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Investigation of Anthramycin Analogs Induced Cell Death in MCF-7 Breast Cancer Cells

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ABSTRACT: 1,5-Benzodiazepines were synthesized using chalcone & o-phenylene diamine. Their structures were characterized using physical & spectral data. These molecules are analogous to anthracin, henceforth evaluated for their anti-breast cancer activity, using in vitro model MTT assay against MCF-7 cell line. Results revealed that these molecules are having potential growth inhibitory effect on the MCF-7 cell line, and certainly better than that of standard cisplatin. Docking studies revealed that these 1,5-benzodiazepine molecules may be working by inhibiting tyrosine kinase receptor, ErbB4 of human breast adenocarcinoma cell line(MCF-7/Michigan Cancer Foundation-7).

KEYWORDS: 1,5-Benzodiazepines; Potassium Aluminium Sulfate Dodecahydrate; MCF-7 Cell Line; Anticancer.

INTRODUCTION

1,5-benzodiazepines are good alternatives to the naturally occurring pyrrolo[2,1-c][1,4-benzodiaepines such as anthramycin which possess properties of DNA alkylation[1]. 1,5-Benzodiazepine is undoubtedly comes under important heterocyclic scaffold, possessing wide array of biological and therapeutic potentials like antitumor[1], antimicrobial[2], analgesic[3], anticonvulsant & hypnotic[4], antioxidant[5] and many more.

In women, breast cancer is the second leading cause of cancer-related death and the fact is well supported by the survey of American chemical society[6]. This infers that design & development of new anticancer drugs, drug combinations and treatment modalities is still the need for effective treatment of breast cancer patients[7].

To the best of our knowledge, very few studies have explored the potential effects of 1,5-benzodiazepines on breast cancer therapy. So keeping all this in mind, we have evaluated anti-breast cancer activity of four analogues of anthramycin with 1,5-benzodiazepine, rather than 1,4-benzodiazepine.

MATERIALS & METHODS

Drugs & Chemicals

Cisplatin and other chemicals were procured from Sigma Aldrich ltd. And Merck. Drugs **RVB-01**, **RVB-04**, **RVB-05**, **RVB-09** were synthesized as per the synthetic procedure[5].

MTT Anticancer Assay[8-10]

MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) is taken up by the viable cells and reduced to formazan by the "Succinate-tetrazolium reductase" system in the mitochondrial respiratory chain of metabolically active cells. Formazan formed, is a purple coloured water-insoluble product that is largely impermeable to cell membranes, thus resulting in its accumulation within the healthy cells which is solubilised by adding DMSO. The optical density (OD) of purple coloured solution developed was read using a conventional ELISA plate reader at 540nm. The ability of cells to reduce MTT provides an indication of the mitochondrial integrity and activity, which in turn, may be interpreted as a measure of viability and/or cell number.

Maintenance of cell lines

MCF-7 cells were grown in 75 cm^2 tissue culture flasks containing Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 1% L- glutamine and 50µg/ml gentamycin sulphate at 37ºC in CO_2 incubator in an atmosphere of humidified 5% CO_2 and 95% air. The cells were maintained by routine sub culturing in 75cm^2 tissue culture flasks.

Procedure

Exponentially growing MCF-7 cells were harvested from 75cm^2 tissue culture flask and a stock cell suspension ($1X10^5$cell/ml) was prepared. A sterile 96-well flat bottom tissue culture plate was seeded with $2x10^3$ cells in 0.1 ml of MEM medium supplemented with 10% FBS and allowed to attach for 24hrs. Cells were treated with different conc. of test compounds and incubated for 24 and 48hrs. The control group cells were treated with only the medium containing 0.1% DMSO. Treatment was performed in triplicates. Drug containing media was removed and washed with 200µl of phosphate buffer saline (PBS) and 100µl of MTT reagent (1mg/ml) was added and incubated for 4 hrs at 37ºC. After 4 hrs of incubation, MTT was removed and the formazan crystals formed in each well were dissolved in 100 µl of DMSO. The absorbance was measured by an ELISA plate reader at 540 nm.

$$\% \text{ Cytotoxicity} = \frac{(\text{Control-blank}) - (\text{test-blank})}{(\text{Control-blank})} \times 100$$

Data Analysis

Data generated after MTT assay was incorporated in the developed formula(% Inhibition & IC_{50} calculations) using Microsoft excel 2007, and graph was developed with the help of Graph Pad Prism 5.0.

Docking Studies

Vlife MDS 4.2 is very robust software with inclusion of all the necessary simulation modules. The structure of anthracin analogs under study have been drawn using ChemDraw Ultra, followed by its conversion into 3D form by using default conversion procedure.

Best conformer with the minimum energy was used for the docking analysis[11]. The PLP function is incorporated by the MDS Vlife Science software in the GRIP docking method which calculates the ligand- receptor binding affinity in terms of the PLP score. The PLP score is designed to enable flexible docking of ligands to perform a full conformational and positional search within a rigid binding site. 1,5-Benzodiazepine derivatives were docked into the active site of 3U9U i.e. ErbB4, Tyrosine kinase receptor by the use of cavity no. 1. The parameters fixed for docking simulation was like this- number of placements: 100, rotation angle: 10^o, exhaustive method, ligand-wise results: 10, scoring function: PLP score. By rotation angle, ligand would be rotated inside the receptor cavity to generate different ligand poses inside the receptor cavity. By placements, the method will check all the 100 possible placements into the active site pocket and will result out best placements out of 100. After docking simulation, the best docked pose of test molecules were then checked for their interactions with targeted proteins like hydrogen bonding, hydrophobic, pi-staking/aromatic, charge and vanderwaal's interactions[12, 13].

RESULTS & DISCUSSION

Four 1,5-benzodiazepine derivatives were synthesized according to the procedure[5](**Figure 1**).

3-(4-1H-Indol-3-yl)-2,3-dihydro-1H-benzo[b][1,5]diazepin-2-yl)-2H-Chromen-2-one (RVB-01): $C_{26}H_{19}N_3O_2$; Mol. Wt. 405.45g/mol; Calcd. Log P: 3.98±1.10; UV(nm) : 275.4 ; LC-ESI-MS : 404.8 m/z$(M)^+$, 426.7 $(M-1+Na)^+$; FT-IR(KBr ,cm^{-1}): 3329.25 (NH), 3055.35,2922.25(Ar-H), 1716.70 (C=O), 1602.90 (C=C), 1226(C-O-C); 1H-NMR (ppm): 3.3(s, 1H, 3^o - CH), 3.9 (s, 1H, Diazepin-NH), 1,225, 2.2161(d, 2H, 2^o- CH), 6.7-8.5 (m, 14H, Ar-H), 10.7(s, 1H, indolyl-NH)[2,5].

3-(4-(1H-indol-3-yl)-2,3-dihydro-1H-benzo[b][1,5]diazepin-2-yl)-8-methoxy-2H-chromen-2-one (RVB-04): $C_{27}H_{21}N_3O_3$; Mol. Wt. 435.47 g/mol; Calcd. Log P: 3.60 ± 1.11; UV(nm): 281.30; FT-IR(KBr, cm^{-1}): 3379.40 (N-H), 3047.63 & 2922.25 (Ar-H), 1703.20 (C=O), 1637.62 & 1577.82(C=C), 1234.48(C-O-C); ^{1}H-NMR(ppm): 9.9267(s, 1H, indolyl-NH), 6.5-8.7(m, 13H, Ar-H), 3.75(3H, methoxy-CH_3), 4.1(1H, aromatic NH), 1.32, 1.98(2H, methylene), 2.3(1H, methine)[5].

2-[4-(1H-indol-3-yl)-2,3-dihydro-1H-1,5-benzodiazepin-2-yl]phenol (RVB-05): $C_{23}H_{19}N_3O$; Mol. Wt. 353.42 g/mol; Calcd. Log P: 2.44 ± 1.10; UV(nm): 217.0 & 280.60; FT-IR(KBr, cm^{-1}): 3396.76(N-H), 3240.52(O-H), 3049.56, 2960.83 & 2920.32 (Ar-H), 1695.49(C=O), 1631.83 & 1577.82(C=C str), 1238.34(C-O-C); ^{1}H-NMR(ppm): 9.9(s, 1H, indolyl- NH), 6.6-8.7(m, 13H, Ar-H), 3.3(s,1H, methylene), 2.0, 2.3(2H, methine), 4.0(1H, aromatic NH), 5.5(1H, OH)[5].

3-(4-(1H-indol-3-yl)-2,3-dihydro-1H-benzo[b][1,5]diazepin-2-yl)-7-methoxy-2H-chromen-2-one (RVB-09): $C_{27}H_{21}N_3O_3$; Mol. Wt. 435.474 g/mol; Calcd. Log P: 3.92 ± 1.11; 3400.62(N-H), 2922 & 2854.74(Ar-H), 1703.20(C=O), 1608.69(C=C str.), 1238.34(C-O-C); ^{1}H-NMR(ppm): 9.99(s,1H, indolyl-NH), 6-8.5(m, 13H, Ar-H), 4.0(1H, Ar-H), 3.7(3H, methoxy-CH3), 3.1(1H, methine), 1.2, 1.9(2H, methylene)[5].

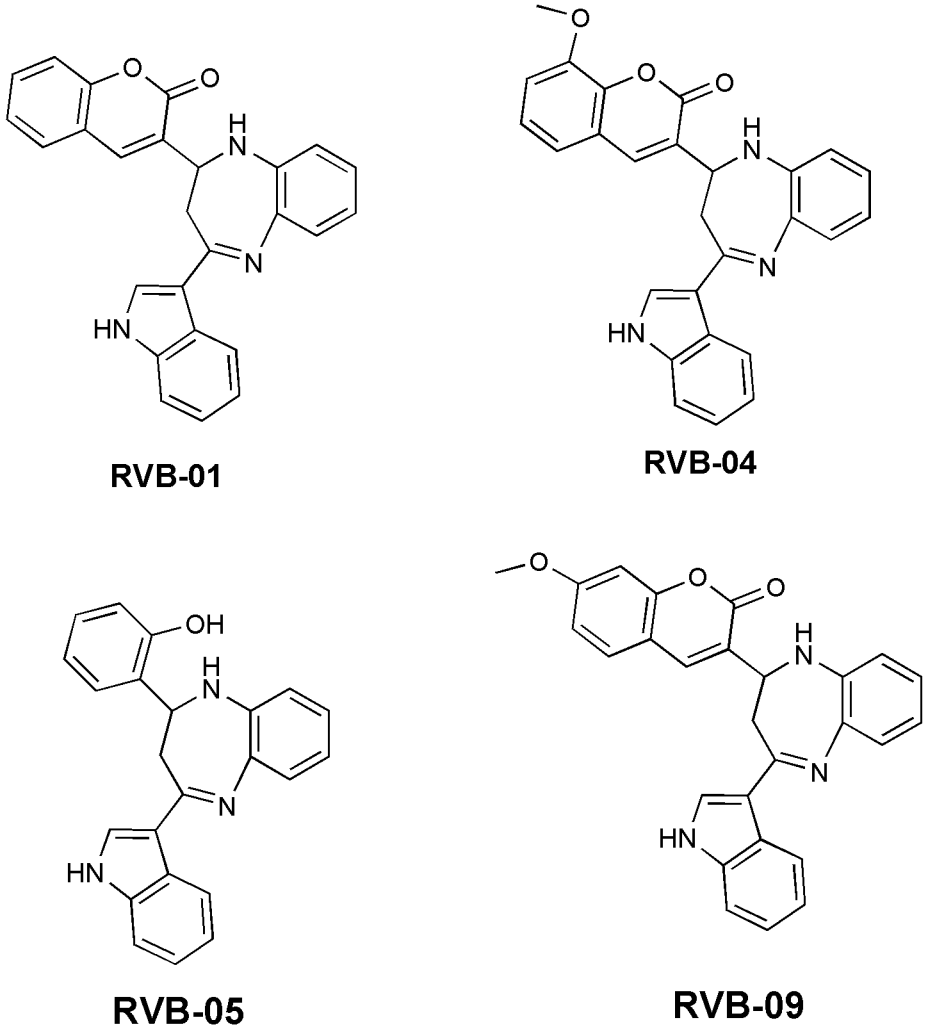

Figure 1 Anthracin Analogs

Table 1 Results of MTT assay against MCF-7Cell Line

Compd. Code	IC50(µg/ml)
RVB-01	1.3
RVB-04	1.22
RVB-05	1.14
RVB-09	1.31
Cisplatin	19.5

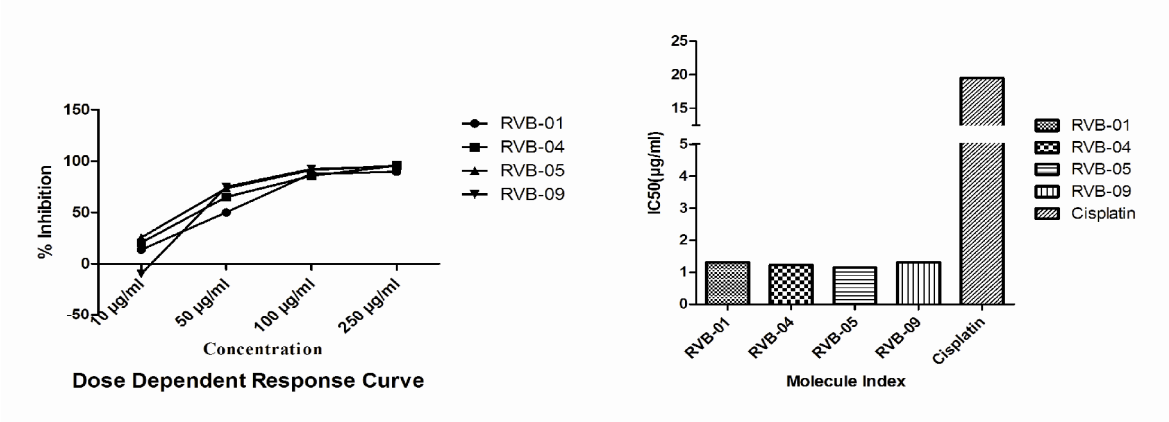

Figure 2 Dose Dependent Response & IC50 value of Anthracin Analogs Against MCF-7Cell Line

Table 2 Docking Results of 1,5-Benzodiazepine Derivatives & Target 3U9U i.e. ErbB4, Tyrosine Kinase Reeceptor. Dock scoring function: PLP Score; Vdw: Vanderwaal's Interations; HYI: Hydrophobic Interactions; Binding Energy = Total Energy- (Energy of Protein + Energy of Ligand).

S.No	Molecule	Pred. Log P	Dock Score	Interactions	Binding Energy (KJ/mol)	Energy of Molecule (KJ/mol)
1	RVB-01	3.74	-61.84	**Vdw:** Gln41, Gly44, Glu154, Pro173, Ala174, Gln43, Lys44, Pro45, Gly46, Gln47, Lys108, Asp170	-5.84	103.67
				HYI: Gly44		
2	RVB-04	3.61	-71.84	**Vdw:** Ser43, Gly44, Pro173, Ala174, Gln43, Lys44, Pro45, Val90, Lys108, Trp168, Thr169, Asp170	118.15	118.66
				HYI: Gly44		
3	RVB-05	3.69	-59.22	**Vdw:** Gly44, Pro173, Gln43, Lys44, Pro45, Gly46, Gln47, Trp168, Thr169, Asp170	-10.53	90.55
				HYI: Gly44, Pro173		
4	RVB-09	3.61	-75.03	**Vdw:** Gln41, Ser43, Gly44, Thr94, Gln43, Lys44, Pro45, Gly46, Gln47, Lys108, Lys147, Trp168, Thr169, Asp170	-1.12	109.89
				HYI: Gly44, Lys147, Trp168		

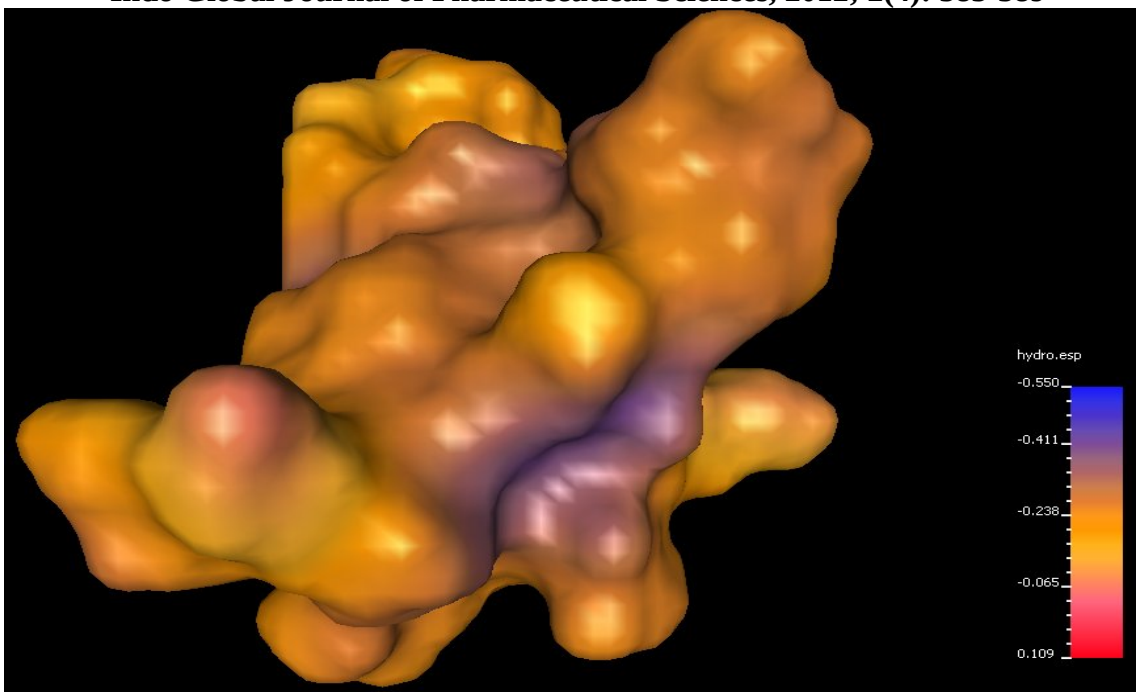

Figure 3 Cavity No. 1 of 3U9U, Tyrosine Kinase Receptor, Surface appearance according to hydrophobicity.

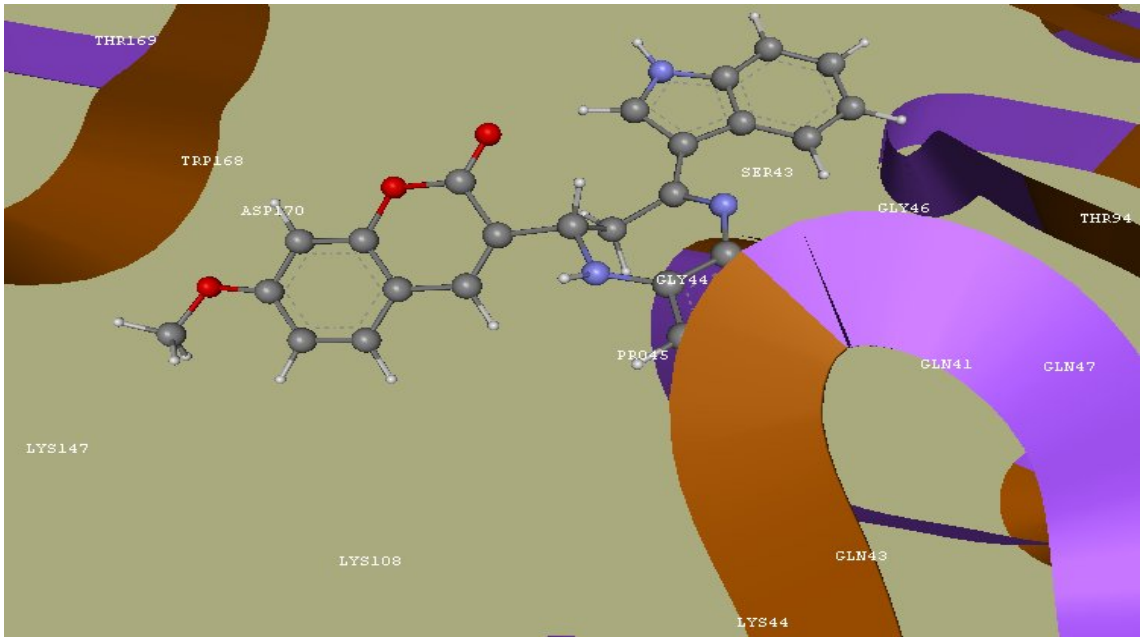

Figure 4 Active Site for RVB-09 on ErbB4, Tyrosine Kinase Receptor.

MTT assay results revealed that these anthracin analogs are having potential cytotoxicity against human breast adenocarcinoma cell line(MCF-07) and that too is dose dependent one(Refer **Figure 2**). All the 1,5-benzodiazepine/anthracin analogs are having 50% inhibitory concentration in the range of 1-1.4 µg/ml. IC50 value of these molecules is much better than the standard cisplatin, which signifies their anti-breast cancer potential(Refer **Figure 2** & **Table 1**). Cavity no. 1 of 3U9U was used for the present docking studies(**Figure 3**). Results of docking study are tabulated in **Table 2**. Active site for RVB-09 inside the cavity no. 1 of 3U9U is represented by **Figure 4.** Docking studies supported the in vitro MTT assay results, and revealed the possible mechanism of their anticancer activity via tyrosine kinase receptor inhibiton. It has been hypothetically claimed that tyrosine kinase receptor blockers

might be potentially more selective inhibitor against the proliferation of breast cancer, that are primarily regulated by hormones and growth factors[14].

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