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DOCKING STUDIES OF INDOLE ASSIMILATED PYRAZOLINE MOLECULAR HYBRIDS: DESIGN, SYNTHESIS AS ANTIINFLAMMATORY AGENTS AND ANTICANCER AGENTS

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ABSTRACT
A series of novel compounds (4a-4l) had been synthesized by Claisen-Schmidt condensation, Schiff's base and Cyclization mechanism. All the molecule structures were affirmed by IR, ^{1}H NMR and Mass spectral data. The synthesized hybrids were screened for *in vivo* anti-inflammatory and *in vitro* anticancer activities. Anti inflammatory activity was performed using carrageenan induced rat paw edema method using Diclofenac as standard drug. The anticancer activity was assessed by using MTT assay against MCF7 and SKOV3 cell lines and Doxorubicin was used as reference standard. Among the compounds compound **4l** exhibited the highest % inhibition of **98.4** when compared with the standard Diclofenac **94.2%**.Compound **4f** exhibited the lowest IC_{50} at concentration of **20.01µg** against MCF7 cell lines and **32.87µg** against SKOV3 cell lines. The molecular docking studies were performed using the Ligprep tool of Schrodinger suite. This study revealed that novel thiazolidne-4-one-pyrazole hybrids (4a-4j) had good interaction with the active site of EGFR receptor. Among the docked compounds, dock score of the compounds ranged from from **-3.186 to -5.212**. The highest score was exhibited by **4f** with -5.212 with Glide binding energy of -34.697 Kcal/mol.

Keywords: Pyrazole, Anti-inflammatory and Anticancer, MTT Assay, Doxorubicin.

1. INTRODUCTION
The complex divergence and biotic importance of nitrogen containing heterocycles contributed as interesting targets for synthesis over a long period of time. Indole derivatives are pharmacologically important moieties with wide range of activities as antifungal, antiviral, antimalarial [1]. Inflammation is a physiological body's natural reaction activated against injury and infection. Inflammation is actively correlated to cancer which plays an important role in tumour growth and progression. Chronic inflammation decreases the immune system by boosting cell proliferation, angiogenesis and metastatis leading to oncogenesis [2]. Many biologically [3-7] important Pyrazoline containing compounds exhibit anticancer, antifungal, antibacterial, antioxidant, antitubercular, anti-inflammatory, and antimalarial activities. Pyrazoline as quiescent heterocyclic moiety when fused with other heterocycles results in enhancement of biological properties and therefore synthesizing these compounds grab the attention of researchers to design the novel drugs [8, 9].

Many of the marketed drugs are containing pyrazole or Indole scaffold as a core functioning unit like Anti-inflammatory, Anticancer and Antimicrobial drugs [10]. Moreover, it was showing the potency of pyrazole scaffolds and also it was evidence for its biological significance. From literature reports, it is known that pyrazoline derivative involves in cancer therapy, due to its huge biological activities and capability of forming hydrogen bonds [11-13]. Pyrazoline also is one of the major potential entities in the field of drug discovery using heterocyclic compounds. Combining pyrazoline moiety with other pharmacophores results enhancement of biological properties and hence, synthesizing such compounds attracts the researchers to discover novel drugs.

2. MATERIAL AND METHODS
The synthesized compounds were screened for anticancer, and anti-inflammatory activities. IR spectra were recorded on Fourier IR spectrophotometer (Shimadzu 8700) in the range of 4000-400cm^{-1} using KBR

pellets and the spectra were interpreted. ^{1}H-NMR spectra were recorded on DPX-200 MHz NMR spectrometer exploiting DMSO-d6 and chemical shifts (δ) were prevalent in parts per million downfield from internal reference Tetramethylsilane (TMS) and the Spectra were interpreted. Mass spectra were catalogued on Mass spectrophotometer (model Shimadzu) by LC-MS and the spectra were interpreted. The precoated silica gel G plates were used to detect the progress of the reaction as well as to assess the purity of the compounds: n-Hexane: Ethyl acetate (7:3). Initial geometrical optimization and strength minimization of molecules had been carried out through the usage of the Ligprep device of Schrodinger suite. Various ionization states were generated out through the usage of the Ligprep module using software EPIK alongside with quite a number of achievable conformers and tautomer's.

2.1. General procedures

2.1.1. Synthesis of Indole -3-carbaldehyde

Freshly distilled dimethyl formamide (3.74 moles) was added to round bottomed flask fitted with an efficient mechanical stirrer. The contents were cooled in an ice salt bath for about 50min and freshly distilled phosphorus oxychloride was subsequently added with stirring to the dimethyl formamide over a period of 50min resulting in pinkish coloured complex. Then100gm of Indole (1.3moles) in 100ml (95gm, 1.3moles) of Dimethyl formamide was added to the yellow solution over a period of one hour during which the temperature should not rise above 10°C. The syrup was continuously stirred for 90min until yellow canary thick paste was obtained. Then 300gm of crushed ice was added with stirring to the flask until cherry red aqueous solution was obtained. This solution was transferred to three necked flask containing crushed ice (200gm) fitted with separating funnel containing 346gm of sodium hydroxide in1 litre of water which was attached with mechanical stirrer, The aqueous base was added dropwise until one third of the solution was completed and then the remaining solution was added with efficient stirring. Then the solution was boiled and then cooled to room temperature which was allowed to stand overnight. Then the product obtained was collected on filter and then it was added to 1litre of water. Most of the product got dissolved leaving a residue which was washed three times with 300ml of water. The compound finally obtained was Indole-3-aldehyde and then recrystallized from ethanol. MP: 196-198°C [14].

2.1.2. Synthesis of Schiff Bases (1a-1b)

A mixture of (0.01 mol) Indole-3-carbaldehyde and (0.01 mol) of substituted aniline derivative were dissolved in 40 ml of ethanol and 2 ml of glacial acetic acid (GAA) (as a catalyst) in a round bottom flask and then whole of the substance was refluxed for about 2-3 hours and then checked for completion by Thin Layer Chromatography (TLC). After refluxing, the whole of the content was poured into cold water. At last, the precipitated product was re-crystallized [15].

2.1.3. Synthesis of N-Acetyl Indole derivative(2a-2b)

A 0.735 gm of compound (1a-1b) was taken into the round bottom flask and to it 5.1 ml of acetic anhydride was added. Then whole of the content was refluxed for about 4 hours and then the solution was poured into beaker containing crushed ice followed by the filtration and drying of the product [16].

2.1.4. Synthesis of Indole chalcone derivative (3a-3f)

To a solution of compound (2a-2b) (0.01 mol) in ethanol (20 mL), substituted benzaldehyde (0.01 mol) was added and cooled to 5-10°C in an ice bath. To this cold solution, sodium hydroxide (30%) was added and stirred magnetically for 1 h and then left overnight or longer, monitored by TLC. The resultant solution was diluted with ice water and acidified with dilute HCl. The chalcone separated as solid was collected by filtration after washing with water and recrystallized from ethanol [17].

2.1.5. Synthesis of Novel pyrazole derivative (4a-4l)

The compound 3a-3f (Indole chalcone derivative) (0.01M) dissolved in ethanol (25 ml) was added to phenyl hydrazine (0.01M). To this aq. KOH/NaOH solution (0.02M) was added. The reaction mixture was refluxed for 8hours, cooled, diluted with water and acidified with conc. HCl. The product was filtered, dried and recrystallized from ethanol [18].

2.2. Anti- inflammatory activity

Albino rats weighing about 180-200gms obtained from Vab biosciences were used. They were acclimated in the of animal house of Sri Venkateshwara College of Pharmacy at ambient temperature of 22°C±2°C and relative humidity one week before use and rats were fed pelleted diet and water *ad libitium*. The experimental protocol was duly approved by IAEC (Indian Animal Ethical Committee) OF CPCSEA (Committee for purpose of control and supervision of Experimentation

on animals) through reference no: IAEC/SVCP/2022/07 dated. The animals were randomly divided into three groups Control, Standard Group Diclofenac and synthesised compounds (4a-4l) (6rats each). At first, 50 µl of1% suspension of carrageenan in saline was prepared 1 hr before experiment and was injected into the plantar surface of right hind paw of rat. Test compounds were administered at a dose level of 100mg/kg to the plantar surface of the right hind paw by gently rubbing with the index finger. Standard Diclofenac was administered at a dose level of 20mg/kg to rats. Carrageenan suspension in saline 1% was administered into planatar surface of right hind paw of rats after one hour after the administration of control, Diclofenac and test compounds (4a-4l). Paw volume was measured immediately after carrageenan injection at 0hr, 1hr, 2hr, 3hr and 4hrs were measured using the mercury displacement technique with the help of plethysmograph. The paw volume was recorded at the different time intervals. The percentage inhibition in paw volume was calculated by using the formula [19].

% inhibition= {Paw volume (control)- Paw volume (test)/ Paw volume (control)} x 100

The results of the studies were expressed as mean±SEM. Data analysis was done by One-way analysis of variance (ANOVA) followed by Dunnets test. Probabilty $P<0.05$values were considered significant when compared to control group.

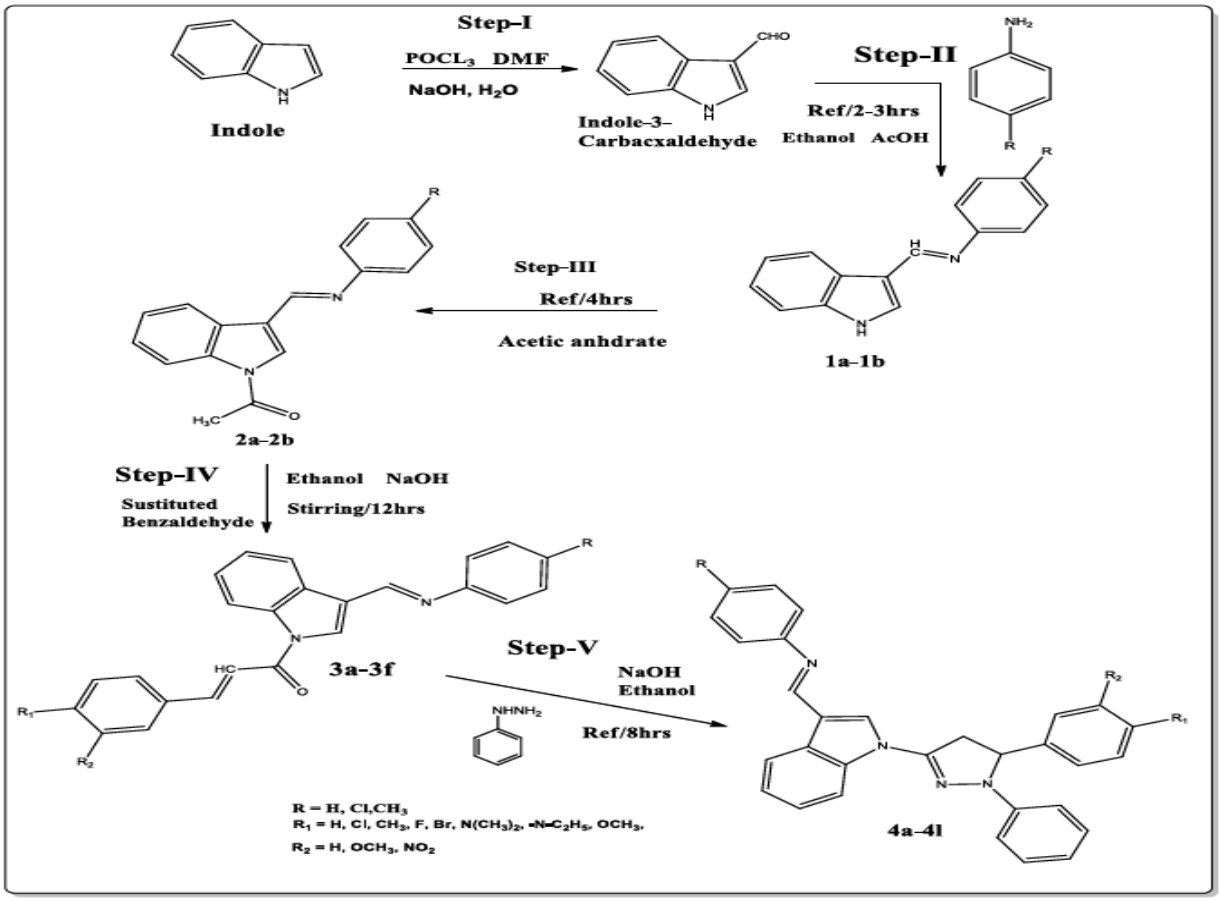

Fig. 1: Scheme-Synthesis of Indole assimilated Pyrazoline derivatives (4a-4l)

2.3. Anticancer activity

The cancer cell lines were purchased from NCCS (National centre for Cell Science), Pune and the cells were maintained in MEM (Modified Eagles Medium) supplemented with 10 % FBS (Fetal Bovine Serum) and the antibiotics penicillin/ streptomycin (0.5 Ml^{-1}), in atmosphere of 5% CO_2/ 95% air at 37°C. Cell viability was evaluated by the MTT Assay 3 (4,5-dimethyl, thiazol-2-yl)2,3 diphenyl tetrazolium bromide with three independent experiments with six concentrations of compounds in triplicates. Cells were trypsinized and preform the trypan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 X 10^3 cells / well in 100 µl media in 96 well plate culture medium and incubated overnight at 37°C. After incubation, take off the old

media and add fresh media 100 µl with different concentrations of test compound in represented wells in 96 plates. After 48 hrs., Discard the drug solution and add the fresh media with MTT solution (0.5 mg / $Ml^{-1)}$ was added to each well and plates were incubated at 37°C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula [20]:

% inhibition= {(Control-Treatment)/Control} x 100

The IC_{50} value was determined by using linear regression equation i.e. y = mx+c. Here, y = 50, m and c values were derived from the viability graph.

2.4. Molecular docking studies

All the synthesized molecules 4a-4l were constructed by Chem Draw Pro 12.0 and the molecular docking process was done by using Ligprep tool of Schrodinger suite. The 2D structures were converted into 3D structure by using potential algorithms and application of high efficient force fields. Various ionization states were generated using Ligprep module making use of special program EPIK along with various possible conformers and tautomer's. The digital structure of the epidermal growth factor receptor (EGFR) was retrieved from the Protein databank website with PDB Id: 1M17 and the structure was optimized by deleting unbound water molecules which are over 1 Å, adding hydrogen atoms to satisfy the valencies, adding missing amino acids to stabilize side chains and energy of the whole structure was minimized using OPLS-2005 force field using Protein Preparation Wizard tool of Schrodinger Suite.

3. RESULTS AND DISCUSSION

3.1. Chemistry

In the present work, Indole reacts with Vilsmeier reagent to form Indole -3-carbaldehyde. This carbaldehyde reacts with aniline to form Schiff's base. Imine undergoes acetylation in the presence of acetyl anhydride to form acetyl indole derivatives. Indole derivatives react with substituted benzaldehyde to form chalcones. Finally, chalcone undergoes cyclisation to form Pyrazoline derivatives. Fused Pyrazoline derivatives were characterised by IR, ^{1}H-NMR and Mass spectral data.

3.1.1. Compound -4a: (Z)-N-pheny-1-[5-phenyl-1-phenyl-4,5-dihydro-1H-pyrazole-3-yl]- 1H -indol-3yl]methanimine

3020(-CH *Str*, Ar-H), 2952, 2828(-CH *Str,* Aliphatic), 1631(-CN *Str*, Imine), 1538(-C=CH *Str*, Ar), 1427(-C=C *Str*, Ar), 1007(-C-N *Str*). ^{1}HNMR (DMSO) δ ppm: 9.7476(s, 1H, Imine proton); 8.3824(d, 2H, Ar-H), 7.9033-7.9006(d, 2H, Ar-H); 7.8784-7.8564(d, 2H, Ar-H); 7.8924-7.8004(d, 2H, Ar-H); 7.7432-7.7067(t, 2H, Ar-H); 7.5983-7.5874(t, 3H, Ar-H); 7.4265-7.4209(t, 3H, Ar-H); 7.4174-7.4109(t, 3H, Ar-H); 6.9854(s, 1H, Ar-H), 3.7823-3.7054(dd, 2H, pyrazole); 3.1539(t, 1H, pyrazole). Mass (LC-MS): m/z 440.20(M), 441.14(M+1).

3.1.2. Compound -4b: (Z)-N-pheny-1-[5-phenyl-1-(4-methylphenyl)-4,5-dihydro-1H-pyrazole -3-yl]- 1H-indol-3yl]methanimine

3012(-CH *Str*, Ar-H), 2933, 2836, 2723(-CH *Str,* Aliphatic), 1622(-CN *Str*, Imine), 1586(-C=CH *Str*, Ar), 1428(-C=C *Str*, Ar), 1077(-C-N *Str*). ^{1}HNMR (DMSO) δ ppm: 9.5643(s, 1H, Imine proton); 7.9987-7.9654(d, 2H, Ar-H), 7.8987-7.8887(d, 2H, Ar-H); 7.7973-7.7903(d, 2H, Ar-H); 7.7898-7.7873(d, 2H, Ar-H); 7.6023-7.6003(d, 2H, Ar-H); 7.5093-7.5002(t, 2H, Ar-H); 7.4872-7.4783(t, 3H, Ar-H); 7.0093(t, 3H, Ar-H); 3.9743-3.9032(dd, 2H, pyrazole); 3.2432 (t, 1H, pyrazole), 2.0932(s, 3H, Ar-CH_3). Mass (LC-MS): m/z 454.22(M), 455.3(M+1).

3.1.3. Compound.-4c:(Z)-N-pheny-1-[5-(4-methoxy-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazole-3-yl]-1H-indol-3yl]methanimine

3082 (-CH *Str*, Ar-H), 2930, 2852(-CH *Str,* Aliphatic), 1615(-CN *Str*, Imine), 1528(-C=CH *Str*, Ar), 1422(-C=C *Str*, Ar), 1127(-C-O- *Str*, OCH_3), 1013(-C-N *Str*). 1HNMR (DMSO) δ ppm: 9.7311(s, 1H, Imine proton); 8.3745-.3112(d, 2H, Ar-H), 8.2156-8.1715(d, 2H, Ar-H); 7.8868-7.8758(d, 2H, Ar-H); 7.8485(s, 1H, Ar-H); 7.6881-7.6586(d, 2H, Ar-H); 7.6575-7.6381(d, 2H, Ar-H); 7.5863-7.5701(t,3 H, Ar-H); 7.5638-7.5545(t, 3H, Ar-H); 3.8245-3.8210(dd, 2H, pyrazole); 3.5561(s, 3H, Ar-OCH_3); 3.1201(t, 1H, pyrazole). Mass (LC-MS): m/z 470.21(M), 471.20(M+1).

3.1.4. Cmpound.4d: : (Z)-N-pheny-1-[5-(4-chloro-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazole -3-yl]-1H-indol-3yl]methanimine

3070(-CH *Str*, Ar-H); 2928, 2755(-CH *Str,* Aliphatic);

1637(-CN *Str*, Imine); 1517(-C=CH *Str*, Ar), 1425(-C=C *Str*, Ar); 1030(-C-N *Str*); 814(C-Cl *Str*, Ar-Cl). ^{1}HNMR (DMSO) δ ppm: 9.9032(s, 1H, Imine proton); 8.0839-8.0802(d, 2H, Ar-H); 7.9823(s, 1H, Ar-H); 7.9763-7.9624(d, 2H, Ar-H); 7.8564-7.8209(t, 3H, Ar-H); 7.7432-7.7204(t, 2H, Ar-H); 7.6432-7.6309(d, 2H, Ar-H); 7.5209-7.5102(d, 2H, Ar-H); 7.4293-7.4092(t, 3H, Ar-H); 7.3983-7.3873(d, 2H, Ar-H); 3.6858-3.6543(dd, 2H, pyrazole); 3.2093(t, 1H, pyrazole). Mass (LC-MS):m/z 474.16(M); 475.14 (M+1); 476.01(M + 2).

3.1.5. Compound 4e:(Z)-N-pheny-1-[5-(4-fluro-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazole-3-yl]- 1H-indol-3yl]methanimine

3024(-CH *Str*, Ar-H); 2978, 2850(-CH *Str,* Aliphatic); 1613(-CN *Str*, Imine); 1485(-C=CH *Str*, Ar), 1418(-C=C *Str*, Ar); 1029(-C-N *Str*); 795(C-F *Str*, Ar-F). ^{1}HNMR (DMSO) δ ppm: 9.8213(s, 1H, Imine proton); 8.1993-8.0988(d, 2H, Ar-H); 7.9943-7.9865(d, 2H, Ar-H); 7.9004(s, 1H, Ar-H); 7.8452-7.8192(d, 2H, Ar-H); 7.7382-7.7230(d, 2H, Ar-H); 7.7094-7.6102(t, 2H, Ar-H); 7.5195-7.5095(d, 2H, Ar-H); 7.4793-7.4465(d, 2H, Ar- H); 7.3589-7.3265(t, 3H, Ar-H); 7.4162-7.4092(d, 2H, Ar-H); 3.7887-3.7632(dd, 2H, pyrazole); 3.1843(t, 1H, pyrazole). Mass (LC-MS):m/z 458.19 (M); 459.21(M+1); 460.02(M + 2).

3.1.6. Compound 4f:(Z)-N-(4-chloropheny)-1-[5-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H -pyrazole-3-yl]- 1H-indol-3yl]methanimine

3023(-CH *Str*, Ar-H); 2980, 2780(-CH *Str,* Aliphatic); 1617(-CN *Str*, Imine); 1573(-C=CH *Str*, Ar), 1481(-C=C *Str*, Ar); 1175(-C-N *Str*); 847(C-Cl *Str*, Ar-Cl). ^{1}HNMR (DMSO) δ ppm: 9.9841(s, 1H, Imine proton); 8.5253-8.5069(d, 2H, Ar-H); 7.9990-7.9871(d, 2H, Ar-H); 7.8263-7.8206(d, 2H, Ar-H); 7.8128-7.8043(t, 2H, Ar-H); 7.7102-7.7047(d, 2H, Ar-H); 7.5127-7.5002(d, 2H, Ar-H); 7.4204-7.4129(d, 2H, Ar-H); 7.4045-7.4001(t, 3H, Ar-H); 7.1342(s, 1H, Ar-H); 3.8431-3.8202(dd, 2H, pyrazole); 3.5643(s, 3H, Ar-OCH_3); 3.2013(t, 1H, pyrazole). Mass (LC-MS):m/z 504.17(M); 505.32(M+1); 506.21(M + 2).

3.1.7. Compound.4g:(Z)-N-(4-chloropheny)-1-[5-(4-chlorophenyl)-1-phenyl-4,5-dihydro -1H-pyrazole-3-yl]- 1H-indol-3yl]methanimine

3003(-CH *Str*, Ar-H); 2914, 2876(-CH *Str,* Aliphatic); 1623(-CN *Str*, Imine); 1523(-C=CH *Str*, Ar), 1465(-C=C *Str*, Ar); 1341(-C-N *Str*); 830(C-Cl *Str*, Ar-Cl). ^{1}HNMR (DMSO) δ ppm: 9.5765(s, 1H, Imine proton); 8.4563-8.3902(d, 2H, Ar-H); 8.1203-8.0034(d, 2H, Ar-H); 7.9654-7.8334(d, 2H, Ar-H); 7.7643-7.6754(t, 2H, Ar-H); 7.4533-7.3894(d, 2H, Ar-H); 7.2983-7.1892(d, 2H, Ar-H); 6.9843-6.9003(d, 2H, Ar-H); 6.8943-6.8102(t, 3H, Ar-H); 6.7833(s, 1H, Ar-H); 3.7873-3.6843(dd, 2H, pyrazole); 3.1982(t, 1H, pyrazole). Mass (LC-MS):m/z 508.12(M); 509.03 (M+1); 510.34(M + 2).

3.1.8. Compound 4h: (Z)-N-(4-chloropheny)-1-[5-(4-methylphenyl)-1-phenyl-4,5-dihydro -1H-pyrazole-3-yl]- 1H-indol-3yl]methanimine

3028(-CH *Str*, Ar-H); 2927, 2805(-CH *Str,* Aliphatic); 1619(-CN *Str*, Imine); 1590(-C=CH *Str*, Ar), 1415-C=C *Str*, Ar); 1252(-C-N *Str*); 794(C-Cl *Str*, Ar-Cl). ^{1}HNMR (DMSO) δ ppm: 9.3827(s, 1H, Imine proton); 8.3762-8.2903(d, 2H, Ar-H); 8.2102-8.2002(d, 2H, Ar-H); 7.9832-7.9024(d, 2H, Ar-H); 7.8963-7.7833(t, 2H, Ar-H); 7.7543-7.6743(d, 2H, Ar-H); 7.5643-7.4563(d, 2H, Ar-H); 7.3452-7.2432(d, 2H, Ar-H); 7.1972-7.0432(t, 3H, Ar-H); 6.9982(s, 1H, Ar-H); 3.9833-3.8943(dd, 2H, pyrazole); 3.3422(t, 1H, pyrazole); 2.0192(s, 3H, Ar-CH_3). Mass (LC-MS):m/z 488.18(M); 489.21(M+1); 490.02(M + 2).

3.1.9. Compound 4i: Z)-N-(4-chloropheny)-1-[5-(4-fluorophenyl)-1-phenyl-4,5-dihydro -1H-pyrazole-3-yl]- 1H-indol-3yl]methanimine

3103(-CH *Str*, Ar-H); 2927, 2876(-CH *Str,* Aliphatic); 1609(-CN *Str*, Imine); 1523(-C=CH *Str*, Ar), 1432(-C=C *Str*, Ar); 1243(-C-N *Str*); 802(C-Cl *Str*, Ar-Cl). ^{1}HNMR (DMSO) δ ppm: 9.8743(s, 1H, Imine proton); 8.3998(d, 2H, Ar-H); 8.3882-8.2998(d, 2H, Ar-H); 8.1654(d, 2H, Ar-H); 7.9854-7.9003(d, 2H, Ar-H); 7.8045-7.7998(d, 2H, Ar-H); 7.6754-7.6584(d, 2H, Ar-H); 7.5784-7.5654(d, 2H, Ar-H); 7.4998-7.4894(t, 3H, Ar-H); 7.1784-7.1564(t, 2H, Ar-H); 3.8365-3.8003(dd, 2H, pyrazole); 3.1325(t, 1H, pyrazole). Mass (LC-MS):m/z 492.15(M); 493.23(M+1); 494.09 (M + 2).

3.1.10. Compound 4j:(Z)-N-pheny-1-[5-(4-nitrophenyl)-1-phenyl-4,5-dihydro -1H-pyrazole -3-yl]-1H-indol-3yl]methanimine

3098(-CH *Str*, Ar-H); 2965, 2898(-CH *Str,* Aliphatic); 1643(-NO_2 Str, Ar-NO_2); 1604(-CN *Str*, Imine); 1423(-C=CH *Str*, Ar), 1398(-C=C *Str*, Ar); 1102(-C-N *Str*). ^{1}HNMR (DMSO) δ ppm: 9.6563(s, 1H, Imine proton); 8.3423-8.2832(d, 2H, Ar-H); 8.1933-

8.1002(d, 2H, Ar-H); 7.9874(s, 1H, Ar-H); 7.8234-7.8043(d, 2H, Ar-H); 7.6744-7.5432(d, 2H, Ar-H); 7.4873-7.3094(t, 2H, Ar-H); 7.2834-7.2032(d, 2H, Ar-H); 7.1832-7.0983(d, 2H, Ar-H); 6.9865-6.8972(t, 3H, Ar-H); 6.8755-6.8032(d, 2H, Ar-H); 3.8972-3.8102(dd, 2H, pyrazole); 3.2832(t, 1H, pyrazole). Mass (LC-MS):m/z 485.19(M); 486.21 (M+1); 487.34 (M + 2).

3.1.11. Compound 4k: (Z)-N-(4-chloropheny)-1-[5-(4-methylphenyl)-1-phenyl-4,5-dihydro - 1H-pyrazole-3-yl]- 1H-indol-3yl] methanimine

3043(-CH *Str*, Ar-H); 2998, 2854(-CH *Str,* Aliphatic); 1614(-CN *Str*, Imine); 1504(-C=CH *Str*, Ar), 1411(-C=C *Str*, Ar); 1198(-C-N *Str*); 799(C-Cl *Str*, Ar-Cl). ^{1}HNMR (DMSO) δ ppm: 9.6543(s, 1H, Imine proton); 8.2738-8.2002(d, 2H, Ar-H); 8.1832-8.0322(d, 2H, Ar-H); 7.9983(d, 2H, Ar-H); 7.8734-7.8302(d, 2H, Ar-H); 7.7833-7.6873(d, 2H, Ar-H); 7.5643-7.4534(d, 2H, Ar-H); 7.3984-7.3021(d, 2H, Ar-H); 7.2983-7.2092(t, 3H, Ar-H); 7.1902-7.0933(t, 2H, Ar-H); 3.7833-3.7231(dd, 2H, pyrazole); 3.3452 (t, 1H, pyrazole); 2-1983(s, 3H, Ar-CH_3). Mass (LC-MS):m/z 488.18(M); 489.32(M+1); 490.02(M + 2).

3.1.12. Compound 4l: (Z)-N-(4-chloropheny)-1-[5-(4-methoxyphenyl)-1-phenyl-4,5-dihydro - 1H-pyrazole-3-yl]- 1H-indol-3yl] methanimine

3102(-CH *Str*, Ar-H); 2976, 2877(-CH *Str,* Aliphatic); 1621(-CN *Str*, Imine); 1521(-C=CH *Str*, Ar), 1406(-C=C *Str*, Ar); 1167(-C-N *Str*); 802(C-Cl *Str*, Ar-Cl). ^{1}HNMR (DMSO) δ ppm: 9.8763(s, 1H, Imine proton); 8.4834-8.3465(d, 2H, Ar-H); 8.2534-8.1982(d, 2H, Ar-H); 8.0322(d, 2H, Ar-H); 7.976-7.9102(d, 2H, Ar-H); 7.8762-7.7897(d, 2H, Ar-H); 7.6754-7.5633(d, 2H, Ar-H); 7.4323-7.4021(d, 2H, Ar-H); 7.3523-7.2093(t, 3H, Ar-H); 7.1002-7.0068(t, 2H, Ar-H); 3.6673-3.5432(dd, 2H, pyrazole); 3.5002(s, 3H, Ar-OCH_3) 3.2983(t, 1H, pyrazole);. Mass (LC-MS):m/z 504.17(M); 505.32(M+1); 506.12(M + 2).

Table 1: Acute anti-inflammatory activity of the test compounds against carrageenan induced acute rat paw oedema model

Group	Treat Ment	Dose mg/kg	Paw oedema volume				
			0 hr	1 hr	2 hr	3 hr	4hr
1	Control	10	0.76±0.23	0.82±0.10	1.23±0.021	1.9±0.232	2.10±0.321
2	Diclofenac	20	0.32±0.03	0.27±0.28	0.21±0.21	0.13±0.03	0.12±0.10
3	4a	100	0.42±0.12	0.38±0.27	0.32±0.21	0.29±0.32	0.20±1.23
4	4b	100	0.49±1.93	0.34±0.54	0.33±0.43	0.28±0.39	0mg/kgm19±0.43
5	4c	100	**0.34±0.20***	**0.28±1.04**	**0.26±1.231**	**0.16±0.32**	**0.15±0.04**
6	4d	100	0.52±0.92	0.43±0.43	0.40±0.22	0.31±1.03	0.28±1.04
7	4e	100	0.44±1.02	0.44±1.32	0.34±0.43	0.28±1.04	0.26±0.43
8	4f	100	**0.36±0.01**	**0.30±1.03**	**0.24±0.02**	**0.18±0.21**	**0.14±0.12**
9	4g	100	0.51±0.33	0.43±0.43	0.37±1.92	0.32±0.43	0.25±0.43
10	4h	100	0.43±0.26	0.35±0.56	0.31±0.43	0.28±1.02	0.21±0.43
11	4i	100	**0.34±0.43**	**0.30±0.54**	**0.25±0.43**	**0.16±1.94**	**0.16±1.04**
12	4j	100	0.39±1.32	0.36±0.43	0.30±2.03	0.21±0.32	0.19±0.68
13	4k	100	**0.35±0.43**	**0.28±1.02**	**0.26±0.32**	**0.19±1.23**	**0.15±0.12**
14	4l	100	0.56±0.32	0.48±0.87	0.40±0.43	0.34±0.56	0.28±0.65

*Data analyzed by one way ANOVA followed by Dunnett's test (n=6). *p<0.05 a*

3.2. *In vivo* Anti-Inflammatory activity

The results for the anti-inflammatory activity of the synthesized compounds 4a-4l were evaluated by carragenan induced rat paw edema method in albino rats using diclofenac as a reference standard. The rat paw volume was measured using plethysmograph and percent inhibition of paw volume was calculated for each test compounds depicted in the table 1. The paw volume is significantly decreased ($p<0.05$) at different time intervals as compared to the control. The test compounds 4a-4l were evaluated and among the compounds compound 4l exhibited the highest % inhibition of 98.4% when compared with the diclofenac (94.2%).

3.3. Evaluation of *in vitro* anticancer activity

The cytotoxicity of six of the synthesized compounds was evaluated against human breast cancer cell lines MCF7 and SKO3 ovarian cancer cell lines using MTT assay with Doxorubicin as reference standard, IC_{50} values were calculated. Doxorubicin exhibited IC_{50} of 12.21µg against MCF cell lines and 15.14 µg against SKOV3 cell lines. All the results were depicted in the table 3 and from the results it was observed that both the cell lines were susceptible to the test compounds. IC_{50} values of the test compounds against MCF-7 cell lines were in the range of 20.01-60.99µg and 32.87-78.43µg. Compound 4f exhibited the lowest IC_{50} at concentration of 20.01µg against MCF7 cell lines and 32.87µg against SKOV3 cell lines.

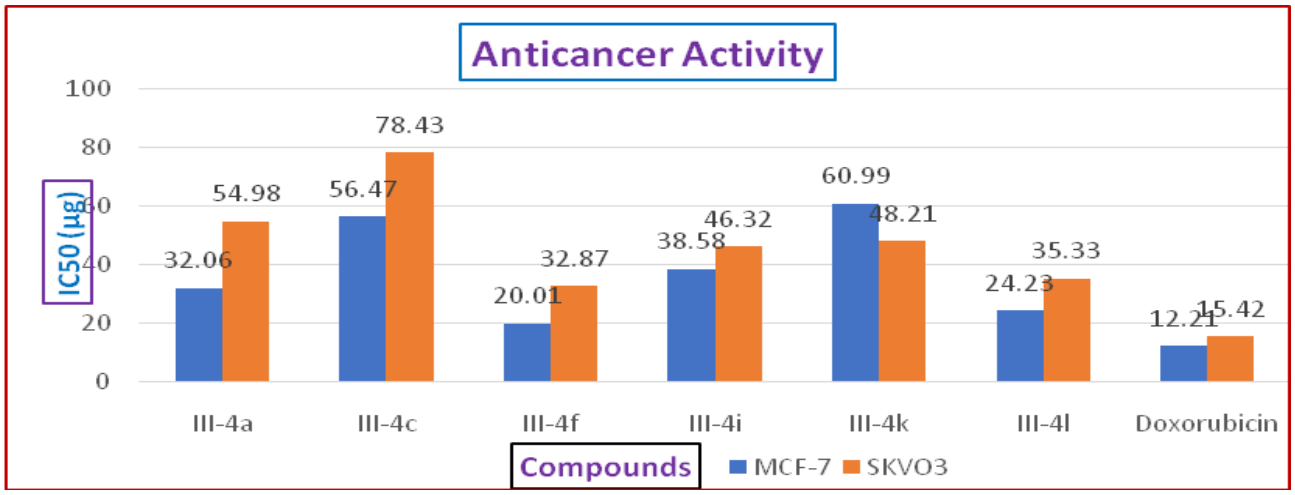

Fig. 2: Graphical representation of novel compunds III-4(a-l) on MCF-7and SKOV3 Cells

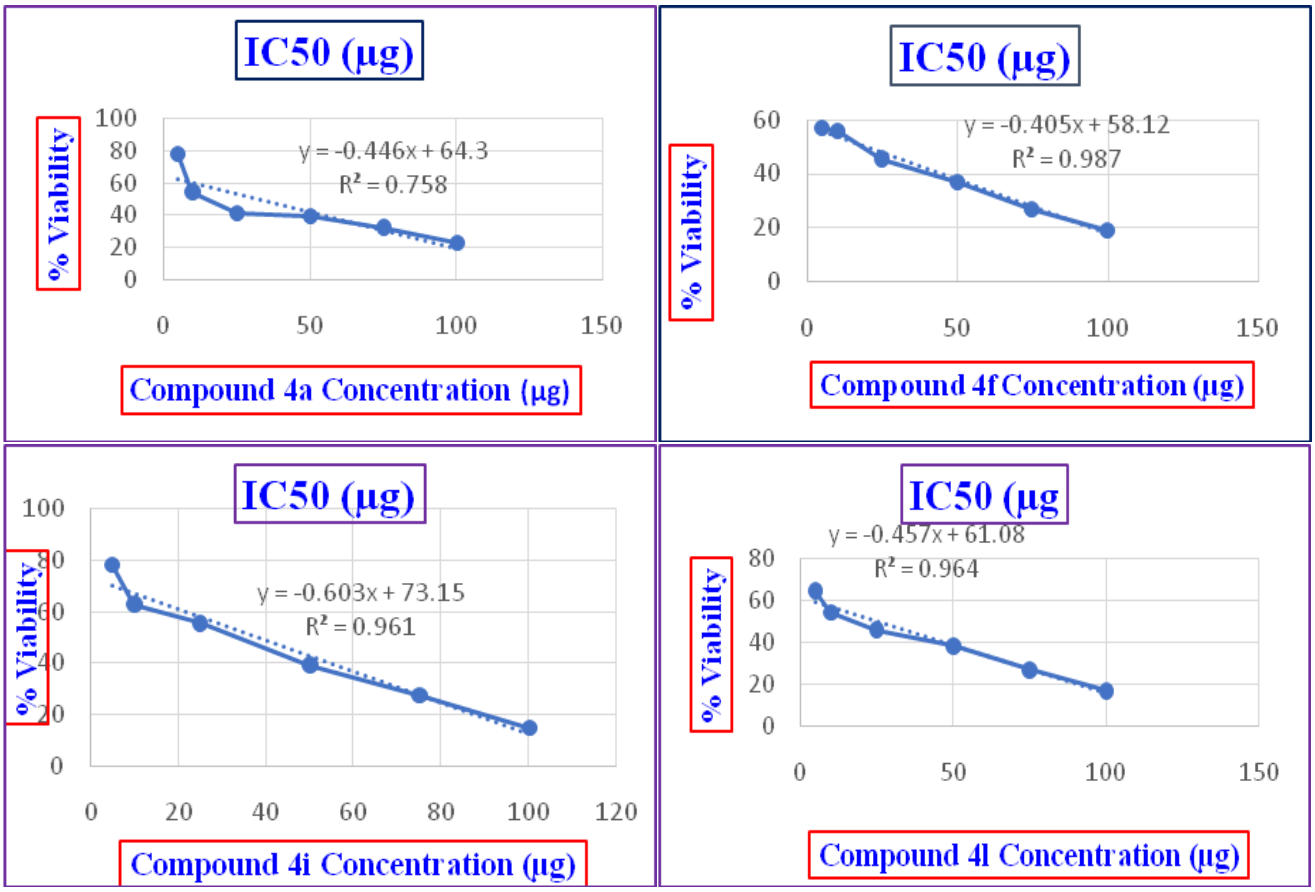

Fig. 3: Graphical representation of indole assimilated pyrazoline -IC_{50} values

Table 2: Acute Anti-inflammatory activity of the test compounds against carrageenan induced acute rat paw oedema model expressed as percent of inhibition of oedema formation at time in 4 hrs

Group	Compound	Dose (mg/kg)	% inhibition
1	Control	10	100
2	Diclofenac	20	94.2
3	4a	100	90.5
4	4b	100	90.1
5	4c	100	**92.9**
6	4d	100	86.7
7	4e	100	87.6
8	4f	100	**93.3**
9	4g	100	88.1
10	4h	100	90
11	4i	100	92.4
12	4j	100	90.9
13	4h	100	**92.9**
14	4l	100	**98.6**

3.4. Molecular Docking

Molecular docking studies were performed in order to find the possible protein ligand interactions of the dataset ligands. Additionally, these also assisted in identifying the conformational changes of the ligand in the protein environment. About 100 different protein-ligand complex conformations for each docked complex were generated through Glide XP module; the confirmation with highest EModel energy was only displayed in the result. Glide dock sores of the dataset ligands were shown in Table 4 along with the interaction amino acids and number of amino acids. Among the docked compounds, dock score of the compounds ranged from 4a-4l from -3.186 to -5.212. The highest score was exhibited by 4f with -5.212 with Glide binding energy of -34.697 Kcal/mol.

Table 3: Anticancer activity of compounds III-4(a-l) on MCF7 and SKOV3 Cell lines

Sample Description	Test Parameters IC_{50} (µg)	
	MCF 7	SKVO3
4a	**32.06**	54.98
4c	56.47	78.43
4f	**20.01**	**32.87**
4i	38.58	46.32
4k	60.99	48.21
4l	**24.23**	**35.33**
Doxorubicin	12.21	15.42

Table 4: Molecular Docking interactions with EGFR Kinase domain

Compound No	Dock Score XP G Score	No of Hydrogen bonds	Interacting aminoacids	H-bond lengths (Å)	Emodel energy	Glide Energy
4a	-4.293	0	-	-	-48.519	-34.943
4b	-3.203	1	LYS 721	2.04	-64.785	-42.057
4c	-4.28	0	-	-	-50.42	-47.844
4d	-4.409	0	-	-	-60.973	-38.624
4e	-3.857	0	-	-	-65.692	-47.713
4f	-5.212	1	MET 769	1.76	-59.978	-34.697
4g	-3.454	1	LYS 721	2.09	-68.043	-45.179
4h	-3.384	1	LYS 721	1.93	-66.964	-44.641
4i	-3.186	1	LYS 721	2.09	-68.806	-44.598
4j	-4.392	1	SER 696	2.80	-70.565	-51.958
4k	-3.497	1	LYS 721	2.07	-67.511	-44.032
4l	-3.981	0	-	-	-57.231	-39.499

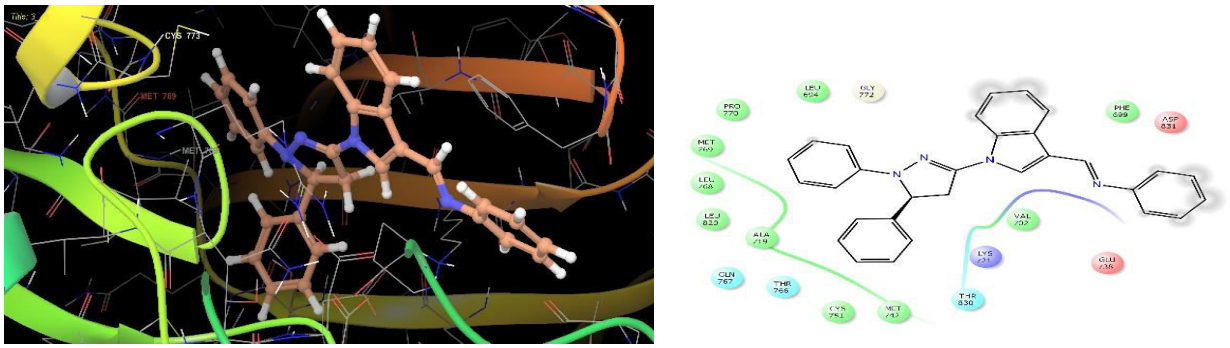

Fig. 4: Docking Pose between the Ligand and the Protein-compound-4a: dock-1 and dock-2

Fig. 5: Docking Pose between the Ligand and the Protein-compound-4d: dock-1 and dock-2

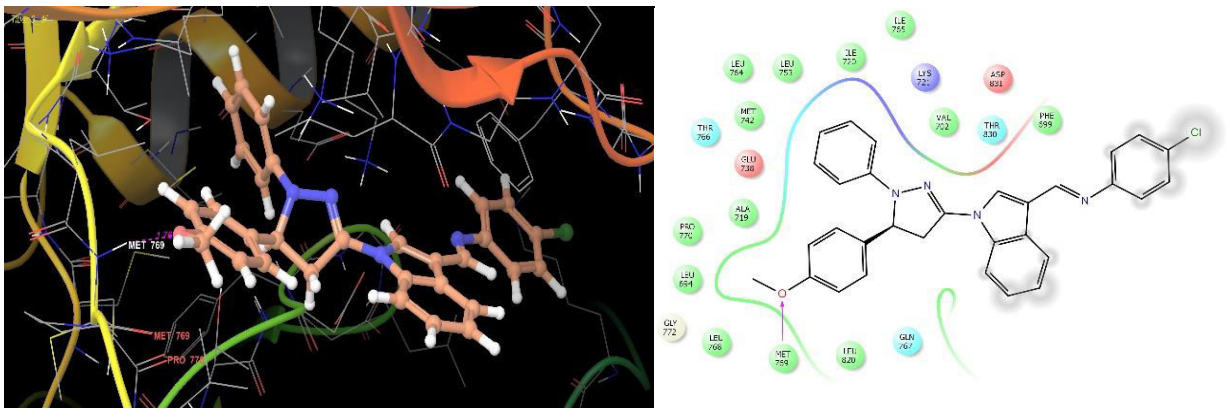

Fig. 6: Docking Pose between the Ligand and the Protein-compound-4f: dock-1 and dock-2

4. CONCLUSION

The study revealed the novel pyrazoline derivative (4a-4l) were synthesized successfully by conventional method. These structures are confirmed by FT-IR, 1H NMR and MASS spectral data. All the hybrids were evaluated for anti-inflammatory and anticancer activities. Most of the synthesized hybrids were found to have good anti-inflammatory activity, among all the active compounds of pyrazole derivative, compound 4l exhibited the highest % inhibition of 98.4 when compared with the standard Diclofenac 94.2%.Compound 4f exhibited the lowest IC_{50} at concentration of 20.01µg against MCF7 cell lines and 32.87µg against SKOV3 cell lines. The molecular docking studies were performed using the Ligprep tool of Schrodinger suite. This study revealed that novel thiazolidne-4-one-pyrazole hybrids (4a-4j) had good interaction with the active site of EGFR receptor.

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Conflict of interest

The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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None declared

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