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3, 4, 10-Trimethoxystilbene Suppresses Human Hepatocellular Carcinoma Cell Proliferation

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Introduction

In Malaysia, the number of people that are newly diagnosed with cancer per year was 37400 cases with incidence of 143.6 in every 100,000 people were recorded.[1] At the same time, people with age of 75 and below had 15% risk of getting cancer and led to almost 21,700 deaths per year. Therefore, many approaches are needed to reduce the occurence of cancer and following deaths mainly via the primary and secondary prevention strategies.[2] The primary prevention strategy is to remove causative agents and modify lifestyle that could lead to the risks of cancer such as smoking cessation, healthy diets and screening tests for detection of precancerous.[3] Cancer chemoprevention which is the secondary prevention, involves the intervention of natural and/or synthetic agents to inhibit the development or spread of malignant tumour through various pathways.[3] One of which is the induction of apoptosis.

However, current cancer chemotherapy agents are cytotoxic, they do not only kill cancer cells but also normal cells that cause adverse effects to patients such as severe vomiting, nausea, stomatitis, alopecia, meory loss and other cognitive changes.[4] Furthermore, drugs of many classes of antineoplastic agents such as anthracyclines, most alkylating agents, platinum-coordiantion complexes, epipodophyllotoxins and camptothecins are capable to induce a high level of oxidative stress in biological systems during apoptosis.[5] Hence the new therapeutic agent that may have less side effects or able to reduce the severity of the adverse effects by the current drug's when used in combination.[6]

Several plant-derived compounds such as vincristine, etoposide, paclitaxel and docetaxel are currently successfully employed in cancer treatment. Resveratrol, a stilbene found abundantly in grapes and red wine, has also been investigated for its potential as a chemopreventive agent. However, resveratrol is associated with low availability and rapid clearance from the circulation. Besides that, it is not stable upon exposure to light and oxygen in the environment.[7] Previuos study has shown that the substitution of hydroxyl groups of resveratrol to methoxy groups can potentiate resveratrol's cytotoxic activity.[8] There are many researches done on new paramagnetic resveratrol analogues that were developed as new antioxidants, more effective than resveratrol itself.[9] Furthermore, several new stilbene derivatives, for instance piceatannol, piceatannol-3'-0-B-D-glucopyranoside (PG) and 3,5,4'-trimethoxystilbene have been synthesized to improve the antioxidant properties of resveratrol.[10]

3 4, 10-Trimethoxystilbene (S2) is a stilbene analogue that has been synthesized through Heck and Wittig reactions in our laboratory with the possibility of reducing the progression of hepatocellular carcinoma and protect normal cells against free radical produced by the current chemotherapy (i.e. doxorubicin) (Figure 1). Therefore, this novel synthetic stilbene might be one of the prevention to cardiotoxicity of doxorubicin that may contribute to the pharmaceutical and nutraceutical industries to enhance quality of life.

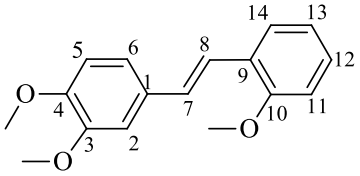

Figure 1 Structure of 3, 4, 10-trimethoxystilbene (S2).[11]

Methods

Antiproliferative activity

Human hepatocytes, Chang (ATCC CCL-13) and human hepatocellular carcinoma, HepG2 (ATCC HB-8065) cell lines were purchased from the American Type Culture Collection (ATCC), USA. Cells were cultured in minimum essential medium Eagle (MEM) supplemented with 10% (v/ v) foetal bovine serum, 50 IU/ mL penicillin and 50 µg/ mL streptomycin. Both cell lines were maintained at 37°C in a 5% CO2 atmosphere with 95% humidity. Briefly, 100 µL of 2×10^4 cells were pipetted on 96-well plates. The cells were incubated for 24 hours. The growth medium was removed and replaced with 200 µL of various concentrations (0.01-1000 µg/ml) of S2 and doxorubicin (drug reference). The 96-well plates were incubated for 48 hours. Later, 20 µl of MTT solution was added to each well and incubated for another 4 hours at 37°C and 5% CO_2. After 4 hours, MTT-containing media was aspirated out and 100 µl DMSO was added to each well. Plate was then shaken gently and read at 520 nm using a microplate reader (Tecan Infinite M200, Switzerland). Percentage of cell viability was calculated using the equation shown below.

$$\text{Cell Viability (\%)} = 100 - \left[\frac{A_{control} - A_{sample}}{A_{control}} \right] \times 100\%$$

Where :

$A_{control}$ = Absorbance of the control (cells)

A_{sample} = Absorbance in the presence of sample (treatment compound)

Then, the graph for percentage of cell viability versus log concentration (µg/ml) was plotted to determine the IC_{50} values for S2 and doxorubicin.

Cytoprotective activity

Chang liver cells were used to determine the cytoprotective effect using MTT assay. After seeding the cells in 96-well plates and incubated for 24 hours, S2 (135 µg/ml) was added and incubated for another one hour. Then, 100 µM of hydrogen peroxide was added to the wells to induce oxidative stress and incubated for 48 hours. Then, 20 µL of MTT solution was added to each well and the plates were incubated for another 4 hours at 37°C and 5% CO_2. DMSO was then put in to solubilize MTT tetrazolium crystal. Finally, the absorbance was measured in each well at 520 nm in a microplate reader. Percentage of viability was calculated.

Acute oral toxicity

Female ICR mice (8 weeks old; 25 – 30 g) were used in this experiment and were supplied by Laboratory of Animal Facility and Management (LAFAM), UiTM, Puncak Alam. They were kept under standard environmental conditions with a 12:12 h light/dark cycle, temperature 22oC (± 3oC) and 50% - 60% relative humidity. Animals were provided with commercial food pellets and water ad libitum. Animals were acclimatized for at least five days prior to commencement of the experiment. Approval from the Ethical Committee on the use and care of animals has been obtained from UiTM (reference number: 10/2012).

The study was conducted as per OECD Guidelines 423. Female ICR mice were randomly divided into two groups of six animals in each. Control group were orally administered with distilled water while treated group received single dose of 2000 mg/kg b. w. of S2 using metal oropharyngeal cannula. The animals were observed closely during the first 30 min, periodically during the first 24 hours and daily for a total of 14 days after dosing for mortality and any sign of toxicity.

Results & Discussion

Antiproliferative activity

MTT assay was performed to determine the antiproliferative activity of S2 and doxorubicin on Chang and HepG2 cells. Based on the results shown in table 1, S2 demonstrated an IC_{50} values of 19.09 ± 3.12 µg/ml and 135.16 ± 12.70 µg/ml against HepG2 and Chang cells, respectively. IC50 values for doxorubicin against HepG2 and Chang cells were 3.00 ± 0.81 µg/ml and 1.70 ± 0.70 µg/ml, respectively. S2 was potent in suppressing the growth of HepG2 cells and had low cytotoxicity on Chang cells. Previous study done on a cis-methoxylated stilbene, (Z)-3,4,40-trimethoxystilbene demonstrated the ability of the compound to induce apoptosis in HepG2 cells. They suggested that the efficacy may be due to the presence of the methoxy groups.[12] Wang et al. (2014) also reported that the substitution of methoxy group exhibits greater cytotoxicity activivies of the resveratodehydes (resveratrol analogues) against MDA-MB-435 and HCT-116 cells compared to resveratrol.[13]

Table 1 The half maximal inhibitory (IC_{50}) values of 3,4,10-trimethoxystilbene (S2) and doxorubicin against HepG2 and Chang cell lines after 48 hours of incubation. Data were expressed as mean ± SD (n=3).

Cell lines	IC_{50} value (µg/ml)	
	S2	Doxorubicin
HepG2	19.09 ± 3.12	3.00 ± 0.81
Chang	135.16 ± 12.70	1.70 ± 0.70

The cytotoxicity selectivity index (SI) of S2 and doxorubicin were calculated using the equation shown below:

$$\text{Selectivity Index (SI)} = \frac{IC_{50} \text{ of Chang}}{IC_{50} \text{ of HepG2}}$$

S2 showed an SI value of 7.08 whilst doxorubicin had an SI value of 0.57 (Table 2). Cytotoxicity SI demonstrates the differential activity of a pure compound against the normal cells to cancerous cells, the greater the SI value is, the more selective it is.[14] SI of more than 2 indicates the selectiveness of a particular compound towards cancerous cells than normal cells in inhibiting cell growth.[15] S2 was more selective than doxorubicin and demonstrated the inability of doxorubicin to target tumour cells and tissues selectively. Many chemotherapeutic agents target both healthy, normal cells as well as cancer cells. The drugs will attack all cells, particularly deleterious to any rapidly proliferating cells such as hair, intestinal epithelial cells, and bone marrow.[16] The most cytotoxic agents are usually the most effective but triggers severe side effects. Doxorubicin, an anthracycline has been used in life-saving chemotherapy but has caused many adverse effects such as, nausea, fatigue, constipation including cardiovascular toxicity.[17]

Table 2 Cytotoxicity Selectivity Index (SI) for 3,4,10-trimethoxystilbene and doxorubicin.

Compounds	Cytotoxicity Selectivity Index (SI)
3,4,10-trimethoxystilbene	7.08
Doxorubicin	0.57

Cytoprotective activity

Based on the results shown in table 3, H_2O_2 markedly decreases the viability of Chang cells. Hydrogen peroxide (100 µM) caused 87.6 ± 0.3 % cell death after 48 hours of exposure. However, pretreatment of S2 significantly inhibits cell injury induced by H_2O_2. S2 ony had 12.1 ± 0.2 % cell death after 48 hours. Our results indicate that while H_2O_2 can cause Chang cell death, S2 pretreatment effectively protects Chang cells from H_2O_2-induced damage.

H_2O_2 has often been used in the oxidative stress injury model with hepatocytes as well as other cell types.[18] Oxidative stress caused by ROS is responsible for a wide variety of cellular damage and is the most validated mechanism of secondary injury.[19] Following oxidative stress, the overproduction of ROS and subsequent tly the depletion of antioxidants resulted in the total breakdown of the endogenous antioxidant defense mechanisms,culminating in failure to protect cells from oxidative damage. In this study, S2 was able to protect the cells either by scavenging the suoeroxide anion radical produced by H_2O_2 or regulating the endogenous antioxidant enzymes.

Table 3 Protective effect of 3,4,10-trimethoxystilbene (S2)against hydrogen peroxide-induced Chang cells. Data were expressed as mean ± SD (n=3).

Treatment	% cell death
Control (+ 100 µM H_2O_2)	87.6 ± 0.3
135 µg/ml S2 (+ 100 µM H_2O_2)	12.1 ± 0.2

Acute oral toxicity

Control and treated mice did not show any changes in behaviour pattern or physical appearance after 24 hours of single oral administration of S2 (Table 4). The times at which the signs of toxicity are present and absent are important to be observed, especially where there is probability of toxic sign to be delayed.[20] No mortality was also recorded. Therefore, further observation was carried out for the next 13 days. No behavioural and physical changes were detected and mortality was recorded after 14 days of the single exposure of S2 (Table 4). Therefore, from this result, S2 is a non-toxic compound.

Table 4 Effects of single dose of 3,4,10-trimethoxystilbene (p.o.) in female mice after 24 hours and 14 days.

Observation	24 hours			14 days		
Dosage (g/kg)	Death/ Total	Mortality latency (h)	Symptoms of toxicity	Death/ Total	Mortality latency (h)	Symptoms of toxicity
0	0/6	-	none	0/6	-	none
2	0/6	-	none	0/6	-	none

Conclusion

As a conclusion, S2 is a safe compound that has very potent antiproliferative activity against hepatocellular carcinoma with cytoprotective effect against hydrogen peroxide-induced oxidative stress. Further study is warranted to determine its molecular mechanism for future development.

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References

1. International International Agency for Research on Cancer. Biennial Report 2012-2013.
2. Kelloff GJ, Crowell JA, Steele VE, Lubet RA, Malone WA, Boone CW, Kopelovich L, Hawk ET, Lieberman R, Lawrence JA, Ali I, Viner JL, Sigman CC Progress in cancer chemoprevention: development of diet-derived chemopreventive agents. Journal of Nutrition. 2000; 130(2S Suppl):467S-471S.
3. Pezzuto JM. Grapes and Human Health: A Perspective. Journal of Agriculture & Food Chemistry. 2008, 56(16), pp 6777–6784. DOI: 10.1021/jf800898
4. Baccarani, M, Saglio, G, Goldman, J, Hochhaus, A, Simonsson, B, Appelbaum, F, Apperley, J, Cervantes, F, Cortes, J, Deininger, M, Gratwohl, A, Guilhot, F, Horowitz, M, Hughes, T, Kantarjian, H, Larson, R, Niederwieser, D, Silver, R, Hehlmann, R. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. Blood. 2006, 108(6) 1809-1820;
5. Conklin KA. Dietary antioxidants during cancer chemotherapy: impact on chemotherapeutic effectiveness and development of side effects. Nutrition and Cancer. 2000, 37(1) 1-18.
6. Conklin KA. (2004). Chemotherapy-associated oxidative stress: impact on chemotherapeutic effectiveness. Integrative cancer therapies. 2004, 3(4), 294-300.
7. Fan GJ, Liu XD, Qian YP, Shang YJ, Li XZ, Dai F, Zhou B. 4, 4′-Dihydroxy-trans-stilbene, a resveratrol analogue, exhibited enhanced antioxidant activity and cytotoxicity. Bioorganic & medicinal chemistry. 2009, 17(6), 2360-2365.
8. Fulda S, Gorman AM, Hori O, Samali A. Cellular stress responses: cell survival and cell death. International Journal of Cell Biology, 2010. doi.org/10.1155/2010/214074
9. Kálai T, Kuppusamy ML, Balog M, Selvendiran K, Rivera BK, Kuppusamy P, Hideg K. Synthesis of N-substituted 3, 5-bis (arylidene)-4-piperidones with high antitumor and antioxidant activity. Journal of Medicinal Chemistry. 2011, 54(15), 5414-5421.
10. Frombaum M, Therond P, Djelidi R, Beaudeux JL, Bonnefont-Rousselot D, Borderie D. Piceatannol is more effective than resveratrol in restoring endothelial cell dimethylarginine dimethylaminohydrolase expression and activity after high-glucose oxidative stress. Free radical research. 2011, 45(3), 293-302.
11. Bunyamin I. Synthesis of natural stilbenoid dimers and unnatural analogues. Universiti Teknologi MARA. 2008
12. Hasiah A, Ghazali AR, Weber J, Velu S, Thomas N, Hussain SI. Cytotoxic and antioxidant effects of methoxylated stilbene analogues on HepG2 hepatoma and Chang liver cells. Human & Experimental Toxicology. 2010, 30, 138-144.
13. Wang J, Cox DG, Ding W, Huang G, Lin Y, Li CThree New Resveratrol Derivatives from the Mangrove Endophytic Fungus Alternaria sp. Marine Drugs. 2014, 12, 2840-2850. doi: 10.3390/md12052840
14. Badisa RB, Darling-Reed SF, Joseph P, Cooperwood JS, Latinwo LM, Goodman CB. Selective Cytotoxic Activities of Two Novel Synthetic Drugs on Human Breast Carcinoma MCF-7 Cells. Anticancer Research. 2009, 29(8), 2993–2996
15. Suffness, M; Pezzuto, JM. Assays related to cancer drug discovery. In Methods in Plant Biochemistry: Assays for Bioactivity; Hostettmann, K, Ed.; Academic Press: London, UK, 1990; 6, 71–133.
16. Feng SS, and Chien S. Chemotherapeutic engineering: Application and further development of chemical engineering principles for chemotherapy of cancer and other diseases. Chemical Engineering Science. 2003, 58(18):4087—4114.
17. de Angelis A, Urbanek K, Cappetta D, Piegari E, Ciuffreda, LP, Rivellino A, Russo R, Esposito G, Rossi F, Berrino L. Doxorubicin cardiotoxicity and target cells: a broader perspective. Cardio-Oncology. 2016, 2:2.
18. Jiang J, Yu S, Jiang ZC, Liang C, Yu W, Li J, Du X, Wang H, Gao X, Wang X. N-Acetyl-Serotonin Protects HepG2 Cells from Oxidative Stress Injury Induced by Hydrogen Peroxide. Oxidative Medicine and Cellular Longevity. 2014 Volume 2014, Article ID 310504, 15 pages http://dx.doi.org/10.1155/2014/310504
19. Wang CC, Fang KM, Yang CS,.Tzeng SF,Reactive oxygen species-induced cell death of rat primary astrocytes through mitochondria-mediated mechanism. Journal of Cellular Biochemistry. 2009, 107(5),.933–943.
20. OECD 423 (2001) OECD Guideline For Testing of Chemicals Acute Oral Toxicity – Acute Toxic Class Method Adopted: 17th December 2001.