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Synthesis, Characterization, Anti-Tumor and Anti-Microbial Activity of Fatty Acid Analogs of Propofol

A. Mohammad[1*], F. B. Faruqi[1] and J. Mustafa[2]
[1]Department of Applied Chemistry, Faculty of Engineering & Technology,
Aligarh Muslim University, Aligarh, India
[2]Department of Pharmacognosy, King Saud University, Riyadh,
Kingdom of Saudi Arabia
*Email: alimohammad08@gmail.com

Abstract

Derivatives of propofol (2, 6-diisopropylphenol) were prepared by coupling with 12-hydroxy-octadec-Z-9-enoic acid and Z-9-octadecenoic acid (oleic acid) with the C1-α-hydroxy function of propofol. Spectroscopic studies confirmed the formation of the desired product. The compounds were then investigated for its in-vitro anticancer activity against a panel of solid human tumor cell lines including human malignant melanoma, human leukemia cells. Their cytotoxicity was also determined against non-cancerous mammalian cells (VERO cells). The analogs were cytotoxic against all cancer cell lines whereas no effect was observed against normal cells. The compounds showed good antimicrobial activity against E. coli and S. albus.

Introduction

Synthesis and biological studies of short chain-length esters of propofol have been reported here. 3 to 8 μg/ml concentrations of propofol were reported to decrease the metastatic potential of human cancer cells, including HeLa, H71080, HOS and RPMI-7951 cells [1]. Siddiqui *et al.* [2] first reported the effect of two omega-3 fatty acids, combined with propofol on a breast cancer cell line in vitro. In view of the significance of long-chain FA in the treatment of cancer, we report here the synthesis and spectral studies of new propofol analogs containing two fatty acids12-hydroxy-octadec-Z-9-enoic acid and oleic acid along with their *in vitro* evaluation against a panel of human cancer cell lines including HeLa, SK-MEL, MCF-7 and HL-60 (human leukemia). Their cytotoxicity was also determined against non-cancerous mammalian cells (VERO cells). Ricinoleic acid (12-hydroxy-octadec-Z-9-enoic acid) is active component of castor oil (85 to 90 %). (www.kristinasoil.com). Castor oil (Cremophor) is a chemomodulator and a MDR reversing agent used in anti-cancer drugs [3]. Oleic acid blocks the action of a cancer-causing oncogene called HER-2/neu which is found in about 30 percent of breast cancer patients. (www.oliveoilfarmer.com).

Experimental

Chemicals and Materials

A thin layer chromatographic applicator (Toshniwal, India), 20 × 3.5 cm glass plates and 24 × 6 cm glass jar were used for performing TLC. Silica Gel "G" (E. Merck, India) was used as a stationary phase. Petroleum ether and diethyl ether (1: 1, vol / vol) was used as a developing solvent. Reaction products on TLC plates were visualized by UV light and by exposure to iodine vapors. Column chromatographic separations were performed using silica gel "G" packing of particle size 60–120 mesh (petroleum ether/diethyl ether, 1: 1, v/v). ^{1}HNMR and ^{13}CNMR spectra were recorded on an Advance DRX-200 Bruker, (Switzerland) NMR Spectrometer. Mass spectra were obtained on a Jeol SX-102 (FAB) spectrometer (JEOL, Tokyo, Japan). FTIR Spectra were recorded in chloroform on a Spectrum RX-1 FTIR, Perkin Elmer Spectrometer. 2, 6-diisopropyl phenol, 4-dimethyl amino pyridine

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(DMAP) was procured from Acros chemicals. The coupling reagent-N, N-dicyclohexylcarbodiimide (DCC) was purchased from Fluka chemical corporation (New York). Oleic acid and – β-mercaptoethanol was purchased from Sigma Aldrich Chemicals and methylene chloride was purchased from CDH Chemicals (Mumbai, India). 12-Hydroxy-octadec-Z-9-enoic acid was isolated from *Ricinus communis* seed oil [4]. All solvents and reagents were of AR or HPLC-grade.

Synthesis of Compounds

Appropriate amounts of Fatty acid (1 mmol) and propofol (1 mmol) were dissolved in dry dichloromethane (5 mL), and DMAP (catalytic amount) was added to this solution. The reaction mixture was stirred at room temperature under nitrogen for 10 min before DCC (1 mmol) was added to it. The reaction mixture was allowed to stir at room temperature. Progress of reaction was monitored on TLC plates. Both coupling reactions showed the formation of single product and were completed in 12 h. The reaction mixture was filtered to remove solid dicyclohexylurea, and the filtrate was evaporated under reduced pressure at 20 °C. The semisolid mass was subjected to column chromatography (petroleum ether/diethyl ether, 1:1, v/v) on silica gel to purify the desired products.

Characterization of 12-Hydroxy-octadec-Z-9-enoic Acid (from Seed Oil of Ricinus Communis)

Viscous oil, R_F = 0.2, isolated yield, 95 %. *IR ($CHCl_3$, cm^{-1}):* 3418.0, 3013.0, 2930.4, 2858.4, 1710.8, 1640.0, 1460.5, 1216.9, 1104.4, 932.6, 763.5, and 668.8. MS-EI found [M+H] $^+$ 298.4638; $C_{18}H_{34}O_3$ [M+H] $^+$ requires 298.4659. ^{1}*HNMR ($CDCl_3$, ∂H, ppm):* 0.89(t, J= 6 Hz, 3H of terminal –CH3), 3.39(s, 1H, CH-OH), 3.62(s, 1H, OH), 5.37(m, 1H, -CH=CH), 5.84(m, 1H, -CH=CH), 3.81–4.47(m, 4H), 2.13(m, 4H), 2.33–2.63(m, 6H), 1.31–1.83(m, 12H). ^{13}*CNMR ($CDCl_3$, ∂c)*:14.03, 22.53, 23.5, 24.6, 25.46, 27.14, 29.12, 31.83, 33.9, 35.86, 37.24, 42.8, 129.07, 130.63, and 179.46.

Spectral Studies of the Compound [1]-2, 6-diisopropyl-[1–12-hydroxy-octa-Z-9]-decenoate

Viscous oil, R_F = 0.5, (petroleum ether/diethyl ether, 1:1 v/v as a developer), isolated yield, 90 %. *IR ($CHCl_3$, cm^{-1}):* 3429.2, 3012.6, 2931.3, 2859.3, 1744.0, 1694.8, 1646.6, 1525.4, 1383.7, 1372, 1216, 1164, 1098.5, 930.7 and 754.7. MS-EI found [M+H] + 458.7287; $C_{30}H_{50}O_3$ [M+H] $^+$ requires 458.7297. ^{1}*HNMR ($CDCl_3$, ∂H, ppm):* 0.88(t, J=6.4 Hz, 3H), 1.182(d, J=6.6 Hz, 6H), 1.27, 1.248(d, J=6.6 Hz, 6H), 2.615(m,6H), 2.40(m,3H), 2.92(m,2H), 3.20(m,2H), 3.615(m,1H), 3.90(m,2H), 4.22(m,2H), 5.38(m,1H), 5.673(m,1H) 6.894(m,1H), 7.042(d, J=7.5 Hz, 1H), 7.230(d, J=8.1 Hz, 1H), 1.308–2.14 (m, 12H). ^{13}*CNMR ($CDCl_3$, ∂c):* 14.47, 22.72, 23.53, 24.95, 25.34, 26.29, 26.98, 27.44, 29.33, 31.46, 32.67, 34.10, 35.77, 37.35, 38.64, 49.65, 55.96, 68.08, 71.61, 73.60, 120.45, 123.30, 123.78, 126.34, 132.5, 149.97, 154.06, 172.34.

Spectral Studies of the Compound [2] – 2, 6-diisopropyl-[1-octa-Z-9]-decenoate

Viscous oil, R_F = 0.4, (petroleum ether/diethyl ether, 1:1 v/v as a developer), isolated yield, 90 %. *IR ($CHCl_3$, cm^{-1}):* 3012.6, 2931, 2859.3, 1744, 1628, 1645.2, 1512.1, 1362, 1243.6, 1160.4, 1089.6, 894.6, 752.6. MS-EI found [M+H] + 442.740; $C_{30}H_{50}O_2$ [M+H] $^+$ requires 442.747.^{1}*HNMR ($CDCl_3$, ∂H, ppm):*0.88(d, J=6.8 Hz, 6H), 1.194(d, J=6.8 Hz, 6H),1.22–1.55(m, 26H), 2.4(t, J=6.5 Hz, 3H), 2.615(t, J=6.5 Hz, 2H), 2.91(m, 1H), 3.197(m, 1H), 5.38(m, 1H, -CH=CH), 5.673(m, 1H, -CH=CH), 6.87(m, 1H), 7.06(d, J=6.8 Hz, 1H), 7.23(d, J=6.8 Hz, 1H).^{13}*CNMR ($CDCl_3$, ∂c):*22.72, 24.06, 24.95, 25.62, 26.32, 27.37, 28.75, 29.06, 34.02, 46.1, 53.39, 56, 114.1, 120.8, 123.3, 123.8, 126.34, 132.5, 140.2, 145.5, 150.0, 157.36, 172.32 and 173.6.

Assay for *in Vitro* Anti-cancer Activity

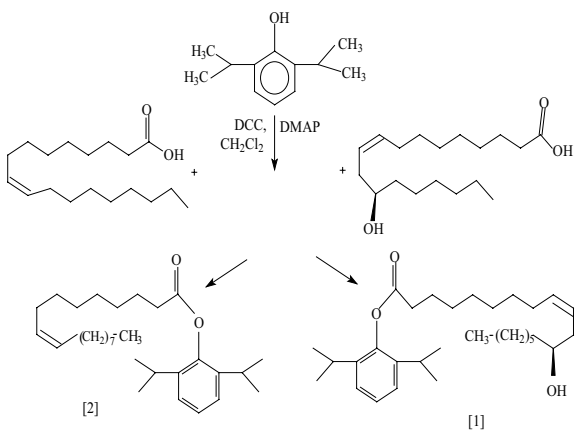
In vitro screening of new drug candidate against human cancer cell line panel is carried out and results are tabulated in Table 1. The assay is the same as we had done earlier [5] The number of viable cells was determined using modified Neutral Red assay [6] procedure. IC_{50} values were calculated from the dose curves generated by plotting % growth v/s the test concentration on a logarithmic scale.

Assay for Antimicrobial Activity

The *in vitro* antimicrobial activity was carried out against *E. coli, S. aureus* and *S. albus.* The assay is the same as we had done earlier [5]. To determine the zone of inhibition cup-plate method was employed [7].

Results and Discussion

After isolation of 12-Hydroxy-octadec-Z-9-enoic acid from seed oil of *Ricinus communis* it was characterized by various spectroscopic techniques. The IR spectra of the compound revealed strong absorption bands at 1710.8 cm^{-1} and 1216.9 cm^{-1} corresponding to C=O and C-O bonds respectively, indicating the presence of carbonyl carbon, showing carbon signal at ∂_C 179.46. Presence of hydroxyl group was confirmed by absorption band at 3418.0 cm^{-1} and its respective carbon signal appeared at ∂_C 71.77 with ∂_H 3.625ppm (s, 1H). IR spectra revealed a sharp band at 1640.0 cm^{-1} indicating the presence of double bond which is further related to chemical shifts at ∂_H 5.37(m, 1H) and 5.84(m, 1H) ppm for the two olefin protons 9H and 10H respectively with ∂_C 129.07 and 130.63. The bands at 2930.4 and 2858.4 cm^{-1} correspond to the aliphatic CH bonds. Some significant signals appeared at ∂_H 0.89(t, J= 6 Hz, 3H of terminal CH_3 group), 3.39(s, 1H, CH-OH) and 1.31–1.83(m, 12H) for the rest of the fatty acid chain length. The efficient synthesis of fatty acid conjugates of propofol is shown in (**Scheme 1**). DCC/DMAP was used to esterify the 1α-hydroxy group of propofol with the carboxylic acid.



(Scheme 1)

The IR spectrum of the compound [1] revealed broad, strong absorption bands at 1744.0 cm^{-1} and 1216 cm^{-1}which are attributable to C=O and C-O bonds ,respectively, and indicate the presence of an ester with their respective carbon signal at ∂_C 172.32. A strong band at 3429.2 cm^{-1} indicate the presence of hydroxyl group with ∂_H 3.615ppm, and its respective carbon signal appeared at ∂_C 71.61. The band at 3012.6 cm^{-1} is characteristic of an aromatic C-H (propofol) and the band at 2931.3 and 2859.3 cm^{-1} is characteristic of aliphatic C-H bonds. A distinct band at 1646.6 cm^{-1} shows the presence of alkene. The two olefin protons, 9'H and 10'H were observed at ∂_H 5.38ppm and 5.673 ppm and correlated with observations at ∂_C 126.34 and 132.5 respectively. The chemical shifts for aromatic protons are moved downfield at ∂_H 6.894 (m, 1H), 7.067 (d, J= 7.5 Hz, 1H), 7.230 (d, J=8.1 Hz, 1H) and their respective carbon signals appeared at ∂_C 120.45, 123.30, 123.78. For 12 protons of the two isopropyl groups, two doublets were observed at ∂_H 1.182(d, J=6.6 Hz, 6H) and 1.248 (d, J=6.6 Hz, 6H) and their respective carbon signals appeared at ∂_C 23.53 and 24.95. The broad and strong absorption bands at 1744 cm^{-1} and 1243.6 cm^{-1} of the compound [2] are attributable to C=0, C-O bands respectively, that indicate the presence of ester group with their respective carbon signals at ∂_C 172.32 and 173.6. The band at 3012.6 cm^{-1} is characteristic of an aromatic C-H and the bands at 2931.0 cm^{-1} and 2859.3 cm^{-1} for aliphatic C-H bonds. A distinct band at 1628 cm^{-1} show the presence of C=C of alkenes. The two olefin protons (terminal alkenes), 9'H and 10'H were observed at ∂_H 5.38 (m, 1H) and 5.673 (m, 1H) which are correlated with ∂_C 126.34 and 132.5 respectively. No O-H band was seen, indicating the absence of nonesterified propofol. The chemical shifts for three aromatic protons are moved downfield at ∂_H 6.87 (m, 1H), 7.06 (d, J= 6.8 Hz, 1H), 7.23 (d, J= 6.8 Hz, 1H) and their carbon signals appeared at 120.8, 123.3 and 123.53 ∂_C values. For 12 protons of the two isopropyl groups, two doublets were observed at ∂_H 0.88(d, J= 6.8 Hz, 6H) and 1.194 (d, J= 6.8 Hz, 6H) and their respective carbon signals appeared at ∂_C 24.06, 24.95.

The compounds were examined for their *in vitro* cytotoxicity against a panel of solid human tumor cell lines. Its cytotoxicity was also determined against non-cancerous mammalian cells (VERO cells) for comparison. The compounds [1] and [2] were cytotoxic against all cancer cell lines where as no effect was observed against normal cells (VERO cells) up to the highest concentration of 15µM in the assay, thus demonstrating selectivity towards the tumor cells. The cytotoxic potency of compounds is expressed in terms of IC_{50} values as shown in Table 1. The significantly higher anti-cancer activity of [1] is attributed to the presence of a methylene interrupted 12-hydroxy and Z-9- monounsaturation in its C-18 fatty acid moi-

ety. Compound [2] also show significant cytotoxicity against all the cancer cell lines especially HL-60 (human leukemia) because of Z-monounsaturation, but its anti-cancer activity is slightly lesser than that of [1], because of the presence of hydroxyl group in [1]- as it has been described earlier that ω-hydroxy and hydroxy fatty acids are potent anti-cancer agents.

Table 1: Anti-cancer activity of compounds

Compound[a]	Cell lines[b] (IC_{50}, μM)			
	MCF-7	HeLa	HL-60	VERO
1	0.42	0.22	0.26	NA
2	0.54	0.30	0.24	NA

[a]The highest concentration tested was 15μM. [b]NA, not active; HeLa, Human cervical epitheloid carcinoma; MCF-7, Human breast adenocarcinoma; HL-60, Human leukemia; VERO, monkey kidney fibroblasts.

The compounds were also screened for their antimicrobial activity against *E. coli, S. aureus* and *S. albus*. Both of the compounds [1] and [2] exhibit significant antimicrobial activity against *E. coli* and *S. albus* while remain not active against S. aureus. Results of anti-microbial screening are reported in **Table 2**.

Table 2: Anti-microbial activity of compounds

	Diameter of zone of inhibition		
Compound	E.coli	S. aureus	S. albus
1	12mm	NA	11mm
2	15mm	NA	9mm
Chloromycetin	22mm	18mm	20mm

DMF used as the control; concentration = 100μg/ml of DMF; NA, not active.

Conclusion

These results suggest that the novel propofol-fatty acid conjugates reported here may be useful for the treatment of cancer as all of them show significant cytotoxicity against a panel of human solid tumor cell lines. Interestingly, none of them showed any cytotoxicity to normal cells. This feature places these products into the class of anticancer agents that possess selectivity toward cancer cells over normal cells. The conjugates also showed significant anti-microbial activity against *E. coli* and *S. albus*.

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