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SYNTHESIS, CHARACTERIZATION, DOCKING STUDIES, AND *IN-VITRO* CYTOTOXIC ACTIVITY OF SOME NOVEL SUBSTITUTED (3,5-DIMETHYL-1H-PYRAZOL-1-YL)(PHENYL)METHANONE DERIVATIVES

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ABSTRACT

The novel set of substituted (3,5-dimethyl-1H-pyrazol-1-yl)(phenyl)methanone derivatives was designed and these compounds were subjected to docking studies against CDK and the result of docking studies revealed that all the compounds possess significant to moderate interaction with the targeted enzyme. Among the docked compounds, compound 17 possesses significant docking score **-8.9** k/cal when compared to standard drug imatinib. And it shows 2 hydrogen bonds between the amino acids THR106 and HIS107. The remaining docked compound shows a docking score range from 7 to 9 k/cal along with one or two hydrogen bond interactions. Based on the docking score the derivatives 2, 4, 5, 6, 12, 14, 15, 16, 17, and 20 were selected for the synthesis by conventional method. These compounds were subjected to *in-vitro* cytotoxicity study by SRB assay method with cell lines MDA-MB-231 cell lines. Among the tested compounds, derivative 16 substituted with Dihydroxy benzene group shows a significant IC_{50} value (65.24 µg/ml) and followed by compound 15 substituted with chlorobenzene derivative (77.35 µg/ml) shows good inhibition in breast cancer cell line. Remaining all other tested compounds shows good to moderate cytotoxic activity on tested cell line.

KEYWORD: (3,5-dimethyl-1H-pyrazol-1-yl) (phenyl)methanone derivatives, docking study, CDK2, SRB assay, MDA-MB-231.

1. INTRODUCTION

One of the major goals of the medicinal chemistry is to treat the life-threatening cancer disease. Cancer is most dangerous to the human health; it causes the second largest death after the cardiovascular diseases. In 2007, 13% of the deaths occurred by cancer over worldwide.[1,2] Cancer has threatened to the developing countries because of changes in the lifestyle. There are about 200 types of cancers that affect the human population. Excessive neovascularization leads to the uncontrolled growth of the cells which is the major reason for cancer and also depends upon the angiogenesis.[3] Ionizing radiations and chemotherapeutics are the two therapies used to therapies to cure the disease and they interfere with cell division and cause the DNA lesions. But they cause damage to the normal cells. Even though much advancement has taken place in cancer research and development of various cancerostatic drugs, but there are two major limitations.[4,5] 1) Lack of selectivity to cancer cells and causes side effects; 2) Development of multidrug resistant anticancer compounds.

The above fact made the scientists to synthesize more and more new target molecules to cure the cancer disease. Now-a-day's extensive research has been devoted to develop the new effective anticancer therapeutics, involving surgical techniques of cancer treatment like radiation and chemotherapy.[6] In the present day's a lot of efforts have been taking place to develop and identify the new novel anti-tumour-specific therapies, which are able to selectively decrease the migration of cancer cells.[7] In cancer biology, many classes of the compounds were tested to get more potent drugs, among them pyrazole derivatives get more attention towards the cancer research. In several attempts, scientists modify's the existing molecules which improve their antitumor activities.[8,9]

In recent years, the chemistry of pyrazole derivatives captivated importance as these compounds have been found to exhibit several biological activities, such as anti-inflammatory, analgesic, antitumor, antibacterial, antihistaminic, schistosomicidal, antiviral, cytotoxic, antiproliferative, CNS depressant, anticonvulsant, HIV-1 reverse transcriptase inhibitor, immunosuppressive agent, pesticidal activity, growth regulator, anti-HIV, antimicrobial, anthelmintic, antileishmanial activity, mixed ligands for serotoninergic 5-HT1a and 5-HT 3 receptors and anti-human rhinovirus (HRV).[10–14] Having such diverse range of pharmacological activities, these classes of compound have attracted medicinal chemists and

consequently a number of strategies were developed based on drug discovery and development have been originated to synthesize them. The **figure 1** shows the newly designed compounds.

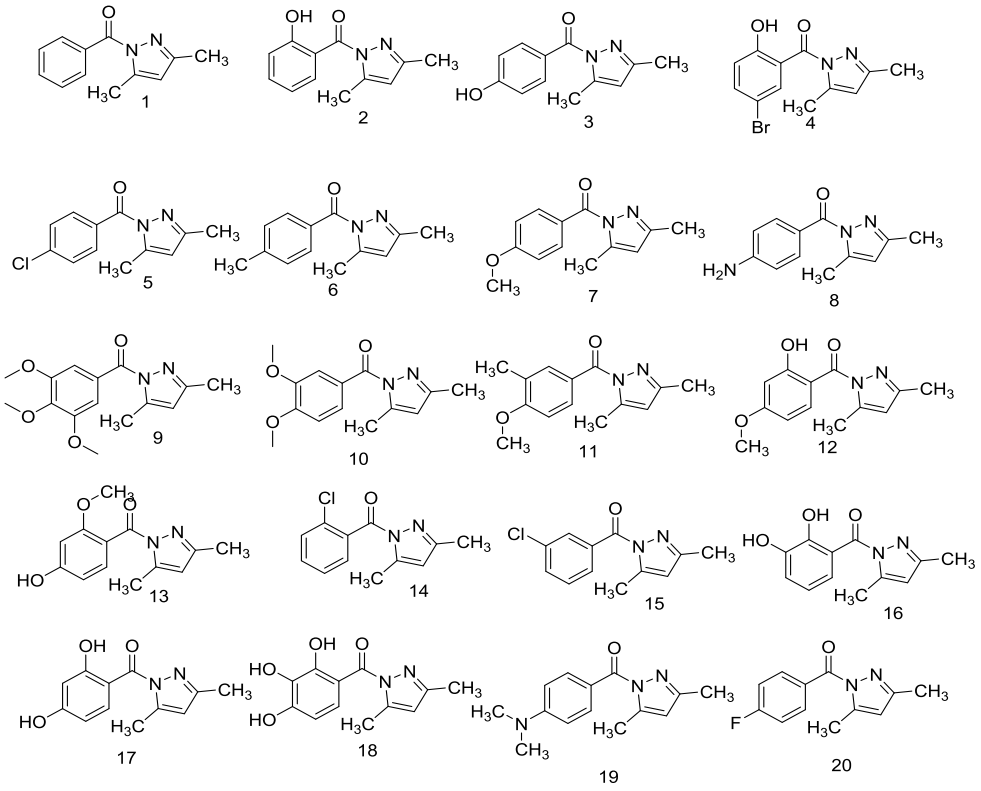


Figure 1: Newly designed compounds.

2. MATERIALS AND METHODS

2.1. Reagents and Instrumentation

Oven dried glass wares were used to perform all the reactions. Procured reagents were of analytical grade and solvents of laboratory grade and purified as necessary according to techniques mentioned in Vogel's Textbook of Practical Organic Chemistry. In an open glass capillary tubes using Veego VMP-1 apparatus, melting points have been determined in ^{0}C and are uncorrected. Ascending TLC on precoated silica-gel plates (MERCK 6 F254) visualized under UV light was utilized to routinely monitor the progress and purity of the synthesized compounds. Solvents used during TLC are n-hexane, ethyl acetate, methanol, petroleum ether, chloroform and dichloromethane. The Infrared Spectra was plotted by Perkin-Elmer Fourier Transform-Infrared Spectrometer and in reciprocal centimetres the band positions are noted. Nuclear magnetic spectra (^{1}H NMR) were obtained from Bruker DRX-300 (300 MHz FT-NMR) spectrophotometer using DMSO as solvent with TMS as the

internal standard ^{13}C NMR have been recorded utilizing Bruker with Dimethyl sulphoxide as solvent. Shimadzu ESI-MS was employed to record Mass Spectra.

2.2. In-silico molecular docking studies

2.2.1. Devices and materials

In the molecular scenario in the modern drug design, the docking is commonly used to understand the interaction between the target ligand-receptor and the target lead molecule's binding orientation with its protein receptor and is quite frequently used to detect the associations between the target components. The research work was done *in-silico* by utilizing bioinformatics tools. Also, we utilize some of the offline programming's like protein data bank (PDB) www.rcsb.org/pdb, PubChem database, Marvin sketch. The molecular docking studies were carried out through Discovery studio.[15]

2.2.2. Preparation of protein

By utilizing the offline program protein data bank (PDB), we take the CDK 2 (PDB ID: 1DI9) which was obtained from PDB website. From the protein we removed the crystal water, followed by the addition of missing hydrogens, protonation, ionization, energy minimization. The SPDBV (swiss protein data bank viewer) force field was applied for energy minimization. Prepared protein is validated by utilizing the Ramachandran plot.[16]

2.2.3. Identification of active sites

Identification of active amino acid present in the protein was detected by using Protein-ligand interaction profile (PLIP) https://plip-tool.biotec.tu-dresden.de/plipweb/plip/index offline tool in google. From this, I found the active amino acid present in the protein.[17]

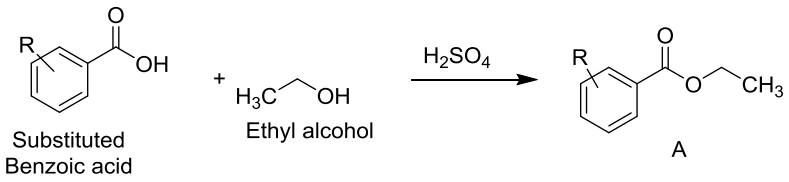
2.2.4. Preparation of Ligands

By utilizing the Marvin sketch tool, the molecules were designed in two and three-dimensional structures. After designed molecule, the structure was optimized in 3D optimization in Marvin sketch and saved as a pdb format.[18]

2.3. Synthesis of designed compounds

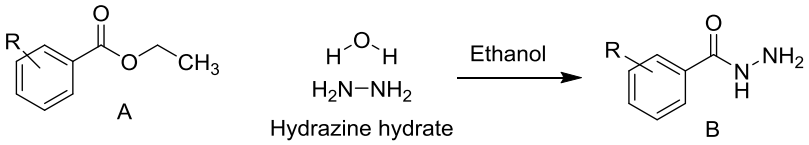
2.3.1. Synthesis of substituted ethyl benzoate derivative (A)

The substituted benzoic acid (0.1mmole) was dissolved in absolute ethanol (60 mL) and to this add 10 mL of H_2SO_4. The reaction mixture was refluxed under water bath for 2hrs. The completion of reaction was monitored by TLC using ethyl acetate and n hexane as a mobile phase.



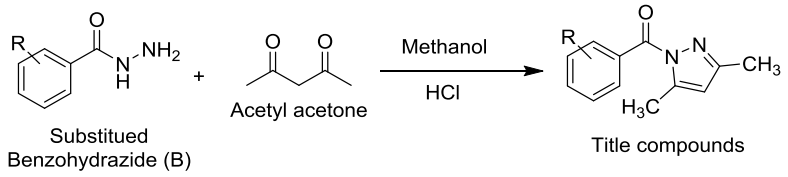
2.3.2. General procedure for the synthesis of substituted benzo hydrazide (B)

Substituted benzoate **A** (0.01 mole) and hydrazine hydrate (0.02 mole) were refluxed in absolute ethanol (50 mL) for 18 hr. The mixture was concentrated, cooled and poured on crushed ice in small portions while stirring and kept for 3-4 hr at room temp. The solid thus separated out was filtered, dried and recrystallized from ethanol.



2.3.3. General procedure for the synthesis of substituted (3,5-dimethyl-1H-pyrazol-1-yl) (phenyl)methanone derivatives

A mixture of acid hydrazide (0.01 mole) and acetyl acetone (0.01 mole) were refluxed in methanol (25 mL), containing concentrated hydrochloric acid (1mL) for 10-12 hr on a water-bath. The resulting solution was then concentrated and cooled at room temp. The solid thus separated was washed with methanol, dried and recrystallized with ethanol.



2.3.3.1. (3,5-dimethyl-1H-pyrazol-1-yl) (2-hydroxyphenyl) methanone (2)

$C_{12}H_{12}N_2O_2$; White solid; MP: 156 – 161^{O}C; % yield: 89%; IR KBr pellet (cm^{-1}): : 3346 (OH stretching); 1676 (C=O stretching ketone); 1455 (C - N bending); 857 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: 7.54 (s, 4H), 7.35 (s, 4H), 7.02 (t, J = 2.7 Hz, 1H), 6.97 (d, J = 37.6 Hz, 7H), 6.67 (s, 4H), 3.78 (s, 4H), 2.43 – 2.39 (m, 12H), 2.27 – 2.23 (m, 12H). ^{13}C NMR (126 MHz, DMSO) δ: 166.02, 139.67, 135.45, 132.00, 129.05, 128.84, 128.14, 124.11, 120.86, 120.75, 8.91. Mass: found 215 m/z; actual 216 m/z.

2.3.3.2. (5-bromo-2-hydroxyphenyl) (3,5-dimethyl-1H-pyrazol-1-yl) methanone (4)

$C_{12}H_{12}BrN_2O_2$; White solid; MP: 162 – 165^{O}C; % yield: 87%; IR KBr pellet (cm^{-1}): 3344 (OH stretching); 1644 (C=O stretching ketone); 1456 (C - N bending); 808 (aromatic ring);

744 (C-Br stretching); ^{1}H NMR (500 MHz, DMSO) δ: 7.77 (s, 1H), 7.53 (s, 1H), 6.82 (s, 1H), 6.67 (s, 1H), 3.74 (s, 1H), 2.43 – 2.39 (m, 3H), 2.25 – 2.21 (m, 3H); ^{13}C NMR (126 MHz, DMSO) δ: 166.02, 164.55, 139.67, 135.45, 133.83, 132.00, 129.05, 128.84, 128.14, 126.08, 124.11, 120.86, 120.75, 24.46, 8.91; Mass: found 294 m/z; actual 294 m/z.

2.3.3.3. (4-chlorophenyl) (3,5-dimethyl-1H-pyrazol-1-yl)methanone (5)

$C_{12}H_{11}ClN_2O$; White solid; MP: 176 – 179^{O}C; % yield: 84%; IR KBr pellet (cm^{-1}): : 3346 (CH stretching); 1678 (C=O stretching ketone); 1455 (C - N bending); 877 (aromatic ring); 678 (C-Cl stretching); ^{1}H NMR (500 MHz, DMSO) δ: 7.75 – 7.60 (m, 2H), 7.54 – 7.40 (m, 2H), 6.76 (s, 1H), 2.45 – 2.41 (m, 3H), 2.29 – 2.25 (m, 3H); ^{13}C NMR (126 MHz, DMSO) δ: 168.50, 156.63, 148.92, 148.39, 131.51, 129.49, 127.56, 126.37, 125.64, 123.24, 121.82, 121.60, 64.38; Mass: found 234 m/z; actual 234 m/z.

2.3.3.4. 3,5-dimethyl-1H-pyrazol-1-yl) (p-tolyl) methanone (6)

$C_{17}H_{15}N_3O_2S$; White solid; MP: 157 – 161^{O}C; % yield: 86%; IR KBr pellet (cm^{-1}): 3194 (CH aromatic); 2985 (CH stretching alkane); 1554 (C=O stretching ketone); 1483 (C - N bending); 810 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: 7.75 – 7.61 (m, 2H), 7.39 – 7.25 (m, 2H), 6.76 (s, 1H), 2.45 – 2.41 (m, 3H), 2.38 – 2.34 (m, 3H), 2.30 – 2.26 (m, 3H); ^{13}C NMR (126 MHz, DMSO) δ: 170.31, 157.06, 155.82, 154.34, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 123.71, 18.59, 11.50; Mass: found 211 m/z; actual 214 m/z.

2.3.3.5. (3,5-dimethyl-1H-pyrazol-1-yl) (2-hydroxy-4-methoxyphenyl) methanone (12)

$C_{13}H_{14}N_2O_3$; White solid; MP: 157 – 161^{O}C; % yield: 89%; IR KBr pellet (cm^{-1}): 3346 (OH stretching); 1644 (C=O stretching ketone): 1418 (C - N bending); 857 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: 7.58 (s, 1H), 6.97 (s, 1H), 6.77 (s, 1H), 6.63 (d, J = 19.4 Hz, 2H), 3.84 – 3.80 (m, 3H), 2.45 – 2.41 (m, 3H), 2.31 – 2.27 (m, 3H); ^{13}C NMR (126 MHz, DMSO) δ: 170.31, 157.06, 155.82, 154.34, 129.99, 129.36, 129.30, 128.79, 128.02, 127.88, 127.26, 127.02, 126.63, 124.45, 123.71, 64.69, 18.59, 11.50; Mass: found 248 m/z; actual 246 m/z.

2.3.3.6. (2-chlorophenyl) (3,5-dimethyl-1H-pyrazol-1-yl) methanone (14)

$C_{12}H_{11}ClN_2O$; White solid; MP: 172 – 174^{O}C; % yield: 81%; IR KBr pellet (cm^{-1}): 3434 (OH stretching); 1615 (C=O stretching ketone): 1433 (C - N bending); 852 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: 9.73 (s, 1H), 7.73 (s, 2H), 7.51 (d, J = 19.8 Hz, 2H), 7.37 (d, J =

22.1 Hz, 1H), 7.20 (t, J = 9.7 Hz, 1H), 7.06 (s, 2H), 6.94 – 6.87 (m, 2H), 5.41 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ: 166.02, 164.55, 135.45, 133.83, 132.00, 129.05, 128.84, 128.14, 126.08, 124.11, 120.86, 120.75, 24.46, 8.91; Mass: found 234 m/z; actual 234 m/z.

2.3.3.7. (3-chlorophenyl) (3,5-dimethyl-1H-pyrazol-1-yl) methanone (15)

$C_{12}H_{11}ClN_2O$; White solid; MP: 176 – 180^{O}C; % yield: 81%; IR KBr pellet (cm^{-1}): 3035 (CH stretching); 1596 (C=O stretching ketone): 1437 (C - N bending); 897 (aromatic ring); 694 (C-Cl stretching).; ^{1}H NMR (500 MHz, DMSO) δ: 9.73 (s, 1H), 7.73 (s, 2H), 7.51 (d, J = 19.8 Hz, 2H), 7.37 (d, J = 22.1 Hz, 1H), 7.20 (t, J = 9.7 Hz, 1H), 7.06 (s, 2H), 6.94 (m, 2H), 5.41 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ: 166.02, 164.55, 135.45, 133.83, 132.00, 129.05, 128.84, 128.14, 126.08, 124.11, 120.86, 120.75, 24.46, 8.91; Mass: found 237 m/z; actual 234 m/z.

2.3.3.8. (2,3-dihydroxyphenyl) (3,5-dimethyl-1H-pyrazol-1-yl) methanone (16)

$C_{12}H_{12}N_2O_3$; White solid; MP: 172 – 174^{O}C; % yield: 81%; IR KBr pellet (cm^{-1}): 3214 (OH stretching); 2948 (CH stretching); 1595 (C=O stretching ketone): 1438 (C - N bending); 897 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: 9.73 (s, 1H), 7.73 (s, 2H), 7.51 (d, J = 19.8 Hz, 2H), 7.37 (d, J = 22.1 Hz, 1H), 7.20 (t, J = 9.7 Hz, 1H), 7.06 (s, 2H), 6.94 (m, 2H), 5.41 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ: 168.50, 156.63, 148.92, 148.39, 125.64, 121.60, 109.19, 106.92, 106.34, 102.06, 21.20; Mass: found 233 m/z; actual 232 m/z.

2.3.3.9. (2,4-dihydroxyphenyl) (3,5-dimethyl-1H-pyrazol-1-yl) methanone (17)

$C_{12}H_{12}N_2O_3$; White solid; MP: 172 – 174^{O}C; % yield: 81%; IR KBr pellet (cm^{-1}): 3217 (OH stretching); 2548 (CH stretching); 1595 (C=O stretching ketone): 1438 (C - N bending); 897 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: 7.41 (s, 1H), 6.68 (s, 1H), 6.49 (d, J = 12.6 Hz, 2H), 4.67 (s, 1H), 3.77 (s, 1H), 2.43 – 2.39 (m, 3H), 2.26 – 2.22 (m, 3H).; ^{13}C NMR (126 MHz, DMSO) δ: 168.50, 156.63, 148.92, 148.39, 125.64, 121.60, 109.19, 106.92, 106.34, 102.06, 21.20; Mass: found 233 m/z; actual 232 m/z.

2.3.3.10. (3,5-dimethyl-1H-pyrazol-1-yl) (4-fluorophenyl) methanone (20)

$C_{12}H_{11}FN_2O_3$; White solid; MP: 172 – 174^{O}C; % yield: 81%; IR KBr pellet (cm^{-1}): 3217 (OH stretching); 2548 (CH stretching); 1595 (C=O stretching ketone): 1438 (C - N bending); 897 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ: 2991 (CH stretching); 1658 (C=O stretching ketone): 1424 (C - N bending); 848 (aromatic ring); 795 (C – F stretching); ^{13}C NMR (126

MHz, DMSO) δ: 170.30, 155.81, 154.37, 134.50, 130.00, 129.31, 128.03, 127.26, 127.03, 124.45, 119.01, 21.72; Mass: found 218 m/z; actual 218 m/z.

2.4. *In vitro* anticancer activity

The *in vitro* cytotoxicity of the synthesized compounds were assessed against MCF cancer cell line using SRB assay. The monolayer culture of the cell line was trypsinized, followed by adjusting the cell count to 1.0 × 105 cells/ mL by means of DMEM medium containing 10% FBS. The diluted cell suspension (0.1 mL) was added to each well of the 96-well microtiter plate. The test wells were added with various concentrations (100 µL) of test samples, and the control wells received media (100 µL). The plates were then incubated at 37 °C for 72hr in 5% CO_2 atmosphere. After this duration, the cultures were fixed with trichloroacetic acid (25 µL, 10% w/v) and stained for 30 min with sulforhodamine B (0.4% w/v) in acetic acid (1% v/v). Unbound dye was cleared by four washes with acetic acid (1% v/v), and protein-bound dye was extracted with 10 mM unbuffered Trisbase [tris (hydroxymethyl) aminomethane]. The optical density of the protein-bound dye was recorded at 540 nm. The percentage cell viability (CV) was calculated using the following formula:

$$\text{Cell viability} = \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

The concentration of test samples required to inhibit cell growth by 50% was tabulated from the dose–response for each cell line.

3. RESULTS AND DISCUSSION

3.1. Molecular docking

Discovery studio software was utilised in this study to dock 25 pyrazole derivatives and imatinib against CDK2 (PDB ID: 1DI9). A single rigid crystal structure of an enzyme is acquired from the Protein Data Bank (PDB) for docking studies, which may be accessed at the URL (http://www.rscb.org/pdb). Before docking the screened ligands into the protein active site, the protein was configured for grid production by removing the substrate cofactor and the crystallographically identified water molecules. The structure of pyrazole derivates was drawn in ChemBio Draw Ultra 12.0 software and loaded into the docking workspace in 'pdb' format. To achieve accurate predictions, the imported structures must be appropriately prepared, which means that the atom connectivity and bond ordering are correct, and partial atomic charges are assigned. PDB files commonly include inaccurate or absent explicit hydrogen assignment, despite the fact that the PDB file format is incapable of storing bond

order information. The binding location specifies the area of interest where the docking technique will look for probable postures (ligand conformations).

The *in-silico* docking study of the 20 designed molecules to the enzyme's active sites were performed by the C dock module of Discovery studio to determine the binding affinities of the ligands. The Docking scores of docking studies against CDK2 (PDB ID: 1DI9) are shown in **Table 1**. From the in-silico docking results, it is evident that the interactions are mainly lipophilic factors due to the presence of aromatic heterocyclic rings. Among the docked compounds, compound 17 possesses significant docking score **-8.9** k/cal when compared to standard drug imatinib. The compound 17 shows 2 hydrogen bonds between the amino acids THR106 and HIS107. The compound 2 shows a significant docking score of – 8.5 k/cal along with 2 hydrogen bonds with amino acids ALA51 and THR106. The remaining docked compound shows a docking score range from 7 to 9 k/cal along with one or two hydrogen bond interactions. **Figures 2 - 10** shows the docking pose of the designed compounds. Based on the docking score the derivatives 2, 4, 5, 6, 12, 14, 15, 16, 17, and 20 are selected for the synthesis by conventional method.

Table 1: Docking score of designed compounds.

Compound code	Binding Affinity
1	-7.8
2	**-8.5**
3	-7.8
4	**-8.2**
5	**-8.1**
6	**-8**
7	-7.9
8	-7.7
9	-7.3
10	-7.7
11	-7.8
12	**-8.3**
13	-7.8
14	**-8.3**
15	**-8.1**
16	**-8.1**
17	**-8.9**
18	-7.8
19	-7.8
20	**-8.1**
Standard	-9.58

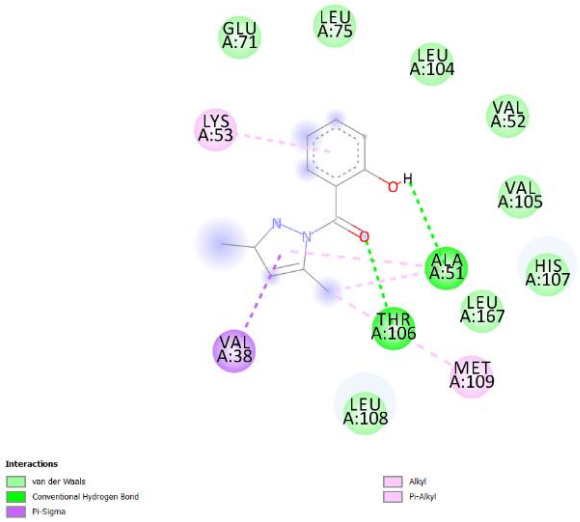


Figure 2: 2D docking pose of compound 2.

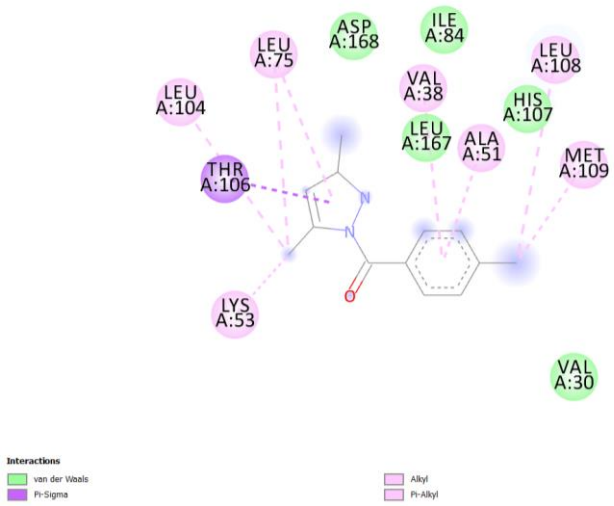


Figure 3: 2D docking pose of compound 5.

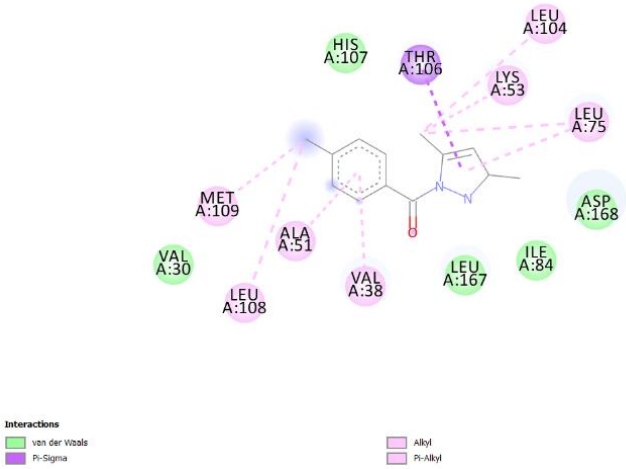


Figure 4: 2D docking pose of compound 5.

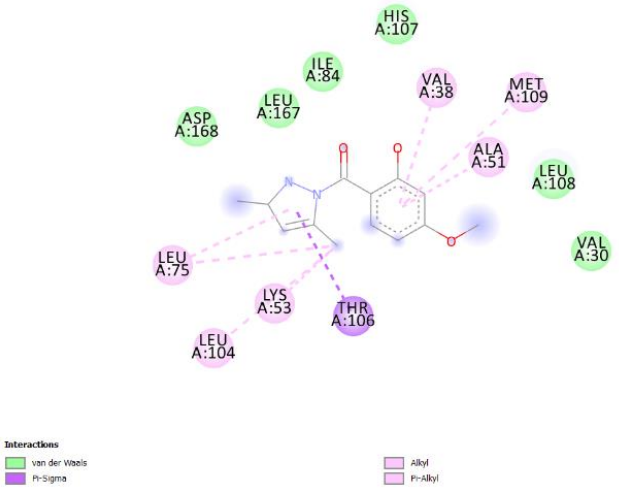


Figure 5: 2D docking pose of compound 12.

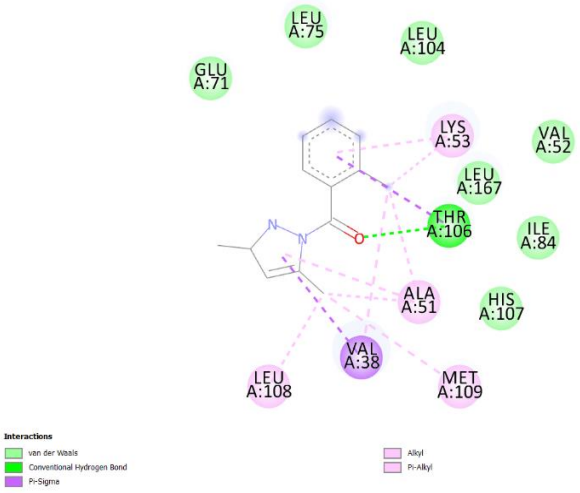


Figure 6: 2D docking pose of compound 14.

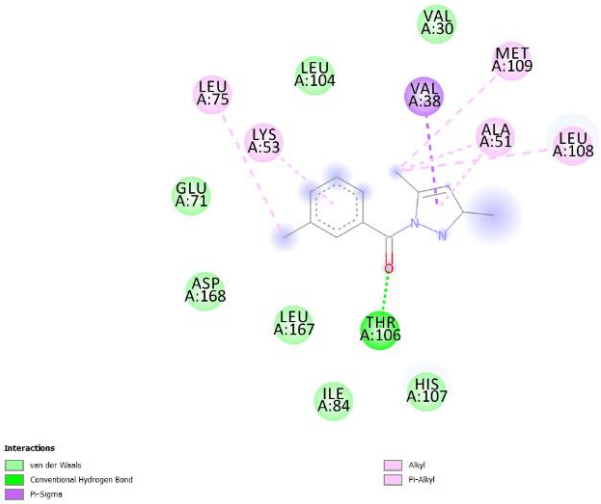


Figure 7: 2D docking pose of compound 15.

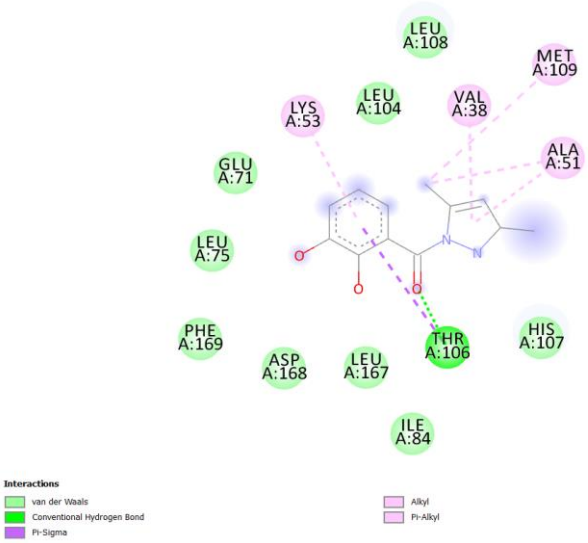


Figure 8: 2D docking pose of compound 16.

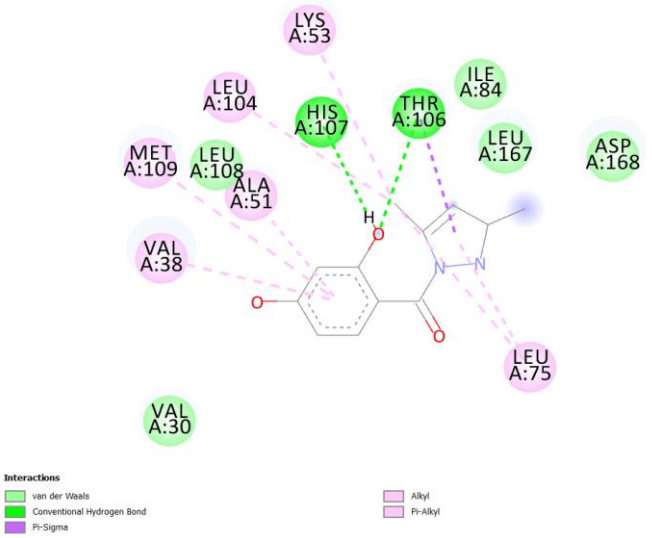


Figure 9: 2D docking pose of compound 17.

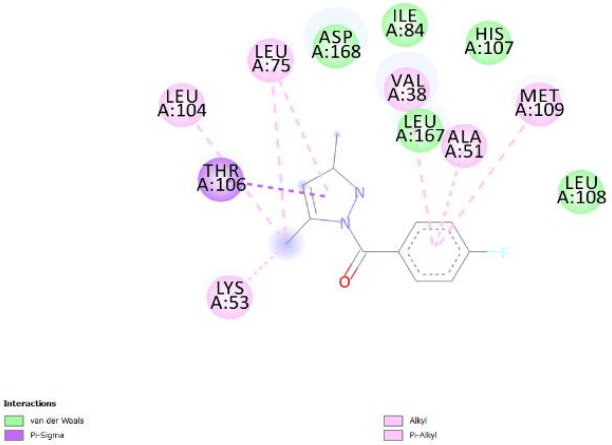


Figure 10: 2D docking pose of compound 20.

3.2. Synthetic work

The substituted benzoic acid (A) was dissolved in absolute ethanol (60ml) to this add 10 mL of H_2SO_4. The reaction mixture was refluxed under water bath for 2hrs. The completion of reaction was monitored by TLC using ethyl acetate and n hexane as a mobile phase. Further compound A react with hydrazine hydrate and it was refluxed in absolute ethanol for 18 hr. The mixture was concentrated, cooled and poured on crushed ice in small portions while stirring and kept for 3-4 hr at room temp. The solid of acid hydrazide (B) was separated out was filtered, dried and recrystallized from ethanol. The compound B react with acetyl acetone (0.01 mole) was refluxed in methanol (25 mL), containing concentrated hydrochloric acid (1mL) for 10-12 hr on a water-bath. The resulting solution was then concentrated and cooled at room temp. The solid corresponding title compounds were obtained. The IR spectrum of the final synthesized compounds showed absorption bands around 2850 – 2950 shows the presence of CH stretching alkane group, the peak 2030 shows presence of CH stretching aromatic and the peak at 1600-1500 cm^{-1} shows the C=O group, 1489–1464 cm^{-1} for NH bending, 800–700 cm^{-1} for aromatic rings, and 750 – 650 cm^{-1} for carbon – halogen stretching group. . These compounds also exhibited appropriate peaks at corresponding ppm in their ^{1}H NMR spectra. The ^{1}H NMR spectra of the synthesized compounds revealed a singlet signal a signal at 7.5-8.5 for H of aromatic ring. The ^{13}C NMR spectra of synthesized compounds revealed a signal at 160 – 175 for carbonyl carbon, and a signal at 120 – 145 for aromatic carbon. The corresponding molecular ion peaks in the LC–MS spectra were in conformity with the assigned structures. All the synthesized compounds were subjected to *in vitro* anti-cancer studies.

3.3. *In-vitro* anticancer activity

Results of anticancer activity of the compounds were expressed as IC_{50} values which were determined by plotting the percentage cell viability versus concentration of sample on a logarithmic graph and reading off the control. The experiments were performed in triplicates, and then, the final IC_{50} values were calculated by taking average of triplicate experimental results. The results of *in-vitro* anti-cancer activity expressed in IC_{50} (µg/mL) are expressed in **table 2** and were compared to Daxorubicin. There are 10 compounds are subjected to *in-vitro* cytotoxicity study by SRB assay method with cell lines MDA-MB-231 cell lines. All the tested compounds displayed an IC_{50} > 125 µg/mL at a concentration range of 30–250 µg/mL. Among the tested compounds, derivative 16 substituted with Dihydroxy benzene group shows a significant IC_{50} value (65.24 µg/ml) and followed by compound 15 substituted with

chloro benzene derivative (77.35 µg/ml) shows good inhibition in breast cancer cell line. Remaining all other tested compounds shows good to moderate cytotoxic activity on tested cell line.

Table 2: Data for *in vitro* cell line study.

Sl No	Compound code	MDA-MB-231 (IC_{50} µg/ml)
1	2	81.49
2	4	90.02
3	5	100.36
4	6	124.61
5	12	98.42
6	14	80.64
7	15	77.35
8	16	65.24
9	17	102.35
10	20	99.65
11	DOX	23.14

4. SUMMARY AND CONCLUSION

The synthesis of titled compounds were achieved by following synthetic routes as shown in the scheme. The synthesis of title compounds were achieved in good yield by simple techniques. It was chemically stable and available in good yield. The sharp melting point and unique spot-on TLC indicated that title compounds were obtained in pure form. The synthesized Compounds were purified by successive recrystallization from the appropriate solvents. These compounds also exhibited appropriate peaks at corresponding δ ppm in their ^{1}H-NMR spectra and corresponding molecular ion peaks in ESI-MS spectra which conformed with the assigned structures. The interpretation of IR, ^{1}H NMR, and ESI-MS spectra confirmed the structure of the title compounds. All the designed compounds were subjected to molecular docking studies using discovery studio software. All the studied compounds showed the significant docking score and which was is compared with standard drug Doxorubicin. All the synthesized compounds were subjected to in vitro anti-cancer activity by SRB assay using MDA-MD-231 cell line studies. It could be concluded from the present investigation that the substituted pyrazole derivative possess the most potent anticancer molecules.

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