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Synthesis and biological evaluation of novel 3-*O*-tethered triazoles of diosgenin as potent antiproliferative agents

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ABSTRACT

Diosgenin, a promising anticancer steroidal sapogenin, was isolated from *Dioscorea deltoidea*. Keeping its stereochemistry rich architecture intact, a scheme for the synthesis of novel diosgenin analogues was designed using Cu (I)-catalysed alkyne-azide cycloaddition in order to study their structure-activity relationship. Both diosgenin and its analogues exhibited interesting anti-proliferative effect against four human cancer cell lines viz. HBL-100 (breast), A549 (lung), HT-29 (colon) and HCT-116 (colon) using [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazoliumbromide] (MTT) assay. Among the synthesized analogues, **Dgn-1** bearing a simple phenyl R moiety attached via triazole to the parent molecule was identified as the most potent analogue against A549 cancer cell line having IC_{50} of 5.54 μM, better than the positive control (BEZ-235). **Dgn-2** and **Dgn-5** bearing o-nitrophenyl and o-cyanophenyl R moieties respectively, displayed impressive anti-proliferative activity against all the tested human cancer cell lines with IC_{50} values ranging from 5.77 to 9.44 μM. The structure-activity relationship (SAR) revealed that the analogues with simple phenyl R moiety or electron withdrawing ortho substituted R moieties seem to have beneficial impact on the anti-proliferative activity.

1. Introduction

Dioscorea deltoidea, commonly known as wild yam, belongs to the family dioscoreaceae. This species is mainly found in India, China, Nepal, Pakistan, Afghanistan, Bhutan and Vietnam. It is a commercial crop of medicinal importance excessively collected in India along Kashmir Himalayas and Assam [1,2]. *D. deltoidea* has been used worldwide in traditional system of medicine as anti-rheumatic, to treat ophthalmic conditions and urinogenital disorders and to get rid of intestinal worms. In Indian folk medicine, the powder from its rhizomes is used to treat dysentery, abdominal pain and piles [3]. In Nepal, the decoction from its rhizomes is used to treat gastric problems [4]. Some preparations from the tubers of *D. deltoidea* have traditionally been used in the treatment of tumours, cardiovascular diseases, central nervous system ailments, dysfunctional changes in the female reproductive system, bone and joint diseases, metabolic disorders, and autoimmune diseases [5,6]. Its rhizome has also been used as an oral medication to get relief from snake bites [7].

The tubers of *D. deltoidea* are rich source of diosgenin (25R-spirost-5-en-3β-ol) (Fig. 1), a steroidal sapogenin, used for the commercial synthesis of sex hormones and corticosteroids which are widely used as anti-inflammatory, androgenic and contraceptive drugs [8]. Diosgenin is structurally similar to cholesterol and other steroids with a great demand in pharmaceutical industry [9]. Several experimental studies have demonstrated the lipid-lowering potential of diosgenin, besides its important role in the control of metabolic diseases such as diabetes and obesity [10–12]. Diosgenin has been extensively studied for its anticancer potential both *in vitro* as well as *in vivo*. Unlike its two structurally identical saponins, hecogenin and tigogenin, diosgenin has been found to trigger cell cycle arrest associated with strong apoptosis *in vitro* against human 1517 osteosarcoma cells [13]. The *in vivo* tumour modulating potential of diosgenin has been studied keenly on a number of tumour models. It has been effectively used to inhibit the formation of colon aberrant crypt foci (ACF), putative precancerous lesions induced by azoxymethane (AOM) in F344 rats [14]. In another study designed to assess the tumour-modulating potential

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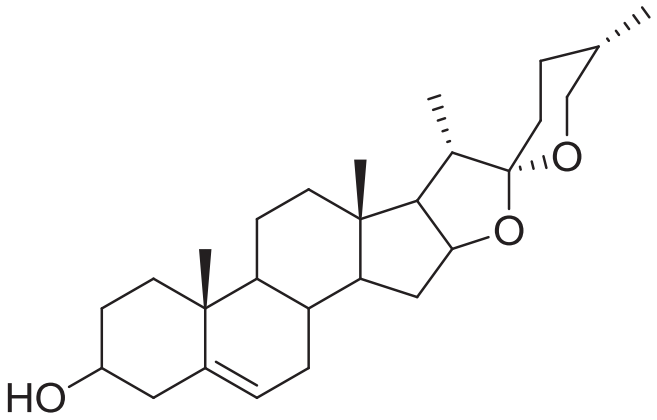

Fig. 1. Structure of Diosgenin (1).

of diosgenin using the AOM-injected F344 rats, it has been reported that 0.1% of diosgenin suppressed the incidence of both invasive and non-invasive colon adenocarcinomas by up to 60% via attenuation of tumour cell proliferation [15]. Diosgenin has been shown to attenuate sub-acute intestinal inflammation and normalized bile secretion in indomethacin-induced intestinal inflammation in rats, which could be extrapolated to its prospective role in carcinogenesis [16]. Diosgenin has been reported effective against experimentally induced inflammation associated with colon carcinogenesis in ICR mice, wherein, it significantly reduced the tumour multiplicity by the alteration of lipid metabolism (reduced serum triglyceride levels by up-regulation of lipoprotein lipase), and the modulation of genes associated with inflammation and multiple signaling pathways [17]. With respect to its anti-breast cancer potential, diosgenin selectively inhibited the growth of tumour xenografts of both MCF-7 and MDA 231 with no toxicity to any of the vital organs in the experimental nude mice [18].

Diosgenin has been studied extensively *in vitro* against various human cancer cell lines wherein it affects different molecular targets critical to tumorigenesis. It has been shown to inhibit the growth of HT-29 and HCT-116 human colon adenocarcinoma cells by induction of apoptosis via inhibition bcl-2 and induction of caspase-3 protein expression [14,19,20]. Additionally, it has been demonstrated that diosgenin operated through involvement of the cholesterol biosynthetic pathway which contributes to its efficacy as an anti-cancer agent [20]. Diosgenin has been reported to induce selective apoptosis in AU565 human breast adenocarcinoma cells through PARP cleavage [21]. Diosgenin has also been reported to display significant anticancer activity against MCF-7 human breast cancer cells via induction of p53 tumour suppressor protein, while as, it is also reported to involve activation of caspase-3 and down-regulation of bcl-2 in its pro-apoptotic mechanism against MDA human breast carcinoma cells [18]. Diosgenin is reported to inhibit proliferation of PC-3 human prostate cancer cells by suppressing cell migration and invasion by reducing the activities and mRNA expression of MMP-2 (matrix metalloproteinase) and MMP-9 [22]. In another study, it has been reported to induce anticancer effects against DU145 human prostate cancer cells by abrogation of hepatocyte growth factor (HGF)-induced cell scattering and invasion, together with inhibition of Mdm2 and vimentin through down-regulation of phosphorylated Akt and mTOR [23]. Diosgenin has been shown to inhibit the proliferation of hepatocellular carcinoma (HCC) at G1 phase of the cell cycle and induced apoptosis through caspase-3 activation leading to PARP cleavage [24]. A novel 26-hydroxy-22-oxocholestanic steroidal derivative from diosgenin has been reported to induce apoptosis in human cervical cancer CaSki cells at non-cytotoxic doses activation of caspase-3 [25]. Cytotoxic effects of diosgenin have also been reported in human cancer cell lines of osteosarcoma [13,26], leukemia [27] and erythroleukemia [28]. *In vivo* studies on the disposition of diosgenin have revealed that diosgenin is poorly absorbed in biological systems and is possibly prone to active biotransformation [29].

The molecular modelling studies have revealed that the necessary structural parameters responsible for its role in apoptosis and anticancer activity include the presence of a hetero-sugar moiety and the 5,6-double bond in its structure [30]. Moreover, structural conformation at C-5 and C-25 carbon atoms have been shown to be important for diosgenin's biological activity [30]. However, further studies are warranted to assess the structure-activity relationship of diosgenin and to understand whether and how synthetic changes brought about could augment its biological activity in favour of its role as a therapeutic agent in the field of cancer chemotherapy. The versatile anticancer activity exhibited by diosgenin indicates that such molecules hold the key to modern drug discovery. Because of its unique biochemical properties, diosgenin and its analogues could be further investigated to yield new leads in the field of anticancer drug development.

Click chemistry of natural products has acquired great reputation in drug development in recent years. Some of the molecules studied include alkaloids [31], coumarins [32,33], saponins [34], steroids [35] and triterpenes [36]. Click chemistry is of great importance in medicinal chemistry and can be used for the synthesis of heterocyclic triazoles with different biological activities such as antiviral, antibacterial, antifungal, anti-tuberculosis, anticonvulsant, antidepressant, anti-inflammatory and anticancer [37]. Triazole based compounds such as anastrozole, letrozole and vorozole are very important antineoplastic drugs, while as, radicicol triazole, indolylsubstituted triazole of novobiocin, lavendustin triazole and combretastatin triazoles are considered as promising anticancer leads [32,38]. Thus, the design and synthesis of novel triazole derivatives of natural products is the prospective direction for the development of novel anticancer agents with better curative effect, lower toxicity, better bioavailability as well as higher selectivity.

While analysing the literature on analogue synthesis of diosgenin, it was noticed that there are no such reports underscoring the structure-activity relationship (SAR) linking the effect of aryl substituents tethered via 1,2,3-triazoles to the parent molecule on the cytotoxic properties of the resultant analogues. Keeping these facts in mind, we directed this work towards the synthesis of a diverse series of novel triazolyl derivatives of biological interest using diosgenin as a key template. All the triazolyl analogues of diosgenin were subjected to MTT [3-(4,5-Dimethylthiazol-yl)-diphenyltetrazoliumbromide] cytotoxicity assay against a panel of four human cancer cell lines namely, breast (HBL-100), lung (A549) and colon (HT-29 and HCT-116) to check their anti-proliferative activity. This work provides the initial report on structure-activity relationship of triazolyl analogues of diosgenin with the aim to prepare the derivatives with better activity, lesser toxicity, enhanced bioavailability and improved selectivity. This strategy would provide an important step towards the rationalisation of lead properties of diosgenin.

2. Experimental

2.1. General methods

All the solvents and reagents for the preparation of extracts, chemical synthesis and biological assays were obtained from Sigma Aldrich. Reactions were carried out in Branson sonicator-3510. All the chemical reactions were monitored by using F_{254} silica gel TLC plates (E. Merck) using ceric ammonium sulphate (char-