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# Synthesis of 2-(Bis(2-chloroethyl)amino)-N-(4-((3-oxobenzofuran-2(3H)-ylidene)methyl)phenyl)acetamide Derivatives on Basis of Benzaldehydes and Acetophenones As Possible Alkylating Anticancer Agents

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## ABSTRACT

A series of 2-(Bis(2-chloroethyl)amino)-N-(4-((3-oxobenzofuran-2(3H)-ylidene)methyl) phenyl)acetamide derivatives were synthesized by fusing aurones with nitrogen mustards. Aurones being synthesized by treating 4-nitrobenzaldehydes with various derivatives of 1-(2-hydroxyphenyl)ethenone. The characterization of the synthesized compounds was done by FTIR, 1H NMR and LCMS spectral studies. The titled compounds were tested for their possible anticancer activities by *in vitro* methods by SRB Assay. These compounds were found to exhibit a moderate anticancer activity.

**Keywords:** Aurone, nitrogen mustard, anticancer, SRB Assay using A-549 and MCF-7 Cell lines

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## INTRODUCTION

4-nitrobenzaldehydes and 1-(2-hydroxyphenyl)ethanones are reasonable and easily available reagents for organic synthesis, It is been reported that benzofuranones can be effectively synthesized by fusing benzaldehydes with acetophenones.[1] Aurones, are a class of flavonoids compound which are available naturally as secondary metabolites and also synthesized in lab sources. They are a class of compounds are known to have a wide range of biological activity.[2-5] Based on our past studies regarding the synthesis of aurones by condensation of benzaldehydes with acetophenones, we synthesized few derivatives of 2-(Bis(2-chloroethyl)amino)-N-(4-((3-oxobenzofuran-2(3H)-ylidene)methyl)phenyl)acetamide.[6] It is well known that nitrogen mustards are used as alkylating neoplastic agents which interferes with cellular proliferation.[7-9] In the present work, we have made an effort to synthesize a series of substituted -(Bis(2-chloroethyl)amino)-N-(4-((3-oxobenzofuran-2(3H)-ylidene)methyl)phenyl)acetamide derivatives from 4-nitrobenzaldehydes and 1-(2-hydroxyphenyl)ethanones to synthesize aurones and further by fusing of nitrogen mustard to the synthesized aurones. The synthesized compounds were subjected to *In-vitro* cytotoxic activity by SRB assay procedure using A-549 and MCF-7 Cell lines.

## MATERIALS AND METHOD

The chemicals and the solvents used in the present project work were purchased from Sigma Aldrich and Merck India. Thin Layer Chromatography (TLC):- Purity of the synthesized compounds and progress of reactions were monitored by Thin layer chromatography using silica gel-G as stationary phase and various mobile phases were used such as n-Hexane: Ethyl acetate (2:1), $CH_2Cl_2$:MeOH (9:1), Hexane: Acetic acid (9:1), Hexane: Ethyl acetate : Acetic acid (various ratio's like 5:4:1, 4:4:2, 3:6:1, 2:7:1), Chloroform : Ethyl acetate : Acetic acid (5:4:1). The spots resolved were visualized using UV and Iodine chamber.

IR Spectra: The IR spectra of the synthesized compounds were recorded on a Fourier Transform IR spectrometer (model Shimadzu 8400S) in the range of 400-4000 by KBr pellet method and the values of Vmax are reported in cm-1. 1H NMR spectra: Nuclear magnetic resonance spectra were obtained on 400 MHz Bruker Supercon using DMSO. The chemical shifts (δ) are reported in parts per million downfield from standard internal reference Tetramethyl silane (TMS). Mass spectra were recorded on a LCMS 2010, SHIMADZU, JAPAN using Auto spectra ionization negative ion mode.

**General Procedure for the synthesis of compounds:**

Step -1: Synthesis of (4-nitrobenzylidene)benzofuran-3(2H)-one (3)derivatives.[10]

To equimolar concentration of (1) 4-nitrobenzaldehyde and (2) substituted acetophenones ($R_1$-$R_7$), 10 mL methanol was added and 800 mg sodium hydroxide was added. The contents were properly

dissolved using sonication for 180 seconds. The mixture was mixed at room temperature for 48 hrs. Reaction was monitored by TLC with solvent system- Hexane: Ethyl acetate (2:1). The mixture was poured into ice to get a yellow precipitate. The precipitate was washed with ice cold water, filtered and recrystallized with ethanol and air dried. The dried recrystallized compound (3) was odorless yellowish cream colour. The mass and IR of the intermediate compound formed was checked.

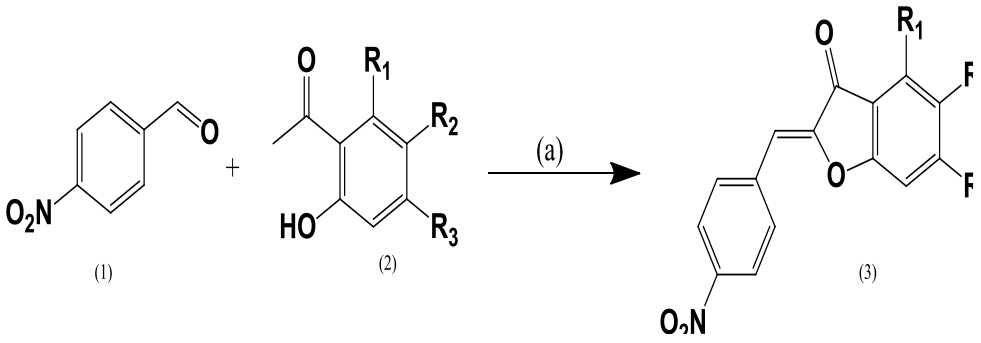


**Figure 01: Synthesis of (4-nitrobenzylidene) benzofuran-3(2H)-one (3)derivatives Reagents and conditions: (a) NaOH, Methanol, rt, 5 hours**

Step 2: Reduction of Nitro group of (4-nitrobenzylidene) benzofuran-3(2H)-one (4)derivatives to amino group.[11]

To the solution of (4-nitrobenzylidene) benzofuran-3(2H)-one derivatives (1mmol) in ethanol 5 mL was added $SnCl_2.2H_2O$ (10mmol). The reaction mixture was exposed to ultrasonic irradiation for 2 h at 30 °C until the reaction was complete as indicated by TLC analysis. The solvent was removed under reduced pressure and the crude residue was partitioned between ethyl acetate and 2M KOH. The aqueous layer was extracted with further portions of ethyl acetate (3 x 25 mL) and the combined organic extracts were washed with brine (2 x 25 mL) and water (3 x 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure to get (4) derivatives of (4-aminobenzylidene)benzofuran-3(2H)-one.

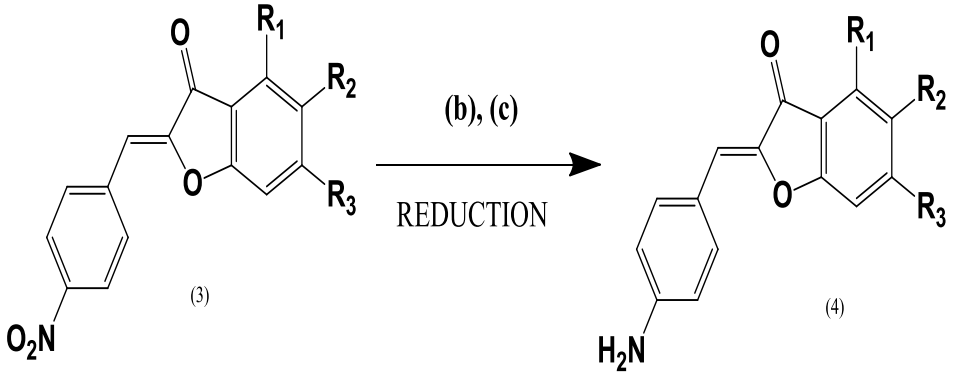


**Figure 02: Reduction of Nitro group of (4-nitrobenzylidene)benzofuran-3(2H)-one (4)derivatives to amino group**

**Reagents and conditions: (b) $SnCl_2.2H_2O$ (c) Ethanol: Glacial Acetic Acid: Water, 5 equivalents, sonication, 30 ºC, 1-2 hours**

Step -3 (Acetylation) Linking of Chloroacetyl chloride at $NH_3$ of (4-amino benzylidene)benzofuran-3(2H)-one (5)derivatives.[12]

(4-aminobenzylidene)benzofuran-3(2H)-one derivatives (0.05 mol) was dissolved in 40 ml of 5% aqueous NaOH in cold and more water was added if necessary. If the solution was highly coloured, charcoal treatment was given. Then the solution was poured into 100 ml stoppered flask and to this was added 0.1 mol of chloro acetyl chloride and the reaction mixture was shaken vigorously until the odour of chloroacetyl chloride disappeared completely. The separated precipitate (5) was filtered, washed with cold water and purified by recrystallization from 90% ethanol.

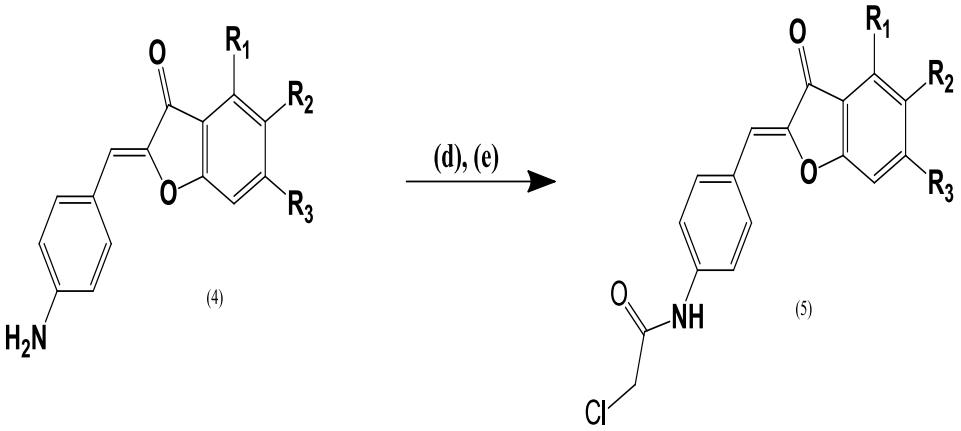


**Figure 03: (Acetylation) Linking of Chloroacetyl chloride at $NH_3$ of (4-amino benzylidene)benzofuran-3(2H)-one (5)derivatives.**

**Reagents and conditions: (d) EtOH, (e) $COClCH_2Cl$**

Step-4: Linking of Bis (2-chloroethyl) amine to the chloro acetyl derivatives.[13]

Chloro acetyl derivative (0.1 mole) was dissolved in Bis (2-chloroethyl) amine (0.012 mole) in pyridine and refluxed for 3 hrs. It was cooled and decomposed in ice water. The product was then filtered, dried and recrystallized using suitable solvent.

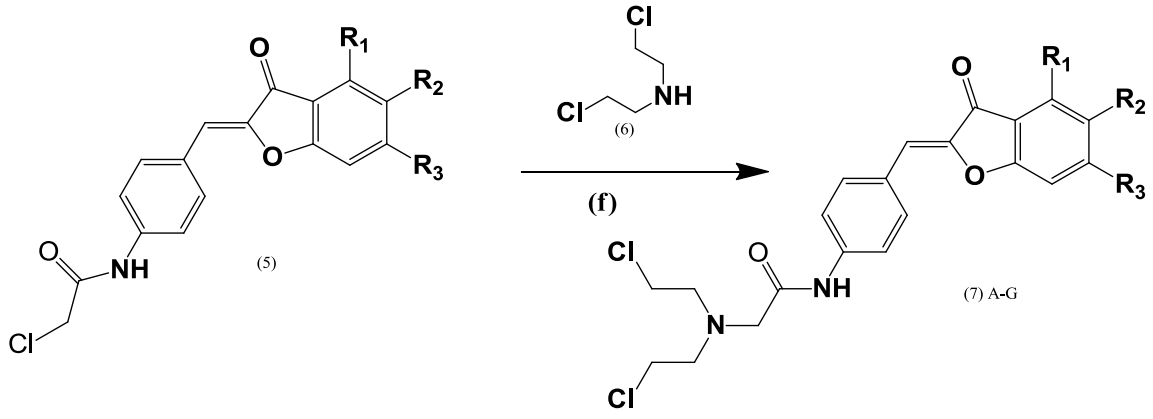

**Figure 04: Linking of Bis (2-chloroethyl) amine to the chloroacetyl derivatives. Reagents and conditions: (f) Pyridine**

**In-vitro cytotoxic studies**[14]

The monolayer cell culture was trypsinized and the cell count adjusted to 1.0 x 105 cells/ml using growth medium. To each well of a 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells/well) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once and 100ml of drug dilution prepared in maintenance media was added per well in microtitre plates. The plates were then incubated at 37 ºC for 3 days in 5% $CO_2$ atmosphere, and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, 25 ml of 50% trichloro-acetic acid was added to the wells gently such that it forms a thin layer over the drug dilutions to form a overall concentration of 10%. The plates were then incubated at 4 ºC for one hour. The plates were flicked; culture was washed five times with tap water to remove traces of medium, drug and serum, and was then air-dried. The air-dried plates were stained with SRB for 30 minutes. The unbound dye was then removed by rapidly washing four times with 1% acetic acid. The plates were then air-dried. 100µl of 10mM Tris base was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 minutes. The absorbance was measured using microplate reader at a wavelength of 540nm.

The percentage growth inhibition was calculated using the formula below:

$$\%\ Growth\ inhibition = 100 - \left[\frac{Mean\ OD\ of\ Individual\ Test\ Group}{Mean\ OD\ of\ Control\ Group}\right] X\ 100$$

## RESULTS AND DISCUSSION

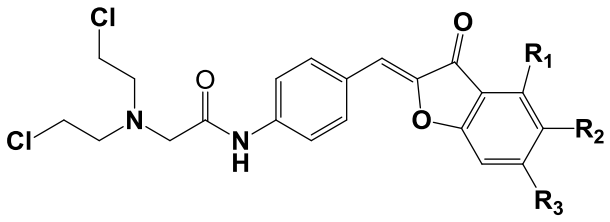


**Figure 05: General Structure of 2-(Bis(2-chloroethyl)amino)-N-(4-((3-oxobenzofuran-2(3H)-ylidene)methyl)phenyl)acetamide**

The Compound codes, R group of the various synthesized compounds, their molecular weight and their m/z details is shown in Table 1

**Table 1: Details of the Synthesized Derivatives**

| Sl No. | Compound Code | $R_1$ | $R_2$ | $R_3$ | Molecular weight | % Yield | m/z |
|---|---|---|---|---|---|---|---|
| 1 | (7) A | OH | H | H | 435.30 | 72 | 435.11 |
| 2 | (7) B | H | OH | H | 435.30 | 76 | 434 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 3 | (7) C | H | H | OH | 435.30 | 70 | 434.08 |
| 4 | (7) D | H | H | H | 419.30 | 75 | 418 |
| 5 | (7) E | H | $CH_3$ | H | 433.33 | 70 | 434 |
| 6 | (7) F | H | Cl | H | 453.75 | 64 | 452 |
| 7 | (7) G | H | Br | H | 498.30 | 68 | 497 |

**Spectral Data**

Compound No (7) A: 2-(Bis(2-chloroethyl)amino)-N-(4-((4-hydroxy-3-oxobenzofuran-2(3H)-ylidene)methyl)phenyl)acetamide; Yellow Solid, 3428.92 (Ar-OH), 3225.17 (CH-CH), 1722.43 (C=O), 1294.11 (C-O-C), 739 .54 (C-Cl), $^1$H NMR ($CDCl_3$): δ 3.46 (s, 1H, Ar-OH), 2.69-3.52 (t, 8H, $(CH_2Cl)_2$), 3.52 (s, 2H, $CH_2C$=O), 5.37 (s, 1H, CNH), 7.23 (s, C=CH), 7.05-7.83 (m, 7H, ArH), m/z: 435.11

Compound No (7) B : 2-(Bis(2-chloroethyl)amino)-N-(4-((5-hydroxy-3-oxobenzofuran-2(3H)-ylidene)methyl)phenyl)acetamide; Yellow Solid, 3327.45 (Ar-OH), 3194.86 (CH-CH), 1792.64 (C=O), 1325.29 (C-O-C), 801.32 (C-Cl), $^1$H NMR ($CDCl_3$): δ 3.58 (s, 1H, Ar-OH), 2.9-3.88 (t, 8H, $(CH_2Cl)_2$), 3.62 (s, 2H, $CH_2C$=O), 6.84 (s, 1H, CNH), 7.29 (s, C=CH), 6.92-7.65 (m, 7H, ArH), m/z: 434

Compound No (7) C : (2-(Bis(2-chloroethyl)amino)-N-(4-((6-hydroxy-3-oxobenzofuran-2(3H)-ylidene)methyl)phenyl)acetamide; Yellow Solid, 3467.34 (Ar-OH), 3261.85 (CH-CH), 1830.26 (C=O), 1398.65 (C-O-C), 821.33 (C-Cl), $^1$H NMR ($CDCl_3$): δ 3.62 (s, 1H, Ar-OH), 2.9-3.89 (t, 8H, $(CH_2Cl)_2$), 3.58 (s, 2H, $CH_2C$=O), 6.51 (s, 1H, CNH), 7.31 (s, C=CH), 6.83-8.09 (m, 7H, ArH), m/z: 434.08

Compound No (7) D : 2-(Bis(2-chloroethyl)amino)-N-(4-((3-oxobenzofuran-2(3H)-ylidene)methyl)phenyl)acetamide; Yellow Solid, IR spectrum ($cm^{-1}$): 3249.27 (CH-CH), 1788.21 (C=O), 1299.19 (C-O-C), 760 (C-Cl), $^1$H NMR ($CDCl_3$): δ 2.99, 3.89 (t, 8H, $(CH_2Cl)_2$), 3.52 (s, 2H, $CH_2C$=O), 8.12 (s, 1H, CNH), 7.25 (s, C=CH), 7.29-7.65 (m, 8H, ArH), m/z: 418

Compound No (7) E : 2-(Bis(2-chloroethyl)amino)-N-(4-((5-methyl-3-oxobenzofuran-2(3H)-ylidene)methyl)phenyl)acetamide ;Yellow Solid, 3229.09 (CH-CH), 1746.15 (C=O), 1322.83 (C-O-C), 787.12 (C-Cl), $^1$H NMR ($CDCl_3$): δ 2.46(s, 3H, $CH_3$), 2.99, 3.88 (t, 8H, $(CH_2Cl)_2$), 3.58 (s, 2H, $CH_2C$=O), 7.9 (s, 1H, CNH), 7.05 (s, C=CH), 7.31-7.65 (m, 7H, ArH), m/z: 434

Compound No (7) F: 2-(Bis(2-chloroethyl)amino)-N-(4-((5-chloro-3-oxobenzofuran-2(3H)-ylidene)methyl)phenyl)acetamide ;Yellow Solid, IR spectrum ($cm^{-1}$): 3320.19 (CH-CH), 1813.32 (C=O), 1384.32 (C-O-C), 812.61 (C-Cl), $^1$H NMR ($CDCl_3$): δ 2.99, 3.89 (t, 8H, $(CH_2Cl)_2$), 3.88 (s, 2H, $CH_2C$=O), 8.12 (s, 1H, CNH), 7.26 (s, C=CH), 7.33-7.65 (m, 7H, ArH), m/z: 452

Compound No (7) G : 2-(Bis(2-chloroethyl)amino)-N-(4-((5-bromo-3-oxobenzofuran-2(3H)-ylidene)methyl)phenyl)acetamide ;Yellow Sticky Solid, 3313.46 (CH-CH), 1799.14 (C=O), 1389.22 (C-O-C), 796.34 (C-Cl), 584.76 (Ar-Br), $^1$H NMR ($CDCl_3$): δ 2.99, 3.89 (t, 8H, $(CH_2Cl)_2$), 3.88 (s, 2H, $CH_2C$=O), 8.15 (s, 1H, CNH), 7.06 (s, C=CH), 7.33-7.59 (m, 7H, ArH), m/z:497

**Cytotoxicity studies**

The cytotoxic properties of all the synthesized compounds is been shown in Table 02 and Figure 06

**Table 2: Cytotoxic Properties (SRB) of The Test Substances on MCF-7 Cells**

| Sl No | Test Substance | SRB (MCF-7) | | | | |
|---|---|---|---|---|---|---|
| | | Test Conc (µg/ml) | % CTC | SEM (±) | CTC 50 (µg/ml) | SEM (±) |
| 1 | (7) A | 1000 | 55.33 | 0.46 | 711.132 | 4.57 |
| | | 500 | 49.42 | 3.54 | | |
| | | 250 | 41.47 | 0.5 | | |
| | | 125 | 38.11 | 2.79 | | |
| | | 62.5 | 16.97 | 5.37 | | |
| 2 | (7) B | 1000 | 59.25 | 0.41 | 604.324 | 7.22 |
| | | 500 | 49.21 | 0.65 | | |
| | | 250 | 44.65 | 1.43 | | |
| | | 125 | 26.43 | 1.91 | | |
| | | 62.5 | 12.23 | 2.74 | | |
| 3 | (7) C | 1000 | 63.18 | 3.22 | 536.157 | 4.91 |
| | | 500 | 48.22 | 0.34 | | |
| | | 250 | 34.54 | 3.11 | | |
| | | 125 | 15.86 | 2 | | |
| | | 62.5 | 9.2 | 3.4 | | |
| 4 | (7) D | 1000 | 52.36 | 1.43 | 488.187 | 5.2 |
| | | 500 | 49.98 | 1.23 | | |
| | | 250 | 38.25 | 2.64 | | |
| | | 125 | 18.28 | 2.48 | | |
| | | 62.5 | 10.41 | 1.23 | | |
| 5 | (7) E | 1000 | 51.23 | 1.92 | 897.645 | 6.39 |
| | | 500 | 40.32 | 1.43 | | |
| | | 250 | 26.64 | 8.42 | | |
| | | 125 | 8.43 | 0.22 | | |
| | | 62.5 | 3.23 | 1.24 | | |
| 6 | (7) F | 1000 | 85.34 | 0.43 | 212.721 | 4.76 |
| | | 500 | 49.45 | 0.64 | | |
| | | 250 | 36.34 | 0.77 | | |
| | | 125 | 26.11 | 0.45 | | |
| | | 62.5 | 17.54 | 1.43 | | |
| 7 | (7) G | 1000 | 84.45 | 0.93 | 511.904 | 5.26 |
| | | 500 | 70.34 | 0.99 | | |
| | | 250 | 58.26 | 0.54 | | |
| | | 125 | 27.42 | 0.83 | | |
| | | 62.5 | 18.32 | 1.43 | | |

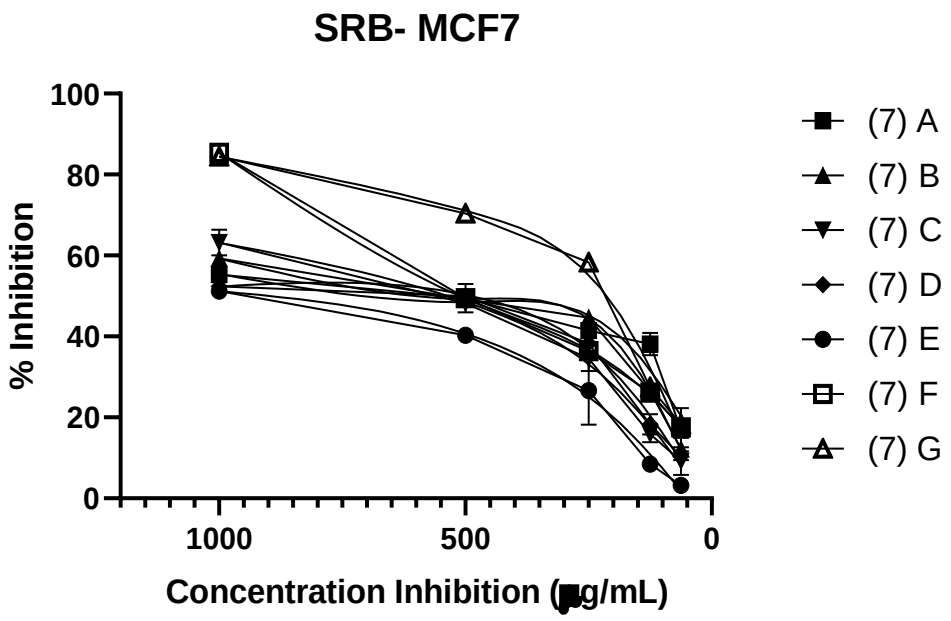


**Figure 6: Cytotoxic Effect of the Test Substances On A-549 Cells.**

## CONCLUSION

A series of compounds with different acetophenone derivatives such as 5-Chloro-2-Hydroxy acetophenone, 5-Bromo-2Hydroxyacetophenone, 3',5'Dichloroacetophenone, 2',4'-Dihydroxyacetophenone, 2',6'-Dihydroxyacetophenone, 2-Hydroxy-5'Methylacetophenone, 2'Hyroxyacetophenone derivatives of 2-(Bis(2-chloroethyl)amino)-N-(4-((3-oxobenzofuran-2(3H)-ylidene)methyl)phenyl)acetamide were prepared and checked for the spectral data as shown above. The derivatives synthesized were found to obtain good yields with satisfactory purity ranging from 78 to 93%. The products obtained were subjected to *in-vitro* cytotoxic activities through SRB assay on MCF-7 cells. All the products synthesized showed mild to moderate cytotoxic activities. The test substances were exposed to different concentrations ranging from 1000µg/ml to 62.5µg/ml to determine the percentage growth inhibition on MCF-7. The test substances (7) A-G has exhibited a CTC50 value which is 711.132 ± 4.57, 604.324 ± 7.22, 536.157 ± 4.91, 488.187 ± 5.2, 897.645± 6.39, 511.904 ± 5.26, 212.721 ± 4.76 µg/ml respectively in MCF-7 cell lines, in which the compound (7) G i.e., 2-(Bis(2-chloroethyl)amino)-N-(4-((5-bromo-3-oxobenzofuran-2(3H)-ylidene)methyl)phenyl) acetamide showed minimum CTC-50 both in MCF-7 cell lines.

## ACKNOWLEDGEMENTS

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