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DESIGN, SYNTHESIS, MOLECULAR DOCKING STUDIES OF NOVEL ISATIN DERIVATIVES AND IN VITRO ANTICANCER ACTIVITY AGAINST EGFR RECEPTOR

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ABSTRACT

The present work deals with the sequence of 5-substituted-3-[2-(phenyl) hydrazinylidene]-1-[(1,2,4-triazol-4-yl) acetyl]-indol-2-one **[(Scheme-I-4(a-o)]** was synthesized by conventional method, and were screened as potential anticancer activity and molecular docking studies. All of the newly synthesized compounds structurally characterized on the basis of IR, ^{1}HNMR and Mass spectral analysis. Further, were screened for anticancer activity against MCF-7 cell lines by MTT assay method. The results showed that some of the compounds **4f** and **4h** exhibited good anticancer activity by comparing standard drug. Additionally, the molecular docking studies of (3Z)-5-substituted-3-[2-(4-methoxyphenyl)hydrazinylidene]-1-[(4H-1,2,4-triazol-4-yl) acetyl]-1,3-dihydro-2H-indol-2-one derivatives was carried out to explain putative bonding interaction between the active site of EGFR enzyme and potent inhibitors by using AUTODOCK VINA with PDB ID: 1M17. All the docked ligands, reported lowest binding energy between **-11.7 to -8.4 Kcal/mol**. Compounds **4b** & **4h** possess excellent bonding score were comparison with other synthesized derivatives.

KEYWORDS: Indole-1,2-dione, Triazol, Phenyl hydrazine, Anticancer Activities, MCF7 Doxorubicin, EGFR and AUTODOCK VINA.

1. INTRADUCTION

Cancer is a major health burden worldwide and it is deemed to be the second leading cause of mankind mortality after cardiovascular diseases. Most of the clinically available anticancer chemotherapeutic agents are not able to discriminate between cancer cells and the rapidly dividing healthy cells.[1] Moreover, the growing increase in drug resistance and undesired side effects of the clinically available cancer chemotherapeutic agents aroused the necessity to search for newer more potent and safer cancer chemotherapeutic candidates.[2]

A heterocyclic compound is a cyclic compound that has atoms of at least two different elements as members of its ring(s). Isatin or Indole-2,3-dione is an aromatic heterocyclic compound with the chemical formula $C_8H_5NO_2$. 1H-Indole-2, 3-dione belongs to the class of organic compounds known as indolines.

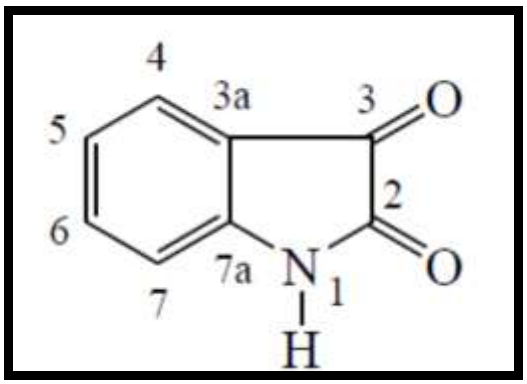


Figure 1: Example for Isatin.

These are compounds containing an indole moiety, which consists of pyrrolidine ring fused to benzene to form 2, 3-dihydroindole. Comprehensive literature survey carried out on Schiff bases are used as substrates in the preparation of a number of industrial and biologically active compounds via ring closure, cycloaddition and replacement reactions. Moreover, Schiff bases derived from various heterocycles have been reported to possess cytotoxic, antimicrobial, antiproliferative, anticancer, anticonvulsant and antifungal activities.[3-5]

The practice of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases. The process of establishing new drugs is exceedingly complex and involves the talents of people from a variety disciplines, including Chemistry, Biochemistry, Physiology, Pharmacology, Pharmaceutics and Medicine.[3-4] Many Indole derivatives find important applications in the field of agriculture, medicine and industry. Indole ring are parts of the larger family of sulphur and nitrogen containing organic

compounds and metal their complexes which display a broad range of biological activity, finding applications ant-tumor, antibacterial, antifungal and antiviral agents.[6-10] Indole is also known as benzopyrrole which contains benzenoid nucleus and has 10 π-electrons (two from lone pair on nitrogen and double bonds provide eight electrons) which makes them aromatic in natur.[10-14] Similar to the benzene ring, electrophilic substitution occurs readily on indole due to excessive π-electrons delocalization.

Molecular docking is generally used to detect the protein-ligand orientation and interaction. The quality of any docking results depends on the starting structure of both the protein and the potential ligand.[14-18] The protein and ligand structure need to be prepared to achieve the best docking results. It includes the following steps, 1. Preparation of receptor & ligand files. 2. Calculation of affinity maps by using a 3D grid around the receptor & ligand. 3. Defining the docking parameters and running the docking simulation.[18-20]

2. EXPERIMENTAL SECTION

2.1. Materials and Methods

The present work is based on Schiff's base and N-acetylation reactions. All the chemicals were used in this research work were obtained from A.R grade and procured from the Merck and LOBA chemicals. The synthesized compounds melting points were determined by Thieles tube method by using liquid paraffin as a solvent. IR spectra were recorded by Thermo Nicolet Nexus 670 FTIR spectrometer and ^{1}HNMR were recorded on Bruker DPX-200 Hz using DMSO-d6 and chemical shifts (δ ppm) are recorded in parts per million downfield from internal reference Tetramethylsilane (TMS). Mass spectra had been recorded by the use of Schimadzu LCMS-8030 mass spectrophotometer and all the spectra had been interpreted. The recoated Silica Gel G plates were used to found the progress of reaction as well as to assessment the purity of the compounds: n-Hexane: Ethyl acetate (7:3). The molecular docking studies were carried out by using AUTODOCK suite of MGL Tools Software.

2.2. General synthetic procedure

Step-I: Synthesis of 5-substituted-1-(chloroacetyl)-indole-2,3-dione 2(a-f): To a solution of substituted isatin (0.016mol) in (30ml) glacial acetic acid, chloroacetyl chloride (3.7g, 0.032mol) was added drop wise with constant stirring. The reaction mixture was refluxed for 3-5 hrs then it was powered onto crushed ice. The precipitated solid that obtained was filtered off, washed with cold water, dried and recrystallized from aqueous ethanol.[21]

Step-II: Synthesis of 5-substituted-1-[(1,2,4-triazol-4-yl) acetyl]-indole-2,3-dione 3(a-f). To a solution of 5-substituted-1-(chloroacetyl)-indole-2,3-dione 2(a-f) (0.016mol) in (30ml) glacial acetic acid, triazole (0.032mol) was added drop wise with constant stirring. The reaction mixture was refluxed for 3-5 hrs then it was powered onto crushed ice. The precipitated solid that obtained was filtered off, washed with cold water, dried and recrystallized from aqueous ethanol.[22]

Step-III: Synthesis 5-substituted-3-[2-(4-substituted-phenyl) hydrazinylidene]-1-[(1,2,4-triazol-4-yl)acetyl]-indol-2-one.4(a-o)

The 5-substituted-1-[(1,2,4-triazol-4-yl) acetyl]-indole-2,3-dione 3(a-f) (0.01 mol) compound was taken in a mixture of 4-substituted phenyl hydrazine (0.01 mol) and glacial acetic acid (5 mL) and Ethanol 30ml, then the reaction mixture was refluxing for 2-3hrs. The progress of the reaction was monitored by TLC (Hexane: EtoAc 7:3). 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.[23]

Compound.4a:3-(2-phenylhydrazinylidene)-1-[(1,2,4-triazol-4-yl)acetyl]-indol-2-one. IR cm^{-1} (KBr): 3420(-NH Str in hydrazinylidene), 3093(-CH Str in Aromatic ring), 2986, 2876(-CH str in aliphatic group), 1710, (-C=O Str in Indole), 1698(-C=O Str in acetyl group), 1568(-C=N Str), 1503(-C=C Str), 1412(-C-N Str), 1358(-C=S Str). **^{1}HNMR (DMSO, δppm):** 9.876(s, 1H, -NH proton), 8.198-8.102(d, 2H, Ar-H), 7.985-7.904(d, 2H, Ar-H), 7.786-7.743(t, 2H, Ar-H), 7.302-7.298(t, 2H, Ar-H), 7.289(s, 1H, triazole), 7.196(s, 1H, triazole ring), 4.345(s, 2H, acetyl protons). **Mass (LC-MS):**m/z 346.2(M+), 347.3(M+1, 100%).

Compound.4b: 5-nitro-3-(2-phenylhydrazinylidene)-1-[(1,2,4-triazol-4-yl)acetyl-indol-2-one. IR cm^{-1} (KBr): 3409(-NH Str in hydrazinylidene), 3054(-CH Str in Aromatic ring), 2945, 2829(-CH str in aliphatic group), 1721 (-C=O Str in Indole), 1702(-C=O Str in acetyl group), 1634(-NO_2 Str in Ar-NO_2), 1556(-C=N Str), 1512(-C=C Str), 1426(-C-N Str), 1329(-C=S Str). **^{1}HNMR (DMSO, δppm):** 9.665(s, 1H, -NH proton), 8.30(s, 1H, Ar-H), 8.0765-8.002(d, 2H, Ar-H), 7.986-7.897(d, 2H, Ar-H), 7.685-7.601(t, 3H, Ar-H), 7.453(s, 1H, triazole), 7.302(s, 1H, triazole ring), 4.281(s, 2H, acetyl protons). **Mass (LC-MS):**m/z 391.1(M+), 392.5(M+1, 100%).

Compound.4c: 5-methyl-3-(2-phenylhydrazinylidene)-1-[(1,2,4-triazol-4-yl)acetyl]-indol-2-one. IR cm^{-1} (KBr): 3419(-NH Str in hydrazinylidene), 3083(-CH Str in Aromatic ring), 2956, 2878(-CH str in aliphatic group), 1718(-C=O Str in Indole), 1696(-C=O Str in acetyl group), 1592(-C=N Str), 1530(-C=C Str), 1431(-C-N Str), 1326(-C=S Str). **^{1}HNMR (DMSO, δppm):** 9.785(s, 1H, -NH proton), 8.298(s, 1H, Ar-H), 8.1093-8.1002(d, 2H, Ar-H), 7.895-7.812(d, 2H, Ar-H), 7.632-7.600(t, 2H, Ar-H), 7.502(s, 1H, triazole), 7.402(s, 1H, triazole ring), 4.211(s, 2H, acetyl protons), 2.092(s, 3H, Ar-CH_3). **Mass (LC-MS):**m/z 360.13(M+), 361.03(M+1, 100%).

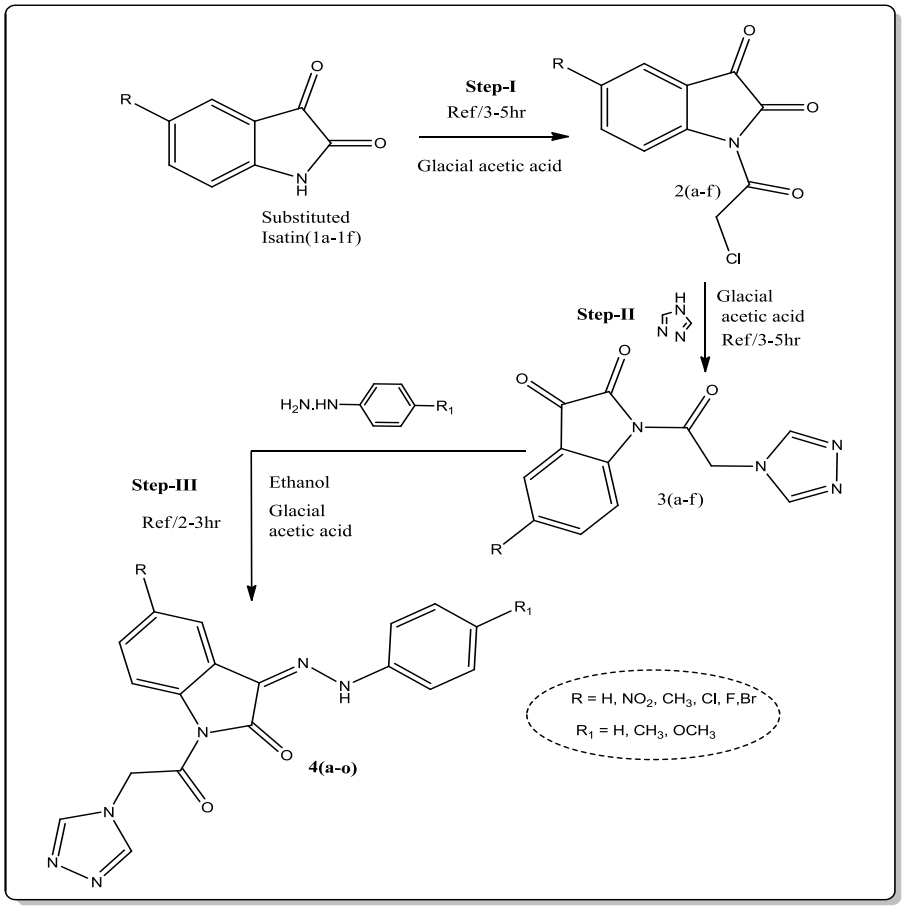


Figure 2: Schematic representation of 5-substituted-3-[2-(4-methoxyphenyl) hydrazinylidene]-1-[(1,2,4-triazol-4-yl)acetyl]-indol-2-one.4(a-o).

Compound.4d: 5-chloro-3-(2-phenylhydrazinylidene)-1-[(1,2,4-triazol-4-yl)acetyl]-indol-2-one: IR cm^{-1} (KBr): 3398(-NH Str in hydrazinylidene), 3104(-CH Str in Aromatic ring), 2976, 2887(-CH str in aliphatic group), 1723(-C=O Str in Indole), 1705(-C=O Str in acetyl group), 1589(-C=N Str), 1523(-C=C Str), 1441(-C-N Str), 1329(-C=S Str), 809(-Cl Str in Ar-Cl). **^{1}HNMR (DMSO, δppm):** 9.832(s, 1H, -NH proton), 8.302(s, 1H, Ar-H), 8.214-

8.209(d, 2H, Ar-H), 8.102-8.098(d, 2H, Ar-H), 7.984-7.932(t, 2H, Ar-H), 7.632(s, 1H, triazole), 7.584(s, 1H, triazole ring), 4.380(s, 2H, acetyl protons). **Mass (LC-MS):**m/z 380.08(M+), 381.021(M+1, 100%), 382.12(M+2, 30%).

Table No. 1: Physical data of 5-substituted-3-[2-(4-methoxyphenyl) hydrazinylidene]-1-[(1,2,4-triazol-4-yl)acetyl]-indol-2-one.4(a-o).

Compound	R	R_1	Molecular Formula	Molecular Weight(gm)	M.P(^{0}C)	%yield
I-4a	-H	-H	$C_{18}H_{14}N_6O_2$	346.2	186-188	83
I-4b	-NO_2	-H	$C_{18}H_{13}N_7O_4$	391.10	171-173	79
I-4c	-CH_3	-H	$C_{19}H_{16}N_6O_2$	360.13	213-215	81
I-4d	-Cl	-H	$C_{18}H_{13}ClN_6O_2$	380.08	237-239	85
I-4e	-CH_3	-CH_3	C_{20} **$H_{18}N_6O_2$**	374.15	210-212	76
I-4f	-F	-H	$C_{18}H_{13}FN_6O_2$	364.11	219-221	85
I-4g	-Cl	-CH_3	$C_{19}H_{15}ClN_6O_2$	394.09	159-161	80
I-4h	-NO_2	--CH_3	$C_{19}H_{15}N_7O_4$	405.12	151-153	79
I-4i	-F	--CH_3	$C_{19}H_{15}FN_6O_2$	378.12	209-211	75
I-4j	-H	-OCH_3	$C_{19}H_{16}N_6O_3$	376.13	183-186	87
I-4k	-Cl	-OCH_3	$C_{19}H_{15}ClN_6O_3$	410.09	167-169	78
I-4l	-CH_3	-OCH_3	$C_{20}H_{18}N_6O_3$	390.14	225-227	84
I-4m	-Br	-H	$C_{18}H_{13}BrN_6O_2$	424.03	189-191	77
I-4n	-Br	-CH_3	$C_{19}H_{15}BrN_6O_2$	438.04	217-219	83
I-4l	-Br	-OCH_3	$C_{20}H_{15}N_6O_3$	374.15	203-205	85

Compound.4e: 5-methyl-3-(2-(4-methylphenyl) hydrazinylidene)-1-[(1,2,4-triazol-4-yl)acetyl]-indol-2-one. IR cm^{-1} (KBr): 3387(-NH Str in hydrazinylidene), 3048(-CH Str in Aromatic ring), 2987, 2838(-CH str in aliphatic group), 1726(-C=O Str in Indole), 1710(-C=O Str in acetyl group), 1567(-C=N Str), 1534(-C=C Str), 1448(-C-N Str), 1338(-C=S Str), 689(-F Str in Ar-F). **^{1}HNMR (DMSO, δppm):** 9.976(s, 1H, -NH proton), 8.204(s, 1H, Ar-H), 8.125-8.200(d, 2H, Ar-H), 7.989(d, 2H, Ar-H), 7.832-7.812(t, 2H, Ar-H), 7.765(s, 1H, triazole), 7.723(s, 1H, triazole ring), 4.187(s, 2H, acetyl protons), 2.034(s,3H, Ar-CH_3), 1.986(s, 3H, Ar-CH_3). **Mass (LC-MS):**m/z 374.15(M+), 375.43(M+1, 100%).

Compound.4f: 5-fluoro-3-(2-phenylhydrazinylidene)-1-[(1,2,4-triazol-4-yl)acetyl]-indol-2-one. IR cm^{-1} (KBr): 3410(-NH Str in hydrazinylidene), 3096(-CH Str in Aromatic ring), 2956, 2885, 2796(-CH str in aliphatic group), 1719(-C=O Str in Indole), 1701(-C=O Str in acetyl group), 1593(-C=N Str), 1534(-C=C Str), 1452(-C-N Str), 1341(-C=S Str). **^{1}HNMR (DMSO, δppm):** 9.976(s, 1H, -NH proton), 8.204(s, 1H, Ar-H), 8.125-8.200(d, 2H, Ar-H), 7.989(d, 2H, Ar-H), 7.832-7.812(t, 2H, Ar-H), 7.765(s, 1H, triazole), 7.723(s, 1H, triazole ring), 4.532(s, 2H, acetyl protons). **Mass (LC-MS):**m/z 374.15(M+), 375.43(M+1, 100%).

Compound.4g: 5-chloro-3-(2-(4-methylphenyl)hydrazinylidene)-1-[(-1,2,4-triazol-4-yl)acetyl]-indol-2-one. IR cm^{-1} (KBr): 3420(-NH Str in hydrazinylidene), 3104(-CH Str in Aromatic ring), 2967, 2887, 2736(-CH str in aliphatic group), 1726(-C=O Str in Indole), 1700(-C=O Str in acetyl group), 1556(-C=N Str), 1527(-C=C Str), 1446(-C-N Str), 1336(-C=S Str), 789(-Cl Str in Ar-Cl). **^{1}HNMR (DMSO, δppm):** 9.786(s, 1H, -NH proton), 8.3097(s, 1H, Ar-H), 8.187-8.126(d, 2H, Ar-H), 7.7865-7.732(d, 2H, Ar-H), 7.798-7.732(d, 2H, Ar-H), 7.687(s, 1H, triazole), 7.756(s, 1H, triazole ring), 4.621(s, 2H, acetyl protons), 2.064(s, 3H, Ar-CH_3). **Mass (LC-MS):**m/z 394.09(M+), 395.3(M+1, 100%), 396.12(M+2, 30%).

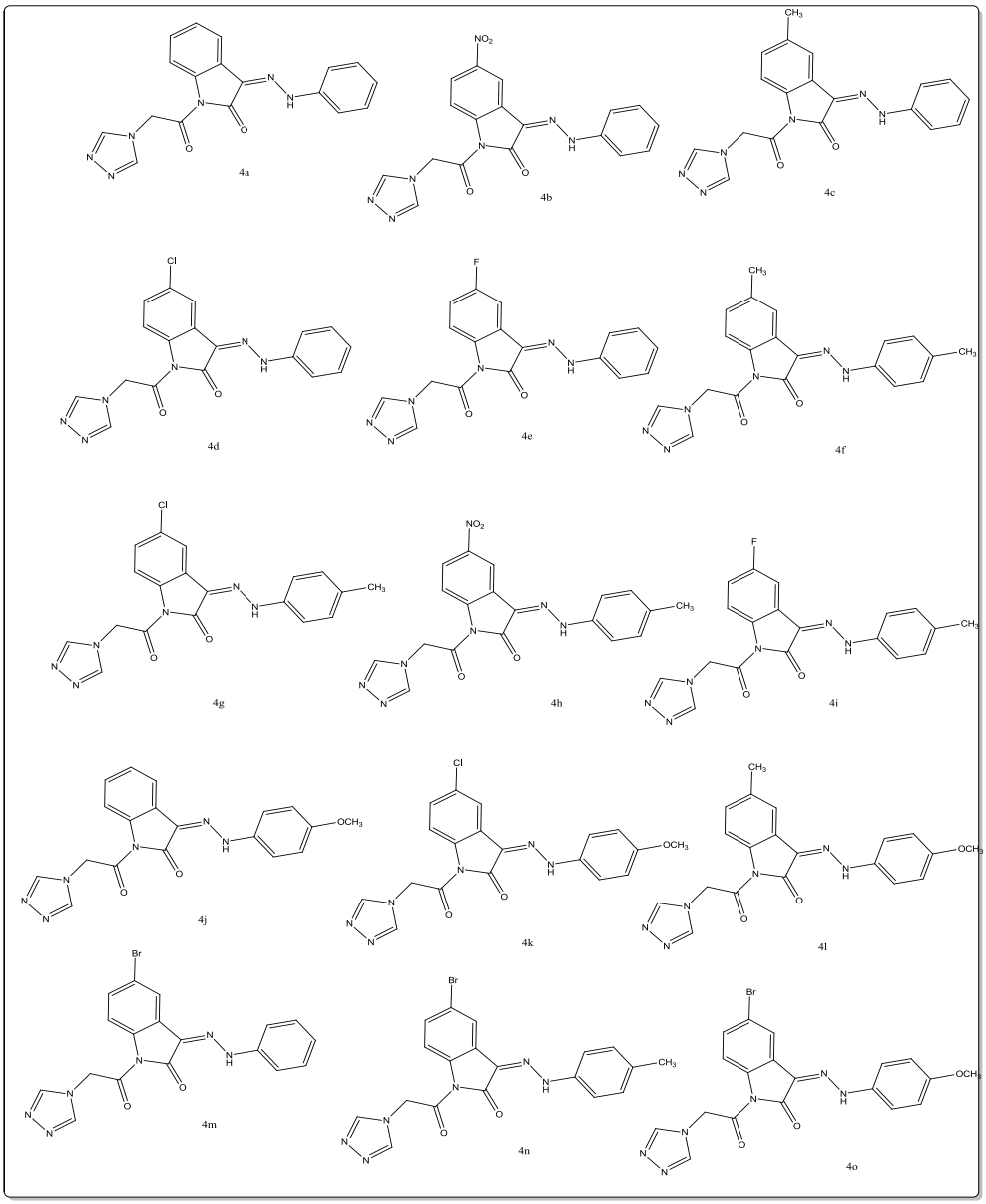


Figure 2: 5-substituted-3-[2-(4-substitutedphenyl) hydrazinylidene]-1-[(1,2,4-triazol-4-yl) acetyl]-indol-2-one.4(a-o).

Compound.4h: 5-nitro-3-(2-(4-methylphenyl) hydrazinylidene)-1-[(1,2,4-triazol-4-yl)acetyl]-indol-2-one. IR cm^{-1} (KBr): 3448(-NH Str in hydrazinylidene), 3096(-CH Str in Aromatic ring), 2976, 2834, 2784(-CH str in aliphatic group), 1712(-C=O Str in Indole), 1697(-C=O Str in acetyl group), 1643(-NO_2 Str in Ar-NO_2), 1551(-C=N Str), 1519(-C=C Str), 1454(-C-N Str), 1329(-C=S Str). **^{1}HNMR (DMSO, δppm):** 9.934(s, 1H, -NH proton), 8.209(s, 1H, Ar-H), 8.095-8.003(d, 2H, Ar-H), 7.984-7.923(d, 2H, Ar-H), 7.765-7.702(d, 2H, Ar-H), 7.687(s, 1H, triazole), 7.498(s, 1H, triazole ring), 4.203(s, 2H, -acetyl), 1.987(s, 3H, Ar-CH_3). **Mass (LC-MS):**m/z 405.12(M+), 406.32(M+1, 100%).

Compound.4i: 5-fluoro-3-(2-(4-methylphenyl) hydrazinylidene)-1-[(1,2,4-triazol-4-yl) acetyl]-indol-2-one. IR cm^{-1} (KBr): 3432(-NH Str in hydrazinylidene), 3103(-CH Str in Aromatic ring), 2967, 2854, 2778(-CH str in aliphatic group), 1727(-C=O Str in Indole), 1707(-C=O Str in acetyl group), 1587(-C=N Str), 1528(-C=C Str), 1453(-C-N Str), 1324(-C=S Str), 678(-F Str in Ar-F). **^{1}HNMR (DMSO, δppm):** 9.896(s, 1H, -NH proton), 8.3043(s, 1H, Ar-H), 8.213-8.210(d, 2H, Ar-H), 7.897-7.832(d, 2H, Ar-H), 7.785-7.723(d, 2H, Ar-H), 7.453(s, 1H, triazole), 7.402(s, 1H, triazole ring), 4.532(s, 2H, acetyl protons)., 1.983(s, 3H, Ar-CH_3). **Mass (LC-MS):**m/z 378.12(M+), 379.32(M+1, 100%).

Compound.4j: 3-(2-(4-methoxyphenyl) hydrazinylidene)-1-[(1,2,4-triazol-4-yl) acetyl]-indol-2-one. IR cm^{-1} (KBr): 3454(-NH Str in hydrazinylidene), 3067(-CH Str in Aromatic ring), 2967, 2843(-CH str in aliphatic group), 1723(-C=O Str in Indole), 1704(-C=O Str in acetyl group), 1589(-C=N Str), 1524(-C=C Str), 1445(-C-N Str), 1339(-C=S Str). **^{1}HNMR (DMSO, δppm):** 9.674(s, 1H, -NH proton), 8.309(s, 1H, Ar-H), 8.208-8.190(d, 2H, Ar-H), 7.894-7.803(d, 2H, Ar-H), 7.764-7.723(t, 2H, Ar-H), 7.589(s, 1H, triazole), 7.421(s, 1H, triazole ring), 4.423(s, 2H, acetyl protons), 3.564(s, 3H, Ar-OCH_3). **Mass (LC-MS):**m/z 376.13(M+), 377.32(M+1, 100%).

Compound.4k: 5-chloro-3-(2-(4-methoxyphenyl) hydrazinylidene)-1-[(1,2,4-triazol-4-yl)acetyl]-indol-2-one. IR cm^{-1} (KBr): 3389(-NH Str in hydrazinylidene), 3010(-CH Str in Aromatic ring), 2967, 2865(-CH str in aliphatic group), 1734(-C=O Str in Indole), 1708(-C=O Str in acetyl group), 1587(-C=N Str), 1534(-C=C Str), 1487(-C-N Str), 1312(-C=S Str), 798(-Cl Str in Ar-Cl). **^{1}HNMR (DMSO, δppm):** 9.892(s, 1H, -NH proton), 8.124(s, 1H, Ar-H), 8.012-8.002(d, 2H, Ar-H), 7.903-7.900(d, 2H, Ar-H), 7.823-7.821(t, 2H, Ar-H), 7.654(s, 1H, triazole), 7.543(s, 1H, triazole ring), 4.287(s, 2H, acetyl protons), 3.854(s, 3H, Ar-OCH_3). **Mass (LC-MS):**m/z 410.09(M+), 411.43(M+1, 100%), 412.43(M+2, 100%).

Compound.4l: 5-methyl-3-(2-(4-methoxyphenyl) hydrazinylidene)-1-[(1,2,4-triazol-4-yl)acetyl]-indol-2-one. IR cm^{-1} (KBr): 3453(-NH Str in hydrazinylidene), 3023(-CH Str in Aromatic ring), 2978, 2887, 2786(-CH str in aliphatic group), 1726(-C=O Str in Indole), 1703(-C=O Str in acetyl group), 1578(-C=N Str), 1554(-C=C Str), 1423(-C-N Str), 1314(-C=S Str). **^{1}HNMR (DMSO, δppm):** 9.786(s, 1H, -NH proton), 8.294(s, 1H, Ar-H), 8.102-8.033(d, 2H, Ar-H), 7.875-7.698(d, 2H, Ar-H), 7.-598(d, 2H, Ar-H), 7.487(s, 1H, triazole), 7.376(s, 1H, triazole ring), 4.612(s, 2H, acetyl protons), 3.685(s, 3H, Ar-OCH_3), 2.054(s, 3H, Ar-CH_3). **Mass (LC-MS):**m/z 390.14(M+), 391.48(M+1, 100%).

Compound.4m: 5-bromo-3-(2-phenylhydrazinylidene)-1-[(1,2,4-triazol-4-yl)acetyl] -indol-2-one. IR cm^{-1} (KBr): 3476(-NH Str in hydrazinylidene), 3076(-CH Str in Aromatic ring), 2976, 2847, 2754(-CH str in aliphatic group), 1721(-C=O Str in Indole), 1698(-C=O Str in acetyl group), 1568(-C=N Str), 1556(-C=C Str), 1432(-C-N Str), 1403(-Br Str in Ar-Br), 1333(-C=S Str). **^{1}HNMR (DMSO, δppm):** 9.879(s, 1H, -NH proton), 8.402(s, 1H, Ar-H), 8.213-8.204(d, 2H, Ar-H), 8.065-8.001(d, 2H, Ar-H), 7.984-7.8912(t, 3H, Ar-H), 7.686(s, 1H, triazole), 7.486(s, 1H, triazole ring), 4.298(s, 2H, acetyl protons). **Mass (LC-MS):**m/z 424.03(M+), 425.03(M+1, 100%), 426.21(M+2, 100%).

Compound.4n: 5-bromo-3-(2-(4-methylphenyl) hydrazinylidene)-1-[(1,2,4-triazol-4-yl)acetyl]-indol-2-one. 3485(-NH Str in hydrazinylidene), 3023(-CH Str in Aromatic ring), 2964, 2893, 2745(-CH str in aliphatic group), 1713(-C=O Str in Indole), 1667(-C=O Str in acetyl group), 1549(-C=N Str), 1549(-C=C Str), 1441(-C-N Str), 1410(-Br Str in Ar-Br), 1338(-C=S Str). **^{1}HNMR (DMSO, δppm):** 9.786(s, 1H, -NH proton), 8.320(s, 1H, Ar-H), 8.198-8.103(d, 2H, Ar-H), 7.989-7.902(d, 2H, Ar-H), 7.843-7.732(d, 2H, Ar-H), 7.598(s, 1H, triazole), 7.367(s, 1H, triazole ring), 4.682(s, 2H, acetyl protons), 2.198(s, 3H in Ar-CH_3). **Mass (LC-MS):**m/z 424.03(M+), 425.3(M+1, 100%), 426.54(M+2, 100%).

Compound.4o: 5-bromo-3-(2-(4-methoxyphenyl) hydrazinylidene)-1-[1,2,4-triazol-4-yl)acetyl]-indol-2-one. 3494(-NH Str in hydrazinylidene), 3054(-CH Str in Aromatic ring), 2993, 2867, 2733(-CH str in aliphatic group), 1719(-C=O Str in Indole), 1683(-C=O Str in acetyl group), 1534(-C=N Str), 1532(-C=C Str), 1451(-C-N Str), 1409(-Br Str in Ar-Br), 1340(-CS Str). **^{1}HNMR (DMSO, δppm):** 9.843(s, 1H, -NH proton), 8.289(s, 1H, Ar-H), 8.098-8.001(d, 2H, Ar-H), 7.854-7.805(d, 2H, Ar-H), 7.734-7.689(d, 2H, Ar-H), 7.487(s, 1H, triazole), 7.203(s, 1H, triazole ring), 4.732(s, 2H, acetyl protons), 3.684(s, 3H in Ar-OCH_3). **Mass (LC-MS):**m/z 438.04(M+), 439.5(M+1, 100%), 440(M+2, 100%).

2.3. Pharmacological activity

Anticancer Activity: Measurement of cell viability and proliferation forms the basis for numerous in vitro assays of a cell population's response to external factors. The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The in vitro anti-cancer activity of test compounds was carried out by MTT Assay against the MCF-7 Cell lines [24]. The cell viability was once appraising by means of the MTT Assay with three impartial experiments with six different concentrations of compounds in triplicates. The novel Azole derivatives were evaluated for cytotoxicity against MCF-7 (human breast cancer cells) cell lines by MTT assay method with Doxorubicin as standard drug and results were mention in the Table.No.2.

Molecular Docking Studies. In drug design, molecular docking studies play an important role in mechanistically by placing a molecule into the binding site of the target molecule. I have docked the synthesized (3Z)-5-substituted-3-[2-(4-methoxyphenyl) hydrazinylidene]-1-[(4H-1,2,4-triazol-4-yl)acetyl]-1,3-dihydro-2H-indol-2-one.4(a-o) compounds into active site of the epidermal growth factor receptor (EGFR) was retrieved from the Protein databank website with PDB Id: 1M17 by using AUTODOCK suite of MGL Tools[25]. The confirmation with lowest binding energy was displayed as the best binding energy. Binding energy of the dataset ligands were shown in Table 1 along with the interaction amino acids and number of amino acids.

3. RESULTS AND DISCUSSION

Synthesis: The series of synthesized (3Z)-5-substituted-3-[2-(4-methoxyphenyl) hydrazinylidene]-1-[(4H- 1, 2, 4-triazol-4-yl) acetyl]-1, 3-dihydro-2H-indol-2-one-4(a-o). compound show satisfactory analysis for the proposed structures, and which were confirmed on the basis of their FT-IR, LC-MASS and ^{1}H NMR spectral data. In this synthetic process, a series of novel Isatin derivatives(4a-4o) were synthesized by three steps. In Step-I, substituted isatin reacts with chloroacetyl chloride in glacial acetic acid to give compounds II(a-f). It can be reacting with triazole to form compound 3(a-f) in step-II. Finally, compound 3(a-f) undergo Schiff's base reaction with substituted phenyl hydrazine in ethanol in the presence of glacial acetic acid to get title compounds Scheme-I, 4(a-o).

Spectral data: Spectral characterization of novel isatin derivatives was performed by IR spectroscopy. Practically, in all the compounds are showing the secondary amine –NH

stretching frequency at around 3380 to 3450 cm^{-1}. The aromatic and aliphatic C-H stretching frequency, as expected is observed at around 3000-3120 cm^{-1} and 2998-2723 cm^{-1}. All the compounds have been show strong absorption in the region of 1680-1743 cm^{-1} is found to be presence of C=O stretching frequency for indole and Acetyl ketone group. All synthesized compounds are showing -C=C stretching of the aromatic ring is around 1585-1545 cm^{-1} respectively. The halogen stretching is observed the strong absorption in the region 650-834 cm^{-1} and some compounds containing $-NO_2$ group shows peaks due to stretching of $–NO_2$ is observed at around 1615-1648 cm^{-1} respectively. Similarly, the ^{1}HNMR (DMSO-d6) spectra of novel isatin derivatives are showed a singlet at 9.500 to 9.983 δ for -NH in hydrazinyl proton and singlet at 4.023 to 4.578 δ for methylene protons. Some of the compounds are showing triplet at 3.543-3.986 δ for $–OCH_3$ in methoxy protons. All these compounds have aromatic protons were found between δ 8.388 to 7.234 δ ppm as singlet, doublet and triplet protons.

Anticancer activity: (3Z)-5-substituted-3-[2-(4-methoxyphenyl) hydrazinylidene]-1-[(4H- 1, 2, 4-triazol-4-yl) acetyl]-1, 3-dihydro-2H-indol-2-one-4(a-o) compounds were screened for cytotoxic activity against human breast cancer cells (MCF7) by using MTT assay method, with doxorubicin as a standard drug. All the results (Table 2) proposed that MCF-7 cell lines were susceptible to the evaluated compounds showed IC_{50}values in the range of **15.99 ± 0.021 µg to 82.56 ± 0.512 µg** against MCF7 cell line. From the results, the compounds **Sindhu-I-4f(15.99±0.021µg)**, **Sindhu-I-4h(19.48±0.011µg)** showed good activity against MCF cell lines, whereas, remaining of the compounds showed moderate activity.

Table.2: Novel isatin derivatives treated with MCF-7 cells showing the IC_{50} values.

S. No.	SAMPLE NAME	IC_{50} (µg)
		MCF7
1	Sindhu-I-4b	32.86±1.03
2	Sindhu-I-4d	36.19±0.23
3	Sindhu-I-4f	**15.99±0.01****
4	Sindhu-I-4h	**19.48±0.001****
5	Sindhu-I-4j	68.26±2.03
6	Sindhu-I-4m	71.37±2.15
7	Sindhu-I-4l	82.56±1.08
8	Sindhu-I-4o	75.13±3.21
9	Doxorubicin	12.83±0.01**

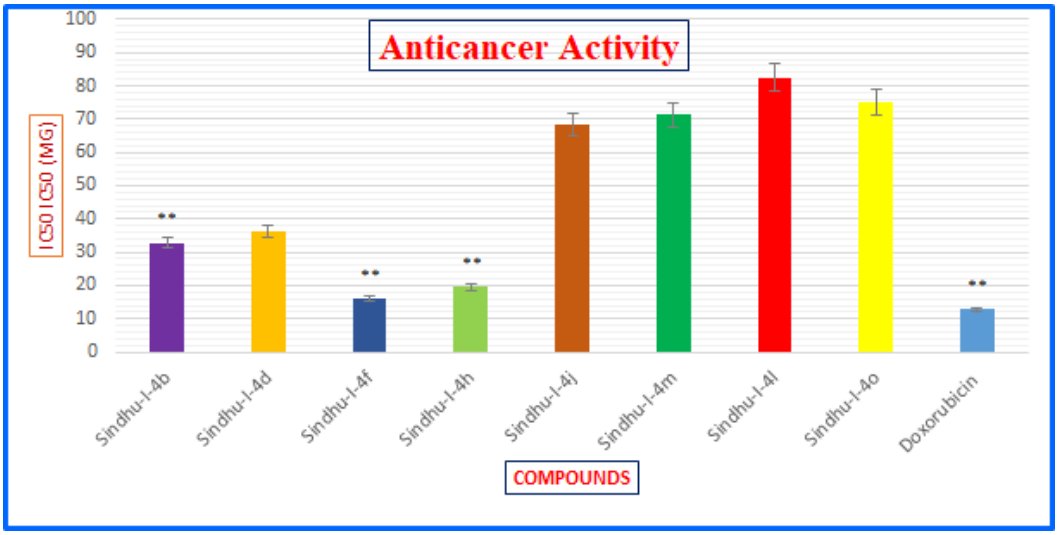
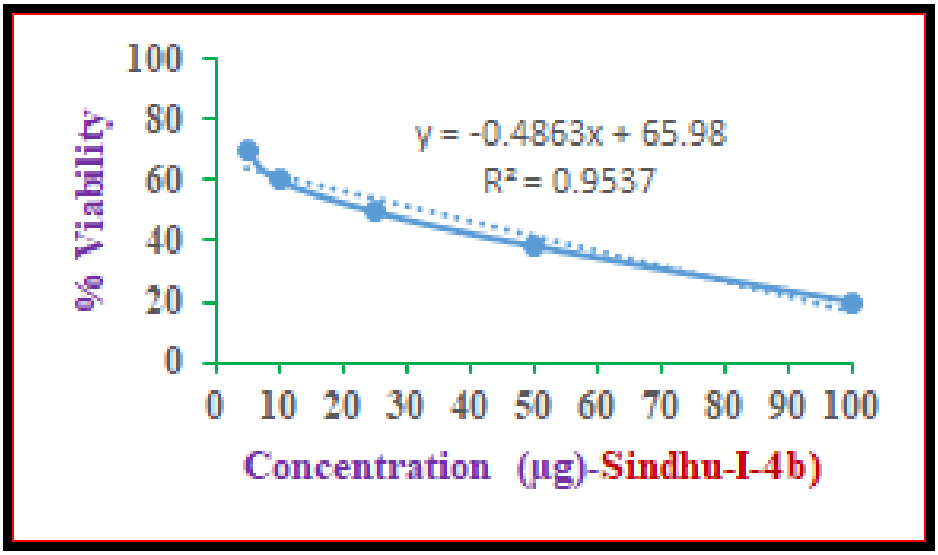
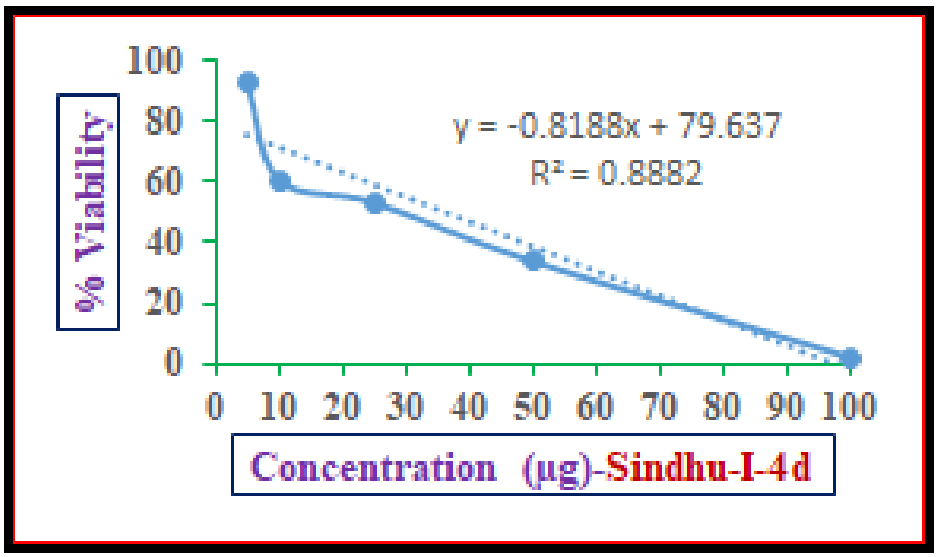
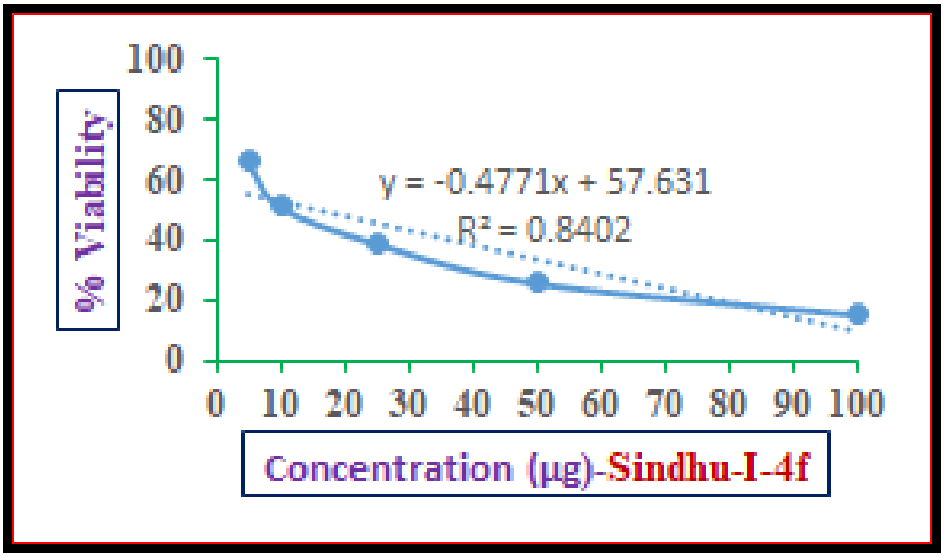
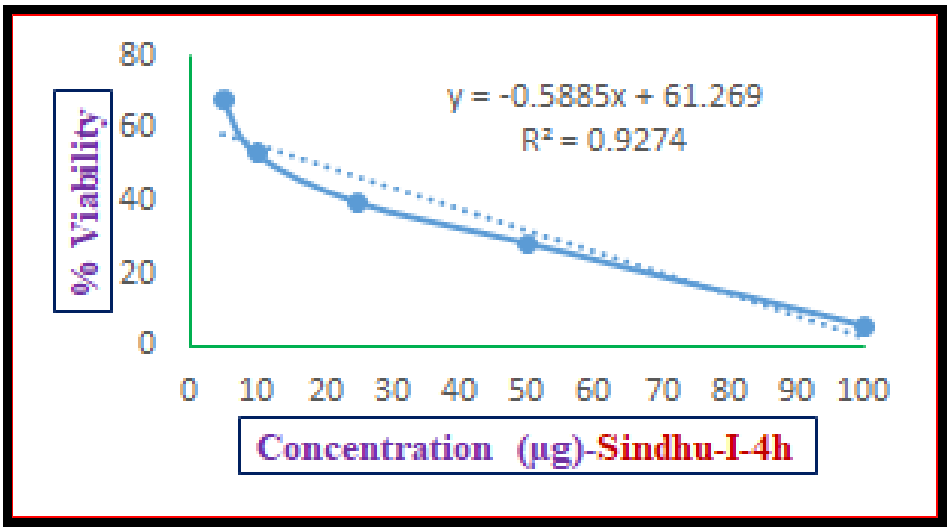


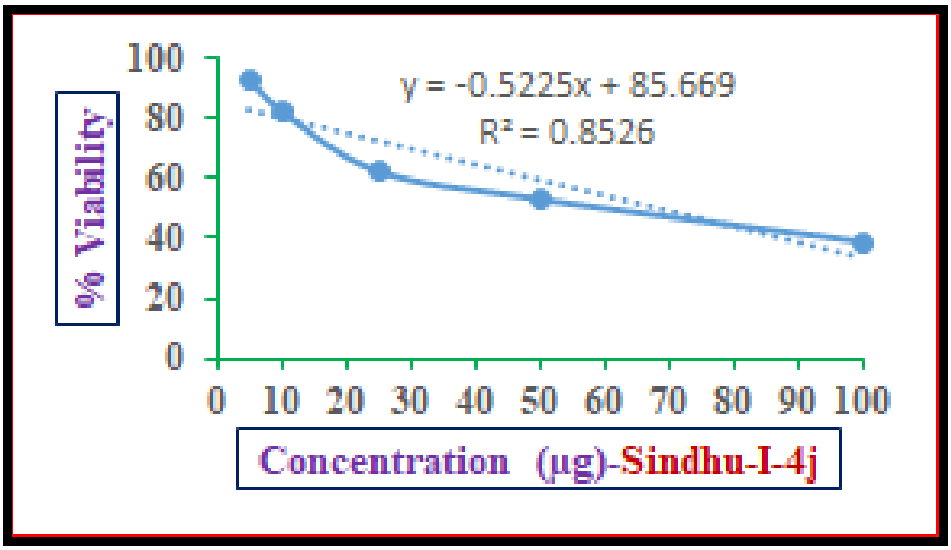
Figure 3: Graphical representation of anticancer activity of Novel isatin derivatives(Sindhu-I-4(a-o) againstMCF-7 cell line.






% Viability
100
80
60
40
20
0
y = -0.4771x + 57.631
R² = 0.8402
0 10 20 30 40 50 60 70 80 90 100
Concentration (µg)-Sindhu-I-4f


% Viability
80
60
40
20
0
y = -0.5885x + 61.269
R² = 0.9274
0 10 20 30 40 50 60 70 80 90 100
Concentration (µg)-Sindhu-I-4h


% Viability
100
80
60
40
20
0
y = -0.5225x + 85.669
R² = 0.8526
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Concentration (µg)-Sindhu-I-4j

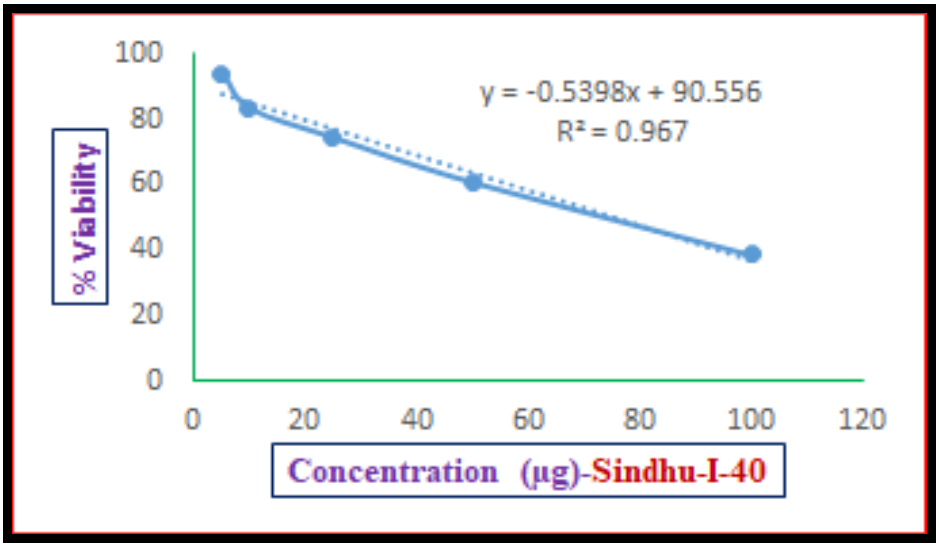


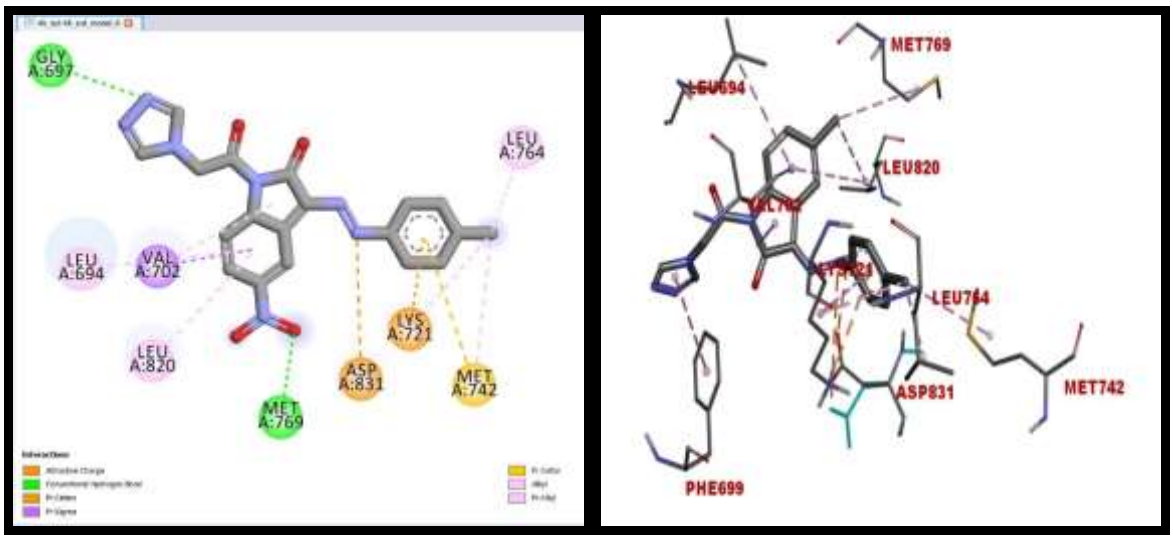
Figure 4: Graphical representation of anticancer activity of Novel isatin derivatives(Sindhu-I-4(a-o)- IC_{50} values.

Molecular Docking studies: All the docked ligands, reported lowest binding energy between **-11.7 to -8.4 Kcal/mol.** Most of the compounds possess one hydrogen bond each with VAL:702, MET:742, LEU:694, MET:769 amino acids. Compounds **Sindhu-I-(4b & 4h)** possess two hydrogen bond interaction. Sindhu-I-(4c,4d,4g, 4i, 4j,4k, 4m, 4o) had one hydrogen bond interaction. Compounds Sindhu-I-(4a,4e, 4f,4n and 4l) no hydrogen bond interaction.

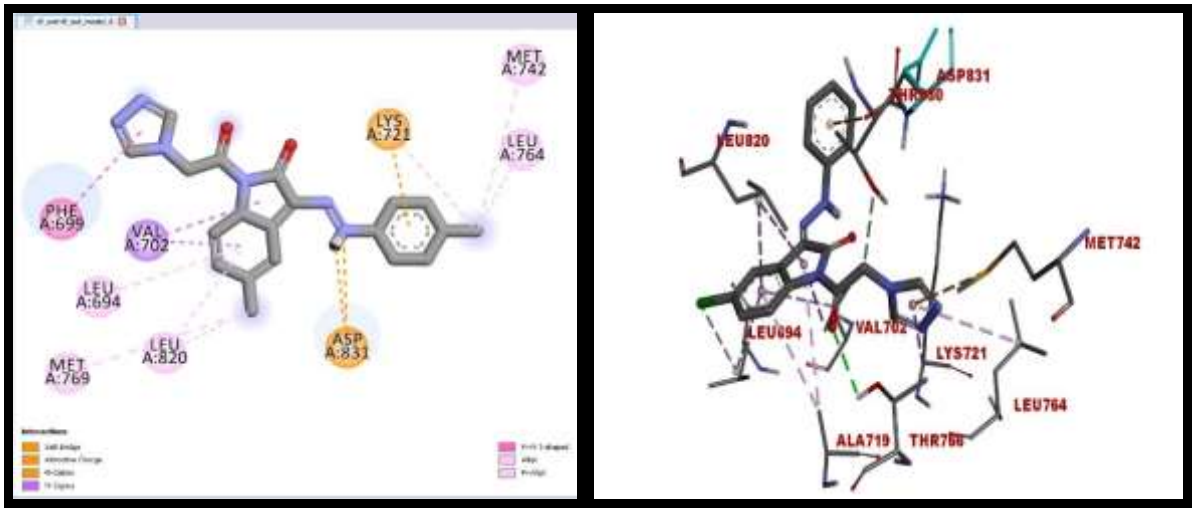
Table No. 5: Insilco EGFR inhibition of novel Isatin derivatives Sindhu-I-(4a-4o)-Binding Energy.

Compound No	Binding Energy (Kcal/mol)	No of H-bonds	Interacting amino acids	H-bond lengths (Å)
4h	-11.7	2	GLY:697, PHE:699, VAL:702, MET:742, LEU:694, MET:769, ALA:719, LEU:820, ASP:831, LYS:721	2.53, 2.81
4f	-11.4	Nil	PHE:699, VAL:702, MET:742, LEU:694, MET:769, ALA:719, LEU:820, ASP:831, LYS:721, LEU:764, LYS:851	Nil
4b	-11.2	2	PHE:699, LEU:694, VAL:702, ALA:719, LYS:721, MET:769, LEU:820, ASP:831	2.61, 2.72
4a	-11.1	Nil	ALA:719, LYS:721, MET:742, LEU:764, CYS:773, LEU:820, THR:830, ASP:831	Nil

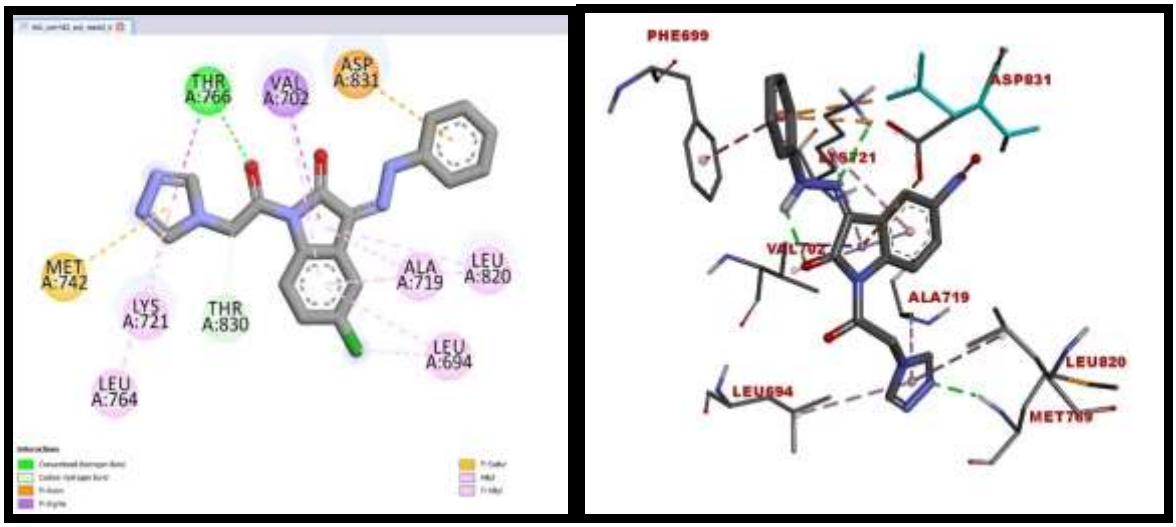
4i	-11.0	1	LEU:764, LEU:764, CYS:773, LEU:820	2.32
4n	-10.9	Nil	THR:766, LEU:694, VAL:702, ALA:719, LYS:721, MET:742, LEU:764, CYS:773,	Nil
4d	-10.8	1	LYS:721, MET:742, LEU:764, CYS:773, LEU:820, THR:830, ASP:831	2.62
4c	-10.5	1	MET:769, ALA:719, LEU:820, ASP:831, LYS:721	1.98
4j	-10.4	1	MET:742, LEU:694, MET:769, ALA:719, LEU:820, LYS:721, LEU:764, LYS:851	2.02
4k	-10.2	1	ALA:719, LEU:820, ASP:831, LYS:721, LEU:764, LYS:851	1.87
4e	-9.7	Nil	PHE:699, VAL:702, MET:742, LEU:694, MET:769, ALA:719,	Nil
4o	-9.5	1	VAL:702, MET:742, LEU:694, MET:769, ALA:719, LEU:820, ASP:831, LYS:721, LEU:764,	2.18
4g	-8.9	1	LEU:694, VAL:702, ALA:719, LYS:721, MET:769, LEU:820	2.01
4l	-8.6	Nil	9, VAL:702, MET:742, MET:769, ALA:719, LEU:820,	Nil
4m	-8.4	1	VAL:702, MET:742, LEU:694, MET:769, LEU:820, LYS:721	1.98



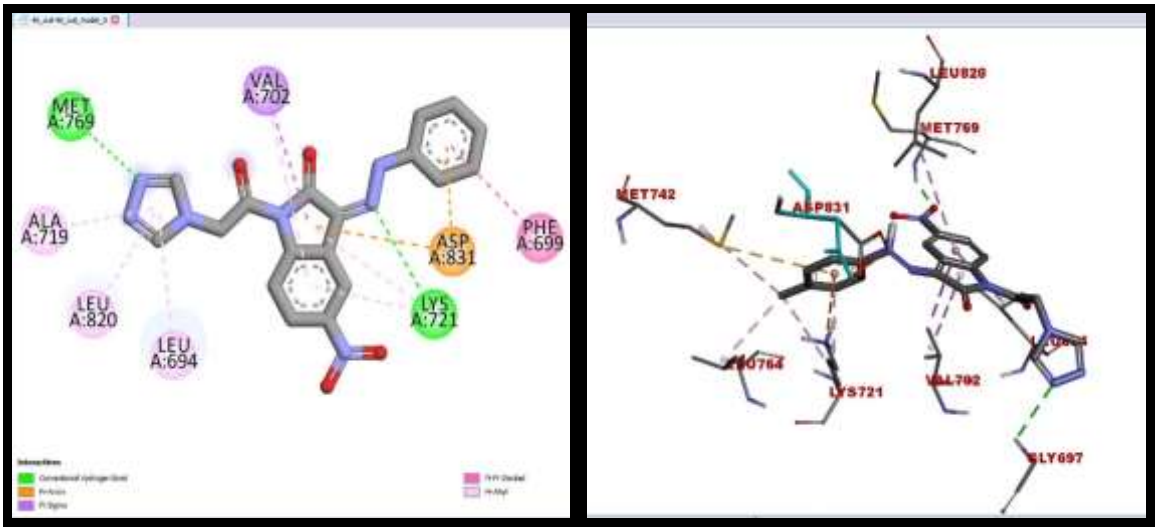
Sindhu-I-4b (Dock1-2d, Dock-2-3d)



Sindhu-I-4d (Dock1-2d, Dock-2-3d)



Sindhu-I-4f (Dock1-2d, Dock-2-3d)



Sindhu-I-4h (Dock1-2d, Dock-2-3d)

Figure 5: Docking Pose between the Ligand and the Protein (Sindhu-I-(4b, 4d, 4f and 4h)-dock-1 2d and dock-2 3d pictures.

5. CONCLUSIONS

The novel 5-substituted-3-[2-(4-methoxyphenyl) hydrazinylidene]-1-[1, 2, 4-triazol-4-yl) acetyl]-indol-2-one-4(a-o) compounds are developed by a three step process under conventional method. The yield of the synthesized compound was found to be in the range from **75-87%.** All novel isatin derivatives (Scheme-I, Sindhu-I-4(a-o)) were elucidated by IR, ^{1}H-NMR, Mass spectroscopy along with physical data. All the synthesized compounds screened for the anticancer activity against MCF-7 ell lines and Molecular docking studies were carried out by AUTODOCK VINA. Most of the compounds are showing good IC_{50} vales were compared with standard.

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