Design, Synthesis and Evaluation and Docking studies of Aryloxybenzylidene-Hydrazinyl-benzoxazoles/Benzothiazoles Analogs for Anticancer Activity.

Running Title:
Evaluation and Docking studies of Aryloxybenzylidene-Hydrazinyl-benzoxazoles/Benzothiazoles Analogs for Anticancer Activity.

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Abstract: Estrogen plays an important role in the development of breast cancer in menopausal women. Aromatase, an enzyme that catalyses the last step in the production of estrogen, has been identified as a promising target for clinical development. A Series of 79 novel, benzothiazole/benzoxazole-phenoxybenzaldehyde derivatives linked with hydrazine bridge as shown in **General Structure-II** containing some electron-withdrawing/electron-releasing substituents at 6th position of benzothiazole/benzoxazole and various substitutions all positions of phenoxy ring system. The synthesized compounds are characterized by different spectroscopic techniques, are evaluated for anti-cancer activity and docking studies were carried out by using AUTODOCK 4.2 by taking the crystal structure of protein The crystal structure of protein *3HY3* (***Structure of human MTHFS with 10-formyltetrahydrofolate***) from *Homo sapiens* was downloaded from protein data bank in one of the critical enzyme involved was retrieved from Protein data bank with a resolution of 0.375A$^{o.}$ Six compounds each of Series-IIa & IIb (Table: 3.2.4) showed less cytotoxicity against MCF-7 (breast cancer) and MCF-10A (Normal breast epithelial cells) with IC_{50} between 9.40±2.47->100 µM compared to 1.86±1.77 and 1.86±0.31 µM shown by the standard cisplatin. All the twelve compounds failed to inhibit the growth of MCF-10A cells at the highest concentration tested (100 µM). Compound **II-7S-2m [X=S;R=NO_2;R_1=CH_3;R_2=$CH_2OCH_3$and R_3=Cl]** and **II-7O-2i [X=O; R= Cl; R_1=H;R_2=H and R_3=Cl]** showed potent activity with IC_{50} of 9.70±2.47 and 11.5±1.45µM against MCF-7 cells. Compound **II-7S-1v** (X=S; R=H; R_1= H; R_2=CH_2COCH_3) and **II-7O-1v** (X=O; R=H; R_1=H;R_2=CH_2COCH_3) showed least toxicity with IC_{50} of 20.37±2.47 and 24.05±2.62 µM against MCF-7 cells.

20 compounds of Series-IIa and 28 compounds of Series-IIb are incorporated in docking studies with the protein of ***human MTHFS with 10-formyltetrahydrofolate*** **(PDB id: *3HY3*).** The compounds **II-7S-1n [X=S; R=CH_3; R1=H; R_2=H and R_3=Cl]; II-7S-1q [X=S;R=CH_3; R_1=H; R_2=CF_3 and**

R_3=H] and **II-7S-1c [X=S; R=OCH_3; R_1=H; R_2=H** and **R_3=H]** of **Series-IIa** showed potent docking scores from -10.98, -10.75 and -10.25 with hydrogen bond interactions Leu56, Leu56 and Leu56, Lys150 one, one, and two respectively. The compounds **II-7S-1i [X=S; R=OCH_3; R_1=H; R_2=Cl** and **R_3=H]**; **II-7S-1k [X=S;R=CH_3;R_1=H; R_2=H** and **R_3= CH_3]** and **II-7S-1m [X=S; R=H; R_1=H, R_2=H** and **R_3=Cl]** of **Series-IIa** showed least potent docking scores with -8.58, -8.56 and -8.55 with one hydrogen bond interaction.

The compounds **II-7O-1d [X=O;R=H; R_1=CH_3;R_2=H** and **R_3=H];** **II-7O-1h [X=O; R=CH_3; R_1=H; R_2=Cl** and **R_3 =H]** and **II-7O-1g [X=O; R=H; R_1=H; R_2=Cl** and **R_3=H]** of Series-IIb showed potent docking scores from -10.24,-10.21 and -9.55 with hydrogen bond interactions Leu56, Leu56 and Tyr83 one, one and one respectively. The compounds **II-7O-1f [X=O;R=OCH_3;R_1=CH_3; R_2=H** and **R_3=H];** **II-7O-1s [X=O; R=H; R_1=H; R_2=Cl** and **R_3=Cl]** and **II-7O-1n [X=O; R=CH_3; R_1=H; R_2=H** and **R_3=Cl]** of Series-IIb showed least potent docking score with -8.04, with one hydrogen bond interaction Tyr83, -7.94 with no hydrogen bond interaction and -7.89 with one hydrogen bond interaction Tyr83 respectively. All the above results indicated that subtituents at 6^{th} position with benzothiazole-phenoxybenzaldehyde derivatives linked with hydrazine bridge have shown potent compounds when compared to substituents at 6^{th} position benzoxazole-phenoxybenzaldehyde derivatives linked with hydrazine bridge.

Key words: Anti-cancer, Aryloxybenzylidene, Benzothiazole, Benzoxazole, Heterocyclic, Hydrazine.

1. Introduction:

Multi-drug resistance is an important obstacle that limits the efficacy of chemotherapy in many cancer types [RahulV.Patel]. Phortress (NSC710305) was the leading compound and demonstrated the activity against breast cancer tumors regardless of oestrogen receptor status against ovarian, renal, lung and colon-cancer [(Sacks.L.V *etal*.,) (Raja Solomon.V *etal*.,)]. Benzothiazole moiety is a versatile moiety that exhibits a wide variety of biological activities including antitubercular [(Jarvest.*etal*.,)(Erskine*etal*.,)(Kim*etal*.,)], antimalarial, anticonvulsant [Farhanullah *etal*.,], antidiabetic [Li *et al*.,], antimicrobial, antifungal and antitumor [(Li*etal*.,)(Rouf*etal*.,)]. Modifications on the benzothiazole have resulted in large number of compounds having diverse biological activities especially phenyl-substituted benzothiazoles[(Chugunova *et al*.,)(Bradshaw *et al*.,)(Hutchinson *et al*.,)(Kashiyama *et al*.,)]. The 2-substituted aminobenzothiazole derivatives have been reported to possess antibacterial, antitumor, anti-tuberculosis, carbonic anhydrase inhibitory and good anticancer properties [(Sanjay *etal*.,)(Jemal *etal*.,)]. 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 activity 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[(Laurence *et al*.,)(Rawat *etal*.,)(Noolvi *etal*.,)(Pireddu *etal*.,)].

Benzoxazole derivatives are key heterocyclic scaffolds in medicinal chemistry that show potent *in vitro* and *in vivo* anticancer activities. They act as strong cytotoxic and antiproliferative agents against various human cancer cell lines (such as MCF-7, HepG2, HCT-116, and A549) often by inducing apoptosis and cell cycle arrest. [(American Society)(Blackadar.,)(Zajaczkowska *etal*.,)(Hitchcock *etal*.,)(Koga *etal*.,)]. Benzoxazole is a prominent structural

motif in medicinal chemistry, and it serves as the basis for many modern pharmacological medicines. Studies have shown that benzoxazole derivatives have anticancer effects *via* a variety of pathways, including DNA intercalation, VEGFR-2 inhibition, topoisomerase I and II inhibition, PARP-poly inhibition, and aromatase inhibition. They also possess significant cytotoxicity against MCF-7 due to its effective inhibition of overexpressed EGFR and aromatase in breast cancer. (Ibrahim H Eissa etal.,) The aminobenzoxazole derivatives shown antitumor action (IC_{50} = 0.72 µM) against RPMI-8226 (leukaemia) by targeting tyrosine kinase. The 2-phenyl-substituted benzoxazole analogues (**leads 1** and **2**) were more cytotoxic to MDA-MB-231, with IC50s of 1.27 and 1.21 µM, respectively (Sandip Gadakh etal.,) . Hydrazones of 1H-indole-2,3-dione have been identified as inhibitors of the protein tyrosine phosphotase Shp2, which plays an important role in cell signaling.(Romagnoli, R. etal.,) New aryloxybenzylidene-hydrazinyl-benzothiazole derivatives, 6-chloro-2-[2-(4-(2,4-dichloro-phenoxy)-benzylidene)-hydrazinyl)-benzothioazole exhibited promising antitubercular activity with MIC value of 15 µg/ml. (telvekar etal., 2012)

In the past few decades, molecularly targeted regimens has become an indespensable 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. ((Romagnoli, R. etal.,) (Meuneir,B etal.,) (Kamal,A etal.,))

In view of the potent antimicrobial, anticancer and antitubercular activity associated with benzothiazole, benzoxazole and diphenyl ether moieties, we here in planned to synthesize the hybrid molecules of benzothiazole/benzoxazole and substituted diphenyl ether moiety joined with hydrazine linkage as shown in **Figure 2.2. General Structure-II**.

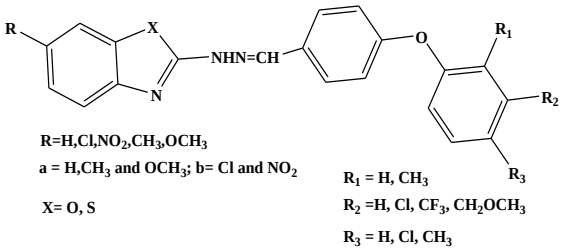
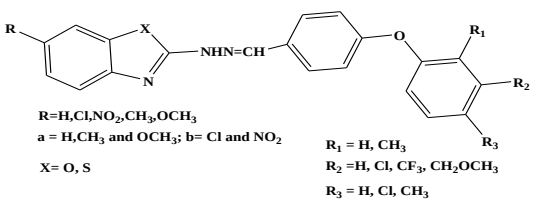


Figure 1. General Structure-II: (aryloxy benzylidene-hydrazinyl-benzothiazole-benzoxazole conjugates) (II-7S-1a-x; II-7S-2a-o and II-7O-1a-x; II-7O-2a-p)

2. NEED FOR THE PRESENT WORK/ Rationale

It is apparent from the foregoing survey that many new benzothiazole/benzoxazole and phenoxy benzaldehyde derivatives have been synthesized as potential antibacterial, antifungal, antitubercular and anticancer agents. As the benzothiazole/benzoxazole and phenoxybenzaldehyde nuclei are among the most extensively studied classes of heterocyclic compounds possessing anticancer, antiinfective, antibacterial and antifungal properties. It is thought worthwhile to synthesize benzothiazole/benzoxazole-phenoxybenzaldehyde derivatives linked with hydrazine bridge as shown in **General Structure-II** containing some electron-withdrawing/electron-releasing substituents at 6^{th} position of benzothiazole/benzoxazole and various substitutions all positions of phenoxy ring system.



General Structure-II: (aryloxy benzylidene-hydrazinyl-benzothiazole / benzoxazole conjugates) (II-7S-1a-x; II-7S-2a-o and II-7O-1a-x; II-7O-2a-p)

3. Experimental section

3.1. Step-1: Preparation of 2-aminobenzothiazoles (I-3a-e)/benzoxazoles (II-3f-j)

A mixture of anilines (**II-1a-e;** 0.01 mol) and potassium thiocyanate (**II-2a**; 0.97g, 0.01 mol)/ potassium cyanate (**II- 2b**; 0.81g, 0.1 mol) were taken into a round bottom flask and 10ml of glacial acetic acid was added then the mixture was cooled in an ice bath and stirred for 10-20 minutes, and then bromine (0.79g, 0.01 mol) in glacial acetic acid (0.571 ml, 0.01 mol) was added drop wise at such a rate to keep the temperature below 10^0C throughout the addition. After completion of addition, the reaction mixture was stirred at room temperature for 2-4 hrs, the hydrobromide (HBr) salt thus separated out was filtered, washed with acetic acid, 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 products,which were purified by passing over silica gel using hexane:ethylacetate (8:2) solvent system to get the compounds **(II-3a-j)** in pure form.

Step-2: Preparation of 2-hydrazinyl-benzothiazoles/ benzoxazoles (II-5a-j)

The 2-amino-benzothiazoles/benzoxazoles **(II-5a-j;** 0.01 mol) were taken in ethylene glycol (0.55 ml) and hydrazine hydrate (**II–4;** 0.641 ml; 0.02 mol) was added. The reaction mixture was refluxed for 4 hrs and resultant crystals thus formed were filtered and dried to obtain corresponding 2-hydrazinylbenzothioazoles/benzoxazoles **(II-5a-j).** (Telvekar *et al.*, 2012).

Step-3: Preparation of 2-(2-(4′-(3″-substituted phenoxy)-benzylidene)-hydrazinyl)-6-substituted benzo[d]thiazoles/benzo[d]oxazoles (II-7S-1a-x, II-7S-2a-o and II-7O-1a-x, II-7O-2a-p)

A mixture of 2-hydrazinyl-benzothiazoles/benzoxazoles (**II-5a-j;** 0.01 mol), phenoxybenzaldehydes **(II–6-a-h;** 0.01mol), were suspended in absolute ethanol (30 ml) and the mixture was refluxed at 80ºC for 6-10 hrs. The reaction mixture was then cooled to room temperature, concentrated under reduced pressure and partitioned between ethyl acetate (50 ml) and water (50 ml). The organic layer was separated, and the aqueous layer was extracted further with ethyl acetate (2×25 ml). The combined organic layers were washed with brine (50 ml), dried over sodiumsulphate (Na_2SO_4), and concentrated under vacuum. The crude products obtained were purified by column chromatography (silica gel), ethyl acetate/hexane: 8:2 as eluent to obtain 2-(2-(4′-(3″-substitutedphenoxy)-benzylidene)-hydrazinyl)-substituted benzo[d]thiazoles /benzo[d]oxazoles **(II-7S** and **II-7O)** in pure form. The physical data of the compounds is presented in **Tables No: 1** and **2.**

Spectral data of 2-(2-(4′-(3″- substituted phenoxy)-benzylidene)-hydrazinyl)-6-substituted benzo[d]thiazoles/benzo[d]oxazoles **(II-7s-1a-x; II-7S-2a-o and II-7O-1a-x, II-7O-2a-p)** is given in **Appendix-A, (Scheme-II, Series-IIa). Appendix-B, (Scheme-II, Series-IIb).**

4. PHARMACOLOGICAL EVALUATION:

A few compounds of the series aryloxy benzothiazoles/benzoxazoles, **Series-IIa** and **Series-IIb (II-7S-1a-x)** & **(II-7S-2a-o)** and **(II-7O-1a-x)** & **(II-7O-2a-p)** were screened for cytotoxic activity against MCF-7 (breast cancer) and MCF-10A (non-cancer breast epithelial cells).

4.1. Cytotoxic activity

The cytotoxic activity of a few compounds from the series: aryloxy benzothiazoles/ benzoxazoles, **Series-IIa** and **Series-IIb (II-7S-1a-x)** & **(II-7S-2a-o)** and **(II-7O-1a-x)** & **(II-7O-2a-p)** was evaluated against human and non-cancer cell lines using MTT assay method. (Venkanna.*et al.*, 2014).The results were represented in **Table No: 3**

MTT assay method:

Cell lines:

MCF-7 (Breast cancer) and MCF-10A (non-cancer breast epithelial cells).

(Cell lines used in this study were purchased from the NCCS, pune).

Methodology:

Preparation of solutions of test compounds:

Test compounds were weighed separately and dissolved in DMSO and diluted with (DMEM) media to make up the final concentration of1 mg/ml. This stock solution was furtherdiluted to get a concentration range from 10 to 100µg/ml (six concentrations of 5, 10, 25, 50, 75, 100 µg/ml).

Standard drug: Cisplatin and doxorubicin were used as reference drugs.

Materials:

DMEM (Dulbecco's modified Eagles medium), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], trypsin, EDTA Phosphate Buffered Saline (PBS) and were purchased from Sigma Chemicals Co.(St. Louis, MO) and Fetal Bovine Serum (FBS) was purchased from Gibco. 25 cm^2 and 75 cm^2 flasks and 96 well plate were purchased from Eppendorf India.

Maintenance of cell line: The MCF-7 breast adenocarcinoma cell line was purchased from NCCS, pune and the cells were maintained in DMEM supplemented with 10 % FBS and the antibiotics penicillin &streptomycin (0.5µg/ml), in atmosphere of 5 % CO_2 /95 % air at 37ºC.

Cell viability by MTT Assay:

Principle: MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by succinate dehydrogenase. The viable cells contain NAD(P)H-dependent oxidoreductase enzymes which reduce the MTT to formazan. The DMSO concentration used does not showed any notable cytotoxicity in all studies. The assay depends both on the number of cells present and on the assumption that dead cells or their products do not reducetetrazolium. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, dark purple coloured formazan crystals. The cells were then solubilized with a DMSO and the released solubilzed formazan reagent was measured spectrophotometrically at 570nm.

Procedure:

The MTT assay is used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity with three independent experiments with six concentrations 5, 10, 25, 50, 75 and 100 µg/mlof compounds in triplicates. MCF-7 cells were trypsinized to know viable cells in cell suspension and preincubated at a concentration of $1X10^6$ cells/ml in culture medium with 1 µg/ ml Cisplatin and Doxorubicin for 3 h at 37 °C and 5-6.5% CO_2.Cells were counted by haemocytometer and seeded at density of 5.0 X 10^3 cells/well in100 µl culture media containing 1 µg/ml Cisplatin and Doxorubicin in 96 well plate culture medium and incubated overnight for 24 hr at 37ºCand 5-6.5% CO_2. After incubation, the old media was taken off and 100 µl fresh media was added with different concentrations of test compound in representive

wells in 96 plates. After 48 hrs, the drug solution discarded and the fresh media with MTT solution 10µl with final concentration of (0.5 mg / ml^{-1}) was added to each well and plates were incubated at 37ºC for 3hrs. Allow the plates to stand overnight in the incubator in a humidified atmosphere (e.g., +37°C, 5-6.5% CO_2). At the end of incubation time, precipitates were formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the formula and the concentration of test drug needed to inhibit cell growth by 50 % is generated from the dose-response curves for each cell line using Origin software. Cisplatin and doxorubicin were used as reference drugs, for comparison. The cytotoxicity was expressed as IC_{50} (µg/ml^{-1}) value along with standard deviation.

The results were summarized in **Table No: 3.**

5. MOLECULAR DOCKING STUDIES:

5.1: DOCKING METHODOLOGY

Docking of all the molecules into the binding site of crystal structures of protein was performed by using autodock 4.2. It is open-source soft ware for drug discovery, molecular docking. The structural interactions between targeted protein structures, with the three series of ligands, aryloxybenzothiazoles **(II-7S-1a-x)** and **(II-7S-2a-o)** and aryloxybenzoxazoles **(II-7O-1a-x)** and **(II-7O-2a-p);** were carried out separately.

Docking studies of the significantly active and weakly active molecules were performed by using AUTODOCK 4.2. All docking calculations were performed using Lamarkian Genetic Algorithm (LGA) method. A scaling factor of 0.8 and a partial atomic charge of less than 0.15 was applied to the atoms of the protein.

AUTODOCK 4.2 docking score was used to determine the affinity, activity, binding orientation of ligand with the target protein and to analyze best conformations. The interactions and ligand conformations, including hydrogen bonds and the bond lengths were analysed.

Validation of the docking protocol

The precision of the docking study was determined by finding the lowest energy conformation of the co-crystallized ligand and resembles an experimental binding mode as determined by X-ray crystallography. The docking procedure was authenticated by removing the co-crystallized ligand from the binding site of the protein and re-docking the same co-crystallized ligand in the active binding site. The hydrogen-bonding interaction and RMSD between the anticipated confirmation and the observed X-ray crystallographic conformation were used for validation.

Ligand preparation

The ligands used as input for docking study were sketched using Tripo's Syby16.7 Chem Draw software and cleaned up the structures for bond alignment, ligands incorporated into the workstation, the energy was minimized 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. This minimization helps to assign bond orders, addition of hydrogens to the ligands and conversion of 2D to 3D structure for further docking studies. The generated output file (best conformations of the ligands) was further used for docking studies.

Protein preparation

Protein was downloaded from protein data bank. Initially the selected crystal structure of a protein was loaded in auto dock, water molecules were removed. Hydrogen atom was added to the proteins and charges were assigned. Generated

Het states using Epik at pH 7.0 ±2.0. Pre-processed the protein and refined, modified the protein by analyzing the workspace, water molecules and other heteroatoms were examined; non-significant atoms were excluded from the crystal structure of the protein. Finally, the protein was minimized by using AMBER force filed. A grid was created by considering the co-crystallized ligand present in the active site of the selected target of enoyl acyl carrier protein reductase from {*Mycobacterium tuberculosis* (PDB id; 3IVX) and *Staphylococcus aureus* (PDB id; 2ZCS)}.

Receptor grid generation

A receptor grid was generated around the protein by picking the inhibitory ligand (X-ray pose of the ligand in the protein). The centroid of the ligand was selected to create a grid box around it, and the Vander waals radius of receptor atoms was scaled to 1.00 Å with a partial atomic charge of 0.25.

20 compounds of **Series-IIa** and 28 compounds of **Series-IIb** are incorporated in docking studies with the protein of ***human MTHFS with 10-formyltetrahydrofolate*** **(PDB id: *3HY3*).** The compounds **II-7S-1n [X=S; R=CH_3; R1=H; R_2=H and R_3=Cl]; II-7S-1q [X=S;R=CH_3; R_1=H; R_2=CF_3 and R_3=H]** and **II-7S-1c [X=S; R=OCH_3; R_1=H; R_2=H** and **R_3=H]** of **Series-IIa** showed potent docking scores from -10.98, -10.75 and -10.25 with hydrogen bond interactions Leu56, Leu56 and Leu56, Lys150 one, one, and two respectively. The compounds **II-7S-1i [X=S; R=OCH_3; R_1=H; R_2=Cl** and **R_3=H]**; **II-7S-1k [X=S;R=CH_3;R_1=H; R_2=H** and **R_3= CH_3]** and **II-7S-1m [X=S; R=H; R_1=H, R_2=H** and **R_3=Cl]** of **Series-IIa** showed least potent docking scores with -8.58, -8.56 and -8.55 with one hydrogen bond interaction.

The compounds **II-7O-1d [X=O;R=H; R_1=CH_3;R_2=H** and **R_3=H]; II-7O-1h [X=O; R=CH_3; R_1=H; R_2=Cl** and **R_3 =H]** and **II-7O-1g [X=O; R=H; R_1=H; R_2=Cl** and **R_3=H]** of Series-IIb showed potent docking scores from -10.24,-10.21 and -9.55 with hydrogen bond interactions Leu56, Leu56 and Tyr83 one, one and one respectively. The compounds **II-7O-1f [X=O;R=OCH_3;R_1=CH_3; R_2=H** and **R_3=H]; II-7O-1s [X=O; R=H; R_1=H; R_2=Cl** and **R_3=Cl]** and **II-7O-1n [X=O; R=CH_3; R_1=H; R_2=H** and **R_3=Cl]** of Series-IIb showed least potent docking score with -8.04, with one hydrogen bond interaction Tyr83, -7.94 with no hydrogen bond interaction and -7.89 with one hydrogen bond interaction Tyr83 respectively. The results of Docking Studies of Series II (IIa and IIb) were given in **Table No: 4** and **5** and **Figure No: 2** and **3** respectively.

Results and Discussion:

In the present investigation the titiled compounds aryloxy benzothiazoles/benzoxazoles **(II-7S-1a-x)** and **(II-7S-2a-o) / (II-7O-1a-x)** and **(II-7O-2a-p)** were synthesized by conventional method and evaluated for their anticancer activity. Further the molecular docking studies were performed to know extent of interactions with the targeted protein molecules. In this regard, the computational docking studies were conducted using AUTODOCK 4.2.

Six compounds each of Series-IIa & IIb (Table No: 3.) showed less cytotoxicity against MCF-7 (breast cancer) and MCF-10A (Normal breast epithelial cells) with IC50 between 9.40±2.47->100 µM compared to 1.86±1.77 and 1.86±0.31 µM shown by the standard cisplatin. All the twelve compounds failed to inhibit the growth of MCF-10A cells at the highest concentration tested (100 µM). Compound **II-7S-2m [X=S;R=NO_2;R_1=CH_3;R_2=$CH_2OCH_3$and R_3=Cl]** and **II-7O-2i [X=O; R= Cl; R_1=H;R_2=H and R_3=Cl]** showed potent activity with IC_{50} of 9.70±2.47 and 11.5±1.45µM against MCF-7 cells. Compound **II-7S-1v** (X=S; R=H; R_1= H; R_2=CH_2COCH_3) and **II-7O-1v** (X=O; R=H; R_1=H;R_2=CH_2COCH_3) showed least toxicity with IC_{50} of 20.37±2.47 and 24.05±2.62 µM against MCF-7 cells.

20 compounds of **Series-IIa** and 28 compounds of **Series-IIb** are incorporated in docking studies with the protein of ***human MTHFS with 10-formyltetrahydrofolate*** **(PDB id: *3HY3*).** The compounds **II-7S-1n [X=S; R=CH_3; R1=H; R_2=H and R_3=Cl]; II-7S-1q [X=S;R=CH_3; R_1=H; R_2=CF_3 and R_3=H]** and **II-7S-1c [X=S; R=OCH_3; R_1=H; R_2=H** and **R_3=H]** of **Series-IIa** showed potent docking scores from -10.98, -10.75 and -10.25 with hydrogen bond interactions Leu56, Leu56 and Leu56, Lys150 one, one, and two respectively. The compounds **II-7S-1i [X=S; R=OCH_3; R_1=H; R_2=Cl** and **R_3=H]**; **II-7S-1k [X=S;R=CH_3;R_1=H; R_2=H** and **R_3= CH_3]** and **II-7S-1m [X=S; R=H; R_1=H, R_2=H** and **R_3=Cl]** of **Series-IIa** showed least potent docking scores with -8.58, -8.56 and -8.55 with one hydrogen bond interaction.

The compounds **II-7O-1d [X=O;R=H; R_1=CH_3;R_2=H** and **R_3=H]; II-7O-1h [X=O; R=CH_3; R_1=H; R_2=Cl** and **R_3 =H]** and **II-7O-1g [X=O; R=H; R_1=H; R_2=Cl** and **R_3=H]** of Series-IIb showed potent docking scores from -10.24,-10.21 and -9.55 with hydrogen bond interactions Leu56, Leu56 and Tyr83 one, one and one respectively. The compounds **II-7O-1f [X=O;R=OCH_3;R_1=CH_3; R_2=H** and **R_3=H]; II-7O-1s [X=O; R=H; R_1=H; R_2=Cl** and **R_3=Cl]** and **II-7O-1n [X=O; R=CH_3; R_1=H; R_2=H** and **R_3=Cl]** of Series-IIb showed least potent docking score with -8.04, with one hydrogen bond interaction Tyr83, -7.94 with no hydrogen bond interaction and -7.89 with one hydrogen bond interaction Tyr83 respectively. All the above results indicated that subtituents at 6^{th} position with benzothiazole-phenoxybenzaldehyde derivatives linked with hydrazine bridge have shown potent compounds when compared to substituents at 6^{th} position benzoxazole-phenoxybenzaldehyde derivatives linked with hydrazine bridge.

Conclusion:

In the present investigation the titiled compounds aryloxy benzothiazoles/benzoxazoles **(II-7S-1a-x)** and **(II-7S-2a-o) / (II-7O-1a-x)** and **(II-7O-2a-p)** were synthesized by conventional method and evaluated for their anticancer activity. Further the molecular docking studies were performed to know extent of interactions with the targeted protein molecules. In this regard, the computational docking studies were conducted using AUTODOCK 4.2.

Six compounds each of Series-IIa & IIb (Table: 3.2.4) showed less cytotoxicity against MCF-7 (breast cancer) and MCF-10A (Normal breast epithelial cells) with IC50 between 9.40±2.47->100 µM compared to 1.86±1.77 and 1.86±0.31 µM shown by the standard cisplatin. All the twelve compounds failed to inhibit the growth of MCF-10A cells at the highest concentration tested (100 µM). Compound **II-7S-2m [X=S;R=NO_2;R_1=CH_3;R_2=$CH_2OCH_3$and R_3=Cl]** and **II-7O-2i [X=O; R= Cl; R_1=H;R_2=H and R_3=Cl]** showed potent activity with IC_{50} of 9.70±2.47 and 11.5±1.45µM against MCF-7 cells.

20 compounds of **Series-IIa** and 28 compounds of **Series-IIb** are incorporated in docking studies with the protein of ***human MTHFS with 10-formyltetrahydrofolate*** **(PDB id: *3HY3*).** The compounds **II-7S-1n [X=S; R=CH_3; R1=H; R_2=H and R_3=Cl]; II-7S-1q [X=S;R=CH_3; R_1=H; R_2=CF_3 and R_3=H]** and **II-7S-1c [X=S; R=OCH_3; R_1=H; R_2=H** and **R_3=H]** of **Series-IIa** showed potent docking scores from -10.98, -10.75 and -10.25 with hydrogen bond interactions Leu56, Leu56 and Leu56, Lys150 one, one, and two respectively.

The compounds **II-7O-1d [X=O;R=H; R_1=CH_3;R_2=H** and **R_3=H]; II-7O-1h [X=O; R=CH_3; R_1=H; R_2=Cl** and **R_3 =H]** and **II-7O-1g [X=O; R=H; R_1=H; R_2=Cl** and **R_3=H]** of Series-IIb showed potent docking scores from -10.24,-10.21 and -9.55 with hydrogen bond interactions Leu56, Leu56 and Tyr83 one, one

and one respectively, indicating that subtituents at 6th position with benzothiazole-phenoxybenzaldehyde derivatives linked with hydrazine bridge have shown potent compounds when compared to substituents at 6th position benzoxazole-phenoxybenzaldehyde derivatives linked with hydrazine bridge.

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 70eV (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 0.06-0.20mm)

Spectral Data of Aryloxy benzylidene hydrazinyl benzothiazoles / benzoxazoles (II-7S-1a-x) to (II-7S-2a-o) δ (II-7O-1a-x) to (II-7O-2a-p)

Appendix-A: Spectral dataof Scheme-II (Series-IIa).

1.2-(2-(4′-phenoxybenzylidene)-hydrazinyl)-benzo[d]thiazole (II-7S-1a)

Molecular Formula: $C_{20}H_{15}N_3OS$ **M.wt**:345.

This compound was obtained as a brown solid in 71% yield. M.p: 210-212ºC

Solubility: DMSO **Rf:**Hexane:Ethylacetate-8:2

IR (KBr, ῡ, cm-1):1306 (C-O), 1533 (C=C), 1590 (C=N)

1H-NMR: (400 Hz; DMSO-d6) δ (ppm): 6.62-7.92(m, 14H, NH, ArH-4, H-5, H-6, H 7, H-2′, H-3′, H-5′, H-6′, H-2″, H-3″, H-4″, H-5″ &H-6″. C-NH,7.08 D2O Exchangeable), 8.67 (s, 1H, CH=N)

Mass (m/z) ESI-MS:$[M+1]^+$ peak is observed at 346.0.

2.2-(2-(4′-(o-tolyloxy)-benzylidene)-hydrazinyl)-benzo[d]thiazole (II-7S-1d)

Molecular Formula: $C_{21}H_{17}N_3OS$ **M.wt:** 359.

This compound was obtained as alight brown solid in 76%yield.M.p.210 212ºC

Solubility: DMSO **Rf**:Hexane:Ethylacetate-9:1

IR (KBr, ῡ, cm-1):1307 (C-O), 1533 (C=C), 1590 (C=N), 2924 (aliphatic C-H), 3447 (NH) **1H-NMR: (400Hz; DMSO-d6)δ (ppm):** 2.38 (s, 3H, CH3),6.95-6.98 (d, 4H, J=8.4, Ar H-2′,H-3′, H-5′ &H-6′), 7.13-7.17 (t, 4H, J= 8.4, Ar, H-5, H-6, H-4″ &H-5″), 7.29-7.42 (m, 6H, ArH-4, H-7, H-2″,H-3″, H-6″, CH=N), 10.70 (s, 1H, C-NH)

Mass (m/z) ESI-MS: $[M+1]^+$ peak is observed at 360.1.

Elemental analysis: Molecular formula: $C_{21}H_{17}N_3OS$ (359.0). **Calculated:** C, 69.54 %, H, 4.38 %, N, 12.17 %, O, 4.63%, S, 9.28 %. **Found:** C, 69.62 %, H, 4.42 %, O,4.72%, N, 12.19 %, S, 9.32 %.

3.6-methyl-2-(2-(4′-(o-tolyloxy)-benzylidene)-hydrazinyl)-benzo[d]thiazole (II-7S-1e)
Molecular Formula: $C_{22}H_{19}N_3OS$ M.wt: 373.

This compound was obtained as a brown solid in 71% yield. M.p.320-322ºC

Solubility: $CDCl_3$ **Rf:** Hexane:Ethylacetate-9:1

IR (KBr, ῡ, cm-1):1632 (C=N), 2952 (aliphatic, C-H), 3427 (NH)

1H-NMR: (400 Hz; CDCl3)δ (ppm): 2.21 (s, 3H, CH3), 2.36 (s, 3H, CH3), 6.88-6.90 (d, 2H, J=8.8, Ar H-3′ &H-5′), 6.95-6.97 (d, 1H, J=8.0, Ar H-3″), 7.10-7.13 (t, 1H, J=6.4, Ar H-4″), 7.16-7.20 (t, 1H, J=8.4, Ar H-5″), 7.28 (s, 1H, C-NH), 7.31-7.32 (d, 1H, J=4.0, Ar H-6″), 7.40-7.42 (d, 2H, J=8.4, Ar H-2′, H-6′), 7.58-7.60 (d, 2H, J=8.8, Ar H-4 &H-5), 7.77 (s, 1H, Ar-H, H-7), 9.68 (s, 1H, CH=N)

Mass (m/z) ESI-MS:$[M+1]^+$ peak is observed at 374.0

4.2-(2-(4′-(3″-Chlorophenoxy)-benzylidene)-hydrazinyl)-6-methylbenzo[d]thiazole (II-7S-1h)

Molecular Formula: $C_{21}H_{16}ClN_3OS$ **M.wt:** 393.

This compound was obtained as a brown solid in 70% yield. M.p. 90-92ºC

Solubility: DMSO **Rf:**Hexane: Diethylether-5:5

IR (KBr, ῡ, cm-1):662 (C-Cl), 1651 (C=N), 2942 (aliphatic, CH), 3026 (aromatic, CH), 3335 (NH)

1H-NMR: (400 Hz; DMSO-d6)δ(ppm): 2.91 (s, 3H, CH3), 6.76 (s, 1H, ArH-2″), 7.05-7.27 (m, 7H, C-NH, ArH-4, H-5, H-7, H-4″, H-5 ″, H-6″,C-NH-D2O Exchangeable), 7.42-7.45 (d, 1H, J=6.9, Ar H-3′), 7.62-7.64 (d, 1H, J=7.2, ArH-5′), 7.89-7.92 (d, 2H, J=6.9, Ar H-2′, H-6′), 8.6 (s, 1H, CH).Signal at δ 7.18(s, 1H, C-NH) is absent (D2O Exchangeable) 13C NMR: (100 MHz, CDCl3δ ppm): 101.28, 114.02, 124.48, 125.27, 125.88, 126.73, 128.14, 128.56, 129.09, 129.35, 131.08, 132.54, 142.08, 152.64, 157.81.

Mass (m/z) ESI-MS:$[M]^+$and $[M+2]^+$ peak observed at 393.0 and 395.0.

5.2-(2-(4′-(p-tolyloxy)-benzylidene)-hydrazinyl)-benzo[d]thiazole (II-7S-1j)

Molecular Formula: $C_{21}H_{17}N_3OS$ **M.wt**:359.

This compound was obtained as a brown solid in 74% yield. M.p: 110 113ºC

Solubility: DMSO **Rf:**Hexane: Ethylacetate-8:2

IR (KBr, ῡ, cm-1):1632 (C=N), 2923 (aliphatic, C-H), 3449 (NH)

1H-NMR: (400 Hz; DMSO-d6)δ (ppm):2.49 (s, 3H, CH3), 6.71-6.76 (d, 1H, J=8.4, Ar-H, H-2″), 7.02-7.27 (m, 12H, C-NH, ArH-3″, H-5″, H-6″, H-2′, H-3′, H-5′, H-6′, H 4, H-5, H-6 &H-7), 8.35 (s, 1H, CH=N)

Mass (m/z) ESI-MS: [M+1]+ is observed at 360.1.

6.6-methyl-2-(2-(4′-(p-tolyloxy)-benzylidene)-hydrazinyl)-benzo[d]thiazole (II-7S-1k)
Molecular Formula: $C_{22}H_{19}N_3OS$ M.wt: 374.

This compound was obtained as a brown solid in 70% yield.M.p. 103-105ºC

Solubility: CDCl3 **Rf:**Hexane:Ethylacetate-8:2

IR (KBr, ῡ, cm-1):1550 (C=C), 1633 (C=N), 3437 (NH)

1H-NMR: (400 Hz; CDCl3)δ (ppm):2.37 (s, 6H, CH3), 6.95-7.05 (m, 6H, Ar-H, C NH, H-2″, H-3″, H-5″, H-6″, H-7), 7.20-7.22 (d, 4H, J=4.4, Ar H-2′, H-3′, H-5′ &H 6′), 7.95-7.98 (d, 2H, J=8.4, Ar H-4 &H-5), 8.97 (s, 1H, CH=N)

Mass (m/z) ESI-MS: $[M+1]^+$ peak is observed at 375.0.

7.6-methoxy-2-(2-(4′-(p-tolyloxy)-benzylidene)-hydrazinyl)-benzo[d]thiazole (II-7S-1l)

Molecular Formula: $C_{22}H_{19}N_3O_2S$ M.wt: 389.

This compound was obtained as a creamish solid in 71% yield. M.p. 160 162ºC

Solubility: DMSO **Rf**:Hexane:Ethylacetate-7:3

IR (KBr, ῡ, cm-1):1463 (C=N), 2924 (aliphatic, C-H), 3438 (NH)

1H-NMR: (400 Hz; DMSO-d6)δ (ppm):2.50 (s, 3H, CH3), 3.33 (s, 3H, OCH3), 7.09 7.12 (d, 10H, J=8.4,Ar H-2″, H-3″, H-5″, H-6″, H-2′, H-3′, H-5′, H-6′, H-4 & H 5), 7.90 (s, 2H, C-NH Ar H-7), 9.91 (s, 1H, CH=N)

Mass (m/z) ESI-MS:$[M+1]^+$peak is observed at 390.0.

8.2-(2-(4′-(3″,4″-dichlorophenoxy)-benzylidene)-hydrazinyl)-6 methoxybenzo[d]thiazole (II-7S-1u)

Molecular Formula: $C_{21}H_{15}Cl_2N_3O_2S$ M.wt: 444.

This compound was obtained as a brown solid in 71% yield. M.p. 95-98ºC

Solubility: DMSO **Rf:**Hexane: Ethylacetate-7:3

IR (KBr, ῡ, cm-1):1603 (C=N), 2923 (aliphatic, C-H), 3414 (NH)

1H-NMR: (400 Hz; DMSO-d6)δ (ppm): 3.53 (s, 3H, OCH3), 6.39-6.42 (d, 1H, J=8.4, Ar H-3′), 7.24-7.26 (d, 2H, J=8, Ar H-5″ &H-6″), 7.34 (s, 2H, C-NH, ArH-2″), 7.42 7.52 (m, 3H, ArH-4, H-5 &H-7), 7.60-7.63 (d, 3H, J=8.4, Ar H-2′, H-5′ &H-6′), 8.51 (s, 1H, CH=N)

Mass (m/z) ESI-MS:$[M]^+$, $[M+2]^+$ and $[M+4]^+$ peak is observed at 444.0, 446.0 and 448.0.

9.6-nitro-2-(2-(4′-phenoxybenzylidene)-hydrazinyl)-benzo[d]thiazole (II-7S-2b)

Molecular Formula:$C_{20}H_{14}N_4O_3S$ **M.wt:** 390.

This compound was obtained as a brown solid in 75% yield. M.P. 200 203ºC

Solubility: CDCl3 **Rf:**Hexane: Ethylacetate-8:2

IR (KBr, ῡ, cm-1):1632 (C=N), 3361 (NH)

1H-NMR: (400 Hz;CDCl3)δ (ppm): 7.02-7.11 (m, 6H, C-NH, Ar H-2″, H-3″, H-4″, H 5″ &H-6″), 7.39-7.43 (m, 3H, Ar H-4, H-5 &H-7), 7.82-7.84 (d, 2H, J=8.0, Ar H-2′ &H-6′), 8.05-8.08(d, 2H, J=8.4, Ar H-3′ &H-5′), 8.65 (s, 1H, CH=N)

Mass (m/z) ESI-MS: $[M+1]^+$ peak is observed at 391.1.

10. 6-nitro-2-(2-(4-(o-tolyloxy)-benzylidene)-hydrazinyl)-benzo[d]thiazole (II-7S-2c)

Molecular Formula: $C_{21}H_{16}N_4O_3S$ **M.wt:**404.

This compound was obtained as a dark brown solid in 72% yield. M.p.200 203ºC

Solubility: CDCl3 **Rf**:Hexane: Ethylacetate-9:1

IR (KBr, ῡ, cm-1):652 (C-Cl), 1631 (C=N), 3447 (NH)

1H-NMR: (400 Hz; CDCl3)δ (ppm): 3.11 (s, 3H, CH3), 7.05-7.13 (m, 5H, C-NH, ArH-3″, H-4″, H-5″, H- 6″), 7.25-7.27 (m, 2H, Ar H-5 &H-7), 7.30-7.31 (d, 1H, J = 4.0, ArH-4), 7.46-7.48 (d, 3H,J=8.0, H-2′, H-3′ &H-5′), 7.58-7.59 (d, 1H, J=4.0, H-6′), 8.65 (s,1H,CH=N) **Mass (m/z) ESI-MS:** $[M+1]^+$ peak is observed at 405.1.

11.6-chloro-2-(2-(4′-(4″-chlorophenoxy)-benzylidene)-hydrazinyl)-benzo[d]thiazole (II-7S-2d)

Molecular Formula: $C_{20}H_{13}Cl_2N_3OS$ **M.wt:** 414.

This compound was obtained as a brown solid in 71% yield. M.p. 180 182ºC **Solubility:**DMSO **Rf:**Hexane:Ethylacetate-8:2

IR(KBr,ῡ,cm-1): 1242 (C-O), 1609 (C=N), 1609 (C=C), 2924 (aliphatic, C-H)

1H-NMR: (400 Hz; DMSO-d6)δ (ppm):7.04-7.09 (m, 6H, Ar-H, N-H, H-2″, 4″, 5″, 6″,7, Benzothiazole-NH), 7.34-7.42 (d,3H, J=4, Ar-H, H-4,5, 5′), 7.83-7.90(d, 3H, J=4, Ar-H, H-2′, 3′, 6′), 8.65 (s, 1H, C-H).

Mass (m/z) ESI-MS: $[M]^+$, $[M+2]^+$ and $[M+4]^+$ observed at 414.0, 416.0 and 418.0.

11. 6-nitro-2-(2-(4′-(p-tolyloxy)-benzylidene)-hydrazinyl)-benzo[d]thiazole (II-7S 2g)

Molecular Formula: $C_{21}H_{16}N_4O_3S$ **M.wt:** 404.

This compound was obtained as a creamish yellow solid in 77% yield. M.p. 165-167ºC **Solubility:** CDCl3 **Rf:**Hexane:Ethylacetate-7:3

IR (KBr,ῡ,cm-1): 612 (C-Cl), 1603 (C=N), 2921 (aliphatic, C-H), 3448 (NH, amine)

1H-NMR: (400 Hz; CDCl3)δ (ppm):2.37 (s, 3H, CH3), 6.95-6.99 (d, 3H, J=12.4, Ar H-2″, H-3″&H-5″), 7.02-7.04 (d, 2H, J= 8.8, Ar H-5′ &H-6″), 7.17-7.22 (m, 3H, C NH,Ar H-2′ &H-H-3′), 7.76-7.78 (d, 1H, J=8.8, H-6′), 7.81-7.83 (d, 2H, J=8.8, H-4 & H-5), 8.61 (s,1H, Ar, H-7), 9.91 (s, 1H, CH=N)

13C-NMR:(400 Hz; CDCl3): δ 32, 117.053, 117.331, 117.560, 119.729, 120.237, 121.967, 127.012, 130.579, 130.718, 131.932, 132.593, 134.226, 152.100, 154.012 155.470, 159.314, 160.474, 166.474, 167.474, 191.487.

Mass (m/z) ESI-MS: $[M+1]^+$ peak is observed at 405.0.

13.6-chloro-2-(2-(4′-(p-chlorophenoxy)-benzylidene)-hydrazinyl)-benzo[d]thiazole (II-7S-2h)

Molecular Formula: $C_{20}H_{13}Cl_2N_3OS$ **M.wt:** 414.

This compound was obtained as a brown solid in 72% yield. M.p: 95-98ºC

Solubility: DMSO **Rf:**Hexane: Ethylacatate-7:3

IR (KBr, ῡ,cm-1): 1240 (C-O), 1610 (C=N), 1606 (C=C), 2923 (aliphatic, C-H)

1H-NMR: (400 Hz; DMSO-d6) δ (ppm): 2.29 (s, 3H, CH3), 6.57-6.60 (d, 2H, J=8.4, ArH-2″ &H-6″), 6.97-7.32(m, 7H, NH, Ar H-2′, H-3′, H-5′, H-6′, H-3″, H-5″ &C-NH, D2O Exchangeable), 7.50 (s, 1H, CH=N), 7.79-7.94 (m, 3H, Ar H-4, H-5 &H-7)

Mass (m/z) ESI-MS: $[M]^+$, $[M+2]^+$, 418.2 $[M+4]^+$ peaks observed at 414.0, 416.2 and 418.2.

14.6-chloro-2-(2-(4′-(3″-trifluoromethyl)-phenoxy)-benzylidene)-hydrazinyl) benzo[d]thiazole (II-7S-2j)

Molecular Formula: $C_{21}H_{13}ClF_3N_3OS$ **M.wt:** 448.

This compound was obtained as a creamish solid in 71% yield. M.p.255 257ºC

Solubility: DMSO **Rf:**Hexane:Ethylacetate-5:5

IR (KBr, ῡ,cm-1): 1260 (C-O), 1536 (C=C), 1585 (C=N), 3303 (NH, amine)

1H-NMR: (400 Hz; DMSO-d6) δ (ppm):7.00-7.06 (m, 3H, C-NH, Ar H-4″ & 5″), 7.42-7.43 (d, 2H, J = 4.8, Ar H-3′ &H-5′), 7.48-7.51 (d, 3H, J = 8.8, Ar H-6′,H- 2″ &H-6″), 7.89-7.91 (d, 3H,J = 8.4, H-4, H-5 &H-2′), 8.30 (s, 1H, Ar H-7), 8.70 (s, 1H, CH=N)

Mass (m/z) ESI-MS:$[M]^+$ and $[M+2]^+$peaks observed at 448.0 and 450.35.

15.6-nitro-2-(2-(4′-(3″-(trifluoromethyl)-phenoxy)-benzylidene)-hydrazinyl) benzo[d]thiazole (II-7S-2k)

Molecular Formula: $C_{21}H_{13}F_3N_4O_3S$ **M.wt:** 458.

This compound was obtained as a light brown solid in 72% yield. M.p. 230-232ºC

Solubility: DMSO **Rf:**Hexane: Ethylacetate-9:1

IR (KBr,ῡ,cm-1): 1239 (C-O), 1611 (C=N), 1605 (C=C), 2920 (aliphatic, C-H), 3409 (NH, amine)

1H-NMR: (400 Hz; DMSO-d6)δ (ppm): 7.18-7.26 (m, 7H, ArH- 7, H-3′,H- 5′, H-2″, H-4″, H-5″ &6″), 7.58-7.74 (m, 6H, C-NH, ArH-4, H-5, H-2′, H-6′ &CH=N, C-NH D_2O Exchangeable)

Mass (m/z) ESI-MS: $[M+1]^+$ is observed at 459.0.

16.6-chloro-2-(2-(4′-(3″,4″-dichlorophenoxy)-benzylidene)-hydrazinyl) benzo[d]thiazole (II-7S-2l)

Molecular Formula: $C_{20}H_{12}Cl_3N_3OS$ M.wt: 449.

This compound was obtained as a yellow solid in 67% yield. M.p. 250 253ºC

Solubility: CDCl3 **Rf:** Hexane:Ethylacetate-7:3

IR (KBr, ῡ, cm-1): 1632 (C=N), 3416 (NH, amine)

1H-NMR: (400 Hz; CDCl3) δ (ppm): 7.04-7.09 (m, 5H, C-NH, Ar H-2″, H-5″, H-6″ & H-7), 7.34-7.37(d, 3H, J=8.4, Ar, H-4, H-5 &H-5′), 7.87-7.90 (d, 3H, J=8.4, ArH-2′, H 3′ &H-6′), 8.65 (s,1H,CH=N)

13C-NMR:(400 Hz; CDCl3): δ 117.453,117.730, 118.057, 118.514, 119.547, 120.473, 121.923, 124.319, 125.478, 128.485, 128.133, 130.212, 131.969, 132.615, 152.100, 155.470, 160.474, 166.474, 167.474, 191.487

Mass (m/z) ESI-MS:$[M]^+$,$[M+2]^+$, $[M+4]^+$ and $[M+6]^+$ peaks observed at 449.0, 451.0, 453.0 and 455.0.

17.2-(2-(4-(3-(methoxymethyl)-2-methylphenoxy)benzylidene)hydrazinyl)-6 nitrobenzo[d]thiazole (II-7S-2m)

Molecular Formula: $C_{20}H_{12}Cl_3N_3OS$ **M.wt:** 448.

This compound was obtained as a brown solid in 72% yield. M.p.200-203ºC

Solubility: DMSO **Rf:** Hexane:Ethylacetate-7:3

IR (KBr,ῡ,cm-1): 747 (C-Cl), 1537 (C=C), 1593 (C=N), 3367 (NH)

1H-NMR: (300 Hz; DMSO-d6) δ (ppm). 7.30-7.33 (d, 4H, J=8.1, Ar H-2′, H-3′, H 5′&H-6′), 7.51-7.69 (m, 6H, Ar H-4, H-5, H-7, H-2″, H-5″ &H-6″), 8.70 (s, 1H, CH=N), 10.10 (s, 1H,C-NH, D2O Exchangeable)

Mass (m/z)ESI-MS: $[M+1]^+$observed at 449.0.

18.6-nitro-2-(2-(4′-phenoxybenzylidene)-hydrazinyl)-benzo[d]thiazole (II-7S-2o)
Molecular Formula: $C_{20}H_{14}N_4O_3S$ **M.wt:** 449.

This compound was obtained as a brown solid in 74% yield. **M.p:** 115 118ºC

Solubility: DMSO **Rf**:Hexane: Ethylacetate-9:4.5

IR (KBr,ῡ,cm-1): 1520 (C=C), 2920 (aliphatic, C-H), 3359 (NH)

1H-NMR: (400 Hz; DMSO-d6) δ (ppm): 6.73(s, 2H, Ar H-2″ &H-7), 7.04-7.81 (m, 9H, Ar H-4, H-5, H-2′, H-3′, H-5′, H-6′, H-4″, H-6″ &C-NH, C-NH-D2O Exchangeable), 8.48 (s, 1H, CH=N)

Mass (m/z) ESI-MS: $[M+1]^+$observed at 450.0.

Appendix-B : Spectral data of Scheme-II Series-IIb:

1. 2-(2-(4′-phenoxybenzylidene)-hydrazinyl)-benzo[d]oxazole (II-7O-1a)

Molecular Formula: $C_{20}H_{15}N_3O_2$ **M.wt:** 329.

This compound was obtained as a brown solid in 71%yield. M.p 210-212ºC

Solubility: DMSO **Rf:** Hexane: Ethylacetate-8:2

IR (KBr, ῡ,cm-1): 1543 (C=C), 3086 (aromatic, C-H), 3313 (NH)

1H-NMR: (300 Hz; DMSO-d6) δ (ppm): 3.5 (s, 1H, C-NH), 6.95- 6.99 (m, 4H, Ar H 2′, H-3′, H-5′ & H-6′), 7.02-7.30 (m, 9H, Ar H-2″, H-3 ″, H-4″, H-5″, H-6″, H-4, H-5, H-6 & H-7), 8.48 (s, 1H, CH=N)

Mass (m/z) ESI-MS:$[M+1]^+$peak observed at 330.0.

Elemental Analysis: Molecular formula: $C_{20}H_{15}N_3O_2$ **Mol.wt:** 329.0 **Calculated:** C, 72.94 %, H, 4.59 %, N, 12.76 %, O, 9.72 %. **Found:** C, 72.99 %, H, 4.63 %, N, 12.85 %, O, 9.80 %

2.6-methyl-2-(2-(4′-phenoxybenzylidene)-hydrazinyl)-benzo[d]oxazole(II-7O-1b)
Molecular Formula: $C_{21}H_{17}N_3O_2$ **M.wt:** 343.

This compound was obtained as a brown solid in 74% yield. **M.p.** 180 182ºC

Solubility: DMSO **Rf:** Hexane: Ethylacetate-8:2

IR (KBr, ῡ,cm-1): 1540 (C=C), 1630 (C=N), 3340 (NH)

1H-NMR: (300 Hz; DMSO-d6) δ (ppm):2.43 (s, 3H, CH3), 3.97 (s, 1H, C-NH), 7.09 7.13(t, 2H, J=6.3, Ar H-3″ &H-4″), 7.26-7.30 (d, 3H, J=9.3, Ar H-2″, H-6″ &H-3′), 7.58-7.62 (t, 1H, J=6.3, Ar H-5″), 7.94-7.96 (d, 3H, J=6.3, Ar H-2′, H-5′ &H-6′), 8.07 8.09 (d, 1H, J=6.3, ArH-4), 8.48 (s, 2H, Ar-H, CH=N, H-7), 8.92-8.94 (d, 1H, J=6.3, Ar H-5)

Mass (m/z) ESI-MS: $[M+1]^+$ peak observed at 344.0.

3.6-methoxy-2-(2-(4′-phenoxybenzylidene)-hydrazinyl)-benzo[d]oxazole (II-7O-1c)

Molecular Formula: $C_{21}H_{17}N_3O_3$ **M.wt:** 359.

This compound was obtained as a creamish solid in 70% yield.M.p: 210 212ºC

Solubility: DMSO **Rf:** Hexane: Ethylacetate-8:2

IR (KBr, ῡ,cm-1): 1514 (C=C), 1589 (C=N), 3310 (NH)

1H-NMR: (300 Hz; DMSO-d6)δ (ppm):3.83 (s, 3H, OCH3), 3.97 (s, 1H, C-NH), 6.75- 6.99 (m, 2H, Ar H-5 &H-7),7.13-7.45 (m, 7H, Ar H-3′, H-5′, H-2″, H-3″, H-4″, H-5″ & H-6″), 7.58-7.62 (d, 3H, J=9.3, Ar H-4, H-2′ &H-6′), 8.49 (s, 1H, CH=N)

Mass (m/z) ESI-MS: $[M+1]^+$ peak is observed at 360.0.

4.6-methyl-2-(2-(4′-(o-tolyloxy)-benzylidene)-hydrazinyl)-benzo[d]oxazole (II-7O-1e)
Molecular Formula: $C_{22}H_{19}N_3O_2$ M.wt: 357.

This compound was obtained as a creamish solidin 71% yield. M.p: 320 322ºC

Solubility: DMSO **Rf:** Hexane: Ethylacetate-9:1

IR (KBr, ῡ,cm-1): 1308 (C-O), 1609 (C=N) 3355 (NH)

1H-NMR: (300 Hz; DMSO-d6) δ (ppm): 2.48(s, 6H, CH3), 3.97 (s, 1H, C-NH), 7.02 7.05 (d, 6H, J=7.5, Ar H-2′, H-3′, H-5′, H-6′, H-3″&H-6″), 7.43-7.46 (t, 2H, J=7.5,Ar H, H-4″ &H-5″), 7.85-7.94 (m, 3H, Ar H-4, H-5 &H-7), 8.69 (s, 1H, CH=N).

Mass (m/z) ESI-MS: $[M+1]^+$ peak is observed at 358.0.

5. 6-methoxy-2-(2-(4′-(o-tolyloxy)-benzylidene)-hydrazinyl)-benzo[d]oxazole(II-7O-1f) Molecular Formula: $C_{22}H_{19}N_3O_3$ **M.wt:**373.

This compound was obtained as a creamish colour solid in 77% yield. **M.p.**180-182ºC **Solubility:** DMSO **Rf:** Hexane: Ethylacetate-9:1

IR (KBr, ῡ,cm-1): 1540 (C=C), 3085 (aromatic, C-H), 2718 (C-H), 3311 (NH)

1H-NMR (400 Hz; CDCl3)δ (ppm): 2.43 (s, 3H, CH3), 3.86 (s,3H, OCH3), 3.99 (s, 1H, C-NH), 6.67-6.68 (d, 1H, J=5.6, Ar H-5), 6.71-6.75 (t, 1H, J=8.4, Ar H-4″), 6.95-6.98 (t, 1H, J=8.4, ArH-5″), 7.11-7.13 (d, 1H, J=8.4, ArH-3″), 7.81-7.84 (d, 1H, J=8.4, Ar H 3′), 8.17-8.19 (d, 1H, J=8.4, Ar H-5′), 8.25-8.27 (d, 1H, J=4.4, ArH-6″), 8.29 (s, 1H,Ar H-7), 8.39-8.41 (d, 1H, J=8.4,Ar H-4), 8.52-8.53 (d, 2H, J=4.4, ArH-2′, H-6′), 8.90 (s, 1H, CH=N)

Mass (m/z) ESI-MS: $[M+1]^+$ peak is observed at 374.1.

6. 6-methyoxy-2-(2-(4′-(p-tolyloxy)-benzylidene)-hydrazinyl)-benzo[d]oxazole (II-7O-1l) Molecular Formula: $C_{22}H_{19}N_3O_3$ **M.wt:**373.

This compound was obtained as a brown solid in 74% yield. **M.p.**115-117ºC **Solubility:**DMSO **Rf:** Hexane: Ethylacetate-8:2

IR (KBr, ῡ,cm-1): 1642 (C=N), 2929 (aliphatic, CH), 3380 (NH, amine)

1H-NMR: (400 Hz; DMSO-d6)δ (ppm): 2.97 (s, 3H, CH3), 3.57(s, 3H, OCH3), 3.74 (s, 1H, C-NH), 6.71-6.72 (d, 1H, J=4.4,Ar H-2″), 6.76-6.78(d, 3H, J=8.8, Ar H-3″, H-5″ &H-6″), 7.06 (s, 1H,Ar H-7), 7.08-7.10 (d, 1H, J=8.4, Ar H-4), 7.16-7.18 (d, 2H, J=8.4, Ar H-3′ &H-5′), 7.64-7.66(d, 3H, J=8.4, H- 5, H-2′ &H-6′), 8.50 (s, 1H, CH=N)

Mass (m/z) ESI-MS:374.0 $[M+1]^+$ peak is observed at 374.0.

7.2-(2-(4′-(3″-chlorophenoxy)-benzylidene)-hydrazinyl)-6-methylbenzo[d]oxazole (II-7O-1n)

Molecular Formula:$C_{21}H_{16}ClN_3O_2$ **M.wt:**378.

This compound was obtained as a brown solid in 72% yield. **M.p:** 190 192ºC

Solubility: DMSO **Rf:** Hexane: Ethylacetate-8:2

IR (KBr,ῡ,cm-1): 699 (C-Cl), 1308 (C-O), 1609 (C=N), 3355 (NH)

1H-NMR: (300 Hz; DMSO-d6)δ (ppm): 2.30 (s, 3H, CH3), 4.85 (s, 1H, C-NH), 6.78 6.99 (m, 2H, Ar H-5 &H-7), 7.02-7.40 (m, 6H, Ar H-3′, H-5′, H-2″, H-4″, H-5″ &H-6″), 7.50-8.58 (m, 4H, Ar H-4, H-2′, H-6′ &CH=N)

Mass (m/z) ESI-MS: $[M]^+$ and$[M+2]^+$peak is observed at 378.0and 380.0

8.2-(2-(4′-(3″-chlorophenoxy)-benzylidene)-hydrazinyl)-6 methoxybenzo[d]oxazole (II-7O-1o)

Molecular Formula: $C_{21}H_{16}ClN_3O_3$ **M.wt:** 394.

This compound was obtained as a brown solid in 76% yield. **M.p:** 215-217ºC **Solubility:**DMSO **Rf:**Hexane:Ethylacetate-8:2

IR (KBr,ῡ,cm-1):1262 (C-O), 1645 (C=N), 3390 (NH)

1H-NMR: (300 Hz; DMSO-d6)δ (ppm): 3.75 (s, 3H, OCH3), 3.83 (s, 1H, C-NH), 6.72 -7.01 (m, 2H, Ar H-5 &H-7), 7.02-7.38 (m, 6H, Ar H-3′, H-5′, H-2″, H-4″, H-5″ & H 6″), 7.50-8.50 (m, 4H, H-4, 2′, 6′ &CH=N)

Mass (m/z) ESI-MS: $[M]^+$ and $[M+2]^+$ peak is observed at 394.0 and 396.0

9.6-methyl-2-(2-(4′-(3″-(trifluoromethyl)-phenoxy)-benzylidene)-hydrazinyl) benzo[d]oxazole (II-7O-1q)

Molecular Formula: $C_{22}H_{16}F_3N_3O_2$ **M.wt:** 411.0.

This compound was obtained as a creamish brown solid in 74% yield. **M.p.** 275-277ºC **Solubility:** DMSO **Rf:**Hexane: Ethylacetate-8:2

IR (KBr, ῡ,cm-1): 1571 (C=N), 2937 (aliphatic, C-H), 3319 (NH)

1H-NMR: (300 Hz; CDCl3) δ (ppm):2.35 (s, 3H, CH3), 3.86 (s, 1H, C-NH), 7.14-7.35 (m, 6H, Ar H-4, H-3′, H-5′, H-4″, H-5″ &H-6″), 7.42-7.66 (m, 5H, Ar H-5, H-7, H 2″,H-2′ &H-6′), 8.45 (s, 1H, CH=N)

Mass (m/z) ESI-MS: $[M+1]^+$peak is observed at 412.0.

10.2-(2-(4′-(3″,4″-dichlorophenoxy)-benzylidene)-hydrazinyl)-6 methylbenzo[d]oxazole (II-7O-1t)

Molecular Formula: $C_{21}H_{15}Cl_2N_3O_2$ **M.wt:** 412.0. This compound was obtained as a brown solid in 71% yield. M.p: 265 268ºC.

Solubility: DMSO **Rf**:Hexane: Ethylacetate-8:2

IR (KBr, ῡ,cm-1): 761 (C-Cl), 1598 (C-O), 1685 (C=N), 2948 (aliphatic, C-H), 3277 (NH) **1H-NMR: (300 Hz; DMSO-d6)δ (ppm):** 2.35 (s, 3H, CH3), 4.0 (s, 1H, C-NH), 7.10 7.68 (m, 8H, Ar H-4, H-5, H-7, H-3′, H-5′, H-2″, H-5″&H-6″), 7.90 - 8.50 (m, 3H, Ar H-2′, H-6′& CH=N)

Mass (m/z) ESI-MS: $[M]^+$, $[M+2]^+$ and $[M+4]^+$peaks are observed at 412.0, 414.0 and 416.0.

11.2-(2-(4′-(3″-(methoxymethyl)-phenoxy)-benzylidene)-hydrazinyl) benzo[d]oxazole (II-7O-1v)

Molecular Formula: $C_{22}H_{19}N_3O_3$ **M.wt:**398.0.

This compound was obtained as a brown solid in 72% yield. **M.p.** 129 131ºC

Solubility: DMSO **Rf:** Hexane: Ethylacetate-7:3

IR (KBr, ῡ,cm-1): 3589 (NH)

1H-NMR: (300 Hz; DMSO-d6): δ 4.5 (s, 1H, C-NH), 6.75-7.52 (m, 6H, Ar-H, H-4, H 5, H-6, H-7, H-2″,H-4″), 7.62-7.64 (s, 1H, Ar-H, H-6″), 7.80-7.89 (dd, 4H, J=6.0, J=6.3 Hz, Ar-H, H-2′, H-3′, H-5′, H-6′), 8.75 (s, 1H, C-H)

Mass (m/z) ESI-MS:$[M+1]^+$peak is observed at 399.0.

12.2-(2-(4′-(3″-(methoxymethyl)-phenoxy)-benzylidene)-hydrazinyl)-6 methylbenzo[d]oxazole (II-7O-1w)

Molecular Formula:$C_{23}H_{21}N_3O_3$ **M.wt:**387.0.

This compound was obtained as a brown solid in 68% yield. **M.p.** 220 222ºC

Solubility: DMSO **Rf:** Hexane: Ethylacetate-7:3

IR (KBr, ῡ,cm-1): 711 (C-Cl), 1262 (C-O), 1645 (C=N), 2930 (aliphatic, C-H), 3293 (NH). **1H-NMR: (300 Hz; DMSO-d6)δ (ppm):**3.75 (s, 1H, C-NH), 7.10 -7.24 (m, 5H, Ar H 4, H-3′, H-5′, H-2″&H-6″), 7.40- 7.45 (m, 4H,Ar H-5, H-6, H-7 &H-4″) 7.89- 8.48 (m, 3H, Ar H-2′, H-6′&CH=N).

Mass (m/z) ESI-MS: $[M+1]^+$peak is observed at 388.0.

12. 6-chloro-2-(2-(4′-(o-tolyloxy)-benzylidene)-hydrazinyl)-benzo[d]oxazole (II 7O-2c) Molecular Formula: $C_{21}H_{16}ClN_3O_2$ **M.wt:** 378.0.

This compound was obtained as a dark brown solid in 74% yield. **M.p:** 210-212ºC. **Solubility:**DMSO **Rf:**Hexane:Ethylacetate-8:2

IR (KBr, ῡ, cm-1): 1531 (C=C), 1637 (C=N), 2923 (aliphatic, C-H), 3256 (NH)

1H-NMR: (400 Hz; DMSO-d6) δ (ppm):2.15 (s, 3H, CH3), 3.76 (s, 1H, C-NH), 7.01 7.25 (m, 6H, Ar H-3′, H-5′, H-3″, H-4″, H-5″&H-6″), 7.27-7.91 (m, 5H, Ar H-4, H-5, H-7, H-2′ &H-6′), 8.48 (s, 1H, CH=N)

Mass (m/z) ESI-MS: $[M]^+$ and $[M+2]^+$peak is observed at 378.0 and 380.0.

13. 6-nitro-2-(2-(4′-(o-tolyloxy)-benzylidene)-hydrazinyl)-benzo[d]oxazole (II-7O 2d) Molecular Formula: $C_{21}H_{16}N_4O_4$ **M.wt**: 388.0.

This compound was obtained as a dark brown solid in 72% yield. **M.p**:200-202ºC

Solubility: DMSO **Rf:** Hexane:Ethylacetate-7:3

IR (KBr, ῡ, cm-1): 1604 (C=C), 1624 (C=N), 2924 (aliphatic, C-H), 3426 (NH)

1H-NMR: (400 Hz; DMSO-d6) δ (ppm): 2.33 (s, 3H, CH3), 4.76 (s, 1H, C-NH), 7.23-7.27 (m, 4H, Ar H-3″,H-4″, H-5″ &H-6″), 7.34-7.36 (d, 4H, J=7.6, H- 2′, H-3′,H 5′ &H-6′), 7.5 (s, 2H, ArH-7, CH=N), 7.85-7.87 (d, 2H, J=8.0, Ar H-4 &H-5)

Mass (m/z) ESI-MS: $[M+1]^+$peak is observed at 389.0.

15.6-Chloro-2-(2-(4′-(3″-Chlorophenoxy)-benzylidene)-hydrazinyl) benzo[d]oxazole (II-7O-2e)

Molecular Formula: $C_{20}H_{13}Cl_2N_3O_2$ **M.wt:** 398.0.

This compound was obtained as a yellow solid in 71% yield. **M.p:**180 182ºC

Solubility: DMSO **Rf:** Hexane:Ethylacetate-4:6

IR (KBr, ῡ, cm-1): 699 (C-Cl), 1308 (C-O), 1609 (C=N), 3355 (NH)

1H-NMR: (300 Hz;DMSO-d6)δ (ppm): 4.85 (s, 1H, C-NH), 6.69-6.76 (m, 4H, Ar H 2″, H-4″, H-5″ & H-6″), 7.30-7.34 (d, 2H, J = 9.3, Ar H-4 &H-5), 7.61-7.64 (d, 4H, J = 9.3, Ar H-2′, H-3′, H-5′&H-6′), 7.80 (s, 1H, Ar H-7), 8.5 (s, 1H, CH=N)

Mass (m/z)ESI-MS: $[M]^+$, $[M+2]^+$ and $[M+4]^+$are observed at 398.0, 400.0 and 402.1.

16.6-chloro-2-(2-(4′-(p-tolyloxy)-benzylidene)-hydrazinyl)-benzo[d]oxazole (II-7O 2g) Molecular Formula: $C_{21}H_{16}ClN_3O_2$ **M.wt:** 378.0.

This compound was obtained as a dark brown solid in 73% yield. **M.p:** 85-87ºC

Solubility: DMSO **Rf:** Hexane: Ethylacetate-4:6

IR (KBr, ῡ, cm-1): 1308 (C-O), 1609 (C=N) 3355 (NH)

1H-NMR: (300 Hz; DMSO-d6) δ (ppm): 2.48 (s, 3H, CH3), 3.85 (s, 1H, C-NH). 6.96 7.01 (d, 6H, J=12.3, Ar H-3′, H-5′,H-2″, H-3″, H-5″&H-6″), 7.27-7.58 (m, 3H,Ar H-4, H-5 &H-7), 7.85-7.87 (d, 2H, J = 6.3, Ar H-2′&H-6′), 8.69 (s, 1H, CH=N)

Mass (m/z)ESI-MS: $[M]^+$ and $[M+2]^+$peaks are observed at 378.0 and 380.0.

17.6-Chloro-2-(2-(4′-(4″-chlorophenoxy)-benzylidene)-hydrazinyl)-benzo[d]oxazole (II-7O-2i)

Molecular Formula: $C_{20}H_{13}Cl_2N_3O_2$ **M.wt:** 398.

This compound was obtained as a brown solid in 72% yield. **M.p:** 110 112ºC

Solubility: DMSO **Rf:** Hexane:Ethylacetate-4:6

IR (KBr, ῡ, cm-1): 1550 (C=C), 1607 (C=N), 3013 (aromatic, CH), 3443 (NH)

1H-NMR: (300 Hz; DMSO-d6) δ(ppm):4.7 (s, 1H, C-NH, D2O Exchangeable), 6.90 7.02 (m, 5H, Ar H-2″, H-3″, H-5″, H-6″, H-7), 7.25-7.35 (d, 4H, J=7.1,Ar, H-2′, H-3′, H-5′, H-6′), 7.48-7.51 (d, 1H, J = 8.7, Ar, H-4), 7.60-7.63 (d, 1H, J=8.7, Ar, H-5), 8.13 (s, 1H, CH=N) **13C-NMR: (400 Hz;DMSO-d6):** δ118.483, 118.639, 118.719, 118.921, 119.027, 119.635, 119.713, 120.572, 121.281, 126.042, 128.206, 128.940, 129.720, 129.917, 130.862, 138.135, 140.283, 152.985, 155.051, 157.344.

Mass (m/z)ESI-MS: $[M]^+$peak observed at 398, $[M+2]^+$ peak observed at 400 and $[M+4]^+$ peak observed at 402.

18.6-nitro-2-(2-(4′-(4″-chlorophenoxy)-benzylidene)-hydrazinyl)-6 nitrobenzo[d]oxazole (II-7O-2j)

Molecular Formula: $C_{20}H_{13}ClN_4O_4$ **M.wt:**409.0.

This compound was obtained as a creamish solid in 73% yield. **M.p:** 225-227ºC

Solubility: DMSO **Rf:** Hexane: Ethylacetate-4:6

IR (KBr, ῡ, cm-1): 1543 (C=C), 3128 (aromatic, C-H)

1H-NMR: (300 Hz; DMSO-d6)δ(ppm): 4.5 (s, 1H, C-NH, D_2O Exchangeable), 6.57-6.59 (d, 2H, J = 8.7, Ar H-3′ &H-5′), 6.70-6.73 (m, 2H, H-7, H-2″), 7.05 -7.27 (m, 3H, Ar H-3″, H 5″&H-6″), 7.42-7.44 (d, 1H, J = 7.5, ArH-4), 7.61-7.64 (d, 1H, J=7.8, Ar H-5), 7.91–7.94 (d, 2H, J = 9.3, Ar H-2′&H-6′), 8.69 (s, 1H, CH=N)

Mass (m/z) ESI-MS:$[M]^+$ and$[M+2]^+$peaks are observed at 409.0 and 411.1.

19.6-chloro-2-(2-(4′-(3″-trifluoromethyl)-phenoxy)-benzylidene)-hydrazinyl) benzo[d]oxazole (II-7O-2k)

Molecular Formula: $C_{21}H_{13}ClF_3N_3O_2$ **M.wt:** 431.

This compound was obtained as a light brown solid in 73% yield. M.p. 110-112ºC

Solubility: DMSO **Rf:** Hexane:Ethylacetate-5:5

IR (KBr, ῡ, cm-1): 1433 (N=CH), 1631 (C=N), 2929 (aliphatic, C-H), 3369 (NH)

1H-NMR: (300 Hz; CDCl3)δ (ppm):3.86 (s, 1H, C-NH), 6.91-7.07 (m, 3H, Ar H-2″,H-4″&H 5″), 7.42-7.66 (m, 5H, Ar H-6″, H-2′, H-3′, H- 5′ &H-6′), 7.97-8.00 (d, 2H, J=11.7, Ar H-4 &H-5), 8.26 (s, 2H, Ar-H, H-7, CH=N)

13C-NMR: (300Hz; CDCl3)δ (ppm):δ 108.558, 111.120, 117.412, 119.120, 119.615, 121.512, 124.412, 124.862, 128.343, 130.212, 134.412, 136.120, 138.110, 138.610, 139.572, 139.818, 148.741, 151.441, 155.841, 156.572, 157.842.

Mass (m/z)ESI-MS: $[M]^+$and $[M+2]^+$ peak is observed at 431.0 and 433.3.

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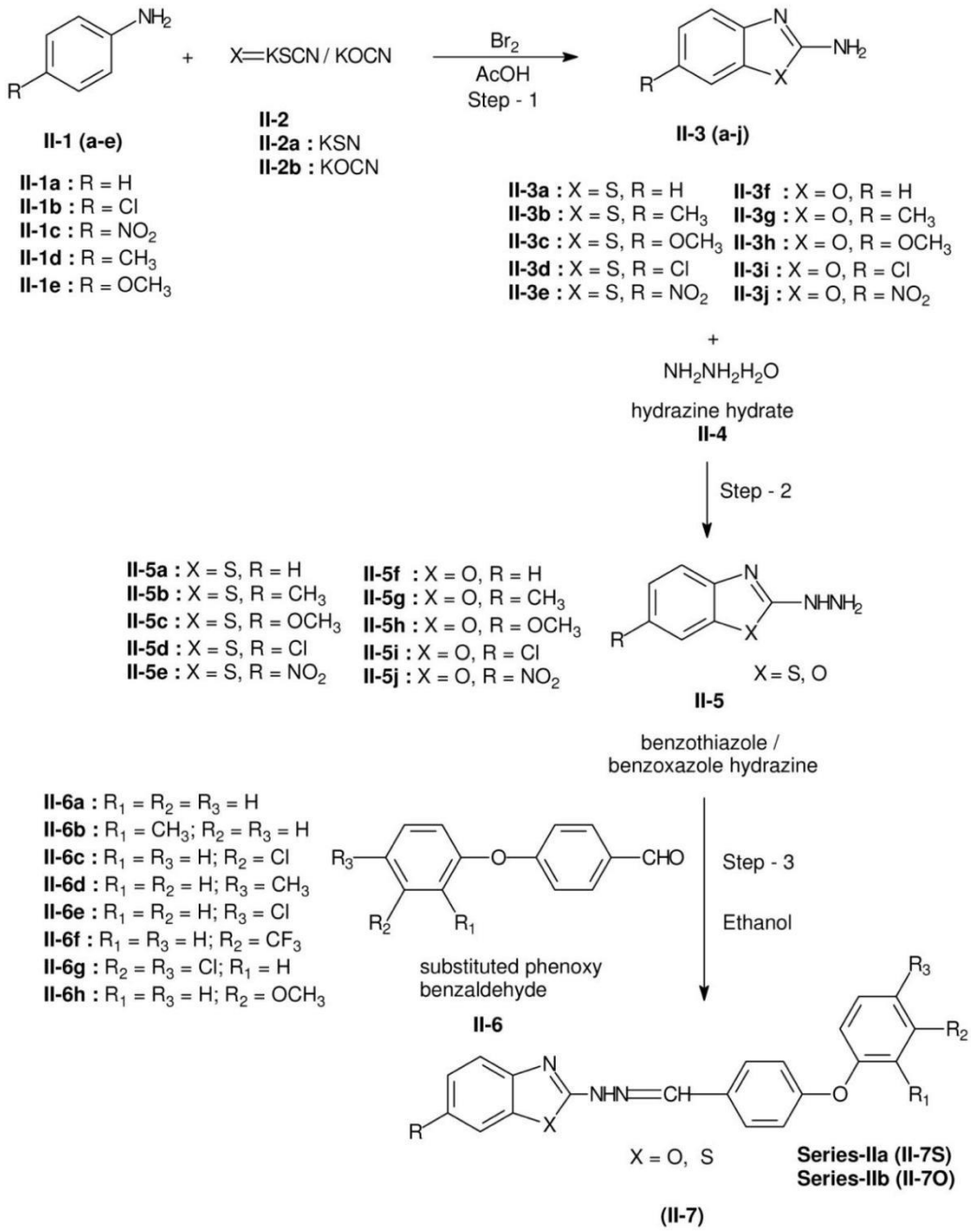
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LIST OF ABBREVIATIONS

ATP	-	Adenosine triphosphate
CAS	-	Catalytic Active Site
CDKs	-	Cyclin-Dependent Kinases
Hep G2	-	Liver Hepatocellular Carcinoma cell line
FDA	-	Food and Drug Administration
MCF-7	-	Michigan Cancer Foundation-7
MIC	-	Minimum Inhibitory Concentration
U937	-	Pro-monocytic, human myeloid leukaemia cell line
DMSO	-	Dimethylsulfoxide
DMF	-	Dimethylformamide
DPPH	-	2,2-diphenylpicrylhydrazyl
HIV	-	Human immunodeficiency virus infection
NCS	-	N-chlorosuccinimide
ROS	-	Reactive oxygen species
THF	-	Tetrahydrofuran
IEC6	-	Epithelial cells
NSCLC	-	Non-small cell lung cancer
MDA-MB 231	-	M.D.Anderson-Metastatic Breast 231
MCF10A	-	Non-tumorigenic epithelial cell line
A431	-	Model human cell line (epidermoid carcinoma)
HCC 827	-	Epithelial cell isolated from the lung of a white, 39-year-old female patient with adenocarcinoma
TLCK	-	Tosyl-L-lysyl-chloromethane hydrochloride
HEK 293	-	Immortalized human embryonic kidney cells. 293 represent Grahams experiment
BT-474	-	Cell line isolated from a solid, invasive ductal carcinoma from a breast cancer patient
BEAS-2B	-	Human bronchial epithelial cell line
DHFR	-	Dihydro folate reductase
IR	-	Infrared
NMR	-	Nuclear magnetic resonance spectroscopy
SAR	-	Structure activity relationship
MW	-	Molecular Weight
Mol	-	Moles
NMDA	-	N-methyl D-asprate
PAS	-	Peripheral Active Site
PDB	-	Protein Databank
ppm	-	Parts per million

TLC	-	Thin-layer chromatography
TMS	-	Tetramethylsilane
CJM 126	-	2-(4-aminophenyl)benzothiazole
ESI-MS	-	Electrospray ionization mass spectroscopy
(Aβ)	-	β-amyloid peptides
PBS	-	Phosphate Buffered Saline
EDTA	-	Ethelenediamino tetraacetic acid
DMSO	-	Dimethyl sulfoxide
min	-	Minutes
hrs	-	Hours
IC50	-	Half maximal inhibitory concentration
µg	-	Microgram
µM	-	Micromole
mM	-	Milli mole
Fig	-	Figure
E	-	Entgegen (Trans)
Z	-	Zusammen (Cis)
R	-	Rectus (Right-handed)
S	-	Sinister (Left-handed)
δ	-	Chemical shift (ppm)
J	-	Coupling constant
Δ	-	Heating/elevated temperature
λ	-	Wavelength
υ	-	Frequency (Hz)
ῡ	-	Wave number
s	-	singlet
d	-	doublet
dd	-	doublet of doublet
t	-	triplet
m	-	multiplet
Bs	-	broad singlet
Hz	-	Hertz
Ppm	-	Parts per million
D_2O	-	Deuterated
m/z	-	Mass/charge
gm	-	Gram
Rf	-	Retardation factor
M.p	-	Melting point
% yield	-	Percent yield
cm^{-1}	-	Per centimeter
DMSO	-	Dimethyl sulfoxide
$CDCl_3$	-	Dueterated chloroform
KBr	-	Potassium bromide

SCHEME-II

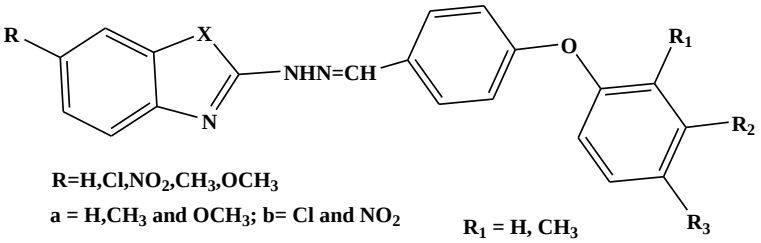


[II-7S-1a-1x; II-7S-2a-2o and II-7O-1a-1x; II-7O-2a-2p]

2-(2-(4-aryloxybenzylidene)hydrazinyl)benzo[d]thiazoles/benzo[d]oxazoles

Reagents and reaction conditions of aryloxybenzylidenehydrazinyl-benzothiazoles/benzoxazoles: ***Step-1:*** *solvent: glacial AcOH, time: 10-20 minutes, R.T.*Br_2*, time:2-4 hrs, temp: below 10ºC.* ***Step-2:*** *solvent: ethylene glycol, hydrazine hydrate, time:4 hrs.* ***Step-3:*** *solvent: ethanol, temp: 80 ºC, time 6-10 hrs.*

Table 1. Physical data of compounds. Series-IIa, (II-7S-1a-x) and (II-7S-2a-o)



R=H,Cl,NO_2,CH_3,OCH_3

a = H,CH_3 and OCH_3; b= Cl and NO_2

X= O, S

R_1 = H, CH_3

R_2 =H, Cl, CF_3, CH_2OCH_3

R_3 = H, Cl, CH_3

General structure –II

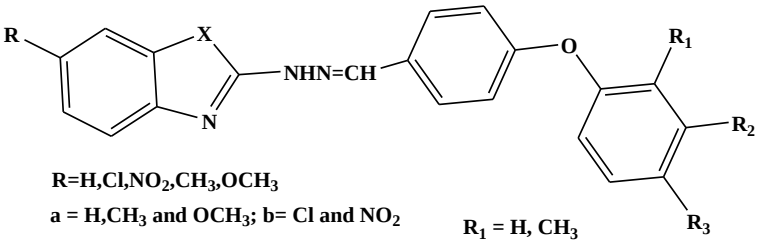
S.No	Compd	X	R	R_1	R_2	R_3	Mol.Wt	*R_f	M.p. Range^{0}C	% yield
1	**II-7S-1a**	S	H	H	H	H	345	0.68	210-212	71
2	**II-7S-1b**	S	CH_3	H	H	H	359	0.38	198-200	74
3	**II-7S-1c**	S	OCH_3	H	H	H	375	0.40	215-217	70
4	**II-7S-1d**	S	H	CH_3	H	H	359	0.62	210-212	76
5	**II-7S-1e**	S	CH_3	CH_3	H	H	373	0.91	320-322	71
6	**II-7S-1f**	S	OCH_3	CH_3	H	H	389	0.91	180-182	77
7	**II-7S-1g**	S	H	H	Cl	H	380	0.68	165-168	73
8	**II-7S-1h**	S	CH_3	H	Cl	H	393	0.77	90-92	70
9	**II-7S-1i**	S	OCH_3	H	Cl	H	410	0.41	215-218	74
10	**II-7S-1j**	S	H	H	H	CH_3	359	0.43	110-113	74
11	**II-7S-1k**	S	CH_3	H	H	CH_3	374	0.56	103-105	70
12	**II-7S-1l**	S	OCH_3	H	H	CH_3	389	0.82	160-162	71
13	**II-7S-1m**	S	H	H	H	Cl	380	0.81	145-148	74
14	**II-7S-1n**	S	CH_3	H	H	Cl	394	0.45	55-58	72
15	**II-7S-1o**	S	OCH_3	H	H	Cl	410	0.75	95-98	71
16	**II-7S-1p**	S	H	H	CF_3	H	413	0.30	240-242	71
17	**II-7S-1q**	S	CH_3	H	CF_3	H	427	0.50	>360	71
18	**II-7S-1r**	S	OCH_3	H	CF_3	H	443	0.43	210-212	70
19	**II-7S-1s**	S	H	H	Cl	Cl	414	0.43	250-252	70
20	**II-7S-1t**	S	CH_3	H	Cl	Cl	428	0.50	265-268	71
21	**II-7S-1u**	S	OCH_3	H	Cl	Cl	444	0.56	95-98	71

Rf (Solvent system: Hexane-ethyl acetate-8:2)** ***Cont'd...

S. No	Compd	X	R	R_1	R_2	R_3	Mol. wt	*Rf	M.p. Range e^0C	% yield
22	**II-7S-1v**	S	H	H	CH_2OCH_3	H	389	0.83	115-117	69
23	**II-7S-1w**	S	CH_3	H	CH_2OCH_3	H	404	0.43	90-92	65
24	**II-7S-1x**	S	OCH_3	H	CH_2OCH_3	H	420	0.58	140-141	70
25	**II-7S-2a**	S	Cl	H	H	H	344	0.57	195-198	71
26	**II-7S-2b**	S	NO_2	H	H	H	390	0.73	200-203	75
27	**II-7S-2c**	S	NO_2	CH_3	H	H	404	0.33	200-203	72
28	**II-7S-2d**	S	Cl	H	Cl	H	414	0.38	180-182	71
29	**II-7S-2e**	S	NO_2	H	Cl	H	425	0.55	90-92	72
30	**II-7S-2f**	S	Cl	H	H	CH_3	394	0.44	85-87	73
31	**II-7S-2g**	S	NO_2	H	H	CH_3	404	0.43	165-167	77
32	**II-7S-2h**	S	Cl	H	H	Cl	414	0.73	95-98	72
33	**II-7S-2i**	S	NO_2	H	H	Cl	425	0.71	100-102	73
34	**II-7S-2j**	S	Cl	H	CF_3	H	448	0.51	255-257	71
35	**II-7S-2k**	S	NO_2	H	CF_3	H	458	0.58	230-232	72
36	**II-7S-2l**	S	Cl	H	Cl	Cl	449	0.31	250-253	67
37	**II-7S-2m**	S	NO_2	CH_3	CH_2OCH_3	H	448	0.33	200-203	72
38	**II-7S-2n**	S	Cl	H	CH_2OCH_3	H	424	0.58	140-142	71
39	**II-7S-2o**	S	NO_2	H	H	H	390	0.71	115-118	74

***Rf (Solvent system: Hexane-ethyl acetate-8:2)**

Table 2. Physical data of compounds. Series-IIb (II-7O-1a-x and **2a-p)**



R=H,Cl,NO_2,CH_3,OCH_3
a = H,CH_3 and OCH_3; b= Cl and NO_2
X= O, S
R_1 = H, CH_3
R_2 =H, Cl, CF_3, CH_2OCH_3
R_3 = H, Cl, CH_3

General Structure–II

S.No	Compd	X	R	R_1	R_2	R_3	Mol. wt	*R_f	M.p. Range ^{0}C	% yield
1	**II-7O-1a**	O	H	H	H	H	329	0.68	210-212	71
2	**II-7O-1b**	O	CH_3	H	H	H	343	0.38	180-182	74
3	**II-7O-1c**	O	OCH_3	H	H	H	359	0.40	210-212	70
4	**II-7O-1d**	O	H	CH_3	H	H	343	0.62	212-214	76
5	**II-7O-1e**	O	CH_3	CH_3	H	H	357	0.91	320-322	71
6	**II-7O-1f**	O	OCH_3	CH_3	H	H	373	0.91	180-182	77
7	**II-7O-1g**	O	H	H	Cl	H	364	0.68	160-162	73
8	**II-7O-1h**	O	CH_3	H	Cl	H	378	0.77	90-92	70
9	**II-7O-1i**	O	OCH_3	H	Cl	H	394	0.41	215-217	74
10	**II-7O-1j**	O	H	H	H	CH_3	343	0.92	90-92	70
11	**II-7O-1k**	O	CH_3	H	H	CH_3	357	0.94	170-172	76
12	**II-7O-1l**	O	OCH_3	H	H	CH_3	373	0.82	115-117	74
13	**II-7O-1m**	O	H	H	H	Cl	364	0.67	200-202	76
14	**II-7O-1n**	O	CH_3	H	H	Cl	378	0.42	190-192	72
15	**II-7O-1o**	O	OCH_3	H	H	Cl	394	0.69	215-217	76
16	**II-7O-1p**	O	H	H	CF_3	H	329	0.66	265-267	71
17	**II-7O-1q**	O	CH_3	H	CF_3	H	411	0.37	275-277	74
18	**II-7O-1r**	O	OCH_3	H	CF_3	H	359	0.76	235-237	72
19	**II-7O-1s**	O	H	H	Cl	Cl	414	0.43	250-252	70
20	**II-7O-1t**	O	CH_3	H	Cl	Cl	412	0.50	265-268	71
21	**II-7O-1u**	O	OCH_3	H	Cl	Cl	373	0.36	>360	71

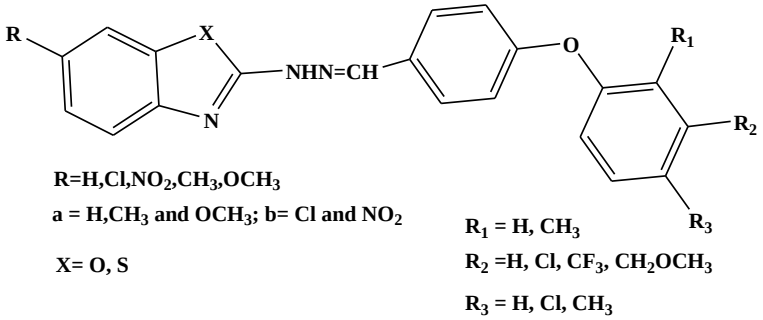
R_f (Solvent system: Hexane-ethyl acetate-8:2)** ***Cont'd...

S.No	Compd	X	R	R_1	R_2	R_3	Mol. wt	*R_f	M.p. Range^{0}C	% yield
22	**II-7O-1v**	O	H	H	CH_2OCH_3	H	398	0.45	129-131	72
23	**II-7O-1w**	O	CH	H	CH_2OCH_3	H	404	0.43	90-92	65

24	**II-7O-1x**	O	OCH_3	H	CH_2OCH_3	H	373	0.46	128-130	70
25	**II-7O-2a**	O	Cl	H	H	H	364	0.57	195-297	71
26	**II-7O-2b**	O	NO_2	H	H	H	375	0.73	200-202	71
27	**II-7O-2c**	O	Cl	CH_3	H	H	378	0.42	210-212	74
28	**II-7O-2d**	O	NO_2	CH_3	H	H	388	0.33	200-202	72
29	**II-7O-2e**	O	Cl	H	Cl	H	398	0.31	180-182	71
30	**II-7O-2f**	O	NO_2	H	Cl	H	425	0.55	90-92	72
31	**II-7O-2g**	O	Cl	H	H	CH_3	378	0.44	85-87	73
32	**II-7O-2h**	O	NO_2	H	H	CH_3	404	0.43	165-167	77
33	**II-7O-2i**	O	Cl	H	H	Cl	398	0.57	110-112	72
34	**II-7O-2j**	O	NO_2	H	H	Cl	409	0.93	225-227	74
35	**II-7O-2k**	O	Cl	H	CF_3	H	432	0.69	110-112	73
36	**II-7O-2l**	O	NO_2	H	CF_3	H	458	0.5	>360	70
37	**II-7O-2m**	O	Cl	H	Cl	Cl	449	0.69	115-117	71
38	**II-7O-2n**	O	NO_2	H	Cl	Cl	459	0.88	130-132	71
39	**II-7O-2o**	O	Cl	H	CH_2OCH_3	H	424	0.75	160-162	65
40	**II-7O-2p**	O	NO_2	H	CH_2OCH_3	H	434	0.35	132-134	70

***R_f (Solvent system: Hexane-ethyl acetate-8:2)**

Table 3. Cytotoxic activity of 2-(2-(4-aryloxybenzylidene)-hydrazinyl)-benzo[d]thiazoles. Series-IIa (II-7S-1a-x and II-7S-2a-o) and2-(2-(4-aryloxybenzylidene)-hydrazinyl)-benzo[d]oxazoles.Series-IIb(II-7O-1a-x and II-7O-2a-p)



R=H,Cl,NO_2,CH_3,OCH_3
a = H,CH_3 and OCH_3; b= Cl and NO_2
X= O, S

R_1 = H, CH_3
R_2 =H, Cl, CF_3, CH_2OCH_3
R_3 = H, Cl, CH_3

General Structure-II

Compound	X	R	R_1	R_2	R_3	MCF -7 (IC_{50} µM= ±S.D)	MCF-10A (IC_{50} µM = = S.D)
II-7S-1d	S	H	CH_3	H	H	11.08±2.46	>100
II-7S-1m	S	H	H	H	Cl	10.69±2.45	>100
II-7S-1v	S	H	H	CH_2OCH_3	H	20.37±2.47	>100
II-7S-2i	S	NO_2	H	H	Cl	12.70±2.50	>100
II-7S-2h	S	Cl	H	H	Cl	10.76±2.44	>100
II-7S-2m	S	NO_2	CH_3	CH_2OCH_3	Cl	9.70±2.47	>100
II-7O-1a	O	H	H	H	H	16.93±1.78	>100
II-7O-1v	O	H	H	CH_2OCH_3	H	24.05±2.62	>100
II-7O-2e	O	Cl	H	Cl	H	14.16±1.40	>100
II-7O-2i	O	Cl	H	H	Cl	11.5±1.45	>100
II-7O-2j	O	NO_2	H	H	Cl	14.86±1.48	>100
II-7O-2k	O	Cl	H	CF_3	H	11.89±1.78	>100
Cisplatin						1.86±0.17	1.86±0.31
Doxorubicin						2.21±0.15	2.21±0.28

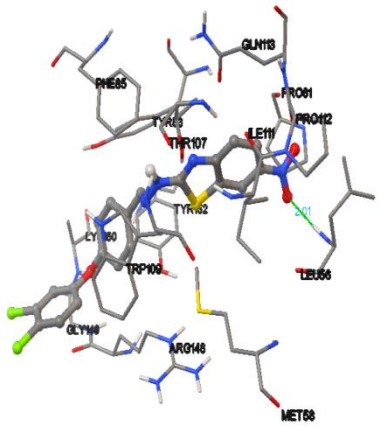
Breast Cancer target

Table-4: Docking studies of Scheme-II Series-IIa

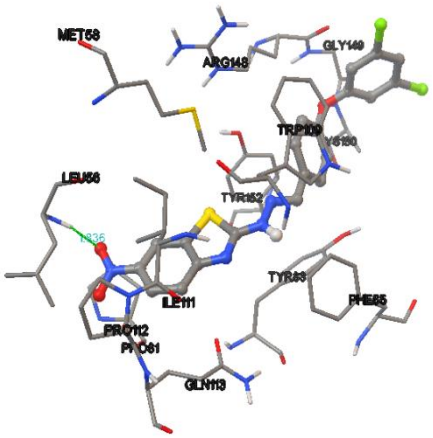
Sl.No	Test Compound	Interacting amino acids	Binding Energy ΔG (Kcal/mol)	Dissociation Constant (kI) (nM)
1	**II-7S-1n**	Leu56	-10.98	9.02
2	**II-7S-1q**	Leu56	-10.75	13.08
3	**II-7S-1c**	Leu56,Lys150	-10.25	30.79
4	**II-7S-1g**	-	-9.87	57.94
5	**II-7S-1f**	-	-9.72	74.84
6	**II-7S-1s**	Tyr83, Tyr152	-9.46	115.96
7	**II-7S-1t**	-	-9.4	129.79
8	**II-7S-1r**	-	-9.37	134.88
9	**II-7S-1e**	Leu56,Lys150	-9.3	151.25
10	**II-7S-1o**	-	-9.28	157.04
11	**II-7S-1b**	Tyr83	-9.23	172.29
12	**II-7S-1l**	Tyr83	-9.12	208.05
13	**II-7S-1h**	Thr107	-9.05	230.96
14	**II-7S-1j**	Thr107	-9.0	251.26
15	**II-7S-1d**	-	-9.0	250.77
16	**II-7S-1a**	-	-8.95	273.33
17	**II-7S-1p**	-	-8.72	403.95
18	**II-7S-1i**	Tyr83	-8.58	510.38

19	**II-7S-1k**	-	-8.56	534.34
20	**II-7S-1m**	-	-8.55	540.8

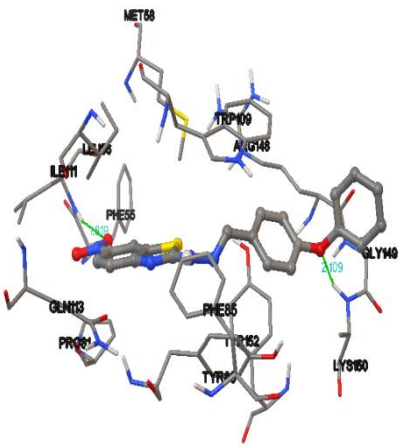
Figure -2: **Docking studies of Scheme-II Series-IIa**



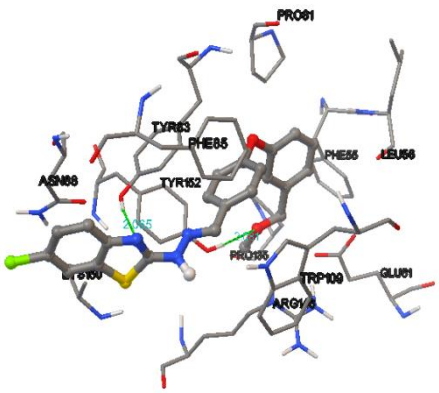
14 - **II-7S-1n**



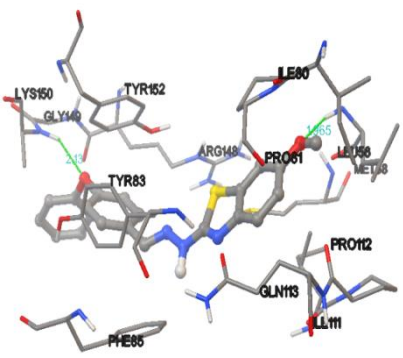
17 - **II-7S-1q**



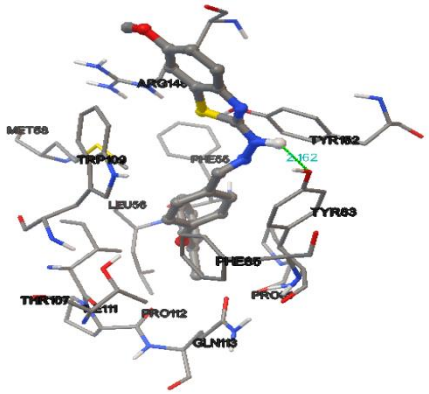
3 - **II-7S-1c**



19 - **II-7S-1s**



5 - **II-7S-1e**



2 - **II-7S-1b**

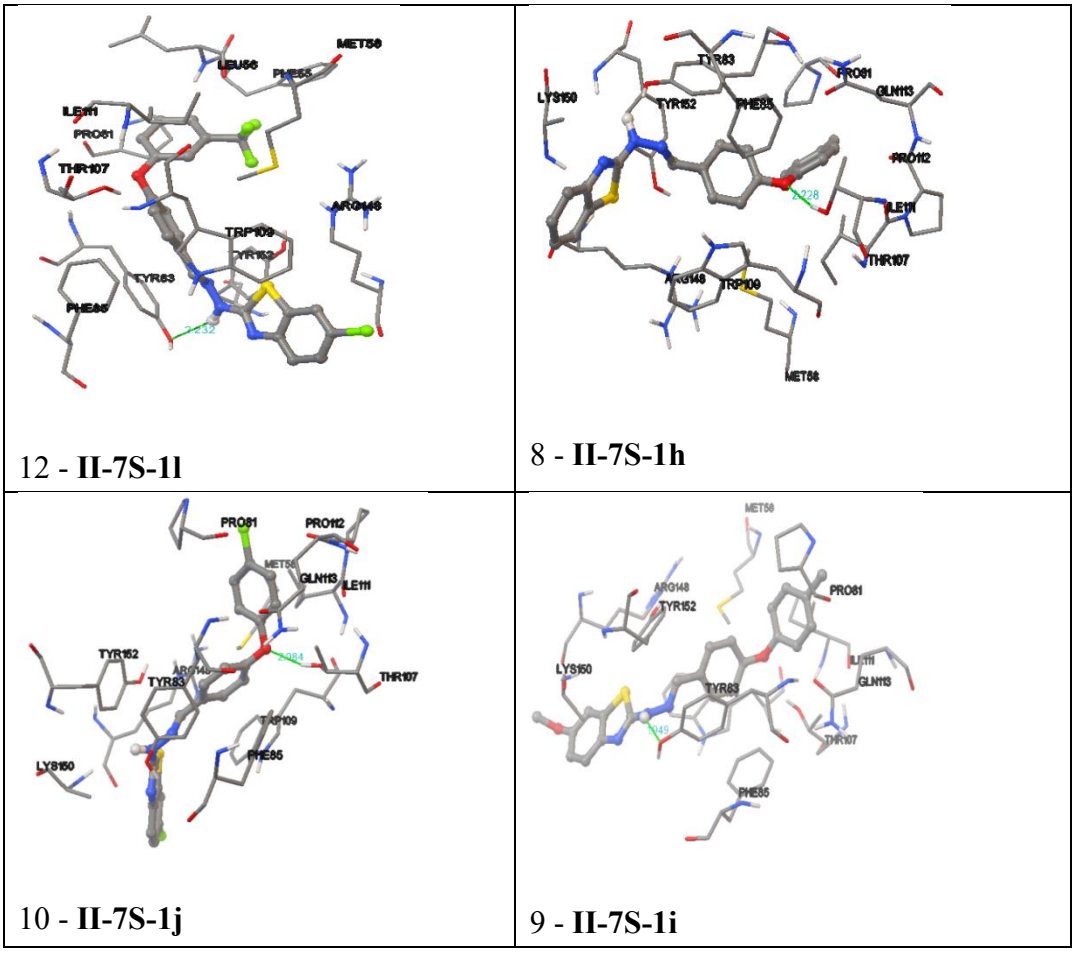
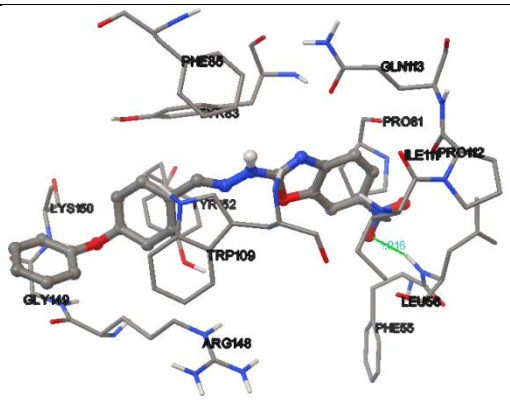


Table-5: Docking studies of Scheme-II Series-IIb

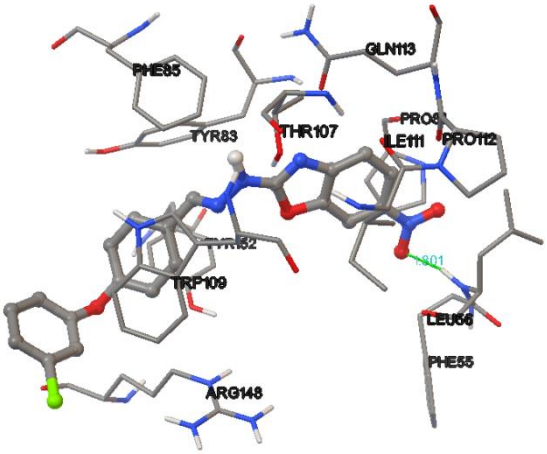
S.No	Test compound	Interacting amino acids	Binding energy, ΔG (Kcal/mol)	Dissociation constant (kI) (nM)
1	**II-7O-1d**	Leu56	-10.24	31.42
2	**II-7O-1h**	Leu56	-10.21	33.05 nM
3	**II-7O-1g**	Tyr83	-9.55	100.53 nM
4	**II-7O-2a**	Leu56	-9.47	114.81 nM
5	**II-7O-1u**	Leu56	-9.47	113.92 nM
6	**II-7O-2b**	-	-9.23	170.39 nM
7	**II-7O-1x**	Tys83	-9.21	178.52 nM
8	**II-7O-2c**	-	-9.17	190.98 nM
9	**II-7O-2d**	-	-9.17	188.92 nM
10	**II-7O-1i**	-	-9.17	188.5 nM
11	**II-7O-1v**	Thr107	-8.96	268.99 nM

12	**II-7O-1j**	-	-8.94	278.04 nM
13	**II-7O-1o**	-	-8.85	328.20 nM
14	**II-7O-1q**	-	-8.69	429.96 nM
15	**II-7O-1a**	Tyr83	-8.63	476.07
16	**II-7O-1w**	Tyr83	-8.63	474.75 nM
17	**II-7O-1c**	Leu56	-8.62	480.26 nM
18	**II-7O-1k**	Tyr83	-8.54	521.31 nM
19	**II-7O-1m**	Tyr83	-8.53	560.21 nM
20	**II-7S-1l**	-	-8.49	595.0 nM
21	**II-7O-1e**	-	-8.47	621.17
22	**II-7O-1b**	Tyr83	-8.45	642.52
23	**II-7O-1t**	-	8.34	766.5 nM
24	**II-7O-1p**	Tyr83	-8.08	1.19 micro M
25	**II-7O-1r**	Tyr83	-8.08	1.2 micro M
26	**II-7O-1f**	Tyr83	-8.04	1.28 microM
27	**II-7O-1s**	-	-7.94	1.52 micro M
28	**II-7O-1n**	Tyr83	-7.89	1.64 micro M

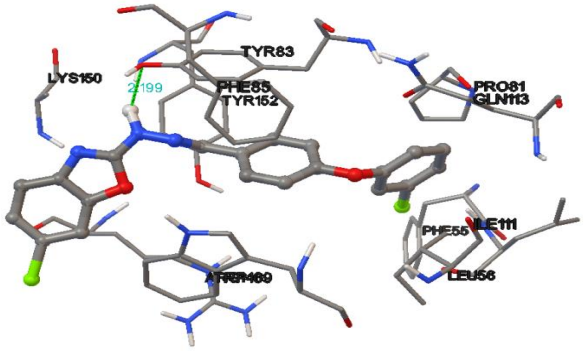
Figure -3: **Docking studies of Scheme-II Series-IIb.**



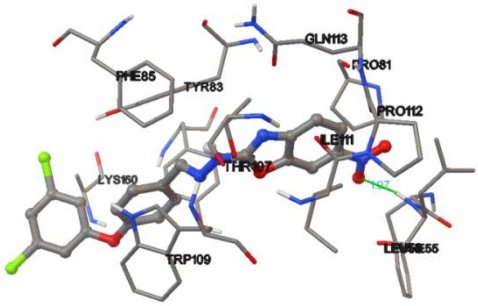
4 - **II-7O-1d**



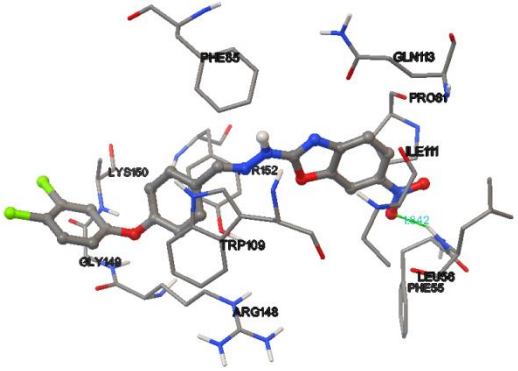
8 -**II-7O-1h**



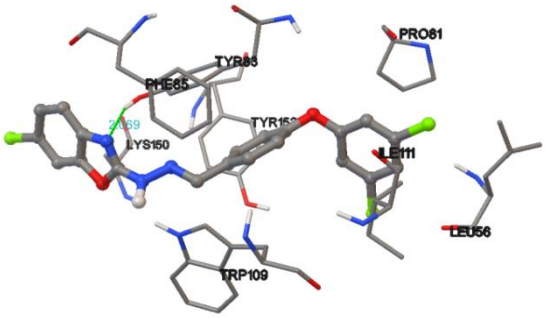
7 - **II-7O-1g**



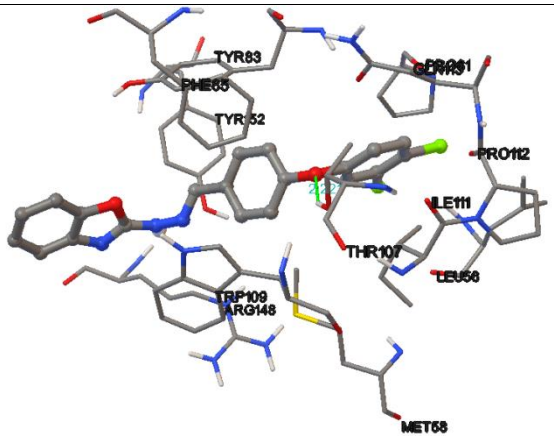
25 - **II-7O-2a**



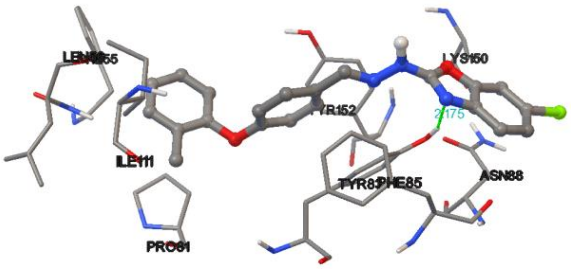
21 - **II-7O-1u**



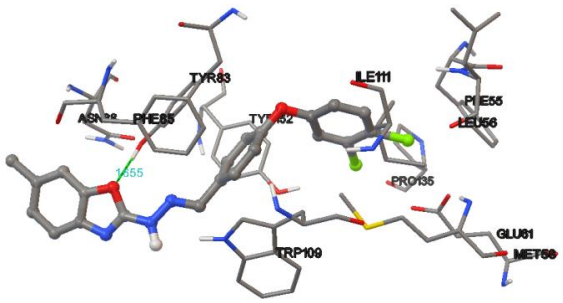
24- **II-7O-1x**



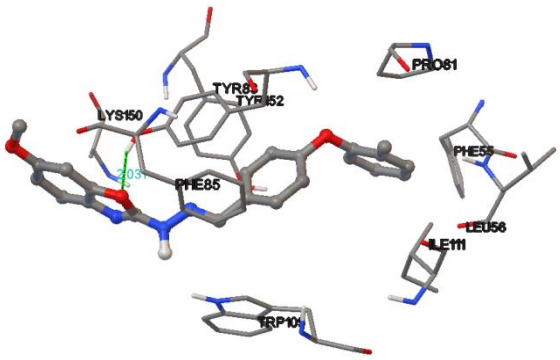
22 - **II-7O-1v**



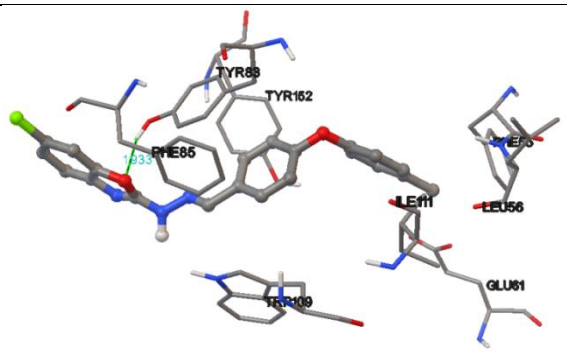
1 - **II-7O-1a**



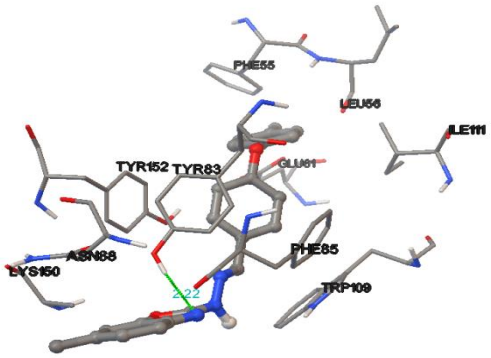
23 - **II-7O-1w**



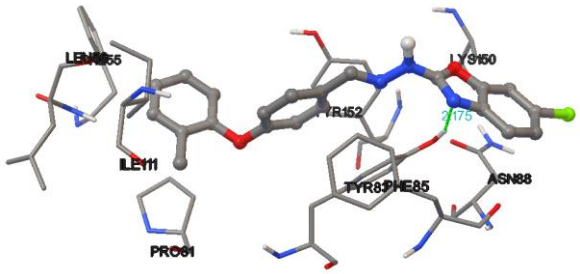
3 - **II-7O-1c**



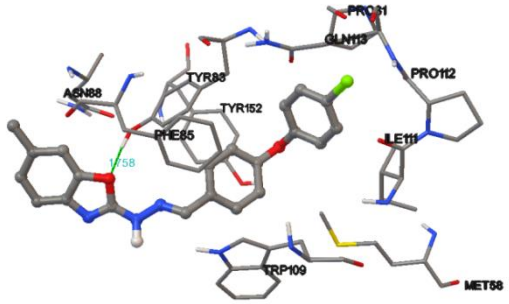
11 - **II-7O-1k**



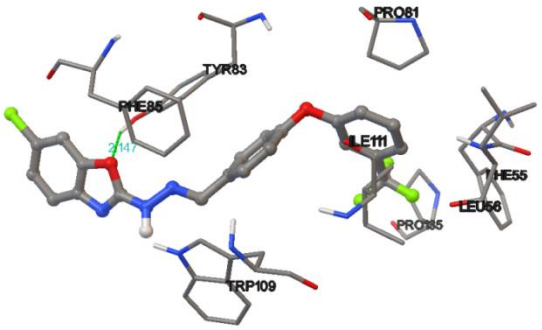
13 - **II-7O-1m**



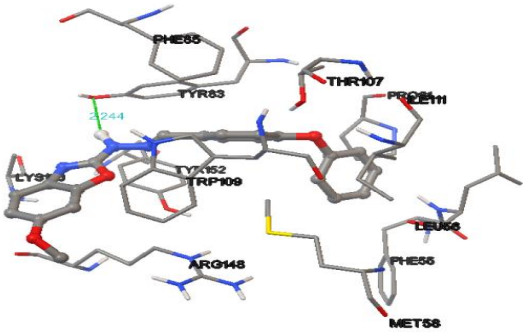
2 - **II-7O-1b**



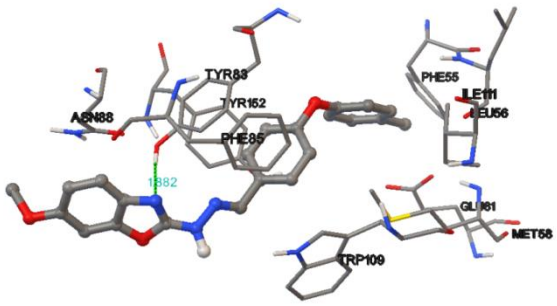
16 - **II-7O-1p**



18 - **II-7O-1r**



6 - **II-7O-1f**



14 - **II-7O-1n**

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