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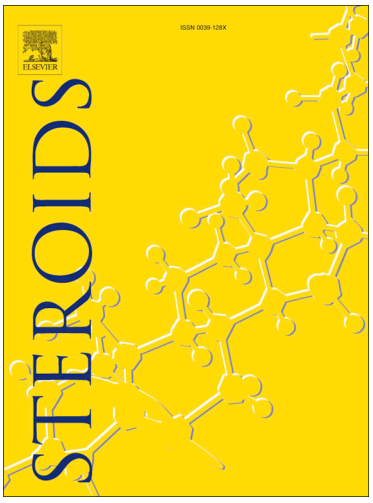

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Synthesis, Antimicrobial Potency with *In Silico* Study of Boc-Leucine-1,2,3-Triazoles

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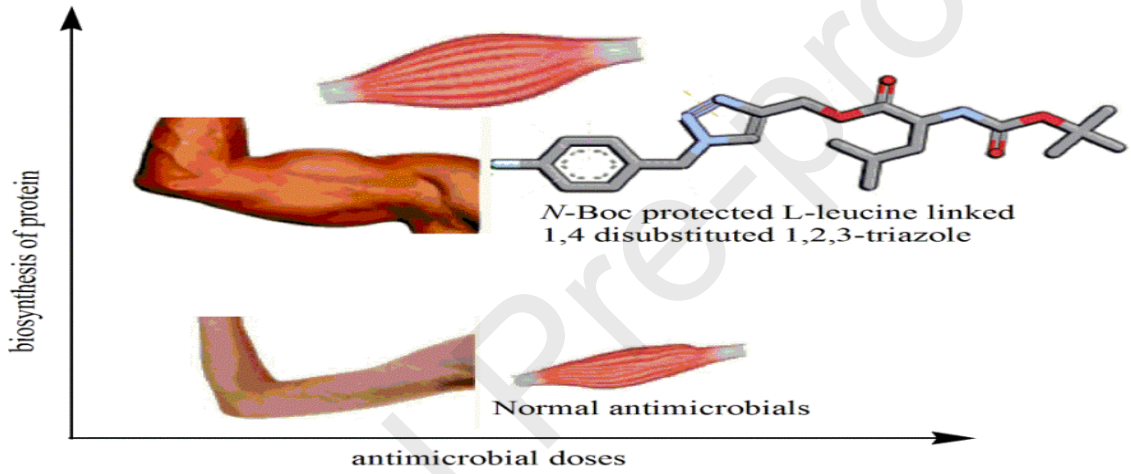


Abstract

A library of *N*-Boc protected Leucine-linked 1,4-disubstituted 1,2,3-triazoles was synthesized and fully characterized, in high yield via copper-catalyzed alkyne-azide cycloaddition (CuAAC) reaction. *In vitro* antibacterial activity showed that compound **4h** found to be more potent than the reference drug Ciprofloxacin (MIC: 0.0196 µmol/mL) against tested bacterial strains *S. entrica*, *B. subtilis, S. aureus, E. coli* and *P. auroginosa* with MIC: 0.0148, 0.0074, 0.0148, 0.0074, and 0.0074 µmol/mL, respectively and antifungal activity with MIC: 0.0148 µmol/mL as compared to reference drug Fluconazole (MIC: 0.0102 µmol/mL) against *A. niger* and *C. albicans* fungal strains. Further, the molecular docking study on **4h** and its predecessor alkyne **3** by choosing *E. coli* topoisomerase II, DNA Gyrase (PDB ID: 1KZN) showed better binding with triazole than alkyne and these results were supported by DFT study using B3LYP/6-311G(d,p) basis set.

Keywords: CuAAC; Boc-Leucine-1,2,3-triazole; Antibacterial activity; Antifungal activity; Molecular docking and DFT/B3LYP-6113G(d,p).

1. Introduction

The day by day increasing graph of microbial infection is causing a serious threat to public health all over the world. The continuous and uncontrolled use of chemotherapeutic drugs has developed multidrug resistance (MDR) to many antibiotics against various bacterial and fungal strains [1]. MDR severely limits the proper treatment of diseases [2]. Due to this, during last few years there was a sharp hike in the frequency of microbial diseases which creates the need for exploration and implementation of second-line antibiotics. So, a multifaceted perspective is required to modify existing and develop some new novel chemotherapeutic agent with a lesser side effect, more potency, better selectivity and broad-spectrum pharmacological activity which can target these MDR-microbes by a different and effective mechanism of action. In search of new antimicrobials, medicinal chemists generally rely on *N*-heterocyclic compounds. In this line, 1,2,3-triazoles have been explored as a significant class of synthetically versatile heterocyclic compounds. They have attracted paramount attention of organic chemists due to their promising and fascinating applications in the drug industry, agricultural chemistry, biological chemistry for the development of antibacterial [3-6], anticancer [7-10], antifungal [11-16], anti-HIV [17-18], antiallergic [19], antitubercular [20-23], anti-inflammatory [24-27], antiviral [28-31], antimalarial [32-33], anticonvulsant [34], antitrypanosomal [35], antileishmania, etc. [36-37]. The efficacy of 1,2,3-triazoles in medicinal chemistry and synthesis of various drugs is because of their stability towards acidic and basic hydrolysis in oxidoreductase environments which resist their metabolic degradation in a diverse range of pH to confirm their aromatic stabilization. Also, the high dipole moment, tendency to show dipole-dipole interaction and H- bonding makes 1,2,3-triazoles capable to effectively bind with different biological targets. Sharpless and Medal have modified the un-catalyzed 1,3-dipolar cycloaddition reaction (DCR) to a new approach of CuAAC reaction with regioselective formation of 1,4-disubstituted 1,2,3-triazoles which avoids unnecessary use of high activation energy of 24-26 kcal mol^{-1} [38-39]. Subsequently, various other methodologies have been proposed to show the improvement of process or product for the synthesis of 1,4-disubstituted 1,2,3-triazoles using various copper-based catalyst under different reaction conditions [40].

The long time use of antibiotics leads to loss of appetite and consequently loss of muscles and health due to unbalanced secretion of digestive juices or suppression in protein biosynthesis. It is known that Leucine, an essential amino acid is important for the protein biosynthesis. It cannot be synthesized in human body. During metabolism the end product of Leucine are acetoacetate, acetyl-CoA and a minor metabolite β-hydroxy β-methylbutyric acid which is responsible for pharmacological action and protein biosynthesis [41]. This year, Hang Wu, Buchang Zhang and co-workers have reported the importance of Leucine-responsive regulatory proteins (Lrps) for various biological processes in bacteria which stimulate transcription of some structural, resistance and regulatory genes [42]. Also, the important role of Leucine was reported recently by Zhang and co-workers as a Leucine-rich repeat and sterile alpha motif containing 1 (LRSAM1) for different cellular activities like antibacterial autophagy and cell adhesion [43]. Considering the importance of Leucine metabolism, current need for the development of advanced antimicrobial, biological potential of 1,2,3-triazoles and our interest in biological active triazoles, we thought to synthesize *N*-Boc protected Leucine-linked 1,4-disubstituted 1,2,3-triazoles as shown in **Figure 1**. Herein, we report the synthesis, *in vitro* antimicrobial activity and *in silico* study of *N*-Boc protected leucine linked 1,4-disubstituted 1,2,3-triazoles.

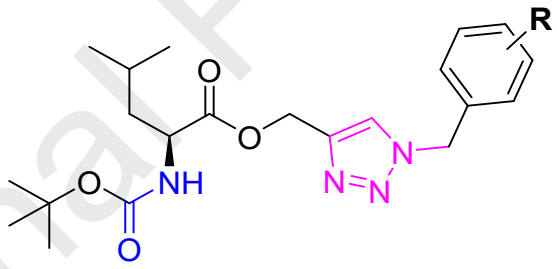


Figure 1. Design of *N*-Boc protected Leucine linked 1,4-disubstituted 1,2,3-triazole.

2. Materials and methods

2.1. General Chemistry

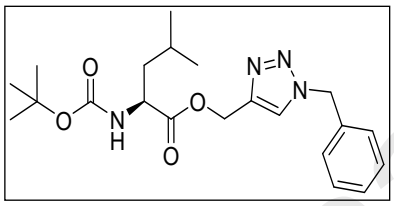
All the commercially available chemicals purchased from Sigma Aldrich. The starting chemicals including solvents were used purification as per the reported protocols. The melting points of all compounds were recorded in open capillaries using LABCO melting point apparatus. The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded using Avance III 400 MHz Bruker spectrometer. Tetramethylsilane (TMS) was used as an internal standard (chemical shift (δ) in ppm, coupling constant (*J*) in Hertz (Hz)). Infrared spectra of compounds were recorded

on SHIMAZDU IR AFFINITY-I FT-IR spectrophotometer. The high- resolution mass spectra (HRMS) were recorded using SCIEX-QTOF spectrometer. Readymade silica gel plates (SIL G/UV254, ALU-GRAM) were used to run thin-layer chromatography (TLC) and visualized in ultraviolet lamp box.

2.2. General procedure for the synthesis of Boc-Leucine linked triazoles (4a-j) [3].

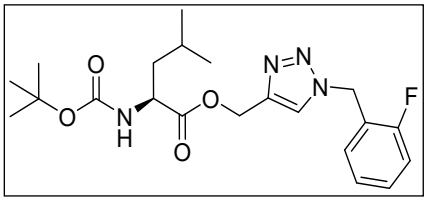
Differently substituted benzyl bromide, NaN_3 & alkyne **3** in THF:water (9:1 v/v) in 1.0 mmol, 3.0 mmol & 1.0 mmol, respectively were taken in a round bottom flask, then 10 mol % $CuSO_4.5H_2O$ and 20 mol % Na-ascorbate were added to it. This reaction solution was allowed to stir for 2-4 hrs at room temperature (i.e. 30˚C). The progression of reaction was studied by using thin layer column chromatography (TLC). The reaction was quenched with the addition of ice-cold water in the reaction mixture, after the completion of reaction. Now, the reaction product was diluted with ethyl acetate and extracted with brine solution followed by washing with aq. NH_4Cl: NH_3 (9:1 v/v) solution. The organic layer of ethyl acetate was evaporated by rotary evaporator under vacuum to obtain the desired triazoles in considerable to good yields.

2.2.1 (1-benzyl-1H-1,2,3-triazol-4-yl)methyl (tert-butoxycarbonyl)-L-leucinate (**4a**)



Appearance: off white solid, Yield: 95 %, m.p: 63-65 ◦C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.38 (d, *J* = 5.8 Hz, 3H), 7.28 (d, *J* = 5.9 Hz, 2H), 5.53 (s, 2H), 5.26 (s, 2H), 4.92 (s, 1H), 4.28 (t, *J* = 11.2 Hz, 1H), 1.64 (dd, *J* = 13.5, 7.0 Hz, 2H), 1.54 (dt, *J* = 10.2, 5.8 Hz, 1H), 1.41 (s, 9H), 0.89 (dd, *J* = 6.4, 3.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.33(s), 155.44(s), 142.98(s), 134.34(s), 129.15(s), 128.85(s), 128.09(s), 123.68(s), 79.91(s), 75.22(s), 58.25(s), 54.25(s), 52.17(s), 41.36(s), 28.27(s), 24.72(s), 22.77(s), 21.76(s). FT IR (KBr): ν_{max} 3387(s), 3142(s), 2955(m), 2872(m), 1749(s), 1688(s), 1504(m), 1391(m). HRMS $[M+H]^+$: m/z for $C_{21}H_{31}N_4O_4$: calculated 403.2345 and found 403.2175

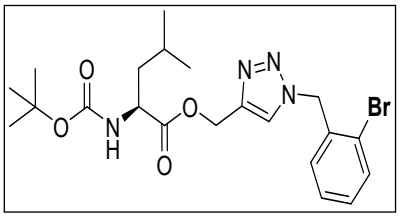
2.2.2 (1-(2-fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl (tert-butoxycarbonyl)-L-leucinate (**4b**)



Appearance: off white solid, Yield: 84%, m.p: 74-76 ◦C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.37 (dd, *J* = 13.5, 7.5 Hz, 1H), 7.29 (t, *J* = 7.2 Hz, 1H), 7.14 (dt, *J* = 14.6, 8.2 Hz, 2H), 5.59 (s, 2H), 5.27 (s, 2H), 4.91 (d, *J* = 8.3 Hz, 1H), 4.29 (t,

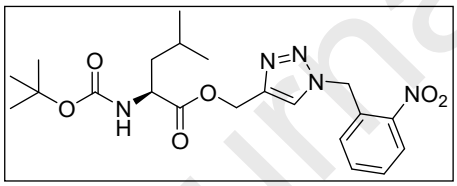
J = 11.2 Hz, 1H), 1.66 (dd, *J* = 14.2, 6.7 Hz, 2H), 1.57-1.49 (m, 1H), 1.42 (s, 9H), 0.89 (dd, *J* = 6.4, 3.4 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.34(s), 161.77(s), 159.31(s), 155.45(s), 142.99(s), 130.84 (d, *J* = 44.9 Hz), 124.87 (d, *J* = 3.7 Hz), 123.85(s), 115.88 (d, *J* = 21.1 Hz), 79.91(s), 58.23(s), 52.17(s), 47.75 (d, *J* = 4.3 Hz), 41.38(s), 28.26(s), 24.72(s), 22.75(s), 21.76 (s). FT IR (KBr): v_{max} 3389(s), 3146(m), 2957(s), 2872(m), 1751(s), 1686(s), 1506(w), 1368(m). HRMS $[M+H]^+$: m/z for $C_{21}H_{30}FN_4O_4$: calculated 421.2251 and found 421.2082.

2.2.3. (1-(2-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl (tert-butoxycarbonyl)-L-leucinate (**4c**)



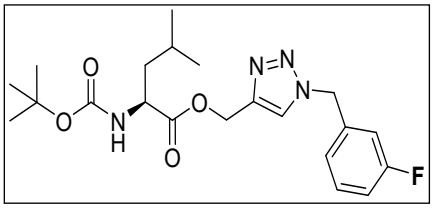
Appearance: off white solid, Yield: 80%, m.p: 83-85 °C.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.32 (t, *J* = 7.4 Hz, 1H), 7.23 (t, *J* = 7.6 Hz, 2H), 7.17 (d, *J* = 7.3 Hz, 1H), 5.66 (s, 2H), 5.27 (s, 2H), 4.92 (s, 1H), 4.29 (t, *J* = 10.7 Hz, 1H), 1.64 (dd, *J* = 13.2, 6.6 Hz, 2H), 1.53 – 1.43 (m, 1H), 1.41 (s, 9H), 0.89 (dd, *J* = 6.2, 3.0 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.34(s), 155.45(s), 142.88(s), 133.91(s), 133.27(s), 130.46 (d, *J* = 9.8 Hz), 128.26(s), 124.07(s), 123.51(s), 79.90(s), 58.24(s), 53.88(s), 52.17(s), 41.40(s), 28.28(s), 24.73(s), 22.78(s), 21.77(s). FT IR (KBr): v_{max} 3383(s), 3149(m), 2959(s), 2870(w), 1747(s), 1691(s), 1519(s), 1367(m)cm^{-1}. HRMS $[M+H]^+$: m/z for $C_{21}H_{30}BrN_4O_4$: calculated 481.1450 and found 481.1246

2.2.4. (1-(2-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl (tert-butoxycarbonyl)-L-leucinate (**4d**)



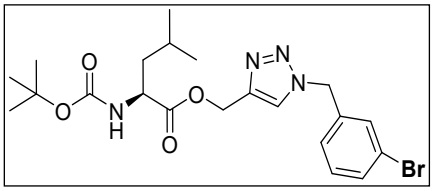
Appearance: off white solid, Yield: 93%, m.p: 80-82 °C.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.80 (s, 1H), 7.65 (t, *J* = 7.6 Hz, 1H), 7.57 (t, *J* = 8.4 Hz, 1H), 7.28 (s, 1H), 5.96 (s, 2H), 5.33 (s, 2H), 4.86 (d, *J* = 10.0 Hz, 1H), 4.33-4.29 (m, 1H), 1.68 (dd, *J* = 13.5, 7.2 Hz, 2H), 1.51 (dt, *J* = 5.8, 2.8 Hz, 1H), 1.43 (s, 9H), 0.93 (dd, *J* = 6.5, 2.5 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.34(s), 155.51(s), 142.82(s), 133.91(s), 133.27(s), 130.50 (d, *J* = 17.4 Hz), 128.26(s), 124.07(s), 123.51(s), 79.95(s), 58.24(s), 53.88(s), 52.26(s), 41.31(s), 28.28(s), 24.73(s), 22.84(s), 21.71(s). FT IR (KBr): v_{max} 3385(s), 3134(m), 3088(m), 2961(s), 1732(s), 1691(s),1522(s), 1368(s). HRMS $[M+H]^+$: m/z for $C_{21}H_{30}N_5O_6$: calculated 448.2196 and found 448.2001.

2.2.5. (1-(3-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl (tert-butoxycarbonyl)-L-leucinate (**4e**)



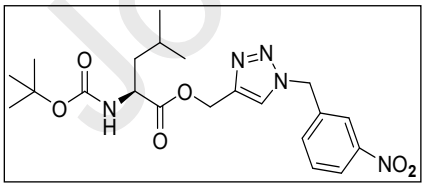
Appearance: off white solid, Yield: 85%, m.p: 69-71 °C.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.36 (dd, *J* = 14.4, 7.4 Hz, 1H), 7.06 (d, *J* = 7.2 Hz, 2H), 6.96 (d, *J* = 9.1 Hz, 1H), 5.53 (s, 2H), 5.28 (s, 2H), 4.91 (s, 1H), 4.32 – 4.26 (m, 1H), 1.64 (dd, *J* = 13.5, 6.6 Hz, 2H), 1.57-1.53 (m, 1H), 1.41 (s, 9H), 0.90 (dd, *J* = 5.7, 2.0 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.38(s), 164.23(s), 161.76(s), 155.47(s), 143.24(s), 136.74 (d, *J* = 7.1 Hz), 130.82 (d, *J* = 8.3 Hz), 123.69 (d, *J* = 20.9 Hz), 115.88 (d, *J* = 21.0 Hz), 115.02 (d, *J* = 22.3 Hz), 79.95(s), 58.23(s), 53.57(s), 52.20(s), 41.31(s), 28.25(s), 24.73(s), 22.75(s), 21.74(s). FT IR (KBr): ν_{max} 3387(s), 3142(m), 2959(s), 2872(m), 1745(s), 1686(s), 1593(m), 1391(m). HRMS $[M+H]^+$: m/z for $C_{21}H_{30}FN_4O_4$: calculated 421.2251 and found 421.2082

2.2.6. (1-(3-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl (tert-butoxycarbonyl)-L-leucinate (**4f**)



Appearance: off white solid, Yield: 91%, m.p: 78-80 °C.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.43 (s, 1H), 7.26 (t, *J* = 7.8 Hz, 1H), 7.20 (d, *J* = 7.8 Hz, 1H), 5.50 (s, 2H), 5.28 (s, 2H), 4.89 (s, 1H), 4.29 (t, *J* = 11.3 Hz, 1H), 1.65 (dd, *J* = 13.5, 7.0 Hz, 1H), 1.50 – 1.44 (m, 2H), 1.41 (s, 9H), 0.90 (dd, *J* = 6.4, 3.7 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.36(s), 155.46(s), 143.28(s), 136.57(s), 132.01(s), 130.87 (d, *J* = 31.3 Hz), 126.58(s), 123.75(s), 123.12(s), 79.93(s), 58.26(s), 53.43(s), 52.19(s), 41.34(s), 28.27(s), 24.74(s), 22.78(s), 21.76(s). FT IR (KBr): ν_{max} 3402(s), 3152(m), 2957(s), 1738(w), 1688(w), 1579(m), 1506(s), 1365(s). HRMS $[M+H]^+$: m/z for $C_{21}H_{30}BrN_4O_4$: calculated 481.1450 and found 481.1246

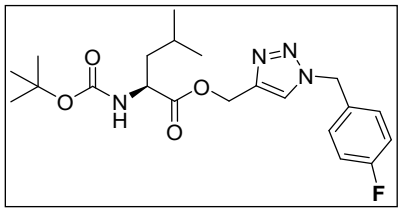
2.2.7 (1-(3-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl (tert-butoxycarbonyl)-L-leucinate (**4g**)



Appearance: off white solid, Yield: 86%, m.p: 68-70 °C.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.80 (s, 1H), 7.65 (t, *J* = 7.6 Hz, 1H), 7.56 (t, *J* = 7.2 Hz, 1H), 7.28 (s, 1H), 5.96 (s, 2H), 5.32 (s, 2H), 4.88 (d, *J* = 8.2 Hz, 1H), 4.33 – 4.30 (m, 1H), 1.67 (dd, *J* = 12.6, 6.4 Hz, 2H), 1.53-1.47 (m, 1H), 1.42 (s, 9H), 0.92 (dd, *J* = 6.5, 2.2 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.37(s), 155.43(s), 147.49(s), 143.20(s), 134.41(s), 130.38(s), 129.75(s), 125.43(s), 124.83(s), 79.96(s), 58.22(s), 52.18(s), 50.89(s), 41.40(s),

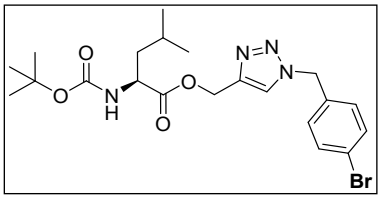
28.27(s), 24.76(s), 22.80(s), 21.78(s). FT IR (KBr): v_{max} 3385(s), 3132(m), 3088(m), 2961(s), 1732(s), 1695(s), 1522(s), 1368(m). HRMS $[M+H]^+$: m/z for $C_{21}H_{30}N_5O_6$: calculated 448.2196 and found 448.2001.

2.2.8. (1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl (tert-butoxycarbonyl)-L-leucinate (**4h**)



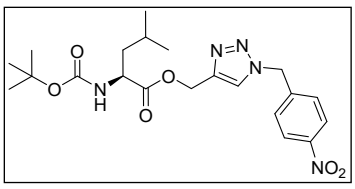
Appearance: off white solid, Yield: 90%, m.p: 77-79 °C.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.28 (t, *J* = 6.8 Hz, 2H), 7.08 (t, *J* = 8.5 Hz, 2H), 5.50 (s, 2H), 5.27 (s, 2H), 4.87 (s, 1H), 4.28 (dd, *J* = 13.3, 8.3 Hz, 1H), 1.69 (d, *J* = 11.0 Hz, 2H), 1.55 (dt, *J* = 14.3, 7.4 Hz, 1H), 1.42 (s, 9H), 0.90 (dd, *J* = 6.4, 3.5 Hz, 6H) ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.35(s), 164.14(s), 161.67(s), 155.43(s), 143.20(s), 129.99 (d, *J* = 8.4 Hz), 123.54(s), 116.18 (d, *J* = 21.8 Hz), 79.91(s), 58.28(s), 53.47(s), 52.18(s), 41.37(s), 28.26(s), 24.73(s), 22.77(s), 21.76(s). FT IR (KBr): v_{max} 3393(s), 3154(s), 2978(s), 2873(), 1734(s), 1688(s), 1512(s), 1367(m). HRMS $[M+H]^+$: m/z for $C_{21}H_{30}FN_4O_4$: calculated 421.2251 and found 421.2082.

2.2.9. (1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methyl (tert-butoxycarbonyl)-L-leucinate (**4i**)



Appearance: off white solid, Yield: 94%, m.p: 98-100 °C.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.16 (d, *J* = 8.3 Hz, 2H), 5.49 (s, 2H), 5.28 (s, 2H), 4.86 (s, 1H), 4.29 (t, *J* = 11.1 Hz, 1H), 1.67 (dd, *J* = 13.3, 5.8 Hz, 2H), 1.50-1.44 (m, 1H), 1.42 (s, 9H), 0.91 (dd, *J* = 6.4, 3.4 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.35(s), 155.43(s), 143.28(s), 133.41(s), 132.35(s), 129.69(s), 123.63(s), 123.05(s), 79.92(s), 58.26(s), 53.53(s), 52.19(s), 41.36(s), 28.26(s), 24.74(s), 22.79(s), 21.77(s). FT IR (KBr): v_{max} 3406(s), 3134(m), 2953(s), 2872(m), 1736(s), 1691(s), 1506(s), 1369(m). HRMS $[M+H]^+$: m/z for $C_{21}H_{30}BrN_4O_4$: calculated 481.1450 and found 481.1246

2.2.10. (1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl (tert-butoxycarbonyl)-L-leucinate (**4j**)



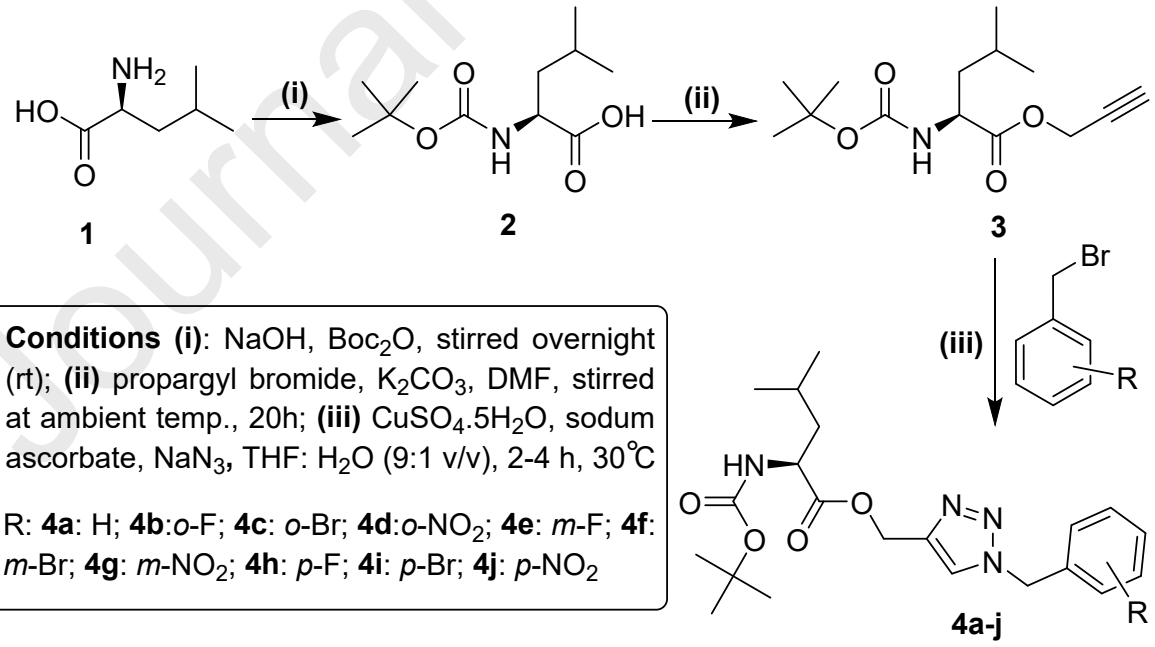
Appearance: off white solid, Yield: 87%, m.p: 88-90 °C.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.16 (d, *J* = 8.3 Hz, 2H), 5.49 (s, 2H), 5.28 (s, 2H), 4.86 (s, 1H), 4.29 (t, *J* = 11.1 Hz, 1H), 1.67 (dd, *J* = 13.3, 5.8 Hz, 2H), 1.50 – 1.44 (m, 1H),

1.42 (s, 9H), 0.91 (dd, J = 6.4, 3.4 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.39(s), 155.47(s), 143.28(s), 133.46(s), 132.35(s), 129.69(s), 123.71(s), 123.05(s), 79.98(s), 58.26(s), 53.53(s), 52.14(s), 41.36(s), 28.26(s), 24.74(s), 22.79(s), 21.77(s). FT IR (KBr): ν_{max} 3350(w), 3144(m), 2961(s), 2872(m), 1744(s), 1705(s), 1526(s), 1348(s). HRMS $[M+H]^+$: m/z for $C_{21}H_{30}N_5O_6$: calculated 448.2196 and found 448.2001.

3. Results and discussion

3.1. Synthesis of Boc-Leucine linked triazoles **4a-j**, **[**3, 44-45**]**.

We have explored the click reaction of C-terminal propargyl esters of *N*-protected L-Leucine with various organic azides. Initially, *N*-Boc protected Leucine **2**, as shown in **Scheme 1**, was prepared by treating Leucine with Boc_2O in aqueous sodium hydroxide (NaOH) solution with the help of reported procedure [44]. The synthesis of alkyne precursor **3** was accomplished by reacting *N*-Boc-Leucine **2**, propargyl bromide, and anhydrous potassium carbonate (K_2CO_3) in dry dimethylformamide (DMF). This solution was stirred for 20 hrs at ambient temperature under inert N_2 atmosphere [45]. The so prepared alkyne **3** was allowed to react in presence of variously substituted organic azides formed *in situ* via reaction of benzyl bromides with NaN_3 in presence of $CuSO_4.5H_2O$ and Na-ascorbate using various combinations of solvent systems under different set of reaction conditions as described in **Table 1**. However, better reaction condition for the form-



Conditions (i): NaOH, Boc_2O, stirred overnight (rt); **(ii)** propargyl bromide, K_2CO_3, DMF, stirred at ambient temp., 20h; **(iii)** $CuSO_4.5H_2O$, sodum ascorbate, NaN_3, THF: H_2O (9:1 v/v), 2-4 h, 30℃

R: **4a**: H; **4b**:*o*-F; **4c**: *o*-Br; **4d**:*o*-NO_2; **4e**: *m*-F; **4f**: *m*-Br; **4g**: *m*-NO_2; **4h**: *p*-F; **4i**: *p*-Br; **4j**: *p*-NO_2

Scheme 1: Synthesis of Boc-Leucine linked-1,2,3 triazoles (**4a-j**).

Table 1. Reaction of **3** with *in situ* generated $PhCH_2N_3$ from $PhCH_2Br$ and NaN_3 using $CuSO_4.5H_2O$/sodium ascorbate in different solvent systems for the formation of triazole **4a**.[a]

Sr. No.	Solvent	Composition	Temp(℃)	Time(h)	4a Yield(%)[b]
1	t-BuOH:H_2O	1:1	30	4.0	77
2	t-BuOH:H_2O	1:1	40	3.5	82
3	t-BuOH:H_2O	9:1	30	3.0	88
4	t-BuOH:H_2O	9:1	40	3.0	86
5	t-BuOH	100%	30-40	24	*c*
6	DMF:H_2O	1:1	30	3.5	79
7	DMF:H_2O	1:1	40	3.0	82
8	DMF:H_2O	9:1	30	2.5	85
9	DMF:H_2O	9:1	40	2.5	82
10	DMF	100%	30-40	24	*c*
11	THF:H_2O	1:1	30	3.0	87
12	THF:H_2O	1:1	40	2.5	88
13	THF:H_2O	9:1	30	2.0	95
14	THF:H_2O	9:1	40	2.0	92
15	THF	100%	30-40	24	*c*
16	DMSO:H_2O	1:1	30	3.5	82
17	DMSO:H_2O	1:1	40	2.5	85
18	DMSO:H_2O	9:1	30	2.0	88
19	DMSO:H_2O	9:1	40	2.0	82
20	DMSO	100%	30-40	24	*c*

[a]**Reaction Condition:** Benzyl azide and alkyne **3** (1 mmol each); $CuSO_4.5H_2O$ (10 mol%) and sodium ascorbate (20 mol%), rt, 2-4 h for S. No. 10,15 & 20 time: 24h. [b]Yield refers to purification by recrystallization. [c]No reaction.

-ation of (1-benzyl-1*H*-1,2,3-triazol-4-yl) methyl (tert-butoxycarbonyl)-L-Leucinate **4a** in high yield of 95% from alkyne **3** required tetrahydrofuran (THF): H_2O (9:1 v/v) mixture as solvent system wherein reaction was completed in 2h after stirring reaction mixture at ambient temperature (30℃).

The triazole **4a** was fully characterized by physical spectroscopic data. For instance, the FT IR spectrum of the representative compound **4a** showed a characteristic band in the region 3142 cm^{-1} due to =C−H stretching of 1,2,3-triazole ring. The N−H stretching band appeared at 3387 cm^{-1}. The band observed at 1749 cm^{-1} was due to the C=O stretching of the ester group. The ^1H NMR spectrum exhibited a characteristic peak at δ 7.56 ppm appeared as singlet equivalent to one proton

of methine of triazole ring to confirm triazole formation. The singlet corresponding to 2H observed at δ 5.26 ppm was assigned to the methylene protons linked to N-1 of triazole ring whereas the singlet observed at δ 5.53 ppm was due to two protons of methylene group attached to ester linkage. A singlet at δ 1.41 ppm integrating for nine protons was assigned to methyl protons of protecting group. Further, a broad singlet at δ 4.92 ppm was attributed to -NH proton. However, the ^{13}C NMR spectrum showed signals due to carbonyl carbon at δ 173.33 ppm (ester) and δ 155.44 ppm (carbamate) and signals at δ 142.98 and δ 134.34 ppm attributed to 4 and 5 carbon atom positions in the triazole ring. Also, two signals at δ 75.22 and δ 58.25 ppm were accounted for the methylene carbons attached to the ester linkage and other methylene carbons linked to N-1 of triazoles, respectively. Finally, the HRMS spectrum of the compound **4a** displayed *m/z* signal due to $(M+H)^+$ at 403.2175 which is in good agreement with the calculated value for $C_{21}H_{31}N_4O_4$: 403.2345.

On the basis of information derived from the standardization of reaction condition for the formation of representative triazole **4a**, other derivatives of N-Boc protected Leucine linked 1,4-disubstituted 1,2,3-triazoles **4b-j** were synthesized under these reaction condition. The complete detail for the formation of *N*-Boc protected L-Leucine linked 1,4-disubstituted-1,2,3-triazoles (**4a-j**) is presented in the **Table 2.** The formation of alkyne **3** and all synthesized triazoles **4a-j** were

Table 2. The reaction of *N*-Boc protected L-Leucine linked alkyne (**3**) for the formation of corresponding 1,4-disubstituted 1,2,3-triazoles (**4a-j**) using CuAAC reaction.[a]

Sr. No.	Compound	R	Time(h)	Yield (%)[b]
1	**4a**	H	2.0	95
2	**4b**	*o*-F	2.5	84
3	**4c**	*o*-Br	3.0	80
4	**4d**	*o*-NO_2	3.0	93
5	**4e**	*m*-F	4.0	85
6	**4f**	*m*-Br	3.5	91
7	**4g**	*m*-NO_2	4.0	86
8	**4h**	*p*-F	2.0	90
9	**4i**	*p*-Br	2.5	94
10	**4j**	*p*-NO_2	2.0	87

[a]**Reaction Condition:** Benzyl azide **4a-j** and alkyne **3** (1 mmol each); $CuSO_4.5H_2O$(10 mol%) and sodium ascorbate (20 mol%), rt, 2-4 h. [b]Yield refers to purification by recrystallization

structurally confirmed on the basis of FT IR, ^{1}H NMR, and ^{13}C NMR data. However, the structures of all new *N*-Boc protected Leucine linked 1,4-disubstituted 1,2,3-triazoles (**4a-j**) were finally established by HRMS data.

3.2. Biological Activities

3.2.1. Antibacterial activity

All the newly synthesized *N*-Boc protected Leucine linked 1,4-disubstituted 1,2,3-triazole (**4a-j**) including alkyne **3** were screened *in vitro* for their antibacterial activity against *S. aureus, S. entrica, B. subtilis* (gram positive bacteria) and *E. coli, P. auroginosa* (gram negative bacteria) by a standard method of serial dilution. In this method, a 100 µg/mL concentration stock solution was prepared using DMSO and commonly used antibacterial drug Ciprofloxacin was used as solvent and reference drug, respectively [46]. The dilution of the initial stock solution of 50 to 3.12 µg/mL concentration was made. Each of these diluted lot was incubated at 37℃ for 24 hours after inoculating with 100 µg/mL suspension of the chosen bacterial strain in sterile saline. A blank experiment under similar conditions with a nutrient medium was also performed to observe the solvent effect on the bacterial growth. A minimum inhibitory concentration, MIC in µmol/mL for alkyne **3**, all the 1,2,3-triazoles **4a-j** are tabulated in **Table 3**.

Table 3. *In vitro* antibacterial activity of *N*-Boc protected L-Leucine linked 1,2,3-triazoles (**4a-j**, MIC in µmol/mL).

Sr. No	Compound	R	*S. entrica*	*B. subtilis*	*S. aureus*	*E. coli*	*P. auroginosa*
1	**3**	**---**	0.0464	0.0929	0.0464	0.0929	0.0464
2	**4a**	H	0.0155	0.0310	0.0310	0.0310	0.0155
3	**4b**	*o*-F	0.0074	0.0148	0.0148	0.0297	0.0148
4	**4c**	*o*-Br	0.0260	0.0130	0.0130	0.0130	0.0130
5	**4d**	*o*-NO_2	0.0139	0.0558	0.0279	0.0558	0.0279
6	**4e**	*m*-F	0.0074	0.0148	0.0297	0.0297	0.0074
7	**4f**	*m*-Br	0.0130	0.0260	0.0260	0.0260	0.0130
8	**4g**	*m*-NO_2	0.0279	0.0558	0.0279	0.0279	0.0558
9	**4h**	*p*-F	0.0148	0.0074	0.0148	0.0074	0.0074
10	**4i**	*p*-Br	0.0130	0.0260	0.0260	0.0130	0.0260
11	**4j**	*p*-NO_2	0.0279	0.0279	0.0279	0.0558	0.0279
12	**Ciprofloxacin**		0.0196	0.0196	0.0196	0.0196	0.0196

By observing antibacterial screening results, it was found that in case of *S. entrica* compounds **4b** and **4e** each having F-moiety on benzene ring with MIC value 0.0074 µmol/mL showed more than two fold better antibacterial activity than the reference drug Ciprofloxacin with MIC: 0.0196 µmol/mL. Similarly, in case of *B. subtilis* among all the synthesized compounds, compound **4h** again having F-moiety on benzene ring with MIC: 0.0074 µmol/mL showed two folds better antibacterial activity than the reference drug Ciprofloxacin with MIC: 0.0196 µmol/mL. Otherwise, all *N*-Boc protected L-Leucine linked 1,4-disubstituted-1,2,3-triazoles containing F-moiety at *ortho*, *meta* and *para* position of the benzene ring i.e. **4b**, **4e** and **4h** are more potent than reference drug Ciprofloxacin (MIC: 0.0196 µmol/mL) for all tested bacterial strains with MIC value either 0.0074 or 0.0148 µmol/mL except **4b** and **4e** showed little higher MIC: 0.0297 µmol/mL for *E. coli* and *S. aureus*. Compounds containing *o*-Br, *m*-Br and *p*-Br are also next more potent than F-moiety containing triazoles *i.e. o*-Br containing compound **4c** for *B. subtilis*, *S. aureus*, *E. coli*, and *P. auroginosa*; *m*-Br containing compound **4f** for *S. entrica* and *P. auroginosa* and *p*-Br containing compound **4i** for *S. entrica* and *E. coli* with MIC: 0.0130 µmol/mL, each are also more potent than reference drug Ciprofloxacin with MIC: 0.0196 µmol/mL. Moreover, compound **4c** and **4h** with MIC: 0.0130 µmol/mL and 0.0148 µmol/mL, respectively were found to be more potent among all triazoles than reference drug Ciprofloxacin against *S. aureus*. Compound **4h** with MIC: 0.0074 µmol/mL was found more potent among all triazoles than reference drug Ciprofloxacin with MIC value 0.0196 µmol/mL against *E. coli*. The activity data revealed that in the case of *P. auroginosa* the compound **4e** and **4h** both with MIC: 0.0074 µmol/mL are more potent than Ciprofloxacin. However, alkyne **3** is least active to all bacterial strains with MIC values ranging from 0.0464 to 0.0929 µmol/mL, as shown in **Figure 2**.

The SAR study which is abbreviated as structure-activity relationship shows that all *N*-Boc-protected-L-Leucine linked 1,2,3-triazoles showed better efficacy than alkyne which confirms that incorporation of 1,2,3-triazole ring improves the antibacterial activity to a large extent. It was also observed that compound containing *o*, *m*, or *p*-F-group in benzyl moiety shows better antibacterial activity than unsubstituted benzyl, *o*, *m*, or *p*-Br- and *o*, *m*, or *p*-NO_2-group substituted benzyl group containing triazoles.

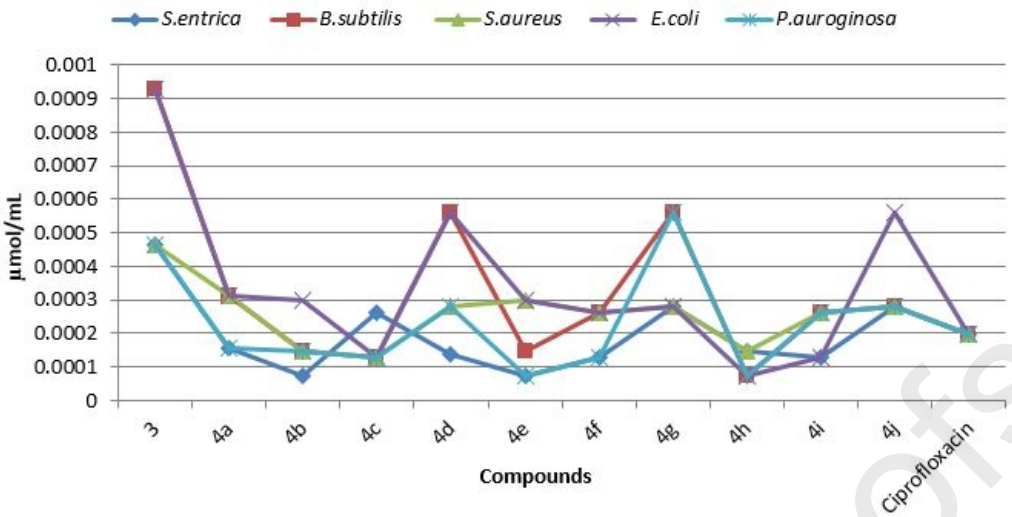


Figure 2. Antibacterial activities of synthesized Leucine linked alkyne **3** & triazoles **4a-j**.

3.2.2. Antifungal activity

All the synthesized *N*-Boc protected L-Leucine linked 1,2,3-triazoles **4a-j** including predecessor alkyne **3** were also screened for *in vitro* antifungal activity by the same standard dilution method (Cappucim and Sherman,1999) against *A. niger* and *C. albicans* fungal strains. The commonly used antifungal drug Fluconazole was used as a reference. The DMSO and Sabouraud dextrose broth were used, respectively as solvent and culture media. The suspension of Aspire was prepared in sterile saline from 24 hours old culture of fungus developed on Sabouraud dextrose broth (SDB, Hi-Media, Mumbai). The samples were incubated at 37℃ and were observed after 2 days for *A. niger*. In case of the *C. albicans* samples were incubated at 25℃ for 7 days. The MIC of all the tested compounds were recorded in µmol/mL as shown in **Table 4**.

The observation of antifungal activity data as shown in **Figure 3**, revealed that all *N*-Boc protected L-Leucine linked 1,2,3-triazoles showed better activity with MIC value in the range of 0.013 to 0.0311 µmol/mL than *N*-Boc protected L-Leucine linked alkyne **3** with MIC: 0.1859 and 0.0929 µmol/Ml for *A. niger* and *C. albicans*, respectively as compared to the Fluconazole (reference drug) with MIC value of 0.0102 µmol/mL [47]. In case of both fungal strains *A. niger* and *C. albicans*, compounds **4c** and **4h** with MIC 0.013 and 0.0148 µmol/mL, respectively exhibited almost comparable activity with the reference drug Fluconazole. Similarly, in the case of *C. albicans* compound, **4c** and **4i** both having MIC values 0.013 µmol/mL are found to have a considerable activity as compared to the reference drug Fluconazole.

Table 4. Antifungal *in vitro* activity results of *N*-Boc protected L-Leucine linked alkyne **3** and corresponding 1,4-disubstituted 1,2,3-triazoles (**4a-j**) in MIC: µmol/mL.

Sr. No	Compound	R	*A. niger*	*C. albicans*
1	**3**	**---**	0.1859	0.0929
2	**4a**	H	0.0311	0.0155
3	**4b**	*o*-F	0.0297	0.0148
4	**4c**	*o*-Br	0.0130	0.0130
5	**4d**	*o*-NO_2	0.0279	0.0139
6	**4e**	*m*-F	0.0297	0.0148
7	**4f**	*m*-Br	0.0260	0.0130
8	**4g**	*m*-NO_2	0.0279	0.0279
9	**4h**	*p*-F	0.0148	0.0148
10	**4i**	*p*-Br	0.0260	0.0130
11	**4j**	*p*-NO_2	0.0279	0.0279
12	**Fluconazole**		0.0102	0.0102

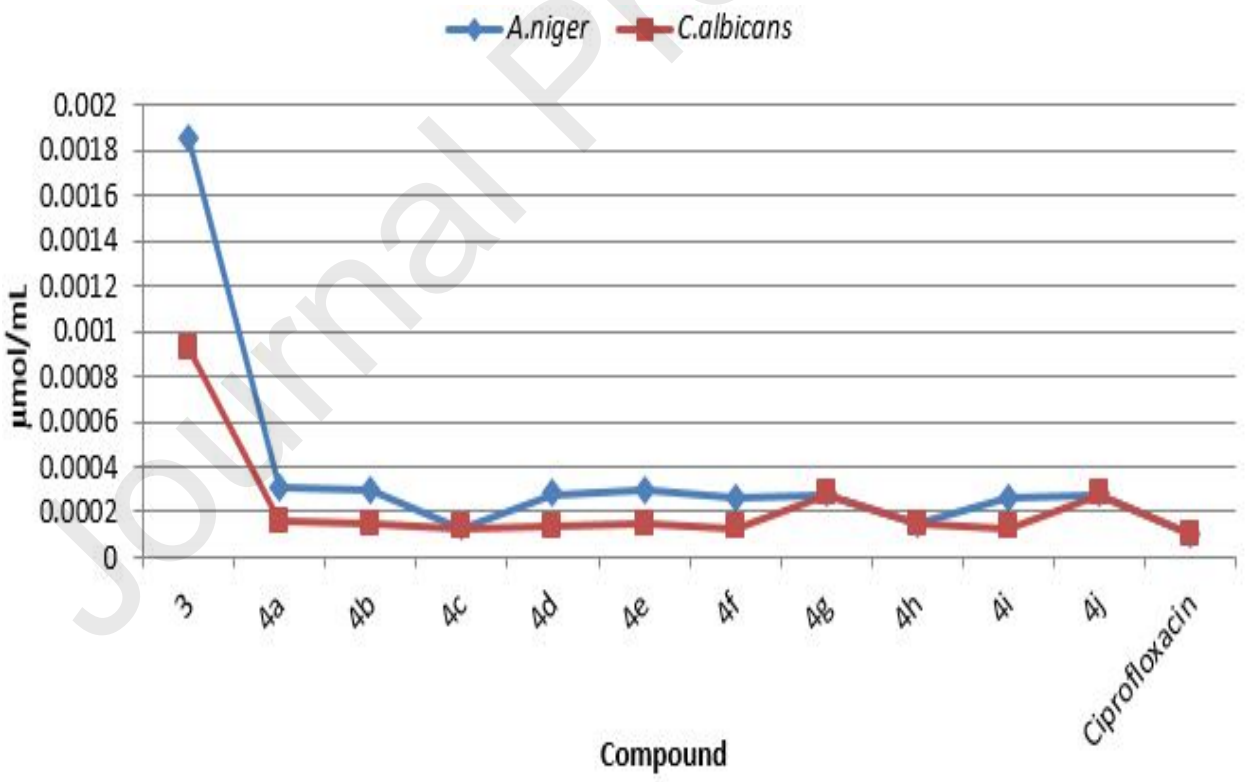


Figure 3. Antifungal activities of synthesized Leucine linked alkyne **3** & triazoles **4a-j**.

3.3. Pharmacokinetics properties for drug-likeness

The drug like property of compounds depends on their absorption, distribution, metabolism, excretion (ADME) in human body. All these processes correlate well with pharmacokinetic properties such as molecular weight, TPSA, permeability, octanol-water coefficient (logP) etc. In the present study, the pharmacokinetic property of *N*-Boc protected L-Leucine linked alkyne **3** and its 1,2,3-triazole derivatives **4a-j** were calculated using web tool of Swiss ADME and are presented in **Table 5** [48].

Table 5. Physicochemical data for drug-likeness score for synthesized *N*-Boc-L-Leucine linked alkyne **3** and its 1,2,3-triazoles **4a-j** calculated using a web tool of Swiss ADME.

Compound	MW[a]	HA[b]	RB[c]	HBA[d]	HBD[e]	MR[f]	TPSA[g]	MLogPh	WS[i]	GIA[j]	LV[k]
3	269.34	19	9	4	1	73.37	64.63	2.14	-2.61	86.70	0
4a	402.49	29	12	6	1	109.41	95.34	2.26	-4.00	76.108	0
4b	420.48	30	12	7	1	109.37	95.34	2.64	-4.17	76.108	0
4c	481.38	30	12	6	1	117.11	95.34	2.86	-4.92	76.108	0
4d	447.48	32	13	8	1	118.23	141.16	1.43	-4.08	60.300	1
4e	420.48	30	12	7	1	109.37	95.34	2.64	-4.17	76.108	0
4f	481.38	30	12	6	1	117.11	95.34	2.86	-4.92	76.108	0
4g	447.48	32	13	8	1	118.23	141.16	1.43	-4.08	60.300	1
4h	420.48	30	12	7	1	109.37	95.34	2.64	-4.17	76.108	0
4i	481.38	30	12	6	1	117.11	95.34	2.86	-4.92	76.108	0
4j	447.48	32	13	8	1	118.23	141.16	1.43	-4.08	60.300	1

[a]molecular weight, [b]heavy atoms, [c]rotable bonds, [d]hydrogen bond acceptors, [e]hydrogen bond donors, [f]molar refractivity, [g]total polar surface area, [h]octanol-water partition coefficient, [i]water solubility, [j]gastrointestinal absorbance, [k]Lipinski's violations

The calculated physicochemical data of synthesized *N*-Boc protected L-Leucine linked alkyne **3** and its 1,4-disubstituted 1,2,3-triazoles **4a-j** as presented in **Table 5**, showed that the low molecular weight of drugs is the primary requirement of any drug to get absorbed in human body. Compounds with higher molecular weights have fewer tendencies to cross the cell membrane and are less likely to be absorbed in the body. Thus, they have fewer tendencies to reach the biological target. Therefore, molecular weights of drugs should be less than equal to 500 (≤500). The distribution, absorption and excretion of compounds depend upon their lipophilicity. The logP value of a molecule is a log value of its partition coefficient between *n*-$C_8H_{17}OH$ and H_2O $\log(c_{octanol}/c_{water})$. It is a measure of lipophilicity of any chemical. The smaller the value of logP

(≤5) more will be the absorption of drug and higher value of logP indicates their less metabolism and fast excretion from the body. All our synthesized triazoles have logP value <5. The TPSA which is abbreviated as topological polar surface area is another factor that correlates well with properties like blood brain barrier penetration and intestinal absorption. If the total area of all the polar atoms exposed to the surface of a molecule is less than 160 $Å^2$**,** then the molecule is likely to pass easily through the cell membrane. In our study**,** TPSA value of all synthesized compounds lies between 64.63-141.16, which shows their permeation and good absorption (% ABS= 66.2998-76.1077). The % absorption (% ABS) can be calculated by % ABS = 109**-**(0.345 × TPSA) formula. The water solubility (WS) of any drug also determines its absorption and distribution properties. Almost 80% of the explored drugs highlighted logS value more than -5. Molecules under study showed WS in a range of -2.61 to -4.92. The number of H-bond donor and acceptor of a good drug must have cut off value ≤5 and ≤10**,** respectively. All the synthesized compounds (**3** and **4a-j**) showed good permeation and absorption, as indicated by unity value of H-bond donors for all compounds and H-bond acceptors value for alkyne is 4 and for triazoles it lies between 6-8 which are less than their cut off value i.e. 5 and 10**,** respectively. It was observed that the majority of orally active drugs (almost 90%) obeys *Rule-of-five* proposed by Lipinski [49]. The parameters of all the tested compounds (**3** and **4a-j**) were found within the limits of the rule. According to this rule, the MW of any ideal drug must be less than and equal to 500 (i.e. ≤ 500), the octanol-water partition coefficient (LogP) must be ≤ 5, H-donors ≤ 5 and H-acceptors ≤10 and number of violations to this rule should be ≤1. All the synthesized molecules fairly obey this *rule-of-five* and these bioactive molecules have good drug like property.

As a general rule, if the bioactivity score of any drug is > -0.5 then the compound is active and if the bioactivity score is < -0.5 then the compound is inactive. The bioactivity score of *N*-Boc protected Leucine linked alkyne **3** and its 1,2,3-triazoles **4a-j** as per the data given in the **Table 6**, showed that the synthesized compounds are active with bioactivity score > -0.5.

Table 6. Bioactivity score of *N*-Boc protected Leucine linked alkyne **3** and 1,2,3-triazoles **4a-j** by Molinspiration Cheminformatics software [50].

Compound	R	GPCR[a]	ICM[b]	KI[c]	NRL[d]	PI[e]	EI[f]
3	----	0.29	0.43	-0.45	0.14	0.66	0.40
4a	H	0.28	0.26	-0.31	-0.12	0.55	0.32
4b	*o*-F	0.28	0.21	-0.30	-0.11	0.52	0.29
4c	*o*-Br	0.12	0.14	-0.39	-0.19	0.38	0.24
4d	*o*-NO_2	0.16	0.21	-0.40	-0.23	0.38	0.18
4e	*m*-F	0.28	0.24	-0.27	-0.09	0.53	0.30
4f	*m*-Br	0.18	0.17	-0.36	-0.23	0.41	0.24
4g	*m*-NO_2	0.12	0.17	-0.40	-0.20	0.37	0.18
4h	*p*-F	0.28	0.23	-0.28	-0.10	0.51	0.29
4i	*p*-Br	0.19	0.18	-0.34	-0.21	0.43	0.25
4j	*p*-NO_2	0.13	0.18	-0.40	-0.20	0.37	0.19

[a]G-protein coupled receptor; [b]ion channel modulator; [c]kinase inhibitor; [d]nuclear receptor ligand; [e]protease inhibitor; [f]enzyme inhibitor

3.4. Molecular docking studies

The docking studies of heterocycles like triazoles were performed and studied by choosing bacterial enzyme DNA gyrase of *E. coli* (topoisomerase II, PDB ID: 1KZN). The present results, considered the docking studies of *N*-Boc-L-Leucine linked alkyne **3** and its most active triazole **4h** on the suitably active sites of DNA gyrase enzyme of *E. coli* bacteria by making use of Autodock Vina [51]. The three-dimensional (3D) structures of alkyne **3** and most active triazole **4h** were generated by Marvin sketch [52]. The binding affinity of alkyne **3** and its most active triazole **4h** showing very good conformations as shown in **Figure 5** and **6**. The ≡CH of alkyne **3** was involved in p-alkyl interaction with ILE A:78 as highlighted by the pink line in dotted form in **Figure 5a**. The alkyl group of leucine in alkyne **3** was also involved in alkyl interaction with ILE A:78. The carbon of **3** was found to be involved in carbon-hydrogen interaction with ASP A:73. The carbonyl oxygen of ester in alkyne **3** showed prevalent H-bond interaction with GLY A:77. But, the carbonyl oxygen of carbamate in **3** exhibited prevalent H-bond association with ARG A:136. The ether oxygen of carbamate in alkyne **3** exhibited prevalent H-bond interaction with ARG A:76. The highest stable conformation of alkyne **3** anchoring with interacting residues is shown in **Figure 5b**. However, the ether oxygen of ester of leucine moiety of compound **4h** showed Van der Waals interaction with PRO A:79. The alkyl group of leucine moiety of **4h** showed alkyl

interaction with ILE A:90. The triazole ring of **4h** showed pi-anion interaction with GLU A:50 and pi-alkyl interaction with ILE A:78. In triazole **4h** benzene ring exhibited p-alkyl association with ALA A:47 also pi-sigma association with THR A:165. The CH_2 of benzyl moiety of **4h** exhibited C and H-bond interaction with ASN A:46. The F-atom substitution on benzyl moiety of the triazole **4h** exhibited conventional H-bond interaction with VAL A:167 and halogen interaction with VAL A:71. The Boc-Leucine linked 1,2,3-triazole **4h** demonstrated a high binding energy of -8.2 kcal/mol as compared to its alkyne precursor **3** (-6.6 kcal/mol). It was therefore revealed that the incorporation of a triazole moiety into its corresponding alkyne improves the antibacterial activity of the resulted hybrid compound. The most stable conformation of N-Boc protected

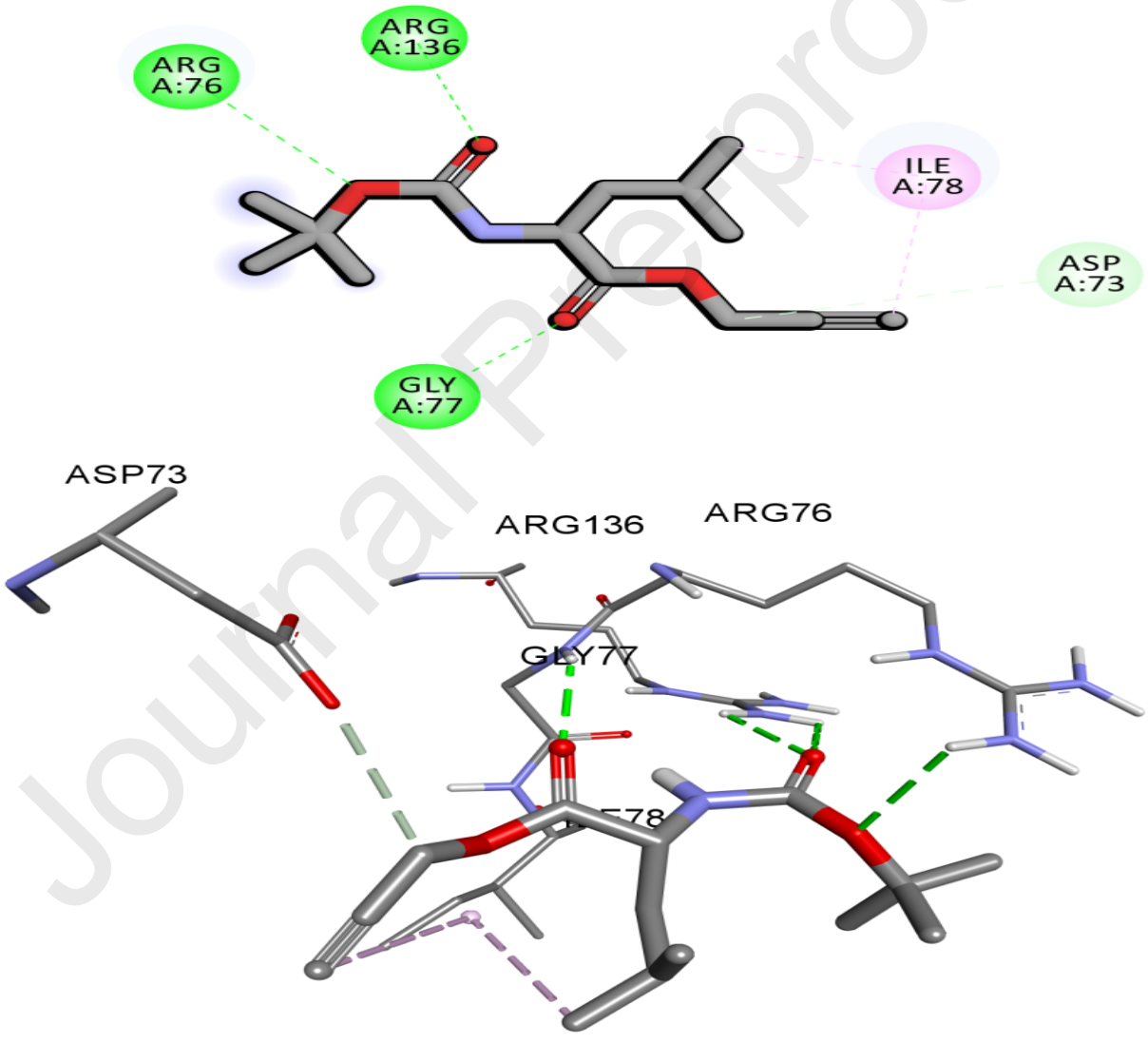


Figure 5a-b. Binding interactions of Boc-Leucine linked alkyne **3** docked with *E. coli* bacterial enzyme (DNA gyrase, topoisomerase II, PDB ID: 1KZN).

leucine linked 1,4-disubstituted 1,2,3-triazole **4** anchoring with interacting residues is shown in **Figure 6b** and both the structures in **Figure 5b** and **6b** were generated by making use of discovery studio visualizer [53].

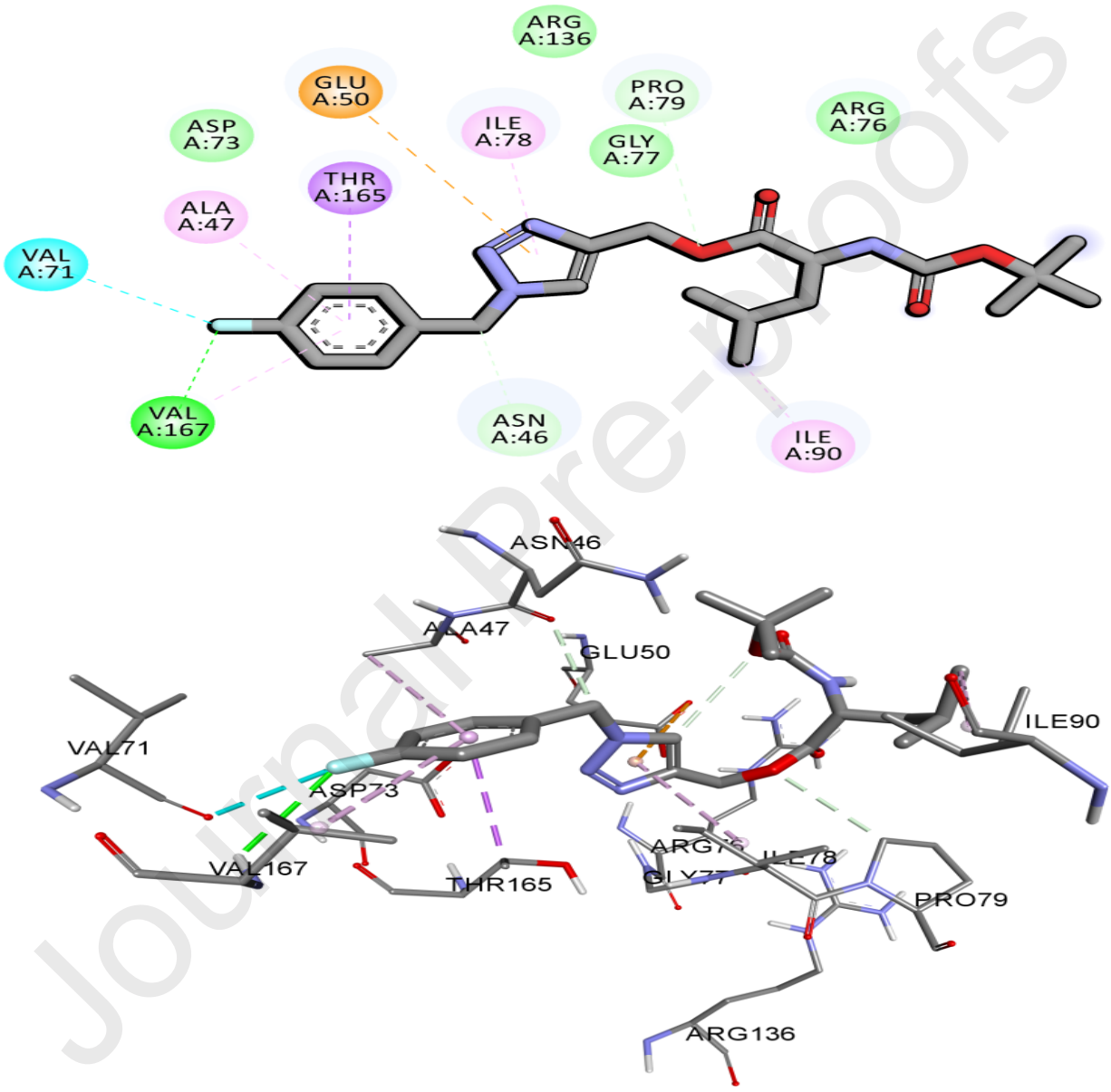
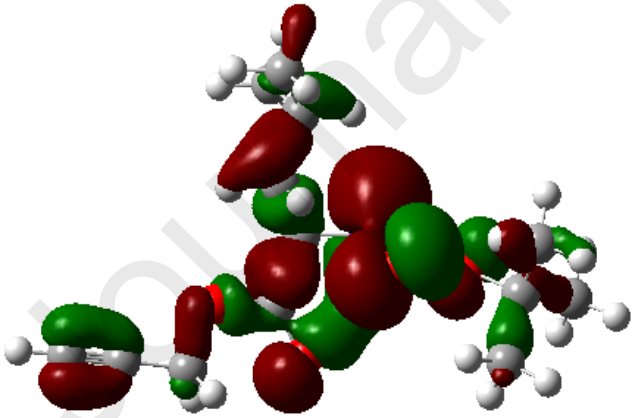


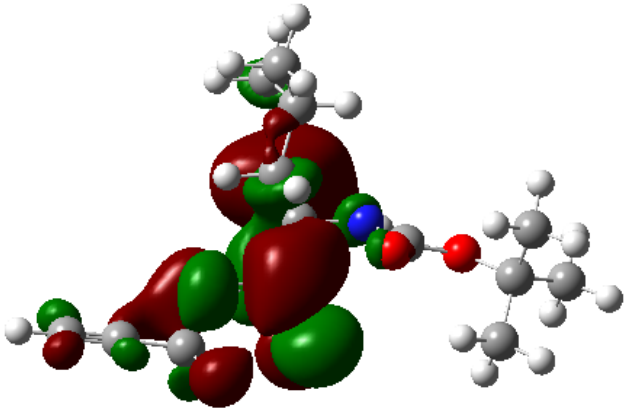
Figure 6a-b. Binding interactions of Boc-Leucine linked 1,2,3-triazole **4h** docked with *E. coli* bacterial enzyme (DNA gyrase, topoisomerase II, PDB ID: 1KZN).

3.5. DFT study

The Density Functional Theory (DFT) calculations on alkyne derivative of N-Boc protected L-Leucine i.e. 2-tert-butoxycarbonylamino-4-methyl-pentanoic acid prop-2-ynyl ester **3** and *N*-Boc protected Leucine-linked 1,4-disubstituted 1,2,3-triazoles (**4a-j**) were performed with the help of B3LYP at 6-311G(d,p) level of theory using a Gaussian 09 package [54]. For convenience, HOMO which is abbreviated as highest occupied molecular orbital and its counter lowest unoccupied molecular orbital (LUMO) of alkyne **3** along with representative and most active triazole **4a** and **4h**, respectively for easy reference are presented in **Figure 7**. However, complete reference to the HOMO and LUMO diagrams of alkyne **3** along with its respective *N*-Boc protected Leucine-linked 1,2,3-triazoles (**4a-j**) are given in **Figure, SI-1**. It is known that frontier orbitals are the important parameter of any chemical compound to design and ascertain their reactivity including pharmacological properties [55-56]. The smaller value of the energy gap ($\Delta E_{LUMO-HOMO}$) indicated easier electron transfer between them and the higher $\Delta E_{LUMO-HOMO}$ revealed better electron-donating and accepting nature, respectively. The calculated energy gap $\Delta E_{LUMO-HOMO}$ values for alkyne **3** and all triazole compounds are presented in **Table 7.** The HOMO and the LUMO energies of *N*-Boc-Leucine-linked alkyne **3** and its triazoles (**4a-j**) were compared to explore the pharmacological activity of triazoles with respect to alkyne, whereby it was observed that the band gap energy of all triazoles **4a-j** (3.95-6.03 eV) is less than alkyne **3** (7.07 eV). The



HOMO (**3**)



LUMO (**3**)

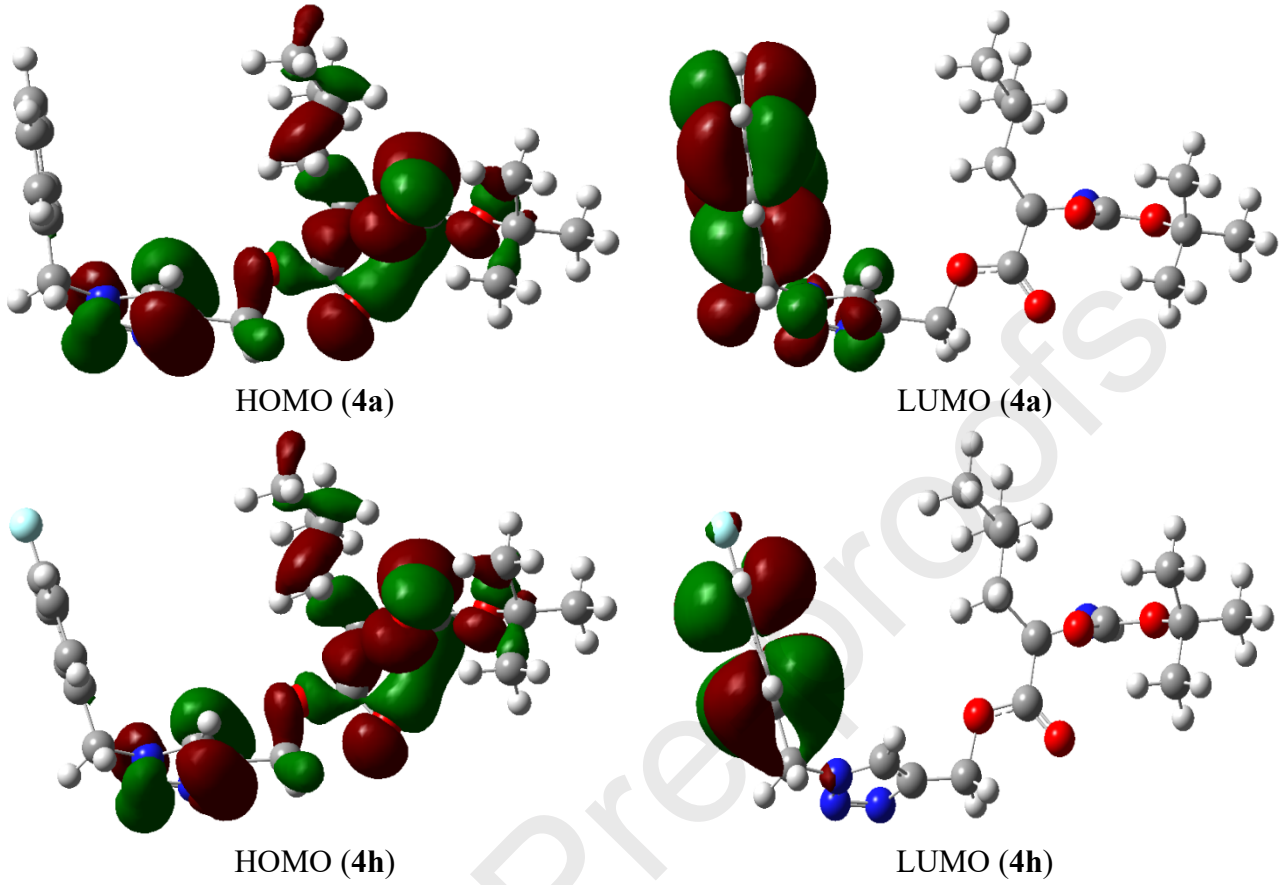


Figure. 7 HOMOs and LUMOs of alkynes **3** and triazoles **4a** & **4h**.

lower value of triazoles than alkyne showed that incorporation of a triazole unit on to the alkyne lowers the band gap energy ($\Delta E_{LUMO-HOMO}$) between respective frontier molecular orbitals (FMOs) and enhances the chemical reactivity and thus enhances the biological activity of the triazoles as compared to the respective alkyne **3** [57]. Also, with the help of chemical reactivity parameters i.e. electronic chemical hardness (η), chemical potential (μ) and electrophilicity (ω), the correlation of pharmacological properties was explored. Wherein, it was observed that all triazoles **4a-j** with lesser chemical hardness values (1.98-3.08 eV) than alkyne **3** (3.54 eV) are softer, hence more reactive. Likewise, the electronic chemical potential (μ) which can be described as the -ive of the electronegativity showed that electronic potential of all the triazoles ranges from -3.92 to -5.01 eV and that of alkyne is -3.61 eV [58]. However, the electrophilicity index (ω) of all triazoles showed values from 2.50-6.34 eV, which is higher than value of alkyne (1.84 eV). Overall, it highlighted that the introduction of 1,2,3-triazole moiety in alkyne lowers the $\Delta E_{LUMO-HOMO}$ along with lower chemical hardness (η) and higher chemical potential (μ) and electrophilicity index (ω) of a molecule.

Table 7. Energy of HOMO and LUMO, energy gap $\Delta E_{LUMO-HOMO}$, chemical hardness (η), chemical potential (μ), and electrophilicity index (ω) of alkyne **3** and its 1,2,3-triazoles **4a-j**.

Compound	E_{HOMO} (eV)	E_{LUMO} (eV)	$\Delta E_{LUMO-HOMO}$ (eV)	η (eV)	μ (eV)	ω (eV)
3	-7.15	-0.07	7.07	3.54	-3.61	1.84
4a	-7.00	-0.84	6.15	3.08	-3.92	2.50
4b	-6.96	-1.16	5.81	2.90	-4.06	2.84
4c	-6.99	-1.23	5.76	2.88	-4.11	2.93
4d	-6.98	-3.03	3.95	1.98	-5.01	6.34
4e	-7.05	-1.06	5.99	3.00	-4.05	2.74
4f	-7.06	-1.16	5.90	2.95	-4.11	2.86
4g	-7.15	-2.86	4.28	2.14	-5.00	5.85
4h	-7.04	-1.01	6.03	3.02	-4.02	2.69
4i	-7.05	-1.16	5.90	2.95	-4.10	2.86
4j	-7.13	-2.69	4.44	2.22	-4.91	5.42

4. Conclusion

A library of *N*-Boc L-Leucine-linked 1,2,3-triazoles was synthesized in high yield via CuAAC reaction and all were characterized by FT IR, ^{1}H NMR, ^{13}C NMR, and MS data. The *in vitro* antibacterial and antifungal activity screening results of Boc protected Leucine-linked alkyne **3** and its 1,2,3-triazoles **4a-j** showed that incorporation of triazole unit enhances the biological activity of the alkynes and among all *N*-Boc-Leucine linked 1,2,3-triazoles **4a-j**, compound **4h** found to be more potent than the reference antibacterial drug Ciprofloxacin against all tested bacterial strains *S. entrica*, *B. subtilis, S. aureus, E. coli* and *P. auroginosa*. Also, antifungal activity was found comparable with MIC: 0.0148 μmol/mL to normally used antifungal drug Fluconazole (MIC: 0.0102 μmol/mL) against *A.niger* and *C.albicans* fungal strains. The molecular docking study of alkyne **3** and its most active 1,2,3-triazole **4h** on DNA Gyrase of *E. coli* (topoisomerase II, PDB ID: 1KZN) revealed better binding with triazole **4h** than precursor alkyne **3** which was supported by DFT study using B3LYP at 6-311G(d,p) basis set.

Conflict of Interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

The supplementary material of this article can be found online, at:

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Highlights of the study:

1) A new library of *N*-Boc-Leucine linked 1,2,3-triazoles was synthesized in high yield via CuAAC reaction.
2) The structures of all newly synthesized compounds were characterized by FT IR, ^{1}H NMR, ^{13}C NMR, and MS data.
3) Compound **4h** to be more potent than the reference drug Ciprofloxacin against all tested bacterial strains *S. entrica*, *B. subtilis, S. aureus, E. coli and P. auroginosa*.
4) Also, compound **4h** exhibited comparable antifungal activity to reference drug Fluconazole against both *A. niger* and *C. albicans* fungal strains.
5) Molecular docking study on *E. coli* topoisomerase II, DNA Gyrase (PDB ID: 1KZN) exhibited better binding with triazole **4h** than alkyne **3**, results supported by DFT study.

Further, we confirm that this article has not been submitted /withdrawn from any other journal.

Yours truly,
Ram Kumar Tittal