CHAPTER II

Synthesis and Characterization of Novel Anticancer Compounds
INTRODUCTION

Fatty acids (FA) are composed of hydrocarbon chains of variable length with an omega (ω) methyl group at one end and a delta (Δ) carboxyl group at the other end of the chain. The biological activity and metabolism of FAs is often determined by both the carbon chain length and the number of unsaturated bonds present within the chain. Functionally, unsaturated FAs act upon the expression pattern of some vital proteins and thereby alter the metabolic status in a manner quite different to that of typically saturated FA. Evidences have shown that ω-3, ω-6 and ω-9 FAs present in natural sources like castor oil, olive oil, almonds, peanuts, red meat, fish oil etc. can control several types of cancer. Preclinical studies reveal that certain unsaturated FAs may actually enhance the cytotoxicity of several antineoplastic agents and the anticancer effects of radiotherapy. The unsaturated FAs show induction of apoptosis in tumor cells both in vitro and in vivo. They can induce apoptosis through both the extrinsic (Fas) and the intrinsic (mitochondrial) molecular pathways, with subsequent activation of caspases and cleavage of their specific substrates resulting in cell death in vitro. PUFAs in the form of dietary supplements in animal models, exhibited greatly increased apoptotic indices on hepatoma, colon and breast tumors. For the present study, among various FAs, ricinoleic acid (RA), oleic acid (OA), arachidonic acid (AA) and linoleic acid (LA) were taken for the synthesis of novel ester compounds and to explore their anticancer prospective.

**Arachidonic Acid (AA):** (all-cis 5,8,11,14-eicosatetraenoic acid) is an ω-6 polyunsaturated fatty acid (PUFA) which is primarily found in fatty parts of red meat and fish and is normally consumed in normal amounts in the regular diet (Li *et al*., 1998). In chemical structure, it is composed of four cis double bonded to 20-carbon chain. The presence of four cis double bonds are primarily responsible for its flexibility, keeping the pure FA liquid, even at subzero temperatures, and helping to impart mammalian cell membranes their correct fluidity at physiological temperature. The double bonds are also the key to the propensity of AA to react with molecular oxygen (Brash, 2001).
Chemical structure of arachidonic acid

AA is one of the essential FAs present in the phospholipids of membranes of the body's cells, and is abundant in the brain and muscles. In addition to being involved in cellular signaling, it is also a key inflammatory intermediate (Baynes, 2005). Contrary to other more abundant unsaturated FAs, levels of unesterified AA is stringently controlled within mammalian cells (Chilton et al., 1996).

Arachidonic acid metabolism, over the past decade, has apparently been observed to play a key role in cancer biology where the function of AA and induction of cancer cell apoptosis has become an interesting tool in cancer therapy. Despite the role of cellular AA in cancer progression the exogenous AA has shown anticancer properties. Studies have shown that diets supplemented with pure AA did not stimulate growth in rodent tumors, even in essential FA-deficient rats (Hillyard and Abraham, 1979). The crucial anticancer role of AA has been supported by the observation that cells resistant to killing by TNFα also fail to produce AA (Neale et al., 1988). DMBA-initiated mouse skin failed to result in a tumor if treated with AA (Conney et al., 1997). HepG2 cells also undergo cytotoxicity and apoptosis when treated with AA (Chen et al., 1997). Moreover, initiation of apoptosis by exogenous AA is reported to be mediated by production of ceramide (Chan et al., 1998). In rat hepatoma AS-30D cells, AA induced apoptosis results in drastic production of reactive oxygen species (Dymkowska et al., 2006).

**Ricinoleic Acid (RA):** Castor oil is obtained from the seeds of the castor bean; *Ricinus communis* L. Approximately 90% of castor oil is composed of a hydroxyl unsaturated FA (ω-9) known as ricinoleic acid. It is the pharmacologically active
constituent produced from hydrolysis of castor oil (Borsotti et al., 2001).

**Chemical structure of ricinoleic acid**

Ricinoleic acid (cis-12-hydroxyoctadeca-9-enoic acid) is a C18 fatty acid with a cis-configured double bond in the 9th position and a hydroxyl group in the 12th position. Unlike other unsaturated FAs presence of one hydroxyl group provide several interesting properties to RA by which it can be converted to useful products.

A study on biological action of RA has shown that it does not exert any hyperalgesic effect towards heat and chemical nociceptive stimuli (Vieira et al., 2000). These properties render RA a promising analgesic and anti-inflammatory agent via its local application. Preliminary experiments have also indicated that RA could have a pro- or anti-inflammatory action following acute or repetitive local application, respectively. Its surfactant properties have been attributed as the cause for showing antibacterial activity (Gaginella et al., 1977). Moreover, its chemical structure has been used as a basis for the development of novel capsaicin-like compounds (Klopman and Li, 1995). RA has also been used for the synthesis of biodegradable polymers developed specifically to be used as implantable drug delivery matrices for the treatment of various pathological conditions (Teomim et al., 1999).

**Oleic Acid (OA):** OA (ω-9) is one of the naturally occurring FA present in greater quantities in plants such as olives, almonds, peanuts, sesame oil, pistachio nuts, cashews, hazelnuts, etc. It has 18-carbon chain with the empirical formula C_{18}H_{34}O_{2} and has only one double bond (monounsaturated) at 9th position which makes it much less susceptible to oxidation thereby helping in imparting high stability and antioxidant action (Owen et al., 2000).
High concentrations of OA are found to lower the levels of cholesterol, risk of heart problems, artherosclerosis and aids in cancer prevention (Rickman, 2004; Win and Au, 2005). Various epidemiological and animal model studies have established that consumption of olive oil, which is rich in OA, reduces the risk of breast cancer (Lipworth et al., 1997; Simonsen et al., 1998; Solanas et al., 2002; Moral et al., 2003). Several in vitro studies examined for anticancer activity of OA, have shown that mainly affected cell lines are those of breast cancer (Noguchi et al., 1997; Rose et al., 1990; 1996; 1999; Hatala et al., 1994). OA has also been observed to synergistically enhance effectiveness of various anticancer agents (Menendez et al., 2005; Reuters, 2005).

**Linoleic acid (LA):** is another member of ω-6 PUFA family and is called 18:2(ω-6). Chemically, it is a carboxylic acid with an 18-carbon chain and two cis double bonds. The first double bond is located at the 6th carbon position and second at 9th position.
LA is one of two essential FAs that humans and other animals must ingest for good health because the body requires them for various biological processes, but can not synthesize them from other food components. It is a PUFA used in the biosynthesis of AA and thus some prostaglandins. It is found in the lipids of cell membranes. It is abundant in many vegetable oils, comprising over half (by weight) of poppy seed, safflower, sunflower, and corn oils. LA by itself has been reported to show anti-tumor activity on various neoplastic cells in culture. It shows antiproliferative effects against human prostrate cancer cells (Robbins et al., 1999). Anticancer effect of LA has also been observed in human breast cancer cells where it is found to be most potent in enhancing the paclitaxel cytotoxicity (Menendez et al., 2001). Rat hepatoma AH-109A cells have also shown increased susceptibility towards cytotoxic action of gamma-LA (Hayashi et al., 1990). LA has also been reported to exert differential effects on the kinetics of anticancer drugs (Ikushima et al., 1990).

Current trends in the treatment of human cancers favor drug combinations. The fact that polyunsaturated FAs inhibit tumor cell proliferation, induce apoptosis and improve sensitivity of cancer cells to other chemotherapeutic agents (Owen et al., 2000; Menendez et al., 2005 Rickman, 2005; Win, 2005) has led to the development of compounds containing FA conjugates. Effectiveness of conjugated-FA has established them as promising molecules in cancer prevention (Moyer et al., 2003; Menendez et al., 2004; Mustafa et al., 2004; Siddiqui et al., 2005). However, FA-substituted propofol analogues have received very little attention despite the fact that such molecules may also emerge as potential anticancer agents.

**Propofol** (diisopropylphenol) is a low molecular weight phenol that is widely used as an intravenous sedative-hypnotic agent in humans and animals (Covington, 1998). Among various advantages its rapid clearance, minimal side effects and non-toxicity to humans at high levels (Coetze et al., 1995; Miller, 2000) make it appropriate for development of anticancer esters. Preliminary studies have demonstrated the toxicity doses
of propofol where LD50 in mouse was 170mg/kg (Moffett et al., 1960) and LD50 in human was 386mg/kg (el-Ebiary et al., 1995). It has a structural analogy with the antioxidant vitamin E, a property that partly explains its antioxidant properties (Eriksson et al., 1992; Aarts et al., 1995). Propofol has a broad range of biological and medical applications. It has been reported to be an anti-emetic (Castano et al., 1995), an anti-epileptic (Kuisma et al., 1995) and an anti-pruritic (Borgeat et al., 1994). Propofol also has significant application as an antioxidant (Sanchez et al., 1994; Sanchez et al., 1995; Aarts et al., 1995). Clinically relevant concentrations of propofol are reported to decrease the metastatic potential of human cancer cells (Mammoto et al., 2002) and have also been shown to induce apoptosis through both extrinsic and intrinsic pathways (Tsuchiya et al., 2002).

In the present study, OA, AA, RA, LA were taken for synthesis of novel compounds with anticancer properties. The FA was conjugated with one of the two isomers of propofol viz. 2,4-diisopropylphenol (2,4-propofol) or 2,6-diisopropylphenol (2,6-propofol) separately. Spectroscopy has been employed as one of the principal techniques to obtain quantitative, physical, chemical, electronic and structural information about molecules. Many scientific techniques exploit spectroscopic phenomena to study crystals and non-crystalline materials and determining the structure of a compound. Therefore, in this phase of study, to establish the structure of the synthesized compounds various analytical methods such as UV spectroscopy, FT-IR, $^1$H NMR, $^{13}$C NMR and high-resolution FAB-MS have been exploited.

**MATERIALS AND METHODS**

**Materials**

Oleic acid, arachidonic acid, ricinoleic acid, linoleic acid, 2,4-diisopropylphenol, 2,6-diisopropylphenol, N, N-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP) were acquired from Sigma Aldrich, USA. Thin-layer chromatographic plates (60A, 0.2mm thick) and silica gel (60-120 mesh) were purchased from Fisher Scientific. Dichloromethane (DCM), methanol, ethanol, chloroform, $n$-hexane, diethyl ether and acetic acid were procured from Merck.
**Synthesis and purification of propofol-FA analogues**

Synthesis of the compounds was accomplished using method of Siddiqui *et al.* (2005) standardised with some modifications. Firstly, 1mmol of respective FA was dissolved in 5ml DCM. Subsequently, 0.45mmol of coupling reagent DCC was added and the reaction mixture was stirred for 10 min at room temperature (23-25°C). Finally, 1mmol of one of the propofol isomer (2,6-propofol/ 2,4-propofol) was dissolved and reaction mixture was esterified in the presence of a catalyst, 0.152mmol DMAP. The reaction mixture was stirred for a period of 10 hr in dark. Reaction was stopped by filtration and the filtrate was concentrated under reduced pressure to yield the product. The synthesis of product was visualized by thin layer chromatography (TLC) in iodine vapour. Finally, the synthesized compound was purified by silica gel (60-120 mesh) column chromatography with solvent system *n*-hexane and diethyl ether (1:1).

**Characterization of propofol-FA analogues**

The formation of new compounds and their structural description was done employing various techniques. Just before use an aliquot of compounds were diluted in less than 0.1% of ethanol (Siddiqui *et al.*, 2005).

**UV spectroscopy**

Presence of propofol in the compounds was assessed by UV spectroscopy on UV Mini-1240 spectrophotometer. The absorption spectrum was measured between 200 to 600 nm. The absorbance was read and spectral scanning curves were made.

**FT-IR spectroscopy**

The infrared spectrum was recorded on Nikolet-6700 FT-IR. Ten microliter (1μg/μl) of the compound was deposited exactly within the cell limit and was run as a thin film after evaporation of solvent. For data acquisition a resolution of 32 was used. The analysis was performed in triplicate with data spacing of 15.428 cm\(^{-1}\).

**NMR spectral studies**

The formation of propofol-FA analogues was elucidated with \(^1\)H NMR and \(^13\)C NMR spectra. The data was recorded on BRUKER AVANCE II 400 NMR at
sophisticated analytical instrumentation facility (SAIF) of Central Drug Research Institute, Lucknow, India.

**FAB-MS studies**

The molecular mass of propofol-FA analogues was determined by mass spectrometry at SAIF of Central Drug Research Institute, Lucknow, India. The FAB-MS spectrum was recorded on a JEOL SX 102 Mass Spectrometer/Data System using Argon/Xenon (6kV, 10mA) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature. M-Nitrobenzoyl alcohol (NBA) was used as the matrix.

**RESULTS**

**Synthesis of propofol-FA analogues**

The chemical synthesis setup resulted in synthesis of ester derivatives of propofol (Scheme 2.1A-D). The follow up on TLC studies (Figure 2.2) confirmed the reaction between respective FA and propofol analogue. The resulting single new product was observed at locations having different RF values where RF of the product was found to be different from both of the parent FA or propofol as substrates. The compounds were synthesized in good yield and were colorless viscous liquid (oily) at room temperature. The nomenclature used for newly synthesized compounds, their simulated models, RF values and % yield are depicted in Figure 2.3A-B.

**Characterization of propofol-FA analogues**

**Compound 1: 2,4-diisopropylphenol-ricinoleic acid (2,4P-RA):**

Yield 65%; 1H NMR (CDCl3, δH): 0.88 (t, J = 9.88 Hz, 3H), 1.19 – 1.48 (m, 30H), 1.77 (m, 2H), 2.07 (q, 2H), 2.23 (t, J = 13.72 Hz, 2H), 2.56 (t, J = 15.0 Hz, 2H), 2.8 (m, 1H), 2.9 (m, 1H), 3.6 (m, 1H), 5.4 (m, 1H), 5.5 (m, 1H), 6.88-6.90 (d, J = 5.76 Hz, 1H), 7.04 (d, J = 8.0 Hz, 1H), 7.12 (s, 1H); 13C NMR (CDCl3, δC): 71.58, 125.24, 133.42, 134.1, 141.02, 150.93, 172.73. FAB-MS: (M)+ 458, (M-17)+ 441.
**Compound 2: 2,6-diisopropylphenol-ricinoleic acid (2,6P-RA):**

Yield 70%; $^1$H NMR (CDCl$_3$, $\delta_H$): 0.80 ($t, J = 6.96$ Hz, 3H), 1.11 – 1.40 (m, 30H), 1.68-1.72 (m, 2H), 1.98 ($q$, 2H), 2.1 ($t, J = 13.6$ Hz, 2H), 2.54 ($t, J = 15.08$ Hz, 2H), 2.8 (m, 2H), 3.5 (m, 1H), 5.3 (m, 1H), 5.5 (m, 1H), 6.80-6.82 ($d, J = 15.44$ Hz, 1H), 6.9 ($t, J = 7.6$ Hz, 1H); $^{13}$C NMR (CDCl$_3$, $\delta_C$) 71.52, 120.6, 123.87, 126.42, 133.67, 139.54, 145.61, 172.40. FAB-MS: (M-17)$^+$ 441

**Compound 3: 2,4-diisopropylphenol-arachidonic acid (2,4P-AA):**

Yield 82%; $^1$H NMR (CDCl$_3$, $\delta_H$; ppm; $J$, Hz): 0.88 (3 H, q, $J 6.8$ Hz, H20), 1.22 (12 H, m, 2Ar-C-(CH$_3$)$_2$), 1.25 to 1.30 (6 H, m, H18 + H19), 1.32-1.35 (2 H, m, H17), 1.85 (2 H, t, $J 7.4$, H3), 2.06 (2 H, dt, $J6.6$, H16), 2.22 (2 H, dd, $J6.2$ and 6.8, H4), 2.58 (2 H, t, $J7.6$, H2), 2.84 (6 H, m, H7 + H10 + H13), 2.87 (2 H, m, Ar-CH-), 5.35-5.44 (8 H, m, H5 + H6 + H8 + H9 + H11 + H12 + H14 + H15), 6.87 (1 H, d, $J8.1$, Ar-H6$^-$) and 7.05 (1 H, dd, $J6.0$, and 2.4, Ar-H5$^-$) and 7.13 (1 H, d, $J2.2$, Ar-H3$^-$); $^{13}$C NMR (CDCl$_3$; $\delta$, ppm): 14.1 (C20), 22.6 (C19), 23.0 (Ar-C-(C)$_2$)$_2^-$, 24.1 (C3), 24.9 (C4), 25.6 (C7), 26.6 (C10), 27.2 (C13)$^|$, 27.5 (Ar-C), 29.3 (C16), 29.3 (C17), 31.5 (C18), 33.7 (Ar-C$_4$-'C), 33.6 (C2), 121.8 (Aromatic carbon C6$^-$), 124.4 (Aromatic carbon C5$^-$), 124.6 (Aromatic carbon C3$^-$), 127.8-130.7 (C5, C6, C8, C9, C11, C12, C14 and C15), 139.5 (Aromatic carbon C2$^-$), 146.0 (Aromatic carbon C4$^-$) 146.5 (Aromatic Cl$^-$) and 172.2 (CO). FAB-MS: m/z:487 (M+Na)$^+$ (6%), m/z: 439 (63%), m/z: 263 (78%), m/z: 261 (16%), m/z: 245 (9%), m/z: 205 (6%), m/z: 178 (100%), m/z:163 (97%), m/z:137 (8%), m/z:135 (28%), m/z:95 (66%), 91 (53%), 82 (34%) and lower mass fragments (less than 6%).

Note: $^*$Asterisks denote assignments that may be interchanged due to unhindered behavior of 2,4-propofol as comparison of 2,6-propofol.

**Compound 4: 2,6-diisopropylphenol-arachidonic acid (2,6P-AA):**

Yield 85%; $^1$H NMR (CDCl$_3$, $\delta_H$; ppm; $J$, Hz): 0.88 (3 H, q, $J 7.0$ Hz, H20), 1.20 (12 H, d, J6.8, 2Ar-C-(CH$_3$)$_2$), 1.25 to 1.36 (6 H, m, H17 + H18 + H19), 1.88 (2 H, t, $J 7.3$, H3), 2.06 (2 H, dis, J6.8, H16), 2.24 (2 H, d, J6.0 and 6.8, H4), 2.63 (2 H, t, $J7.4$, H2), 2.85 (6 H, m, H7 + H10 + H13), 2.88 (2 H, m, Ar-CH-), 5.35-5.91 (8 H, m, H5 + H6 + H8 + H9 + H11 + H12 + H14 + H15), 7.15 (1 H, t, J6.4, Ar-H4$^-$) and 7.20 (2 H, d, J6.0, Ar-H3$^-$ and H5$^-$); $^{13}$C NMR (CDCl$_3$; $\delta$, ppm): 14.1 (C20), 22.6 (C19), 22.7, (C3), 24.9
(C4), 25.6 (C7), 26.7 (C10), 27.1 (C13), 27.2 (Ar-C-(C)2-), 27.5 (C16), 29.3 (Ar-C), 29.7 (C17), 30.9 (C18), 33.6 (C2), 123.4-123.9 (Aromatic carbon C3' and C5'), 126.7 (Aromatic carbon C4') 127.8-130.7 (C5, C6, C8, C9, C11, C12, and C14), 133.6 (C15), 140.3 (Aromatic carbon C2' and C6'), 145.5 (Aromatic C1') and 172.2 (CO). FAB-MS: m/z:510 (M+2Na)$^+$ (3%), m/z: 418 (74%), m/z: 401 (13%), m/z: 375 (68%), m/z: 373 (6%), m/z: 333 (29%), m/z: 285 (6%), m/z: 271 (100%), m/z:269 (48%), m/z:237 (13%), m/z:178 (87%), m/z:163 (42%), m/z:137 (35%), m/z:135 (13%), m/z:111 (10%), 95 (16%), 91 (Characteristics peak), 82 (13%) and lower mass fragments (less than 6%).

**Compound 5: 2,4-diisopropylphenol-oleic acid (2,4P-OA):**

Yield 85%; $^1$H NMR (CDCl$_3$, δ$_H$): 0.88 (t, J = 3.76 Hz, 3H), 1.20 (d, J = 6.92 Hz, 12H), 1.25 – 1.46 (m, 22H), 1.79 (p, J = 7.32 Hz, 2H), 2.0 (q, 2H), 2.6 (t, J = 7.64 Hz, 2H), 2.8 (t, J = 6.68 Hz, 1H), 2.9 (m, 1H), 5.3 (m, 2H), 7.14 (d, J = 2.2 Hz, 1H), 7.16 (s, 1H), 7.20 (d, J = 2.88 Hz, 1H); $^{13}$C NMR (CDCl$_3$, δ$_C$): 14.1, 22.6, 25.06, 25.66, 27.23, 27.53, 29.18–29.63, 31.55, 34.22,123.87, 126.42, 127.91, 128.12, 130.02, 130.25, 140.32, 145.62, 172.38. FAB-MS: (M)$^+$ 442.

**Compound 6: 2,6-diisopropylphenol-oleic acid (2,6P-OA):**

Yield 89%; $^1$H NMR (CDCl$_3$, δ$_H$): 0.88 (t, J = 3.92 Hz, 3H), 1.19 – 1.43 (m, 34H), 1.76 (m, 2H), 2.0 (m, 2H), 2.57 (t, J = 7.48 Hz, 2H), 2.9 (m, 2H), 5.35 (m, 2H), 6.89 (d, J = 5.96 Hz, 2H), 7.0 (t, J = 6.08 Hz, 1H); $^{13}$C NMR (CDCl$_3$, δ$_C$) 14.23, 22.71, 24.31, 24.97, 27.22, 27.50, 29.15–29.79, 33.56, 34.13,121.90, 124.08, 124.37, 130.06, 130.25, 140.32, 146.52, 172.68. FAB-MS: (M)$^+$ 442.

**Compound 7: 2,4-diisopropylphenol-linoleic acid (2,4P-LA):**

Yield 84%; $^1$H NMR (CDCl$_3$, δ$_H$): ppm; J, Hz): 0.88 (3 H, q, J 6.8 Hz, H20), 1.22 (12 H, m, 2Ar-C-(CH$_3$)$_2$),1.44 to 1.33 (14 H, m, 7×CH$_2$), 1.78-1.75 (2 H, t, J 7.6, -COCH$_2$CH$_2$-), 2.07 (4 H, q, J 6.8,2-CH$_2$-CH=), 2.58 (2 H, t, J6.4, -COCH$_2$-), 2.79 (2 H, t, J6.4, =CH-CH$_2$-CH=), 3.00 and 2.90 (2 H, t and t, Ar-2CH-), 6.87 (1 H, d, J8.1, Ar-H6') and 7.05 (1 H, dd, J6.0, and 2.4, Ar-H5') and 7.13 (1 H, d, J2.2, Ar-H3'); $^{13}$C NMR (CDCl$_3$; δ, ppm): 25.0 (C3'), 29.1-29.7 (C4, C5, C6, C7, C14, C15,), 27.2 (C8), 130.2
(C9), C10 (128.1), C11 (25.6), C12 (127.9), C13 (130.0), C16 (31.5), C17 (22.6), 14.1 (C18), 33.8 (Ar-C-(C)2-)*, 27.5 and 23.0 (Ar-2C)*, 121.9 (Aromatic carbon C6'), 124.3 (Aromatic carbon C5'), 124.6 (Aromatic carbon C3'), 139.5 (Aromatic carbon C2'), 146.0 (Aromatic carbon C4') 146.5 (Aromatic C1') and 172.2 (CO). Note: *Asterisks denote assignments that may be interchanged due to merging of peaks.

**Compound 8: 2,6-diisopropylphenol-linoleic acid (2,6P-LA):**

Yield 86%; $^1$H NMR (CDCl$_3$, $\delta$H; ppm; J, Hz): 0.88 (3 H, q, J 6.3 Hz, H20), 1.20 (12 H, d, J6.8, 2Ar-C-(CH$_3$)$_2$), 1.35 to 1.26 (14 H, m, 7×CH$_2$), 1.88 (2 H, q, J 7.5, Hz - COCH$_2$CH$_2$-), 2.06 (4 H, q, J 6.8, 2-CH$_2$-CH=), 2.63 (2 H, t, J 7.6, -COCH$_2$-), 2.24 (2 H, d, J6.8, =CH-CH$_2$-CH= ), 3.18 (2 H, m, Ar-CH-), 7.15 (1 H, t, J6.4, Ar-H4') and 7.20 (2 H, d, J6.0, Ar-H3' and H5'); Ar-H6') and 7.05 (1 H, dd, J6.0, and 2.4, Ar-H5') and 7.13 (1 H, d, J2.2, Ar-H3'); $^{13}$C NMR (CDCl$_3$; $\delta$, ppm): 25.0 (C3*), 29.2-29.7 (C4, C5, C6, C15, C17), 31.9 (C7), 27.2 (C8), 130.6 (C9), 128.1 (C10), C11 (27.1), C12 (127.9), C13 (130.2), C14 (29.2), 14.1 (C18), 27.5 (Ar-C-(C)2-)*, 26.5 (Ar-C)*, 123.4-123.8 (Aromatic carbon C3' and C5'), 126.7 (Aromatic carbon C4'), 140.3 (Aromatic carbon C2' and C6'), 145.6 (Aromatic C1') and 172.4 (CO).

**UV spectroscopy**

*Propofol-RA analogues:*

In Figure 2.4A, the spectra of parent RA showed two absorption peaks in the range of 270-275 nm. Whereas 2,6-propofol and 2,4-propofol showed absorption peak at 292.5 and 281.3 nm, respectively. These peaks were shifted to 286 and 284 nm, for 2,6P-RA and 2,4P-RA compounds, respectively.

*Propofol-AA analogues:*

In Figure 2.4B, the spectra of parent AA showed absorption peak at 272 nm whereas 2,6-propofol and 2,4-propofol showed absorption peak at 293 and 281.5 nm, respectively. These peaks were shifted to 282 and 280 nm, for 2,6P-AA and 2,4P-AA compounds, respectively.
**Propofol-OA analogues:**

In Figure 2.4C, the spectra of parent OA showed absorption peak at 275 nm. Whereas 2,6-propofol and 2,4-propofol showed absorption peak at 292.8 and 281 nm, respectively. These peaks were shifted to 270 and 269 nm, for 2,6P-OA and 2,4P-OA compounds, respectively.

**Propofol-LA analogues:**

In Figure 2.4D, the spectra of parent LA showed absorption peak at 267 nm. Whereas 2,6-propofol and 2,4-propofol showed absorption peak at 292 and 281 nm, respectively. These peaks were shifted to 268 and 267 nm, for 2,6P-LA and 2,4P-LA compounds, respectively.

**FT-IR spectroscopy**

The infrared absorption spectra of propofol-FA analogues are illustrated in Figure 2.5A-D. In the following write-up respective resulting spectroscopic values for 2,4Propofol-FA analogues are given in bracket.

**Propofol-RA analogues:**

Both FT-IR spectra showed two strong absorption bands at 1749.07 (1735.60) and 1171.16 (1174.75) cm\(^{-1}\) which are attributable to \(\nu(C=O)\) and \(\nu(C–O)\) bond, respectively, indicating the presence of an ester. The spectra also revealed a short band at 2934.54 (2955.49) cm\(^{-1}\) which is characteristic of an aromatic C–H bond (propofol), and the band at 2869.63 (2874.76) cm\(^{-1}\) is characteristic of aliphatic C–H bonds. Since the hydroxyl (–OH) bond of RA remains free therefore, its absorption band was seen at 3038.90 (3035.12). However, single peak indicates the absence of peak of non-esterified propofol (Figure 2.5A).

**Propofol-AA analogues:**

Both FT-IR spectra showed two strong absorption bands at 1750.23 (1756.21) and 1143.30 (1134.42) cm\(^{-1}\) which are attributable to \(\nu(C=O)\) and \(\nu(C–O)\) bond, respectively, indicating the presence of an ester. The spectra also revealed a short band at 2957.69 (2949.86) cm\(^{-1}\) which is characteristic of an aromatic C–H bond (propofol), and the band at 2908.39 (2913.53) cm\(^{-1}\) is characteristic of aliphatic C–H bonds. No hydroxyl (–OH)
absorption band was seen, indicating the absence of non-esterified propofol (Figure 2.5B).

**Propofol-OA analogues:**

Both FT-IR spectra showed two strong absorption bands at 1757.49 (1758.73) and 1142.30 (1137.92) cm\(^{-1}\) which are attributable to \(\nu(C=O)\) and \(\nu(C-O)\) bond, respectively, indicating the presence of an ester. The spectra also revealed a short band at 3023.31 (3018.76) cm\(^{-1}\) which is characteristic of an aromatic C–H bond (propofol), and the band at 2863.75 (2860.14) to 2929.83 (2926.43) cm\(^{-1}\) is characteristic of aliphatic C–H bonds. No hydroxyl (–OH) absorption band was seen, indicating the absence of non-esterified propofol (Figure 2.5C).

**Propofol-LA analogues:**

FT-IR spectra of 2,6P-LA showed two strong absorption bands at 1757.85 (1755.51) and 1137.00 (1147.02) cm\(^{-1}\) which are attributable to \(\nu(C=O)\) and \(\nu(C-O)\) bond, respectively, indicating the presence of an ester. The spectra also revealed a short band at 2931.26 (2925.99) cm\(^{-1}\) which is characteristic of an aromatic C–H bond (propofol), and the band at 2866.02 (2858.87) cm\(^{-1}\) is characteristic of aliphatic C–H bonds. No hydroxyl (–OH) absorption band was seen, indicating the absence of non-esterified propofol (Figure 2.5D).

**NMR studies**

The formation of propofol-FA analogues was confirmed by \(^1\)H and \(^{13}\)C NMR spectra. Assignments of the signals were based on the chemical shift and intensity pattern of specific spectrum.

**Propofol-RA analogues:**

In \(^1\)H NMR spectrum (Figure 2.6I-A and 2.6II-A), the two olefinic protons were resonating as multiplet in the range \(\delta_H 5.3-5.5\) ppm (Magrioti and Constantinou-Kokotou, 2002). Due to the proximity of the hydroxy group to the double bond; the signal of the olefinic protons was split, with the downfield signal assigned to the \((k\text{ and }i)\) proton at C-10 and the upfield signal assigned to the \((l\text{ and }j)\) proton at C-9. The –OH proton is exchangeable with deuterium and might not always be visible as in our case. A triplet
was observed at 0.88 ppm corresponding to the terminal methyl protons (q and p) of the
FA chain. A strong multiplet appeared in the range 1.19-1.48 ppm was corresponds to the
methylene protons of the FA chain and the signal corresponding to methyl protons of
isopropyl chain which were merged with the methylene protons. The methylene protons –
CH₂–CH=CH–CH₂–CH(OH)– were resonating as quartet in the range 1.98-2.07 ppm
implying the partial drift of electron cloud of olefinic bond. The methylene protons –
CH=CH–CH₂–CH(OH)– were observed in the range 2.1-2.23 ppm as triplet with a
coupling of 13.6-13.72 Hz. The –CH₂–CH(OH)–CH₂– proton resonate in the range 3.5-
3.62 ppm as multiplet, since it is attacked to the -OH group, therefore is shifted downfield
from the others. The –COCH₂– protons adjacent to carbonyl group resonate in the range
2.54-2.56 ppm as triplet with a coupling of 15 Hz, while the -CO-CH₂-CH₂– protons
resonate in the range 1.72-1.77 ppm as multiplet. Ar–CH< gives two multiplet signals at
2.8 ppm (e) and 2.9 ppm (d) in the case of 2,4-propofol while for 2,6-propofol it gives a
single signal at 2.8 ppm. The aromatic protons appear in the usual range 6.80–7.20 ppm.

¹³C NMR confirmed the presence of the following carbons: carbonyl carbon at
around 172.73 ppm (C18) (3), a double bond between 133.42 (C10) and 125.24 ppm (C9)
(1,2), and one –CH(OH)– carbon at 71.58 ppm. The terminal methyl groups gave signals
in 14–15 ppm range. Assignment of other significant carbon signals has been shown in
the Figure 2.6I-B and 2.6II-B.

Propofol-AA analogues:

In ¹H NMR spectrum (Figure 2.7I-A and 2.7II-A), the absence of signal of
phenolic proton as well as hydroxyl proton of carboxylic group at δ 4.99 and 10.5-13.5
ppm confirmed the synthesis of 2,4P-AA and 2,6P-AA, respectively. The 6H-allylic
protons and 8H-olefinic protons were observed as a multiplet at δH 2.75 and 5.29 whereas
a quartet and pentate were observed at 0.88 ppm due to long range coupling and coiling
nature of conjugated or non-conjugated FA corresponding to the terminal methyl protons
of the FA chain. A strong multiplet appeared in the range 1.18-1.30 ppm corresponding
to the methylene protons of the FA chain. The methyl protons of isopropyl chain of the
ring of 2,6-propofol derivative were observed as a doublet in case of 2,6-propofol and as
a multiplet in case of 2,4-propofol at δH 1.20 and 1.19-1.24 ppm. The –COCH₂– protons
resonated in the range 2.61-2.65 ppm while Ar–CH< were resonating as a multiplet
mixed with allylic protons at 2.85-2.91 ppm range. The aromatic protons appeared in the usual range of 6.80–7.30 ppm.

The $^{13}$C NMR spectrum of 2,4P-AA and 2,6P-AA (Figure 2.7I-B and 2.7II-B, respectively), further support the formation of ester compounds. The assignments of the $^{13}$C signal for $\text{>C}=\text{O}$ group of ester was quite straightforward and observed at 172.4 ppm. The terminal methyl group was appeared at 14.1 ppm. Olefinic and allylic carbons were recorded in the range of 127.5-130.5 and 25.7 to 27.3 ppm. A few other significant carbon signals for the FA chain were recorded at $\delta_C$ 33.8 (C-2), 33.7 (Ar-C), 31.5 (C-18) and others including Ar-C-(C)2 at 24.9 to 22.6 ppm.

Propofol-OA analogues:

In $^1$H NMR spectra (Figure 2.8I-A and 2.8II-A), the absence of signal of hydroxyl group confirms the synthesis of 2,4P-OA and 2,6P-OA compounds, respectively. The two olefinic protons were observed as a multiplet at $\delta_H$ 5.36 (Magrioti and Constantinou-Kokotou, 2002). A triplet was observed at 0.88 ppm corresponding to the terminal methyl protons of the FA chain. A strong multiplet appeared in the range 1.25-1.46 ppm was corresponds to the methylene protons of the FA chain. The methyl protons of isopropyl chain of the ring of 2,4P-OA were observed as a doublet at 1.20 ppm while the signal corresponding to methyl protons of isopropyl chain of 2,6P-OA was merged with the methylene protons appearing as a strong multiplet at 1.19-1.43 ppm range. The two methylene groups attached to the olefinic carbons were resonating as a quartet at 2.0 ppm implying the partial drift of electron cloud of olefinic bond. The $\text{-COCH}_2-$ protons resonate in the range 2.50–2.60 ppm, while the $\text{-CO-CH}_2\text{-CH}_2-$ protons were resonating in the range 1.75–1.85 ppm. In the case of 2,4P-OA, Ar–CH< gives two signal at 2.9 and 2.6 ppm (Klemm et al., 1980) while for 2,6P-OA, it gives a single signal at 2.9 ppm (Brown et al., 2009; Malkov et al., 2008; Zhang et al., 2008). The aromatic protons appear in the usual range 6.80–7.30 ppm.

In the $^{13}$C NMR spectra (Figure 2.8I-B and 2.8II-B), the assignments of the $^{13}$C signal for $\text{>C}=\text{O}$ group of ester is quite straightforward and were observed in the range of 172.0–173.0 ppm (Davletbakova et al., 2001). The terminal methyl groups gave signals in 14–15 ppm range. Olefinic carbons were recorded at 130–131 ppm (Mustafa et al.,
A few other significant carbon signals for the FA chain were recorded at $\delta_C$ 34.22 (C-17), 25.0 (C-8 and C-11).

**Propofol-LA analogues:**

In $^1$H NMR spectrum (Figure 2.9I-A and 2.9II-A), the absence of signal of phenolic proton as well as hydroxyl proton of carboxylic group at $\delta$ 4.99 and 10.5-13.5 ppm confirm the synthesis of 2,4P-LA and 2,6P-LA, respectively. The 4H- olefinic protons were observed as a multiplet at $\delta_H$ 5.3.2-5.28 ppm whereas a quartet and pentate were observed at 0.88 ppm due to long range coupling and flexible nature of the chain of the fatty acid corresponding to the terminal methyl protons of the FA chain. A strong multiplet appeared in the range 2.93-2.86 ppm was correspond to the diallylic methylene (=$CH-CH_2-CH=$) protons of the FA chain. The methyl protons of isopropyl methylene (ring of 2,6-propofol derivative were observed as a doublet in case of 2,6-propofol and as a multiplet in case of 2,4-propofol at $\delta_H$ 1.20 and1.25-1.19 ppm. The –COCH$_2$– protons resonated in the range 2.63-2.59 ppm while Ar–CH< were resonating as a multiplet at 3.21-2.91 ppm range. The aromatic protons appeared in the usual range of 6.80–7.30 ppm.

The $^{13}$C NMR spectrum of 2,4P-LA and 2,6P-LA (Figure 2.9I-B and 2.9II-B) further support the formation of above compounds. The assignments of the $^{13}$C signal for >C=O group of ester was quite straightforward and observed at 172.4 ppm. The terminal methyl group was appeared at 14.1 ppm. Olefinic carbons were recorded in the range of 127.5-130.5. A few other significant carbon signals for the FA chain were recorded at $\delta_C$ 34.8 (C-2), 33.7 (Ar-C), and others including Ar-C-(C)2 at 24.9 to 22.6 ppm.

**FAB-MS studies**

**Propofol-RA analogues:**

The (+)FAB-MS of 2,4P-RA and 2,6P-RA shown in Figure 2.10I-A and 2.10II-A and fragmentation pattern have been given in Figure 2.10I-B and 2.10II-B, respectively. Compound 2,4P-RA has prominent ions peak at $m/z$ = 163, 177 and 281 which are all fragments about the ester group. The molecular ion ($m/z$ = 458) was easily distinguished. There is a signal at (M – 17)$^+$ ($m/z = 441$) indicating the cleavage of the hydroxyl group. The base peak was observed at $m/z = 177$ with its isotopic peak at $m/z = 178$ and
represents a cleavage in ester group. There is a signal at \( m/z = 163 \) representing loss of one of the terminal methyl group of one of isopropyl chain of propofol (Christie, 1998).

For 2,6P-RA compound, molecular ion peak was not observed however, a signal at \((M - 17)^+ (m/z = 441)\) indicating the cleavage of the hydroxyl group was obtained. Two adduct signals were also observed at \( m/z = 549 \) & 577 corresponding to \(((M - 17)^+ + 108)\) & \(((M - 17)^+ + 136)\) respectively. These adducts were formed by the conjugation of matrix ions and \((M - 17)^+\). The base peak was observed at \( m/z = 177 \) with its isotopic peak at \( m/z = 178 \) and represents a cleavage in ester group. There is a signal at \( m/z = 163 \) representing loss of one of the terminal methyl group of one isopropyl chain of propofol. Other possible fragments have been shown in above mentioned figures.

**Propofol-AA analogues:**

The (+)FAB-MS of the 2,4P-AA (Figure 2.11I-A) showed molecular ion peak at \( m/z:487 \) (M+Na)+ followed by fragment ion peaks at \( m/z:81, m/z:91, m/z:95, m/z:135, m/z:137, m/z:163, m/z:178\) and \( m/z:205 \) etc. It is believed that the fatty acid with four or more double bond as in propofol give molecular ion with very low abundance. It is important to note that 2,4-propofol show different fragments pattern due to unhindered nature of phenyl having two isopropyl moiety at the position (2 and 4) as compared to 2,6-propofol having moieties at 2,6 position creating hindrance. The base peak at \( m/z:178 \) \((M'+H)^+\) clearly indicate that phenyl derivative incorporate with fatty acid and represent a cleavage ester linkage. There is another peak at \( m/z:163 \) representing loss of terminal methyl from base peak of propofol. Moreover, the peak at \( m/z:163 \) again cleaved and gave peak at \( m/z:135 \) due to loss of CO from phenyl moiety. The spectrum of compound follow McLafferty and subsequently loss of methyl give characteristic peak at \( m/z:205 \). Other peaks at different \( m/z \) show polyunsaturated fatty acids fragmentation patterns as mentioned in the scientific literature. The fragmentation pattern of 2,4P-AA is diagrammatically represented in Figure 2.11I-B.

The (+)FAB-MS of the compound, 2,6P-AA (Figure 2.11II-A) gave molecular ion peak at \( m/z:510 \) (M+2Na)+ followed by some characteristics fragment ion peaks such as \( m/z:91, m/z:178, m/z:219, \) and \( m/z:271 \) which correspond to 2,6P-AA. The molecular ion peak at \( m/z: 510 \) is not very abundant, but diagnostic ion for the \( \omega-6 \) moiety at \( m/z:373 \) (M+2Na)+ (M’=stand for daughter ions) does stand out, although the fragment is
small but distinctive. The another important feature of spectrum is that the FAs with four or more double bonds, even when no conjugated, a tropylion ion at m/z:91 becomes more important component of the spectrum. The base peak at m/z:271 (M^-2H)^+ arise due to the fragmentation of 6, 7 carbon chain position. The McLafferty ion at m/z:219 (M^-H)^+ is always small. The another peak of this compound following McLafferty ion shows at m/z:178 (M+H)^+ which indicates the cleavage of ester linkage and presence of propofol moiety. There is a peak at m/z:163 representing loss of the terminal methyl group from base peak of propofol. The fragmentation pattern of 2,6P-AA is diagrammatically represented in Figure 2.11II-B.

Propofol-OA analogues:

The (+)FAB-MS of 2,4P-OA (Figure 2.12I-A) revealed ions peak at m/z = 163, 177 and 265, which are all fragments about the ester group. The base peak was observed at m/z = 177 with its isotopic peak at m/z = 178 and represents a cleavage in ester group. There is an ion at m/z = 163 representing loss of the terminal carbon as methylene group from base peak of propofol (Christie, 1998). The molecular ion (m/z = 442) is easily distinguished. Fragmentation pattern of 2,4P-OA as generated on basis of (+)FAB-MS is illustrated in Figure 2.12I-B, respectively.

The (+)FAB-MS of 2,6P-OA (Figure 2.12II-A) revealed ions peak at m/z = 163, 178 and 264, which are all fragments about the ester group. The base peak was observed at m/z = 178 with its isotopic peak at m/z = 179 and represents a cleavage in ester group. There is an ion at m/z = 163 representing loss of the terminal carbon as methylene group from base peak of propofol (Christie, 1998). The molecular ion (m/z = 442) is easily distinguished. Fragmentation pattern of 2,6P-OA as generated on basis of (+)FAB-MS is illustrated in Figure 2.12II-B, respectively.

Propofol-LA analogues:

The (+)FAB-MS of the 2,4P-LA (Figure 2.13I-A) showed molecular ion peak at m/z:440 (M)^+ followed by fragment ion peaks at m/z:95 m/z135, m/z:137, m/z:163 and m/z:178 etc. It is important to note that the 2,4-propofol show different fragments pattern due to unhindered nature of phenyl having two isopropyl moiety at the position (2 and 4) as compared to 2,6-propofol having moiety at (2 and 6) position creating hindrance. The
base peak at m/z:178 (M+H)^+ clearly indicate that phenyl derivative incorporate with fatty acid and represent a cleavage ester linkage. There is another peak at m/z:163 representing loss of terminal methyl from base peak of propofol. Moreover, the peak at m/z:163 again cleaved and gave peak at m/z:135 due to loss of CO from phenyl moiety. The spectrum of compound follow McLafferty and subsequently loss of methyl give characteristic peak at m/z:205 (M+2H)^+. Other peaks at different m/z show ω-6 PUFA fragmentation patterns (Christie, 1998). The fragmentation pattern of 2,4P-LA is diagrammatically represented in Figure 2.13I-B. The important fragments and their abundance are arranged systematically: m/z:440 (M)^+ (46.8%), m/z: 335 (9.3%), m/z: 281 (15.6%), m/z: 264 (62.5%), m/z: 262 (12.5%), m/z: 246 (6.2%), m/z: 221 (12.5%), m/z:207 (18.7%), m/z:191 (31.5%), m/z:178 (100%), m/z:163 (93.7%), m/z:147 (53.1%), m/z:137 (68.7%), m/z:135 (53.1%), m/z:121 (30.5%), m/z:62.5 (62.5%) and lower mass fragments (less than 6%).

The (+)FAB-MS of the compound, 2,6P-LA (Figure 2.13II-A) gave molecular ion peak at m/z:440 (M)^+ followed by some characteristics fragment ion peaks such as m/z:95, m/z:163, m/z:178, m/z:179, and m/z:264 which correspond to 2,6P-LA. The molecular ion peak at m/z: 440 is not very abundant, but diagnostic ion for the ω-6 PUFA moiety at m/z:263 does stand out, although the fragment is small but distinctive. The base peak at m/z:178 arise due to the fragmentation of aromatic moiety at position C1 of carbon chain position. The McLafferty ion at m/z:264 (M+2Na-H)^+ is in moderate intensity (69.1%). The another peak of this compound following McLafferty ion shows at m/z:178 (M+H)^+ and m/z:205 which indicates the cleavage of ester linkage and presence of propofol moiety. There is a peak at m/z:426 (M+H)^+ representing loss of the terminal methyl group from the parental peak of the synthesized compounds. The fragmentation pattern of 2,6P-LA is diagrammatically represented in Figure 2.13II-B. The important fragments and their abundance are arranged systematically: m/z:440 (M)^+ (69.1%), m/z: 356 (2.5%), m/z:355 (2.7%) m/z: 314 (2.5%), m/z: 305 (2.7%), m/z:290 (3.6%), m/z: 264 (69%), m/z: 263 (6.1%), m/z: 246 (6.1%), m/z:245 (5.1%), m/z:231 (5.1%), m/z:205 (5.1%), m/z:179 (61%), m/z:178 (100%), m/z:163 (86%), m/z:137 (52%), m/z:135 (27%), m/z:105 (25%), 95 (41%), lower mass fragments (less than 6%).
Scheme 2.1A: Schematic representation of chemical synthesis of propofol-RA analogues.
Scheme 2.1B: Schematic representation of chemical synthesis of propofol-AA analogues.
Scheme 2.1C: Schematic representation of chemical synthesis of propofol-OA analogues.
Scheme 2.1D: Schematic representation of chemical synthesis of propofol-LA analogues.
Figure 2.2: Thin layer chromatography analysis of synthesis of eight novel propofol-FA analogues. Lanes designated as Co-spot shows the reaction mixture + respective propofol.
Figure 2.3A: Simulated models of four novel propofol-FA analogues. Their $RF$ values and % yield are shown in bracket.
Figure 2.3B: Simulated models of four novel propofol-FA analogues. Their RF values and % yield are shown in bracket.
Figure 2.4A: UV absorbance spectra of propofol-RA analogues viz. 2,4P-RA and 2,6P-RA. Absorbance of parent controls is also shown.
Figure 2.4B: UV absorbance spectra of propofol-AA analogues viz. 2,4P-AA and 2,6P-AA. Absorbance of parent controls is also shown.
Figure 2.4C: UV absorbance spectra of propofol-OA analogues viz. 2,4P-OA and 2,6P-OA. Absorbance of parent controls is also shown.
Figure 2.4D: UV absorbance spectra of propofol-LA analogues viz. 2,4P-LA and 2,6P-LA. Absorbance of parent controls is also shown.
Figure 2.5A: The FT-IR spectra of propofol-RA analogues.
Figure 2.5B: The FT-IR spectra of propofol-AA analogues.
Figure 2.5C: The FT-IR spectra of propofol-OA analogues.
Figure 2.5D: The FT-IR spectra of propofol-LA analogues.
Figure 2.6I: $^1$H NMR (A) and $^{13}$C NMR (B) spectrum of 2,4P-RA.
Figure 2.6II: $^1$H NMR (A) and $^{13}$C NMR (B) spectrum of 2,6P-RA.
Figure 2.7I: $^1$H NMR (A) and $^{13}$C NMR (B) spectrum of 2,4P-AA.
Figure 2.7II: $^1$H NMR (A) and $^{13}$C NMR (B) spectrum of 2,6P-AA.
Figure 2.8I: $^1$H NMR (A) and $^{13}$C NMR (B) spectrum of 2,4P-OA.
Figure 2.8II: $^1$H NMR (A) and $^{13}$C NMR (B) spectrum of 2,6P-OA.
Figure 2.9: $^1$H NMR (A) and $^{13}$C NMR (B) spectrum of 2,4P-LA.
Figure 2.9II: $^1$H NMR (A) and $^{13}$C NMR (B) spectrum of 2,6P-LA.
Figure 2.10I: FAB-MS spectrum (A) and mass fragmentation pattern (B) of 2,4P-RA.
Figure 2.10II: FAB-MS spectrum (A) and mass fragmentation pattern (B) of 2,6P-RA.
Figure 2.111: FAB-MS spectrum (A) and mass fragmentation pattern (B) of 2,4P-AA.
Figure 2.11II: FAB-MS spectrum (A) and mass fragmentation pattern (B) of 2,6P-AA.
Figure 2.12I: FAB-MS spectrum (A) and mass fragmentation pattern (B) of 2,4P-OA.
Figure 2.12II: FAB-MS spectrum (A) and mass fragmentation pattern (B) of 2,6P-OA.
Figure 2.13I: FAB-MS spectrum (A) and mass fragmentation pattern (B) of 2,4P-LA.
Figure 2.13II: FAB-MS spectrum (A) and mass fragmentation pattern (B) of 2,6P-LA.
DISCUSSION

Most of the presently available anticancer drugs are associated with toxicity (Miller and McLeod, 2007) and other related adverse effects including neurotoxicity (Sarah et al., 2008). Beside intrinsic toxicity, widespread systemic distribution of the administered drug in the recipient and rapid elimination of these anticancer agents further restrict their usage as potential therapeutic agents. Keeping into consideration various untoward issues for most of the presently available anticancer drugs, it’s desirable to opt for suitable substitutes for them. There have been many epidemiological reports (Prentice et al., 1989; Severson et al., 1989) and animal studies (Welsch and O’Connor, 1989; Reddy and Sugie, 1988) concerning role of dietary FAs in relation to tumors. Interestingly, clinical applications of such compounds in cancer treatment, has also been reported (McIlmurray and Turkic, 1987; Van der Merwe et al., 1987). The ω-hydroxy FA is reported to prevent cancer metastasis and inhibit the occurrence and reoccurrence of cancer while having few side effects (Ito et al., 2002). It has also been suggested that exogenous unsaturated FA may increase anticancer activity of cancer chemotherapeutic agents (Miyazawa et al., 2000; Menendez et al., 2002). However, the effectiveness of dietary FAs remains unconvincing.

Earlier studies involving chemically modified FA molecules have demonstrated their less toxicity, more specificity and potent biological activity against range of therapeutic targets (Tronstad et al., 2003). Since last decade several attempts have been made to synthesize novel anticancer compounds by conjugating FAs with various anticancer drugs (Takahashi et al., 1997; Bradley et al., 2001; Mustafa et al., 2004; 2005; Siddiqui et al., 2005). Among unsaturated FAs, OA is one of the important FA which is found to be more effective and less toxic. Also, its strong hydrophobic nature helps in facilitating rapid uptake by cells (Dratz et al., 1986; Salem et al., 1986). Among PUFAs, exogenously supplied ω-6 PUFA has been shown to kill malignant cells selectively (Begin et al., 1986, 1989). There are indications that AA, one of the important ω-6 PUFA, exerts antitumor activity where it causes loss of cell viability in different types of human cancer cells (Begin et al. 1986; Wu and Cederbaum, 2001). Nonsteroidal anti-inflammatory drugs (NSAIDs), which are among the few agents that can effectively
prevent neoplasias (Baron and Sandler, 2000), induce apoptosis through elevation of the intracellular levels of AA, and their effects can be mimicked by the addition of exogenous AA (Chan et al., 1998). AA is emerging a very important player in the pathways to cell death. As far as LA is concerned, it is by itself has been reported to show anti-tumor activity on various neoplastic cells in culture (Robbins et al., 1999; Hayashi et al., 1990; Menendez et al., 2001).

Often a major obstacle to the successful use of a drug is its ability to be taken up and retained by cells. Either the drug must have its target on the outer membrane surface or it must cross the plasma membrane through either an existing transport system or by simple diffusion to affect intracellular targets. One approach to overcome the problem of cell entry and retention has been to link water-soluble drugs to lipophilic carriers (Takahashi et al., 1997; Bradley et al., 2001; Zerouga et al., 2002). Several attempts have been made in the past to synthesize novel compounds by conjugating FAs with drugs. For example, chlorambucil-FA conjugates (Chl-FA) were synthesized and tested on human lymphoma cell lines (Takahashi et al., 1997). These studies found that the conjugates selectively affect neoplastic lymphocytes, with minimal effect on quiescent lymphocytes. The cell toxicity observed with Chl-arachidonic acid and Chl-docosahexaenoic acid against lymphoma cells was observed to be equal to or higher than the individual toxic potential of either chlorambucil or the FAs, whereas the Chl-oleic acid conjugate was much less toxic than Chl alone. Moreover, Bradley and colleagues synthesized the DHA-paclitaxel conjugate demonstrating antitumor activity in lung tumors mice (Bradley et al., 2001). In the M109 mouse tumor model, DHA-paclitaxel is found to be less toxic to animals than paclitaxel alone and cured all tumor-bearing animals; in contrast to unconjugated paclitaxel.

During the present investigation, another approach was used to synthesize a class of novel compounds by directly conjugating FA with propofol. Respective two isomers of propofol were conjugated with selected FAs. Synthetic methodology was based on the coupling of the hydroxyl function (1C-OH) present in the propofol with the terminal carboxylic group of FA to synthesize a specific ester. Quantitative formation of the compounds was achieved with the help of DCC as a coupling reagent and DMAP as a
catalyst (Figure 2.1A-D). As a catalyst, DMAP is essential for esterification, especially in concentration range of 10 mole%. The DCC/DMAP method is a more suitable synthetic route with mild reaction conditions, higher yield and convenient product purification.

Results presented in Figures 2.3A-B shows that the synthetic process produced the propofol-FA analogues. Separations by TLC (Figure 2.2), UV absorption (Figure 2.4A-D), infrared spectroscopy (Figure 2.5A-D), NMR (Figure 2.6-2.9) and mass spectroscopy (Figure 2.10-2.13) confirm the identity of the compounds. The absorption peaks of the two compounds were quite distinct from their parent controls. Also, in infrared absorption spectra the presence of an ester bond, aromatic C-H absorbance, and absence of free -OH group absorbance, all confirmed the formation of the compounds. The presence of carbon and hydrogen ions in structure of new compounds was determined by $^1$H NMR and $^{13}$C NMR spectra. When NMR signal in compounds were compared with the parent propofol (Siddiqui *et al*., 2005), the hydroxyl proton signal at about 4.82 ppm in 2,6-propofol was not observed in the spectra of compounds. The absence of signal of hydroxyl group in $^1$H spectra of the compounds confirms the synthesis of new products different from parent compounds. Overall shift in signals ($^{13}$C NMR spectra) indicated that the ester bond was formed at C-19 (in propofol analogues RA/OA compounds) hydroxyl group and at C1' (in propofol analogues AA/LA compounds) hydroxyl group of the propofol. Results of mass spectra identified products with molecular mass values which were very close to the calculated molecular mass values of 442.47Da, 464.47Da, 458Da, 440Da for propofol analogues-OA/AA/RA/LA compounds, respectively.

Conclusively, chemical synthesis has yielded eight novel propofol-FA analogues. The novel compounds are colorless viscous liquid (oily) at room temperature, conforming to the common physical state of unsaturated FA esters. The structural characterization of synthesized products has also been achieved successfully.