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

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ABSTRACT
In connection with our research program on the development of novel Isatin **(Scheme-II, II-3(a-j)** based anticancer agents, here in we report the design and synthesis of different Isatin derivatives II-3(a-j). The structures of all new compounds were identified by FT-IR, ^{1}HNMR, Mass spectroscopic techniques. Cell viability assays (MTT) for the tested novel Isatin derivatives were performed and IC_{50} values of the compounds were calculated after a 24h treatment. All isatin derivatives test results showed a promising anticancer activity against MCF-7 cells as compared with the standard drug doxorubicin. Compounds **II-3e, II-3h,** and **II-3j** were the most potent derivatives with IC_{50} values of **19.35±0.004, 25.70±0.412**, and **16.53± 0.021µg**. Furthermore, molecular docking studies were carried out by AUTODOCK VINA against EGFR with PDBID:1M17 to investigate the binding patterns of the designed compounds. The designed molecules showed binding energies ranging from -**9.54 to -7.2 Kcal/mol.**
KEYWORDS: Isatin, Benzene sulphonamide, Anticancer Activities, MCF-7 cell line, Doxorubicin, EGFR and AUTODOCK VINA.

1. INTRADUCTION

Isatin is an important class of nitrogen containing heterocyclic aromatic compounds, found in plants, human blood and tissue and acts as important specie for the synthesis of various heterocyclic compounds especially, Indolic and quinolinic compounds [1]. Isatinbased multicomponent reactions are one of the preferred strategies for the one pot synthesis of spirooxindole fused heterocycles [2]. Isatin (1H-indole-2,3-dione) derivatives show a broad spectrum of biological activities such as antibacterial, antifungal, antiviral, anti-inflammatory, anti-convulsant and anticancer drug

[3,4]. Due to the increase in cancer incidence, scientific research on cancer morbidity and mortality and improvement in the quality of life of cancer patients is increasing rapidly.

Cancer is a serious health concern in all societies, regardless of wealth or social status. In 2018, 18.1 million people worldwide suffered from cancer and 9.6 million patients died from the disease. By 2040, these figures will almost double, and the largest increase will be noticed in low- and middle-income countries where more than two-thirds of world cancer cases occur [5]. Over the last two decades, hydrazide–hydrazine derivatives have been known as one of the most important groups in medicinal chemistry [6-8] and they are present in a large number of compounds due to their diverse biological properties [9-12]. Furthermore, hydrazide–hydrazones are increasingly considered to be a valuable core in medicinal chemistry [13-16]. However, Isatin is a natural product discovered in the early 19th century in the genus Isatis and in Couroupita guianensis Aubl. Although the developments in chemotherapy increase the fight against cancer, numerous side effects of the drugs used pose serious problems in cancer treatment. A great number of drugs have been used to treat cancer, such as cis-platinum and ruthenium compounds, but good treatable values have not been achieved with existing drugs. To eliminate these disadvantages, increasing numbers of studies are continuously being carried out in this area.

Docking is widely used to anticipate the alignment of small molecule therapeutic compounds concerning their protein targets in anticipating the small molecule's affinity and activity [17]. Docking plays a critical role in rational drug design. Considering the biological and pharmacological importance of docking studies, much effort has been made to improve the algorithms for docking prediction. Docking is a mathematical technique that anticipates the preferable orientation of one molecule relative to another when they are linked together to create a stable complex. Using scoring functions, it is possible to estimate the strength of the connection or binding affinity across two compounds based on their preferential orientation.

We have also synthesized and tested anticancer activities of many heterocyclic compounds against MCF-7 cell lines. The aim of this study was to examine the effect of the Chlorine, Nitro and methyl group at different positions of the Isatin and aromatic ring of novel isatin derivatives on cytotoxic properties and also to try to improve their solubility. We synthesized 2-{5-substituted-3-[2-(4-substitutedphenyl) hydrazinyl idene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl-acetamide-3-(a-j) via the reaction of substituted isatin, phenyl hydrazine with substituted benzaldehyde in the presence of glacial acetic acid according to the Schiff's and N-alkylation method.

2. EXPERIMENTAL SECTION

2.1. Materials and Methods

The starting materials and reagents used in the reactions were supplied commercially by Merck and LOBA chemicals. The present work is based on Schiff's base and N-alkylation reactions. 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 (8:2). The molecular docking studies were carried out by using AUTODOCK suite of MGL Tools Software.

2.2. General synthetic procedure

Step-I: (3Z)-5-substituted-3-[2-(4-substituedphenyl) hydrazinyldene]-1,3-dihydro-2H-indol-2-one(1a-1e): The 5-substituted Isatin (0.01 mol) compounds 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 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 [18].

Step-II: Synthesis of N-(benzene sulfonyl)-2-chloroacetamide(2a): To a solution of Benzene sulphonamide (0.032mol) in (30ml) glacial acetic acid, the chloracetyl chloride (0.016mol) was added drop wise with constant stirring. The reaction mixture was refluxed for 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 [19].

Step-III: 2-{5-substituted-3-[2-(4-substitutedphenyl) hydrazinylidene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl-acetamide. To a solution of (3Z)-5-substituted-3-[2-(4-substituedphenyl) hydrazinyldene]-1,3-dihydro-2H-indol-2-one(1a-1e) (0.032mol) in (30ml) glacial acetic acid, the N-(benzene sulfonyl)-2-chloroacetamide(2a) (0.016mol) was added drop wise with constant stirring. The reaction mixture was refluxed for 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[20].

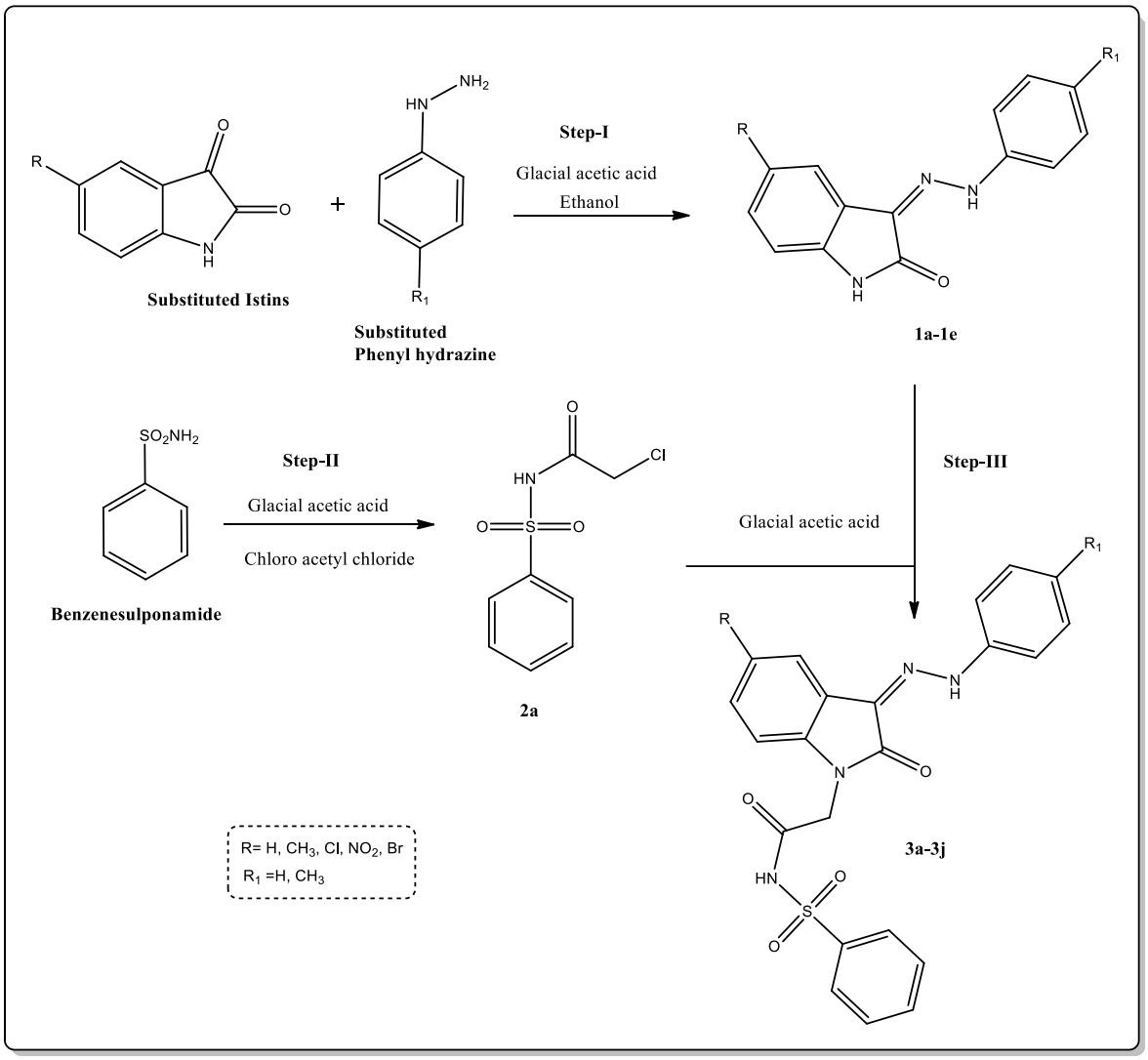


Fig.No.1. Schematic representation of 2-{5-substituted-3-[2-(4-substitutedphenyl) hydrazinyl idene-2-oxo-2,3-dihydro-1H -indol-1-yl] -N-benzene-1-sulfonyl- zacetamide-3-(a-j).

Compound.3a:**2-{3-[2-phenyl-hydrazinylidene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl-acetamide. IR cm^{-1} (KBr):** 3412(-NH Str in Sulphonamide); 3276(-NH Str in NH-Aromatic); 3012(-CH Str in Aromatic); 2974, 2867(-CH Str in alkyl); 1702(-CO Str in Indole); 1667(-CO Str in Sulphonamide); 1529(-C=N Str); 1449(-C=C Str); 1386(-C-C Str); 1128(-SO_2 Str). **^{1}HNMR (DMSO, δppm):** 10.2035(s, 1H, NH-aromatic proton); 9.8933(s, 1H, -NH-SO_2 proton); 8.0231-0024(d, 2H, Aromatic protons); 7.8454-7.8102(d, 2H,

Aromatic); 7.6458-7.6034(d, 2H, Aromatic); 7.4984-7.4803(t, 2H, Aromatic); 7.4456-7.4209(t, 3H, Aromatic); 7.3987-7.3503(t, 3H, aromatic); 4.4043(s, 2H, -N-alkyl proton).

Mass (LC-MS): m/z 434.10(M+); 435.21(M+1, 100%).

Compound.3b: 2-{5-methyl-3-[2-phenyl-hydrazinylidene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl-acetamide. IR cm^{-1} (KBr): 3351(-NH Str in Sulphonamide); 3310(-NH Str in NH-Aromatic); 3035(-CH Str in Aromatic); 2991, 2851(-CH Str in alkyl); 1715(-CO Str in Indole); 1655(-CO Str in Sulphonamide); 1575(-C=N Str); 1437(-C=C Str); 1317(-C-C Str); 1189(-SO_2 Str). **^{1}HNMR (DMSO, δppm):** 10.5653(s, 1H, NH-aromatic proton); 9.6843(s, 1H, -NH-SO_2 proton); 8.2093(s, 1H, Aromatic protons); 8.0980-8.0723(d, 2H, Aromatic); 7.8243-7.8109(d, 2H, Aromatic); 7.7987-7.7802(d, 2H, Aromatic); 7.6907-7.6290(t, 3H, Aromatic); 7.5903-7.5209(t, 3H, aromatic); 4.5021(s, 2H, -N-alkyl proton), 2.0543(s, 3H, Ar-CH_3). **Mass (LC-MS):** m/z 448.12(M+); 449.03(M+1, 100%).

Compound.3c: 2-{5-Chloro-3-[2-phenyl-hydrazinylidene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl-acetamide. **IR cm^{-1} (KBr):** 3400(-NH Str in Sulphonamide); 3260(-NH Str in NH-Aromatic); 3016(-CH Str in Aromatic); 2928, 2750(-CH Str in alkyl); 1715(-CO Str in Indole); 1667(-CO Str in Sulphonamide); 1515(-C=N Str); 1458(-C=C Str); 1386(-C-C Str); 1277(-SO_2 Str), 814(-Cl Str in Ar-Cl). **^{1}HNMR (DMSO, δppm):** 10.8964(s, 1H, NH-aromatic proton); 9.8212(s, 1H, -NH-SO_2 proton); 8.2065(s, 1H, Aromatic protons); 8.1654-8.1502(d, 2H, Aromatic); 8.0453-8.0102(d, 2H, Aromatic); 7.8874-8402(d, 2H, Aromatic); 7.7124-7.7023(t, 3H, Aromatic); 7.6102-7.6005(t, 3H, aromatic); 4.6895(s, 2H, -N-alkyl proton). **Mass (LC-MS):** m/z 468.07(M+); 469.32(M+1, 100%), 470.2, 30%).

Compound.3d: 2-{5-nitro-3-[2-phenyl-hydrazinylidene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl-acetamide. **IR cm^{-1} (KBr):** 3424(-NH Str in Sulphonamide); 3287(-NH Str in NH-Aromatic); 3023(-CH Str in Aromatic); 2965, 2804(-CH Str in alkyl); 1720(-CO Str in Indole); 1682(-CO Str in Sulphonamide); 1628(-NO_2 Str in Ar-NO_2); 1521(-

C=N Str); 1467(-C=C Str); 1345(-C-C Str); 1221(-SO_2 Str). **^{1}HNMR (DMSO, δppm):** 10.8964(s, 1H, NH-aromatic proton); 9.8212(s, 1H, -NH-SO_2 proton); 8.2120(s, 1H, Aromatic protons); 8.0654-8.0502(d, 2H, Aromatic); 7.8453-7.8102(d, 2H, Aromatic); 7.6874-6402(d, 2H, Aromatic); 7.6124-7.6023(t, 3H, Aromatic); 7.4102-7.4005(t, 3H, aromatic); 4.6895(s, 2H, -N-alkyl proton). **Mass (LC-MS):** m/z 468.07(M+); 469.32(M+1, 100%), 470.2, 30%).

Table.No.1: **Physical Data of Synthesized 2-{5-substituted-3-[2-(4-substitutedphenyl) hydrazinyl idene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl-acetamide-3-(a-j).**

Compound	R	R_1	Molecular Formula	Molecular Weight(gm)	M.P (°C)	%Yield	Rf Value
II-3a	H	H	$C_{22}H_{18}N_4O_4S$	434.10	209-211	84	0.67
II-3b	CH_3	H	$C_{23}H_{20}N_4O_4S$	448.12	201-203	78	0.72
II-3c	Cl	H	$C_{22}H_{17}ClN_4O_4S$	468.07	176-178	86	0.82
II-3d	NO_2	H	$C_{17}H_{17}N_5O_6S$	479.09	187-189	81	0.63
II-3e	Br	H	$C_{22}H_{17}BrN_4O_4S$	512.02	236-238	76	0.76
II-3f	H	CH_3	$C_{23}H_{20}N_4O_4S$	448.12	261-263	79	0.62
II-3g	CH_3	CH_3	$C_{24}H_{22}N_4O_4S$	462.14	159-161	82	0.83
II-3h	Cl	CH_3	$C_{23}H_{19}ClN_4O_4S$	482.08	229-231	81	0.73
II-3i	NO_2	CH_3	$C_{23}H_{19}N_5O_6S$	493.11	247-249	76	0.67
II-3j	Br	CH_3	$C_{23}H_{19}BrN_4O_4S$	526.03	139-141	87	0.84

Compound.3e: 2-{5-bromo-3-[2-phenyl-hydrazinylidene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl-acetamide. IR cm^{-1} (KBr): 3456(-NH Str in Sulphonamide); 3301(-NH Str in NH-Aromatic); 3089(-CH Str in Aromatic); 2952, 2889(-CH Str in alkyl); 1719(-CO Str in Indole); 1696(-CO Str in Sulphonamide); 1531(-C=N Str); 1484(-C=C Str); 1323(-C-C Str); 1218(-SO_2 Str), 686(-Br Str in Ar-Br). **^{1}HNMR (DMSO, δppm):** 10.4874(s,

1H, NH-aromatic proton); 9.6843(s, 1H, -NH-SO_2 proton); 8.3023(s, 1H, Aromatic protons); 8.0564-8.0022(d, 2H, Aromatic); 7.8654-7.8343(d, 2H, Aromatic); 7.6453-7.6213(d, 2H, Aromatic); 7.5094-7.4903(t, 3H, Aromatic); 7.2893-7.2198(t, 3H, aromatic); 4.8203(s, 2H, -N-alkyl proton). **Mass (LC-MS):** m/z 512.02(M+); 513.32(M+1, 100%).

Compound.3f: 2-{3-[2-(4-methylphenyl)hydrazinylidene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl-acetamide. **IR cm^{-1} (KBr):** 3423(-NH Str in Sulphonamide); 3298(-NH Str in NH-Aromatic); 3067(-CH Str in Aromatic); 2956, 2869(-CH Str in alkyl); 1717(-CO Str in Indole); 1682(-CO Str in Sulphonamide); 1520(-C=N Str); 1463(-C=C Str); 1332(-C-C Str); 1201(-SO_2 Str). **^{1}HNMR (DMSO, δppm):** 10.2873(s, 1H, NH-aromatic proton); 9.5654(s, 1H, -NH-SO_2 proton); 8.3012(s, 1H, Aromatic protons); 7.9875-7.9023(d, 2H, Aromatic); 7.7864-7.7002(d, 2H, Aromatic); 7.5684-7.534(d, 2H, Aromatic); 7.4784-7.4123(t, 3H, Aromatic); 7.2283-7.2034(t, 3H, aromatic); 4.6875(s, 2H, -N-alkyl proton), 2.0943(s, 3H, Ar-CH_3). **Mass (LC-MS):** m/z 448.12(M+); 449.03(M+1, 100%).

Compound.3g: 2-{5-methyl-3-[2-(4-methylphenyl)hydrazinylidene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl-acetamide. IR cm^{-1} (KBr): 3412(-NH Str in Sulphonamide); 3243(-NH Str in NH-Aromatic); 3017(-CH Str in Aromatic); 2965, 2845(-CH Str in alkyl); 1719(-CO Str in Indole); 1692(-CO Str in Sulphonamide); 1518(-C=N Str); 1459(-C=C Str); 1314(-C-C Str); 1220(-SO_2 Str). **^{1}HNMR (DMSO, δppm):** 10.5874(s, 1H, NH-aromatic proton); 9.8934(s, 1H, -NH-SO_2 proton); 8.1564(s, 1H, Aromatic protons); 7.8934-7.8205(d, 2H, Aromatic); 7.7394-7.7094(d, 2H, Aromatic); 7.4023-7.4012(d, 2H, Aromatic); 7.2903-7.2093(d, 2H, Aromatic); 7.0943-7.0102(t, 3H, aromatic); 4.8024(s, 2H, -N-alkyl proton), 2.1432(s, 3H, methyl proton in Indole), 1.909(s, 3H, Ar-CH_3). **Mass (LC-MS):** m/z 462.14(M+); 463.32(M+1, 100%).

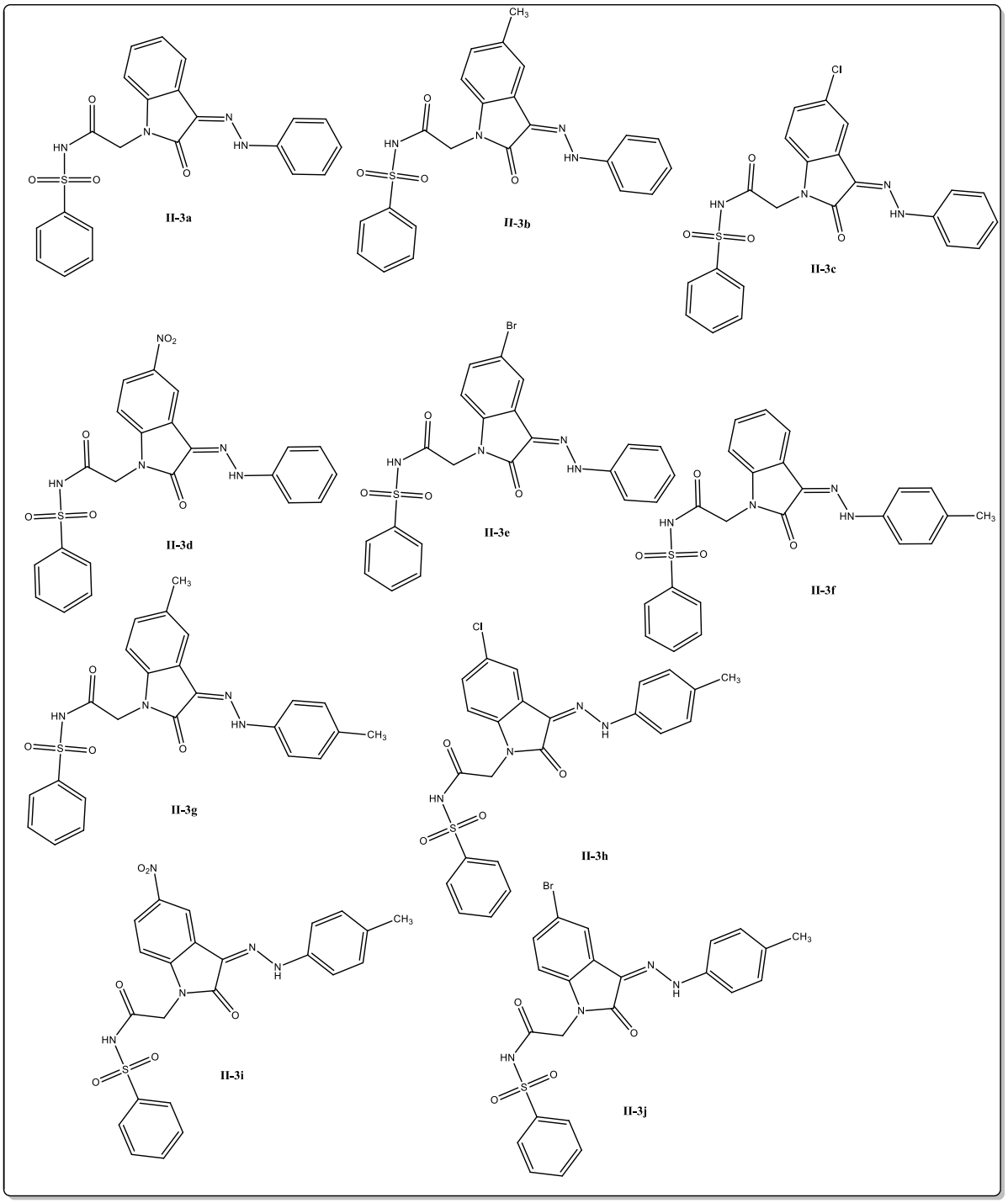


Figure.. 2-{5-substituted-3-[2-(4-substitutedphenyl) hydrazinyl idene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl-acetamide-3-(a-j).

Compound.3h: 2-{5-Chloro-3-[2-(4-methylphenyl)hydrazinylidene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl-acetamide. IR cm^{-1} (KBr): 3412(-NH Str in Sulphonamide); 3243(-NH Str in NH-Aromatic); 3017(-CH Str in Aromatic); 2965, 2845(-CH Str in alkyl); 1719(-CO Str in Indole); 1692(-CO Str in Sulphonamide); 1518(-C=N Str);

1459(-C=C Str); 1314(-C-C Str); 1220(-SO_2 Str), 810(-Cl Str in Ar-Cl). **^{1}HNMR (DMSO, δppm):** 10.5874(s, 1H, NH-aromatic proton); 9.8934(s, 1H, -NH-SO_2 proton); 8.1564(s, 1H, Aromatic protons); 7.8934-7.8205(d, 2H, Aromatic); 7.7394-7.7094(d, 2H, Aromatic); 7.4023-7.4012(d, 2H, Aromatic); 7.2903-7.2093(d, 2H, Aromatic); 7.0943-7.0102(t, 3H, aromatic); 4.8024(s, 2H, -N-alkyl proton), 2.1432(s, 3H, methyl proton in Indole), 1.909(s, 3H, Ar-CH_3). **Mass (LC-MS):** m/z 482.08(M+); 483.06(M+1, 100%), 484.05(M+2, 30%).

Compound.3i: 2-{5-nitro-3-[2-(4-methylphenyl)hydrazinylidene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl-acetamide. IR cm^{-1} (KBr): 3454(-NH Str in Sulphonamide); 3252(-NH Str in NH-Aromatic); 3034(-CH Str in Aromatic); 2984, 2856(-CH Str in alkyl); 1721(-CO Str in Indole); 1687(-CO Str in Sulphonamide); 1612(-NO_2 Str in Ar-NO_2); 1512(-C=N Str); 1429(-C=C Str); 1305(-C-C Str); 1218(-SO_2 Str). **^{1}HNMR (DMSO, δppm):** 10.3874(s, 1H, NH-aromatic proton); 9.6754(s, 1H, -NH-SO_2 proton); 8.3092(s, 1H, Aromatic protons); 7.9234-7.9032(d, 2H, Aromatic); 7.8240-7.8109(d, 2H, Aromatic); 7.6543-7.6109(d, 2H, Aromatic); 7.3154-7.3092(d, 2H, Aromatic); 7.1902-7.1002(t, 3H, aromatic); 4.6854(s, 2H, -N-alkyl proton), 2.2093(s, 3H, Ar-CH_3). **Mass (LC-MS):** m/z 493.11(M+); 494.21(M+1, 100%).

Compound.3j: 2-{5-bromo-3-[2-(4-methylphenyl) hydrazinylidene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl) acetamide. IR cm^{-1} (KBr): 3434(-NH Str in Sulphonamide); 3265(-NH Str in NH-Aromatic); 3109(-CH Str in Aromatic); 2976, 2846(-CH Str in alkyl); 1734(-CO Str in Indole); 1693(-CO Str in Sulphonamide); 1623(-NO_2 Str in Ar-NO_2); 1534(-C=N Str); 1432(-C=C Str); 1312(-C-C Str); 1234(-SO_2 Str), 686(-Br Str in Ar-Br). **^{1}HNMR (DMSO, δppm):** 10.5764(s, 1H, NH-aromatic proton); 9.8203(s, 1H, -NH-SO_2 proton); 8.3092(s, 1H, Aromatic protons); 7.8976-7.8023(d, 2H, Aromatic); 7.7443-7.7342(d, 2H, Aromatic); 7.5643-7.5023(d, 2H, Aromatic); 7.4832-7.4213(d, 2H, Aromatic); 7.293-

7.2093(t, 3H, aromatic); 4.8243(s, 2H, -N-alkyl proton), 2.1893(s, 3H, Ar-CH_3). **Mass (LC-MS):** m/z 526.03(M+); 527.05(M+1, 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 [21]. 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 2-{5-bromo-3-[2-(4-methylphenyl) hydrazinylidene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl) acetamide 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. Molecular docking studies were performed in order to find the possible protein ligand interactions of the dataset ligands. The potential active site amino acids of 1M17 complex were predicted using CASTp. The target protein and inhibitors were geometrically optimized. All the 10 compounds were docked against active site of target protein using AUTODOCK VINA. Additionally, these also assisted in identifying the conformational changes of the ligand in the protein environment. About 100 different protein-ligand complex conformations for each docked complex were generated through AUTODOCK suite of MGL Tools, the confirmation with lowest binding energy was displayed as the best binding energy. Binding energy of the dataset ligands were shown in Table 3 along with the interaction amino acids and number of amino acids [22].

3.RESULTS AND DISCUSSION

3.1. Synthesis: The series of synthesized novel 2-{5-bromo-3-[2-(4-methylphenyl) hydrazinylidene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl) acetamide derivatives (II-3(a-j) 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**(II-3a-j)** were synthesized by three steps. In Step-I, substituted Isatin reacts with phenyl hydrazide in glacial acetic acid to give compounds a-Ie. In step-II, benzene sulphonamide reacts with Chloroacetyl chloride in the presence of glacial acetic to give compound 2a. It can be reacting with compounds 1a-1e to form title compounds II-3(a-j).

3.2. Spectral data: Spectral characterization of 2-{5-bromo-3-[2-(4-methylphenyl) hydrazinylidene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl) acetamide derivatives was performed by IR spectroscopy. All synthesized compounds are showing -C=C stretching of the aromatic ring is around 1530-1545 cm^{-1} respectively. The halogen stretching is observed the strong absorption in the region 635-828 cm^{-1} and some compounds containing -NO_2 group shows peaks due to stretching of –NO_2 is observed at around 1610-1637 cm^{-1} respectively. Practically, in all the compounds are showing the secondary amine –NH stretching frequency at around 3220 to 3467 cm^{-1}. The aromatic and aliphatic C-H stretching frequency, as expected is observed at around 3000-3109 cm^{-1} and 2921-2743 cm^{-1}. All the compounds have been show strong absorption in the region of 1650-1725 cm^{-1} is found to be presence of C=O stretching frequency for indole and sulphonamide ketone groups respectively. Similarly, the ^{1}HNMR (DMSO-d6) spectra of novel isatin derivatives are showed a singlet at 10.0234-10.8504 δ for –NH proton in sulphonamide and 9.3545-9.8743 δ for -NH in Phenyl hydrazine proton. All these compounds have aromatic protons were found between

δ 8.248 to 7.003 δ ppm as singlet, doublet and triplet protons. All the compounds were showing singlet around at 4.0342 to 4.8743 δ for methylene protons acetyl group.

3.3. Anticancer activity: 2-{5-bromo-3-[2-(4-methylphenyl) hydrazinylidene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl) acetamide derivatives were screened for anticancer activity against by MTT assay with MCF-7 to predict the IC_{50} values. From the resulting table.3 proposed that MCF-7 cell lines were susceptible to the evaluated compounds showed IC_{50}values in the range of **16.53 ± 0.021 µg to 51.69±2.107 µg** against MCF7 cell line. Compounds **II-3e, II-3h,** and **II-3j** were the most potent derivatives with IC_{50} values of **19.35±0.004, 25.70±0.412**, and **16.53± 0.021µg** showed good activity. Whereas, remaining of the compounds showed moderately potent.

Table.2: Novel isatin derivatives(II-3a-3j)-Anticancer activity with IC_{50} values.

S. No	SAMPLE NAME	IC_{50} (µg)
		MCF7
1	**II-3a**	45.97± 0.204
2	**II-3b**	40.44±0.301
3	**II-3e**	**19.35±0.004****
4	**II-3h**	**25.70±0.412****
5	**II-3j**	**16.53± 0.021****
6	**II-3l**	48.42±0.0206
7	**II-3m**	50.57±0.127
8	**II-3o**	51.69±2.107
9	**Doxorubicin**	12.83±0.011

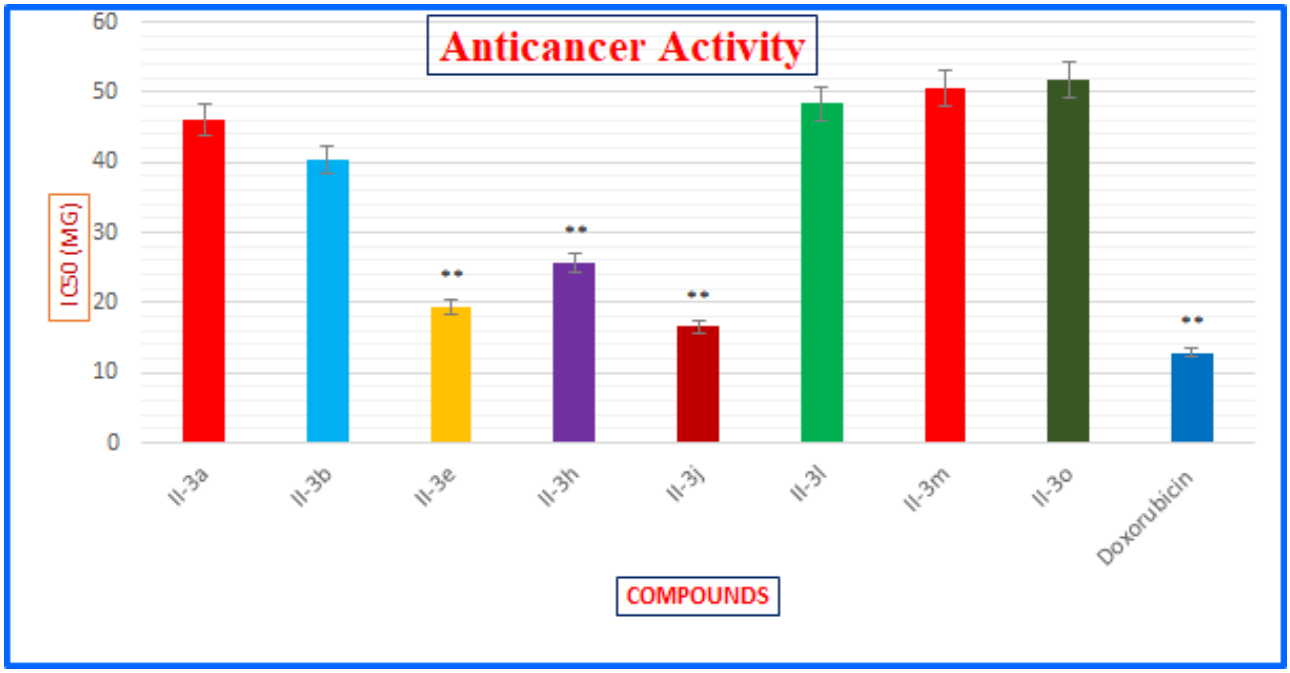
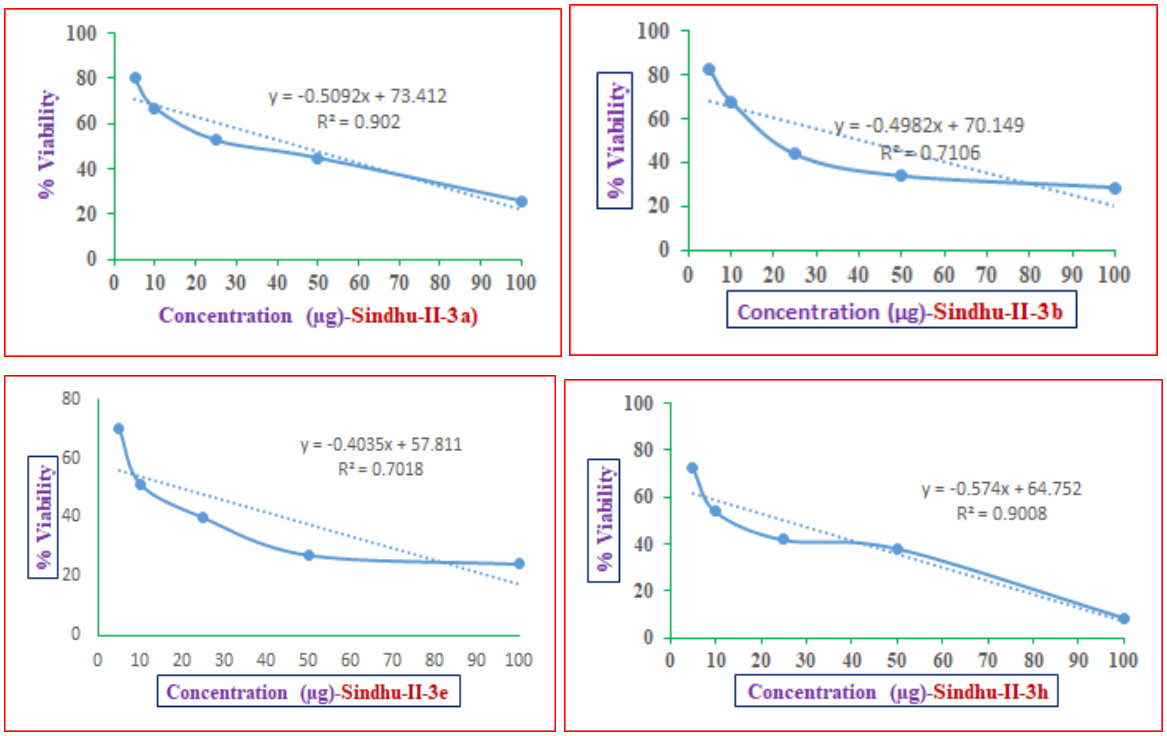


Figure. 3: Graphical representation of anticancer activity of Novel isatin derivatives(II-3(a-j) againstMCF-7 cell line.



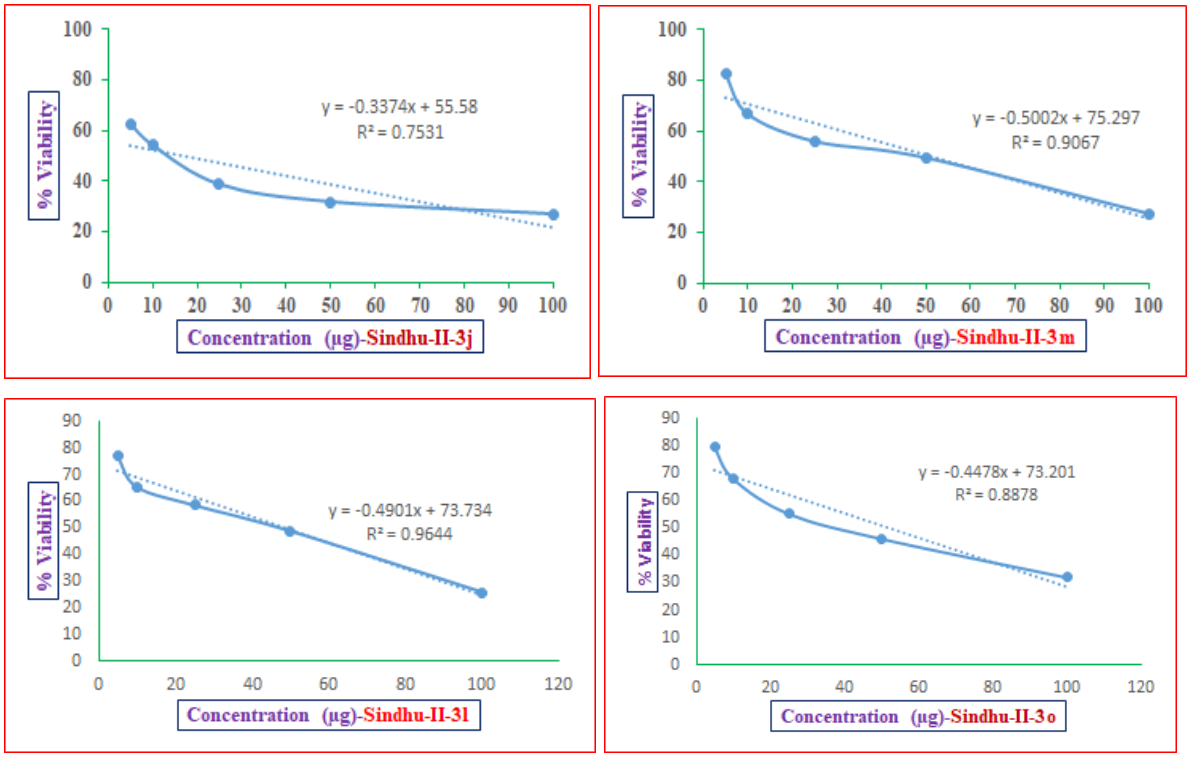


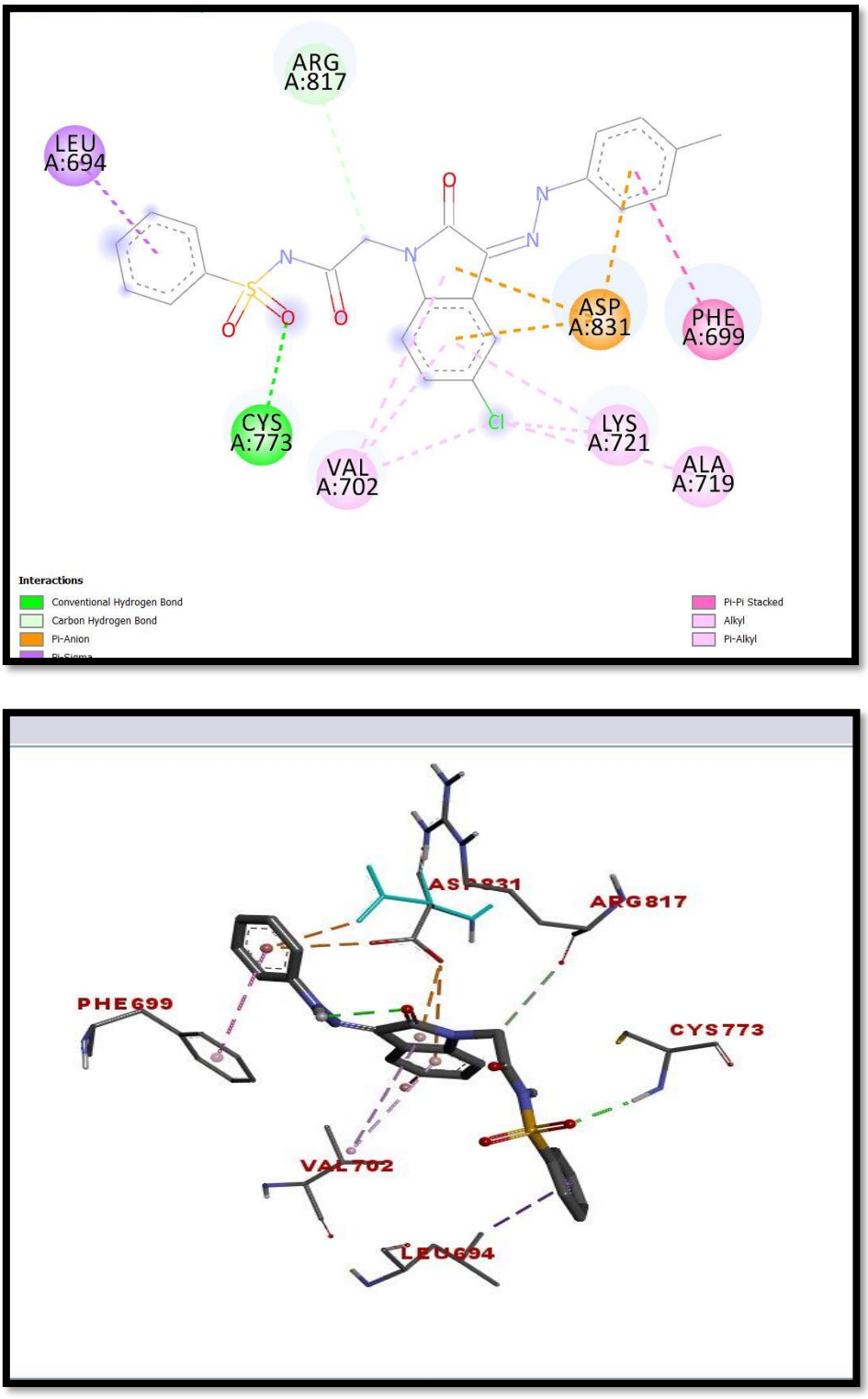
Figure. 4: Graphical representation of anticancer activity of Novel isatin derivatives(II-3(a-j)- IC_{50} values.

3.4. Molecular Docking studies: Molecular docking studies were carried out by AUTODOCK VINA against EGFR with PDBID:1M17 to investigate the binding patterns of the designed compounds. The designed molecules showed binding energies ranging from **-9.54 to -7.2 Kcal/mol.** Compounds **II-(3h, ii-3j, ii-3e, ii-3a & ii-3c)** has lowest binding energy when compared with other compounds. Most of the synthesized ligands possess one hydrogen bond each with VAL:702, MET:742, LEU:694, MET:769 amino acids.

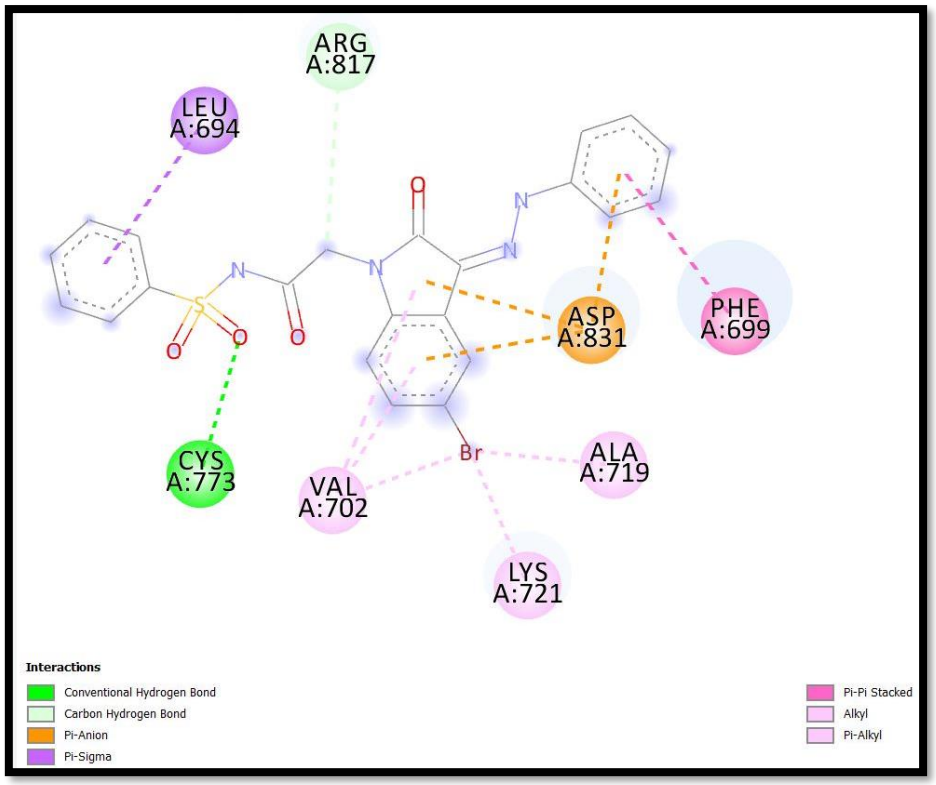
Table.No-3: Insilco EGFR inhibition of novel Isatin derivatives II-(4a-4o)-Binding Energy.

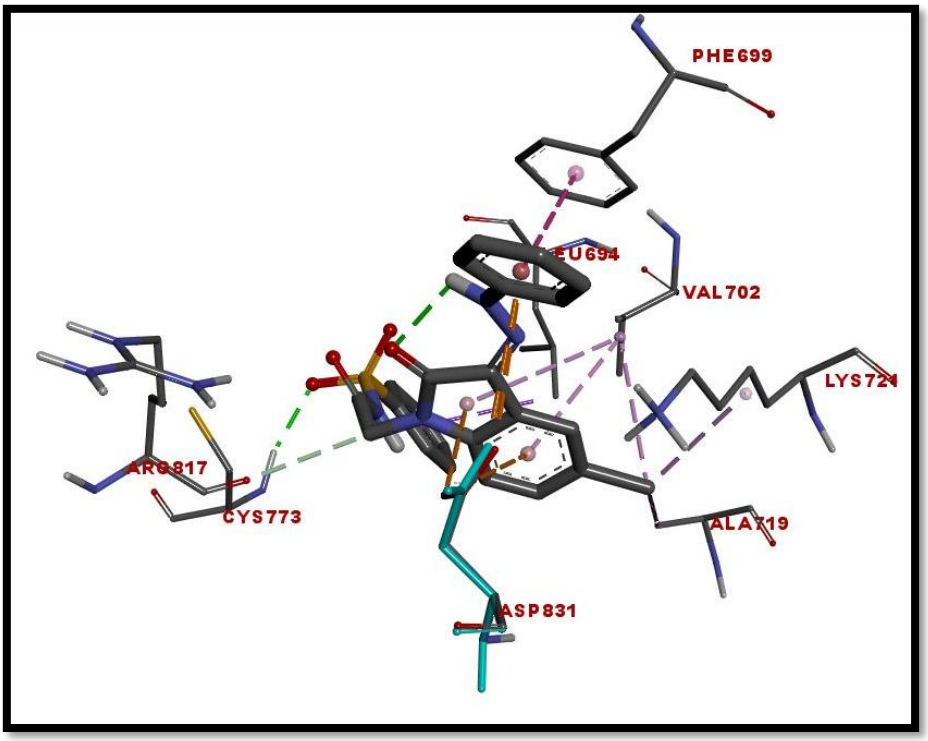
Compound No	Binding Energy (Kcal/mol)	No of H-bonds	H-bond lengths (Å)	Hydrogen bond interactions	Other Interacting amino acids
II-3a	-9	2	2.40, 2.92	ARG: 817, ASP: 831	PHE: 699, ALA:719, LYS:721, LEU: 764, MET: 742, CYS: 773
II-3b	-7.2	1	2.15	ASP: 831	LYS:721, LEU: 764, CYS: 773

II-3c	-9	2	2.07, 2.30	CYS:773, ARG: 817	PHE: 699, VAL: 702, ALA:719, LYS:721, GLY: 772, MET: 742, ASP: 831, LEU: 694
II-3d	-9.	2	2.04, 2.06	ALA:719, CYS	PHE: 699, ALA:719, LEU: 764, CYS: 773
II-3e	-9.1	2	2.09, 2.46	LYS: 721, CYS:773	PHE: 699, VAL: 702, GLY: 772, ASP: 831, LEU: 694
II-3f	-7.5	2	2.21, 2.18	CYS:773, ARG: 817	VAL: 702, LYS: 721, GLY: 772
II-3g	-8.3	1	2.32	PHE: 699, CYS:773	VAL: 702, GLY: 772, ASP: 831
II-3h	-9.5	2	2.09, 2.29	ARG: 817, CYS:773	PHE: 699, ALA:719, LYS:721, VAL: 702, GLY: 772, ASP: 831, LEU: 694
II-3i	-7.8	NIL	NIL	NIL	VAL: 702, GLY: 772, , LEU: 694
II-3j	-9.2	NIL	NIL	NIL	LEU: 694, PHE: 699, VAL: 702, ALA:719, LYS:721, LEU: 764, MET: 742, ASP: 831

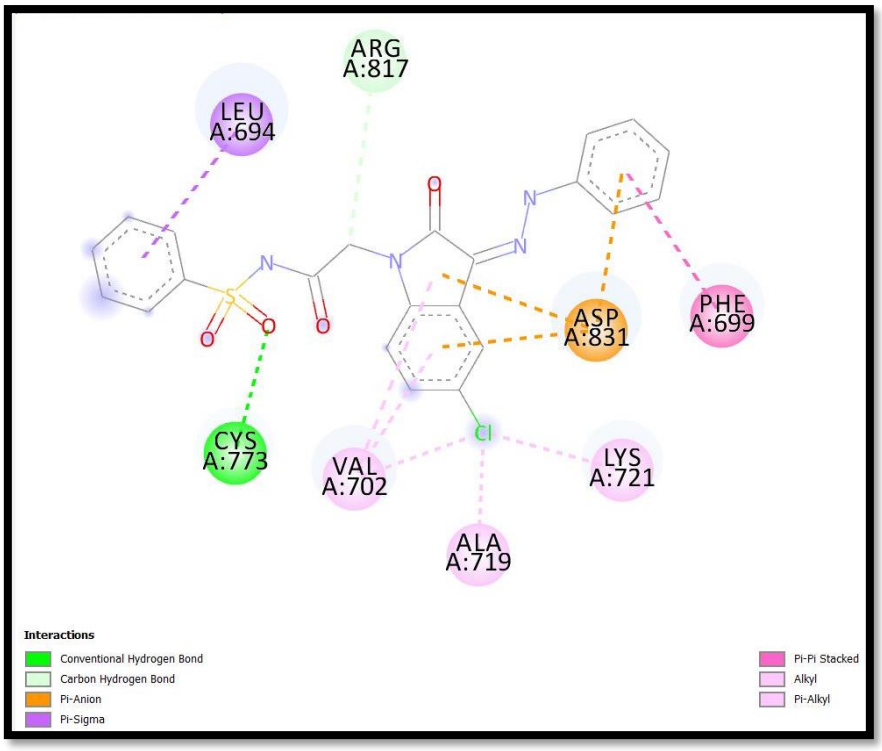


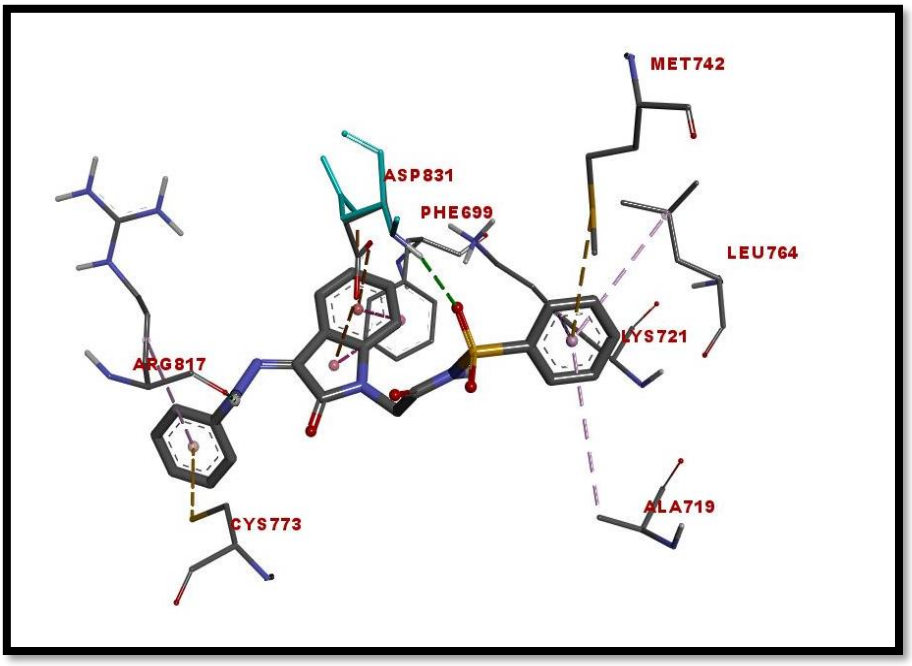
II-3a (Dock1-2d, Dock-2-3d)



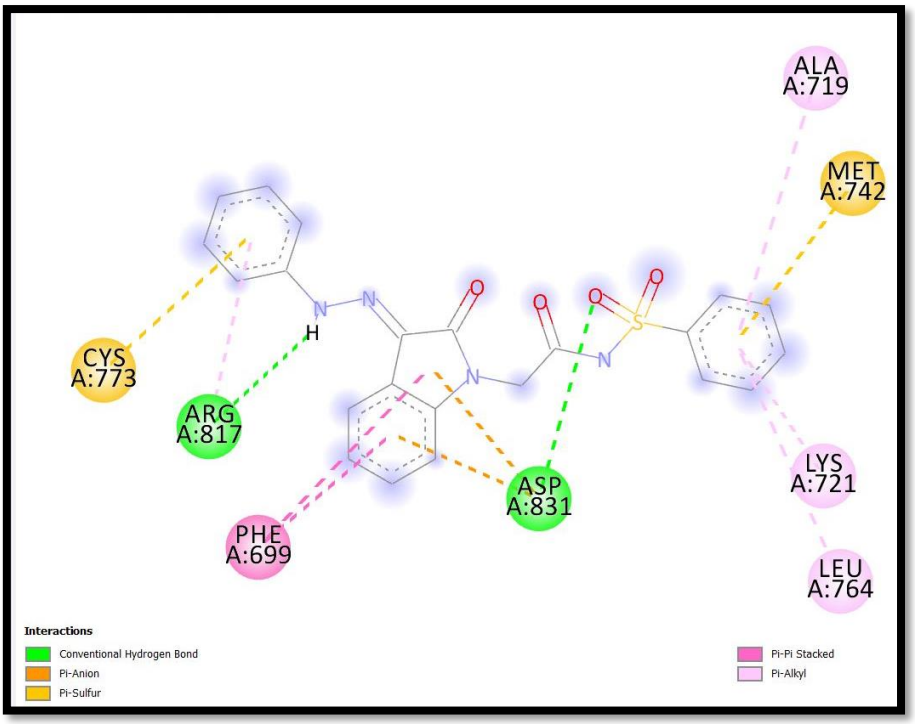


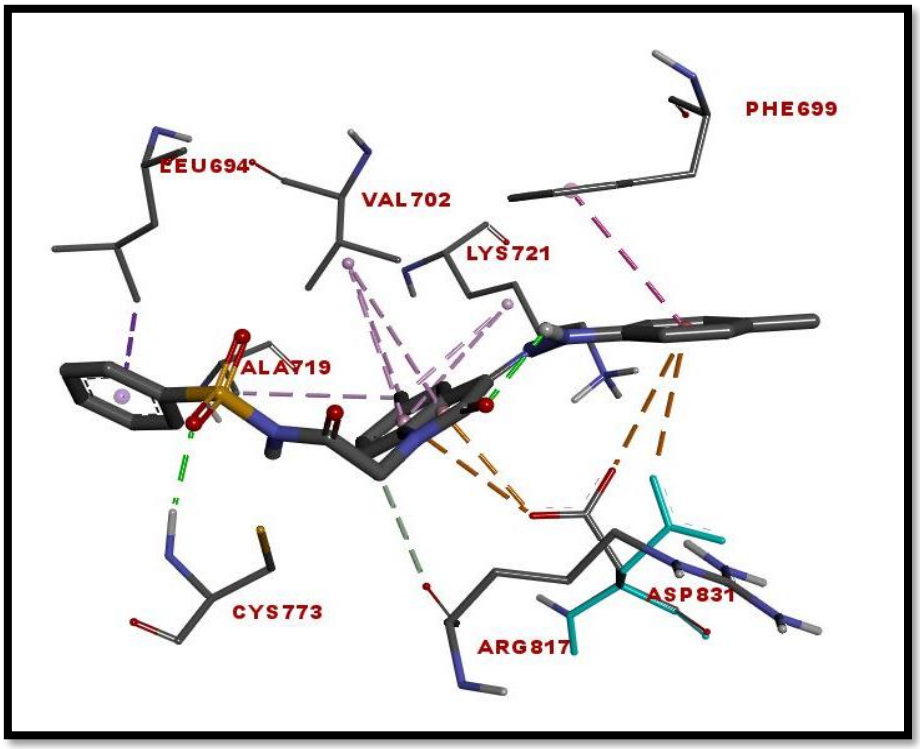
II-3c (Dock1-2d, Dock-2-3d)



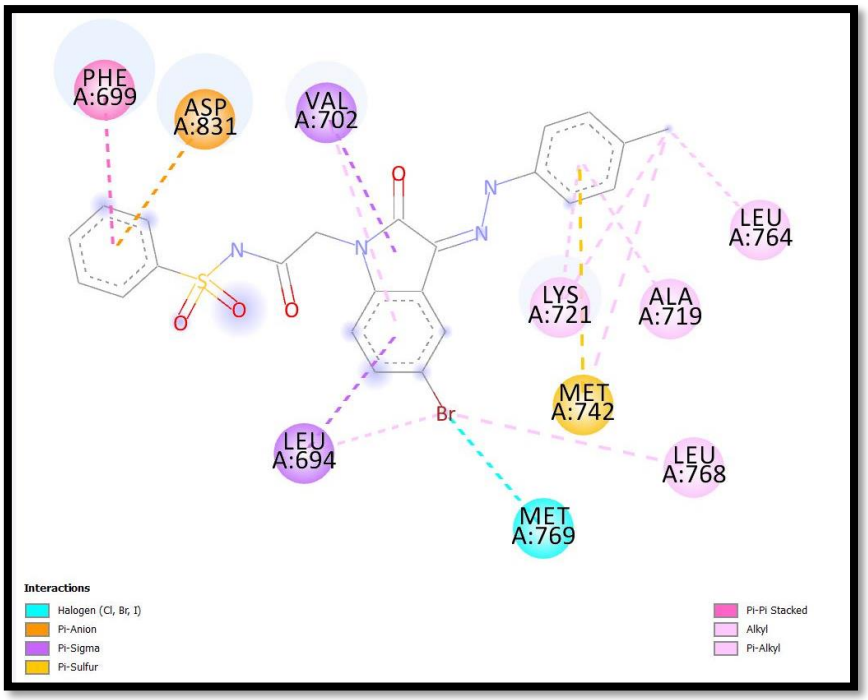


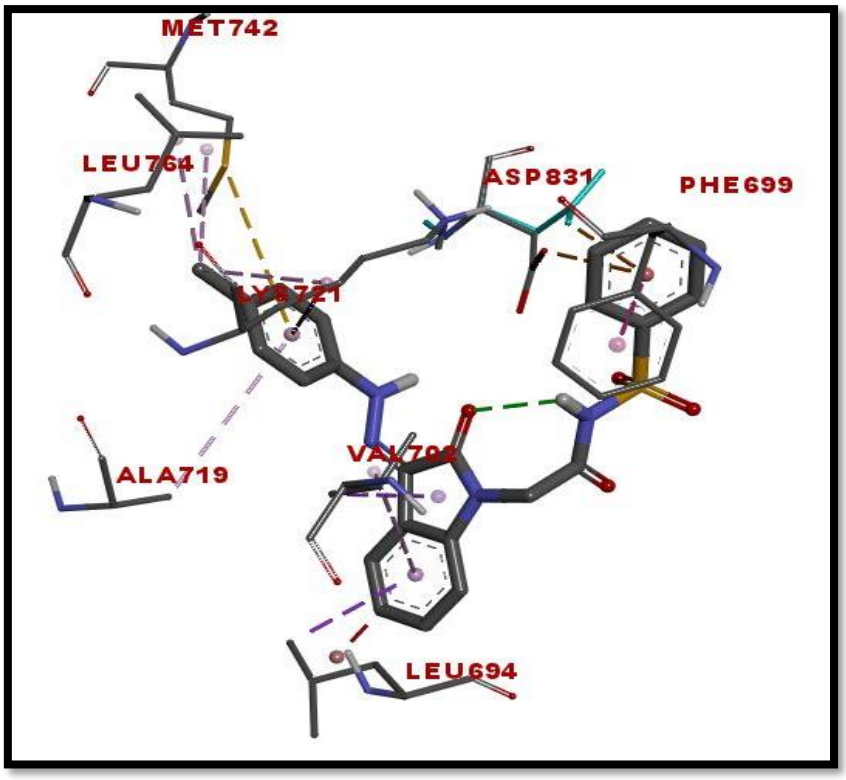
II-3e (Dock1-2d, Dock-2-3d)





II-3h (Dock1-2d, Dock-2-3d)





II-3j (Dock1-2d, Dock-2-3d)

Figure.5. Docking Pose between the Ligand and the Protein-II-(3b, 3c, 3e, 3h and 3j)-dock-1 2d and dock-2 3d pictures.

5. CONCLUSIONS:

In conclusion, we are synthesized 2-{5-bromo-3-[2-(4-methylphenyl) hydrazinylidene-2-oxo-2,3-dihydro-1H-indol-1-yl]-N-benzene-1-sulfonyl) acetamide derivatives (II-3(a-j)) compounds are prepared by Schiff's base and N-alkylation mechanism by conventional method. The yield of the synthesized compound was found to be in the range from 76-87%. All the synthesized compounds structure were confirmed by IR, ^{1}H-NMR, Mass spectroscopy along with physical data All isatin derivatives test results showed a promising anticancer activity against MCF-7 cells as compared with the standard drug doxorubicin. Compounds II-3e, II-3h, and II-3j were the most potent derivatives with lower IC_{50} values. In molecular docking studies, all the ligands were showed binding energies ranging from -9.54 to -8.23 Kcal/mol.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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