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Improved cellular uptake, enhanced efficacy and promising pharmacokinetic profile of docetaxel employing glycine-tethered C_{60}-fullerenes

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ABSTRACT

Water dispersible fullerenes were synthesized by tethering with glycine. The glycinated fullerenes were conjugated to docetaxel and characterized using FT-IR and NMR. The nanometric drug-loaded carriers were able to release drug at cancer cell pH, but resisted drug release at plasma pH. The cytotoxicity in MDA MB-231 cells was substantially enhanced as well as the system was well tolerated by erythrocytes. The confocal laser scanning microphotographs confirmed the substantial drug delivery to cytosol as well as nuclei of cancer cells. The developed system not only increased the circulation time of drug, but also decreased its protein binding and substantially enhanced AUC. The glycinated fullerenes can serve as promising "cargo vehicles" for delivery of anti-cancer drugs in safe and effective manner.

1. Introduction

Despite the advancements in the medical field, cancer is still a big threat to humanity [1]. The world's cancer affected population is predicted to be 7.5 billion by 2020. Nearly 15.0 million new cancer cases are expected to be diagnosed by that period [2]. Out of numerous cancers, breast cancer is the commonest malignancy type diagnosed in women. It is the major cause for death in women of the age 40 to 50 years [3]. Drugs like tamoxifen and docetaxel are beneficial to treat early-stage invasive breast cancer. These drugs also helps to get rid of any cancer cells that may be left behind after surgery and also, to reduce the risk of re-occurrence of cancers [4]. Docetaxel (DTX) is commercially synthesized from the naturally occurring compound, [(*N*-debenzoyl-*N*-*tert*-butoxy-carbonyl)-10-deacetyl paclitaxel] which is extracted from the yew plants (*Taxus brevifolia*) [5]. It is one of the potent anti-cancer drugs, which shows the efficacy against cancers, which fail to respond to other powerful anti-cancer agents. It is a potent mitotic inhibitor which acts through improvement of microtubule polymerisation and inhibition of tubulin depolymerisation. However, lower aqueous solubility and poor tissue permeability makes it poorly bioavailable, BCS class IV drug [6]. On the other hand, dose dependent side effects also pose challenge in proper utilization of the drug [7]. These lacunae are the driving forces for researchers to improve DTX chemotherapy. In recent past, various attempts have been made to deliver the drug by means of novel drug delivery carriers like polymeric nanoparticles [8], liposomes [9], polymeric micelles [10] and microspheres [11]. Inorganic materials like multi-walled carbon nanotubes [12], carboxylated C_{60} fullerenes [6] and graphene oxide [13] have also been used.

Fullerenes within nanometric size alter both pharmacological and therapeutic effects of drug molecules. Due to their small size, these novel drug delivery systems offer superior advantages, such as altered pharmacokinetic behavior and improved payload, over traditional large-scale systems [14]. Thus it can be concluded that fullerenes are used in drug delivery because of: (i) Efficient drug incorporation and release, (ii) formulation stability and shelf life (iii) biocompatibility, (iv) biodistribution and targeting, (v) functionality [15]. In addition, the relative ease in modifying their surface chemistry permits the attachment of targeting and therapeutic molecules for specific therapeutic

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applications. However, tethering of fullerenes with biocompatible glycine and its exploration in drug delivery of DTX is still unexplored. Henceforth, it was aimed to chemically tether glycine with C_{60}-fullerenes and subsequently conjugate with DTX for the promises and potentials in delivery of DTX to cancerous cells.

2. Material and methods

Docetaxel (DTX) was purchased from M/s Thermo Fisher Scientific India Co., Ltd., (Acros Organics) Noida, India. C_{60}-fullerenes were supplied by M/s Bucky USA, Houston, USA. 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT), D-chloroform and D_6-dimethyl sulphoxide were procured from M/s Sigma–Aldrich, Bangalore, India. HPLC-acetonitrile, HPLC-water and sodium dodecyl sulphate were procured from M/s Spectrochem Co., Ltd., Mumbai, India. Glycine was provided by M/s Thermo Fisher Scientific India [Pvt] Ltd., Mumbai, India. The solvents employed in the study were procured from M/s Thermo Fisher Scientific India, Co., Ltd., Mumbai, India. Ethanol was obtained from M/s Jai Chemical Pharma Works, Jaipur. The buffer ingredients and tetrahydrofuran (THF) were purchased from M/s CDH Co., Ltd., New Delhi, India. Dialysis membrane was procured from M/s Himedia laboratories Co., Ltd., Mumbai, India. MDA-MB-231 cell lines were procured from The National Centre for Cell Science, Pune, India. Distilled water was employed throughout the studies and the chemicals/reagents were used as such without further purification.

2.1. Glycination of C_{60}-fullerenes

C_{60} fullerene in the presence of amino acid undergoes hydroamination reaction. In this reaction, amine (—N—H) group of glycine reacts with carbon (—C═C—) multiple bond in fullerene [16]. For functionalization of C_{60}-fullerenes, 100 mg of glycine and 20 mg of sodium hydroxide were added to 3 mL water, followed by addition of 15 mL of ethanol. The resulting solution was added to a 50 mg C_{60}-fullerene solution in 60 mL of toluene. To the solution, 5 drops of tetrabutylammonium hydroxide were added, followed by stirring for 60 h at room temperature, under nitrogen atmosphere. After the

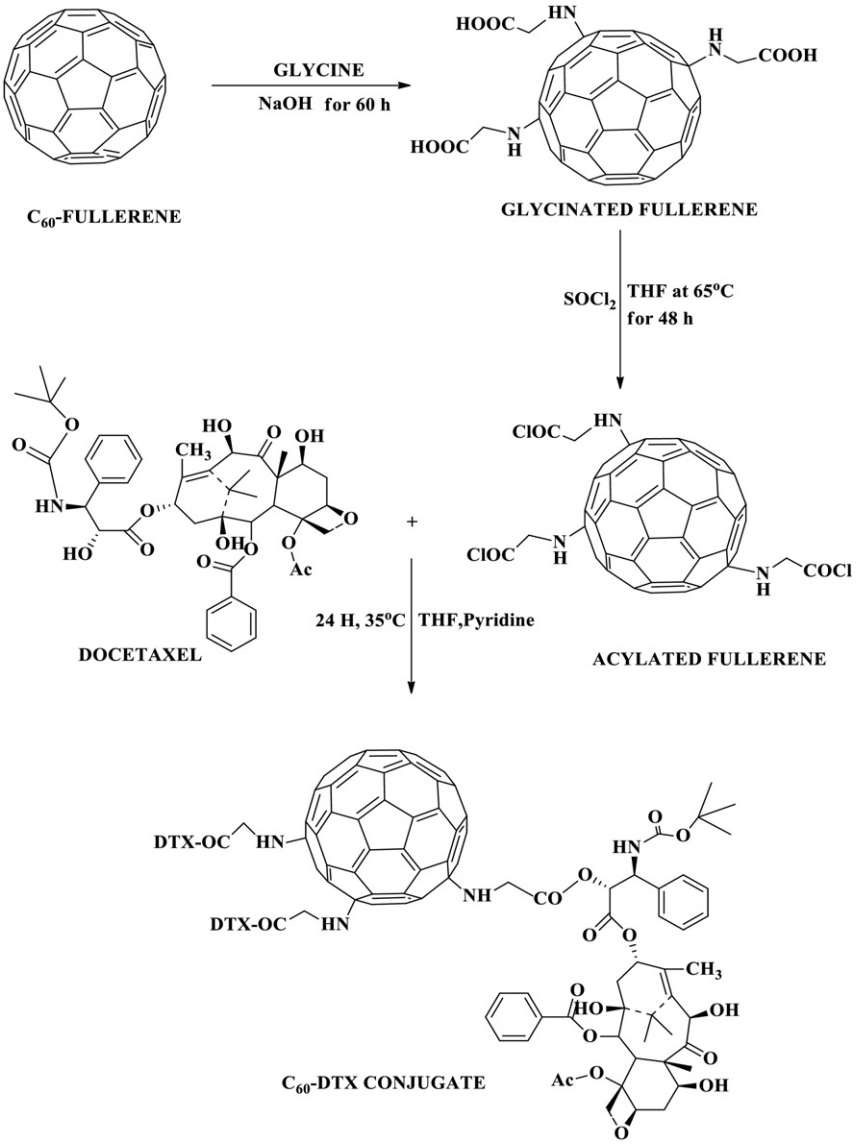

Fig. 1. Representation of schematic scheme for synthesis of C_{60}-DTX conjugate.