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Research paper

Esterification and in vitro test of derivatives eugenol from clove (syzygies aromatic) as growth inhibitor of human colorectal cancer cell line HCT-116

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Abstract

Colorectal cancer occupies the third position in the world (more than 940,000 cases). Natural compounds are widely used as anti-cancer such as salicylic acid and eugenol isolated from clove (Syzygium aromaticum). The study of the qualitative relationship of eugenol-derived activity structure in the benzene position associated with modification shows that the compound is active as an invitro cancer therapy compound. This research is carried out chemical synthesis by condensation between eugenol and salicylic acid that produce new compound and conducted invitro biological activity test on colorectal cancer cell HCT-116. This research aims to obtaine a new compound derived eugenol that can inhibit the colorectal cancer cell HCT-116. The result of synthesis showed that condensation between eugenol isolates with salicylic acid (ESA) have yield 75% of white needle crystal with melting point 60-65oC. The result of in-vivo test showed that compound ESA have IC50 of 20.98 ± 2.1 µg / ml and the gossypol standard of natural compounds have IC50 14.22 ± 1.1 µg / ml. The results of this test indicate that the results of this new compound need to be developed further so have a high potential as a colorectal anticancer compound.

Keywords: *Esterification; Eugenol Isolates; Syzygium Aromatic; Anti Colorectal Cancer; HCT-116 Cell Line.*

1. Introduction

CRC is the third most commonly diagnosed cancer in males and the second in females, with 1.65 million new cases and almost 835,000 deaths in 20151. In accordance with the National Comprehensive Cancer Network (NCCN) guidelines, if the treatment of colorectal cancer of lines 1 and 2 is unsuccessful, then it is not possible to succeed, according to the National Comprehensive Cancer Network (NCCN) guidelines. conducted target therapy. Cancer therapy with a target mechanism using natural materials such as taxol, gosypol and other polyphenols2, 3. Natural ingredients that have been studied for cancer therapy include eugenol. Eugenol is a compound found in cloves (Syzygium aromaticum).

The modification of eugenol derivatives by the nitration reaction of the hydroxy group is capable of inhibiting prostate cancer cells 4,5. Eugenol modification can be done by cluster method by considering aromatic groups having the same properties, including esterification with salicylic acid. Salicylic acid is used as colorectal anticancer that can induce apoptosis and reduce the growth of colon cancer cells SW480, HT29 and HCT116 6.

Synthesis of derivatives is more effective than random synthesis which is difficult, expensive and time consuming7. The advantages of this derivative synthesis include a high probability to produce a more active compound of the guide compound 8,9. This study conducted synthesis with esterification Mitsunobu reaction with catalyst diethylazodicarboxylate (DEAD) and triphenylphosphine (TPP) which condensation between two active compounds eugenol with salicylic acid 10. The results of the synthesis compound were tested for activity with MTT assay. It is expected that the synthesized compound has the potential as an anti-colorectal cancer compound.

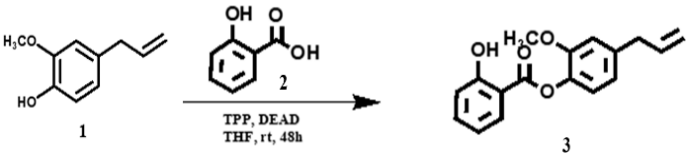


Fig. 1: Esterification of Eugenol (1) With Salicylic Acid (2).

2. Materials and methods

2.1. Esterification

Eugenol (1.0 equiv) was added to a solution of salicylic acids (1.1 equiv) and triphenylphosphine reagent (TPP) (1.1 equiv) in THF at 0 °C. The resulting suspension was added with diethylazodicarboxylate (DEAD) (1.1 equiv) and the reaction mixture was continued stirring at temperature room for 48 hours up to completion of the reaction. The solvent was evaporated and the residue dissolved in ether, the TPP oxide precipitated and was filtered and then the filtrate evaporated with evaporator. The product was purified by column chromatography on silica gel with elution gradient hexane–ethyl acetate, 20: 1 until 10: 1 to get pure products[10].

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2.2. In-vitro test

Cell culture is taken from the stock stored in the liquid tank placed in the locator at -196 ° C. Cell culture was thawed in water ± 37.7 ° C, then the cell was grown in several (2-3) small tissue culture flasks and incubated in an incubator at 37 ° C with a flow of 5% CO_2 and 95% O_2. After 24 hours, the media was replaced and the cells are grown until confluent and the amount is enough for the assay. After a sufficient or confluent cell count (± 70%), the medium is replaced with a new RPMI 1640 medium of 5 ml. Cells were drawn as much as 3 x 104 cells / 100 µl of the media by counting through the haemocytometer chambers[11].

2.3. Cytotoxicity test

The 100 µl suspension cell HCT-116 with a density of 3 x 104 cells / 100 µl of media distributed into wells at 96-well plate and incubated for 24 hours. After incubation, into the well, 100 µl of the test solution was added at various concentration series. As a positive control 100 µl of Gossypol and cisplatin were added to various series of concentrations into wells containing 100 µl of cell suspense. As a cell control, 100 µl of culture medium was added to the well containing 100 µl of cell suspense and 100 µL of DMSO was added to the solvent in a well containing 100 µl of suspension of cells with suitable delusions in the delusions of the concentration of the test solution, then incubated for 24 hours in incubator with 5% CO_2 and 95% O_2 flow. At the end of incubation, the culture medium is discarded and 10 µl of MTT solution is added (5 mg / mL PBS), then the cell is incubated for 3-4 hours. The MTT reaction was discontinued with the addition of an SDS stopper reagent (100 µl). The microplate contains a suspended cell suspension ± 5 minutes then wrapped with aluminum foil and incubated for 1 night at room temperature. Live cells react with MTT to form a purple color. Test results are read with ELISA reader at 595 nm wavelength[12].

Inhibiton rate (%) = 1 – (absorbance of treatment group/absorbance of control group) x 100%.

The 50% inhibitory concentrations (IC_{50}) of the 48 hours are calculated with Bliss assay. Data interpretation Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely, a higher absorbance rate indicates an increase in cell proliferation.

3. Results and discussion

The Mitsunobu reaction, converts an alcohol into a variety of other functional groups including esters, and this method could generate esters in excellent yield (90%) via the condensation of a carboxylic acid and alcohol with a mixture of triphenylphosphine (TPP) and diethyl azodicarboxylate (DEAD)[9]. More specifically, the Mitsunobu reaction is highly stereospecific and selective; therefore, it is appropriate for preparing some products or derivatives with sensitive groups. In the Mitsunobu reaction, the alcohol was usually activated towards nucleophilic attack from the carboxylic acid, and this activation was achieved by the reaction with a phosphine, typically TPP, and a dialkyl azodicarboxylate. In recent years, a number of reports have focused on generating other azo dicarboxylates such as diisopropyl azodicarboxylate, di-2-methoxyethyl azodicarboxylate, azodicarbonyl dimorpholide [13-16].

The Mitsunobu reaction in this study was used to change eugenol with salicylic acid using triphenylphosphine and azodicarboxylate such as diethyl azodicarboxylate (DEAD) or diisopropyl azodiccarboxylic (diad), with alcohols having stereochemical inversion, being EAS compounds. EAS purification results white solid, yield (75%), TCL: Rf = 0.72 (n-hexane–ethyl acetate, 4:1, v/v), (c=2, CH3Cl). IR (CCl4, σ cm−1): 3403 (C-OH), 1683 (C=O), 1324 (C-O), 2956 (C-H), 1587 (C=Caroma), 1HNMR (CDCl3, 500 MHz) δ: 7,06 – 7,08 (d, 2H, J= 8,0 Hz, C3, C5) 7,51 – 7,56 (m, 1H, C4,), 8,11 – 8,12 (dd, 1H, J= 8,0 ; 4,0 Hz, C6), 6,84 – 6,85 (d, 2H, J= 8,0 Hz, C3', C5'), 7,02 – 7,04 (d, 1H, J= 8,0 Hz, C6'), 3,40 – 3,42 (d, 1H, J= 6,9 Hz, C7'), 5,91 – 6,05 (m, 1H, C8'), 5,08 – 5,19 (m, 1H, C9'), 3,82 (s, 1H, C10'). 13C NMR (CDCl3, 500 MHz): 111.8, 161,7, 119,3, 136.0, 130.5, 168.5, 136.7, 151.5, 113.6, 139.5, 122.4, 40.0, 137.0, 116.2, 65.0, MS (ESI+) m/z: 284,1 m/z. The results of the analysis of GCMS, FTIR, 1H-NMR spectrometer and 13C-NMR, the eugenol derivative code ESA is a 4'-allyl-2'-methoxyphenyl 2-hydroxybenzoate compound as Figure 2.

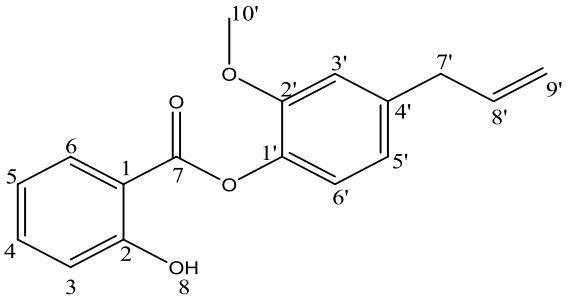


Fig. 2: Structure of 4'-Allyl-2'-Methoxyphenyl 2-Hydroxybenzoate (ESA).

Mitsunobu's reaction in drug synthesis produces simple molecules containing ethyl aryl. The Mitsunobu reaction is usually used to combine the functional groups of secondary carboxylic and alcoholic acids with configuration inversion. In this research, it has been successful in combining the carboxylic group of salicylic acid with alcohol which is bound to the benzene ring of eugenol using the reaction of Mitsunobu to produce compounds as shown. Undoubtedly, Mitsunobu's optimized products will be adopted as drug chemist strategies to obtain biologically active compounds that have high activity against a target disease. in this study, new compounds were tested on HT-116 cells as colorectal anti-cancer.

Table 1: MTT Assay of New Compound With HCT-116 Cell Line

Compounds	Inhibition of Compounds with concentration						IC_{50}
	3,125	6,25	12,5	25	50	100	
ESA	38,32	46,34	42,24	45,45	55,97	87,34	20,98
EU	43,49	47,95	44,74	45,45	58,11	83,77	25.06
SA	40,28	41,89	48,66	56,38	67,38	95,00	26.88
EU+SA	38,13	45,09	45,99	48,66	57,93	83,60	39,57
Cispl	39,04	42,78	37,25	41,18	52,41	73,44	10.47
Gossy	38,50	42,25	39,04	43,31	52,94	77,89	14.22

ESA: 4'-allyl-2'-methoxyphenyl 2-hydroxybenzoate; EU: Eugenol; SA: Salicylic Acid; Cispl: Cisplatin; Gossy: Gossypol

The result of cytotoxicity test of the compound of eugenol derivative synthesis as Table 1, was further processed by linear regression analysis method, by making a graph showing the relationship between% inhibition and log of sample concentration (µg / mL). The graph shows the data of linear equations and correlation (r). The value of IC50 can be determined by passing the value y = 50 on the graph to get the value of x. IC50 value is an antilog of x value. Graph of the relationship between% inhibition with log of maximum concentration (µg / mL) of eugenol derivatives Figure 3.

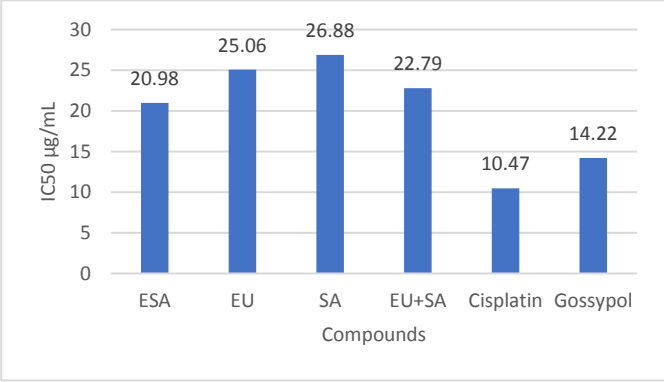


Fig. 3: Cytotoxicity of ESA And Standard (ESA: 4'-Allyl-2'-Methoxyphenyl 2-Hydroxybenzoate; EU: Eugenol; SA: Salicylic Acid).

In vivo test of ESA showed that the IC_{50} 20.98 ± 2.1 µg / ml the gossypol standard of 14.22 ± 1.1 µg / ml. The results of this test indicate that this new compound need to be developed further to be potential as a colorectal anticancer compound. Thus, it is imperative to search for new alternatives to colon cancer prevention agents.
This study shows that the new compound produced by eugenol and salicylic acid is a phenolic compound, it is hoped that this compound has activities such as other natural compounds including gossypol piperine or cinnamaldehyde is an anti-inflammatory antioxidant widely consumed, that compounds has strong anticancer activity against human cancer cell lines in vitro assay. Gossypol is used as an agent for BCL2 inhibition of apoptosis from the mechanism of cancer. This is new Bcl-2 functional interface Bcl-2 in colorectal carcinoma cells. A similar study was carried out on piperine induced mitochondrial dysfunction in HCT-116 human colon cancer triggers, responsible for caspase-dependent apoptosis pathways, and elevated ratio of Bcl-2 is likely to be involved in this effect[16]. The inhibitory effect of ESA may be a potentil chemotherapeutics or a chemopreventive agent based on its ability to induce apoptotic in cancer cell with relatively low toxicity.

4. Conclusion

In conclusion the result of the present study indicated that condensation of eugenol and salicylic acid can combined with esterification with Mitsunobu reaction. This derivatives Eugenol modification can be done by cluster method was considering aromatic groups having the same properties as contribute and potent effects as anticancer activities to colorectal cancer cell line HCT-116. This new compound ESA need to be developed further to increase activity as a new colorectal anti-cancer.

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