SYNTHESIS OF LACTONE COMPOUND AS A CANDIDATE ANTI-CANCERAGENT: 4-PHENYLCHROMAN-2-ONE USING ACID CATALYST

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Abstract

Coumarin derivatives compounds have a bioactivity that is beneficial to health. They arehave biological activities such as anti-inflammatory, anti-oxidant, and anti-cancer. One of the activity for coumarin derivative compound such as 4-aryl dihydrocoumarin have been used as treatment of disease and infection in China and Japan. Here, we have performed the synthesis of lactone compounds: 4-phenylchroman-2-one as a candidate anti-cancer agent using p-toluene sulfonic acid as catalyst at temperature of 120 ^{o}C for 4 hours. Elucidation of lactone compound was done using ^{1}H-NMR and ^{13}C-NMR.The compound of 4-phenylchroman-2-one showed a candidate anti-cancer inhibitor with toxicity BSLT (Brine Shrimp Lethality Test) is Lc_{50} = 112.2 µg/mL.

Keywords : *Coumarin, synthesis, 4-phenylchroman-2-one, ^{1}H-NMR and ^{13}C-NMR*

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INTRODUCTION

Coumarin (*2H*-chromen-2-one) is a chemical compound in the benzopyrone chemical class, found in many plants such as *Anthoxanthumodoratum*, *Galiumodoratum*, *Hierochloeodorata, Cinnamomumaromaticum*, etc. Coumarin derivatives have shown a wide range of biological activities. They are approved for few medical uses such as anti-tumor, anti-hypertension, anti-inflammatory, anti-osteoporosis, analgesic. It also used in the treatment of astma [1,2,3].

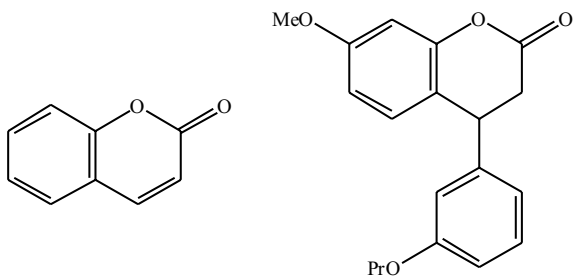

Figure 1. Structure of Coumarin and 7- methoxy-4-aryl coumarin

Coumarin is used in the pharmaceutical industry as a precursor in the synthesis.The pharmacological and therapeutic applications of coumarin derivatives depend upon the pattern substitution. Currently, coumarin is used in the development of new drugs because of their diverse pharmacological properties. Among these properties, their cytotoxic effects were most extensively examined. Creation and development of coumarin derivative of nature-based materials is done by synthesizing the compound of cinnamic acid into compounds that have bioactivity. To be able to obtain compounds that have activity, it is needed to study quantitative structure-activity relationship relationships (QSAR) which is the process by which chemical structure is quantitatively correlated with biological activity/chemical reactivity. Therefore, many synthetic methods for 3,4-dyhydrocoumarin have been reported [4,5,6]. But, commonly of these methods use of large excess of expensive transition metal catalyst such as $Yb(OTf)_5$, Ru(III), $Pd(Oac)_2$, etc [7,8,9].The preparation of a compounddihydrocoumarin have beenwidely applied.Ofsome of theexisting literature, there areseveralways togetdihydrocoumarinderived compounds. One of them isthroughthe use ofLewisacidswithphenolsandacrylonitrile(Pechmanmethod), it could be throughcatalytichydrogenationof coumarins, it also hydrolation of cinnamicacid withacidic media, and theactivation of2-hydroxy benzaldehydewithCH-acid compounds(Knoevenagelmethod) [10,11,12].

Inthispaper, wereport thesynthesis oflactone compounds: 4-Phenylchroman-2-oneusinghydroarylation of cinnamic acid with phenol. This reaction was used *p*-toluensulfonicacid (p-TsOH) as acid catalyst and solvent-free condition.

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 cinnamatewas used as starting material for the synthesis of 4-Phenylchroman-2-one.*p*-TsOH was used as acid catalyst. Phenol was used as reagent.

Instruments

^1H and ^{13}C NMR spectra were recorded on JEOL 5NM–LA for 500 MHzin 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 ^1H NMR and ä 77.0 for ^{13}C NMR as internal standard, and coupling constant are reported in Hertz.

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METHODS

Synthesis ofLactoneCompoundasa Candidate Anti-CancerAgent:
4-Phenylchroman-2-one

To a 100 mL round-bottomed flask equipped were charged phenol (0.008mol), cinnamic acid (0.006mol), and *p*-toluenesulfonic acid (0.007mmol). The reaction mixture was heated to 125 ^{o}C for 3h. 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.

Hatching the brine shrimp

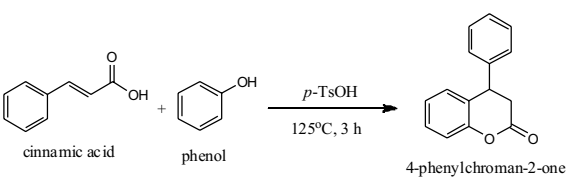
Brine shrimp eggs (*Artemiasalina)* were hatched in artificial sea water prepared from commercial sea salt.The hatching process was done under light regime condition. After 48 hours incubation at room temperature (25-29°C), nauplii (larvae) were collected by pipette from the lighted side whereas their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

Bioassay

The procedure for BSLT was modified from the assay described by Solis et al. (1993) [13]. 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

Treatment cinnamic acid with phenol in the presence of *p*-toluenesulfonic acidin the case of solvent free condition at 125 ^{o}C for 3 hours led to the formation of the corresponding 4-phenylchroman-2-one (Scheme 1). This reaction was reported as an inter-molecular reaction type. Themechanism ofthisreaction, the reaction betweencinnamicacidandphenolsto formphenolicesterandsubsequentlyfollowed byintermolecularFriedelCrafttype cyclizationto form4-phenylchroman-2-one. The advantage of this method is the use inexpensive agent and less demand for the decrease of entropy, so this reaction of both efficiency and selectivity.



Scheme 1. Synthesis of 4-phenylchroman-2-one in the presence of *p*-toluenesulfonic acid

The structure of compound 4-phenylchroman-2-one was deduced from their ^{1}H-NMR and ^{13}C-NMR data. In the ^{1}H NMR spectra data of 4-phenylchroman-2-one exhibited in lactone ring; two protons in position 3 *doublet doublet* at 3.05 – 3.077 (2H, dd), one triplet in position 4 at 4.35 (1H, t), exhibited in aromatic ring; one doubletin position 5 at 6.98 (1H, d, J=7.8 Hz), two tripletsin position 6 and 7 at 7.33(1H, t) 7.35 (1H, t), one doublet in position 8 at 7.17 (1H, d, J=7.8 Hz) exhibited in phenyl ring; two doublets in position 10, 10' at

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7.29 (2H, d, J=7.8 Hz), three triplets in position 11, 11', 12 at 7.15, 7.15(2H, t, J=7.8 Hz), 7.08 (1H, t). In the ^{13}C-NMR spectra data of 4-phenylchroman-2-one exhibited in lactone ring; C-lactone at 167.83, CH_2 at 37.18, CH at 40.83, C in position 5' at 129.31, C in position 5" at 151.87, exhibited in aromatic ring; CH in position 5 at127.74, CH in position 6 at 124.83, CH in position 7at 128.51, CH in position 8 at117.30, exhibited in phenyl ring; C in position 9 at140.42, CH in position 10, 10' at127.84, CH in position 11, 11' at129.31and CH in position 12 at125.93. The ^{1}H NMR and ^{13}C NMR data are presented in table 1.

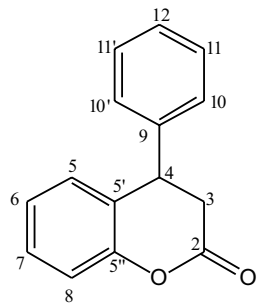

Figure 2. Structure of 4-phenylchroman-2-one

Table 1. The ^{1}H NMR (500 MHz) and ^{13}C NMR Data for 4-phenylchroman-2-one in $CDCl_3$

Position	^{1}H	^{13}C
1		
2		167.83
3	3.05, 3.07 (2H, dd)	37.18
4		40.835
5	4.35(1H, t)	127.74
5'	6.98 (1H, d)	129.31
5"		151.87
6		124.83
7	7.33 (1H, t)	128.51
8	7.35 (1H, t)	117.30
9	7.17 (1H, d)	140.42
10/10'		127.84
11/11'	7.29 (2H, d)	129.31
12	7.15 (2H, t)	125.93
	7.08 (1H, t)	

Brine shrimp lethality activity of the compound4-phenylchroman-2-one was LC_{50}= 112.2 µg/mL, respectively. Compounds resulting in LC_{50} values of less than 250 ìg/mL were considered significantly active and had the potential for further investigation (Rieser et al., 1996) [14].

Conclusion

The selective one-pot reaction synthesis of4-phenylchroman-2-one is obtained from cinnamic acid by *p*-toluenesulfonic acid, respectively. The advantage of this method is the use inexpensive agent and wide versatility of this reaction to be used for dihydrocoumarin derivative natural product synthesis.The compound of 4-phenylchroman-2-one showed a candidate anti-cancer inhibitor with BSLT (Brine Shrimp Lethality Test) assay is LC_{50} = 112.2 µg/mL.

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REFFERENCES

D.M.X. Donnelly, G.M. Boland, in: J.B. Harborne (Ed.), *The flavonoids*: advances in research since 1986, Chapman and Hall, London, **1993**.

H. Krawczyk, Q. Albrecht, J. Wojciechowski, W.M. Wolf, *Tetrahedron63*, **2007**, 12583.

Farinola, Nicholas; Piller, Neil, "Pharmacogenomics: Its Role in Re-establishing Coumarin as Treatment for Lymphedema". *Lymphatic Research and Biology3*(2): 81–86, **2005**.

C.E. Rodrigues-Santos, A. Echevarria, *Tetrahedron Lett*. *48*.**2007**, 4505.

I. Yavari, M. Sabbaghan, M. Ghazanfarpour-Darjani, Z. Hossaini, *J. Chem. Res*. (S). **2007**, 392.

K. Li, L.N. Foresee, J.A. Tunge, *J. Org. Chem*. 70 .**2005**, 2881.

E. Fillion, A.M. Dumas, B.A. Kuropatawa, N.R. Malhotra, T.C.Sitler, *J. Org. Chem*. *71* . **2006**, 409.

S.W. Youn, S.J. Pastine, D. Sames, *Org. Lett*. *6*.**2004**, 581.

C. Jia, T. Kitamura, Y. Fujiwara, *Acc. Chem. Res*. *34*. **2001**, 633.

Jagdale, R. Arun and SudalaiArumugam, *Tetrahedron Letters*, *48*. **2007**, 4895-4898.

Jeon. Jae-Ho, Yang.Deok-Mo, Jun. Jong-Gab, *Bull. Korean Chem. Soc*. **2011**, Vol. 32, No.1, 65-70.

Rezayan. Ali Hossein, C. R. Chimie, **2012**, 1-5.

Solis, P. N., Wright, C. W., Anderson, M. M., Gupta, M. P., &Phillipson, J. D.**1993**. A microwell cytotoxicity assay using *Artemiasalina*(brine shrimp). *PlantaMedica, 59*, 250-252.

Rieser, M. J., Gu, Z.-M., Fang, X.-P., Zeng, L., Wood, K. V., & McLaughlin,J. L. (1996). Five novel mono-tetrahydrofuran ring acetogenins from the seeds of *Annonamuricata. Journal of Natural Products*, 59, 100-108.