Discovery of Novel Triazole-Containing Pyrazole Ester Derivatives as Potential Antibacterial Agents

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Abstract: To develop new antibacterial agents, a series of novel triazole-containing pyrazole ester derivatives were designed and synthesized and their biological activities were evaluated as potential topoisomerase II inhibitors. Compound 4d exhibited the most potent antibacterial activity with Minimum inhibitory concentration (MIC) values of 4 µg/mL, 2 µg/mL, 4 µg/mL, and 0.5 µg/mL against Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, and Salmonella gallinarum, respectively. The in vivo enzyme inhibition assay 4d displayed the most potent topoisomerase II (IC₅₀ = 13.5 µg/mL) and topoisomerase IV (IC₅₀ = 24.2 µg/mL) inhibitory activity. Molecular docking was performed to position compound 4d into the topoisomerase II active site to determine the probable binding conformation. In summary, compound 4d may serve as potential topoisomerase II inhibitor.

Keywords: pyrazole; triazole; ester; antibacterial; topoisomerase II inhibitor

1. Introduction

Bacterial type II topoisomerases (DNA gyrase and topoisomerase IV) are ubiquitous and essential enzymes that have critical roles in the fundamental biological processes of replication, transcription, recombination, repair, and chromatin remodeling [1,2]. The action of topoisomerases is to change the spatial structure of DNA by catenation and decatenation of duplex DNA rings, relaxation of supercoiled DNA, and in the use of DNA topoisomerase II introduction of negative supercoils into DNA in an energy-dependent reaction [3]. Therefore, bacterial DNA topoisomerase II has drawn much attention as a selected target for finding potent antibacterial agents [4–6]. For example, a lot of synthetic quinolone antibacterial agents have been marketed and are now widely used for the treatment of bacterial infectious diseases [7,8]. Specifically, sparfloxacin has proven to be a very successful inhibitor of bacterial DNA topoisomerase II [9]. However, the abuse of antibacterial has led to an increase in bacterial resistance, especially in immunocompromised patients [10]. Our current research efforts are to find novel antibacterial agents as DNA topoisomerase II inhibitors.

Hybridization was a common method of drug development and design [11]. It was based on combining two or more different biologically active moieties in a single molecule to obtain the corresponding conjugated hybrid molecules [12]. These hybrid molecules may exhibit better activities...
than their precursors [13]. The novel synthesized heterocyclic molecules can work by the same or different mechanisms of action compared to the precursors [14]. Nitrogen containing heterocyclic may be potential antibacterial drugs which inhibit bacterial topoisomerases [15], such as triazoles [16], quinolones [17], oxazolopyridines, aminopyrazinamides, and pyrazole [18–20]. Recently, Plech [21] and Lal [22] reported triazole derivatives as potential topoisomerase II inhibitors, which exhibited greater antibacterial activities than ciprofloxacin (Figure 1).

![Figure 1. Recently discovered triazole derivatives as DNA topoisomerase inhibitors.](image)

As is well known, prolonged use of antibacterial agents can lead to environmental problems and residual toxicity [23,24]. However, compound containing ester groups, can easily be hydrolyzed to produce low toxicity compounds. In addition, it can increase its soluble fat in vivo and enhance antimicrobial activity. Furthermore, it has multiple charges, enabling them to interact with the bacterial cell surface more strongly than their monomeric counterparts. Recently, Haldar et al. [25] reported that two compounds were linked by an ester group, and the antibacterial activities of the new compounds were improved compared with the previous compounds. In the previous research work [8], we discovered a novel Topoisomerase II (Topo II) inhibitors compound 3, which showed considerable inhibitory activity than ciprofloxacin. In the search for a class of new Topoisomerase II inhibitors, we chose pyrazole as primer molecule, introduced a triazole structure, and used the ester group as a linker to obtain a new scaffold (Figure 2) [26,27]. After that, a series of novel triazole-containing pyrazole ester derivatives were designed and synthesized. Initially, all of the synthesized compounds were used against two fungi, but the results of the antifungal activity were not ideal. Therefore, four bacteria were used to test the antibacterial activity of these compounds, two Gram-positive bacteria, namely *Staphylococcus aureus* and *Listeria monocytogenes*, and two Gram-negative bacteria, namely *Escherichia coli* and *Salmonella gallinarum*. In addition, molecular docking analysis studies were performed on all derivatives to determine critical structural factors responsible for their antibacterial efficacy.
2. Results and Discussion

2.1. Chemistry

A series of triazole-containing pyrazole ester derivatives were prepared and the route listed in Scheme 1. We dissolved substituted phenylhydrazine with ethanol, then added ethylacetoacetate. Preparation of 1a–d was with Vilsmeier–Haack Reagent \((N,N\text{-Dimethylformamide}/\text{POCl}_3)\). Then, we added KMnO\(_4\) as an oxidation reagent to obtain 2a–d. Compounds 3a–d were obtained from phenylhydrazine hydrochloride and urea, we used formic acid under acidic conditions. The preparation of the target compounds was carried out using the reported method [28]. We analyzed all prepared compounds using spectral and elemental methods, which showed that all compounds fully complied with the structure in Table 1.

![Scheme 1. General synthesis of compounds 4a–m. Reagents and conditions: (a) H\(_2\)O, ethanol, 60 °C; (b) N,N-Dimethylformamide(DMF), POC\(_4\), 90 °C, 5h; (c) KMnO\(_4\), 70–80 °C; (d) HCl, 135 °C, H\(_2\)SO\(_4\), 90 °C; (e) Dicyclohexylcarbodiimide(DCC), 4-dimethylaminopyridine(DMAP), DMF, rt.](image-url)
Table 1. Minimum inhibitory concentration (MIC) of all compounds against bacteria.

| Compounds | MIC (µg/mL) | S.a * | L.m * | E. c * | S.e * |
|-----------|-------------|-------|-------|--------|-------|
| 2a        | H           | 64    | 32    | 32     | 64    |
| 2b        | F           | 64    | >128  | >128   | 64    |
| 2c        | Cl          | 64    | 64    | >128   | >128  |
| 2d        | CH₃         | 16    | 32    | 16     | 4     |
| 3a        | H           | 32    | 32    | 16     | 16    |
| 3b        | F           | >128  | 32    | 64     | 64    |
| 3c        | Cl          | 64    | >128  | 64     | 64    |
| 3d        | CH₃         | 32    | 64    | 16     | 32    |
| 4a        | H           | 16    | 16    | 8      | 16    |
| 4b        | F           | H     | >128  | >128   | 64    |
| 4c        | Cl          | 64    | >128  | 64     | 64    |
| 4d        | CH₃         | H     | 4     | 2      | 4     |
| 4e        | H           | F     | 64    | 32     | 32    |
| 4f        | F           | F     | >128  | >128   | >128  |
| 4g        | Cl          | F     | 64    | 64     | 64    |
| 4h        | CH₃         | F     | 8     | 16     | 32    |
| 4i        | F           | Cl    | >128  | 64     | >128  |
| 4j        | Cl          | Cl    | 64    | 64     | 64    |
| 4k        | H           | CH₃   | 4     | 8      | 4     |
| 4l        | F           | CH₃   | 32    | 64     | 32    |
| 4m        | Cl          | CH₃   | 64    | 32     | 64    |
| DMSO      |             |       | >128  | >128   | >128  |

* Abbreviations: Staphylococcus aureus (ATCC-12600); Listeria monocytogenes (ATCC-15313); Escherichia coli (ATCC-25922); Salmonella gallinarum (ATCC-9184). b Ciprofloxacin.

2.2. Antibacterial Activity and Structure-Activity Relationships (SAR) Discussion

In vitro antimicrobial activity of the prepared compounds against four bacteria was evaluated by the conventional agar-dilution method. Ciprofloxacin was selected as a reference standard. The results of the in-vitro antibacterial activity screening of the test compounds are summarized in Table 1. The results revealed that most of the synthetic compounds exhibited antibacterial activities Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, and Salmonella gallinarum, demonstrating the rationality of our design strategy. As can be seen from Table 1, among all the intermediate compounds, compound 2d exhibited the most potent antibacterial activity with MIC values of 16 µg/mL against Staphylococcus aureus, but among the tested target compounds, three compounds (i.e., 4d, 4g, and 4k) were found to display improved antibacterial activities against Staphylococcus aureus compared with (16 mg/L), with MIC values ranging from 4 to 8 mg/L. The eye-catching finding was that the target products greater excellent antibacterial activity than intermediate compounds. Among them, compounds 4d and 4k displayed most potent activity with MIC values of 4, 2, 4, and 0.5 µg/mL and 4, 8, 4, and 4 µg/mL against Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, and Salmonella gallinarum, which were similar to the broad-spectrum antibiotic Ciprofloxacin), indicating that they possess antibacterial activity. Compound 4a showed antibacterial activities against Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, and Salmonella gallinarum, Then, we introduced different substituent groups on benzene ring in 4a, the introduction of methyl at R¹ displayed significantly enhanced effects compared with that of other compounds. Compound 4d exhibited best antibacterial activity than others. When R² position was substituted with fluorine, methyl substituted at the R¹ site was beneficial to antibacterial activity of the synthesized compounds (e.g., 4h). When the R² position was substituted with methyl, electron-donating substituents (e.g., 4k) at R¹ showed more potent activities than those with electron-withdrawing (e.g., 4l, 4m). When R¹ and R² were simultaneously substituted by electron-withdrawing, their activities were poor compared
with other compounds. (e.g., 4j, 4f, 4i). Considering the above SAR results, these findings suggest that the antibacterial potency of designed compounds could be ascribed to a combination of factors, such as the R^1 position was substituted with electron-donating groups, which may enhance antibacterial activity, and that the R^2 position was substituted with hydrogen and the compound may enhance antibacterial activity.

2.3. Inhibitory Effects against DNA Gyrase and Topoisomerase IV

In order to determine the relationship between compounds and antibacterial activity, the inhibitory activity of selected compounds (4d and 4k) against DNA gyrase and topoisomerase IV isolated from *Escherichia coli* was examined. As shown in Table 2, 4d showed more potent inhibition than 4k against the two enzymes. The same exhibited the same tendency as the MIC data, suggesting that compound 4d may serve as a potential topoisomerase II inhibitor.

Table 2. Inhibitory effects of selected compounds against DNA gyrase and topoisomerase IV.

| Compd. | IC_{50} (µg/mL) | Gyrase | Topo IV |
|--------|-----------------|--------|---------|
|        |                 | a      | b       |
| 4d     | 13.5            | 24.2   |
| 4k     | 25.7            | 33.1   |
| CIP    | 0.25            | 6.9    |

*a* Topoisomerase II supercoiling activity; *b* Topoisomerase IV decatenation activity. *c* Ciprofloxacin.

2.4. Docking Analysis

In order to gain a better understanding on the potency of the synthesized compounds and guide further structure, we conducted activity relationships studies. All of the derivatives were docked into the active site of Topo II (PDB entry: 2xcs) in the binding model of compound 4d and Topo II. The skeleton of compound 4d was embedded in the binding pocket, showing that the pose of 4d into the Topo II-binding site had a suitable shape that was complementary to the binding pocket, which means that our design strategy was rational. E:DC11, F:DC11, and F:Dg10 established π–π interactions with a benzene ring of compound 4d (distance: 5.01 Å, 4.18 Å, 5.31 Å), E:DC11 established π–π interactions with triazole ring of compound 4d (distance: 4.60 Å), Arg 1122 established cation–π interactions with pyrazole ring of compound 4d (distance: 5.26 Å) (Figure 3).

![Figure 3](image-url)  
*Figure 3.* (A) Binding model of 4d (2D diagram). (B) The π–π interactions are displayed as yellow solid lines. The cation–π interactions are displayed as green solid lines.
3. Experimental Section

3.1. Materials and Methods

All chemicals were purchased from Energy, Meryer, and Aladdin Chemicals and were used as received. $^1$H-NMR spectra analyses were carried out using an Agilent DD2 600 Hz spectrometer with CDCl$_3$ as the solvent and tetramethylsilane as the internal standard. ESI-MS (Electrospray Ionisation Mass Spectrometry) spectra were carried out on a Mariner System 5304 mass spectrometer. Elemental analyses were performed on a CHN–O–Rapid instrument. Molecular docking was performed with Discovery Studio 3.5 [29]. The $^{13}$C-NMR and ESI-MS spectrum of compounds can be found in the Supplementary Materials.

3.1.1. General Procedure for the Synthesis of 5-Chloro-1-aryl-3-methyl-1H-pyrazole-4-carboxylic Acids 2a-d

Intermediates 2a–d were obtained from Reference [30]. Dissolve para-substituted phenyl hydrazine (0.025 mol) with anhydrous ethanol ethyl acetoacetate (0.025 mol) was added portion wise, stirred, and refluxed for 5 h, then the solution was rotary evaporated to form a solid, which was dissolved in DMF (25 mL) and phosphorus oxychloride (20 mL) of cold mixed solution and stirred at 85 °C for 2 h. The resulting product was poured into ice-cold water and the solid was isolated by filtration to give a yellow solid, which was oxidized by KMnO$_4$ solution and stirred at 70–80 °C. After cooling to room temperature, the pH was adjusted to alkaline by the addition of 10% NaOH solution, and the solution was filtered, HCl solution was added to the solution and solid 2a–d eventually separated out. The resulting crude product was recrystallized from anhydrous ethanol to give the pure product.

3.1.2. General Pathway for Prepare of 1-phenyl-1H-1,2,4-triazol-3-ol 3a–d

Intermediates 3a–d were synthesized according to Bin sun et al. [31]. Phenylhydrazine (0.2 mol) and urea (0.25 mol) were dissolved in water (50 mL) and 30% hydrochloric acid (64.8 g) was added for 4 h at 135 °C. Add formic acid (0.3 mol) and 98% concentrated sulfuric acid (4.8 g) at 90 °C for 6 h. Finally, the product was cooled to room temperature, filtered, and washed with water to neutral and dried to give solid 3a–d.

3.1.3. General Procedure for Synthesis of 1-phenyl-1H-1,2,4-triazol-3-yl 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylate 4a–m

Compounds 2a–d (1 mmol) was stirred with triethylamine (1.5 mmol) into DMF (10 mL) medium, then a mixture of DCC (1 mmol) and DMAP (1 mmol) was added in the reaction system, stirred at 25 °C for 1 h. The mixture of intermediate 3 (1.2 mmol) and DMF (6 mL) was added in the reaction, stirred at 25 °C for 3 h. Finally, the product was extracted from chloroform with water, hydrochloric acid (0.2 mol/L), sodium hydroxide (2 mol/L), saturated sodium chloride successively, and then dried, concentrated, and purified by preparative thin layer chromatography (PE: EA = 8:1) [27].

3.1.4. 1-phenyl-1H-1,2,4-triazol-3-yl5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylate (4a)

White solid, yield 62%; mp 138–140 °C; $^1$H-NMR (600 MHz, CDCl$_3$) δ 8.00 (s, 1H, Triazole-H), 7.53 (s, 6H, Ph-H), 7.08 (d, $J$ = 8.0 Hz, 1H, Ph-H), 6.98 (t, $J$ = 8.6 Hz, 1H, Ph-H), 6.93 (d, $J$ = 4.4 Hz, 1H, Ph-H), 6.88 (d, $J$ = 8.2 Hz, 1H, Ph-H), 2.55 (s, 3H, CH$_3$). MS (ESI) calculated for C$_{19}$H$_{15}$ClN$_5$O$_2$ [M + H]$^+$, 380.1, found 380.1; Anal. Calcd for C$_{19}$H$_{14}$ClN$_5$O$_2$: C, 60.09; H, 3.72; N, 18.44%; Found: C, 60.12; H, 3.79; N, 18.33%.
3.1.5. 1-phenyl-1H-1,2,4-triazol-3-yl5-chloro-1-(4-fluorophenyl)-3-methyl-1H-pyrazole-4-carboxylate (4b)

White solid, yield 54%; mp 166–168 °C; 1H-NMR (600 MHz, CDCl3) δ 7.95 (s, 1H, Triazole-H), 7.51 (dd, J = 8.8, 4.7 Hz, 2H, Ph-H), 7.28 (s, 1H, Ph-H), 7.25 (s, 1H, Ph-H), 7.22 (t, J = 8.4 Hz, 2H, Ph-H), 6.98–6.91 (m, 3H, Ph-H), 2.54 (s, 3H, CH3). MS (ESI) calculated for C19H13ClF3N2O2 [M + H]+, 398.1, found 398.1; Anal. Calcd for C19H13ClF3N2O2: C, 57.37; H, 3.29; N, 17.61%; Found: C, 57.41; H, 3.31; N, 17.73%.

3.1.6. 1-phenyl-1H-1,2,4-triazol-3-yl5-chloro-1-(4-chlorophenyl)-3-methyl-1H-pyrazole-4-carboxylate (4c)

White solid, yield 57%; mp 172–173 °C; 1H-NMR (600 MHz, CDCl3) δ 7.92 (s, 1H, Triazole-H), 7.49 (d, J = 2.3 Hz, 4H, Ph-H), 7.28 (s, 1H, Ph-H), 7.25 (s, 1H, Ph-H), 6.98–6.90 (m, 3H, Ph-H), 2.54 (s, 3H, CH3). MS (ESI) calculated for C19H13ClF3N2O2 [M + H]+, 414.1, found 414.1; Anal. Calcd for C19H13ClF3N2O2: C, 55.09; H, 3.16; N, 16.91%; Found: C, 55.13; H, 3.24; N, 17.05%.

3.1.7. 1-phenyl-1H-1,2,4-triazol-3-yl5-chloro-3-methyl-1-(p-tolyl)-1H-pyrazole-4-carboxylate (4d)

White solid, yield 52%; mp 154–156 °C; 1H-NMR (600 MHz, CDCl3) δ 8.38 (s, 1H, Triazole-H), 7.57 (d, J = 8.4 Hz, 6H, Ph-H), 7.49 (d, J = 8.3 Hz, 1H, Ph-H), 7.31 (t, J = 8.2 Hz, 2H, Ph-H), 2.62 (s, 3H, CH3), 2.42 (s, 3H, CH3). MS (ESI) calculated for C20H17ClF3N2O2 [M + H]+, 394.1, found 394.1; Anal. Calcd for C20H17ClF3N2O2: C, 61.00; H, 4.10; N, 17.78%; Found: C, 61.05; H, 4.18; N, 17.68%.

3.1.8. 1-(4-fluorophenyl)-1H-1,2,4-triazol-3-yl5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylate (4e)

White solid, yield 47%; mp 187–189 °C; 1H-NMR (600 MHz, CDCl3) δ 7.97 (s, 1H, Triazole-H), 7.58–7.43 (m, 6H, Ph-H), 6.98–6.91 (m, 3H, Ph-H), 2.55 (s, 3H, CH3). MS (ESI) calculated for C19H13ClF3N2O2 [M + H]+, 398.1, found 398.1; Anal. Calcd for C19H13ClF3N2O2: C, 57.37; H, 3.29; N, 17.61%; Found: C, 57.43; H, 3.30; N, 17.57%.

3.1.9. 1-(4-fluorophenyl)-1H-1,2,4-triazol-3-yl5-chloro-1-(4-fluorophenyl)-3-methyl-1H-pyrazole-4-carboxylate (4f)

White solid, yield 75%; mp 69–71 °C; 1H-NMR (600 MHz, CDCl3) δ 8.03 (s, 1H, Triazole-H), 7.89 (d, J = 8.2 Hz, 2H, Ph-H), 7.51 (dd, J = 8.8, 4.7 Hz, 2H, Ph-H), 7.35 (d, J = 8.1 Hz, 2H, Ph-H), 7.20 (d, J = 8.2 Hz, 2H, Ph-H), 2.54 (s, 3H, CH3). MS (ESI) calculated for C19H13ClF3N2O2 [M + H]+, 416.1, found 416.1; Anal. Calcd for C19H13ClF3N2O2: C, 54.89; H, 2.91; N, 16.84%; Found: C, 54.96; H, 3.10; N, 16.77%.

3.1.10. 1-(4-fluorophenyl)-1H-1,2,4-triazol-3-yl5-chloro-1-(4-chlorophenyl)-3-methyl-1H-pyrazole-4-carboxylate (4g)

White solid, yield 66%; mp 253–254 °C; 1H-NMR (600 MHz, CDCl3) δ 8.43 (s, 1H, Triazole-H), 7.65 (dd, J = 8.2, 4.0 Hz, 2H, Ph-H), 7.54–7.48 (m, 4H, Ph-H), 7.21 (t, J = 8.2 Hz, 2H, Ph-H), 2.61 (s, 3H, CH3). MS (ESI) calculated for C19H13ClF3N2O2 [M+H]+, 432.0, found 432.0; Anal. Calcd for C19H13ClF3N2O2: C, 52.80; H, 2.80; N, 16.20%; Found: C, 52.87; H, 2.77; N, 16.28%.

3.1.11. 1-(4-fluorophenyl)-1H-1,2,4-triazol-3-yl5-chloro-3-methyl-1-(p-tolyl)-1H-pyrazole-4-carboxylate (4h)

White solid, yield 71%; mp 99–101 °C; 1H-NMR (600 MHz, CDCl3) δ 8.41 (s, 1H, Triazole-H), 7.66 (dd, J = 8.1, 4.5 Hz, 2H, Ph-H), 7.42 (d, J = 7.6 Hz, 2H, Ph-H), 7.31 (d, J = 7.9 Hz, 2H, Ph-H), 7.21 (t, J = 8.0 Hz, 2H, Ph-H), 2.60 (s, 3H, CH3), 2.43 (s, 3H, CH3). MS (ESI) calculated for C20H15ClF3N2O2 [M + H]+, 412.1, found 412.1; Anal. Calcd for C20H15ClF3N2O2: C, 58.33; H, 3.67; N, 17.01%; Found: C, 58.41; H, 3.59; N, 17.09%.
3.1.12. 1-(4-chlorophenyl)-1H-1,2,4-triazol-3-yl5-chloro-1-(4-fluorophenyl)-3-methyl-1H-pyrazole-4-carboxylate (4i)

White solid, yield 51%; mp 84–86 °C; ¹H-NMR (600 MHz, CDCl₃) δ 8.46 (s, 1H, Triazole-H), 7.64 (d, J = 8.6 Hz, 2H, Ph-H), 7.55–7.54 (m, 2H, Ph-H), 7.50 (d, J = 8.6 Hz, 2H, Ph-H), 7.23–7.20 (m, 2H, Ph-H), 2.61 (s, 3H, CH₃). MS (ESI) calculated for C₁₉H₁₂Cl₂FN₅O₂ [M + H]⁺, 432.0, found 432.0; Anal. Calcd for C₁₉H₁₁Cl₂FN₅O₂: C, 52.80; H, 2.80; N, 16.20%; Found: C, 52.86; H, 2.74; N, 16.11%.

3.1.13. 1-(4-chlorophenyl)-1H-1,2,4-triazol-3-yl5-chloro-1-(4-chlorophenyl)-3-methyl-1H-pyrazole-4-carboxylate (4j)

White solid, yield 56%; mp 72–74 °C; ¹H-NMR (600 MHz, CDCl₃) δ 8.42 (s, 1H, Triazole-H), 7.55–7.53 (d, J = 3.1 Hz, 4H, Ph-H), 7.31 (dd, J = 8.9, 4.7 Hz, 2H, Ph-H), 7.20 (t, J = 8.4 Hz, 2H, Ph-H), 2.42 (s, 3H, CH₃). MS (ESI) calculated for C₁₉H₁₃Cl₂FN₅O₂ [M + H]⁺, 448.0, found 448.0; Anal. Calcd for C₁₉H₁₂Cl₂FN₅O₂: C, 50.86; H, 2.70; N, 15.61%; Found: C, 50.91; H, 2.64; N, 15.58%.

3.1.14. 1-(p-tolyl)-1H-1,2,4-triazol-3-yl-5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylate (4k)

White solid, yield 72%; mp 135–138 °C; ¹H-NMR (600 MHz, CDCl₃) δ 8.43 (s, 1H, Triazole-H), 7.57–7.46 (m, 7H, Ph-H), 7.31-7.40 (d, J = 8.2 Hz, 2H, Ph-H), 2.62 (s, 3H, CH₃), 2.42 (s, 3H, CH₃). MS (ESI) calculated for C₂₀H₁₇ClN₅O₂ [M + H]⁺, 394.1, found 394.1; Anal. Calcd for C₂₀H₁₆ClN₅O₂: C, 61.00; H, 4.10; N, 17.78%; Found: C, 61.08; H, 4.15; N, 17.84%.

3.1.15. 1-(p-tolyl)-1H-1,2,4-triazol-3-yl5-chloro-1-(4-fluorophenyl)-3-methyl-1H-pyrazole-4-carboxylate (4l)

White solid, yield 46%; mp 141–143 °C; ¹H-NMR (600 MHz, CDCl₃) δ 8.43 (s, 1H, Triazole-H), 7.55–7.53 (m, 4H, Ph-H), 7.31 (d, J = 8.2 Hz, 2H, Ph-H), 7.23-7.21 (t, J = 8.5 Hz, 2H, Ph-H), 2.61 (s, 3H, CH₃). MS (ESI) calculated for C₂₀H₁₆ClFN₅O₂ [M + H]⁺, 412.1, found 412.1; Anal. Calcd for C₂₀H₁₅ClFN₅O₂: C, 58.33; H, 3.67; N, 17.01%; Found: C, 58.42; H, 3.74; N, 17.07%.

3.1.16. 1-(p-tolyl)-1H-1,2,4-triazol-3-yl5-chloro-1-(4-chlorophenyl)-3-methyl-1H-pyrazole-4-carboxylate (4m)

White solid, yield 49%; mp 174–176 °C; ¹H-NMR (600 MHz, CDCl₃) δ 7.97 (s, 1H, Triazole-H), 7.53 (d, J = 4.2 Hz, 4H, Ph-H), 7.49 (dd, J = 8.9, 4.5 Hz, 1H, Ph-H), 7.00–6.91 (m, 3H, Ph-H), 2.55 (s, 3H, CH₃). MS (ESI) calculated for C₂₀H₁₅Cl₂N₅O₂ [M + H]⁺, 428.1, found 428.1; Anal. Calcd for C₂₀H₁₄Cl₂N₅O₂: C, 56.09; H, 3.53; N, 16.35%; Found: C, 56.13; H, 3.61; N, 16.44%.

3.2. In Vitro Antibacterial Activity

3.2.1. Medium

The solid media Luria-Bertani (LB)-Broth-Agar-Medium (tryptone 10 g/L, yeast 5 g/L, NaCl 10 g/L, agar 15 g/L, and distilled water 1000 mL, adjusted to pH 7.4 was used for testing the antibacterial activity.

3.2.2. Minimum Inhibitory Concentration (MIC)

The in vitro antibacterial activity for synthesized compounds 4a–m were evaluated using the agar-dilution method [32–34]. Two-fold serial dilutions of the compounds and reference drugs (ciprofloxacin) was prepared in LB-Broth-Agar-Medium. Drugs (10.0 mg) were dissolved in DMSO (1 mL) and the solution was diluted with water (9 mL). Further progressive double dilution with melted LB-Broth-Agar-Medium was performed to obtain the required concentrations of 128, 64, 32, 16, 8, 4, 2, 1, 0.5 µg/mL, and the MIC values were calculated separately. The bacterial inocula were prepared by suspending 24 h-old bacterial colonies from LB-Broth-Agar-Medium in 0.85% saline. The inocula were adjusted to 0.5 McFarland Standard (1.56108 CFU/mL). The suspensions were then
diluted in 0.85% saline to give 107 CFU/mL. Petri dishes were spot-inoculated with 1 µL of each of the prepared bacterial suspensions (104 CFU/spot) and incubated at 37 °C for 24 h. At the end of the incubation period, the MIC was determined, which is the lowest concentration of the test compound that resulted in no visible growth on the plate. A control test was also performed with test medium supplemented with DMSO at the same dilutions as used in the experiment in order to ensure that the solvent had no influence on bacterial growth.

3.3. Enzyme Inhibition Experimental

The in vitro antibacterial activity of the target compounds was carried out on by the methods of Sato et al. [35] and Peng and Marians [36]. First, the *E. coli* suspension is extracted to obtain a crude enzyme solution. After the purification step, the selected compounds are determined by gel electrophoresis to obtain data of different gradient concentrations, thereby calculating the IC₅₀ values.

3.4. Molecular Docking

The crystal structures of bacterial DNA topoisomerase II (PDB entry: 2xcs) was downloaded from the RCSB (Research Collaboratory for Structural Bioinformatic) Protein Data Bank. The molecular docking procedure was performed using CDOCKER protocol for receptor–ligand interactions section of DS 3.5 [29].

4. Conclusions

A series of novel triazole-containing pyrazole ester derivatives 4a–m were synthesized and evaluated for their biological activities. Compound 4d showed significant antibacterial activities with MIC values of 4, 2, 4, and 0.5 µg/mL against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella gallinarum*. Furthermore, compound 4d displayed the most potent Topo II (IC₅₀ = 13.5 µg/mL) and topoisomerase IV (IC₅₀ = 24.2 µg/mL) inhibitory activity. This study suggests that compound 4d can be used as a potential topoisomerase II inhibitor and this finding will lay the foundation for further structural modification and development of novel topoisomerase II inhibitors.

Supplementary Materials: The following are available online.

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