Synthesis of a Potential Anti-Cancer Inhibitor Compound: Methyl 2-Cinnamamido-3-Hydroxy Propanoate

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Abstract

Cinnamic acid derivative compound was investigated forthe anti-cancer inhibitory activity. To be able to obtain compounds that have bioactivity as above, it is needed to study quantitative structure-activity relationship (QSAR) which is the process by which the chemical structure is quantitatively correlated with biological activity/chemical reactivity. Chemical methods used in synthesizing the chemical of methyl trans-cinnamate derivatives are tailored to match their targeted bioactivities. Here, weinvestigated the anti-cancer inhibitor compound with the method amidation of cinnamic acid derivative compounds. In this reaction we use two steps to get the target product.Firstly, we hydrolize of methyl trans-cinnamate to cinnamic acid. That reaction has a yield 85.5 % and secondly, we amidate of cinnamic acid to metil 2-cinnamamido-3-hydroxy propanoatehas a yield of 31.5%. The compound of metil 2-cinnamamido-3-hydroxy propanoate showed a BSLT assay with Lc_{50} *= 91.20 µg/mL.*

Keywords : *methyl trans-cinnamate,cinnamic acid, isolation, synthesis, bioactivity, amidation, anti-cancer*

ISBN : 978-979-18458-5-4

INTRODUCTION

Cinnamic acids are abundant in various natural resources. Cinnamic acid and its natural analogues are unique as anticancer agents.Cinnamic acid amide derivatives and related compounds, like cinnamic acid ester, form a class of anticancer agents. It also use as a flavourings [1]. Ekmekcioglu*et al.* investigated the effect of cinnamic acid on cell proliferation and on the differentiation markers alkaline phosphatase, sucrase and aminopeptidase N in human colon adenocarcinoma cells. Cinnamic acid inhibits the DNA synthesis of growing cells [2].Recently, a lot of cinnamido compounds were also synthesized and their anticancer abilities were evaluated. 2-Methyl cinnamide isolated from a fermentation beer of *Streptomyces griceoluteus*, showed significant anti-invasive or anti-metastatic effects [3]. It also 2-methyl cinnamide was pretreatment of maglinant melanoma cells (C8161 and A375 M *in vitro*) caused a dose and time-dependent reversible reduction (IC_{50}= 12.5ìg/mL) of invasion. Similarly, lung colonization was significantly inhibited when tumor cells were pretreated *in vitro* with the same prior to intravenous injection (P<0.05). Here, we focus to synthesize of themethyl 2-cinnamamido-3-hydroxy propanoatecompoundhas potential as ananti-cancer agent.

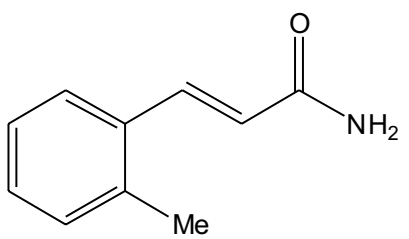

Figure 1. 2-Methyl Cinnamide

MATERIALS AND METHOD

Materials

All solvents were dried and distilled according to standard procedure. Analytical thin layer chromatography (TLC) was performed on Merck silica gel plates (Kiesel gel $60F_{254}$ 0.25 mm) and preparative TLC was carried out on Merck silica gel plates (Kiesel gel $60F_{254}$ 0.5 mm). Silica gel column chromatography was carried out on Daisogel IR-60. Cinnamicacid isolated from hydrolisis of methyl cinnamate was used as starting material for the synthesis of metil 2-cinnamamido-3-hydroxy propanoate. L-serine methyl ester was used as reagent. 1,3Dicyclohexylcarbodiimide and 4-Dimethylamino pyridine was used as catalyst and pyridine was used as solvent.

Instruments

^{1}H and ^{13}C NMR spectra were recorded on JEOL 1NM–LA for 500 MHz in deuterio chloroform unless otherwise specified. Chemical shifts (ä) are reported in parts per million (ppm) downfield from tetramethylsilane (ä 0.00) or $CDCl_3$ (ä 7.26) for ^{1}H NMR and ä 77.0 for ^{13}C NMR as internal standard, and coupling constant are reported in Hertz.

METHODS

Synthesis of a Potential Anti-Cancer Inhibitor Compound:
Methyl 2-cinnamamido-3-hydroxy propanoate

To a 100 mL round-bottomed flask equipped were charged cinnamic acid (0.005 mol), L-serin methyl ester (0.01mmol) in pyridine 20 mL was added 1,3 dicyclohexylcarbodiimide (0.011 mol) and 4-dimethylamino pyridine (0.002 mol). The reaction mixture was heated to 60^{o}C for 4h. After completion (monitored by TLC), the reaction mixture was cooled and quenched with water (50 mL) and extracted with ethyl acetate (3x50 mL). The organic phase was washed with water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to afford the crude product, which was purified by column chromatography over silica gel using hexane and ethyl acetate as eluent.

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Hatching the brine shrimp

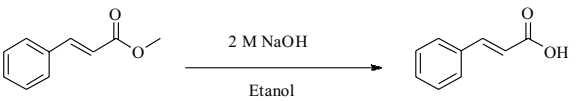
Brine shrimp eggs (*Artemiasalina)* were hatched in artificial sea water prepared from commercial sea salt.The hatching process was done under light regime condition. After 48 hours incubation at room temperature (25-29°C), nauplii (larvae) were collected by pipette from the lighted side whereas their shells were left in another side.

Bioassay

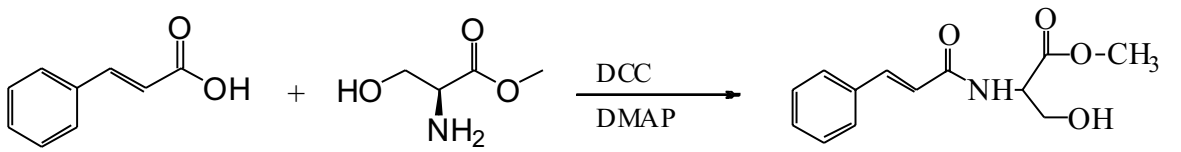
The procedure for BSLT was modified from the assay described by Solis et al. (1993) [4]. Ten milligrams of the sample were made up to 2 mg/ml in artificial sea water except for water insoluble compounds which were dissolved in DMSO. 50 ìl prior to adding sea water.Serial dilutions were made in the wells of 96-well microplatesin triplicate in 120 ìl sea water. Control wells with DMSO were included in each experiment. A suspension of nauplii containing 10 organisms (100 ìl) was added to each well. The plates were covered and incubated at room temperature (25-29°C) for 24 hours. Plates were then examined under the binocular steromicroscope and the numbers of dead (non-motile) nauplii in each well were counted. One hundred microlitrs of methanol were then added to each well to immobilize the nauplii and after 15 minutes the total numbers of brine shrimp in each well were counted. Analysis of the data was performed by probit analysis on a Finney computer program to determine the lethal concentration to half of the test organisms (LC_{50}).

RESULT AND DISCUSSION

Methyl 2-cinnamamido-3-hydroxy propanoate synthesized from simple and readily available chemicals usingcinnamic acid .Cinnamic acid obtained from hydrolysis of methyl *trans*cinnamate (Scheme 1), in this reaction was produce a product of cinnamic acid in 85 % yield, respectively. Esterification of cinnamic acid and L-serin methyl ester with 1,3dicyclohexylcarbodiimide and 4-dimethylamino resulted methyl 2-cinnamamido-3-hydroxy propanoate in pyridine at 60 ^{o}C for 4 hours (Scheme 2).The esterification reaction was produce methyl 2-cinnamamido-3-hydroxy propanoatein 31 % yield.



Scheme1. Hydrolysis of methyl *trans*cinnamate to cinnamic acid



Scheme 2. Synthesis of Methyl 2-cinnamamido-3-hydroxy propanoate

The ^{1}H NMR and ^{13}C NMR data are presented in table 1 below. In the ^{1}H NMR spectra data of methyl 2-cinnamamido-3-hydroxy propanoate, one methoxy signaläH 3.71 (3H, s) and one protonsäH 3.93(1H, t) were detected along with –NH groups, two protons äH 4.02, 4.04 (2H,d), one proton was detected along olefinäH 6.54 (1H, d), olefin proton äH 7.54 (1H, d) and aromatic proton äH 7.41 (2H, d), äH 7.27 (2H, t), äH 7.28 (1H, t). In the ^{13}C NMR spectra data of methyl 2-cinnamamido-3-hydroxy propanoate, one methoxy signal 52.74, one ester signal 171.33, carbon was bonded to-NH group 55.06, carbon was bonded to –OH group 62.94, one amide signal 166.55, olefin signals 120.16, 141.86, and aromatic signals 134.66, 127.99, 128.85 1nd 129.89.

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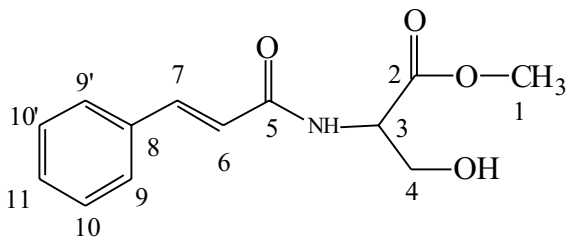

Figure 2. Structure of Methyl 2-cinnamamido-3-hydroxy propanoate

Table 1. The ^{1}H NMR (500 MHz) and ^{13}C NMR Data for Methyl 2-cinnamamido-3-hydroxy propanoate in CDl_3

Position	^{1}H	^{13}C
1	3.71	52.74
2		171.33
3	3.93	55.06
4	4.02, 4.04	62.94
5		166.55
6	6.54	120.16
7	7.54	141.86
8		134.66
9	7.41	127.99
9’	7.41	127.99
10	7.27	128.85
10’	7.27	128.85
11	7.28	129.89

Toxicity tests of methyl 2-cinnamamido-3-hydroxy propanoate conducted with BSLT method. BSLT assay showed that this compound have LC_{50}= 91.20 µg/mL.The valueof thisactivityis oneindicationthat themethyl 2-cinnamamido-3-hydroxy propanoatecompoundhas potential as ananti-cancer agent.

Further research will be conductedof methyl 2-cinnamamido-3-hydroxy propanoatecompound trials against cancer cells.

CONCLUSION

Synthesis amide of cinnamic acid was done because it lesser known attributes towards anti-cancer activity. Herein, we synthesize of methyl 2-cinnamamido-3-hydroxy propanoatefrom cinnamic acid, respectively.The compound of methyl 2-cinnamamido-3-hydroxy propanoateshowed a candidate anti-cancer inhibitor with BSLT (Brine Shrimp Lethality Test) assay is LC_{50} = 91.20 µg/mL.

ACKNOWLEDGMENT

We are gratefully acknowledge the financial support from the Competitive Project of Indonesian Institutes of Sciences (LIPI).

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