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Original article

Click chemistry inspired synthesis and bioevaluation of novel triazolyl derivatives of osthol as potent cytotoxic agents

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ABSTRACT

A new series of diverse triazoles linked through the hydroxyl group of lactone ring opened osthol (**1**) were synthesized using click chemistry approach. All the derivatives were subjected to 3-(4,5-Dimethylthiazol-yl)-diphenyl tetrazoliumbromide (MTT) cytotoxicity screening against a panel of seven different human cancer cell lines viz. colon (colo-205), colon (HCT-116), breast (T47D), lung (NCI-H322), lung (A549), prostate (PC-3) and Skin (A-431) to check their cytotoxic potential. Interestingly, among the tested molecules, most of the analogs displayed better cytotoxic activity than the parent osthol (**1**). Of the synthesized triazoles, compounds **8** showed the best activity with IC_{50} of 1.3, 4.9, 3.6, 41.0, 35.2, 26.4 and 7.2 μM against colon (Colo-205 and HCT-116), breast (T47D), lung (NCI-H322 and A549), prostate (PC-3) and Skin (A-431) cancer lines respectively. Compound **8** induced potent apoptotic effects in Colo-205 cells. The population of apoptotic cells increased from 11.4% in case of negative control to 24.1% at 25 μM of **8**. Compound **8** also induced a remarkable decrease in mitochondrial membrane potential ($\Delta\Psi m$) leading to apoptosis of cancer cells used. The present study resulted in identification of broad spectrum cytotoxic activity of analogs bearing electron withdrawing substituents, besides the enhanced selective activity of analogs with electron donating moieties.

1. Introduction

Natural products (NPs) have been used to treat human disease for thousands of years and play an increasingly important role in drug discovery and development. In fact, the majority of anticancer and anti-infectious agents are of natural origin [1,2]. NPs with a coumarinic moiety have been reported to have multiple biological activities including anticancer [3,4], antioxidant [5,6], anti-inflammatory [7], antimicrobial [8], antiviral [9] and enzymatic inhibitory activities [10]. Natural coumarins and their derivatives are of great interest due to their widespread pharmacological properties, and this has attracted many medicinal chemists for further derivatization and screening them as novel therapeutic agents. The coumarin ring system, present in a large number of natural products (such as the anticoagulant, Warfarin), having interesting pharmacological properties [11,12] has intrigued chemists for decades to explore the natural coumarins or their synthetic derivatives for their applicability as drugs.

Many intersecting molecules having coumarin based ring systems have been synthesized utilizing novel synthetic methodologies. Some new derivatives bearing coumarin ring including the furanocoumarins (imperatorin), pyranocoumarins (seselin) and coumarin sulfamates (coumates) have been found to be useful in photochemotherapy, antitumour and anti-HIV therapy [13,14]. Among the diverse biological activities of coumarins, the notable one being their effect against breast cancer and sulfatase and aromatase inhibitory activity [15,16].

The naturally occurring coumarin osthol **1** (Fig. 1) has been thoroughly investigated during the past years for its promising pharmacological properties, particularly in the field of cancer [17,18]. It is clinically ingested as an important component of medicinal plants and herbs in Tradition Chinese Medicine (TCM) [19–21], and it exhibits a host of biological activities [22–26]. Osthol has been found to be a promising agent for the treatment of

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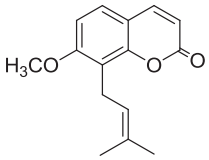

Fig. 1. Structure of osthol (**1**).

osteoporosis [27] and in reproductive system improvement properties by activation of the central cholinergic neuronal system [28]. Physiological [29], bacteriostatic and antitumour activities make these compounds attractive for further backbone derivatization and screening as novel therapeutic agent/s [30]. From the literature scan, both *in vitro* and *in vivo* studies revealed that osthol possesses anticancer effect by inhibiting human cancer cell growth and inducing apoptosis [31]. The studies on cytostatic activity in human cancer cell line MCF-7 (breast carcinoma) revealed that osthol displays some estrogenic activity by preventing the synthesis and action of estrogens (ER antagonists) indicating its potential to become a breast cancer treatment reagent [32]. This compound reportedly possesses anticancer effects by inhibiting cancer cell growth, metastasis and inducing cell apoptosis [33]. It induces G2/M arrest and apoptosis by modulating the PI3K/Akt pathway and suppresses migration and invasion through inhibition of matrix metalloproteinase-2 and matrix metallopeptidase-9 in human lung adenocarcinoma A549 cells [34]. Osthol has also been found to be effective in inhibiting the migration and invasion of breast cancer cells by wound healing and transwell assays [35]. The biological studies of osthol carried out in last few years have provided an additional dimension to the bioactivity profile of the title compound. The potential of osthol has not been fully exploited despite its biological importance; therefore, more efforts are invested towards the building of diverse libraries around its chemical structure and their biological profiles are in demand.

Click chemistry of natural products has acquired great importance in recent years. Some of the molecules studied include alkaloids [36,37], coumarins [38], saponins [39], steroids [40] and triterpenes such as betulinic acid [41–43]. Triazoles and their derivatives are of great importance in medicinal chemistry and can be used for the synthesis of numerous heterocyclic compounds with different biological activities such as antiviral, antibacterial, antifungal, anti-tuberculosis, anticonvulsant, antidepressant, anti-inflammatory and anticancer [44,45]. Triazole based compounds such as anastrozole, letrozole and vorozole are very important antineoplastic drugs (Fig. 2) [46]. Thus, the design and synthesis of novel triazole derivatives is the prospective direction for the development of novel anticancer agents with better curative effect, lower toxicity as well as higher selectivity.

Based on the above cited findings and inspiration from the potential anticancer activity of triazoles, we directed this work towards the synthesis of a diverse series of novel triazolyl derivatives of biological interest using osthol (**1**) as a key starting material. All the newly synthesized compounds were subjected to MTT [3-(4,5-Dimethylthiazol-yl)-diphenyl tetrazolium-bromide] assay against a panel of seven different human cancer cell lines viz. prostate colon (Colo-205 and HCT-116), breast (T47D), lung (NCI-H322 and A549), prostate (PC-3) and skin (A-431) to check their cytotoxic potential. This work provides the initial report on structure–activity relationship of triazolyl analogs of lactone ring opened coumarins in general and osthol (**1**) in particular.

2. Result and discussion

2.1. Chemistry

Osthol exhibits comparatively weak activity, low water solubility and limited permeability, and these properties may lower its absorption and bioavailability upon oral administration [19,20,47]. To overcome these lacunas it becomes imperative to introduce more hydrogen bond donors through its lactone ring opening and simultaneous introduction of heterocyclic ring system to improve its solubility and activity for better drug-likeness. The incorporation of heterocyclic moieties either as substituent group or as a fused component into parent coumarin nucleus alters its properties and converts it into a more useful product [48]. Keeping in view the interesting pharmacological activities of coumarins, click chemistry inspired approach involving union of terminal alkynes with organic azides has been taken up for the synthesis of regiospecific novel OH linked triazolyl derivatives of osthol in excellent yields. These click reactions exhibit remarkably broad scope and exquisite selectivity and have contrasting applications in chemistry, biology, and materials science. The target compounds **3**–**22** were synthesized as depicted in Scheme 1.

In the present study, osthol (**1**), isolated from the root parts of *Prangos pabularia* was used as the starting material. It was subjected to lactone ring opening in DMSO using NaOH as base yielding a *cis* (*Z*) product which simultaneously undergoes alkylation at OH group in presence of propargyl bromide to form (*E*)-3-(4-methoxy-3-(3-methylbut-2-en-1-yl)-2-(prop-2-yn-1-yloxy)phenyl)acrylic acid **2**. The proposed structure (**2**) was confirmed by spectral data analysis. From elemental analysis and HR-ESIMS (*m/z* 301.1459) data, this compound was assigned the molecular formula $C_{18}H_{20}O_4$. Proton singlets at δ 2.52 and 4.45 (integrating for one and two protons respectively) were assigned to terminal alkyne proton and two methylenic protons of propargylic moiety respectively. The *cis* (*Z*) behaviour of the protons of α,β-unsaturated system was depicted by the presence of two doublets at δ 5.92 and 7.26 with the coupling constant (*J*) of 12.4 Hz each. Compound **2** was allowed to undergo 1,3-dipolar cycloaddition reaction typically called Huisgen cycloaddition with various aromatic azides under sharpless click chemistry conditions ($CuSO_4.5H_2O$ and sodium ascorbate in *t*-BuOH:H_2O (1:1)) to afford regioselectively 1,4-disubstituted-1,2,3-triazoles (**3**–**22**) in good to excellent yields (Scheme 1). Under click conditions a series of such analogs was synthesized to look for structure–activity relationship studies. The structures of all the synthesised triazolyl derivatives were characterised by analytical and spectral data analysis. Formation of products could easily be confirmed by a downfield H-5 proton singlet (almost around 8.0 ppm) and other proton resonances in the aromatic region. Further characterisation of all the products was done using ^{13}C NMR-DEPT and HR-ESIMS as well as HRESI-MS.

2.2. Biology

2.2.1. Anti-proliferative activity

There are innumerable number of synthetic drugs to treat the diseased condition, but the treatment is not satisfactory, often due to their severe adverse effects, making provision for the synthesis of new and safer ones. Presently, scientists are keen to evaluate drugs from plant origin, due to their specific curative properties, healthy action, and safe and non-toxic effects. The biological studies of coumarins and related molecules containing coumarin ring; carried out in last few years have provided an additional dimension to the bioactivity. The potential of coumarins has not been fully exploited despite their biological importance; therefore, more efforts towards building the diverse libraries around its chemical structure and