‘’Design, Synthesis , Docking Studies and Evaluation of Benzothiazole and Indole based analogs for anti-cancer agents.’’

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Abstract:

A series of 20 compounds were prepared by condensation of hydrazinylbenzothiazole derivatives (R= -H, -Cl, $-CH_3$, $-CF_3$, $-NO_2$) with substituted indole (R′= -H, $-NO_2$, -Br, $-CH_3$). Hydrazinylbenzothiazole derivatives were prepared by treating 2-aminobenzothiazole with hydarzinehydrate in presence of ethyleneglycol. The synthesized compounds are characterized by different spectroscopic techniques, are evaluated for anti-cancer activity and docking studies are also further carried by using AUTODOCK 4.2 to find out the molecules with optimum interactions. All the derivatives showed IC_{50} values in the range of 8.53-12.6µM against MCF-7 cells. The compound (**20**) **R′=NO_2 & R=CF_3** exhibited highest potency with value of **IC_{50}=8.53µM** whereas compound **(10) R′=H & R=Cl** without any substituent on indole nucleus showed least potency with IC_{50} value of **IC_{50}=12.6µM.** However, all six compounds were less potent than the standard cisplatin, which showed IC_{50} of **1.18µM** and doxorubicin, which showed IC_{50} of **2.21µM**. Significantly all the six derivatives were found to be less toxic to MCF-10A cells also when compared to the standard cisplatin. Docking study is also done by taking crystal structure of protein 3HY3, structure of human MTHFS with 10-formyltetrahydrofolate) from Homo sapiens using AUTODOCK 4.2. The results showed that the compound **(8) R′ = NO_2, R=NO_2** exhibited highest binding energy of **-10.97 kcal/mol** with interacting Lys 150 anf Gln 113. Nine molecules were shown interacting with Arg148 and 7 molecules were interacting with Arg 148 and Thr 152.

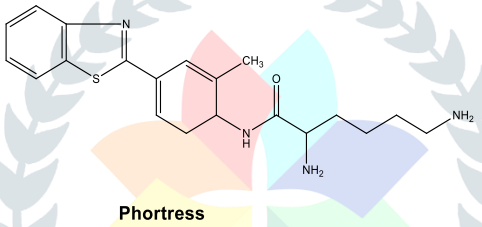
Key words: Indole-benzothiazole hybrids, Docking Studies, Anticancer activity.

1.Introduction:

Multi-drug resistance is an important obstacle that limits the efficacy of chemotherapy in many cancer types[1]. Phortress (NSC7 10305) was the leading compound and demonstrated the activity against breast cancer tumors regardless of oestrogen receptor status against ovarian, renal, lung and colon-cancer [2,3].Indole ring system is one of the most ubiquitous heterocycles in nature has been becoming an important structural component in many pharmaceutical agents, such as antidepressant[4], anticonvulsant[5], antifungal[6], antiviral[7], anti-inflammatory[8] particularly indole-based small molecules potent new antimicrobial, antimycobacterial and anti-cancer[9,10] agents. Sunitinib (Fig.1) a multi-targeted receptor tyrosine kinase inhibitor containing indolin-2-one moiety. 2-oxoindole derivatives of SU-5416 (semaxanib) and SU-11248 were reported having tyrosine kinase inhibitory and antiangiogenic properties[11]. Su9516 was reported a potential inhibitor of cyclin-dependant kinases that can induce apoptosis in colon carcinoma cells[12].Based on this several 2-indoloneimino derivatives were synthesized to examine their antitumor and antiangiogenic properties[13]. It is reported that Halogenated Indole derivatives induces the anticancer activity[14]. Benzothiazole moiety is a versatile moiety that exhibits a wide variety of biological

activities including antitubercular[15,16,17], antimalarial[18], anticonvulsant[19], antidiabetic[20], antimicrobial, antifungal and antitumor[20,21]. Modifications on the benzothiazole have resulted in large number of compounds having diverse biological activities especially phenyl-substituted benzothiazoles[22,23,24,25]. The 2-substituted aminobenzothiazole derivatives have been reported to possess antibacterial, antitumor, anti-tuberculosis, carbonicanhydrase inhibitory activity and good anticancer properties[26,27]. The development of anticancer drug is more difficult than discovering cures for bacterial infection as there are very few biochemical differences between cancerous cell and normal cells. Moreover, the increase in acitivity of many drugs is limited to their toxicity to the normal rapidly growing cells in the intestinal and bonemarrow areas. Also the cancerous cells which are initially suppressed by a specific drug may develop a resistance to that drug. Riluzole, a blocker of excitatory aminoacids mediated neurotransmission is also reported[28-31]. Hydrazones of 1H-indole-2,3-dione have been identified as inhibitors of the protein tyrosine phosphatase Shp2, which plays an important role in cell signaling[32]. In the past few decades, molecularly targeted regimens has become an indispensable part of medicinal chemistry research. The hybridization of two or more bioactive drug fragments with complementary pharmacophoric functions or different mechanisms of action into a single molecule is a unique approach that often results in synergistic activity and enhanced drug efficacy[33-36].

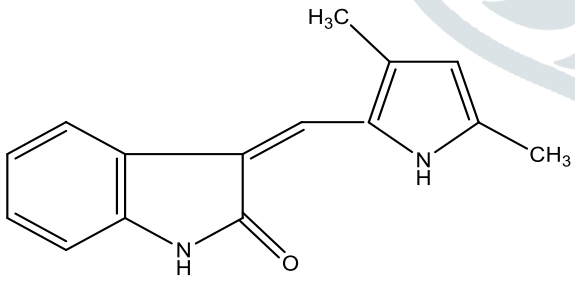
2.Structure of Benzothiazole:



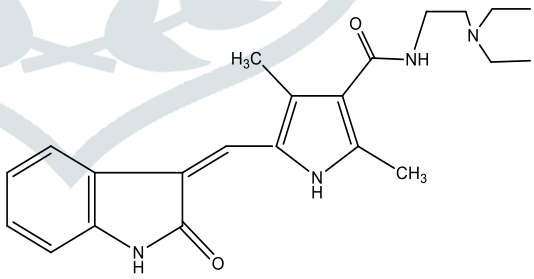
Phortress

(anti-cancer activity).

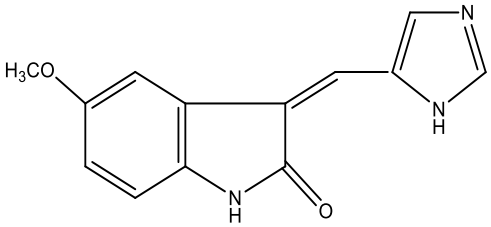
Structures of Indole:



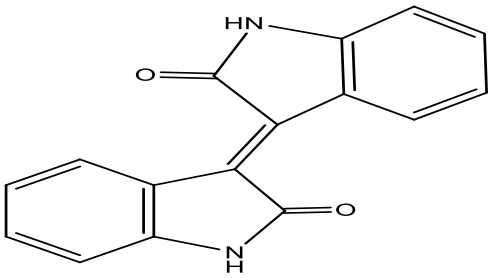
SU - 5416, Semaxanib (anti-cancer activity).



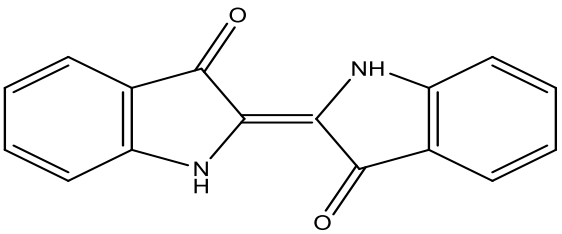
SU - 11248,Sunitinib (anti-cancer activity).



SU - 9516 (anti-cancer activity).



Isoindigo (anti-cancer activity).



Indigo (anti-cancer activity).

(Figure:1)

3.Rationale:

Literature Survey reveals that 2-oxoindole derivatives of SU-5416 (Semaxanib) and SU-11248 (Sunitinib) as shown in figure.1 are reported tyrosine kinase inhibitory properties. Benzothiazoles are known to exhibit a wide range of biological properties including anticancer, antimicrobial, antidiabetic, anti-convulsant, anti-inflammatory, antiviral, antitubercular activities, etc. Benzothiazole nucleus also possesses a potent anticancer activity against Human cancer. Phortress (NSC 710305) is the lead compound from this work. Based on these observations, it is proposed to synthesize Indole-benzothiazole analogs containing both substituted indoles and substituted benzothiazoles and hydrazine at 2nd position of benzothiazole and evaluate them for the anticancer activity.

4.1.Chemistry: Hybrid Pharmacophore Approach:

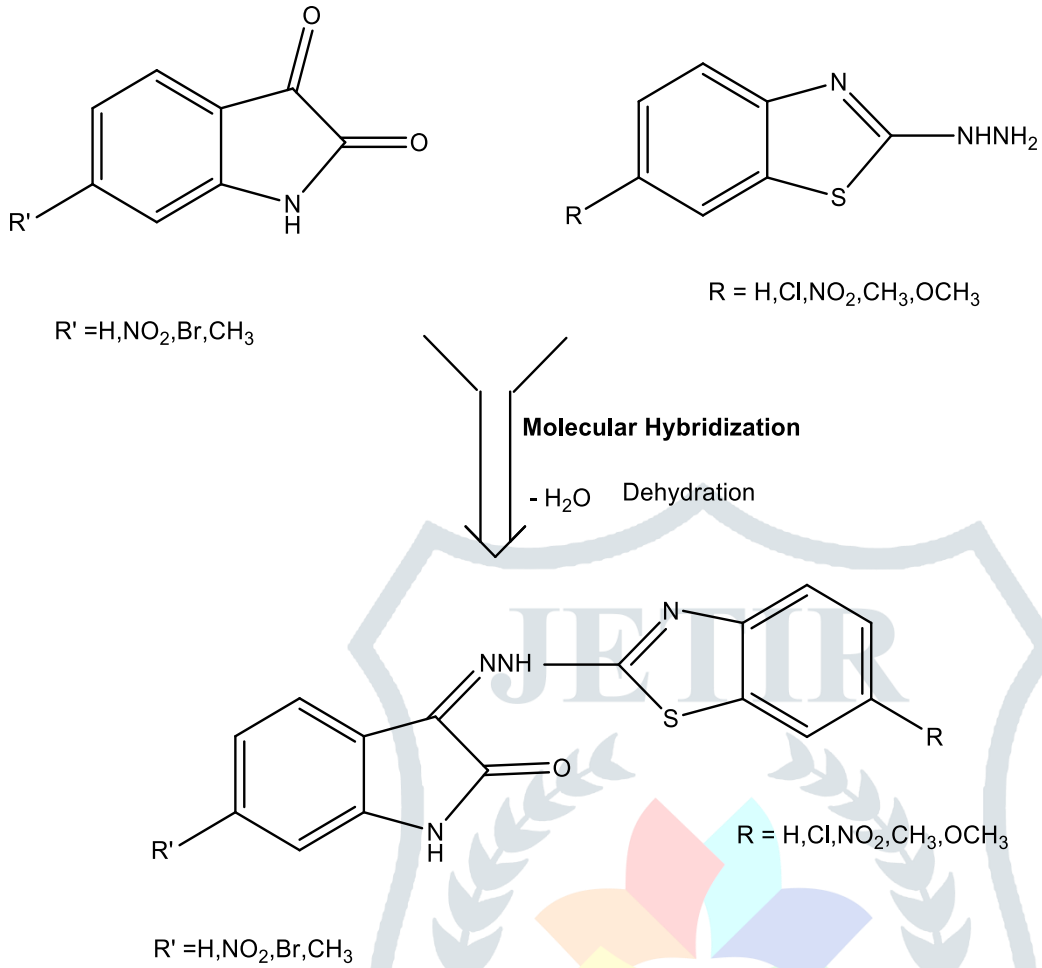


Figure: A design for synthesis of Benzothiazole – indole analogs (Scheme-I, 1-20).

4.2 Procedure:

Step-1: Synthesis of substituted benzothiazoles[37].

A mixture of 0.1 mol of 4-substituted aniline and 0.1 mol of potassium thiocyanate (KCNS) in 100ml glacial acetic acid (AcOH) was cooled in an ice bath and stirred for 10-20 min, and then 0.1 mol bromine in glacial acetic acid was added dropwise at such a rate to keep the temperature below 10^{o}C throughout the addition.The reaction mixture was stirred at room temperature for 2-4h, the hydrobromide(HBr) salt thus separated out was filtered, washed with aceticacid, dried, dissolved in hot water and basified to pH 11.0 with ammonia solution(NH_4OH) and the resulting ppt was filtered, washed with water and dried to get the desired product. The progress of the reaction was monitored by Thin layer chromatography using hexane:ethylacetate (8:2) solvent system.

Step-2: Synthesis of substituted benzothiazolehydrazines[37].

2-aminobenzothiazole derivatives which on treatment with hydrazinehydrate in presence of ethylene glycol resulted in the formation of 2-hydrazinyl benzothiazole derivatives.

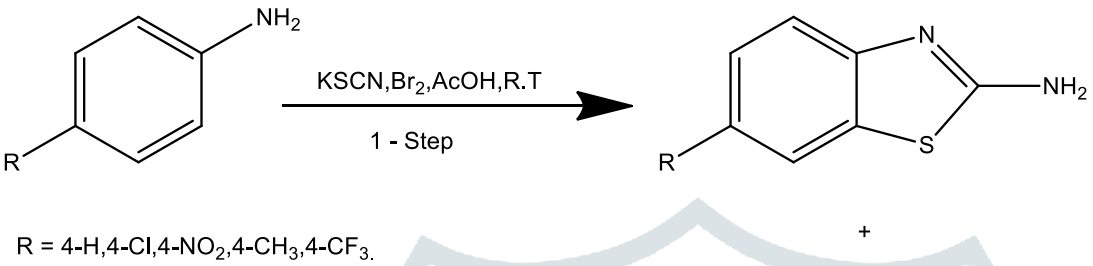
Step-3: Synthesis of benzothiazole and indole analogs [38].

5-{4-(Aminophenyl)-1,3,4-oxadiazol}2-thiol (1.93g, 0.01 mol) and indole (1.47g, 0.01 mol) were refluxed in 20 ml of ethanol in the presence of a catalytic amount of glacial acetic acid (2-3 drops) for 5-6h and

cooled.The solid separated was filtered and washed with cold alcohol and the product obtained was recrystalized from methanol (yield 2.41g,75% m.p-268-270^{o}C).

Scheme - I

1.Synthesis of Substituted benzothiazoles:

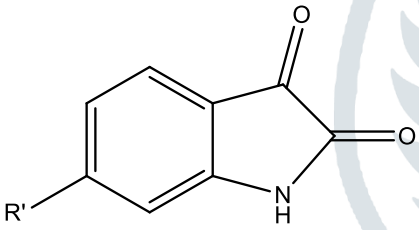


+

$HNHNH_2 .H_2O$

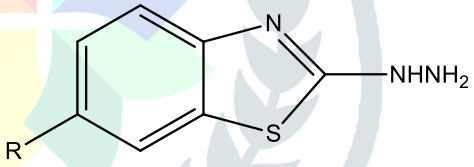
2 -Step

3.Synthesis of Isatin - Benzothiazole Anologs:



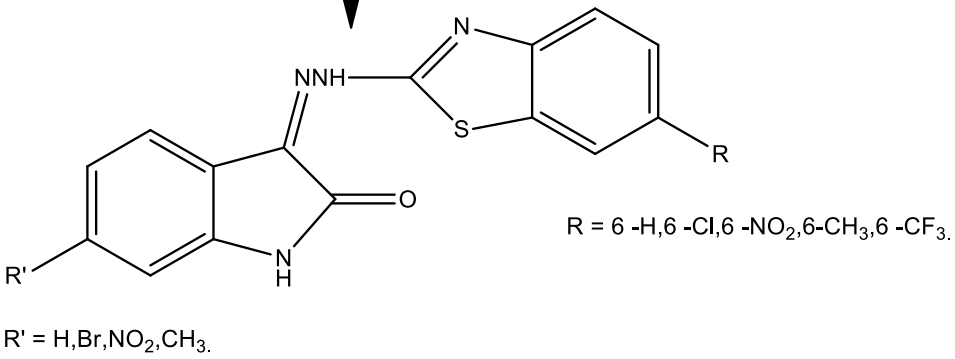
+

2.Synthesis of Substituted Benzothiazolehydrazines



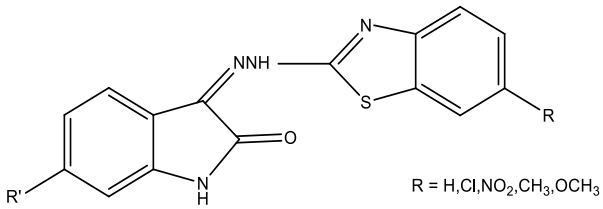
Ethanol, - H_2O

3 - Step



Scheme - I

Table-I : Physical data of Benzothiazole and Indole analogs:



S.No	R'	R	Molecular Formula	Molecular weight	R_f	Melting point (°C)	% yield.
1.	Br	H	$C_{15}H_9BrN_4OS$	373.23	0.86	206-209°C	73%
2.	H	H	$C_{15}H_{10}N_4OS$	294.33	0.80	195-197°C	72%
3.	CH_3	H	$C_{16}H_{12}N_4OS$	308.36	0.76	177-180°C	69%
4.	NO_2	H	$C_{15}H_9N_5O_3S$	339.33	0.67	220-221°C	58%
5.	Br	NO_2	$C_{15}H_8BrN_5O_3S$	418.22	0.70	225-228°C	64%
6.	H	NO_2	$C_{15}H_9N_5O_3S$	339.33	0.66	221-222°C	58%
7.	CH_3	NO_2	$C_{16}H_{11}N_5O_3S$	353.36	0.64	195-200°C	59%
8.	NO_2	NO_2	$C_{15}H_8N_6O_5S$	384.33	0.57	219-235°C	55%
9.	Br	Cl	$C_{15}H_8BrClN_4OS$	407.67	0.73	185-190°C	68%
10.	H	Cl	$C_{15}H_9ClN_4OS$	328.78	0.68	201-204°C	69%
11.	CH_3	Cl	$C_{16}H_{11}ClN_4OS$	342.80	0.65	198-200°C	70%
12.	NO_2	Cl	$C_{15}H_8ClN_5O_3S$	373.77	0.63	250-255°C	65%
13.	Br	CH_3	$C_{16}H_{11}BrN_4OS$	387.25	0.90	195-200°C	65%
14.	H	CH_3	$C_{16}H_{12}N_4OS$	308.36	0.87	148-150°C	63%
15.	CH_3	CH_3	$C_{17}H_{14}N_4OS$	322.38	0.85	144-146°C	65%
16.	NO_2	CH_3	$C_{16}H_{11}N_5O_3S$	353.36	0.65	195-200°C	60%
17.	Br	CF_3	$C_{16}H_8BrF_3N_4OS$	441.23	0.92	216-219°C	62%
18.	H	CF_3	$C_{16}H_9F_3N_4OS$	362.33	0.86	185-190°C	70%
19.	CH_3	CF_3	$C_{17}H_{11}F_3N_4OS$	376.36	0.82	191-193°C	69%
20.	NO_2	CF_3	$C_{16}H_8F_3N_5O_3S$	407.33	0.80	240-45°C.	59%

5. In vitro cytotoxicity:

The Benzothiazole and indole compounds are reported for various biological activities such as antibacterial, antifungal and antimycobacterial activity . Till now there is no evidence in the literature Benzothiazole and indole compounds have anti-breast cancer activity. Therefore, we synthesized 20 benzothiazole and indole analogs, studied for docking studies and then examined their potential as anticancer drugs.

All the compounds synthesized were evaluated for their cytotoxic effects on one breast cancer cell line, MCF7 and one non-cancer breast epithelial cell line MCF10A. Each compound were weighed separately and dissolved in DMSO. With media make up the final concentration to 1mg/ml and the cells are treated with series of concentrations from 10 to 100µg/ml. Cells are treated with each compound for 48h, followed by measuring cell growth rates by MTT based spectrophotometry. The reading of MTT staining is known to accurately reflect the levels of total cellular growth proliferation. The GI_{50} concentration for each compound was calculated with reference to a control sample, which represents the concentration that results in a 50% decrease in cell growth after 48h incubation in the presence of the drug. For each compound, 50% growth inhibition (GI_{50}) was calculated from

Sigmoidal dose-response curves and presented. The data for cisplatin and doxorubicin were recorded as reference. The resultant data showed that Benzothiazole and indole compounds had significant cytotoxic effects on the breast cell line examined. All the six compounds evaluated for cytotoxic activity against MCF-7 and MCF-10A exhibited significant activity against both cell lines (cancer and normal cells). All the derivatives showed IC_{50} values of 33.23-40.69µg/ml against MCF-7 cells. Compound (**12**) ($R'=NO_2$, R=Cl) substituents exhibited highest potency (IC_{50} =33.2µg/ml), whereas Compound (**10**) (R' = H, R = Cl) without any substituent on indole nucleus showed least potency among the series (IC_{50} = 40.6µg/ml). However, all the compounds were least potent than the standard Cisplatin, which showed IC_{50} of 5.59µg/ml and Doxorubicin, whose IC50 value is 12.06µg/ml. Significantly all the six derivatives found to be less toxic to MCF 10A cells also when compared to the standard Cisplatin and doxorubicin. The differences in the GI_{50}values may be attributable to such factors as the nature of the substitution on the 6th position of Indole ring and the substitution on the 6th position of Benzothiazole ring and the genetic and biochemical nature of the cell lines.

5.1.Materials and methods:

5.1.1. Cell lines:

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 MCF-7 breast adenocarcinoma cancer cell line was purchased from NCCS, Pune and the cells were maintained in DMEM supplemented with 10% fetal bovine serum and the antibiotics pencillin/streptomycin (0.5mL^{-1}), in atmosphere of 5% CO_2 /95% air at 37^{o}C.

5.1.2. Reagents:

5.1.2. Reagents:

Dulbecco's modified Eagles medium, (MTT) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, trypsin, EDTA Phosphate Buffered Saline and Cisplatin are purchased from Sigma Chemicals Co. and Fetal Bovine Serum are purchased Gibco. 25 cm^2 and 75 cm^2 flask and 96 well plated purchased from eppendorf India. All the compounds were dissolved in 10-20mM dimethyl sulfoxide (DMSO) and stored at -20^{o}C until use. The stock solution was diluted in culture medium (0.1-100µM) immediately before use. The final concentration of DMSO in the MTT- based cytotoxicity assays did not exceed 0.1%. To rule out that the DMSO concentration used may effect cell cytotoxicity, culture medium containing equivalent concentration of DMSO was used as a negative control in all experiments.

5.2: MTT assay:

Cytotoxic effects were determined by a MTT-based protocol. MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethylthiazol- The DMSO concentration used does not showed any notable cytotoxicity in all studies.2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The assay depends both on the number of cells present and the dead cells or their products which do not reduce tetrazolium. The MTT entered the cells and passed into the mitochondria where it gets reduced to an insoluble, dark purple coloured formazan crystals. The cells are then solubilized with a DMSO and the released, solubilized formazan reagent is measured spectrophotometrically at 570nm.

MCF-7 cells were trypsinised and perform the tryphan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0×10^3 cells/well in 100µl media in 96 well plate culture medium and incubated overnight at 37^{o}C. After incubation, take off the old media and add fresh media 100µl with different concentrations of test compound in representative wells in 96 plate. After 48hrs, discard the drug solution

and add the fresh medic with MTT solution (0.5 mg/mL $^{-1}$) was added to each well and plates were incubated at 37^{o}C for 3 hrs. At the end of incubation time, precipitates were formed as a result of the reduction of the MTT salt to chromophore formazan crystals in DMSO was measured at 570nm on a microplate reader. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% values is generated from the dose-response curves for each cell line.

Table-2: In vitro anticancer screening of Scheme-I against human breast cell line MCF-7.

Compound				Concentration(µM)				IC_{50}(µg/ml)	
				Surviving fraction (Mean ±S.E.)[a]					
S.No	Code	R'	R	10	25	50	100	IC_{50} (µg/ml)	IC_{50} (µM)
1.	09	Br	Cl	3.19±0.015	2.602±0.0005	2.259±0.0026	0.733±0.00152	35.92	8.81
2.	17	Br	CF_3	3.297±0.00152	2.595±0.00251 6	2.153±0.00152	0.4973±0.00115	38.13	8.60
3.	10	H	Cl	3.54±0.001	2.57±0.0005	2.069±0.0020	0.727±0.001	40.69	12.3
4.	18	H	CF_3	3.923±0.00665	2.5963±0.001	1.793±0.0005	0.6963±0.00057	37.26	10.30
5.	20	NO_2	CF_3	3.188±0.00173	2.584±0.00057	1.532±0.00152	0.459±0.00152	34.71	8.53
6.	12	NO_2	Cl	3.30±0.0011	2.586±0.00057	1.759±0.00208	0.2603±0.00057	33.23	8.89
Cisplatin								**5.59**	**1.86**
Doxorubicin								**12.06**	**2.21**

Table-3:In vitro anticancer screening of Compounds against human breast cell line MCF-10A.

Compound				Concentration(µM)					
				Surviving fraction (Mean ±S.E.)[a]					
S.No	Code	R'	R	10	25	50	100	IC_{50} (µg/ml)	IC_{50} (µM)
1.	09	Br	Cl	5.029±0.00057735	4.635±0.00305505	3.891±0.00057735	2.569±0.0005773	90.35	22.16
2.	17	Br	CF_3	4.580±0.00057735	4.420±0.00057735	4.072±0.00665832	3.406±0.0208166	151.71	34.46
3.	10	H	Cl	4.658±0.00251661	4.401±0.00152752	3.952±0.001	2.876±0.001	107.27	32.62
4.	18	H	CF_3	4.733±0.05084617	4.639±0.001	4.377±0.00152752	3.136±0.0015275	119.83	33.19
5.	20	NO_2	CF_3	4.902±0	4.663±0.00057735	4.047±0.00057735	2.867±0.0066583	102.2	25.12
6.	12	NO_2	Cl	4.663±0.00057735	4.427±0.00057735	3.645±0.00115470	2.609±0.0005773	90.67	24.25
Cisplatin								**5.59**	**1.86**
Doxorubicin								**12.06**	**2.21**

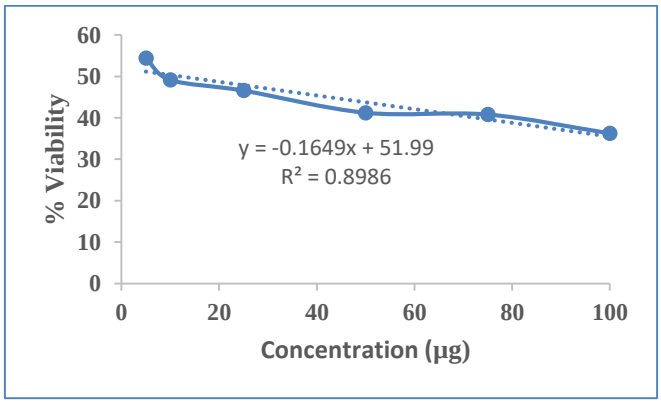


Figure 2: Cytotoxic Effect of Doxorubicin on MCF 7 Cell Line

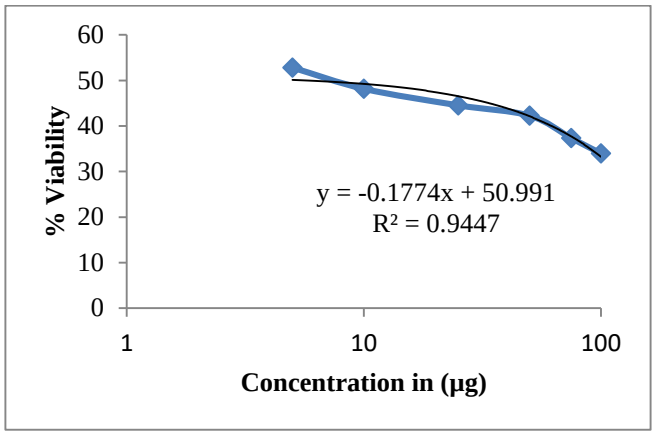
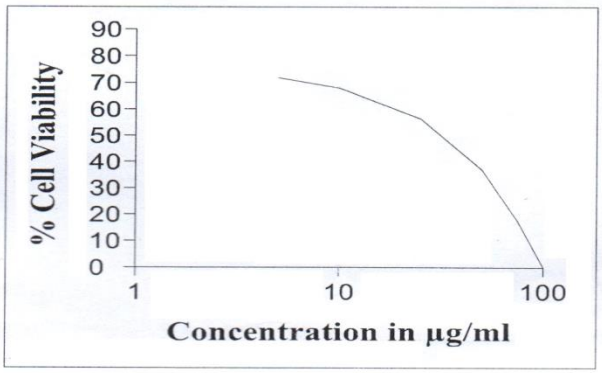
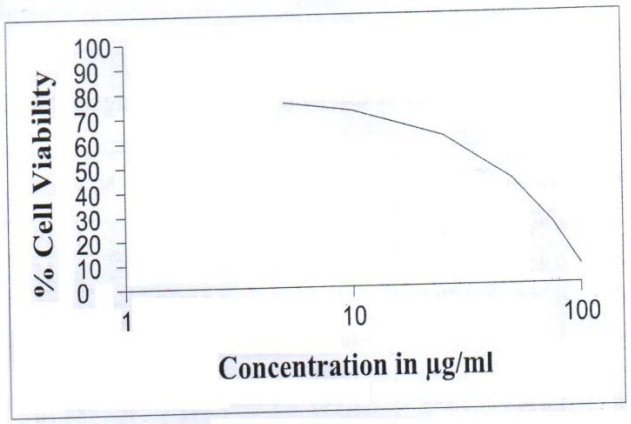


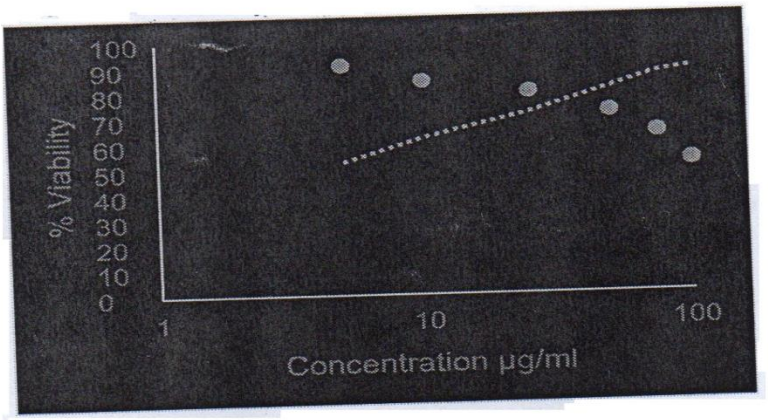
Figure 3: Cytotoxic Effect of Cisplatin on MCF 7 Cell Line



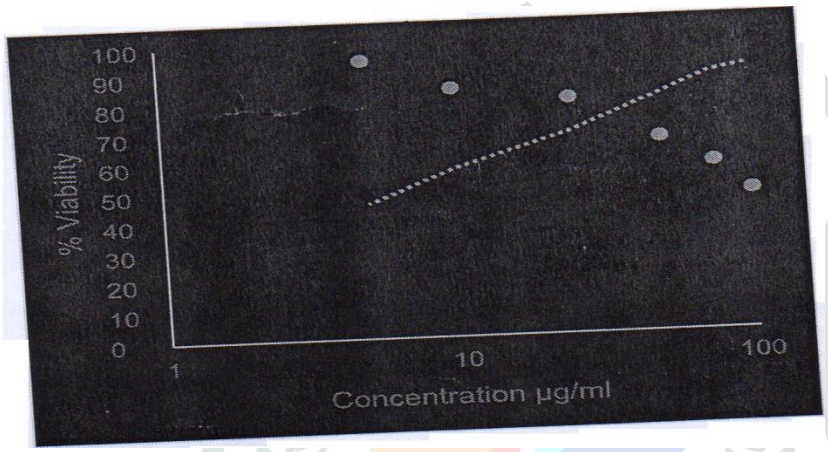
Cytotoxic effect of compound-12 against MCF-7 cell line.



Cytotoxic effect of compound-10 against MCF-7 cell line.



Cytotoxic effect of compound-12 against MCF-10A cell line.



Cytotoxic effect of compound-10 against MCF-10A cell line.

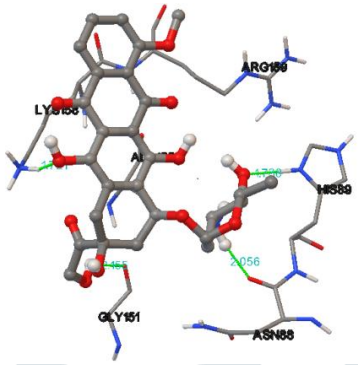
6: Molecular docking model analysis of compounds with the active site of 3HY3.

To investigate the binding effects between protein and ligand molecular docking study was performed.Here we selected "Structure of human MTHFS with 10-formyltetrahydrofolate" (pdb id: 3HY3) for molecular docking study. The binding models of compounds are depicted.In the binding model, the interactions of human MTHFS with 10-formyltetrahydrofolate protein and ligand conformations, including hydrogen bonds and the bond length were analyzed. Estimating the binding affinity of the complex is a significant part of the structure based drug design process.The structural interactions between PDB with 20 inhibitors were docked separately.

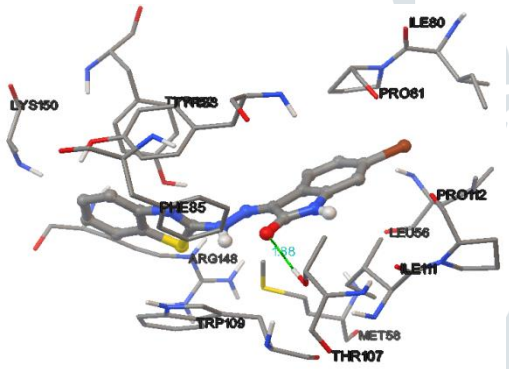
Preparation of Protein Structure: All the synthesized molecules were sketched in Tripo's Syby16.7 and minimized the molecules by adding Gasteiger-Huckel charges to give the exact conformation, stability and lowest energy. After energy minimization each and every molecule was saved in .mol2 format. Protein-ligand interaction studies were performed by using Autodock 4.2. The crystal structure of protein 3HY3 (Structure of human MTHFS with 10-formyltetrahydrofolate) from Homo sapiens was downloaded from protein data bank(PDB). Initially the selected crystal structure of a protein was loaded in autodock, water molecules were removed and hydrogens were added and saved it in PDBQT format. The molecule was then loaded; torsions were set and saved it in PDBQT format.Autodock uses genetic algorithm. A grid box with the dimensions 60×60×60 and selected coordinates of X:5.375, Y:10.714 and Z:2.793A^0, with a default grid spacing of 0.375A^0 was used.

Molecular docking is the most extensively used method for the calculation of protein-ligand interactions. It is an efficient method to predict the potential ligand interactions.Experimental activities and predicted values by Lamarckian Genetic Algorithm dockings of the 20 compounds are summarized in Table 4.Except compound 3 all the other nineteen molecules showed good interactions and better lower free energy values, indicating more

thermodynamically favoured interaction. Compound eight showed highest binding energy of -10.97 Kcal/mol with interacting Leu56, Thr107 and Lys150. The compound 6 and 7 exhibited binding energy of -10.69 Kcal/mol with interacting Lys150 and Gln113. Out of 20 compounds 9 molecules are showed interactions with Gln113. Seven ligands are interacting with Arg148 and Thr152.Five molecules are showing good binding energy of less than -10.00.All the compound interactions are shown in Table-4.



Standard drug: Doxorubicin.



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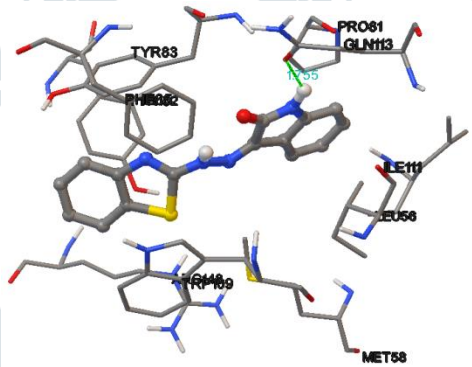

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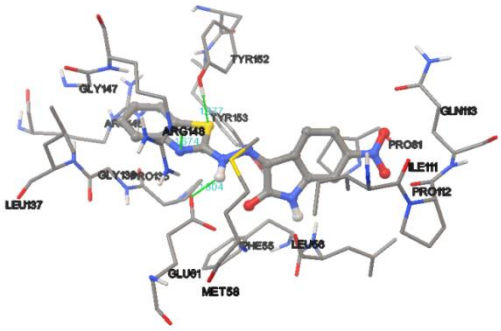

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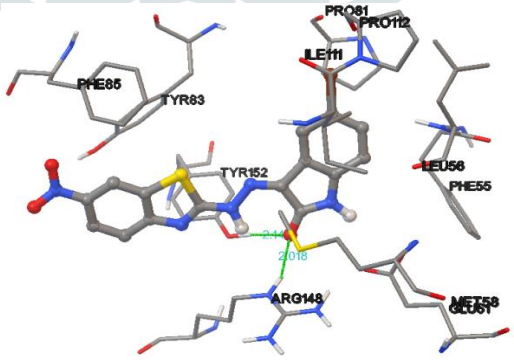

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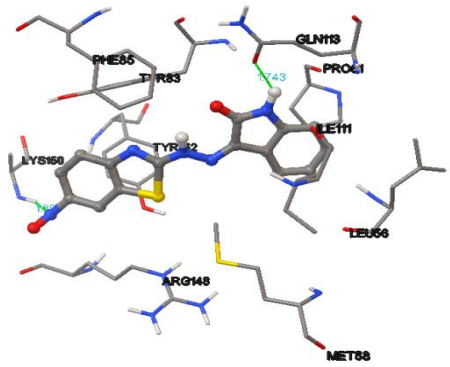

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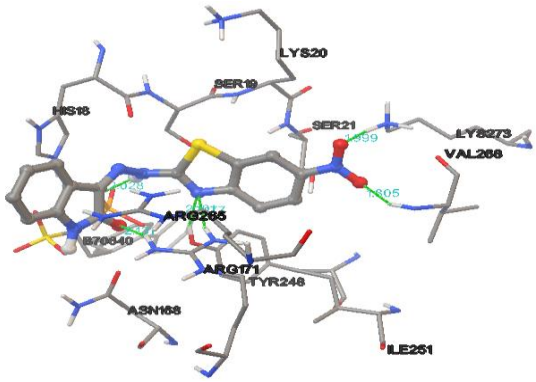

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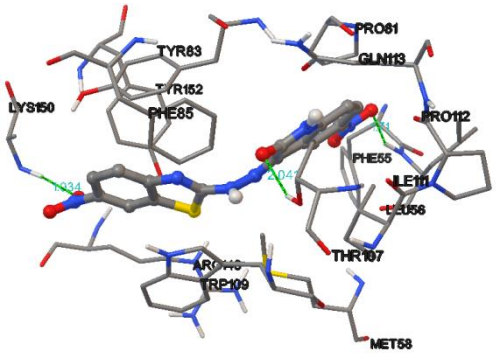

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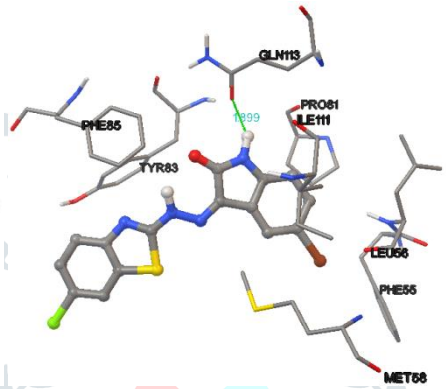

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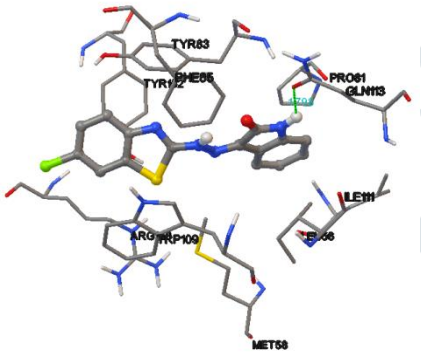

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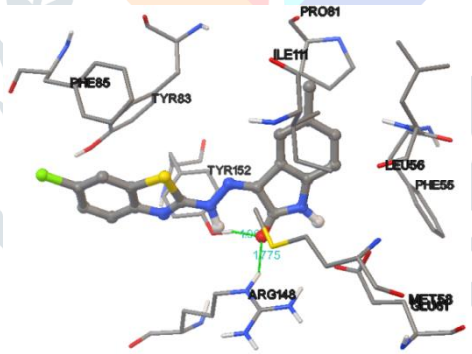

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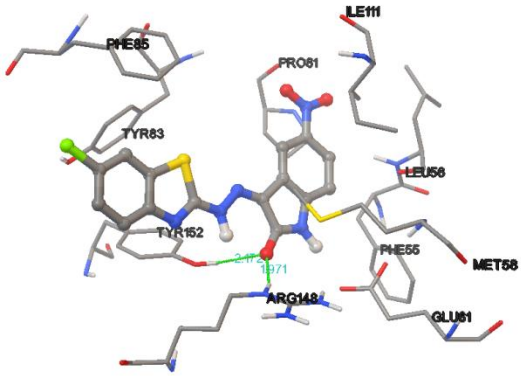

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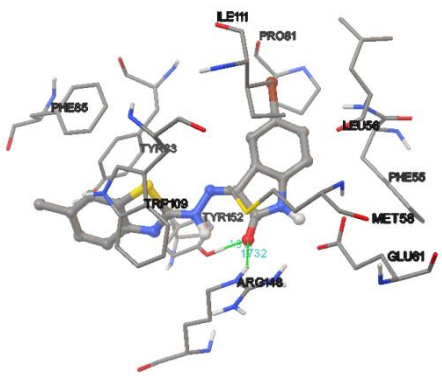

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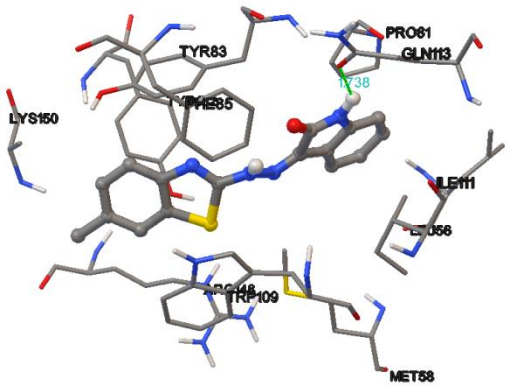

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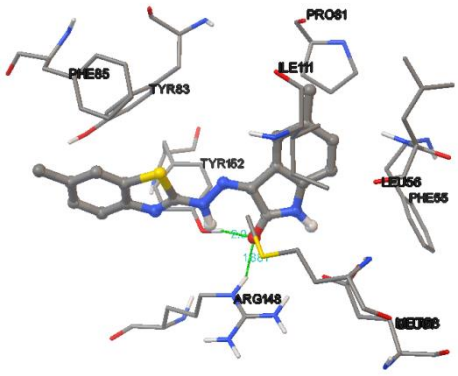

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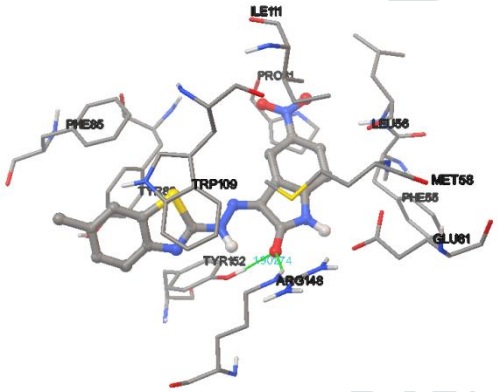

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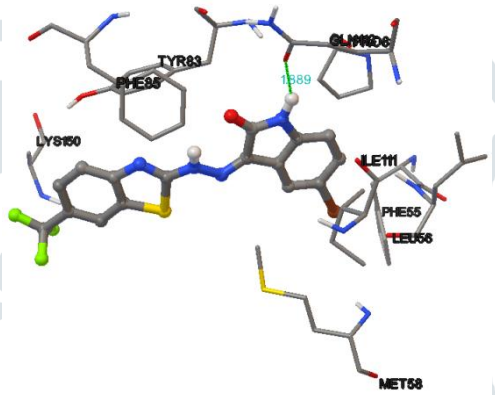

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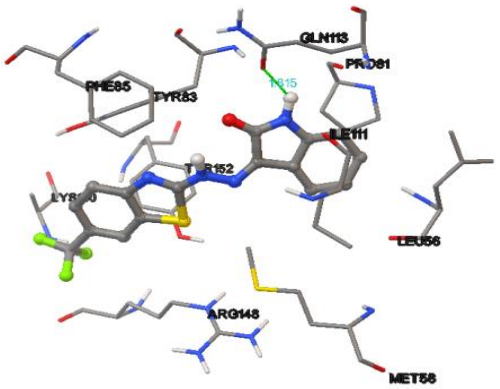

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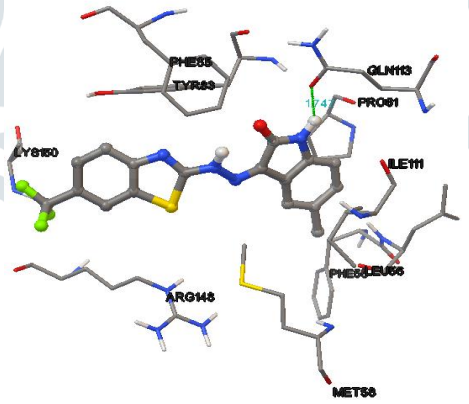

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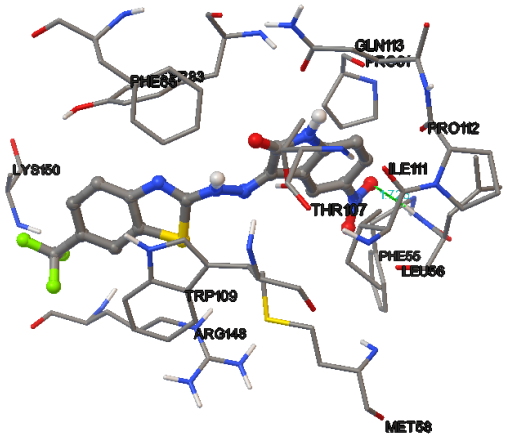

20.

4: Docking Studies of compounds (1-20).

S.No	R'	R	Interacting aminoacids	Binding energy, ΔG (Kcal/mol)	Dissociation constant (kI) (nM)
1	Br	H	Thr 107	-9.08	221.72
2	H	H	Gln113	-8.75	383.99
3	CH_3	H	-	-9.24	168.95
4	NO_2	H	Thr152, ARg148, Glu61	-5.88	49.04
5	Br	NO_2	Arg 148,Try152	-9.61	89.91
6	H	NO_2	Lys150,Gln 113	-10.69	40.13
7	CH_3	NO_2	Lys 150,Gln113	-10.69	39.84
8	NO_2	NO_2	Leu56, thr 107, Lys 150	-10.97	9.09
9	Br	Cl	**Gln 113**	**-9.08**	**219.67**
10	H	Cl	**Gln 113**	**-9.24**	**169.65**
11	CH_3	Cl	Arg148,Tyr 152	-9.34	142.40
12	NO_2	Cl	**Arg148, Tyr 152**	**-10.02**	**45.40**
13	Br	CH_3	Arg148, Tyr 152	-9.79	66.63
14	H	CH_3	Gln113	-9.10	213.83
15	CH_3	CH_3	Arg148, Tyr 152	-9.23	170.30
16	NO_2	CH_3	Arg148, Tyr152	-10.28	34.43
17	Br	CF_3	**Gln113**	**-8.65**	**456.05**
18	H	CF_3	**Gln113**	**-6.00**	**252.05**
19	CH_3	CF_3	Gln113	-8.87	314.78
20	NO_2	CF_3	**Leu 56**	**-9.29**	**154.59**
Doxorubicin			**Asn88, His89, Gly151, Lys158**	**-4.61**	**417.1**

7.Results and Discussion:

All the six compounds evaluated for cytotoxic activity against MCF-7 (cancer cells) and MCF-10A (normal cells) exhibited significant activity against both cell lines. All the derivatives showed IC_{50} values of **(8.53-12.3µM)** against MCF-7 cells. Compound (**20**) (R'=NO_2, R=CF_3) substituents exhibited highest potency

(IC_{50}=8.53µM), whereas Compound (**10**) (R'=H, R= Cl) without any substituent on indole nucleus showed least potency among the series **(IC_{50}=12.3µM)**. However, all the six compounds were found to be less toxic to MCF 10A cells also when compared to the standard Cisplatin **(IC_{50}=1.86µM)** and Doxorubicin **(IC_{50}=2.21µM)**. The compound (**6**) (R'=H, R=NO_2) exhibited highest binding energy of -10.69 kcal/mol with interacting Lys 150 and Gln 113. Nine molecules were shown interacting with Arg 148 and seven molecules were interacting with Arg 148 and Thr 152. Five molecules (**6**) (R'=H, R=NO_2), -10.69, (**7**) (R'=CH_3, R=NO_2),-10.09, (**8**) (R'= NO_2, R= NO_2), -10.97, (**12**) (R' = NO_2, R=Cl), -10.02 and (**16**) (R'=NO_2, R=CH_3), -10.28 were shown good binding energy of above -10.00 when compared to that of the standard drug doxorubicin -4.61 interacting with the Asn88, His89, Gly151 and Lys158.

8.Conclusion:

All the derivatives showed IC_{50} values of **(8.53-12.6µM)** against MCF-7 cells. Compound IBNCF_3 (R'=NO_2, R=CF_3) substituents exhibited highest potency **(IC_{50}=8.53µM)**, whereas Compound IBHCl (R'=H, R= Cl) without any substituent on indole nucleus showed least potency among the series **(IC_{50}=12.6µM)**. However, all the six compounds were found to be less toxic to MCF 10A cells also when compared to the standard Cisplatin **(IC_{50} =1.86µM)** and Doxorubicin **(IC_{50}=2.21µM)**. The compound IBHN (R'=H, R=NO_2) exhibited highest binding energy of -10.69kcal/mol with interacting Lys 150 and Gln 113. Nine molecules were shown interacting with Arg 148 and seven molecules were interacting with Arg 148 and Thr 152. Five molecules IBHN (R'=H, R=NO_2), -10.69, IBMN (R'=CH_3, R=NO_2), -10.09, IBNN (R'= NO_2, R= NO_2), -10.97, IBNCl (R' = NO_2, R=Cl), -10.02 and IBNM (R'=NO_2, R=CH_3), -10.28 were shown good binding energy of above -10.00.

9.Experimental:

All chemicals which are used as starting materials and reagents are purchased from Merck (India) and Sigma-Aldrich which are of reagent grade. All melting points were taken in open capillaries on the Electrothermal 1A 9100 apparatus (Shimadzu, Japan): IR spectra are recorded on Bruker ALPHA FT-IR spectrometer. The 1H NMR spectra are determined on a Bruker (200, 400 MHz) spectrometer using CDCl3 and DMSO-d6 as solvent. The chemical shifts are reported as parts per million tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on 70 eV(EI Ms-QP 1000EX,Shimadzu, Japan). The progress of the reaction was monitored on readymade silica-gel plates(merck) using hexane/ethylacetate (8:2) as solvent. Iodine was used as a developing agent or by spraying with the Dragendorff's reagent. Chromatography purification was performed over a silica gel 60 (particle size 0.06e 0.20mm).

Procedure[38].

5-{4-(Aminophenyl)-1,3,4-oxadiazol}2-thiol (1.93g, 0.01 mol) and indole (1.47g, 0.01 mol) were refluxed in 20 ml of ethanol in the presence of a catalytic amount of glacial acetic acid (2-3 drops) for 5-6h and cooled. The solid separated was filtered and washed with cold alcohol and the product obtained was recrystalized from methanol.

Spectral data of Scheme-I Compounds (1-20):

1.3-(2-(benzo[d]thiazol-2-yl)hydrazono)-6-bromoindolin-2-one (1).

This compound was obtained as a brown solid in **73%** yield. M.p 206-209°C. **IR (KBr, cm^{-1}):** C=O-1752, C=N-1702, N-H-3296, C-H-935. **^{1}H NMR (DMSO d_6, 300Hz)**: δ 6.83-6.95 (m,3H,Ar-H), 7.05-7.11 (m,2H,Ar-H), 7.25-7.30 (m,2H,Ar-

H), 7.38-7.43 (dd,2H,Ar-H). **D_2O Exchange (DMSO d_6, 300Hz):** 6.84-7.0 (m,3H,Ar-H), 7.09-7.14 (m,2H,Ar-H), 7.26-7.31 (m,2H,Ar-H), 7.40-7.43 (d,2H,J=9.6Hz). **FAB-Ms m/z**: 372.55.

2.3-(2-(benzo[d]thiazol-2-yl)hydrazono)indolin-2-one (2).

This compound was obtained as a brown solid in **72%** yield. M.p:195-197^oC.**IR (KBr, cm^{-1}):** C=O–1728, C=N- 1616, N-H–3192, C-H-1331.**^{1}H NMR (DMSO d_6, 400 MHz):** δ 7.21 (s,1H,Ar-H), 7.36-7.59 (m,6H,Ar-H), 7.70-7.72 (d,2H,Ar-H,J=7.6Hz), 8.86 (s,1H,Ar-H), 9.97 (s,2H,N-H). **FAB-Ms m/z**: 294.

3.3-(2-(benzo[d]thiazol-2-yl)hydrazono)-6-methylindolin-2-one (3).

This compound was obtained as a brown solid in **69%** yield. M.p: 177-180^oC. **IR (KBr, cm^{-1}):** C=O-1728, C=N -1616, N-H-3192, C-H-945.**^{1}H NMR (DMSO d_6, 400MHz):** δ 2.35 (s,3H,CH_3), 6.88-6.93 (m,3H,Ar-H), 7.12-7.16 (t,2H,Ar-H,J=2.8Hz), 7.47-7.93 (m,3H,Ar-H), 11.01 (s,2H,N-H). **FAB-Ms m/z**: 293.

4. 3-(2-(benzo[d]thiazol-2-yl)hydrazono)-6-nitroindolin-2-one (4).

This compound was obtained as a brown solid in **58%** yield. M.p: 220-221^oC. **IR (KBr, cm^{-1}):** C=O-1735, C=N-1630, N-H st-3369, NO_2-1371, C-H-1245.34. **^{1}H NMR (DMSO d_6, 300MHz):** δ 6.60-6.67 (t,1H,Ar-H,J=10.5Hz), 6.80-6.83 (d,2H,Ar-H,J=9.3Hz), 7.84-7.97 (t,1H,Ar-H,J=31.2Hz), 8.00-8.03 (d,2H,Ar-H,J=8.4Hz), 8.47 (s,2H,Ar-H), 8.64 (s,1H,Ar-H). **D_2O Exchange (DMSO d_6, 300MHz):** δ 6.53-6.63 (t, 1H,Ar-H,J= 6.3Hz), 6.79-6.82 (d,2H,Ar-H,J=6.9Hz), 7.27-7.87 (t,1H,Ar-H,J=27.6Hz), 8.01-8.03 (d,1H,Ar-H,J=5.7Hz), 8.40 (s,2H,Ar-H), 8.59 (s,1H,Ar-H). F**AB-Ms m/z:** 338.7.

5.6-bromo-3-(2-(6-nitrobenzo[d]thiazol-2-yl)hydrazono)indolin-2-one (5).

This compound was obtained as a brown solid in **64%** yield. M.p.225-228^oC. **IR (KBr, cm^{-1})**: C=O-1727, C=N-1615, N-H-3192, NO_2-1460, C-Br-479. **^{1}H NMR (DMSO d_6, 400MHz)**: δ 7.60-7.69 (m,5H,Ar-H), 7.82-7.90 (m,2H,Ar-H), 7.93 (s,1H,Ar-H), 8.11 (s,1H,Ar-H), 11.2 (s,1H,N-H). **FAB-Ms m/z**: 418.22.

6. 3-(2-(6-nitrobenzo[d]thiazol-2-yl)hydrazono)indolin-2-one (6).

This compound was obtained as an orange solid in **58%** yield. M.p. 221-222^oC. **IR (KBr, cm^{-1}):** C=O-1632, C=N-1616, N-H-3416, NO_2-1364, C-H-1460.**^{1}H NMR (DMSO d_6, 400MHz):**δ 2.04 (s,3H,CH_3), 7.00-7.02 (d,2H,Ar-H,J= 7.6Hz), 7.26-7.30 (t,2H,Ar-H,J=7.6Hz), 7.56-7.58 (d, 2H, Ar-H,J=8.0Hz), 9.90 (s, 3H, N-H,Ar-H). F**AB-Msm/z**: 339.33.

7.6-methyl-3-(2-(6-nitrobenzo[d]thiazol-2-yl)hydrazono)indolin-2-one (7).

This compound was obtained as a yellowish solid in **59%.** yield. M.p:195-200^oC. **IR (KBr, cm^{-1}):**C=O- 1682, C=N-1647, N-H-3164, NO_2-1406.**^{1}H NMR (DMSO d_6, 300MHz) :** δ 2.5 (s,3H,CH_3), 6.83-6.85 (d,1H,Ar-H,J=7.5Hz), 7.33-7.35 (d,1H,Ar-H,J=7.5Hz), 9.51-9.55 (d,1H,Ar-H,J=14.1Hz), 10.50-10.67 (m,4H,Ar-H,N-H). **D_2O Exchange (DMSO d_6, 300MHz)**: δ 2.5 (s,3H,CH_3), 6.85-6.88 (d,1H,Ar-H,J=7.5Hz), 7.32-7.35 (d,1H,Ar-H,J=7.2Hz). **FAB-Msm/z**: 353.36.

8.6-nitro-3-(2-(6-nitrobenzo[d]thiazol-2-yl)hydrazono)indolin-2-one (8).

This compound was found as an orange solid in **55%** yield. M.P.219-225^oC. **IR (KBr, cm^{-1})**: C=O-1702 , C=N -1752, N-H-3296, NO_2-1358, NO_2-1389, C-H-818. **^{1}H NMR (DMSO d_6, 400MHz)**: δ 6.57-6.59 (d,2H,Ar-H,J=8.4Hz), 6.96 (s,3H,Ar-H), 7.90-7.93 (d,2H,Ar-H,J=8.4Hz), 10.15 (s,1H,N-H).**FAB-Ms m/z**: 384.33.

9.6-bromo-3-(2-(6-chlorobenzo[d]thiazol-2-yl)hydrazono)indolin-2-one (9).

This compound was obtained as an orange solid in **68%** yield. M.P: 185-190^{o}C. **IR (KBr, cm^{-1}):** C=O-1752, C=N-1702, N-H-3296, C-Cl-781, C-Br-598. **^{1}H NMR (CDCl$_3$,400MHz)**:δ 6.76 (s,1H, Ar-H), 6.97-6.98 (d,2H, Ar-H,J=6Hz), 7.31-7.38 (m,3H,Ar-H,J=12Hz), 7.51 (s,1H,Ar-H), 8.12 (s, 1H, Ar-H).**FAB-Ms m/z**: 407.0

10. 3-(2-(6-chlorobenzo[d]thiazol-2-yl)hydrazono)indolin-2-one (10).

This compound was obtained as an orange solid in **69%** yield .M.p:201-204^{o}C. **IR (KBr, cm^{-1}):** C=O- 1616, C=N-1728, N-H-3192, C-Cl-770. **^{1}H NMR (DMSO d$_6$, 300MHz):** δ 6.67-6.70 (d,1H,Ar-H,J=8.4Hz), 6.89-6.92 (d,2H,Ar-H,J=7.8Hz), 7.01-7.06 (t,1H,Ar-H,J=7.5Hz), 7.16 (s,1H,Ar-H), 7.46-7.49 (t,1H,Ar-H,J=7.2Hz), 7.54-7.58 (t,1H,Ar-H,J=7.5Hz), 11.10 (s,1H,N-H). **D$_2$O Exchange (DMSO d$_6$, 300MHz):** δ 6.67-6.70 (d,2H,Ar-H,J=7.5Hz), 6.89-6.92 (d,2H,Ar-H,J=7.8Hz), 7.01-7.06 (t,1H,Ar-H,J=9Hz), 7.16 (s,1H,Ar-H), 7.46-7.49 (d,2H,Ar-H,J=7.2Hz), 7.54-7.58 (t,1H,Ar-H,J=6.9Hz). F**AB-Ms m/z**: 329.

11. 3-(2-(6-chlorobenzo[d]thiazol-2-yl)hydrazono)-6-methylindolin-2-one (11).

This compound was obtained as a brown solid in **70%** yield. M.p: 198-200^{o}C. **IR (KBr, cm^{-1}):** C=O-1631, C=N-1752, N-H-3369, C-CH$_3$-1433, C-H-1043, C-Cl- 616.**^{1}H NMR (DMSO d$_6$,300MHz):** δ 2.24 (s,3H,CH$_3$), 6.74-6.76 (d,1H,Ar-H,J=7.2Hz), 7.12-7.15 (d,3H,Ar-H,J=7.5Hz), 7.76 (s,3H,Ar-H), 10.7 (s,1H,N-H). **D$_2$O Exchange (DMSO d$_6$,300MHz):** δ 2.24 (s,3H,CH$_3$), 6.74-6.76 (d,1H,Ar-H,J=7.2Hz), 7.12-7.15 (d,3H,Ar-H,J=7.5Hz), 7.76 (s,3H,Ar-H) .FA**B-Ms m/z**: 343.80.

12. 3-(2-(6-chlorobenzo[d]thiazol-2-yl)hydrazono)-6-nitroindolin-2-one (12).

This compound was obtained as a yellowish brown solid in **65%** yield. M.p. 250-255^{o}C. **IR (KBr, cm^{-1}): C=O**-1712, C=N-1616, N-H-3192, C-NO$_2$-1331, C-Cl-770. **^{1}H NMR (DMSO d$_6$,400MHz):** δ 6.57-6.59 (d,4H,Ar-H,J=8.4Hz), 6.98 (s,1H,Ar-H), 7.11 (s,1H,Ar-H), 8.5 (s,1H,Ar-H), 10.10 (s,1H,N-H), 11.37 (s,1H,N-H). F**AB-Ms m/z**: 394.0. **C-13**: 61.356, 110.531, 112-251, 115.521, 120.304,121.966, 124.954,126.304, 128.534, 135.522, 142.304, 143.994, 144.114, 147.974, 155.664.

13. 6-bromo-3-(2-(6-methylbenzo[d]thiazol-2-yl)hydrazono)indolin-2-one (13).

This compound was obtained as a yellow solid in **65%** yield. M.p: 195-200^{o}C. **IR (KBr, cm^{-1}):** C=O- 1752, C=N-1702, N-H-3296, C-Br-506, C-CH$_3$-1429. **^{1}H NMR (CDCl$_3$, 400MHz):** δ 2.35 (s, 3H,CH$_3$), 6.88-6.99 (m, 5H, Ar-H), 7.67 (s, 1H, Ar-H), 7.75-7.78 (d, 1H, Ar-H,J=8.4Hz), 8.61 (s, 1H, N-H).**FAB-Ms m/z: 385.98.**

14. 3-(2-(6-methylbenzo[d]thiazol-2-yl)hydrazono)indolin-2-one (14).

This compound was obtained as an orange solid in **63%** yield. M.p: 148-150^{o}C. **IR (KBr, cm^{-1}):**C=O- 1728, C=N-1616, N-H-3192, C-H-945, C-CH$_3$-1460. **^{1}H NMR (CDCl$_3$, 400MHz):** δ 2.37 (s,3H,CH$_3$), 6.90-6.92 (d,2H,Ar-H,J=8Hz), 7.11-7.15 (t,1H,Ar-H,J=7.6Hz), 7.55-7.63 (m,3H,Ar-H), 8.03 (s,3H,Ar-H). **FAB-Ms m/z**: 331.0. **C-13:** 112.155, 114.195, 117.769, 119.155, 121.155, 121.342, 122.714, 124.631, 126.667, 129.125, 130.105, 133.115, 138.324, 150.676, 159.308.

15. 6-methyl-3-(2-(6-methylbenzo[d]thiazol-2-yl)hydrazono)indolin-2-one (15).

This compound was obtained as a creamish solid in **65%** yield. M.p: 144-146^{o}C. **IR (KBr, cm^{-1}):** C=O- 1728, C=N-1616, N-H-3192, C-CH$_3$-1460. **^{1}H NMR (DMSO d$_6$, 400MHz): δ** 1.24 (s ,3H,CH$_3$), 2.26 (s, 3H,CH$_3$), 7.09-7.11 (d,2H,Ar-H,J=8Hz), 7.25-7.27 (d,1H,Ar-H,J=6.4Hz), 7.83 (s,2H,Ar-H), 10.54 (s,1H,N-H. **FAB-Ms m/z**: 323.25. **C-13**: 109.808, 116.367, 117.118, 122.118, 126.811, 127.055, 130.643, 131.472, 134.274, 139.791, 143.911, 146.118, 150.233, 165.247, 177.133.

16. 3-(2-(6-methylbenzo[d]thiazol-2-yl)hydrazono)-6-nitroindolin-2-one (16).

This compound was obtained as a brown solid in **60%** yield. M.p: 195-200^{o}C. **IR (KBr, cm^{-1}):** C=O-1647, C=N-1682, N-H-3025, C-CH_3-660. **^{1}H NMR (DMSO d_6, 400MHz)**: δ 3.5 (s, 3H, CH_3), 6.57-6.59 (d, 2H, Ar-H, J=8.4Hz), 6.96 (s, 3H, Ar-H), 7.90-7.93 (d, 2H,Ar-H, J=8.4Hz), 10.01 (s ,1H,N-H). **FAB-Ms m/z**: 352.7.

17.6-bromo-3-(2-(6-(trifluoromethyl)benzo[d]thiazol-2-yl)hydrazono)indolin-2-one (17).

This compound was obtained as a brown solid in **62%** yield. M.p: 216-219^{o}C. **IR (KBr, cm^{-1}):** C=O-1727, C=N-1615, N-H-3192, C-CF_3-1096, C-Br-479. **^{1}H NMR ($CDCl_3$, 400MHz):** δ 6.88-6.99 (m,4H,Ar-H), 7.67 (s,1H,Ar-H), 7.75-7.78 (d,1H,Ar-H,J=8.4Hz), 8.61 (s,2H,Ar-H). **FAB-Ms m/z**: 441.23.

18.3-(2-(6-(trifluoromethyl)benzo[d]thiazol-2-yl)hydrazono)indolin-2-one (18).

This compound was obtained as a yellow solid in **70%** yield. M.p: 185-190^{o}C. **IR (KBr, cm^{-1}):** C=O -1728, C=N-1616, C-H-1331, C-CF_3-1201.**^{1}H NMR ($CDCl_3$, 400MHz):** δ 7.10-7.13 (t, 1H, Ar-H,J=7.2Hz), 7.16-7.24 (t, 1H, Ar-H, J=7.6Hz), 7.28 (s,1H,Ar-H), 7.40-7.42 (d,2H, Ar-H,J=8.4Hz), 7.58-7.60 (d, 2H, Ar-H, J=8.8Hz), 7.77 (s,1H, Ar-H), 9.03 (s,1H,Ar-H), 9.68 (s, 1H, N-H).**FAB-Ms m/z**: 361. **C-13**:24.161, 110.083, 117.416, 119.417, 121.337, 122.257, 124.737, 126.220, 127.009, 129.417, 131.329, 133.729, 138.729, 156.567, 162.766, 177.673.

19.6-methyl-3-(2-(6-(trifluoromethyl)benzo[d]thiazol-2-yl)hydrazono)indolin-2-one (19).

This compound was obtained as a brown solid in **69%** yield. M.p:191-193^{o}C. **IR (KBr, cm^{-1}):** C=O-1728, C=N-1616, N-H-3192, C-CF_3-1201, C-CH_3-1460. **^{1}H NMR (DMSO d_6, 400MHz):** δ 2.25 (s, 3H, CH_3), 6.8-7.0 (m, 3H,Ar-H), 10.9-11.1 (s, 2H, N-H).**FAB-Ms m/z**: 376.06. **C-13**: 111.045, 112.170, 117.760, 118.954, 122.461, 122.714, 122.899, 124.625, 128.581, 138.323, 150.689, 159.314, 184.346.

20.6-nitro-3-(2-(6-(trifluoromethyl)benzo[d]thiazol-2-yl)hydrazono)indolin-2-one (20).

This compound was obtained as a brown solid in **59%** yield. M.p: 240-245^{o}C. **IR (KBr, cm^{-1}):** C=O-1647, C=N-1682, C-H-660, C-CF_3-1008, C-NO_2-1406. **^{1}H NMR (DMSO d_6, 400MHz):** δ 7.00 (s, 1H, Ar-H), 7.13 (s, 1H, Ar-H), 7.25-7.32 (m, 4H, Ar-H), 10.12 (s, 2H, N-H). **FAB-Ms m/z**: 407.33. **C-13**: 110.890, 112.471, 118.123, 119.546, 120.275, 122.565, 126.122, 127.665, 130.133, 131.257, 133.035, 142.576, 150.053, 155.168, 159.859, 182.341.

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