**RESEARCH PAPER**

# Design, Synthesis, Characterization, Molecular Docking Studies and Evaluation of Anticancer Activities of Pyrazole Fused Novel Indole-2-One Derivatives

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## ABSTRACT
In this study, a new series of pyrazole fused novel Indole-2-one derivatives (II-VI_(a-o)) by conventional method via Schiff's and Mannich base mechanism. Novel 3-(3-((1H-pyrazol-4-yl)imino)-2-oxoindolin-1-yl)-N,3-diphenylpropanamide derivatives were prepared by two steps. In step-1, substituted Isatins were reacted with 4-amino pyrazole via Schiff's base reaction to give pyrazol-4-yl)imino)-5-chloroindolin-2-one(IV). It can undergo Mannich base reaction with phenylacetamide and substituted benzaldehyde(V) to get title compounds. The synthesized compound's structure was confirmed by IR, $^1$H NMR, and Mass spectroscopy. Anticancer activity against two cancer cell lines (MCF-7 and SKOV3) were evaluated using MTT assay method. Compounds II-VI_c and II-VI_f showed broad spectrum anticancer activity on the two tested cell lines with $IC_{50}$ values compared with standard. Doxorubicin was used as a standard reference drug. The anticancer activity results shown that compound II-VI_c with $IC_{50}$ values of 20.21 ± 0.021 µg (MCF-7) and 25.97 ± 0.023 µg (SKOV3) and compound II-VI_f with $IC_{50}$ values of 37.26 ± 0.001 µg (MCF-7) and 24.56 ± 0.002 µg (SKOV3) respectively. Additionally, Molecular docking studies were conducted to investigate the binding mode, amino acid interactions and free binding energy of these potent derivatives. Notably compounds II-VI_c and f exhibited highest amino acid bonding interactions like PHE: 699, LYS:721, LEU: 764, LEU:694, ASP:831, VAL:702, CYS:773, ARG:817.

**Keywords:** Pyrazole, Indole-2-one, Anticancer activity, Molecular docking, MCF-7 and SKOV3 Cell lines, Doxorubicin.
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## 1. INTRODUCTION

Indole or Indole-2-one, a heterocyclic compound with the molecular formula $C_8H_7N$, is a planar molecule with ten pi electrons and a fused benzene ring and pyrrole ring. The molecule is weakly basic because of the the delocalization of the lone pair electrons on the nitrogen atom [1]. Indole derivatives demonstrated an extensive variety of pharmacological activities, among them antihypertensive (reserpine, yohimbine, pindolol)[4, antihistamines (zafirlukast, ondansetron, dolasetron), anticancer (vincristine, adozelesin, carzelesin, intoplicine), antidepressant (binedaline, trandolapril, siramesine, amedalin), antipsychotic (psilocybin), and many other functions have been identified reported with this compound [2-6]. Proteins, amino acids, and alkaloids (ajmalicine, strychnine) were natural sources of indole derivatives [7, 8]. Cancer has become one of the top two causes of death worldwide mainly because of ecological shifts and degradation in the environment [9].Owing to

the tremendous toxicity and resistance of existing therapies, there is an urgent need for the development of specific and effective anticancer medicines. Current research mainly concentrates on therapeutic targets involved in cell proliferation since anticancer drugs often exert their biological effect through a number of intracellular targets [10-12].One of the challenges though, is identifying the exact target for a given class of chemicals [13]. According to predictions, breast cancer is one of the most common cause of cancer-related deaths for women around the world and has the highest diagnosis rate of any malignancy [14].However, at the present time, gene expression analyses significantly affect the understanding of the biology and molecular analysis of breast cancer, contributing to information that is therapeutically useful and targeted therapy [15]. Indole derivatives was given a lot of attention in the field of pharmaceutical/medicinal chemistry [16-18] owing to their natural occurrence and pharmacological activities, among which are antitumor, anticonvulsant, anti-inflammatory, antioxidant, antibacterial, insecticidal, anti-tuberculosis, antihistaminic, antifungal, anti-HIV, lipoxygenase inhibitor, and cardiovascular. Several indole alkaloids are thought of as one of the most rapidly developing classes of marine invertebrate metabolites owing to their broad spectrum of biological activity.

In previous decades, the technique of identifying new drugs included lengthy, in-vivo investigations and time-consuming, non-costly synthetic trials. The public requires a more cost-efficient approach to drug design in order to be given safer, more effective medications, and insilico drug designing constitutes a breakthrough in this regard [19].High-throughput screening (HTS), structure-based (target structure, pharmacophore modeling, ligand docking, de novo design, molecular dynamics), and ligand-based CADD (QSAR, ligand-based virtual screening) constitute a few of the computational methods that are used in these in-silico investigations.The structure-based drug design technique known as "molecular docking" includes determining the conformation of a small molecule with biological proteins and the binding energy of interactions between molecules. Knowing that the interaction of a compound at its molecular levels is made accessible by the development of computational methods that introduced a range of algorithms that can generate a larger number of sample ligands for the development, supported by a suitable scoring function that estimates the binding affinity of the biomolecule with sample ligands, and the involvement of different docking methodologies (rigid docking and flexible docking) [20]. In drug discovery, a wide range of computational techniques have been used to analyze a prospective drug molecule, includes drug-likeness, fragment-based virtual screening, bioactivity prediction, and toxicity studies that have to be performed by several software programs.

## 2. MATERIALS AND METHODS

Chemdraw was utilized to draw the chemical structure, and Chem3D Pro was employed to optimize that by setting the Minimum RMS Gradient set 0.010. ACD/Chemsketch software has been employed to generate the SMILES notation. All solvents and reagents were freshly distilled and purified according to standard procedures. All melting points are recorded on digital Gallen Kamp MFB-765 instrument. Thin-layer chromatography was utilized to track their development during the synthesis and test for impurities in the synthesized derivative. Because this method employs TLC plate, Aluminum-backed Silica gel 60 F254 sheet and mobile phase (n-Hexane, ethyl acetate, 8:2). Column chromatography was performed using 100-200 mesh silica gel. The IR spectra (KBr) $cm^{-1}$ were measured on a Thermo Nicolet Nexus 670 spectrophotometer.Mass spectrometry was performed using a Shimadzu LCMS-8030 with electron spray ionization.$^{1}$H NMR spectra (δ, ppm) were obtained in DMSO-$d_6$(deuterated dimethyl sulfoxide) at 500 MHz, it was recorded on a Bruker/TopSpin 3.2 spectrometer.

**General procedures**

**Step-I: Synthesis of -((1H-pyrazol-4-yl)imino)-5-chloroindolin-2-one(IV):** The compound substituted Isatin (0.01 mol) was taken in a mixture of 4-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 (Hexane: EtoAc 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 [21].

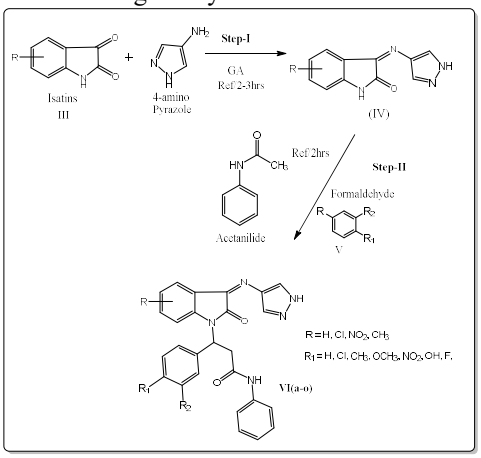


**Figure. No.1. Schematic representation of pyrazole fused novel Indole-2-one derivatives-II-V_(a-o)**

**Step-II: Synthesis of Pyrazole fused novel Indole-2-one derivatives VI(a-l):** To prepare the mixture of substituted benzaldehyde (0.01mol), Compound III (0.01 mol) and phenylacetamide (0.01 mol) in ethanol (30ml), the reaction mixture was refluxed for 2-3 h. The reaction mixture was cooled and poured into ice-cold water. The precipitate was collected by filtration. The precipitate was dried and recrystallized from absolute ethanol. The above procedure was followed by all the remaining compounds [22].

## 3.Results and Discussion

### Chemistry:

The design of pyrazole fused novel Indazole-2-one was based on the EGFR inhibitors to evaluate ther anticancer activity. Novel 3-(3-((1H-pyrazol-4-yl)imino)-2-oxoindolin-1-yl)-N,3-diphenylpropanamide derivatives were prepared by two steps. In step-1, substituted Isatins were reacts with 4-amino pyrazole via Schiff's base reaction to give pyrazol-4-yl)imino)-5-chloroindolin-2-one(IV). It can be undergone mannich base reaction with phenylacetamide and substituted benzaldehyde(V) to get title compounds.

**Table. No. 1. Physical characterization of Pyrazole fused Novel Indole-2-one derivatives-II-VI(a-o)**

| Compounds | Mol. Formula | R | R1 | R2 | Mol. Weight(g) | M.P($^0$C) | Rf value | % Yield |
|---|---|---|---|---|---|---|---|---|
| **II-VI-a** | $C_{27}H_{25}N_5O_2$ | H | H | H | 451.20 | 213-215 | 0.67 | 82 |
| **II-VI-b** | $C_{27}H_{24}ClN_5O_2$ | Cl | H | H | 485.16 | 187-189 | 0.72 | 78 |
| **II-VI-c** | $C_{27}H_{24}N_6O_4$ | $NO_2$ | H | H | 496.19 | 235-237 | 0.69 | 76 |
| **II-VI-d** | $C_{28}H_{27}N_5O_2$ | $CH_3$ | H | H | 465.22 | 217-219 | 0.76 | 84 |
| **II-VI-e** | $C_{28}H_{26}ClN_5O_2$ | $CH_3$ | Cl | H | 499.15 | 237-239 | 0.66 | 79 |
| **II-VI-f** | $C_{29}H_{29}N_5O_2$ | $CH_3$ | $CH_3$ | H | 479.23 | 175-177 | 0.81 | 83 |
| **II-VI-g** | $C_{28}H_{26}N_6O_4$ | $CH_3$ | $NO_2$ | H | 510.21 | 209-211 | 0.72 | 81 |
| **II-VI-h** | $C_{28}H_{26}FN_5O_2$ | $CH_3$ | F | H | 483.21 | 165-167 | 0.83 | 75 |
| **II-VI-i** | $C_{28}H_{27}N_5O_3$ | $CH_3$ | OH | H | 481.22 | 201-203 | 0.74 | 83 |
| **II-VI-j** | $C_{29}H_{29}N_5O_3$ | $CH_3$ | $OCH_3$ | H | 495.23 | 231-233 | 0.76 | 80 |
| **II-VI-k** | $C_{28}H_{27}N_5O_3$ | H | $OCH_3$ | H | 481.21 | 213-215 | 0.66 | 77 |
| **II-VI-l** | $C_{28}H_{27}N_5O_2$ | H | $CH_3$ | H | 465.22 | 210-212 | 0.73 | 81 |
| **II-VI-m** | $C_{27}H_{22}ClN_5O_2$ | Cl | $CH_3$ | H | 483.14 | 239-24 | 0.67 | 76 |

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|---|---|---|---|---|---|---|---|---|
| | | | | | | 1 | | |
| **II-VI-n** | $C_{27}H_{22}ClN_5O_3$ | Cl | $OCH_3$ | H | 499.12 | 227-229 | 0.72 | 82 |
| **II-VI-o** | $C_{28}H_{25}N_5O_4$ | H | $OCH_3$ | $OCH_3$ | 495.19 | 247-249 | 0.82 | 78 |

**Spectral Characterization**

**Compound-II-VI_a:(Z)-3-(3-((1H-pyrazol-4-yl)imino)-2-oxoindolin-1-yl)-N,3-diphenylpropanamide.**IR($Vcm^{-1}$):3326(-NH Str in pyrazole); 3025(-CH Str in Ar-CH); 2965, 2865(-CH Str in Aliphatic-CH); 1705(-CO Str in Indole-CO); 1603(-C=N Str in Imine); 1421(-C=C Str in Ar-C); 1056(-C-N Str). $^1$HNMR(DMSO)δpp: 12.5647(s., 1H, Pyrazole-NH proton); 9.0352(s, 1H, Acetamide-NH); 8.2873-8.2019(s, 2H, pyrazole-H); 8.0453-8.0021(d, 2H, Ar-H); 7.9854-7.9342(d, 2H, Ar-H); 7.8433-7.7983(d, 2H, Ar-H); 7.5965-7.5340(t, 2H, Ar-H); 7.3874-7.3054(t, 3H, Ar-H); 7.1378-7.0032(t, 3H, Ar-H); 4.5232-4.4893(d, 2H, Acetamide-$CH_2$); 2.2832-2.2056(t, 1H, N-CH proton). Mass (LC-MS); m/z 451.20(M); 452.32(M+1).

**Compound-II-VI_b:Z)-3-(3-((1H-pyrazol-4-yl)imino)-5-chloro-2-oxoindolin-1-yl)-N,3-diphenylpropanamide:** IR($Vcm^{-1}$):3423(-NH Str in pyrazole); 3052(-CH Str in Ar-CH); 2965, 2887(-CH Str in Aliphatic-CH); 1726(-CO Str in Indole-CO); 1610(-C=N Str in Imine); 1434(-C=C Str in Ar-C); 1076(-C-N Str); 798(-Cl Str in Ar-Cl). $^1$HNMR(DMSO)δpp: 12.9743(s., 1H, Pyrazole-NH proton); 9.3452(s, 1H, Acetamide-NH); 8.3452-8.3023(s, 2H, pyrazole-H); 8.0432(s, 1H, Ar-H); 7.9546-7.9196(d, 2H, Ar-H); 7.8543-7.8102(d, 2H, Ar-H); 7.6754-7.6109(d, 2H, Ar-H); 7.3984-7.3128(t, 3H, Ar-H); 7.2903-7.2001(t, 3H, Ar-H); 4.2783-4.2187(d, 2H, Acetamide-$CH_2$); 2.3874-2.3185(t, 1H, N-CH proton). Mass(LC-MS); m/z 485.16(M); 485.32(M+1); 486.31(M+2).

**Compound-II-VI_c:Z)-3-(3-((1H-pyrazol-4-yl)imino)-5-nitro-2-oxoindolin-1-yl)-N,3-diphenylpropanamide.** IR($Vcm^{-1}$):3387(-NH Str in pyrazole); 3016(-CH Str in Ar-CH); 2969, 2874(-CH Str in Aliphatic-CH); 1709(-CO Str in Indole-CO); 1645(-NO Str in Ar-$NO_2$); 1615(-C=N Str in Imine); 1423(-C=C Str in Ar-C); 1086(-C-N Str). $^1$HNMR(DMSO)δpp: 11.9873(s., 1H, Pyrazole-NH proton); 9.6594(s, 1H, Acetamide-NH); 8.3097-8.302(s, 2H, pyrazole-H); 8.1872(s, 1H, Ar-H); 7.8976-7.8263(d, 2H, Ar-H); 7.7654-7.7283(d, 2H, Ar-H); 7.5987-7.5231(d, 2H, Ar-H); 7.4035-7.4003(t, 3H, Ar-H); 7.3674-7.3162(t, 3H, Ar-H); 4.3564-4.32187(d, 2H, Acetamide-$CH_2$); 2.4233-2.4052(t, 1H, N-CH proton). Mass (LC-MS); m/z 496.19(M); 497.18(M+1).

**Compound-II-VI_d:(Z)-3-(3-((1H-pyrazol-4-yl)imino)-5-methyl-2-oxoindolin-1-yl)-N,3-diphenylpropanamide:** IR($Vcm^{-1}$):3287(-NH Str in pyrazole); 3065(-CH Str in Ar-CH); 2946,2895, 2765(-CH Str in Aliphatic-CH); 1715(-CO Str in Indole-CO); 1608(-C=N Str in Imine); 1413(-C=C Str in Ar-C); 1078(-C-N Str). $^1$HNMR(DMSO)δpp: 12.0093(s., 1H, Pyrazole-NH proton); 9.5674(s, 1H, Acetamide-NH); 8.2983-8.2155(s, 2H, pyrazole-H); 8.0954(s, 1H, Ar-H); 7.9564-7.9034(d, 2H, Ar-H); 7.8954-7.8167(d, 2H, Ar-H); 7.6743-7.6176(d, 2H, Ar-H); 7.3984-7.3712(t, 3H, Ar-H); 7.2674-7.2104(t, 3H, Ar-H); 4.4994-4.4102(d, 2H, Acetamide-$CH_2$); 2.3984-2.3812(t, 1H, N-CH proton); 2.0132(s, 3H, Ar-$CH_3$). Mass(LC-MS); m/z 465.22(M); 466.87(M+1).

**Compound-II-VI_e: (Z)-3-(3-((1H-pyrazol-4-yl)imino)-5-methyl-2-oxoindolin-1-yl)-3-(4-chlorophenyl)-N-phenylpropanamide:** IR($Vcm^{-1}$):3420(-NH Str in pyrazole); 3065(-CH Str in Ar-CH); 2941, 2856, 2795(-CH Str in Aliphatic-CH); 1723(-CO Str in Indole-CO); 1613(-C=N Str in Imine); 1423(-C=C Str in Ar-C); 1093(-C-N Str); 802(-Cl Str in Ar-Cl). $^1$HNMR(DMSO)δpp: 12.3244(s., 1H, Pyrazole-NH proton); 9.6823(s, 1H, Acetamide-NH); 8.3422-8.3021(s, 2H, pyrazole-H); 8.0956(s, 1H, Ar-H); 7.8797-7.8453(d, 2H, Ar-H); 7.6843-7.6322(d, 2H, Ar-H); 7.5673-7.5203(d, 2H, Ar-H); 7.4533-7.4032(d, 2H, Ar-H); 7.3103(t, 3H, Ar-H); 4.3875-4.3210(d, 2H, Acetamide-$CH_2$); 2.2897-2.2564(t, 1H, N-CH proton); 2.0956(s, 3H, Ar-$CH_3$). Mass (LC-MS); m/z 499.15(M); 500.32(M+1); 501.04(M+2).

**Compound-II-VI_f:(Z)-3-(3-((1H-pyrazol-4-yl)imino)-5-methyl-2-oxoindolin-1-yl)-N-phenyl-3-(p-tolyl) propenamide:**

IR($Vcm^{-1}$):3486(-NH Str in pyrazole); 3027(-CH Str in Ar-CH); 2968, 2854, 2791(-CH Str in Aliphatic-CH); 1717(-CO Str in Indole-CO); 1604(-C=N Str in Imine); 1428(-C=C Str in Ar-C); 1058(-C-N Str). $^1$HNMR(DMSO)δpp: 11.9562(s., 1H, Pyrazole-NH proton); 9.5923(s, 1H, Acetamide-NH); 8.2892-8.2187(s, 2H, pyrazole-H); 8.1673(s, 1H, Ar-H); 7.9543(d, 2H, Ar-H); 7.7853-7.7213(d, 2H, Ar-H); 7.6932-7.6432(d, 2H, Ar-H); 7.3984-7.3093(d, 2H, Ar-H); 7.2873-7.2197(t, 3H, Ar-H); 4.4865-4.4274(d, 2H, Acetamide-$CH_2$); 2.4093-2.4001(t, 1H, N-CH proton); 2.005-1.9934(s, 6H, Ar-$CH_3$). Mass (LC-MS); m/z 479.23(M); 480.31(M+1).

**Compound-II-VI_g:(Z)-3-(3-((1H-pyrazol-4-yl)imino)-5-methyl-2-oxoindolin-1-yl)-3-(4-nitrophenyl)-N-phenylpropanamide:**IR($Vcm^{-1}$):3482(-NH Str in pyrazole); 3025(-CH Str in Ar-CH); 2973, 28612(-CH Str in Aliphatic-CH); 1721(-CO Str in Indole-CO); 1632(-$NO_2$ Str in Ar-$NO_2$); 1607(-C=N Str in Imine); 1442(-C=C Str in Ar-C); 1038(-C-N Str). $^1$HNMR(DMSO)δpp: 12.3874(s., 1H, Pyrazole-NH proton); 9.4893(s, 1H, Acetamide-NH); 8.1987-8.1563(s, 2H, pyrazole-H); 8.0934(s, 1H, Ar-H); 7.8965-7.8342(d, 2H, Ar-H); 7.6984-7.6453(d, 2H, Ar-H); 7.5974-7.5013(d, 2H, Ar-H); 7.4035-7.3956(d, 2H, Ar-H); 7.2973-7.2164(t, 3H, Ar-H); 4.6435-4.5934(d, 2H, Acetamide-$CH_2$); 2.5894-2.4895(t, 1H, N-CH proton); 2.1873(s, 3H, Ar-$CH_3$). Mass (LC-MS); m/z 510.21(M); 511.32(M+1).

**Compound-II-VI_h:(Z)-3-(3-((1H-pyrazol-4-yl)imino)-5-methyl-2-oxoindolin-1-yl)-3-(4-fluorophenyl)-N-phenylpropanamide.**IR($Vcm^{-1}$):3386(-NH Str in pyrazole); 3092(-CH Str in Ar-CH); 2968, 2887(-CH Str in Aliphatic-CH); 1711(-CO Str in Indole-CO); 1618(-C=N Str in Imine); 1453(-C=C Str in Ar-C); 1265(-F Str in Ar-F); 1076(-C-N Str). $^1$HNMR(DMSO)δpp: 13.0243(s., 1H, Pyrazole-NH proton); 9.5634(s, 1H, Acetamide-NH); 8.3764-8.3209(s, 2H, pyrazole-H); 8.1253(s, 1H, Ar-H); 8.0354-8.0012(d, 2H, Ar-H); 7.8543-7.8102(d, 2H, Ar-H); 7.6874-7.6102(d, 2H, Ar-H); 7.5432-7.5023(d, 2H, Ar-H); 7.1072-7.0043(t, 3H, Ar-H); 4.3984-4.3109(d, 2H, Acetamide-$CH_2$); 2.6543-2.6102(t, 1H, N-CH proton); 2.2093(s, 3H, Ar-$CH_3$). Mass (LC-MS); m/z 483.21(M); 484.32(M+1); 485.18(M+2).

**Compound-II-VI_i:(Z)-3-(3-((1H-pyrazol-4-yl)imino)-5-methyl-2-oxoindolin-1-yl)-3-(4-hydroxyphenyl)-N-phenylpropanamide.**IR($Vcm^{-1}$):3389(-NH Str in pyrazole); 3026(-CH Str in Ar-CH); 2976, 2874(-CH Str in Aliphatic-CH); 1726(-CO Str in Indole-CO); 1617(-C=N Str in Imine); 1452(-C=C Str in Ar-C); 1063(-C-N Str). $^1$HNMR(DMSO)δpp: 12.5633(s., 1H, Pyrazole-NH proton); 9.5872(s, 1H, Acetamide-NH); 8.2984-8.2109(s, 2H, pyrazole-H); 8.0932(s, 1H, Ar-H); 7.9954-7.9023(d, 2H, Ar-H); 7.7984-7.7342(d, 2H, Ar-H); 7.6342-7.6132(d, 2H, Ar-H); 7.4873-7.4032(d, 2H, Ar-H); 7.2873-7.2012(t, 3H, Ar-H); 5.8726(s, 1H, Ar-OH); 4.5853-4.4732(d, 2H, Acetamide-$CH_2$); 2.4897-2.4234(t, 1H, N-CH proton); 1.9983(s, 3H, Ar-$CH_3$). Mass (LC-MS); m/z 481.29(M); 482.35(M+1).

**Compound-II-VI_j:(Z)-3-(3-((1H-pyrazol-4-yl)imino)-5-methyl-2-oxoindolin-1-yl)-3-(4-methoxyphenyl)-N-phenylpropanamide.** IR($Vcm^{-1}$):3402(-NH Str in pyrazole); 3016(-CH Str in Ar-CH); 2967, 2872, 2791(-CH Str in Aliphatic-CH); 1706(-CO Str in Indole-CO); 1611(-C=N Str in Imine); 1423(-C=C Str in Ar-C); 1072(-C-N Str). $^1$HNMR(DMSO)δpp: 11.8943(s., 1H, Pyrazole-NH proton); 9.3874(s, 1H, Acetamide-NH); 8.2893-8.2109(s, 2H, pyrazole-H); 8.1092(s, 1H, Ar-H); 7.8934-7.8013(d, 2H, Ar-H); 7.6830-7.6102(d, 2H, Ar-H); 7.5643-7.5342(d, 2H, Ar-H); 7.3846-7.3214(d, 2H, Ar-H); 7.1982-7.1034(t, 3H, Ar-H); 4.6322-4.6031(d, 2H, Acetamide-$CH_2$); 3.5645(s, 3H, Ar-$OCH_3$); 2.5633-2.5198(t, 1H, N-CH proton); 2.0564(s, 3H, Ar-$CH_3$). Mass (LC-MS); m/z 481.29(M); 482.35(M+1).

**Compound-II-VI_k:(Z)-3-(3-((1H-pyrazol-4-yl)imino)-2-oxoindolin-1-yl)-3-(4-methoxyphenyl)-N-phenylpropanamide:** IR($Vcm^{-1}$):3421(-NH Str in pyrazole); 3091(-CH Str in Ar-CH); 2986, 2854, 2761(-CH Str in Aliphatic-CH); 1714(-CO Str in Indole-CO); 1623(-C=N Str in Imine); 1421(-C=C Str in Ar-C); 1068(-C-N Str). $^1$HNMR(DMSO)δpp: 11.7644(s., 1H, Pyrazole-NH proton); 9.5412(s, 1H, Acetamide-NH); 8.1983(s, 2H, pyrazole-H); 8.0873-8.0012(d, 2H, Ar-H); 7.9453-7.9023(d, 2H, Ar-H); 7.7893-7.7102(d, 2H, Ar-H); 7.5983-7.5032(d, 2H, Ar-H); 7.3874-7.3023(t, 2H, Ar-H); 7.0943-7.0032(t, 3H, Ar-H); 4.5876-4.5219(d, 2H, Acetamide-

$CH_2$); 3.6865(s, 3H, Ar-$OCH_3$); 2.4987-2.4187(t, 1H, N-CH proton). Mass (LC-MS); m/z 481.21(M); 482.35(M+1).

**Compound-II-VI_l:(Z)-3-(3-((1H-pyrazol-4-yl)imino)-2-oxoindolin-1-yl)-N-phenyl-3-(p-tolyl)propenamide.**IR($Vcm^{-1}$):3402(-NH Str in pyrazole); 3078(-CH Str in Ar-CH); 2948, 2819, 2795(-CH Str in Aliphatic-CH); 1726(-CO Str in Indole-CO); 1619(-C=N Str in Imine); 1427(-C=C Str in Ar-C); 1093(-C-N Str). $^1$HNMR(DMSO)δpp: 12.2873(s., 1H, Pyrazole-NH proton); 9.5843(s, 1H, Acetamide-NH); 8.2093(s, 2H, pyrazole-H);7.9843-7.9034(d, 2H, Ar-H); 7.8563-7.8213(d, 2H, Ar-H); 7.7984-7.7104(d, 2H, Ar-H); 7.4985-7.4274(d, 2H, Ar-H); 7.2933-7.2109(t, 2H, Ar-H); 6.9844-6.9023(t, 3H, Ar-H); 4.6983-4.6124(d, 2H, Acetamide-$CH_2$); 2.5643-2.5102(t, 1H, N-CH proton); 2.0453(s, 3H, Ar-$CH_3$). Mass (LC-MS); m/z 465.12(M); 466.32(M+1).

**Compound-II-VI_m:(Z)-3-(3-((1H-pyrazol-4-yl)imino)-5-chloro-2-oxoindolin-1-yl)-N-phenyl-3-(p-tolyl)propenamide:** IR($Vcm^{-1}$):3391(-NH Str in pyrazole); 3078(-CH Str in Ar-CH); 2967, 2816, 2751(-CH Str in Aliphatic-CH); 1709(-CO Str in Indole-CO); 1620(-C=N Str in Imine); 1403(-C=C Str in Ar-C); 1078(-C-N Str); 799(-Cl Str in Ar-Cl). $^1$HNMR(DMSO)δpp: 12.9673(s., 1H, Pyrazole-NH proton); 9.6784(s, 1H, Acetamide-NH); 8.2893(s, 2H, pyrazole-H); 8.1045(s, 1H, Ar-H); 7.8975-7.8154(d, 2H, Ar-H); 7.7203-7.7102(d, 2H, Ar-H); 7.5674-7.5024(d, 2H, Ar-H); 7.3984-7.3174(d, 2H, Ar-H); 7.1093-7.0043(t, 3H, Ar-H); 4.4536-4.3984(d, 2H, Acetamide-$CH_2$); 2.6754-2.6102(t, 1H, N-CH proton); 1.9974(s, 3H, Ar-$CH_3$). Mass (LC-MS); m/z 483.14(M); 484.31(M+1); 485.11(M+2).

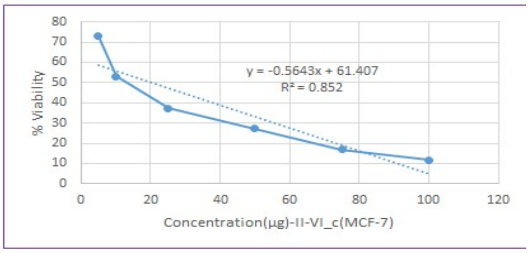
**Compound-II-VI_n:(Z)-3-(3-((1H-pyrazol-4-yl)imino)-5-chloro-2-oxoindolin-1-yl)-3-(4-methoxyphenyl)-N-phenylpropanamide.**IR($Vcm^{-1}$):3419(-NH Str in pyrazole); 3071(-CH Str in Ar-CH); 2951, 2873, 2797(-CH Str in Aliphatic-CH); 1718(-CO Str in Indole-CO); 1613(-C=N Str in Imine); 1429(-C=C Str in Ar-C); 1069(-C-N Str); 814(-Cl Str in Ar-Cl). $^1$HNMR(DMSO)δpp: 11.9845(s., 1H, Pyrazole-NH proton); 9.6893(s, 1H, Acetamide-NH); 8.3884-8.3021(s, 2H, pyrazole-H); 8.1873(s, 1H, Ar-H); 8.0834-8.0015(d, 2H, Ar-H); 7.9345-7.9012(d, 2H, Ar-H); 7.6547-7.6102(d, 2H, Ar-H); 7.3564-7.3213(d, 2H, Ar-H); 7.2093-7.1982(t, 3H, Ar-H); 4.5987-4.5253(d, 2H, Acetamide-$CH_2$); 3.6923(s, 3H, Ar-$OCH_3$); 2.5647-2.5102(t, 1H, N-CH proton). Mass (LC-MS); m/z 499.13(M); 500.31(M+1); 501.43(M+2).

**Compound-II-VI_o:(Z)-3-(3-((1H-pyrazol-4-yl)imino)-2-oxoindolin-1-yl)-3-(3,4-dimethoxyphenyl)-N-phenylpropanamide:** IR($Vcm^{-1}$):3389(-NH Str in pyrazole); 3026(-CH Str in Ar-CH); 2956, 2873, 2719(-CH Str in Aliphatic-CH); 1725(-CO Str in Indole-CO); 1621(-C=N Str in Imine); 1453(-C=C Str in Ar-C); 1073(-C-N Str). $^1$HNMR(DMSO)δpp: 12.3452(s., 1H, Pyrazole-NH proton); 9.6864(s, 1H, Acetamide-NH); 8.2873-8.2109(s, 2H, pyrazole-H); 8.1820-8.1043(d, 2H, Ar-H); 7.9453(d, 2H, Ar-H); 7.6784-7.6109(d, 2H, Ar-H); 7.4763-7.3998(d, 2H, Ar-H); 7.2873-7.2109(t, 2H, Ar-H); 6.8965-6.8023(t, 3H, Ar-H); 4.6894-4.6023(d, 2H, Acetamide-$CH_2$); 3.5763-3.5103(s, 6H, Ar-$OCH_3$); 2.4875-2.4109(t, 1H, N-CH proton). Mass (LC-MS); m/z 495.19(M); 496.21(M+1).

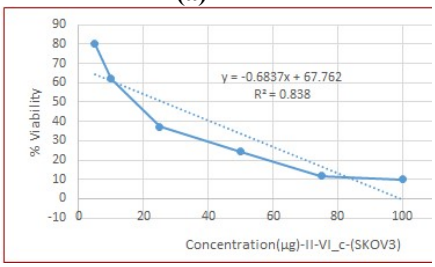
### 3.2. Anticancer Activity

The MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay has been employed for assessing these therapies' anticancer efficacy in vitro against human breast cancer (MCF-7) and ovarian (SKOV3) cell lines (Table.2). The new indole-2-one derivatives such as II-VI_c and II-VI_f displayed stronger anticancer activates towards both cancer cell lines(MCF-7 and SKOV3), according to the findings. From screening results compounds sowing **II-VI_c** with $IC_{50}$ values of 20.21±0.021 µg(MCF-7) and 25.97±0.023 µg(SKOV3) and compound **II-VI_f** with $IC_{50}$ values of 37.26±0.001µg (MCF-7) and 24.56±0.002µg (SKOV3) respectively.

**Table. No.2. Anticancer activity of Pyrazole fused novel Indole-2-one derivatives**

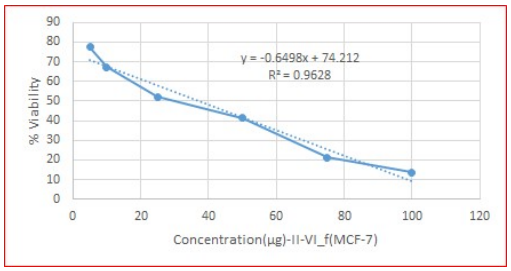



| Compounds | IC50 Values | |
|---|---|---|
| | MCF-7 | SKOV3 |
| II-VI_c | 20.21±0.021** | 25.97±0.023** |
| II-VI_f | 37.26±0.001** | 24.56±0.002** |
| II-VI_g | 47.52±2.032 | 60.47±4.212 |
| II-VI_k | 57.45±3.023 | 43.38±2.982 |
| II-VI_m | 44.58±2.763 | 46.29±1.983 |
| Doxorubicin | 18.2±0.001 | 22.23±0.002 |




(a)

(b)

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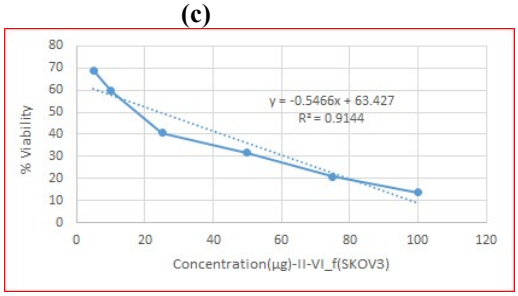


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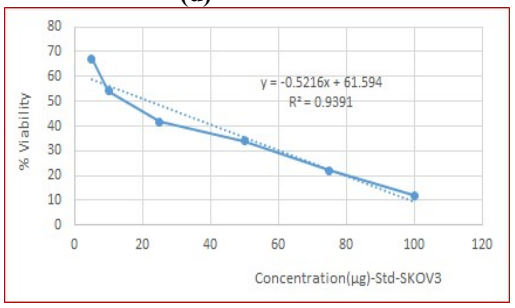



(e)

**Figure No.2. Linear graphs depicting comparative cellular viability. (a, b) Compound-II-VI_c against MCF-7and SKOV3 (c,d)Compound-II-VI_f against MCF-7 and SKOV3. (d,e) Standard drug-Doxorubicin against MCF-7 and SKOV3 cell lines**

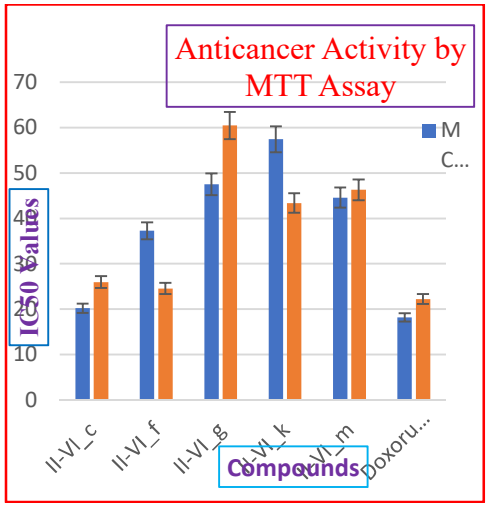


**Figure. No.3.Graphical representation of Pyrazole fused novel Indole-2-one derivatives-Anticancer activity.**

### 3.3. Molecular Docking studies.

Molecular docking studies were carried out using Auto Dock Vina software to investigate the binding interactions of the biologically active compounds-II-VI_(a-o) with EGFR tyrosine kinase enzyme. The receptor grid that was generated will helps in locating the protein active site and preparing the grid for the ligands to be docked in the shape and properties of the receptor are represented on a grid by many different sets of fields that provide progressively more precise scoring of the ligand poses. The binding energies of mentioned analogs, further clarify the design of potential drug candidates against Pokeweed Antiviral Protein.Binding energy of the dataset ligands were shown in Table 1 along with the interaction amino acids and number of amino acids.

**Table.No. 3. Molecular docking studies of Compound's-II-VI(a-o)**

| Compound No | Binding Energy (Kcal/mol) | No of H-bonds | H-bond lengths (Å) | Hydrogen bond interactions | Other Interacting amino acids (TARGET : 1M17) |
|---|---|---|---|---|---|
| **II-VI-a** | **-9.2** | **1** | **2.19** | **LYS:702** | ASP:699, ALA:719, LEU:764, VAL:702, CYS:773 |
| **II-VI-b** | **-8.7** | **NIL** | **NIL** | **NIL** | LYS:721, LEU:764, ASP:831, ARG:817 |
| **II-VI-c** | **-10.3** | **NIL** | **NIL** | **NIL** | PHE: 699, LYS:721, LEU: 764, LEU:694, ASP:831, VAL:702, CYS:773, ARG:817 |
| **II-VI-d** | **-9.4** | **NIL** | **NIL** | **NIL** | ASP:699, ARG:817, LEU:764, VAL:702 |
| **II-VI-e** | **-8.9** | **1** | **2.03** | **ASP:831** | ASP:699, ALA:719, LEU:764, ARG:817, CYS:773 |
| **II-VI-f** | **-10.1** | **NIL** | **NIL** | **NIL** | PHE: 699, ALA:719, LYS:721, LEU: 764, LEU:694, ASP:831, VAL:702, CYS:773, ARG:817 |
| **II-VI-g** | **-9.7** | **1** | **2.45** | **ARG: 817** | LYS:721, LEU: 764, LEU:694, ASP:831, VAL:702 |
| **II-VI-h** | **-8.8** | **NIL** | **MIL** | **NIL** | LYS:721, LEU: 764, LEU:694, ASP:831, VAL:702, ARG:817 |
| **II-VI-i** | **-9.6** | **1** | **2.18** | **LEU:694** | PHE: 699, ALA:719, LYS:721, LEU: 764, ASP:831, VAL:702 |
| **II-VI-j** | **-9.1** | **1** | **2.80** | **CYS:773** | PHE: 699, LYS:721, LEU:694, ASP:831, VAL:702, ARG:817, MET:742 |
| **II-VI-k** | **-9.7** | **NIL** | **NIL** | **NIL** | PHE: 699, ALA:719, LYS:721, , LEU:694 |
| **II-VI-l** | **-8.6** | **NIL** | **NIL** | **NIL** | LYS:721, LEU: 764, LEU:694, ASP:831, VAL:702, ARG:817 |
| **II-VI-m** | **-9.8** | **3** | **2.14, 2.20, 2.42** | **LYS:721, ASN:818, SER:696** | PHE: 699, LEU:820, ASP:831, VAL:702, ARG:817, GLY:772 |
| **II-VI-n** | **-8.7** | **NIL** | **NIL** | **NIL** | LEU: 764, LEU:694, ASP:831 |

| | | | | | |
|---|---|---|---|---|---|
| **II-VI-o** | **-9.1** | **NIL** | **NIL** | **NIL** | ALA:719, LYS:721, LEU: 764, ASP:831 |

Most of the compounds interact with the protein target 1M17 through a combination of hydrogen bonds, hydrophobic interactions, and ionic/polar interactions. All compounds form at least one hydrogen bond with main residues such as SER:696, ASP831, or LYS721, contributing to their binding stability. Hydrophobic interactions with residues like PHE699, LEU694, GLY:772, and VAL702 are consistent across all compounds, further stabilizing the binding. The strongest binding affinity is observed with compound II-VI-c and f, likely due to its ideal combination of hydrogen bonding and hydrophobic interactions, while II-VI-l has the weakest binding affinity, possibly due to fewer or less effective interactions.

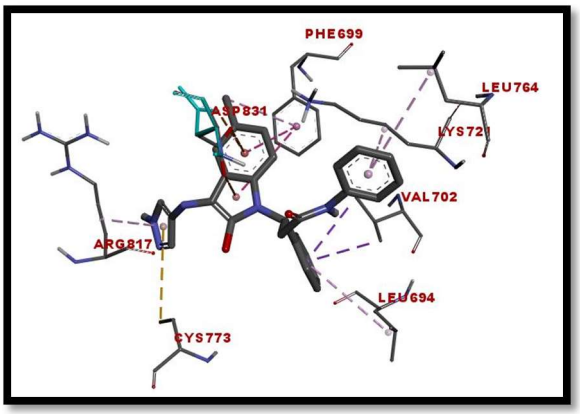
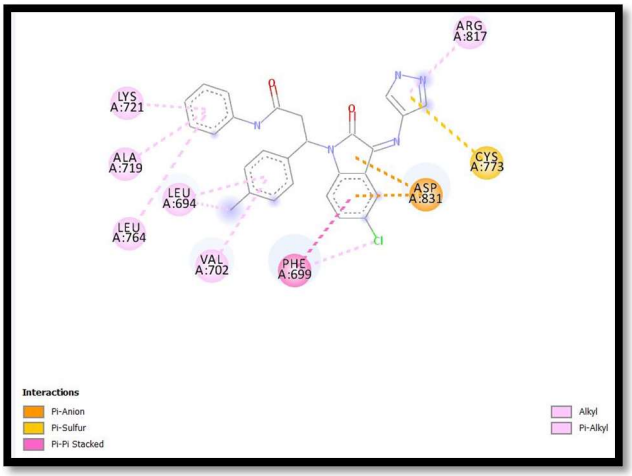
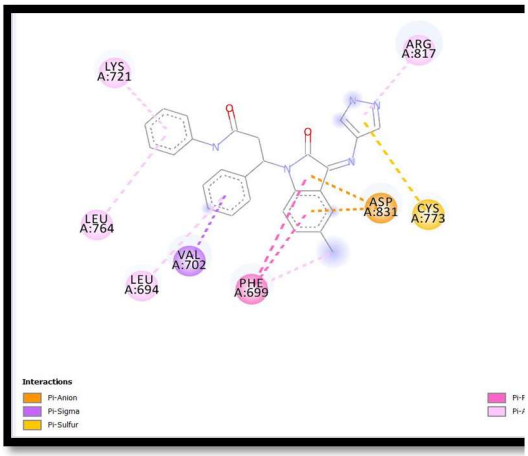



**Figure. No.4.Compound-VI-c_2D and 3D docking possess**



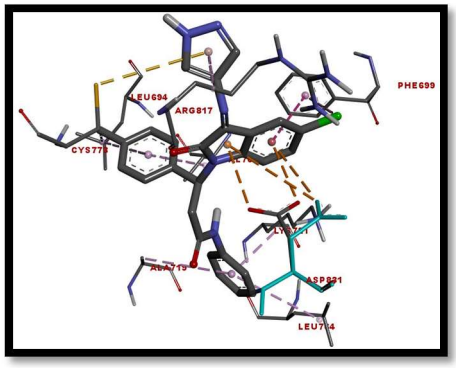




**Figure. No. 5.Compound-VI-f_2D and 3D docking possess**

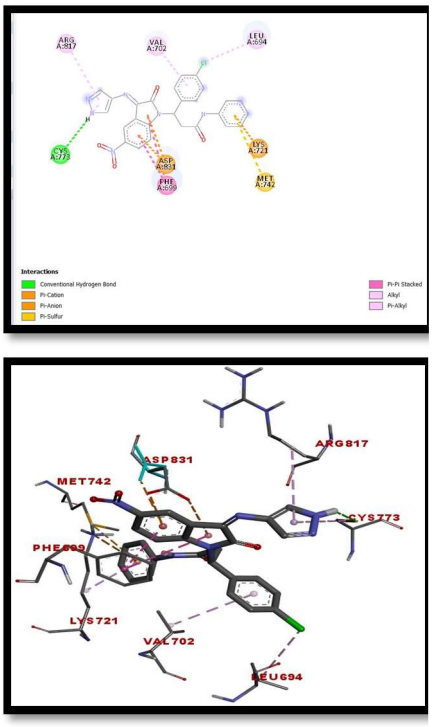



**Figure. No.6.Compound-II-VI-j_2D and 3D docking possess**

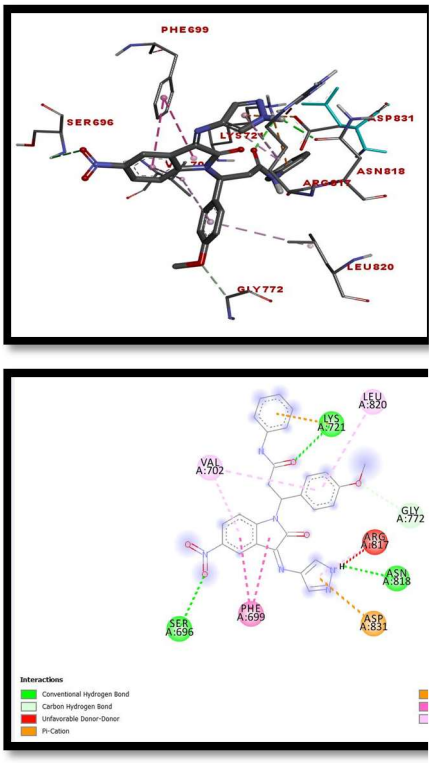



**Figure. No.7.Compoiund-II-VI-m_2D and 3D docking possess**

## 4.CONCLUSION

The synthesis of pyrazole fused novel Indole-2-one scaffold by Schiff's and Mannich mechanism and evaluation of their biological activity has been addressed in a highly efficient. The compound II-VI_c and f displayed promising anticancer activity against MCF-7 and SKOV3 cell lines. In further investigation, molecular docking studies were conducted to predict the interaction modes between the ligands and EGFR receptor with PDB ID:1M17. Notably, the results demonstrate that the synthesized compounds have strong protein binding affinities.

## CONFLICT OF INTEREST

The authors reveal that they have no conflict of interest and the author's solitary are answerability for content and writing of the paper.

## ACKNOWLEDGEMENT

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