Design, synthesis, characterization, and antimicrobial studies of novel 1, 3, 5-Trisubstituted Pyrazoline derivatives

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Abstract

Molecular docking is a well-known computational technique for predicting the interaction energy between two molecules and is commonly used to better understand drug-receptor interactions. Six Pyrazoline derivatives with substituted Pyrazole moiety (**6a-f**) were synthesised in this study. Spectral studies were used to characterise the structures of the newly synthesised substances (UV, IR, and NMR). Antibacterial and antifungal activity were tested on the compounds. When compared to the standard medicine and other test compounds, Compound (**6b**) displayed better antibacterial activity (15mm) against *k.pneumonia* at 200 µg/ml and good antifungal activity (13mm) against *c.albicans* at 200 µg/ml. All of the compounds were subjected to molecular docking experiments using autodock vina software. The results of the in silico molecular docking analysis revealed that all of the synthesised compounds have a low binding energy and a high affinity for the active pocket, making them excellent inhibitors of bacterial and fungal activity.

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1. Introduction

Molecular docking is a well-known computer approach for estimating the energy of a two-molecule contact. This technology employs algorithms such as molecular dynamics, Monte Carlo simulation, and fragment-based search approaches. Molecular docking studies are performed to determine how two molecules interact and to determine the optimal ligand orientation for building a complex with the least amount of energy[1]. The ligand is a small molecule that fits into the cavity of a protein, which the search algorithm anticipates. When these protein cavities come into contact with external molecules, they become active and are known as active sites[2-3]. Docking is a technique for predicting

the binding orientation, affinity, and activity of small molecule therapeutic candidates to their protein targets. As a result, docking plays an important role in rational drug design. Given the importance of molecular docking in biology and pharmacology, major efforts have been made to enhance docking prediction systems[4-5]. The data is analysed using a statistical scoring technique that converts interacting energy into numerical values known as the docking score and calculates the interaction energy. Various visualization tools, like Pymol, Rasmol, and others, can be used to examine the 3D pose of the bound Ligand, which can help determine the optimum ligand match[6-7]. By assuming the active site of the protein molecule, it is possible to predict the mode of

protein ligand interaction. Molecular docking also plays an important part in medication design and discovery. Antimicrobial drug research has lately intensified due to an urgent need for novel antimicrobial medicines to combat these potentially fatal invasive diseases[8-9]. In this century, antimicrobial resistance has evolved, prompting the development of innovative antimicrobial drugs that are more selective, potent, and less toxic than currently available antibiotics in clinical therapy[10-11]. Heterocyclic compounds have been used to create pharmaceutically relevant chemicals such as pyrazolines, isoxazolines, imidazoles, benzimidazoles, furans, benzofurans, triazoles, and tetrazoles. These substances were discovered to have substantial medicinal promise.

The compounds having pyrazoline structure are a significant group in the heterocyclic chemistry. These compounds are scaffold target compounds in the field of medicinal and synthetic chemistry[12]. These compounds were used in the development of drug research and agricultural products. In the previous studies, it was indicated that these compounds have significant pharmacological and biological activities[13-14]. Many pyrazoline compounds are reported to possess a broad spectrum of biological activities such as antimicrobial, antidepressant, neuroprotective, anticonvulsant, anti-inflammatory, analgesic, antitubercular, local anesthetic, hypoglycemic, hypotensive, insecticide, herbicide, and molluscicidal[15-23]. The pyrazoline derivatives were used as efficient and potent inhibitors of glycogen synthase kinase and they also were used as a selective inhibitor capable of causing bacterial cell death and DNA gyrase[24-26]. Some known samples including the synthetic pyrazoline dipyrone having feature analgesics are used for preoperative pain[27]. Propifenazon has been developed for antipyretic anti-inflammatory drugs. Palerol is used as a novel spasm-analgesic in obstetrics and gynecology[28-29]. Nifenazone is utilized for treating rheumatic and analgesic and muzolimine is used for the treatment of hypertension and novel N1-substituted 3,5-diphenyl pyrazoline compounds have been synthesized for evaluation as anti-Helicobacter pylori agents[30].

. In view of the diverse biological activities of the heterocyclic compounds, it is planned to synthesize substituted Pyrazolines and to evaluate them for antimicrobial activities and compare experimental results with docking scores.

2. Materials and methods

Melting points were determined in open capillaries using an uncorrected Tempo melting point equipment. Analytical UV-VIS Spectrometer was used to record the UV spectra. FT-IR spectrophotometer was used to record the IR spectra (in KBr pellets). TMS was used as the internal standard for NMR spectra obtained on a Bruker (400 MHz). The reaction was completed using thin layer chromatography (TLC) on silica gel coated aluminium sheets. Without additional filtration, commercial grade solvents and reagents were utilized.

3. Experimental

Synthesis of N-(3-acetyl phenyl)-2-chloroacetamide (3)

3-amino acetophenone and glacial acetic acid were taken, stirred well at room temperature. A solution of chloroacetyl chloride in glacial acetic acid was added drop wise to the above reaction mixture with gradual stirring. After addition was complete, stirring was continued for 30 minutes then added 0.4 M Sodium acetate solution, the precipitate obtained was cooled in an ice water bath for 5 minutes, washed with water. Crude product was re-crystallized from ethanol
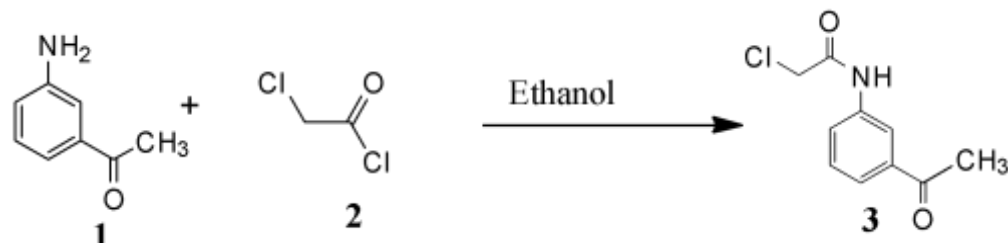
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Synthesis of 2-(4-acetamidophenoxy)-*N*-(3-acetyl-phenyl)acetamide (4)

To a stirred solution of Potassium carbonate in *N,N*-dimethylformamide ,a solution of *N*-(3-Acetyl-phenyl)-2-chloro-acetamide (**3**) in *N,N*-dimethylformamide was added dropwise at room temperature. The reaction mixture was stirred for 30 min and then N-(4-Hydroxyphenyl)acetamide

(**4**) was added. The reaction mixture was refluxed for 3 hours. Progress of the

reaction was monitored by TLC. Upon completion, the reaction mixture was poured into crushed ice. The precipitated solid was filtered, washed with water, and dried. The product was crystallized from ethanol.

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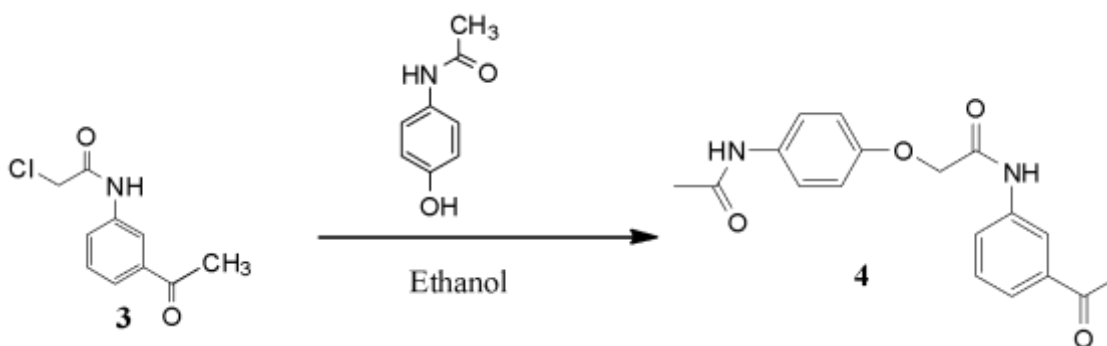
General procedure for the synthesis of Substituted chalcones (5a-f)

Equimolar quantity of 2-(4-Acetamidophenoxy)-*N*-(3-Acetyl-phenyl)acetamide and various substituted benzaldehydes were dissolved in ethanol and 20%

NaOH solution was added slowly with constant stirring. After addition was complete stirring was continued for 6 hours and kept overnight. The reaction mixture was poured into crushed ice and acidified with 10 % HCl to obtain product. Crude product was re-crystallized from ethanol.
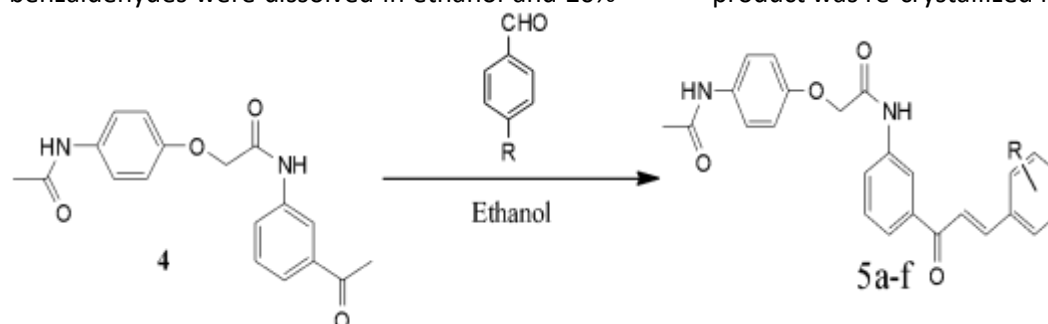
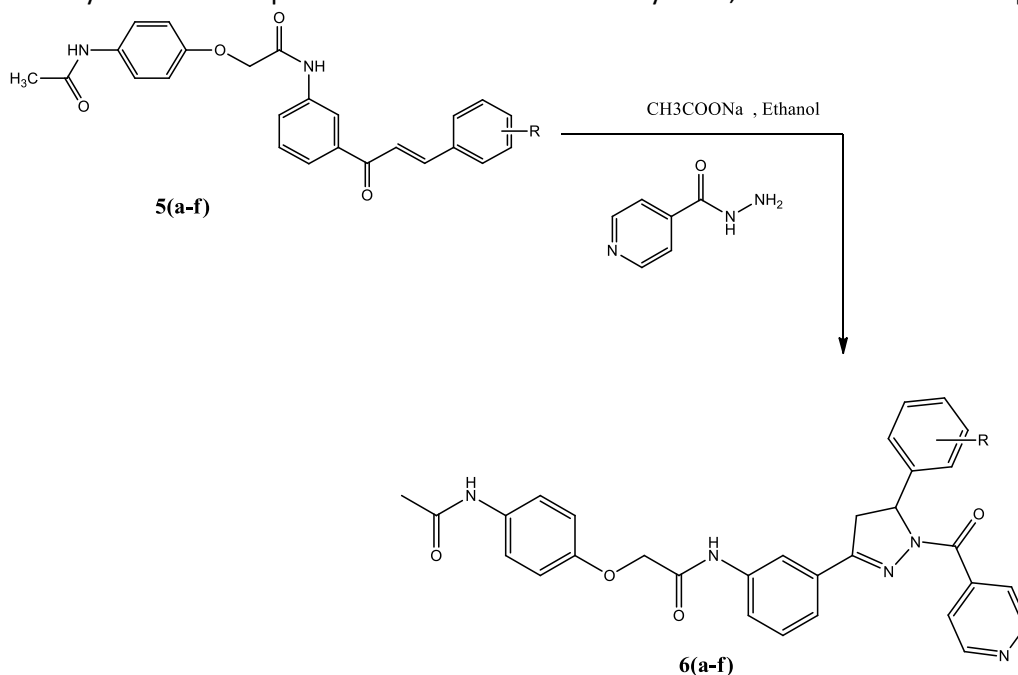

Synthesisof 2-(4-acetamidophenoxy)-N-(3-(5-(4-subsitutedphenyl)-1-isonicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)acetamide (6a-f).

Cmds	5a	5b	5c	5d	5e	5f
R	-H	4-Cl	3-Br	4-CH₃	4-OCH₃	4-N(CH3)₂

The (E)-2-(4-acetamidophenoxy)-N-(3-cinnamoylsubsitutedphenyl)acetamide (**5a-f**) were treated with

isonicotinic hydrazide in the presence of sodium acetate and ethanol as solvent to yield 2-(4-acetamidophenoxy)-N-(3-(5-(4-subsitutedphenyl)-1-isonicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)acetamide **(6a-f)**. The final product separated and purified by ethanol recrystallization[1]**.** The structures of the synthesized compounds **6a-f** are characterized by FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy.

Cmds	6a	6b	6c	6d	6e	6f
R	-H	4-Cl	3-Br	4-CH$_3$	4-OCH$_3$	4-N(CH$_3$)$_2$

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4. Results and discussion

(E)-2-(4-acetamido phenoxy)-N-(3-cinnamoyl subsituted phenyl)acetamide **(5a-f)** were prepared by following the standard protocol and were reacted with isonicotinic hydrazide to yield 2-(4-acetamidophenoxy)-N-(3-(5-(4-subsitutedphenyl)-1-isonicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)acetamide **(6a-f)**.The

synthetic route of compound **(6a-f)** and the physical data along with yield of pyrazoline derivatives were reported in **Table 1.**The assigned structure and molecular formula of the newly synthesized compound**s** were confirmed and supported by ^{1}H NMR, ^{13}CNMR and IR data, which was in full agreement with proposed structures.

Table 1: Melting point and elemental analysis for compounds 6a-f

C.No	Substitution	Molecular formula	M.Wt	% Yield	m.pt $^{(0)}$C
6a	-H	C$_{31}$H$_{27}$N$_5$O$_4$	533.59	67	190
6b	4-Cl	C$_{31}$H$_{26}$ClN$_5$O$_4$	568.03	65	215
6c	3-Br	C$_{31}$H$_{26}$BrN$_5$O$_4$	612.48	43	232
6d	4-CH$_3$	C$_{32}$H$_{29}$N$_5$O$_4$	547.62	74	187
6e	4-OCH$_3$	C$_{32}$H$_{29}$N$_5$O$_5$	563.61	79	164
6f	4-N(CH$_3$)$_2$	C$_{33}$H$_{32}$N$_6$O$_4$	577.66	70	209

The IR spectra of condensed product **(6a)** displayed characteristic absorption band at around 1600 cm^{-1} due to C=N of pyrazoline. The compounds also exhibit bands at around 3221 cm^{-1}confirmsthepresence of − NHgroup. Synthesized compounds showed weak C-H stretching bands near around 3052 cm^{-1}, C=C skeletal vibrations near around 1503 cm^{-1} for aromatic and absorption around 2915 cm^{-1} for aliphatic nature of compounds/substituents[2-3]. The IR spectra of compound **(6a)** displayed characteristic absorption band at around 1653 cm^{-1} due to C=O of Pyrazoline. The ^{1}H NMR spectra of compound **(6a)** showed characteristic ABX system due to geminal-vicinal multiple coupling between -CH$_2$ and -CH protons. The high field two doublets at δ 3.55 ppm and δ 3.80 ppm due to H$_A$ and H$_B$ respectively of C-4 protons and low field δ 5.52 ppm due to Hx at C-5 are characteristics signals due to vicinal coupling with the two magnetically nonequivalent protons of methylene group at position 4 of the pyrazolines ring. Singlet at δ 9.47 ppm corresponding to the NH group. In the compound absorption as a multiplet at δ 6.90 – 8.68 ppm was assigned to aromatic protons[4-5]. In ^{13}C NMR spectra of compound **(6a),** it is observed that C4 and C$_5$ carbon of pyrazolines resonated at δ 40.3 and 67.4 ppm, respectively. The carbon of –O-CH$_2$ in compound **(6a)** resonates at δ 64.2 ppm, respectively[6]. The carbon of (C=O) displayed signals at δ 152.7, 165.4, 169.1. The compound showed signals at δ 116.5 –

140.6 ppm were assigned to the aromatic carbon. The compound **(6a)** showed a signal at δ 150.7 ppm assigned to (C=N). All other derivatives **(6b-f)** showed the same trend in IR, ^{1}H NMR and ^{13}C NMR spectroscopy.

5. Antibacterial and antifungal activity

All the newly synthesized pyrazoline derivatives incorporated with electron withdrawing and donating groups were examined for antibacterial and antifungal activities using invitromodel[7]. Screening at the Preliminary level was taken out for all the compounds amounting the antifungal activity in sabouraud agar medium against two fungal strains and antibacterial activity in the nutrient agar medium against four bacterial (two gram positive and two gram negative) strains

by disk diffusion method at a concentration of 100 and 200 μg/ml. The zone of inhibition (mm) of each derivative was ascertained and compared with streptomycin and fluconazole taken as a standard drug for bacteria and fungi respectively. DMSO was used to prepare stock solutions of tested derivatives. The findings of antimicrobial evaluation presented that most of the compounds have comparable activity against the bacterial and fungal strains. It was conceivable that in Table 2 and Figure 1 to 2, among tested compounds (**6a-f**), compound **6d** (14mm/200μg/ml) and **6f** (14mm/200μg/ml**)** were found to exhibited potent antifungal activity in comparison to standard drug fluconazole.

The outcome of antimicrobial activity evaluation of the synthesized compounds revealed that these compounds possessed antibacterial and antifungal activities. From antimicrobial activity data (Table 2), Compound (**6b**) displayed better antibacterial activity (15mm) against *k.pneumonia* at 200 μg/ml and good antifungal activity (13mm) against *c.albicans* at 100 μg/ml. Primary microbiological screening results showed that compounds **6b** (4-Cl), **6c** (3-Br) and **6f**(-N(CH$_3$)$_2$ possessed moderate activity (12mm, 13mm) against E. coli.at 100 μg/ml. The antibacterial activity against E. coli improved when the substitution pattern was changed[8-9] by the installation of electron withdrawing groups in compounds **6a**. Compound **6b** (4-Cl) possessed good activity (14mm) against *bacillus subtilis* at 200 μg/ml, while compounds **6c**, **6d**, **6e** and **6f** showed moderate activity against *bacillus subtilis* at 200 μg/ml.

Moreover, when we introduced chloro group as a substituent at para position in compound **6a** , the activity was enhanced and showed better activity against *bacillus subtilis* at 200 μg/ml. In case of S. aureus, the electron withdrawing group at 3rd position like in **6c** (3-Br) showed moderate activity at 100 μg/ml, while electron donating groups at 4th position like in **6d** (-CH3) displayed lesser activity[10-11] . It was observed from Table 2, all the compounds possessed moderate activity against k.pneumonia, while compounds **6c** (-Br) and **6f** (-N(CH$_3$)$_2$) showed lesser activity against k.pneumonia at 100 μg/ml.
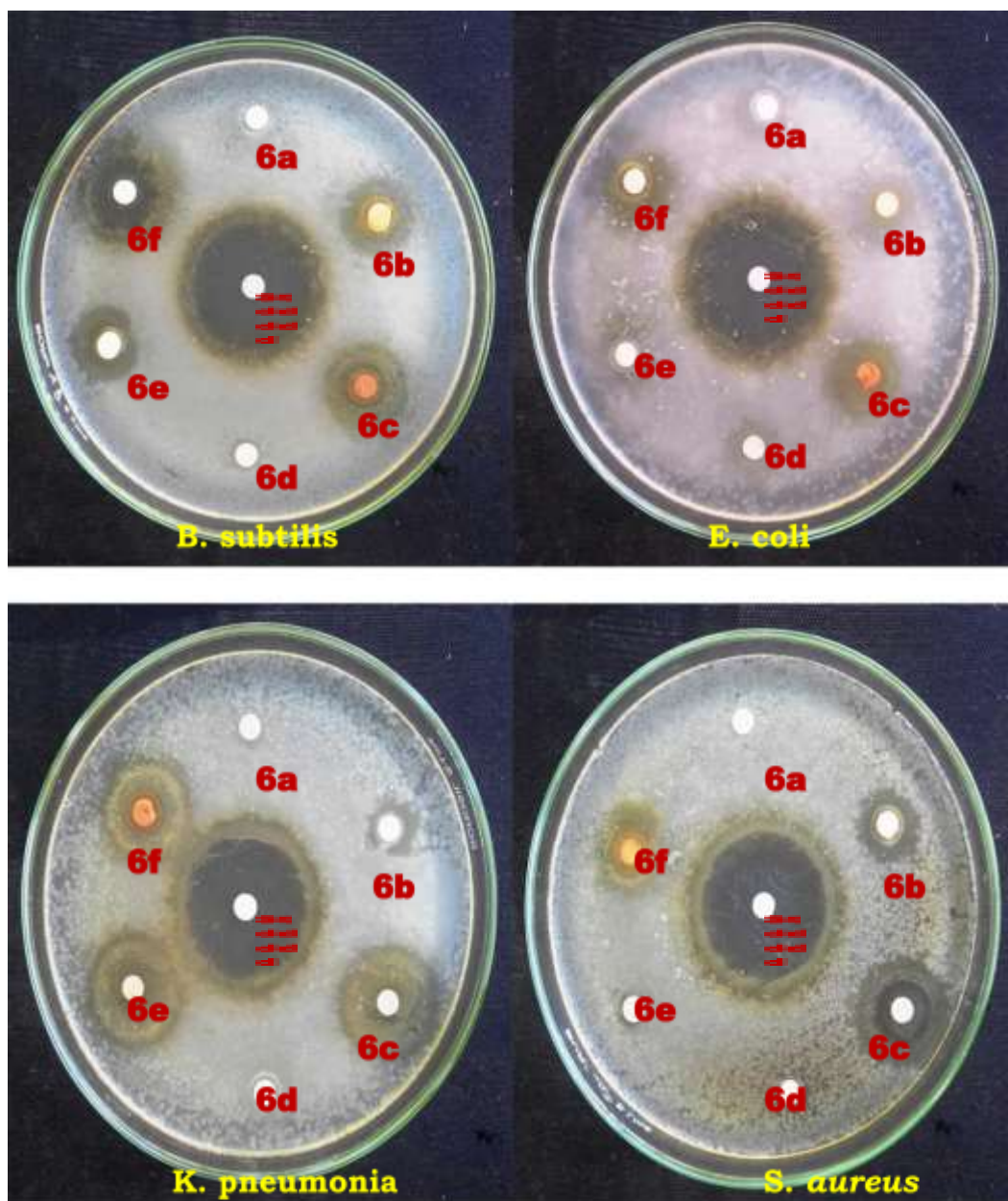
Fig 1: Antibacterial activities of compounds 6a-f by disc diffusion method
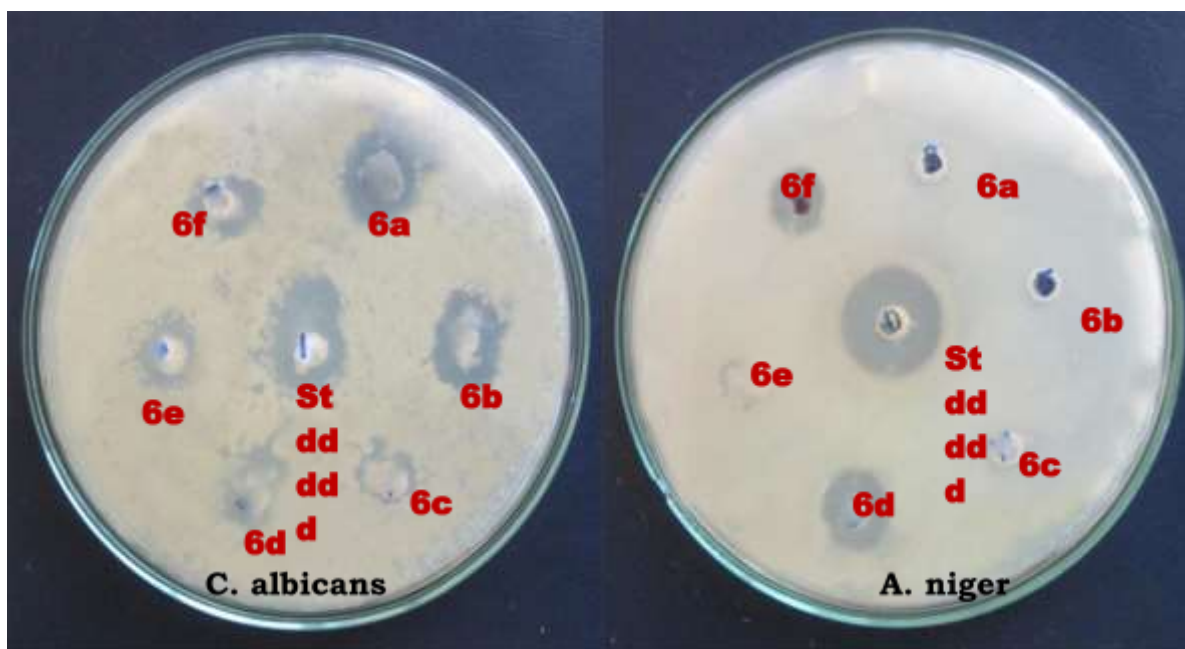
Fig 2: Antifungal activities of compounds 6a-f by disc diffusion method

6. Molecular docking studies of synthesized pyrazoline derivatives (6a-f)

Downloaded 3D structure of 1T15 protein was converted into pdbqt format from its protein data bank (PDB). With a binding energy of -8.65 kcal/mol for **6b** and -8.18 for **6a,** these two compounds are among the best for binding to 1T15 proteins. Against the 1T15 protein, docking scores of **6c** and **6f** were also higher (-7.89 & -7.83 k.cal/mol). Others have docking scores ranging from -7.62 to 7.76 k.cal/mol. Human trial drugs like streptomycin (-9.12k.cal/mol), fluconazole (-8.44 k-cal/mol) and clotrimazole (-8.05.k-cal/mol) were used to compare the docking results. The docking scores of **6b, 6a, 6c,** and **6f** are nearly identical to those of the standard drugs.

Four hydrogen bonds were found in the docked complex of 1T15-6b. oxygen atoms of amino acid residues arg1699, lys1690, val1696 and gly1656 formed hydrogen bonds with amide and pyrazoline ring hydrogen atoms at distance 2.63Å, 2.55Å, 2.56Å and 3.20Å. Three hydrogen bonds were found in the docked complex of 1T15-6a. Hydrogen atoms of amino acid residues Lys1702, Gln1779 And Ile1680 formed hydrogen bonds with

amide and ketonic oxygen atoms at distance 2.56Å, 2.99Å and 4.12Å. Besides hydrogen bonding, other interactions such as hydrophobic, alkyl-pi, van der Waal, and polar / electrostatic interactions were also found in the other compounds studied.

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Table 2: Antibacterial and Antifungal activities of compounds 6a-f by disc diffusion method

Antibacterial activity								
S. No.	Bacteria	Streptomycin	Zone of inhibition mm in diameter 100μg/ml					
			6a	6b	6c	6d	6e	6f
1	*Bacillus subtilis*	24	0	12	14	6	12	16
2	*Escherichia coli*	24	11	12	13	10	11	13
4	*Staphylococcus aureus*	24	0	11	16	4	4	9
5	*K. pneumoniae*	24	10	16	5	15	15	4
S. No.	Bacteria	Streptomycin	Zone of inhibition mm in diameter 200μg/ml					
			6a	6b	6c	6d	6e	6f
1	*Bacillus subtilis*	20	11	14	13	13	12	12
2	*Escherichia coli*	20	12	11	10	10	12	13
4	*Staphylococcus aureus*	20	10	13	12	12	11	12
5	*K. pneumoniae*	18	14	15	14	13	14	13
Antifungal activity								
S. No.	Fungal	Fluconazole	Zone of inhibition mm in diameter 100μg/ml					
			6a	6b	6c	6d	6e	6f
1	*C.albicans*	18	14	13	10	10	11	12
2	*A.niger*	14	8	0	0	12	0	10
S. No.	Fungal	Fluconazole	Zone of inhibition mm in diameter 200μg/ml					
			6a	6b	6c	6d	6e	6f
1	*C.albicans*	22	12	9	14	14	13	14
2	*A.niger*	22	8	11	12	12	6	10

Table 3 :Docking score of synthesized pyrazoline derivatives (6a-f)

C.No	Substitution	Docking score with 1HNJ	Docking score with 1KZN	Docking score with 1T15
6a	-H	-7.57	-8.34	-8.18
6b	4-Cl	-7.09	-7.7	-8.65
6c	3-Br	-8.2	-7.43	-7.89
6d	4-CH$_3$	-6.55	-7.29	-7.76
6e	4-OCH$_3$	-7.11	-6.97	-7.62
6f	4-N(CH$_3$)$_2$	-7.66	-7.38	-7.83
Streptomycin		-8.24	-8.77	-9.12
Fluconazole		-7.85	-7.55	-8.44
Clotrimazole		-7.72	-7.68	-8.05

Downloaded 3D structures of the 1HNJ protein were then converted into pdbqt format from the protein data bank (PDB). The binding energies of 6c and 6f with 1HNJ proteins were -8.20 and -7.66k.cal/mol, respectively, making them stand out in the compound sequence. Similarly, docking scores for 6a and 6e were lower against the 1HNJ protein (-7.56k.cal/mol and -7.11k.cal/mol, respectively).

The docking scores of other compounds range from -6.55 to -7.09. Human trial drugs like streptomycin (-8.24 k.cal/mol), fluconazole (-7.85 k.cal/mol), and clotrimazole (-7.72 k.cal/mol) were used to compare the docking results. In terms of docking scores, the standard drugs are very similar to **6c, 6f, 6a,** and **6e.** Tables 3 to 6 and Figures 3 to 5 show the docking results.

Four hydrogen bonds Arg151 (Hydrogen atom of amino acid – ketonic oxygen atom of pyrazoline), his244(Hydrogen atom of residue – Oxygen atom of amide in **6c**), Phe304(Oxygen atom of residue – Hydrogen atom of amide in **6c**), arg151(Oxygen atom of residue – Hydrogen atom of amide in **6c**) with the distances of 2.48Å, 3.31Å, 2.97Å and 1.95Å in the docked complex of 1HNJ-**6c**. The docked complex of 1HNJ-**6f** was found to have three hydrogen bonds in it. Amino acid residues met207 and gly152 forms hydrogen bond using its oxygen atoms with hydrogen atom of pyrazoline derivative at distance 2.37Å and 2.49Å, asn274 formed hydrogen bonds with the oxygen atom of pyrazoline with distance of 3.37Å. There were other types of interactions found in addition to hydrogen bonding in the other compounds, including hydrophobic, alkyl-pi, van der Waal, and polar/electrostatic.

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Table 4 : Docking interactions of synthesized pyrazoline derivatives and with receptor protein 1HNJ

Compound 6c			Compound 6f		
Types of Interaction	Participating Residue and Atoms	Distance (A⁰)	Types of Interaction	Participating Residue and Atoms	Distance (A⁰)
Hydrogen Bond	A:ARG151:NE - 6c:O29	2.48091	Hydrogen Bond	A:ASN274:ND2 - 6f:O3	3.37619
Hydrogen Bond	A:HIS244:ND1 - 6c:O39	3.31061	Hydrogen Bond	6f:H46 - A:MET207:SD	2.37244
Hydrogen Bond	6c:H69 - A:PHE304:O	2.97177	Hydrogen Bond	6f:H61 - A:GLY152:O	2.49375
Hydrogen Bond	6c:H60 - A:ARG151:O	1.95908	Electrostatic	A:ARG249:NH2 - 6f	3.56218
Other	A:MET207:SD - 6c	2.73527	Hydrophobic	A:PHE213 - 6f	4.65719
Hydrophobic	A:TRP32 - 6c	5.54034	Hydrophobic	A:ALA246 - 6f	4.50213
Hydrophobic	A:TRP32 - 6c	4.4356	Hydrophobic	A:ILE250 - 6f	5.28543
Hydrophobic	A:ILE156 - 6c	3.58266	Hydrophobic	6f - A:MET207	5.3636
Hydrophobic	6c:Br41 - A:ARG151	5.16322	Hydrophobic	6f - A:ALA216	4.43568
Hydrophobic	6c - A:ARG151	4.5975	Hydrophobic	6f - A:ALA246	3.44165
Hydrophobic	6c - A:ARG151	5.27477	Hydrophobic	6f - A:ILE250	5.11168

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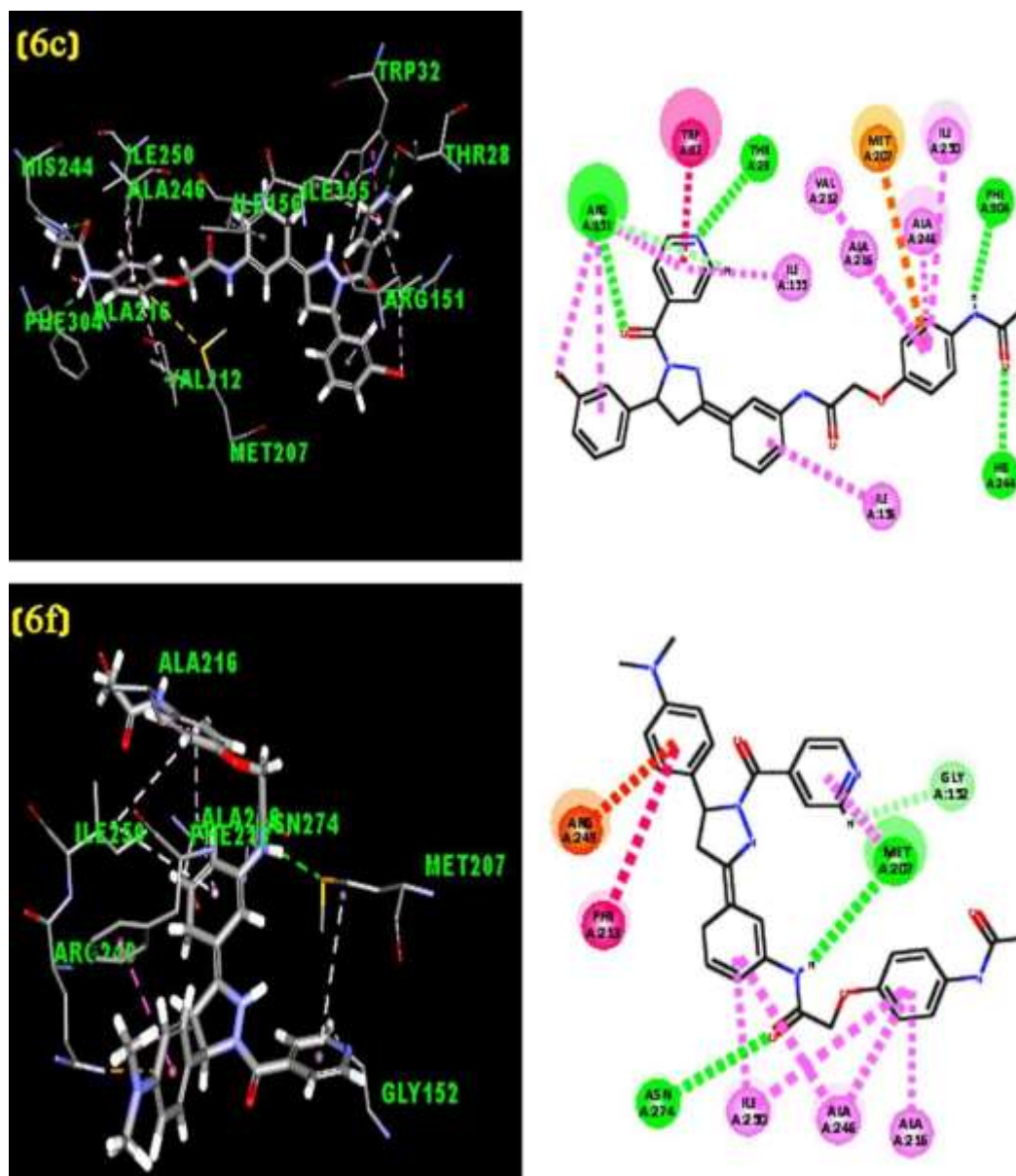**Table 5 : Docking interactions of synthesized pyrazoline derivatives and with receptor protein 1KZN**

Compound 6a			Compound 6b		
Types of Interaction	Participating Residue and Atoms	Distance (A⁰)	Types of Interaction	Participating Residue and Atoms	Distance (A⁰)
Hydrogen Bond	A:ARG76:NH1 - 6a:O3	2.98489	Hydrogen Bond	A:ALA96:N - 6b:O39	3.18384
Hydrogen Bond	6a:H69 - A:ASP73:OD1	3.04495	Hydrogen Bond	6b:H53 - A:ASN46:O	2.53795
Electrostatic	A:GLU42:OE1 - 6a	3.81992	Hydrogen Bond	6b:H60 - A:GLY77:O	2.95995
Electrostatic	A:GLU50:OE1 - 6a	4.53337	Electrostatic	A:ARG76:NH1 - 6b	4.03748
Hydrogen Bond	A:THR165:OG1 - 6a	3.65254	Electrostatic	A:GLU50:OE1 - 6b	4.11203
Hydrophobic	A:GLY77:C,O;ILE78:N - 6a	3.69569	Hydrophobic	6b:Cl41 - A:VAL43	5.44649
Hydrophobic	6a - A:ILE90	5.08337	Hydrophobic	6b:Cl41 - A:VAL167	3.7047
Hydrophobic	6a - A:ILE78	4.54146	Hydrophobic	6b - A:ILE78	5.09794
			Hydrophobic	6b - A:ARG76	5.30932
			Hydrophobic	6b - A:PRO79	4.52757

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Table 6 : Docking interactions of synthesized pyrazoline derivatives and with receptor protein 1T15

Compound 6b			Compound 6a		
Types of interactions	Participating amino acid & Atoms	Distance (A^0)	Types of interactions	Participating amino acid & Atoms	Distance (A^0)
Hydrogen Bond	6b:H50 - A:ARG1699:O	2.6357	Hydrogen Bond	A:LYS1702:N - 6a:O3	2.56601
Hydrogen Bond	6b:H69 - A:LYS1690:O	2.5545	Hydrogen Bond	A:GLN1779:NE2 - 6a:O39	2.99477
Hydrogen Bond	6b:H69 - A:VAL1696:O	2.56404	Electrostatic	A:GLU1698:OE2 - 6a	3.10002
Hydrogen Bond	A:GLY1656:CA - 6b:O30	3.2058	Hydrogen Bond	A:ILE1680:N - 6a	4.12393
Electrostatic	A:GLU1698:OE1 - 6b	4.17298	Hydrophobic	A:ILE1680:CG2 - 6a	3.60912
Other	A:MET1775:SD - 6b	5.76754	Hydrophobic	6a - A:LYS1690	5.22526
Hydrophobic	6b - A:LYS1690	4.26996	Hydrophobic	6a - A:LEU1679	5.32391
			Hydrophobic	6a - A:LEU1701	5.33634
			Hydrophobic	6a - A:LYS1702	4.87866

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Fig 3: 2D and 3D interactions of synthesized pyrazoline derivatives (Top two binding energy) with receptor protein 1HNJ

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Fig 4: 2D and 3D interactions of synthesized pyrazoline derivatives (Top two binding energy) with receptor protein 1KZN
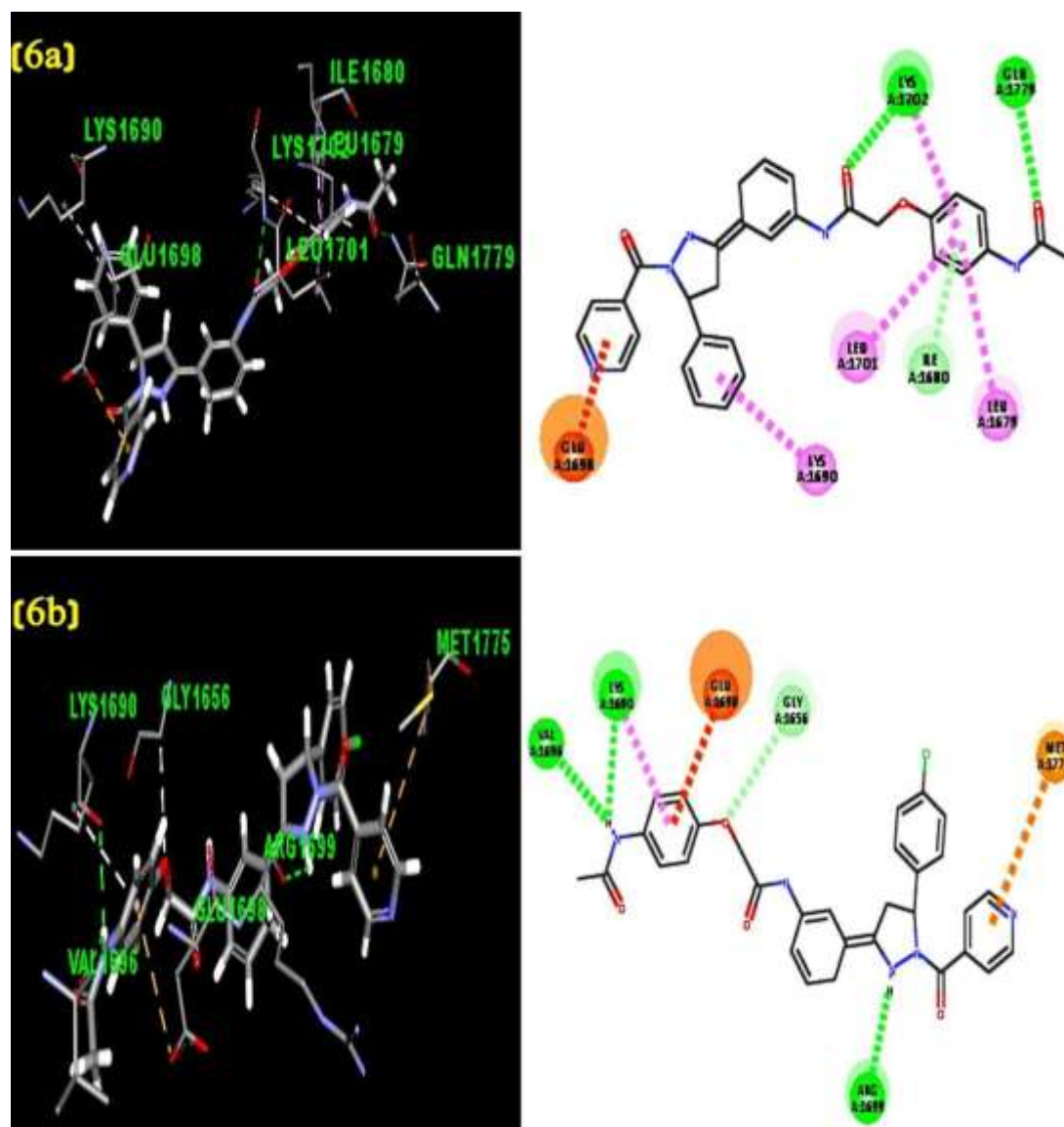
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Fig 5 : 2D and 3D interactions of synthesized pyrazoline derivatives (Top two binding energy) with receptor protein 1T15

The 3D structures of the 1KZN protein were downloaded from the protein data bank and then converted into pdbqt format (PDB). It stands out in the compound sequence because the binding energies of **6a** and **6b** to 1KZN proteins were -8.34 and -7.70 kcal/mol, respectively. There were similarly higher docking scores for the **6c** and **6f** proteins (-7.43 kcal/mol and 7.38 kcal/mol, respectively) when docked against the 1HNJ protein. Other compounds 6e and 6d have docking scores between -6.97 and -7.29. The docking results were compared using human trial drugs like streptomycin (-8.24 kcal/mol), fluconazole (-7.85 kcal/mol), and clotrimazole (-7.72 kcal/mol). **6a, 6b, 6c,** and **6f** have very similar docking scores to the standard drugs.

This 1KZN-6a complex has two hydrogen bonds between the Arg76 (NH(amino acid)-------ketonic O (pyrazoline), distance: 2.98Å) and asp73 (O (amino acid)-------amide H (pyrazoline)distance: 3.04Å) There were three hydrogen bonds found in the docked complex of 1KZN-6b,ALA96 (NH(amino acid)-------ketonic O (pyrazoline), distance: 3.18Å) and Asn46 (O (amino acid)   H (pyrazoline)distance: 2.53Å) and gly77 (O (amino acid)------- H (pyrazoline)distance: 2.95Å) Hydrogen bonding was not the only type of interaction found in the other compounds; hydrophobic, alkyl-pi, van der Waal, and polar/electrostatic interactions were also found.

7. Conclusion

In conclusion, the *in-vitro* antibacterial and antifungal activity results shows that the compound **6b, 6c** and **6f** have shown strong antibacterial and antifungal activity. However, all the synthesized pyrazoline compounds displayed moderate antibacterial and fungal activity compared to those of the standard drugs. The results showed that the binding energies of **6a-f** were lower than that of streptomycin in its interaction with three protein receptors 1HNJ, 1KZN and 1T15. The docking outcome disclosed that the compound **6c** in 1HNJ and **6a** in 1KZN have higher docking score of −8.20 and −8.34 kcal mol^{-1}. It was due to the presence of a 3-bromo in the phenyl ring. Compound **6b** shows a good binding score (-8.65 kcal mol^{-1}) against 1T15 with 4- chloro substitution on phenyl ring.

Reference

1. Bissantz, C. (2002). *Development and application of new methods for the virtual screening of chemical databases* (Doctoral dissertation, ETH Zurich).

2. Yuan, Y., Pei, J., & Lai, L. (2013). Binding site detection and druggability prediction of protein targets for structure-based drug design. *Current pharmaceutical design*, *19*(12), 2326-2333.

3. Tripathi, A., & Kellogg, G. E. (2010). A novel and efficient tool for locating and characterizing protein cavities and binding sites. *Proteins: Structure, Function, and Bioinformatics*, *78*(4), 825-842.

4. Eweas, A. F., Maghrabi, I. A., & Namarneh, A. I. (2014). Advances in molecular modeling and docking as a tool for modern drug discovery. *Der Pharma Chemica*, *6*(6), 211-228.

5. Rudrapal, M., & Chetia, D. (2020). Virtual screening, molecular docking and QSAR studies in drug discovery and development programme. *Journal of Drug Delivery and Therapeutics*, *10*(4), 225-233.

6. Shehu, Z., Uzairu, A., & Sagagi, B. (2018). Quantitative structure activity relationship (QSAR) and molecular docking study of some pyrrolones antimalarial agents against plasmodium falciparum. *Journal of the Turkish Chemical Society Section A: Chemistry*, *5*(2), 569-584.

7. Ferreira, L. G., Dos Santos, R. N., Oliva, G., & Andricopulo, A. D. (2015). Molecular docking and structure-based drug design strategies. *Molecules*, *20*(7), 13384-13421.

8. Ooms, F. (2000). Molecular modeling and computer aided drug design. Examples of their applications in medicinal chemistry. *Current medicinal chemistry*, *7*(2), 141-158.

9. Geromichalos, G. D. (2007). Importance of molecular computer modeling in anticancer drug development. *Journal of BUON*, *12*(1), S101-S118.

10. Brown, E. D., & Wright, G. D. (2016).

NeuroQuantology|August 2022|Volume20|Issue10|Page 3277-3294|doi:10.14704/nq.2022.20.10.NQ55326

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Antibacterial drug discovery in the resistance era. *Nature*, *529*(7586), 336-343.

11. Jackson, N., Czaplewski, L., & Piddock, L. J. (2018). Discovery and development of new antibacterial drugs: learning from experience?. *Journal of Antimicrobial Chemotherapy*, *73*(6), 1452-1459.

12 Turkan, F.; Cetin, A.; Taslimi, P.; Karaman, H.S.; Gulçin, İ. Synthesis, characterization, molecular docking and biological activities of novel pyrazoline derivatives. Archiv der Pharmazie 2019, 352, 1800359.

13 Fernández-García, E.; Carvajal-Lérida, I.; Jarén-Galán, M.; Garrido-Fernández, J.; Pérez-Gálvez, A.; Hornero-Méndez, D. Carotenoids bioavailability from foods: From plant pigments to efficient biological activities. Food Research International 2012, 46, 438–450.

14 Strobel, G.; Daisy, B. Bioprospecting for microbial endophytes and their natural products.
Microbiology and molecular biology reviews 2003, 67, 491–502.

15

16 Rostom, S.A.; Badr, M.H.; Abd El Razik, H.A.; Ashour, H.M.; Abdel Wahab, A.E. Synthesis of some pyrazolines and pyrimidines derived from polymethoxy chalcones as anticancer and antimicrobial agents. Archiv der Pharmazie 2011, 344, 572–587.

17 Manna, K.; Banik, U.; Ghosh, P.S.; Das, M. A review on synthesis and pharmacological diversity of isoxazoles and pyrazolines. Nirma Univ J Pharm Sci 2014, 1, 37–49.

18 Jainey, P.J.; Bhat, I.K. Antitumor, Analgesie, And Anti-Inflammatory Activities Of Synthesized Pyrazolines. Journal of Young Pharmacists 2012, 4, 82–87.

19 Kumar, S.; Bawa, S.; Drabu, S.; Kumar, R.; Gupta, H. Biological activities of pyrazoline derivatives-A recent development. Recent patents on anti-infective drug discovery 2009, 4, 154–163.

20 Yerragunta, V.; Suman, D.; Swamy, K.; Anusha, V.; Patil, P.; Naresh, M. Pyrazole and its biological activity. PharmaTutor2014, 2, 40–48.

21 Marella, A.; Ali, R.; Alam, T.; Saha, R.; Tanwar, O.; Akhter, M.; Shaquiquzzaman, M.; Mumtaz Alam, M. Pyrazolines: a biological review. Mini reviews in medicinal chemistry 2013, 13, 921–931.

22 Shaaban, M.R.; Mayhoub, A.S.; Farag, A.M. Recent advances in the therapeutic applications of pyrazolines. Expert opinion on therapeutic patents 2012, 22, 253–291.

23 Turkan, F.; Cetin, A.; Taslimi, P.; Karaman, H.S.; Gulçin, İ. Synthesis, characterization, molecular docking and biological activities of novel pyrazoline derivatives. Archiv der Pharmazie 2019, 352, 1800359.

24 Zhao, P.-L.. Synthesis, fungicidal, and insecticidal activities of β-methoxyacrylate-containing N-acetyl pyrazoline derivatives. Journal of agricultural and food chemistry 2008, 56, 10767–10773.

25 Peng, X.-M.; LV Damu, G.; Zhou, H. Current developments of coumarin compounds in medicinal chemistry. Current pharmaceutical design 2013, 19, 3884–3930.

26 Zinad, D.S.; Mahal, A.; Mohapatra, R.K.; Sarangi, A.K.; Pratama, M.R.F. Medicinal chemistry of oxazines as promising agents in drug discovery. Chemical Biology & Drug Design2020, 95, 16–47.

27 Islan, G.A.; Durán, Nanopharmaceuticals as a solution to neglected diseases: Is it possible? Acta tropica 2017, 170, 16–42.

28 da Silva, D.P.; Florentino, I.F.; da Silva, D.M.; Lino, R.C.; Cardoso, C.S.; Moreira, L.K.; Vasconcelos, G.A.; Vinhal, D.C.; Cardoso, A.C.; Villavicencio, B. Molecular docking and pharmacological/toxicological assessment of a new compound designed from celecoxib and paracetamol by molecular hybridization. Inflammopharmacology 2018, 26, 1189–1206.

29 Sheha, M.; Khedr, A.; Elsherief, H. Biological and metabolic study of naproxen–propyphenazone mutual prodrug. European journal of pharmaceutical sciences 2002, 17, 121–130.

30 Brambilla, G.; Martelli, A. Genotoxicity and carcinogenicity studies of analgesics,

3293

anti-inflammatory drugs and antipyretics. Pharmacological research 2009, 60, 1–17.

31 Burdan, F. Developmental effects of propyphenazone in analgesic and antipyretic combination with caffeine or paracetamol. Human & experimental toxicology 2004, 23, 235–244.