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Synthesis, spectroscopic and anti-cancer studies of fatty acid analogue of 2, 6-Diisopropylphenol

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Abstract

10-Undecenoic acid possessed anti-fungal, anti-bacterial, antiviral and anti-diabetic activities, but, there are a few reports indicating that the derivatives of this compound may also affect cellular processes related to cancer. Propofol (2, 6-diisopropylphenol) is a potent intravenous hypnotic agent which is widely used for the induction and maintenance of anesthesia and for sedation in the intensive care unit. Propofol also possess anti-cancer properties in addition to its sedative effects. The present study describes the synthesis, characterization and evaluation of a novel anticancer conjugate, 1-isopropyl phenol undece-10'-noate.

Keywords: propofol, anesthetic, sedative, undecylenic acid, anti-cancer conjugate.

INTRODUCTION

Mostly a variety of fatty acids present in lipids of fatty oils are great nutritional beneficial compounds and have recently being recognized as candidates of potential advantages of their incorporation into human diets as essential functional ingredients. Essential fatty acids (EFAs), linoleic acid (LA), and α-linolenic acid (ALA) are essential for humans, and are freely available in the diet. Hence, EFA deficiency is extremely rare in humans. To derive the full benefits of EFAs, they need to be metabolized to their respective long-chain metabolites, i.e., dibromo-γ-linolenic acid (DGLA), and arachidonic acid (AA) from LA; and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from ALA. Some of these long-chain metabolites not only form precursors to respective prostaglandins (PGs), thromboxanes (TXs), and leukotrienes (LTs), but also give rise to lipoxins (LXs) and resolvins that have potent anti-inflammatory actions. Furthermore, EFAs and their metabolites may function as endogenous angiotensin-

converting enzyme and 3-hdroxy-3-methylglutaryl coenzyme A reductase inhibitors, nitric oxide (NO) enhancers, anti-hypertensive, and anti-atherosclerotic molecules. Recent studies revealed that EFAs react with NO to yield respective nitroalkene derivatives that exert cell-signaling actions via ligation and activation of peroxisome proliferators-activated receptors. The metabolism of EFAs is altered in several diseases such as obesity, hypertension, diabetes mellitus, coronary heart disease, schizophrenia, Alzheimer's disease, atherosclerosis, and cancer. Thus, EFAs and their derivatives have varied biological actions and seem to be involved in several physiological and pathological processes [1].

Dietary supplementation with (n-3) fatty acids was evaluated in several clinical trials, and the results suggest some benefits to cancer patients [2, 3]. Kubota et al [4] suggested ketol-type unsaturated fatty acids as anti cancer agents and studied their enzymic manufacture. Nakamura et al, [5] reported higher fatty acids as anticancer agents and anticancer agent enhancers. Pohl et al [6] examined the role of hydroxyl group-containing fatty acids in anti-inflammatory action, neuroprotection, bactericide and anticancer defense.

Antitumor activity of some unsaturated fatty acids and the ester derivatives was tested with Ehrlich ascites carcinoma in mice. [7] Undecylenic acid (10-undecenoic acid) is an eleven-carbon monounsaturated fatty acid, a substance found naturally in the body (occurring in sweat); undecylenic acid is produced commercially by the vacuum distillation of castor bean oil, via the pyrolysis of ricinoleic acid. It is used in the manufacture of pharmaceuticals, cosmetics and perfumery including anti-dandruff shampoos, anti-microbial powders and as musk in perfumes and aromas. It is an inexpensive source for the synthesis of pheromones of cotton pests, peach tree borer and cherry tree borer [8]. It is an economical antifungal agent and is the active ingredient in many topical over-the-counter antifungal preparations. [9] Undecylenic acid has long been known to be fungicidal against Candida albicans, thus helping achieve a healthy balance of normal vaginal and intestinal flora. [10] It is FDA approved in over the counter medications for skin disorders or problems. It is the active ingredient in medications for skin infections, and relieves itching, burning and irritation. It is also used in the treatment of psoriasis [11]. 10-Undecenoic acid is also proven to have anti-bacterial and anti-viral properties that are effective on viral skin infections such as the Herpes simplex virus [12, 13]. It has the bifunctionality, odd carbon number and the position of its unsaturated double bond at the end of the chain, makes it a versatile tool for chemical synthesis. Although most studies of undecylenic acid have concentrated on its anti-fungal, anti-bacterial, antiviral activities, there are a few reports indicating that the derivatives of this compound may also affect cellular processes related to cancer **[**14, 15**].**

2, 6-diisopropylphenol or Propofol (fig.1), is a hypnotic alkyl phenol derivative. It is the most extensively used general anesthetic-sedative agent [16, 17]. At high levels, it is non-toxic to humans (3 to 8μg/ml; 20 to 50μM). [18]. this agent is associated with minimal respiratory depression and has a short half life with duration of action of 2-10 minutes. It is a global central nervous system depressant. It decreases cerebral oxygen consumption, reduces intracranial pressure and has potent anti-convulsant properties [19]. It is a potent antioxidant [20-23] and has been shown to stimulate protein kinase C [24, 25], inhibit calcium entry in muscle cells [26] and increase the calcium sensitivity of myofilaments in ventricular myocytes [27]. It is a potent bronchodilator and has anti-inflammatory properties. As a consequence of these properties,

propofol is being increasingly used in the management of traumatic head injury, status epilepticus, delirium tremens, and status asthmaticus and in critically ill septic patients [28]. 3 to 8µg/ml concentrations of propofol were reported to decrease the metastatic potential of human cancer cells, including HeLa, H71080, HOS and RPMI-7951 cells [29]. Propofol was reported to inhibit pulmonary metastasis of murine osteosarcoma (CM8) cells in mice through the modulation of Rho A [30]. All of the above studies suggest that propofol possesses anti-cancer properties in addition to its sedative effects.

Rafat et al [31], was the first, who studied the effect of two omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), combined with propofol on a breast cancer cell line in vitro. The results of the study show that propofol and DHA or EPA has a much more significant effect on cancer cells when used in combination, as conjugates, than when used alone. The conjugates inhibit cancer cell adhesion by 15% and 30% respectively, reduce cell migration by 50% and increase apoptosis by 40% [32].

The present study describes the synthesis, characterization and evaluation of a novel anticancer conjugate, propofol-undecenoate. The results indicate that this novel conjugate might represent a new class of anticancer agents.

RESULTS AND DISCUSSION

The broad and strong absorption bands at 1758.6cm^{-1} and 1243.6cm^{-1}of the compound are attributable to C=0, C-O bands respectively, that indicate the presence of an ester. The carbon signals at ∂_C 172.25 and 173.6, further confirmed the presence of an ester group. The band at 3036.0cm^{-1} is characteristic of an aromatic C-H (propofol) and the bands at 2927.0cm^{-1}and 2851.6cm^{-1}is characteristic of aliphatic C-H bonds. A distinct band at 1628cm^{-1} show the presence of C=C of alkenes. The two olefin protons (terminal alkenes), 10'H and 11'H were observed at ∂_H 4.936(m, 1H) and 5.807(m, 1H) which are correlated with observations at ∂_C 126.26 and 133.7 respectively. No O-H band was seen, indicating the absence of non-esterified propofol. The chemical shifts for three aromatic protons are moved downfield at ∂_H 6.896(m, 1H), 7.15(d, J= 6.8Hz, 1H), 7.193(d, J= 6.8Hz, 1H) and their carbon signals appeared at 120.5, 123.26 and 123.72 ∂_C values. For 12 protons of the two isopropyl groups, two doublets were observed at ∂_H 0.892(d, J= 6.8Hz, 6H) and 1.787(d, J= 6.8Hz, 6H) and their respective carbon signals appeared at ∂_C 24.06, 24.86. Two multiplets were observed at ∂_H 2.91(m, 1H) and 3.197(m, 1H) for protons of carbon adjacent to C-2 and C-6 respectively, and their respective carbon signals appeared at ∂_C 25.62 and 26.32.

Fig.2. show effect of DMSO, undecenoic acid, propofol and propofol-undecenoate on cancer cells. The cells (10^6 per well) were cultured in serum free RPMI-1640 medium supplemented with antibiotic solution (60 mg/L), at 37°C, 95% humidity, 5% CO_2 and sub cultured every 2–3 day using standard cell culture techniques. Cell growth was assayed using trypan blue assay as described in the Experimental section. Fig. 2 depicts that the compound 1-isopropyl phenol undece-10'-noate is far more active than the parent compounds i.e. 10-undecenoic acid and 2, 6-diisopropyl phenol, and possess unique anticancer activity. Undecenoic acid possess some anti-cancer activity but Propofol, exhibit significant anti-cancer activity as it is clear from the figure

that on increasing concentration, a significant decrease in the number of viable cells is found, but, on conjugating with undecenoic acid, it exhibit good amount of anti-cancer activity.

These results suggest that the propofol-undecenoic acid conjugate is far more effective at inducing apoptosis in HeLa cancer cells than are the unconjugated parent compounds i.e. 2, 6-diisopropyl phenol and 10-undecenoic acid.

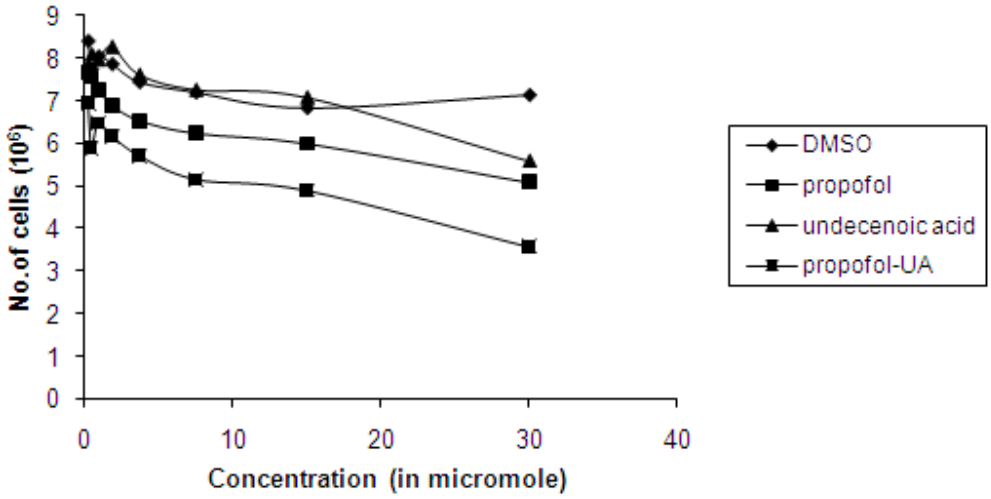


Fig. 2. Variation of number of viable cells at different concentrations

MATERIALS AND METHODS

Purity of the compound was checked by TLC on Silica Gel "G" (E. Merck, India). Petroleum ether 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). H^1NMR and C^{13}NMR spectra were recorded on Advance DRX-200 Bruker, (Switzerland) NMR Spectrometer. Molecular weights were determined by MS route JMS-600H, Jeol (Japan) Mass Spectrometer. FTIR Spectra were recorded in chloroform on a Spectrum RX-1 FTIR, Perkin Elmer Spectrometer. 2, 6-diisopropyl phenol (propofol), dimethyl amino pyridine (DMAP) were procured from Acros chemicals. The coupling reagent- cyclohexyl carbodiimide (DCC) was purchased from Fluka chemical corporation (New York), 10-undecenoic acid was purchased from Aldrich Chemicals and methylene chloride was purchased from CDH Chemicals (Mumbai, India).

Preparation of 1-isopropyl phenol undece-10'-noate

Equimolar amounts of propofol (1mmol) and 10-undecenoic acid (1mmol) were dissolved in dry dichloromethane (5ml), and DMAP (catalytic amount) was added to this solution. The reaction mixture was stirred at room temperature under nitrogen for 10 minutes before DCC (1mmol) was added to it. The reaction mixture was allowed to stir at room temperature. Progress of reaction was monitored on TLC plates. This coupling reaction showed the formation of one product and was completed in 10 hours. The reaction mixture was filtered to remove solid dicyclohexylurea,

and the filtrate was evaporated under reduced pressure at 200C. The semisolid mass was passed through a silica gel column 60-120 mesh particle size, (E. Merck, India, petroleum ether/ diethyl ether, 1:1, v/v; R_f 0.5) to obtain a sticky and viscous colorless oil (yield 90%). The reaction scheme is shown below.

Reaction Scheme

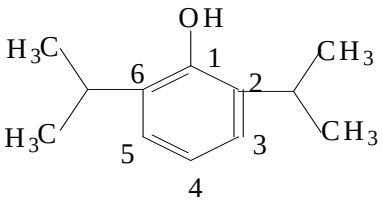
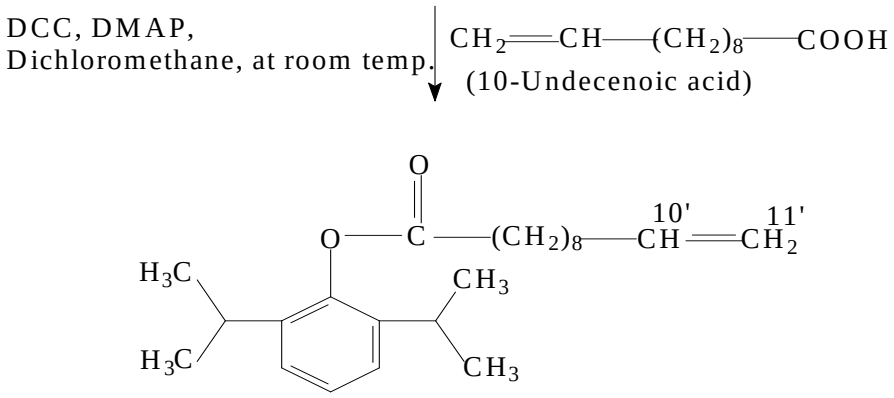


fig.1: 2,6-diisopropyl phenol (propofol)



1-Isopropyl phenol undece-10'-noate (S-1)

Spectral data of 1-isopropyl phenol undece-10'-noate S-1
IR Spectra ($CHCl_3$, cm^{-1}): 3036, 2927.0, 2851.6, 1758.6, 1628, 1243.6, 1160.4, 1089.6, 894.6; *FAB-MASS Spectra:* $[M]^+$ 344; *H^1NMR Spectra (400MHz, $CDCl_3$, ∂_H, ppm):* 0.892(d, J= 6.8Hz, 6H), 1.194(d, J= 6.8Hz, 6H), 1.559(m, 2H), 1.659(m, 2H), 1.787(m, 2H), 1.902(m, 2H), 2.037(m, 2H), 2.293(m, 2H), 2.378(m, 2H), 2.612(t, J=6.5Hz, 2H), 2.91(m, 1H), 3.197(m, 1H), 4.936(m, 2H), 5.80(m, 1H), 6.896(m, 1H), 7.15(d, J= 6.8Hz, 1H), 7.193(d, J= 6.8Hz, 1Hz); *C^{13}NMR Spectra ($CDCl_3$):* 22.97, 24.06, 24.86, 25.62, 26.32, 27.37, 28.75, 29.06, 33.63, 34.02, 40.1, 48.4, 53.39, 56, 114.1, 115.90, 120.5, 123.26, 123.72, 126.26, 133.7, 138.9, 140.2, 145.52, 149.9, 157.36, 172.25.

Assay for Anti-cancer activity
The compound 1-isopropylphenolphenol-undec-10'-enoate was examined for its *in vitro* cytotoxicity against human cancer cell line HeLa cell lines. The cells were cultured in serum free RPMI-1640 medium supplemented with antibiotic solution (60 mg/L), at 37°C, 95% humidity,

5% CO_2 and sub cultured every 2–3 day using standard cell culture techniques. For the assay, cells from different cell lines were seeded in 6-well plates at a density of 1×10^6 cells/well and were allowed to grow undisturbed for 24 h before addition of the test compound. Different concentrations of compound were added and cells were further incubated for 24 h. After the incubation time the samples were diluted in RPMI-1640 medium at 37°C. Cytotoxicity was made by using the trypan blue assay [33] to determine cell viability. The results are summarized in Fig. 2.

CONCLUSION

These results suggest that the novel propofol-undecanoate conjugate reported here may be useful for the treatment of cancer as it show significant cytotoxicity against human HeLa cancer cell lines. The conjugate is more active than the parent compounds and possess unique anticancer activity.

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REFERENCES

[1] N.D. Undurti. *Biotechnology Journal*, **2006**, 1(4), 420-439.
[2] C.A. Gogos, P. Ginopoulos, B. Salsa, E. Apostolidou, N.C. Zoumbos & F. Kalfarentzos. *Cancer*, **1998**, 82, 395–402.
[3] S.J. Wigmore, J.A. Ross, J.S. Falconer, C.E. Plester, M.J. Tisdale, D.C. Carter & K.C. Fearon. *Nutrition*, **1996**, 12 (Suppl 1), 7–30.
[4] A. Kubota, K. Sato, J. Oki, A. Ejima, F. Saito, H. Kuga, N. Ishihara. *Jpn. Kokai Tokyo Koho*, **1993**, **JP05279252.**
[5] S. Nakamura, Y. Nishimura, N. Miwa. *Jpn. Kokai Tokyo Koho*, **1992**, **JP04295428 A19921020.**
[6] E. Pohl, Elener, C. Volt, M. Anna, A. Rupprecht. *Biochimica et Biophysica Acta, Biomembranes*, **2008**, 1778(5), 1292-1297.
[7] Y. Nishikawa, K. Yoshimoto, Okabemidori and F. Fukuoka. *Chemical and Pharmaceutical Bulletin*, **1976,** 24(4), 756-762
[8] S. Archana, C. Subrata. *Molecules*, **1997**, 2, 87-90.
[9] A.L. Shapiro, S. Rothman. *Arch Dermatol Syphilol*, **1945**; 52: 166-171.
[10] N. Mc Lain, R. Ascanio, C. Baker. Antimicrob. Agents Chemother, **2000**; 44: 2873-2875.
[11] Undecylenic acid in Psoriasis. *CMAJ.*
[12] N. Bourne, J. Ireland, L.R. Stanberry, D.I. Bemstein. *Antiviral Res*. **1999**, 40(3): 139-44.
[13] S.D. Shafran, S.L. Sacks, F.Y. Aoki. *J. Infect Dis*. **1997**, 176: 78-83.
[14] V.P.M. Rahman, S. Mukhtar, W.H. Ansari and G. Lemiere. *Eur. J. Med. Chem*. **2005**, 40, 173-184.
[15] T. Yutaka, K. Masaru, R. Takao, Y. Shusaku, Y. Kenji. *Chem. Lett*. **2007**, 17: 0960-894X.
[16] H. Covington. *Crit Care Nurse*. **1998**, 18: 34-39.
[17] R.D. Miller. Local Anesthetics. 5 New York: Churchill Livingstone; **2000**, pp.491-521.

[18] J.F. Coetzee, J.B. Glen, C.A.William, L. Boshoff. *Anesthesiology*. **1995**, 82: 1328-1345.
[19] Y. Shin, Uranus, Endot. *Chem. Pharm Bull*. **2005,** 53(3): 344-346.
[20] O. Eriksson, P. Pollesello, N.E. Seris. *Biochem Pharmacol*. **1992**, 44: 391-393.
[21] P.G. Murphy, D.S. Myers, M.J. Davies, N.R. Webster, J.G. Jones. Br. J. Anesth. **1992,** 68: 613-618.
[22] M. Tsuchiya, A. Asada, K. Maeda, Y. Ueda, E.F. Sato, M. Shindo, M. Inove. *Am J Respir Crit Care Med.* **2001**, 163: 26-31.
[23] L. Aarts, R. Van der Hee, I. Dekker, J. de Jong, H. Lange meijer, A. Bast . *FEBS Lett*. **1995**, 357: 83-85.
[24] H.C. Hemming, A.I. Adamo. *Anesthesiology*, **1994**, 81: 147-155.
[25] N. Kanaya, B.Gable, P.A. Murray, D.S. Damron. *Anesthesiology*, **2003**, 98: 1363-1371.
[26] M. Horibe, I. Kondo, D.S. Damron, P.A. Murray. *Anesthesiology*, **2001**, 95: 681-688.
[27] N. Kanaya, P.A. Murray, D.S. Damron. *Anesthesiology*, **2001**, 94: 1096-1104.
[28] P.E. Marik. *Curr Pharm Des*. **2004**, 10(29): 3639-49.
[29] T. Mammoto, M. Mukai, A. Mammoto, Y.Yamanaka, Y. Hayashi, T. Mashimo, Y. Kishi, H. Nakamura. *Cancer Lett*. **2002**, 184: 165-170.
[30] K. Atsuko, I. Takefumi, S. Koh. *Immuno Pharmacology and Immunotoxicology*, **2004**, 29(3, 4), 477-486.
[31] S.A. Rafat, Z. Mustapha, W. Min, C. Alicia, H. Kevin, Z.P. Gary and S. William. *Breast Cancer Res.* **2005,** 7(5): R645-R654.
[32] P.R. Roper and B. Drewinko. *Cancer Research*, **1976**, 36: 2182-2188.