The studies on modified β-carboline molecules and their anti-cancer activity - Their role in topoisomerase inhibition

The β-carboline molecules are the alkaloids found in nature. The β-carboline alkaloids are the interesting class of molecules with a wide variety of medicinal properties including anticancer activity. Small molecules with β-carboline scaffold such as Harman, Harmine, Norharman and Harmaline are isolated from *Peganum harmala* (Zygophillaceae, Syrian Rue). The β-carboline scaffolds contain a tricyclic planner structure that is capable of intercalating with DNA and inhibit topoisomearse effectively. The anticancer activities of these molecules are known to be due to interaction with DNA either through intercalation or through external binding, topoisomerase inhibition, kinesin spindle protein (KSP) inhibition etc., however, in cancer therapy, the DNA intercalative topoisomerase inhibition has attained significant importance in cancer therapy. The anticancer activity of several synthetic compounds displays these mechanisms of action.

Hence, in several of our studies where modified β-carboline molecules were used are assayed for both topo I and topo II inhibition activity. Since, the molecule's interaction with double stranded and topoisomerase inhibition are considered as a crucial, few podophyllotoxin linked β-carboline and β-carboline-bisindole congeners were synthesised and assayed for its potential to interact with DNA and inhibit topoisoemerase using biophysical and biochemical approaches.

Broadly, the topoisomerases are classified into two of types, topo-I and topo-II. Since topo-I It is involved in replication and proliferation process, over production of topo I was noticed in cancer cells compared to normal cells. In general, topo I controls the changes in the DNA structure by cutting a single stranded DNA and re-joining the phosphate backbone during the normal cell cycle. Mechanism of topo I inhibition is known to occur in two ways, these inhibitors may bind topoisomerase directly or they may bind to DNA and alter its structure, so that it cannot be recognized by topoisomerases. The assay showing the inhibition of topo- I by few synthetic β-carboline- bisindole compounds tested by us are shown below in figure 1.

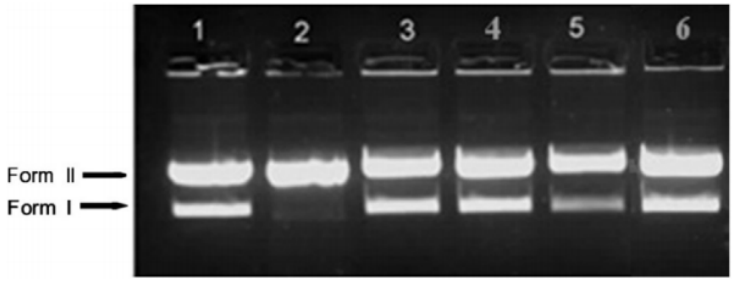


Figure 1: Agarose gel picture showing the inhibition of topo I by β-carboline- bisindole compounds. Lane1- pBR322 along; Lane2- pBR322 with topo I; Lane3,4 and 5- with different β-carboline- bisindole compounds; Lane6- pBR322+topoI +Camptothecin.

Where as, topoisomerase II is a nuclear enzyme that controls the DNA structure by catalysing the DNA cleavage and re-ligating the phosphodiester bonds. Recently, topoisomerase II inhibitors have gained importance as most of them interfere with the re-ligation process. The effect of synthetic β-carboline compounds on topoisomerase II inhibition was assayed by treating the mixture containing of synthetic compounds and topoisomerase II with kinetoplastid DNA (kDNA). kDNA is a form of macromolecular DNA structure in which several circular DN A structures are catenated to each other to form a network of interlocked rings.

When kDNA is incubated different concentrations of β-carboline molecules that inhibit topo II activity, and subjected to electrophoresis, relatively fast migrating bands were observed, indicating the complete conversion of catenated DNA form to decatenated forms like nicked circular, linear and relaxed forms. If the DNA is not cut by the enzyme, the bands corresponding to relaxed DNA will not be seen. However, most of kDNA remained as catenated form and remain near the wells. Since catenated kDNA is highly supercoiled, it remains in the well without entering the gel. There are two varieties of topo II inhibitors like ITD and IFP. The observational results obtained from our studies indicate that couple of synthetic β-carboline molecules (namely 7i and 7j) Catalytic Inhibitory Compound or CIC. The results obtained from various synthetic β-carboline molecules used in our study are shown below (figure 2)

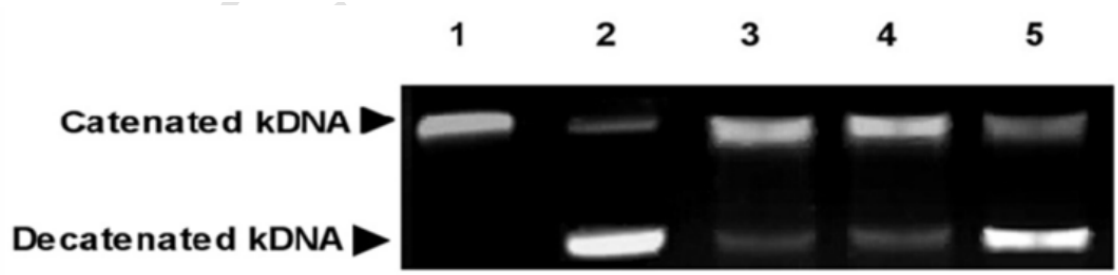


Figure 2: Agarose gel picture showing the inhibition of topo II by podophyllotoxin linked β-carboline compounds. Lane1- Catenated kDNA alone; Lane2- Catenated kDNA with topo II (5 units); Lane 3 and 4 - kDNA+ 5 units of topo II with different β-carboline-podophyllotoxin compounds; Lane6- kDNA+ 5 units of topoII +Etoposide.

The β-carboline- bisindole compounds were found to break DNA by generating free radicals. To study the effect of UV-light on the extent of DNA photocleavage by the free radicals generated by β-carboline- bisindole compounds, was studied in the presence and absence of UV-light. In the presence of UV-light (first gel picture) the β-carboline- bisindole compound generate more of free radicals and cleave supercoiled plasmid DNA (pBR322) (form I) to relaxed form of DNA (form II) where as in the absence of UV-light (in dark, second gel picture) the effect of β-carboline- bisindole compounds on supercoiled pBR322 plasmid DNA is not seen (the band intensity of pBR322 remained same throughout, indicating less or no effect of free radicals in the absence of UV light). These results indicate that β-carboline-bisindole compounds are suitable for photodynamic therapy (PDT). The results are shown in the gel below (figure 3)

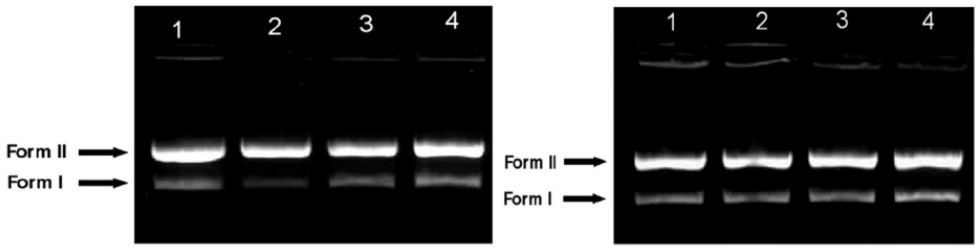
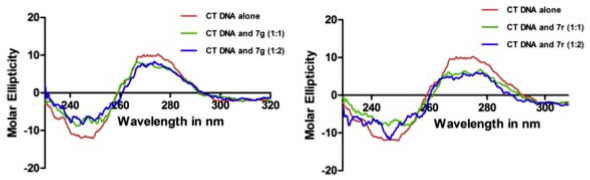
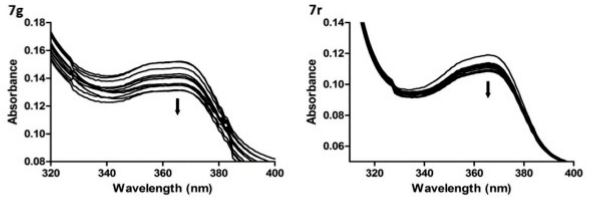


Figure 3: Gel electrophoresis picture showing the photo cleavage of pBR322 DNA by β-carboline -bisindole compounds in the presence and absence of UV light.

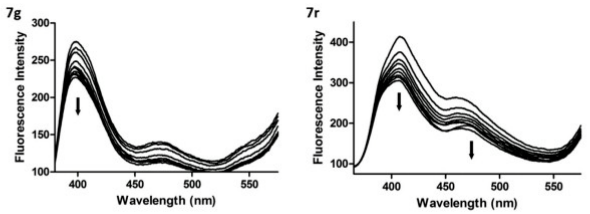
The role of β-carboline scaffolds synthesised and assayed in our laboratory indicate that they have very good binding interaction with double stranded DNA. In most of the cases, they will intercalate with dsDNA and few cases, they may show combilexin type of interaction with DNA (i.e both intercalation and external binding). Since most of the cell biological studies are linked to DNA, the information on the nature of β-carboline scaffolds interaction with dsDNA will through light on the manner in which β-carboline scaffolds interact with DNA and exhibit anti-cancer activity. DNA binding studies are done using spectroscopic techniques like, UV-vis, Fluorescence, and Circular dichroism and Viscosity studies. Molecular modelling studies were carried out to understand the nature of small molecular interaction with macromolecules. The data spectroscopy data collected using β-carbolinebisindole compounds (**7g** and **7r**) is shown below. The data indicates that these molecules have combilexin type interactions with dsDNA. The various spectra obtained from biophysical experiments are shown below (figure 4)



Circular dichroism spectra represents the CT-DNA (10 μM) at 10.0 μM and 20 μM of compounds **7g** and **7r**.



UV–Visible spectra indicates the interaction of compounds **7g** (1 ml of 25.0 μM) and **7r** (1 ml of 25.0 μM) with the addition of 5 μl of DNA (25.0 μM) each time at 25 °C.



. Fluorescence spectra depicts the interaction of compounds **7g** (10 μM) and **7r** (10 μM) with increasing concentrations of CT-DNA (multiples of 0.5 μM) at 25 °C.

Figure 4: β-carbolinebisindole compounds (**7g** and **7r**) interaction with dsDNA (CT-DNA).

Cell biology studies include MTT, comet, cell cycle and apoptosis assays. With β-carboline - bisindole and Podophyllotoxin linked β-carboline compounds, it was identified that they inhibit Topo I and Topo II enzymes. Both the compounds inhibit cancer cell growth much effectively compared to normal cells. Cell cycle assay indicate that both type of β-carboline compounds studied were inhibiting the cell cycle at G2/M phase in cancer cells compared to normal cells. Both varieties of β-carboline compounds induce apoptosis among cancer cells. With Podophyllotoxin linked β-carboline compounds, comet assay was performed to assess the extent of DNA damage occurred with the addition of Podophyllotoxin linked β-carboline compounds. Double-strand DNA in DU-145 cells was cleaved by Podophyllotoxin linked β-carboline compounds similar to the control, Etoposide. In the present assay Propidium Iodide, a fluorescent DNA-binding molecule was used to detect the extent of DNA damage. DU-145cells were treated with 10μM of Podophyllotoxin linked β-carboline compounds and Etoposide, and after lysis the samples were allowed to undergo electrophoresis. Cells grown in the absence of any congeners is considered as control. It was observed that the DNA damage is taking place in presence of Podophyllotoxin linked β-carboline compounds and Etoposide (details were shown in figure 5). However, there is no DNA damage observed in the control. Etoposide is well known topoisomerase II inhibitor which exhibits this inhibition activity through stabilizing the DNA cleavable complex. It was known earlier that Etoposide will induce the formation of open circular and linear DNA through the topoisomerase-DNA cleavable complex formation. We speculate that Podophyllotoxin linked β-carboline compounds may also effectively damage the DNA like Etoposide and inhibit the activity of topoisomerase II. The results obtained indicate that Podophyllotoxin linked β-carboline compounds are having potential to damage the cellular DNA effectively and stop the growth of cancer cells. The effect was found to be less with normal cells.

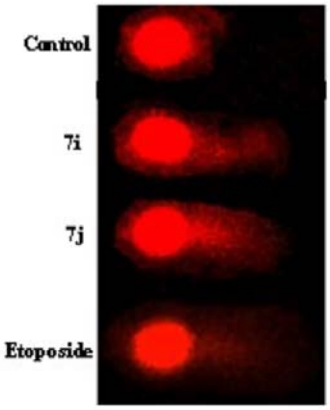


Figure 5: Comet assay showing the extent of DNA damage occurred with Podophyllotoxin linked β-carboline compounds are treated to DU-145 cells. Control; Podophyllotoxin linked β-carboline compound-1 (**7i**, 10 μM); Podophyllotoxin linked β-carboline compound -2 (**7j**, 10μM); Etoposide.

Cell cycle and apoptosis assay with β -carbolinebisindole compounds (**7g** and **7r**) at two different concentrations like 1µM and 2µM (to see dose effect on the cell cycle progressing and apoptosis). It was noticed that β -carbolinebisindole compounds stop cell cycle at G2/M phase and induce apoptosis in dose dependant manner. The results obtained are shown in figure 6 and 7.

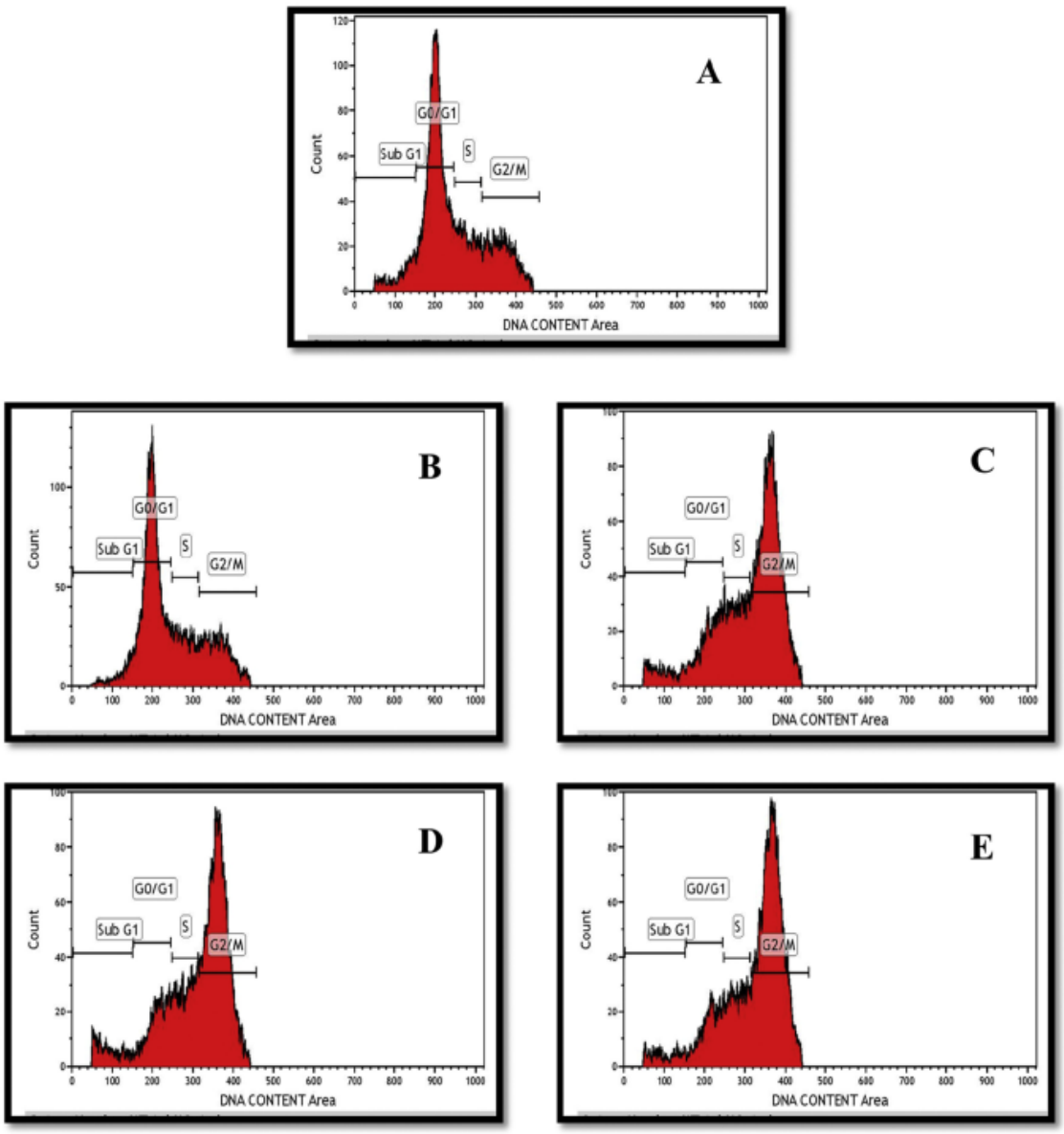


Figure 6: Flow cytometric analysis in DU-145 cells after treatment with two different β -carbolinebisindole compounds at 1 and 2 mM concentrations for 48 h. A: Control cells, B: β -carbolinebisindole compound, **7g** (1 mM), C: β -carbolinebisindole compound, **7g** (2 mM), D: β -carbolinebisindole compound-2, **7r** (1 mM) and E: β -carbolinebisindole compound-2,**7r** (2 mM). Cell cycle inhibition at G2/M phase was noticed on addition of β -carbolinebisindole compounds.

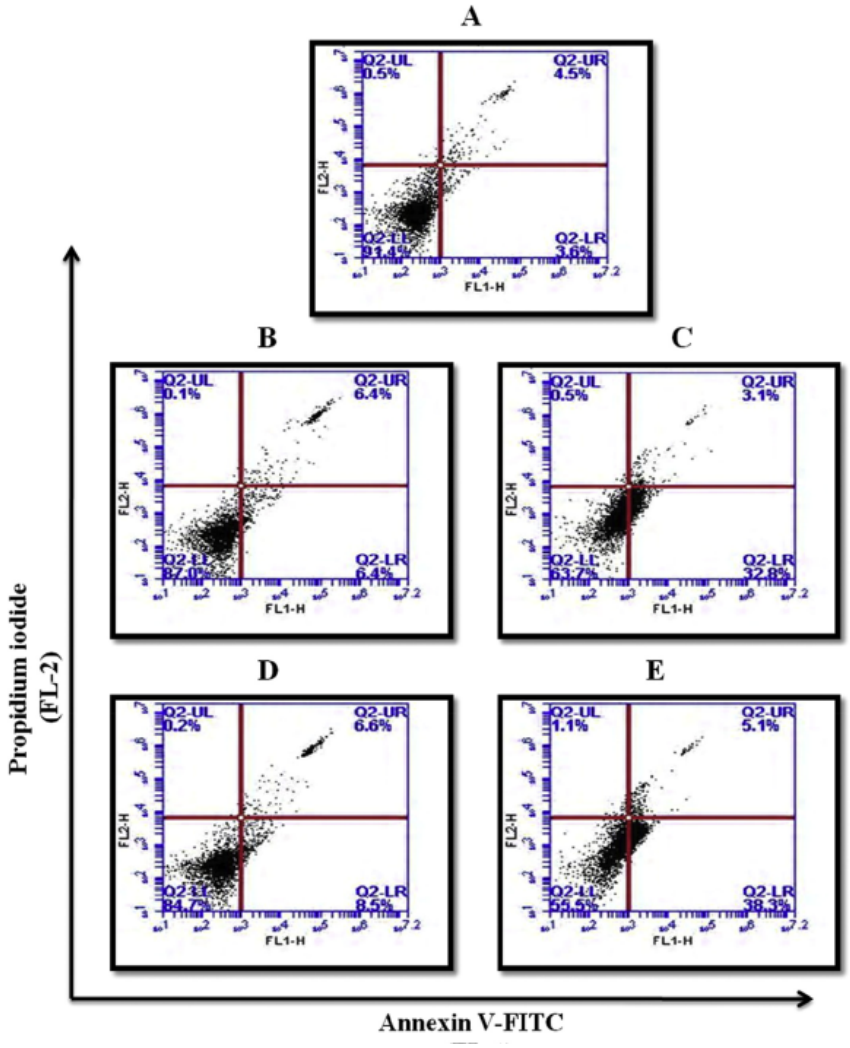


Figure 7: Annexin V-FITC/PI (AV/PI) dual staining assay: Quadrants; Upper left (necrotic cells), Lower left (live cells), Lower right (early apoptotic cells) and Upper right (late apoptotic cells). A: Control cells (DU-145), β -carbolinebisindole compound,**7g** (1 mM), C: β -carbolinebisindole compound,**7g** (2 mM), D: β -carbolinebisindole compound-2,**7r** (1 mM) and E: β -carbolinebisindole compound-2,**7r** (2 mM).

The above mentioned studies with β-carboline linked molecules were carried out and the data obtained from these experiments were analysed and published in two international peer reviewed journals. The reference for each paper is provided below.

References of the papers in which the details of research was published:

1. Design, synthesis and biological evaluation of new b-carbolinebisindole compounds as DNA binding, photocleavage agents and topoisomerase I inhibitors. (2018) Jeshma Kovvuri , Burri Nagaraju, V. Lakshma Nayak, Ravikumar Akunuri, M.P. Narasimha Rao , Ayyappan Ajitha , Narayan Nagesh *, Ahmed Kamal *. European Journal of Medicinal Chemistry, 143, 1563-1577.(IF- 6.5).

2. Synthesis of podophyllotoxin linked β-carboline congeners as potential anticancer agents and DNA topoisomerase II inhibitors. (2018) M. Sathish, B. Kavitha, V.L. Nayak, Tangella Yellaiah , Ayyappan Ajitha, S. Nekkanti, A. Alarifi, N. Shankaraiah*, N. Nagesh*, Kamal A*. Eur J Med Chem.,144, 557-571. doi: 10.1016/j.ejmech.2017.12.055. (IF- 6.5).