***In-vitro* ANTICANCER ACTIVITY, ANTIMICROBIAL AND *In-silico* STUDIES OF NAPHTHYL PYRAZOLE ANALOGUES**

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ABSTRACT

A new sequence of new pyrazoline derivatives (**5a-5h**) was synthesized from 2-naphthylstyryl chalcone to react with hydrazine hydrate in the presence of n-butyric acid using the cyclization method. Synthesized compounds (**5a-5h**) chemical structure was elucidated by FT-IR, Proton and Carbon NMR Spectral data and CHN analysis. All the compounds (**5a-5h**) were subjected to in-vitro biological activity using disk diffusion method. The electron-withdrawing fluoro substituted compound **5b** was better antibacterial activity against the bacterial strain of *Pseudomonas aeruginosa* and also the electron-withdrawing compound like bromo substitution **5d** was excellent activity against bacterial strain of *Escherichia coli* and the fluoro substituted compound **5b** was shown excellent activity against the gram-positive bacterial strain of *Streptococcus pyogenes* which was compared with the standard drug (Ciprofloxacin). The fluoro substituted compound **5b** shown good antifungal activity against *candida albicans*. After that synthesized compounds (**5a-5h**) were subjected to molecular docking studies using bacterial protein and breast cancer protein. From this result, the synthesized compounds (**5a-5h**) have a high binding affinity score compared with the standard drug ciprofloxacin. Based on the high binding affinity score, compound **5b** was subjected to in-vitro anticancer activity by MTT assay method against MDA MB-231 Cell line. From this result, the fluoro substituted compound **5b** showed good activity at low concentration (6.25 µg/ml). The LC 50 value of this compound **5b** is 27.76 ± 0.003 µg/ml. Compounds **5b** and **5h** are the most important compound and there is only needed to develop new antibacterial agents. The compounds (**5a-5h**) are also subjected to ADME (druglikeness) property using Osiris program, a result that **5c** has the best druglikeness property and 0.51 drug score with 32.67 Å total polar surface area.

Keywords: Conventional method, 2-Naphthylstyryl Ketone, n-Butyric acid, Hydrazine hydrate, Antimicrobial activity; Anticancer activity(MDA MB-231 Cell line).

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INTRODUCTION

For centuries, Cancer has been prevailing as the most serious disease and its incidence is rising day-to-day in the world.^{1,2} The literature review is clearly explained more than 90% of cancer patients die due to chronic tumor metastases. Despite the presence of a large number of anticancer drugs, there is no currently available agents can destroy completely cancer cells without harming normal tissues.³⁻⁵ The development of a new anticancer agent's future is very important in the major engrossment in many industrial research laboratories and academics across the world to develop more potent molecules with higher explicitness and minimized toxicity.^{6,7}

Heterocyclic compounds particularly those with oxygen, nitrogen and sulphur atoms have been identified to have the most comprehensive spectrum of biological activities.⁸ In the synthesis of heterocyclic compounds, chalcones are used as an intermediate and it should have good biological activity as well as it plays an important role in medicinal chemistry and drug discovery.⁹⁻¹¹ The pyrazoline derivatives are the most important classes of the heterocyclic compound and it is a versatile lead molecule in the agrochemical and pharmaceutical field. The pyrazoline derivatives have various biological activities such as antimicrobial¹², antitumor¹³, antibacterial¹⁴, anti-inflammatory¹⁵, anticancer¹⁶, antidiabetic¹⁷, pharmacological¹⁸, free radical scavenging^{19,20} activities. Among the reported activities, it is important to

note that pyrazoline is not only useful in the treatment of various cancer types, including brain, bone, mouth, esophagus, stomach, liver, bladder, pancreas, cervix, lung, breast, colon, rectum and prostate cancers, but some of them act as cancer chemopreventive agents.²¹ Most of the pyrazole compounds have a lot of industrial applications and anticorrosive inhibition property.²²

On the other hand, pyrazoline derivatives were carried out of the focus of drug discovery; like the studies drug-ability and bioactivity of the compounds. The concept of drug-ability is defined as the prospect to find a compound with high potency, drug-like properties, as well as measured properties concerning undesirable side effects, metabolism, and intestinal absorption. It was observed that about 30% of oral drug fail in development due to poor pharmacokinetics studies.²³ It is worth to note that lack of *in vivo* effectiveness of a drug candidate might be due to poor physicochemical properties of the drug candidate itself.²⁴ Also binding activity can be predicted by studying whether the tested compounds are complementary with the binding sites on biological molecules in terms of topology, volume and physiochemical properties.²⁵ That's to say, it is useful to estimate the probability that a molecule can bind a given protein with sufficient affinity to modify its activity.²⁶ So, Computation screening of new compounds, ie *in-silico* prediction of druglikeness and bioactivity, has been proved to be very important in the early stage of drug discovery to subject the most suitable compounds to further optimization and to find drug candidate for further clinical development.^{27,28}

Fig.-1: The Structure of Some Drugs bearing the Pyrazole Moiety

Prompted by the aforementioned findings and in the continuation of our ongoing research in the field of design, synthesis, and biological evaluation of pyrazoline derivatives, herein we described the synthesis and spectral characterization of a new series of substituted pyrazoline as potential anticancer agents against (3,4-methylenedioxyamphetamine) MDA MB 231 Breast cancer cell line study by MTT (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay method.²⁹⁻³¹ Molecular docking studies

were carried out against bacterial proteins and breast cancer protein. Finally, the synthesized compounds were screened for antimicrobial activity against the disc diffusion method. New anticancer drugs are previously used in clinical research, some of the anticancer drugs are not efficiently and intolerable side effects³². Based on the above study, we need to development of new drugs against anticancer and antimicrobial activities. Therefore, we were led to identifying new approaches of pyrazoline derivatives as well as test the antimicrobial and anticancer activity.

EXPERIMENTAL

Chemicals and Reagents

All the chemicals were purchased from variable sources, used without purification. The synthesized compounds were checked by TLC on Silica gel plates (E-Merk, Mumbai, India). The melting points of the synthesized compounds are carried out by an open capillary method and it was uncorrected. The FT-IR spectrum was recorded in KBr pellets on an FT-IR Shimadzu 8400s spectrometer in the range of 400-4000 cm^{-1} . ^1H NMR and ^{13}C NMR spectra were recorded by Bruker 400 MHz and Bruker 100 MHz spectrometer (in δ ppm) using TMS as an internal standard. CHN analyses were recorded on the Perkin Elmer CHN analyzer. By adopting the literature precedent; chalcones (3a-3h) are prepared⁵.

Synthesis of Pyrazoline Butanone Derivatives (5a-5h)

Naphthyl Chalcones (3a-3h) (0.01 mol), hydrazine hydrate (0.01 mol), anhydrous sodium acetate (0.01 mol) and Butyric acid (Propyl formic acid) (20 ml) are taken in a round bottom flask and reaction mixture was refluxed until the products are formed. The reaction is monitored by TLC. The time required for the formation of various pyrazole is 6-9 hrs. The reaction mixture is poured into crushed ice and left overnight. The precipitate is separated by filtration, washed well with water, dried and the obtained solids are purified by recrystallization using rectified spirit which affords the title compounds (5a-5h) in moderate yield. The experimental procedure was followed by the reference method.³³

Molecular Docking Study

The molecular docking studies have been carried out using the Auto dock Tools (ADT) version 1.5.6 and Auto dock version 4.2.5.1 docking program⁵.

Preparation of the Protein

The bacterial protein and breast cancer protein were downloaded from Protein Data Bank with PDB id: 1UAG and 1OQA.

Ligand Preparation

2D structure of the target compounds is drawn using Chemdraw Ultra 8.0 (Chemoffice 2002). After Chem 3D Ultra 8.0 was used to convert the 2D structure into the 3D structure, and the energy is minimized using the semi-empirical AM1 method. All the structures are saved as pdb file format for input to ADT. Finally, all the ligand structures are saved as PDB file format to carry out molecular docking in Auto dock Vina.

Grid Formation

A grid box with a dimension of 40 X 40 X 40 $\text{\AA}^3$ in 0.375 $\text{\AA}$ spacing and centered on 30.473, 47.997, 9.563 has created around the binding site of protein using ADT. The center of the box was set at ligand center and grid energy calculations have been carried out.

Docking Protocol

The Auto dock calculation, are such as default parameters have been used and 10 docked conformations are generated for each compound. The energy calculation is done using genetic algorithms. The outputs are exported to Chimera 1.10 and discovery studio 4.5 for visual inspection of the binding modes and interaction of the compounds with amino acid residues in the active site. The procedure was followed by the literature method.³⁴

Pharmacological Activity

Antibacterial Screening

The antibacterial screening for synthesized pyrazoline compounds was determined by the agar disk diffusion method against gram-positive bacterial strains of *Staphylococcus aureus*, *Streptococcus pyogenes* and gram-negative bacterial strains of *Escherichia coli*, *Pseudomonas aeruginosa*. The zone of inhibition was measured after 24h incubation at 37 °C. The biological screening experimental procedure was adopted by the literature survey method.³⁵⁻³⁷

Antifungal Screening

The antifungal screening for synthesized pyrazoline compounds was determined by the agar disk diffusion method against *Candida albicans* strain. The zone of inhibition was measured after 24 h incubation at 37 °C.

In-silico Druglikeness Property

ADME property is done by Osiris online software program. All synthesized compounds (5a-5h) subjected to druglikeness property to predict the solubility, absorption, Lipinski rule violation, and surface area of the compounds. The compound which has molecular weight ≤ 500 , n-HA ≤ 10 , n-HD ≤ 5 , logP ≤ 5 and Molar refractivity ≤ 140 obey the Lipinski rule of five. Also, compounds have a polar surface area (7 to 200), solubility value and drug score (above 0.5). All the above properties are predicted with the help of the Osiris program.

Anticancer Activity

MTT Assay

MDA MB-231 (Human Breast Adenocarcinoma) cell was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified eagle's medium, DMEM (Sigma Aldrich, USA).

The cell line was cultured in 25cm² tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and an antibiotic solution containing: penicillin (100 U/ml), Streptomycin (100 µg/ml), Streptomycin (100 µg/ml), and Amphotericin B (2.5 µg/ml). Cultured cell lines were kept at 37 °C in a humidified 5% CO₂ incubator (NBS Eppendorf, Germany).

The viability of cells was evaluated by direct observation of cells by an inverted phase-contrast microscope and followed by MTT assay method.

Cells Seeding in 96 Well Plate

Two days old confluent monolayer of cells trypsinized and the cells were suspended in 10% growth medium, 100 µl cell suspension (5×10^4 cells/well) was seeded in 96 well tissue culture plate and incubated at 37 °C in a humidified 5% CO₂ incubator.

Preparation of Compound Stock

1mg of the sample was weighed and dissolved in 1 ml DMEM using a cyclomixer. The sample solution was filtered through 0.22 µm Millipore syringe filter to ensure sterility.

Anticancer evaluation

After 24 h the growth medium was removed, freshly prepared each compound in 5% DMEM was five times serially diluted by two-fold dilution (100, 50, 25, 12.5, 6.25 µg in 500 µl) of 5% DMEM and each concentration of 100 µl was added in triplicates to the respective wells and incubated at 37 °C in a humidified 5% CO₂ incubator. Non treated control cells were also maintained⁵.

Anticancer Assay by MTT Method

15 mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 h of the incubation period, the sample content in wells was removed and 30 µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken

well, then incubated at 37 °C in a humidified 5% CO₂ incubator for 4 h. After the incubation period, the supernatant was removed and 100 µl of MTT solubilization solution (DMSO, Sigma Aldrich, USA) was added and the cells were mixed gently by pipetting up and down to solubilize the formazan crystals. The absorbance values were measured by using a microplate reader at a wavelength of 540 nm³⁸.

The percentage of growth incubation was calculated using the following formula:

$$\% \text{ of Viability} = [\text{Mean OD Samples} \times 100] / [\text{Mean OD of Control Group}]$$

RESULTS AND DISCUSSION

Synthesis and Characterization of Pyrazoline Analogs

The pyrazoline derivatives (**5a-5h**) were synthesized from 2-naphthylstyryl chalcone react with Hydrazine hydrate in the presence of propyl formic acid using the cyclization process via the conventional method. Before that, the 2-naphthyl styryl chalcone derivatives (**3a-3h**) were synthesized from 2-naphthylstyryl ketone react with different substituted aromatic aldehydes in the presence of a strong base. The synthetic route of pyrazoline derivatives represented in Scheme-1.

Scheme-1: Synthetic Route of the Isolated Compounds (**5a-5h**)

Spectral Characterization

The isolated compound's chemical structures were elucidated using FT-IR, ¹H NMR, ¹³C NMR and elemental analysis. The compound **3a** was confirmed by FT-IR spectral data showing the sharp band at 1660.70 cm⁻¹ which indicates the presence of carbonyl group (C=O). The aromatic CH stretching frequency was observed as a sharp peak at 3059.10 cm⁻¹. The aromatic C=C stretching frequency was observed at 1593.20 cm⁻¹. Other chalcone derivative's physical and chemical properties are shown in Table-1. The pyrazoline derivatives (**5a-5h**) chemical structures were confirmed using FT-IR spectral data. From this result, compound **5a** shows the strong absorption frequency at 1658.78 cm⁻¹, which indicates the presence of a carbonyl group of butane moiety. The C=N stretching frequency was observed

at 1402.25 cm^{-1} . The aromatic CH stretching frequency was observed at 3140.11 cm^{-1} . Other compounds IR results were shown in Table-2.

Fig.-2: FT-IR Spectral Data of Isolated Compound 5a.

Table-1: FT-IR Spectral Data of Synthesized Compound (3a-3h) cm^{-1}

Entry	C=O	C=C	Ar-CH	Others
3a	1660.7	1593.20	3059.10	819.75, 752.24
3b	1624.06	1579.70	3068.75	823.60, 752.24
3c	1664.57	1597.06	3059.10	819.25, 756.10, 705.95
3d	1651.07	1600.92	3051.39	815.89, 750.31, 709.80, 680.87
3e	1651.07	1602.85	3051.39	750.31, 706.96, 813.36
3f	1654.92	1597.06	3051.39	815.83, 759.95, 746.45
3g	1660.71	1600.92	3055.24	813.96, 752.24,
3h	1658.87	1589.32	3067.41	813.73, 759.67

Table-2: FT-IR Spectral Data of Isolated Compound (5a-5h) cm^{-1}

Entry	C=O	C=N ¹	Ar-CH
5a	1658.78	1402.25	3140.11
5b	1664.57	1400.32	3155.54
5c	1658.78	1404.18	3140.11
5d	1652.14	1407.13	3110.14
5e	1659.70	1403.38	3115.41
5f	1655.42	1405.89	3119.82
5g	1651.92	1401.01	3142.33
5h	1654.89	1402.04	3188.79

The Proton NMR spectrum of compound **5a** shows the three doublets of doublet 3.25, 3.85 and 5.59ppm respectively, the doublet of a doublet at in the downfield region of the 5.59 ppm and it has the coupling constant value $J_{5a, 4a} = 11.6\text{ Hz}$ and $J_{5a, 4b} = 4.4\text{ Hz}$, which indicates the presence of H-5a proton of pyrazole moiety. The H-4a proton of the pyrazole moiety exhibited the downfield shift that appeared at the doublet of a doublet at 3.25 ppm and the coupling constant value $J_{4a, 4b} = 17.4\text{ Hz}$ and $J_{4a, 5a} = 4.2\text{ Hz}$. The H-4b proton of pyrazole moiety in appeared the doublet of a doublet at 3.85 ppm and the coupling constant value $J_{4b, 4a} = 17.2\text{ Hz}$ and $J_{4b, 5a} = 12\text{ Hz}$. The aromatic protons have appeared at the range between 7.19 – 8.09 ppm. The methyl group of butane moiety in pyrazoline compound appears triplet at the range is 1.008-1.026 ppm. The methylene proton of butane moiety in the pyrazoline compound exhibited a multiplet at in the range of 2.75 ppm – 2.90 ppm is due to the presence of neighboring protons of butane moiety. Another CH_2 proton of butane moiety in pyrazoline compound exhibits the multiplet at in the range of 1.725 ppm – 1.780 ppm. The compounds (**5a-5h**) Proton-NMR spectral values are shown in Table-3. From the proton NMR spectral studies of the synthesized compound; the structure has been unambiguously confirmed.

Fig.-3: Proton-NMR Spectrum of Isolated Compound 5a

Table-3: ¹H NMR Spectral Data for Isolated Compound (5a-5h) ppm (δ)

Entry	5a	5b	5c	5d	5e	5f	5g	5h
H-4a	3.25	3.18	3.22	3.21	3.26	3.14	3.18	3.24
H-4b	3.85	3.77	3.83	3.89	3.75	3.78	3.80	3.84
H-5a	5.59	5.97	5.78	5.64	5.79	5.94	5.75	5.83
Ar-H	7.19- 8.09	7.08- 8.08	7.12- 8.18	7.06- 7.99	7.12- 8.01	7.09- 8.05	7.04- 8.02	7.16- 8.12
CH ₂	1.725- 1.780	1.582- 1.821	1.625- 1.820	1.658- 1.825	1.710- 1.792	1.728- 1.801	1.710- 1.808	1.732- 1.810
CH ₂	2.756- 2.900	2.815- 2.983	2.764- 2.938	2.782- 2.938	2.782- 2.899	2.689- 2.912	2.710- 2.855	2.764- 2.882
CH ₃	1.008- 1.026	1.055- 1.073	1.006- 1.028	1.038- 1.022	1.008- 1.062	-	1.012- 1.052	1.028- 1.052
CH ₃	-	-	-	-	2.35	-	-	-
OCH ₃	-	-	-	-	-	3.73	-	-

The ¹³C NMR spectrum of compound **5a** shows that the ¹³C resonance observed at 171.61 ppm is attributed to C=O group of butane moiety in the pyrazoline compound. The ¹³C resonance observed at 153.52 ppm which indicates the presence of C=N group of pyrazole ring in the pyrazoline compound. The ¹³C resonance observed at 59.63 ppm is due to the presence of C-5 carbon of pyrazole ring in pyrazoline

compound. The ^{13}C resonance observed at 41.95 ppm which is attributed to the presence of C-4 carbon of pyrazole ring in pyrazoline compound. The ^{13}C resonance observed at 36.17 ppm which indicates the presence of C-2' carbon of pyrazole ring in pyrazoline compound. The ^{13}C resonance observed at 18.39 ppm which is unambiguously assigned the presence of C-3' carbon of butane moiety in pyrazoline compound. The ^{13}C resonance observed at 14.04 ppm which is due to the presence of CH_3 group of butane moiety in pyrazoline compound. The aromatic carbons are appeared at in the range of 123.39 – 129.10 ppm. The other ^{13}C signals 133.02, 133.40, 134.28 and 140.40ppm are the presence of *ipso* carbons. From the Carbon-NMR spectral results, the structures of the synthesized compounds were confirmed. The other compounds (**5a-5h**) ^{13}C NMR spectral data values are shown in Table-4.

Fig.-4: Carbon-NMR Spectral Data of Isolated Compound 5a

Table-4: ^{13}C NMR Spectral Data of Isolated Compound (5a-5h)ppm(δ)

Entry	5a	5b	5c	5d	5e	5f	5g	5h
C=O	171.61	171.55	172.66	172.18	171.98	172.04	171.87	171.02
C=N	153.52	153.96	154.25	153.98	153.75	153.99	153.84	153.78
C-5	59.63	58.36	58.98	59.42	59.18	59.36	59.61	59.58
C-4	41.95	41.28	41.89	41.91	41.57	41.82	41.39	41.67
C-2'	36.17	36.20	36.34	36.84	36.73	36.02	36.54	36.32
C-3'	18.39	18.65	18.93	18.82	18.57	18.63	18.35	18.52
CH_3	14.04	14.16	14.08	14.25	14.12	14.29	17.08	14.19
Ar-C	123.39- 129.10	123.26- 131.80	121.03- 128.97	119.34- 128.69	123.07- 131.58	124.09- 132.47	120.17- 131.47	122.04- 131.17

CH ₃	-	-	-	-	24.22	-	-	-
OCH ₃	-	-	-	-	-	56.87	-	-

Table-5: Physical Characterization of Isolated Compound (5a-5h)

Entry	M.F	M.W	Yield %	Elemental analysis	
				Calculated	Found
5a	C ₂₃ H ₂₂ N ₂ O	342.43	58	C, 80.66; H, 6.42; N, 8.17; O, 4.66	C, 80.67; H, 6.48; N, 8.18; O, 4.67
5b	C ₂₃ H ₂₁ FN ₂ O	360.42	54	C, 76.64; H, 5.82; F, 5.26; N, 7.76; O, 4.43	C, 76.64; H, 5.87; F, 5.27; N, 7.77; O, 4.44
5c	C ₂₃ H ₂₁ ClN ₂ O	376.88	59	C, 73.29; H, 5.57; Cl, 9.40; N, 7.42; O, 4.24	C, 73.30; H, 5.62; Cl, 9.41; O, 4.25
5d	C ₂₃ H ₂₁ BrN ₂ O	421.33	56	C, 65.56; H, 4.98; Br, 18.96; N, 6.64; O, 3.79	C, 65.57; H, 5.02; Br, 18.96; N, 6.65; O, 3.80
5e	C ₂₄ H ₂₄ N ₂ O	356.46	60	C, 80.86; H, 6.73; N, 7.85; O, 4.48	C, 80.87; H, 6.79; N, 7.86; O, 4.49
5f	C ₂₄ H ₂₄ N ₂ O ₂	372.46	64	C, 77.38; H, 6.44; N, 7.51; O, 8.58	C, 77.39; H, 6.49; N, 7.52; O, 8.59
5g	C ₂₃ H ₂₁ N ₃ O ₃	387.43	62	C, 71.29; H, 5.42; N, 10.84; O, 12.38	C, 71.30; H, 5.46; N, 10.85; O, 12.39
5h	C ₂₃ H ₂₀ Cl ₂ N ₂ O	411.32	65	C, 67.15; H, 4.86; Cl, 17.23; N, 6.80; O, 3.88	C, 67.16; H, 4.90; Cl, 17.24; N, 6.81; O, 3.89

Antibacterial Activity

Antibacterial screening of synthesized derivatives (5a-5h) against Gram-positive bacterial species: *Staphylococcus aureus*, *Streptococcus pyogenes* and gram-negative species *Escherichia coli*, *Pseudomonas aeruginosa*. Antibacterial activity results (Table-6) particularly; compound 5b (Fluoro substitution) exhibited good zone of inhibition (20 mm) against *Staphylococcus aureus* and Compound 5d (Bromo substitution) exhibited excellent zone of inhibition (24 mm) as compared with standard drug ciprofloxacin against *Streptococcus pyogenes*. Compound 5b (Fluoro substitution) exhibited an excellent zone of inhibition (24 mm) as compared with standard drug ciprofloxacin against *E.coli*. Compound 5b has an excellent zone of inhibition (25 mm) compared with the standard drug against *Pseudomonas aeruginosa*. Finally, the antibacterial result, the electronegativity group (F and Br) has excellent activity. The reason is due to the presence of the electronegativity group directly attached to the pyrazole ring moiety of compound 5b and 5d. The electron-withdrawing group attached to the pyrazoline ring portion increased the antibacterial potential against *S.pyogenes*.

Fig.-5: Chemical Structure of Compound 5b and 5d

Table-6: Antibacterial Activity of Isolated Compound (5a-5h) at 1.0 mg/ml.

Entry	Bacteria			
	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	<i>Escherichia Coli</i>	<i>Pseudomonas aeruginosa</i>
5a	15	21	18	16

5b	20	22	24	25
5c	14	18	16	17
5d	16	24	20	16
5e	16	19	18	-
5f	17	18	17	16
5g	16	16	17	20
5h	13	15	13	10
Ciprofloxacin	26	17	19	22

Fig.-6: Antibacterial Activity of Isolated Compound (5a-5h)

Antifungal Activity

Antifungal screening of synthesized derivatives (5a-5h) against *Candida albicans* species was done by agar disk diffusion method. Antifungal results indicated (Table-7) particularly compound 5b shown more promising antifungal activity (14 mm) as compared with standard drug Clotrimazole (24 mm) while other derivatives are moderately active. The electron-withdrawing group directly attached with pyrazole ring portion of compound 5b increased the antifungal potential against *C. albicans*.

Table-7: Antifungal Activity of Isolated Compound (5a-5h)

Entry	<i>Candida albicans</i>
5a	-
5b	14
5c	12
5d	13
5e	-
5f	-
5g	11
5h	10
Clotrimazole	24

In-silico Molecular Docking Study

Pyrazoline Compounds (5a-5h), Docked with Bacterial Protein

Molecular modeling studies were accomplished to investigate the possible bind mode of the synthesized eight pyrazoline derivatives targeting the crystal structure of the bacterial protein 1UAG using Auto dock docking program. From this Table-8, the docking score, conventional hydrogen bond, and hydrophobic contacts such as alkyl and pi-alkyl stacking were provided. Most of the synthesized pyrazoline compounds showed very good interaction with the studied protein. Our synthesized compounds (5a-5h) all are exhibited good binding affinity scores compared with standard drugs. Among the 8 pyrazoline compounds studied with the protein 1UAG, compound 5b showed a high binding affinity score -9.4 kcal/mol compared with standard drug ciprofloxacin -7.9 kcal/mol. Compound 5b has three conventional

hydrogen bond contact with the residue such as SER A: 415, PHE A: 422, LYS A: 115. The C=O group of compound **5b** interacted with the active pockets of the enzyme forming hydrogen bonds with SER A: 415 and PHE A: 422 at different distances (2.06 Å, 2.70 Å). Also, 4-f substitution benzene ring of compound **5b** interacted with the active pockets of the enzyme forming a hydrogen bond with LYS A: 115 at 2.95 Å distance. The pyrazole ring and benzene ring of compound **5b** interacted with the active pockets of the enzyme forming hydrophobic contact with ALA A: 414 and LEU A: 416 at different distances of 4.70 Å, 4.67 Å respectively. Docking score, hydrophobic contact of the derivatives are shown in Table-8. 2D and 3D image of compound **5b** is shown in Fig.-8.

Fig.-7: Antifungal Activity of Isolated Compound (5a-5h)

Fig.-8: 2D and 3D Images of Compound 5b

***In-vitro* Anticancer Activity**

The anticancer activity of synthesized pyrazoline derivatives (**5a-5h**) was carried out for docking studies using breast cancer protein 1OQA and the results are presented in Table-9. From this result, synthesized compounds (**5a-5h**) all have a good binding affinity score. Especially, Compound **5b** has a higher binding affinity score when compared with the other derivatives. The benzene ring of compound **5b** interacted with the active pockets of the enzyme forming hydrophobic contact with the residues PRO A: 59 and PRO A: 103 at different distances (4.62 Å, 5.28 Å). The other compounds docking score, H-bond, and hydrophobic contacts are given in Table-9. The 2D and 3D images of compound **5b** shown in Fig.-9.

Table 8: Molecular Docking Result of Isolated Compound (5a-5h) using Bacterial Protein.

Entry	Docking results	Hydrogen interaction	Length of Hydrogen bond Å	Hydrophobic contact	Hydrophobic contact bond length Å
5a	-7.9	HIS A: 267	1.99	ARG A: 302	5.06
5b	-9.4	SER A: 415, PHE A: 422, LYS A: 115	2.06, 2.70, 2.95	ALA A: 414, LEU A: 416	4.70, 4.67
5c	-8.7	SER A: 415, PHE A: 422	2.06, 2.70	ALA A: 414, LEU A: 416	4.64, 5.29
5d	-8.6	SER A: 415, PHE A: 422	2.05, 2.69	ALA A: 414, LEU A: 416	4.64, 5.29
5e	-8.7	SER A: 415, PHE A: 422	2.08, 2.71	ALA A: 414, LEU A: 416	4.66, 5.24
5f	-8.9	SER A: 415, PHE A: 422, LYS A: 319	2.07, 2.71, 2.53	ALA A: 414, LEU A: 416	4.70, 5.23
5g	-8.8	SER A: 415, PHE A: 422, LYS A: 115, LYS A: 319	2.08, 2.70, 2.40, 2.63	ALA A: 414, LEU A: 416	4.70, 5.27
5h	-7.9	-	-	PHE A: 422, ARG A: 186	5.08, 5.01, 5.11
Ciprofloxacin	-7.8	HIS A: 267, ASN A: 113	2.07, 2.15, 2.81	ALA A: 328	4.70

Table-9: Molecular Docking Studies Performed for Pyrazoline Compound (5a-5h) Using Breast Cancer Protein 1OQA.

Entry	Docking results	Conventional H-Bond	H-B Bond length Å	Hydrophobic Contact Å	Hydrophobic Bond length Å
5a	-7.1	-	-	PRO A: 59, PRO A: 103, ILE A: 102	4.65, 5.31, 5.48
5b	-7.9	-	-	PRO A: 59, PRO A: 103	4.62, 5.28
5c	-7.3	-	-	PRO A: 59, ILE A: 103	4.61, 5.30
5d	-7.3	-	-	PRO A: 59, PRO A: 103, ILE A: 102	4.64, 4.62, 4.42
5e	-7.4	-	-	PRO A: 59, PRO A: 103, ILE A: 102	4.58, 5.28, 5.46
5f	-7.2	-	-	PRO A: 59, PRO A: 103, ILE A: 102	4.50, 5.37, 5.43
5g	-7.6	ASP A: 65	2.34	PRO A: 103, PRO A: 59	4.60, 5.21
5h	-6.9	-	-	PRO A: 59, PRO A: 103, ILE A: 102	4.59, 4.58, 4.66

Fig.-9: 2D and 3D Images of Compound 5b

In-silico Druglikeness Study

The target molecules (**5a-5h**) subjected to druglikeness property with the help of Osris program. The values from the program said that the solubility, MW, TPSA, log P o/w and drug score. All the compounds, the target **5c** which have positive value 1.21 in the druglikeness property, 0.51 as a drug score with 32.67Å TPSA and zero violation in Lipinski's rule. All the pyrazoline derivatives have zero violations in Lipinski's rule, have good TPSA and have solubility value between -7 to -5. The values are predicted from the software program, it was tabulated and given below in Table-10.

In-vitro Anticancer Activity**MTT Assay**

According to the docking results. The compound **5b** was performed *in-vitro* anticancer activity against the human breast cancer cell line(MDA MB-231) using MTT assay method at various concentrations (100, 50, 25, 12.5, 6.25 µg/ml) and the results are presented in Fig.-10 and 11. Anticancer screening results revealed that in general pyrazoline derivatives exhibited good anticancer potential against human breast cancer cell line, especially, compound 5b act as a moderate activity in all concentrations except 6.25 µg/ml showed good activity and the LC₅₀ value of this compound is 27.76 ±0.003 µg/ml. Triplicate experimental method was followed and then the mean value was calculated.

Fig.-10: *In-vitro* Anticancer Activity Screening for Compound 5b at Low Concentration

Table-10: Druglikeness, Toxicity, Surface Area and Lipinski Rule Values of Compound 5a-5h

Compound	Log P	MW	n-HA	n-HD	n-violation in rule of five	TPSA	Solubility logS	Druglikeness	Drug Score
5a	5.33	342.4	3	0	0	32.67	-5.76	-0.60	0.45

5b	5.43	360.4	3	0	0	32.67	-6.08	-0.34	0.45
5c	5.94	376.8	3	0	0	32.67	-6.50	1.21	0.51
5d	6.06	421.3	3	0	0	32.67	-6.60	-1.85	0.31
5e	5.68	356.4	3	0	0	32.67	-6.11	-1.88	0.35
5f	5.26	372.4	4	0	0	41.90	-5.78	-0.39	0.46
5g	5.42	386.4	4	1	0	72.98	-6.22	-0.82	0.41
5h	6.54	411.3	3	0	0	32.67	-7.23	-1.09	0.32

Fig.-11: *In-vitro* Anticancer Activity of Compound 5i. MDA-MB-231 Cells were incubated for 48 hours in presence of (i) 5i at 6.25 $\mu\text{g/ml}$ (ii) 5i at 12.5 $\mu\text{g/ml}$ (iii) 5i at 25 $\mu\text{g/ml}$ (iv) 5i at 50 $\mu\text{g/ml}$ (v) 5i at 100 $\mu\text{g/ml}$

CONCLUSION

Summarizing, Eight novel propanone pyrazole compounds were synthesized and characterized by phiochemical and spectral data was found in good agreement with the assigned molecular structures. The in-vitro antimicrobial screening of synthesized compounds indicated **5b** and **5d** exhibited appreciable antimicrobial potential. The anticancer screening results demonstrated that compound **5b** ($\text{LC}_{50} = 27.76 \pm 0.003 \mu\text{g/ml}$) is the most active one against MDA-MB-231 cancer cell line. Molecular docking studies indicated the compound **5b** being the most active molecule has the maximum hydrogen bond interaction(three) and pi-pi stacking network among pyrazoline derivatives. The synthesized compounds may exhibit their antimicrobial and anticancer activity by the inhibition of enzymes 1OQA and MDA-

MB-231 respectively. Finally, the obtained antimicrobial and anticancer data makes such type of compounds a fruitful matrix for further development of more potent and selective anticancer and/or antimicrobial agents. In particular the compound **5b** could be considered as a possible dual antimicrobial-anticancer candidate that deserves further investigation and derivatization to explore the scope and limitation of its biological activities.

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