African Journal of Microbiology Research Vol. 6(34), pp. 6319-6324, 6 September, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.297
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Evaluation of the anti-bacterial and anti-tumour activity of two Chroman -4- one derivatives

Golamreza Dehghan Noudeh[1,2]*, Mohammad Hassan Moshafi[1,2], Ali Dehghan-Noodeh[3] and Mehdi Rezapour [1]

[1]Pharmaceutics Research Centre, Kerman University of Medical Sciences, Kerman, Iran.
[2]Department of pharmaceutics and School of pharmacy, Kerman University of Medical Sciences, Kerman, Iran.
[3]Department of life sciences, Anglia Ruskin University, East Road, Cambridge, CB1 1PT, UK.

Accepted 20 February, 2012

In this study, the antibacterial activities of two synthetic chalcones were investigated against *Ecsherichia coli, Bacillus subtilis*, *Staphylococcus aureus, Pseudomonas aeroginosa, Klebsiella pneumonia* and *Micrococus luteus* by agar dilution method. The potato disc method was used to investigate the antitumor activity of the compounds. This assay is accepted as a primary general screening assay of antineoplastic activity of compounds. Different amount of compounds were utilized including: 1.56, 3.125, 6.25, 12.5, 25 and 50 µg/disc. After incubating for 20 days at 25ºC, induced tumors were counted and compared with controls. The inhibition was expressed as percentages of tumor onset discs versus controls. Minimum inhibitory concentration (MIC) of 3-(2-chlorobenzylidene) chroman -4- one against *K. pneumonia* and *B. Subtilis* was determined as 25 and 100 µg/ml, respectively. 3-(2,4 dichlorobenzylidene) chromon -4- one showed antibacterial activity against *K. pneumonia* and *E.coli* with MIC=50 and 100 µg/ml, respectively. The result of this study showed that these synthetic chalcones have more inhibitory effects on Gram negative bacteria. Based on this study antitumor activity of 3-(2-chlorobenzylidene) chroman -4- one and 3-(2,4 dichlorobenzylidene) chromon -4- one were determined as 52.3 and 53.9% inhibition of the growth of tumors respectively in the highest concentration (50 µg/disc). The results indicated a possible strong anticancerous effect of chromans and similar compounds.

Key words: Antibacterial activity, potato disc method, chroman - 4 - one.

INTRODUCTION

Nowadays, there are many different medicines and drugs for the one of the most important disease, cancer, in the world. Considering the high mortality rate in different types of cancer, more research needs to be done about it. One of the most important types of research is to design and study for the effective compounds on cancer (Avila et al., 2008). The anticancer compounds are able to slow the progress of cancer and increase the recovery in patients (Boonkaew and Camper, 2005; Santoyo et al., 2005). Chalcones are a series of aromatic compounds with plant origin which act as intermediate components in the biosynthesis route of flavonoids in some plants and widely considered as the useful compound with the variety of biological activities such as anti-oxidant, anti-cancer, anti-viral, anti-inflammatory, anti-ulcer, anti-fungal and also act as potential modulators in multidrug resistance (Sugamoto et al., 2008; Rao et al., 2009). These compounds have a core with many biological characteristics, which are interested because of their wide spectrum of effects. Flavonoids represent an important class of naturally occurring compounds which from them, the prenylated derivatives are quite rich in structural variety and pharmacological activity. In addition to the properties listed above, the antibacterial activity of chalcones is being increasingly documented (Avila et al., 2008). Depending on the substitution of the two aromatic rings, the chalcones can display different spectra activity.

*Corresponding author. E-mail: ghr_dehghan@kmu.ac.ir. Tel: ++98-341-3205014. Fax: ++98-341-3205215.

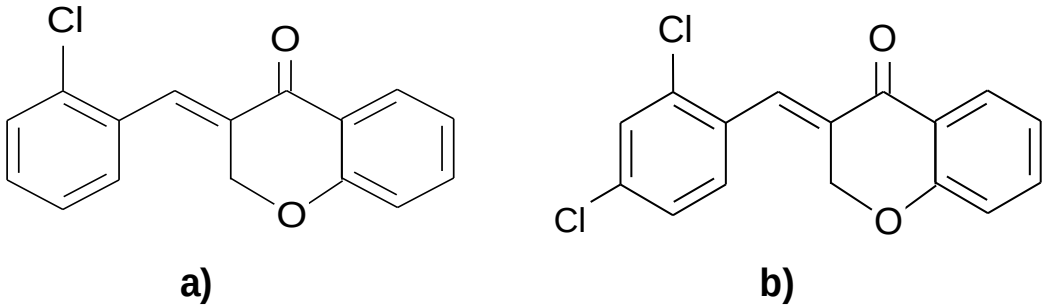

Figure 1. a) Structure of 3-(2-chlorobenzylidene) chroman -4- one and **b)** 3-(2,4 dichlorobenzylidene) chroman -4- one.

These features are more obvious and more considerable in their synthetic derivatives. The most recent effects of these compounds which have been pointed out in several investigations are anti-inflammatory effects and preventing effects, such as preventing Interleukin 5(IL5) activities.

2,3-Dihydro-4H-1-benzopyran-4-ones (chroman-4-ones) are widely distributed in nature. 2-Arylchroman-4-ones (flavanones) and 3-arylchroman-4-ones (isoflavanones) many exhibit interesting and useful biological activities. Chroman-4-ones are important synthetic intermediates for chromans, chromenes and chromanols which themselves possess diverse pharmacological properties such as b-blockade, anticonvulsant, antiestrogen, antitumor and antimicrobial (Draper et al., 2000). One of the simple tests to evaluate the anti-tumor effect of certain compounds is measuring the inhibition of crown form tumors on potato disks, the so-called potato disc test. This test was developed by Coalsky and Wilsey in 1980 (Galsky et al., 1980). In this experiment, compounds with anti-tumor activity inhibit the growth of the plant crown form tumor, caused by *Agrobacterium tumefaciens.* These compounds have not shown anti-bacterial effects in their anti-tumor concentration range. Therefore their anti-tumor effects are independent from their anti-bacterial effects (Coker et al., 2003; Dehghan noudeh et al., 2010; Groot et al., 1998).

The crown form tumor cells of plants are made by the plasmid of *A. tumefaciens.* In fact this microorganism has a large plasmid called Ti plasmid, which carries the genetic information. If these plasmids transform their DNA to the plant cells, they will be converted to independent tumor cells. A part of the plasmid called transferred DNA (T-DNA) is transferred from the *Agrobacterium* plasmid to the plant cells. This DNA makes unnecessary amino acids such as opines. There is no opines in plant cells and *Agrobacterium* utilizes it as a source of nitrogen and carbon. Ti plasmid codes the ability for production and consumption of opines. Mean while, transformed Ti plasmid codes some enzymes which produce oxcyins and cytokines in plant cells. These hormones play important roles in the growth and contamination of adjacent cells (Galsky et al., 1980; Dehghan noudeh et al., 2010).

In this study the biological activity of two chalcone derivatives, from the chromans group were studied. These two chalcones were included: 3-(2-chlorobenzylidene) chroman -4- one with chemical formula of $C_{16}H_{11}O_2Cl$ and 3-(2,4 dichlorobenzylidene) chroman -4- one with chemical formula of $C_{16}H_{10}O_2Cl_2$ (Figure 1).

MATERIALS AND METHODS

Bacteria used in this study were obtained from the Persian Type Culture Collection (PTCC), Tehran, Iran (Table 1).

Anti-bacterial activity

After synthesis of the desired compounds, different dilutions were made by water and dimethyl sulfoxide (DMSO) (3:1) as the selected solvents. In this study different dilutions used including 3.125, 6.25, 12.5, 25 and 100 µg/ml. Agar dilution method was used for assessment of antibacterial properties of synthesized compounds. In this method a certain dilution of anti-bacterial solution was mixed with a certain volume of melted agar at 45-50ºC, then spread on ordinary laboratory plates (Muller Hinton agar currently used for anaerobic cultures). Thus a series of plates containing different concentrations of anti-bacterial compounds and without any antibacterial compounds were prepared. The plates were incubated at 35°C for 18 h. After the incubation period, minimum inhibitory concentration (MIC) was determined.

Potato disk preparation

Healthy white potatoes without any budding and white brain were washed with tap water and sterilized. Before sterilization, the potatoes were kept in sodium hypochlorite 1% (w/w). Potatoes were disinfected by savlon solution and carried out to laminar flow cabinet. For the sterilization of the potatoes, the head and the bottom of potatoes were cut and cylinders were made by a sterile ramrod. One centimetre from each end of these cylinders was cut by surgical blade, and removed, and then pieces with one centimetre diameter and 1.5 cm diagonally were made from the centre of the cylinder disks. The disks were kept in sterile distilled

Table 1. Bacteria Names.

Number	Bacterium name	PTCC	Categorized
1	*Staphylococcus aureus*	1112	Gram positive cocci
2	*Escherishia coli*	1330	Gram negative bacillus
3	*Bacillus subtilis*	1023	Gram positive bacillus
4	*Pseudomonas aeroginosa*	1074	Gram negative bacillus
5	*Micrococcus luteus*	1110	Gram positive cocci
6	*Klebsiella pneumoniae*	1053	Gram negative bacillus

water before placing on media containing agar. The disks were transferred on plates containing 20 ml of 2% agar by sterile forceps. Each plate contained five potato disks, one in the center and the rest around it (Mclaughlin et al., 1993; Turker and Camper 2002).

Potato disk test

For preparation of our desired dilutions (62.5, 125, 250, 500, 1000 and 2000 µg/ml), the volume of 0, 0.25, 0.5, 1, 2, 4, and 8 µg/ml of the compounds were dissolved in 1 ml of DMSO. Then the solution was mixed with 1 ml of sterile distilled water and 2 ml of $10^8 – 10^9$ CFU/ml bacteria suspension, next the final solution was used for our experiments. For preparing negative control, 1 ml of the solvent was mixed with 1 ml of bacteria suspension. Vincristine was used as positive control; to make final dilution (160 µg/ml) of vincristine 1 mg ampoule was mixed with 6.66 ml of the solvent. Then 1 ml from this dilution was mixed with 1 ml of bacteria suspension. Sine the volume of injection into the disks was 25 µl, the volume of vincristine was 2 µg per disk (Mclaughlin et al., 1993; Turker and Camper, 2002).

The samples were injected by a 25 µl pipette in a sterile atmosphere between two gas lights. The interval time from disk preparation and injection was less than 30 min (Galsky et al., 1980). Four plates for each dilution were considered. Each plate contained five potato disks, the central disk was considered as positive control, three disks contain main sample, and the last disk was considered as negative control. After injection, the plates were sealed by parafilm and incubated at 25°C for 20 days. In this study, this test was repeated three times. The numbers of tumors were counted 20 days after injection. For this purpose, lugol solution (10% iodide potassium and 5% iodine) was used. The inhibition percentage of growth of crown tumors was calculated using this equation:

$$\text{Percent inhibition} = 100 - \frac{Average\ number\ of\ tumors\ of\ sample}{Average\ number\ of\ tumors\ of\ control} \times 100$$

The intact potato cells contained starch and they appeared purple, while the tumor cells did not contain any starch and therefore appeared colorless. A significant point was that the disks were kept moist all the time through the test including cutting, injection and incubation time (Coker et al., 2003). The analysis of variance (ANOVA) test was used for analyzing the data and comparison of results by SPSS software (Ver. 11).

RESULTS

The anti-bacterial effect of two chalcons was very weak in different dilutions. The 3-(2-chlorobenzylidene) chroman -4- one showed the most inhibition of growth of *Klebsiella pneumoniae*, and there was no bacterial growth in dilutions of 25, 50 and 100 µg/ml (MIC= 25 µg/ml). There was no growth of *Bacillus subtilis* in the highest dilutions of 100 µg/ml, while there was few growth in dilution of 50 µg/ml (MIC= 100 µg/ml). The other bacteria grew in all dilutions (MIC > 100 µg/ml) (Tables 2 and 3).

After adding tetrazulium and incubating for 30 min, the pink colour intensity was seen less than the negative control when both compounds were used in the highest dilution (8 mg/ml). This means *A. tumefaciens* was relatively inhibited in these dilutions. The pink colour was very clear in the lower dilutions which mean both compounds have no anti-bacterial effects. Therefore the study of the number of tumors in dilutions less than 8 mg/ml was more reliable. There was no colour change in the gentamycine control, which showed no bacterial growth. There was no colour change on the sterilized control, which confirms the standard conditions of the experiments.

The obtained results showed that 3-(2-chlorobenzylidene) chroman -4- one, exhibited more anti-tumor activity when different dilutions were used, but there was not significant difference between tested chalcons. This compound inhibited the growth of tumors in dilutions of 0.08 mg/ml. There was a significant effect on the inhibition of tumor formation in all dilutions in comparison with negative controls. Neither 3-(2-chlorobenzylidene) chroman -4- one nor 3-(2,4 dichlorobenzylidene) chroman -4- one, were as effective as vinchristine on inhibiting plant tumors (Figures 2 and 3).

DISCUSSION

Based on the results, 3-(2-chlorobenzylidene) chroman -4- one, showed higher anti-bacterial effect on *K. pneumoniae* and *B. subtilis* with MIC = 25 and 100 µg/ml respectively. No inhibition effect was seen against

Table 2. Effect of inhibition of different of dilutions of 3-(2-chlorobenzylidene) chroman -4- one, on the growth of bacteria.

Bacteria name	Dilution (µg/ml) Sample number	3.125	6.25	12.5	25	50	100	Positive control	Negative control	Sterilized control	MIC (µg/ml)
Staphylococcus aureus	1	++	++	+	+	+	+	-	+	-	> 100
	2	++	++	+	+	+	P	-	++	-	
	3	++	++	++	+	+	+	-	+	-	
Bacillus subtilis	1	++	+	+	+	P	-	-	++	-	= 100
	2	++	+	+	+	+	-	-	++	-	
	3	+	+	+	+	P	-	-	++	-	
Escherishia coli	1	++	++	++	++	+	+	-	+	-	> 100
	2	++	++	++	++	+	P	-	+	-	
	3	++	++	++	++	+	+	-	+	-	
Klebsiella pneumoniae	1	+	+	+	-	-	-	-	++	-	= 25
	2	+	+	+	-	-	-	-	+	-	
	3	++	+	+	+	-	-	-	+	-	
Pseudomonas aeroginosa	1	++	++	++	++	++	+	-	++	-	> 100
	2	++	++	++	++	++	++	-	++	-	
	3	++	++	++	++	++	+	-	++	-	
Micrococcus luteus	1	++	+	+	+	+	P	-	++	-	> 100
	2	++	++	++	+	+	P	-	+	-	
	3	++	+	+	+	+	+	-	++	-	

++: Full growth, +: Medium growth, -: No growth, P: Weak growth.

Staphylococcus aureus, Pseudomonas aeroginosa, Escherishia coli, and *Micrococcus luteus* for this compound in different dilutions. The 3-(2,4 dichlorobenzylidene) chroman -4- one, was used for its antibacterial effects, it showed more inhibition effect against *K. pneumoniae* and *E. coli* with MIC= 50 and 100 µg/ml. No inhibition effect was observed on the growth of *S. aureus, P. aeroginosa, E. coli,* and *M. luteus* for this compound in tested dilutions. These two chalcons are from chroman derivatives, which show more inhibition effect on Gram negative bacteria rather than Gram positive bacteria. However, 3-(2,4 dichlorobenzylidene) chroman -4- one, showed more inhibition effect on the other bacteria than the 3-(4-chlorobenzylidene) chroman -4- one, which completely inhibited the growth of *K. pneumoniae* and *E. coli*, meanwhile, *S. aureus, and B. subtilis,* but *M. luteus* showed low susceptibility in dilution of 100 µg/ml.

In this study, biological activity was found as a factor which is influenced the anti-bacterial activity as well as anti-tumor activity. Therefore, using of neutral solvents like water was preferable. However DMSO is known as a solvent, for many chemical compounds and plant extracts. In this study, due to a low volume of the synthesized material, a mixture of water and DMSO (3:1) was used as a solvent. DMSO has anti-bacterial effect, and it is able to haemolyse the cell membrane of bacteria in the high dilution (Hang et al., 2007). However, DMSO used in our mixture showed no inhibition effect on the growth of bacteria. This was studied by a control solvent, for each series of our experiments. In another case, only the anti-bacterial activity of our compounds was studied and compared.

Comparison of the outcome of this study with similar compounds and with the other chroman derivatives indicated that chalcon derivatives showed a very close concentration range for growth inhibition. The MIC for fluro- chromans's group against *Salmonella typhimurium* is 100 - 500 µg/ml.

The anti-tumour effect in potato disk method is defined on the basis of reduction of 20% or more in the number of tumors caused by *A. tumefaciens* in comparison to a negative control disk in at least two direct tests. However it should be considered that the used dilutions of tested compounds exhibited no anti-bacterial effect, otherwise there will be false negative results. It also was shown that

Table 3. Effect of inhibition of different dilutions of 3-(2,4 dichlorobenzylidene) chroman -4- one, on the growth of bacteria.

Bacteria name	Dilution (µg/ml) Sample number	3.125	6.25	12.5	25	50	100	Positive control	Negative control	Sterilized control	MIC (µg/ml)
Staphylococcus aureus	1	++	++	+	+	+	P	-	+ +	-	> 100
	2	++	++	+	+	+	+	-	+ +	-	
	3	++	++	+	+	+	+	-	+ +	-	
Bacillus subtilis	1	++	++	++	+	P	P	-	+	-	> 100
	2	++	++	++	+	+	P	-	+	-	
	3	++	++	++	+	P	+	-	++	-	
Escherishia coli	1	+	+	+	+	P	-	-	+	-	=100
	2	+	+	+	+	+	-	-	++	-	
	3	++	+	+	+	+	P	-	++	-	
Klebsiella pneumoniae	1	+	+	+	P	-	-	-	++	-	=50
	2	+	+	+	P	P	-	-	+	-	
	3	+	+	+	P	-	-	-	++	-	
Pseudomonas aeroginosa	1	++	++	++	++	+	P	-	++	-	> 100
	2	++	++	++	++	++	+	-	++	-	
	3	++	++	++	++	+	+	-	++	-	
Micrococcus luteus	1	+	+	+	+	P	P	-	++	-	> 100
	2	+	+	+	+	+	P	-	++	-	
	3	++	++	+	+	+	P	-	++	-	

++: Full growth, +: medium growth, -: no growth, p: weak growth.

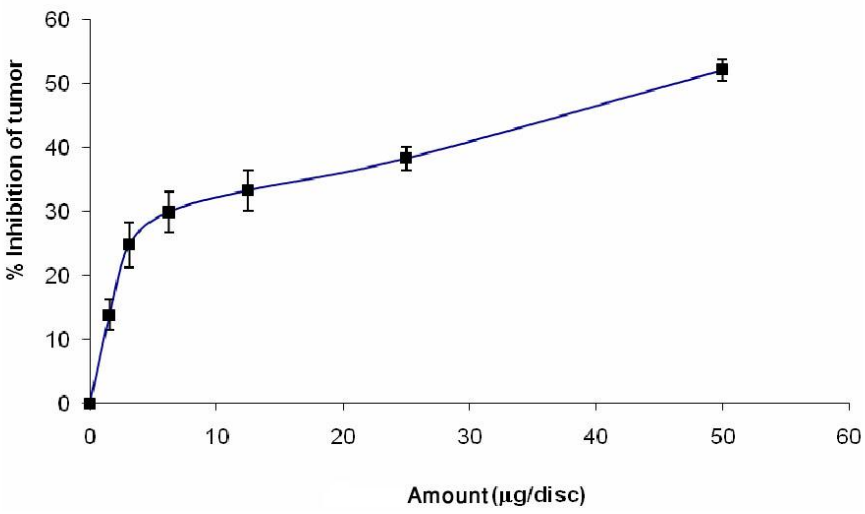

Figure 2. Percentage inhibition of making tumors using 3-(2-chlorobenzylidene) chroman -4- one.

there would be more inhibition on the growth of crown form tumor cells, if a higher concentration of compounds is used. Alternatively there is a direct correlation between the used dilution of the compounds and the growth of the

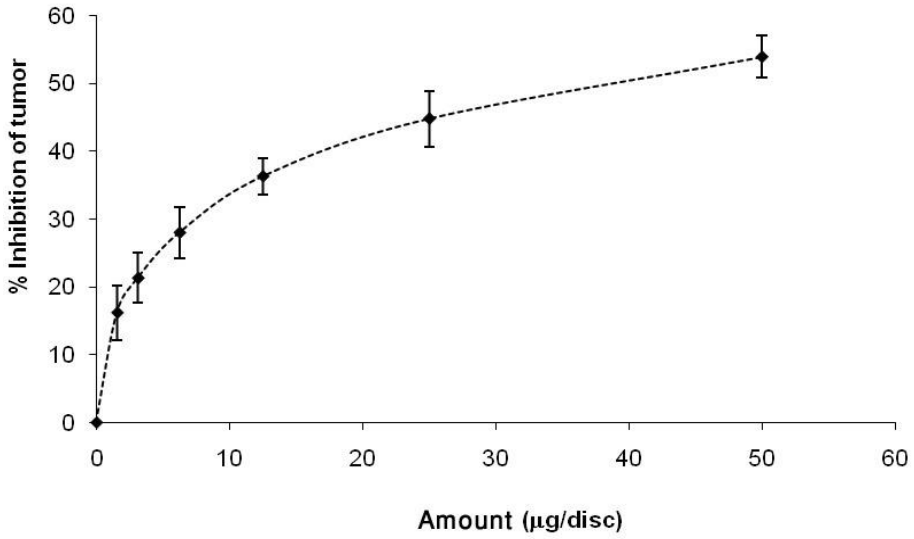

Figure 3. Percentage of inhibition of tumor formaing of 3-(2,4 dichlorobenzylidene) chroman -4- one.

crown form tumor cells. Both compounds showed similar anti-tumor activity and there was not any significant difference between them.

ACKNOWLEDGMENT

This work was taken from a pharmacy student thesis in Kerman University of Medical Sciences (Thesis No.560). This research was supported by a grant (No. 68/92) from Vice President for Research of Kerman University of Medical Sciences.

REFERENCES

Avila HP, Smania EFA, Monache FD, Junior AS (2008). Structure–activity relationship of antibacterial chalcones. Bioorg. Med. Chem. 16: 9790-9794.

Boonkaew T, Camper ND (2005). Biological activities of Ginkgo extracts. Phytomedicine 12:318–323.

Coker PS, Radecke J, Guy C, Camper ND (2003). Potato disc tumor induction assay: A multiple mode of drug action assay. Phytomedicine 10: 133-138.

Dehghan noudeh GR, Sharififar F, Dehghan noodeh A, Moshafi MH, Ahmadi afzadi M, Behravan E, Aref M, Sakhtianchi R (2010). Antitumor and antibacterial activity of four fractions from *Heracleum persicum* Desf. and *Cinnamomum zeylanicum* Blume. J. Med. Plant. Res. 4(21):2176 - 2180.

Draper RW, Hu B, Iyer RV, Li X, Lu Y, Rahman M, Vater EJ (2000). An Efficient Process for the Synthesis of trans-2,3-Disubstituted-2,3-dihydro-4*H*-1-benzopyran-4-ones (Chroman-4-ones). Tetrahedron 56: 1811–1817.

Galsky AG, Wilsey JP, Powell RG (1980). Crown gall tumor disc bioassay; a possible aid in the detection of compounds with antitumor activity. Plant Physiol. 65:184-185.

Groot MJA, Bundock P, Hooykaas PJJ, Beijersbergen AGM (1998). *Agrobacterium tumefaciens*-mediated transformation of filamentous fungi. Nature Biotechnol. 16:839-842.

Hang W, Liu MZ, Li Y, Tan Y, Yang GF (2007) Design, S‿ntesis and antitumor activity of novel chromane and aurone derivatives. Bioorg. Med. Chem. 15(15):5191-5197.

McLaughlin JL, Chang C, Smith DL (1993). Simple bench top assays (brine shrimp and potato disc) for the discovery of plant antitumor compounds. In: Klinghorn, AD, Balandrin, MF (Eds.), ACS symposium series. Human Med. Agents Plants 534:112–137.

Rao GV, Swamy BN, Chandregowda V, Reddy GC (2009). Synthesis of (±) abyssinone I and related compounds: their anti-oxidant and cytotoxic activities. Eur. J. Med. Chem. 44(5):2239-2245.

Santoyo S, Cavero S, Jaime L, Ibǔez E, Seǒrǔns FJ, Reglero G (2005). Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. essential oil obtained via supercritical fluid extraction. J. Food Protec. 68(4):790-795.

Sugamoto K, Kurogi C. Matsushita Y, Matsui T (2008). Synthesis of 4-hydroxyderricin and related derivatives. Tetrahedron Lett. 49:6639–6641.

Turker AU, Camper ND (2002). Biological activity of common mullein a medicinal plant. J. Ethnopharmacol. 82:117-125.