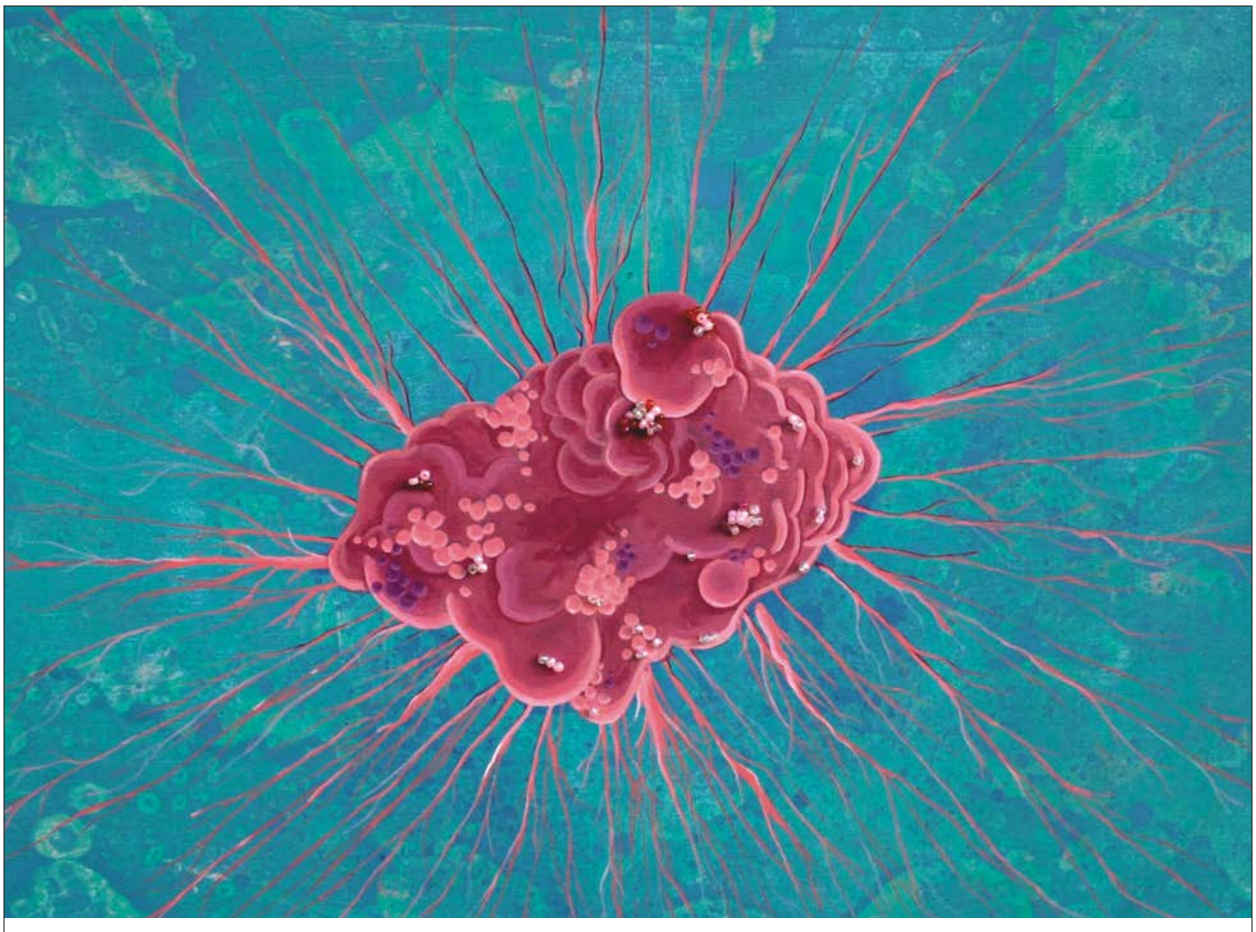

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Anti-Tumor Activities of Analogues Derived from the Bioactive Compound of *Zingiber zerumbet*

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Abstract: The aim of this study is to evaluate some derivatives of Zerumbone for their anti-tumor effects towards human cervical cancer cell lines (HeLa). The MTT tetrazolium salt colorimetric assay was utilized to evaluate the cytotoxic effects of ZER, cisplatin and the derivatives were n-Butylbenzene (compound 5), 1,1'-(4-Chlorobutylidene) bis(4-fluorobenzene) (compound 6), alpha, alpha-Diphenyl-gamma-butyrolactone (compound 7) and (1,4'-Bipiperidine)-4'-carboxaminde (compound 8). The results of this study showed that derivatives of ZER have shown lesser anti-tumor effects towards HeLa cancer cells compared to the principal compound (ZER).

Key words: Zerumbone, analogues, human cervical cancer cell line

INTRODUCTION

Zingiber zerumbet Smith locally known as lempoyang wild ginger belongs to Zingiberaceae family. It is native to South East Asia but has been widely cultivated plant in village gardens throughout the tropical and subtropical area around the world and has naturalized in some areas for its medicinal properties (Perry, 1980; Nharet Somchit and Nur Shukriah, 2003; Yu *et al*., 2008). *Zingiber zerumbet* is used in local traditional medicine as a cure for swelling, sores and loss of appetite. Besides that the juice of the boiled rhizomes has also been used as a medicine for worm infestation in children. The volatile oils of the rhizomes have been shown to contain zerumbone, humulene and camprene (Hasnah, 1991; Jang *et al*., 2005).

Zerumbone (ZER), a monocyclic sesquiterpene from rhizomes of edible plant *Zingiber zerumbet* Smith. ZER has recently been found to suppress tumor promoter 12-0-tetradecanoylphorpol-13 acetate (TPA)-induced Epstein-Barr virus activation in a patent manner (Murakami *et al*., 2002). ZER is known to be a potent suppressant of cyclooxygenase (COX)-2 and inducible nitric oxide synthase expression (Murakami *et al*., 2003). ZER is a food phytochemical that has a distinct potential for use as an effective anticancer agent (Matthes *et al*., 1980), markedly suppresses free radical generation, proinflammatory protein production (Murakami *et al*., 2002), possibly by its apoptosis-inducing, antiproliferative influences (Kirana *et al*., 2003) and activates phase II drug metabolizing enzymes (Nakamura *et al*., 2004).

However, studies on the synthetic ZER derivatives for their cytotoxic ability in cervical cancer remains unresolved. Therefore, the objective of this study is to investigate the cytotoxic effects of several synthetic ZER derivatives compare to ZER and cisplatin in human cervical cancer cells.

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MATERIALS AND METHODS

ZER was extracted and was isolated from *Zingiber zerumbet* by Ms. Nirmala Devi Thailan, Department of Biomedical Science, Faculty of Medicine and Health Science, Universiti Putra Malaysia (UPM). Synthetic derivatives of ZER (Fig. 1), were a generous gift by Assoc. Prof. Dr. Muhd Nazrul Hakim Abdullah (IBS, UPM), were n-Butylbenzene (compound 5), 1,1'-(4-Chlorobutylidene) bis (4-fluorobenzene) (compound 6), alpha, alpha-Diphenyl-gamma-butyrolactone (compound 7) and (1,4'-Bipiperidine)-4'-carboxaminde (compound 8).

Cell Culture

HeLa, cervical cancer cells obtained from ATCC were grown in RPMI 1640 supplemented with 10% fetal calf serum, 1% penicillin-streptomycin and 1% amphostat. Flasks were incubated at 37°C in a humidified incubator with 5% CO_2, 95% air. Cultures were regularly examined using inverted microscope. Compounds were added to the cells in different concentrations and left for 72 h.

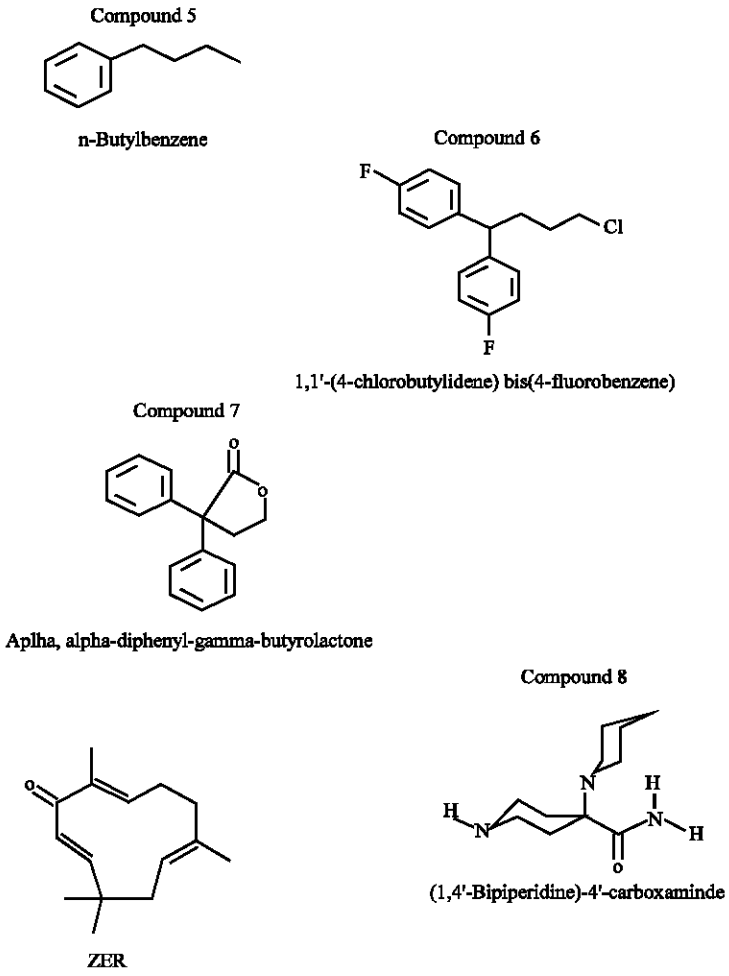

Fig. 1: Structure of several synthetic ZER derivatives

MTT (Microculture Tetrazolium) ASSAY

After incubated for 68 h, 20 μL MTT solution was added under dark condition, with gentle shaking. After 4 h, content in the wells was aspirated and 100 μL DMSO was added with gentle shaking. Finally, the wells were read using ELISA reader at 450 nm and Optical Density (OD) was recorded. The percentage of the cytotoxicity was calculated using the equation below:

Calculation of Percentage of the Cytotoxicity

$$\text{Cell viability (\%)} = (\text{Average OD}_{\text{sample}}/\text{Average OD}_{\text{control}}) \times 100\%$$

$$\text{Od}_{\text{sample}} = \text{Optical Density of sample (treated cells)}$$

$$\text{Od}_{\text{control}} = \text{Optical Density of control (untreated cells)}$$

A dose-response curve was drawn using Microsoft Excel, with cell viability versus ZER concentration. The Inhibition Concentration, IC_{50}, which is the drug concentration that inhibits 50% of HeLa cells growth, was determined from the graph. The experiment was conducted in triplicates.

Statistical Analysis

The values of IC_{50} from each batch were recorded in table and were analyzed using One-way Analysis of Variance (ANOVA) to compare with Cisplatin, the positive control. The values of IC_{50} from each batch were expressed as the mean ±SE of mean. DUNCAN test was conducted to compare the differences between groups mean value. The statistical analysis was done using SPSS (15.0) with $p<0.05$ as significant.

RESULTS

IC_{50} obtained from MTT assay was used to determine the viability of HeLa cells. The higher IC_{50} value, the more viable cells remaining after treatment, which indicates the lesser effective the compound was, for cytotoxicity. The synthetic analogues of ZER were screened at concentration ranged from 10 to 100 μM. Based on the Cytotoxicity Screening Index from the National Cancer Institute Chemotherapeutic Standard (Geran *et al*., 1972), the IC_{50} value less than 18 μM is considered very significant whilst between 18 and 137.6 μM is considered significant. The finding (Fig. 2) indicated that IC_{50} of the analogues felt between 18 and 137.6 μM, which is considered significant.

From Fig. 2, the IC_{50} for the analogues obtained were 70.0±2.5 μM in n-Butylbenzene, 79.4±4.1 μM 1, 1'-(4-Chlorobutylidene) bis(4-fluorobenzene), 87.6±4.6 μM in alpha,alpha-Diphenyl-gamma-butyrolactone and 80.0±7.8 μMin (1,4'-Bipiperidine)-4'-carboxaminde. The finding indicated significant different ($p<0.05$) in IC_{50} among analogues. In comparison within the analogues as seen in the Fig. 2, n-Butylbenzene shown significant lower value of IC_{50}, while alpha, alpha-Diphenyl-gamma-butyrolactone shown significant higher value of IC_{50}. Among the screened synthetic compounds, n-Butylbenzene considered most cytotoxic compound as its lowest IC_{50}.

Parent compound, ZER and commercial drug, cisplatin were previously established their IC_{50} which were 20.7±0.9 and 5.3±1.3 μM. This study found that the analogues were incompetent to neither ZER nor cisplatin for cytotoxicity in HeLa cells as their IC_{50} were higher (Fig. 2). Thus, ZER was the most active natural compound, comparing to its synthetic analogues and comparable to cisplatin as well.

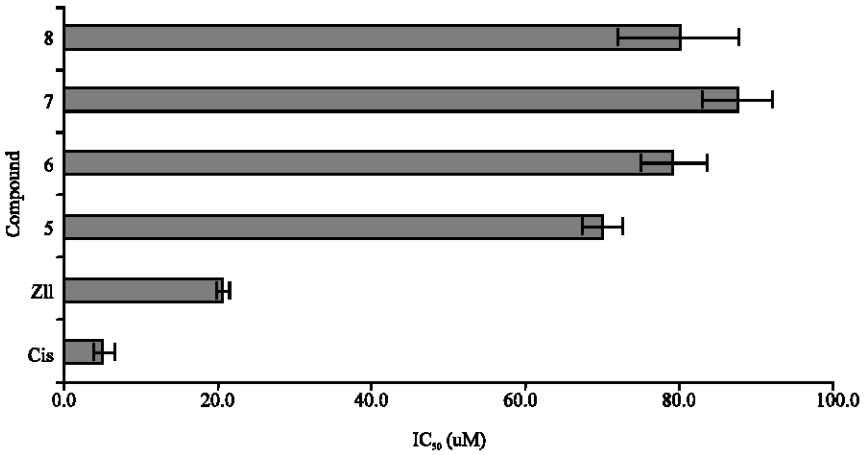

Fig. 2: The concentration-response curve of human cervical cancer cell (HeLa) derived from MTT cytotoxicity assay performed after 72 h exposures with compound 5, 6, 7, 8 ZER and cisplatin are presented as Means±SD (n = 3)

DISCUSSION

Natural products possess a pedigree to justify quality and appreciation in drug discovery and development (Nielsen, 2002; Bogevik *et al*., 2008). Currently, there is rapid increase in application of natural products in combinatorial chemistry and vice versa (Koehn, 2008). The therapeutic areas of oncology are emerging (Saif, 2008). Natural products or intermediates have served as building blocks or scaffolds in the synthesis of complex natural products, bioactive analogues or designed hybrid molecules (Ortholand and Ganesan, 2004). Finally, structural motifs from the biologically active parent molecule have been identified and have served for design of natural product mimicry, which improves the knowledge and research in this area (Ertl *et al*., 2008).

ZER is a crystalline sesquiterpene derived from the wild ginger, *Z. zerumbet*. This bioactive component has its unique structure, with a cross-conjugated ketone in an 11-membered ring, as well as remarkable biological activity (Kitayama *et al*., 2003). Antiproliferative activity of *Z. zerumbet* is mainly modulated by the ZER component which is the main cytotoxic compound that constitutes about 37% of the whole *Z. zerumbet* content (Murakami *et al*., 2002). Terpenoids, including mono-, sesqui-, di- and triterpenoids, are biosynthesized by tandem reactions of the phosphorylated isoprene unit consisting of five carbons and are ubiquitously found in the plant kingdom. Some of these dietary terpenoids have anti-carcinogenic activities in a variety of rodent experiments and clinical trial results also demonstrated that their potential of treating cancers without major toxicity (Damodaran and Dev, 1968; Crowell, 1999; Rabi and Gupta, 2008). The further understanding of their biological and physiological mechanisms may lead to the identification of more effective compounds in this category for the prevention and treatment of targeted cancer types.

Studies demonstrated that ZER inhibited the proliferation of human colonic adenocarcinoma cell lines in a dose-dependent manner, while the growth of normal human dermal and colon fibroblasts was less affected. Intriguingly, a-humulene (HUM), a structural analogue lacking only the carbonyl group in ZER, was virtually inactive in all experiments conducted, indicating that the a; b-unsaturated carbonyl group in ZER may play some pivotal roles in interactions with unidentified target molecule(s) (Murakami *et al*., 2002). Thus, second-generation chemicals may be synthesized to mimic naturally occurring compounds, ZER but with greater specificity and less toxicity.

The MTT tetrazolium salt colorimetric assay previously described by Mosmann (1983); to measure cytotoxicity and cell proliferation. The level of MTT cleavage by viable cells of various origins was found to be directly proportional to the number of cells (Gerlier and Thomasset, 1986).

The synthetic analogues of ZER screened in this study, were n-Butylbenzene, 1, 1'-(4-Chlorobutylidene) bis(4-fluorobenzene), alpha,alpha-Diphenyl-gamma-butyrolactone and (1,4'-Bipiperidine)-4'-carboxaminde. The synthetic analogues were shown in the result, to have cytotoxic effect towards cervical cancer cells. Yet, these analogues were not as effective as ZER and Cisplatin, if compare to their MTT findings established previously in the laboratory. Since IC_{50} of the analogues were high, we suggest that it may be used for anti-proliferative studies in order to investigate it preventive potential in cancer development. Nevertheless, the screening stage of this study concluded that ZER remains the most active cytotoxic natural compound on cervical cancer cells. A future chemical synthesis is required to produce more analogues, however, this approach must be followed by preserving the most functions biological active groups in ZER.

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