



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 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. The synthesized compound's structure was confirmed by IR, ¹H 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 IC₅₀ vales compared with standard. The Doxorubicin used as a standard reference drug. The anticancer activity results shown that compound II-VI_c with IC₅₀ values of 20.21±0.021 μg(MCF-7) and 25.97±0.023 μg(SKOV3) and compound II-VI_f with IC₅₀ values of 37.26±0.001μg (MCF-7) and 24.56±0.002μg (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.

1. INTRODUCTION

Indole or Indole-2-one, a heterocyclic compound with the molecular formula C₈H₇N, 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, lipoxigenase 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. ^1H 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].

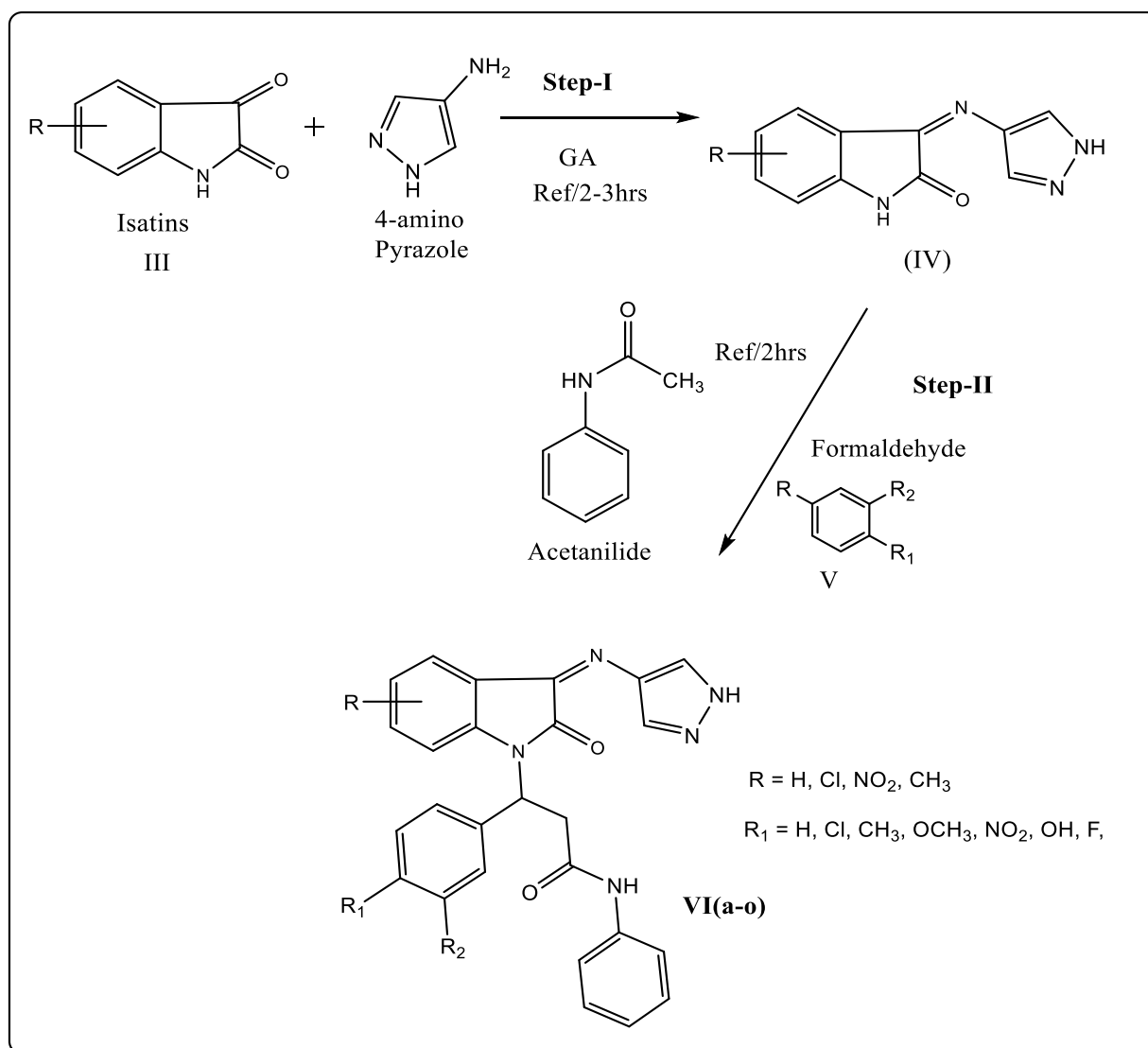


Figure. No.1. Schematic representation of pyrazole fused novel Indole-2-one derivatives-II-V_(a-o)

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 their anticancer activity. Novel 3-(3-((1H-pyrazol-4-yl)imino)-2-oxindolin-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 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 ₅ O ₂	H	H	H	451.20	213-215	0.67	82
II-VI-b	C ₂₇ H ₂₄ ClN ₅ O ₂	Cl	H	H	485.16	187-189	0.72	78
II-VI-c	C ₂₇ H ₂₄ N ₆ O ₄	NO ₂	H	H	496.19	235-237	0.69	76
II-VI-d	C ₂₈ H ₂₇ N ₅ O ₂	CH ₃	H	H	465.22	217-219	0.76	84
II-VI-e	C ₂₈ H ₂₆ Cl N ₅ O ₂	CH ₃	Cl	H	499.15	237-239	0.66	79
II-VI-f	C ₂₉ H ₂₉ N ₅ O ₂	CH ₃	CH ₃	H	479.23	175-177	0.81	83
II-VI-g	C ₂₈ H ₂₆ N ₆ O ₄	CH ₃	NO ₂	H	510.21	209-211	0.72	81
II-VI-h	C ₂₈ H ₂₆ FN ₅ O ₂	CH ₃	F	H	483.21	165-167	0.83	75
II-VI-i	C ₂₈ H ₂₇ N ₅ O ₃	CH ₃	OH	H	481.22	201-203	0.74	83
II-VI-j	C ₂₉ H ₂₉ N ₅ O ₃	CH ₃	OCH ₃	H	495.23	231-233	0.76	80
II-VI-k	C ₂₈ H ₂₇ N ₅ O ₃	H	OCH ₃	H	481.21	213-215	0.66	77
II-VI-l	C ₂₈ H ₂₇ N ₅ O ₂	H	CH ₃	H	465.22	210-212	0.73	81
II-VI-m	C ₂₇ H ₂₂ ClN ₅ O ₂	Cl	CH ₃	H	483.14	239-241	0.67	76
II-VI-n	C ₂₇ H ₂₂ ClN ₅ O ₃	Cl	OCH ₃	H	499.12	227-229	0.72	82
II-VI-o	C ₂₈ H ₂₅ N ₅ O 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). ¹HNMR(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-CH₂); 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). ¹HNMR(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-CH₂); 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-NO₂); 1615(-C=N Str in Imine); 1423(-C=C Str in Ar-C); 1086(-C-N Str). ¹HNMR(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-CH₂); 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). ¹HNMR(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-CH₂); 2.3984-2.3812(t, 1H, N-CH proton); 2.0132(s, 3H, Ar-CH₃). 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). ¹HNMR(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-CH₂); 2.2897-2.2564(t, 1H, N-CH proton); 2.0956(s, 3H, Ar-CH₃). 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). ¹HNMR(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-CH₂); 2.4093-2.4001(t, 1H, N-CH proton); 2.005-1.9934(s, 6H, Ar-CH₃). 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(-NO₂ Str in Ar-NO₂); 1607(-C=N Str in Imine); 1442(-C=C Str in Ar-C); 1038(-C-N Str). ¹HNMR(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-CH₂); 2.5894-2.4895(t, 1H, N-CH proton); 2.1873(s, 3H, Ar-CH₃). 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). ¹HNMR(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-CH₂); 2.6543-2.6102(t, 1H, N-CH proton); 2.2093(s, 3H, Ar-CH₃). 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). ¹HNMR(DMSO) δ pp: 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-CH₂); 2.4897-2.4234(t, 1H, N-CH proton); 1.9983(s, 3H, Ar-CH₃). 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). ¹HNMR(DMSO) δ pp: 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-CH₂); 3.5645(s, 3H, Ar-OCH₃); 2.5633-2.5198(t, 1H, N-CH proton); 2.0564(s, 3H, Ar-CH₃). 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). ¹HNMR(DMSO) δ pp: 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-CH₂); 3.6865(s, 3H, Ar-OCH₃); 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). ¹HNMR(DMSO) δ pp: 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-CH₂); 2.5643-2.5102(t, 1H, N-CH proton); 2.0453(s, 3H, Ar-CH₃). 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). ¹HNMR(DMSO) δ pp: 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-CH₂); 2.6754-2.6102(t, 1H, N-CH proton); 1.9974(s, 3H, Ar-CH₃). 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). $^1\text{H-NMR(DMSO)}\delta_{\text{pp}}$: 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-CH₂); 3.6923(s, 3H, Ar-OCH₃); 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). $^1\text{H-NMR(DMSO)}\delta_{\text{pp}}$: 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-CH₂); 3.5763-3.5103(s, 6H, Ar-OCH₃); 2.4875-2.4109(t, 1H, N-CH proton). Mass (LC-MS); m/z 495.19(M); 496.21(M+1).

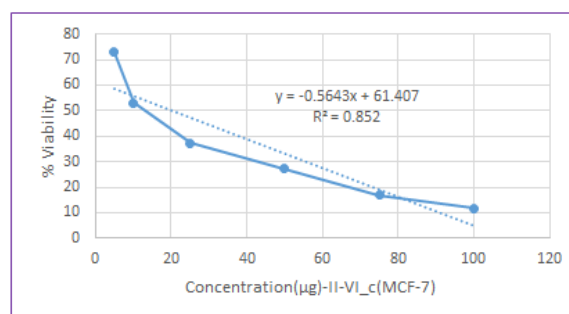
3.2. Anticancer Activity

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 IC₅₀ values of 20.21±0.021 μg(MCF-7) and 25.97±0.023 μg(SKOV3) and compound **II-VI_f** with IC₅₀ values of 37.26±0.001μg (MCF-7) and 24.56±0.002μg (SKOV3) respectively.

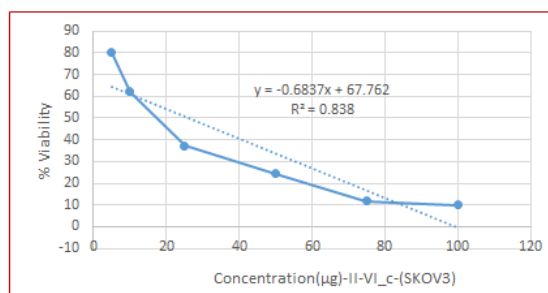
Table. No.2. Anticancer activity of Pyrazole fused novel Indole-2-one derivatives

IC50 Values

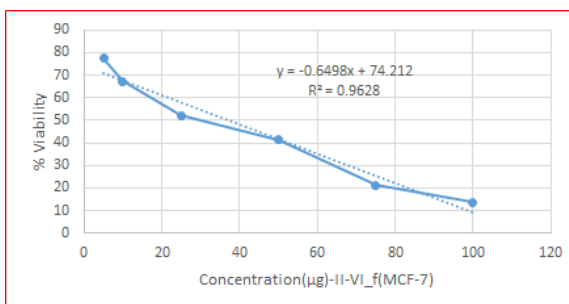
Compounds	MCF-7	SKOV3
II-VI_c	20.21±0.021**	25.97±0.023**
II-VI_f	37.26±0.001**	24.56±0.002**
II-VI_g	47.52±2.032	60.47±4.212
II-VI_k	57.45±3.023	43.38±2.982
II-VI_m	44.58±2.763	46.29±1.983
Doxorubicin	18.2±0.001	22.23±0.002



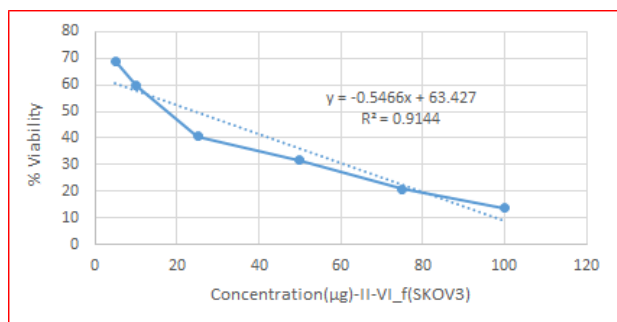
(a)



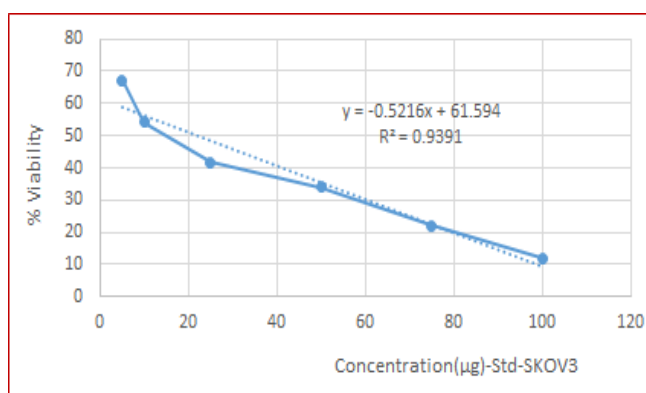
(b)



(c)



(d)



(e)

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

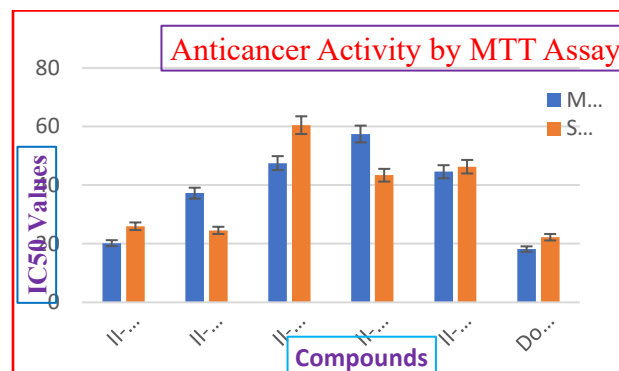


Figure. No.3. Graphical representation of Pyrazole fused novel Indole-2-one derivatives-Anticancer activity.

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 help 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.

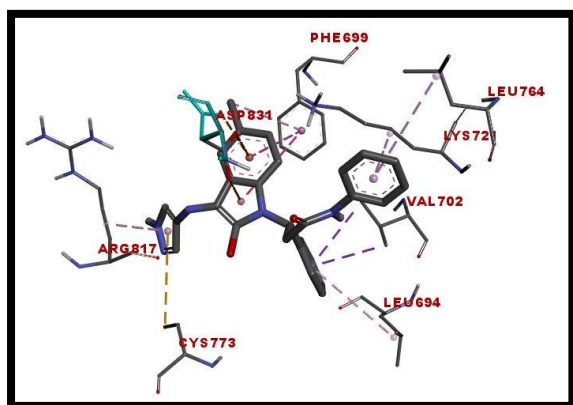
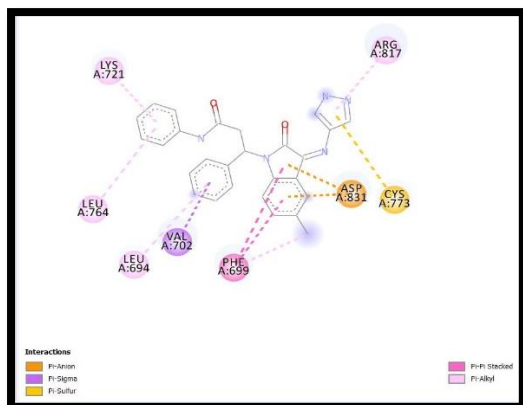


Figure. No.4.Compound-VI-c_2D and 3D docking possess

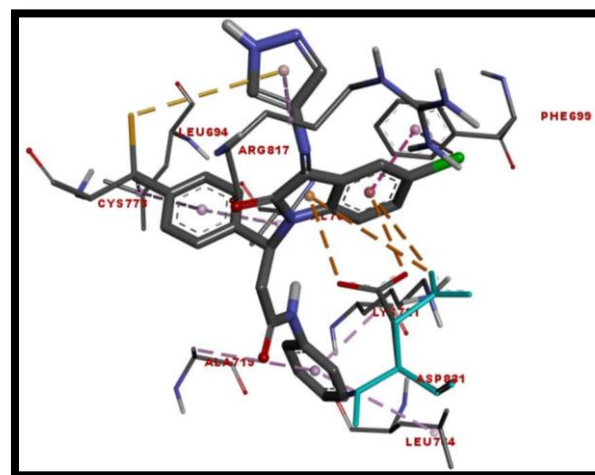
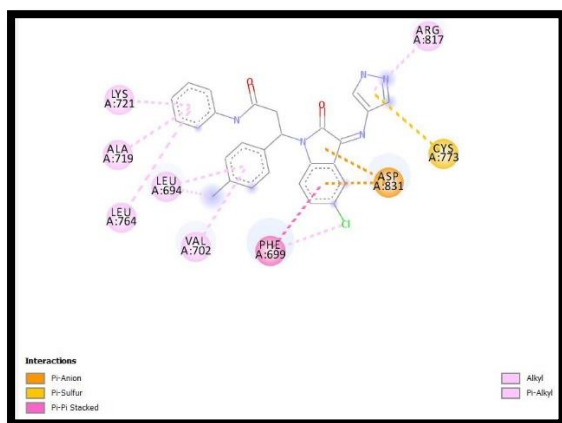


Figure. No. 5.Compound-VI-f_2D and 3D docking possess

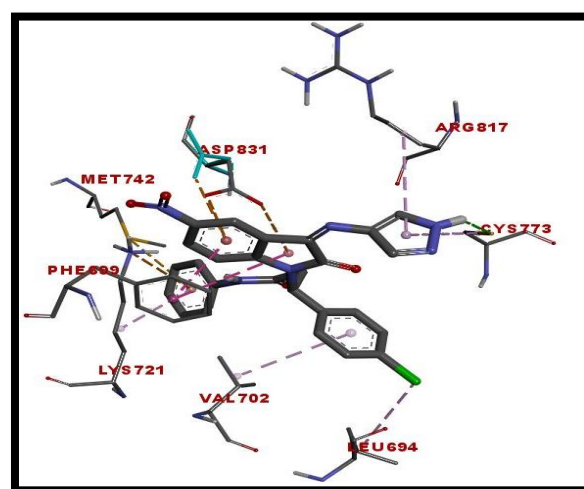
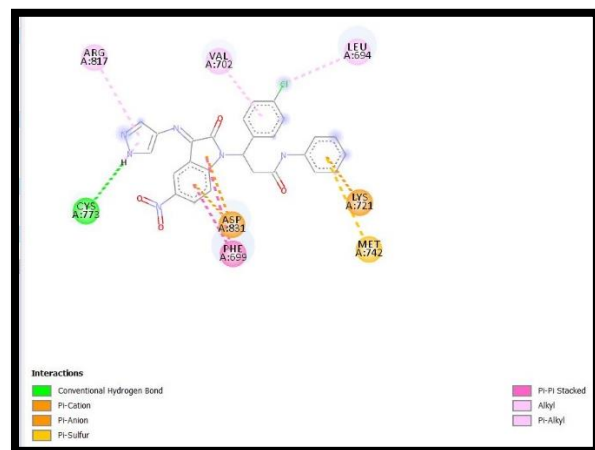


Figure. No.6.Compound-II-VI-j_2D and 3D docking possess

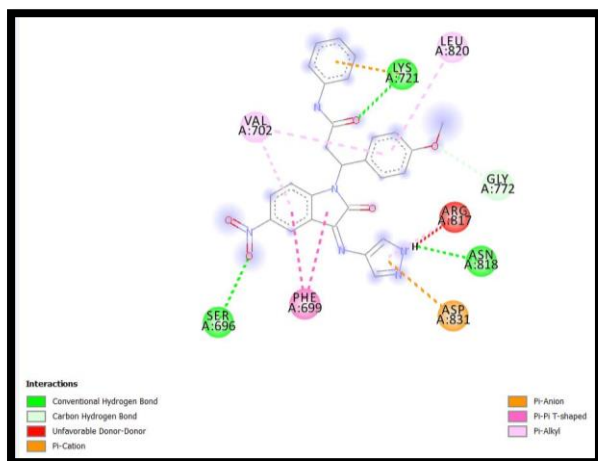
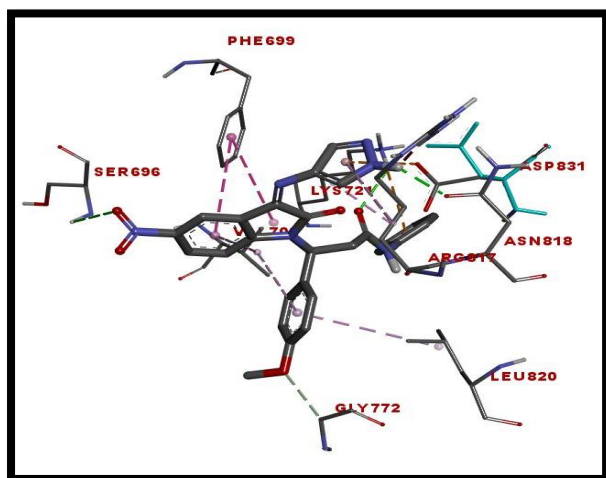


Figure. No.7. Compound-II-VI-m_2D and 3D docking possess

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.

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