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Molecular docking and Invitro cytotoxicity activity of styryl compound against the HeLa cancer cell line from the extract of crotolaria medicaginea

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ABSTRACT

Background: *Stilbene compounds occur in some type of plant species, they preventing many disorder and illness. Recently focus a number of researches in medicine and plant physiology similarly to have emerged as promising molecules that probably have an effect on human health.*

Methods: *In these review, the aim of present study evaluated the stilbene compound from the methanolic extract of plant, crotolaria medicaginea. The compound was isolated and evaluated for their molecular docking studies using BIO-VIA Discovery studio and cytotoxicity activity towards human cervical cancer cell line (HeLa) by using MTT colorimetric assay techniques.*

Results: *The IC50 (half maximal inhibitory concentration) value of the controlled compound was determined. It confirmed a percentage of cell viability of 97% at 35.64µg/ml. The docking studies reveal that the isolated compound inside 4j96 protein showed a minimum CDOCKER score. The structure had been elucidated on the basis of FT-IR, 1H and 13C NMR spectral studies, the results of these studies have been confirmed the isolated compound.*

Conclusion: *the present investigation concluded that the extracted compound has shown to be a potent invitro anticancer activity against the human cervical cancer Hela cell line and docking studies.*

Keywords: Stilbene, Crotolaria medicaginea, Anticancer, Human cervical cancer cell line, Cytotoxicity, CDOCKER energy.

INTRODUCTION

Cervical cancer is the most common female genital tract malignancy, and it is the third most common malignant tumor next to breast cancer and colorectal cancer in the global women[1], especially in under developed and developing countries[2]. This cancer slowly develops; ranging from a malignant tumor abnormally selected cervical intraepithelial pathologic processes that will additional develop to invasive cervical cancer [3]. This cancer starts within the cervix, the lower slender a part of the uterus and mostly the cases of cervical cancer are infected by high risk type of Human Papilloma virus (HPV). Primarily the patients were from the generative age group [4].

Stilbenes are natural compounds found in some types of plants. Stilbene derivatives are synthesized comparatively easily, are usually thermally, and chemically stable [5]. Synthesis and bioactivity analysis of changed stilbene derivatives received abundant attention and interest in medicinal chemistry [6]. Stilbene derivatives plays a crucial role in preventing several disease and illness, such as inflammation [7-10], cancer [11,12,9] and heart diseases [13,14], various reviews have summarized the effects of resveratrol treatment on breast, colorectal, liver, pancreatic and prostate cancer. Many studies found the analysis of respective stilbene derivatives could also contain anticancer activities. Methoxylation has been significantly improving the antitumor potential of compounds [15, 16].The greater the number of methoxy group's presence, the better the anti-tumor activity [17]. Some experimental evidence shows that, methoxy substituted stilbenes are having good cancer chemo-preventive agent and most of methoxy resveratrol derivatives exhibit potent cytotoxic and pro-apoptotic activity against cancer cells [8-11]. Polymethoxy stilbenes are able to interact with biomembrane models and have a higher bioavailability than resveratrol [18-20]. A various literature survey has been suggested that the position of methoxy group had a significant impact on the biological activities and oral pharmacokinectic profiles of methoxy stilbenes. Pharmacokinetics plays a highly imortant role in drug discovery and development [21]. Recently, the pharmacokinetic profiles of several methylated resveratrol analogues were reported [22,23]. Our studies suggested that the positions of the methoxy groups of stilbenes were important. In this study, methoxy stilbene derivatives were isolated from the crude extract of the plant crotolaria medicaginea (Figure 1). The styryl compound was extracted and evaluated for their cytotoxcity activity towards human cervical cancer cell line (HeLa) by using MTT assay techniques. These findings recommended that stilbene derivative is apparently related to a significant reduction in the chance of cervical cancer. A study has reported that substituted stilbene derivative can inhibit the proliferation of HeLa cells have better inhibitory activities against human cervical carcinoma HeLa cells.

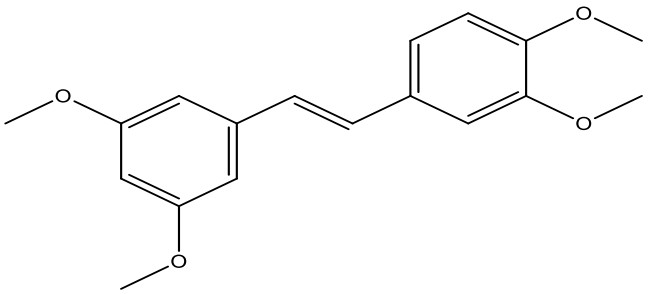

Figure 1: Chemical structure of (E)-4-(3, 5-dimethoxystyryl)-1, 2-dimethoxybenzene

EXPERIMENTAL

Materials and Methods

All the chemicals and solvents were used in this experiment were purchased from sigma-Aldrich. Infra red spectroscopy were recorded on Aglient Pro FT-IR spectrometer using KBr pellet method.1H NMR (400MHz) and 13C NMR (100 MHz) spectra were obtained in CDCl3 with a Bruker 400MHz NMR spectrometer using tetramethylsilane (TMS) as a internal standard and the chemical shift value (δ) were reported in parts per million (ppm) scale.

Extraction and isolation

Air-dried and powdered of the plant was extracted with methanol in a soxhlet apparatus for 2 days. The methanolic extract of the plant was further isolated with petroleum ether, benzene, chloroform and ethyl acetate. The ethyl acetate fraction was distilled under reduced pressure to yield a dark colored product. The compound was separated by column chromatography on 100-200 mesh silica gel eluted with a stepwise gradient of using petroleum ether: ethyl acetate. The column fractions were monitored by TLC [24].

Spectral Studies of (E)-4-(3, 5-dimethoxystyryl)-1,2-dimethoxybenzene

Dark color, Yield: 44%, Fourier transform (FT)-IR (KBr, cm-1); 2835 (-OCH3 Ali), 2999,3068(-CH Str(Ar)),1593(C=C); 1H- NMR (400MHz, DMSO): (δ,ppm), 3.862(s, 6H, -OCH3), 3.815(s, 3H, -OCH3), 3.743 (s, 3H, -OCH3), 6.9 (d, 2H), 6.301-6.932 (m, 6H); 13C-NMR (100MHz, DMSO) (δ,ppm), 161.0, 150.43, 149.05, 139.59, 136.15, 126.60, 120.05, 111.22, 108.79, 104.36, 99.73, 55.38; For C18H20O4: C 71.98 %, H 6.71 %, O 21.31 %.

Invitro anticancer activity

Cell line

HeLa cervical cancer cell line was obtained from the National centre for cell science, Pune. These cells cultures were maintained in Eagles minimum Essential Medium (EMEM) containing 10% fetal bovine serum medium at 370C in 5% CO2, 95% air and 100% relative humidity. Protection cultures were the passages weekly, and the culture medium was changed twice a week.

Cell Treatment Procedure

The obtained monolayer cell culture were segregated with trypsin-ethylene diamine tetra acetic acid to create viable cells and single cell suspensions were counted by using hemocytometer and medium containing 5% fetal bovine serum, to create a end density of 1X105 cells/well. 100µl/well of cell suspension have been seeded independently in 96-well culture plates at a plating density of 10,000 cells per well and incubated at 370C in a humidified atmosphere with 5% CO2 and 95% air. After 24 hours, the cells were exposed with different concentrations of the test samples. The sample was first dissolved or distributed in DMSO, and the solution becomes diluted to two times the required last test concentration containing serum free medium. Extra 4 dilutions were making ready to provide a whole of 5 sample concentrations. Following additions, the plates were incubated for an additional 48 hours at 370C in a humidified atmosphere with 5% CO2 and 95% air. All the assays were performed in manipulate and triplicate was well kept for entire concentrations [24].

MTT (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide) Assay

MTT(3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide) is a colorimetric assay based on a enzymatic reduction of mitochondria succinate dehydrogenase in living cells to reduce yellow colored water soluble tetrazolium salt of MTT into insoluble purple formazan. The quantity of cell viability is directly proportional to the activity of enzyme formazan produced, and inversely proportional to the cell inhibition. After 48 hrs of incubation, 10µl of phosphate buffered saline in 15µl of MTT solution was pipetted out every well and the plates were incubated at 370C for four hours. The plates were shaken gently at room temperature and then 100µl of DMSO was added to the each well to dissolve the formazan crystals. The optical density was absorbed at 570nm by using micro plate reader [25].

Statistical Analysis

The experimental results expressed as the mean ± standard deviation (n=3) analyzed by one way analysis of variance ANOVA followed by post hoc Dunnett's test. The non linear regression graph was plotted between % cell inhibition and log10 concentration and IC50 was determined using the

graph pad prism program

Calculation of IC50 and % Inhibition

IC50 value is a concentration that inhibits half of the cells in vitro. The half maximal inhibitory concentration (IC50) of the control compound was calculated. The MTT assay results were expressed as the percent inhibition according to the following formula:
% inhibition = [1- (Absorbance of treatments/ Absorbance of DMSO) x 100]

Molecular docking studies

Molecular docking studies were completed to analyze the binding mode between the extracted compound and the FGF Receptor2 the use of CDOCKER docking protocol of discovery Studio 2016 (CHARMm based docker) is molecular dynamics based docking algorithm [26]. The protein selecting for the present investigation is one of the most crucial aspects. The 3D X-ray crystal structure of the FGFR2 (PDB ID: 4j96) were retrieved from Protein Data Bank (PDB) based on good resolution of 2.297Å. 4j96 protein is selected as target receptor because to its significant role in anticancer studies [27, 28]. Thereafter, the protein is once selected, according to energy minimization with the useful resource of using CHARMm force field until the best gradient tolerance is received. The 3D structure of the extracted compound becomes received by Chem Draw Ultra 12.0 and Chem Draw 3D Pro 12.0 Softwares. CDOCKER is a grid-based molecular docking method that employs CHARMm [29].The default parameters have been used within the docking stimulations with CDOCKER [30]. Different poses of protein-ligand complex is acquired after docking approach with their unique CDOCKER energy and CDOCKER interaction scores. The ligand poses were analyzed and interaction of ligand molecule with the 4j96 protein structure was studied on the basis of H-bonding made by the poses to the receptor molecule and Vander Waals forces between the poses and receptor molecule.

RESULTS AND DISCUSSION

In Vitro Cytotoxicity Activity of 3, 5, 3 ', 4'-tetramethoxystilbene

In vitro confirmation of their cytotoxicity on cervical cancer cell line (HeLa) was studied using MTT colorimetric assay. The cytotoxicity activity was distributed for extracted compound. The compound was screened for its cytotoxicity against human cancer cell line at completely different concentrations. To calculate the IC50 value by MTT assay, the toxicity of compound was found to be 6.25, 12.5, 25, 50 and 100µg/ml at a concentration of 7.30, 13.80, 48.43, 96.35 and 100 respectively. IC50 value of µg/ml was obtained for cervical cancer cell line. It showed a good cytotoxicity than HeLa cell. The percentage cell growth inhibition was found to be increasing with increasing concentration of isolated compound. For instance, the maximum cell was dying within the concentration of 50, and 100µg/ml, as well as 96.35% cell died at the concentration of 50µg/ml, and these results discovered dose-dependent response (Table 1). The slope value of percentage inhibition indicating that, with increasing of concentration, a purposeful changeability should occur in the inhibition with logarithmic regression equation, which estimate a highly significant impact at 50µg/ml, as well as, strong correlation coefficient have been reported between the studied factors with highly significant at 25µg/ml, also the long-term trend between the two factors, % cell inhibition and concentration, which that a highly responding is accounted with % cell inhibition upto 6.25µg/ml (Figure.2). A compound having methoxy substituent attached to the phenyl ring is the reason of potential anticancer activity of the compound. In summary, the excising examine established that compound have an efficient antitumor compound with an IC50 of 7.30µg/ml such as growth inhibition in the human health cervical cancer cells. The results disclosed that the compound possesses the inhibition effect on the growth of HeLa tumor cell line and this effect seems clearly with the rise within the compound concentration.

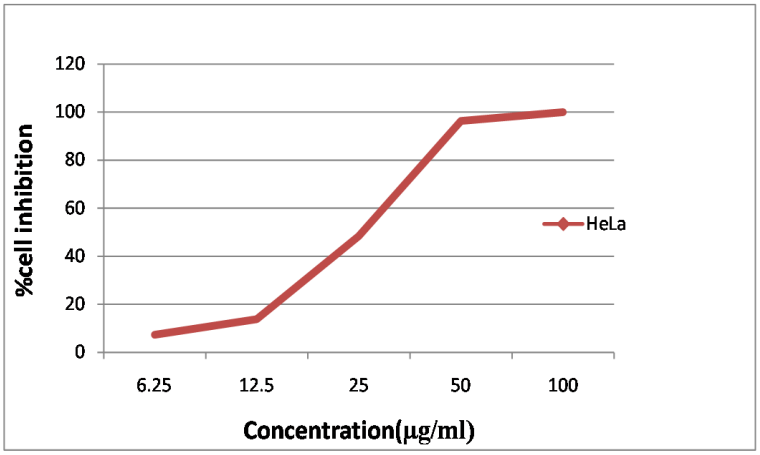

Figure 2: Percentage cell inhibition against human cervical cancer cell line

Morphological Changes of HeLa Cells Treated with the Compound

The effects of different concentrations (6.25-100µg/ml) of compound in HeLa cell were observed using phase contrast of an inverted microscope. The phase comparison observations in HeLa cells discovered that the morphology [31-34] of cell varies considerably, with a rise in concentration. The Untreated control HeLa cells were discovered showing their regular form and a lesser sort of degrading cells. However, cells treated with various different concentrations of isolated compound exhibited morphological changes like nuclear fragmentation, finally leading to the formation of apoptotic bodies. The representative image of HeLa is shown in Figure.3.

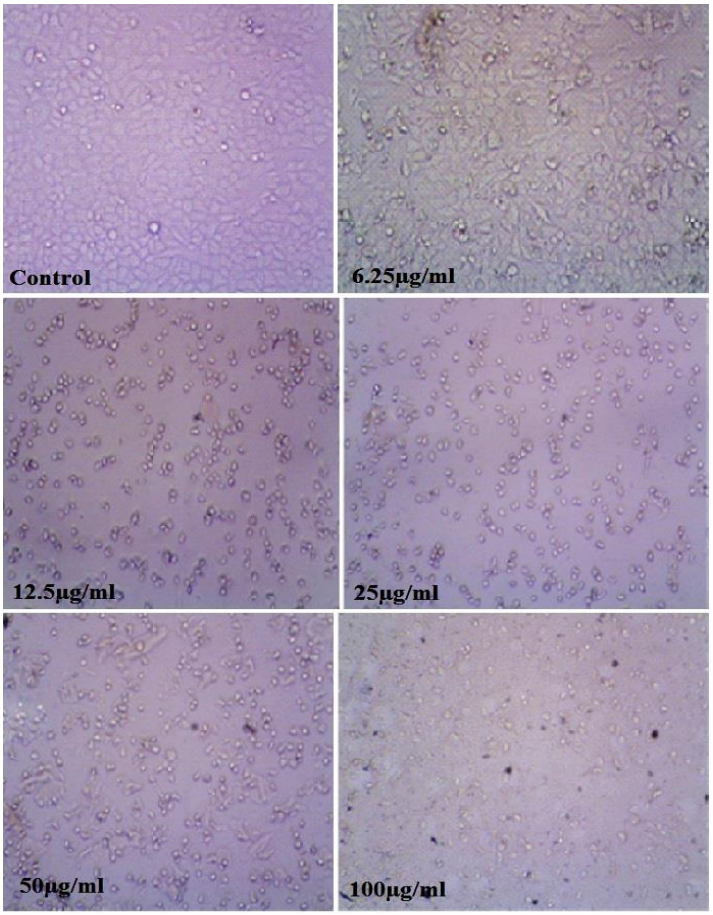

Figure 3: Proliferation of HeLa human cervical cancer cell line.

Molecular Docking Results

The structure was analyzed for identifying the possible binding site of FGFR2. Docking of this optimized compound against FGFR2 (4j96) structure at the catalytic active site residues were performed by discovery studio. To locate the appropriate binding orientations and conformations of ligand on 4j96, molecular docking was performed by the use of CDOCKER. The theoretical binding mode between the compound and FGFR2 was shown in Figure 4. The docking results reveal that the isolated compound shows a minimum cdocker energy (12.3728 kcal/mol) and cdocker interaction energy (31.9969 kcal/mol). The 2D structure of the synthetic ligand is produced using discovery studio (v 16.1.0.15350) Figure 5. In the 2D structure, the green dotted lines represent the Vander Waals interaction, blue dotted lines represent the water-Hydrogen bond and pink dotted lines represent the pi-Alkyl interactions. Docking of this optimized compound against a 4j96 structure of the active site residues is performed by discovery studio Figure 6. The structure of compound shows interaction with the residues ASP A: 644, LEU A: 633, VAL A: 495, HOH W: 71 and HOH W: 13. The interaction of the docked structure is shown in the fig. The docking compound shows a minimum CDOCKER energy with the amino acid residues of the receptor molecule. The CDOCKER interaction energy between the isolated ligand and protein receptor 4j96 was finally computed.

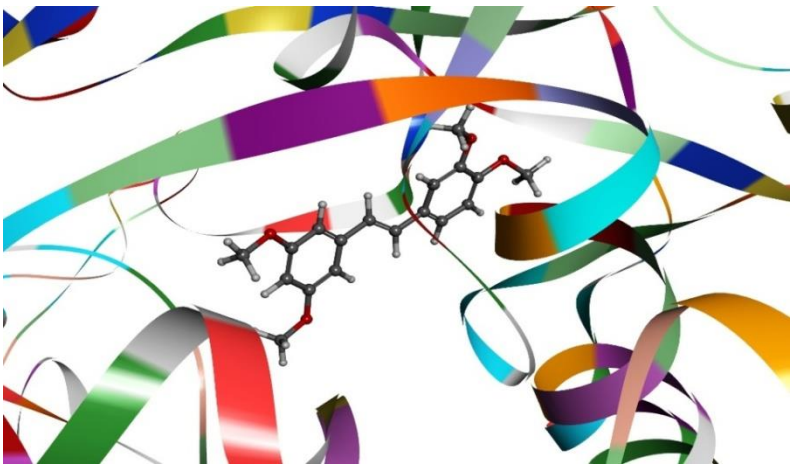

Figure 4: The structure of FGFR2 (4j96) protein with isolated compound.

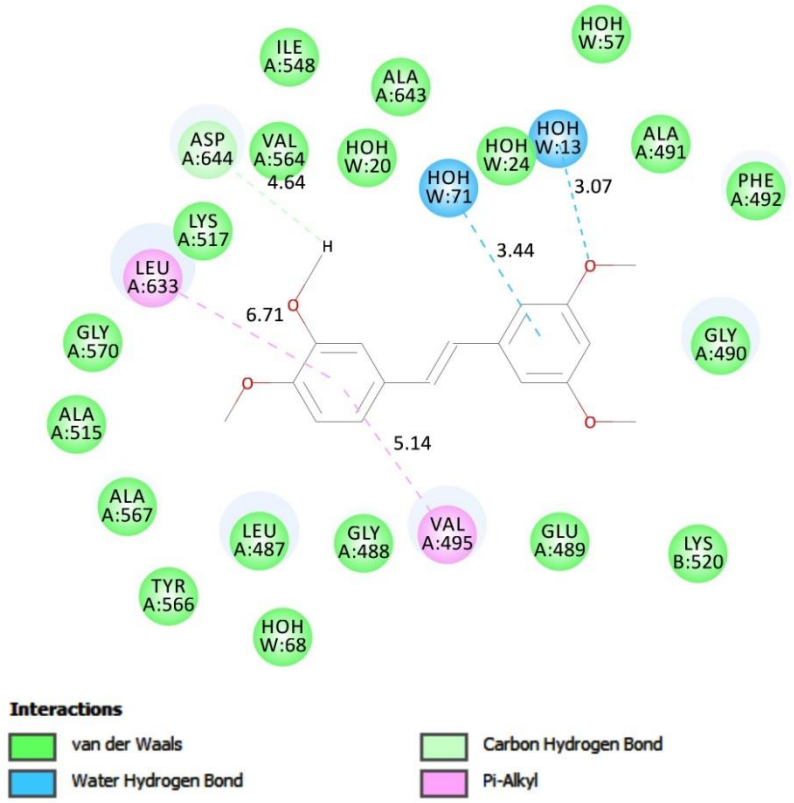

Figure 5: Two-Dimensional diagram of compound docked with 4j96 protein

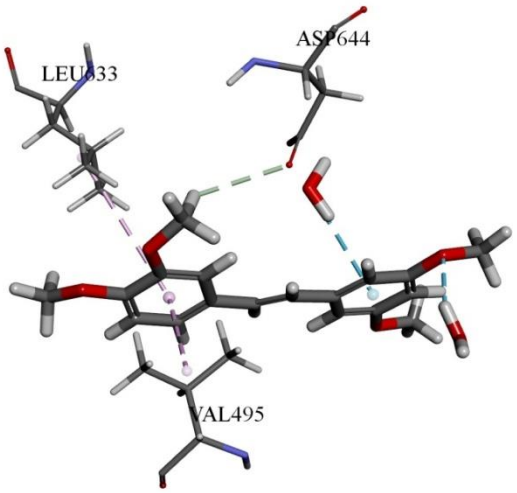

Figure 6: Docking interaction of FGFR2 (4j96) protein with isolated compound.

CONCLUSION

In conclusion, the presented study revealed that the methanolic extract from plant crotalaria medicaginea possesses invitro anticancer activity against the human cervical cancer cell line. A study has reported that substituted stilbene compound can inhibit the proliferation of HeLa cells having better inhibitory activities against human cervical carcinoma HeLa cells. It has shown that moderate cytotoxicity activity towards the HeLa cell line at IC50 concentrations. Docking results reported that the extracted ligand is well docked with target protein 4j96, it shown to a minimum CDOCKER scores. The structure of isolated styryl compound was characterized by FT-IR, 1H and 13C NMR spectroscopic techniques.

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