Original Article

Synthesis, Characterization, Molecular Docking and Biological studies on Novel Pyazole fused Indole Derivatives.

Padmaja V[1]*, M. Sumakanth[2], P.Shashikala[3]

*[1,2]Department of Pharmaceutical Chemistry, RBVRR Women's College of Pharmacy, Hyderabad, Telangana.
[3]Department of Pharmacy, University College of Technology, Osmania University, Hyderabad, Telangana.
E-mail: pv.oct29@gmail.com

***Corresponding Author: -** Padmaja V

*Department of Pharmaceutical Chemistry, RBVRR Women's College of Pharmacy, Hyderabad, Telangana.
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Abstract

The present work deals with the sequence of Novel Pyrazole fused Indole derivatives**(4a-4l)** were synthesized by simple conventional method and screened for anthelmintic, antibacterial and anticancer activities. The molecular docking studies were also performed. All of the newly synthesized compounds were structurally characterized on the basis of IR, ^{1}HNMR and Mass spectral analysis. Further, all of the newly prepared pyrazole fused indole derivatives were screened for anthelmintic activity by using Albendazole as standard drug. The antibacterial activity was carried out by agar diffusion (Cup plate) method by using Streptomycin as standard and anticancer activity against MCF-7 cell lines by MTT assay method. The results showed that some of the compounds **4b, 4c** and **4f** exhibited good anticancer activity. The compounds **4c**, **4f**, **4j showed good** anthelmintic activity and **4b,4h** and **4i** exhibited potential antibacterial activity by comparing with standard drug. Additionally, the molecular docking studies of novel pyrazole fused Indole derivatives was also carried out to explain putative bonding interaction between the active site of EGFR enzyme and synthesized Novel Pyrazole fused Indole derivatives by Schrodinger suite.

Keywords: Isatin,5-methyl-3-amino pyrazole, Anthelmintic, Antibacterial and Anticancer Activities, MCF-7, Doxorubicin, Albendazole, EGFR and Schrodinger suite.

INTRODUCTION

Presently, different fused heterocyclic rings are being discovered exponentially due to their remarkable use in biomedical therapeutics[1]. Medicinal chemistry is a chemistry-based discipline, involving aspects of biological, medical and pharmaceutical science. It is concerned with the invention, discovery, design, identification and preparation of biologically active compounds, the interpretation of their mode of action at the molecular level and the construction of the relationship between chemical structure and pharmacological activity. In recent years, indole derivatives have acquired conspicuous significance due to their wide spectrum of biological activities[2].Indole-2-one derivatives are an important class of heterocycliccompounds known for their potential pharmaceutical applications like effective as antimicrobial[3],antischistosomal activity, antifungal, anti-inflammatory, antimalarial[4], antiviral, antidiabetic, and anticancer agents[5].The literature also revealed some fused heterocyclic rings with Indole-2,3-dione with different biological properties. Several methods for the synthesis of Indole-2-one derivatives have been developed, the most widely used being the Fisher indole synthesis, which uses phenyl hydrazine followed by rearrangement reaction.

The Insilco approach have become a decisive part for determining the molecular properties, bioavailability and identification of targets of designed compounds. The Computer-aided drug design is a universally used term that represents computational tools, enables the development, modification and optimization of design process and Molecular docking studies has been acknowledged with significant attention among all virtual screening methods.

In this surveillance, according to reported literature Indole nucleus were designed and evaluated for Potentially active compounds by predicting the dock score by using the Insilco molecular modelling tool Schrödinger software[7]. As per the data collected from this software of all Novel pyrazole fused Indole derivatives has examined thoroughly and most Potent compounds are selected. Thus, in our present work, predicted potent molecules are synthesized and screened for anthelminthic, antibacterial and anticancer activities.

MATERIAL AND METHODS

The 2D structures of the novel pyrazole fused Indole derivatives (4a-4l) **5-Substituted-3-((5-methyl-1H-pyrazol-3-yl)imino)-1-(4-substituted benzyl) indolin-2-one** were converted to 3D using potential algorithms and application of high-efficient force fields. Initial geometrical optimization and energy minimization of molecules were performed by using the Ligprep tool of Schrodinger suite. The whole structure was minimized using OPLS-2005 force field using Protein Preparation Wizard tool of Schrodinger Suite. All chemicals and reagents used for synthesis are LR grade, which

were procured from Sigma-Aldrich, Merck, SD fine and Avra company and solvents of LR grade were used and were purified before use.The FT-IR(Fourier-transform infrared spectroscopy absorption spectra were obtained in the range 4000-400 cm-1 on Alpha Bruker FT-IR instrument, the ^{1}H-NMR spectra were recorded on BrukerUx-NMR instrument and the samples was made in $CDCl_3$ using tetra methyl silane (Me4Si) as the internal standard and chemical shifts were expressed in parts per million (PPM). The Mass spectra have been recorded on SHIMADZU spectrometer using chemical ionization technique and the melting point (MP) of the all the newly synthesized pyrazole fused indole derivatives were recorded on Thieles tube method by using liquid Paraffin as a solvent.

EXPERIMENTAL PROCEDURE.

Step: I. Synthesis of nitrosoacetanilides from substituted anilines: 9 gm of Chloral hydrate was taken into the round bottom flask and dissolved in 120 ml water. To that 13 gm of sodium sulphate, a solution of 5.4 gm of substituted aniline in 30 ml of water containing 5.12 gm of concentrated hydrochloric acid (4.34 ml) to dissolve the amine and solution of 11 gm of hydroxylamine hydrochloride in 50 ml of water were added. Flask was heated vigorously and the completion of reaction was monitered by TLC. After it, the solution containing beaker was cooled in running water followed by the filtration of reminder crystallized product with suction pump and air dried[8].

Step: II. Synthesis of substituted Isatin from nitrosoacetanilides (2a-2f): 18.4 gm of concentrated sulphuric acid (10.0 ml) was warmed to 50^{0}C and 2.5 gm of dry nitrosoacetanilide was added in such a rate so as to keep the temperature between 60-70^{0}C but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly after the addition of isonitroso compound was finished. The solution was heated to 80^{0}C and kept at this temperature for about 10 min to complete the reaction. Then the reaction mixture was cooled to room temperature and poured it into ten times its volume of cracked ice. After standing for 90 min, the final product was filtered with suction pump followed by washing with cold water to remove excess of sulphuric acid and dried in air[9].

Step: III. N-Benzyl indole 2, 3- dione (N-(4-Substituted-Benzyl Isatin) (3a-3l): In the round bottomed flask take substituted indole-2,3-dione (substituted Isatin) 0.8gm (3.37mM) and equimolar quantity of benzyl chloride (benzyl, 4-methyl benzyl) i.e. 6.5ml (3.37mM), mix with 20ml of DMF and to this mixture add 2gm of K_2CO_3. After gentle mixing of this reaction mixture, reflux for 2 hr, cool and pour to 100 ml of ice cold water. The resultant orange red ppt. collected wash with water and dried and recrystallized from acetonitrile[10].

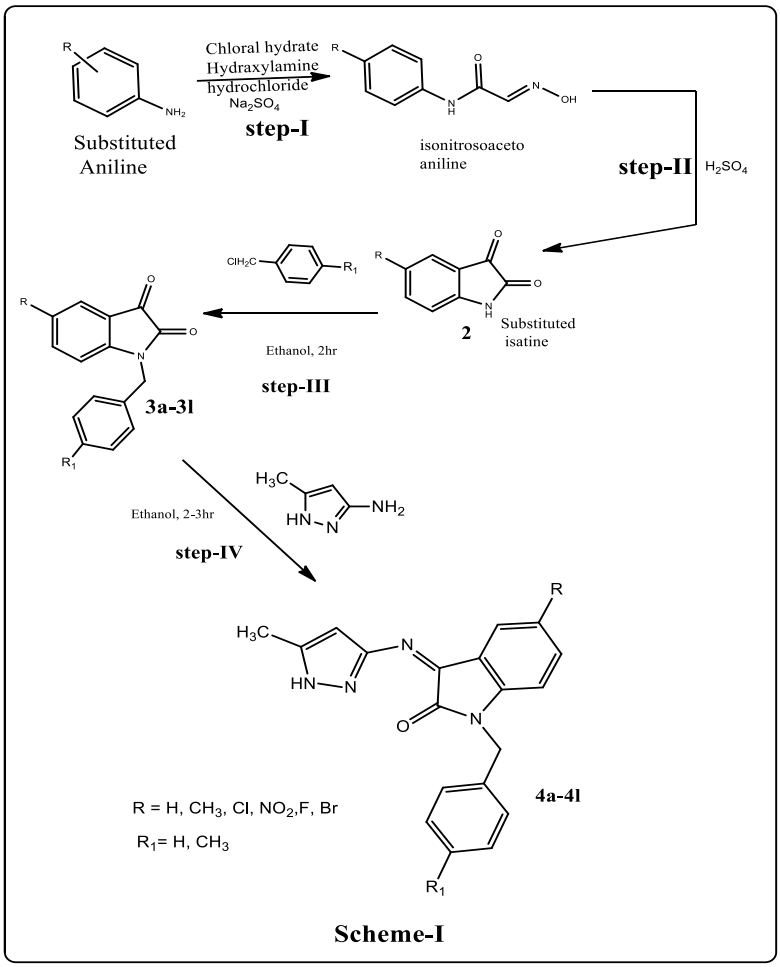

Fig.No.1. Scheme-I Synthesis of 5-Substituted-3-((5-methyl-1H-pyrazol-3- yl)imino)-1-(4-substituted benzyl) indolin-2-one (4a-4l).

Step: IV. 5-Substituted-3-((5-methyl-1H-pyrazol-3-yl)imino)-1-(4-substituted benzyl) indolin-2-one (4a-4l). Compound 3a-3l (0.01 mol) was taken in a mixture of 5-methyl-3-amino pyrazole (0.01 mol) and glacial acetic acid (5 ml) and Ethanol 30ml, then the reaction mixture was refluxing for 2hrs. The progress of the reaction was monitored by TLC (n-Hexane: EthylAcetate 8:2). The reaction mixture was cooled to room temperature. A solid was obtained, which was filtered off and washed with hexane and recrystallized from methanol to give crystalline solid[11].

BIOLOGICAL ACTIVITIES.

***Anthelmintic activity*[12]:** Anthelmintic activity screening of the pyrazole fused novel Indole derivatives was done at 0.1%, 0.2%, 0.5% by using Indian earth warms. The Albendazole was used as the standard drug, the paralysis and death time was recorded in min (Table 2)

***Antibacterial activity*[13]:** All the synthesized compounds are screened for antibacterial activity was performed by cup plate method (Agar diffusion technique). The standard drug and all synthesized derivatives (4a–4l) were dissolved and adjusted and made up the volume with distilled water to get 100µg/ml concentration. The Streptomycin used as a standard drug against *Staphylococcus aureus*, *Bacillus subtilis* (Gram positive) and *Escherichia coli*, *Salmonella paratyphi*, *Pseudomonas* (Gram negative) and finally to measure the zone of inhibition. (Table 3).

***Anticancer activity*[14]:** Evaluation of the viability was carried out by the MTT assay with three independent experiments against human breast cancer cells (MCF7) with six concentrations of Novel Pyrazole fused Indole derivatives in triplicates and the doxorubicin as a standard drug. The percentage growth inhibition was calculated by using standard formula and concentration of test drug needed to inhibit cancer cell growth by 50 % values (IC_{50}) was generated from the dose- response curves for cell line using origin software and all the results are mentioned in (Table 4).

***Molecular Docking Studies*[15]:** All the synthesized pyrazole fused Novel Indole derivatives are docked into active site of the EFGR was retrieved from the Protein databank website with PDB Id: 1M17 by using the Ligprep tool of Schrodinger suite and consequently to rationalize the obtained pharmacological data. Furthermore, structurally optimized protein shape was once used to observe protein-ligand interactions of the dataset ligands by the use of Glide Xp docking protocol. Initially, a 3D grid used to be set up to the binding pocket (active site) of the protein, into which all the dataset ligands had been docked into. Binding interactions have been calculated effectively in phrases of Glide Score and it is a mixture of hydrophilic, hydrophobic, metal binding groups, Vander Waals energy, freezing rotatable bonds and polar interactions with receptor.

RESULTS.

The Physical properties of the synthesized Novel pyrazole fused Indole derivatives are mention in table no.1

Table.No.1. Physical Data(4a-4l)
(TLC Profile: n-Hexane: Ethyl acetate (8:2))

Compound	Molecular Formula	R_1	R	Molecular Weight (gms)	M.P (^{0}C)	%Yield
4a	$C_{19}H_{16}N_4O$	H	H	316.13	206-208	73
4b	$C_{20}H_{18}N_4O$	H	CH_3	330.15	213-215	68
4c	$C_{19}H_{15}N_4ClO$	H	Cl	350.09	235-237	77
4d	$C_{19}H_{15}N_5O_3$	H	NO_2	361.12	243-245	80
4e	$C_{19}H_{15}N_4OF$	H	F	334.12	215-217	76
4f	$C_{19}H_{13}N_4OBr$	H	Br	394.04	177-179	70
4g	$C_{21}H_{20}N_4O$	CH_3	CH_3	344.16	227-229	69
4h	$C_{20}H_{18}N_4O$	CH_3	H	330.15	163-165	76
4i	$C_{20}H_{17}N_4ClO$	CH_3	Cl	364.11	205-207	71
4j	$C_{20}H_{17}N_5O_3$	CH_3	NO_2	375.13	211-214	66
4k	$C_{20}H_{17}N_4FO$	CH_3	F	348.14	>270	75
4l	$C_{20}H_{17}N_4BrO$	CH_3	Br	408.16	231-233	83

SPECTRAL DATA.

Compound.4a:1-benzyl-3-((5-methyl-1H-pyrazol-3-yl)imino)indolin-2-one: 3204(NH *Str*, Pyrazole), 3059(C-H *Str*, Ar), 2912, 2887(C–H *Str*, Aliphatic), 1703(C=O *Str*, Indole), 1616(-C=N*Str*), 1536(C=CH *Str*), 1344(C=C *Str*, Ar), 1046(C-N *Str*).^{1}H-NMR (DMSO) $\delta\delta$ ppm: 12.3839(s, 1H, -NH in pyrazole ring), 9.6338(s, 2H, Benzyl), 8.4438(s, 1H, pyrazole), 8.1677-8.0544(d, 2H, Ar-H), 7.9696-7.5096(d, 2H, Ar-H), 7.4961-7.4490(t, 2H, Ar-H), 7.4309-7.3959(t, 3H, Ar-H), 1.9809(s,3H, -CH_3). Mass (LC-MS): m/z 316.13(M), 317.32(M + 1, 100%).

Compound.4b: 1-benzyl-5-methyl-3-((5-methyl-1H-pyrazol-3-yl)imino)indolin-2-one. 3285(NH *Str*, Pyrazole), 3082(C-H *Str*, Ar), 2914, 2873(C–H *Str*, Aliphatic), 1700(C=O *Str*, Indole), 1656(-C=N*Str*), 1548(C=CH *Str*), 1358(C=C *Str*, Ar), 1024(C-N *Str*).^{1}H-NMR (DMSO) $\delta\delta$ ppm: 10.5930(s, 1H, -NH in pyrazole ring), 9.3006(s, 2H, Benzyl), 7.8019-7.7830(s, 1H, pyrazole), 7.6533-7.5200(d, 2H, Ar-H), 7.4895-7.4338(d, 2H, Ar-H), 7.3957-7.3895(t, 3H, Ar-H),2.1638-2.1603(s,6H, -CH_3). Mass (LC-MS): m/z 330.15(M), 331.52(M + 1, 100%).

Compound.4c:5-chloro-3-((5-methyl-1H-pyrazol-3-yl)imino)-1-(benzyl)indolin-2-one. 3249(NH *Str*, Pyrazole), 3068(C-H *Str*, Ar), 2937, 2802(C–H *Str*, Aliphatic), 1701(C=O *Str*, Indole), 1657(-C=N *Str*), 1444(C=CH *Str*), 1249(C=C *Str*, Ar), 1153(C-N *Str*), 892(C-Cl*Str*).^{1}H-NMR (DMSO) $\delta\delta$ ppm: 10.1312(s, 1H, -NH in pyrazole ring), 9.0406-9.0401(s, 2H, Benzyl), 8.3529(s, 1H, pyrazole), 8.0673-8.0477(s, 1H, Ar-H), 7.9452-7.9201(d, 2H, Ar-H), 7.8287-7.7573(d, 2H, Ar-H), 7.1731-6.8618(t, 3H, Ar-H), 2.0416(s,3H, -CH_3). Mass (LC-MS): m/z 350.09(M), 351.21(M + 1, 100%), 352.16(M + 2, 30%).

Compound.4d: 5-chloro-3-((5-nitro-1H-pyrazol-3-yl)imino)-1-(benzyl)indolin-2-one. 3225(NH *Str*, Pyrazole), 3022(C-H *Str*, Ar), 2991, 2842(C–H *Str*, Aliphatic), 1713(C=O *Str*, Indole), 1636(NO_2 Str Ar-NO_2), 1591(-C=N *Str*), 1542(C=CH *Str*), 1225(C=C *Str*, Ar), 1189(C-N *Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 11.0987(s, 1H, -NH in pyrazole ring), 9.8928-9.8913(s, 2H, Benzyl), 7.9720-7.9005(s, 1H, pyrazole), 7.8771(s, 1H, Ar-H), 7.8487-7.7813(d, 2H, Ar-H), 7.6963-7.6068(d, 2H, Ar-H), 7.5913-7.4883(t, 3H, Ar-H), 2.0281(s,3H, -CH_3). Mass (LC-MS): m/z 361.12(M), 362.31(M + 1, 100%).

Compound.4e: 5-Fluoro-3-((5-methyl-1H-pyrazol-3-yl)imino)-1-(benzyl)indolin-2-one. 3252(NH *Str*, Pyrazole), 3082(C-H *Str*, Ar), 2903, 2837(C–H *Str*, Aliphatic), 1701(C=O *Str*, Indole), 1616(-C=N *Str*), 1405(C=CH *Str*), 1252(C=C *Str*, Ar), 1119(C-N *Str*), 794(C-F*Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 11.1131(s, 1H, -NH in pyrazole ring), 9.8913(s, 2H, Benzyl), 8.3771(s, 1H, pyrazole), 7.8991-7.8458(s, 1H, Ar-H), 7.7951-7.7808(d, 2H, Ar-H), 7.6991-7.6806(d, 2H, Ar-H), 7.5949-7.4883(t, 3H, Ar-H), 2.1123(s,3H, -CH_3). Mass (LC-MS): m/z 334.12(M), 335.26(M + 1, 100%), 336.19(M + 2, 30%).

Compound.4f: 5-bromo-3-((5-methyl-1H-pyrazol-3-yl)imino)-1-(benzyl)indolin-2-one. 3243(NH *Str*, Pyrazole), 3067(C-H *Str*, Ar), 2998, 2889(C–H *Str*, Aliphatic), 1712(C=O *Str*, Indole), 1623(-C=N *Str*), 1502(C=CH *Str*), 1276(C=C *Str*, Ar), 1089(C-N *Str*), 789(C-Br*Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 12.0932(s, 1H, -NH in pyrazole ring), 9.9093(s, 2H, Benzyl), 8.5642(s, 1H, pyrazole), 7.9083-7.8123(s, 1H, Ar-H), 7.6894-7.5832(d, 2H, Ar-H), 7.4852-7.3092(d, 2H, Ar-H), 7.2883-7.1093(t, 3H, Ar-H), 2.0543(s,3H, -CH_3). Mass (LC-MS): m/z 394.03(M), 395.12(M + 1, 100%), 396.03(M + 2, 30%).

Compound.4g:5-methyl-3-((5-methyl-1H-pyrazol-3-yl)imino)-1-(4-methylbenzyl)indolin-2-one. 3289(NH *Str*, Pyrazole), 3088(C-H *Str*, Ar), 2983, 2857, 2798(C–H *Str*, Aliphatic), 1719(C=O *Str*, Indole), 1634(-C=N *Str*), 1522(C=CH *Str*), 1289(C=C *Str*, Ar), 1103(C-N *Str*).^{1}H-NMR (DMSO) $\delta\delta$ ppm: 11.0932(s, 1H, -NH in pyrazole ring), 9.5986-9.4894(s, 2H, Benzyl), 8.4973(s, 1H, pyrazole), 8.2034-8.0832(d, 2H, Ar-H), 7.9094-7.8023(d, 2H, Ar-H), 7.7896-7.6980(t, 2H, Ar-H), 7.4785-7.3933(d, 2H, Ar-H), 2.1093-2.0034(s,6H, -CH_3). Mass (LC-MS): m/z 344.16(M), 345.32(M + 1, 100%).

Compound.4h:3-((5-methyl-1H-pyrazol-3-yl)imino)-1-(4-methylbenzyl)indolin-2-one. 3354(NH *Str*, Pyrazole), 3029(C-H *Str*, Ar), 2978, 2887, 2789(C–H *Str*, Aliphatic), 1706(C=O *Str*, Indole), 1634(-C=N *Str*), 1529(C=CH *Str*), 1288(C=C *Str*, Ar), 1099(C-N *Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 11.9064(s, 1H, -NH in pyrazole ring), 9.6753-9.4567(s, 2H, Benzyl), 8.3421(s, 1H, pyrazole), 8.1083-8.0522(s, 1H, Ar-H), 7.9743-7.8211(d, 2H, Ar-H), 7.6754-7.4534(d, 2H, Ar-H), 7.2091-7.1127(t, 3H, Ar-H), 2.2903-2.0943(s,9H, -CH_3). Mass (LC-MS): m/z 330.15(M), 331.28(M + 1, 100%).

Compound.4i:5-chloro-3-((5-methyl-1H-pyrazol-3-yl)imino)-1-(4-methylbenzyl)indolin-2-one. 3290(NH *Str*, Pyrazole), 3081(C-H *Str*, Ar), 2992, 2878, 2793(C–H *Str*, Aliphatic), 1710(C=O *Str*, Indole), 1621(-C=N *Str*), 1534(C=CH *Str*), 1292(C=C *Str*, Ar), 1103(C-N *Str*), 8023(C-Cl*Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 12.0932(s, 1H, -NH in pyrazole ring), 9.3674-9.1902(s, 2H, Benzyl), 8.4532(s, 1H, pyrazole), 8.2093-8.1652(s, 1H, Ar-H), 7.8932-7.7903(d, 2H, Ar-H), 7.5893-7.3903(d, 2H, Ar-H), 7.2091-7.0883(d, 2H, Ar-H), 2.1903-2.0032(s, 6H, -CH_3). Mass (LC-MS): m/z 364.11(M), 365.21(M + 1, 100%), 366.09(M + 2, 30%).

Compound.4j:5-nitro-3-((5-methyl-1H-pyrazol-3-yl)imino)-1-(4-methylbenzyl)indolin-2-one. 3304(NH *Str*, Pyrazole), 3077(C-H *Str*, Ar), 2978, 2893, 2783(C–H *Str*, Aliphatic), 1721(C=O *Str*, Indole), 1654(C-NO_2 *Str*), 1632(-

C=N *Str*), 1554(C=CH *Str*), 1309(C=C *Str*, Ar), 1093(C-N *Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 11.9043(s, 1H, -NH in pyrazole ring), 9.6743-9.57765(s, 2H, Benzyl), 8.4532(s, 1H, pyrazole), 8.3045-8.2743(s, 1H, Ar-H), 7.9983-7.8754(d, 2H, Ar-H), 7.7643-7.7042(d, 2H, Ar-H), 7.1032-7.1002(d, 2H, Ar-H), 1.9332-1.9083(s, 6H, -CH_3). Mass (LC-MS): m/z 375.13(M), 376.09(M + 1, 100%).

Compound.4k:5-fluoro-3-((5-methyl-1H-pyrazol-3-yl)imino)-1-(4-methylbenzyl)indolin-2-one. 3304(NH *Str*, Pyrazole), 3095(C-H *Str*, Ar), 2984, 2890, 2788(C–H *Str*, Aliphatic), 1705(C=O *Str*, Indole), 1623(-C=N *Str*), 1554(C=CH *Str*), 1309(C=C *Str*, Ar), 1093(C-N *Str*), 802(C-F*Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 12.1282(s, 1H, -NH in pyrazole ring), 9.5883(s, 2H, Benzyl), 8.3664(s, 1H, pyrazole), 8.2093-8.2003(s, 1H, Ar-H), 7.9854-7.9003(d, 2H, Ar-H), 7.6023-7.5234(d, 2H, Ar-H), 7.3094-7.2093(d, 2H, Ar-H), 2.1043-2.0023(s, 6H, -CH_3). Mass (LC-MS): m/z 348.14(M), 349.34(M + 1, 100%), 350.18(M + 2, 30%).

Compound.4l:5-bromo-3-((5-methyl-1H-pyrazol-3-yl)imino)-1-(4-methylbenzyl)indolin-2-one. 3328(NH *Str*, Pyrazole), 3091(C-H *Str*, Ar), 2999, 2879, 2782(C–H *Str*, Aliphatic), 1715(C=O *Str*, Indole), 1604(-C=N *Str*), 1564(C=CH *Str*), 1312(C=C *Str*, Ar), 1087(C-N *Str*), 821(C-Br*Str*). ^{1}H-NMR (DMSO) $\delta\delta$ ppm: 12.3040(s, 1H, -NH in pyrazole ring), 9.7632(s, 2H, Benzyl), 8.4834(s, 1H, pyrazole), 8.3984-8.2283(s, 1H, Ar-H), 7.8754-7.6733(d, 2H, Ar-H), 7.4953-7.3923(d, 2H, Ar-H), 7.2094-7.1022(d, 2H, Ar-H), 2.3092-2.1223(s, 6H, -CH_3). Mass (LC-MS): m/z 408.06(M), 409.12(M + 1, 100%), 410.16(M + 2, 30%).

Anthelmintic activity: Anthelmintic activity screening of the pyrazole fused novel Indole derivatives was done at 0.1%, 0.2%, 0.5% by using Indian earth warms. The Albendazole was used as the standard drug, the paralysis and death time was recorded in min (Tables 2). A closer revelation of results from this table indicated that the synthesized compound 4c, 4f and 4j (Scheme-I) showed good Anthelmintic activities whereas others showed significant activities.

Table.No.2. Antihelmintic activity of compounds [4a-4l].

S.No.	Compound	Time in minutes					
		For paralysis % Concentration			For death % Concentration		
	Concentration	0.1	0.2	0.5	0.1	0.2	0.5
	Control	-	-	-	-	-	-
	Albendazole	19	14	9	41	30	23
1	4a	38	29	20	58	40	38
2	4b	37	23	22	56	46	37
3	4c	21	19	12	38	34	29
4	4d	39	32	25	49	37	35
5	4e	40	37	23	58	45	39
6	4f	20	18	13	37	32	26
7	4g	40	33	21	55	43	33
8	4h	39	27	20	57	48	33
9	4i	37	29	23	61	50	39
10	4j	22	20	16	40	30	25
11	4k	34	28	23	57	42	37
12	4l	44	33	22	46	37	33

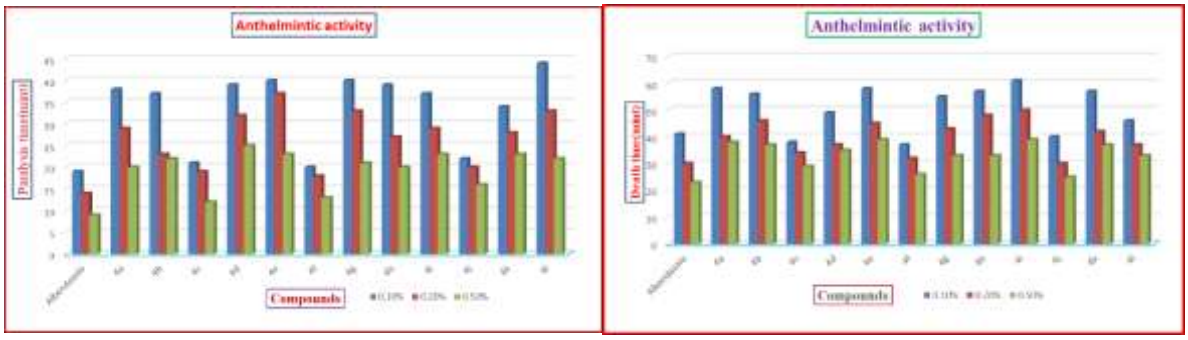

Fig.No.2. Anthelmintic activity of compounds[4a-4l]-Paralysis and Death time.

Antibacterial activity: The antibacterial activity of all the synthesized compounds (4a-4l) was performed by cup plate method (diffusion technique). The streptomycin used as a standard drug against Staphylococcus aureus, Bacillus subtilis (Gram positive) and Escherichia coli, Salmonella paratyphi, Pseudomonas (Gram negative). Most of the synthesized compounds like **4b, 4h** and **4i** were exhibited potential activity by comparing the standard drug.

Table.3. Antibacterial activity of Compounds[4a-4l].

Microorganism	Zone of Inhibition (in mm)										
	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**	**Streptomycin**
Staphylococcus aureus	12	11	09	14	10	09	16	10	15	10	30
Bacillus subtilis	12	**21**	14	09	12	15	10	13	**21***	15	32
E.Coli	13	14	15	14	10	10	10	16	09	18	32
Pseudomonas	09	**23***	12	10	16	10	15	**20***	16	19	29
Salmonella paratyphi	09	10	12	10	09	13	10	15	12	11	32

All values are expressed as Zone ofInhibition; Bore size = 6mm;Concentrationof test compounds is 100µg/ml.

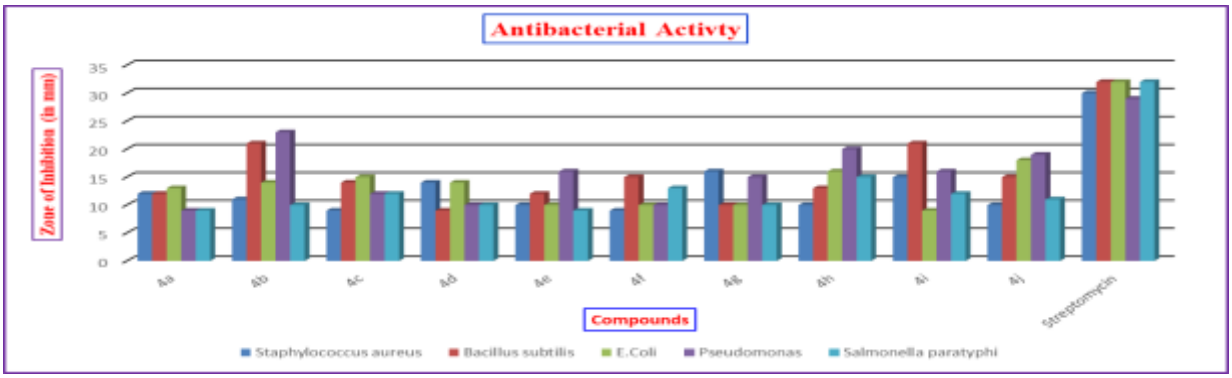

Fig.No.3. Antibacterial activity of compounds[4a-4l]-Zone of Inhibition.

Anticancer activity: Pyrazole fused novel Indole derivatives were screened for cytotoxic activity against human breast cancer cells (MCF7) by using MTT assay method, with doxorubicin as a standard drug. All the results (Table 4) proposed that cell lines were susceptible to the evaluated compounds showed IC_{50}values in the range of 0.047µM to 0.13µM (Scheme-I) against MCF7 cell line. From the results, the compounds 4b(0.08µM), 4c(0.06µM) and 4f(0.04µM)(Scheme-I) showed good activity against MCF7 cell line, whereas remaining compounds showed moderate activity .

Table.4. Anticancer activity of Pyrazole fused Indole derivatives on MCF-7 Cell lines.

	SAMPLE NAME	**IC_{50} (µM)**
1	4b	0.08
2	4c	0.06
3	4f	0.04
4	4g	0.10
5	4i	0.13
6	4l	0.09
13	**Doxorubicin**	0.02

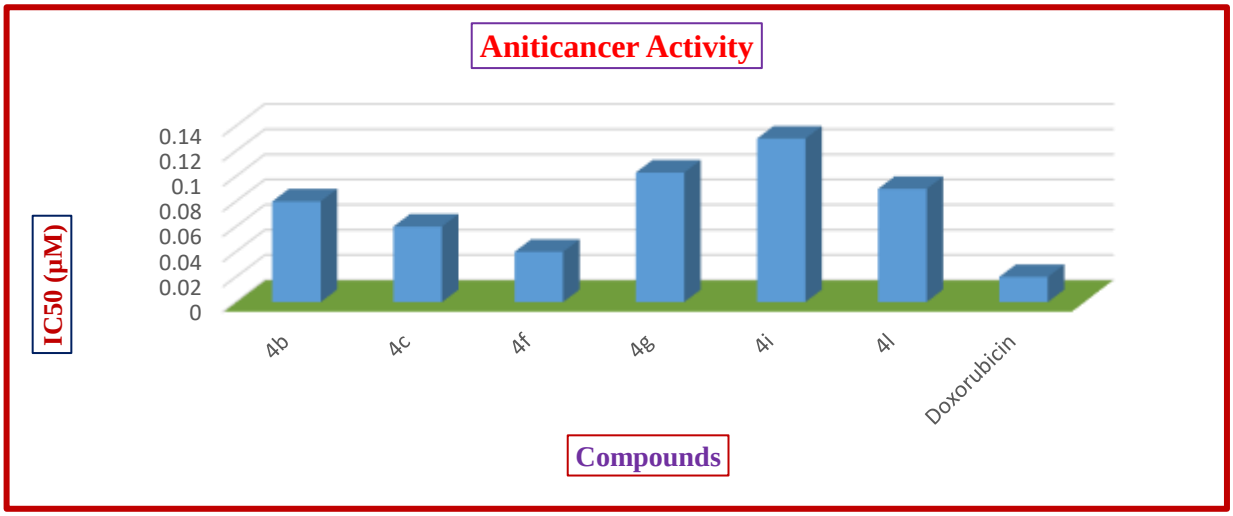

Figure.4. Anticancer activity of Pyrazole fused Indole derivatives on MCF-7 Cells.

Molecular Docking studies: Molecular docking research has been carried out by using the Ligprep tool of Schrodinger suite. Based on the E Model energy, solely one was once displayed in the result. Among the docked ligands, compound 4f reported highest dock score of -6.836 with Glide binding energy of -38.49 Kcal/mol. Dock scores of all the compounds ranged from -6.836 (compound 4f) to -5.435 (compound 4d). MET 769 and ASP 831 are the most common amino acids with H-bonds. Pi-Pi stacking was observed between compounds 4f or 4b and PHE 699.

Table.No.5: Insilco EGFR inhibition of pyrazole fused novel Indole Derivatives-Glide dock scores of the dataset ligands.

Compound No	Dock score XP GScore	No of H-bonds	Interacting amino acids	H-bond lengths (Å)	Emodel energy	Glide energy
Doxorubicin	-10.04	3	GLN 165 MET 165 ASN 142	1.97 2.03 3.152	-46.5	-28.43
4f	-6.836	1	MET 769	1.96	-55.93	-38.49
4c	-6.74	1	MET 769	1.97	-57.464	-38.845
4a	-6.697	1	MET 769	1.88	-53.595	-37.132
4h	-5.9	2	MET 769 GLN 767	2.07 2.31	-52.072	-39.256
4b	-5.719	1	MET 769	1.87	-49.429	-32.031
4j	-5.536	1	ASP 831	2.00	-57.871	-41.187
4l	-5.495	1	ASP 831	2.05	-56.081	-40.748
4d	-5.435	1	ASP 831	2.07	-53.779	-40.084

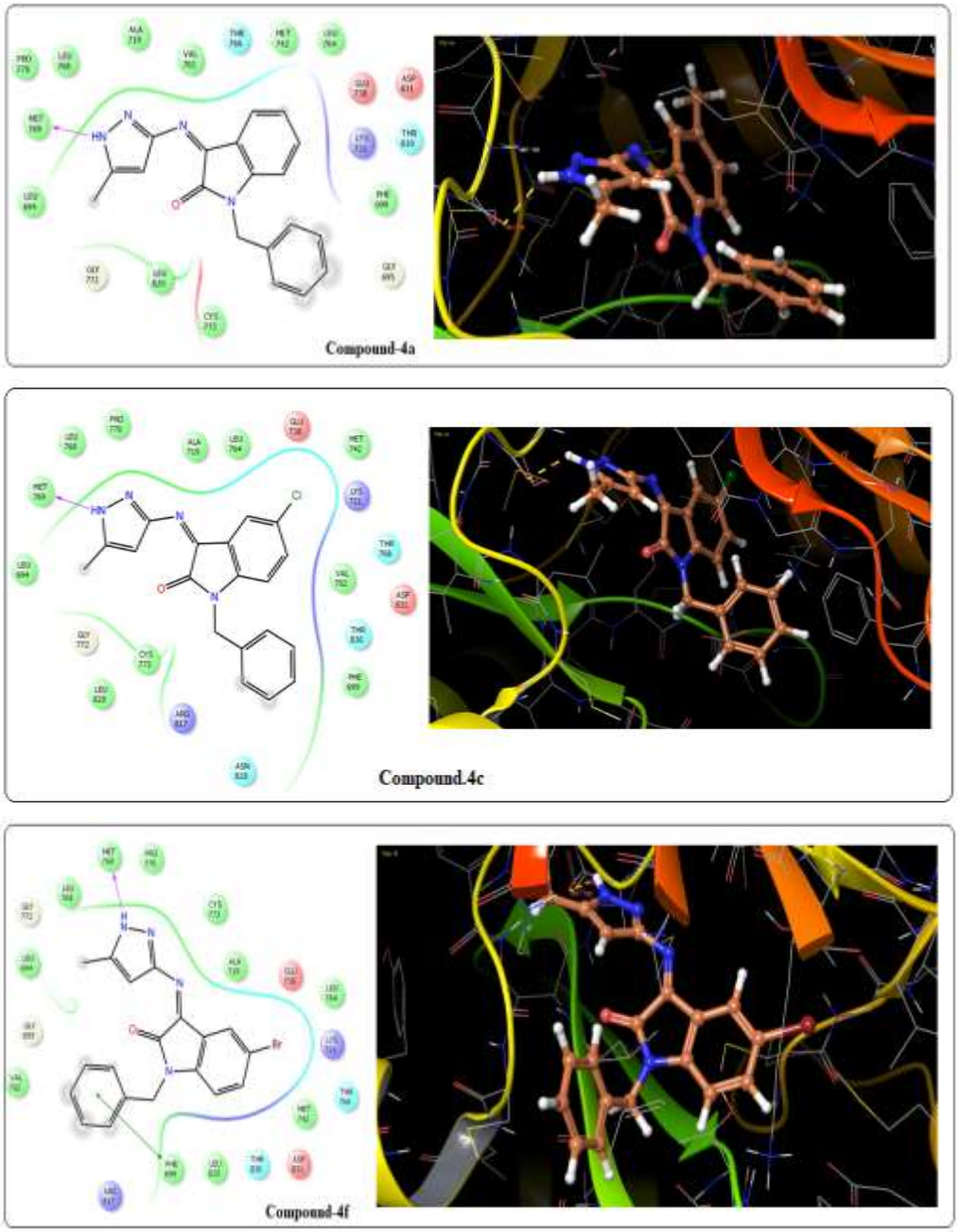

Figure. 5. Docking Pose between the Ligand and the Protein (Dock1 and Dock-2)

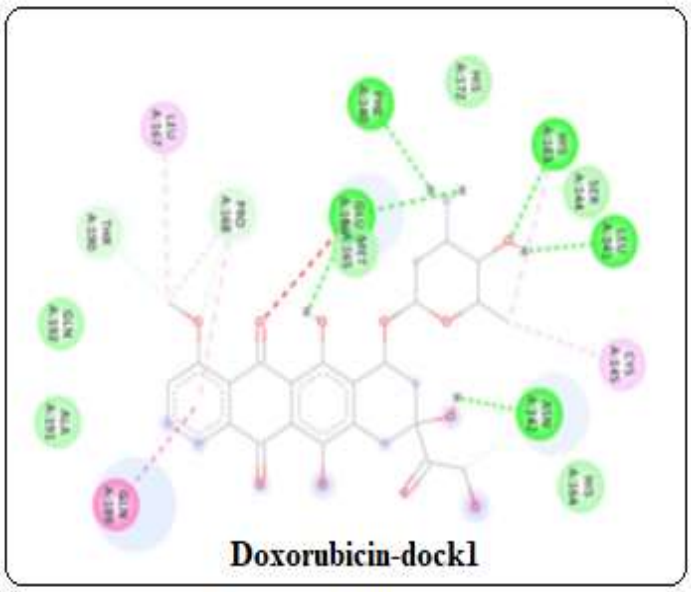

Figure.6. Docking Pose between the Ligand and the Protein (Dock1)

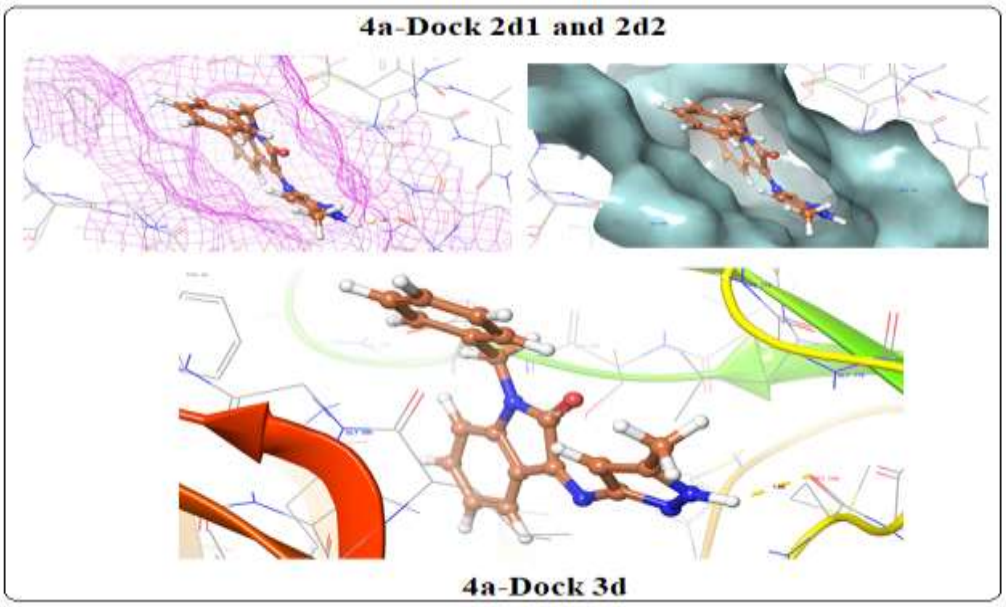

Figure.7. Docking Pose between the Ligand and the Protein-Compound.4a -Dock 2d1, 2d2 and 3d

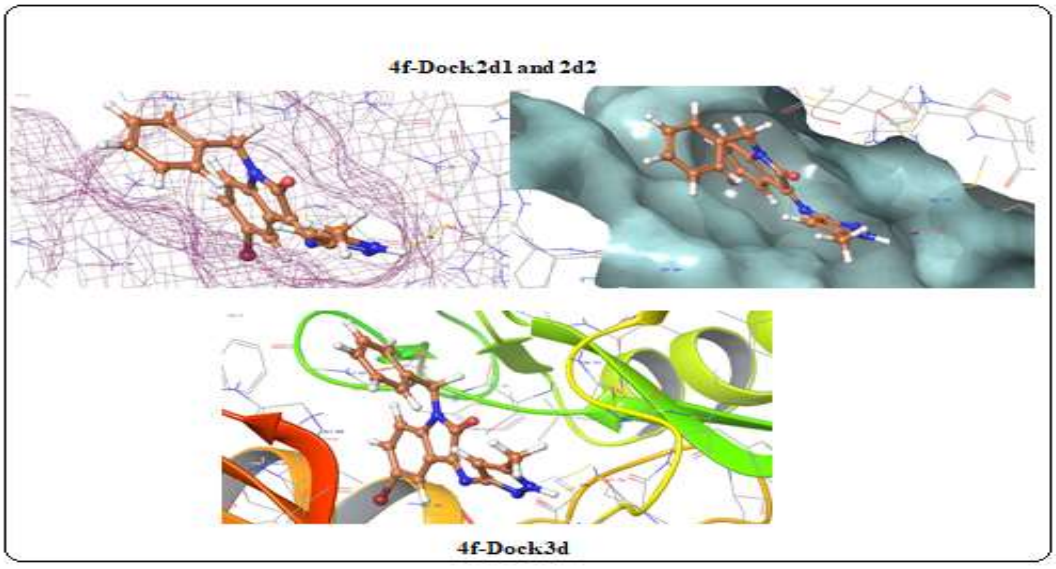

Figure.8. Docking Pose between the Ligand and the Protein-Compound. 4f-Dock 2d1, 2d2 and 3d

DISCUSSION

The yield of the synthesized compound was found to be in the range from 68-87 %. All these molecules were characterized by FTIR, H^1-NMR and Mass spectral analysis along with physical data.Spectral characterization of the pyrazole fused novel Indole derivatives was performed by IR spectroscopy. Practically, in all the compounds are showing the aromatic and aliphatic C-H stretching frequency, as expected is observed at around 3000-3098 cm-1 and 2910-2732 cm^{-1}. All the compounds have been show strong absorption in the region of 1700-1725 cm^{-1} was found to be presence of C=O stretching frequency and in most of the compounds the C=C stretching of the aromatic ring is around 1545-1535cm^{-1} respectively. The Ar-Cl stretching is showing the strong absorption in the region 790-825 cm-1 and few compounds containing -NO_2 group shows peaks due to stretching of –NO_2 group is observed at around 1625-1650 cm-1 respectively. Similarly, the ^{1}HNMR (DMSO-d6) spectra of pyrazole fused novel Indole derivatives showing a singlet at 10.00-12.032 for -NH in pyrazole proton and singlet at 9.02-9.698 for Benzyl protons. All these compounds have aromatic protons were found between δ 8.65-6.80 ppm as singlet, doublet and triplet protons.

Anthelmintic activity screening of the novel pyrazole fused Indole derivatives was done at 0.1%, 0.2%, 0.5% by using Indian earth warms. The Albendazole was used as the standard drug. A closer revelation of results from this table indicated that the synthesized compound **4c, 4f** and**4j** showed good Anthelmintic activities whereas others showed significant activities. For antibacterial activity, streptomycin used as a standard and most of the synthesized compounds like **4b, 4h** and **4i** showed significant antibacterial activity. Pyrazole fused novel Indole derivatives were screened for cytotoxic activity against one cancer cell like human breast cancer cells (MCF7) by using MTT assay method and doxorubicin used as a standard drug. All the results proposed that cell line were susceptible to the evaluated compounds showed IC_{50}values in the range of 0.04µM to 0.13µM against MCF7 Cell line. From the results, the compounds 4b(0.08µM), 4c(0.06µM) and 4f(0.04µM) showed good activity whereas, remaining of the compounds showed moderate activity against MCF7 cell line.Molecular docking research has been carried out by using the Ligprep tool of Schrodinger suite. Based on the E Model energy, solely one was once displayed in the result. Among the docked ligands, compound 4f reported highest dock score of -6.836 with Glide binding energy of -38.49 Kcal/mol. MET 769 and ASP 831 are the most common amino acids with H-bonds. Pi-Pi stacking was observed between compounds 4f or 4b and PHE 699.

CONCLUSION

In summary we have synthesized a novel pyrazole fused Indole derivatives by conventional method possessing biologically potent pyrazole fused indole nucleus with good to percentage yield and purity. The synthesized compounds showed good anthelmintic, antibacterial and anticancer activities.

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