Section: Research Paper
ISSN 2063-5346

Design, Synthesis, Characterization, antibacterial, *In Vitro* and *In silico* anti-cancer evaluations of some novel 4-(benzylideneamino)-N-(5-methyl-1*H*-pyrazol-3-yl) benzamide derivatives

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Abstract:

We synthesized a new series of some novel 4-(benzylideneamino)-N-(5-methyl-1H-pyrazol-3-yl) benzamide derivatives **KI-(3a-3j)** by conventional method. In step one 4-amino-benzoyl chloride reacts with 3-amino-5-methyl pyrazole by Scatton Baumann mechanism and followed by Schiff base mechanism to form compound 2. In step two the compound 2 reacts with substituted benzaldehyde via Schiff's base mechanism to form title compounds. All structures were confirmed by IR, 1HNMR and Mass spectral analysis and Physical properties. The yield of the synthesized compounds was found to be in the range from **75-86%** and studied for their in vitro antibacterial and anticancer activity. The antibacterial activity was carried by Agar disc plate method against gram-positive *Bacillus substillus*, and *Staphylococcus aureus* and gram-negative *Escherichia coli and Klebsiella pneumoniae* species. The anticancer activity was performed by MTT assay againist MCF-7 cell line. From the results compound **KI-3c, KI-3d** and **KI-3i** are more antibacterial activity and compound **KI-3j** were showing better anticancer activity. Finally, a docking studies was carried out for anticancer activity via EGFR receptor with PDB ID:1M17. Among the docked ligands, Compound **KI-3j** reported highest docking score **-9.10KJ/mol.**

Keywords: 3-amino-5-methyl pyrazole, antibacterial and anti-cancer activities, MCF-7 Cell line, Molecular docking.

1. Introduction

Medicinal chemistry is the application of chemical research techniques to the synthesis of pharmaceutical compounds. During the early stages of medicinal chemistry development, scientists were primarily concerned with the isolation of medicinal agents found in plants. Today, scientists in this field are also equally concerned with the creation of new synthetic drug compounds. Medicinal chemistry is almost always geared toward drug discovery and development [1]. Heterocyclic compounds are a class of organic compounds whose molecules contain one or more ring of atoms with at least one heteroatom being an element other than carbon, most frequently oxygen, nitrogen or sulphur. Heterocyclic compounds probably constitute the largest and most varied family of organic compounds [2-3]. The most common heterocycles are those having five- or six-membered rings and containing

heteroatoms of nitrogen (N), oxygen (O), or sulphur (S). Some of the common heterocycles are mentioned below.

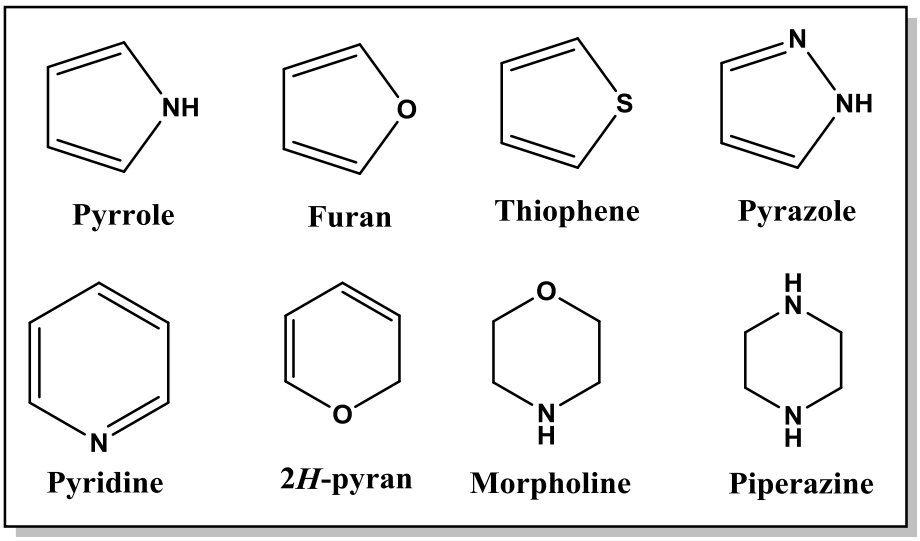

Figure.1: Structures of Heterocyclic rings

The chemistry of heterocyclic compounds is one of the most complex and important stream of organic chemistry. Because of the diverse properties, easily accessible path and the wide range of biological activities, these are centre of attraction for organic chemist to synthesise various heterocyclic compounds. The literature survey reveals the importance of pyrazole contain benzamide derivatives as an intermediate in the medicinal chemistry [4]. Pyrazole fused benzamide and their substituted derivatives are interesting potential pharmaceuticals. They exhibit wide variety of biological and pharmaceutical activities. Therefore, they play important role in medicinal chemistry. The pyrazole and its fused molecules has been reported to possess a wide spectrum of biological properties such as anti-inflammatory, antibacterial, analgesic, antifungal, antiviral, antibacterial, CNS depressant, antitumor, potent local anaesthetics etc. [5-10]. Keeping in view, the importance of heterocyclic compounds were synthesised according to Schotten Baumann and Schiff's base mechanism. The newly synthesised compounds were screened for their antimicrobial, anticancer activities and Molecular docking studies.

2. Material and Methodology

The innovation of pyrazole fused some novel benzamide derivatives was screened for antimicrobial, anticancer activities. Fourier Transform IR spectrometer (model Shimadzu 8700) in the range of 400-4000 cm-1 Using KBr pellets and values are reported in cm-1 and the spectra were interpreted. ^{1}H-NMR spectra were recorded on DPX-200 MHz NMR spectrometer exploiting DMSO-d6 and chemical shifts (δ) are 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. Precoated Silica Gel G plates were used to observe the progress

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of reaction as well as to assessment the purity of the compounds: n-Hexane: Ethyl acetate (8:2). The molecular docking studies were carried out by using AUDOC VINA soft wear.

2.1.General Procedure:

Step-1: Synthesis of 4-amino-N-(5-methyl-1H-pyrazol-3-yl)benzamide (1): In conical flask prepare 10% of sodium hydroxide solution and dissolve in it 0.03 mole of 3-amino-5-methyl pyrazole. Add 0.03mole of 4-amino-benzoyl chloride in 5 portions to the solution. Stopper the vessel and shake vigorously after each addition until all the chloride has reacted. Transfer the solution to a beaker and rinse the conical flask with a little water. Place a few pieces of crushed ice to the solution and add slowly 5 mL of HCl with stirring until the mixture is acid to Congo red paper. Collect the resulting crystalline precipitate of 4-methoxy-benzylglycine. Filter the product on Buchner funnel, and dry on air on Petri dish [12].

Step II: Synthesis of (E)-4-(benzylideneamino)-N-(5-methyl-1H-pyrazol-3-yl) benzamide (3a-3l): A mixture of equimolar quantity of 4-amino-N-(5-methyl-1H-pyrazol-3-yl)benzamide (1) (0.01mol) and Substituted benzaldehyde (0.01mol) was dissolved in 20ml of ethanol, refluxed for 2-3hrs in the presence of few drops of (2-5ml) glacial acetic acid. The progress of the reaction was monitored by TLC (n-Hexane: EtoAc 8:2). The reaction mixture was cooled to room temperature and keep in refrigerator for overnight to get precipitate. A solid was obtained, which was filtered off and recrystallized from methanol or ethanol to give crystalline solid [12-13].

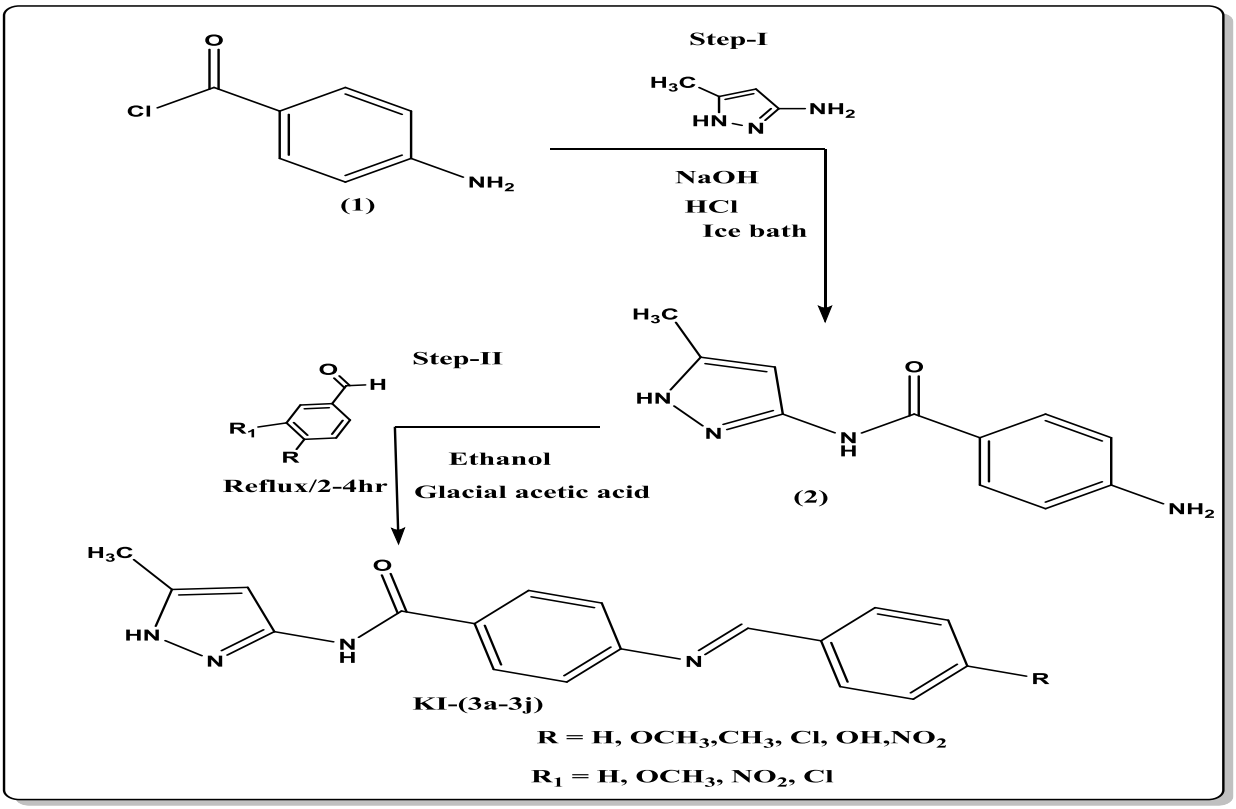

Figure 2: Scheme-I

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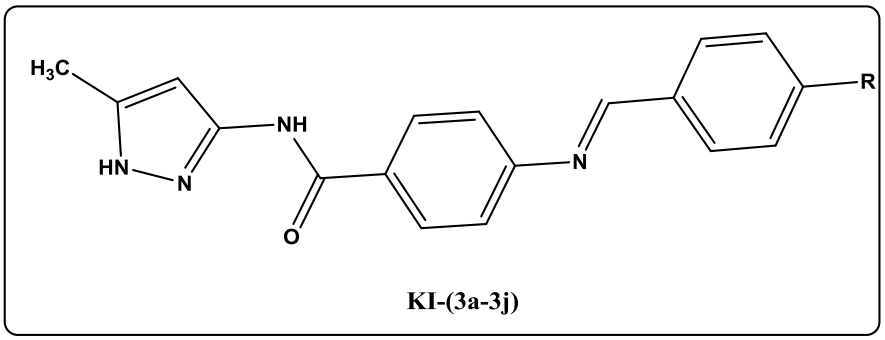
Table.1: Physical Characterization of Compounds-KI(3a-3j)



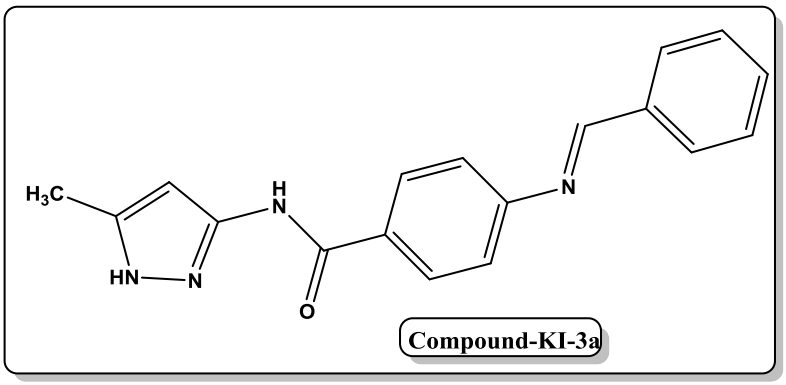
KI-(3a-3j)

Compounds	Molecular Formula	R	Melting Point(^{0}C)	Rf Value	%Yield	Physical state and Solubility
KI-3a	$C_{18}H_{16}N_4O$	H	187-189	0.67	86	White solid & Ethanol, DMSO
KI-3b	$C_{18}H_{15}ClN_4O$	4-Cl	216-218	0.56	78	Light green solid & Ethanol, DMSO
KI-3d	$C_{19}H_{18}N_4O$	4-CH_3	161-163	0.63	88	Creamish white solid & Ethanol, DMSO
KI-3d	$C_{18}H_{15}N_5O_3$	4-NO_2	253-255	0.78	84	Pale Yellow solid & Ethanol, DMSO
KI-3e	$C_{19}H_{18}N_4O_2$	4-OCH_3	190-192	0.59	77	White solid & Ethanol, DMSO
KI-3f	$C_{18}H_{16}N_4O_2$	4-OH	213-215	0.82	81	Brown solid & Ethanol, DMSO
KI-3g	$C_{20}H_{20}N_4O_3$	3,4-$(OCH_3)_2$	237-239	0.77	84	White solid & Ethanol, DMSO
KI-3h	$C_{18}H_{15}ClN_4O$	3-Cl	159-161	0.61	80	Green solid &

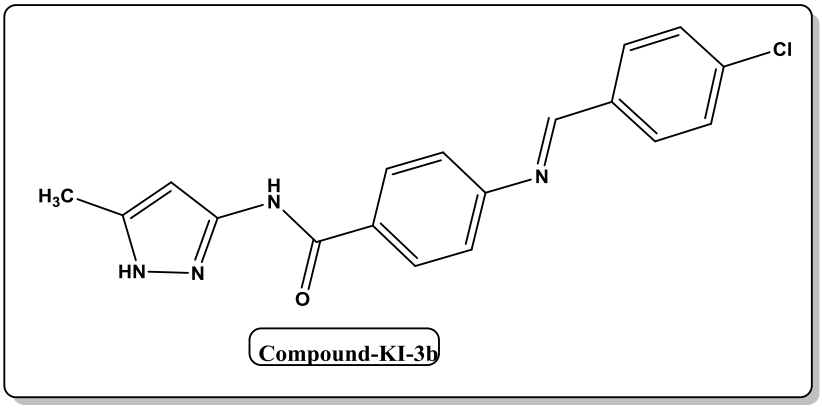
						Ethanol, DMSO
KI-3i	$C_{18}H_{16}N_4O_2$	3-OH	219-221	0.82	83	Brown solid & Ethanol, DMSO
KI-3j	$C_{18}H_1N_5O_3$	3-NO_2	349.12	0.66	78	Yellow solid & Ethanol, DMSO

(E)-4-(benzylideneamino)-N-(5-methyl-1*H*-pyrazol-3-yl) benzamide 3a:

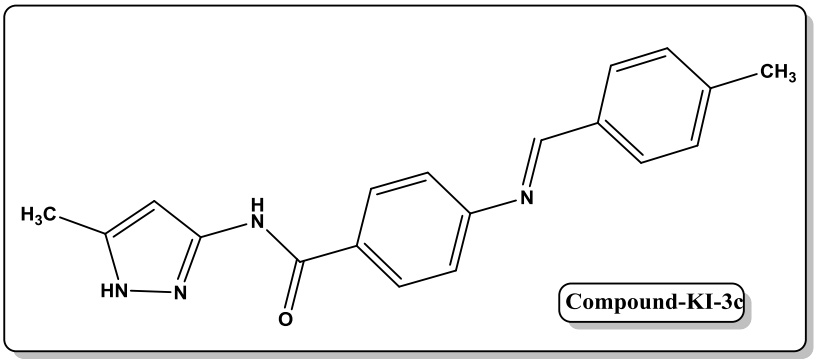
IR (ν cm−1): 3420(NH *Str*, pyrazole); 3350(-NH *Str*, Amide); 3023(-C-H *Str*, Aromatic ring); 2965, 2857(-CH *Str*, Aliphatic group); 1710(-CO *Str*, Amide group); 1502(-C=CH *Str*, Aromatic group); 1423(-C=C *Str*, Aromatic group); 1095(-C-N *Str*). ^{1}H-NMR (DMSO) δ ppm: 12.1009(s, 1H, Pyrazole proton); 9.8998(s, 1H, Imine proton); 8.8956(s, 1H, Amide proton); 7.9432-7.9032(d, 2H, Aromatic protons); 7.7476-7.7287(d, 2H, Aromatic proton); 7.6789-7.6674(d, 2H, Aromatic protons); 7.5092-7.5009(t, 3H, Aromatic protons), 6.9234(s, 1H, Pyrazole proton); 1.9998(s, 3H, -CH_3 protons on Pyrazole ring). Mass (LC-MS): 339.21(M + 1).



(E)-4-((4-chlorobenzylidene) amino)-N-(5-methyl-1*H*-yrazol-3-yl) benzamide 3b: IR (ν cm−1): 3409(-NH *Str*, pyrazole); 3287(-NH *Str*, Amide); 3010(-C-H *Str*, Aromatic ring); 2903, 2866(-CH *Str*, Aliphatic group); 1703(-CO *Str*, Amide group); 1510(-C=CH *Str*, Aromatic group); 1432(-C=C *Str*, Aromatic group); 1087(-C-N *Str*), 810(-C-Cl Str, Ar-Cl group). ^{1}H-NMR (DMSO) δ ppm: 12.2834(s, 1H, Pyrazole proton); 9.8387(s, 1H, Imine proton); 9.1293(s, 1H, Amide proton); 8.0231-8.0032(d, 2H, Aromatic protons); 7.7085-7.7004(d, 2H, Aromatic proton); 7.6998-7.6902(d, 2H, Aromatic protons); 7.5543-7.5430(d, 2H, Aromatic protons), 7.2509(s, 1H, Pyrazole proton); 2.0080(s, 3H, -CH_3 protons on Pyrazole ring).Mass (LC-MS):339.12(M + 1).



(E)-N-(5-methyl-1*H*-pyrazol-3-yl)-4-((4-methylbenzylidene)amino) benzamide 3c: IR (*ν* cm−1): 3398(-NH *Str*, pyrazole); 3289(-NH *Str*, Amide); 3098(-C-H *Str*, Aromatic ring); 2935, 2889(-CH *Str*, Aliphatic group); 1714(-CO *Str*, Amide group); 1498(-C=CH *Str*, Aromatic group); 1410(-C=C *Str*, Aromatic group); 1109(-C-N *Str*). ^{1}H-NMR (DMSO) *δ* ppm: 12.4328(s, 1H, Pyrazole proton); 9.9093(s, 1H, Imine proton); 9.1208(s, 1H, Amide proton); 8.0387-8.0254(d, 2H, Aromatic protons); 7.8987-7.8786(d, 2H, Aromatic proton); 7.6983-7.6924(d, 2H, Aromatic protons); 7.5998-7.4905(d, 2H, Aromatic protons), 7.2783(s, 1H, Pyrazole proton); 2.0548(s, 3H, -CH_3 protons on Pyrazole ring); 1.9843(s, 3H, Ar-CH_3 protons). Mass (LC-MS): 319.21(M + 1).



(E)-N-(5-methyl-1*H*-pyrazol-3-yl)-4-((4-nitrobenzylidene)amino) benzamide 3d: IR (*ν* cm−1): 3403(-NH *Str*, pyrazole); 3276(-NH *Str*, Amide); 3109(-C-H *Str*, Aromatic ring); 2920, 2876(-CH *Str*, Aliphatic group); 1713(-CO *Str*, Amide group); 1615(-NO_2 *Str*, Ar-NO_2); 1503(-C=CH *Str*, Aromatic group); 1421(-C=C *Str*, Aromatic group); 1121(-C-N *Str*). ^{1}H-NMR (DMSO) *δ* ppm: 12.3653(s, 1H, Pyrazole proton); 9.8874(s, 1H, Imine proton); 9.0932(s, 1H, Amide proton); 7.8103-7.8034(d, 2H, Aromatic protons); 7.7023-7.7004(d, 2H, Aromatic proton); 7.5863-7.5806(d, 2H, Aromatic protons); 7.4999-7.4803(d, 2H, Aromatic protons), 7.1602(s, 1H, Pyrazole proton); 2.0973(s, 3H, -CH_3 protons on Pyrazole ring)Mass (LC-MS): 350.03(M + 1).

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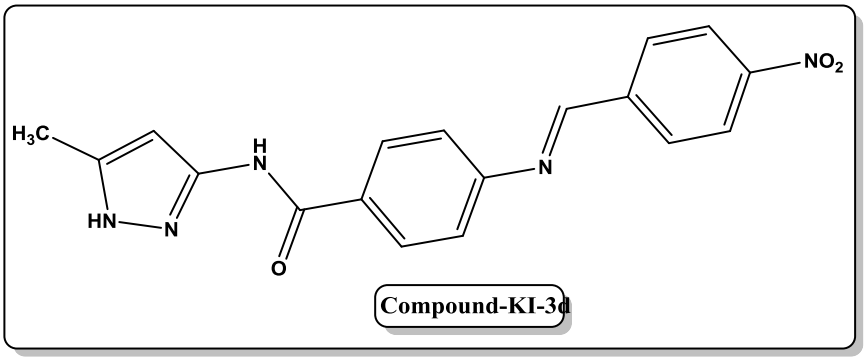
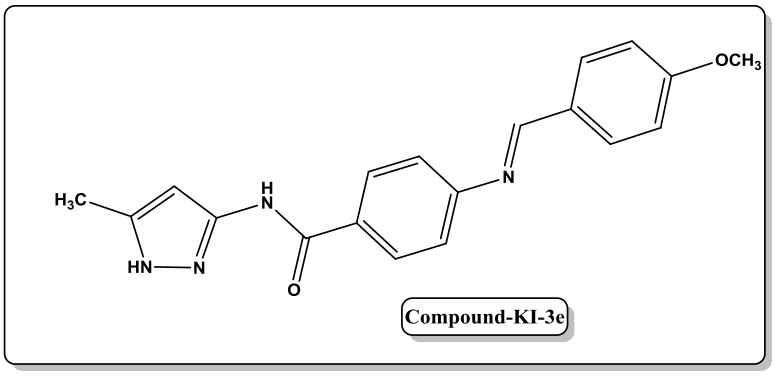


(E)-4-((4-methoxybenzylidene) amino)-N-(5-methyl-1*H*-pyrazol-3-yl)benzamide 3e: IR (*ν* cm−1): 3422(-NH *Str*, pyrazole); 3302(-NH *Str*, Amide); 3094(-C-H *Str*, Aromatic ring); 2976, 2883(-CH *Str*, Aliphatic group); 1702(-CO *Str*, Amide group); 1518(-C=CH *Str*, Aromatic group); 1432(-C=C *Str*, Aromatic group); 1124(-C-N *Str*); 1043(-C-O *Str*, Methoxy group). ^{1}H-NMR (DMSO) *δ* ppm: 12.2873(s, 1H, Pyrazole proton); 9.9543(s, 1H, Imine proton); 9.293(s, 1H, Amide proton); 8.0432-8.0032(d, 2H, Aromatic protons); 7.8943-7.8021(d, 2H, Aromatic proton); 7.4843-7.3904(d, 2H, Aromatic protons); 7.2093-7.2003(d, 2H, Aromatic protons), 6.9043(s, 1H, Pyrazole proton); 3.8654(s, 3H, -OCH_3 protons on Aromatic ring); 2.1093(s, 3H, -CH_3 protons on Pyrazole ring). Mass (LC-MS): 335.21(M + 1).



(E)-4-((4-hydroxybenzylidene)amino)-N-(5-methyl-1*H*-pyrazol-3-yl)benzamide 3f: IR (*ν* cm−1): 3584(-OH *Str*, Aromatic OH group); 3402(-NH *Str*, pyrazole); 3343(-NH *Str*, Amide); 3056(-C-H *Str*, Aromatic ring); 2962, 2881(-CH *Str*, Aliphatic group); 1705(-CO *Str*, Amide group); 1509(-C=CH *Str*, Aromatic group); 1428(-C=C *Str*, Aromatic group); 1102(-C-N *Str*); 1033(-C-OH *Str*, Hydoxy group on Aromatic ring). ^{1}H-NMR (DMSO) *δ* ppm: 12.5873(s, 1H, Pyrazole proton); 9.8732(s, 1H, Imine proton); 9.0532(s, 1H, Amide proton); 8.1092-8.1002(d, 2H, Aromatic protons); 7.9843-7.9032(d, 2H, Aromatic proton); 7.6754-7.6032(d, 2H, Aromatic protons); 7.4120-7.3908(d, 2H, Aromatic protons), 7.0233(s, 1H, Pyrazole proton); 5.8654(s, 1H, Hydroxy proton on Aromatic ring); 2.0032(s, 3H, -CH_3 protons on Pyrazole ring).Mass (LC-MS): 321.03(M + 1).

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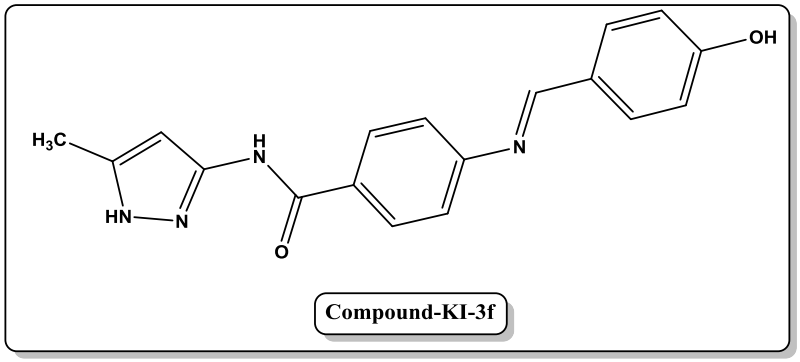
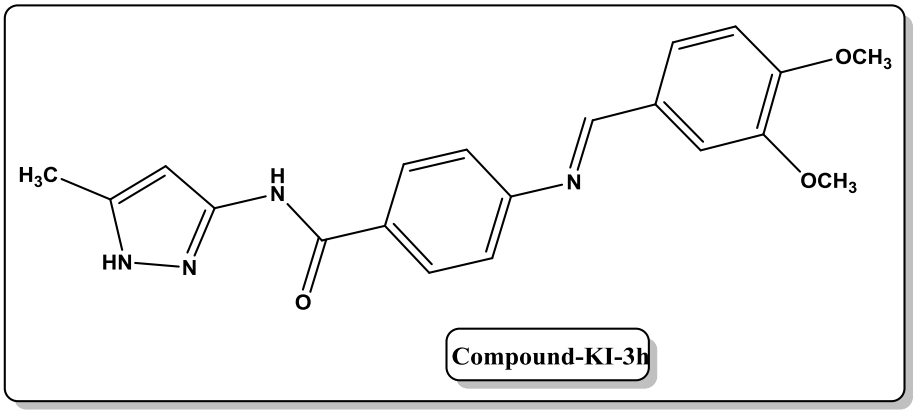


(E)-4-((3,4-dimethoxybenzylidene)amino)-N-(5-methyl-1*H*-pyrazol-3-yl) benzamide 3g: (IR (*ν* cm−1): 3414(-NH *Str*, pyrazole); 3320(-NH *Str*, Amide); 3039(-C-H *Str*, Aromatic ring); 2961, 2893(-CH *Str*, Aliphatic group); 1718(-CO *Str*, Amide group); 1511(-C=CH *Str*, Aromatic group); 1429(-C=C *Str*, Aromatic group); 1132(-C-N *Str*); 1065(-C-O *Str*, Methoxy group). ^{1}H-NMR (DMSO) *δ* ppm: 12.4932(s, 1H, Pyrazole proton); 9.8945(s, 1H, Imine proton); 9.1093(s, 1H, Amide proton); 8.1092-8.1003(d, 2H, Aromatic protons); 7.8873-7.8002(d, 2H, Aromatic proton); 7.7833-7.7023(d, 2H, Aromatic protons); 7.4883-7.4023(d, 2H, Aromatic protons), 6.9983(s, 1H, Pyrazole proton); 3.9833-3.9022(s, 6H, -OCH_3 protons on Aromatic ring); 2.0943(s, 3H, -CH_3 protons on Pyrazole ring). Mass (LC-MS): 365.04(M + 1).



(E)-4-((3-chlorobenzylidene)amino)-N-(5-methyl-1*H*-yrazol-3-yl) benzamide 3h: IR (*ν* cm−1): 3419(-NH *Str*, pyrazole); 3302(-NH *Str*, Amide); 3055(-C-H *Str*, Aromatic ring); 2983, 2862(-CH *Str*, Aliphatic group); 1708(-CO *Str*, Amide group); 1519(-C=CH *Str*, ^{1}H-NMR (DMSO) *δ* ppm: 12.3092(s, 1H, Pyrazole proton); 9.9032(s, 1H, Imine proton); 9.0954(s, 1H, Amide proton); 8.0234-8.0005(d, 2H, Aromatic protons); 7.8954-7.8334(d, 2H, Aromatic proton); 7.5986-7.5034(d, 2H, Aromatic protons); 7.4985-7.4100(d, 2H, Aromatic protons), 7.2009(s, 1H, Pyrazole proton); 2.1093(s, 3H, -CH_3 protons on Pyrazole ring). Mass (LC-MS): 339.12(M + 1).

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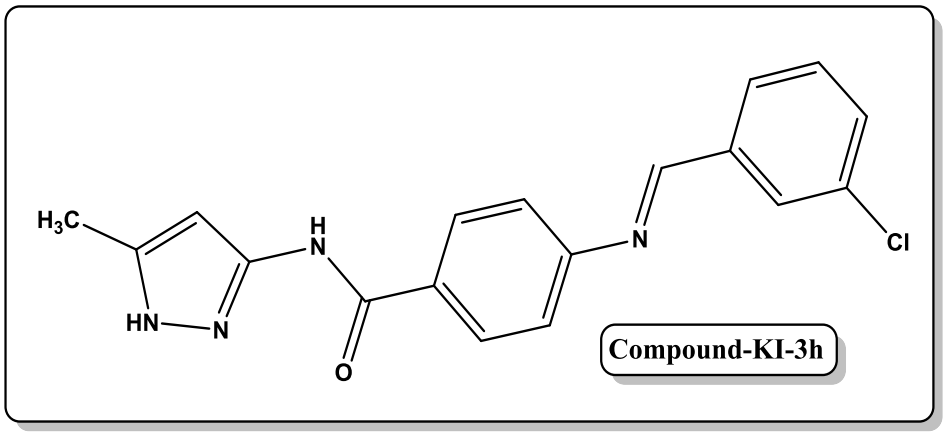
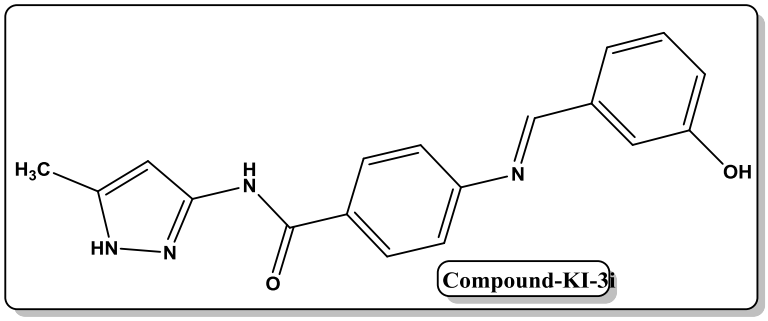
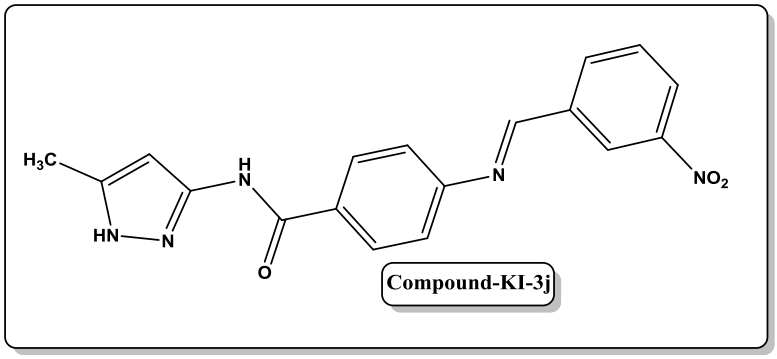


(E)-4-((3-hydroxybenzylidene) amino)-N-(5-methyl-1*H*-pyrazol-3-yl) benzamide3i. IR (*ν* cm−1): 3598(-OH *Str*, Aromatic OH group); 3409(-NH *Str*, pyrazole); 3302(-NH *Str*, Amide); 3062(-C-H *Str*, Aromatic ring); 2979, 2897(-CH *Str*, Aliphatic group); 1701(-CO *Str*, Amide group); 1512(-C=CH *Str*, Aromatic group); 1430(-C=C *Str*, Aromatic group); 1108(-C-N *Str*); 1039(-C-OH *Str*, Hydoxy group on Aromatic ring). ^{1}HNMR (DMSO) *δ* ppm: 12.4855(s, 1H, Pyrazole proton); 9.9843(s, 1H, Imine proton); 9.1023(s, 1H, Amide proton); 8.2093-8.2002(d, 2H, Aromatic protons); 7.8893-7.8232(d, 2H, Aromatic proton); 7.7845-7.7102(d, 2H, Aromatic protons); 7.3984-7.3489(d, 2H, Aromatic protons), 7.0098(s, 1H, Pyrazole proton); 5.7673(s, 1H, Hydroxy proton on Aromatic ring); 2.1092(s, 3H, -CH_3 protons on Pyrazole ring). Mass (LC-MS): 321.03(M + 1).



(E)-N-(5-methyl-1*H*-pyrazol-3-yl)-4-((3-nitrobenzylidene)amino) benzamide KI-3j.IR (*ν* cm−1): 3412(-NH *Str*, pyrazole); 3267(-NH *Str*, Amide); 3084(-C-H *Str*, Aromatic ring); 2978, 2843(-CH *Str*, Aliphatic group); 1709(-CO *Str*, Amide group); 1612(-NO_2 *Str*, Ar-NO_2); 1510(-C=CH *Str*, Aromatic group); 1419(-C=C *Str*, Aromatic group); 1134(-C-N *Str*). ^{1}HNMR (DMSO) *δ* ppm: 12.2633(s, 1H, Pyrazole proton); 9.9033(s, 1H, Imine proton); 9.1023(s, 1H, Amide proton); 7.9843-7.9034(d, 2H, Aromatic protons); 7.8754-7.8323(d, 2H, Aromatic proton); 7.6743-7.6674(d, 2H, Aromatic protons); 7.4102-7.4003(d, 2H, Aromatic protons), 7.0983(s, 1H, Pyrazole proton); 2.1092(s, 3H, -CH_3 protons on Pyrazole ring). Mass (LC-MS): 350.03(M + 1).



Compound-KI-3j

2.2.Biological Activities:

2.2.1. Antibacterial activity.

2.2.2. ***Anticancer activity.*** MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The assay depends both on the number of cells present and, on the assumption, that dead cells or their products do not reduce tetrazolium. Cell viability was evaluated by the MTT Assay 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 ^{0} 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 medic with MTT solution (0.5 mg / $Ml^{-1)}$ was added to each well and plates were incubated at 37 ^{0} 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 [14].

$$\% \text{ Inhibition} = \frac{100\ (\text{Control} - \text{Treatment})}{\text{Control}}$$

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.

2.2.3. Molecular docking studies. The 2D structures of 10 compounds were generated from the ACD/Chemsketch Software [15]. The generated ligands cleaned and performed 3D optimization then saved in the MDL molfile format. The ligands were then converted to a PDBQT file format using the Open Babel chemistry toolbox. The three-dimensional (3D) structure of Epidermal Growth Factor Receptor tyrosine kinase (PDB ID:1M17) was downloaded from Brook Heaven Protein Data Bank (https://www.rcsb.org) and saved as a Brookhaven protein data bank file and the structure was optimized by deleting unbound water molecules which are over 1 Å, adding hydrogen atoms to satisfy the valences, adding missing amino acids to

stabilize side chains and energy of the whole structure was minimized using AUTODOCK suite of MGL Tools. Auto dock Vina was used for molecular docking studies.

3. Results and Discussion:

3.1.Synthesis: The synthesized a new series of some novel 4-(benzylideneamino)-N-(5-methyl-1*H*-pyrazol-3-yl) benzamide derivatives **KI-(3a-3j)** by conventional method. In step one 4-amino-benzoyl chloride reacts with 3-amino-5-methyl pyrazole by Scatton Baumann mechanism and followed by Schiff base mechanism to form compound 2. In step two the compound 2 reacts with substituted benzaldehyde via Schiff's base mechanism to form title compounds. All structures were confirmed by IR, 1HNMR and Mass spectral analysis and Physical properties. The yield of the synthesized compounds was found to be in the range from **75-86%.**

3.2.Biological activity.

3.3.1. Antibacterial activity. Agar diffusion (Disk Plate) method was employed to test the antibacterial activity of the synthesised novel Pyrazole contain benzamide derivatives [KI-(3a-3j)] [16]. This antibacterial activity was carried out against the *Bacillus substillus*, and *Staphylococcus aureus* and gram-negative *Escherichia coli and Klebsiella pneumoniae* as test organisms. In this method the petridishes were filed with inoculated liquefied agar medium to uniform thickness the bores were made using core borer which filled with test drug and a Standard drug(Streptomycin) and inoculated at 37 ± 1^0 C hrs[15]. The drug will diffuse in to the agar medium are prevents the growth of microbes and produce a clear zone of inhibition **Table.2.**

Table. 2: Antibacterial activity by zone of inhibition (in mm) Novel Pyrazole contain benzamide derivatives KI-(3a-3j).

Entry	**Zone of Inhibition (in mm)**			
	Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Klebsiella pneumoniae***
Streptomycin (100µg/ml)	32	30	32	34
KI-3a	19	9	24	19
KI-3b	17	15	19	16
KI-3c	13	18	**25***	12
KI-3d	16	**25***	18	20
KI-3e	12	20	13	10

KI-3f	18	12	15	12
KI-3g	13	23	10	14
KI-3h	24	16	12	10
KI-3i	18	**26***	10	**27***
KI-3j	**12**	**25**	15	**12**

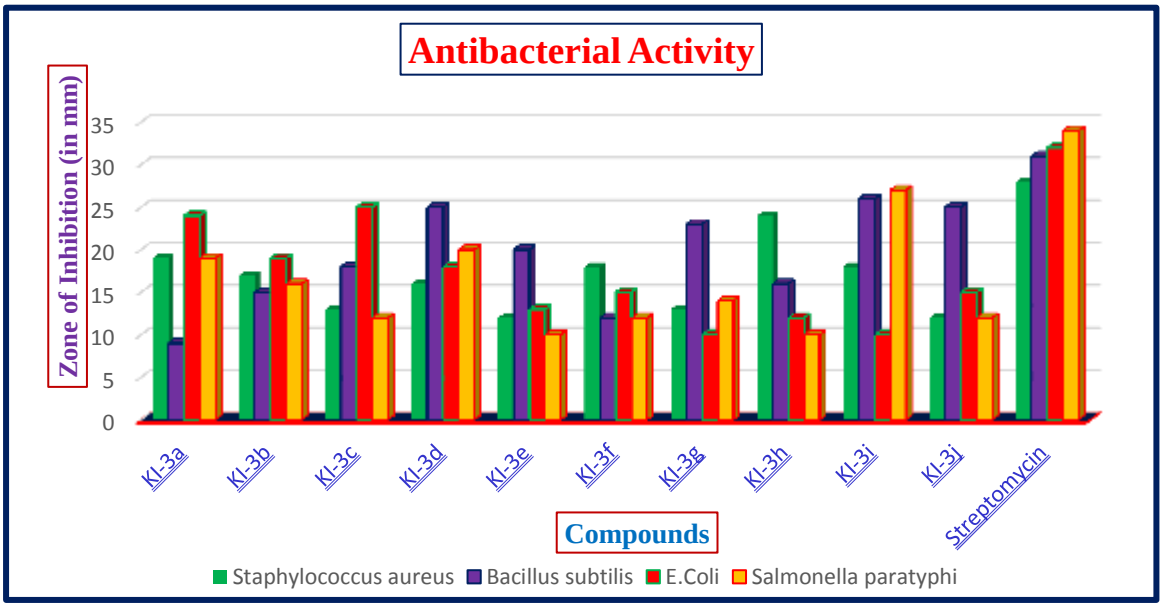


Figure. No.3.: Graphical representation of antibacterial activity of Novel Pyrazole contain benzamide derivatives [KI-(3a-3j)].

3.3.2. Anticancer activity.

The novel Pyrazole contain benzamide derivatives [KI-(3a-3j)] was tested for their anticancer activity against two cancer cell line like MCF-7 by using MTT assay method and doxorubicin as a standard drug. The results of anticancer screening of novel Pyrazole contain benzamide derivatives [KI-(3a-3j)] were expressed as IC_{50} values are summarized in **Table 3**.

Table.3. Anticancer activity of Novel Pyrazole contain benzamide derivatives KI-(3a-3j) on MCF-7 Cell line.

S. No	Sample Name	IC_{50} (µg)
1	KI-3a	52.76

2	**KI-3b**	**44.76**
3	KI-3e	52.52
4	KI-3g	81.67
5	**KI-3j**	**24.1**
6	**Doxorubicin**	**16.32**

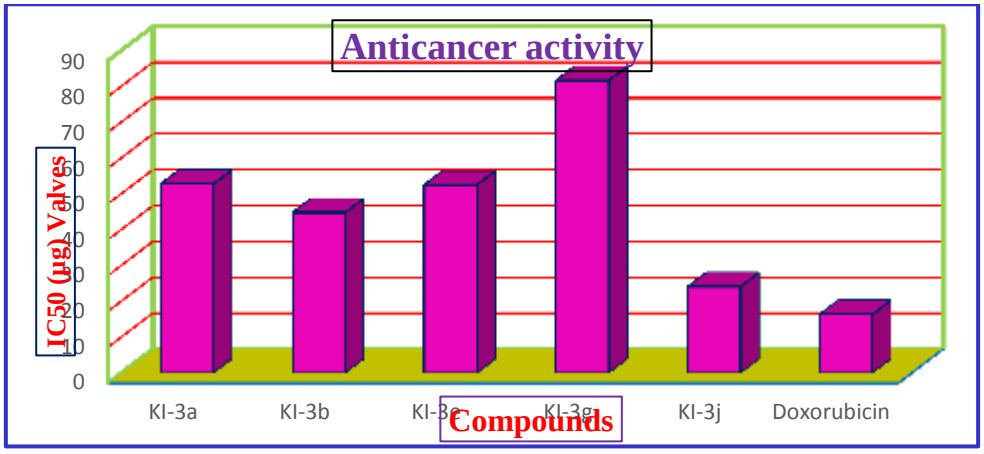
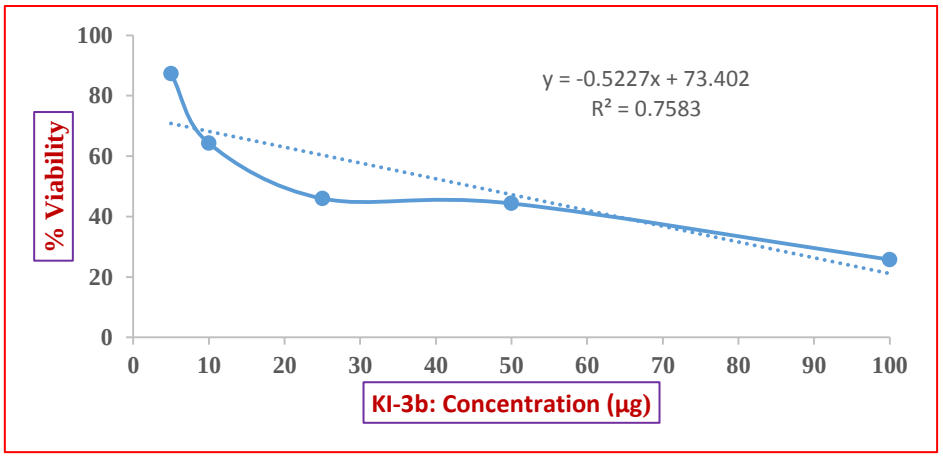


Figure. No.4: Graphical representation of anticancer activity of novel Pyrazole contain benzamide derivatives KI-3a, KI-3b, KI-3e, KI-3g, KI-3j.



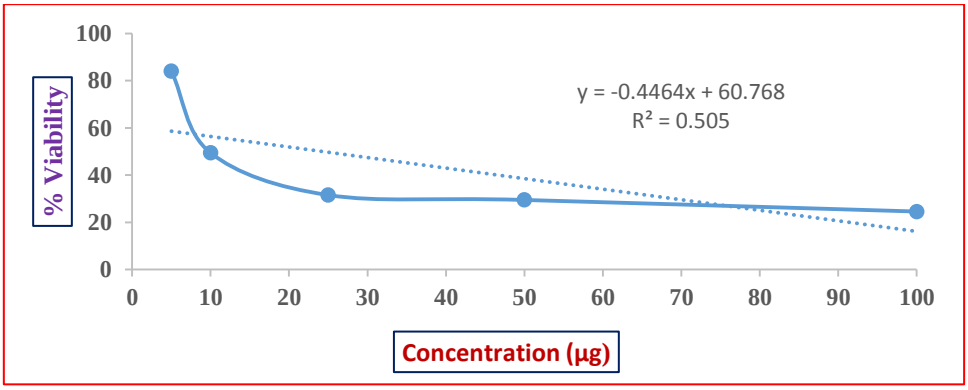


Figure. 5-Graphical representation of novel Pyrazole contain benzamide derivatives-KI-(3b and 3j)-IC_{50} values.

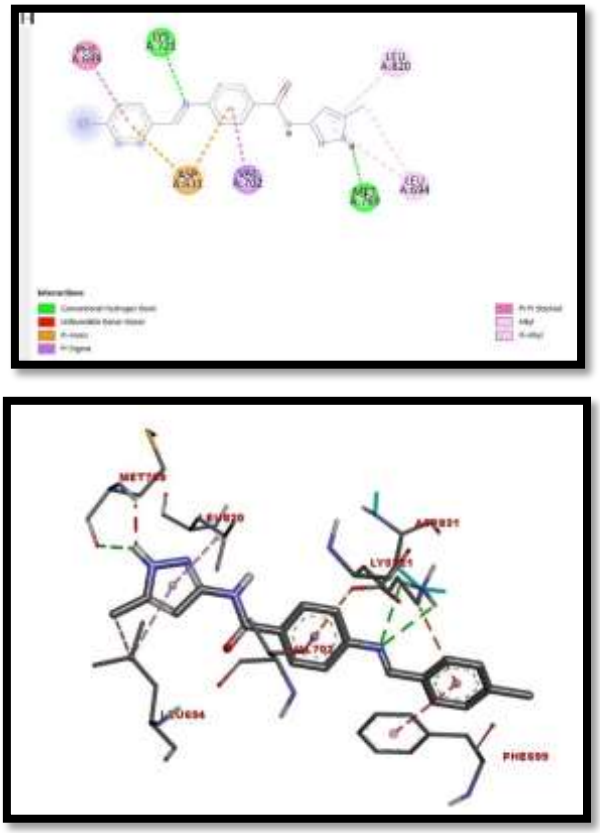
3.3.3. Molecular docking studies.

The molecular docking models was applied to investigate the binding mode of target molecules via selected proteins (EGFR receptor) [17-18] with PDB ID (171M) for the protein active pocket of the modelled Fgb1. I have docked 10 ligands like novel Pyrazole contain benzamide derivatives KI-(3a-3j). In to active site of the Fgb1 protein using AUTODOCK suite of MGL Tools. Glide dock score of the dataset ligand were showed in Table.4, along with the interaction amino acids like LEU:694, PHE:699, VAL:702, ALA:719, LYS:721, GLU:738, LEU:820, ASP:831, MAT:742, CYS:773, MET:769. From the results with Fgb1 protein, compound 4a, 4d, and 4h (-9.8, -9.7, -9.7). The docking score of the ligands ranged from **-9.01**(compound **KI-3j**) to—**6.8**(Compound **KI-3a).** Except compound KI-3a, KI-3c, KI-3d, KI-3 and KI-3i all compound one hydrogen bond with most of the amino acids. The compound **KI-3j** has three hydrogen bond with most of the amino acids like LEU: 694, PHE: 699, VAL: 702, LYS: 721, ASP: 831, MET: 742.

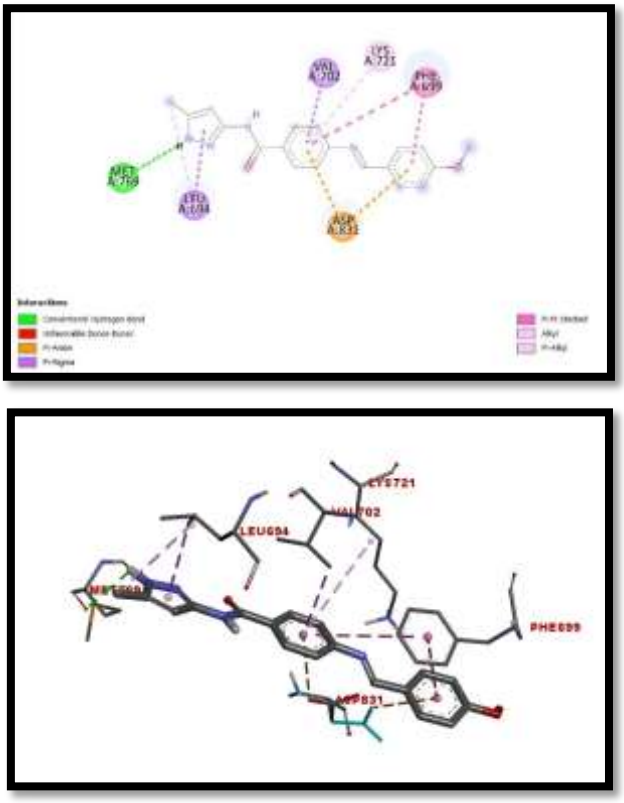
Table.No.4: Insilico EGFR inhibition of novel Pyrazole contain benzamide derivatives KI-(3a-3j). dock scores of the dataset ligands.

Entry	Binding Energy (Kcal/mol)	No of H-bonds	Interacting amino acids	H-bond lengths (Å)
3a	-6.8	Nil	PHE:699, VAL:702, LYS:721, MET:769, LEU:820	-
3b	-8.6	2	LEU:694, PHE:699, VAL:702, LYS:721, MET:769, LEU:820, ASP:831	2.25, 2.95
3c	-7.4	Nil	LEU:694, LYS:721, GLU:738, MET:769, ASP:831	-
3d	-7.6	Nil	PHE:699, VAL:702, LYS:721, LEU:820, ASP:831	-
3e	-8.3	1	LEU:694, PHE:699, VAL:702, LYS:721, MET:769, ASP:831	2.61

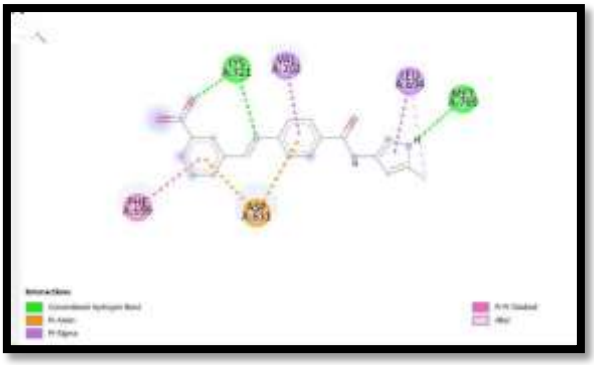
3f	-7.2	Nil	VAL:702, LYS:721, GLU:738, MET:769, ASP:831	-
3g	-8.7	2	LEU:694, PHE:699, VAL:702, LYS:721, GLU:738, MET:769, ASP:831	2.71 3
3h	-8.2	1	LEU:694, PHE:699, VAL:702, LYS:721, MET:769	2.12
3i	-8.1	Nil	LEU:694, VAL:702, LYS:721, GLU:738, ASP:831	-
3j	-9.01	3	LEU:694, PHE:699, VAL:702, LYS:721, ASP:831, MET: 742	2.32, 2.33, 2.89

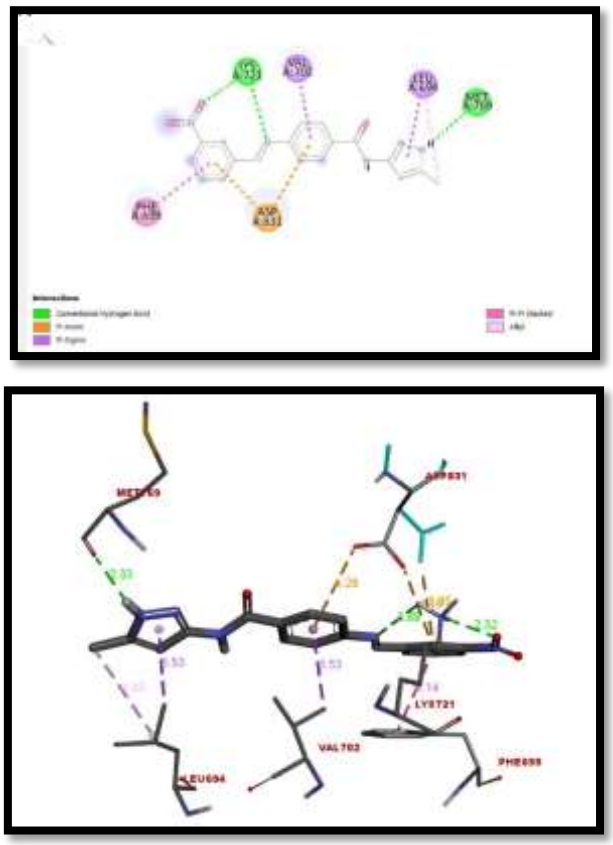


Compound-KI-3b: 2D and 2D docking Poses



Compound-KI-3e: 2D and 2D docking Poses





Compound-KI-3j: 2D and 2D docking Poses

Figure.6. Docking Pose between the Ligand and the Protein (Dock 1 and Dock-2) - Compounds-KI-(3a-3j)

4. CONCLUSION

In this study, we synthesized ten novel Pyrazole contain benzamide derivatives KI-(3a-3j) derivatives by simple conventional method via Shcotten Baumann and Schiff's base mechanism. All these compounds were eco-friendly, non-hazardous and biologically active. The yield of the synthesized compounds was found to be in the range from 75-86% and studied for their in vitro antibacterial and anticancer activity. The antibacterial activity was investigated for their inhibitory action on the growth of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella paratyphi* bacteria, respectively. The results indicated, the compound KI-3c, KI-4d, KI-3i, and KI-3j are showing more activity by comparison with standard drug and the compound KI-3j are showing maximum anticancer activity against MCF-7 cell line. The docking score of the ligands ranged from -9.01 KI-3j to-6.8 KI-3a.

Acknowledgment

Author are thankful to the Director Vishnu Chemicals, Hyderabad for providing spectral analysis including copies.

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