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Research paper

Design, synthesis and anticancer properties of novel oxa/azaspiro[4,5] trienones as potent apoptosis inducers through mitochondrial disruption

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ABSTRACT

A series of twenty seven oxa/azaspiro[4,5]trienone derivatives were synthesized and their anticancer properties have been explored. GI_{50} values of all these compounds were evaluated against four types of human cancer cell lines, i.e. MCF-7 (breast), DU-145 (prostate), A549 (lung) and HepG2 (liver). Five compounds of the series exhibited good anticancer potential against MCF-7 with GI_{50} values less than 2 μM. Detailed biological studies of the two representative compounds **9b** and **9e** revealed that they arrest cell cycle in G0/G1 phase and induce mitochondria mediated apoptosis, that was further confirmed by measurement of mitochondrial membrane potential (ΔΨm), intracellular ROS generation, caspase 9 activity and Annexin V-FITC assay. Furthermore, western blot analysis suggested that these compounds up-regulate the levels of p53, p21, p27 and Bax, and down-regulate the level of Bcl-2 confirming the apoptosis inducing properties.

1. Introduction

Identification of novel scaffolds with improved pharmaceutical properties is always exciting to medicinal chemists towards the development of new chemical entities (NCEs). There are several scaffolds of significant synthetic interests whose pharmaceutical properties need to be explored [1]. The newly designed molecules, mimicking drug skeletons with additional functionalities, can display improved or reduced pharmaceutical potential depending on their interactions with biological system. Hence, a detailed mechanistic investigation and structure activity relationship (SAR) studies of the new skeletons are required for developing pharmacophores with improved efficacy [2]. With the exceeding cases of cancer patients every year and lesser success in anticancer drug development, new scaffolds need to be identified as potential leads [3]. In our efforts to study the pharmaceutical potential of new scaffolds, we have identified novel oxaspiro[4,5]trienones; **II** possessing anti-proliferative properties [4]. These spiro[4,5]trienones were designed as constrained tamoxifen; **I** mimic to develop new selective estrogen receptor modulators (SERMs), however *in vitro* screening of these compounds revealed that they are not selective towards ER positive breast cancer cell line (MCF-7) and were found to be equally potent against ER negative breast cancer cell line (MDA-MB-231) [Fig. 1] [4]. In continuation to our previous work, herein we report the detailed biological profiling of oxaspiro[4,5] trienones; **II** and azaspiro[4,5]trienones; **III** along with their structure activity relationship (SAR) studies. The literature survey shows that structurally related antitumor compounds bearing enone moiety may facilitate ROS production which in turn can induce apoptosis [Fig. 1] [5–8]. Anthracenyl amino acid **IV** [5], Dehydroaltenusin **V** [6]; a natural product isolated from a fungus *Alternaria tennuis*, and its tautomer **VI**, inhibit DNA polymerase α and induce apoptosis. Increase in ROS production by small molecules triggers the cancer cell death [7], for example, a dienone containing natural product derivative Deoxynyboquinone **VII**

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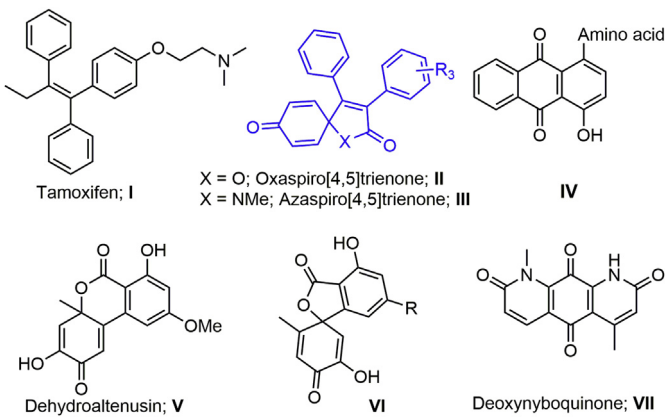


Fig. 1. Recently reported apoptosis inducing agents and our designed prototypes.

exhibits excellent tumoristatic potential in animal models through ROS generation [8].

ROS production in cancer cells regulates cellular growth, cell signaling and synthesis of important substances. Furthermore, mitochondria plays an important role for the survival of cancer cells, hence disrupting mitochondrial function by small molecules induces apoptosis [9,10]. Considering these findings from literature, we became interested to examine the apoptosis inducing properties of oxa/azaspiro[4,5]trienones.

2. Results and discussion

2.1. Chemistry

As reported in our previous communication, oxaspiro[4,5]trienones **9a-g** were obtained by coupling of compound **6** with various boronic acids; **8** under Suzuki conditions [4]. Compound **9h** was prepared by Heck reaction of **6** with methylacrylate. Azaspiro [4,5]trienones **10a-o** were synthesized from *N*-methyl-*p*-anisidine; **2** by following similar synthetic strategy. *N*-methyl-*p*-anisidine; **2** was coupled with 3-phenylpropiolic acid; **3** to yield amide; **5** which was subjected to iodine mediated *ipso*-iodocyclization for the construction of compound **7**. Suzuki coupling of **7** with various boronic acids; **8** produced **10a-o** in good yields [Scheme 1]. All the compounds were characterized by ^{1}H, ^{13}C NMR and HRMS analysis. Purity of all the newly synthesized compounds was checked by HPLC.

2.2. Biology

2.2.1. Anticancer activity

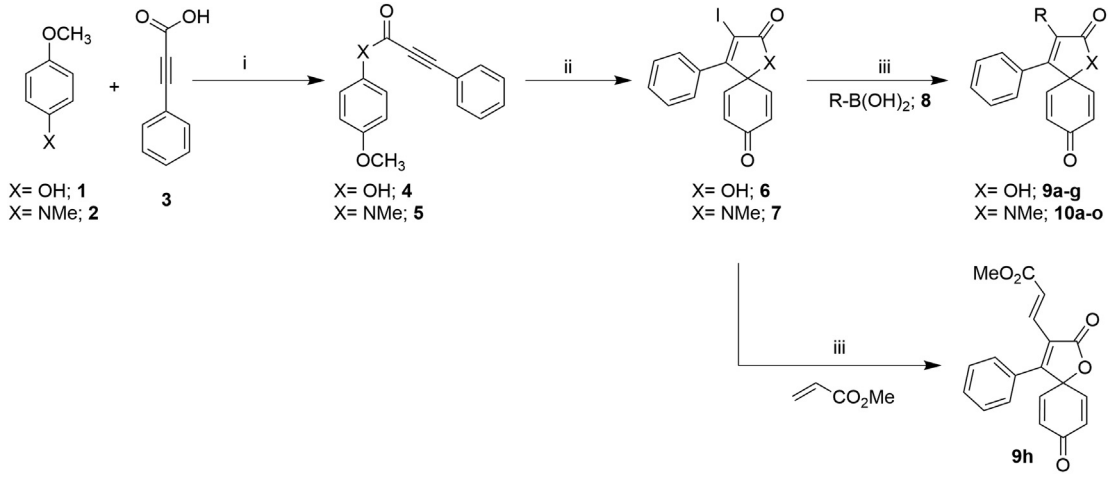
In vitro cytotoxicity of compounds **9a-h** and **10a-o** were evaluated against four types of human cancer cell lines; MCF-7 (Breast-ER positive), DU-145 (Prostate), A549 (Lung) and HepG2 (Liver) by using sulforhodamine B (SRB) method [11]. There are several rapid colorimetric assays described for *in vitro* chemo sensitivity testing of tumor cell lines. While tetrazolium [MTT; 3-(4,5-dimethylthiazolyl-2)-2, 5 diphenyltetrazoliumbromide] assay being the most widely used, recently the US National Cancer Institute (NCI) recommended use of the sulforhodamine B (SRB) protein stain for *in vitro* chemo sensitivity testing. The SRB assay appeared to be more sensitive than MTT assay, with better linearity with cell number and higher reproducibility [12]. The compounds exhibiting $GI_{50} \leq 10$ μM were considered to be active against the respective cancer cell lines. The growth inhibition data (expressed as GI_{50}) of compounds **9a-h** and **10a-o** are shown in Table 1. The results of the cytotoxicity assay showed that compounds **9b** and **9e** possess significant anti-proliferative properties against the human breast cancer cell line MCF-7. The promising activity of **9b** and **9e** prompted us to examine their pharmacological properties in detail.

2.2.2. Cell cycle analysis

In general, anticancer agents prevent cell division at various checkpoints of cell cycle, thereby decreasing the growth and proliferation of cancerous cells [13]. Cell cycle analysis after treatment with potent anticancer agents shows the distinguish cells in different phases of the cell cycle. In this study, MCF-7 cells were treated with compound, **9b** and **9e** at 0.5 and 1 μM concentrations for 48 h. The data obtained clearly indicated that these compounds (**9b** and **9e**) showed G0/G1 cell cycle arrest when compared to untreated control (Fig. 2, Table 2).

2.2.3. Measurement of mitochondrial membrane potential ($\Delta\Psi m$)

The maintenance of mitochondrial membrane potential ($\Delta\Psi m$) is important for mitochondrial integrity and bioenergetic function



Reagents and Conditions: i) DIC, HOBt, DCM; ii) I_2, $NaHCO_3$, CH_3CN; iii) $Pd(OAc)_2$, K_2CO_3, DMF, 80 °C

Scheme 1. Synthesis of spirotrienediones.