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Synthesis and cytotoxic activities of semisynthetic zearalenone analogues

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ABSTRACT

Zearalenone is a β-resorcylic acid macrolide with various biological activities. Herein we report the synthesis and cytotoxic activities of 34 zearalenone analogues against human oral epidermoid carcinoma (KB) and human breast adenocarcinoma (MCF-7) cells as well as noncancerous Vero cells. Some zearalenone analogues showed moderately enhanced cytotoxic activities against the two cancer cell lines. We have discovered the potential lead compounds with diminished or no cytotoxicity to Vero cells. Preliminary structure–activity relationship studies revealed that the double bond at the 1′ and 2′ positions of zearalenone core was crucial for cytotoxic activities on both cell lines. In addition, for zearalenol analogues, the unprotected hydroxyl group at C-2 and an alkoxy substituent at C-4 played key roles on cytotoxic effects of both cell lines.

Zearalenone (**1**) is a β-resorcylic acid macrolactone originally isolated from the fungus *Gibberella zeae* (*Fusarium graminearum*) by Baldwin et al. in 1962 (Fig. 1).[1] Compound **1** has been shown to exhibit various biological activities[2] particulary estrogen agonistic properties[3] and anabolic activities.[4] Several members of this class of metabolites are potent protein kinase inhibitors, which could potentially be developed as anticancer drugs[5].

There have been a number of literature precedents for biological activity studies of zearalenone analogues obtained either from commercial sources or simple semisyntheses; however, there are only a few reports on cytotoxic activities of zearalenone derivatives. In 2010, Banjerdpongchai and co-workers reported that zearalenone exhibited weak cytotoxic activities against human leukemia HL-60 cells with an IC_{50} of 138.21 μM and discovered that **1** induced leukemic cell apoptosis by endoplasmic stress and mitochondrial pathway.[6] In 2011, Oberlies et al. disclosed studies on cytotoxic and NF-κB inhibitory activities of 12 resorcylic acid lactones including zearalenone analogues.[7] In their studies, zearalenone (**1**) and analogues: α-zearalenol, β-zearalenol, α-zearalanol and β-zearalanol were assayed against three cancer cell lines: MCF-7 human breast carcinoma, NCI-H460 human large cell lung carcinoma and SF-268 human astrocytoma. These compounds exhibited very weak cytotoxicities against the three cancer cell lines. Only β-OH analogues (β-zearalenol, **2** and β-zearalanol, **3**) displayed moderate cytotoxicity against H460 cell line (IC_{50} = 15.4 and 9.5 μM, respectively) (Fig. 2). Several structure–activity relationship (SAR) studies of related resorcylic acid lactones for protein kinase inhibition have been disclosed. These studies suggested that the *Z*-enone moiety at the 7′–8′ positions was important for highly potent kinase inhibitory activity. In addition, reduction of the enone functionality to allylic alcohol led to reduction in potency of kinase inhibition.[8] In 2011, Murphy and co-workers found that isosteric replacement of the *Z*-enone in RALS with the *E*-enone (**4**) resulted in more potent kinase inhibitors of a subset of kinases containing a conserved cysteine (Fig. 2). Chai et al. also explored SAR for the inhibition of protein kinases related to cancer pathways by aigialomycin D (**5**) and a series of its analogues, which led to discovery that an unprotected resorcinol motif and a double bond at the 1′ and 2′ positions are crucial for such activity (Fig. 2).[9]

As part of ongoing search for biologically active compounds from fungi, our group has found that zearalenone is a major secondary metabolite produced by the seagrass-derived fungus *Fusarium* sp. PSU-ES123 and the para rubber-derived fungus *Fusarium* sp. PSU-H266. Due to therapeutic potentials and availability in a large quantity of zearalenone, we pursued the chemical structure modification of zearalenone obtained from these fungi and evaluated cytotoxic activities of zearalenone analogues against

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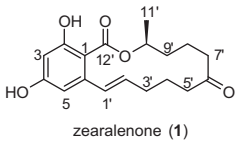


Figure 1. Structure of zearalenone (**1**).

two cancer cell lines in comparison with noncancerous Vero cells (African green monkey kidney fibroblasts).

The first series of zearalenone analogues were synthesized by methylation and acylation as shown in Scheme 1. Monomethylation of the hydroxyl group at the 4-position and bismethylation of the 2- and 4-hydroxyl groups of **1** were performed by treatment with 4 equiv and 10 equiv of iodomethane in the presence of K_2CO_3 in acetone at room temperature to deliver 4-methoxyzearalenone (**6**) and 2,4-dimethoxyzearalenone (**7**), respectively. Acetylation of the hydroxyl group at the 4-position or both hydroxyl groups was achieved by reacting **1** with 1 and 3 equiv of acetic anhydride in pyridine in CH_2Cl_2 to give acetylated analogues **8** and **9**. Similarly, propionylated derivatives **10** and **11** were prepared by a similar procedure using 1 and 2 equiv of propionyl chloride in the presence of Et_3N in THF, respectively. The 5-chlorinated analogues **12**–**14** were also synthesized by treatment of compounds **1**, **6** and **7** with *N*-chlorosuccinimide in DMF at room temperature[10] in order to evaluate the effect of a Cl atom which has been previously shown to enhance cytotoxicity of some RALs against MCF-7 breast cancer cells.[11] In addition, zearalanone (**15**) could be obtained by catalytic hydrogenation of **1**. Methylated and bismethylated derivatives of **15** (**16** and **17**) were also prepared using the same reaction conditions for preparation of **6** and **7**.

The second series of zearalenone analogues were the corresponding alcohols (Scheme 2). Reduction of the ketone functionality of **1** with $NaBH_4$ in methanol at 0 °C led to the formation of a mixture of alcohol diastereomers which could be separated by reverse phase column chromatography (70% MeOH/H_2O as an eluent) to afford β- (**2**) and α-zearalenol (**18**) in 58% and 29% yield, respectively. Alkylation of the 4-hydroxy group of **2** and **18** was achieved using iodomethane, iodoethane or iodopropane in the presence of K_2CO_3 in acetone at room temperature or 1-bromooctane and K_2CO_3 in refluxing acetone to provide derivatives **19**–**23** and **26**–**30**, respectively. Acetylation and propionylation of the hydroxyl group at the 4-position of **2** and **18** were accomplished utilizing the same procedure for preparation of compounds **8** and **10** to give compounds **24**–**25** and **31**–**32**. In order to identify the importance of the double bond at 1′ and 2′ positions of zearalenol derivatives, both β- (**3**) and α-zearalanols (**35**) and their methylated analogues **33**–**34** and **36**–**37** were prepared via catalytic hydrogenation and methylation of the corresponding parent compounds (Scheme 3). With the aforementioned chemical transformations, 34 zearalenone analogues were obtained.

Zearalenone and its analogues **2**, **3** and **6**–**37** were evaluated for cytotoxic activities against two cancer cell lines: human oral epidermoid carcinoma (KB) and human breast adenocarcinoma (MCF-7) cells using the resazurin microplate assay (REMA)[12] as well as against noncancerous Vero cells (African green monkey kidney fibroblasts) using the green fluorescent protein (GFP)-based assay.[13] Ellipticine, doxorubicin and tamoxifen were used as

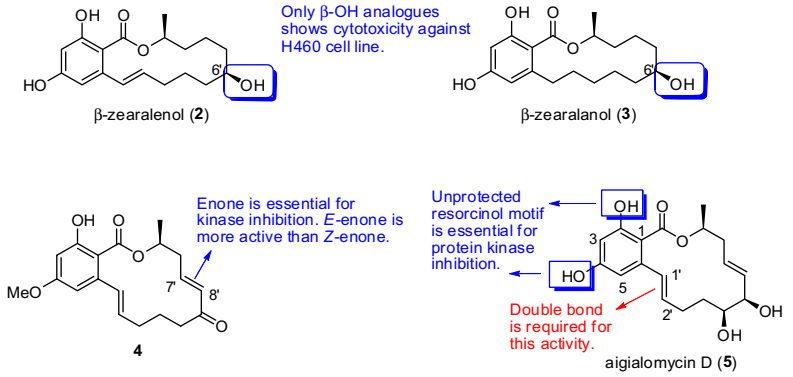
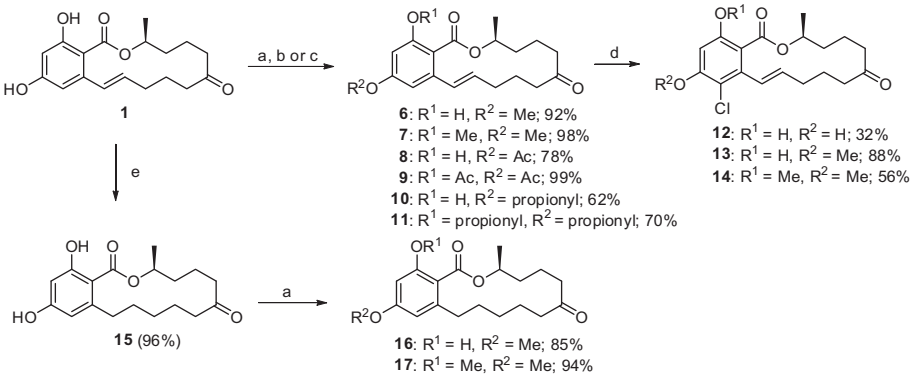


Figure 2. Structural effects of zearalenone analogues on cytotoxicity against H460 cell line and on protein kinase inhibition.



Scheme 1. Reagents and conditions: (a) iodomethane, K_2CO_3, acetone, rt; (b) Ac_2O, pyridine, CH_2Cl_2, 0 °C to rt; (c) propionyl chloride, Et_3N, THF, 0 °C to rt; (d) *N*-chlorosuccinimide, DMF, rt; (e) H_2, Pd/C (4 mol %), EtOH, rt.