
Antibacterial agents
Molecular docking
Thymidylate kinase (TMPK) inhibitors

ABSTRACT

Antimicrobial resistance (AMR) compelled scientists in general while pharmacists, chemists and biologists in specific to believe that we could always remain ahead of the pathogens. The pipeline of new drugs is running gasping and the inducements to develop new antimicrobials to address the global problems of drug resistance are weak. In this pursuit, effective endeavours to prepare new anti-bacterial entities is highly wished. The present study demonstrates successful synthesis of a library of 1,4-disbustituted 1,2,3-triazoles (**3a-3k**) using Click-chemistry concept and anti-their bacterial potential. In this 1,3-dipolar cycloaddition, the 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde (1) was used as alkyne partner which was synthesized from vanillin and propargyl bromide and further reacted with differently substituted arylpropoxy azides (**2a-k**) to furnish series of mono and *bis*1,4-disubstituted-1,2,3-triazoles. All the synthesized compounds were characterized spectroscopically and were evaluated for their initial antimicrobial activity. Preliminary results of antibacterial screening revealed that the synthesized compounds have the highest inhibitory effects compare to the control ciprofloxacin. The compounds **3b** and **3g** were found to be the most active (MIC: 5 μg/mL, MIC: 10 μg/mL respectively) against various strains of gram-positive and gram-negative bacteria. The molecular docking study against 4GQQ protein with synthesized ligands was performed to see the necessary interactions responsible for anti-bacterial activity. The docking analysis of the most potent compound **3g** supported the antibacterial activity exhibiting high inhibition constant and binding energy.

1. Introduction

Antimicrobial resistance (AMR) is the major threat to human health growing in the field of infectious diseases mainly caused by bacteria, fungi, viruses and parasites. According to a survey in 2016, around 490,000 people have multidrug-resistant Tuberculosis (MDR-TB) worldwide, and the drug resistance is commencing to complicate the battle against HIV as well as malaria [1]. With the passage of time the need for effective antimicrobial has become essential to control the spread of "superbugs": microorganisms that develop antimicrobial resistance are sometimes referred to as superbugs [2].

The triazoles have grabbed significant attention of organic and medicinal chemists since the inception of copper catalyzed azide-alkyne cycloaddition (CuAAC) [3]. This method has superiority over conventional thermal method [4] where reaction of an alkyne and azide would generate a mixture of both 1,4- and 1,5-disubstituted 1,2,3-triazoles. While in CuAAC exclusively 1,4-disubstituted 1,2,3-triazoles are obtained [5] in excellent yields which have very promising role in pharmaceutical industry [6].

The importance of triazoles in medicinal chemistry is inevitable with respect to both biological and pharmaceutical applications [7]. Recently 1,2,3-triazoles have emerged as active components in the agrochemical

* Corresponding author.
** Corresponding author.
E-mail addresses: aamersaeed@yahoo.com (A. Saeed), aaliya.shehzadi@iiu.edu.pk (S.A. Shehzadi).

https://doi.org/10.1016/j.heliyon.2019.e02812
Received 18 July 2019; Received in revised form 13 October 2019; Accepted 7 November 2019

[8], pesticidal, polymer [9] and materials chemistry fields [10, 11]. 1,2,3-Triazoles also know to exhibit applications as optical brighteners [12], corrosion inhibitors [13], photostabilizers for fibres, plastics or dyes [14], and UV-screens for the protection of human skin [15]. In addition, several compounds of the 1,2,3-triazole family have shown a broad spectrum of biological properties such as antibacterial [16], anti-HIV activity,[17] anticancer [18], antifungal [19], herbicidal [20], anti-allergic [21] and anti-tuberculosis [22]. 1,2,3-Triazole moiety is present in many available drugs [23] and lately 1,2,3-triazole hybrids are getting attention of many pharmacists partly due to their ease of formation by coupling two desired structural cores. There are several reports on the antibacterial activity of triazoles [24, 25]. Recently Zhang reported a comprehensive review on the recent advances of 1,2,3-triazole hybrids as potential anti-bacterial agents [26].

Some representative examples of 1,2,3-triazole hybrids with significant anti-bacterial potential are shown in Fig. 1 [27].

As part of our ongoing research on synthesis and biological investigation of 1,2,3-triazoles [7c], it was hypothesized that combining the structural features of vanillin and triazoles could lead to effective anti-bacterial candidates. In the present study we report the synthesis of novel derivatives of 1,2,3-triazoles *via* click strategy using copper catalyzed azide-alkyne cycloaddition (CuAAC). Using this strategy mono and homo-dimers of 1,2,3-triazoles were synthesized in search of new anti-bacterial agents. The modifications involved the introduction of vanillin as main structural core to explore its anti-bacterial potential because to the best of our knowledge vanillin has not been used before as anti-bacterial agent and propyloxy group to control lipophilic character. A variety of aromatic substituents are utilized to delineate the pharmacophoric features established for inhibition of bacterial DNA synthesis and cellular growth. The synthesized compounds were investigated for potential antibacterial activity against different gram positive and gram negative bacterial strains. Molecular docking analysis to selected bacterial Thymidylate kinase (TMPK) enzyme were adopted to aid understanding the possible binding modes and interactions between the synthesized compounds and protein target.

2. Results and discussion

2.1. Chemistry

Various alkyl azides **2a-k** were prepared according to previously reported procedure [7c]. For this purpose various bromopropoxy arenes were refluxed with sodium azides to get 3-azidopropoxy arenes **2a-f** as shown in Scheme 1. Some *bis*(azidopropoxy)aryl compounds **2g-k** were also synthesized. The alkyne partner **1a** namely 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde was synthesized starting from easily available vanillin while **1b** *bis*(3-methyl-4-(prop-2-yn-1-yloxy)phenyl)methane from 4,4′-methylene*bis* (2-methylphenol) by propargylation in the presence of potassium carbonate as base (Scheme 1). The synthesized azides **2a-k** were then reacted with alkyne partner **1** in the presence of Cu(I) catalyst which was generated *in situ* by the reduction of $CuSO_4.5H_2O$ with sodium ascorbate to deliver 3-methoxy-4-((1-(3-arylpropyl)-1*H*-1,2,3-triazol-4-yl)methoxy)benzaldehyde **3a-k** in good yield (Scheme 1). All the synthesized triazoles were structurally characterized by IR, NMR, Mass spectrometry and by elemental analysis.

2.2. Spectroscopic analysis

The ^{1}H NMR spectra of all the products showed a characteristic signal as singlet in the region of 9.8 ppm due to the proton of CHO of aldehyde, while a characteristic signal due to C=C*H*N proton of triazole ring appeared at 8.20–8.25 ppm (Figs. S1-S8). IR spectra showed characteristic bands at 1700-1705 cm^{-1} for C=O functional moiety while absorption in the range of 2100–2200 was observed for C=C (Figs. S9-S12).

2.3. Biological activity

The synthesized series of triazoles **3a-k** was subjected to antibacterial activity and were screened to determine their *in vitro* ability to inhibit the growth of selected pathogens by well diffusion method using Mueller Hinton agar. The antibacterial inhibition was tested against eight Gram-positive bacterial strains such as *Bacillus subtilis, Methicillin-resistant Staphylococcus aureus* (MRSA) (NCTC 10442), *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus pyogenes, Enterococcus faecalis, Vancomycin-resistant Enterococcus;* and five Gram-negative bacterial strains such as *Escherichia coli, Pseudomonas aeruginosa, Shigella, Klebsiella pneumonia and Enterobacteriaceae.*

The % inhibition of all the compounds against gram positive bacteria are given in Table 1, while for gram negative bacteria are given in Table 2. The zone of inhibition is calculated in mm using standard agar well diffusion method[28]. The values are compared using a control (Ciprofloxacin) which was prepared in the same way as the plates using DMSO as a solvent and all experiments were run in a triplicate. The minimum inhibitory concentration (MIC) values were calculated by agar dilution method [29].

2.3.1. In vitro antibacterial activity

The synthesized library of triazoles **(3a-k)** showed promising activity against most of the strains of bacteria. From these results a structure activity relationship (SAR) can be deduced for the tested triazoles. The antibacterial screening was explored by varying the differently substituted aromatics at propyl group on position one of 1,2,3-triazoles **(3a-k)**. Different electron donating (-CH3) and electron withdrawing (-Br, -Cl and -NO2) substituents on the aromatic rings of azide partner were tried to unravel the antimicrobial potency. Overall *bis*-1,2,3-triazoles **(3g-k)** were found to be more potent against gram positive bacterial strains (Table 1). Compound **3g** with benzene ring as aromatic linker between both triazoles showed promising activity against *Bacillus subtitles, Methicillin-resistant Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus* and *Vancomycin-resistant*

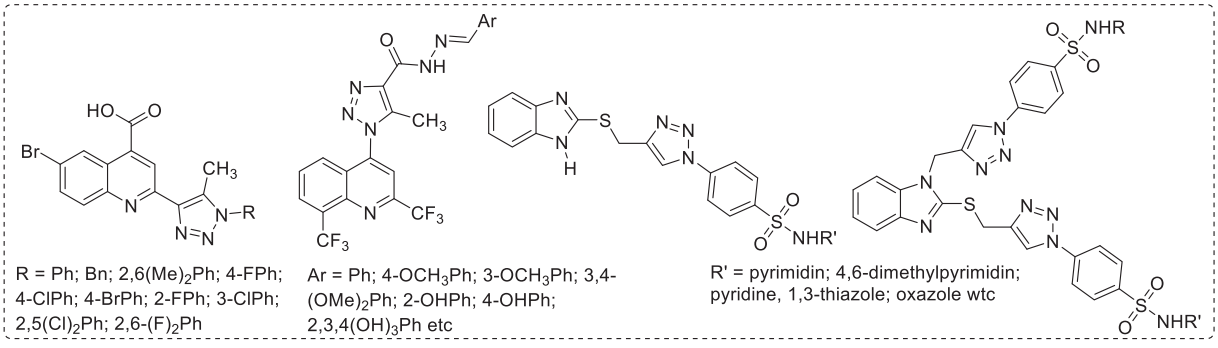
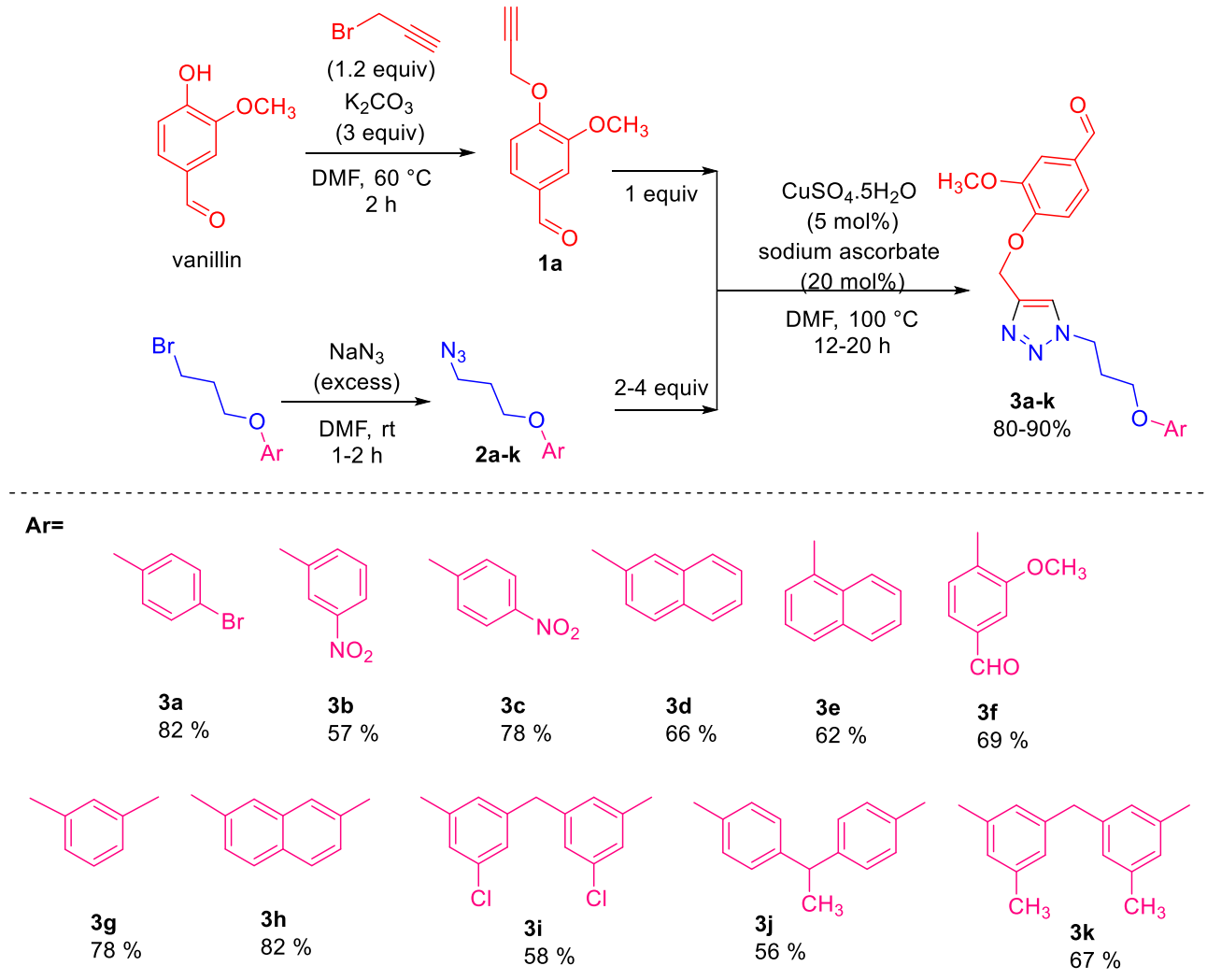


Fig. 1. Some representative examples of 1,2,3-triazole hybrids having anti-bacterial potential.



Scheme 1. Synthesis of 1,2,3-triazoles (3a-f) and *bis*-triazoles (3g-k) using click reaction.

Table 1
In vitro antibacterial screening of synthesized 1,2,3-triazoles (3a-k) against gram positive bacterial strains as Minimum Inhibitory Concentrations (MIC, μg/mL) with SD ± 0.02 and zone of inhibition (mm) with SD ± 2.

Gram positive Bacterial strains[a] MIC±0.02 (Zone of inhibition±2)								
Compound	*B. subtilis*	*S. aureus*		*S. epidermidis*	*S. saprophyticus*	*S. pyogenes*	*E. Faecalis*	*VRE*
		MRSA N315	NCTC 10442					
3a	5 (22)	5 (19)	15 (20)	5 (22)	5 (20)	10 (13)	10 (19)	15 (14)
3b	5 (10)	10 (4)	10 (18)	15 (10)	10 (22)	10 (12)	5 (17)	10 (18)
3c	5 (22)	5 (23)	15 (13)	-[b]	-	5 (15)	10 (22)	-
3d	5 (12)	15 (18)	15 (10)	-	10 (22)	5 (18)	-	10 (15)
3e	10 (10)	15 (21)	15 (14)	15 (10)	5 (20)	15 (12)	10 (22)	5 (14)
3f	10 (5)	15 (19)	-	5 (25)	5 (20)	10 (15)	15 (13)	-
3g	5 (22)	5 (22)	10 (18)	5 (22)	5 (20)	10 (13)	10 (16)	5 (22)
3h	15 (10)	10 (22)	15 (20)	5 (22)	-	15 (14)	10 (20)	15 (15)
3i	15 (10)	10 (22)	10 (21)	10 (18)	10 (20)	15 (17)	5 (22)	5 (21)
3j	10 (20)	10 (22)	-	10 (25)	15 (14)	15 (18)	-	-
3k	5 (10)	10 (22)	10 (18)	15 (10)	15 (16)	15 (20)	5 (18)	-
Control (Ciprofloxacin)	5 (18)	10 (20)	5 (20)	10 (15)	15 (22)	15 (21)	5 (22)	5 (20)

[a] *B. subtilis, Bacillus subtilis* (ATCC6633); *MRSA, Methicillin-Resistant Staphylococcus aureus* (N315); *S. aureus, Staphylococcus aureus* (ATCC25923); *S. epidermidis, Staphylococcus epidermidis* (ATCC12228); *S. saprophyticus, Staphylococcus saprophyticus* (ATCC15305); *S. pyogenes, Streptococcus pyogenes* (ATCC19615); *E. faecalis, Enterococcus faecalis* (MTCC439); *VRE, Vancomycin-resistant Enterococcus.*

[b] (–): Inactive (no inhibition).

Table 2
Antibacterial screening of synthesized 1,2,3-triazoles (3a-k) against gram negative bacterial strains as Minimum inhibitory concentrations (MIC, μg/mL) with SD ± 0.02 and zone of inhibition (mm) with SD ± 2.

Gram negative Bacterial strains[a] MIC±0.02 (Zone of inhibition±2)				
Compounds	*E. coli*	*P. aeruginosa*	*S. dysenteriae*	*K. pneumonia*
3a	10 (13)	10 (15.5)	5 (19.5)	5 (20)
3b	15 (17)	15 (19)	5 (20)	10 (15.5)
3c	10 (15)	10 (18)	5 (20)	5 (20)
3d	5 (18)	10 (22)	5 (22)	10 (20)
3e	15 (11.5)	10 (18.5)	10 (15.5)	5 (16)
3f	15 (15)	15 (18)	10 (18)	5 (20)
3g	5 (24)	5 (20)	10 (12)	15 (10)
3h	10 (14)	10 (22)	-[b]	-
3i	10 (15.5)	15 (20.5)	15 (19)	10 (16.5)
3j	10 (17)	15 (19)	10 (20)	15 (12)
3k	10 (13)	15 (15.5)	10 (19.5)	15 (20)
Control (Colistine)	5 (20)	10 (15)	10 (18)	15 (15)

[a] *E. coli, Escherichia coli* (JM109); *P. aeruginosa, Pseudomonas aeruginosa* (ATTC15692); *S. dysenteriae, Shigella dysenteriae* (ATCC13313); *K. pneumoniae, Klebsiella pneumoniae* (ATCC33495).
[b] (–): Inactive (no inhibition).

Enterococcus. In the first two strains it inhibited the growth even more then the positive control ciprofloxacin. A comparison of inhibition potential of compound **3g** with control against gram-positive bacterial strains has been shown in figure 2. When linker was replaced with bulky naphthyl or diphenylmethyl groups, the decrease in activity against various gram-positive strains was observed (Table 1, compounds 3h-k).

Against gram-negative strains most of the compounds showed promising susceptibility and compound **3g** showed good inhibition activity against growth of *Escherichia coli* and *Pseudomonas aeruginosa* while in case of *Klebsiella pneumonia* and *Shigella dysenteriae* it was moderately potent. Compound **3b** bearing at propoxy phenyl ring also appeared antbacterial against *Pseudomonas aeruginosa & Shigella dysenteriae*. Changing the 3-nitro group as 4-nitro in **3c** showed similar activity not only for these two but also for *Klebsiella pneumonia*. Compounds **3d** to **3f** were moderate to good inhibitors of bacterial growth. Compound **3h** did not show any activity against *Shigella dysenteriae* and *Klebsiella pneumonia* but it was intermediate inhibitor for *E. coli* and *P. aeruginosa*.

A broader pattern was observed for activity of other triazoles and bis-triazoles against gram negative bacterial strains, so a generalized SAR could not be established. Although some compounds such as **3a** was quite potent against *Klebsiella pneumonia*. While compound **3g** was more potent against *P. aeruginosa*. Overall most of the compounds showed good to moderate activity against various strains of bacteria.

2.4. Molecular docking

The mechanism of anti-bacterial drugs generally includes the inhibition of cell wall synthesis, inhibition of protein synthesis, inhibition of nucleic acid (DNA) synthesis and anti-metabolism [30]. In general, the antibiotics inhibit these routes by interacting with specific cell proteins which are responsible for specific activity. Thymidylate kinase (TMPK), a nucleotide 50-monophosphate kinase, contains an evolutionarily conserved essential enzyme which catalyzes the biosynthetic pathway that generates dTTP used for DNA synthesis of bacterial cells [31]. Since ciprofloxacin acts by inhibiting the DNA gyrase which is necessary to separate the bacterial DNA resulting in the cell division inhibition therefore, we took the TMPK as target protein to explore the molecular interactions between synthesized ligands and target protein which are responsible for the antibacterial activity of triazoles.

2.4.1. Binding energy evaluations

In general, to evaluate the prediction of accuracy of binding affinity between ligands and target protein, the binding free energies (ΔG) for the crystal structures and the docking models are calculated. The lower

Table 3
Docking energy values (ΔG in Kcal/mol) of synthesized compounds (**3a-k**).

Compound	Docking energy (Kcal/mol)
3a	-7.4
3b	-8.6
3c	-7.9
3d	-8.6
3e	-8.4
3f	-7.6
3g	-7.3
3h	-9.5
3i	-9.4
3j	-8.7
3k	-9.8

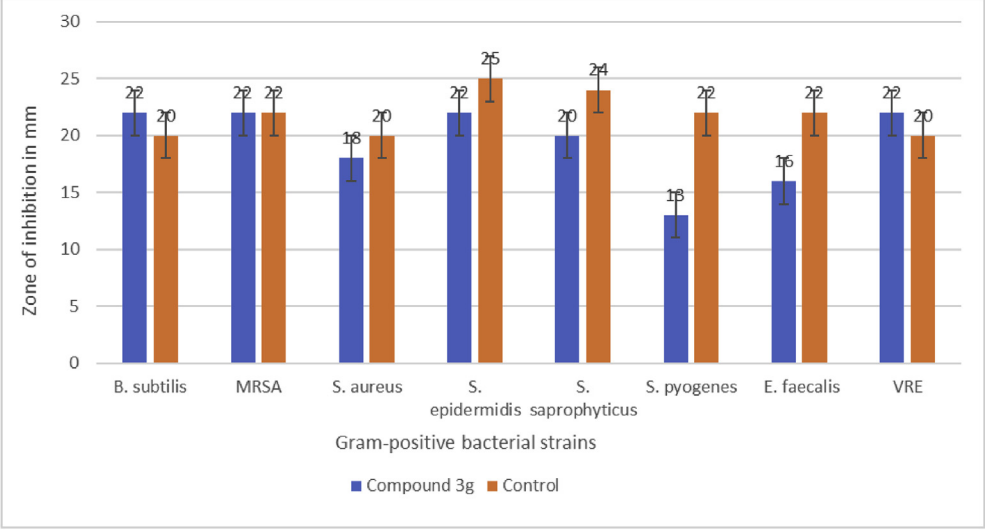


Fig. 2. Antibacterial activity of compound 3g and ciprofloxacin (control) against gram-positive bacterial strains (comparison of zone of inhibition).

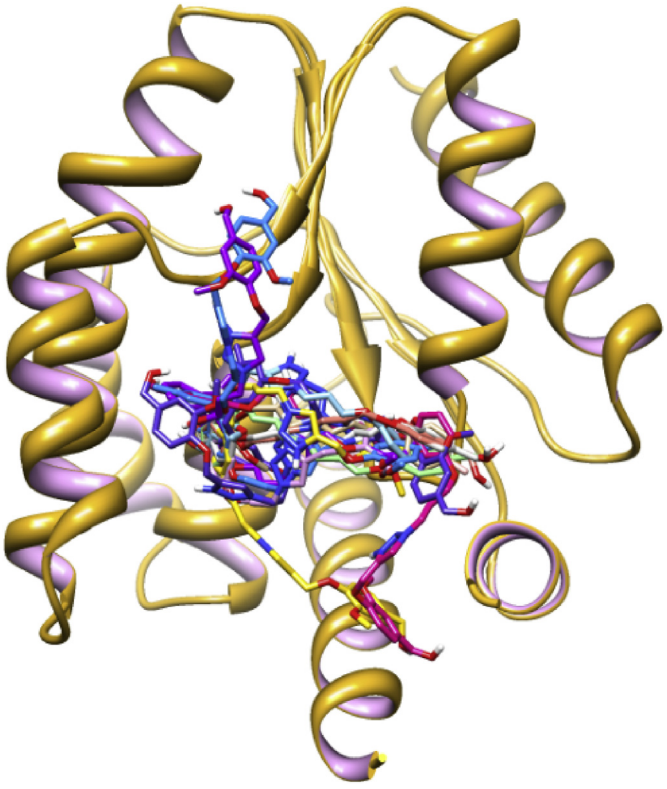


Fig. 3. Docked poses of all the synthesized triazole (3a-k) in the active site of target protein.

values of binding energy indicate the binding strength of the ligands. Therefore, to predict the binding strength of our synthesized ligands, the ligand-protein docked complexes were analyzed based on minimum binding energy values and ligand interaction (hydrogen/hydrophobic) pattern.

Docking results justified that compounds (**3a-k**) possessed good binding energy values (-9.8 to -7.3 kcal/mol), as mentioned in Table 3. It has been observed that since the structural skeleton of compounds **3a-g** are comparable of **3h-k**, therefore, the binding energy values were also in similar pattern. In all docking affinity values, the predicted energy values were not higher than 2.5 kcal/mol indicating that the synthesized ligands were fitted well in the active pocket of the targeted protein. Therefore, the *in vitro* results were focused to check their binding profile. The highest ranked poses were extracted from each binding site and were used as starting points for the molecular dynamics (MD) calculations without further modifications.

2.4.2. Binding pocket and structure activity relationship (SAR) analysis

The docking study explored the actual binding pattern within the active site of the target protein. Most of the ligands confined within the active site of the target protein having different conformational positions as shown in Fig. 3. All the compounds were further analysed through structure-activity relationship (SAR) analysis and their docked complexes in the binding pocket of target protein are provided in the supplementary data (Figs. S13-S23).

Based on the experimental results, the most active compound **3g** was used to interpret the binding behavior of newly synthesized ligands against target protein. The binding pocket residues were observed from PDB and literature data [32, 33]. Binding pocket analysis showed that compound **3g** binds within the active region of target protein (Fig. 4 A, B).

Based on bacterial activity results, **3g** was selected to check their binding profile against target protein. Results justified that **3g** firmly binds with active binding residues of TMPK which functionally participate in the inhibition of DNA synthesis (Fig. 5A). It has been observed that couple of hydrogen bonds were observed at Ser68 and Glu10 against **3g** with different bonding distances. The oxygen atom at benzene ring formed hydrogen bond with Ser68 with bond length 1.93 Å, whereas, amino group at five membered ring structure was involve in hydrogen bond at Glu10 through bond length 2.97 Å (Fig. 5B).

Moreover, **3g**-docking complex in 2D format has been mentioned in supplementary data (Figure S19). Two amino acids Asp146 and Asp90 were binds with 3g through electrostatic interaction. Moreover, Ile41 binds with 3g though Pi-sigma bonding. These interactions along with hydrogen bonding showed good stability in **3g**-docking complex. Prior research also showed that π-π stacking is essential for the favorable electron correlation, whereas cation-π contacts produce further electrostatic contributions [34]. The **3g**-docking complex and 4QGG proteins were superimposed to check the ligands imposition (Figure S24). The data showed that benzene ring with oxygen atom in **3g** showed their superimposition with compound embedded in TMPK structure. The 2D depiction of docking interactions for all other compounds are mentioned in Figs. S13-S23 in the supplementary data. The predicted results showed that our designed ligand could be used as novel therapeutic agent against bacterial infections.

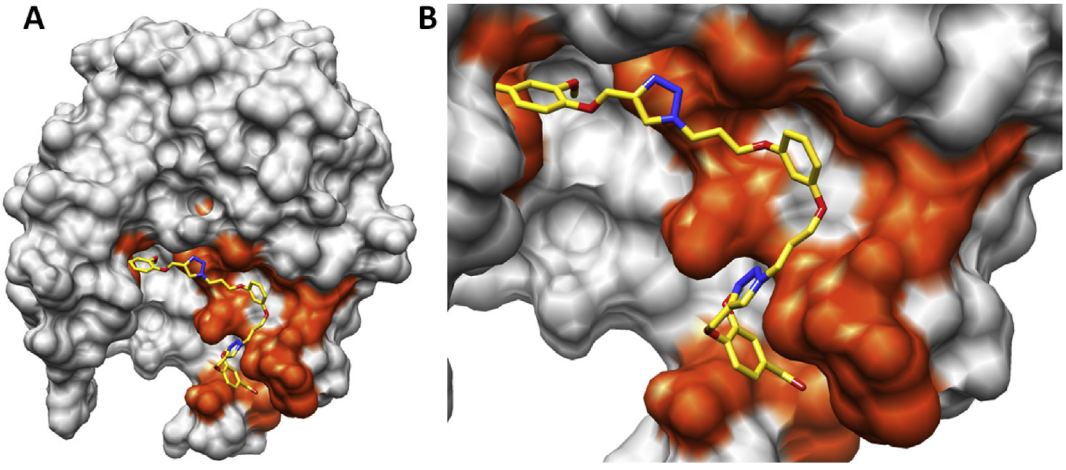


Fig. 4. Binding pose of compound 3g in the active site of target protein (A) and conformational behavior within the active site of the target protein (B).

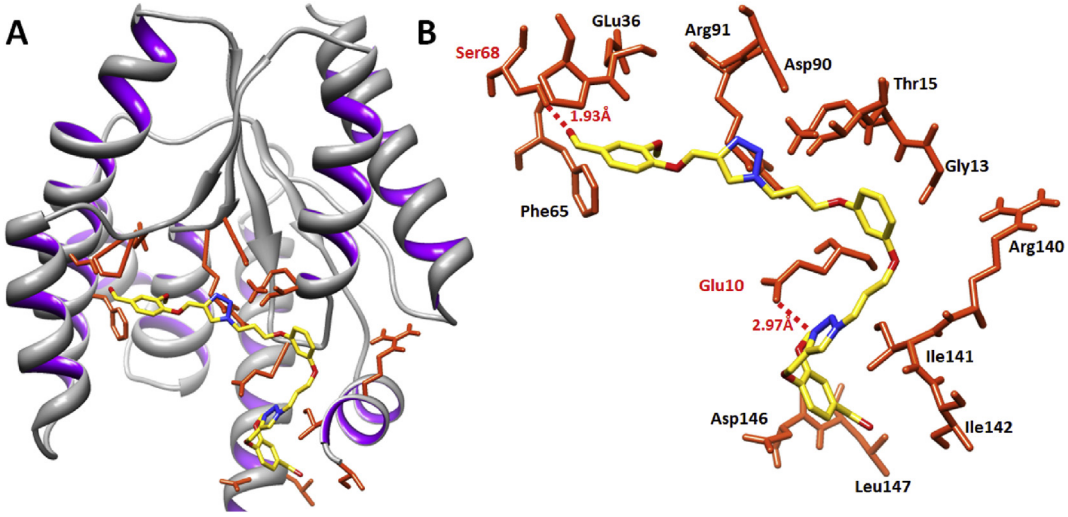


Fig. 5. Docking of 3g with TMPK. (A) The docking complex of 3g-TMPK is mentioned in light gray and purple color in ribbon format. Whereas, active binding residues and ligand is represented in orange and yellow color respectively. (B) The closer view of docking complex to justify the interaction behavior of 3g against target protein. The red color labelled residues are actively participated in hydrogen bonding and their distances were mentioned in angstrom (Å).

3. Conclusion

In summary a series of new vanillin-derived 1,2,3-triazoles and *bis* 1,2,3-triazoles substituted with different aromatic rings were synthesized *via* [3 + 2] cycloaddition of arylpropoxy azides and terminal alkynes. Structural confirmation of compounds was performed by spectroscopic and elemental analysis. Their anti-microbial activity was evaluated against gram-positive strains including *B. subtilis, Methicillin-resistant S. aureus, S. aureus, S. epidermidis, S. saprophyticus, S. pyogenes, E. faecalis, Vancomycin-resistant Enterococcus* as well as gram-negative strains such as *E. coli, P. aeruginosa, Shigella* and *K. pneumonia* using agar well diffusion method. The preliminary anti-bacterial screening of synthesized triazoles manifested moderate to good *in vitro* anti-microbial potency. Among mono 1,2,3-triazoles, compound **3a** and **3b** containing electron withdrawing -Br & -NO2 groups at 3- and 4-position of aryl group were more active against gram positive bacteria (MIC: 5 μg/mL) while **3c-3f** exhibited moderate activity (MIC: 5–15 μg/mL). The compound **3g** among *bis* 1,2,3-trizoles **(3g-k)** was the most active in the series for most of the gram-positive and gram-negative strains (MIC: 5 μg/mL). To explore the mechanism of action of potential compounds **(3a-k)** theoretically, further computational studies were performed. The molecular docking studies buoyed that compound **3g** can work as lead inhibitor of bacterial DNA synthesis due to conformational fitting in the active site of targeted protein.

These results demonstrated that vanillin-derived 1,2,3-triazoles **(3a-3f)** and *bis*-1,2,3-triazoles **(3g-3k)** could be of biological significance, specially *bis*-1,2,3-triazoles have the perspective as new members of anti-microbial agents.

4. Experimental

4.1 General remarks and instrumentation

All the chemicals and solvents of analytical grade were purchased from commercial sources and were used as such without further purification. The reactions were performed under normal atmospheric conditions. Thin layer chromatography (TLC) was performed on precoated silica gel plates (F254 grade - 0.2 mm thickness) and were visualized under UV light at 256 nm & 365 nm. Manual column chromatography was performed on SiO_2 gel 80–100 mesh with positive air pressure. The melting points were recorded on electrothermal 1101D Mel-Temp digital melting point apparatus. IR spectra of were recorded on a ThermoScientific Nicolet 6700 FTIR spectrometer using potassium bromide (KBr) disks. The disks were prepared from powdered samples (2 mg) mixed with dry KBr and spectra were recorded in absorbance mode from 4000 to 400 cm^{-1}. ^{1}HNMR spectra were recorded on 300 MHz Bruker spectrometer in deuterated dimethyl sulphoxide (DMSO-d6) containing TMS as internal standard. Chemical shift values are given in parts per million (ppm). The mass spectra were recorded using MAT CH-5 spectrophotometer at 70 eV EI. The above triazole compounds were synthesized according to literature.[7c]

4.2 Chemistry

4.2.1. Synthesis of 1a

To a stirred solution of Vanillin (0.76 g, 5.0 mmol) in dry DMF (15 mL), anhydrous potassium carbonate (15.0 mmol) was added and heated to 55–60 °C for 30 min under inert atmosphere. The mixture was then cooled to room temperature and propargyl bromide (80% solution in toluene) (1.02 g, 8.62 mmol) was added dropwise through a septum using syringe. The mixture was stirred for 2 h at 60 °C and poured on the ice water with stirring. Stirring was continued for 10 min, the solid separated was filtered and dried under vacuum to afford the desired compound **1a**.

4.2.2. Synthesis of alkyl or arylpropoxy azides (2a-k)

To a stirred solution of arylpropoxy bromide (5.0 mmol), a slight excess of sodium azide (10.0 mmol) was added in the presence of dimethylformamide as solvent (15 mL). The reaction mixture was stirred at room temperature for 1–2 h. Afterwards dimethylformamide was removed by large dilution with water and extraction with chloroform. The organic layers were collected, dried with anhydrous Na_2SO_4 and filtered. The filtrate was evaporated to get arylpropoxy azides **(2a-k)**. These azides were used in further reactions without any purification.

4.2.3General experimental procedure for the synthesis of 1,4-disubstituted 1,2,3-triazoles (3a-f)

In a 100 mL round bottomed flask containing stirred solution of 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde **1a** (1.0 g, 5.3 mmol) in 20 ml DMF was added appropriate 1-(3-azidopropoxy)aryl (**2a-k**) (10.6 mmol, 2 equiv). After formation of clear solution, 5 mol % copper (II) sulphate pentahydrate (0.05 equiv) and 20 mol % sodium ascorbate (0.2 equiv) was added under constant stirring. The reaction was heated at 100 °C for 12 h (monitored by TLC, solvent system; EtOAc:*n*-Hexane). After

completion of reaction the color of reaction mixture was changed from green to brown. DMF was separated by freeze drying and filtered through filter paper. Reaction mixture was dissolved in dichloromethane and solvent was evaporated under reduced pressure. The products were purified by silica gel column chromatography using ethyl acetate:*n*-hexane (3:7) as eluent.

4.2.4. Synthesis and characterization data of 1,2,3-triazoles (3a-f)

4.2.4.1. 4-((1-(3-(4-bromophenoxy)propyl)-1H-1,2,3-triazol-4-yl) methoxy)-3-methoxybenzaldehyde (3a). According to general procedure 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde **1a** (1.0 g, 5.3 mmol) was reacted with 1-(3-azidopropoxy)-4-bromobenzene **2a** (2.7 g, 10.6 mmol) in the presence of copper (II) sulphate pentahydrate (0.07 g, 0.27 mmol) and 20 mol % sodium ascorbate (0.21 g, 1.06 mmol) for 12 h in DMF. After workup and purification **3a** was obtained in 82% Yield; m.p. 101–102 °C; IR (KBr) ν_{max}/cm^{-1}, 1700 (C=O), 1670, 2285 (C=C); ^{1}H NMR (300 MHz, DMSO-d6) δ: 9.8 (s, 1H, Ar-C*H*O), 8.3 (s, 1H, *H*-triazole), 7.5 (m, 2H, Ar–*H*), 7.4 (dd, J = 10.9, 7.3 Hz, 2H, Ar–*H*), 7.2 (d, J = 6.8 Hz, 2H, Ar–*H*), 6.8 (d, J = 7.1 Hz, 1H, Ar–*H*), 5.2 (s, 2H, OCH_2), 4.5 (t, J = 5.7 Hz, 2H, OCH_2), 4.0 (s, 3H, OCH_3), 3.8 (t, J = 6.6 Hz, 2H, N_3CH_2), 2.3–2.2 (m, 2H, ArOCH$_2$CH_2CH$_2$); $^{EI-}$MS m/z (%): 445 (M^+, 100); Anal. Calcd for $C_{20}H_{20}BrN_3O_4$ (445): C, 58.82; H, 8.51; N, 9.41; O, 23.26 % Found: C, 58.80; H, 8.50; N, 9.51; O, 22.26 %.

4.2.4.2. 3-methoxy-4-((1-(3-(3-nitrophenoxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)benzaldehyde (3b). According to general procedure 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde **1a** (1.0 g, 5.3 mmol) was reacted with 1-(3-azidopropoxy)-3-nitrobenzene **2c** (2.3 g, 10.6 mmol) in the presence of copper (II) sulphate pentahydrate (0.07 g, 0.27 mmol) and 20 mol % sodium ascorbate (0.21 g, 1.06 mmol) for 12 h in DMF. After workup and purification **3b** was obtained in 57% Yield; m.p. 129–130 °C; IR (KBr) ν_{max}/cm^{-1}, 1130 (C–O), 1677, 2189 (C=C); ^{1}H NMR (300 MHz, DMSO-d6) δ: 8.23 (s, 1H, *H*-triazole), 7.8 (d, J = 7.3 Hz, 1H, Ar–*H*), 7.6 (s, 1H Ar–*H*), 7.5 (t, J= 8.2 Hz, 1H, Ar–*H*), 7.3 (dd, J = 10.9, 7.3 Hz, 1H, Ar–*H*), 7.0–6.8 (m, 4H, Ar–*H*), 5.1 (s, 2H, OCH_2), 4.5 (t, J = 5.8 Hz, 2H, OCH_2), 4.2 (t, J = 6.6 Hz, 2H, N_3CH_2), 3.7 (s, 3H, OCH_3), 2.3–2.2 (m, 2H, ArOCH$_2$CH$_2$CH_2N); EI-MS m/z (%):526 (M+, 100): Anal. Calcd for $C_{30}H_{30}N_4O_5$ (526); C, 58.17; H, 7.10; N, 10.60; O, 23.28 % Found: C, 58.20; H, 7.11; N, 10.90 O, 24.00 %.

4.2.4.3. 3-methoxy-4-((1-(3-(4-nitrophenoxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)benzaldehyde (3c). According to general procedure 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde **1a** (1.0 g, 5.3 mmol) 1 was reacted with 1-(3-azidopropoxy)-4-nitrobenzene **2c** (2.3 g, 10.6 mmol) in the presence of copper (II) sulphate pentahydrate (0.07 g, 0.27 mmol) and 20 mol % sodium ascorbate (0.21 g, 1.06 mmol) for 12 h in DMF. After workup and purification **3c** was obtained in 78% Yield; m.p. 105–106 °C; IR (KBr) ν_{max}/cm^{-1}, 1700 (C=O), 1670, 2210 (C=C, CN); ^{1}H NMR (300 MHz, DMSO-d6) δ: 9.6 (s, 1H, Ar-C*H*O), 8.1 (s, 1H, *H*-triazole), 7.5 (d, J = 7.1 Hz, 2H, Ar–*H*), 7.4 (d, J = 7.5 Hz, 2H, Ar–*H*), 7.3 (d, J = 6.9 Hz, 2H, Ar–*H*), 6.9 (d, J = 7.8 Hz, 1H, Ar–*H*), 5.1 (s, 2H, OCH_2), 4.4 (t, J = 5.4 Hz, 2H, OCH_2), 4.0 (s, 3H, OCH_3), 3.6 (t, J = 6.6 Hz, 2H, N_3CH_2), 2.3–2.2 (m, 2H, ArOCH$_2$CH_2CH$_2$); EI-MS m/z (%): 412.14 (M^+, 100), 413.14 (23.6); Anal. Calcd for $C_{20}H_{20}N_4O_6$ (412.14): C, 58.25; H, 4.89; N, 13.59; O, 23.28 % Found: C, 56.26; H, 5.90; N, 14.60; O, 22.26 %.

4.2.4.4. 3-methoxy-4-((1-(3-(naphthalen-2-yloxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy) benzaldehyde (3d). According to general procedure 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde **1a** (1.0 g, 5.3 mmol) 1 was reacted with 2-(3-azidopropoxy)naphthalene **2d** (2.4 g, 10.6 mmol) in the presence of copper (II) sulphate pentahydrate (0.07 g, 0.27 mmol) and 20 mol % sodium ascorbate (0.21 g, 1.06 mmol) for 12 h in DMF. After workup and purification **3d** was obtained in 66% Yield; m.p. 111–112 °C; IR (KBr) ν_{max}/cm^{-1}, 1700 (C=O), 1670, 2285 (C=C, CN); ^{1}H NMR (300 MHz, DMSO-d6) δ: 9.6 (s, 1H, Ar-C*H*O), 8.3 (s, 1H, *H*-triazole), 7.9 (d, J = 7.1 Hz, 2H, Ar–*H*), 7.6 (d, J = 7.4 Hz, 1H, Ar–*H*), 7.5 (d, J = 7.5 Hz, 1H, Ar–*H*), 7.4 (dd, J = 9.9, 7.3 Hz, 1H, Ar–*H*), 7.3 (d, J = 7.8 Hz, 2H, Ar–*H*), 7.1 (d, J = 7.2 Hz, 2H, Ar–*H*), 6.9 (d, J = 7.3 Hz, 1H, Ar–*H*), 5.2 (s, 2H, OCH_2), 4.2 (t, J = 5.7 Hz, 2H, OCH_2), 4.1 (s, 3H, OCH_3), 3.8 (t, J = 6.7 Hz, 2H, N_3CH_2), 2.3–2.1 (m, 2H, ArOCH$_2$CH_2CH$_2$); EI-MS m/z (%): 417.2 (M^+, 100); Anal. Calcd. for $C_{24}H_{23}N_3O_4$ (417): C, 61.05; H, 5.55; N, 10.00; O, 23.66. % Found: C, 61.50; H, 5.51; N, 10.30; O, 23.11 %.

4.2.4.5. 3-methoxy-4-((1-(3-(naphthalen-1-yloxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy) benzaldehyde (3e). According to general procedure 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde **1a** (1.0 g, 5.3 mmol) 1 was reacted with 1-(3-azidopropoxy)naphthalene **2e** (2.4 g, 10.6 mmol) in the presence of copper (II) sulphate pentahydrate (0.07 g, 0.27 mmol) and 20 mol % sodium ascorbate (0.21 g, 1.06 mmol) for 12 h in DMF. After workup and purification **3e** was obtained in 62% Yield; m.p. 109–110 °C; IR (KBr) ν_{max}/cm^{-1}, 1700 (C=O), 1670, 2315 (C=C, CN); ^{1}H NMR (DMSO-d6) δ: 9.60 (s, 1H, Ar-C*H*O), 8.3 (s, 1H, *H*-triazole), 7.7 (s, 2H, Ar–*H*), 7.6 (s, 1H, Ar–*H*), 7.5 (d, J = 7.2 Hz, 2H, Ar–*H*), 7.4 (d, J = 7.3 Hz, 1H, Ar–*H*), 7.3 (dd, J = 7.2, 5.1 Hz, 1H, Ar–H), 7.1 (d, J = 7.3 Hz, 2H, Ar–*H*), 6.9 (d, J = 7.5 Hz, 1H, Ar–*H*), 5.1 (s, 2H, OCH_2), 4.4 (t, J = 5.8 Hz, 2H, OCH_2), 4.0 (s, 3H, OCH_3), 3.8 (t, J = 6.6 Hz, 2H, N_3CH_2), 2.3–2.2 (m, 2H, ArOCH$_2$CH_2CH$_2$); EI-MS m/z (%): 417 (M^+, 100); Anal. Calcd. for $C_{24}H_{23}N_3O_4$ (412.14): C, 61.05; H, 5.55; N, 10.07; O, 22.89 % Found: C, 61.22; H, 5.41; N, 10.29; O, 23.11 %.

4.2.4.6. 4-(3-(4-((4-formyl-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)propoxy)-3-methoxy benzaldehyde (3f). According to general procedure 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde **1a** (1.0 g, 5.3 mmol) 1 was reacted with 4-(3-azidopropoxy)-3-methoxybenzaldehyde **2f** (2.5 g, 10.6 mmol) in the presence of copper (II) sulphate pentahydrate (0.07 g, 0.27 mmol) and 20 mol % sodium ascorbate (0.21 g, 1.06 mmol) for 12 h in DMF. After workup and purification **3f** was obtained in 69% Yield; m.p. 114–115 °C; IR (KBr) ν_{max}/cm^{-1}, 1700 (C=O), 1670, 2180 (C=C); ^{1}H NMR (300 MHz, DMSO-d6) δ: 9.5 (s, 1H, Ar-C*H*O), 9.0 (s, 1H, Ar-C*H*O), 8.3 (s, 1H, *H*-triazole), 7.7 (s, 1H, Ar–*H*), 7.5 (d, J = 7.1 Hz, 2H, Ar–*H*), 7.1 (d, J = 7.2 Hz, 2H, Ar–*H*), 6.9 (d, J = 6.8 Hz, 1H, Ar–*H*), 5.2 (s, 2H, OCH_2), 4.0 (t, J = 5.8 Hz, 2H, OCH_2), 3.7 (t, J = 6.7 Hz, 2H, N_3CH_2), 3.6 (s, 3H, ArOCH_3), 3.5 (s, 3H, ArOCH_3), 2.3–2.2 (m, 2H, ArOCH$_2$CH_2CH$_2$); EI-MS m/z (%): 425 (M^+, 100); Anal. Calcd. for $C_{22}H_{23}N_3O_6$ (425.43): C, 62.11; H, 5.45; N, 9.88; 22.99. % Found: C, 62.10; H, 5.46; N, 9.86; 23.11 %.

4.2.5 General experimental procedure for the synthesis of bis-triazoles (3g-k)

In a 100 mL round bottomed flask containing stirred solution of 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde **1a** (1.0 g, 5.3 mmol) in 20 ml DMF was added appropriate *bis*1-(3-azidopropoxy)aryl **(2g-k)** (16.8 mmol, 3 equiv). After formation of clear solution, 5 mol % copper (II) sulphate pentahydrate (0.05 equiv) and 20 mol % sodium ascorbate (0.2 equiv) was added under constant stirring. The reaction was heated at 100 °C for 12 h (monitored by TLC, solvent system; EtOAc:*n*-Hexane). After completion of reaction the color of reaction mixture was changed from green to brown. DMF was separated by freeze drying and filtered through filter paper. Reaction mixture was dissolved in dichloromethane and solvent was evaporated under reduced pressure. The products were purified by silica gel column chromatography using ethyl acetate:*n*-hexane (3:7) as eluent.

4.2.6. Synthesis and characterization data of bis-triazoles **(3g-k)**

4.2.6.1. 4,4'-(((((1,3-phenylenebis(oxy))bis(propane-3,1-diyl))bis(1H-1,2,3-triazole-1,4-diyl))bis (methylene))bis(oxy))bis(3-methoxybenzaldehyde) (3g). According to general procedure 3-methoxy-4-(prop-2-yn-1-

yloxy)benzaldehyde **1a** (1.0 g, 5.3 mmol) 1 was reacted with 1,3-*bis*(3-azidopropoxy)benzene **2g** (4.6 g, 16.8 mmol) in the presence of copper (II) sulphate pentahydrate (0.07 g, 0.27 mmol) and 20 mol % sodium ascorbate (0.21 g, 1.06 mmol) for 12 h in DMF. After workup and purification **3g** was obtained in 78% Yield; m.p. 122–123 °C; IR (KBr) ν_{max}/cm^{-1}, 1700 (C=O), 1670, 1700 (C=C, C=N); ^{1}H NMR (300 MHz, DMSO-d6) δ: 9.4 (s, 1H, Ar-C*H*O), 8.3 (s, 1H, *H*-triazole), 7.4 (d, J = 7.1 Hz, 2H, Ar–*H*), 7.3 (d, J = 7.4 Hz, 2H, Ar–*H*), 7.2–7.0 (m, 2H, Ar–*H*), 6.5 (d, J = 7.3 Hz, 1H, Ar–*H*), 5.2 (s, 2H, ArOC*H*$_2$), 4.1 (t, J = 5.7 Hz, 2H, OC*H*$_2$), 3.9 (t, J = 56.6 Hz, 2H, N$_3$C*H*$_2$), 3.6 (s, 3H, ArOC*H*$_3$), 2.3–2.2 (m, 2H, ArOCH$_2$C*H*$_2$CH$_2$); ESI-MS m/z (%): 656 (M$^+$, 100): Anal. Calcd for $C_{34}H_{36}N_6O_8$ (656.26); C, 62.19; H, 5.53; N, 12.80; O, 19.49 %. Found: C, 62.20; H, 5.60; N, 12.81; O, 19.40 %.

4.2.6.2. 4,4'-(((((naphthalene-2,7-diylbis(oxy))bis(propane-3,1-diyl)) bis(1H-1,2,3-triazole-1,4-diyl))bis(methylene))bis(oxy))bis(3-methoxybenzaldehyde) (3h). According to general procedure 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde **1a** (1.0 g, 5.3 mmol) 1 was reacted with 2,7-*bis*(3-azidopropoxy)naphthalene **2h** (5.5 g, 16.8 mmol) in the presence of copper (II) sulphate pentahydrate (0.07 g, 0.27 mmol) and 20 mol % sodium ascorbate (0.21 g, 1.06 mmol) for 12 h in DMF. After workup and purification **3h** was obtained in 82% Yield; m.p. 148–149 °C; IR (KBr) ν_{max}/cm^{-1}, 1721 (C=O), 1677, 2365 (C=C); ^{1}H NMR (300 MHz, DMSO-d6) δ: 9.7 (s, 1H, Ar-C*H*O), 8.4 (s, 1H, *H*-triazole), 7.7–7.2 (m, 2H, Ar–*H*), 7.3 (d, J = 7.4 Hz, 2H, Ar–*H*), 6.9 (d, J = 7.1 Hz, 2H, Ar–*H*), 5.2 (s, 2H, OC*H*$_2$), 4.1 (t, J = 5.7 Hz, 2H, OC*H*$_2$), 3.9 (t, J = 6.6 Hz, 2H, N$_3$C*H*$_2$), 3.6 (s, 3H, ArOC*H*$_3$), 2.3–2.2 (m, 2H, ArOCH$_2$C*H*$_2$CH$_2$). ESI-MS (m/z) (%): 706 ($C_{38}H_{38}N_6O_5$), 433 ($C_{24}H_{23}N_3O_5$). Anal. Calc. For $C_{38}H_{38}N_6O_8$ (706.28): C, 64.57; H, 5.43; N, 11.88; O, 18.12 %. Found: C, 64.60; H, 5.44; N, 11.90; O, 18.22 %.

4.2.6.3. 4,4'-(((1,1'-(((methylenebis(4-chloro-2,1-phenylene))bis(oxy)) bis(propane-3,1-diyl))bis(1H-1,2,3-triazole-4,1-diyl))bis(methylene)) bis(oxy)) bis(3-methoxybenzaldehyde) (3i). According to general procedure 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde **1a** (1.0 g, 5.3 mmol) 1 was reacted with *bis*(2-(3-azidopropoxy)-5-chlorophenyl) methane **2i** (7.3 g, 16.8 mmol) in the presence of copper (II) sulphate pentahydrate (0.07 g, 0.27 mmol) and 20 mol % sodium ascorbate (0.21 g, 1.06 mmol) for 12 h in DMF. After workup and purification **3i** was obtained in 58% Yield; m.p. 124–125 °C; IR (KBr) ν_{max}/cm^{-1}, 1700 (C=O), 1670, 2275 (C=C); ^{1}H NMR (DMSO-d6) δ: 9.6 (s, 2H, Ar-C*H*O), 8.2 (s, 2H, *H*-triazole), 7.4 (d, J = 7.2 Hz, 2H, Ar–*H*), 7.3 (d, J = 7.3 Hz, 2H, Ar–*H*), 7.2 (s, 1H, Ar–*H*), 6.8 (d, J = 6.9 Hz, 1H, Ar–*H*), 5.1 (s, 2H, OC*H*$_2$), 3.9 (s, 2H, Ar-C*H*$_2$-Ar), 3.8 (t, J = 6.6 Hz, 2H, N$_3$C*H*$_2$), 3.7 (s, 3H, OC*H*$_3$), 2.2–2.1 (m, 2H, ArOCH$_2$C*H*$_2$CH$_2$); ESI-MS m/z (%): 814.2 (M$^+$,100): Anal. Calcd for $C_{41}H_{40}Cl_2N_6O_8$ (815.70); C, 60.37; H, 4.94; Cl, 8.69; N, 10.30; O, 15.69 %. Found: C, 60.40; H, 5.00; Cl, 8.70; N, 11.39; O, 15.70 %.

4.2.6.4. 4,4'-((((((ethane-1,1-diylbis(4,1-phenylene))bis(oxy))bis(propane-3,1-diyl))bis(1H-1,2,3-triazole-1,4-diyl))bis(methylene))bis(oxy)) bis(3-methoxybenzaldehyde) (3j). According to general procedure 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde **1a** (1.0 g, 5.3 mmol) 1 was reacted with 2,2'-(ethane-1,1-diyl)*bis* ((3-azidopropoxy)benzene) **2j** (6.4 g, 16.8 mmol) in the presence of copper (II) sulphate pentahydrate (0.07 g, 0.27 mmol) and 20 mol % sodium ascorbate (0.21 g, 1.06 mmol) for 12 h in DMF. After workup and purification **3j** was obtained in 56% Yield; m.p. 107–108 °C; IR (KBr) ν_{max}/cm^{-1}, 1700 (C=O), 1670, 2198 (C=C); ^{1}H NMR (300 MHz, DMSO-d6) δ: 9.60 (s, 2H, Ar-C*H*O), 7.59 (s, 1H, *H*-triazole), 7.4 (d, J = 7.1 Hz, 2H, Ar–*H*), 7.3 (d, J = 7.5 Hz, 2H, Ar–*H*) 7.2 (m, 4H, Ar–*H*), 6.9 (d, J = 7.0 Hz, 4H, Ar–*H*), 5.2 (s, 2H, OC*H*$_2$), 4.0 (t, J = 5.7 Hz, 2H, OC*H*$_2$), 4.0 (s, 1H, Ar-C*H*-Ar), 3.8 (s, 3H, OC*H*$_3$), 2.3–2.1 (m, 2H, ArOCH$_2$C*H*$_2$CH$_2$); ESI-MS m/z (%):760.3 (M$^+$, 100): Anal. Calcd for $C_{42}H_{44}N_6O_8$ (760.3); C, 66.30; H, 5.83; N, 11.05; O, 16.82 %. Found: C, 66.22; H, 5.80; N, 11.00; O, 16.80 %.

4.2.6.5. 4,4'-((((((methylenebis(5-methyl-3,1-phenylene))bis(oxy))bis(propane-3,1-diyl))bis(1H-1,2,3-triazole-1,4-diyl))bis(methylene))bis(oxy)) bis(3-methoxybenzaldehyde) (3k). According to general procedure 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde **1a** (1.0 g, 5.3 mmol) 1 was reacted with 1-(3-azidopropoxy)-2-(2-(3-azidopropoxy)-4-methylbenzyl)-4-methylbenzene **2k** (6.6 g, 16.8 mmol) in the presence of copper (II) sulphate pentahydrate (0.07 g, 0.27 mmol) and 20 mol % sodium ascorbate (0.21 g, 1.06 mmol) for 12 h in DMF. After workup and purification **3k** was obtained in 67% Yield; m.p. 129–130 °C; IR (KBr) ν_{max}/cm^{-1}, 1700 (C=O), 1670, 2195 (C=C); ^{1}H NMR (DMSO-d6) δ: 9.6 (s, 2H, Ar-C*H*O), 8.2 (s, 2H, *H*-triazole), 7.4 (d, J = 7.3 Hz, 2H, Ar–*H*), 7.3 (s, 2H, Ar–*H*), 6.9 (s, 2H, Ar–*H*), 6.8 (d, J = 7.5 Hz, 2H, Ar–*H*), 6.7 (d, 2H, Ar–*H*), 5.2 (s, 4H, Ar-C*H*$_2$), 3.8 (s, 6H, ArO-C*H*$_3$), 3.9 (s, 2H, Ar-C*H*$_2$-Ar), 2.3 (s, 6H, Ar-C*H*$_3$), 2.1 (m, 2H, ArOCH$_2$C*H*$_2$CH$_2$); ESI-MS m/z (%): 774.3 (M$^+$, 100); Anal. Calcd for $C_{43}H_{46}N_6O_8$ (774.3): C, 66.65; H, 5.98; N, 10.85; O, 16.52 %. Found: C, 66.60; H, 5.90; N, 10.90; O, 16.52 %.

4.3. Antimicrobial activity

The synthesized compounds (**3a-3k**) were tested against series of eight gram-positive bacterial strains including *B. subtilis* (ATCC6633)*; MRSA* (N315); *S. aureus* (ATCC25923)*; S. epidermidis (*ATCC12228*); S. saprophyticus (*ATCC15305*); S. pyogenes (*ATCC19615*); E. faecalis (*MTCC439*); VRE* and four gram-negative strains such as *E. coli* (JM109)*; P. aeruginosa* (ATTC15692)*; S. dysenteriae* (ATCC13313); *K. pneumoniae* (ATCC33495) by using standard protocol of agar well diffusion method on Mueller-Hinton agar (MHA). The nutrient agar of Merk was used and agar plates containing 24 h old culture of bacterial strains to get 10^4–10^6 colony forming units (CFU) in media. In general agar plates were inoculated by spreading these species on whole culture surface. A hole with a diameter of 6–8 mm was punched aseptically with a sterile cork borer at 25 mm distance from each other then 10 μL volume of the tested compounds in DMSO was introduced into the wells. The plates were then incubated at 37 °C for 24 h. A DMSO control (5% v/v) was also screened to ensure that the solvent did not inhibit bacterial growth. The inhibition zones were measured to the nearest whole millimeter using a meter ruler and all experiments were run in triplicate. The minimum inhibitory concentration (MIC) values were also determined by agar dilution method where MIC represented the lowest concentration that inhibited the bacterial growth. Stock solution was diluted to 2, 5, 10 and 15 μg/mL concentrations. No inhibition was observed with >5 μg/mL concentration.

4.4. Computational methodology

4.4.1. Selection of target protein from PDB

The crystal structures of Thymidylate kinase (TMPK), having PDB ID as 4QGG was accessed from Protein Data Bank (PDB) (www.rcsb.org). The TMPK, an essential enzyme in bacterial DNA biosynthesis, is an attractive therapeutic target for the development of novel antibacterial agents. The selected target protein was minimized with Amber force field by employing conjugate gradient algorithm in UCSF Chimera 1.10.1 [35].

4.4.2. Designing of ligands and molecular docking

The synthesized ligands were sketched in drawing ACD/ChemSketch tool and minimized by UCSF Chimera 1.10.1. Molecular docking experiment was employed on all the synthesized ligands against TMPK by using PyRx docking tool [36]. The grid box parameters values for TMPK were adjusted as center_x = 31.4238, center_y = 7.4971, center_z = -22.8764 while size_x = 41.8523, size_y = 49.3614, and size_z = 44.2556, respectively. The default exhaustiveness value = 8 was adjusted in both docking to maximize the binding conformational analysis. The docking poses for each docking were adjusted to obtain the best docking results. The docked complexes were evaluated on lowest binding energy (Kcal/mol) values and structure activity relationship analyses. The

graphical depictions of all the docking complexes were carried out using Discovery Studio (2.1.0) [37].

Declarations

Author contribution statement

Mumtaz Hussain, Tahir Qadri: Performed the experiments.

Zahid Hussain, Pervaiz Ali Channar: Analyzed and interpreted the data.

Aamer Saeed: Conceived and designed the experiments.

Syeda Aaliya Shehzadi: Conceived and designed the experiments; Wrote the paper.

Mubashir Hassan: Performed the experiments; Analyzed and interpreted the data.

Fayaz Ali Larik, Tarique Mahmood, Arif Malik: Contributed reagents, materials, analysis tools or data.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2019.e02812.

References

[1] N.R. Naylor, R. Atun, N. Zhu, K. Kulasabanathan, S. Silva, A. Chatterjee, G.M. Knight, J.V. Robotham, Estimating the burden of antimicrobial resistance: a systematic literature review, Antimicrob. Resist. Infect. Cont. 7 (2018) 58.

[2] J. O'Neill, Tackling a crisis for the health and wealth of nations, Rev. Antimicrob. Resist. 1 (2014) 20.

[3] (a) H.C. Kolb, M.G. Finn, K.B. Sharpless, Diverse chemical function from a few good reactions, Angew. Chem. Int. Ed. 40 (2001) 2004;
(b) Q. Wang, S. Chittaboina, H.N. Barnhill, Highlights in organic chemistry advances in 1,3-dipolar cycloaddition reaction of azides and alkynes-a prototype of "click" chemistry, Lett. Org. Chem. 2 (2005) 293;
(c) V.D. Bock, R. Perciaccante, T.P. Jansen, H. Hiemstra, J.H. Maarseveen, Click chemistry as a route to cyclic tetrapeptide analogues: synthesis of cyclo-[Pro-Val-ψ (triazole)-Pro-Tyr], Org. Lett. 8 (2006) 919;
(d) M.I. Garcia-Moreno, D. Rodriguez-Lucena, C.O. Mellet, J.M.G. Fernandez, Pseudoamide-type pyrrolidine and pyrrolizidine glycomimetics and their inhibitory activities against glycosidases, J. Org. Chem. 69 (2004) 3578.

[4] R. Huisgen, Kinetics and mechanism of 1,3-dipolar cycloadditions, Angew. Chem. Int. Ed. 2 (1963) 633. http://www.chem.ccu.edu.tw/.

[5] (a) L. Liang, D. Astruc, The copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) "click" reaction and its applications. An overview, Coord. Chem. Rev. 23 (2011) 2933;
(b) J.E. Moses, A.D. Moorhouse, The growing applications of click chemistry, Chem. Soc. Rev. 36 (2007) 1249.

[6] (a) H.C. Kolb, B.K. Sharpless, The growing impact of click chemistry on drug discovery, Drug Discov. Today 8 (2003) 1128;
(b) F. Pagliai, T. Pirali, E.D. Grosso, Rapid synthesis of triazole-modified resveratrol analogues via click chemistry, J. Med. Chem. 49 (2) (2006) 467–470;
(c) A. Dondoni, The keystone in glycosylated molecular architectures constructed by a click reaction,, Chem. Asian J. 2 (2007) 700.

[7] (a) D. Dheer, V. Singh, R. Shankar, Medicinal attributes of 1,2,3-triazoles: current developments, Bioorg. Chem. 71 (2017) 30;
(b) P. Rani, 1,2,3-Triazole and its applications in various fields, Int. Refereed J. Rev. Res. (2014) 2. International Manuscript ID: 23482001V2I6112014-04;
(c) F. Jabeen, S.A. Shehzadi, M.Q. Fatmi, S. Shaheen, L. Iqbal, N. Afza, S.S. Panda, F.L. Ansari, Synthesis, in vitro and computational studies of 1, 4-disubstituted 1,2,3-triazoles as potential α-glucosidase inhibitors, Bioorg. Med. Chem. Lett. 26 (2016) 1029.

[8] I.K. Body, G.G. Briggs, R.P. Harrison, T.H. Jones, H.J. Mahony, I.D. Marlow, B.G. Roberts, The synthesis and insecticidal activity of a series of 2-aryl-1, 2, 3-triazoles, J. Pestic. Sci. 48 (1996) 189.

[9] (a) K.H. Buechel, H. Gold, P.E. Frohberger, H. Kaspers, Chem. Abstr. 96 (1981) 52509;
(b) D. Fournier, R. Hoogenboom, U.S. Schubert, Clicking polymers: a straightforward approach to novel macromolecular architectures, Chem. Soc. Rev. 36 (2007) 1369;
(c) J.F. Lutz, 1, 3-Dipolar cycloadditions of azides and alkynes: a universal ligation tool in polymer and materials science, Angew. Chem. Int. Ed. 46 (2007) 1018.

[10] (a) T. Jin, S. Kamijo, Y. Yamamoto, Copper-catalyzed synthesis of *N*-unsubstituted 1,2,3-triazoles from nonactivated terminal alkynes, Eur. J. Org. Chem. 18 (2004) 3789;
(b) A. Poulain, D. Canseco-Gonzalez, R. Hynes-Roche, Synthesis and tunability of abnormal 1, 2, 3-triazolylidene palladium and rhodium complexes, Organometallics 30 (2011) 1021.

[11] (a) D. Liu, Y. Zheng, W. Steffen, M. Wagner, H.J. Buttand, T. Ikeda, Glycidyl 4-functionalized-1, 2, 3-triazole polymers, Macromol. Chem. Phys. 214 (2013) 56–61;
(b) A. Coelho, P. Diz, O.E. Caamano, E. Sotelo, Polymer-supported 1,5,7-triazabicyclo [4.4.0] dec-5-ene as polyvalent ligands in the copper-catalyzed Huisgen 1,3-dipolar cycloaddition, Adv. Synth. Catal. 352 (2010) 1179.

[12] D. Guenther, H.J. Nestler, G. Roesch, E. Schinzel, Chem. Abstr. 93 (1980) 73786.

[13] (a) A.M.S. Abdennabi, A.I. Abdulhadi, S.T. Abu-Orabi, H. Saricimen, The inhibition action of 1(benzyl) 1-H-4,5-dibenzoyl-1, 2,3-triazole on mild steel in hydrochloric acid media, Corrosion Sci. 38 (1996) 1791;
(b) K.K. Nippon, Chem. Abstr. 96 (1981) 56298.

[14] J. Rody, M. Slongo, Chem. Abstr. 95 (1981) 187267.

[15] A.F. Strobel, M.L. Whitehouse, Benzotriazole auxiliary in the synthesis of heterocyclic compounds and amino acid derivatives, Chem. Abstr. 76 (1971) 99674. UFE0011594.

[16] X.L. Wang, K. Wan, C.H. Zhou, Synthesis of novel sulfanilamide-derived 1,2,3-triazoles and their evaluation for antibacterial and antifungal activities, Eur. J. Med. Chem. 45 (2010) 4631.

[17] A. San-Féalix, R. Alvarez, S. Veláazquez, E. De Clercq, J. Balzarini, M.J. Camarasa, Synthesis and anti-HIV-1 activity of 4- and 5-substituted 1,2,3-triazole-TSAO derivatives, Nucl. Nucleot. 14 (1995) 595.

[18] (a) B.F. Abdel-Wahab, H.A. Mohamed, G.E.A. Awad, Synthesis and biological activity of some new 1,2,3-triazole hydrazone derivatives, Eur. Chem. Bull. 3 (2014) 1069;
(b) B.F. Abdel-Wahab, H.A. Mohamed, G.E.A. Awad, J. Mod, Med. Chem. 2 (2014) 70.

[19] I.F. da Silva, P.R.C. Martins, E. Da Silva, S.B. Ferreira, V.F. Ferreira, R.C. Da Costa, M.C. De Vasconcellos, E.S. Lima, F.C. Da Silva, Synthesis of 1H-1,2,3-triazoles and study of their antifungal and cytotoxicity activities, Med Chem. 9 (2013) 1085.

[20] (a) F. Reisser, Chem. Abstr. 96 (1981) 69006. British Patent 8101239;
(b) H.R. Krueger, U. Schroeer, D. Baumert, H. Joppien, Chem. Abstr. 96 (1981) 52509. German Patent 2936951.

[21] H.A. Mohamed, E. Abdel-Latif, B.F. Abdel-Wahab, G.E.A. Awad, Novel antimicrobial agents: fluorinated 2-(3-(benzofuran-2-yl) pyrazol-1-yl)thiazoles, Int. J. Med. Chem. 2013 (2013) 986536.

[22] (a) A. Aziz, D. Ali, A.K. Gogoi, A.K. Chaliha, P. Buragohain, P.J. Trivedi, P.S. Saikia, A. Gehlot, V. Kumar, V. Chaturvedi, D. Sarma, Synthesis and biological evaluation of novel 1,2,3-triazole derivatives as anti-tubercular agents, Bioorg. Med. Chem. Lett. 27 (2017) 3698;
(b) Y. Sajja, S. Vanguru, H.R. Vulupala, R. Bantu, P. Yogeswari, D. Sriram, L. Nagarapu, Med. Chem. Lett. 27 (2017) 3698.

[23] (a) E.A.M. El-Zahany, A.M.A. El-Seidy, S.A. Drweesh, N.S. Youssef, B.F. Abdel-Wahab, A. El-Beih, Synthesis, characterization and biological activity of some transition metal complexes of pyrrolidine derivatives, J. Appl. Sci. Res. 9 (2013) 2268;
(b) A. Dondoni, Chem. Asian J. 2 (2007) 700.

[24] (a) C. Dayakar, B.S. Kumar, G. Sneha, G. Sagarika, K. Meghana, S. Ramakrishna, R.S. Prakasham, B.C. Raju, Synthesis, pharmacological activities and molecular docking studies of pyrazolyltriazoles as anti-bacterial and anti-inflammatory agents, Bioorg. Med. Chem. 25 (2017) 5678;
(b) S. Gatadi, J. Gour, M. Shukla, G. Kaul, S. Das, A. Dasgupta, S. Malasala, R.S. Borra, Y.V. Madhavi, S. Chopra, S. Nanduri, Synthesis of 1,2,3-triazole linked 4(3H)-quinazolinones as potent antibacterial agents against multidrug-resistant staphylococcus aureus, Eur. J. Med. Chem. 157 (2018) 1056;
(c) H.M. Savanur, K.N. Naik, S.M. Ganapathi, K.M. Kim, R.G. Kalkhambkar, Click chemistry inspired design, synthesis and molecular docking studies of coumarin, quinolinone linked 1,2,3-triazoles as promising anti-microbial agents, ChemistrySelect 3 (2018) 5296.

[25] (a) K.K. Angajala, S. Vianala, R. Macha, M. Raghavender, M.K. Thupurani, P.J. Pathi, Synthesis, anti-inflammatory, bactericidal activities and docking studies of novel 1,2,3-triazoles derived from ibuprofen using click chemistry, SpringerPlus 5 (2016) 423;
(b) S. Archana, M. Dinesh, R. Ranganathan, A. Ponnuswamy, P. Kalaiselvi, S. Chellammal, G. Subramanian, S. Murugavel, Water mediated one-pot synthesis and biological evaluation of 1,2,3-triazolyl-1,4-dihydropyridine hybrids, Res. Chem. Intermed. 43 (2017) 187.

[26] B. Zhang, Eur. J. Med. Chem. 15 (2019) 357.

[27] (a) S. Senthil, R. Gopi, N-Substituted-1,2,3-triazoles: synthesis, characterization and antimicrobial activity studies, Der. Pharma. Chemica. 7 (2015) 15;
(b) V. Sumangala, B. Poojary, N. Chidananda, J. Fernandes, N.S. Kumari, Synthesis and antimicrobial activity of 1,2,3-triazoles containing quinoline moiety, Arch. Pharm. Res. 33 (2010) 1911;
(c) F.F. Al-blewi, M.A. Almehmadi, M.R. Aouad, S.K. Bardawee, P.K. Sahu, M. Messali, N. Rezki, E.S.H. El Ashry, Design, synthesis, ADME prediction and pharmacological evaluation of novel benzimidazole-1,2,3-triazole-sulfonamide hybrids as antimicrobial and antiproliferative agents, Chem. Cent. J. 12 (2018) 110.

[28] J.D. MacLowry, M.J. Jaqua, S.T. Selepak, Detailed methodology and implementation of a semiautomated serial dilution microtechnique for antimicrobial susceptibility testing, Appl. Microbiol. 20 (1970) 46.
[29] H.M. Ericson, J.C. Sherris, Antibiotic sensitivity testing. Report of an international collaborative study, Acta Pathol. Microbiol. Scand. B Microbiol. Immunol. 217 (1971) 1.
[30] (a) R.B. Ghooi, S.M. Thatte, Inhibition of cell wall synthesis-is this the mechanism of action of penicillins? Med. Hypotheses 44 (1995) 127;
(b) C.O. Gualerzi, L. Brandi, A. Fabbretti, C.L. Pon (Eds.), Antibiotics: Targets, Mechanisms and Resistance, Vol. 1, Wiley VCH, 2013;
(c) G. Kapoor, S. Saigal, A. Elongavan, Action and resistance mechanisms of antibiotics: a guide for clinicians, J. Anaesthesiol. Clin. Pharmacol. 33 (2017) 300.
[31] Q. Cui, W.S. Shin, Y. Luo, J. Tian, H. Cui, D. Yin, An old topic brings new perspectives, Curr. Med. Chem. 20 (2013) 1286.
[32] S.P. Kawatkar, T.A. Keating, N.B. Olivier, J.N. Breen, O.M. Green, S.Y. Guler, M.F. Hentemann, J.T. Loch, A.R. McKenzie, J.V. Newman, L.G. Otterson, G. Martinez-Botella, Antibacterial inhibitors of gram-positive thymidylate kinase: structure–activity relationships and chiral preference of a new hydrophobic binding region, J. Med. Chem. 57 (2014) 4584.
[33] A. Barakat, A.M. Al-Majid, B.M. Al-Qahtany, M.A.M. Teleb, M.H. Al-Agamy, S. Naz, Z. Ul-Haq, Synthesis, antimicrobial activity, pharmacophore modeling and molecular docking studies of new pyrazole-dimedone hybrid architectures, Chem. Cent. J. 12 (2018) 29.
[34] (a) M. Brylinski, Aromatic interactions at the ligand–protein interface: implications for the development of docking scoring functions, Chem. Biol. Drug. Des. 91 (2018) 380–390;
(b) D.D. Boehr, A.R. Farley, G.D. Wright, J.R. Cox, Analysis of the π-π stacking interactions between the aminoglycoside antibiotic kinase APH(3′)-IIIa and its nucleotide ligands, Chem. Biol. 9 (2002) 1209.
[35] E.F. Pettersen, T.D. Goddard, C.C. Huang, UCSF chimera—a visualization system for exploratory research and analysis, J. Comput. Chem. 25 (2004) 1605.
[36] S. Dallakyan, A.J. Olson, Small-molecule library screening by docking with PyRx, Methods Mol. Biol. 1263 (2015) 243.
[37] Accelrys Software Inc. D. Studio, Version 4.1, Accelrys Software Inc, San Diego, CA, 2007.