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Design, Synthesis, Characterizations and Biological Evaluations of Substituted 2-((3-Chloro-2-Methylphenyl) ((1-Phenyl-1H-1,2,3-Triazol-4-Yl) Methyl) Amino) Benzoic Acid Derivatives

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Abstract

A series of novel substituted 2-((3-chloro-2-methylphenyl) ((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)amino) benzoic acid (5a-l) derivatives were synthesized using a multi-step synthetic route involving the click chemistry approach. The structures of the synthesized compounds were confirmed using various spectroscopic techniques, including NMR, IR, Mass and elemental analysis. The antibacterial and antifungal activities of the synthesized compounds were evaluated against a panel of bacterial strains (including *B. Subtilis*, *B. Sphaericus*, and *S. Aureus*, *P. Aeruginosa*, *K. Aerogenes* and *C. violaceum*) and fungal species (such as *C. albicans*, *A. Fumigatus, T. Rubrum* and *T. mentagrophytes*). The results revealed significant inhibitory effects, with some derivatives showing superior activity compared to standard drugs. Additionally, the cytotoxicity of these compounds was assessed against human cancer cell lines (MCF-7, PC-3, and HeLa) using MTT assays. Several derivatives exhibited potent cytotoxic effects, indicating their potential as anticancer agents. Molecular docking studies of newly synthesized derivatives 5a-l, along with Doxorubicin and Tamoxifen, against enoyl reductase (PDB ID: 1QSG) indicate potential inhibitors for lipid biosynthesis in cancer therapy.

Keywords: Click Chemistry, Antimicrobial Activities, MTT Assay, Docking Studies.

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1. INTRODUCTION

Heterocyclic chemistry plays a crucial role in researching and developing new bioactive compounds. Medicinal chemistry, a key area within pharmaceutical and medical sciences, is primarily focused on designing and creating biologically active drug compounds [1, 2]. Heterocyclic molecules containing oxygen and nitrogen have demonstrated significant biological activity. Many synthetic substances exhibit a wide range of pharmacological effects [3]. Despite ongoing challenges, medicinal chemists continue to search for novel agents [4]. The synthesis of nitrogen-rich heterocyclic systems has surged, driven by their applications in propellants, explosives, pyrotechnics, and particularly chemotherapy [5, 6]. Triazoles and their fused derivatives have garnered significant attention due to their biological and synthetic importance [7-9]. The biological activities of azolic derivatives, including thiadiazole, triazole, oxadiazole, and thiazole, have been extensively studied, revealing their pharmacological potential [10-12]. The synthesis of 1,2,3-triazoles can be achieved through a variety of methods, with one of the most popular being the "click chemistry" approach using the copper-catalyzed azide-alkyne cycloaddition (CuAAC), a notable pharmacophore within nitrogen-containing heterocycles [13-16], this moiety can facilitate a variety of non-covalent interactions with proteins, enzymes, and receptors, such as dipole-dipole interactions, hydrogen bonds, and van der Waals forces. Additionally, it serves as a flexible linker connecting different pharmacophoric elements [17-19].

1,2,3-Triazole derivatives have garnered significant attention in the field of medicinal chemistry due to their unique structural attributes and versatile biological activities [20-23]. The 1,2,3-triazole scaffold is known for its broad spectrum of biological activities, making it a valuable pharmacophore in drug design and development [24-27]. These derivatives exhibit potent anticancer [28], antimicrobial [29], antiviral [30], anti-inflammatory [31], antioxidant [32], and immunomodulatory [33], properties. Their ability to interact specifically with biological targets, such as enzymes and receptors, enhances their therapeutic

potential and allows for the development of drugs with high efficacy and minimal side effects [34-37].

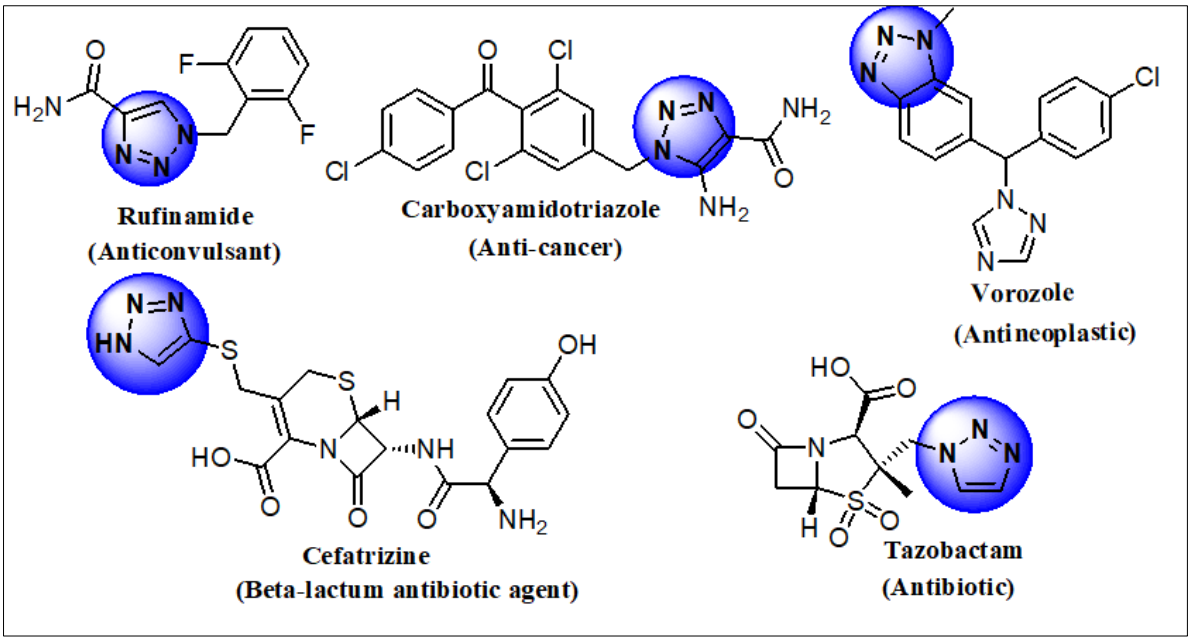


Fig. 1: Structures of 1,2,3 triazole moiety-containing drugs

The current study focused on the multi-step synthesis of novel substituted 2-((3-chloro-2-methylphenyl)((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)amino)benzoic acid (5a-l) derivatives using click chemistry. Their structures were confirmed by various spectroscopic methods. The synthesized compounds were evaluated through antibacterial, antifungal, cytotoxicity, and docking studies.

2. MATERIALS AND METHODS

2-(3-chloro-2-methylphenylamino)benzoic acid, propargyl bromide, potassium carbonate, aromatic substituted anilines, copper sulfate pentahydrate, sodium ascorbate, sodium azide, sodium nitrate, con HCl, DMF, dichloromethane, ethyl acetate, and petroleum ether were purchased from BLD Company Hyderabad, and all are of reagent grade and used as obtained without doing further purification.

3. Experimental Section

Proton (^{1}H) NMR spectra were recorded using a SA-Agilent spectrometer at 400 MHz. Carbon (^{13}C) NMR spectra were obtained with a Varian spectrometer; also, at 400 MHz. IR spectra were measured with a Perkin-Elmer 100S spectrometer using KBr pellets. Mass spectra (ESI) were acquired on a Jeol JMSD-300 spectrometer.

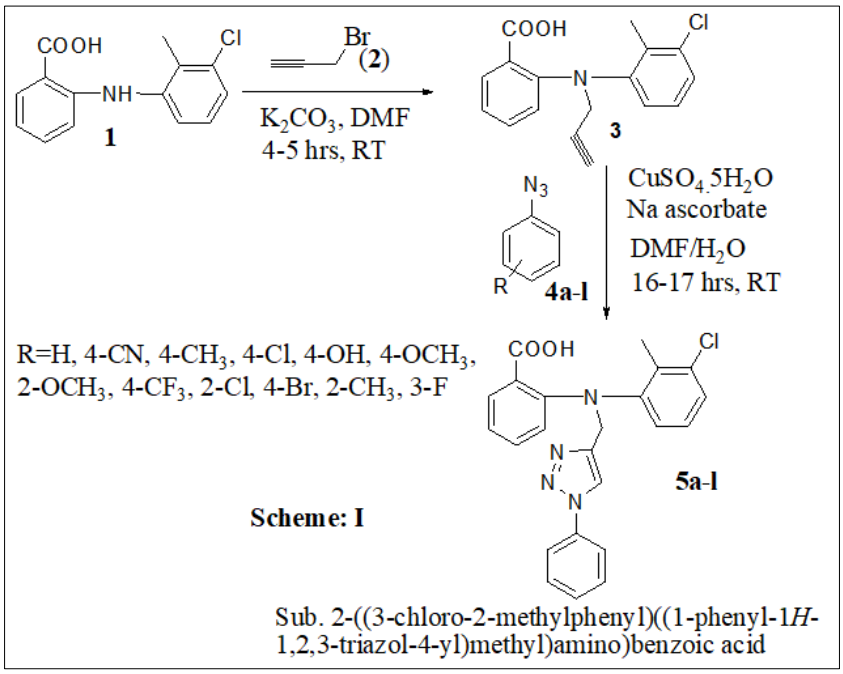
3.1.1 Synthesis of Propargylated Compound 3

Add the starting material compound (1 eq), DMF (10 mL), and K_2CO_3 (2.5 eq) to a clean, dry 100 mL RBF at 30°C under inert conditions. Stir the mixture for 5 minutes, and then add propargyl bromide (1.2 eq) at RT under inert conditions. Continue stirring the reaction mixture at RT for 4-5 hours, checking the progress with TLC. Once the reaction is complete, quench the mixture with cold water. Filter the resulting solid and wash with water. Yield: 70%.

3.1.2 General Synthesis Procedure of Substituted Aromatic Azide Derivatives (4a-l)

Aromatic substituted anilines (1 eq) in DCM (5 vol) were cooled to 0°C and mixed with 5N HCl solution, then agitated for 30 minutes at 0°C. Aqueous sodium nitrite (1.2 eq) was slowly added while maintaining 0°C. Aqueous sodium azide (1.2 eq) was then introduced at 0°C, and the mixture was agitated at 25-30°C for two hours. The mixture was separated into organic and aqueous layers, and the organic layer was washed with $NaHCO_3$ solution and brine. After solvent evaporation, the resulting aromatic substituted azides (**4a-l**) were obtained and used without further purification.

3.1.3 General Synthesis Procedure of 1,2,3-Triazole Moiety Derivatives (5a-l)



Scheme: 1: Synthesis Pathway for Substituted 1,2,3-Triazole moiety derivatives (5a-l)

Add 1 eq of 2-((3-chloro-2-methylphenyl)(prop-2-ynyl)amino)benzoic acid (**3**) and DMF (5mL) to a clean, dry 100 mL round-bottom flask. Then, add aqueous copper sulfate (3mL) and aqueous sodium ascorbate (3mL) solutions. Stir the reaction mixture for 5-10 minutes, then add aromatic substituted azides (**4a-l**) and continue stirring at room temperature for 16-17 hours. Check the reaction progress with TLC. Once the reaction is complete, quench the mixture with crushed ice. Filter the resulting solid compounds and wash with water and hexane. To obtain desired substituted 2-((3-chloro-2-methylphenyl)((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)amino)benzoic acid derivatives (**5a-l**). Yield: 74-82%.

3.2 Biological Studies

3.2.1 Antibacterial Assay

A novel series of **5a-l** compounds were evaluated for antibacterial activity against six bacterial strains, including three Gram- positive (*B. Subtilis*, *B. Sphaericus* and *S. Aureus*) and three Gram- negative (*P. Aeruginosa*, *K. Aerogenes* and *C. violaceum*). The disc diffusion method was employed for this evaluation. Standard inoculums of 1-2×10^7 colony-forming units per milliliter (c.f.u/mL), prepared from 0.5 McFarland standards, were evenly spread onto sterile agar plates using a glass spreader. Sterile 6.26 mm diameter discs made from Whatman No. 1 filter paper were sterilized at 140 °C for 1 hour and then immersed in known concentrations of the test compounds.

3.2.2 Antifungal Studies

The antifungal activity of compounds **5a-l** was evaluated using the disc diffusion method against *C. albicans*, *A. fumigatus*, *T. rubrum*, and *T. mentagrophytes*. The compounds were dissolved in dimethyl sulfoxide (DMSO), with amphotericin B used as the reference standard. The effectiveness of the drugs was assessed by measuring the Mean Zone of Inhibition (MZI) values, which are presented in Table 2 along with control values.

3.2.3 In Vitro Cytotoxicity

3.2.3.1 Cell Culture

The cell lines MCF-7, PC-3, and HeLa were cultured in RPMI-1640 medium with 10% FBS, whereas MCF-7 cells were also grown in MEM medium with 10% FBS. Cultures were maintained under a humidified atmosphere with 5% CO_2 at 37°C. Stock solutions of the synthesized **5a-l** compounds were made in DMSO and administered to the cultures at the required concentrations. The DMSO concentration in the final culture was kept below 1:1,000.

3.2.3.2 MTT Assay

Cytotoxic activities of the phenanthridine derivatives were determined using the MTT assay. Dilutions of stock solutions of the synthesized derivatives were prepared in culture medium. Cells were seeded into 96-well plates at 5×10^4 cells per well and grown until they reached 90–95% confluence. Each well received 100 µL of medium with the desired derivative concentrations and was incubated for 48 hours. Afterward, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 more hours. The medium was then removed, and 200 µL of DMSO was added to each well. Absorbance was measured at 490 and 630 nm using a microplate reader (MODEL). Cell growth

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inhibition percentages were calculated according to the equation.

% inhibition = [1– (Sample group OD490 – Sample group OD630) / (Control group OD490-Control group OD630)] × 100%

IC_{50} values were calculated with the aid of GraphPad Prism software. The standard deviations for these values were determined based on results from at least three independent experiments.

3.2.4 Docking Studies

The protein structure was acquired from the RCSB PDB Database. Water molecules and additional non-protein components were removed using Discovery Studio Visualizer. The molecular structures of compounds **5a-l** and the standard drug Doxorubicin were drawn with ChemDraw software. Docking studies were performed using PyRx, and the resulting interactions between the ligands and the target protein were visualized with Pymol and Discovery Studio Visualizer.

4. RESULTS AND DISCUSSIONS

Consequently, Scheme I illustrates the ultimate synthesis route for the 2-((3-chloro-2-methylphenyl)((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)amino)benzoic acid derivatives (5a-l). Step-1, Add the starting material compound (1 eq), DMF (10 mL), and K_2CO_3 (2.5 eq) to a clean, dry 100 mL RBF at 30°C under inert conditions. Stir the mixture for 5 minutes, and then add propargyl bromide (1.2 eq) at RT under inert conditions. Continue stirring the reaction mixture at RT for 4-5 hours. Step-2, Aromatic substituted anilines (1 eq) in DCM (5 vol) were cooled to 0°C and mixed with 5N HCl solution, then agitated for 30 minutes at 0°C. Aqueous sodium nitrite (1.2 eq) was slowly added while maintaining 0°C. Aqueous sodium azide (1.2 eq) was then introduced at 0°C, and the mixture was agitated at 25-30°C for two hours. The resulting aromatic substituted azides (4a-l) were obtained. Final step Add 1 eq of 2-((3-chloro-2-methylphenyl)(prop-2-ynyl)amino)benzoic acid (3) and DMF (5mL) to a clean, dry 100 mL round-bottom flask. Then, add aqueous copper sulfate (3mL) and aqueous sodium ascorbate (3mL) solutions. Stir the reaction mixture for 5-10 minutes, then add aromatic substituted azides (4a-l) and continue stirring at room temperature for 16-17 hours. To obtain desired substituted 2-((3-chloro-2-methylphenyl)((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)amino)benzoic acid derivatives (5a-l). Yield: 74-82%.

4.1 Spectral Characterizations

2-((3-chloro-2-methylphenyl)((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)amino)benzoic acid (5a):

M.F: $C_{23}H_{19}ClN_4O_2$. Colour: Off white. MP: 239-241 ^{0}C. Yield: 67 %. ^{1}H NMR (400 MHz, DMSO) δ : 9.42 (s, 1H, COOH), 8.62-8.64 (d, 2H, J= 5.2 MHz), 8.60-8.62 (d, 1H, J= 6.8 MHz), 8.40-8.42 (d, 1H, J= 8.8 MHz), 8.38 (s, 1H, Trizole-CH), 7.96-7.99 (m, 2H), 7.78-7.80 (d, 1H, J= 9.2 MHz), 7.58-7.62 (m, 1H), 7.18-7.21 (m, 1H), 6.98-7.02 (m, 1H), 6.86-6.89 (m, 1H), 6.49-6.51 (d, 1H, J= 7.2 MHz), 5.26 (s, 2H, CH_2), 1.32 (s, 3H, CH_3). ^{13}C NMR (400 MHz, DMSO) δ: 188.03 (COOH), 161.03 (=C-N), 157.50, 154.38, 151.31, 149.05, 141.95, 134.31, 130.53, 128.48, 128.46, 128.42, 127.32, 126.20, 119.16 (Triazole-CH), 114.67, 113.52, 103.16, 61.38 (CH_2), 10.84 (CH_3). IR [ν, cm$^{-1,}$ KBr)]: 3357 (COOH), 3066 (C=C-H, str) 2979 (C-C-H Str), 1455 (N-CH2, str), 807(C-H, Ar), 739 (C-H, Alip). Mass: 420.37 $[M+1]^+$. E. analysis: Found; C, 65.90; H, 4.61; Cl, 8.38; N, 13.31; O, 7.67. Cald. C, 65.95; H, 4.57; Cl, 8.46; N, 13.38; O, 7.64.

2-((3-chloro-2-methylphenyl)((1-(4-cyanophenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzoic acid (5b): M.F: $C_{24}H_{18}ClN_5O_2$. Colour: Pale yellow. MP: 227-229 ^{0}C. Yield: 70 %. ^{1}H NMR (400 MHz, DMSO) δ : 9.43 (s, 1H, COOH), 8.41-8.43 (d, 1H, J= 7.6 MHz), 8.26 (1H, Traizole-CH), 8.12-8.14 (d, 1H, J= 8.4 MHz), 7.82-7.84 (d, 1H, J= 8.8 MHz), 7.713-7.742 (m, 1H), 7.58-7.61 (m, 3H), 7.41-7.45 (m, 2H), 7.36-7.39 (m, 1H), 6.75-6.77 (d, 1H, J= 7.6 MHz), 6.48-6.50 (d, 1H, J= 7.6 MHz), 5.23 (s, 2H, CH_2), 1.07 (s, 3H, CH_3). ^{13}C NMR (400 MHz, DMSO) δ: 192.34 (COOH), 170.02 (=C-N), 167.02, 163.62, 159.35, 151.35, 149.04, 142.74, 139.54, 129.88, 126.23, 123.36, 121.87, 119.19 (triazole-CH), 114.88, 114.66, 113.42, 110.95, 103.11, 61.55 (CH_2), 5.85 (CH_3). IR [ν, cm$^{-1,}$ KBr)]: 3434 (COOH), 2251 (C=C-H, str) 2124 (C-C-H Str), 1659 (N-CH2, str), 1049 (CN), 821(C-H, Ar), 759 (C-H, Alip). Mass: 444.5 $[M+1]^+$. E. analysis: Found; C, 64.88; H, 4.02; Cl, 7.83; N, 15.71; O, 7.21. Cald. C, 64.94; H, 4.09; Cl, 7.99; N, 15.78; O, 7.21.

2-((3-chloro-2-methylphenyl)((1-p-tolyl-1H-1,2,3-triazol-4-yl)methyl)amino)benzoic acid (5c):

M.F: $C_{24}H_{21}ClN_4O_2$. Colour: Off white. MP: 231-233 ^{0}C. Yield: 72 %. ^{1}H NMR (400 MHz, DMSO) δ : 9.47 (s, 1H, COOH), 8.59-8.61(d, 1H, J= 7.6 MHz) , 8.21 (s, 1H, Triazole-CH), 8.02-8.06 (m, 1H), 7.81-7.83 (d, 1H, J= 7.6 MHz), 7.60-7.68 (m, 4H), 7.38-7.40 (d, 1H, J= 8.4 MHz), 7.11-7.15 (m, 2H), 6.47-6.49 (d, 1H, J= 7.6 MHz), 5.22 (s, 2H, CH_2), 1.21 (s, 3H, CH_3), 0.98 (s, 3H, CH_3). ^{13}C NMR (400 MHz, DMSO) δ: 187.38 (COOH), 166.83 (=C-N), 163.02, 159.92, 151.65, 149.04, 141.64, 130.89,127.18, 126.24, 125.85, 125.52, 120.90, 119.22 (triazole-CH), 114.66, 113.42, 113.00, 103.08, 61.45 (CH_2), 12.10 (CH_3), 5.69 (CH_3). IR [ν, cm$^{-1,}$ KBr)]: 3395 (COOH), 2949 (C=C-H, str) 2870 (C-C-H Str), 1609 (N-CH2, str), 808 (C-H, Ar), 755 (C-H, Alip). Mass: 433.6 $[M+1]^+$. E. analysis: Found; C, 66.51; H, 4.94; Cl, 8.13; N,12.90; O, 7.44. Cald. C, 66.59; H, 4.89; Cl, 8.19; N, 12.94; O, 7.39.

2-((3-chloro-2-methylphenyl)((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzoic acid (5d): M.F: $C_{23}H_{18}Cl_2N_4O_2$. Colour: Off white. MP: 237-239 ^{0}C. Yield: 75 %. ^{1}H NMR (400 MHz, DMSO) δ : 9.58 (s, 1H,

COOH), 8.07-8.09 (d, 1H, J= 8.8 MHz), 8.02 (s, 1H, Triazole-CH), 7.81-7.83 (d, 1H, J= 8.8 MHz), 7.72-7.77 (m, 2H) , 7.62-7.66 (m, 2H), 7.41-7.47 (m, 1H), 7.34-7.36 (d, 2H, J= 8.8 MHz), 7.18-7.24 (m, 1H), 6.47-6.49 (d, 1H, J= 8.4 MHz), 5.24 (s, 2H, CH_2), 1.28 (s, 3H, CH_3). ^{13}C NMR (400 MHz, DMSO) δ: 191.38 (COOH), 162.70 (=C-N), 157.48, 153.62, 151.39, 149.11, 141.96, 136.13, 133.03, 131.40, 129.94, 127.06, 126.75, 126.25, 126.06, 119.22 (triazole-CH), 114.72, 113.59, 103.18, 61. 57 (CH_2), 17.42 (CH_3). IR [ν, $cm^{-1,}$ KBr)]: 3369 (COOH), 2924 (C=C-H, str) 2853 (C-C-H Str), 1615 (N-CH2, str), 831 (C-H, Ar), 746 (C-H, Alip). Mass: 454. $[M+1]^+$. E. analysis: Found; C, 60.90; H, 4.05; Cl, 15.58; N, 12.31; O, 7.09. Cald. C, 60.94; H, 4.00; Cl, 15.64; N, 12.36; O, 7.06.

2-((3-chloro-2-methylphenyl)((1-(4-hydroxyphenyl)-1H-1,2,3-triazol-4yl)methyl)amino)benzoic acid (5e):M.F: $C_{23}H_{19}ClN_4O_3$. Colour: Brown. MP: 244-246 ^{0}C. Yield: 73 %. ^{1}H NMR (400 MHz, DMSO) δ : 9.58 (s, 1H, COOH), 9.23 (s, 1H, OH), 8.16-8.18 (d, 1H, J= 8.8 MHz), 8.08 (s, 1H, Triazole-CH), 7.95-8.00 (m, 1H), 7.81-7.83 (d, 2H, J= 8.4 MHz), 7.59-7.64 (m, 2H), 7.37-7.39 (d, 2H, J= 8.4 MHz), 7.18-7.20 (d, 1H, J= 8 MHz), 6.99-7.01 (d, 1H, J= 9.2 MHz), 6.48-6.50 (d, 1H, J= 8.4 MHz), 5.26 (s, 2H, CH_2), 1.51 (s, 3H, CH_3). ^{13}C NMR (400 MHz, DMSO) δ: 197.16 (COOH), 162.39 (=C-N), 158.21, 153.98, 151.32, 149.08, 143.21, 138.19, 136.78, 130.52, 128.36, 124.59, 123.70, 119.58, 119.19 (triazole-CH), 114.71, 113.49, 103.22, 61. 54 (CH_2), 10.88 (CH_3). IR [ν, $cm^{-1,}$ KBr)]: 3412 (OH), 3360 (COOH), 2927 (C=C-H, str) 2858 (C-C-H Str), 1611 (N-CH2, str), 834 (C-H, Ar), 741 (C-H, Alip). Mass: 435.6 $[M+1]^+$. E. analysis: Found; C, 63.48; H, 4.45; Cl, 8.09; N, 12.83; O, 11.00. Cald. C, 63.52; H, 4.40; Cl, 8.15; N, 12.88; O, 11.04.

2-((3-chloro-2-methylphenyl)((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4yl)methyl)amino)benzoic acid (5f): M.F: $C_{24}H_{21}ClN_4O_3$. Colour: Off white. MP: 233-235 ^{0}C. Yield: 78 %. ^{1}H NMR (400 MHz, DMSO) δ : 9.62 (s, 1H, COOH), 8.58-8.60 (d, 1H, J= 8MHz), 8.32 (s, 1H, Triazole-CH), 7.96-8.05 (m, 1H), 7.79-7.81 (d, 2H, J= 8.8 MHz), 7.62-7.66 (m, 1H), 7.38-7.40 (d, 1H, J= 9.2 MHz) , 7.11-7.13 (d, 1H, J= 8.8 MHz), 6.94-6.96 (d, 1H, J= 8.4 MHz), 6.75-6.79 (m, 1H), 6.46-6.48 (d, 1H, J= 8.8 MHz), 5.24 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3), 1.40 (s, 3H, CH_3). ^{13}C NMR (400 MHz, DMSO) δ: 180.29 (COOH), 163.55 (=C-N), 161.11, 151.21, 149.00, 137.57, 131.86, 131.77, 126.16, 123.51, 119.11, (triazole-CH), 116.07, 115.61, 115.40, 114.63, 113.42, 103.16, 61. 43 (CH_2), 25.80 (OCH_3),11.28 (CH_3). IR [ν, $cm^{-1,}$ KBr)]: 3354 (COOH), 2921 (C=C-H, str) 2849 (C-C-H Str), 1602 (N-CH2, str), 839 (C-H, Ar), 734 (C-H, Alip). Mass: 449.3 $[M+1]^+$. E. analysis: Found; C, 64.17; H, 4.78; Cl, 7.85; N, 12.51; O, 10.63. Cald. C, 64.21; H, 4.72; Cl, 7.90; N, 12.48; O, 10.69.

2-((3-chloro-2-methylphenyl)((1-(2-methoxyphenyl)-1H-1,2,3-triazol-4yl)methyl)amino)benzoic acid (5g): M.F: $C_{24}H_{21}ClN_4O_3$. Colour: Off white. MP: 227-229 ^{0}C. Yield: 80 %. ^{1}H NMR (400 MHz, DMSO) δ : 9.61 (s, 1H, COOH), 8.37-8.39 (d, 1H, J= 9.2 MHz), 8.26 (s, 1H, s, 1H, Triazole-CH), 8.07-8.14 (m, 1H), 7.93-8.03 (m, 4H), 7.81-7.85 (m, 1H), 7.66-7.71 (m, 1H), 7.44-7.46 (d, 1H, J= 8.4 MHz), 7.35-7.37 (d, 1H, J= 8.4 MHz), 6.69-6.71 (d, 1H, J= 7.6 MHz). 5.64 (s, 2H, CH_2), 3.56 (s, 3H, OCH_3) , 1.41 (s, 3H, CH_3). ^{13}C NMR (400 MHz, DMSO) δ: 196.20 (COOH), 173.83 (=C-N), 169.60, 165.65, 162.30, 159.19, 156.20, 152.48, 146.92, 143.23, 135.26, 133.18, 132.54, 131.16, 129.94, 123.11, 121.90, 118.38, (triazole-CH), 113.41, 110.97, 62. 13 (CH_2), 26.53 (OCH_3), 8.59 (CH_3). IR [ν, $cm^{-1,}$ KBr)]: 3347 (COOH), 2927 (C=C-H, str) 2842 (C-C-H Str), 1610 (N-CH2, str), 833 (C-H, Ar), 737 (C-H, Alip). Mass: 449.3 $[M+1]^+$. E. analysis: Found; C, 64.18; H, 4.74; Cl, 7.85; N, 12.51; O, 10.64. Cald. C, 64.21; H, 4.72; Cl, 7.90; N, 12.48; O, 10.69.

2-((3-chloro-2-methylphenyl)((1-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzoic acid (5h): M.F: $C_{24}H_{18}ClF_3N_4O_2$. Colour: Light green. MP: 234-236 ^{0}C. Yield: 80 %. ^{1}H NMR (400 MHz, DMSO) δ : 9.53 (s, 1H, COOH), 8.97-8.89 (d, 1H, J= 7.6 MHz), 8.39 (s, 1H, Triazole-CH), 8.08-8.14 (m, 1H), 7.93-8.01 (m, 4H), 7.61-7.63 (d, 1H, J= 8.4 MHz), 7.26-7.34 (m, 1H), 7.17-7.19 (d, 1H, J= 8.4 MHz), 6.99-7.05 (m, 1H), 6.91-6.93 (d, 1H, J= 9.2 MHz), 5.36 (s, 2H, CH_2), 1.24 (s, 3H, CH_3). ^{13}C NMR (400 MHz, DMSO) δ: 196.21 (COOH), 165.65 (=C-N), 162.49, 159.26, 157.90, 154.28, 150.96, 146.83, 142.85, 135.86, 132.55, 131.16, 129.88, 128.58, 123.57 (CF_3), 122.85, 122.06, 116.08, (triazole-CH), 113.42, 62. 26 (CH_2), 10.53 (CH_3). IR [ν, $cm^{-1,}$ KBr)]: 3343 (COOH), 2920 (C=C-H, str) 2847 (C-C-H Str), 1607 (N-CH2, str), 1299 (CF_3), 836 (C-H, Ar), 729 (C-H, Alip). Mass: 487.5 $[M+1]^+$. E. analysis: Found; C, 59.17; H, 3.70; Cl, 7.22; F, 11.76; N, 11.47; O, 6.57. Cald. C, 59.21; H, 3.73; Cl, 7.28; F, 11.71; N, 11.51; O, 6.57.

2-((3-chloro-2-methylphenyl)((1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzoic acid (5i): M.F: $C_{23}H_{18}Cl_2N_4O_2$. Colour: Pale yellow. MP: 244-246 ^{0}C. Yield: 82 %. ^{1}H NMR (400 MHz, DMSO) δ : 9.42 (s, 1H, COOH), 8.42-8.43 (d, 1H, J= 8.4 MHz), 8.22 (s, 1H, Triazole-CH), 8.02-8.11 (m, 4H), 7.92-7.99 (m, 1H), 7.72-7.77 (m, 1H), 7.54-7.60 (m, 1H), 7.42-7.44 (d, 1H, J= 8.8 MHz), 7.19-7.21 (d, 1H, J= 8.8 MHz), 6.99-7.01 (d, 1H, J= 9.2 MHz), 5.40 (s, 2H, CH_2), 1.39 (s, 3H, CH_3). ^{13}C NMR (400 MHz, DMSO) δ: 185.70 (COOH), 167.84 (=C-N), 163.70, 160.03, 146.80, 144.62, 138.79, 134.93, 130.94, 129.60, 128.62, 128.15, 127.88, 127.65, 127.46, 125.44, 124.66, 121.38, 118.43, (triazole-CH), 63. 96 (CH_2), 15.98 (CH_3). IR [ν, $cm^{-1,}$ KBr)]: 3331 (COOH), 2934 (C=C-H, str) 2872 (C-C-H Str), 1596 (N-CH_2, str), 841

(C-H, Ar), 738 (C-H, Alip). Mass: 453.4 $[M+1]^+$. E. analysis: Found; C, 60.89; H, 4.05; Cl, 10.64; N, 12.31; O, 7.09. Cald. C, 60.94; H, 4.00; Cl, 15.64; N, 12.36; O, 7.06.

2-(((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)(3-chloro-2-methylphenyl)amino)benzoic acid (5j): M.F: $C_{23}H_{18}BrClN_4O_2$. Colour: Brown. MP: 239-241 ^{0}C. Yield: 76 %. ^{1}H NMR (400 MHz, DMSO) δ : 9.42 (s, 1H, COOH), 8.16-8.18 (d, 1H, J= 8.4 MHz), 7.98 (s, 1H, Triazole-CH), 7.78-7.83 (m, 1H), 7.59-7.61 (d, 2H, J= 8 MHz), 7.47-7.49 (d, 1H, J= 9.2 MHz), 7.33-7.38 (m, 1H), 7.16-7.20 (m, 2H), 6.99-7.01 (d, 2H, J= 8.4 MHz), 6.71-6.73 (d, 1H, J= 8.4 MHz), 5.43 (s, 2H, CH_2), 1.39 (s, 3H, CH_3). ^{13}C NMR (400 MHz, DMSO) δ: 192.36 (COOH), 170.37 (=C-N), 167.60, 164.49, 159.98, 146.80, 145.87, 138.68, 135.98, 133.09, 129.62, 127.68, 127.42, 125.41, 124.69, 122.68, 122.02, 120.79 (triazole-CH), 63. 99 (CH_2), 8.96 (CH_3). IR [ν, $cm^{-1,}$ KBr)]: 3328 (COOH), 2939 (C=C-H, str) 2868 (C-C-H Str), 1598 (N-CH2, str), 835 (C-H, Ar), 732 (C-H, Alip). Mass: 498.2 $[M+1]^+$. E. analysis: Found; C, 55.43; H, 3.69; Br, 16.01; Cl, 7.16; N, 11.19; O, 6.43. Cald. C, 55.50; H, 3.64; Br, 16.05; Cl, 7.12; N, 11.26; O, 6.43.

2-((3-chloro-2-methylphenyl)((1-o-tolyl-1H-1,2,3-triazol-4-yl)methyl)amino)benzoic acid (5k):

M.F: $C_{24}H_{21}ClN_4O_2$. Colour: Off white. MP: 234-236 ^{0}C. Yield: 73 %. ^{1}H NMR (400 MHz, DMSO) δ : 9.93 (s, 1H, COOH), 8.17-8.19 (d, 1H, J= 8.8 MHz), 7.93 (s, 1H, Triazole-CH), 7.61-7.64 (m, 1H), 7.53-7.55 (d, 2H, J= 6.4 MHz), 7.34-7.39 (m, 1H), 7.19-7.25 (m, 1H), 6.98-7.01 (d, 1H, J= 9.2 MHz), 6.79-6.83 (m, 1H), 6.59-6.61 (d, 1H, J= 8.4 MHz), 5.21 (s, 2H, CH_2), 1.59 (s, 3H, CH_3), 1.41 (s, 3H, CH_3). ^{13}C NMR (400 MHz, DMSO) δ: 196.76 (COOH), 162.84 (=C-N), 159.98, 146.77, 145.97, 138.74, 138.65, 137.48, 130.46, 129.65, 128.71, 127.65, 127.46, 125.43, 124.90, 124.81, 124.71, 120.97, 119.78 (triazole-CH), 116.37, 64. 02 (CH_2), 17.83 (CH_3), 14.90 (CH_3). IR [ν, $cm^{-1,}$ KBr)]: 3322 (COOH), 2934 (C=C-H, str) 2861 (C-C-H Str), 1591 (N-CH2, str), 830 (C-H, Ar), 741 (C-H, Alip). Mass: 433.3 $[M+1]^+$. E. analysis: Found; C, 66.59; H, 4.89; Cl, 8.19; N, 12.94; O, 7.39. Cald. C, 66.59; H, 4.89; Cl, 8.19; N, 12.94; O, 7.39.

2-((3-chloro-2-methylphenyl)((1-(3-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)amino)benzoic acid (5l): M.F: $C_{23}H_{18}ClFN_4O_2$. Colour: Pale yellow. MP: 230-232 0C. Yield: 77 %. ^{1}H NMR (400 MHz, DMSO) δ: 9.60 (s, 1H, COOH), 8.38-8.40 (d, 1H, J= 8.8 MHz), 8.20 (s, 1H, Triazole-CH), 7.99-8.04 (m, 1H), 7.86-7.91 (m, 1H), 7.73-7.77 (m, 3H), 7.55-7.60 (m, 1H), 7.29-7.31 (d, 1H, J= 8.4 MHz), 6.92-6.94 (d, 1H, J= 7.6 MHz), 6.69-6.71 (d, 1H, J= 8.8 MHz), 5.29 (s, 2H, CH_2), 1.17 (s, 3H, CH_3). ^{13}C NMR (400 MHz, DMSO) δ: 196.74 (COOH), 155.41 (=C-N), 152.33, 146.29, 139.99, 138.60, 137.48, 133.04, 130.36, 129.54, 128.90, 128.72, 128.56, 128.24, 128.06, 126.62, 124.85, 120.96, 119.87, 119.15 (triazole-CH), 117.34, 63. 23 (CH_2), 10.31 (CH_3). IR [ν, cm^{-1}, KBr)]: 3318 (COOH), 2927 (C=C-H, str) 2853 (C-C-H Str), 1594 (N-CH2, str), 835 (C-H, Ar), 718 (C-H, Alip). Mass: 437 $[M+1]^+$. E. analysis: Found; C, 63.19; H, 4.19; Cl, 8.07; F, 4.31; N, 12.86; O, 7.29. Cald. C, 63.23; H, 4.15; Cl, 8.12; F, 4.35; N, 12.82; O, 7.32.

4.2 Biological Evaluations

4.2.1 Anti-Bacterial Activity

The antibacterial activity of the synthesized compounds 5a-l was assessed against six bacterial strains, with the findings detailed in Table 1. The activity of these compounds varies considerably, with 5c, 5h, and 5i demonstrating the highest potency. These compounds show strong antibacterial effects against both Gram-positive and Gram-negative bacteria, nearing the effectiveness of the standard antibiotic, Streptomycin. These promising results indicate that compounds 5c, 5h, and 5i could be potential leads for developing new antibacterial agents. Further research, including optimization and mechanistic studies, is needed to improve their antibacterial properties and elucidate their mechanisms of action. Compounds 5a, 5b, and 5g exhibit moderate antibacterial activity, with MZI values lower than the top-performing compounds but still notable. Conversely, compounds 5d, 5e, 5f, 5k, and 5l show the least antibacterial activity, with MZI values significantly below those of Streptomycin.

Table 1: Antibacterial activity of 5a-l Compounds (Mean Zone of Inhibition at 100µg/mL)

Compound	Mean Zone Inhibition (MZI) in 100 µg/mL					
	B. Subtilis	B. Sphaericus	S. Aureus	P. aeruginosa	K. aerogenes	C. violaceum
5a	24	21	22	19	17	18
5b	25	23	24	23	21	23
5c	**31**	**29**	**30**	**28**	**26**	**28**
5d	20	18	19	17	16	17
5e	18	16	17	15	14	15
5f	18	16	20	19	16	19
5g	22	19	20	18	17	21
5h	**30**	**28**	**29**	**27**	**25**	**27**
5i	**29**	**27**	**28**	**26**	**24**	**26**
5j	23	21	20	18	17	18
5k	17	19	20	17	16	18

5l	18	15	16	14	13	17
Streptomycin	**32**	**30**	**31**	**29**	**28**	**30**

4.2.2 Antifungal Activity

Table 2 displays the Mean Zone Inhibition (MZI) values of various final analogues (5a-l) tested at a concentration of 100µg/mL against four fungal strains: *C. albicans*, *A. fumigatus*, *T. rubrum*, and *T. mentagrophytes*. MZI values indicate the diameter of inhibition zones, reflecting the compounds' antifungal efficacy.

Compounds 5b, 5g, and 5h exhibit the highest MZI values across all tested fungal strains. These compounds show superior antifungal activity, with their inhibition zones comparable to those of the reference drug, Amphotericin B. This suggests that these compounds are highly effective at preventing fungal growth, positioning them as strong candidates for further development. Compounds 5d, 5e, 5j, 5k, and 5l display moderate inhibition against the fungal strains. While their MZI values are lower than those of the top-performing compounds (5b, 5g, and 5h), they still show significant antifungal activity. This indicates their potential for effectiveness, although they may not be as potent as the best candidates. Compounds 5a, 5c, and 5i exhibit the lowest MZI values among the tested compounds. Their smaller inhibition zones suggest that they are the least effective in preventing fungal growth. These compounds show relatively weaker antifungal activity compared to the others, indicating limited potential for their use as antifungal agents.

Table: 2. MZI values for a series of final analogues 5a-l derivatives

Compound	Mean zone inhibition (MZI)[a] in 100 µg/mL			
	C. albicans	A. fumigatus	T. rubrum	T.mentagrophytes
5a	17	15	14	18
5b	**26**	**24**	**24**	**25**
5c	16	13	15	16
5d	21	19	17	19
5e	22	20	18	19
5f	18	16	15	20
5g	**27**	**26**	**25**	**27**
5h	**25**	**24**	**23**	**25**
5i	18	16	15	18
5j	20	17	18	19
5k	23	21	20	21
5l	23	21	19	20
Amphotericin B	**28**	**27**	**26**	**28**

4.2.3 Cytotoxicity Studies

In cancer research, assessing how compounds inhibit cell growth across different cell lines is essential for discovering potential treatments. Table 3 below details the growth inhibition percentages for three cancer cell lines: MCF-7, PC-3, and HeLa when exposed to various compounds at concentrations of 5 and 10 µM. This table offers important information about the relative efficacy of the compounds, highlighting those with significant potential for advancement as cancer therapies.

Compounds 5h, 5i, and 5j are the most effective, showing the highest growth inhibition percentages across all cell lines and concentrations, similar to or exceeding the effect of Doxorubicin. Compounds such as 5a, 5d, 5f and 5l also exhibit significant inhibitory effects, particularly at the higher concentration (10µM), but are generally less effective compared to 5h, 5i, and 5j. Compounds like 5b, 5c, 5e, 5g and 5k show lower inhibition percentages and appear less effective compared to others.

Table 3: % of growth inhibitory values of MCF-7, PC-3 and HeLa cell lines

Compound Code	MCF-7		PC-3		HeLa	
	5µM	10µM	5µM	10µM	5µM	10µM
5a	71	80	68	77	69	78
5b	58	66	56	65	57	67
5c	54	63	55	62	61	69
5d	68	76	60	66	64	70
5e	56	63	53	61	58	67
5f	71	80	58	67	64	72
5g	60	69	51	57	57	65
5h	**86**	**93**	**78**	**87**	**79**	**88**

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5i	**85**	**91**	**77**	**86**	**78**	**86**
5j	**87**	**94**	**79**	**88**	**80**	**89**
5k	63	72	61	70	62	71
5l	69	79	66	75	67	75
Doxorubicin	**87**	**95**	**80**	**89**	**81**	**90**
Control	**10**	**10**	**10**	**10**	**10**	**10**

Table 4 presents the IC_{50} values for a series of 5a-l final analogues and the standard Doxorubicin across three cancer cell lines. The IC_{50} value, or the half-maximal inhibitory concentration, indicates the concentration of a compound required to inhibit 50% of cell viability, providing a measure of the compound's potency against cancer cells. Lower IC_{50} values signify higher potency.

The data indicates that several of the 5a-l analogues exhibit potent inhibitory effects across the three cell lines, with compounds 5h, 5i, and 5j demonstrating consistently low IC_{50} values, making them comparable or even superior to Doxorubicin in some cases. The variations in IC_{50} values among different compounds and cell lines highlight the need for further investigation to understand the mechanisms of action and optimize these compounds for potential therapeutic use.

Table 4: IC_{50} values of 5a-l final analogues with standard Doxorubicin

Compound Code	IC_{50} (µM±SEM)		
	MCF-7	**PC-3**	**HeLa**
5a	4.09±0.05	4.88±0.09	4.73±0.05
5b	6.88±0.07	8.64±0.09	8.34±0.06
5c	8.12±0.05	9.79±0.12	9.32±0.11
5d	6.29±0.09	7.49±0.12	7.41±0.05
5e	7.01±0.05	9.88±0.11	9.32±0.09
5f	4.29±0.07	5.50±0.07	5.200±0.08
5g	6.46±0.12	8.25±0.12	8.01±0.11
5h	3.21±0.05	4.19±0.09	4.03±0.14
5i	3.69±0.07	4.60±0.08	4.11±0.09
5j	3.10±0.03	3.91±0.05	3.38±0.05
5k	6.52±0.05	7.94±0.03	7.81±0.02
5l	4.89±0.06	6.677±0.05	6.46±0.07
Doxorubicin	3.12±0.09	3.97±0.08	3.42±0.10

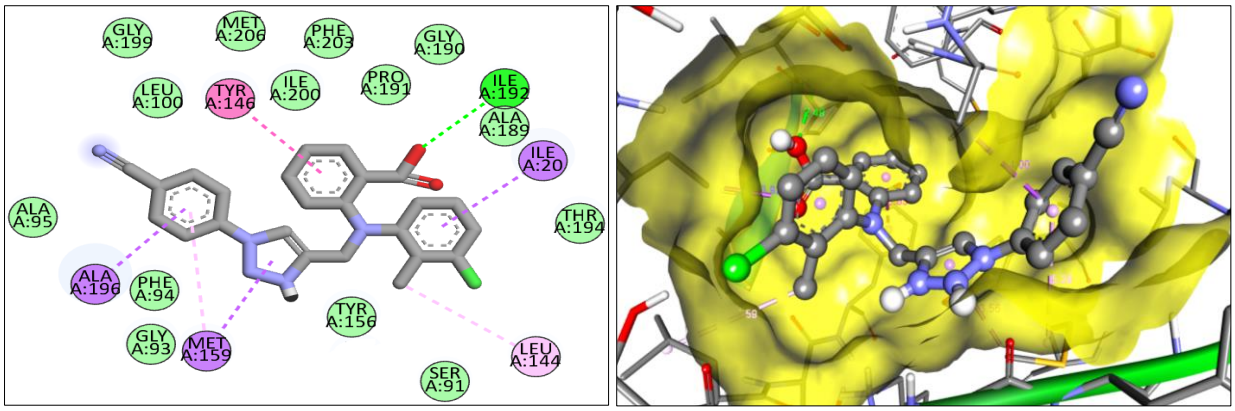
4.2.4 Molecular Docking Assays

Molecular docking studies were conducted on novel derivatives **5a-l**, the standard drug doxorubicin, and Tamoxifen against Enoyl reductase (PDB ID: 1QSG). Enoyl-ACP reductase is a crucial enzyme in the fatty acid synthesis pathway, essential for lipid biosynthesis, supporting the rapid growth and proliferation of cancer cells. This enzyme is overexpressed in various cancers, including breast, prostate, and lung cancers, and is linked to tumor aggressiveness and poor prognosis. Inhibiting enoyl-ACP reductase can disrupt cancer cell metabolism by limiting essential fatty acids, thereby inhibiting tumor growth and inducing apoptosis, making it a promising therapeutic target for novel anti-cancer agents. Tamoxifen, a selective estrogen receptor modulator (SERM), is commonly used to treat and prevent breast cancer.

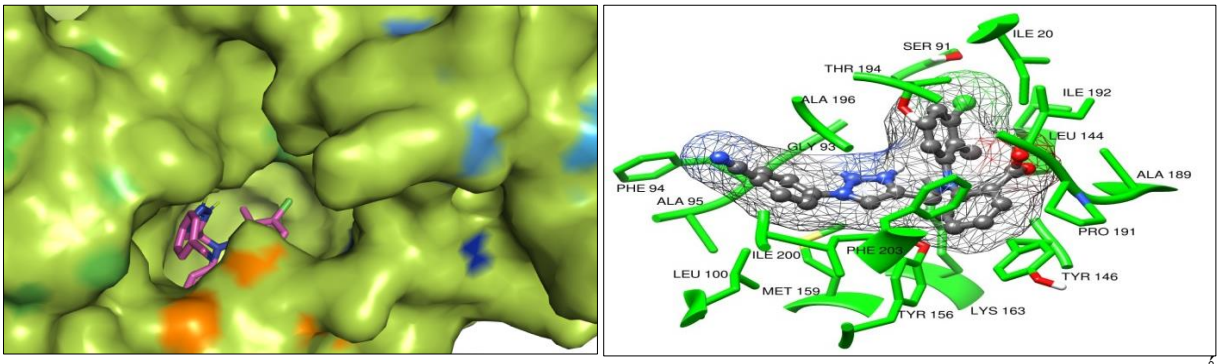
The binding energies of compounds **5a-l** ranged from -9.5 to -10.4 kcal/mol, surpassing the binding energies of doxorubicin (-8.5 kcal/mol) and Tamoxifen (-8.4 kcal/mol). Notably, compounds **5b** and **5h** exhibited binding energies of -10.2 kcal/mol and -10.4 kcal/mol, respectively, indicating superior binding affinities. This is due to the various interactions, including conventional hydrogen bonding, carbon-hydrogen bonding, pi-sigma, pi-anion, pi-cation, pi-alkyl, and Van der Waals interactions, between the novel compounds and Enoyl reductase.

Table 5: Binding Energies and Interacting Amino Acid Residues of Novel Derivatives 5a-l, Tamoxifen, and Doxorubicin with Enoyl Reductase (PDB ID: 1QSG)

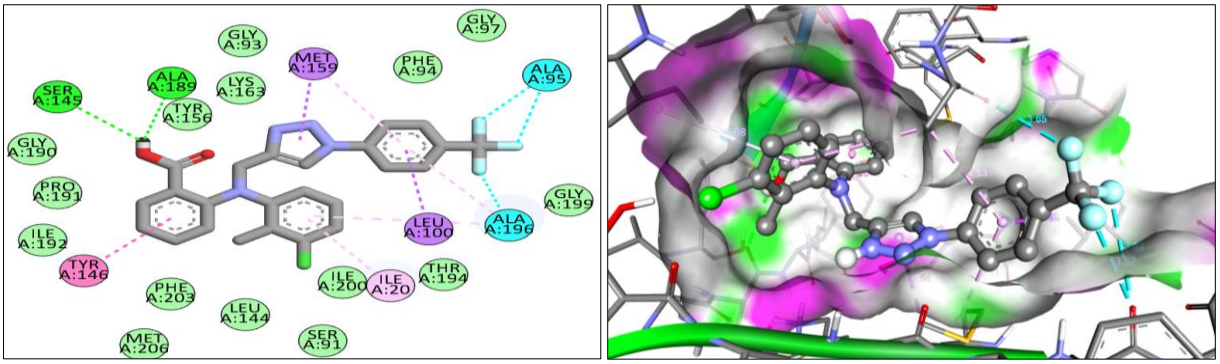
Molecule	B. E	Interacting A.A Residues	
		H-Bonding	Other types of Interactions
5a	**-9.7**	SER 145	ILE 20, GLY 93, PHE 94, ALA 95, LEU 100, LEU 144, TYR 146, TYR 156, MET 159, ALA 189, GLY 190, PRO 191, ILE 192, THR 194, ALA 196, ALA 197, ILE 200, PHE 203, MET 206.
5b	**-10.2**	ILE 192	ILE 20, SER 91, GLY 93, PHE 94, ALA 95, LEU 100, LEU 144, TYR 146, TYR 156, MET 159, ALA 189, GLY 190, PRO 191, THR 194, ALA 196, GLY 199, ILE 200, PHE 203, MET 206.
5c	**-10.0**	SER 145	ILE 20, GLY 93, PHE 94, ALA 95, LEU 100, LEU 144, TYR 146, TYR 156, MET 159, ALA 189, GLY 190, PRO 191, ILE 192, THR 194, ALA 196, ALA 197, GLY 199, ILE 200, PHE 203, MET 206.
5d	**-9.9**	SER 145	ILE 20, GLY 93, PHE 94, ALA 95, GLY 97, LEU 100, LEU 144, TYR 146, TYR 156, MET 159, ALA 189, GLY 190, PRO 191, ILE 192, THR 194, ALA 196, ALA 197, GLY 199, ILE 200, PHE 203, MET 206.
5e	**-9.9**	ALA 95, SER 145	ILE 20, SER 91, GLY 93, PHE 94, ALA 95, GLY 97, LEU 100, SER 120, LEU 144, TYR 146, TYR 156, MET 159, GLY 190, PRO 191, THR 194, ALA 196, ALA 197, ILE 200, PHE 203, MET 206.
5f	**-9.7**	SER 145	ILE 20, SER 91, GLY 93, PHE 94, ALA 95, GLY 97, LEU 100, LEU 144, TYR 146, TYR 156, MET 159, GLY 190, PRO 191, ILE 192, THR 194, ALA 196, GLY 199, ILE 200, PHE 203, MET 206.
5g	**-9.6**	NIL	ILE 20, SER 91, GLY 93, PHE 94, ALA 95, LEU 100, LEU 144, TYR 146, TYR 156, MET 159, ALA 189, GLY 190, PRO 191, THR 194, ALA 196, ILE 200, PHE 203, MET 206.
5h	**-10.4**	SER 145, ALA 189	ILE 20, SER 91, GLY 93, PHE 94, ALA 95, GLY 97, LEU 100, LEU 144, TYR 146, TYR 156, MET 159, LYS 163, GLY 190, PRO 191, ILE 192, THR 194, ALA 196, GLY 199, ILE 200, PHE 203, MET 206.
5i	**-9.5**	SER 145	ILE 20, GLY 93, PHE 94, ALA 95, LEU 100, LEU 144, TYR 146, TYR 156, MET 159, ALA 189, GLY 190, PRO 191, ILE 192, THR 194, ALA 196, ALA 197, ILE 200, PHE 203, MET 206.
5j	**-9.9**	SER 145, ALA 189	ILE 20, GLY 93, PHE 94, ALA 95, LEU 100, LEU 144, TYR 146, TYR 156, MET 159, GLY 190, PRO 191, ILE 192, THR 194, ALA 196, ALA 197, GLY 199, ILE 200, PHE 203, MET 206.
5k	**-10.0**	ILE 192	ILE 20, SER 91, GLY 93, PHE 94, ALA 95, LEU 100, LEU 144, TYR 146, TYR 156, MET 159, ALA 189, GLY 190, PRO 191, THR 194, ALA 196, ALA 197, ILE 200, PHE 203, MET 206.
5l	**-9.9**	ALA 189	ILE 20, GLY 93, PHE 94, ALA 95, LEU 100, SER 120, LEU 144, SER 145, TYR 146, TYR 156, MET 159, 190, PRO 191, ILE 192, THR 194, ALA 196, ALA 197, ILE 200, PHE 203, MET 206.
Tamoxifen	**-8.4**	NIL	SER 19, ILE 20, ALA 21, SER 91, GLY 93, PHE 94, ALA 95, LEU 100, LEU 144, TYR 146, TYR 156, MET 159, LYS 163, ALA 189, GLY 190, PRO 191, ILE 192, THR 194, ALA 196, ALA 197, GLY 199, ILE 200, PHE 203, MET 206
doxo	**-8.5**	ILE 20, GLY 93, LEU 195	GLY 13, VAL 14, ALA 15, SER 16, SER 19, ALA 21, GLN 40, LEU 44, CYS 63, ASP 64, VAL 65, SER 91, ILE 92, PHE 94, ILE 119, LEU 144, LYS 163, THR 194, ALA 196



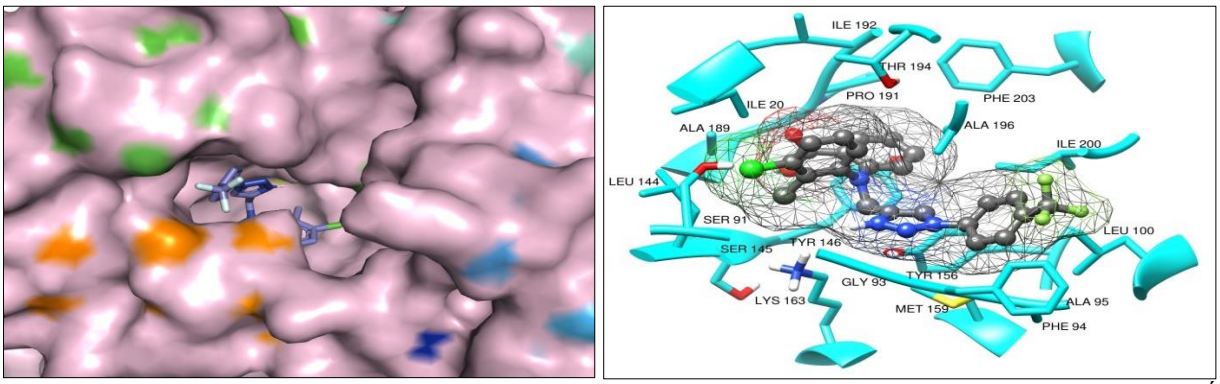
2D Interaction of '**5b**' with the Enoyl Reductase 3D Interaction of '5b' with the Enoyl Reductase



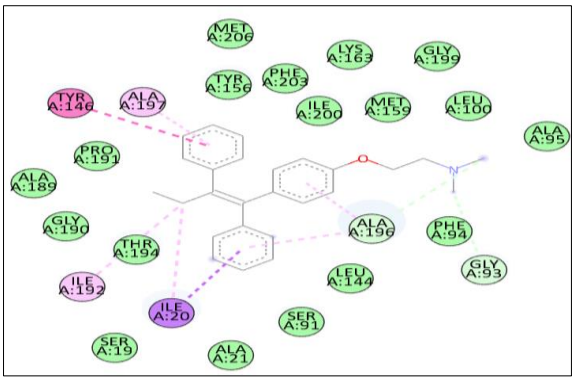
'**5b**' in the binding pocket of Enoyl Reductase Interaction of '**5b**' with the residues of Enoyl Reductase within 4 Å

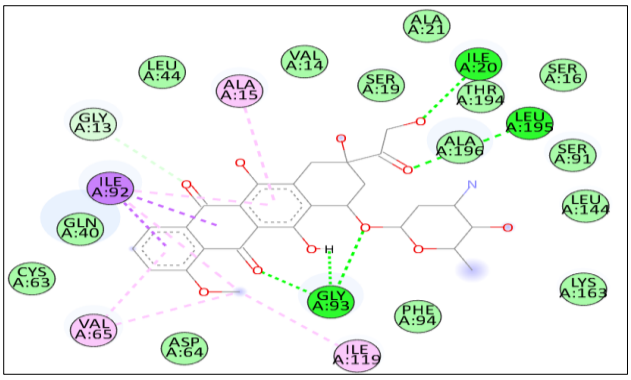


2D Interaction of '**5h**' with the Enoyl Reductase 3D Interaction of '**5h**' with the Enoyl Reductase

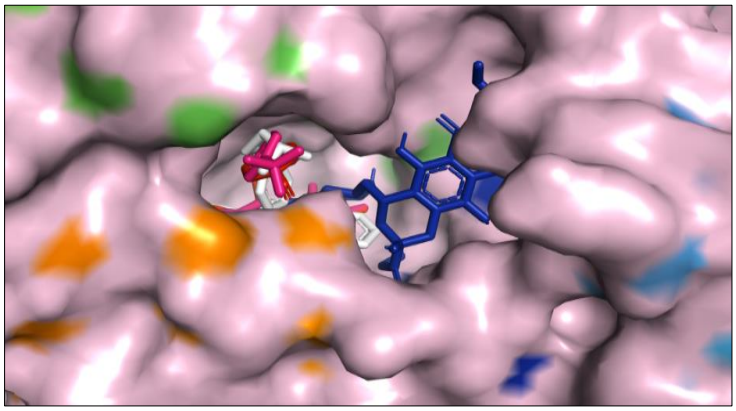


'**5h**' in the binding pocket of Enoyl Reductase Interaction of '**5h**' with the residues of Enoyl Reductase within 4 Å





2D Interaction of 'Tamoxifen' with the Enoyl Reductase 2D Interaction of 'doxorubicin' with the Enoyl Reductase



Molecule '**5b**' (Red), '**5h**' (Pink), 'tamoxifen' (white) with doxorubicine (blue) in the Same binding pocket of enoyl Reductase

Fig: 2: Molecular Docking assays results of Compounds 5b, 5h, Tamoxifen, and Doxorubicin with Enoyl Reductase

5. CONCLUSIONS

A series of 2-((3-chloro-2-methylphenyl)((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)amino)benzoic acid derivatives (5a-l) were successfully synthesized using a multi-step process incorporating click chemistry. The structural and purity of these derivatives were confirmed through comprehensive spectral analyses, including NMR, IR, and mass spectrometry. These findings establish the compounds' suitability for further biological testing. Antibacterial Activity of Compounds 5c, 5h, and 5i exhibit the highest antibacterial potency against both Gram-positive and Gram-negative bacteria. Antifungal Activity of compounds 5b, 5g, and 5h display the strongest antifungal activity with inhibition zones comparable to Amphotericin B. Cancer Cell Growth Inhibition of compounds 5h, 5i, and 5j demonstrate the highest growth inhibition across MCF-7, PC-3, and HeLa cell lines, comparable to or exceeding Doxorubicin. IC_{50} Value of compounds 5e, 5f, and 5j exhibit low IC_{50} values across the cell lines, suggesting potent activity comparable to or superior to Doxorubicin. Further research is needed to optimize these compounds for therapeutic use. Docking studies of Compounds 5a-l showed superior binding energies (-9.5 to -10.4 kcal/mol) compared to doxorubicin and Tamoxifen. Compounds 5b and 5h had the highest binding affinities, suggesting strong potential as inhibitors of enoyl reductase.

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