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# SYNTHESIS, CHARACTERIZATION, MOLECULAR DOCKING OF SOME NOVEL BENZOFURAN CHALCONE DERIVATIVES AND THEIR EVALUATION OF *INVITRO* ANTI CANCER ACTIVITY

**[1]*Kathiravan M., [2]Umarani G. and [3]Abdul Hassan Sathali**

Department of Pharmaceutical Chemistry, College of Pharmacy, Madurai Medical College, Madurai—625020.

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***Corresponding Author**
**Kathiravan M.**
Department of Pharmaceutical Chemistry, College of Pharmacy, Madurai Medical College, Madurai-625020.

## ABSTRACT

A series of Novel benzofuran chalcone derivatives have been synthesised in multiple steps with the suitable claisen schmidt condensation reaction procedures and then well characterized by various analytical techniques. All the synthesised compounds were evaluated for their invitro anticancer activity against two human cell lines includes Human breast cancer cells (MCF-7) and human liver cell lines (HepG2) at various concentrations. The results were shown in terms of percentage cell viability reduction and IC50 values. Amoung all the five synthsesied compounds, Compounds **5a** and **5b** has showed potent activity against breast cancer cells **(MCF-7)** And the synthesised compounds **5c** and **5d** has showed significant activity against Liver cells **(HepG2).** Furthermore, the structure and anticancer activity relationship was supported by molecular docking study of the major potent compounds 5a, 5b and 5c.The compounds 5a, 5b and 5c were dock against the two major target proteins. The PDB id of 1MOX (lung cancer) and 2DSQ (breast epithelian cancer) were selected and obtained from protein data bank (PDB) selected for INSILICO study and the protein-ligand binding site was analysed and visualized. Its results majorly compounds 5a and 5b are the best binding pose with targets.

**KEYWORDS:** Benzofuran chalcone derivatives, Claisen schmidt condensation reaction, *Invitro*- anticancer activity, *Insilico* molecular docking study.

## INTRODUCTION

Cancer is one of the leading causes of death worldwide, accounting for death of 82 lakh people in the year 2012. Top three death-causing cancers are lung cancer, liver cancer, and stomach cancer which killed 15.9 lakh, 7.45 lakh, and 7.23 lakh people, respectively, in the same year. On gender-wise, lung cancer is the leading cause of deaths in males while breast cancer in females, killing 10 lakh males and 5.21 lakh females in single year, respectively.

In India, 6.82 lakh people died because of various types of cancer during the same period, of which 48,697 males and 70,218 females died because of lung cancer and breast cancer, respectively. Worldwide, lung cancer is the foremost cause of cancer-related deaths, whereas in India, breast cancer is the topmost cause. Higher incidence of lung cancer can be accrued to increased air pollution or smoking while the reasons for breast cancer in India are late diagnosis due mainly to lack of awareness on early detection, barriers to health services or change in lifestyle such as obesity, late pregnancy, hormone replacement therapy, and lower lifetime duration of breast feeding.[1]

Chalcones an important intermediate of flavonoid synthetic pathway, has been shown to exhibit diverse biological and pharmacological activities. Chalcones are unsaturated ketone containing the reactive keto ethylenic group –CO CH=CH. These are coloured compounds because of the presence of chromophore. Chalcones are also called as benzalacetophenone or benzylidine acetophenone or phenyl styryl ketone. Chalcone derivative have wide variety of biological activities reported for these compounds include anti-inflammatory, anti-fungal, antibacterial, antimalarial and antitumor activity.[2]

Benzofuran derivatives are an interesting class of heterocyclic compounds. Benzofuran derivatives are of great interest in medicinal chemistry and have drawn remarkable attention due to their biological activities with **chemotherapeutic properties**. Some benzofurans bearing various substituents at the C-2 position are greatly distributed in nature; for example, ailanthoidol, a neolignan derivative, has been reported to have antiviral, antioxidant, and antifungal activities. Furthermore, most of the compounds prepared from 2- acetylbenzofuran have antimicrobial**, anticancer, antitumor**, anti-inflammatory, and antitubulin activities and are also used for treatment of cardiac arrhythmias.[3]

**Molecular Docking**

Molecular docking provides useful information about drug receptor interactions. It analyzes the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule.

Docking is considered to be a powerful simulation of the molecular recognition process. It is used to illustrate the probable molecular interaction of a designed ligand with the protein of interest, predict the affinity and activity of the ligand, and identify the energy of the interaction between the ligand and protein. It is an invaluable tool in the field of molecular biology, computational structural biology, computer-aided drug designing, and pharmacogenomics.

In present work, we describe the synthesis of Benzofuran chalcone derivatives (5a-e). in addition to this, all these compounds were evaluated for their invitro anticancer against 2 cells, viz Human breast carcinoma (MCF-7) and Human lung cancer (Hep G2) was performed at various concentrations. The results of cell viability interms of IC50 values. Furthermore, all these pharmacologically effective compounds were explored for molecular docking studies.

## MATERIALS AND METHODS

***2.1 Materials:*** All the reagents and solvents purchased from commercially available sources dimethyl sulfoxide (DMSO) fetal bovine serum (FBS) and DMEM (Dulbecco's Modified Eagle Medium). Double distilled water as used at all stages of experiments. Samples of chalcone compounds for testing were prepared at various concentrations. Analytical TLC was performed on Merck Silica Gel Precoated plates and visualization by I2 Vapours/UV light.

***2.2Characterization Techniques:*** Melting points were determined in open capillary tubes on ce100 labtronics. NMR spectra were determined on a Bruker AC 400 (400MHz) spectrometer with tetramethylsilane (TMS) as the internal standard in DMSOd6 or CDCl3 as solvents. FT-Infrared (FT-IR) spectra were recorded as KBr pellets on a Perkin-Elmer Spectrum One FTIR spectrometer.

***Synthesis of 1-(1-benzofuran-2-yl)ethanone***

A mixture of salicylaldehyde (1g, 4.97mmol) and potassium carbonate (0.69 g, 4.97mmol) in dry acetone (10 mL) was stirred at 25°C for 1 hour. Reaction mixture was cooled at 0–5°C,

and then chloroacetone (4 mL, 4.97mmol) was added dropwise. Reaction mixture was stirred at room temperature for ten minutes and then refluxed. Progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was poured on crashed ice. The precipitated solid was filtered, washed with water, and dried. The product was crystallized from ethanol.[23]

***General Procedure for Synthesis of Chalcones (5a–e)***

A solution of 1-(1-benzofuran-2-yl) ethanone (1 g, 4.18mmol) and Benzaldehyde (4.18mmol) in MeOH (10 mL) was cooled at 0–5°C and then 6mL of aqueous NaOH (1 mol/L) was added to this solution and stirred at room temperature for 3 hours. The reaction mixture was poured on crushed ice. The precipitated solid was filtered after neutralization with diluted HCl and was washed several times with water and then dried. The product was recrystallized from ethanol.[4]

**(2*E*)-1-(1-benzofuran-2-yl)-3-phenylprop-2-en-1-one *(5a)*.** Yield: 74%; M.p. 146°C; FT-IR (KBr, cm−1): 1566(C=O), 1647 (C=C); 1H-NMR (400MHz, DMSO-d6), ppm:7.79 (s, 1H, 5-H), 8.12 (s, 1H, 7-H), 7.92–7.84 (m, 4H, 13-H, 17-H, 10-H, 11-H), 7.84–7.65 (m, 2H, 3-H, 2-H), 7.65–7.40 (m, 3H, 16-H, 15-H, 14-H); 13C-NMR (400MHz, DMSO-d6): 178.96, 154.59, 154.55, 144.46, 134.76, 131.63, 131.46, 129.64, 129.50, 129.46, 126.49, 122.18, 116.77, 114.88, 114.56.

**(2*E*)-1-(1-benzofuran-2-yl)-3-(4-chlorophenyl)prop-2-en-1-one** *(5b)*. Yield: 76%; M.p. 149°C; FT-IR (KBr, cm−1): 1658 (C=O), 1652 (C=C); 1H-NMR (400MHz, DMSO-d6), ppm: 8.29 (s, 1H, 5-H), 8.15 (s, 1H, 7-H), 8.00– 7.62 (m, 8H, 3-H, 2-H, 17-H, 16-H, 14-H, 13-H, 10-H, 11-H); 13C-NMR (400MHz, DMSO-d6): 178.90, 154.58, 154.52, 143.18, 134.04, 132.47, 131.78, 131.68, 131.43, 129.62, 126.59, 124.98, 122.87, 116.84, 114.98.

**(2*E*)-1-(1-benzofuran-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one** *(5c)*. Yield: 72%; M.p. 154°C; FT-IR (KBr, cm−1): 1577 (C=O), 1650 (C=C); 1H-NMR (400MHz,DMSO-d6), ppm: 8.78 (s, 1H, 5-H), 8.37–8.27 (m, 13-H, 15-H, 17-H), 8.13 (s, 1H, 7-H), 8.08 (d, 1H, *J* = 15.6Hz, 11-H), 7.92 (d, 1H, *J* = 16Hz, 10-H), 7.78–7.68 (m, 3H, 3-H, 2-H, 16-H); 13C-NMR (400MHz, DMSO-d6): 178.73, 154.67, 154.40, 148.95, 141.86, 136.66, 135.70, 131.87, 130.92, 129.57, 126.59, 125.43, 124.88, 123.51, 116.85, 115.39, 114.93.

**(2*E*)-1-(1-benzofuran-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one** *(5d).* Yield: 69%; M.p. 138°C; FT-IR (KBr, cm−1): 1566 (C=O), 1658 (C=C); 1H-NMR (400MHz, DMSO-d6), ppm: 8.10 (s, 2H, 5-H, 7-H), 7.78–7.5 (m, 6H, 3-H, 2-H, 13-H, 17-H, 10-H, 11-H), 6.77 (d, 1H,*J* = 2.8Hz, 14-H), 6.75 (d, 1H, *J* = 3.6Hz, 16-H), 3.02 (s,6H, CH3); 13C-NMR (400MHz, DMSO-d6): 178.52, 155.35, 154.31, 152.75, 145.69, 131.61, 131.10, 129.85, 126.24, 122.00,116.64, 115.98, 114.83, 112.98, 112.22, 40.56–39.31.

**(2*E*)-1-(1-benzofuran-2-yl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one** *(5e).* Yield: 65%; M.p. 144°C; FT-IR (KBr, cm−1): 1604 (C=O), 3400 (C-OH); 1H-NMR (400MHz, DMSO-d6), ppm:8.03–7.96 (m, 3H, 5-H, 7-H, 15-H), 7.74–7.62 (m, 3H, 3-H, 2-H, 11-H), 7.55–7.40 (d, 1H, *J* = 15.2Hz, 10-H), 7.14 (s, 1H, 13-H), 6.71 (s, 1H, 14-H); 13C-NMR (400MHz, DMSO-d6):178.56, 154.54, 154.41, 151.34, 147.15, 131.48, 130.78, 129.65, 126.37, 118.73, 118.50, 116.72, 114.85, 114.57, 113.77.

### *2.3 Invitro Anticancer Activity*

**Principle**

**MTT**, a yellow tetrazole, is reduced to purple formazan in living cells. A solubilization solution (usually either dimethyl sulfoxide, an acidified ethanol solution, or a solution of the detergent sodium dodecyl sulfate in diluted hydrochloric acid) is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. The degree of light absorption depends on the solvent.


3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide **(MTT)**

(*E*,*Z*)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan **(Formazan)**

Tetrazolium dye reduction is dependent on NAD(P)H-dependent oxido reductase enzymes largely in the cytosolic compartment of the cell. Therefore, reduction of MTT and other tetrazolium dyes depends on the cellular metabolic activity due to NAD(P)H flux. Cells with a low metabolism such as thymocytes and splenocytes reduce very little MTT. In contrast, rapidly dividing cells exhibit high rates of MTT reduction.

**MTT Assay Method**

The MTT assay is a standard colorimetric non-radioactive assay for measuring viable cell and cytotoxicity through increased metabolism of the tetrazolium salt. Cancer cells (1 x $10^{-5}$ cells/ml) were seeded into 96 well plates and incubated for 24 hours incubation. Then the cells were treated with different concentration of drug formulation (10-200µg/ml). then, the cells were incubated in the presence of 5% CO2 at $37^0$C for 24 hours. After incubation, MTT (0.5 mg/mL) was added to the incubated cells. Then cells were incubatedfor another 4 hours. Then 100µL of DMSO were added into each well and mixed well. Absorbance was measured in a multimode reader at 570 nm.[3]

***2.4 Insilico Docking Analysis***

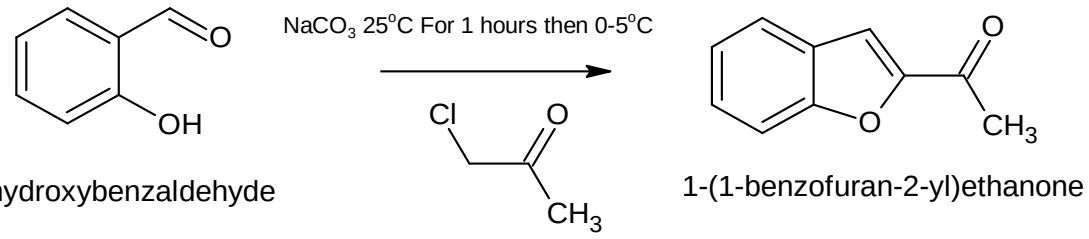
Geometry optimized molecular structures for all synthesized derivatives were obtained using AUTODOCK 4.0 (A Generic Evolutionary Method for molecular docking) automated docking program. AUTODOCK 4.0 is software used for integrated structure based virtual screening, molecular docking, post screening analysis and visualization step. The 3-dimension (3D) coordinates of four cancer target proteins were selected and obtained from protein data bank (PDB). The PDB id of 1MOX (lung cancer) and 2DSQ (breast epithelian cancer) were selected for INSILICO study. The 3D structure coordinates of each therapeutic target protein and ligand molecules were implemented through the AUTODOCK 4.0 graphical environment interface. Before doing docking analysis, the output path was set. AUTODOCK 4.0.

**RESULT AND DISCUSSION**

***3.1 Chemistry:*** The synthetic route for the desired (2E)-1-benzofuran-2-yl)-3-phenylprop-2-en-1-one derivatives (5a-e) were summarized in scheme. The synthetic compound derivatives were constructed by commercially available salicylaldehyde (1) and chloroacetone (2) in presence of Sodium carbonate at 5℃ for one hour it forms (3). The intermediate compound (3) with aromatic aldehyde (4a) in presence of methanol was cooled at 0-5℃ and then 6ml of aqueous NaOH at room temperature for 3 hours upon claisen Schmidt condensation reaction lead to final product.

**SCHEME**

**STEP 1**



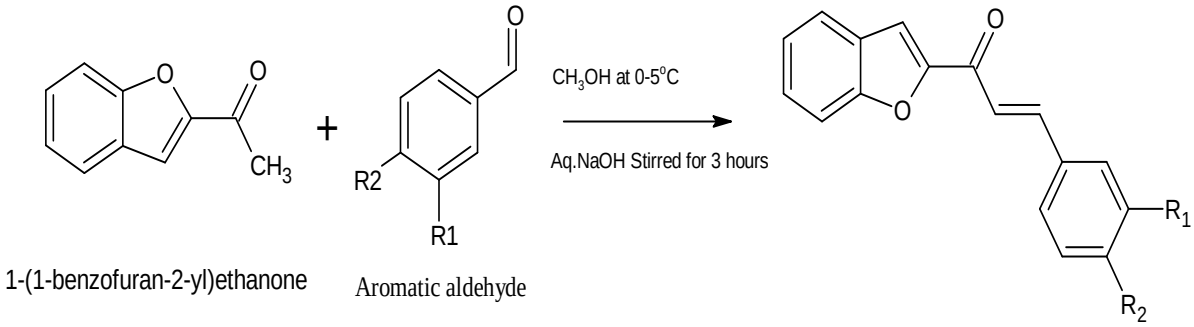

2-hydroxybenzaldehyde

1-chloropropan-2-one

1-(1-benzofuran-2-yl)ethanone

**(1)** **(2)** **(3)**

**STEP-2**




1-(1-benzofuran-2-yl)ethanone

Aromatic aldehyde

**(3)** **(4)** **(5a-e)**

**SUBSTITUTIONS**

| COMPOUND | $R_1$ | $R_2$ |
|---|---|---|
| 3a | H | H |
| 3b | H | Cl |
| 3c | H | $OCH_3$ |
| 3d | H | $NO_2$ |
| 3e | $CH_3$ | OH |

**Scheme:** Conditions (1) NaCO3 for 1 hr then reflux (2) CH3OH with aqueous NaOH stirred for 3hrs at RT.

***3.2 Structural Characterization:*** All the synthesized compounds were confirmed by analytical and spectroscopic data. In the FT-IR spectra of the compounds (5a-e) were showed characteristic bands between 1564 and 1604 $cm^{-1}$ (C=O Stretching at chalcone) and between 1647 1nd 1658 $cm^{-1}$ (C=C Strectching at chalcone). The compound (5a-e) confirmed by representative signals in 1H NMR, observed at 8.29-8.03 ppm(B3-H at Benzofuran ring) and at 7.80-7.40 ppm (α-H and β-H of chalcone moiety) The carbonyl carbon was observed at about 178ppm in the 13C NMR Spectra of 5a-e.These were further confirmed by mass spectrum of the compounds (5a-e) molecular mass 248,282,278,293,294 respectively.

***3.3. Anticancer Activity:*** synthesized novel benzofuran chalcone derivatives 5a-e were evaluated for their invitro anticancer activity against a panel of Human breat cancer and lung cancer cell lines MCF-7 and Hep G2 By MTT Colorimetric assay as per ATCC protocol and the results were performed in table-1 as percentage of inhibition and interms of IC50 Values. Cell viability of all the two human cell lines were also presented separately as shown in Fig 1-5 demonstrates cell viability on Hep G2; Fig 6-10 reveals the cell viability on MCF-7 cells at various concentrations.

From the results, the synthesized compound 5a and 5b were potent activity against breast cancer cells (MCF-7) and the synthesized compound 5c and 5d shown significant activity against liver cells (Hep G2).

**Table 1.**

| S.No | Compound | IC50 Values | |
|---|---|---|---|
| | | Hep G2 | MCF-7 |
| 1 | 5a | 88.23 | 53.39 |
| 2 | 5b | 90.76 | 56.41 |
| 3 | 5c | 73.63 | 69.65 |
| 4 | 5d | 72.95 | 80.22 |
| 5 | 5e | 123.45 | 84.36 |

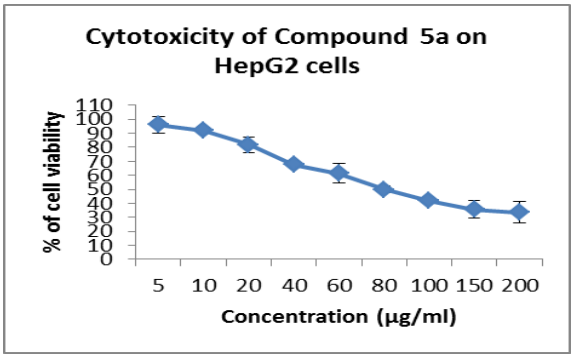



**Fig-1**

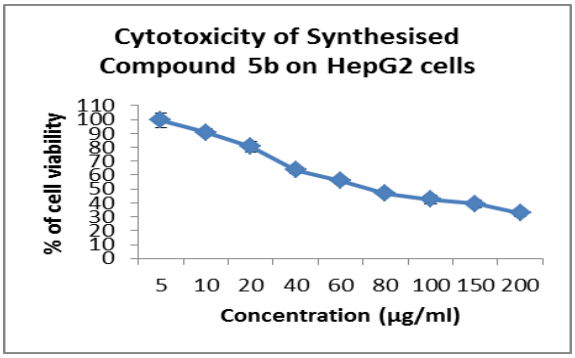



**Fig-2**

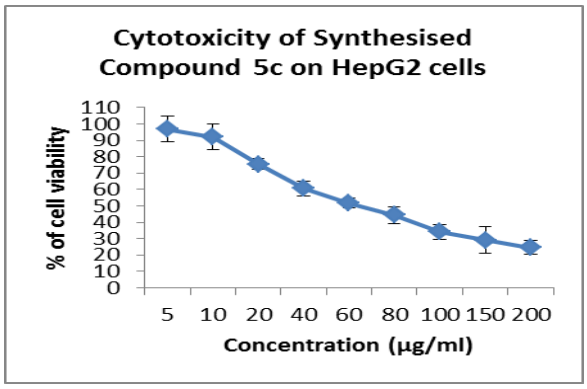



**Fig-3**

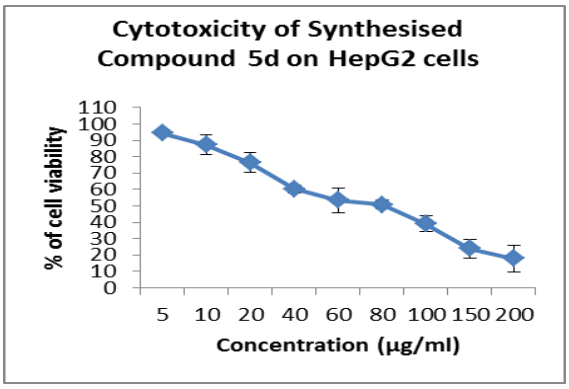



**Fig-4**

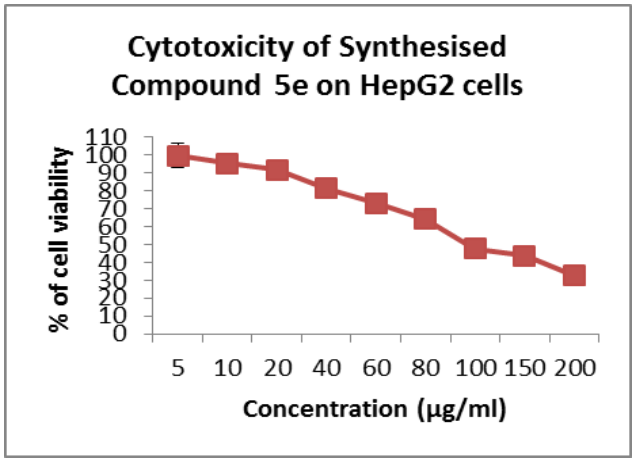



**Fig-5.**

**Fig 1-5: Invitro anticancer activity of compounds 5a-e measured by MTT Aaasay on Human lung cancer (Hep G2) with various concentrations for 48 hours.**

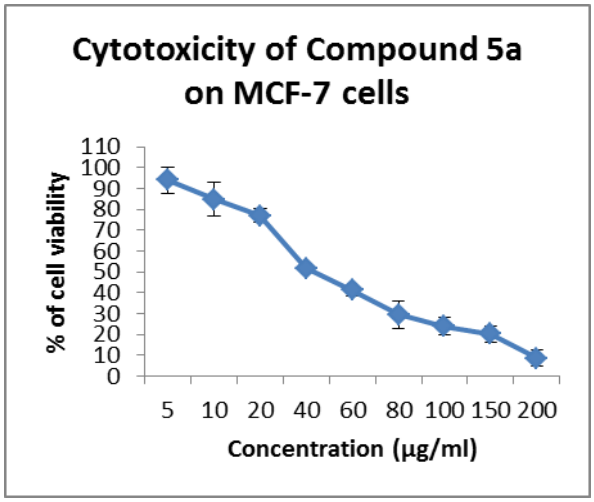



**Fig-6**

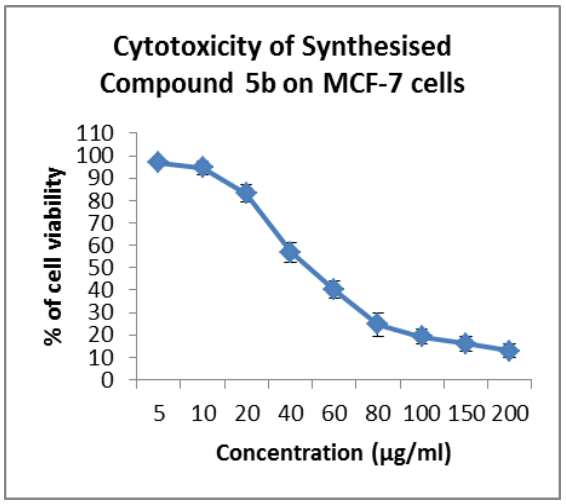



**Fig-7**

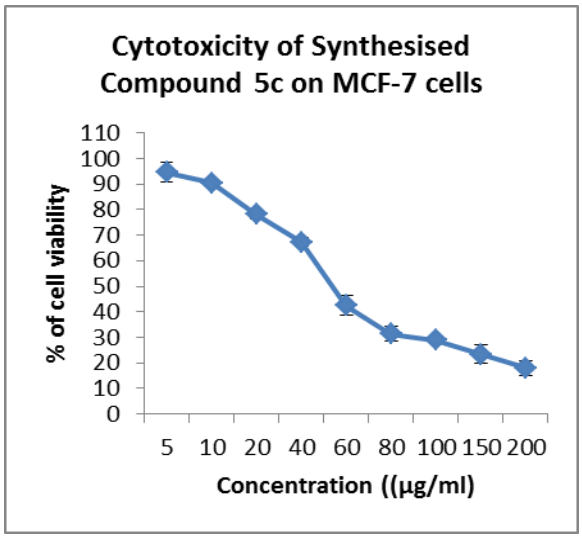



**Fig-8**

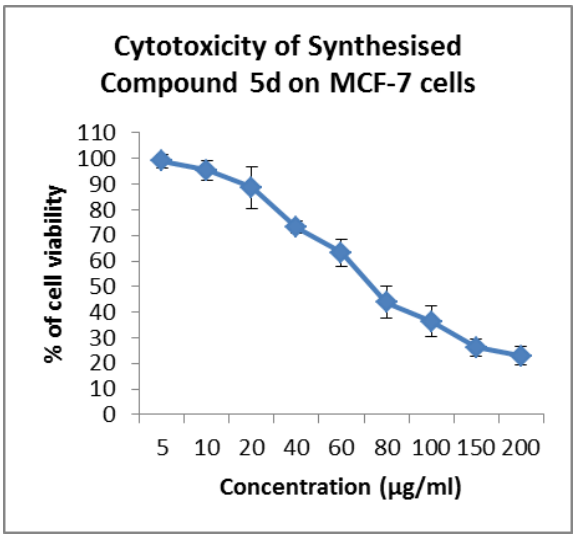



**Fig-9**

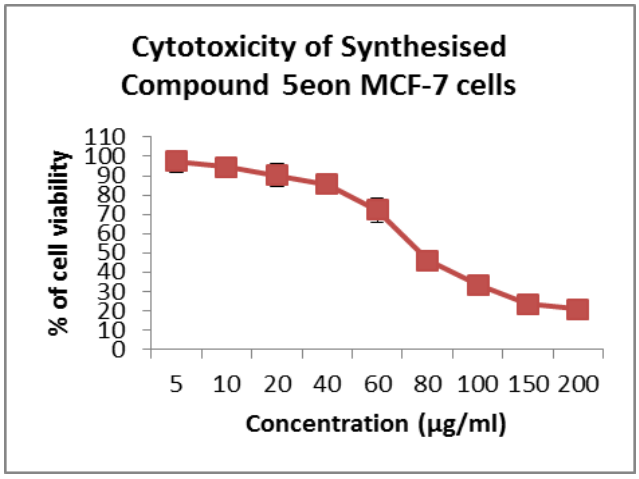



**Fig-10.**

**Fig 6-10: Invitro anticancer activity of compounds 5a-e measured by MTT Aaasay on Human breast cancer (MCF-7) with various concentrations for 48 hours.**

***Docking studies***

From the docking studies, the PDB id of 1MOX (Lung cancer) and 2DSQ (Breast epithelium cancer) were selected and obtained from protein data bank (PDB), In the post docking screening the best binding pose and total energy of each ligand was analysed and visualized. Its results majorly compounds 5a and 5b are the best binding pose with targets. Fig A, B,C represents the docking poses with ligand interaction diagram of compound 5a,5b,5c molecules in the target of 1MOX (Lung cancer) Fig D, E,F represents the docking poses with ligand interaction diagram of compound 5a,5b,5c molecules in the target of 2DSQ (Breast epithelial cancer) And the docking scores with binding energies were given in table 2 & 3.

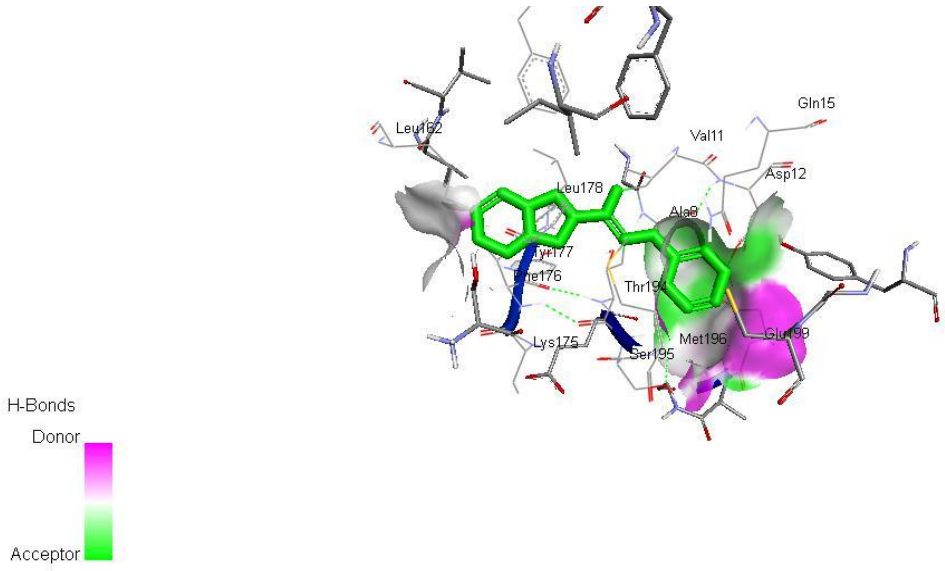



**Fig (a) Binding of Compound 5a with 2DSQ.**

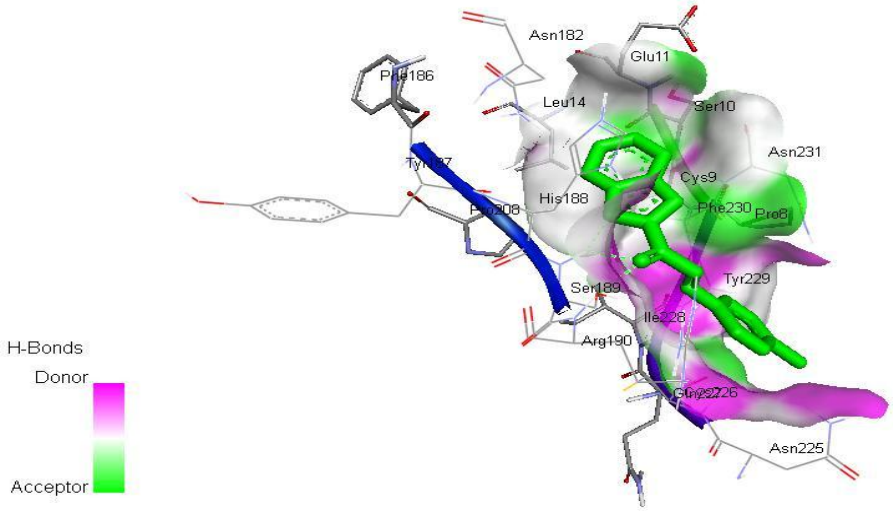



**Fig (b): Binding of Compound 5b with 2DSQ.**

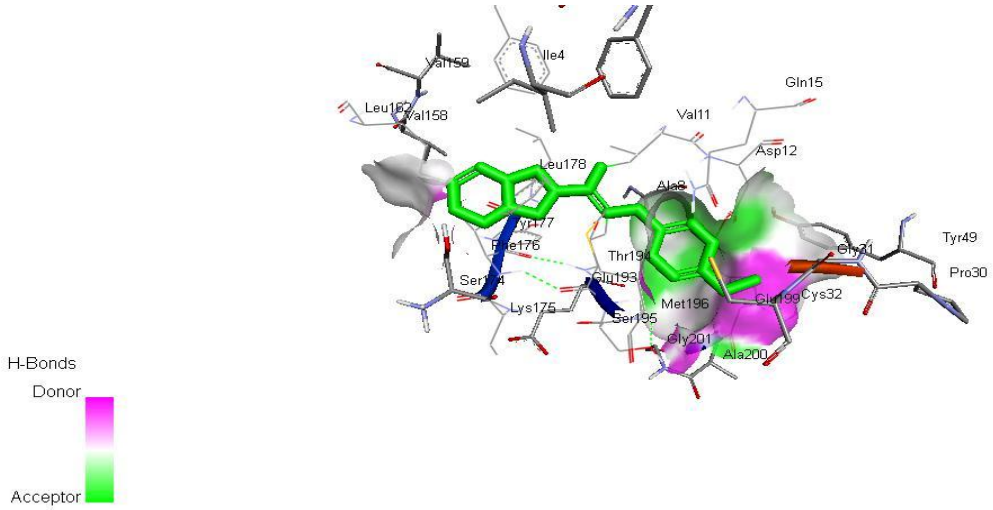



**Fig (c): Binding of Compound 5c with 2DSQ.**

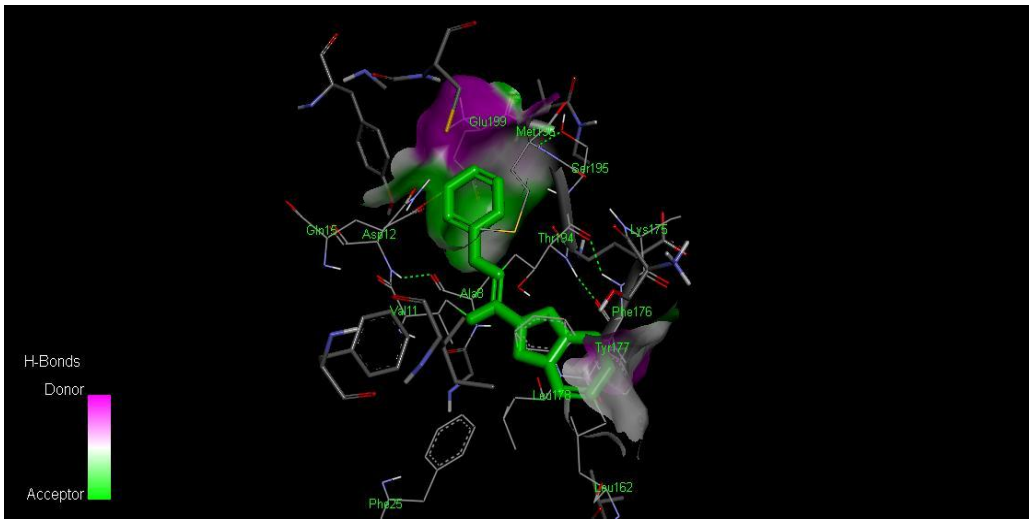



**Fig (d): Binding of Compound 5a with 1MOX.**

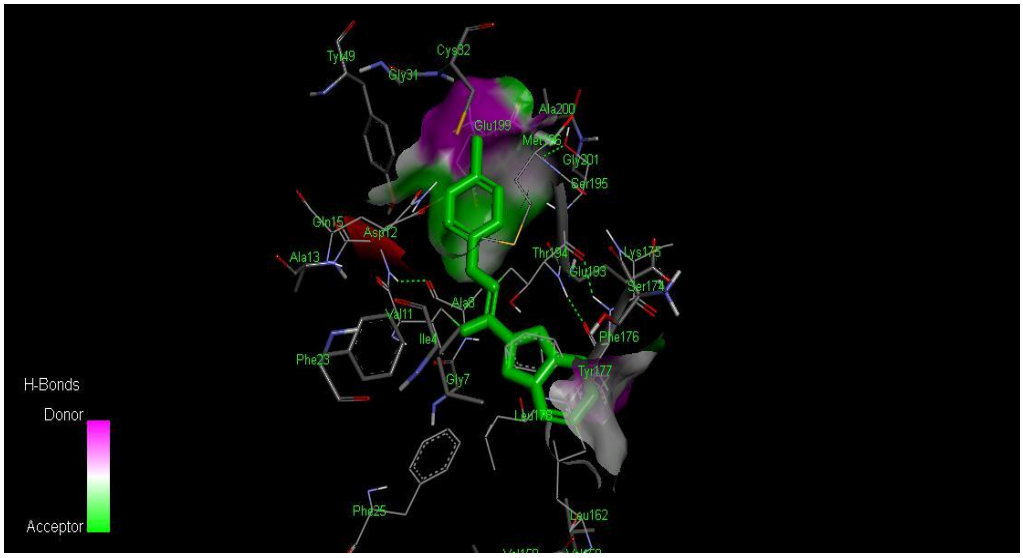



**Fig (e): Binding of Compound 5b with 1MOX.**

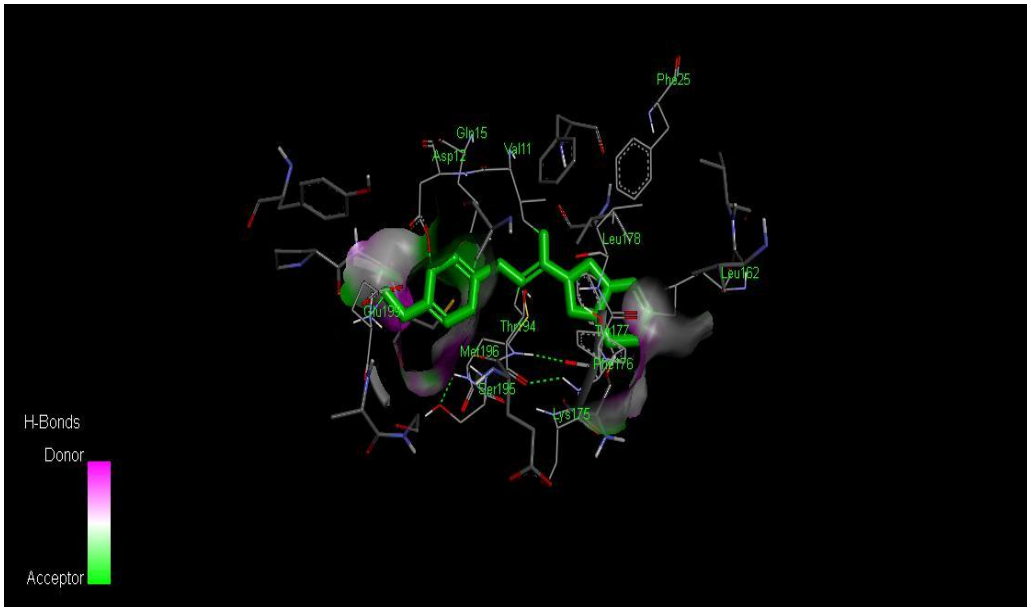



**Fig (f): Binding of Compound 5c with 1MOX.**

**Table 2: With Breast epithelian cancer-2DSQ.**

| Compound | Target protein | Binding energy |
|---|---|---|
| 5a | 2DSQ | -10.63 |
| 5b | 2DSQ | -7.14 |
| 5c | 2DSQ | -6.83 |

**Table 3: With Lung cancer-1MOX.**

| Compound | Target protein | Binding energy |
|---|---|---|
| 5a | 1MOX | -6.13 |
| 5b | 1MOX | -10.74 |
| 5c | 1MOX | -4.11 |

## CONCLUSION

A series of novel (2E)-1-(1-Benzofuran-2-yl)-3-phenyl prop-2-en-1-one and derivatives were synthesized and well characterized by using various spectral techniques. All the synthesized compounds were examined for their anticancer activity against human cell lines viz (HepG2 and MCF-7) at various concentrations and the results were performed by means of cell viability. Among all the compounds, Compound 5a, 5b and 5c showed potent activity against MCF-7 and compound 5c and 5d showed significant activity against HepG2. Based on the anticancer activity, docking and molecular design studies, the synthesized molecule can be considered as promising lead molecules for the development of new anticancer medicines.

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