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# *In vitro* bioassessment of novel $\delta$- carboline derivatives as an antiproliferative agent

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ABSTRACT

Several carboline derivatives are anticancer agents and studied for antiproliferative action against various cancer cells. Based on the preliminary analysis using *insilico* strategies, we have selected eight compounds for the study. All compounds have been synthesised and characterised for their purity and chemical composition. Antiproliferative activity was assessed by insilico Carcinogenicity assay, Cytotoxicity analysis by sulphorhodamine B, Antiproliferation assay and DNA damage analysis. The cytotoxic effects of the CH5, CH17, CH29, CH34, CH37, CH39, CH42 and CH47 on Vero, HeLa, A549, BRL3A, HCT116, and MCF7 were determined using the SRB assay. CH5, CH34, CH37 and CH42 was the most potent cytotoxic towards HCT116 cells with $CTC_{50}$ value of 62.1±0.19, 47.1±0.41, 78.5±1.26 and 32.1±1.11 $\mu$g/ml respectively. The assay revealed a noticeable reduction in cell number for CH5 and CH37 tested except CH34 and CH42. CH5 and CH37 observed cytotoxic effects were found to destroy the cells according to time, and cell viability decreased with that time length. To learn their role in cell death, CH5 and CH37 were therefore taken up for a further screening. This study suggested that CH5 and CH37 had a separate mechanism of action to kill and that in the cell line. Such results will provide enrichment of scientific knowledge on the molecular mechanism and target therapies of CH5 and CH37, thereby potentially helpful for patients with Colon cancer.

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## INTRODUCTION

The protein bromodomain (BRD4) can serve as an effector of histone acetylation because it is capable of detecting acetylated residues in histone tails. Over the last decade, it has been shown that inhibitors that block the interaction of BRD4 with acetylated residues have therapeutic potential (Muller *et al.*, 2011). BRD4 protein inhibitors have been identified as critical antiproliferative agents in many cancer cells, and most of the compounds are in clinical trials. These inhibitors mechanism of action is to block the expression of oncogenes associated with enhancers with a very high level of histone acetylation known as super-enhancers (SE) and thus prevent oncogene-driven

cancer cell propagation (Garcia-Carpizo *et al.*, 2019; Wu *et al.*, 2017; Xiang *et al.*, 2018; Zhang *et al.*, 2019). Based on the previous study using *insilico* methods, eight compounds belonging to carboline derivatives were selected and utilised against different cancer cells to see that these compounds could stop cell proliferation. It has been estimated that half of all therapeutic agent consists of tetracyclic heteroaromatic ring compounds. There are various biologically active molecules containing different heteroatoms such as nitrogen and oxygen that have often drawn the attention of chemists over the year, primarily due to their biological significance. Carbolines are a group of heterocyclic compounds with a wide range of biological activity. $\delta$-Carbolines are the less-known group of compounds compared to their analogues of $\alpha$, $\beta$ and $\gamma$. Effective drugs have not yet been found among this class of heterocycles.

Nevertheless, for several compounds of this heterocycle class, a vast spectrum of biological activity has been identified. A broad group of analogue $\delta$-Carbolines has demonstrated high antimuscarinic, antihyperglycemic, antimalarial, antiplasmodial, antifungal, anticryptococcal, antiviral, and antitumor activity. Recently, cryptolepine and its analogues have been identified as cytotoxic to B16 melanoma cells and M109 Madison lung cancer cells (Queiroz *et al.*, 2006).

## EXPERIMENTAL METHODS

### Chemical and Reagents

All of the glassware's were dried on the oven before use. All the major chemicals had been acquired from Sigma Aldrich Co., Ltd. All solvents used in the study have been of acceptable quality and have been used as such without further purification. African green monkey Kidney cell (Vero), Human cervical cell line (HeLa), lungs cancer cell line (A549), Liver Cell line (BRL3A), human-colon-cancer cell line (HCT116) and breast cancer cell line (MCF7) were collected from National Centre for Cell Science, Pune, India.

### Synthesis and characterisation

The compounds which were used in this entire study were identified through *insilico* receptor-based drug discovery. BRD4 was used as a receptor in the previous study. This BRD4 is known for his histone acetylation and Post Transcriptional modification in the cell. More than 50 compounds were taken in the prior research against BRD4 protein. Based on the findings, we selected eight compounds Table 1 for antiproliferation activity. These selected compounds were then synthesised, purified and characterised by IR, NMR and Mass their structure based on the various spectra. The characterised compounds have now been used in an *in vitro* proliferation study.

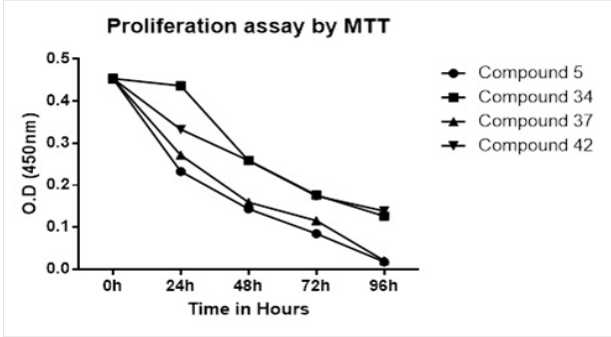


**Figure 1: Antiproliferation properties of synthesised compounds**

### Carcinogenicity activity of compounds

The synthesised compounds have been used for the production of carcinogenicity prediction models using CarcinoPred-EL (Zhang *et al.*, 2017). The synthesised eight compounds were used as a data set to construct and validate predictive models. The purpose of the study was to establish various molecular fingerprints and machine learning methods to predict the carcinogenicity of multiple compounds. That also describes the structural characteristics of the carcinogenic effects. Five-fold cross-validation assessed the efficiency of predictive model's, which is commonly used in *insilico* methods. The models are used to detect possible carcinogens in the early phases of drug development—a free carcinogenicity prediction online tool (CarcinoPred-EL) used for this purpose.

### Cytotoxicity study of compounds on the cancer cell line

The cytotoxicity study was conducted on eight cancer cells, such as Vero, HeLa, A549, BRL3A, HCT116, and MCF7 for all eight compounds using an SRB assay method. The monolayer of various cultures of cancer cells was trypsinised, and the number of cells was balanced to $1x10^5$ cells/ml using a 10 % NBCS medium. Added 100 $\mu$l (approximately 10,000 cells) of diluted suspension of cells into 96 microtitre plate. The partial layer was formed after 24 hours, then the supernatant was washed, and the cells in the microtitre plates were added with 100 $\mu$l of different compound concentrations, then incubated at 37°C for 72 h. After 72 h of incubation, 25 $\mu$l containing 50% of TCA was added gently to the wells to form a layer over the dilutions of the synthesised compounds to create a total concentration of 10 % and incubated for 1h at 4°C. Then the plates were washed with distilled water, then air-

**Table 1: Selected synthesised compound for the study**

| Compounds | Molecular Weight | Molecular Name | Molecular Formula |
|---|---|---|---|
| CH5 | 251.24 | 8-methylpyrido[3,2-b]pyrrolo[3,4-f]indole-7,9(5H,8H)-dione | C14H9N3O2 |
| CH17 | 265.27 | 8-ethylpyrido[3,2-b]pyrrolo[3,4-f]indole-7,9(5H,8H)-dione | C15H11N3O2 |
| CH29 | 279.29 | 8-propylpyrido[3,2-b]pyrrolo[3,4-f]indole-7,9(5H,8H)-dione | C16H13N3O2 |
| CH34 | 319.36 | 8-cyclohexylpyrido[3,2-b]pyrrolo[3,4-f]indole-7,9(5H,8H)-dione | C19H17N3O2 |
| CH37 | 313.31 | 8-phenylpyrido[3,2-b]pyrrolo[3,4-f]indole-7,9(5H,8H)-dione | C19H11N3O2 |
| CH39 | 327.34 | 8-benzylpyrido[3,2-b]pyrrolo[3,4-f]indole-7,9(5H,8H)-dione | C20H13N3O2 |
| CH42 | 314.30 | 8-(pyridin-2-yl)pyrido[3,2-b]pyrrolo[3,4-f]indole-7,9(5H,8H)-dione | C19H10N4O2 |
| CH47 | 314.30 | 8-(pyridin-3-yl)pyrido[3,2-b]pyrrolo[3,4-f]indole-7,9(5H,8H)-dione | C19H10N4O2 |

**Table 2: Carcinogenicity level of synthesised compounds on various system**

| Compound | Algae | Ames Test | Carcino Mouse | Carcino Rat | Daphnia | hERG Inhibition | Medaka | Minnow |
|---|---|---|---|---|---|---|---|---|
| CH5 | 0.2 | mutagen | negative | positive | 1.0 | Medium risk | 1.5 | 1.1 |
| CH17 | 0.2 | mutagen | negative | negative | 0.7 | Medium risk | 0.7 | 0.6 |
| CH29 | 0.1 | mutagen | negative | negative | 0.4 | Medium risk | 0.3 | 0.2 |
| CH34 | 0.0 | mutagen | negative | positive | 0.1 | Medium risk | 0.0 | 0.0 |
| CH37 | 0.1 | mutagen | negative | positive | 0.1 | Medium risk | 0.0 | 0.0 |
| CH39 | 0.0 | mutagen | negative | negative | 0.1 | Medium risk | 0.0 | 0.0 |
| CH42 | 0.1 | mutagen | negative | positive | 0.2 | Medium risk | 0.1 | 0.1 |
| CH47 | 0.2 | mutagen | negative | positive | 0.4 | Medium risk | 0.4 | 0.3 |

**Table 3: Carcinogenicity level of synthesised compounds on various system**

| Compounds | TA100 10RLI | TA100 NA | TA1535 10RLI | TA1535 NA |
|---|---|---|---|---|
| CH5 | positive | negative | negative | positive |
| CH17 | Positive | Positive | Negative | Positive |
| CH29 | positive | positive | negative | positive |
| CH34 | positive | positive | negative | positive |
| CH37 | positive | positive | negative | positive |
| CH39 | positive | negative | negative | positive |
| CH42 | positive | positive | negative | positive |
| CH47 | positive | negative | negative | negative |

**Table 4: Cytotoxic profile of compounds**

| Compounds | Cytotoxicity of the compounds against various cancer cell lines ($\mu$g/ml) | | | | | |
|---|---|---|---|---|---|---|
| | Vero | HeLa | A549 | BRL3A | HCT116 | MCF7 |
| CH5 | 170.1+0.11 | 241.1+1.07 | 173.3+0.77 | 222+10.21 | 194.5+0.98 | 232+0.87 |
| CH17 | 111.6+1.20 | 112.9+1.13 | 124+1.01 | 196.1+2.31 | 129.2+1.06 | 140.1+1.28 |
| CH29 | 121.2+0.38 | 182.2+3.4 | 181.5+0.96 | 218.1+2.34 | 310+1.81 | 152.1+0.61 |
| CH34 | 181.5+0.87 | 118.1+0.48 | 131+0.81 | 183.8+0.43 | **62.1+0.19** | 195.7+0.97 |
| CH37 | 159.4+0.52 | 186.6+1.20 | 141.8+1.71 | 191.1+1.22 | **47.1+0.41** | 186.6+0.19 |
| CH39 | 149.5+0.23 | 147.1+1.15 | 186.6+0.62 | 151+0.18 | **78.5+1.26** | 222.3+0.51 |
| CH42 | 132.3+1.22 | 218.1+0.56 | 150.7+0.59 | 158.2+1.51 | **32.1+1.11** | 167.2+1.18 |
| CH47 | 172.9+0.05 | 266.3+0.68 | 232.2+1.47 | 262.3+0.57 | 175.4+0.19 | 266.2+1.52 |

dried and stained for 30 min with MTT dye. The remaining dye was removed by washing with a 1 % acetic acid further air-dried. Then wells were added with 100 $\mu$l of 10 mM tris base to solubilise the dye. The absorbance was measured at a wavelength of 540 nm (Bannister and Kouzarides, 2011).

### Antiproliferation assay on the colorectal cell line

Cell proliferation assay was conducted on colorectal (HCT116) cell line for an all the four compounds using SRB assay method. The procedure described here was optimised in a 96-well model for the proliferation assay of compounds to adherent cells. The monolayer was made on tissue culture flask according to the MTT assay described above (Bannister and Kouzarides, 2011). This setup helps the flask to assess the ability of the selected compounds for antiproliferative activity. After incubation, cell monolayers were fixed with 10 % trichloroacetic acid and stained with SRB for 30 min; then, the excess dye was removed by repeatedly washing the cells with 1 % acetic acid. Ten mM Tris base was added to dissolve the dye and measured at 510 nm (Orellana and Kasinski, 2016; Vanicha Vichai and Kirtikara, 2006).

### Measurement of DNA damage

We selected four compounds by SRB assay for further cytotoxicity. The DNA damage caused by the compounds was assessed during this process. The cells were suspended in 10 mm Tris HCl and 10 mm EDTA in alkaline pH. The cells were incubated and treated with proteinase-K. The combination was incubated (37°C/3h) in phenol: chloroform: isoamyl (25:24:1) for DNA extraction. The extracted DNA was treated with 20 mg/ml of DNase and RNase at 4°C for one hour and precipitated with sodium acetate and ethanol (1:3). The Gel electrophoresis method was used to measure the DNA damage for the synthesised compounds. The mixture was prepared by adding 10 $\mu$g of DNA from selected cancer cells on a 2 % agarose gel containing ethidium bromide and visualised at 100 V for 45 min under the Gel Doc method (Thangam *et al.*, 2014).

## RESULTS AND DISCUSSION

### Carcinogenicity nature of the compounds

Carcinogenicity is one of the cell-killing property of the chemical compound. To know the carcinogenicity nature of the compound CarcinoPred-EL was used (Mady *et al.*, 2018; Roman *et al.*, 2019). The results showed that all the compound are mutagenic in Amis test either by point frameshift mutation (Table 2 and Table 3), and none of the com-

pounds showed positive mutagenicity on a carcinoma mouse model. Compounds CH5, CH34, CH37, and CH42 were shown positive mutation on carcino rat except for compound CH17, CH29, CH39, and CH47. Inhibition of these prolongs $QT_C$ intense along with the risk of cardiac arrhythmias all the compounds showed a medium risk of hERG inhibition (Table 2 and Table 3).

### *In vitro* Cell Killing property of selected compounds

Based on the *in silico* study, eight compounds were selected for *in vitro* cell killing property against various cancer cell line such as Vero, HeLa, A549, BRL3A, HCT116, and MCF7 to know their cell-killing property. Cell-based assays, particularly those using human cancer cell lines, gives a fast and inexpensive way of assessing compounds *in vitro* activity. To differentiate the cytostatic and cytotoxic effects of a compound SRB assay is the best of choice. It measures cell density based on the amount of cellular protein. Such tests are less sensitive to metabolic errors and measure the overall concentration of cells directly by the amounts of protein present (Aslantürk, 2018; Gordon *et al.*, 2018). The cytotoxic effects of the CH5, CH17, CH29, CH34, CH37, CH39, CH42 and CH47 on Vero, HeLa, A549, BRL3A, HCT116, and MCF7 were determined using SRB assay Table 4. As displayed in Table 4, CH5, CH34, CH37 and CH42 was the most potent cytotoxic towards HCT116 cells with $CTC_{50}$ value of $62.1\pm0.19$, $47.1\pm0.41$, $78.5\pm1.26$ and $32.1\pm1.11$ $\mu$g/ml respectively.

### Inhibition of cancer cell proliferation using reduction assay

Based on the cell-killing property of the cell, four compounds were selected for the study. These four compounds were taken for further research. *In vitro* cell proliferation of CH34, CH37, CH39 and CH42 against HCT116 were assessed by MTT reduction assay. Some recent studies have shown that chemicals can inhibit cell proliferation, modulate the detention of the cell cycle and trigger apoptosis in cancer cells but not in healthy cells. The assay revealed a noticeable reduction in cell number for CH5 and CH37 tested except CH34 and CH42. CH5 and CH37 were observed to kill the cells in a time-dependent manner. We also found that cell viability was indirectly proportional to time Figure 1. Hence, CH5 and CH37 were taken up for further screening to know their mechanism in cell death.

### Measurement of DNA damage

Selected two compounds CH5 and CH37, were used against PBR322 Plasmid DNA to check their activity on Raw DNA sample. The results showed no fragmentation on Plasmid DNA after addition of the compounds. This study indicated that those two compounds had a different mechanism of action in killing and reduction of the cell line in *invitro*. Several scientists found that the compound that displayed anticancer activity did not show DNA damaging and apoptosis (Bernstein *et al.*, 2013; Jackson and Bartek, 2009). So to know the mechanism, various anticancer assays were taken into consideration to reveal the mechanism of action of the two compounds.

## CONCLUSION

Our study demonstrates the nature of carcinogenicity, properties of cell killing, antiproliferation activity, and DNA damage assessment of synthesised derivatives of $\delta$-carboline. Among the eight compounds, CH5 and CH37 showed promising cell-killing properties and halted HCT116 cell line proliferation at a lower concentration. These findings can provide enrichment of scientific information about the molecular mechanism and target therapies of CH5 and CH37, which is therefore potentially beneficial to Colon cancer patients.

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### Conflict of Interest

The author declares that there is no conflict of interest.

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