CHAPTER III

Anticancer Potential of Novel Propofol-FA Analogues
INTRODUCTION

In the last decade, basic cancer research has produced remarkable advances in our understanding of cancer biology and cancer genetics. Among the most important of these advances is the realization that apoptosis and genes that control it have profound effect on the malignant phenotype. It is now well documented that most cytotoxic anticancer agents induce apoptosis, raising the intriguing possibility that defects in apoptotic programs contribute to treatment failure. Apoptosis, or programmed cell death, is a highly organized cell death process, characterized by loss of plasma membrane phospholipid asymmetry, enzymatic cleavage of the DNA into oligonucleosomal fragments, and segmentation of the cells into membrane-bound apoptotic bodies (Kidd, 1998). Apoptosis is a fundamental process essential for normal regulation of cell homeostasis (Raff et al., 1994; Thompson, 1995; Wyllie, 1997). The understanding of apoptosis has provided the basis for novel targeted therapies that can induce death in cancer cells or sensitize them to established cytotoxic agents and radiation therapy. All of the anticancer drugs used in clinic can induce apoptosis in various cell lines but the mechanisms by which these drugs activate programmed cell death are just beginning to be examined (Hickman, 1996).

Genetic changes resulting in loss of apoptosis or derangement of apoptosis-signaling pathways in the transformed cells are critical components of carcinogenesis (Kastan et al., 1995; Schulte-Hermann et al., 1997). Interestingly, induction of apoptosis of cancer cells is recognized as a valuable tool for management of cancer (Kornblau, 1998). In general, the cells execute apoptosis by caspase-3, one of a family of cysteine proteases (Thornberry and Lazebnik, 1998). This in turn is thought to be regulated by Bax and Bcl-2. Proapoptotic Bax form pores in the outer mitochondrial membrane, releasing cytochrome c while anti-apoptotic Bcl-2 prevents the opening of mitochondrial transition pore by binding with Bax (Zamzami et al., 1995; Antonsson et al., 1997). Reactive oxygen species have been suggested to act as an upstream signal for caspase-3 activation (Jacobson, 1995; Kim et al., 2007). Some types of cancers, such as B-cell chronic lymphocytic leukemia, follicular lymphoma (Tsujimoto et al., 1985) and tumors infected by human T-cell leukemia/lymphoma virus-1 (Hengartner, 2000) are
characterized by defects in apoptosis leading to immortal clones of cells. Other malignancies have defects in the apoptotic regulatory pathways such as p53, the nuclear factor kappa B (NFκB), or phosphatidylinositol 3-Kinase (PI3K)/Akt leading to defects in apoptosis (Kaufmann and Hengartner, 2001).

The impact of unsaturated FAs on the sensitivity to antineoplastic agents has been investigated in several neoplastic cell lines of laboratory animal and human origin. Some recent studies have revealed that OA, blocks the action of a cancer-causing HER-2/neu oncogene, which is found in about 30% of breast cancer patients (Menendez et al., 2005). Also, co-exposure of OA synergistically enhances efficacy of trastuzumab towards Her-2/neu over-expressors by promoting DNA fragmentation associated with apoptotic cell death. AA has also been reported to cause loss of cell viability in different types of human cancer cells (Begin et al., 1985). Many investigators have demonstrated the role of LA, GLA, DGLA, AA, ALA, EPA, and DHA, in growth inhibition and increase cytotoxicity in cancer cells in vitro (Begin et al., 1985, 1986, 1988; Fujiwara et al., 1986; Chow et al., 1989; Finstad et al., 1998; Hawkins et al., 1998; Das, 1991) Interestingly, several investigators, utilizing human cervical carcinoma (HeLa) cells (Das et al., 1998; Sangeetha and Das, 1993), human breast carcinoma cells, (Germain et al., 1998; Neades et al., 1991) and lymphoma cells (Kinsella et al., 1993) have shown enhanced cytotoxicity of doxorubicin when above mentioned FAs were added to the culture medium. Enhance cytotoxicity of cisplatin in HeLa cells (72μM GLA or 33μM EPA), (Das et al., 1998; Sangeetha and Das, 1993) cisplatin-sensitive human ovarian cells (18-36μM GLA), (Plumb et al., 1993) and human neuroblastoma cells (108μM GLA) (Ikushima et al., 1990) is observed when they were simultaneously exposed to PUFAs. However, as of now RA has not been explored for its anti-tumor activities despite the fact that such molecules may emerge as potential pharmaceutical molecules (Teomim et al., 1999).

In the present study, the cytotoxic effect of novel propofol-FA analogues was elucidated on a panel of cancer cell lines viz., HepG2, MDA-MB-361, SK-MEL-1, A549, HL-60. The effect was also studied on normal HFL1 cell line. Further in vitro anticancer
evaluation was accomplished by assessing the induction of apoptosis on treated cancer cells.

MATERIALS AND METHODS

Materials

Fetal calf serum (FCS) was procured from Bio-Whittaker. RPMI-1640 medium, Eagle's Minimum Essential Medium (EMEM); 2-[4-(2 hydroxyethyl)piperazine-1-yl]ethane sulfonic acid (HEPES); phenylmethylsulfonyl fluoride (PMSF); EDTA; dithiothreitol (DTT); 3-4, 5-dimethylthiazol-2-yl-2, 5-diphenyl-tetrazolium bromide (MTT) and bicinchoninic acid (BCA) protein estimation kit were acquired from Sigma Aldrich, USA. Sucrose, KCl, MgCl₂ and dimethyl sulfoxide (DMSO) were procured from Merck.

Anti-caspase-3 antibody, anti-cytochrome-c antibody, anti-β-actin antibody was from BD Biosciences (San Diego, CA) and a goat anti-mouse IgG-HRP secondary antibody was from Amersham Pharmacia Biotech. Nitrocellulose membranes were from BD Biosciences (San Diego, CA). Chemiluminescence detection kit was purchased from GE healthcare.

Cell lines and culture conditions

Cell lines SK-MEL-1 (ATCC# HTB-67), HepG2 (ATCC# HB-8065), MDA-MB-361 (ATCC# HTB-27), A549 (ATCC#CCL-185), HL 60 (ATCC#CCL-240) and non-cancerous HFL1 (ATCC# CCL-153) were obtained from American Type Culture Collection (Rockville, MD). SK-MEL-1 and HepG2 cell lines were maintained in EMEM whereas MDA-MB-361, A549, HL-60 and HFL1 were maintained in RPMI-1640 medium. To make the complete growth medium, both types of media were supplemented with 10% (v/v) heat-inactivated FCS, 2mM L-glutamine, 100U/ml penicillin and 100μg/ml streptomycin. All cells were maintained at 37°C in a 95% humidified atmosphere containing 5% CO₂. Cells were screened periodically for mycoplasma contamination.
**Growth inhibition in cancerous cell lines by propofol-FA analogues**

The novel compounds were examined for their cytotoxicity against five types of cancer cell lines SK-MEL-1 (human skin malignant melanoma), HepG2 (human liver hepatocellular carcinoma), MDA-MB-361 (human ductal carcinoma, breast), A549 (human lung carcinoma), HL-60 (human leukemia, acute promyelocytic) as well as non-cancerous HFL1 (human lung fibroblast) using a standard MTT reduction assay. Cells in exponential growth were seeded into 96-well plates at a concentration of 5×10⁵ cells /200µl/well and allowed to grow in RPMI-1640 medium containing 5% FCS. After 24 hr, cells were treated with different concentrations of test compound or parent controls (FA only/ propofol only) at a concentration range of 0-15µM. Vehicle control (ethanol only) and positive control (doxorubicin) cells were cultured using the same conditions. Following 94 hr incubation, the medium was removed and replaced with fresh medium. MTT (5mg/ml in PBS) was added to each well at a volume of 1:10 and incubated for 2–3 hr at 37°C. After treatment, 100µl of DMSO was added to each well after carefully aspirating the supernatants. Absorbance was measured at 620 nm in a multi-well plate reader. Triplicate samples were used in each experiment. For each treatment, cell viability was evaluated as a percentage using the following equation: \((A_{620} \text{ of treated sample}/ A_{620} \text{ of untreated sample}) \times 100\). Dose-response curves were plotted as percentages of the cell absorbances. Drug sensitivity was expressed in terms of the concentration of drug required for a 50% reduction of cell viability (IC₅₀).

**Post-nuclear fraction for apoptosis assay**

Cancer cells (1×10⁷ cells per well) were grown in 6-well plates in serum free culture medium (respective to the cell line used) in a humidified CO₂ incubator at 37°C. After 24 hr, cells were treated with control (ethanol only) or parent control (FA only/ propofol only) or test compound and incubated for further 24 hr. Following incubation, cells were harvested by trypsinization and washed twice in PBS. The cells were suspended in 50µL of ice-cold buffer containing 20mM HEPES (pH 7.4), 10mM KCl, 1.5mM MgCl₂, 0.25mM sucrose, 0.2mM EDTA, 1mM DTT and 0.1mM PMSF and homogenized in a Teflon homogenizer. A post-nuclear fraction was prepared by centrifugation for 5 min at 2,000 r.p.m. at 4°C. The supernatant was further centrifuged
for 20 min at 10,000 r.p.m. at 4°C and the resultant cytosolic fraction was used for
detecting the expression of two apoptotic factors; cytochrome c and caspase-3.

**Western blotting**

Equal amounts of protein in SDS sample buffer were subjected to 10% SDS-
polyacrylamide gel electrophoresis (Laemmli, 1970). Immunoblotting of resolved
proteins was done on nitrocellulose membranes. Non-specific binding on the
nitrocellulose filter paper was minimized by blocking for 1 hr at room temperature with
PBS-T [PBS (pH 7.5) and 0.05% Tween-20] containing 5% (w/v) non-fat dry milk. The
treated filters were washed in PBS-T and then incubated overnight at 4°C with specific
primary antibodies (monoclonal anti-cytochrome-c or monoclonal anti-caspase-3) in
PBS-T containing 5% (w/v) BSA. The membranes were again washed in PBS-T, goat
anti-mouse IgG-HRP secondary antibodies in PBS-T were added for 1 hr, and
immunoreactive bands were detected by enhanced chemiluminescence detection (ECL)
kit. Blots were re-probed with an antibody for β-actin to control for equal protein loading
and transfer. Densitometric values of protein bands were quantified using Alpha Image
Analysis software on Alpha Image Gel Documentation System.

**Protein determination**

Protein content was determined with the BCA method (Stoscheck, 1990). The
mixture of solutions A and B (1:49) of BCA reagent was added to the protein sample and
then incubated at 37°C for 45 min. The absorbance was measured at 562 nm and the
protein concentration was calculated using a standard curve of BSA.

**Statistical data analysis**

Results are expressed as the mean ± SD of three experiments for each treatment
and were plotted accordingly. Individual treatments were tested against the control by
using Student-t test. Significant differences from control were considered at p<0.05.
Analyses were conducted with SPSS version 13.0.
RESULTS

Cytotoxic effect of propofol-FA analogues on cancer cells

The cytotoxic effects were examined by evaluating the metabolic status of cancer cells after exposing them with increasing concentrations of various synthesized compounds (0, 2.5, 5, 7.5, 10, 12.5, 15µM). Inhibition of cancer cell growth was observed to be in a dose-dependent manner (Figure 3.1-3.4, A-C). All of the tested compounds showed significant (p<0.05) anticancer activity in comparison with vehicle control. Compounds were more potent, less potent or equipotent than the reference drug (Table I). It was observed that introduction of the 2,4-propofol/ 2,6-propofol moiety resulted in an increase in the activity of tested compounds when compared to the parent FA. The effectiveness of compounds however varied with respect to cell lines where inhibition was seen in the order:

2,4P-RA/ 2,6P-RA: SK-MEL-1>HL-60>HepG2>A549>MDA-MB-361 (Figure 3.1A-C)
2,4P-AA/ 2,6P-AA: HepG2>HL-60>A549>MDA-MB-361>SK-MEL-1 (Figure 3.2A-C)
2,4P-OA/ 2,6P-OA: MDA-MB-361>HepG2>HL-60>A549>SK-MEL-1 (Figure 3.3A-C)
2,4P-LA/ 2,6P-LA: HepG2>MDA-MB-361> SK-MEL-1>A549>HL-60 (Figure 3.4A-C)

When cancerous cells were tested against parent controls a slight growth inhibition in dose-dependent manner was observed for individual FAs. Only RA did not inhibit the growth of cancer cells. Both propofol isomers inhibited cancer cell growth to ~10-15%. However, they were non toxic on normal HFL1 cells.

Results on normal HFL1 cell line were observed to be quite distinct where propofol-OA/LA/RA compounds showed no killing of normal cells. At the lowest and highest concentrations (0 and 15µM) used in this study these compounds did not produce any structural alterations in normal HFL1 cells (Figure 3.1, 3.3, 3.4 (C) thereby indicating their non toxic nature on normal living cells. In contrast, propofol-AA analogues showed ~15% killing which were although less potent than parent AA (Figure 3.2C).

Note: Further in-vitro evaluation of anticancer efficacy of newly synthesized compounds was done on those cell lines which were found to be most susceptible to the
cytotoxic effect. Therefore, SK-MEL-1 (human skin malignant melanoma) for propofol-RA analogues, HepG2 (human liver hepatocellular carcinoma) for propofol-AA analogues, MDA-MB-361 (human ductal carcinoma, breast) for propofol-OA analogues and HepG2 (human liver hepatocellular carcinoma) for propofol-LA analogues was used.

**Apoptosis induction by propofol-FA analogues**

Protein fractions of cancer cells after incubation with FA or propofol alone or with test compounds individually were screened by Western blotting after 24 hr of treatment. All the test compounds were able to induce apoptosis in cancer cells at 15µM concentration whereby significant increase in expression of cytochrome c and caspase-3 was distinctly visualized (Figure 3.5, 3.7, 3.8, lanes 4-5). Although, propofol-AA analogues were able to initiate the release of cytochrome c but no expression of caspase-3 was recorded (Figure 3.6, lanes 4-5). However, 2,6-propofol analogues showed enhanced induction of apoptotic factors when compared to their 2,4-propofol counterparts. Mean densitometric values of individual bands of cytochrome c and caspase-3 were quantified where each value was mean of three experiments. At 15µM tested concentration, increased expression of cytochrome c as well as caspase-3 was recorded with slight variations in the activity of respective compounds of same FA group. In propofol-RA analogues; 27.4% and 28.7% cytochrome c release and 31.4% and 31.6% caspase-3 expression was observed after treatment with 2,4P-RA and 2,6P-RA, respectively (Figure 3.5B-C). In propofol-AA analogues; 24.2% and 25% cytochrome c release was observed after treatment with 2,4P-AA and 2,6P-AA, respectively (Figure 3.6B). In propofol-OA analogues; 40% and 42.4% cytochrome c release and 41.4% and 43.4% caspase-3 expression was observed after treatment with 2,4P-OA and 2,6P-OA, respectively (Figure 3.7B-C). The propofol-LA analogues induced 35.2% and 36.6% cytochrome c release and 32.6% and 33.7% caspase-3 expression after treatment with 2,4P-LA and 2,6P-LA, respectively (Figure 3.8B-C). Moreover, distinct increase in degree of apoptosis induced by all test compounds was observed when compared to their respective parent controls.
Figure 3.1A: Viability assessment of HepG2 and MDA-MB-361 cancer cells after treatment with propofol-RA analogues. Effect of novel compounds along with parent RA and 2,4-/2,6-propofol are expressed as the mean ± SD of three experiments.
Figure 3.1B: Viability assessment of SK-MEL-1 and A549 cancer cells after treatment with propofol-RA analogues. Effect of novel compounds along with parent RA and 2,4-/2,6-propofol are expressed as the mean ± SD of three experiments.
Figure 3.1C: Viability assessment of HL-60 cancer cells and HFL1 normal cells after treatment with propofol-RA analogues. Effect of novel compounds along with parent RA and 2,4-/2,6-propofol are expressed as the mean ± SD of three experiments.
Figure 3.2A: Viability assessment of HepG2 and MDA-MB-361 cancer cells after treatment with propofol-AA analogues. Effect of novel compounds along with parent AA and 2,4-/2,6-propofol are expressed as the mean ± SD of three experiments.
Figure 3.2B: Viability assessment of SK-MEL-1 and A549 cancer cells after treatment with propofol-AA analogues. Effect of novel compounds along with parent AA and 2,4-/2,6-propofol are expressed as the mean ± SD of three experiments.
Figure 3.2C: Viability assessment of HL-60 cancer cells and HFL1 normal cells after treatment with propofol-AA analogues. Effect of novel compounds along with parent AA and 2,4-/2,6-propofol are expressed as the mean ± SD of three experiments.
Figure 3.3A: Viability assessment of HepG2 and MDA-MB-361 cancer cells after treatment with propofol-OA analogues. Effect of novel compounds along with parent OA and 2,4-/2,6-propofol are expressed as the mean ± SD of three experiments.
Figure 3.3B: Viability assessment of SK-MEL-1 and A549 cancer cells after treatment with propofol-OA analogues. Effect of novel compounds along with parent OA and 2,4-/2,6-propofol are expressed as the mean ± SD of three experiments.
Figure 3.3C: Viability assessment of HL-60 cancer cells and HFL1 normal cells after treatment with propofol-OA analogues. Effect of novel compounds along with parent OA and 2,4-/2,6-propofol are expressed as the mean ± SD of three experiments.
Figure 3.4A: Viability assessment of HepG2 and MDA-MB-361 cancer cells after treatment with propofol-LA analogues. Effect of novel compounds along with parent LA and 2,4-/2,6-propofol are expressed as the mean ± SD of three experiments.
Figure 3.4B: Viability assessment of SK-MEL-1 and A549 cancer cells after treatment with propofol-LA analogues. Effect of novel compounds along with parent LA and 2,4-/2,6-propofol are expressed as the mean ± SD of three experiments.
Figure 3.4C: Viability assessment of HL-60 cancer cells and HFL1 normal cells after treatment with propofol-LA analogues. Effect of novel compounds along with parent LA and 2,4-/2,6-propofol are expressed as the mean ± SD of three experiments.
Table I: Cytotoxicity of propofol-FA analogues (1-8) in a panel of cell lines.

<table>
<thead>
<tr>
<th>PROPOFOL-FA ANALOGUES</th>
<th>CELL LINES (IC₅₀, µM)</th>
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<tbody>
<tr>
<td></td>
<td>HepG2</td>
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<tr>
<td>2,4-diisopropylphenol-ricinoleic acid (2,4P-RA)</td>
<td>4.5</td>
</tr>
<tr>
<td>2,6-diisopropylphenol-ricinoleic acid (2,6P-RA)</td>
<td>4.4</td>
</tr>
<tr>
<td>2,4-diisopropylphenol-arachidonic acid (2,4P-AA)</td>
<td>3.7</td>
</tr>
<tr>
<td>2,6-diisopropylphenol-arachidonic acid (2,6P-AA)</td>
<td>3.1</td>
</tr>
<tr>
<td>2,4-diisopropylphenol-oleic acid (2,4P-OA)</td>
<td>3.5</td>
</tr>
<tr>
<td>2,6-diisopropylphenol-oleic acid (2,6P-OA)</td>
<td>2.9</td>
</tr>
<tr>
<td>2,4-diisopropylphenol-linoleic acid (2,4P-LA)</td>
<td>3.2</td>
</tr>
<tr>
<td>2,6-diisopropylphenol-linoleic acid (2,6P-LA)</td>
<td>2.75</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>2.17</td>
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</table>

The highest concentration tested was 15µM. Values are means of three observations. LA, less active; NA, not active; SK-MEL-1, human skin malignant melanoma; HepG2, human liver hepatocellular carcinoma; MDA-MB-361, ductal breast carcinoma; A549, human lung carcinoma; HL-60, human leukemia; HFL1, human lung fibroblast.
Figure 3.5A: Immunoblots showing relative distribution of cytochrome c, caspase-3 and β-actin (loading control) in SK-MEL-1 (human skin malignant melanoma) cells after treatment with (1) control; (2) ricinoleic acid; (3) propofol; (4) 2,4P-RA; (5) 2,6P-RA.

B-C: Densitographs showing relative density of cytochrome c and caspase-3 respectively, in the nitrocellulose blots. Results are mean values ± SD in triplicates.
Figure 3.6A: Immunoblots showing relative distribution of cytochrome c and β-actin (loading control) in HepG2 (human liver hepatocellular carcinoma) cells after treatment with (1) control; (2) arachidonic acid; (3) propofol; (4) 2,4P-AA; (5) 2,6P-AA.

B: Densitograph showing relative density of cytochrome c, in the nitrocellulose blots. Results are mean values ± SD in triplicates.
Figure 3.7A: Immunoblots showing relative distribution of cytochrome c, caspase-3 and β-actin (loading control) in MDA-MB-361 (human ductal carcinoma, breast) cells after treatment with (1) control; (2) oleic acid; (3) propofol; (4) 2,4P-OA; (5) 2,6P-OA.

B-C: Densitographs showing relative density of cytochrome c and caspase-3 respectively, in the nitrocellulose blots. Results are mean values ± SD in triplicates.
Figure 3.8A: Immunoblots showing relative distribution of cytochrome c, caspase-3 and β-actin (loading control) in HepG2 (human liver hepatocellular carcinoma) cells after treatment with (1) control; (2) linoleic acid; (3) propofol; (4) 2,4P-LA; (5) 2,6P-LA.

B-C: Densitographs showing relative density of cytochrome c and caspase-3 respectively, in the nitrocellulose blots. Results are mean values ± SD in triplicates.
DISCUSSION

The effects of FAs upon tumor cells have been attributed to the induction of programmed cell death by apoptosis. The induction of apoptosis by unsaturated FAs has been extensively studied in many different tumor types both in vitro and in vivo and was recently reviewed by Serini et al. (2009). Many studies have investigated apoptosis in breast and colon cancer in an attempt to identify the mechanisms behind PUFA-induced cell death (Jiang et al., 1998; Dupertuis et al., 2007). It has been observed that apoptosis is induced by unsaturated FAs through both the extrinsic (Fas) and the intrinsic (mitochondrial) molecular pathways, with the subsequent activation of caspases and cleavage of their specific substrates resulting in cell death in vitro (Hawkins et al., 1998; Colquhoun et al., 2001). The improve outcome of chemotherapy is now well recognized with co-administration of antioxidants in subjects with cancer (Chinery et al., 1997). As an example, co-administration of pyrrolidine dithiocarbamate (PDTC) or vitamin E with 5-FU or doxorubicin inhibits the growth of colorectal cancer tumors in mice. Propofol, which functions as antioxidant (Eriksson et al., 1992; Aarts et al., 1995), is useful in the treatment of cancer or as adjuvant in the treatment of cancer. Co-administered with chemotherapeutic agents, they enhance cytotoxicity, thereby inhibiting the growth of tumors. In addition, they also inhibit oxidative damage that generally accompanies use of anticancer agents (Hendler et al., US Patent 6254853, 2001). Propofol can be used to prevent or ameliorate the effects of chemotherapeutic agents that have oxidative damage as a significant side effect.

Keeping in view the fact, that unsaturated FA plays a role in tumor growth suppression and propofol enhance the cytotoxicity of anticancer agent, all the eight novel propofol-FA analogues were investigated for their anticancer efficacy, in vitro. After obtaining the compounds, the cytotoxic interactions between the FA and propofol and their effects on a panel of cancer cells were examined. The significant growth inhibition (Figure 3.1-3.4, A-C) of cancer cells in a dose-dependent manner indicates the test compounds to possess potent anticancer properties. Difference in activity of compounds and selectivity towards cancer cell was, however, observed where the compounds of propofol-AA/LA analogues showed the preference towards liver carcinoma (HepG2),
propofol-OA analogues towards breast carcinoma (MDA-MB-361) and propofol-RA analogues towards skin melanoma (SK-MEL-1). Nevertheless, the novel compounds were able to inhibit the metastatic potential of cancer cells at concentration where parent reactant controls alone were not effective. Interestingly, the synthesized test compounds (except propofol-AA analogues) did not induce any structural alterations in non cancerous HFL1 cells under experimental conditions while they were able to inhibit cancer cells, indicating that toxicity is not due to FA metabolism per se but rather due to the effect of the ester based compounds. Overall, the inhibitory effects of novel compounds were more or less similar to the results obtained for DHA-propofol esters (Harvey et al., 2010).

The effects of FAs upon tumor cells have been attributed to the induction of programmed cell death by apoptosis. In present study, role of the novel compounds on induction of apoptotic factors was also analyzed by determining the release of cytochrome c and activation of caspase-3. Cytochrome c has a function in the intrinsic pathway of apoptosis and leads to the activation of caspase-3, which is a downstream enzyme in the apoptosis process and is involved in the execution phase of the death pathway (Stennicke et al., 1998). Results (in Figure 3.5-3.8) show that all compounds were able to induce apoptosis (immunoblotting method) in cancer cells. Slight induction of apoptosis was found after treatment with parent propofol. Also, difference in inhibition activity was observed within compounds of same FA where both 2,6-propofol-FA were found to be more potent than 2,4-propofol-FA analogues. In contrast, both forms of RA (2,4P-RA and 2,6P-RA) were comparatively similar in their activity (Figure 3.5). Overall, the compounds were able to induce apoptosis in cancer cell lines confirming that these compounds induce a pathway for apoptosis that eventually leads to the death of cancer cells. As, apoptotic cell death is accompanied by the release of cytochrome c from mitochondria to the cytosol as well as, activation of caspase-3, indicating to the mitochondrial mode of programmed cell death.

Present in vitro study demonstrate that the increased antiproliferating effect of novel compounds can be attributed to their unique structures wherein FA of strong
hydrophobic nature is rapidly translocated against the plasma membrane and propofol, being a partly lipophilic agent, also facilitates intake of the chemotherapeutic agent. Volatile nature and a very short plasma half-life of propofol also help in its rapid removal from membranes (Miller, 2000). Conjugation of propofol with selected FAs will result in increase in lipid-solubility, bioavailability, enhance its activity, and decrease its side-effects. Moreover, the two moieties of the compound, which should be released in the cell via enzymatic actions, were expected to have synergistic effect so that an optimum increase in their chemotherapeutic index is possible.