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Review Article

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Synthesis, biological activities and therapeutic properties of esculetin and its derivatives

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ABSTRACT

Esculetin is the main active ingredient of Cortex Fraxini, its chemical name is 6,7-dihydroxycoumarin. This review covered recent studies on the synthesis, pharmacological activities and mechanism of esculetin and its derivatives over the past decade. With a variety of novel esculetin derivatives being continuously synthesized, the development and clinical application of Cortex Fraxini and its main ingredient esculetin will be more prospective.

Key words: Esculetin, Coumarin, Derivatives, Synthesis, Pharmacological activities

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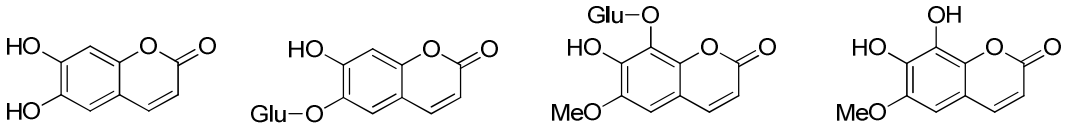
INTRODUCTION

Chinese herbal medicine has been widely used for centuries for the treatment of different diseases. Cortex Fraxini is a commonly used traditional Chinese medicine. According to the Chinese medicine, there are four species classified under Cortex Fraxini, namely *Fraxinus rhynchop hylla Hance*, F. *chinensis Roxb.*, F. *sz aboana Lingelsh.* and F. *stylosa Lingelsh.* It has been indicated that Cortex Fraxini possess various pharmacological effects, including anti-pathogenic microorganism[1], anti-inflammatory[2], antitumor[3] and neuroprotection[4]. There are many active ingredients in Cortex Fraxini. Especially, esculetin **1**, esculin **2**, fraxin **3** and fraxetin **4** found in Cortex Fraxini been investigated as major pharmacologically active ingredients



As the main active ingredient of Cortex Fraxini, esculetin has been widely used in expectorant, antitussive and other aspects, such as anti-inflammatory effects, antioxidant, antibacteria, antitumor and so on. Because of multiple pharmacological effects, esculetin has broad prospect of developing effective drugs. The 6,7 two phenolic hydroxyl and 3,4-double bond of the structure are the vital reactive sites, which could obtain novel structure esculetin derivatives by chemical reaction. This review covered recent studies on the synthesis, pharmacological activities and mechanism of esculetin and its derivatives over the past decade.

1.Pharmacological effects of esculetin

1.1. Anti-inflammatory effects

Experiments reveal that esculetin has anti-inflammatory effects. On one hand, esculetin reduces the secretion of NO to regulate blood vessels and eases the organs tissues damage of inflammation; on the other hand, esculetin inhibit

the secretion of soluble intercellular adhesion molecule (sICAM-1), which can reduce the adhesion reaction of leukocytes and endothelial cells to reduce the inflammatory[5]. Esculetin can significantly reduce the expression of MMP-1 in cartilage and levels of NO and PGE2 in synovial fluid, and postpone the occurring of osteoarthritis[6]. In the experiment, esculetin can protect myocardial from ischemia reperfusion injury by reducing systemic inflammatory responses[7].

Obesity is closely related to chronic low-grade inflammation of adipose tissue. Esculetin exhibites anti-inflammatory properties by inhibiting the production of proinflammatory cytokines in the interaction between adipocytes and macrophages through HO-1 expression. Esculetin may have the potential to improve chronic inflammation in obesity[8].

1.2 Inhibition the proliferation of vascular smooth muscle cell

The proliferation of vascular smooth muscle cells (VSMCs) induced by injury to the intima of arteries is a vital pathogenic factors in vascular proliferative disorders including atherosclerosis and restenosis. Esculetin can effectively inhibit the proliferation of rVSMCs in vitro in a dose- and time- dependent manner. The main mechanism may the antiproliferative effect is mediated by inhibiting the activation of Ras-Raf-MEK-ERK/ MAPK and Ras-PI3K Akt[9]. The experiment reveals that esculetin blocks cell proliferation via the inhibition of an upstream influence of Ras and downstream events, such as p42/44 MAPK activation, PI 3-kinase activation, immediate early gene expression, as well as NF-kappaB and AP-1 activation. It also blocks intimal hyperplasia after balloon vascular injury in the rat, showing the curative potential for treating restenosis after arterial injury[10]. Another experiment reveals that esculetin can activate PPAR - ɣ and promote ABCA1 and ABCG1 expression, thereby inhibits the formation of smooth muscle - derived foam cells[11]. Besides, esculetin has neuroprotective effects on cerebral ischemia/reperfusion (I/R) injury in a middle cerebral artery occlusion model in mice[12]. Esculetin reduces cleaved caspase 3 level, a marker of apoptosis; and esculetin exerts its anti-apoptotic activity by up-regulating the expression of Bcl-2 and down-regulating the expression of Bax. In view of its clinical use as an anticoagulant and its safety profile, esculetin may have a medicinal potential for the treatment of stroke in the future.

1.3 Antioxidative effects

Free radicals and reactive oxygen species (ROS), which are generated by ultraviolet irradiation, may cause serious skin diseases. Esculetin is a potent agent to protect cells against ROS-mediated Abeta-damage[13]. Esculetin shows the strong scavenging activity against DPPH radicals[14]. In the experiment, esculetin is effective in protecting cells against DNA damage induced by oxidative stress [15]. Another experiment also illustrates the esculetin shows strong scavenging activities on DPPH radical[16]. The free radical activities are related to the concentration and time of esculetin mixed with DPPH. The ability of scavenging free radicals is positively correlated with time and concentration.

1.4 Hepatoprotective effect

Esculetin is found to possess anti-hepatotoxic activity and the presence of this compound in Cichorium intybus and Bougainvllra spectabillis may explain the folkloric use of these plants in liver damage[17].

More and more evidence relate to free radical-generating agents and inflammatory processes suggests that cumulation of reactive oxygen species can cause hepatotoxicity. A short-chain analog of lipid hydroperoxide, t-butyl hydroperoxide (t-BHP), can be metabolized to free radical intermediates by cytochrome P-450 in hepatocytes, which conversely can initiate lipid peroxidation, affect cell integrity and lead to cell damage. Histopathological evaluation of the rat livers revealed that esculetin reduced the incidence of liver lesions induced by t-BHP, including liver cell swelling, leukocyte infiltration, and necrosis[18]. Esculetin may play a prevention role via reducing oxidative stress in living systems.

1.5 Antidiabetes activities

Diabetes mellitus is the most common serious metabolic disorder and it is considered to be one of the most important reason leading to death in the world. Diabetes also result in many diabetes complications such as harm to the human heart, brain, kidneys, blood vessels, nerves, skin and so on. Hyperglycemia-mediated oxidative stress plays a crucial role in diabetic complications. Based on the research of Prabakaran, esculetin treatment exerts a protective effect in diabetes by attenuating hyperglycemia-mediated oxidative stress and antioxidant competence in hepatic and renal tissues [19]. In the experiment, esculetin exerts a pronounced antihyperglycemic effect against streptozotocin-induced diabetic rats [20].

1.6 Antibacterial properties

The human pathogen Escherichia coli O157:H7 is thought to be spread by direct or indirect contact with infected animal or human faeces. E. coli O157:H7 is the most common cause of hemorrhagic colitis, and no effective therapy

exists for E. coli O157:H7 infection. The addition of esculetin to human faecal slurries and in vitro continuous-flow fermenter models simulating conditions in the human colon and rumen caused marked decreases in the survival of an introduced strain of E. coli O157 [21]. Another experiment proved that esculetin can repress Shiga-like toxin gene stx2 in E. coli O157:H7 and weaken its virulence in vivo in the nematode Caenorhabditis elegans [22].

1.7 Antitumor properties

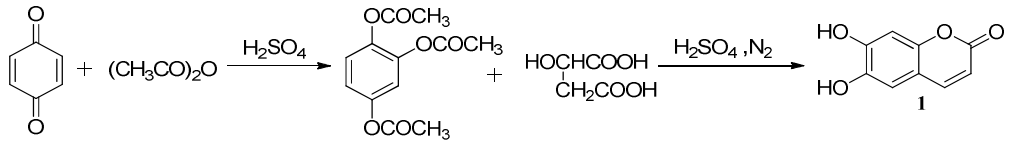
Esculetin is a phenolic compound that is found in natural plant products and induces apoptosis in several types of human cancer cells. Esculetin has been shown to selectively induce tumor apoptosis in several kinds of cancers and is considered as a promising chemotherapeutic agent. Acute promyelocytic leukemia (APL) is a type of cancer, in which immature cells called promyelocytes proliferate abnormally. Esculetin is found to inhibit the survival of human promyelocytic leukemia cells in a concentration-dependent and time-dependent manner[23-24]. Other research reported that esculetin exerts its anti-proliferative action on cultured human monocytic leukemia U937 cells[25-27]. ERK pathway is key regulators of apoptosis in response to esculetin in human leukemia U937 cells. Another experiment proved that esculetin significantly suppress the growth of oral cancer SAS cells in a dose-dependent manner [28]. Esculetin enhances TRAIL-induced apoptosis primarily through upregulation of DR5, combination of esculetin and TRAIL may be a new therapeutic strategies for oral cancer

1.8 Suppressing adipogenesis

The quality of the adipose tissue is determine by the number of fat cells and it is subjected to homeostatic regulation involving cell death mechanisms. Esculetin mediates adipocyte apoptosis involves the mitochondrial pathway [29]. Esculetin decreases adipocyte number by initiating apoptotic process in 3T3-L1 adipocytes. Another experiment indicated that esculetin has anti-adipogenic effects through modulation of PPARγ and C/EBPα via the AMPK signaling pathway [30].

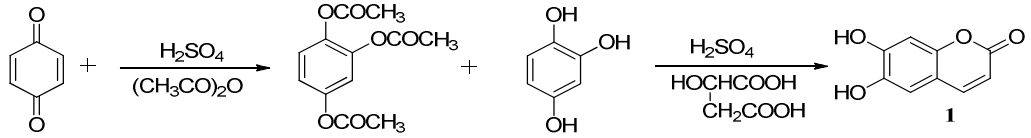
2. Synthesis of esculetin

Cao[31] et al. studied the synthesis of esculetin. P - benzoquinone, acetic anhydride and sulfuric acid were used as the raw material to produce 1, 2, 4 - phloroglucinol triacetate intermediate and then combined with concentrated sulfuric acid and malic acid to synthesize aesculetin. The yield reached 80. 3%. (Scheme 1)



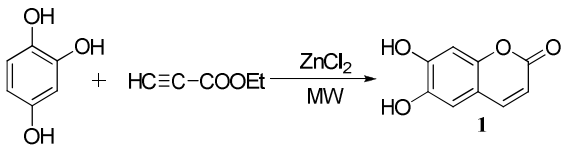
Scheme 1

Zhang[32] designed the synthesis ways of esculetin and optimized the reaction conditions by unifactor and multifactor orthogonal experiment. The optimal reaction conditions are that the amounts of P - benzoquinone: acetoacetate: concentrated sulfuric= 1: 3: 0.15(mol ratio), at 3h and 45□, the yield reached 89.2%; and the reaction temperature is about 120□and time is 2 h, the volume of concentrated sulfuric acid is 10 mL, the amount of l,2,4-benzenetriol is 5 g, the amount of malic acid is about 5.6 g, the selectivity of esculetin could reach about 80%. (Scheme 2)



Scheme 2

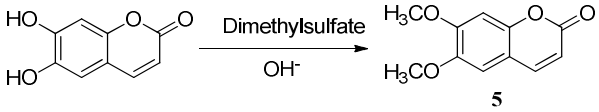
Yang[33] et al. used the cyclization of 1, 2, 4-benzenetriol and ethyl propionate to synthesis esculetin under microwave irradiation and used $ZnCl_2$ as catalyst. The optimum reaction conditions were as follows: n(1, 2, 4-benzenetriol) : n (ethyl propionate) = 1. 0 : 1. 0, 3. 5 g $Zncl_2$, 10 min, 105 □ and microwave power 400W. The yield reached 87. 4% . (Scheme 3)



Scheme 3

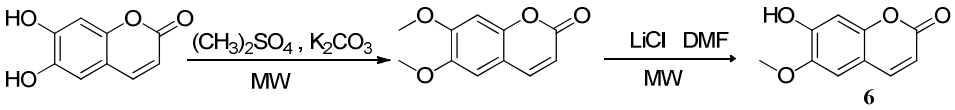
3. Synthesis and pharmacological activities of esculetin derivatives

Zhang[34] et al. reported the synthesis of 6,7-dimethoxycoumarin **5** by methylating from 6, 7- dihydroxycoumarin with an overall yield of 74. 4%. (Scheme 4) Zhao[35] et al. used the same way to synthesis 6,7-dimethoxycoumarin and catalysts in the methylation progress of 6,7-dihydroxycoumarin were investigated and chosen. The research compared the catalytic effects in the methylation process with [BMIm][BF_4]、[BMIm]Cl、[BMIm]Br、and [BMIm][PF_6]. The results showed that the catalytic effect of N5N-dialkylimidazolium-based ionic liquid is better than PTC. Besides, using imidazolium ionic liquids as catalysts not only can increase the reaction yields, improve selectivity and the reactation under the low temperature, but also the catalysts can be reused. Another research[36] reported that 6,7-dimethoxycoumarin had potently inhibitory activity against Clostridium histolyticum collagenase (ChC).

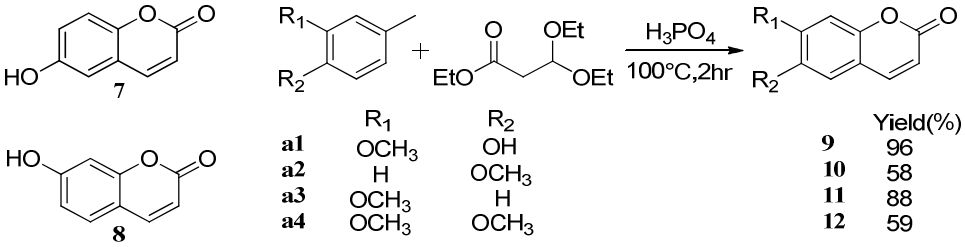


Scheme 4

Compared with traditional methods[37,38], using assisted microwave irradiation to synthesize scopoletin **6** could apparently shorten the reaction time, increase the reaction selectivity and the yield, and also suitable for industrial production. Yield increased to 73% from the traditional 46% [39].(Scheme 5) Morina et al.[40] synthesized several coumarin derivatives whose chemical structures are similar to scopoletin **6**. Scopoletin **6** showed the strongest termiticidal activity among the compounds tested, followed by 6-methoxycoumarin (**10**), 6-hydroxycoumarin (**7**), and umbelliferone(**8**).



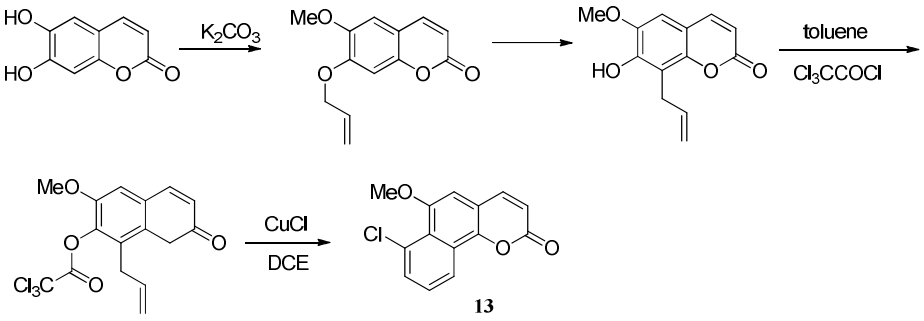
Scheme 5



	R_1	R_2	Yield(%)
a1	OCH_3	OH	**9** 96
a2	H	OCH_3	**10** 58
a3	OCH_3	H	**11** 88
a4	OCH_3	OCH_3	**12** 59

Scheme 6

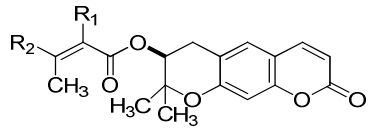
Bull et al.[41] put forward a new method to the synthesis of the 6H-benzo[d]naphtha[1,2-b]pyran-6-one ring system. The key of this method to synthesis coumarin derivatives is the application of a new benzannulation strategy (the BHQ Reaction), which easier to make ortho-allylaryl trichloroacetates transforme into naphthalene derivatives via a cascade of reactions which involves an initial ATRC reaction followed by the extrusion of CO_2. The isolation of the coumarin **13** in 84% after chromatography . (Scheme 7)



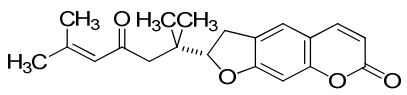
Scheme 7

Nemoto et al. [42] successively reported using catalytic asymmetric epoxidation of an enone as the key step to synthesis a series of coumarin derivatives , including the enantioselective total syntheses of (+)-decursin **14** and related natural dihydropyranocoumarins (-)-prantschimgin **16**, (+)-decursinol **17**, and (+)-marmesin **18** for the first time. The new asymmetric catalyst generated from La(O-i-Pr)$_3$, BINOL, and Ph_3As=O in a 1:1:1 ratio epoxide in

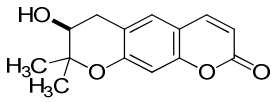
94% yield and 96% ee could effectively promote catalytic asymmetric epoxidation of the enone, recrystallized the product can give optically pure epoxide.



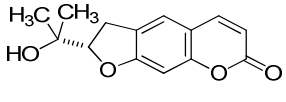
(+)-decursin(**14**):R_1=H,R_2=CH_3
(+)-decursinol angelate(**15**):R_1=CH_3,R_2=H



(-)-prantschimgin(**16**)

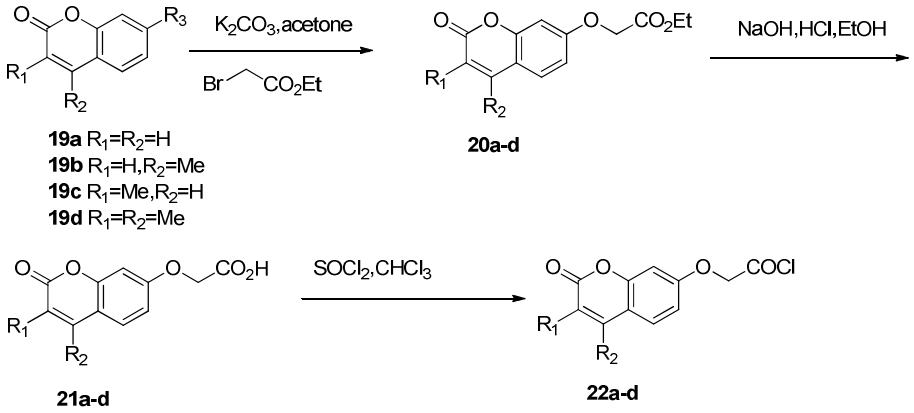


(+)-decursinol(**17**)



(+)-marmesin(**18**)

Chimichi et al.[43] developed a new synthetic route to coumarinyloxyaldehydes starting from hydroxycoumarins, which a convenient procedure leading to 7-(2-oxoethoxy)coumarins **23a-d** that could easier get target compound in much higher yields. These compounds, intermediates in the preparation of natural products including geiparvarin and psoralens, now are available in good yields with a simple post-processing program, and the reported route has been applied to dihydroxycoumarins. (Scheme 8)



19a R_1=R_2=H
19b R_1=H,R_2=Me
19c R_1=Me,R_2=H
19d R_1=R_2=Me

20a-d

21a-d

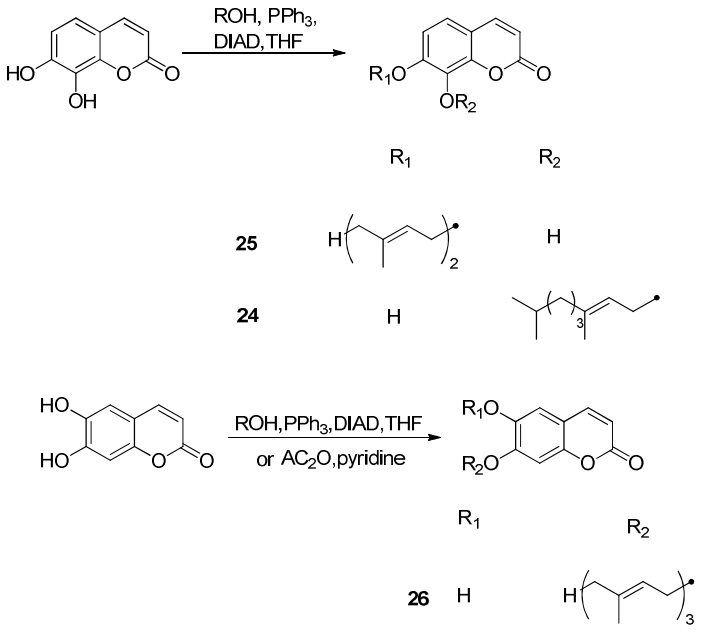
22a-d

23a-d

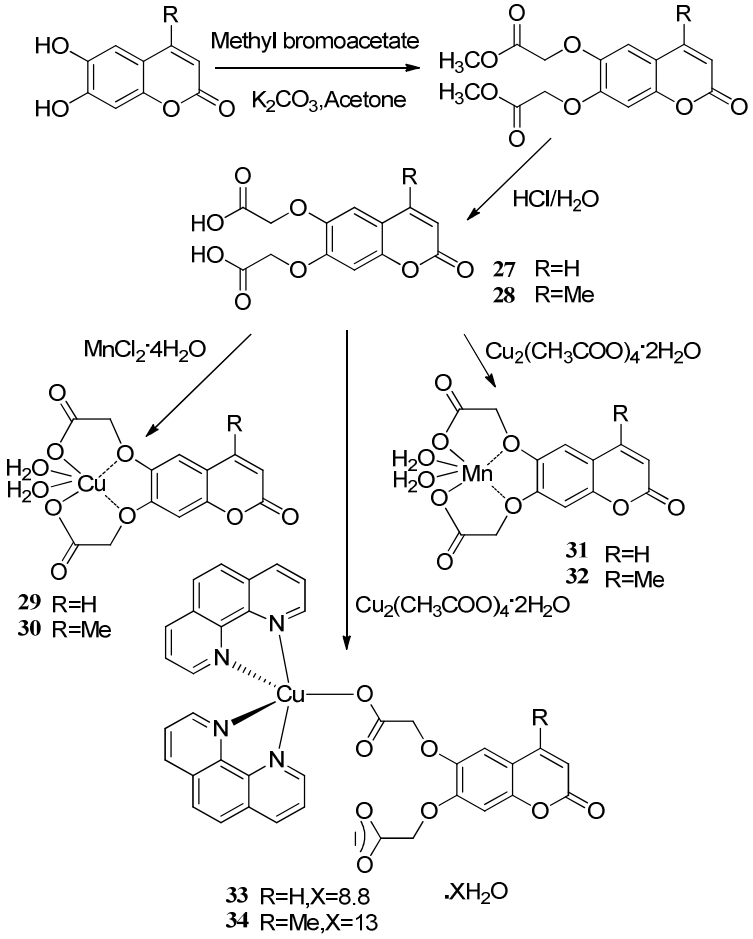
Scheme 8

Cravotto et al.[44] developed the monoalkylation of natural dihydroxycoumarins under sonochemical conditions. This method led to several compounds with pharmacological activities or precursors thereof. The phytoestrogen ferujol **24** isolated from *Ferula jaeschkeana*[45] for the first time. Besides, a simple methylation of compound **25** could produce the anti-virus and anti-platelet aggregation agent collinine (from Zanthoxylum schinifolium)[46], a selective epoxidation of 7-farnesylesculetin **26** could produce some new hopene squalene cyclase inhibitors. (Scheme 9).

In 2007, Creaven et al. [47] used two novel coumarin-based ligands, coumarin-6,7-dioxyacetic acid **27** (cdoaH_2) and 4-methylcoumarin-6,7-dioxyacetic acid **28** (4-MecdoaH_2) to react with copper(II) and manganese(II) salts. The complexes carried out the antimicrobial activity against some microbial species, including methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* and *Candida albicans*. The metal-free ligands **27** and **28** were effectively against all of the microbial species. Complexes **29**-**32** had no apparent activity while the phen adducts **33** and **34** were active against MRSA (MIC80 = 12.1 μM), E. *Coli* (MIC80 = 14.9 μM) and *Patonea agglumerans* (MIC80 = 12.6 μM). Complex **33** also had anti-*Candida* activity,which was (MIC80 = 22 μM) comparable to the commercially antifungal agent ketoconazole (MIC80 = 25 μ M). (Scheme 10).

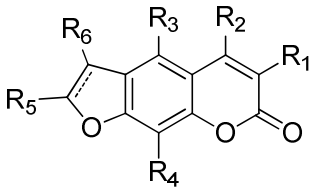


Scheme 9



Scheme 10

Hu[48] et al. reported a series of furanocoumarin derivatives **35**. **35** may be used as membrane transfer of GLUT4 agonists and GLUT4 protein expression agonists, and therefor can be used in the therapeutic of diabetes and its complications(wherein R_1, R_2, R_5, and R_6=independently H, alkyl, alkenyl, or phenyl; R_3 and R_4=independently H, alkyl, alkenyl, Ph, hydroxy, or alkoxy).



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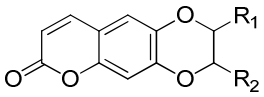
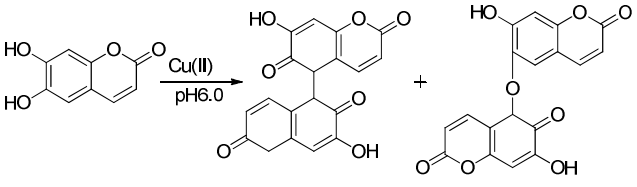
Bhardwaj et al. [49] investigated the catalyst for oxidative coupling of coumarin with propenyl phenols and alkene substrates. This led to some novel coumarinolignans **36-39** by dimerization of the two through C-O-C linkage.



36 R_1=CH_2OH,R_2=C_6H_3-3-OMe-4-OH,52%
37 R1=CO_2Et,R_2=C_6H_3-3-OMe-4-OH,60%
38 R_1=H,R_2=OCOMe,61%
39 R_1=H,R_2=CO_2H,62%

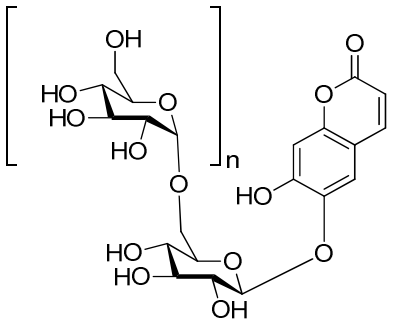
Esculetin was found to have the ability to cause the reduction of Cu(II) to Cu(I) with formation of ESC oxidation products **40,41** [50]. Castaldi et al. tested the ability of malic acid and ESC to mobilize the Cu(II) ions accumulated in a Ca-polygalacturonate matrix (Ca-PGA) used as a model of the root apoplasm, in aqueous phase. At pH 5.0 and 6.0, malic acid mobilizes about 22% and 34% of the Cu(II) accumulated, while esculetin around 12% and 25%. The research of Cu (II) -esculetin also showed that with the formation of semiquinonic radicals, one molecule of esculetin reduces one Cu(II) ion, and the speed was faster in the presence of malic acid. (Scheme 11)



40 **41**

Scheme 11

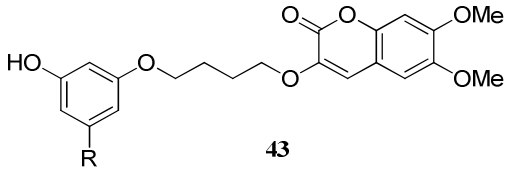
Aurioll et al. reported the invention relates to [6)-O-a-D-Glcp-(1→]$_n$-6-O-b-D-Glcp-(1→-phenolic derivatives **42[**51]. These new derivatives have useful applications in cosmetic, nutrition and pharmaceutical compositions, such as treatment or prevention of oxidative stress, cancer, cardiovascular disease, bacterial infections, viral infections, fungal infections, UV-induced erythema, an allergy, a metabolism. Disorders, diabetes, obesity, hormonal disorders, bone disorders, pain, cerebrovascular disease, oral or dental diseases, inflammatory or immune disorders.



42

Pisani [52] et al. synthesized a large group of substituted coumarins linking through a spacer to 3-hydroxy-N,N-dimethylanilino or 3-hydroxy-N,N,N-dialkyl benzaminium moieties. These coumarins derivatives were evaluated as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitors. The highest AChE inhibitory potency in the 3-hydroxy-N,N-dimethylanilino series was discovered with a 6,7-dimethoxy-3-substituted coumarin derivative **43**. **43** had outstanding affinity (IC50=0.236 nm) and exhibited remarkable AChE/BChE

selectivity (SI>300 000).



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CONCLUSION

Numerous studies have investigated chemical synthesis and pharmacological activities of esculetin and esculetin derivatives. Further clinical applications of esculetin may be developed on the basis of these pharmacological mechanisms found both in *vivo* and in *vitro*.

Acknowledgements
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REFERENCES

[1] Yang, T. M.; Ge, X.; Wang, X. N., *Medical Journal of National Defending Forces in Northwest China*. **2003,** 24 (5), 387.
[2] Liu, S. Q.; He, L.;Peng, H.;Liu, J., *Chinse Journal of Clinical Rehabilitation.* **2005,** 9 (6), 150.
[3] Kaneko, T.; Tahara S.; Takabayashi, F., *Biol Pharm Bull.* **2007,** 30 (11), 2052-2057.
[4] Zhao, D. L.; Zou, L. B.; Lin, S; Shi,J. G.; Zhu, H. B., *Neuropharmacology.* **2007,** 53(6), 724.
[5] Duan, H. Q.; Zhang, Y. D.; Fan, K., Suo Z. W.; Hu, G.; Mu, X., *Chinese Journal of Veterinary Medicine.* **2007**,43(9), 45-46.
[6] Liu, S. Q.; He, L.; Peng, H., *Medical Journal of Wuhan University.* **2004,** 9(5), 567-570.
[7] Wang, Z. Q.; Xia, Y., *Journal of Chengdu Medical College.* **2011,** 6 (1), 49-51.
[8] Kim, Y.; Park, Y.; Namkoong, S.; Lee, J., *Food Funct.* **2014,** 5(9), 2371-2317.
[9] Dai, R.; Zheng, Q. L.; XU, Q. S.; Zhu, W., *Acta Med Univ Sci Technol Huazhong*. **2009,** 38 (2), 239-242.
[10] Pan, S. L.; Huang, Y. W.; Guh, J. H.; Chang, Y. L.; Peng, C. Y.; Teng, C. M., *Biochem Pharmacol*. **2003,** 65 (11), 1897-1905
[11] He, C.; Huang, Y.; Li, B. S.; Zhou, J. J.; Hu, L.; Guo, X. M., *GuangDong Medical Journal.* **2012,** 33 (22), 3368-3371.
[12] Wang, C.; Pei, A.; Chen, J.;Yu, H,.;Sun, M. L.; Liu, C. F.; Xu, X., *J Neurochem.* **2012,** 121 (6), 1007-10013.
[13] Lin, H. C.; Tsai, S. H.; Chen, C. S; Chang, Y. C., Lee, C. M.; Lai, Z. Y.; Lin, C. M., *Biochem Pharmacol.* **2008,** 75(6), 1416-1425
[14] Lee, B. C.; Lee, S. Y.; Lee, H. J.; Sim, G. S.; Kim, J. H.; Kim, J. H.; Cho, Y. H.; Lee, D. H.; Pyo, H. B.; Choe, T. B.; Moon, D. C.; Yun, Y. P.; Hong, J. T., *Arch Pharm Res.* **2007,** 30 (10), 1293-1301.
[15] Kaneko, T.; Tahara. S.; Takabayasi, F., *Biol Pharm Bull.* **2003,** 26(6), 840-844.
[16] Liang, M., *Science and Technology of Food Industry.* **2006,** 27 (3), 64-66.
[17] Gilani, A. H.; Janbaz, K. H.; Shah, B. H., *Pharmacol Res.* **1998,** 37 (1), 31-35.
[18] Lin, W. L.; Wang, C. J.; Tsai, Y. Y.; Liu, C. L.; Hwang, J. M.; Tseng, T. H., *Arch Toxicol.* **2000,**74 (8), 467-472.
[19] Prabakaran, D.; Ashokkumar, N., *Biochimie.* **2013**, 95 (2), 366-73.
[20] Prabakaran, D.; Ashokkumar N., *Journal of Functional Foods.* **2012,** 4 (4), 776-783.
[21] Duncan. S. H.; Leitch, E. C.; Stanley, K. N.; Richardson, A. J.; Laven, R. A.; Flint, H. J.; Stewart, C. S., *Br J Nutr.* **2004,** 91 (5), 749-755.
[22] Lee, J. H.; Kim, Y. G.; Cho, H. S.; Ryu, S. Y.;Cho, M. H.; Lee, J., *Phytomedicine.* **2014,** 21 (8-9), 1037-1042.
[23] Rubio, V.; Calviño, E.; García-Pérez, A.; Herráez, A.; Diez, J. C., *Chem Biol Interact.* **2014,** 220:129-139.
[24] Chu, C. Y.; Tsai, Y. Y.; Wang, C J.; Lin, W. L.; Tseng, T. H., *Eur J Pharmacol.* **2001,** 416 (1-2), 25-32.
[25] Park, C.; Jin, C. Y.; Kwon, H. J.; Hwang, H. J.; Kim, G. Y.; Choi, I. W.; Kwon, T. K.; Kim, B. W.; Kim, W. J.; Choi, Y. H., *Toxicol In Vitro.* **2010,** 24 (2), 486-494.
[26] Lee, S. H.; Park, C.; Jin, C. Y.; Kim, G. Y.; Moon, S. K.; Hyun, J. W.; Lee, W. H.; Choi, B. T.; Kwon, T. K.; Yoo, Y. H.; Choi, Y. H., *Biomed Pharmacother.* **2008,** 62 (10), 723-729.
[27] Park, C.; Jin, C. Y.; Kim, G. Y.; Choi, I. W.; Kwon, T. K.; Choi, B. T.; Lee, S. J.; Lee, W. H.; Choi, Y. H., *Toxicol Appl Pharmacol.* **2008,** 227 (2), 219-228.
[28] Kok, S. H.; Yeh, C. C.; Chen, M. L.; Kuo, M. Y., *Oral Oncol.* **2009,** 45 (12), 1067-1072.
[29] Yang, J. Y.; Della-Fera M. A.; Baile, C. A., *Apoptosis.* **2006 ,** 11 (8),1371-1378
[30] Kim, Y.; Lee, J., Esculetin, *Journal of Functional Foods.* **2015,** 12, 509-515.

[31] Cao, W. Q.; Xue, J. F.; Shi, C. M.; Ding, C. F.; Zhu, X. G.; Liu, F.; Zhou, X. J., *Fine Chemical Intermediates.* **2013,** 43 (3), 39-41.
[32] Zhang, T.,Synthesis of 6,7-dihydroxyeoumarin. *Nanjing University of Science and Technology.***2007.**
[33] Yang, X. J.; Gao, H. H., *Applied Chemical Industry.* **2011,** 40 (4), 627-629
[34] Zhang, S. F.; Ma, J. H.; Chen, S. R.; Li, H. Y.; Xin, J. F., *Journal of Hebei University of Science and Technology.* **2007,** 28 (1), 24-25.
[35] Zhao, L. J., Synthesis and research of 6,7-dimethoxycoumarin. *Lanzhou Jiaotong University.* **2012.**
[36] Oshima, N.; Narukawa, Y.; Takeda T.; Kiuchi, F., *Journal of Natural Medicines.* **2013,** 67 (1), 240-245.
[37] Sun, W. J.; Yang, X. M., *The medicine science and technology press of China.* **1999,** 367-369.
[38] Demyttenaere, J.; Vervisch, S.; Debenedetti, S.; Coussio, J.; Maes, D.; Kimpe, N. D., *Synthesis*. **2004,** 11, 1844-1848.
[39] Fang, Z.; He, G. L., He, L., *West China Journal of Pharmaceutical Sciences.* **2007,** 22 (3), 302-303.
[40] Adfa, M.; Yoshimura, T.; Komura, K.; Koketsu M., *Journal of Chemical Ecology.* **2010**, 36 (7), 720-726.
[41] Bull, J. A.; Lujan, C.; Hutchings, M. G.; Peter, Q., *Tetrahedron Letters.* **2009,** 50 (26), 3617-3620.
[42] Nemoto, T.; Ohshima, T.; Shibasaki, M., *ChemInform.* **2003,** 59 (35), 6889-6897.
[43] Chimichi, S.; Boccalini, M.; Cosimelli, B., *Tetrahedron.* **2002,** 58 (24), 4851-4858.
[44] Cravotto, G.; Chimichi, S.; Robaldoa, B.; Boccalinib, M., *Tetrahedron Letters.* **2003,** 44 (46),8383-8386.
[45] Singh, M. M.; Gupta, D. N.; Wadhwa, V.; Jain, G.K.; Khanna, N. M.; Kamboj, V. P., *Planta Med.* **1985,** 3, 268-270;
[46] (a)Chang, C. T.; Doong, S. L.; Tsai, I. L.; Chen, I. S., *Phytochemistry.* **1997,** 45, 1419-1422; (b)Tsai IL, Lin WY, Teng CM, Ishikawa T, Doong SL, Huang MW, Chen YC, Chen IS. *Planta Med.* **2000,** 66 (7), 618-623.
[47] Creaven, B. S.; Egan, D. A.; Karcz, D.; Kavanagh, K.; McCann, M.; Mahon, M.; Noble, A.; Thati, B.; Walsh, M., *Journal of Inorganic Biochemistry.* **2007,** 101 (8), 1108-1119.
[48] Hu, L. H.; Shen, X.; Jiang, H. L., Zhang, Y.; Ma, L., *Faming Zhuanli Shenqing.* 200710040733.8,**2007**-5-16.
[49] Bhardwaj, S.; Mishra, A. K.; Kaushik, N. K., *Trends in Applied Sciences Research.* **2006,** 1 (2), 115-122.
[50] Castaldi, P.; Garau, G.; Palma, A.; Deiana S., *Journal of Inorganic Biochemistry.* **2012,** 108 (1), 30-35.
[51] Auriol, D.; Aurelie, G.; Fabrice L.; Renaud, N., *PCT Int. Appl.* PCT2009067736,**2011**-10-20.
[52] Pisani, L.; Catto, M.; Giangreco I.; Leonetti, F.; Nicolotti, O.; Stefanachi, A.; Cellamare, S.; Carotti, A., *Chem Med Chem.* **2010,** 5 (9), 1616-1630.