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ORIGINAL RESEARCH

Synthesis, Biological Evaluation and Molecular Docking Studies of Novel Series of Bis-1,2,4-Triazoles as Thymidine Phosphorylase Inhibitor

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Introduction: Heterocyclic compounds have diverse biological activities and potential in drug development. This study aims to synthesize novel compounds with two 1,2,4-triazole cores and evaluate their biological properties, particularly their inhibitory activity against thymidine phosphorylase (TP), an enzyme involved in various physiological processes.

Methods: The compounds were synthesized by reacting 5,5'-butane-bis-1,2,4-triazole derivatives with prenyl bromide. Characterization involved various techniques, including spectroscopy and elemental analysis. Antimicrobial potential was evaluated against bacteria and fungi, with comparative antibiotics as references. Inhibitory activity against TP was assessed, and molecular docking studies were conducted.

Results: Six compounds were successfully synthesized and their structures confirmed. The synthesized triazole derivatives exhibited high biological activity, with compounds 2 and 6 showing the most promising TP inhibition. Molecular docking studies revealed interactions between compound 2 and TP, involving nine amino acids.

Discussion: The synthesis of novel compounds with two 1,2,4-triazole cores contributes significantly to bis-triazole research. These compounds have potential as anti-tumor agents due to their inhibitory activity against TP, a crucial enzyme in tumor growth and metastasis. Comparative evaluation against antibiotics highlights their potency. Docking results provide insights into their interactions with TP, supporting their potential as potent TP inhibitors. Further research should focus on evaluating their efficacy in biological models, understanding their mechanisms of action, and optimizing their activities.

Conclusion: The synthesized compounds with two 1,2,4-triazole cores exhibit significant biological activity, including strong TP inhibition and broad-spectrum antimicrobial effects. These findings emphasize their potential as anti-tumor agents and the need for further exploration and optimization. Future research should focus on evaluating their efficacy in biological models, understanding their mechanisms of action, and developing more potent bis-triazole derivatives for drug discovery efforts. The combined results from assays and docking studies support the therapeutic potential of these compounds as anti-tumor agents.

Keywords: alkylations, antitumor agents, heterocycles, hydrogen bonds, molecular modelling

Introduction

The field of heterocyclic chemistry offers a wide range of valuable compounds, with biological activity being a key indicator of their significance.[1,2] In fact, the incorporation of heterocyclic moieties in drug compositions has become increasingly prevalent, with approximately 90% of all drugs containing such components. The triazole ring is no exception to this trend,[3–5] as it is known for its biological properties, pharmaceutical effects, and diverse synthesis pathways.[6–9] Mononuclear and fused derivatives of 1,2,4-triazole have proven to be particularly valuable compounds, finding applications as antifungal, anticancer, and hypotensive agents (Figure 1).

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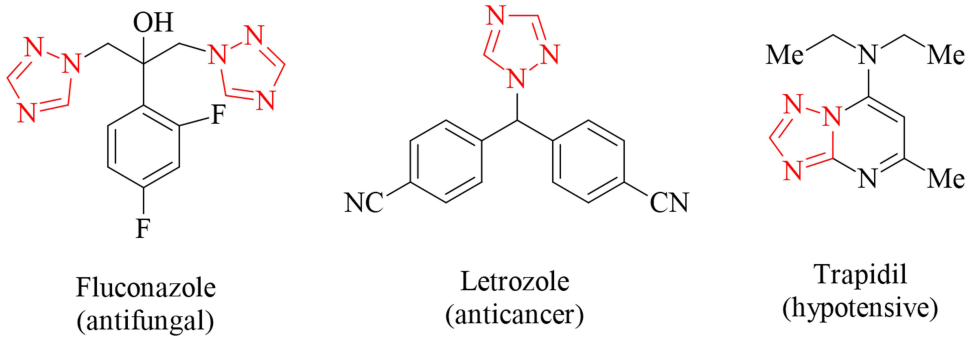


Figure 1 Examples of 1,2,4-triazole derivatives used in drugs. The red color serves as a visual indication of the 1,2,4-triazole cores.

Despite the proven anticancer[10] and antifungal[11] activities, as well as their potential application as chemosensors,[12] bis-triazoles have not been extensively explored in the pharmaceutical field.

In our previous studies, we successfully synthesized fused 1,2,4-triazolium salts[13–16] using a low-cost, efficient, and easy workup electrophilic cyclization methodology.[17] Building upon this work, our current aim is to obtain new derivatives of 5,5'-butane-bis-1,2,4-triazole, considering the potential enhancement of their biological properties due to the presence of both the butane bridge and the triazole moiety.[18] Furthermore, we seek to investigate their inhibitory activity against thymidine phosphorylase (TP).

Thymidine phosphorylase is an angiogenic enzyme that plays a role in pyrimidine salvage to repair RNA and DNA degradation.[19] It is known to be associated with various conditions, such as bone loss, sepsis,[20] disseminated intravascular coagulation,[21] and even as a diagnostic marker for COVID-19.[22] Recent investigations[23–28] have revealed that many different heterocyclic compounds exhibit effective inhibition of thymidine phosphorylase and demonstrate potent anti-tumor activity.[25,29–33]

In line with these findings, our research focuses on the synthesis of novel compounds containing two 1,2,4-triazole cores. We aim to evaluate their biological properties, specifically their inhibitory activity against thymidine phosphorylase, through a comprehensive analysis. Additionally, molecular docking studies will be conducted to gain insights into the interactions between the synthesized compounds and the thymidine phosphorylase enzyme.

These combined approaches and techniques will allow us to assess the significance of the obtained compounds and facilitate the design of further transformations aimed at increasing their biological activity. Ultimately, our goal is to explore the potential of these compounds as anti-tumor agents.

Materials and Methods

Chemistry

All reagents were obtained from commercial suppliers and used without any further purification, only hexyl-, heptyl- and octyl-isothiocyanates were obtained according to the protocol described in.[34] The melting points were determined on Stuart SMP30 instrument. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in $(CD_3)_2SO$ as a solvent and TMS as an internal standard on Varian VXR 400. Elemental analyses were performed on Elementar Vario MICRO cube analyzer.

General Procedure for the Synthesis of Compounds 1–6

To a solution of 5,5'-Butane-1,4-diyl(4-substituted-4*H*-1,2,4-triazole-3-thioles (10.0 mmol) in ethanol (20 mL) was added 12.0 mmol of potassium hydroxide. The mixture was heated. Prenyl bromides (12.0 mmol) in ethanol (5 mL) were added to the cooled solution of triazole. The mixture was boiled for 1 h. After cooling, the precipitated product was filtered, washed with deionized water, and purified by crystallization from ethanol.

5,5'-Butane-1,4-Diylbis(4-Methyl-3-[(3-Methylbut-2-en-1-yl)sulfanyl])-4*H*-1,2,4-Triazole (1)

White powder (recrystallized from EtOH), Yield: 87%; MW 420.64 g/mol; m.p. 106–107 °C; 1H NMR (400 MHz, DMSO) δ [ppm] 1.44 (6H, s, N–$\underline{CH_3}$), 1.62 (6H, s, =C–$\underline{CH_3}$), 1.71 (6H, s, =C–$\underline{CH_3}$), 2.73 (4H, s, - $\underline{CH_2}$-$\underline{CH_2}$), 3.44 (4H, s,

- $\underline{CH_2}$-$\underline{CH_2}$), 3.61 (4H, d, J = 7.8, - $\underline{CH_2}$-CH=), 5.26 (2H, t, J = 7.5, - CH_2-$\underline{CH}$=). 13C NMR (75MHz, DMSO) δ [ppm] 17.66, 24.50, 25.79, 26.11, 30.42, 32.26, 119.40, 137.10, 148.89, 156.16; Anal. found for $C_{20}H_{32}N_6S_2$: C, 56.95%, N, 19.74%, H, 7.52%. Calculated: C, 57.11%, H, 7.67%, N, 19.98%, S, 15.25%.

5,5'-Butane-1,4-Diylbis(4-Hexyl-3-[(3-Methylbut-2-en-1-yl)sulfanyl])-4*H*-1,2,4-Triazole (2)

White powder (recrystallized from EtOH), Yield: 92%; MW 560.90 g/mol; m.p. 114–115°C; 1H NMR (400 MHz, DMSO) δ [ppm] 0.96 (6H, s), 1.25 (6H, s), 1.52 (14H, m), 1.64 (6H, s), 1.78 (6H, s), 2.73 (4H, s), 3.70 (4H, d, J = 7.8, - $\underline{CH_2}$-CH=), 3.83 (4H, t, J = 7.2), 5.29 (2H, t, J = 7.5, -CH_2-$\underline{CH}$=). 13C NMR (75MHz, DMSO) δ [ppm] 14.24, 17.76, 22.40, 24.40, 25.76, 26.03, 26.49, 29.96, 31.13, 32.21, 43.54, 119.35, 137.26, 148.78, 155.51; Anal. found for $C_{30}H_{52}N_6S_2$: C, 64.08%, N, 15.04, H, 9.27%. Calculated: C, 64.24%, H, 9.34%, N, 14.98%, S, 11.43%.

5,5'-Butane-1,4-Diylbis(4-Heptyl-3-[(3-Methylbut-2-en-1-yl)sulfanyl])-4*H*-1,2,4-Triazole (3)

White powder (recrystallized from EtOH), Yield: 94%; MW 588.96 g/mol; m.p. 119–120 °C; 1H NMR (400 MHz, DMSO) δ [ppm] 0.85 (6H, s), 1.24 (20H, s), 1.58 (6H, m), 1.71 (6H, m), 2.33 (4H, s), 2.70 (4H, m), 3.69 (4H, d, J = 7.9), 3.89 (4H, s), 5.29 (2H, t, J = 7.5). 13C NMR (75MHz, DMSO) δ [ppm] 14.24, 17.76, 22.40, 24.40, 25.76, 26.03, 26.49, 27.17, 27.30, 29.96, 31.13, 32.21, 43.54, 119.35, 137.26, 148.78, 155.51; Anal. found for $C_{32}H_{56}N_6S_2$: C, 65.18%, N, 14.33%, H, 9.61%. Calculated: C, 65.26%, H, 9.58%, N, 14.27%, S, 10.89%.

5,5'-Butane-1,4-Diylbis(4-Octyl-3-[(3-Methylbut-2-en-1-yl)sulfanyl])-4*H*-1,2,4-Triazole (4)

White powder (recrystallized from EtOH), Yield: 90%; MW 617.01 g/mol; m.p. 120–122 °C; 1H NMR (400 MHz, DMSO) δ [ppm] 0.86 (6H, s), 1.24–1.32 (20H, m), 1.65–1.75 (20H, m), 2.85 (4H, t, J = 7.3), 3.51 (4H, d, J = 6.95), 3.94 (4H, t, J = 7.3), 5.61 (2H, t, J = 6.95). 13C NMR (75MHz, DMSO) δ [ppm] 14.0, 17.9, 22.6, 25.8, 25.9, 26.2,27.1, 27.2, 29.3, 29.4, 31.8, 35.2, 50.1, 119.2, 138.0, 143.2, 150.9; Anal. found for $C_{34}H_{60}N_6S_2$: C, 66.21%, N, 13.68%, H, 9.77%. Calculated: C, 66.18%, H, 9.80%, N, 13.62%, S, 10.39%.

5,5'-Butane-1,4-Diylbis(4-Phenyl-3-[(3-Methylbut-2-en-1-yl)sulfanyl])-4*H*-1,2,4-Triazole (5)

White powder (recrystallized from EtOH), Yield: 97%; MW 544.78g/mol; m.p. 136–137 °C; 1H NMR (400 MHz, DMSO) δ [ppm] 1.45 (4H, s), 1.53 (6H, s), 1.63 (6H, s), 2.43 (4H, s), 3.65 (4H, d, J = 7.6), 5.22 (2H, t, J = 7.0), 7.32 (4H, s), 7.54 (6H, s). 13C NMR (75MHz, DMSO) δ [ppm] 17.93, 24.61, 25.80, 31.14, 119.13, 127.72, 130.25, 133.69, 137.42, 149.97, 155.55; Anal. found for $C_{30}H_{36}N_6S_2$: C, 66.09%, N, 15.46%, H, 6.63%. Calculated: C, 66.14%, H, 6.66%, N, 15.43%, S, 11.77%.

5,5'-Butane-1,4-Diylbis(4-(4-Nitrophenyl)-3-[(3-Methylbut-2-en-1-yl)sulfanyl])-4*H*-1,2,4-Triazole (6)

Yellow powder (recrystallized from EtOH), Yield: 93%; MW 634.77g/mol; m.p. 141–142 °C; 1H NMR (400 MHz, DMSO) δ [ppm] 1.49 (4H, s), 1.55 (6H, s), 1.62 (6H, s), 3.65 (4H, d, J = 8.0), 5.18 (2H, t, J = 7.6), 5.57 (1H, s), 6.62 (2H, m), 6.84 (2H, m), 7.72 (2H, d, J = 8.8), 7.92 (1H, d, J = 9.1), 8.39 (4H, d, J = 8.8). 13C NMR (75MHz, DMSO) δ [ppm] 17.86, 24.58, 25.58, 25.73, 31.63, 112.82, 118.87, 125.46, 126.69, 128.00, 129.38, 137.67, 138.94, 148.22, 149.59, 155.39; Anal. found for $C_{30}H_{34}N_8O_4S_2$: C, 56.73%, N, 17.59%, H, 5.42%. Calculated: C, 56.76%, H, 5.40%, N, 17.65%, O, 10.08%, S, 10.10%.

Biological Section

Comparative antibiotics were commercially obtained from India («HiMedia», India). Dimethyl Sulfoxide (DMSO) was used to prepare stock solutions of test compounds. Bacterial strains (*Staphylococcus aureus* Ks-02, *Pseudomonas aeruginosa* Kp-02, *Escherichia coli* Ke-06, *Proteus mirabili* Km-02, *Enterococcus faecalis* Ke-05, *Salmonella enterica* Ksa-32, *Enterobacter cloacae* Kc-12, *Klebsiella pneumonia* Kk-12, *Candida albicans* Kl-03, *Saccharomyces cerevisiae* Ksc-22) were obtained from surgically drained soft tissue abscesses from patients of the hospital of the Transcarpathian Regional Clinical Center of Neurosurgery and Neurology, Uzhhorod, Ukraine. Subsequent confirmation of the cultures was done using conventional microbiological techniques including Gram staining, microscopic examination, mannitol fermentation, β-hemolysis assay using 5% sheep's blood agar, catalase test using 3% hydrogen peroxide and coagulase test (PlivaLaChema, Chezh Republic). Conventional characterization was further validated by PCR amplification and

DNA sequencing of 16srRNA gene (Agilent Technologies, USA). Only those isolates were subjected to molecular characterizations which were confirmed as being Multidrug Resistant (MDR) after screening through Kirby-Bauer Disk Diffusion Assay.

All clinical strains used in this study are preserved in the collection of microorganisms at the Research and Educational Center for Molecular Microbiology and Immunology of Mucous Membranes at Uzhhorod National University. The Ethics Committee of the State Higher Educational Establishment «Uzhhorod National University» approved the study protocol No. 7/3 on 12/22/2021.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) of selected antibiotics and the test compounds against test organisms (0.25–512 µg/mL) was determined by broth microdilution method in accordance with CLSI guidelines (CLSI, 2019). In this assay, antibiotics and test compounds were serially diluted using Mueller Hinton Broth (MHB) (Sigma-Aldrich, USA) in such a way that each well of 96 well microtiter plate (DeltaLab, Spain) contained half the concentration of antibiotic present in preceding well. Finally, 5×10^6 of bacterial cells were inoculated into each well except for wells labeled negative control after 24 h incubation. To enhance the clarity of the growth end points, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT dye) was added to each well at a concentration of 0.5 µg/mL. The sealed plates were then incubated on a shaking incubator at 37°C and 80 rpm for 2–3 hours and subsequently examined for any discernible color changes.

Determination of TP Inhibition Activity

TP/PD-ECGF (*E. coli* TP (Sigma T6632)) activity was determined by measuring the absorbance at 290 nm spectrophotometrically (DeNovix Inc., USA). The original method is reported in Krenitsky and Bushby.[35] In 96 wells, flat bottom, microplate with each well capacity 200 µL, reaction mixture of 200 µL was prepared which contained 145 µL of potassium phosphate buffer (pH 7.4), 20 µL of 1.5 mM Thymidine-5'-monophosphate solution as substrate, 30 µL of enzyme (*E. coli* TP (Sigma T6632)) at concentration 0.05 and 0.002 U, respectively, were incubated with 5 µL of test materials for 10 min at 25°C in temperature controlled incubator before taking readings by microplate reader ELx800 (*BioTek*, USA) at 290 nm. The wells containing a reaction mixture without substrate served as blanks, and the mean optical density (OD) of these blank wells was subtracted from wells containing the reaction mixture with substrate. The readings were taken continuously after 10, 20, and 30 min using a microplate reader. All assays were performed in triplicate.

Molecular Docking

Molecular docking studies of molecule **2** that were docked into the crystal structures of thymidine phosphorylase in *E. coli* with PDB_ID: 4LHM were carried out using Autodock Vina software,[36] an open-source molecular docking software. First, we optimized the structure of the enzyme using BIOVIA Discovery Studio 2021 software (https://discover.3ds.com/discovery-studio-visualizer-download). We utilized this software for adding polar hydrogens to the enzyme structure and performing energy minimization. Additionally, we employed HyperChem 7 (http://www.hypercubeusa.com/) to optimize the structure of compound **2**. We have generated a grid box with desired parameters around the active site of thymidine phosphorylase (PDB_ID: 4LHM) as center: x = 31.889, y = −12.619, z = 4.741 and grid box size: x = 20, y = 20, z = 20 with 1Å grid spacing. We used the advanced Genetic algorithm method in Vina Protein/TP to generate 10 conformations in each docking output. The molecule input preparations and docking output analysis were carried out using Discovery Studio software.

Results

Synthesis

The starting bis-triazoles were prepared using adipic acid dihydrazide. The synthesis involved refluxing the dihydrazide with the corresponding isothiocyanates in ethanol for one hour, leading to the formation of 5,5'-butane-bis-1,2,4-triazole-3-thiones **1**–**6**. Subsequently, the thiosemicarbazides were subjected to prolonged heating in an aqueous solution of

potassium hydroxide, resulting in the formation of triazoles. These triazoles were then treated with a double excess of prenyl bromide, leading to the excellent yield of the alkylated triazoles **1**–**6** (Scheme 1).

The NMR spectra of the synthesized compounds exhibited signals that fully matched the assigned structures, confirming the success of the synthesis and the purity of the products. Specifically, the chemical shifts of the aromatic and aliphatic protons were consistent with the predicted structures, and no unexpected peaks were observed. This analysis provided further evidence for the accurate synthesis and high quality of the compounds.

In vitro Antibacterial Activity

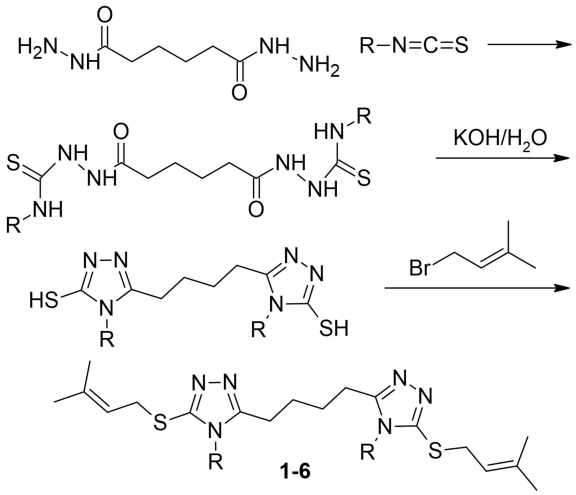
To assess the biological activity of the synthesized compounds, we conducted a comprehensive evaluation using a panel of 10 different bacteria, including both Gram-positive and Gram-negative strains. The results obtained from these assays revealed that the alkylated derivatives **1**–**6** exhibited moderate levels of biological activity. Notably, compounds **1**–**4**, which incorporate alkyl substituents at the 4 position of the 1,2,4-triazole moiety, displayed higher levels of biological activity compared to compounds with aromatic substituents at the same position.

In fact, several of the tested compounds demonstrated comparable or even greater activity when compared to the reference antibiotic. Specifically, compound **2** exhibited notable activity against *Escherichia coli*, while compound **5** showed significant inhibitory effects against *Saccharomyces cerevisiae*. Additionally, compounds **1**, **2**, **5**, and **6** displayed promising activity against *Pseudomonas aeruginosa*. An overview of all the obtained results is presented in Table 1, providing a clear comparison of the biological activity of each compound.

The observed trends in the biological activity of the alkylated derivatives highlight the importance of the specific substitution pattern at the 4 position of the 1,2,4-triazole moiety. The presence of alkyl substituents in this position appears to enhance the compounds' antimicrobial potential, underscoring the significance of structural modifications in modulating their biological activity. These findings contribute to a better understanding of the structure–activity relationship and guide future optimization efforts to develop more potent compounds with improved antimicrobial properties.

The evaluation of the synthesized compounds against a diverse panel of bacteria provides valuable insights into their potential as \antimicrobial agents. While the observed biological activity is moderate, it establishes a promising starting point for further optimization and development of more potent derivatives. Subsequent investigations should focus on elucidating the mechanisms of action and conducting additional structure–activity relationship studies to unlock the full therapeutic potential of these compounds as antimicrobial agents.

In order to facilitate a meaningful comparison of the obtained results, we have converted the data for compounds **1**–**6** into IC_{50} values in micromolar (μM) concentrations, as presented in Table 2.



R = CH_3 (**1**), C_6H_{13} (**2**), C_7H_{15} (**3**), C_8H_{17} (**4**), Ph (**5**), 4-NO_2-Ph (**6**)

Scheme 1 Synthetic route for obtaining target compounds **1**–**6**.

Table 1 Comparison of Minimum Inhibitory Concentration (MIC (μg/mL)) of Compounds **1–6** Against Gram-Positive and Gram-Negative Bacteria

Compound / Microorganism	1	2	3	4	5	6	CLT	PCN	CFZ
Pseudomonas aeruginosa	128	64	ND	256	16	64	ND	256	2
Staphylococcus aureus	256	64	ND	256	ND	64	ND	4	4
Escherichia coli	ND	16	ND	64	128	32	-	8	16
Proteus mirabilis	64	256	64	128	32	256	ND	0.25	2
Enterococcus faecalis	64	ND	128	128	128	ND	-	16	16
Salmonella enterica	128	64	128	128	64	ND	ND	4	32
Enterobacter cloacae	ND	64	ND	32	32	16	-	4	8
Klebsiella pneumoniae	ND	256	16	ND	32	256	ND	16	8
Candida albicans	32	64	16	ND	256	32	0.25	ND	ND
Saccharomyces cerevisiae	16	ND	32	16	64	32	2	ND	64

Note: "-" – corresponding test have not been provided.
Abbreviations: CLT, clotrimazole; PCN, penicillin; CFZ, cefazolin; ND, no detected activity at 256 μg/mL.

Table 2 Comparison of Minimum Inhibitory Concentration (IC_{50} (μM)) of Compounds **1–6** Against Gram-Positive and Gram-Negative Bacteria

Compound / Microorganism	1	2	3	4	5	6
Pseudomonas aeruginosa	304	114	ND	415	29	101
Staphylococcus aureus	608	114	ND	415	ND	101
Escherichia coli	ND	29	ND	104	235	50
Proteus mirabilis	152	456	109	207	59	403
Enterococcus faecalis	152	ND	217	207	235	ND
Salmonella enterica	304	114	217	207	117	ND
Enterobacter cloacae	ND	114	ND	52	59	25
Klebsiella pneumoniae	ND	456	27	ND	59	403
Candida albicans	76	114	27	ND	470	50
Saccharomyces cerevisiae	38	ND	54	26	117	50

Based on the antimicrobial activity data of the synthesized compounds, a notable trend can be observed. Compounds **1**–**4** exhibited greater activity against Gram-positive bacteria, while compounds **5** and **6** displayed enhanced activity against Gram-negative bacteria. This distinction in activity against different bacterial types underscores the potential versatility and broad-spectrum nature of the synthesized compounds.

Overall, the results presented in Table 2 indicate the promising antimicrobial activity of compounds **1**–**6**, with distinct preferences for different types of bacteria. These findings contribute to our understanding of the compounds' potential as antimicrobial agents and pave the way for future research and development efforts in this field.

Thymidine Phosphorylase Inhibitory Activity

The inhibitory activity of prenyl-alkylated triazoles **1–6** against thymidine phosphorylase was determined using a spectrophotometric protocol,[37] and the results are presented as IC_{50} values. Notably, all the tested compounds demonstrated moderate to excellent inhibitory activity against thymidine phosphorylase.

Among the series of compounds tested, compound **2**, which contains a hexyl substituent, exhibited the highest inhibitory activity with an IC50 value of 28.74 ± 0.59 μM. This fact indicates that compound **2** is the most potent inhibitor in the series. Additionally, compound **6**, which features a *p*-nitrophenyl group in the 4th position, also showed significant inhibitory activity with an IC_{50} value of 34.95 ± 0.73 μM, further highlighting its potential as an effective inhibitor.

The results presented in Table 3 demonstrate the efficacy of the tested compounds in inhibiting thymidine phosphorylase. Further investigations can explore their mechanisms of action, conduct structure–activity relationship studies, and evaluate their efficacy in relevant biological models.

Molecular Docking Study

The leading compound **2** was chosen for the molecular docking study to analyze its interaction with the protein target. For this investigation, the crystallographic structure of thymidine phosphorylase (TP) in *E. coli*, specifically the 4LHM (pdb), was selected as the protein of interest. The selection of this target protein is based on the essential role of TP in *E. coli* bacteria, making it an intriguing choice to explore the interaction characteristics between the synthesized compounds and the bacterial enzyme.

The affinity value of the interaction between the optimized conformation of compound **2** and the active site of the protein 4LHM was determined to be −6.9 kcal/mol, indicating a favorable binding affinity. This interaction involved nine amino acids in the binding process. Notably, Lys190 participated in the interaction with the triazole ring through hydrogen bonding and electrostatic interactions. Val177 and Phe210 formed hydrophobic bonds with the triazole group, while Met211 engaged in a pi-sulfur bond interaction. His85 was observed to interact with both the hexyl group and the sulfur atom at the 5 position of the triazole ring through pi-alkyl and pi-bonds, respectively. Leu117 contributed to the interaction by forming a hydrophobic bond with the alkyl part of the prenyl group and the hexyl substituent. Additionally, hydrophobic interactions were observed between Tyr168 and the triazole ring, as well as between Ile183 and the butane bridge, and Ile187 and the hexyl group (Figure 2).

Obtained results provide valuable insights into the specific interactions between compound **2** and the active site of thymidine phosphorylase, shedding light on the potential mechanisms of action and aiding in the understanding of the compound's inhibitory properties.

Discussion

The variation in the biological activity of the synthesized compounds can be attributed to structural differences in the cell wall of the bacteria. Gram-negative bacteria are characterized by the presence of an outer lipid membrane, which serves

Table 3 Inhibition of Thymidine Phosphorylase by Triazoles **1–6**

Compound	IC_{50} μM
1	113.28±1.06
2	28.74±0.59
3	124.69±0.47
4	68.29±0.87
5	79.35±1.16
6	34.95±0.73

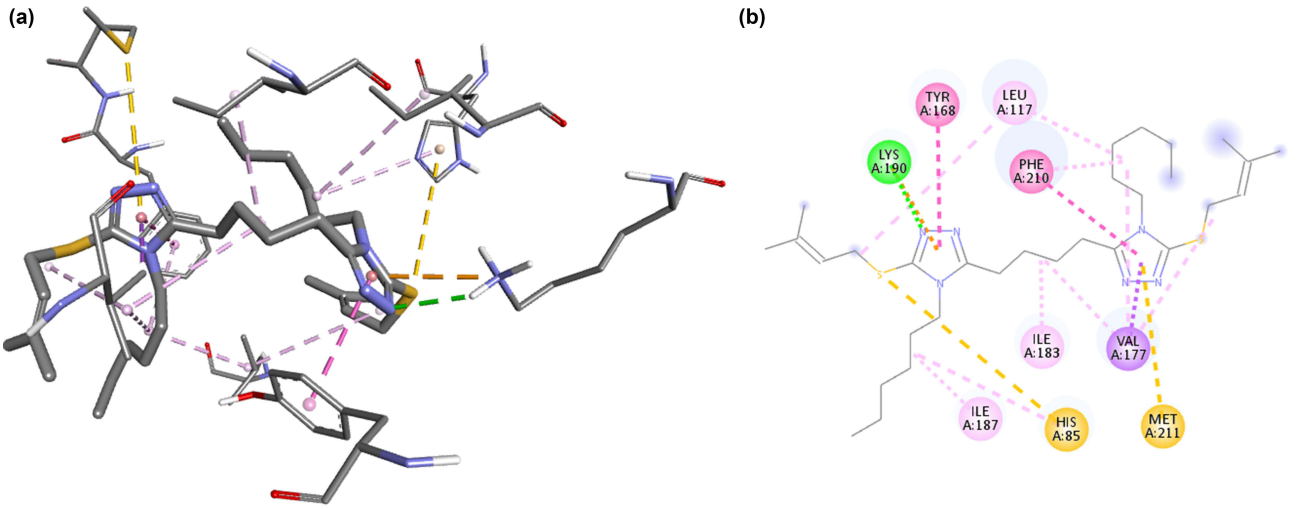


Figure 2 Ligand interaction diagram of compound 2 in the active site of 4LHM in 3D (**a**) and 2D (**b**) visualization.

as a selective permeation barrier. This outer membrane restricts the entry and access of substances to the periplasmic space, thereby influencing the interaction and activity of the compounds.[37] Based on the presence of a *p*-nitrophenyl substituent at the 4 position of the triazole nucleus in compound **6**, it can be inferred that this fragment plays a role in enhancing the compound's activity against Gram-negative bacteria. The beneficial influence of a *p*-nitrophenyl moiety on biological properties has been previously described, further supporting its positive impact in this context.[38]

Our research not only uncovered the antibacterial activity of the synthesized compounds against both Gram-positive and Gram-negative bacteria but also highlighted their potential as antifungal agents. Specifically, our findings indicated that derivatives **1**, **2**, **3**, and **6**, which contain methyl, hexyl, heptyl, and *p*-nitrophenyl substituents at the 4th position of the triazole moiety, exhibited enhanced antifungal activity against *Candida albicans*. Additionally, compounds **1**, **3**, **4**, **5**, and **6**, comprising methyl, heptyl, octyl, phenyl, and *p*-nitrophenyl fragments, respectively, demonstrated activity against the yeast *Saccharomyces cerevisiae*. These results align with previously published studies that have reported on the antibacterial and antifungal properties of similar compounds.[39]

Based on the obtained results, it is evident that the synthesized triazole derivatives have the potential to serve as valuable agents for the design and development of novel therapeutic drugs to combat bacterial resistance.

Particularly noteworthy is the fact that compounds **2** and **6** exhibited greater TP inhibition activity compared to the standard 7-deazaxanthine. This highlights their promising prospects in targeting and inhibiting the activity of thymidine phosphorylase, an enzyme involved in various physiological processes, and provides evidence that these substances are suitable candidates for further clinical studies as potent anti-tumor drugs.[40]

The notable correlation between the minimum inhibitory concentration (MIC) values against the *E. coli* strain and the corresponding TP inhibitory activity values can be attributed to the fact that the crystalline structure of the TP enzyme was obtained from the same bacterial species. This correlation reinforces the potential significance of targeting thymidine phosphorylase as a strategy for combating tumor growth.[41–44]

Conclusion

We have conducted a comprehensive investigation on a novel series of *bis*-1,2,4-triazoles, exploring their potential as antibacterial and fungicidal agents, efficient inhibitors of thymidine phosphorylase (TP), and promising anti-tumor compounds. Our results demonstrate that most of the synthesized compounds possess significant biological activity against various bacteria. Notably, the alkylated derivatives displayed excellent TP inhibition, with two compounds exhibiting higher inhibitory activity than the standard reference, 7-deazaxanthine (IC_{50} = 41.0 ± 1.63 μM). Besides, molecular docking analysis revealed a specific interaction between the lead compound **2** and Lys190 through a hydrogen

bond. These results provide valuable insights for the design of advanced compounds with enhanced inhibitory activity against the TP enzyme, thus offering potential prospects for their utilization as effective anti-tumor agents.

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Disclosure

The authors report no conflicts of interest in this work.

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