Thesis at a glance

Estrogen and its receptor are classically involved in a majority of cancers of gynecological origin. The estrogen receptor (ER), a nuclear hormone receptor, is expressed in estrogen-responsive organs such as ovary, uterus and mammary glands. Estrogen and estrogen-bound ER possess both genomic and non-genomic functions. Since, the functional expression of the ER is confined to the initial stages of neoplastic transformation; the design of compounds that interfere with ER function is expected to be an effective strategy in preventing these types of cancer.

Cancer treatment needs multi-modal strategies to restrain its aggressive malignancy. Strategies that include simultaneous detection/sensing and treatment should be specific for cancer to avoid any unwanted side-effects. Among tumour-detecting molecular probes, intrinsically fluorescent small molecules are preferably and widely employed as they can enter live cells easily and offer screening through visual detection.

Tumour-selective ligands have been used to construct novel drug conjugates. Vitamin folic acid (FA), displays high affinity for the folate receptor (FR), a glycosylphosphatidylinositol-linked membrane protein that captures its ligands from the extracellular milieu and transports them inside the cell via a non-destructive, recycling, endosomal pathway. Owing to the fact that its expression is largely absent from normal tissues, FR is also a recognized tumor antigen/biomarker. Because of this, diagnostic and therapeutic methods which exploit the FR's function have been developed to treat cancer.

The present work involves, therefore, the exploration of highly potent, ER-selective, anti-breast-cancer oxindole-conjugated bis-phenols. With intrinsic fluorescence, this bis-arylideneoxindole derivative upon conjugation with known ROS generator betulinic acid, is then further employed for selective detection and killing of cancer cells. The most potent derivative in the library, is further preceded towards folate receptor targeted chemotherapy. Biological evaluation of this conjugate is underway to expand our knowledge in optimizing the pharmacophore with improved potential.
In chapter 1, we have presented a general and concise background on the mechanistic aspects of cancer i.e. the role of estrogen and estrogen receptors in cancer pathology.

In chapter 2, we have reported a new family of bis-arylideneoxindole derivatives, that show highly selective estrogen receptor (ER)-mediated anticancer activity at low-nanomolar concentrations in ER-positive (ER+) breast cancer cells. In terms of cell growth inhibition, IC$_{50}$ values for these compounds in ER+ breast cancer cells are two to three orders of magnitude lower than in ER-negative (ER-) breast cancer cells and non-cancer cells. In comparison with known bis-arylidene drugs, these compounds are at least three orders of magnitude more toxic than tamoxifen and 1.5–4 fold more toxic than 4-hydroxytamoxifen in ER+ MCF-7 cancer cells. The uncompromised ER-targeting or anti-estrogenic properties are equivocally strengthened by the introduction of oxindole moieties in arylidenes. Serendipitously, N-4-methoxybenzyl protection of oxindoles rather reinforced ER-mediated anti-breast-cancer activity. These oxindoles inhibit ER-transactivation and their anticancer activities are inhibited in ER-depleted MCF-7 cells. Some of these nonsteroidal molecules also exhibit essential properties of selective ER down-regulation. From the development of two series of bis-arylideneoxindole based compounds, we report a new series of anticancer agents for estrogen-responsive breast cancer.

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\text{HO} \quad \text{N} \quad \text{O} \quad \text{R} \\
\text{N} \quad \text{O} \quad \text{H} \\
\text{R= H, } n=3, \ (2) \\
\text{R= H, } n=2, \ (2a) \\
\text{R= 4-methoxybenzyl, } n=3, \ (3) \\
\text{IC$_{50}$} < 35 \text{ nM} \\
\text{HO} \quad \text{O} \quad \text{R} \\
\text{N} \quad \text{O} \quad \text{H} \\
\text{R= } \alpha\text{-OH (13b)} \\
\text{R= p-COMe (13c)} \\
\text{IC$_{50}$} < 70 \text{ nM}
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In chapter 3, hybrid of cancer cell-selective, ROS generator betulinic acid and bis-arylideneoxindole with amino propyl-linker, is developed. With intrinsic fluorescence, the molecule exhibits cancer cell-specific residence. Further, it generates ROS, triggers apoptosis and exhibits potent cytotoxicity in cancer cells selectively. The study demonstrates the first example among isatin derivatives, conjugated with natural product betulinic acid, as a new anticancer therapeutic with dual role of selective detection and killing of cancer cells via apoptosis.
In chapter 4, bis-arylidineoxindole is further preceded towards folate receptor targeted chemotherapy. Literature precedence shows that, therapeutic methods, which exploit the FR’s function, have been developed to treat cancer. Molecular payloads that range in size from radionuclides to large DNA and liposomal constructs have successfully been delivered inside cancer cells via the FR pathway. One eminently related approach involves the attachment of FA to potent chemotherapeutic compounds to form small molecule drug conjugates, or SMDCs. In this section, we have synthesized a “Folate-PEG-Isafen” conjugate. Biological evaluation is underway to expand our knowledge in optimizing the pharmacophore with improved potential.

"Folate-PEG-Isafen" conjugate