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Research Article

Synthesis, Molecular Docking and Antimicrobial Evaluation of Octadecanoic Acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl) phenyl]-amide pyrazoline Derivatives

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ABSTRACT

A new series of octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl]-amide pyrazoline derivatives were designed synthesized, and characterized by spectral analysis (IR, MS, 1H-NMR, 13C-NMR). The compounds were screened for in vitro antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. All the compounds exhibited significant antibacterial activity with minimum inhibitory concentrations ranging from 1.2 to 2.5 mg/mL. The newly synthesized compounds were also found to have good anti-fungal activity against *Fusarium oxysporum* and *Penicillium italicum,* with all the compounds showing MIC of 2.5 mg/mL for both the fungal strains. Molecular docking studies were also incorporated to elucidate the possible interactions of the synthesized compounds with bacterial and fungal proteins.

Introduction

Heterocycles are a unique class of compounds that plays an important role in medicinal chemistry. They can be acidic as well as basic, which can interact with nucleophilic and electrophilic reactants.[1] Pyrazoles are an important class of heterocycles characterized by a 5-membered ring with three carbon atoms and two nitrogen atoms. Ludwig Knorr first coined the term pyrazole in 1883. They are also known as alkaloids owing to their composition and unique pharmacological effects on humans. The first pyrazole present in nature was isolated from seeds of watermelons in 1959.[2] They are very rare in nature due to their difficulty in forming N-N bonds by living organisms.[3] Naturally, pyrazoles are extracted from Houttuynia cordata, which is a common plant from tropical Asia.[4] Pyrazoles are also found in natural products such as vitamins, hormones, and alkaloids.[5] Pyrazolines are the partially reduced forms of pyrazole, and the completely reduced form is pyrazolidine.[6] The substituted pyrazoles are synthesized by two classical methods, which involve approaches on intermolecular [3+2] cycloadditions of 1,3-dipoles to alkynes or by condensations of hydrazines with 1,3-dicarbonyl compounds or their 1,3-dielectrophile equivalents.[7] Pyrazoles are the backbones for many compounds for widespread applications as building blocks of a large number of compounds, agrochemicals, catalysis, and medicine.[8] Pyrazole and its derivatives played an important role in the field of medicinal chemistry with a long history of applications in pharmaceutical, agrochemical[2] as well as a chemical industry[9] and made up the core part of many compounds and have been reported to show a wide range of activities such as antimicrobial, antiviral, antitumor, anti-depressant, insecticides, fungicides,[10] anti-histaminic,[11] anti-fungal, anti-convulsant, anti-viral8,

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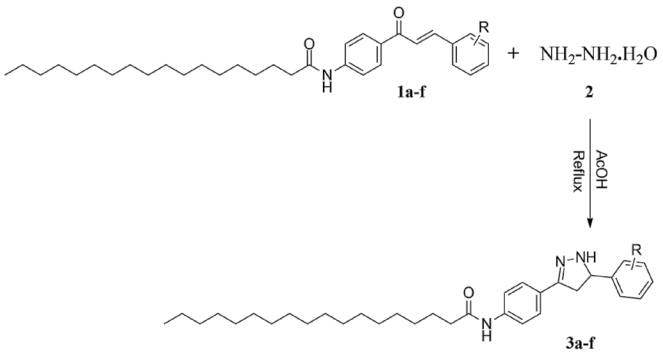
antitubercular, anti-inflammatory,[12] anticancer, analgesic, antihypertensive and CNS activity like an antiepileptic, antidepressant, etc.[13]

At present, antimicrobial resistance is a vital concern that poses a major threat to public health, and therefore, it is necessary to develop new antibiotic analogues to combat the drug resistance. Pyrazole ring fused in chalcone structure have drawn considerable attention as antimicrobial agents to fight against the resistance towards anti-fungal and antibacterial strains.[14] Henceforth, in the present study, we report the design and synthesis of a series of octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl) phenyl]-amide pyrazoline derivatives (3a-f). Further considering the potential antibacterial properties of pyrazole derivatives, the synthesized compounds were tested for their antibacterial activity against *E. coli* and *S. aureus* and anti-fungal activity against *F. oxysporum* and *P. italicum*. Molecular docking studies were performed to understand the possible interaction between the receptor protein and the synthesized compounds. Bacterial protein DNA gyrase belongs to the topoisomerase family which is involved in replication and transcription by catalysing the negative supercoiling of bacterial circular DNA. Since its blockage induces bacterial death it is considered a suitable target for antibacterial agents. Dihydrofolate reductase (DHFR) is a vital target in many therapeutic areas like cancer and anti-infectives where it is used to generate anti-fungal, antibacterial, and antiparasitic agents.[15,16]

Material and Methods

All the reagents and solvents were purchased from commercially available sources and used without further purification. Melting points were recorded in open capillaries using IKON melting point apparatus and are uncorrected. FTIR spectra of the compounds were recorded on Perkin-Elmer spectrophotometer (Spectrum-Two) using KBr disk, and values are expressed in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra for the compounds were recorded using Bruker 300 MHz spectrophotometer in $CDCl_3$ as a solvent and TMS as an internal standard; values are given in parts per million (ppm). Mass spectra for the compounds were recorded on Advion Expression (S)CMS system. Progress of the reactions was monitored by Thin Layer Chromatography (TLC) with silica gel plates (Merck) using ethyl acetate and n-hexane (3:7) as a solvent system and visualized under UV-light/iodine vapours.

General procedure for the synthesis of octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl]-amide pyrazoline derivatives (3a-f): To a mixture octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide 1a (0.489 g, 1 mmol), hydrazine hydrate 2 (0.150 g, 10 mmol) in ethanol was added AcOH and the reaction mixture was stirred under reflux conditions for 24 h. TLC monitored the progress of the reaction. After completion of the reaction, crushed ice was added and filtered. The solid product



Scheme 1: Synthesis of octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl]-amide pyrazoline derivatives

was washed with water (3-4 times) to obtain the pure octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl]-amide pyrazoline (3a). The same method was followed for the synthesis of other compounds (3b-f) (Scheme 1).

N-(4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl) phenyl)stearamide (3a)

Brown solid, Yield: 85%, MP: 102-105°C. FT-IR (KBr, cm^{-1}): 3275.27(N-H), 3027.99(Ar-CH), 1695.17(C=O), 1593.31(C=N), 1249.61(C-N), 1026.43(N-N). ^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm): 7.72-7.67 (m, 2H, Ar-H), 7.60-7.57 (m, 2H, Ar-H), 7.46-7.42 (m, 1H, Ar-H), 7.36-7.28 (m, 2H, Ar-H), 7.22-7.20 (m, 2H, Ar-H), 5.61-5.55 (dd, 1H, -CH), 3.77-3.67 (dd, 1H, -CH_2), 3.17-3.10 (dd, 1H, -CH_2), 2.36-2.32 (t, 2H, CH_2), 1.72-1.61 (q, 2H, CH_2), 1.25 (s, 28H, $(CH_2)_{14}$), 0.89-0.85 (t, 3H, CH_3). ^{13}C-NMR (300 MHz, $CDCl_3$), δ (ppm): 178.90, 171.89, 169.10, 153.82, 141.91, 140.17, 129.03, 127.80, 127.63, 126.98, 126.39, 125.64, 120.23, 119.60, 60.10, 42.53, 34.19, 32.06, 29.81, 29.51, 29.49, 29.40, 29.25, 24.93, 22.82, 22.06, 14.24. MS (m/z): [M+1]; 504.8.

N-(4-(5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)stearamide (3b)

Pale yellow solid, Yield: 75%, MP: 70-74 oC. FT-IR (KBr, cm^{-1}): 3311.04(N-H), 3050.54(Ar-CH), 1685.84(C=O), 1598.75(C=N), 1255.05(C-N), 1007.77(N-N). 1H-NMR (300 MHz, CDCl3), δ (ppm): 7.78-7.58 (m, 3H, Ar-H), 7.44-7.34 (m, 1H, Ar-H), 7.26-7.18 (m, 3H, Ar-H), 7.12-7.10 (m, 1H, Ar-H), 5.57-5.51 (dd, 1H, -CH), 3.78-3.68 (dd, 1H, -CH2), 3.14-3.07 (dd, 1H, -CH2), 2.37-2.32 (t, 2H, CH2), 1.74-1.61 (q, 2H, CH2), 1.25 (s, 28H, (CH2)14), 0.89-0.85 (t, 3H, CH3). 13C-NMR (300 MHz, CDCl3), δ (ppm): 178.79, 172.03, 169.26, 167.81, 153.83, 143.88, 140.37, 134.93, 130.36, 128.05, 127.67, 125.81, 123.96, 119.67, 59.62, 42.42, 34.29, 32.05, 29.80, 29.60, 29.48, 29.41, 29.27, 24.99, 22.81, 22.00, 20.68, 14.23. MS (m/z): [M+1] ; 539.0.

N-(4-(5-(p-tolyl)-4,5-dihydro-1H-pyrazol-3-yl) phenyl)stearamide (3c)

Pale yellow solid, Yield: 71%, MP: 80-82 °C. FT-IR (KBr, cm^{-1}): 3270.60(N-H), 3038.10(Ar-CH), 1694.40(C=O),

1601.86(C=N), 1258.94(C-N), 1012.44(N-N). ^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm): 9.11 (s, 1H, NH, D_2O exchangeable), 7.69-7.54 (m, 3H, Ar-H), 7.44-7.34 (m, 1H, Ar-H), 7.25-7.19 (m, 1H, Ar-H), 7.12-7.10 (m, 3H, Ar-H), 5.57-5.51 (dd, 1H, -CH), 3.75-3.65 (dd, 1H, -CH_2), 3.15-3.08 (dd, 1H, -CH_2), 2.37-2.33 (t, 2H, CH_2), 2.0 (s, 3H, Ar-CH_3), 1.74-1.63 (q, 2H, CH_2), 1.25 (s, 28H, $(CH_2)_{14}$), 0.89-0.85 (t, 3H, CH_3). ^{13}C-NMR(300 MHz, $CDCl_3$), δ (ppm): 178.39, 172.12, 170.95, 169.11, 168.03, 154.04, 144.79, 140.34, 138.96, 137.47, 129.65, 127.56, 125.55, 119.62, 59.90, 42.55, 37.82, 32.03, 29.79, 29.59, 29.46, 29.40, 29.27, 25.63, 22.79, 22.04, 14.22. MS (m/z): [M+1] ; 518.7.

N-(4-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)stearamide (3d)

Pale yellow solid, Yield: 81%, MP: 75-79°C. FT-IR (KBr, cm^{-1}): 3256.60(N-H), 3038.10(Ar-CH), 1688.95(C=O), 1587.86(C=N), 1259.72(C-N), 1003.11(N-N). ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 9.20 (s, 1H, NH, D_2O exchangeable), 7.77-7.65 (m, 2H, Ar-H), 7.60-7.53 (m, 2H, Ar-H), 7.44-7.41 (m, 2H, Ar-H), 7.26 (m, 1H, Ar-H), 7.11-7.08 (m, 1H, Ar-H), 5.54-5.49 (dd, 1H, -CH), 3.77-3.67 (dd, 1H, -CH_2), 3.10-3.05 (dd, 1H, -CH_2), 2.36-2.31 (t, 2H, CH_2), 1.71-1.60 (q, 2H, CH_2), 1.25 (s, 28H, $(CH_2)_{14}$), 0.89-0.85 (t, 3H, CH_3). ^{13}C-NMR (300 MHz, $CDCl_3$), δ (ppm): 178.15, 172.25, 171.05, 169.21, 168.15, 167.86, 154.00, 140.85, 140.53, 132.10, 128.61, 127.58, 127.45, 126.49, 121.64, 119.66, 59.57, 42.34, 34.20, 32.01, 29.78, 29.57, 29.49, 29.44, 29.38, 29.24, 22.77, 21.97, 14.19. MS (m/z): [M^+] ; 582.4.

N-(4-(5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)stearamide (3e)

Yellow solid, Yield: 72%, MP: 86-89°C. FT-IR (KBr, cm^{-1}): 3332.82(N-H), 3036.30(Ar-CH), 1696.67(C=O), 1601.36(C=N), 1249.24(C-N), 1029.50(N-N). ^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm): 7.66-7.60 (m, 3H, Ar-H), 7.26-7.13 (m, 1H, Ar-H), 6.96-6.82 (m, 2H, Ar-H), 6.29-6.24 (m, 2H, Ar-H), 5.54-5.51 (dd, 1H, -CH), 3.84-3.68 (t, 3H, -CH_3), 3.15-3.09 (dd, 1H, -CH_2), 2.40-2.35 (t, 2H, CH_2), 2.16-2.05 (dd, 1H, -CH_2), 1.71-1.64 (q, 2H, CH_2), 1.25 (s, 28H, $(CH_2)_{14}$), 0.89-0.85 (t, 3H, CH_3).^{13}C-NMR (300 MHz, $CDCl_3$), δ (ppm): 178.72, 171.82, 169.02, 159.16, 153.77, 140.08, 134.18, 128.55, 127.62, 127.02, 126.37, 119.59, 114.50, 114.36, 59.58, 55.48, 42.44, 37.97, 34.13, 32.06, 29.81, 29.60, 29.50, 29.41, 29.24, 25.63, 24.92, 22.82, 14.25. MS (m/z): [M-1] ; 532.3.

N-(4-(5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)stearamide (3f)

Pale yellow solid, Yield: 78%, MP: 60-63°C. FT-IR (KBr, cm^{-1}): 3216.33(NH), 3044.25(Ar-CH), 1699.31(C=O), 1593.41(C=N), 1265.12(C-N), 1034.79(N-N). ^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm): 7.69-7.66 (m, 1H, Ar-H), 7.62-7.48 (m, 2H, Ar-H), 7.40-7.31 (m, 2H, Ar-H), 7.21-7.18 (m, 1H, Ar-H), 7.06-7.03 (m, 1H, Ar-H), 6.94-6.91 (m, 1H, Ar-H), 5.93-5.87 (dd, 1H, -CH), 3.87-3.77 (dd, 1H, -CH_2), 3.07-2.99 (dd, 1H, -CH_2), 2.37-2.32 (t, 2H, CH_2), 1.68-1.58 (q, 2H, CH_2), 1.25 (s, 28H, $(CH_2)_{14}$), 0.89-0.85 (t, 3H, CH_3). ^{13}C-NMR (300 MHz, $CDCl_3$), δ (ppm): 179.38, 169.25, 168.99, 154.35, 140.31, 138.50, 131.84, 131.17, 130.14, 128.94, 127.62, 127.40, 125.93, 119.62, 57.87, 41.59, 32.05, 29.80, 29.57, 29.48, 29.38, 29.22, 24.88, 22.81, 14.23. MS (m/z): [M+12] ; 550.5.

Biological Assay

Antibacterial Activity

The antibacterial activity of the newly synthesized compounds was tested against gram-negative bacteria *E. coli* and gram-positive bacteria *S. aureus*. The antibacterial activity was studied by determining the minimum inhibitory concentration (MIC) of the compounds. MIC is the lowest concentration of the test compounds, which prevents the growth of bacteria.[17] The bacterial strains were grown in 250 mL of nutrient broth overnight at 37°C, and the freshly grown culture broth was further centrifuged at 3000 rpm for 15 minutes. The bacterial cells were further resuspended in sterile Phosphate buffered solution (PBS) to obtain approximately 108 CFU/mL cell count. MIC was determined by taking 10mg/mL initial concentration of the compounds prepared using DMSO as the solvent. Each compound was further serially diluted two-fold with nutrient broth, inoculated with 200 µL of ~108 CFU/mL of *E. coli* cells, and incubated at 37°C for 24 hours. The antibiotic Streptomycin (1 mg/mL) was taken as the standard reference. The lowest concentration of the compounds at which no visible growth was observed was recorded as the MIC. All experiments were performed in triplicates.

Anti-fungal Activity

The same compounds were also tested for anti-fungal activity against *F. oxysporum* and *P. italicum* by following the same two-fold dilution method to determine the MIC. Fresh fungal cultures were grown in potato dextrose broth at 27°C ± 2°C for around 48 hours. Initially, each compound was taken 10 mg/mL, and the test compounds were further serially diluted two-fold using potato dextrose broth. Around 200 µL of the freshly grown fungal cultures were inoculated on each tube and were incubated at 27°C for 48 hours. The lowest concentration, which showed no growth, was considered as the MIC for each drug. Antibiotic Fluconazole was used as a standard drug for anti-fungal activity. All experiments were performed in triplicate to confirm reproducibility.

Molecular Docking Studies

Molecular docking studies were conducted using Molegro Virtual Docker (MVD). In this study, the methodology is based on grid cavity prediction algorithm to determine the potential binding sites. The compounds or ligands were docked against three target protein structures of enzymes dihydrofolate reductase (Pdb id: 3SRW) as gram-positive bacteria target, DNA Gyrase B (Pdb id: 1KZN)

as gram-negative bacteria target and dihydrofolate reductase (Pdb id: 1AI9) as fungi target. All the ligands were drawn using ChemDraw Ultra 12.0 and Chem3D pro 12.0 optimized by MM2 force field method. For molecular docking simulation, all the water molecules were removed and charges assigned. MVD was used for predicting the cavities and the binding cavity set at the site X: -1.03, Y: -31.45, Z: 7.57 with a sphere radius 13 Å, X: 19.88, Y: 26.73, Z: 39.56 with a sphere radius 15 Å, X: 30.13, Y: -6.63, Z: 4.28 with a sphere radius 19 Å for enzymes dihydrofolate reductase (Pdb id: 3SRW), DNA Gyrase B (Pdb id: 1KZN) and dihydrofolate reductase (Pdb id: 1AI9), respectively. All the ligands were docked against the three target protein with 30 independent runs for each ligand and the top pose of the ligand from the docking score was selected for molecular interaction study.[18,19]

Results and Discussion

The present study reports the synthesis of octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl]-amide pyrazoline derivatives (Scheme 1). The reaction was carried out starting from octadecanoic acid [4-(3-phenyl-acryloyl)-phenyl]-amide chalcone derivatives 1a-f with hydrazine hydrate 2 in the presence of Acetic acid (AcOH) in ethanol solution refluxing for 24 hours. The newly synthesized pyrazoline derivatives were obtained in good yield and characterized by a spectroscopic technique such as ^{1}H-NMR, ^{13}C-NMR, IR, and Mass.

Table 1: Antimicrobial activity of the tested compounds

	Minimum inhibitory concentration (mg/mL)			
Compound	*E. coli*	*S. aureus*	*F. oxysporum*	*P. italicum*
3a	2.5	2.5	2.5	2.5
3b	1.25	2.5	2.5	2.5
3c	2.5	2.5	2.5	2.5
3d	1.25	1.25	2.5	2.5
3e	1.25	1.25	2.5	2.5
3f	1.25	1.25	2.5	2.5
Streptomycin	0.07	0.15	-	-
Flucanazole	-	-	0.06	0.03

The Infrared spectrum of the newly synthesized pyrazoline derivatives 3a-f shows a new absorption band at 3275 and 1593 cm^{-1} for NH and C=N groups, respectively. The N-N group also appear at 1026 cm^{-1}. The 1H-NMR showed the -CH_3 group as a triplet at 0.89-0.85 δ ppm, and the -CH_2 protons of the pyrazoline ring resonated as a pair of doublet of doublets at δ 3.17-3.10 ppm, 3.77-3.67 ppm and 5.61-5.55 ppm. In ^{13}C-NMR, it was also observed that the characteristic chemical shift values appear at δ 42.55-41.59 ppm, 60.10-55.48 ppm, 154.35-153.77 ppm for pyrazoline rings carbons CH_2, CH, and C=N, respectively. The antimicrobial study of the newly synthesized compounds was performed against gram-negative bacteria *E. coli* and gram-positive bacteria *S. aureus* and two different fungi *F. oxysporum* and *P. italicum*. The standard antibiotics, namely streptomycin, and fluconazole, were used as a positive control for bacteria and fungi. The antimicrobial activity was recorded for each tested compound as the minimum inhibition concentration (MIC). The results of the antimicrobial activity of the newly synthesized compounds are presented in Table 1. The results show that all the compounds exhibited considerable antibacterial activity against both the tested strains. The MIC of compounds 3d, 3e, and 3f was 1.25 mg/mL for both the tested bacterial strains. Compounds 3a and 3c showed MIC at 2.5 mg/mL against both *E. coli* and *S. aureus*, while compound 3b showed activity at 1.25 mg/mL and 2.5 mg/mL for *E. coli* and *S. aureus,* respectively. For anti-fungal activity, it was found that all the compounds showed the same MIC at 2.5mg/mL for both the fungal strains, *F. oxysporum* and. *P. italicum*. From the molecular docking studies, the binding mode of ligand (3a-f) with the three target protein structures of enzymes dihydrofolate reductase (Pdb id: 3SRW) as gram-positive bacteria target, DNA Gyrase B (Pdb id: 1KZN) as gram-negative bacteria target and dihydrofolate reductase (Pdb id: 1AI9) as fungi target were evaluated. The top pose from each ligand was then selected for ligand-protein interaction energy analysis, as shown in Tables 2–4. Fig. 1a, 1b and 1c depicts the possible mode of interaction between the ligand and the target protein's active site. Tables 5, 6 and 7 show the

Table 2: Docking score of the compounds with 1KZN

Ligand	*MolDock Score*	*Rerank Score*	*Interaction*	*Internal*	*HBond*	*LE1*	*LE3*
3a	-145.476	-71.2202	-164.777	19.3016	-1.40248	-3.93178	-1.92487
3b	-150.295	-33.9811	-170.541	20.2463	-3.52455	-3.95513	-0.89424
3c	-152.504	-40.9473	-173.785	21.2812	-2.54496	-4.01326	-1.07756
3d	-149.581	-9.48633	-162.948	13.3674	-3.25912	-3.93634	-0.24964
3e	-106.747	-41.5115	-125.545	18.7989	-2.97058	-2.73709	-1.0644
3f	-147.282	-79.7796	-175.631	28.3486	-1.37037	-3.87585	-2.09946
Streptomycin	-111.289	-103.256	-156.693	45.4042	-9.19529	-2.78222	-2.58141

Table 3: Docking score of the compounds with 3SRW

Ligand	*MolDock Score*	*Rerank Score*	*Interaction*	*Internal*	*HBond*	*LE1*	*LE3*
3a	-173.371	-73.9621	-186.148	12.7778	-3.49061	-4.68569	-1.99898
3b	-159.917	-100.526	-169.882	9.96486	-1.62162	-4.20834	-2.64543
3c	-153.707	-93.6604	-183.33	29.6229	-2.19789	-4.04492	-2.46475
3d	-157.351	-50.9853	-179.902	22.5502	-1.61141	-4.14082	-1.34172
3e	-156.937	-28.6536	-179.205	22.2676	-3.19522	-4.02403	-0.73471
3f	-161.157	-111.949	-181.687	20.5294	-2.5	-4.24098	-2.94602
Streptomycin	-125.724	-113.962	-183.652	57.9273	-15.6688	-3.14311	-2.84905

Table 4: Docking score of the compounds with 1AI9

Ligand	*MolDock Score*	*Rerank Score*	*Interaction*	*Internal*	*HBond*	*LE1*	*LE3*
3a	-160.069	-115.946	-208.812	48.7422	-2.6774	-4.3262	-3.13368
3b	-153.085	-119.312	-175.238	22.1533	-2.5	-4.02854	-3.13978
3c	-179.668	-127.828	-205.144	25.4766	-3.69616	-4.7281	-3.36389
3d	-174.102	-134.016	-208.953	34.8501	-2.50859	-4.58164	-3.52674
3e	-148.034	-112.771	-174.822	26.7877	-4.1113	-3.79575	-2.89157
3f	-165.004	-74.1873	-180.004	15.0006	-2.69913	-4.34221	-1.9523
Fluconazole	-125.68	-96.2464	-134.019	8.33914	-13.0705	-5.71273	-4.37483

Table 5: Molecular interaction analysis of the compounds with the active site of 1KZN

Compound	*Interaction (Protein-Ligand)*	*Interaction Energy (kJ/mol)*	*Interaction Distance(Å)*	*Hybridization (Protein Atom)*	*Hybridization (Ligand Atom)*
3a	Gly77(O)---N(26)	-0.402	3.16	Sp^2(Acceptor)	Sp^2(Donor)
	Arg136(NH1)---N(27)	-1.231	3.35	Sp^2(Donor)	Sp^2(Acceptor)
	Gly77(N)---N(27)	-0.080	3.38	Sp^2(Donor)	Sp^2(Acceptor)
3b	Gly77(O)---N(26)	-2.498	2.66	Sp^2(Acceptor)	Sp^2(Donor)
	Gly77(N)---N(27)	-1.026	3.09	Sp^2(Donor)	Sp^2(Acceptor)
3c	Gly77(O)---N(26)	-1.475	2.84	Sp^2(Acceptor)	Sp^2(Donor)
	Gly77(N)---N(27)	-1.069	2.96	Sp^2(Donor)	Sp^2(Acceptor)
3d	Gly77(O)---N(26)	-0.342	3.34	Sp^2(Acceptor)	Sp^2(Donor)
	Gly77(N)---N(27)	-0.069	3.54	Sp^2(Donor)	Sp^2(Acceptor)
3e	Arg136(NH1)---O(37)	-2.5	2.89	Sp^2(Donor)	Sp^3(Acceptor)
	Arg136(NH2)---O(37)	-1.097	3.23	Sp^2(Donor)	Sp^3(Acceptor)
3f	Gly77(N)---N(27)	-0.249	3.35	Sp^2(Donor)	Sp^2(Acceptor)
	Gly77(O)---N(26)	-0.832	3.12	Sp^2(Acceptor)	Sp^2(Donor)
	Asp73(OD1)---N(19)	-0.289	2.66	Sp^3(Acceptor)	Sp^2(Donor)
Streptomycin	Asn46(O)---O(11)	-2.5	2.69	Sp^2(Acceptor)	Sp^2(Both)
	Glu50(N)---O(11)	-0.069	3.55	Sp^2(Donor)	Sp^3(Both)
	Gly77(O)---N(2)	-0.555	3.18	Sp^2(Acceptor)	Sp^2(Donor)
	Asn46(ND2)---O(33)	-2.5	2.77	Sp^2(Donor)	Sp^3(Both)
	Asn46(ND2)---O(14)	-2.5	2.66	Sp^2(Donor)	Sp^3(Acceptor)
	Lle90(O)---O(34)	-1.392	3.32	Sp^2(Acceptor)	Sp^3(Both)
	Val120(N)---O(34)	-0.759	2.87	Sp^2(Donor)	Sp^3(Both)
	Ser121(N)---O(34)	-2.5	2.85	Sp^2(Donor)	Sp^3(Both)
	Ser121(OG)---O(34)	-1.668	3.27	Sp^3(Both)	Sp^3(Both)
	Ala96(N)---O(35)	-1.346	2.94	Sp^2(Donor)	Sp^3(Both)
	Val93(O)---O(35)	-0.793	3.44	Sp^2(Acceptor)	Sp^3(Both)

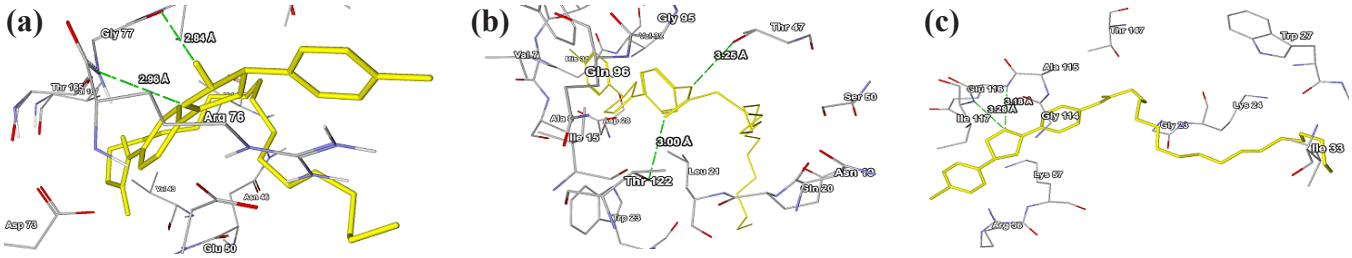


Fig. 1: Molecular interaction of the compound 3c at the active pockets of the protein 1KZN (a) and compound 3d at the active pockets of the protein 3SRW(b) and 1AI9(c)

Table 6: Molecular interaction analysis of the compounds with the active site of 3SRW

Compound	*Interaction (Protein-Ligand)*	*Interaction Energy (kJ/mol)*	*Interaction Distance(Å)*	*Hybridization (Protein Atom)*	*Hybridization (Ligand Atom)*
3a	Ala8(O)---N(26)	-1.393	3.26	Sp^2 (Acceptor)	Sp^2 (Donor)
	Thr47(N)---O(18)	-0.670	3.17	Sp^2 (Donor)	Sp^2 (Acceptor)
	Thr47(OG1)---O(18)	-1.201	2.44	Sp^3 (Both)	Sp^2 (Acceptor)
	Gly95(N)---O(18)	-0.366	3.33	Sp^2 (Donor)	Sp^2 (Acceptor)
3b	Thr47(OG1)---O(18)	-0.273	3.28	Sp^3 (Both)	Sp^2 (Acceptor)
	Ser50(OG)---O(18)	-2.5	2.85	Sp^3 (Both)	Sp^2 (Acceptor)
3c	Thr47(OG1)---N(19)	-2.197	3.16	Sp^3 (Both)	Sp^2 (Donor)
3d	Thr122(OG1)---O(18)	-2.5	3.00	Sp^3 (Both)	Sp^2 (Acceptor)
	Thr47(OG1)---N(19)	-1.611	3.25	Sp^3 (Both)	Sp^2 (Donor)
3e	Ala8(O)---N(26)	-2.151	3.17	Sp^2 (Acceptor)	Sp^2 (Donor)
	Thr122(OG1)---O(18)	-1.938	3.01	Sp^3 (Both)	Sp^2 (Acceptor)
	Asn19(N)---O(18)	-1.043	3.30	Sp^2 (Donor)	Sp^2 (Acceptor)
3f	Ser50(OG)---O(18)	-2.5	2.76	Sp^3 (Both)	Sp^2 (Acceptor)
Streptomycin	Thr47(O)---N(39)	-1.340	3.29	Sp^2 (Acceptor)	Sp^2 (Donor)
	Asn19(OD1)---O(20)	-1.769	2.51	Sp^2 (Acceptor)	Sp^3 (Both)
	Asn19(N)---O(22)	-0.441	3.42	Sp^2 (Donor)	Sp^2 (Acceptor)
	Ile15(O)---O(11)	-2.5	2.64	Sp^2 (Acceptor)	Sp^3 (Both)
	Thr97(OG1)---O(35)	-1.283	3.34	Sp^3 (Both)	Sp^3 (Both)
	Lys46(NZ)---O(35)	-1.166	3.37	Sp^3 (Donor)	Sp^3 (Both)
	Phe93(O)---N(2)	-1.095	3.38	Sp^2 (Acceptor)	Sp^2 (Donor)
	Phe93(O)---O(12)	-1.331	3.33	Sp^2 (Acceptor)	Sp^3 (Both)
	Thr47(OG1)---O(12)	-2.5	2.66	Sp^3 (Both)	Sp^3 (Both)
	Thr47(OG1)---O(13)	-0.242	3.55	Sp^3 (Both)	Sp^3 (Acceptor)
	Thr47(OG1)---O(14)	-2.5	2.93	Sp^3 (Both)	Sp^3 (Acceptor)
	Leu98(N)---O(33)	-2.5	2.92	Sp^2 (Donor)	Sp^3 (Both)
	Thr97(N)---O(33)	-0.515	3.29	Sp^2 (Donor)	Sp^3 (Both)
	Thr97(N)---O(24)	-0.526	3.49	Sp^2 (Donor)	Sp^3 (Acceptor)
	Gly95(N)---O(24)	-0.394	3.30	Sp^2 (Donor)	Sp^3 (Acceptor)
	Gly96(N)---O(24)	-0.514	2.69	Sp^2 (Donor)	Sp^3 (Acceptor)

ligand-protein interaction, interaction energy, interaction distance, and hybridization. It can be observed from the results that the compounds (3a-f) possessed favorable ligand-protein interaction energy values of -2.5 to -0.069 kJ/mol, -2.5 to -0.242 kJ/mol and -2.5 to -0.043 kJ/mol at the binding cavity of 1KZN, 3SRW, and 1AI9, respectively. A common molecular interaction of the compounds and streptomycin was observed with the Gly77 for enzymes DNA Gyrase B (Pdb id: 1KZN), Gly95, Thr47, and Asn19 for enzymes dihydrofolate reductase (Pdb id: 3SRW). Also, both compounds and fluconazole revealed a common molecular interaction with Leu77, Arg56, Glu116, Lys57,

Table 7: Molecular interaction analysis of the compounds with the active site of 1AI9

Compound	*Interaction (Protein-Ligand)*	*Interaction Energy (kJ/mol)*	*Interaction Distance(Å)*	*Hybridization (Protein Atom)*	*Hybridization (Ligand Atom)*
	Leu77(O)---N(26)	-0.678	2.49	Sp^2 (Acceptor)	Sp^2 (Donor)
3a	Arg56(N)---N(27)	-1.594	3.08	Sp^2 (Donor)	Sp^2 (Acceptor)
	Glu116(N)---O(18)	-0.405	3.23	Sp^2 (Donor)	Sp^2 (Acceptor)
3b	Ser61(OG)---N(19)	-2.5	2.88	Sp^3 (Both)	Sp^2 (Donor)
	Glu116(OE1)---N(26)	-0.101	3.43	Sp^2 (Acceptor)	Sp^2 (Donor)
3c	Thr58(OG1)---N(19)	-1.094	2.73	Sp^3 (Both)	Sp^2 (Donor)
	Ala115(N)---O(18)	-2.5	2.89	Sp^2 (Donor)	Sp^2 (Acceptor)
3d	Glu116(N)---N(27)	-0.985	3.18	Sp^2 (Donor)	Sp^2 (Acceptor)
	Ile117(N)---N(27)	-1.522	3.28	Sp^2 (Donor)	Sp^2 (Acceptor)
	Ile19(O)---N(19)	-1.604	2.97	Sp^2 (Acceptor)	Sp^2 (Donor)
3e	Asp146(OD1)---N(26)	-2.286	2.73	Sp^3 (Acceptor)	Sp^2 (Donor)
	Thr147(OG1)---N(26)	-0.221	3.00	Sp^3 (Both)	Sp^2 (Donor)
	Thr147(OG1)---N(27)	-2.5	2.72	Sp^3 (Both)	Sp^2 (Acceptor)
3f	Thr58(OG1)---N(27)	-2.5	2.67	Sp^3 (Both)	Sp^2 (Acceptor)
Fluconazole	Gly114(N)---N(12)	-0.043	3.57	Sp^2 (Donor)	Sp^2 (Acceptor)
	Ile117(N)---N(12)	-1.318	3.34	Sp^2 (Donor)	Sp^2 (Acceptor)
	Glu116(N)---N(12)	-1.431	2.79	Sp^2 (Donor)	Sp^2 (Acceptor)
	Arg56(N)---O(3)	-1.557	2.82	Sp^2 (Donor)	Sp^3 (Both)
	Leu77(O)---O(3)	-1.427	3.31	Sp^2 (Acceptor)	Sp^3 (Both)
	Arg79(NH1)---N(7)	-2.450	3.11	Sp^2 (Donor)	Sp^2 (Acceptor)
	Arg79(NH2)---N(7)	-2.369	2.99	Sp^2 (Donor)	Sp^2 (Acceptor)
	Arg56(NH2)---N(5)	-0.592	2.98	Sp^2 (Donor)	Sp^2 (Acceptor)
	Lys57(NZ)---N(5)	-2.303	3.14	Sp^3 (Donor)	Sp^2 (Acceptor)
	Lys57(NZ)---N(4)	-2.5	2.96	Sp^3 (Donor)	Sp^2 (Acceptor)

and Ile117 for enzymes dihydrofolate reductase (Pdb id: 1AI9).

Conclusions

A series of novel octadecanoic acid [4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl]-amide pyrazoline derivatives (3a-e) were synthesized and characterized by various spectroscopic methods such as IR, ^{1}H-NMR, ^{13}C-NMR and MS. All the newly synthesized compounds (3a-f) exhibited appreciable antibacterial activity against *E. coli* and *S. aureus*. Besides, the compounds also showed activity against two fungal strains, *F. oxysporum* and *P. italicum*. Molecular docking studies revealed a common interaction for both the synthesized compounds and standard drugs with the target protein. Thereby, future studies on these compounds might increase their potency and thus, may be used as a potential lead for developing novel antimicrobial agents.

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