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Original article

Development of new estradiol-cationic lipid hybrids: Ten-carbon twin chain cationic lipid is a more suitable partner for estradiol to elicit better anticancer activity

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ABSTRACT

The present study illustrates the synthesis and anticancer evaluation of six, ten, twelve and fourteen carbon chain containing cationic lipidated-estradiol hybrids. Previously, we have established the lipidation strategy to introduce anticancer activities in various pharmacophores including estradiol (ES). In this structure activity study the length of the carbon chain is narrowed down between C6–C14 to screen out the most potent anticancer molecule among the class. Among the newly developed ES-cationic lipid conjugates, ten-carbon chain containing derivative, ES-C10 (**5c**) exhibited 4–12 folds better anticancer activity than the previously established derivative, ES-C8 (**5b**) in various cancer cells of different origin. Moreover cytotoxicity of this molecule was not observed in non-cancer cells. Notably, in spite of bearing estrogenic moiety, ES-C10 exhibited anticancer activity irrespective of estrogen receptor (ER) expression status. ES-C10 exhibited prominent sub-G_0 arrest of cancer cells with concomitant induction of apoptosis and demonstrated significant inhibition of tumor growth in mouse melanoma model. Collectively, ES-C10 exemplifies the development of an anticancer agent with broader activity against cancer cells of different origins.

1. Introduction

Cancer with its resilience evolves several mechanisms for regeneration, survival and proliferation. Hence, combinational approaches that use a blend of therapeutic agents targeting multiple pathways are more effective for treatment than a single agent [1]. There are several clinical examples which illustrate the importance of multiple agent usage in curative cancer therapy [2–6].

Molecular hybridization technique can produce the effect of combination therapy while using a single agent [7]. Combining two different pharmacophores (i.e., the concept of Molecular hybridization) to combat multifunctional and complex disorders like cancer is an intelligent strategy in medicinal chemistry. Unlike physical combination of parent compounds, this strategy bestows distinct biological activities to the new hybrids [8]. Besides,it confers more efficacy and affinity to the hybrids in comparison to the individual entities.

Cationic lipids are extensively studied non-viral vectors for the delivery of genetic cargoes, including p-DNA, siRNA etc. [9]. The hydrophobic long chain moieties of these amphiphilic molecules are generally one or two fatty acid chains, cholesterol derived moieties etc. Cationic lipids are mainly recognized for their delivery potentials but unlike other small molecules their medicinal properties are under-recognized. Cationic lipids are known to activate several cellular pathways like pro-apoptotic and pro-inflammatory cascades [10], generate reactive oxygen species (ROS) in macrophages or immature B cells [11,12], activates prolonged calcium $[Ca^{2+}]_i$ release from intracellular calcium stores sensitive to thapsigargin [13] etc. Cationic lipids are also known to induce apoptosis, a well programmed biochemical event, in cancer cells [14–16]. Cationic lipids in conjugation with various pharmacophores such as glycosides, estradiol, haloperidol, benzamide also induce prominent apoptosis through generation of ROS, activation of several kinases (PKC, MAPK), modifications of the mitochondrial

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membrane potential, release of cytochrome c and caspases, modulation of m-TOR kinase, etc. [17–20].

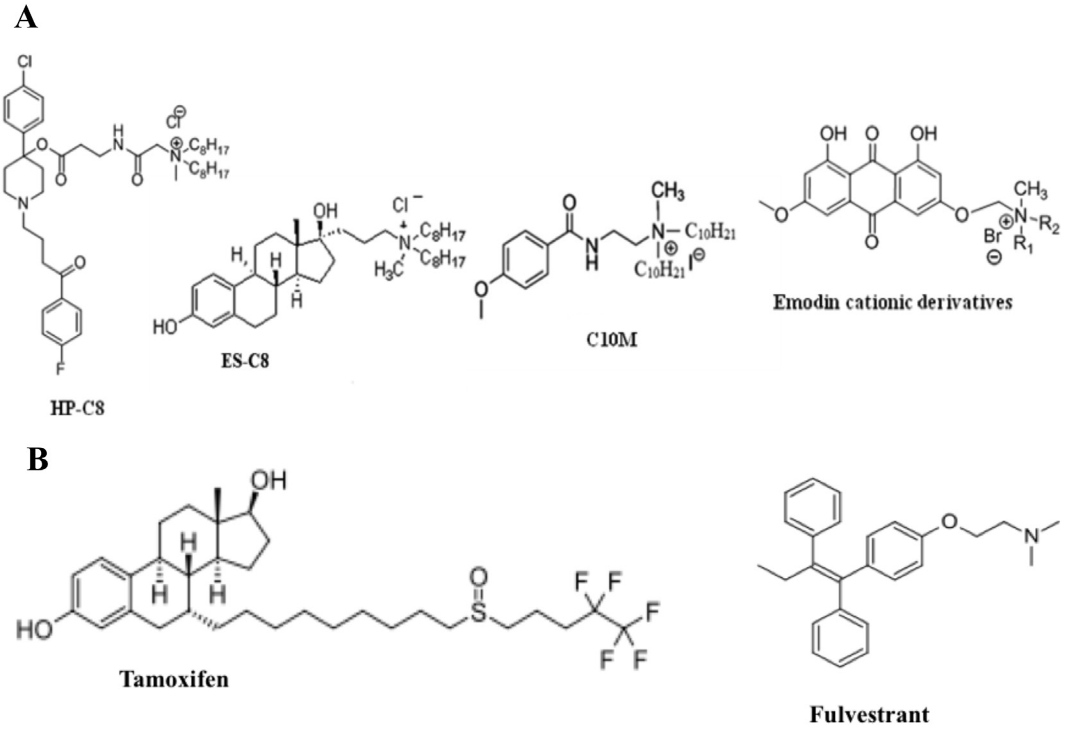
From the above discussion it is clear that the pharmacologic potential of the cationic lipids in cancer research is immense. This rationalise, among other possibilities, generation of cationic lipid-based anticancer hybrids. Recently we have reported different classes of cationic lipidated small molecules (Scheme 1a)) that include cationic lipid conjugates of estradiol [18], haloperidol [19] and benzamides [20]. Others have utilized this hybridization concept towards developingemodin-based anticancer agents [21]. From these reports it is obvious that cationic lipid modification of small molecule pharmacophores bestows potent anticancer activity to parent molecules. However, improved biological activity is highly dependent on both small molecule as well as lipid-chain length. Interestingly, not all the combinations of ligands and cationic lipid chain-lengths exhibit anticancer activity. Evidently, there needs to be a specific combination of these two in order to generate potential anticancer agent.

Earlier we have developed cationic lipid estradiol hybrids among widely varying carbon chain lengths. The optimization was done among three molecules, starting from two (ES-C2), eight (ES-C8) and sixteen (ES-C16) carbon chain lengths. Among the three compounds, ES-C8 was found to exhibit potent anti-breast cancer activity [18]. Since ES-C8 is an estradiol-associated molecule we expected that like other estrogen-based drugs (Scheme 1b) this molecule would exhibit usual anti-breast cancer activity against only ER-positive cancers [22–24]. But the molecule shows activity in breast cancer cells independent of their ER-expression status. We questioned: Does C8 the optimal carbon chain length, which when conjugated with estradiol provide most potent anticancer molecule? The present report describes the synthesis and *in vitro* & *in vivo* anticancer evaluation of the newer derivatives of cationic lipid-modified estradiol while varying the carbon chain length between C6 to C14. Our findings disclose that the C10 analogue is a more suitable partner than the existing potent C8 analogue towards imparting selective anticancer activity. The anticancer activity of C10 analogue, i.e., ES-C10 is not only pronounced against ER ± breast cancer cells but also effective against ER + melanoma and pancreatic cancer cells, thereby exhibiting its general utility against cancers of various lineage.

2. Results

2.1. Chemistry

The structures and general synthetic strategies adopted for preparing cationic estradiol conjugates are outlined in Scheme 2. The distinctive novel structural features common to the cationic estradiol derivatives **5a**–**e** disclosed in the present investigation include: (a) The presence of hydrophobic groups which are directly linked to the positively charged nitrogen atom and (b) the presence of ER-binding 17β-estradiol group. The details of the synthetic procedures for the cationic estradiol conjugates **5a**–**e**, as per our previous report [18], are shown in Scheme 2 and are described in the experimental section. More specifically, 3- hydroxyl group of estrone was protected as tertiary butyl dimethyl silyl ether, alkylation at C-17-keto group of protected estrone using ally bromide and Mg yielded the 17-α allyl derivative of estrone. The allyl moiety was converted to primary alcohol with Oxidative hydroboration (BH_3.DMS; NaOH, H_2O_2) and subsequently oxidized with IBX to give aldehyde. To obtain 3-tert-butyldimethylsilyloxy-17α-[3-N, N dialkyl (amino) propane] − 17β-estradiol, one-pot reductive amination of aldehyde with secondary amine and sodium cyanoborohydride was performed. Subsequent quaternization of nitrogen with methyl iodide followed by chloride ion exchange produced the final cationic estradiol derivatives. All compounds were characterized using ^{1}H NMR, ^{13}C NMR and mass spectrometry. The purities of all the final compounds were ascertained by HPLC (Figure S2) and they are consistently found to be ≥98%.



Scheme 1. a) Cationic lipid modified compounds. b) Breast cancer drugs acting through ER.