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SYNTHESIS, *IN VITRO* ANTICANCER AND MOLECULAR DOCKING STUDIES OF NEW BISISATINS AS ANTICANCER AGENTS

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ABSTRACT

Fifteen symmetrical bis-schiff base of isatin derivatives were synthesized by reaction of succinic acid hydrazide with various isatins and the synthesized compounds were characterised by spectral analysis. The substances were further subjected to in vitro cytotoxicity evaluatation against A549 and MCF-7 cell lines with MTT assay. All the synthesized compounds shown significantly inhibited the growth of MCF-7 cells over the A549 cells and the IC_{50} values of all the compounds were found between 10.24 and 28.65 µM. The compound **6j** has resulted highest cytotoxicity in the entire series studied, in addition **6d**, **6c, 6e** and **6n** were shown to display moderate activity. Further, molecular docking studies of the ligand's were done on EGFR using GRIP batch docking method. The compounds **6c, 6d, 6e, 6i, 6j** and **6l** exhibited good docking (PLP) scores with receptor having Hydrogen, Hydrophobic and Vander Waal's interactions.

KEYWORDS: Anticancer; Isatin; Molecular docking; Succinohydrazide; MTT Assay.

INTRODUCTION

Cancer is defined as a group of diseases characterized by uncontrolled growth and the spread of abnormal cells which if left untreated may lead to death.[1] Cancer continues to be a major health problem worldwide and more than ten million new cancer cases occur annually, roughly half of which is prevalent in the developed countries and the disease causes over six million deaths a year.[2] Till date chemotherapy has been the mainstay of cancer therapy. However the use of available chemotherapeutics causes undesirable side effects, due to lack

of selectivity against cancerous cells which include bone marrow depression, alopecia, drug-induced cancer, hepatotoxicity, along with a limited choice of available anti-cancer drugs.[3]

Receptor tyrosine kinases (RTKs) are high affinity cell surface receptors were proved to be a viable target for anticancer drug development.[4-6] They are the second most important drug targets after G protein coupled receptors (GPCR's).[7] Kinases are involved in many pathophysiological problems especially cancers where their over expression can lead to different types of malignancies.[8-9] In addition, EGFR-TK is one of the most important kinases that play a fundamental role in signal transduction pathways.[10] EGFR and its ligands, epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) have been implicated in numerous tumours of epithelial origin.[11] Therefore, the design of inhibitors that target EGFR-TK is an attractive approach for the development of new therapeutic agents.[12-13]

The indole-2, 3-dione (isatin) pharmacophore has attracted, and still attracts, much attention from medicinal chemists because of great importance in their biological as well as synthetic approach of medicinal chemistry. Isatin is an endogenous compound isolated in 1988[14] and reported to possess a wide range of central nervous system activities.[15-16] Various derivatives of isatin are known to possess a wide range of pharmacological properties.[17] Among the important pharmacological properties, antibacterial[18], anticonvulsant[19], anti-inflammatory[20],), anitidepressant[21], anxiolytic[22], analgesic[23], antiviral[24], antitubercular[25] and cytotoxic.[26]

In continuation to our study on biologically active isatin derivatives[27-28], here, we report the synthesis and spectroscopic characterization of some (N^1Z,N^4Z)-N^1,N^4-bis(2-oxoindolin-3-ylidene) succinohydrazides depicted in **Scheme-1** and evaluated the anticancer activity against the A549 and MCF-7 cell lines. We have also tried to dock the synthesised compounds with the crystal structure of EGFR (Pdb: 4ICZ) to explore the possible anticancer mechanism of our compounds. It maybe helps to the development of more potent anticancer agents.

MATERIALS AND METHODS

General

Human breast cancer (MCF-7) and human lung cancer (A549) cell lines were purchased from National Cancer Center for Cell Science (NCCS), Pune, India. All the reagents and solvents

used were of laboratory grade. The melting point of synthesized compounds was determined by open capillary method and was uncorrected. The purity of the compounds was checked by thin layer chromatography (TLC). IR spectra were obtained Bruker FT-IR Spectrophotometer by using Kbr disc. 1H-NMR spectra were recorded on Bruker Avance 300MH_Z Spectrometer; tetramethylsilane (TMS) as the internal standard in DMSO-d_6. The chemical shifts (δ) are reported in parts per million.

Chemistry

***Procedure for the Synthesis of Isonitrosoacetanilides* (2)**

In a 5 lit. R.B. Flask were placed chloral hydrate (0.54 mol) and 1200 ml of water. To this solution, were then added crystallized sodium sulphate (1300 gm) followed by a solution of appropriate Aniline (**1**) in 300 ml of water and concentrated Hydrochloric acid (0.52 mol). Finally, a solution of hydroxylamine HCl (1.58 mol) in 500 ml of water was added. The contents of the flask were heated over a wire-guage by a mecker burner so that vigorous boiling began in about 45 minutes. After 1 to 2 minutes of vigorous boiling the reaction was completed. During the heating period itself the crystals of isonitrosoacetanilides started separating out. On cooling under the current of water, the entire product was solidified. It was filtered under suction, air dried and purified by recrystallization from suitable solvent(s).

***Procedure for the Synthesis of Indole-2, 3-diones* (3)**

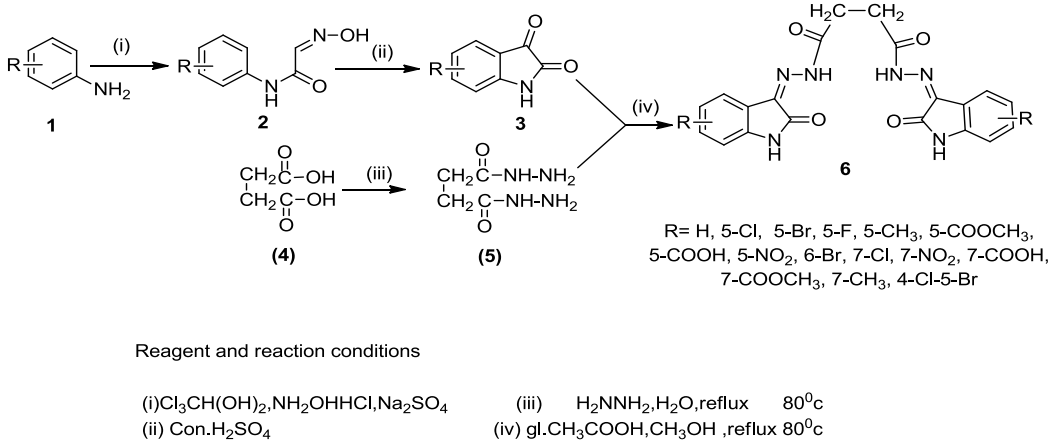
Sulphuric acid (326 ml) was warmed at 50^0C in a one liter R.B. flask fitted with an efficient mechanical stirrer and to this, finely powdered appropriate isonitrosoacetanilide (**2**, 0.46 mol) was added at such a rate so as to maintain the temperature between 60°C to 70°C but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly. After the addition of isonitroso compound was completed the temperature of the solution was raised to 80°C and maintained at that temperature for 10 minutes to complete the reaction. Then the reaction mixture was cooled to room temperature and poured on to crushed ice (2.5 kg) while stirring. After standing for about half-an-hour, the product separated was filtered, washed several times with small portions of cold water and dried. Purification of the compound was effected by the recrystallization from methanol.[29]

Procedure for the synthesis of succinohydrazide (5)

Diethylsuccinate (**4**, 0.1 mol) in alcohol was refluxed with hydrazine hydrate (99.9%, 0.2 mol) for 15 minutes. The resulting compound was cooled and the solvent was removed by distillation. The product thus obtained was recrystallized from ethanol.

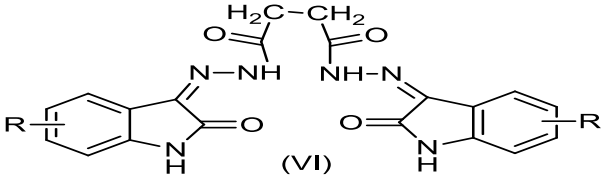
Procedure for synthesis of (N^1,N^4)-N^1,N^4-bis(2-oxoindolin-3-ylidene)succinohydrazide (6)

A mixture of an appropriate indole2, 3-dione (**3**,0.02 mol) and Succinohydrazide (**5**, 0.01mol) in methanol (50 ml) presence of glacial acetic acid was refluxed for 12 hours. The solvent was removed by distillation and resulting coloured solid was collected, dried and recrystallized from methanol. Physical properties of the compounds listed in **Table-1.**



***Scheme-1*: Schematic steps of (N^1,N^4)-N^1,N^4-bis(2-oxoindolin-3-ylidene) succinohydrazide derivatives.**

Table-1: Physical Data of (N'^1,N'^4)-N'^1,N'^4-bis(2-oxoindolin-3-ylidene) succinohydrazide derivatives (VIa-o).



S.No.	Compounds	R	Mol formula	Mol.wt.	M.P.(°C)	Yield(%)
1	6a	H	$C_{20}H_{16}N_6O_4$	404	275-276	60
2	6b	5-CH_3	$C_{22}H_{20}N_6O_4$	432	279-280	70
3	6c	5-Cl	$C_{20}H_{14}Cl_2N_6O_4$	473	290-292	55
4	6d	5-Br	$C_{20}H_{14}Br_2N_6O_4$	562	308-309	50
5	6e	5-F	$C_{20}H_{14}F_2N_6O_4$	440	290-293	50
6	6f	5-COOH	$C_{22}H_{12}N_6O_4$	492	305-308	60
7	6g	5-NO_2	$C_{20}H_{14}N_8O_8$	494	310-312	40
8	6h	5-$COOCH_3$	$C_{24}H_{20}N_6O_8$	520	270-272	40
9	6i	6-Br	$C_{20}H_{14}Br_2N_6O_4$	562	265-268	40
10	6j	4-Cl 5-F	$C_{20}H_{12}F_2Cl_2N_6O_4$	509	280-282	45
11	6k	7-CH_3	$C_{22}H_{20}N_6O_4$	432	285-286	50
12	6l	7-NO_2	$C_{20}H_{14}N_8O_8$	494	295-296	50
13	6m	7-COOH	$C_{22}H_{12}N_6O_4$	492	300-302	50
14	6n	7-Cl	$C_{20}H_{14}Cl_2N_6O_4$	473	290-292	50
15	6o	7-$COOCH_3$	$C_{24}H_{20}N_6O_4$	520	265-267	50

Spectral data of the synthesized compounds

(N^1, N^4)-N^1, N^4-bis (2-oxoindolin-3-ylidene) succinohydrazide (6a)

IR(KBr) cm^{-1}: 3364(NH), 3152 (NH), 2976 (C-H), 1765 (C=O), 1683(C=O), 1615(C=O), 1442(C=N), 1389(C=N).^{1}HNMR(DMSO d6) (ppm): 10.5(s, 2H,NH), 10.0(s, 2H,NH), 7.8(d, 2H, ArH), 7.7(d,2H, ArH), 7.5(t, 2H, ArH), 7.2(t, 2H, ArH), 2.2(s, 4H, 2(CH_2). LC–MS (ESI, m/z):404 (M+).

(N^1, N^4)-N^1, N^4-bis (5-methyl-2-oxoindolin-3-ylidene)succinohydrazide (6b)

IR(KBr) cm^{-1}: 3362(NH), 3142 (NH), 2976 (C-H), 1764 (C=O), 1685(C=O), 1616(C=O), 1444(C=N), 1392(C=N). ^{1}HNMR(DMSO d6) (ppm):10.6(s, 2H,NH), 10.0(s, 2H,NH), 8.2(s, 2H, ArH), 7.1(d, 2H, ArH), 6.6(d, 2H, ArH), 2.2(s, 4H, 2(CH_2), 2.3(s, 6H, 2(CH_3). LC–MS (ESI, m/z):432 (M+).

(N^1, N^4)-N^1, N^4-bis (5-chloro-2-oxoindolin-3-ylidene)succinohydrazide (6c)

IR(KBr) cm^{-1}: 3363(NH), 3143 (NH), 2973 (C-H), 1763 (C=O), 1683(C=O), 1613(C=O), 1443(C=N), 1393(C=N). ^{1}HNMR(DMSO d6) (ppm): 10.6(s, 2H,NH), 10.2(s, 2H,NH), 7.9(s, 2H, ArH), 7.8(d, 2H, ArH), 7.5(d, 2H, ArH), 2.1(s, 4H, 2(CH_2). LC–MS (ESI, m/z):474 (M+1),475(M+2).

(N^1, N^4)-N^1, N^4-bis (5-bromo-2-oxoindolin-3-ylidene)succinohydrazide (6d)

IR(KBr) cm^{-1}: 3363(NH), 3143 (NH), 2973 (C-H), 1763 (C=O), 1683(C=O), 1613(C=O), 1443(C=N), 1393(C=N). ^{1}HNMR(DMSO d6) (ppm): 10.4(s, 2H,NH), 10.2(s, 2H,NH), 8.1(s, 2H, ArH), 7.7 (d, 2H, ArH), 7.4(d, 2H, ArH), 2.1(s, 4H, 2(CH_2). LC–MS (ESI, m/z):562 (M+).

(N^1, N^4)-N^1, N^4-bis (5-fluoro-2-oxoindolin-3-ylidene)succinohydrazide (6e)

IR(KBr) cm^{-1}: 3353(NH), 3133 (NH), 2963 (C-H), 1753 (C=O), 1693(C=O), 1623(C=O), 1453(C=N), 1383(C=N). ^{1}HNMR(DMSO d6) (ppm): 10.6(s, 2H,NH), 10.0(s, 2H,NH), 7.8 (d, 2H, ArH), 7.7(s, 2H, ArH), 7.3(d, 2H, ArH), 2.1(s, 4H, 2(CH_2). LC–MS (ESI, m/z):441 (M+1).

(N^1, N^4)-N^1, N^4-bis (5-carboxy-2-oxoindolin-3-ylidene)succinohydrazide(6f)

IR(KBr) cm^{-1}: 3361 (NH), 3142 (NH), 2973 (C-H), 1764 (C=O), 1683(C=O), 1634(C=O), 1464(C=N), 1373(C=N). ^{1}HNMR(DMSO d6) (ppm): 12.7 (s,2H, OH), 10.5(s, 2H,NH),

10.2(s, 2H,NH), 8.5 (s, 2H, ArH), 8.3(d, 2H, ArH), 8.0(d, 2H, ArH), 2.6(s, 4H, 2(CH_2). LC–MS (ESI, m/z):492 (M+).

(N^1, N^4)-N^1, N^4-bis (5-nitro-2-oxoindolin-3-ylidene)succinohydrazide (6g)

IR(KBr) cm^{-1}: 3363 (NH), 3144 (NH), 2975 (C-H), 1766 (C=O), 1685(C=O), 1635(C=O), 1466(C=N), 1375(C=N). ^{1}HNMR(DMSO d6) (ppm): 10.4(s, 2H,NH), 10.1(s, 2H,NH), 8.5 (s, 2H, ArH), 8.1(d, 4H, ArH), 2.6(s, 4H, 2(CH_2). LC–MS (ESI, m/z):494 (M+).

(N^1, N^4)-N^1, N^4-bis (5-carbomethoxy-2-oxoindolin-3-ylidene)succinohydrazide(6h)

IR(KBr) cm^{-1}: 3363 (NH), 3144 (NH), 2975 (C-H), 1766 (C=O), 1685(C=O), 1635(C=O), 1466(C=N), 1375(C=N). ^{1}HNMR(DMSO d6) (ppm): 10.6(s, 2H,NH), 10.0(s, 2H,NH), 8.5 (s, 2H, ArH), 8.1(d, 2H,ArH), 7.9(d, 4H, ArH), 3.9(s, 6H, 2((CH_3)$_2$), 2.6(s, 4H, 2(CH_2). LC–MS (ESI, m/z):521 (M+1).

(N^1, N^4)-N^1, N^4-bis (6-bromo-2-oxoindolin-3-ylidene)succinohydrazide (6i)

IR(KBr) cm^{-1}: 3363(NH), 3143 (NH), 2973 (C-H), 1763 (C=O), 1683(C=O), 1613(C=O), 1463(C=N), 1353(C=N). ^{1}HNMR(DMSO d6) (ppm): 10.6(s, 2H,NH), 10.2(s, 2H,NH), 8.5(s, 2H, ArH), 7.7 (d, 2H, ArH), 7.4(d, 2H, ArH), 2.4(s, 4H, 2(CH_2). LC–MS (ESI, m/z):563 (M+1).

(N^1, N^4)-N^1, N^4-bis (4-chloro-5-fluoro-2-oxoindolin-3-ylidene)succinohydrazide(6j)

IR(KBr) cm^{-1}: 3382(NH), 3163 (NH), 2984 (C-H), 1763 (C=O), 1686(C=O), 1613(C=O), 1492(C=N), 1393(C=N). ^{1}HNMR(DMSO d6) (ppm): 11.6(s, 2H,NH), 10.8(s, 2H,NH), 8.5 (d, 2H, ArH), 8.2(d, 2H, ArH), 2.8(s, 4H, 2(CH_2). LC–MS (ESI, m/z):509 (M+),511(M+2).

(N^1, N^4)-N^1, N^4-bis (7-methyl-2-oxoindolin-3-ylidene)succinohydrazide(6k)

IR(KBr) cm^{-1}: 3365 (NH), 3147 (NH), 2980 (C-H), 1772 (C=O), 1692(C=O), 1643(C=O), 1475(C=N), 1385(C=N). ^{1}HNMR(DMSO d6) (ppm): 11.6(s, 2H,NH), 10.6(s, 2H,NH), 7.6(d, 2H, ArH), 7.3(d, 2H,ArH), 7.1(t, 2H, ArH), 2.5(s, 4H, 2(CH_2)), 2.1(s, 6H, 2(CH_3). LC–MS (ESI, m/z):432 (M+).

(N^1, N^4)-N^1, N^4-bis (7-nitro-2-oxoindolin-3-ylidene)succinohydrazide(6l)

IR(KBr) cm^{-1}: 3361 (NH), 3144 (NH), 2978 (C-H), 1771 (C=O), 1692(C=O), 1644(C=O), 1477(C=N), 1388(C=N). ^{1}HNMR(DMSO d6) (ppm):11.6(s, 2H,NH), 10.6(s, 2H,NH), 8.3(d, 2H, ArH), 8.2(d, 2H,ArH), 7.5(t, 2H, ArH), 2.5(s, 4H, 2(CH_2). LC–MS (ESI, m/z):494 (M+).

(N^1, N^4)-N^1, N^4-bis (7-carboxy-2-oxoindolin-3-ylidene)succinohydrazide(6m)

IR(KBr) cm^{-1}: 3362 (NH), 3148 (NH), 2984 (C-H), 1781 (C=O), 1684(C=O), 1656(C=O), 1490(C=N), 1392(C=N). ^{1}HNMR(DMSO d6) (ppm): 12.7(s, 2H, OH),11.6(s, 2H,NH), 10.6(s, 2H,NH), 8.3(d, 2H, ArH), 8.1(d, 2H,ArH), 7.4(t, 2H, ArH), 2.5(s, 4H, 2(CH_2). LC–MS (ESI, m/z):492 (M+).

(N^1, N^4)-N^1, N^4-bis (7-chloro-2-oxoindolin-3-ylidene)succinohydrazide(6n)

IR(KBr) cm^{-1}: 3358 (NH), 3135 (NH), 2978 (C-H), 1774 (C=O), 1678(C=O), 1645(C=O), 1480(C=N), 1381(C=N). ^{1}HNMR(DMSO d6) (ppm): 11.8(s, 2H,NH), 10.4(s, 2H,NH), 7.7(d, 2H, ArH), 7.5(d, 2H,ArH), 7.2(t, 2H, ArH), 2.3(s, 4H, 2(CH_2). LC–MS (ESI, m/z):473 (M+1),475(M+2).

(N^1, N^4)-N^1, N^4-bis (7-carbomethoxy-2-oxoindolin-3-ylidene)succinohydrazide(6o)

IR(KBr) cm^{-1}: 3364 (NH), 3139 (NH), 2989 (C-H), 1782 (C=O), 1685(C=O), 1652(C=O), 1485(C=N), 1385(C=N).^{1}HNMR(DMSO d6) (ppm): 11.6(s, 2H,NH), 10.5(s, 2H,NH), 8.2(d, 2H, ArH), 8.0(d, 2H,ArH), 7.4(t, 2H, ArH), 3.9 (s, 6H, 2(CH_3))2.4(s, 4H, 2(CH_2). LC–MS (ESI, m/z):521 (M+1).

Invitro anticancer activity

Growth of breast cancer and lung cancer cells were quantitated by the ability of living cells to reduce the yellow MTT to purple formazan products.[30] The amount of formazan product formed is directly proportional to the number of living cells.

Synthesized compounds were prepared as stock solutions, dissolved in DMSO. MCF-7 human breast cancer and A 549 lung cancer cells were cultivated at 37◦C in an atmosphere of 5% CO2 in Dubecco's modified Eagle's minimal medium (DMEM) supplemented with 3.0mM l-glutamine with 10% fetal bovine serum were routinely subculture twice weekly to maintain in continuous logarithmic growth. Cells were trypsinized for the passage into the well plate and plated at 10,000 cells/well in 100 µl of medium in 96-well plates. Cells were allowed to adhere to the surface of well plates. After 24 h, medium was removed and series of drug solutions were added into the wells. 100 µl of fresh medium without cells was added as control. Other wells were used for each concentration of drug solution. The total drug exposure was 48 h. After 48 h, contents of the well were removed and 20 µl of MTT solution (5mg in 1mL of phosphate buffer saline) was added to each well. Incubation at 37^0C for 4 h allowed reduction of MTT by mitochondrial dehydrogenase to an insoluble formazan

product. Well contents were removed and the formazan product was solubilised by addition of 100 µl DMSO. The purple colour was produced. Cisplain used as reference drug. The absorbance at 570 nm was measured on a UV Visible spectrophotometer and IC_{50} values were determined from plot: % inhibition (from control) Vs concentration.

Molecular docking studies

The involvement of the EGFR family of tyrosine kinases in cancer proliferation suggests that an inhibitor which blocks the tyrosine kinase activity of the entire EGFR family, could have significant therapeutic potential.[31] So we selected EGFR as a target for docking studies of all synthesized compounds and learn the mechanism of activity. The X-ray crystal structure of EGFR kinase domain in complex with an irreversible inhibitor (PDB ID: 4ICZ) was obtained from the protein data bank[32] (www.rcsb.org). The structure was cleaned using V Life MDS 4.3.program (www.vlifesciences.com). The refinement of the crude PDB structure of receptor was done by completing the incomplete residues. The co-crystallized ligand lying within the receptor was modified by assigning missing bond order and hybridization states. The side chain hydrogens were then added to the crystal structure and their positions were optimized up to the rms gradient 1 by aggregating the other part of the receptor. The optimized receptor was used for docking simulation.

The 2D structure of the compounds were built and then converted into the 3D with the help of VLife MDS 4.3(www.vlifesciences.com). The 3D structures were then energetically minimized up to the rms gradient of 0.01 using Merck Molecular Force Field (MMFF). Conformers of all the synthesized ligands were then generated by Monte Carlo method. In doing so, all rotatable bonds of the ligands were selected and number of seeds used for searching the conformational space was set as 5. All conformers were then energetically minimized up to the rms gradient of 0.01 and then saved in separate folder. The active site selection was done by choosing the cavity having maximum hydrophobic surface area. The docking simulation was done using GRIP batch docking. In this, all generated conformers of one ligand were put as one batch in GRIP docking wizard. Likewise, the batches for all other ligands were put. All the conformers were virtually docked at the defined cavity of the receptor. The parameters fixed for docking simulation was like this – number of placements: 30, rotation angle: 30◦, exhaustive method, scoring function: dock score. By rotation angle, the ligand gets rotated for different poses. By placements, the method will check all the 30 possible placements into the active site pocket and will result out few best placements out of

30. For each ligand, all the conformers with their best placements and their dock score will be saved in output folder. The method also highlights the best placement of best conformer of one particular ligand which is having best (minimum) PLP Score. After completion of the docking process, the minimum interaction energy between each ligand and EGFR protein for the best ligand pose inside the receptor cavity was obtained as the Piecewise Linear Pair wise Potential score (PLP).[33] The PLP scores were compared with gefitinib (Pao et al 2004), an EGFR inhibitor used for certain breast, lung and other cancers. Gefitinib is like erlotinib, which interrupts signalling through the epidermal growth factor receptor (EGFR) in target cells. In the results of docking, we have listed only best conformers and the dock score for each ligand in **Table 3**.

RESULTS AND DISCUSSION

The (N^1,N^4)-N^1,N^4-bis(2-oxoindolin-3-ylidene) succinohydrazide derivatives were prepared by condensation reaction of appropriate isatins and succinohydrazide in methanol presence of gl. Acetic acid (scheme 1). The reaction completion and purity of the synthesized compounds was monitored by TLC using silica gel G and visualization was done using iodine vapour. Their structures were confirmed by FT- IR, 1H NMR and Mass spectra.

Invitro anticancer activity

The anticancer activity of (N^1,N^4)-N^1,N^4-bis(2-oxoindolin-3-ylidene) succinohydrazide derivatives evaluated, using MTT assay with cisplatin as positive control. The IC50 values of MCF-7 and A549 cells (listed in **Table 2)** was calculated from the measured absorbance of formazan product produced by living cells. All the synthesized compounds shown significantly inhibited the growth of MCF-7 cells over the A549 cells and the IC50 values of all the compounds were found between 10.24 and 28.65 µM. The compound **6j** showed highest cell growth inhibition, while **6d, 6c, 6e** and **6n** showed moderate inhibition and **6k** and **6a** showed least inhibition. These results indicated that compounds having electron withdrawing groups are shown significant anticancer activity.

Table-2: IC 50 (µM) values of (N^1,N^4)-N^1,N^4-bis(2-oxoindolin-3-ylidene) succinohydrazide derivatives and cisplatin on A 549 and MCF-7 cell lines.

IC 50 µM				
S.No	**Compounds**	**R**	**Lung cancer cells (A549)**	**breast cancer cells (MCF-7)**
1	6a	H	24.65	19.52
2	6b	5-CH_3	21.14	20.58
3	6c	5-Cl	14.98	14.2
4	6d	5-Br	14.18	13.52
5	6e	5-F	15.72	12.56
6	6f	5-COOH	NA	NA
7	6g	5-NO_2	19.76	21.5
8	6h	5-$COOCH_3$	NA	NA
9	6i	6-Br	16.32	15.53
10	6j	4-Cl, 5-F	11.54	10.24
11	6k	7-CH_3	28.65	25.2
12	6l	7-NO_2	18.62	21.71
13	6m	7-COOH	NA	NA
14	6n	7-Cl	16.34	19.25
15	6o	7-$COOCH_3$	NA	NA
16	STD	Cisplatin	6.63	8.54

NA= No Activity.

Conc. of test compounds 100 µM;

Docking studies

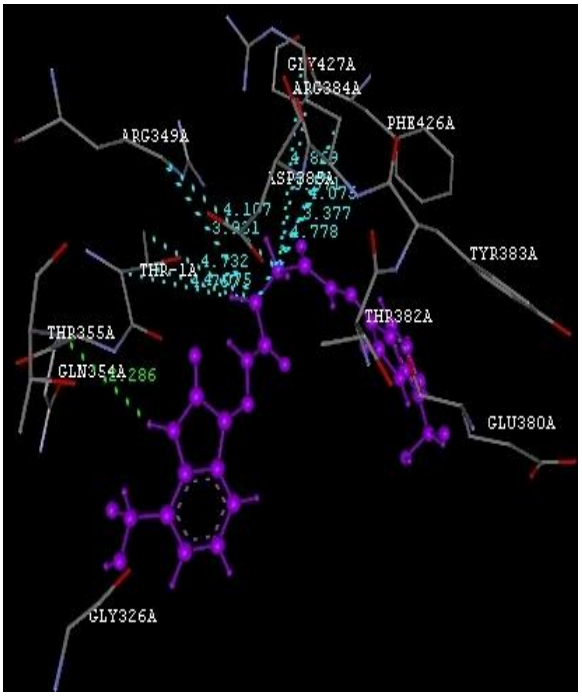
All the synthesized ligands were docked into highly hydrophobic portion of cavity 1 of 4ICZ protein by using VLife Sciences 4.3 programme. To validate the docking simulations, gefitinib was used as the reference ligand for the docking studies. The reference ligand gefitinibl score was obtained **-71.54,** confirming the ability of the method to accurately predict the binding confirmation. All ligands exhibited negative docking scores and were comparable with the gefitinib. From the dock score, all the compounds were found to have highest negative dock score ranging from **-71.46** to **-48.32** (**Table 3**). Among that the compounds **6c, 6d, 6e, 6i, 6j** and **6l** formed most stable drug-receptor complex. All the docked compounds were analyzed for various types of interactions such as hydrophobic, hydrogen, aromatic and van der Waal's interactions and no compound was found to exhibit an aromatic bonding with the receptor (**Table 4**). **Figure- 1** shows the docking of ligands **6c, 6d, 6e, 6i, 6j** and gefitinib with the EGFR protein. All ligands exhibited hydrophobic (cyan blue doted lines), hydrogen (green doted lines) and van der Waal's (red doted lines) interactions were as gefitinib exhibited hydrogen, charge, hydrophobic and van der Waal's interactions with dock score of -71.54.

Table 3: Dock scores [PLP] of (N^1,N^4)-N^1,N^4-bis(2-oxoindolin-3-ylidene) succinohydrazide (VIa–o) and gefitinib.

S.No	Compounds	R	PLP Scores(Dock scores)
1	6a	H	-58.47
2	6b	5-CH_3	-57.03
3	6c	5-Cl	**-63.33**
4	6d	5-Br	**-71.46**
5	6e	5-F	-61.12
6	6f	5-COOH	-57.9
7	6g	5-NO_2	-48.32
8	6h	5-$COOCH_3$	-58.93
9	6i	6-Br	**-69.09**
10	6j	4-Cl 5-F	**-62.97**
11	6k	7-CH3	-58.9
12	6l	7-NO2	**-61.24**
13	6m	7-COOH	-58.85
14	6n	7-Cl	-56.45
15	6o	7-$COOCH_3$	-58.42
Std	Gifitinib	-	**-71.54**

Table-4: Binding interactions of (N^1,N^4)-N^1,N^4-bis(2-oxoindolin-3-ylidene) succinohydrazides with EGFR Protien.

			Hydrophobic bonds		Hydrogenbods	
Compounds	**R**	**PLP Scores**	**Distance**	**Amino acids**	**Distances**	**Amino acids**
6c	5-Cl	-63.33	4.008	ASN 381A	1.181	GLY326
			4.361	THR 382A		
			4.693	ARG 384A		
6d	5-Br	-71.46	4.016	ASN 381A	1.181	GLY326
			4.237	THR 382A		
			4.693	ARG 384A		
6i	6-Br	-69.09	3.525	ASN 381A	2.334	GLY427
6j	4-Cl 5-F	-62.97	4.369	THR 382A	1.909	THR355
			3.611	ARG384A	2.15	ASP385
			4.362	ASP385A		
6l	7-NO2	-61.24	3.931	ARG349A	2.286	THR355
			4.075	THR1A		
			3.571	ARG384A		
Gifitinib		-71.54	3.542	GLY427A[a]	3.938	ASN381
			3.581	PHE426A[a]		
			4.635	ARG384A		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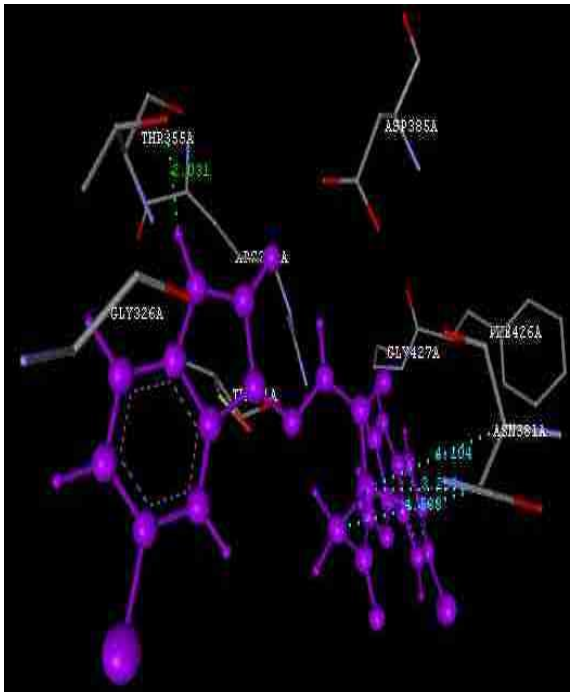
GLY326A

6c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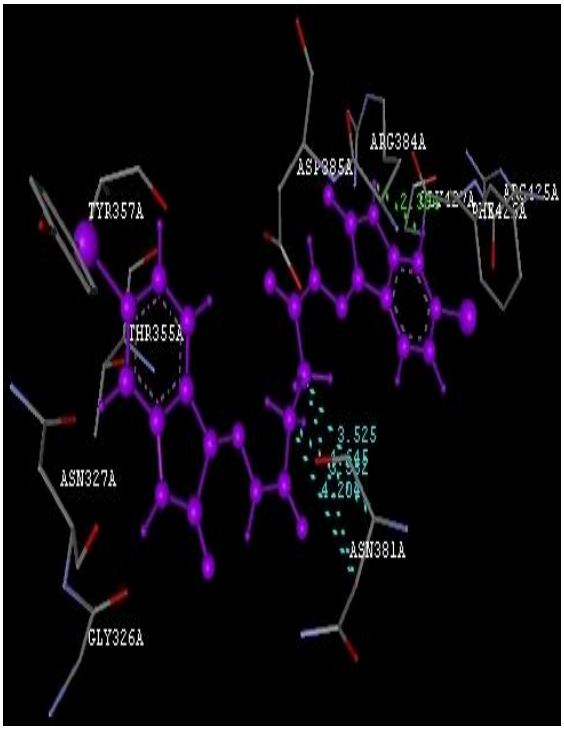
6d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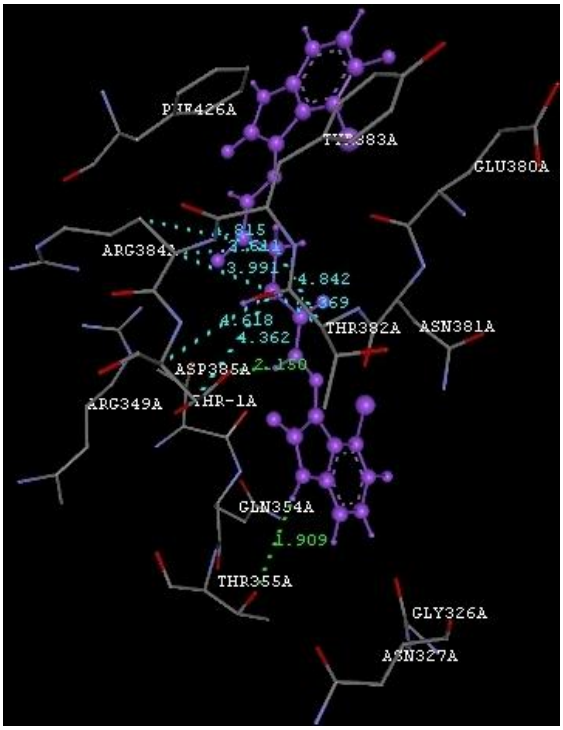
6i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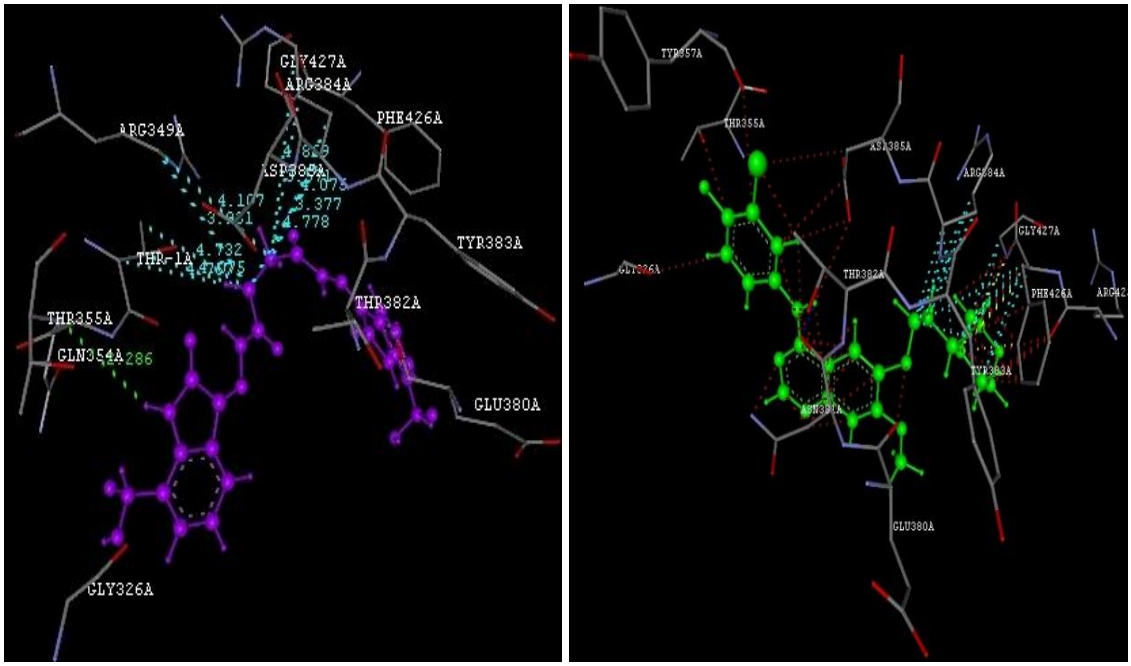
ASN327A

6j



6l **Gifitinib**

Fig-1: 3D representations of docking of ligands 6c,6d,6i,6j,6l and Gifitinib showing hydrogen (green dotted line), hydrophobic (cyan dotted lines) and van der Waal's (red dotted lines) interactions with EGFR protein.

CONCLUSIONS

In the present study, the synthesis, characterization, invitro anticancer and molecular docking of a novel (N^1,N^4)-N^1,N^4-bis(2-oxoindolin-3-ylidene) succinohydrazides derivatives have been described. The synthesis of title compound were obtained by the condensation of various isatins with succinohydrazide. All the compounds are subjected for the evaluation of *in vivo* anticancer activity.. Among all, the compounds **6j** showed highest cell growth inhibition, while **6d, 6c, 6e** and **6n** showed moderate inhibition and **6k** and **6a** showed least inhibition. Electron withdrawing groups seemed to be necessary factors in providing higher antiinflammatory activity.

The molecular docking studies further help in uderstanding the various interactions between the ligands and enzyme active sites indetail and thereby help to design novel potent inhibitor. The docking experiments was carried out for all the synthesized compounds on EGFR protien and compared the docking score with references gifitinib. The compounds **6c, 6d,6i and 6j** showed higher binding score. It was observed that the docking score of the compounds could be correlated with the in vitro anticancer activity. In conclusion it can be stated that the electron withdrawing groups seemed to be necessary factors in providing

higher anticancer activity. which are further supported to the invivo activity of these compounds.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of manuscript.

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