CHAPTER 4

CELLULAR EFFECTS OF DEOXYBENZOINS AND
ISOFLAVONES

This chapter presents the effects of novel DOBs and IFs designed from in silico analysis, on human cancer cell lines, PC3, MCF-7 and Hep2, and discusses these cellular effects in detail.

The estrogenic compounds can modulate the cell cycle through activation/deactivation of several genes which may promote cell proliferation or cytotoxicity (Jagannathan and Robinson-Rechavi, 2011; Nilsson and Gustafsson, 2010). In addition to causing cell proliferation or cytotoxicity, the active ligands may cause cellular responses like cytoskeletal remodeling.

MTT assay was used to quantify the cytoproliferative/cytotoxic effects of the compounds (DOB and IF) on cells which express differential levels of ERα and ERβ. These effects were expressed in quantitative terms (EC25 and IC50) so as to enable the comparison of potencies. Fluorescence microscopy and phase-contrast microscopy were employed to visualize the cellular changes like nuclear fragmentation and cytoskeletal remodeling.

4.1 EFFECT OF DOBs AND IFs ON GROWTH OF MCF7 AND PC3

The MTT assay is an indirect method for assessing the effect of test compounds on cell viability (Mosmann, 1983). In viable cells, the functional mitochondrial dehydrogenase reduces the MTT to form a formazan product, which is purple in colour, on incubation at the culture conditions for 3 to 5 hours. The concentration of this formazan is a measure of the activity of dehydrogenase in the functional mitochondria in viable cells. MTT assay is
reported to differ by 5-10% from the actual viable cell counts as estimated by methods such as Trypan Blue dye exclusion method (Wang et al., 2010). However, MTT assay is more convenient when a large number of samples and replicates are analysed (Mosmann, 1983), and hence this method was used in this study for estimation of cell viability. MTT assay results with less than 10% change from the control culture wells were omitted from calculation of EC$_{25}$ or IC$_{50}$. In this study, three human cancer cell lines were used to determine the cytotoxic/proliferative effect of the test compounds.

i) MCF7 cells: human breast adenocarcinoma cells which expresses predominantly ER$\alpha$ subtype (Katzenellenbogen et al 1987).

ii) PC3 cells: human prostate cancer cells which expresses predominantly ER$\beta$ subtype (Li and Sarkar, 2002).

iii) Hep2 cells: human larynx cancer cells which expresses low levels of ER$\alpha$ and ER$\beta$.

4.1.1 Effect of DOBs

The effect of the DOBs on the proliferation of MCF7 and PC3 cells after 72 hours of exposure are shown in Figure 4.1 to Figure 4.3. The error bar represents the standard deviation of the mean (n=3).

The DOBs tested in this study, CMPD3, CMPD6, and CMPD 9, caused proliferation of MCF7 cells and caused growth inhibition of PC3 cells. It is known that predominant the ER in MCF7 is ER$\alpha$ (Payne et al 2000) and ER$\beta$ in PC3 cells (Leung et al 2006). Thus, this observation was consistent with the well known effect of estrogenic agonist-like molecules on ER$\alpha$ (cell proliferation) and on ER$\beta$ (growth inhibition and apoptosis). Both these
response curves had sigmoidal profile, suggesting a receptor mediated response.

**Figure 4.1** Dose-response to CMPD3 on proliferation of MCF7 cells and PC3 cells

**Figure 4.2** Dose-response to CMPD6 on proliferation of MCF7 cells and PC3 cells
Figure 4.3  Dose-response to CMPD9 on proliferation of MCF7 cells and PC3 cells

Figure 4.4  Dose-response curves for Estradiol on MCF7 cells and Genistein on PC3 cells
All the three DOBs showed similar activity profile (Figure 4.1 to Figure 4.3) and similar EC\textsubscript{25} values on MCF7 cells and IC\textsubscript{50} values on PC3 cells, with CMPD6 being the least potent amongst the three DOBs (Table 4.1, Table 4.2).

**Table 4.1 EC\textsubscript{25}, relative potency and efficacy of DOBs on MCF7**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>EC\textsubscript{25} (µM)</th>
<th>Relative potency\textsuperscript{a}</th>
<th>Efficacy\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>1.4 x 10^{-5}</td>
<td>1.00</td>
<td>177 ± 3</td>
</tr>
<tr>
<td>CMPD3</td>
<td>1.17</td>
<td>1.19 x 10^{-5}</td>
<td>142 ± 4</td>
</tr>
<tr>
<td>CMPD6</td>
<td>2.76</td>
<td>0.50 x 10^{-5}</td>
<td>132 ± 3</td>
</tr>
<tr>
<td>CMPD9</td>
<td>2.98</td>
<td>0.46 x 10^{-5}</td>
<td>143 ± 3</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Relative potency was calculated by taking the ratio between the EC\textsubscript{25} of estradiol to the EC\textsubscript{25} of the test compound.

\textsuperscript{b} Efficacy of cell proliferation (% of the control) ± SD, in response to estradiol (1 nM) or the compound (100 µM).

**Table 4.2 IC\textsubscript{50}, relative potency and efficacy of DOBs on PC3**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>IC\textsubscript{50} (µM)</th>
<th>Relative potency\textsuperscript{a}</th>
<th>Efficacy\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genistein</td>
<td>5.49</td>
<td>1.00</td>
<td>42 ± 3</td>
</tr>
<tr>
<td>CMPD3</td>
<td>12.00</td>
<td>0.45</td>
<td>76 ± 3</td>
</tr>
<tr>
<td>CMPD6</td>
<td>14.84</td>
<td>0.36</td>
<td>84 ± 5</td>
</tr>
<tr>
<td>CMPD9</td>
<td>10.63</td>
<td>0.51</td>
<td>80 ± 3</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Relative potency was calculated by taking the ratio between the IC\textsubscript{50} of genistein to the IC\textsubscript{50} of the test compound.

\textsuperscript{b} Efficacy of cytotoxicity (% of the control) ± SD, in response to genistein or the compound at 100 µM.
The IC\textsubscript{50} values and EC\textsubscript{25} values of the DOBs were in the range of potencies reported for other weak xenoestrogens like genistein and daidzein on PC3 (Li and Sarkar 2002; Pavese et al 2010) and on MCF7 (Hsieh et al 1998; Miodini et al 1999; Pavese et al 2010).

The DOBs were also reported to act as weak to moderate ER\textbeta agonists (Fokialakis et al 2004; Papoutsi et al 2007). This may explain the cytotoxic effect of the DOB on ER\textbeta+ prostate cell (PC3) observed in this study (Figure 4.1 to 4.3, Table 4.2), which was similar (but weaker) to the effect produced by genistein on PC3 cells (Figure 4.12). The relative inhibitory efficacy of the compounds on PC3 was approximately 30-50% of the inhibitory effect of genistein (Table 4.2).

From this study, it was observed that CMPD3 showed the maximum proliferative potency among the DOBs, while CMPD9 had the maximum inhibitory potency. Also, it was observed that the differences in the relative potencies were not significantly different, among the DOBs used in this study.

### 4.1.2 Effect of IFs

The effect of the IFs on the proliferation of MCF7 and PC3 after 72 hours of exposure are shown in the Figure 4.5 to Figure 4.11. The error bars represents the standard deviation (n=3).
Figure 4.5  Dose-response to CMPD1 on proliferation of MCF7 cells and PC3 cells

Figure 4.6  Dose-response to CMPD2 on proliferation of MCF7 cells and PC3 cells
Figure 4.7  Dose-response to CMPD4 on proliferation of MCF7 cells and PC3 cells

Figure 4.8  Dose-response to CMPD5 on proliferation of MCF7 cells and PC3 cells
Figure 4.9  Dose-response to CMPD8 on proliferation of MCF7 cells and PC3 cells

Figure 4.10  Dose-response to CMPD10 on proliferation of MCF7 cells and PC3 cells
Figure 4.11 Dose-response to CMPD11 on proliferation of MCF7 cells and PC3 cells

Figure 4.12 Dose-response to CMPD7 on proliferation of MCF7 cells and PC3 cells. Notice the lack of sensitivity of MCF7 to CMPD7
This study evaluated eight IFs for the estrogenic effects on the MCF7 and PC3 cells. Among this, CMPD1 showed the maximum proliferative effect on MCF7 cells (Figure 4.5) while CMPD7 showed the maximum apoptotic effect on the PC3 cells (Figure 4.12).

Table 4.3 EC$_{25}$, relative potency and efficacy of IFs on MCF7

<table>
<thead>
<tr>
<th>Ligand</th>
<th>EC$_{25}$ (µM)</th>
<th>Relative Potency$^a$</th>
<th>Efficacy$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>1.4 x10(^{-5})</td>
<td>1.00</td>
<td>177 ± 3</td>
</tr>
<tr>
<td>CMPD1</td>
<td>0.43</td>
<td>3.29 x10(^{-5})</td>
<td>122 ± 2</td>
</tr>
<tr>
<td>CMPD2</td>
<td>ND</td>
<td>ND</td>
<td>108 ± 0</td>
</tr>
<tr>
<td>CMPD4</td>
<td>ND</td>
<td>ND</td>
<td>110 ± 1</td>
</tr>
<tr>
<td>CMPD5</td>
<td>ND</td>
<td>ND</td>
<td>105 ± 3</td>
</tr>
<tr>
<td>CMPD7</td>
<td>ND</td>
<td>ND</td>
<td>102 ± 1</td>
</tr>
<tr>
<td>CMPD8</td>
<td>ND</td>
<td>ND</td>
<td>108 ± 1</td>
</tr>
<tr>
<td>CMPD10</td>
<td>ND</td>
<td>ND</td>
<td>111 ± 1</td>
</tr>
<tr>
<td>CMPD11</td>
<td>ND</td>
<td>ND</td>
<td>110 ± 1</td>
</tr>
</tbody>
</table>

ND, Not determined since the response was insignificant (less than 10% change with respect to control)

$^a$ Relative potency was calculated by taking the ratio between the EC$_{25}$ of estradiol to the EC$_{25}$ of the test compound.

$^b$ Efficacy of cell proliferation (% of the control) ± SD, in response to estradiol (1 nM) or compound (100 µM).
Table 4.4 IC$_{50}$, relative potency and efficacy of IFs on PC3

<table>
<thead>
<tr>
<th>Ligand</th>
<th>IC$_{50}$ (µM)</th>
<th>Relative Potency$^a$</th>
<th>Efficacy$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genistein</td>
<td>5.49</td>
<td>1</td>
<td>42 ± 3</td>
</tr>
<tr>
<td>CMPD1</td>
<td>ND</td>
<td>ND</td>
<td>96 ± 1</td>
</tr>
<tr>
<td>CMPD2</td>
<td>ND</td>
<td>ND</td>
<td>96 ± 2</td>
</tr>
<tr>
<td>CMPD4</td>
<td>ND</td>
<td>ND</td>
<td>100 ± 3</td>
</tr>
<tr>
<td>CMPD5</td>
<td>ND</td>
<td>ND</td>
<td>102 ± 3</td>
</tr>
<tr>
<td>CMPD7</td>
<td>5.09</td>
<td>1.07</td>
<td>54 ± 2</td>
</tr>
<tr>
<td>CMPD8</td>
<td>ND</td>
<td>ND</td>
<td>96 ± 1</td>
</tr>
<tr>
<td>CMPD10</td>
<td>ND</td>
<td>ND</td>
<td>98 ± 0</td>
</tr>
<tr>
<td>CMPD11</td>
<td>ND</td>
<td>ND</td>
<td>95 ± 1</td>
</tr>
</tbody>
</table>

ND, Not Determined since the response was insignificant (less than 10% change with respect to control)

$^a$ Relative potency was calculated by taking the ratio between the IC$_{50}$ of genistein to the IC$_{50}$ of the test compound.

$^b$ Efficacy of cytotoxicity (% of the control) ± SD, in response to genistein or the compound at 100 µM.

This study showed that majority of the IFs, except CMPD1 and CMPD7, showed negligible activity on the cells. Except for CMPD1 and CMPD7, the IFs failed to produce any significant change (<10%) in the cell population of either MCF7 or PC3.

The effect of CMPD1 on MCF7 was weak and the EC$_{25}$ value of 0.43 µM was at least 3x10$^3$ times lesser than the estradiol (Table 4.3). CMPD1 did not produce significant cytotoxic effect on the PC3 cells (Figure 4.13, Table 4.4). This differential action suggested that CMPD1 may
have weak estrogenic effect on ERα+ cells like MCF7, when compared to ERβ+ cells like PC3. Nevertheless, the efficacy of CMPD1 was weak (25-30% that of 1 nM estradiol) when compared to the efficacy of estradiol on MCF7.

CMPD10 and CMPD11 were also weakly proliferative on MCF7 with CMPD11 showing weak cytotoxic effect on the PC3 cells (Figure 4.13). The weak estrogenic effects of CMPD11 were surprising since, CMPD11 is 2’-ethyl genistein and is structurally very similar to genistein (Table 1.6). This 2’ modification resulted in a drastic decrease in the estrogenic activity of genistein which may be due to the reduced affinity of the molecule to the ERs. Docking scores also indicated that the CMPD11 may have reduced affinity for the ERs, when compared to genistein (Table 3.2). Receptor binding studies and X-ray crystallographic studies will be required to provide insights for the alterations in the binding affinity and binding orientation of this molecule, when compared to genistein.

CMPD7 showed strong cytotoxic effect on PC3 cells while showing no significant activity towards MCF7 proliferation across the tested concentration range (Figure 4.8). CMPD7 is reported in US patent 20040147551 (Heaton et al 2004), with evidence for cytotoxicity towards ERβ+ cells. A similar cytotoxic behavior was observed in this study, partially confirming the ERβ selective agonist activity of this molecule. The relative potency of CMPD7 was similar (1.07) to that of genistein. The efficacy of CMPD7 was 75-80% that of genistein. The comparable values of efficacy and IC₅₀ of CMPD7 suggested that this compound may bind and cause functional changes in the ERβ, similar to genistein.

The compounds with ethyl substitution on the R1 position of the isoflavone nucleus (CMPD2, CMPD8, CMPD10 and CMPD11) had
significantly weaker activity compared to IFs unsubstituted at this position (CMPD1 and CMPD7) as shown in Figure 4.13 and Tables 4.1 and 4.2.

In case of CMPD4, which carries a pentyl chain at the R1 position, the long aliphatic pentyl chain may make it a poor agonist for the ER leading to the lack of activity towards MCF7 and PC3 cells (Figure 4.6, Figure 4.13, Table 4.1, Table 4.2). A similar lack of activity was observed with CMPD5, which contains an ethyl group at R position and a pentyl group at the R1 position (Figure 4.7, Figure 4.13, Table 4.1, Table 4.2).

The differential binding modes of the compounds in the LBD of the ERs, as discussed in Chapter 3, may cause significant repositioning of the crucial aminoacids and thereby affect the functionality of the receptor. A consequence of this could be the lower efficacy of the active compounds (CMPD1, CMPD3, CMPD6, CMP9 and CMPD7), when compared to estradiol and genistein (Table 4.1 to Table 4.4).

Effects of several IFs on cell viability and proliferation are well studied (Andres et al., 2011; Kalaiselvan et al., 2010; Zhang et al., 2012c). IFs such as genistein affects the cell proliferation mainly through the ER mediated effects (Banerjee et al., 2008; Pavese et al., 2010; Peterson, 1995). In addition to the slow, ER-mediated effects, genistein also shows rapid effects through other cellular receptors such as tyrosine kinases (Akiyama et al., 1987; Doerge and Sheehan, 2002; Peterson, 1995).

Genistein acts as an ER agonist and shows ER subtype specificity, with approximately 40 times more affinity for ERβ when compared to ERα (Banerjee et al., 2008; Manas et al., 2004a; Zhu et al., 2006). This specificity of genistein causes the effect of genistein to be dependent upon the differential expression levels of ERα and ERβ in a cell system.
In cells with higher ERα expression, such as MCF7 cells, genistein causes a concentration dependent proliferative effect (Hsieh et al 1998; Miodini et al 1999). In cells with a higher differential expression of ERβ, such as prostate cells, genistein causes suppression of proliferation and apoptosis (Bhamre et al., 2010; Li and Sarkar, 2002; Pavese et al., 2010). Genistein is reported to induce proliferation in the MCF7 cells, at the same concentration at which it induces cytotoxicity in PC3 cells (Fioravanti et al 1998).

In addition to genistein, several other structurally related IFs shows similar estrogenic effects (Andres et al., 2011; Handayani et al., 2006; Kalaiselvan et al., 2010; Tomar and Shiao, 2008; Zhu et al., 2006). This differential effect of IFs on cells can be utilized for achieving tissue or organ specific proliferation/inhibition. The growth modulating effect is particularly of pharmacological relevance, due to effect of these compounds on tissues and organs which show sensitivity to endogenous estrogens (Gallo et al., 2012; Nilsson and Gustafsson, 2010).

At high levels (>100 – 200 µM), genistein produces cytotoxicity in several cell types irrespective of their ER expression level (Beard et al., 2005; Maggiolini et al., 2001; Morito et al., 2001), which may be due to the tyrosine kinase inhibition (Akiyama et al 1987; Cos et al 2003) or by activation of the apoptosis pathways (Kyle et al 1997; Pavese et al 2010).

4.1.3 Effect of DOBs and IFs at High Concentration

At higher concentration (500 µM), all the test compounds (except CMPD1 and CMPD4) showed significant cytotoxicity on MCF7 and PC3 cells (Figure 4.14). Such cytotoxicity on MCF7 and PC3 cells are reported for genistein and estradiol also, and is thought to be contributed largely by non-genomic effects such as inhibition of tyrosine kinases (Akiyama et al 1987) or activation of apoptotic pathways (Morito et al 2002). The lack of cytotoxicity
for CMPD1 was an interesting observation, as it was shown to have a proliferative effect on MCF7 at low concentrations (≤100 µM) but negligible cytotoxicity at high (500 µM) concentration. Similarly, the cytotoxic effect of CMPD5 at high concentration was also interesting and suggested cellular targets other than the ERs.

**Figure 4.13** Effect of the test compounds on the cell proliferation of PC3 and MCF7 cells at 500 µM. Error bars represent standard deviation (n=3)

It should be noted that the high concentration (500 µM) used in this *in vitro* study may not pharmacologically significant, because such concentrations in serum can be achieved only at very high doses of the compounds. For example, concentration of CMPD7 (mw. 268) required to achieve a serum concentration 500 µM will be 134 mg/liter of serum.
4.2 ETHIDIUM BROMIDE: ACRIDINE ORANGE STAINING OF MCF7 AND PC3 CELLS TREATED WITH DOBs AND IFs

Fluorescent microscopy, with appropriate dyes, can reveal the morphological changes which accompanies the cell cycle. The use of ethidium bromide:acridine orange (EB/AO) dyes allows the distinct visualization of the nucleus and cytoplasm. Acridine orange is taken up by all cells and emits green fluorescence (526 nm) while the ethidium bromide is taken up only by the dead cells and fluoresces in orange (590 nm) when illuminated by 480-500 nm light. In case of apoptosis induced by cytotoxic agents, the nucleus becomes fragmented, blebbing appears on the cell membrane and the cell disintegrates into vesicles (Renvoizé et al 1998). Therefore when stained with EB/AO and observed under a fluorescent microscope, the apoptotic cells show bright green nucleus with condensed or fragmented chromatin, while in necrotic cell, the nuclear structure is intact and shows orange fluorescence (Renvoizé et al 1998; Ribble et al 2005).

It was observed in this study that in PC3 cells treated with DOBs, the cellular changes associated with apoptosis, namely ‘blebbing’ of the cells and fragmentation of the nucleus occurred (Figure 4.15). These events were evident after 24 hours of treatment at concentration near the IC$_{50}$, suggesting that the apoptotic events mediated through induced expression/activation of apoptotic and cell cycle arrest proteins.
Figure 4.14  The EB/AO staining of PC3 cells treated with vehicle (A) and treated with 100 µM CMPD3 (B) for 24 hours.

Arrow in panel (B) shows a typical apoptotic cell with ‘blebbing’ of cell membrane and diffused nucleus. Fluorescent microscopy at 20X magnification.

MCF7 cells treated with concentrations of DOBs which found to be cytotoxic to PC3 cells did not show any signs of toxicity. In case of MCF7 cells, the cells showed proliferation in presence of DOB, which was similar to the proliferative response to estradiol.

4.3  PHASE CONTRAST MICROSCOPY OF MCF7 CELLS

The MCF7 cells showed very evident proliferative responses such as shorter doubling times and cytological changes such as podosome-like and lamellipodia-like structures on treatment with DOBs or CMPD1 (Figure 4.15). Podosome and lamellipodia are actin-rich, protease-rich structures
which are involved in cell plasticity, spreading and invasion (Linder, 2007; Yamaguchi et al., 2006). In MCF7 cells, estrogenic stimulation is reported to promote podosomes formation (Goicoechea et al. 2008) which may contribute to the invasive nature of ERα breast cancer cells. The formation of podosome-like structures and lamellipodia-like structures in MCF7 cells on treatment with DOBs and CMPD1 was an indication that the DOBs and CMPD1 were capable of producing estrogenic responses in MCF7 cells.

![Figure 4.15](image)

**Figure 4.15** Formation of (A) podosomes and (B) lamellipodia in MCF7 cells treated with CMPD1. Phase contrast images at 40X magnification.

### 4.4 EFFECT OF DOBs AND IFs ON HEP2 CELLS STABLY EXPRESSING EITHER ERα OR ERβ

In case of the Hep2 cells stably expressing either ERα or ERβ, the cells mimicked the proliferative / apoptosis behavior of the MCF7 and PC3 respectively on exposure to estrogens (Figure 4.16).
Figure 4.16 Effect of the IFs, DOBs and estradiol on Hep2 cells and Hep2 cells stably expressing ERα (Hep2_ERα) or ERβ (Hep2_ERβ). Error bars represents standard deviation (n=3)

Several studies (Bake et al., 2008; Maminta et al., 1991; Wu et al., 2011) have shown that introduction and exogenous elevated expression of ERα and or ERβ in cell types inherently expressing low levels of that ER can alter the proliferation patterns of these cell types on exposure to ER agonists or antagonists. The stable over-expression of ERβ and its subsequent stimulation by agonists is anti-proliferative and apoptotic in ERβ cells as expected by its growth inhibitory role (Lazennec et al 2001b; Wu et al 2011).

Paradoxically, the effect of stable ERα overexpression and subsequent stimulation is reported to be also growth inhibitory in ERα cell lines (Bake et al., 2008; Maminta et al., 1991). However, Bake et al (2008) reported that the effect of stable expression of ERα on cells is highly dependent of the levels of ERα expression; with ERα agonists being mitogenic and stimulatory at low levels of ERα expression and being growth
inhibitory at higher expression levels of ERα. In this study, it was observed that the stably transformed Hep2 clones showed expected effects on cell viability, viz. stimulation and inhibition, with over-expression of ERα and ERβ respectively.

In Hep2 cells over expressing ERα (Hep2_ERα+), estradiol, CMPD1, CMPD3, CMPD6 and CMPD9 induced a proliferative effect. In Hep2 cells over expressing ERβ (Hep2_ERβ+), genistein, CMPD7, CMPD3, CMPD6 and CMPD9 induced apoptotic effect. The effects caused by these compounds were similar that on MCF7 and PC3 showing that the observed effect on the cell growth/apoptosis were mediated by estrogen receptors.

4.5 SUMMARY OF CELLULAR EFFECTS

The results presented in this chapter indicated that DOBs and IFs evaluated in this study were weakly to moderately estrogenic in their biological actions. Several groups have reported the cellular effects of DOBs (Fokialakis et al., 2004; Papoutsi et al., 2007) and IFs (Beard et al., 2005; Hsieh et al., 1998; Li and Sarkar, 2002) on breast and prostate cancer cells. In general, DOBs and IFs with ERα agonistic effect cause proliferative effect on ERα+ cells like MCF7 (Fokialakis et al., 2004; Hsieh et al., 1998; Papoutsi et al., 2007), and cytotoxic effect on ERβ+ cells like PC3(Kyle et al., 1997b). The effects (proliferative or cytotoxic) and extend of the effects, reported for the various compounds used in these studies depended on the ER affinity and the selectivity of the compounds. It should be noted that most of the published reports contains data for active compounds, and seldom on compounds tested and found to be inactive.

This study found that out of the eleven compounds (3 DOBs and 8 IFs) tested for their cellular effects, only five compounds (CMPD3, CMPD6, CMPD9, CMPD1 and CMPD7) showed moderate to moderately high activity.
The remaining compounds were either not active (CMPD4 and CMPD5) or very weakly active (CMPD2, CMPD8, CMPD10 and CMPD11). Both classes of compounds tested were less potent than estradiol and genistein. DOBs were more estrogenic than all the IFs, except for CMPD7. DOBs did not show significant ER subtype specificity. Among the IFs, the CMPD1 showed moderate agonist effect on cells with higher levels of ERα (MCF7 and Hep2_ERα); while CMPD7 showed strong agonist effect on cells with higher expression of ERβ (PC3 and Hep2_ERβ).

Although therapeutically not relevant, the nonspecific cytotoxicity shown by majority of the test compounds at high concentration (Figure 4.13) showed the ability of these compounds to act on cellular targets other than ER. These targets may include receptor tyrosine kinases or GPER, as similar cytotoxic effects have been reported for genistein (Akiyama et al., 1987) and estradiol (Acconcia et al., 2005), especially at high concentrations.

Taken together, these results indicated that the active compounds may potentially modulate the functioning of tissues or organs which shows differential levels of the ERs. These effects may be pharmacologically beneficial (eg: cytotoxic effect on prostate cancer) or harmful (eg: estrogenic / proliferative effect on breast cancer cells). More studies involving suitable animal models are required to get more insights into the detailed pharmacodynamics of these compounds.